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Assessment of Heavy Metals in Common Smokeless Tobacco Products From North Maharashtra Region: An Analytical Study

[1]Dr. Divya Ghune, Post Graduate Student, Department of Public Health Dentistry, JMF's ACPM Dental College, Dhule, Maharashtra

[2]Dr. Prashanth VK, Professor, Department of Public Health Dentistry, JMF's ACPM Dental College, Dhule, Maharashtra

[3]Dr. Arun Dodamani, HOD, Department of Public Health Dentistry, JMF's ACPM Dental College, Dhule, Maharashtra

[4]Dr. Snehal Chintale, Post Graduate Student, Department of Public Health Dentistry, JMF's ACPM Dental College, Dhule, Maharashtra

[5]Dr. Sujata Chhabile, Post Graduate Student, Department of Public Health Dentistry, JMF's ACPM Dental College, Dhule, Maharashtra

[6]Dr. Snehal Patil, Post Graduate Student, Department of Public Health Dentistry, JMF's ACPM Dental College, Dhule, Maharashtra

[7]Dr. Swapnali Patil, Post Graduate Student, Department of Public Health Dentistry, JMF's ACPM Dental College, Dhule, Maharashtra

Corresponding Author: Dr. Divya Ghune, Post Graduate Student, Department of Public Health Dentistry, JMF's ACPM Dental College, Dhule, Maharashtra.

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Abstract

Introduction & Objectives: Pudi is an immorally vended smokeless tobacco product (STP) generally used by youths and teenagers in the North Maharashtra region. This study aimed to assess Pudi's composition, which is believed to contain numerous carcinogens and poisonous factors. In particular, Pudi's heavy metal content includes cadmium (Cd), Copper (Cu), lead (Pb), and nickel (Ni).

Methods: This study was conducted between September and October 2023. Two samples of *Pudi* and V-1 were first dried and also ground to form a homogenous mixture. The mixture was digested before the toxic metal analysis by inductively coupled plasma-mass spectrometry (ICPMS).

Results: Pudi and V-1 were shown to have high situations of all toxic metals (Cd, Cu, Pb, and Ni) which were detected in amounts above respectable

International limits. The estimated diurnal input of heavy metals from Pudi was also above the maximum admissible limit when compared with transnational norms.

Interpretation & Conclusions: The results of this study showed that Pudi and V-1 contain several toxic metals that may beget health problems. Thus, critical regulation of the illegal trade of Pudi is demanded in North Maharashtra along with a crusade to address public health education and mindfulness of Pudi and its health pitfalls.

Keywords: Cadmium, Heavy metals, Lead, Nickel, Smokeless tobacco

Introduction

Tobacco is the most fluently accessible, fairly available addicting substance which contributes significantly to death and long-term suffering and is also a major threat factor for cardiovascular conditions, obstructive pulmonary conditions, cancers, reproductive issues, and oral conditions.[1]

As per Global Adult Tobacco Survey- India(GATS2) 2 India is home to over 28.6 of all tobacco druggies and worldwide it's the second largest patron and consumer of tobacco products.[3] Available estimates in India show that tobacco smoking and smokeless tobacco(SLT) redounded in 930, 000, and 350,000, periodic deaths independently together counting for about deaths per time or roughly 3500 deaths every day.[4, 5] In addition, tobacco also impacts the profitable development of the country, and as per studies conducted by the Health Ministry, the total profitable costs attributable to tobacco use from all conditions and deaths in the time 2011 was INR 104,500 crores, which is a huge burden for a developing country like India.[6] Tobacco and several chemicals in tobacco suppress the exertion of different types of vulnerable cells that are involved in general and targeted vulnerable responses.[7]

Marketed SLTs vary vastly in form and other constituents, piecemeal from nicotine numerous dangerous constituents include heavy poisonous essence. Numerous scientific studies show a clinical association between heavy poisonous essence and its effect on health. Nearly 4000 chemicals of tobacco were classified into different groups according to their health hazard indicator.[14] The International Agency for Research on Cancer(IARC) has designated cadmium(Cd) and chromium (Cr)(VI) as group 1 carcinogenic to humans. also, lead(Pb) is an IARC Group 2 A probable mortal carcinogen.[15] Lead exposure is dangerous for the youngish age group, as habitual exposure results in the lowering of IQ, and its poisoning effect on the brain may not be reversible.[10, 11,12] Arsenic exposure can beget skin saturation and cancer problems, ulcerations of the mouth, low hemoglobin, leukemia, acute renal failure, seizures, and whim-whams damage [13] and it's also an implicit carcinogen.[14] inordinate boluses of Cd are known to beget lung and bone damage, increased blood pressure [15], and occasion of cardiovascular complaint.[16, 17,18]The influence of heavy essence being poisonous through tobacco was studied and established way back among smokers [21] After numerous attempts and efforts to discourage the smoking habit, it has been most prevalent in the North Maharashtra region since last many decades. North Maharashtra (Uttar Maharashtra) is a geographical region of Maharashtra State, India. The region is composed of Nashik, Dhule, Nandurbar, and Jalgaon districts.[23]

