

The Role of Soil as Trace Evidence in Forensic Science: Methodologies and Applications

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Abstract

In forensic science soil as trace evidence can play a vital role of establishing connection between the suspect, victims, any object with a particular area based on the characteristics of soil. Forensic soil investigation is the analysis of soil evidence in the service of crime solving, environmental monitoring, and geologic exploration. Analysts use a combination of geological, ecological, biological, or chemical to identify soil origin, link evidence to crime scenes, or reconstruct events. This study aims to conduct a comprehensive review of existing literature on soil as trace evidence, with a focus on its forensic applications. This covers various aspects of forensic soil investigation, including Physical methods (grain size analysis, density gradient measurement), chemical processes (organic matter analysis; loss on ignition (LOI), pH), biological methods (DNA analysis, microbial community analysis), mineralogical analysis (SEM-EDX, XRD), for soil analysis are evaluated for their applicability and limitations. It concludes by arguing the importance of assembling multiple disciplines for effective forensic soil investigation. It provides a helpful resource for forensic scientists and researchers, as well as for investigators and practitioners in forensic science.

Keywords: Forensic soil analyses, Trace evidence, Physical methods, Chemical methods Interdisciplinary collaboration

Introduction

Soil is unprocessed mineral and organic material on the Earth's surface, featuring complex physical, chemical, and biological properties from interactions between organisms and non-organisms. In forensics, it serves as crucial background evidence, as its geographical source can be identified and applied

in various forensic contexts ¹we hypothesized that soils can be forensically distinguished through the analysis of their clay fraction alone, and that samples of the same soil type can be consistently distinguished according to the distance they were collected from each other. To test these hypotheses 16 Oxisol samples were collected at distances of between 2 m and 1.000 m, and 16 Inceptisol samples were collected

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at distances of between 2 m and 300 m from each other. Clay fractions were extracted from soil samples and analyzed for hyperspectral color reflectance (HSI). Soil contains plant debris, microorganisms, and minerals that can link individuals or objects to crimes. Therefore, soil analysis is crucial for environmental forensics, applied investigations, and criminal and counter-terrorism efforts².

Soil, rocks, minerals, and man-made particles like bricks are crucial in linking suspects to crime scenes. In Corryn Rayney's 2007 Perth murder, synchrotron XRD matched red brick particles from her body to her home, suggesting she was attacked in her front yard, providing crucial evidence³. The case study involves a hit-and-run where two suspects fled. Control and alibi soil samples were analyzed using morphological, microscopic, XRD, and DRIFT methods to determine if the suspects crossed the crime trail.⁴

This review covers methodologies in forensic soil investigation, including physical, chemical, biological, and mineralogical analyses. Physical methods like grain particle size analysis and density gradient measurements help define soil texture and structure. Chemical methods, such as organic matter analysis, loss on ignition (LOI), and pH, determine soil elemental content. Biological techniques, including

DNA and microbial analysis, add complexity by focusing on living microbes in soil. Therefore, the purpose of this current review is to perform a comprehensive review of existing literature on soil as trace evidence with consideration to forensic aspects. Consequently, forensic soil analysis is a complex discipline involving different methods for better analysis of soil and its evidence. When integrated, these methods allow a direct linking of soil samples to a crime scene as well as resources for solving a case and acquiring useful forensic conclusions.

Physical Methods

In forensic soil investigations, the physical properties of soil like grain size distribution and density measurements are important of identification of a specific soil sample⁵. Grain size analysis is a key method in forensic soil investigation, allowing forensic geologists to sort and compare soil samples based on particle size distribution⁶. Grain size analysis uses sieving and sedimentation. Sieving involves sorting soil particles using mesh-sized sieves, expressing coarse material in size fractions for easier assessment⁷. However, Sieving effectively quantifies soil composition, with grain size analysis being more useful for descriptions and exclusion rather than diagnostic purposes⁸.

