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FROM THE GROUND UP: EXAMINING THE ROLE OF POLLEN AND SOIL ANALYSIS IN FORENSIC INVESTIGATIONS

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ABSTRACT

Forensic soil science and forensic botany have experienced a significant rise in interest and recognition in recent times. In forensic soil science, molecular biology, chemistry, geophysics, mineralogy, and soil morphology are applied to legal issues. Forensic botany examines plant anatomy, behavioural characteristics, growth patterns, reproductive cycles, and categorization systems for judicial purposes. Acceptance of botanical evidence in legal systems requires adherence to specific requirements. Taking into account their potential as crucial court evidence, this review highlights the importance of forensic soil science and botanical analysis in criminal investigations. Several scientific fields are discussed, including soil morphology, chemistry, geophysics, and plant anatomy. While countries such as New Zealand and the United Kingdom have proven the effectiveness of these techniques, they are underutilised globally. Case studies highlight the importance of pollen analysis and soil evidence in determining geolocation, identifying drug manufacturing sites, and connecting suspects to crime scenes. To encourage their integration into international forensic practices, the necessity of more knowledge and training in these areas is stressed.

Keywords: Forensic Botany, Pedology, Palynology, Case Studies.

I. INTRODUCTION

Forensic soil science deals with research and study of the soil. The study involves an interdisciplinary approach, integrating morphology, chemistry, geophysics, mineralogy, biology, and molecular biology. Its main application lies in responding to legal theories, concerns and problems (Fitzpatrick et al., 2009). Soils imply various things to various individuals. Some people refer to the soil as "dirt" or "mud" since it makes them "dirty" when they come into touch with it. There are currently a variety of confusing terminology for the application of these fields to forensic science for both forensic scientists and geologists/geoscientists. For e.g.: Forensic Pedology (soil science); Forensic Geology; Forensic Geoscience; Geoforensics, Soil Forensics, and Environmental Forensics.(Ruffell, 2010)

Forensic botany is a blend of different sciences that ultimately lead to their use in legal situations. Forensic palynology is often used to obtain legal evidence by examining the spores and pollen that are around the crime spot or any evidence that could be of legal importance to the case study. Although a highly beneficial and potential method, it is not often given much importance except in a few countries like New Zealand and the United Kingdom. There are several theories as to why this technique is under-utilised around the world. (Bryant, 2016) Anatomy of the plant, behavioural patterns, growth and development patterns, reproductive cycle, dynamics of their population, as well as the classification system for the identification of the species are the elements of plants that are studied well under forensic botany. The forensic component requires knowledge of what botanical evidence is required for it to be accepted as a form of evidence in our legal systems. This is followed by preservation, recording and maintenance of the evidence, knowledge of the methods used for testing the samples, validating the new techniques as well as the admissibility standards are all required to be presented in the court. There have been numerous criminal cases such as murder, sexual assault, child abuse, hit-and-run accidents, kidnapping, drug enforcement, determining the death time as well as verification of an alibi where plants were used as strong evidence. Additionally, using plant materials in forensics as "tracers" to help identify missing persons, monitor the way the drug gets distributed and connect the corpses to the secondary location that it might have been disposed of in.(Milne et al., 2005).

A solid grasp of Locard's exchange principle is the cornerstone of crime reconstruction according to Dr John Thornton, a criminalist in practise and a former instructor in forensic science at the University of California (UC) at Berkeley. Edmund Locard, the head of the first crime laboratory in Lyon, France, put out this theory at the beginning of the 20th century. According to Locard's trade Principle, whenever two objects come in contact, tiny material will trade. This obviously comprises fibres, but it also includes other microscopic components like soil, pollen, paint and hair. Dr. Locard proposed that criminals may be located and afterwards linked to specific locations, pieces of evidence and people (i.e., victims) by identifying, cataloguing, and assessing the nature and extent of evidentiary trails and exchanges in a crime scene. He viewed this hypothesis as both obvious and old, and he compared the identification and investigation of trace evidence to a hunting strategy that has existed from the dawn of time. In the usual course of drinking at a watering hole, the prey, for instance, leaves tracks, spoor, and other clues that reveal its presence and location; the hunter purposefully looks for these clues, takes up the trail and pursues. Every interaction leaves a trail that can be found and deciphered (Moran, 2011; Gallagher & Thornton, 2011; Chisum & Turvey, 2012).

Plants can and have been considered as biological evidence in many situations such as seeds trapped in the cuffs of clothes after an assault, smudges of grass, or other vegetation snagged against vehicle parts or tyres used in crime, using pollen grains to estimate the date of buried skeletal remains(Alotaibi et al., 2020). Such evidence helps to connect humans to an object, an individual to the crime scene, or a criminal to a deceased. "Every criminal leaves a 'trace' (proof)," as the saying goes. That "trace" might be biological plant material (Milne et al., 2005). The United States is a great location for using forensic pollen data across both civil and criminal proceedings. There are a variety of main ecotonal zones ranging from Arctic tundra to blistering deserts to tropical rain forests, in addition to hundreds of microhabitats, each with its own distinct combination of plants (Bryant & Jones, 2006) Botanists and ecologists have described the country's vegetational richness in several thorough studies. Another significant advantage of the United States is its lengthy and rich record of pollen research, which originated with the very first pollen study in North America (Bryant & Jones, 2006).