It borders the state of Gujarat to the northwest, Paschim Maharashtra to the south, Konkan to the west, and the Vidarbha and Marathwada regions of Maharashtra to the east. Nashik comprised (25.61%), Dhule (31.55%),

Nandurbar (69.3%), Jalgaon (14.28%), and Ahmednagar (8.32%) tribal populations which are involved in the manufacturing and processing of loose tobacco products which are locally known as 'Pudi'. These loose tobacco products have started to gain attention in the North Maharashtra region because of their low price, easy vacuity as well as lack of awareness about their troubles, especially among young people. They are sold in the local pan shops without proper labeling of manufacturing company, manufacturing date, expiry date, and warning signs. Another popular smokeless tobacco product after Pudi is V-1 which is a marketed Smokeless Tobacco Product brand.

As far as our knowledge, till now no studies are available regarding the levels of heavy metals in the loose form of SLT vended unlawfully in the North Maharashtra region. This handed the motivation to assess and compare the common heavy metals (Cd, Pb, Ni, Cu) among the most commonly available smokeless tobacco products in North Maharashtra by the ICP- MS system.

Methodology

The present study is an in vitro study conducted in the Department of Public Health Dentistry ACPM Dental College, Dhule, and Technischer Uberwachungsverein (TUV) India Pvt Ltd, Pune, Maharashtra. Institutional Ethical Committee (EC/ NEW/ INST/ 2022/ 2959/ 2022/ 047) Clearance was obtained before conducting the Study

Selection and coding of the sample

The samples were collected from different belts of the North Maharashtra Region. Tobacco was taken into consideration based on its popularity, and widespread use among the people. The study was conducted over for15 days.

Preparation of material for metal determination

2-3 packets of Pudi were unpacked and segregated to remove gross impurities using a magnifying glass and were ground to form a mixture of uniform-sized particles. The samples were weighed according to 100 mg of tobacco content and were sealed in air-tight plastic pouches with labeling. The samples were then transported to the study center in hygienic and moisture-proof conditions.

Processing

ICP-MS (inductively coupled plasma-mass-spectrometry) is a technique to determine low concentrations (range: ppb = parts per billion = µg/l) and ultra-low concentrations of elements (range: ptt = parts per trillion= ng/l). Atomic elements are lead through a plasma source where they become ionized Then, these ions are sorted on account of their mass. The advantages of the ICP-MS technique above AAS (Atomic Absorption Spectroscopy) or ICP-OES (inductively coupled plasma optical emission spectrometry) are:

- Extremely low detection limits
- A large linear range.

Possibilities to detect isotope composition of elements The ICP-MS technique has a multi-element character and a high sample throughput, like ICP-OES, but it allows one to perform more sensitive measurements.

Disadvantages and weaknesses of the ICP-MS detection are the occurrence of spectral and non-spectral interference and the high costs.

Apparatus

As for the ICP-OES, the sample solution is introduced into the device employing a peristaltic pump. There it becomes nebulized in a spray chamber. The resulting aerosol is injected into an argon plasma that has a temperature of 6000-8000 K. Inside the plasma torch,

the solution is removed from the sample and also atomization and ionization occur.

Only a small amount part of the ions produced in the plasma further penetrate the mass-spectrometer part.

Requirement

Reagent/reference materials

- Concentrated. Nitric Acid
- Hydrogen Peroxide (30 %)
- Suprapure/ Ultrapure grade Nitric acid
- Deionized Water
- 1000 µg/ml stock solution of Respective element standard
- ICP Standards Traceable to NIST

Apparatus

- Micropipette 0.1 to 1 ml & 0.02 to 0.2 ml
- Volumetric Flask-50 ml
- What man filter paper no. 41
- Plastic Beaker, funnel.

