Measurement of soil characteristics for forensic applications

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Soils may constitute evidence that connects a person or object to a particular location. The value of soil stems from its ubiquity and transferability to objects or persons. Due to the complexity of soil, the analysis of its inorganic and organic components can provide complementary and independent types of information about its geological origin, dominant vegetation, management and environment. We present an overview of a range of soil characterisation methods including chemical analysis, mineralogy and palynology, along with new approaches such as DNA profiling and profiling of other digital data such as that obtained from X-ray powder diffraction, infrared spectroscopy and organic marker analysis.

Individual analytical techniques have different scales of resolution and relevance depending on the nature of the criminal case and context. Each method has its strengths and weaknesses. As more methods have become digital and quantitative, their use in combination as digital profiles will help to characterise soils more accurately and at different scales. These new approaches can be tested using existing soil databases, and database development and use will help to refine and narrow probable origin of a questioned sample in police intelligence, as well as giving increasingly robust sample comparisons for evidence. Copyright © 2010 John Wiley & Sons, Ltd.

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Introduction

Soils are complex materials. They consist of both inorganic and organic components in varying proportions. These components may be naturally occurring or introduced by the activities of man, and so soils contain a wealth of information of potential forensic use. In addition, the particulate nature of most soil components and the customary contact of people and objects with the ground surface create numerous opportunities for the transfer and subsequent recovery of soil as potential evidential material. Thereafter, any of the biogeochemical characteristics of soil found on potential evidential items, i.e. the ‘questioned’ soil, may be used to indicate its provenance or to compare it with other samples of known provenance. As such, soil may be used for investigative/intelligence purposes during enquiry or for evaluative/comparative purposes which culminate in the presentation of soil as evidence in courts of law.

There is a wide range of analytical approaches that can be used for the forensic investigation of soil, and many different parameters that can be measured. Traditionally, the inorganic (i.e. mineral) components have been most commonly represented in soil forensic investigations,[1] while, apart from palynology,[2] the organic (plant and animal derived) components found as physical evidence[3] have been given much less attention. There may also be rare or unusual components, either naturally occurring or anthropogenic, that are held within the soil sample. Such components may considerably increase the level of discrimination or association of samples for forensic comparison. More often, however, the forensic soil sample does not contain rare or unusual components. Identification of source, or linking to a crime scene, is then reliant upon the specific characterisation of the common inorganic and organic constituents of soil samples in ways in which they can be compared precisely and contrasted with other samples. Most of the methods and techniques that find current application in forensic soil science are bulk methods. Surfaces, however, abound in soils and the principles that guide the application of bulk methods should be equally applicable to methods that potentially are capable of characterising the surface properties of soil components for forensic comparisons.

The concept of using soil as evidence has a long history, dating back to the writings of Sir Arthur Conan Doyle between 1887 and 1893 through his ‘Sherlock Holmes’ book series, and there are many examples throughout history where soil has been used as physical evidence.[4–7] High profile cases such as the work of the FBI in the Camarena case,[8] the laboratory of the Garda Siochana in the Lord Mountbatten case[9] and G Lombardi in the Aldo Moro case[9] have contributed to the general recognition that soil as physical evidence can make significant and important contributions to criminal investigations. In our opinion, however, there is still a widespread lack of awareness among the legal profession and police forces as to the true potential of soil as evidence. Against this background, the widespread application of modern soil analytical methods in forensic investigations is a relatively recent development, so much so that the merits of one method compared to another are barely known, and the choice of methods used is often made on a rather ad hoc basis, sometimes having more to do with availability in a given laboratory. Most of the properties of soil components are continuous variables and, in a forensic context, account must be taken of the scale at which such properties vary and the availability of comparative information. In addition, there are technological limitations on the amount of material required for many methods and this represents
a common constraint on choice as well as sequence of analytical measurements.

According to Murray,\cite{10} in the United States the common methods currently applied in forensic laboratories for the analysis of soil trace evidence are colour (before and after heating), macroscopic observation, low-power stereo-microscopic observation, determination of anionic composition by capillary electrophoresis and the determination of elemental (chemical) compositions of soil particles by scanning electron microscopy using energy dispersive X-ray spectroscopy. Other than palynomorph identification, soil biological components have received limited recognition in forensic soil science. In Australia, in addition to the common features of soil, the use of wider soil characteristics is now being implemented\cite{11} such as may be obtained by the analysis of soils by diffraction and spectroscopic methods.

Currently, soil analyses for investigative intelligence gathering are generally only performed in cases of serious crime, whereas they may be used in the evidential stage of many less serious cases. There is an opportunity, however, for the development of a soil forensic approach that would permit a greater use of soil information in the intelligence phase of police operations as well as the evidential phase.\cite{12} In this context, soil comparisons may be used not only to associate but to eliminate areas of land and/or suspects from further police enquiries, thus permitting the reassigning of limited resources.

An outline of where traditional methods, as well as new approaches, have been used in a forensic context will now be presented, followed by a brief perspective as to possible future directions and legal considerations. The nature of the forensic process means that it is difficult to predict which methods of soil analysis will be the most appropriate and useful for a given sample of soil and case context. As a result, the forensic investigation of soil typically starts with an initial examination stage where the sample is assessed and possibly sub-sampled. Methods that are frequently integral to this initial assessment are described first, along with methods that are usually applied to soil material as a whole. This is followed by a commentary on more specific methods that in research are traditionally applied to either the inorganic or the organic components of soil, although some are quite widely applicable to both. Following an emerging new paradigm of digital soil typing, one important future direction is the potential integration of multiple digital analytical measurements, and the concept of this approach is highlighted. Ultimately, the methods of choice depend on the size and condition of the soil sample and whether it is being studied for the purpose of a sample comparison as evidence or in providing clues in intelligence for police search and detection. In practice, the choice of methods often also depends upon the instruments available for analysis and, of course, their costs. Consideration has also to be given to the destructive nature of an analysis technique and, consequently, the logical order in which a series of measurements are to be made.

**Initial Sampling, Examination and Description**

Collection of the forensic soil sample, often by its removal from an object, commonly dictates that some properties or characteristics of the sample must be recorded in this preliminary stage. In addition, there are also key requirements for collection of comparator samples from a scene of crime (SOC) that must be considered at the outset of any investigation. Ideally, the scientists conducting the analyses should carry out all the soil sampling, but in practice, samples are often taken by the SOC officer. Although it may seem obvious, effective and relevant sampling assumes a basic knowledge of soils and how they may be transferred to objects. For example, with burials, soil sampling may need to consider the different soil horizons present in a soil profile.\cite{13} Likewise, an evidential sample may consist of several different soil layers, and careful dissection may be required to sample the layers independently. When footwear marks are evident at the SOC, either samples can be taken directly from these marks (after photography has taken place), or soil can be recovered afterwards from plaster casts of the marks, taking care to avoid contamination with plaster. If an area of bare soil is exposed within the area around the crime scene, such as a flowerbed below a window through which forced entry has taken place, samples should be taken from these areas at the points where an offender is most likely to have stood\cite{14} and likely made contact with the soil. In addition, if paths/routes of entry/exit can be identified, sampling should take place along these routes.

