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TITLE: Morphologically-directed Raman Spectroscopy for forensic soil analysis

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Abstract

Morphologically-Directed Raman Spectroscopy (MDRS) is a novel yet reliable analytical technique that can be used for a variety of forensic applications, enabling scientists to gain more information from samples than they obtain using more traditional methods. In soil forensics, MDRS delivers particle size distribution and microscopic morphological characteristics for the particles present, and at the same time allows secure mineral identification. In this article, we explore the benefits of utilizing soil in forensic investigations, and demonstrate the value of applying MDRS. Two case studies illustrate the real-life potential and applications of this technology.

Keywords

Soil Analysis, Morphologically Directed Raman Spectroscopy, Raman Spectroscopy

Introduction

In his 1893 manual for examining magistrates, Hans Gross, a forensic scientist credited with being a founder of the field of criminalistics, wrote that ‘dirt on shoes can often tell us more about where the wearer of those shoes had last been than toilsome inquiries’ [1]. No particular case was mentioned in this document, but generations of criminalists have gone on to evaluate and embrace the use of soil forensics – by the early 1900s, cases where the resulting soil evidence proved pivotal were already being reported [1]. *Today, the analysis of soil represents an important area of criminal investigation, but can be challenging due to the complexity of soil samples. However, it is this same complexity that makes soil analysis so revealing, since it enables such a high level of discrimination between samples.*

A defining tenet of forensic science is that every contact leaves a trace, commonly referred to as Locard’s Exchange Principle. This is amply demonstrated by the Margarethe Filbert case of 1908, where three distinct layers of soil were removed from a shoe and analyzed to determine the route taken by the criminal over the course of a day, thus robustly associating him with the sequence of

events leading to a murder [1]. Today, soil evidence is collected routinely by forensic investigators from tire and shoe treads in order to understand the connection of a person with a location, or from, for example, a shovel, to link a suspect to a burial site. Such investigations rely on comparative analyses but soil samples can also be analyzed simply for investigative information, intelligence or identification. Though soil evidence is often considered to be 'class evidence', because multiple sites may have the same type of soil, the variety of soil characteristics that can now be analyzed means that a case can be made for greater specificity and individualization.

Murray and Tedrow defined soil (within a forensics context) as 'earth material that has been collected deliberately or accidentally and has some association with the matter under investigation' [1]. Such material typically contains: minerals, oxides, organic matter, microorganisms, pollen and spores, fossils, and artificial materials. This complexity of components makes forensic soil analysis relatively complicated, but it is this very diversity of composition and morphology that enables the differentiation of samples with such high discriminating power, as long as analytical techniques that robustly deliver sufficient analytical detail can be identified.

Modern physical methods applied by forensic soil analysts include: color and texture matching, particle size distribution analysis, density gradient measurement, mineral identification, and DNA profiling [1,2]. Techniques which are non-destructive – in order to preserve evidence – sensitive, specific, easy-to-use and cost-effective are particularly valued, so advances in these areas are prized. For example, geological sieves have traditionally been used for the determination of particle size, but more reproducible, precisely resolved, and consequently discriminating, data can be acquired using a laser diffraction particle size analyzer [3]. Furthermore, such analysis is complete in a fraction of the time and requires far less manual input.

The mineral content of a soil sample can be particularly illuminating in terms of determining its source, providing significant points of comparison between samples that enhance evidential value. Although thousands of minerals exist in nature, only around 40 are commonly encountered in soil specimens, with a typical sample containing just 3 to 5 [1, 2]. This narrows the analytical challenge, though techniques that are able to detect a rare or unusual mineral can provide pivotal evidence when such materials are identified.

Forensic mineral identification has traditionally been carried out using Polarized Light Microscopy. Microscopy techniques are vital in forensic mineral analysis because of the information that can be gathered from morphological characterization. These are complemented by techniques such as differential thermal analysis, X-ray diffraction and X-ray fluorescence, which provide insight into the crystal structure of the mineral and/or its chemical composition. Raman microspectroscopy is useful for the investigation of molecular chemistry, and has the added benefits of being rapid, reliable and non-destructive. It is used to analyze a plethora of forensic science samples including, but not limited to: drug pharmaceutical materials, geological materials, fibers, paints, inks and explosives, and can

be extremely useful when facing any unknown component. In soil samples, Raman can be used not only to identify minerals but also to detect and confirm the identity of, for example, paint flakes and various biological materials.

The technique of Morphologically-Directed Raman Spectroscopy (MDRS) combines automated particle imaging and Raman spectroscopy. Particle imaging determines particle size and shape distributions for each component in a sample, yielding detailed morphological information, while Raman spectroscopy can be applied to probe the molecular chemistry of specific particles of interest. In forensic soil analysis, MDRS is therefore able to non-destructively identify the types of minerals present and also provide morphological information about individual mineral grains. Particle size distributions can be generated for the entire sample and/or for each mineral present, along with quantitative information on the relative amount of each type of particle. In this work, we present case study data describing the application of MDRS to a number of different soil samples, highlighting the analytical protocols applied and the information generated and its value in forensic studies.

Material and methods

Case study 1

Soil samples from three different geographical sites were analyzed with the technique of MDRS, using a Morphologi G3-ID (Malvern Instruments, Malvern, UK). The samples were taken from close to the Malvern Instruments offices in: Malvern, UK; Westborough, USA; and Tokyo, Japan. To prepare a sample for analysis, it was first washed with deionized water, dried, then washed once more with deionized water and sieved. The 25 μm – 75 μm sieve fraction was separated from the rest of the sample, dried and then dispersed at low energy for analysis using the integrated sample dispersion unit.

