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Determination of trace metals in commercially available Khat (*Catha edulis* Forsk) in Addis Ababa.

BY

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Determination of trace metals in commercially available Khat (*Catha edulis* Forsk) in Addis Ababa

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By

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Dedication

To my beloved father the late

Tilahun Woldemariam

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Table of Contents

Page

List of Tables

Page

List of Figures

Abstract

Khat (Catha edulis Forsk) is a plant that grows in certain areas of East Africa and the Arab Peninsula. A large number of people in Ethiopia chew khat leaves because of its pleasurable and stimulating effects. In the present study the level of selected Trace metals (Cu, Zn, Mn, Ni, Co, Cr, Cd and Pb) in four different kinds of khat sampled from different khat shops in Adiss Abeba were analyzed. Known weights of oven dried Khat samples were digested by wet digestion using 3 mL of HNO_3 ,1 mL of $HCLO_4$ and 1 mL of H_2O_2 by setting the temperature first to 60 ^0C for 30 min and then increased to 210 ^0C for the next 2 h and 30 min. The contents of the minerals in the digests were analysed using flame atomic absorption spectrometry. The following concentration ranges in dry weight (μg/g) were obtained in the khat samples: Cu (19.2 - 24.4); Zn (24.1 – 46.9); Mn (14.92 – 20.6); Ni (4.7 – 7.7); Co (3.1 – 7.76); Cr (3.1 – 6.76); Cd (1.3 – 2.9) and Pb (4.8 – 9.1). Among the essential metal analyted Zn was the most abundant one followed by Cu,Mn,and Ni Whereas Co and Cr were less than Pb. Generally 'Bahirdar' Khat contained higher concentration compared to Gelemso, Gurage and Wondo for the majority of the mineral nutrients identified. The tested four kinds of khat proved high variability ($p<0.05$) in their content of trace metals. The possible uptake by the human body from fresh khat has also been determined. Except Cd and Pb the amount of metal that one may take through consumption of fresh khat alone were found to be below the recommended daily allowance

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Key Wordes: Khat (Catha edulis Forsk), Bahirdar, Gelemso, Gurage, Wondo,

Adiss Abeba, Trace metal, Flame Atomic Absorption Spectrometry

1. Introduction

The chewing of khat (or qat) leaves (*Catha edulis Forsk*.) is widely practised in East Africa and parts of the Middle East, such as The Yemen where it forms a deep-rooted social and cultural function [1]. This habit has now spread to ethnic communities in the rest of the World, including Britain such as the Somali Communities in South Wales and London [2]. The pleasure derived from khat chewing is attributed to the euphoric actions of its content of (-)-S-cathinone, a sympathomimetic amine with properties described as similar to those of amphetamine [3 - 5]. Although (-)-S-cathinone is restricted under international convention and in the UK under the Misuse of Drugs Act 1971, khat is not controlled and its possession and use are not restricted in the UK [2].

1.1. Plant origin, history and geographic distribution

Some oral traditions claim that khat originated from Yemen, however the literature indicates that khat originated from Ethiopia, specifically in Hararghe with a gradual expansion to different parts of Ethiopia, Yemen and other parts of the world [6]. 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 khat in tropical Africa extends from north Arabia to South Africa. In Africa it is well established in Ethiopia, Eritrea, Somalia, Kenya, Tanzania, Uganda, Burundi, Rwanda, Democratic Republic of Congo, Zambia, Zimbabwe, Southern Rhodesia and South Africa, despite efforts of the respective governments to discourage its cultivation. In East Africa it grows in the range of 1500-2500 metres above sea level. Outside Africa it is planted in the Arabian Peninsula, Yemen, Afghanistan, India and Sri Lanka for consumption and in the USA, UK and France for experimental purposes.

1.1.1. The plant

Khat is an evergreen perennial shrub plant that belongs to the Celastraceae family. There are several names for the plant, depending on its origin:chat-Ethiopia,qat-Yemen [7,8], qaad/jaad-Somalia [9], qu`t, Catha, gat, tohat, and muraa [10].The dried leaves of khat are known as Abyssinian tea or Arabian tea [11].

Khat usually grows up to 7 meters but occasionally reaches as high as 15 to 25 meters. The khat plant is polymorphic and the branches have either opposite or alternate leaves. The leaves are 2–5 cm wide and 5–10 cm long. The shapes of the leaves are elliptical and have serrated edges. Old leaves are leathery in texture, highly polished on their upper surface and deep green in colour. The leaf peduncle is around 3–7 mm long [12]. The leaf odour is faintly aromatic and the taste is astringent and slightly sweet [3]. The buds and leaves contain an alkaloid and are 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 stem is straight and slender; the bark has different colours depending on the variety and age of the stem and branches. The young branches are smooth and green to pinkish but grey and sometimes rougher and darker on older branches and stems. The root system can grow as deep and as long as 3 -5 m.

1.1.2. Distribution in Ethiopia

The total area of land under khat cultivation in Ethiopia in the year 1997/98 was estimated at 78,570 hectare [13]. Oromia, mainly East and West Hararghe zones, is the most important centre of khat production (East Hararghe zone alone contributes 53.4% of the total production area) in Ethiopia. Hararghe is considered to be the most important producer of quality khat in the world [14]. Despite silent support and objection against the crop by development institutions, khat is cultivated and expanding in different parts of Ethiopia (Fig. 1). It can be grown rain fed and/or irrigated, though the later covers less than 20% of the total khat area. The crop could be planted both in home garden and in the field [8].

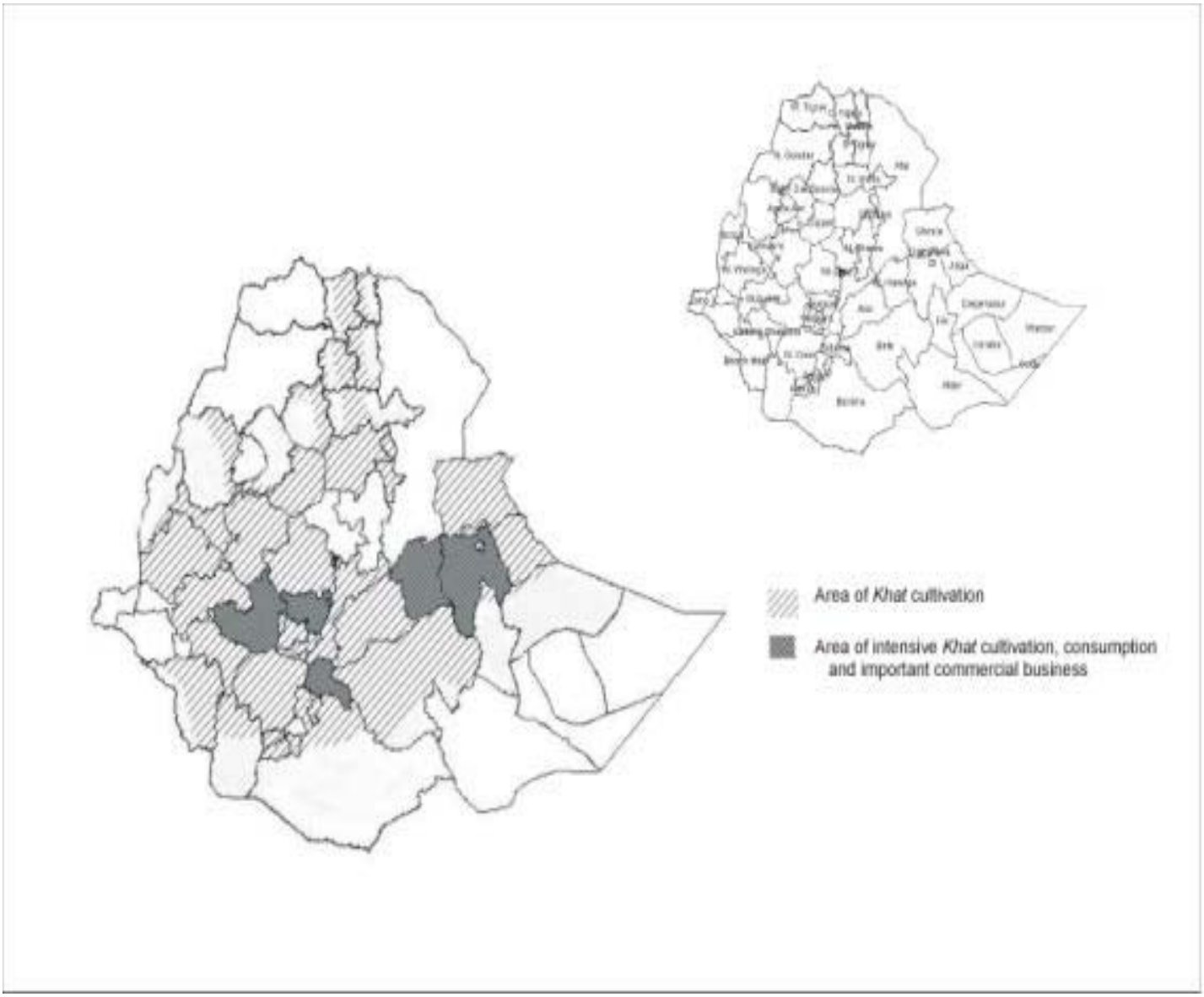


Fig 1 Map showing (rough) distribution of khat in Ethiopia

1.1.3. Soils and topography

Soil with high clay content is not suitable for khat production. The crop requires well drained dark red-brown, sandy loam with a low percentage of clay and medium to high amounts of total nitrogen, organic matter, available phosphorus, calcium, potassium and magnesium [15]. Khat performs best on soils with a pH of 6.0-8.2. Nevertheless, once established, khat grows well under a wide range of soil types and climatic conditions. The optimal altitude for growing khat ranges

from 1500-2100 m. In Ethiopia khat is extensively grown and thrives best in mid-land (1500 - 2500 m), but it can also be grown with irrigation down to 1000 m if the area is free of frost. At the early development stage of the plant, water supply is more critical than soil type. The field should be well manured and drained for good crop performance [8].

1.1.4. Harvesting

From planting a stand of khat to regular harvesting normally takes 2 to 3 years though Krikorian and Amare (1973) state five to eight years, although it requires a further 6–7 years for the tree to attain maturity [16]. A healthy tree will continue to give good harvests for another 50 years. It appears that khat is not damaged to any extent of economic significance by any known pathogens [12]. Farmers of the crop consider the presence of a tiny green leaf hopper (Empoasca species) to be beneficial because it causes older tips to wilt and die off for the eventual emergence of the new shoots which can be harvested. However, prior to harvesting, the hopper is removed by dusting the plants with very fine dust particles. This appears to be effective by preventing the hopper from attaching itself to the tree. Recently, some farmers have used an insecticide to get rid of the hopper. The compound is mixed with plant juice which is consumed by the hopper. Although the use of the insecticide leads to better branches and leaf growth, there are concerns relating to the safety to humans. Indeed, many consumers look for khat bundles, that have not been treated with the insecticide. As a result, many farmers are returning to the fine dust treatment to achieve customer satisfaction. Khat is harvested by breaking off the young branches from the main branches and trimming it to about 40-cm. Depending on growth stage of the harvestable products there are different types of khat products. Young and soft shoots are detached with the bare hands, while hardy shoots are cut off by hand tools. Each harvestable product is locally given name (s). Khat has short shelf-life and cannot be kept for longer than 2-3 days. Mature khat should be harvested and marketed without delay; otherwise the quality deteriorates and loses market value [17].

1.2. Processing methods and Marketing

There are a number of procedures and processes employed to ensure the marketable value of the harvested material of khat. The consumable part is harvested and put in shawls or plastic sacks at farm level and taken home for sorting and grading by plucking off the leathery leaves and trimming the long stems. The selected material and the unfit/ unmarketable portion, locally called garaaba, are separated. The unfit part is set aside for animal feed and as compost material for later use as manure. The selected and marketable part is tied into haqara/bundle (40-60 selected slender twigs) and splashed with water to keep the product wet and fresh. It is then wrapped with fresh leaves and twigs of different plants and grasses Fig 2 [8]. At best, the leaves can remain in an acceptable condition for up to 5 days. However, it is an important commercial fact that the value of the leaves drops dramatically after the first day of harvesting. This reflects the instability of the main active constituents in the isolated leaf, which was appreciated by the consumer a long time before the scientist [18]. (-)-S-Cathinone, the main effective constituent of the khat leaves, is relatively unstable and decomposes into (-)-norpseudoephedrine and norephedrine within a few days of picking or if the leaf is dried. Thus, only freshly picked leaves have the full efficacy. Norpseudoephedrine and norephedrine are slowly absorbed and then excreted mainly in the unchanged form within about 24 h [19]. The major metabolites of (-)-S-cathinone after ingestion are (-)-R,S-norephedrine and (-)-R,R-norpseudoephedrine [19].

