Levels and Health Risk Assessments of Heavy Metals in Khat and Its Support Soil in Algesachi, Ilu Ababor, Ethiopia

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ABSTRACT

BACKGROUND: Khat (*Catha edulis Forsk*) is a stimulant plant grown in East Africa and the Arabian Peninsula. Heavy metal pollution has been a global concern due to its acute and chronic health effects and the major route of exposure is the consumption of contaminated foods. In this study, the determination and health risk assessment of heavy metals (Mn, Cu, Zn, Ni, Cr, Cd, and Pb) in khat and its support soil samples was carried out.

MATERIALS AND METHODS: Khat and its support soil were analyzed for the levels of 7 toxic heavy metals by Atomic Absorption Spectrophotometer. Samples were randomly collected from 3 districts of khat farming kebeles and digested using mixture of strong acids.

RESULTS: The concentrations (mg/kg) of analytes in soil and khat samples were: Cu (6.78-35.80); Zn (24.30-199.02); Mn (7.59-1855.40); Ni (6.37-64.80); Cr (0.82-169.20); Cd (14.2-38.8), and Pb (ND). Among the analyzed heavy metals in soil, Mn was with the highest concentration, followed by Zn, Cr, Cu, Ni, and Cd while that of Zn was the maximum followed by Cu, Mn, and Cr in khat. The levels of Zn, Cr, and Cd in soil samples from all study sites and detected concentrations of Cr in khat samples exceeded the recommended FAO/WHO levels.

CONCLUSION: The hazard index (HI) of metals in khat from study areas was less than 1 indicating a less likelihood of non-carcinogenic toxicological health effects. However, the presence of these toxic chemicals in soil and khat indicates product contamination and needs extensive further investigation involving other heavy metals.

KEYWORDS: Soil, khat, heavy metals, health risk assessment, transfer factor, AAS

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Introduction

Khat (*Catha edulis*) is a leafy green herbal plant in Celastraceous family. It is commonly grown in East Africa and the Arabian Isthmus, particularly in Yemen.[1] An alkaloid named cathinone, accountable for most of the khat's stimulating effects, is found in its fledgling leaves and stalk tips.[2] Its consumption is both a communal and traditional activity and would promote societal interactions. In Ethiopia, it is chewed by teenagers and older people.[3,4]

Khat is consumed for its stimulating and ecstatic effects, spiritual events and therapeutic purposes. Airway diseases such as cough, asthma and flu are treated by the extracts of khat`s roots and leaves.[5] It is also treats ulcers and premature ejaculation in men. Khat`s use is rampant in Middle East, and sub-Saharan African countries including Yemen, Saudi Arabia, Uganda, Eritrea, Ethiopia, Djibouti, Kenya.[6]

However, regular consumption of khat, particularly in the form of chewing, has been reported to be closely associated with increased susceptibility to hypertension, increased heart rate, and liver problems.[7,8] It is also linked to decreased libido, cancer, depression, dental abnormalities, liver damage, and high blood pressure. The psychoactive ingredients cathine and cathinone are believed to be proven precursors of cancer (oral and esophageal) in people who regularly chew khat.[9] It is also important to remember that the presence of high levels of toxic metal ions in khat is dangerous and can potentially damage important organs of the body such as the liver and kidneys.[8] Since agrochemicals like pesticides are applied to boost productivity, residues on khat leaves may cause cancer and genotoxicity.[10]

In Ethiopia, khat is consumed daily.[11,12] Khat varieties depend on the geographical area of cultivation that includes climate and soil chemistry,[1] color and the leaves' and entire plants size and height.[13] Furthermore, it was previously estimated that the high profits of this crop surpasses the earnings from other cereals by almost 2.7-fold.[14,15] It is currently one of the top 5 sources of export revenue, and its income for the budgetary year that ended in 2021/22 was $392 million.

Khat is referred to as "green gold" in northern Madagascar as it is a major income source to cover expenditures the expenditure of other items. In most countries like Djibouti, khat consumption is more prevalent in males than females attributing to detrimental effects on the socio-economic circumstances of the general public. A frequent use of khat can cause oral hygiene deterioration necessitating the use of dental plasters. It is worth noting that about 90% of the country's population regularly consumes khat.[16] The prevalence of khat consumption in Ethiopia is 23.22%, especially among which could be endorsed by family chewing behaviors, alcohol intake, and peer pressure. Furthermore, a lifetime incidence of khat munching of 33.2% was reported in the Jazan region of Saudi Arabia.[16]

Heavy metals are inorganic minerals which are not decomposed by bacteria or other living organisms. Their persistence in the environment can cause acute and chronic poisonousness particularly in animals and humans. The sources of these toxicants in khat could be consistent use of agrochemicals like pesticides and fertilizers and combustion of untreated metal wastes. Heavy metals can also be transferred from polluted soil by geogenic and anthropogenic activities to khat plants. Eventually, lasting risks of health from exposure to heavy metals, both at workplace and environmental, include multiorgan toxicity, including disorders of the interactions between brain, spinal cord and nerves, mental and body energy state processes, body`s ability to fight diseases, ontogenesis, and possibly lasting disability.[17] It is reported that Ethiopian khat is polluted by toxic heavy metals including iron, chromium, copper, cobalt, and zinc.[18] A study in Yemen has also indicated the presence of zinc, copper, lead, and cadmium. Use of cadmium polluted khat may lead to elevated blood pressure, though its mode of action is not yet clear[19] while lead causes nervous breakdown, high blood pressure, cardiovascular disease and cancer. For example, blood with Pb levels between 40 and 60 µg/dL leads to serious health problems if urgent therapeutic measures are not considered.[6]

