

Presence of the heavy metal lead in samples of tobacco (*Nicotiana tabaccum*) snuff in Nigeria

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Abstract

Background: Tobacco snuff is a finely ground powered substance obtained by crushing the leaves of the plant *Nicotiana tabaccum*. It is sometimes contaminated with some heavy metals such as lead, which can accumulate in the body following prolonged use, resulting in adverse health conditions.

Objective: To evaluate for the presence of the heavy metal lead in different samples of tobacco snuff obtained from various geopolitical zones of Nigeria, to compare it with the imported samples, and to assess their conformation to permissible limits by the Food and Agricultural Organization/World Health Organization (FAO/WHO) recommendation.

Materials and Methods: The basket protocol sample collection method was used to collect 30 samples of tobacco snuff obtained from various geopolitical zones of Nigeria. The wet acid digestion method was used to determine the lead (Pb) content of each sample, using the atomic absorption spectrophotometer.

Result: The study showed that 25 (83%) of the samples exceeded the FAO/WHO lead violation level whereas 5 (17%) were below the limit. Also, 27 (90%) of the samples were found to exceed the daily permissible consumption levels of lead when compared to the provisional tolerable intake levels determined by the FAO/WHO. Besides, the foreign tobacco samples were found to contain more lead than the locally made (Nigeria) samples.

Conclusion: The study showed that samples of tobacco snuff obtained from various parts of Nigeria were contaminated with the heavy metal lead. This constitutes a major health risk to the users as prolonged usage could result in lead toxicity that can be life threatening.

KEY WORDS: Presence, lead, tobacco snuff, Nigeria

Introduction

Tobacco snuff is one of the varieties of tobacco products that are not smoked (smokeless tobacco). It consists of finely

ground leaves of the tobacco plant (*Nicotiana tabaccum*), which is prepared into dry or moist forms, and is sometimes packaged in ready-to-use pouches. Dry snuff is usually sniffed or swallowed, whereas moist snuff is placed between the gum and the lips or cheek where it is slowly dissolved and absorbed.^[1]

Tobacco snuff contains more than 19 known carcinogens and at least 30 metallic compounds comprising some heavy metals such as lead (Pb), chromium (Cr), nickel (Ni), cadmium (Cd), selenium (Se), and mercury (Hg).^[2] Tobacco is a rich source of these heavy metals because they get preferentially absorbed by the leaves of tobacco during plant growth.^[3]

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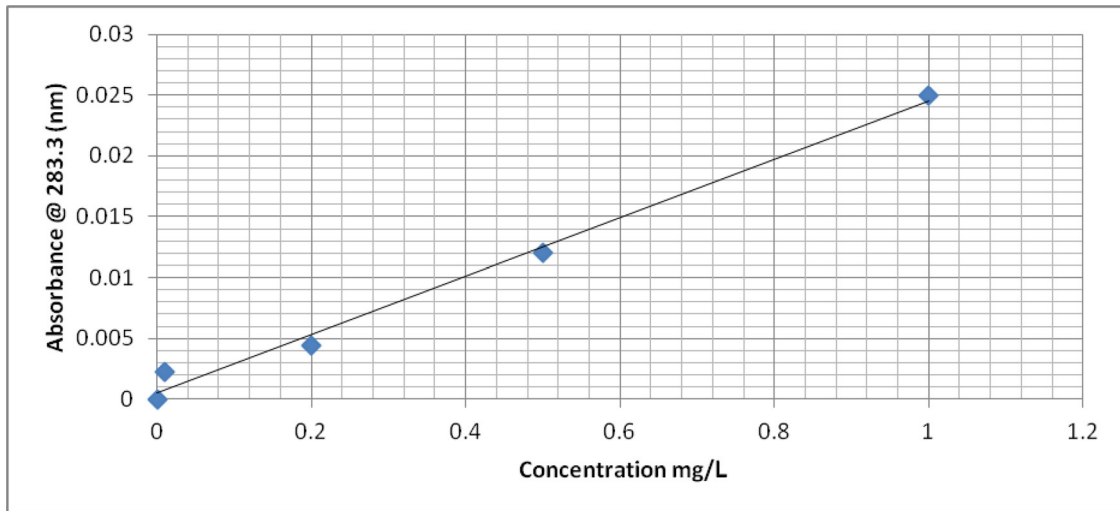


Figure 1: AAS calibration curve for lead concentration (mg/L) against absorbance at 283.3 nm.

