Chapter 4
Forensic Soil Analysis: Case Study of Looting at a Roman-Visigothic Burial Vault

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Abstract The Guardia Civil Crininalistic Service (Spain) recognises the high value of soil evidence in criminal investigations, as well as its strength as forensic evidence in judicial proceedings, due to its capacity to link a crime scene with a suspect. There is an average of 15 cases per year related to the forensic analysis of soils, which is conducted in our laboratory in the course of criminal investigations. One example is the case of looting at a Roman-Visigothic burial vault which took place in Moron de la Frontera (Seville, Spain), where the Guardia Civil Nature Protection Service (SEPRONA) collected samples from the burial site and from the boots and tools found inside the boot of a suspect's vehicle.

This chapter illustrates the methodology used for forensic analysis of soil samples related to the looting of this burial site (colour, particle size distribution, X ray powder diffraction, major and trace element composition, electrical conductivity, pH, anion concentration and comparison of phylogeny of microorganisms). The results were used to identify similarities between soil samples collected from the tools and boots found in the suspect's vehicle and soil samples from the Roman-Visigothic burial vault.

4.1 Introduction

There are a large number of laboratories that perform analyses of soils and other geological materials found on a variety of materials such as shoes, tools and vehicle tyres and undercarriages, etc. for forensic purposes in cases of homicide, rape, robbery and terrorism, etc. These analyses are considered as evidence in police investigations. The wide variability in the distribution and properties of soils and the techniques applied make it possible to characterise and discriminate different soils,

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rendering them an important tool in the forensic field. In Spain, there are numerous police laboratories or forensic units that analyse the soil remains found at crime scenes in the course of criminal investigations.

Most of the trace evidence collected for analysis consists primarily of sediments and soil particles, materials which are easily transferred between objects. This geological evidence can be classified into seven groups, which have more or less overlapping boundaries: (1) rocks, (2) sediments, (3) soils, (4) dust, (5) minerals, (6) fossils and (7) particles (Pye 2007).

This paper focuses on comparative analyses of soil, since this is the principal geological material transferred at crime scenes and thus forms the bulk of the material studied in forensic laboratories. Generally, a soil constitutes a complex matrix composed of minerals, organic matter and living and decomposing organisms, and its composition depends on the bedrock, the climate, the organisms present, the topography and the time. From a forensic point of view however, soils are defined according to those elements of a soil which constitute relevant physical evidence in a criminal investigation.

Many different techniques are used in the forensic analysis of soils, all of which have the capacity to characterise a soil sample and compare it with another related to a criminal act. The most frequently employed techniques can be divided into three groups, the first of which concerns the identification of a soil according to its morphological and physical characteristics (e.g., colour, particle size, consistency and so on), as well as determination of the properties of the various minerals present in the soil (for example, particle shape and surface texture). A second group encompasses mineralogical and chemical techniques (for instance, X-ray diffraction, chromatographic and spectroscopic techniques, etc.), whilst the third group involves the analysis of biological traces (e.g., microbiology, pollen, etc.).

It is necessary to employ a comprehensive range of techniques in order to obtain sufficient data for effective characterisation of the samples to compare. Selection of the different analytical techniques to employ mainly depends on the availability of such techniques in the laboratory and their power of discrimination. It is also necessary to take other factors into account, such as sample size, appropriate choice of the fractions (particle size), destructive/non-destructive techniques and the collection of quantitative or qualitative data.

The minimum amount of sample required for analysis is a very important factor that determines selection of the techniques to be used, since in most cases in the forensic field, only a small quantity of soil is recovered from the objects, occasionally limited to just a few milligrams. It is also important to select a suitable particle size in order for the results to be representative and comparable with other soil samples, since although a soil may be mineralogically homogeneous, its chemical composition varies significantly according to particle size and the presence of silica, which is more abundant in the coarser fractions, and trace metals, which are mainly concentrated in fine sand, silt and clay (Pye and Blott 2004). Other factors to consider include the use of non-destructive techniques which will enable further

analyses at a later date, and quantification, which provides comparable numerical data through the use of statistical methods. In most cases where a sufficient amount of sample is available, the preferred method of soil comparison would be based on a combination of methods that combine qualitative and quantitative analyses.