Fig. 2 Khat wrapped with banana leaf

In Ethiopia khat is used for direct consumption, local sale and for export. It is estimated that 85 to 90% of khat production is sold, the rest is used for local consumption. In Ethiopia, the full range of means to transport khat are employed including: head load, human shoulder, packing animals, small automobiles, trucks, trains and aeroplanes, progressing from the farm level through to regional and international markets [8].

1.2.1. Kinds of marketable khat products and Forms of consumption

When plants grown under different climatic characteristics were subjected to quantitative analysis, it was found that the chemical profile of khat leaves grown in Yemen was largely determined by the environment in which it grows rather than by the cultivators [12]. Therefore the type of khat in Ethiopia is referred to by its geographic area of cultivation. Thus, there are more than 15 types of khat, from different geographic areas of the country. Fig 3 shows the four kinds of khat in Ethiopia, which studies under this paper.

Khat is grown for its tender leaves and twigs/stems, which are chewed for their mild stimulating effect. They taste sweet to bitter when fresh, based on the type of khat consumed. Although the most common way of obtaining the stimulating effect of khat is by chewing fresh leaves and soft twigs, consuming dried and pounded materials, in the form of tea, infusion and smoking is also seen [8].

Baherdar

Gurage

wondo

Gelemso

Fig 3 Four kinds of Khat

1.3. Uses

Economic use: In Ethiopia khat is an important and potentially profitable cash crop. The employment opportunity created through the cultivation of khat is very high in that a large number of people are involved in growing, harvesting, sorting, packing, transporting, loading and unloading the commodity. The wood of the plant is commonly used for fuel and due to its resistance to termite is used in the construction of houses and fencing. It is also used for making rafters, handles of farm tools (hammers and chisels) and handles of household articles such as pots and pans, rolling pins, and to make forks, combs, spoons and for rulers [20].

Medicinal use: Processed leaves and roots are used to treat influenza, cough, gonorrhea, asthma and other chest problems. The root is also used for stomach ache and an infusion is taken orally to treat boils [21].

Social value: Khat has considerable social value. It is served to welcome and entertain guests, in mourning, weddings and circumcision ceremonies and collective labour works. Khat chewing has its own associated ceremonies like smoking of incense, cigarettes and use of drinks (soft drinks, tea and milk) [17].

Environmental value: In Ethiopia khat is grown in an intensive production system. It is planted in rows on hillsides along terraces in association with different food crops, mainly annuals, and oriented against slopes. As such, khat cultivation plays key role in controlling soil erosion, which is a major threat in most area of Ethiopia due to the undulated topography and intensive deforestation for farmland expansion and hence khat culture is considered to be the best agroforestry system practiced by farmers. Had it not been for the cultivation of khat, the erosion of topsoil would have been severe and possibly disastrous in few area of Ethiopia [8].

1.4. Khat chewing

Fresh leaves from khat trees are chewed daily by over 20 million people on the Arabian Peninsula and East Africa [17, 22]. Recent reports suggest that 80–90% of male adult and 10–60% of female adult populations in East Africa consume khat on a daily basis [23]. New patterns of khat consumption, including morning chewing sessions and khat parties, have emerged in these East African countries [23].Traditionally khat has been chewed by Muslims during religious ceremonies and during prayer to facilitate contact with Allah [24]. Today chewing is common among other religions and in most parts of the country [20]. A recent survey of one region in rural Ethiopia placed the prevalence of current use at about 50% of the total population [25]. Some of the effects of khat chewing are enhanced concentration, feelings of euphoria and suppression of hunger and sleep. Khat is also chewed to increase work efficiency and facilitate social interactions [21].

The effect of khat varies from person to person. Some people mention that the main reason for chewing is an enhancement of socialisation and that the ceremony is a nice way of spending time with friends, not as much the physical effect of khat such as staying awake or getting ”high”. Different production areas and varieties of the khat are reported to give dissimilar effects on the same chewer [20].

The active ingredient in khat is the alkaloid cathinone, sometimes called “natural amphetamine” [26]. The khat is preferably chewed within two days after harvesting, after that the leaves loose much of the desired effect.

Even though khat is mainly a social drug, it is taken to treat illnesses such as malaria [27]. The medical use of khat has a long history and in Harar region people consider khat to treat more than 500 ailments. Historically, khat has also been used as medicine to treat symptoms of depression and melancholia and there are sources telling about Alexander the Great using khat to cure ill soldiers [24]. In the eastern parts of Ethiopia khat is known to treat influenza as well as coughs and asthma [21]. The effect of khat chewing varies according to the type of khat and according to the person. Such as, euphoric, cheerful sensation and excitement stage, which last about 1–2 hour [28, 29].

1.4.1. Addiction

Most khat chewers consider that khat sessions represent an important social occasion to meet other people and for the exchange of ideas and information. [30, 31]. The use of khat often starts at a young age and can develop into a compulsive daily habit lasting a lifetime and practiced by both men and women [32 - 34]. Khat taking behaviour depends not only on the reinforcing psychostimulant action of khat, but also on deeply rooted cultural factors [35]. Habitual use of khat is in many instances compulsive, as indicated by the tendency of khat chewers to secure their daily supply of the leaves at the expense of vital needs and their behaviour at the markets [3, 35]. This is described as a psychological dependence by many authors [36]. In eastern African countries the prevalence of chat dependence is estimated to be 5–15% of the population [35]. The

first documented case of khat addiction was that of Amda Tsion, an Ethiopian ruler in the early 14th century, and his subjects in the city of Mar'ade (Dillmann, 1884 as quoted by Giannini et al., 1992). Giannini reported two cases of khat addiction which were effectively treated with bromocriptine using a protocol developed for cocaine addiction [37]. From their description it could be inferred that both cases satisfy the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) [38] criteria for substance abuse. It is postulated that khat could have a higher dependence potential than amphetamine [36] because of its less aversive nature [39], higher rate of self administration, and rapid onset of action in discrimination experiments [40] when compared to amphetamine in various operant experiments. Although cathinone is known to cause sensitization upon repeated administration similar to amphetamines, there are reports of tolerance to the CNS stimulating effects of chat chewing. This is alleged to be due to the physical limits on the amount that can be chewed rather than the inherent property of khat leaves [41]. There are conflicting opinions regarding the existence of a withdrawal syndrome. Generally it is believed that there is no physical withdrawal syndrome as experienced with alcohol, morphine or barbiturates [42, 43]. Abandoning the khat-chewing habit however is followed by symptoms including lassitude, anergia, nightmares, slight trembling, and depression [9, 25]. Indeed, habitual users report that they have no serious difficulties when moving to an area where khat cannot be obtained [36]. However, there are reports of social withdrawal symptoms after cessation of the habit, described as 'experiences of deprivation of joys and camaraderie which khat session almost unfailingly provides'. As in the case of drug abuse in industrialized societies, khat use is associated with simultaneous use of other drugs especially cigarette and alcohol [44]. Cigarette smoking is believed to enhance the effect obtained by chewing khat and to reduce its bitter taste while alcohol is widely used to counteract the stimulatory effects of khat. The concomitant use of psychotropic agents such as hypnotics is also common [44].

1.4.2. Legal considerations

Khat circulates freely in Yemen, Ethiopia (despite the Orthodox Tewahdo Church prohibiting its use), Somalia (though briefly banned during the six months rule of the United Islamic Courts in Mogadishu in 2006) and some other East African countries [25, 29]. Almost every small kiosk in Addis Ababa, the Ethiopian capital, openly sells khat, and in small cities and towns all over the

country it is brought to market as produce, where people publicly chew it and offer it to visitors as a mark of hospitality [45]. In Yemen and Ethiopia there have been attempts to curtail the habit for social and economic reasons but these have met with little success [1, 9]. One reason for this is that in Yemen [17] and in some parts of Ethiopia it is consumed by government officials, making its regulation very difficult. Although the active alkaloids of khat, namely cathinone and cathine have been labeled as Schedule I and Schedule III substances respectively by WHO since 1971 [46], its status in European countries is not uniform [36]. For example, khat is prohibited in Ireland, France, Switzerland, Sweden and Norway [22, 29] whilst it is legal in the U.K. and in the Netherlands [47, 48]. Outside of Europe, it is illegal in the U.S.A. and Canada but permissible in Australia [22]. Recently, the WHO Committee reviewed the data on khat and determined that the potential for abuse and dependence is low and the threat to public health is not significant enough to warrant international control, and did not recommend the scheduling of khat [49].

1.5. The toxicological potential of khat

Chewing its fresh leaves is a widespread habit in the local populations, with several million people consuming khat regularly in social sessions that often last for hours [17]. Users of khat report increased levels of energy, alertness and self-esteem, a sensation of elation, enhanced imaginative ability and a higher capacity to associate ideas. These effects have been attributed to the khat's content in cathinone, a sympathomimetic amine with properties similar to those of amphetamine [5], although other less potent stimulant substances may also be present, namely norpseudoephedrine (cathine) and norephedrine [17]. Like amphetamine, the pharmacological profile of cathinone, has been shown to result from the increased release of monoamine neurotransmitters from nerve terminals [3]. Taking into account the structural and pharmacological similarities between cathinone and amphetamine it might be expected that the toxic effects could also be alike.

In humans, adverse effects common with those of amphetamine have also been observed in khat users, namely a depressive stage which usually comes at the end of the khat session (which depends on the potency of the khat), mydriasis, irritability, anorexia, hyperthermia, insomnia and endocrine disturbances .The "next morning" set of symptoms, which include lethargy, sleepy state

and crave for khat leaves, are also characteristic [17]. The oral consumption of khat by people of Arab Peninsula has been linked with oesophageal carcinoma [50]. Accordingly, human genotoxicity caused by khat chewing was ascertained by the occurrence of micronucleus in exfoliated buccal and bladder cells of khat consumers [51]. The chronic use of khat has also been associated with the development of hypertension, psychosis, cognitive impairment and disabling neurological illness [52], as well as an increased incidence of acute coronary vasospasm and myocardial infarct [53].The possible effects of sustained oxidative stress induced by khat consumption may lead to oxidative damage of cellular macromolecules such as DNA, lipids and proteins contributing to the development of several pathologies, notably cancer, hepatotoxicity, nephrotoxicity, cardiovascular toxicity and neurodegenerative diseases.

The most obvious effect of khat-use can be seen on the digestive system where gastritis is fairly common among regular chewers due to the effects of the acid tannin in khat. Daily intake can also cause chronic constipation. Hyperactivity, insomnia and hypertension are other side effects of khat, as well as dental and oral problems [9]. Khat is said to have reproductive toxicity in human beings and several studies have shown that khat use can lead to a decrease in sperm quality, low birth weight and inhibition of lactation in mothers [20]. Khat should not be taken during pregnancy [27].

Khat in itself is rarely considered to cause psychological disorders, but in persons already prone to mental illness, intake of khat can lead to psychosis or schizophrenic reactions. There are reports though, on regular chewers who become psychotic when they increase their intake of khat. More common are withdrawal symptoms such as nervousness, nightmares, lethargy and “absent-mindedness”[20].

1.6. Chemical aspect of khat

1.6.1. The chemical profile of khat

The leaves and young shoots of *Catha edulis*, a species of the plant family Celastraceae, are usually referred to as khat (Family: *Celastraceae*, genus: *Catha*, and Species *Catha edulis*).Most taxonomists consider that the genus Catha consistes of the single species Catha edulis [54]. The environment and climate condition determine the chemical profile of khat leaves. In Yemen Arab Republic about 44 different types of khat exists originating from different geographic areas of the country [17].

Khat contains a lot of chemical components that may have different effect on the body system.Such as, alkaloids, terpenoids,flavonoids sterols,glycosides,tannins,more than 10 amino acids including tryptophan, glutamic acid, glycine, alanine and threonine [10, 55],trace quantities of vitamins including ascorbic acid, riboflavin, niacin, and carotene [55], negligible amount of fluoride [56] and Elementes including copper, Zinc, and toxic metals like lead and cadmium [57].