Harmful effects on human health are associated with prolonged exposure to these heavy metals.^[4] Lead poisoning results in the interference with a variety of body processes, and lead is toxic to many organs and tissues including the heart, bones, intestines, kidneys, and reproductive and nervous systems.^[5,6] It is also unsafe in pregnancy.^[7]

Symptoms of lead poisoning include abdominal pain, confusion, headache, anemia, irritability, kidney failure, and in severe cases seizures, coma, and death.^[8–10] Children can inhale tobacco snuff indirectly from parent users or as it is being prepared leading to toxicity over a prolonged period. Lead is particularly toxic to children, causing potentially permanent learning and behavioral disorders.^[11]

This study was specifically directed at evaluating the presence of the heavy metal lead in samples of tobacco (*N. tabaccum*) snuff in Nigeria.

Materials and Methods

Study location: The study was conducted at the Department of Pharmacy, Madonna University, Elele Campus, Rivers State, Nigeria, from January to July 2013. **Materials/apparatus:** Petri dish, digestion bottle, test tube, beaker, filter paper, separator funnel. **Chemicals:** De-ionized water, dilute 0.1-M nitric acid, distilled water, standard solution of lead. **Equipment/instruments (atomic absorption spectrophotometer [AAS]):** Electronic weighing balance (model 2610; Ohaus), centrifuge, atomic absorption spectrophotometer machine (SP 2900 model; Pye Unicam) [Figure 1].

Sample Collection

Thirty samples of tobacco snuff were obtained from five geopolitical areas in Nigeria. The date of purchase, name of

manufacturer, method of preparation, and place of purchase were all noted. Different brands were collected from each area. Samples of the same brand were mixed together to obtain a representative sample. Brand names were not disclosed because of legal requirement. The breakdown of sample, geographical area, and type are shown in Table 1.

Sample Pretreatment

Two grams of the dry snuff sample was weighed using an electrical weighing balance for specific analysis and pretreated as per standard procedure for different metals. This was done for each of the 30 samples.

Wet Acid Digestion Method

A known quantity of sample (2.0 g) was transferred into a digestion bottle. The sample was wet digested with 50 mL dilute nitric acid. This enhances the extraction of the metals in the tobacco snuff. The oven was set at a temperature of 110°C. The digested sample was kept in the oven for 2 h.

After removal from the oven, the digested sample was filtered using a filter paper to ensure that the residue obtained after digestion is free from organic matter, which otherwise acts as impurities during the metal analysis.

After filtration, the filtrate was further analyzed using AAS.

Sample Analysis

The filtrate obtained was further analyzed to detect the concentration of heavy metals using AAS. This is sensitive for the determination of heavy metals at low concentrations. It is based on the fact of absorption of radiation of definite wavelength characteristic of the element. Its high sensitivity is exemplified by the fact that most metals can be determined at the part per million and part per billion concentration level.

Table 1: Breakdown of sample geographical area

Sample	Geographical area	Town	Type/name
1	North central	Babangida Market, Suleja	Foreign snuff
2	North central	Babangida Market, Suleja	Medicated
3	North central	Kuje Market, Abuja	Local
4	Southwest	Iseyin Market, Oyo	Local
5	Southwest	Iseyin Market, Oyo	Local
6	Southwest	Iseyin Market, Oyo	Local
7	Southwest	Iseyin Market, Oyo	Local
8	Southwest	Agbeni Market, Ibadan	Local
9	Southwest	Agbeni Market, Ibadan	Local
10	Southwest	Illeshiro Market, Ibadan	Local
11	Southwest	Illeshiro Market, Ibadan	Local
12	Southeast	Main Market Onitsha	UAC & Campbell foreign
13	Southeast	Main Market Onitsha	Foreign
14	Southeast	Main Market Onitsha	Local
15	Southeast	Main Market Onitsha	Campbell, KTC, & Maxwell (foreign)
16	Southeast	Main Market Onitsha	KTC (foreign)
17	Southeast	Main Market Onitsha	Broken (local)
18	Southeast	Main Market Onitsha	Medicated
19	Southeast	Main Market Onitsha	Maxwell (foreign)
20	Northwest	Aitiken Road, Sabon Gari Kano	Local
21	Northwest	Yoruba Road, Sabon Gari Kano	Local
22	Northwest	Yoruba road, Sabon Gari Kano	Medicated
23	Southeast	Ochanga Market, Onitsha	Medicated
24	Southeast	Ochanga Market, Onitsha	Local
25	Southeast	Ahia Ohuru Market, Aba	Local
26	Southeast	Ahia Ohuru Market, Aba	Medicated
27	Southeast	Umuahia Market	Local
28	Southeast	Umuahia Market	Broken
29	Southeast	King's Palace, Aba	Local
30	Southeast	King's Palace, Aba	Medicated

Atomic Absorption Spectroscopy

Atomic absorption spectroscopy is used principally in limit test for metals in drugs before their incorporation into formulation. The sample is generally dissolved in 0.1 M nitric acid to avoid formation of metal hydroxides from heavy metals, which are relatively volatile and suppress the AAS reading.