Equipment

- Microwave digester
- Weighing Balance (Range 0.1 mg to 200 gm)
- ICP-MS (AGILENT 7700)

Sample Preparation for Food Products (By Microwave Digestion)

- Weight 0.1 to 1.0 g of Homogenized sample for metal analysis on ICP-MS & ICP-OES. Transfer the Sample into a microwave digester tube.
- Add 6 ml Supraure grade conc. Nitric acid for sample preparation and 0.3 ml 30 % H2O2 solution add 7 ml deionized water and keep the microwave digester tube in the microwave digester.

Table 1: Microwave digester program for food samples.

Power	1500 W
Ramp	15 min
Hold	25 min
Temperature	170-degree celsius
Cooling	15 min

- After completion of the digestion remove the tube from the digester and cool it to room temperature.
- Observe the condition of the digested sample, it should be clear. If any presence of particulate matter is observed, then again add conc. Nitric acid and H2O2 solution and reediest sample.
- Transfer the clear digested solution to a volumetric flask and make volume with Deionized water.
- If any insoluble is observed, filter the solution through what man filter paper.
- This solution is ready for aspiration. Run Sample on ICP as per the working instructions.

Table 2: ICP-MS operating conditions.

Plasma (Ar) gas flow	15 L min^{-1}
Carrier (Ar) gas flow	0.8-1.0 L min^{-1}
Collision (He or H2) gas flow	4.5-5.0 L min^{-1}
S/C temp	2 degree celsius
Sampler and skimmer cons	Ni
Plasma Power	1500 W
Sample depth	6-8 min
Reflected power	Less than 20 W

Method Critical Points

- Polypropylene wares should be properly cleaned with acid to avoid cross-contamination.
- While volume makeup, care should be taken to avoid spillage of solution from the tube during transfer into the flask.
- After digestion, the solution should be clear and if any particulate matter is observed, then add conc. Nitric acid and H2O2 solution and re-digest sample.
- After re-digestion, if still, the solution is insoluble (generally in the case of silica), filter the solution through Whatman filter paper.

- If the aspirated concentration of the sample is higher than the linearity range, then dilute further to get the aspirated concentration in the linearity range. Triplication was done to maintain the accuracy and minimize the experimental error.

Result and Discussion

The concentrations of the heavy metals (Cr, Ni, Cd, and Pb) in 100mg of Pudi and V-1 are listed in Table 3 and Table 4. When tobacco consumers were informally asked about their Pudi and V-1 use, most of them stated that they consumed a minimum of five doses per day; however, use patterns are also dependent on the level of addiction and the needs of the user. Considering the assumption that the average pinch-sized dose of Pudi is 2 g, the minimum estimated daily intake of heavy metals in *Pudi* is shown in Table 5. These were compared with the permissible daily limits set by the WHO and FAO.[19]

Table 3: The concentrations of heavy metals in the tested Pudi Sample

Sn.	Heavy Metals	Results	Unit	LOQ	Test Method
Sample Name: Sample 1 (Pudi)				CA NO: 0102302084	
Discipline: Chemical				Product Category: Food and Agriculture	
1	Cadmium	0.49	mg/kg	0.1	Determined by ICP-MS
2	Lead	0.98	mg/kg	0.1	Determined by ICP-MS
3	Nickel	2.26	mg/kg	0.25	Determined by ICP-MS
4	Copper	9.92	mg/kg	0.25	Determined by ICP-MS

Cd = cadmium; Pb = lead;Ni = nickel;Cu = copper; LOQ = Limit of Quantification

Table 4: The concentrations of heavy metals in the tested V-1 Sample

Sn.	Heavy Metals	Results	Unit	LOQ	Test Method
Sample Name: Sample 2 (V-1)				CA NO: 0102302083	
Discipline: Chemical			Product Category: Food and Agriculture		
1	Cadmium	0.42	mg/kg	0.1	Determined by ICP-MS
2	Lead	0.97	mg/kg	0.1	Determined by ICP-MS
3	Nickel	3.31	mg/kg	0.25	Determined by ICP-MS
4	Copper	12.57	mg/kg	0.25	Determined by ICP-MS

Cd = cadmium; Pb = lead; Ni = nickel; Cu = copper; LOQ = Limit of Quantification

Table 5: Permissible daily intake limits for the human consumption of heavy metals according to the Food and Agriculture Organization and World Health Organization and the estimated minimum daily intake of metals in a single dose of the tested Pudi and V-1

