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Assessment of Source of Contamination of Heavy Metals and Estimation of Metals Risk in Smokeless Tobacco Products

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The aim of this study is to measure the level of heavy metals As, Cd, Cr, Cu, Ni, Pb, Se, Co, Fe and Mn in smokeless tobacco products and ingredients used in the preparation of smokeless tobacco products and to determine the source of contamination and the potential risk by calculating the daily intake (DI metals) and target hazard quotients. As, Cr, Se, Fe and Mn found in high concentration while Cu, Ni, Pb and Co observed in low concentration in smokeless tobacco products as compared to the ingredients of smokeless tobacco products. The average daily intake metals were estimated 187.72, 18.23, 255.83, 421.63, 148.14, 146.31, 27.79, 31.83, 911.04 and 6.34 mg/day for As, Cd, Cr, Cu, Ni, Pb, Se, Co, Mn and Fe, respectively. The total target hazard quotients (TTHQ) of six categories of smokeless tobacco products exceeded which indicates the health risk associated with their use.

Keywords: Smokeless tobacco products, Heavy metals, Target hazard quotients.

INTRODUCTION

The smokeless tobacco products (STPs) included a great variety of products and used as an alternative in place of smoking tobacco products because the people believe that STPs have least health risk [1]. Although there are more than 2000 compounds identified in the smokeless tobacco products which includes the natural constituent of tobacco and also contains other chemical compounds added during the processing of products. Major classes of compounds recognized in tobacco include aliphatic and aromatic hydrocarbons, alcohols, aldehydes, ketones, amines, amides, phenols, alkaloids, metals and radioactive elements [2].

The STPs used in subcontinent are manufactured in small scale without any standard operating procedure which causes fermentation and the variability in products. The additions of additives, less care of smokeless tobacco products and poor quality packing are the main reason of serious health issues [3]. In Pakistan the use of its larger quantity, continuous habit and due to uncheck of the respective department also increase the risk of products.

In Pakistan the selling of STPs is restricted by the Government. That's why most of the smokeless tobacco products manufacture in very small extent and home industry and do not obey any requirement of food safety. Pakistan produces more than 75 million kilograms of tobacco in which 45 to 50 million utilized in Pakistan. According to the survey of Pakistan Medical Research Council in 2013, there are 22 million smokers in Pakistan which consist of 36 % adult men and 9 % of adult women [4].

Metals concentration in the smokeless tobacco products are the concern of this study due to the greater health risk. The metals contaminations in STPs are either environmental contaminates of tobacco leaf and other constituents (absorption of metals from soil, water *etc.*) or due to the methods used for the manufacturing of STPs *i.e.* process, use of chemical additives and also the use of metallic tools. The level of metals contaminations differs with the difference of tobacco products. Sniffing tobacco (naswar) contains Fe, Al and Mn as major elements, smoking products have Al and Fe while gutka products contain Cu and Si in higher proportion [5]. Among the metals, Al affects the calcium metabolism in the brain. The International Agency for Cancer (IARC) has declared Ni includes in group one cancer producing agent [6]. Lead exposure cause impaired fetal development and lower the intelligence quotient (IQ) [7]. Arsenic toxicity causes serious health problems such as mouth ulcerations, low hemoglobin, leukemia, acute renal failure, skin cancer and nerve damages. Upper cadmium level leads to the formation of cardiovascular diseases [8].

The present study contains the assessment of heavy metals in STPs as well as in the major ingredients used in the preparation of STPs, also included the multivariate analysis of data to assist the types of contamination. In this work, consumption of STPs determine practically by conducting survey while other studies based on consideration of the consumption of STPs. Therefore this study deals with clear picture of daily intake estimate of metals and heavy metal hazards.

EXPERIMENTAL

Almost 237 samples of smokeless tobacco products (STPs) and its ingredients were purchased from different markets of Karachi city (Pakistan). Samples were divided into two main classes' raw products and smokeless tobacco products and each sample was collected from three different locations. Raw products (RP) includes those entire samples which are used as ingredients in the preparation of STPs, they are also further divided into eight sub classes *i.e.* betel leaf, areca nut, catechu, tobacco leaf, lime, ash, natural and commercial sweetening and flavouring agents (Table-1). While smokeless tobacco products included all the available brands of STPs in local market; it is further divided into eight groups *i.e.* tobacco brands, pan, zarda, wet gutka, dry gutka, mawa, sniffing products (sniffing naswar) and dipping products.

Sample preparation and digestion: The reagents used were of analytical grade obtained from Merck, Germany. All glassware used were soaked in commercial detergent solution, rinsed and soaked in 10 % (v/v) HNO_3 overnight. They were rinsed thrice with bi-distilled water and then with deionized water. The water used throughout this research work was deionized water. Deionized water was used for the preparation of all solutions. Samples that were in the raw form were washed thoroughly and dried in the oven at 100 °C for 30 to 60 min. While STPs and finishing products were dried at 100 °C without washing. They were then grounded into the powdered form. About 1 g of each powdered sample was weighed into 50 mL digestion tube and added 15 mL of 65 % nitric acid. Samples were allowed to stand for about 24 h at room temperature to enable the foam that formed to settle. They were then heated in a digestion block on a hot plate for about 2 h at 150 °C. The digested samples were allowed to cool and prepared the triplicates of each sample by diluted to 50 mL, 100 mL and 150 mL. Blanks were also prepared alongside. The samples were further stored for analysis.