II. RESULTS AND DISCUSSION

A. Pollen

Forensic palynology uses pollen and spores to help solve crimes (Bryant, 2013). It has been used by experts in the field to provide forensic evidence and expertise in particular instances of law for at least the last three decades. It has benefited both civil and criminal cases since the 1950s. (Milne et al., 2005; Mildenhall et al., 2006; Morgan et al., 2014). Because of their variety of dispersal processes, quantity, resistance to mechanical and chemical deterioration, shape, and microscopic size, pollen and spores are useful in forensic investigations. The gross pollen and spore makeup of a sample in addition to the pollen type ratios must be recorded by forensic palynologists. High levels of analytical precision are produced as a result of this combination. The longevity of pollen, spanning several thousand to millions of years, as indicated by earlier studies, is frequently observed in association with charcoal and remnants of common fires. (Morales-Molino et al., 2012; Sniderman and Haberle, 2012). Pollen grains provide great forensic trace elements due to their small size, wide variety, and prevalence of items that have been subjected to or come into contact with the air. Pollen can be extracted by forensic analysis from a variety of materials, including soil, clothing, textiles, medications, air purifiers, vegetation, and animal and human remains (Milne et al., 2005).

In most cases, forensic pollen experts present their conclusions in terms of probability and then draw on their knowledge. Pollen analysis is a crucial technique for determining the source plant groups, source environment, and possible regions of origin for archival material (Traverse, 1988). A match can be made by combining pollen identification records (sometimes down to the genus level) with quantitative proportions of every pollen and spore taxon. It becomes infeasible or excessively expensive to require close fits of a pollen type to an accurate species level of identification for most concerns, especially in genera having numerous species, like *Clematis*, which has over 220 recognised species. Fourth, most pollen and spores have a high level of resistance to deterioration. Evidence gathered from crime scenes, such as spores and pollen, can endure for several hundred, thousand, or even more years. Trapped pollen and spores can be recovered and used as evidence in investigations for a long time with proper and secure processing of crime scene materials. An additional reason why pollen analysis is a significant area of forensic research since pollen grains can survive in the intestines for up to 21 days (Arguelles et al., 2015). There are certain exceptions, such as regions where soil is frequently wet and dried or in ploughed fields, wherein pollen and spores decompose very readily (Bryant, 2022).

When attempting to evaluate forensic samples, it is crucial to be aware of the laws that control pollen production and dispersal. Several factors significantly impact the pollen spectral range of any sample. These include the varying speeds at which distinct pollen and spore types settle from the ambient air (plunging speed) and the resilience of diverse pollen and spore types over time. Additionally, there are distinct indicators suggesting the repurposing of pollen grains post-deposition (Jackson and Lyford, 1999). Pollen rain is created when atmospheric pollen and spores fall to the ground, and the amount and types of pollen and spores that are included in the pollen rain determine the pollen print that can be retrieved from any given location or province (Tauber, 1965). For instance, the pollen grains of *Cannabis sp.*, *Alnus sp.*, *Juniperus sp.*, and *Betula sp.* are all exceptionally tiny and light in load, sinking in still air at rates of about 1-2 cm s^{-1}. As a result, the presence of a handful of pollen grains in a forensic specimen from an unidentified source might not be sufficient to demonstrate that the plants in question are present in the region where the sample was taken (Jackson and Lyford, 1999). Instead, it's possible that the pollen grains in the sample came from distant plants growing over a great distance. Conversely, if a sample contains sizeable and dense anemophilous pollen types like those from *Zea sp.*, *Cedrus sp.*, *Picea sp.*, *Pseudotsuga sp.*, or Fir (Abies), it typically means that the object or sample originated from a location where all these plants were growing nearby. Due to the larger pollen types' rapid sinking rates, which cause them to reappear at a rate of 6–12 cm s^{-1}, it is possible to rely on this generalisation. Some of these pollen types are only ever scattered far from the plant because of the quick sinking rate. More or less every airborne pollen grain sinks at a different rate, causing some types to spread over a smaller area while others may spread over a larger one. Pollen grains of various masses and sizes would be subject to scouring in additament to sinking rates when they are struck by droplets of water or when they come into contact with obstacles of sizes ranging, including such bits of wood, leaves, or person-made objects. For every surveyed location or any forensic subset of the population of unknown origin, all of these considerations must be made (Tauber, 1965).