Morphological data were gathered for all particles within the scan area (over 19,000 particles in total), using 5x magnification and diascopic measurement conditions to generate statistically relevant particle size and shape distributions. Morphological analysis took approximately 30 minutes. One-thousand particles from each sample were then selected for Raman analysis using a coupled Kaiser optical systems RamanRxn1 spectrometer using a 785 nm semiconductor laser with a power of < 500 mW, and a 3 micron spot size. A 30 second exposure time on a low laser power was used to avoid fluorescence. Additionally, this study examined samples from different sites worldwide, leading to greater variation in data, thus a longer exposure time was implemented to ensure quality of spectral results. Raman analysis was performed overnight, taking approximately 9 hours. As this study was seeking only general geographical variations, multiple measurements were not performed.

These particles were selected evenly from the complete database of particle images for the sample without morphological classification. The spectra gathered for these particles were compared to

Raman spectral reference libraries using KnowitAll® software from Bio-Rad to identify the minerals present.

A significant number of different minerals were detected across the three samples but five were found to be particularly prevalent: pyrophyllite, orthoclase, quartz, calcite, and hematite. The reference spectra for these minerals were imported into the spectral library of the instrument software, and each of the 1,000 Raman-analyzed particles was then classified as one of these minerals based on the best match to the reference spectra, provided this exceeded a baseline correlation score of 0.5. Material that was not classified as one of these five minerals on this basis was ignored for the purposes of this comparative primary analysis of the soil samples.

Case study 2

For the second case study, soil samples were collected from four different sites along a single road (approximately 300 meters apart from one another) in Connecticut, USA. Sample preparation proceeded via an analogous process of washing and sieving but, for this study, it was the 60 µm -120 µm mineral size fraction that was separated out for analysis. This mineral size fraction was chosen because this range is commonly used for forensic microscopical examinations of soil minerals in the United States and is the standard employed by the laboratory that performed this analysis.

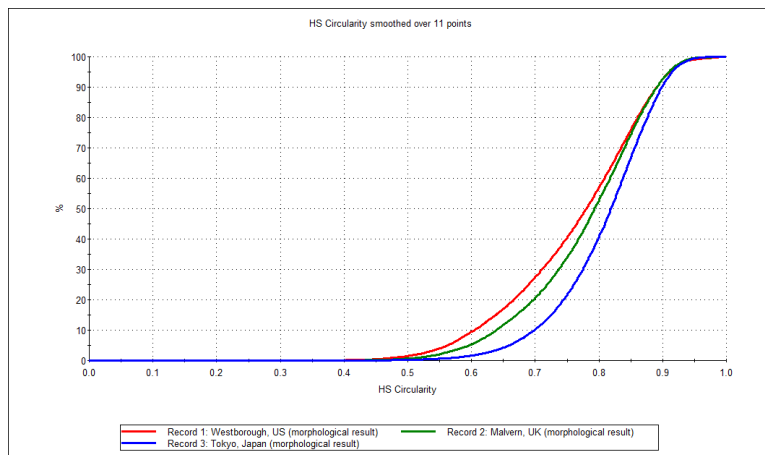
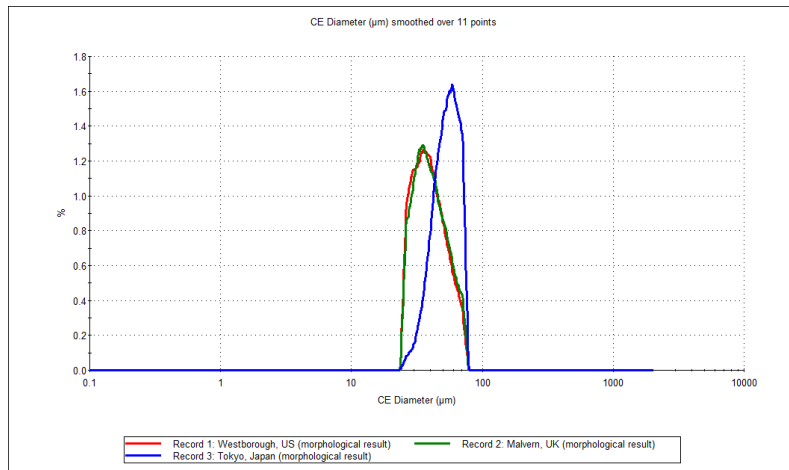
For each analysis, 7 mm³ of sample was prepared for measurement using a process of evaporative dispersion. Triplicate analysis for each of the four samples was performed, with separate dispersal and measurements (morphological and spectral) for each. The experiment used 10x objectives for the capture of over 150,000 particle images. Of these particles, Raman spectra were collected for up to 3000 particles per sample replicate, and only particles with a circle equivalent diameter (CED) greater than 7.0 µm and solidity greater than 0.75 were tagged for chemical analysis. Raman spectra were collected in the same manner as in Case Study 1, except for a shorter Raman exposure time of 2 seconds. The soil geology of the region was known prior to analysis, and it was determined that quality Raman spectra was obtainable with a short exposure. This also enabled the greater number of particles to be analyzed in a relatively short time, with analysis taking less than 3 hours.

A correlation algorithm was used for mineral identification, comparing the gathered spectra with reference spectra from the RRUFF database [4]. Different databases were used in the two case studies due to their availability by the two research centres. This demonstrates the robustness of mineral Raman spectral in that more than one database can be employed for mineral identification. A minimum hit quality above 0.85 was required to identify a particle as being a certain mineral.