A detailed review on prevalence of khat use in different countries, its heavy metal content and associated adverse health effects are available in literature.[6] Oyugi et al[20] determined the levels of heavy metals in khat using inductively coupled plasma atomic emission spectroscopy (ICP-AES). The mean levels of Pb, Cd, and Cr topped WHO allowable limits. In a study conducted in Ethiopia, the highest level of iron, Fe (37.2-90.3 mg/kg) in khat samples followed by Zn (5.1-10.6 mg/kg), Mn, Ni, Cu and Co while Pb and Cd were undetected.[21] Soil samples from the same study sites contained metals in the order of Fe > Mg > Ca > Mn > Zn > Co > Ni > Cu. Cd was detected at levels of 0.73 to 1.23 mg/kg. The Cd, Pb, Cu, and Zn contents of Yemeni Khat were determined by Matloob[22] and the levels were in the decreasing order of Zn > Cu > Pb > Cd. The results from a study by State et al[23] discovered that the levels of potentially toxic metals are in the order of Mn > Pb > Zn > Ni > Cd > Cu in and soil samples.

Khat cultivation is legal and popular in the Sembeto, Nado, and Chekorsa Doyu kebeles. It is used as a recreational drug on holidays, weekends, cultural ceremonies and other social events. The prevalence of khat consumption by Ethiopian University students was reported to be 23.22%.[24] It is also an important source of income. The employment created by khat cultivation is significant, as it involves a large number of people in cultivation, collecting, storage, transport, and sales. Many literatures are existing about the history, taxonomy, pharmacology, chemistry, and socio-economical aspects of Khat,[25] but the researches related to the quantification of heavy metals in Khat are limited. However, the concentration of toxic heavy metals and their associated health risks in the prevalently used khat product in the study area is not addressed. Therefore, the objective of the current study was to quantify selected heavy metals in khat and its support soil and assess the risk of khat consumption on human health.

Materials and Methods

Description of study area

Alge sachi district is located in the Ilu Aba Bora zone in Southwestern Ethiopia, located 675 km from Addis Ababa. It is a geographically bordered on the north by the Nole Kabba district (West Wollaga), in the South by the Bilo Nopa district, in the Western by the Darimu district, and Eastern by Mako district (Buno Bedelle). The total area coverage of Alge Sachi district is 84 013 hectares and the total population is around 102 679. The district consists of twenty-eight (28) rural and four (4) towns. Khat leaf and soil samples were randomly collected from 3 khat producing kebeles in the district, namely, Sambo, Chekorsa, Doyu, and Nado.

Reagents chemicals, and materials

Nitric acid (HNO_3, 68% (w/w)), sulfuric acid (H_2SO_4, 98% (w/w)), perchloric acid ($HClO_4$ 70% (w/w)), and hydrogen peroxide (H_2O_2, 30% (w/w)) were chemicals used for sample digestion. Nitrate salts of Manganese (Mn), Nickel (Ni), Lead (Pb), Zinc (Zn), Copper (Cu), Chromium (Cr), and Cadmium (Cd), were used to prepare standards and were analytical grade supplied from Merck, USA, through Addis Ababa, Ethiopia representative. Distilled water was used throughout the analysis.

Apparatuses and instruments

Sterile plastic bags, stainless steel knife, digital analytical balance, ceramic mortar, and pestle, microwave (Model: STRAT D 134348, EVISA), flame atomic absorption spectrophotometer (FAAS) (Buck Scientific, Model 210 VGP, East Norwalk, USA) and drying oven (Model: DHG-9123A) were used in this study. Volumetric flasks, graduated cylinders, and digestion flasks of various volumes were cleaned with cleansing agent, rinsed with distilled water, sodden in 8% HNO_3 for 24 hours, rinsed 5 times with double distilled water and dried in an oven.

Sample collection

Composite samples (1 kg) from each sampling site containing 7 subsamples were randomly collected from the 3 kebeles. The khat samples were rinsed a number of times with distilled water after being transported to the laboratory and dried in sunlight for 48 hours to decrease the moisture. Furthermore, they were dried in an oven at 60ºC following peeling and chopping with a Teflon knife followed by grinding to a fine powder using a pestle and mortar. The homogenized samples were kept in air-tight plastic containers and stored until analysis.

Collection of soil samples was carried out beneath the khat at 10 to 30 cm using an auger. A composite sample weighing approximately 2 kg was made by mixing 7 subsamples collected from each farms. After drying for 5 days at room temperature, the grinded and homogenized samples were sieved to pass through a 500 µm mesh. A further oven drying at 60°C occurred for 24 hours and then kept in a clean polyethylene bag till needed for analysis.

Preparations of working standard solutions

Nitrate salts of each metal was used to prepare a 1000 mg/L stock solution. Then 10 mg/L standard solution was prepared, from which 5 different concentrations were made for calibration of FAAS. Dilution was used for the preparation of other working solutions.

Sample digestion

Digestion of khat samples. A 0.5 g of crushed khat powder samples were weighed and transferred to a clean crucible with the sample number. The wet aching was done in a muffle furnace by gradually increasing the temperature to 500°C at which ashing took 5 hours. Then the samples were removed and cooled carefully under a fume hood. The ash was moistened with a little distilled water, then 7 mL of conc. HNO_3, 1 mL $HClO_4$, and 2 mL H_2O_2 in ratio (7:1:2) for each khat sample were added into 100 mL round-bottomed ground-bottom flasks with reflux condenser to digest the samples on the hot plate block digester. After cooling, the digestion, product was filtered into 100 mL reagent bottles using Whatman filter paper #42. The flask was rinsed with filtered distilled water and the samples were then diluted to 100 mL and stored in reagent bottles and stored at 4°C to prevent degradation prior to determination by FAAS.