4.2 Case of Looting at A Roman-Visigothic Burial Vault

Looting of archaeological sites is a criminal activity that contributes to the deterioration and destruction of Spain's national heritage and is classified in the Spanish Criminal Code as an offence against Historical Heritage. Although such criminal activities occur throughout Spain, a country rich in archaeological remains, one of the provinces where this type of crime is committed most and where most objects have been seized is Seville. The case study presented in this paper concerns the looting of a Roman-Visigoth burial vault in Moron de la Frontera (Seville, Spain).

Because the Guardia Civil has jurisdiction over most of the areas where archaeological sites are located, our units conduct numerous investigations into this type of crime. Where the investigations bear fruit and the perpetrators are located, the soil remains adhering to the recovered objects or the digging tools used to plunder the site can be compared. There are three types of looters: occasional ones who enjoy searching for archaeological remains to add to their personal collection and who use rudimentary means. Then there are the people who engage regularly in this activity for economic reasons and who possess technical and material means, and lastly, there are the so-called local scholars, consisting of "archaeology enthusiasts who see themselves as the saviours of local culture but know nothing of modern excavation techniques" (www.guardiacivil.es/patrimonio/activ_princip.jsp).

The Guardia Civil Nature Protection Service (SEPRONA) initiated a police investigation in relation to the looting of a burial vault (Roman-Visigothic) in Moron de la Frontera (Sevilla, Spain). This investigation resulted in the arrest of some suspects who a few days earlier had been prowling around the area where these archaeological remains appeared, and various tools which might have been used to carry out the criminal act were confiscated from the boot of their vehicle. The objects found in the boot included a pair of green boots, a pair of brown boots, a spade, a large mattock and another smaller one, with traces of soil attached. Given the evidence gathered by the Guardia Civil Unit, it was decided to collect soil profile samples at the entrance to the vault which, together with those taken from the vehicle, were sent to the Guardia Civil Criminalistic Service for soil comparison. During sampling of the burial vault, three samples were taken corresponding to one at 120 cm depth, another at 60 cm depth and a last one at surface level (<5 cm depth). The order of sampling was from the bottom up to avoid cross contamination, and samples were collected under sterile conditions (Figs. 4.1 and 4.2).

Fig. 4.1 Roman visigothic burial vault

Fig. 4.2 Green boots inside the suspect's vehicle

4.3 Methodology

Each of the samples provided sufficient soil to employ a variety of analytical techniques and perform a very thorough comparison, enabling us to determine the similarity or dissimilarity between the evidence and reference samples received in the laboratory.

The first step was to identify exotic materials that could characterise the samples, using stereo-binocular light microscopy. The samples were then dried at room temperature and sample separation was performed for subsequent analysis using two complementary methods. Thus, a subsample of 1 g was taken for microbiological analysis and the remainder was used for geochemical analysis, including the physical and chemical studies required to characterise the samples.

The analytical techniques used to analyse the bulk properties of soils for forensic comparison purposes are as follows: colour, particle size distribution, elemental analysis, major and trace element composition, anion concentration, electrical conductivity, pH, rDNA 16 s sequencing and comparison of microbial communities.

4.3.1 Soil Colour Using the Munsell Soil Colour Charts

The soil property of colour has been applied in forensic investigation and this characteristic is a potentially powerful method for sample discrimination (Pye and Croft 2004). Basically, two identification methods can be used: the Munsell Soil Colour Charts and spectrophotometry.

The different standard colours in the Munsell Soil Colour Charts (Munsell 1994) are expressed through a combination of three parameters: Hue (H), which indicates the relationship to red, yellow, green, blue and purple; Value (V), which indicates lightness; and Chroma (C), which indicates strength. However, this system may be subjective due to differences in colour perception on the part of the observers.

Colour spectrophotometry permits quantification, and thus represents a reproducible technique which is free of the subjectivity inherent in the use of Munsell tables. The spectrophotometers are based on the L*a*b colour system, where L represents luminance and a* and b* are the chromaticity coordinates.