Results and discussion

Case study 1: Comparing soil samples from three of Malvern's global sites using MDRS.

Examples of the morphological data generated for the three soil samples from Malvern sites across the globe are shown in Figure 1, which displays particle size, circularity and elongation distribution data respectively. The particle sizing metric reported is circular equivalent diameter, which is defined as the diameter of a circle with the same 2D area as the particle. Circularity is a normalized shape metric that illustrates how close the particle is to completely spherical, with a value close to one indicating that the particle is highly circular. Elongation is a normalized parameter determined from the ratio of particle width to length, with higher numbers associated with needle-shaped particles. Number-based distributions are generated for each size and shape parameter.



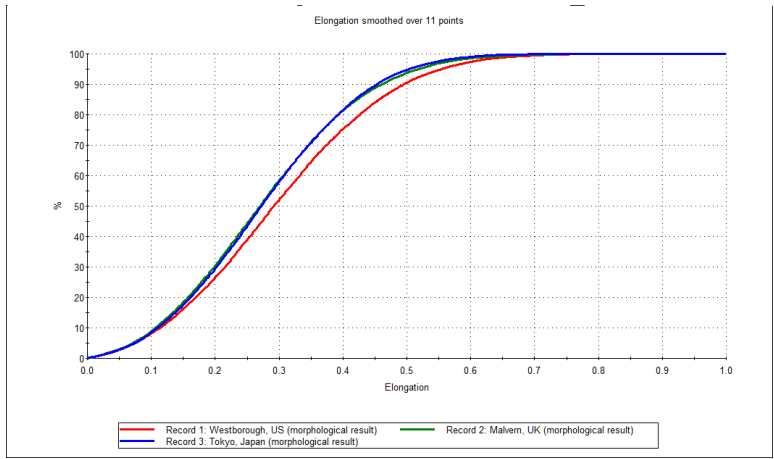


Figure 1: Morphological data for the soil samples shows clear differences between them in terms of particle size distribution (a); circularity (b); and elongation (c).

These data show that the samples from the USA and UK locations are closely similar in terms of size distribution. However, the sample collected from the Japanese site is markedly different, containing more particles bigger than 30 μm , and fewer particles smaller than 30 μm , compared to the other two site samples. A comparison of shape is also differentiating. The sample from Japan is the most circular while that from the US has the lowest circularity. The elongation data shows that the samples from the UK and Japan are very similar in terms of circularity, whereas the sample from the USA contains more particles that are needle-like in nature, a finding reflected in its low circularity score.

A further way in which particles can be physically differentiated is on the basis of their transparency, a parameter generated from measurements of the intensity/amount of light that can pass through the particle (see Figure 2). This comparison reveals that, in general, the soil particles from Japan are much darker than those from the other two locations, letting less light pass through them. The most transparent particles are those from the UK.

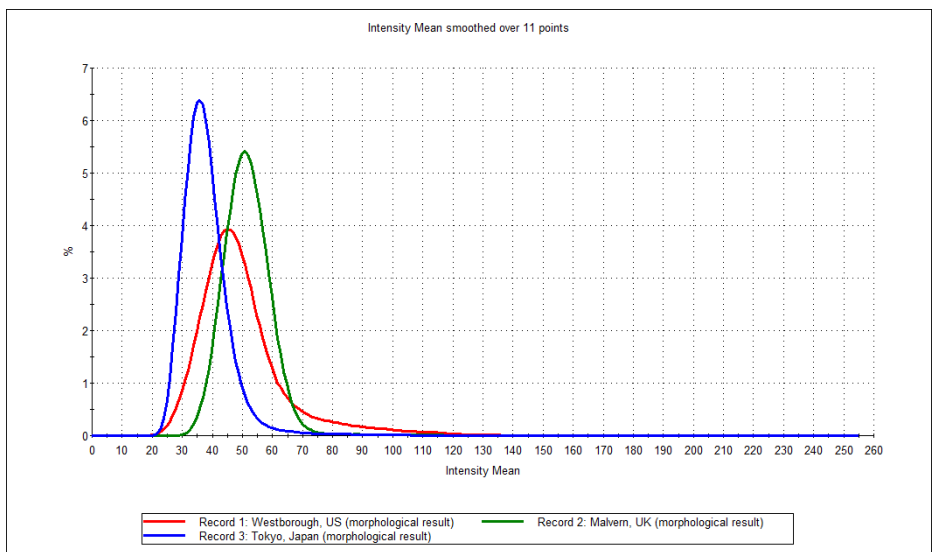


Figure 2: Transparency data indicates that the soil particles in the Japanese samples are darker than those from the other two locations.

With MDRS, it is possible to select particles for Raman analysis simply on a subjective basis, by looking through the database of images and manually choosing particles of interest. It is also possible to classify the sample based on any of the parameters already discussed: particle size, particle shape and/or transparency, to identify or group a population with specific morphological characteristics for chemical analysis. However, in this instance, the particles selected for Raman analysis were simply an even distribution of 1,000 from the 19,000 captured images.

Across the samples from the three locations, five minerals were found to be most abundant: pyrophyllite, orthoclase, quartz, calcite, and hematite. Classification of the particles from each sample generated comparative compositional data for the soils, which is shown in Figure 3. These results show that quartz is the dominant mineral across all the samples examined. However, there are distinct differences between the samples in terms of their quartz content, with samples from the UK and USA containing a much higher proportion of quartz than the sample from Japan, which in contrast contains a higher proportion of orthoclase. Interestingly, pyrophyllite was found only in the sample from the US, while calcite and haematite were completely absent from the US sample but present in those from both Japan and the UK.

