Henk Kars
Lida van den Eijkel
Editors

Soil in Criminal and Environmental Forensics

Proceedings of the Soil Forensics Special, 6th European Academy of Forensic Science Conference, The Hague

Springer

Soil Forensics

Series editor
Henk Kars
Faculty of Earth and Life Sciences
VU University Amsterdam
Amsterdam, The Netherlands

To be a forum for all (scientific) workers in the rather fragmented field of Soil Forensics. This fragmented character is intrinsic to multidisciplinary research fields and a common platform for the exchange of knowledge and discussion is therefore heavily needed. To promote the field of Soil Forensics in academia, in forensic research institutes, legal profession/jurisdiction organisations and for the general public (science sections in newspapers). To contribute to a high scientific standard of the field. To be attractive for publishing in the series it is peer reviewed in order to be competitive with journals such as Forensic Science International.

More information about this series at http://www.springer.com/series/11807

Henk Kars • Lida van den Eijkel
Editors

Soil in Criminal and Environmental Forensics

Proceedings of the Soil Forensics Special, 6th European Academy of Forensic Science Conference, The Hague

Springer

Editors
Henk Kars
Faculty of Earth and Life Sciences
VU University Amsterdam
Amsterdam, The Netherlands

Lida van den Eijkel
Netherlands Forensic Institute
The Hague, The Netherlands

ISSN 2214-4293 ISSN 2214-4315 (electronic)
Soil Forensics
ISBN 978-3-319-33113-3 ISBN 978-3-319-33115-7 (eBook)
DOI 10.1007/978-3-319-33115-7

Library of Congress Control Number: 2016951948

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG Switzerland

Preface

In this first volume of a newly established series on soil forensics, the reader will find a collection of papers based on contributions to the soil forensics sessions that were a part of the 6th triennial conference of the European Academy of Forensic Sciences in the Hague in 2012. They represent a cross section of the many contributions: 34 oral presentations, a workshop and a forum in a total of 12 sessions, and, in addition, another 18 posters on display throughout the conference.

The soil sessions of the conference, also the 4th meeting of the world-wide Soil Forensics International (SFI) network, attracted contributors from all corners of the globe, reflecting the fact that everywhere soils are recognized as a source of meaningful forensic information.

Together the contributors showed the multiple uses of soil forensics in the different areas of law enforcement these days. In criminal investigation, soil is studied as trace evidence and as a place where victims are buried and decay. In environmental investigations, the quality of soil as such is studied, since everywhere soil is protected by law from pollution and mismanagement. All forensic soil examinations however share common ground: fieldwork at crime scenes, sampling procedures, and laboratory analysis to gather data, followed by the difficult task of interpreting the obtained results given the enormous complexity of soil with its many functions, the multitude of processes that take place in it, and its big variability in space and time.

During the conference, much experience with this complicated material was shared among all participants, both practitioners doing casework and academic researchers involved in the fundamental development of soil forensics. Finally, in a forum, questions and insights that had emerged during the sessions were discussed, leading to some recommendations for the community of forensic soil scientists to work on in the future. For instance, what's in a name? Soil forensics deals with the study of soils as depicted above, and it is, as is the case with the whole field of forensic science, an inter- to multidisciplinary discipline, with extremely important transdisciplinary aspects. It was felt, therefore, that a widely accepted common terminology for all aspects of this field is urgently needed for the research community and end users in law enforcement.

Another concern was related to the fragmentary nature of the current practice of forensic soil science. It is an applied science, but the field covers the whole range of research, from service-on-demand work at one end to fundamental interdisciplinary research at the other. The research community of soil forensics however is composed of numerous small groups or even individual researchers all over the world. It is a challenge of vital importance to this community to create larger research groups and interaction on (inter)national levels. Cooperation and exchange will help to be more successful with funding bodies and will further improve the quality of soil forensics and keep it up to date – all with the final goal of increasing its strength of evidence for the end users in law enforcement.

This book gives the reader a broad view on the current practice of soil forensics in case work and the research that is taking place internationally to further develop this field. The contents can of course be studied from a specialist point of view, focusing on the particular aspect that one is interested in, but for forensic applications of soil science, it is essential to keep in mind and elaborate on the themes as discussed by the forum. The aim of this book is to contribute substantially to the importance of soil forensics as a truly forensic expertise.

Amsterdam, The Netherlands — Henk Kars
The Hague, The Netherlands — Lida van den Eijkel

Contents

Part I
Criminal Soil Forensics: The Examination of Traces and Legal Context

Chapter 1
Forensic Palynology: Checking Value of Pollen Analysis as a Tool to Identify Crime Scene in Semiarid Environments

M. Munuera-Giner and J.S. Carrión

Abstract Taphonomic variables affecting pollen content of soil are especially relevant in semiarid localities, which could limit the potential of palynology as a source of evidence in courts. A number of positive experiences have so far been carried out in humid climates, but not in semiarid environments. Here we aim at comparing pollen spectra from soil surface samples and footwear sediment infill in order to evaluate the possibility of using palynology as associative evidence in a theoretical crime scene occurring in a semiarid environment. To check if any "handy forensic correspondence" can be found, five areas of the region of Murcia in south-eastern Spain, different in flora, vegetation and biogeography, were selected.

1.1 Introduction

Plants release pollen grains that mostly settle on the ground, where, if appropriate conditions, they can persist even for millennia; those pollen grains can be extracted from soil and analyzed, showing particular assemblages and giving precise information about the vegetation in the surrounding areas (Erdtman 1969; Moore et al. 1991). As a consequence, palynology has a potential as a source of evidences in solving legal issues, as was firstly proposed by Locard (1930) and evidenced by Wilhelm Klaus in 1959 (Erdtman 1969).

The theoretical principles of forensic palynology have been amply described by different authors and a number of methods and examples have been displayed, showing that palynology can be a valuable forensic tool at least for over 50 years and emphasizing potentiality of this "blooming science" (Palenik 1982; Mildenhall 1988; Bryant and Mildenhall 1990; Mildenhall 1990; Brown and Llewellyn 1991;

M. Munuera-Giner (✉)
Faculty of Agricultural Engineering, Department of Agricultural Science and Technology, Technical University of Cartagena (UPCT), Cartagena (Murcia), Spain
e-mail: Manuel.Munuera@upct.es

J.S. Carrión
Faculty of Biology, Department of Plant Biology, University of Murcia, Murcia, Spain

H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics,* Soil Forensics, DOI 10.1007/978-3-319-33115-7_1

Mildenhall 1992; Stanley 1992; Szibor et al. 1998; Bruce and Dettmann 1996; Eyring 1997; Bryant and Mildenhall 1998; Horrocks and Walsh 1999, 2001; Mildenhall 2004; Milne 2004; Bryant and Jones 2006; Mildenhall 2006; Mildenhall et al. 2006; Wiltshire and Black 2006; Bertino 2008; Bryant 2009; Dobrescu et al. 2011). Unfortunately, the full potential of forensic palynology remains neglected in most countries in spite of its proved versatility in many kinds of criminal inquiries.

Forensic palynology is not an exact science due to the diversity of factors that control whether pollen grains and spores are or not finally present in a given place, and in which proportions they occur, that is, because the existence of diverse taphonomic variables (Mildenhall et al. 2006; Wiltshire and Black 2006). Precisely because of the taphonomic variability affecting palynomorphs' presence in soils (and other surfaces too), it must be assumed a certain unpredictability of the spatial patterning of pollen spectra as well as great heterogeneity of pollen and spore assemblages (Wiltshire and Black 2006), but, even so, strong correlations have been shown between soil samples obtained from footwear or clothes and soil surface samples from a precise site (Bruce and Dettmann 1996; Horrocks et al. 1998, 1999; Brown et al. 2002; Bull et al. 2006; Riding et al. 2007).

Regardless its undeniable validity and with relations to those taphonomic questions above-referred must be considered that reported examples connecting soil surface samples and soil from footwear/clothes by their palynological assemblages are mostly related with mud in more or less humid climates (Horrocks et al. 1998, 1999; Bull et al. 2006; Mildenhall et al. 2006; Riding et al. 2007), but no experiences in forensic palynology have been carried out in arid or semiarid, Mediterranean environments. That is significant because mud and wet soils effectively trap pollen and easily stick to footwear and clothes in considerable amounts, unlike dry sediments, which easily lose pollen and hardly stick to surfaces.

1.1.1 Why Semiarid Sites Are Special?

In richly vegetated regions transport of pollen by winds, rivers and other factors has a subordinate effect on pollen spectra from soil samples but are of prime importance in arid areas (Horowitz 1992), and can lead to an over-representation of anemophilous taxa and even to a scarce presence of pollen grains and types. In addition, the oxic conditions in those dry environments usually involve a poor preservation or even complete disintegration of pollen grains, specially those having thin walls. For instance, modern surface samples from the arid south-western USA generally record less than 40 pollen types of which only five, namely *Pinus*, *Juniperus*, Poaceae, Chenopodiaceae, and Asteraceae, may account for 90 % of the pollen counts (Hall 1985). In these habitats, the anemophilous pollen percentages can be considerably higher than zoophilous ones even when anemophilous elements are less represented than zoophilous (El Ghazali and Moore 1998).

In spite of this, palynological study of surface soil samples is a suitable tool to register vegetation differences in arid environments (Carrión 2002), and seems to have a potential in forensic sciences (Guedes et al. 2011). Certainly, because the

influence of a number of factors the pollen content of a soil could show not an "exact/correct picture" of the surrounding vegetation. Nonetheless, its particular pollen spectrum could be useful for comparison purposes (linking persons/objects with possible crime scenes), making necessary to test the existing correspondence in pollen content between soil surface samples and soil forensic samples from clothes, fabrics and footwear. This work is aimed to check if any "handy forensic correspondence" can be found between soil pollen spectra and pollen content of soil samples from shoes in a semiarid environment as southeastern Spain.

1.2 Materials and Methods

Five localities showing a diversity of plant communities were selected within the region of Murcia (Fig. 1.1). Details about location, climate, bioclimatic belt and vegetation of the sites are shown in Table 1.1. At each locality, clean outdoor boots

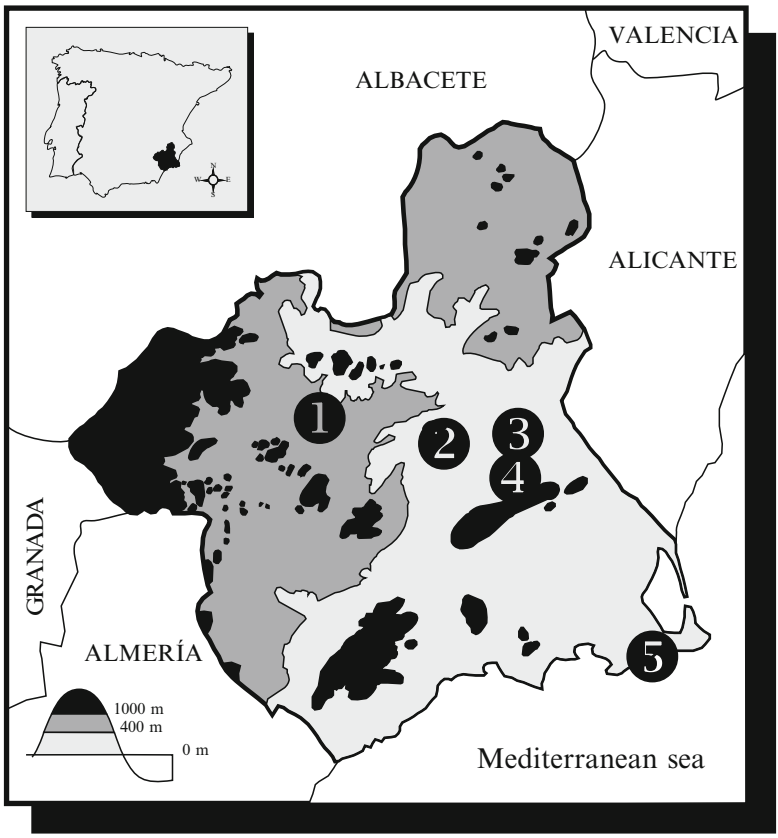


Fig. 1.1 Location of sampling sites in Murcia Region (Spain). *1* Carrascalejo; *2* Albudeite; *3* Espinardo; *4* La Alberca; *5* Cartagena

Table 1.1 Main characteristics of the sampling sites

Site	Altitude m.a.s.l.	Coordinates	Bioclimatic belt	Ombro-climate	Short description	Predominate species
Carrascalejo	620	38° 03′ 38″ N 01° 42′ 40″ W	Meso-Mediterranean	Dry	Small stream with special deciduous/ evergreen forest gallery	Trees: *Quercus faginea, Populus nigra* and *Populus alba* near the stream and *Pinus halepensis, Quercus rotundifolia, Fraxinus angustifolia* and *Olea europaea* nearby
						Shrubs: *Quercus coccifera, Daphne gnidium, Pistacia terebinthus, Pistacia lentiscus, Rhamnus lycioides, Genista scorpius, Ulex parviflorus, Rosmarinus officinalis, Thymus vulgaris, Sideritis leucantha, Satureja obovata*
Albudeite	193	38° 01′ 24″ N 01° 23′ 42″ W	Upper thermo-Mediterranean	Semiarid	Stream (usually dry) with marly-saline-nitrified soils rich in Chenopodiaceae	Shrubs: *Suaeda vera, Anabasis hispanica, Atriplex halimus, A. glauca, Salsola genistoides, Tamarix boveana, T. canariensis, Limonium caesium, Capparis spinosa, Anthyllis cytisoides, Lygeum spartum, Stipa capensis, Helianthemum squamatum*

Espinardo	95	38° 01′ 11″ N 01° 10′ 06″ W	Upper thermo-Mediterranean	Semiarid	Landscaped area in Campus of University of Murcia	Trees: in the selected garden *Morus alba, Phoenix dactylifera, Ph. canariensis, Schinus molle, Citrus limon, C. aurantium;* and *Acacia farnesiana, Robinia pseudoacacia, Pinus halepensis, Ceratonia siliqua* and *Ulmus pumila* nearby
La Alberca	58	37° 56′ 32″ N 01° 09° 07″ W	Upper thermo-Mediterranean	Semiarid	*Eucalyptus* wood in anthropic area with nitrified soils	Trees: *Eucalyptus camaldulensis* Shrubs: *Oxalis pes-caprae, Marrubium vulgare, Sisymbrium irio, Moricandia arvensis, Capsella bursa-pastoris, Piptatherum miliaceum, Hyparrhenia sinaica, Hordeum vulgare, Silybum marianum, Chrysanthemum coronarium, Malva parviflora*
Cartagena	40.	37° 34′ 33″ N 00° 57′ 53″ W	Lower thermo-Mediterranean	Semiarid	Coastal site with special *Periploca* thicket	Trees: *Periploca angustifolia, Chamaerops humilis* Shrubs: *Asparagus albus, Genista umbellata, Calicotome intermedia, Thymelaea hirsuta, Salsola oppositifolia*

with a maximum tread of 6 mm deep was "normally" walked around (that is, not using exaggerated force in order to deliberately entrain material into the boot tread) for 3 min, in random directions over an area of approximately 25 m^2. After that and by using a small clean spatula and a clean brush, all sediment in the boots soles was removed and saved in a new sterile plastic bag. Finally, a composite sample was collected as a control and consisting of 12–15 subsamples of soil taken at a depth of 1–2 mm with a clean spatula and put together in a sterile plastic bag to be thoroughly homogenized before pollen analysis.

After deflocculation by using sodium pyrophosphate, soil samples were prepared for pollen analysis according to the KOH, hydrofluoric acid and hydrochloric acid method, including flotation in zinc chloride (Dimbleby 1957, 1961; Frenzel 1964; Bastin and Couteaux 1966; Girard and Renault-Miskovsky 1969; Juvigné 1973). Pollen mounted in glycerol was identified and quantified at X400–X1000 by light microscopy.

1.3 Results and Discussion

As expected and with the exception of the locality at Cartagena, pollen spectra show relatively low diversity and dominance of anemophilous types (Hall 1985; El Ghazali and Moore 1998). Except for Carrascalejo, all selected sites are semiarid and, as expected, pollen spectra from soil samples (Fig. 1.2) depict five well-differentiated habitats and correlate quite well with main vegetation in their surrounding areas. After microscopic examination, a total of 57 pollen types (54 Magnoliophyta and 3 Pinophyta), 10 spore types (2 Bryophyta, 5 Algae and 3 Fungi) and one Oribatida species (moss mites) were identified.

For each study case, the pollen diagram (Fig. 1.2) shows a close resemblance between soil surface samples and those from footwear dust, not only in main pollen types but also in rare types and fungal and algal spores. Between 8 and 37 different types were identified in sites (Table 1.2). Maximum diversity was found in Cartagena and Espinardo but relative diversity in soil surface samples was higher than in shoe samples in Espinardo and Albudeite, and lower in samples from Carrascalejo, La Alberca and Cartagena. The proportion of taxa present both in soil and shoe samples moves around 45 % in Albudeite, Espinardo and Cartagena, reaching 61 % in Carrascalejo and almost 90 % in La Alberca (Table 1.2). Even though results are summarized in Figs. 1.2, 1.3 and Table 1.2 a short analysis for every site is done.

- **Carrascalejo.** A total of 23 pollen types was identified, 61 % of them both in soil and shoe samples. In spite of some differences in percentages, pollen spectra from soil and shoes correlate quite well. According with its dominance in the surroundings, *Pinus* is the dominant type, being *Quercus*, Chenopodiaceae, Asteraceae, *Populus* and Cistaceae other important elements characterizing the site. Noteworthy is the presence of fungal spores (*Glomus*, Sordariaceae and *Tilletia*) and algae zygospores and aplanospores (*Zygnema, Rivularia,*

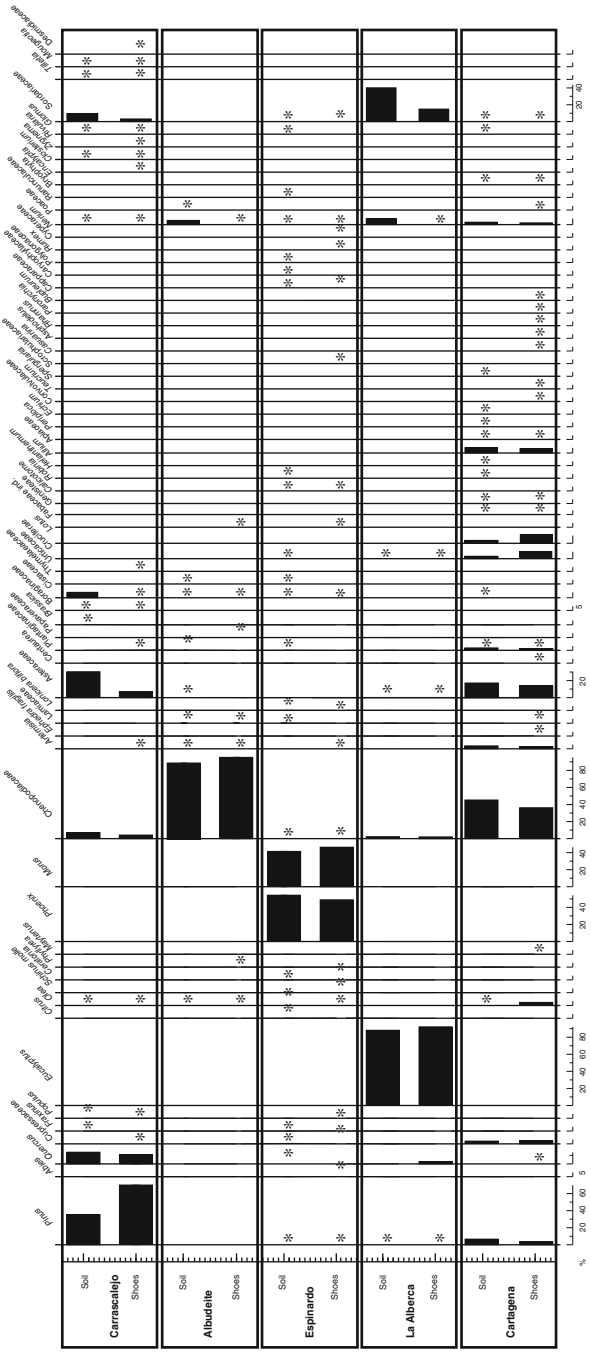


Fig. 1.2 Pollen diagram showing percentages for soil surface and shoe samples. Percentages below 2 % showed as *

Table 1.2 Number of pollen/spore types found in sites

	Total	Soil	Shoes	Both	Both
Carrascalejo	23	16	21	14	60.9 %
Albudeite	13	10	9	6	46.1 %
Espinardo	32	25	22	15	46.9 %
La Alberca	8	7	8	7	87.5 %
Cartagena	37	23	30	16	43.2 %

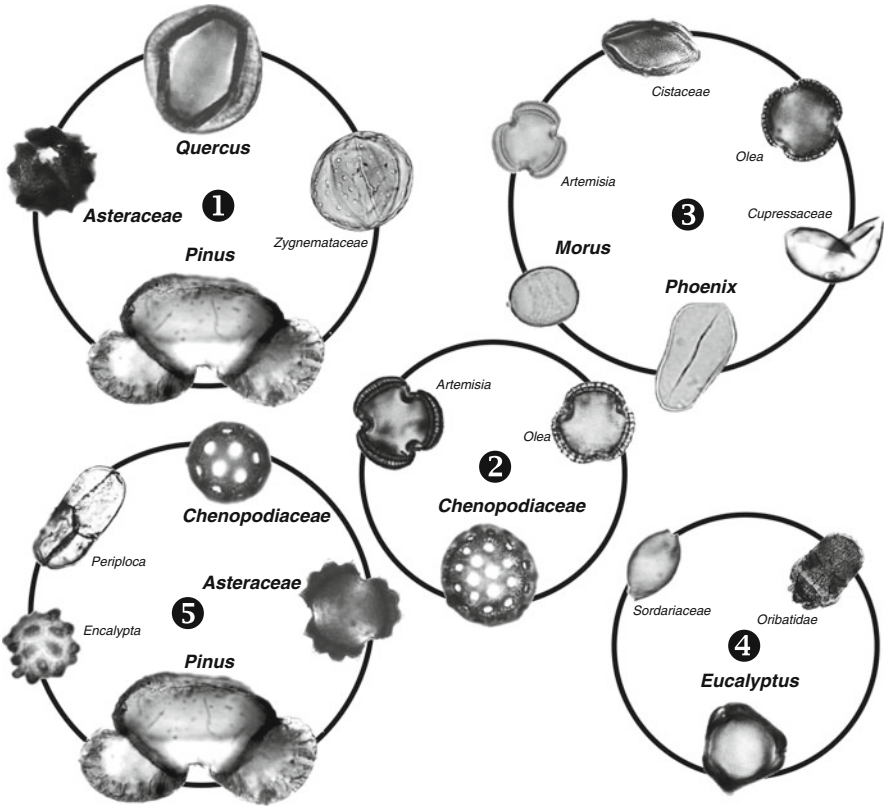


Fig. 1.3 Summary chart of the discrimination of the five sites on the basis of the pollen percentages both in shoes and soil samples. *1* Carrascalejo; *2* Albudeite; *3* Espinardo; *4* La Alberca; *5* Cartagena

Desmidiaceae, Closterium and *Mougeotia*). Such a number of particular occurrences are probably due to a footstep on a wet site near the stream.

- **Albudeite.** Chenopodiaceae, Poaceae and *Tamarix* are the more abundant plants on this site (Table 1.1), but in pollen counts Chenopodiaceae reach by itself 88.6 % in soil surface samples and 95.3 % in shoe samples. *Artemisia*, Lamiaceae, Cistaceae and Poaceae are other characteristic elements. Only 13 pollen types were identified in Albudeite, six of them both in soil and shoe samples.

- **Espinardo.** Although other trees are frequent in the selected area (*Citrus*, *Schinus*, *Fraxinus*, *Robinia*; Table 1.1), their pollen grains are scarce in samples while pollen from *Phoenix* and *Morus* exceeded 95 % of the pollen found both in soil surface and shoe samples, probably because *Morus* was just finishing blooming and *Phoenix* blooms through the whole year. Presence of *Lonicera* and low percentages of *Pinus* and *Cupressaceae* are noteworthy, especially the last ones, which are "highly under-represented" having in mind their significant presence in the vicinity of the garden, its high production of pollen and its anemophilous dispersal.
- **La Alberca.** With the only exception of *Quercus* (only found in shoes), the same taxa are found in soil surface and shoe samples. A low diversity characterizes samples from La Alberca, which are dominated by *Eucalyptus* (88–92 %), a pollen type totally absent in the other sites. The high presence of Sordariaceae agrees with the use of the area as grazing land.
- **Cartagena.** In spite of being the driest location, shows the highest diversity with a total of 37 taxa, 30 of them found in shoe samples. Correlation of taxa between soil surface and shoe samples reaches 43 %. Both spectra match very well and show the main elements of the surrounding area, including characteristic, entomophilous taxa like *Periploca*, *Maytenus*, *Calicotome* and *Rhamnus*

1.4 Conclusion

The forensic use of palynology is challenging when dealing with semiarid regions, principally due to the particularities of pollen taphonomy and, in addition, because of the limited possibilities of adherence of dry soil to footwear. Here we have compared pollen assemblages in soil surface samples with those from soil samples in footwear walked, and found remarkable correlation. However, this is a preliminary study and a more complete, wide-ranging research is still needed. This new study should be orientated towards a thorough investigation of the effect of time (weeks, months) on the pollen spectra so as to elucidate when the control samples will stop being valid as evidential samples due to the biases caused by differential preservation of pollen grains and spores.

References

Bastin B, Couteaux M (1966) Application de la méthode de Frenzel à l'extraction des pollens dans les sédiments archéologiques pauvres. L'Anthropologie 70:201–203

Bertino A (2008) Forensic sciences: fundamentals and investigations, 1st edn. South-western Cengage Learning, Mason

Brown AG, Llewellyn P (1991) Traces of guilt: science fights crime in New Zealand. Collins Publishers, Aukland

Brown AG, Smith A, Elmhurst O (2002) The combined use of pollen and soil analyses in a search and subsequent murder investigation. J Forensic Sci 47:614–618
Bruce RG, Dettmann ME (1996) Palynological analysis of Australian surface soils and their potential in forensic science. Forensic Sci Int 81:77–94
Bryant VM Jr (2009) Forensic palynology: why it works? Trace evidence symposium. Clearwater Beach, Florida
Bryant VM Jr, Jones GD (2006) Forensic palynology: current status of a rarely used technique in the United States of America. Forensic Sci Int 163:183–197
Bryant VM Jr, Mildenhall DC (1990) Forensic palynology in the United States of America. Palynology 14:193–208
Bryant VM Jr, Mildenhall DC (1998) Forensic palynology: a new way to catch crooks. In: Bryant VM, Wrenn JW (eds) New developments in palynomorphs sampling, extraction, and analysis, American association of stratigraphic palynologists foundation, contributions series 33, pp 145–155
Bull PA, Parker A, Morgan RM (2006) The forensic analysis of soils and sediments taken from the cast of a footprint. Forensic Sci Int 162:6–12
Carrión JS (2002) A taphonomic study of modern pollen assemblages from dung and surface sediments in arid environments of Spain. Rev Palaeobot Palynol 120:217–232
Dimbleby GW (1957) Pollen analysis of terrestrial soils. New Phytol 56:12–28
Dimbleby GW (1961) Soil pollen analysis. J Soil Sci 12:1–11
Dobrescu EM, Olteanu GI, Sima E (2011) Defining the elements of new scientific disciplines – Palynoforensics. Int J Criminal Investig 1:87–94
El Ghazali GEB, Moore PD (1998) Modern lowland pollen spectra and contemporary vegetation in the eastern Sahel Vegetation Zone, Sudan. Rev Palaeobot Palynol 99:235–246
Erdtman G (1969) Handbook of palynology. An introduction to the study of pollen grains and spores. Hafner, New York
Eyring MB (1997) Soil pollen analysis from a forensic point of view. Microscope 44:81–97
Frenzel B (1964) Zur pollenanalyse von Lössen. Eiszeit Gegenw 15:5–39
Girard M, Renault-Miskovsky J (1969) Nouvelles techniques de preparation en palynologie appliques a trois sediments du Quaternaire final de l'Abri Corneille (Istres-Bouches-du-Rhone). Bulletin de l'Association Française pour l'Etude du Quaternaire 4:275–284
Guedes A, Ribeiro H, Valentim B, Rodrigues A, Sant'Ovaia H, Abreu I, Noronha F (2011) Characterization of soils from the Algarve region (Portugal): a multidisciplinary approach for forensic applications. Sci Justice 51:77–82
Hall SA (1985) Quaternary pollen analysis and vegetational history of the Southwest. In: Holloway RG, Bryant VM (eds) Pollen records of late-quaternary North American sediments. American Association of Stratigraphic Palynologists Foundation, Dallas, pp 95–123
Horowitz A (1992) Palynology of arid lands. Elsevier, Amsterdam
Horrocks M, Walsh KAJ (1999) Fine resolution of pollen patterns in limited space: differentiating a crime scene and alibi scene seven meters apart. J Forensic Sci 44:417–420
Horrocks M, Walsh KAJ (2001) Pollen on grass clippings: putting the suspect at the scene of the crime. J Forensic Sci 46:947–949
Horrocks M, Coulson SA, Walsh KAJ (1998) Forensic palynology: variation in the pollen content of surface samples. J Forensic Sci 43:320–323
Horrocks M, Coulson SA, Walsh KAJ (1999) Forensic palynology: variation in the pollen content on shoes and in shoeprints in soil. J Forensic Sci 44:119–122
Juvigné E (1973) Une méthode de séparation des pollens applicable aux sédiments mineraux. Ann Soc Géol Belg 96:253–262
Locard E (1930) The analysis of dust traces. Part II. Am J Police Sci 1:401–418
Mildenhall DC (1988) Deer velvet and palynology: and example of the use of forensic palynology in New Zealand. Tuatara 30:1–11
Mildenhall DC (1990) Forensic palynology in New Zealand. Rev Palaeobot Palynol 64–65:227–234

Mildenhall DC (1992) Pollen plays part in crime-busting. Forensic Focus 11:1–4
Mildenhall DC (2004) An example of the use of forensic palynology in assessing an alibi. J Forensic Sci 49:1–5
Mildenhall DC (2006) An unusual appearance of a common pollen type indicates the scene of the crime. Forensic Sci Int 163:236–240
Mildenhall DC, Wiltshire PEJ, Bryant VM Jr (2006) Forensic palynology: why do it and how it works. Forensic Sci Int 163:163–172
Milne LA (2004) A grain of truth: how pollen brought a murderer to justice. New Holland Publishers Pty Limited, New Holland
Moore PD, Webb JA, Collinson ME (1991) Pollen analysis, 2nd edn. Blackwell Scientific Publications, Oxford
Palenik S (1982) Microscopic trace evidence – the overlooked clue: Part II, Max Frei – Sherlock Holmes with a microscope. The Microscope 30:163–168
Riding JM, Rawlins BG, Coley KH (2007) Changes in soil pollen assemblages on footwear at different sites. Palynology 31:135–151
Stanley EA (1992) Application of palynology to establish the provenance and travel history of illicit drugs. Microscope 40:149–152
Szibor R, Schubert C, Schöning R, Krause D, Wendt U (1998) Pollen analysis reveals murder season. Nature 395:449–50
Wiltshire P, Black S (2006) The cribriform approach to the retrieval of palynological evidence from the turbinates of murder victims. Forensic Sci Int 3:224–230

Chapter 2
Forensic Palynology: How Pollen in Dry Grass Can Link to a Crime Scene

Martina Weber and Silvia Ulrich

Abstract This chapter describes a homicide case of a baby and the forensic potential of pollen in dry grass. Dry grass is a good source for pollen. Pollen analysis gave a very characteristic pollen assemblage, dominated by grass pollen and a fungal spore. The dry grass in which the baby's corpse was embedded could be traced back to the crime scene. An accompanying investigation of various dry grass samples showed that each one had a unique pollen assemblage. This case reintroduced Forensic Palynology to Austria.

2.1 Introduction

Forensic palynology is the use of pollen and spores in legal cases (Bryant and Mildenhall 1998; Mildenhall et al. 2006). Pollen and spores have characteristics that make them an excellent tool for forensic investigations (Bryant et al. 1996; Miller Coyle 2005; Mildenhall 2008; Morgan et al. 2008; Walsh and Horrocks 2008). They are microscopically small (approx. 20–200 μm) and thus invisible to the naked eye. The pollen wall is, depending on preservation conditions, mechanically as well as chemically extremely resistant to decay. Their morphology (mainly ornamentation of the walls and aperture types) allows correlation with specific plant taxa and thus vegetation types. Moreover, pollen and spores are omnipresent and each site is unique in its pollen/spore assemblage (Wiltshire 2006a). In a large number of cases pollen has been the key for solving crime. This has been demonstrated in various types of crimes: homicide (Brown et al. 2002; Mildenhall 2004; Wall et al. 2004; Wiltshire 2006b), burglary (Mildenhall 1989; Mildenhall 2006a), rape and violent assault (Mildenhall 2006b), war crimes (Szibor et al. 1998; Brown 2006), forgery (Bryant and Mildenhall 1998; Bryant 2007) and illicit and counterfeit drugs (Bryant et al. 1990; Stanley 1992; Newton et al. 2008).

M. Weber (✉) • S. Ulrich
Department of Structural and Functional Botany, Faculty Centre of Biodiversity, University of Vienna, Rennweg 14, A–1030 Vienna, Austria
e-mail: martina.weber@univie.ac.at

H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics*, Soil Forensics, DOI 10.1007/978-3-319-33115-7_2

This chapter reports the results of a forensic palynological study on dry grass, which was carried out in the course of a homicide inquiry. Results demonstrate the importance of close cooperation between palynologists and crime scene officers.

2.2 The Case

On April 5th, 2009 the corpse of a new-born babygirl was found inside a sealed white box by two huntsmen. The box was deposited next to a field path on the countryside in Lower Austria (Fig. 2.1a). The region is a typical agricultural area, with rather small fields and shelter belts (Fig. 2.1b). Inside the box, the dead body was embedded in dry grass, which we got for investigation (Fig. 2.1c).Before the arrival of the crime scene officers the huntsmen had opened and emptied the box, so that the corpse and the dry grass got into contact with soil. A reference sample from the surface soil, at the scene where the body was found (crime scene 1) and two other samples (loose surface material from the surrounding area) were taken by us on April 8th, 2009. Additionally, we secured a little tussock of dry grass, which was supposed to belong to the dry grass from inside the box (Fig. 2.1d). In the adjacent

Fig. 2.1 (**a & b**) Scene in lower Austria where the baby's body was found; (**c**) Dry grass in which the corpse was embedded, (**d**) Precise scene where body was found, white arrow indicates a tussock of dry grass, exposed to environment for 2 days. Picture (**a**) kindly provided by Landespolizeidirektion Niederösterreich, Landeskriminalamt

area to crime scene 1 no other sources for dry grass were documented. At first, police wanted to know, whether the pollen grains within the dry grass indicate a specific location - a possible crime scene.While analysing the forensic pollen samples police found a young mother, who confessed killing her baby. In the room, at her home, where the baby was killed after birth (crime scene 2) police officers secured another dry grass sample, for palynological investigation. To complete the evidence, we were commissioned to analyse whether the dry grass from the box found at crime scene 1 and the dry grass from the crime scene 2 had the same origin.

2.3 Material and Methods

The dry grass samples were thoroughly washed in distilled (pollen-free) water. The liquid fraction (including pollen) was then transferred into test tubes and centrifuged at 3000 rpm for 2 min, in order to settle pollen grains and other residues at the bottom of the test tubes. After decanting the water, samples were dehydrated with concentrated acetic acid, centrifuged and the acid decanted. For the acetylation step (Erdtman 1960; Brown 2008), the samples were put into a mixture of nine parts acetic anhydride and one part concentrated sulphuric acid and heated to 100 °C for approximately 5 min. After the mixture had been centrifuged and the liquid fraction decanted, the residue was rinsed in acetic acid and water. Glycerine was then added to the sample to form a suspension which was then transferred to a glass slide for light microscopic investigation. Pollen grains were identified, counted and assigned to specific plant taxa.

Pollen types in the graphs are listed according to their frequency within each sample. In each graph 13 pollen types (taxa) are listed. All others are summarized in the grey bar (right end). Excluded from the final graphs is *Pinus*-pollen, as saccate pollen gets lost in different amounts during preparation. For a better overview each pollen type is represented by the same colour in all graphs.

2.4 Results

Pollen analysis of the dry grass samples shows very characteristic pollen assemblages. As shown in Fig. 2.2, graph 1 the dry grass from the box is dominated by grasses (Poaceae, Fig. 2.2a), a fern spore (*Dryopteris*, Fig. 2.2d) and daisies (Asteraceae). The Asteraceae pollen mainly belongs to the liguliflorous type (Fig. 2.2b). Additionally, buttercup (Ranunculaceae, Fig. 2.2c), oaks (*Quercus*, Fig. 2.2h) and few other plant taxa are found. A characteristic of this specific dry grass is a fungal spore (Fig. 2.2i), which is found in large quantities (Fig. 2.2, graph 1, large blue bar).

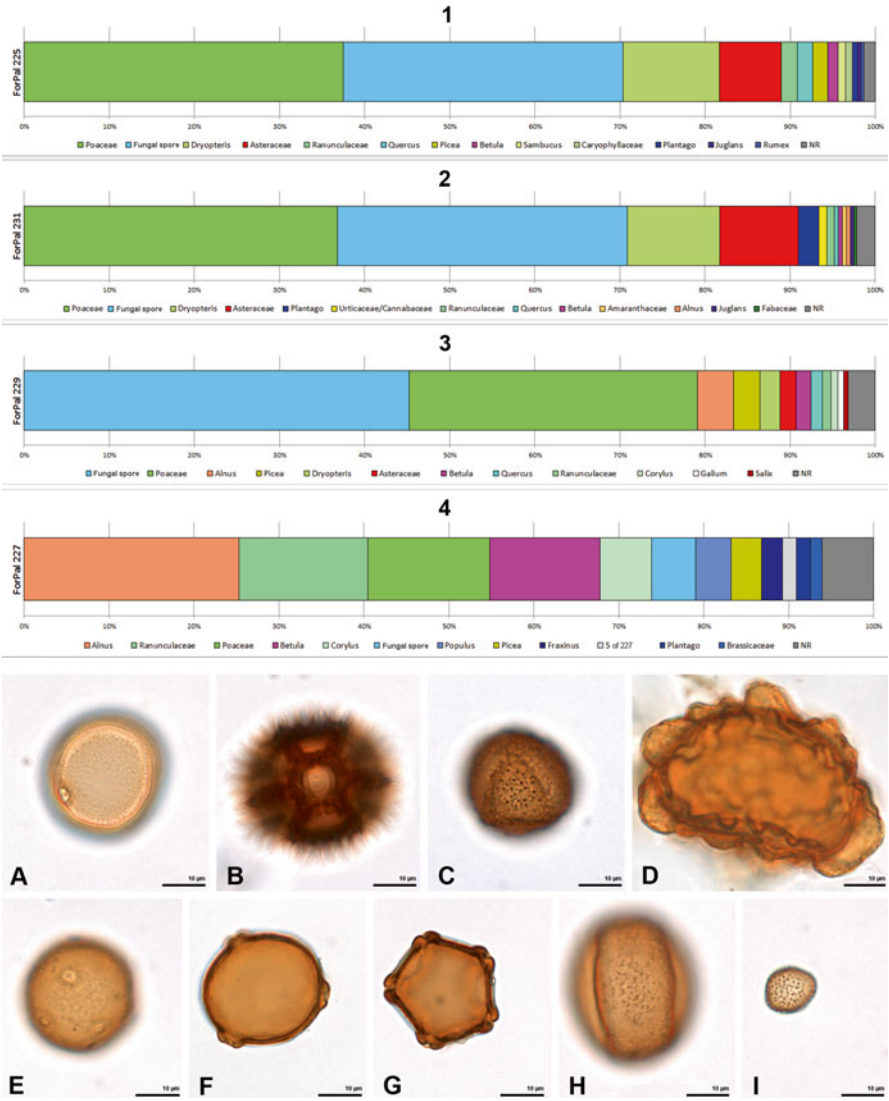


Fig. 2.2 (1–4) Graphs showing pollen analysis of dry grass samples and a soil surface sample. (1) Dry grass in which the corpse was embedded, (2) Dry grass from the crime scene, (3) Dry grass exposed 2 days to environment, (4) Surface soil sample; (**a**–**h**) Most frequent pollen and spore types in dry grass samples: (**a**) *Poaceae*, (**b**) *Liguliflorous Asteraceae*, (**c**) Ranunculaceae, (**d**) *Dryopteris*, (**e**) *Plantago lanceolata*, (**f**) *Betula*, (**g**) *Alnus*, (**h**) *Quercus*, (**i**) Characteristic, unidentified fungal spore. NR = identified, non-relevant pollen types

The dry grass sample found at the crime scene 2 location (Fig. 2.2, graph 2) is almost identical to the sample from inside the box, including the specific fungal spore. Within both samples the pollen types and the amount of each type are nearly identical, including characteristic markers, like the fungal spores. There is no doubt, that these two samples have the same origin. That applies also to the dry grass-tussock, as shown in Fig. 2.2, graph 3. Additionally, this graph illustrates that 2 days exposure to the environment lead to contamination. In this case the sample got contaminated by two typical wind pollinated species *Alnus* (orange bar) and *Betula* (pink bar).

To prove, that the dry grass sample involved in the crime has a unique combination of pollen types, other dry grass samples were investigated, selected according to various aspects (see Fig. 2.3). Sample 5 is dry grass bought in a pet shop, samples 6 & 7 represent gardens at a distance of 70 km away from each other (sampling time May 2009), samples 8 & 9 are two adjacent meadows (sampling time May 2009), samples 10 & 11 represent the same meadow, but time of sampling is different (April 2009 and July 2009), samples 12 & 13 are from the same garden, but different sampling times (May 2009 and autumn 2008).

As shown in Fig. 2.3 each sample has a characteristic combination of pollen types. Remarkably this also applies to the two meadows (samples 8 & 9) which are in immediate vicinity. In sample 8 Asteraceae pollen is dominating (85 %), while in sample 9 grasses and oak pollen are predominant.

Additionally, each dry grass sample was characterized by a specific combination of fungal spores (not included in the graphs) and other palynomorphs, like fungal hyphae, which give each sample a characteristic marker.

2.5 Discussion

Forensic palynology is based on two principles: (1) pollen and spores are omnipresent and (2) a specific location at a specific time has a specific combination of pollen, spores and other palynomorphs. Thus, each area has a unique palynological signature (Wiltshire 2006a). The current investigation shows, that these principles also apply to dry grass samples. Even those samples from adjacent areas had different and significant pollen assemblages. The differences in the dry grass samples are due to the particular combination of plant species growing and flowering in the meadow and the surrounding vegetation before mowing. Moreover, pollen is deposited on dry grass while drying.

In the investigated surface soil sample from crime scene 1, the wind pollinated plants (*Alnus*, *Betula*) dominate. Both species were still flowering or just finished flowering season. According to Montali et al. (2006) and Szibor et al. (1998) pollen grains recently released can be closely linked to a flowering season, although surface soil samples may represent years of pollen accumulation, at least a mixture of several month (Montali et al. 2006).

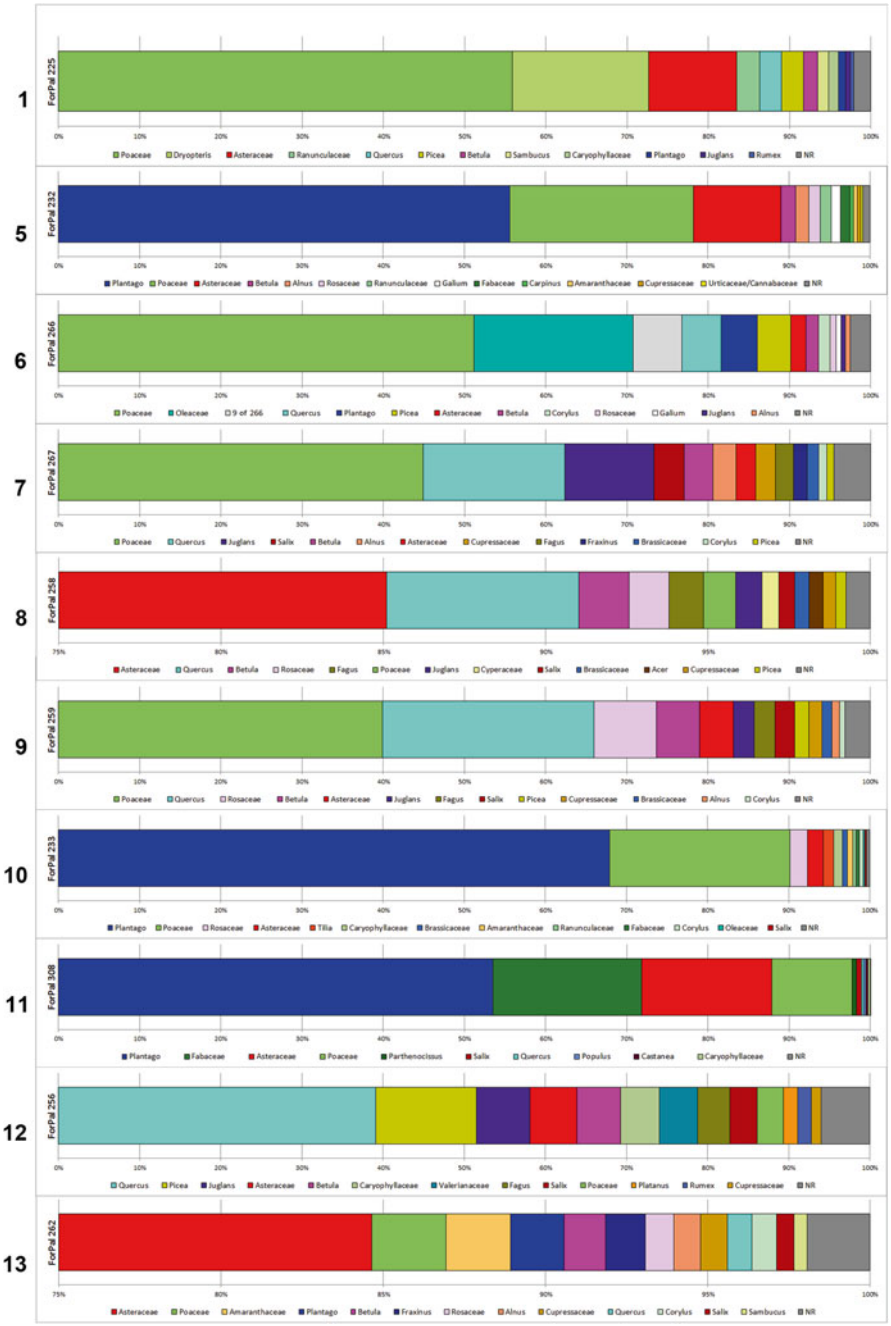


Fig. 2.3 Graphs showing pollen analysis of various dry grass samples in comparison with the dry grass involved in the crime. Each sample is different and has a unique pollen assemblage. (1) Dry grass in which the corpse was embedded (without fungal spores), (5) Dry grass sample bought in a pet shop, (6 & 7) dry grass samples collected in May 2009 from two gardens 70 km away from each other, (8 & 9) adjacent meadows (sampled in May 2009), (10 & 11) same meadow, different time of sampling (April 2009 and July 2009), (12 & 13) same meadow, different sampling time (May 2009 and autumn 2008); *Note*: In graphs (8) and (13) a different scale was used, as in these samples Asteraceae pollen were dominating by more than 75 %. NR = identified, non-relevant pollen types

The need to take pollen samples as soon as possible is shown by the pollen assemblage of sample 3. The tussock of dry grass found at crime scene 1 was exposed to environment for 3 days. *Alnus* and *Betula* are found in a much higher amount than in the dry grass from the box that was not exposed. Thus, the contamination with these wind-pollinated species is a result of either contact with the soil and/or the actual pollen rain.

So far there are no forensic palynological reports about dry grass. In a case report by Horrocks and Walsh (2001) pollen grains on grass clippings were investigated and finally linked the suspect to the crime scene. Dry grass samples have the same potential as surface soil samples, as each sample had a different pollen assemblage, even from the same plant community and of adjacent areas. Horrocks et al. (1998) investigated several soil surface samples from localized areas of similar vegetation types within the same geographic region. The samples showed significantly different pollen assemblages.

Besides pollen and spores, other palynomorphs like fungal hyphae, shells of testate amoeba and diatoms can be useful markers within a forensic sample (Wiltshire 2006b).

Fungal spores were also found in the investigated dry grass samples, which turned out to be good makers. Most fungi are associated with particular plants. For example, grasses are host plants for various smut fungi (Vánky 2012). As shown by Hawksworth and Wiltshire (2011) mycology can provide useful evidence in legal cases.

Crucial for forensic palynological investigations is the method and time of sampling. As shown in Fig. 2.2, graph 3 contamination occurs very fast. This requires an intensive collaboration with crime scene officers (Wiltshire 1993). In Austria police and palynologists have been working together since 2007 on several cases (including crime scene work). This allows both sides to learn and gain experience from each other (e. g. collection of suitable samples, proper sampling methods, documentation of the surrounding vegetation at a crime scene). This cooperation with crime scene officers is of high importance for further successful forensic palynological research in Austria.

In this homicide case pollen grains did not solve the crime itself, but it was a good example to demonstrate to prosecution and the crime scene officers in Austria how forensic palynology can be used in criminal investigations. This case reintroduced forensic palynology to Austria, the first country worldwide where a murder case in 1959 was solved by using pollen grains. To date there are only few countries (including the United Kingdom, New Zealand and Australia) where forensic palynology is well established. In several others countries (e.g. Portugal, Italy, Thailand, Spain) more and more scientists and police officers are becoming aware of the usefulness of pollen.

Acknowledgments The authors would like to acknowledge the open-mindedness and assistance for forensic palynology by the police and in particular the crime scene officers from "Landespolizeidirektion Niederösterreich, Landeskriminalamt". We are very grateful for good collaboration, which plays an important role in re-establishing forensic palynology in Austria. We would also like to thank Mag. Philpp Preusche providing us excellent tools for graphic data output and for discussions. Thanks are also due to all the collectors of dry grass for their prompt sampling.

References

Brown AG (2006) The use of forensic botany and geology in war crimes investigations in NE Bosnia. Forensic Sci Int 163:204–210

Brown CA (2008) Palynological techniques, 2nd ed. In: Riding JB, Warny S (eds). American Association of Stratigraphic Palynologists Foundation, Dallas, TX, 137 p

Brown AG, Smith A, Elmhurst O (2002) The combined use of pollen and soil analyses in a search and subsequent murder investigation. J Forensic Sci 47:614–618

Bryant VM (2007) A honey of a deal. American Association of Stratigraphic Palynologists. Newsletter 40:18–19

Bryant VM, Mildenhall DC (1998) Forensic palynology: a new way to catch crooks. In: Bryant VM, Wrenn JW (eds) New developments in palynomorph sampling, extraction, and analysis, Contribution series 33. American Association of Stratigraphic Palynologists. Foundation, Houston, pp 145–155

Bryant VM, Jones JG, Mildenhall DC (1990) Forensic palynology in the United States of America. Palynology 14:193–208

Bryant VM, Jones JG, Mildenhall DC (1996) Chapter 23: 23G – forensic studies in palynology. In: Jansonius J, McGregor DC (eds) Palynology: principles and applications, vol 3. American Association of Stratigraphic Palynologists Foundation, Texas, pp 957–959

Erdtman G (1960) The acetolysis method. Sven Bot Tidskr 54:561–564

Hawksworth DL, Wiltshire PEJ (2011) Forensic mycology: the use of fungi in criminal investigations. Forensic Sci Int 206:1–11

Horrocks M, Walsh KAJ (2001) Pollen on grass clippings: putting the suspect at the scene of the crime. J Forensic Sci 46:947–949

Horrocks M, Coulson SA, Walsh KAJ (1998) Forensic palynology: variation in the pollen content of soil surface samples. J Forensic Sci 43:320–323

Mildenhall DC (1989) Deer velvet and palynology: an example of the use of forensic palynology in New Zealand. Tuatara 30:1–11

Mildenhall DC (2004) An example of the use of forensic palynology in assessing an alibi. J Forensic Sci 49:312–316

Mildenhall DC (2006a) Hypericum pollen determines the presence of burglars at the scene of the crime: an example of forensic palynology. Forensic Sci Int 163:231–235

Mildenhall DC (2006b) An unusual appearance of a common pollen type indicates the scene of the crime. Forensic Sci Int 163:236–240

Mildenhall DC (2008) Civil and criminal investigations The use of spores and pollen. SIAK J 4:35–52

Mildenhall DC, Wiltshire PEJ, Bryant VM (2006) Forensic palynology: why do it and how it works. Forensic Sci Int 163:63–172

Miller Coyle H (ed) (2005) Forensic botany. Principles and applications to criminal casework. CRC Press, Boca Raton, 318 p

Montali E, Mercuri AM, Grandi GT, Accorsi CA (2006) Towards a “crime pollen calendar”- Pollen analysis on corpses throughout one year. Forensic Sci Int 163:211–223

Morgan RM, Allen E, Lightowler ZL, Freudiger-Bonzon J, Bull PA (2008) A forensic geoscience framework and practice. Policing 2:185–195

Newton PN, Fernandez FM, Placon A, Mildenhall DC, Green MD, Li Z-Y, Christophel EM, Phanouvong S, Howells S, McIntosh E, Laurin P, Blum N, Hampton CY, Faure K, Nyadong L, Soong CWR, Santoso B, Wang Z-G, Newton J, Palmer K (2008) A collaborative epidemiological investigation into the criminal fake artesunate trade in South East Asia. PLoS Med 5(2), e32. doi:10.1371/journal.pmed.0050032

Stanley EA (1992) Application of palynology to establish the provenance and travel history of illicit drugs. Microscope 40:149–152

Szibor R, Schubert C, Schöning R, Krause D, Wendt U (1998) Pollen analysis reveals murder season. Nature 395:449–450

Vánky K (2012) Smut fungi oft the world. APS Press, American Phytopathological Society, St. Paul, MI, 1458 p
Wall NA, Reinhard KJ, Okoye MI (2004) Forensic analysis of a recent homicide case: usage of palynology to help determine time and location of death. Polen 14:115
Walsh KAJ, Horrocks M (2008) Palynology: its position in the field of forensic science. J Forensic Sci 53:1053–1060
Wiltshire PEJ (1993) Environmental profiling and forensic palynology: background and potential value to the criminal investigator. Handbook for the national crime and operations faculty with the British association for human identification
Wiltshire PEJ (2006a) Consideration of some taphonomic variables of relevance to forensic palynological investigation in the United Kingdom. Forensic Sci Int 163:173–182
Wiltshire PEJ (2006b) Hair as a source of forensic evidence in murder investigations. Forensic Sci Int 163:241–248

Chapter 3
Geological Analysis of Soil and Anthropogenic Material. Three Case Studies

Rosa Maria Di Maggio

Abstract In a judicial investigation, the evidence provided by pedological materials can have significant evidential value. The forensic geologist is able, in many instances, to obtain compatible results from pedological materials, and anthropogenic fragments in soil, by combining a variety of analytical methods. However, it must be noted that soil trace evidence can be modified during its transfer to an object, and after its deposition. This aspect needs to be considered when processing and interpreting the analytical data. In this paper, three cases are presented which exemplify the use of geological traces and anthropogenic materials in a criminal investigation.

The first case involves damage to a coachbuilder shop where soil samples contained unique anthropogenic fragments frequently used in the automobile industry and manufacturing. The presence of these in soil adhering on the suspects' shoes compared well with the kind of work carried out in such workshops, and what was known about the nature of the crime. This allowed a link to be established between the footwear and the coachbuilder shop where the damage had taken place.

The second case is that of the homicide of a young girl: her burned body was found in the countryside near the town of Misilmeri, in Sicily. Soil found adhering to the roots of *Cyperus alternifolius* (umbrella papyrus) was found at the deposition site even though the plant was not growing there. This soil, and that found on the victim's partially-burned shoes, was very similar to that attached to dried plants in the garden of the suspect's home. The similarity of the soils, the presence of *C. alternifolius* at the suspect's home, and the anomalous presence of the same plant at the deposition site, provided convincing evidence that the suspect had had contact with the place where the girl's body was found.

The last case is the damage of Jewish graves in the Verano Monumental Cemetery in Rome. Tools belonging to a group of gardeners, who had been working illegally in the cemetery, showed traces of white material that could have been transferred from a damaged headstone during impact. Evidence from several independent analytical methods suggested that the gardeners had been involved in the offence.

R.M. Di Maggio (✉)
Geoscienze Forensi Italia ®, Viale Mediterraneo, 77, 00122 Rome, Italy
e-mail: rosamaria.dimaggio@gmail.com

H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics,*
Soil Forensics, DOI 10.1007/978-3-319-33115-7_3

These examples suggest how the data can be useful only if they are suitably placed in the context of the investigation. However, for better discrimination for studying soil samples in a judicial investigation, the analysis of the organic fraction of soil (e.g. botanical elements) is essential, especially in those cases where soil samples come from area with homogeneous pedology, or when it is necessary to distinguish the temporal deposition of a soil trace.

3.1 Introduction

In a judicial investigation, the evidence provided by pedological materials, such as samples of soil or sand, can have significant evidential value. Pedological materials include three fractions which have very variable reciprocal ratios: inorganic, organic, and anthropogenic (Lombardi et al. 1983; Murray 2004; Di Maggio et al. 2009; Bergslien 2012). The latter is an assemblage of various materials that have been brought into a soil by human activity. The inorganic fraction is composed of fragments of rock and minerals and, where the landscape geology is relatively homogeneous, the mineralogy can be very similar over large areas. In such cases, the organic and anthropogenic fractions greatly facilitate identification of the location and origin of a soil.

The organic fraction may include living plant roots, algae, protists and animals, as well as functioning microbial communities. After death, organisms become decomposed to fragments, and are eventually converted into humus, and a complex of humic acids. Thus, the organic fraction is represented at both molecular and particulate levels, and its quantity and quality reflects both the regional and micro-environmental factors prevailing during pedogenesis. The anthropogenic fraction can include fragments of various materials (e.g. paper, glass, plastic, fibres, paint, metal, bricks, baked clay, cements), and chemical substances such as precipitates, solvents, general scoria (Di Maggio et al. 2009). These materials can provide valuable trace evidence to link a soil to its place or origin.

There is little doubt that excellent results can be achieved with this kind of micro-trace evidence when the information is correctly viewed within the context of the criminal investigation. The forensic geologist is able, in many instances, to obtain compatible results from pedological and anthropogenic materials by combining a variety of analytical methods (Murray 2004; Pye 2007; Ruffell and McKinley 2008). However, it must be noted that, like any other type of evidence, soil traces can be modified during and after transfer to an object. In this case, the inherent nature of the pedological material, meteorological conditions before the collection, and the force and direction of energy involved in transfer and deposition, are all factors to be considered when processing and interpreting the analytical data (Di Maggio et al. 2009).

After deposition onto an object, soil particles can disperse or be lost, and new material(s) may be added. Newly added material may be deposited on top of, or mixed with, the original trace material. Furthermore, surfaces and fabrics can vary in their ability to retain trace evidence, so transference will involve an element of selectivity. This can be a function of clast size and/or mass. Dispersion, or loss of particles after transference, also affects the degree of comparison possible with a reference sample. For example, losses from the wheel of a vehicle are correlated with the distance of journeys, and the speed of wheel revolution. Mixing is an important consideration in the temporal aspects of deposition. A soil can accumulate on a shoe, or on a vehicle's foot well mat, both before and after soil from the crime scene has been transferred to the item. The crime scene soil can, therefore, be "sandwiched" between other deposits which are not of interest to the investigator.

With repeated use, and absence of cleaning, there is sequential deposition of trace evidence into a car foot well from footwear. Very few mineralogical species can reflect season or weather (Lombardi et al. 1983), and this obviously imposes limitation on the interpretation of mineralogical data. Thus, from the minerogenic component alone, it may be difficult to distinguish even the most recently deposited material from that originally accumulated at the time of an offence. However, because of the predictability of flowering and sporing times, analysis of plant macro-remains, plant spores, pollen and fungal spores, might give more information on temporal aspects of deposition.

Three cases are presented here which exemplifies the use of pedological and anthropogenic material in criminal investigation.

3.2 Case 1: The Damage to a Coachbuilder Shop

Early one morning in 2010, both the interior and exterior of a coachbuilder's shop was damaged. Windows were broken, and some of the machines inside the workshop had been severely damaged. The identity of the perpetrators of the damage was unknown. However, some days before the incident, the owner of the shop had been offered protection from harm to his property in exchange for money. Thus, the inquiry became one of extortion. A few days later, police identified two suspects who already had a criminal record of extortion, and shoes which had traces of soil were seized from them.

The scene of crime was a front yard, adjacent to the shop, and this was surveyed by the police. There was a large area of bare, exposed, moist soil littered with small fragments, possibly of paint and glass. There were tyre tracks and footprints in the soil of the yard and it was obvious that vehicles were often parked there. The tyre impressions and footprints were poorly preserved, and not suitable for comparison with existing manufacturer databases, but the police collected soil samples for investigation by a forensic geologist.

3.2.1 Aims

The aim of the analyses was to establish whether there was a link between the footwear and the yard soil. A high degree of similarity between the two soils might indicate contact between the footwear and the yard.

3.2.2 Methods

The forensic geologist collected five samples of soil from the front yard adjacent to the shop. Fresh tire and footprints were targeted. The soils over the yard were relatively homogeneous so, for simplicity, only one sample is reported here (designated YARD). A soil sample was also collected from each of the suspects' footwear (designated SHOE 1 and SHOE 2).

Soil samples were dried at 60 °C, weighed into tubes, and disaggregated in an ultrasonic bath. These were wet-sieved with sieve mesh sizes spaced at one-phi intervals between 2000 and 4 μm. Samples were viewed with stereo-binocular microscope (within a range of 10x-50x), and colour of clay fractions assessed with Munsell Colour Charts (Macbeth 2000). Samples were embedded in resin, and thin sections of the sand particles were examined by polarizing light microscopy. To determine the volume fraction percentage of identifiable constituents in the thin sections, semi-quantitative analysis was carried out by point counting and size measurement. This was performed using a manual, mechanical stage with mm-graduated x-y stage translation controls for moving the thin section.

The whole of each soil sample was subjected to X-ray diffraction (XRD analysis) by means of a Philips PW 1800 diffractometer, with radiation Cu-Kα generated at 40 kV and 40 mA. Each of the XRD analysis charts was drawn within an angular value range of 5–80°, at a step size of 0.01, and at a time per step of 0.9 s.

The anthropogenic fragments in the soils were subjected to the non-destructive techniques of stereoscopic microscopy and Raman spectroscopy. Raman analysis was by a Horiba LabRAM Aramis. Each of the Raman analyses were carried out within the following instrumental conditions: laser wavelengths 785 nm, objective 10× with a working distance of 0,38 mm, hole 200 μm, 600 I/mm grating, and slit 200 μm.

There is extensive literature dealing with examination of materials such as paint and glass, using Raman spectroscopy (e.g. Long 2002; Edwards 2004) and X-ray diffraction (e.g. Kugler 2003). However useful overviews of these techniques applied to anthropogenic materials in a forensic investigation are provided by Claybourn (2004), and Ruffell and Wiltshire (2004). These suggest how data can be useful only if they are suitably placed in the context of the investigation.

3.2.3 Results

The detailed examination by stereoscopic observation and Raman spectroscopy revealed that they all contained particles of anthropogenically-derived hyaline microspheres, blue fragments, and white fragments embedding hyaline microspheres.

Soil The soil samples were very pale brown (10YR 8/2). Stereoscopic microscopy revealed that all the soil samples had particles of very similar morphology. They were irregularly shaped, ranging from angled to sub-angled. X-ray diffraction showed all the samples consisted of the same crystalline phases, namely quartz, plagioclase and calcite (Fig. 3.1). Thin section observations by polarizing light microscopy revealed that all the soil samples had the same suites of minerals (quartz, plagioclase, calcite, serpentine, biotite, and magnetite), and rock fragments (sandstone, limestone, flints, quartzites, phyllites, and micaschists).

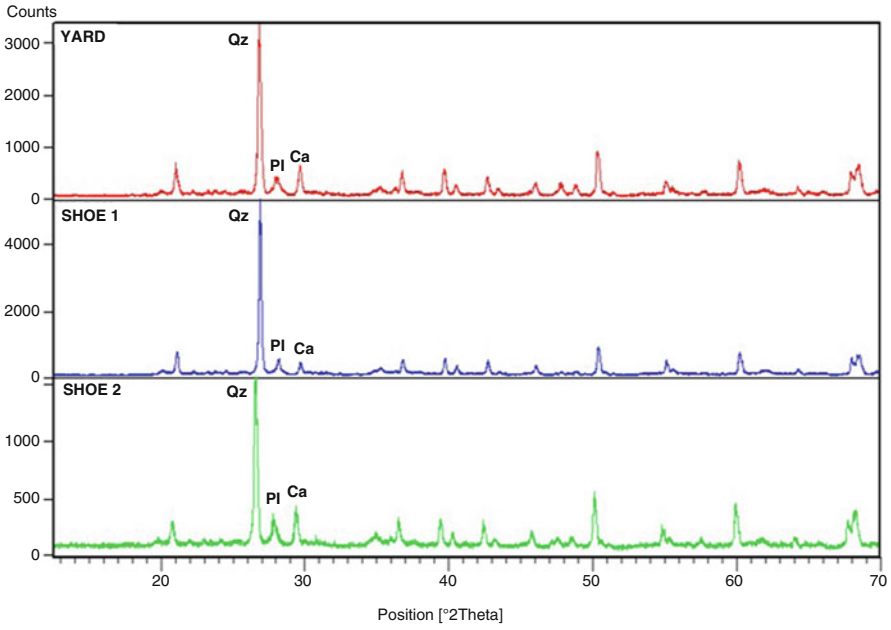


Fig. 3.1 X-ray diffractograms of soil samples SHOE1, SHOE2, and YARD. The comparison among all the spectra shows the similarity between the soil samples. The critical peaks are labelled with the initials of minerals. *Ca* calcite, *Pl* plagioclase, *Qz* quartz

Table 3.1 Values of the relative percentages of sialic and femic minerals, and sedimentary and metamorphic rocks in the samples SHOE1, SHOE2, and YARD

Sample	SHOE 1	SHOE 2	YARD
Sialic minerals	11.5	12.0	10.2
Femic minerals	4.9	4.3	5.0
Sedimentary rock	69.2	67.3	68.2
Metamorphic rock	14.0	16.4	16.6
	100	100	100

A semi-quantitative analysis was performed to estimate the abundance of sialic[1] and femic[2] minerals, and sedimentary and metamorphic rock, in both the yard comparator samples and those from the footwear. The relative percentages are shown in Table 3.1.

There was little difference in the results of grain size analysis between the footwear and that of the crime scene soil, although soil from both shoes showed a greater abundance of the fine fractions. Grain size was, therefore, of little probative value and this emphasised the importance of the anthropogenic materials.

Hyaline Microspheres The hyaline microspheres had dimension between 250 and 500 μm (Fig. 3.2a, b). Raman spectroscopy showed that these spheres were common glass (Fig. 3.3a).

Blue Fragments The blue fragments were between 100 and 500 μm (Fig. 3.2c, d). Raman spectroscopy showed that they consisted of a pigment called Hostaperm blue (Fig. 3.3b).

White Fragments Embedding Hyaline Microspheres The white fragments embedding the hyaline microspheres were between 50 μm 100 μm (Fig. 3.2e, f). Raman spectroscopy revealed that the white material to be of anatase and rutile (Fig. 3.3c), and the microspheres were of common glass.

3.2.4 Discussion and Conclusion

The various analyses demonstrated that both the comparator soil from the yard, and from the suspects' shoes were similar with respect to colour, grain morphology, mineralogy, and petrography. There was a slightly greater abundance of the finer soil fraction in the shoe samples than the yard sample. This could be a function of clast size in that the coarser fraction is probably more easily lost during wear.

[1] The assemblage of minerals, rich in silica and alumina, that comprise the continental portions of the upper layer of the earth's crust.

[2] Minerals having one or more normative, dark-colored iron, magnesium, or calcium-rich as the major components of the norm.

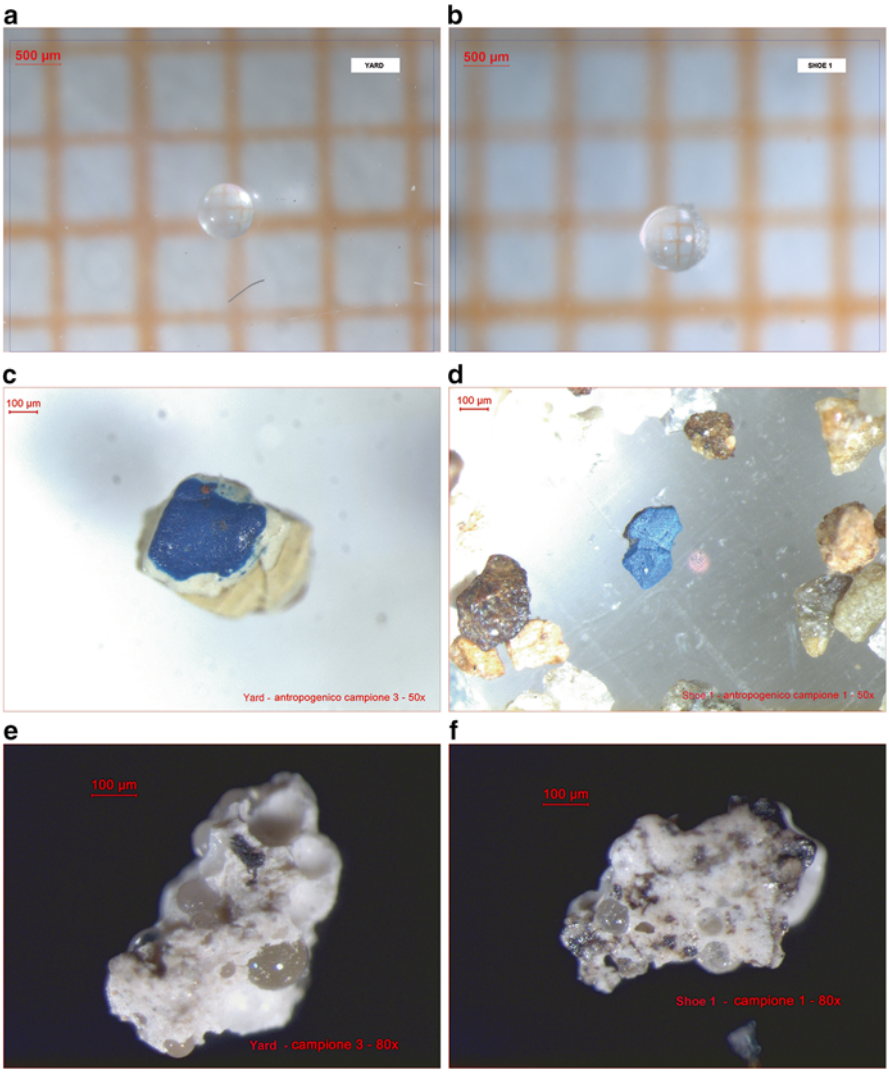


Fig. 3.2 Anthropogenic fragments in the soil samples: (**a**) hyaline microsphere in sample YARD; (**b**) hyaline microsphere in sample SHOE1; (**c**) blue fragment in sample YARD; (**d**) blue fragment in sample SHOE1; (**e**) white material embedding hyaline microspheres in sample YARD; (**f**) white material embedding hyaline microspheres in sample SHOE1

However, the three kinds of anthropogenic fragments found in all the soils were more informative. Raman analysis revealed that the three kinds of material in all the soil samples had the same nature: glass microspheres, blue fragments of pigment, and a white material, consisting of anatase and rutile, which had glass microspheres embedded within it.

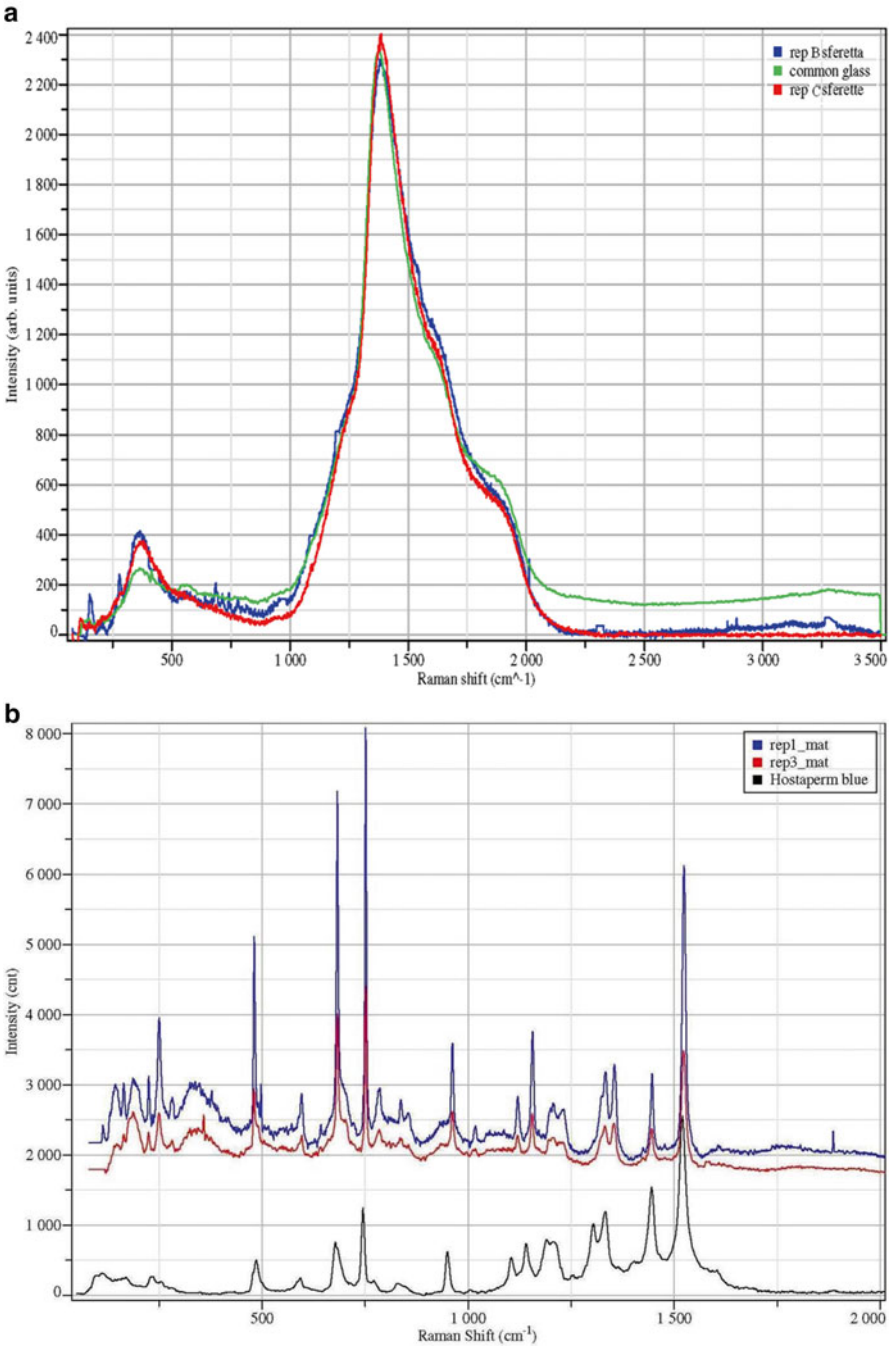


Fig. 3.3 (**a**) Raman spectra of hyaline microspheres: in blue the spectrum of microsphere in sample SHOE2, in red the spectrum of microsphere in sample YARD, in green the database spectrum of common glass; (**b**) Raman spectra of blue fragments: in blue the spectrum of fragment in sample SHOE1, in red the spectrum of fragment in sample YARD, in black the database spectrum of Hostaperm blue pigment; (**c**) Raman spectrum of white fragment embedding hyaline microspheres (*blue line*); in green and red the database spectra of anatase and rutile

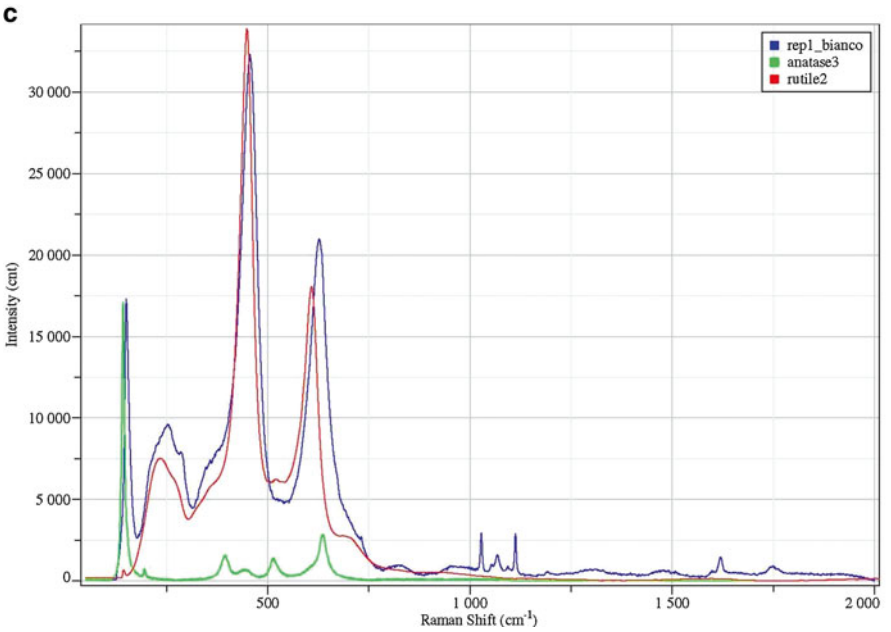


Fig. 3.3 (continued)

Glass microspheres were probably derived from shot peening, which although used in various industrial processes, is also used for cleaning and polishing metal gearings, and in treating metal bodywork. The dimension of the spheres used in the process depends on the level of finishing required.

The blue Hostasperm pigment is used in the manufacture of high performance paints for application to metal, resin, and PVC. Its quality of high resistance makes it suitable for the automobile industry.

Rutile and anatase are frequently used for the production of paints. Paints embedding glassy microspheres are called A-way paints; they have reflective properties and are used for painted road signs.

These three materials are not ubiquitous, and are likely to be found only in an environment associated with specific activities such as those carried out in the damaged workshop. Although the gross soil characteristics were less helpful in identifying a specific link between the footwear and the soil of the yard, the anthropogenic fragments added a high level of specificity. They related to the trade carried out at the crime scene, and their presence in the shoe soil samples indicated contact between the yard and the suspects' footwear.

3.3 Case 2: The Homicide of Nike Adekunle

In 2012, the murdered, semi-burned body of Nike Adekunle, a Nigerian prostitute, was found in the northern Sicilian countryside, near the village of Misilmeri. It was the latest in a series of crimes in which Nigerian prostitutes were involved and, early in the investigation, police addressed their enquiries to members of criminal organisations known to manage prostitution between Africa and Italy. However, a few days after the body had been found, they turned their attention to a man who, according to witnesses, had been the girl's last customer.

During the *post mortem* examination traces of soil were found on the victim's shoes and, when the deposition site was being evaluated by the police and forensic geologist, dried plant roots with some associated soil were noted and collected. The roots were identified by a botanist to be of *Cyperus alternifolius*. It is grown as an ornamental around the world, and its presence at the deposition site was considered anomalous since there was no evidence of the plant growing anywhere in the environment of the place where the body was found. The geologist subsequently visited the suspect's home and collected soil samples from inside and outside his garden. During this visit, dried roots, as well as a stack of dried and fresh plants of *C. alternifolius*, were found in the backyard.

From this information, investigators postulated that the suspect had killed the girl in his home, put her body into his off-road vehicle, and then covered it with some of the dried plant remains of *C. alternifolius* from his garden. He then drove a distance of 5 km, dumped the body, and burned it with petrol.

3.3.1 Aims

The aims were: (a) to compare soil from the victim's shoes with that from the suspect's home to ascertain the likelihood of common origin, (b) to compare soil adhering to the plant root material found at the deposition site with that from the suspect's garden to establish that they were associated, and to show that the roots were not derived from the deposition site itself.

3.3.2 Methods

At the suspect's home, the geologist collected soil samples from inside and outside the garden. This was considered necessary in order to ascertain whether or not the garden offered a unique pedological microenvironment. If this were the case, its soil could be specifically distinguished from that of other locations. Eight soil samples were collected to ascertain their spatial heterogeneity within the garden. The soil proved to be remarkably homogeneous so, for brevity, only one of the garden samples is presented (designated HOME). The soils adhering to the plant roots found at

the deposition site, and from the suspect's home are designated P1 and P2. The soil from the victim's shoes and the deposition site are designated SHOE and SITE respectively.

The protocols and methods used in this case were the same as those described in Case 1. Each of the XRD analysis charts were drawn within an angular value range of 3–80°, at a step size of 0.01, and at a time per step of 1 s.

The anthropogenic fragments in soil were subjected to stereoscopic microscopy and FT-IR spectroscopy (Fourier Transform Infrared Spectroscopy) (Griffiths and De Haseth 2007). FT-IR analysis involved using a Thermo Scientific Nicolet iS 50 FT-IR spectrometer, with a laser wavelength of 780 nm. A useful overview of this technique applied to anthropogenic materials in a forensic investigation is provided by Beauchaine et al (1988).

The dried root material was identified by a botanist, using fresh material collected from the suspect's home as reference material.

3.3.3 Results

There were three components to the scientific examination: (a) identification of the plant material (b) analysis of red fragments of anthropogenically-derived material, and (c) analysis of gross soil characteristics.

Dried Plant Material The plant proved to be *Cyperus alternifolius*. It has several common names including "umbrella papyrus, umbrella sedge, and umbrella palm".

Red Fragments The soil on the root material from the deposition site and the suspect's garden contained the same dark red fragments (Fig. 3.4a, b). The FT-IR charts showed very similar spectra for the red fragments in soil samples P1 and P2, suggesting they were made from the same materials (Fig. 3.5a). Comparison with appropriate databases indicated that these were anthropogenically-derived, and likely to consist of clay coating and alchydic paint (Fig. 3.5b).

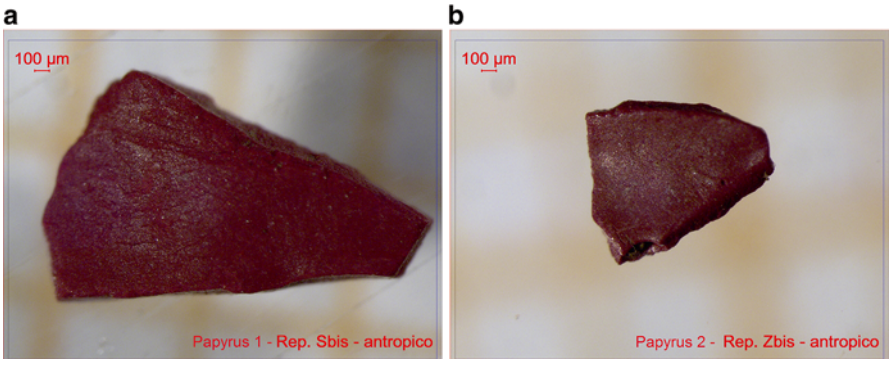


Fig. 3.4 Anthropogenic fragments in the soil samples from the roots of papyrus: (**a**) red fragment in sample P1; (**b**) red fragment in sample P2

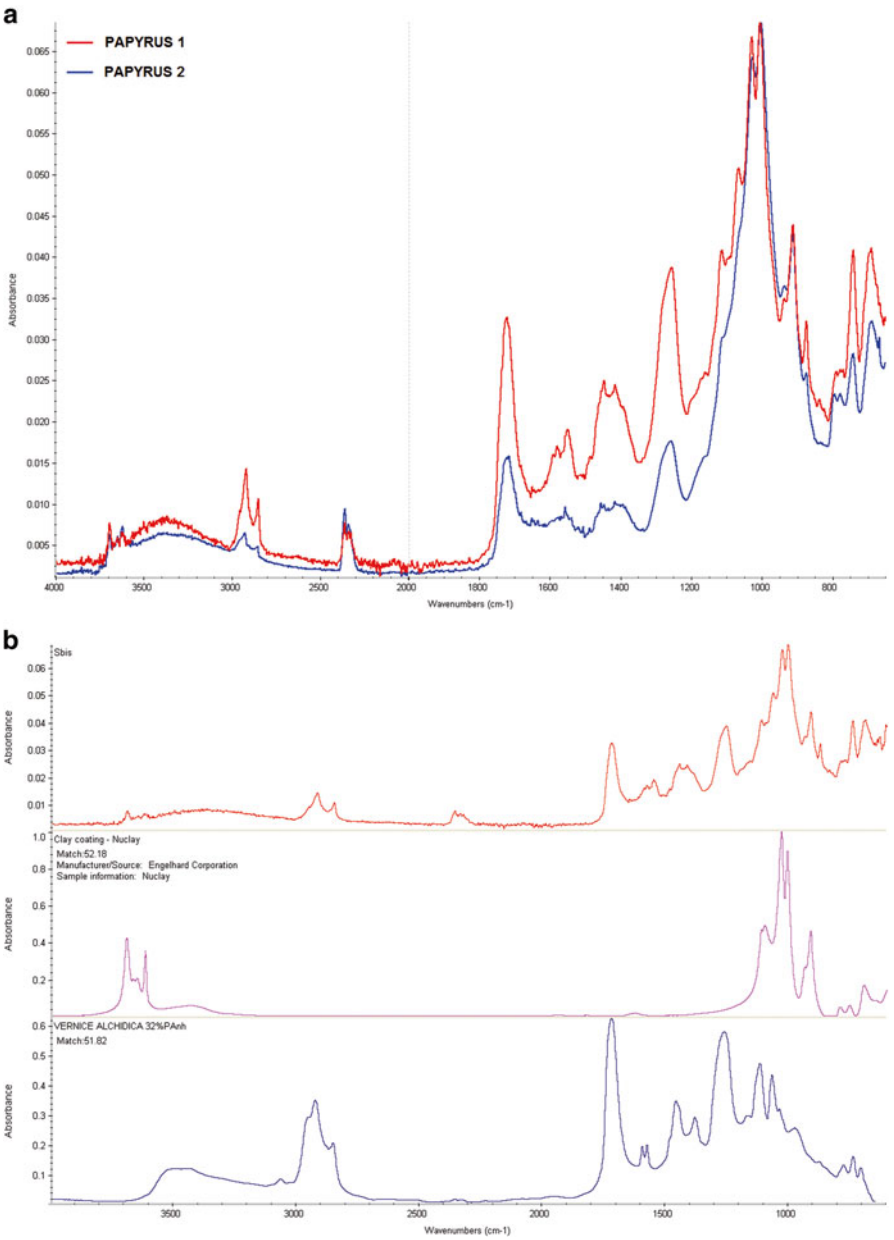


Fig. 3.5 FT-IR spectra: (**a**) red fragments found in sample P1 (*red line*) and P2 (*blue line*); (**b**) comparison between the database FT-IR spectra of clay coating and alchydic paint

Soil Stereoscopic microscopy showed that in all samples, soil grains were similar in typology and shape (from sub-angled to sub-rounded). With regard to colour, the soil on all the exhibits (P1, P2, SHOE, HOME) was light reddish brown (2,5YR 6/4) while that from the deposition site (SITE) was pink (5YR 7/4). The colour and grain size distributions were similar for all the soils except for that from the deposition site.

X-ray diffraction of the deposition site soil showed that its mineralogy was dominated by calcite, dolomite, kaolinite, and quartz. However, although the soil mineralogy of the plant roots, the victim' shoes, and the suspect's garden also contained dolomite, kaolinite, and quartz, it was notable that calcite was absent.

Observation of thin sections under polarized light confirmed that, with respect to mineralogy and petrology, the deposition site soil was different from all the others. It contained the minerals calcite, quartz, and magnetite, and rock fragments of quartz-arenite, lithic arenite, quartzites, limestone, and organogenic limestone. The soil from the garden, shoe, and plant roots had the minerals quartz, dolomite, sanidine, and magnetite, while the rock fragments were of quartz-arenite, quartzites, limestone, and micaschists (Table 3.2).

A semi-quantitative analysis of the abundance of sialic minerals, sedimentary rocks, and metamorphic rock in all the samples was also performed. The relative percentages are shown in Table 3.3.

Table 3.2 Minerals and rock fragments in the samples SITE, HOME, SHOE, P1, and P2

Sample	SITE	HOME	SHOE	P1	P2
Calcite	+	–	–	–	–
Lithic arenite	+	–	–	–	–
Organogenic lim	+	–	–	–	–
Dolomite	–	+	+	+	+
Sanidine	–	+	+	+	+
Micaschists	–	+	+	+	+
Magnetite	+	+	+	+	+
Quartz-arenite	+	+	+	+	+
Limestone	+	+	+	+	+
Quartzites	+	+	+	+	+
Quartz	+	+	+	+	+

Table 3.3 Values of the relative percentages of sialic minerals, and sedimentary and metamorphic rocks in the samples SITE, HOME, SHOE, P1, and P2

Sample	SITE	HOME	SHOE	P1	P2
Sialic minerals	25.3	20.5	19.9	17.2	17.4
Sedimentary rock	57.8	72.2	72.9	71.0	70.3
Metamorphic rock	16.9	7.3	7.2	11.8	12.3
	100	100	100	100	100

3.3.4 Discussion and Conclusion

The various analyses demonstrated that, in terms of colour, grain size distribution and morphology, mineralogy, and petrology, the soil from the exhibits (HOME, SHOE, P1, P2) were similar enough to indicate a common provenance. However, the profiles showed an accentuated similarity between the samples HOME and SHOE, and between P1 and P2 (the plant roots). This can be accounted for by the soil from the garden and the shoe having been from the surface, while that from the plant roots would have been from a deeper soil profile (10–15 cm deeper). Physico-chemical characteristics of soils can differ both laterally and vertically over small distances, and this is emphasized by the contrast between the soil from the exhibits and that from the deposition site.

The high level of similarity between the soil from the garden and the victim's shoe suggests that little, if any, loss of original soil, or accumulation of new material, had occurred. This might indicate that the girl's feet had not contacted the ground outside the suspect's garden or, indeed, at the deposition site. The presence of the clay coating and alchydic paint fragments (possibly derived from building materials such as roofing tiles) in the garden soil, and in the detached roots of *C. alternifolius* at the deposition site, suggests a link between the two places. The absence of the red fragments in the deposition soil itself indicates that the garden had been the source.

The plant roots themselves were of considerable importance to this case in that *Cyperus alternifolius* is not native to northern Sicily. It requires cultivation and does not spread as an adventive. Its presence at the deposition site and in the garden of the accused, provided significant, additional evidence of a link between the suspect and the place where the girl's body was found.

The geological findings corroborated the police's hypothesis outlined above

3.4 Case 3: Damage to Jewish Tombs

The day before a Jewish religious celebration, Jewish tombs in the Verano Monumental Cemetery in Rome were severely damaged. Headstones, made from limestone marls and travertine, had been broken, and some graves had been opened (Di Maggio and Nuccetelli 2013).

Police investigators suspected that a group of gardeners, who were doing illegal gardening and repair work in the cemetery, might be involved. The Police carried out a judicial site survey of the scene of crime, and seized gardening equipment which they found in a box inside the cemetery. This box belonged to the gardeners under investigation and the tools included picks and iron bars.

The gardeners claimed they had used these tools to restore some partition walls in the graveyard with white mortar few days before the damage. The walls were unrefined and made from tufaceous bricks, with dark mortar filling the gaps; some gaps had been roughly filled with easily removable, and brittle, white material.

3.4.1 Aims

The aims of the investigation were to compare samples of trace marks on the tools with material from the damaged headstones and white material from the walls. If the results of inorganic analyses showed a greater concordance between the marks on the tools and the headstones than to material from the wall, the testimony of the workmen could be challenged.

3.4.2 Methods

The grave headstones were constructed either of travertine or of limestone marl. Samples were taken from them in areas of fresh breaks in the stone since these probably represented the points of impact with the tools. White material from the partition walls was also taken for comparison with the small marks on the tools. Because of the small amount of material on the pick and bar, sampling was carried out very carefully to maximise retrieval.

Non-destructive techniques were used for analysis of all the samples. These were stereoscopic microscopy, X-ray diffraction (XRD), and elemental study with SEM-EDX (Pye 2004). Each of the XRD analysis charts were drawn within an angular value range of 5–80°, at a step size of 0.01 and at a time per step of 0.5 s. SEM-EDX analysis involved using a Tescan Vega microscope, at variable pressure, and EDAX microanalysis. Each of the EDX analysis charts were done by counting 100s.

There is extensive literature on the use of SEM-EDX and XRD in examining rock material, and industrial products such as bricks and concrete (Pye and Krinsley 1984; Allen et al. 2000; Evans and Tokar 2000; Schiavon 2002).

3.4.3 Results

The detailed stereoscopic microscopic examination of the white marks on the tools revealed that they were doughy and compact, but easily removed. They also featured micrometric stripes on the surface. The position of the marks, and orientation of the stripes, on the pick and bar were consistent with an up and down movement of the tools.

Headstones The headstones made from limestone marl gave diffraction patterns typical of pure calcite. The only other peak identified was attributed to a small quantity of quartz. The headstone made from travertine consisted of calcite (Fig. 3.6). Quantitative analysis showed the elements in this headstone were dominated by calcium with smaller quantities of aluminium and silicon (Table 3.4).

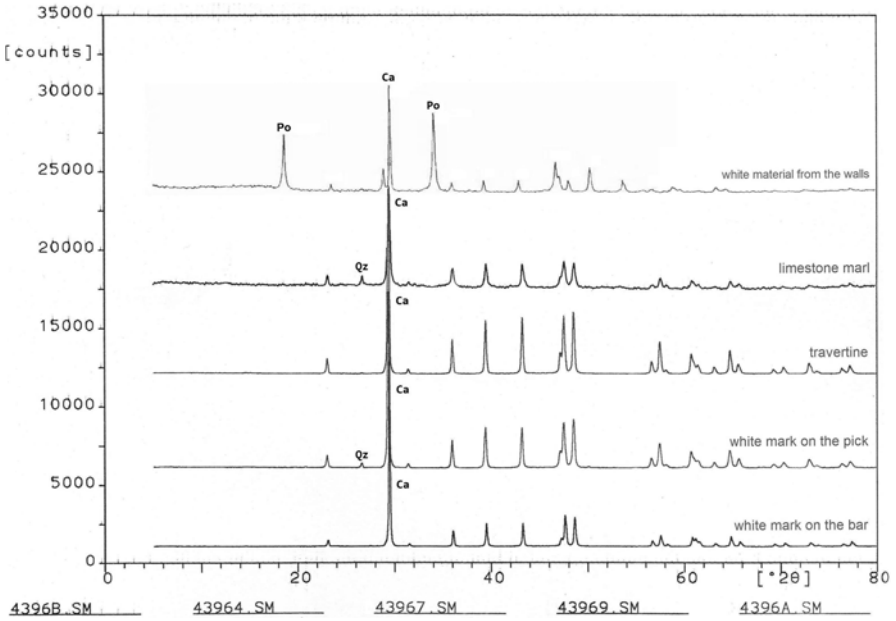


Fig. 3.6 X-ray diffractograms of headstones (limestone marl and travertine), marks on the pick and the bar, and white material from the walls. The critical peaks are labelled with the initials of minerals. *Ca* calcite, *Po* portlandite, *Qz* quartz

Table 3.4 Values of the percentages of calcium, silicon and aluminium in the samples LIMESTONE, PICK, TRAVERTINE, BAR, and WALL

Sample	LIMESTONE	PICK	TRAVERTINE	BAR	WALL
Ca	87.0	88.2	93.7	92.9	100
Si	9.4	8.3	3.9	4.0	–
Al	3.5	3.4	2.3	3.1	–
	99.9	99.9	99.9	100	100

Pick and Bar The pick gave similar results to those of the limestone marl headstones in that calcite and small quantity of quartz were present. The marks on the bar consisted of the same crystalline phase as that of the travertine gravestone, namely calcite (Fig. 3.6). The quantitative data suggested a similarity between the travertine and the mark on the bar, while the limestone marl signature was similar to that on the pick (Table 3.4).