Soil collected for comparative purposes must be relevant to the soil that was removed. In most cases, this means the surface topsoil. Consequently, care needs to be taken in avoiding contamination of the soil surface with deeper soil horizons, particularly for biological components. If soil is adhering to a shoe, then the whole shoe should be wrapped with the soil intact and carefully transported to the laboratory.\cite{15} If soil is adhering to materials or objects, then the whole item should be collected and bagged, and examined in situ with an appropriate technique for the amount of soil available. Sample preparation is also of vital importance. For example, it is imperative that moist (i.e. not air-dried) samples do not stay for long in a sealed container, since microbial activity may alter the microbial/organic component signature of the soil. Attention also has to be given to sample representation, measurement uncertainty (instrument and sample preparation) and the extent of natural variation.

Soil examination generally starts with a visual comparison and microscopic analysis, gathering information on characteristics such as structure, texture, colour and organic matter content.\cite{5} Typically, a stereo binocular microscope is used to carefully examine the soil sample and identify and record any foreign objects held within the sample, such as fibres, metals, paint, glass and plastics, which other specialists might examine. Any individual seeds or leaves can be recorded and sub-sampled at this time. When unusual or rare particles are found in soil, precise and rapid discrimination may be achieved.\cite{16} For this reason, Sugita and Maruma\cite{16} suggest that microscopy is still the most useful initial technique.

Although no standard forensic soil examination method exists, the identification of soil differences using various morphological attributes (colour, consistency, texture and structure) on whole soil samples is an important first step for using soil information to help police investigators at crime scenes.\cite{11} However, it should be borne in mind that many of the conventional soil characteristics such as particle size distribution and pH may require about 1 g of soil, which is seldom available in a forensic investigation. Also, measurements such as texture and consistency require the scientist to touch and hold the sample, which is not generally possible for forensic evidence.

Colour has the advantage that it is one attribute commonly recorded in soil databases, although it is usually recorded as a field-moist measurement. However, as colour varies with moisture content, and is often characterised using the semi-subjective
comparison to Munsell soil colour charts,[17] care has to be taken in its applicability in forensic casework. In a study of 73 soil samples,[18] multiple colorimetric features such as colour after air-drying, after wetting, after organic matter decomposition, after iron oxide removal and after ashing were used. Although only about 70% of the soils could be differentiated by comparing the colours of air-dried samples, combining colour measurements on soils after air-drying and wetting, with colour on the clay fraction after organic matter decomposition and iron oxide removal enabled them to differentiate 97% of soil samples. When combined with particle size analysis by sieving, it was then possible to discriminate 99.5% of the soil samples. Instrumental methods have now been applied to soil colour analysis with increased resolving power, e.g. the Minolta CM2600d spectrophotometer. Results of research and casework using colour have been presented,[19] which show the spectrophotometer to provide a precise and rapid method for soil sample comparison. However, care has to be taken in sample preparation such as in milling; and colour would never be used as the only measurement on a forensic soil sample in the United Kingdom.

Particle size is a physical property of any soil that can provide important clues to the nature and provenance of a sample. However, in forensic comparative analysis an important point to bear in mind is that, because of transfer and selective persistence, physical methods such as particle size distribution may be altered. For example, sand may not be retained on a shoe or a vehicle as readily as clay, but the sand fraction will still be present in the control sample. Soil traces adhering to boots, shoes and clothes have been compared with control samples using particle size analysis in the past. For example, in a study,[20] a loss of the coarse fraction was observed on most of the suspect samples, even though they were derived from soils having different distributional patterns. Experimental results also suggested that equal weights of soils should be compared in the determinations of both control and questioned samples.[21]

The ‘field’ conditions at the time the soil is collected (as a control or contact trace), the pre-treatment of the soil prior to sieving and the form of sieving (wet or dry) may also influence the particle distribution. For forensic work, the particle size distribution of sometimes very small samples requires precise determination using a rapid and precise high-resolution method. Recently, particle size analysers have been used for the fine particle fraction.[22] The Malvern Mastersizer 2000 laser granulometer, for example, offers rapid and precise sizing of particles in the range 0.02–2000 µm for a variety of sample types, including soils, unconsolidated sediments, dusts, powders and other particulate materials. It is possible to use this technique for sample weights of just 50 mg, although the minimum soil weight limit to produce reproducible results is strongly affected by the size distribution, with coarser grained materials requiring a larger sample weight. Discrimination between samples is performed on the basis of the shape of the particle size curves and statistical measures of the size distributions. There are, however, many factors that need to be considered when making particle size measurements, and in the forensic context it is essential that any factors that may affect the precision of the measurements are fully understood and controlled.

**The Inorganic Soil Component**

The value of an inorganic characterisation is that the inorganic fraction is generally inert and not affected by time or sample storage. The inorganic component reflects not only the underlying bedrock geology, via rock fragments, minerals and weathering products, but also anthropogenically introduced products, which may be particularly useful for site characterisation. However, because of issues of selective transfer it can be difficult to be fully quantitative in comparison and important that, as far as possible, a ‘like with like’ comparison is made when comparing an evidential sample with a control. In this regard, it is often a particular size fraction that is analysed for the inorganic characterisation.[11]

**Detailed examination**

Examination of soil samples with conventional light microscopes can provide a wealth of information but electron microscopes such as the SEM provide a resolution not possible with lower power optical microscopes and so allow for much more detailed examinations to be made (Fig. 1). Depending on the size of the forensic object in question, it may also be possible to examine soils by SEM without removing them from the object in question. SEMs have been more commonly used in forensic science for the identification of fibres, hair, paint, fossils and any other ‘unusual’ objects, but many of the more common mineral particles of soil may also display a variety of distinctive attributes, e.g. size, shape, surface texture and chemical composition, that enable them to be compared from one sample to another. Additionally, the individual particles in a soil are frequently associated into aggregates, and these associations and other textural data can be observed. Energy dispersive spectroscopy (EDS) in the SEM is frequently used to characterise the elemental composition of particles, and backscattered electron (BSE) imaging and X-ray mapping provides another means of locating unusual particles and of mapping their distribution, which may be of diagnostic or discriminatory importance. The SEM has been also used in the forensic analysis of quartz grain surface textures, which uses the ubiquitous nature of quartz grains along with the natural variability of surface shape and texture to compare sites.[23–25] Transmission electron microscopes (TEMs) have seldom been
used in soil forensics but they have the advantage that extremely small amounts of material (a few micrograms) can be examined in great detail. Furthermore, TEM has been widely applied in soil mineralogy, providing a wealth of background information on characteristics that may be recorded with the TEM. Criticism has been voiced at the potentially unrepresentative nature of many of these methods, with too few particles being examined, its subjectivity and over-reliance on qualitative evaluation.