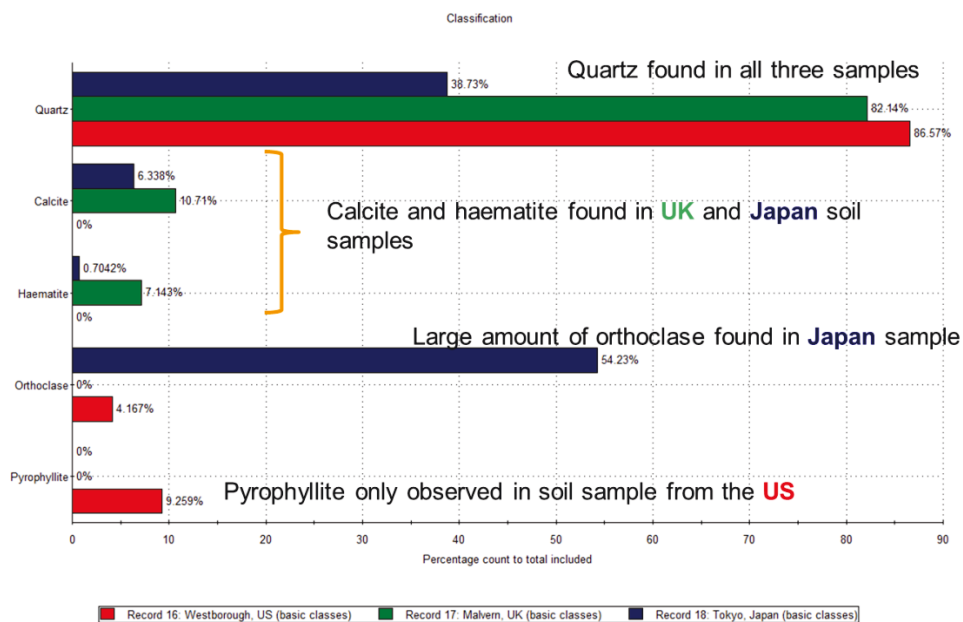


Figure 3: Classifying particles as one of five principal components reveals clear differences in the mineral composition of the soil samples from the different sites.

It is then possible to focus the investigation on particular chemical classes of interest. Taking the quartz class as an example, the morphology of the quartz particles detected at each site can be

compared to add an additional layer of information about the nature and properties of the soil samples being analysed. Here, the analysis (Figure 4) reveals that the quartz in the sample from Japan tends to have a slightly larger particle size than that of the other two samples. In terms of shape, the quartz particles in the US sample are more needle-like and less circular than those in the other two samples.

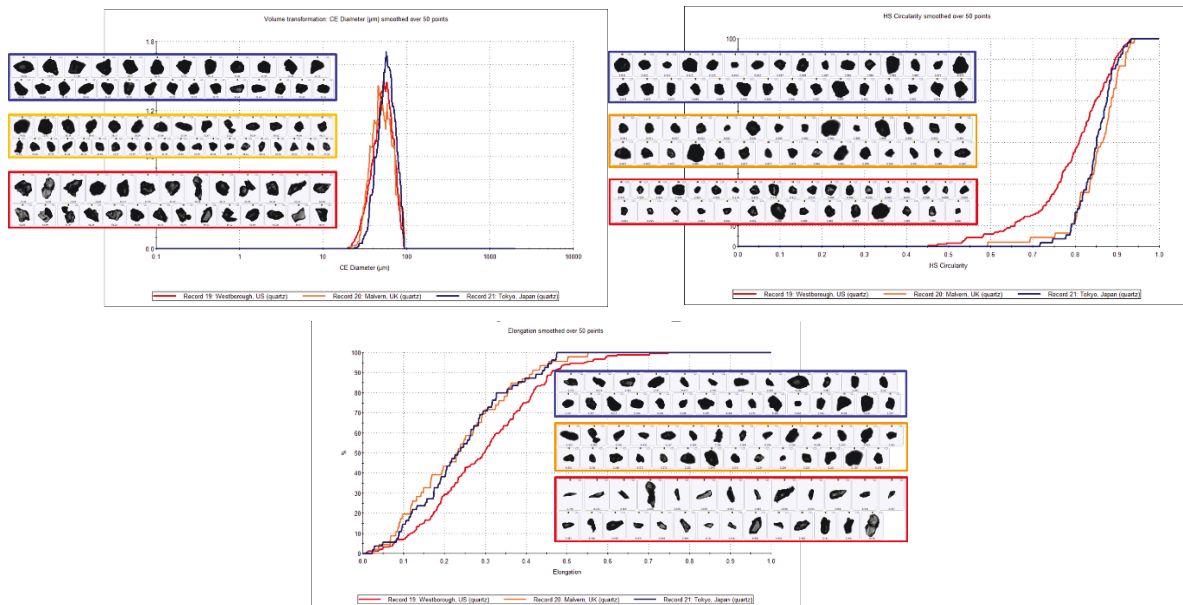


Figure 4: Morphological analysis of the quartz from different sites reveals that there are clear differences between the size (a) and shape (b) and (c) of the quartz particles detected at different locations

In a final analysis, a more detailed chemical investigation of the sample from Japan was carried out to see if other materials could be successfully detected from the Raman spectra gathered for the sample. This analysis revealed particles of dolomite, starch, ilmenite, diopside, stilbite, talc and more, generating the more complete component analysis shown in Figure 5, which indicates a relatively high abundance of bayerite. It shows how MDRS can be used to drill down into the detail of a sample and reliably identify even trace components which may provide useful evidence.

