Chapter 5
Soil Comparisons Using Small Soil Traces, A Case Report

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Abstract In forensic investigations, soil traces from pieces of evidence (e.g. shoes, shovels) can be compared to each other or to soil samples from the crime scene. A case report is presented on an attempted rape case using bacterial terminal restriction fragment length polymorphism (tRFLP) profiling and pollen analysis. From both the victim's and the suspect's clothing soil stains were sampled. No samples from the crime scene were supplied. For both the bacterial profiles and the pollen spectra of the soil samples Bray-Curtis distances were calculated and interpreted using databases. For the pollen spectra palynological knowledge on the frequency of the pollen types was also taken into account. The Bayesian approach was used to express the evidential value of the combined results in which multiple common characteristics were used as opposed to only rare characteristics.

Keywords Forensics • Soil comparison • Bacteria • Pollen

5.1 Introduction

Soil traces are often present in criminal case work and can play an important role in linking suspects or objects to a crime scene. To compare soil samples various parameters can be used, such as pollen spectra, visual characteristics, grain size distribution, elemental composition, bacterial terminal restriction fragment length polymorphism (tRFLP) profiles or infrared spectra (Brown et al. 2002; Mildenhall 2004; Horrocks and Walsh 1999; Bull et al. 2006; Horsewell et al. 2002; Quaak and Kuiper 2011; Pasternak et al. 2012; Cox et al. 2000). The evidential value of a soil comparison can be improved when multiple parameters are combined.

At the Netherlands Forensic Institute (NFI) visual inspection is the first step in forensic soil investigations. It is used to determine color, morphology, possible mixtures, additives (plastics, iron, etc.), sample size and condition. After visual

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H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics*, Soil Forensics, DOI 10.1007/978-3-319-33115-7_5

inspection of the samples the technique(s) which can be applied for sample comparisons are determined. At the NFI elemental composition analysis (Energy Dispersive X-Ray Fluorescence, ED-XRF), bacterial tRFLP profiling and pollen analysis are used for soil comparisons.

These techniques have been chosen because they focus on different fractions of the soil; the abiotic (mineral), organic (dead) fraction and living components. This ensures that the resulting data is (conditionally) independent which is important when combining results.

All three techniques have been validated and their spatial resolutions and discriminative powers were shown to be very suitable for casework. For these techniques, databases are necessary to reliably interpret the results and these were constructed.

Both bacterial tRFLP and pollen analyses are (semi-) destructive to the soil sample, but can be applied to very small samples. The elemental composition analysis used at the NFI is non-destructive but requires a relatively large amount of sample (>1 g).

Soil comparisons consist of three stages: (i) calculating the degree of similarity between questioned soil samples for each technique (elemental-, bacterial- or pollen analysis); (ii) determining the evidential value for each degree of similarity; (iii) evaluating the evidential value of the combined result.

Ideally, all three techniques are applied to all samples. However, this is not always possible, because the soil samples can be too small for elemental composition analysis, the condition of the sample can be unfit for use for bacterial tRFLP profiling or the soil sample can contain too few pollen grains for a comparison.

In this paper a case is presented in which bacterial tRFLP profiling and pollen analysis were used for a soil comparison. The elemental composition could not be determined in this case, because not enough soil was recovered.

5.2 Case Description

A 7 months pregnant woman was walking home from the metro when she was dragged into the bushes by a male teenager who had followed her through the park. While threatening her with a knife, he tried to rape her, but her screaming chased him away. She immediately reported the incident to the police and they were able to apprehend the suspect later in the metro using her description of the assailant. The police recovered the suspect's shoes and trousers and the victim's tights as evidence.

On the victim's tights soil traces around the knees and crotch were clearly visible (Fig. 5.1). The suspect's trousers had only vague stains on the lower end of the legs, while the shoes had clear soil traces on the side of the sole (Fig. 5.1).

Unfortunately, the victim could not recall precisely where the attempted rape had taken place. As a result the police was unable to sample the crime scene. In order to link both victim and suspect to the same (unknown) crime scene, the police requested a soil comparison between the soil traces on the victim's tights and the suspect's shoes and trousers.

Fig. 5.1 Visible soil traces on the victim's tights (*left*) and the suspect's left shoe (*right*)

5.3 Materials and Methods

The soil traces on the pieces of clothing were inspected and collected from the knees of the victim's tights and the side of the sole of the suspect's left shoe. Because only vague stains were present on the suspect's trousers no further visual inspection was possible and a small part containing the stain was cut out of these trousers.

