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ORIGINAL ARTICLE

Elemental Profiling of Smokeless Tobacco Samples Using Inductively Coupled Plasma-Mass Spectrometry, their Chemometric Analysis and Assessment of Health Hazards

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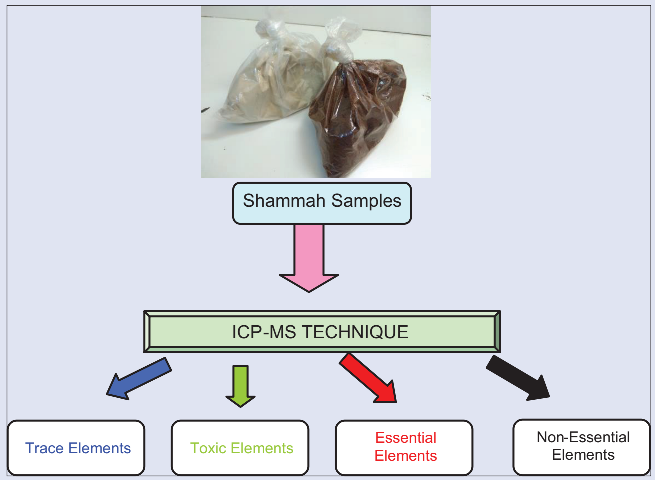
ABSTRACT

Background: Smokeless tobacco (ST), locally called shammah, is a form of tobacco that is widely used in Middle Eastern countries, including Saudi Arabia. **Objective:** A total of 21 ST samples were collected from the southern province of Jazan for elemental analysis. **Materials and Methods:** These samples were analyzed by inductively coupled plasma mass spectrometry to determine their element concentrations. Chemometric multivariate analysis such as hierarchical cluster analysis, Pearson correlation analysis, and principal component analysis were performed for better understanding and interpretation of the data. Concentrations obtained were used to determine the users' daily and weekly intake of elements and were compared with the acceptable daily intake and provisional tolerable weekly intake. **Results:** Metal ions present in maximum concentrations were strontium (11608.71 μg/kg) and manganese (3543.10 μg/kg), whereas those with minimum concentrations were silver (53.90 μg/kg) and chromium (62.33 μg/kg). **Conclusion:** Although the concentrations of all the elements fell under the safe limit, the concentrations of many toxic elements were significantly high and resulted in various health hazards on the intake of these elements with other sources.

Key words: Elements, heavy metals, inductively coupled plasma-mass spectroscopy, Jazan, Shammah, smokeless tobacco

SUMMARY

- Twenty one different varieties of Smokeless Tobacco (Shammah) samples were collected from various regions of Jazan, Saudi Arabia
- Qualitative and quantitative determination of different types of elements was performed by ICP-MS
- Total intake of elements by means of tobacco consumption was calculated and compared with the reference doses provided by international agencies and their hazard quotient were calculated
- Various Chemometric statistical analyses were performed to analyze the correlation between elements present in samples.



Abbreviations used: ST: Smokeless tobacco; EDI: Estimated daily intake; ADI: Acceptable daily intake; PTWI: Provisional tolerable weekly intake; HQ: Hazard Quotient; R_fD = Oral reference dose; ICH: International Council for Harmonisation; ICP-MS: Inductively coupled plasma-mass spectroscopy; AAS: Atomic absorption spectroscopy; CTA-OTL-1: Oriental Tobacco Leaves; % R. S. D.: % Relative standard deviation; ppb: Parts per billion; LOD: Limit of detection; SD: Standard Deviation; FAO: Food agriculture organization; WHO: World Health Organization; JECFA: Joint Expert Committee on Food Additives.

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INTRODUCTION

Smokeless tobacco (ST), also known as chewing, spitting, dipping, or snuffing tobacco, is a form of tobacco that is used other than smoking. ST is generally used by placing tobacco into the oral cavity, mostly between the lips or cheeks and gums, and ingesting the saliva produced thereafter. The general types of ST include the leaves of *Nicotiana tabacum*, *Nicotiana rustica*, *Nicotiana glauca*, or *Nicrophorus nepalensis*.[1,2] Although, the trade of ST is illegal in some countries such as Saudi Arabia, Japan, Hong Kong, Singapore, Australia, and New Zealand,[3] its use is continuously increasing with time. ST has over 300 million users worldwide, and most of these users are from

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South Asian and Middle Eastern countries.[4] The use of ST is more prevalent in the southern part of Saudi Arabia (Jazan Province) due to its proximity to Yemen, where it is legal to use and trade.

ST is associated with a range of adverse health issues, including cancer and cardiovascular diseases. Some harmful substances are reported to be found in ST, including at least 28 chemicals as potential carcinogens, of which nitrosamines are the most harmful.[5] There are some trace elements in the soil that are absorbed by tobacco plant which accumulates them in its leaves in large quantities. Some of the elements are too toxic to the human body even in minute quantities.[6-9] The concentrations of these trace elements in tobacco largely depend on the genotype; type and pH of water and soil; fertilizers and pesticides used; and various other environmental factors. Some of these elements are responsible for different types of ailments and diseases when consumed in quantities beyond the tolerable limits.

The regions of Jazan Province, where the use of ST (Shammah) was most prevalent, were selected for sample collection. These regions were Jazan, Sabya, Abu Areesh, Ahd Al Masaraha, and Samtah. Different types of tobacco samples collected were Green, Black Adani 1, Black Adani 2, Black Sudani, Suhail, Areeshi, and Special. A total of 21 different types of samples were collected and subsequently analyzed to investigate their metal ion concentrations using inductively coupled plasma mass spectrometry (ICP-MS). Different types of metal ions analyzed in this study were lithium (Li), beryllium (Be), vanadium (V), chromium (Cr), manganese (Mn), cobalt (Co), copper (Cu), zinc (Zn), gallium (Ga), arsenic (As), selenium (Se), rubidium (Ru), strontium (Sr), silver (Ag), cadmium (Cd), cesium (Cs), barium (Ba), thallium (Tl), lead (Pb), and uranium (U).

ICP-MS was used to determine the metal ion concentrations in tobacco samples because it is a robust, sensitive, and accurate method having high detection capability and can be used to accurately detect concentrations at parts per quadrillion level. Moreover, the advantage of ICP-MS over other elemental detection techniques, such as atomic absorption spectroscopy (AAS), is that the concentration of a single element can be measured in AAS at a specific time, whereas, all elements can be determined simultaneously in ICP-MS.

The present study was aimed to select various tobacco samples from different regions of Jazan Province and to determine the metal ion concentrations in their leaves by using ICP-MS and their comparison with the provisional tolerable weekly intake (PTWI) to assess the health hazards that they pose.

MATERIALS AND METHODS

Sample collection

A total of 21 samples were collected from five different cities of Jazan Province: Khudra Jazan (Jazan Green; Sample no. T-1), Areeshi Jazan (Sample no. T-2), Adani Jazan (Jazan Black Adani type-1; Sample no. T-3), Adani Jeeban (Jazan Black Adani type-2; Sample no. T-4), Sudani Sabya (Sabya Black Sudani; Sample no. T-5), Suhail Sabya (Sample no. T-6), Khudra Sabya (Sabya Green; Sample no. T-7), Areeshi Sabya (Sample no. T-8), Khususi Masna Sabya (Sabya Special; Sample no. T-9), Adani Barid Abu Areesh (Abu Areesh Black Cool type; Sample no. T-10), Adani Haar Abu Areesh (Abu Areesh Black Hot type; Sample no. T-11), Khususi Abu Areesh (Abu Areesh Special; Sample no. T-12), Areeshi Abu Areesh (Sample no. T-13), Ahd Al Masaraha Areeshi (Sample no. T-14), Ahd Al Masaraha Khudra (Ahd Al Masaraha Green; Sample no. T-15), Ahd Al Masaraha Khususi (Ahd Al Masaraha Special; Sample no. T-16), Adani Baheel Ahd Al Masaraha (Ahd Al Masaraha Black Adani with Cardamom, Sample no. T-17), Adani Ahd Al Masaraha (Ahd Al Masaraha Black Adani; Sample no. T-18), Khudra Abu Areesh (Abu Areesh Green; Sample no. T-19), Areeshi Khudra Samtah (Samtah Green Areeshi; Sample no. T-20), and Khususi Samtah (Samtah Special; Sample no. T-21). The five cities of Jazan Province, from which the samples were collected, included Sabya, Abu Areesh, Ahd Al Masarha, and Samtah. The use of ST is most prevalent in these cities. The samples were collected and stored in dry condition away from direct sunlight until further analysis.