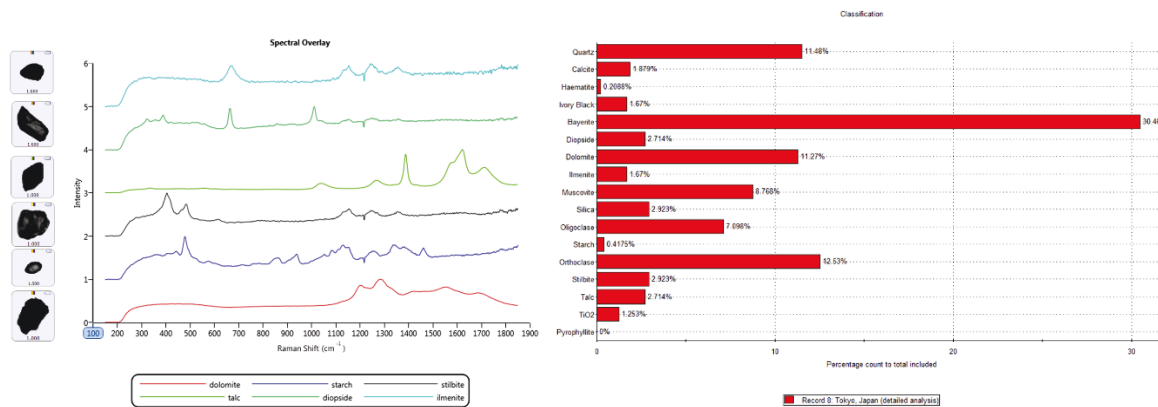


Figure 5: Broadening the chemical classification of the particles in the sample from Japan reveals particles of a wider range of minerals and a relatively high abundance of bayerite.

Case study 2: Comparing four soil samples from one road in Connecticut, USA, using MDRS

As expected, all four samples from the Connecticut road were found to be very similar in terms of their morphology. Images of quartz particles from two of the four sites are shown in Figure 6. Because of their close similarity, differentiation on the basis of quartz morphology, as in the preceding analysis, is not feasible.

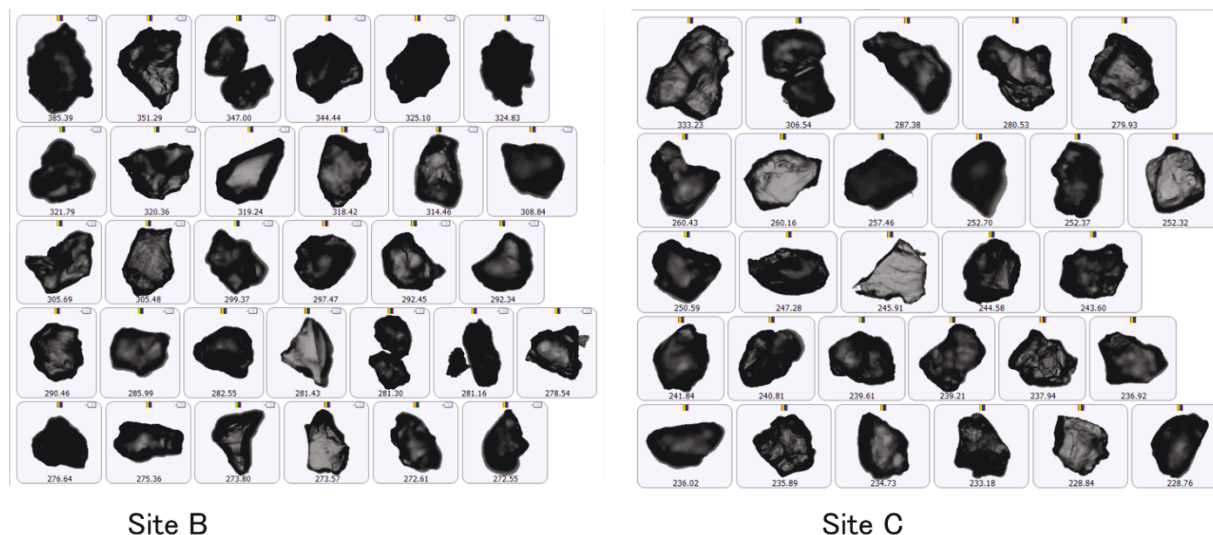


Figure 6: Morphological data for quartz in soil samples B and C shows similarities between the samples.

However, the graph below (Figure 7) illustrates that although the particle sizes of the two samples were similar, the sample from site C has a broader particle size distribution, showing some potential for differentiation between the samples. Incidentally, this particle size distribution data also shows that the sieving process was not working properly, since there are particles present in the range 120 μm –

400 μm , whereas the intention was to select particles between 60 μm – 120 μm . Here then, the particle size data provides some statistical data with which to differentiate the samples.