Elemental/chemical analysis

One inorganic criterion used for soil sample comparison is the composition of major and trace elements, either in the bulk sample or in one or more separated fractions. Analytical method development has meant that increasingly smaller sizes of sample can be analysed in terms of their elemental composition. X-ray fluorescence (XRF), atomic absorption spectroscopy (AAS), inductively coupled plasma (ICP) spectrometry, neutron activation analysis (NAA) and energy and wavelength dispersive X-ray (EDX and WDX) microanalysis are the most widely employed methods used to measure elemental composition.

In a study of soil samples collected from 110 different sites in the Kyoto district, Japan, analysed quantitatively using X-ray fluorescence spectroscopy, the amounts of elements, such as Sr, Rb, K and Fe, allowed classification of the soils into nine types that showed good agreement with geological features. Probabilities of correct identification, by comparing unknown soils with control datasets, were about 71%, according to the systematic discrimination that was derived from multivariate analysis and a geochemical survey map of soils.

ICP spectrometry has been used to measure the abundance of a broad range of elements, but requires dissolution of samples. ICP-OES (optical emission spectrometry, sometimes called ICP-AES) and ICP-MS (mass spectrometry) are the main two types of analysis, providing concentration data for around 60 elements. Important considerations in using this type of information is how the values in the suspect sample may reflect a different fraction of the whole, and, indeed, how it compares with every other sample. Uncertainty increases as concentrations approach the limit of detection. A study of three soils using small sample sizes (0.05 g) showed that between-sample variability had an effect on uncertainty in the result, with uncertainty increasing as the concentrations approach the lower limit of detection. Although analysis of major and trace element data has been used in forensic comparison of soils, and ICP can provide concentration data for a wide range of major and trace elements relatively quickly and at a reasonable cost, it has been suggested that it should only be used in combination with other methods.

Geochemical techniques, using isotope ratios, and geochemical signatures have also been utilised in forensic work. The use of isotope ratio mass spectrometry in the linking of forensic drug and soil samples from crime scene to suspected point of origin was recently adopted. These methods, however, do not always discriminate samples effectively on their own. (Used in combination with other analytical techniques, and indeed coupled with other elemental analyses, isotopic analysis may prove to be a useful tool in a forensic context. In a study to compare single source and primary transfer soil samples, four analytical techniques (spectrophotometric colour determination, laser diffraction particle size analysis, stable isotope analysis and chemical element analysis) were used. Four soil types and five footwear types were investigated. All the four techniques showed excellent precision and good resolving power between the soil types. Only relatively small differences were obtained between source and transferred soil samples in terms of colour, stable carbon and nitrogen isotope ratios and elemental chemistry, while significant differences were found in grain size, indicating that the primary transfer process is to some extent grain-size-selective.

The lead contents of 206 soil samples determined by AAS indicated that such determination can provide a useful parameter for soil comparison and discrimination in forensic science. Soil investigations near a former smelter in Colorado, USA, revealed that historic use of arsenical pesticides has contributed significantly to anthropogenic background concentrations of arsenic on certain residential properties. A variety of techniques including spatial analysis, arsenic speciation and calculation of metal ratios was successful in the separation of smelter impacts from pesticide impacts but such an application would tend to be limited to specific locations.

Mineralogy

Most soils are composed predominately of minerals, many of which are derived from the geological parent materials. Fundamentally, the elemental composition of the inorganic fraction of soils is determined by the mineralogical composition, but there is more to mineralogy than just chemical compositions. A mineral is a solid material consisting of fixed proportions of various chemical elements arranged and bonded together into a regular structure, known as a crystal structure. Thus, different kinds of minerals not only have distinctive elemental compositions but also have a great variety of distinct physical and chemical properties determined by their different structures. There are many methods for studying minerals and measuring their properties. In principle, any of these methods could find application in the forensic examination of minerals in soil.

Soil is generally developed on residual or transported geological material and so may always be traced back to the parent rocks from which it has formed. This means that all of the minerals that occur in rocks may also occur in soils, in addition to those formed by soil-forming (weathering) processes. There are thousands of different minerals but in most soils the main minerals and groups of minerals; encountered above trace concentrations (<1%) consist of around 20 or 30 common types, including quartz, feldspars, amphiboles, pyroxenes, iron oxides, aluminium oxides, sulfates, carbonates, zeolites and clay minerals. Feldspar, for example, is a name for a group of minerals, and plagioclase and potassium feldspars represent a further subdivision of feldspars. Similarly, where plurals are used for clay minerals, e.g. smectites, this refers to a group of minerals which may be further subdivided. An understanding of how minerals are identified and classified is important because minerals may be distinguished from each other at different levels of detail. Furthermore, even examples of the same mineral species can still possess many features that distinguish one occurrence from another. In other words, different examples of the same minerals are rarely identical in detail, and therefore the forensic scientist must be aware of the level to which minerals are differentiated when assessing the significance of mineralogical data.

In nature, the common set of soil-forming minerals is typically further restricted over wide geographic areas because of the influence, or otherwise, of various soil-forming factors, the most important of which are parent material and degree of weathering. A typical soil will contain a suite of around six to ten different
major minerals. (A major mineral may be defined as one that is present at a concentration of a few percent or more, at which it will be readily detectable by routine techniques such as X-ray powder diffraction (XRPD) or microscopic examination, either optical or electron.) It is also quite usual for several other less common minerals to be present in any given soil but usually in amounts that put them below the routine detection limits of many techniques. Nonetheless, these ‘accessory’ or trace minerals can often be concentrated by some means that separates the soil sample into different physical or chemical fractions, thus effectively lowering the detection limits for trace minerals. For example, there are many procedures for obtaining the so-called heavy mineral fractions from soils. These fractions consist of minerals with densities greater than those of the more common silicate minerals such as quartz and feldspars. Once identified, trace minerals are potentially more distinctive than the more common minerals that make up the bulk of the soil.

One of the simplest, oldest and most widely used techniques for the identification of minerals is polarised light microscopy. A skilled microscopist can quickly obtain a wealth of mineralogical information. It has been suggested that the examination of around 2–5 mg of the 120–140 mesh fraction obtained by sieving is the most appropriate.[37] Of course, any soil size fraction could be examined but it is important that samples are compared on a like-for-like basis, and this is aided by the selection of specific size fractions.

A key issue with all forms of microscopy is that much depends on the skill of the operator. This applies both in terms of recognising the rare or unusual features of a sample and in terms of the judgments that must be made when attempting to characterise and/or quantify the more mundane components for comparative analysis. With the development of automation of such procedures, such as in the QEMSCAN®, an automated SEM electron microprobe system, analysis can be largely operator independent.[38] The QEMSCAN technology,[38] successfully used in the mining industry, has considerable potential in forensic geoscience and can analyse thousands of mineral grains in a matter of hours to produce statistically reproducible modal mineral analyses, as well as recording other aspects such as grain shape. Its use, however, is optimal when the sample can be presented with a polished surface. With such systems, however, it should be borne in mind that mineralogy is determined indirectly from data on chemical composition and there are also limitations due to the inability to measure organic components. Its application to samples presented as grain mounts with rough three-dimensional surfaces will also undoubtedly introduce the same uncertainties that affect accurate elemental analysis of such materials in conventional SEM/EDS.[39] Furthermore, the analysis and precise identification of clay minerals is also likely to pose some difficulties, as most clay minerals will be smaller than the sample volume excited by the electron beam, so that the elemental compositions recorded will often represent mixtures rather than individual clay minerals. Nonetheless, automatic analysis systems are clearly very attractive because of their precise and quantitative output.