Most of our present knowledge on the constituents of khat is derived from studies in the late 1970s and 1980s following recommendation by the UN Commission on Narcotic Drugs [58]. The phenylalkylamines and the cathedulins are the major alkaloids. Szendrei (1980) at the UN Narcotics Laboratory, together with Schorno and Steinegger at the University of Berne, Switzerland, isolated and identified the phenylalkylamine, (−)-α-aminopropiophenene, later named as S-(−)-cathinone as a major active constituent in fresh khat [55]. The plant contains the (–)-enantiomer of cathinone only [49, 59] which has the same absolute configuration as S-(+)-amphetamine (see Fig. 4) [36, 60]. During maturation, cathinone is enzymatically converted to cathine [(+) norpseudoephedrine] and (−)- norephedrine [61]. Sunlight-induced or heat-induced degradation to cathine and norephedrine also occurs during extraction of cathinone in the laboratory [62]. Indeed, to slow down the degradation process, the khat leaves are usually wrapped in banana leaves immediately after picking to retain their moisture.

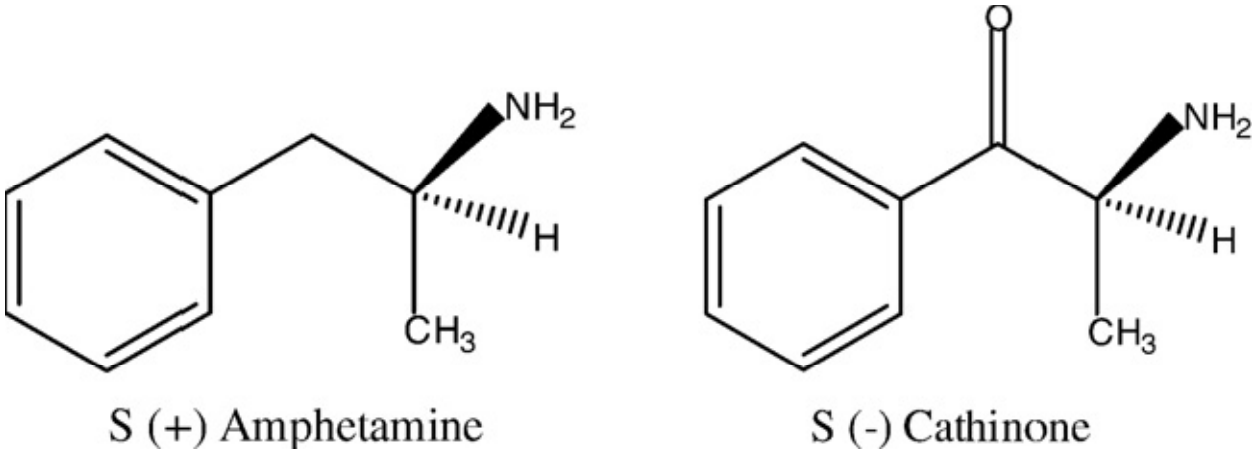


Fig.4. Chemical structure of S-(+)-amphetamine and S-(−)-cathinone

Other alkaloids of phenylalkylamines in khat leaves include the phenylproanolamine diastereoisomers of cathine [1S, 2S-(+) norpseudoephedrine or (+) norpseudoephedrine], and norephedrine [1R, 2S-(−) norephedrine]. Cathine and norephedrine occur mainly in older plants and is also formed by reduction of cathinone during drying and storage. Cathinone is transformed mainly to cathine in khat leaves and mainly to norephedrine by human metabolism (see Fig. 5) [63]. The environment, climate conditions, as well as local traditions connected with cultivation and harvesting determine the chemical profile and general appearance of khat leaves [54]. The phenylalkylamine content of khat leaves varies widely. In certain khat samples, the phenyalkylamine fraction consisted of up to 70% of (−) cathinone and that the (−) cathinone content is correlated with the market price of khat (see Table 1) [64]. Accordingly, analyses of khat samples from Kenya and Ethiopia have shown that the commercial value of the material correlates with its cathinone content [65].

Another class of phenylalkylamine alkaloids found in khat leaves are the phenylpentenylamines; merucathinone, pseudomerucathine and merucathine, which are mainly detected in khat leaves originating from Kenya [66] (Fig. 6). They seem to contribute less to the stimulant effects of khat [36] and their concentration is relatively low [66]. Other classes of alkaloid in khat are the cathedulins, classified according to their relative molecular mass [58]. Recently, 62 different cathedulins derived from fresh khat leaves were characterized [58]. Though there has been much

research on the phenyalkalymines, there has been little investigation of the cathedulins to date [67].

Table 1 Concentration of khat alkaloids from fresh khat leaves of various origins

Cathinone	Cathine	Norephedrine	Origin	References
0.74±0.40	1.49±0.51	0.9±0.16	Kenya	[68]
1.09±0.8 3	60±1.9	0.25±1.8	Ethiopia, Kenya, Yemen and Madagascar	[65]
1.02±0.11	0.86±0.06	0.47±0.05	Kenya	[29]

Data given in mg per gram of khat leaf expressed as mean ± SD;

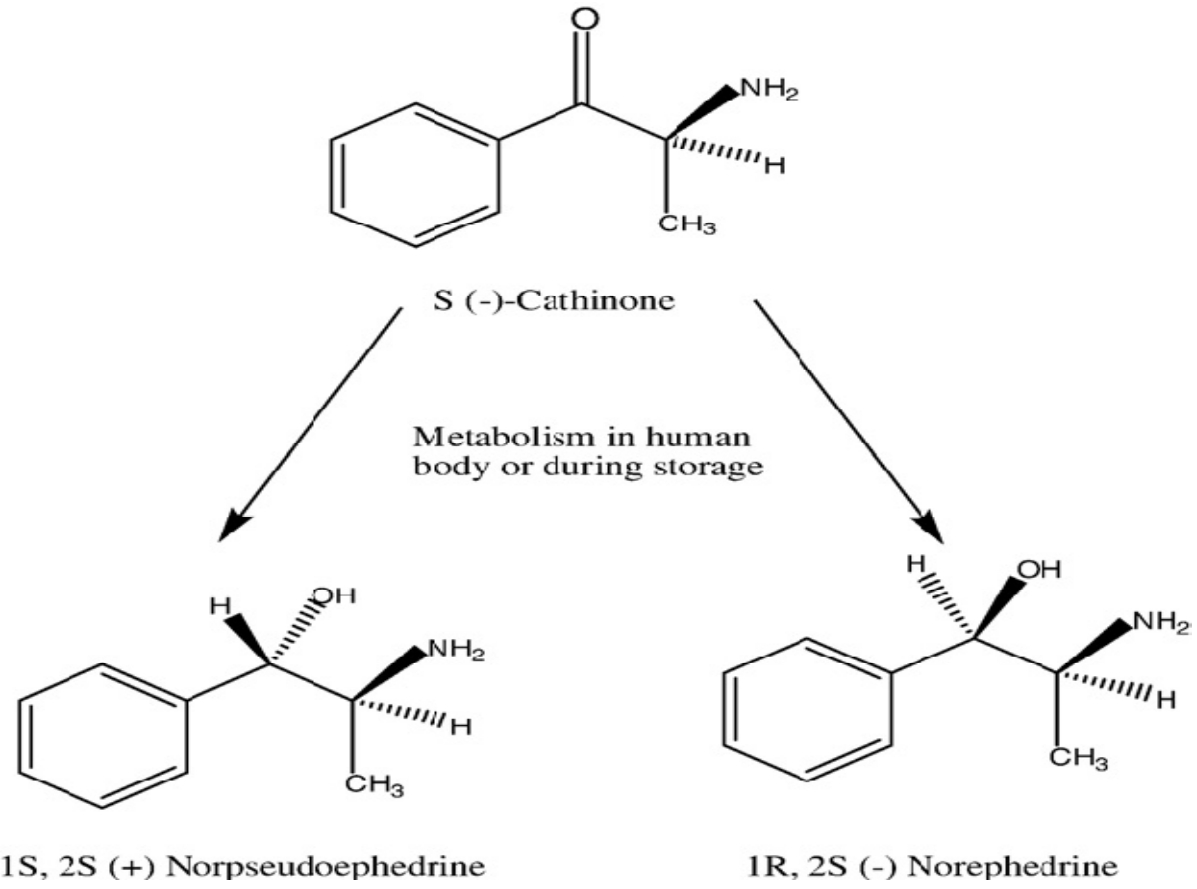


Fig. 5 Chemical structures of S-(−)-cathinone, S, S-(+)-norpseudoephedrine (cathine) and R, S-(−)-norephedrine.

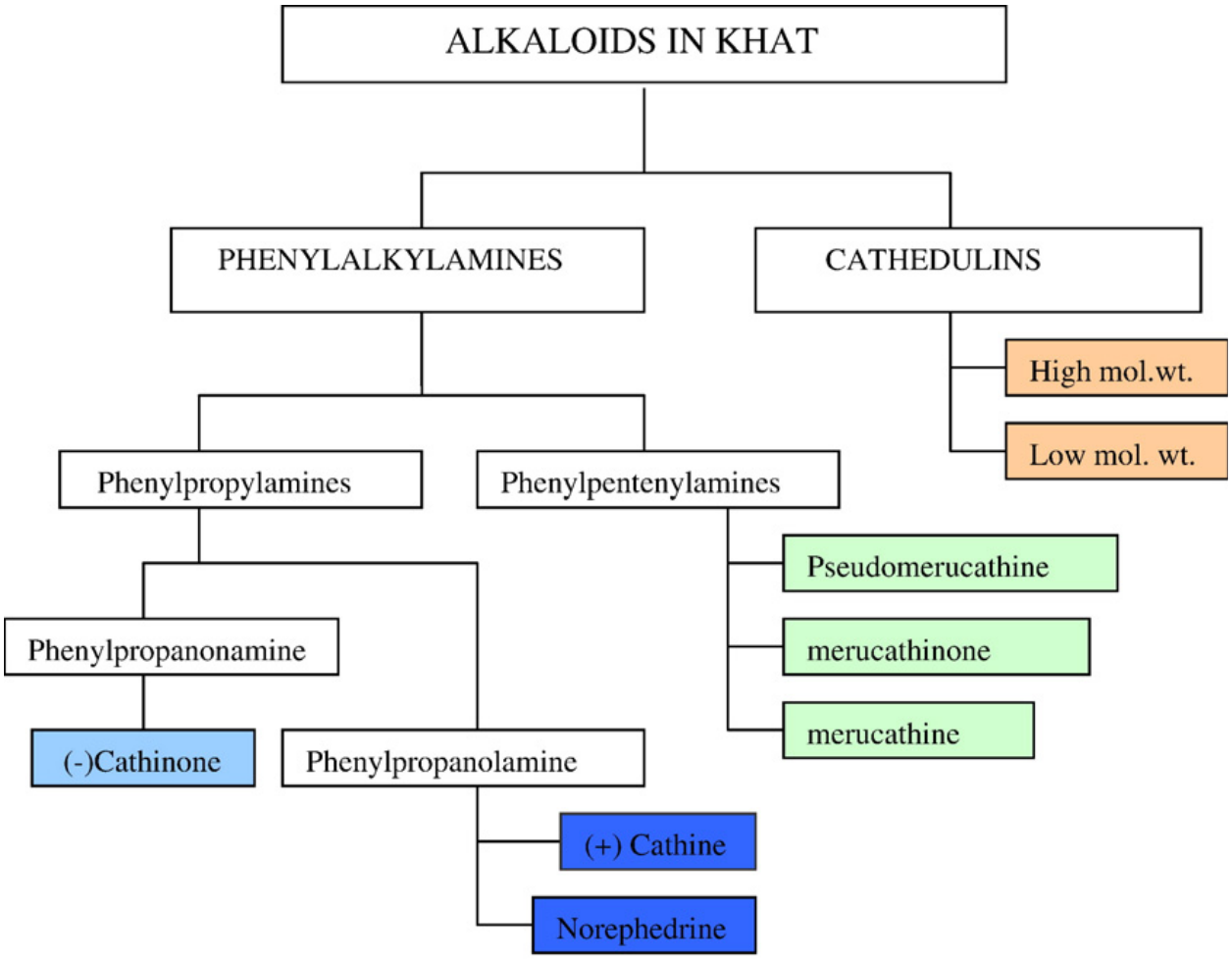


Fig. 6. Summary of class of different alkaloids in khat leaves.

1.6.2. Pharmacology of khat

By chewing the khat leaves, cathinone is effectively extracted into the saliva and directly absorbed in to the body fluid. The absorption of the constituents of khat is said to have two phases, the first being at the oral mucosa, plays a major role in the absorption of alkaloids [69]. The second phase is following swallowing of the juice, at the stomach and/or small intestine [69]. For cathinone,maximal plasma concentrations are reached 2 to 2.5 hours after the start of a session; for cathine, after 2.6 hours. Cathinone has a mean terminal elimination half-life of 3 h; for cathine it is approximately 5 hours [69].