Metals analysis: All the standards and samples were analyzed in triplicate by using atomic absorption spectrophotometer (Perkin Elmer AAnalyst-700). The calibration standards were prepared in the range from detection limits to 1, 10, 20, 30, 40, 50 and 60 ppm. The calibration curves for all the elements are constructed by using six to eight standards where the value of linearity (r) is in an order of $1 > r > 0.998$, while the limit of detection is determined by detecting the intensity of deionized water and the solution of 1 ppm of each elements were recorded [5]. The limit of detection for As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Se were 2.703, 6.865, 642.9, 1.759, 28.44, 1.635, 1.141, 0.706, 0.232 and 8.746 (μg/L), respectively.

Daily intake of metal estimation: The daily intake of metals was calculated by using the following equation with little modification, according to Dhawar *et al.* [9].

$$\text{Daily intake (μg/day)} = C_{metal} \times W_{analysis} \times D_{intake}$$

where C_{metal} is the concentration of elements in determine in the STPs in μg/g, $W_{analysis}$ is the weight of pouch of respective STPs (g/pouch), D_{intake} the number of pouch consumed per day (pouch/day) which determine by conducting a general survey by questionnaire, contains general information about gender, age, types of STP they used and quantity consumed per day.

Determination of target hazard quotient (THQ) and total target hazard quotient (TTHQ): Risk to the human health by the usage of metal contaminated STPs was characterized by target hazard quotient, determined by the methodology of USEPA [10]. Reference dose (RfD) values for metal used for analysis of THQ as As (0.0003 mg/kg/day) [11], Cd (0.001 mg/Kg/day) [11], Cr (1.5 mg/Kg/day) [11], Cu (0.04 mg/Kg/day) [11], Ni (0.02 mg/Kg/day) [11], Pb (0.0035 mg/Kg/day) [12], Fe (0.700 mg/Kg/day) [13] and Mn (0.014 mg/Kg/day) [13] while there is no consensus about the reference dose for

TABLE-1
SAMPLING INFORMATION AND COMPOSITION OF RAW PRODUCTS AND SMOKELESS TOBACCO PRODUCTS

Class	Name	Major constituents	Types of sample	Number of sample
Raw products (RP)	Betel leaf	-	5	15
	Areca nut	-	4	12
	Catechu	-	6	18
	Tobacco leaf	-	3	9
	Limes	-	3	9
	Ash	-	3	9
	Natural sweeting/Flavour agents	Aniseed, Coconut, Coriander, Dried Date, Clove, Cardamom, Fennel	7	21
	Commercial flavour & sweetening products	Powder, cream, liquid, Vermicelli, *etc.*	6	18
Smokeless tobacco products (STPs)	Tobacco brands	Tobacco + Flavour	-	30
	Pan	Tobacco + Betel Leaf + Areca Nut + Catechu + lime + Flavour/sweeting agents	-	9
	Zarda	Tobacco + $Ca(OH)_2$ + Moister	-	12
	Wet gutkha	Areca Nut (large) + Tobacco + Catechu + Water	-	21
	Dry gutkha	Areca Nut (small) + Tobacco + Catechu + Lime	-	30
	Mawa	Nut + Tobacco + Lime	-	9
	Sniffing products (sniffing niswar)	Tobacco + Ash + Lime	-	12
	Dipping product	Tobacco + Lime	-	12

Co, then the oral reference dose was taken as 0.043 mg/Kg/day [14]. Target hazard quotient of metals was determined based on the formula given by Orisakwe *et al*. [15].

$$THQ = (EF_r \times ED_{tot} \times SIR \times C \times 10^{-3})/(RfD_o \times BW_a \times AT_n)$$

where EF_r is the exposure frequency = 312 days/year, equivalent to 6 days per week; ED_{tot} is the exposure duration = 70 years, SIR is the smokeless tobacco consumption rate determine by survey; C is the amount of metal in the STP (µg/g); RfD_0 is the oral reference dose average life time (mg/Kg/day); BW_a is the average body weight (60 Kg) and ATn is the average exposure time in days (312 days/year × 70 years); 10^{-3} is the conversion factor. The total THQ (TTHQ) of each category of STPs was calculated by sum of individual THQ of all metals [16].

$$TTHQ = THQ_{(toxicant\ 1)} + THQ_{(toxicant\ 2)} \ldots\ldots + THQ_{(toxicant\ n)}$$

Statistical analysis: Data of metal analysis reported as mean ± standard deviation. All data were analyzed by one-way analysis of variance (ANOVA) and Tukey's HSD multiple comparison to excess the significant difference between means at 95 % confidence level. Person correlation and agglomerative hierarchical cluster (AHC) analyses were performed by using XLSTAT® version 2014.5.03 to find difference between the STPs and its ingredients.

RESULTS AND DISCUSSION

Level of metals in the ingredients (raw products) and smokeless tobacco products (STPs): In the ingredient of smokeless tobacco products (Table-2), iron was the major element in almost all samples and was found in the range of 9.00 -372.42 µg/g, although in the samples of lime and ash, Fe was the third most abundant element among all analyzed elements. Nickel found as second most common elements in raw products samples but in ash Ni was the major element, these samples also contained the greater quantity of Co and Se 3.74 ± 0.12, 2.96 ± 0.57 µg/g, respectively while in rest of the samples Co and Se present <1.00 µg/g. High concentration of Co was also observed in catechu (4.88 ± 1.26). Mn, Cu and Pb found in the range of 1.04 to 28.25 µg/g. In case of As and Cd, there is no significant difference among all the categories of ingredients and found in the range of 0.00-0.99 µg/g. The mean value of all analyzed samples from each category of ingredients (raw products) was calculated for various elements. The order of elemental concentration can be summarized as Fe > Ni > Cu > Mn > Pb > Cr > Co > Se > Cd > As.