As mentioned previously, the mineralogical composition of soil is generally a function of particle size. The smallest particles in a soil sample comprise the clay fraction. The term ‘clay’ is used both in the sense of a particle size fraction (<2 μm) and in a mineralogical sense to refer to a specific group of minerals; the so-called clay minerals. Most soils contain clay minerals and some consist mainly of clays. Traditionally, soil mineralogists have studied the clay minerals of soils by analysis of a clay-sized fraction[26,40] and isolated them by particle size separation from the non-clay minerals such as quartz and feldspars, which occur predominantly in larger particle size fractions. Forensic scientists have also focussed attention on the clay fraction of soil, perhaps in part because the properties of clays and clay minerals make them more likely than any other soil fraction to be transferred and persist after contact.[15] Various instrumental methods are used for clay mineral identification, but XRPD is of paramount importance.[26,41] Fig. 2 illustrates an example of an X-ray diffraction pattern of soil with peaks that are used to identify the different mineral components. Clay mineralogical analysis is a specialised discipline with most analyses conducted in terms of quantitative representation.[41] The potential variability of clay mineralogy from one soil type to another is at the core of its potential application in forensic science.

XRPD is also a very useful tool for the identification of all the major minerals present in soils, not just clay minerals. In the United States, X-ray diffraction data are accepted as legitimate ‘signatures’ of the provenance of samples.[42] Recently, there have been many developments in the use of XRPD data for quantitative analyses of materials.[43] It has been commented that ‘Quantitative x-ray diffraction could possibly revolutionise forensic soil examination’[43] and with developments to miniaturise sample requirements this will considerably expand the application of this approach. As with many analytical methods, sample preparation for XRPD is of paramount importance, particularly with regard to particle size requirements and preferred orientation. Inadequately controlled, either of these may cause severe imprecision in the intensity of diffraction. In many instances, the amount of soil available for study in a forensic investigation may preclude the use of conventional powder diffraction sample preparation. In such instances, one can use an X-ray diffractometer fitted with a system for analysis of samples loaded into thin glass capillaries. The amount of material needed for analysis by XRPD in a capillary is of the order of a few to a few tens of milligrams. The capillary is usually spun around its axis during analysis and is second only to spray drying[44] as a method of producing near-random powders and hence reproducible diffraction data. Combined with new position-sensitive detectors such as the X’celerator from Panalytical or Bruker’s Vantec, such systems may become the configuration of choice for forensic XRPD of bulk soil. XRPD has also been used effectively for the in situ non-destructive screening of ‘questioned’ samples,[45] thus dealing with some of the arguments about homogenisation of sample[46] and potential loss of valuable trace amounts of material.

Other chemical methods – inorganic and organic

Some methods are equally applicable to both inorganic and organic components and foremost among these methods is infrared spectroscopy. Fourier transform infrared spectroscopy (FTIR) gives detailed soil signatures from minute samples (1 mg), thus making it a very useful tool for forensic work. FTIR gives an overall chemical fingerprint of the main organic and mineral components in soils (Fig. 3). It can also identify non-soil components/contaminants which may be of crucial importance and which may not be readily identified by any other analytical technique; also it can distinguish soil patterns under different vegetation and soil conditions as well as giving some information on the range of minerals present in a sample. In addition, information from this technique can be extended by comparing the spectrum both prior to and after pyrolysis (with and without organic matter), obtaining an increased
discrimination. Soils that had identical Munsell colour values could be discriminated by this subtractive FTIR method. A new ancillary method using thermal gravimetric analysis in addition to IR analysis on samples prior to and after pyrolysis has been applied to soils and could give additional valuable information for the discrimination of soils. IR also has the advantage that it is usually non-destructive, and can be used on trace amounts of sample. As a digital technique, subtraction of background signatures is also possible, enabling soil spectra to be obtained from evidential material such as splashes to jean material (Fig. 4). Subtle differences in the spectral pattern also can indicate the presence of rare mineral or organic phases or specific features of more common ones such as Fe substitution in clay minerals; such characteristics are not easily accessible by other methods.

The use of Raman spectroscopy of materials can also be regarded as a signature of composition, and can describe resins, waxes
Soil forensic characteristics

Figure 4. The use of FTIR for the in situ characterisation of soil adhering to denim fabric. (a) FTIR spectrum of denim with soil adhering, (b) spectrum of denim fabric alone, (c) spectrum of soil adhering to fabric and (d) IR spectrum of soil on fabric (solid line) compared directly to IR spectrum of soil from the crime scene (dotted line).

and gums, along with minerals while not requiring detachment of the material. However, whereas many laboratories have IR facilities, Raman facilities currently tend not to be as widely available.

The Organic Soil Component

Botanical fragments

Botanic evidence is useful in part due to the diversity of plant species that exist. Forensic soil samples often contain plant fragments, which until recently have received little attention. They potentially can adhere to footwear or clothing or be transferred to areas of contact.

The species identification of plant fragments present in a sample can be performed using traditional microscopic methods or the DNA sequencing of specific genes. The technique of plant DNA analysis is achievable (for tracing of illegal drugs such as marijuana) and has been accepted in the British courts.

Amplified fragment length polymorphism (AFLP) analysis of botanical forensic evidence has been shown to provide a reproducible DNA profile in a relatively short period of time in species (Acer rubrum in this case) for which no sequence information is available. Within a closed set of 40 A. rubrum plant samples, 93.8% of 14 blind samples were correctly identified.

In an experiment on transfer to footwear, the possibility of using bryophyte characterisation in forensic studies was examined. Bryophyte fragments became attached to shoes, where they remained even after a post-transfer activity on a dry road for several hours. In another experiment, fresh material of nine bryophyte species was kept in a shed in adverse conditions for 18 months, after which the DNA was extracted, and the bryophyte DNA stayed intact, allowing DNA profiling. It was concluded that bryophytes are among the most usable plants to provide botanical evidence for forensic investigations.

However, it is more difficult to determine whether or not a plant sample has come from a specific plant (individualisation) or a group of clonal plants, although recent research on pine and silver fir has utilised microsatellite fingerprinting to compare fine roots collected from soil samples to individual trees in woodland. Microsatellites are regions of high variability in the DNA and can help distinguish between individuals of the same species within a population. In the above-mentioned study, a fingerprint for each of the individual trees was first created by polymerase chain reaction (PCR) amplifying DNA collected from foliar tissue using primers targeting specific microsatellite regions in either plastid or genomic DNA (respectively). PCR products from two or more such microsatellite loci per tree result in a unique set of DNA fragments amplified for each individual tree. A similar approach may allow

matching of plant fragments at the SOC with material on an object or suspect to allow confirmation of alibi or linking to the SOC.

