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A Review on Chemical Composition and Forensic Analysis of Tobacco and Ash in Cigarettes and Bidis

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Introduction:

With regard to tobacco products, India ranks second in the world for consumption, third in terms of production, and fifth in terms of exports (1). Smoke is released during cigarette combustion, leaving behind an ash column that contains chopped tobacco residue encased in cigarette paper ash. Thus, three key areas of cigarette combustion study may be identified: the combustion process, smoke, and ash (2). The tobacco plant, Nicotiana tabacum, may concentrate trace elements in its leaves by absorbing them from the soil. Numerous factors, including pesticides, soil pH, type, and genotype, affect the amount and presence of metal in tobacco (3). These days, there is no shortage of cigarette-related debris anywhere you look, even at crime scenes. Cigarette ash has not received much attention in forensics, despite the fact that cigarette butts—which reveal the brand name and may contain a smoker's DNA—have long been used in criminal investigations. Trace-metal concentrations contain information that could help identify the brand from which the ash originated, which could help identify suspects at crime scenes or estimate the number of persons who may have attended. However, not much work has been done to make use of this information (4).

In contrast to the cigarette industry, which is dominated by three corporations with a 94 percent market share, the SLT and bidis sector is somewhat more complex.

The bidi market is primarily unorganized; companies work with a huge number of unregulated, small-scale local producers that have a big number of insignificant home manufacturing units and are distributed by up to one million retailers (1).As we talk about smoking tobacco (Nicotiana tabacum L.), we usually mean inhaling and exhaling the smoke that is released as the plant material burns. There is a broad variety of tobacco products on the market anywhere in the world. Cigarettes, cigarillos, cigars, pipes, bidis, kreteks, and waterpipes (or hookah) are among the most popular choices. In many parts of the world, people have also traditionally used smokeless tobacco

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products as snuff, which is inhaled directly via the nose, or as chewing tobacco preparations, which involve inserting a wad in the mouth between the gums and cheeks (5).

Bidis were small, flavorless cigarettes that first appeared in India in 1905. Modern bidis are made of hand-rolled. Oriental tobacco flakes that have been sun-dried and covered in leaf material from ebony trees of to the family Ebenaceae Gu¨rke, such as Diospyros ebenum, Diospyros melanoxylon, Diospyros ebenaster, and Diospyros ismailii. Bidis marketing in tempting candy-like tastes (strawberry, grape, chocolate, clove, etc.) and vividly colored packs that improve visibility and product appeal are partly responsible for their attraction to young people in America (6). An essential first step in locating and gathering tangible evidence that might be connected to the crime is conducting a crime scene investigation. Physical evidence of an offense can be traced or minute, ranging in size from macroscopic to microscopic . In order to reconstruct the crime scene and identify the culprits, an investigating officer uses and analyzes various evidences, such as bodily fluids soil cosmetic stains , nail clippings , etc. Another sort of supporting evidence that may be discovered in trace amounts at any crime scene, including ones involving murder, suicide, rape, dacoity, or other assaults, is cigarette ash (7).

Any place that may be connected to a crime that has been committed and where forensic evidence may be obtained is referred to as a crime scene (8).
The process of investigating a crime scene involves documenting the scene as it is the initial point of contact, as well as identifying and gathering all physical evidence that may be useful in solving the case. Identification and retrieval of tangible evidence mark the start of the inquiry at the spot. Following the examination and development of the retrieved physical evidence, papers, and witness statements, all conclusions are presented to the court of law (9).

2. Crime Scene Investigation (CSI):

Any physical location where an offense has been committed and where an investigating officer may find evidence is referred to as a "crime scene." It typically comprises a structure, a car, a person's body, and items discovered there or outside. The term "crime scene examination" describes an investigation in which tangible evidence of a crime is gathered and preserved using scientific or forensic methods. Crime scenes can be reconstructed through the analysis and assessment of scene patterns and the physical evidence analysis (10).

2.1 Crime Scene Search Patterns:

The searching for the evidences can be performed in many ways, which entirely depends upon the nature of crime, size of crime scene, location of crime scene and other conditions. Though, the most popular choices fore searching of evidences applied by the investigating officers include: strip, wheel, zone, spiral, grid methods. After searching for the potential evidences, the IO collects those evidences and label them accurately with all the description mentioned on the evidence. Then, the evidence is forwarded to Forensic science Laboratories for further analysis.

3. Types of Tobacco Products:

The residue left over after tobacco is completely burned is called tobacco ash. It is found in amphorous steel gray powder that tastes somewhat salty. It reacts alkaline, is insoluble in water and alcohol, and is only partially soluble in diluted acid.