B. Methods

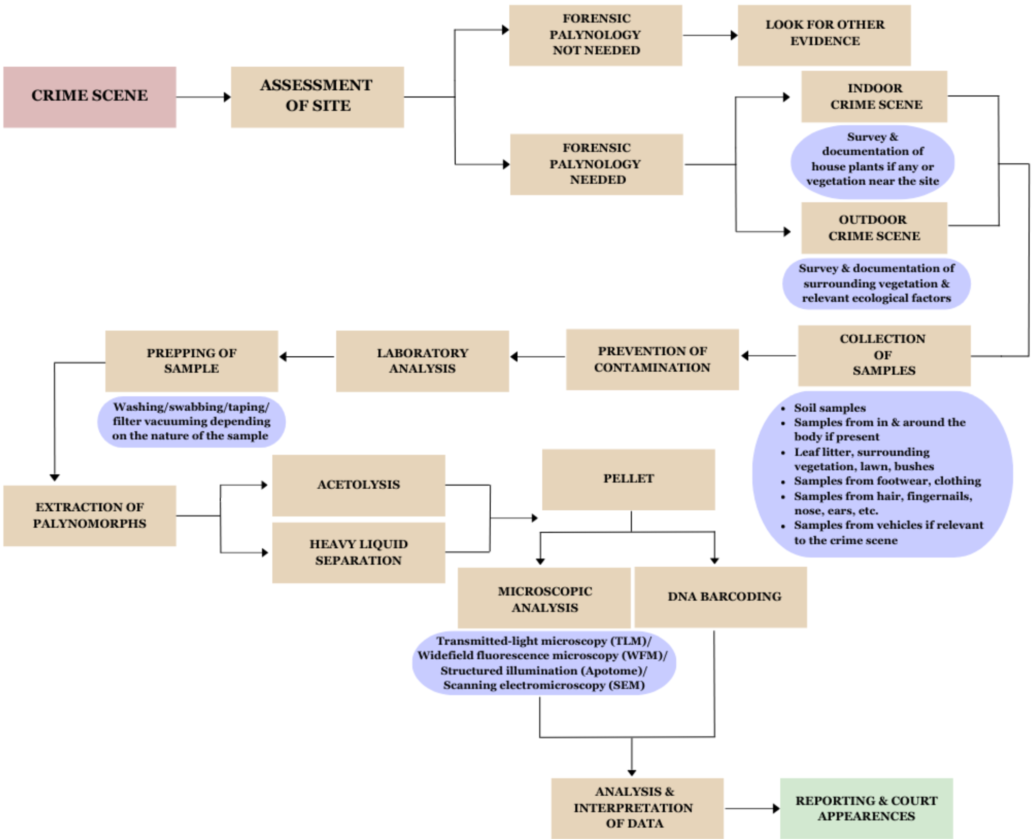

Fig. 1. Overview of the methods employed for forensic analysis of pollen

1. Traditional Methods

Palynology specimens from different plant species include a wide variety of microscopic plant parts as well as various kinds of pollen and spores. The word "pollen assemblage," also known as a palynomorph assemblage, refers to the total amount of identifiable pollen and spores in a sample and is used to describe this mixture of pollen and spores. Since each place has its own unique mixes of plant species and other environmental conditions, this assemblage acts as a distinctive "fingerprint" for that particular area. As a result, investigating and examining these pollen assemblages can provide important information about the flora and ecology of certain geographic areas (Milne et al., 2005). Traditionally, morphology has been used to identify pollen. Conventional techniques, like scanning electron microscopy (SEM), were used to identify palynomorphs. Due to the higher degree of detail it offers in analysing pollen grains, this method has been employed in routine testing since the seventies. It serves as a valuable tool for the preliminary classification of palynomorph kinds. It is, however, time-consuming and labour-intensive, making it unsuitable for regular evaluation (Walsh and Horrocks, 2008). Transmitted-light microscopy (TLM), widefield fluorescence microscopy (WFM), and structured illumination (Apotome) microscopy are the three techniques for acquiring good images of pollen grains. These three techniques are semi-automated versions of older, manual techniques for finding pollen grains (Johnsrud et al., 2013).

Forensic palynologists must identify pollen not just with a microscope but also by consulting extensive reference collections including various taxa from all over the world. Martin and Harvey founded the Global Pollen Project (GPP) in response to this demand (Martin & Harvey, 2017). The GPP's major goal is to create a centralised and vast digital database for pollen identification. This unique effort encourages the upload and exchange of photos and metadata in a standardised format, facilitating the digitization of pollen reference materials. The GPP supports collaboration and knowledge from global participants by leveraging the power of crowdsourcing, resulting in a dynamic, peer-reviewed pollen database. GPP unites effortlessly with external services like the Global Biodiversity Information Facility and the Neotoma Palaeoecology Database, giving valuable botanical descriptions and occurrence statistics for each taxon, as well as complete pollen photos and information. Pollen grains must be cleaned to remove the pollen covering and enable for a thorough examination of their morphological properties before proper identification can take place. Acetolysis, followed by alkali and acid treatment are the standard methods. High- and low-temperature switched treatment procedures, on the other hand, have been established, resulting in clean and undamaged pollen samples (Gonzalez-Cruz et al., 2018).

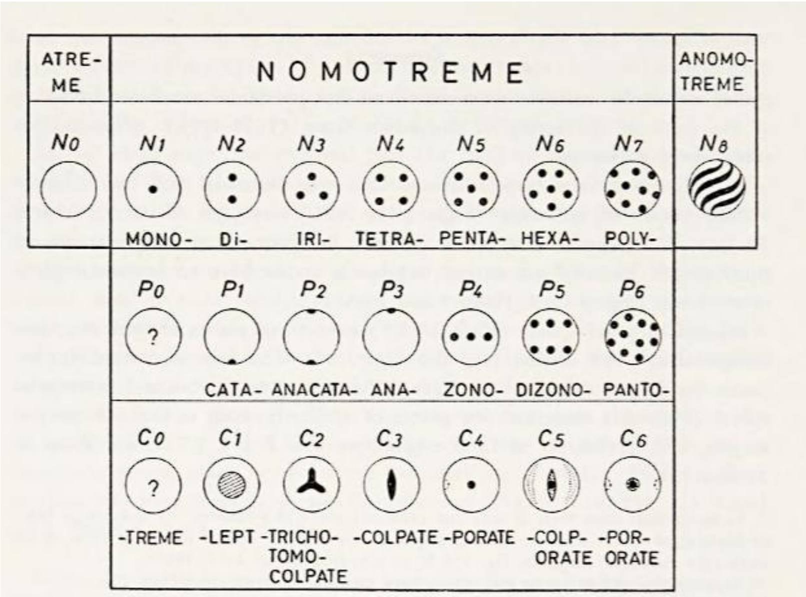

Fig. 3. The NPC-system. – Diagram showing aperture number (N), position (P), and character or form (C).