Wall Quantitative analysis showed that the major element in the white wall material was calcium (Table 3.4), while further SEM analysis (at a magnification of 8.00 K X) revealed well-formed hexagonal plates of about 10 μm. These corresponded to portlandite, a calcium hydroxide, which is the major bonding agent in

cement and concrete (Fig. 3.6). Portlandite ($Ca(OH)_2$) is formed during the hydration of lime (CaO) and reaction with atmospheric CO_2 to form calcite (carbonation).

Wall mortar should normally contain quartz and/or other minerals as inert material, but none of these was found. The XRD data suggested that the white wall material could be an hydrated lime paste. The diffraction pattern showed close intensities of the peaks of calcite and portlandite. The dimensions of portlandite crystals were compatible with a hydrated lime paste of about 3 months old (Colantuono et al. 2011; Callebaut et al. 2000). Even if the carbonation process was slow, and the sample fresh, it has been shown that in some lime deposits, the ratio of portlandite to calcite is low (Hughes and Swann (1998).

3.4.4 Discussion and Conclusion

XRD analysis showed the material from the walls was different from the white marks on the tools. Furthermore, the peaks of calcite had similar intensities in headstone samples and in the marks, while the peak of calcite in the white material from the wall did not fully correspond with those of the headstone or the marks.

The quantitative data suggested a similarity between the travertine and the mark on the bar, and between the limestone marl and the mark on the pick. The silicon and the aluminum in the limestone marl linked up with the presence of small quantities of quartz and aluminum silicate hydrate. The presence of small quantities of silicon and aluminum in the travertine were consistent with the inclusion of impurities during its genesis. The material from the partition walls consisted of calcium, but no aluminium and/or silicon were found. The total absence of these elements in this sample, as supported the XRD data, suggested again that the material from the wall could be a hydrated lime. Furthermore, the dimension of the well-formed hexagonal crystals of portlandite suggested the hydrated lime paste had been used about three months before. This time did not correspond with the gardeners' claim.

The SEM-EDX data provided further evidence of the similarity of the gravestone with the marks on the tools and the difference of those with the material from the wall. The analysis of the material from the walls revealed that it was not a conventional mortar but, more likely, an hydrate lime paste (made from portlandite and calcite), which had been improperly used to restore the partition walls. Indeed the mineralogical and chemical data did not show the presence of sand or pozzolana which are invariably used as inert material in mortar. However, analysis using stereo-microscopy, X-ray diffraction, and SEM-EDX showed that, although the white material from the walls was different, the headstones contained the same minerals and elements as the marks on the tools.

These combined results suggested that the white marks on the tools were derived from the damaged headstones rather than the hydrated lime of the wall mortar.

3.5 Final Remarks

The three case studies illustrated in this paper showed that the successful application of geology in forensic analysis depends on several factors: (a) obtaining all pertinent samples early in the investigation, (b) accurate interpretation of events at a crime scene, (c) accurate contextualization of information and evidence in the specific investigation, the crime, and its dynamics, (d) the application of techniques suitable for analysis of micro-traces, and (e) appropriate data analysis. The practitioner should also be aware that trace evidence can be modified before and after collection.

It must be noted that analysis of the inorganic fraction alone may fail to discriminate between soils at a degree of resolution that would satisfy the requirements of a case. This is because in any given area, minerals and rocks can be ubiquitous, or certainly very widespread. However, if analysis of anthropogenic inclusions, and organic particulates, such as pollen and spores, is included, there can be a higher degree of resolution in establishing provenance. This is because although spatially large areas may yield similar pedological and geological characteristics, plant communities, and the by-products of human activity are more confined and can, thus, confer specificity.

Forensic geology can be most effective in a criminal investigation when the geologist is regarded as part of the investigative team early in the case. If the geologist is given appropriate information on the case history, and on the dynamics of the crime, sampling can become effectively targeted to maximise the retrieval of critical evidence.

Acknowledgements The author wish to thank Dr. Patricia Wiltshire for her help in writing this article; Dr. Antonia Pavan, Judge Prosecutor of Criminal Court of Law of Termini Imerese, Dr. Katia Marino, Judge Prosecutor of Criminal Court of Law of Reggio Emilia, and Dr. Adelchi D'Ippolito, Judge Prosecutor of Criminal Court of Law of Rome, for the permission at delivering the detailed scientific data on the cases.

References

Allen GC, Hallam KR, Radonjic M, Elton NJ, Farey M, Ashurst J (2000) X-ray diffraction and environmental electron microscopy of hydrate limes. In: Bartos P, Groot C, Hughes JJ (eds) Historic mortars: characteristics and tests. Proceedings of the international RILEM workshop. RILEM Publications S.A.R.L

Callebaut K, Elsen J, Van Balen K, Viaene W (2000). Historical and scientific study of hydraulic mortars from the 19th century. In: Bartos P, Groot C, Hughes JJ (eds) Historic mortars: characteristics and tests. Proceedings of the international RILEM workshop. RILEM Publications S.A.R.L

Claybourn M (ed) (2004) Raman spectroscopy in forensic science. Humana Press, New York

Colantuono A, Del Vecchio S, Marino O, Mascolo G, Mascolo MC, Vitale A (2011) Evoluzione del grassello in calce con il tempo di stagionatura. Forum Italiano Calce News 2:1–7

Beauchaine JP, Peterman JW, Rosenthal RJ (1988) Application of FT-IR/microscopy in forensic analysis. Microchimica Acta 94:133–138

Bergslien E (2012) An introduction to forensic geosciences. Wiley-Blackwell, Chichester

Di Maggio RM, Nuccetelli L (2013) Analysis of geological trace evidence in a case of criminal damage to graves. In: Pirrie D, Ruffell A, Dawson LA (eds) Environmental and criminal geoforensics, Special Publications 384. Geological Society, London. Updated version online May, 2013, http://dx.doi.org/10.1144/SP384.2

Di Maggio RM, Maio M, Nuccetelli L (2009) Geologia Forense. In: Picozzi M, Intini A (eds) Scienze Forensi: Teoria e Prassi dell'Investigazione Scientifica. UTET, Torino, pp 255–266

Edwards HGM (2004) Forensic application of Raman spectroscopy to the non-destructive analysis of biomaterials and their degradation. In: Pye K, Croft DJ (eds) Forensic geoscience. Principles, techniques and application, Special publication 232. Geological Society, London, pp 159–170

Evans JE, Tokar FJ (2000) Use of SEM/EDX and X-ray diffraction analyses for sand transport studies, Lake Eire, Ohio. J Coast Res 16:926–933

Griffiths P, De Haseth JA (2007) Fourier transform infrared spectrometry. Wiley, Chichester

Hughes D, Swann S (1998) Hydraulic limes – a preliminary investigation. Lime News 6:41–52

Kugler W (2003) X-ray diffraction analysis in the forensic science: the last resort in many criminal cases. Adv X-ray Anal 46:1–16

Lombardi G, Fucci P, Gualdi G (1983) La petrografia e le indagini medico-legali criminalistiche. La Giustizia penale 88:276–285

Long DA (2002) Raman spectroscopy. Wiley, Chichester

Munsell (2000) Soil color charts, Revised Edition by Greta G. Macbeth, New York

Murray RC (2004) Evidence from the earth. Mountain Press Publishing Company, Montana

Pye K (2007) Geological and soil evidence: forensic applications. CRC Press, Boca Raton

Pye K (2004) Forensic examination of rocks, sediments, soils and dust using scanning electron microscopy and X-ray chemical microanalysis. In: Pye K, Croft DJ (eds) Forensic geoscience. principles, techniques and application, Special publication 232. Geological Society, London, pp 103–122

Pye K, Krinsley DH (1984) Petrographic examination of sedimentary rocks in the SEM using backscattered electron detectors. J Sediment Res 54:877–888

Ruffell A, McKinley J (2008) Geoforensics. Wiley, Chichester

Ruffell A, Wiltshire P (2004) Conjunctive use of quantitative and qualitative X-ray diffraction analysis of soil and rocks for forensic analysis. Forensic Sci Int 145:13–23

Schiavon N (2002) Biodeterioration of calcareous and granitic building stones in urban environment. In: Siegesmund S, Weiss T, Vollbrecht A (eds) Natural stone, weathering phenomena, conservation strategies and case studies, Special Publication 205. Geological Society, London, pp 195–205

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

Enrique Santillana, Jose C. Cordero, and Francisco Alamilla

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,

E. Santillana (✉) • J.C. Cordero • F. Alamilla
Chemistry and Environment Department, Guardia Civil Criminalistic Service,
Guzmán el Bueno110, 28003 Madrid, Spain
e-mail: ejsantillana@guardiacivil.es; jccordero@guardiacivil.es; falamilla@guardiacivil.es

H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics,*
Soil Forensics, DOI 10.1007/978-3-319-33115-7_4

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

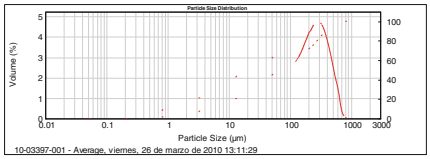
First, a similarity was observed between the colour of samples taken at depth (60–120 cm) and that of the soil sample adhering to the spade (Table 4.1). These deep samples should correspond to the same soil horizon. No differences were observed between the colour of samples taken at depth (60–120 cm) 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.

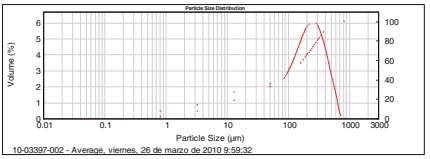
The mineralogical results (Table 4.3) indicated the presence of the smectite clay group (montmorillonite) in the soil samples taken at depth (60–120 cm) and the soil adhering to the spade and green boots, which was not present in the other samples. In these samples, montmorillonite clay was more abundant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

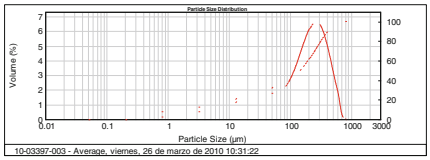
Sample	Colour <0.5 mm	Colour <2 μm	pH 1:5	Conductivity mS/cm
Surface level	7.5 YR 5/3	10 YR 3/2	7.57	312.0
60 cm depth	7.5 YR 7/2	10 YR 4/2	7.99	260.7
120 cm depth	7.5 YR 7/2	10 YR 4/2	8.38	185.6
Spade	7.5 YR 7/2	10 YR 4/2	7.68	277.1
Green boots	7.5 YR 6/3	10 YR 6/2	6.91	450.0
Brown boots	7.5 YR 5/3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



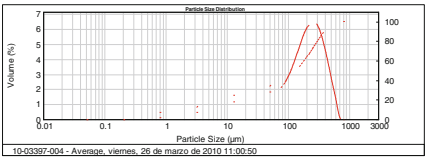
Surface level



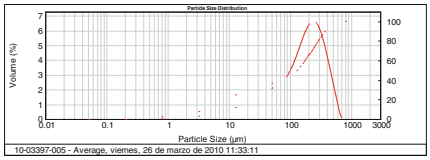
60 cm depth



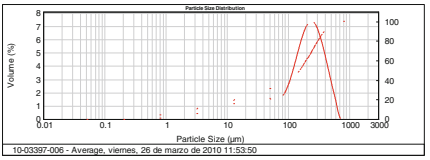
120 cm depth



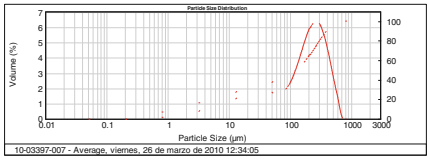
Spade



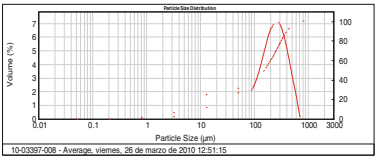
Green boots



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

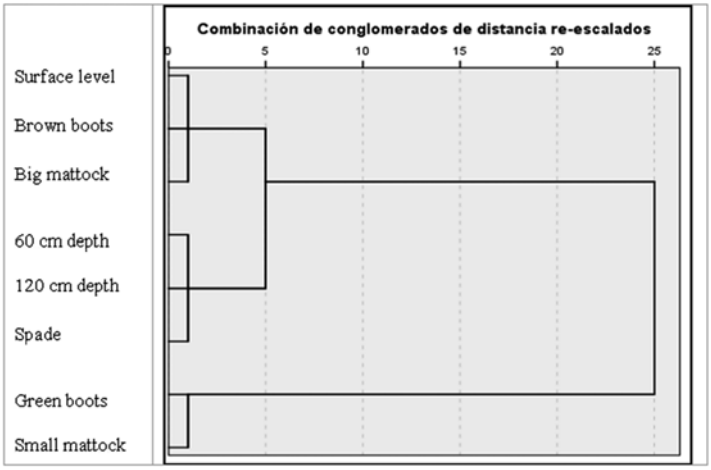


Fig. 4.4 Historical cluster dendrogram (Ward method) combining particle size distribution and chemical analysis

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

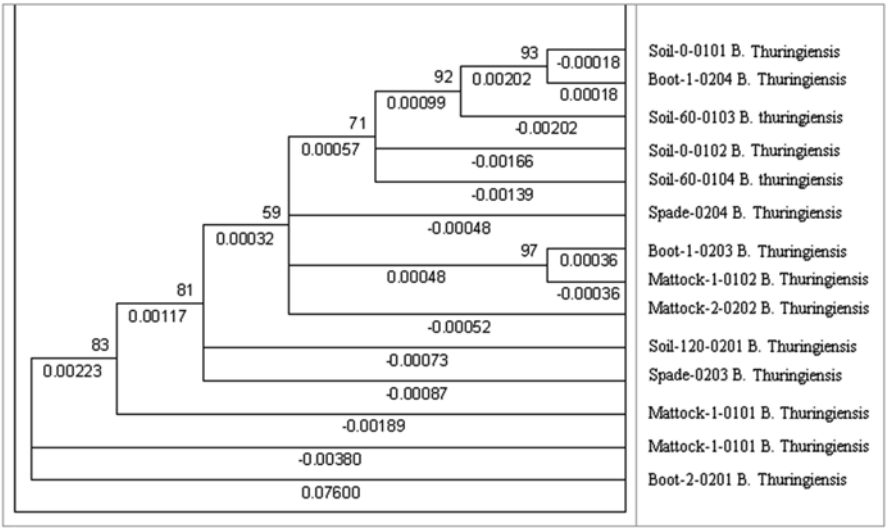
The results of the qualitative and quantitative analyses indicated that the samples taken at depth (60–120 cm) and the soil adhering to the spade presented similarities as regards colour, distribution profile (granulometry), mineralogy and chemical composition. The dendrogram resulting from this classification 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.

References

Blott SJ, Croft DJ, Pye K, Saye SE, Wilson HE (2004) Particle size analysis by laser diffraction. Forensic geoscience: principles, technique and applications. Geol Soc 232:63–73

Bommarito CR, Sturdevant AB, Szymanski DW (2007) Analysis of forensic soil sample via high-performance liquid chromatography and ion chromatography. J Foren Sci 52(1)

Color M (1994) Munsell soil color charts. Gretag Macbeth, New Windsor

Croft DJ, Pye K (2004) Colour theory and the evaluation of an instrumental method of measurement using geological samples for forensic application. Forensic geoscience: principles, technique and applications. Geol Soc 232:183–196

Fitzpatrick FW, Raven MD, Forrester ST (2009) A systematic approach to soil forensics: criminal case studies involving transference from crime scene to forensic evidence. In: Ritz K, Dawson L (eds) Criminal and environmental soil forensics. Springer, London, pp 105–127, Chapter 8, ISBN 978-1-4020-9203-9

Guedes A, Ribeiro H, Valentim B, Noronha F (2009) Quantitative colour analysis of Beach an dune sediments for forensic applications: a Portuguese example. Forensic Sci Int 190:42–51

Pirrie D, Power MR, Rollinson GK, Wiltshire PEJ, Newberry J Campbell HE (2009) Automated sem-eds (Qemscan) mineral analysis in forensic soil investigation: testing instrumental reproducibility. Criminal Environ Soil Forensic, 411–429

Pye K (2007) Geological and soil evidence. CRC Press, Boca Raton

Pye K, Blott SJ (2004) Compararison of soil and sediments using major and trace element data. Forensic geoscience: principles, technique and applications. Geol Soy, 183–196

Pye K, Croft DJ (2004) Forensic geoscience: introduction and overview. Forensic geoscience: principles, technique and applications. Geol Soc 232:1–5

Pye K, Croft DJ (2007) Fosensic analysis of soil and sediment traces by scanning electron microscopy and energy-dispersive X-ray analysis: an experimental investigation. Forensic Sci Int 165:52–63

Pye K, Blott SJ, Croft DJ, Carter JF (2006) Forensic comparison of soil samples: Assessment of small-scale spatial variability in elemental composition, carbon and nitrogen isotope ratios, colour, and particle size distribution. Forensic Sci Int 163:59–80

Ruffell A, Wiltshire P (2004) Conjunctive use of quantitative and qualitative X-ray diffraction analysis of soils and rocks for forensic analysis. Forensic Sci Int 145:13–23
Sugita R, Marumo Y (1996) Validity of color examination for forensic soil identification. Forensic Sci Int 183:201–210
UNE 77305 Determinación del pH (1999)
UNE 77308 Determinación de conductividad eléctrica específica (2001)
www.guardiacivil.es/patrimonio/activ_princip.jsp

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

Stefan Uitdehaag*, Frederike Quaak*, and Irene Kuiper

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

*Both Stefan Uitdehaag and Frederike Quaak contributed equally to this publication.

S. Uitdehaag (✉) • F. Quaak • I. Kuiper
Netherlands Forensic Institute, Laan van Ypenburg 6, 2497 GB The Hague, The Netherlands
e-mail: s.uitdehaag@nfi.minvenj.nl

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

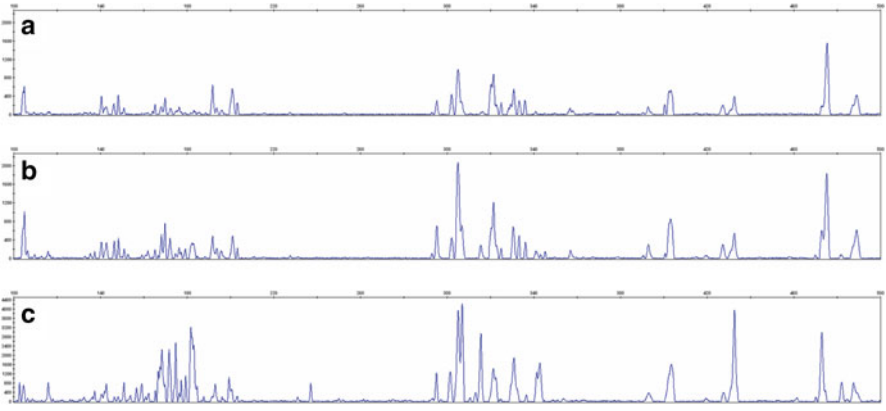


Fig. 5.2 Bacterial tRFLP profiles from (**a**) soil sample from suspect's shoe, (**b**) soil sample from victim's tights and (**c**) from non-related soil sample (positive control)

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.

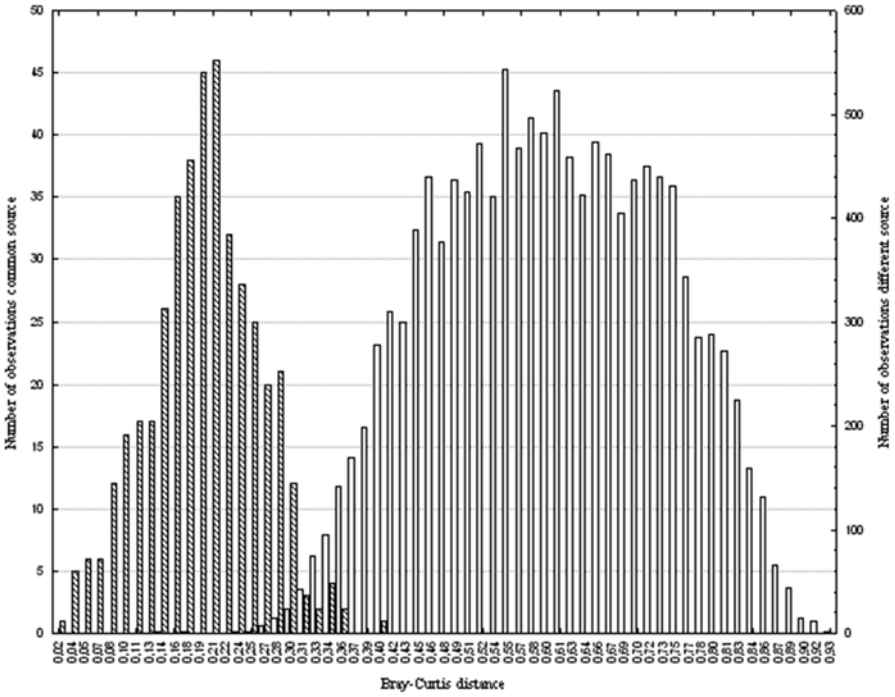


Fig. 5.3 Histogram with distribution of 13,366 Bray-Curtis (BC) distances (x-axis) calculated between 164 profiles of 50 soil samples. The number of observations of BC distances is indicated on the y-axes. Shaded bars represent distances calculated between profiles originating from the same source (420 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.

References

Aitken C, Berger CEH, Buckleton JS, Champod C, Curran J, Dawid AP, Evett IW, Gill P, Gonzalez-Rodriguez J, Jackson G (2011) Expressing evaluative opinions: a position statement. Sci Justice 51:1–2

Association of Forensic Science Providers (2009) Standards for the formulation of evaluative forensic science expert opinion. Sci Justice 49:161–164

Beals EW (1984) Bray-Curtis ordination: an effective strategy for analysis of multivariate ecological data. Adv Ecol Res 14:1–55

Berger CEH, Buckleton J, Champod C, Evett IW (2011) Evidence evaluation: a response to the court of appeal judgement in R v T. Sci Justice 51:43–49

Brown AG, Smith A, Elmhurst O (2002) The combined use of pollen and soil analyses in a search and subsequent murder investigation. J Forensic Sci 47(3):614–618

Bull PA, Parker A, Morgan RM (2006) The forensic analysis of soil and sediment taken from the cast of a footprint. Forensic Sci Int 162:6–12

Bull PA, Morgan RM, Freudiger-Bonzon J (2008) Letter to the editor – a critique of the present use of some geochemical techniques in geoforensic analysis. Forensic Sci Int 178:e35–e40

Cox RJ, Peterson HL, Young J, Cusik C, Espinoza EO (2000) The forensic analysis of soil organic by FTIR. Forensic Sci Int 108:107–116

Faegri K, Iversen J (1989) Textbook of pollen analysis, 4th revised edition. The Blackburn Press. ISBN 1-930665-016, 328 p

Horrocks M (2004) Technical note: sub-sampling and preparing forensic samples for pollen analysis. J Forensic Sci 49(5):1–4

Horrocks M, Walsh KAJ (1999) Fine resolution of pollen patterns in limited space: differentiating a crime scene and alibi scene seven meters apart. J Forensic Sci 44:417–420

Horsewell J, Cordiner SJ, Maas EW, Martin TM, Sutherland KBW, Speir TW, Nogales B, Osborn AM (2002) Forensic comparison of soils by bacterial community DNA profiling. J Forensic Sci 47:350–353

Kerkhoff W, Stoel RD, Mattijssen EJAT, Hermsen R (2013) The likelihood ratio approach in cartridge case and bullet comparison. AFTE J 45(3):284–289

McQuiston-Surrett D, Saks MJ (2008) The testimony of forensic identification science: what expert witnesses say and what factfinders hear. Law Hum Behav 33(5):436–453

Mildenhall DC (2004) An example of the use of forensic palynology in assessing an alibi. J Forensic Sci 49(2):312–316

Munsterman D, Kestholt S (1996) Sodium polytungstate, a new non-toxic alternative to bromoform in heavy liquid separation. Rev Palaeobot Palynol 91:417–422

Nordgaard A, Ansell R, Drotz W, Jaeger L (2012) Scale of conclusions for the value of evidence. Law, Probab Risk 11(1):1–24

Pasternak Z, Al-Ashhab A, Gatica J, Gafny R, Avraham S, Frenk S, Minz D, Gillor O, Jurkevitch E (2012) Optimization of molecular methods and statistical procedures for forensic fingerprinting of microbial soil communities. Int Res J Microbiol 3(11):363–372

Quaak FCA, Kuiper I (2011) Statistical data analysis of bacterial t-RFLP profiles in forensic soil comparisons. Forensic Sci Int 210:96–101

Riding JB, Kyffin-Hughes JE (2004) A review of the laboratory preparation of palynomorphs with a description of an effective non-acid technique. Rev Bras de Paleontol 70:13–44

Saks MJ, Koehler JJ (1991) What DNA 'fingerprinting' can teach the law about the rest of forensic science. Cardozo Law Revies 13:361–372

Saks MJ, Koehler JJ (2005) The coming paradigm shift in forensic identification science. Science 5(309):892–895

Chapter 6
Forensic Comparison of Soil Samples

Jisook Min, Kiwook Kim, Sangcheol Heo, and Yurim Jang

Abstract As a preliminary experiment to test the discriminating ability of forensic soil analysis techniques and obtain area-specific information, soil samples were collected from eight areas near the eastern branch of the National Forensic Service (NFS) located in Gangwondo, an eastern province of South Korea. The soil samples were collected from five spots within each sample area using a small-scale (1 m^2) soil sampling technique; for each of these five spots, two samples were collected from two places in each spot, (i) one from the surface and (ii) another from 30 cm below the surface. For each sample, the color of the sample with particle size in the range 53–500 μm and the major constituents were determined using a spectrophotometer and X-ray fluorescence spectrometer (XRF), respectively. The carbon content and carbon isotope ratio of the part of the sample of particle size below 53 μm were measured using an element analyzer-isotope ratio mass spectrometer (EA-IRMS). The canonical discriminant and XRF analyses showed an excellent color discriminating ability of 87.5 % and 88.8 %, respectively, with respect to the major constituents. The EA-IRMS results showed that the soils obtained from a 30-cm depth below the surface were generally more enriched in $\delta^{13}C$ (0/00) than the surface soils, and that the surface soils contained a higher carbon amount (%). The canonical discriminant analysis confirmed 100 % discriminating ability when all three soil characteristics (i.e., color, composition, and content) were used in the analysis. Out of the two functions obtained from the analysis, Function 1 exhibited greater potential for explaining the SiO_2, Fe_2O_3, and TiO_2; thus, Area 6 and 7 could be more easily differentiated than the other areas using this function. Function 2 exhibited greater potential for explaining color factor b* ($\delta^{13}C$ and C content), and could more efficiently differentiate Area 2 and 5. However, different results were obtained within the same area based on the soil depth. Therefore, when performing a comparative sampling analysis in forensic science, due care should be taken to prevent the mixing of adjacent soils from various depths. Better results can be achieved by collecting soil samples from different spots within the same area.

J. Min (✉) • K. Kim • S. Heo • Y. Jang
National Forensic Service, 331-1 Shinwol7-Dong Yangcheon-Gu, 158-707 Seoul, Korea
e-mail: minjs@korea.kr

H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics*,
Soil Forensics, DOI 10.1007/978-3-319-33115-7_6

6.1 Introduction

Soil samples are most frequently used in forensic investigations associated with a wide variety of criminal acts and incidences such as abandonment of corpse, rape, robbery, and traffic accidents. The most frequently encountered difficulties in soil analyses are the extreme complexity of soil composition and minuscule sample volume. Moreover, apart from the identification of comparing samples, it is often required to obtain the area-specific soil information of a given type of soil. However, because of the lack of systematically developed techniques, such area-specific information data obtained from existing techniques do not meet the actual information needs. To improve this situation, there is an urgent need for establishing measurement techniques that are capable of analyzing minuscule samples and studying area-specific features using the state-of-the-art equipment (Junger 1996). In this study, the discriminating abilities of various soil-analysis methods were tested using various techniques, and the results of area-specific information obtained was reviewed.

Some of the forensic soil analysis methods are as follows: mineral morphology and particle state analysis (Graves 1979), hydrometer-assisted mineral morphological analysis (Chaperlin and Howarth 1983), color inspection (Guedes et al. 2011), size and classification inspection, and pollen analysis. The comparative analysis of biotic components found in soil can be performed, among others, using chromatography, Fourier transform infrared (FT-IR) spectroscopy, and Raman spectroscopy (Wheals and Noble 1972; Reuland and Trinler 1981; Cox et al. 2000: Thanasoulias et al. 2002). The investigation methods for comparative analyses of the ratio of rare-earth elements to major mineral constituents, using XRF (Fitton 1997) and X-ray diffraction (XRD), inductively-coupled plasma (ICP), and laser ablation inductively-coupled plasma mass spectrometry (LA-ICP-MS), have also been reported (Petraco et al. 2000; Bull et al. 2006; Pye et al. 2007; Petraco et al. 2008; Pye and Blott 2009; Murray 2012). Furthermore, the most recent addition to this list of techniques is the determination of carbon and nitrogen contents and isotope ratios (Meyers 1994; Pye et al. 2006).

When different soils are compared, one of the most important discriminating factors is color. Color differences are the results of different compositions of biotic and abiotic materials in soil. They provide cumulative information such as the original place of the soils, decomposition residues of native plants and animals in that place, and other organic matters such as excrementitious matters. In many cases, the surface of a soil particle consists of iron, aluminum, organic matter, clay, and other substances, and gives important information on the history of the soil sample. Brown, yellow, or red color of the soil indicates a high concentration of iron oxides in the soil. Brown color soils predominate in warm and humid weather conditions, yellow color soils predominate in water environment, and soils turn grey when their iron content migrates to groundwater or other water sources.

Red color soils indicate high temperature or oxidizing conditions existing in soil, and the red color of soil becomes stronger not only as the iron content increases, but also as the oxidation advances. The iron components in soils exist in the forms of hematite, limonite, goethite, lepidocrocite, etc. The black mineral tone of soil is generally associated with the presence of manganese or iron-manganese composites. The color of soils is also influenced by biotic matters irrespective of the presence of abiotic matters. The organic materials on soil surface usually appear black. The humus infiltrated into mineral soil layers is dark in color, and the soil with iron and humic acid has a dark blackish red-brown color. Although such information based on the color of soil can be used as a primary discriminating factor in forensic soil comparison, given the fact that the data would be presented in a criminal court, it is important to perform scientifically the color investigation using a spectrophotometer instead of a rough visual examination.

In particular, when the soil evidences have similar colors, more sophisticated forensic analysis techniques are required. In such cases, besides morphological investigation using a spectrophotometer and stereo microscope, high-precision analyses should be performed, such as elemental analysis using a XRF, comparison of carbon contents and carbon isotope ratios using an EA-IRMS, and mineral identification using a XRD.

Therefore, the soil samples were collected from eight areas in Wonju, (Gangwondo, South Korea) and the following analyses were performed: color inspection using a spectrophotometer, elemental analysis using a XRF, and measurement of carbon content and carbon isotope ratio using an EA-IRMS. With the help of these quantitative analyses, the discriminating abilities of various techniques used for soil identification were evaluated, and their abilities to obtain area-specific information were ascertained. Furthermore, statistical analyses of the abovementioned samples were conducted using SPSS 18.0 and soft independent modeling of class analogy (SIMCA).

6.2 Materials and Methods

6.2.1 Area Description and Sample Collection

The soil samples were collected from eight areas (Fig. 6.1 and Table 6.1) in Wonju (Gangwondo, South Korea) for this study. The soil sampling was carried out on five spots in each of the areas as follows: a center point (c) and four corner points (a, b, d, and e) of a square (Fig. 6.2).

From each spot, the soil samples were collected from the surface (S) and 30-cm depth (D) below the surface, and then dried for at least 5 days in a well-ventilated shady place. To ensure the homogeneity of the dried samples, they were separated using 500 and 53-μm-mesh sieves.

Fig. 6.1 Locations of the eight soil sampling areas

Table 6.1 Soil sampling plots and their locations

Area	Location
1area	Empty lot in the NFS eastern branch
2area	Near Moonmak Industrial Complex
3area	Under a river bridge
4area	Residential area near streets
5area	Fishing zone near a dam
6area	Roadside pasturage near factories
7area	River beach sands
8area	Soil near the river

6.2.2 Color Determination of Soils Using Spectrophotometer

The particles in soils are classified into coarse sand, medium sand, fine sand, silt, and clay in decreasing order of size. As the size decreases, red or red-brown color becomes increasingly apparent, while as the size increases, grey and yellow colors become more and more dominant. Therefore, sieves were used to separate the soils in specific sizes (53–500 μm) prior to determining their color using a

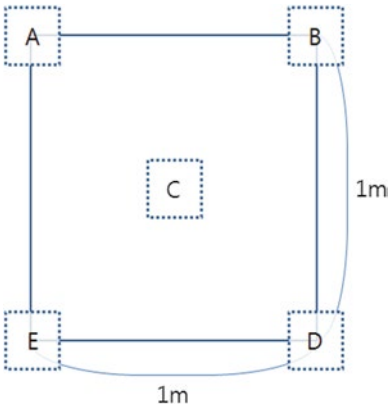


Fig. 6.2 Soil sampling areas

spectrophotometer (Spectrophotometer CM-5, Konika Minolta, Japan), which can measure the wavelength range between 360 and 740 nm. Before using the spectrophotometer, a black-and-white color calibration was carried out, and each sample was measured for five times. The results of color measurement were expressed according to the CIE L^*, a^*, b^* (CIELAB) color space.

6.2.3 *Elemental Analysis of Soils Using X-ray Fluorescence Spectrometer*

Beads were made from the soils ranging from 53 to 500 μm and subjected to an XRF analysis for determining the contents of major elements. Using K2 Prime (Katanax, Canada), beads of 30-mm diameter were fabricated from the soil dough mixed at the ratio of soil:flux (lithium tetraborate):remover (lithium bromide) = 0.5 g:4.5 g:0.03 g. The SiO_2, Al_2O_3, Fe_2O_3, K_2O, CaO, and TiO_2 contents were analyzed using an XRF spectrometer (M4 Tornado, Bruker, Germany). Each sample was measured for five times.

6.2.4 *Carbon Isotope Ratio and Carbon Content of Soils Using EA-IRMS*

In this measurement, samples smaller than 53 μm were used. They were wrapped in tin capsules after the weight check, and their $\delta^{13}C$ (0/00) and carbon contents (%) were measured using CH-6 and urea as the reference standards. Each sample was measured in triplicates. Our attempt to perform measurements for nitrogen isotope ratio and carbon content was aborted because $\delta^{15}N$ could not be measured even with as much as 20 mg sample. Given the usual forensic settings in which the sample

analyses should be often performed with relatively small amounts of sample, the nitrogen-related measurements are considered inadequate. The elemental analyses were performed using an elemental analyzer (Euro EA 3000, Euro Vector, Italy), and Isoprime (GV instrument, UK) was used as the mass spectrometer. The degree of analytical precision checked against the reference standard material (CH-6) by a multiple analysis was within a 0.1‰ range.

6.2.5 Statistical Analysis

At least five measurements were carried out on the ten sub-samples collected within a 1-m^2 range for each of the eight sampling areas, and their average values were used in all the statistical analyses. The analysis of variance (ANOVA) and canonical discriminant analysis were performed using SPSS 18.0, and other analyses such as partial least square (PLS) data analysis were performed using SIMCA. The level of reliability was kept at 95 % for all the analyses.

6.3 Results and Discussion

The average values resulting from the three experiments performed five times each on a total of 80 sub-samples (S and D of five spots [a, b, c, d, and e] of each of the eight areas) are listed in Tables 6.2 and 6.3.

6.3.1 Color Determination

A spectrophotometer furnishes the required data by quantifying the color tones with an accuracy better than that of human eye. The spectrophotometer used in this study can measure the wavelengths between 400 and 700 nm, measures the color attributes as Munsell's hue, value, and chroma in L^*, a^*, and b^* (CIELAB index), respectively, and provide spectra along with reflectance data. The CIELAB color space, which was specified by the Commission Internationale de l'eclairage (CIE), has been internationally used as the standard color scale. It shows the color difference in delta E (ΔE) unit. In the CIELAB color space, L^* represents luminance (brightness), and hue and intensity are expressed as a^* and b^* values. With a^* and b^*, the direction of a color is indicated: $+a^*$ indicates the direction toward red,$-a^*$ toward green, $+b^*$ toward yellow, and$-b^*$ toward blue (Fig. 6.3). The color intensity increases as the given value increases. While the human eye can usually detect color difference from 3 (ΔE) onwards, the spectrophotometer was calibrated to detect the color difference smaller than or equal to 1 (ΔE). The calibration was performed again for every analysis.

Table 6.2 The average data from 8 areas in Gangwon-do (*S*: on the surface, *D*: from a depth of 30 cm) using XRF

Data label	XRF																	
	SiO_2(%)			Al_2O_3(%)			Fe_2O_3(%)			K_2O(%)			CaO(%)			TiO_2(%)		
	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD
1-1-S	54.78	1.42	2.60	16.74	2.66	15.90	11.88	0.51	4.33	4.90	0.17	3.56	5.00	0.14	2.89	1.40	0.05	3.72
1-2-S	57.45	1.75	3.04	14.55	2.89	19.88	12.02	0.64	5.32	5.45	0.18	3.28	3.83	0.14	3.73	1.38	0.05	3.83
1-3-S	53.82	0.88	1.63	15.09	1.67	11.10	12.08	0.41	3.40	5.08	0.10	1.91	5.08	0.14	2.67	1.50	0.08	5.01
1-4-S	54.04	1.24	2.29	15.22	2.08	13.70	12.66	0.58	4.57	5.18	0.15	2.90	4.59	0.12	2.71	1.42	0.06	3.94
1-5-S	54.26	1.37	2.52	16.23	2.32	14.29	13.10	0.61	4.66	5.12	0.12	2.42	4.98	0.14	2.79	1.47	0.06	3.91
1-1-D	55.99	2.13	3.81	16.18	3.87	23.91	11.57	0.71	6.12	5.17	0.25	4.76	5.10	0.22	4.21	1.34	0.06	4.60
1-2-D	55.55	1.33	2.40	18.32	2.03	11.08	11.15	0.39	3.47	5.24	0.19	3.58	3.90	0.11	2.79	1.27	0.04	3.31
1-3-D	56.28	2.28	4.06	14.76	4.90	33.20	11.92	1.17	9.80	5.13	0.35	6.73	5.30	0.39	7.37	1.44	0.14	9.58
1-4-D	57.06	1.37	2.39	13.86	3.18	22.93	12.63	0.80	6.35	5.37	0.23	4.24	4.52	0.24	5.35	1.46	0.07	4.53
1-5-D	55.28	1.41	2.55	16.19	2.26	13.94	11.76	0.41	3.50	5.11	0.17	3.25	5.02	0.14	2.79	1.50	0.07	4.81
1Area	55.45	1.25	2.26	15.71	1.29	8.18	12.08	0.57	4.74	5.18	0.15	2.98	4.73	0.51	10.85	1.42	0.07	5.13
2-1-S	55.16	1.18	2.14	15.82	2.30	14.51	10.15	0.40	3.89	4.79	0.16	3.33	4.14	0.17	4.02	1.35	0.05	3.96
2-2-S	58.19	2.23	3.83	12.18	4.28	35.09	11.50	0.95	8.28	5.40	0.31	5.80	4.47	0.32	7.22	1.51	0.11	7.37
2-3-S	55.98	2.11	3.76	13.15	4.48	34.06	11.05	0.83	7.51	4.86	0.27	5.56	4.37	0.28	6.40	1.37	0.08	5.62
2-4-S	55.49	1.35	2.44	14.26	2.53	17.77	11.14	0.48	4.27	4.85	0.18	3.68	4.50	0.16	3.54	1.44	0.03	2.12
2-5-S	58.12	0.88	1.52	16.81	2.00	11.92	9.32	0.44	4.72	5.39	0.24	4.51	4.17	0.18	4.29	1.30	0.06	4.28
2-1-D	54.52	1.57	2.89	15.69	3.38	21.55	12.87	0.84	6.56	4.35	0.19	4.31	5.46	0.28	5.05	1.63	0.10	6.29
2-2-D	57.47	0.74	1.30	13.51	1.35	9.99	11.83	0.29	2.46	4.72	0.14	2.93	5.79	0.17	3.02	1.47	0.03	2.05
2-3-D	57.49	1.88	3.27	16.00	4.39	27.45	10.22	0.98	9.62	5.09	0.41	8.12	4.39	0.35	7.89	1.28	0.12	9.64
2-4-D	58.00	0.76	1.32	13.60	2.70	19.83	10.42	0.87	8.37	5.23	0.25	4.82	4.70	0.28	5.95	1.41	0.07	5.09
2-5-D	62.14	1.78	2.86	11.99	3.22	26.83	8.65	0.45	5.24	6.26	0.30	4.81	4.03	0.25	6.30	1.16	0.06	5.25
2Area	57.26	2.18	3.81	14.30	1.69	11.81	10.71	1.23	11.49	5.09	0.52	10.27	4.60	0.58	12.55	1.39	0.13	9.35

(continued)

Table 6.2 (continued)

Data label	XRF																	
	SiO_2(%)			Al_2O_3(%)			Fe_2O_3(%)			K_2O(%)			CaO(%)			TiO_2(%)		
	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD
3-1-S	60.19	2.36	3.91	14.05	4.36	31.07	7.86	0.53	6.74	5.40	0.30	5.54	2.05	0.08	4.09	1.22	0.10	8.45
3-2-S	63.16	2.17	3.43	13.05	3.62	27.75	6.26	0.42	6.69	6.57	0.35	5.40	2.59	0.18	7.04	0.94	0.05	4.85
3-3-S	61.33	1.72	2.80	13.84	3.15	22.76	6.92	0.39	5.67	6.68	0.34	5.11	2.78	0.17	6.10	1.04	0.05	4.99
3-4-S	59.39	1.77	2.97	12.47	3.13	25.11	8.97	0.47	5.24	6.05	0.19	3.21	2.87	0.12	4.17	1.15	0.05	4.29
3-5-S	64.55	1.69	2.61	13.07	2.88	22.01	6.60	0.33	5.03	7.51	0.38	5.07	2.44	0.13	5.17	1.07	0.07	6.98
3-1-D	64.62	1.07	1.66	11.25	2.41	21.42	6.81	0.41	5.97	7.14	0.24	3.40	3.14	0.19	5.92	1.03	0.04	4.36
3-2-D	64.84	1.01	1.56	11.36	2.33	20.55	5.14	0.26	5.10	6.56	0.21	3.18	2.50	0.11	4.60	0.76	0.04	5.59
3-3-D	64.81	2.81	4.34	11.52	4.88	42.41	6.96	0.64	9.20	6.86	0.50	7.26	3.05	0.25	8.17	1.06	0.07	6.77
3-4-D	61.88	1.88	3.04	15.02	2.77	18.44	6.33	0.30	4.77	6.45	0.33	5.10	2.97	0.09	3.04	0.98	0.06	5.72
3-5-D	60.82	0.80	1.32	15.93	1.23	7.74	6.80	0.17	2.49	6.36	0.14	2.20	2.94	0.08	2.78	1.03	0.04	3.50
3Area	62.56	2.09	3.35	13.16	1.58	12.01	6.86	1.01	14.66	6.56	0.58	8.79	2.73	0.34	12.29	1.03	0.12	11.89
4-1-S	53.31	1.51	2.84	15.91	3.14	19.74	11.79	0.64	5.45	4.89	0.20	4.01	4.41	0.21	4.73	1.43	0.08	5.25
4-2-S	54.49	2.35	4.31	14.52	5.19	35.74	13.06	1.23	9.44	4.88	0.40	8.11	4.09	0.32	7.76	1.35	0.09	6.66
4-3-S	64.14	1.64	2.56	14.34	2.52	17.58	4.24	0.22	5.20	7.96	0.30	3.76	0.96	0.07	7.08	0.73	0.03	4.45
4-4-S	53.15	0.95	1.79	17.70	1.78	10.06	12.81	0.38	2.94	4.78	0.14	2.95	3.97	0.09	2.28	1.39	0.01	1.07
4-5-S	54.14	0.97	1.79	16.98	2.16	12.70	12.53	0.66	5.26	5.16	0.22	4.32	3.47	0.10	2.86	1.45	0.06	4.27
4-1-D	59.93	2.43	4.06	13.72	4.70	34.25	9.90	0.95	9.58	6.12	0.41	6.75	3.56	0.28	7.97	1.31	0.12	9.47
4-2-D	61.44	2.00	3.25	14.52	3.59	24.74	7.32	0.53	7.27	6.94	0.38	5.42	2.35	0.12	5.13	1.02	0.07	6.37
4-3-D	65.54	1.53	2.34	13.61	2.36	17.31	3.61	0.16	4.43	7.95	0.30	3.74	0.85	0.04	4.71	0.67	0.05	6.81
4-4-D	61.81	2.40	3.88	11.76	4.55	38.68	8.84	0.71	8.08	6.62	0.41	6.16	2.64	0.22	8.22	1.13	0.10	8.61
4-5-D	56.88	1.77	3.11	15.52	3.54	22.83	12.02	0.90	7.48	5.15	0.26	4.97	3.54	0.20	5.79	1.51	0.09	6.00
4Area	58.48	4.67	7.99	14.86	1.74	11.68	9.61	3.53	36.70	6.05	1.26	20.84	2.98	1.26	42.27	1.20	0.30	25.22
5-1-S	64.23	1.38	2.14	15.17	2.16	14.22	4.67	0.23	4.98	8.28	0.23	2.84	0.91	0.05	5.71	0.71	0.04	5.63

5-2-S	66.62	2.38	3.58	10.44	3.83	36.63	5.24	0.31	5.85	8.69	0.35	4.04	0.88	0.03	3.41	0.78	0.08	10.65
5-3-S	61.67	1.12	1.82	16.33	2.02	12.38	4.63	0.27	5.75	7.39	0.38	5.11	1.08	0.16	14.58	0.72	0.04	5.21
5-4-S	65.21	1.77	2.72	13.07	3.22	24.60	5.09	0.36	7.16	8.58	0.50	5.77	0.80	0.06	7.82	0.75	0.05	6.27
5-5-S	62.79	2.53	4.02	16.34	4.12	25.24	5.05	0.40	7.87	7.92	0.53	6.70	1.06	0.06	5.43	0.81	0.05	6.62
5-1-D	64.78	1.98	3.06	14.10	3.30	23.44	4.59	0.26	5.71	7.94	0.38	4.84	0.79	0.03	4.40	0.69	0.04	6.43
5-2-D	63.74	2.10	3.29	17.29	3.15	18.21	4.76	0.33	6.85	8.03	0.39	4.84	0.81	0.03	3.54	0.68	0.05	6.90
5-3-D	64.20	2.30	3.59	12.93	4.04	31.25	4.69	0.38	8.17	8.00	0.40	5.05	0.83	0.07	8.34	0.73	0.07	9.27
5-4-D	65.87	3.06	4.64	13.04	4.70	36.07	4.85	0.46	9.46	8.50	0.57	6.67	0.86	0.06	7.28	0.73	0.07	9.06
5-5-D	64.97	2.65	4.07	11.84	4.81	40.59	4.89	0.37	7.52	8.13	0.55	6.75	0.85	0.11	13.37	0.75	0.08	10.19
5Area	64.41	1.44	2.24	14.05	2.19	15.60	4.85	0.22	4.51	8.15	0.38	4.70	0.89	0.10	11.71	0.74	0.04	5.27
6-1-S	56.49	1.36	2.40	16.28	2.98	18.33	7.84	0.35	4.50	6.56	0.26	4.02	1.39	0.06	4.23	1.23	0.06	4.84
6-2-S	56.05	3.05	5.43	14.92	5.93	39.76	9.62	0.99	10.30	6.01	0.43	7.18	1.62	0.16	9.62	1.37	0.12	8.74
6-3-S	57.83	2.61	4.51	13.76	4.94	35.91	8.70	0.67	7.67	6.60	0.38	5.84	1.27	0.08	6.01	1.38	0.11	8.06
6-4-S	55.51	2.35	4.24	17.53	4.14	23.64	8.17	0.48	5.93	6.39	0.21	3.29	1.39	0.09	6.50	1.22	0.08	6.87
6-5-S	57.81	1.50	2.59	16.41	3.09	18.81	7.83	0.36	4.61	7.20	0.36	4.97	0.89	0.07	8.35	1.19	0.08	6.75
6-1-D	61.23	1.93	3.16	13.21	3.22	24.36	7.44	0.36	4.78	7.86	0.36	4.60	0.76	0.05	6.05	1.17	0.05	4.37
6-2-D	58.15	1.94	3.33	17.52	3.44	19.65	8.64	0.57	6.55	7.79	0.40	5.17	0.73	0.06	7.70	1.34	0.08	6.22
6-3-D	60.36	1.11	1.84	14.24	1.73	12.15	7.67	0.27	3.54	7.79	0.24	3.13	0.70	0.05	7.59	1.20	0.03	2.13
6-4-D	60.41	1.89	3.13	13.44	3.43	25.51	8.27	0.52	6.26	7.91	0.33	4.23	0.86	0.04	4.10	1.32	0.10	7.36
6-5-D	61.41	1.87	3.04	13.58	3.56	26.21	9.08	0.65	7.17	8.33	0.45	5.37	0.75	0.04	5.23	1.40	0.09	6.71
6Area	58.53	2.19	3.74	15.09	1.70	11.28	8.33	0.68	8.20	7.24	0.80	11.04	1.04	0.34	33.20	1.28	0.09	6.83
7-1-S	63.14	2.52	4.00	13.96	3.68	26.36	6.07	0.37	6.05	5.71	0.27	4.75	3.38	0.20	5.81	0.88	0.06	6.65
7-2-S	62.46	1.60	2.56	14.85	2.68	18.02	6.40	0.32	5.03	5.62	0.21	3.74	3.29	0.14	4.26	0.90	0.04	4.67

(continued)

Table 6.2 (continued)

Data label	XRF																	
	SiO_2(%)			Al_2O_3(%)			Fe_2O_3(%)			K_2O(%)			CaO(%)			TiO_2(%)		
	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD
7-3-S	67.51	1.84	2.73	9.37	3.00	32.04	5.57	0.30	5.40	5.97	0.34	5.73	3.38	0.16	4.71	0.95	0.06	6.83
7-4-S	62.59	2.19	3.50	11.01	4.42	40.12	7.94	0.66	8.37	5.76	0.37	6.41	3.71	0.22	5.81	1.07	0.08	7.62
7-5-S	66.04	1.34	2.02	10.49	3.78	36.05	6.41	0.65	10.06	6.37	0.41	6.37	3.46	0.26	7.65	0.94	0.09	9.67
7-1-D	65.98	1.09	1.64	12.23	1.49	12.17	5.14	0.15	2.86	6.07	0.12	1.97	3.22	0.09	2.92	0.92	0.02	2.66
7-2-D	66.14	1.84	2.78	12.68	3.11	24.52	4.78	0.27	5.65	5.48	0.27	4.97	3.14	0.22	6.96	0.89	0.07	7.45
7-3-D	67.74	2.62	3.86	6.94	4.77	68.63	7.03	0.63	8.89	6.78	0.48	7.14	3.18	0.19	6.10	0.91	0.04	4.48
7-4-D	66.26	2.55	3.86	9.94	4.12	41.51	5.99	0.44	7.28	6.40	0.34	5.25	3.22	0.23	7.08	0.89	0.06	6.45
7-5-D	66.33	0.92	1.39	10.34	2.17	21.02	5.73	0.18	3.07	6.56	0.18	2.79	2.96	0.14	4.88	0.81	0.02	2.31
7Area	65.42	1.96	2.99	11.18	2.32	20.77	6.11	0.92	14.99	6.07	0.44	7.24	3.29	0.20	6.20	0.92	0.07	7.43
8-1-S	55.39	1.25	2.25	16.51	2.36	14.32	11.03	0.41	3.76	5.17	0.17	3.27	3.78	0.11	3.01	1.30	0.03	2.60
8-2-S	56.19	1.29	2.30	13.14	2.88	21.94	11.65	0.63	5.42	5.46	0.24	4.41	4.10	0.21	5.05	1.35	0.08	5.54
8-3-S	55.61	0.51	0.92	12.86	1.44	11.16	11.64	0.33	2.84	5.45	0.15	2.81	4.14	0.17	4.23	1.35	0.06	4.15
8-4-S	55.58	1.63	2.93	14.87	3.00	20.19	11.21	0.52	4.65	5.18	0.20	3.85	4.21	0.17	4.05	1.31	0.05	3.66
8-5-S	56.47	1.89	3.35	14.46	3.50	24.23	10.99	0.59	5.38	5.40	0.25	4.59	4.35	0.19	4.40	1.33	0.06	4.78
8-1-D	60.83	2.11	3.46	13.09	3.50	26.73	9.16	0.53	5.74	5.84	0.32	5.54	3.54	0.13	3.53	1.15	0.06	4.83
8-2-D	62.52	1.90	3.04	12.02	3.25	27.02	8.42	0.50	5.95	6.33	0.31	4.95	3.46	0.15	4.28	1.10	0.06	5.28
8-3-D	61.87	1.20	1.93	12.01	2.63	21.92	8.62	0.51	5.94	6.16	0.30	4.88	3.81	0.20	5.19	1.17	0.06	4.79
8-4-D	60.64	1.91	3.14	13.00	3.05	23.47	9.08	0.57	6.28	6.07	0.21	3.48	3.82	0.18	4.60	1.21	0.07	5.66
8-5-D	62.17	2.44	3.92	12.44	4.61	37.02	8.56	0.75	8.73	6.16	0.42	6.89	3.70	0.26	7.00	1.20	0.11	9.35
8Area	58.73	3.10	5.28	13.44	1.43	10.61	10.04	1.37	13.66	5.72	0.44	7.65	3.89	0.29	7.57	1.25	0.09	7.37

Table 6.3 The average data from 8 areas in Gangwon-do(*S*: on the surface, *D*: from a depth of 30 cm) with a spectrophotometer and EA-IRMS

Data label	SpectraMagic NX									EA-IRMS					
	L*(D65)			a*(D65)			b*(D65)			$\delta^{13}C$(‰)			C amount(%)		
	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD
1-1-S	48.64	0.00	0.00	6.63	0.00	0.00	19.87	0.01	0.03	−25.85	0.21	−0.81	0.38	0.01	2.84
1-2-S	50.02	0.00	0.00	6.90	0.01	0.08	20.09	0.01	0.05	−26.23	0.20	−0.76	0.31	0.01	2.56
1-3-S	48.71	0.01	0.01	6.23	0.00	0.00	19.52	0.00	0.00	−26.56	0.05	−0.18	0.65	0.01	1.31
1-4-S	48.91	0.01	0.01	6.55	0.00	0.00	19.92	0.01	0.05	−25.58	0.05	−0.18	0.69	0.02	3.29
1-5-S	48.54	0.40	0.81	6.00	0.03	0.57	19.24	0.12	0.62	−25.89	0.20	−0.76	0.63	0.04	6.03
1-1-D	51.37	0.02	0.04	6.85	0.01	0.08	20.31	0.03	0.14	−25.06	0.42	−1.68	0.19	0.01	3.92
1-2-D	50.03	0.11	0.23	6.61	0.06	0.92	19.18	0.12	0.65	−26.25	0.23	−0.89	0.25	0.01	3.30
1-3-D	49.91	0.38	0.75	6.20	0.02	0.36	19.11	0.12	0.63	−26.59	0.19	−0.73	0.44	0.04	8.88
1-4-D	46.03	0.12	0.26	6.48	0.01	0.15	18.75	0.07	0.36	−25.57	0.30	−1.18	0.24	0.01	2.59
1-5-D	47.14	0.25	0.54	6.46	0.04	0.62	18.95	0.02	0.08	−25.37	0.25	−0.99	0.14	0.01	9.93
1Area	48.93	1.54	3.14	6.49	0.28	4.36	19.49	0.53	2.71	−25.89	0.51	1.98	0.39	0.20	51.44
2-1-S	45.13	0.14	0.31	4.83	0.02	0.31	16.27	0.05	0.33	−23.82	0.37	−1.57	3.21	0.03	0.92
2-2-S	50.51	0.13	0.26	5.93	0.02	0.39	19.59	0.07	0.33	−21.33	0.12	−0.56	1.12	0.01	0.66
2-3-S	44.31	0.08	0.18	4.72	0.01	0.21	15.94	0.00	0.00	−23.54	0.14	−0.60	3.63	0.09	2.43
2-4-S	47.39	0.11	0.23	4.99	0.03	0.50	17.33	0.03	0.15	−24.07	0.04	−0.16	2.86	0.05	1.88
2-5-S	50.30	0.21	0.41	5.53	0.02	0.38	19.02	0.05	0.26	−22.18	0.53	−2.37	1.87	0.02	1.32
2-1-D	49.88	0.23	0.47	6.42	0.01	0.18	20.85	0.06	0.29	−23.04	0.26	−1.13	0.74	0.04	5.18
2-2-D	50.31	0.21	0.41	5.74	0.05	0.80	19.50	0.15	0.76	−21.42	0.21	−1.00	0.48	0.01	3.10
2-3-D	50.22	0.04	0.07	5.74	0.01	0.10	19.26	0.01	0.06	−22.89	0.09	−0.39	1.28	0.01	0.81
2-4-D	50.50	0.09	0.17	5.39	0.04	0.65	19.01	0.06	0.32	−23.97	0.02	−0.09	1.40	0.02	1.54
2-5-D	54.01	0.05	0.10	5.75	0.04	0.61	20.01	0.02	0.09	−22.53	0.11	−0.51	0.55	0.00	0.31
2Area	49.25	2.87	5.83	5.50	0.53	9.63	18.68	1.62	8.69	−22.88	1.01	4.40	1.71	1.14	66.54

(continued)

Table 6.3 (continued)

Data label	SpectraMagic NX									EA-IRMS					
	L*(D65)			a*(D65)			b*(D65)			$\delta^{13}C$(‰)			C amount(%)		
	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD
3-1-S	47.89	0.17	0.34	4.74	0.03	0.63	16.01	0.08	0.50	−25.93	0.07	−0.27	3.27	0.01	0.25
3-2-S	49.06	0.00	0.00	4.64	0.00	0.00	15.91	0.00	0.00	−25.74	0.10	−0.37	2.73	0.08	2.98
3-3-S	49.62	0.01	0.01	4.41	0.01	0.13	15.44	0.01	0.06	−25.63	0.08	−0.33	2.11	0.06	2.81
3-4-S	48.45	0.09	0.19	4.53	0.04	0.89	15.72	0.09	0.57	−25.28	0.43	−1.70	2.17	0.08	3.52
3-5-S	50.12	0.15	0.29	4.45	0.01	0.13	15.45	0.03	0.19	−25.64	0.02	−0.09	2.15	0.04	2.07
3-1-D	49.08	0.03	0.06	4.61	0.04	0.76	16.22	0.06	0.36	−25.28	0.19	−0.75	1.44	0.02	1.72
3-2-D	49.76	0.01	0.01	4.78	0.01	0.12	16.76	0.01	0.03	−24.99	0.07	−0.27	1.10	0.02	2.00
3-3-D	50.58	0.16	0.32	4.86	0.05	1.04	16.86	0.07	0.44	−25.14	0.07	−0.30	0.99	0.03	2.98
3-4-D	49.49	0.21	0.43	4.60	0.01	0.13	16.35	0.07	0.44	−25.21	0.04	−0.15	1.51	0.03	1.66
3-5-D	48.31	0.05	0.10	4.55	0.02	0.34	16.11	0.02	0.09	−25.16	0.06	−0.22	1.67	0.06	3.38
3Area	49.23	0.84	1.71	4.62	0.14	3.11	16.08	0.49	3.02	−25.40	0.31	1.22	1.91	0.72	37.44
4-1-S	48.70	0.55	1.13	6.65	0.04	0.61	19.67	0.23	1.19	−25.40	0.34	−1.35	3.20	0.13	4.10
4-2-S	49.04	0.05	0.10	7.53	0.06	0.74	21.12	0.20	0.92	−24.35	0.08	−0.33	1.60	0.04	2.33
4-3-S	47.11	0.43	0.90	4.62	0.13	2.71	15.05	0.42	2.80	−21.01	0.11	−0.53	3.82	0.25	6.65
4-4-S	49.18	0.05	0.10	7.52	0.02	0.31	21.16	0.04	0.17	−24.96	0.51	−2.02	1.31	0.03	2.49
4-5-S	47.51	0.15	0.32	7.63	0.07	0.86	20.87	0.10	0.46	−27.45	0.14	−0.52	1.80	0.08	4.57
4-1-D	51.01	0.27	0.53	6.72	0.11	1.67	19.99	0.16	0.78	−20.63	0.06	−0.31	1.26	0.06	5.09
4-2-D	49.88	0.03	0.06	5.80	0.01	0.20	18.36	0.04	0.19	−21.42	0.08	−0.37	1.83	0.04	2.34
4-3-D	51.45	0.01	0.01	5.32	0.01	0.11	17.45	0.01	0.03	−20.39	0.05	−0.23	2.08	0.13	6.01
4-4-D	51.80	0.08	0.16	6.46	0.03	0.41	20.16	0.06	0.29	−21.08	0.08	−0.40	1.34	0.06	4.83
4-5-D	54.51	0.29	0.53	8.61	0.06	0.64	23.45	0.14	0.60	−26.54	0.12	−0.47	0.56	0.03	5.45
4Area	50.02	2.23	4.45	6.68	1.20	17.98	19.73	2.32	11.74	−23.32	2.69	11.54	1.88	0.97	51.37
5-1-S	46.45	0.01	0.01	4.67	0.01	0.21	14.73	0.01	0.04	−24.69	0.10	−0.41	2.71	0.05	1.85

5-2-S	47.55	0.11	0.23	4.74	0.05	1.12	15.00	0.13	0.85	−25.15	0.06	−0.23	2.46	0.04	1.47
5-3-S	46.74	0.00	0.00	4.78	0.01	0.12	15.04	0.01	0.04	−25.52	0.04	−0.17	2.48	0.05	2.19
5-4-S	45.97	0.45	0.98	4.61	0.04	0.88	14.48	0.11	0.79	−24.24	0.07	−0.30	2.70	0.06	2.05
5-5-S	47.20	0.05	0.11	4.88	0.01	0.24	15.43	0.01	0.06	−25.23	0.07	−0.27	2.69	0.10	3.72
5-1-D	47.11	0.00	0.00	4.93	0.01	0.12	15.14	0.01	0.04	−25.38	0.06	−0.24	2.51	0.06	2.26
5-2-D	48.16	0.27	0.56	4.98	0.07	1.45	15.72	0.15	0.97	−25.32	0.03	−0.12	1.99	0.07	3.49
5-3-D	48.75	0.01	0.01	4.99	0.01	0.12	15.64	0.01	0.07	−25.42	0.13	−0.52	1.92	0.07	3.67
5-4-D	47.22	0.07	0.15	4.75	0.03	0.53	14.85	0.03	0.17	−25.28	0.06	−0.23	2.51	0.04	1.41
5-5-D	47.37	0.00	0.00	4.83	0.01	0.12	14.88	0.01	0.04	−25.46	0.06	−0.24	2.66	0.07	2.54
5Area	47.25	0.80	1.69	4.82	0.13	2.67	15.09	0.40	2.65	−25.17	0.40	1.59	2.46	0.28	11.55
6-1-S	48.05	0.01	0.01	5.41	0.01	0.11	17.16	0.01	0.03	−25.07	0.05	−0.21	2.21	0.04	1.90
6-2-S	49.18	0.07	0.13	5.24	0.02	0.29	17.34	0.02	0.09	−25.11	0.08	−0.31	2.18	0.09	4.35
6-3-S	46.93	0.38	0.80	5.30	0.05	0.93	17.08	0.14	0.83	−24.78	0.34	−1.39	2.66	0.06	2.32
6-4-S	47.78	0.22	0.45	5.14	0.02	0.34	17.08	0.04	0.24	−24.87	0.34	−1.36	1.77	0.05	2.98
6-5-S	48.22	0.01	0.01	5.85	0.00	0.00	17.66	0.01	0.03	−24.04	0.04	−0.16	1.72	0.01	0.44
6-1-D	50.42	0.00	0.00	6.88	0.01	0.08	19.67	0.01	0.03	−22.66	0.16	−0.69	0.92	0.04	4.22
6-2-D	49.50	0.13	0.26	6.80	0.03	0.44	19.62	0.06	0.28	−23.41	0.38	−1.62	1.15	0.02	1.44
6-3-D	51.17	0.00	0.00	7.10	0.01	0.08	20.60	0.01	0.03	−23.05	0.19	−0.81	0.96	0.02	1.99
6-4-D	49.63	0.06	0.11	6.22	0.02	0.33	18.98	0.04	0.19	−24.07	0.05	−0.21	1.33	0.04	3.37
6-5-D	49.54	0.06	0.11	7.01	0.03	0.46	19.68	0.04	0.19	−23.01	0.05	−0.20	0.93	0.06	6.27
6Area	49.04	1.29	2.63	6.10	0.80	13.15	18.49	1.36	7.34	−24.01	0.93	3.86	1.58	0.62	39.26
7-1-S	53.99	0.22	0.40	3.87	0.13	3.27	15.26	0.08	0.49	−24.91	0.16	−0.63	0.76	0.01	1.77
7-2-S	54.24	0.30	0.56	3.98	0.06	1.45	15.52	0.04	0.28	−25.12	0.31	−1.25	0.95	0.08	8.39

(continued)

Table 6.3 (continued)

Data label	SpectraMagic NX									EA-IRMS					
	L*(D65)			a*(D65)			b*(D65)			$\delta^{13}C$(‰)			C amount(%)		
	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD	Ave	Std	RSD
7-3-S	54.00	0.08	0.14	3.94	0.07	1.69	15.54	0.20	1.31	−24.92	0.11	−0.43	0.74	0.04	5.36
7-4-S	53.25	0.03	0.05	4.06	0.09	2.29	15.71	0.16	1.01	−25.24	0.12	−0.47	1.13	0.05	4.68
7-5-S	53.07	0.13	0.24	4.01	0.03	0.66	15.61	0.02	0.11	−25.04	0.07	−0.28	0.75	0.05	6.39
7-1-D	54.16	0.32	0.58	4.03	0.11	2.74	15.88	0.22	1.36	−25.76	0.19	−0.76	0.84	0.06	7.26
7-2-D	55.21	0.87	1.58	4.15	0.03	0.74	16.00	0.10	0.63	−25.81	0.24	−0.94	0.62	0.03	5.22
7-3-D	48.37	0.42	0.86	4.42	0.01	0.23	16.36	0.05	0.32	−25.78	0.08	−0.31	1.22	0.11	8.68
7-4-D	51.77	0.00	0.00	4.49	0.01	0.22	16.54	0.01	0.03	−25.71	0.07	−0.29	1.36	0.10	7.04
7-5-D	51.38	0.01	0.01	4.17	0.00	0.00	16.46	0.00	0.00	−25.96	0.14	−0.53	1.61	0.14	8.76
7Area	52.94	1.98	3.74	4.11	0.20	4.93	15.89	0.44	2.78	−25.43	0.41	1.63	1.00	0.32	32.15
8-1-S	50.14	0.10	0.20	4.60	0.03	0.55	16.98	0.05	0.28	−25.76	0.45	−1.75	1.04	0.02	2.13
8-2-S	50.84	0.14	0.28	4.37	0.03	0.61	16.34	0.05	0.28	−25.30	0.16	−0.65	1.17	0.07	5.92
8-3-S	51.12	0.12	0.24	4.38	0.02	0.35	16.04	0.08	0.51	−24.73	0.16	−0.64	0.68	0.02	3.44
8-4-S	51.21	0.28	0.54	4.57	0.04	0.88	16.46	0.05	0.28	−24.95	0.16	−0.66	0.76	0.02	2.49
8-5-S	52.44	0.27	0.51	4.56	0.05	1.05	16.47	0.06	0.35	−24.72	0.05	−0.21	0.70	0.02	3.14
8-1-D	49.18	0.00	0.00	4.92	0.01	0.12	17.00	0.00	0.00	−26.11	0.07	−0.28	1.67	0.03	1.84
8-2-D	49.51	0.00	0.00	4.73	0.01	0.12	16.98	0.01	0.03	−25.63	0.17	−0.66	1.18	0.06	5.04
8-3-D	48.69	0.00	0.00	4.53	0.01	0.13	16.65	0.01	0.03	−25.93	0.11	−0.41	1.25	0.01	0.89
8-4-D	49.60	0.21	0.41	4.93	0.03	0.59	17.04	0.09	0.51	−25.73	0.08	−0.31	1.09	0.03	2.65
8-5-D	49.85	0.19	0.37	4.50	0.03	0.71	16.38	0.14	0.83	−26.03	0.07	−0.25	1.35	0.01	1.08
8Area	50.26	1.13	2.25	4.61	0.20	4.24	16.63	0.35	2.09	−25.49	0.53	2.08	1.09	0.31	28.66

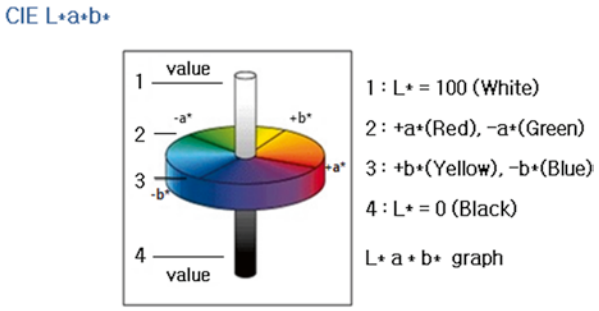


Fig. 6.3 CIELAB color space

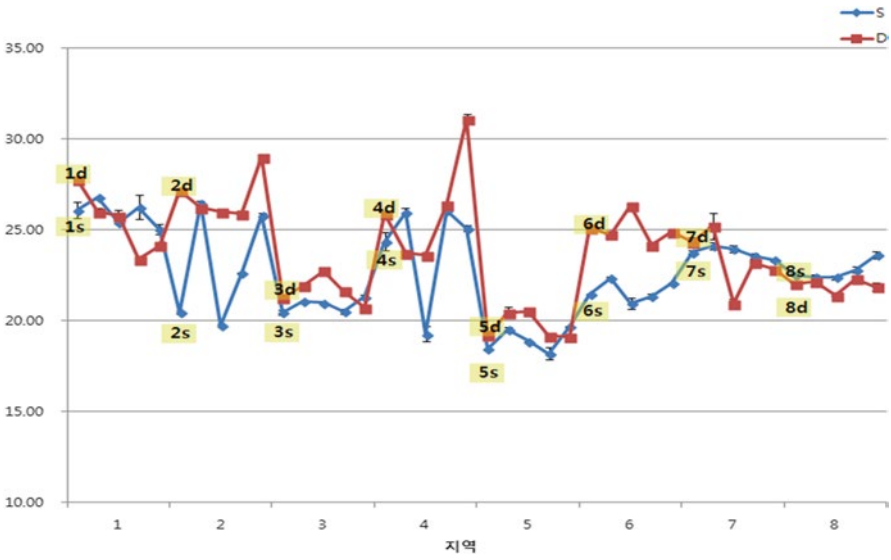


Fig. 6.4 Color differences between surface (*S*) and 30-cm depth (*D*)

While the color changes between the surface (S) and 30-cm depth (D) show similar patterns, as shown in Fig. 6.4, one area showed an S–D difference as large as 2.8 (ΔE). On the other hand, in some cases, similar color data could be obtained from different areas. For example, Area 1 and 2 are approximately 1 km apart from each other on either side of the river; however, 1-S, 1-D, and 2-D samples demonstrated strikingly similar color tones (Table 6.4). This implies that color tone should not be the sole factor for the identification of forensic soil samples.

As shown in Table 6.3, the areas which demonstrated the largest differences in color data between maximum and minimum values are Area 2 (L^*) and Area 4 (a^*, b^*): L^* (brightness) of Area 2 ranged from 44.31 to 54.01, and a^* and b^* of Area 4 ranged from 4.62 to 8.61 and from 15.05 to 23.45, respectively.

Table 6.4 Results of color measurements using spectrophotometer

		S		D				S		D	
Area	Label	Ave	Std.	Ave	Std.	Area	Label	Ave	Std.	Ave	Std.
1	1-1	26.09	0.44	27.75	0.01	5	5-1	18.43	0.01	19.19	0.01
	1-2	26.77	0.00	26.02	0.18		5-2	19.50	0.13	20.44	0.29
	1-3	25.39	0.00	25.79	0.32		5-3	18.87	0.01	20.52	0.01
	1-4	26.23	0.67	23.39	0.12		5-4	18.18	0.33	19.17	0.02
	1-5	25.02	0.28	24.13	0.12		5-5	19.67	0.03	19.10	0.01
2	2-1	20.47	0.08	27.15	0.18	6	6-1	21.44	0.01	25.12	0.01
	2-2	26.45	0.13	26.21	0.22		6-2	22.35	0.06	24.75	0.12
	2-3	19.75	0.04	25.98	0.03		6-3	20.95	0.31	26.32	0.00
	2-4	22.62	0.04	25.89	0.07		6-4	21.35	0.14	24.15	0.05
	2-5	25.80	0.10	29.00	0.05		6-5	22.06	0.01	24.89	0.07
3	3-1	20.46	0.15	21.28	0.05	7	7-1	23.76	0.11	24.33	0.24
	3-2	21.04	0.00	21.92	0.01		7-2	24.13	0.20	25.20	0.72
	3-3	20.97	0.01	22.74	0.04		7-3	23.96	0.19	20.93	0.28
	3-4	20.50	0.12	21.62	0.19		7-4	23.55	0.15	23.19	0.01
	3-5	21.31	0.12	20.72	0.04		7-5	23.35	0.07	22.81	0.00
4	4-1	24.36	0.48	25.87	0.07	8	8-1	22.50	0.10	22.02	0.01
	4-2	25.97	0.21	23.72	0.05		8-2	22.41	0.12	22.14	0.01
	4-3	19.26	0.42	23.60	0.01		8-3	22.38	0.11	21.37	0.00
	4-4	26.07	0.07	26.40	0.09		8-4	22.79	0.16	22.30	0.19
	4-5	25.08	0.17	31.08	0.29		8-5	23.61	0.20	21.85	0.23

Area 6 showed large deviations for a^* and b^* in both S and 30-cm D, whereas Area 1 showed the least deviation owing to a high degree of soil homogeneity.

The paired *t*-test on the results of color measurements confirmed that S and D samples showed significant differences in all L^*, a^*, and b^* ($p \leq 0.05$).

A canonical discriminant analysis was performed using the color attributes (L^*, a^*, and b^*) obtained from the above spectrophotometric analysis. Prior to the analysis, Box's M test was conducted to check the homogeneity of the multivariate normal covariance matrices of the three independent color variables (L^*, a^*, and b^*) for each group. Because the significance probability was estimated to be 0.000 indicating the lack of between-group homogeneity of covariance matrices, individual-group covariance matrices were utilized when applying the classification method.

The canonical discriminant analysis was performed using a stepwise method for selecting variables to be included in the analysis, where the significance of the variables entering in each step was tested using Wilks' lambda and exact F statistics. Therefore, all the variables were confirmed to have significance. The order of entrance was a^* (red direction) ⇨ b^* (yellow direction) ⇨ L^*.

Out of the two canonical discriminant functions that were obtained in the discriminant analysis, the eigenvalue of Function 1 was 4.345, accounting for 54.6 % of the total discriminating power. Thus, 100 % discriminating ability was achieved by adding the discriminating ability of Functions 2 and 3 to that of Function 1.

These discriminant functions were subjected to significance tests and proved their significance, their significance probability being 0.000 each with respect to Wilks' lambda values and Chi-square statistic.

When referenced to the standardized canonical-discriminant-function coefficients, discriminant functions 1, 2, and 3 are most closely related to a^*, b^*, and L^*, respectively.

In the case of unstandardized coefficients, the standardized canonical discriminant functions were used to calculate the discriminant scores of individual samples, thereby acquiring the group centroids by entering the average value of each group.

The three discriminant functions utilized are as follows:

$$D1 = 11.152 - 0.175L^* + 4.037a^* - 1.378b^*$$

$$D2 = -4.555 - 0.440L^* - 3.837a^* + 2.683b^*$$

$$D3 = -26.317 + 0.596L^* + 1.691a^* - 0.703b^*$$

The centroids thus obtained demonstrated that Function 1 was effective in discriminating Area 7 and 4 from the other areas, and Function 2 was effective in discriminating Area 5 from others.

While the discrimination performed using the canonical discriminant functions confirmed a perfectly accurate classification of Area 2, 5, 6, and 8, Area 1, 3, 4, and 7 showed 2, 2, 5, and 1 classification errors out of 10, respectively, resulting in an overall classification accuracy of 87.5 %.

Figure 6.5 is a scatter plot of discriminant scores calculated by using the canonical discriminant functions. The concentrated distribution of pink-colored plots in Area 8 demonstrates its relatively high classification accuracy. In particular, a clustering tendency is observed according to area-specific characteristics as follows: Area 3, 7, and 8 near the river; Area 1 and 2 near the industrial complex; Area 6 in the pasturage near factories; and Area 5 in the fishing zone. The broadest dispersion of discriminant scores is shown by Area 4, the roadsides near the residential areas, represented by violet color on the scatter plot.

Color factor b^* is related to yellow (Fig. 6.6). The color factor has a high degree of discriminating ability. Because soils of different colors are found even in an area as small as 1 m^2, samples should be taken from many different spots within the determined range, where due attention should be paid to different colors by a rough visual examination.

6.3.2 Elemental Analysis

The XRF analyses revealed the differences in soil elements from a sampling spot to another within the same area. The data are listed in Table 6.2.

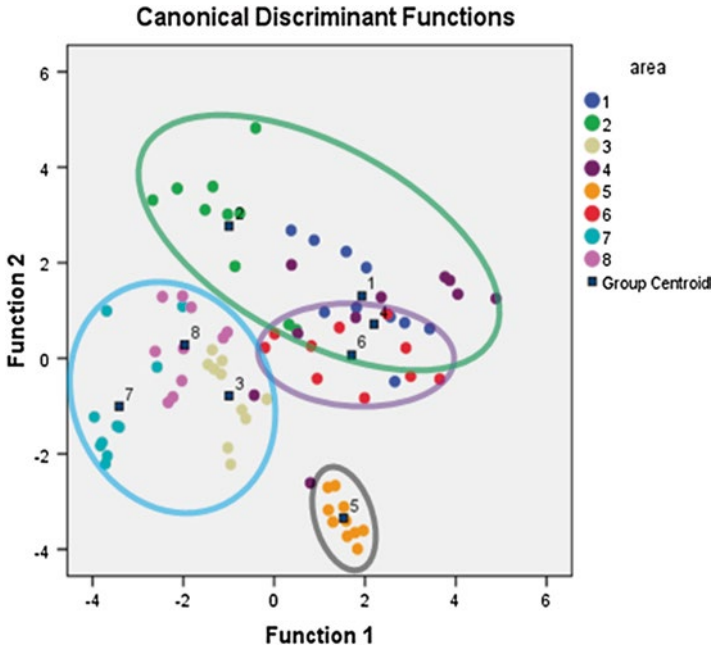


Fig. 6.5 Discriminant scores calculated with canonical discriminant functions

The smallest and largest differences in the distribution of elements for each sampling area were shown by SiO_2 in Area 4 with 53.15–65.54 %, Fe_2O_3 in Area 4 with 3.61–13.06 %, and Al_2O_3 in Area 7 with 6.94–14.85 %, respectively.

Significant S–D differences in element distribution were also confirmed among the surface and 30-cm depth samples within 1-m^2 range of the same area. The comparisons of the S–D data indicated that Area 8 contained 55.8 % and 61.6 % SiO_2 on average in its surface and depth soils, respectively, and 11.3 % and 8.7 % Fe_2O_3 in its surface and depth soils (Fig. 6.7).

The results of the paired *t*-test performed on the soil elements of both S and D samples confirmed that the Al_2O_3 content exhibited a significant depth-dependent difference.

A canonical discriminant analysis was then performed using the values of SiO_2, Al_2O_3, Fe_2O_3, K_2O, CaO, and TiO_2 obtained from the elemental analyses.

Box's M test, which was conducted to test the homogeneity of the between-group covariance matrices of the six independent variables, namely, SiO_2, Al_2O_3, Fe_2O_3, K_2O, CaO, and TiO_2, gave a significance probability of 0.000. Since it signifies that there is no homogeneity of covariance matrices among the groups, the individual-group covariance matrices were utilized when applying the classification method.

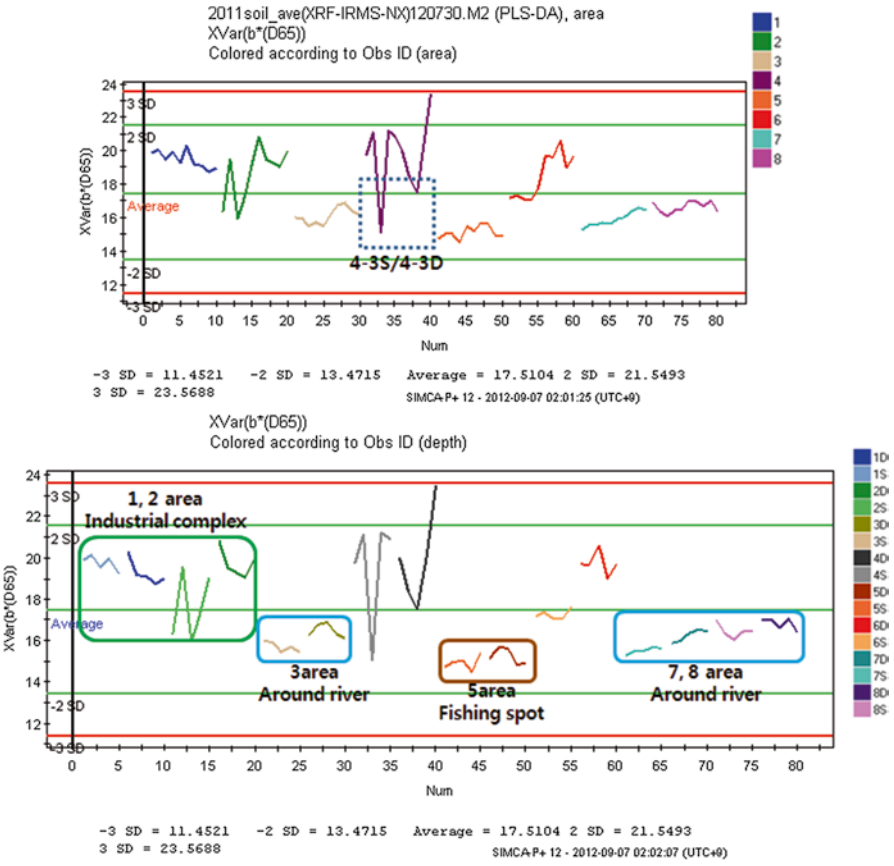


Fig. 6.6 Distribution diagram of color factor b* by sampling area

The canonical discriminant analysis was performed using a stepwise selection method, where the significance of the variables entering in each step was tested using Wilks' lambda and exact F statistics. According to the results of significance tests, CaO was entered first, followed by TiO_2 and Fe_2O. It is also shown that the variables entered had not been removed because of high values of F statistic.

Out of the two canonical discriminant functions that were obtained from the discriminant analysis, the eigenvalue of Function 1 is 9.915, thereby accounting for 71.3 % of the total discriminating power. Thus, 100 % discriminating power is possible by adding the discriminating power of Functions 2 and 3 to that of Function 1. The significance tests, which were performed on these discriminant functions, confirmed their significance probability as being 0.000 each with respect to Wilks' lambda values and Chi-square statistic.

The standardized canonical-discriminant-function coefficients show that the discriminant functions 1, 2, and 3 are most closely related to CaO, TiO_2, and Fe_2O_3, respectively.

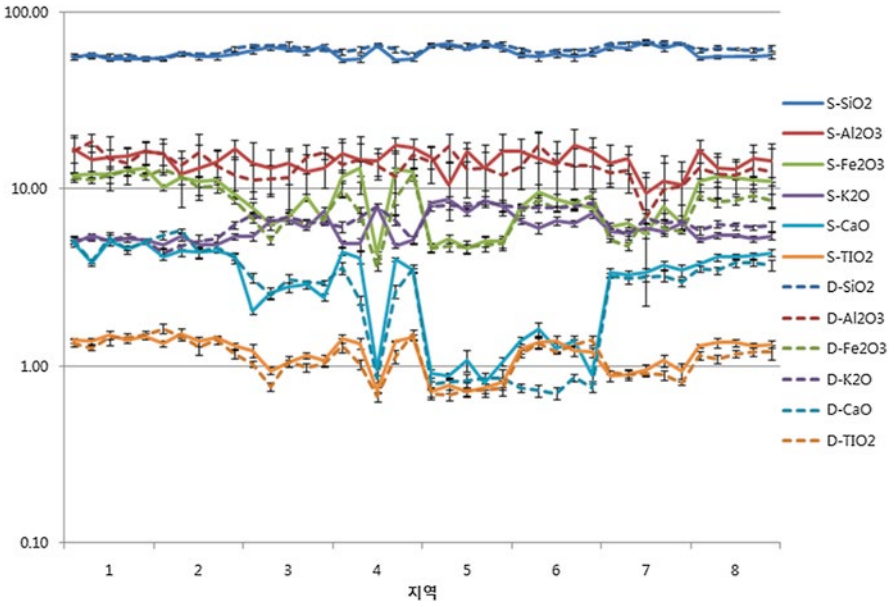


Fig. 6.7 Depth-dependent differences in soil element compositions (*s*: solid line, *d*: dash line)

The three discriminant functions utilized are as follows:

$$\mathrm{D1} = 0.355 - 0.158\,\mathrm{Fe_2O_3} + 2.813\,\mathrm{CaO} - 6.502\,\mathrm{TiO_2}$$

$$\mathrm{D2} = -9.396 - 0.367\,\mathrm{Fe_2O_3} - 0.141\,\mathrm{CaO} + 11.251\,\mathrm{TiO_2}$$

$$\mathrm{D3} = 3.165 + 1.718\,\mathrm{Fe_2O_3} - 0.743\,\mathrm{CaO} - 13.579\,\mathrm{TiO_2}.$$

According to the centroid values, Function 1 was effective in discriminating Area 6 from other areas. Function 1 is easily influenced by CaO, and soils in Area 6, the pasture grounds near a factory, have relatively low CaO values compared to the other areas. The TiO_2-sensitive Function 2 is effective in discriminating Area 5 from the other areas because Area 5 is the fishing areas located near the dam that has soils with a low TiO_2 content.

The discrimination tests performed using the canonical discriminant functions confirmed a perfect classification of Area 5, 6, 7, and 8; however, revealed 2, 2, 1, 4 classification errors in Area 1, 2, 3, and 4, respectively, yielding an overall classification accuracy of 88.8 %.

Figure 6.8 shows a scatter plot of the discriminant scores of the eight areas calculated by using the canonical discriminant functions. The distributions patterns

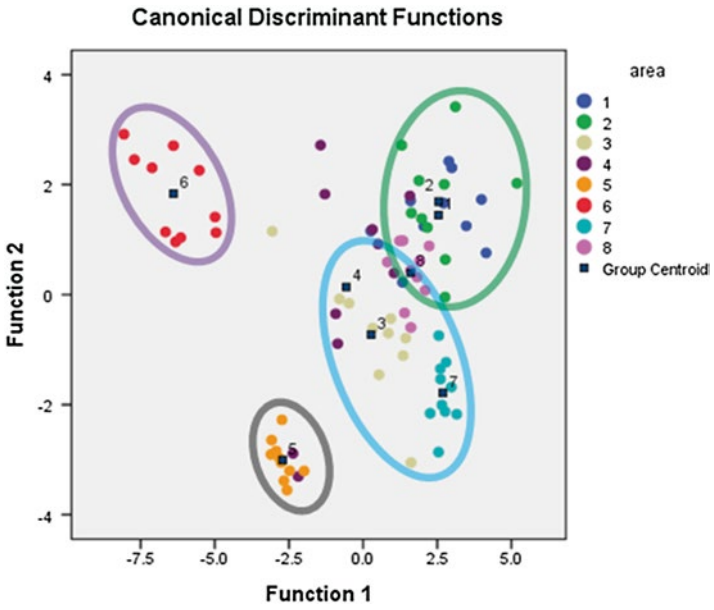


Fig. 6.8 A scatter plot illustrating the distribution of soil elements in the eight sampling plots

show a clear area-to-area discrimination. As shown in the results of color test, a clustering tendency is observed according to area-specific characteristics: Area 3, 7, and 8 near the river; Area 1 and 2 near the industrial complex, Area 6 near the pasturage of the factories, and Area 5 near the fishing zone of the dam.

The Fe_2O_3 content was found to be low in water areas. TiO_2 was found to be abundant in Area 1, 2, and 6. The SiO_2 content was found to be higher in Area 3, 5, 7, and 8-D than in other areas. It is noteworthy that the areas 1, 2, and 8-S showed similar distributions (Fig. 6.9). This indicates that the depth of soil influences the soil characteristics even within the same area.

In the SIMCA analysis according to depth (Fig. 6.10), the loading plot quadrants are divided between 8S (3rd quadrant) and 8D (4th quadrant). Either side of the y-axis is occupied by the manufacturing and industrial complex (1st and 4th quadrants) and the water areas (2nd and 3rd quadrants). Moreover, y-axis is a dividing line for the areas influenced by SiO_2, Fe_2O_3, and TiO_2 contents.

Water areas are typically formed with alluviums and considered to have high SiO_2 content. (Alluviums are unsolidified sediments shaped by the accumulation of soils consisting of gravel, sand, clay, etc. through recent river activities. Sand has mainly rock-forming mineral particles containing SiO_2 or Si; however, calcareous sands are also formed from limestones.)

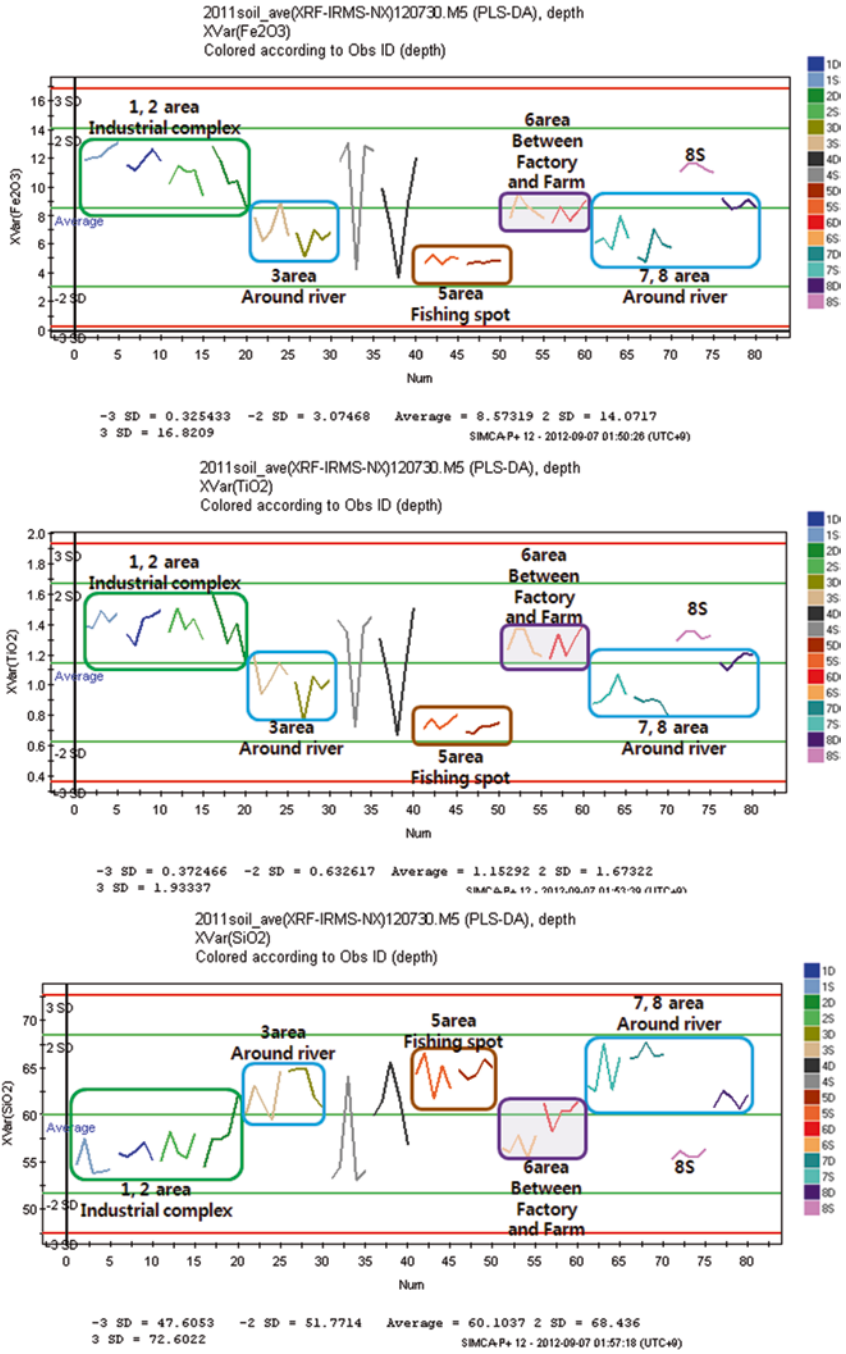


Fig. 6.9 Distribution diagrams of XRF measurement values by sampling area

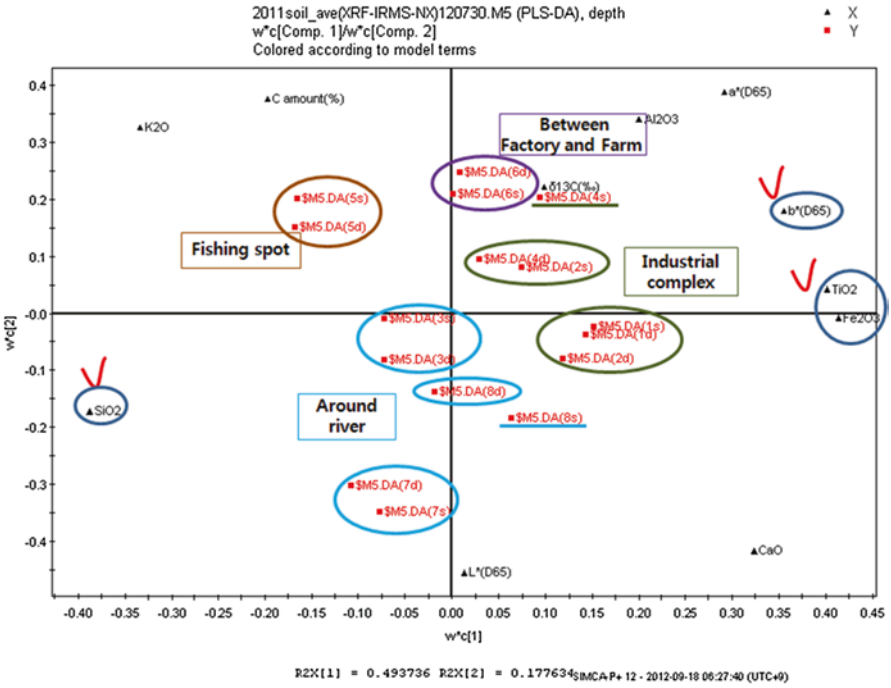


Fig. 6.10 Results of the SIMCA analysis

6.3.3 Carbon Isotope Ratio and Carbon Content

Figure 6.11 shows the carbon content and carbon-isotope ratio. A rough contrast between the river and industrial areas is observed. This contrast in carbon-isotope ratio distribution is also shown in the ANOVA. Group A is the water area consisting of Area 3, 5, 7, and 8. Consequently, the soils in the water area showed enrichment in isotope ratio compared to those in the industrial area.