It should be borne in mind that colour variations depend on particle size, the amount of organic material and the moisture content, and thus various authors have proposed measuring colour after carrying out different treatments (drying, calcination, removal of organic material or oxides) (Sugita and Marumo 1996) and in different fractions. Of the techniques proposed, and that which best discriminates between samples, is measurement of a dry, unsieved sample together with a dried sample sieved to <150 μm (Croft and Pye 2004; Guedes et al. 2009).

Determination of soil colour was performed using the *Munsell Soil Colour Charts*, which entail a degree of subjectivity due to assessment by the observer; this method was employed because a colour spectrophotometer was not available at the time.

Colour measurements were conducted using two soil fractions. These consisted of dry samples sieved to <0.5 mm and <2 μm, the latter corresponding to the sample extracted for analysis of the clay fraction using oriented aggregate mounts for X ray powder diffraction.

4.3.2 Particle Size Distribution by Laser Granulometry

Various methods have been used to determine particle size distribution, including direct observation (microscope), image analysis, dry and wet sieving and the hydrometer method, etc. However, the most reproducible of these is laser diffraction, especially in the case of fractions of sand, silt and clay, and the most frequently used fraction is <150 μm (Pye et al. 2006). To determine the proportion of sand and gravel, it is best to use dry or wet sieving.

Samples were analysed for particle size distribution using a Mastersizer 2000 with a Hydro 2000G liquid dispersion module, employing the Mie model.

In this case, the samples were processed using a <500 μm size fraction, which provides the highest level of reproducibility, although it was at the expense of limiting the potentially valuable and discriminatory information in the coarser part of the particle size (Blott et al. 2004).

Three subsamples were taken, of approximately 400 mg each, from each of the samples under study, previously sieved and homogenised. These were placed in an Erlenmeyer flask to which we added 50 ml of 2 % sodium hexametaphosphate dispersant solution for 1 h. Water and ultrasound were used as dispersant for 90 s prior to measurement.

To discriminate between samples, we used the following statistical measures: the percentage of sand, silt and clay, mean, median and $D_{10} - D_{90}$ (Blott et al. 2004), in addition to observing the particle size distribution curves of simple and cumulative percentage curves.

4.3.3 Qualitative Analysis from Bulk and Clay Mineralogy by X-ray Powder Diffraction (XRD)

X-ray diffraction (XRD) is one of the most important techniques used for identification of crystalline substances, the advantages of which are that it is a non-destructive technique, it can be used to analyse small samples, minimal sample preparation is required, and elements and their oxides can be differentiated, as well as polymorphic forms and mixtures of crystalline substances. Qualitative and quantitative tests can be conducted, and it is thus an extremely valuable tool for soil discrimination (Fitzpatrick et al. 2009).

X-ray diffraction analyses are performed on the unsieved fraction or on different fractions. It can also be used to identify the clay minerals present in the soil. The following groups are found within the clays: (1) the serpentine-kaolinite group (serpentine, chrysotile, kaolinite, dickite and halloysite), (2) the illite-mica group (illite polytypes, mica and glauconite), (3) the chlorite group (chamosite and clinochlore), (4) the smectite group (montmorillonite, nontronite and saponite),

(5) the vermiculite group, (6) interstratified minerals (e.g. illite-smectite, chlorite-smectite, etc.), (7) the sepiolite-palygorskite group, (8) the talc-pyrophyllite group, and (9) the imogolite-allophane group (Pye et al. 2007). The qualitative analysis of clays is performed after examining the changes in the 001 interplanar distances caused by saturation with the alkaline earth elements Mg^{2+} and K^{+} and organic compounds solvation (glycerol, ethylene glycol and DMSO). Heat treatment is also necessary to differentiate certain clay minerals, in which there are variations in the basal spacing or disappearance of diffraction lines.

The advantage of quantification by X-ray diffraction is the possibility it provides of statistical analysis and comparison of the results for evidence and reference samples. However, the ideal approach would be a combination of qualitative and quantitative diffraction (Ruffell et al. 2004).

X-ray diffraction analyses (Bruker Advance) were performed on the <2 and <0.5 mm (random powder) fractions. The diffractograms were obtained using a vertical goniometer and scanning from 3 to 70° at 0.05° 2θ/min. The diffractometer was operated in reflection mode, 40 Kv and 40 mA.

