

## RESEARCH ARTICLE

# Determination of Metal Levels in Shamma (Smokeless Tobacco) with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Najran, Saudi Arabia

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### Abstract

**Objective:** The use of Shamma (smokeless tobacco) by certain groups is giving rise to health problems, including cancer, in parts of Saudi Arabia. Our objective was to determine metals levels in Shamma using inductively coupled plasma mass spectrometry (ICP-MS). **Methods:** Thirty-three samples of Shamma (smokeless tobacco) were collected, comprising four types: brown Shamma (n = 14.0), red Shamma (n = 9.0), white Shamma (n = 4.0), and yellow Shamma (n = 6.0). All samples were collected randomly from Shamma users in the city of Najran. Levels of 11 elements (Al, As, Cd, Co, Cr, Cu, Li, Mn, Ni, Pb, and Zn) were determined by ICP-MS. **Results:** A mixed standard (20 ppb) of all elements was used for quality control, and average recoveries ranged from 74.7% to 112.2%. The highest average concentrations were found in the following order: Al (598.8–812.2 µg/g), Mn (51.0–80.6 µg/g), and Ni (23.2–53.3 µg/g) in all four Shamma types. The lowest concentrations were for As (0.7–1.0 µg/g) and Cd (0.0–0.06 µg/g). **Conclusions:** The colour of each Shamma type reflects additives mixed into the tobacco. Cr and Cu were showed significant differences ( $P < 0.05$ ) among Shamma types. Moreover, Pb levels are higher in red and yellow Shamma, which could be due to use (PbCrO<sub>4</sub>) as yellow colouring agent and lead tetroxide, Pb<sub>3</sub>O<sub>4</sub> as a red colouring agent. The findings from this study can be used to raise public awareness about the safety and health effects of Shamma, which is clearly a source of oral exposure to metals.

**Keywords:** Najran- Shamma- Smokeless tobacco- ICP-MS- Metal

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### Introduction

Tobacco is believed to have originated in Central America, where it was first discovered in both Peru and Mexico in 3,500BC. The history of tobacco plant cultivation dates back to fifty centuries (IARC 2007). Smokeless tobacco is known under different names worldwide and is used for chewing and snuff. Nicotine is the main tobacco constituent related to its addictive properties (Haustein, 2010) Some local names for smokeless tobacco are Toombak (Sudan), Afzal (Oman), Qat and Snuff (Yemen), Shammah/Shamma (Kingdom of Saudi Arabia), moist snuff and Alaskan Iqmik (USA), areca nut and betel quid (Pakistan, India, Bangladesh, Sri Lanka, and Taiwan), Snus (Sweden), and Masher/Mishri (India) (Idris et al., 1998; Pappas et al., 2008; Chaudhary, 2010; Akhtar et al., 2012; Abbas and Ahmed, 2013; Al-Mukhaini et al., 2014; Alsanosy 2014; Rahmi et al., 2014; Makrami et al., 2015; Al-Jaber et al., 2016).

In this paper, we focus on Shamma used in the KSA, specifically in the city of Najran, where it is also known as Yemeni snuff. Shamma is known among young people in Najran under the local name Bardaga. Shamma use has gained widespread prevalence in recent years; in the

1980s and prior, Shamma use was limited to the Yemeni community in Najran.

Shamma consists of leaves of specific tobacco species, such as *Nicotiana rustica* and *Nicotiana glauca* (Alsanosy 2014). Shamma use is prevalent in the southern region of Saudi Arabia, in the areas of Najran, Jizan, Dhahran, and ahad-Almsarha. The plant is cut, dried, and milled; other materials are then added to it. Additives include salt, ash, cement, henna, flour, and glass powder. Components and quantities vary among Shamma types, and may also include black pepper, flavourings, carbonate of lime, and oils. The Shamma is placed in the mouth as a quid in the labial vestibule or in the buccal between the lower lip and gums (IARC 2007; Alsanosy 2014).

There are four types of Shamma, based on their colours. White Shamma (Shamma Beda) is a lighter mixture, containing only wheat flour as an additive. New users typically select white Shamma. Red Shamma owes its colour to henna. It has a moderate effect on users. Yellow Shamma (Safra Bardaga and Safra Suhaila) is pure tobacco without additives; most users prefer yellow Shamma. Brown Shamma (Shamma Gabra) is a mixture of yellow and white Shamma, and is also popular.