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).

Microwave digestion

To determine the total concentration of different metals, 0.1 g of dry and fine ST samples were taken and digested in a microwave digestion system ethos I in Teflon vessels with 4 mL of nitric acid. The digestion parameters were as follows: The programmable microwave power ranged from 800 W to 1400 W, temperatures ranged from 50°C to 200°C, ramp time was set to 10 min, and the total time of digestion was 70 min. On completion of the digestion process, Teflon vessels were allowed to cool at room temperature for 30 min. Thereafter, the Teflon vessels were opened under the hood and kept for 5 min until all fumes evaporated. The digested samples (0.5 mL) were then diluted to 10 mL with ultrapure deionized water and analyzed using the ICP-MS. Blanks were digested and prepared by following the same procedure.

Inductively coupled plasma-mass spectroscopy analysis

The metal concentrations of the ST were analyzed using ICP-MS 7500 (Agilent, Germany). All samples, blanks, and standards were analyzed in triplicate. Instrument quality control and tuning were performed using an instrument tuning solution at 1 ppb in 2% HNO_3.

Preparation of calibration curve and validation

A six-point calibration curve of the multi-element standard was plotted at 1, 5, 10, 20, 50, and 100 ppb. Excellent linearity was obtained in case of all elements with a correlation coefficient (r) value > 0.99. The digestion and analytical methods were validated using standard reference tobacco leaves (CTA-OTL-1). Percentage relative standard deviation (% R. S. D.) was calculated by using the recovered value of each element from the selected method and certified value. The percentage recovery of each metal was calculated as:

% Recovery = 100 × (value of selected method/certified value)

To determine the precision and accuracy of the selected method five quality control samples of concentrations 5, 10, 25, 50, and 90 ppb were analyzed interday and intraday before doing the real-time analysis. % Accuracy was calculated by the following formula:

% Accuracy = 100 × (practically obtained concentration/theoretical concentration)

Limit of Detection (LOD) values were determined from the calibration curve according to the ICH guidelines of validation (Q2R1). The standard deviation of several blank samples (SD_{blank}) was determined and was used in the following formula:

$LOD = 3.3 \times (SD_{blank}/\text{slope of calibration curve})$

Human exposure assessment

Considering that a user/dipper holds the tobacco in his/her pinch and places it between his/her gums and lower lip, then the quantity of consumed tobacco at one time varies between 0.5 g and 1.0 g. In this study, the quantity of consumed tobacco for all calculations was set at 0.5 g. If on an average, a dipper consumes tobacco 10 times daily, then the total quantities of tobacco consumed daily and weekly are 5 and 35 g, respectively. From this quantity, the amounts of elements consumed through tobacco per week were calculated by using Equation 1:

$$\text{Amount of elements consumed per week} = \frac{C_{element} \times C_w}{1000} \quad \text{(Eq. 1)}$$

Where

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

C_w = amount of Tobacco consumed per week = 35 g.

Other assessment parameters, namely, estimated daily intake (EDI) and hazard quotient (HQ), were investigated. EDI refers to the concentration of elements consumed per kg body weight per day by a user. EDI (μg/kg bw/day) is calculated using Equation 2

$$\text{EDI} = C_{element} \times \left(\frac{C_d}{bw}\right) \quad \text{(Eq. 2)}$$

Where

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

C_d = amount of tobacco consumed per day = 5 g = 0.005 kg,

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

HQ is calculated by dividing EDI by the established R_fD values. The significance of HQ is that if the value of HQ is <1, then the substance is not hazardous. HQ is calculated using Equation 3

$$\text{HQ} = \frac{\text{EDI}}{R_f D} \quad \text{(Eq. 3)}$$

Where

EDI = estimated daily intake,

$R_f D$ = reference dose of the substance.

Statistical analyses

The analytical data were analyzed using SPSS statistical program 20.0 (IBM, SPSS, Armonk, NY). Three multivariate statistical analyses were employed for better interpretation of data. There were 20 rows expressing various elements and 21 columns indicated different ST samples. Hierarchical cluster analysis was performed to determine if there were any significant groups of samples with the same characteristics. Pearson correlation analysis was utilized to show the correlation between variables and is useful in determining divergence and coherence of data. The third multivariate technique performed was a principal component analysis on the whole data to check the variability on a scale by dividing variables into principal components.

RESULTS

Validation of the method

All the method validation parameters were assessed according to the ICH guidelines (Q2R1), and the results are presented in Table 1. Multi-element calibration curve was constructed for the quantification of elements and excellent linearity (1> r > 0.99) was observed for all elements. % Recovery, % Accuracy, % R. S. D. and LOD values were calculated for each element, and the results are summarized in Table 2. % Recovery values were obtained to be in a range of 92%–105%, % Accuracy was obtained in a range of 96%–122% and % R. S. D. values were <2.0 in case of all elements. All the obtained values were within the prescribed limit as stated in the ICH guidelines.

Elemental analysis

All 20 elements were analyzed simultaneously for each sample in triplicate, and the concentrations were determined in micrograms per kilograms (μg/kg). All the obtained results are summarized in Table 2. The highest concentration was found for Sr (average concentration = 11608.71 μg/kg), whereas the lowest concentration was for Cr (average concentration = 62.33 μg/kg) in all the samples. The concentration values of all the elements in different samples were almost similar with few exceptions. For example, Cr was present in sample T-1 in relatively high quantity (528.4 μg/kg), but it was below detection limit (BDL) in most samples. Li was present in the concentration range of 151.7–708.4 μg/kg with an average of

Table 1: Validation parameters of the selected method

Elements	Correlation coefficient (*r*)	Percentage recovery	Percentage accuracy	LOD (ng/L)	Precision (% RSD)
Li	0.9992	101.3	99.2	0.76	0.89
Be	0.9989	94.5	106.7	0.43	0.17
V	0.9996	102.5	117.4	0.37	0.18
Cr	0.9989	94.6	115.5	0.03	1.92
Mn	0.9997	92.5	118.7	0.15	0.09
Co	0.9997	96.2	118.6	0.46	0.39
Cu	0.9991	100.3	97.2	0.22	0.30
Zn	0.9449	103.2	99.36	0.05	0.54
Ga	0.9990	99.3	96.8	0.43	0.43
As	0.9989	100.4	107.5	0.33	0.29
Se	0.9915	91.4	101.5	0.17	1.89
Rb	0.9996	104.5	117.1	0.09	0.61
Sr	0.9997	105.3	117.8	0.43	0.24
Ag	0.9995	93.4	119.4	0.04	0.97
Cd	0.9980	92.9	111.8	0.17	0.13
Cs	0.9996	96.4	116.3	0.22	0.59
Ba	0.9990	99.4	119.9	0.07	0.39
Tl	0.9994	101.6	119.4	0.68	0.27
Pb	0.9998	103.5	118.2	0.91	0.39
U	0.9993	94.3	122.6	0.16	0.15

LOD: Limit of detection; RSD: Relative Standard Deviation

Table 2: Concentrations (µg/kg) of various elements in different tobacco samples as measured by inductively coupled plasma mass spectrometry