The following chemical compounds make up its composition: Potassium in K20 20–25 percent Phosphates, such as P205 3–5% CaO is the symbol for calcium. 25–30% MgO is the symbol for magnesium. 5–7% Na2O is sodium. around 1% Chlorine: around 2% Sulfates as SO: approximately 6% (11). Gujarat, Andhra Pradesh, Karnataka, Uttar Pradesh, Tamil Nadu, Bihar, and West Bengal are the main tobacco-producing states in India. Together, the states of Andhra Pradesh, Gujarat, Karnataka, and Uttar Pradesh produce about 90% of the nation's tobacco (12).

3.1 Bidis:

Indian and other Southeast Asian countries are the main importers of bidis, or "bee-dees," which are tiny, thin hand-rolled cigarettes. Tightened with a colorful string at one or both ends, they are made of tobacco wrapped in the leaves of tendu or temburni, which are native plants to Asia. Bidis comes in two flavors: unflavored and flavored (such chocolate, cherry, or mango). There is a correlation between bidding and smoking that increases the risk of heart attacks, coronary heart disease, lung, stomach, and esophageal cancer, as well as chronic bronchitis (13). As a result, bidi smoking is deeply rooted in Indian society, which has influenced attitudes toward smoking. Bidis are widely accessible and reasonably priced in India, especially for those with lower socioeconomic standing. They are also substantially less expensive than manufactured cigarettes (14).

3.2 Cigarettes:

A cigarette is a roll or stuffing of cured and finely chopped tobacco, reconstituted tobacco, and additional ingredients into a cylinder wrapped in paper. On one end of many cigarettes is a filter. Tobacco and tobacco smoke include almost 4,000 distinct compounds. There are about 60 compounds among them that are known to cause cancer (15). Filters made of cellulose acetate, which are intended to partially preserve particle smoke components, are found in over 97% of cigarettes . Nicotine, polycyclic aromatic hydrocarbons (PAHs), substances unique to the Solanaceae family, nicotiana alkaloids, and catechols are the main harmful substances (16).

3.3 Cigars, Cigarillos and Little Cigars

One variety of air-cured or dried tobacco is used to make the majority of cigars. Before being fermented in a series of steps that can take three to five months, cigar tobacco leaves are first matured for approximately a year. The tobacco is altered by fermentation due to bacterial and chemical processes. Cigars taste and smell different from cigars because of this. Conventional cigars lack a filter and are bigger than cigarettes. Filters, pipe tobacco, and little cigars, often known as cigarillos, are features that make them quite similar to cigarettes in size and shape. Flavored cigars, such as chocolate, cherry, apple, or mango, are common. Just like cigarettes, they can be purchased singly or in packs of twenty. Compared to cigarettes, cigars have a higher nicotine content. When a cigar smoker inhales, the nicotine enters their lungs much like it does when they smoke cigarettes. The nicotine is absorbed more gradually through the mouth lining in non-inhalers(17).

3.4 Kreteks:

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Some people refer to Kreteks—pronounced "cree-techs"—as clove cigarettes. Indonesian imports of kreteks are known for their mixing of tobacco, cloves, and additional ingredients. Similar to bidis, kreteks provide higher levels of nicotine, carbon monoxide, and tar than regular cigarettes, according to established machine-smoking assessments. Smoking kretek is linked to a higher risk of acute lung injury, particularly in those who are more vulnerable due to respiratory illnesses or asthma. Studies reveal that individuals who smoke regularly with Kretek have a 13–20 times higher chance of impaired lung function as compared to nonsmokers (18).

3.5 Hookah:

A hookah is a pipe used to smoke shisha, which is made of heated tobacco mixed with fruit or vegetable ash, which is then filtered through water. The components of the hookah are the head, body water bowl, and hose. In a hookah, charcoal is typically used to heat the tobacco or shisha. An hour of hookah smoking can expose a user to 100–200 times the amount of smoke that one cigarette produces, according to a World Health Organization guideline. Tobacco smoke still has a lot of harmful substances in it, such as heavy metals, carbon monoxide, and chemicals that cause cancer (carcinogens), even after it has been through water. Significant amounts of nicotine, the addictive ingredient in tobacco, are also released when smoking a hookah (19)

4. Constituents present in Cigarette and Bidi:

4.1 Organic Composition of the Unburnt Flavoured Cigarettes and Bidis :

The component of the filler and wrapper material that is actually consumed while smoking is called the burnable portion. For this study, Gas Chromatography- Mass Spectrometer (GC-MS) coupled with Selected Ion Mode (Sim) was used to identify the chemical constituents present in the fillers of cigarette. SPME was used to inject the sample in the sample headspace. High concentrations of compounds associated to flavor can be found in the tobacco and leaf wrapper of the entire bidi. Azad regular featured just menthol out of the two unflavored bidi brands [51.0-192 µg/cigarette (µg/cig)]. and Gold Kishen had no analytes in it. All of the flavored bidi brands, even the ones

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without menthol, included menthol. All five of the clove-flavored bidi products included eugenol, the primary flavor-related component of cloves (20).

