

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

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ABSTRACT

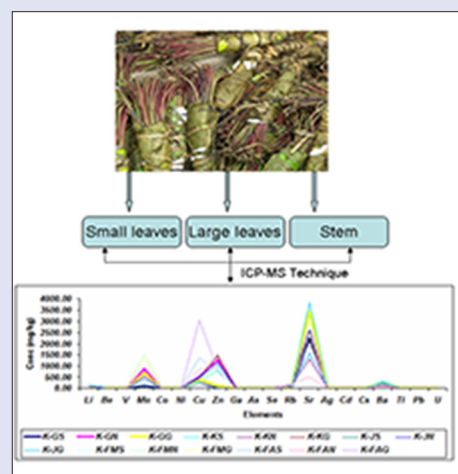
Background: Khat (*Catha edulis*, family: *Celastraceae*) is a plant that is native to Africa and Arab peninsula and is used for their amphetamine-like properties. Although the use of Khat is banned in Saudi Arabia, people particularly in southern Jazan province manage to get it from Yemen, and the use is increasing steadily. **Objective:** Five most commonly used varieties of Khat namely Gaifi, Kofat, Jahasha, Faqarah Menjed, and Faqarah Aswad were selected for the study. **Materials and Methods:** Metal ion concentrations were determined using inductively coupled plasma-mass spectroscopy. Since Khat is available as one bundle consisting of three parts of the plant, metal ions in all three parts were determined separately for comparison purpose. The concentrations (mg/kg) of 20 metal ions were determined in Nwaif leaves (new and smaller in size), Gafra leaves (old and larger in size), and stem of the plant and compared with the Provisional Tolerable Weekly Intake (PTWI) and acceptable daily intake (ADI) of metal ions to study the health hazards posed by them. **Results:** The non-essential metal ion Strontium (Sr) was present in highest abundance in all the samples with a concentration range of 498.6 ± 18.9 – 3837 ± 52.1 mg/kg followed by Copper (215.4 ± 12.3 – 3054 ± 45.2 mg/kg), Zinc (23.17 ± 0.4 – 1490 ± 32.6 mg/kg), and Manganese (108 ± 5.8 – 1357 ± 18.6 mg/kg). Several toxic heavy metal ions including Arsenic, Lead, and Cadmium were also present in trace concentrations in many samples. **Conclusion:** Many metal ions were observed to be present in concentrations much higher than their PTWI and ADI which further allude to the extremely hazardous nature of Khat plants. Multicomponent variate analyses were also performed using chemometric methods to establish the possible correlation between samples.

Key words: Acceptable daily intake, chemometric methods, inductively coupled plasma-mass spectroscopy, Khat, metal ions, provisional tolerable weekly intake

SUMMARY

- Five commonly used Khat varieties from Jazan Province, Saudi Arabia were selected for the study
- Qualitative and quantitative determination of various trace metals present in small leaves, large leaves as well as in stem of all Khat varieties was performed by inductively coupled plasma-mass spectroscopy technique.
- Several metal ions such as strontium, Cu, Zn, MN, and Ba were found to be in significantly high concentrations.
- Various parameters of human exposure assessment were calculated as per the guidelines of the WHO to evaluate the health hazards

- Statistical chemometric methods were used to establish the correlation between samples on the basis of trace metal concentrations.



Abbreviations used: AOAC: Association of official analytical chemists; EDI: Estimated daily intake; ADI: acceptable daily intake; PTWI: Provisional tolerable weekly intake; THQ: Target Hazard Quotient; HI: Hazard Index; R_{ED}: Oral reference dose; ICH: International Council for Harmonisation; ICP-MS: Inductively coupled plasma-mass spectroscopy; % R. S. D.: % Relative standard deviation; ppb: Parts per billion; LOD: Limit of detection; LOQ: Limit of quantitation; SD: Standard Deviation; FAO: Food agriculture organization; WHO: World Health Organization; JECFA: Joint Expert Committee on Food Additives.

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INTRODUCTION

Khat (*Catha edulis*) is a plant which is grown in some parts of South-Western Arabian peninsula and East Africa. Among the Arabian Peninsula, it is commonly grown in Yemen, and due to its proximity to the southern part of Saudi Arabia, it is also used by the Saudi people especially in the Jazan Province despite the fact that the use and cultivation of Khat is banned in Saudi Arabia. In general, the leaves of the Khat plant are chewed on a daily basis by more than 20 million people on the South-Western Arabian Peninsula and Eastern part of Africa.^[1,2] Five varieties of Khat are commonly used in South-Western Saudi Arabia which are Gaifi, Kofat, Jahasha, Faqarah Menjed, and Faqarah Aswad out of which Gaifi, Kofat, and Jahasha are originally from Yemen whereas, Faqarah Menjed and Faqarah Aswad are grown on the mountains of Jazan Province at the border of Yemen.

A large number of people chew Khat leaves owing to its amphetamine-like properties that have a variety of pleasurable and stimulating effects.^[3] In general, one bundle of around 350 g of mixed fresh Khat leaves and stems are chewed by the user per day depending on the variety, nature of the user and availability in the market. This amount usually increases on certain occasions such as cultural/social festivals and ceremonies.^[4]

The stimulating and euphoric effects of Khat are due to the presence of alkaloids cathine and cathinone. These alkaloids are categorized as sympathomimetic amines, the category similar to amphetamine. They result in the feeling of well-being and mental alertness as well as the excitement in users, but they have a number of untoward effects as well due to which their uses are banned in many countries including Saudi Arabia. Khat is often found to be associated with various medical conditions including dental diseases, oral cancer, cardiovascular complications, hepatic disorders, sexual problems, sleep issues, constipation, and decreased appetite. The mental problems associated with Khat include psychosis, depression, frequent mood swings, and violent behavior that lead to social and family problems.

Extensive literatures are present about the history, taxonomy, pharmacology, chemistry, and socio-economical aspects of Khat,^[5,6] but the studies related to the determination of metal ions present in Khat are scarce. These metal ions especially the toxic metal ions are expected to have a major share of toxicities caused by Khat if they are present in higher than the acceptable limit. Few studies have been carried out to determine the metal ions in Khat samples in other countries such as Ethiopia, Kenya using atomic absorption spectroscopy. To the best of our knowledge, no study is performed on Khat varieties from Jazan province using inductively coupled plasma-mass spectroscopy (ICP-MS), a technique which has many advantages of being simple, accurate, robust, and sensitive as it can measure the concentrations at parts per billion (ppb) level, and a number of metal ions can be determined at the same time.

The knowledge of the concentration of toxic metal ions in foodstuffs is of prime significance as it is directly related to the quality and safety. Green leafy vegetables that are consumed as such are supposed to be one of the major sources of these metal ions.^[7,8] Their chemical and elemental profile needs to be determined to reduce the intake of harmful chemicals and elements as much as possible. The actual chemical profile of Khat and their role in the distribution of trace metals in body fluids and tissues are less studied. Moreover, there was no comparative study on the determination of toxic metals in different varieties of Khat, particularly in Jazan Province. These varieties were expected to have different metal ion concentrations depending on their geographical locations, environmental conditions, types of soil and water used, and the fertilizers and pesticides used. Comparative analysis of these samples would lead to

the identification of Khat varieties that have more concentration of toxic metal ions and have more health hazards than the others.