A study was also conducted of the clay fraction (<2 μ), previously extracted by decantation and oriented aggregate mounts. Clays were analysed with respect to modifications in 001 interplanar distances as a result of saturation with the alkaline earth elements Mg^{2+} and K^{+} and organic compounds (ethylene glycol), and heat treatment (400–550 °C). Prior to studying the clays, carbonates were eliminated using a 1 N acetic acid/sodium acetate trihydrate buffer at pH = 5. The diffractograms were obtained using a vertical goniometer and scanning from 2 to 30° at 0.05° 2θ/min. The diffractometer was operated in reflection mode, 40 Kv and 40 mA.

4.3.4 Elemental Analysis by Scanning Electron Microscopy (SEM-EDX)

Analysis by scanning electron microscopy provides information about the size, shape, surface texture and elemental composition of individual particles, which may be less than 3 μm in size, in addition to a general analysis of the sample to determine the elemental composition of the minerals present.

This type of analysis does not require a large amount of sample, since a small quantity of fine sand contains hundreds of particles from which hundreds of items of quantitative data can be obtained for comparison. The most suitable fraction which presents least variability is that of <150 μm (Pye et al. 2007; Pirrie et al. 2009).

In this case, the elemental composition was obtained for the <0.5 mm sample used for X-ray diffraction, which had previously been crushed. The analysis was conducted using a accelerating voltage of 25 Kv, emission current of 60 μA, 5.10^{e-6} mbar, area scan at 500X magnification and 10 spot mode analyses taken on an area of 200 μm^2.

4.3.5 Major and Trace Element Composition by Inductively Coupled Plasma Spectrometry (ICP-OES)

Major and trace elements were analysed by optical emission spectrometry (ICP-OES) using a Perkin Elmer Optima 3200 DV device, with an online internal standard (Sc) for calibration and analysis following microwave assisted acid digestion of the samples using the <150 μm fraction, which in most cases is the most suitable fraction for discriminating between samples (Pye and Blott 2004; Pye et al. 2006). Three replicates were performed for each sample, using 100 mg for each one.

Digestion of the soil sample (<150 μm) was performed with ultra trace grade nitric acid, using Milestone Ethos One equipment at 180 °C for 15 min. All material was previously washed with a solution of 0.5 % nitric acid and then rinsed with distilled water.

4.3.6 Anion Concentration by Ion Chromatography (IC)

The concentration in soils of anions such as chlorides, sulphates, nitrates, bicarbonates, phosphates, fluorides and bromides provides data for environmental or land use studies, but can also be used to compare soils in a criminal investigation. In general, the most suitable fraction for routine soil analysis and the one with the greatest power of discrimination is that of <250 μm (Bommarito et al. 2007).

Determination of anions in the soil samples was carried out by ion chromatography (Dionex LC20) after leaching with milli-Q water in a ratio of 1:5, centrifuging at 1500 rpm for 10 min and filtering the supernatant through a 0.20 μm PTFE filter. The mineral fraction used corresponded to <250 μm.

4.3.7 Other Analytical Techniques

Other techniques frequently used for the characterisation of soils which can also be used as comparison parameters include: (1) pH, which indicates the soil type (basic or acidic) and (2) electrical conductivity (EC), which measures the concentration of ions in solution (dissolved salts). Determination of the pH and conductivity of the 1:5 mass/volume extract was conducted in accordance with UNE 77305 and UNE 77308.

4.3.8 rDNA 16S Sequencing and Comparison of Microbial Communities

Using samples from soils, boots, the spade and the mattocks, microbiological analyses were performed employing the pre-enriching technique in non-selective culture (peptone saline – Cultimed© – 0.1 %) incubated for 24 h at 36 ± 2 °C and subsequent sowing for extension of 20 µl per plate in general culture media (nutritive agar-Cultimed ©) incubated another 24 h at 36 ± 2 °C to obtain bacterial isolates. Individualised diagnosis was performed through bacterial rDNA 16S, in accordance with the extraction, amplification and sequencing protocols established by the Fast Microseq 500 © Kit of Applied Biosystems. An ABI PRISM 3130 Genetic Analyzer© sequencer was used for automatic detection. Identification of the obtained sequences was carried out with the help of Microseq ID Manager© Software.