The paired *t*-test on the results of carbon content and carbon-isotope ratio measurements of the S and D samples confirmed that the carbon content showed significant depth-dependent differences (0.000), whereas the carbon isotope ratio (0.64) was not influenced.

The results of depth analysis show that the carbon-isotope ratios were enriched in the D samples compared to the S samples in Area 1, 2, 3, 4, and 6, and the opposite was true for Area 5, 7, and 8.

The carbon content was higher in the S samples of Area 1–6, and in the D samples of Area 7 and 8. This contradictory result for Area 7 and 8 compared to all the other areas may be attributed to the influence of water flowing right next to the areas.

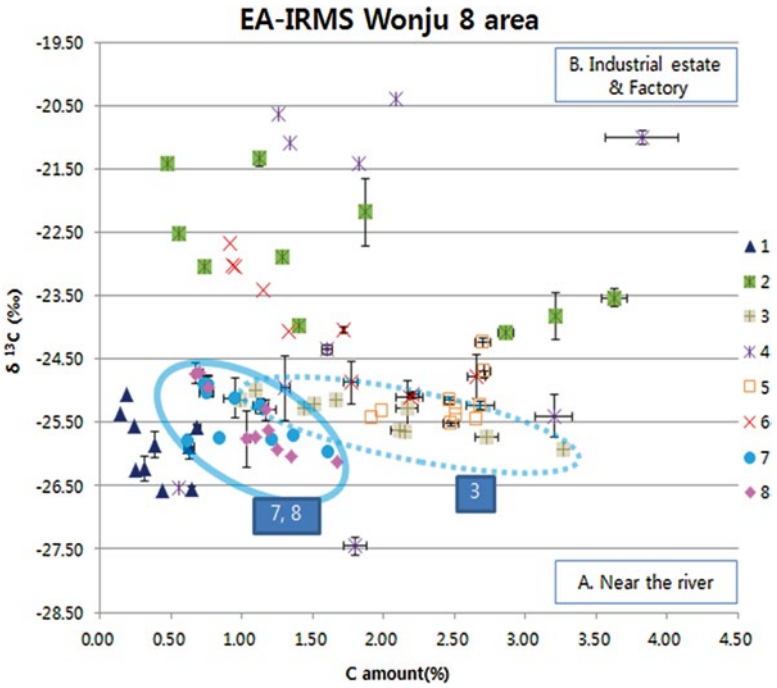


Fig. 6.11 Carbon amount (%) and carbon isotope ratio

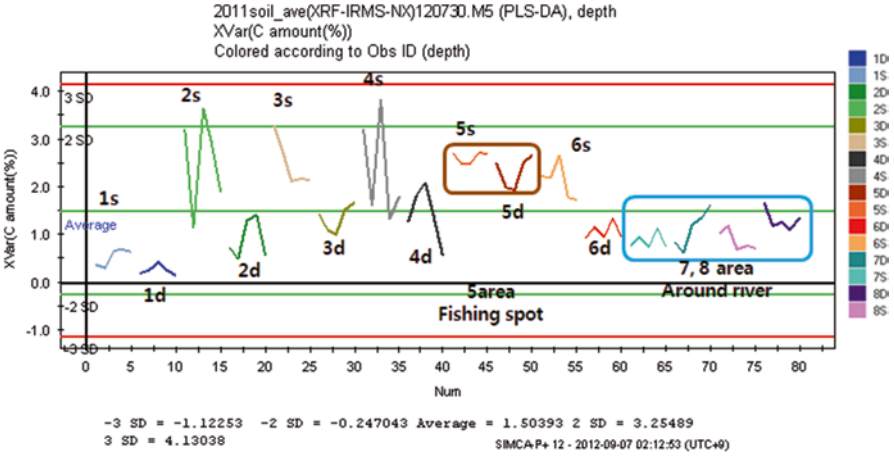


Fig. 6.12 Variable line plot of carbon amount (%) by area and depth

Figure 6.12 shows the carbon amount (%) in variable line plot that gives the measurement values of analysis factors grouped by areas (1–8) and depths (S and D) in different colors.

Because not only area-to-area, but also surface-to-depth differences were observed in all the measurements, therefore, when collecting comparative samples, due care should be taken to prevent the mixing of adjacent soils from the depths other than the expected depth.

6.3.4 Total Statistics

Principal Component Analysis (PCA)

PCA algorithm is a 2nd-order statistics that utilizes the statistical characteristics of mean values and sampling distributions. It searches for a series of orthonormal subsets indicating each direction of the maximum covariance for the input data. PCA is a standard tool for dimension reduction of correlated multivariate data while keeping the highest possible number of variables. This enables to create a new combination of variables, namely, a set of principal components (PC) converted from a set of variables. The PCs are linearly uncorrelated, and the first several ones are defined to carry out most of the changes that occurred in the original variables. PCA aims to extract and interpret a small number of PCs independent of one another by carrying out an adequate linear transformation of correlated variables in the cases where a direct interpretation of correlations among a number of images is difficult. The coordinates of PCs obtained from dimension reduction serve as input data for statistical analyses, thus playing an intermediate role in a series of analysis process (Park et al. 2011). Figure 6.13 shows the results of PCA-X statistics.

Each area is differentiated well according to its characteristics. In order to determine the area-specific determinant factors more accurately, a partial least squares-discriminant analysis (PLS-DA) was performed.

PLS-DA of Soils

The PLS-DA maximizes the covariance between the predicted data set (X block: unlimited number of soil factors) and the data to be predicted (Y block: class assignment). The fractions of the Y variables designed by the selected component and those predictable by the component determined by the cross validation were plotted, and the PLS-DA model thus constructed was validated. The predictions shown on the PLS-DA scatter plot are selected according to the significance rule specified by SIMCA-P software, where Q^2 of a significant component should be >0.05 for 100 observations or less and zero for more than 100 observations. Moreover, PLS-DA plots are displayed by the superposition of the two highest latent variables (t[1]/t[2] or p[1]/p[2] as x- and y-axes). The high coefficient values of R^2Y and Q^2Y indicate good discriminating power.

The soil discriminant factors can be obtained by observing the clustering of the input factors on a score plot using the PLS-DA multivariate analysis, thereby

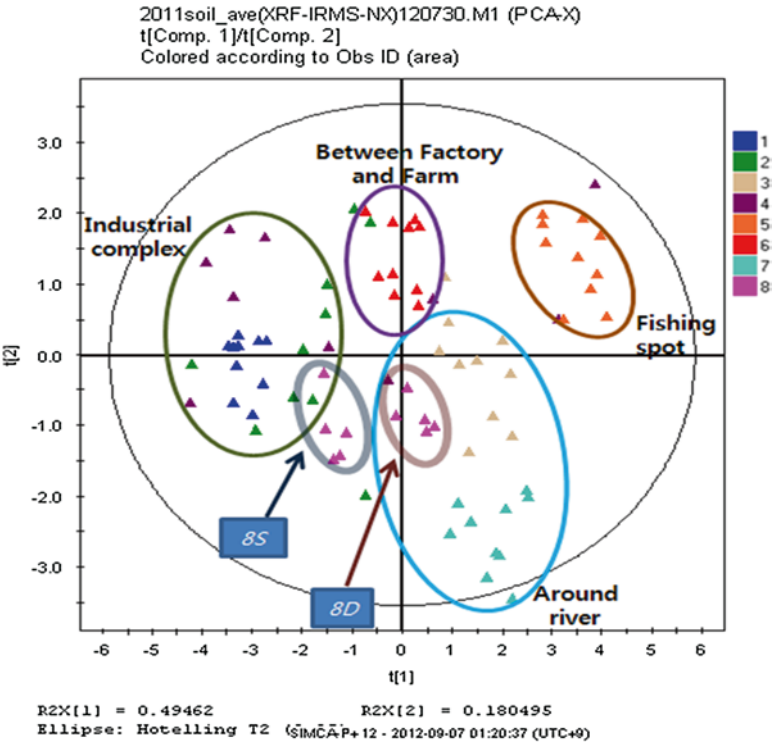


Fig. 6.13 Results of PCA-X statistics(*S*: on the surface, *D*: from a depth of 30 cm)

investigating the factors that are likely to be the indicators displayed on the loading plot, and finally having their significances confirmed using variable importance in the projection (VIP) (Kim 2007).

The degree of between-group discriminating accuracy can be assessed by comparing the spots within the 95 %-level confidence ellipses on the score plot. The results of the analyses of the soil samples of the eight areas in Wonju (Gangwondo, South Korea) were plotted on a PLS-DA score plot (Fig. 6.14), and the clustering patterns were observed. Therefore, they were classified into four groups according to the area-specific characteristics, whereby Area 8 was divided into two different groups because of the S–D differences.

After the results of the score plot were analyzed, the loading plot was then examined to identify the determinant factors by quadrant that displayed the distribution of each area.

Based on the results obtained through the loading plot (Fig. 6.15), significant factors whose average VIP score is ≥1 were identified using the VIP method. The VIP scores reflect the degree of importance with respect to X and Y variables. The average of squared VIP scores is equal to 1 when the VIP scores are equalized. The VIP plot displays the order of importance representing an intergroup priority ranking when the groups are classified. In the samples collected from Wonju

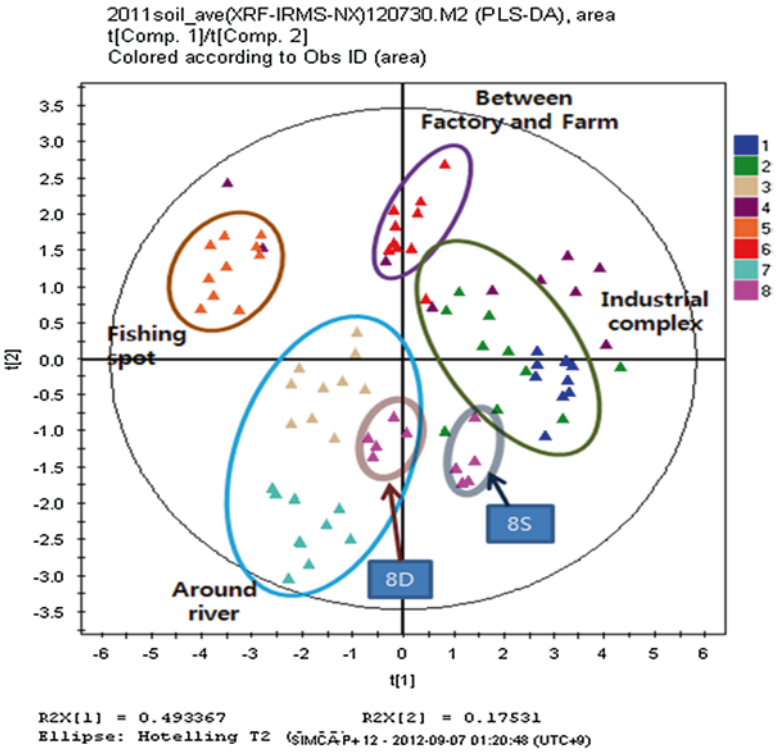


Fig. 6.14 PLS-DA score plot(*S*: on the surface, *D*: from a depth of 30 cm)

(Gangwondo, South Korea), the factors whose average VIP score was ≥1 were identified as SiO_2, Fe_2O_3, TiO_2, and a* color factor (see Fig. 6.16 and Table 6.5).

In the factors whose average VIP score is ≥1, the top four factors are shown on the loading plot (Fig. 6.10). As shown on the loading plot, they are distributed along the far ends of the quadrants divided by the y axis.

Comparison of the Statistical Analysis Results: SPSS Multivariate Analysis vs. Simca PLS-DA

A stepwise selection method was used in the SPSS discriminant analysis, where the significance tests using Wilks' lambda and exact F statistics on the variables entering in each step confirmed the entering order of CaO and TiO_2. In the SIMCA loading plot, K_2O and CaO are on extreme edge of the 2nd and 4th quadrant, respectively.

According to standardized canonical-discriminant-function coefficients that furnish the explanatory power of discriminant functions, Function 1 exhibited a high explanatory power for the components SiO_2 (Fe_2O_3, TiO_2), and discriminant Function 2 exhibited a high explanatory power for color factors B* ($\delta^{13}C$, carbon content).

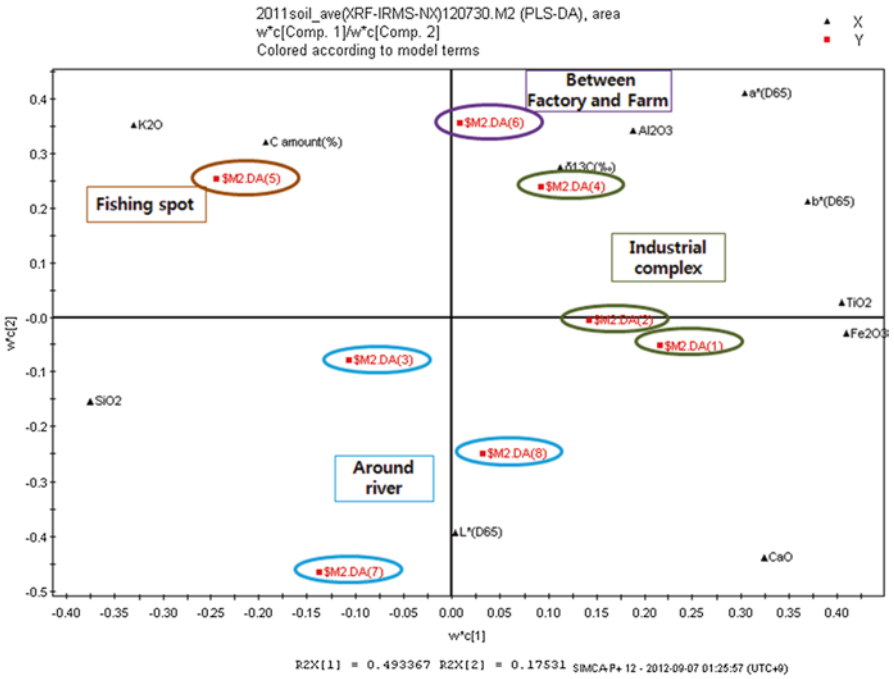


Fig. 6.15 Loading plot

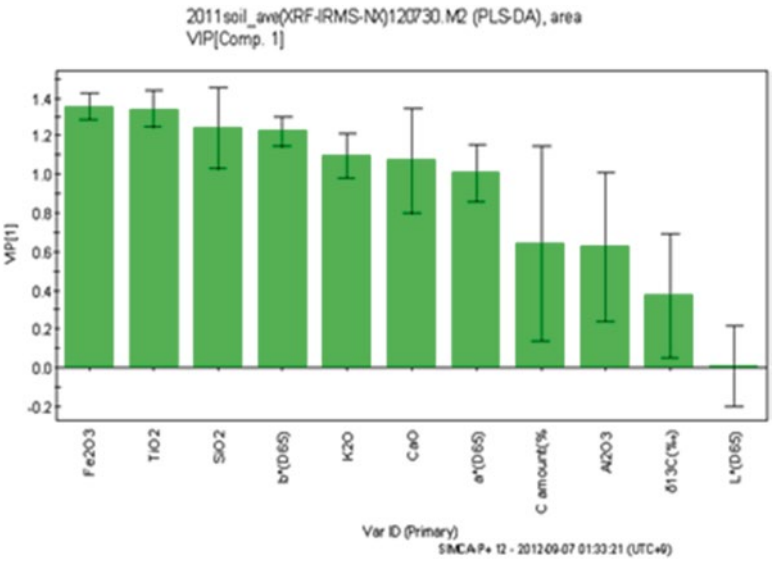


Fig. 6.16 VIP plot

Table 6.5 Determinant factors (VIP ≥1)

Var ID (Primary)	M2.VIP[1]	1.89456 * M2.VIP[1] cvSE
Fe2O3	1.35649	0.0661687
TiO2	1.34238	0.100236
SiO2	1.24434	0.213469
b*(D65)	1.22531	0.0779343
K2O	1.09671	0.11571
CaO	1.0738	0.273555
a*(D65)	1.00653	0.147745
C amount (%)	0.63997	0.505365
Al2O3	0.624347	0.382421
δ13C(‰)	0.373935	0.319269
L*(D65)	0.00906133	0.205676

$\delta^{13}C$ and carbon content can be considered as the factors with a high explanatory power whereas their SIMCA VIP scores are lower than 1. From this result, it may be inferred that these factors are dependent more on depth rather than area.

Discriminant Analysis

A discriminant analysis that was performed using all the data obtained (color, XRF, IRMS) confirmed 100 % discriminating power.

In Box's M test, which was conducted to test the homogeneity of the between-group covariance matrices of 11 independent variables, homogeneity in between-group covariance matrices was not observed, individual-group covariance matrices were utilized when applying the classification method.

The discriminant analysis was performed using a stepwise method for selecting variables, where the significance of the variables entering in each step was tested using Wilks' lambda and exact F statistics. The results of the significance tests are listed in Table 6.6. The order of entrance was CaO and TiO_2.

Canonical correlation (CANCOR) reflects the degree of association between a discriminant function and a set of variables. The closer it is to 1, the higher is the discriminating power of the function. The correlation coefficients of Functions 1 and 2 obtained in this study are 0.975 and 0.961, respectively, which can be considered as excellent results. Moreover, a higher eigenvalue signifies a proportionally higher discriminating ability of a function. A function is then considered to have an excellent discriminating power.

Out of the two canonical discriminant functions obtained in the discriminant analysis in this study (Table 6.7), the eigenvalue of Function 1 was 19.625, which accounted for 49.1 % of the total discriminating power, and the discriminating power acquired by adding functions 2–7 to that of Function 1 was 100 %.

Table 6.6 Results of the significance tests using Wilk's lambda statistics and exact F statistics

		Wilks' Lambda											
						Exact F				Approximate F			
Step	Entered	Statistic	df1	df2	df3	Statistic	df1	df2	Sig.	Statistic	df1	df2	Sig.
1	CaO	.134	1	7	72.00	66.300	7.00	72.00	.00				
2	TiO_2	.024	2	7	72.00	55.512	14.00	142.00	.00				
3	a*(D65)	.008	3	7	72.00					40.99	21.00	201.55	.00
4	b*(D65)	.003	4	7	72.00					34.71	28.00	250.21	.00
5	C amount (%)	.001	5	7	72.00					32.94	35.00	288.48	.00
6	$\delta^{13}C$	.000	6	7	72.00					31.94	42.00	317.71	.00
7	L*(D65)	.000	7	7	72.00					27.99	49.00	339.49	.00
8	Fe_2O_3	.000	8	7	72.00					24.96	56.00	355.35	.00
9	SiO_2	.000	9	7	72.00					23.27	63.00	366.56	.00
10	K_2O	.000	10	7	72.00					22.06	70.00	374.17	.00

At each step, the variable that minimizes the overall Wilks' Lambda is entered

[a]Maximum number of steps is 22

[b]Minimum partial F to enter is 3.84

[c]Maximum partial F to remove is 2.71

[d]F level, tolerance, or VIN insufficient for further computation

Table 6.7 Canonical correlation (CANCOR) of the discriminant functions

Function	Eigenvalue	% of variance	Cumulative %	CANCOR
1	19.625[a]	49.1	49.1	.975
2	12.045[a]	30.1	79.2	.961
3	4.481[a]	11.2	90.4	.904
4	2.674[a]	6.7	97.1	.853
5	.633[a]	1.6	98.7	.623
6	.484[a]	1.2	99.9	.571
7	.051[a]	.1	100.0	.220

[a]First seven canonical discriminant functions were used in the analysis

Table 6.8 Significance probability of discriminant functions

Test of function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 7	.000	667.218	70	.000
2 through 7	.001	455.361	54	.000
3 through 7	.020	275.572	40	.000
4 through 7	.107	156.485	28	.000
5 through 7	.393	65.387	18	.000
6 through 7	.642	31.069	10	.001
7	.952	3.460	4	.484

As shown in Table 6.8, the significance probability of both Wilks' lambda value and Chi-square statistic for these discriminant functions is 0.000, which proves their significance. The only function that cannot be considered significant is Function 7 (0.484). Because Wilks' lambda tests the average homogeneity between the groups, its null hypothesis is "H0: i.e., there is no difference in the average between the groups." As the Wilks' lambda value decreases, the explanatory power of the corresponding function increases. Moreover, the significance probability of $\chi 2 = 667.218$ being 0.000 (<0.005), the null hypothesis is discarded and the discriminant function proves to be significant.

A standardized canonical discriminant function coefficient furnishes the explanatory power of a discriminant function; Function 1 has a high explanatory power for elements SiO_2 (Fe_2O_3, TiO_2), and Function 2 for color factors B* ($\delta^{13}C$, carbon content); see Table 6.9.

Table 6.10 lists the unstandardized canonical discriminant function coefficients. The discriminant score of each individual item was obtained by multiplying the value of each independent variable by the coefficient and subsequently adding the whole. The calculation modules of discriminant functions 1 and 2 are as follows:

$$\begin{aligned} D1 = &-44.260 + 0.675\,SiO_2 + 0.893\,Fe_2O_3 - 1.303\,K_2O + 1.385\,CaO - 8.031\,TiO_2 \\ &- 0.452\delta^{13}C - 0.298\,C\,amount + 0.294\,L^* - 0.479\,a^*(D65) - 0.768\,b^*(D65) \end{aligned}$$

Table 6.9 The standardized canonical discriminant function coefficients of functions 1–7

	Function						
Variables	1	2	3	4	5	6	7
SiO_2	1.733	.382	.048	.964	−.469	.032	−.098
Fe_2O_3	1.360	−.194	.720	.194	.179	2.797	−.249
K_2O	−.848	−.709	.218	−.967	1.040	.375	.601
CaO	.783	.806	.918	−.713	.500	−.555	.347
TiO_2	−1.103	−.066	−1.822	.139	−.108	−1.335	.467
δ13C	−.508	1.067	.122	.292	.103	.078	−.736
Carbon conc. (%)	−.196	1.116	.862	1.008	.492	.357	.966
L*(D65)	.507	−.046	.476	.627	−.361	.764	.550
a*(D65)	−.271	−.210	2.688	−.515	−1.035	−.741	−.033
b*(D65)	−.894	1.284	−1.988	.758	.747	.303	.324

Table 6.10 The unstandardized canonical discriminant function coefficients of functions 1–7

	Function						
Variables	1	2	3	4	5	6	7
SiO_2	.675	.149	.019	.375	−.182	.013	−.038
Fe_2O_3	.893	−.128	.473	.128	.117	1.838	−.163
K_2O	−1.303	−1.090	.335	−1.487	1.598	.576	.923
CaO	1.385	1.426	1.624	−1.261	.883	−.982	.613
TiO_2	−8.031	−.481	−13.262	1.011	−.789	−9.719	3.398
δ13C	−.452	.950	.109	.260	.092	.069	−.655
C amount (%)	−.298	1.698	1.312	1.533	.748	.544	1.470
L*(D65)	.294	−.027	.276	.364	−.210	.443	.319
a*(D65)	−.479	−.371	4.747	−.909	−1.829	−1.309	−.058
b*(D65)	−.768	1.103	−1.709	.651	.642	.261	.278
(Constant)	−44.260	.122	−5.417	−32.157	8.310	−24.585	−46.649

$$\begin{aligned} D2 = {} & 0.122 + 0.149\,SiO2 - 0.128\,Fe_2O_3 - 1.090\,K_2O + 1.426\,CaO - 0.481\,TiO_2 \\ & + 0.950\delta^{13}C + 1.698\,C\,amount - 0.027\,L^* - 0.371\,a^*(D65) + 1.103\,b^*(D65) \end{aligned}$$

Table 6.11 lists the discriminant scores produced by replacing independent variables with their means. The group centroids thus acquired indicate that Function 1 has a high discriminating power for Area 1, 3, 7, 8, and 6 than the other areas, and Function 1 has a high discriminating ability for Area 2, 4, and 5.

The classification using the canonical discriminant functions (D1 and D2) resulted in 100.0 % discriminating accuracy for all the eight areas.

Table 6.11 Unstandardized canonical discriminant functions evaluated at group means

	Function						
Area	1	2	3	4	5	6	7
1	.256	1.118	.652	−3.581	−.747	−.442	−.029
2	−.183	6.046	−1.017	.482	1.018	−.457	−.211
3	1.873	−1.350	−.247	.549	.455	−.839	.454
4	−3.259	3.679	2.946	1.256	−.651	.684	.154
5	−1.152	−5.009	3.075	.014	.857	−.048	−.210
6	−7.966	−2.558	−2.944	.607	−.486	−.083	−.057
7	7.200	−1.155	−.562	1.700	−1.017	−.181	−.187
8	3.231	−.772	−1.903	−1.028	.570	1.365	.086

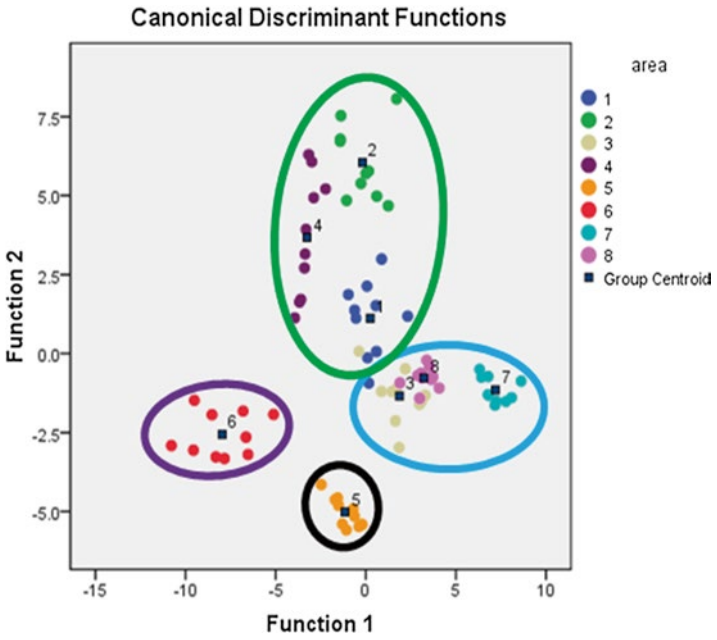


Fig. 6.17 Scatter plot displaying the discriminant scores

Figure 6.17 shows a scatter plot displaying the discriminant scores of the classification of all sub-samples performed by applying D1 and D2. Area 6 and 7 are well discriminated from other areas with D1 (horizontal axis), and Area 2 and 5 are well discriminated from other areas with D2 (vertical axis). A clustering tendency is observed according to area-specific characteristics: Area 3, 7, and 8 are located near the water, Area 1 and 2 near the industrial complex, Area 6 near the pasturing area and factories, and Area 5 in the fishing zone near the dam.

6.4 Conclusions

As a preliminary experiment to test the discriminating power of forensic soil analysis and obtain area-specific information, the soil analyses with respect to color, elements, and carbon-isotope ratio were performed. The results of analyses of the soil samples collected from the eight areas located in Munmak-eup (Wonju, Gangwondo, South Korea) are as follows.

1. The discriminating power regarding color, elements, and carbon content and isotope ratio was improved when all the three factors were combined compared to the discriminating power by individual data. In particular, the discriminating power for color and elemental analyses was excellent, and the results regarding carbon content and isotope ratio showed significant differences depending on soil depth.
2. The paired *t*-test on the results of carbon content and carbon isotope ratio measurements of the S and D samples confirmed that the samples had significant depth-dependent differences in carbon content.
3. The results of SIMCA PLS-DA performed on the samples collected from the eight areas in Wonju (Gangwondo, South Korea) showed that the Fe_2O_3, TiO_2, SiO_2, and a* color factors had an average VIP score greater than 1.
4. From the ANOVA tests, the null hypotheses for all the factors were rejected except that for Al_2O_3.
5. The canonical-discriminant analysis on three soil characteristics (color, composition, and content) showed that Function 1 exhibited a greater explanatory power for SiO_2 (Fe_2O_3, TiO_2), and could differentiate Area 6 and 7 more easily than the other areas. Function 2 had greater implications for color factor b* (δ13C and C content), and could differentiate Area 2 and 5 more efficiently. However, different results were obtained even within the same area, depending on the soil depth. Area 7 near the river, mainly formed with alluviums, has a high SiO_2 content and a relatively low Fe_2O_3 content.
6. During the elemental analysis, Al_2O_3 was categorized as an element without discriminating power owing to its extremely high RSD within the sub-sample. However, this may be attributed to the suppressed separation in the Br and XRF phase during the process of bead production. Therefore, in the case Al analysis using XRF, the beads should be made without using the remover, or pellets should be made instead of beads. If the remover is added, the sample should be analyzed with other methods such as LA-ICP-MS or ICP-AES.
7. Future studies should be performed for the analysis of Zn, Cu, and platinum group elements (PGE) (Pt, Pd, and Rh) in order to obtain the vehicle-related information. Further, Zn, Cu, Pb, Sn, and Ag elements should be analyzed. The nitrogen-related tests should be reconsidered, given its ability to discriminate the soil's origin in water environment.
8. In order to obtain more useful information, the results obtained in this study should be compared with those performed on samples collected from the areas at some distance from the experimental areas. Furthermore, by performing experiments in other regions, a more concrete and systematic region-specific information can be established.

References

Bull PA, Parker A, Morgan RM (2006) The forensic analysis of soil and sediment taken from the cast of a footprint. Forensic Sci Int 162:6–12

Chaperlin K, Howarth PS (1983) Soil comparison by the density gradient method – a review and evaluation. Forensic Sci Int 23:161–177

Cox RJ, Peterson HL, Young J, Cusik C, Espinoza EO (2000) Forensic analysis of soil organic by FTIR. Forensic Sci Int 108:107–116

Fitton G (1997) X-ray fluorescence spectrometry. In: Gill R (ed) Modern analytical geochemistry: an introduction to quantitative chemical analysis techniques for earth, environmental and materials scientists. Addison Wesley Longman, London, pp 87–115

Graves WJ (1979) A mineralogical soil classification technique for the forensic scientist. J Forensic Sci 24:323–338

Guedes A, Ribeiro H, Valentim B, Rodrigues A, Sant'Ovaia H, Abreu I, Noronha F (2011) Characterization of soils from the Algarve region (Portugal): a multidisciplinary approach for forensic applications. Sci Justice 51:77–82

Junger EP (1996) Assessing the unique characteristics of close-proximity soil samples: just how useful is soil evidence? J Forensic Sci 41:27–34

Kim K (2007) Metabolic profiling of hair from patients with skin appendage diseases by gaschromatography-mass spectrometry, Yonsei University

Meyers PA (1994) Preservation of elemental and isotopic source identification of sedimentary organic matter. Chem Geol 114:289–302

Murray RC (2012) Forensic examination of soils. In: Kobilinsky L (ed) Forensic chemistry handbook. Wiley, Hoboken

Park HH, Ok CS, Kang SS, Ko SJ, Choi SY (2011) Recognition for lung cancer using PCA in the digital chest radiography. J Korean Inst Inf Commun Eng 15(7):1573–1582

Petraco N, Kubic T (2000) A density gradient technique for use in forensic soil analysis. J Forensic Sci 45(4):872–873

Petraco N, Kubic TA, Petraco NDK (2008) Case studies in forensic soil examinations. Forensic Sci Int 178(2):e23–e27

Pye K, Blott SJ (2009) Development of a searchable major and trace element database for use in forensic soil comparisons. Sci Justice 49:170–181

Pye K, Blott SJ, Croft DJ, Carter JF (2006) Forensic comparison of soil samples: assessment of small-scale spatial variability in elemental composition, carbon and nitrogen isotope ratios, colour, and particle size distribution. Forensic Sci Int 163:59–80

Pye K, Blott SJ, Croft DJ, Witton SJ (2007) Discrimination between sediment and soil samples for forensic purposes using elemental data: an investigation of particle size effects. Forensic Sci Int 167:30–42

Reuland DJ, Trinler WA (1981) An investigation of the potential of high performance liquid chromatography for the comparison of soil samples. Forensic Sci Int 18:201–208

Thanasoulias NC, Piliouris ET, Kotti M-SE, Evmiridis NP (2002) Application of multivariate chemometrics in forensic soil discrimination based on the UV–vis spectrum of the acid fraction of humus. Forensic Sci Int 130:73–82

Wheals BB, Noble W (1972) Forensic applications of pyrolysis gas chromatography. Chromatography 5(9):553–557

Chapter 7
Reinstating Soil Examination as a Trace Evidence Sub-discipline

Brenda Woods, Chris Lennard, K. Paul Kirkbride, and James Robertson

Abstract In the past, forensic soil examination was a routine aspect of forensic trace evidence examinations. However, the apparent need for soil examinations has diminished and with it the capability of forensic laboratories to carry out soil examination has been eroded. In recent years, due to soil examinations contributing to some high profile investigations, interest in soil examinations has been renewed. The need for, and suggested pathways to, the reinstatement of soil examinations as a trace evidence sub-discipline within forensic science laboratories is presented in this chapter. An examination procedure is also proposed that includes: appropriate sample collection and storage by qualified crime scene examiners; the preliminary examination of soils by trace evidence scientists within a forensic science laboratory; and the higher-level examination of soils by specialist geologists and palynologists. Soil examinations conducted by trace evidence scientists will be facilitated if the examinations are conducted using the instrumentation routinely used by these examiners. Trace evidence scientists routinely use a microspectrophotometer (MSP) for the colour analysis of forensic samples, including paint, fibres, inks and toners. This chapter also presents how a microspectrophotometer can be used to objectively measure the colour of forensic-sized soil samples as a demonstration as to how the proposed examination procedure can incorporate both trace evidence scientists within a forensic laboratory and specialist soil scientists.

B. Woods (✉)
Chemical Criminalistics Team, Forensics, Australian Federal Police, Canberra, ACT, Australia

National Centre for Forensic Studies, University of Canberra, Canberra, ACT, Australia
e-mail: brenda.woods@afp.gov.au

C. Lennard
School of Science and Health, Western Sydney University, Richmond, NSW, Australia

K.P. Kirkbride
Faculty of Science and Engineering, Flinders University, Adelaide, SA, Australia

J. Robertson
National Centre for Forensic Studies, University of Canberra, Canberra, ACT, Australia

H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics*, Soil Forensics, DOI 10.1007/978-3-319-33115-7_7

7.1 Introduction

Soil for use as forensic evidence may be encountered in many different situations, for example: shoes from a suspect alleged to have stepped in a garden bed prior to committing a burglary; dirty shovels recovered from a suspect's house alleged to have been used to bury illicit materials; and soil from a suspect's vehicle that may have been at a burial site. As with all trace forensic evidence, the transfer of soil from a scene to an object or person is governed by what is commonly referred to as Locard's exchange principle. This principle has been summarised as follows:

> Whenever two objects come into contact, there is always a transfer of material. The methods of detection may not be sensitive enough to demonstrate this or the decay rate may be so rapid that all evidence of transfer has vanished after a given time. Nonetheless, the transfer has taken place (Palenik 1988).

Ultimately, soil can be used as evidence to exclude a suspect, a victim or an object from a particular scene, identify the scene of a crime, or contribute to forensic intelligence.

An early example of the use of soil analysis for forensic purposes is described in the work of Professor Ehrenberg of Berlin in 1856. He was consulted regarding the substitution of a barrel of silver coins for sand. Prof. Ehrenberg collected samples of sand along the railway line on which the barrel of silver was being transported and identified the station from where the sand had come. Using this soil information, suspects were identified and charged (Anonymous 1856). The use of soil as forensic evidence was further advanced by Hans Gross and Georg Popp. In 1893, Austrian Magistrate Gross wrote his "Handbook for Examining Magistrates, Police Officials, Military Policemen etc." in which he stated that "Dirt on shoes can often tell us more about where the wearer of those shoes had last been than toilsome inquires" (Murray and Tedrow 1975). Popp was a German scientist who applied the concepts of Gross in 1904, when he studied minerals on a handkerchief from a crime scene to assist with a murder investigation (Collins 1993).

From these beginnings, the forensic analysis of soils developed into a trace evidence sub-discipline that was commonly carried out in forensic laboratories. Many forensic laboratories employed a specialist geologist and applied a suite of geological methods to examine and compare soils. By the 1970s, the geological methodology for the analysis of forensic soil samples included: the use of Munsell colour charts to determine colour of soil and ash; density gradient distributions to observe the distribution of soil particles and aggregates; elemental composition of soils as determined by a number of instrumental techniques including neutron activation analysis, atomic emission spectroscopy and atomic absorption spectroscopy; particle size determination by sieving or using a Coulter Counter; pH of the soil; and general mineral identification using a microscope (Collins 1993; Andrasko 1981; Thornton 1986; Murray 1982).

In Australia from the late 1980s and through the 1990s, with the introduction of biometrics such as DNA profiling and the use of computers for the automation of fingerprint searching, interest in the examination of traditional trace evidence

waned, including the forensic analysis of soils. As a result, when the practicing forensic geologists employed by forensic laboratories retired or left the laboratory, no succession planning was undertaken resulting in their specialist skills not being replaced. As the capability to carry out meaningful analyses on soil samples in mainstream forensic laboratories diminished, awareness of the potential value of soil examinations to contribute to investigations also decreased.

More recently, however, the use of forensic soil examinations has seen a revival of interest, with soil examinations contributing to the investigation of terrorist incidents and other high profile investigations. The void left by the loss of expertise in forensic laboratories has been filled by experienced soil scientists from government and private agencies as well as universities. These scientists, while very familiar with the process of examining soils for geological purposes, are generally less familiar with the requirements of forensic analyses or the presentation of evidence in courts of law.

Australian scientists have established the Centre for Australian Forensic Soil Science (CAFSS; www.clw.csiro.au/cafss/) as a partnership involving the forensic industry and specialist geologists within the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Similarly, in other countries very productive relationships have emerged to bring together soil specialists and forensic scientists. As a result of this newly-available expertise and contributions to high profile investigations, interest from investigators has been renewed to the point where demand for services is greater than the available capacity. It is unlikely that the soil science teams from government agencies and universities will be able to expand to fill the increasing gap in capacity versus demand. Therefore, the forensic community needs to determine how to contribute to the forensic examination of soils if it wishes to see this evidence type as a sustainable and important trace evidence sub-discipline for the long term.

7.2 The Role of a Trace Evidence Scientist

We would argue that for soil examinations to regularly contribute to the investigation of criminal matters, soil science needs to be reinstated as a core trace evidence sub-discipline in forensic laboratories. This may be done in one of two ways:

1. Forensic laboratories employ specialist geologists; and/or
2. Trace evidence scientists up-skill to assist with soil examinations.

Unfortunately, forensic laboratories are unlikely to incur the expense of employing specialist geologists unless demand for services was sufficient or the specialists could be trained in, or have expertise in, other forensic science disciplines. Additionally, the geologist would not be in a professional space that would allow for technical review of their casework or for the exchange of ideas vital for the examination of complex casework. Forensic laboratories are more likely to incur the expense of up-skilling already highly skilled trace evidence scientists to undertake

the examination of simple forensic soil casework and for the screening of cases that might require outsourcing to an external specialist. This is also more likely to occur if the existing instrumentation within these facilities could be adapted for soil analyses (rather than additional equipment expenses being incurred).

Trace evidence scientists in forensic laboratories routinely analyse very small samples, from single textile fibres to individual layers of microscopic paint chips. The instrumentation available for such forensic analyses is specifically designed for very small samples. The basis for these examinations is to observe differences between two samples to determine whether or not they could be from the same source. The soil submitted for forensic analysis is usually very small in size – often milligram quantities – significantly less than the quantities usually available in a geology laboratory and much less than required for traditional geological analyses. Forensic soil samples may also be complex, comprising different soil types, some of which may not be relevant to the incident under investigation.

Forensic analysts are also trained to write statements and reports for court and to present their evidence in court when required, ensuring that the evidence presented can be easily understood by a lay person. They are required to regularly undergo proficiency testing to ensure that the examinations conducted are accurate and reliable. Finally, Australian and many overseas forensic laboratories are accredited against international standards (ISO 17025) to ensure that the examinations conducted follow sound forensic and scientific principles. As such, trace evidence scientists are well placed to provide assistance with the examination of soil samples for forensic intelligence and criminal investigations.

Reinstating forensic soil examinations in a trace evidence laboratory is more likely to occur if the trace evidence examiners are able to apply techniques already in use for other trace evidence examinations, such as paint, fibre and miscellaneous chemical analysis. As an example of the application of existing technology, the remainder of this chapter describes a method used in forensic trace evidence laboratories for colour analysis and the application of this technique to forensic soil samples. Additionally, a model is presented for how the trace evidence scientist (working within a standard forensic facility), in partnership with specialist geologists and soil scientists, could contribute to ensuring a sustainable future for forensic soil examinations.

7.3 Colour Theory and Colour Examinations

Colour examination of soils is usually one of the first screening tests conducted by forensic geologists as a means of eliminating non-relevant soils samples from further testing (Morrisson et al. 2009). It is common for forensic geologists to determine the colour of dry soil by examining it relative to the Munsell colour charts (Fitzpatrick 2009). The Munsell colour system uses three variables to describe colour (Thornton 1986):

1. hue – the spectral colour of the sample;
2. value – the amount of lightness or darkness in the sample; and
3. chroma – the brightness of the sample relative to neutral grey.

The Munsell Soil Colour Charts are swatches of colour with combinations of hue, value and chroma over intervals of 2.5 degrees of hue. The observer visually compares the dry soil sample to the Munsell colour chart to provide a Munsell colour formula that can be used for comparison purposes across soil samples.

As previously mentioned, the instrumentation available in a trace evidence laboratory is specially engineered for the analysis of microscopic and complex forensic samples. Trace evidence scientists regularly use a microspectrophotometer (MSP) for the objective measure of colour for typical forensic samples – from single fibres, to individual paint layers and inks and toners (Zięba-Palus and Trzcińska 2012).

A microspectrophotometer consists of a microscope equipped with, a spectrophotometer unit (Stoecklein 2001). Trace forensic samples are usually presented to the MSP on a glass slide and analysed either using transmitted or reflected light. Where it can be applied, transmitted light is the preferred method of illumination as, when reflected light is used, the effects of surface observation angle, surface texture and sample curvature may hinder colour comparisons (Scientific Working Group on Materials Analysis (SWGMAT) 2000).

The microscope is used to produce a magnified image of a microscopic object using lenses and mirrors. The objective of the microscope is used to collect the light from the sample and focus it on the spectrophotometer aperture. The spectrophotometer, which generally operates over a spectral range from the ultraviolet through to the near-infrared (e.g., 300–800 nm), separates the light into wavelengths and measures the intensity of each wavelength. The result is a spectrum that displays the intensity of light relative to its wavelength (Craic Technologies 2012).

In 1931, the CIE (Commission International de l'Éclairage) attempted to standardise colour examinations by establishing three colour-matched wavelength responses corresponding to red, green and blue lights. These responses were assigned x, y and z values for each wavelength and were called the CIE tristimulus values. The 1931 CIE colour space system is non-linear because it relates to the human visual response; it was subsequently adapted using a mathematical equation so that equal numerical steps are also visually equal. This adapted colour space is composed of the colour scale L*, a* and b*, where: L* correlates to the amount of lightness in a sample; a* correlates to the amount of red/green in a sample; and b* correlates to the amount of yellow/blue in a sample (Stoecklein 2001). A visual depiction of the CIE L*a*b* colour space is provided in Fig. 7.1.

Using a mathematical algorithm, which is beyond the scope of this chapter to detail, a microspectrophotometry spectrum can be converted into L*, a*, and b* chromaticity co-ordinates. When interpreting MSP spectra, both the L*a*b* co-ordinates and the spectrum itself should be examined as some samples with different spectral curves may have identical chromaticity co-ordinates (Craic Technologies 2012).

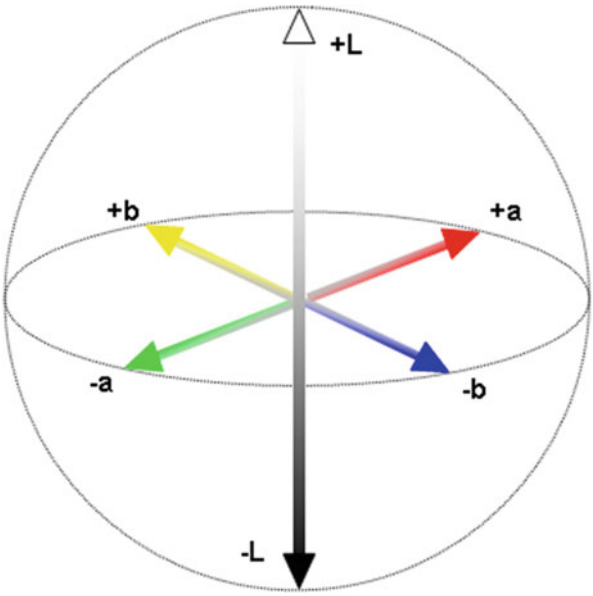


Fig. 7.1 A representation of the CIE L*a*b* colour space (Code Project 2011). L* correlates to the lightness; a* correlates to the amount of *red*/*green*; and b* correlates to the amount of *yellow*/*blue* in a sample

7.4 Colour Examinations of Forensic Soil Samples

The use of Munsell colour examinations is a well-established technique within geological communities as a simple morphological descriptor (Fitzpatrick 2009); however, it relies on the observer's subjective visual comparison of the soil sample against the Munsell Soil Colour Chart to arrive at a colour formula. Variations in visual colour measurements between different observers can be significant enough to result in different Munsell colour formulae being assigned (Islam et al. 2004). Munsell Soil Colour Charts may have been printed at different times or may have faded (Sánchez-Marañón et al. 2005), again potentially resulting in different Munsell colour formulas. Additionally, the moisture content of the soil and the lighting conditions employed for the examination will affect the accuracy and precision of the Munsell colour assigned (Bhadra and Bhavanarayana 1996). Further, the very small questioned sample usually present in a forensic case makes it difficult for comparisons against large amounts of reference soil. Ultimately, the court is presented with a subjective opinion of colour, which may have been used by the forensic geologist to discriminate soil samples.

To overcome the subjective difficulties of the Munsell colour system, a study was undertaken utilising the MSP and CIE-L*a*b* process as a means of objectively measuring colour in forensic soil samples. The soil samples used in this study were collected from six sites around the Canberra area. The soils were rudosols and chromosols from volcanic mountains, alluvial fans, granitic material and metasediments (New South Wales Department of Land and Water 2000). At each site, surface debris and leaf litter was removed then the surface soil (0–5 cm depth) and sub-surface soil (5–10 cm depth) collected, resulting in a total of 12 soil samples being obtained.

The soil samples were oven dried for 24–48 h at 40 °C prior to storage. The soil samples were crushed using a mortar and pestle then sieved. The < 2 mm fraction was then milled to a powder XRD consistency (with a resulting particle size approximately 5–10 μm). No attempt was made to remove any organic content. This sample preparation procedure was utilised as, in Australia, it is common for soil samples to be treated in this manner prior to instrumental analysis (Fitzpatrick 2009).

The following methods were evaluated as a means of presenting the soil samples to the MSP:

1. Potassium bromide (KBr) disks;
2. Polyethylene disks;
3. Pure soil disks; and
4. Pure soil in a holder.

7.5 Potassium Bromide and Polyethylene Disks

Given that transmitted light is the preferred method of illuminating the sample for MSP analysis, the soil was first prepared to enable this mode of illumination. The first method attempted was the creation of potassium bromide (KBr) disks. There are many benefits of KBr disks, including the following: the resulting disk is transparent, making it suitable for transmission MSP; only a very small soil sample is required; the disk can also be used for mid-infrared analyses; and the disc can be archived for subsequent re-examination by the laboratory or by defence experts.

Approximately 1.5 mg of soil was added to approximately 200 mg of spectrophotometric-grade KBr. This sample-to-KBr ratio was used to ensure a transparent, indurated disk. The mixture was homogenised using an agate mortar and pestle and then placed in a 13 mm die. The sample was compressed using a press with approximately eight tonnes of pressure. The resulting KBr soil disk was placed on a glass slide for analysis using the MSP. Unfortunately, the ratio of soil to KBr resulted in insufficient soil in the disk to allow for the collection of a satisfactory MSP spectrum. Various soil-to-KBr ratios were trialled, resulting in opaque disks not suitable for transmission MSP or disks too friable to be of use. Powdered polyethylene was trialled in place of KBr given the possibility that the polyethylene could be used to create an indurated disk with an increased soil content. Similarly to the KBr disks, the amount of soil sample required in the disk to collect a suitable MSP spectrum resulted in opaque and friable disks.

7.6 Pure Soil Disks

Unfortunately, without a means to present a transparent soil sample to the MSP, transmission MSP could not be utilised. Pure soil samples were compressed into 13 mm disks with a view to using the MSP in the reflectance mode. Adequate spectra were collected for these samples (see Fig. 7.3, for example). However, this method

required a relatively large quantity of soil sample, being approximately 200 mg. This quantity of soil is beyond what would normally be encountered in a forensic soil sample. The pure soil disks were therefore considered an impractical method for the MSP analysis of forensic soil samples.

7.7 Pure Soil in Holder

Diffuse Reflectance Infrared Fourier Transform spectroscopy (DRIFTS) is a method of infrared analysis commonly used on powder samples that requires limited or no sample preparation. The powder is commonly presented to the DRIFTS instrument using a sample holder or cup. An example of a DRIFTS sample holder can be seen in Fig. 7.2.

The milled < 2 mm fraction pure soil samples were placed in the DRIFTS sample holders and very lightly compressed to ensure a flat examination surface. These samples were then presented to the MSP in the DRIFTS sample holders and satisfactory reflectance spectra recorded. The DRIFTS sample holders used in this study contained approximately 6 mg of soil sample – a sample size that is more realistic for forensic soil samples.

Full details on sample preparation and spectral acquisition parameters have been provided in a more detailed publication on colour measurement of soil samples for forensic application (Woods et al. 2014a). MSP spectra were obtained for all 12 samples previously described.

As can be seen from Fig. 7.3, the MSP spectra for the Canberra soil samples collected in this study did not contain many features. The peaks, shoulders and inflections were very broad, with no distinct maxima or minima. To aid the interpretation of the MSP results, the spectra were converted to CIE L*a*b* chromaticity coordinates. Three sub-samples from each of the 12 soil samples were analysed. Figure 7.4 is a matrix plot of the CIE L*a*b* chromaticity coordinates of the three sub-sample replicates examined for each of the 12 milled soil samples from the Canberra area.

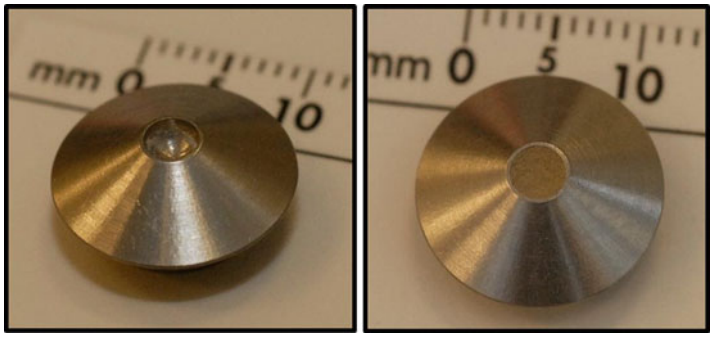


Fig. 7.2 A picture of a DRIFTS sample holder empty (*left*), and containing a soil sample (*right*)

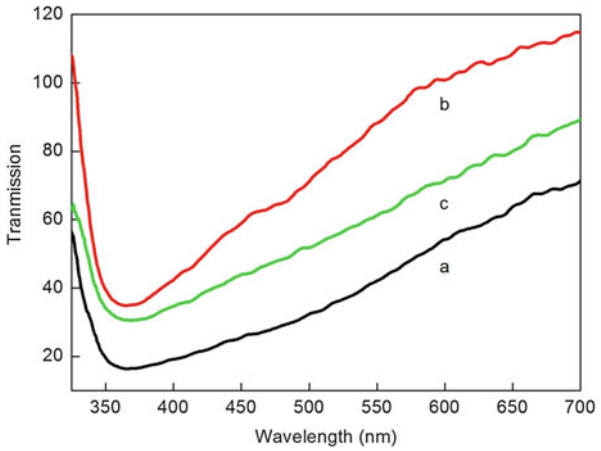


Fig. 7.3 Microspectrophotometer (MSP) spectra for soil samples from site 2 surface (spectrum a), site 1 surface (spectrum b) and site 2 sub-surface (spectrum c). Using the MSP spectra and the CIE L*a*b* chromaticity coordinates the surface soil sample from site 1 (spectrum b) can be differentiated from the soils of site 2. The surface and sub-surface soil of site 2 can not be differentiated using this technique

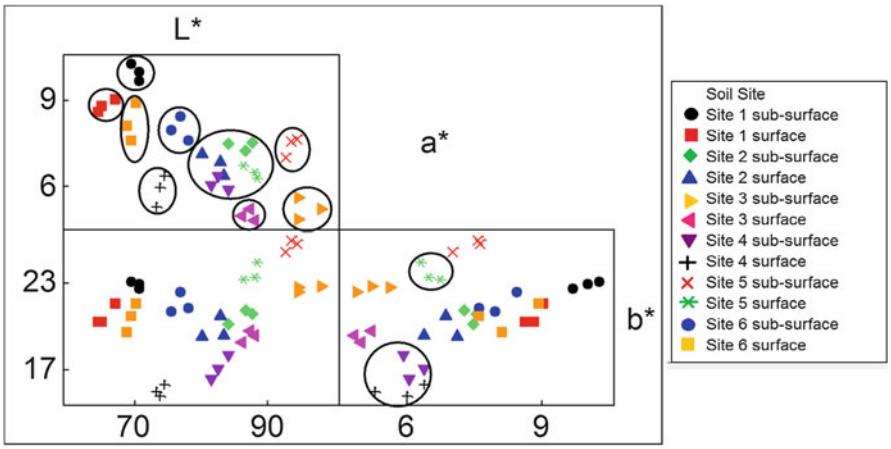


Fig. 7.4 Matrix plot of the CIE L*a*b* chromaticity coordinates for three sub-sample replicates of the 12 milled soil samples from the Canberra area

In Fig. 7.4, it can be seen from the sub-sample replicates for each of the 12 Canberra area soil samples that the intra-sample variation is low. Further, there is sufficient inter-sample variation to differentiate some samples using the MSP spectral results and the CIE L*a*b* chromaticity coordinate data. The 12 soil samples from the Canberra area can be readily classified into 11 groups based on the MSP results, refer to Table 7.1.

Table 7.1 Classification of the 12 Canberra area milled soil samples into 11 groups based on MSP spectra and CIE L*a*b* chromaticity coordinates

Group 1	Site 2 surface
	Site 2 sub-surface
Group 2	Site 1 surface
Group 3	Site 1 sub-surface
Group 4	Site 3 surface
Group 5	Site 3 sub-surface
Group 6	Site 4 surface
Group 7	Site 4 sub-surface
Group 8	Site 5 surface
Group 9	Site 5 sub-surface
Group 10	Site 6 surface
Group 11	Site 6 sub-surface

Based on MSP spectra and CIE L*a*b* chromaticity coordinates, the surface and the sub-surface samples from site 2 could not be differentiated.

Although milling a soil sample to a 5–10 μm particle size is routine in geological applications there are some drawbacks when considered in forensic context. For example, if the starting soil samples had different initial fraction ratios present then the milled samples may also be different. Although only limited studies have been conducted on the transfer and persistence of soil particles (Fitzpatrick 2009; Morgan et al. 2010; French et al. 2012) it is clear that larger soil particles are preferentially lost after transfer. In a real forensic casework scenario it is not possible to assume that the recovered/ questioned soil and reference soil samples will have the same starting particle size distribution. Hence, having shown the potential of MSP to differentiate Canberra soils using milled samples, a forensically more realistic approach was developed aimed at producing 'comparable' starting known and case samples.

Initially the soil samples from the six sites around the Canberra area were dry sieved, and the < 63 μm fraction mounted for MSP analysis. However, due to the relatively large size of the particles in the soil, no reproducible MSP results could be obtained. The intra-sample variations were too large for inter-sample comparisons to be meaningful. Hence the 12 Canberra area soil samples were further dry sieved, with the < 38 μm soil fractions mounted for MSP analysis. The MSP results for these fractions were converted to CIE L*a*b* chromaticity coordinates. A matrix plot of the sieved < 38 μm soil fraction samples is provided in Fig. 7.5.

As can be seen from Fig. 7.5, the intra-sample variation of the MSP results for the < 38 μm soil fraction is larger than the intra-sample variations observed for the milled soil samples (Fig. 7.4). However, while the intra-sample variation is larger, there is still sufficient inter-sample variation to allow for classification into 6 groups. Refer to Table 7.2 for the classification of the dry sieved < 38 μm soil fractions based on MSP spectral results and CIE L*a*b* chromaticity coordinates data.

Based on MSP spectra and CIE L*a*b* chromaticity coordinates, the surface and sub-surface samples from sites 2 and 4 could not be differentiated from the

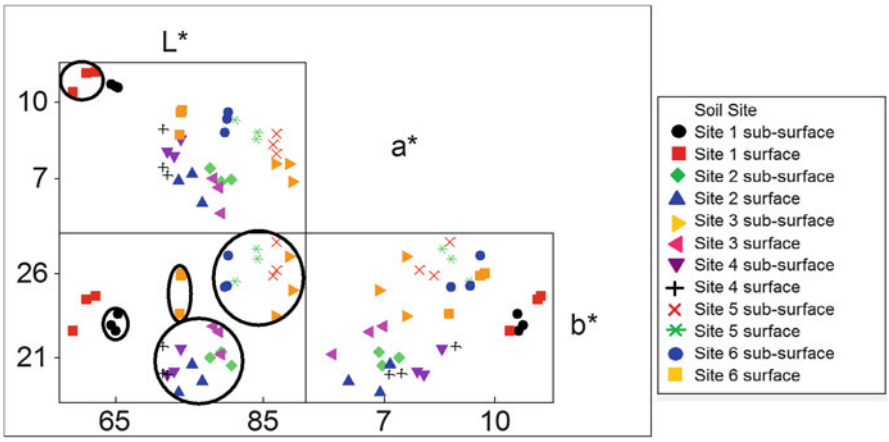


Fig. 7.5 Matrix plot of the CIE L*a*b* chromaticity coordinates for three sub-sample replicates of the < 38 μm soil fraction from the 12 soil samples from the Canberra area

Table 7.2 Classification of the sieved < 38 μm fractions from the 12 Canberra area soil samples into 5 groups based on MSP spectra and CIE L*a*b* chromaticity coordinates

Group 1	Site 2 surface
	Site 2 sub-surface
	Site 3 surface
	Site 4 surface
	Site 4 sub-surface
Group 2	Site 3 sub-surface
	Site 5 surface
	Site 5 sub-surface
	Site 6 sub-surface
Group 3	Site 1 surface
Group 4	Site 1 sub-surface
Group 5	Site 6 surface

surface soil of site 3. Furthermore, the surface and sub-surface soil from site 5 could not be differentiated from the sub-surface soil of site 3 and the sub-surface soil from site 6. The inability to differentiate the < 38 μm soil fractions to the same extent as the milled soil samples is due to the increased intra-sample variations observed. The increased intra-sample variation observed is due to the larger particle size resulting in more variable MSP results.

Colour analysis of soils is a relatively quick and easy examination. As demonstrated here, the colour analysis of such samples can be conducted by trace evidence scientists, in an objective fashion, utilising a microspectrophotometer. This screening technique has a good level of discrimination and the proposed examination procedure of utilising the < 38 μm soil fraction will result in soil samples that can be further examined by the expert soil scientist.

Reinstating soil examinations into trace evidence laboratories will reduce the number of soil cases that need to be examined by experts. Samples that can be readily differentiated by colour analysis, for example, would not require further examination. Soil experts would therefore be able to concentrate on complex cases where samples cannot be differentiated by preliminary analyses. It is important to also note that the colour of soil samples used in this study represented a very narrow range of visual colours. The technique has been applied to a broader visual colour range of soils and has been shown to produce expected greater levels of discrimination. Of course colour is only one characteristic of soil, a number of other aspects of soils which can be analysed using routine techniques employed by trace evidence examiners have also been investigated (Woods et al. 2014b, 2016).

7.8 Conclusions

Forensic science is science applied to the law. Crime scene examiners record, collect and preserve a large variety of evidence from crime scenes on a daily basis. The trace evidence examiner is in a unique position to be able to assist and enhance current soil analysis procedures. They are familiar with the reporting requirements for the criminal justice system, the requirements for expert opinion in court, and the accreditation requirements for a forensic laboratory.

To increase the use of soil examinations as a tool for forensic intelligence-led investigations and for presentation in court, there is a need for a partnership between trace evidence laboratories and forensic geological experts. We have proposed the following procedure, utilising both trace evidence scientists and geological experts, to enable best practice for the examination of forensic soil samples. The procedure would consist of four levels of soil examination:

Level 1 – Reference soil sample collection and preservation by crime scene examiners.

Level 2 – Questioned soil sample collection and preservation by trace evidence chemists. Preliminary screening of questioned and reference soil samples for differences using instrumentation currently available in trace evidence laboratories, including microspectrophotometry, infrared spectroscopy and elemental analysis. At the conclusion of Level 2 examinations, comparison data could be used for investigative support, intelligence purposes or as exclusionary examinations for court reports.

Level 3 – Up-skilling trace forensic examiners with basic mineralogical identification skills to be used in conjunction with instrumentation currently available in a trace forensic chemistry laboratory. At the conclusion of Level 3 examinations, comparison data could be used as inclusionary examinations for court reports.

Level 4 – Geological or palynological examination by an external expert for casework that is complex; requires provenancing; when the questioned sample is particularly small; or when additional instrumental expertise is required.

The purpose of the research reported in this chapter is to develop level 2 skills to promote the value of soil examination and certainly not to discourage the broader use of the Munsell soil colour chart system, which is an established technique that is robust when in the hands of experienced individuals who understand the context for its application. The nature of soil samples that have to be examined in a forensic context present specific issues for the appropriate use of the Munsell system, specifically the size of the evidentiary specimen and the difficulty in comparing 'like with like.' Samples recovered in case situations and reference samples against which these will be compared may be different in gross appearance as a result of factors such as the transfer and persistence of soil particles. Recovered samples may also be very small in total weight. In this context we have examined the use of microspectrophotometry, or MSP, typically used in forensic laboratories to measure colour of microscopic trace materials such as textile fibres or paint fragments.

We have studied how best to treat soil samples and present them to the MSP instrument. Intentionally we chose a range of soils in the Canberra region that displayed a very limited visual colour range and we pre-treated samples using established protocols used in geology. The latter included milling our samples. Using milled samples we showed that MSP spectra with appropriate use of CIE L*a*b* chromaticity coordinates achieved good levels of discrimination between our samples. However, as milling was seen as being a potential issue in forensic context we investigated less intrusive approaches to sample pre-treatment. Using dry sieving to produce a <38 μm fraction enabled similar levels of discrimination although it was less than that obtained with milled samples.

This study has shown that MSP as used in forensic laboratories can be applied to the examination of small, forensically relevant samples with minimal pre-treatment of these samples. MSP is only the first of a number of techniques that have been investigated for their application to the examination of soil samples in forensic laboratories by trace evidence examiners.

The proposed soil procedure would ensure integration of the phases undertaken by the trace evidence examiner in a forensic science laboratory and the higher-level analyses that could be performed by geological and/or palynological experts. The procedures applied within the forensic laboratories would be conducted in such a way as to enhance the results of the soil expert, resulting in additional techniques being available to assist in the exploitation of soil samples as forensic evidence.

References

Andrasko J (1981) Soil. In: Maehly A, Strömberg L (eds) Chemical criminalistics. Springer, Berlin, pp 171–181

Anonymous (1856) Science and Art: curious use of the microscope. Scientific American, p 240

Bhadra SK, Bhavanarayana M (1996) Estimation of soil colour through spectral refectance characteristics. J Indian Soc Remote Sens 24(1):1–10

Code Project (2011) http://www.codeproject.com/Articles/243610/The-Known-Colors-Palette-Tool-Revised. Accessed 6 May 2013

Collins B (1993) Soil and related substances. In: Freckelton I, Selby H (eds) Expert evidence, vol 8. The Law Book Company Ltd, North Ryde, pp 5401–5613
Craic Technologies (2012) http://www.microspectra.com/support/service-contracts/microspectrophotometer-design. Accessed 6 May 2013
Fitzpatrick RW (2009) Soil: forensic analysis. In: Jamieson A, Moenssens A (eds) Wiley encyclopedia of forensic science. Wiley, Chichester, pp 2377–2388
French JC, Morgan RM, Baxendell P, Bull PA (2012) Multiple transfers of particulates and their dissemination within contact networks. Sci Justice 52(1):33–41
Islam K, McBratney AB, Singh B (2004) Estimation of soil colour from visible reflectance spectra. Paper presented at the supersoil 2004, Sydney, Australia
Morgan RM, French JC, O'Donnell L, Bull PA (2010) The reincorporation and redistribution of trace geoforensic particulates on clothing: an introductory study. Sci Justice 50(4):195–199
Morrisson A, McColl S, Dawson LA, Brewer M (2009) Characterisation and discrimination of urban soils: preliminary results from the soil forensics university network. In: Ritz K, Dawson L, Miller D (eds) Criminal and environmental soil forensics. Springer, Dordrecht/London
Murray RC (1982) Forensic examination of soil. In: Saferstein R (ed) Forensic science handbook. Prentice Hall, New Jersey, pp 653–671
Murray RC, Tedrow JC (1975) Forensic geology – earth sciences and criminal investigation. Rutgers University Press, New Jersey
New South Wales Department of Land and Water (2000) Conservation survey map H8727
Palenik S (1988) Microscopy and microchemistry of physical evidence. In: Saferstein R (ed) Forensic science handbook, vol 2. Prentice Hall, Englewood Cliffs
Sánchez-Marañón M, Huertas R, Melgosa M (2005) Colour variation in standard soil-colour charts. Aust J Soil Res 43(7):827–837
Scientific Working Goup on Materials Analysis (SWGMAT) (2000) Forensic paint analysis and comparison guidelines
Stoecklein W (2001) The role of colour and microscopic techniques for the characterisation of paint fragments. In: Caddy B (ed) Forensic examination of glass and paint. Taylor & Francis, London, pp 143–163
Thornton JI (1986) Forensic soil characterization. In: Maehly A, Williams RL (eds) Forensic science progress, vol 1. Springer, Berlin, pp 1–35
Woods B, Lennard C, Kirkbride KP, Robertson J (2014a) Soil examination for a forensic trace evidence laboratory – part 1: spectroscopic techniques. Forensic Sci Int 245:187–194. doi:10.1016/j.forsciint.2014.08.009
Woods B, Kirkbride KP, Lennard C, Robertson J (2014b) Soil examination for a forensic trace evidence laboratory – part 2: elemental analysis. Forensic Sci Int 245:195–201. doi:10.1016/j.forsciint.2014.10.018
Woods B, Lennard C, Kirkbride KP, Robertson J (2016) Soil examination for a forensic trace evidence laboratory – part 3: a proposed protocol for the effective triage and management of soil examinations. Forensic Sci Int 262:46–55. doi:10.1016/j.forsciint.2016.02.034
Zięba-Palus J, Trzcińska BM (2012) Comparing the colour of forensic traces. Anal Lett 45(11):1333–1346

Chapter 8
Methodology of Forensic Soil Examination in Russia and a View on the World Standardization Process

Olga Gradusova and Ekaterina Nesterina

Abstract A survey is given of the current status of forensic soil examinations in the Russian Federation, emphasizing the uniformity of the methodology that has been developed and implemented in Russia. The methodology is outlined, as well as the training of forensic scientists to work according to this methodology. Next attention is paid to the difference in the interpretation and presentation of the results of forensic examinations in Russia and elsewhere. In Russia courts do not accept probabilistic evidence and results are to be given in a categorical form. To further elucidate the consequences of this the separate stages in the process of conducting a forensic soil examination are depicted. Finally the practice of soil forensics in the Russian Federation is illustrated by presenting the questions asked and the answers given in six real cases.

8.1 Introduction

Experts and expert activity differ greatly from what we can see on TV screens in different shows and serials. To our minds the real expert activity is best of all described by Collins and Johll (2006). In their publication on forensic chemistry they state that forensic scientists do not directly solve crimes but simply analyze the physical evidence, that is combined with all the other evidence by the detective assigned to the case. It is the detective who attempts to solve the crime, forensic scientists do not work for the defense nor for the prosecution but are advocates of the truth under all circumstances. As we see it this view can be adopted as a manifest for forensic scientists.

O. Gradusova (✉) • E. Nesterina
Federal Budget Institution Russian Federal Centre of Forensic Science,
Building 13/2, Khokhlovsky Lane, 109028 Moscow, Russian Federation
e-mail: bio_soil@rambler.ru

H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics,*
Soil Forensics, DOI 10.1007/978-3-319-33115-7_8

8.2 Methodology of Forensic Science in Russia

In our country the status of forensic examination and forensic experts (rights and duties) are given in the Codes of Criminal and Civil Procedure of the Russian Federation. Not long ago a special Federal Law on State forensic activity, which covers all state and private expert activity within the Russian Federation, was adopted. By now a number of State Forensic Laboratories of the Ministry of Justice cooperate in a network, with the Russian Federal Centre of Forensic Science in Moscow as its parent organization.

Already in 1980 a discipline of theoretical forensics was developing markedly in Russia, providing a uniform approach in the formalization of concepts and working out a general methodology for forensic examinations. From the first days of its foundation the Russian Federal Centre of Forensic Science seeks to develop manuals and training programs, to provide quality assurance systems and to harmonize methods and techniques in all laboratories for all forensic disciplines.

In accordance with the *uniform* requirements, adopted by the Ministry of Justice, special training programs and a special proficiency testing procedure along with a number of manuals were developed for applicants for a state forensic expert job for different forensic disciplines. In addition *uniform* requirements for forensic expert reports were developed and adopted by the Ministry of Justice.

We want to outline the uniform system of management for providing forensic soil examination that exists in Russia since the 1980s. Below let us see its main components.

8.3 Training Programs: Certification and Recertification of the Expert

The training program focuses on the development of theoretical and practical knowledge, skills and abilities that are necessary to the forensic examination of objects of soil origin. The training usually lasts about 6–12 months depending on the education, skill and ability the applicant already possesses.

All persons employed should have at least a bachelor's degree in soil science or geology and it is very desirable for them to be skillful in the examination of macro and micro soil structure, soil classification, soil mineralogy and microscopic analysis.

The program comprises a whole number of topic areas concerning general forensic science theory, criminalistics legal documents and standard acts as well as special knowledge about the examination and analysis of soil. It also includes guidance on forensic science procedures and working as an expert. A list of references for self-learning is given to the trainee.

The training process consists of the following consecutive stages in which the applicant is carefully guided to learn working as an independent forensic expert:

- Self-learning of the literature as specified in the training program.
- A course of lectures on the theory of forensic science and ABC of criminalistics
- Test work: writing a review of methodological approaches in forensic soil examination on one of several suggested topics.
- Contact training hours in a laboratory setting.
- Control tasks.
- A proficiency test.

The goal of "Contact training" is to give applicants the specific skills required of the forensic soil scientists. For this purpose it is considered to be very desirable that an applicant is involved in a real case examination process together with an experienced soil expert.

To demonstrate that the ability to carry out an examination independently has been acquired, an applicant should fulfill control tasks (mock cases) and present them as expert reports according to the requirements for reports as adopted by the Ministry of Justice.

Control tasks mimic real case scenarios as closely as possible. Each applicant should complete at least five control tasks involving questions about the provenance of questioned soil samples and their relation to a specific source that is relevant to the crime.

Then an applicant must pass an exam, where he must answer questions on three topics: general forensic science theory, the ABC of criminalistics and his specialty (i.c. forensic soil examination). After passing such exam successfully an applicant will get the diploma of the additional special education on forensic soil examination and will become the certified forensic soil examiner.

Recertification of an expert takes place every 5 years. This procedure involves an external reviewing of five expert reports that were made in the last 5 years. The review has to be positive for the expert to pass the proficiency test and be recertified.

8.4 Manuals for Forensic Soil Examination

The first manual on forensic soil examination was published in 1978. It was revised and republished in three parts in the beginning of the 1990s. The first part was devoted to theoretical aspects: "The Basic Scientific Principles of Complex Criminalistic Soil Examination (A Manual for Experts, Case Investigators and Judges)" and the following chapters are included:

- Objects of forensic soil examination.
- Subject of forensic soil examination.
- Classification of forensic soil examination tasks.
- Forensic soil examination: management and procedures.
- Collection and packing of materials. Recommendations for sampling.

– The work of an expert on a scene of crime.
– Sorting of soil characteristics in complexes (“ensembles”) as being generic, grouping or individualizing.
– Analysis and collection of results, interpretation of results.
– Conclusions (the actual formulation of the answers to the questions posed).

The two other books are “Forensic Soil Examination. Methods of Complex Criminalistic Soil Examination (A Manual for Experts, Case Investigators and Judges)”. They are devoted to the examination of mineral constituents and organic constituents (plant materials and organic matter). In these two books are described a wide variety of special techniques for analyzing small soil samples by simple routine methods as well as by the most modern physicochemical techniques of the time. The methods described in the manual were newly developed or borrowed and modified from different fields of science.

These books till now remain the main manuals for all forensic soil experts in Russia.

As stated in the Federal Law about expert activity, the forensic expert has to conduct an examination using special knowledge, techniques and equipment to answer questions posed by the case investigator. Experts have the right to use any method and procedure that they deem necessary, but they must provide the reasons for their choices in their examination reports. All forensic investigations begin with a careful study of case papers, especially reports from the scene of crime. Sometimes it is necessary to visit a crime scene to examine its physical environment (e.g. relief, nature and homogeneity of the soil and the vegetation). When a visit is not possible, experts usually request photographs which were made during the examination of a crime scene. Then the expert examines items and chooses a scheme according to which he will conduct an investigation. Of course, every case is very individual, but after many years of experience a number of the most typical schemes were described and adopted for forensic soil examination in our laboratory. These schemes are depicted in the above mentioned manuals.

8.5 International Forensic Science Communities and Standardization Process

Nowadays a number of international communities all over the world are actively involved in drawing up best practice manuals, setting up collaborative tests and education and training programs and are working towards increased harmonization and standardization of methods and techniques in forensic science. In Table 8.1 the most important networks involved in this process are summarized.

The standardization process and the harmonization of national standards with global standards nowadays proceeds markedly in Russia. We very much want to keep up with the global standards. In the context of the globalization process our organization attempts to contribute to global consistency. Our organization has

Table 8.1 Forensic science networks

Full name	Acronym	Website	Orientation
European Network of Forensic Science Institutes	ENFSI	www.enfsi.eu	Europe
American Society of Crime Laboratory Directors	ASCLD	www.ascld.org	USA/Global
Senior Managers Australian and New Zealand Forensic Laboratories	SMANZL	www.nifs.com.au	Australia/ New Zealand
Academia Iberoamericana de Criminalistica y Estudios Forenses	AICEF	www.2itad.or2	Spanish-speaking countries
Scientific Working Group for Materials Analysis	SWGMAT	www.swgmat.org	USA/Global
Asian Forensic Sciences network	AFSN	www.asianforensic.net	Asia
The International Union on Geological Sciences (IUGS) Initiative on Forensic Geology	IUGS-IFG	www.forensicgeologyinternational.com	Global

always been a member of ENFSI working groups, such as Animal, Plant and Soil traces (APST), Textile and hair, Document, Handwriting and some others. We are very glad, that the APST working group of ENFSI and the International Initiative group on Forensic Geology were founded and it is very important for us to be involved and that our organization and Russia as a whole, takes part in these global initiatives.

Considerable work has been done by international communities in developing different documents to this moment. A review of periodic literature and documents highlighted that the procedure of forensic examinations and the techniques and methods used in various countries are practically the same in general. The major difference between the approaches in Russia and in other countries is found in the way the results of examinations are presented in courts or in how the strength of evidence is expressed.

The probabilistic approach is used in a number of countries. In that approach the value of the likelihood ratio could quantify the degree of probability or the strength of the evidence. It is well known that a large part of forensic soil examinations is based on examination of morphological (pattern) characteristics and there is still no uniform doctrine on how soil experts should interpret them and present the obtained data in reports. In most cases the interpretation and presentation look like a subjective opinion of an examiner which cannot be easily formalized and valued with the help of likelihood ratio. We fully agree with Aitken (2009) that there are a lot of

difficulties to develop a procedure using likelihood ratios for soil forensic analyses in the nearest future. Even in instrumental analysis where statistical analysis seems to be possible, an expert encounters difficulties in estimating accuracy. Statistical estimation in most cases is also a puzzle that still needs to be solved. It differs completely from that in DNA analysis. Usually only semi quantitative or even qualitative data can be obtained for soil traces. That is the consequence of having small amounts of substance under consideration that originate from a large, divers and heterogeneous soil cover.

Since the numerical form of the likelihood ratio cannot be easily calculated or interpreted for the court, translating it into verbal scales according to the subjective expert's opinion was proposed by the Association of Forensic Science Providers (Standards for the formulation of evaluative forensic science expert opinion 2009) and is adopted in a number of European countries (for example The Netherlands, Ireland and Sweden). We much appreciate the tremendous work of the ENFSI group on development the "ENFSI guideline for evaluative reporting in forensic science", and consider it to be a great step forward.

In Russia courts do not accept any probabilistic evidence. This leads to the fact that the evidence should be expressed in the categorical way, or in other words the answers to the questions posed by the case investigator should be given in the categorical form. If results are given in the probabilistic form to our Courts that is *de jure* equal to the answer that it is impossible to solve the task of forensic identification.

Though probabilistic results are not accepted by Russian courts, the answers given in the probabilistic form, sometimes, may be very useful for case investigators in the inquisition process, in the search process to evaluate leads and for verification of the reliability of somebody's testimony.

8.6 Forensic Identification and Inference of Identity in Soil Forensics in Russia

We have described once very briefly the basic tasks in soil examination in relation to the forensic context (Gradusova and Nesterina 2009), but in this work we would like to pay attention to the *identification task* (sometimes in literature called *individualization*) since it is the most complicated task to solve as well as to present in courts. The ultimate aim of all forensic identification science is the inference of identity. Two broken fragments of glass that physically fit together and were once one piece are known to be the classical example of identity.

In the late 1970s the method of "identification of the whole by parts" in the absence of an interface or a common boundary line has been developed in Russia by Mitrychev (1976). This method was borrowed from forensic medicine, forensic portrait examination and archeology. It became the uniform method in the practice of forensic science in different disciplines where identification of different objects (including those of natural origin) should be established.

It is considered that a source can be examined like a set being composed of a number of subsets (structural components) which are identically equal with known samples. When comparing known soil samples with questioned soil samples an expert should state the identity of a source. It is accepted that a source is identically equal to known samples. Then, questioned samples are accounted to be an identifiable object and known samples are accounted to be an identifying object.

The principle of forensic soil trace investigation is based on the following. A forensic examiner should identify the scene of crime (top soil, soil covering or burial) or other appointed place (the whole) by structured examination of the parts (for example, soil layers taken from items which might depict the features of the whole, and/or complex of different constituents of soil traces).

The identification is considered to be a multistage process. It can be discontinued in every stage when:

- There are no soil traces
- There are soil traces, but they are unusable for examination
- Individual characteristics are absent or a complex of individual characteristics is absent

Identity may be established only when the soil (or soil covering) at the crime scene possesses individual characteristics or an individual ensemble of characteristics and they have been depicted in the traces.

An expert can never be sure that a characteristic or complex of characteristics is individual until he has observed all relevant objects (places), or knows the frequency of their appearance. Of course it is virtually impossible to inspect large territories. It will enlarge the time of investigation significantly. Therefore, as a rule, the frequency of appearance of such complexes is unknown and in those cases experts rely on their experience and skill and also on different data taken from literature.

After the investigation process begins the process to establish identity. Here all results should be thoroughly analysed, summarized and interpreted and finally, conclusions are to be made.

Stoney (1991) imagined the mechanism of the identity establishing process as a "leap of faith". Sierps and Berger (2012) wrote: "The reasoning process leading to a conclusion often requires more than just 'common sense' and basic logic". As a joke we say that in the result of investigation first of all "an examiner himself should be satisfied that he has arrived to the truth" before he begins the inferential process, writing an expert report the end point of which is to answer the questions posed by the customer (the case investigator).

8.7 Questions and Answers in Forensic Soil Examinations

Questions posed by a case investigator are usually as follows:

- Are there any soil traces on the questioned items?
- If "yes", then: do they have a general group belonging with the crime scene (soil or top soil on the crime scene)?
  or

– If “yes”, can they originate from that place?

Conclusions can be as follows:

– There are, or there are no soil traces on the items.

If the identity is established, then:

– The soil traces on the items are derived from the place appointed by the case investigator and characterised by comparison samples

BUT, usually (in the majority of cases) there are no individualizing characteristics for soil matter in any place. In these cases we give conclusions like:

– The soil traces have general group belonging with the soil covering or “soil material” on the place (crime scene) appointed by the case investigator.
– To answer the question whether the soil traces originate exactly from that place is not possible due to the absence of individualizing features or an individualizing ensemble of characteristics.

Experts should explain or clarify in the experts report (not in the conclusions!) what it means “to have a general group belonging”. **General group belonging** does mean that “A soil trace on a questioned item is derived as the result of contact of the item with the top soil or soil material on that place or on another place with the same ensemble of characteristics”. It is just the same as to say “The fact that the soil traces on the item really originate from that place can not be excluded”.

When there are no similar or common characteristics at all, or there is a significant difference in the ensemble of characteristics, the conclusion should be:

– The soil traces on the questioned items are not derived from that place.

If an expert has conducted an examination but could not come to any of the above mentioned conclusions, then the following answer should be given:

– It is not possible to answer this question for the reasons described previously in the research part of the report.

8.8 The Structure of the Expert’s Report

According to the instruction, developed by the Ministry of Justice every expert report should consist of the following main parts: a written undertaking (on a separate page), Introduction, Research, Comparative study, Summary (Results and discussion), Conclusions.

Every part, mentioned above, by-turn should comprise the following points respectively.

8.8.1 A Written Undertaking

- Name, title, education, qualification, data of the initial and the last accreditation of an expert
- Corresponding chapters and points of the Criminal (Civil) Code which should be signed by the expert
- A date and the expert's signature

8.8.2 Introduction Part

- Identification number of the report
- Identification number of a criminal case
- Day, year, time when an examination was begun
- Day, year, time when an examination was completed
- Data, when it was received and registered
- Identification of a case investigator/customer
- List of items which were presented and are to be examined
- List of materials (photos, reports from the scene of crime and so on) which were presented along with a request
- Questions posed by a case investigator/customer
- Short case story, if necessary to explain the further examination procedure

8.8.3 Research Part

- Description of wrappings, labels, seals and so on
- Description of the consistency of wrappings
- Description and photos of exhibits if necessary
- Localization of traces on the items and photos if necessary
- Ascertainment of the soil nature of these traces
- If there are objects of another nature (paint, fibers, glass, etc.) the expert should inform the case investigator and, if necessary (if there is a question of a customer/case investigator), make a complex examination together with an expert of the appropriate other competence
- Recovering of the questioned soil substance/soil traces from the items if possible or/and if necessary
- Selection of a scheme to carry out the comparative examinations
- Citation on literature which was used during the examination

8.8.4 Comparative Study

Examination of the bulk comparative sample(s):

- Determination of characteristics

Examination of the questioned object:

- Determination of characteristics

During the separate examination of comparative and questioned soil samples the expert should determine as many independent characteristics as necessary and enough to discriminate questioned and comparative soil samples, or to determine group belonging of the questioned sample to the source, or to determine the identity of the source. In every case a whole ensemble of independent characteristics has to be established and compared to each other.

8.8.5 Generalizing Part (Results and Discussion)

When generalizing the results of the comparative study, the expert should ascertain the discriminations and similarities in ensembles of characteristics of both questioned and reference soil samples. The expert should describe the characteristics he considers to be in a group or individual and explain why that is so.

8.9 Conclusions

Conclusions should be formulated exactly in compliance with the questions posed by a case investigator.

The written expert report must be verified by the head of the laboratory and then by the Assistant Director on expert work.

The investigation should have a real scientific base, nevertheless the expert report should be written very clearly in simple language. Explanations and interpretations of results must be understandable also for the non-specialist. We consider the interpretation of results and the formulation of answers or conclusions to be one of the most complicated problems in forensic soil examinations. The ability to analyze and interpret characteristics of the soil during forensic soil investigation is a skill, gained by training and testing and by many years of experience.

To our mind however, the mechanism of the identification process in forensic soil examination can hardly be formalized and standardized any time soon. So, as we see it, the assessment of what is, and what is not meaningful or significant and whether an ensemble of characteristics is individual or not, depends mostly on the quality of the forensic examiner.

Owing to national, traditional peculiarities in legal systems and therefore in forensic examination procedures, reporting and presenting results, we deem that verification of professional competence of forensic soil examiners in different countries nowadays may be fulfilled best of all only by their participation in collaborative trials and also by accreditation according to ISO/IEC 17025 and ILAC-G19:08/2014.

We hope very much, that international communities all together will work out a uniform strategy and develop a uniform quality assurance system which will aid to strengthen the evidence of forensic soil examinations and contribute to the acceptance of forensic soil examinations by courts and protect experts from distractions that can be made by lawyers.

Very briefly by giving examples from our practice we'll try to demonstrate the way we come to conclusions and the forms of conclusions we give in our reports.

8.9.1 CASE I Attacks of People of Non–slavic Appearance by a Group of Guys Armed with Pocket Knifes

Two men were attacked in broad daylight by a criminal group of guys armed with pocket knives. It happened in two different sites of one local region. One man died virtually at once on the crime scene from fatal hemorrhage and another was terribly injured but survived.

As we later learned (after requesting the materials of the case) a group of young perpetrators, so called fascists, was walking along one region in the south-west of Moscow looking for victims of non–Slavic appearance.

The surviving person was from Uzbekistan, but he refused to give any witness account and very soon left Russia. The autopsy revealed multiple stab wounds on the body of the murdered man, but only one of them was considered to be mortal. Many people witnessed the criminal fact but all of them affirmed they could not make out the assailant's appearance. One of the guys seriously injured his arm when he was working with his knife and applied to a clinic for a medical advice. All clinics had been informed about the criminal assault to that moment and the young man was detained right in the clinic. Neither knife nor blood or any other evidence that could have been subjected to DNA analysis was found. The case investigator brought us the sport boots taken from the apprehended person and four comparative soil samples, taken nearby the murdered man. Questions posed by the case investigator were:

– Are there soil traces on the boots presented for investigation?
– If so, have they general group belonging with the soil on the scene of crime (the place of murder)?
– Were these soil traces generated at the scene of crime?

Twelve larch (Larix) tree needles and fragments of moss in the questioned soil sample were found. We visited the region where it happened and found out that it was a rather large territory with asphalt roads between multi-storey housing and places with open soil covering. Somewhere soil was covered with grass and moss. Different trees were growing there in the gardens near by the houses. Fourteen larch trees grew along all the way the group of young perpetrators had taken, one of them stood very close to the place of the murder. Samples of needles from the 14 larch trees and samples of moss which was found along the way were collected. Fourteen additional reference soil samples (only samples consistent with the questioned sample in color and granulometric composition) were collected along the entire route of the criminal group.

The characteristics which were taken into account in the comparative investigation were: soil color, texture, degree of carbonate activity (calcareous or not calcareous soil), granulometric composition, mineralogical composition, composition of the pollen-spore complex, anthropogenic particle composition (we call them 'inclusions'), plant remains and plant fragments and DNA analysis of the larch needles.

The results of the comparative study demonstrated that all 14 reference samples were similar in the biggest part of the characteristics with the questioned sample. Two reference samples, one of which was taken just from the scene of crime, had a completely similar ensemble of characteristics as the questioned traces. We made an attempt to differentiate sites with the aid of DNA analysis of the larch needles, but unfortunately DNA analysis gave poor results. So there was no individualizing ensemble of characteristics.

The following answers were given in the report:

– Yes, there are soil traces on the boots presented for investigation.
– he soil traces on the boots have general group belonging with the soil on the scene of crime (the place where the murder took place).
– It is not possible to answer the question "were they generated from the scene of crime?" because of the absence of individualizing characteristics.

8.9.2 CASE II Rape of an 11 years Old Little Girl

According to a girl's testimony she was walking nearby her house in the garden. A young man whom she did not know before and whose appearance she could not remember asked her for a mobile phone to make a very important call. Then he told her that he would return the mobile phone if the girl would follow him. When they reached a remote place near by train tracks and heat supplying pipes, the man threw the girl on the pipes and hit her on the head. The girl blacked out and couldn't remember what had happened after it.

The items for examination were the girl's clothing. The posed question was:

– Are there soil traces on the girl's clothing?
– If so, do they originate from the crime scene?

– What is the localization of the traces?

We requested photos from the scene of crime and analyzed them. Then an investigation was conducted and the following answers were given:

– There are no soil traces on the clothing. There are a lot of small fragments of glass, fibers and fragments of insulating materials.
– It is not possible to answer the question "did the traces originate from the crime scene?" for the reason that such small particles are widespread on urban territories.
– The localization of the traces is very unusual for normal use of clothing and is shown on photos (see the attachment to the report).

8.9.3 CASE III Fall of a Young Lady from the Eighth Floor

A young lady was in the company of two guys. According to the guys testimony the company was sitting in the kitchen. The young lady was drinking a lot. Then the guys went out to the balcony and left the lady alone. When they returned, the kitchen was empty.

The guys found the lady lying facedown on the ground near by the wall of the house and close to the tree. Brunches of the tree were broken and some of them were lying over the body. The lady was dead. Questions posed by case investigator were:

– Are there any soil traces on the lady's clothing? If so, do the traces originate from the place where she was found?
– Are there any soil traces of another origin, if so, what is the region where they might have originated from?

After examination we concluded that the traces on the dress might have been formed as a result of falling through tree foliage, however the quantity of the soil traces was not enough for comparative study. This was all depicted in the research part of the report. The answers given were as follows:

– There are traces of soil and plant nature on the lady's clothing.
– It is not possible to answer the questions "do the traces originate from the place where the girl was found?" and "are there any soil traces of another origin and what is the region where they might have been originated from?" for the reasons described in the research part.

8.9.4 CASE IV The Rape of a 16 years Old Girl

It happened in one of the Moscow regions. A 16 years old girl, covered in mud, came to the police and stated that she was raped when returning home from her friends in another village in the late evening. The girl told that when she was

walking along the road near by a forest somebody attacked her from the backside, grappled her neck and dragged her into the forest. Then the malefactor threw her down on the earth and raped her. To check the girl's words the police officer asked the girl to show him the place where the accident has happened and clarify how she was lying on the earth. The questions posed by the police officer were:

– Are there soil traces on the girl's clothing (jacket and jeans)?
– If yes, then, what is their localization?
– If yes, do they have a general group belonging with the top soil on the crime scene?

The items were thoroughly examined and it was stated that there were really soil traces on the girls clothing. Their localization fully corresponded with the girl's testimony. The quantity of soil traces was very small, but we could make a comparison on a number of characteristics, including a very specific ensemble of small particles, which was extracted from them. It was established that the set of characteristics of the reference sample was similar with those of the soil traces. We could not confirm that the whole complex of determined characteristics was individual. So the following answers were given:

– Yes, there are soil traces on the girl's clothing (jacket and jeans).
– The localization of the soil traces is described in the research part of the report and is shown on photos that were included as an attachment to the report. The localization of traces on the girl's clothing fully correspond with her testimony.
– Soil traces on the girls clothing have a general group belonging with top soil on the crime scene.

8.9.5 CASE V An Auto Theft with the Murder of the Owner

Three years ago, in the beginning of June, a woman came to a police officer and said that her husband went to a lake in his car for fishing and disappeared. A month later he was found murdered. The corpse was found in a pit, which was situated in a forest on the slope of a hill. The case investigator got information that the man was the victim of criminal gang members that stole expensive autos for spare parts and killed their owners. Soon two offenders were detained. A shovel and an axe with soil traces and plant fragments were found in their garage.

The questions posed by the case investigator were:

– Are there soil traces on the shovel and the axe?
– If yes, do they generate from the pit where the murdered man was found?

When visiting the crime scene we examined the pit where the corpse of the victim was found. We took reference soil samples from the walls of the pit at different depths. Also we examined the vegetation and noticed that the pit was dug near a birch tree. We saw that roots of this birch tree were damaged very specifically, most

likely as a result of using a shovel and axe as tools for digging. Soil traces and soil samples taken from the scene of crime were similar in the whole ensemble of characteristics: color, micro texture, lack of carbonate activity, granulometric composition (very specific and typical for wash-inwash soils), mineralogical composition, content of decomposed organic matter, chemical composition of the clay fraction. Also they were similar in the composition of plant fragments and pollen and spore spectra. The parts of roots of birch tree in the traces had the same type of damage as the roots in the pit.

Though we had a wide specter of similar independent characteristics we could not establish identity in that case, because the offenders denied the murder of the man. They insisted that they did not see the victim at all, but saw only a car with open doors and a murdered dog lying near by it. They said they used shovel and axe when burying the dog's corpse. The dog's burial place has not been found. So it was impossible to prove or disprove this declaration. The following answers were given:

– Yes, there are soil traces on the shovel and axe.
– To answer the question "do the traces generate from the pit where the murdered man was found?" is not possible because there are no known samples from the dog's burial place.