MATERIALS AND METHODS

Collection and nomenclature of samples

Bundles of five different Khat varieties Gaifi (KG), Kofat (KK), Jahasha (KJ), Faqarah Menjed (KFM; Faqarah variety obtained from Menjed mountains), and Faqarah Aswad (KFA; Faqarah variety obtained from Black mountains) were procured from different cities of Jazan province. Smaller Nwaif leaves, larger Gafra leaves, and stems were separated from each bundle, washed with deionized water, and dried at room temperature. The dried Khat samples were powdered to fine size and stored at 4°C till digestion is performed. Fifteen samples were prepared on the basis of varieties and parts of the plant. They were named and classified as Khat-Gaifi-Stem (K-GS), Khat-Gaifi-Nwaif (K-GN), Khat-Gaifi-Gafra (K-GG), Khat-Kofat-Stem (K-KS), Khat-Kofat-Nwaif (K-KN), Khat-Kofat-Gafra (K-KG), Khat-Jahasha-Stem (K-JS), Khat-Jahasha-Nwaif (K-JN), Khat-Jahasha-Gafra (K-JG), Khat-Faqarah Menjed-Stem (K-FMS), Khat-Faqarah Menjed-Nwaif (K-FMN), Khat-Faqarah Menjed-Gafra (K-FMG), Khat-Faqarah Aswad-Stem (K-FAS), Khat-Faqarah Aswad-Nwaif (K-FAN), and Khat-Faqarah Aswad-Gafra (K-FAG).

Chemicals and reagents

All the chemicals used were of AR grade and were purchased from Sigma Aldrich (Germany). Deionized water (18 MΩ) purchased from Sigma Aldrich (Germany) was used throughout the study. Microwave Digestion System Ethos I (Milestone, USA) was used for the digestion of tobacco samples. The elemental concentrations of the digested samples were analyzed using ICP-MS 7500 (Agilent, Germany).

Optimization of digestion method

The digestion method of Khat samples was optimized for different parameters such as digestion time, temperature, reagent volume, and ratio. Trial and error method was used for the optimization by varying one parameter and keeping others constant. At the end, lesser reaction time and temperature with the least use of reagent volume and ratio was selected as an optimized procedure and was utilized for the digestion of all Khat samples.

Digestion of Khat samples

All the samples were digested according to the procedure suggested by the Association of Official Analytical Chemists^[9] with slight modification. About 1.0 g of the previously stored dried and meshed Khat samples were completely dried in hot air oven at 105°C followed by cooling in a desiccator to remove the moisture content. For the determination of total concentration of elements of interest, 1 g of each dried part was pretreated with 8 mL conc. HNO₃ and 1 mL of 30% Hydrogen peroxide (H₂O₂) and was predigested for 16 h in fume hood at 30°C.^[10] Thereafter, all samples were digested in a microwave digestion system ethos I (Milestone, USA) in Teflon vessels.

The digestion parameters set was as following: programmable for time and microwave power from 800 to 1400 W and programmable temperature from 50°C to 200°C, ramp time 10 min, and total time of digestion 70 min. On completion of the digestion process, teflon vessels were allowed to cool at room temperature for 30 min. These were then kept opened under the hood for 5 min until all the fumes evaporated. A volume of 1 mL of digested samples were drawn and filtered into 15 mL polypropylene tubes, diluted with 9 mL of ultrapure deionized water and then analyzed by ICP-MS instrument directly. The blank was digested and filtered using a similar procedure.

ICP-MS analysis

Metal ion concentrations in the all Khat plant samples were analyzed with an ICP Mass Spectrometer ICP-MS 7500 (Agilent, Germany). All samples, blanks and standards, were prepared and analyzed in triplicate. An instrument quality control and tuning was performed using instrument tuning solution at 1 ppb in 2% HNO₃. An 8-point calibration curve of multi-element standard was drawn at 0, 5, 10, 20, 40, 60, 80, and 100 ppb in absorbance mode and was used to determine the concentration of metal ions present in all samples. The ICP-MS method developed was validated according to the International Council for Harmonisation (ICH)-Q2B guideline parameters for linearity, precision, accuracy, and robustness.

Determination of method detection limit and recovery

The calibration curves were plotted using absorbance readings against the corresponding concentrations of standards and correlation coefficients (*r*) were calculated using MS-Excel 2007 software. Standard deviation (SD) of the values and slope of the curve were obtained, and the method detection limits (LOD) were calculated using the following Eq. 1 and the limit of quantitation was calculated using Eq. 2.

$$\text{Method Detection Limit} = 3.3 \times \frac{\text{SD of blank readings}}{\text{Slope}} \quad (\text{Eq. 1})$$

$$\text{Limit of Quantitation} = 10 \times \frac{\text{SD of blank readings}}{\text{Slope}} \quad (\text{Eq. 2})$$

To determine the accuracy and precision of the method, 10 mL aliquot of 5 µg of each analyte metal ion were spiked into 1 g of the Khat sample followed by the same digestion procedure for spiked as well as nonspiked samples. The concentrations of metal ions in spiked and nonspiked samples were determined by ICP-MS, and the percentage recovery values were calculated using the following Eq. (3).

$$\% \text{ Recovery} = \frac{\text{Conc. in spiked sample} - \text{Conc. in non-spiked sample}}{\text{Amount added}} \quad (\text{Eq. 3})$$

Five quality control samples of concentrations 5, 10, 25, 50, and 90 ppb were analyzed interday and intraday to determine the percentage accuracy of the method. % Accuracy was calculated by the following Eq. (4).

$$\% \text{ Accuracy} = 100 \times \frac{\text{Practically obtained concentration}}{\text{Theoretical concentration}} \quad (\text{Eq. 4})$$

Human exposure assessment

Khat is supplied in bundles consisting of leaves and stem of the plant and on an average, a user consumes one bundle of Khat in a day. Leaves generally are of two types – new leaves which are smaller in size and old leaves which are larger in size. New leaves generally are present on the apex of the bunch and are consumed more by the user as it contains a larger amount of cathinone than other parts. In general, one bundle of Khat weighed around 350 g, and the weights of all three parts were almost equal. The weights of small and large leaves were roughly around 120 g each, and the weight of stems was slightly lesser and was 110 g. As far as consumption of Khat is concerned, it largely depends on the consumer as some people consume only small leaves from the bundle, whereas others consume both small and large leaves but not the stem. On the other hand, some users consume small leaves, large leaves as well as half of the stems which are softer and easy to chew. Therefore, the amount of Khat consumed varies between 120 and 300 g in 1 day. For calculation purpose, we considered 300 g as the weight of Khat consumed per day

by a user and the weekly intake to be 2.1 kg. From this quantity, the total weekly intakes (TWI, mg) of elements through Khat per week were calculated using Eq. (5).

$$\text{TWI} = C_{\text{element}} \times C_w \quad (\text{Eq. 5})$$

Where,

C_{element} = concentrations of elements (mg/kg) in different samples

C_w = amount of Khat consumed per week = 2.1 kg

Other assessment parameters, namely estimated daily intake (EDI) and target hazard quotient (THQ), were investigated. EDI refers to the intake of elements per kg body weight per day by a user. EDI (mg/kg bw/day) is calculated using Eq. (6).

$$\text{EDI} = C_{\text{element}} \times \left[\frac{C_d}{b_w} \right] \quad (\text{Eq. 6})$$

Where,

C_{element} = concentrations of elements (mg/kg) in different samples,

C_d = weight of Khat consumed per day = 300 g = 0.3 kg,

b_w = average body weight (kg) of target population = 70 kg.

THQ values for all the metal ions were calculated by dividing EDI values by the established oral reference dose (R_fD) values. THQs were calculated using the following Eq. (7).

$$\text{THQ} = \frac{\text{EDI}}{R_fD} \quad (\text{Eq. 7})$$

Where

EDI = estimated daily intake,

R_fD = oral reference dose of the substance.

The hazard index (HI) was calculated from the THQ values of all metal ions and can be expressed as the sum of the THQs.^[11] It may be calculated from the following Eq.(8).

$$\text{HI} = \text{THQLi} + \text{THQBe} + \text{THQV} + \text{THQMn} + \text{THQCo} + \text{THQNi} + \text{THQCu} + \text{THQZn} + \text{THQGa} + \text{THQAs} + \text{THQSe} + \text{THQRb} + \text{THQSr} + \text{THQAg} + \text{THQcd} + \text{THQCs} + \text{THQBa} + \text{THQTl} + \text{THQPb} + \text{THQU} \quad (\text{Eq. 8})$$

Statistical analyses

The obtained analytical data were analyzed using SPSS statistical program 20.0 (IBM, Armonk, NY, USA). Three widely used multivariate statistical analyses were employed to interpret the data considering various elements expressing 20 rows and Khat samples constituting 15 columns. In order to determine significant groups of samples having the same characteristics, hierarchical cluster analysis was performed. To analyze the correlation present between variables, Pearson correlation analysis was performed which was also useful to determine divergence and coherence among the data. Another very important multivariate technique, principal component analysis with varimax rotation was also performed on the whole data based on eigenvalue <1 by dividing variables into various principal components to check the variation of the data.

