Analysis of cadmium, nickel, and lead in commercial moist and dry snuff used in Pakistan

Tasneem Gul Kazi • Sadaf Sadia Arain • Hassan Imran Afridi • Naeemullah • Kapil Dev Brahman • Nida Fatima Kolachi • Moina Akhtar Mughal

Received: 7 August 2012 / Accepted: 2 October 2012 / Published online: 14 October 2012 © Springer Science+Business Media Dordrecht 2012

Abstract The extent to which smokeless tobacco endangers human health is an ongoing subject of debate. In this study, concentrations of toxic metals, cadmium (Cd), lead (Pb), and nickel (Ni), were measured in different snuff products (dry brown and black and moist green and brown), available and consumed in Pakistan. Concentrations of Cd, Pb, and Ni were determined in 23 samples of various brands of snuff by electrothermal atomic absorption spectrometry, after microwave-

T. G. Kazi · S. S. Arain (⊠) · H. I. Afridi · Naeemullah · K. D. Brahman · N. F. Kolachi National Centre of Excellence in Analytical Chemistry, University of Sindh, Jamshoro 76080, Pakistan e-mail: ssadiashafi@gmail.com

T. G. Kazi e-mail: tgkazi@yahoo.com

H. I. Afridi e-mail: hassanimranafridi@yahoo.com

Naeemullah e-mail: khannaeemullah@ymail.com

K. D. Brahman e-mail: kr_brahman@yahoo.com

N. F. Kolachi e-mail: nidafatima6@gmail.com

M. A. Mughal

Dr. M.A. Kazi Institute of Chemistry, University of Sindh, Jamshoro 76080, Pakistan e-mail: moina virgo@hotmail.com assisted acid digestion. The reliability of methodology was assured by analyzing certified reference material. The resulted data of toxic metals in different snuff products are comparable to the existing information with limited exceptions. It was estimated that 10 g intake of different types of snuff could contribute 14–68, 17–47, and 20–73 % of the provisional maximum tolerable daily intake for Cd, Ni, and Pb, respectively.

Keywords Cadmium \cdot Nickel \cdot Lead \cdot Dry and moist snuff \cdot Smokeless tobacco \cdot Microwave-assisted acid digestion

Introduction

Smokeless tobacco (SLT) is linked with cancers of the cheek, gum, and inner surface of the lips (Schildt et al. 1998). Many epidemiologic studies addressing the risk of SLT use for cancers of the oral cavity and adjacent sites are being reviewed (Bouquot 1991; Vijayan and Kumar 2005). Cancers caused by SLT often begin as leukoplakia or erythroplakia. Erythroplakia is generally more severe than leukoplakia and has a higher chance of becoming cancerous over time (US Department of Health and Human Services 1986). Even nasal, esophageal, laryngeal, and stomach cancer can be facilitated by SLT (Muir and Zaridze 1986).

The use of SLT is limited to some areas in all continents. In the USA, SLT is available in a variety

of forms, such as dry or moist snuff and loose or plug chewing tobacco leaves (FTC 2003). More than 7.7 million Americans use SLT products (Substance Abuse and Mental Health Services Administration 2006). Certain snuff products have been gaining popularity, especially among adolescent males (CDC 2000). It was reported in 2004 that 6.0 % of US high school students and 2.9 % of middle school students used SLT products (CDC 2005).

In Sweden, oral snuff was used by 20 % of all males in 1996 (National Institute of Public Health 2000). Snuffed tobacco consists of moist particles of tobacco treated with diverse flavors, such as mint or menthol, and placed between the lip or cheek and the gum (IARC 1985; WHO 1988; National Institute of Public Health 2000). SLT also generates systemic alteration such as increase in blood pressure and tachycardia (Schroeder and Chen 1985) while addiction is behind all these harmful effects. A 12-year follow-up longitudinal study in Sweden indicated that more than 100,000 construction workers showed a higher hypertension prevalence in oral snuff users than among nonusers and a 2-fold greater relative risk of death due to cardiovascular disease (versus 3-fold greater among smokers compared with never users of tobacco; p < 0.001) (Bolinder 1997).

Tobacco is known to contain numerous classes of carcinogenic substances, and tobacco-specific nitrosamines, which are often regarded as a major factor in SLT-related carcinogenesis (Hecht and Hoffmann 1988; Stepanov and Hecht 2005). While the carcinogenesis, more likely results from the combined exposure of nitrosamines and other classes of organic and inorganic substances, including toxic metals and metalloids (Colak et al. 2005; Sesli et al. 2008).

It was reported in literature that heavy metals in different environmental and biological samples are classified as group 1 human carcinogens (IARC 2006a; Narin and Soylak 1999) while lead (Pb) compounds are classified as group 2A, a probable human carcinogens (IARC 2006b; Uluozlu et al. 2009).