Palynology

Palynology (spore/pollen analysis) is a sub-discipline of botanical ecology and it has been shown to have great benefit to the criminal investigator.[2] Palynology has been tested in court and has provided evidence for contact between object and place as well as location of clandestinely disposed human remains, estimated times of body deposition, differentiated murder sites from deposition sites and has provided provenance for objects and materials. Pollen grains are produced in the anthers of flowers and can be characterised using microscopic techniques; they can differ in many ways. Pollen and spores (plant and fungal) provide clues as to the source of items and the characteristics of the environments from which the material on them is sourced. Their usefulness is due to their abundance, dispersal mechanisms, resistance to mechanical and chemical destruction, microscopic size and morphology. Their often complex morphology (Fig. 5) allows identification to an individual parent plant taxon, which can be related to a specific ecological habitat or a specific scene. Pollen and spore assemblages characterise different environments and scenes and, due to their microscopic size, they can easily be picked up and transported away from scenes of interest without providing any visual clue to a suspect as to what has occurred.[54]

A detailed procedure for the preparation of samples for pollen analysis has been presented.[55] As in all forensic analyses, the importance of minimising risks of laboratory and cross-sample contamination during sub-sampling and preparation is recognised. Palynomorphs can provide excellent trace evidence, fulfilling the requirements relating to the transfer, persistence and detection of such evidence. Palynological evidence can also provide very powerful investigative and associative evidence. However, palynology has generally been applied to forensic problems in a largely unstructured way, resulting in a lack of formalised discussion of the underlying principles. In most countries, it is extremely underutilised probably because it is labour-intensive, needs considerable expertise and experience and requires appropriate controlled and timely sample collection.[56]

Pollen composition in a trace sample can also be used to disassociate origin. For example, an investigation of a small amount of biological material isolated from a tubular component of the fuel assembly of a private plane that had crashed in New Mexico in 1989 showed that, consistent with other biological, chemical and other soil evidence, the biological material was a post-crash accumulation and was unrelated to the accident.[57] Some cases in New Zealand, with ropes, soil samples and illicit drugs, have received wide publicity and helped increase the profile of palynology as a forensic tool.[58] In a murder case, a man was found shot in the back on Mount Holdsworth in the Tararua Ranges north of Wellington. Police investigations pinpointed one individual who had been seen in the area, knew, and had the means and motive to kill the victim but his alibi was that he never had been in the area. However, pollen of Nothofagus menziesii, a mountain plant, found on his clothing suggested that his alibi of never being at such a site was untrue and that the clothing had been in mountains where N. menziesii was growing.[59]

It has been suggested that a database of pollen/spore types be initiated as a reference collection to be used for expert witness evidence in this field.[60] Increasingly, statistical approaches are linked to palynological evidence: for example, by using the likelihood ratio and considering how frequently the pollen assemblage occurs.[61] However, results showed that localised areas of similar vegetation type, even within the same geographic region, have significantly different pollen assemblages which can be used to improve discrimination.[62] In a case of alleged sexual assault, the pollen content of samples of grass clippings and soil from the suspect’s clothing and shoes were compared to that of a sample of grass clippings from the alleged crime scene (a grassy area) to determine whether or not the suspect had been at the scene. The clothing and shoe samples showed a very strong

Figure 5. Images of pollen grains found in New Zealand soil from a range of plant species differentiated by shape and form using a high-power microscope.
correlation with each other and with pollen on the sample from the alleged crime scene in the combination of the different types of pollen present, very strongly supporting the contention that the suspect had been at the scene. Nevertheless, some plant families such as the Poaceae and the Rosaceae are difficult to differentiate using this technique.

The pollen assemblages on footwear that had been worn at different rural sites in the East Midlands of England, United Kingdom, were studied. They examined the transfer of pollen to pristine boots and to boots that were previously worn at other localities. Samples of adherent soil from these items of footwear and control samples were analysed palynologically. With the exception of one sample, the pollen adherent to footwear had a characteristic signature, supporting the view of a general distinctiveness of pollen from individual sites and the concept of widespread palynological heterogeneity. The data from this study also show that when mixing occurs from wearing footwear at different sites, the pollen/spore content of the footwear predominantly reflects that of the last site visited. This emphasises the importance of seizing footwear belonging to suspects as soon as possible after a crime has been committed, as footwear will have a palynomorph load accumulated from a range of different places. However, relatively few pollen grains are picked up from paved surfaces, although lichen spores can be transferred from lichens on pavements. The most significant assemblages of palynomorphs are picked up from bare soil, mud, leaf litter organic debris and vegetation. Nevertheless, even if discrete soil patches can be identified on soles, it is rare for perfectly uncontaminated samples to be obtained and as a consequence the palynological profile will contain a mixed assemblage.

Microorganisms

The soil microbial community responds to soil type, land management and environmental conditions and soils contain a large number of diverse microorganisms. These microorganisms assemble in communities that may be specific to a location. The large microbial diversity found in soils, their functions and adaptability to changing conditions make them attractive biomarkers, i.e. the structure of these communities may be used for classification for forensic uses through the analysis of marker genes. This is compounded with the ability to characterise microbial profiles of small samples (≤0.2 g). Although the possibility of applying molecular microbial methods to soil evidence for forensic purposes is relatively unexplored, knowledge of the soil microbial ecology can be used to provide clues as to likely origin of an unknown sample, i.e. grassland rather than woodland, while the evidentiary approaches focus upon a comparison of the questioned samples with a relevant population.

The development of molecular methods such as the nucleic acid technologies as well as the use of signature lipid biomarkers have overcome the problems associated with methods which rely on culturing, and there are now a variety of techniques being used extensively in soil science to examine the diversity and ecology of soil microorganisms. Nucleic acid techniques are those that target analysis of the genetic information of organisms encoded in their DNA and RNA. The highest precision of these techniques is the complete analysis of DNA sequences, but this is invariably very slow. A lower level of resolution can be obtained by PCR fingerprinting techniques that amplify the small amounts of DNA/RNA into more measurable quantities. General procedures for investigating microbial communities involve the extraction and purification of the RNA/DNA from the sample and then amplification using the PCR followed by analysis of the nucleotide sequence. PCR is central to most nucleic acid techniques and there are numerous variations and modifications that can alter the targeting of the probes and selectivity of the amplification; the choice of primers at this stage is therefore crucial.

