
Assessment of Selected Nutrients and Toxic Chemicals in Ethiopian Khat

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Abstract: Heavy metal pollution is among the leading health concerns all over the world because of their long-term cumulative effects. Khat (*Catha edulis* Forsk.), a plant used as a stimulant is grown in certain areas of East Africa and the Arab Peninsula. This day it is a known cash crop in Ethiopia. Due to increased demand and value, many farmers have not only begun growing it but have also adopted modern farming methods which include application of fertilizers, pesticides, compost manure, and irrigation. Yet some of these agricultural practices such as application of fertilizers and pesticides are known to increase the concentration of heavy metals such as Cd, Pb, Zn and Cu in the soil. Some of these heavy metals such as Pb and Cd are toxic even at low concentrations while Zn, Cu, Fe and Cr though essential in the body, are toxic at high levels. This call for monitoring to make sure that the levels of heavy metals in khat do not exceed the threshold limits recommended by WHO due to their adverse health effects to man. This study therefore assesses selected chemical nutrients and toxic metal in khat that is available in Ethiopia. Khat samples were collected from 16 sites of the three main khat growing regions namely Oromiya, South Nation and Nationality and Amhara regional state. Known weights of oven dried khat samples were digested using nitric and perchloric acids. The digests were analyzed for selected heavy metals using flame atomic absorption spectrophotometer. The following concentration ranges in dry weight ($\mu\text{g/g}$) were obtained in khat: Zn (25.15-73.95), Cu (0.10-41.80), Cr (ND-39.50), Cd (ND-0.90) and Pb (0.50-13.00). Cd was only detected in khat samples from Oromiya. Children are more susceptible to adverse effects of Pb than adults. The results suggested that there was significant difference ($p < 0.05$) in the levels of heavy metals between khat from various regions. Levels of studied heavy metals in khat were below the maximum limits recommended by WHO except for Pb and Cr. Therefore children should be discouraged from chewing khat since they are more susceptible to adverse effects of Pb than adults.

Keywords: Khat, Heavy Metal, Toxic Chemicals, Essential Nutrients

1. Introduction

Khat (*Catha edulis* Forsk.) is an evergreen flowering shrub whose fresh leaves are chewed for their central stimulating effect [1-3]. Khat plants known by different names in different countries: gat, Khat, Qat (Arabia), chat (Amarigan), Jimaa (Afanoromo), Jaat, Qat (Somalia), Bushmentea (South Africa), Khattea, Abyssinia tea (East Africa) [4]. Varieties of Khat can be found in East Africa and Yemen, where its use is deeply anchored on the Yemeni tradition. The khat tree was first described by Forskal (1736-1763), a Swedish botanist who had travelled with his friend the geographer Karsten Niebuhr (1736-1815) on an expedition to Egypt and Yemen organized by King Frederick V of Denmark, [5]. Among the

many things collected was khat, which Forskal described as *Catha edulis* in the family Celastraceae. Karsten Niebuhr was the only survivor of the five members of the expedition and in memory of his friend, he called khat (*Catha edulis*) Forskal; this was published in the botanical papers in 1775 [5]. Khat (*Catha edulis*) is a natural stimulant from the *C. edulis* plant found in the flowering evergreen tree or large shrub of the Celastraceae family, which grows mainly in Yemen, Ethiopia, Somalia, Kenya, Saudi Arabia, and at high altitude areas in South Africa and Madagascar Weir, [4]. In Ethiopia legend has it that 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 strength [6].

The fresh leaves of Khat (*Catha edulis* Forsk.) a bush native to East Africa, have been used as a stimulant in the middle East Africa and the Arabian peninsula for at least 1,000 years. The amphetamine like effects of Khat have long been recognized, and although efforts to isolate the active ingredient were first undertaken in the 19th century, has only been in the past decade that cathinone [5-(α -amino - β -phenone or 5-amino - 1-phenyl)- 1-propanone] was shown to be the substance responsible. Cathinone has most of the central nervous system and peripheral action of amphetamine and appears to have the same mechanism of action [7].

Cathinone produces sympathomimetic and central nervous system stimulation analogous to the effects of amphetamine. These effects include elevated blood pressure, mydriasis, hyperthermia, anorexia, insomnia, alertness, elevated mood, and over talkativeness. Subjective pleasurable effects such as the ability to concentrate, euphoria, confidence and friendliness. Contentment and flow of ideas have also been reported [8].

Despite efforts have been made by scientists to illuminate the chemical and pharmacological aspects of Khat, little is known reported that leaves and twigs of young Khat contain different groups of Alkaloid (Cathine, cathinone and cathidine) [8].

In Ethiopia, khat chewing is popular in Hararge, Wkite and Jimma but now a day, chewing Khat is common practice among many individuals of all age groups of the country and it is used socially and even prestigiously [9]. Depending upon the type of Khat availability on the market and the maturity of the person, up to 500g of fresh edible portion of the leaf can be chewed per day. The amount of Khat chewed may increase on occasions.