8.9.6 CASE VI A Murdered Man on a Snow Cover

A murdered young man with a fractured skull was found in winter lying on a snow cover nearby an asphalted road in the Moscow region. Soil traces were easily seen on his jeans, though there was no place with soil covering around the corps. These soil traces seemed to be very unusual for that season. Questions posed by the case investigator were:

– Are the traces on the jeans really soil traces, if so, what are the characteristics of the soil covering on that place?
– How did the traces on the jeans get there?

After examination we gave the following answer:

– The soil traces might be the result of the jeans contact with a wet soil, contaminated with indoor anthropogenic particles.

Two years passed and the case investigator found two suspects and the place where it might have happened. The fact of the matter was as follows. Three men were sitting in the kitchen in the cottage of the future victim drinking spirit. After they drank a lot they began to quarrel. One of them took a bottle and hit the owner of the cottage on the head. The man fell down on the floor and seemed to be dead. The fellows were frightened and hid him till night in the cellar, the entrance to which was situated in the floor of the kitchen. At night they brought out the body to the forest by car and threw it on the snow. The posed question was:

– Do the soil traces on the jeans really originate from the cellar of the cottage situated at the appointed address?

Apart from soil traces small particles of a different nature were determined in the traces on the questioned jeans. Forensic experts of different disciplines were involved in the process of this examination. A complex, interdisciplinary examination was conducted. It was stated that soil samples, taken from the cellar and soil traces taken from the questioned jeans had the same complex of particles with the same characteristics. It was possible to state that this complex of characteristics was individual and the following answer was given:

– Yes, the soil traces on the jeans originate from the cellar of the cottage situated at the appointed address.

Literature

Collins D, Johll ME (2006) Investigating chemistry in the laboratory. W.H. Freeman, New York, p 202

Federal Law on State forensic activity in the Russian Federation (№73 – FZ, May 2003)

Association of Forensic Science Providers (2009) Standards for the formulation of evaluative forensic science expert opinion. Sci Justice 49:161–164

Aitken CGG (2009) Some thoughts on the role of probabilistic reasoning in the evaluation of evidence. In: Ritz K et al (eds) Criminal and environmental soil forensics. Springer, Netherlands, pp 33–47

Gradusova O, Nesterina E (2009) The current status of forensic soil examination in the Russian Federation. In: Ritz K et al (eds) Criminal and environmental soil forensics. Springer, Netherlands, pp 61–73

Mitrychev VS (1976) Criminalistic identification of the whole in parts. In: Theory and practice of identification of the whole in parts. Collection of research works 24. VNISE, Ministry of Justice, Moscow, pp 3–111

Stoney DA (1991) What made us ever think we could individualize using statistics? J Forensic Sci Soc 31(197):199

Sjerps MJ, Berger CE (2012) How clear is transparent? Reporting expert reasoning in legal cases. Law Probab Risk 11:317–329

Part II
Environmental Soil Forensics: Tools for Spatial and Chemical Analysis

Chapter 9
Geographical Information Systems – A Working Example in the Brazilian Federal Police for Fighting Environmental Crime

Daniel Araujo Miranda and Daniel Russo

Abstract A Geographical Information System (GIS) can store and publish information that can be of great use for soil forensics experts. Aerial imagery, weather data and location of previous forensic activities are but some examples of information that may be hard or impossible to obtain with just a field trip, and can be easy to access using GIS.

This article presents the usefulness of GIS for soil forensics. It describes a few alternatives to specify the desired data, most likely places to locate it and ways to access and use the information. Brief considerations are provided to help the reader decide if it makes sense to build a GIS inside his or her organization.

An example is provided of the Inteligeo system in the Brazilian Federal Police. Inteligeo is a country-wide database for information that is used for several types of forensic activities and administrative tasks, mostly regarding environmental crime. It concentrates information from several government sources, including data produced by the Federal Police Forensics, and provides tools to analyze the information and make searches and reports.

9.1 Introduction

Geographical Information Systems (GIS) are used to store, distribute and provide analysis tools for geographical data. They can be of great use to a forensics expert to gather information beyond what can be obtained by examining a crime scene. Not all relevant information can be obtained with a field trip and laboratory tests. It is possible to determine, for example, the nature of a soil contaminant by examination,

D.A. Miranda (✉) • D. Russo
Inteligeo – Geographic Intelligence, National Institute of Criminalistics,
Brazilian Federal Police, Setor Policial Sul, 70610-200 Brasilia – DF, Brazil
e-mail: miranda.dam@dpf.gov.br; russo.dr@dpf.gov.br

H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics,*
Soil Forensics, DOI 10.1007/978-3-319-33115-7_9

but the analyst would be hard pressed to determine whether a previous incident had already been investigated in the area or if there was a legal permit for the use of that chemical in a neighboring chemical plant. Such information can be useful for the forensics work, and even though it is not available from the scene or is hard to obtain, it can be sourced from other parties. For example:

- The location and characteristics of nearby geographical features like rivers, valleys and slopes.
- The forensic reports concerning previous incidents in the region.
- The legal status of the area (currently and previously licensed activities, property boundaries, and special protection status)
- Geological data of ground water distribution.
- Weather data of seasonal rain and floods.
- Demographics of the affected population (quantity, sanitary conditions, age distribution)
- Satellite or aerial imagery from before and after an event.

The relevance of the examples above in a soil pollution scenario is straightforward. Some of the data will allow the examiner to estimate specific time frames for the events, use other reports as reference, quantify the victims and recommend further sanctions against the offender (for example, for using a chemical without a permit, repeating an offense or operating in a protected area). There are many other types of data that are shared by institutions with the aid of GIS. It is important for the forensics expert to be aware of the available sources and have the knowledge to handle the information from them.

If an institution makes extensive use of GISs from external sources or produces geographical information itself, it may make sense for it to build its own GIS. The Brazilian Federal Police has a working implementation of a geographical intelligence system that is used in the entire country, mostly by forensic experts in the environmental area. The system aggregates information from several sources, including internally produced data, and provides tools for searching, analyzing and exporting.

9.2 Specifying, Locating and Using Information

The first step to procure information is to specify the requirements. Sometimes multiple sources, formats and versions are available for the same information, for example satellite imagery and topographic data. It is, therefore, important to decide not only which information is required, but also details such as spatial resolution, date of generation and presentation. Topographical data, for example, may be presented as an ASTER DEM or SRTM grid, which have a spatial resolution between 30 m (ASTER, SRTM over US) and 90 m (SRTM outside US) and are available as GeoTIFF files. Another possible presentations are a “spot elevation” map, which displays points and their elevations, a contour line map, which displays lines of equal elevation, and a slope grid, which may be a color coded raster image

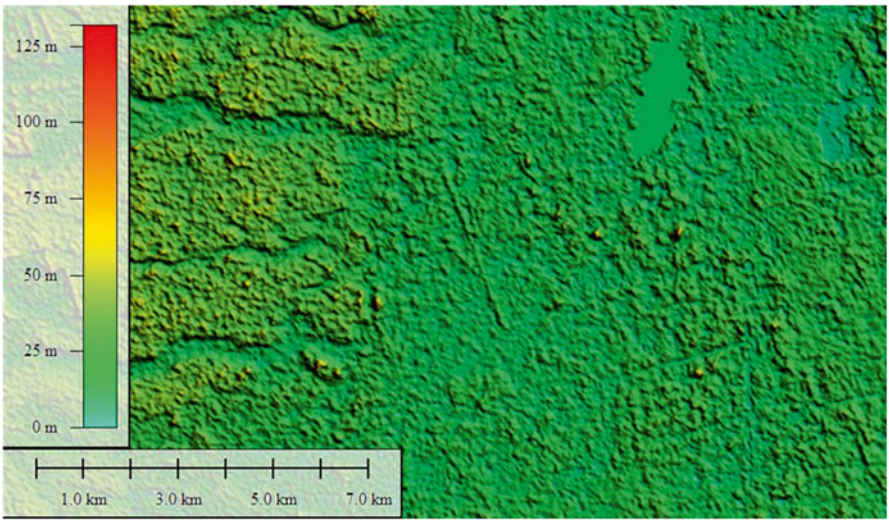


Fig. 9.1 Aster GDEM elevation map of a small region around 20°S, 40°W

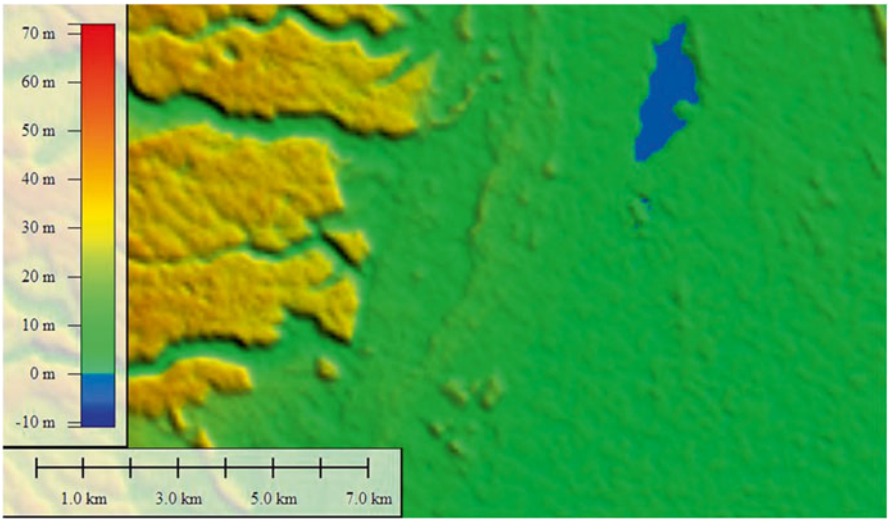


Fig. 9.2 SRTM elevation map of the same region of Fig. 9.1

representing the average terrain slope. Examples are given in Figs. 9.1, 9.2, and 9.3, comparing different sources and representations for elevation data on the same area. Satellite images may also differ greatly depending on the sensor and mode of operation, the most fundamental factor being which part of the electromagnetic spectrum is used. Figures 9.4 and 9.5 show the difference between an image from the optical sensor AVNIR of the ALOS satellite and an image from the synthetic aperture radar PALSAR from the same satellite.

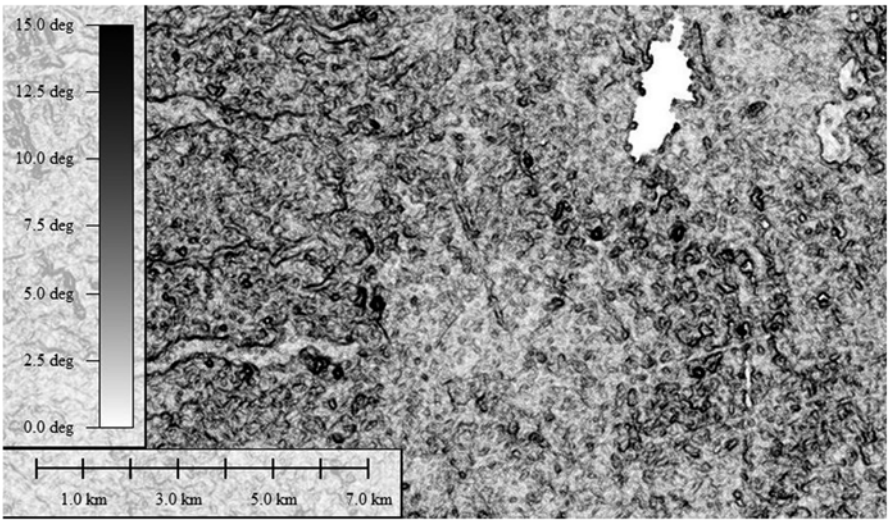


Fig. 9.3 ASTER GDEM slope map of the same region of Fig. 9.1

Fig. 9.4 ALOS AVNIR Image of the city of Brasilia using bands 1, 2 and 3 mapped to the B, G and R channels respectively

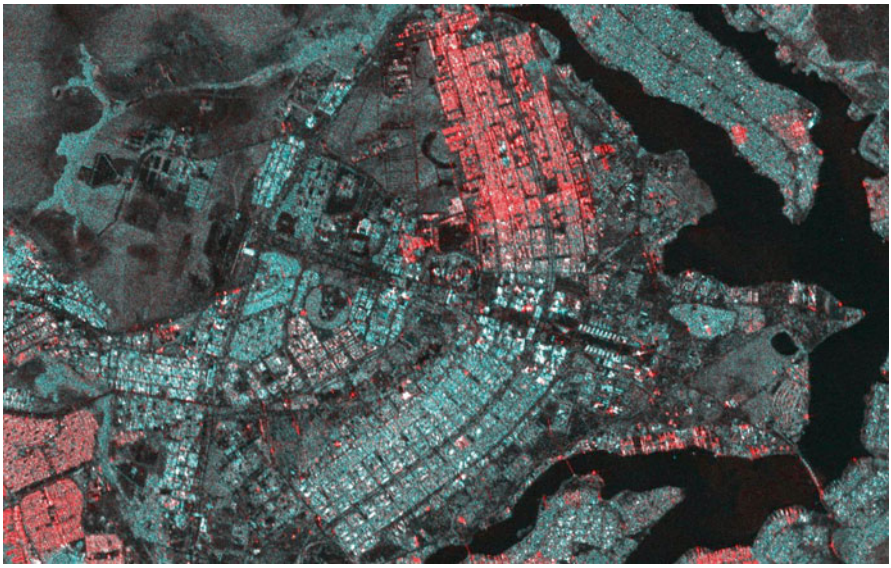


Fig. 9.5 ALOS PALSAR synthetic aperture radar image of the city of Brasilia using bands HH and HV mapped to the R and G/B channels respectively

Once the basic requirements are known, a suitable source must be found. Brazil has a centralized metadata catalog, INDE's GeoNetwork portal, in which this information may be searched.

In the Brazilian Federal Police experience, these sources fall mostly in three categories:

- Government agencies produce and share the largest amount of geographical data. Efforts have been made to standardize the presentation of the information such as the European Union's INSPIRE initiative and Brazil's INDE, but there are many data publishers and not all of them comply with the standards, where standards exist. Information from government sources is usually available freely on the Internet.
- Private institutions also hold and sometimes share geographical data. Examples of popular sharing interfaces are the map portals of Google and Microsoft; Digital Globe provides a delivery portal called Global Basemap for paid content. The usage of that data may be limited in several ways, including copying, modification and/or production of derived works (such as an illustration in a forensic report) or use for commercial purposes. On the other hand, the data may be available for purchase and the customer may be able to ask for a custom product.
- Crowd sourced databases [wikimapia, openstreetmap] are updated by the general public and contain much information found nowhere else. Since there are no guarantees about the quality of that information, their usefulness for soil forensics is limited. They may be helpful for intelligence and logistics, but other sources should be used when correctness is required.

Ideally, the data should not need to be downloaded entirely. Some sources display it in a convenient web map with a few tools, others provide web services so that you can use your preferred desktop application to connect to the data, and others provide means to download all or part of the data to use it locally. To understand the best way to use the information, one may ask: Is a web map provided? Will only a small portion of it be needed? Will it be cross-referenced with other data? Does the user have proficiency with geographical desktop software such as ArcGIS or QGIS? Is a numerical analysis necessary or is it sufficient to only look at the data? The user can then make a choice based on the answers to these questions.

If enough people in an organization have to go through these steps, it may make sense for the organization to have its own GIS, even if only for storing updated, organized and translated data from other parties. A centralized repository avoids work replication, maintains a more consistent representation of external data inside the organization, allows better quality control and more specialized handling of the input data, and provides a single point where the user can find the most used data. If the organization itself produces geographical information, that system can be used to publish the data.

9.3 Inteligeo – A Working Solution in the Brazilian Federal Police

The Brazilian Federal Police fights crimes of national concern. It is responsible for international borders, coast patrol, gun control, drug traffic repression and other crimes of federal competence. As of June of 2016, it has about 12,000 policemen of which 1,140 are forensic experts in several areas (civil engineering, environmental, electronics, chemistry, computers, CSI, medical, document forgery and others). The Federal Police investigates many cases of environmental damage, and about 3000 forensic reports are generated every year on environmental issues alone. The most frequent crimes are deforestation in protected areas, water and ground pollution, mining without a license and trafficking of animals.

Environmental forensics demands a high level of situational awareness of the region of interest. The amount of environmental damage depends on features like climate, presence of water bodies, soil characteristics, human occupation, etc. On the other hand the legal sanctions depend on features like the presence of environmental reserves, indigenous reservations, protected areas, mining licenses, etc. To make a forensic analysis, all this information from several sources needs to be available and gathering this kind of information is very labor intensive in Brazil. To help solve this problem on a national scale, the Brazilian Federal Police developed a system called Inteligeo.

Inteligeo is a modern and powerful Web GIS system that supports forensic analysis and integrated information management in Brazilian Federal Police. Several data sources from Brazilian government institutions are integrated into the system and

the data is published to all forensic experts using the police's internal network. The main sponsors of the project where JICA (Japan International Cooperation Agency), UNODC (United Nations Office on Drugs and Crime) and FINEP (Brazilian Fund for Research and Projects), which provided training, consulting, equipment and funds for the implementation of the system. More than 800 information "themes" are provided, of which some of the most prominent are enumerated below and displayed in Figs. 9.6, 9.7, 9.8, 9.9, 9.10, 9.11, 9.12, and 9.13:

* Location and content of previous forensic reports
* Federal Police installations location and jurisdiction limits
* Roads, rivers and water bodies
* Environmental Protection Areas
* Indigenous lands
* Mining licenses
* Political limits
* Satellite images (stored in the Federal Police and of images stored in other agencies)
* Rural properties boundaries and owners
* Unofficial roads and landing strips
* Statistical maps of drug seizures, pesticide forensics and of public works fraud

The system uses a web interface to display the information on top of a map. The user can switch "layers" on and off, change the background map, and use several tools to analyze and query the data. One of the tools allows the user to search for a "layer" by name. There is an advanced search tool that performs searches within a polygon with an attribute filter and the output can be exported as a spreadsheet, KML file or used as an input to other search. By clicking on the map a window is displayed with information about the features that were clicked upon (see Fig. 9.14). There are also tools for drawing and measuring that can be customized with color, transparency and annotations for reporting purposes (Fig. 9.15). A user can load files in KML, Garmin GPX and Shapefile formats directly on the web interface, perform edits and then export the data to KML format. Two analysis tools stand apart: the graphics/dashboard tool (see Image Fig. 9.16) and the density map; both are of little use for the forensics expert on the field, but they produce invaluable information for management. Almost any data can be used to plot a graphic and any point data can be used to plot a density map (see Fig. 9.17).

A typical use case of a Federal Police forensic expert would be to first identify if a forensic report already exists about the area, and if there is, to access the content of the report by clicking on an icon. The expert then checks if there is a valid mining permit and displays the authorized activity, the name of the party and the expiration date, checks if the area is inside an environmental reserve, indigenous land or other protected area. The expert would then check the access paths to be used to get to the area, including rivers and unofficial roads. After a field trip for examining the area, the expert would then use the drawing and measuring tools to annotate the map and export the image to use in the forensic report.

Fig. 9.6 Inteligeo web interface displaying the location of the installations, jurisdictional limits and political limits

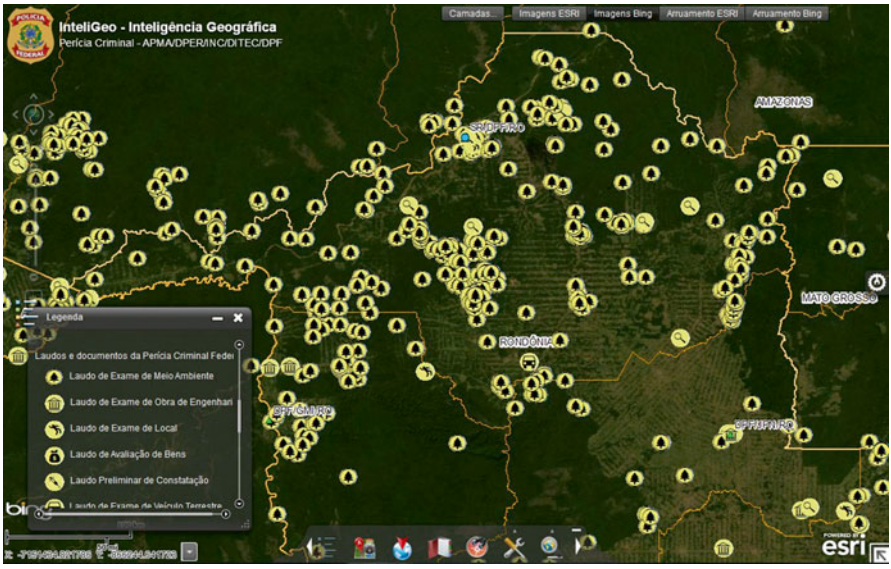


Fig. 9.7 Inteligeo web interface displaying the location of the forensic reports produced in the region of Rondônia

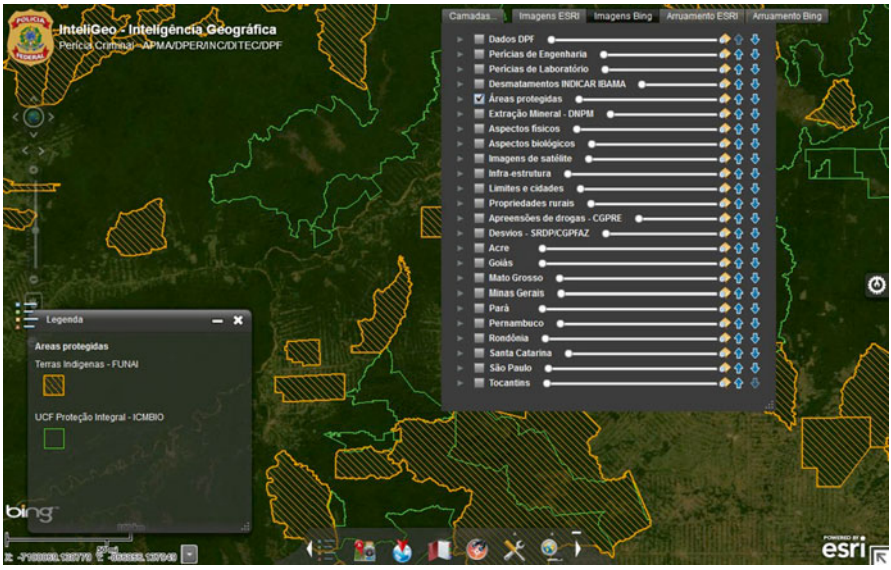


Fig. 9.8 Inteligeo web interface displaying the location of indigenous lands and environmental protection areas in the region of Rondônia

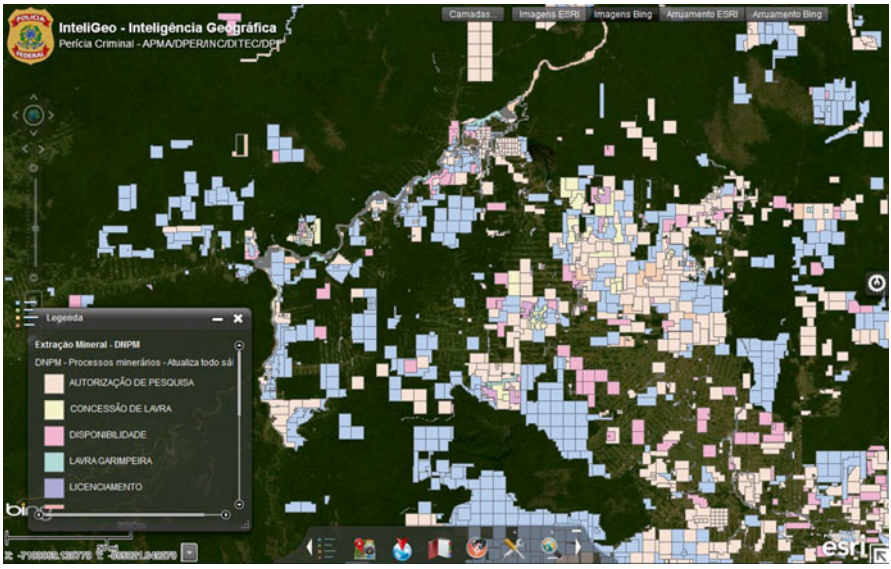


Fig. 9.9 Inteligeo web interface displaying the mining licenses in the region of Rondônia

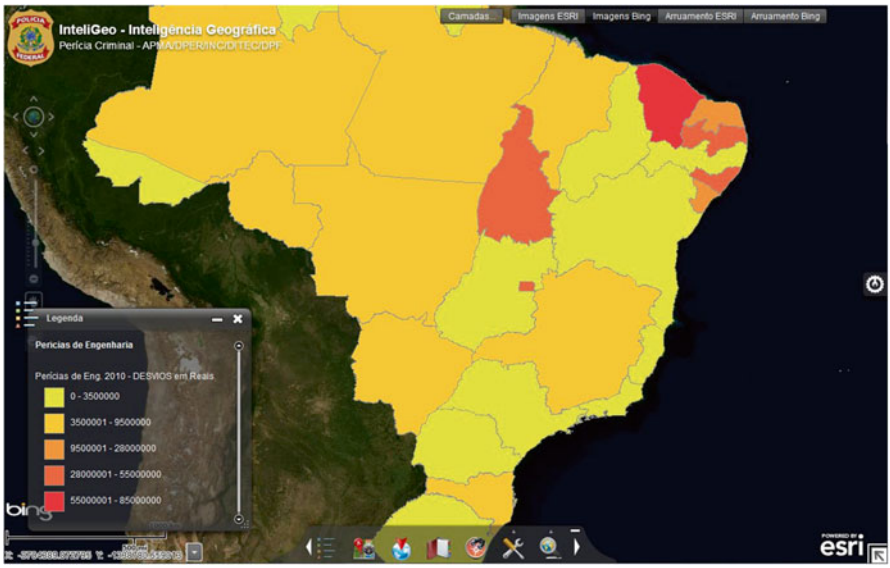


Fig. 9.10 Inteligeo web interface displaying the amount of fraud in public works in the year of 2010 detected by the Civil Engineering Forensics of the Federal Police

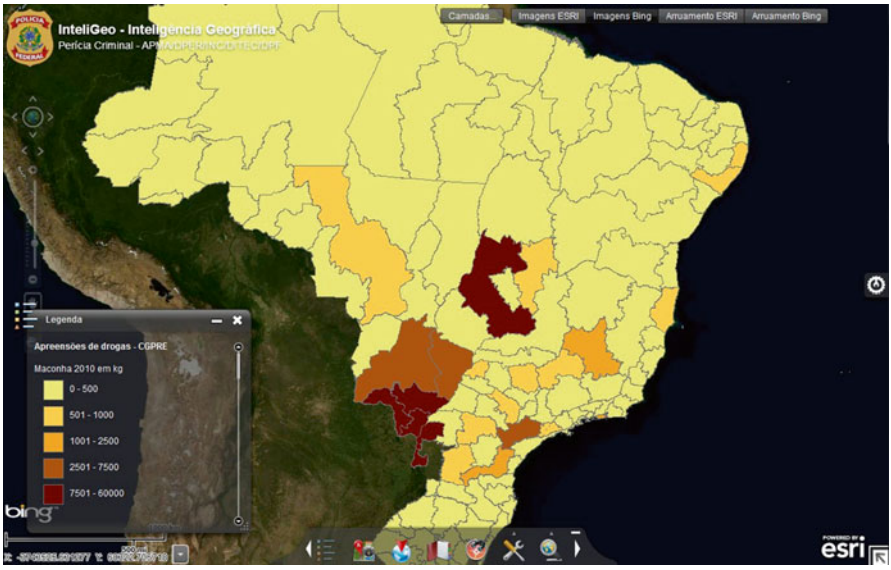


Fig. 9.11 Inteligeo web interface displaying the amount of Cannabis Sativa seized by the Federal Police in the year of 2010 in each jurisdiction

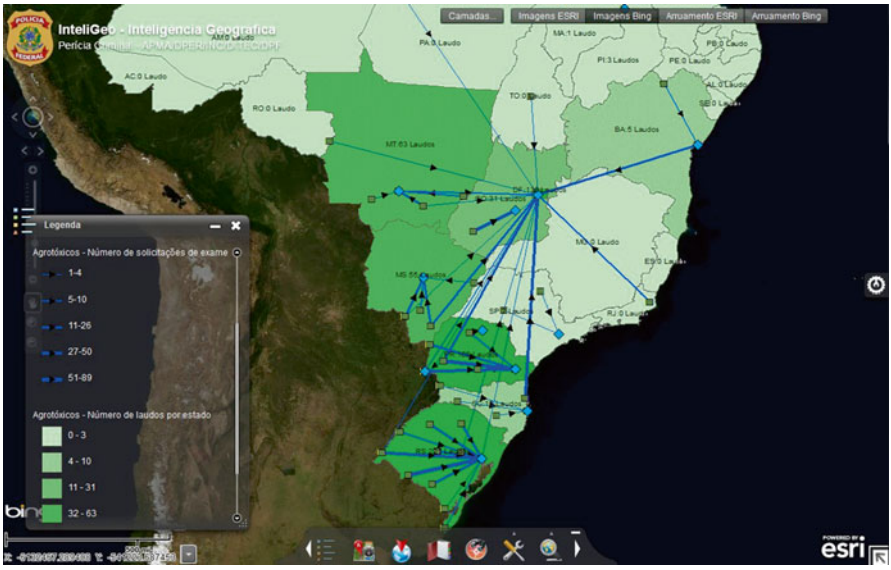


Fig. 9.12 Inteligeo web interface displaying the amount of forensic reports of pesticides produced in the year 2012 and the flow between units of material to be analyzed

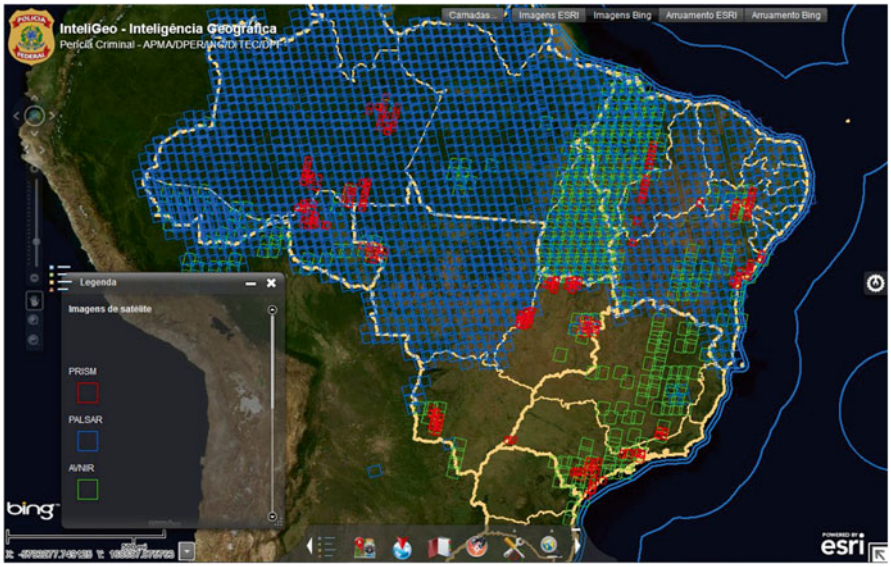


Fig. 9.13 Inteligeo web interface displaying the footprints of the ALOS satellite images stored in the Federal Police. To download the images the user clicks on the footprint and then in the download link

Fig. 9.14 Inteligeo web interface showing information about features clicked on the map

Fig. 9.15 Inteligeo web interface showing drawings, annotations, measurements and the customization window of the drawing tool

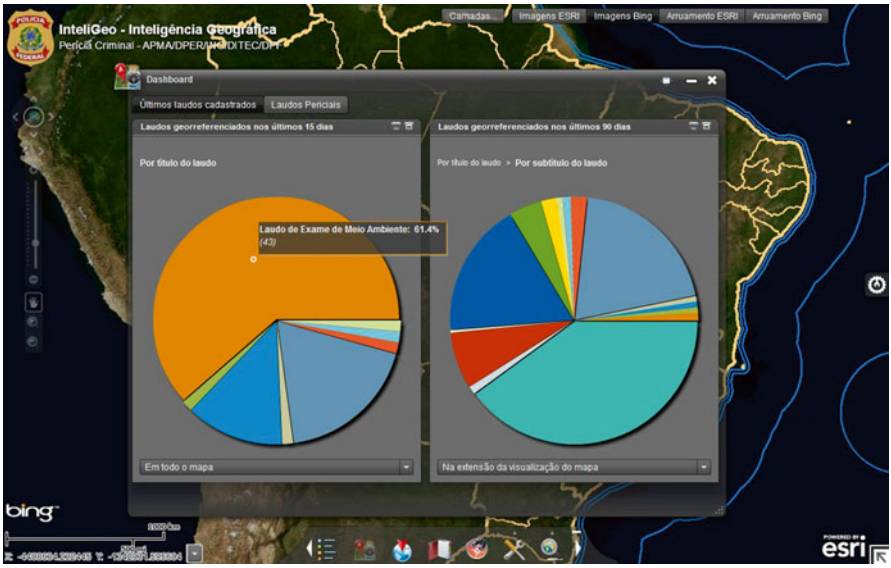


Fig. 9.16 Inteligeo web interface showing statistics about all forensic reports produced in the last 15 days and last 90 days. It is possible to define a polygon in the map for the graphics and they will be recalculated in real time using only the data inside the polygon. Clicking on the "slices" will "drill down" and present a more detailed graphic about the data subset selected

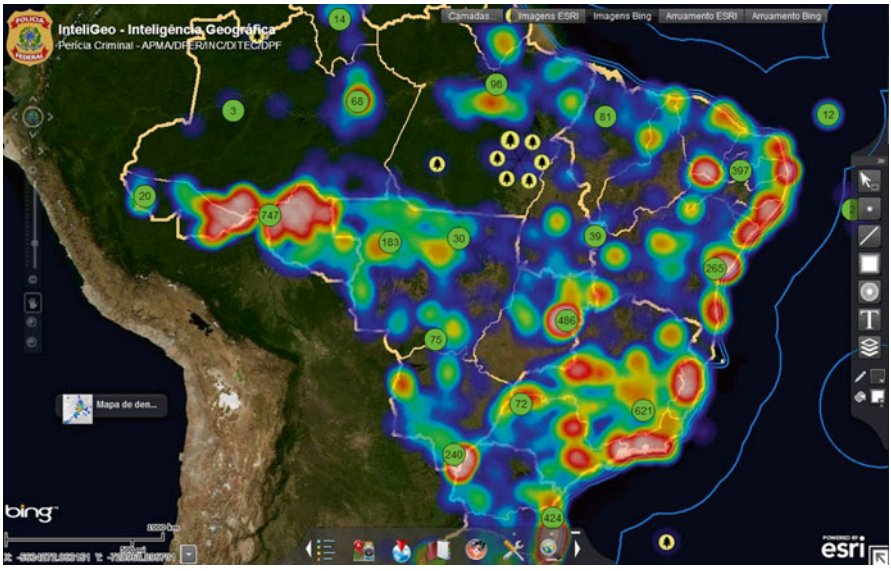


Fig. 9.17 Inteligeo web interface showing a density map of all the environmental crime forensic reports. The *green circles* show the amount of samples in each group. Hovering the mouse over a small group will pop up the icons of the reports in that group allowing the user to retrieve information for each one

A number of technologies are used as the foundation of the system. Most important, the data is stored in an entirely open source structure which is of paramount importance to maintain control of the information. The maps are generated from the raw data and displayed using proprietary software and a web interface. This closed-source middleware is also responsible for processing some of the spatial operations provided by the web portal.

With the continuous improvement and inclusion of new tools, it is already possible to use the system to aid in management decisions. It is possible, for example, to identify areas of intense forensics demand and request satellite images proactively. There are already several statistic reports available on the data set such as the drug seizure and the public works fraud maps.

The system had a good reception among users. Most of them have an opinion that the interface is good looking but still a bit awkward. It has greatly eased the access to commonly used data and has transferred the quality control and translation of information tasks to the core maintenance team. The experts now have and increased spatial awareness and access to historical data regarding environmental forensic reports. Other areas in the police are starting to use the system to display their own data: Engineering forensics, drug repression and logistics are among them.

Chapter 10
Forensic Characterization of Gasoline Releases Impacting the Environment

Gil Oudijk

Abstract Many different environmental-forensic techniques are needed when investigating the spillage of gasoline to soil or groundwater. An important laboratory analytical technique is known as a PIANO analysis, which can identify hundreds of different hydrocarbons in gasoline. Through interpretation of these results, the origin and age of the gasoline can often be determined and, hence, responsibility for the release can may be identified.

What is environmental forensics? One definition is that it is the use of environmental expertise to resolve disputes, whether or not those disputes might be environmental in nature. But why is this field important in the United States or in any other industrialized country? The United States manufactures about 300,000,000 gallons (about 1,200,000,000 L) of gasoline per day (Dickson et al. 1987), whereas Canada, Europe and Japan are not far behind on a per capita basis. Even if the spillage rate is only 0.01 % of the quantity manufactured, the amount of gasoline released to the environment would still be more than 30,000 gallons (>100,000 L) per day, just for the United States and no one wants to pay for those cleanups. The problem in modern society is that environmental cleanups are quite expensive and no one will pay for them unless they are forced to. It is from this problem that forensic scientists earn their living. Might there be other responsible parties to help pay for these cleanups? Regulatory agencies, who are often left holding the bill for these cleanups, often employ these techniques to help find additional contributors. The forensic techniques described herein can be helpful if other responsible parties do exist.

In general, there are two important types of forensic studies being performed nowadays on environmental cases, especially the ones involving the spillage of gasoline (Oudijk 2005):

- *Fingerprinting*: What type of gasoline was released? Regular? Premium? How was it manufactured? Who manufactured the gasoline? (This question is often very difficult to answer because gasoline is a fungible commodity, meaning that it can be bought, sold and traded between the refining companies) (Oudijk 2007).

G. Oudijk (✉)
Triassic Technology, Inc., 57 Hamilton Avenue, Hopewell, NJ 08525, USA
e-mail: goudijk@triassictechnology.com

H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics*,
Soil Forensics, DOI 10.1007/978-3-319-33115-7_10

For example, the gasoline found in an underground tank owned by Chevron was not necessarily produced by Chevron, and

- *Age dating*: When was the gasoline manufactured and, hence, how old is the release? One should keep in mind that the "shelf life" of gasoline (or how long it might be stored) is commonly less than 2 weeks and the date of manufacture for a gasoline is commonly fairly close to when it was released.

This above information often allows us to determine who was responsible for gasoline releases.

In the United States, the majority of the environmental cases that end up in litigation are civil cases and not criminal cases. In my experience, the majority of the criminal cases are resolved before they ever reach a courtroom. However, the methods explained in this paper should be applicable whether the case is civil or criminal.

10.1 The PIANO Analysis

A PIANO analysis is a laboratory procedure through gas chromatography coupled with mass spectroscopy. PIANO is an acronym for *n*-paraffins (P), *iso*-paraffins (I), aromatics (A), naphthenes (N) and olefins (O), what this analysis can detect (Peters and others 2005). Oxygenates can also be identified through this analysis.

Many or most of our fingerprinting and age-dating questions can be answered through an interpretation of a PIANO analysis. This procedure allows us to identify and quantify 500–1000 different hydrocarbons in gasoline and they are presented in either weight%, volume% or molar concentration. Many of these hydrocarbons would not be identified on the normal scans used for regulatory compliance.

The five hydrocarbon classes in the PIANO analysis are (Kaplan and others 1997; Kaplan 2003):

- paraffins (P) or "normal alkanes": straight-chain saturated ("aliphatic") hydrocarbons (C_nH_{2n+2});
- *iso*-paraffins (I) or "i*so*-alkanes": branched-chain saturated hydrocarbons (C_nH_{2n+2});
- aromatics (A): carbon chains with a double bound after every second carbon (C_nH_{2n-6});
- naphthenes (N) or "*cyclo*-alkanes": saturated carbon chains (C_nH_{2n+2});
- olefins (O) or "alkenes": unsaturated hydrocarbons with double bounds (C_nH_{2n}).

These are the predominant hydrocarbons in gasoline (plus the oxygenates). What does the laboratory need to perform this analysis? Separate-phase gasoline, but more than a sheen and at least a film of gasoline, preferably a layer. Can the analysis be performed on soil samples? Yes, the analysis can be performed, but because of hydrocarbon adsorption, it will probably adversely impact the subsequent interpretation of the results.

The laboratory methods used with a PIANO analysis are similar to those used for volatile and semi-volatile analyses that are often required by regulatory agencies. With both, a gas chromatograph is employed equipped with a mass spectrometric detector (GC/MS).

10.2 Data Interpretation

Through the PIANO data, we normally look at three factors impacting the chemical composition of the gasoline:

- the magnitude of environmental weathering;
- refining characteristics: the types of methods used, the grade of gasoline, etc., and
- compliance with regulatory requirements (in the United States: now known as reformulated gasoline or "RFG").

All of this information very often will help us to characterize the gasoline and, hopefully, identify its origin.

Normally, we look at several ratios of hydrocarbons obtained from the PIANO scan. Commonly, they are hydrocarbons of the same molecular weight, but with different environmental characteristics, such as volatility, solubility or biodegradability (Kaplan et al. 1997; Peters et al. 2005). It is commonly helpful to perform the PIANO analysis on known gasoline samples for comparison purposes. For example, samples of premium- and regular-grades of gasoline obtained from a nearby service station.

Provided in the paragraphs below are some of the important ratios of hydrocarbons obtainable from a typical PIANO analysis. These ratios were prepared for gasoline manufactured in the United States, but these principles should be fairly close for other countries where modern refining techniques are implemented and similar environmental regulations are in place.

10.3 Biodegradation Ratios

- *(iso-paraffins + naphthenes)/n-paraffins*: Gives us an approximation of the magnitude of biological alteration ("biodegradation"). The ratio normally ranges from about 2 to 10 in fresh gasoline, but it increases as biodegradation proceeds because the *iso*-paraffins and naphthenes (*cyclo*-alkanes) are more resistant to biological alteration compared to the *n*-paraffins, and
- *methyl-cyclo-hexane/n-C_7 (n-heptane):* Normally, this ratio is around 0.5–2.0 in fresh gasoline, but it can vary. The ratio increases as biodegradation proceeds because methyl-c*yclo*-hexane is more resistant to biological alteration compared to *n*-heptane.

10.4 Dissolution Ratios

- *[Benzene + Toluene]/[Ethylbenzene + o,m,p-xylenes]:* In fresh gasoline, this ratio normally ranges from about 0.8 to 1.1 (Kaplan et al. 1997). The ratio then declines as dissolution of the aromatics proceeds because benzene and toluene are more water soluble compared to ethylbenzene and the *o,m,p*-xylenes. However, this ratio is highly dependent on the geochemical conditions or the type of microbes present (Alvarez et al. 1998). Under anaerobic conditions, the ratio could reverse;
- *Benzene/cyclo-hexane:* This ratio provides an approximation of the magnitude of dissolution or "water washing". The ratio is normally, between 0.5 and 2.0 in fresh gasoline and it declines as dissolution proceeds because benzene is more water soluble compared to *cyclo*-hexane, and
- *Toluene/methyl-cyclo-hexane:* Normally, the ratio ranges from about 2 to 10 in fresh gasoline. The ratio declines as dissolution proceeds because toluene is more water soluble compared to methyl-*cyclo*-hexane.

10.5 Evaporation Ratios

- *n-pentane (n-C_5)/n-heptane (n-C_7):* This ratio provides an approximation of the magnitude of evaporation of the gasoline. Normally, the ratio is around 0.5–2 in fresh gasoline and it declines as evaporation proceeds because *n*-pentane is more volatile compared to *n*-heptane, and
- *2-methylpentane/2-methylheptane:* Normally, the ratio is between about 3 and 8 in fresh gasoline. The ratio declines as evaporation proceeds because 2-methylpentane is more volatile compared to 2-methylheptane.

With the evaporation ratios, we can often assess whether the gasoline release was aboveground or underground; for example, from (1) an underground tank or subsurface pipeline or (2) an aboveground tank. Gasoline from releases that had any residence time in contact with the atmosphere will normally be more evaporated, especially near sea level where the atmospheric pressure could be much higher. Based on this knowledge, better insight into who may be responsible for a particular spill event may be more easily achieved.

One should compare the results to a PIANO analysis of a local and recent gasoline for that particular area (keep in mind that the octane rating of gasoline and, hence, the chemical composition will be dependent on elevation). For example, do not compare the recent results from a sea-level site to 1980 gasoline from Switzerland (in the mountains) (Table 10.1). The original chemical compositions of these gasolines could be radically different and this type of comparison would not tell us much.

Table 10.1 Example of a comparison of PIANO analyses of gasoline separate-phase samples from two gasoline service station sites in 2008 to a sample collected from a spill site in New Jersey

Method	Formula	NAPL sample MW - 8	*Compariso:* 2008 Exxon regular unleaded gasoline[1]	2008 Gulf regular unleaded gasoline[2]	Significance	Explanation
Environmental:						
Biodeg	$(iso \text{ paraffins} + \text{naphthenes}) / n \text{ paraffins}$	4.61	2.29	8.54	Biodegradation minimal	Ratio increases s biodegradation proceeds because the *iso*-paraffins and naphthenes are more resistant to biological alteration compared to the *n*-paraffins.
Biodeg	$\text{MCH} / n-\text{C}_7$	0.70	0.57	0.84	Biodegradation minimal	Ratio increases as biodegradation proceeds because methyl-*cyclo*-hexane is more resistant to biological alteration compared to *n*-heptane.
R_b	$(\text{B}+\text{T})/(\text{E}+\text{X})$	0.04	1.12	1.07	Dissolution significant	The ratio declines as dissolution of the aromatics proceeds because benzene and toluene are more water soluble compared to ethylbenzene and the *o*, m, p-xylenes.

(continued)

Table 10.1 (continued)

Method	Formula	NAPL sample MW - 8	*Compariso:* 2008 Exxon regular unleaded gasoline[1]	2008 Gulf regular unleaded gasoline[2]	Significance	Explanation
Water wash	B / *cyclo* – hexane	0.00	0.45	1.41	Dissolution significant	The ratio declines as dissolution proceeds because benzene is more water soluble compared to *cyclo*-hexane.
Water wash	toluene/MCH	0.22	9.25	5.6	Dissolution significant	The ratio declines as dissolution proceeds because toluene is more water soluble compared to methyl*cyclo*- hexane.
Evap	n – pentance / n heptane	0.00	0.13	0.23	Evaporation significant	The ratio declines as evaporation proceeds because *n*-pentane is more volatile compared to *n*-heptane.
Evap	2 - methylpentane/2 - methylheptane	0.62	7.49	4.83	Evaporation significant	The ratio declines as evaporation proceeds because 2-methylpentane is more volatile compared to 2-methylheptane.

Table 10.2 Conventional versus reformulated parameters in the United States and Canada

	Limit	US	Canada
Benzene	<1 %	1995	1998
Oxygenates	>2 %	1995	1998
Total aromatics	<35 %*	1995	1998

These parameters are for North America only. There are similar regulations in Europe.
* Not a regulation, but vapor-pressure limit normally cannot be met with total aromatics at >35 %.

- *2,2,4-tmp/(2,2,4-tmp + 2,2,3-tmp + 2,3,4-tmp + 2,3,3-tmp):* Alkylation is the transfer of an alkyl group from one molecule to another. The most important alkylation process is the reaction with a catalyst (normally sulfuric or hydrofluoric acid) of *iso*-butane with olefins to produce *iso*-octane (Beall et al. 2002). Values for this ratio of between 0.54 and 0.73 represent hydrofluoric acid (HF) alkylation, whereas values between 0.39 and 0.45 represent sulfuric acid (H_2SO_4) alkylation. Alkylation is a refining technique used to produce high-octane *iso*-alkanes, like *i*-C_8 and has been used in refining for several decades, and

10.6 A Comparison to Reformulated Gasoline

In the United States, the chemical composition of gasoline was altered in 1995 to lessen air pollution. This gasoline is known as reformulated or "RFG" in the United States. Similar regulations were enacted in Europe and Canada at around the same time (Table 10.2). For the most part, these regulations lowered the vapour pressure of the gasoline, to lessen volatilization to the atmosphere. Furthermore, concentrations of aromatics (in particular, benzene) and olefins were decreased and the concentrations of oxygenates were increased, although there were numerous particular changes.

10.7 Refining Ratios

By identifying several ratios, the types of refining techniques employed to produce the gasoline may be assessed (Gary and Handwerk 1984):

- *2,2,4-trimethylpentane (iso-octane)/methyl-cyclo-hexane:* Values greater than 5 normally represent a premium grade of gasoline (such as 92 octane), whereas values less than 5 normally represent a regular grade. The *iso*-alkane, 2,2,4-trimethylpentane, which is the same as *iso*-octane (*i*-C_8), is the basis for the octane rating index; gasoline with 100 % *i*-C_8 would exhibit an octane rating of 100, whereas gasoline with 100 % *n*-C_7 (*n*-heptane) would have an octane rating of 0;

- *Octane index (OI) = (2,2,4-trimethylpentane + toluene)/(n-C_7 + n-C_8):* The OI value increases with octane rating; however, toluene, *n*-C_7 and *n*-C_8 are susceptible to environmental weathering (Schmidt et al. 2002, 2003). As the gasoline becomes more evaporated, it becomes more difficult to identify the octane rating, which is accomplished through a graph given in the Schmidt et al. (2003) article;
- *n-C_4/(n-C_4 + iso-C_4): Iso*-butane is often removed in modern gasolines to be used as a feedstock in chemical manufacturing; however, both butanes are susceptible to volatilization in the subsurface;
- *iso-C_5/(iso-C_5 + n-C_5):* Isomerization is the conversion of straight-chain molecules to higher-octane branched molecules for blending into gasoline or as a feed into alkylation units. It normally imparts on the gasoline a pentane ratio of >0.70;
- *naphthalene/nC_{12}:* This ratio is normally between 1 and 3 in fresh gasoline. Reforming is a refining process used to produce aromatics. Higher values indicate that high concentrations of aromatics are or were in the gasoline.

10.8 Organo-Metallic Compounds

Organo-metallic compounds are not normally identified with a PIANO analysis, but if there is a chance that the gasoline could be older than around 2000, these compounds should also be searched for. Organic lead was banned in automotive gasoline in the United States in 1996, in 1990 in Canada and generally before 2000 in Europe (Oudijk 2010, 2012); however, organic lead is still permitted in aviation gas, racing gas and many off-road fuels. Therefore, the presence of these compounds can be quite helpful in estimating when the gasoline was manufactured.

The organo-metallic compounds commonly detected in gasoline include the organo-leads (tetraethyl lead, tetramethyl lead and several physical and reaction mixes) plus methyl-cyclo-pentadienyl manganese tricarbonyl (MMT).

10.9 Conclusions

The composition of automotive gasoline has changed over the years, but these changes have been known and documented. By identifying the chemical composition of gasoline spilled into the environment, we can often estimate when a particular gasoline was manufactured, which usually helps in identifying responsible parties. In addition to changes brought by the modernization of refining techniques, the composition of gasoline changed because of regulations. The purpose of the regulations was normally to reduce air pollution. By comparing the chemical composition of spilled gasoline to these known changes in regulation, we can further estimate the time frame of when a gasoline was manufactured. To properly identify the chemical composition of spilled gasoline, a PIANO analysis is often quite

helpful. This analysis often identifies hydrocarbons that would not be found with the scans normally required by the regulatory agencies.

References

Alvarez PJJ, Heathcote RC, Powers SE (1998) Caution against interpreting gasoline release dates based on BTEX ratios in ground water. Ground Water Monit Rem 19:69–76

Beall PW, Stout SA, Douglas GS, Uhler AD (2002) On the role of process forensics in the characterization of fugitive gasoline. Environ Claims J 14:487–505

Dickson CL, Woodward PW, Bjugstad PL (1987) Trends of petroleum fuels fuels: Topical report. (No. NIPER-224). National Inst. for Petroleum and Energy Research, Bartlesville, OK (USA)

Gary GE, Handwerk JH (1984) Petroleum refining: technology and economics, 2nd edn. Marcel Dekker, Inc., New York

Kaplan IR (2003) Age dating of environmental organic residues. Environ Forensic 4:95–141

Kaplan IR, Galperin Y, Lu S-T, Lee R-P (1997) Forensic environmental geochemistry: differentiation of fuel types, their sources and release time. Org Geochem 27:289–317

Oudijk G (2005) Fingerprinting and age dating of gasoline releases. Environ Forensic 6:2–10

Oudijk G (2007) The use of alkyl leads in gasoline age-dating investigations: new insights, common investigative techniques, limitations & recommended practices. Environ Claims J 19(1/2):68–87

Oudijk G (2010) The rise and fall of organometallic additives in automotive gasoline. Environ Forensic 11(1):17–49

Oudijk G (2012) The history of leaded petrol in North America and Europe. In: Morrison RD, O'Sullivan G (eds) Environmental forensics: proceedings of the 2011 INEF conference. Royal Society of Chemistry, Cambridge, pp 49–63

Peters KE, Walters CC, Moldowan JM (2005) The biomarker guide: volume 1, biomarkers and isotopes in the environment and human history. Cambridge University Press, Cambridge

Schmidt GW, Beckmann DD, Torkelson BE (2002) A technique for estimating the age of regular/mid-grade gasolines released to the subsurface since the early 1970s. Environ Forensic 3(2):145–162

Schmidt GW, Beckmann DD, Torkelson BE (2003) Gas chromatography technique for estimating the octane rating of fresh and weathered gasolines released to soils and groundwater. Environ Forensic 4:75–80

Chapter 11
A General Overview of Pesticides in Soil: Requirement of Sensitive and Current Residue Analysis Methods

Sevcan Semen, Selda Mercan, and Munevver Acikkol

Abstract Pesticides are chemical agents used to destroy or control pests, both in agriculture and in public health. Despite the beneficial effects associated with the usage of them, these chemicals may cause adverse effects to humans and to the nature. In addition, many pesticides are persistent and may therefore bioaccumulate in the environment; also some of them are important carcinogens and mutagens. In the world, alarming levels of pesticides have been detected in air, water, soil, as well as in foods and biological materials. Because of the special character as sink and source of contaminants soil is a critical medium, and as an environmental contaminant that comes into contact with soil intensively, pesticides are one of the important issues of environmental soil forensics. The different classes and wide range of pesticides and environmental mediums containing them have made essential the development of sensitive and current methods for the analysis of pesticide residues for environmental monitoring and forensic investigations. This chapter describes pesticides, historical background of pesticide usage, pesticides classification, environmental impacts and fate of pesticides, misuse and overuse of them, and provides a general brief overview on the soil sampling and pre-treatment, the basic principles of the conventional and also modern extraction approaches (including their advantages and disadvantages), and the chromatographic-based determination techniques used for pesticide residue analysis in soil.

11.1 Introduction

The preservation of the environment and human health from exposure to persistent organic pollutant is nowadays a priority objective in the whole world. In this sense, pesticides constitute a very important group of target compounds owing to their persistence, bioaccumulation, toxicity, long-range environmental transport ability,

S. Semen (✉) • S. Mercan • M. Acikkol
Institute of Forensic Sciences, Istanbul University, Istanbul 34098, Cerrahpasa, Turkey
e-mail: sevcansemen@gmail.com; mercans@istanbul.edu.tr; acikkolm@istanbul.edu.tr

H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics*,
Soil Forensics, DOI 10.1007/978-3-319-33115-7_11

and unavoidable usage. Because of that pesticides are of concern to both scientists and environmental quality managers or policy makers (Richter et al. 2003; Li et al. 2011).

As the presence of trace amounts of pesticide residues could be potential health hazards, UN organization has formed specialized groups: World Health Organization (WHO) and Food Agriculture Organization (FAO), with the aim to establish restrictive measures to protect the environment against pollution. These organizations and their experts groups on annual meetings summarize international achievements in pesticides domain, establish legislation and make recommendations obligating member states to act in accordance with international standards (Durovic and Dordevic 2011).

Since World War II, it has been impossible to imagine agriculture without the use of pesticides. Pesticides and their metabolites can be found everywhere: in fresh water, groundwater, soil, bottom sediments, food and even faraway oceans (den Hond et al. 2003). Among them soil has a different significance. Because, soil acts as a sink/receptor of the effects of human activities or environmental phenomena and it is an interface between earth, air and water, and additionally hosts most of the biosphere. Therefore, any contamination also affects other environmental media and ecosystems. Also it should not be forgotten that soil is considered as a non-renewable resource because of extremely low formation process (Commission of the European Communities 2006). Based on these reasons, the 6th Environmental Action Programme, published by the European Commission (EC) in 2001, established the basis for further actions to protect soil against adverse impacts on a European level. For this purpose, in 2002 a communication from the EC to the Council and the European Parliament, entitled: "Towards a Thematic Strategy for Soil Protection", was developed and ratified by the 15 ministers of environment of the European Union in 2002. The Soil Thematic Strategy brings soil to a higher level of importance for water managers, policy makers and researchers (Blum et al. 2004).

Development of environmental regulations over the past few decades led to the need for analytical methods that determine qualitatively and quantitatively pesticides in the environment. This need is critical for forensic sciences that require sensitive and selective analytical methods to be useful in litigation (Wait 2000). Originally, the purpose of pesticide laws and regulations was to protect consumers. But, the focus now has shifted to the protection of health and the environment. The determination of pesticide residues is a requirement to support the enforcement of legislation, ensure trading compliance, conduct monitoring residue programs in environmental samples, and study their mode of action and movement within the environment (US EPA 2012a; Pico et al. 2004).

The use of pesticides is still increasing and since soil monitoring plays an important part in the assessment of impacts on environmental quality as well as forensic sciences, pesticide residues in soil continue to be studied more than any other environmental contaminant. This chapter aims to provide a general brief overview of pesticides, their environmental impacts and the main features of pesticide residue analysis in soil.

11.2 Pesticides

According to the Food and Agriculture Organization (FAO) pesticide means any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies (FAO 2002).

There are 920 active ingredients used as pesticides worldwide, mostly in agriculture, and they are currently formulated in thousands of different commercial products (MacBean 2012).

11.2.1 Historical Background of Pesticides Usage

Since before 2000 BC, humans have used pesticides to protect their crops. However, the use of modern pesticides in agriculture and public health is dated back to the after World War II. The first generation pesticides were highly toxic compounds, such as arsenic, mercury, lead, and hydrogen cyanide. The second-generation pesticides included synthetic organic compounds. The first important synthetic organic pesticide was an organochlorine: dichlorodiphenyltrichloroethane (DDT). DDT was discovered in 1939 by a Swiss chemist Paul Muller. In its early days, it was hailed as a miracle because of its effectiveness against a wide range of insects, persistence, low cost and easiness of production. During the 1940s manufacturers began to produce large amounts of synthetic pesticides and their use became widespread. Consequently, in 1948, Dr. Paul Muller won the Nobel Prize in Medicine for discovering its insecticidal properties (Muir 2012).

However, even though the governments, universities and the public were hailing DDT as a miracle, by the mid-1940s some toxicological problems associated with it were being reported. In 1962, Rachel Carson published her best selling book "Silent Spring". In this book, she alerted the public to the potential problems of pesticide misuse, and predicted massive destruction of the planet's fragile ecosystems unless more was done to halt what she called the "rain of chemicals". Afterwards, public confidence in pesticide use was shaken and the modern environmental movement started (Jarman and Ballschmiter 2012).

These concerns, and the resulting public outcry prompted the US Environmental Protection Agency (EPA) to cancel the registration of DDT in the US in 1972. Research activities concentrated on finding new pesticides, which have greater selectivity and better environmental and toxicological profiles. Organochlorines were replaced by organophosphates and carbamates by 1975. Then, pyrethroids have become the dominant insecticides (Unsworth 2010).

In 2004, the Stockholm Convention on Persistent Organic Pollutants (POPs), an international environmental treaty that aims to eliminate or restrict the production and use of POPs entered into force. At that time, the restricted compounds included nine POC pesticides: aldrin, chlordane, dieldrin, endrin, heptachlor, hexachlorobenzen, mirex, toxaphene and DDT. In 2009, α-hexachlorocyclohexane (HCH), β-HCH and γ-HCH were added to the restricted list (http://chm.pops.int).

The early 1990s a new kinds of pesticide entered the European market. The group of these pesticides are called neonicotinoids and presented as "modern". Initially neonicotinoids were praised for their low-toxicity to many beneficial insects, including bees; however this claim has come into question. Since about 2006 there has been a world-wide dramatic rise in the number of hive losses and a reduction of wild bees. Recent research has suggested a potential toxicity to bees and other beneficial insects through low levels of contamination of nectar and pollen with neonicotinoid insecticides used in agriculture (Goulson 2013). Eventually, in 2013 three of them (clothianidin, imidacloprid and thiametoxam) have been temporarily banned by the European Commission, based on the growing scientific evidence regarding the negative effects they have on bees (Di Prisco et al. 2013).

Today, due to the adverse impact of chemical pesticides, there was resurgence in academic and industrial research related to biopesticide development (Fountain and Wratten 2013). And with the rapid expansion of organic agriculture during the past decade, adoption rates have rapidly increased. Biopesticides offer more sustainable solution to pest control than synthetic alternatives but still only make up a small percentage of pest control products (Glare et al. 2012). Also, limited scientific literature is available on the use and environmental impact of them and serious questions remain about the safety of biopesticide products from both a human and ecosystem health standpoint. Current regulations do not go nearly far enough in evaluating systemic broader impacts of biopesticides (Romero-Gonzalez et al. 2011; Chandler et al. 2008).

11.2.2 Classification of Pesticides

Pesticides can be classified or grouped in many different ways; according to the pests they control, their mode of action or their chemical structure.

According to the type of pest they control, pesticides are named after the name of target pest group as shown in Table 11.1.

Under the classification that according to the mode of action, pesticides are classified based on how they work. Contact pesticides generally control a pest as a result of direct contact. They do not penetrate plant tissues. On the other side, systemic pesticides are pesticides, which are absorbed by plants or animals and transported to untreated tissues. Systemic pesticides penetrate the plant tissues and move through the leaves, stems or roots. Stomach poisons kill animal pests after ingestion and so they have to be eaten. Fumigants are chemicals that are applied as toxic gas or as a solid or liquid which forms a toxic gas. The gas penetrates cracks and

Table 11.1 Classification of pesticides according to target organism

Class	Target organism	Usage area
Herbicides	Unwanted plants and weed	Agriculture, forestry, pasture, control of wildlife habitats, and cleaning of waste grounds, industrial sites, and railways
Insecticides	Insects and other arthropods	Agriculture and public health: used in all stages of growth; egg, larva, and insect
Fungicides	Fungi and fungal spores	Agriculture and livestock
Bactericides	Bacteria	Used as disinfectant, antiseptic or antibiotic
Others	Algicides, Antifouling agents, Attractants, Biopesticides, Biocides, Disinfectants and sanitizers, Fumigants, Miticides, Microbial pesticides, Molluscicides, Nematicides, Ovicides, Pheromones, Repellents, Rodenticides, Defoliants, Desiccants, Insect growth regulators, Plant growth regulators	

crevices of structures or soil or the spaces between products stored in containers and kill pests (Zacharia 2011).

Another way of classification is using their active ingredient. The chemical classification at the same time gives information about physical and chemical properties of pesticides so more useful for researchers. According to this, major chemical groups are organoclorines, organophosphates, carbamates and pyrethriods.

Organochlorine pesticides were commonly used in the past in agriculture and public health as insecticides, but many have been removed from the market due to their health and environmental effects, and their persistence (e.g. DDT and chlordane). Organoclorines act as central nervous system disruptors. Furthermore, due to their tendency to accumulate in fatty tissues of organisms they can stay in the body for a long time (US EPA 2012b).

Organophosphate pesticides affect the nervous system by disrupting the acetylcholinesterase enzyme (AChE) that regulates acetylcholine (a neurotransmitter) and stops nerve transmission. Most organophosphates are insecticides. They were developed during the early nineteenth century, but their effects on insects, which are similar to their effects on humans, were discovered in 1932. Organophosphates are efficiently absorbed by inhalation, ingestion, and skin penetration. They are highly toxic to bees, wildlife, and humans. Commonly used organophosphates have included malathion, parathion, chlorpyrifos and diaznon (Gupta et al. 2011; US EPA 2012b).

Carbamate pesticides are derivatives of carbamic acid. The mode of action of carbamates is very similar to that of the organophosphates as they suppress AChE. However, they differ in action from the organophosphate compounds in that the inhibitory effect on cholinesterase is brief. Thus, even though organophosphates inhibit AChE irreversibly, whereas carbamates inhibit AChE reversibly. They are relatively unstable compounds that break down in the environment within weeks or months. Some of the common used carbamates include aldicarb, carbofuran and carbaryl (Gupta et al. 2011).

The last major chemical group, pyrethroid pesticides, are synthetic derivatives of naturally occurring pyrethrins which are obtain from pyrethrum produced by chrysanthemum flowers. They have been modified to increase their stability in the environment. They act as contact poisons, affecting the insect's nervous system but they are not cholinesterase inhibitors like organophosphates or carbamates. Their primary mode of action is inhibition of voltage-sensitive sodium channels. Pyrethroids have relatively low toxicity in humans but they are highly toxic to fish and aquatic invertebrates. They have an extremely low pesticide movement rating because of their tendency to bind the soil particles. The most widely used synthetic pyrethroids include cypermethrin, permethrin and deltamethrin (Acikkol et al. 2012; Zacharia 2011).

11.2.3 Environmental Impact of Pesticides

Despite the beneficial effects of pesticides, which include crop protection, preservation of food and materials and prevention of vector born diseases, their extensive applications have raised serious concerns about entire environment in general and the health of humans and over the years, more and more problems associated with the use of pesticides have shown up (Muir 2012).

In fact, it has been estimated that less than 0.1 % of the pesticide that applied to crops actually reaches the target pest; the rest enters environment gratuitously and contaminating soil, sediment, water, and air, where it can affect non-target organisms (Arias-Esteves et al. 2008). Besides being toxic to the pests they are intended to control, pesticides are also toxic to non-target species including different birds, fish species, animals, and humans (Henny et al. 1985; Stroud 1998; Jett 2011). Also, most of the pesticide residues were found to accumulate in human and biological food chain (Dewailly et al. 2000). Moreover, many studies presented that the low-level long-term exposure to pesticides can result in chronic effects like cancer and other genetic disorders, liver and kidney damage, disorders of the nervous system, damage to the immune system, endocrine disruption, and birth defects (Fortes and Aprea 2011; Mostafalou and Abdollahi 2013; Landau-Ossondo et al. 2009).

Furthermore, pesticides kill not only the pests but also the natural enemies of these pests. That means natural control mechanisms are disrupted and it allows the pest populations to rapidly build up again to levels that can cause serious crop damage (Hardin et al. 1995).

Also eventually, after repeated and more intensive use of the same pesticide to the same pest population, the pesticide becomes ineffective. Accordingly, an increasing number of fungi, weeds and insects have become resistant to the action of individual as well as groups of chemically related active ingredients. Indeed, some observers have noted a preserve effect of the general use of pesticides, namely that crop losses due to insect invasion have actually increased with increasing pesticide use (den Hond et al. 2003).

11.2.4 Environmental Fate and Persistence of Pesticides in Soil

When a pesticide is used in the environment, it becomes distributed among four major compartments: soil, water, air, and biota. In addition, part of it goes to the air or to surface waters, due to emission or drift. Once on the target site, the pesticide may drain into surface waters or volatilize into the air. From the air it may deposit on humans, wildlife or plants or on the soil. From the animals or plants where it was applied the pesticide may leak into groundwater. Pesticides in surface water may go into aquatic organisms, and by sedimentation into other organisms that remain in the sediment (Linde 1994).

Careful consideration of these fate processes and their interactions is necessary to evaluate the risk to groundwater and surface water. All pesticides in groundwater, and most residues present in surface water enter by way of the soil, through surface runoff and leaching. In the case of pesticides presence in soil is mainly product of crop protection, reaching it in many ways: direct treatments, by aerial spraying, and vegetable waste after harvest (Arias-Estevez et al. 2008; Agrawal et al. 2010).

Soil is generally defined as the top layer of the earth's crust, formed by mineral particles, organic matter, water, air, and living organisms. Over 320 major soil types have been identified in Europe and within each there are enormous variations in physical, chemical and biological properties (Commission of the European Communities 2006). The persistence and mobility of pesticides in soil depends on: soil factors (soil composition, soil chemistry, and microbial activity); pesticide properties (water solubility, vapor pressure, and the molecule's susceptibility to chemical or microbial alteration or degradation); climatic factors (moisture, temperature, and sunlight); site conditions (elevation, slope, aspect, geographical conditions, presence of pollutants, tillage, irrigation, etc.); and application features (method, time, frequency, and amount) (Curran 1998; Kerle et al. 1994; Hao et al. 2008).

11.2.5 Misuse and Overuse of Pesticides

In the regulation of pesticides application, government bodies have an important and major role because both producers and users are not likely to limit themselves in the sales and use of pesticides. The weak enforcement of laws and regulations governing pesticide use results in misuse and overuse of pesticides, and consequently, increased environmental contamination and human exposure (Abhilash and Singh 2009; Grovermann et al. 2013).

Surveys show that farmers have overused pesticides in many developing countries including Turkey, Thailand, Bangladesh etc. as many farmers believe that the level of protection derived from pesticides is proportional to the amount applied. Further, they tend to mix more than two types of pesticides that should not be mixed (Abhilash and Singh 2009; Ali et al. 2012; Demircan and Yılmaz 2005). The use of

unprescribed pesticides in inappropriate doses is not only disturbing the soil conditions but also destroying the healthy pool of biocontrol agents that normally coexist with the vegetation and affecting whole ecosystem. Therefore, after application of agrochemicals should be monitor closely by government authority and experts to minimize the health hazard towards human and environment (Ali et al. 2012).

Another problem beside the misuses and overuses is illegal use of pesticides. Despite the prohibition process and public announcements regarding the bans, numerous reports reveal continued widespread use of banned pesticides even today. Because of the effectiveness in controlling pests and low cost, they are still in high demand from farmers (Panuwet et al. 2012; Abhilash and Singh 2009; Rahman 2013).

Governments should emphasize on the issue of misuses and improper sale of pesticides among suppliers and farmers. These criminal activities must be observed in order to preserve the safety of consumer, human body, animal, growing crops and the conservation of ecosystem (Ali et al. 2012).

A vital component of investigating pesticide misuse is the collection of environmental forensic samples such as soil, air, water or any other medium that come into contact with a pesticide. The analysis for pesticide residue is an important aspect of many investigations. Monitoring of pesticide residues and enforcing the MRLs (maximum residue limits) are challenging for the responsible regulatory agencies particularly when relying on the use of non-quantitative and sensitive techniques. So, it is important to establish proper protocols for sampling and use sensitive and selective techniques for monitoring to ensure enforcement actions against companies and other individuals (Saxton and Engel 2005; Panuwet et al. 2012).

11.3 Analytical Procedures

Since soil is an extremely complex and variable medium, the analysis of pesticides in soil is a complicated procedure involving many steps; field sampling, soil pretreatment, extraction, clean up (if necessary), and determination.

11.3.1 Soil Sampling

The first step in the process is to determine how soil samples would be taken in the field, packed, and transported to laboratory. Collection of environmental forensic sample such as soil is a vital component of investigating pesticide misuse (Saxton and Engel 2005).

The main objective in any soil sampling strategy is to obtain a representative portion of the sample. Because of its heterogenic structure soil sampling is very difficult and an effective sampling strategy must be include sample location, sample volume, sample number, sampling depth, sampling approach (random, systematic, judgmental or a combination of these), sample handling, transport and storage. Besides, it is necessary to collect proper blank samples from the same site as the

samples. Blank samples are matrices that have no measurable amount of the analyte of interest so they must be free of the pesticide and all conditions will be carried out as the actual samples (Dean 1998).

After sampling, since probably soil samples are analyzed after some delay, care should be taken to preserve them from contamination and degradation, both during transportation and storage.

11.3.2 Soil Pre-treatment

Collected soil samples are commonly dried, ground and sieved through a mesh. Soil drying is necessary to limit microbial growth and other soil processes to provide protection of samples, and also to enable better homogenisation. The most used method in drying is a thin layer of soil air-dried at room temperature and protected from direct sunlight. Afterwards dry soil samples are grounded and passed through a sieve (the conventional 2 mm sieve has generally been accepted) (Theocharopoulos et al. 2001). Grounding allows the homogenisation and analyses of the soil sample to be carried out under standard conditions with the most physico-chemically active fine particles. The sieving will mostly reduce the fraction of the soil that is largely chemically inert such as coarse-grained, feldspar and carbonate minerals, and will increase the components active in pollutant enrichment. After sieving, obtained fully homogenized sample mechanically or manually mixing performed and homogenized powder is stored in brown glass bottles until chemical analysis (Andreu and Pico 2004; Theocharopoulos et al. 2004).

11.3.3 Extraction

Extraction aims to remove as much as possible of the analyte from the matrix, so it is important to select the appropriate extraction method and optimize the extraction parameters. The sample extraction step, which takes most of the total analysis time, is still the weakest link and the time-determining step in the whole analytical step and also the main reason of errors and differences between laboratories. Ideally, a sample extraction should be rapid, simple, low cost, environmentally friendly and provide clean extracts. For the isolation of pesticides from soil samples various extraction methods have been proposed.

Liquid-Solid Extraction

Conventional methodology frequently involves liquid-solid extraction (LSE). LSE can be sub-divided into approaches that utilize heat and those do not. The use of heat is typified by Soxhlet extraction and methods which no heat is added, but utilise

some form of agitation i.e. shaking or sonication are shake-flask and ultrasonic solvent extraction (USE) (Dean 1998).

Baron von Soxhlet introduced Soxhlet extraction in the mid-nineteenth century. Soxhlet extraction normally requires large volumes (up to 150 ml per sample) of solvent and takes time 6–24 h. Also, only one sample can be extracted per set of apparatus. On the other hand, shake-flask and ultrasonic extraction require smaller volumes of organic solvent (20–100 ml) and are relatively fast (10–60 min). Besides, they allow multiple extractions to be carried out by the use of the simple laboratory mechanical shakers and ultrasonic bath or prob (Dean and Xiong 2000; Pozo et al. 2001; Babic et al. 1998).

These methods are inexpensive and easy to handle but they are laborious, time-consuming, requires large volumes of organic solvents and subject to problems arising from evaporation of large volumes of solvent and loss of some analyte quantity. As a result, modern sample extraction procedures based on instrumental techniques have been developed and applied to overcome the disadvantages of the traditional approaches (Fuentes et al. 2007).

Instrumental Techniques

The first of these new types of extraction techniques appeared almost 20 years ago in the form of supercritical fluid extraction (SFE). This technique makes use of the gas-like and liquid-like properties of a supercritical fluid (a fluid is any substance above its critical temperature and pressure), typically carbon dioxide, to extract organic analytes from solid environmental matrices at temperatures >31.1 °C and 74.8 atm. Initial limitations of the technique centered around its inability to extract polar molecules. By using combinations of CO_2 mixed with an organic modifier, e.g. methanol and acetone, it is possible to extract a range of molecules of different polarity (Forero-Mendieta et al. 2012). All SFE systems contain six basic components, namely the supply of high purity CO_2, a supply of high purity organic modifier, the pumps, the oven for the extraction cell, the pressure outlet or restrictor, and the collection vessel. In general, SFE lasts less than 2 h and requires low solvent volumes (10–40 ml). Besides these, it does not allow multiple extractions and has high cost of the equipment (Dean 1998).

The second of the instrumental techniques is microwave-assisted extraction (MAE). The first use of MAE for the extraction of analytes with organic solvents appeared in 1986. MAE utilizes electromagnetic radiation to desorb desired components from the matrix. In MAE, organic solvent and the sample are subjected to radiation from a magnetron in sealed vessels. In order to heat a solvent, part of it must be polar with high dielectric constant to absorb microwave energy efficiently, if it is not certain amount of water or a polar solvent must be added. MAE is a promising technique for soil samples, so in last years, applications of MAE for extracting pesticides from soil have increased rapidly (Paiga et al. 2008). For the optimization of the MAE procedures, several parameters such as volume and solvent composition, extraction temperature and time are usually studied. The high sample throughput (up to 14 vessels can be extracted simultaneously), need for minimum sample

amount (2–5 g), low solvent consumption (10–40 ml), fast extraction, high level of automation and efficiency make this technique attractive (Dean and Xiong 2000; Durovic and Dordevic 2011). But due to its limited selectivity and simultaneous co-extraction of soil components together with the target analytes, it often requires a further clean-up step (Lesueur et al. 2008). Also, an alternative application of MAE using micellar media as extractans (MAME) to completely avoid the use of organic solvents has been reported and offers advantages, such as low toxicity and compatibility with aqueous-organic mobile phase in liquid chromatography (Padron-Sanz et al. 2005).

The final instrumental technique is pressurised liquid extraction (PLE), available commercially in the form of accelerated solvent extraction (ASE). This technique, which first appeared commercially in 1995, uses small amounts of water and organic solvents to sequentially extract analytes from the sample matrix under elevated temperature (up to 200 °C) and pressure (up to 20 MPa). ASE is an automated instrument capable of sequentially extracting up to 24 samples. A typical extraction time per sample is 12 min (Dean and Xiong 2000; Luo et al. 2010). The combination of high temperature and pressure results in better extraction efficiency, thus minimizing solvent use. For all that, high temperatures may lead to degradation of thermo labile analytes and also to the co-extraction of interfering species. Obvious ASE advantage is that it requires much less solvent and shorter extraction times than conventional techniques. Additionally, ASE is reduced both, waste levels and analysts exposure to harmful solvents. However, limited by high cost, its application is still not widespread (Durovic and Dordevic 2011).

Despite mentioned disadvantages related to conventional solvent extraction methods, they are still the most popular methods for routine analysis. To overcome the disadvantages of these methods, new approaches in pesticide residues analysis have appeared. In 2003, Anastassiades et al. developed a method for the multi-class, multi-residue extraction of pesticides in fruits and vegetables. This method was called QuEChERS, which stands for Quick, Easy, Cheap, Rugged and Safe, and it is based on dispersive solid phase extraction (dSPE). In dSPE analytes are extracted with an aqueous miscible solvent with a high amount of salt ($MgSO_4$) and/or buffering agents, in order to induce liquid phase separation and stabilize acid and base pesticides (Pinto et al. 2010). In the recent studies, the QuEChERS method applied for the determination of pesticides from soil successfully (Lesueur et al. 2008; Drozzdzynski and Kowalska 2009). The QuEChERS advantages are the high recovery, accurate results, and low solvent and glassware usage. Besides, the main QuEChERS disadvantage is requirement of concentration of the final extract to provide the necessary sensitivity (Rouviere et al. 2012).

Miniaturized Techniques

Modern trends in analytical chemistry are towards the simplification and miniaturization of sample preparation, as well as the minimization of organic solvent used. In view of this aspect, several newer miniaturized procedures are being developed in order to reduce the analysis step, increase the sample throughput and to improve

the quality and the sensitivity of analytical methods (Lambropoulou and Albanis 2007). Liquid-phase micro-extraction (LPME), solid-phase microextraction (SPME), and matrix solid-phase dispersion (MSPD) are some of the most representative procedures for pesticide analysis from soil.

One of the emerging techniques in this area is liquid-phase micro-extraction (LPME). LPME involves the use of a small amount (3 µl) of organic solvent impregnated in a hallow fiber membrane, which is attached to the needle of a conventional gas chromatography (GC) syringe. It is quick, inexpensive and can be automated but only a limited number of studies have performed on soil samples (Hou and Lee 2004; Lambropoulou and Albanis 2007).

Solid-phase microextraction (SPME) is a newly developed solvent-free analytical technology, which allows the simultaneous extraction and pre-concentration of analytes from a sample. It involves the use of a fiber coated with an extracting phase, that can be a liquid (polymer) or a solid (sorbent), which extracts different kinds of analytes (including both volatile and non-volatile) from different kinds of media (Möder et al. 1999). Several disadvantages related to fiber stability and sensitivity has been pointed out. Yet, only a few references on the application of SPME for the determination of pesticides in soil samples are available (Hernandez et al. 2000; Bouaid et al. 2001; Moreno et al. 2006). Recently, headspace SPME (HS-SPME) has also been used to determine pesticide compounds in soil. Sampling in the headspace presents a significant advantage in terms of selectivity because only volatile and semivolatile organic compounds can be released into the headspace (Doong and Liao 2001).

Matrix solid-phase dispersion (MSPD) is a relatively developed extraction–clean-up technique characterized by simplicity and sensitivity. In MSPD, extraction and clean-up are carried out in the same step, which can avoid the general disadvantages of other traditional methods, such as the use of a large amount of solvent and glassware, the laborious extraction procedure and the occurrence of troublesome emulsions (Li et al. 2002; Salemi et al. 2012).

These new techniques seem to provide good results but there are still few reports to establish their usefulness and to compare them with other techniques. Therefore, further study is required.

11.3.4 Clean-Up

Because of the complexity of the matrix, during the extraction step many interfering components (lipids, pigments or cholesterol and its derivatives) are co-extracted from soil samples together with target analytes. Clean-up stage requires removing these substances that could disturb determination and quantitation of target analytes (Shen et al. 2006).

There are several approaches for extract clean-up: liquid-liquid partitioning; solid liquid adsorption chromatography using long open columns packed with alumina, Florisil, ion-exchange resins, silica gel, and many silica-based sorbents;

solid-phase extraction (SPE) on disposable cartridges packed with C_{18}, NH_2, or CN modified silica or graphitized carbon; thin layer chromatography; and gel permeation (GPC) chromatography (Yasin et al. 1996; Dabrowska et al. 2003; Andreu and Pico 2004).

Some other matrix components that falsify the results are non-organic components of the extract, such as elemental sulphur. They also should be eliminated in order to protect the column. To remove sulphur generally copper is used in different grain-size forms (Esteve-Turillas et al. 2004).

11.3.5 Determination

Most of the analytical methods for the single or multiresidue determination of pesticides in soil are based on chromatographic techniques; gas chromatography (GC), liquid chromatography (LC), and thin layer chromatography (TLC). Chromatography is based on separation and then identification and quantification of components in extracts. Separation is achieved by using differences in equilibrium constants of components between mobile phase (a liquid or gaseous) that tends to transport them and stationary phase (column or plate) that tends to retain them (Theocharopoulos et al. 2004; Chen and Wang 1996).

Pesticide residue analyses in soil are conducted often by GC with different detectors. Electron-capture detector (ECD) is specific for halogen containing compounds and is used to determine some of the organoclorine, organophosphorus, and pyrethroid pesticides (Sun et al. 2009; Ozcan et al. 2009; Wang et al. 2008). Nitrogen-phosphorus detection (NPD) is also used for many pesticides (Forero-Mendieta et al. 2012; EL-Saeid and AL-Dosari 2010). In addition, for the analysis of non-halogen containing pesticides flame ionization detection (FID) can be applied; however, detection limits are not sensitive enough for residue analysis (Naeeni et al. 2011).

Moreover, GC has been coupled to mass spectrometry (MS) to provide a highly sensitive detector, which also gives information on the molecular structure of the analytes and selective detectors have progressively been replaced by GC-MS, mainly using electron impact (EI) and chemical ionization (CI). Also in recent years, the use of ion-trap tandem MS (MS/MS) has allowed improvement in the selectivity and the sensitivity of GC-MS methods for analysis of pesticides in soil (Santos and Galceran 2002). Besides, GC is not suitable for thermo-labile, low volatility, and strongly polar compounds.

Vice versa LC is ideally suited for the analysis of polar compounds. In comparison with GC, LC has relatively low sensitivity. However, the development in the LC including the introduction of high-performance (HP) columns and the improvement of new detectors (UV, Fluorescence, and MS) have broadened the application of it in the pesticide residue analysis. HPLC does not have the same limitations of GC with regard to compounds of low volatility and low thermal stability (Theocharopoulos et al. 2004; Chen and Wang 1996). In the last years, there has been an increase of the scientific publications dealing with LC–MS and LC-MS/MS for the determination

of pesticides in soil (Dagnac et al. 2005; Li et al. 2013). Reversed-phase LC is the technique most widely used, especially for acidic pesticides (Hogendoorn et al. 2001).

Furthermore, recent years, new active ingredients have been developed through more specific reactions. These new pesticides can be produced by the synthesis of more complex molecules, which normally cannot be analyzed by GC, but better respond to analysis by LC. The same holds true for their, usually even more polar, transformation /degradation products or metabolites (Pizzutti et al. 2007).

TLC is less widely used compared to GC and LC in recent years, due to the low detection limits. The development of modern, instrumantalized HPTLC, that perform the final determination by measuring the UV absorbance with TLC scanner, makes the TLC application more promising (Acikkol et al. 2012; Babic et al. 1998).

11.4 Conclusions

Due to intensive and widespread usage, pesticides residues have become an unavoidable part of the environment and soil is an important medium that is closely associated with humans and their health. As a result, the development of new analytical methods for the determination of pesticides in soil is currently a high interest research area.

Pesticides are extremely diverse with nearly a thousand active ingredients currently in use and comprise a great variety of compounds, mainly insecticides, herbicides and fungicides, as well as their metabolites, with extremely diverse physico-chemical characteristics and large differences in polarity, volatility and persistence. Moreover, newly developed pesticide products are being introduced in the market consistently. The monitoring of conventional priority pesticides, such as DDT, which have long been recognized as posing risks to human health and persistent in the environment, follows long established standards and certified methods. But sensitive and current analytical methods for environmental monitoring and forensic investigations are not available for all pesticides. The need for the detection of low levels of a wide variety of pesticide residues in soil samples both in individual and simultaneously, and on the other hand the complexity of the soil matrix (because of the strong diversity and heterogeneity) makes the development of efficient and reliable analytical methods quite a challenge.

References

Acikkol M, Semen S, Turkmen Z et al (2012) Determination of α-cypermethrin from soil by using HPTLC. JPC-J Planar Chromatogr-Mod TLC 25:48–53

Abhilash PC, Singh N (2009) Pesticide use and application: an Indian scenario. J Hazard Mater 165:1–12

Agrawal A, Pandey RS, Sharma B (2010) Water pollution with special reference to pesticide contamination in India. J Water Resour Prot 2:432–448

Ali A, Noah RM, Malik SA (2012) Legal implications on sales and purchase, uses and misuses of agro chemicals in smallholders' agro production in Malaysia. Procedia Soc Behav Sci 68:156–163

Anastassiades M, Lehotay SJ, Stajnbaher D et al (2003) Fast and easy multiresidue method employing acetonitrile extraction/partitioning and ''Dispersive solid-phase extraction" for the determination of pesticide residues in produce. J AOAC Int 86:412–431

Andreu V, Pico Y (2004) Determination of pesticides and their degradation products in soil: critical review and comparison of methods. TrAC-Trend Anal Chem 23:772–789

Arias-Estevez M, Lopez-Periago E, Martinez-Carballo E et al (2008) The mobility and degradation of pesticides in soils and the pollution of groundwater resources. Agric Ecosyst Environ 123:247–260

Babic S, Petrovic M, Kastelan-Macan M (1998) Ultrasonic solvent extraction of pesticides from soil. J Chromatogr A 823:3–9

Blum WEH, Büsing J, Montanarella L (2004) Research needs in support of the European thematic strategy for soil protection. TrAC-Trends Anal Chem 23:680–685

Bouaid A, Ramos L, Gonzalez MJ et al (2001) Solid-phase microextraction method for the determination of atrazine and four organophosphorus pesticides in soil samples by gas chromatography. J Chromatogr A 939:13–21

Chandler D, Davidson G, Grant WP et al (2008) Microbial biopesticides for integrated crop management: an assessment of environmental and regulatory sustainability. Trends Food Sci Tech 19:275–283

Chen ZM, Wang YH (1996) Chromatographic methods for the determination of pyrethrin and pyrethroid pesticide residues in crops, foods and environmental samples. J Chromatogr A 754:367–395

Commission of the European Communities (2006) Thematic strategy for soil protection. Brussels, 22.9.2006 COM(2006)231 final

Curran WS (1998) Persistence of herbicides in soil, Agronomy facts 36. Penn State College of Agricultural Science, The Pennsylvania State University, Pennsylvania

Dabrowska H, Dabrowski L, Biziuk M et al (2003) Solid-phase extraction clean-up of soil and sediment extracts for the determination of various types of pollutants in a single run. J Chromatogr A 1003:29–42

Dagnac T, Bristeau S, Jeannot R et al (2005) Determination of chloroacetanilides, triazines and phenylureas and some of their metabolites in soils by pressurised liquid extraction, GC–MS/MS, LC–MS and LC–MS/MS. J Chromatogr A 1067:225–233

Dean JR (1998) Extraction methods for environmental analysis. Wiley, Chichester

Dean JR, Xiong G (2000) Extraction of organic pollutants from environmental matrices: selection of extraction technique. Trends Anal Chem 19:553–564

Demircan V, Yılmaz H (2005) The analysis of pesticide use in apple production in Isparta province in terms of economy and environmental sensitivity perspective (in Turkish). Ekoloji 14:15–25

den Hond F, Groenewegen P, van Straalen N (eds) (2003) Pesticides: problems, improvements, alternatives. Blackwell, London

Dewailly E, Ayotte P, Bruneau S et al (2000) Susceptibility to infections and immune status in inuit infants exposed to organochlorines. Environ Health Perspect 108:205–211

Di Prisco G, Cavaliere V, Annoscia D et al (2013) Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees. PNAS 110(46):18466–18471

Doong RA, Liao PL (2001) Determination of organochlorine pesticides and their metabolites in soil samples using headspace solid-phase microextraction. J Chromatogr A 918:177–188

Drozzdzynski D, Kowalska J (2009) Rapid analysis of organic farming insecticides in soil and produce using ultra-performance liquid chromatography/tandem mass spectrometry. Anal Bioanal Chem 394:2241–2247

Durovic R, Dordevic T (2011) Modern extraction techniques for pesticide residues determination in plant and soil samples. In: Stoytcheva M (ed) Pesticides in the modern world – trends in pesticides analysis. InTech, Rijeka, Croatia, pp 221–246

EL-Saeid MH, AL-Dosari SA (2010) Monitoring of pesticide residues in Riyadh dates by SFE, MSE, SFC, and GC techniques. Arab J Chem 3(3):179–186
Esteve-Turrillas FA, Aman CS, Pastor A et al (2004) Microwave-assisted extraction of pyrethroid insecticides from soil. Anal Chim Acta 522:73–78
FAO (2002) International code of conduct on the distribution and use of pesticides. Food and Agriculture Organization of the United Nations, Rome
Forero-Mendieta JR, Castro-Vargas HI, Parada-Alfonso F et al (2012) Extraction of pesticides from soil using supercritical carbon dioxide added with methanol as co-solvent. J Supercrit Fluid 68:64–70
Fortes C, Aprea C (2011) Cancer risks from residential exposure to pesticides. In: Nriagu JO (ed) Encyclopedia of environmental health, vol 1. Elsevier, Burlington, pp 489–497
Fountain ED, Wratten SD (2013) Conservation biological control and biopesticides in agricultural? Reference module in earth systems and environmental sciences. doi:10.1016/B978-0-12-409548-9.00539-X
Fuentes E, Báez ME, Labra R (2007) Parameters affecting microwave-assisted extraction of organophosphorus pesticides from agricultural soil. J Chromatogr A 1169(1–2):40–46
Glare T, Caradus J, Gelernter W et al (2012) Have biopesticides come of age? Trends Biotechnol 30(5):250–258
Goulson D (2013) An overview of the environmental risks posed by neonicotinoid insecticides. J Appl Ecol 50:977–987
Grovermann C, Schreinemachers P, Berger T (2013) Quantifying pesticide overuse from farmer and societal points of view: an application to Thailand. Crop Prot 53:161–168
Gupta RC, Malik JK, Milatovic D (2011) Organophosphate and carbamate pesticides. In: Gupta RC (ed) Reproductive and developmental toxicology. Elsevier, London, pp 471–486
Hao H, Sun B, Zhao Z (2008) Effect of land use change from paddy to vegetable field on the residues of organochlorine pesticides in soils. Environ Pollut 156:1046–1052
Hardin MR, Benrey B, Coll M et al (1995) Arthropod pest resurgence: an overview of potential mechanisms. Crop Prot 14:3–18
Henny CJ, Blus LJ, Kolbe EJ et al (1985) Organophosphate insecticide (famphur) topically applied to cattle kills magpies and hawks. J Wildl Manage 49:648–658
Hernandez F, Beltran J, Lopez FJ et al (2000) Use of solid-phase microextraction for the quantitative determination of herbicides in soil and water samples. Anal Chem 15(72):2313–2322
Hogendoorn EA, Huls R, Dijkman E et al (2001) Microwave assisted solvent extraction and coupled-column reversed-phase liquid chromatography with UV detection: use of an analytical restricted-access-medium column for the efficient multi-residue analysis of acidic pesticides in soils. J Chromatogr A 938:23–33
Hou L, Lee HK (2004) Determination of pesticides in soil by liquid-phase micro-extraction, and gas chromatography–mass spectrometry. J Chromatogr A 1038:37–42
Jarman WM, Ballschmiter K (2012) From coal to DDT: the history of the development of the pesticide DDT from synthetic dyes till silent spring. Endeavour 36:131–142
Jett DA (2011) Neurotoxic pesticides and neurologic effects. Neurol Clin 29:667–677
Kerle EA, Jenkins JJ, Vogue PA (1994) Understanding pesticide persistence and mobility for groundwater and surface water protection. Oregon State University, Corvallis
Lambropoulou DA, Albanis TA (2007) Liquid-phase micro-extraction techniques in pesticide residue analysis. J Biochem Biophys Methdos 70:195–228
Landau-Ossondo M, Rabia N, Jos-Pelage J et al (2009) Why pesticides could be a common cause of prostate and breast cancers in the French Caribbean Island, Martinique. An overview on key mechanisms of pesticide-induced cancer. Biomed Pharmacother 63:383–395
Lesueur C, Gartner M, Mentler A et al (2008) Comparison of four extraction methods for the analysis of 24 pesticides in soil samples with gas chromatography–mass spectrometry and liquid chromatography–ion trap–mass spectrometry. Talanta 75:284–293
Li J, Lu Y, Shi Y et al (2011) Environmental pollution by persistent toxic substances and health risk in an industrial area of China. J Environ Sci 23:1359–1367

Li Y, Dong F, Liu X et al (2013) Development of a multi-residue enantiomeric analysis method for 9 pesticides in soil and water by chiral liquid chromatography/tandem mass spectrometry. J Hazard Mater 250–251:9–18

Li ZY, Zhang ZC, Zhou QL et al (2002) Fast and precise determination of phenthoate and its enantiomeric ratio in soil by the matrix solid-phase dispersion method and liquid chromatography. J Chromatogr A 977:17–25

Linde CD (1994) Physico-chemical properties and environmental fate of pesticides. Environmental Protection Agency, Sacramento

Luo L, Shao B, Zhang J (2010) Pressurized liquid extraction and cleanup procedure for the determination of pyrethroids in soils using gas chromatography/tandem mass spectrometry. Anal Sci 26:461–465

MacBean C (ed) (2012) The pesticide manual: a world compendium, 16th revised edn. British Crop Protection Council, Alton

Moreno DV, Ferrera ZS, Rodriguez JJS (2006) Microwave assisted micellar extraction coupled with solid phase microextraction for the determination of organochlorine pesticides in soil samples. Anal Chim Acta 571:51–57

Mostafalou S, Abdollahi M (2013) Pesticides and human chronic diseases: evidences, mechanisms, and perspectives. Toxicol Appl Pharm 268:157–177

Möder M, Popp P, Eisert R et al (1999) Determination of polar pesticides in soil by solid phase microextraction coupled to high-performance liquid chromatography electrospray/mass spectrometry. Fresenius J Anal Chem 363:680–685

Muir P (2012) A history of pesticide use. Oregon State University. http://people.oregonstate.edu/~muirp/pesthist.htm. Accessed 21 June 2012

Naeeni MH, Yamini Y, Rezaee M (2011) Combination of supercritical fluid extraction with dispersive liquid–liquid microextraction for extraction of organophosphoruspesticides from soil and marine sediment samples. J Supercrit Fluids 57:219–226

Ozcan S, Tor A, Aydin ME (2009) Application of miniaturised ultrasonic extraction to the analysis of organochlorine pesticides in soil. Anal Chim Acta 640:52–57

Padron-Sanz C, Halko R, Sosa-Ferrera Z et al (2005) Combination of microwave assisted micellar extraction and liquid chromatography for the determination of organophosphorous pesticides in soil samples. J Chromatogr A 1078:13–21

Paíga P, Morais S, Correia M et al (2008) A multiresidue method for the analysis of carbamate and urea pesticides from soils by microwave-assisted extraction and liquid chromatography with photodiode array detection. Anal Lett 41:1751–1772

Panuwet P, Siriwong W, Prapamontol T et al (2012) Agricultural pesticide management in Thailand: status and population health risk. Environ Sci Policy 17:72–81

Pico Y, Blasco C, Font G (2004) Environmental and food applications of LC–tandem mass spectrometry in pesticide-residue analysis: an overview. Mass Spectrom Rev 23:45–85

Pinto CG, Laespada MEF, Martín SH et al (2010) Simplified QuEChERS approach for the extraction of chlorinated compounds from soil samples. Talanta 81:385–391

Pizzuttia IR, de Kok A, Zanella R et al (2007) Method validation for the analysis of 169 pesticides in soya grain, without clean up, by liquid chromatography–tandem mass spectrometry using positive and negative electrospray ionization. J Chromatogr A 1142:123–136

Pozo O, Pitarch E, Sancho JV et al (2001) Determination of the herbicide 4-chloro-2-methylphenoxyacetic acid and its main metabolite, 4-chloro-2-methylphenol in water and soil by liquid chromatography-electrospray tandem mass spectrometry. J Chromatogr A 923:75–85

Rahman S (2013) Pesticide consumption and productivity and the potential of IPM in Bangladesh. Sci Total Environ 445–446:48–56

Richter P, Sepulveda B, Oliva R et al (2003) Screening and determination of pesticides in soil using continuous subcritical water extraction and gas chromatography–mass spectrometry. J Chromatogr A 994:169–177

Romero-Gonzalez R, Garrido Frenich A, Martínez Vidala JL et al (2011) Simultaneous determination of pesticides, biopesticides and mycotoxins in organic products applying a quick, easy, cheap, effective, rugged and safe extraction procedure and ultra-high performance liquid chromatography–tandem mass spectrometry. J Chromatogr A 1218:1477–1485

Rouvière F, Buleté A, Cren-Olivé C et al (2012) Multiresidue analysis of aromatic organochlorines in soil by gas chromatography–mass spectrometry and QuEChERS extraction based on water/dichloromethane partitioning. Comparison with accelerated solvent extraction. Talanta 93:336–344

Salemi A, Shafiei E, Vosough M (2012) Optimization of matrix solid phase dispersion coupled with gas chromatography electron capture detection for determination of chlorinated pesticides in soil. Talanta 101:504–509

Santos FJ, Galceran MT (2002) The application of gas chromatography to environmental analysis. TrAC-Trend Anal Chem 21:672–685

Saxton GN, Engel B (2005) A survey of soil sample handling procedures of state pesticide regulatory agencies. Environ Forensic 6:105–108

Shen X, Cai J, Gao Y et al (2006) Determination of organophosphorus pesticides in soil by MMSPD-GC-NPD and confirmation by GC-MS. Chromatographia 64:71–77

Stroud RK (1998) Wildlife forensics and the veterinary practitioner. Semin Avian Exot Pet 7:182–192

Sun K, Zhao Y, Gao B et al (2009) Organochlorine pesticides and polybrominated diphenyl ethers in irrigated soils of Beijing, China: levels, inventory and fate. Chemosphere 77:1199–1205

Theocharopoulos SP, Wagner G, Sprengart J et al (2001) European soil sampling guidelines for soil pollution studies. Sci Total Environ 264:51–62

Theocharopoulos SP, Mitsios IK, Arvanitoyannis I (2004) Traceabilty of environmental soil measurements. TrAC-Trends Anal Chem 23:237–251

Unsworth J (2010) History of pesticide use. http://agrochemicals.iupac.org/index.php?option=com_sobi2&sobi2Task=sobi2Details&catid=3&sobi2Id=31. Accessed 21 June 2012

US EPA (2012a) Agricultural pesticides. US Environmental Protection Agency. http://www.epa.gov/oecaagct/ag101/croppesticideuse.html. Accessed 10 Dec 2012

US EPA (2012b) Types of pesticides. US Environmental Protection Agency. http://www.epa.gov/pesticides/about/types.htm#chemical. Accessed 21 June 2012

Yasin M, Baugh PJ, Bonwick GA et al (1996) Analytical method development fort he determination of synthetic pyrethroid insecticides in soil by gas chromatography–mass spectrometry operated in negative-ion chemical-ionization mode. J Chromatogr A 754:235–243

Wait AD (2000) Evolution of organic analytical methods in environmental forensic chemistry. Environ Forensic 1:37–46

Wang X, Zhao X, Liu X et al (2008) Homogeneous liquid-liquid extraction combined with gas chromatography-electron capture detector for the determination of three pesticide residues in soils. Anal Chim Acta 620:162–169

Zacharia TJ (2011) Identity, physical and chemical properties of pesticides. In: Stoytcheva M (ed) Pesticides in the modern world – trends in pesticides analysis. InTech, Rijeka, pp 1–18

Part IIIa
Searches: Cooperation, Strategies and Techniques

Chapter 12
A Study of pH as an Influencing Factor in the Survival of Human Remains at Sites Investigated by the Independent Commission for the Location of Victims Remains

N.A. McCullagh

12.1 Introduction

In a search for buried human remains there are two possible outcomes: the remains are located or they are not located. In case of the latter, the question is whether the remains were never there to start with or whether they have completely disintegrated and nothing could be found. Usually buried human remains can survive for at least decades, if not thousands of years under the right circumstances. But what if you consider the least favourable circumstances? Is it possible that there may be nothing found after 30 years?