In atomic absorption spectroscopy, the metal atoms are volatilized in a flame and radiation is passed through the flame. The volatilized atoms that are mainly in their ground state and not emitting energy will absorb radiation with an energy corresponding to the difference between their ground state and excited state.

Instrumentation of AAS

An AAS consists of the following components that enable effective analysis of heavy metals:

- Light source:* This is made up of a hollow cathode lamp coated with the element being analyzed.
- Flame:* This is usually air/acetylene providing a temperature of 250°C; nitrous oxides/acetylene may be used to produce temperatures up to 300°C, which

are required to volatilize salts of elements such as aluminum or calcium.

- Monochromator:* This is used to narrow down the width of the band of radiation being examined and is thus set to monitor the wavelength being emitted by the hollow cathode lamp. This cuts out interference by radiation emitted from the flame from the filter gas in the hollow cathode lamp and from other elements in the sample.

- Detector:* This is a photosensitive cell.

Data Analysis

Daily Intake

The daily intake was calculated using the following equation of Dhaware *et al.*^[9]:

$$DI (\mu\text{g/day}) = C_{\text{metal}} \times W_{\text{analyses}} \times D_{\text{intake}}$$

where C_{metal} is the metal concentration in tobacco snuff sample (TSS) taken for analysis (in $\mu\text{g/g}$), W_{analyses} are the weight of TSS taken for analysis (2.0 g in this study), and D_{intake} is the daily intake (assuming 10 pouches per day, 5.0 g/pouch).

Table 2: Concentrations of lead in each of the tobacco snuff samples by geopolitical zone

Geopolitical zone	Sample	Lead (µg/g)	Mean ± SD	F-test/p-value
North central	TSS 1	1.4125	1.013 + 0.4	F = 2.91 p < 0.05
	TSS 2	1.0612		
	TSS 3	0.4536		
	TSS 4	1.1252		
Southwest	TSS 5	0.6349	1.11 + 0.69	
	TSS 6	1.0882		
	TSS 7	0.4536		
	TSS 8	1.1720		
	TSS 9	0.5974		
	TSS 10	2.4770		
	TSS 11	1.3421		
Southeast	TSS 12	0.6942	0.58 + 0.52	
	TSS 13	0.4893		
	TSS 14	1.2571		
	TSS 15	0.6529		
	TSS 16	1.3694		
	TSS 17	0.7241		
	TSS 18	0.5059		
	TSS 19	1.0972		
	TSS 23	0.8053		
	TSS 24	0.0291		
	TSS 25	0.0072		
Northeast	TSS 20	1.1610	0.43 + 0.4	
	TSS 21	0.0219		
	TSS 22	0.1067		

Table 3: Daily intake and percentage violation of each TSS from the WHO/FAO standard

Sample	Pb (µg/kg/day)	Percentage violation from the WHO/FAO standard (@ 5 µg/kg/day)
TSS 1	141.0	2820
TSS 2	106.0	2120
TSS 3	45.0	900
TSS 4	113.0	2260
TSS 5	63.0	1260
TSS 6	109.0	2180
TSS 7	45.0	900
TSS 8	117.0	2340
TSS 9	60.0	1200
TSS 10	248.0	4960
TSS 11	134.0	2680
TSS 12	69.0	1380
TSS 13	49.0	980
TSS 14	126.0	2520
TSS 15	65.0	1300
TSS 16	137.0	2740
TSS 17	72.0	1440
TSS 18	51.0	1020
TSS 19	110.0	2200
TSS 20	116.0	2320
TSS 21	2.0	40
TSS 22	11.0	220
TSS 23	81.0	1620
TSS 24	3.0	60
TSS 25	1.0	20
TSS 26	1.0	20
TSS 27	0.5	10
TSS 28	12.0	240
TSS 29	7.0	140
TSS 30	148.0	2960