In 1980, the World Health Organization (WHO) classified Khat as a drug of abuse that can produce mild to moderate psychological dependence (less than tobacco or alcohol). The high prevalence use of Khat has some immediate effects and long term effects on human beings. The immediate effects are such as increased heart rate, breathing rate, body temperature, blood pressure, increased alertness, excitement and energy, loss of appetite and some long term effects are: - Increased severity of psychological problems (such as anxiety, irritation and more severe psychological, gastrointestinal tract problems such as constipation, inflammation of mouth and other parts of the oral cavity, oral cancer [10]. Presence of heavy metals in the environment is a major concern because of their essentiality, toxicity and threat to human life and environment. Atomic absorption spectroscopy techniques are extensively employed for the quantification of heavy metals. Flame atomic absorption spectrophotometer (FAAS) presents desirable characteristics such as low cost, cooperation facilities, high analytical frequency and good selectivity among the techniques [11].

Khat (*Catha edulis* Forsk.) is an evergreen perennial shrub plant that belongs to the Celastraceae family. It is widely cultivated in East Africa and the Arabian Peninsula more

specifically in Yemen [12]. Its young leaves and stem tips contain higher proportions of cathinone, which is responsible for much of the stimulant effect of khat [13]. Khat chewing is highly prevalent in East African and some Middle Eastern countries. It 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 [14], and more than 30% of Ethiopians have been reported to use khat [9].

In Ethiopia, khat is sold commercially in many parts of the country and where it is easily accessible to even primary school children. Chewing khat is a common recreation activity among many individuals of all age groups. In order to meet its ever increasing 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 as application of phosphate fertilizers, pesticides and refuse derived composts can be an important source of heavy metals in the soil [15]. Continuous 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 [16]. Excessive content of these metals in food is associated with a number of diseases, especially those of the cardiovascular, renal, nervous and skeletal systems [17-20].

The World Health Organization (WHO) classified Khat as a drug of abuse that can produce mild to moderate psychological dependence (less than tobacco or alcohol) [21]. In the developing world, Khat consumption is rising by 500 g per day. The sole aim of this research is to identify selected chemical nutrients and toxic metals in khat that is available in Ethiopia specifically it was to:

- i Assess the impact of toxic chemicals or metals that are found in Khat samples.
- ii Identify what are the major and minor metals found in it.
- iii Determine the levels of Zn, Cu, Pb, Cd and Cr in khat from three regional states of Ethiopia.

2. Materials and Methods

2.1. Study Area

The khat leaves were collected from sixteen main khat growing regions in the country namely Wendo, Indibir, Gelemso, Bahirdar, Aweday, Haramaya, Yibrs, Sike, Chengie, Bole, Debo, Damile, Mekanisa, Sebete, Bonga and Anferara were selected because most of the khat for the domestic market is distributed from these places. Aweday area was selected because it has a long history of khat as part of their traditions.

Awaday is also considered to be the most important producer of quality khat in Ethiopia for both domestic consumption and for export

Table 1. Sampling site of Khat with their varieties.

Sampling area	Region/ province	Trade name of Khat varieties
Yibrs	SNNP	Yibrs
Sike	SNNP	Sike
Chengie	SNNP	Chengie
Bole	SNNP	Bole
Debo	Oromia / SNNP	Debo
Damile	Oromia / SNNP	Damile
Mekanisa	Oromia / SNNP	Mekanisa
Sebeta	Oromia / region	Sebeta
Aleta –wondo	SNNP	Gerbicho
Bonga	SNNP	Gebeli
Anferara	Oromia	Anferara/dole
Wondogenet(belechie)	SNNP	Belechie
Wondogenet(basha)	Oromia / SNNP	Basha
Aweday	Oromiya	Aweday
Haramaya	Oromiya	Adale
Bahirda	Amahara	Bahirdar
Indibir	SNNP	Gurage

2.2. Method

The study is expected to delivery preliminary data on the levels of metal Khat plants grown in Ethiopia and provides use full information for future studies which would be conducted on agronomy and physiology of the Khat, fertilized application and nutritional, medicinal and toxicological effects in relation to the Khat leaves grown in Ethiopia. Therefore, in this study we were try to review different analytical method and lastly design the research analysis by using Atomic Absorption spectroscopy

2.3. Reviewed Methods Used to Assess Different Nutrients in Khat

2.3.1. Flame Atomic Absorptions Spectroscopy (FAAS)

The atomic absorptions spectroscopy techniques are extensively employees for the quantification of heavy metals. Flame atomic absorptions spectroscopy (FAAS) presents desirable characteristics such as low cost, operational facilitates, high analytical frequency and good sensitivity among the techniques [11].