Cadmium (Cd) is an inhibitor of enzymes with sulphydryl groups and disrupts the pathways of oxidative metabolism (Miccadei and Floridi 1993; Verougstraete et al. 2003). Catalysis of peroxidative reactions by Cd may be a major contributor to the toxic effects of this metal. Various studies have shown that administration of toxic metals to experimental animals results in the production of lipid peroxidation in brain and hepatic tissues (Stohs and Bagchi 1995; Verougstraete et al. 2003). Moist snuff consists of fire- and air-cured dark tobacco that is finely cut. It is now the most popular form of SLT in the USA and Sweden; sales of this product have increased by 77 % over the past 15 years (FTC 2001). One reason for the popularity of moist snuff in both countries is that it has become more user friendly. Traditional moist snuff users place a "pinch" of the finely ground tobacco between the gingival and buccal mucosa. Recently, moist snuff products are sold in small, pre-portioned pouches similar to teabags. These products remain stationary in the mouth and generate very little juice.

The dry brown snuff (DB) is composed of tobacco, ash, cotton, or sesame oil (US Department of Health and Human Services 1986) whereas gum is often included. The moist brown snuff (BM) contains roasted tobacco leaves, gum, calcium oxide, and water. The main constituents of dry black snuff (DBK) are tobacco leaves, ash, slaked lime, and indigo, while in moist green snuff (GM), fresh tobacco leaves, oil, menthol, and water are used.

Snuff was popular among all social classes from the aristocracy to the miner and spread to all corners of the world from Europe to Asia. In Pakistan, snuff is locally called "naswar" but we use the word snuff which is its international name. In most of the small confectionary shops, snuff are available in small pouches containing about 5–10 g of the material. Due to general opinion against tobacco smoking, these products have gained acceptance even among women and teenagers who generally avoid the smoking and other tobacco habits. Thus, due to lack of knowledge about the toxic metals concentration of locally produced snuff, there is an urgent need to investigate their levels in different snuff products consumed frequently.

Atomic absorption spectrometry (Silici et al. 2008) and inductively coupled plasma optical emission spectrometry (Altundag and Tuzen 2011) techniques are frequently used for the specific determination of very low elemental concentrations in environmental and biological samples. At present, the mineralization methods frequently employed for the analysis of biological and food samples are wet digestion with concentrated acids using either convective systems or microwave ovens (Demirel et al. 2008; Soylak et al. 1993). The main advantage of microwave-assisted sample pretreatment is its requirement of a small amount of mineral acids and a reduction in the production of nitrous vapors (Soylak et al. 2007; Saracoglu et al. 2009).

In the present study, the toxic metals (Cd, Ni, and Pb) concentration in different types of snuff (dry brown and black and moist green and brown) available and consumed in Pakistan, were investigated. The intake of understudied toxic metals via consumptions of different types of snuff products was also estimated. Results were compared with the existing data on different snuff from elsewhere.

Materials and methods

Study population

A survey was carried out about the snuff chewing and inhaling habits of both genders, age ranged 15–60 years, residing in the different cities of Pakistan. Before the start of this study, all snuff users were informed about the aim of the study and all agreed to participate and signed the form. A questionnaire was administered to them to collect details regarding physical data, ethnic origin, health, duration and frequency of snuff use, age, and consent. This study was approved by the ethics committee of NCEAC, University of Sindh. The aim of the present study was to investigate the chewing

Fig. 1 a Brown moist snuff. **b** Green moist snuff. **c** Dry brown snuff. d Dry black snuff

habits of different types of snuff among the people in the south-eastern province of Pakistan. From the analysis of 537 questionnaires, we found that more than 50 % of people consumed both brown and green moist snuff, 30 % consumed only moist brown snuff (mostly laborers and drivers), while 20 % used dry brown and dry black snuff. About 20 % of these participants were also smokers. The ratio of male/female was 5:1 for all snuff products. Nowadays, all these products become more popular among teenagers and young people.

Sampling

A total of 23 brands of moist snuff (brown (Fig. 1a) and green (Fig. 1b)) and dry snuff (brown (Fig. 1c) and black (Fig. 1d)) were purchased from local markets of the different cities of Pakistan as per their availability over a 1-year period (January 2011-December 2011). The samples were packed in their original packing and placed in prewashed dried plastic bags separately and stored at 4 °C until tested. Ten composite samples of each snuff brands were prepared by homogenizing the mixture after removing the wrappers. Care was taken to avoid any source of contamination, and this preparation was carried out in a clean environment. All samples



were dried at 80 °C. The dried samples were ground with agate mortar and pestle, sieved through nylon sieves with mesh sizes of 125 μ m, and then stored in the labeled sample bottles.