In all the samples of smokeless tobacco products (Table-3) Fe is the major element among all and were found in the range of 43.22-479.72 µg/g, which is less than the reported value [5]. Manganese found as the second most abundant element in all categories of STPs excluding tobacco brands, pan and zarda. While Cr, Cu, Ni and Pb were present in the range of 1.32 to19.99 µg/g. As and Cd found in similar quantity, ranges from 0.04-0.69 while the quantity of As is significantly greater in STP than the raw products samples. The metals concentration in smokeless tobacco products was Fe > Mn > Cu > Cr > Ni > Pb > Co > Se > As > Cd, almost same pattern was reported by Syed *et al*. [5]. While the quantity of Pb found more than observed by Orisakwe *et al*. [15].

The sample of STPs as compared to the ingredients (raw products) contained high concentration of As, Cr, Se, Fe and Mn while low concentration of Cu, Ni, Pb and Co, respectively. There is no difference of the concentration of Cd was observed between the ingredients and STPs. The high concentration of As, Cr, Se, Fe and Mn mainly related to the anthropogenic activities, it's maybe due to the processing or due to the addition of unknown substance in the STPs. While source of contamination of Cu, Ni, Pb and Co associated with the ingredients (raw products).

Multivariate analyses: The relation among metals and possible source of contamination in the ingredients (raw products) and STPs was assessed using Pearson correlation matrix (Tables 4 and 5) and Agglomerative hierarchical cluster analysis (Fig. 1), respectively.

TABLE-2
AVERAGE METALS AND METALLOIDS CONTENTS IN THE RAW PRODUCTS (INGREDIENTS OF SMOKELESS TOBACCO PRODUCTS)[d]

	n[e]	As[f,g]	Cd	Cr	Cu	Ni	Pb	Se	Co	Fe	Mn
Betel leaf	5	0.021 ± 0.01^{bB}	0.058 ± 0.06^{aB}	0.46 ± 0.30^{bB}	2.70 ± 0.66^{cdB}	15.01 ± 9.20^{aA}	1.04 ± 0.01^{bB}	0.039 ± 0.03^{bcB}	0.561 ± 0.46^{abB}	22.63 ± 13.69^{bcA}	1.14 ± 1.31^{aB}
Areca nut	4	0.030 ± 0.02^{bB}	0.128 ± 0.03^{aB}	0.35 ± 0.03^{bB}	9.41 ± 4.04^{cAB}	19.62 ± 10.60^{aAB}	2.23 ± 0.1^{bB}	0.061 ± 0.05^{bcB}	0.161 ± 0.13^{abB}	34.23 ± 31.28^{bcA}	2.98 ± 1.85^{aB}
Catechu	6	0.032 ± 0.02^{bB}	0.015 ± 0.01^{aB}	2.93 ± 1.16^{bB}	5.87 ± 4.17^{cdB}	28.25 ± 12.99^{aB}	6.02 ± 3.04^{bB}	0.043 ± 0.08^{cB}	4.882 ± 6.12^{abB}	250.00 ± 71.86^{abA}	3.85 ± 0.64^{aB}
Tobacco leaf	3	0.029 ± 0.01^{abB}	BDL[h]	10.42 ± 2.45^{aB}	11.06 ± 4.82^{cB}	7.93 ± 2.95^{aB}	1.66 ± 0.39^{bB}	0.008 ± 0.00^{bcB}	0.695 ± 0.17^{abB}	372.42 ± 81.97^{aA}	6.14 ± 4.97^{aB}
Lime	3	BDL	0.067 ± 0.03^{aF}	5.92 ± 0.24^{abA}	34.89 ± 0.56^{aB}	27.05 ± 0.29^{aC}	13.80 ± 1.06^{aD}	0.140 ± 0.00^{bcF}	0.840 ± 0.11^{abF}	9.00 ± 0.60^{bcE}	3.78 ± 3.28^{aF}
Ash	3	BDL	0.57 ± 0.16^{aF}	8.55 ± 0.47^{aA}	26.08 ± 1.99^{bCD}	28.07 ± 1.22^{aB}	7.10 ± 3.93^{abF}	2.961 ± 0.57^{aF}	3.740 ± 0.12^{aC}	22.30 ± 0.32^{abcDE}	11.75 ± 10.64^{aEF}
Natural sweeting/flavour agents	7	0.050 ± 0.03^{aB}	0.998 ± 0.86^{aB}	7.64 ± 4.05^{aB}	5.67 ± 3.89^{cdB}	26.27 ± 16.22^{aAB}	4.03 ± 1.49^{bB}	0.439 ± 0.23^{bB}	0.317 ± 0.42^{bB}	53.79 ± 32.47^{bcA}	7.84 ± 4.87^{Ab}
Commercial flavour/sweeting products	6	0.044 ± 0.02^{bB}	0.030 ± 0.02^{aB}	0.15 ± 0.06^{bB}	1.74 ± 1.25^{dB}	0.08 ± 0.00^{aB}	1.05 ± 0.34^{bB}	0.332 ± 0.31^{bcB}	0.044 ± 0.04^{bB}	12.83 ± 9.48^{cA}	1.64 ± 0.22^{aB}

[d]Result are expressed as mean ± standard deviation, [e]types of samples, [f](a-c) Means in the same column that do not share a common letter differ significantly ($P < 0.05$), [g](A-F) Means in the same row that do not share a common letter differ significantly ($P<0.05$), [h]BDL = below detection limits.