2.3.2. Chromatographic Identification of Different Khat

In guidelines for future investigation which were formulated by the UN export on the Botany and chemistry Khat in 1979 an urgent and need for specific qualification means of khat material and quantification identification of the important constituents was emphasized.

2.3.3. HPLC Identification of Khat Sample

High performance liquid chromatography (HPLC) method for the quantization of all known Khat has already been proposed [22] to our knowledge no simple and reliable test for identification of Khat sample has been published until how.

2.3.4. Rapid Thin Layer Chromatographic Identification of Different Khat Plants

Furthermore the proposed thin layer chromatography (TLC) system results in inefficient separation of complex Khat extracts (example, MeOH extracts). A macroscopic and histochemical examination of Khatleaves does not show enough characteristics and in appropriate for forensic purpose. Therefore, our aim was to establish a specific and sensitive test for Khat based on a rapid, simple and inexpensive TLC method. The proposed TLC method was developed and tested by repeated analysis of numerous catha edulis (Khat) samples of different type, origin and age which have been screened before for Khat by HPLC and GC profiling

2.3.5. Gas Chromatographic – Mass Spectrometric Identification of Khat Samples

A gas chromatographic – mass spectrometric (Ge-MS) detection method of Cathinone (Khat) and method of Cathinone (CAT) in was developed. The compounds were detected as 4- carboethoxyhexaflourbutyric derivatives. Three ions for the drugs and two ions for the internal standards were monitored. The concentrations were measured by using amphetamine d6 as internal standard for Cathinone one and methamphetamine –d9 as internal standard for meth Cathinone and were liner over the range of 25-500 ng/ml for Cathinone and 12.5-5000 ng/ml for meth Cathinone. The overall recoveries of Cathinone and methcathinone were 86% and 78% respectively.

In generally, TLC, GC, Ge/MS and HPLC are used for Khat plants. The TLC method was originally proposed as rapid qualitative screening test, example for the presumptive identification of confiscated Khat samples basing on the fact that cathinone has only been found in catha edulis (Khat). GC and Ge/MS required a careful cleanup and derivatization of phenyl alkyl amine extracts and the incidence of artifacts is higher than with HPLC. Ge/MS is an excellent method identification of forensic Khat samples, but less suited for the acquisition of quantitative profile odd complex Khat extracts. The HPLC method use for the quantization of Khat samples, based on a normal phase system, required a very time – consuming extraction procedure of large sample amounts and was not sensitive enough for detection of low concentrations of nor pseudo ephedrine / nor ephedrine (NPE/NE) Recently a specific and sensitive method for the analysis of confiscated samples, using HPLC with photodiode array detection (HPLC- DAD), was established.

2.4. Research Design

The experimental design which was used in Assessing of selected chemical nutrients and toxic metal in khat that is available in Ethiopia sample from sixteen main khat growing regions namely wendo, Indibir, Gelemso, Bahirdar, Aweday, Haramaya, Yibrs, sike, chengie, Bole, Debo, Damile, Mekanisa, Sebeta, Bonga and Anferara Samples were obtained from different farms randomly selected in each region, then dried, weighed and digested before the digests

were analyzed for the chosen elements using AAS.

2.4.1. Reagents

The nitric acid and perchloric acid used in this study were analytical grade and supplied by Hopkin and Williams Ltd, England. Commercial 1000ppm standard solutions of Zn, Cu, Cr, Cd and Pb were purchased from Sigma chemical company. Distilled water was also used for sample preparation, dilution and rinsing apparatus prior to analysis.

2.4.2. Cleaning of Glassware and Sample Containers

All glassware were cleaned with detergent and hot water, rinsed several times with tap water and then soaked for 12 hours in 10% analytical grade nitric acid solution. Finally they were rinsed with distilled de-ionized water and dried in the oven at 105°C. The plastic containers were cleaned with detergent and tap water, soaked in 1:1 nitric acid and water overnight and rinsed thoroughly with distilled de-ionized water. They were then dried in an open rack and stored safely in a locked dust free storage area.

2.4.3. Sample Collection and Digestion

Khat leaves or tips ready for harvesting were sampled from khat trees grown sixteen subsamples from each farm were then combined to form a representative sample of the farm weighing about half a kilogram. A total of 135 khat samples from three regions were collected and transferred in to the laboratory where they were washed with tap water and then rinsed several times with de-ionized water. They were then dried in an oven at 70°C for 24 hour. After cooling the samples were ground to fine powder and packed in clean, labeled and decontaminated plastic containers awaiting further analysis.

The samples were digested following the procedure recommended by official methods of analysis [24]. One gram of dried sample was placed in 250 ml digestion tube and 10 ml of concentrated nitric acid added. The mixture was boiled gently for 30-45 minute. After cooling, 5 ml of 70% perchloric acid was added and the mixture boiled gently until dense white fumes appeared. Then 20 ml of distilled water was added and the mixture was boiled further to release any fumes. The solution was cooled further and filtered through what man No. 42 filter paper into a 50 ml volumetric flask. The filtrate was made to the mark using distilled water.