Reagents and glassware

Chemicals used were of analytical grade. Ultrapure water obtained from ELGA Labwater System (Bucks, UK) was used throughout the work, and 65 % nitric acid purchased from Merck (Darmstadt, Germany) was also used. Certified reference material (CRM) Virginia tobacco leaves (ICHTJ-cta-VTL-2) was purchased from the International Atomic Energy Agency, Vienna (Austria). Moreover, matrix modifiers were employed to analyze Cd, Pb, and Ni (0.001 mg Pd+0.0015 mg Mg (NO₃)₂, 0.2 mg NH₄H₂PO₄, and 0.05 mgMg(NO₃)₂ were prepared from NH₄H₂PO₄, Mg(NO₃)₂, and 99.999 % Pd, respectively; Sigma, St. Louis, MO). Standard solutions of Cd, Ni, and Pb were prepared by dilution of certified standard solutions (1,000 mg/l) obtained from Fluka Kamica (Bush, Switzerland) of corresponding metal ions. Glassware and polyethylene containers were soaked in 10 % (ν/ν) HNO₃ for 24 h, washed with distilled water and finally with de-ionized water, and then dried in such a manner as to ensure that no sign of any contamination from glassware occurs.

Instrumentation

The determination of Cd, Ni, and Pb in digests was carried out by means of a double-beam Perkins-Elmer Atomic Absorption Spectrometer Model 700 (Norwalk, CT) equipped with the graphite furnace HGA-400, pyrocoated graphite tubes with integrated platform, and an autosampler AS-800 and deuterium lamp as background correction system. Hollow cathode lamps (Perkin Elmer) were used as radiation sources, and they were operating at a recommended current for all cases. All instrumental conditions were used according to the manufacturer's recommendation. Portions of both standard or sample and modifier were transferred into autosampler cups, and 20 µl (standard or sample volume of 10 µl and 10 µl of modifier in each case) were injected to electrothermal graphite atomizer. A horizontal flask electrical shaker (220/60 Hz, Gallenkamp, England) was used for shaking the samples. The pH was measured by a pH meter (781pH meter, Metrohm). A PEL domestic microwave oven (Osaka, Japan), programmable for time and microwave power from 100 to 900 W, was used for digestion of samples.

Determination of pH

To determine the pH of the different types of snuff product, take 1 gm sample of each type in 10 ml of ultrapure water in flask (100 ml capacity) and shake in an electrical shaker at 30 rpm for 30 min, and then filter the solution through Whatman No. 42 filter paper; the extracts were used to determine the pH.

Microwave-assisted acid digestion

A microwave-assisted acid digestion procedure was carried out, in order to achieve a shorter digestion time; 0.2 g replicate of six samples of CRM (Virginia tobacco leaves) and 0.2 g duplicate (dry weight) of different types of snuff products were weighed and placed directly into polytetrafluoroethylene (PTFE) flasks (25 ml in capacity). For further validation, spike recovery study of each metal in a real snuff sample at three concentration levels were also carried out; 2 ml of a concentrated HNO₃ was added to each flask and kept at room temperature for 10 min, then the flasks were placed in a covered PTFE container. It was then heated at 80 % of the total power (900 W) at time intervals of 3–5 min. After the digestion, the flasks were left to cool and the resulting solution was evaporated to a semidried mass to remove excess acid. About 10 ml of 0.1 mol/l nitric acid was added to the residue and filtered through a Whatman No. 42 filter paper. Blanks and standard solutions were prepared in a similar acid matrix.

Quality assurance-quality control

The linear range of the calibration curve reached from the detection limit up to 10, 50, and 100 µg/l for Cd, Pb, and Ni, respectively. The detection and quantitation limits were calculated by $\text{LOD} = 3 \times \frac{s}{m}$ and $\text{LOQ} = 10 \times \frac{s}{m}$, respectively, where "*s*" is the standard deviation of ten measurements of a reagent blank and "*m*" is the slope of the calibration graph corresponding to Cd, Ni, and Pb. The calculated LOD and LOQ for Cd, Ni, and Pb were of 0.12, 0.289, and 0.307 and 0.4, 0.964, and 1.02 µg/l, respectively. The proposed microwave-assisted acid digestion method was assured by the analysis of duplicate samples, reagent blank, procedural blanks, CRM, and spike recovery study in a real sample. The precision of

CRM/metals	Certified values (µg/g)	CDM	% recovery	MAD	% recovery	Paired t test ^a ($t_{Experimental}$)
Cd	$1.52{\pm}0.17^{b}$	1.51±0.11 (7.28) ^c	99.0	1.50±0.10 (6.66)	98.4	0.826
Ni	$1.98 {\pm} 0.21$	1.96±0.14 (7.14)	98.9	1.95±0.11 (5.64)	98.2	0.823
Pb	22.1 ± 1.2	21.8±0.82 (3.77)	98.6	21.7±0.62 (2.86)	98.1	0.709

Table 1 Validation of methods for Cd, Ni, and Pb in certified reference material (CRM) Virginia tobacco leaves

*t*_{Critical} at 95 % confidence limit=2.26

^a Paired *t* test between CDM versus MAD; degree of freedom n-1=5

^bMean±SD

^c Values in parenthesis are RSD

the methods, expressed as the relative standard deviation of five independent analyses of the same sample, provided values ranging from 2.86 to 7.28 % as a function of the metals considered and its concentration level.