After ingestion,S-(-)-cathinone is reduced to its main metabolites R,S-(-)-norephedrine and S,S-(+)-norpseudoephedrine [69]. Numerous laboratory studies have confirmed that cathinone resembles amphetamine in chemical structure and that it affects the central and peripheral nervous system [17] cathinone is about 50% less potent and cathine 7–10 times less potent than amphetamine, and both compounds are subject to rapid decay [70]. Johanson and Schuster (1981) also investigated the relative potency of cathinone and amphetamine in monkeys and found that amphetamine was two to four times more potent than cathinone . Therefore, any risk assessment based on the amphetamine analogy would be inadequate and unjustified. Thus, the ongoing debate around this prompted us to identify the need to review the scientific evidence for the association between khat and mental illness [40].

In the Central Nervous System (CNS), cathinone provokes the release of catecholamines, especially dopamine,at the presynaptic storage sites, and is about half as potent as amphetamin; it inhibits re-uptake and can produce depletion of cenreral dopamine [62].Cathine has a much smaller effect in the release of central catecholamines. Peripherally, cathinone and cathine are equipotent in the release of noradrenaline at the presynaptic storage sites; thus,both have sympathicomimetic effects. The short term physiological effects of chat reflect the sympathicomimetic and central dopaminergic activity; e.g. increased heart rate and elevated blood pressure [69].The immediate emotional effects in experimental human settings are euphoria and a subsequent depressive reaction. Thus, it has been argued that the depressive phase after the end of the chat session motivates the user to continue to chew [17].

1.7. The classification of trace element

The concentration of major and minor elements in living tissues can be expressed in grams per kilograms. On the other hand, the concentration of trace elements in living tissues varies between 0.01 and 100 mg Kg^{-1} . It may not be appropriate to classify them as essential or toxic elements. It is logically wrong to establish a category of “toxic” elements, because any element may be potentially toxic and this property is but a function of concentrations to which humans are exposed. Essentiality of the trace elements is established when a further reduction below the range of tolerable levels, better known as ‘rang of safe and adequate intakes’, results in a consistent and

reproducible impairment of a physiological function[71]. All major miner elements are important; besides that, some of the trace elements Cr, Fe, Co, Cu, Zn, Se, Mo, Mn and I are essential trace elements; and some of them; Ni and V are probably essential trace elements; and further some possibly essential may also be toxic in animals and humans if ingested at sufficiently high levels and for a long enough period [71].

Essential trace elements are required by man in amounts ranging from 50μg day^{-1} to 20 mg day^{-1}. The organism can neither grow nor complete its life cycle without the element in question. The element should have a direct influence on the organism and be involved in its metabolism. The effect of the essential elements cannot be wholly replaced by any other elements [72]. The bioavailability of essential elements depends on their chemical form, the composition of the diet and health situation of the individuals. Thus, establishment of the optimum daily requirements and determinations of actual daily intake of essential elements are important problems of trace elements in nutrition [72]. The physiological role of Cu, Zn, Mn, Ni, Co, Cr, Cd and Pb are briefly described below.

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 haemoglobin. 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 intak of copper can cause vomiting, nervous system disorder and wilso's diseases [72, 73].

Zinc (Zn)

Zinc is an essential element found in the tissue of animals and plants even at normal ambient concentration. However, if plants and animals are exposed to large concentration of bioavailable Zn, significant bioaccumulations can result, with possible toxic effects. Zinc is the most ubiquitous of the trace element involved in human metabolism. More than one hundred specific

enzymes require zinc for their catalytic function. If zinc removed from the catalytic site, activity is lost; replacement of zinc restores activity [73].

Zinc participants in all major biochemical pathways and plays multiple roles in the perpetuation of genetic material, including transcription of DNA, translation of RNA, and ultimately cell division. When the supply of dietary zinc is insufficient to support these functions, biochemical abnormalities and clinical signs may develop. Studies in individuals with acrodermatities enteropathica, a genetic disorder with zinc mal-absorption resulting in sever deficiency; have provided much insight into the functional outcomes of zinc deficiency. These include impairments of dermal, gastrointestinal, neurologic and immunologic systems [73].

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 physiologic effects, 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 [71, 73].

Cobalt (Co)

Cobalt is an essential element that is part of vitamin B_{12}, or cobalamin, a coenzyme that is essential in the formation of proteins, nucleic acids, and red blood cells. Although cobalt poisoning is not common, excessive levels can be harmful. Most cases of human exposure to toxic levels of cobalt have occurred through inhalation in the workplace. Many exposures have been suffered by workers working with hard metal alloys of cobalt and tungsten carbide, where very

fine particles of the alloy produced from grinding it were inhaled. The adverse effects of cobalt inhalation have been on the lungs, including wheezing and pneumonia as well as allergic asthmatic reactions and skin rashes. Lung fibrosis has resulted from prolonged exposures. Human epidemiology and animal studies suggest an array of systemic toxic effects of cobalt, including, in addition to respiratory effects, cardiovascular, hematological hepatic, renal, ocular, and body weight effects.

Exposure to cobalt is also possible through food and drinking water. An interesting series of cobalt poisonings occurred in the 1960s when cobalt was added to beer at levels of 1 to 1.5 ppm to stabilize foam. Consumers who drank excessive amounts of the beer (4 to 12 L per day) suffered from nausea and vomiting, and in several cases, heart failure and death resulted [73].

Nickel (Ni)

It is strongly suspected of being an essential trace element for human nutrition, although definitive evidence has not yet established its essentiality to humans. A nickel-containing urease metalloenzyme has been found in the jack bean. Toxicologically, nickel is important because it has been established as a cause of respiratory tract cancer among workers involved with nickel refining. Of course, the potential importance of nickel in human nutrition is not limited to deficiency. Like other mineral elements, nickel ingested in high amounts can have adverse effects. However, because of excellent homeostatic regulation, life-theatening toxicity of nickel through oral intake is unlikely. Generally, greater than 250 μgg-1 of diet are required to produce signs of nickel toxicity (such as depressed growth and anaemia) in animals. But the generality “nickel is relatively nontoxic” cannot be made for humans [73, 74].

Cadmium (Cd)

Cadmium has no known nutritional value, and it is highly toxic to both plants and animals. The biochemical effects of Cd in humans include interference with enzymatic activity, the ability of interacting with nucleic acids and damaging kidney, hypertension and anosmia (absence of smell).

Cadmium is known to accumulate in the kidney. This kidney damage leads to calcium deficiency in the rest of the body, particularly in the skeleton [73].

Manganese (Mn)

Manganese is an essential element to both plants and animals. It is necessary for normal bone metabolism and important enzyme reactions. It also helps to maintain normal nerve, brain and thyroid function 50 .While a deficiency of this mineral is uncommon, it is often lost in processed foods [74].A deficiency of manganese may affect brain health, glucose tolerance, normal reproduction, and skeletal and cartilage formation. Grains and cereal products are the best food sources of manganese, while animal products are the poorest. Toxicity from manganese is uncommon. Exposure to high level of Mn can caus both mental and emotional disturbance, along with increased slowness and clumsiness of the body movements. This disease is called manganism. Any brain injury due to the accumulation of Mn in the brain is permanent [74].

Chromium (Cr)

Chromium exists in three main forms: metallic state, trivalent and hexavalent forms. While hexavalent chromium is recognised as an industrial toxin linked to lung cancer, trivalent chromium is acknowledged as an essential nutrient. The latter is known to improve insulin sensitivity and, therefore, to influence carbohydrate, fat and protein metabolism. Diabetes and coronary heart disease are associated with low chromium concentration in human tissue [71, 73].

Several spectrometry techniques have been used for macro and trace elements determination in plants or biological materials. Flame atomic absorption spectrometry (FAAS) is the most widely used technique for the metal determinations because most of the concentrations of metals in environmental samples are readily determined using this technique [75]. Furthermore AAS is cheap and its usage is easier than other instruments.

1.8. Purpose and scope

Khat-related studies, in Ethiopia and elsewhere, have so far mainly emphasized the chemical properties, the chewing culture and its social implications, drug abuse and marketing aspects [17, 26, 76]. In Ethiopia, [20] has studied the contribution made by khat to both household and regional economies, while [77] examine the effects of khat expansion on the farming system, as well as economic and social conditions.

During the last two decades, important progress has been made in understanding the pharmacological and social effects of khat, but less attention has been paid to the concentration of trace elements in khat. Since trace metal have role in disturbing trace element levels in human tissues and body fluids [48].

Ethiopia is one of the main consumer countries of khat. It is the country where khat plantation is ever increasing. Different types of khats are grown in different regions of the country. The environment and climate condition determine the chemical profile of khat leaves. Therefore it is necessary to determine the level of trace metals in different kinds of Ethiopian khat in order to know health effect. Moreover, the findings of this study will provide adequate information on the distribution of trace metals in Ethiopia khat.

1.9. Specific objectives

1. To develop suitable methods for the digestion of khat samples.
2. To determine the levels of both essential and toxic trace metals (Zn, Cu, Mn, Ni, Cr, Co, Pb and Cd) in khat collected from Addis Ababa by FAAS.
3. To compare the levels of metals between the four kinds of khat sold in Addis Ababa.
4. To compare the levels of metals in the Ethiopian khat with literature data.

2. Experimental

2.1. Apparatuses and reagents

2.1.1. Apparatuses

Ceramic pestle and mortar were used to grind and homogenize the dried khat sample. A drying oven (DIGITHEAT, J.P.SELECTA,S.a, Spain) was used to dry khat samples. A digital analytical balance (Mettler Toledo, Model AG204, Switzerland) with ± 0.0001 g precision was used to weigh chat samples. A 100 mL round bottomed flasks fitted with reflux condensers were used in Kjeldahl apparatus hot plate to digest the dried and powdered khat samples. **BUCK SCIENTIFIC MODEL 210 VGP** (East Norwalk, USA) atomic absorption spectrophotometer equipped with deuterium ark back ground correctors was used for analysis of the analyte metals (Mn, Ni, Pb, Zn, Cu, Co, Cr and Cd) using air-C_2H_2 flame.

2.1.2. Cleaning apparatus

Apparatus such as volumetric flasks, measuring cylinder and digestion flasks were washed with detergents and tap water, rinsed with deionised water, soaked in 8 % nitric acid for 24 hr, rinsed with deionised water five times, dried in oven and kept in dust free place until analysis begins.

2.1.3. Reagents and chemicals

Reagents that were used in the analysis were all analytical grade. (69-72 %) HNO_3 (Research lab. Fine Chemical industries, Mumbai), (70%) $HClO_4$(Research lab. Fine Chemical industries, Mumbai) and (30 %) H_2O_2 (Scharlaw Chemie.S.A) were used for digestion of Khat samples. Stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals Mn, Ni, Pb, Zn, Cu, Co, Cr, Cd (BUCK SCIENTIFIC PURO-GRAPHIC^{tm}) were used for the preparation of calibration standards and in the spiking experiments. Deionized water was used throughout the experiment for sample preparation, dilution and rinsing apparatus prior to analysis.

2.2. Procedures

2.2.1. Description of sampling sites

For the collection of khat Saris, Mercato, 6 Kilo and Piazza were chosen among sites in Addis Ababa. The reason for selection of these places was based on two things. First, most of the khat sold in Addis Ababa is distributed from these places, So that fresh khat can be obtained. Secondly, commercially available Khat in urban markets particularly Addis Ababa may mix fresh khat with khat which have stayed more than 24 hr longer after harvesting or with different kinds, Like low cost with high cost Khat . So, in order to have accurate information, the samples were collected from those places where the khat samples are sold without mixing.

2.2.2. Khat Sampling

Sixteen (16) Khat samples i.e. four kinds (Wondo, Baherdar, Gurage and Gelemso),which are sold in Addis Ababa, were bought from the Khat Shops of different sites. There are two reasons for selecting the above four kinds of khat among others. First, they are found everywhere in Addis Ababa, so they can be easily accessed by consumers. Secondly, the origins of the plants are located in four corner of Addis Ababa. Fifty grams of a particular khat from each Khat shop, which are from four different sites, were bought for the purpose of random sampling. Each sample was collected in a pre-cleaned polyethylene bag, washed thoroughly with distilled water and oven dried at 80^0C for 24 hr. The dried sample was grind and homogenized. Finally four bulk samples were prepared for analysis.