Extraction. Each of the different morphotypes obtained from the microbial colonies, isolated in nutritive Agar culture media, was placed in a 1.5 ml centrifuge tube using an inoculation loop, after adding 100 µl of the extraction reagent (*Ultra Prepman* ©-Applied *Biosystems)*. These samples were boiled for 10 min and then centrifuged for 3 min at a speed of 13000 rpm. Subsequently, 5 µl of supernatant was diluted to a final volume of 500 µl (1:100 dilution factor) with previously autoclaved deionised water.

rDNA 16S (500 bp) amplification. 15 µl of the above-mentioned 1:100 dilution plus 15 µl of amplification reagent (FAST Microseq 500®) were taken to obtain a final volume of 30 µl in sterile plastic centrifuge tubes with 0.2 ml capacity. The amplification protocol (cycling programme) was defined as: 10′ at 95 °C; 30 cycles of: 0″ at 95 °C and 15″ at 64 °C; and finally 1′ at 72 °C. The PCR products thus obtained were purified with Centrisep® filtration columns in accordance with the manufacturer's instructions.

The sequencing reaction required a final volume of 20 µl for each sample: 13 µl sequencing kit (Microseq 500-Sequencing Kit®) plus 7 µl taken from the amplified and purified PCR products. At this point, samples were duplicated to take one fraction for the Forward and another for the Reverse reaction. The sequencing protocol consisted of: 1′ at 96 °C; 25 cycles of: 10′ at 96 °C, 5″ at 50 °C and 1′15″ at 60 °C; and finally 0″ at 60 °C. Products from the sequencing reaction were purified as described in the preceding paragraph.

Each well of the ABIPRISM 3130- Applied Biosystems® was filled with 10 µl of the purified sequencing product, plus the same volume of formamide, to obtain a final volume of 20 µl.

This procedure enabled us to genetically identify each bacterial colony grown in the nutritive Agar culture media, after having been isolated from the original samples.

Sequences were processed and analysed following the procedures described previously. Sequences were removed from the analysis if they were less than 450 bp in length, had a quality score of less than 25 or did not contain a minimum identity of 99 %. For the statistical analysis of relationships and for the creation of family trees, we used the MEGA 4.0.1 program. Representative sequences were aligned using CLUSTAL 1.6©. A phylogenetic tree was inferred using Clearcut with the Maximum Composite Likelihood model. Taxonomy was assigned with a minimum support threshold of 60 %.

4.4 Results and Discussion

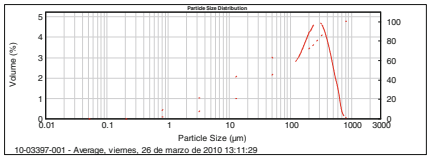
First, a similarity was observed between the colour of samples taken at depth (60–120 cm) and that of the soil sample adhering to the spade (Table 4.1). These deep samples should correspond to the same soil horizon. No differences were observed between the colour of samples taken at depth (60–120 cm) and that of the remnants of soil adhering to the spade. Differences between deep soil samples (60–120 cm) were however evident in the conductivity values (Fig. 4.3). pH analyses do not yield results with the power to discriminate between soil samples, although they can be useful for screening.

Particle size distribution curves for simple and cumulative percentage curves are presented in Fig. 4.3. The laser granulometry analysis showed that the particle size distribution and cumulative curves were very similar to each other, with the exception of the sample obtained at surface level. This difference was also observed in the higher percentage of silt and the D_{50} value obtained for the surface sample (Table 4.2). In samples taken from the profile of the burial vault, particle size distribution was observed to be very homogeneous at depth, and differed from the surface area.