The accuracy of analytical method was performed with CRM Virginia tobacco leaves and spiking the standards of all three metals in a real sample of brown moist snuff (BM3). Recoveries of target elements were computed by comparison of microwave-assisted method data against values of CRM values and the results obtained from a reference analytical method using electric hot plate digestion on same CRM as reported in a previous work (Arain et al. 2008). Statistical analysis showed that there was no significant difference between the two methods using paired *t* test at 95 % confidence level with five degrees of freedom (Tables 1 and 2).

Results

The pH of all snuff products was highly basic, found in the range of 8.4–8.7, which flavors the formation of tobacco-specific amines thus making the product potentially toxic. Since we analyzed multiple samples for each brand of snuff, therefore, only the mean concentration along with the standard deviation for each brand are provided (Table 3).

The Cd level was found in the range of 0.865–2.03 and 0.658-1.21 µg/g in BM and GM snuff, respectively, while the levels of Cd in DB and DBK snuff products were found in the range of 0.741-1.43 and $0.426-0.997 \mu g/g$, respectively. The concentration of Cd in different snuff products was found in increasing order as: BM>DB>>GM>DBK. The concentration of Ni in BM and GM snuff samples was found in the range of 10.7-14.1 and 7.56-11.4 µg/g, respectively, while the levels of Ni in DB and DBK snuff products were found in the range of 8.49-10.5 and 5.29-9.29 µg/g, respectively. The concentration of Ni in different snuff products was found in increasing order as: BM>GM>DB>DBK. The Pb level was found in the range of 8.46–15.7 μ g/g and 7.10–10.1 μ g/g in BM and GM snuff, respectively, while the level of Pb in DB and DBK snuff products were observed in the range of 4.31-6.10 and 6.02-8.62 µg/g, respectively.

Table 2 Spike recovery study in a brown moist snuff sample (added Cd/Ni/Pb in a moist snuff sample (BM3); in micrograms per gram)

Cd		Ni		Рb	
Mean±SD (µg/g)	% recovery	Mean±SD (µg/g)	% recovery	Mean±SD (µg/g)	% recovery
1.85±0.14	_	13.5±0.61	_	14.8±0.42	_
2.31 ± 0.11	98.3	14.3 ± 0.74	98.6	16.5±0.59	98.2
2.80±0.15	98.2	15.2 ± 0.87	98.1	19.6±0.72	99.0
$3.81 {\pm} 0.21$	99.0	$18.2 {\pm} 0.96$	98.4	24.5 ± 1.42	98.8
	Cd Mean±SD (μg/g) 1.85±0.14 2.31±0.11 2.80±0.15 3.81±0.21	$\begin{tabular}{ c c c c } \hline Cd & & & & \\ \hline Mean\pm SD~(\mu g/g) & \% \ recovery & \\ \hline 1.85\pm 0.14 & - & & \\ 2.31\pm 0.11 & 98.3 & & \\ 2.80\pm 0.15 & 98.2 & & \\ 3.81\pm 0.21 & 99.0 & & \\ \hline \end{tabular}$	$\begin{tabular}{ c c c c c } \hline Cd & Ni \\ \hline Mean \pm SD (\mu g/g) & \% \ recovery & Mean \pm SD (\mu g/g) \\ \hline 1.85 \pm 0.14 & - & 13.5 \pm 0.61 \\ 2.31 \pm 0.11 & 98.3 & 14.3 \pm 0.74 \\ 2.80 \pm 0.15 & 98.2 & 15.2 \pm 0.87 \\ 3.81 \pm 0.21 & 99.0 & 18.2 \pm 0.96 \\ \hline \end{tabular}$	$\begin{tabular}{ c c c c c } \hline Cd & Ni & \\ \hline Mean\pm SD (\mu g/g) & \% \ recovery & Mean\pm SD (\mu g/g) & \% \ recovery & \\ \hline 1.85\pm 0.14 & - & 13.5\pm 0.61 & - & \\ 2.31\pm 0.11 & 98.3 & 14.3\pm 0.74 & 98.6 & \\ 2.80\pm 0.15 & 98.2 & 15.2\pm 0.87 & 98.1 & \\ 3.81\pm 0.21 & 99.0 & 18.2\pm 0.96 & 98.4 & \\ \hline \end{tabular}$	$ \begin{array}{c c c c c c c c c c c c c c c c c c c $