TABLE-3
AVERAGE METALS AND METALLOIDS CONTENTS IN VARIOUS SMOKELESS TOBACCO PRODUCTS (STPs)[e]

	n[f]	As[g,h]	Cd	Cr	Cu	Ni	Pb	Se	Co	Fe	Mn
Tobacco brands	30	0.67 ± 0.04^{bB}	0.39 ± 0.22^{bB}	7.78 ± 4.35^{bcB}	8.35 ± 2.71^{aB}	5.72 ± 4.41^{aB}	3.80 ± 1.36^{aB}	1.86 ± 0.78^{aB}	0.98 ± 0.43^{bB}	204.00 ± 112.51^{bcA}	2.85 ± 0.60^{cB}
Pan	9	0.42 ± 0.01^{aB}	0.69 ± 0.07^{aB}	2.18 ± 0.34^{bcB}	11.94 ± 5.81^{aB}	4.37 ± 0.00^{abB}	4.84 ± 2.95^{aB}	0.60 ± 0.35^{bB}	0.13 ± 0.09^{bB}	52.10 ± 13.73^{cdA}	0.93 ± 0.40^{abcB}
Zarda	12	0.26 ± 0.02^{bcB}	0.28 ± 0.23^{bcB}	8.24 ± 5.10^{bcB}	6.80 ± 3.02^{aB}	9.60 ± 0.96^{aB}	2.21 ± 1.24^{aB}	2.28 ± 1.13^{aB}	1.19 ± 0.96^{bB}	176.77 ± 112.38^{bcdA}	2.11 ± 0.29^{bcB}
Dry gutkha	30	0.37 ± 0.00^{bcB}	0.05 ± 0.02^{cB}	1.77 ± 1.06^{cB}	10.12 ± 7.92^{aB}	1.40 ± 0.82^{bB}	1.53 ± 0.78^{aB}	0.68 ± 0.42^{bB}	0.48 ± 0.26^{bB}	51.66 ± 41.12^{dA}	35.99 ± 28.14^{abA}
Wet gutkha	21	0.12 ± 0.01^{cB}	0.05 ± 0.02^{cB}	2.02 ± 1.38^{cB}	3.67 ± 1.94^{aB}	BDL[i]	1.32 ± 1.12^{aB}	0.60 ± 0.36^{bB}	1.00 ± 0.16^{bB}	43.22 ± 29.77^{dA}	11.25 ± 1.20^{abcB}
Mawa	9	0.36 ± 0.06^{bcB}	0.04 ± 0.01^{cB}	1.94 ± 0.9^{bcB}	6.89 ± 2.35^{aB}	1.66 ± 0.31^{abB}	1.59 ± 0.19^{aB}	0.49 ± 0.00^{bB}	0.52 ± 0.21^{bB}	47.11 ± 31.00^{cdA}	25.70 ± 16.75^{abcAB}
Sniffing products	12	0.31 ± 0.00^{bcB}	0.11 ± 0.06^{bcB}	19.09 ± 4.12^{aB}	9.99 ± 2.72^{aB}	4.55 ± 1.77^{abB}	3.10 ± 2.47^{aB}	BDL	1.19 ± 0.98^{bB}	479.72 ± 101.57^{aA}	46.24 ± 18.8^{aB}
Dipping Product	12	0.51 ± 0.02^{bcB}	0.18 ± 0.07^{bcB}	11.40 ± 1.92^{abB}	5.72 ± 0.94^{aB}	5.24 ± 0.65^{abB}	3.75 ± 1.45^{aB}	0.42 ± 0.00^{bB}	3.24 ± 1.81^{aB}	300.17 ± 54.72^{abA}	45.04 ± 41.05^{abB}

[e]Result are expressed as mean ± standard deviation , [f]number of samples, [g](a-d) Means in the same column that do not share a common letter differ significantly ($P<0.05$), [h](A-B) Means in the same row that do not share a common letter differ significantly ($P<0.05$), [i]BDL = below detection limits.