Table 1: Grain size distribution USDA(United States Department of Agriculture) and ISSS (International Society of Soil Science) classifications:

Classification	USDA Grain Size (mm)	ISSS Grain Size (mm)	Difference
Sand	0.05 - 2.0	0.02 - 2.0	The key difference lies in the sand-silt boundary: 0.05 mm (USDA) and 0.02 mm (ISSS)
Silt	0.002 - 0.05	0.002 - 0.02	
Clay	<0.002	<0.002	Both classifications define clay similarly

Despite its drawbacks, sieving remains crucial in forensic soil analysis, aiding in understanding soil gradation and comparing crime scene samples to suspects⁹. Forensic science also employs geological methods to differentiate soil samples by texture and composition¹⁰. Thus, while time-consuming, sieving is still a fundamental tool in forensic soil analysis⁹.

Goin and Kirk introduced the density gradient method into forensics for the first time in 1947 as a way to distinguish between various soils densities.

since been used as a common forensic test standard in determining the origins of soils¹¹. Linear density gradients could be established in 30 cm glass tubes employing bromoform/bromobenzene solutions. The grades varied between 1.5 and 2.9 g/mL and could be relied on in the separation of soil samples that were difficult to differentiate based on their physical appearance¹². In a study, 241 soil samples from 133 Southeast England locations were analyzed using density gradient tubes containing bromoform

and bromobenzene. While density gradient analysis is efficient, it is time-consuming and may require fine-tuning¹¹. The density gradient tube technique examined soil samples from the same area at different depths using bromoform (2.9 g/mL) and bromobenzene (1.5 g/mL). Seven layers formed overnight, revealing significant differences in color, density, texture, and consistency¹³.

A density gradient tube is a glass tube filled with liquids of varying densities, increasing towards the base. Heavy liquids like bromoform (2.89 g/mL) or tetrabromoethane (2.96 g/mL) are combined with lighter liquids such as bromobenzene (1.50 g/mL) or ethanol (0.789 g/mL). Soil samples are crushed and placed in the tube¹⁴. The density gradient range has been expanded from 2.89-1.50 g/mL to 4.24-1.00 g/mL using Clerici's solution and distilled water, allowing analysis of a broader range of soil mineral particle densities¹⁵.

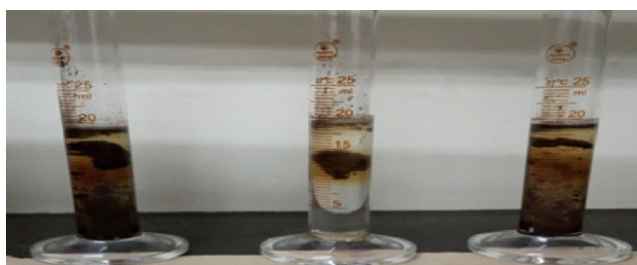


Figure 1: A density gradient tube for soil sample

Table 2. Temperatures and heating times for different types of soil^{21,22}

Soil type	Temperature (°C)	Heating Time (hours)	Purpose	
Sandy Soil	105	1	Drying	
Loamy Soil	105	1.5		
Silty Soil				
Organic Soil				
Clay Soil				110
Peaty Soil	105	2		
Sandy Soil	450	2-3	Ashing/Organic Matter Removal	
Loamy Soil	500			
Silty Soil	550			
Clay Soil				3
Peaty Soil				4
Organic Soil	600			4
	650			2-4

Soil pH measurement is crucial in forensic analysis for distinguishing soil samples based on chemical traits. Using small samples (2.5g to

analysis¹⁶

Chemical Methods

Organic matter is crucial in forensic soil analysis, providing provenance details. Variations indicate environmental differences and human activity, aiding criminal justice and environmental studies^{17,18}. Loss on Ignition (LOI) method is a cheap and easy technique for assessing organic matter, but may take time if used only at specific temperatures¹⁹ but is time-consuming and provides information only for specific, pre-determined temperatures. It also requires relatively large sample sizes and is destructive. Thermogravimetric analysis (TGA). The LOI method measures soil sample loss when heated in a muffle furnace. In a study in Manchester Township, NJ, a 2.0 g soil sample lost 4.319% of organic content, while samples from Connecticut fell between 13.747% and 13.309%²⁰.