Digestion of soil samples. Two grams of soil (dry weight) was weighed using a digital weighing scale and the homogenized samples were poured into a 250 mL refluxing block digester. A mixture of HNO_3 (70%), H_2SO_4 (98%), and H_2O_2 (30%) was added in a volume ratio of 6:2:2 followed by mild heating for 2:45 hours at 100°C. Then the temperature was set to 150°C and heating continued for 2 hours until a clear solution observed. Then, the samples were cooled, filtered with Whatman No.42 filter paper and diluted to 100 mL by distilled water.

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

Evaluation of the Analytical Methods

Linearity. The method`s linearity was validated by measuring the absorbance of standard solutions of the analytes. It was considered linear when a signal versus concentration gave an $R^2 > .99$ (see Table 1).

Recovery test. For the recovery tests, 2 mg/L of Mn, Pb, and Cu, 4 mg/L of Cr and Ni, 3 mg/L of Zn, and 2.5 mg/L of Cd were spiked with a 0.5 g khat in 7 round bottom flasks. Then, digestion was carried out with similar experimental conditions

Table 1. Instrument working conditions and correlation coefficients for the determination of metals in soil and khat samples by FAAS.

METALS	WAVELENGTH (NM)	SLIT WIDTH (NM)	INSTRUMENT DETECTION LIMIT (MG/L)	CONC. USED FOR THE CALIBRATION CURVE (MG/L)	CORRELATION COEFFICIENT (R^2)
Mn	279.5	0.2	0.002	2, 4, 6, 8, and 10	.998
Cu	324.8	0.5	0.003	2, 4, 6, 8, and 10	.997
Zn	213.9	1.0	0.001	0.3, 0.6, 0.9, 1.2, and 1.5	.996
Cr	357.9	0.2	0.006	2, 4, 6, 8, and 10	.998
Pb	283.2	0.7	0.010	2, 4, 6, 8, and 10	.995
Ni	232.0	0.2	0.010	2, 4, 6, 8, and 10	.993
Cd	228.9	0.7	0.002	0.5, 1, 1.5, 2, and 2.5	.993

and reagents used for unspiked samples. Spiking experiments were carried out in triplicate for all analytes. The same approach was followed for soil samples.

Limit of detection (LoD) and limit of quantification (LoQ). The detection limits for each metal were calculated from 6 reagent blank readings. Three times the standard deviations of triplicate absorbance readings of reagent blank ($3S_B$) were considered as the LoD and $10S_B$ as LoQ.

Precision. Precision is the degree to which the measured values agree with each other. Repeatability was assessed by estimating the relative standard deviation (% RSD) of the percent recovery (%R) at all spiked concentration.

Human exposure assessment

The human exposure assessment was based on the levels of the metals in khat samples. Khat comes in packs comprising of leaves and stems, and the user consumes an average of 1 pack per day.

Estimated daily intake (EDI). The amount of khat consumed in 1 day was determined using equation (1)[26] with slight modification.

$$EDI = \frac{E_f \times ED \times F_{IR} \times C_m \times C_f \times 0.001}{B_w \times TA} \quad (1)$$

where E_f is frequency of consumption (365 day/year); ED is the duration of chewing (65 years), F_{IR} is the estimated amount of khat used per day (200 g/person/day), C_M is the level of the metal (mg/kg dry weight); C_f is concentration conversion factor (0.085).[27]

Target hazard quotient (THQ). The health adverse effects of consuming khat polluted with heavy metals was calculated using equations (1) and (2).[28]

$$THQ = \frac{E_f \times ED \times F_{IR} \times C_m \times C_f \times 0.001}{RfD \times B_w \times TA} \quad (2)$$

0.014, 0.001, 0.3, 0.04, 0.02, 0.001, and 0.003 were taken as the oral reference doses, RfD for Mn, Cd, Zn, Cu,Ni, Cd, and Pb (mg/kg of body weight per day), respectively. B_W (70 kg) was taken as the average body weight of an adult and TA is the total khat chewing duration in life time (65 year × 365 day/year).

Results and Discussion

Method validation

Linearity. The correlation coefficients (R^2) of the calibration curve for the analysis of Cd, Cr, Mn, Cu, Zn, Pb, and Ni in soil and khat leaves by FAAS were in the range of .995 to .998, indicating a significant positive correlation between metal levels and absorbance. The FAAS was calibrated with 5 standards. The concentrations used for the calibration, instrument working parameters and the correlation coefficients of the calibration curve for each of the metals are shown in Table 1.

Recovery test. The concentrations of the 7 metals (Zn, Cu, Pb, Cd, Ni, Mn, and Cr) in khat and its support soil samples collected from farms in the 3 main khat growing areas in the Alge-Sachi woredas were determined in triplicate using FAAS. The validity of the method was evaluated by spiking the samples with standards of known levels and calculating the percent recovery expressed as mean ± standard deviation (SD) (n = 3). Percent recoveries for soil and khat leaves were (90.00%-95.00%) and (90.29%-101.59%), respectively, as shown in Tables 2 and 3. The percentage accuracies of the method were also determined by dividing the practically obtained metal concentrations by their respective spiked concentrations.