2.5. Method Detection Limit and Recovery Test

The calibration curves were established by a plot of absorbance readings against the corresponding concentration of ideal standards. The absorbance readings and concentration of ideal standards were used to calculate the correlation coefficients (r). The method detection limits were calculated using the equation below.

Method detection limit = 3 x Standard deviation of blank readings /slop

The accuracy of the analytical procedure was investigated by spiking a 10 ml aliquot of 5 μ g of each analyte metal into conical flask containing 1.0g of the khat sample. Then same digestion procedure was followed for non-spiked and spiked

samples side by side. Each sample was analyzed for their respective spiked metals by atomic absorption spectrophotometer and the percentage recovery calculated using the following equation.

% Recovery = $\frac{\text{Conc. in spiked sample} - \text{conc. in non-spiked sample}}{\text{amount added}}$

2.6. Determination of the Selected Heavy Metals Using AAS

Determination of (Ca, Mn, Fe, Mg, Cu, Zn, Co, Ni, Cd, and Pd) concentration Khat plants

Zn, Cu, Pb, Cd and Cr were done in replicates using computerized Varian Atomic Absorption Spectrometer model AA-10 (Varian manufacturing co. Ltd, Australia). The calibration of the instrument using standards and blank was frequently done between samples to ensure stability of the base line.

2.7. Data Analysis

Mean values obtained for Zn, Cu, Pb, Cd and Cr from the three regions were compared by One-Way ANOVA at 95% level using SPSS 21 for windows assuming that there were significant differences among them when the statistical comparison gives $p < 0.05$. Pearson's correlation analysis was used to investigate the existence of linear relationship between metal concentrations in khat assessed by spiking of samples with standards of known levels and calculating percentage recoveries.

3. Results and Discussion

The levels of Zn, Cu, Pb, Cd and Cr in soil and Khat sample obtained from Ethiopia were determined by using computerized Varian Atomic Absorption spectrophotometer. The validity of the atomic absorption spectroscopy (AAS) results was assessed by spiking of samples with standards of known levels and

Calculating percentage recoveries

3.1. Recovery Test

The recovery test for all samples was performed in triplicates and the results are as indicated in table 2.

Table 2. Recovery test results for the metals (percentage).

Metal	Conc. In sample (μ g/g)	Amount added (μ g/g)	Conc. in spiked sample (μ g/g)	Recovery (%)
Cu	10.3 \pm 0.01	5.0	15.26 \pm 0.04	99.2 \pm 0.02
Zn	37.0 \pm 0.02	5.0	41.89 \pm 1.2	97.8 \pm 0.03
Cr	15.2 \pm 0.01	5.0	20.12 \pm 0.12	98.4 \pm 0.02
Cd	0.47 \pm 0.02	5.0	5.38 \pm 0.13	98.2 \pm 0.01
Pb	7.4 \pm 0.02	5.0	12.36 \pm 0.2	99.2 \pm 0.01

As shown in table 2 the percentage recovery for khat samples lie in the range 97.8-99.2%, which are within the acceptable range for metals [28]. This confirms that the method is of good precision and accuracy.

3.2. Method Detection Limits and Equations of the Calibration Curves

The detection limits, the correlation coefficients, and the

equations of the calibration curves for the determination of metals in both soil and khat samples by AAS are given in table 3

Table 3. Detection limits, correlation coefficients and equations of the calibration curves.

Element	Method detection limit($\mu\text{g/ml}$)	Correlation coefficient of calibration curve	Equations for calibrationCurve
Zn	0.02	0.999	$Y=0.018x+0.007$
Cu	0.027	0.998	$Y=0.01x+0.004$
Cr	0.03	0.999	$Y=0.018x+0.001$
Pb	0.016	0.998	$Y=0.001x+0.005$
Cd	0.008	0.997	$Y=0.001x+0.002$

Table 3 indicates that the correlation coefficients of all calibration curves were ≥ 0.997 , which shows that there was a very high positive correlation between concentration and absorbance. The method detection limits for all the selected metals were $< 0.1\mu\text{g/g}$ indicating that the method is applicable for the determination of heavy metals at trace levels. The performance of AAS spectrophotometer used in this study was therefore good and reliable to warrant its use in the analysis of the selected heavy elements in the samples

3.3. Mean Concentration Levels of Selected Heavy Metals in Khat Samples

The mean levels of Zn, Cu, Cd, Pb and Cr in khat samples from khat growing areas of Oromiya, South Nation and Nationality and Amara are discussed in the following subsections

3.3.1. Concentration Levels of Zinc in Khat

The mean levels of Zn in khat samples are presented in table 4.