2.2.3. Digestion of Khat Samples

Applying the optimized procedure, 0.5 g of dried and homogenized khat samples were transferred into a 150 mL round bottomed flask. To this 5 mL of a mixture of HNO_3 (69- 72%), $HCLO_4$(70%) and H_2O_2 (30%)with a volume ratio of 3:1:1 and the mixture was digested on a micro Kjeldahl digestion apparatus by setting the temperature first to 60 ^{0}C for 30 min and then increased to 210 ^{0}C for the next 2 h and 30 min then after the digested solution was allowed to cool for 5 min

without dismantling the condenser from the flask and for 10 min after removing the condenser. To the cooled solution 8 mL of deionized water was added to dissolve the precipitate formed on cooling and to minimize dissolution of filter paper by the digest residue while filtering with Whatman®, (110 mm, diam), filter paper. The round bottom flask was rinsed with 8 mL deionized water and the solution was filled to the mark (50 mL) with deionized water. Triplicate digestions were carried out for each bulk sample. The digested samples were kept in the refrigerator, until the level of all the metals in the sample solutions were determined by FAAS. Six blank solutions were prepared following the same digestion procedure as the sample.

2.2.4. Determinations of trace metals in khat samples

Secondary standard solutions containing 10 mg/L were prepared from standard stock solutions that contained 1000 mg/L. These secondary standards were diluted with deionized water to obtain four working standards for each metal of interest. Cu, Zn, Mn, Ni, Co, Cr, Cd, and Pb were analyzed with FAAS (BUCK SCIENTIFIC MODEL 210GP) equipped with deuterium arc background corrector and standard air-acetylene flame system using external calibration curve after the parameters (burner and lamp alignment, slit width and wavelength adjustment) were optimized for maximum signal intensity of the instrument. Three replicate determinations were carried out on each sample. Hallow cathode lamp for each metal operated at the manufacturer's recommended conditions were used at its respective primary source line. The acetylene and air flow rates were managed to ensure suitable flame conditions. The eight elements were determined by absorption/concentration mode. The same analytical procedure was employed for the determination of elements in six-digested blank solutions. The operating conditions for FAAS employed for each analyte are given in Table 2.

Table 2 Instrumental operating condition for the determination of metals in Khat Samples using flame atomic absorption spectrophotometery.

Element	Wavelength (nm)	Detection limit (mg/L)	Slit width (nm)	Lamp current (mA)	Energy
Cu	324.7	0.020	0.7	1.5	3.775
Zn	213.9	0.005	0.7	2.0	3.001
Mn	279.5	0.010	0.7	3.0	3.863
Ni	232.0	0.040	0.2	7.0	3.762
Co	240.7	0.050	0.2	4.5	3.338
Cr	357.9	0.050	0.7	2.0	3.623
Cd	228.9	0.005	0.7	2.0	3.065
Pb	283.2	0.100	0.7	2.0	3.507

2.2.5. Recovery test

The efficiency of the optimized procedure was checked by adding known concentration of each metal in 0.5 g sample. The procedure was as follow: 0.5 µg of 1000 mg/L. Ni, Co, Cr, Cd and Pb were spiked at once in to 0.5 g of Khat sample and the remaining metals 5 µg of Zn and 2.5 µg of Mn and Cu were spiked at once in to the same round bottomed flask containing 0.5 g of Khat and the same digestion process as sample was followed. The sample was determined for their spiked metals by atomic absorption spectrophotometery. Recovery test for the sample was performed in triplicates.

2.2.6. Method detection limit

Six blank samples were digested following the same procedure as the samples and each of the samples were determined for the elements of interest (Cu, Zn, Mn, Ni, Co, Cr, Cd, and Pb) by atomic absorption spectrophotometery. The standard deviation for each element was calculated from the six blank measurements to determine method detection limit.

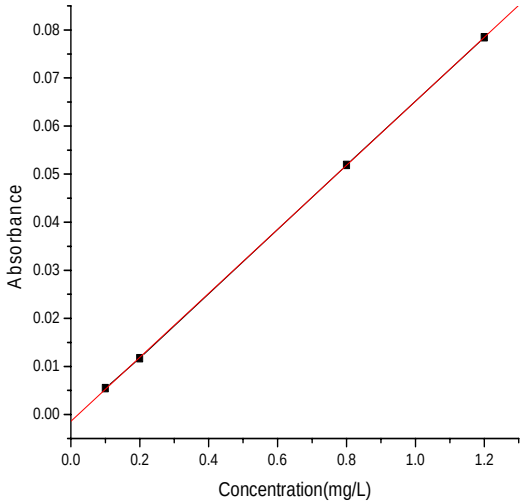
3. Results and Discussion

3.1. Instrument Calibration

The qualities of results obtained for trace metals analysis using AAS are seriously affected by the calibration and standard solution preparations procedures. The instrument was calibrated using four series of working standards. Concentrations of the intermediate standards, working standards and value of correlation coefficient of the calibration graph for each of the metals are listed in Table 3. The calibration graph of each of metals of interest is shown in Figure 7.

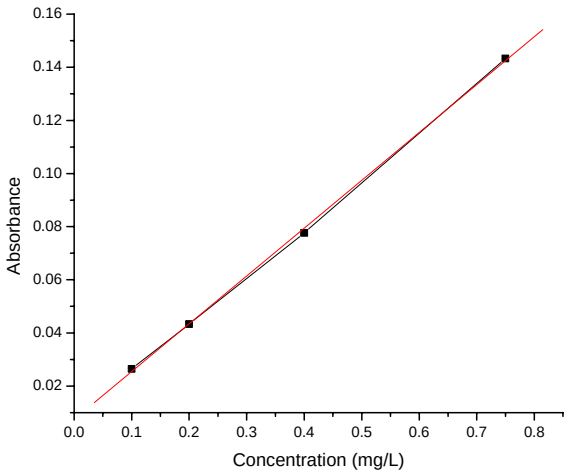
Table 3 Working standards and correlation coefficients of the calibration curves for determinations of metals using flame atomic absorption spectrophotometer

No	Metal	Concentration of intermediate standard (mg/L)	Concentration of standards, in mg/L	Correlation coefficient of calibration curves
1	Cu	10	0.1, 0.2, 0.8,1.2	0.9999
2	Zn	10	0.1, 0.2,0.4,0.75	0.9997
3	Mn	10	0.05,0.2, 0.4, 0.8	0.9998
4	Ni	10	0.05, 0.1, 0.2,0.4	0.9997
5	Co	10	0.05, 0.2, 1,2	0.9999
6	Cr	10	0. 08, 0.16,0.36, 0.72	0.9999
7	Cd	10	0.01,0.04,0.2,0.8	0.9999
8	Pb	10	0.1, 0.3, 0.6, 1.2	0.9998



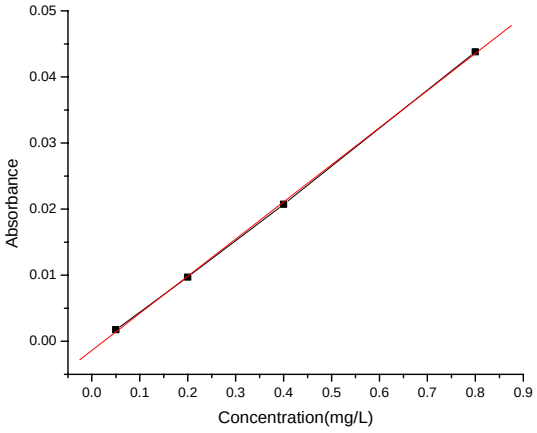
Y = -0.00139+ 0.06654X
R = 0.9997

Figure 7- a. Calibration graph of Cu
Standard solution



Y = 0.00743 + 0.18005X
R = 0.99999

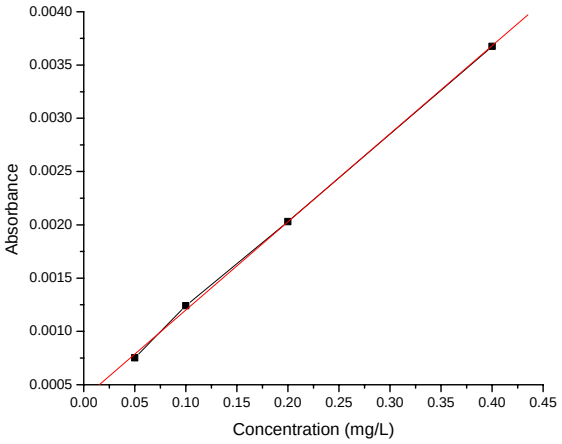
Figure 7-b. Calibration graph of Zn
Standard solution



Y = -0.00138+ 0.0562X
R = 0.9997

Figure 7-c. Calibration graph of Mn

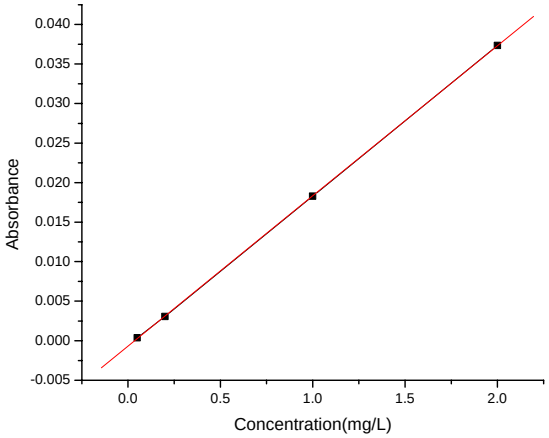
Standard solution



Y = 3.74717E-4+ 0.00827X
R = 0.9998

Figure 7-d. Calibration graph of Ni

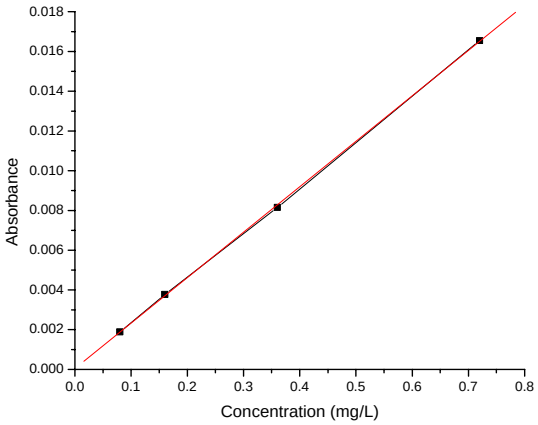
Standard solution



Y = -6.63805E-4 + 0.01899X

R = 0.9999

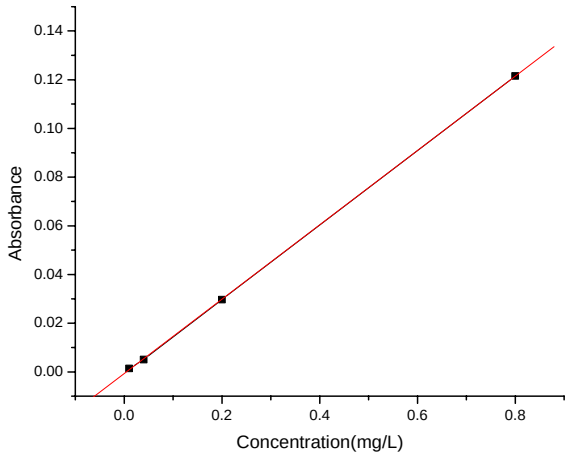
Figure 7-e. Calibration graph of Co Standard solution



Y = 5.18985E-5 + 0.02285X

R = 0.9999

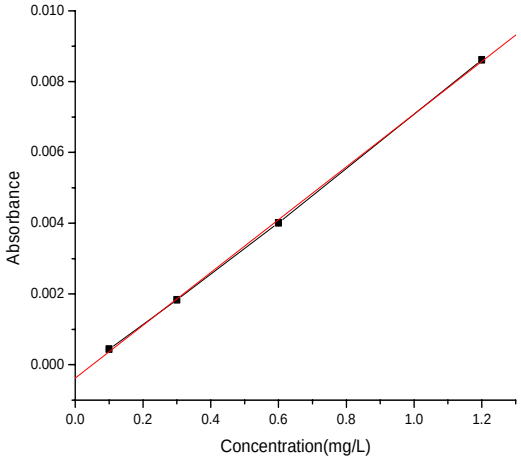
Figure 7-f. Calibration graph of Cr Standard solution



Y = -6.88574E-4 + 0.15272X

R = 0.9999

Figure 7-g. Calibration graph of Cd Standard solution



Y = -3.73384E-4 + 0.00745X

R = 0.9998

Figure 7-h. Calibration graph of Pb Standard solution

3.2. Optimization of Digestion procedure of Khat Samples

One of the basic requirements for sample preparation for analysis is to get an optimum condition for digestion. The optimum condition is the one which leads: Minimum reagent volume consumption, Minimum digestion time, Minimum residue (clear solution) and Ease of simplicity.