The brand of bidi that had the most eugenol was Azad clove bidi. The amount of eugenol in clove-flavored bidis differed considerably between brands and within a single brand of cigarettes. The maximum value for a single cigarette under the Azad clove brand was 14% higher than the mean value for that brand, but the maximum level for the Shiv Clove brand was 45% higher than the mean level. The majority of the clove-flavored bidis also contained anethole, myristicin, and elemicin in addition to eugenol, indicating common flavoring methods. These chemicals (eugenol, anethole, myristicin, and elemicin) were rarely found in any brand other than the clove-flavored bidis, with the exception of the herbal bidi that included numerous target analytes. The herbal bidi did not contain tobacco, but it did contain significant concentrations of a number of flavor-related chemicals that are also present in bidis that contain tobacco. Azad herbal bidis had the highest concentrations of anethole, methyleugenol, myristicin, elemicin, estragole, and safrole of all the bidi brands (20).

Several chemicals found in the grape-flavored bidi were absent from the other items examined in the study. While cis-EMPG was present in significantly smaller amounts, some grape bidis had milligrams of methyl anthranilate and diphenyl ether per cigarette. Since this was the only grape-flavored bidi that was tested, we are unsure if these substances are also present in other grape-flavored bidi products. Bidis flavored with menthol and cinnamon included a restricted quantity of the analytes that we had chosen. All of them included menthol, however we only found pulegone and eugenol as other flavor-related chemicals. Contrarily, a variety of flavor-related chemicals were present in clove, herbal, and grape bidis (20).

Table 4.1 Amount of Flavor-Related Compounds Detected in the Burnable Portion of 10 Flavored Bidi Cigarette and 3 U.S. Cigarette Brands

	analyte range, burnable portion[a] (μg/cig)								
	anethole	eugenol	methyl-eugenol	safrole	myristicin	elemicin	menthol	methyl anthranilate	diphenyl ether
LOD (μg)	20.8	42.9	6.3	1.8	4.1	1.2	32.5	27.0	10.9
bidis									
Kailas clove[b]	293–647	148–560	ND[c]	ND	ND–18.2	ND–7.35	186–403	ND	ND
Shiv clove	ND	984–2790	ND	ND	4.36–26.0	ND–8.24	35.3–80.8	ND	ND
Irie clove	40.3–94.7	1230–3230	ND	ND	6.13–10.3	1.76–2.68	68.8–101	ND	ND
Darshan clove	73.1–217	2390–4000	ND	ND	ND	ND	50.7–95.3	ND	ND
Azad clove	ND–43.3	6210–7130	ND–7.52	ND	4.98–34.7	1.47–12.8	58.3–260	ND	ND
Azad herbal[d]	489–665	1670–2470	27.7–36.6	32.4–34.4 (2)	170–247	101–109	758–1380	ND	ND
Darshan grape[e]	ND–88.0	ND	ND	ND	ND	ND	67.0–471	154–2360	200–3550
Shiv cinnamon	ND	ND–89.0	ND	ND	ND	ND	ND–59.8	ND	ND
Shiv menthol[f]	ND	ND	ND	ND	ND	ND	129–305	ND	ND
Azad menthol	ND	ND	ND	ND	ND	ND	930–2770	ND	ND
U.S. cigarettes									
filter menthol	ND	ND	ND	ND	ND	ND	3580–6950	ND	ND
filtered	ND	ND	ND	ND	ND–7.62	ND	ND–95.0	ND	ND
nonfiltered	ND	ND	ND	ND	ND	ND	ND–72.3	ND	ND

Source: STEPHEN B. STANFILL,* CANDACE R. BROWN,XIZHENG (JANE)YAN, CLIFFORD H. WATSON, AND DAVID L. ASHLEY (2006). Quantification of Flavor-Related Compounds in the Unburned Contents of Bidi and Clove Cigarettes. J. Agric. Food Chem 54, 8580–8588

4.1.1 Flavor-Related Analytes in Bidis: A High-Resolution Mass Analysis

High-resolution mass spectrometry (GC-HRMS) was also employed in this investigation to verify the existence of substances such safrole, methyleugenol, and diphenyl ether in bidis.

4.1.2 Quantification of Analytes Associated with Flavor in Kretek Filler

The three Kretek brands under investigation have an increased eugenol content. Kreteks has ten times more eugenol than the five bidi brands with clove flavor. Together, myristicin and elemicin were found in one kretek brand. Furthermore, kreteks have larger concentrations of coumarin than bidis.