The samples from the left shoe and the tights were used to generate bacterial tRFLP profiles and pollen spectra. For bacterial tRFLP profiling 100 mg of each soil sample was used. DNA extraction, PCR, bacterial tRFLP profiling and data analysis was performed according to Quaak and Kuiper (2011).

For pollen analysis the remaining part of the soil samples from the left shoe (0.4 g) and from the tights (1.0 g) were used. In addition the sampled part of the trousers was also analyzed. Pollen grains were extracted from the soil samples using a standard method (Faegri and Iversen 1989) with two additional steps; sodiumpyrophosphate dispersion (Riding and Kyffin-Hughes 2004) and polytungstate separation (Munsterman and Kestholt 1996). *Lycopodium* sp. spore tablets were added for quality control. Pollen grains from the trousers were extracted using the method described by Horrocks (2004), also modified with the addition of polytungstate separation and *Lycopodium* sp. spore tablets. For each pollen spectrum at least 250 pollen grains were identified using pollen keys and pollen collections for North West Europe.

To determine the similarity between relative (to the total peak area) bacterial tRFLP profiles or relative (to the total pollen sum) pollen spectra, Bray-Curtis (BC) distances (Beals 1984), a statistical distance measure commonly used in ecology, were calculated. For each parameter a database of BC distances calculated between soil samples from the NFI collection was used to support the interpretation of the results. The database can be used to interpret a calculated distance (between two samples) on a continuous scale. In general the database separates the distances in three groups; common source, different source and inconclusive (area of overlap between the other two groups) (Quaak and Kuiper 2011).

The method used for bacterial tRFLP profiling does not identify different bacterial species, therefore the database is essential to determine the value (Likelihood Ratio (LR)) of a comparison. However, the identified pollen types in the pollen spectra can be linked to their parent plants with associated ecology, method of distribution and relative rarity. The BC distance database for pollen spectra is limited as it only contains 81 pollen spectra and 3,046 comparisons (of which 55 comparisons for a common source). As a result the calculated BC distance between the samples under investigation is mainly used to get an indication of the evidential value of the calculated similarity. Subsequently, palynological knowledge is used to determine the final evidential value. In this last stage the non-pollen-palynomorphs (such as fungal spores) are included, taking conditions of the samples and differences in storage conditions into account.

5.4 Results

Only a small amount of soil (0.5 g for the left shoe and 1.1 g for the tights) was recovered from the pieces of evidence, limiting the visual inspection to general description of color and soil type. Both soil samples consisted of yellow sandy clay.

For both samples the obtained tRFLP profiles had more than 50 peaks exceeding the lower limit of detection, the sum of peak areas was larger than 100,000 and the intensity of the peaks was evenly distributed throughout the profile (Fig. 5.2). A BC-distance of 0.22 was calculated as described in Quaak and Kuiper (2011) between the profiles from the left shoe and the tights.

In each sample more than 250 pollen grains could be identified and a robust and reliable comparison between the pollen spectra was made. A BC distance of 0.18 was calculated between the pollen spectra from the left shoe and the tights. Between the tights and the trousers a higher BC distance of 0.34 was calculated. In all three samples, the following pollen types were identified in relatively equal and high amounts: *Alnus* sp., *Betula* sp., *Pinus* sp., *Quercus robur-pubescens*-type and

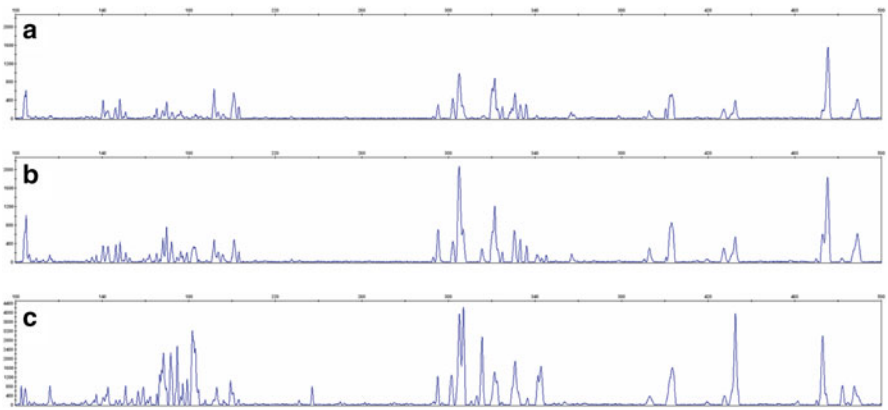

Fig. 5.2 Bacterial tRFLP profiles from (**a**) soil sample from suspect's shoe, (**b**) soil sample from victim's tights and (**c**) from non-related soil sample (positive control)

Poaceae wild-type. These are pollen types that are commonly found in the upper soil layer in The Netherlands. In addition to these types, the pollen spectrum of the trousers also contained relatively high numbers of uncommon pollen from garden plants and horticulture (*Solanum nigrum*-type, *Sedum* type, *Olea europaea*, Campanulaceae).