Table 4. Mean concentration levels ($\mu\text{g/g}$) of zinc in khat samples.

Sample		Amara (Bahirdar)	Oromiya(Aweday)	South Nation (Blachu)	P-value
khat	mean \pm SE	40.77 \pm 1.58 ^b	42.93 \pm 1.28 ^b	36.49 \pm 1.40 ^a	0.006
	Range	28.55-73.95	31.05-60.10	25.15-57.85	

Note: mean \pm SE values followed by the same small letters within the same row are not significantly different at $\alpha=0.05$

The Zn levels in khat samples indicated in table 4 range from 25.15 $\mu\text{g/g}$ to 73.95 $\mu\text{g/g}$. The highest mean concentrations of Zn in khat samples were found in Oromiya (Aweday) with a mean of 42.93 \pm 1.28 $\mu\text{g/g}$ is attributed to its high concentration in the soil [12]. The value was not significantly different ($p < 0.05$) from the mean value obtained in Amhara (Bahirdar) since these are adjacent regions it could be due to the farmers in the two regions having similar agricultural practices such as application of Zn based insecticides. The mean value of Zn reported in Amara was significantly ($p < 0.05$) different from mean values obtained in South Nation and nationality and Oromiya. The values of Zn in this study are within the normal levels of Zn in most crops which range 10-100 $\mu\text{g/g}$ DW [28]. Lower levels of Zn, 24.1-46.9 $\mu\text{g/g}$ was reported in Addis Ababakhat [12]. The reason for this variation may be

due to cultivation of different khat species in different areas of the countries with different geographical variation. Zinc plays essential metabolic roles in the plant, of which the most significant is its activity as a component of a variety of enzymes, such as dehydrogenase, proteinases, peptidases and phosphohydrolases [27] while excess Zn causes toxicity whose symptoms included chlorosis in young leaves, browning of coralloid roots, and serious inhibition on plant growth [25]. Zinc levels in this study are below permissible values of 60 $\mu\text{g/g}$ in vegetables [26] implying that there was no Zn contamination in khat.

3.3.2. Concentration Levels of Copper in Khat

The levels of copper in khat samples were calculated and compared as shown in table 5.

Table 5. Mean concentration levels ($\mu\text{g/g}$) of copper in khat samples.

Sample		Oromiya	Amara	South	p-value
Khat	Mean \pm SE	4.46 \pm 0.57 ^a	13.73 \pm 1.66 ^c	8.85 \pm 0.44 ^b	0.0003
	Range	0.10-13.10	5.55-41.80	3.45-14.80	

Note: mean \pm SE values followed by the same small letters within the same row are not significantly different at $\alpha=0.05$.

The concentration levels of Cu in khat ranged from 0.10 $\mu\text{g/g}$ to 41.80 $\mu\text{g/g}$ as indicated in table 5. The mean levels from the three regions differ significantly ($P < 0.05$) with the highest recorded in Aweday. High Cu levels in khat samples from Aweday could be due to application of Cu based insecticides that are used for spraying coffee. Furthermore, farmers in this region irrigate khat from streams which pass through coffee

farms. These streams are likely to be contaminated with agrochemical residues from coffee farms. Agricultural use of Cu products accounts for 2% of Cu released to the soil [28]. Khat from Bahirdar had the lowest levels of Cu which is attributed to its low concentrations in the soil [29, 31]. Found that Cu concentration in the shoots was significantly influenced by Cu concentration in soil and increased markedly

with an increase in the soil Cu concentration. The levels of Cu reported in this study are below the maximum allowable limits of Cu in vegetables of 40µg/recommended by [26]. Therefore khat was not contaminated with Cu.

3.3.3. Concentration Levels of Lead in Khat Samples

The mean concentration levels of Pb obtained in khat samples from the three khat growing regions was as shown in table 6.

Table 6. Mean concentration levels of Pb in Khat samples.

Sample		Oromiya	Amara	South	p-value
Khat	Mean±SE	3.28±0.22 ^a	8.36±0.25 ^b	7.89±0.47 ^b	ˆ0.001
	Range	0.50-6.00	5.00-12.50	1.50-13.00	

Note: mean±SE values followed by the same small letters within the same row are not significantly different at $p < 0.05$

Concentration levels of lead in khat. Inkhat samples concentration levels of Pb ranged from 5.00 µg/g to 119.00 µg/g as shown in table 6. Khat from Aweday had the highest concentration mean value which was not significantly ($p < 0.05$) different from the mean value obtained in Bahirdar. Low soil pH values in Aweday and Bahirdar accounts for relatively high levels of Pb in the two regions. The solubility of heavy metals is generally greater as pH decreases [30-31] Low levels of Pb reported in south are due to its low levels in the soil and the soil type [32], noted that the uptake, transport and accumulation of Pb by plants are strongly depended on concentration, soil type, soil properties and plant species. Similar results of Pb in khat of range 4.8-9.1 µg/g were reported in Addis Ababa [12].