^a Added cadmium concentration

^b Added nickel content

^c Added lead concentration

Snuff	Cd	Cd			Pb	
	$\overline{x} \pm ts/\sqrt{n}^{\mathrm{a}}$	$\mu g/10 \ g^b$	$\overline{x} \pm ts/\sqrt{n}^{\mathrm{a}}$	$\mu g/10 g^b$	$\overline{x} \pm ts/\sqrt{n}^{\mathrm{a}}$	$\mu g/10 \ g^b$
Moist snuff						
BM1 ^d	1.61 ± 0.23	13.5–18.5 ^c	12.2±0.72	111-127	12.6±0.26	119–129
BM2	1.04 ± 0.25	8.72-11.9	13.2±0.51	124-138	15.2±0.48	144–157
BM3	1.85 ± 0.14	16.9-20.3	13.5±0.61	129-141	14.8 ± 0.42	143-153
BM4	1.03 ± 0.16	8.65-11.6	11.9±0.31	114-123	12.1±0.38	117-126
BM5	1.32 ± 0.21	10.1-15.8	12.5±0.32	118-127	14.5±0.77	139–153
BM6	$1.05 {\pm} 0.26$	9.83-13.1	11.3 ± 0.72	107-119	10.9 ± 0.46	98.9–111
BM7	1.23 ± 0.24	9.78-13.9	11.9±1.2	109-133	9.33±0.62	84.6-98.5
GM8 ^e	0.77±0.12	7.46-8.01	8.03 ± 0.38	75.6-83.5	$9.89 {\pm} 0.30$	95.0-101
GM9	$0.874 {\pm} 0.16$	7.09-10.1	9.48±0.51	88.9-101	7.72±0.52	72.4-84.6
GM10	$0.825 {\pm} 0.24$	6.58-10.2	10.8 ± 0.38	105-113	8.55±0.79	74.6-92.4
GM11	0.925±0.18	7.23-12.1	10.7 ± 0.67	101-114	$7.46 {\pm} 0.17$	72.1-75.9
GM12	$0.746 {\pm} 0.14$	7.09-8.80	9.68±0.99	85.7-104	$8.46 {\pm} 0.68$	76.9–90.1
GM13	$0.815 {\pm} 0.15$	6.89-9.46	9.09±0.75	81.3-98.1	$7.89 {\pm} 0.75$	71.0-84.5
GM14	$0.824 {\pm} 0.13$	6.99-9.64	10.4 ± 0.99	92.9-113	$8.89 {\pm} 0.92$	78.7–99.4
Dry snuff						
$DB1^{f}$	1.13 ± 0.19	9.34-13.2	9.08±0.51	85.7-95.9	5.97±0.11	56.7-60.1
DB2	1.01 ± 0.17	8.51-11.4	9.81±0.38	93.1-105	4.72 ± 0.24	43.1-49.9
DB3	1.15 ± 0.28	8.69-14.3	9.54±0.68	88.1-101	5.73 ± 0.41	52.8-61.0
DB4	0.991 ± 0.25	7.41-12.1	9.71±0.83	84.9-103	5.91±0.12	58.0-60.9
DBK5 ^g	$0.632 {\pm} 0.24$	4.26-8.51	7.89 ± 0.72	70.1-85.4	6.24±0.23	60.2-65.3
DBK6	$0.741 {\pm} 0.28$	5.11-9.97	$8.29 {\pm} 0.86$	73.5-90.8	$7.37 {\pm} 0.16$	71.5-76.2
DBK7	$0.880 {\pm} 0.11$	7.69–9.87	8.69±0.51	81.0-92.9	8.42±0.12	82.3-86.2
DBK8	0.693 ± 0.14	5.49-8.41	$5.99 {\pm} 0.68$	52.9-65.7	7.21±0.25	67.8–74.9
DBK9	$0.645 {\pm} 0.12$	5.19-7.56	$6.58 {\pm} 0.83$	56.7-73.9	$8.23 {\pm} 0.13$	81.1-85.9

 Table 3
 Toxic metal concentrations in dry and moist snuff samples—intake of toxic metals by consuming 10 g of each snuff products (in micrograms per gram)

^a Average value \pm confidence interval (p=0.05)

^b Intake of toxic metals/10 g of each snuff products

- ^c Range
- ^d Brown moist
- e Green moist
- ^fDry brown
- g Dry black

The concentration of Pb in different snuff products was observed in increasing order as: BM>GM> DBK>DB.

The intra-product variation in all three toxic metals content was found to be higher in all snuff products. The resulted data of toxic metals in different snuff products are compared with the existing metal data on snuff products from different studies.

Discussion

The dried tobacco leaves are the major raw material for local snuff production. The tobacco grown in various geographical regions or under different agricultural conditions will have different levels of the heavy metals. So in tobacco product (snuff), significant variation in metal contents would be expected, because the levels of toxic metals in tobacco samples all over the world are not Fig. 2 Concentration of Cd in different types of brown and green moist snuff and brown and black dry snuff products (in micrograms per gram)



apparent (Asta et al. 2003). Though SLT is described as a group 1 carcinogen by the International Agency for Research on Cancer, little is known regarding bioavailability, absorption, and toxicological effects of toxic and carcinogenic inorganic substances from them.