Precision test. The reproducibility of the results was assessed using the relative standard deviation of 3 samples (n = 3) with triplicate readings for each sample. The relative standard deviation (% RSD) for the soil and khat leaves were (0.00722-1.786)% and (0.17-5.114)%, respectively. An analytical method with relative standard deviation (% RSD) <15% and percent recovery (%*R*) of 70% to 120% is considered valid. Tables 2 and 3 show the % RSD of each metal in each sample.

LoD and LoQ. For the determinations of LoD and LoQ, duplicate analyses were performed for 3 blank samples for all analytes and the pooled standard deviation was calculated. As indicated in Table 4, the LoD for each element is higher than the FAAS detection limit (IDL) but lower than the levels of metals detected signifying the method's appropriateness for quantification of the analytes at trace levels. The differences in the LoD and LoQ's of the khat and soil was due to the differences in the standard deviations of the blank signals measured at different times.

Determinations of heavy metals in khat and its support soil. The concentration of selected heavy metals (Mn, Zn, Ni, Cd, Pb, Cu, and Cr) in khat and soil of the 3 khat growing areas of Sembeto, Chokorsa Doyu, and Nado kebeles were determined with FAAS. The mean levels of the studied metals are depicted in Table 5.

Levels of heavy metals in khat samples. As it can be seen in Table 5, Mn, Cu, and Zn were present in all khat samples from the 3 kebeles with level ranges (mg/kg) of 7.59 ± 0.95 to 10.48 ± 0.84, 6.78 ± 0.71 to 25.68 ± 0.49, and 24.30 ± 0.65 to 29.89 ± 1.02, respectively. The levels of metals in khat leaves from Sambato kebele follows the order Zn > Cu > Mn > Cr while Pb and Cd couldn't be detected by the employed method. The low copper level in samples from Nado kebele compared to samples from Chokorsa Doyo and Sambato kebeles could

Table 2. Recovery and precision results for the determination of metals in soil samples from Sambato kebele.

METALS	UNSPIKED SAMPLE (N=3)	CON. SPIKED (MG/L)	SPIKED RECOVERY (N=3)	RECOVERY (%)	ACCURACY (%)	% RSD
Mn	1724.60 ± 0.52	2	1726.3 ± 0.15	95.00	99.90	0.007
Cu	29.00 ± 0.94	2	30.87 ± 0.55	93.33	93.94	1.460
Zn	199.02 ± 0.73	3	201.87 ± 0.40	94.89	98.59	0.163
Cr	66.60 ± 0.64	4	70.2 ± 0.30	90.00	94.87	0.349
Pb	ND	2	1.9 ± 0.04	94.5	95.00	1.786
Ni	40.2 ± 0.59	4	43.83 ± 0.07	90.75	91.71	0.134
Cd	14.2 ± 0.94	2.5	16.49 ± 0.22	91.73	86.11	1.123

Table 3. Recovery and precision test results for the determination of the metal in khat samples (mean ± SD) from Sambato Kebele.

METALS	UNSPIKED SAMPLE	CON. SPIKED	SPIKED RECOVERY	RECOVERY (%)	ACCURACY (%)	RSD (%)
Mn	7.59 ± 0.95	2	9.58 ± 0.03	97.93	100.10	0.22
Cu	23.18 ± 0.12	2	24.99 ± 0.19	90.29	92.75	0.17
Zn	25.09 ± 0.84	3	28.14 ± 0.25	101.59	89.16	0.73
Cr	0.82 ± 0.81	4	4.82 ± 0.03	99.90	82.98	0.52
Pb	ND	2	1.94 ± 0.03	95.50	97.00	1.28
Ni	ND	4	3.92 ± 0.03	98.08	98.00	0.73
Cd	ND	2.5	2.32 ± 0.14	93.07	92.8	5.11

Table 4. IDL, LoD, and LoQ of the analytes.

METALS	SOIL (MG/KG)				KHAT (MG/KG)		
	STD	IDL	LOD	LOQ	STD	LOD	LOQ
Mn	0.004	0.002	0.013	0.042	0.007	0.022	0.070
Cu	0.159	0.003	0.497	1.586	0.010	0.031	0.099
Zn	0.006	0.001	0.019	0.062	0.061	0.191	0.609
Cr	0.052	0.006	0.164	0.523	0.005	0.016	0.052
Pb	0.023	0.010	0.069	0.230	0.049	0.147	0.490
Ni	0.051	0.010	0.153	0.510	0.064	0.192	0.640
Cd	0.028	0.002	0.084	0.280	0.035	0.105	0.350

Abbreviations: IDL, instrument detection limit, LoD, limit of detection, LoQ, limit of quantification; STD, standard deviation.

be due to the form of copper in the soil, pH, organic matter content and clay composition. Nevertheless, there is a research indicating the presence of Pb and Cd in Yemeni khat leaves with concentration of 0.066 to 0.7 mg/kg and 0.007 to 0.018 mg/kg, respectively.[29] The samples from Chokorsa Doyu kebele follow the same trend except the presence of Ni and absence of Cr. Khat leaves from Nado sampling sites detected positive for Zn, Mn, Cu, and Cr with concentrations of 24.30 ± 0.65, 8.42 ± 0.59, 6.78 ± 0.71, and 3.49 ± 0.15 mg/kg, respectively. The highest levels of Mn (10.48 ± 0.84 mg/kg), Cu (25.68 ± 0.49 mg/kg), Zn (29.89 ± 1.02 mg/kg), and Ni (6.37 ± 0.24 mg/kg) were recorded in samples from Chokorsa Doyu and that of Cu (35.80 ± 0.88 mg/kg) and Cr (3.49 ± 0.15 mg/kg) were from Nado kebele. From the studied

Table 5. Levels (mg/kg) of heavy metals in soil and khat in the 3 kebeles.