TABLE-4
CORRELATION BETWEEN METALS IN INGREDIENT (RAW PRODUCTS)

Variables	As	Cd	Cr	Cu	Ni	Pb	Se	Co	Fe	Mn
As	**1**									
Cd	0.146	**1**								
Cr	-0.198	0.227	**1**							
Cu	**-0.413**	0.080	**0.407**	**1**						
Ni	0.228	-0.201	0.050	0.273	**1**					
Pb	-0.012	0.070	0.071	**0.647**	0.109	**1**				
Se	-0.146	0.245	**0.384**	**0.400**	0.166	0.102	**1**			
Co	-0.062	0.036	0.116	0.265	**0.351**	0.025	**0.429**	**1**		
Fe	0.049	-0.033	0.130	-0.064	-0.038	0.144	-0.197	-0.127	**1**	
Mn	0.058	**0.473**	0.221	0.210	0.000	**0.349**	**0.367**	0.134	-0.004	**1**

Values in bold are different from 0 with a significance level $\alpha = 0.05$

TABLE-5
CORRELATION BETWEEN METALS IN SMOKELESS TOBACCO PRODUCTS

Variables	As	Cd	Cr	Cu	Ni	Pb	Se	Co	Fe	Mn
As	**1**									
Cd	0.130	**1**								
Cr	0.051	**0.296**	**1**							
Cu	**0.416**	0.241	0.135	**1**						
Ni	0.054	**0.554**	**0.662**	0.115	**1**					
Pb	0.136	**0.481**	**0.407**	**0.386**	**0.343**	**1**				
Se	0.220	**0.345**	-0.095	0.000	0.240	0.016	**1**			
Co	0.039	0.113	**0.473**	-0.040	**0.326**	**0.412**	-0.038	**1**		
Fe	0.244	0.200	**0.699**	0.119	**0.447**	**0.336**	0.095	**0.479**	**1**	
Mn	0.111	**-0.351**	0.182	0.068	-0.129	-0.109	**-0.454**	0.104	0.154	**1**

Values in bold are different from 0 with a significance level $\alpha = 0.05$

In the sample of ingredients (Table-4), Cu showed strong positive correlation with Pb (r = 0.647 and Cr (r = 0.407). Mn had positively correlated with Cd (r = 0.473). Se shows positive correlation with Mn (r = 0.473), Cu (r = 0.400), Cr (r = 0.384) and Co (r = 0.351). Other positive significant correlation were found between Ni and Co (r = 0.351), Mn and Pb (r = 0.349) while Fe showed no appropriate correlation to any of the element analyzed. Significant negative correlation was only existed between Cu and As (r = -0.413).

In STPs (Table-5) Cr showed significant positive correlation with Fe (r = 0.699) and Ni (r = 0.662). Ni had significant positive correlation with Cd (r = 0.554). Lead shows significant positive correlation with Cd (r = 0.481), Co (r = 0.412), Cr (r = 0.407), Cu (r = 0.386), Ni (r = 0.343) and Fe (r = 0.336). Other significant positive correlation were found between Co and Fe (r = 0.479), Cr and Co (r = 0.473), Ni and Fe (r = 0.447), As and Cu (r = 0.416), Cd and Se (r = 0.345) and between Ni and Co (r = 0.326) while only Mn shows a negative significant correlation with Se (r = -0.454) and Cd (r = -0.351).

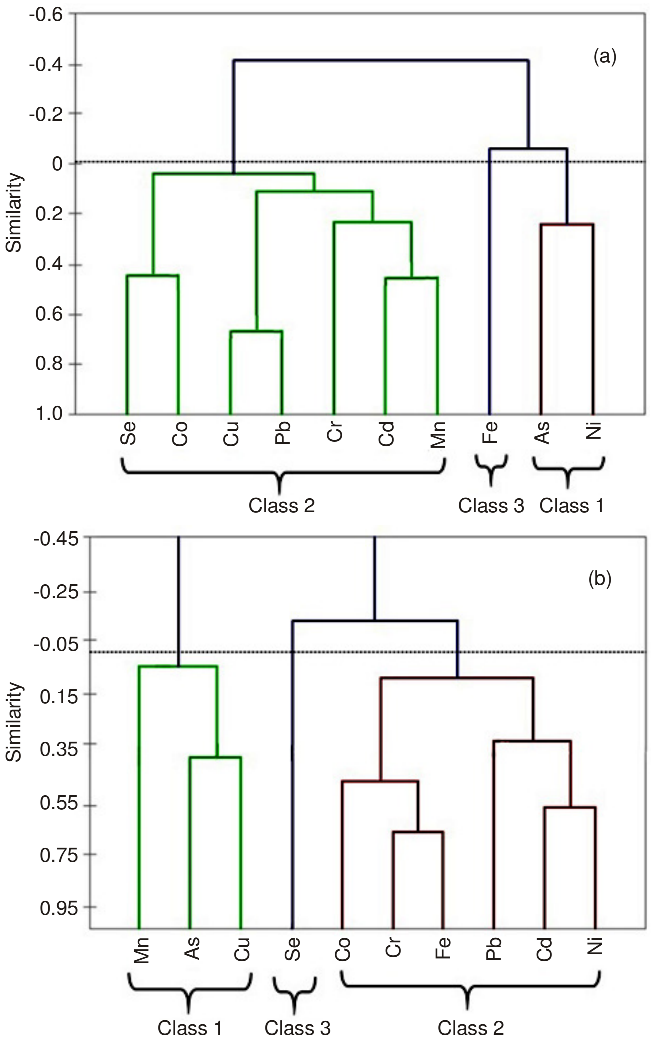

Fig. 1. Hierarchical diagram of studied metals in (a) ingredients of STPs (RP) and (b) STPs

The agglomerative hierarchical cluster analysis (Fig. 1) also had shown closed association between studied metals as indicated by correlation analysis. Three groups of metals were identified Group 1: As and Ni; Group 2: Cd, Cr, Cu, Pb, Se, Co and Mn; Group 3: Fe in ingredients (raw products). The elements in STPs were grouped by agglomerative hierarchical cluster (Fig. 1B) as Group 1: As, Cu and Mn; Group 2: Cd, Cr, Ni, Pb, Co and Fe; Group 3: Se.