Element	Concentration (µg/kg)										
	T 1	T 2	T 3	T 4	T 5	T 6	T 7	T 8	T 9	T10	T 11
Li$^{*,\tau,\eta}$	623.1±6.7	496.4±5.3	386.6±4.1	451.3±4.8	151.7±1.6	830.3±8.9	446.7±4.8	667.6±7.2	700.7±7.5	329.2±3.5	367.6±3.9
Be$^{\tau,\eta}$	109.2±1.0	108.3±1.0	106.6±1.0	107.5±1.0	106.3±0.9	108.6±1.0	107.8±1.0	109±1.0	109±1.0	106.8±0.9	107.5±1.0
V$^{*,\eta}$	104.8±0.3	92.32±0.2	51.7±0.1	95.3±0.3	48.38±0.1	128.1±0.4	131.6±0.4	160.4±0.4	163.7±0.5	62.22±0.2	71.3±0.2
Cr$^{*,\varrho}$	528.4±7.1	237.2±3.2	BDL	BDL	BDL	BDL	BDL	111.8±1.5	134.2±1.8	BDL	BDL
Mn$^{*,\tau,\varrho}$	3577±12.8	3329±11.9	1664±5.9	2370±8.5	1570±5.6	4972±17.8	4156±14.9	4983±17.9	5151±18.5	1685±6.0	1902±6.8
Co$^{*,\varrho}$	68.61±0.4	67.7±0.4	48.4±0.3	57.4±0.3	45.5±0.2	97.89±0.6	95.07±0.6	105±0.6	104.6±0.6	50.11±0.3	52.61±0.3
Cu$^{*,\tau,\varrho}$	423.4±0.8	367.9±0.7	356.4±0.7	445.5±0.9	289.7±0.5	465.1±0.9	385.4±0.7	428.4±0.8	423.4±0.8	347.4±0.7	346.6±0.6
Zn$^{*,\tau,\varrho}$	826.8±4.3	741.2±3.9	583.9±3.0	670.8±3.5	747.6±3.9	869±4.6	1263±6.6	728.3±3.8	871±4.6	662±3.5	755.9±4.0
Ga$^{\eta}$	87.05±0.6	82.04±0.5	75.1±0.5	79.2±0.5	74.58±0.5	85.79±0.6	85.35±0.5	89.79±0.6	90.05±0.6	76.39±0.5	77.21±0.5
As$^{*,\tau,\eta}$	115.8±0.5	114.7±0.5	99.6±0.4	106.4±0.5	100.3±0.4	122.1±0.5	122.3±0.5	128.9±0.5	128.8±0.6	104.8±0.4	107.4±0.5
Se$^{*,\varrho}$	309.4±5.7	305±5.7	296.9±5.5	298.7±5.5	299±5.5	306.8±5.7	303.7±5.6	305.3±5.7	305.8±5.7	293.6±5.4	297.6±5.5
Rb$^{*,\eta}$	165.2±0.4	148.3±0.3	207.4±0.5	136.6±0.3	177±0.4	139±0.3	179.3±0.4	157.2±0.3	164.1±0.4	225.5±0.5	243.4±0.6
Sr$^{*,\eta}$	14,750±19.1	9731±12.6	7822±10.1	13,430±17.4	6566±8.5	16350±21.2	11,270±14.6	14650±19.0	15,450±20.0	6874±8.9	8002±10.4
Ag$^{\tau,\eta}$	59.1±0.2	55.3±0.2	54.2±0.1	54.4±0.2	53.4±0.2	54±0.2	53.5±0.1	53.5±0.2	53.5±0.1	53.3±0.2	53.3±0.1
Cd$^{\tau,\eta}$	97.78±0.9	97.5±0.9	93.8±0.8	93.6±0.8	95.9±0.8	102.7±0.9	97.6±0.8	96.49±0.8	99.03±0.9	93.28±0.8	93.51±0.8
Cs$^{\eta}$	71.69±0.5	70.2±0.5	68.9±0.5	70±0.5	68.9±0.5	69.8±0.5	69.86±0.6	70.1±0.5	70.17±0.5	68.94±0.5	69.15±0.5
Ba$^{\tau,\eta}$	1348±0.9	1147±0.8	548.2±0.4	1096±0.7	1066±0.7	1854±1.2	1154±0.8	1379±0.9	1469±1.0	520.4±0.3	850.5±0.6
Tl$^{\tau,\eta}$	109.8±0.3	109.5±0.3	109.6±0.3	109.7±0.3	109.6±0.3	109.6±0.3	109.6±0.3	109.5±0.3	109.6±0.3	109.4±0.3	109.3±0.3
Pb$^{\tau,\eta}$	188±0.6	184.4±0.6	175.3±0.6	184.6±0.6	183.2±0.6	183.2±0.6	208.2±0.7	191.4±0.6	188.9±0.6	176.4±0.6	178.8±0.6
U$^{\eta}$	133.3±0.7	126.6±0.7	120.4±0.6	123.5±0.6	119.6±0.6	127.2±0.7	125.3±0.6	127.8±0.7	127.8±0.7	120.6±0.6	120.9±0.6

Element	Concentration (µg/kg)										
	T 12	T 13	T 14	T 15	T 16	T 17	T 18	T 19	T 20	T 21	Average
Li$^{*,\tau,\eta}$	637.1±6.8	708.4±7.6	699.6±6.3	551.5±5.4	572.2±6.0	323.3±3.4	416.1±4.2	508.2±5.3	582.6±6.4	496.6±5.1	521.3±5.6
Be$^{\tau,\eta}$	107.6±1.0	109.7±1.0	109.9±0.9	107.5±0.8	109.4±0.9	107.5±0.8	108.8±0.7	107.7±0.6	108.7±0.8	108.7±0.7	108.2±1.0
V$^{*,\eta}$	73.4±0.2	147.9±0.4	116.1±0.3	92.2±0.2	110.1±0.4	179.4±0.9	227.8±1.4	69.9±0.2	90.8±0.5	72.9±0.2	109.1±0.3
Cr$^{*,\varrho}$	139.9±1.9	105.6±1.4	4.596±0.4	BDL	BDL	BDL	47.18±0.7	BDL	BDL	BDL	62.33±0.8
Mn$^{*,\tau,\varrho}$	3477±12.5	5886±21.1	4831±17.4	3558±11.8	3848±13.2	3494±12.7	4718±16.7	2959±9.4	3469±10.9	2806±8.5	3543.1±12.7
Co$^{*,\varrho}$	66.04±0.4	106.4±0.6	85.64±0.3	64.51±0.2	75.43±0.4	72.22±0.4	85.96±0.3	63.61±0.2	66.36±0.3	66.04±0.3	73.58±0.4
Cu$^{*,\tau,\varrho}$	404±0.8	587.5±1.1	427.5±0.8	373±0.7	358.3±0.6	316.4±0.4	396.2±0.5	323±0.4	313.1±0.3	280.6±0.5	383.7±0.7
Zn$^{*,\tau,\varrho}$	721.4±3.8	835.4±4.4	714.9±3.5	680.1±2.9	660.7±2.3	670.9±2.7	918.4±4.3	551.4±2.1	535.3±1.8	533.4±1.6	740.1±3.9
Ga$^{\eta}$	79.84±0.5	89.76±0.6	86.89±0.5	81.39±0.4	85.15±0.5	86.53±0.6	90.4±0.7	80.14±0.4	81.97±0.5	80.98±0.4	83.12±0.5
As$^{*,\tau,\eta}$	120.2±0.5	132.6±0.6	126.4±0.6	112±0.5	123.8±0.6	108.3±0.6	113.1±0.7	109.3±0.6	110±0.5	114.3±0.6	115.3±0.5
Se$^{*,\varrho}$	303.8±5.6	306.4±5.7	304.2±5.5	303.8±5.2	303±5.2	293.8±4.8	292.5±5.0	299±4.3	294.3±4.7	295.5±4.3	300.9±5.6
Rb$^{*,\eta}$	132±0.3	195.4±0.4	212.5±0.5	151.5±0.3	176.7±0.5	158.5±0.4	189.3±0.6	166.2±0.4	157.4±0.4	149.2±0.3	173.0±0.4
Sr$^{*,\eta}$	11,850±15.4	15,440±20.0	15,720±17.4	13,440±14.5	12,780±11.7	7220±6.8	10,090±10.2	10,730±9.6	11,890±14.2	9728±9.5	11,608.7±15.0
Ag$^{\tau,\eta}$	53.3±0.2	53.4±0.2	53.7±0.1	53.4±0.2	53.4±0.5	53.1±0.5	53.4±0.4	53.6±0.5	53.5±0.4	53.12±0.5	53.9±0.2
Cd$^{\tau,\eta}$	96.43±0.8	99.75±0.9	100.2±1.0	96.58±0.9	99.05±1.0	93.54±0.9	94.68±0.7	97.1±1.1	96.66±0.7	95.52±0.6	96.7±0.8
Cs$^{\eta}$	69.15±0.5	70.9±0.5	71.25±0.5	69.88±0.4	70.54±0.4	72.4±0.5	73.71±0.6	69.92±0.5	70.52±0.6	70.09±0.6	70.3±0.5
Ba$^{\tau,\eta}$	1212±0.8	1499±1.0	1664±1.2	1332±0.9	1475±1.2	1001±0.7	1327±1.2	1218±0.8	1147±0.7	946.2±0.5	1202.5±0.8
Tl$^{\tau,\eta}$	109.4±0.3	109.5±0.3	109.5±0.2	109.5±0.2	109.5±0.3	109.6±0.3	109.7±0.5	109.4±0.7	109.4±0.6	109.4±0.5	109.5±0.3
Pb$^{\tau,\eta}$	180.1±0.6	189.9±0.6	194.3±0.7	185.9±0.6	189±0.7	198.9±0.8	211.5±0.7	177.7±0.6	184.2±0.9	180.6±0.7	187.3±0.6
U$^{\eta}$	127.1±0.7	128.1±0.7	130.8±0.8	128±0.7	128.5±0.7	120.1±0.6	122±0.7	124.8±0.7	127.1±0.8	124.9±0.7	125.4±0.6