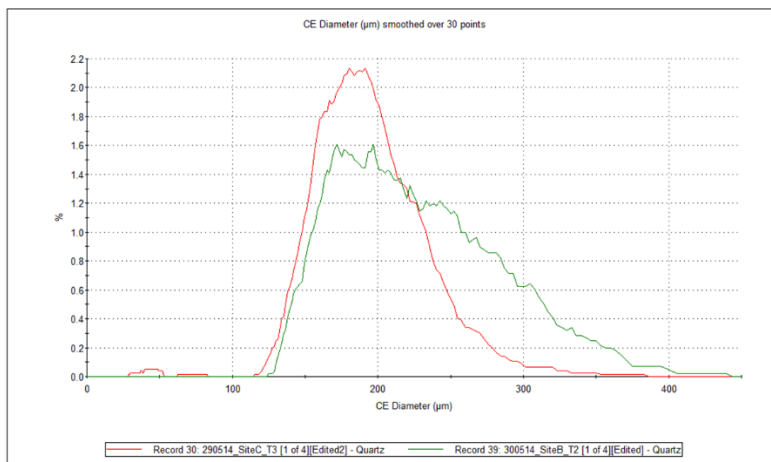
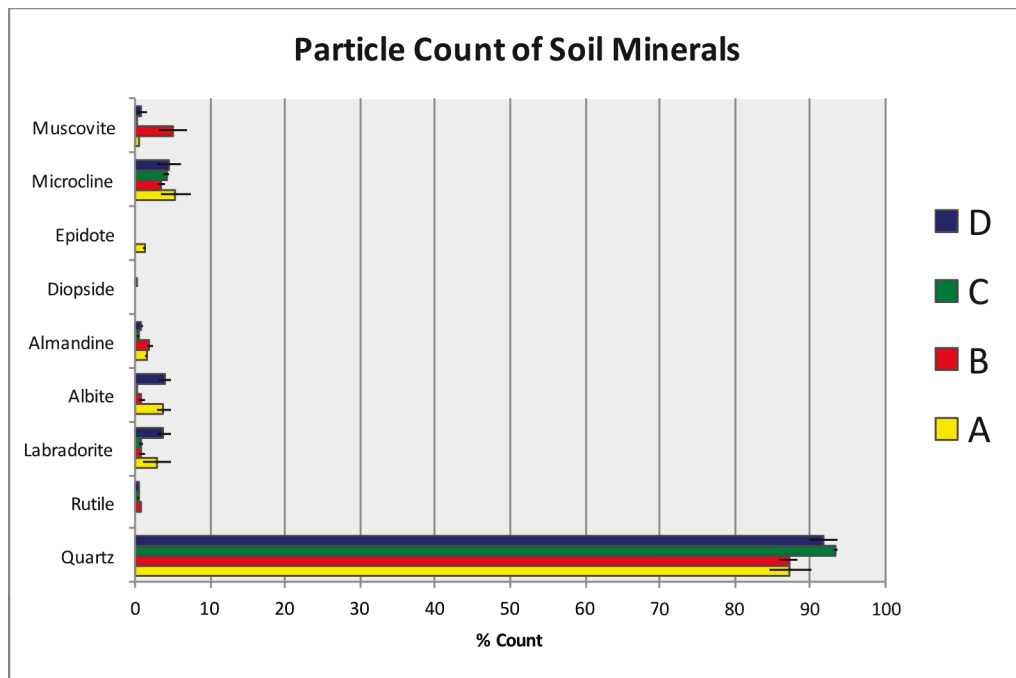


Figure 7: Shows the differences in particle size distribution between the samples from site B and site C, illustrating that samples from site C had a broader particle size distribution.

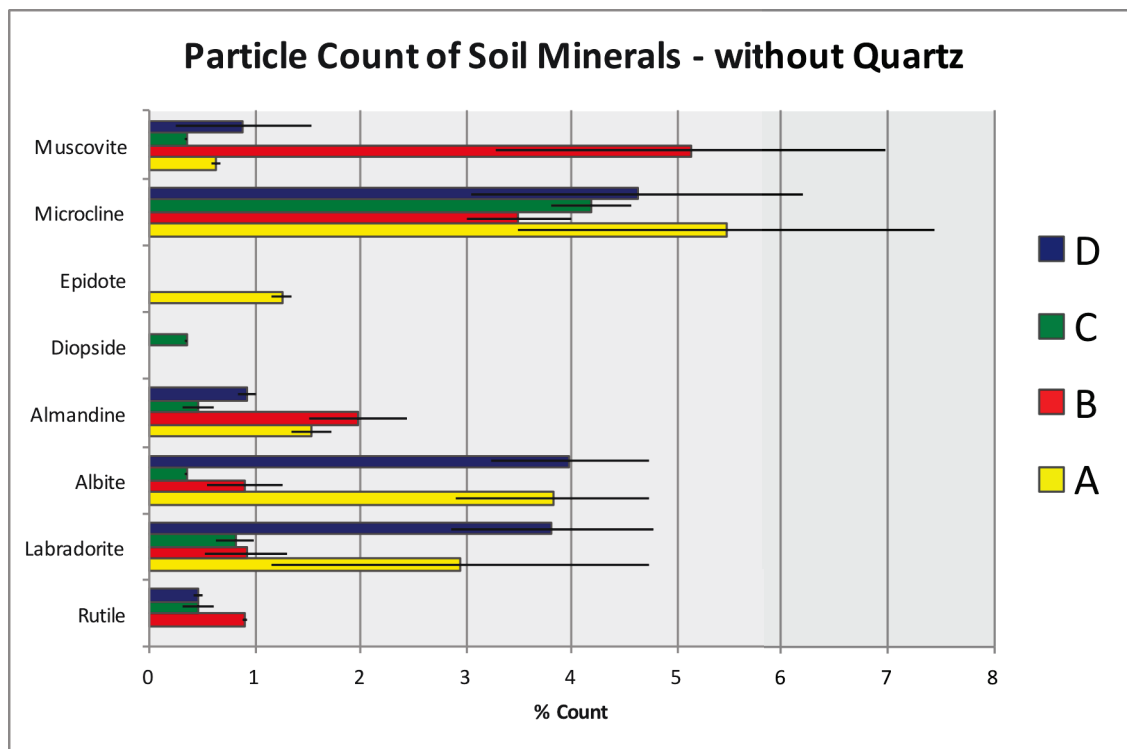
Further analysis was conducted to identify the minerals present in the sample from each location (Table 1 and Figure 8). Qualitative comparisons between the samples were conducted using the particle counts for each mineral, and these proved highly differentiating. Quartz was found to be the major component in all four samples, but epidote was only found at site A, diopside was uniquely associated with site C, and a significantly higher percentage of muscovite was found at site B than at any other site.