5.5 Bayesian Approach

Since DNA typing has been introduced in forensic science, more attention has been given to the data interpretation and testimony in court by older, more established, forensic disciplines (e.g. fingerprint or shoe print analysis) (Saks and Koehler 1991, 2005). In these forensic disciplines it was often concluded that the samples under investigation were a 'conclusive match', but this leads to a misunderstanding of the evidential value (McQuiston-Surrett and Saks 2008).

In scientific experiments it is common practice to falsify (exclude) hypotheses. For this reason it is sometimes advised to only report exclusions (Bull et al. 2008). Inclusions would then be reported as 'can not be excluded'. However, in cases where an inclusion is found, the evidential value of the inclusion is usually very important as this is often incriminating evidence. If the forensic scientist does not provide the evidential value, this is left to the fact finders in the case. In most cases fact finders are less equipped to interpret the findings than the forensic scientist.

A commonly accepted approach for evaluating the evidential value of an exclusion or inclusion in forensic science is the Bayesian approach (Aitken et al. 2011) (mainly used in the UK, Europe, Australia and Asia). In this approach the conclusion is given as the Likelihood Ratio (LR) of the results given two hypotheses (see Berger et al. 2011 for an introduction) and it specifically does not address the likelihood of the hypotheses themselves.

At the NFI, the Bayesian approach is used for soil comparisons. An example of the difficulties involved in the process of implementing the Bayesian approach in casework has been published for firearms comparison (Kerkhoff et al. 2013). Results of the soil comparison are reported as a likelihood ratio given one hypothesis put forward by the prosecutor (in this case report: the soil originated from the same site in the park) and a second hypothesis put forward by the defense (the soil originated from another place than the park). Although we would prefer to report the LR, each case involving soil traces has different hypotheses and technical challenges which would require a different database for each case. Therefore, we use a verbal scale: equally likely, somewhat more likely, more likely, much more likely, very much more likely. To support consistency each verbal scale has a corresponding range of LRs (e.g. (Association of Forensic Science Providers 2009; Nordgaard et al. 2012)).

5.6 Case Resolution

For the case described in this report the combined comparison of the results is mainly based on bacterial DNA profiling and pollen analysis. The results from the visual inspection lacked discriminative power due to the limited amount of material. The BC distance of 0.22 calculated between the tRFLP profiles from the shoe and the tights has been calculated 24 times for samples originating from the same source (5.7 % of 420 distances) in our BC database and 1 time for samples originating from different sources (0.08 % of 12,496 distances) (Fig. 5.3). The LR is calculated by dividing these percentages; (5.7 %/0.08 %) resulting in a LR of 740.

Bacterial profiles have a high spatial resolution, which means large differences (i.e. low similarity) can be found between samples taken in close proximity to each other. High similarity values have only been calculated between samples from the same source. Calculating these values between questioned samples will therefore result in high evidential values in favor of the ‘same source’ hypothesis.