*Trace elements; $^{\tau}$Toxic elements; $^{\varrho}$Essential elements; $^{\eta}$Nonessential elements. Values are presented as mean±SEM (n=3). BDL: Below detection limit; SEM: Standard error of mean

521.28 μg/kg. Similarly, Be (106.3–109.9; 108.2), V (48.38–227.8; 109.08), Cr (0–528.4; 62.33), Mn (1570–5886; 3543.1), Co (45.5–106.4; 73.58), Cu (280.6–587.5; 383.75), Zn (533.4–1263; 740.07), Ga (74.58–90.41; 83.12), As (99.6–132.6; 115.29), Se (292.5–309.4; 300.86), Rb (132–225.5; 172.94), Sr (6566–16350; 11608.71), Ag (53.15–59.13; 53.90), Cd (93.28–102.7; 96.7), Cs (68.9–73.71; 70.29), Ba (520.4–1854; 1202.54), Tl (109.3–109.8; 109.53), Pb (175.3–211.5; 187.36), and U (119.6–133.3; 125.45) were found to be in significant quantities in all the samples. Figure 1 shows the comprehensive chart of various elements present in different tobacco samples for comparison.

The amounts of elements consumed per week [Table 3] by the user were calculated using Equation 1, and the resulting values were compared with the PTWI based on the guidelines set by the Food Agriculture Organization/World Health Organization and Joint Expert Committee on Food Additives (JECFA). The PTWI for some elements, such as Li, V, Cr, Mn, Co, Cu, Zn, As, Se, Cd, and Pb were set to be 600, 500, 200, 4000, 19, 1500, 15, 35, 7, and 25 μg/kg body weight, respectively.[10-12] Therefore, for these elements, the tolerable limits per week for a 70 kg person would be 42, 35, 14, 280, 1.33, 245, 105, 1.05, 2.45, 0.49, and 1.75 mg for Li, V, Cr, Mn, Co, Cu, Zn, As, Se, Cd, and Pb from all sources, respectively.

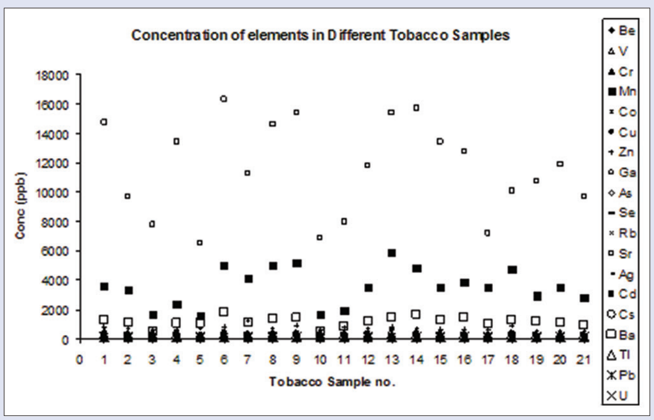

Figure 1: Comprehensive chart showing the concentrations of various elements in different tobacco samples (T1–T21)

Human exposure assessment

These limits were compared with the consumption of elements per week using ST, and the results showed a significant share in the total dietary intake for tobacco users. The results for the estimated consumption of elements per week are summarized in Table 3. It shows the amounts of elements found in 35 g sample, which is the approximate weekly intake of tobacco by the user for each sample. Although all the results were under the PTWI, they are still alarming because they constitute a major part of the total dietary intake of these elements by all means. These elements are also present in other food sources, and together, they can cross the PTWI, which could be detrimental for the user.

The results for EDI and HQ are summarized in Table 4. The EDI for each element was calculated for different tobacco samples which gives the concentration of various elements consumed daily by the user per kg body weight. This result can be compared with acceptable daily intake (ADI) and PTWI, which were previously established along with R_fD by JECFA[13] and the United States Environmental Protection Agency (U. S. EPA).[14] The HQ values of various elements were calculated by dividing the EDI with Oral R_fD is the dose in μg that can be taken orally per kilogram body weight per day. If the HQ of an element is more than one, that is, if the EDI exceeds R_fD, then the element is considered toxic. Similarly, if the HQ is <1, that is, the ADI is less than the R_fD, then the element is not considered hazardous. All the HQ values were <1, indicating that the overall intake of elements from ST was within safe limits.

Statistical analysis

Hierarchical cluster analysis

By performing the cluster analysis, for the elements, we could determine that there were four clusters of samples based the clustering method used. The cluster solution was seen as a sudden jump (gap) in the distance coefficient. The solution before the gap indicates the good solution. Samples T-2,-7,-12,-18,-19,-20, and -21 were distributed in the same cluster. Where sample T-3,-5,-10,-11, and -17 have the same characteristics to be include in the same cluster. Distribution of the samples based on their elemental concentrations in form of a dendrogram is shown in Figure 2.