Microbial fingerprinting methods such as denaturing gradient gel electrophoresis (DGGE) and single strand conformation polymorphism (SSCP) use the PCR product to look at microbial communities. After amplification, the PCR products can be separated as bands by gel electrophoresis on denaturing (DGGE) or temperature gradient gel electrophoresis (TGGE). The banding pattern itself is a genetic fingerprint of the microbial community and can be analysed using multivariate methods. From a soil forensic point of view, the nature of the microbial communities is often not important per se, and it is simply another type of signature. Methods such as DGGE involve significant operator skill, and variation in the gradient of gels can influence the results. The use of TGGE somewhat overcomes some of the latter problems by using a temperature gradient to denature the DNA instead of a chemical gradient. SSCP is also a gel-based system but is simpler and faster with high reliability and can have higher sensitivity, but it is only just starting to be applied to environmental studies. A third popular method is terminal restriction fragment length polymorphism (TRFLP), which uses restriction enzymes to cut the PCR products into fragments that can be analysed on a sequencer to obtain an electropherogram. During the PCR, fluorescent primers are used so that the terminal end of the cut fragment is labelled and can be detected in the DNA sequencer. The ability to determine the fingerprint in this way offers greater opportunities for standardisation, may have greater sensitivity and more easily produces numerical output for ease of statistical analysis. The interpretation of fragment patterns can be more problematic but are suitable for comparison. Multiplex-terminal restriction fragment length polymorphism has been shown to give excellent results on soil. TRFLP has been used in at least one preliminary forensic study, where the soil bacterial community DNA profile obtained from a small sample of soil recovered from both the sole of a shoe and from soil stains on clothing were recovered and the profiles were shown to be representative of the site of collection. This method is subject to international patent applications for a range of forensic applications.

The specificity of all these techniques depends, however, on the PCR conditions and primers used, and in many cases may detect only the dominant members of the microbial community. Consequently, for forensic purposes there is a significant amount of work still to be done to ensure that the right balance is achieved between resolution and sensitivity to small-scale variation in soil. A further important consideration is that compared to human DNA as evidence where the human target is a discrete entity with a unique DNA profile, fixed at conception, the soil target is not so clearly defined.

Some fundamental issues concerning soil DNA, related to forensic context, require to be resolved to ensure that the tools that are developed provide robust and reliable data. These issues include consideration of likely sample condition, storage and handling and its consequential influence on the DNA profile, the selection of suitable microbial targets and ensuring quality control is in place, particularly in samples that may have been stored for prolonged periods. The drying of soil samples is likely to be of particular importance where soil evidence is recovered following a temporal delay or where samples may have been stored for...
prolonged periods (e.g. in cold case investigations). DNA profiling in soil ecological studies is generally carried out on fresh or frozen soil to minimise any alteration in the original community structure. However, soil forensic evidence may already be (indeed is likely to be, due to the uncertainties involved) in a dried condition and it would be helpful to stabilise the evidential samples as soon as possible following collection. Furthermore, air-drying soil represents a simple means of stabilisation commonly applied to forensic soil samples for subsequent mineralogical and chemical analysis.

However, many factors can affect the microbial community composition, and alter their structure, i.e. temporal, or weather, or spatial, and reflect the heterogeneity of the soil environment or of its vegetative cover. Moreover, many soils are managed (e.g. for agriculture) and their microbial communities may also be altered by such practices. These changes may render the comparison of soil samples, as required in forensic investigations, difficult, subject to much interpretation and consequently lead to a reduction in forensic value. Several studies have examined the potential use of soil microbial community profiles to provide provenance-dependent soil DNA profiles for forensic application.[72,75] Soil DNA analyses offer an attractive alternative to conventional soil examination, as many standard forensic laboratories are already suitably equipped and would be able to provide a relatively quick and routine comparison of the soil microbial community. Studies reporting the potential use of soil DNA profiles for forensic purposes have focussed on bacteria.[72,76] However, other target taxa such as fungi are commonly characterised in soil ecological literature. Fungal DNA profiles have been shown to be stable with soil air-drying, and soil fungi may represent a more robust target for the development of soil forensic methods compared to bacteria.[75] Therefore, while the potential for the forensic use of soil microbial communities is apparent, the practice and its limitations have yet to be established. A multi-taxa (e.g. bacteria, fungi and archaea) approach[77] illustrated with an example trace from a grassland soil (Fig. 6) is likely to provide greater resolution in identifying originating site characteristics relating to an unknown sample (investigative intelligence) and greater confidence when comparing soil samples to soil reference samples (evidential value). However, further research and validation is required before microbial community profiling methods can be used directly as a tool for the forensic analysis of soil evidence. The interplay and interconnection between the analytical method used and the patterns observed in the microbial communities result in challenges still to be resolved in this area.[74]

Signature lipid biomarkers (SLBs) or fatty acid profiling is another molecular approach that has become widely used to characterise the diversity of microbial communities.[78] Like nucleic acid methods, it is not dependent on the growth or morphology of organisms but relies on the direct extraction of lipids from cells in the soil. The most common extraction procedure for fatty acid profiling of soil communities is the one in which the phospholipid fraction is separated by fractionation and the fatty acids measured by gas chromatography. This method is considered to be a sensitive and reliable measure of microbial communities and it is possible to extract and analyse further fatty acid classes. Phospholipid fatty acid (PLFA) analysis has been used to study land use and management effects[79] and vegetation cover[80] and to biologically fingerprint windblown soil that had contaminated adjacent areas.[81] A U.S. patent covers its use for locating the origin of soils.[82] Measuring the concentrations of different PLFAs extracted from soils can, therefore, provide a biochemical fingerprint of the soil microbial community and the

![Image](www.interscience.wiley.com/journal/sia)
(<10 years). The humic fraction comprises a variety of organic materials (complex polysaccharides, lipid and wax compounds, humic acids, suberin, cutin, etc.) constituting the largest pool of organic matter. These compounds are considered relatively resistant to decomposition and can persist for thousands of years. Compounds with high turnover rates, such as most carbohydrates, proteins and nucleic acids, are likely to be less useful for forensic comparison than the compounds with slower turnover rates, which are likely to provide a more robust profile. While the complex nature of polymeric materials such as humic compounds and tannins in soil may be appropriate for describing the organic matter, they prove difficult to characterise and analyse quantitatively. Analytical methods which involve fragmenting the polymeric substances into complex mixtures of lower molecular weight compounds are available and have the potential to provide highly specific profiles of the organic component of soil. These procedures include pyrolysis and thermally assisted hydrolysis as well as methylation with tetramethyl ammonium hydroxide. Discrimination of soil samples in forensic science using organic components in the soil was investigated by Curie-point pyrolysis gas chromatography (PyGC). Pyrograms of soils under the conditions of pyrolysis temperature and time showed various contrasting patterns. In addition, 15 constituents of phenolic aromatics in pyrolysis products were identified by gas chromatography–mass spectrometry (GC–MS) (PyGC–MS). The amounts of toluene and phenol, derived from lignin, and those of 2-methylfuran and furfural derived from polysaccharide in the soil showed quantitative differences. The comparison of these pyrolysis products may be useful for the structural analysis of organic matter in the soil, and can be useful for the discrimination of soils in forensic science. The soil UV–vis absorbance spectrum of the acid fraction of soil humus was used to discriminate soils and achieved good discrimination (85% correct classification) provided multivariate statistical techniques were also applied.