RESULTS

Method validation

All the method validation parameters were assessed according to the ICH guidelines (Q2B). Multi-element calibration curve was constructed for the quantification of elements and excellent linearity ($1 > r > 0.99$) was observed for all elements which showed the high positive correlation between values of concentration and absorbance. % Recovery, % Accuracy, % relative standard deviation (R. S. D.), and LOD values were calculated for each element, and the results are summarized in Table 1.

% Recovery values were obtained to be in a range of 90%–105%, % Accuracy was obtained in a range of 95%–120% and % R. S. D. values were <2.0 in case of all elements. All the obtained values were within the prescribed limits as stated in ICH guidelines. The method detection limit (LOD) values for all the detected metals were <0.21 µg/kg which indicated the applicability of the method for determining elements even at trace levels.

Elemental analysis

The three edible parts of the Khat plant were separated for all five varieties and samples were prepared separately using the same digestion

procedure. The concentrations of 20 metal ions (mg/kg) were determined in all 15 samples of Khat in triplicate, and the data are presented as Mean ± SD [Table 2]. The abundance of these elements in different Khat varieties is further depicted in Figure 1 which shows the average elemental concentration in mg/kg dry weight in the five Khat varieties. As shown in the figure, highest peaks were obtained for elements Mn, Cu, Zn, strontium (Sr), and Ba. The highest concentration among all elements was found to be of Sr which showed unusual presence in the range of 498.6 ± 18.9–3837 ± 52.1 mg/kg in all Khat samples. It was followed by 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), Ba (80.52 ± 14.3–320.4 ± 22.5 mg/kg),

Table 1: Method validation parameters obtained for all elements

Elements	Correlation coefficient (r)	Percentage recovery	Percentage accuracy	LOD (µg/kg)	Precision (percentage RSD)
Li	0.9930	102.4	95.4	0.17	0.21
Be	0.9964	92.3	112.4	0.09	0.34
V	0.9976	100.6	104.5	0.11	0.92
Mn	0.9912	97.3	112.8	0.14	0.43
Co	0.9989	91.8	97.7	0.09	0.12
Ni	0.9932	98.9	119.6	0.10	0.98
Cu	0.9997	99.6	98.2	0.06	1.34
Zn	0.9424	99.3	102.5	0.12	1.45
Ga	0.9946	104.4	97.4	0.04	0.27
As	0.9965	102.4	110.4	0.05	0.11
Se	0.9977	101.5	95.9	0.03	0.53
Rb	0.9987	93.2	106.5	0.09	0.44
Sr	0.9986	91.6	116.7	0.11	1.83
Ag	0.9921	98.3	119.8	0.05	0.86
Cd	0.9954	100.3	113.4	0.15	0.23
Cs	0.9934	93.5	118.6	0.08	0.11
Ba	0.9994	94.5	97.4	0.21	0.26
Tl	0.9936	93.5	99.8	0.13	0.31
Pb	0.9963	104.4	107.1	0.06	0.63
U	0.9975	91.7	117.5	0.13	0.79

LOD: Limit of detection; RSD: Relative standard deviation

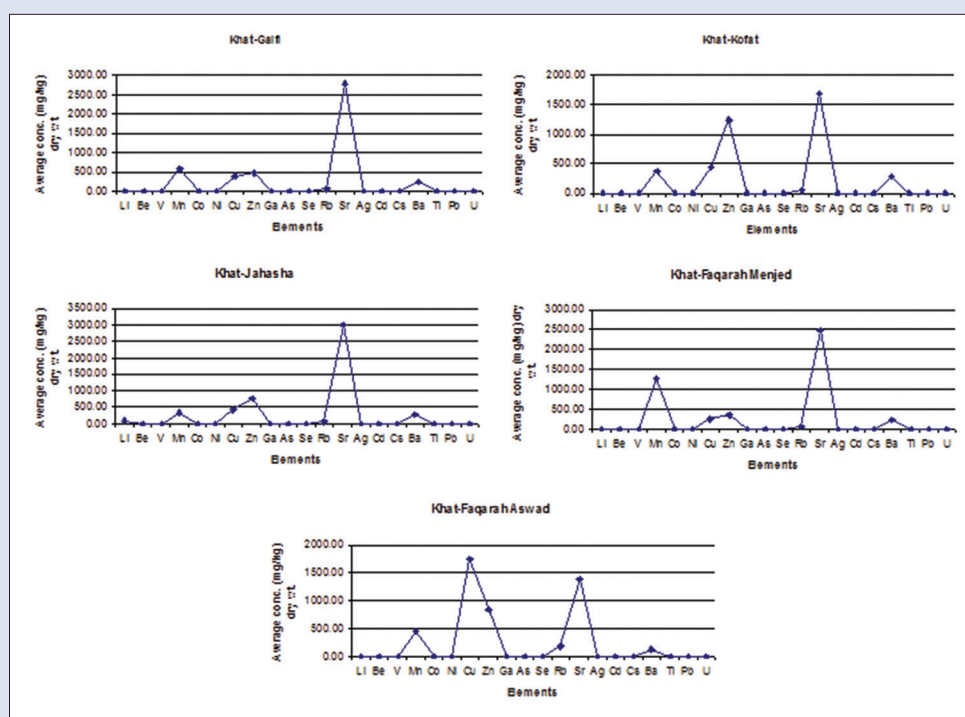


Figure 1: Line graphs showing average elemental concentration in different Khat varieties in mg/kg dry weight

Table 2: Concentrations of elements detected in fifteen Khat samples (mean±standard deviation)