Table 3: Amounts of elements (μg) consumed per week for different samples

Elements	Amount Consumed (μg) per week																					
	T 1	T 2	T 3	T 4	T 5	T 6	T 7	T 8	T 9	T 10	T 11	T 12	T 13	T 14	T 15	T 16	T 17	T 18	T 19	T 20	T 21	Average
Li	21.8	17.4	13.5	15.8	5.3	29.0	15.6	23.3	24.5	11.5	12.8	22.3	24.7	24.5	19.3	20.0	11.3	14.5	17.8	20.4	17.4	18.2
Be	3.8	3.8	3.7	3.8	3.7	3.8	3.8	3.8	3.8	3.7	3.7	3.8	3.8	3.8	3.7	3.8	3.7	3.8	3.77	3.8	3.8	3.8
V	3.6	3.2	1.8	3.3	1.7	4.5	4.6	5.6	5.7	2.1	2.5	2.6	5.2	4.0	3.2	3.8	6.2	7.9	2.4	3.18	2.5	3.8
Cr	18.5	8.3	0	0	0	0	0	3.9	4.7	0	0	4.9	3.7	0.2	0	0	0	1.6	0	0	0	2.2
Mn	125.2	116.5	58.2	83.0	55.0	174	145.5	174.4	180.3	58.9	66.5	121.7	206	169.1	124.5	134.7	122.3	165.1	103.6	121.4	98.2	124.0
Co	2.4	2.4	1.7	2.00	1.6	3.4	3.3	3.7	3.6	1.7	1.8	2.3	3.7	3.0	2.2	2.6	2.5	3.0	2.2	2.3	2.3	2.6
Cu	14.8	12.9	12.5	15.6	10.1	16.3	13.5	15.0	14.8	12.1	12.1	14.1	20.5	14.9	13.0	12.5	11.07	13.8	11.3	10.9	9.8	13.4
Zn	28.9	25.9	20.4	23.5	26.2	30.4	44.2	25.5	30.5	23.1	26.4	25.2	29.2	25.0	23.8	23.1	23.5	32.1	19.3	18.7	18.6	25.9
Ga	3.0	2.9	2.6	2.8	2.6	3.0	3.0	3.1	3.1	2.7	2.7	2.8	3.1	3.0	2.8	3.0	3.0	3.1	2.8	2.9	2.8	2.9
As	4.0	4.0	3.5	3.7	3.5	4.3	4.3	4.5	4.5	3.7	3.7	4.2	4.6	4.4	4.0	4.3	3.8	4.0	3.8	3.8	4.0	4.0
Se	10.8	10.7	10.4	10.4	10.5	10.7	10.6	10.7	10.7	10.3	10.4	10.6	10.7	10.6	10.6	10.6	10.3	10.2	10.5	10.3	10.3	10.5
Rb	5.8	5.2	7.3	4.8	6.2	4.9	6.3	5.5	5.7	7.9	8.5	4.6	6.8	7.4	5.3	6.2	5.5	6.6	5.8	5.5	5.2	6.0
Sr	516.3	340.6	274	470.1	229.8	572.3	394.5	512.8	540.8	240.6	280.1	414.8	540.4	550.2	470.4	447.3	252.7	353.2	375.6	416.2	340.5	406.3
Ag	2.0	1.9	1.9	1.9	1.8	1.9	1.8	1.8	1.8	1.8	1.8	1.8	1.9	1.9	1.9	1.8	1.8	1.9	1.9	1.9	1.9	1.9
Cd	3.4	3.4	3.3	3.3	3.3	3.6	3.4	3.4	3.4	3.3	3.2	3.3	3.5	3.5	3.4	3.4	3.3	3.3	3.4	3.4	3.3	3.4
Cs	2.5	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.5	2.5	2.4	2.5	2.5	2.6	2.4	2.4	2.4	2.5
Ba	47.2	40.1	19.2	38.3	37.3	64.9	40.4	48.3	51.4	18.2	29.8	42.4	52.5	58.2	46.6	51.6	35.0	46.5	42.6	40.1	33.1	42.1
Tl	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8
Pb	6.6	6.4	6.1	6.5	6.4	6.4	7.3	6.7	6.6	6.2	6.2	6.3	6.6	6.8	6.5	6.6	7.0	7.4	6.2	6.5	6.3	6.6
U	4.7	4.4	4.2	4.3	4.2	4.4	4.4	4.5	4.5	4.2	4.2	4.5	4.5	4.6	4.5	4.5	4.2	4.3	4.4	4.5	4.4	4.4

Table 4: Estimated daily intake and hazard quotient values of various elements for different tobacco samples

Samples	Li	Be	V	Cr	Mn	Co	Cu	Zn	Ga	As	Se	Rb	Sr	Ag	Cd	Cs	Ba	Tl	Pb	U
T 1																				
EDI	0.045	0.008	0.007	0.038	0.256	0.005	0.030	0.059	0.006	0.008	0.022	0.012	1.054	0.004	0.007	0.005	0.096	0.008	0.013	0.010
HQ	0.022	0.004	0.001	0.013	0.002	0.000	0.001	0.000	NA	0.028	0.004	NA	0.002	0.001	0.007	NA	0.001	0.000	NA	0.003
T 2																				
EDI	0.035	0.008	0.007	0.017	0.238	0.005	0.026	0.053	0.006	0.008	0.022	0.011	0.695	0.004	0.007	0.005	0.082	0.008	0.013	0.009
HQ	0.0177	0.0038	0.0007	0.0056	0.0016	1.612E-05	0.0006	0.0001	NA	0.0273	0.0043	NA	0.0011	0.0007	0.0069	NA	0.0011	3.91E-05	NA	0.0030
T 3																				
EDI	0.028	0.008	0.004	0.000	0.119	0.003	0.025	0.042	0.005	0.007	0.021	0.015	0.559	0.004	0.007	0.005	0.039	0.008	0.013	0.009
HQ	0.0138	0.0038	0.0004	0	0.0008	1.152E-05	0.0006	0.0001	NA	0.0237	0.0042	NA	0.0009	0.0007	0.0067	NA	0.00055	3.91E-05	NA	0.0028
T 4																				
EDI	0.032	0.008	0.007	0.000	0.169	0.004	0.032	0.048	0.006	0.008	0.021	0.010	0.959	0.004	0.007	0.005	0.078	0.008	0.013	0.009
HQ	0.0161	0.0038	0.0007	0	0.0012	1.367E-05	0.0007	0.0001	NA	0.0253	0.0042	NA	0.0015	0.0007	0.0066	NA	0.0011	3.92E-05	NA	0.0029
T 5																				
EDI	0.011	0.008	0.003	0.000	0.112	0.003	0.021	0.053	0.005	0.007	0.021	0.013	0.469	0.004	0.007	0.005	0.076	0.008	0.013	0.009
HQ	0.0054	0.0037	0.0003	0	0.0008	1.083E-05	0.0005	0.0001	NA	0.0238	0.0042	NA	0.0007	0.0007	0.0068	NA	0.0010	3.91E-05	NA	0.0028
T 6																				
EDI	0.059	0.008	0.009	0.000	0.355	0.007	0.033	0.062	0.006	0.009	0.022	0.010	1.168	0.004	0.007	0.005	0.132	0.008	0.013	0.009
HQ	0.0296	0.0038	0.0010	0	0.0025	2.331E-05	0.0008	0.0002	NA	0.0290	0.0043	NA	0.0019	0.0007	0.0073	NA	0.0018	3.91E-05	NA	0.0030
T 7																				
EDI	0.032	0.008	0.009	0.000	0.297	0.007	0.028	0.090	0.006	0.009	0.022	0.013	0.805	0.004	0.007	0.005	0.082	0.008	0.015	0.009
HQ	0.0159	0.0038	0.0010	0	0.002	2.264E-05	0.0006	0.0003	NA	0.0291	0.0043	NA	0.0013	0.0007	0.0069	NA	0.0011	3.91E-05	NA	0.0029
T 8																				
EDI	0.048	0.008	0.011	0.008	0.356	0.008	0.031	0.052	0.006	0.009	0.022	0.011	1.046	0.004	0.007	0.005	0.099	0.008	0.014	0.009
HQ	0.0238	0.0038	0.0012	0.0026	0.0025	0.00002	0.0007	0.0001	NA	0.0306	0.0043	NA	0.0017	0.0007	0.0068	NA	0.0014	3.91E-05	NA	0.0030
T 9																				
EDI	0.050	0.008	0.012	0.010	0.368	0.007	0.030	0.062	0.006	0.009	0.022	0.012	1.104	0.004	0.007	0.005	0.105	0.008	0.013	0.009
HQ	0.0250	0.0038	0.0012	0.0031	0.0026	2.49E-05	0.0007	0.0002	NA	0.0306	0.0043	NA	0.0018	0.0007	0.0070	NA	0.0014	3.91E-05	NA	0.0030
T 10																				
EDI	0.024	0.008	0.004	0.000	0.120	0.004	0.025	0.047	0.005	0.007	0.021	0.016	0.491	0.004	0.007	0.005	0.037	0.008	0.013	0.009
HQ	0.0117	0.0038	0.0004	0	0.0008	1.193E-05	0.0006	0.0001	NA	0.0249	0.0041	NA	0.0008	0.0007	0.0066	NA	0.0005	3.91E-05	NA	0.0028
T 11																				
EDI	0.026	0.008	0.005	0.000	0.136	0.004	0.025	0.054	0.006	0.008	0.021	0.017	0.572	0.004	0.007	0.005	0.061	0.008	0.013	0.009
HQ	0.0131	0.0038	0.0005	0	0.0009	1.253E-05	0.0006	0.0001	NA	0.0255	0.0042	NA	0.0009	0.0007	0.0066	NA	0.0008	3.9E-05	NA	0.0028
T 12																				
EDI	0.046	0.008	0.005	0.010	0.248	0.005	0.029	0.052	0.006	0.009	0.022	0.009	0.846	0.004	0.007	0.005	0.087	0.008	0.013	0.009
HQ	0.0227	0.0038	0.0005	0.0033	0.0017	1.572E-05	0.0007	0.0001	NA	0.0286	0.0043	NA	0.0014	0.0007	0.0068	NA	0.0012	3.91E-05	NA	0.0030
T13																				
EDI	0.051	0.008	0.011	0.008	0.420	0.008	0.042	0.060	0.006	0.009	0.022	0.014	1.103	0.004	0.007	0.005	0.107	0.008	0.014	0.009
HQ	0.0253	0.0039	0.0011	0.0025	0.0030	2.533E05	0.0010	0.0001	NA	0.0315	0.0043	NA	0.0018	0.0007	0.0071	NA	0.0015	3.91E-05	NA	0.0030
T 14																				
EDI	0.050	0.008	0.008	0.000	0.345	0.006	0.031	0.051	0.006	0.009	0.022	0.015	1.123	0.004	0.007	0.005	0.119	0.008	0.014	0.009
HQ	0.0249	0.0039	0.0009	0.0001	0.0024	2.039E-05	0.0007	0.0001	NA	0.0300	0.0043	NA	0.0018	0.0007	0.0071	NA	0.0016	3.91E-05	NA	0.0031
T 15																				
EDI	0.039	0.008	0.007	0.000	0.254	0.005	0.027	0.049	0.006	0.008	0.022	0.011	0.960	0.004	0.007	0.005	0.095	0.008	0.013	0.009
HQ	0.0196	0.0038	0.00072	0	0.0018	1.536E-05	0.0006	0.0001	NA	0.0266	0.0043	NA	0.0016	0.0007	0.0068	NA	0.0013	3.91E-05	NA	0.0030
T 16																				
EDI	0.041	0.008	0.008	0.000	0.275	0.005	0.026	0.047	0.006	0.009	0.022	0.013	0.913	0.004	0.007	0.005	0.105	0.008	0.014	0.009
HQ	0.0204	0.0039	0.0008	0	0.0019	1.796E-05	0.0006	0.0001	NA	0.0294	0.0043	NA	0.0015	0.0007	0.0070	NA	0.0015	3.91E-05	NA	0.0036
T 17																				