Very little research has been carried out on the intake of toxic metals from different types of snuff by the population of all age groups in Asian countries including Pakistan. It was observed that people who consumed different types of snuff, inhaled about 1-2 g of snuff through the nose while 2–3 g are used between the gingival and buccal mucosa, both habits are repeated 2–15 times a day depending upon individual mood.

The results indicated that the levels of Cd, Ni, and Pb were found to be significantly higher (p < 0.01), in commercially available brands of brown moist snuff as compared with green moist snuff as shown in Table 3. The mean values of Cd, Ni, and Pb in different types of snuff are shown as Box plots (Figs. 2 and 3)

It is important to note that the different types of snuff are processed differently from the raw tobacco leaves and other additives. Moreover, different brands of snuff products are produced in different companies with their own processing type/formula. It is well established that tobacco plants selectively enrich some of the heavy metals from soil (Arain et al. 2008). The overall order of concentration of toxic metals determined in the present study in different types of snuff products are Pb>Ni>Cd.

The World Health Organization/Joint Expert Committee on Food Additives (WHO-JECFA) has established provisional maximum tolerable weekly intake of Cd, Ni, and Pb to be 3.5, 35, and 25 μ gkg⁻¹ body weightweek⁻¹, respectively (Baars et al. 2001; FAO/

Fig. 3 Concentration of Ni and Pb in different types of brown and green moist snuff and brown and black dry snuff products (in micrograms per gram)



Smokeless tobacco	Cd		Ni		Pb		References	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range		
Creamy snuff	0.07-0.15		_		0.13-0.59		Dhaware et al. (2009)	
Snuff	$0.48{\pm}0.08$	0.41-0.62	9.13±3.39	6.12-13.1	$3.10{\pm}0.81$	1.76-6.1	Verma et al. (2010)	
Ghanian snuff	1.08	1.06-1.11	_		_		Addo et al. (2008)	
Moist snuff	$1.40 {\pm} 0.31$	0.66-1.88	$2.28 {\pm} 0.36$	1.39-2.73	$0.45 {\pm} 0.13$	0.28-0.85	Pappas et al. (2008)	
Snuff	-		~3.0		~7.8		Shaikh et al. (2002)	
Snuff	_		2.1		8.0		Mishra and Shaikh (1986)	

Table 4 Metals concentration level in snuff products as reported in literature (in micrograms per gram)

WHO 1989, 1993; WHO 1989; Bhupander and Mukherjee 2011).

Table 3 shows the following: (1) the intake of toxic metals by consumption of different types of snuff products; (2) the intake of Cd via consumption of 10 g of different types of snuff product found in the range 8.65-20.3, 6.58-12.1, 7.41-14.3, and 4.26-9.97 µg day⁻¹ person⁻¹ for BM, GM, DB, and DBK respectively, contributing 14-68 % of the provisional maximum tolerable daily intake (PMTDI) for Cd in adults (60 kg); (3) the intake of Ni via 10 g consumption of each snuff product, BM, GM, DB, and DBK, corresponding to the range of 107-141, 75.6-114, 84.9-105, and 52.9-92.9 µgday⁻¹ person⁻¹, respectively, contributing 17– 47 % of the PMTDI for adults (60 kg); and (4) the Pb intake via 10 g consumption of different types of snuff product observed in the range of 84.6-157, 71-101, 43.1–61.0, and 60.2–86.2 μ gday⁻¹ person⁻¹ for BM, GM, DB, and DBK respectively, contributing 20-73 % of the PMTDI for Pb in adults (60 kg).

It is evident from Table 4 that Cd content in understudy snuff products, BM, GM, and DB samples are higher as compared with previous studies by Dhaware et al. (2009), Verma et al. (2010), and Addo et al. (2008) while the Cd contents are lower in DBK snuff products than values reported in previous studies by Pappas et al. (2008) and Addo et al. (2008). In our study, BM and GM have higher content of Ni as compared with that reported by previous works of Shaikh et al. (2002), Mishra and Shaikh (1986), and Pappas et al. (2008) while DB and DBK having a lower content of Ni is equal to the values of Ni reported by Verma et al. (2010).

Results show that the concentrations of Pb in BM, GM, and DBK samples are much higher and are indicated in previous studies by Mishra and Shaikh (1986), Shaikh et al. (2002), Dhaware et al. (2009), Verma et al. (2010), Addo et al. (2008), and Pappas et al. (2008) and Pb level is found to be lower in DB snuff than the values reported in previous research works by Mishra and Shaikh (1986) and Shaikh et al. (2002).

The results of the present study indicated a lifetime cancer risk from Cd, Pb, and Ni contents in snuff products. Greater incidence of oral cavity and esophagus cancers were reported in individuals using snuff. It is also pertinent to note that oral cancer attributably caused by the use of snuff and other SLT products accounts for 50 % of the total oral cancer cases reported in Pakistan. Similarly, the consumption of snuff has also been correlated with higher incidence of peptic ulcer disease.