Table 4.2 Amount of Flavor-Related Compounds Detected in the Burnable Portions of Three Clove Cigarette Brands

	analyte range, burnable portion[a] (μg/cig)					
	anethole	eugenol	estragole	myristicin	menthol	coumarin
LOD (μg)	20.8	42.9	2.0	4.1	32.5	9.9
Djarum Super 16[b]	39.2–261	15400–34300	ND[c]	6.21–15.7 (2)	130–259	60.5–146
Gudang Garam Kings	284–384	47500–78900	12.5–23.9	ND	271–558	180–260
Wismalak Gelora Djaja	ND –23.1(2)	45200–51300	ND	ND	314–376	172–383

Source: **STEPHEN B. STANFILL,* CANDACE R. BROWN,XIZHENG (JANE)YAN, CLIFFORD H. WATSON, AND DAVID L. ASHLEY (2006). Quantification of Flavor-Related Compounds in the Unburned Contents of Bidi and Clove Cigarettes. J. Agric. Food Chem 54, 8580–8588**

4.2 Composition in Tobacco by ICP-OES:

The quantification limits and permitted limits of heavy metals, as well as the analytical wavelength, linear range, and instrumental detection limit for the detection of heavy metals (Cd, Cr, Cu, Fe, Mn, Pb, and Zn) by ICP-OES were carried out.

4.2.1 Concentration of heavy metals in tobacco products, cigars, and sheesha:

Since tobacco's moisture content is regarded as a crucial quality attribute, the average stated concentration of tobacco in a cigarette is 0.62 g with a moisture content of 15%. The current investigation found that the target element concentrations in cigarettes, cigars, and shisha (waterpipe) ranged from 0.09 to 3.98 mg/kg on average, with Fe having the highest concentration (245.55 mg/kg) in shisha products. In contrast, a Turkish study found that several imported and Indian cigarette brands were used in Turkey, with Cd values ranging from 0.44 to 1.55 mg/kg, Cu contents from 10.36 to 30.47 mg/kg, Fe contents from 306.03 to 595.42 mg/kg, and Pb contents from 0.16 to 7.37 mg/kg. The average values reported in this study for Cd, Cu, and Fe are marginally lower than the concentrations reported in the study from Turkey; however, Pb contents were comparable, suggesting that the concentrations may vary depending on the area, agronomic practices, soil conditions, and processing technology.

I. Concentration of Pb in Cigarette Fillers:

The study's mean value of Pb in tobacco filler for cigarettes was found to be lower than the maximum certified limit suggested by WHO/FAO, which is 25 mg/kg for weekly ingestion of tobacco leaves and 5 mg/kg for daily use.

Pb values in the range of 5.43–10.55 mg/kg were discovered in another similar study carried out in the Nigerian state of Nasarawa, which was higher than the advised limit by FAO/WHO. A different study found that the Pb concentration in five different brands of cigarettes ranged from 2.76 mg/kg to 3.20 mg/kg, exceeding the WHO's recommended maximum allowable limit of 0.05 mg/kg done by Kalicanin and Velimirovic´, 2012. ElMohr et al. (2020) found that the mean concentration of lead in a subsequent study was 0.0304 mg/L, which was lower than the value found in the current investigation conducted by Dahlawi et al., 2021. Nonetheless, it is clear that the Pb levels (0.389 mg/kg) found is above than the WHO's recommended level of 0.05 mg/kg.

II. Concentration of Cd in cigarette

The current study's mean Cd content was found to be in the range of 0.093 mg/kg, which is similar to the findings of a study conducted in the Nigerian district of Zaria regarding the Cd concentrations in different brands of cigarettes (0.06– 0.40 mg/kg (Velikovic´ and Kalicćanin, 2012). While the concentrations of Cd in four different brands of cigarettes ranged from 0.53 to 0.59 mg/kg in another investigation, the concentrations of Cd in the former study were higher than the concentration of Cd reported in the current study (Engida and Chandravanshi, 2017). Another study reported concentrations of Cd in the range of 1.3–7.6 mg/g. Additionally, it is evident that all of these investigations' reported Cd concentrations above the Cd threshold levels.

Comparably, a study carried out in Pakistan found that locally branded cigarettes had Cd concentrations between 1.66 and 2.96 mg/g, which is greater than WHO's recommended threshold of 0.05 mg/kg.

III. Concentration of Cr in cigarette

The typical range for the concentration of chromium found in mainstream cigarette smoke is 0.0002–0.5 mg. The study found that the Cr concentration was 0.663 mg/kg, falling within the WHO's recommended range of maximum allowable levels (0.01–1.2 mg/kg or 0.5 mg/kg). Five different brands of cigarettes were found to have Cr levels of 12.30, 17.86, 13.44, 14.58, and 16.10

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mg/kg in one investigation. These levels were found to be much higher than those found in this study and also above the WHO acceptable limit.