Contd...

Table 4: Contd...

Samples	Li	Be	V	Cr	Mn	Co	Cu	Zn	Ga	As	Se	Rb	Sr	Ag	Cd	Cs	Ba	Tl	Pb	U
EDI	0.023	0.008	0.013	0.000	0.250	0.005	0.023	0.048	0.006	0.008	0.021	0.011	0.516	0.004	0.007	0.005	0.072	0.008	0.014	0.009
HQ	0.0115	0.0038	0.0014	0	0.0017	1.72E-05	0.0005	0.0001	NA	0.0257	0.0041	NA	0.0008	0.0007	0.0066	NA	0.0010	3.91E-05	NA	0.0028
T 18																				
EDI	0.030	0.008	0.016	0.003	0.337	0.006	0.028	0.066	0.006	0.008	0.021	0.014	0.721	0.004	0.007	0.005	0.095	0.008	0.015	0.009
HQ	0.0148	0.0038	0.0018	0.0011	0.0024	2.047E-05	0.0007	0.0002	NA	0.0269	0.0041	NA	0.0012	0.0007	0.0067	NA	0.0013	3.92E-05	NA	0.0029
T 19																				
EDI	0.036	0.008	0.005	0.000	0.211	0.005	0.023	0.039	0.006	0.008	0.021	0.012	0.766	0.004	0.007	0.005	0.087	0.008	0.013	0.009
HQ	0.0181	0.0038	0.0005	0	0.001	1.515E-05	0.0005	0.0001	NA	0.0260	0.0042	NA	0.0012	0.0007	0.0069	NA	0.0012	3.91E-05	NA	0.0029
T 20																				
EDI	0.042	0.008	0.006	0.000	0.248	0.005	0.022	0.038	0.006	0.008	0.021	0.011	0.849	0.004	0.007	0.005	0.082	0.008	0.013	0.009
HQ	0.0208	0.0038	0.0007	0	0.001	0.00001	0.0005	0.0001	NA	0.0261	0.0042	NA	0.0014	0.0007	0.0069	NA	0.001	3.91E-05	NA	0.0030
T 21																				
EDI	0.035	0.008	0.005	0.000	0.200	0.005	0.020	0.038	0.006	0.008	0.021	0.011	0.695	0.004	0.007	0.005	0.068	0.008	0.013	0.009
HQ	0.0177	0.0038	0.0005	0	0.0014	1.572E-05	0.0005	0.0001	NA	0.0272	0.0042	NA	0.0011	0.0007	0.0068	NA	0.0009	3.91E-05	NA	0.0029
R_fD	2	2	9	3	140	300	40	300	NA	0.3	5	NA	600	5	1	NA	70	200	NA	3
ADI	1000	0.42	18	NA	140	20	500	300	NA	2.14	5	NA	600	5	1	NA	200		3.6	3

EDI: Estimated daily intake; HQ: Hazard quotient; R_fD: Reference dose; ADI: Acceptable daily intake; NA: Not available

Table 5: Pearson correlation matrix for 20 elements

Variables	Li	Be	V	Cr	Mn	Co	Cu	Zn	Ga	As	Se	Rb	Sr	Ag	Cd	Cs	Ba	Tl	Pb	U
Li	1																			
Be	0.761**	1																		
V	0.302	0.535*	1																	
Cr	0.295	0.337	0.099	1																
Mn	0.777**	0.814**	0.763**	0.203	1															
Co	0.710**	0.729**	0.759**	0.138	0.955**	1														
Cu	0.655**	0.520*	0.426	0.300	0.688**	0.661**	1													
Zn	0.119	0.154	0.485*	0.191	0.450*	0.551**	0.424	1												
Ga	0.623**	0.817**	0.887**	0.322	0.930**	0.901**	0.566**	0.452*	1											
As	0.809**	0.820**	0.531*	0.246	0.901**	0.901**	0.669**	0.423	0.803**	1										
Se	0.688**	0.472*	0.081	0.564**	0.547*	0.530*	0.624**	0.403	0.422	0.689**	1									
Rb	−0.336	−0.094	−0.104	−0.202	−0.226	−0.179	−0.026	0.078	−0.181	−0.166	−0.278	1								
Sr	0.916**	0.752**	0.365	0.308	0.781**	0.718**	0.736**	0.243	0.663**	0.790**	0.748**	0.35	1							
Ag	0.164	0.189	−0.085	0.876**	−0.032	−0.095	0.183	0.103	0.108	−0.034-	0.445*	−0.14	0.239	1						
Cd	0.790**	0.662**	0.211	0.197	0.717**	0.664**	0.515*	0.316	0.531*	0.741**	0.750**	−0.25	0.767**	0.133	1					
Cs	0.130	0.548*	0.796**	0.249	0.521*	0.395	0.185	0.186	0.718**	0.242	−0.103-	−0.06	0.199	0.195	0.079	1				
Ba	0.771**	0.734**	0.500*	0.195	0.829**	0.733**	0.568**	0.326	0.718**	0.757**	0.669**	−0.38	0.846**	0.098	0.864**	0.379	1			
Tl	0.007	0.093	0.436*	0.419	0.209	0.205	0.307	0.428	0.364	0.011	0.239	−0.25	0.247	0.555**	0.101	0.474*	0.253	1		
Pb	0.037	0.383	0.830**	0.030	0.579**	0.578**	0.223	0.673**	0.707**	0.386	0.030	−0.02	0.185	−0.06	0.132	0.744**	0.378	0.482*	1	
U	0.831**	0.777**	0.163	0.567**	0.637**	0.521*	0.487*	0.134	0.567**	0.722**	0.780**	−0.33	0.852**	0.472*	0.730**	0.207	0.719**	0.133	0.111	1

**Correlation is significant at the 0.01 level (two-tailed); *Correlation is significant at the 0.05 level (two-tailed)

Pearson correlation analysis

For correlation analysis, values obtained that were higher than 0.50 were considered to correlate various data points and the results are summarized in Table 5. Values in bold indicated significant correlation between variables at higher confidence interval (**$P < 0.01$). If the values obtained were positive, it showed positive correlation among the variables, whereas, the negative values signified negative correlation. The correlation values near to "zero" indicated poor and nonsignificant positive or negative correlation and on the other hand, values closer to "one" indicated significant correlation among variables. Interestingly, no any pair of elements displayed significant negative correlation among themselves at both the confidence levels ($P = 0.01$ and $P = 0.05$). Rubidium (Rb) showed an interesting behavior by not showing any significant positive or negative correlation with any of the element. Another element, Zinc (Zn), showed positive significant correlation with only one element (Co). Therefore, these two elements were placed in two distinct groups. The remaining elements were placed in three different groups following the interpretation of the correlation matrix. Group I consisted of elements such as Li, As, Sr, Cd, U, Ba, Mn, Be, Se, Co, and Cu. Group II comprised Cs, V, Pb, and Ga whereas, elements such as Ag, Cr, and Tl were placed in group III. Group IV and Group V consisted of Zn and Pb, respectively. This grouping of elements was further analyzed through another multivariate analysis, i.e., principal component analysis.