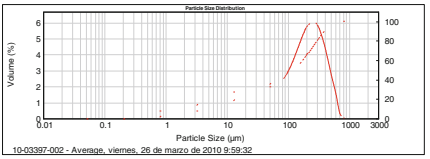
The mineralogical results (Table 4.3) indicated the presence of the smectite clay group (montmorillonite) in the soil samples taken at depth (60–120 cm) and the soil adhering to the spade and green boots, which was not present in the other samples. In these samples, montmorillonite clay was more abundant than other types, whereas in the remaining samples illite was the most abundant type. This difference as

Table 4.1 Summary of results for soil colour, pH and conductivity

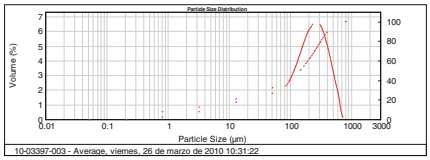
Sample	Colour <0.5 mm	Colour <2 μm	pH 1:5	Conductivity mS/cm
Surface level	7.5 YR 5/3	10 YR 3/2	7.57	312.0
60 cm depth	7.5 YR 7/2	10 YR 4/2	7.99	260.7
120 cm depth	7.5 YR 7/2	10 YR 4/2	8.38	185.6
Spade	7.5 YR 7/2	10 YR 4/2	7.68	277.1
Green boots	7.5 YR 6/3	10 YR 6/2	6.91	450.0
Brown boots	7.5 YR 5/3	10 YR 6/2	7.39	422.0
Large mattock	7.5 YR 5/3	10 YR 6/2	–	–
Small mattock	7.5 YR 6/4	10 YR 6/2	7.17	624.0



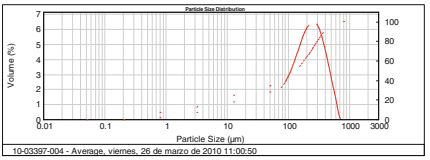
Surface level



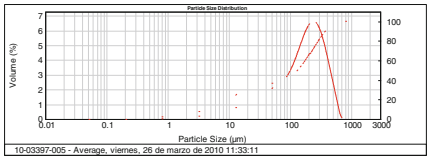
60 cm depth



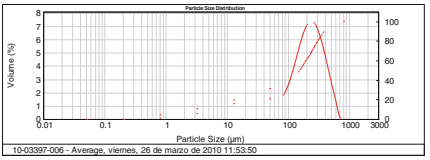
120 cm depth



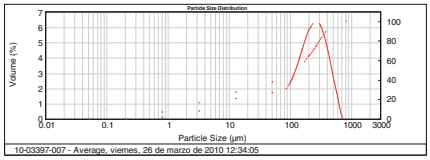
Spade



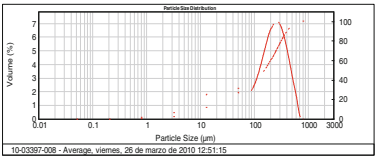
Green boots



Brown boots



Large mattock



Small mattock

Fig. 4.3 Particle size distribution curves and cumulative percentage curves

Table 4.2 Results of particle size parameters determined by laser granulometry

Sample	Mean (µm)	D_{10} (µm)	D_{50} (µm)	D_{90} (µm)	$D_{90} - D_{10}$ (µm)	% sand	% silt	% clay
Surface level	139.8	4.8	68.0	377.7	372.9	55.8	39.7	4.5
60 cm depth	162.3	4.9	123.2	390.9	386.0	64.9	30.1	5.0
120 cm depth	176.4	4.8	146.0	409.6	404.8	68.0	26.7	5.3
Spade	165.2	5.2	133.5	388.2	383.0	66.0	29.1	4.9
Green boots	162.6	11.0	131.5	371.8	360.8	68.8	29.6	1.6
Brown boots	175.0	6.6	155.2	390.5	383.9	69.0	27.4	3.6
Large mattock	161.5	4.3	126.3	389.3	385.0	62.7	32.2	5.1
Small mattock	181.3	11.4	156.5	407.5	396.1	69.1	29.7	1.2

Table 4.3 Mineralogy results obtained by x ray diffraction

Sample	Mineralogy				
Surface level	Quartz	Calcite	Illite	kaolinite	
60 cm depth	Quartz	Calcite	Illite	kaolinite	Montmorillonite
120 cm depth	Quartz	Calcite	Illite	kaolinite	Montmorillonite
Spade	Quartz	Calcite	Illite	kaolinite	Montmorillonite
Green boots	Quartz	Calcite	Illite	kaolinite	Montmorillonite
Brown boots	Quartz	Calcite	Illite	kaolinite	
Large mattock	Quartz	Calcite	Illite	kaolinite	
Small mattock	Quartz	Calcite	Illite	kaolinite	Dolomite