IV. Concentration of Cu in cigarette

The findings of this study reported mean concentration of Cu was 2.61 mg/kg. Significantly higher amounts have been recorded in a few earlier investigations (than the present study). For example, concentrations ranged from 6.02 to 15.85 mg/kg g in commonly smoked cigarette brands and local snuff retailed in the Nigerian market (Vincent et al., 2011); 14.53–21.8 mg/kg in a study conducted in Ghana (Sebiawu et al., 2014); 20 to 50 mg/g in a study conducted on Indian cigarette tobacco (Shaikh et al., 2002); 10.2 to 21.8 mg/g in another study conducted in Ghana (Engida and Chandravanshi, 2017); 2.80 to 25 mg/g in a study on tobacco products in Ethiopia (Engida and Chandravanshi, 2017). This notable variation in the reported Cu values suggests that metal concentrations can fluctuate significantly between nations and locations, and that soil conditions play a major role in this variability. All of the studies' levels of Cu, however, are below the 100 mg/kg and 500 mg/kg daily and weekly permitted limits for copper set by the WHO and FAO, respectively.

V. Concentration of Zn in Cigarette

The current study's mean zinc concentration was 1.641 mg/kg, which is substantially less than the 100 mg/kg maximum allowed for zinc in cigarettes (Poorolajal et al., 2020). Nonetheless, Zn concentrations in the range of 8.5–23.18 mg/kg (Kalicˇanin and Velimirovic´, 2012) and 27.75–39.50 mg/kg (Engida and Chandravanshi, 2017) have been found in two different studies. These changes may be caused by a variety of biotic and abiotic factors, including the state of the soil, the area used for tobacco farming, the presence of heavy metals in soils, and others.

Table 4.3 Heavy metals analysis of tobacco obtained from different shisha products. The values were presented in mg/kg.

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Brands	Cd	Cr	Cu	Fe	Mn	Pb	Zn
A	0.15 ± 0.00^{AB}	0.55 ± 0.01^{D}	4.87 ± 0.00^{B}	44.22 ± 0.56^{D}	4.04 ± 0.01^{D}	1.18 ± 0.04^{A}	2.52 ± 0.02^{B}
B	0.07 ± 0.00^{CDE}	0.46 ± 0.00^{E}	1.09 ± 0.00^{G}	23.95 ± 0.00^{EF}	7.89 ± 0.01^{A}	0.11 ± 0.03^{G}	1.51 ± 0.02^{C}
C	0.04 ± 0.00^{EF}	0.23 ± 0.00^{G}	0.17 ± 0.00^{J}	3.138 ± 0.04^{H}	1.44 ± 0.02^{I}	0.29 ± 0.01^{EF}	0.56 ± 0.02^{FG}
D	0.07 ± 0.01^{CD}	0.21 ± 0.04^{GH}	1.41 ± 0.00^{D}	20.75 ± 0.05^{FG}	2.75 ± 0.01^{F}	0.23 ± 0.04^{F}	0.87 ± 0.03^{E}
E	0.09 ± 0.01^{C}	0.24 ± 0.00^{G}	1.06 ± 0.00^{G}	18.19 ± 0.33^{G}	4.39 ± 0.02^{D}	0.15 ± 0.01^{G}	1.35 ± 0.06^{CD}
F	0.09 ± 0.00^{C}	0.18 ± 0.00^{HI}	1.28 ± 0.01^{E}	25.39 ± 0.37^{E}	3.12 ± 0.06^{E}	0.11 ± 0.00^{G}	1.21 ± 0.02^{D}
G	0.19 ± 0.00^{A}	2.12 ± 0.00^{B}	17.60 ± 0.04^{A}	1075.50 ± 5.03^{B}	6.53 ± 0.04^{B}	0.37 ± 0.03^{D}	2.58 ± 0.34^{B}
H	0.06 ± 0.01^{DEF}	2.22 ± 0.01^{A}	1.19 ± 0.03^{F}	1087.90 ± 2.39^{A}	6.50 ± 0.56^{B}	0.31 ± 0.02^{E}	2.65 ± 0.19^{B}
I	0.04 ± 0.00^{F}	1.07 ± 0.00^{C}	1.73 ± 0.00^{C}	633.21 ± 1.45^{C}	5.52 ± 0.01^{C}	0.57 ± 0.01^{B}	3.88 ± 0.02^{A}
J	0.06 ± 0.00^{DE}	0.32 ± 0.01^{F}	0.23 ± 0.06^{J}	3.08 ± 0.05^{H}	1.88 ± 0.09^{GH}	0.26 ± 0.01^{EF}	0.39 ± 0.02^{G}
K	0.19 ± 0.00^{A}	0.22 ± 0.04^{G}	0.46 ± 0.00^{H}	6.58 ± 0.06^{H}	2.13 ± 0.00^{G}	0.58 ± 0.06^{B}	0.68 ± 0.05^{EF}
L	0.05 ± 0.04^{DEF}	0.15 ± 0.00^{I}	0.24 ± 0.00^{J}	4.69 ± 0.07^{H}	1.64 ± 0.00^{HI}	0.44 ± 0.01^{C}	1.48 ± 0.05^{C}
Average	0.09 ± 0.01	0.66 ± 0.01	2.61 ± 0.01	245.55 ± 0.87	3.98 ± 0.07	0.38 ± 0.02	1.64 ± 0.07