The study found that temperature and heating time significantly impact soil mass loss. Sandy soil exhibited greater mass loss at temperatures between 350°C and 550°C, while silt loam soils showed gradual increases in LOI values across all temperatures. Higher heating times resulted in LOI values ranging from 2.22 at 350°C to 6.60 at 650°C.²¹ It is essential to control parameters such as furnace type, sample mass, integration time, temperature, and soil clay composition during loss on ignition analysis²¹

50mg), researchers noted significant pH variations across different soil colors, with a specificity of about 0.4²³.

Table 3. Soil pH values, and types of soil reactions²⁴.

PH Range	Soil Reaction Rating
<4.6	Extremely acid
4.6-5.5	strongly acid
5.6-6.5	moderately acid
6.6-6.9	slightly acid
7.0	neutral
7.1-8.5	moderately alkaline
>8.5	strongly alkaline

Soil pH is typically measured using a calibrated pH meter, which is reliable for forensic context analysis and field research in soil samples²⁵. The study found that measuring soil pH in suspensions with deionized water and 1M calcium chloride provided useful differentiation between samples, particularly in the fall, despite some inconsistencies²⁶. Four colorimetric methods were evaluated for forensic use, with the 2.5:1 water-to-soil ratio and centrifuge separation method being the most satisfactory, improving pH measurement reliability and accuracy²³.

Biological methods

DNA sequencing and microbial population's analysis adding forensic scientists obtain data when traditional soil analysis fail to differentiate between comparable samples.²⁷ Metabarcoding accurately identifies microflora, plants, metazoans, and protozoans, enhancing forensic investigations by distinguishing similar samples from different geographical locations. This DNA-based method involves extracting DNA, amplifying genetic regions, and sequencing to quickly and accurately identify species in environmental samples like soil.²⁸

High-throughput sequencing and DNA extraction methods have shown promising findings in forensic science, revealing clear distinctions even in mixed soil samples, making it highly useful²⁹. Amplified Ribosomal DNA Restriction Analysis (ARDRA) is a forensic soil analysis technique that uses ribosomal DNA amplification and restriction enzyme digestion to identify microbial communities. This method helps correlate soil with crime scenes, increasing the accuracy of soils as forensic evidence.³⁰ Despite potential for forensic investigations, microbial profiling methods face challenges due to environmental factors affecting including, pH, land

management, climate, soil texture, nutrient levels, bacteria and archaea, making accurate interpretation challenging³¹

Microbial profiling, a method used in forensic science, has shown efficiency and reproducibility in identifying soil types, as demonstrated in study was conducted in Miami-Dade³²

Mineralogical analysis

SEM-EDX is a non-invasive method that retains the sample's admissibility for legal purposes as well as offering definitive qualitative and quantitative information³³. The technique utilized in forensic examinations for high definition imaging and chemical compositional analysis as well as providing detailed information on surface topography³⁴. SEM-EDX is a reliable and reproducible forensic examination method for criminal and environmental cases, enabling comparison of soil samples and analysis of rocks, sediments, dust, and soils³⁵ continues supporting its forensic applicability by using the clay and soil sources to solve the double murder case³⁶.

In a study developed a new semi-automated SEM-EDX that indicates how well it can distinguish samples from different parent populations in forensic soil analysis³⁷. additionally the importance of automated mineralogical profiling undertaken with SEM-EDX to identify a bedrock lithology that may help in Geolocation and comparison of forensic samples³⁸.

SEM-EDX used in geographical investigations such as comparing Canterbury (UK), Dubai (UAE), and Kerala (India) with their samples of soil texture, color, and composition³⁹. Another study comparing the effect of homogenizations at elemental composition level on the 17 sample collected in Istanbul using SEM-EDX⁴⁰ and have some characteristics because of the natural effects and transfers made by human and other living beings in time. So that forensic examination of soil is not only concerned with the analysis of naturally occurring rocks, minerals, vegetation, and animal matter. It also includes the detection of such manufactured materials such as ions from synthetic fertilizers and from different environments (e.g., nitrate, phosphate, and sulfate).

XRD, to determine crystalline phases of soil sample, XRD is shown capable of analyzing soil's mineralogy to resolve a real-world murder investigation³⁶. XRD can classify samples based on the composition of minerals, whereas in the Swan Coastal Plain study quartz sand coatings were incorporated in order to identify sandy soils⁴¹. The review discusses the effectiveness of XRD in distinguishing minerals and confirms its importance in forensic soil examination⁴².