Principal component analysis

Principal component analysis was performed on the whole data and the results obtained are summarized in Table 6. It shows the rotated component matrix of the principal component analysis. All the variables are grouped in five principal components (PC1 to PC5). Data in bold showed the absolute values >0.50. Elements of Group I were found to give major contribution to principal component 1. Similarly, Group II elements of the correlation matrix were the major contributor to principal component II. Three major principal components were observed as shown in Figure 3 that depicts the component plot in rotated 3D-space. It shows the exact placement of elements in their principal components according to their absolute values.

DISCUSSION

All the detected elements were classified into (a) trace, (b) toxic, (c) essential, and (d) nonessential elements based on their nature and abundance and their presence in tobacco samples were studied. Various trace elements present were Li, Zn, Cu, Cr, Co, V, As, Rb, Sr, Mn, and Se, whereas toxic elements such as As, Pb, Cd, Be, Cu, Li, Mn, Ag, Zn, Ba, and Tl were also present. These elements are considered to be toxic when consumed even in smaller quantities for a long period. Arsenic (As) is known to cause cancer and diabetes, whereas, Pb is responsible for anemia, plambism, and encephalopathy. Cadmium (Cd) causes high blood pressure, lung cancer, and osteomalacia, whereas, Be leads to cardiovascular disorders. Similarly, other elements have various harmful effects on the body when consumed regularly. Essential elements that were present in the tobacco samples were Co, Cu, Cr, Mn, Se, and Zn. These elements were present in trace quantities in all the samples. Furthermore, the presence of nonessential elements was also observed. These elements were V, As, Ba, Be, Cd, Ga, Ag, Pb, Li, Rb, Sr, Cs, Tl, and

Table 6: Component matrix obtained for principal component analysis showing placements of elements in five principal components (PC1-PC5)

	Rotated component matrix[a] Component				
	PC1	PC2	PC3	PC4	PC5
Li	**0.942**	0.054	0.086	−0.0106	0.110
As	**0.902**	0.276	−0.063	0.150	−0.090
Sr	**0.902**	0.124	0.171	0.063	0.179
Cd	**0.870**	−0.011	0.035	0.140	0.142
U	**0.832**	0.053	0.428	−0.143	0.097
Ba	**0.820**	0.298	0.025	0.108	0.284
Mn	**0.806**	**0.543**	−0.070	0.164	0.038
Be	**0.783**	0.480	0.161	−0.229	−0.137
Se	**0.778**	−0.207	0.397	0.336	0.105
Co	**0.759**	0.493	−0.148	0.306	−0.001
Cu	**0.679**	0.140	0.165	0.377	−0.145
Cs	0.054	**0.942**	0.221	−0.119	0.029
V	0.270	**0.898**	−0.081	0.240	0.035
Pb	0.077	**0.845**	−0.054	0.423	0.013
Ga	**0.623**	**0.748**	0.089	0.123	0.006
Ag	0.066	−0.029	**0.982**	0.025	0.060
Cr	0.241	0.073	**0.901**	0.031	−0.008
Tl	−0.074	0.423	**0.549**	0.474	0.355
Zn	0.214	0.284	0.067	**0.870**	−0.108
Rb	−0.227	−0.025	−0.087	0.082	**−0.920**

Extraction method: Principal component analysis; Rotation method: Varimax with kaiser normalization. [a]Rotation converged in 6 iterations. Values in bold indicates the absolute values >0.50

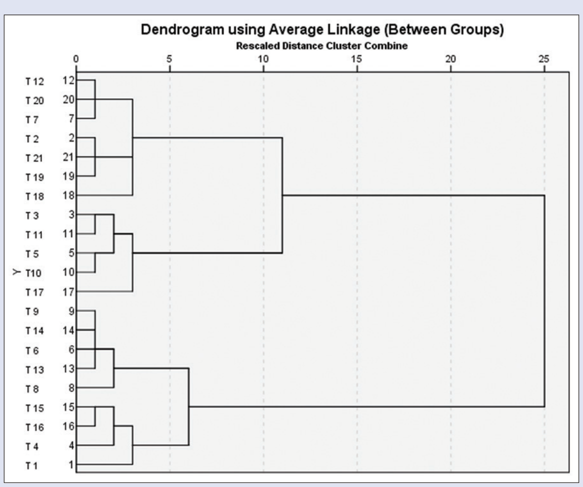

Figure 2: Hierarchical cluster analysis – dendrogram for 21 samples

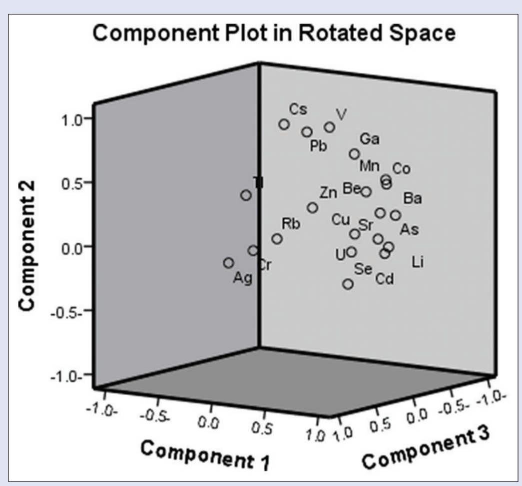

Figure 3: Component plot obtained after Principal component analysis showing the placement of elements in three major principal components

U which were present in low-to-high concentrations.

Metals, such as Co, Cu, Cr, Mn, Se, and Zn, are referred to as essential nutrients because these are required for various physiological and biochemical functions of the body.[15] The insufficiency of the supply of these elements results in various diseases or syndromes. These elements have important roles as constituents of several enzymes and take part in several redox reactions in biological systems.[15] For example, Cu is an essential cofactor for many oxidative stress-related enzymes, such as peroxidase, cytochrome c oxidases, catalase, monoamine oxidase, etc.[16-18] Moreover, Cu has toxic properties too as it can exhibit transitions between Cu (II) and Cu (I), which generate reactive oxygen species (ROS), including superoxide and hydroxyl radicals.[16-19] Excessive Cu intake has been reported to cause cellular damage, which leads to Wilson disease in humans.[18,19] Similar to Cu, other essential elements that are present in tobacco are required for biological functioning. However, excessive amounts of these metals produce damage to tissues and cells, resulting in various harmful effects and diseases in humans. Cu along with Cr has a very narrow range of concentrations between beneficial and toxic effects.[19,20]

Other elements, such as As, Ba, Be, Cd, Ga, Pb, Li, Ag, Sr, Tl, V, and Uranium (U), which are present in all tobacco samples in high to medium concentrations, do not have any established roles in biological functions and are thus considered non-essential elements.[20] Strontium (Sr) was found to be present in highest concentrations in all the samples that is similar in properties and uses to calcium (Ca), but its use is restricted nowadays because of its harmful effects on the body including various cardiovascular disorders.[21] After Sr, the other highly abundant metal found in tobacco samples was Mn, which comes under the essential metal ions category. Mn has an important role in maintaining human health and is essential for the development and metabolism in humans. However, its excessive intake causes a set of disorders called manganism, which is a neurodegenerative disorder that affects the dopaminergic nerves and leads to a condition similar to Parkinsonism.[22-23]