HEAVY METALS	SAMPLE	CONCENTRATION OF METALS IN SAMPLES (N=3)		
		SEMBETO (MG/KG)	CHOKORSA DOYU (MG/KG)	NADO (MG/KG)
Mn	Soil	1724.60 ± 0.52	1855.40 ± 0.72	1197.60 ± 0.48
	Khat	7.59 ± 0.95	10.48 ± 0.84	8.42 ± 0.59
Cu	Soil	29.00 ± 0.94	31.40 ± 0. 35	35.80 ± 0.88
	Khat	23.18 ± 0.12	25.68 ± 0.49	6.78 ± 0.71
Zn	Soil	199.02 ± 0.73	87.90 ± 0.19	87.66 ± 1.42
	Khat	25.09 ± 0.84	29.89 ± 1.02	24.30 ± 0.65
Cr	Soil	66.60 ± 0.64	92.20 ± 0.82	169.20 ± 2.17
	Khat	0.82 ± 0.81	ND	3.49 ± 0.15
Pb	Soil	ND	ND	ND
	Khat	ND	ND	ND
Ni	Soil	40.2 ± 0.42	52.6 ± 0.61	64.8 ± 0.86
	Khat	ND	6.37 ± 0.24	ND
Cd	Soil	14.2 ± 0.24	26.6 ± 1.10	38.8 ± 0.95
	Khat	ND	ND	ND

analytes, only the concentrations of Cr in khat samples from Sambato and Nado sites exceeded the recommended level (0.25 mg/kg) by FAO/WHO.

A study in Saudi Arabia determined the highest concentrations of Cu (215.4 ± 12.3-3054 ± 45.2 mg/kg), Mn (108 ± 5.8-1541 ± 24.7 mg/kg), Zn (23.17 ± 0.4-1490 ± 32.6 mg/kg), Pb (0.18 ± 0.87 mg/kg), and Cd (0.00 ± 0.08 mg/kg) in khat leaves.[30] The level of chromium in the soil and its likely human health risk on the chewers was also evaluated.[31] A 71.01 ± 12.05 to 317.55 ± 23.14 mg/kg was obtained for soil while khat leaves contained 6.5 ± 1.76 to 30.01 ± 2.91 mg/kg total chromium, a value that comprises the present study.[31] The levels of Pb, Cd, Zn, and Cu in Ethiopian khat were 0.18 to 0.87 mg/kg, 0.15 ± 0.90, 0.10 to 41.80, and 25.15 to 73.95 µg/g, respectively.[11] FAAS/OES GFAAS using an external calibration curve was used in analysis of 13 metals and the levels (mg/kg) were reported as Cu: 5.11 to 9.55, Cd: 0.03 to 6.54, Pb: non-detectable to 1.57, Zn: 4.15 to 89.3, Mn: 6.45 to 20.[32] A study conducted by Fentie Tadesse[18] analyzed khat cultivated Gidolle, Konso and Koyira and reported the results (µg/g) as: Fe (180-222), Zn (25.23-33.69), Cu (12.64-15.74), Cr (4.01-4.53), while Pb and Cd were not detected.

In a study conducted by Oyugi et al[20] in Kenya, the mean heavy metal concentrations (mg/kg) in dry khat samples of 6 toxic heavy metals were Cd (7.81 ± 1.56), Cr (15.98 ± 2.22), Cu (15.81 ± 2.84), Fe (97.35 ± 32.67), Ni (0.37 ± 0.02), and Pb (32.36 ± 9.95) in which the levels of Pb, Cd, and Cr surpassed WHO acceptable bounds. In a study done by Matloob[22] on khat samples, 20.3 ± 10.3, 233.6 ± 141.5, 5308 ± 1888, 6622 ± 1822 µg/kg were obtained for Cd, Pb, Cu, and Zn, respectively. The levels of essential and non-essential metals in Ethiopian khat samples in fresh-weight basis were reported in the order: Ca (1038-2173 µg/g) > Mg (478.2-812.3 µg/g) > Fe (53.95-82.83 µg/g) > Zn (5.18-9.40 µg/g) > Mn (6.98-8.66 µg/g) > Cu (1.85-5.53 µg/g) > Cr (0.66-3.47 µg/g) > Co (0.41-0.80 µg/g).[33] The variation between the current studies from results in the literature could be differences in the nature and type of soil as these are considered the most key feature that governs the incidence of heavy metals as it serves as the retention and binding site for several toxic materials. Moreover, the type and dose of fertilizer and pesticide usage affect the levels of these metals in khat samples.

Levels of heavy metals in soil. The contents of plant elements are conditional, and they depend on soil properties and plant capacity to selectively accumulate certain metals. It is also known that these elements can have a detrimental effects on plant growth, soil cover and, fertility.[34] From the studied 7 heavy metals, all of them were detected in soil samples except Pb. The levels follow different order in the samples from 3 kebeles. However, Mn was found with the highest concentration in all samples from the study sites. The reported levels (mg/kg) of the metals from Sambato kebele follow the order: Mn (1724.60 ± 0.52) > Zn (199.02 ± 0.73) > Cr (66.60 ± 0.64) > Ni (40.2 ± 0.42) > Cu