Some users place the Shamma plug under the tongue,

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while others place it between the gums and lower lip. The amount of Shamma to used is always the quantity pinched between three fingers (thumb, index finger, and middle finger), less than 1.0 g (typically about 0.5 g).

Shamma is known to cause gum cancer, osteoporosis, and rheumatism. Studies have reported effects such as oral cancer, leukoplakia, immune dysfunction, periodontal diseases, decreased sperm viability, and perinatal mortality (Hannan et al., 1986; Allard et al., 1999; Alsanosy 2014; Makrami et al., 2015).

Levels of metals in tobacco have been characterized in multiple studies. Metals tested have included Cu, Mn, Na, K, As, B, Se, Hg, Sb, Co, Rb, Cr, Zn, Sc, Ba, Be, Cd, Pb, Ni, and Fe, in both cigarettes and smokeless tobacco (Ahmad et al., 1979; Pappas et al., 2008; Al-Mukhaini et al., 2014). A review by Pappas (2011) summarized the contributions of metals from tobacco to observed pathologies

The toxicity of some metals found in tobacco has also been reported. Al can reach the brain (Perl and Good, 1987), as can cause cardiovascular diseases (Lee et al., 2003). Oral ingestion of Ba can cause hypertension and tachycardia (Atsdr 2007a). Cd in smokeless tobacco has been shown to be associated with arterial disease (Navas-Acien et al, 2004; Pappas et al., 2008;). Both Ni and Co cause dermatitis and oral allergic sensitizations (Rüegger 1995; Kelleher et al., 2000). Cu may cause free radical-induced lung injury, while Pb levels have been associated with elevated blood pressure (Atsdr 2007b). Mn was shown to cause pulmonary inflammation in rats (Rice et al., 2001). Oral allergic contact dermatitis is known to be caused by Cr(VI) (Moller et al., 1986). Li is a well-established psychiatric medication, and its occurrence in tobacco may contribute to addiction through its mood-regulating action (Houas et al., 2016).

To the best of our knowledge, this is the first study to look at metal contents in Shamma used in Najran, KSA. We used inductively coupled plasma mass spectrometry (ICP-MS) to determine the levels of 11.0 metals in the Shamma types common to Najran. Analysis of metal levels in Shamma is an important first step in raising awareness about adverse health effects caused by Shamma, and in assessing the risks related to oral absorption of metals from Shamma.

## Materials and Methods

This section includes seven subsections are Shamma sample collection, Shamma samples preparation (digestion), elemental measurement, chemicals reagents, analytical method, quality control and statistical analysis.

### *Shamma samples collection*

In total, 33 samples of different types of Shamma based on colour (brown, red, white, and yellow) were collected from Najran. The samples were collected randomly and directly from Shamma users. These four types are the most commonly used in Najran. The samples were collected in zip-close plastic bags and stored in a dry place prior to sample preparation. Collected Shamma samples from

Najran included four types: Brown Shamma (n = 14.0), red Shamma (n = 9.0), white Shamma (n = 4.0) and yellow Shamma (n = 6.0).

### *Shamma sample digestion*

All collected Shamma samples were digested; approximately 0.5 g from each sample was used for the digestion process. The weighed sample was mixed with 4.0 mL of 69.0% nitric acid (HNO<sub>3</sub>) (Sigma-Aldrich) and 2.0 mL of hydrogen peroxide (30% H<sub>2</sub>O<sub>2</sub>) (Sigma-Aldrich). The mixture was digested for 40 min using a microwave digester at a total pressure of 20 bars and a maximum temperature of 125°C (Anton-Paar Multiwave 3000 Microwave Sample Preparation System, Graz, Austria). The digested solution was diluted to 10 mL in volumetric flasks with deionized water prior to analysis. This digestion process was reported previously by Al-Rmalli (2012). All digested samples were kept in polyethylene bottles and stored in a refrigerator until analysis. Thereafter, the digested samples were diluted (10-fold) for the analysis.

### *Elemental measurement*

Concentrations of Al, As, Cd, Co, Cr, Cu, Li, Mn, Ni, Pb, and Zn in the digested sample solutions (n = 33.0) were determined by ICP-MS (iCAP Q, Thermo Fisher Scientific, Waltham, MA, USA).