However, such profiles can be very complex and difficult to interpret, and are dependent on the specific conditions used for analysis, bringing into question analytical reproducibility which may limit forensic applications.

Plant wax compounds

Within the humic fraction, plant wax compounds are found, which are complex mixtures of lipids consisting mainly of aliphatic compounds with relatively long carbon chains (C$_{20}$ – C$_{60}$) and comprise a number of classes. The most common and widely studied class is the hydrocarbons, including n-alkanes, as well as free and esterified long-chain fatty alcohols and fatty acids. Most plant wax components can be quantitatively analysed as individual compounds by GC or GCMS, after solvent extraction and separation into different compound types by liquid chromatography, most conveniently by using solid-phase extraction columns. Such analytical methods are reliable and relatively straightforward, and facilities are commonly available in most forensic laboratories. They also have the advantage that a series of individual compounds can be analysed in a single chromatographic run. Depending on the sample matrix, extracts prepared for GC or GCMS analysis may contain compounds not necessarily of plant wax origin, but with similar properties, which can also be separated and quantitatively analysed, and can be useful, particularly for urban soil characterisation and comparison; for example, odd-chain aliphatic ketones (2-alkanones) may be analysed together with long-chain fatty alcohols (Fig. 7).

Plant wax compounds are found primarily on the surfaces of plant leaves and, although they only amount to around 1% of the SOM, they are useful markers due to persistence. Patterns of organic compounds in soil, originating from plant waxes (n-alkanes and long-chain fatty alcohols), are the same as the patterns found in associated vegetation. The lipid profile of a soil largely represents the product of the synthesis, polymeric and degradative processes on the vegetation, all of which are determined by the soil environment. Figure 8 shows the characteristic profile of a grassland soil, reflecting the profile of the overlying vegetation. This method has been validated by conventional pollen analysis but could also overcome some of its limitations (e.g. wind drift and poor identification of certain species, such as grasses). Evidence for the long-term survival of plant wax marker patterns in soil has been provided by samples of buried soil layers (14C-dated to 5000 to 6000 years BP); n-alkane and alcohol analysis data indicated the presence of heather in a buried horizon, matching independent evidence from pollen identification.

Profiling soil plant wax compounds can be useful for both investigative intelligence, aiming to use landscape-level indicators within plant wax compound profiles to indicate land use or vegetation characteristics, and for evaluative comparison of a questioned soil sample to reference samples taken from the SOC. Soil information pertaining to vegetation history at a given location can identify individual agricultural fields (data unpublished), and analyses can be performed on samples containing less than 5 mg of organic matter. Their potential in discriminating small patches of soil in urban gardens and in helping to ascertain the extent of soil contamination by pollutant hydrocarbons has been shown. Carbon isotope signatures can also assist in community-level discrimination (less negative ratios for C$_4$ plant-dominated and more negative ratios for C$_3$ plant-dominated) and has potential for development as a linked methodology.

Future Directions

Combined approaches

One future direction in the forensic analysis of soils is likely to be an increase in the combined use of very different but complementary methods to enhance the evidential value of soil information. The value of combining methods is essentially that of increased discrimination or association. Differences in the spatial...
scale at which some methods may discriminate samples, as well as differences in the manner a measured property varies spatially, both contribute to additional discriminatory potential and this provides the added value of a combined approach. For example, petrology combined with palynology was used in a search in a murder investigation, on soil samples from a car believed to have been used by the suspect in a missing persons case. The soil inorganic characteristics were used to redefine the search area using geology and soils maps, while the organic characteristics (pollen and vegetative remains) were used to target woodlands with a particular species mix. As a result, two bodies were located and the environmental evidence was used in the subsequent trial. In this case, the history of the vehicle was well known and the wheel arches and footwells acted as reliable soil traps.\(^{[94]}\)

Many mineralogical and geochemical characteristics of soil vary at a regional scale, generally reflecting variations in geological parent materials, while the organic component of soil is influenced by associated vegetation and soil management decisions that occur or are made over more local scales and timeframes. Owing to these differences, and the relatively independent information that they can provide, SOM profiles and mineralogical techniques used together are often very complementary in the investigation of forensic problems.

Another type of combination is where two independent aspects of evidence are present in crime samples. For example, in a rape case in America, three flowerpots were tipped over and spilled on the floor during a struggle. It was shown that potting soil on the suspect's shoe had a high degree of similarity with a sample collected from the floor and represented soil from one of the pots. In addition, small clippings of blue thread were identified to be present in both the flowerpot sample and on the shoe of the suspect. The thread provided additional trace evidence that supplemented the soil evidence.\(^{[10]}\) Other trace materials such as paint, glass, fibres and hair can also be present in a soil sample and can indicate contact and association. When coupled with the soil information can enhance overall evidential value.

**Digital soil profiling**

Many of the methods described in this article provide digital signatures or ‘profiles’. In soil science, there is an emerging paradigm that seeks to determine the properties of soil and to type soil by digital means.\(^{[95]}\) In the forensic context, if precise digital signatures which are reproducible can be generated, then their expert interpretation is not a necessary step that has to precede their comparison. It is easiest to illustrate this concept with an example. An XRPD pattern of a soil may be interpreted by an expert in terms of the qualitative mineralogical composition of that soil and subsequently analysed further to provide a quantitative mineralogical analysis of that soil. Alternatively, or additionally, the raw digital signature, or profile, represented by the XRPD pattern may simply be compared with the XRPD patterns from other samples, perhaps using statistical techniques, and expert interpretation of the signature, in this case a diffraction pattern, is not necessarily required. This concept may be applied to any method that generates a digital signature or profile, some obvious examples being XRPD patterns, IR spectra and GC traces. The combined approach, identified above as a future direction, has a logical extension to digital soil typing, and together these approaches may progress to comparisons against reference profiles stored in relevant soil databases.

**Databases**

Contextual information is very important in forensic analysis. In practice, direct contextual information on soils is often limited to specific comparator samples and is otherwise in the realm of the experience and opinions of experts. Databases of soil information are already in existence, but the application in soil forensics is comparatively untested. Development of searchable databases\(^{[96]}\) for use in forensic soil comparisons will provide useful information which can assist police search investigations and provide valuable contextual information aiding the evidential assessment of soil evidence when used in court. Database comparisons, particularly those based on digital soil typing or profiling, will allow probabilities of a ‘match’ to be calculated and, with the associated metadata for the reference soil (land use, geology and location), provide additional clues to soil origin. It will also assist in the elimination of areas of land with associated uncertainties from further investigation. However, in many forensic cases not enough soil material may be available from the immediate location to permit a representative sample to be measured, which could limit the confidence in comparing control samples with samples from a regional soil survey. Unknown soils in Japan have been compared with control datasets giving a 71% ‘match’, according to the systematic discrimination that was derived from multivariate analysis of soil elements, including trace elements, and a geochemical survey map of soils.\(^{[97]}\) In a regional soil survey in eastern England, samples over the same parent material were discriminated on the basis of multi-element chemistry but, although 99% could be discriminated from each other, there were limitations due to the sample size required for elemental analysis.\(^{[98]}\) A database of particle size characteristics in addition to ICP-AES has also been developed for coastal dune sediments in England and Wales.\(^{[99]}\) Based on these variables, it was found that not all dune fields were unique but was suggested.
that it would be more useful if it had been used in combination with mineralogical and biological data. In the future, extended types of analyses and spatial statistics could be incorporated in further database comparisons, and soil databases will increasingly play a role in the forensic analysis of soil and in the research assessment of new methodologies.