(29.00 ± 0.94) > Cd (14.2 ± 0.24). For the soil samples analyzed from Chokorsa Dayu, the results (mg/kg) were: Mn (1855.40 ± 0.72) > Cr (92.20 ± 0.82) > Zn (87.90 ± 0.19) > Ni (52.6 ± 0.61) > Cu (31.40 ± 0.35) > Cd (26.6 ± 1.10). As can also be seen from Table 5, the concentrations (mg/kg) of the 6 metals in soil from Nado kebele were, in decreasing order 1197.60 ± 0.48, 169.20 ± 2.17, 87.66 ± 1.42, 64.8, 38.8, and 35.80 ± 0.88 for Mn, Cr, Zn, Ni, Cd, and Cu, respectively. Similarly, the highest levels of 4 of the 6 metals were determined in soil from Nado kebele as follows: Cu (35.80 ± 0.88), Cr (169.20 ± 2.17), Ni (64.8 ± 0.86), and Cd (38.8 ± 0.95). A 1855.40 ± 0.72 and 199.02 ± 0.73 mg/kg were recorded as the maximum concentrations of Mn and Zn from Chokorsa Dayo and Sambato kebeles, respectively. The levels of Zn, Cr, and Cd in soil samples from all study sites are above the recommended FAO/WHO levels set at 50, 50, and 1 mg/kg, respectively.

In a study done in Tanzania on soil, Fe was found with the highest concentration followed by Zn, Pb, and Cu. The concentrations (mg/100 g dry soil) of the metals were 2760.1 to 2833.07, 15.5 to 20.13, 305.95 to 308.25, 224.48 to 230.39 for Fe, Cu, Zn, and Pb, respectively.[35] Similarly, in the soil samples irrigated with wastewater in and around Asmara, Eritrea, heavy metals were detected in the concentration order of Mo < Cd < Co < Cu < Pb < V < Cr ≈ Zn < Mn < Fe < Al indicating that Fe, Mn, and Pb has exceeded FAO/WHO limits.[36]

In the present study, the mean concentrations of the 6 metals were compared using ANOVA. There were no significant differences ($P = .54$, $\alpha = .05$) among the mean levels of each metals in soil between the 3 kebeles. However, the levels of the different metals (within each 3 kebeles differ significantly ($P = 8.3 \times 10^{-7}$, $\alpha = .05$). The same trend was also revealed by for metal concentrations of khat samples within and between the kebeles. The calculated *P*-values of metals concentration variations for the within and between kebeles were .000108 and .206585, respectively. The variation of the metal contents from different sampling sites might be attributed to regular use of fertilizers, incineration of scrap of metals and application of pesticides.[32,37] Heavy metals can also be absorbed from contaminated soils.

Determination of transfer quotients. Transfer quotient is the ratio of metal's concentration in khat to soil with the assumption that metals in khat come from soil. The transfer coefficient exhibited reduced concentration with values of 0.004 to 0.818, as shown in Table 6. The finding indicates that Cu has the highest transfer coefficient followed by Zn, Ni, Cr, and Mn. The uptake and bioaccumulation of heavy metals depend on the bioavailability, levels in soil, the chemical form, plant uptake capabilities, and plant species growth rate.[38] The higher transfer coefficients of Cu and Zn might be attributed to their higher mobility but lower retention with in soil than other metals. It is also reported that high value transfer factor indicates low retention capacity.[39] Similarly, TF exceeding 1 shows hyperaccumulation, particularly in soils[17] but TF of 0.1 showed that the plant was excluding metals from its tissues, while the TF values of 0.2 indicated the likelihood of metal pollution by anthropogenic undertakings.[39]

Human health risk assessment

The human health risk associated with consumption of meat contaminated khat were assessed using estimated daily intake, hazard quotient and hazard indexes. The results are shown in Table 7.

Table 6. Soil-Khat transfer coefficient (%) of heavy metals.

METALS	SAMPLING SITES		
	SAMBATO	CHOKORSA DOYU	NADO
Mn	0.4	0.6	0.7
Cu	79.9	81.8	7.7
Zn	12.6	34.0	27.7
Cr	1.2	-	2.1
Pb	-	-	-
Ni	-	12.1	-
Cd	-	-	-

Table 7. Estimated daily intake (mg/kg bw) and hazard quotient of various elements for different Khat varieties.

KHAT/METALS		MN	CU	ZN	CR	NI	CD	HAZARD INDEX (HI)
Sambato	EDI	0.0018	0.005	0.06	0.0002	-	-	0.47
	THQ	0.13	0.14	0.2	0.0001	-	-	
Chokorsa Doyu	EDI	0.003	0.006	0.007	-	0.002	-	0.43
	THQ	0.18	0.15	0.024	-	0.076	-	
Nado	EDI	0.0018	0.002	0.006	0.0008	-	-	0.21
	THQ	0.144	0.041	0.02	0.0006	-	-	

As shown in Table 7, for the 7 heavy metals studied, all their corresponding EDIs were less than the corresponding provisional tolerable daily intakes, and did not approach the doses. The finding showed the highest estimated daily intake values of zinc in all samples fooled by Cu and Mn. The EDI value of Cr is higher in samples from Sambato than in Nado and Ni had the lowest EDI than other metals for samples from Chokorsa Doyu. For samples from Sambato kebele, the THQ follows the order: Zn > Cu > Mn > Cr while it is Mn > Cu > Ni > Zn for Chokorsa Doyu khat samples. Nado samples had THQ of 0.144, 0.041, 0.02, and 0.0006 for Mn, Cu, Zn, and Cr, respectively. According to Vinet and Zhedanov,[40] THQ > 1 indicates a higher possibility of occurring for non-cancer health disorders. Since the THQ of the metals for all samples is less than 1, chewing of khat has less likelihood to cause non-cancerous adverse health effects with respect to the studied heavy metals. The hazard index (HI) calculated as the sum of the THQs of all metal ions is also less than 1 indicating less likelihood of non-carcinogenic toxicological health effects. However, the results of EDI, THQ and HI were calculated considering amount of khat used per day to be 200g, and the average body weight of an adult as 70 kg. These values are estimations and the mass of khat consumed per day could be higher and the considered weight could also be lower as this plant is even commonly used by young generations. But it's possible for chronic and synergistic effects to occur even when the estimated daily intake (EDI) of heavy metals is less than 1. The concept of EDI primarily considers the average daily intake of a substance over a given period. However, chronic and synergistic effects can result from long-term exposure to low levels of multiple heavy metals, even if the intake of each metal individually remains below a certain threshold. Furthermore, the toxicity of heavy metals can be influenced by various factors such as the route of exposure, duration of exposure, individual susceptibility, and interactions with other chemicals or pollutants present in the environment.