### *Chemicals and reagents*

Deionized water (18 Ω cm<sup>-1</sup>) was used throughout the study. Single-stock solutions of Al, As, Co, Cd, Ni, Li, Cr, Cu, Zn, Mn, and Pb were prepared (1000 µg/mL, ULTRA Scientific, North Kingstown, RI, USA). A single-stock solution of an internal standard of Sc was also prepared (1,000.0 µg/mL, ULTRA Scientific).

A mixture of 29.0 analytes (Al, As, Ba, Be, Bi, Cd, Ca, Ce, Cr, Co, Cu, Ga, In, Fe, Pb, Li, Mg, Mn, Ni, K, Rb, Se, Ag, Na, Sr, Tl, U, V, and Zn) was also obtained from ULTRA Scientific, at a concentration of 10.0 ± 0.05 µg/mL.

The mixture of 29.0 analytes was used to prepare fresh daily standards for analysis. All stock solutions were prepared in 1.0% HNO<sub>3</sub>. A concentration of 100.0 µg/L of Sc was used as an internal standard for the total elements analysis.

### *Analytical method*

Elements in the various Shamma samples were determined by ICP-MS. The operating conditions are as follows: RF Power 1550 watt; Cool gas flow 14.1 L/min; Nebuliser gas flow 0.94 L/min; Auxiliary gas flow 0.79 L/min; Dwell Time 0.01s; Number of repeat per sample 3.0; Total time for each sample measurement 3min. Internal standard was introduced to the sample stream via the T-piece. Multi-element calibration standards were used and included the 11 metals at concentrations of 0.0, 1.0, 5.0, 10.0, 20.0 and 40.0 µg/L.

### *Quality control*

Prior to daily analysis, ICP-MS instrument performance was checked for sensitivity using tune B iCAP solution

containing U, In, Li, and Co for the background signal in standard mode. The tune solution concentration was 1.0 µg/L for each element in 2.0% HNO<sub>3</sub> and 0.5% HCl.

The limits of detection (LOD) for the 11.0 elements were calculated by using Qtegra software (Thermo Fisher Scientific) from the calibration curve of each element. The calculated LODs (µg/L) and correlation coefficient (R<sup>2</sup>) values for each element are as follows: Al (LOD = 0.4, R<sup>2</sup> = 0.99); As (LOD = 0.0, R<sup>2</sup> = 0.99); Cd (LOD = 0.01, R<sup>2</sup> = 0.99); Co (LOD = 0.1, R<sup>2</sup> = 0.99); Cr (LOD = 0.1, R<sup>2</sup> = 0.9); Cu (LOD = 0.0, R<sup>2</sup> = 0.99); Li (LOD = 0.0, R<sup>2</sup> = 0.99); Mn (LOD = 0.1, R<sup>2</sup> = 0.99); Ni (LOD = 0.1, R<sup>2</sup> = 0.99); Pb (LOD = 0.0, R<sup>2</sup> = 0.99) and Zn (LOD = 0.9, R<sup>2</sup> = 0.9).

A quality control (QC) test was performed for each run by using a continuing calibration verification (CCV) test. The method was validated by measuring 20.0 µg/L of a mixed standard of all measured elements after each set of 10 runs. Recoveries for each element in a session after 10 runs were: Al, 203.9%; As, 102.1%; Cd, 124%; Co, 98.5%; Cr, 93.8%; Cu, 103.5%; Li, 67%; Mn, 99.3%; Ni, 98%; Pb, 135.6%; Zn, 105.5%.

**Statistical analysis**

Statistical comparisons of heavy metal levels between Shamma types (brown, red, white, and yellow) were performed using Statistical Analysis System (SAS) software (SAS Institute, Cary, NC, USA). The SAS was used for calculating analysis of variance to identify significant differences (p < 0.05) among Shamma types related to each measured element. Multiple comparisons to separate means of the Shamma types were calculated using Duncan's multiple range test.