Conclusions

Based on the present study on toxic metals, viz., Ni, Pb, and Cd in 23 samples of various brands of snuff product, all three toxic metals content were found to be higher in moist brown snuff than other types of snuff which are frequently consumed by people of every age in Pakistan. The level of toxic metals in snuff and its contribution to the daily intake was also established; the consumption of 10 g of different types of snuff samples provides 14-73 % of the provisional maximum tolerable daily intake for all three toxic metals in adults (60 kg). We consider that people consuming any type of snuff, especially those consuming more than 10 g/day, are at risk of developing ill health. Public health policy makers should seriously consider implementing policies to reduce or eliminate the consumption of snuff in populations where this practice is prevalent.

Conflict of interest statement The authors declare that there are no conflicts of interest.

References

- Addo, M. A., Gbadago, J. K., Affum, H. A., Adom, T., Ahmed, K., & Okley, G. M. (2008). Mineral profile of Ghanaian dried tobacco leaves and local snuff: a comparative study. *Journal* of *Radioanalytical and Nuclear Chemistry*, 277, 517–524.
- Altundag, H., & Tuzen, M. (2011). Comparison of dry, wet and microwave digestion methods for the multi element, determination in some dried fruit samples by ICP-OES. *Food* and Chemical Toxicology, 49, 2800–2807.
- Arain, M. B., Kazi, T. G., Jamali, M. K., Jalbani, N., Afridi, H. I., Kandhro, G. A., et al. (2008). Hazardous impact of toxic metals on tobacco leaves grown in contaminated soil by ultrasonic assisted pseudo-digestion: multivariate study. *Journal of Hazardous Material*, 155, 216–224.
- Asta, J., Guillard, E., Tissut, M., Gaude, T., & Ravanel, P. (2003). Heavy metal transfer from atmosphere to plants. *Journal of Physics*, 107, 65–67.
- Baars, R. J., Theelen, R. M. C., Janssen, P. J. C. M., Hesse, J. M., Apeldoorn, M. E., Van Meijerink, M. C. M., et al. (2001). *Re-evaluation of human-toxicological maximum permissible risk levels*. Bilthoven: National Institute for Public Health and the Environment.
- Bhupander, K., & Mukherjee, D. P. (2011). Assessment of human health risk for arsenic, copper, nickel, mercury and zinc in fish collected from tropical wetlands in India. *Advances in Life Science and Technology*, 2, 13–24.
- Bolinder, G. (1997). Overview of knowledge of health effects of smokeless tobacco. Increased risk of cardiovascular diseases and mortality because of snuff. *Loekartidningen*, 94, 3725–3731.
- Bouquot, J. E. (1991). Reviewing oral leukoplakia. Clinical concepts for the 1990s. *Journal of the American Dental* Association, 122, 80–82.
- CDC. (2000). Tobacco use among middle and high school students—United States. *Centers for Disease Control and Prevention*, 1999, 300–303.
- CDC. (2005). Tobacco use, access, and exposure to tobacco in media among middle and high school students—United States. *Centers for Disease Control and Prevention*, 2004, 297–301.
- Colak, H., Soylak, M., & Turkoglu, O. (2005). Determination of trace metal content of various herbal and fruit teas produced and marketed from Turkey. *Trace Elements and Electrolytes*, 22, 192–195.
- Demirel, S., Tuzen, M., Saracoglu, S., & Soylak, M. (2008). Evaluation of various digestion procedures for trace element contents of some food materials. *Journal of Hazardous Materials*, 152, 1020–1026.
- Dhaware, D., Deshpande, A., Khandekar, R. N., & Chowgule, R. (2009). Determination of toxic metals in Indian smokeless tobacco products. *The Scientific World Journal*, 9, 1140–1147.
- FAO/WHO (1989). Expert Committee on Food Additives: toxicological evaluation of certain food additives and contaminants.

33rd Meeting of the Joint FAO/WHO. Ser. 24. World Health Organization, Geneva.