The research reported in this paper was undertaken for the Independent Commission for the Location of Victims Remains (ICLVR) at the close of the Active Search Phase (2006–2011). Its immediate context are the restrictions, embodied in the legislation setting up the Commission, on forensic testing of human remains or any items associated with the discovery of a body (Northern Ireland (Location of Victims' Remains) Act 1999, s.4, Criminal Justice (Location of Victims' Remains) Act 1999, s. 5). This restriction is justified by the fact that the sole purpose of the ICLVR is the recovery and repatriation of remains rather than the prosecution of those involved in the disappearance of the individuals whose remains are being sought.

The acidity of the ground in which the human remains are buried is a contributing factor to the preservation of those remains and it can be easily tested. For this reason a full scale analysis of pH values at all sites under consideration was chosen. It enabled the search team to establish the role that the biochemical properties of soil at the sites of excavation may have contributed to the presence or absence of human remains. All sites excavated since 2006 were sampled to allow comparison

N.A. McCullagh (✉)
Independent Commission for the Location of Victims Remains, Dublin, Ireland
e-mail: n.a.mccullagh@gmail.com

H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics*, Soil Forensics, DOI 10.1007/978-3-319-33115-7_12

Table 12.1 ICLVR site sampling catalogue

Site	Site type	Human remains found	No. of samples
A	Saturated peat – fen bog	Yes	32
B	Desiccated peat – Mountainous	Yes	42
C	Saturated peat – fen bog	Yes	71
D	Coastal – sand	Yes	19
E	Coastal – sand	Yes	29
F	Woodland – sand/gravel	No	27
G	Desiccated peat – blanket bog	No	171
H	Desiccated peat – former blanket bog	No	96
I	Disturbed peat – raised bog	No	122
		Total samples	**609**

between sites where human remains were located and those where remains were not found (Table 12.1).

This chapter has a number of sections. In the first section the rationale for pH sampling the will be set out, drawing on the findings from the study of forensic taphonomy. This will be followed by an outline of the methodology and sampling strategy used, the results of the pH testing at each site will then be presented and discussed together with relevant illustrations. Finally, by way of conclusion the overall significance of the results will be interpreted to provide some biochemical basis for possible future excavation work.

12.2 Rationale for Ph Testing – Initial Considerations

The importance of soil and edaphic conditions in relation to a burial environment, and the ability to design an effective search strategy, cannot be underestimated. If there is little chance of skeletal remains (or elements that may be associated with a deliberate burial) being found, then search strategies must be adapted to find a grave cut. And if human remains do survive in good condition, then we must ask why this has happened and what are the implications for subsequent identification. These are questions that need to be addressed during the design of a forensic search strategy. The more knowledge an investigator has about the nature of a site and what can be expected from the location, the more informed decisions can be made increasing the likelihood that even the slightest traces of a clandestine grave will be recognised for what they are.

The ICLVR was set up in 1999 and went through a number of phases in its work. One of these was an intense period of search activity from 2006 to 2011. After this the field team was temporarily scaled down and it was necessary to reconsider the remaining cases and their associated sites. Sites where human remains had not been located were re-examined to determine if there was potential or fruitful purpose in conducting further physical searches at these locations.

During the course of this reassessment of sites the Forensic Archaeology team conducted a survey of pH levels across all sites – those where human remains were located and those where they had not been found. Pre-search research had established key environmental data for every site; rainfall, average temperatures and soil typology. However it was determined that pH information was necessary to provide a more complete and comprehensive picture.

While this study of pH levels cannot be directly compared to controlled laboratory experiments of burial environments (e.g. Schotsmans et al. 2014; Carter et al. 2010; Pringle et al 2010; Haslam and Tibbett 2009; Carter and Tibbett 2008; Turner-Walker and Peacock 2008; Nielsen-Marsh et al 2007; Forbes et al 2005) it represents a more realistic application of soil science to forensic investigation in the conditions that prevailed for the work of the ICLVR. The results cannot be comparable with the kinds of laboratory controlled experiments that feature largely in the scientific literature, but they do provide real time results that can contribute to real time site investigation.

12.3 Rationale for Ph Testing – Further Elaboration

In the field of taphonomy and forensic taphonomy in particular, the detailed study of the situational decay of biological remains is relatively young and still developing. A number of elements have been identified as important for the analysis of the preservation and decay of biological remains in the burial environment (Carter and Tibbett 2008; Janaway et al. 2009; Plunkett et al. 2009; Schotsmans et al. 2011; Stodkilde-Jorgensen 2008; Turner-Walker 2008; Turner-Walker and Peacock 2008; Wilson et al. 2007). It has however been firmly established that the differential survival of human remains is site and situation dependent (see for example Manifold 2012; Nord et al. 2005; Küpper et al. 2001; Nielsen-Marsh and Hedges 2000).

The rate of decomposition of buried human remains can be directly attributed to a number of environmental factors, among them pH, redox and ambient temperatures (Pringle et al. 2010). It has been observed that when analysing human remains temperature and moisture are the two most influential factors contributing to the rate of decomposition (Janaway et al. 2009; Manifold 2012). When examined in more detail it is found that within the study of forensic soil science it has been acknowledged that soil pH is significantly influential (Lange 2008). Dry or wetland soils, gravels and clays, and the relative acidity or alkalinity of a site will promote or support other influential factors such as temperature and site hydrology. These in turn will have a direct effect on features such as microbial activity that in turn will have direct consequences on the preservation or decay of human remains.

The most effective approach to the searching of such sites and landscapes is to understand as succinctly as possible the exact nature of a site. Due to the complex nature of most sites and fluctuating water tables over the timeframe of most cases, pH was deemed the most relatively reliable and ultimately the most directly quantifiable factor. While localised pH levels will be altered by the introduction of human

remains studies show that this will be for a limited amount of time and pH levels will return to their original values after some time (Haslam and Tibbett 2009).

The choice of pH is of course not without its difficulties as pH can be altered by extrinsic factors such as pollutant run off in the case of low-lying sites. Each site has a complex history, the course of which is influenced and altered by local circumstance, environmental inevitabilities and previous searches. For example at Site C, what was at the time of burial a well drained site, at the time of search, due to the absence of maintenance of drainage, had become a completely saturated waterlogged landscape. This will have affected the grave site over the passage of time with moisture levels varying. Also the impact of local landuse becomes a factor as the water table rises to meet the grave and levels of farming in the vicinity are increased, altering the biochemical properties of the newly saturated environment over a prolonged period.

12.4 Site Typology

Nine sites were examined in this study, all of which had been excavated by the ICLVR at various times since its inception in 1999. Human remains had been successfully located at five of the sites and the remaining four sites had been subjected to search but no human remains or any evidence of a clandestine burial had been discovered. For the purposes of this publication sites will not be named but will be referred to as Site(s) A-I.

It has been shown that soil type has a considerable effect on the decomposition of buried human remains (e.g. Haslam and Tibbett 2009). There are a number of classifications of site 'type' on these nine sites that the ICLCR has excavated. While the majority of sites are referred to colloquially as 'bog' they do not necessarily possess the true characteristics of what would be classified as a bog or wetland site. Other sites excavated include an uphill mountain site that was also referred using the generic term 'bog' and a coastal site. These site descriptions have led to a belief that human remains would survive inordinate periods of time in these particular 'bog' environments, when in fact each site displays a unique typology that will have direct consequences on the survival or degradation of human remains. It is important that this perception is deconstructed as it leads to the false expectation that sites with similar characteristics would also contain human remains.

An example of this can be found with the case of Site I. It is specifically classified as a raised bog, recognised as being an excellent environment for the survival of human remains (Fischer 1998). However, significant earth movement at this location, redistribution of the top 1 m of the surface of the site, caused potential dispersion of the burial. In this case the pH study would assist in the assessment of whether these dispersed remains would survive in their redistributed form, after being subjected to the increased degradation factors that burial disturbance leads to e.g. exposure to oxygen and moisture.

The specific site types can more accurately be broken down into the following classifications: saturated peat, desiccated peat and coastal sand (see Table 12.1). A more detailed description of the individual environment of each site will be provided in the results section.

At sites where human remains have been found, as previously specified, due to the nature of the legislation governing the ICLVR testing of human remains is not permitted. It was not possible, nor would it have been appropriate, to sample grave contents. The level of degradation or preservation of human remains found was established through a general visual identification. In terms of microbiology and biochemistry issues such as bone digenesis and keratotic degradation were not examined in detail.

12.5 Methodology

The nine sites that were excavated were sampled by a Forensic Archaeologist. Eight of these sites had been excavated in an Active Search Phase begun in 2006. One of the sites, a coastal site, had been excavated in 2002 and was included in the sampling strategy for comparison and control purposes.

A total of 609 samples were taken across all sites. These sample numbers are broken down in Table 12.1 above. As can be seen the number of samples taken at locations where no remains have been found to date far outweigh sites where remains have been found. This was to ensure a maximum return of raw data as a knowledge base for hypotheses and possible future work at these locations.

Sample locations from sites where remains have been found were focussed in and around the core area to acquire comparative data that may provide an insight into the survival of human remains at each location. It must of course be acknowledged that this is retrospective sampling as no other option existed.

12.6 Sampling Method

All samples were taken using a Russian Core (Fig. 12.1), rinsed in-between samples with di-ionised water to reduce contamination. Each sample was given its own unique identification number and was placed in a clear grip seal plastic bag, 90x115 mm. Each bag was labelled and its location was recorded on the site map. Samples were taken, where possible, from different depths at each sample point location; at depths which were designed to correspond to the burial depth. Control samples were also taken at various points in unexcavated ground up to and including depths of 2.5 m, where possible.

These samples were then taken to an in-house laboratory for processing. It had been hoped that onsite processing would be a possibility as it is recognised that this is the most effective way to process samples, to reduce the impact of temperature

Fig. 12.1 Sampling with a Russian Core

fluctuations (Jones 2011). However, due to unpredictable weather conditions and the increased probability of contamination associated with outdoor processing it was decided that indoor laboratory processing would yield the most secure results.

An equal quantity of de-ionised water was added to each sample, the sample was stirred with a glass rod and allowed to sit for 1 h before a pH reading was taken. All readings were taken with an Orion three Star waterproof portable pH meter and an Orion Ross Ultra Triode. PH is a conventional quantity and values are based on the Bates-Guggenheim convention (Buck et al. 2002) thus all samples were recorded to two decimal places (0.00). Sample readings were controlled by regular buffering with predefined solutions of pH 4.00, 7.00 and 10.00. Results were recorded and plotted into charts using Microsoft Excel. These charts are presented along with sample results.

12.7 Results

In an effort to aid interpretation the sites have been divided into two parts: part one, sites where human remains were located and part two, those where remains currently have not been found. Each site is accompanied by a chart illustrating the results of the pH tests.

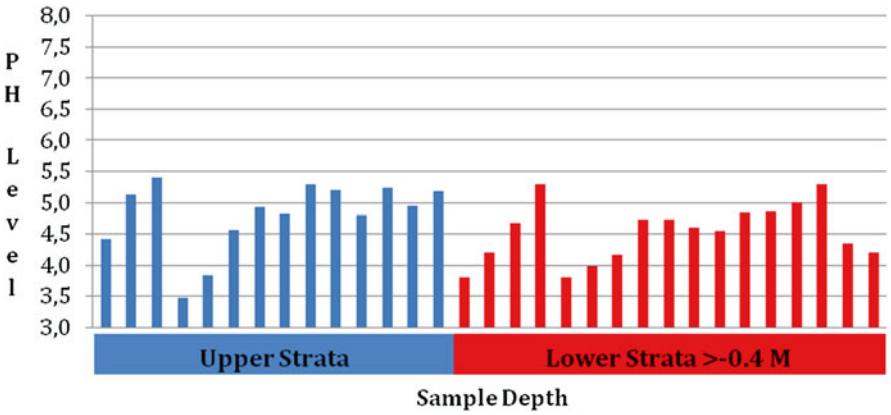


Fig. 12.2 pH Sampling results for Site A

12.7.1 Part 1: Sites Where Human Remains Have Been Located

Site A

Site A may be best classified as a saturated peat bog sitting on bedrock. There was a large amount of peat extraction undertaken here in the past. This site has been retired as a working landscape for over six decades and has become egregiously waterlogged in this time. Drainage was inserted for the purposes of excavation but it has since been allowed to return to its saturated state. The amount of samples retrieved at this location was largely dictated by access, made more restrictive by this water logging.

The minimum pH reading at Site A was pH 3.48 and the maximum was pH 5.39. The mean for the samples taken in the upper strata, at the surface of the site, was pH 4.68, the mean pH return for samples taken at sub surface depths was 4.63. Standing water on the site was a neutral 7.23. Skeletal remains survived at this site alongside treated natural fibres (Fig. 12.2).

Site B

Site B is in a mountainous location. There was some peat extraction in the vicinity in the past, however in the immediate surroundings of the find location the site cannot be described as a peat bog. There is a thin covering of dark humic soil followed by gravelly sand on a loose bedrock.

The minimum pH reading for Site B was 3.64 and the maximum was 5.33. The mean pH value across the site was 4.52 and the range at the centre point of the find location was between 4.40 at the surface, 4.31 at 0.4 m and 4.89 at 0.8 m. There was no particular trend identified on the horizontal or vertical plane, most likely explained by the fact that this area had been totally excavated to a uniform depth and

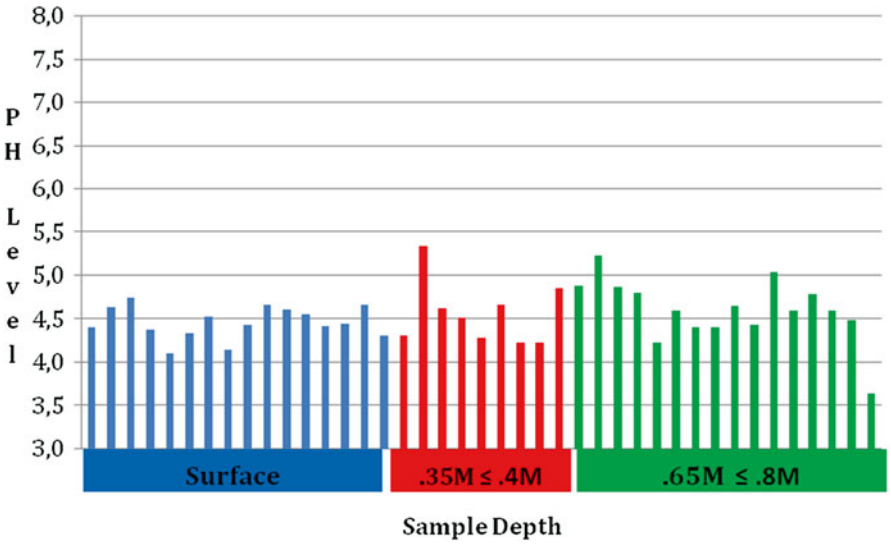


Fig. 12.3 pH Sampling results for Site B

subsequently reinstated, producing a completely disturbed search area. Highly degraded and fragmentary skeletal remains were found at this site alongside treated natural fibres (Fig. 12.3).

Site C

Site C is a saturated peat bog, more specifically a fen bog, sitting on a pure clay or marl base. There are many retired peat cuttings in this location and the site has become completely waterlogged in the last half a century due to the lack of maintenance of drainage.

A sample line was taken across Site C to represent the original waterlogged conditions prior to ICLVR drainage and excavation. Along this line the minimum value returned was pH 3.74 and the maximum was pH 7.28. The mean pH close to the surface was pH 6.30 and the mean return for 1 m below the surface was 6.17. A number of 'bog holes' were examined in close proximity to where human remains had been located. The minimum pH value at these locations was pH 5.49 and the maximum was pH 7.00. The stratigraphy was divided into upper, mid and lower and the respective mean return values for these locations were pH 6.10, pH 6.48 and pH 6.75. These results are more pH neutral than was expected, thus supporting the preservation of human remains. Skeletal remains in a good state of preservation survived at this location with some soft tissue alongside the survival of synthetic and natural treated fibres (Fig. 12.4).

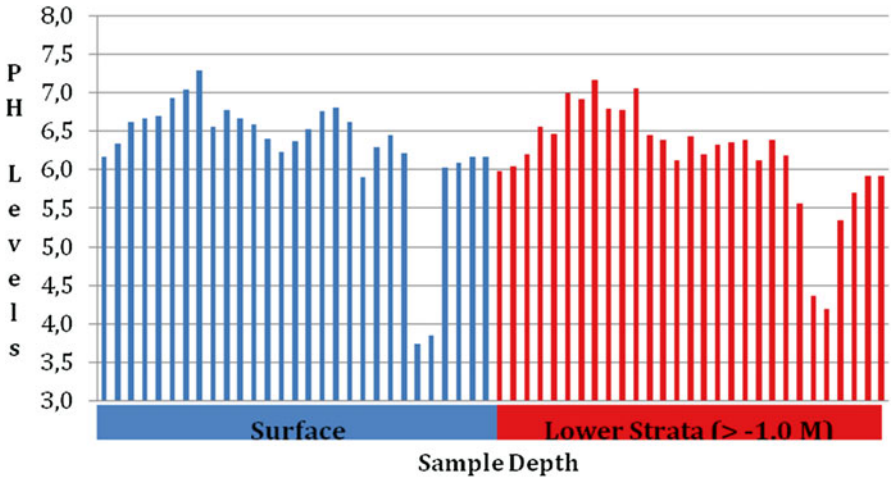


Fig. 12.4 pH Sampling results for Site C

Site D

Site D is a coastal location. The specific find location was at the base of a sand dune bank, well out of tidal range and within dry sand.

The minimum and maximum pH values for Site D were pH 7.06 and pH 9.02 respectively. The mean pH was a neutral pH 7.89. There was very little fluctuation in results and the trend was firmly in this neutral pH zone. PH return in the tidal zone was slightly less alkaline with a mean of 7.73, the slight reduction in value likely due to the repeated contact with saline water. Skeletal human remains alongside synthetic and natural treated fibres had been found at this location in 2002 (Fig. 12.5).

Site E

Site E is a coastal location. The specific find location is at the interface between the beach area and the sand dunes. While the location was free from the tidal zone, it had been affected by retreating and advancing dunes in the decades since burial. It was evident on site that the depth at which the remains were located had, over time, been subject to tidal activity.

The minimum pH at Site E was pH 7.39 and the maximum was pH 9.47, the overall mean pH return was an alkaline pH 8.78. From the three depths that samples were taken at, surface, 0.5 and 1 m it was found that the general alkalinity increased with depth. The mean return for the surface was pH 8.36, pH 8.96 at 0.5 m and pH 9.17 at 1 m depth. Skeletal human remains survived at this location along with treated natural fibres. These results compare closely with the results at Site

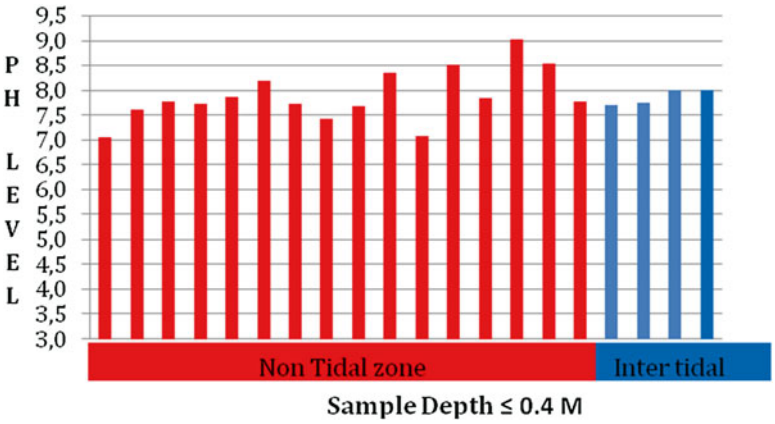


Fig. 12.5 pH Sampling results for Site D

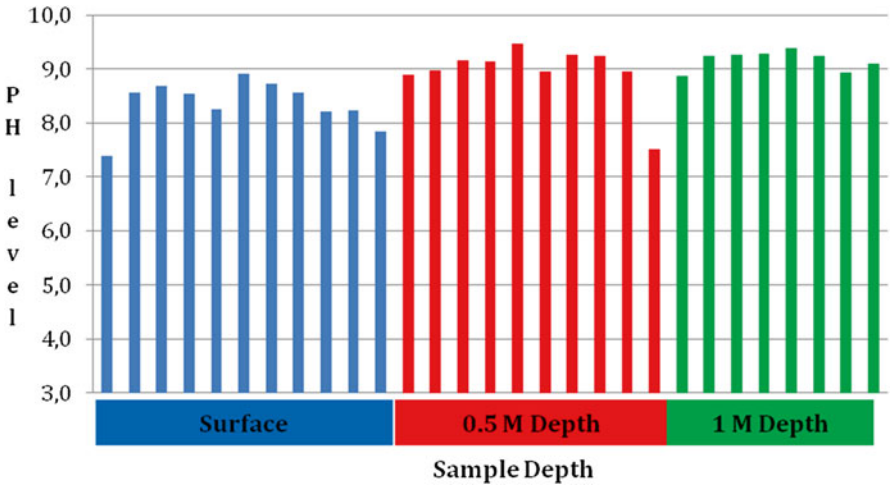


Fig. 12.6 pH Sampling results for Site E

D. However a visual assessment of the remains suggested that there was slightly better preservation of biological elements at Site E. This may be due to a variety of factors relating to the treatment of remains prior to burial (Fig. 12.6).

12.7.2 Part 2: Sites Currently in Absence of Human Remains

A sample 'mix' was analysed for sites where human remains were not found. This mix sample is also presented in an attempt to generically mimic what the pH of a grave 'fill' from these locations may resemble.

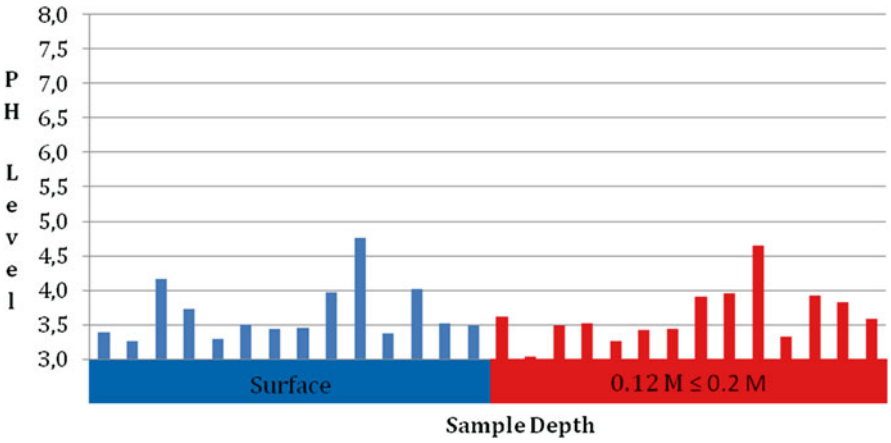


Fig. 12.7 pH Sampling results for Site F

Site F

Site F is typically characteristic of Spruce forest, a mature and managed woodland.

The minimum pH for Site F was a quite acidic 3.04 and the maximum a 4.76. No human remains were located at this site and it is widely accepted that the information leading to the search of this site was misleading. Further analysis of the results would not be beneficial as it is not likely that the ICLVR will return to search the area. Samples were taken for comparative and control purposes (Fig. 12.7).

Site G

The initial area of excavation at Site G was originally a typical blanket bog, sitting on a pure clay or marl base. Since 2000 this site has been subject to large scale disturbance and excavation. The area of interest has since moved to the periphery of this bog where the peat is desiccated, highly compacted and shallow (0.2–0.6 m), and sitting on a clay or marl base. This area is now inhabited by commercial Spruce woodland.

The minimum pH for this area was an acidic pH 3.39 in the upper stratigraphy and the maximum was a more neutral pH 7.11 in the lower stratigraphy. The mean return for the upper stratigraphy was pH 4.37, the mid layers was pH 4.12 and the lower clay stratigraphy was pH 4.98. The trend for this site is for the pH values to increase with depth. The surface is quite acidic and the clay slightly less so, however the soil still falls within the acidic soil range (Fig. 12.8).

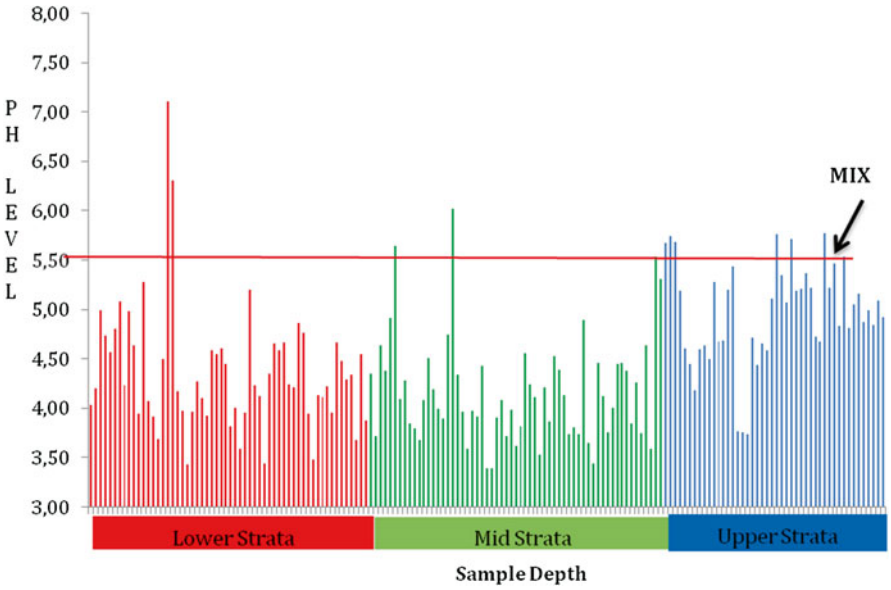


Fig. 12.8 pH Sampling results for Site G

Site H

Site H is a former blanket bog, it has been subject to reclamation and drainage over the last number of decades and now stands as shallow desiccated peat lying on a gravel base. The heavy covering of fern prior to excavation indicated the highly acidic nature of this site.

The minimum pH value at Site H was an acidic 2.99 and the maximum was 7.29. However a pH value above 4 was a rare occurrence, as is demonstrated by the mean pH of 3.98. When the site was divided into the stratigraphic layers upper, mid and lower a more insightful picture is gleaned from the results. The mean value for the upper and mid stratigraphy was pH 3.42 and pH 3.47 respectively. However as depth increased so did the pH value with a mean for the lower stratigraphy of pH 5.04, less acidic than the upper stratigraphy.

These results become more significant in light of the survival of canine remains at the base of a birch tree at pH 4.30. The state of the remains were fragmentary skeletal, highly degraded, with the survival of keratotics in the form of hair also present. Roots growing in and around the remains suggested that the dog had been placed here during the lifetime of the tree. A rudimentary tree ring analysis showed a possible age of a maximum of 15 years for the tree. This highly speculative interpretation would support a hypothesis that human remains could survive a period of 30 years but would be highly degraded. Skeletal elements may not in themselves survive but it would be expected that some suggestion of their presence would be evident such as a grave cut or clothing of synthetic origin (Fig. 12.9).

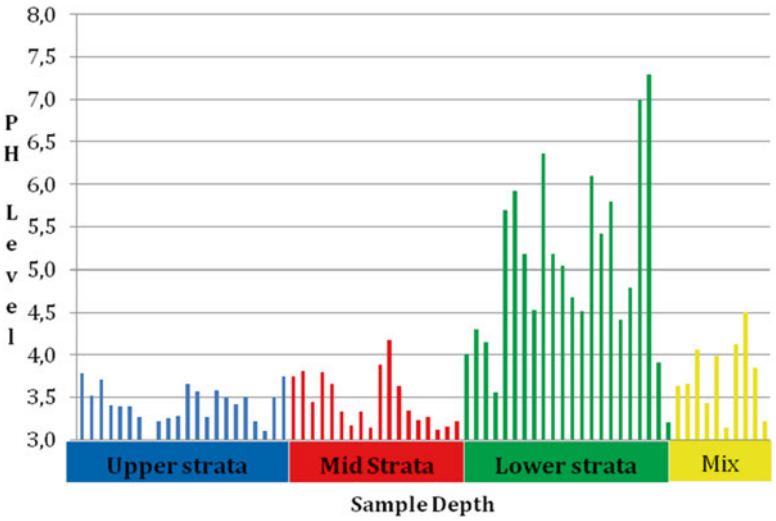


Fig. 12.9 pH Sampling results at Site H

Site I

Site I is one of the few raised bogs that survives in the local county. In the area of interest to the ICLVR this bog has however lost its original stratigraphy. It has been subject to major phases of disturbance throughout the last century. The most significant of these was in the 1980s when the surface of the peat banks was restructured on a large scale. Thus the original profile of this bog is retained only in the peat bank faces that lie to the north of the excavated areas. These were sampled individually to provide a more coherent picture of the original pH of this site.

Eleven banks were sampled to provide results that would be relevant to future work. A prescribed surface area of six of these banks had been excavated by the ICLVR, a prescribed distance on three of the banks had been excavated by An Garda Síochána (the Irish Police force) c. 1999 and two of the banks, to our knowledge, had never been excavated. Samples were taken in excavated and unexcavated areas.

The minimum pH value returned across all the samples was pH 3.23 and the maximum was pH 5.12. The mean for the site in total was 4.02. When the excavated areas are extracted from the unexcavated areas the mean for excavated areas is pH 4.15 while for unexcavated areas it is a more acidic pH 3.84. However there is evidence to suggest that disturbance to human remains during interment will accelerate decomposition (Carter and Tibbett 2008).

Samples were taken in the eleven bank sections. All show the trend of increasing pH as depth increases, thus a reduction in acidity as depth increases. Based on information received for this site these pH results would support the hypotheses that remains have degraded in the more acidic upper stratigraphy (0.0 m–0.5 m) where the mean pH across all banks at this depth was pH 3.69 (Fig. 12.10).

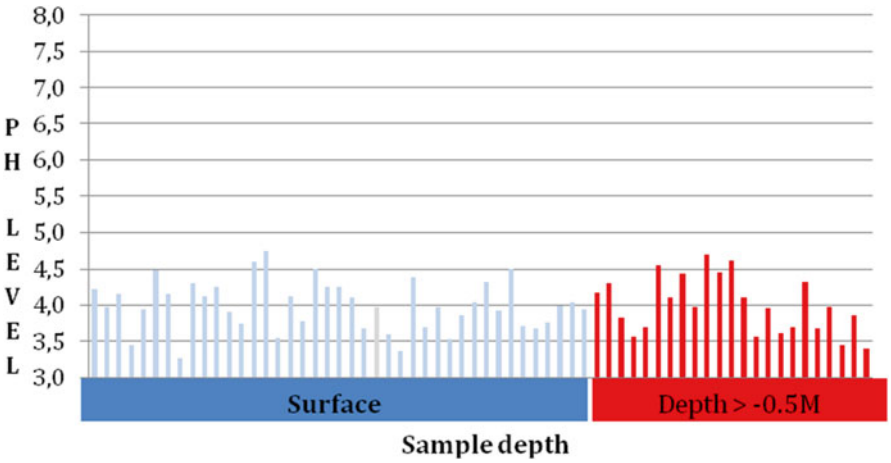


Fig. 12.10 pH Sampling results from Site I

12.8 Discussion

This study has shown that the human remains located by the ICLVR have survived in both acidic and alkaline conditions (see Table 12.2). The minimum pH value on sites where remains were found was pH3.48 and the maximum was pH 9.47. At first these would seem to be similar to the results retrieved from sites where remains have not been located, a minimum of pH 3.04 and a maximum of pH 7.11. The specifics of the site locations however are very different when examined more closely; each site has demonstrated its own unique factors that may have influenced forensic taphonomy and the survival of human remains.

The access of oxygen to buried human remains is a large contributing factor to the preservation state, the soil type, burial depth, water level and wrapping have to be taken into consideration.

It is worth considering what is different about these sites when compared to sites where human remains have been found and to consider this alongside their similarities in pH. The peat bog locations of Site H and Site I have a history that is potted with major soil disturbance and drainage events. When compared with Site A and Site C, these sites have remained undisturbed and dormant for the last half a century. This has caused them to become extremely waterlogged and saturated. The pH of this water table has been shown to be neutral and it may be suggested that this more favourable feature has allowed the preservation of biological elements at these locations. The peat itself may be acidic but it is the presence of more pH neutral water that has supported some form of preservation. It is not possible to determine the timeframe of this location being an anaerobic environment.

While Site H and Site I produced indications of an extremely acidic sub-surface environment some evidence of a clandestine burial should have survived. Be it in the form of an empty a grave cut or clothing of various fibres. These features may

Table 12.2 Data showing that human remains did survive in acidic as well as in alkaline environments

Site	Soil type	Burial depth	Ground-water level	Time elapsed between burial and excavation	Preservation state	pH	Wrapping in plastic/ clothing
A	Peat on bedrock	?	High	30 years?	?	Acidic	Natural fibres
B	Gravelly sand with thin humic layer on surface	?	Low?	?	Bad	Acidic	Natural fibres
C	Peat on clay	?	High	?	Good	Neutral	Plastic?
D	Fine sand	?	Low	?	?	Alkaline	Plastic?
E	Sand	?	Fluctuating?	?	Good	Alkaline	plastic?
F	?	N/A	Low?	N/A	N/A	Acidic	N/A
G	Peat (0,2–0,6 m) on clay	N/A	?	N/A	N/A	Acidic	N/A
H	Peat	N/A	low?	N/A	N/A	Acidic	N/A
I	Peat on gravel	N/A	?	N/A	N/A	Acidic	N/A

well have been lost at Site I due to the significant disturbance event, conversely at Site H, however discrete the surviving features were, they should have been apparent to the trained observer. The wider parallel that may be drawn here is that in very broad terms during this Active Search Phase human remains were located at sites where there had been no prior excavation (excluding Site B for operational reasons), and as stated above, it has been demonstrated that disturbance accelerates decomposition of human remains.

Information at Site G indicates that human remains were placed in the less acidic clay or marl in the lower stratigraphy. Despite the fact that it is reasonable to assume that the marl and acidic peat would have been mixed in the grave fill again the possible presence of remains should be identifiable to a trained observer. If skeletal elements do not survive the presence of a grave cut and any disturbance to the pure grey clay or marl should be apparent.

12.9 Conclusion

On average pH returns on sites where human remains were found were a minimum of c. pH 3.6 in peaty soils and a maximum of a more alkaline pH 9.0 in sandy soils. For sites where no remains were located the minimum pH was a slightly more acidic

pH 3.30 and a maximum of pH 6.00. In short, there is limited difference between the sites where remains have been located and where they have not. Conditions suggest that if human remains would have been buried at sites F to I, they would have been found, as their state of preservation would be at least reasonable. The lack of remains on these sites must therefore be attributed to other factors, such as the reliability of the information from sources or the site history since burial.

Acknowledgements Sincere thanks are due to Geoff Knupfer and Jon Hill for their consistent support and innate willing to push boundaries, without them this chapter would not have been possible. The author would also like to acknowledge the Commissioners Sir Ken Bloomfield and Mr Frank Murray and our dedicated team of field staff who make this work a reality. Thank you to Aidan Harte for sampling assistance in this survey.

References

Buck RP, Rondinini S, Covington AK, Baucke FGK, Brett CMA, Camões MF, Milton MJT, Mussini T, Naumann R, Pratt KW, Spitzer P, Wilson GS (2002) Measurement of pH, definition, standards and procedures (IUPAC recommendations 2002). Pure Appl Chem 74(11):2169–2200

Carter DO, Tibbett M (2008) Does repeated burial of skeletal muscle tissue (Ovis Aries) in soil affect subsequent decomposition? Appl Soil Ecol 40:529–535

Carter DO, Yellowlees D, Tibbett M (2010) Moisture can be the dominant environmental parameter governing cadaver decomposition in soil. Forensic Sci Int 200:60–66

Criminal Justice (Location of Victims' Remains) Act (1999) s. 5, http://www.irishstatutebook.ie/1999/en/act/pub/0009/index.html

Fischer C (1998) Bog bodies of Denmark and north-western Europe. In: Cockburn A, Cockburn E, Reyman A (eds) Mummies disease and ancient cultures. Cambridge University Press, Cambridge, pp 237–262

Forbes SL, Dent DB, Stuart BH (2005) The effect of soil type on adipocere Formation. Forensic Sci Int 154:35–43

Haslam TCF, Tibbett M (2009) Soils of contrasting pH affect the decomposition of buried Mammalian (Ovis Aries) skeletal muscle tissue. J Forensic Sci 54:900–904

Janaway RC, Percival SL, Wilson AS (2009) Decomposition of human remains. In microbiology and aging. In: Percival SL (ed) Microbiology and aging: clinical manifestations. Springer Science & Business Media, New York

Jones DM (ed) (2011) Environmental archaeology: a guide to theory and practice of methods, from sampling and recovery to post excavation. English Heritage Publishing, Swindon

Küpper L, Heise HM, Bechara FG, Stucker M (2001) Micro-domain analysis of skin samples of moor-mummified corpses by evanescent wave infrared spectroscopy using silver halide fibers. J Mol Struct 565–566:497–504

Lange TB (2008) The Effects of Soil Chemistry on Skeletal Preservation at the Voegtly Cemetery. MSc thesis, Michigan State University

Manifold BM (2012) Intrinsic and extrinsic factors involved in the preservation of non-adult skeletal remains in archaeology and forensic science. Bull Int Assoc Paleodontol 6:51–68

Nielsen-Marsh C, Hedges REM (2000) Patterns of diagenesis of bone I: the effects of site environments. J Archaeol Sc 27:1139–1151

Nielsen-Marsh CM, Smith CI, Jans MME, Nord A, Kars H, Collins MJ (2007) Bone digenesis in the European Holocene II: taphonomic and environmental considerations. J Archaeol Sc 34:1523–1531

Nord AG, Tronner K, Mattsson E, Borg GC, Ullen I (2005) Environmental threats to buried archaeological remains. Ambio 34:256–260

Northern Ireland (Location of Victims' Remains) Act 1999, s.4, http://www.legislation.gov.uk/ukpga/1999/7/contents

Plunkett G, Whitehouse NJ, Hall VA, Charman DJ, Blaauw M, Kelly E, Mulhall I (2009) A multiproxy palaeoenvironmental investigation of the findspot of an Iron Age body from Oldcroghan, Co. Offaly. Ireland J Archaeol Sci 36:265–277

Pringle JK, Casella JP, Jervis JR (2010) Preliminary soilwater conductivity analysis to date clandestine burials of homicide victims. Forensic Sci Int 198:126–133

Schotsmans EMJ, Van de Voorde W, De Winne J, Wilson AS (2011) The impact of shallow burial on differential decomposition to the body: a temperate case study. Forensic Sci Int 206:e43–e48

Schotsmans EMJ, Fletcher JN, Denton J, Janaway RC, Wilson AS (2014) Long-term effects of hydrated lime and quicklime on the decay of human remains using pig cadavers as human body analogues: field experiments. Forensic Science International 238:141.e1–141.e13. doi:10.1016/j.forsciint.2013.12.046

Stodkilde-Jorgensen H, Jacobsen NO, Warncke E, Heinemeier J (2008) The intestines of a more than 2000 years old peat-bog man: microscopy, magnetic resonance imaging and ^{14}C-dating. J Archaeol Sci 35:530–534

Turner-Walker G (2008) The chemical and microbial degradation of bones and teeth. In: Pinhasi R, Mays S (eds) Advances in palaeopathology. Wiley, Chichester, pp 3–29

Turner-Walker G, Peacock EE (2008) Preliminary results of bone diagenesis in Scandinavian bogs. Palaeogeogr Palaeoclimatol Palaeoecol 266:151–159

Wilson AS, Janaway RC, Holland AD, Dodson HI, Baran E, Pollard AM, Tobin DJ (2007) Modelling the buried human body environment in upland climes using three contrasting field sites. Forensic Sci Int 169:6–18

Chapter 13
Interdisciplinary Approaches to the Search and Location of Buried Bodies: A United Kingdom Context

Karl Harrison, Lorna Dawson, and Gaille Mackinnon

Over the past 20 years, the discipline of forensic archaeology has established itself in the United Kingdom as a primary method of detection for buried human remains. This has been achieved predominantly through the recognition of specific variations, patterning and disturbances in landscape, geological, botanical and ground signatures. These interpretations have subsequently been greatly enhanced by an increasingly sophisticated understanding, adoption and utilisation of geophysical search equipment and search techniques. In addition, the application of traditional archaeological excavation methodologies to criminal investigations that involve buried human remains can be seen to have become an important milestone in optimising an investigating team's ability to elucidate and extract evidence from the grave and burial environment (Hunter et al. 1995; Groen et al. 2015).

As a consequence, forensic archaeology has negotiated an important position within criminal investigation, existing as it does between the outdoor crime scene, normally controlled by the Crime Scene Manager and Police Search Advisor, the mortuary setting, typically dominated by the Forensic Pathologist and Anthropologist, and the laboratory environment of the Forensic Scientist.

It has been our experience that a multidisciplinary and strategy-led approach to the search for human remains offers by far the highest chance of success for the subsequent location and recovery of remains, whilst also maintaining effective control of the search area and preserving the integrity of the crime scene and any associated evidence contained therein (Harrison and Donnelly 2008). Within the United Kingdom forensic archaeology generally tends to be regarded as one of the disciplines of forensic ecology, which includes; sedimentology, soil science, botany, palynology (pollen), diatom analysis, entomology, stable isotope studies, radiocar-

K. Harrison (✉) • G. Mackinnon
Alecto Forensics, PO BOX 1339, Fulwood Park, Preston, UK
e-mail: karl.harrison@alectoforensics.com

L. Dawson
James Hutton Institute, Invergowrie, Dundee, UK

H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics*,
Soil Forensics, DOI 10.1007/978-3-319-33115-7_13

bon and other dating techniques, archaeology and anthropology (Roberts and Marquez-Grant 2012).

This paper presents a number of case studies that demonstrate what can be achieved when a comprehensive and integrated range of multidisciplinary search, ecological profiling and forensic solutions is applied in a complementary and synergistic way in order to best support criminal investigations.

In considering the search for and location of clandestine burials, it is crucial to understand the range of responses that may be considered by the investigative authorities. Depending on the nature and veracity of directed intelligence, it is often the case that the enquiry team will identify specific sites of interest. Where such sites can be defined within specific investigative parameters, they are often treated as crime scenes from the outset (ACPO 2006). Such a decision may be regarded as a high risk one; the deployment of staff onto a crime scene associated with a major enquiry entails the production of a forensic strategy and the consideration of extensive sequential evidence collection, to say nothing of the potential financial implications that such an approach will necessarily incur. It must also be noted that deployment to major crime scenes, including those that involve murder, fatal fires and sexual assaults takes precedence over the attendance volume crime scenes.

The confirmation of suspected scenes of major crime will initially be the responsibility of uniformed police response teams, who in relation to this role are referred to as First Officers Attending (FOA). Initial attendance at the major scene and ongoing examination would generally be undertaken by Crime Scene Investigators (CSIs). Any CSIs deployed to a major scene would be managed directly by a Crime Scene Manager (CSM) who has a responsibility to ensure that the forensic strategy is complied with, and that findings from the crime scene are communicated back to the Police Incident Room. Whilst the CSM is deployed to the scene with CSIs, the Crime Scene Coordinator[1] (CSC) has overall responsibility for deploying staff to scenes; coordinates the examination strategies of numerous CSMs, and ensures integration between the forensic strategy and the overall investigation directed by the Senior Investigating Officer (SIO). Because of the close relationship between the SIO and CSC, there is an expectation that crime scene coordination should be managed from the Incident Room. As such there is generally no requirement for CSCs to deploy to crime scenes, as this would compromise their pivotal management role in the major crime investigation.

All actions carried out by CSIs within the context of the major crime investigation should be guided by a written forensic strategy. This strategy is outlined in some detail at the highest level by the CSC, who ensures that the overall forensic strategy document is agreed by the SIO and integrates with other aspects of the investigation. This strategy can then be disseminated down to the lead examiners at each of the identified scenes; a large, complex scene with numerous opportunities for evidence gathering would be controlled by a CSM, because the CSC's strategy may require adapting to suit the challenges of the scene[2]. A relatively small and simple scene with a restricted range of forensic potential (such as a prisoner in custody) may well be entrusted to a lone CSI supported by a fairly proscriptive examination strategy.

In contrast with the fixed framework associated with the definition and examination of a major crime scene, it is more common that the no-body murder investigation is characterised by a period of search. Where directed intelligence associated with specific identified sites is absent or uncorroborated, a Senior Investigating Officer (SIO) would be unlikely to consider speculative forensic examination. More probably, a Police Search Adviser (PolSA), normally a middle-ranking police officer with specialist training in search coordination, would be given responsibility for identifying anomalous locations that might be associated with body deposition within a wider landscape of potential search possibilities. Such coordination would usually comprise both the deployment of search-trained police officers, as well as the integration of specialist services, such as findings derived from forensic telephonic examination, aerial reconnaissance, or attendance by forensic ecologists, in particular forensic archaeologists.

Whether the investigation is focused primarily on sequential forensic examination in the first instance, or on wider intelligence and search operations, it is fundamental that the contracted expert recognises the investigative priorities established by the SIO and the Incident Room. Whilst it is crucial that scientific procedure is deployed in the most robust way possible, it must also form part of an integrated inquiry which is ultimately initiated and directed by police action. Investigating detectives rarely attend crime scenes associated with major offences. Developments in DNA recovery, concerns about scene contamination, and tighter strategic control of detective actions via the Home Office Large Major Enquiry Suite (HOLMES; www.holmes2.com) has largely removed the requirement for a detective to be present at the scene. For similar reasons, it is preferred that a single ecological expert is present at the scene and can be trained to take samples for other experts such as botanists, soil scientists and entomologists to subsequently work on.

13.1 Case Study 1

A UK police force was investigating a suspected no-body murder in which a settled member of a traveling community had gone missing. Proof of life enquiries were centered on mobile phone and bank activity which added further confidence to suggest that the missing male was deceased. Witness intelligence from the male's family implicated a second male in the disappearance. The potential scene most strongly associated with the suspect as a site of body deposition was a pig farm owned by the suspect's family.

The investigative team had initially considered the successful recovery of human remains from a site such as a pig farm as being practically impossible, due to the belief that hard tissue fed to pigs would be entirely digested leaving to evident trace; the capacity of pigs to smell buried items and disturb them; and the extensive sewage system lying under the pig stalls which carried large quantities of water, effluent, feed and bedding. Despite these significant challenges, on seeking the opinion of the forensic archaeologist it was suggested that pig digestion would be unlikely to

completely destroy human bone without further processing, and that stalled pigs would be unlikely to have an opportunity to detect and disturb deeper burials, or indeed to interfere with burials located elsewhere on the farm site, such as the extensive raised earth embankments that functioned as a windbreak around much of the farm area.

The search phase of the operation was initiated on the arrest of the suspect. A search of the farm revealed large areas that the investigative team were happy to be of low significance, such as the farmhouse itself and the concreted animal stalls. Of more immediate concern were a large pile of dead and decomposing pig carcasses which had been set on fire prior to police arrival and was still smoldering at the initiation of the search operation. This suspicious area could only be searched by hand-raking out the animal remains in order to confirm the absence of any intermixed or commingled human remains.

Ultimately, the search was partial and inconclusive. Whilst much of the farm area was searched by eye, there was no systematic sequence of 'least to most' invasive searching, which would be consistent with strategies of search and forensic examination. The pile of decomposing pigs was subjected to a partial search, but as the archaeological excavation grew deeper, safety concerns about collapse of the newly dug trenches grew greater, and ultimately the search was discontinued. Prosecutors advanced a 'no body murder' case against the two defendants that was based on a hypothesis that the body of the missing male had been fed to the pigs and as such could not be found. The jury consequently delivered a not guilty verdict on both of the charged males.

13.2 Case Study 2

An extensive search for a missing female by a UK police force ultimately ended in a successful conclusion through a mix of investigative skill, technical expertise and the use of specialist search techniques. The female in question had been missing for just over a year, during which time police enquiries had established the likelihood of her being deceased. The investigative team has subsequently prioritised a male suspect with whom she had had a relationship in the months immediately prior to her disappearance.

The male suspect had been arrested, questioned and subsequently released, during which time technical intelligence had been gathered from his communication devices suggesting the location of a potential area of search. This area remained rather expansive, encompassing a number of fields on either side of a small country road located a short distance outside the town where the male and female both lived.

Whilst this area remained too wide to consider any form of comprehensive search solution, it did allow for the targeted use of geophysical and geochemical search techniques to attempt to elucidate an area of burial. This technical approach was combined with overt, high-visibility police searches of field boundaries and specific areas of interest within the site parameters.

Ultimately, the high visibility techniques of police search proved more successful by indirect means. As the search progressed across the fields and got increasingly closer to the actual burial location, which had been correctly assumed to lie in a field next to the country lane, the suspect decided to attempt to move the female's body in order to prevent its discovery. This attempt by the suspect failed, and resulted in the partial uncovering and disturbance of the body before the suspect opted to hand himself in to police custody and thus trigger the securing of the scene and ultimately the recovery of the human remains and associated forensic evidence.

The search phase of this case study was fully integrated within the criminal investigation and saw the deployment of a planned sequence of police and specialist search techniques, which ultimately prompted a failed attempt by the suspect to move the remains prior to their imminent discovery by the police. Ultimately the suspect was tried and found guilty of murder.

13.3 Case Study 3

A search of an extensive detached house with an adjacent industrial yard, located in a rural area close to a busy main road, was initiated following a report that the sole female occupant of the house and majority partner in the yard's business had gone missing. Questioning of the female's business partner suggested that he may have been complicit in her disappearance, and as a consequence a search of the house and ground was instigated.

The search area was controlled by the PolSA, but with close reference to the requirements of the CSM, who ensured that the needs of the forensic strategy, particularly with regard to anti-contamination procedures and the sequential recording and examination of areas of concerted searching by police search officers. The potential for burial as an act of concealment was considered by the investigative team at an early stage in the examination, partly because of the general nature of the site, and specifically because of the presence of a mechanical excavator at the yard around the material times of the female's disappearance, and the apparent movement of earth from large piles of spoil present in the industrial yard. As a consequence, the support and advice of the forensic archaeologist was sought at a relatively early stage by the investigation team.

The nature of the site suggested a clear priority search area, which was centered on a derelict branch railway line and platform that formed part of the farthest boundary of the industrial yard. This platform was closely associated with a large deposit of soil formed into a linear embankment, which was believed to conceal beneath it a brick-lined shaft contemporary with the construction of the railway dated to the mid-late Victorian period. The soil embankment posed challenges for the deployment of specialist search techniques, in that it was comprised of uneven made ground with rubble inclusions which was poorly suited to geophysical survey, and that the ground level was raised to the extent that venting the embankment with an

augur would largely fail to penetrate the underlying 'natural' ground surface to facilitate the release of volatiles and subsequent use of cadaver search dogs. As a consequence, controlled machine and hand excavation by a police search team operating under the supervision of both the forensic archaeologist and the CSM was utilized.

Despite this clear initial focus, wider consideration of the site by the PolSA and CSM remained crucial, and a planned strategy was prepared to prioritise subsequent areas of searching, should the embankment area prove not to feature concealed human remains. This strategic planning proved to be redundant when the top of the brick shaft was first located, followed some time later by the discovery of the deposition of the body of the missing female who was located at a considerable depth at the base of the shaft.

While early identification of the industrial area to the rear of the house had been primarily driven by suspect and witness interview and by the preliminary findings of associated property searches, the development of the police search and forensic examination at the scene was the shared responsibility of the PolSA and CSM. Within this partnership arrangement, it was tacitly understood that primacy of responsibility lay with the PolSA until the location of human remains was confirmed, at which point the seniority of roles was reversed, with the CSM acting as senior decision-maker.

This search was proactive and sequential, with a clear order of investigation both across the scene as a whole, as well as with regard to specific actions taken at the primary site of interest by the railway platform. The nature of the deposition site was such that the non- or semi-invasive search techniques of geophysical survey and cadaver dog use were largely ineffective, and controlled excavation had to be almost exclusively relied upon. Crucially, this development only served to increase the importance of appropriate oversight by a forensic archaeologist, rather than preclude it.

13.4 Case Study 4

Soil analysis can be a very useful technique in both determining provenance in a law enforcement/police search and also as physical evidence to determine if an offender or item was associated with a crime or location (Dawson and Hillier 2009; Dawson and Mayes 2014). This case illustrates an example where soil information assisted in the search for a grave and also provided evidence that was presented in court. This case is an excellent example of effective team work, and illustrates interdisciplinary integration, which included police investigators, police search teams, a forensic geology ground search specialist, forensic archaeologists and soil scientists, all with particular complementary expertise.

Operation Sorrento was a large scale, high profile 'No-body Murder' investigation which lasted over several months, stretching from County Durham to West Yorkshire, in England, UK. During the winter of 2013, the sons of a middle-aged

woman called the Police reporting that they had not seen or heard of their mother for 5 days. Initial enquiries revealed that the last person to have seen her was her boyfriend. Due to some concerns from a comparison of the boyfriend's initial account to the Police with the account he had given to the missing woman's family, a Homicide investigation was quickly commenced, running alongside a Missing Person enquiry (MisPER). This developed through the phases of arrest, painstaking search, recovery of evidence and, in particular, the eventual recovery of the remains of the victim, to full trial and ultimately conviction.

Passive data from the suspect's mobile phone indicated wide areas of moorland which the suspect had visited in the times between the last sighting of the missing woman and the report of her being missing from home. The search and passive data strategies were intrinsically linked with other pieces of work on-going, including for example Closed Circuit Television (CCTV), forensic searches, 'house to house' and witness interviews.

There was also significant time pressure on the investigation team as early Crown Prosecution Service (CPS) charge authority had been sought and granted for a murder charge on the male only 3 days after the victim's disappearance. The search for the missing woman was one of the largest search operations ever undertaken by Durham Constabulary, supported by specialist search resources from West Yorkshire. South Yorkshire Police, Greater Manchester Police, CAST (UK Home Office, Centre for Applied Science and Technology) and other underwater assets being among the specialist teams deployed. The objective of the search was to find her body and/or evidence relating to it. A preliminary conceptual appreciation of the geology was suggested for the suspected burial site located in the central Pennines of northern England. Here, the geology is dominated by coarse, strong, well-jointed and cross-bedded sandstones of Kinderscout Grit, of Namurian age. These were interbedded with weaker shales and mudrocks that have undergone weathering and erosion to form distinct escarpments, rolling hills in a moorland topography setting. These are covered by a veneer of peat, which has accumulated over the past c. 10,000 years. The peat may reach up to about 2–3 m depth in places. In the context of a burial site, the distinct weathering of the slopes and rock exposures potentially enables navigation across the moorland landscape to perhaps facilitate the location of a possible grave. The peat can be dug with ease and a shallow grave may be dug and the peat/sub-soils and the displaced soils reinstated in a very short period of time, as determined in other search areas in this same range of hills (Donnelly and Harrison 2013). Geophysical techniques potentially deployable in these type of geological settings were advised to include ground penetrating radar, magnetic and electromagnetic methods.

This case presented particular challenges with regard to the scope of the potential area of search. While mobile phone evidence suggested an upland location close to Cragg Vale, West Yorkshire, other intelligence suggested the possibility of a link with a reservoir site. Consequently a reconnaissance report was prepared by the forensic scientists that considered geological characteristics and geomorphological features across a number of target areas in the Halifax area. In addition, the search

report identified key exhibits taken from the suspect's car that might retain linking soil traces.

Production of a reconnaissance report by the forensic archaeologist was followed by targeted soil sampling at sites supported by both case-specific intelligence and generalised models of body deposition behaviour. Target landscapes were characterised by a variety of potential contact materials (as is found on the moorland around Turvin Clough, which featured highly organic upland peat bog overlying deposits of Kinderscout Grit).

Ultimately, intelligence-led targeting of soil samples helped provide a focus for searching efforts that led to the discovery of the remains of the missing woman in a clandestine grave. Field observations by a skilled member of South Yorkshire Police's Victim Recovery Dog Team, near to the roadside, fine-tuned the geographic position of the grave. The location highlighted was a layby, which offered easy access to the moors via a wooden gate. On the right hand side of the gate was a natural steam flowing towards the roadside. The moorland was open and directly accessible on foot and had little natural visual protection from the roadside. The deposition site was dug into the banking of the stream further into the moor about 700 m away from the parking area. The location of the deposition site on the stream bank gave concealment from the road side allowing the perpetrator to cut into the banking and conceal the body without being compromised by any passers-by.

Using a dual soil analysis approach, by analysing both the inorganic (mineralogy) and the organic characteristics (hydrocarbon and alcohol fractions) of the soil, we were able to exclude certain areas as the source of the material found on the gloves from the boot of the male's vehicle (home area, other areas identified as locations where he had been parked during the initial period the woman went missing). The forensic soil scientists were also able to home in on this area as being the location that most matched the characteristics of the material on the male's gloves found in the boot of his car. In parallel to this, field search teams identified anomalies in the edge of the road that indicated disturbance with unusually Kinderscout Grit sandstone near the soil surface. The Victim Recovery Dog (VRD) Handler also noticed rocks, clay and soil, which had been deposited in the stream near to the site. On examining the distribution of the clay and boulders, these were spread over a short distance, in an arc shaped pattern as if thrown from one location. He also noticed that an elongate rock appeared to have been moved from a patch of Moss less than a metre away (it appeared to have different weathering patterns to others in the area and no lyceum was present offering a clue to the experienced PolSA). The grass and foliage around the boulder appeared to be relatively dry, compared to the surrounding foliage. The officer then took an auger sample near to the site (without interfering with the site) and noticed that the core was disrupted with subsoil located where the topsoil should have been. He then put his dog over the area and got a positive indication.

From the soil organic marker analysis it was also clear that of the reference samples collected and analysed, one sample was very similar to that sample collected from the glove brushings. Furthermore, the organic marker characteristics (alkanes, alcohols, aldehydes, ketones) of the sample from the glove differed greatly from the

other reference samples. On the basis of soil mineralogy, a number of sample locations were excluded as locations for the source of the material examined from the glove. There were also clear differences in mineralogy between both sample locations and the victim's house at Chester-le-Street; these two locations were subsequently excluded as being the source of the material on the gloves found in the boot of the male's vehicle and these were eliminated from the search as being the source of soil on the gloves. One sample location was the location found to be very similar in terms of mineralogy and organic characteristics to the soil on glove. The missing woman's body was found within 700 m of this location by police dogs once the area was further narrowed down through field observations. Soil samples were then collected at the grave, including contact point samples where the perpetrator would have stood and would have dug. The suspect vehicle was forensically sampled and traces of soil were found in the foot wells of the suspect's car. These evidential soil samples were analysed in the same way as were the samples examined in the search phase of this operation.

In court, under the UK adversarial system with a judge and a jury, the strength of evidence that the soil-like material found on the gloves originated from the grave site location was moderately strong for both organic and mineral soil characteristics. This evidence suggests that the person who was wearing the gloves had been in contact with an upland moorland environment in northern England, as was characterised at the grave site location. In addition, the strength of the evidence that the soil-like material found in some of the footwell mats of the suspect's vehicle originated from both the organic and mineral horizons of the grave site location was moderately strong for organic characteristics. Signs of mottling and deciduous *Molinia* grass also provided additional common characteristics between the soil from the vehicle and the grave site location.

The soil and plant evidence suggested that there was transfer of soil to the suspect's vehicle from an upland moorland environment as was characterised at the grave site location. In addition, evidence was presented that indicated that the suspect's fingerprint had been transferred to flowers found within the grave, and CCTV imagery and phone records linked to the dates and times of the missing woman's disappearance.

The suspect was found guilty of manslaughter and was sentenced to 18 years in prison.

13.5 Conclusion

Over the past 20 years, the numerous types of forensic scientist involved in the location of buried and concealed remains have grown used to considering themselves part of a multidisciplinary effort. Forensic geoscientists, palynologists, entomologists, geophysical search specialists and forensic archaeologists are generally happier to conceive of themselves as forming part of a suite of methods broadly classified under the banner of 'forensic ecology', and that rather than any one

specialism offering superior capability, their functions are frequently complementary and are at their most effective when coordinated as an ensemble (Davenport et al. 1992). Each case is individual and depending on that case context, a different suite of specialists will be required.

Whilst the application of these various natural sciences to forensic search scenes is not new[3], their formal affiliation with one another in the syncretic science of forensic ecology is new, and not always straightforward, despite their shared stated goals. Despite this, the various disciplines of forensic ecology have made progress in consolidation, particularly in the last decade[4]. This development appears to be two-pronged; deriving simultaneously from a small number of academic authors with an interest in forensic search (Ruffell and McKinley 2004; Roberts and Márquez-Grant 2012; Pirrie et al. 2013) and from the maintenance of a range of forensic ecology provision solutions offered by a number of UK Forensic Service Providers (FSPs).

There is another side to multidisciplinary working which has thus far attracted less attention from either academic authors or FSPs, but which this chapter has attempted to consider and exemplify in some detail; the integration of the forensic ecologist within the structure of the police investigation and specifically alongside police search officers and CSIs in the context of no-body murder search and location operations. It is arguable that the forensic ecologist present at such scenes has longer and more in-depth contact with the police than their counterparts who work primarily as laboratory-based analysts. No body murder cases frequently represent some of the longest and most complex investigations, with greater initial reliance on human and technical intelligence than most other responses to major crime outside of the arena of counter-terrorism. Within this context, the forensic ecologist must retain their independence and objectivity to ensure they meet the robust requirements of the court whilst being able to facilitate the specialist needs of the police investigation.

As a consequence of this, it behoves the forensic ecologist engaged in body search and location to not only possess an intimate understanding of the general processes of forensic examination, but in addition to have an awareness of the requirements and capabilities of police search professionals, and furthermore to understand the processes that underpin the workings of the Major Incident Room. The optimization of confidence in any police search supported by forensic ecology requires a sequential, analytical approach informed by scientific best practice as a *sine qua non*, and the continuing development of scene and laboratory quality standards in forensic science will assist greatly in underpinning this axiom. Further to this, however, a secondary challenge to optimal searches lies in the manner in which the various agents of police major investigations interact with and reflect on the advice and work of the forensic ecologist, and *vice versa*. Such a complex and multifaceted challenge is not so easily nor so comprehensively addressed through academic writing or forensic service provision as the conceptualisation and branding of the science of forensic ecology, but it remains just as critical to its eventual maturity. Effective cooperation between the forensic experts, the investigation teams and the criminal justice system should better enable justice to prevail.

References

Association of Chief Police Officers (ACPO) (2006) Murder investigation manual. National Centre of Policing Excellence, Wyboston

Davenport GC, France DL, Griffin TJ, Swanburg JG, Lindemann JW, Tranunell V, Armbrust CT, Kondrateiff B, Nelson A, Castellano K, Hopkins D (1992) A multidisciplinary approach to the detection of clandestine graves. J Forensic Sci 37:1445–1458

Dawson LA, Hillier S (2009) Measurement of soil characteristics for forensic applications. Surf Interface Anal 42:363–377

Dawson LA, Mayes RW (2014) Criminal and environmental soil forensics: soil as physical evidence in forensic investigations. In: Murphy BL, Morrison RD (eds) Introduction to environmental forensics, 3rd edn. Academic, Oxford, pp 457–486

Donnelly LJ, Harrison M (2013) Geomorphological and geoforensic interpretation of maps, aerial imagery, conditions of diggability and the colour coded RAG prioritisation system in searches for criminal burials. In: Pirrie D, Ruffell AR, Dawson L (eds) Environmental and criminal geoforensics, vol 384, Special publication. Geological Society of London, London, pp 173–194

Groen WJM, Marquez-Grant N, Janaway R (2015) Forensic archaeology: a global perspective. Wiley-Blackwell, Oxford

Harrison M, Donnelly L (2008) Buried homicide victims: applied geoforensics in search to locate strategies. J Homicide Major Incident Investig 4(2):71–86

HOLMES2: The future of crime management technology. www.holmes2.com. [Online. Accessed 19 Oct 2015]

Hunter J, Roberts C, Martin A (1995) Studies in crime: an introduction to forensic archaeology. Routledge, London

Pirrie D, Ruffell A, Dawson LA (eds) (2013) Environmental and criminal geoforensics. Geological Society of London Special Publications, London

Roberts J, Márquez-Grant N (2012) Forensic ecology handbook: from crime scene to court. Wiley-Blackwell, London

Ruffell A, McKinley J (2004) Forensic geoscience: applications of geology, geomorphology and geophysics to criminal investigations. Earth-Sci Rev 69:235–247

Chapter 14
Forensic Geophysics: How the GPR Technique Can Help with Forensic Investigations

P.M. Barone, C. Ferrara, E. Pettinelli, and A. Fazzari

Abstract In addition to traditional techniques, a broad range of science and technological expertise is applied in forensics, including geophysics. Forensic geophysics is used to locate, map and study buried objects or targets beneath soil or water using geophysical tools. Various geophysical techniques are used in forensic investigations, in which the buried targets may include varied items, such as weapons, metal barrels, human remains and bunkers. Geophysical methods have the capability to aid investigators in searching for and recovering such targets because large areas can be investigated non-destructively and rapidly. An area of interest may be the location of an illegal burial or where a suspect has attempted to hide a target item underground. When there is a contrast between the physical properties of a target and those of the material in which it is buried, it is possible to accurately locate the target item. Geophysical methods can also be used to recognize evidence from humans or excavation activities, both recent and older.

Ground penetrating radar (GPR) is one of the most useful geophysical tools for investigating targets beneath the soil. This technique uses radio waves to map structures and features buried in the ground. A radar-transmitting antenna emits an electromagnetic impulse that can be reflected or scattered by a dielectric discontinuity in the ground and gathered by a receiving antenna.

This technique has yielded impressive results in various applications, such as archaeology, environmental science and engineering; it has recently been applied to forensics with good results, but the technique can still be improved.

P.M. Barone (✉) • C. Ferrara
Archaeology and Classics Program, The American University of Rome,
Via Pietro Roselli, 4, 00153 Rome, Italy
e-mail: p.barone@aur.edu

E. Pettinelli
Department of Physics, University of Roma Tre,
Via della Vasca Navale, 84 00146 Rome, Italy

A. Fazzari
Sensors & Software, Inc., 1040 Stacey Court, Mississauga, ON, Canada

H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics*,
Soil Forensics, DOI 10.1007/978-3-319-33115-7_14

The aim of this chapter is to highlight the potential of GPR, specifically how it may be helpful during forensic investigations to quickly provide high-quality results. The method is very useful, but it is necessary to understand its underlying principles and procedures to obtain the best results. In this paper, laboratory-scale and field-scale experimental data are presented to provide a better understanding of the strengths and weaknesses of GPR.

14.1 Introduction

Forensic geophysics is a scientific technique that is useful in forensic applications. Forensic geophysics is used to locate, map and study buried objects or targets beneath soil or water using geophysical tools. Various geophysical techniques are used in forensic investigations in which the buried targets may include such varied items as weapons, metal barrels, human remains and bunkers. Geophysical methods have the capability of aiding investigators to search for and recover such targets because large areas can be investigated non-destructively and rapidly. When there is a contrast between the physical properties of a target and those of the material in which it is buried, it is possible to accurately locate the target item. It is also possible to recognize, for example, evidence of human presence on the soil or excavation activities, both recent and older (Calkin et al. 1995; Ruffell and McKinley 2005, 2008, and literature therein).

Ground penetrating radar (GPR) allows investigators to find buried artifacts and identify soil disturbances, making it a powerful tool for police forensic investigations, as described in several papers (Strongman 1992; Calkin et al. 1995; Roark et al. 1998; Davenport 2001; Freeland et al. 2002, 2003; Cheetham 2005; Ruffell and McKinley 2005, 2008; De Souza 2009; Harrison and Donnelly 2009). Applications are wide ranging, including the search for ancient and modern graves (Bevan 1991; France et al. 1992, 1997; Mellet 1992; King et al 1993; Owsley 1995; Miller 1996; Nobes 2000; Miller et al. 2002; Schultz et al. 2002; Powell 2004; Scott and Hunter 2004; Ruffell 2005; Barone et al. 2007; Schultz 2007; Congram 2008; Pringle et al. 2008; Ruffell et al. 2009a, b; Novo et al. 2011; Schultz and Martin 2011; Dirkmaat and Schultz 2012; Pringle et al. 2012), experimental tests (Buck 2003; Schultz et al. 2006; Schultz 2008; Balsi et al. 2010; Solla et al. 2012) and very specific GPR forensic investigations, such as locating mass graves (Witten et al. 2000; Ruffell et al. 2009a, b), identifying illegally buried toxic waste (Ruffell and Kulessa 2009) and locating victims of the 1918 Spanish Flu (Davis et al. 2000).

As described in the literature, police regularly use GPR to uncover buried caches of drugs, money and weapons and to locate unmarked graves or clandestine burials while avoiding costly and intrusive excavations. However, it is not always easy to use the technique in a forensic investigation without a strong background in geophysics. The method is very useful, but it is necessary to understand its principles and procedures to obtain the best results.

In this chapter, we provide examples of the application of GPR to specific forensic problems. In particular, certain tests are used to identify the electromagnetic signature of a buried metallic handgun., We show how the GPR signal is affected by the disturbed soil in graves to provide an understanding of the way in which the technique can be helpful during forensic investigations in terms of quickly providing high-quality results.

14.2 Theory and Methods

GPR functions by sending pulses of radio waves into the ground and measuring the travel time of the returning reflection. Reflections result when the GPR wave encounters a change in electrical properties as it travels through the host material. This change may be caused by a localized target (a gun or box of money) or an area with contrasting soil conditions.

The signals reflected from subsurface interfaces or buried objects are received by the receiver antenna. The receiving electronics amplify and digitize the reflected signals, which are stored on a disk or tape for complete post-processing.

The primary factors that affect the performance of the GPR technique are as follows: (i) the contrast in relative permittivity (dielectric constant) between the target and the surrounding ground, (ii) the conductivity of the ground, (iii) the shape of the target and its orientation with respect to the radar antenna and (iv) the density of scattering bodies within the ground that produce reflections similar to those from the target (Annan 2003).

The depth of penetration of the radar energy depends on the conductivity of the materials being probed, which, in turn, is primarily governed by the water content and the concentration of salts in solution.

The ability of the radar to detect a target will depend to a large extent on the contrast in the dielectric properties of the materials being penetrated. These properties can be expressed in terms of relative permittivity (K), which is a measure of the dielectric constant of the ground versus that of free space.

Both conductivity and relative permittivity are, in general, frequency dependent. Moreover, the addition of water causes the relative permittivity to rise. In most cases, the greatest changes occur in soils with high porosity. Relative permittivity is sensitive to moisture content and pore water chemistry. Conductivity varies in a much wider range, approximately 20 orders of magnitude, and is sensitive to moisture content and pore water chemistry.

At their best, radar surveys reveal a greater richness of detail of underground features than does any other type of geophysical survey. Radar surveys also detect a wider variety of features than other surveys do. Because so many features can be identified with a radar survey, the technique may be less able to furnish an identification of particular items because various features may tend to look similar in a radar image. However, the value of radar for identification is in the depth information that it provides. By collecting continuous lines of data over an area, investigators

are able to detect and map anomalies and focus on those areas rather than excavating the entire field in search of evidence. When radar profiles are constructed along parallel lines, a rough approximation of the three-dimensional form of a buried feature can be determined from the plan maps. This form or shape information is usually the best guide to the identification of the features that are detected by the radar (Jol 2009). In general, GPR involves a trade-off between penetration depth and resolution. Higher-frequency transducers will provide better resolution, whereas lower-frequency transducers will achieve deeper penetration into the ground.

After acquiring the data, the first step in the interpretation of a radar survey is a determination of the velocity of the radar pulse in the material. With this information, it is possible to estimate the depth of the features that are detected. These radar echo arcs are mathematical hyperbolas; they are simply the result of the changing distance from the radar antenna to the reflector. The radar antennas send a broad beam of radiation into the soil; therefore, the antenna detects a feature before it has traveled directly above the feature. As the antenna passes over the feature, the distance to the object decreases, and it increases again as the antenna moves away from the object. Conventional GPR data collection involves moving the GPR across the area of interest to produce a cross-section (or radargram) view. This image is visible in real-time on the screen of the data logger, making it possible to analyze the data in the field and mark areas of interest (Pettinelli et al. 2011).

A typical GPR system is shown in Fig. 14.1, with the primary transducer unit mounted on a cart, suspended slightly above the ground, and equipped with a GPS (global positioning system). The data acquisition is controlled by means of an odometer wheel, which triggers the radar at set intervals. The data are collected and displayed on the digital video logger, which acts as a weatherproof computer in the field. Certain situations (tight areas or wall scanning) may require a different setup, such as a handheld system.

14.3 Lab-Scale Experiments

One of the best ways to determine whether GPR can provide the investigator with the desired data is to observe how GPR performs in laboratory tests before using it in the field.

From lab-scale experiments conducted in the Geophysics Laboratory, Department of Mathematics and Physics at the University of Roma Tre, it is possible to study the electromagnetic signal of an object buried in a host material. This return signal is normally affected by various soil conditions (e.g., wet, disturbed, excavated) that may increase or decrease the "visibility" of the target.

A simple host material, such as glass beads ($\varepsilon = 3.1$), allows for a perfect reconstruction of not only the depth but also the geometry of a metallic object (e.g., a handgun). This experiment provides data on the detectability of such a buried object under optimal conditions and the resolution of a multi-profile GPR acquisition.

Fig 14.1 A typical GPR system during a forensic investigation: the main transducer unit mounted on a cart, a DVL, and equipped with a GPS

The GPR system used in this experiment was the bistatic TR1000 (Sensors & Software, Inc., Mississauga, Canada) with 1 GHz antennas, a time window of 25 ns, the capability of stacking four return signals and a step interval of 0.5 cm. As shown in Fig. 14.2a, the metallic gun (with a length of approximately 25 cm and a height of approximately 11 cm) was buried 9 cm deep in this homogeneous material, with the weapon lying flat. The GPR acquisition was performed following a plastic grid (60×60 cm) with orthogonal lines set 5 cm apart.

The acquisition was performed repeatedly but using a line spacing of 10 cm and then 15 cm to observe the decreasing spatial resolution as the line spacing increases. As shown in Fig. 14.2b, with the burial depth of the gun remaining unchanged, the GPR map with the 5 cm line spacing clearly yielded better resolution of the buried gun than did the 10 and 15 cm line spacings, and the 5 cm line spacing also showed the gun geometry very clearly.

Although the tightest line spacing seems to be the best way to obtain high-quality GPR data, it is worth noting that the time component is also very important during a forensic investigation. To strike a balance between time efficiency and spatial resolution in a field-scale acquisition, a multi-profile GPR survey should be performed using a spacing interval of 10 cm.

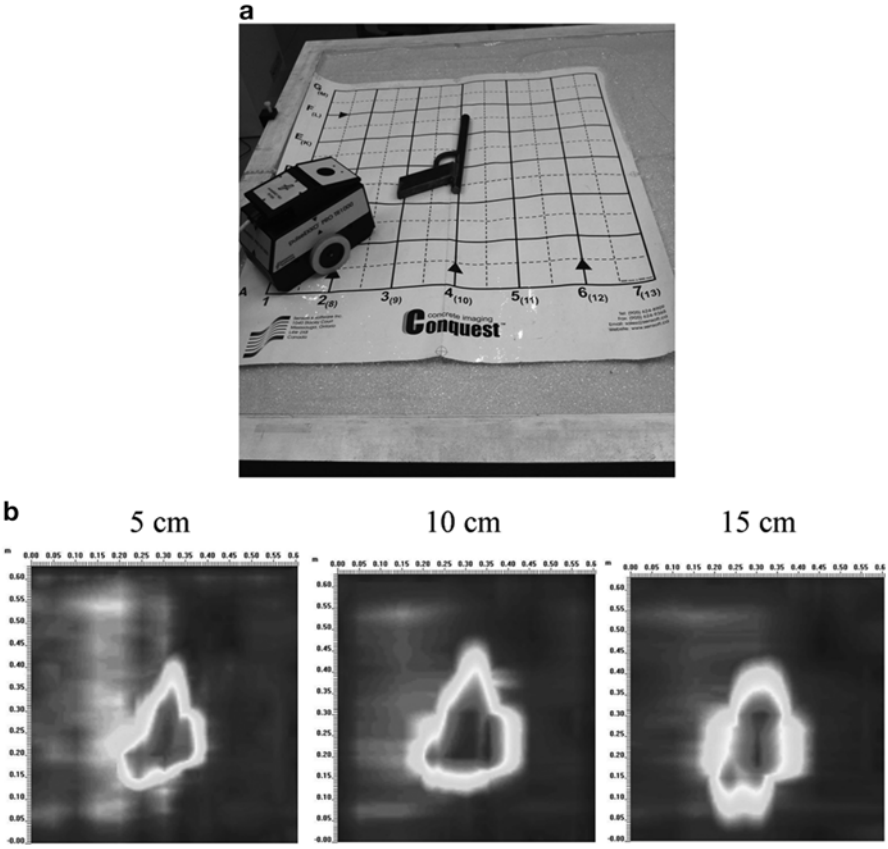


Fig 14.2 (**a**) shows the GPR system used, the orientation of the metallic gun and the grid; (**b**) depicts three GPR maps acquired using three different line spacings (5, 10 and 15 cm) (Note that the 5 cm map is better in terms of spatial resolution and the geometrical reconstruction of the buried gun)

14.4 Field-Scale Experiments

14.4.1 Buried Gun

Generally, the walls of a backfilled excavation (i.e., a negative interface) are visible even centuries after backfilling, and archaeological stratigraphy has confirmed that GPR can identify both modern and ancient subsurface structures (Crist 2001; Hunter and Cox 2005; Schultz and Dupres 2008). A buried metallic weapon, however, is not always detectable, depending on the orientation of the GPR survey, any correction for the effects of survey geometry and the spatial distribution of energy (i.e., migration) and the merger of individual responses into a single response as the separation between the two objects decreases (Annan 2003; Jol 2009).

GPR responses vary greatly depending on the target being sought and the host material. This field-scale test contributes to an understanding of this variability, which allows the investigator to select a correct approach and minimize errors during an actual investigation.

The same gun used in the lab-scale test was now buried in real soil, simulating a typical condition during a forensic GPR survey. In particular, the weapon was buried at a depth of 30 cm in a non-coherent tufa soil ($\varepsilon = 5$). GPR multi-profile data were acquired in an area of 100×100 cm, as shown in Fig. 14.3a. As is typical in an open-air crime scene investigation, the GPR system used was a bistatic hand-towed pulseEKKO PRO (Sensors & Software, Inc.), equipped with 1 GHz antennas. The data collection was rapid, acquired using a line spacing of 10 cm, a step interval of 1 cm and a time window of 60 ns. Only Y orientation profiles were surveyed, and data processing was performed in the field.

If the data collection is rapid, then more care is needed during the data processing. In fact, as shown in Fig. 14.3b, no anomaly due to the buried gun was detected unless the spatial deconvolution process, also called migration, was used. This process is an attempt to remove source and receiver directionality from the reflection data (Fischer 1992; Jol 2009). Moreover, this anomaly seemed to be "weak" in terms of recognizability in the GPR map. For this reason, another GPR acquisition was performed, changing the orientations of the profiles by switching from the Y to the X direction. As shown in Fig. 14.3c, the anomaly seems to be more defined in terms of its "visibility" both in the unmigrated and migrated GPR maps.

Moreover, overlapping the two surveys using the migration process demonstrated that higher-resolution GPR results can be obtained by acquiring data in both the X and Y directions (Fig. 14.3d).

Graves

Depending on the geology, rainfall and how well the soil drains or holds water, a "scar" is often present at a gravesite in two different forms, visible and invisible, even long after burial and backfilling. The visible form is a depression in the earth, however slight, or a different pattern of vegetation on the surface; the invisible form is buried in the subsoil and is the interface (a "cut") between the in-place soil and the backfill. These scars are present because not all of the soil that was removed is replaced, nor is it replaced with the original orientation or degree of compaction.

The area is disturbed relative to the surrounding soil, indicating that an activity of some type has taken place. Certain backfill soils can retain their disturbed characteristics for months, years and centuries (Barone et al 2012).

Locating unmarked graves is a common application of GPR, especially in archaeology (Bevan 1991; Conyers and Goodman 1997; Barone et al. 2007), but most of the forensic literature using GPR investigations to identify graves (Mellet 1992; Miller 1996; Nobes 2000; Miller et al. 2002; Schultz et al. 2002, 2006; Ruffell 2005; Schultz 2007; Congram 2008; Pringle et al. 2008; Schultz and Martin 2011; Dirkmaat and Schultz 2012) is focused on the identification of the buried body and

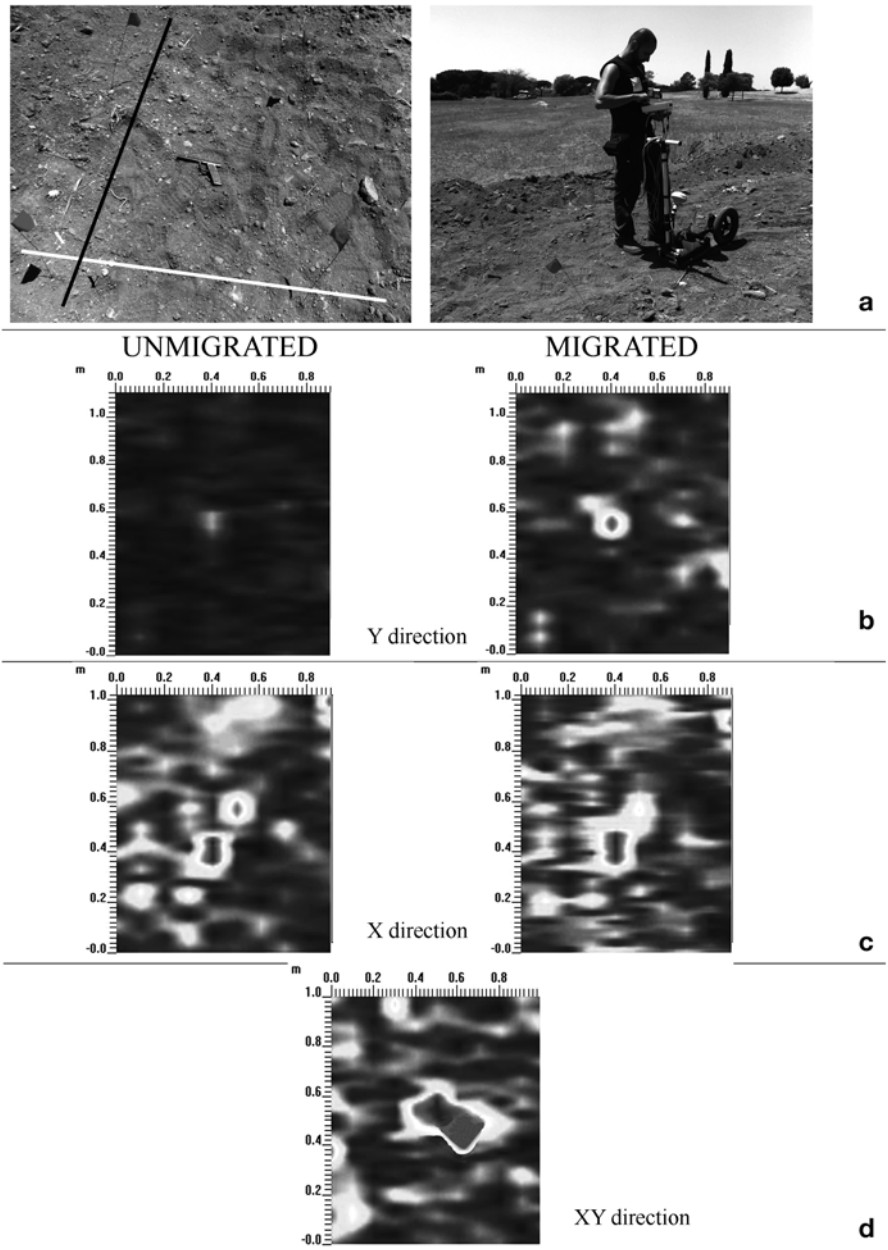


Fig 14.3 (**a**) shows the area in which the gun was buried and the GPR system used; (**b**) and (**c**) illustrate, respectively, the unmigrated and migrated GPR maps in the Y and X directions; (**d**) depicts the XY GPR maps in which the anomaly due to the gun is more recognizable

its stage of decomposition. In this section, we focus on the detectability of the interface between the in-situ soil and the backfill (the so-called "cut," made to bury a body or an object) and the best way to acquire and process the GPR data.

A grave is a relatively well-defined target: the size is typically 0.5×2 m, and the depth is normally less than 2 m. In addition, the act of excavating the soil for burial radically disturbs the natural soil structure. Therefore, the identification of a body beneath the soil is complicated by numerous physical and chemical factors. In particular, if a body has been buried for several centuries, its detection may be difficult.

At an archaeological site near Rome, Italy, an extensive GPR survey was conducted to delineate the stratigraphy of an ancient Roman city. In an area in the middle of the excavation, the GPR radargram detected a typical anomaly due to a negative interface between a zone of backfill and in-situ soil. As shown in Fig. 14.4a, two strong oblique reflectors are generated when the GPR crosses perpendicular to the "cut" in the soil; a hyperbolic signal in between these reflectors is produced by a buried target (e.g., a body or coffin). This anomaly can also be reconstructed by creating a GPR map, highlighting a rectangular geometry at a certain depth.

The surrounding area did not exhibit any other relevant anomalies, which allowed for a clear interpretation of the target as a grave. After subsequent archaeological exploration at this particular location, at the depth determined by using the GPR, the archaeologists exposed an ancient lead coffin (Fig. 14.4b).

Starting from such archaeological examples in which the walls of an ancient backfilled trench are made "electromagnetically" visible (Unterberger 1992), it is possible to explore how a precise study of the buried target starting from the GPR data can help forensic investigations.

The most commonly selected GPR systems for detecting buried victims are 500 MHz SmartCart (Sensors & Software, Inc.) configurations. The 500 MHz NOGGIN system provides the correct balance between spatial resolution and exploration depth and a sufficiently large footprint to maximize area coverage.

The next case study describes the results of a police training exercise in which training investigations were conducted on controlled, pre-placed targets. The target in the following example was a pig carcass buried at the bottom of a trench 1.2 m deep. The carcass was buried for 1.5 months before the GPR survey was performed.

Trainees used two modes to search for zones of interest. The first mode is most effective when results are needed quickly and forensic dig teams are immediately available to follow up. The trainees surveyed lines using the NOGGIN SmartCart and identified zones of interest directly from the radargrams on the display screen (Fig. 14.5a–b). Using the back-up arrow capability of the SmartCart, zones were pinpointed and marked directly on the ground, and the depth of the target was noted.

The second mode uses systematic grid surveying to completely blanket an area. This approach is most effective when there are complex subsurface conditions. The trainees established a regular grid of perpendicular lines (X and Y directions) to span the area to be investigated (several grids can be used if the area is large and irregularly shaped). Each line was surveyed using the NOGGIN 500 SmartCart

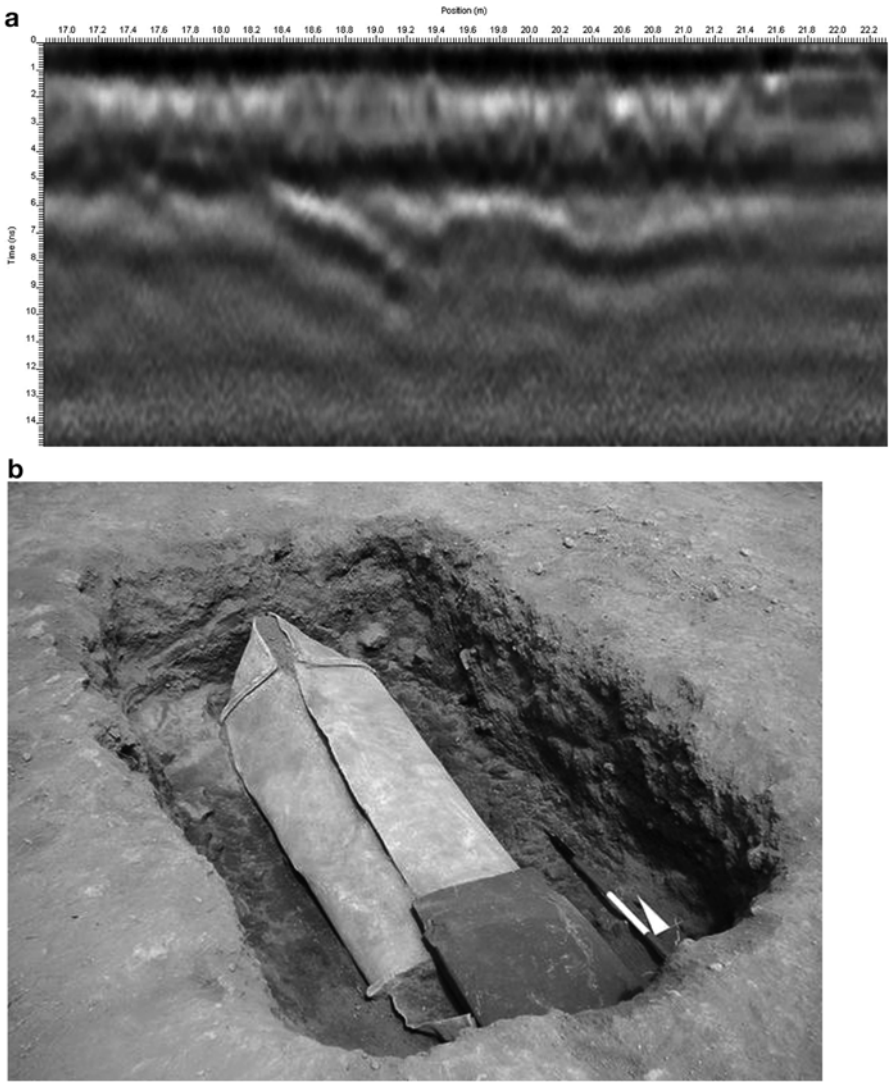


Fig 14.4 (**a**) shows a detail of a radargram that clearly shows the anomaly due to a buried target (two oblique reflectors with a hyperbolic signal in the middle); (**b**) depicts the ancient coffin exposed after the archaeological excavation

configuration. The step size was set to 2 cm, and the lines were spaced 25 cm apart. The data were then transferred to a field computer.