Daily ingestion rate of STPs and daily intake of metals: On the basis of survey results the STPs consumers were classified into three age groups *i.e.* below 20, between 21- 40 and above 40 years. Dry gutka (36.0 %) and mawa (23.5 %) were the popular products in the age group of below 20 years, in age group of between 21 to 40 mawa (31 %) and wet gutka (26.8 %) while consumer above 40 years used sniffing products (22.7 %) and pan (18.4 %) more frequently among all other categories. Fig. 2 represents the average daily consumption (g/day) of different categories of STPs.

Daily metals intake estimate for each category of STPs are shown in Table-6. Cr plays an important role in human body, it takes part in insulin activity and DNA transcription less than 20 µg/day can decrease the cellular response to insulin [21]. Chromium in every category of STPs exceeds from the

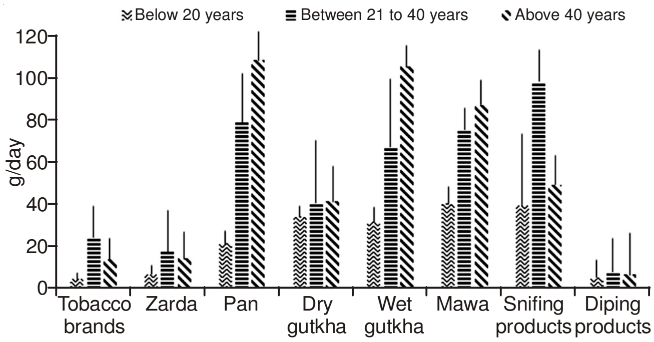

Fig. 2. Consumption pattern of STPs (g/day) with respect to different age groups. Error bars represented the SD

upper trouble daily intakes (UL) level (35 µg/d) in all age groups. IARC has been declared Cd included in group I cancer-genes. The excessive use induces kidney dysfunction, osteomalacia and reproductive deficiency [17]. In samples of pan Cd (74.83 µg/d) and Pb (524.92 µg/d) exceeded the upper trouble daily intakes level (64 and 240 µg/d, respectively) in age group of above 40 years. Lead known as a multiorgan toxicant, cause renal tumor and increase blood pressure in adult [15,17], in age group of between 21 to 40 years daily intake of Pb found greater than the upper trouble daily intakes in category of pan and sniffing products (383.23 and 305.51 µg/d, respectively). Ni works as a co-factor for some microbial intestine enzyme. The RDA for Ni is 1.00 mg/day; excess of Ni may cause damages to DNA and cellular structure [21]. Daily intake of Ni in wet gutka and Se in sniffing products were not determine because of respective metals found below detection limits in the mention categories. The content of As, Pb and Cu intake found as lower that reported in literature, which ranged between 1.1-109.0, 0.3-680 and 0.12-6560 (µg/d), respectively [9,15]. Intake of Cd, Cr, Ni and Co were found greater than the literature, ranges from 0.1-32.0, 430-1140, 2.0-7.0 and 1.0-3.0 (µg/d) [9,15-20].

Target hazard quotient (THQ): Target hazard quotient consider a reliable method of risk estimation although hazard quotient (HQ)-based risk assessment method only provided the indication of health effects due the exposure of pollutant [15]. The hazard index (THQ) greater than 1 indicates the risk associated with the respective samples while lower than 1 that indicates safety of the sample [26]. Fig. 3 shows the THQ of individual element in STPs and total THQ (TTHQ) in all categories of STPs. The THQ of As exceeds from 1.0 in the pan, mawa and sniffing products. As is a widely distributed metalloid, present in rock, soil, water and air. The excessive exposure produced gastrointestinal symptoms, severe disturbance of the cardiovascular and central nervous system and eventually death [22]. Manganese is co-factor for arginase also associated with astrocyte-specific enzyme glutamine synthetase, high level of Mn causes neurodegenerative damage [23]. Dry gutkha, mawa and sniffing products shows THQ > 1.0 in case of Mn. While total target hazard quotient (TTHQ) of all categories of STPs exceeds from 1.0 except zarda and dipping products.

Conclusion

The average metal concentration and multivariate analysis indicate that the source of As, Cr, Se, Fe and Mn may be