Conclusion and Recommendation

The content of some selected heavy metals Mn, Ni, Cu, Zn, Pb, Cd, and Cr in khat and soil samples from the 3 kebeles of Alge Sachi Woredas was determined using FAAS. The developed analytical method was validated and acceptable values of parameters were obtained. The levels of Zn, Cr, and Cd in soil samples from all the study sites and detected concentrations of Cr in khat samples exceeded the recommended FAO/WHO levels. However, the health risk assessments indicated that the hazard index (HI) of metals in khat from study areas was less than 1 indicating a less likelihood of non-carcinogenic toxicological health effects. The high TF values (>20%) of Cu and Zn show the likelihood of metal contamination by anthropogenic activities. It is recommended that a better understanding of the health effects and chemical composition of khat should be established through a multidisciplinary approach involving khat producers as most of them use fertilizers and pesticides to increase product yield without considering their detrimental health effect on consumers. Moreover, a study encompassing large number of samples from all districts in the Ilu Ababor Zone and ICP-MS as a detection technique considering other toxic heavy metals may lead to a more comprehensive and representative report.

Author Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Shibiru Damana, Ibrahim Umer, and Abiyot Kelecha. The first and final draft of the manuscript was written by Abiyot Kelecha and all authors commented on both versions of the manuscript. All authors read and approved the final manuscript.

Ethical Approval

Not applicable.

Consent to Participate

Not applicable.

Consent for Publication

All the authors give their consent for publication of the manuscript.

REFERENCES

1. Masresha Woldamanuel M. Assessment of selected nutrients and toxic chemicals in Ethiopian khat. *Sci J Chem*. 2019;7:26.
2. Silva B, Soares J, Rocha-Pereira C, et al. Khat, a cultural chewing drug: a toxicokinetic and toxicodynamic summary. *Toxins*. 2022;14:1-12.
3. Rather RA, Berhanu S, Abaynah L, Sultan M. Prevalence of khat (*Catha edulis*) chewing and its determinants: a respondent-driven survey from Hossana, Ethiopia. *Subst Abuse Rehabil*. 2021;12:41-48.
4. Mohamed Abdoul-Latif F, Ainane A, Houmed Aboubaker I, et al. Chemical composition of the essential oil of *Catha edulis* Forsk from Djibouti and its toxicological investigations in vivo and in vitro. *Processes*. 2023;11:1324.
5. Yimer A, Khan M. Determination of iron, cobalt, chromium and copper metals in commercially available khat (*Catha edulis* forsk) in Arba Minch, Ethiopia. *Int J Eng Res Appl*. 2015;5:66-74.
6. Oyugi AM, Kibet JK, Adongo JO. A review of the health implications of heavy metals and pesticide residues on khat users. *Bull Natl Res Cent*. 2021;45-67. doi:10.1186/s42269-021-00613-y
7. Alrobaian M, Arida H. Assessment of heavy and toxic metals in the blood and hair of Saudi Arabia smokers using modern analytical techniques. *Int J Anal Chem*. 2019;2019:1-8.
8. Glomb T, Świątek P. Antimicrobial activity of 1,3,4-oxadiazole derivatives. *Int J Mol Sci*. 2021;22:6979-7002. doi:10.3390/ijms22136979
9. Omare MO, Kibet JK, Cherutoi JK, Kengara FO. Contemporary trends in the use of khat for recreational purposes and its possible health implications. *OALib*. 2020;07:1-22.
10. Atnafie SA, Muluneh NY, Getahun KA, Tsegaw Woredekal A, Kahaliw W. Pesticide residue analysis of khat leaves and health risks among khat chewers in the Amhara region, northwestern Ethiopia. *J Environ Public Health*. 2021;2021:1-8.
11. Geta TG, Woldeamanuel GG, Hailemariam BZ, Bedada DT. Association of chronic khat chewing with blood pressure and predictors of hypertension among adults in gurage zone, southern Ethiopia: a comparative study. *Integr Blood Press Control*. 2019;12:33-42. doi:10.2147/IBPC.S234671
12. Hunde AD, Demissie DB, Garado TS, et al. Caffeine consumption, khat chewing, and associated factors among pregnant mothers in Illu Aba Bor Zone, South West Ethiopia. *Int J Afr Nurs Sci*. 2023;18:100559-100568.