Metal	Permissible daily intake limit* in µg/kg/day	The permissible Daily intake limit for a 60kg individual in µg/day	Estimated daily intake of metals in a single dose of tested Pudi per day in mg/kg	
			Pudi	V-1
Pb	5	300	0.98	0.97
Cd	0.4-2	60	0.49	0.42
Ni	0.2	12	2.26	3.31

Pb = lead; Cd = cadmium; Cr = chromium; Ni = nickel. *Source: Joint Food and Agriculture Organization and World Health Organization Expert Committee. Evaluation of certain food additives and contaminants. For this calculation, a pinch-sized single dose of Pudi and V-1 was considered to be 2 g.

Pudi consumption in the North Maharashtra Region has increased in the last two decades, especially among young people. This STP contains several heavy metals. Some of these toxic metals are carcinogenic.[18] Several studies have reported the presence of heavy metals in various types of STPs, similar to snuff and Indian smokeless tobacco.[21] To the authors' knowledge, this is the first analysis of its kind in India that is performed on illegally sold tobacco. The results of this study give veritably useful information about the attention of four toxic metals in Pudi and V-1. A comparison of the contents of different STPs from various regions may vary extensively due to the diversity of the tobacco shops, different processing and manufacturing ways and storage conditions, and the flavoring agents used.

In a similar study of wettish snuff and Alaskan iqmik conducted in the USA, the order in which the rudiments passed from topmost to least was as follows Ni(2.28 µg/ g), Cr(2.04 µg/ g), Cd(1.40 µg/ g) and Pb(0.45 µg/ g).[20] It seems that the STPs in this study from the USA have lower levels of toxic metals than the Pudi sample of the current study. These types of variations are to be anticipated due to the forenamed factory diversity or variations in processing criteria around the world. The results of the current study can be used to formulate an idea about the circular input of that essence by STP druggies. This was done by estimating their diurnal input and also assessing whether the circular input of toxic metals was harmonious with the admissible and respectable diurnal situations set by the WHO and the FAO.[19] Estimating the minimum levels of toxic metals in one cure of the named Pudi sample can help people understand the possible health pitfalls associated with using loose forms of tobacco, particularly in boluses lesser than the estimated input. Unfortunately, the factual volume of heavy essence ingested by a stoner may be more advanced than the minimal diurnal input estimated in this study, due to variations in the mix of Pudi as well as the stoner's existent preferences. A single cure of Pudi cannot be assigned a standard weight of 1 – 2 g like the tea-bag-shaped Swedish snus; each cure depends on the stoner's position of dependence. In

addition, the rate of birth of the essence from the STPs by the slaver will vary according to the existence. Still, the wettish form of the product, fortunately, has a lower toxic metal content than that set up in the dry samples anatomized. Nonetheless, the frequent use of this brand of STP allows the accumulation of nondegradable and dangerous rudiments in the stoner's body.[20] Likewise, banning other implicit sources of diurnal heavy essence input similar to environmental and salutary sources, frequent use of Pudi may put druggies at threat of exposure to combined essence. Heavy metals have implicit poisonous and carcinogenic goods and some of them can beget severe health problems, indeed in trace amounts.[17]

The present study has its limitations concerning the number of samples tested due to financial burden. We could not exactly estimate the exact form and nature of the heavy metals that were behind the objective of our study. Further studies are to be needed with larger samples and various brands available in the market with emphasis on the form of heavy metals like Nickel, whether it is volatile or particulate, whether it is inorganic or methylated, and whether it is bioavailable or not. The higher metal concentration coupled with addictive nicotine in various S LT products is a health risk when the toxicity potential from dietary, as well as other environmental exposure, is taken into consideration. Considering all these facts the authors feel that the STPs should be labeled with levels of toxic metals as per international guidelines and that their production and promotion should be adhering to the stricter government guidelines.

Conclusion

This study gives a better understanding of the levels of selected heavy metals in the most commonly consumed Loose tobacco product (Pudi) of the North Maharashtra Region. Unfortunately, the estimated daily intake of the tested metals exceeded the allowable safe limits recommended by the FAO and the WHO, rendering users at high risk of being poisoned or developing other undesirable side effects. It is therefore recommended that the sale of illegal STPs such as Pudi be urgently regulated in North Maharashtra. Furthermore, education programs aimed at the youth must be implemented to raise awareness of the health risks and dangers of using this product. There is an urgent need to reign in these products under mandatory quality control.

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