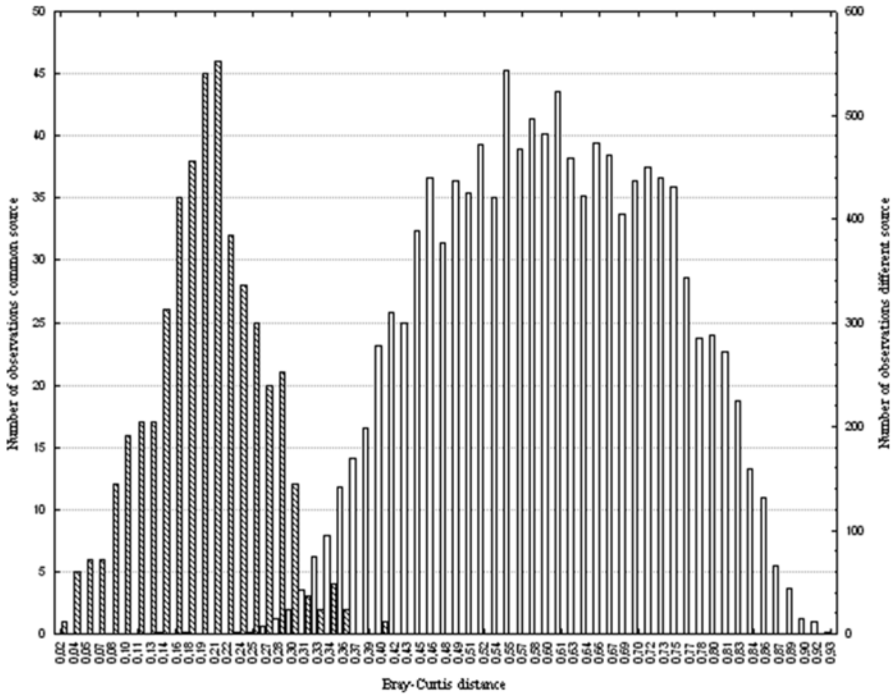

Fig. 5.3 Histogram with distribution of 13,366 Bray-Curtis (BC) distances (x-axis) calculated between 164 profiles of 50 soil samples. The number of observations of BC distances is indicated on the y-axes. Shaded bars represent distances calculated between profiles originating from the same source (420 distances). Open bars represent distances calculated between profiles originating from a different source (12,946 distances) (Source: Quaak and Kuiper (2011))

The BC distance of 0.18 between the pollen spectra of the shoe and the tights is usually calculated between soil samples originating from the same source, and reflects a LR of 200 in our pollen database. The calculated BC distance of 0.34 between the tights and the trousers fits within the inconclusive group of the pollen database.

The pollen spectra of the soil on the shoe and the tights were very similar, but contained mostly common pollen types. This was interpreted as an indication for a common source, but with a lower evidential value than if it would have contained uncommon pollen types as well. The pollen spectrum of the stain on the trousers was partly different from the other two spectra. This pointed to either a different source or a mixture, possibly with garden soil.

Combining all results and taking the different spatial resolutions of the techniques into account, we reported to the police that the results of the soil comparison using pollen spectra and bacterial tRFLP profiles were much more likely if the soil traces from the suspect's shoe and the soil traces from the victim's tights originated from the same location than if these soil traces originated from different locations.

The possible mixture of pollen in the stain on the trousers was also reported. In response to this finding the police provided the information that the suspect worked in a commercial glass greenhouse. Therefore, the relatively uncommon pollen grains appeared to be less useful than the common ones for this particular case.

Before going to court, but after the results were reported, the suspect confessed to the crime. He was convicted in court for attempted rape and was sentenced to juvenile involuntary commitment to a state facility.

5.7 Discussion

Many forensic soil comparisons focus on a few rare and unique particles or parameters that by chance happen to be in the soil samples under investigation. In general The Netherlands has almost no natural outcropping of rock and a low variation in soil types, most of the soil has been deposited by a few major rivers. In addition large amounts of soil are transported daily throughout The Netherlands for all types of (re)construction activities (houses, dikes, beaches, etc.). The vegetation is heavily influenced by urbanization, agriculture and landscaping and often crime scenes are found in urban areas with relatively high numbers of garden plants. All these factors make it very difficult to find and assign a certain "unique" characteristic in the soil to a specific location. However, there are large numbers of common characteristics in soil in varying combinations and concentrations.

At the NFI multiple common characteristics of soil are used to distinguish between various locations. This has the advantage that soil comparisons can be used more often in casework, since the common characteristics are by definition more common than the rare and "unique" characteristics. In addition, rare characteristics have often not been studied extensively and therefore less is known about their actual rarity and distribution. Depending on the frame of reference, a rarity for one researcher can be relatively common for the other, which makes it difficult to develop quantitative and objective comparative methods based on rare characteristics. In our experience when training new investigators, it is easier to teach to recognize the common characteristics instead of all possible rare characteristics.

5.8 Conclusion

This case report shows that small soil samples can be used in forensic casework and that the combination of results from (independent) techniques can yield a higher evidential value. We also show that common characteristics can be used in the comparisons. Even in cases where rare characteristics are available we advise to use common characteristics as well and where possible combine the results. The samples under investigation will never be exactly the same, thus differences will always be present. The Bayesian approach is well suited for reporting probabilistic results and can also be used when results from different techniques are combined.

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