This could be attributed to the mobility of Pb after its absorption. Majority of Pb is easily taken up by plants from the soil and accumulate in roots while only a small fraction is translocated upward to the shoots [33]. The presence of Pb in khat is due to application of fertilizers and manure containing

Pb [34]. Therefore Pb emitted from vehicles exhaust could have also contributed to the presence of Pb in khat [35]. Reported high accumulation of Pb, Cr, and Cd in leafy vegetables due to atmospheric deposition in the form of metal containing aerosols. These aerosols can enter the soil and be absorbed by plants, or alternatively be deposited on leaves and then adsorbed. Lead is a toxic element that can be harmful to plants, although plants usually show ability to accumulate large amounts of lead without visible changes in their appearance or yield [36]. Excess Pb causes a variety of toxicity symptoms in plants [37-38]. The mean levels of Pb in khat exceeded the permissible values for vegetables (0.3 µg/g) recommended by the FAO/WHO [26] implying that khat considered in this study was contaminated by lead

3.3.4. Concentration Levels of Cadmium in Khat Samples

Concentration levels of Cd in khat samples were compared at $\alpha = 0.05$ level as shown in table 7

Table 7. Mean concentration levels (µg/g) of cadmium in khat samples.

Sample		Amara /Bahirdar /	Oromiya /Aweday/	South/Wondogenet/	p-value
Khat	Mean±SE	*ND	0.48±0.02	*ND	0.743
	Range		0.15-0.90		

Note: mean±SE values followed by the same small letters within the same row are not significantly different at $\alpha = 0.05$, *ND =below method detection limit.

Cadmium was only reported in khat samples from Aweday which ranged from 0.15 to 0.90 µg/g and a mean value of 0.48±0.02 µg/g as indicated in table 7. This mean value of 0.48±0.02 µg/g exceeds 0.2 µg/g, the maximum allowable limits of Cd in vegetables [26] implying that there was Cd contamination in khat. This could be due to use of manure and phosphate fertilizers that are reported to contain traces of Cd from phosphaterocks [39] reported that fertilizers contain 0.1-190 µg/g of Cd while nitrogen fertilizer contain 0.1-9 µg/g of Cd. [43]., found that pig, dairy cow, and chicken manures contained high Cd due to its presence in their feeds while [40]. reported that addition of manure increased Cd uptake by plants. Since some khat farmers apply phosphate fertilizer while others apply daily cow and chicken manure or both on their farms to varying levels, this could have

contributed to variation in Cd concentration in the soil affecting its bioavailability for plant absorption. The presence of Cd in khat from Aweday is also related to its high concentration in the soil while the absence of Cd in khat samples from Bahirdar and Wondogenet may be attributed to its weak adsorptive nature in the soil [41]. The levels of Cd in this study, 0.15-0.90 µg/g are lower than 1.3-2.9 µg/g reported in khat samples in Addis Ababa [12] and 0.50-2.33 µg/g reported in fresh tobacco leaf in Kenya [42]. The reason for this may be due to cultivation of different khat species in the sample area and type of soil.

3.3.5. Concentration Levels of Chromium in Khat Samples

The concentration levels of Cr in khat from the three khat growing areas are presented as shown in table 8.

Table 8. Mean concentration levels (µg/g) of Cr in khat samples.

Sample		Oromiya /Aweday/	Amara /Bahirdar /	South/Wondogenet/	p-value
Khat	Mean±SE	18.62±1.48	*ND	18.01±1.12	
	Range	5.00-39.50		3.50-30.00	

Note: mean±SE values followed by the same small letters within the same row are not significantly different at $\alpha = 0.05$, *ND =below method detection limit.

Chromium in khat samples was only detected in Aweday and Wendognet with mean levels of 18.62 ± 1.48 $\mu\text{g/g}$ and 18.01 ± 1.12 $\mu\text{g/g}$ respectively as shown in table 8. These values are higher than the maximum allowable limits in vegetables 2.3 $\mu\text{g/g}$ [26]. Chromium was not detected in Bahirdar and this may be attributed to its low concentration levels in the soil, the type and nature of the soil as well as its chemical form in the soil. Suggested that the availability of the soil Cr to the plant depends on the oxidation state of Cr, pH, and the presence of colloidal binding sites and Cr-organic complexes that would influence its total solubility [44].

Therefore, only a very small fraction of total Cr content in the soil found to be extractable is available to the plant [46]. Low soil pH in both Aweday and Wendognet may also have attributed to the presence of Cr in khat from these regions since Cr behavior in soil is controlled by soil pH and redox Potential [46].