**Results**

Element concentration levels did not differ significantly between the four Shamma types. Concentrations of all 11.0 measured elements in the four types followed a similar order. Al concentrations were highest and Cd concentrations were lowest in all four types. Mean

Table1. Summary of Significance (P.value<0.05) Level of Shamma Type in Elements Measured, Means, and Concentration Ranges

Element	P	Mean	Range of Concentrations	
			Low	High
Al	0.87	635.1	103.9	1,813.1
Co	0.78	4.5	0.4	26.2
As	0.98	0.9	0.2	7.2
Li	0.39	14.1	nd	187.2
Pb	0.6	4.5	nd	26.2
Zn	0.08	2.4	nd	6.2
Cu	0.03	2.8	0.9	9.3
Mn	0.27	61.7	6.0	118.0
Cd	0.27	0.0	nd	0.3
Cr	0.0	3.7	nd	7.4
Ni	0.85	40.6	0.6	267.0

\*range of mean concentrations for all four Shamma types and pooled averages between brackets # dipping tobacco \$ cultivated tobacco plants

Element	Al	As	Cd	Co	Cr	Cu	Li	Mn	Ni	Pb	Zn	Reference
KSA*	598-8-812.2 (635.1)	0.7-1.0- (0.9)	0.0-0.03 (0.03)	2.9-6.0 (4.5)	2.1-5.4 (3.7)	1.8-3.92 (2.8)	1.71-28.4 (14.1)	50.9-80.6 (61.7)	23.18-53.29 (40.6)	1.6-6.2 (4.5)	1.41-3.03 (2.4)	This study
USA	699-0-1,200.0	<1	nr	<0.01-0.9	<0.1-3.4	nr	nr	155.0-400.0	<2-400	nr	16.8-30.5	Chiba & Masironi (1992)
USA	nr	0.2-0.4	1.0-1.7	0.4-1.1	1.3-3.2	nr	nr	131.0-245.0	2.2-3.9	0.6-1.2	nr	Fresquez et al (2013)
UK	nr	<0.2-0.7	0.5-0.8	nr	1.3-3.1	11.7-16.2	nr	151.0-172.0	1.1-2.7	0.4-0.9	26.0-40.0	Stephens et al (2005)
UKS	nr	<0.1-2	<0.1-47.8	nr	<0.3-1.0	8.0-28.6	nr	nr	<0.4-1.4	nr	7.3-62.3	Campbell (2014)
Paki- stan#	670-0-5,600.0	0.4-3.1	0.1-2.0	0.4-2.9	2.1-78.8	11.3-37.9	nr	36.6-189.9	1.2-14.2	0.5-4.9	15.4-66.9	Musharrat et al (2012)
India	nr	nr	0.4-1.4	nr	nr	0.02-28.9	nr	nr	nr	1.5-5.1	9.8-29.2	Prabhakar et al (2013)
Ghana	3,006.0-5,160.0	0.1-0.2	1.1-1.1	0.1-0.2	0.9-1.4	18.5-27.7	nr	121.0-139.0	nr	nr	nr	Addo et al (2008)
Ethiopia	nr	nr	1.7	nr	1.7	8.9-12.8	nr	nr	2.3	nr	92.7	Regassa & Chandravanshi (2016)
Oman	nr	nr	1.8	nr	15.7	nr	nr	nr	1.6	1.6	nr	Al-Mukhaini et al (2014)

Table 3. Ranges and mean concentrations of elements measured in the four Shamma types.

Shamma type	No. of samples	Ranges (mean) (µg/g)										
		Al	As	Cd	Co	Cr	Cu	Li	Mn	Ni	Pb	Zn
Brown Shamma	14	117.1-1,813.1 (602.3)	0.2-7.2 (1.0)	nd-.26 (0.1)	0.4-10.2 (3.9)	nd-6.7 (2.1)	2.7-9.3 (3.9)	0.4-187.2 (28.4)	5.9-118.0 (51.0)	0.6-110.5 (36.6)	nd-10.2 (3.8)	nd-6.2 (3.0)
Red Shamma	9	15.7-1,348.5 (631.5)	0.4-1.3 (0.9)	nd-0.02 (0.02)	1.1-16.0 (5.0)	1.2-7.4 (4.7)	2.7-9.3 (1.9)	nd-19.15 (4.6)	8.2-99.9 (60.01)	3.4-163.9 (47.2)	nd-16.0 (6.2)	0.02-2.6 (1.4)
White Shamma	4	103.9-1,173.1 (812.2)	0.3-0.9 (0.7)	nd-0.01 (0.003)	1.8-3.7 (2.9)	2.6-6.7 (5.4)	1.0-3.4 (2.5)	0.1-2.9 (1.7)	15.1-112.9 (74.6)	21.1-33.8 (23.2)	0.6-4.3 (1.6)	0.8-2.8 (2.0)
Yellow Shamma	6	227.5-1,034.3 (598.8)	0.6-1.6 (0.9)	nd-0.1 (0.1)	1.7-26.2 (6.0)	1.4-6.8 (4.8)	0.9-2.3 (1.8)	0.3-9.4 (3.0)	27.9-104.0 (80.6)	3.5-267.0 (53.3)	0.5-26.2 (5.6)	1.6-5.2 (2.7)