Optimizing of the digestion procedure involved some changes of parameters such as reagent volume, digestion temperature and digestion time. Accordingly, thirteen procedures were tested for digestion of khat (Table 4). Based on the above listed criteria, the optimal digestion procedure chosen was the one that fulfilled the selected criteria for complete digestion of 0.5 g of the dry sample powders, with 3 mL of HNO_3 (62- 79 %), 1 mL $HClO_4$ (70 %) and 1 mL H_2O_2 (30 %) for a total of 3 hours. The mixture was digested smoothly by setting the temperature first to 60 ^{0}C for 30 min and then increased to 210 ^{0}C for the next 2 h and 30 min then after the digested solution was allowed to cool for 5 min without dismantling the condenser from the flask and for 10 min after removing the condenser. The procedures that required higher reagent volume longer digestion time and colored digest solution were rejected.

Table 4 Procedures tested during optimization of method for digestion of khat samples

No	Sample size	Reagent added	[a]Intial Temp.	Final Temp.	[b]Digestion time	Nature of the Digest After Filtration
1	0.5 g	2 mL HNO_3 (69 – 72 %) 1 mL H_2SO_4 2 mL $HClO_4$ (70 %)	60 ^{0}C	180 ^{0}C	3 hr	Clear and Yellowish color
2	0.5 g	3 mL HNO_3 (69 – 72 %) 1 mL $HClO_4$ (70 %)	60 ^{0}C	180 ^{0}C	3 hr	Clear and Yellowish color
3	0.5 g	3 mL HNO_3 (69 – 72 %) 0.5 mL H_2SO_4 1 mL $HClO_4$ (70 %)	60 ^{0}C	180 ^{0}C	3 hr	Clear and Pale Yellow color
4	0.5 g	3 mL HNO_3 (69 – 72 %) 1 mL H_2SO_4 3 mL $HClO_4$ (70 %)	60 ^{0}C	180 ^{0}C	3 hr	Clear and Pale Yellow color
5	0.5 g	3 mL HNO_3 (69 – 72 %) 1 mL $HClO_4$ (70 %) 1 mL H_2O_2 (30 %)	60 ^{0}C	180 ^{0}C	3 hr	Clear and Pale Yellow color

6	0.5 g	3 mL HNO_3 (69 – 72 %) 0.5 mL H_2SO_4 1 mL $HClO_4$ (70 %)	60 ^{0}C	180 ^{0}C	4 hr	Clear and almost colorless
7	0.5 g	3 mL HNO_3 (69 – 72 %) 1 mL $HClO_4$ (70 %) 1 mL H_2O_2 (30 %)	60 ^{0}C	180 ^{0}C	4 hr	Clear and Colorless
8	0.5 g	2 mL HNO_3 (69 – 72 %) 1 mL H_2SO_4 2 mL $HClO_4$ (70 %)	60 ^{0}C	180 ^{0}C	4 hr	Clear and Pale Yellow color
9	0.5 g	2 mL HNO_3 (69 – 72 %) 2 mL $HClO_4$ (70 %) 1 mL H_2O_2 (30 %)	60 ^{0}C	180 ^{0}C	4 hr	Clear and Yellowish color
10	0.5 g	3 mL HNO_3 (69 – 72 %) 1 mL $HClO_4$ (70 %)	60 ^{0}C	180 ^{0}C	4 hr	Clear and pale Yellow color
11	0.5 g	3 mL HNO_3 (69 – 72 %) 0.5 mL H_2SO_4 1 mL $HClO_4$ (70 %)	60 ^{0}C	210 ^{0}C	3 hr	Clear and pale yellow color
12	0.5 g	3 mL HNO_3 (69 – 72 %) 1 mL $HClO_4$ (70 %) 1 mL H_2O_2 (30 %)	60 ^{0}C	210 ^{0}C	3 hr	Clear and Colorless Optimum
13	0.5 g	2 mL HNO_3 (69 – 72 %) 2 mL $HClO_4$ (70 %) 1 mL H_2O_2 (30 %)	60 ^{0}C	210 ^{0}C	3 hr	Clear and Yellowish color

[a] Initial temperature for 30 minutes

[b] The total time in hour for digestion including smoothly heating for 30.

3.3. Evaluation of Analytical Figures of Merit

3.3.1. Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at two levels: repeatability and reproducibility. For these guidelines, a simple assessment of repeatability will be acceptable. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

In this study the precision of the results were evaluated by the pooled standard deviation, and relative standard deviation of the results of three samples (n = 3) and triplicate readings for each sample meaning that a total of 9 measurements for a given bulk sample. These parameters are useful in estimating and reporting the probable size of indeterminate error. The results of the present analysis are reported with corresponding pooled standard deviation of nine measurements for a bulk sample and triplicate reading per sample and relative standard deviation. Table 7 - 10 shows % RSD of each metal in each khat sample.

3.3.2. Method detection limit

Method detection limit is the lowest analyte concentration that produces a response detectable above the noise level of the system, typically three times the noise level but not necessarily quantitated as an exact value. For the present study, replicate analyses for six blank samples for all elements were performed and the pooled standard deviation of the six blank samples was calculated. The detection limits were obtained by multiplying the pooled standard deviation of the reagent blank by three. As can be seen from Table 5, the method detection limit of each element is above the instrument detection limit.

Table 5 Method detection limit for khat samples (n=6)

Element	MDL (mg/g)	Instrument detection limit (mg/L)
Cu	0.004	0.020
Zn	0.002	0.005
Mn	0.003	0.010
Ni	0.002	0.040
Co	0.001	0.050
Cr	0.001	0.050
Cd	0.0007	0.005
Pb	0.002	0.100

MDL = method detection limit

3.4. Evaluation of Analytical Method

Method validation is the process of providing that analytical method is acceptable for its intended purpose. Therefore, analysts are increasingly encouraged to validate analytical procedures and to estimate uncertainty associated to the results. Since there is no certified reference material (Khat) in our laboratory, the validity of the optimized digestion procedure for Khat were checked by carrying out with a lower level of traceability, such as spiked samples. As shown in Table 6 the percentage recovery for khat samples are lie in the range 91 - 102 % , which are within the acceptable range for all metals was obtained.

Table 6 Recovery test for Khat samples (dry sample)

Metal	[a]Conc.in sample (µg/g)	Amount added (µg/g)	[b]Conc.in spiked sample (µg/g)	[c]Recovery (%)
Cu	20	5.0	24.96 ± 0.04	99.2 ± 3
Zn	37	10	46.1 ± 1.2	91 ± 9.6
Mn	16.8	5.0	21.52 ± 0.45	96.8 ± 5.6
Ni	4.7	1.0	5.67 ± 0.18	97 ± 3
Co	4.9	1.0	5.91 ± 0.13	101 ± 5
Cr	3.2	1.0	4.11 ± 0.12	91 ± 9
Cd	2.3	1.0	3.23 ± 0.01	93 ± 5.4
Pb	7.4	1.0	8.42 ± 0.2	102 ± 3.2

[a] average value of four analyzed samples (µg/g).
[b] values are mean ± SD of triplicate readings of triplicate analyses.
[c] values are mean ± SD of triplicate readings of triplicate analyses.

3.4.1 Levels of Metals in Khat

The concentration of eight trace elements (Zn, Mn, Pb, Co, Ni, Cd, Cu, and Cr) in the digested and diluted solutions of Khat were identified with flame AAS. The levels of total metal contents of the four kinds of khat samples show that khat could be a source of nutrients in addition to its use as a stimulant. The level of the essential and non-essential metals in the khat samples differ widely along with geographical location. The concentrations of the metals in each of the four kinds of the commercially available Ethiopian khat are reported and discussed in the following subtopics.

3.4.1.1 Level of metals in 'Bahirdar' Khat

The levels of the metals in Bahirdar Khat and Relative standard deviation (%RSD) are given in Table 7.The results shows that out of the six analyzed essential metals Cu,Zn,Mn and Ni were found in larger amount compared to Co and Cr. Zn is the largest amount with dry weight of 46.9 ± 1.6 µg/g followed by Cu 19.2 ± 0.46 µg/g.Mn and Ni was found to be 17.5 ± 0.46 µg/g and 7.7 ± 0.2 µg/g respectively. The nonessential heavy metals Cd and Pb were also found in the sample. The amount of Pb in the sample was larger than even some of essential metals (Co,Cr and Ni) with concentration of 9.1 ± 0.4 µg/g. But Cd 1.3 ± 0.12 µg/g was the smallest. The amount of Co and Cr are 3.1 ± 0.2 µg/g and 3.3 ± 0.2 µg/g respectively. The amount of the analyzed metals in the khat samples could be arranged in an increasing order of Zn>Cu>Mn>Pb>Ni>Cr>Co>Cd. Figure 8 shows the level of trace metals.

Table 7 Level of metals in 'Bahirdar' Khat (dry weight) and % RSD

Metal	Cu	Zn	Mn	Ni
[a]Concentration (µg/g)	19.2 ± 0.46	46.9 ± 1.6	17.5 ± 0.46	7.7 ± 0.2
%RSD	4.6	3.5	2.7	2.6

Metal	Co	Cr	Cd	Pb
[a]Concentration (µg/g)	3.1 ± 0.2	3.3 ± 0.2	1.3 ± 0.12	9.1 ± 0.4
%RSD	7	6	9	4

[a] values are mean ± SD of triplicate readings of triplicate analyses.

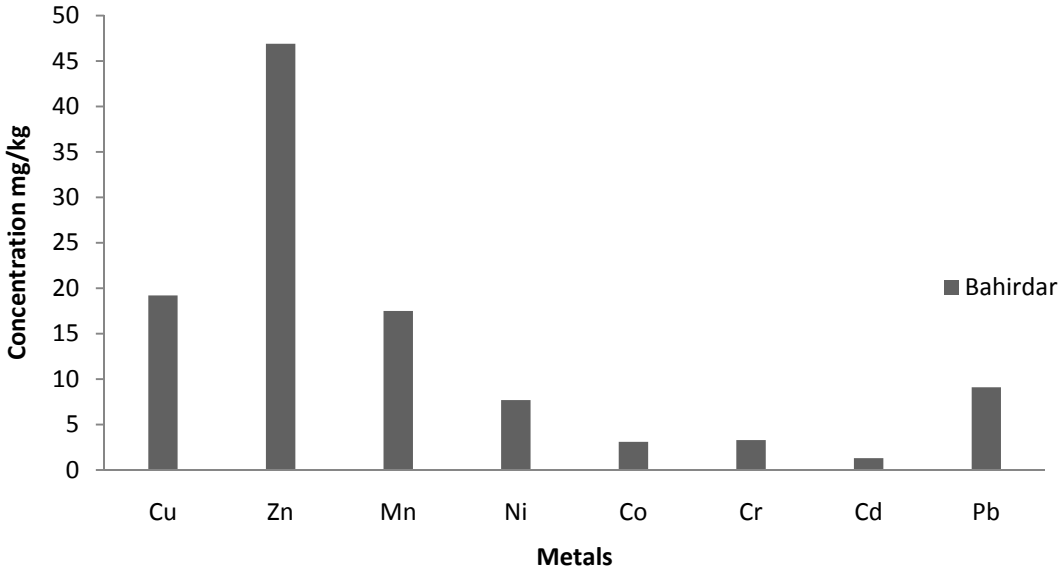


Fig.8. Levels of Trace Metals in 'Bahirdar' Khat

3.4.1.2. Level of metals in 'Gelemso' Khat

Table 8 presents the level of the analyzed metals in Gelemso khat. The results show that Zn, 37 ± 2.4 µg/g was the largest of the eight metals. Cu and Mn were found in larger amount next to Zn with values of 20 ± 0.4 µg/g and 16.8 ± 0.3 µg/g respectively. Out of the nonessential Pb 7.4 ± 0.3 µg/g was found to be in larger amount while Cd, 2.3 ± 0.18 µg/g was the least of all. Ni,Co and Cr were found to be 4.7 ± 0.2 µg/g, 4.9 ± 0.24 µg/g and 3.2 ± 0.2 µg/g respectively. The increasing trend was found to be Zn>Cu>Mn>Pb>Co>Ni>Cr>Cd. Figure 9 shows the level of heavy metals in decreasing order.