Fig. 2. NPC system used to classify and identify pollen (Erdtman, 1969)
(Abbreviations: N- aperture number, P- position, C- character or form.)

Images of certain pollen grains with unusual frameworks that can be utilized for analysis are provided below (Bryant, 2014).

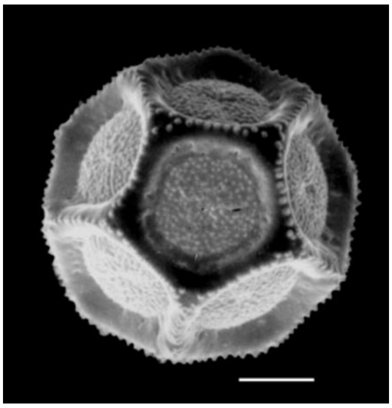

Fig. 3. SEM image of *Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae). Scale is 5-μm. [Gretchen D. Jones, PhD, and Ester F. Wilson, USDA-ARS, APMRU.]

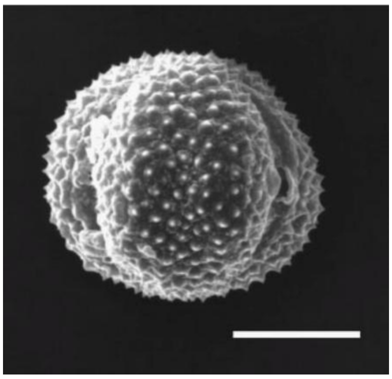

Fig. 4. SEM image of Artemisia californica (Less.) (Asteraceae). Scale is 10-μm. [Gretchen D. Jones, PhD, and Ester F. Wilson, USDA-ARS, APMRU.]

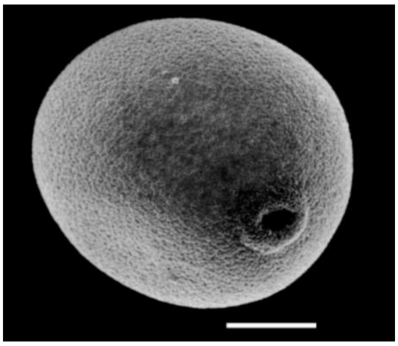

Fig. 5. SEM image of Arundinaria gigantea (Walter) Muhl. (Poaceae). Scale is 10-μm. [Gretchen D. Jones, PhD, and Ester F. Wilson, USDA-ARS, APMRU.]

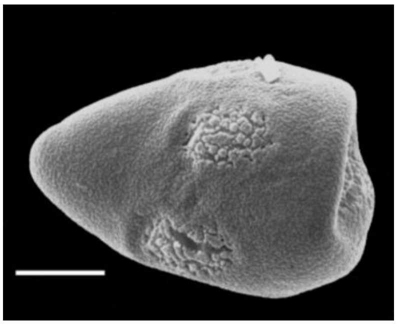

Fig. 6. SEM image of Carex microdonta Torr. & Hook. (Cyperaceae). Scale is 10-μm. [Gretchen D. Jones, PhD, and Ester F. Wilson, USDA-ARS, APMRU.]

2. DNA Barcoding

In forensic applications, DNA barcoding has substantial advantages. For starters, it provides a consistent procedure that can be used to multiple taxonomic categories, removing the requirement for specialist expertise for each species identification. Second, DNA barcoding enables the identification of components that lack distinguishing physical characteristics. This skill is especially useful in circumstances involving trace evidence like pieces or pollen. When paired with high-throughput sequencing, DNA barcoding allows for the forensic analysis of complex mixtures, improving investigative accuracy and speed (Bell et al., 2016). DNA barcoding has been extensively employed in plant identification with the chloroplast genome regions rbcL, matK, and trnH-psbA, along with the nuclear ribosomal ITS region (ITS2), being widely used either individually or in combination (Fazekas et al., 2008; CBOL Plant Working Group, 2009; Fazekas and Kesanakurti, 2009; Chen et al., 2010; Hollingsworth et al., 2011; Bell et al., 2016).

The study of pollen DNA from multiple locales and/or time frames allows for the assessment of numerous possibilities in forensic casework. Pollen grains contain haploid DNA that is shielded by an exterior wall structure that is very resistant to environmental and chemical alterations. However, DNA extraction necessitates the destructive sampling of pollen grains. The exine is disrupted after sample collection to allow for DNA extraction. Exine disruption methods include mortar and pestle grinding and bead-beating pulverisation. Petersen et al. were the first to use the genetic markers rbcL and ITS to amplify DNA from single pollen grains of mountain rye (*Secale strictum*) and barley (*Hordeum vulgare*) retrieved by bursting the grain (Bell, Burgess, et al., 2016; Bell, De Vere, et al., 2016; Parducci et al., 2005, 2017; Petersen et al., 1996).