\*nd = not detected

concentrations of 11 elements in 33 Shamma samples are presented in Table 3. Decreasing order for concentrations of the elements in each Shamma type are as follows: Brown Shamma order: Al>Mn >Ni>Li>Co>Cu>Pb>Zn>Cr>As>Cd; Red Shamma Order: Al>Mn >Ni>Pb>Co>Cr>Li>Cu>Zn> As>Cd; White Shamma order: Al>Mn>Ni>Cr>Co>Cu>Zn> Li>Pb>As>Cd and Yellow Shamma order: Al>Mn>Ni>Cr>Zn>Co>Pb>Li>Cu>As>Cd. The statistical analyses are displayed in Tables 1 with Duncan's test for differences. Table 2 showed the comparison between concentrations of measured metals in Shamma and reported concentrations in the literature.

Table 3 presented ranges and mean concentrations (µg/g) of 11 elements in 33 Shamma samples stratified by type (brown = 14, red = 9, white = 4, yellow = 6)

## Discussion

Although there was a slight variation in the distribution of metals among the four types of Shamma, most element concentrations followed a common tendency (Figure 1). In all samples, the highest concentrations followed the order Al > Mn > Ni, while the lowest concentrations followed the order Cd < As. These findings are in agreement with previous studies (Chiba and Masironi, 1992; Bhisey 2012). The other elements followed the order Cr > Co > Cu > Pb > Li > Zn, with some exceptions as shown in results sections. High Al concentrations in filler tobacco, ranging from 333 to 546.0 µg/g, were reported in a previous study (Kazi et al., 2009). Levels of Al, Mn, and Ni in this study were similar to those in a previous study (Chiba and Masironi, 1992), where concentrations measured in cigarette tobacco of 12 American brands were 699–1,200, 155.0–400, and 2–400 µg/g, respectively.

As and Cd levels in this study were similar to those measured by Bhisey (2012), where concentrations in smokeless tobacco were 0.1–1.5 and 0.0–0.5 µg/g, respectively. For Cr, our findings were similar to those reported by Pappas et al, (2008), who found a range of 0.9–3.2 µg/g. Mn levels in our study were similar to those reported by Addo et al, (2008).

The unusual finding in this study is the similar order of concentration levels in all four types of Shamma. This may imply that the only difference between Shamma types is the source of additives. These additives could contribute to the colour of each Shamma type; the minor variation in elements' concentration order may be attributable to the Shamma treatment process.

This study confirmed that some various heavy metals were found in Shamma. This finding may reflect soil contamination or air pollution where Shamma tobacco is grown (Golia et al., 2016). Other possible source of these heavy metals in Shamma may be pollution in the water used in tobacco fields or additives for enticing users.

This study also confirmed that Shamma is a source of oral exposure to metals. Our findings concur with those of previous studies that found metals in tobacco (Dhaware et al., 2009; Talhout et al., 2011; Fresquez et al., 2013; Al-Mukhaini et al., 2014; Pappas et al., 2015).

The occurrence of metals in Shamma is a matter of

concern for users, as the exposure could lead to adverse health effects. Effects from metal absorption, such as neurotoxicity, cardiovascular diseases, and hypertension, have been reported (Perl and Good, 1987; Lee et al., 2003; Atsdr 2007a). Frequency and duration of use could combine to lead to accumulation of these metals in Shamma users' bodies. The main concern is the manner of use; Shamma is placed between the gum and lower lip and is easily absorbed by buccal cells. This could lead to local and systemic damage.

General toxicity and carcinogenicity are characteristic of heavy metals (Tchounwou et al., 2012). Al can reach the brain in humans (Perl and Good, 1987; Pappas et al., 2011). As is carcinogenic and can be absorbed orally (Lee et al., 2003; Pappas et al., 2011). Because of their high toxicity, Cd, Cr, and Pb are ranked among the foremost metals of public health concern (Tchounwou et al., 2014).