Table 4.4 Major and trace elements determined by ICP/OES (mg/Kg) and isotopic relationship determined by ICP/MS

Sample	Fe	Al	Mn	Zn	Ba	Cu	Cr	Ni	Pb	Cd	Co
Surface level	1200	2290	394	29.5	99.5	86.6	22.5	<10.0	17.7	<10.0	<10.0
60 cm depth	9760	18,400	435	15.8	102	19.3	15	<10.0	12.7	<10.0	<10.0
120 cm depth	10,200	17,300	496	15.4	159	17.1	14.1	<10.0	11.0	<10.0	<10.0
Spade	11,000	18,800	465	18.7	115	15.5	16	20.7	11.8	<10.0	<10.0
Green boots	7850	11,200	214	47.5	64.3	61.4	11.3	<10.0	<10.0	<10.0	<10.0
Brown boots	12,600	23,700	320	39.7	86.7	43	23.3	12.5	15.1	<10.0	<10.0
Large mattock	7340	7720	153	55.0	28.5	28.3	<10.0	<10.0	<10.0	<10.0	<10.0
Small mattock	13,500	2003	317	11.2	74.8	39.4	11.6	<10.0	11.2	<10.0	<10.0

regards the presence of an expansive clay (montmorillonite) distinguished these four samples from the rest.

The results obtained by ICP/OES indicated that the concentrations of the elements Mn, Zn and Cu had a greater power of discrimination regarding the deep soil samples (60–120 cm) and that attached to the spade compared with the rest of the samples (Table 4.4).

In the light of the results obtained by IC, it was concluded that the surface level sample could be excluded from the other evidence (Table 4.5). These results were used to generate a hierarchical cluster dendrogram.

For a statistical analysis capable of sample discrimination, a hierarchical cluster analysis was performed using SPSS version 18.0. To this end, a combination of the quantitative data obtained was employed, including particle size, trace metal, anions, pH and conductivity. The number of clusters was determined using the Euclidean distance as a distance measure and the Ward method as a linking method.

Table 4.5 Inorganic anion results obtained by IC (mg/l)

Sample	F	Cl	NO_2	NO_3	PO_4	SO_4
Surface level	<2	54.5	13	66	12.5	<20
60 cm depth	<2	289	>0.35	<10	<10	<20
120 cm depth	<2	<15	<0.35	<10	<10	<20
Spade	<2	38	<0.35	<10	<10	34
Green boots	<2	<15	<0.35	<10	114	206.5
Brown boots	<2	159.5	<0.35	<10	<10	68.5
Large mattock	–	–	–	–	–	–
Small mattock	<2	256.5	2.5	<10	175	547.5

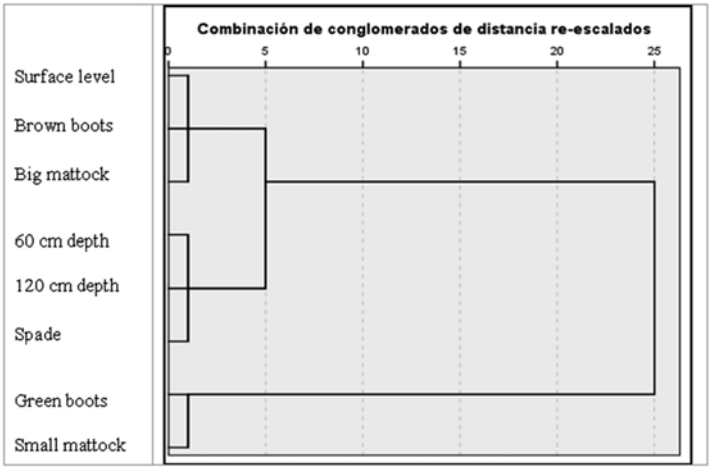


Fig. 4.4 Historical cluster dendrogram (Ward method) combining particle size distribution and chemical analysis

The dendrogram resulting from this classification of samples into relatively homogeneous and heterogeneous groups (cluster analysis) clearly demonstrates their similarity (Fig. 4.4).