Target Hazard Quotient

For the assessment of health risks arising from the indirect intake of heavy metals through the consumption of TSSs, target hazard quotient (THQ) was calculated in accordance to the methodology described by the USEPA.^[12] Target Hazard Quotient is one of the methods of estimating risks based on the noncarcinogenic effects of the toxicant and the reference dose.^[4] THQ was determined based on the formula (modified) given by Chien *et al.*^[4]:

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

where EFr is the exposure frequency = 312 days/year, equivalent to average lifetime; TSIR is the tobacco snuff ingestion rate = 50.0 g/day, equivalent to 10 pouches/day, 50.0 g/pouch of TSS; C is the concentration of metal in TSS in µg/g; RfD_o is the oral reference dose in mg/kg/day; BW_a is the average body weight, adult = 60 kg; AT_n is the average exposure time for noncarcinogens in days (EFr(312 days/year) × ED_{tot} (number of exposure years, assuming 70 years in this study); and 10⁻³ is the unit of conversion.

Result

Table 2 shows the comparison of concentration of lead in each sample collected from the four geopolitical zones of purchase. The daily intakes based on the different geographical areas from the table show variation in lead concentration. Southwest region shows the highest mean lead concentration in snuff samples, followed by north central, southeast. Northwest shows the least concentration of lead. This difference in mean lead concentration in the four zones is statistically significant (p < 0.05).

Table 3 shows the levels of the metal intake through the daily consumption of various TSSs and the percentage Food and Agricultural Organization/World Health Organization (FAO/WHO) violation. The daily intakes were calculated based on the consumption of 10 pouches per day. The various daily concentrations of lead in each of the samples were compared to that of the FAO/WHO standard, which is 5 µg/kg/day. The table shows that all the TSSs exceeded the permissible limit by the WHO except for TSSs 21, 24, 25, 26,

Table 4: Permissible intake levels as per the FAO/WHO recommendations

Metal	Provisional tolerable weekly intake ($\mu\text{g}/\text{kg}/\text{week}$)	Per day intake ($\mu\text{g}/\text{kg}/\text{day}$)	For a 60-kg individual ($\mu\text{g}/\text{day}$)	Reference
Pb	25.0	5.0	300.0	FAO/WHO

Table 5: Target hazard quotient for lead from consumption of TSSs

TSS	Frequency	THQ
1, 16	2	0.0008
2, 4, 6, 19, 20	5	0.0006
3, 7, 9, 13, 18	5	0.0003
5, 12, 15, 17	4	0.0004
8, 11, 14	3	0.0007
10	1	0.0014
21, 24–27, 29	6	0.0000
22, 28	2	0.0001
23	1	0.0005
30	1	0.2167

and 27, which had concentrations of lead below the permissible limit stipulated by the FAO/WHO.

Table 4 gives the daily intake of the assayed metal, compared with the provisional tolerable weekly intake and the proposed maximum permissible level suggested by the FAO/WHO.

Table 5 shows the results of THQ calculations to assess the potential health risk in the consumption of the TSSs. These THQs were calculated using the oral reference

doses ($\text{mg}/\text{kg}/\text{day}$) (Pb – 1.5) of the metal as stipulated by the United State Environmental Protection Agency (USEPA).^[12]

The mean concentration of lead from the chart shows that the foreign made products had higher concentration of lead when compared to the Nigerian or locally made products [Figure 2].

Ethical Concentration

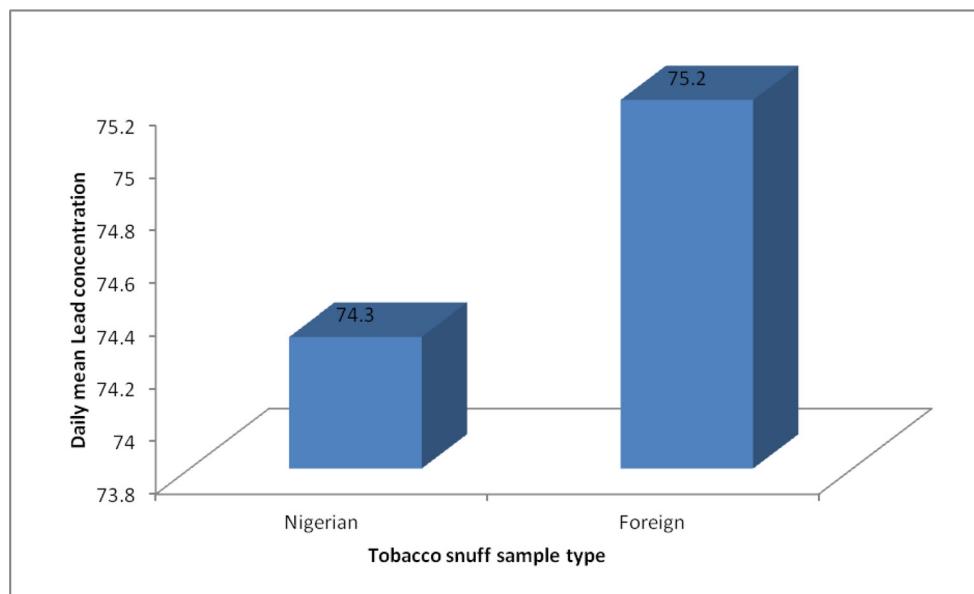
Permission was obtained from the ethics and research committee of Madonna University, Elele Campus, Rivers State, Nigeria, before the study was conducted.