Elements	Concentration (mg/kg) ^a														
	K-GS	K-GN	K-GG	K-KS	K-KN	K-KG	K-JS	K-JN	K-JG	K-FMS	K-FMN	K-FMG	K-FAS	K-FAN	K-FAG
Li	0.52±0.04	2.36±0.1	3.28±0.4	0.36±0.03	1.36±0.2	1.43±0.1	1.92±0.2	73.73±1.4	160±4.5	0.67±0.04	0.67±0.03	0.44±0.05	0.13±0.02	0.28±0.02	0.33±0.03
Be	BDL	0.02±0.001	0.06±0.001	BDL	BDL	0.02±0.002	BDL	BDL	0.02±0.001	BDL	0.01±0.001	0.03±0.002	BDL	BDL	0.04±0.003
V	0.13±0.03	2.95±0.5	2.11±0.3	0.40±0.07	2.91±0.3	2.64±0.2	0.45±0.07	3.6±0.4	3.29±0.2	8.50±0.6	7.11±0.4	4.7±0.3	1.04±0.2	2.46±0.4	3.13±0.4
Mn	124.2±8.5	860.4±9.4	677.6±6.4	116±4.6	537±6.2	449.2±6.9	108±5.8	454.6±9.3	456.3±6.8	955.3±11.5	1541±24.7	1357±18.6	334.4±7.4	403±18.9	578.5±16.3
Co	BDL	0.68±0.2	0.53±0.1	BDL	0.59±0.1	0.52±0.1	BDL	0.53±0.2	0.48±0.2	1.72±0.5	1.79±0.7	1.68±0.6	0.65±0.1	0.76±0.2	1.26±0.4
Ni	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Cu	258.2±9.3	494.9±13.6	390.8±15.2	333.4±12.5	502.4±12.5	499.5±21.3	272.9±16.7	524±32.1	448.7±24.6	242.8±16.7	215.4±12.3	305.6±14.3	1383±34.2	835.1±21.4	3054±45.2
Zn	23.17±0.4	1276±23.5	102.1±5.9	771.8±14.7	1490±32.6	1461±32.8	39.86±9.2	1105±23.9	1057±24.7	42.12±12.5	47.52±9.2	966.2±18.9	717.8±23.1	982.6±28.4	800.8±32.7
Ga	BDL	0.17±0.04	0.13±0.01	0.01±0.004	0.21±0.04	0.16±0.06	BDL	0.25±0.04	0.2±0.07	0.32±0.06	0.26±0.06	0.19±0.01	0.01±0.004	0.14±0.05	0.14±0.02
As	0.13±0.04	0.28±0.02	0.25±0.03	0.04±0.002	0.19±0.07	0.18±0.02	BDL	0.14±0.08	0.29±0.02	0.39±0.03	0.40±0.07	0.36±0.09	0.07±0.05	0.19±0.02	0.24±0.04
Se	0.50±0.1	0.42±0.07	0.45±0.05	0.62±0.07	0.72±0.1	1.17±0.6	BDL	BDL	BDL	2.25±0.4	2.41±0.6	2.9±0.8	1.1±0.2	BDL	1.42±0.03
Rb	6.56±0.9	115.8±12.3	50.96±12.3	28.55±14.5	108.6±14.6	30.01±9.7	103±13.5	104.1±11.2	8.04±0.7	83.09±12.5	6.89±1.4	81.32±16.8	159.5±21.6	212.4±12.4	143.7±9.8
Sr	22.44±22.1	2583±32.5	3447±36.7	1557±12.4	1315±14.7	2155±34.7	2614±43.8	2496±37.8	3837±52.1	2436±32.7	2516±34.5	2463±33.2	2080±25.6	498.6±18.9	1623±28.9
Ag	0.04±0.002	0.06±0.001	0.06±0.003	0.04±0.006	0.06±0.002	0.05±0.001	0.05±0.002	0.07±0.002	0.08±0.002	0.05±0.001	0.09±0.004	0.06±0.001	0.08±0.002	0.03±0.005	0.06±0.001
Cd	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.08±0.006	BDL	BDL
Cs	BDL	0.01±0.004	0.01±0.002	BDL	0.02±0.006	0.03±0.004	BDL	0.04±0.007	0.03±0.005	0.04±0.003	0.04±0.009	0.03±0.001	0.001±0.0006	0.02±0.005	0.01±0.003
Ba	187.6±12.5	256.5±22.6	286±18.7	266.2±17.4	265.6±12.8	320.4±22.5	251±18.7	230.2±28.5	311.6±22.6	207.1±18.9	232.8±20.1	206.6±17.6	150.8±12.3	80.52±14.3	146.9±15.6
Tl	0.002±0.0004	BDL	BDL	BDL	0.005±0.0003	0.001±0.0004	BDL	0.001±0.0005	0.004±0.001	0.02±0.004	0.01±0.008	0.02±0.003	BDL	0.001±0.0004	0.005±0.001
Pb	0.03±0.004	0.3±0.07	0.22±0.06	0.04±0.007	0.36±0.07	0.59±0.09	0.04±0.005	0.94±0.1	0.33±0.09	0.93±0.2	0.90±0.1	0.78±0.3	0.29±0.05	0.33±0.004	0.42±0.09
U	BDL	0.01±0.002	0.01±0.005	0.001±0.0002	0.01±0.006	0.02±0.007	BDL	0.04±0.002	0.03±0.001	0.06±0.005	0.06±0.007	0.03±0.008	0.004±0.0003	0.01±0.005	0.02±0.007

^aSamples were analyzed in triplicate and the data is presented as mean±SD of the concentrations obtained. BDL: Below detection limit; SD: Standard deviation; K-GS: Khat-Gaifi; K-GN: Khat-Gaifi; K-FMS: Khat-Faqarah; K-FMN: Khat-Faqarah; K-FMG: Khat-Faqarah; K-FAS: Khat-Faqarah; K-FAN: Khat-Faqarah; K-FAG: Khat-Faqarah; K-KS: Khat-Kofat-Nwaif; K-KN: Khat-Kofat-Nwaif; K-KG: Khat-Kofat-Nwaif; K-JS: Khat-Jahasha-Stem; K-JN: Khat-Jahasha-Nwaif; K-JG: Khat-Jahasha-Nwaif; K-FMS: Khat-Faqarah; K-FMN: Khat-Faqarah; K-FMG: Khat-Faqarah; K-FAS: Khat-Faqarah; K-FAN: Khat-Faqarah; K-FAG: Khat-Faqarah; K-KS: Khat-Kofat-Nwaif; K-KN: Khat-Kofat-Nwaif; K-KG: Khat-Kofat-Nwaif; K-JS: Khat-Jahasha-Stem; K-JN: Khat-Jahasha-Nwaif; K-JG: Khat-Jahasha-Nwaif; K-FMS: Khat-Faqarah; K-FMN: Khat-Faqarah; K-FMG: Khat-Faqarah; K-FAS: Khat-Faqarah; K-FAN: Khat-Faqarah; K-FAG: Khat-Faqarah

and Rb (6.56 ± 0.9 – 212.4 ± 12.4 mg/kg). In general, with few exceptions, it was observed that the concentrations of elements were least in stem of the plant, and the highest concentrations were present in the Nwaif (small) leaves. Several hazardous heavy metals were also found to be present in Khat samples including as (0.04 ± 0.002 – 0.40 ± 0.07 mg/kg), Pb (0.03 ± 0.004 – 0.94 ± 0.1 mg/kg), Li (0.13 ± 0.02 – 160 ± 4.5 mg/kg), and U (0.001 ± 0.0002 – 0.06 ± 0.007 mg/kg). When different Khat varieties were compared for the presence of metal ions, it was observed that the highest concentrations of metal ions were present in KJ variety followed by KFA and KFM, whereas, it was lesser in the other two varieties KG and KK. The Nwaif leaves of KJ variety showed the highest concentrations of Cs and Pb, whereas, the Gafra leaves had Li and Sr in highest concentrations when compared with parts of other varieties. Similarly, Nwaif leaves of KFM variety showed highest concentrations of Mn, Co, As, Ag, and U, whereas, Se was present in highest concentration in the Gafra leaves. KFM variety also showed the highest concentrations of V, Ga, and Tl in stem as compared to other samples. Cu was present in highest concentration in Gafra leaves of KFA variety, and the Nwaif leaves showed highest concentration of Rb. As far as, KK and KG varieties are concerned, the concentration of Zn and Ba was found to be highest in Nwaif and Gafra leaves of KK variety, respectively, whereas, Gaifi variety showed the presence of Be in highest concentration in Gafra leaves.

Human exposure assessment

Since some users consume all the three parts of the plant equally; the average concentrations of all the elements were calculated for each variety. This average concentration was then utilized to calculate the TWI of elements (mg) by the user which was compared with the provisional tolerable weekly intake (PTWI) of elements as per the guidelines set by the Food Agriculture Organization/World Health Organization (WHO) and Joint Expert Committee on Food Additives.^[12-14] Results obtained are summarized in Table 3.

From Table 3, weekly intake of elements such as Mn, Cu, and Zn not only exceeded the PTWI values set by the WHO but were many folds higher. For example, the weekly intake of Mn was 10 times (KJ) to 40 times (KFM) higher than the prescribed PTWI. Similarly, the weekly intake of Cu was observed to be two times (KFM) to 15 times (KFA) and for Zn, it was two times (KG) to five times (KK) higher than the PTWI of respective elements. This indicated the extremely hazardous level of intake of these elements by means of Khat. Other elements such as Li was also found to be present in KJ variety which was almost four times higher than the PTWI values. Co and Se were also detected in both KFM and KFA varieties in concentrations higher than the PTWI values.