The presence of heavy metal ions in tobacco samples was also investigated, and their concentrations were compared with the prescribed limits. The heavy metals present in these tobacco samples were As, Cu, Cr, Co, Zn, Ag, Cd, Pb, Se, and Tl. Although, except Zn, the presence of other heavy metal ions was comparatively less but cannot be ignored. Heavy metals in biological systems are known to have pronounced effects on cellular components and various enzymes involved in metabolism and repair.[24] They interact with the cellular components and cause conformational changes that damage these components and lead to cancer and apoptosis.[24,25] Heavy metals, such as As,[26-28] Cd,[29] Cr,[30,31] and Pb,[32,33] are known to produce carcinogenic effects due to their capability to produce ROS in biological systems. These elements are among those that pose a significant hazard to human health. These elements are all systemically toxic and cause damage to many organs, even at low concentrations. Various experimental and epidemiological studies have reported that these elements are placed under the category "known" carcinogens by the U. S. EPA and the International Agency for Research on Cancer.

CONCLUSION

The concentrations of various elements in all 21 tobacco samples were measured successfully by ICP-MS. The tobacco samples were of different varieties, color, and origin, and most of the elements in these samples showed some variation in concentration. Elements were found in either significantly high or relatively low concentrations. Different types of heavy and toxic metals, which could lead to various health hazards like cancer and cardiovascular diseases, were found in all tobacco samples. Further studies should be conducted to discover other toxic substances that are present in these tobacco samples to justify their potential in causing diseases.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Winter JC. Tobacco Use by Native North Americans: Sacred Smoke and Silent Killer. Chicago: University of Oklahoma Press; 2000.
2. Valdes de la Cruz M, Gonzalez C, Lara RM, Roman MI, Hernandez Y, Hernandez RM, *et al.* Genetic diversity of wild species of *Nicotiana* genus I: Characterization using biochemical markers. Rev Prot Veg 2010;25:88-97.
3. Klus H, Kunze M, Konig S, Poschl E. Smokeless tobaccoan overview. Beitr Tabak Contr Tob Res 2009;23:125-8.
4. National Cancer Institute and Centers for Disease Control and Prevention. Smokeless Tobacco and Public Health: A Global Perspective. Bethesda, MD: US Department of Health and Human Services, Centers for Disease Control and Prevention and National Institutes of Health, National Cancer Institute; 2014.
5. International Agency for Research on Cancer. *Smokeless Tobacco and Some Tobacco-Specific N-Nitrosamines*. Vol. 89. Lyon, France: World Health Organization International Agency for Research on Cancer, International Agency for Research on Cancer Monographs on the Evaluation of Carcinogenic Risks to Humans; 2007.
6. Repace JL, Lowrey AH. Indoor air pollution, tobacco smoke, and public health. Science 1980;208:464-72.
7. Wu D, Landsberger S, Larson SM. Determination of the elemental distribution in cigarette components and smoke by instrumental neutron activation analysis. J Radioanal Nucl Chem 1997;217:77-82.
8. Smith MV, Sneddon J. Simultaneous determination of cadmium and lead in cigarettes and cigarette smoke by impaction-graphite furnace and graphite furnace atomic absorption spectrometry. Spectrosc Lett 1999;32:615-21.
9. Csalari J, Szantai K. Transfer rate of cadmium, lead, zinc and iron from the tobacco-cut of the most popular Hungarian cigarette brands to the combustion products. Acta Aliment 2002;31:279-88.
10. Food and Agriculture Organization/World Health Organization, Joint Expert Committee on Food Additives. Evaluation of Certain Food Additives and Contaminants. Vol. 837. Geneva: World Health Organization Technical Report series, World Health Organization; 1993.
11. Food and Agriculture Organization/World Health Organization, Joint Expert Committee on Food Additives. Evaluation of Certain Food Additives and Contaminants. Vol. 940. Geneva: World Health Organization Technical Report Series, World Health Organization; 2007.
12. Food and Agriculture Organization/World Health Organization, Joint Expert Committee on Food Additives. Summary report of the Seventy-Second Meeting of Joint Expert Committee on Food Additives. Geneva: World Health Organization; 2010.
13. Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives. Summary and Conclusions of the 61st Meeting of the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives. Rome, Italy: Proceedings of JECFA/61/SC; 2003. p. 1-22.
14. United States Environmental Protection Agency. Risk-Based Concentration Table. Philadelphia, USA: United States Environmental Protection Agency; 2011.
15. World Health Organization/Food and Agriculture Organization/International Atomic Energy Agency. Trace Elements in Human Nutrition and Health. Switzerland, Geneva: World Health Organization; 1996.
16. Stern BR. Essentiality and toxicity in copper health risk assessment: Overview, update and regulatory considerations. J Toxicol Environ Health A 2010;73:114-27.
17. Harvey LJ, McArdle HJ. Biomarkers of copper status: A brief update. Br J Nutr 2008;99 Suppl 3:S10-3.

18. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Copper, Centers for Disease Control. Atlanta, GA: Division of Toxicology; 2002.
19. 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.
20. Chang LW, Magos L, Suzuki T, editors. Toxicology of Metals. Boca Raton, Florida, USA: CRC Press; 1996.
21. Medicines and Healthcare products Regulatory Agency. Strontium Ranelate: Cardiovascular Risk – Restricted Indication and New Monitoring Requirements. United Kingdom: European Medicines Agency; 2014.
22. Emsley J. Manganese. Nature's Building Blocks: An A-Z Guide to the Elements. Oxford, UK: Oxford University Press; 2001. p. 249-53.
23. 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.
24. Wang S, Shi X. Molecular mechanisms of metal toxicity and carcinogenesis. Mol Cell Biochem 2001;222:3-9.
25. Beyersmann D, Hartwig A. Carcinogenic metal compounds: Recent insight into molecular and cellular mechanisms. Arch Toxicol 2008;82:493-512.
26. Yedjou CG, Tchounwou PB. Oxidative stress in human leukemia (HL-60), human liver carcinoma (HepG2), and human (JURKAT-T) cells exposed to arsenic trioxide. Met Ions Biol Med 2006;9:298-303.
27. Yedjou CG, Tchounwou PB. *In vitro* cytotoxic and genotoxic effects of arsenic trioxide on human leukemia (HL-60) cells using the MTT and alkaline single cell gel electrophoresis (Comet) assays. Mol Cell Biochem 2007;301:123-30.
28. Tchounwou PB, Centeno JA, Patlolla AK. Arsenic toxicity, mutagenesis, and carcinogenesis – A health risk assessment and management approach. Mol Cell Biochem 2004;255:47-55.
29. Tchounwou PB, Ishaque AB, Schneider J. Cytotoxicity and transcriptional activation of stress genes in human liver carcinoma cells (HepG2) exposed to cadmium chloride. Mol Cell Biochem 2001;222:21-8.
30. Patlolla AK, Barnes C, Hackett D, Tchounwou PB. Potassium dichromate induced cytotoxicity, genotoxicity and oxidative stress in human liver carcinoma (HepG2) cells. Int J Environ Res Public Health 2009;6:643-53.
31. Patlolla AK, Barnes C, Yedjou C, Velma VR, Tchounwou PB. Oxidative stress, DNA damage, and antioxidant enzyme activity induced by hexavalent chromium in Sprague-Dawley rats. Environ Toxicol 2009;24:66-73.
32. Yedjou CG, Tchounwou PB. N-acetyl-l-cysteine affords protection against lead-induced cytotoxicity and oxidative stress in human liver carcinoma (HepG2) cells. Int J Environ Res Public Health 2007;4:132-7.
33. Tchounwou PB, Yedjou CG, Foxx DN, Ishaque AB, Shen E. Lead-induced cytotoxicity and transcriptional activation of stress genes in human liver carcinoma (HepG2) cells. Mol Cell Biochem 2004;255:161-70.