Discussion

The study showed that the levels of lead in 27 of the 30 samples exceeded the daily recommended limit of $5 \mu\text{g}/\text{day}$ set by the FAO/WHO.^[13] Thus, a continuous intake of snuff could lead to bioaccumulation of this heavy metal, with the resulting negative health implications.

With respect to the individual percentage violation limit, all the samples except samples 21 and 24–27 exceeded the FAO/WHO violation limit. The concentration of lead in the TSSs in Nigeria ranged from 0.41 to $1.13 \mu\text{g}/\text{g}$ for north central, 0.45 to $2.48 \mu\text{g}/\text{g}$ for southwest, 0.01 to $1.48 \mu\text{g}/\text{g}$ for southeast, and 0.02 to 1.16 for northwest. This showed that the highest concentration of the heavy metal lead was found in the samples obtained from southwest Nigeria ($0.45\text{--}2.48 \mu\text{g}/\text{g}$).

Lagos, Ibadan, and Sango Otta, which are some of the major industrial hub of the country, lie in the southwest Nigeria. A lot of land pollution from industrial waste and effluents released by paint, cement, cosmetic, and other factories may have been the reason for this. Heavy metals

**Figure 2:** Daily mean concentration of lead ($\mu\text{g}/\text{kg}/\text{day}$) in Nigerian and foreign TSSs.

in the polluted soils are easily absorbed and retained by the leaves of *N. tabaccum*.^[3] Incidentally, the Nigeria Tobacco company, the major producer of tobacco products in Nigeria, is located in the southwest.

The THQ for each of the sample was less than 1 (<1.0), which makes them nonhazardous as per the USEPA.^[12] However, the level of lead violated the permissible levels of the FAO/WHO. Cumulatively, the THQ might be exceeded considering the fact that tobacco snuff is used chronically, thus leading to adverse effects on health.^[14]

The study also showed that all the foreign TSSs (1, 2, 12, 13, 15, 16, and 18) exceeded violation limit for lead recommended by the FAO/WHO, in contrast to some of the locally produced TSSs (20, 22, and 23–27), which did not exceed the violation limit. This showed that foreign tobacco snuffs were more contaminated with lead and indeed other heavy metals than the locally made snuffs. Foreign countries are more industrialized than Nigeria, hence the tendency for their soils to be more heavily contaminated with chemical pollutants, which are then absorbed by the leaves of tobacco plant in the farms.^[14]

Also, crop farmers in foreign countries have more access to fertilizers and pesticides than Nigeria farmers, hence the tendency for their tobacco plants to be exposed more easily to lead and other heavy metals that are thereafter retained in the leaves, which will undergo eventual processing to snuff and other tobacco product.^[14,15]

Conclusion

The study showed varying levels of the heavy metal lead in different brands of tobacco snuff obtained from various geopolitical zones of Nigeria. This evaluation shows that the majority of the tobacco snuffs in Nigeria is highly contaminated with lead and therefore constitutes major health risk to the local population. It also showed that foreign tobacco snuffs have a relatively higher concentration of lead than the local ones. This means a more profound adverse health effects on our population as our people often seem to prefer foreign goods to the locally made ones, including snuff.

Recommendation

The Federal Ministry of Health (FMOH) should regulate further with a stricter measure the production and use of all tobacco products in Nigeria. Also, health-care workers should enlighten their patients and the general populace on the obvious dangers inherent in the use of tobacco snuffs.

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