Other exposure assessment parameters such as estimated daily intake (EDI) of Khat and THQ were calculated for all Khat varieties using the R_pD for each element, and the results are summarized in Table 4. R_pD is the maximum acceptable oral dose of a toxic substance and is set by the United States Environmental Protection Agency. THQ or Hazard Quotient is defined as the ratio of the potential exposure of a toxic substance to the level at which no adverse effects are expected. It is highly significant indicator of the hazards associated with a substance as if the values of THQ are <1, it indicates no adverse effects expected, but if it is more than 1, adverse effects are possible.

As shown in the table, 8 out of 20 elements had THQ values more than 1 in one or more Khat varieties. Surprisingly, five elements Sr, Mn, Cu, Zn, and Ba showed extremely high THQ values in all the Khat varieties and were much higher than 1. Li also showed THQ values more than 1 in all the Khat varieties except KFA, whereas, in case of Se, the THQ values were higher in KFM and KFA varieties. The THQ values of V were also higher than 1 in KJ, KFM, and KFA varieties. Although all other elements detected were present in higher concentrations in all the

Table 3: Average elemental concentrations in Khat varieties, their weekly intake and comparison with provisional tolerable weekly intake

Elements	Khat varieties												PTW/a (mg)			
	KG			KK			KJ			KFM				KFA		
	Average concentration (mg/kg)	Weekly intake (mg)	Weekly intake (mg)	Average concentration (mg/kg)	Weekly intake (mg)	Weekly intake (mg)	Average concentration (mg/kg)	Weekly intake (mg)	Weekly intake (mg)	Average concentration (mg/kg)	Weekly intake (mg)	Weekly intake (mg)		Average concentration (mg/kg)	Weekly intake (mg)	Weekly intake (mg)
Li	2.05	4.31	2.20	1.05	2.20	164.95	78.55	0.59	1.24	0.25	0.52	42				
Be	0.04	0.08	0.04	0.02	0.04	0.05	0.02	0.02	0.04	0.04	0.08	NA ^b				
V	1.73	3.63	4.16	1.98	4.16	5.14	2.45	6.79	14.26	2.21	4.64	35				
Mn	554.07	1163.54	771.54	367.40	771.54	713.23	339.63	1284.43	2697.31	438.63	921.13	68.6				
Co	0.60	1.27	1.17	0.56	1.17	1.06	0.51	1.73	3.63	0.89	1.86	1.33				
Ni	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.245				
Cu	381.30	800.73	934.71	445.10	934.71	871.92	415.20	254.60	534.66	1757.37	3690.47	245				
Zn	467.09	980.89	2605.96	1240.93	2605.96	1581.90	753.29	351.95	739.09	833.73	1750.84	490				
Ga	0.15	0.32	0.27	0.13	0.27	0.47	0.22	0.26	0.54	0.10	0.21	NA				
As	0.22	0.46	0.28	0.13	0.28	0.46	0.22	0.38	0.81	0.17	0.35	1.05				
Se	0.46	0.96	1.76	0.84	1.76	0.00	0.00	2.55	5.36	1.26	2.64	2.45				
Rb	57.77	121.32	117.01	55.72	117.01	150.60	71.71	57.10	119.91	171.87	360.92	NA				
Sr	2758.00	5791.80	3518.90	1675.67	3518.90	6262.90	2982.33	2471.67	5190.50	1400.53	2941.12	NA				
Ag	0.06	0.12	0.10	0.05	0.10	0.14	0.07	0.07	0.14	0.06	0.12	NA				
Cd	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.18	0.49				
Cs	0.01	0.03	0.06	0.03	0.06	0.07	0.04	0.04	0.08	0.01	0.02	NA				
Ba	243.37	511.07	596.54	284.07	596.54	554.96	264.27	215.50	452.55	126.07	264.75	NA				
Tl	0.00	0.00	0.01	0.00	0.01	0.01	0.00	0.01	0.03	0.00	0.01	NA				
Pb	0.18	0.38	0.69	0.33	0.69	0.92	0.44	0.87	1.83	0.35	0.73	1.75				
U	0.01	0.02	0.02	0.01	0.02	0.07	0.03	0.05	0.11	0.01	0.02	NA				

^aPTWI: Intake calculated in mg for a 70 kg body weight person; ^bNA: Data not available; values in bold are the values exceeding reported PTWI values showing hazardous intake. PTWI: Provisional tolerable weekly intake; KG: Khat varieties Gaiifi; KK: Kofati; KJ: Jahasha; KFM: Faqarah Menjed; KFA: Faqarah Aswad

Khat varieties, their THQ values were <1 indicating safe intake of these elements. HI was calculated by adding all the THQ values of different elements to compare the hazardous nature of all the Khat varieties. KJ variety showed extremely high HI values (231.53) followed by KFA (95.41), KFM (82.27), KG (66.64), and KK (64.97). It indicated that all the Khat varieties are associated with significantly high metal intake and the KJ variety topped the list.

Statistical analysis

Pearson correlation analysis

For Pearson correlation analysis, correlation coefficient values higher than 0.50 were considered to be statistically significant to correlate various data points and the results obtained are summarized in Table 5. Values given in bold indicate significant correlation between variables (concentration of metal ions) at different confidence intervals (**P* < 0.01, ***P* < 0.05). The positive values indicated a positive correlation among the variables, whereas, the negative correlation coefficient values showed a negative correlation. If the correlation values are near to “zero,” it indicated weak or nonsignificant correlation, and on the other hand,

if the values are closer to “one,” it indicated highly significant correlation among the two variables. Few elements such as Cu and Cs showed an unusual behavior of not showing any significant positive or negative correlation with any other element, which suggested that the source for these metal ions are different from other elements. Two elements V and U showed the highest number of positive correlations with other elements suggesting the multiple source of contamination and multiple origins for these elements. Sr, the most abundant element in all Khat samples showed correlations with Li, Rb, Ag, and Ba, but the correlation was not strong enough and the correlation coefficient values near to 0.5 (**P* < 0.05). It suggested that the source for Sr is different from most of the elements. Li and Ag showed positive significant correlations with only one element Sr; therefore, they were placed in a distinct group. Following the interpretation of the correlation matrix, other elements were further placed in three different groups. Elements such as V, Pb, Mn, and U were placed in group I, whereas, Group II consisted of Co, Ga, and As. Group III comprised of Se, Rb, and Sr. This elemental grouping was further analyzed through principal component analysis.

Table 4: Estimated daily intake and hazard quotient of various elements for different Khat varieties

Khat varieties	Li	Be	V	Mn	Co	Ni	Cu	Zn	Ga	As	Se	Rb	Sr	Ag	Cd	Cs	Ba	Tl	Pb	U	H.I.	
KG																						
EDI	0.01	0.0002	0.01	2.37	0.003	0.00	1.63	2.00	0.001	0.001	0.002	0.25	11.82	0.0002	0.00	0.0001	1.04	0.00	0.001	0.00004	66.64	
THQ	4.40	0.09	0.82	16.96	0.09	0.00	11.67	6.67	NA	0.31	0.39	NA	19.70	0.05	0.00	NA	5.22	0.00	0.22	0.05		
KK																						
EDI	0.004	0.0001	0.01	1.57	0.002	0.00	1.91	5.32	0.001	0.001	0.004	0.24	7.18	0.0002	0.00	0.0001	1.22	0.00001	0.001	0.00004	64.97	
THQ	2.25	0.04	0.94	11.25	0.08	0.00	13.63	17.73	NA	0.19	0.72	NA	11.97	0.04	0.00	NA	6.09	0.0001	0.00	0.05		
KJ																						
EDI	0.34	0.0001	0.01	1.46	0.002	0.00	1.78	3.23	0.001	0.001	0.00	0.31	12.78	0.0003	0.00	0.00	1.13	0.00001	0.002	0.0001	231.53	
THQ	168.32	0.05	1.17	10.40	0.07	0.00	12.71	10.76	NA	0.31	0.00	NA	21.30	0.06	0.00	NA	5.66	0.0001	0.54	0.18		
KFM																						
EDI	0.003	0.0001	0.03	5.50	0.01	0.00	1.09	1.51	0.001	0.002	0.01	0.24	10.59	0.0003	0.00	0.0002	0.92	0.0001	0.004	0.0002	82.27	
THQ	1.27	0.04	3.23	39.32	0.25	0.00	7.79	5.03	NA	0.55	2.19	NA	17.65	0.06	0.00	NA	4.62	0.0003	0.0002	0.27		
KFA																						
EDI	0.001	0.0002	0.01	1.88	0.004	0.00	7.53	3.57	0.0004	0.001	0.01	0.74	6.00	0.0002	0.0004	0.00004	0.54	0.00	0.001	0.00005	95.41	
THQ	0.53	0.08	1.05	13.43	0.13	0.00	53.80	11.91	NA	0.24	1.08	NA	10.00	0.05	0.36	NA	2.70	0.0001	0.0001	0.06		
RfD	0.002	0.002	0.01	0.14	0.03	0.05	0.14	0.30	NA	0.003	0.01	NA	0.60	0.01	0.001	NA	0.20	0.20	0.004	0.001		
ADI	1.00	0.004	0.02	0.20	20.00	5.00	0.50	0.50	NA	2.14	0.01	NA	40.00	0.01	0.001	NA	0.20	0.20	0.03	0.001		