TABLE-6

DAILY METALS INTAKE ESTIMATE (DAILY INTAKE METALS) OF SMOKELESS TOBACCO PRODUCTS

Element		Daily intake		Tobacco brands	Zarda	Pan	Dry gutkha	Wet gutkha	Mawa	Sniffing products	Dipping products
	RDA[a] ND[b] [Ref. 24]	DI1[a]$_{(As)}$	Mean	2.90	0.26	0.42	0.37	0.12	0.36	0.31	0.51
			Range	2.01-4.02	1.3-2.08	3.6-14.38	3.74-23.4	2.53-5.06	7.25-21.76	6.11-18.33	1.3-3.91
As (μg/day)		DI2[b]$_{(As)}$	Mean	14.74	3.84	33.26	14.98	6.33	27.27	30.55	3.91
			Range	10.05 -21.44	2.6-7.02	10.79-50.33	7.49-22.47	2.53-15.18	14.5-43.51	24.44-36.66	2.61-5.21
	UL[c] ND [Ref. 24]	DI3[c]$_{(As)}$	Mean	8.26	3.12	45.55	15.29	12.65	31.47	15.28	3.26
			Range	6.7-11.39	2.6-5.2	35.95-57.52	13.11-17.79	10.12-15.18	21.76-43.51	12.22-18.33	2.61-3.91
	RDA 0.0 [Ref. 25]	DI1$_{(Cd)}$	Mean	1.69	1.87	14.77	2.12	1.58	1.61	4.34	0.92
			Range	1.17-2.34	1.4-2.24	5.91-23.63	0.51-3.16	1.05-2.11	0.81-2.42	2.17-6.5	0.46-1.38
Cd (μg/day)		DI2$_{(Cd)}$	Mean	8.58	4.13	54.63	2.02	2.64	3.03	10.84	1.38
			Range	5.85-12.48	2.8-7.56	17.72-82.69	1.01-3.04	1.05-6.33	1.61-4.83	8.67-13.01	0.92-1.84
	UL 64.0 [Ref. 25]	DI3$_{(Cd)}$	Mean	4.81	3.36	74.83	2.07	5.27	3.5	5.42	1.15
			Range	3.9-6.63	2.8-5.6	59.06-94.50	1.77-2.4	4.22-6.33	2.42-4.83	4.34-6.5	0.92-1.38
	RDA ND [Ref. 24]	DI1$_{(Cr)}$	Mean	33.69	54.96	46.65	75.01	63.9	78.16	752.53	58.25
			Range	23.34-46.68	41.2-65.92	18.66-74.64	17.91-111.95	42.6-85.2	39.08-117.24	376.26-1128.79	29.13-87.38
Cr (μg/day)		DI2$_{(Cr)}$	Mean	171.16	121.54	172.61	71.65	106.5	146.95	1881.32	87.38
			Range	116.7-248.96	82.4-222.48	55.98-261.25	35.82-107.47	42.6-255.61	78.16-234.49	1505.06-2257.58	58.25-116.51
	UL 35.0 [Ref. 24]	DI3$_{(Cr)}$	Mean	95.93	98.88	236.43	73.13	213.01	169.61	940.66	72.82
			Range	77.8-132.26	82.4-164.8	186.61-298.57	62.69-85.08	170.41-255.61	117.24-234.49	752.53-1128.79	58.25-87.38
	RDA 900 [Ref. 24]	DI1$_{(Cu)}$	Mean	36.16	45.36	255.52	428.86	116.1	277.6	393.81	29.23
			Range	25.05-50.1	34-54.4	102.21-408.83	102.41-640.09	77.4-154.8	138.8-416.4	196.9-590.71	14.61-43.84
Cu (μg/day)		DI2$_{(Cu)}$	Mean	183.7	100.3	945.41	409.66	193.5	521.88	984.51	43.84
			Range	125.25-267.2	68-183.6	306.62-1430.89	204.83-614.49	77.4-464.4	277.6-832.79	787.61- 1181.42	29.23-58.46
	UL [Ref. 24] 10,000	DI3$_{(Cu)}$	Mean	102.96	81.6	1294.96	418.11	387	602.39	492.26	36.54
			Range	83.5-141.95	68-136	1022.06-1635.3	358.45-486.47	309.6-464.4	416.4-832.79	393.81-590.71	29.23-43.84
	RDA 500 [Ref. 24]	DI1$_{(Ni)}$	Mean	24.77	64.03	93.52	59.33	ND	66.88	179.36	26.78
			Range	17.16-34.32	48-76.8	37.41-149.63	14.17-88.55		33.44-100.32	89.68-269.04	13.39-40.16
Ni (μg/day)		DI2$_{(Ni)}$	Mean	125.84	141.6	346.02	56.67	ND	125.74	448.4	40.16
			Range	85.8-183.04	96-259.2	112.22-523.70	28.34-85.01		66.88-200.64	358.72-538.08	26.78-53.55
	UL 1000 [Ref. 24]	DI3$_{(Ni)}$	Mean	70.53	115.2	473.95	57.84	ND	145.13	224.2	33.47
			Range	57.2-97.24	96-192	374.07-598.52	49.59-67.3		100.32-200.64	179.36-269.04	26.78-40.16
	RDA 0.0 [Ref. 25]	DI1$_{(Pb)}$	Mean	16.45	14.74	103.58	64.84	41.76	64.06	122.2	19.16
			Range	11.4-22.8	11.05-17.68	41.43-165.72	15.48-96.77	27.84-55.68	32.03-96.09	61.1-183.3	9.58-28.74
Pb (μg/day)		DI2$_{(Pb)}$	Mean	83.6	32.6	383.23	61.93	69.6	120.43	305.51	28.74
			Range	57-121.6	22.1-59.67	124.29-580.03	30.97-92.9	27.84-167.03	64.06-192.18	244.4-366.61	19.16-38.33
	UL [Ref. 25] 0.240	DI3$_{(Pb)}$	Mean	46.85	26.52	524.92	63.21	139.19	139.01	152.75	23.95
			Range	38-64.6	22.1-44.2	414.3-662.89	54.19-73.55	111.36-167.03	96.09-192.18	122.2-183.3	19.16-28.74
	RDA 45 [Ref. 24]	DI1$_{(Se)}$	Mean	8.05	15.21	12.84	28.82	18.98	19.74	ND	2.15
			Range	5.58-11.16	11.4-18.24	5.14-20.54	6.88-43.01	12.65-25.31	9.87-29.61		1.07-3.22
Se (μg/day)		DI2$_{(Se)}$	Mean	40.92	33.63	47.51	27.53	31.64	37.12	ND	3.22
			Range	27.9-59.52	22.8-61.56	15.41-71.9	13.76-41.29	12.65-75.92	19.74-59.23		2.15-4.29
	UL [Ref. 24] 400	DI3$_{(Se)}$	Mean	22.93	27.36	65.07	28.09	63.27	42.84	ND	2.68
			Range	18.6-31.62	22.8-45.6	51.36-82.18	24.09-32.69	50.62-75.92	29.61-59.23		2.15-3.22
	RDA DNA[g]	DI1$_{(Co)}$	Mean	4.24	7.94	2.78	20.34	31.64	20.95	46.91	16.56
			Range	2.94-5.88	5.95-9.52	1.11-4.45	4.86-30.36	21.09-42.18	10.48-31.43	23.45-70.36	8.28-24.83
Co (μg/day)		DI2$_{(Co)}$	Mean	21.56	17.55	10.29	19.43	52.73	39.39	117.27	24.83
			Range	14.7-31.36	11.9-32.13	3.34-15.58	9.72-29.15	21.09-126.54	20.95-62.85	93.82-140.73	16.56-33.11
	UL DNA	DI3$_{(Co)}$	Mean	12.08	14.28	14.10	19.83	105.45	45.46	58.64	20.7
			Range	9.8-16.66	11.9-23.8	11.13-17.8	17-23.07	84.36-126.54	31.43-62.85	46.91-70.36	16.56-24.83
	RDA 2300 [Ref. 24]	DI1$_{(Mn)}$	Mean	12.34	14.07	19.9	1525.17	355.89	1035.45	1822.78	230.15
			Range	8.55-17.1	10.55-16.88	7.96-31.84	364.22-2276.37	237.26-474.53	517.73-1553.18	911.39-2734.17	115.08-345.23
Mn (μg/day)		DI2$_{(Mn)}$	Mean	62.7	31.12	73.64	1456.88	593.16	1946.65	4556.95	345.23
			Range	42.75-91.2	21.1-56.97	23.88-111.45	728.44-2185.31	237.26-1423.58	1035.45-3106.36	3645.56-5468.34	230.15-460.31
	UL [Ref. 24] 11,000	DIc$_{(Mn)}$	Mean	35.14	25.32	100.86	1486.92	1186.31	2246.93	2278.48	287.69
			Range	28.5-48.45	21.1-42.2	79.61-127.37	1274.77-1730.04	949.05-1423.58	1553.18-3106.36	1822.78-2734.17	230.15-345.23
	RDA [Ref. 24] 8.1	DI1$_{(Fe)}$	Mean	0.88	1.18	1.12	2.19	1.37	1.9	18.91	1.53
			Range	0.61-1.22	0.88-1.41	0.45-1.78	0.52-3.27	0.91-1.82	0.95-2.85	9.46-28.37	0.77-2.3
Fe (mg/day)		DI2$_{(Fe)}$	Mean	4.49	2.61	4.13	2.09	2.28	3.57	47.28	2.30
			Range	3.06-6.53	1.77-4.77	1.34-6.24	1.05-3.14	0.91-5.47	1.9-5.69	37.82-56.73	1.53-3.07
	UL [Ref. 24] 45	DI3$_{(Fe)}$	Mean	2.52	2.12	5.65	2.13	4.56	4.12	23.64	1.92
			Range	2.04-3.47	1.77-3.54	4.46-7.14	1.83-2.48	3.65-5.47	2.85-5.69	18.91-28.37	1.53-2.3