Using mapping software, the data were rendered as GPR response maps at various depths. Depth slices were analyzed to find the zones of interest, which were then marked out and prioritized for following up. Although the results are less

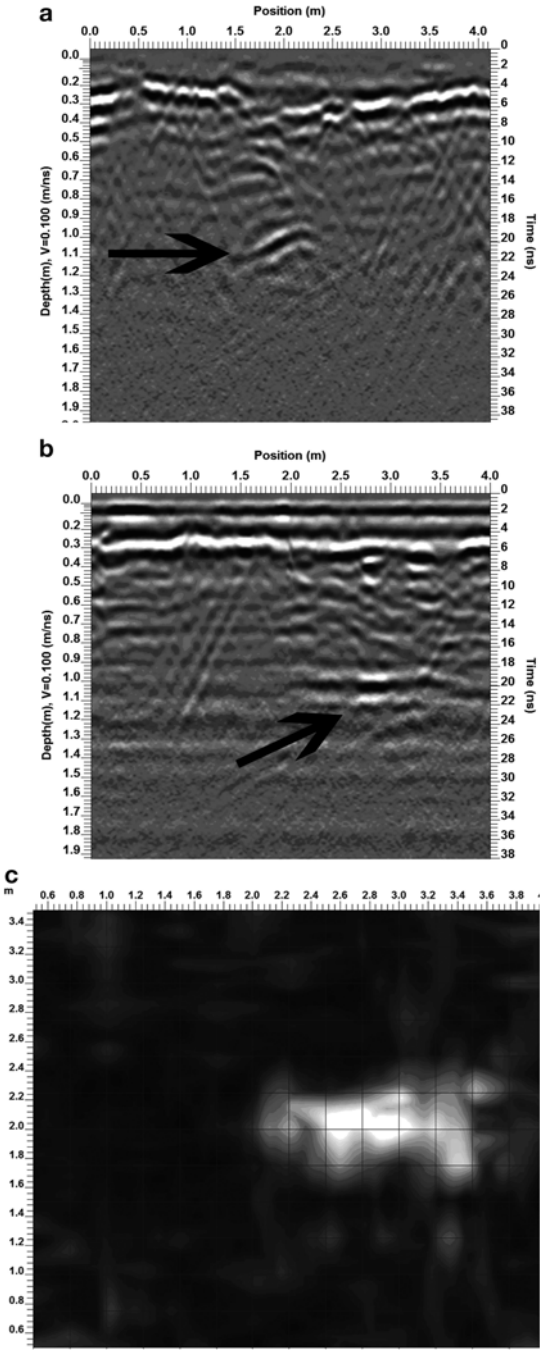


Fig 14.5 (**a**) and (**b**) show a radargram collected over a pig carcass using alternate survey orientations (X and Y); (**c**) shows a depth slice image from a grid collected over the same pig carcass. The depth slice image provides a better idea of the orientation of the carcass

immediate, grid surveying reduces false alarms and improves the likelihood that significant targets will be detected.

The depth slice images also give a better understanding of the geometry of the target and its relationship to the surroundings (i.e., the cut in the soil) (Fig. 14.5c). The systematic approach and automated data processing delivers results that are less dependent on operator skill and experience.

In both survey modes, the GPR data can be recorded and saved in digital form. If the GPR is augmented with GPS positioning, the data can be geo-referenced for use with a variety of software mapping tools such as ArcGIS, AutoCAD and Google Earth. This capability can be valuable in cases in which data are needed for expert witness testimony at a trial.

14.5 Discussion and Conclusions

As is clear from the above examples, the capabilities of this geophysical technique include the ability to rapidly survey areas of interest and to receive an immediate real-time image and printout of what lies underground. The great advantage in forensics is that GPR is noninvasive and nondestructive and provides investigators with enormous amounts of information about what lies underground before excavation commences. Moreover, because the equipment is portable, it can be carried to the survey area.

Nevertheless, its use should be supervised by geophysical experts due to the high possibility of making mistakes while collecting, processing and interpreting the data.

The laboratory-scale and field-scale experiments demonstrate that under certain circumstances, the GPR will not work well, and it is necessary to know how and when it is possible to increase the resolution to obtain better results. Typically, poor conditions for GPR surveying include where the soil contains a high percentage of clay (clay is electrically conductive and will absorb and dissipate the radar energy), which prevents the detection of deep targets (Annan 2003; Jol 2009). In clay soils, however, any near-surface disturbance that may indicate the presence of a target is still detectable (Barone et al 2013, and references therein).

International police departments should frequently employ or improve the use of the GPR technique to help search for evidence in forensic cases. The ability of GPR to non-intrusively detect disturbed soil conditions and shallow buried objects makes it an ideal method to aid forensics investigations.

Forensic investigations call for the follow-up of many leads based on sparse information and often ambiguous eyewitness reports. Searching for evidence in the case of buried targets can be extremely challenging. With no visual information available and often extensive areas to search, being able to pinpoint zones of interest quickly is important. Often, time-limited search warrants demand the rapid deployment of limited resources. Efficient search methods that minimize the use of time and manpower resources, such as these GPR systems, are needed.

Acknowledgements We thank the participants in the forensic collaboration between Sensors & Software, Inc., and the OPP (Ontario Provincial Police), Canada.

References

Annan AP (2003) Ground penetrating radar: principles, procedures & applications. Sensors & Software Inc., Mississauga

Balsi S, Esposito F, Frezza P et al (2010) GPR measurements and FDTD simulations for landmine detection. In: Abstracts of the 13rd international conference on Ground Penetrating Radar, Lecce, Italy. doi:10.1109/ICGPR.2010.5550213

Barone PM, Ferrara C, Pettinelli E, Annan AP, Fazzari A, Redman D (2012) Forensic Geophysics: how GPR could help police investigations. In: Abstracts of EAGE international conference on Near Surface Geoscience 2012, Paris, France

Barone PM, Graziano F, Pettinelli E, Ginanni Corradini R (2007) Ground-penetrating radar investigation on the constructive techniques of the Concordia Temple (Agrigento, Sicily). Archaeol Prospect 14:47–59. doi:10.1002/arp.300

Barone PM, Mattei E, Pettinelli E (2013) Non-invasive archaeological exploration in stratigraphically complex rural settings: an example from Ferento (Viterbo, Italy). Archaeol Anthropol Sci. doi:10.1007/s12520-013-0138-3

Bevan BW (1991) The search for graves. Geophysics 56:1310–1319

Buck SC (2003) Searching for graves using geophysical technology: field tests with ground penetrating radar, magnetometry, and electrical resistivity. J Forensic Sci 48:5–11

Calkin SF, Allen RP, Harriman MP (1995) Buried in the basement – geophysics role in a forensic investigation. In: 8th EEGS symposium on the application of geophysics to engineering and environmental problems, April 23–26, 1995, Orlando, USA. pp 397–403

Cheetham P (2005) Forensic geophysical survey. In: Hunter J, Cox M (eds) Forensic archaeology: advances in theory and practice. Routledge, Abingdon, pp 62–95

Crist TAJ (2001) Bad to the bone? Historical archaeologists in the practice of forensic science. In: Connor M, Scott DD (eds) Archaeologist as forensic investigators: defining the role. Historical Archaeology, 35 SI: 39–56

Congram DR (2008) A clandestine burial in Costa Rica: prospection and excavation. J Forensic Sci 53(4):793–796

Conyers LB, Goodman D (1997) Ground penetrating radar: an introduction for archaeologists. AltaMira Press, Walnut Creek

Davenport GC (2001) Remote sensing applications in forensic investigations. Hist Archaeol 35:87–100

Davis JL, Heginbottom JA, Annan AP et al (2000) Ground penetrating Radar surveys to locate 1918 Spanish Flu victims in Permafrost. J Forensic Sci 45:68–76

De Souza T (2009) Ground penetrating radar for forensic. CINDE J 30(4):16–17

Dirkmaat DC, Schultz JJ (2012) The application of ground-penetrating radar for forensic grave detection. In: Dirkmaat DC (ed) A companion to forensic anthropology. Wiley-Blackwell Publishing, Hoboken, NJ, USA. pp 85–100. doi:10.1002/9781118255377.ch4

Fisher E, McMechan GA, Annan AP, Cosway SW (1992) Examples of reverse-time migration of single-channel, ground penetrating radar profiles. Geophysics 57:577–586

France DL, Griffin TJ, Swanburg JG et al (1992) A multidisciplinary approach to the detection of clandestine graves. J Forensic Sci 37(6):1445–1458

France DL, Griffin TJ, Swanburg JG et al (1997) NecroSearch revisited: further multidisciplinary approaches to the detection of Clandestine graves. In: Haglund WD, Sorg MH (eds) Forensic taphonomy: the postmortem fate of human remains. CRC Press, Boca Raton, pp 497–509

Freeland RS, Miller ML, Yoder RE et al (2003) Forensic application of FMCW and pulse radar. J Environ Eng Geophys 8(2):97–103
Freeland RS, Yoder RE, Miller ML et al (2002) Forensic application of sweep-frequency and impulse GPR. In: Proceedings of the 9th international conference on ground penetrating radar, Santa Barbara, California, USA, pp 533–538
Harrison M, Donnelly LJ (2009) Locating concealed homicide victims: developing the role of geoforensics. In: Ritz K, Dawson L, Miller D (eds) Criminal and environmental soil forensics. Springer, Dordrecht, pp 197–219
Hunter J, Cox M (eds) (2005) Forensic archaeology: advances in theory and practice. Routledge, Abingdon
Jol HM (2009) Ground penetrating radar: theory and applications. Elsevier Science, Burlington
King JA, Bevan BW, Hurry RJ (1993) The reliability of geophysical surveys at historic-period cemeteries: an example from the Plains Cemetery, Mechanicsville, Maryland. Hist Archaeol 27:4–16
Mellet JS (1992) Location of human remains with ground penetrating radar. In: Hanninen P, Autio S (eds) 4th International conference on ground penetrating radar, Rovaniemi, Finland, Geological Survey of Finland, special paper 16, pp 359–365
Miller PS (1996) Disturbances in the soil: finding buried bodies and other evidence using ground penetrating radar. J Forensic Sci 41:648–652
Miller ML, Freeland RS, Koppenjan SK (2002) Searching for concealed human remains using GPR imaging of decomposition. In: Proceedings of the 9th international conference on ground penetrating radar, Santa Barbara, California, USA, pp 539–544
Nobes DC (2000) The search for "Yvonne": a case example of the delineation of a grave using near surface geophysical methods. J Forensic Sci 45:715–721
Novo A, Lorenzo H, Rial FI et al (2011) 3D GPR in forensics: finding a clandestine grave in a mountainous environment. Forensic Sci Int 204:134–138
Owsley DW (1995) Techniques for locating burials, with emphasis on the probe. J Forensic Sci 40:735–740
Pettinelli E, Barone PM, Di Matteo A et al (2011) Radio waves technique for non-destructive archaeological investigations. Contemp Phys 52:121–130. doi:10.1080/00107514.2010.545208
Powell K (2004) Detecting human remains using near-surface geophysical instruments. Expl Geophys 35:88–92
Pringle JK, Holland C, Szkornik K et al (2012) Establishing forensic search methodologies and geophysical surveying for the detection of clandestine graves in coastal beach environments. Forensic Sci Int 219:e29–e36. doi:10.1016/j.forsciint.2012.01.010
Pringle JK, Jervis J, Cassella JPN et al (2008) Time-lapse geophysical investigations over a simulated urban clandestine grave. J Forensic Sci 53(6):1405–1416
Roark M, Strohmeyer J, Anderson N et al (1998) Applications of the ground-penetrating radar technique in the detection and delineation of homicide victims and crime scene paraphernalia. In: Symposium on the application of geophysics to engineering and environmental problems, pp 1063–1071. doi:10.4133/1.2922490
Ruffell A (2005) Searching for the IRA "disappeared": ground-penetrating radar investigation of a churchyard burial site, Northern Ireland. J Forensic Sci 50:1–6
Ruffell A, Donnelly C, Carver N et al (2009a) Suspect burial excavation procedure: a cautionary tale. Forensic Sci Int 183:11–16
Ruffell A, Kulessa B (2009) Application of geophysical techniques in identifying illegally buried toxic waste. Environ Forensic 10:196–207
Ruffell A, McCabe A, Donnelly C, Sloan B (2009b) Location and assessment of an historic (150–160 years old) mass grave using geographic and ground penetrating radar investigation, NW Ireland. J Forensic Sci 54:382–394
Ruffell A, McKinley J (2005) Forensic geoscience: applications of geology, geomorphology and geophysics to criminal investigations. Earth Sci Rev 69:235–247

Ruffell A, McKinley J (2008) Geoforensics. Wiley, Chichester
Schultz JJ (2007) Using ground-penetrating radar to locate clandestine graves of homicide victims. Homicide Stud 11(1):15–29
Schultz JJ (2008) Sequential monitoring of burial containing small pig cadavers using ground-penetrating radar. J Forensic Sci 53(2):279–287
Schultz JJ, Collins ME, Falsetti AB (2006) Sequential monitoring of burials containing large pig cadavers using ground-penetrating radar. J Forensic Sci 51(3):607–616
Schultz JJ, Dupres TL (2008) The contribution of forensic archaeology to homicide investigations. Homicide Stud 12:399–413. doi:10.1177/1088767908324430
Schultz JJ, Falsetti AB, Collins ME et al (2002) The detection of forensic Burials in Florida using GPR. In: Proceedings of the 9th international conference on ground penetrating radar, Santa Barbara, California, USA, pp 443–448
Schultz JJ, Martin MM (2011) Controlled GPR grave research: comparison of reflection profiles between 500 and 250 MHz antennae. Forensic Sci Int 209(1–3):64–69. doi:10.1016/j.forsciint.2010.12.012: 60–62
Scott J, Hunter JR (2004) Environmental influences on resistivity mapping for the location of clandestine graves. In: Pye K, Croft DJ (eds) Forensic geoscience: principles, techniques and applications, vol 232. Geological Society of London Special Publication, pp 33–38
Solla M, Riveiro B, Alvarez MX et al (2012) Experimental forensic scenes for the characterization of ground-penetrating radar wave response. Forensic Sci Int 220(1–3):50–58. doi:10.1016/j.forsciint.2012.01.025
Strongman KB (1992) Forensic applications of ground penetrating radar. In: Pilon J (ed) Ground penetrating radar, Paper 90–4. Geological Survey of Canada, pp 203–211
Unterberger RR (1992) Ground penetrating radar finds disturbed earth over burials. Geol Surv Finland 16:351–357
Witten A, Brooks R, Fenner T (2000) The Tulsa race riot of 1921: a geophysical study to locate a mass grave. Lead Edge 20:655–660

Chapter 15
Filter Paper Adsorption and Ninhydrin Reagent as Presumptive Test for Gravesoil

Martien H.F. Graumans, Tim C.W. van der Heijden, Aleksandra Kosinska, Maarten J. Blom, and Ben M. de Rooij

Abstract In the field of forensic science, the investigation of soil for the determination of decomposition by geophysical, biological and chemical methods, is a fast growing area. During decomposition different substances are formed as a result of breakdown and dissolution. The analysis of ninhydrin reactive nitrogen (NRN: ammonium and organic amines) is helpful in the investigation of the decomposition process, because such products may result from degradation of proteins and amino acids. During this research a new application form of the well known ninhydrin reagent was developed and applied to soil and gravesoil.

Soil samples were collected in the vicinity of a decomposing pig (*Sus domesticus*), after approximately 2 months. The samples were supplied by the University of Central Lancashire and transported to the laboratory on dry ice. In previously published work, NRN is extracted from soil using an aqueous extraction, whereof the liquid is used for a modified ninhydrin reaction. During this study, the samples were moisturised, and subsequently adsorbed to a filter paper. The paper was treated with ninhydrin reagent.

The soil samples taken in the direct vicinity of the cadaver, gave positive results in the form of a deep purple colour. Soil samples taken in the same area resulted in a slight purple colour change, indicating low concentrations of NRN as well.

This ninhydrin reagent application is not validated yet and may need some further development. Nevertheless, this rapid test may complement the analytical methods described in the literature and contribute, as a presumptive test, to the field of forensic taphonomic research.

M.H.F. Graumans (✉) • T.C.W. van der Heijden • A. Kosinska • M.J. Blom • B.M. de Rooij
Forensic Laboratory Science, Avans University of Applied Sciences School of Life Science, Lovensdijkstraat 61-63, 4818 AJ Breda, The Netherlands
e-mail: martiengraumans@hotmail.com

H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics*, Soil Forensics, DOI 10.1007/978-3-319-33115-7_15

15.1 Introduction

In fatal crimes the perpetrator often tries to hide the corpse to avoid detection (Pringle and Jervis 2010; Pringle et al. 2010). The most common approach for hiding the corpse is a quickly dug grave, which is normally dug into or nearby the topsoil surface. These burials are defined as clandestine graves which will give a confounding effect on the used area. To locate a clandestine grave, several techniques can be applied to find the physical and chemical changes that are caused by the decomposition of the buried corpse (Hopkins et al. 2000; Hunter and Cox 2005; Carter et al. 2008; Tibbett and Carter 2008; Van Belle et al. 2009; Pringle and Jervis 2010; Pringle et al. 2010). During a field search, experts in the areas of forensic archaeology and forensic anthropology are very closely involved. Forensic archaeology mainly involves; location and recovery of (clandestine) graves where forensic anthropology mainly focuses on the investigation of the human skeleton. A discipline that provides the overlap in these two areas is forensic taphonomy. Forensic taphonomy is an applied expertise that focuses on the reconstruction of events before, during and after the death (Broeders and Muller 2008; Tibbett and Carter 2008).

For the reconstruction, material of the natural environment is collected in the grave or in the vicinity of the cadaver. During the scene of crime investigation of a clandestine grave, several traces can be found in the form of botanical (plant residues, pollen etc. (Gunn 2006), geological (minerals, rocks, including remains of earth in different shape and texture (Hunter and Cox 2005; Campbell et al. 2008; Tibbett and Carter 2008), and entomological traces (insects in various stages of development (Gunn 2006). Using a field based search combined with laboratory techniques, an indication can be given of how long someone has been deceased, also an estimation can be made as to whether or not the decomposition has occurred in the localised grave. During the field investigation numerous (geo-)scientific techniques are applied in the form of destructive and non destructive techniques. The techniques mainly used are; soil probing, cadaver dogs and excavations. Also non-destructive field mapping techniques can be used in the form of thermal cameras (infrared red), metal detectors, remote sensing (aerial photography) and ground penetrating radar (GPR) (Pringle et al. 2012). Each method has its own advantages and disadvantages that still may be specific to the target location (Hunter and Cox 2005; Broeders and Muller 2008; Tibbett and Carter 2008; Pringle and Jervis 2010; Pringle et al. 2010; Swann et al. 2010).

The surface of the earth can be divided into different layers. These layers are very important for the maintenance of life on earth. The topsoil is the uppermost layer and consists of a very complex ecosystem. The complexity of this ecosystem is formed over time by climatic, mineralogical, biological, chemical and physical conditions (Swann et al. 2010). The topsoil is mainly composed of organic and inorganic components, such as; soil, plants, bacteria and fungi, which all play a major role in the formation of the nitrogen cycle (Van der Schoot and Leegwater 2005;

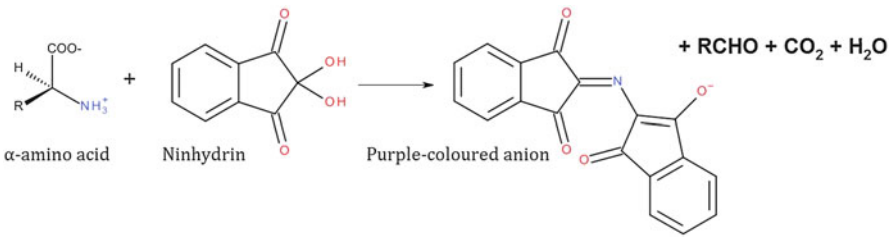


Fig. 15.1 Chemical reaction of ninhydrin reagent with an α-amino acid, two ninhydrin molecules bind with the nitrogen group, forming a purple-coloured anion (Ruhemann's purple), an aldehyde, CO_2 and water (According Wade (2009))

Johll 2007; Campbell et al. 2008). The main sources of nitrogen on earth are; the atmosphere (N_2), the biomasses of living organisms (nitrogenous organic compounds in the form of; amino acids, proteins, deoxyribonucleic acid (DNA) and chlorophyll) and surface areas such as; soil, sediments, lakes rivers and oceans (Campbell et al. 2008). When animals or humans die, the physical and chemical composition of their bodies will change completely and ammonifying bacteria, which live in the humus-rich topsoil, covert the releasing organic nitrogen compounds in forms of proteins, (poly-)peptides and amino acids into nitrogenous minerals. Besides the influence of ammonifying bacteria, also environmental influences, climate changes and the attraction of other organisms affect the decomposition process (Dent et al. 2004; Forbes et al. 2005; Gunn 2006; Tibbett and Carter 2008).

Two research studies (Carter et al. 2008; Van Belle et al. 2009), have shown that ninhydrin could be an useful chemical reagent for the determination of these nitrogenous minerals, in the form of; proteins, (poly-)peptides, amino acids and ammonium (NH_4^+) in soil. These nitrogenous minerals are therefore better described as ninhydrin reactive nitrogen (NRN). Ninhydrin is a well recognised reagent in forensic science for the detection of latent fingerprints on (porous-) surfaces such as paper and cardboard (Odén and von Hofsten (1954). The reaction mechanism of ninhydrin manly involves a reaction with α-amino acids. α-Amino acids contain a primary amino group that can react with ninhydrin to form a binding site of two ninhydrin molecules with the nitrogen group. This binding of two ninhydrin molecules with a nitrogen group results in a purple-coloured anion known as Ruhemann's purple including an aldehyde, CO_2 and water (Fig. 15.1) (Wade 2009). In cases where it is suspected that a clandestine grave is found, a 'rapid on the scene' method can be useful to indicate whether or not a further (chemical-/biological) investigation is needed. This study shows a possible presumptive test that can be used before or after forensic geo-scientific techniques to contribute to the detection of possible decomposition in clandestine graves, while presenting a new application of the ninhydrin reagent.

15.2 Experimental

15.2.1 Reagents and Standards

The amino acid analytes phenylalanine and D-leucince were obtained from Fisher Scientific (Landsmeer, the Netherlands) and Acros Organics (Geel, Belgium) respectively. Pre-prepared ninhydrin reagent was purchased from BVDA (Haarlem, the Netherlands), solid ninhydrin reagent and hydrindantin hydrate were both acquired from Sigma Aldrich (Steinheim, Germany). The chemicals dimethyl sulfoxide and sodium acetate, were both ordered from Boom laboratory supplies (Meppel, the Netherlands). Masonry sand was purchased from a local do it yourself-shop Karwei (Breda, the Netherlands) and all aqueous standards and reagents were prepared by using deionised water obtained from the laboratory.

15.2.2 Decomposition Samples

Research samples were a kind gift from the *University of Central Lancashire*, Preston UK. Soil samples were taken in a $n = 1$ replicate with a total of twelve soil samples ($n = 12$). A pig *(Sus domesticus)* was laid down on the soil surface and samples were collected in the direct vicinity (~10 cm) or under the cadaver, after a decay period of 2 months and placed into falcon tubes of 50 mL. Samples were all labelled following the position collected; hind leg, head and abdomen, including the 12, 6 and 9 o'clock positions (Labelled code was following digital clock time; e.g. C16 18.00, pig code 6 o'clock position) (Fig. 15.2). The soil samples were then posted on dry ice, and upon arrival in the Netherlands immediately stored at −70 °C. Control soil samples were all collected in the area of Breda (NL) and spiked control samples were prepared by mixing 3.5 and 43.0 mg solid phenylalanine into 10.0 mg of soil.

15.2.3 Filter Paper Method

In the research articles of Carter et al. (2008) and Van Belle et al. (2009), the usage of ninhydrin as detection reagent for decomposition was conducted as a quantitative measurement of NRN using spectrophotometry. Inspired by this approach, the method described in this paper uses a simplified application that can be used as a qualitative measurement for the detection of NRN. The filter paper method was carried out by spraying deionised water onto a soil samples and adsorbing the moisture onto a cellulose filter paper from Schleicher & Schuell, Ø70 mm (Shifferstadt, Germany). The filter paper was subsequently sprayed with pre-prepared ninhydrin

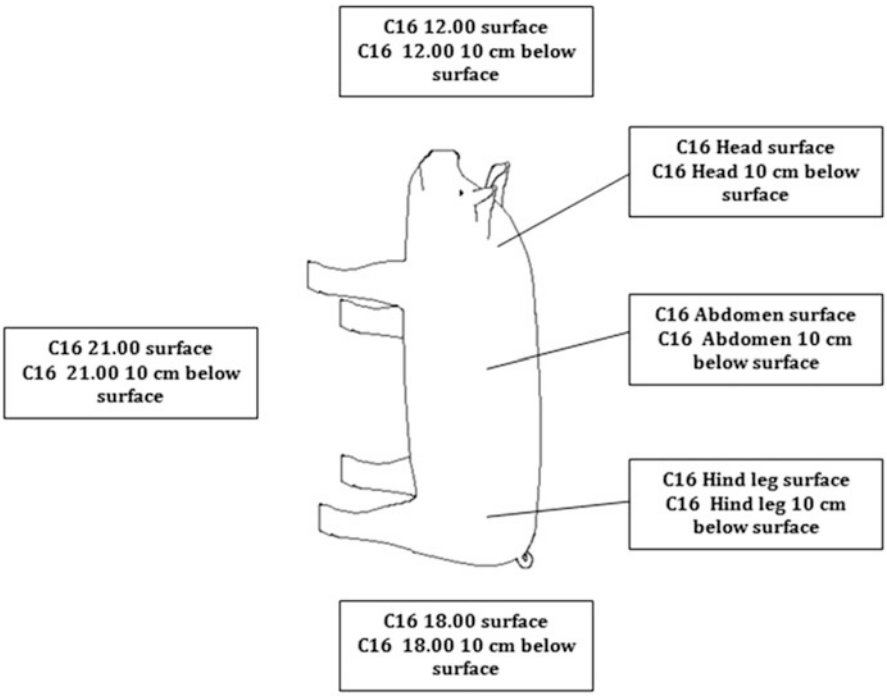


Fig. 15.2 Sample coding for single taken soil samples $n = 1$, with a total of $n = 12$. The samples C16 12.00, 18.00 and 21.00 o'clock positions are taken in the direct vicinity, ~10 cm away, of the decomposing *Sus domesticus*. C16 head, abdomen and hind leg samples are taken directly under the decomposing pig after a decay period of 2 months

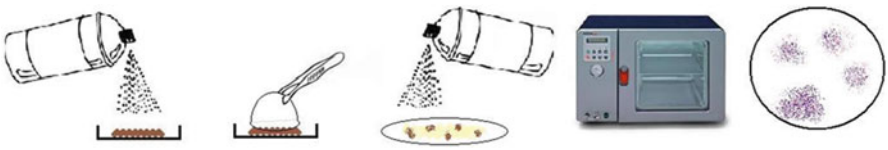


Fig. 15.3 Schematic representation of the new ninhydrin application, where the soil is moisturised using deionised water. A filter paper is than pressed onto the moisturised soil and subsequently sprayed with ninhydrin reagent. The treated filter paper was allowed to dry and then placed for approximately 10 min at 80 °C in an oven, so the result could be interpreted afterwards

reagent (ninhydrin dissolved in ethanol, acetic acid, petroleum ether and MTBE), and incubated for approximately 10 min at 80 °C (Fig. 15.3). Prior to applying the ninhydrin reagent to real decomposition samples, positive and negative controls were carried out for the pre-prepared ninhydrin reagent, using fingerprint-touched and untouched filter papers.

15.2.4 Filter Paper Development

Further development of the ninhydrin reagent and application of the qualitative technique was done by testing different filter paper approaches such as; ninhydrin saturation, ninhydrin spraying and ninhydrin pipetting. Modified ninhydrin reagent was freshly prepared before each experiment following the method described by Van Belle et al. (2009). To summarise this method, 800 mg of ninhydrin and 120 mg of hydrindantin hydrate, were weighed and dissolved in 30 mL of dimethyl sulfoxide and 10 mL of 4 M-sodium acetate. The prepared ninhydrin reagent was then covered by aluminium foil and allowed to stand at 4 °C until analysis.

A simulation study was carried out using different concentrations; 20, 40 and 80 µg/mL of freshly prepared D-leucine stock standard (500 µg/mL), spotted onto a filter paper and simulation soil using masonry sand.

15.3 Results and Discussion

15.3.1 Result Interpretation

Colour developments of presumptive test results are very subjective and therefore the interpretation can be biased or inaccurate. To simplify and classify the colour change interpretation, different criteria are established and given in Table 15.1.

In order to determine the test results, the simulation study with D-leucine standard have resulted in an accurate representation where colour change is correlated to amino acid concentration (Fig. 15.4). Supported by the results described in the papers of Carter et al. (2008) and Van Belle et al. (2009) it is shown that colour intensity will increase in proportion to higher presence of amino acids (Fig. 15.4). However, it is noteworthy that positive results observed after testing 2 month old decomposition samples (Fig. 15.5), show a decreased intensity in deeper soil samples. This difference in intensity is according the concentration amino acids transferred into the filter paper. Looking at the samples; C16 Abdomen 10 cm below

Table 15.1 Useful criteria for the result determination

Score	Description	Colour
−	No colour change	Nothing visible (0)[a]
+−	Average colour change	Rose-light purple (5RP 5/10) [a]
+	Good colour change	Purple (5P 5/10)[a]
++	Excellent colour change	Purple- deep purple (5 PB 5/10) [a]

[a]Interpretation of the colours done according Munsell colour chart, using the hues; Red (R), Yellow (Y), Green (G), Blue (B) and Purple (P), including the lightness and brightness of the colour (the value and chroma)

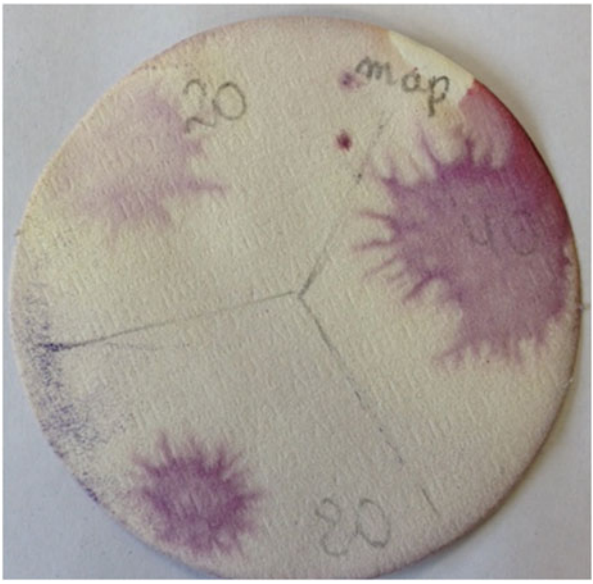


Fig. 15.4 Filter paper showing 20, 40 and 80 μg/mL of D-leucine spots, the paper was pre-treated with ninhydrin reagent before spotting the amino acid

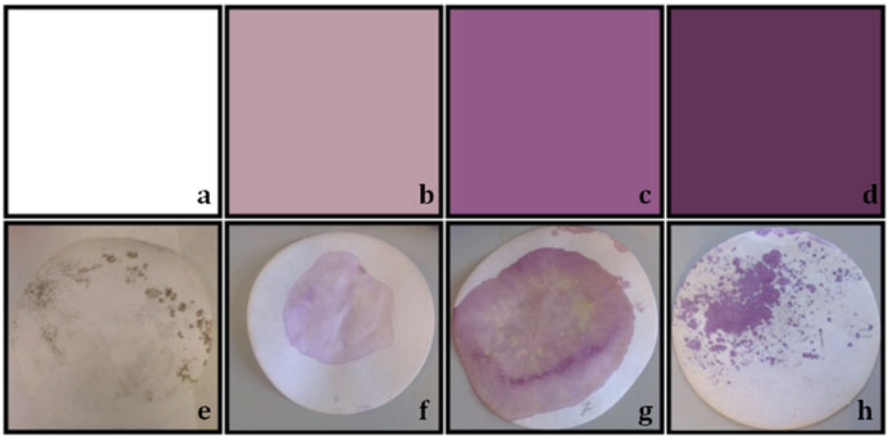


Fig. 15.5 A theoretical colour chart (**a, b, c, d**) compared to positive results obtained from: (**e**) Blank soil sample; (**f**) C16 Abdomen 10 cm below surface; (**g**) C16 Abdomen surface; (**h**) Control sample spiked with 43.0 mg phenylalanine in 10.0 g soil

surface and C16 Abdomen surface, a light purple colour and strong purple colour development are observed (Fig. 15.5f and g). Comparing these results with the filter paper showing different concentrations of known D-leucine standard (Fig. 15.4), it is demonstrated that the amino acid presences for the C16 Abdomen 10 cm below surface (Fig. 15.5f) is lower than C16 Abdomen surface. Nonetheless, it should be mentioned that the applied test is indicative for the presence of amino acids, and

therefore no actual conclusion can be made about the concentration amino acids present, mainly since no quantitative measurement is carried out to confirm this observation.

15.3.2 Decomposition Samples

Several control and decomposition samples were investigated while using the ninhydrin spray test application. During the test, it became clear that the negative controls had not reacted with the ninhydrin reagent. The prepared spiked control samples indicated on the other hand, a very strong purple colour change. This colour change can be interpreted following Table 15.1 as 'excellent colour change'. Also the tested decomposition samples have shown positive colour indications which ranged from 'average colour change' to 'good colour change'. Mainly the soil samples taken at the surface areas; head, abdomen and hind leg showed a positive indication for the presence of amino acids. The samples taken on these areas 10 cm below the surface, resulted in an 'average colour change' or even in a negative test result, Table 15.2, which indicates that the presence of amino acids derived from decay is lower for areas less close to the actual decomposition source.

However it is found that ninhydrin indicates a positive test result in the presence of amino acids, it should be noted that amino acids do occur naturally in soil

Table 15.2 Test results control- and decomposition samples

Sample code	Result
Blank filter paper (negative control)	–
Blank soil sample (negative control)	–
Filter paper with fingerprint (positive control)	+
3.5 mg phenylalanine in 10 mg soil (spiked control)	++
43.0 mg phenylalanine in 10 mg soil (spiked control)	++
C16 12.00 surface	+–
C16 12.00 10 cm below surface	+–
C16 Head	+
C16 Head 10 cm below surface	–
C16 Abdomen surface	+
C16 Abdomen 10 cm below surface	+–
C16 Hind leg surface	+
C16 Hind leg 10 cm below surface	+
C16 18.00 surface	+–
C16 18.00 10 cm below surface	+–
C16 21.00 surface	+
C16 21.00 10 cm below surface	+–

(Sowden and Ivarson 1966; Campbell et al. 2008; Jämtgård 2009). Also since there is no quantitative test carried out as a control measurement for the presence of naturally occurring amino acids, it is impossible to conclude whether these compounds have contributed to the ninhydrin reaction. Also important to notice is that the control soil samples taken in Breda (the Netherlands) do not show any colour change, which might indicate that amino acids can be present in low concentrations beneath the visual limit of detection of the ninhydrin reagent.

It must be taken into account although the applied method yielded a positive result for some decomposition samples it is still presumptive in nature. Application of the current method will depend on the circumstances of the case, and evidential value will be increased when applying this current described method previously or afterwards in combination with other existing techniques to determine decay.

15.3.3 Filter Paper Development

During the filter paper development using D-leucine standard 80 μg/mL, three different approaches were carried out to determine the best visible test result on a cellular filter paper. In the first method D-leucine standard was directly pipetted onto a clean filter paper and treated with ninhydrin reagent while dropping it onto. This application shows that D-leucine present on the filter paper is likely to migrate. This migration arises in a poor observable test result, since the colour change is less identifiable and appears as a big blurred spot. Also the spraying method, previously described in this paper was evaluated, and resulted in a spatter pattern. This spatter pattern occurs since the ninhydrin reagent seems not to be equally distributed and could be difficult to interpret, since there is no homogenous colour development present. However it is thought that this can be overcome by using a better spray bottle or airbrush to distribute the ninhydrin reagent more dense. A third application method gave the most optimal test results, since the spots of D-leucine were added in the centre of the filter paper onto a pre-treated ninhydrin reagent filter paper. As shown in the results (Figs. 15.4 and 15.6), the spots with D-leucine standard are less likely to migrate through the paper and the colour observation of the different shades

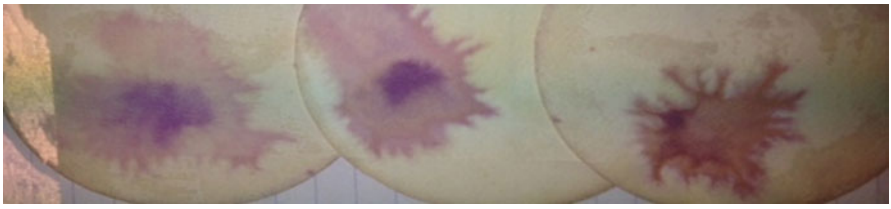


Fig. 15.6 Three filter papers are saturated in modified-ninhydrin reagent, dried at room temperature, and later used to pipette directly D-leucine amino acid standard onto the pre-prepared filter papers. Colour development will occur after placing the paper in a oven at 80 °C for approximately 10 min

of purple are easier and more clearly visible than in the other used methods. Since the filter paper saturation is only carried out with D-leucine standard, it is important to mention, that further analysis is needed for the detection of NRN present in complex matrices.

15.4 Conclusions

Ninhydrin reactive nitrogen (NRN) compounds are released into soil during the decomposition of a corpse. To demonstrate NRN compounds directly in soil, a new application form of the well known ninhydrin reagent was successfully developed. The emerging method used filter paper adsorption from moisturised soil samples prior to the colour reaction and was applied to soil and gravesoil. Results were determined by interpretation of the purple colour change and have shown that ninhydrin is a useful reagent for the detection of decomposition.

Also further development of the pre-treated filter paper, saturated in ninhydrin reagent could be useful for the detection of NRN, since results have shown that NRN compounds are less likely to migrate through the paper.

Since the nitrogen cycle will bring the value of NRN compounds back to a normal value (Van der Schoot and Leegwater 2005; Campbell et al. 2008, it is worth to mention that the concentration of NRN could fall beneath the limit of detection, and therefore this test application could be out of use in some cases. To overcome these circumstances the usage of other amino-acid reagents might be considered.

The ninhydrin test presented in this paper, used at the realistic decomposition samples, is still a draft for the development of a 'rapid on the scene test' for the detection of suspected clandestine graves. After method validation, the designed concept method could be a complementary test to the current used testing approaches, such as; cadaver dogs, puncture probe and Ground Penetrating Radar. The results have shown that no sophisticated extraction methods are required and that the colour development will appear within 10 min after using an oven. Also it is important to mention that this technique is relatively cheap in comparison to other methods and advanced detectors.

Published by previously research studies (Hopkins et al. 2000; Carter et al. 2008; Van Belle et al. 2009; Pringle and Jervis 2010; Pringle et al. 2010, 2012) it is shown that the decomposition of domestic pigs is a good model for human decay. As described by Van Belle et al. (2009), the adult *Sus domestica* is equal to a human torso by biochemistry, physiology and fat to muscle ratio. The used decomposition samples provided by the University of Central Lancashire have shown the release of NRN compounds, since these are detected by using the new ninhydrin application form. After this positive test result it is still important to mention that the technique described in this study, is just an indicative test. To confirm the obtained colour change, it is vital to use more advanced (analytical laboratory) confirmation techniques.

To summarise; the analysis of ninhydrin reactive nitrogen in decomposition samples is possible after 2 months of decomposition. Also the first results have shown that further development of the ninhydrin saturated filter paper may lead to a point of care testing paper, which can provide an indicative test result.

15.5 Recommendation-Future Perspective

The development of this new application of the ninhydrin reagent, described as in this study, is still going on at Avans University of applied sciences. At the moment different research approaches are carried out in order to validate the method within the forensic standard recommendations.

Acknowledgement The authors would like to thank Dr. T. Simmons and Mr. P. Cross of the University of Central Lancashire (Preston, UK) for providing the valuable soil samples.

References

Broeders APA, Muller ER (2008) Forensische wetenschap. Kluwer, Deventer [Dutch]
Campbell NA, Reece JB, Urry LA, Cain ML, Wasserman SA, Minorsky PV, Jackson RB (2008) Biology, 8th edn. Pearson Education, San Francisco
Carter DO, Yellowlees D, Tibbett M (2008) Using ninhydrin to detect gravesoil. J Forensic Sci 53:397–400
Dent BB, Forbes SL, Stuart BH (2004) Review of human decomposition processes in soil. Environ Geol 45:576–585
Forbes SL, Dent BB, Stuart BH (2005) The effect of soil type on adipocere formation. Forensic Sci Int 154:35–43
Gunn A (2006) Essential forensic biology. Wiley, Chichester
Hopkins DW, Wiltshire PEJ, Turner BD (2000) Microbial characteristics of soils from graves: an investigation at the interface of soil microbiology and forensic science. Appl Soil Ecol 14:283–288
Hunter J, Cox M (2005) Forensic archaeology advances in theory and practice. Tayolor & Francis Group, London
Jämtgård S (2009) The occurrence of amino acids in agricultural soil and their uptake by plants. Doctoral thesis, Faculty of Natural Resources and Agricultural Sciences Department of Agricultural Research for Northern Sweden, Umeå
Johll ME (2007) Investigating chemistry, a forensic science perspective, 2nd edn. W.H. Freeman and Company, New York
Odén S, von Hofsten B (1954) Detection of fingerprints by the ninhydrin reaction. Nature 173:449–450
Pringle JK, Jervis JR (2010) Electrical resistivity survey to search for a recent clandestine burial of a homicide victim, UK. Forensic Sci Int 202:1–7
Pringle JK, Cassella JP, Jervis JR (2010) Preliminary soilwater conductivity analysis to date clandestine burials of homicide victims. Forensic Sci Int 198:126–133
Pringle JK, Holland C, Szkornik K, Harrison M (2012) Controlled GPR grave research: comparison of reflection profiles between 500 and 250 MHz antennae. Forensic Sci Int 219:e29–e36
Sowden FJ, Ivarson KC (1966) The 'free' amino acids of soil. Can J Soil Sci 48:109–114

Swann LM, Forbes SL, Lewis SW (2010) Analytical separations of mammalian decomposition products for forensic science: a review. Anal Chim Acta 682:9–22

Tibbett M, Carter DO (2008) Soil analysis in forensic taphonomy. Taylor & Francis Group, Boca Raton

Van Belle LE, Carter DO, Forbes SL (2009) Measurement of ninhydrin reactive nitrogen influx into grave soil during aboveground and belowground carcass (Sus domesticus) decomposition. Forensic Sci Int 193:37–41

Van der Schoot EJ, Leegwater AN (2005) Samengevat Biologie, 3rd edn. Thieme/Meulenhoff, Utrecht/Zutphen [Dutch]

Wade LG (2009) Organic chemistry, 7th edn. Pearson Higher Education/Prentice Hall, Upper Saddle River

Part IIIb
Burial Sites: Decomposition and Degradation Processes

Chapter 16
Changes in Soil Microbial Activity Following Cadaver Decomposition During Spring and Summer Months in Southern Ontario

Heloise A. Breton, Andrea E. Kirkwood, David O. Carter, and Shari L. Forbes

Abstract Bodies are often disposed of clandestinely in environments allowing direct contact with soil yet the impact of cadaver decomposition on the surrounding environment remains generally poorly studied. The microbial load associated with a decomposing body is substantial and it is believed that decomposition has a notable impact on the surrounding soil microbiology. During 2011 and 2012 a study consisting of four experimental trials was undertaken at the University of Ontario Institute of Technology decomposition facility located in Southern Ontario. The study documented the decomposition of human analogues (pig carcasses) and the subsequent microbiological impacts on the soil within the decomposition islands created. Two trials were conducted per year, one in the spring and one in the summer to account for seasonal variations. For each trial, soil samples were collected from three experimental sites and three controls sites over a 3 month period. Sample analysis included soil pH, moisture and microbial activity using a fluorescein diacetate assay. Microbial activity levels between control and experimental samples were compared on each sampling day and overall for all trials. An increase in microbial activity was observed on multiple occasions during the Spring 2011, Summer 2011 and Spring 2012 trial. However, a decrease in microbial activity was observed during the Summer 2012 trial. Soil pH and soil moisture underwent similar fluctuations in control samples and experimental samples pointing to environmental conditions having a strong influence on both these soil parameters.

H.A. Breton (✉) • A.E. Kirkwood
University of Ontario Institute of Technology, 2000 Simcoe St. N., Oshawa, ON, Canada
e-mail: heloise.breton@hotmail.com

D.O. Carter
Laboratory of Forensic Taphonomy, Division of Natural Sciences and Mathematics, Chaminade University of Honolulu, 3140 Waialae Avenue, Honolulu, HI, USA

S.L. Forbes
University of Ontario Institute of Technology,
2000 Simcoe St. N., Oshawa, ON L1H 7K4, Canada

University of Technology Sydney, 15 Broadway, Ultimo,
NSW 2007, Australia

H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics*,
Soil Forensics, DOI 10.1007/978-3-319-33115-7_16

16.1 Introduction

The application of soil science in forensic casework mainly consists of chemical analyses or particle comparisons to link evidence, suspects or victims and locations. Taphonomic processes often take place in terrestrial environments and soil analyses within this field are routine. Cadaver decomposition in terrestrial environments is known to produce an influx of nutrients, notably nitrogen, phosphorus and sodium, which alter the chemical composition of soil (Parmenter and MacMahon 2009). This change is known to affect surrounding vegetation (Bornemissza 1957; Towne 2000) and soil microbial communities (Hopkins et al. 2000; Carter et al. 2007; Parkinson et al. 2009). Decomposition is also believed to introduce a new microbial inoculum into the soil environment (Moreno et al. 2011). The possibility of monitoring and documenting the changes that soil microbial communities undergo as a result of decomposition suggests that alterations of the soil microbial profile can be used as indicators of clandestine graves or to aid in the estimation of post-mortem intervals (Carter et al. 2007).

Studies investigating the changes to soil microbial communities as a result of cadaver decomposition have mainly been conducted within the laboratory where environmental variables are controlled (Carter and Tibbett 2006; Haslam and Tibbett 2009). Although such studies provide an insight to the effects on soil during decomposition, they are not representative of the conditions typically observed in forensic casework. When factors such as natural variation in ambient temperature, rainfall, insect activity and scavenging are not taken into consideration it is difficult to apply laboratory results to casework.

During decomposition a body will undergo breakdown via two major processes: (1) autolysis, an intrinsic breakdown of cellular components and (2) putrefaction, the active breakdown of tissue by microorganisms (Forbes 2008). These processes lead to the liquefaction of soft tissue and the production of decomposition fluids. These fluids are high in microbial content, mainly originating from the gastrointestinal tract and are purged from the body through orifices and ruptures following bloat (Knight 1996). Native soil microbial communities are thought to react to the presence of a cadaver within the first 24 h of deposition (Carter et al. 2008) and increases in soil microbial activity have been reported in the area immediately surrounding the cadaver following purging of fluids (Carter and Tibbett 2008; Carter et al. 2010). Over time cadaver decomposition is believed to increase fertility within the decomposition island created around the body (Towne 2000; Carter et al. 2007).

Although the microbial load in soil where decomposition takes place may increase as a result of a new source of inoculum, soil microbial communities may also be hindered by the presence of a decomposing substrate. Decomposition is known to be associated with large influxes of ammonia into the soil environment (Hopkins et al. 2000) which may be toxic to some microorganisms. Taphonomic processes are also known to discolour the soil, cause vegetation death and displace the natural soil fauna (Bornemissza 1957; Towne 2000). These events suggest that decomposition has a harmful effect on organisms within close proximity of decomposing carcasses or cadavers.

Soil pH fluctuations as a result of cadaver decomposition have been well documented. Most studies report the alkalinisation of the soil following decomposition (Vass et al. 1992; Towne 2000; Hopkins et al. 2001; Carter and Tibbett 2006; Carter et al. 2008; Haslam and Tibbett 2009). This effect has been shown to endure in soils that see repeated decomposition activity over the course of many years (Damman et al. 2012). Chemical processes associated with decomposition will likely alter soil pH, either increasing acidity or alkalinity. As such, soil pH must be taken into account when attempting to understand soil microbial dynamics. Microorganisms are typically well adapted to survive within a specific range of pH values. A small change in pH can alter the availability of nutrients in the environment as well as the microorganisms' ability to utilize these nutrients (Bååth and Arnebrant 1994; Aciego Pietri and Brookes 2008).

Soil moisture content can also alter microbial activity and survival rates. When soil moisture is high, the concentration of certain nutrients may decrease through dilution limiting their availability (Stark and Firestone 1995). If there is too little moisture, nutrients become bound to soil particles and unavailable to microbes. Increased water content in the soil may also render the environment anoxic, causing a shift from aerobic to anaerobic microbial metabolism. As decomposition is thought to be associated with high levels of moisture and the seeping of fluids into the surrounding environment, moisture content in the soil may change when decomposition takes place. If such a change occurs rapidly, a decrease in microbial activity may ensue and long lasting effects on soil microbial community composition may be observed (Schimel et al. 1999).

The data presented in this chapter is part of a study investigating the changes that take place in soil microbial communities following deposition of a cadaver on the soil surface. The experimental design allowed natural fluctuations in environmental variables (i.e. ambient temperature and rainfall) and their impact on decomposition and soil parameters to be investigated. Soil microbial activity was measured throughout decomposition to determine whether the presence of a cadaver increased or decreased microbial activity. Fluorescein diacetate (FDA) hydrolysis was used to measure soil total microbial activity. This method has proven useful in characterising changes in soil microbial activity in a variety of soils (Schnürer and Roswall 1982) and is commonly used to characterize the effects of various soil treatments. Soil pH and soil moisture were measured to understand how these variables influenced soil microbial activity in the presence or absence of decomposition.

16.2 Method

16.2.1 Experimental Design

To study the effects of decomposition on soil from the fresh stage through to the dry remains stage a total of 4 trials were conducted over 2011 and 2012. Each year, one trial was started in the late spring and a second trial started in the summer. All trials

were carried out at the University of Ontario Institute of Technology decomposition facility located in Oshawa, Ontario, Canada (43.948 °N, 78.900°W). Soil at the facility has been characterized as a gravely sandy loam (pH 7.78 ± 0.26) by the University of Guelph Agriculture and Food Laboratory. Ambient temperature and rainfall within the facility were monitored using a Hoboware® weather station (Onset, Cape Cod, USA).

For each experiment three pig carcasses (*Sus scrofa*) weighing approximately 23 kg were used as human cadaver analogues. Each pig ingested the same diet as they were reared together on the same farm. The pigs were killed at a local abattoir according to the guidelines set out by the Ontario Ministry of Agriculture, Food and Rural Affairs on the morning of each trial (day 0) and immediately transported to the decomposition facility. The pig carcasses were deposited on the soil surface a minimum of 2 m apart and covered with wire cages to prevent scavenging. Soil samples were collected from below the head, torso and hind quarters for all the pigs during all trials. Sample collection occurred on days 0, 2, 4, 6, 8, 11, 14, 17, 20, 27, 34, 41, 48, 62, 90. Three sites located within the facility but having had no contact with decomposing carcasses were used as controls. These sites were situated a minimum of 5 m from the decomposition sites and measured 4 m^2. Each control was sampled three times on each day and the locations of sample collection rotated so that the same area was not repeatedly sampled. All soil samples were obtained using a sterilised stainless steel scoopula that was inserted 3 cm into the soil and used to produce soil cores which were stored in glass scintillation vials fitted with Teflon lined caps. Samples were immediately transported to the laboratory where analyses requiring fresh soil (i.e. measures of microbial activity) were carried out. Remaining soils were stored at −20 °C.

The decomposition stages described by Payne (1965) and adapted by Anderson and Van Laerhoven (1996) were used to categorize the stage carcasses had reached at each sampling day. These stages are: fresh, where the body appears the same as before death with some slight discolouration; bloated, during which the body becomes distended due to the proliferation of gut bacteria and the accumulation of gases within the body; active decay, during which the majority of soft tissue will be broken-down; advanced decay, typically observed when the rate of soft tissue breakdown is slowed and the body becomes weathered; dry remains; at which point all that remains are bones, hair and dried tissue.

Accumulated degree days (ADD) were calculated using the temperature data to compare rates of decomposition between trials based on ambient temperatures or heat units. ADD is calculated by obtaining daily average temperatures for each experimental day up to a given time point and calculating the sum of these temperatures (Edwards et al. 1987).

16.2.2 Soil pH and Soil Moisture

Soil pH was measured using a 1:5 w/v suspension of soil in distilled water (pH 7.5). Soil samples and water were placed in 20 ml vials, shaken vigorously and left to settle for at least 30 min before pH measurements were taken using a UltraBasic Benchtop pH-meter (Denver Instruments, Bohemia, NY, USA) coupled with an Accumet double junction gel filled pH electrode (Cole Palmer, Montreal, Canada).

Soil water holding capacity (WHC) was used as a measure of soil moisture content. This measure allows for soil matrix characteristics to be taken into consideration by expressing soil moisture as a percent of the full water content a soil sample could theoretically contain. Soil porosity was taken to represent the amount of space available for water within a soil sample and was calculated by measuring particle density and bulk density of soil at the experimental sites (Hao et al. 2007). Soil water content of samples collected was determined using the gravimetric method (Clarke Topp et al. 2007) and then expressed as a percentage of the total water holding capacity.

16.2.3 Soil Total Microbial Activity

Microbial activity levels in soil samples were measured using a fluorescein diacetate assay protocol adapted from Green et al. (2006). This method has been shown to measure microbial activity by measuring the hydrolysis of fluorescein diacetate by many enzymes including esterases, proteases and lipases (Schnurer and Roswall 1982). FDA measures were taken immediately after sampling. For each experimental and control sample, 2 g of sieved soil was weighed and placed in a 50 ml Falcon tube to which 15 ml of a potassium phosphate buffer (pH 7.6) and 200 µl of fluorescein diacetate stock solution prepared in acetone were added. The tubes were vortexed and heated in a water bath at 30 °C for 20 min. After incubation, 20 ml of a 2:1 chloroform: methanol solution was added to each tube to inhibit further breakdown of fluorescein. Tubes were centrifuged at 2000 rpm for 3 min. A 2 ml aliquot of the top phase containing the fluorescein product was filtered using Whatman filter paper no. 42. The absorbance of the final product was measured using a Genesys 10S UV–VIS spectrophotometer (Thermo Scientific) at $\lambda = 490$ nm. A blank was produced for each set of samples analysed and consisted of buffer and fluorescein stock solution only.

16.2.4 Statistical Analyses

Data was tested for normal distribution by the Shapiro-Wilk's test and for equal variance. Student's *t*-test was used to determine if significant differences existed between daily measures of soil pH, soil moisture and microbial activity from control samples and experimental samples. When the normality test failed, a Mann–Whitney Rank sum test was performed. Statistically significant differences among controls and experimental treatments for each trial were analyzed by one way repeated measure analysis of variance on ranks. To determine if soil pH, soil moisture or ambient temperature could be correlated with the measures obtained for microbial activity, Pearson product–moment correlations were conducted. All data was analyzed using the SigmaPlot 12.0 software package (Systat Software Inc., San Jose, USA).

16.3 Results and Discussion

It was hypothesised that experiments conducted in the spring would produce slower rates of decomposition and that rainfall might affect levels of soil moisture in both control and experimental sites. Trials undertaken during the summer were expected to produce faster rates of decomposition due to higher ambient temperatures. Since precipitation is more sporadic in the summer months, it was expected that soil at the experimental site would become intermittently dry. It was also anticipated that soil in contact with decomposing carcasses would be subjected to an increase in moisture as a result of tissue liquefaction and purging of decomposition fluids. Despite the varying effects of season on decomposition rates, an overall increase in microbial activity at sites where decomposition occurred was anticipated due to both increased nutrient release and the influx of microorganisms from the carcasses during decomposition.

16.3.1 Environmental Conditions and Stages of Decomposition

Average air temperatures for spring trials were 21.0 °C (2011) and 20.6 °C (2012). As is consistent with the seasonal changes observed in Southern Ontario the temperature gradually increased over the course of both spring trials reaching a maximum daily average temperature on day 49 (30.3 °C) in 2011 and day 46 (30.2 °C) in 2012 (Fig. 16.1). Both summer trials commenced during the warmest period of the season and temperatures steadily decreased overtime as the seasons changed from summer to fall. Overall average temperatures for trials conducted in the summer were 20.7 °C (2011) and 14.0 °C (2012). The fall of 2012 was cool with daily average temperatures at the end of the experiment nearing 0 °C.

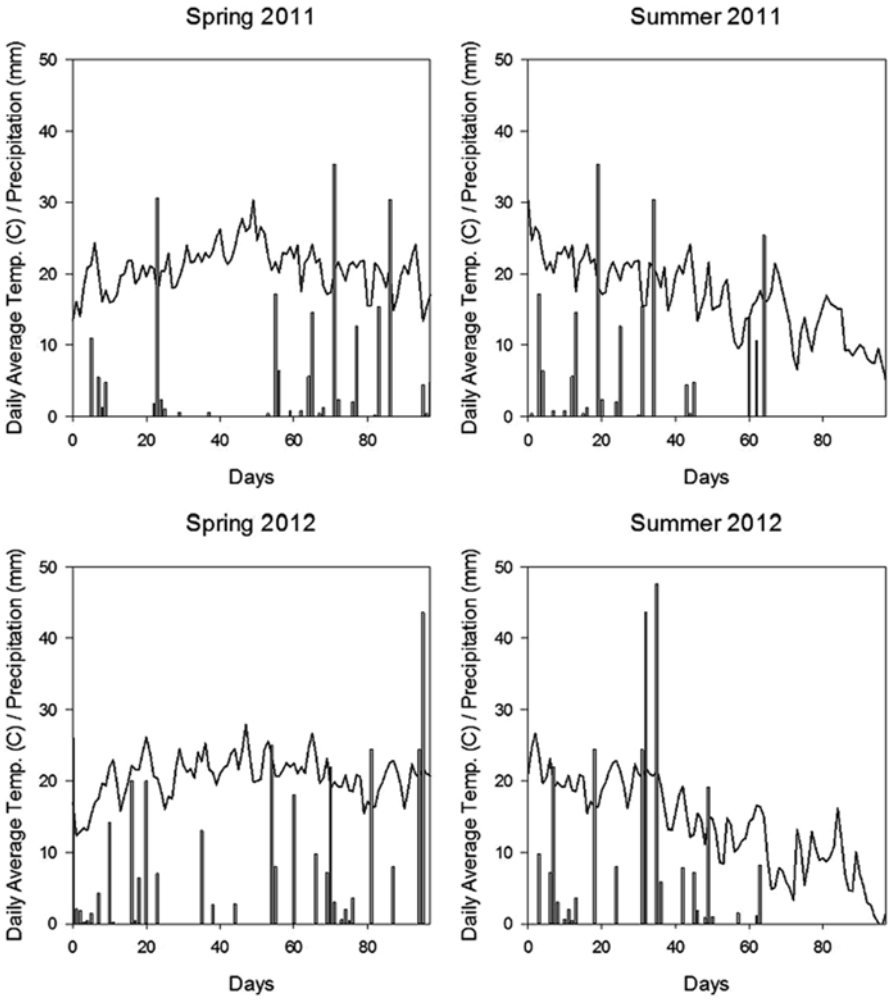


Fig. 16.1 Average daily temperatures (°C) and precipitation (mm) for the Spring 2011, Summer 2011, Spring 2012 and Summer 2012 experimental trials

Spring 2011 was characterized by high precipitation levels during the first week of the experiment and a gap in precipitation between days 30 and 50. The lack of rainfall caused drought like conditions in the region where the experiment took place. Rainfall was sporadic after day 40 of this trial which coincided with the early days of the Summer 2011 trial. Spring 2012 saw below normal temperatures during the first few experimental days with temperatures averaging 15 °C rather than the seasonal average of 20 °C. After day 10, seasonal temperatures were observed. Precipitation was recorded on a regular basis during the spring and summer 2012 trials.

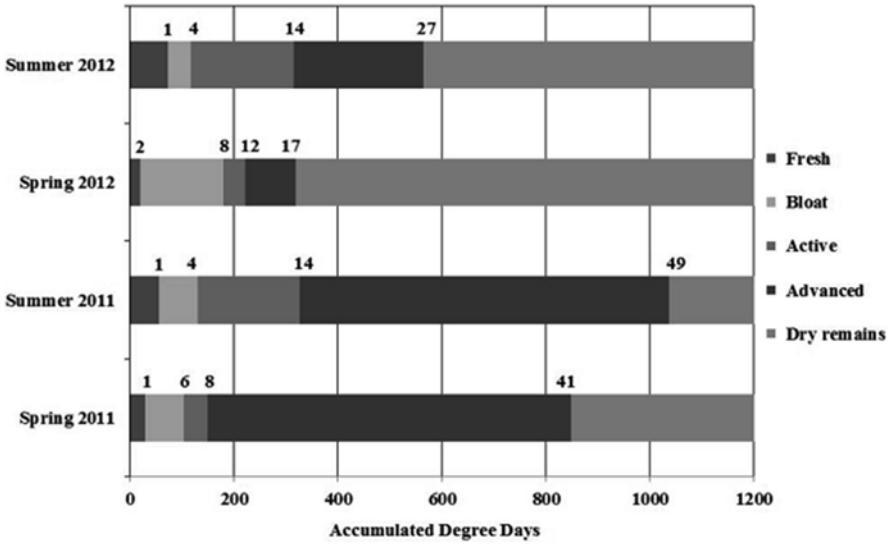


Fig. 16.2 Decomposition stages for the Spring 2011, Summer 2011, Spring 2012 and Summer 2012 experimental trials expressed in accumulated degree days (ADD). The length of each stage in experimental days is shown above the bar

Each trial was subjected to a unique set of temperatures and precipitation, which likely contributed to varied rates of decomposition. To allow decomposition stages to be compared between trials, the length of each stage per trial was expressed in accumulated degree days or heat units (Fig. 16.2). In all trials the fresh stage was observed on days 0 and 1. When accumulated degree days were calculated, the end of the fresh stage and beginning of bloat was noted to begin at 74.4 ADD (Spring 2011), 52.2 ADD (Summer 2011), 20.0 ADD (Spring 2012) and 51.3 ADD (Summer 2012). Bloat was observed by day 2 in all cases although the classification of bloat was more ambiguous for the Spring 2012 trial with full bloat not being recorded until day 6. Active decay was characterised by maggot masses covering the carcasses and deflation of the torso. The pig carcasses were characterised as being in active decay on day 6 at 128.4 ADD (Spring 2011), day 4 at 150.2 ADD (Summer 2011), day 8 at 179.3 ADD (Spring 2012) and day 4 at 115.6 ADD (Summer 2012). The stage of advanced decay was distinguished by the migration of maggots away from the carcasses. During the Spring 2011 trial, all maggots present on the pig carcasses migrated away from the bodies on day 8, earlier than was expected based on previous experiments. Soft tissue consumption by the larvae was limited resulting in much of the tissue still being present on the carcass. It is believed that higher than normal amounts of precipitation during the first few days of decomposition may have been responsible for initiating early maggot migration. A relationship between rain and maggot dispersal has previously been suggested by Lewis and Benbow (2011). Based on the criteria used to distinguish decomposition stages,

Fig. 16.3 Carcass decomposition on experimental day 42 of the Spring 2011, Summer 2011, Spring 2012 and Summer 2012 trials

carcasses from the Spring 2011 trial were considered as being in the advanced decay stage on day 8 at 164.6 ADD. The onset of advanced decay for the other trials occurred on day 14 at 346.8 ADD (Summer 2011), day 12 at 221.9 ADD (Spring 2012) and day 14 at 315.4 ADD (Summer 2012). The carcasses were characterised as dry remains by day 41 at 699.2 ADD (Spring 2011), day 49 at 384.3 ADD (Summer 2011), day 17 at 76 ADD (Spring 2012) and day 27 at 269.7 ADD (Summer 2012).

The onset of the first three stages of decomposition (i.e. fresh, bloat, active decay) occurred below 200 ADD in all four trials. The onset of advanced decomposition and dry remains varied greatly between trials with the distinction between the two stages often being difficult to determine. Pig carcasses from the spring and summer 2011 trials had significant amounts of soft tissue remaining once they had reached the stage of advanced decomposition (Fig. 16.3). The presence of residual soft tissue at this later stage of decomposition made it difficult to determine the beginning of the dry remains stage for these two trials. It was observed that remaining soft tissue became rehydrated following rainfall making it appear that the carcasses had not yet reached the dry remains stage. Soft tissue was efficiently removed

from the carcasses in both trials carried out in 2012 and skeletonisation was more pronounced. The beginning of the dry remains stage was observed earlier for both of these trials.

Throughout this study larvae were a major factor of decomposition and were responsible for the majority of soft tissue removal. Increased rates of decomposition as a result of maggot activity have been well documented by Simmons et al. (2010a, b). The early dispersal of larvae in the spring 2011 trial greatly affected the progress of decomposition. During this study, early maggot migration was thought to explain the difference in decomposition rates observed between the two spring trials. Maggots migrated on day 8 in the Spring 2011 trial versus day 12 in the Spring 2012 trial. Both summer trials produced similar decomposition rates and the length of each decomposition stage when measured in ADD was comparable. These results were in agreement with the hypothesis that temperature will dictate the progression of decomposition and the transition from one stage of decomposition to the next. Slight differences between larval colonisation rates across triplicates were observed. These variations appeared to be a result of differences in the opening of the mouth and eyes as well as the presence or absence of feces. Although pig carcasses were chosen based on their similarity in weight, some carcasses were smaller than others. Soft tissue from smaller carcasses was removed slightly more rapidly than that of larger carcasses. Body constitution is known to influence maggot activity (Campobasso et al. 2001).

The onset of the advanced stage was associated with the formation of a crust on the soil surface in all of the trials conducted. It is believed that this crust forms through the mixing of decomposition fluids, remnants of broken down tissue and soil particles. This phenomenon was previously reported in entomological studies (Bornemissza 1957; Forbes and Dadour 2010) suggesting it may be a common phenomenon in cases where maggots are present on the decomposing body. In order to obtain soil samples it was necessary for the crust to be broken or lifted to gain access to the soil beneath. It was also noted that the presence of the crust caused water to pool on the surface of experimental sites after rainfall events. Consequently, soil below the crust remained slightly drier than surrounding soil due to the barrier created by the crust on the soil surface.

16.3.2 Soil pH

Average soil pH values of control and experimental sites were compared on each sampling day. It was found that soil pH values were only significantly different ($p<0.05$) on a few days during each trial; specifically days 14, 20 and 62 in Spring 2011; days 2, 6 and 34 in Summer 2011; days 48 and 62 in Spring 2012; and days 0, 6, 8, 11 and 62 in Summer 2012. No relationship could be found between the occurrence of these pH shifts and decomposition stages. Soil pH values obtained over the course of each trial were compared for overall significant differences between control and experimental samples but no significant difference was identified (Table 16.1).

Table 16.1 Statistical summary of results from repeated measures ANOVA on ranks used to determine overall significant differences ($p<0.05$) (highlighted in *bold*) between control measures and experimental measures of microbial activity, soil moisture and soil pH during the Spring 2011, Summer 2011, Spring 2012 and Summer 2012 trials

	Spring 2011		Summer 2011		Spring 2012		Summer 2012	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Soil pH	17.30	0.240	17.90	0.211	23.20	0.057	18.80	0.173
Soil moisture	22.60	0.067	21.49	0.064	23.65	0.051	**25.96**	**0.026**
Microbial activity	14.00	0.442	23.78	0.049	13.05	0.523	14.10	0.442

Overall, pH values followed a similar trend as each trial progressed (Fig. 16.4). Initial soil pH averages were between 7.0 and 8.5 and gradually decreased during the first 2 weeks of the experiments often declining below 7.0. This change suggests a slight acidification of all soils, although the change was relatively short and soils gradually increased before plateauing at a circumneutral pH slightly lower than the initial soil pH. Control and experimental samples underwent similar fluctuations suggesting that the pH fluctuations observed in experimental soils was not a direct result of decomposition. The acidification of the experimental soils appears slightly more pronounced than that of control soils although no significant difference overall was found (Table 16.1).

Published studies investigating the changes that occur in soil following taphonomic events indicate that a localised increase in pH can be expected as a result of cadaver decomposition (Vass et al. 1992; Towne 2000; Hopkins et al. 2000; Carter and Tibbett 2006; Carter et al. 2008; Haslam and Tibbett 2009). This was not observed in any of the four trials conducted in this study and correlates with studies conducted by Van Belle et al. (2009) in the same environment. The acidification of the soil observed in all experiments coincides with rainfall and increases in soil moisture, suggesting that rain may be the cause for the observed changes in soil pH. In most experiments, it is possible that increases in alkalinity as a result of decomposition were masked by the effects of rain. Soil pH at the experimental facility is naturally alkaline with high buffering potential, which may explain why soil did not become more alkaline with decomposition. Initial soil pH has been shown to influence the soil pH changes observed following decomposition (Haslam and Tibbett 2009).

Acidification of the soil later in the decomposition process has been reported by Vass et al. (1992) and by Carter and Tibbett (2008). This change is thought to result from cations being released following bone decomposition. However the decrease in pH observed in this study occurred in the early stages of decomposition. Although the changes in soil moisture were more pronounced during the spring, rainfall was logged on multiple days during the first weeks of all four trials. It is possible that the acidification observed is a result of rainfall which is generally known to have an acidic pH of approximately 5.6 and can become even more acidic in urban areas (Charlson and Rhode 1982).

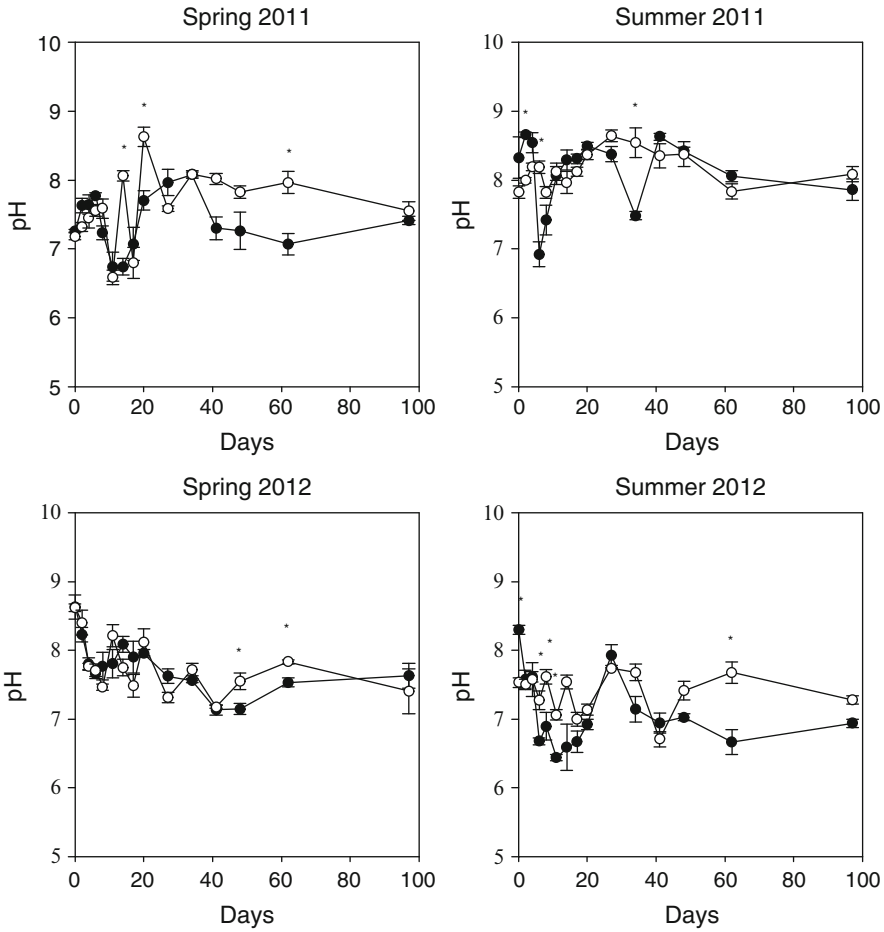


Fig. 16.4 Soil pH measurements for experimental (-●-) and control (-○-) samples collected on each sampling day during the Spring 2011, Summer 2011, Spring 2012, and Summer 2012 trials. * indicates ignificant differences ($p < 0.05$) between experimental and control samples

16.3.3 Soil Moisture

Seasonality appeared to play an important role in soil moisture levels. An increase in soil moisture was observed in both spring studies following extended periods of rain. Experiments which commenced in the summer months were subjected to drier soil conditions during the first few weeks with soil moisture gradually increasing as the trial progressed (Fig. 16.5). Temperature was found to be negatively correlated with soil moisture in all four trials. Increases in temperature were seen to correspond to decreases in soil moisture suggesting rates of evaporation played an important role in regulating soil moisture levels.

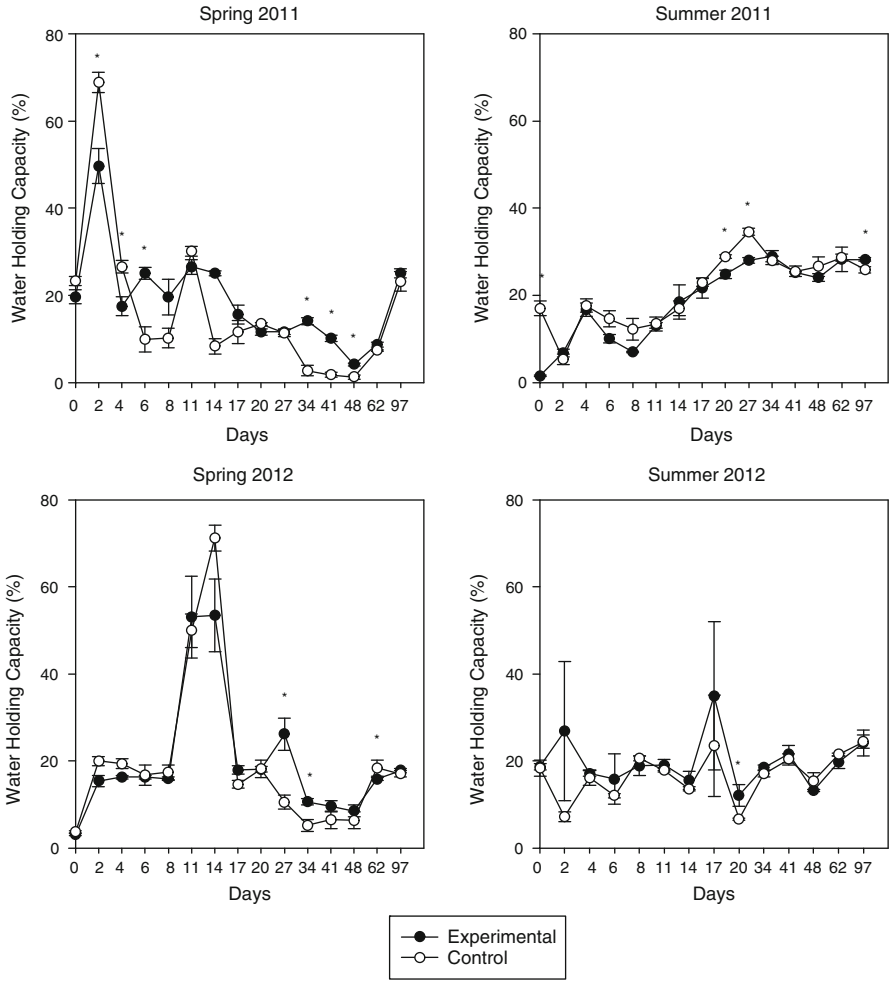


Fig. 16.5 Soil water holding capacity for experimental (-●-) and control (-○-) soil samples collected on each sampling day during the Spring 2011, Summer 2011, Spring 2012, and Summer 2012 trials

During the Spring 2011 trial, soil moisture was significantly higher ($p<0.05$) in control soils on days 2 and 4 but significantly higher in experimental soils on days 6, 14, 34, 41 and 48. For the Summer 2011 trial, soil moisture was significantly higher in control soil on days 20 and 27. The Spring 2012 trial showed that soil moisture was significantly higher ($p<0.05$) in experimental soil on days 27 and 34. During Summer 2012, soil moisture was significantly higher ($p<0.05$) in experimental soils on days 20 and 27. Summer 2012 was the only trial to show a significant

difference between soil moisture values for control sites and experimental sites overall (Table 16.1).

Throughout this study, it was observed that when rainfall occurred, an increase in soil moisture would follow and was typically more pronounced in control soils than at experimental sites. It is believed that soils below the pig carcasses were protected from rainfall either by the pigs and their remains or by the crust formed by decomposition products later in the decomposition process.

Soil moisture levels were expected to fluctuate in the experimental soils as a result of decomposition fluids being purged from the body and the liquefaction of soft tissue. This was expected to occur immediately following bloat and at the beginning of active decay when purging and liquefaction are most noticeable. A significant ($p < 0.05$) increase in experimental soil moisture that could be associated with these decomposition events was only observed on day 6 of the Spring 2011 trial.

Soil moisture was significantly higher overall in experimental samples versus control samples during the Summer 2012 trial. This is in accordance with the hypothesis that decomposition increases moisture at the site of decay. During the Summer 2012 trial, the pigs were deposited on the soil surface in the western portion of the decomposition facility. Although all experimental sites were cleared of vegetation prior to the deposition of the carcasses to facilitate sampling, vegetation in this area was observed to be denser than elsewhere in the facility. It is believed that this difference in vegetation may have resulted in higher water retention and increased soil fertility.

16.3.4 Soil Total Microbial Activity

Average microbial activity for control and experimental sites on each sampling day of the four trials can be seen in Fig. 16.6. Statistical analyses of these results are presented in Table 16.2. During Spring 2011, significantly higher microbial activity levels were observed for the decomposition sites on eight of the fourteen sampling days (2, 6, 11, 14, 17, 34, 41 and 48). These days fell within three different stages of decomposition: bloat, advanced decay and dry remains, however the increase during the bloat stage is unlikely to be related to decomposition processes. The Summer 2011 trial produced five instances where experimental soils showed significantly higher microbial activity levels. These occurrences fell within the bloat stage (day 2), active decay stage (day 8) and advanced decay stage (days 27, 34 and 41). During the Spring 2012 trial, microbial activity was found to be significantly higher in experimental soils during the bloat stage (days 6), active stage (day 11) and dry remains stage (days 27 and 62). During the Summer 2012 trial, microbial activity was significantly higher in experimental soils during the fresh stage (day 0). It is unlikely that this increase relates to decomposition activity as no changes at the soil level are observed at the time of deposition. Furthermore, microbial activity of experimental soils was significantly lower than control soils on most days of the

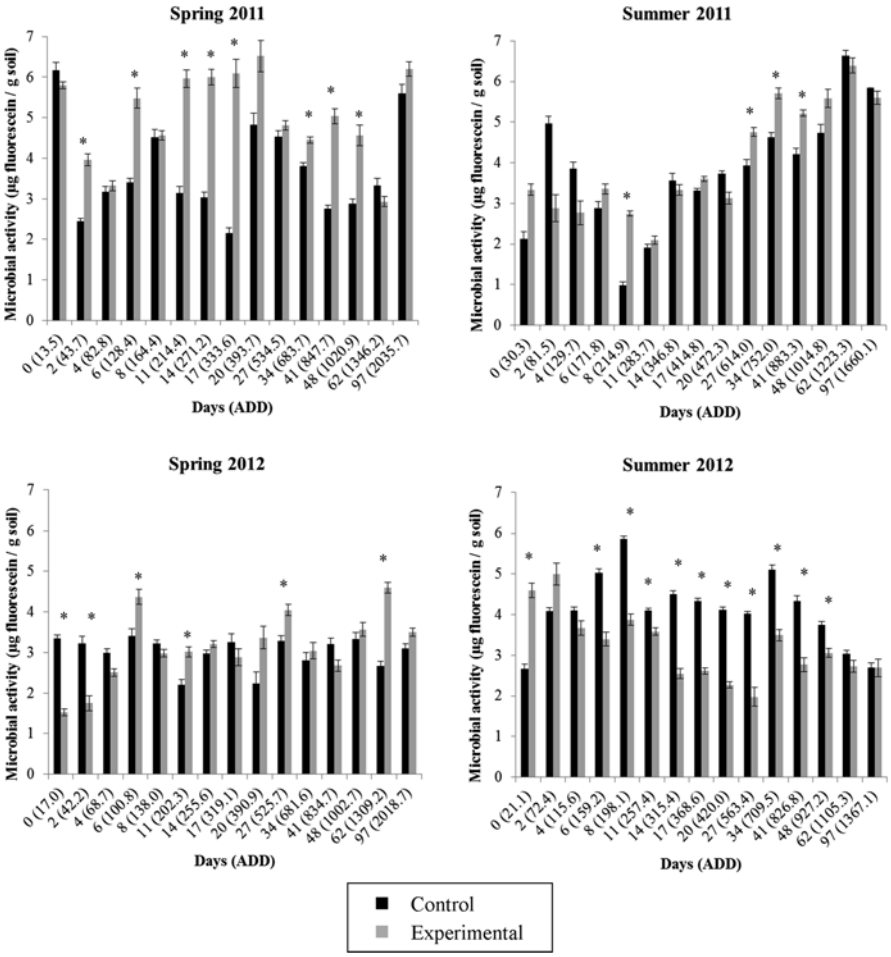


Fig. 16.6 Average measures of total microbial activity for control sites and experimental sites collected on each sampling day during the Spring 2011, Summer 2011, Spring 2012, and Summer 2012 trials. * indicates ignificant differences ($p < 0.05$) between experimental and control samples

summer 2012 with the exception of days 0, 2, 4, 62 and 97. Comparison of microbial activity for control and experimental samples for each trial overall, demonstrated no significant differences.

The results suggest that decomposition can result in increased soil microbial activity although the effect differed between seasons and years. During the Spring 2011 trial, early dispersal of maggots was observed which in turn meant that soft tissue removal was slow and the amount of liquefaction was reduced. Summer 2011 and Spring 2012 data showed several days where microbial activity appeared to increase as a result of decomposition while Summer 2012 data pointed to

Table 16.2 Statistical summary of Student's t-tests or Mann-Whitney rank sum tests (*) used to determine significant differences ($p < 0.05$) (highlighted in *bold*) between average microbial activity levels of control samples and experimental samples for each day sampled during the Spring 2011, Summer 2011, Spring 2012 and Summer 2012 trials

	Spring 2011		Summer 2011		Spring 2012		Summer 2012	
Day	*t*	*P*	*t*	*P*	*t*	*P*	*t*	*P*
0	−0.898	0.382	−1.199	**0.017**	4.913	**<0.001**	−4.528	**<0.001**
2	4.742	**<0.001**	2.812	**0.013**	2.151	**0.047**	63.00*	0.052
4	0.421	0.680	1.610	0.127	1.287	0.216	1.125	0.277
6	3.887	**0.001**	−1.184	0.254	−2.150	**0.047**	120.00*	**0.003**
8	0.107	0.916	−8.864	**<0.001**	0.807	0.431	6.383	**<0.001**
11	5.283	**<0.001**	−0.721	0.481	−3.660	**0.002**	2473	**0.025**
14	6.360	**<0.001**	0.516	0.613	−1.1–6	0.285	6.749	**<0.001**
17	5.419	**<0.001**	−1.798	0.091	0.772	0.451	8.565	**<0.001**
20	1.767	0.096	103.00*	0.133	−1.828	0.086	8.460	**<0.001**
27	0.737	0.472	−2.201	**0.043**	−2.225	**0.041**	4.385	**<0.001**
34	2.895	**0.011**	−3.100	**0.007**	−0.480	0.638	4.653	**<0.001**
41	5.662	**<0.001**	−3.070	**0.007**	1.261	0.225	3.752	**0.002**
48	3.026	**0.008**	−1.353	0.195	−0.462	0.651	2.429	**0.027**
62	0.925	0.369	0.522	0.609	−4.528	**<0.001**	0.907	0.381
97	1.068	0.301	0.464	0.649	−1.211	0.244	0.007	0.995

decomposition having an inhibiting effect on microbial activity. During the Summer 2011, Spring 2012 and Summer 2012 experiments, maggot masses completely covered the carcasses, soft tissue removal was accelerated and products of liquefaction leached into the decomposition sites. The varied rate of decomposition across all trials coupled with the seasonal climatic differences is thought to have produced varying rates of entry of decomposition products into the soil. When decomposition products were abundant, the accumulation of toxic products and the formation of an anoxic environment may have negatively impacted soil microbial communities.

The presence of larvae may also have an impact on the microbial population within the carcass and in the soil due to anti-microbial activities. As maggots feed on a cadaver, consumed tissues pass through the digestive system of the larvae and are effectively disinfected. This is thought to occur through the production of antibacterial peptides (Bexfield et al. 2004) and the alkaline pH of the maggots' secretions (Mumcuoglu et al. 1998). When maggot masses are substantial, it may be possible that the majority of the microbial load originating from the cadaver or carcass never makes its way into the surrounding environment.

Data was analysed to see if correlations existed between microbial activity and soil pH, soil moisture or ambient temperature (Table 16.3). A significant negative correlation was identified in the Spring 2011 trial between microbial activity and soil moisture. The correlation existed for both control and experimental samples when considered separately. Although soil moisture was not statistically correlated to microbial activity in each trial, it was noted that spikes in soil moisture were often

Table 16.3 Statistical summary of Pearson product moment correlation analyses used to determine significant correlations ($p<0.05$) (highlighted in *bold*) between soil microbial activity measures and soil pH, soil moisture and ambient temperature during the Spring 2011, Summer 2011, Spring 2012 and Summer 2012 trials

	Environmental parameter					
	Soil pH		Soil moisture		Ambient temperature	
	R	*P*	*r*	*P*	*R*	*P*
Spring 2011	0.300	0.107	**−0.390**	**0.033**	0.267	0.153
Summer 2011	**−0.459**	**0.014**	−0.075	0.697	0.116	0.550
Spring 2012	0.384	0.0360	−0.0329	0.863	0.456	0.0113
Summer 2012	−0.304	0.116	−0.420	0.261	0.271	0.164

associated with decreases in microbial activity. It is believed that in these instances, soil became saturated with water creating anoxic conditions which were unfavourable to soil microbial communities. Previous ecological studies have shown that changes to soil moisture, i.e. from very dry to very moist, can significantly affect soil microbial activity and alter soil microbial communities for prolonged periods of time (Schimel et al. 1999). During the Summer 2011 trial, a negative correlation between soil pH and microbial activity was observed. During this trial, soil pH in both control and experimental soils became slightly alkaline during which time a decrease in microbial activity was observed. When pH values returned to their initial state, microbial activity increased which is in agreement with the well-known effect pH can have on soil microbial properties (Aciego Pietri and Brookes 2008).

16.4 Conclusions

The purpose of this chapter was to present results of four taphonomic studies conducted during two different seasons over two years using pig carcasses as human analogues. As was predicted, the rates of decomposition observed in the spring were slightly slower than those observed in the summer. This can be attributed to the higher temperatures to which the carcasses are exposed during the summer, which will favor microbial activity as well as larval development. Both 2012 trials demonstrated an increase in soft tissue removal when compared to the 2011 trials which may be due to increased colonisation of the carcasses by carrion insects.

Larvae were responsible for soft tissue removal throughout this study with their absence clearly slowing down the decomposition process. Observations made over the course of all four trials indicate that larvae may also play a role in the microbial response observed in soil following decomposition. The presence of larvae accelerated soft-tissue removal and liquefaction of the carcass, thus influencing the rate at which decomposition products entered the surrounding environment. When the pulse of decomposition products is strongest, soil microorganisms may have difficulty adapting to the extreme change in their environmental conditions. If an

influx of microorganisms originating from the body does enter the soil, the influence of this new microbial load may be counterbalanced by the loss of the original soil microbial community.

The trials conducted as part of this study demonstrated an increase in microbial activity as a result of decomposition in some instances. The Spring 2011 trial produced multiple days where microbial activity was significantly higher in experimental soils when compared to control soils. This coincided with limited maggot activity and slow removal of soft tissue. It is believed that the rate of decomposition will affect the soil microbial response. Where decomposition is slower, microorganisms are able to better adapt and utilise the nutrients which are slowly entering the soil environment. When decomposition is accelerated and liquefaction is rapid, the influx of decomposition products into the environment may be overwhelming and potentially toxic for soil microorganisms resulting in decreased soil microbial activity. The prolonged effect of decomposition on soil microorganisms remains to be shown.

During this study, soil pH did not become more alkaline following decomposition. Changes in soil pH such as a slight acidification were observed over the course of the experiments and appear to be related to environmental changes rather than decomposition events. Rainfall and vegetation cycles may play an important role in the regulation of soil pH and need to be accounted for when attempting to understand the impact that decomposition may have on soil pH and soil microbial communities.

Decomposition was expected to create an influx of moisture into the decomposition environment yet this was not consistently observed during this study. Soil moisture in both control samples and experimental samples appeared to fluctuate comparably for the majority of all four trials indicating that rainfall, as well as rates of evaporation, were the major factors that affected soil moisture. Soil texture is also believed to play a role in the effect of decomposition on soil microbial communities. The soil used during this study consisted of sandy loam. This soil type has relatively low water holding capacity, allowing good drainage of excess water. This may explain why decomposition did not appear to increase soil moisture within the decomposition site. This soil type may also have been favourable for the formation of the crust that was observed on the surface of the soil beneath the carcasses.

Data generated during this study showed no clear trends in soil microbial activity or environmental parameters. Although soil moisture and soil pH could not be related to fluctuations in microbial activity in experimental soil samples, correlations did exist in control soil samples. It appears that the microbial response to decomposition is a complex process with multiple factors requiring further consideration in order to fully understand microbial dynamics. Total organic content may be a better predictor for microbial activity measures throughout the decomposition process and should be considered in future studies.

Results presented in this chapter are part of a larger study investigating the dynamics of soil microbial communities during the decomposition process. Subsequent analyses will investigate the succession of microorganisms present in soils during decomposition with the aim of identifying microbial indicators of

PMI. Studying soil microbial communities is becoming increasingly feasible with the development of profiling methods that do not require microorganisms to be cultured in the laboratory. Increased access to the technology and tools necessary for the study of soil communities as well as a growing number of collaborations between forensic scientists and microbial ecologists has also changed the way microorganisms are studied within the field of taphonomy. However the need remains for reproducible microbial data from taphonomic studies across different soil types, soil depths, seasons and geographical regions. This will be crucial for the application of microbiological analyses within forensic casework.

Acknowledgements Research capacity and infrastructure at the University of Ontario Institute of Technology is supported by NSERC and the Canada Research Chair program. Research capacity and infrastructure at Chaminade University of Honolulu is supported by NIH-BRIC P20MD006084.