Values in bold are the values of target hazard quotient exceeding 1 suggesting hazardous intake. EDI: Estimated daily intake; THQ: Target hazard quotient; NA: Data not available; HI: Hazard index; RfD: Oral reference dose in mg/kg bw/day; ADI: Acceptable daily intake; KG: Khat varieties Gaifi; KK: Kofat; KJ: Jahasha; KFM: Faqarah Menjed; KFA: Faqarah Aswad

Table 5: Pearson correlation matrix obtained for elements

	Li	V	Mn	Co	Cu	Zn	Ga	As	Se	Rb	Sr	Ag	Cs	Ba	Tl	Pb
Li	1															
V	0.054	1														
Mn	-0.125	0.822**	1													
Co	-0.370	0.820**	0.831**	1												
Cu	-0.099	-0.116	-0.128	-0.001	1											
Zn	0.249	-0.114	-0.085	-0.577*	0.174	1										
Ga	0.221	0.872**	0.599*	0.470	-0.318	-0.149	1									
As	0.066	0.859**	0.879**	0.765**	-0.169	-0.271	0.760**	1								
Se	-0.468	0.782**	0.775**	0.916**	-0.037	-0.194	0.552	0.707*	1							
Rb	-0.277	-0.090	-0.128	-0.132	0.0509	0.275	-0.207	-0.171	-0.020	1						
Sr	0.547*	0.158	0.212	-0.068	-0.326	-0.282	0.267	0.394	0.093	-0.590*	1					
Ag	0.419	0.363	0.475	0.091	0.086	0.019	0.205	0.328	0.380	-0.211	0.540*	1				
Cs	-0.264	-0.041	0.296	0.341	0.117	0.181	-0.151	0.202	0.466	0.428	-0.356	-0.433	1			
Ba	0.354	-0.008	0.012	-0.311	-0.489	0.164	0.148	0.101	-0.297	-0.708**	0.625*	0.252	0.162	-0.507	1	
Tl	-0.148	0.794**	0.399	0.570	-0.103	-0.601	0.763*	0.638*	0.319	-0.124	0.162	0.162	-0.428	0.015	1	
Pb	0.099	0.870**	0.721**	0.639*	-0.070	0.099	0.799**	0.612*	0.889**	-0.022	0.096	0.413	-0.010	-0.027	0.421	1
U	0.198	0.933**	0.668*	0.691*	-0.255	-0.466	0.842**	0.728**	0.798**	-0.361	0.336	0.389	-0.187	0.047	0.701*	0.892**

*Correlation is significant at the 0.05 level (two-tailed); **Correlation is significant at the 0.01 level (two-tailed). Values in bold are >0.5 and are statistically significant

Principal component analysis

Principal component analysis with varimax rotation followed by Kaiser Normalization was performed on the whole data set and the results obtained are summarized in Table 6. It shows the factor loadings for rotated principal component analysis of all Khat samples by keeping the concentration of metal ions as variables and calculating the Eigenvalues. It was applied to establish the association of elements in different factors which would give information regarding the source and distribution of metal ion and its contamination. The factor loadings were determined by the analysis, and the highest values were marked bold, which represents the placement of variable in the factor or component. There were four factors obtained for the data, and the elements were divided into these factors according to their characteristics. Factor 1 had the highest loadings of elements with Mn, Co, As, Se, Sr, Cs, and Pb in it. It also accounted for the most variance (55.92%) and hence had the highest Eigenvalues (9.506) suggesting the influence of one factor and one source of contamination for these elements. Factor 2 accounted for 20.71% of the total variance and was composed of elements such as V, Zn, Ga, Tl, and U. These elements again might get deposited by another factor with a different source of contamination. Factor 3 shows 13.296% of the variance and showed the presence of Li, Cu, Rb, and Ba, whereas Factor 4 accounted for 6.864% variance and consisted of high loading of only one element, Ag. Component plot in rotated three-dimensional space of metal data in soil samples are shown in Figure 2 which shows the placement of elements in various principal components as per their absolute values.

Hierarchical cluster analysis

Hierarchical cluster analysis was performed to determine the similarity between the variables (Khat samples) by dividing them into clusters. The dendrogram tree obtained is depicted in Figure 3 which shows the presence of four different clusters based on their elemental concentration. The cluster solution was seen as a sudden jump (gap) in the distance coefficient, and the solution before the gap indicated good solution. The first cluster comprised of samples KGS, KGG, KJS, and KJG with lesser distances showing the similarity between the samples. Samples coming in the same cluster signify that these are similar to each other as far as their elemental concentrations are concerned. The gaffra leaves and stem of Gaifi and Jahasha varieties were similar to each other. Out of these samples, KGS, KJS, and KGG showed much similarity as

they were linked to each other with a very short distance (~ 1), whereas, KJS and KJG were linked at a comparatively larger distance (~ 3). The other cluster had all three Fakharah Menjed samples, i.e., KFMS, KFMN, and KFMD showing common characteristics between them. The third cluster being the largest one consisted of KKS, KJN, KKG, KGN, KKN, and KFAS whereas, the last cluster was the smallest one consisting of only two samples, KFAN, and KFAG. The lesser the distance between the variables, the lesser is the dissimilarity between them.

DISCUSSION

This study was aimed to determine the concentration of metal ions in different Khat varieties consumed in Jazan, Saudi Arabia as it remains one of the biggest sites of Khat consumption in the Kingdom. Five main varieties of Khat were identified on the basis of consumption and

Table 6: Factor loadings for Varimax with Kaiser Normalization and rotated Principal Component Analysis of Khat samples

Elements	Factor 1	Factor 2	Factor 3	Factor 4
Li	0.628	0.170	0.735	0.166
V	0.469	0.871	0.053	0.124
Mn	0.798	0.270	0.080	0.489
Co	0.760	0.507	0.309	0.262
Cu	0.309	0.273	0.841	0.050
Zn	0.413	0.806	0.214	0.344
Ga	0.198	0.911	0.234	0.000
As	0.750	0.602	0.063	0.249
Se	0.955	0.261	0.038	0.132
Rb	0.247	0.158	0.804	0.449
Sr	0.791	0.365	0.355	0.115
Ag	0.185	0.094	0.013	0.960
Cs	0.753	0.518	0.318	0.198
Ba	0.319	0.192	0.925	0.067
Tl	0.040	0.993	0.096	0.050
Pb	0.706	0.616	0.293	0.127
U	0.474	0.812	0.112	0.292
Eigenvalues	9.506	3.521	2.260	1.167
Percentage of variance	55.920	20.710	13.296	6.864
Cumulative variance	55.920	76.630	89.926	96.790

Values in bold indicates the absolute values >0.5 and are statistically significant