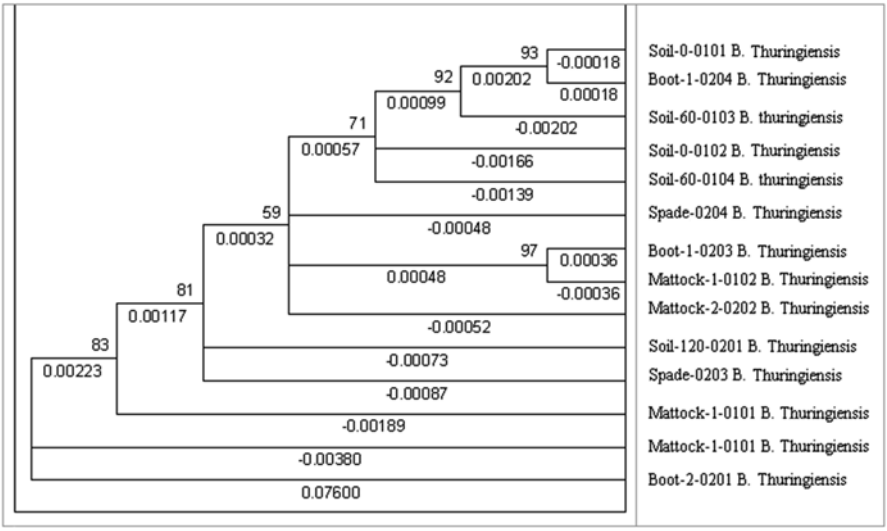
The results of the qualitative and quantitative analyses indicated that the samples taken at depth (60–120 cm) and the soil adhering to the spade presented similarities as regards colour, distribution profile (granulometry), mineralogy and chemical composition. The dendrogram resulting from this classification of samples into relatively homogeneous and heterogeneous groups (cluster analysis) clearly demonstrates their similarity.

The principal results in support of this conclusion include the same chromatic colour (7.5 YR 7/2 pinkish gray) and mineralogy, since these samples presented an expansive clay (montmorillonite) in the deep layers of the burial vault profile.

Table 4.6 Results of the bacterial phylogenetic analysis

Sample	Identification
Surface level	Bacillus thuringiensis (ATCC10792; DSM6091).
60 cm depth	Bacillus thuringiensis (ATCC10792; DSM6091); Enterobacter sp.
120 cm depth	Bacillus thuringiensis (ATCC 33679).
Spade	Citrobacter braakii (ATCC 51113); Bacillus thuringiensis (ATCC 33679).
Green boots	Escherichia coli(ATCC53503); Bacillus thuringiensis (ATCC 33679; ATCC10792).
Brown boots	Bacillus thuringiensis (ATCC 33679; DSM6110).
Large mattock	Bacillus thuringiensis (DSM6110).
Small mattock	Bacillus thuringiensis (DSM6110).

Table 4.7 Phylogenetic relationships among the 27 specimen isolates studied



Coincident bacterial isolates were identified to species level in several of the samples. A bacterial phylogenetic analysis indicated statistically significant relationships between *Bacillus thuringiensis* isolates obtained from the surface level sample and the green boots (93 %), and from the 60 cm depth sample and the same boots (92 %). A close relationship (97 %) was also observed between isolates of *B. thuringiensis* obtained from the green boots and those from the small mattock (Tables 4.6 and 4.7).

The remaining site sample could not be excluded as a possible source of the soil recovered from the tools used to loot the burial site. Samples presented a strong match in terms of all comparison criteria used.

4.5 Conclusions

This case study illustrates the forensic analysis of soil carried out by the Guardia Civil Criminalistic Service. It also shows the importance of this analysis for the study of trace evidence in criminal investigations, due to its capacity to link a crime scene with the object used.

In soil forensics, where samples taken at the crime scene are compared with samples adhering to different objects in the possession of a suspect, the use of a combination of different geochemical and biological techniques is of great importance, in addition to the statistical treatment of the data obtained, in order to arrive at a conclusion about the relationships between samples. It should also be noted that the data obtained will depend on the amount of sample available and use of the most suitable techniques in order to obtain sufficient data to establish such relationships.

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