References

Aciego Pietri JC, Brookes PC (2008) Relationships between soil pH and microbial properties in a UK arable soil. Soil Biol Biochem 40:797–802

Anderson GS, VanLaerhoven SL (1996) Initial studies on insect succession on carrion in Southwestern British Columbia. J Forensic Sci 41:617–625

Bååth E, Arnebrant K (1994) Growth rate and response of bacterial communities to pH in limed and ash treated forest soils. Soil Biol Biochem 26:995–1001

Bexfield A, Nigam Y, Thomas S, Ratcliffe NA (2004) Detection and partial characterisation of two antibacterial factors from the excretions/secretions of the medicinal maggot Lucilia sericata and their activity against methicillin-resistant Staphylococcus aureus. Microbe Infect 6:1297–1304

Bornemissza GF (1957) An analysis of arthropod succession in carrion and the effect of its decomposition on the soil fauna. Aust J Zool 5:1–12

Campobasso CP, Di Vella G, Introna F (2001) Factors affecting decomposition and Diptera colonisation. Forensic Sci Int 120:18–27

Carter DO, Tibbett M (2006) The decomposition of skeletal muscle tissue (Ovis aries) in a sandy loam soil incubated at different temperatures. Soil Biol Biochem 38:1139–114

Carter DO, Yellowlees D, Tibbett M (2007) Cadaver decomposition in terrestrial ecosystems. Naturwissenschaften 94:12–24

Carter DO, Tibbett M (2008) Does repeated burial of skeletal muscle tissue (Ovis aries) in soil affect subsequent decomposition? Appl Soil Ecol 40:529–535

Carter DO, Yellowlees D, Tibbett M (2008) Temperature affects microbial decomposition of cadavers (Rattus rattus) in contrasting soils. Appl Soil Ecol 40:129–137

Carter DO, Tibbett M, Yellowlees D (2010) Moisture can be the dominant environmental parameter governing cadaver decomposition in soil. Forensic Sci Int 200:60–66

Charlson JR, Rhode H (1982) Factors controlling the acidity of natural rain water. Nature 295:683–685

Clarke Topp G, Parkin GW, Ferré TPA (2007) Soil water content. In: Carter MR, Gregorich EG (eds) Soil sampling and methods of analysis, 2nd edn. CRC Press, Boca Raton, pp 939–962

Damman FE, Tanittaisong A, Carter DO (2012) Potential carcass enrichment of the University of Tennessee anthropology research facility: a baseline survey of edaphic features. Forensic Sci Int 222:4–10

Edwards R, Chaney B, Bergman M (1987) Temperature developmental units for insects. Pest Crop Newsl 2:5–6

Forbes SL (2008) Decomposition chemistry in a burial environment. In: Tibbett M, Carter DO (eds) Soil analysis in forensic taphonomy. CRC Press, Boca Raton

Forbes SL, Dadour I (2010) The soil environment and forensic entomology. In: Byrd JH, Castner JL (eds) Forensic entomology: the utility of arthropods in legal investigations. CRC Press, Boca Raton, pp 207–425

Green VS, Stott DE, Diack M (2006) Assay for fluorescein diacetate hydrolytic activity: optimization for soil samples. Soil Biol Biochem 38:693–701

Haslam TCF, Tibbett M (2009) Soils of contrasting pH affect the decomposition of buried mammalian (*Ovis aries)* skeletal muscle tissue. J Forensic Sci 54:900–904

Hao X, Ball BC, Cullery JLB, Carter MR, Parkin GW (2007) Soil density and porosity. In: Carter MR, Gregorich EG (eds) Soil sampling and methods of analysis, 2nd edn. CRC Press, Boca Raton, pp 743–759

Hopkins DW, Wiltshire PEJ, Turner BD (2000) Microbial characteristics of soils from graves: an investigation at the interface of soil microbiology and forensic science. Appl Soil Ecol 14:283–288

Knight B (1996) Forensic pathology, 2nd edn. Arnold, New York

Lewis AJ, Benbow ME (2011) When entomological evidence crawls away: *phormia Regina* en-masse larval dispersal. J Med Entomol 48:1114–1119

Moreno LI, Mills D, Fetscher J, John-Williams K, Meadows-Jants L, McCord B (2011) The application of amplicon length heterogeneity PCR (LH-PCR) for monitoring the dynamics of soil microbial communities associated with cadaver decomposition. J Microbiol Methods 84:388–393

Mumcuoglu KY, Ingber A, Gilead L, Stessman J, Friedmann R, Schulman H, Bichucher H, Ioffe-Uspensky I, Miller J, Galun R, Raz I (1998) Maggot therapy for the treatment of diabetic foot ulcers. Diabetes Care 21:2030–2031

Parkinson RA, Dias K-R, Horswell J, Greenwood P, Bannin N, Tibbett M, Vass AA (2009) Microbial community analysis of human decomposition on soil. In: Ritz K, Dawson L, Miller D (eds) Criminal and environmental soil forensics. Springer, New York, pp 379–394

Payne JA (1965) A summer carrion study of the baby pig Sus scrofa Linnaeus. Ecology 46:592–602

Parmenter RR, MacMahon JA (2009) Carrion decomposition and nutrient cycling in a semiarid shrub-steppe ecosystem. Ecol Monogr 79:637–661

Schimel JP, Gulledge JM, Clein-Curley JS, Lindstrom JE, Braddock JF (1999) Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga. Soil Biol Biochem 31:831–838

Schnurer J, Rosswall T (1982) Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Appl Environ Microbiol 43:1256–1261

Simmons T, Adlam RE, Moffatt C (2010a) Debugging decomposition data – comparative taphonomic studies and the influence of insects and carcass size on decomposition rate. J Forensic Sci 55:8–13

Simmons T, Cross PA, Adlam RE, Moffatt C (2010b) The influence of insects on decomposition rate in buried and surface remains. J Forensic Sci 55:889–892

Stark JM, Firestone MK (1995) Mechanisms for soil moisture effects on activity of nitrifying bacteria. Appl Environ Microbiol 61:218–221

Towne EG (2000) Prairie vegetation and soil nutrient responses to ungulate carcasses. Oecologia 122:232–239

Van Belle LE, Carter DO, Forbes SL (2009) Measurement of ninhydrin reactive nitrogen influx intro gravesoil during aboveground and belowground carcass (*Sus domesticus*) decomposition. Forensic Sci Int 193:37–41

Vass AA, Bass WM, Wolt JD, Foss JE, Ammons JT (1992) Time since death determinations of human cadavers using soil solution. J Forensic Sci 37:1236–1253

Chapter 17
Soil Fauna and Their Effects on Decomposition Within Coniferous and Deciduous Tree Soil Samples

Rebecca J. Camplin, Damian Evans, and Iain D. Green

Abstract Soils are dynamic environments that undergo constant change due to activities of the community that lives within and upon them. This dynamic system as a medium for corpse decomposition is little understood. This study investigated the decomposition of mice buried for 21 days in tubs containing freshly dug soils obtained from beneath the canopies of Scots Pine (*Pinus sylvestris)* and Maple (*Acer platanoides*). These two soils contrasted in the type of humus and pH, which affects the activity of soil microfauna. Despite this, soil type had no significant effect on the decomposition rate of mouse cadavers. This could mean that differences in the soil microbiology have little impact on the rate of cadaver decomposition. However, the mass of soil significantly decreased in the soil from under the *P. sylvestris* canopy, suggesting increased oxidation of soil organic matter. This was attributed to the low C:N ratio of the cadaver stimulating microbial activity in the mor humus forming under *P. sylvestris* due to the flush of available N and alkaline substances released from the cadaver. It is also possible the relatively low microbial activity associated with mor humus may have been negated by the increased numbers of mesofauna associated with the mor humus. The role of soil mesofauna in decomposition processes has received scant attention, but clearly requires investigation.

17.1 Introduction

Decomposition of human remains on and within soils has received much attention (Dent et al. 2004; Rodriguez 1997; Rodriguez and Bass 1983; Bass et al. 1990; Vass et al. 1992). Some of this work has investigated the effects of scavenging by large animals and fauna such as flies and beetles. The majority of these studies have concentrated on soil as a surface or a carrier and relatively little has been written on

R.J. Camplin (✉) • D. Evans • I.D. Green
The Forensic and Biological Sciences Group, The School of Applied Sciences, Bournemouth University, Fern Barrow, Wallisdown, Poole, Dorset, UK, BH12 5BB
e-mail: rebeccajanecamplin@hotmail.com

H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics*, Soil Forensics, DOI 10.1007/978-3-319-33115-7_17

the soil as a medium for the decomposition process of human remains. A particular gap in the knowledge is the difference in the rate of decomposition in soils forming under contrasting tree canopies. Forensic scientists currently operate on the assumption that the microbial metabolism of soil is not associated with the early stages of decomposition (Carter et al. 2008), but this has not been established sufficiently.

17.1.1 Soil Functions

From tree roots to the carbon cycle, from man-made adaptations to environmental disasters, soils are a dynamic environment. As the floral and faunal community colonise the detritus of soils, they modify the content. This is turn changes many parameters and can consequently affect the rate of decomposition (Ajwa and Tabatabai 1994; Gerrard 2000; White 2006).

The organic matter of soil is made up of two important chemical elements; these are carbon (C) and nitrogen (N). These two elements are accessed by the microbial community through decomposition of plant residues and other organic matter. The sources of C available within soil is of importance as it determines the composition and size of the microbial biomass, as it used by the microorganisms for energy and cell synthesis (Sylvia et al. 2005). However, microorganisms also require N to grow and reproduce. The relationship between the two elements is of importance, the C:N ratio, as it varies between soils. In deciduous soils the C:N ratio is less than 20, so net mineralisation of N is facilitated; when organic matter is oxidised by the microbial community there is an excess of N, which is released in plant available forms. In coniferous soils the opposite occurs, the C:N ratio is greater than 25, there is insufficient N for the microbial community to use all the available C. Consequently, the microbial community rapidly utilises any available N, immobilising N within their cells. Carbon tends to build up in these soils, which the microbial community can rapidly exploit if available N (such as cadaver) is added to the soil.

17.1.2 Soil Biota

Soil is inhabited by a community of microorganisms that includes bacteria, fungi, algae and archaea, they are the smallest (μm-nm) of the soil biota. Soil also contains meso and macro fauna such as nematodes, mites, springtails and earthworms as well as plants (Trudgill 1947; Gerrard 2000; Byrd and Castner 2001; White 2006). Not all of the soil biota lives permanently within the soil, some live part of their lives out of soil. The sizes of these organisms can restrict the location in which they dwell. The smaller organisms, such as the aquatic nematodes live within the thin water layer of soils, which is created by the adsorption of water to soil particles. These nematodes prey on smaller organisms such as Amoebas. Such is the nature of the soil biota, other organisms such as those in the mesofauna play a key role by

reducing materials into smaller pieces which provides greater access for the microfauna (Sylvia et al. 2005).

The invertebrate activity within soils is a factor that needs careful consideration. Soil animals (such as earthworms) incorporate organic matter from the surface down into the deeper soil horizons (Hopkins et al. 2000; Hopkins 2008) and will feed on detritus within the soil layers. Other organisms can be categorised as decomposers, those which utilise carbon from the organic matter and producers which utilise carbon (CO_2) from the atmosphere during photosynthesis. There are over 4 million insects such as eelworms, 60,000 other insects such as larvae, ants and beetles as well as 150 earthworms m^{-3} of soil (Gerrard 2000).

17.1.3 Decomposition of Soils and Corpses

The breakdown of organic matter within soils is principally a biological process, due to the fact that it is the soil dwelling organisms that perform the breakdown processes both chemically and physically. The physical process of decomposition involves the physical fragmentation of the organic matter, principally by the invertebrate soil biota. This organic matter also undergoes chemical alteration, which releases mineral nutrients that will be used as energy by organisms.

The process of breaking down the organic matter occurs immediately after the organism (or part of it) dies. Microfauna use enzymes to oxidise the organic matter to produce energy and C. Macrofauna such as earthworms and mesofauna, such as mites and springtails, integrate the organic matter into the subsurface soil through fragmentation and this increases the surface area, allowing for a larger colonisation by the microorganisms and therefore aides the process of decomposition. It is this removal and changing of organic matter that plays a key role in the activity of invertebrates in decomposition (Gray et al. 1937; Trudgill 1947; Rodriguez and Bass 1983; Galloway et al. 1989; Bass et al. 1990; Ajwa and Tabatabai 1994; Galloway 1997; Gerrard 2000; Carter and Tibbett 2008; Forbes 2008).

Decomposition of human remains is also a chemical process that is almost inevitable, even refrigerated remains will show some form of chemical change (Micozzi 1991; Haglund and Sorg 1997; Pinheiro 2006; Carter et al. 2008; Byers 2011). Chemical breakdown of cells happens almost immediately after death occurs. The chemical breakdown of human remains progresses through three chemical processes autolysis, putrefaction and decay. Autolysis is the degeneration of tissues within the body by the digestive system (Maples and Browning 1994; Haglund and Sorg 1997; Carter et al. 2008), the body essentially is being digested by its own enzymes. The appearance of autolysis is evident by blistering of the skin, although it is not apparent until a few days after death has occurred. Putrefaction follows autolysis and is the breakdown of the body by bacteria and the releasing of gaseous compounds such as ammonia, nitrogen and carbon dioxide causing the abdomen to expand as it fills with gases. Putrefaction is also responsible for marbling of the skin due to the breakdown of haemoglobin into sulphaemoglobin, which results in the

greenish brown discolouration of the skin as it is a green derivative of haemoglobin (Vass et al. 1992). Decay is the final stage where the remains soft tissues and muscles are broken down until it is fully skeletonised (Bass et al. 1990; Micozzi 1991; Haglund and Sorg 1997; Galloway 1997; Ubelaker 1997; Pinheiro 2006; Carter et al. 2008; Forbes 2008).

The aim of this current study is to determine whether there was an association between decomposition rates and activity of inhabiting organisms of soils taken from under the tree canopies of two different trees. The objectives of this study were to determine whether there was a difference in the rate of decomposition in soils forming under coniferous and deciduous trees by using soils from under Scots Pine (*Pinus sylvestris*) and Maple (*Acer platanoides*). The secondary objective of this study was to identify whether differences between the soils were due to soil fauna. It is hoped that this research will provide greater knowledge of the activities of the organisms within soils during decomposition. The study was performed using mice cadavers as mice have been used as models of human decomposition (Huff 2004), are easily obtained from ethical sources and present a cadaver of convenient size that still contains intact organ systems and microbial community.

17.2 Methodology

17.2.1 Soils

An area of mature, semi-natural mixed coniferous and deciduous woodland situated in Wallisdown, Dorset, UK was chosen as the sources of the soils. Samples were taken within a 20 m diameter to minimise differences soil parameters caused by the homogeneity in the underlying parent material. Thus, the main differences in the soils sampled were due to the input of leaf litter. For the purposes of this project, the selected soils received litter from Scots Pine (*Pinus sylvestris* L.) or Maple (*Acer platanoides* L.). The soil receiving *P. sylvestris* litter showed a typical mor humus. This consisted of a L layer of undecomposed plant litter; a F layer of fermenting, partially decomposed material with abundant mites and springtails; a H layer of humic material that was almost completely decomposed. This formed a horizon > 10 cm deep with a pH of 5.5 (measured in 1:2.5 soil water suspension) at the start of the experiment. In contrast, the soil receiving litter from *A. platanoides* exhibited mull type humus with a thin (<2 cm) litter layer above the A horizon and a pH of 7.5.

Shallow cuts were made in the soil under each tree canopy and a sample measuring the length of a container measuring 19.5 cm × 16 cm × 8.5 cm was collected by digging a spade in to the pre-cut area and carefully moving the soil into the tub without disturbing the layers of the soil. This was then repeated three times for each species of tree. The soil invertebrate community was initially assessed visually as the soil samples were taken. This showed little evidence of invertebrates. Soils were

carefully sifted and a more intensive visual search made at the end of the experiment as it was anticipated that the community at this point would reflect the taxa that benefited from the food sources provided by the cadaver and were hence the taxa engaged in the decomposition of the cadaver.

Figure 17.1 shows how samples were taken from points around the tree base and as close to the tree trunk as possible without disturbing the roots of the tree or the local fauna surrounding it (distances of 1–1.5 m from the base of the tree).

Once the soil samples were collected, the soil moisture content was measured (Tramex Compact Moisture Meter for Wood; Table 17.1). Moisture content was re-measured at the end of the experiment to ensure moisture content had remained constant.

17.2.2 Cadavers

Frozen mice (*Mus musculus)* were purchased from a commercial supplier (Reptiles Plus, Bournemouth, UK). It has been shown that frozen specimens are suitable for the study of decomposition in soil as there are no differences compared to fresh specimens (Tibbett et al. 2009). The second stage was to place six weighed mice into individual mesh litter bags made of nylon measuring 9 × 8 cm with meshes of

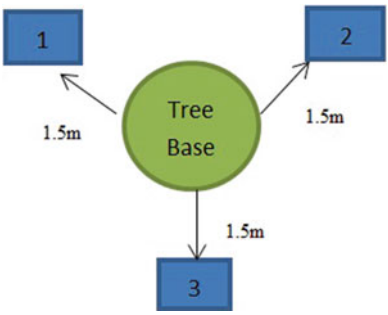


Fig. 17.1 The collection method of soils

Table 17.1 Soil moisture content of soil samples at the start and after 21 days of mouse burial (*M* Maple, *P* Pine)

Specimen No. (Mice)	Start soil moisture content (%)	End soil moisture content (%)	Difference +/−
M1	26	26	0
M2	26	27	+1
M3	26	26	0
P1	25	25	0
P2	24	25	+1
P3	24	25	+1

2000 μm to aid recovery. Mice cadavers were then placed into the containers of soil by carefully making a cut into the soil layer and inserting the mesh bag. Lids were then placed on top of these containers (to contain the invertebrates and prevent cross colonisation between treatments), holes were made in the side to maintain an aerobic environment around the soil. Each soil sample was replicated three times.

17.2.3 Environment Chamber

The containers with the buried mice were placed into a Conviron Adaptis with IN (incubator adaptor) controlled environment chamber. The environment chamber was pre-set to the below variables (both levels were pre-set at 12 h each to mimic day/night patterns).

Lights on (Level 1):	Lights off (Level 0):
Temperature 20 °C	Temperature 20 °C
Relative Humidity: 50 %	Relative humidity: 40 %

The chamber was monitored daily for 10 days in order to ensure that equilibrium was reached and that the chamber was working effectively. The samples were left for 21 days. This interval was determined to be a suitable time period for the incubation of the samples, as long as they were carefully monitored. Based on the findings of Carter et al. (2008) for *Rattus rattus*, it was anticipated that an incubation period longer than 21 days would result in skeletonised mice cadavers, whilst a shorter period may have been insufficient for external changes to the cadavers to be evident. After 21 days, litter bags containing the mice remains were reclaimed from the soil and the mice remains weighed to determine mass loss and hence the extent of decomposition.

17.3 Results

17.3.1 Mass Loss

After 21 days, the soil samples and the mice were removed and analysed. For the purposes of this research project the soil moisture content and mass weight were recorded and compared to the start of the experiment. Figure 17.2 shows the change in mass of the soil samples. The soil receiving litter from *P. sylvestris* lost almost three times the mass of the soil receiving *A. platanoides* litter. A Mann–Whitney *U* test was conducted to evaluate the statistical significance of this, which demonstrated that there was indeed a significant difference ($Z = -1.96$, $P < 0.05$). There was no difference in the moisture content of the soils over the course of the experiment (Table 17.1), suggesting the differences in the soil mass was due to the burial of the mice.

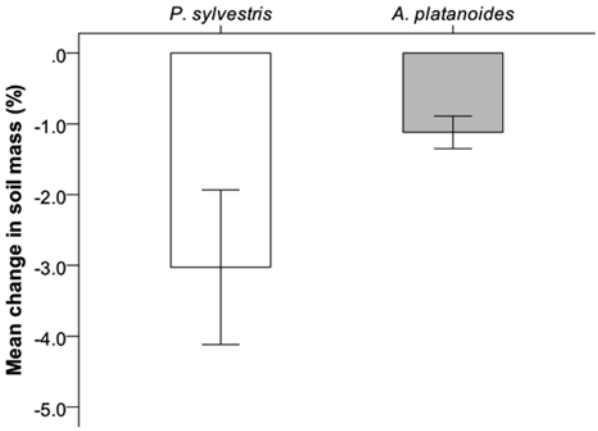


Fig. 17.2 Decomposition induced changes in the mass of soil under the canopies of two contrasting tree species

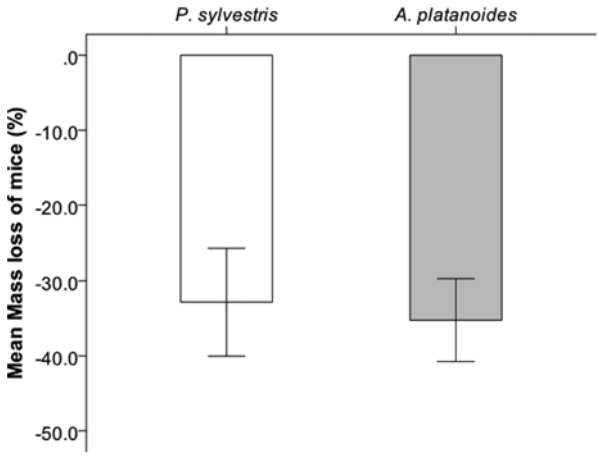


Fig. 17.3 Mean change in mouse mass after burial for 21 days in soils receiving litter from contrasting tree types

The mean percentage mass loss in the mice buried in the soil receiving *A. platanoides* litter was slightly higher than for mice buried in the soil receiving *P. sylvestris* litter (Fig. 17.3; 35.2 % compared to 32.8 %). However, a Mann Whitney *U* test showed that there was no statistical difference in the mass lost from the mice cadavers (Z = –0.22, P = 0.83).

17.3.2 Faunal and Floral Activity

The array of invertebrates found at the time of the mice's removal from the soil samples was recorded by means of identifying and noting the species and abundance within the given sample. Clear differences in observable meso and macro

Table 17.2 Observed occurrence of invertebrate group, plants and fungi in soils after the decomposition of mice

Specimen	Invertebrates	Growth of flora
*A. cam*1	Basidiomycete fungus, woodlice, localised mite distribution, oribatidae, molluscs	Clearly evidence, grass growing on top
A. cam 2	Basidiomycete fungus, woodlice, localised mite distribution, oribatidae, molluscs	Fresh ivy shoots
A. cam 3	Basidiomycete fungus, woodlice, localised mite distribution, oribatidae, molluscs	Fresh ivy shoots
P. syl 1	Springtails, *tipula oleracea* larvae, large mite distribution	None
P. syl 2	Springtails, *tipula oleracea* larvae, large mite distribution	None
P. syl 3	Springtails, *tipula oleracea* larvae, large mite distribution	None

fauna were observed between the two soils (Table 17.2). In soil containing *P. sylvestris* litter, mites were common and widely distributed and in some cases completely covered all the soil layers. By contrast, soils receiving *A. platanoides* litter showed a more localised colony of mites on or near the mice. Springtails were also found in the *P. sylvestris* soil, but not in the soil receiving *A. platanoides* litter. Fungal and plant growth were both evident in the soil receiving *A. platanoides* litter, but were not observed in the soil receiving *P. sylvestris* litter.

17.4 Discussion

The nature of soil humus is greatly dependant on the chemistry of the plant litter entering the soil. Mor humus typically forms from coniferous or ericaceous litter, whilst mull humus typically forms from deciduous tree litter. Soil with mor type humus typically exhibits a high C:N ratio (typically >30), low pH and low invertebrate and microorganism activity. In contrast, soils with mull type humus have a low C:N ration (<20), high invertebrate and microorganism activity. These properties were broadly reflected in the soils used in the present study. The soil receiving *P. sylvestris* litter and thus possessing mor humus had an acidic pH (5.5), lower diversity of invertebrates and no visible evidence of fungi. The soil receiving *A. platanoides* litter possessed mull humus with a slightly alkaline pH (7.5), a greater diversity of invertebrates and clear evidence of extensive fungal growth. However, the level of mesofauna such as mites and springtails, which are capable of passing through the litter bag mesh, were higher in the soil under *P. sylvestris*.

The effect of soil pH on cadaver decomposition is still not clear, but acidic soils may lead to slower decomposition (Carter et al. 2008). The present, limited, trial found no difference in decomposition in mouse cadavers over a 21 day period, despite a 2 pH unit difference between the two soils. However, as decomposition

proceeds, the pH of soil may become more alkaline as ammonification proceeds and base cations are released from tissues (Haslam and Tibbett 2009). Moreover, the cadaver also represents a nutrient source for soil microorganisms (Carter et al. 2007). Animal tissues typically have a C:N ration between 3 and 6. Thus, as a cadaver decomposes, ammonification releases available N into the soil. It is possible therefore, that the lower microbial activity associated with mor humus is overcome by an increase microbial activity during putrefaction resulting from favourable change in the soil pH and release of available N. The significantly greater decrease in the soil mass of the soil containing mor humus certainly indicates that mouse cadaver affected the oxidation of organic matter, which in term strongly suggests that microbial activity was enhanced by the presence of a decomposing cadaver through the release of available N.

A second possibility explaining a lack of an expected effect on decomposition due to the lower microbial activity in a soil containing mor humus is that the presence of higher numbers of mites and springtails in this soil. Species of both invertebrates groups may be necrophagus and are found on cadavers (Goff 2009). Consequently, it is also possible the relatively low microbial activity associated with mor humus may have been negated by the increased numbers of mesofauna that may have directly consumed the cadaver.

This limited study has demonstrated that whilst contrasting litter inputs into soils have little effect on the rate of decomposition of mouse cadavers, the process of decomposition can have an effect on soils with mor type humus. This could be potentially useful in determining whether an empty excavation has contained a body, how long a body has lain on the soil surface or how long a body has been buried in a shallow grave. Nevertheless, the work needs to be extended to more fully investigate these processes before useful evidence can be gathered in a forensic investigation. More detailed soil chemical and microbiological monitoring is especially required to quantify changes, particularly those of carbon, nitrogen, organic matter and microbial activity in the soil. A greater range of conditions including soil types, temperatures and precipitation is also required to extrapolate beyond the controlled conditions used in the present study to the natural environment. Finally, a greater understanding of the role of mesofauna in decomposition is required.

17.5 Conclusion

Large differences in the type of humus and pH of the soil had no effect on the decomposition of mouse cadavers over 21 day. This could mean that differences in the soil microbiology have little impact on the rate of cadaver decomposition. However, the low C:N ratio of the cadaver appeared to stimulate increased oxidation of soil organic matter to a greater extent in the mor humus and it possible that the flush of available N and alkaline substances released from the cadaver increased the microbial activity in the mor humus to a level similar to that of the mull humus. It is also possible the relatively low microbial activity associated with mor humus may

have been negated by the increased numbers of mesofauna associated with the mor humus. The role of soil mesofauna in decomposition processes has received scant attention, but clearly requires investigation.

References

Ajwa HA, Tabatabai MA (1994) Decomposition of different organic materials in soils. Biol Fertil Soils 18:175–182

Bass W, Meadows L, Mann R (1990) Time since death and decomposition of the human body: variables and observations in case and experimental field studies. J Forensic Sci 35:103–111

Byers S (2011) Introduction to forensic anthropology, 4th edn. Pearson Education, Harlow

Byrd J, Castner J (2001) Forensic entomology: the utility of anthropods in legal investigations. CRC Press, New York

Carter DO, Tibbett M (2008) Cadaver Decomposition and Soil Processes. In: Soil analysis in forensic taphonomy. CRC Press, London, pp 29–51

Carter DO, Tibbett M, Yellowlees D (2008) Temperature affects microbial decomposition of cadavers (Rattus rattus) in contrasting soils. Appl Soil Ecol 40:129–137

Carter DO, Yellowlees D, Tibbett M (2007) Cadaver decomposition in terrestrial ecosystems. Naturwissenschaften 94:12–24

Dent BB, Forbes SL, Stuart BH (2004) Review of human decomposition processes in soil. Environ Geol 45:576–585

Forbes S (2008) Decomposition chemistry in a burial environment. In: Soil analysis in forensic taphonomy. CRC Press, London, pp 203–223

Galloway A (1997) The process of decomposition in: a model from the Arizona-Sonoran desert. In: Forensic taphonomy: the postmortem fate of human remains. CRC Press, Boca Raton, pp 139–150

Galloway A et al (1989) Decay rates of human remains in arid environments. J Forensic Sci 34:607–616

Gerrard J (2000) Fundamentals of soil. Routledge, London

Goff ML (2009) Early postmortem changes and stages of decomposition in exposed cadavers. Exp Appl Acarol 49:21–36

Gray T, Parkinson D, Williams S (1937) Methods for studying the ecology of soil micro-organisms. Blackwell Scientific Publishing, Oxford

Haglund WD, Sorg MH (1997) Forensic taphonomy: the postmortem fate of human remains. CRC Press, Boca Raton

Haslam TCF, Tibbett M (2009) Soils of contrasting pH affect the decomposition of buried mammalian (Ovis aries) skeletal muscle tissue. J Forensic Sci 54:900–904

Hopkins D (2008) The role of soil organisms in terrestrial decomposition. In: Soil analysis in forensic taphonomy. CRC Press, Boca Raton, pp 53–62

Hopkins DW, Wiltshire PE, Turner BD (2000) Mircobial characteristics of soils from graves: an investigation at the interface of soil microbiology and forensic science. Appl Soil Ecol 14:283–288

Huff BL (2004) Postmortem polyamine degradation in mice. http://trace.tennessee.edu/utk_chanhonoproj/749. University of Tennessee Honors Thesis Projects

Maples WR, Browning M (1994) Dead men do tell tales: the strange and fascinating cases of a forensic anthropologist. Broadway Books, New York

Micozzi MS (1991) Postmortem change in human and animal remains: a systematic approach. C.C. Thomas, Springfield

Pinheiro J (2006) Decay process of a Cadaver. In: Forensic anthropology and medicine. s.l.:Humana Press, pp 85–116

Rodriguez W (1997) Decomposition of buried and submerged bodies. In: Forensic taphonomy: the postmortem fate of human remains. CRC Press, Boca Raton, pp 459–468
Rodriguez W, Bass W (1983) Insect activity and its relationship to decay rates of human cadavers in East Tennessee. J Forensic Sci 28:423–432
Sylvia DM, Fuhrmann JJ, Hartel PG, Zuberer DA (2005) Biological control of plant pathogens and nematodes. In: Principles and applications of soil microbiology. Pearson Education, Upper Saddle River, pp 427–446
Tibbett M, Stokes KL, Forbes SL (2009) Freezing skeletal muscle tissue does not affect its decomposition in soil: evidence from temporal changes in tissue mass, microbial activity and soil chemistry based on excised samples. Forensic Sci Int 183:6–13
Trudgill S (1947) Soil and vegetation systems. Clarendon, Oxford
Ubelaker D (1997) Taphonomic applications in forensic anthropology. In: Forensic taphonomy: the postmortem fate of human remains. CRC Press, London, pp 77–90
Vass AA et al (1992) Time since death determinations of human cadavers using soil solution. J Forensic Sci 38:1236–1253
White R (2006) Principles and practice of soil science, 4th edn. Blackwell Publishing, Oxford

Chapter 18
Analysis of Decomposition Fluid Collected from Carcasses Decomposing in the Presence and Absence of Insects

Jenna L. Comstock, Helene N. LeBlanc, and Shari L. Forbes

Abstract Most decomposition studies investigate soft tissue degradation in the presence of insects, however several studies have shown that when insect activity is excluded from carcasses, the rate of decomposition slows down. The goal of this study was to explore the effect of insect activity on the chemical properties of decomposition fluid. Fluid was collected from pig (*Sus scrofa)* carcasses over the course of two summer trials (2011 and 2012) conducted in southern Ontario, Canada. The pH and conductivity were measured and fatty acids were analysed using Attenuated Total Reflectance- Infrared (ATR-IR) spectroscopy. Results were compared between insect inclusion, partial exclusion, and complete exclusion carcass groups. The results indicate that the presence of insects increases the pH and decreases the conductivity of decomposition fluid. Spectral fatty acid results did not appear to vary greatly between experimental groups. The overall levels were not sufficiently different between carcass groups to conclude that the presence of insects played an important role in the fatty acid degradation process.

18.1 Introduction

Decomposition, when allowed to progress to completion, will result in the complete disintegration of soft tissue, leading to partial or complete skeletonisation (Evans 1963; Dent et al. 2004; Goff 2009). Soft tissue can be biochemically degraded

J.L. Comstock • H.N. LeBlanc
University of Ontario Institute of Technology,
2000 Simcoe St. N, Oshawa, ON L1H 7K4, Canada
e-mail: jenna.comstock@gmail.com

S.L. Forbes (✉)
University of Ontario Institute of Technology,
2000 Simcoe St. N, Oshawa, ON L1H 7K4, Canada

University of Technology Sydney, 15 Broadway, Ultimo,
NSW 2007, Australia
e-mail: Shari.Forbes@uts.edu.au

H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics*,
Soil Forensics, DOI 10.1007/978-3-319-33115-7_18

through autolysis and putrefaction (Gill-King 1997; Dent et al. 2004; Janaway et al. 2009), or physically removed by the feeding of vertebrate and invertebrate scavengers (Amendt et al. 2007; O'Brien et al. 2007; Goff 2009). Decomposition can be characterized using different stages, but the process is typically categorized using the stages first established by Payne (1965): fresh, bloat, active decay, advanced decay, dry, and remains.

There are many factors that influence the rate of decomposition, however studies have found that when temperature is accounted for, insects play the most significant role (Simmons et al. 2010a). In addition, it has been observed that carcasses decompose at a significantly accelerated rate in the presence of insects when compared to those which are excluded from insect activity (Simmons et al. 2010a). Few studies have explicitly investigated the decomposition process in the absence of insects. The first study to do so was conducted by Payne (1965), where foetal pig carcasses were used to monitor the progression of decomposition. Payne (1965) observed that these carcasses exhibited different decomposition patterns from those colonized by insects, and established an alternative set of decomposition stages to characterize the process: fresh, bloating and decomposition, flaccidity and dehydration, mummy stage, and desiccation and disintegration. It was also observed that the carcasses decomposed more slowly than their insect-colonized counterparts (Payne 1965). It was hypothesized that insects accelerate the rate of decomposition by distributing bacteria throughout the carcasses in the fluids they secrete, and through mechanical burrowing as they feed (Payne 1965).

In a more recent study, the effect of insect exclusion on the decomposition process was investigated using rabbit carcasses that were either buried or deposited on the ground surface and protected by screens (Simmons et al. 2010b). The results showed that the most important influence on the rate of decomposition, when time and temperature were accounted for using accumulated degree days (ADD), was the presence of insects (Simmons et al. 2010b). The exclusion carcasses were observed to decompose slower than those accessed by insects, regardless of the method of exclusion (Simmons et al. 2010b).

The study of decomposition-related products, such as soil, mammalian soft tissue, and fluid, is a prominent focus of researchers who study cadaver decomposition. One of the main goals in studying these decomposition products is to develop methods that identify chemical biomarkers that can be used to estimate post-mortem interval (PMI) or correlate the findings with specific decomposition stages (Swann et al. 2010c).

Many studies have concentrated on identifying potential chemical biomarkers in soil and tissue, however few studies have focused on their detection in decomposition fluid. Decomposition fluid is a challenging matrix to study since it is often a complex chemical mixture that contains insects, microorganisms, and other debris from the surrounding environment (Swann et al. 2010c). Previous research using decomposition fluids has focused on examining short chain volatile fatty acids and long chain fatty acids from pork rashers, stillborn piglets, and adult pig carcasses (*Sus scrofa*) in an attempt to determine their utility in estimating PMI (Swann et al. 2010a, b). The same authors also developed a method using capillary zone electrophoresis to detect selected biogenic amines and amino acids in decomposition fluid (Swann et al. 2010d).

Adipose tissue is composed of 60–85 % lipids, 90–99 % of which are triglycerides (Reynold and Cahill 1965). During decomposition, the degradation of lipids results in the hydrolysis of the triglycerides and other neutral lipids (Fiedler and Graw 2003; Dent et al. 2004; Forbes et al. 2005b), including diglycerides and phospholipids (Kramer and Hulan 1978). Intrinsic lipases work to free the fatty acids from the glycerol backbone, leading to a mixture of free saturated and unsaturated fatty acids (Dent et al. 2004; Forbes et al. 2004; Janaway et al. 2009; Notter et al. 2009). Following hydrolysis, anaerobic bacteria present within the body will promote hydrogenation of unsaturated fatty acids, thus converting them into their saturated counterparts (Evans 1963; Notter et al. 2009). It is therefore expected that, during decomposition, the levels of free fatty acids in the body will initially increase with the hydrolysis of neutral lipids, shown by an increase in saturated fatty acids and a concomitant decrease in unsaturated fatty acids. The examination of fatty acid degradation products in decomposition fluid therefore has the potential to demonstrate a correlation with the post-mortem period which may be valuable in estimating post-mortem interval.

Insects, like other living organisms, contain fatty acids to serve various biological purposes. Most of their fatty acids, as in mammals, are bundled in triglycerides for metabolic energy (Stanley-Samuelson et al. 1988). They contain many of the major and most commonly reported fatty acids: palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic, which account for approximately 98 % of the fatty acids in whole lipid extracts (Stanley-Samuelson and Dadd 1983). More specifically, the dominant fatty acid detected in triglyceride fractions of Diptera is palmitoleic acid (Fast 1966; Stanley-Samuelson et al. 1988) and more than 50 % of the fatty acids in neutral lipids are less than 18 carbons long (Fast 1966). Long chain polyunsaturated fatty acids are also a regular component of insect tissues, but they are mainly found in phospholipid fractions (Fast 1966; Stanley-Samuelson and Dadd 1983; Stanley-Samuelson et al. 1988). Very little is known about whether insect fatty acids are transferred to remains as they feed, and how insects affect the fatty acid degradation profile of soft tissue, if at all.

The objective of this study was to identify trends in the chemical properties of decomposition fluid, including pH, conductivity, and lipid degradation products, and compare these between insect- included and excluded carcasses to determine their effect on the chemical properties of decomposition by-products.

18.2 Materials & Methods

18.2.1 Experimental Site

The current research was conducted in Oshawa, Ontario, Canada, 68 km northeast of Toronto (43°94 N, 78°90 W). The fenced field site was located in a temperate open grassland. A forest was located outside of the facility on the north side, but did

not provide cover or shade for any of the experimental subjects. This southern Ontario location experiences warm summers (17 °C) and cool winters (−5 °C) (Bernhardt n.d.). The experiments were conducted during the summer months, from June-September 2011 (Trial 1) and 2012 (Trial 2).

18.2.2 Weather Data

A HOBO® Micro Station Data Logger was placed within the confines of the experimental site and recorded hourly temperatures, rainfall, and relative humidity for the duration of the studies. Data was subsequently retrieved from the data logger using HOBOware® Pro Graphing and Analysis Software, Version 3. Temperature data was used to calculate accumulated degree days (ADD), which is calculated by averaging daily temperatures (above 0 °C) and adding it to the summed average of the previous day (Megyesi et al. 2005). The use of ADD as a time scale allows studies conducted in different environments and at different times to be more accurately related to one another by accounting for temperature. It therefore accounts for most of the variability observed between carcasses, in terms of the rate of decomposition (Michaud and Moreau 2011). However, one recent study showed that the use of ADD may not accurately predict the rate of decomposition in certain environments (Myburgh et al. 2013) and thus would prevent comparison of studies between different geographical regions. Additional factors may also influence the rate of decomposition, even when temperature is accounted for in a given environment (Myburgh et al. 2013) hence it must be used with caution. Since the current study was replicated in the same environment and during the same season it was determined to be the most applicable method for comparing data across both trials.

18.2.3 Experimental Set-Up

Pig carcasses (*Sus scrofa domesticus)* were used as human analogues due to the ethical restrictions of using human cadavers for decomposition research in Canada. Pig carcasses are appropriate models for human decomposition because they have a similar internal anatomy, fat distribution, and are relatively hairless (Schoenly et al. 2006). They are also omnivorous, leading to the belief that they will share similar gut flora (Anderson and VanLaerhoven 1996).

Six sub-adult pig carcasses, weighing between 50 and 60 lbs, were used in each trial. All subjects were killed on the same day by captive headbolt, following regulated procedures at a local abattoir. All carcasses were transported to the research facility in sealed storage containers, to prevent the entry of insects. Three carcasses were used in each of the control groups (accessible to insects) and experimental groups (excluded from insects). The carcasses that were accessible to insects were used as controls since most decomposition studies are conducted in outdoor environments where insects are available to aid in the decomposition process. Much

knowledge is therefore available in terms of the expected visible changes carcasses will exhibit when decomposing in an outdoor environment. Any visible differences or differences in chemical properties of collected fluid for the exclusion carcasses placed in the same environment could therefore be attributed to the lack of insect activity, as opposed to other environmental factors.

Each carcass was placed in a clear, plastic container, on top of a plastic resin shelf containing holes. The shelf served to raise the carcasses above the base of the containers so that the carcasses were not decomposing in a pool of decomposition fluids. The holes in the shelves allowed for the fluids to drain away from the carcasses. The containers were also placed on a slight angle, to allow the fluids to drain to one side for collection. The containers in which the control carcasses were placed were shallow ($34\frac{5}{8}'' \times 18\frac{3}{4}'' \times 7''$), to allow the full exposure of the carcasses to available insects. The experimental carcasses were placed into deeper containers ($34\frac{5}{8}'' \times 18\frac{3}{4}'' \times 12\frac{1}{2}''$), to prohibit accessibility of insects. A double layer of mosquito netting was placed over the containers and sealed with Velcro along the perimeter of the opening to prevent the entry of insects for the experimental carcasses. All carcasses were protected from vertebrate scavengers by placing 3 ft × 2 ft × 1 ft cages with 1 cm mesh over the containers. The cages used to protect the experimental carcasses were also covered with another layer of mosquito netting to further inhibit the entry of insects. Petroleum jelly was used along the bottom perimeter of the containers to prevent the entry of crawling insects.

18.2.4 Sampling Regime

Photographs and observations were collected once a day each sampling day, beginning the end of May (2012, Trial 2) or beginning of June (2011, Trial 1). Fluid was also collected once daily on each sampling day. Serological pipettes attached to a vacuum bulb were used to collect available fluid. A new pipette was used for the collection of fluid from each carcass. If fluids were too viscous to collect with pipettes, scoopulas were used to collect the fluid. The scoopulas were rinsed with denatured alcohol between samples. Collected fluid was placed in glass jars and stored at −4 °C until analysis. Any remaining fluid in the containers was removed using siphons. This was to prevent the mixing of fluids released at different time periods and to ensure an accurate chemical degradation profile was determined for each day of decomposition. The frequency of fluid collection depended on the rate of decomposition and the presence of fluid in the containers.

The exclusion of insects was further necessary during visual observations and sample collection of the experimental subjects. A mosquito netting-lined tent was placed over the researcher and the carcass following removal of the anti-scavenging cage. The double layer of mosquito netting that covered the containers was only removed on one corner of the container to allow for the collection of fluid. Any Diptera that entered the tent were subsequently removed. Once fluid collection was complete, the netting and cage were promptly replaced over the container.

Table 18.1 Description of decomposition stages used to characterize the inclusion carcasses

Decomposition stage	Characteristics
Fresh	No discolouration, minimal change
Bloat	Inflation of the abdomen and raised limbs
	Marbling/skin discolouration
Active decay	Extensive maggot feeding, formation of masses
	Strong ammonia odour
	Exposed skin appears leathery and may be discoloured (orange-red)
Advanced decay	Little cadaveric tissue remains
	Few insects present on the carcass
	Some exposure of bones
	Gradual loss of moisture from remaining tissue
Dry	Dry skin, cartilage, and bones
	Bones may appear dirty
Remains	Only hair, parts of skin, bones, and teeth remain
	Teeth and bones are bleached

18.2.5 Stages of Decomposition

The six stages of decomposition initially established by Payne (1965) were used to characterize the process among the inclusion (control) carcasses. The characteristics used to identify each stage can be found in Table 18.1. The intent of the experimental group was to completely exclude insects for the entire period of study however insects did eventually gain access to most of the carcasses in the experimental group. Delayed colonization was observed among these carcasses, compared to the controls, and since access was limited, noticeably fewer insects were able to feed on the available soft tissue (i.e. they were unable to completely colonize the carcass and were restricted to feeding where they initially gained access). Succession patterns were not observed because attempts were continually made to prevent the entry of insects, even after they gained access. These carcasses were subsequently called partially-excluded carcasses and five stages were used to characterize the decomposition process (Table 18.2). Only one carcass remained completely excluded from insects for the period of study and four stages were used to describe the decomposition process of this carcass (Table 18.3). Different stages of decomposition were required to categorize each set of carcasses due to the variable processes observed.

18.2.6 pH & Conductivity Analyses

Approximately 3 mL of fluid was transferred to disposable culture tubes for pH and conductivity analysis. The pH values were measured using a digital pH meter (Denver Instrument, Ultra Basic pH Meter, Bohemia, NY). The pH meter was calibrated

Table 18.2 Description of decomposition stages used to characterize the partially excluded carcasses

Decomposition stage	Characteristics
Fresh	No discolouration, minimal change
Bloat	Inflation of the abdomen and raised limbs
	Marbling/skin discolouration
Localized tissue removal	Few, distinct maggot masses (mainly in the head and dorsal regions)
	Exposed skin appears leathery and may be discoloured (orange-red)
	Ammonia odour
Dry decomposition	Large amount of soft tissue remains (body form still relatively intact)
	Skin appears dry and leathery, but underlying tissues retain moisture
	Microbial decomposition still occurring
	Maggots have migrated and there is no further insect activity
	Few bones exposed, if any
Desiccation	Overall dry appearance
	Skin and underlying tissues dry; skin is hard
	Exposed bones may become bleached

Table 18.3 Description of decomposition stages used to characterize the completely excluded carcass

Decomposition stage	Characteristics
Fresh	No discolouration, minimal change
Bloat	Inflation of the abdomen and raised limbs
	Marbling/skin discolouration
Deflation	Deflation of abdomen, limbs fall limp over belly
	Carcass begins to flatten
	Tissues retain moisture
	Odours similar to domestic livestock
Dry decomposition	Substantial amount of soft tissue present (body form still intact)
	Dry skin and underlying tissue
	Microbial decomposition still occurring
	Hair remains on almost entire body
	Some skin discolouration

using buffer solutions with pH values of 4, 7, and 10 (Fisher Scientific, New Jersey, NY) prior to measuring the pH of the samples. The conductivity measurements were carried out using a digital dual pH-conductivity meter (Mettler-Toledo Seven Multi conductivity meter, Switzerland). The conductivity meter was calibrated using a 0.01 M KCl solution (>95 % purity, Fisher Scientific, New Jersey, NY).

18.2.7 Infrared Spectroscopy

One to five mL of fluid was transferred to 20 mL centrifuge tubes, depending on the degree of dilution resulting from precipitation. Lipids were extracted from fluid samples using a modified Folch method (Stuart et al. 2005) for analysis using infrared spectroscopy. Six mL of a chloroform-methanol (2:1 v/v) solution (both HPLC grade, Fisher Scientific, New Jersey, NY) was added to each tube and sealed. Samples were placed in a FS110D Sonicator (Fisher Scientific, Waltham, MA) for three 10 min intervals. One mL of deionized water was added to each of the samples, followed by 5 min of centrifuging at 3000 rpm. The organic layer from each sample was transferred to scintillation vials by pipette and subsequently dried using a Savant SC210A speedvac concentrator, attached to an RVT4104 refrigerated vapour trap (Thermo Electron Corporation, Madison, WI). Samples were either analysed immediately or stored at −4 °C until analysis. The concentrated product was directly applied to the germanium ATR crystal of a Nicolet 4700 Fourier transform infrared spectrometer, equipped with an ATR Smart Performer (ThermoFisher Scientific, Waltham, MA) for analysis. The spectra were scanned over the frequency range of 4000–500 cm^{-1}, with a resolution of 4 cm^{-1}. One twenty eight scans were recorded and collected using Omnic software. Relative band absorptions were calculated using the saturated C-H stretching band (2926–2913 cm^{-1}) as the reference band.

18.3 Results

18.3.1 Weather Data

Average daily temperatures and daily sums of precipitation were calculated for each trial (Fig. 18.1). The mean temperatures experienced during both trials were similar: 20.1 °C in Trial 1 and 19.3 °C in Trial 2. The temperature trends observed were also comparable between the two trials, in that they peaked after approximately 40 days, and then decreased until the end of the study (Fig. 18.1). However, the temperatures were lower for the first week of Trial 2, compared to those of Trial 1 (Fig. 18.1).

Trial 1 experienced a greater amount of precipitation (225.0 mm) than Trial 2 (190.4 mm) overall. However, during the first week of Trial 2 a greater amount of precipitation was recorded (28.4 mm) than in Trial 1 (14.5 mm). For a period of approximately 3 weeks during Trial 1 (from days 28 to 52), no precipitation was recorded (Fig. 18.1), which led to very hot and dry conditions. After this point (mainly between days 53 and 75), the majority of the precipitation for Trial 1 was recorded (Fig. 18.1). In contrast, precipitation regularly occurred throughout Trial 2 (Fig. 18.1).

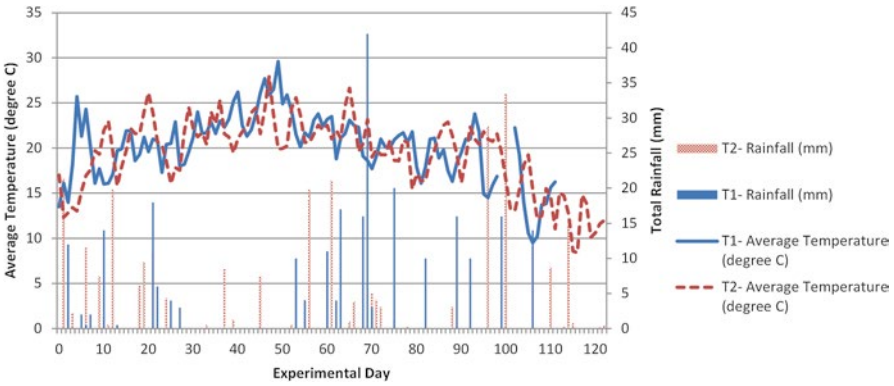


Fig. 18.1 Temperature and rainfall data during Trial 1 (2011) and Trial 2 (2012)

18.3.2 Stages of Decomposition

Although every attempt was made to completely exclude insects during both trials, a limited number of insects did gain access to all experimental carcasses in Trial 1 (on experimental day 7; 153 ADD) and two of the experimental carcasses in Trial 2 (on experimental days 10 and 11; 179 and 202 ADD, respectively) however their presence was localised to specific regions of the carcass (Table 18.2). These carcasses were subsequently referred to as partially excluded. One carcass was completely excluded from insects during Trial 2.

The fresh stage persisted for 2 days (29 ADD) in all carcasses in both trials (Figs. 18.2, 18.3 and 18.4). Following this stage, the patterns of decomposition diverged between carcass groups and the rate of decomposition differed between years (Figs. 18.2, 18.3 and 18.4).

18.3.3 pH Measurements

The pH values in Trial 1 were initially neutral for both the control and partial exclusion groups (Fig. 18.5a). The pH of the partially excluded group gradually increased thereafter, until approximately day 20 (399 ADD; early dry decomposition), after which point the values decreased for the remainder of the study (Fig. 18.5a). The final pH values recorded were approximately 7.5 (Fig. 18.5a). Many of the values for the inclusion group could not be measured due to the inability to collect fluid between experimental days 6–9 (133–178 ADD; active decay) and 13–15 (256–298 ADD; early advanced decay) due to the large numbers of maggots present. It is therefore difficult to determine whether this trend also took place for these samples. Following day 18 (358 ADD), the inclusion carcass values appear lower than those

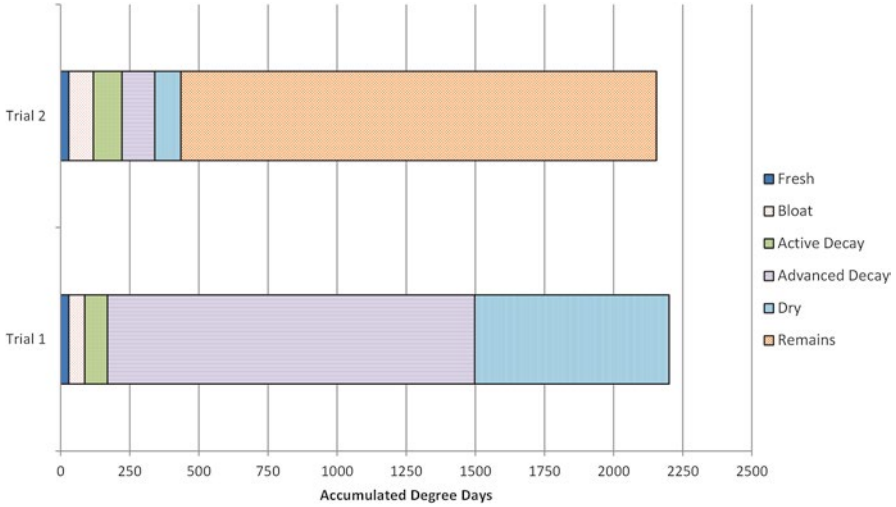


Fig. 18.2 Onset of the decomposition stages for the inclusion carcasses in Trials 1 (2011) and 2 (2012)

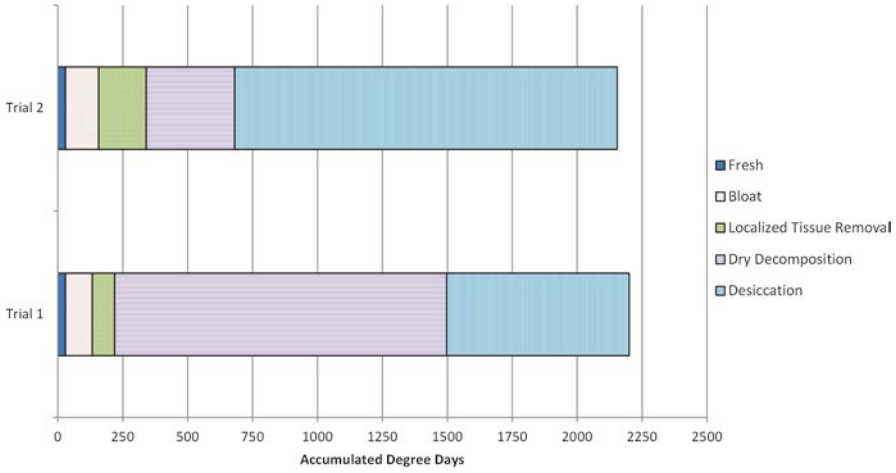


Fig. 18.3 Onset of the decomposition stages for the partially excluded carcasses in Trials 1 (2011) and 2 (2012)

from the partially excluded carcasses (Fig. 18.5a), however there were no significant differences between any of the measurements ($p > 0.001$).

The pH values in Trial 2 displayed more fluctuations than observed in Trial 1 and there was the addition of the completely excluded carcass. The control and completely excluded carcasses yielded fluids with pH values around 7.5 initially, while the partially excluded carcasses displayed pH values between 8.5 and 9 (Fig. 18.5b). All pH values decreased below 7 on day 6 (101 ADD) which coincided with the late bloat stage for the controls and the mid bloat stage for the experimental groups. The pH values then increased to values between 7 and 7.5 until day 9 (157 ADD) which

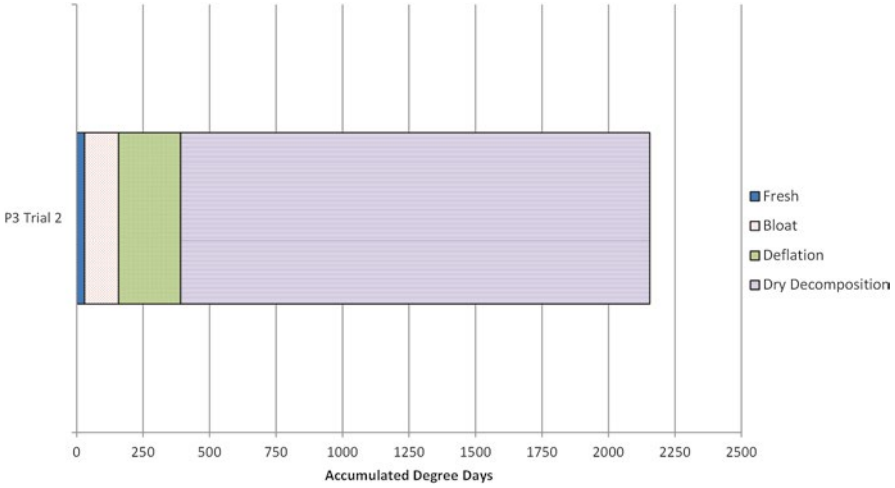


Fig. 18.4 Onset of the decomposition stages for the completely excluded carcass in Trial 2 (2012)

coincided with the beginning of the active decay stage for the controls and the end of the bloat stage for the experimental group. After this point, the trends and pH values diverged between carcass groups. The values increased for the inclusion and partially excluded groups, with the inclusion group having more alkaline values than the partial exclusion group. These values subsequently decreased after day 13 (238 ADD; beginning of advanced decay for the inclusion carcasses; during localized tissue removal for the partially excluded carcasses) until the end of the study, with final values recorded between 7.5 and 8. After day 9 (157 ADD; end of bloat), the completely excluded carcass decreased in pH until day 15 (275 ADD; mid deflation), after which point it increased and followed similar trends as the partially excluded group, with final values between 7.5 and 8. There were no significant differences between the inclusion and partial exclusion groups ($p > 0.001$). Statistics could not be performed on the completely excluded carcass because there were no replicates, so it is undetermined whether there were significant differences between the completely excluded group and the other groups.

18.3.4 Conductivity Measurements

At the beginning of Trial 1, the conductivity measurements of each group followed very similar trends (Fig. 18.6a). The lowest values were recorded on day 3 (62 ADD; early bloat) (Fig. 18.6a). The values then steadily increased until day 8 (169 ADD; end of active decay for the inclusion carcasses and early localized tissue removal for the partially excluded carcasses), at which point the values decreased until day 13 (256 ADD; early advanced decay for the inclusion carcasses and end of localized tissue removal for the partially excluded carcasses). The partial exclusion group then demonstrated another cycle of increasing and decreasing conductivity values until the end of the study. The control group exhibited similar trends

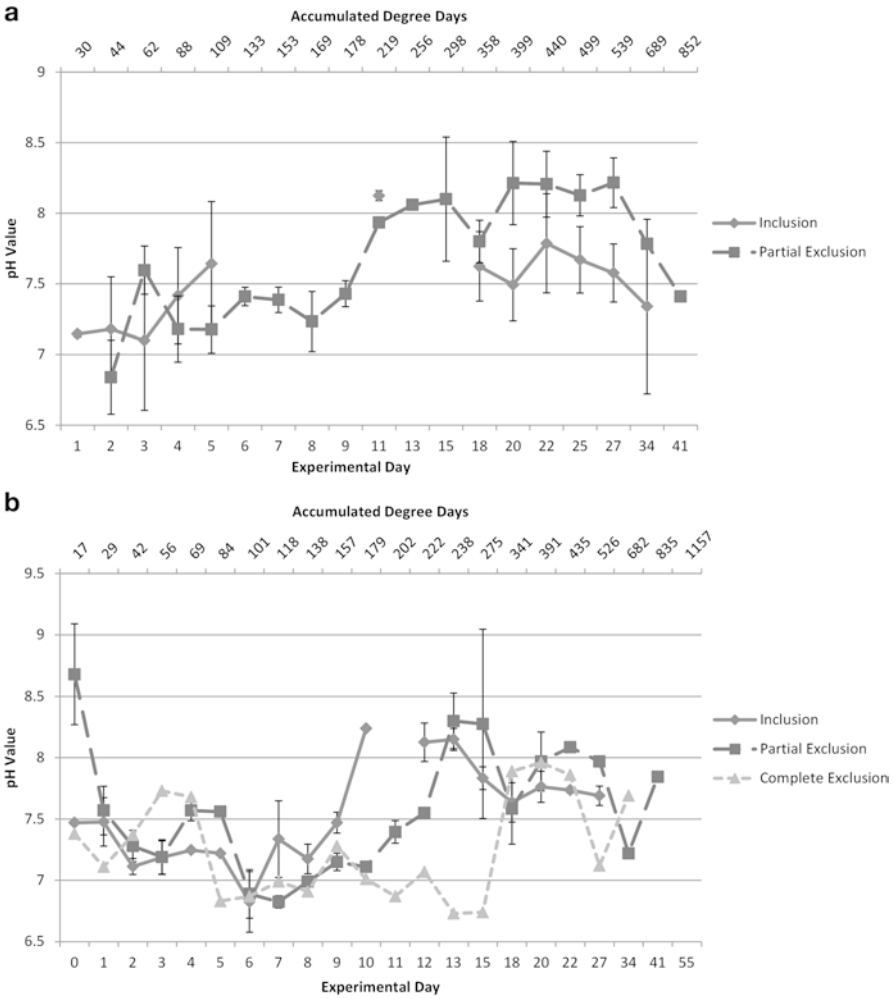


Fig. 18.5 (**a**) pH values of decomposition fluid collected from inclusion and partial exclusion carcasses during Trial 1 (2011); (**b**) pH values of decomposition fluid collected from inclusion, partial exclusion, and complete exclusion carcasses during Trial 2 (2012)

following day 18 (358 ADD; early advanced decay), however the fluctuation in values was less evident. Fluid samples from the controls were not collected between days 6 and 9 (133–178 ADD; active decay) and 13–15 (256–298 ADD; early advanced decay) and it is therefore unclear whether they followed the same trends as the partial exclusion group during that time. There were no significant differences between any of the measurements ($p > 0.001$).

The conductivity measurements of each carcass group in Trial 2 also displayed similar values and trends during early decomposition (Fig. 18.6b). The values were initially low, with the lowest values recorded on days 2 and 3 (42 and 56 ADD; beginning of bloat). The values of all groups showed increasing trends until day 9

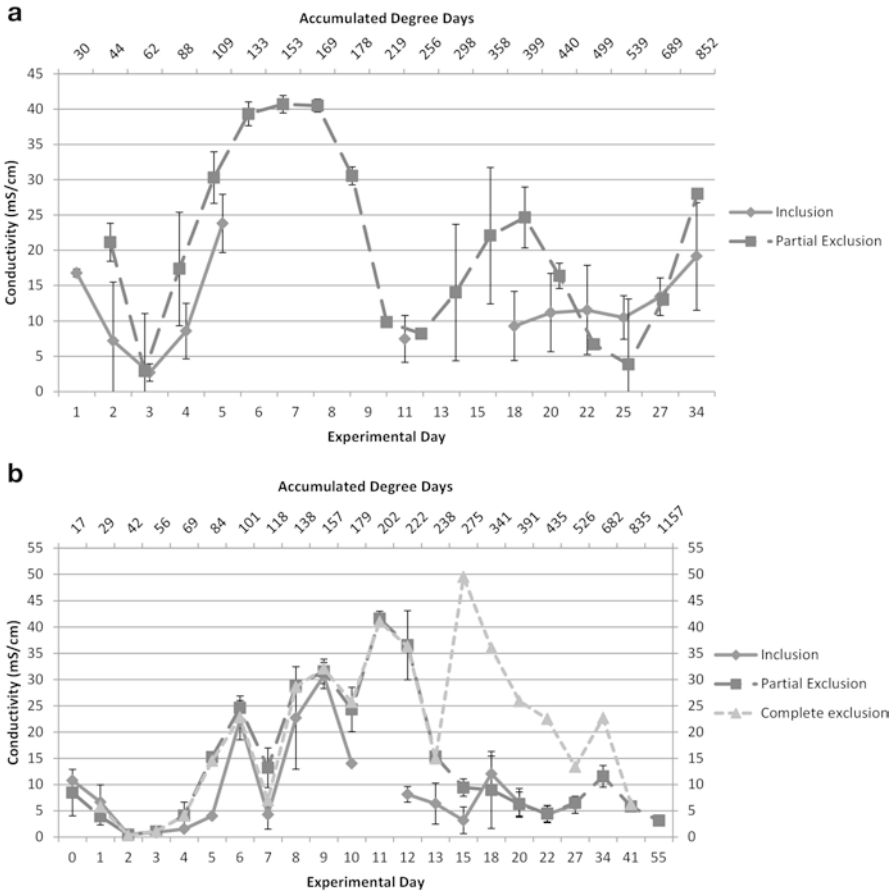


Fig. 18.6 (**a**) Conductivity measurements of decomposition fluid collected from inclusion and partial exclusion carcasses during Trial 1 (2011); (**b**) Conductivity measurements of decomposition fluid collected from inclusion, partial exclusion, and complete exclusion carcasses during Trial 2 (2012)

(157 ADD; beginning of active decay for the controls, and end of bloat for the experimental groups), with decreased values on day 7 (118 ADD; late bloat) and day 10 (179 ADD; early active decay for the inclusion carcasses, beginning of localized tissue removal for the partially excluded carcasses, and beginning of deflation for the completely excluded carcass). After day 9 (157 ADD), the trends and conductivity measurements from each carcass group diverged. The values from the inclusion group decreased and remained low, while the exclusion groups remained high. The partial exclusion group subsequently decreased and remained low, while the decrease in values from the complete exclusion carcass was delayed, but remained higher in value until the last measurement. There were no significant differences between any of the values for the inclusion and partial exclusion groups ($p > 0.001$). Statistics could not be performed using the completely excluded group because of a lack of replicates.

Table 18.4 The major infrared stretching bands detected in decomposition fluid

Wave number (cm^{-1})	Assignment
3150–3000	Unsaturated fatty acid = C–H stretching
2950–2800	C–H stretching
1730–1700	Saturated fatty acid C=O stretching
1680–1620	Unsaturated fatty acid C=C stretching
1576–1540	Salts of fatty acids

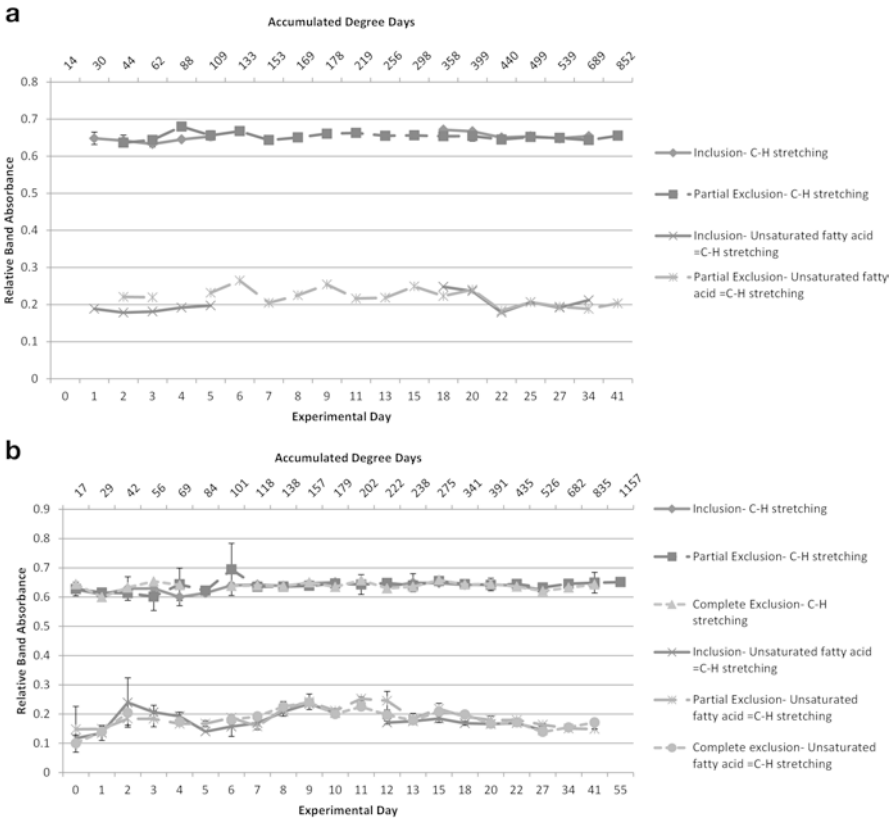


Fig. 18.7 ATR-IR spectroscopy results for early C–H stretching and unsaturated fatty acid =C–H stretching bands from: (**a**) Trial 1 (2011) and (**b**) Trial 2 (2012)

18.3.5 ATR-IR Spectroscopy

Several infrared stretching bands were consistently observed in all samples and are listed in Table 18.4. Stretching bands between 3150 and 3000 cm^{-1} were consistently low, while those between 2950 and 2800 cm^{-1} were consistently high (Fig. 18.7). Neither bands fluctuated noticeably over the course of Trial 1 or 2. The absorbance of the saturated fatty acid C=O stretching bands (1730–1700 cm^{-1}) was typically higher than the absorbance resulting from the unsaturated fatty acid C=C stretching bands (1680–1620 cm^{-1}) in both Trials 1 and 2 (Fig. 18.8).

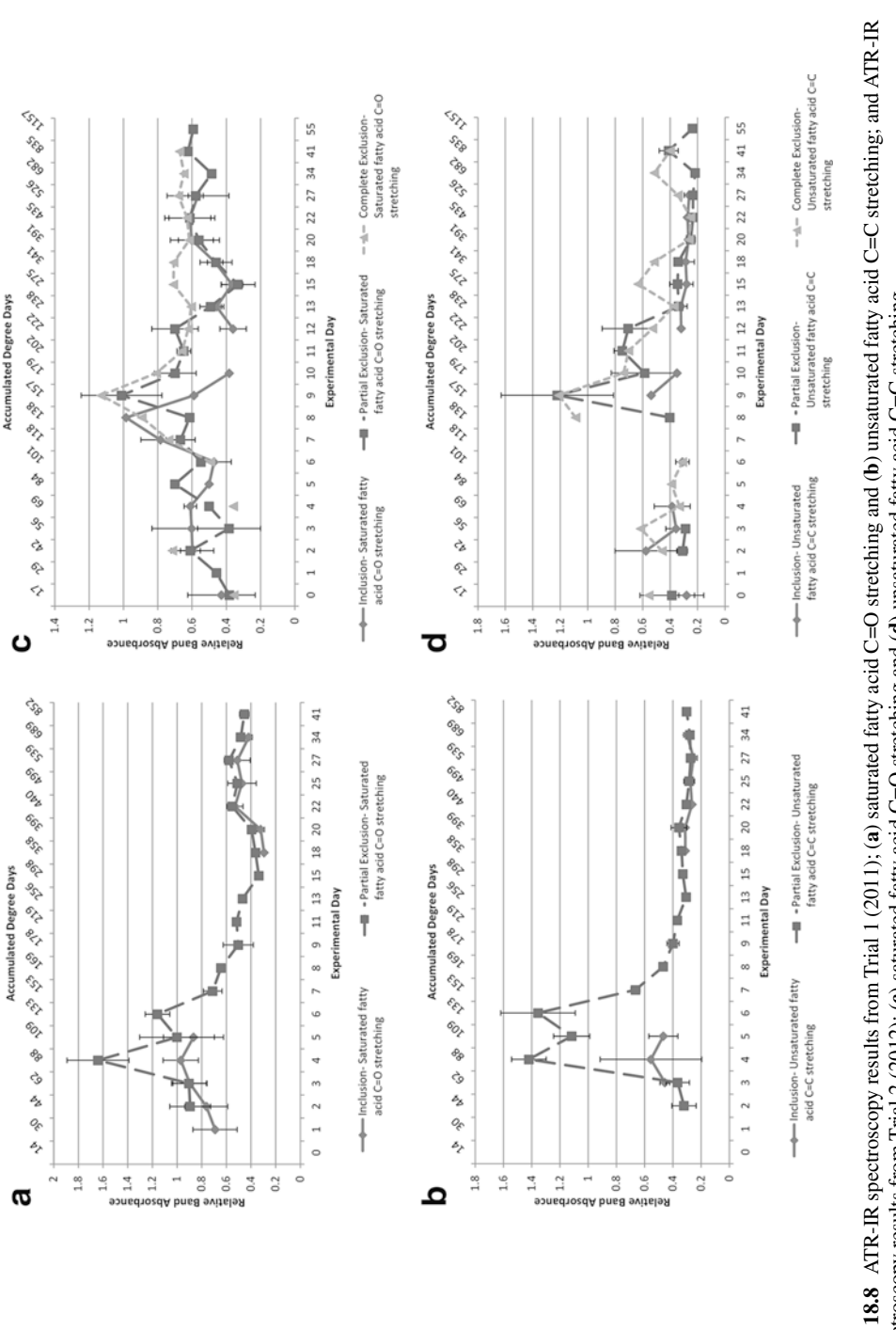


Fig. 18.8 ATR-IR spectroscopy results from Trial 1 (2011); (**a**) saturated fatty acid C=O stretching and (**b**) unsaturated fatty acid C=C stretching; and ATR-IR spectroscopy results from Trial 2 (2012): (**c**) saturated fatty acid C=O stretching and (**d**) unsaturated fatty acid C=C stretching

The saturated fatty acid stretching bands between 1730 and 1700 cm^{-1} and the unsaturated fatty acid stretching bands between 1680 and 1620 cm^{-1} displayed similar trends during Trial 1 (Fig. 18.8a and b). Sharp increases in levels in the partial exclusion group occurred after day 4 (88 ADD; mid bloat), which subsequently decreased and shared similar values with the inclusion group after day 18 (358 ADD; early advanced decay for the inclusion group and early dry decomposition for the partially excluded group) (Fig. 18.8a and b). The levels of saturated fatty acids decreased to values that were below the initial absorbance, while the levels of unsaturated fatty acids were similar to the initial levels following the increase during early decomposition (Fig. 18.8a and b).

In Trial 2, the absorbance values from all bands appeared to fluctuate more than in Trial 1. All carcass groups shared similar absorbance values at the beginning of the study for both the saturated C=O (1730–1700 cm^{-1}) and unsaturated C=C (1680–1620 cm^{-1}) fatty acid stretching bands (Fig. 18.8c and d). Sharp increases in the levels of saturated fatty acids were displayed by all groups around days 8–9 (138–157 ADD; beginning of active decay for the control group and end of bloat for the experimental groups) and in the exclusion groups for the unsaturated fatty acids (Fig. 18.8c and d). The sharp increase in unsaturated fatty acids among the exclusion groups was followed by a gradual decrease until the end of the collection period (Fig. 18.8d). The values for saturated fatty acids were higher at the end of the study, compared to initial values, while the unsaturated fatty acids were slightly lower (Fig. 18.8c and d).

The absorbance values for saturated and unsaturated fatty acids displayed by the inclusion groups were always lower than the other groups (Fig. 18.8). The completely excluded carcass typically exhibited the highest absorbance values for these free fatty acids (Fig. 18.8c and d).

The bands indicative of fatty acid salts (1576–1540 cm^{-1}) from the partial exclusion group showed a sharp increase early in the study during Trial 1, similar to the saturated and unsaturated fatty acid stretching bands, but fluctuated over time (Fig. 18.9a). The absorbance levels from the inclusion group were initially lower than the partial exclusion group, but reached similar values at the end of the study (Fig. 18.9a). During Trial 2, similar values were observed among all carcass groups following day 6 (101 ADD; end of bloat for the control group and mid bloat for the experimental groups) (Fig. 18.9b). Prior to that time, the complete exclusion carcass exhibited sharp increases (Fig. 18.9b).

18.4 Discussion

Fluids collected from all carcasses during both trials initially had pH values close to 7. The fluid collected was predominantly blood which, in a living individual, should have a pH between 7.35 and 7.45 (Waugh and Grant 2010). When the pH of blood is outside this narrow range, it indicates a disruption to the normal physiological and biochemical processes (Waugh and Grant 2010). Death leads to the cessation of regulatory mechanisms within the body (Janaway et al. 2009) and as a result, the pH of cadaver blood will be slightly outside the normal range.

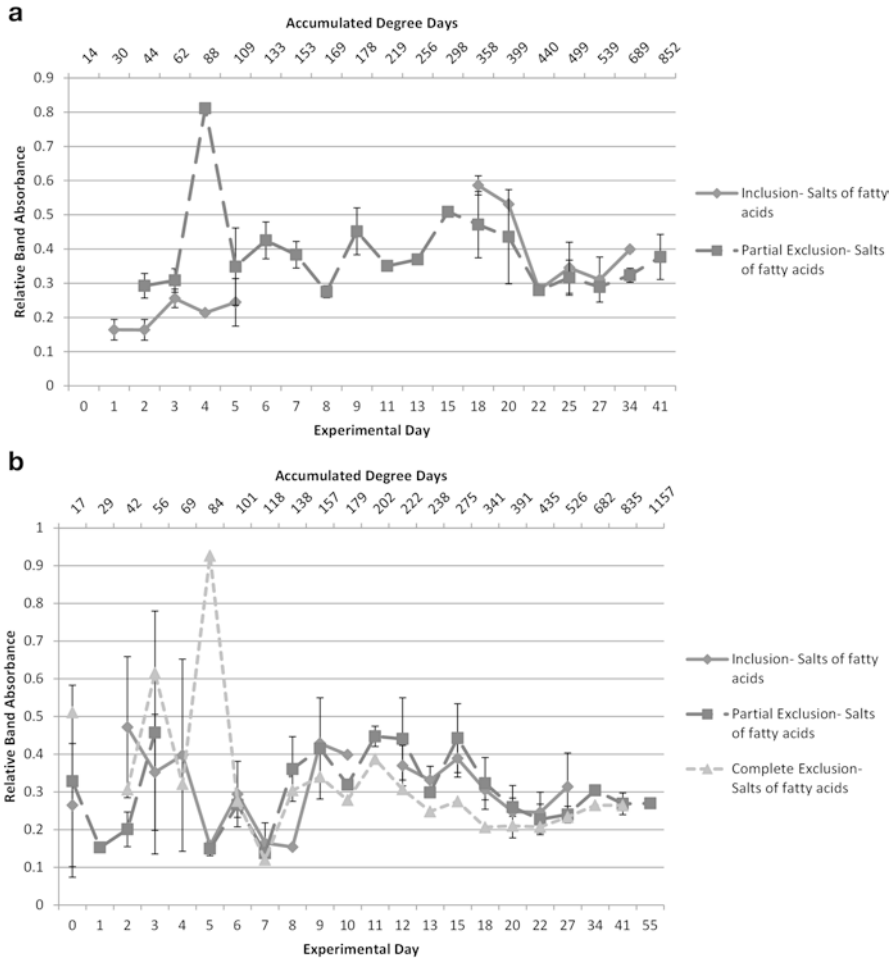


Fig. 18.9 ATR-IR spectroscopy results for salts of fatty acids from **a** Trial 1 (2011) and **b** Trial 2 (2012)

The pH values of collected fluid displayed similar trends and values between groups, based on the available data for the inclusion group in Trial 1 and during early decomposition in Trial 2. The pH values began to increase in all carcass groups near the end of the bloat stage. This may have been caused by the onset of proteolysis, which has been observed to lead to an increase in intracellular pH levels (Gill-King 1997). However, once the active decay and localized tissue removal stages began, the pH values diverged between groups. This was especially evident following experimental day 9 (157 ADD) in Trial 2. The fluid from the inclusion group exhibited higher pH values than the partial and complete exclusion groups and displayed a sharp increase in pH. Studies have found that high ammonium (NH_4^+) levels contribute to high pH values (Hopkins et al. 2000). Maggots release high levels of ammonia in their exudates as they feed (Turner 2005), which can be converted to ammonium and subsequently increase pH. The partially excluded group displayed

an increase in pH following day 10 (179 ADD), which correlated with the beginning of the localized tissue removal stage, when maggots were present to contribute ammonium. Fewer maggots were present on these carcasses which may explain why the pH increased at a later ADD relative to the inclusion group. The completely excluded carcass displayed a decrease in pH, which may be due to the lack of maggots and ammonium ions present in that environment.

During late decomposition, the pH values for the inclusion and partially excluded groups began to decrease, while the values for the completely excluded carcass increased to reach similar values as the partially excluded group. The inclusion values were always lower than those from the partially excluded groups during this time. It is possible that the nature of the remains contributed to the differences observed between the inclusion and the exclusion (partial and complete) groups. During late decomposition, only bones and skin remained among the inclusion carcasses, while large amounts of soft tissue were still present among the partially and completely excluded groups, due to the limited or lack of feeding by maggots. Microbial-driven decomposition was still taking place among the partial and complete exclusion carcasses during the later stages of decomposition (as evidenced by changes in soft tissue and fluid) and these changes may have maintained higher levels of pH among these groups. During the later decomposition stages, it was unclear whether the inclusion carcasses were indeed purging fluids or precipitation was causing fluids to accumulate in the containers. The latter may explain the lower pH values observed, since rainwater is considered to be relatively acidic and has been found to have a pH between 4 and 7 (Ferguson and Jeffries 2012). A study conducted in north-eastern United States found that the average annual precipitation had pH values ranging from 4.05 to 4.3 (Likens et al. 1996). Therefore, if the collected fluid was predominantly precipitation from the inclusion carcasses, it follows that the pH values would be lower than those from the partial and complete exclusion groups.

The conductivity values and trends exhibited by the carcass groups within each trial were very similar during early decomposition. All groups displayed a general increase in conductivity during the fresh and bloat stages. Vass et al. (1992) showed that several ions (mainly NH_4^+, K^+, Cl^-, and SO_4^{2-}) increase sharply during early decomposition and continue to increase over time. These ions would have been released by all carcasses and likely influenced the increase in conductivity observed. All carcass groups also displayed noticeable decreases on the same days within each trial. In Trial 1, this occurred on experimental day 3 (62 ADD) and in Trial 2, on days 2 (42 ADD), 3 (56 ADD), 6 (101 ADD), 9 (157 ADD), and 12 (222 ADD). These represent days that either received, or followed days that received, large amounts of precipitation. Water dilutes fluids and will dilute the amount of total dissolved ions present in a given fluid, thus decreasing its conductivity. A significant decrease in conductivity was observed in a study that measured the conductivity in soil solution collected from a burial site and it was attributed to the increased rainfall received during that time (Pringle et al. 2010).

Differences in conductivity were observed between groups following the active decay and localized tissue removal stages in Trial 2. The conductivity of the inclu-

sion carcasses decreased after this point and remained low, while the conductivity of the partial and complete exclusion groups remained high and continued to increase. The partially excluded group decreased a few days after and remained low, while the completely excluded carcass remained higher in value than the other two groups and gradually declined until the end of the study. This difference between groups must have been caused by the presence of maggots. Electrolytes rapidly leach out of soft tissue promoting an increase in conductivity, however bacteria have been shown to use many ions for metabolism (Vass et al. 1992) which would lead to a subsequent decrease in conductivity. It is possible that the maggots contributed bacteria or allowed the already present bacteria to access the released ions (mainly HPO_4^{2-}, HCO_3^-, and NO_3), thus leading to the decrease in conductivity in the inclusion and partially excluded carcasses. The delayed decrease observed in the complete exclusion carcass may have been due to fewer ionic compounds being released from the decaying carcass as time progressed, which can also explain why the values for the other groups remained low even after maggots had migrated away from the carcasses.

Strong –C–H stretching bands in the region 2950–2800 cm^{-1} and a small shoulder in the region 3150–3000 cm^{-1}, attributable to=C–H stretching of unsaturated fatty acids, were consistently observed and the absorbance did not fluctuate over time. These bands are commonly observed in infrared spectra resulting from fatty acid analysis of pork tissue or soil collected from beneath decomposing pigs (Flatten et al. 2005; Forbes et al. 2005a, b, 2011).

The fatty acid absorbance levels exhibited by the inclusion group in both trials were typically lower than the other groups, however they appeared to reach similar levels during the later stages of decomposition. Further, a divergence in trends between groups was observed for the saturated fatty acid C=O stretching band after day 8 (138 ADD) in Trial 2, which was the onset of the active decay stage. The inclusion group decreased sharply and remained low. The feeding of maggots removed soft tissue and liquefied tissues that contained fatty acids, thus decreasing the levels released into fluid. This resulted in lower levels being detected in the purged fluids. Similarly, a decrease in absorbance was also exhibited by the partial exclusion group at the beginning of the localized tissue removal stage. However, the completely excluded carcass decreased at the same time (for both the saturated fatty acid C=O stretching and the unsaturated fatty acid C=C stretching). This indicates that maggots are not the only factor involved in the decrease of saturated fatty acid levels. It is likely that fatty acids are also chemically degraded, however insects may accelerate the process and lead to increased lipid degradation.

The absorbance levels of all groups appear to reach similar values near the end of the collection period, despite different degradation pathways during earlier stages. This was especially true in the saturated fatty acid C=O stretching band in Trial 2, whereby the inclusion and partial exclusion group levels increased to reach the same values as the completely excluded carcass. A slight increase was also observed at the end of the collection period in Trial 1. The unsaturated fatty acid C=C stretching band displayed a decreasing trend in all carcass groups near the end of the study. This results not only from the natural degradation processes but also as

a result of hydrogenation, which converts unsaturated fatty acids into their saturated counterparts (Dent et al. 2004; Janaway et al. 2009; Notter et al. 2009). This process may explain the slight increase in absorbance of the saturated fatty acid C=O stretching bands and the low levels of the unsaturated fatty acid C=C stretching bands during the later stages of decomposition.

A study conducted by Swann et al. (2010b) investigated long chain fatty acids in decomposition fluid collected from adult pig carcasses. They observed that the levels of fatty acids displayed an increasing trend over time and reached a maximum after 14 days (310 ADD) (Swann et al. 2010b). Unfortunately, the study was terminated at this time, so further trends could not be observed. The increase in fatty acid content (exhibited by the saturated fatty acid C=O and unsaturated fatty acid C=C stretching bands) peaked after 4 days (88 ADD) in Trial 1 and after 8–9 days (138–157 ADD) in Trial 2, which is much earlier than observed by Swann et al. (2010b). The authors explained a cycle in fatty acid content observed in fluid collected from pork rashers as being caused by the level of fly activity, the feeding cycle of maggots, and the adipose tissue content of the carcass (Swann et al. 2010b). It was hypothesised that as maggots fed on tissues, the production of long chain fatty acids steadily increased, and once they migrated from the carcasses to pupate the levels decreased (Swann et al. 2010b). This is contrary to what was observed in the current study, whereby in Trial 2, the peak in fatty acid content correlated with the onset of the maggot feeding stages and decreased throughout this stage and subsequent stages. Similar trends were also observed by the completely excluded carcass, which was not exposed to any insect activity.

The bands indicative of salts of fatty acids exhibited absorbance levels that were similar between all groups. However, the completely excluded carcass displayed higher levels during the early stages of decomposition. The fresh and bloat stages took place during this time, which are stages exhibited by all carcasses. Similar chemical changes would therefore have taken place within each carcass. Further, maggots were not present during this stage and the differences between groups cannot be attributed to insect activity. It is unclear as to why the completely excluded carcass displayed higher fatty acid salt levels compared to the other groups, especially since all carcass groups displayed similar levels following day 9 (157 ADD) in Trial 2, which was when the absorbance levels typically diverged between groups. It can be concluded that the fatty acid salt levels are not influenced by insect activity.

18.5 Conclusions

Few studies have examined the decomposition process in both the presence and absence of insects, and to the best of our knowledge, only one study has performed fatty acid analysis using decomposition fluid. The current study combined these two knowledge gaps in an attempt to determine the effect of insect activity on the chemical properties of decomposition fluid. It was observed that the pH and conductivity of fluid appear to be influenced by maggot activity. The feeding of maggots caused

increases in pH among the inclusion and partial exclusion groups, although the partial exclusion group displayed a more gradual increase. The presence of maggots likely led to higher levels of ammonium, thus increasing the alkalinity of the fluid. In contrast, the presence of maggots led to decreases in conductivity. It is hypothesized that bacteria from maggots utilised the electrolytes that were released by the degradation of macromolecules, thereby decreasing the levels of dissolved ionic compounds. There were differences observed in the absorbance levels of fatty acids detected using ATR-IR, however the overall levels were not sufficiently different between carcass groups to accurately determine whether the presence of insects played an important role in the fatty acid degradation process. Qualitative and quantitative methods are currently being performed using gas chromatography-mass spectrometry (GC-MS), in an attempt to better identify specific fatty acid trends in decomposition fluid.

References

Amendt J, Campobasso CP, Gaudry E, Reiter C, LeBlanc HN, Hall MJR (2007) Best practice in forensic entomology–standards and guidelines. Int J Legal Med 121:90–104

Anderson G, VanLaerhoven S (1996) Initial studies on insect succession on carrion in southwestern British Columbia. J Forensic Sci 41:617–625

Bernhardt T (n.d.) Canada's ecozones. http://canadianbiodiversity.mcgill.ca/english/ecozones/index.htm. Accessed 20 Dec 2012

Dent BB, Forbes SL, Stuart BH (2004) Review of human decomposition processes in soil. Environ Geol 45:576–585

Evans WED (1963) The chemistry of death. Thomas, Springfield

Fast PG (1966) A comparative study of the phospholipids and fatty acids of some insects. Lipids 1:209–215

Ferguson HL, Jeffries DS (2012) Acid rain. http://www.thecanadianencyclopedia.com/articles/acid-rain. Accessed 9 May 2013

Fiedler S, Graw M (2003) Decomposition of buried corpses, with special reference to the formation of adipocere. Naturwissenschaften 90:291–300

Flatten A, Bryhni E, Kohler A, Egelandsdal B, Isaksson T (2005) Determination of C22:5 and C22:6 marine fatty acids in pork fat with Fourier transform mid-infrared spectroscopy. Meat Sci 69:433–440

Forbes SL, Dent BB, Stuart BH (2005a) The effect of soil type on adipocere formation. Forensic Sci Int 154:35–43

Forbes SL, Stuart BH, Dadour IR, Dent BB (2004) A preliminary investigation of the stages of adipocere formation. J Forensic Sci 49:566–574

Forbes SL, Stuart BH, Dent BB (2005b) The effect of the burial environment on adipocere formation. Forensic Sci Int 154:24–34

Forbes SL, Wilson MEA, Stuart BH (2011) Examination of adipocere formation in a cold water environment. Int J Leg Med 125:643–650

Gill-King H (1997) Chemical and ultrastructural aspects of decomposition. In: Haglund WD, Sorg MH (eds) Forensic taphonomy: the postmortem fate of human remains. CRC Press, Florida, pp 93–105

Goff ML (2009) Early post-mortem changes and stages of decomposition in exposed cadavers. Exp Appl Acarol 49:21–36

Hopkins DW, Wiltshire PEJ, Turner BD (2000) Microbial characteristics of soils from graves: an investigation at the interface of soil microbiology and forensic science. Appl Soil Ecol 14:283–288

Janaway RC, Percival SL, Wilson AS (2009) Decomposition of human remains. In: Percival SL (ed) Microbiology and aging. Humana Press, Totowa, pp 313–334
Kramer JM, Hulan HW (1978) A comparison of procedures to determine free fatty acids in rat heart. J Lipid Res 19:103–106
Likens G, Driscoll C, Buso D (1996) Long-term effects of acid rain: response and recovery of a forest ecosystem. Science 272:244–246
Megyesi MS, Nawrocki SP, Haskell NH (2005) Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. J Forensic Sci 50:618–626
Michaud J-P, Moreau G (2011) A statistical approach based on accumulated degree-days to predict decomposition-related processes in forensic studies. J Forensic Sci 56:229–232
Myburgh J, L'Abbé EN, Steyn M, Becker PJ (2013) Estimating the postmortem interval (PMI) using accumulated degree-days (ADD) in a temperate region of South Africa. Forensic Sci Int 229:165.e1–165.e6
Notter SJ, Stuart BH, Rowe R, Langlois N (2009) The initial changes of fat deposits during the decomposition of human and pig remains. J Forensic Sci 54:195–201
O'Brien RC, Forbes SL, Meyer J, Dadour IR (2007) A preliminary investigation into the scavenging activity on pig carcasses in Western Australia. Forensic Sci Med Pathol 3:194–199
Payne JA (1965) A summer carrion study of the Baby Pig *Sus scrofa* Linnaeus. Ecology 46:592–602
Pringle JK, Cassella JP, Jervis JR (2010) Preliminary soilwater conductivity analysis to date clandestine burials of homicide victims. Forensic Sci Int 198:126–133
Reynold A, Cahill G (1965) Handbook of physiology: adipose tissue. American Physiological Society, Washington, DC
Schoenly KG, Haskell NH, Mills DK, Bieme-Ndi C, Larsen K, Lee Y (2006) Recreating death's acre in the school yard: using pig carcasses as model corpses to teach concepts of forensic entomology & ecological succession. Am Biol Teac 68:402–410
Simmons T, Adlam RE, Moffatt C (2010a) Debugging decomposition data–comparative taphonomic studies and the influence of insects and carcass size on decomposition rate. J Forensic Sci 55:8–13
Simmons T, Cross PA, Adlam RE, Moffatt C (2010b) The influence of insects on decomposition rate in buried and surface remains. J Forensic Sci 55:889–892
Stanley-Samuelson DW, Dadd RH (1983) Long-chain polyunsaturated fatty acids: patterns of occurrence in insects. Insect Biochem 13:549–558
Stanley-Samuelson DW, Jurenka RA, Cripps C, Blomquist GJ, De Renobales M (1988) Fatty acids in insects: composition, metabolism, and biological significance. Arch Insect Biochem Physiol 9:1–33
Stuart BH, Craft L, Forbes SL, Dent BB (2005) Studies of adipocere using attenuated total reflectance infrared spectroscopy. Forensic Sci Med Pathol 1(3):197–201
Swann L, Chidlow GE, Forbes S, Lewis SW (2010a) Preliminary studies into the characterization of chemical markers of decomposition for geoforensics. J Forensic Sci 55:308–314
Swann L, Forbes S, Lewis SW (2010b) Observations of the temporal variation in chemical content of decomposition fluid: a preliminary study using pigs as a model system. Aust J Forensic Sci 42:199–210
Swann LM, Forbes SL, Lewis SW (2010c) Analytical separations of mammalian decomposition products for forensic science: a review. Anal Chim Acta 682:9–22
Swann LM, Forbes SL, Lewis SW (2010d) A capillary electrophoresis method for the determination of selected biogenic amines and amino acids in mammalian decomposition fluid. Talanta 81:1697–1702
Turner B (2005) Blowfly maggots: the good, the bad and the ugly. Clin Pathol 14(2):81–85
Vass AA, Bass WM, Wolt JD, Foss JE, Ammons JT (1992) Time since death determinations of human cadavers using soil solution. J Forensic Sci 37:1236–1253
Waugh A, Grant A (2010) Introduction to the chemistry of life. In: Premdas N, Hewat C (eds) Ross and Wilson anatomy and physiology in health and Illness, 11th edn. Churchill Livingstone, Toronto, 21

Chapter 19
Forensic Analysis of Volatile Organic Compounds from Decomposed Remains in a Soil Environment

Sonja Stadler, Jean-François Focant, and Shari L. Forbes

Abstract The detection of clandestine graves or concealed remains can pose a challenge to investigators. Research into the chemical signatures of decomposition, including volatile organic compounds (VOCs), can aid in the development of improved methods for the detection of remains and can further the understanding of decomposition processes. Over the last decade a number of studies have investigated decomposition VOCs from a variety of soil environments. However due to the variety of environments and methods used during these investigations a consistent odour signature remains elusive. This paper will discuss the complexity of decomposition odour and the current knowledge base of decomposition VOCs within soil environments including the impact of the entire death assemblage on the production of VOCs. The use of advanced instrumentation such as comprehensive two dimensional gas chromatography – time-of-flight mass spectrometry for the characterisation of decomposition odour is proposed. Incorporating advanced instrumentation and data handling tools into the analysis of decomposition odour will facilitate the comparison of odour profiles and generation of a consistent decomposition odour signature.

S. Stadler
Faculty of Science, University of Ontario Institute of Technology,
2000 Simcoe St N., Oshawa, ON L1H 7K4, Canada

J.-F. Focant
Organic and Biological Analytical Chemistry Group – CART, Chemistry Department,
University of Liège, Allée du six aout, Quartier Agora 11,
B6c Sart-Tilman, B-4000 Liège, Belgium

S.L. Forbes (✉)
University of Ontario Institute of Technology,
2000 Simcoe St. N., Oshawa, ON L1H 7K4, Canada

University of Technology Sydney, Broadway, 123, Ultimo,
NSW 2007, Australia
e-mail: Shari.Forbes@uts.edu.au

H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics*,
Soil Forensics, DOI 10.1007/978-3-319-33115-7_19

19.1 Introduction

Forensic taphonomy is a multidisciplinary field within forensic science that investigates the chemical processes that occur after death and their impact on the surrounding environment. The changes to the visible appearance, chemical properties and biodiversity of the soil and surrounding environment are the result of soft tissue decomposition (France et al. 1997; Carter et al. 2007; Carter and Tibbett 2008). Investigators can utilize the changes that occur at a deposition site in order to aid in the detection and recovery of remains. During a forensic investigation or in the event of a mass disaster a variety of conditions may be encountered, therefore the method for detection of victims must be able to efficiently and accurately locate human remains (Statheropoulos et al. 2006, 2007, 2011). One potential method is the use of the chemical signature, particularly the volatile organic compounds (VOCs) produced by decomposition (Statheropoulos et al. 2011). Decomposition odour has only been studied over the last decade and the variety of studies conducted has identified a range of odour profiles from different environments. Therefore this work will explore the current state of knowledge in this area and highlight the application of advanced instrumentation to this growing field.

19.2 Decomposition VOCs

Over the last decade there has been an increase in research aimed at identifying the chemical components of decomposition odour. This work aims to understand the volatile compounds that elicit a response in cadaver dogs and attracts carrion insects to remains. Additionally this research can be applied to the development of portable instrumentation for the detection of remains as well as provide insight into the chemical processes of decomposition.