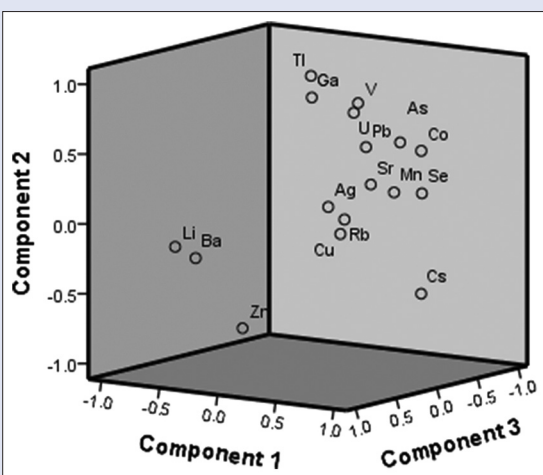


Figure 2: Component plot in rotated space of elemental data in Khat samples showing the placement of elements in three major principal components

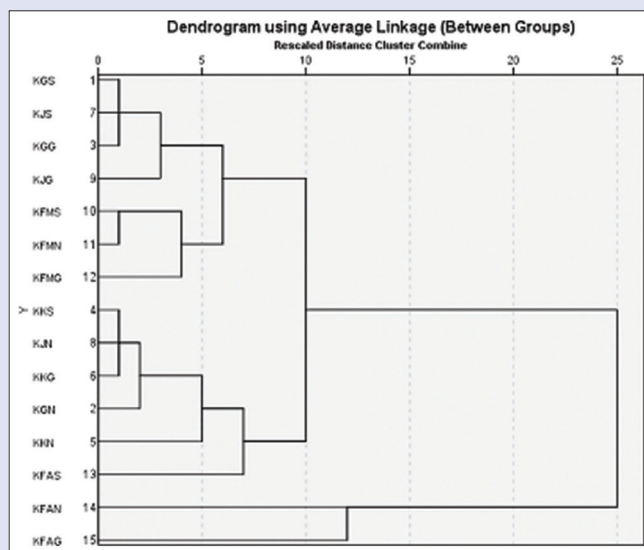


Figure 3: Dendrogram obtained after Hierarchical cluster analysis of 15 Khat samples

popularity among the users and subjected to elemental analysis using ICP-MS. All the Khat varieties were grown in different environmental conditions and were expected to have different concentrations of metal ions. Metal ions, especially the heavy metal ions in Khat plants, are absorbed and accumulated due to many reasons including the presence of heavy metal ions in soil as well as in atmosphere and the vegetation phase.^[15-18] In addition to it, some other sources are also responsible for their presence that includes waste/dirty water irrigation and use of fertilizers and pesticides containing heavy metals.^[19-21] The nature and type of soil are believed to be the most important factor which determines the presence of heavy metals as it remains the retention and binding site for various toxic materials.^[18-20] These metal ions are transferred to different parts of the plants from soil intrinsically and are also present on the surface of leaves being present in soil, pesticides, and water. Some users chew the Khat leaves without washing as they believe that washing Khat leaves before chewing reduces its potency. It leads to ingestion of more metal ions, harmful chemicals, and pesticides. This is nevertheless an additive factor associated with their health hazards.

Several metal ions are required by the body for its important biological processes. Food remains one of the major sources of these ions in the body. The elements that are essential to the human body are accumulated inside the plants along with the elements that are of no use to plants. These elements include Cd, Co, and Ag.^[22,23] Some highly toxic metals such as Cd and Pb are almost always present in trace quantities in most of the foodstuffs.^[23] These trace elements from soil, water, fertilizer, and pesticide move to the human body through plants and they serve as intermediate reservoirs. Some micronutrients which are necessary for biological systems such as Cu, Co, and Mn cause toxicity to the body, if the intake increases than the tolerable amount. Among these elements, heavy metals are believed to be the most dangerous causative factor for various health hazards in humans.^[24,25] There are several toxic heavy metals that are nonbiodegradable and remain present inside the food chain and pass from environment to plants, to animals, to humans, and so on. These toxic metals include Cd, Pb, Ni, and Al and they have already been identified as serious threat to the living beings if consumed in higher concentrations. Excessive intake can always lead to chronic toxicities in humans and animals.

The results obtained in this study indicated the presence of several metal ions in much higher concentrations in all the Khat varieties including Sr, Mn, Zn, Cu, and Ba. Sr is a ubiquitous element present in almost all living organism in low-to-high concentrations. In most plants, it is reported to be present in concentrations from 1 to 169 ppm (average 36 ppm) dry weight, but in some plants which grow on Sr rich soils can reach concentrations up to 26,000 ppm.^[26] The average whole body content in a 70 kg human being was determined to be 320 mg.^[27] In general, Sr is considered to have low toxicity, and till now, no reports have been published by the WHO on the toxicology of Sr, but its capacity to substitute Ca from the bones is a cause of concern. It can also substitute Ca in maintaining excitability of nerve and muscle membranes and can enhance acetylcholine release from myoneural junctions at low Ca concentrations but at higher Ca concentrations, it can inhibit the release. It can also substitute Ca in the excitation and contraction of cardiac and skeletal muscles.^[26] Sr has been used in the prevention and treatment of osteoporosis and even high doses (1700 mg/day) have shown no clear evidence of toxicity in humans.

Elements such as Mn, Zn, and Cu come under the category of essential micronutrients and are required by plants and animals for their normal biological functions. The insufficiency of the supply of these elements results in various diseases or syndromes. These elements are the constituents of several enzymes and play important roles in redox reactions occurring in the biological systems.^[28] For instance, Cu acts

as an essential cofactor for many oxidative stress-related enzymes, such as cytochrome C oxidases, peroxidases, monoamine oxidases, and catalases.^[29-31] Nevertheless, the toxic properties of Cu is because of the fact that it can exhibit transitions between Cu (II) and Cu (I) generating reactive oxygen species which include superoxide and hydroxyl radicals.^[29-32] The excessive intake of Cu leads to cellular damage and Wilson disease in case of humans.^[31,32] Cu along with Cr has a very narrow window of concentrations between beneficial and toxic effects.^[32,33] Mn, on the other hand, plays an important role in maintaining human health and is essential for metabolic processes in humans. However, its excessive intake causes a set of disorders called manganism, which is a neurodegenerative disorder affecting dopaminergic nerves and leads to a condition similar to Parkinsonism.^[34,35]

Similarly, Zn has also been shown to possess toxic effects when ingested in overdose. Apart from causing minor adverse effects such as nausea, vomiting, cramps, and diarrhea, it is also involved in the suppression of absorption of Cu and Fe inside the body.^[36] When ingested in concentrations above Recommended Dietary Allowance (RDA; 100–300 mg/day), it leads to anemia, neutropenia, impaired immune functions, and higher low-density lipoprotein/high-density lipoprotein ratio.^[36] Elemental concentrations were determined for different parts of the five Khat varieties including Nwaif (small) leaves, Gafra (old) leaves and Stem. Since Khat is supplied in the form of a bunch of these parts, and the consumption varies with the choice of users. Some users who are newer consume only Nwaif leaves, whereas others consume both Nwaif and Gafra leaves. However, some very addictive users consume all the three parts of the plant in the whole day. Therefore, the concentrations in all three parts were determined separately to compare the elemental intake depending on the consumption. In general, it was observed that the stem of the plant contained lesser concentrations of metal ions than Nwaif and Gafra leaves. Some elements were present in higher concentration in Nwaif leaves, and some were present more in Gafra leaves. Nevertheless, this concentration depends on the soil, and other environmental factor such as water for irrigation, pesticides and surrounding air and this is the reason for different concentrations in various parts of the plant. The intake of metal ions would be lesser if the user consumes only Nwaif leaves followed by if he consumes both Nwaif and Gafra leaves and the maximum intake would be when the user consumes all three parts.