Mean concentrations of all nine elements measured showed a correlation among all four Shamma types (Table 2). The analysis showed a significant effect of Shamma type for only two metals, Cu and Cr (Table 2;  $p = 0.03$  and  $0.001$ , respectively). In contrast, the effect of type on Zn concentration was marginally insignificant ( $p = 0.077$ ) and highly insignificant for Al, Co, As, Li, Pb, Mn, Cd, and Ni ( $p = 0.87, 0.78, 0.98, 0.39, 0.60, 0.27, 0.27, \text{ and } 0.85$ , respectively). The analysis of the samples for each of the 11 elements showed a wide range of concentrations within and among the four types. The widest range was observed in Al, followed by Ni, Li, and Mn (Table 2).

As mentioned above, the effect of Shamma type on Al, Co, As, Li, Pb, Zn, Mn, Cd, and Ni was not significant, and the differences among the means of the four types were not significant. However, the differences among the means of Cu and Cr were significant. Cu levels were significantly higher in the brown Shamma than in the yellow one, and Cr levels were significantly lower in yellow Shamma than in the other types (Table 2).

Brown Shamma contained higher average Li concentrations compared with other types. This suggests that brown Shamma may be more addictive than other types because of its Li content (Houas et al., 2016). Higher means of Al, found in brown Shamma, may harm the brain (Perl and Good, 1987). Red and yellow Shamma contained higher levels of Pb compared with the other types.

Our results in this study are comparable with previous studies as shown in Table 2. However, no record was observed in all other studies for Li. Lower levels of Cd and Zn were observed in comparison to other values. Slight higher concentrations of Co and Cr were observed; excluding Oman's study showed the highest level for Cr. Therefore, we can conclude that Shamma is a source of oral exposure to some metals. The potential diverse health effect from Shamma is different to other tobacco products in other countries based on variations showed in Table 2.

The important finding of this study was the higher levels of Pb in red and yellow Shamma compared with other Shamma types (Table 3). This finding could explain the colour of red Shamma, which may include lead tetroxide (Pb<sub>3</sub>O<sub>4</sub>) as a red colouring agent. Likewise, the colour of yellow Shamma may due to addition of Lead

(II) chromate (PbCrO<sub>4</sub>) as yellow colouring agent. This will lead us to say using of inorganic dyes including As, Cd, Pb and Cr as colouring agents in Shamma will be a matter of concern for Shamma users. Our future study will be focused on investigation of inorganic dyes in Shamma.

Exposure estimation of metals in Shamma can be evaluated based on provisional tolerable weekly intake (PTWI) set Food Agriculture Organization /World Health Organization (FAO/WHO) and Expert Committee on Food Additives (JECFA). The PTWI ( $\mu\text{g}/\text{kg bw}$ ) for some metals; Al, As, Pb, Ni, Cu, Zn and Cd are 0-700, 15, 25, 35, 3500, 7000 and 7, respectively (FAO/WHO, 1982; FAO/WHO, 1993; FAO/WHO, 2007; FAO/WHO, 2010). Daily dietary guideline for Co, Cr, Mn and Li are 0.11-0.019, 0.05-0.2, 4 mg/day and Li 200-600  $\mu\text{g}/\text{day}$ , respectively (Watson, 2001; Preedy, 2013; Bogden and Klevay, 2000). Estimated weekly intake of Pb in a single dose (0.5g) of red and yellow Shamma (ten times/day- 60 Kg bw), calculated based on mean value in Table 3, is 3.4  $\mu\text{g}/\text{kg bw}$ , and for Al (mean values in Table 3) in all Shamma types is 386  $\mu\text{g}/\text{kg bw}$ . The PTWI ( $\mu\text{g}/\text{kg bw}$ ) for all other metals were same as for Pb and Al, and the values were found as follows: As (0.32), Co (8.4 (0.02 mg/day)), Li (37.2 (47.3 mg/day)), Zn (2.2), Cu (2.7), Mn 38.8 (0.33 mg/day)), Cd (0.1), Cr (2.5 (0.02 mg/day)) and Ni (23.4). In calculations, we anticipated that Shamma user ingested whole saliva of a single dose used Shamma. These results showed lower values of exposure for all metals in all Shamma types than those set by FAO/WHO in food. Only Co showed that calculated value was equivalent to the daily guideline value.

In conclusion concentrations of all 11.0 elements measured followed a similar order in all four Shamma types. Pb levels showed high levels in red and yellow Shamma compared with other types. Shamma could have different potential diverse health effect than other tobacco products. Further studies are needed to explore inorganic dyes in Shamma, Further studies are needed to explore the effects of Shamma on health of users.

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