Source: Dahlawi, S., Abdulrahman Al Mulla, A., Saifullah, Salama, K., Ahmed Labib, O., Tawfiq Aljassim, M., … Khalid, N. (2021). Assessment of different heavy metals in cigarette filler and ash from multiple brands retailed in Saudi Arabia.

5. Ash at Crime Scene:

To identify the brand of cigarettes, look for the motif or connotation of the name on the end of the butt. But, it's possible that a cigarette was burned all the way through to the tip, or that the smoker deliberately destroyed the cigarette to hide who they were. The ash in this instance is considered for analysis. This might have been in an ashtray or on the floor of the murder site. Due to a dearth of studies in this area, cigarette ash is generally disregarded in the forensic context rather than being a potential piece of evidence. The influence of cigarette ash's toxicity on human health and the environment is being investigated in medical and environmental contexts by an analysis of its trace metal composition. Analysis of the metal composition of cigarettes has also been used to distinguish and identify the illicit or counterfeit origin of some brands. Researchers found that, except for a few that volatilize because of the way tobacco burns at different temperatures, cigarette ash retains roughly 70–80% of the original concentration of all components found in tobacco (7).

5.1 Concentration of metals in Tobacco Ash using ATR-FTIR:

The work carried out by Sharma A. et al., 2022 uses chemometric PCA and PLS-DA models in conjunction with ATR-FTIR (Attenuated Total Reflectance – Fourier Transform Infrared) spectroscopy to distinguish between different cigarette brands sold locally in Indian markets and to differentiate cigarette ash samples in order to improve the characterization and discrimination of cigarette ash and their brands. The three main goals of this research are as follows: the

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examination of cigarette ash to distinguish it from other samples of ash because the crime scene may contain ash from many materials, such as paper, cloth, tandoor, etc.; cigarette ash samples can be distinguished from bidi ash samples because they both include tobacco and are frequently discovered at crime scenes. Lastly, by analyzing the ash using an ATR assembly in the mid-IR spectral range (4000–450 cm− 1), it will be possible to discriminate between three different cigarette brands.

To distinguish between the two groups, spectral characteristics of cigarette ash and other ash samples were recorded using ATR-FTIR spectroscopy. This investigation was performed in the 4000–450 cm− 1 mid-IR band. The fingerprint region of the spectra was named after the range of 1800 to 450 cm−1, which included several unique peaks associated with chemical composition. notable peaks in the wavenumber region 512 cm −1, 565 cm −1, 615 cm − 1,712 cm −1, 874 cm −1, 1045 cm − 1, 1109 cm − 1, 1407 cm − 1, 1736 cm − 1. The peak detected at 1736 cm−1 is ascribed to C–O stretching in aromatic compounds, cyclopentanone, and ester bonds; meanwhile, the presence of polyethylene glycol is responsible for the peak at 1407 cm− 1. The presence of α-unsaturated and cyclic tertiary alcohols, as well as C–C–O stretching vibrations in aromatic compounds, is indicated by the peaks around 1109 cm−1 and 1045 cm−1, respectively. The existence of C–H out-of-plane bending vibrations, C–H deformations in alkynes, and mono-substituted aromatic bending vibrations is indicated by the peaks at 874 cm−1, 712 cm−1, and 615 cm−1, respectively. The C-N-S bending vibrations and the metal-oxygen bending vibrations are represented by the peaks at around 565 cm−1 and 512 cm−1, respectively. Some of the samples also show other small peaks, which serve as the foundation for differentiating them from one another (7).