Table 1: The average percent particle counts for each mineral for the four soil sample locations.

	Quartz	Rutile	Labradorite	Albite	Almandine	Diopside	Epidote	Microcline	Muscovite
A	87.33	0.00	2.95	3.82	1.53	0.00	1.26	5.46	0.63
B	87.12	0.91	0.92	0.90	1.98	0.00	0.00	3.49	5.14
C	93.27	0.46	0.81	0.35	0.46	0.35	0.00	4.19	0.35
D	91.80	0.47	3.81	3.98	0.93	0.00	0.00	4.63	0.89



(A)



(B)

Figure 8: Bar graphs, with and without quartz (A and B, respectively), of the particle counts for minerals in each sample, with the corresponding exact figures in Table 1. Error bars are included showing the standard deviation of replicate samples. Quartz was found to be a main component in all samples but key differences in mineral content effectively differentiated the samples.

Principal component analysis (PCA) is a powerful statistical tool that can be used to emphasize variation, thereby highlighting relevant information from complex datasets. In chemometric analysis, PCA can be usefully applied to spectral or chemical data to reveal structure in the datasets, and it is

consequently a valuable tool for MDRS analysis. Multivariate statistical analysis was performed with the R statistical computing and graphics software (© The R Foundation), using R code initially written by N.D.K. Petraco, Ph.D. and subsequently modified by B.W. Kammrath, Ph.D. For this study, PCA on the autoscaled particle counts revealed that 87.7% of the variation in the data was captured in the first three principle components, showing a good separation between the four datasets, as illustrated in Figure 9. It is evident from analysis of the loadings plots (Figures 10A and B) that rutile, labradorite, albite, microcline, and epidote were all important for PC1, while quartz, almandine, diopside, and muscovite were the most influential minerals for PC2. The biplot of PC1 and PC2 (Figure 10C) informs the important minerals for discrimination of each of the samples. Soil C had the largest amounts of quartz and diopside, which are the two minerals important for its discrimination, while rutile was important for the distinction of soil B. Rutile, almandine and epidote are important for the discrimination for soils A and D. This research demonstrated the ability of the mineral counts generated by MDRS for use in sample differentiation, even with very closely related samples.

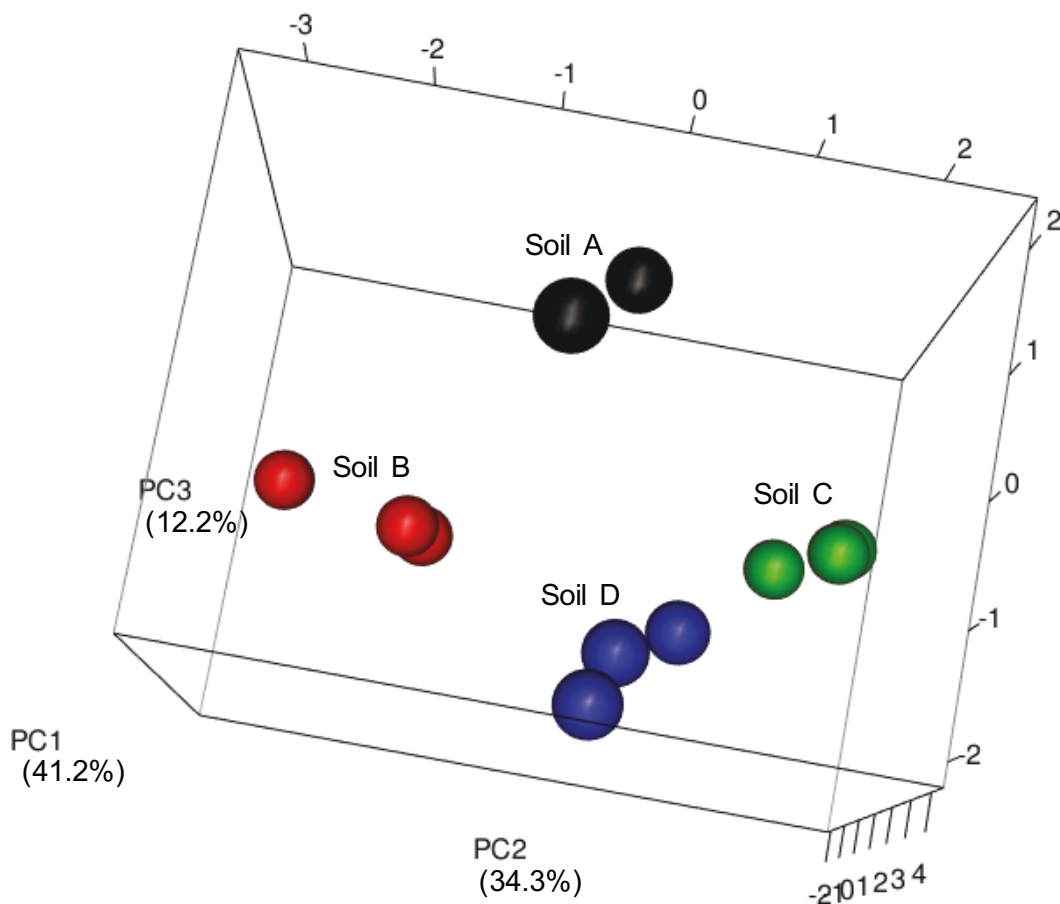
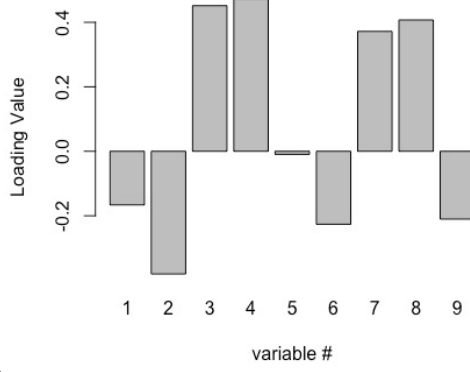
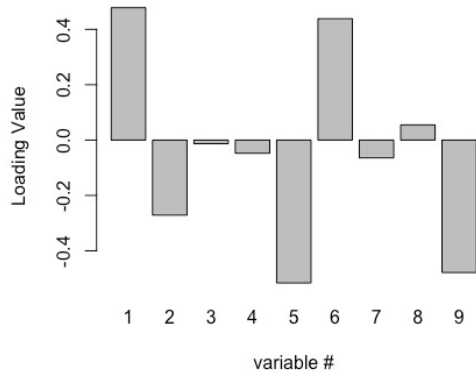