The levels of Cr obtained in this study are higher than those obtained from khat in Addis Ababa which ranged from 3.1 to 6.76 $\mu\text{g/g}$ [12] and 1.5 - 6.6 $\mu\text{g/g}$, found in vegetable leaves irrigated with waste water in the city of Harare [47]. This is because different crops vary in their ability to accumulate Cr in their tissues [48] as well as cultivation of different khat species in the two countries and application of different methods of farming.

3.4. Estimation of Heavy Metal Intake Through Consumption of Khat

The estimated heavy metal intakes through consumption of khat in relation to the PMTDI/PMTWI are presented in table 13. Khat from Aweday was used since it had all the selected heavy metals studied.

Table 9. Estimation of heavy metal intakes (for 70 kg adult) through consumption of khat.

Element	Intake from khat in mg/100g khat (FW)	Maximum limits	PMTDI/PMTWI
Zn	1.073 mg/day	70 mg/day	PMTDI
CU	0.343 mg/day	35 mg/day	PMTDI
Cr	0.466 mg/day		NR
Cd	0.012 mg/day	0.49 mg/week	PMTWI
Pb	0.209 mg/day	1.750 mg/week	PMTWI

NR=FAO/WHO has not recommended PMTDI or PMTWI for Cr. Source: (FAO/WHO, 1999)

Zinc is an essential mineral which is involved in numerous aspects of cellular metabolism and is required for catalytic activity of approximately 100 enzymes [49-50]. The mean daily intake of zinc by khat consumers is estimated to be 1.073 mg for 100 g of khat (since fresh khat is chewed for its stimulating property, the result in terms of dry weight basis is converted to fresh (wet) weight basis using a conversion factor of 0.25). These values may not pose a health risk according to PMTDI of 70 mg/day [51]. No adverse, health effects are thus expected from Zn intake from khat unless other additional and richer sources of this heavy metal in the diet contribute significantly to its intake.

Copper is an essential trace element, vital to the healthy life of many animals and plants. The average daily intake of Cu from khat is 0.343 mg for 100 g of fresh weight. This value is far much below the current PMTDI of Cu of 35 mg/day [51]. Therefore, no adverse health effects are thus expected from Cu intake from khat.

Chromium is another essential mineral found in khat. The availability of this metal in human is important for many biological activities [54]. It regulates blood sugar, therefore reducing medication and insulin needs in diabetic patients [52] and also plays a role in the management of heart diseases by regulating fat and cholesterol synthesis in the liver [53]. The average daily intake of Cr by khat consumers of 100 g of khat per day is 0.466 mg fresh weight. The WHO has not recommended PMTDI or PMTWI for Cr. Therefore, no adverse health effects are thus expected from Cr intake from khat. Unless other additional and richer sources of this heavy metal in the diet contribute significantly to its intake. Copper is an essential trace element, vital to the healthy life of many animals and plants.

The average daily intake of Cu from khat is 0.343 mg for 100g of fresh weight. This value is far much below the current PMTDI of Cu of 35 mg/day [51]. Therefore, no adverse health effects are thus expected from Cu intake from khat. Chromium is another essential mineral found in khat. The availability of this metal in human is important for many biological activities [54]. It regulates blood sugar, therefore reducing medication and insulin needs in diabetic patients [52]. and also plays a role in the management of heart diseases by regulating fat and cholesterol synthesis in the liver [53]. The average daily intake of Cr by khat consumers of 100 g of khat per day is 0.466 mg fresh weight. The WHO has not recommended PMTDI or PMTWI for Cr. Therefore, no adverse health effects are thus expected from Cr intake from khat. Lead is the most significant toxin of heavy metals and the inorganic forms are absorbed through ingestion by food [55]. while cadmium has no known bio-importance in human biochemistry and physiology and consumption even at very low concentrations can be toxic [56-57]. The consumption of khat contributes 0.012 mg for 100 g fresh weight of Cd daily and 0.209 mg for 100 mg fresh weight of Pb daily.

The FAO/WHO has recommended a PMTWI of Cd and Pb of 0.49 mg/week and 1.750 mg/week respectively [51]. According to these values, the daily intake of Cd and Pb by Kenyan consumer for 100 g of khat alone is below the PMTWI levels. However, if above 100 g of khat is consumed and if other sources of Cd and Pb are included then the intake may exceed the recommended levels and continuous exposure to Cd and Pb may result in their gradual accumulation in human vital organs which may cause profound biochemical and neurological changes in the body. The results of the present study indicate that intakes of the selected heavy metals due to consumption of khat, pose no health risk as the values are lower than the respective permissible intakes. The daily metal intake from khat can however be minimized by reducing the amount of khat.

consumed.

The sympathomimetic and central nervous system (CNS) stimulating effects produced by Khat chewing are due to mainly to Cathinone, the natural amphetamine present in fresh Khat. Compared to amphetamine, however, Khat seems to have less potential of inducing tolerance or toxic psychosis.