Case Studies

- The first documented application of pollen and spore analysis to a forensic investigation occurred in Austria in 1959 (Erdtman, 1969). An Austrian man went missing while cruising down the Danube River. His body could never be tracked down. The victim's business partner and friend were under investigation and were charged and arrested for his murder. But as the body was missing and the suspects pleaded innocent, a strong case could not be registered. A pair of soiled boots, however, were discovered in the man's cabin amid investigation. This gave a new lead in the investigation, Wilhelm Klaus, from the University of Vienna, was requested to give an expert's view on the mud sample that was scraped from those boots. He found a mixture of different pollen grains in the sample which included spruce, adler and willow and some old fossil pollen as well. This kind of mixture was consistent with only one particularly small region in the north of Vienna. When confronted, the defendant broke down and admitted to have murdered the man. He then led the investigators to the location where he buried the body and it was indeed the region that was chosen by Klaus
- Numerous studies including palynological studies have been conducted on the Shroud of Turin, which some claim was the fabric utilised to enshroud the body of Jesus before burial. Max Frei examined the cloth in great detail and discovered 49 different kinds of pollen grains woven into its fibres. The Shroud's pollen range corresponded to similar pollen types found in the western Mediterranean, especially in the desert-like flora that were produced there. While some pollen types, like beech pollen, were more characteristic of central Europe, others, like those discovered in Turkey, were similar to those there as well. Max Frei argued that the majority of the pollen on the Shroud came from plants in the western Mediterranean and Turkey based on his findings (Wilson, 1979).
- In a cornfield close to Caledonia, New York, a young woman, possibly 15 years old, was found dead in 1979. The investigation went cold for years since there were no distinguishing characteristics and no ties to missing person reports. Detective John York made the decision in 2006 to investigate a novel investigative strategy called "criminological dust investigation," which he learned about while conducting an internet search. The researcher who invented this method received the girl's clothes, which were still contained in evidence bags. Her original clothing was carefully vacuumed, revealing numerous forms of dust, the majority of which could be connected to the plants growing in the cornfield where she was discovered. However, there were also some dust particles from tropical plants that were not native to New York but instead might be prevalent in Southern Florida and Southern California. This significant piece of information suggested that the victim had most likely recently visited or lived in either Florida or California before her passing. A new examination concentrated on these southern regions as a result of the dust information. Tammy Jo Alexander, a 16-year-old who had fled her home in southern Florida in 1979, was ultimately identified as the little girl in 2015 (Bryant, 2016).

- In a gorge along a rural route in the United States, a murdered victim's corpse was found. The victim's head had been beaten, and all of their clothing and identity had been taken. In order to prevent the victim from being recognised by their prints or fingerprints, their hands and feet were severed. Since none of the blood had seeped into the ground where the corpse was found, there was no way to pinpoint the scene of the murder or the possible residence of the suspect, leaving the authorities unsure of the best course of action. The victim's shirt, socks, pants and shoes were examined for forensic pollen samples. Four different surface dirt samples were also taken from the scene where the body was discovered. Each sample of surface soil included similar levels and varieties of pollen. However, the collective pollen "fingerprint" found in the victim's various pieces of clothes did not exactly match. The victim might have resided some distance from the scene of the body's discovery based on the minor variations in the samples (clothing and soil). A closer investigation of the native pollen found nearly an exact match between the deceased's garments and the pollen, suggesting the victim most likely resided at a distance of 150 mi north of the site of the corpse's discovery (and may have been killed). Thanks to this information, the police were able to identify the victim and find his murderer (Bryant et al., 1990).

Limitations And Potential Sources Of Error

Although pollen analysis is a useful method in forensic science, there are some restrictions and possible sources of inaccuracy that should be taken into account. It's important to keep in mind that pollen analysis still has applications in forensic investigations regardless of its limitations. By taking these restrictions into account and adhering to strict processes, pollen analysis in criminal investigations can be made more accurate.

- Limited evidential weighting: Forensic palaeontology currently receives little evidence weighting in casework, despite its potential. Pollen analysis's credibility as evidence may not be as strong as that of other forensic methods owing to its circumstantial nature which may affect the court's willingness to accept it (Mildenhall, 1990).
- Lack of qualified palynologists: In addition to the loss of skilled palynologists who possess not only in-depth botanical knowledge and training experience, but who can also withstand the challenges of rebuttal in courts, there aren't many qualified palynologists who specialise in forensic cases. The utilisation of pollen analysis in forensic investigations may be constrained by the specialised knowledge needed to correctly identify and examine pollen grains under a microscope (Kumari et al., 2017).
- Laborious nature of analytical procedures: Analytical processes can be time- and labour-intensive, especially when analysing pollen. It entails gathering, preparing, and examining samples under a microscope, which takes a lot of time and money. This can prevent an investigation from moving further and cause delays in getting results (Bryant et al., 1990).
- Sample Collection: According to Bryant et al., 1990, Sample collection is a big issue. The usefulness of forensic pollen samples that were erroneously collected or corrupted after being obtained is quite negligible.
- Assessing the Uniqueness of Pollen Assemblages: Court proceedings are complicated by the distinctiveness of pollen assemblages used in forensic palynology. Due to time and financial constraints, it is challenging to determine if samples from different sources might fit the crime scene. In contrast to DNA fingerprinting, which can produce precise probabilities, proving the uniqueness of each pollen assemblage calls for challenging mathematical problems, time-consuming, expensive pollen grain and spore counts, and the use of numerous control samples. As a result, it is challenging to determine with certainty the likelihood that a certain pollen assemblage would appear on a given population (Mildenhall, 1990).
- Taxonomic resolution: Analyses of fossil and contemporary pollen assemblages are essential for grasping the dynamics of the vegetation in a given region. However, because there aren't many reference collections available for comparison and because it can be difficult to identify plants to the level of their genus or species using pollen, doing palynological study can be difficult. Since pollen from the same genus or family frequently shows little morphological difference, there is a clear need for improved taxonomic resolution. In order to solve this, computer-based solutions hold promise since they can detect minute but systematic morphological variations better than the human eye, resulting in improved taxonomic resolution. Since the amount of material that needs to be analysed exceeds human capabilities, this technology is also desired for general taxonomy and species identification (Holt & Bennett, 2014; Khansari et al., 2012; Martin & Harvey, 2017).