13. Atlabachew M, Chandravanshi BS, Zewge F, Redi M. Fluoride content of Ethiopian khat (*Catha edulis* Forsk) chewing leaves. *Toxicol Environ Chem*. 2011;93:32-43.
14. Feyissa AM, Kelly JP. A review of the neuropharmacological properties of khat. *Prog Neuropsychopharmacol Biol Psychiatry*. 2008;32:1147-1166.
15. Gezon L. Beyond (anti)utilitarianism: khat and alternatives to growth in northern Madagascar. *J Polit Ecol*. 2017;24:582-594.
16. Al-Maweri SA, Al-Soneidar WA, AlQahtani KW. Evaluation of khat (*Catha edulis*) use as a risk factor of cancer: A systematic review (Chong et al., 2020). *Asian Pac J Cancer Prev*. 2020;21:2181-2182.
17. Offor SJ, Orish CN, Chidi Eze E, Frazzoli C, Orisakwe OE. Blood donation and heavy metal poisoning in developing nations: any link? *Transfus Apher Sci*. 2021;60:103067-103078.
18. Fentie Tadesse S. Determination of the level of selected heavy metals from khat leaves (*Catha edulis* forsk) grown in Gidolle, Konso and Koyira, southern Ethiopia. *Sci J Anal Chem*. 2015;3:115.
19. Garner RE, Levallois P. Associations between cadmium levels in blood and urine, blood pressure and hypertension among Canadian adults. *Environ Res*. 2017;155:64-72.
20. Oyugi AM, Kibet JK, Adongo JO. Analysis of the concentration of heavy metals in khat grown in Meru County and the assessment of their associated health risks. *Int J Anal Chem*. 2024;2024:1-12.
21. Atlabachew M, Chandravan BS, Redi M. Profile of major, Minor and toxic metals in soil and khat (*Catha edulis* forsk) cultivars in Ethiopia. *Trends Appl Sci Res*. 2011;6:640-655.
22. Matloob MH. Determination of cadmium,lead,copper and zinc in Yemeni khat by anodic stripping voltammetry. *East Mediterr Health J*. 2003;9:28-36.
23. State J, Sagagi B, Bello A, Danyaya H. Transfer factors and potential ecological risk index of potentially toxic metals in soil, irrigation water and vegetables along Gada river bank of Jigawa State, Nigeria. *ChemSearch J*. 2022; 13:23-30.
24. Gebrie A, Alebel A, Zegeye A, Tesfaye B. Prevalence and predictors of khat chewing among Ethiopian university students: a systematic review and meta-analysis. *PLoS One*. 2018;13:e0195718-NaN15.
25. Toennes SW, Harder S, Schramm M, Niess C, Kauert GF. Pharmacokinetics of cathinone, cathine and norephedrine after the chewing of khat leaves. *Br J Clin Pharmacol*. 2003;56:125-130.
26. Chary NS, Kamala CT, Raj DS. Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. *Ecotoxicol Environ Saf*. 2008;69:513-524.
27. Napier B, Rhoads K, Strenge D. A compendium of transfer factors for agricultural and animal products. Pacific Northwest National Laboratory Richland, Washington, 2003.
28. FAO, WHO. Report of the fifth session of the Codex Committee on Contamination in Foods. Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission Thirty-fourth Session. *Codex*. 2011:4-9. www.codexalmentarius.net (REP11/CF Joint FAO/WHO Food Standards Programme)
29. Uddin AH, Khalid RS, Khan UAK, Abbas S. A review of heavy metals contamination in traditional medicinal products. *J Appl Pharm*. 2013;5:41-49.
30. Ahsan W, Al Bratty M, Alhazmi H, et al. Determination of trace metal concentrations in different parts of the khat varieties (*Catha edulis*) using inductively coupled plasma-mass spectroscopy technique and their human exposure assessment. *Pharmacogn Mag*. 2019;15:449-458.
31. Alemu A, Tegegne A. Assessment of chromium contamination in the soil and khat leaves (*Catha edulis* forsk) and its health risks located in the vicinity of tannery industries: a case study in Bahir dar City, Ethiopia. *Heliyon*. 2022;8:11914-11920.
32. Ashenef A, Birhanu G, Engidawork E. Levels of essential and toxic metals in Ethiopian khat, (*Catha edulis* forsk.). *Ethiop J Environ Stud Manag*. 2014;7:289-297.
33. Atlabachew M, Chandravanshi BS, Redi M. Concentration levels of essential and non-essential metals in Ethiopian khat (*Catha edulis* forsk). *Biol Trace Elem Res*. 2010;138:316-325.
34. Yeshiwas Y, Tadele E. Review on heavy metal contamination in vegetables grown in Ethiopia and its economic welfare implications. *J Biol Agric Healthc*. 2017;7:31-44.
35. Kacholi DS, Sahu M. Levels and health risk assessment of heavy metals in soil, water, and vegetables of dar es Salaam, Tanzania. *J Chem*. 2018;2018:1-9.
36. Kfle G, Asgedom G, Goje T, et al. The level of heavy metal contamination in selected vegetables and animal feed grasses grown in wastewater irrigated area, around Asmara, Eritrea. *J Chem*. 2020;2020:1-15.
37. Atlabachew M, Chandravanshi BS, Redi M. Selected secondary metabolites and antioxidant activity of khat (*Catha edulis*Forsk) chewing leaves extract. *Int J Food Prop*. 2014;17:45-64.
38. Tinker PB. Levels, distribution and chemical forms of trace elements in food plants. *Philos Trans R Soc Lond B Biol Sci*. 1981;294:41-55.
39. Nirmal Kumar JI, Soni H, Kumar RN, Bhatt I. Hyperaccumulation and mobility of heavy metals in vegetable crops in India. *J Agric Environ*. 2009;10:34-45.
40. Vinet L, Zhedanov A. A "missing" family of classical orthogonal polynomials. *J Phys A Math Theor*. 2011;44:1-15.