- FAO/WHO (1993). Expert Committee on Food Additives: evaluation of certain food additives and contaminants. 41st Meeting of the Joint FAO/WHO. Ser. 837. World Health Organization, Geneva.
- FTC (2001). Report to Congress for the years 1998 and 1999, Federal Trade Commission.
- FTC. (2003). FTC report to congress shows increases in smokeless tobacco revenues and advertising and promotional expenditures. Washington, DC: Federal Trade Commission.
- Hecht, S. S., & Hoffmann, D. (1988). Tobacco-specific nitrosamines, an important group of carcinogens in tobacco and tobacco smoke. *Carcinogenesis*, 9, 875–884.
- IARC. (1985). Tobacco habits other than smoking: betel-quid and areca-nut chewing; and some related nitrosamines. Lyon: International Agency for Research on Cancer.
- IARC. (2006a). Monographs on the overall evaluations of carcinogenicity to humans (pp. 1–95). Lyon: International Agency for Research on Cancer.
- IARC. (2006b). Monographs on the evaluation of carcinogenic risks to humans, inorganic and organic lead compounds (p. 87). Lyon: International Agency for Research on Cancer.
- Substance Abuse and Mental Health Services Administration (2006). Results from the 2005 National Survey on Drug Use and Health: Detailed Tables. Office of Applied Studies.
- Miccadei, S., & Floridi, A. (1993). Sites of inhibition of mitochondrial electron transport by cadmium. *Chemico-biological interactions*, 89, 156–167.
- Mishra, U. C., & Shaikh, G. N. (1986). Simultaneous multielement determination of chewing and snuff tobaccos used in India by INAA. *Journal of Radioanalytical and Nuclear Chemistry*, 98, 297–301.
- Muir, C. S., & Zaridze, D. G. (1986). Smokeless tobacco and cancer: an overview. In: Tobacco a major international health hazard. *International Agency for Research on Cancer Scientific Publication*, 74, 35–44.
- Narin, I., & Soylak, M. (1999). Monitoring trace metal levels in Nigde, Turkey: nickel, copper, manganese, cadmium and cobalt contents of the street dust samples. *Trace Elements* and Electrolytes, 16, 99–103.
- National Institute of Public Health (2000). 11th World Conference on Tobacco or Health.
- Pappas, R. S., Stanfill, S. B., Watson, C. H., & Ashley, D. L. (2008). Analysis of toxic metals in commercial moist snuff and Alaskan Iqmik. *Journal of Analytical Toxicology*, 32, 281–291.
- Saracoglu, S., Tuzen, M., & Soylak, M. (2009). Evaluation of trace element contents of dried apricot samples from Turkey. *Journal of Hazardous Materials*, 167, 647–652.
- Schildt, E. B., Eriksson, M., Hardell, L., & Magnuson, A. (1998). Oral snuff, smoking habits and alcohol consumption in relation to oral cancer in a Swedish case-control study. *International Journal of Cancer*, 77, 341–346.
- Schroeder, K. L., & Chen, M. S., Jr. (1985). Smokeless tobacco and blood pressure. New England Journal of Medicine, 312, 919.
- Sesli, E., Tuzen, M., & Soylak, M. (2008). Evaluation of trace metal contents of some wild edible mushrooms from Black sea region, Turkey. *Journal of Hazardous Materials*, 160, 462–467.

- Shaikh, A. N., Negi, B. S., & Sadasivan, S. (2002). Characterization of Indian cigarette tobacco and its smoke aerosol by nuclear and allied techniques. *Journal of Radioanalytical and Nuclear Chemistry*, 253, 231–234.
- Silici, S., Uluozlu, O. D., Tuzen, M., & Soylak, M. (2008). Assessment of trace element levels in Rhododendron honeys of Black Sea Region, Turkey. *Journal of Hazardous Materials*, 156, 612–618.
- Soylak, M., Elci, L., & Dogan, M. (1993). Determination of some trace metals in dialysis solutions by atomic absorption spectrometry after preconcentration. *Analytical Letters*, 26, 1997– 2007.
- Soylak, M., Tuzen, M., Souza, A. S., Korn, M. G. A., & Ferreira, S. L. C. (2007). Optimization of microwave assisted digestion procedure for the determination of zinc, copper and nickel in tea samples employing flame atomic absorption spectrometry. *Journal of Hazardous Materials*, 149, 264–268.
- Stepanov, I., & Hecht, S. S. (2005). Tobacco-specific nitrosamines and their pyridine-N-glucuronides in the urine of smokers and smokeless tobacco users. *Cancer Epidemiology Biomarkers* and Prevention, 14, 885–891.
- Stohs, S. J., & Bagchi, D. (1995). Oxidative mechanisms in the toxicity of metal ions. *Free radical biology and medicine*, 18, 321–336.

- Uluozlu, O. D., Tuzen, M., Mendil, D., & Soylak, M. (2009). Assessment of trace element contents of chicken products from turkey. *Journal of Hazardous Materials*, 163, 982– 987.
- US Department of Health and Human Services (1986). *Health* consequences of using smokeless tobacco: a report of the Advisory Committee to the Surgeon General. Bethesda, Maryland.
- Verma, S., Yadav, S., & Singh, I. (2010). Trace metal concentration in different Indian tobacco products and related health implications. *Food and Chemical Toxicology*, 48, 2291–2297.
- Verougstraete, V., Lison, D., & Hotz, P. (2003). Cadmium, lung and prostate cancer: a systematic review of recent epidemiological data. *Journal of Toxicology and Environmental Health, Part B: Critical Review, 6*, 227–255.
- Vijayan, V. K., & Kumar, R. (2005). Tobacco cessation in India. The Indian Journal of Chest Disease and Allied Sciences, 47, 5–8.
- WHO. (1988). *Smokeless tobacco control*. Geneva: World Health Organization.
- WHO (1989). Toxicological evaluations of certain food additives and contaminants: arsenic. 33 Report of the JEFCA, World Health Organization WHO food additive series.