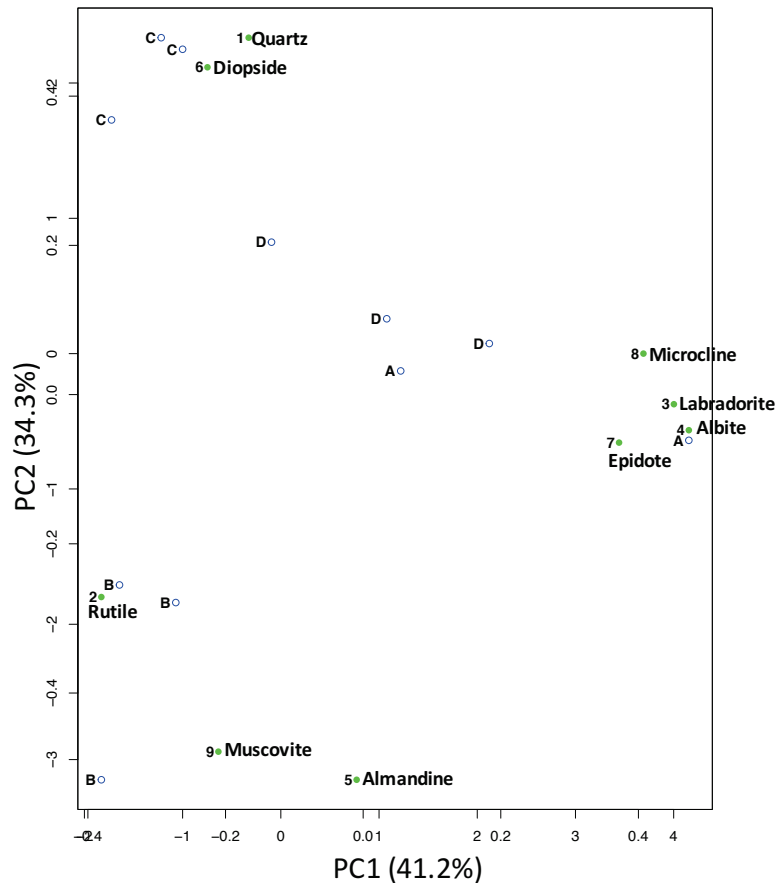
Figure 9: PCA results of the particle count data for the minerals in the four soil samples. The three-dimensional PCA plot shows clear differentiation of the four locations in addition to the good clustering for replicate samples within each sampling location.



(A)



(B)



(C)

Figure 10: (A) loadings plot of PC1 and (B) loadings plot of PC2, and (C) biplot of the particle count data for the minerals in the four soil samples. The variables #1-9 correspond to the minerals quartz, rutile, labradorite, albite, almandine, diopside, epidote, microcline, and muscovite, respectively.

Conclusion

The case studies presented here demonstrate the potential of MDRS as a powerful tool for forensic soil analysis. MDRS is a non-destructive, relatively fast and automated way to collect both morphological and chemical information for individual mineral particles within a soil sample. Both types of data can be helpful in determining and differentiating the provenance of a sample. The data presented in case study 2 suggests that MDRS may prove to be sensitive enough to detect subtle differences in mineral content between soil samples collected from nearby locations. Further research to evaluate this potential for MDRS using different soil types should be completed. Case study 1, on the other hand, shows the striking differences between samples from across the globe, not only in their mineral content, but also in terms of circularity and transparency. The experiments also show the ability of MDRS to robustly detect even trace elements in the sample which can be pivotal in linking a soil sample to a specific site. These studies suggest that routinely employing MDRS in soil analysis may allow criminalists to efficiently obtain detailed information to support their investigations.

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