Table 8 Level of metals in 'Gelemso' Khat (dry weight) and % RSD

Metal	Cu	Zn	Mn	Ni
[a]Concentration (µg/g)	20 ± 0.4	37 ± 2.4	16.8 ± 0.3	4.7 ± 0.2
%RSD	5.8	6	1.8	4

Metal	Co	Cr	Cd	Pb
[a]Concentration (µg/g)	4.9 ± 0.24	3.2 ± 0.2	2.3 ± 0.18	7.4 ± 0.3
%RSD	4.8	6.7	8	4.7

[a] values are mean ± SD of triplicate readings of triplicate analyses.

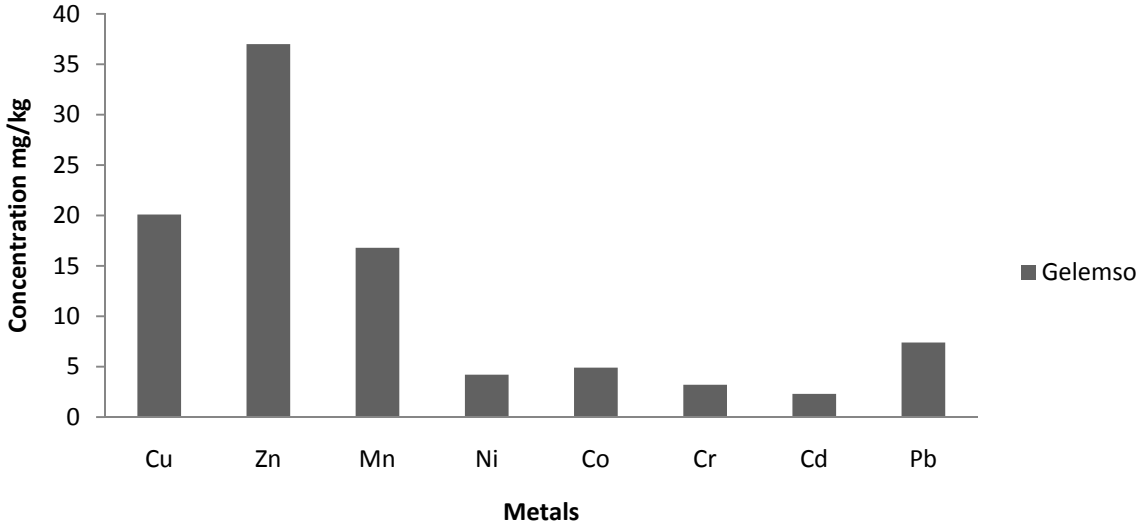


Fig.9. Levels of Trace Metals in 'Gelemso' Khat

3.4.1.3. Levels of metals in 'Gurage' Khat

The third kind of khat i.e.'Gurage', was also analyzed for its total metal concentration and levels of the metals found are given in Table 9. Like the previous two kind of khat, Gurage also was found to contain the largest amount of Zn, 24.1 ± 0.3 µg/g. Cu, 22.5 ± 0.46 µg/g and Mn, 14.92 ± 0.3 µg/g were found to be next to Zn. Ni,7.01 ± 0.21 µg/g was found to be larger than Pb, 6.3 ± 0.37 µg/g. Co, 6.1 ± 0.32 µg/g was found to be around two fold of Cr, 3.1 ± 0.32 µg/g.

The smallest was Cd 1.9 ± 0.14 µg/g. The increasing trend was found to be Zn> Cu>Mn> Ni> Pb>Co >Cr>Cd. Figure 10 shows the levels of heavy metals in Gurage khat.

Table 9 Levels of metals in 'Gurage' Khat (dry weight) and % RSD

Metal	Cu	Zn	Mn	Ni
[a]Concentration (µg/g)	22.5 ± 0.46	24.1 ± 0.3	14.92 ± 0.3	7.01 ± 0.21
%RSD	6	1.2	2	2.9

Metal	Co	Cr	Cd	Pb
[a]Concentration (µg/g)	6.1 ± 0.32	3.1 ± 0.32	1.9 ± 0.14	6.3 ± 0.37
%RSD	5	8.7	7.7	6

[a] values are mean ± SD of triplicate readings of triplicate analyses.

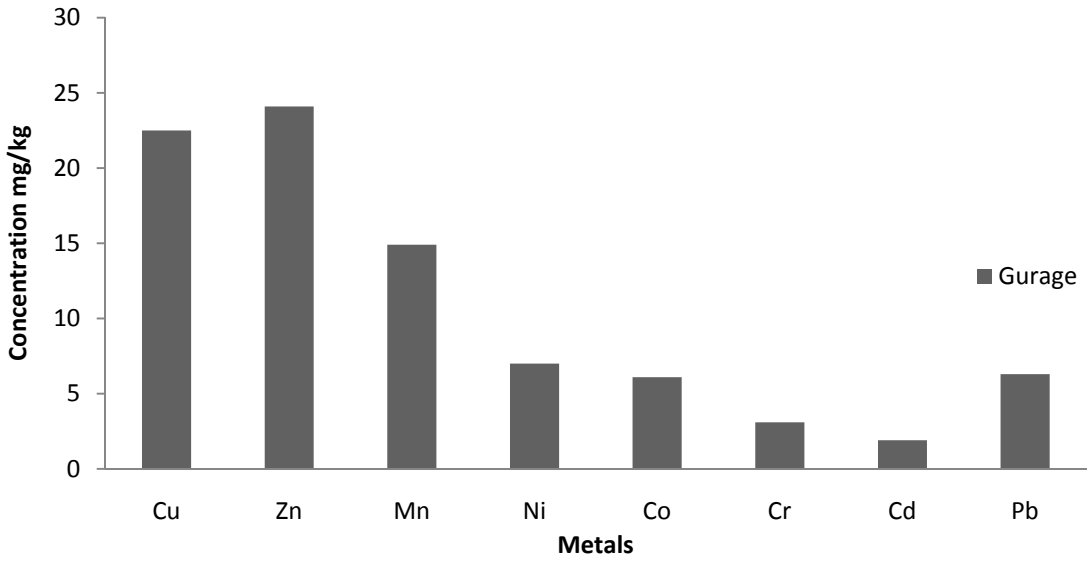


Fig.10. Levels of Trace Metals in 'Gurage' Khat

3.4.1.4. Levels of metals in 'Wondo' Khat

The last kind of khat ('Wondo') was analyzed for its eight metals content and the levels of the metals are shown in Table 10. All the essential metals were found in larger amounts than nonessential metals. Like in the previous kinds Zn is present in the largest amount of all metals, with the concentration of 26 ± 0.4 µg/g .Cu 24.4 ± 1 µg/g and Mn 20.6 ± 0.5µg/g were found to be next to Zn. Unlike others kinds the essential metal Co and Cr are larger, with concentration of 7.76 ± 0.31 µg/g and 6.76 ± 0.3 µg/g respectively. Ni, 5.2 ± 0.24 µg/g was found to be larger than either Pb 4.8 ± 0.2 µg/g or Cd 2.9 ± 0.15 µg/g. The increasing trend was found to be Zn>Cu>Mn>Ni>Co>Cr>Ni>Pb>Cd. Figure 11 shows the levels of heavy metals in Wondo khat.

Table 10 Levels of metals in 'Wondo'(dry weight) and % RSD

Metal	Cu	Zn	Mn	Ni
[a]Concentration (µg/g)	24.4 ± 1	26 ± 0.4	20.6 ± 0.5	5.2 ± 0.24
%RSD	6.8	1.5	2.4	4.6

Metal	Co	Cr	Cd	Pb
[a]Concentration (µg/g)	7.76 ± 0.31	6.76 ± 0.3	2.9 ± 0.15	4.8 ± 0.2
%RSD	4	3.8	5	3.6

[a] values are mean ± SD of triplicate readings of triplicate analyses.

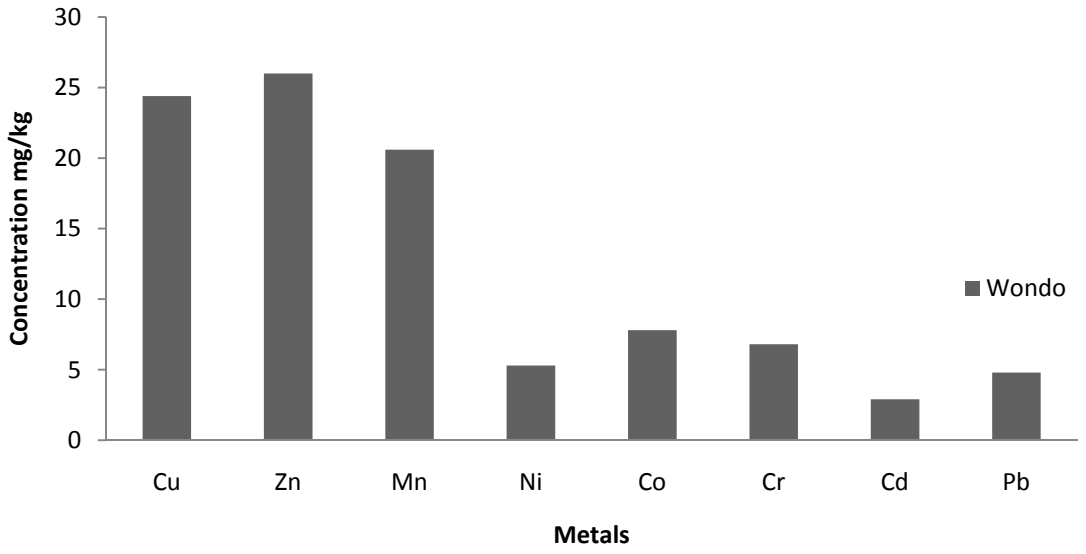


Fig.11. Levels of Trace Metals in 'Wondo' Khat

3.4.2. Comparison of the levels of metals in the four kinds of khat

When plants grown under different climatic characteristics were subjected to quantitative analysis, it was found that the chemical profile of khat leaves was largely determined by the environment in which it grows rather than by the cultivators [12]. As with all plants, the mineral composition of Khat varies from place to place depending on the climate, the soil mineral content and its pH, age of the plant being harvested.

Knowing the distribution of metals in a given food stuff is generally important to recommend it as a source of certain nutrient, fortify or supplement deficient nutrient or enhance or stop the use of the food as a staple or co-staple food. In the following section, the comparative study of the mineral contents of the four kinds of Khat is presented.

When the concentration of trace metals in four kinds of Khat were compared, Bahirdar has relatively higher essential metal concentrations similarly it also has relatively higher nonessential metal concentration. The overall comparative result between Khat samples are presented in Figure 12.

For most of the metals Bahirdar has relatively more metal concentration followed by Wondo and Gelemso, while Gurage contain the least metal concentration. The concentration of Zn is higher in all the four samples followed by Cu. From Figure 12, the trend for Zn is: Bahirdar > Gelemso > Wondo > Gurage. For Manganese the trend is reveirsed for wondo meaning that Wondo has higher amount of Mn followed by Bahirdar and Gelemso followed by Gurage. For Cr the trend is similar to that of Mn. Whereas Cu and Co, the trend is Wondo> Gurage> Gelemso> Bahirdar. Lead follows the reveres trend of Cu and Co. For Ni the trand is similar with Pb except Gelemso, it has the least. The trend for Cd is Wondo>Gelemso>Gurage>Bahirdar. The overall concentration range of all metals is given in Table 11.