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- Lack of pollen geodatabase: The identification of pollen in forensic investigations has been transformed by DNA metabarcoding, which has also provided invaluable insights into prospective suspects and crime scene linkages. However, creating a thorough geodatabase of pollen origin still poses a substantial issue. Our forensic understanding will be improved if pollen can be precisely linked to particular locations and periods. Researchers would be able to identify suspects and enhance investigations by establishing the spatial and temporal context of pollen samples obtained at crime scenes using the geodatabase. By overcoming this obstacle, pollen forensics would become an even more potent instrument in the investigation of criminal cases (Helderop et al., 2021).

a. Soil

Owing to its pervasiveness on the surface of the earth, the soil is a significant source of evidence that can be used to connect the link between the suspect and the crime scene. There are several methods for the investigation. Because of the forensic purpose's distinctiveness and the complexity of soil, several methods for forensic soil investigation have been developed and explored. Examiners must pick a mix of approaches that are appropriate for the unique characteristics of the soil evidence in order for soil identification to be successful. As a vital element of airborne dust, soil particles are frequently transferred when an accused person comes into proximity with a dusty surface like an entrance, window ledge, etc. (Marumo, 2003; Fitzpatrick, 2004).

One of the earliest forms of corroborating analysis to be used in criminal proceedings was soil. Unfortunately, it seems to be a piece of evidence that is frequently ignored today. However, it can offer useful connections between victims, perpetrators, and/or crime scenes when it is recognised and gathered. The geomorphologic and ecological historical narratives, as well as anthropogenic activities at each location, all have an impact on the expansive range of useful characteristics of soils that can be compared. German researcher Georg Popp analysed soil obtained from the slacks of a person of interest in 1904, marking the first documented incident of soil being used to assist in a criminal investigation (Murray & Tedrow, 1975). Two distinct soil samples were collected from the trousers. One sample had the same mineral composition as the soil where the murder victim was discovered. The soil taken from the route between the crime site and the domicile of the suspect was identical to the soil in the second sample. The suspect admitted to the crime after being shown how the soil evidence linked him to the murder (Murray & Tedrow, 1975; Randle, 2023)

b. Methods

The current method of forensic soil evidence analysis focuses on chemical profiling to determine the soil's likely origin. This focus causes police to seldom photograph soil trace or pattern evidence on offenders' or victims' garments at the scene of the crime, before transporting the corpse or taking the garments off. Instead, soil proof is often ignored until particular elements are selected for examination by judicial forensic analysts or forensic soil experts. After enough soil has been taken off the clothes fabric, this soil analysis commences. For this removal, it might be necessary to shake dirt off garments into evidence bags or maybe even cut out sections of the filthy material (Murray, 2017). The technique of soil analysis employed in the field of forensics is determined by the size of the sample and the intended use of the analytical results. The methods used for comparing the samples must be adaptable to both small and large samples, feasible (using conventional methods), affordable, and accurate (Fitzpatrick et al., 2009).

The vast majority of soil forensic analysis is done in the lab, and it usually starts with the sampling and characterization of three different groupings of samples, as follows (Fitzpatrick & Raven, 2012):

- Questioned soil samples - These samples, with origins often unknown or disputed, are typically collected from suspects or victims.
- Control soil samples - These samples have a known origin, often obtained from locations such as the crime scene.
- Alibi soil samples - these samples have a known origin and aid in determining how distinctive the questioned and control samples are, allowing for a more thorough study of the aimed comparator samples and a more precise understanding of within-site heterogeneity.

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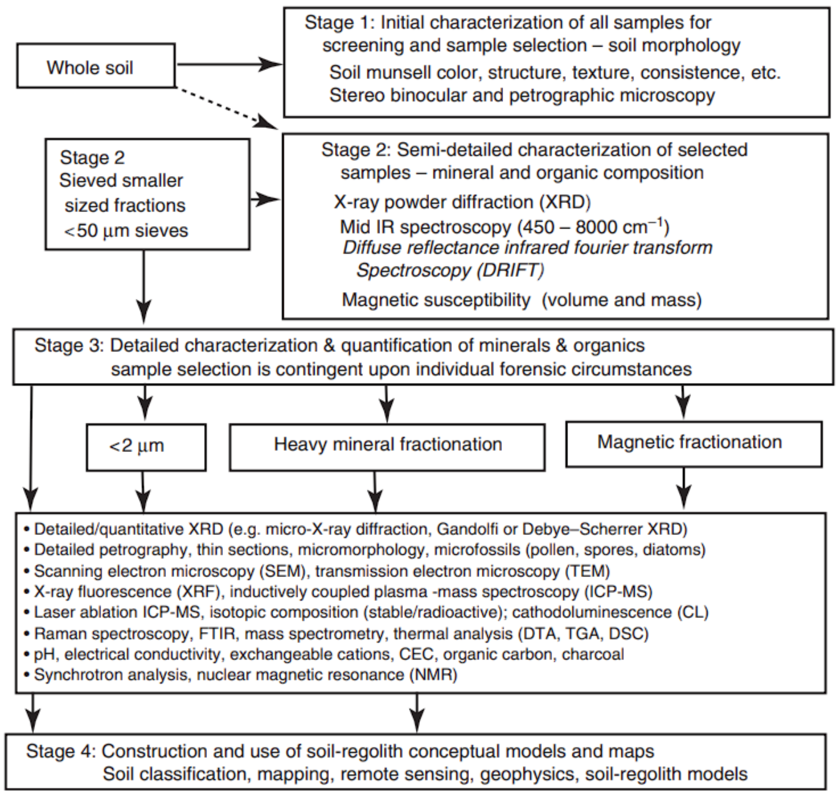