Table – 5.1 Representation of several wavenumber peaks and their corresponding band allocations for various ash samples

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Peak (Wavenumber)	Assignment
3780–3700 cm^{-1}	O–H stretching, free oxime/ free alcohol
3370 cm^{-1}	Intra-molecular hydrogen bonding, Amino group, Polyethylene glycol
2965–2874 cm^{-1}	N–H stretching in amine salts; C–H stretching in aldehyde(doublet)
2512 cm^{-1}	Stretching vibrations in paraffin compounds, aliphatic compounds and –CH groups
2165 cm^{-1}	-N-C = S asymmetric stretching
1982 cm^{-1}	C = C = N stretching in ketonimine
1794–1735 cm^{-1}	C = O stretching in cyclopentanone, Ester bonds & aromatic compounds
1633 cm^{-1}	C = C stretching
1407 cm^{-1}	Polyethylene glycol
1217 cm^{-1}	C-O stretching in vinyl ether
1109 cm^{-1}	α-unsaturated and cyclic tertiary alcohols
1088 cm^{-1}	C–H bending vibrations in aromatic compounds
1045 cm^{-1}	C–C-O stretching vibrations
874 cm^{-1}	C–H out-of-plane bending vibrations
712 cm^{-1}	C–H deformations in alkynes
670 cm^{-1}	C–H out-of-plane bending vibrations
615 cm^{-1}	Mono-substituted aromatic bending vibrations
601 cm^{-1}	C-X stretching; broad absorption band of titanium oxide
565–570 cm^{-1}	C-N-S bending vibrations
512 cm^{-1}	Metal-oxygen bending vibrations

Source: Akanksha Sharma, Vishal Sharma (2022). Forensic analysis of cigarette ash using ATR-FTIR spectroscopy and chemometric methods

5.2 Concentration of Tobacco Ash using ICP-OES:

The concentration of each heavy metal found in the ash of the various tobacco products sold in the Kingdom of Saudi Arabia was also reported in this study. The results showed that the levels of Cd, Cr, Cu, Fe, Mn, Pb, and Zn were 37.39, 1.81, 13.92, 239.69, 75.01, 312.78, and 20.23 mg/kg, respectively. For Fe, Mn, and Pb, these values are similar to those found in a study that involved

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Marlboro cigarettes (filter) imported from nine different countries and the Russian Belomorkanal "papierosy" (filter-less), where the ash still retained 71–86% of the original elemental component. Ren et al. (2017) conducted a study that was similar to the current study in that it also found high metal concentrations in ash. The concentrations of different heavy metals ranged from 55.60 to 125.99 mg/g. These levels of toxic metal contents are fairly comparable to those found in the ash of various tobacco products (1.81–312.78 mg/kg).

Table- 5.2 Heavy metals comparison in different brands of shisha, cigarettes, and cigars. The values were presented in mg/kg.

Heavy Metals	Cd	Cr	Cu	Fe	Mn	Pb	Zn
Tobacco filler	0.09 ± 0.01	0.66 ± 0.01	2.61 ± 0.01	245.55 ± 0.87	3.98 ± 0.07	0.38 ± 0.02	1.64 ± 0.07
Ash	37.39 ± 0.09	1.81 ± 0.07	13.92 ± 0.14	239.69 ± 0.20	75.01 ± 0.22	312.78 ± 0.22	20.23 ± 0.18
With filter	173.22 ± 0.12	1.19 ± 0.07	1.66 ± 0.05	5.05 ± 0.24	0.20 ± 0.09	4.01 ± 0.07	0.48 ± 0.05
Without filter	11.78 ± 0.11	1.83 ± 0.15	1.37 ± 0.07	32.86 ± 0.08	0.63 ± 0.06	0.65 ± 0.04	0.77 ± 0.03

Source: Dahlawi, S., Abdulrahman Al Mulla, A., Saifullah, Salama, K., Ahmed Labib, O., Tawfiq Aljassim, M., … Khalid, N. (2021). Assessment of different heavy metals in cigarette filler and ash from multiple brands retailed in Saudi Arabia.

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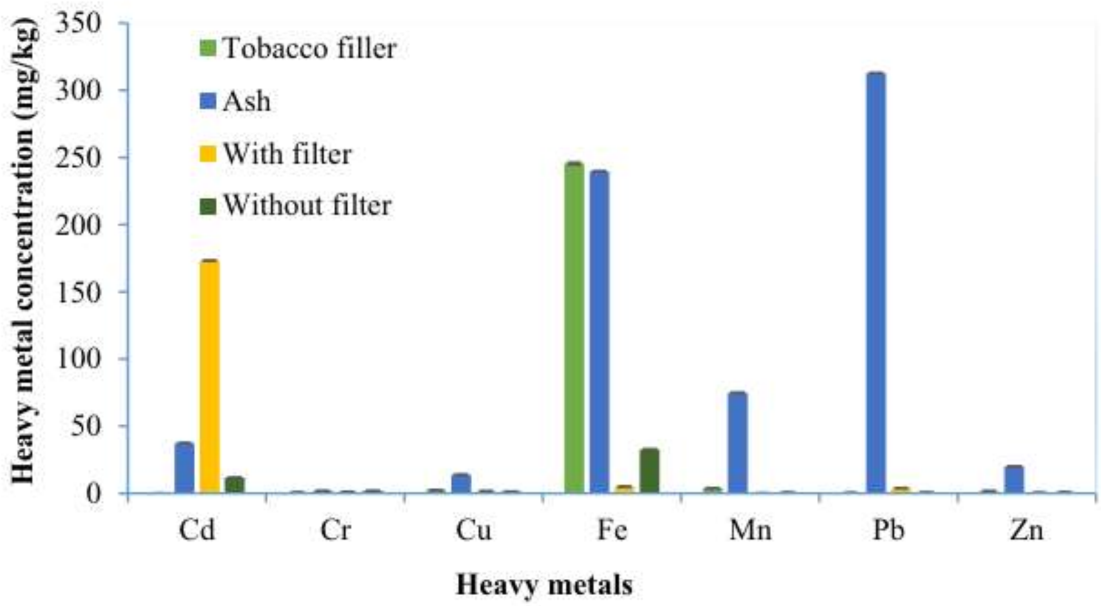