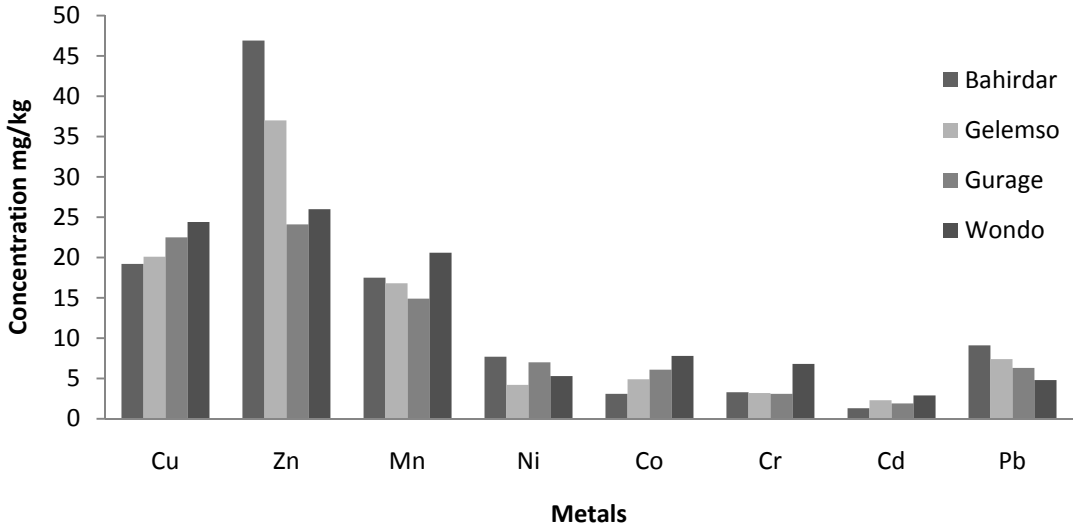


Fig.12. The overall comparative result between Khat samples

Table 11 Concentration rang of metals in Khat sample

Metals	range (μg/g)
Cu	19.2 - 24.4
Zn	24.1 – 46.9
Mn	14.92 – 20.6
Ni	4.7 – 7.7
Co	3.1 – 7.76
Cr	3.1 – 6.76
Cd	1.3 – 2.9
Pb	4.8 – 9.1

According to Matloob, the level of metals in khat compared to other vegetables collected from the same uncontaminated agricultural areas was found to be high in the level of essential metal (Cu, Zn) and comparable level of toxic element (Cd, Pb). In the present study the sample were collected from khat shops in Addis Ababa and the determination was done by Atomic Absorption Spectroscopy. As shown in Table 12, the level of Cu, Zn, Cd and Pb was higher in all Ethiopian khat compared to the Yemani khat determined by Anodic Stripping Voltammetry [57]. The reason for this variation may be; first, in the present study the sample were collected from khat market in Addis Ababa, so there are high possibilities of contamination during transportation from the harvesting area to market. Second, according to Dechassa [8] most of Ethiopian farmers use fertilizer and pesticide in order to get good yield and protect from pests. Third, geographical variation of two countries. The trend of the level of these metals is similar to the present study Zn>Cu>Pb>Cd.

Table 12 Comparison of metal level in Ethiopian and Yemani khat (mg/kg) Fresh weight

Metals	Cu	Zn	Cd	Pb
Ethiopian	4.8 - 6.1	6.03 - 11.73	0.325 - 0.7	1.2 - 2.28
Yemani	3.32 - 5.19	4.8 - 8.44	0.01 - 0.031	0.921 - 2.1

3.4.3. The level of trace metal in Ethiopian khat in relation to the Recommended Daily Allowance.

The pollution of air, soil, food and water is widely anticipated and reported in industrialized and densely populated areas. In countries like Ethiopia, there is no enough industry to cause substantial pollution. The major source of contamination comes from agricultural activities, such as the application of fertilizers and pesticides and the use of sewage sludge from biological wastewater treatment plants. National and international regulations on food quality have lowered the maximum permissible levels of toxic metals in human food; hence, an increasingly important aspect of food quality should be to control the concentrations of trace metals in food.

The geometric mean concentration levels of Cu and Zn in khat samples ranges from 19.2 to 24.4 mg/kg and 24.1 to 46.9 mg/kg dry weight, respectively (Table 11). Zn and Cu are two trace minerals essential for important biochemical functions and necessary for maintaining health throughout life. Zn deficiency results in a variety of immunological defects whereas Cu deficiency is characterized by anaemia and skeletal abnormalities [72, 73].

The average quantity of khat chewed by Ethiopians ranges from 100 g to 200 g daily. The mean daily intake of Zn by khat consumers is estimated to be 0.603 mg to 1.173 mg for 100 g khat fresh weight (a fresh weight to dry weight conversion factor of approximately 0.25 was applied when necessary). These values may not pose a health risk according to the 1989 recommended daily allowance (RDA) levels (15 mg/day for males and 12 mg/day for females) [78]. The average daily intake of Cu by khat chewers ranged from 0.48 mg to 0.61 mg for 100 g fresh weight. The current estimated safe and adequate daily intake range of Cu is 2 to 3 mg/day [78]. If other Cu sources are included, the daily intake would substantially exceed the recommended level. Negative consequences of excessive Cu intake have been extensively documented [72, 73]. Liver cirrhosis typically develops from toxic intake and abnormalities in red blood cell formation also occur.

The mean Mn and Ni content of khat ranges from 14.9 to 20.6 mg/kg and 4.7 to 7.7 mg/kg dry weight respectively (Table 11). Both are essential trace elements, Mn deficiency may affect brain health, glucose tolerance, normal reproduction and skeletal and cartilage formation. However, high level of manganese also has effects like mental and emotional disturbance [74]. Similarly

deficiency of Ni also affects enzymatic activity. In addition, its high level causes anemia and depressed growth [73, 74].

The mean daily intake of Mn by khat consumers is estimated to be 0.374 to 0.516 mg for 100 g fresh weight. These values may not pose a health risk according to the 1989 recommended daily allowance levels (3 mg/day) [78].The mean daily intake of Ni by khat consumers being 0.175 to 0.19 mg for 100 g fresh weight, is less than recommended daily allowance (< 250 mg/kg). If other source of this metal is available the amount may go beyond the recommended daily intake.

The mean Cr and Co in khat ranges from 3.1 to 6.76 mg/kg and 3.1 to 7.76 mg/kg dry weight respectively (Table 11).The availability of these metals in human are important for many biological activities [73]. The deficiency of chromium cause Diabetes and coronary heart diseases and the deficiency of cobalt is associated with deficiency of vitamin B_{12} [73].

The average daily intake of Cr and Co by consumers of 100 g/day is 0.0775 to 0.169 mg fresh weight and 0.0775 to 0.199 mg fresh weight respectively. When compared with RDA (Cr 0.5 mg/day and Co 0.3 mg/day) [79] the amount of chromium is below RDA value but for Co when consumed 200 g the amount exceed RDA value. So both metals can be a source of health effect when together with other source of those metals.

The mean Cd and Pb content of khat ranges from 3.61.3 to 2.9 mg/kg and 4.7 to 9.1 mg/kg dry weight respectively (Table 11). Compared with the literature, these levels are normal for uncontaminated areas. Which means the value is higher compared with value in the literature for uncontaminated area. Surprisingly, Bahirdar has high Pb level.

The consumption of khat contributes 0.0325 to 0.07 mg for 100 g fresh weight of Cd daily and 0.12 to 0.2275 mg for 100 g fresh weight of Pb daily. The Food and Agriculture Organization /World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives has recommended a provisional maximum tolerable daily intake of Cd and Pb from all sources (food, air and water) of 0.001 to 0.0012 mg/kg body mass and 0.0035 to 0.004 mg/kg body mass respectively [80]. These values correspond to a provisional daily intake of 0.06 mg to 0.072 mg and 0.21 mg to 0.24 mg (assuming an average Ethiopian weight of 60 kg). According to these directives, the daily intake of Cd by Ethiopian consumers from 100 g khat alone is below the

FAO/WHO provisional tolerable daily intakes. However, if above 100 g of chat is consumed the value both toxic metals (Cd and Pb) exceeds the recommended value. Therefore, the consumers are in high risk of contamination by both metals. In addition, other Pb and Cd sources are included and if khat is digested without washing (as mostly the case in Ethiopia), the daily intake may exceed the recommended levels and continuous exposure to Cd and Pb results in their gradual accumulation in human vital organs, which may cause profound biochemical and neurological changes in the body.

3.5. Statistical analysis

In this study samples were collected from Addis Ababa town from different randomly selected khat shop. Each sample was mixed thoroughly and one representative bulk sample was taken for each Kind of khat sample. From each bulk sample three aliquots taken, digested and analyzed. During this processes a number of random errors might be introduced in each aliquots and in each replicate measurements. There may be differences in results of the analysis between different khat samples within group and between groups. Therefore depending upon the type and the nature of results at hand, there are different statistical methods used to check whether there is a difference in results of analysis or not; and if there is a difference, statistical analysis will tell us whether the difference is significant or not. Of these, analysis of variance (ANOVA) is the best method [81].

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Analysis of variance (ANOVA) is used to test hypothesis about differences between two or more means. For the present study, the significance of variation within sample and between samples has been studied using one-way ANOVA. SPSS is used to calculate the presence or absence of significant difference in mean concentration of each metal between four kinds of khat samples. The following results are obtained.

A one way ANOVA shows that except Ni ($p=0.212$) the concentration of Cu ($p=0.00$), Zn ($p=0.00$),Mn($p=0.00$),Co($p=0.00$),Cr ($p=0.001$),Cd ($p=0.001$),Pb ($p=0.00$) has overall significant. When multiple comparisons were made for Cu and Cr, significant difference is between the means of Wondo and each of the other three khat samples (Bahirdar, Gurage and Gelemso). For Zn, Co and Pb the mean value of each sample was significantly different from each other. That means the

P-value of the comparison of each mean is less than 0.05 ($p<0.05$).The presence of significant difference between the means may indicate that they are in different geographical location and they grow in different climatic conditions.

No significance difference ($P>0.05$) at 95% confidence interval is observed in Ni mean concentration between each samples. However, a significant difference in Mn concentration between Bahirdar and Gurage, Wondo and Gelemso and Bahirdar and Wondo is observed. Similarly significant difference was observed between Gurage and Gelemso, Gurage and Wondo. But no significance difference in Mn concentration of Bahirdar and Gelemso ($p>0.05$).

There was no significant diference in Cd mean concentration between Gelemso and gurage, Gelemso and Wondo. But significant difference between Bahirdar and Gelemso,Wondo and Bahirdar,Bahirdar and Gurage and was found between Gurage and Wondo.

The result of the comparison of the level of metal in khat samples between washed and unwashed khat is found to be significantly higher in the later one.

Absence of insignificant difference in some mineral nutrient in the four kind of khat samples may indicates that, since plant tissue concentrations of some metals such as Cr, Ni Cu and Zn are not related to their respective amounts in the soil, even if soil pH and soil texture are considered. The differences in some metal contents of khat and other plants have been reported to be heavily dependent upon plant growth properties and species [82]. Similarly presence of significant difference in concentration for some minerals indicates that either of the studied sample area contains higher concentration of mineral nutrient in the soil or a well aged plant has been harvested and well managed precautions have been followed during processing.

4. Conclusion and Recommendations

In this study commercially available four kinds of Khat were analyzed for their contents of Cu, Zn, Mn, Ni, Co, Cr, Cd and Pb. The optimized wet digestion method for Khat analysis was found effective for all of the minerals and as it was evaluated through the recovery experiment, a good percentage recovery was obtained (91-102) for the minerals identified.

In this study the levels of eight elements in four different kinds of khat, commercially available in Addis Ababa, were analyzed. The result showed that all of them contain considerable concentration of trace metals. For the essential and non essential metals, the concentration is higher in Bahirdar compared to the other three kinds. In Wondo the level of all essential metals were higher than non essential metals, so it becomes best source of minerals. The ANOVA results suggest that there are significant variations in the level of some elements between the khat samples, which could be attributed to different factors such as environment and climate condition, which determine the chemical profile of khat leaves [17]. For some elements the variations are insignificant, since plant tissue concentrations of some metals such as Cr, Ni Cu and Zn were not related to their respective amounts in the soil, even if soil pH and soil texture are considered, but dependent upon plant growth properties and species [82].

The results of Cu, Zn, Cd and Pb in this study were compared with the corresponding Yemani khat reported by Matloob [57] and a significant difference was found in these metal concentrations. Specifically the amount in the present study is higher than the literature value. Indeed, there is difference in the sampling procedure and sampling area.

According to the National Herbarium, AAU, Khat has only one species. The khat load of metals in Ethiopia is generally critical; therefore, the continuous exposure to heavy metals does require attention. Daily metal intake can be minimized by washing khat properly and reducing the amount of khat digested.

It is recommended that a better understanding of the health related effect and chemical profile of Ethiopian khat, be developed through a multidisciplinary approach with the full involvement of

khat growers. Because most Ethiopian farmers uses fertilizer and pesticides to get good and attractive crop without considering its side effect on consumers; since khat is consumed directly after harvesting. The findings derived from such an approach should then be counter balanced with a realistic understanding of the negative health effect of khat use or abuse.

Generally speaking, in Ethiopia the numbers of khat chewers is increasing through time so is, its economic importance. The available reports simply focus on negative and positive aspects of khat on economic, social, cultural and agricultural value of the country. Moreover, a few researches are conducted on its chemical aspect, especially, the organic part. But no study is conducted on mineral profile of khat. In relation to the ever increasing human consumption of khat and the benefits from this crop, an overall investigation of its chemical profile and disadvantage remains an immediate issue of scholarly attention.

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