Fig. 7. A diagrammatic representation of the overall workflow during soil analysis given by Fitzpatrick, Raven and McLaughlin (Fitzpatrick et al., 2006) and modified by Fitzpatrick et al., 2009

The two main steps of the approach for soil analysis are descriptive (morphological) and analytical (Fitzpatrick et al., 2009).The screening method begins with quickly categorising soil particles in composite entire soil or bulk samples. In order to compare samples via bulk morphology, mineralogy, and organic matter characterisation, soil morphology, low magnification light microscopy, X-ray diffraction (XRD), Diffuse Reflectance Infrared Fourier Transform (DRIFT) spectroscopy, and magnetic susceptibility (volume and mass) are used. After sample selection, size separation, and intensive inorganic and organic matter investigations, the second stage entails rigorous classification and quantification of the aggregate and individual soil particles using advanced analytical methods. At this stage, individual minerals and organic compounds in the soil samples are identified and quantified using methods including scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), X-ray fluorescence (XRF), and stable isotope analysis.

Various techniques are used in contemporary forensic soil analysis to contrast and distinguish soil samples. Colour and texture matching is one of the main strategies, which compares the visual properties of the soils to find similarities or differences between the questioned and control samples. Munsell's approach, which bases comparison on three factors—hue (base colour), chrome (colour intensity), and value (lightness) is used (Kuehni, 2002). Particle size distribution analysis looks at the relative abundance of various-sized mineral particles in soils using techniques like dry or wet sieving (Robertson et al., 1984), laser diffraction (Wanogho et al., 1987), or the Coulter counter technique (Dudley, 1976). Understanding the density differences in soil samples which can provide important hints about their origin and history takes the help of density gradient measurement. The mineral content of the soils is further examined using mineral identification methods including X-ray diffraction and spectroscopy, which facilitate the differentiation process.

Automated particle imaging and Raman spectroscopy are used in the method known as morphologically-directed Raman spectroscopy (MDRS) (Kammrath et al., 2018). Raman spectroscopy is a technique that analyses the molecular chemistry of specific particles that are of interest. Particle imaging, on the other hand, is a technique that determines the size and shape of each particle in a sample and provides detailed information about their morphology.. Therefore, MDRS can non-destructively classify the minerals present in forensic soil analysis while additionally providing morphological details about specific mineral grains. Along with quantitative data on the proportionate amount of each type of particle, particle size distributions can be created for the total sample and/or for each mineral that is present.

Case Studies

- In a civil case, the complainant claimed that a contractor had tossed soil that was contaminated with asphalt on his private property from the newly constructed highway. The environmental laboratory conducting the investigation received six soil samples from various locations. All the six samples were examined and analysed to determine which sample was from a different location. The examination was done as a blind test and the examiner was not told of the origin of the soil sample. From the table it can be seen that sample S1 was from a different place as compared to the other samples S2-S6. It was also noted that samples S3 and S6 and samples S2, S4 and S5 have similar soil compositions which indicates that it has originated from the same soil. These were confirmed with the laboratory manager and they were correct (Petraco et al., 2008).

Results of the soil comparisons conducted in this case

Specimen	Soil color[a]	Construction debris	Vegetation	Asphalt[b]
S1	7.5 YR 5/4	None	(–)	(–)
S2	10 YR 5/2	Plaster, concrete, and architectural glass	(+)	(+)
S3	2.5 Y 5/4	Plaster and concrete	(–)	(++)
S4	10 YR 5/2	Plaster and concrete	(+)	(+)
S5	10 YR 4/4	Plaster, brick, and concrete	(+)	(+)
S6	2.5 Y 5/2	Plaster and concrete	(–)	(+++)

[a] For color assessment methods see Munsell® Soil Color Charts [10], Antoci and Petraco [11], and Petraco and Kubic [12].
[b] Asphalt contents: (+) light, (++) medium, and (+++) heavy.