The weekly intake of several elements such as Li, Mn, Co, Cu, Zn, Se, Sr, and Pb were much higher and above the PTWI suggesting very hazardous intake and possible toxicities due to these elements. Cu, being the highest, showed 15 folds higher intake than the recommended value which is a major cause of concern associated with the consumption of Khat. It was further confirmed by determining another assessment parameter THQ that showed values higher than 1 in case of several metal ions. These elements, if consumed in this much quantity, would surely have extremely deleterious effects on various biological systems and may lead to deadly diseases including cancer and cardiovascular disorders.

CONCLUSION

On the basis of above results, it may be concluded that Khat not only shows toxicity because of various hazardous chemical constituents present in it, but it also has high concentrations of toxic heavy metal ions which is equally responsible for its capability to cause dreadful diseases. Many toxic elements were present in much higher concentrations than the tolerable limits which is mainly due to environmental factors in which Khat plants are grown. Type of soil, water for irrigation, and surrounding environment has direct influence on the concentration of metal ions which should be taken care while growing Khat. Users of Khat should be educated by the concerned authorities regarding the adverse

effects and plausible toxicities associated with its use which would help in the cessation program.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Al-Motarreb A, Baker K, Broadley KJ. Khat: Pharmacological and medical aspects and its social use in Yemen. *Phytother Res* 2002;16:403-13.
- Saha S, Dollery C. Severe ischaemic cardiomyopathy associated with khat chewing. *J R Soc Med* 2006;99:316-8.
- Expert Committee on Drug Dependence. World Health Organization Technical Report Series. Expert Committee on Drug Dependence; 4 April, 2006. Available from: https://www.who.int/medicines/areas/quality_safety/4.4KhatCritReview.pdf. [Last accessed on 2018 Sep 18].
- Feyisa H, Aune JB. Khat expansion in the Ethiopian highlands, effects on the arming system in Habro district. *Mt Res Dev* 2003;23:185-9.
- 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;56:125-30.
- Al-Hebshi NN, Skaug N. Khat (*Catha edulis*)-an updated review. *Addict Biol* 2005;10:299-307.
- Ministry of Agriculture, Fisheries and Food. 1997 Total Diet Study: Aluminium, Arsenic, Cadmium, Chromium, Copper, Lead, Mercury, Nickel, Selenium, Tin and Zinc, Food Surveillance Information Sheet No. 191. Ministry of Agriculture, Fisheries and Food; 1999.
- Hagos T, Redi M, Taddese A. Correlation Between Khat (*Catha edulis* Forsk) Leaves and Soil Composition in Hararghe Region. Ethiopia: Haramaya University; 2010.
- Association of Official Analytical Chemists. AOAC Official Methods of Analysis. 15th ed. Arlington: Association of Official Analytical Chemists; 1990.
- Retka J, Maksymowicz A, Karmasz D, editors. Determination of Cu, Ni, Zn, Pb, Cd by ICP-MS and Hg by AAS in plant samples. Proceedings of 15th International Conference on Heavy Metals in the Environment. Department of Analytical Chemistry, Chemical Faculty, Gdansk University of Technology, Gdansk, Poland; 19-23 September 2010. p. 1071.
- United States Environmental Protection Agency. USEPA Regional Screening Level (RSL) Summary Table. Available from: <http://www.epa.gov/regshwmd/risk/human/Index.htm>. [Last updated on 2011 Nov 04; Last accessed on 2018 Sep 20].
- Food and Agriculture Organization/World Health Organization. Joint Expert Committee on Food Additives. Evaluation of Certain Food Additives and Contaminants. WHO Technical Report Series. Vol. 837. Geneva: World Health Organization; 1993.
- Food and Agriculture Organization/World Health Organization. Joint Expert Committee on Food Additives. Evaluation of Certain Food Additives and Contaminants. WHO Technical Report Series. Vol. 940. Geneva: World Health Organization; 2007.
- Food and Agriculture Organization/World Health Organization. Joint Expert Committee on Food Additives, Summary Report of the Seventy-Second Meeting of JECFA. Geneva: World Health Organization; 2010.
- NJF, editor. Essential Trace Elements for Plants, Animals and Humans. Proceedings of the NJF Seminar No. 370. Reykjavik, Iceland: 15-17 August, 2005. p. 1-94.
- Kabata-Pendias A. Soil plant transfer of trace elements-an environmental issue. *Geoderma* 2004;122:143-9.
- Anke MK. Transfer of macro, trace and ultratrace elements in the food chain. In: Marian EA, Anke M, Steoppler M, editors. Elements and their Compounds in the Environment: Occurrence, Analysis and Biological Relevance. Weinheim: Wiley-VCH Verlag and Co.; 2004. p. 1499-522.
- Nouri J, Khorasani N, Lorestani B, Karami M, Hassani AH, Yousefi N. Accumulation of heavy metals in soil and uptake by plant species with phytoremediation potential. *Environ Earth Sci* 2009;59:315-23.
- Prabu PC. Impact of heavy metal concentrations of Akaki River of Ethiopia on soil and metal toxicity on cultivated vegetable crop. *Electr J Environ Agric Food Chem* 2009;8:818-27.
- Hart AD, Oboh CA, Barimalaa IS, Sokari TG. Concentrations of trace metals (Pb, Fe, Cu and Zn) in crops harvested in some oil processing locations in River state, Nigeria. *Afr J Food Nutr Sci* 2005;5:1-21.
- Nwazhuku OI. Contaminant source as factor of soil heavy metal toxicity and bioavailability to plants. *Environ Res J* 2008;2:322-6.
- Grusak MA, DellaPenna D. Improving the nutrient composition of plants to enhance human nutrition and health. *Annu Rev Plant Physiol Plant Mol Biol* 1999;50:133-61.
- Kabata-Pendias A, Pendias F. Trace Elements in Soils and Plants. 3rd ed. Boca Raton, Florida, USA: CRC Press; 2001.
- Ajasa AO, Bello MO, Ibrahim AO, Ogunwande IA, Olowore NO. Heavy trace metals and macronutrients status in herbal plants of Nigeria. *Food Chem* 2004;85:67-71.
- Voutsas D, Grimanis A, Samara C. Trace elements in vegetables grown in an industrial area in relation to soil and air particulate matter. *Environ Pollut* 1996;94:325-35.
- Beliles RP. The metals - Section on strontium. In: Clayton GD, Clayton FE, editors. Patty's Industrial Hygiene and Toxicology. Wiley Online Library. New York: John Wiley & Sons; 1994. p. 2216-27.
- Tipton IH, Cook MJ. Trace elements in human tissue. II. Adult subjects from the United States. *Health Phys* 1963;9:103-45.
- World Health Organization, Food and Agriculture Organization, International Atomic Energy Agency. Trace Elements in Human Nutrition and Health. Switzerland, Geneva: World Health Organization; 1996.
- Stern BR. Essentiality and toxicity in copper health risk assessment: Overview, update and regulatory considerations. *J Toxicol Environ Health A* 2010;73:114-27.
- Harvey LJ, McArdle HJ. Biomarkers of copper status: A brief update. *Br J Nutr* 2008;99 Suppl 3:S10-3.
- Agency for Toxic Substances and Disease Registry. Toxicological Profile for Copper. Atlanta, GA: Centers for Disease Control; 2002.
- Tchounwou PB, Newsome C, Williams J, Glass K. Copper-induced cytotoxicity and transcriptional activation of stress genes in human liver carcinoma (HepG (2)) cells. *Met Ions Biol Med* 2008;10:285-90.
- Chang LW, Magos L, Suzuki T. Toxicology of Metals. Boca Raton, Florida, USA: CRC Press; 1996.
- Emsley J. Manganese, Nature's Building Blocks: An A-Z Guide to the Elements. Oxford, UK: Oxford University Press; 2001. p. 249-53.
- Avila SD, Puntel LR, Aschner M. Manganese in health and disease. In: Astrid S, Helmut S, Sigel RK, editors. Interrelations between Essential Metal Ions and Human Diseases, Metal Ions in Life Sciences. Vol. 13. Basel, Switzerland: Springer; 2013. p. 199-227.
- Fosmire GJ. Zinc toxicity. *Am J Clin Nutr* 1990;51:225-7.