[a]RDA = Recommended daily intake, [b]ND = not determine, [c]UL= upper tolerable daily intake level, [d]DI1 = Represent age group below 20 years, [e]DI2 = Represent age group Between 21 to 40 years, [f]DI3 = Represent age group Above 40 years, [g]DNA = Data not available

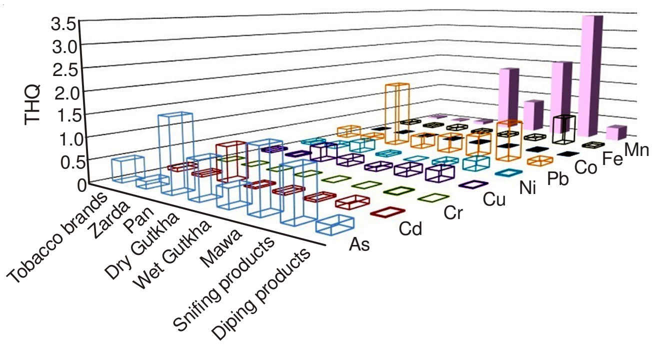

Fig. 3. Target harzard quotient (THQ) values of various metals caused by consumption of different classes of smokeless tobacco products (STPs)

associated with processing of STPs or addition of unknown ingredients. While source of contamination of Cu, Ni, Pb and Co associated with ingredients (absorption of metals from soil, water or air) or may be contaminated from other source. The daily intake metals of Cr, Cd and Pb found greater than the upper trouble daily intakes and total target hazard quotient (TTHQ) of six categories of STPs exceeded which indicates the health risk associated with their use while high consumption rate demand the need to implement more strict rules and regulation.

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