Fig. 8. Results of the soil comparisons done in the above case study (Petraco et al., 2008)

- Near midtown Manhattan, the body of a young adult girl was found floating in the East River. The body was seen by joggers on the sidewalk bobbing in the river. The primary suspect in the investigation was ultimately revealed to be the victim, a missing sworn officer who was in the process of getting divorced from her estranged husband. Crime scene investigators discovered a water-stained men's shoe in the bedroom closet of the suspect's upstate home. They examined the shoe and found a small amount of sand stuck to the heel. They sent the pair of shoes for additional analysis as a result of their discovery. A stereo microscope was used to closely inspect the shoe's heel sand, which was then analysed using chemical and X-ray diffraction techniques. Its discovery of sodium and chloride ions suggested that it was similar to known salty residue discovered at a nearby beach. According to the prosecution's argument, the suspect brought the deceased to this shore where he shot her using her very own revolver—which was never found—and fled the scene. The suspect responded to the investigation by asserting that the crime scene investigators had put his foot into a saltwater fish tank he had once owned in his home but had just disassembled. A known sample of sand was taken from a nearby beach and compared to the sample of sand from the shoe in order to confirm the location of the sand. The level of similarity between the two samples was confirmed by the analysis. Additional East River sand samples were gathered, and they were compared to the sample from the shoe. The differences between all other samples confirmed the link to the neighbourhood beach crime scene. In order to aid the jury in reconstructing the chain of events leading up to the murder, this critical forensic evidence—including the thorough sand analysis—was given during the trial. The connection between the sand sample and the neighbourhood beach was vital in proving the suspect's involvement in the crime scene and helped lead to the case's successful conclusion (Petraco et al., 2008).
- Soil analysis was important in connecting Colin Duffy, a well-known republican in Northern Ireland, to the killings of two soldiers. Two gunmen shot and killed two British soldiers outside Massereene Barracks in Northern Ireland on March 7, 2009. As proof, an abandoned automobile was found. 2011 saw the release of fresh soil sample evidence purportedly connecting Colin Duffy to the killings of the two soldiers. Samples of the soil retrieved from Duffy's shoes and the crime scene were compared. Given that the soil samples from the crime scene and those from Duffy's shoes matched, the analysis proved that Duffy had been present when the crime was committed. Duffy and Brian Shivers were accused of carrying out the killings ("Soil Evidence 'Links Colin Duffy to Sappers' Murders'," 2011).

Limitations and potential sources of error

It is significant to remember that soil analysis is still a useful technique in forensic investigations despite its limitations. The accuracy of soil analysis in criminal investigations can be improved by taking these limitations into account and using stringent protocols.

- Contamination: During collection, handling, and analysis, soil samples are prone to contamination, which could jeopardise data integrity and produce false results. To lessen the possibility of contamination, it is essential to develop strict protocols and use the right handling techniques (Peverill, 2005).
- Lack of Standardisation: Forensic investigations involving soil analysis lack standardised techniques and procedures. Inconsistencies in the interpretation of data may result from the use of various methodologies and comparison criteria by various laboratories (Fitzpatrick, 2009).
- Anthropogenic Sources: Analysing soil from anthropogenic sources, like building sites or contaminated areas, can be challenging. The data interpretation may be hindered by the presence of additional materials or pollutants. The movement of people, vehicles, wind, and water further mixes the loose debris, possibly introducing foreign substances (Ruffell et al., 2013).
- Sample Size and Representativeness: The accuracy of the analysis might be impacted by the size and representativeness of soil samples taken from crime scenes. The credibility of the results may be limited by insufficient or unrepresentative samples that do not give a complete picture of the soil composition (Rawlins & Cave, 2004; McKinley, 2013)
- Raman spectroscopy: Raman spectroscopy comes with a number of benefits, but it also has certain downsides. The possible interference from fluorescence, whose initiation probability is larger than that of Raman scattering, is a severe constraint. Raman spectra may be obscured by this interference, particularly when using visible laser wavelengths. Another flaw is its sensitivity, which requires costly and extremely sensitive equipment for reliable readings due to the Raman effect's poor conversion efficiency. Additionally, when working with delicate or light-absorbing materials, intense laser exposure during investigation can result in sample harm or laser-induced degradation. These disadvantages can be lessened by deploying several lasers with various excitation wavelengths, choosing the right laser wavelengths and employing skilled analysts (Buzzini et al., 2006; Colthup et al., 1990; Vaskova, 2011).
- Results Interpretation: It takes knowledge and experience to interpret the findings of a soil analysis. Forensic soil scientists who are trained in the identification and comparison of soil characteristics must be able to accurately analyse and interpret the data. The analysis may contain errors due to insufficient expertise or interpretation that is subjective.

III. CONCLUSION

A crime scene can be better understood when both pollen analysis and soil analysis are combined. For instance, soil analysis can provide details about the unique features of the soil, such as mineral composition or pH levels, while pollen analysis can indicate the sorts of plants present in the area. Investigative teams can increase the ecological evidence in criminal investigations by combining these strategies to learn more about a crime's geographic origin, migration patterns, and linkages. In forensic science, soil analysis and pollen analysis work in tandem. In forensic science, soil analysis and pollen analysis work in tandem. While soil analysis sheds light on the properties of the soil, pollen analysis sheds light on plant species and seasonality. Investigators can better grasp a crime scene, establish associations, and give important evidence for criminal investigations through utilising the aforementioned strategies. This paper has provided a concise explanation of the process and justification for forensic soil and pollen investigations and comparisons, which have been employed for many years with great success in forensic casework. Although the forensic analysis of soil and pollen is still relatively new and is not commonly practised in many regions of the world, it has demonstrated to have significant forensic promise. More and more law enforcement organisations are beginning to understand the importance of examining the nearby soil, pollen, and vegetation. Additionally, this causes a rise in demand for institutions that train these professionals as well as for individuals with the appropriate training.

DECLARATION

This paper features Anushka Agarwal and Harshwardhan Gupta as co-first authors. The contributions of the co-first authors were deemed equivalent, and therefore they are listed in alphabetical order.

CONFLICT OF INTEREST

We hereby declare that there is no potential for conflict of interest among the authors and that the work complied with all applicable ethical guidelines.

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