Graph- 5.1 Heavy metals comparison in different brands of shisha, cigarettes, and cigars, before and after removing filters. Source: **Dahlawi, S., Abdulrahman Al Mulla, A., Saifullah, Salama, K., Ahmed Labib, O., Tawfiq Aljassim, M., … Khalid, N. (2021). Assessment of different heavy metals in cigarette filler and ash from multiple brands retailed in Saudi Arabia.**

6. Conclusion:

In conclusion, the analysis of tobacco products reveals significant insights into their chemical composition and the implications for forensic investigations. India's substantial role in tobacco consumption and production underscores the importance of understanding the trace metal content in cigarette ash. The study of heavy metals in tobacco, such as Pb, Cd, Cr, Cu, and Zn, highlights variations based on regional agricultural practices and soil conditions. Forensic applications can benefit from the unique metal signatures in cigarette ash, potentially aiding in crime scene investigations by linking ash samples to specific brands. Despite the variety of tobacco products, including bidis, kreteks, and hookahs, the consistent presence of harmful substances emphasizes the health risks associated with tobacco use. The findings advocate for more rigorous forensic research and public health policies to address the widespread use and environmental impact of tobacco products. The present study's heavy metal contents (Cd, Cr, Cu, Fe, Mn, Pb, and Zn) were about equivalent to those found in 36 well-known brands of cigars, cigarettes, and water pipes that are sold in Saudi Arabian markets. All tobacco samples had greater concentrations of the two most carcinogenic metals—cadmium and lead—than the threshold. Moreover, the ratio of metal content added during the manufacture of the cigarettes can be ascertained by comparing the ash composition of burned cigarettes and tobacco leaves.

One kind of corroborative evidence that is occasionally discovered at crime scenes is cigarette ash. Investigators may be able to reduce the number of potential suspects by knowing the number of people that were at the crime scene. Cigarette ash is often disregarded in forensic situations due to a paucity of study in this field. ICP-AES and ICP-MS, two destructive techniques, were formerly employed in forensic situations to analyze cigarette ash. However, these methods require a high sample size and take time due to the acid-digestion sampling stage. The three main objectives of the study are to separate cigarette ash from other ash samples, separate cigarette and bidi ash samples, and separate three distinct cigarette brands from the ash samples. After smouldering a variety of cigarette and bidi stick samples, the obtained ash samples and additional ash samples were immediately examined on the ATR crystal. Cigarette ash found at crime scenes can be effectively discriminated against using ATR-FTIR spectroscopy combined with statistical methods. In a study, PLS-DA analysis demonstrated strong separation between cigarette ash and other types of ash, with a cross-validation balanced error rate (BER) of 3% and an AUC value of 1 for component 6. The model showed high sensitivity and accuracy (1 and 0.96, respectively) and correctly predicted 100% of unknown samples. Similarly, for differentiating between cigarette and bidi ash, the PLS-DA model achieved perfect accuracy and sensitivity with a BER of 0. Further, distinguishing between cigarette ash from three different brands yielded high classification accuracy, with the lowest BER at component 7 and sensitivity and accuracy rates of 1 and 0.833, respectively. This approach is superior to previous methods (ICP-AES and ICP-MS) as it is non-destructive, quick, and non-invasive, without requiring sample pre-treatment. Despite a limited sample size, reliable inferences were drawn, making this method excellent for forensic applications. The study suggests that cigarette ash analysis can provide critical forensic information, and the creation of a comprehensive database of cigarette ash from various brands could aid in criminal investigations, although such a database is not currently available.The analysis of cigarette ash using ATR-FTIR spectroscopy and PLS-DA offers a robust, non-destructive method for forensic investigations, capable of distinguishing between various types of ash and identifying cigarette brands. This method's high accuracy and reliability underscore its potential to significantly enhance crime scene analysis and aid in criminal identification. Developing a comprehensive database of cigarette ash profiles could further bolster forensic capabilities, although such a resource is yet to be established.

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