As cathinone has only been found in *Catha edulis* a positive identification of this compound is a specific indication for Khat. Nevertheless, positive results should be confirmed by alternative techniques such as high performance liquid chromatography (HPLC), gas chromatography/ Mass spectrometry (GC/MS) [22]

The total content of Khatamine's and especially the concentration ratios of cathinone, nor pseudoephedrine and nor ephedrine are dependent on the Khat type, origin, time of harvesting, drying method, age and storage conditions [22-23].

Dried and old Khat samples usually contain only small but still thin layer chromatograph (TLC) detectable amounts of cathinone due to enzymatic reduction of cathinone to nor pseudoephedrine and nor ephedrine [22-23].

In such neither plant material nor pseudoephedrine is the dominating Katharine [22-23]. But in any case only a quantitative analysis of a major and minor Khat, for example by high performance liquid chromatography (HPLC) profiling, provides more information about the "history" and the psychotropic potency of given Khat samples.

The result of total contents of the studied nutrients and toxic metals in Khat (*Catha edulis* forsk) variety show the ability to accumulate high amount of the both macro – and micronutrients elements the most abundant metal among the microelement analyzed was Ca followed by Mg whereas Fe content of Khat leaves was the predominant among different micronutrient heavy metals followed by Zn, Mn, Cu, Ni, and Co.

It can be deduced from the levels of all the metals in the studied Khat varieties of all the sampling sites, that the concentration of the macro and the micronutrient metals followed similar trend for most of the samples. In general ranges of concentrations of the studied macronutrient and micronutrient metals could be arranged according to their levels on the Khat varieties of all the sampling sites in the following order on dry weight basis: Ca (3850-8750 mg kg⁻¹) > Mg (1670-3070 mg kg⁻¹) > Fe (121-336 mg kg⁻¹) > Zn (18.7 -40.6 mg kg⁻¹) > Mn (11.4 -24.4 mg kg⁻¹) > Cu (4.81-1.39 mg kg⁻¹) > Ni (4.43-10.2 mg kg⁻¹) > Co (0.67 mg kg⁻¹). The same trend has been reported by [4, 58].

The higher levels of Ca and Mg in the studied Khat plants is obvious since these nutrients are highly mobile in the plant tissue and are translocated from old leaves to young leaves.

The concentration of Fe, Zn, and Mn were higher than entire trace metal in the khat samples. Among this iron was

the most abundant trace metal in Khat samples. This suggested the ability of Khat plants to accumulate heavy metals like Cu and Zn which are comparable to present analysis. Fortunately, the concentrations of toxic heavy metals [58]. Pb and Cd in the studied Khat leaves cultivars were too low to be detected by the analytical technique used in this study. In fact the concentration of the toxic heavy metals are expected to be very low in Ethiopian Khat since these metals are related to environmental pollution caused by different industrial activities.

Comparing level of metals among the analyzed Khat varieties, it is impossible to have a common trend for the distribution of all the metals in all the Khat varieties.

Table 10. Correlation between major, minor and trace metals in Khat samples.

	Ca	Mg	Mn	Fe	Zn	Co	Cu	Ni
Ca	1.00							
Mg	0.39	1.00						
Mn	-0.245	0.043	1.00					
Fe	0.218	0.426	0.429	1.00				
Zn	0.427	0.335	0.418	0.137	1.00			
Co	-0.286	-0.271	-0.214	-0.177	0.197	1.00		
Cu	0.127	0.426	0.443	0.296	0.294	-0.400	1.00	
Ni	0.55	0.05	-0.370	-0.02	0.020	-0.38	0.300	1

Generally, based on the current status, chewing Ethiopian Khat is addition to its stimulating property, it contributed appreciable amount of macro and trace metals for daily requirements of the individuals and are free from toxic heavy metals. Particularly, Khat could be good source of Fe and Zn for individuals who are chewing this plant regularly.

4. Conclusion

In this study the levels of five heavy metals Zn, Cu, Cr, Cd and Pb in khat from three main khat growing regions in Ethiopia were analyzed but the levels of studied heavy metals in khat were below the maximum limits recommended by WHO except for Pb and Cr. Therefore children should be discouraged from chewing khat since they are more susceptible to adverse effects of Pb than adults. The result also shows that Khat from Bahirdar had the lowest levels of heavy metals as compared with Aweday and Wondogenet not only in Aweday in Wondogenet, the levels of Zn, Cu and Cr in khat samples is highly significant. The results showed the ability of Khat to accumulate relatively higher amount of Ca, Mg and Fe among the determined micro and macro nutrient metals, respectively. Heavy metals Cu and Co was found to be comparatively at lower levels in most of analyzed Khat with respect to the Khat varieties, the plants showed no significant difference in their pattern of accumulation of the studied metals.

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