Application of Forensic Soil Analysis

Forensic soil analysis is essential for crime investigation, geological exploration, border security, and disaster management. It identifies suspects; maps pollution sources, detects smuggling, and locates mineral deposits. Additionally, it supports disaster management, archaeological surveys, and climate change studies by linking evidence to crime scenes and employing advanced analytical techniques^{4,43,44}

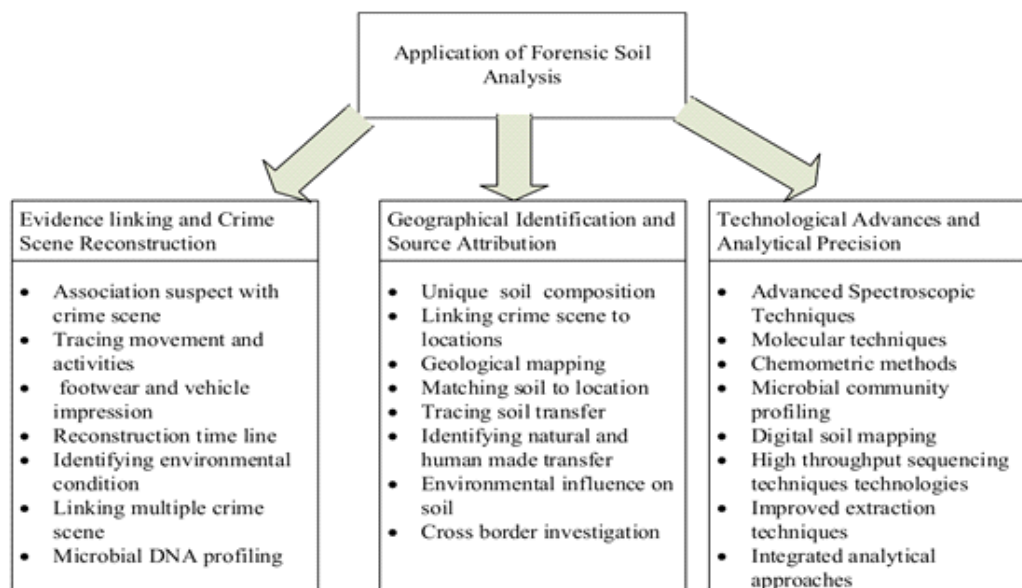


Figure.2 Application of forensic soil analysis^{4,45,46}

Challenges and Future Directions

The chemical method of soil analysis has limitations, especially when used in isolation, due to sample-related factors and expert intervention, resulting in errors in conclusions⁴⁷. Influence of microbial populations in soil samples and database created quite a challenge in relating collected soil samples to the very specific position³⁰ Mishandling by introducing contaminants, personal protective equipment (PPE), cleaning, and decontamination of working surfaces are critical prevention activities within forensic settings⁴⁸

Soil is heterogeneous in nature, and there are no uniform process controls for comparison, which makes forensic soil analysis challenging¹⁷.

However, several practical issues, including small sample sizes, time constraints, and funding issues, also limit comprehensive research and

the capacity to deliver clearly forensic results⁴⁹. Combining analytical techniques with improved statistical tools and reference databases can boost the confidence and probative value of soil analysis in forensic investigations⁵⁰

Conclusion

Soil is vital in forensic science as trace evidence, linking suspects, victims, and crime scenes. Its composition of minerals, organic materials, and pollutants can be analyzed to connect samples to specific locations or objects. Forensic soil analysis aids in crime investigations, geological surveys, border security, contamination assessment, and disaster victim identification. This review discusses various methodologies, including physical, chemical, biological, and mineralogical analyses, emphasizing the need for interdisciplinary collaboration. However, challenges like contamination, sample heterogeneity, and standardization persist.

The review encourages continued research on novel DNA analysis techniques that complement traditional physical and chemical approaches. Technological advancements are improving methodologies and expanding the capabilities of forensic soil analysis, ultimately serving as valuable resources for forensic scientists and practitioners.

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