Legal and Theoretical Considerations

Among the legal systems in the world, there are high expectations as to the information that forensic analysis can provide. Interpretation of findings and the most appropriate means of communicating their significance remain widely debated subjects across the board of all forensic disciplines.  It is currently under discussion that the probability of occurrence and the significance of forensic findings should be considered within a Bayesian framework, rather than solely through attempts to apply conventional frequentist statistics.  Soil evidence is derived from a source that is a continuum rather than a discrete entity, and is subject to spatial and temporal variations that operate on different scales, depending on the analytical measurement.  Source heterogeneity (physical and biochemical) and susceptibility to post-transfer fractionation or mixing with pre- and post-transfer sources cannot always be easily estimated or accommodated using conventional frequentist statistics. It has sometimes been argued that the ethos behind evidential comparison should be one of elimination, since the goal of matching a questioned sample to its origin is fundamentally flawed.

Various statistical approaches look at data in different ways, and, if inconsistent differences cannot be explained and understood, then the evidence based on that analytical profiling is open to challenge in court.  Any analysis carried out for presentation in court must be of a high standard and levels of uncertainty must be minimised. Providing robust analysis with known confidence levels is essential when supporting a legal argument.  If results are to be used as evidence, then analyses should be carried out preferably by an accredited laboratory using accredited methods, and the use of statistical tests should be carried out by those who not only have a proper understanding of statistical analytical procedures, but also understand when the resulting numbers have true statistical significance. However, while statistical results are useful (indeed essential) for scientists, they should generally be avoided in presentation to the jury. In addition, to provide accurate evidence, it is important that forensic ‘soil specialists’ interpret the data. Another concern raised at the moment is the risk of erroneous false positives or false negatives.  In the law, such outcomes have devastating and untenable consequences.

Conclusions

Due to the unique nature of any investigation, it is difficult to prescribe approaches in regard to relevant spatial scales of interest. However in general terms, initial interest in an intelligence role may lie at a broad scale, while evidential stages demand that measured parameters can be used to refine relevant local scales, perhaps a few tens of meters apart. This distinction is likely to be helpful in defining the scale of interest, and therefore inform the intended focus, choice of method and design of research validation studies. Other key considerations for soil forensic research and application relate to factors that influence the representative nature of a questioned sample compared to the soil comparator samples, transfer and persistence of the material of interest and the risk that the observed analytical results reflect multiple soil sources. The questioned soil sample is also likely to be of a small size, and not necessarily representative of its source, and as sampling error increases, the confidence in comparison decreases. The extent to which transfer and persistence issues influence the comparison between a ‘questioned’ soil sample and a set of reference samples is poorly understood. A greater understanding of the expected variability introduced when soil is transferred in different ways to various evidentiary types would help to guide how best to account for the associated uncertainty in the analytical observation. Choice of appropriate and robust approaches to geo-forensic analysis continues to be increasingly debated.

Forensic examinations involve identification of soil components and comparison of samples to determine a likely common source and to provide clues to aid investigations. The future will hopefully see an increased use of soil as evidence, more new, automated methods of examination, increasing resolution and miniaturisation of techniques, in situ sampling and analysis, improved training of those who collect samples and research on the diversity and variability of soils and on how, when and what parts of soils are transferred during various types of contact. In court, quantitative methodologies will increasingly be required as evidence, as will the reference to reliable databases, to set appropriate contextual information. In an analogy to the use of human DNA database material, when similar links are established for soil material, it will provide good and reliable estimates of probability. Consequently, the use of soil as physical evidence in sample comparison and as a search tool should escalate. There is a continuum of development in techniques, and new opportunities will arise in parallel with new scientific developments in research, ensuring the scientists keep ahead of the criminal mind.

Soil is clearly a complex material, and analyses of the different components provide different types of information. Individual analytical techniques will have different degrees of importance depending on the nature of the criminal case in question. Each method has its strengths for different situations and there is great need to give more guidance on how to deploy the appropriate techniques for a given situation. As many more methods become quantitative, their use in combination will help to characterise the soil more broadly and thus help to refine and narrow its probable origin as well as give increasingly robust sample matches with probabilities that can be quantified. The digital soil typing paradigm may be the way forward in this respect.

The complexity and variability of soil properties is both an advantage and a hindrance. Complexity means that many different characterisations can be used to provide high-resolution signatures but, equally, the variability in this complexity creates a problem of ensuring that reference samples are representative and that sampling accounts for the expected variation. The suite of techniques reviewed here, which includes the chemical, mineralogical and molecular fingerprinting of soils, can both complement conventional forensic methods and provide new investigative or matching tools where previously none existed. There is no general consensus as to the best protocol or best methodologies for the forensic examination of soil samples; indeed, the method of choice tends to vary dependant upon availability of instruments and national preferences.

At present there is no one reference ‘population’ for soils to judge any soil analysis against. There are, however, significant sources of data and archived soils around the world that have
been gathered by agricultural and environmental institutes for other purposes. It seems obvious there is an opportunity to bring together and use some of these sources to generate the appropriate population data needed to test existing and new methods for their accuracy and resolution, and to establish what is required to define probability and certainty for the most promising methods. This, however, does not eliminate the necessity to adequately represent the variability at the specific SOC for case sample comparison.

Another major challenge for advancement in the area of soil forensics is that the methods require rigorous testing and standardisation. Although this is important for agricultural and environmental research, in the forensic arena the validity and rigour can be subjected to unprecedented scrutiny. DNA fingerprint of human specimens is widely perceived as an accepted and standardisation. Although this is important for agricultural methods. This, however, does not eliminate the necessity to bring together and use some of these sources to generate the other purposes. It seems obvious there is an opportunity to establishment of validation-type studies, further experimentation types in the current legal environment, there is a need for the certainly be the case for soil characterisation methods also. As there efforts across the forensic and science communities and this will caution about sample size, representation, context and analysis, it is likely that new and exciting approaches will be brought to bear on the analysis of soil materials in forensics; approach for soil analysis, using complementary and independent analysis, it is likely that new and exciting approaches will be brought to bear on the analysis of soil materials in forensics; however, caution about sample size, representation, context and transfer and persistence issues will first have to be fully resolved.

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