During decomposition, the putrefactive breakdown of macromolecules produces a variety of decomposition gases including methane, carbon dioxide and hydrogen sulfide as well as volatile organic compounds (VOCs) (Gill-King 1997; Dent et al. 2004; Statheropoulos et al. 2005; Boumba et al. 2008). VOCs can be generally defined as any compound with an appreciable vapour pressure and can have a variety of chemical properties (ASTM International 2004). The degradation of soft tissue produces hundreds of decomposition VOCs from numerous chemical classes including alcohols, aldehydes, alkanes/alkenes, aromatics, carboxylic acids, esters, ethers, halogens, ketones, nitrogen and sulfur compounds (Vass et al. 2004; Statheropoulos et al. 2005, 2007; Boumba et al. 2008; Vass et al. 2008; Dekeirsschieter et al. 2009; DeGreeff and Furton 2011; Paczkowski and Schutz 2011; Statheropoulos et al. 2011; Brasseur et al. 2012; Cablk et al. 2012; Dekeirsschieter et al. 2012; Stadler et al. 2013). Although similar chemical classes have been reported in a variety of studies the complexity of these samples has precluded the complete

characterisation of decomposition odour (Statheropoulos et al. 2011; Dekeirsschieter et al. 2012).

Decomposition is a highly complex process and characterising decomposition odour is a challenge. Many studies have determined that decomposition odour is dynamic and changes its composition over time (Vass et al. 2004; Dekeirsschieter et al. 2009; Statheropoulos et al. 2011; Cablk et al. 2012; Dekeirsschieter et al. 2012; Stadler et al. 2013). All stages of decomposition produce VOCs, however outside the use of human analogues; few studies have monitored decomposition headspace throughout soft tissue decomposition. The rapid loss of soft tissue and perceived odour associated with remains during the active and advanced decay stages correlates with the number and variety of compounds identified during these stages (Anderson and VanLaerhoven 1996; Dekeirsschieter et al. 2009, 2012; Stadler et al. 2013). The overall process of decomposition in a soil environment can be characterised by a combination of chemical classes. The early stages are characterised by higher levels of polysulfides and alcohols, the transition to the later stages produces increased levels of aromatics and carboxylic acids which persist through the later stages of decay along with aldehydes and ketones (Dekeirsschieter et al. 2009, 2012; Stadler et al. 2013). Fresh and dry / skeletonized remains produce fewer VOCs, however cadaver dogs are known to locate remains shortly after death and after prolonged post-mortem intervals (Komar 1999; Rebmann et al. 2000; Lasseter et al. 2003; Oesterhelweg et al. 2008). In order to locate human remains, these canines rely on odorous compounds available for olfaction, therefore sufficient VOCs must be produced during these stages and additional research is required, including trace analysis, in order to elucidate the chemical composition of decomposition odour during these stages.

The chemical pathways and exact origins of these compounds are uncertain however it is clear that the decomposition environment and the bacteria present during decomposition will affect their production (Boumba et al. 2008; Dekeirsschieter et al. 2009; Paczkowski and Schutz 2011; Statheropoulos et al. 2011). Currently there is a large amount of variability in the compounds reported (DeGreeff and Furton 2011; Paczkowski and Schutz 2011). Studies have identified tens to hundreds of decomposition VOCs, however very few compounds have been reported in all studies, with the polysulfides being the most commonly cited (Stadler et al. 2013). The high degree of variability in the reported compounds is in part due to the range of decomposition environments studied. Decomposition VOCs have been analysed from buried remains and grave soil (Vass et al. 2004, 2008; Brasseur et al. 2012; Vass 2012), deposition on the soil surface (Dekeirsschieter et al. 2009, 2012; Stadler et al. 2013), case studies from aqueous environments (Statheropoulos et al. 2005, 2007;), enclosed spaces (Statheropoulos et al. 2011) and individual tissue samples (Hoffman et al. 2009; Cablk et al. 2012;). Each of these environments has a unique set of environmental conditions that will affect the production and availability of VOCs. The complete odour profile is complex and dynamic requiring the interaction of all aspects of the decomposition assemblage including the entomological fauna and micro-organisms.

19.3 Impact of the Environment on Decomposition VOCs

Abiotic factors such as temperature, humidity, availability of oxygen and pH affect the process of decomposition (Mann et al. 1990; Clark et al. 1997; Gill-King 1997; Dent et al. 2004) and therefore the production of VOCs. However, the interaction between VOCs and the environment also dictates which compounds are available within the headspace of decomposition. The majority of decomposition VOC studies have been conducted within soil environments (Vass et al. 2004, 2008; Dekeirsschieter et al. 2009; Brasseur et al. 2012; Dekeirsschieter et al. 2012; Stadler et al. 2013) however the impact of the soil on the overall profile has received little attention. In a burial situation, the compounds produced by the remains must travel through the soil column in order to be available at the surface for olfaction/detection (Vass et al. 2004; Brasseur et al. 2012). The migration of compounds is affected by the properties of the soil such as soil type, moisture content and pH but also by the properties of the compound itself (Vass et al. 2004; Statheropoulos et al. 2007; Brasseur et al. 2012; Vass 2012). The polarity of a compound indicates the likelihood the compound will migrate through the soil versus becoming bound to the soil particles, with less polar compounds such as hydrocarbons being detected near or at the surface (Brasseur et al. 2012). Decomposition soil is an integral part of the death assemblage and taphonomic studies have demonstrated the exchange of material between remains and the environment by identifying a variety of decomposition by-products within soil (Vass et al. 1992, 2002; Dent et al. 2004; Carter et al. 2007, 2008; Benninger et al. 2008; Van Belle et al. 2009; Swann et al. 2010a,b,c). Several of these compounds have also been identified within the headspace of decomposition (Dekeirsschieter et al. 2012; Stadler et al. 2013) and indicate that soil may act as a scent reservoir for decomposition odour (Brasseur et al. 2012). A challenge in the analysis of decomposition odour from soil environments is the odour signature from the soil itself. Soil VOCs have not been studied directly however, through the use of control samples and reference soils, it is clear that soil produces its own volatile signature consisting of equal complexity and diversity (Brasseur et al. 2012; Dekeirsschieter et al. 2012). This volatile signature will vary between environments and seasons and needs to be taken into consideration when interpreting decomposition VOC profiles.

In addition to the abiotic conditions of the decomposition environment, the entire ecosystem must be considered. The deposition of remains represents a large input of nutrients that will consequently alter the composition of the soil, flora and fauna of the area (Carter et al. 2007; Benninger et al. 2008; Janaway et al. 2009). Little is known about the microflora involved with decomposition, however it is known to be a complex combination of intrinsic bacteria from the respiratory and digestive systems and extrinsic bacteria from soil and the surrounding environment (Carter et al. 2007; Janaway et al. 2009). This diverse ecosystem encompasses both aerobic and anaerobic bacteria as well as fungi (Janaway et al. 2009), whose metabolic pathways degrade proteins, lipids and carbohydrates to produce numerous breakdown products, including VOCs (Boumba et al. 2008; Dekeirsschieter et al. 2009;

Paczkowski and Schutz 2011). This metabolism may be the result of several bacteria or may be products of a sequential food chain with one organism working on the products of the next (Boumba et al. 2008; Paczkowski and Schutz 2011). Microbial modification of VOCs may also occur within soil as compounds migrate from buried remains (Vass et al. 2004). By altering the decomposition environment including the micro-organisms present, a different odour profile is created (Dekeirsschieter et al. 2009). The odour profile may also be affected by the entomological fauna that colonize the remains as it has been demonstrated that many decomposition VOCs are present within the headspace of isolated blowfly larvae and pupae (Frederickx et al. 2012). An investigation into the decomposition VOCs from three different environments was able to demonstrate that although the overall odour profile varies between environments there was a common core of VOCs (Dekeirsschieter et al. 2009).

The complexity of decomposition odour is further elucidated when the headspace of individual tissues are examined. Hoffman et al. (2009) examined a selection of tissue samples commonly utilized as canine training aids. None of the 14 samples analysed produced the same odour profile, and no single compound was found in common between all samples (Hoffman et al. 2009). Removal of the environmental influences and separation of tissues from the decomposition processes that occur within whole remains produces a sub-set of the profile of decomposition odour. This can be problematic when trying to conduct comparisons between individuals or to human analogues. Compounds that have been reported as being absent from the headspace of human analogue tissue such as pig carcasses and therefore unique to human remains have in fact been identified when the headspace from the entire death assemblage is analysed (Stadler et al. 2013). Development of a method for remains detection needs to consider the entire death assemblage (i.e. the remains, intrinsic and extrinsic micro flora, entomological fauna, plants and soil) in order to be an accurate representation of remains deposition encountered by investigators.

19.4 Signature of Decomposition Odour

Recently, decomposition odour was presented as evidence for the prosecution in the State of Florida v. Casey Marie Anthony (case no. 48-2008-CF-15606-O). Decomposition VOCs were utilized to identify a decomposition event of human origin, however the odour signature of human decomposition is not generally accepted in the scientific community and currently the analysis of decomposition VOCs has not been validated as a method for identifying the presence of human remains (Perry 2011).

Characterising the signature of human decomposition odour has been confounded by the variability in the compounds reported in the literature as well as some ambiguity surrounding the precise goal of these investigations. It has been proposed that there are two complimentary but opposing goals in the analysis of decomposition VOCs (Cablk et al. 2012). First is the goal of developing portable

instrumentation for the detection of human remains. This instrumentation would detect compounds unique to humans, these compounds need not be products of decomposition but could include anthropogenic compounds that as humans we are uniquely exposed to (Cablk et al. 2012). In contrast is the characterisation of decomposition odour in order to determine the compounds required for odour recognition by cadaver dogs (Cablk et al. 2012).

Cadaver dogs are currently the best detectors for human remains. They are able to differentiate human from animal remains and locate human remains in a variety of conditions including submerged, buried and skeletal (Komar 1999; Lasseter et al. 2003; Lorenzo et al. 2003; Oesterhelweg et al. 2008). Their ability to differentiate these complex odours may be due to their ability to chromatographically separate odorants (Lawson et al. 2012), however the mechanism of odour recognition and the key compounds identified remains unclear. Research on the odour signatures of static odorants such as explosives, have indicated that canines may be responding to the most abundant compounds in an odour profile (Johnston 1999; Lorenzo et al. 2003). Recognition of complicated and dynamic target odours such as decomposition may require a variety of compounds in addition to the dominant ones as many of the compounds identified within the headspace of decomposition are not unique to decomposition and can be found from a variety of sources (Vass et al. 2008). In order to gain a better understanding of decomposition odour and evaluate the uniqueness of the entire profile, an in depth analysis of these compounds from a variety of death assemblages is required.

19.5 Analysis of Decomposition VOCs

Over the last decade this area of study has gained more attention and numerous methods have been explored. A variety of collection methods are available including solid-phase microextraction (SPME) (Hoffman et al. 2009; Kalinova et al. 2009; DeGreeff and Furton 2011; Cablk et al. 2012), thermal desorption (TD) (Vass et al. 2004; Statheropoulos et al. 2005, 2007; Vass et al. 2008; Stadler et al. 2013) and solvent desorption (Brasseur et al. 2012; Dekeirsschieter et al. 2009, 2012). Although all of these sampling methods utilize a solid sorbent to trap the VOCs they each have particular advantages and disadvantages.

SPME is a passive sampling device and is comprised of a small fused silica fibre with a sorbent coating which acts as an integrated system for both sample extraction and sample introduction. It is a fast and simple sample collection method however competition between analytes for adsorptive sites within the fibre can limit the accuracy and precision of the results (Augusto et al. 2001). SPME is generally utilized to sample smaller volumes of headspace and requires that an equilibrium status be reached between the sample matrix, the headspace and the fiber (Agelopoulos and Pickett 1998; Augusto et al. 2001). This equilibrium condition is not always feasible in a field setting especially when sampling whole remains.

The field portability and variety of sorbents available for sorbent tubes makes them a popular alternative. The choice of sorbent is dictated by the compounds of interest, however the stainless steel or glass tubes can be packed with multiple sorbents in order to increase the volatility range of compounds that can be sampled (McClenny 1999; Statheropoulos et al. 2011). This trait is particularly desirable for the analysis of decomposition VOCs and multi-sorbent tubes have been utilized in a number of studies (Vass et al. 2004; Statheropoulos et al. 2005, 2007; Vass et al. 2008; Statheropoulos et al. 2011; Stadler et al. 2013). Desorption of these sample tubes can be done either thermally or through the use of a solvent. Solvent desorption allows retention of a liquid sample that can be stored and analysed at a later date. However, the large solvent peak that is generated may mask many of the early eluting compounds (Agelopoulos and Pickett 1998; Brasseur et al. 2012; Dekeirsschieter et al. 2012). Conversely thermal desorption (TD) is a solvent free system that offers many advantages including increased sensitivity and applicability to complex samples with a wide range of compound volatility and polarity (Agelopoulos and Pickett 1998; Ribes et al. 2007). TD analysis generally utilizes the entire sample however many TD systems can overcome the 'one-shot' nature of this analysis by running the instrument in split mode and collecting the split flow prior to sample injection. TD instrumentation can also provide improved peak shape and compound resolution via a focusing step in the desorption sequence (McClenny 1999; Sanchez and Sacks 2006). TD is preferable for sampling large volumes of headspace, e.g. collecting VOCs above decomposed remains; whereas SPME is preferred for sampling small volumes of headspace e.g. collecting VOCs from blood or decomposition fluid used as cadaver dog training aids.

Despite the range of sampling methods available the analysis of VOCs has been primarily carried out using conventional gas chromatography – mass spectrometry (GC-MS). Chromatographic efficiency is influenced by numerous parameters, including temperature programming, flow rate and primarily, column selection. Published studies on the analysis of decomposition VOCs have utilized general purpose capillary columns suitable for the analysis of a variety of compound classes and volatile organics. These include non-polar 100 % dimethyl polysiloxane (Vass et al. 2004, 2008), low polarity 5 % phenyl/95 % dimethyl polysiloxane (Hoffman et al. 2009; Cablk et al. 2012) and slightly polar cyanopropylphenyl/ dimethyl polysiloxane (Statheropoulos et al. 2005, 2007; Dekeirsschieter et al. 2009; Statheropoulos et al. 2011). These studies were able to identify tens to hundreds of compounds using retention times and mass spectral data. However, chromatograms published from these studies illustrate the difficulty in achieving compound resolution (Statheropoulos et al. 2005; Dekeirsschieter et al. 2009;). The complexity of these samples in terms of the large number of components, the combination of compound classes and the significant dynamic range across the decomposition process presents an analytical challenge.

In 2011, Statheropoulos et al. explored more advanced instrumentation for the analysis of decomposition VOCs and utilized thermal desorption – gas chromatography – time-of-flight mass spectrometry (TD-GC-TOFMS) (Statheropoulos et al. 2011). In contrast to other studies that utilized mass spectrometers such as

quadrupoles or ion traps (Vass et al. 2004; Statheropoulos et al. 2005, 2007; Vass et al. 2008; Dekeirsschieter et al. 2009; Hoffman et al. 2009; Cablk et al. 2012), TOFMS is a non-scanning instrument and collects full mass spectra during each acquisition (Cochran 2002; Semard et al. 2009). These nonskewed spectra ensure that consistent ion ratios are maintained as a peak elutes into the detector (Erickson et al. 1990). The nonskewed spectra allow for software algorithms to perform peak finding and spectral deconvolution of overlapping peaks (Focant et al. 2004). The advantage of utilizing hyphenated techniques for the analysis of these complex samples was noted (Statheropoulos et al. 2011) and the benefit of exploring additional technologies became evident. The spectral deconvolution of the TD-GC-TOFMS system was able to identify numerous decomposition VOCs (Statheropoulos et al. 2011) however this only provides a starting point for the full characterisation and non-target analysis of these complex samples.

The goal of non-target analysis is to confidently identify all components within the sample. To achieve this goal improvements in the chromatographic resolution in addition to the spectral resolution are required. Conventional GC systems offer high peak capacities and generally provide sufficient resolution of compounds. However when analysing highly complex samples with a variety of components such as decomposition odour, the peak capacities offered by such systems are not able to sufficiently resolve all components (Dalluge et al. 2003; Dekeirsschieter et al. 2012). Comprehensive two dimensional gas chromatography (GC×GC) is an emerging technique that provides the additional peak capacity required for the analysis of complex samples, however its wide range of potential applications are not well represented in the literature (Dalluge et al. 2003). Recent studies into decomposition odour have shown the applicability of this analytical technique to this complex sample type (Kalinova et al. 2009; Brasseur et al. 2012; Dekeirsschieter et al. 2012; Stadler et al. 2013).

19.6 Comprehensive Two Dimensional Gas Chromatography – Time-of-Flight Mass Spectrometry

GC×GC has been developed for the trace analysis or in-depth investigations of complex samples and matrices. In these situations, the peak capacity of conventional GC systems might not be adequate to achieve efficient separation of sample components. GC×GC-TOFMS provides multi-dimensional information about the entire sample in approximately the same amount of time as conventional one dimensional GC systems and can be combined with a variety of sample types and injection systems (Semard et al. 2009).

Multidimensional GC techniques started as heart-cut GC (GC-GC) where individual fractions of the column effluent were subjected to a secondary separation. The additional peak capacity gained from this analysis can be represented as the sum of the two columns (first dimension (^{1}D) peak capacity + second dimension

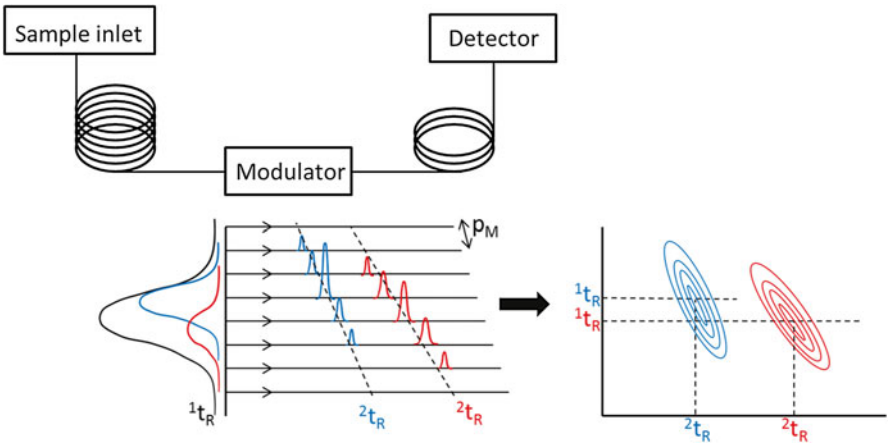


Fig. 19.1 Schematic of GC×GC system. Sample inlet can be liquid injector or thermal desorption apparatus, detector is typically a TOFMS. The *black trace* represents the coelution of the *red* and *blue* peak on a conventional GC system. ^1t_R: first dimension retention time, ^2t_R: second dimension retention time, p_M: modulation period (Figure adapted from Semard et al. 2009)

(^{2}D) peak capacity) (Dalluge et al. 2003). This is in contrast to *comprehensive* two dimensional GC (GC×GC) where all the column effluent from the ^{1}D undergoes separation on a second GC column in the ^{2}D (Dalluge et al. 2003; Schoenmakers et al. 2003). In this case, the overall peak capacity can be represented as the product of the two columns (^{1}D peak capacity × ^{2}D peak capacity) (Giddings 1987; Venkatramani et al. 1996; Dalluge et al. 2003). Comprehensive GC×GC provides a distinct advantage because the entire sample undergoes thorough analysis and therefore the maximum amount of information is gained.

The first and second dimension columns of GC×GC are connected in series (Fig. 19.1) and are selected to ensure the orthogonal separation of components i.e. the compounds are separated by two different properties (Semard et al. 2009). As two compounds are not likely to have the same boiling point and the same 2nd dimension column interaction, they can be efficiently resolved. The key feature of the GC×GC instrumentation is the modulator. The modulator is situated between the two columns and serves several functions; to continuously trap and re-focus fractions of effluent from the first dimension column, and to re-inject these fractions onto the second dimension column (Dalluge et al. 2003). As a peak elutes from the ^{1}D column the modulator collects small fractions of it at a time, producing multiple slices across the peak (Fig. 19.1). The peaks resulting from the two dimensional separation and modulation are quite narrow (100–600 milliseconds) and require fast detectors in order to reconstruct the two dimensional chromatograms. Time of flight mass spectrometry (TOFMS) fulfils this requirement with its acquisition rates of >50 Hz (Dalluge et al. 2003). The combination of the chromatographic resolution provided by GC×GC along with the analytical or mass spectral resolution provides an extremely powerful tool for the analysis of complex samples as each sample

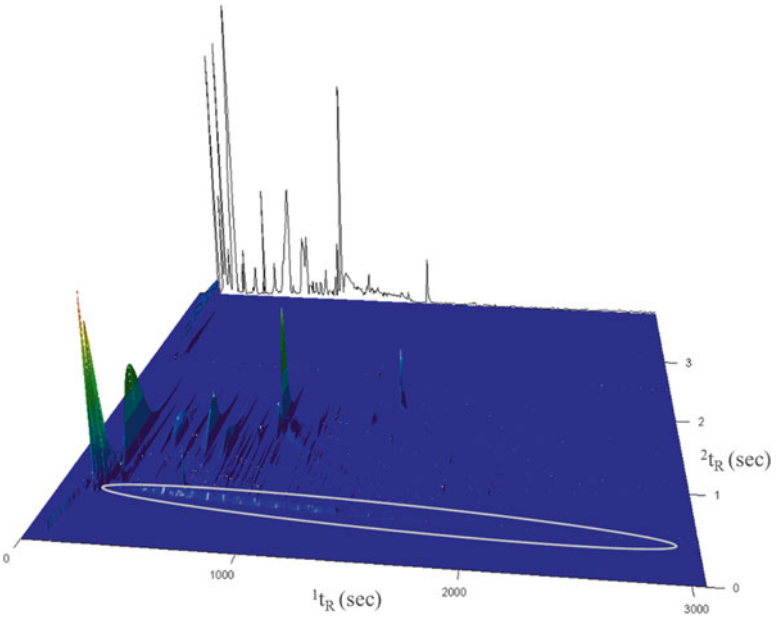


Fig. 19.2 Sample output of comprehensive two dimensional chromatography – time-of-flight mass spectrometry of decomposition headspace from human analogue in a soil environment. Linear trace at rear of image is a one dimensional projection of the chromatogram similar to what would be produced from the analysis with conventional GC-MS systems. The *grey oval* outlines the baseline of the GC×GC chromatogram. $^1t_{R:}$ first dimension retention time, $^2t_{R:}$ second dimension retention time

component will be described by its first dimension retention time (^1t_R), second dimension retention time (^2t_R) and mass spectra.

The initial data processing required to generate an output for GC×GC-TOFMS analysis requires extensive computing power and integrated software tools (Dalluge et al. 2002, 2003). The raw output of the TOFMS is a linear trace of the modulated peaks or slices. Each slice has four pieces of information associated with it; the ^1t_R, ^2t_R, a mass spectrum and signal intensity. A reconstructed peak matrix is then plotted by their first and second retention times. In a 3D surface plot the intensity of a peak is shown on the z-axis. A contour plot is a 2D plot of the data and is essentially a birds-eye view of the 3D surface plot (Fig. 19.2). In both, the colour scale indicates the intensity of a peak. Once processed, the data may also be presented as an apex plot. In this case there is no scaling to indicate the intensity of the peak and the two retention times of the peak apex are plotted as one point. The contour plot can be overlaid with the apex plot for further clarity (Dalluge et al. 2002).

Few taphonomic studies have utilized this technique however the application of this advanced instrumentation to the analysis of decomposition odour has shown several benefits over conventional GC-MS. Utilization of this method for the analysis of decomposition headspace demonstrated that the increased peak capacity is capable of producing a more detailed profile by separating and identifying up to ten

times the components (Dekeirsschieter et al. 2012). The enhanced peak capacity and separation of sample components is also enhanced by the utilization of the large chromatographic space available in GC × GC (Stadler et al. 2013). Figure 19.2 demonstrates the separation of all sample components including large overloading compounds from smaller peaks. Component peaks that align in the 2nd dimension of Fig. 19.2 would have co-eluted in conventional chromatography as shown by the linear trace along the rear of the figure and thus their identification may have been inhibited. Optimization of the two dimensional method to ensure that the majority of the chromatographic space is utilized allows the sample components to be separated from the instrumental background, shown by the oval outline in Fig. 19.2, thus ensuring better compound detection and identification (Dekeirsschieter et al. 2012; Stadler et al. 2013). This is particularly advantageous when characterising samples such as fresh or skeletonized remains, that have lower or trace amounts of compounds which would be otherwise masked within the baseline (Kalinova et al. 2009; Dekeirsschieter et al. 2012).

The non-target analysis of decomposition odour by GC × GC-TOFMS allows for the complete characterisation and comparison of these samples. This approach produces large datasets that can be difficult to manage, however there are several data handling tools that make reduction of the data set and cross-sample comparisons fast and efficient (Brasseur et al. 2012; Dekeirsschieter et al. 2012; Stadler et al. 2013). *Scripting* is a data handling tool that utilizes characteristic mass spectral features to extract all peaks from a particular chemical class (Brasseur et al. 2012). The unique aspects of the fragmentation pattern are written in a computer language and applied to the entire data set, peaks whose mass spectra match that of the script are highlighted allowing for a large number of compounds to be extracted simultaneously (Brasseur et al. 2012). Scripts for multiple chemical classes can be applied to a data set facilitating the extraction of the decomposition odour signature (Brasseur et al. 2012). In addition to scripting, statistical analysis tools are available to facilitate the comparison of samples. By comparing the peak tables generated from initial data processing the unknown chemical variations between sample groups i.e. experimental and control, can be determined. Specifically this tool allows for the alignment of multiple chromatograms and the statistical comparison of the peak tables from groups or classes of samples (Stadler et al. 2013). This approach to non-target analysis and the identification of all components within a sample facilitates the extraction of decomposition products from environmental VOCs producing more accurate and consistent profiles of decomposition odour (Brasseur et al. 2012; Dekeirsschieter et al. 2012; Stadler et al. 2013). This can be of additional benefit when working with difficult matrices, such as soil (Brasseur et al. 2012). Due to the complex nature of both the soil matrix and decomposition signatures, sophisticated data handling tools can be used to quickly screen raw data sets and extract particular compounds or classes of interest (Brasseur et al. 2012). With ongoing research these tools can be used to generate profiles from various environments and decomposition scenarios thereby facilitating the production of a more holistic profile and improving our understanding of decomposition assemblages and their volatile signatures.

19.7 Application of GCxGC-TOFMS to Human Analogue Decomposition in a Soil Environment

A field study was conducted at the Geoforensic Research Facility in Oshawa Ontario, Canada during the summer months (July–August 2011) to investigate the application of GCxGC-TOFMS for the chemical analysis of decomposition products. Pig carcasses (*Sus scorfa domesticus*) were utilized as human analogues and were placed on a soil surface on top of light grassy vegetation. Wire scavenging cages were placed over the carcasses between sampling periods in order to prevent avian and mammalian scavenging. Within the Geoforensic Research Facility, control sites of similar vegetation were delineated and the headspace was sampled following the same schedule and procedure as the experimental sites which contained the carcasses. At the time of sampling, a stainless steel hood (100 cm x 70 cm x 40 cm) was placed over the remains for a period of 30 min in order to develop a headspace. A multi-sorbent (Tenax GR & Carbopack B) thermal desorption tube was connected to a sampling port and 1 L of headspace was collected.

The decomposition headspace was analysed using TD-GCxGC-TOFMS. Thermal desorption was carried out by a Markes International Ltd. (Llantrisant U.K.) Unity 2 series thermal desorber, which combines sample desorption, focusing and injection. The two dimensional chromatography and spectroscopy was completed with an Agilent 7890 GC (Palo Alto, CA, USA) equipped with a secondary oven and modulator which was coupled with a Pegasus 4D GCxGC TOFMS from LECO Corporation (St. Joseph, MI, USA). An Rxi5Sil-MS column (30 m × 0.25 mm × 0.25 μm) was utilised in the first dimension and a BPX-50 column (1.2 m × 0.1 mm × 0.1 μm) was placed in the second dimension. A detailed description of the methods and analytical parameters utilised have been described previously (Stadler et al. 2013).

The non-target analysis of the decomposition headspace revealed thousands of peaks, including instrumental background, environmental VOCs and compounds of interest. The comparison of the control and experimental samples using sophisticated software and statistical techniques identified 300 VOCs within the headspace of decomposition throughout the decay process. The compounds were from a variety of chemical classes and the odour profile formed was dynamic across decomposition. The dominant compounds of the various chemical classes are shown in Table 19.1. The key chemical families, alcohols, sulfides, aromatics, and carboxylic acids characterized the continuous process of decomposition and the dominant compounds (e.g., 1-butanol, 2-and 3-methyl butanoic acid, DMDS, DMTS, phenol, and indole) were identified as potential target odorants of decomposition. In addition to these major chemical classes the aldehydes, ketones and nitrogen compounds further characterized the complete profile.

A major challenge in the analysis of complex samples such as decomposition headspace is the combination of trace compounds with large overloading peaks. Using conventional GC-MS systems, this large dynamic range can cause compounds to be lost due to co-elution or misidentification of compounds due to con-

Table 19.1 The dominant compounds of the various chemical classes identified with TD-GCxGC-TOFMS from carrion decomposition in a soil environment

Compound class	Compound name	Literature citation
Alcohol	1-Propanol	Dekeirsschieter et al. (2012) and Stadler et al. (2013)
	1,2-Propanediol	Statheropoulos et al. (2011) and Stadler et al. (2013)
	1-Butanol	Dekeirsschieter et al. (2012), Stadler et al. (2013), Dekeirsschieter et al. (2009), Statheropoulos et al. (2005, 2011)
	1-Butanol,3-methyl	Dekeirsschieter et al. (2009) and Stadler et al. (2013)
	2-Butanol	Dekeirsschieter et al. (2009), Stadler et al. (2013) and Statheropoulos et al. (2011)
	1-Pentanol	Dekeirsschieter et al. (2009, 2012), Hoffman et al. (2009) and Statheropoulos et al. (2005)
	1-Hexanol	Dekeirsschieter et al. (2012), Stadler et al. (2013), Hoffman et al. (2009), Statheropoulos et al. (2011) and Statheropoulos et al. (2005)
	1-Heptanol	Dekeirsschieter et al. (2012) and Stadler et al. (2013)
	1-Octanol	DeGreeff and Furton (2011), Stadler et al. (2013), Dekeirsschieter et al. (2012) and Hoffman et al. (2009)
	1-Octen-3-ol	Hoffman et al. (2009), Stadler et al. (2013) and Statheropoulos et al. (2011)
Aldehyde	Butanal, 3-methyl	Statheropoulos et al. (2007), Stadler et al. (2013) and Statheropoulos et al. (2011)
	Pentanal	Dekeirsschieter et al. (2012), Stadler et al. (2013) and Statheropoulos et al. (2005)
	Pentanal, 2-methyl	Stadler et al. (2013)
	Hexanal	Dekeirsschieter et al. (2009), Stadler et al. (2013), Hoffman et al. (2009) and Statheropoulos et al. (2005)
	Heptanal	Hoffman et al. (2009), Stadler et al. (2013) and Dekeirsschieter et al. (2012)
	2-Heptenal	Hoffman et al. (2009) and Stadler et al. (2013)
	Octanal	Dekeirsschieter et al. (2012), Stadler et al. (2013) and Hoffman et al. (2009)
	2-Octenal	Dekeirsschieter et al. (2012), Stadler et al. (2013) and Hoffman et al. (2009)
	2-Decenal	DeGreeff and Furton (2011) and Stadler et al. (2013)

(continued)

Table 19.1 (continued)

Compound class	Compound name	Literature citation
Aromatic	Benzaldehyde	Brasseur et al. (2012), Stadler et al. (2013), DeGreeff and Furton (2011), Dekeirsschieter et al. (2012), Hoffman et al. (2009), Statheropoulos et al. (2011) and Vass et al. (2004)
	Benzonitrile	Brasseur et al. (2012), Stadler et al. (2013), DeGreeff and Furton (2011) and Vass et al. (2004)
	Benzenesulfonic acid,4-hydroxy	Dekeirsschieter et al. (2012) and Stadler et al. (2013)
	Indole	Brasseur et al. (2012), Dekeirsschieter et al. (2012), Hoffman et al. (2009), Stadler et al. (2013)
	Indole,3-methyl	Dekeirsschieter et al. (2012) and Stadler et al. (2013)
	Phenol	Brasseur et al. (2012), Stadler et al. (2013), DeGreeff and Furton (2011), Dekeirsschieter et al. (2009, 2012), Statheropoulos et al. (2007, 2011)
	Phenol,4-methyl	Dekeirsschieter et al. (2012), Stadler et al. (2013) and Statheropoulos et al. (2007)
Carboxylic acid	Acetic acid	DeGreeff and Furton (2011), Stadler et al. (2013) and Statheropoulos et al. (2011)
	Propanoic acid	Dekeirsschieter et al. (2009), Hoffman et al. (2009) and Stadler et al. (2013)
	Propanoic acid, 2-methyl	Dekeirsschieter et al. (2009, 2012) and Stadler et al. (2013)
	Butanoic acid	Dekeirsschieter et al. (2012), Stadler et al. (2013), Dekeirsschieter et al. (2009) and Hoffman et al. (2009)
	Butanoic acid,2-methyl	Dekeirsschieter et al. (2012), Stadler et al. (2013) and Dekeirsschieter et al. (2009)
	Butanoic acid,3-methyl	Dekeirsschieter et al. (2012), Stadler et al. (2013), Dekeirsschieter et al. (2009) and Statheropoulos et al. (2011)
	Petanoic acid	Dekeirsschieter et al. (2012), Stadler et al. (2013), Dekeirsschieter et al. (2009) and Hoffman et al. (2009)
	Hexanoic acid	DeGreeff and Furton (2011), Stadler et al. (2013), Dekeirsschieter et al. (2012), Dekeirsschieter et al. (2009) and Hoffman et al. (2009)
Hydrocarbon	1-Octene	Stadler et al. (2013)
	Octane	Dekeirsschieter et al. (2012), Stadler et al. (2013), Statheropoulos et al. (2007) and Statheropoulos et al. (2011)
	Nonane	Dekeirsschieter et al. (2012), Statheropoulos et al. (2011) and Stadler et al. (2013)
	1,11-Dodecadiene	Stadler et al. (2013)
	1-Undecene	Statheropoulos et al. (2007) and Stadler et al. (2013)

(continued)

Table 19.1 (continued)

Compound class	Compound name	Literature citation
Ketone	2-Butanone	Statheropoulos et al. (2007), Stadler et al. (2013), Statheropoulos et al. (2011) and Statheropoulos et al. (2005)
	2-Butanone,3-methyl	Statheropoulos et al. (2011) and Stadler et al. (2013)
	2-Pentanone	Statheropoulos et al. (2011), Statheropoulos et al. (2005) and Stadler et al. (2013)
	2-Heptanone	Dekeirsschieter et al. (2012), Stadler et al. (2013), Hoffman et al. (2009) and Statheropoulos et al. (2005)
	2-Octanone	Dekeirsschieter et al. (2012) and Stadler et al. (2013)
	1-Octen-3-one	Dekeirsschieter et al. (2012) and Stadler et al. (2013)
	2-Nonanone	Dekeirsschieter et al. (2012) and Stadler et al. (2013)
	2-Decanone	Dekeirsschieter et al. (2012) and Stadler et al. (2013)
	2-Undecanone	Dekeirsschieter et al. (2012) and Stadler et al. (2013)
	3-Octanone	Dekeirsschieter et al. (2012)
	3-Octen-2-one	Stadler et al. (2013)
Nitrogen	Trimethylamine	Dekeirsschieter et al. (2012), Dekeirsschieter et al. (2009) and Statheropoulos et al. (2011)
	Azidrine	Stadler et al. (2013)
	Ethanamine,N-methyl	Stadler et al. (2013)
	Ethylenimine	Stadler et al. (2013)
	Octodrine	Stadler et al. (2013)
	Hexanitrile	Stadler et al. (2013)
	Pyrazine,2,6-dimethyl	Dekeirsschieter et al. (2012) and Stadler et al. (2013)
Sulphide	Dimethyl Disulfide	Brasseur et al. (2012), Stadler et al. (2013), DeGreeff and Furton (2011), Dekeirsschieter et al. (2012), Dekeirsschieter et al. (2009), Hoffman et al. (2009), Statheropoulos et al. (2007), Statheropoulos et al. (2011), Statheropoulos et al. (2005), Vass et al. (2008) and Vass et al. (2004)
	Dimethyl Trisulfide	Brasseur et al. (2012), Stadler et al. (2013), DeGreeff and Furton (2011), Dekeirsschieter et al. (2012), Dekeirsschieter et al. (2009), Statheropoulos et al. (2007), Statheropoulos et al. (2011), Statheropoulos et al. (2005), Vass et al. 2008 and Vass et al. (2004)
	Dimethyl Tetrasulfide	Statheropoulos et al. (2011) and Stadler et al. (2013)

centration effects within the mass spectrometer. Decomposition odour has the highest complexity during the active decay (Dekeirsschieter et al. 2012; Stadler et al. 2013), including the presence of large overloading compounds which increase the potential for compound co-elution (Fig. 19.2). The linear trace along the rear of Fig. 19.2 represents a chromatogram obtained using a one dimensional GC. It is evident that the larger peaks would mask many of the smaller components. However the additional peak capacity provided by the two dimensional chromatography allows for the separation of sample components across the chromatographic plane thereby decreasing the amount of co-elution within the sample. The improved compound resolution along with the TOFMS utilized in this work compensated for the sample complexity and dynamic range. Compound identifications were made by a mass spectral library search. The TOFMS generated higher quality non-skewed spectra which facilitated the identification of decomposition VOCs by correcting for concentration effects as overloading peaks elute into the detector.

This profile generated from the non-target analysis of decomposition headspace was able to characterize the process of soft tissue decomposition within a surface soil environment (Stadler et al. 2013). The ability to identify numerous compounds of interest in order to characterize these complicated samples illustrates the main benefit of multidimensional chromatography. The production of large data sets facilitates the use of a number of data mining strategies to generate meaningful results (Brasseur et al. 2012). Currently there exists a large amount of variation in the VOC profiles reported within the literature; however the application of GCxGC-TOFMS to the comprehensive analysis of decomposition odour can generate more consistent profiles of decomposition odour. The odour profile identified in this work was consistent with another study on carrion decomposition within a surface soil environment conducted in a separate geographical location (Stadler et al. 2013). The use of GCxGC-TOFMS for the non-target analysis of decomposition VOCs allows for the comparison of VOC profiles from different experimental treatments and is able to generate a comprehensive and accurate decomposition odour profile.

19.8 Summary

VOCs are an integral part of decomposition; they are responsible for the attraction of forensically relevant insects and are utilized by cadaver dogs to locate human remains. The profile generated by the current literature indicates the major chemical families; polysulfides, alcohols, aromatics, carboxylic acids and aldehydes. The polysulfides are dominant compounds within decomposition odour however, the consistent identification of other profile components has been elusive. If the identification of compounds can be improved a decomposition odour fingerprint can be developed. This odour profile of decomposition can be further utilized to develop improved methods for remains detection as well as further our understanding of the chemistry of decomposition. In order to determine the components of human decomposition odour, extensive research into the chemical composition of

decomposition headspace needs to be conducted utilizing instrumentation capable of the non-target analysis of complex samples such as thermal desorption coupled to comprehensive two dimensional gas chromatography – time-of-flight mass spectrometry. Non-target analysis is capable of identifying all chemical components within samples and can be used for the comparison of sample profiles. This level of analysis may also aid in the production of more consistent volatile profiles.

Using this instrumentation, investigations into the decomposition odour from a variety of geographical locations and decomposition environments will facilitate the development of a comprehensive odour signature. Studies isolating the variables that affect the production of decomposition VOCs including micro-organisms, and identifying the resultant changes in the VOCs produced would provide an expanded odour profile that would provide valuable information about this complex target odour and advance our knowledge of decomposition chemistry. A major limitation to this area of research is the difficulties associated with accessing human remains however this can be alleviated through the use of human analogues, such as pig carcasses. In addition to the analysis of soft tissue decomposition, research into skeletonised remains as well as potential scent reservoirs such as decomposition soil are required.

References

Agelopoulos NG, Pickett JA (1998) Headspace analysis in chemical ecology: effects of different sampling methods on ratios of volatile compounds present in headspace samples. J Chem Ecol 24:1161–1172

Anderson GS, VanLaerhoven SL (1996) Initial studies on insect succession on carrion in southwestern British Colombia. J Forensic Sci 41:617–625

ASTM International (2004) D 6345 standard guide for selection of methods for active, integrative sampling of volatile organic compounds in air. ASTM Standards, West Conshohocken

Augusto F, Koziel J, Pawliszyn J (2001) Design and validation of portable SPME devices for rapid field air sampling and diffusion-based calibration. Anal Chem 73:481–486

Benninger LA, Carter DO, Forbes SL (2008) The biochemical alteration of soil beneath a decomposing carcass. Forensic Sci Int 180:70–75

Boumba VA, Ziavrou KS, Vougiouklakis T (2008) Biochemical pathways generating post-mortem volatile compounds co-detected during forensic ethanol analyses. Forensic Sci Int 174:133–151

Brasseur C, Dekeirsschieter J, Schotsmans EMJ, de Koning S, Wilson AS, Haubruge E, Focant JF (2012) Comprehensive two dimensional gas chromatography-time-of-flight mass spectometry for the forensic study of cadaveric volatile organic compounds released in soil by buried decaying pig carcasses. J Chromatogr A 1255:163–170

Cablk ME, Szelagowski EE, Sagebiel JC (2012) Characterization of the volatile organic compounds present in the headspace of decomposing animal remains, and compared with human remains. Forensic Sci Int 220:118–125

Carter DO, Tibbett M (2008) Cadaver decomposition and soil: processes: chemical and biological effects of buried human remains. In: Tibbett M, Carter DO (eds) Soil analysis in forensic Taphonomy. CRC Press, New York, pp 29–51

Carter DO, Yellowlees D, Tibbett M (2007) Cadaver decomposition in terrestrial ecosystems. Naturwissenschaften 94:12–24

Carter DO, Yellowlees D, Tibbett M (2008) Using ninhydrin to detect gravesoil. J Forensic Sci 53:397–400

Clark MA, Worrell MB, Plessm JE (1997) Postmortem changes in soft tissues. In: Haglund WD, Sorg MH (eds) Forensic taphonomy: the postmortem fate of human remains. CRC Press, New York, pp 151–164

Cochran JW (2002) Fast gas chromatography-time-of-flight mass spectrometry of polychlorinated biphenyls and other environmental contaminants. J Chromatogr Sci 40:254–268

Dalluge J, Vreuls RJ, Beens J, Brinkman UA (2002) Optimization and characterization of comprehensive two-dimentional gas chromatography with time-of-flight mass spectometric detection (GCxGC-TOF MS). J Sep Sci 25:201–214

Dalluge J, Beens J, Brinkman UA (2003) Comprehensive two-dimensional gas chromatography: a powerful and versatile analytical tool. J Chromatogr A 1000:69–108

DeGreeff LE, Furton KG (2011) Collection and identification of human remains volatiles by non-contact, dynamic airflow sampling and SPME-GC/MS using various sorbent materials. Anal Bioanal Chem 401:1295–1307

Dekeirsschieter J, Verheggen FJ, Gohy M, Hubrecht F, Bourguignon L, Lognay G, Haubruge E (2009) Cadaveric volatile organic compounds released by decaying pig carcasses (Sus domesticus L.) in different biotopes. Forensic Sci Int 189:46–53

Dekeirsschieter J, Stefanuto PH, Brasseur C, Haubruge E, Focant JF (2012) Enhanced characterization of the smell of death by comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GCxGC-TOFMS). PLoS ONE 7(6), e39005

Dent BB, Forbes SL, Stuart BH (2004) Review of human decomposition processes in soil. Eviron Geo 45:576–585

Erickson ED, Enke CG, Holland JF, Watson JT (1990) Application of time array detection to capillary column gas chromatography/conventional time-of-flight mass spectrometry. Anal Chem 62:1079–1084

Focant JF, Sjodin A, Turner WE, Patterson DG Jr (2004) Measurement of selected polybrominated diphenyl ethers, polybrominated and polychlorinated biphenyls, and organochlorine pesticides in human serum and milk using comprehensive two-dimensional gas chromatography isotope dilution time-of-flight mass spectrometry. Anal Chem 76:6313–6320

France DL, Griffin TJ, Swanburg JG, Lindemann JW, Davenport GC, Trammell V, Travis CT, Kondratieff B, Nelson A, Castellano K, Hopkins D, Adair T (1997) NecroSearch revisited: further multidisciplinary approaches to the detection of clandestine graves. In: Haglund WD, Sorg MH (eds) Forensic taphonomy: the postmortem fate of human remains. CRC Press, New York, pp 497–509

Frederickx C, Dekeirsschieter J, Brostaux Y, Wathelet JP, Verheggen FJ, Haubruge E (2012) Volatile organic compounds released by blowfly larvae and pupae: new perspectives in forensic entomology. Forensic Sci Int 219:215–220

Giddings JC (1987) Concepts and comparisons in multidimensional separation. J High Resolut Chromatogr 10:319–323

Gill-King H (1997) Chemical and ultrastructural aspects of decomposition. In: Haglund WD, Sorg MH (eds) Forensic taphonomy: the postmortem fate of human remains. CRC Press, New York, pp 93–108

Hoffman EM, Curran AM, Dulgerian N, Stockham RA, Eckenrode BA (2009) Characterization of the volatile organic compounds present in the headspace of decomposing human remains. Forensic Sci Int 186:6–13

Janaway RC, Percival SL, Wilson AS (2009) Decomposition of human remains. In: Percival SL (ed) Microbiology and aging. Springer Science + Business Media, New York, pp 313–334

Johnston JM (1999) Canine detection capabilities: operational implications of recent R & D Findings. Institute for Biological Detection Systems, Auburn

Kalinova B, Podskalska H, Ruzicka J, Hoskovec M (2009) Irresistible bouquet of death—how are burying beetles (Coleoptera: Silphidae: Nicrophorus) attracted by carcasses. Naturwissenschaften 96:889–899

Komar D (1999) The use of cadaver dogs in locating scattered, scavenged human remains: preliminary field test results. J Forensic Sci 44:405–408

Lasseter AE, Jacobi KP, Farley R, Hensel L (2003) Cadaver dog and handler team capabilities in the recovery of buried human remains in the southeastern United States. J Forensic Sci 48:617–621

Lawson MJ, Craven BA, Paterson EG, Settles GS (2012) A computational study of odorant transport and deposition in the canine nasal cavity: implications for olfaction. Chem Senses 37:553–566

Lorenzo N, Wan T, Harper RJ, Hsu YL, Chow M, Rose S, Furton KG (2003) Laboratory and field experiments used to identify Canis lupus var. familiaris active odor signature chemicals from drugs, explosives, and humans. Anal Bioanal Chem 376:1212–1224

Mann RW, Bass WM, Meadows L (1990) Time since death and decomposition of the human body: variables and observations in case and experimental field studies. J Forensic Sci 35:103–111

McClenny EAWWA (1999) Compendium method TO-17: determination of volatile organic compounds in ambient air using active sampling onto sorbent tubes. U.S. Environmental Protection Agency, Cincinnati

Oesterhelweg L, Krober S, Rottmann K, Willhoft J, Braun C, Thies N, Puschel K, Silkenath J, Gehl A (2008) Cadaver dogs—a study on detection of contaminated carpet squares. Forensic Sci Int 174:35–39

Paczkowski S, Schutz S (2011) Post-mortem volatiles of vertebrate tissue. Appl Microbiol Biotechnol 91:917–935

Perry B (2011) State of Florida v. Casey Marie Anthony – Order denying motion to exclude unreliable evidence. 9th Judicial Circuit

Rebmann A, David E, Sorg MH (2000) Cadaver dog handbook: forensic training and tactics for the recovery of human remains. CRC Press, New York

Ribes A, Carrera G, Gallego E, Roca X, Berenguer MA, Guardino X (2007) Development and validation of a method for air-quality and nuisance odors monitoring of volatile organic compounds using multi-sorbent adsorption and gas chromatography/mass spectrometry thermal desorption system. J Chromatogr A 1140:44–55

Sanchez JM, Sacks RD (2006) Development of a multibed sorption trap, comprehensive two-dimensional gas chromatography, and time-of-flight mass spectrometry system for the analysis of volatile organic compounds in human breath. Anal Chem 78:3046–3054

Semard G, Mohamed A, Focant J-F (2009) Basic Instrumentation for GCxGC. In: Comprehensive Analytical Chemistry, vol 55. Elsevier, pp 15–48

Schoenmakers P, Marriott P, Beens J (2003) Nomenclature and conventions in comprehensive multidimensional chromatography. Coupling Matters, Ellesmere Port

Stadler S, Stefanuto PH, Brokl M, Forbes SL, Focant JF (2013) Characterization of volatile organic compounds from human analogue decompsoition using thermal desorption coupled to comprehensive two-dimensional gas chromatography - time-of-flight mass spectrometry. Anal Chem 85:998–1005

Statheropoulos M, Spiliopoulou C, Agapiou A (2005) A study of volatile organic compounds evolved from the decaying human body. Forensic Sci Int 153:147–155

Statheropoulos M, Mikedi K, Agapiou A, Georgiadou A, Karma S (2006) Discriminant analysis of volatile organic compounds data related to a new location method of entrapped people in collapsed buildings of an earthquake. Anal Chim Acta 566:207–216

Statheropoulos M, Agapiou A, Spiliopoulou C, Pallis GC, Sianos E (2007) Environmental aspects of VOCs evolved in the early stages of human decomposition. Sci Total Environ 385:221–227

Statheropoulos M, Agapiou A, Zorba E, Mikedi K, Karma S, Pallis GC, Eliopoulos C, Spiliopoulou C (2011) Combined chemical and optical methods for monitoring the early decay stages of surrogate human models. Forensic Sci Int 210:154–163

Swann L, Chidlow GE, Forbes S, Lewis SW (2010a) Preliminary studies into the characterization of chemical markers of decomposition for geoforensics. J Forensic Sci 55:308–314

Swann L, Forbes S, Lewis SW (2010b) Observations of the temporal variation in chemical content of decomposition fluid: a preliminary study using pigs as a model system. Aust J Forensic Sci 42:199–210

Swann LM, Forbes SL, Lewis SW (2010c) Analytical separations of mammalian decomposition products for forensic science: a review. Anal Chim Acta 682:9–22

Van Belle LE, Carter DO, Forbes SL (2009) Measurement of ninhydrin reactive nitrogen influx into gravesoil during aboveground and belowground carcass (Sus domesticus) decomposition. Forensic Sci Int 193:37–41

Vass AA (2012) Odor mortis. Forensic Sci Int 222:234–241

Vass AA, Bass WM, Wolt JD, Foss JE, Ammons JT (1992) Time since death determinations of human cadavers using soil solution. J Forensic Sci 37:1236–1253

Vass AA, Barshick SA, Sega G, Caton J, Skeen JT, Love JC, Synstelien JA (2002) Decomposition chemistry of human remains: a new methodology for determining the postmortem interval. J Forensic Sci 47:542–553

Vass AA, Smith RR, Thompson CV, Burnett MN, Wolf DA, Synstelien JA, Dulgerian N, Eckenrode BA (2004) Decompositional odor analysis database. J Forensic Sci 49:760–769

Vass AA, Smith RR, Thompson CV, Burnett MN, Dulgerian N, Eckenrode BA (2008) Odor analysis of decomposing buried human remains. J Forensic Sci 53:384–391

Venkatramani CJ, Xu J, Phillips JB (1996) Separation orthogonality in temperature-programmed comprehensive two-dimensional gas chromatography. Anal Chem 68:1486–1492

Chapter 20
GC×GC-TOFMS, the Swiss Knife for VOC Mixtures Analysis in Soil Forensic Investigations

Pierre-Hugues Stefanuto and Jean-François Focant

Abstract The study of the 'smell of death' is an important part of the thanatochemistry, the chemistry of death. Since 2004 (Vass et al. 2004), an increasing number of studies have been conducted to understand the body decomposition process by measuring the Volatile Organic Compounds (VOCs) released by decaying bodies. However, the chemical profile of the decomposition odor is still far for from being resolved. Indeed, the complexity of the VOC mixture makes it difficult to be carried out by classical GC-MS. A better understanding of the decomposition process could thus possibly be achieved using a multidimensional technique such as Comprehensive Two Dimensional Gas Chromatography coupled to time of flight mass spectrometry (GC×GC-TOFMS). The high peak capacity of this multidimensional technique combined with the visualization power of multivariate statistical methods allows a deeper understanding of complex VOC matrices.

20.1 Introduction

Forensic science is a dynamic area. Police officers always need advanced analytical methods to help them in their investigations. For several years, researchers have been working on volatile evidence analysis. Many cases of investigation are linked with volatile organic compound (VOC) mixtures including drug, contraband, and terrorism. Nowadays, police dogs are mainly used for searching for volatile evidence in investigations (Lorenzo et al. 2003; Oesterhelweg et al. 2008). However, the dog detection mechanism is not yet completely understood. Indeed, it is not yet known which compounds police dogs react to during the detection of drugs,

P.-H. Stefanuto (✉) • J.-F. Focant
Organic and Biological Analytical Chemistry Group – CART, Chemistry Department, University of Liège, Allée du six aout, Quartier Agora 11, B6c Sart-Tilman, B-4000 Liège, Belgium
e-mail: phstefanuto@ulg.ac.be

H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics*, Soil Forensics, DOI 10.1007/978-3-319-33115-7_20

explosives and bodies. Lorenzo et al. studied the olphactometric fingerprint for some illicit products. These results assisted with dog training improvement (Lorenzo et al. 2003).

Another area of investigation for forensic volatiles is decomposition odor analysis. People start to study VOC originating from decomposition in 2004. A decomposition VOC database was created during this study containing approximately 400 compounds (Vass et al. 2004). Several additional studies tried to elucidate the process of decomposition following the release of VOCs (Statheropoulos et al. 2005, 2007, 2011; Vass et al. 2004, 2008; Paczkowski and Schütz 2011; Pandey and Kim 2011; Hoffman et al. 2009; DeGreeff and Furton 2011; Dekeirsschieter et al. 2009). Allmost all of these studies were based on gas chromatography (GC) methods coupled with different types of sampling methods.

Unfortunately, VOC mixtures from decaying bodies belong to the most complex volatile matrixes from Life Sciences. Indeed, the decomposition process is dynamic and the VOC concentration profile is changing over time. However, a better understanding of this process could help dog trainers to improve their training techniques. Improvement of cadaver dog training would not only help in crime solving but also improve the efficiency in finding trapped people after a natural disaster (Statheropoulos et al. 2006). In the past, gas chromatography – mass spectrometry (GC-MS) methods were used for this kind of forensic VOC investigation. Nevertheless, the resolution limits has not yet allowed a full understanding of the decomposition VOC profile.

In 2011, GC×GC was applied to the forensic field for the first time. Mitrevski et al. used it for the analysis of the volatile profile of ecstasy to determine the drug's origin. de Vos et al. also applied GC×GC for environmental forensic analysis (de Vos et al. 2011). They used GC×GC for Persistent Organic Pollutants (POPs) analysis in developing countries that do not have access to GC systems coupled to high-resolution mass spectrometry (HRMS). Their study shows that even though GC×GC-TOFMS is a non-target method, it allows screening of the compounds that are present in the environment and it can provide a good estimation of the concentration levels (de Vos et al. 2011). GC×GC was also successfully applied for complex matrix analysis in different metabolomics studies (Seeley and Seeley 2013). Hence, it is considered as a good approach to solve the many co-elution problems often encountered in classical GC methods.

In 2012, Brasseur et al. used multidimensional gas chromatography (MDGC) to analyze grave soil samples in order to overcome the 1D GC limitations (Brasseur et al. 2012). In subsequent years, several decomposition studies were conducted using GC×GC-TOFMS. Dekeirsshieter et al. monitored the VOC profile from pig carcasses during the different stages of decomposition. This study demonstrated that the list of compounds in the decomposition database is not exhaustive. Therefore, a method with high separation power is required (Dekeirsschieter et al. 2012). Stadler et al. conducted different studies on decomposition VOCs. The first study analyzed the VOC profile of synthetic training aids used for cadaver dog training solution analysis. That study determined that these training aids are very simple mixtures when compared to the true VOC profile from a decaying body. However,

dogs trained with these solutions are able to locate human remains (Stadler et al. 2012). The second study was focused on the comparison of the decomposition process between different environments in Canada and Belgium. They used TD-GC×GC-TOFMS. This method combined the sampling advantage of TD (Thermal Desorption) and the strong separation capability of GC×GC. This research demonstrated for the first time the similarities between two different decomposition trials conducted in different countries (Stadler et al. 2013).

An additional chromatographic dimension is helpful to improve the resolution of individual VOCs. Moreover, data treatment with multivariate statistical methods, such as Principal Component Analysis (PCA), can improve the data visualization and simplify the interpretation. These kinds of statistical methods were first applied to decomposition studies in 2006 by Statheropoulos et al. (2006). The aim of this current paper is to demonstrate the advantage of combining the chromatographic resolution power of GC×GC-TOFMS and the visualization enhancement of multivariate statistics for data handling. This combination of methods is applied to grave soil VOC analysis to demonstrate the different aspects of these tools and their value to forensic investigators.

20.2 Comprehensive Two Dimensional Gas Chromatography

Comprehensive Two Dimensional Gas Chromatography is a multidimensional approach that offers the possibility to isolate and identify compounds present in complex mixtures (Seeley and Seeley 2013). GC×GC was introduced almost 25 years ago by Liu and Phillips (Liu and Phillips 1991). This method is based on two chromatographic separations that separate the compounds on a chromatographic plane. GC×GC allows the screening of large numbers of compounds in one GC run. The main advantages of this method are the increase in peak capacity and the possibility to obtain a structured chromatogram (Dallüge et al. 2003).

The majority of hardware equipment in a GC×GC system is the same as in classical GC except the addition of a secondary column and the presence of a special device, i.e. a modulator (Seeley and Seeley 2013). The modulator is the interface between the two dimensions of separation (Ryan and Marriott 2003). The design of efficient modulators was crucial for GC×GC development. The key of a GC×GC application is the ability to rapidly pulse segments of effluent from the first to the second dimension (Phillips and Beens 1999). The modulator samples peaks eluting from the first dimension and injects them into the second dimension. The co-eluting peaks from the first dimension will be sent together into the second column where they will separate according to the selectivity of the second dimension. This additional separation contributes to cleaner mass spectra but the ultimate aim is, of course, to correctly resolve all the peaks on the chromatographic space to use the mass spectra for identification purposes. The slicing process also improves the deconvolution of the co-eluting peaks. The peak height is increased by the compression process in the modulator. This process increases the peak intensity and the limit

of detection compared with classical GC (Patterson et al. 2011; Shellie et al. 2001). There are two major types of modulator: thermic and valve systems. Both types must serve three functions: (1) Continuously trap small adjacent fractions of the eluent from the primary dimension; (2) Refocus the trapped sections; and (3) Inject the refocused trapped slices into the secondary dimension (Dallüge et al. 2003).

The selection of the best columns combination for a specific analysis is not obvious to achieve a proper comprehensive separation. There are three important parameters to take into account to ensure a comprehensive separation that were developed from the Giddings's rules (Giddings 1987). First, all peaks must pass through the two dimensions. Second, the two dimensions of separation must be orthogonal (meaning that the process of separation is entirely different on each column). Finally, the second dimension must be in fast GC condition.

Nowadays, there are numerous GC columns available on the market. Column manufacturers are introducing new kinds of stationary phases, either to increase the existing polarity range or using completely different separation principles, e.g. with liquid crystalline phases. Recently, Supelco® introduced the ionic liquid columns (de Boer et al. 1992). These columns allow GC users to obtain very high polarity selectivity (Armstrong et al. 2009). The constant development of new columns is really helpful to determine the best separation conditions for a specific analysis. This wide selection of phases offers a bench of possibilities to tune the separation. A suitable column combination will ensure a full separation (Dallüge et al. 2003; Ryan et al. 2005; Ryan and Marriott 2003). In multidimensional methods, each added dimension gives additional information that can be used for identification (Dallüge et al. 2003). The column set must be carefully selected taking into account the sample composition (Dallüge et al. 2003; Ryan et al. 2005). For example, in polychlorinated biphenyls (PCBs) congeners analysis, a structured chromatograms can give important additional information that is helpful for the identification (Korytar et al. 2002; Focant et al. 2004). More recently, Seeley et al. combined two semi-polar columns to obtain a specific separation for the FAME compounds in diesel samples (Seeley et al. 2012). These studies show the importance of the structure in multidimensional chromatography.

The last Giddings's rule is very important as the separation obtained in the first dimension must be maintained in the secondary column. An important consideration is the length ratio between the two columns. The first column length is similar to that used in classical GC. Thus, the separation is similar to a 1D GC temperature program analysis. The second dimension must be a series of fast GC isothermal separations. The second column has to be smaller to fit with this restriction. The combination of these two separations gives a GC×GC two dimensional chromatogram (e.g. Fig. 20.1) (Seeley and Seeley 2013). Additionally, the modulation period must be chosen carefully to obtain the best space occupation on the two dimensional separation space (Ryan et al. 2005).

Peaks elute from the second dimension with a Full Width at Half Maximum (FWHM) in the order of 100–300 ms. This represents less than 10 % of the peak width in conventional GC analysis. Hence, GC×GC detectors need to have a fast response (Seeley and Seeley 2013). There are several detectors that can reach the

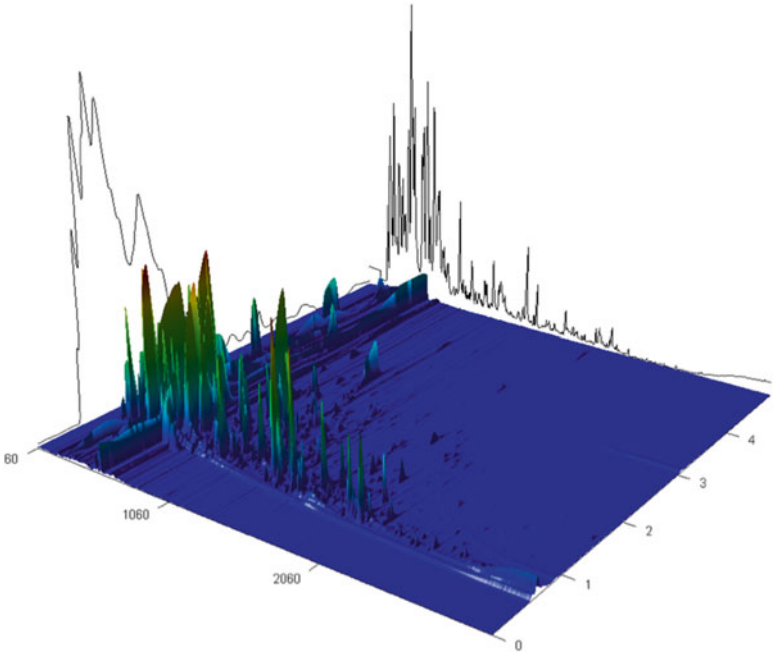


Fig. 20.1 3D chromatogram of the VOCs trapped in the headspace of the grave soil. The two retention axes are in seconds and the 1D traces for each dimension are shown. The peak occupation is important due to the sample complexity. Without a multidimensional approach, the complete resolution of this mixture is not possible

acquisition rate required for GC×GC. There are electron capture detectors (ECD, μECD), flam ionization detectors (FID) and other elemental detectors that do not allow full identification (Dallüge et al. 2003). But GC×GC systems can also be connected with a mass spectrometer. The mass spectra can be compared with databases (e.g. NIST and Wiley) to obtain peak identification. Due to the acquisition speed required, quadrupole MS is not suitable. A Time of Flight Mass Spectrometer (TOFMS) is the most viable technology available to provide this rapid acquisition capability (Ryan and Marriott 2003; Dallüge et al. 2003). The absence of concentration skewing ensures spectral continuity and allows for effective mass spectral deconvolution of the co-eluting peaks characterized by different fragmentation patterns (Cochran 2002; Focant et al. 2004). Recently, High Resolution Time of Flight Mass Spectrometers (HRTOFMS) have also become available coupled to GC×GC systems. These new instruments will improve the mass spectra dimension without losing the required acquisition rate. HRMS provides exact masses for the ions that can be linked to their chemical formula improving the identification capabilities (Ochiai et al. 2011; Ieda et al. 2011).

For sample injection, GC×GC systems can be linked to all the classical injection devices that are used for classical GC. All the methods usually used in 1D GC can be implemented on 2D GC. The most used one is probably the liquid injection but

to study VOC mixtures Thermal Desorption (TD) methods are more efficient (Barro et al. 2009; Ramírez et al. 2010; Brokl et al. 2013).

One of the major drawback of the GC×GC technique is the number of parameters that can affect the separation. The additional parameters make the optimization steps more complicated. GC×GC users need to choose the best column combination and set the best modulation period… (Dallüge et al. 2003). Condition optimization is important to ensure optimal separation by GC×GC (Semard et al. 2011; Mostafa et al. 2012).

The data treatment software is another critical part in GC×GC. The commercial availability of GC×GC instruments was strongly linked to the development of dedicated software. Due to the raw data complexity, GC×GC instruments must be linked to powerful software for the data processing and to obtain the final chromatogram (Fig. 20.1). The chromatogram obtained from the detector must be transformed to obtain a 2D chromatogram and as a result, the data process follows different steps. A raw (1D) chromatogram is cut in several slices due to the modulation process. Based on the modulation period (P_M), the software will divide the 1D traces into different pieces. Each piece will be rotated by 90° to obtain the 2D space chromatogram. After that, each modulation slice will be combined to reconstruct the real GC peaks. If a mass spectrometer is used for the detection, a mass spectra comparison is applied to determine if two consecutive slices are part of the same peak and have to be combined. The slice-to-slice combination also improves the deconvolution capabilities. Slices from the same peak must be separated by one P_M and the mass spectra of each slice must be the same. The deconvolution improvement is critical for very complex samples because even with the increased peak capacity, co-elutions can remain. Finally, a 2D chromatogram is obtained with two retention times, one on the x-axis and one on the y-axis. The z-axis is used to show the peak intensity based on the detector response (see Fig. 20.1). If a mass detector is used, a mass library identification can be performed to identify the compound linked to each peak (Dimandja 2004; Seeley and Seeley 2013; Ramos 2009; Mondello et al. 2008).

When the 2D chromatogram is obtained, different data comparison tools are available. To compare a set of data robust comparison tools are recommended (Almstetter et al. 2011; Castillo et al. 2011). The LECO® ChromaTOF software contains a Statistical Compare (SC) option. This feature allows chromatogram alignment and Fisher Ratio (FR) calculations. SC aligns the chromatograms based on the two retention times and on a mass spectra comparison. When the chromatogram is aligned, the FR can be calculated. A Fisher Ratio is the ratio of "between class" variance to "within class" variance. This factor identifies the compounds that are significantly different from one class to another (Almstetter et al. 2011). This tool assists with finding biomarkers that are responsible for group segregation when different compounds have to be compared (e.g. different origin of drugs).

Chromatogram comparison is like DNA analysis. It helps to identify the links that exist between different samples. Each peak is comparable to a gene and the complete peak pattern represents the genetic fingerprint of the sample. If different samples have some groups of peaks in common, it means that they are probably linked.

20.3 GC×GC and Multivariate Statistics: A Helpful Combination!

As explained before, GC×GC offers a real solution for complete separation of complex samples. However, this improvement of separation is linked to an increase in the data complexity. Dallüge notes that "The amount of data generated per run is overwhelming and data handling is, consequently, rapidly becoming the real analytical problem" (Dallüge et al. 2003). To solve this problem, GC×GC users need tools that can reduce the amount of data. A way to achieve this is the use of multivariate statistics.

The combination of GC×GC resolution power and robust statistical tools can be helpful when a large number of different samples need to be compared. GC×GC separation provides all the information about the composition of one sample. It is its chemical fingerprint. The comparison of this chemical signature with databases or other samples can identify the origin of one substance. The combination of comprehensive GC and multivariate statistic has been employed to analyze the VOC profile from beers and olive oils (Cajka et al. 2010a, b). These products are much appreciated by customers all over the world and their geographic origin can have a significant impact on the prices. Economic fraud by incorrect labeling is sometimes observed and should be avoided. Thus, analytical methods that can verify the traceability of these products are important to the producers. In these two studies, the traceability was studied using GC×GC and multivariate analysis (Cajka et al. 2010a, b). This combination of multidimensional chromatography and statistics was also applied in 2011 to a forensic application. Mitrevski et al. used GC×GC-TOFMS to analyze the VOC profiles of ecstasy from different countries. Based on the volatile fingerprint, Principal Component Analysis (PCA) was applied to observe clustering of the different kind of drugs according to their country of origin. The improvement of peak capacity and signal enhancement due to the use of GC×GC method allowed the detection of low level markers of the drug's origin (Mitrevski et al. 2011).

These studies are the result of the combination of the chromatographic resolution from GC×GC analysis and the data visualization power of multivariate statistic.

20.4 Application for Grave Soils Analysis

In this study, TD-GC×GC-TOFMS was used in combination with multivariate statistics to monitor the VOC profile from grave soil samples collected above a grave at different depths. The method was based on a study by Brasseur et al., which used GC × GC for grave soil investigations. The study identified different kinds of biomarkers with the highest number coming from the soil below the pig. Notably, branched alkane compounds were found through all grave soil depths above the pig carcass up to the surface. Based on a scripting method, an algorithm was developed

to search the different peaks and identify the branched alkanes. The scripts were based on the specific fragmentation pattern of alkanes in electron ionization source (Brasseur et al. 2012).

The research was conducted by trapping VOCs on tubes and desorption using a liquid solvent. However, Brokl et al. demonstrated that the volatile profile is influenced if the sampling is conducted using liquid extraction versus thermal desorption. Moreover, the number of peaks detected in the headspace were two times higher using thermal desorption (Brokl et al. 2013). Based on this result, it was decided to investigate the volatile organic compound profile of grave soil samples using TD-GC × GC-TOFMS. This experiment follow the pig were the same as in a previous study realized by Brasseur et al. (Brasseur et al. 2012). The pigs for the two studies were buried at the same time but the excavation took place after a different delay (17 months). Three hundred grams of the soil above the pig carcass was sampled every 10 centimeters from the surface to the carcass. The VOCs were trapped using the same kind of pumping device as Brasseur reported (Brasseur et al. 2012) but the sorbent tubes were chosen to be more polyvalent. A combination of Tenax® and Carbopack B® wad used to trap a higher number of VOCs. The efficiency of these sorbent tubes has been previously reported in other decomposition studies (Stadler et al. 2013).

The TD-GC×GC-TOFMS system used was a Markes® Unity 2 TD (Llantrisant, U.K) linked to a LECO® Pegasus 4D (St. Joseph, MI). The separation was conducted on a reverse column set: a polar ionic liquid (SLB-IL-111; 30 m × 0.25 mm × 0.25 μm) column in the first dimension and a non-polar polysiloxane (Rtx-1; 1 m × 0.1 mm × 0.08 μm) column in the second dimension. This particular configuration was chosen because most of the decomposition biomarkers found in previous studies are polar or semi-polar compounds. These compounds are better separated on a reverse column combination (polar – non-polar) (Dimandja et al. 2003).

The chemical composition of the headspace was analyzed at each depth. The results are displayed on Fig. 20.2. It shows that the VOCs from the top of the grave are mostly hydrocarbon compounds. This observation is consistent with the result obtained by Brasseur et al. in the headspace of a grave. At a depth of 10 cm, the most abundant chemical class is the carboxylic acids. Carboxylic acids likely result from the degradation of amino acids and lipids. At a depth of 30 and 34 cm, the most abundant class is that of the sulfur compounds. Sulfides are typically the most abundant compounds in the headspace of decomposition. Dimethyl disulfide and dimethyl trisulfide are predominantly responsible for this abundance (Stadler et al. 2013).

The Statistical Compare feature of the LECO Software (see section above) was also used to determine which compounds are different between grave soil samples and control soil samples. Fig. 20.3 shows on the top PCA that the grave soil samples (red) are correctly separated from the control soil samples (blue). The principal component axis responsible for this segregation is PCA (1). On the lower plot, the PCA (1) component was plotted vs. the different chemical families (the variables of the multivariate analysis). The green points represent the classes that have a medium influence on the PCA (1). The three points have different colors and demonstrated the greatest impact. These points correspond to the chemical classes with the highest

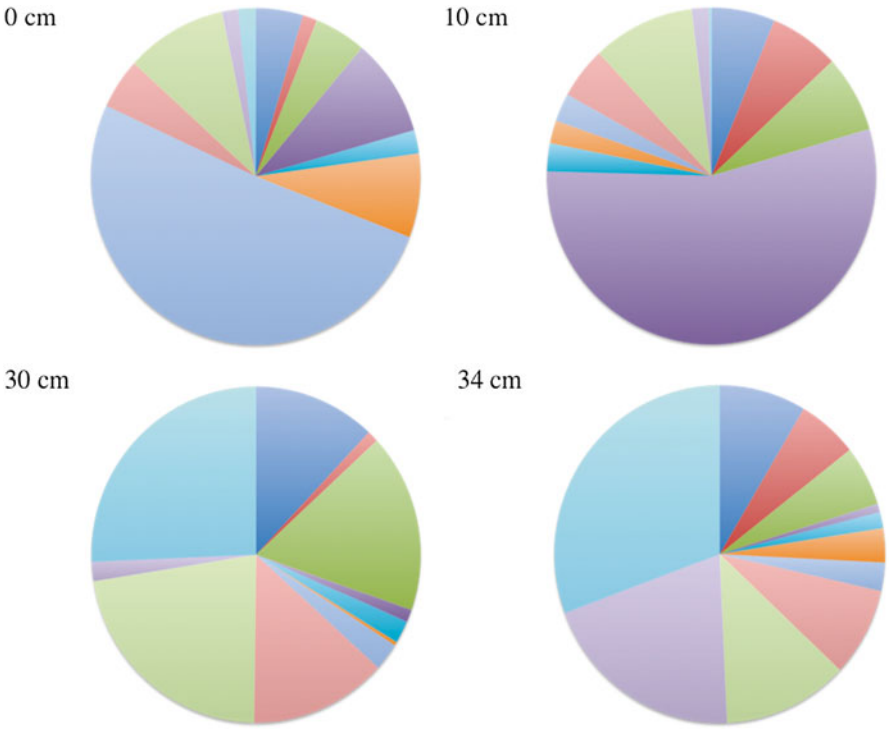


Fig. 20.2 Chemical families present in the different depths of the grave: Alcohol, Aldehyde, Aromatic, Carboxylic acid, Ester, Halogen, Hydrocarbon, Ketone, Nitrogen, Sulfur, Other

impact on the separation. This observation is consistent with the conclusion from Fig. 20.2. The chemicals, which are significantly different between the grave soil and the control, are present in greater quantity.

The major advantage of the Statistical Compare approach is the speed of the data processing. It allows direct alignment of all the samples. Moreover, the comparison of all the peaks through the different samples allows multivariate statistics to visualize how the different classes are clustering and which variables (i.e. compounds) are responsible for this separation.

20.5 Conclusion

A comprehensive decomposition VOC profile is still unknown due to the complexity and the dynamic nature of the decomposition process. Moreover, the decomposition markers are often linked to other complex matrices (e.g. soils). Since 2004, an increasing number of studies have investigated the VOCs profile of decaying bodies.

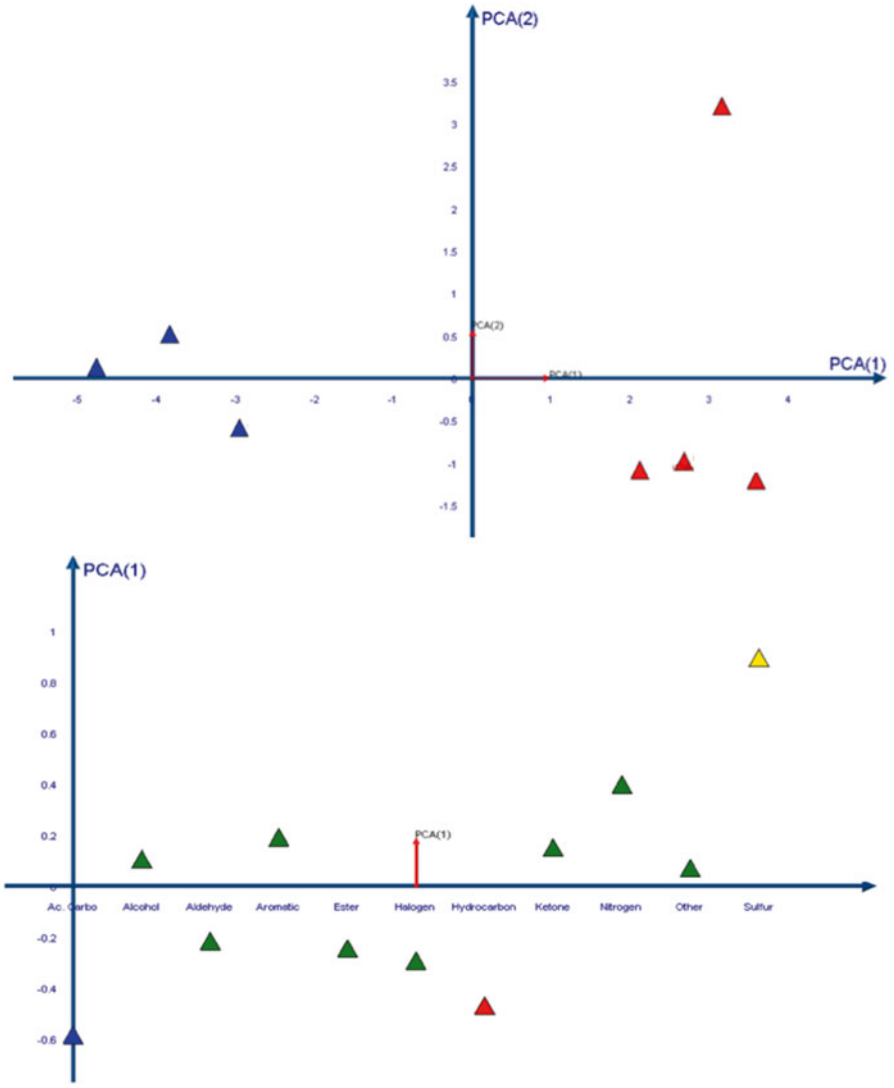


Fig. 20.3 The top figure displays the Principal Component Analysis of the VOC profiles from a grave (*red*) and a control (*blue*). The first axes clearly separates the two samples. The plot at the bottom shows which chemical families are responsible for this separation: the carboxylic acids (*blue*), the hydrocarbons (*red*) and the sulfur compounds (*yellow*)

However, a robust analytical method is required to ensure a comprehensive analysis of decomposition samples.

This study aimed to demonstrate the real analytical improvements offered by GC×GC-TOFMS. The high resolution of separation can isolate the markers from the matrix and the mass spectral data can provide an identification of the com-

pounds. The implementation of multivariate analysis (e.g. Principal Component Analysis) in the data processing is helpful to obtain a better visualization of the results. This visualization tool helps to reduce the data dimensionality of GC×GC-TOFMS analysis.

This combination of multidimensional chromatography and multivariate statistic offers solutions to overcome the analytical limitations of decomposition studies. Moreover, it offers solutions for VOC profiling in different areas of forensic sciences.

Acknowledgement We wish to thank Restek® Corp., SGE® and Supelco Sigma-Aldrish® for providing GC phases and various GC consumables. We would also like to thank JSB® and LECO® for the technical support on the system. We also want to thank the University of Bradford, the University of Ontario Institute of Technology, University of Technology Sydney and Gembloux agro biotech (University of Liège) for their contribution on the sampling.

References

Almstetter MF et al (2011) Comparison of two algorithmic data processing strategies for metabolic fingerprinting by comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry. J Chromatogr A 1218(39):7031–7038

Armstrong DW, Payagala T, Sidisky LM (2009) The advent and potential impact of ionic liquid stationary phases in GC and GC and GCXGC. LC GC Eur 22(9):459–461

Barro R et al (2009) Analysis of industrial contaminants in indoor air: Part 1. Volatile organic compounds, carbonyl compounds, polycyclic aromatic hydrocarbons and polychlorinated biphenyls. J Chromatogr A 1216(3):540–566

Brasseur C et al (2012) Comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry for the forensic study of cadaveric volatile organic compounds released in soil by buried decaying pig carcasses. J Chromatogr A 1255:163–170

Brokl M et al (2013) Analysis of mainstream Tobacco smoke particulate phase using comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry. J Sep Sci 36(6):1037–1044

Cajka T, Riddellova K, Tomaniova M et al (2010a) Recognition of beer brand based on multivariate analysis of volatile fingerprint. J Chromatogr A 1217(25):4195–4203

Cajka T, Riddellova K, Klimankova E et al (2010b) Traceability of olive oil based on volatiles pattern and multivariate analysis. Food Chem 121:282–289

Castillo S, Mattila I, Miettinen J (2011) Data analysis tool for comprehensive Two-dimensional Gas chromatography/time-of-flight mass spectrometry – analytical chemistry (ACS publications). Anal Chem 83(8):3058–3067

Cochran JW (2002) Fast gas chromatography-time-of-flight mass spectrometry of polychlorinated biphenyls and other environmental contaminants. J Chromatogr Sci 40(5):254–268

Dallüge J, Beens J, Th UAB (2003) Comprehensive two-dimensional gas chromatography: A powerful and versatile analytical tool. J Chromatogr A 100:69–108

de Boer J, Dao QT, van Dortmond R (1992) Retention times of fifty one chlorobiphenyl congeners on seven narrow bore capillary columns coated with different stationary phases. J High Resolut Chromatogr 5(4):249–255

de Vos J et al (2011) Comprehensive two-dimensional gas chromatography time of flight mass spectrometry (GC×GC-TOFMS) for environmental forensic investigations in developing countries. Chemosphere 82:1230–1239

DeGreeff LE, Furton KG (2011) Collection and identification of human remains volatiles by non-contact, dynamic airflow sampling and SPME-GC/MS using various sorbent materials. Anal Bioanal Chem 401:1295–1307

Dekeirsschieter J et al (2009) Cadaveric volatile organic compounds released by decaying pig carcasses (Sus domesticus L.) in different biotopes. Forensic Sci Int 189(1–3):46–53

Dekeirsschieter J et al (2012) Enhanced characterization of the smell of death by comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GCxGC-TOFMS) PLoS ONE 7(6) p.e39005

Dimandja J-M (2004) Comprehensive 2-D GC provides high-performance separations in terms of selectivity, sensitivity, speed, and structure. Anal Chem 76(9):167A–174A

Dimandja J-M, Clouden G, Colón I (2003) Standardized test mixture for the characterization of comprehensive two-dimensional gas chromatography columns: the Phillips mix. J Chromatogr A 1019:261–272

Focant J-F, Sjödin A, Patterson DG (2004) Improved separation of the 209 polychlorinated biphenyl congeners using comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry. J Chromatogr A 1040(2):227–238

Giddings JC (1987) Concepts and comparisons in multidimensional separation. J High Resolut Chromatogr 10(5):319–323

Hoffman E, Curran A, Dulgerian N (2009) Characterization of the volatile organic compounds present in the headspace of decomposing human remains. Forensic Sci Int 186(1–3):6–13

Ieda T et al (2011) Environmental analysis of chlorinated and brominated polycyclic aromatic hydrocarbons by comprehensive two-dimensional gas chromatography coupled to high-resolution time-of-flight mass spectrometry. J Chromatogr A 1218(21):3224–3232

Korytar P et al (2002) High-resolution separation of polychlorinated biphenyls by comprehensive two-dimensional gas chromatography. J Chromatogr A 958:203–218

Liu Z, Phillips JB (1991) Comprehensive two-dimensional gas chromatography using an on-column thermal modulator interface. J Chromatogr Sci 29(6):227–231

Lorenzo N et al (2003) Laboratory and field experiments used to identify Canis lupus var. Familiaris active odor signature chemicals from drugs, explosives, and humans. Anal Bioanal Chem 376(8):1212–1224

Mitrevski B et al (2011) Chemical signature of ecstasy volatiles by comprehensive two-dimensional gas chromatography. Forensic Sci Int 209(1–3):11–20

Mondello L et al (2008) Comprehensive two-dimensional gas chromatography-mass spectrometry: a review. Mass Spectrom Rev 27(2):101–124

Mostafa A, Edwards M, Górecki T (2012) Optimization aspects of comprehensive two-dimensional gas chromatography. J Chromatogr A 1255:38–55

Ochiai N et al (2011) Stir bar sorptive extraction and comprehensive two-dimensional gas chromatography coupled to high-resolution time-of-flight mass spectrometry for ultra-trace analysis of organochlorine pesticides in river water. J Chromatogr A 1218(39):6851–6860

Oesterhelweg L et al (2008) Cadaver dogs–a study on detection of contaminated carpet squares. Forensic Sci Int 174(1):35–39

Paczkowski S, Schütz S (2011) Post-mortem volatiles of vertebrate tissue. Appl Microbiol Biotechnol 91(4):917–935

Pandey SK, Kim K-H (2011) Human body-odor components and their determination. Trends Anal Chem 30(5):784–796

Patterson DG et al (2011) Cryogenic zone compression for the measurement of dioxins in human serum by isotope dilution at the attogram level using modulated gas chromatography couple to high resolution magnetic sector mass spectrometry. J Chromatogr A 1218:3274–3281

Phillips JB, Beens J (1999) Comprehensive two-dimensional gas chromatography: a hyphenated method with strong coupling between the two dimensions. J Chromatogr A 856:331–347

Ramírez N et al (2010) Comparative study of solvent extraction and thermal desorption methods for determining a wide range of volatile organic compounds in ambient air. Talanta 82(2):719–727

Ramos L (2009) Comprehensive two dimensional gas chromatography. Elsevier, Amsterdam
Ryan D, Marriott P (2003) Comprehensive two-dimensional gas chromatography. Anal Bioanal Chem 376(3):295–297
Ryan D, Morrison P, Marriott P (2005) Orthogonality considerations in comprehensive two-dimensional gas chromatography. J Chromatogr A 1071(1–2):47–53
Seeley JV, Seeley SK (2013) Multidimensional gas chromatography: fundamental advances and new applications. Anal Chem 85(2):557–578
Seeley JV et al (2012) Stationary phase selection and comprehensive two-dimensional gas chromatographic analysis of trace biodiesel in petroleum-based fuel. J Chromatogr A 1226:103–109
Semard G et al (2011) Comparative study of differential flow and cryogenic modulators comprehensive two-dimensional gas chromatography systems for the detailed analysis of light cycle oil. J Chromatogr A 1218(21):3146–3152
Shellie R, Marriott P, Morrison P (2001) Concepts and preliminary observations on the triple-dimensional analysis of complex volatile samples by using GC×GC−TOFMS. Anal Chem 73(6):1336–1344
Stadler S et al (2012) Analysis of synthetic canine training aids by comprehensive two-dimensional gas chromatography-time of flight mass spectrometry. J Chromatogr A 1255:202–206
Stadler S et al (2013) Characterization of volatile organic compounds from human analogue decomposition using thermal desorption coupled to comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry. Anal Chem 85(2):998–1005
Statheropoulos M et al (2005) Preliminary investigation of using volatile organic compounds from human expired air, blood and urine for locating entrapped people in earthquakes. J Chromatogr B Anal Technol Biomed Life Sci 822(1–2):112–117
Statheropoulos M et al (2006) Discriminant analysis of volatile organic compounds data related to a new location method of entrapped people in collapsed buildings of an earthquake. Anal Chim Acta 566(2):207–216
Statheropoulos M et al (2007) Environmental aspects of VOCs evolved in the early stages of human decomposition. Sci Total Environ 385(1–3):221–227
Statheropoulos M et al (2011) Combined chemical and optical methods for monitoring the early decay stages of surrogate human models. Forensic Sci Int 210(1–3):154–163
Vass AA et al (2004) Decompositional odor analysis database. J Forensic Sci 49(4):1–10
Vass AA et al (2008) Odor analysis of decomposing buried human remains. J Forensic Sci 53:384–391

Chapter 21
An Investigation of the Degradation of Polymeric Grave Goods in Soil Environments

C. Sullivan, B.H. Stuart, and P.S. Thomas

Abstract Plastic materials are a source of items that may be located in clandestine grave sites. Knowledge of their type and state of preservation or deterioration may provide a valuable resource for the identification of a victim or perpetrator. This study involves an examination of the effect of the nature of the soil environment on the structural properties of two common polymers, poly(vinyl chloride) and nylon, over a period of 18 months. These polymers represent common types of plastic sheeting and carpet material that may be used to wrap a body. Infrared spectroscopy and scanning electron microscopy have been used to monitor the structural changes that occur to these polymers in a soil environment and degradation mechanisms are proposed.

21.1 Introduction

A clandestine burial may be accompanied by materials such as clothing, other textile material, tools, weapons or even plastic or paper products. Such items may provide valuable information that establishes the identity of a victim or a perpetrator. The nature of a burial environment affects the state of preservation of grave goods (Janaway 1996, 2008). Such materials may be exposed to a variety of conditions, such as differing soil types, climate or exposure to body fluids. The environment to which these materials are exposed may be responsible for the varying degrees of preservation or the rate at which materials degrade in a burial environment. The acceleration or inhibition of the material degradation processes affects the interpretation of a burial site. There have been relatively few reported forensic studies about how the structure of various material types may be specifically affected by soil burial, with much of the reported work involving physical descriptions of the degraded materials.

C. Sullivan (✉) • B.H. Stuart • P.S. Thomas
Centre for Forensic Science, University of Technology, Sydney, NSW 2007, Australia
e-mail: Clare.Sullivan@uts.edu.au

H. Kars, L. van den Eijkel (eds.), *Soil in Criminal and Environmental Forensics*,
Soil Forensics, DOI 10.1007/978-3-319-33115-7_21

Synthetic polymers are used for a variety of purposes and for this study two examples of polymers that might potentially be found at a clandestine burial site are examined. Polymers is in sheet form may be used to wrap a body prior to disposal or burial and a number of case studies have reported the use of plastic shower curtains as a means of covering a body. Shower curtains are commonly manufactured using the polymer poly(vinyl chloride) (PVC). Carpets manufactured using synthetic polymer fibres also provide an example of material used for the disposal of human remains. Nylon is the most common synthetic material used in the manufacture of carpet with nylon 6 and nylon 6,6 being the particular structural types of this polyamide that are widely used for carpet production (Anton and Baird 2005).

For the current study, the effects of various environmental factors on the structural and chemical properties of polymers that can be encountered as grave goods are being investigated. Buried materials have been systematically exhumed from model soil environments, with parameters including soil type and moisture content being controlled. Analytical techniques, including infrared spectroscopy and scanning electron microscopy are being employed to examine the exhumed specimens.

21.2 Materials and Methods

Model soil environments were prepared in sealed polyethylene boxes with ventilation holes inserted above soil level. Five environments were prepared for the current study: loam soil, a 50:50 clay: loam soil mixture, sand, wet soil and dry soil. Commercially obtained loam soil, builder's clay and river sand were used to produce the model environments. The reference soil environment was established using loam soil at pH 7 and 'as received' moisture content. The wet soil environment was created by the addition of distilled water and this was monitored using a moisture meter with additional portions of distilled water been added when necessary. The dry soil environment was established by placing loam soil in a vacuum oven at 60 °C for 12 h. Commercial plasticised PVC sheeting of 70 μm thickness was cut into 5 × 20 cm pieces and buried in each soil environment. A preliminary study of nylon carpet (comprised of 0.4 mm × 10 mm fibre bundles) was carried out on the burial of 5 × 20 cm pieces in a wet environment. All specimens were buried at a depth of 5 cm below the surface (total depth 10 cm). The boxes were stored at room temperature in the dark. Sampling was carried out on a 3 month basis for a period of 18 months. Exhume specimens were rinsed with distilled water to remove residual soil. Following air-drying, the specimens were stored in polyethylene bags prior to analysis.

PVC specimens were analysed using a Nicolet Magna IR 6700 Fourier transform infrared spectrometer equipped with a liquid nitrogen cooled mercury cadmium telluride detector. The specimens were examined using an attenuated total reflectance (ATR) sampling accessory. The spectra were recorded over a range of 4000–500 cm^{-1} and 128 scans were collected with a resolution of 4 cm^{-1}. Samples were repeated in duplicate. Nylon samples were analysed using a Cary 630 Fourier transform infrared

spectrometer and sampled using a diamond ATR accessory. The spectra were recorded over a range of 4000–600 cm^{-1} and 32 scans were collected with a resolution of 2 cm^{-1}. Samples were repeated in duplicate.

A FEI Quanta 200 ESEM and a Zeiss Evo SEM was used for the qualitative analysis of the surface morphology of the polymer specimens. Specimens were secured onto the sample holder using a carbon tab. Both instruments had an accelerating voltage of 20 kV using H_2O as the chamber gas. The FEI Quanta 200 was used with a spot size of 4.0 and pressure of 130 Pa while the Zeiss Evo was used with a spot size of 5.0 and pressure of 105 Pa.

21.3 Results and Discussion

21.3.1 Poly(Vinyl Chloride)

Figure 21.1a illustrates the infrared spectrum of the PVC film prior to burial. The spectrum shows representative bands associated with the polymer itself, as well as distinctive bands associated with the plasticiser content. Typically PVC sheeting contains about 10–30 % plasticiser content in order to introduce flexibility into the polymer. The spectrum shows bands associated with bis(2-ethylhexyl) phthalate, a common phthalate ester plasticiser. Characteristic plasticiser bands are observed at 1070, 1120, 1465, 1578 and 1595 cm^{-1} and a strong band due to C=O stretching at 1720 cm^{-1} (Marcilla et al. 2008; ASTM Standard D2124 2011; Muralisrinivarasan 2012). Infrared analysis of an extract of the plasticiser from the PVC into tetrahydrofuran was used to confirm the identity of the plasticiser as bis(2-ethylhexyl) phthalate as observed in Fig. 21.1b. The fingerprint region of the plasticised PVC spectrum below 1500 cm^{-1} is complex with overlapping plasticiser and polymer bands,

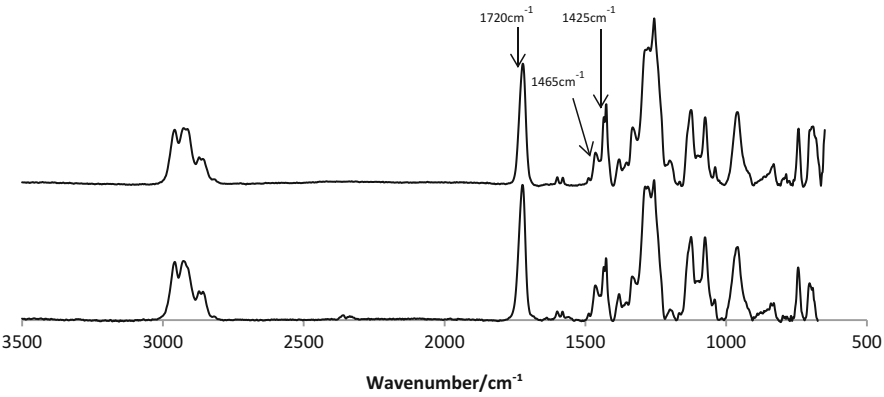


Fig. 21.1a FTIR spectra of PVC prior to burial (*bottom*) and after burial in a wet soil environment for 12 months (*top*)

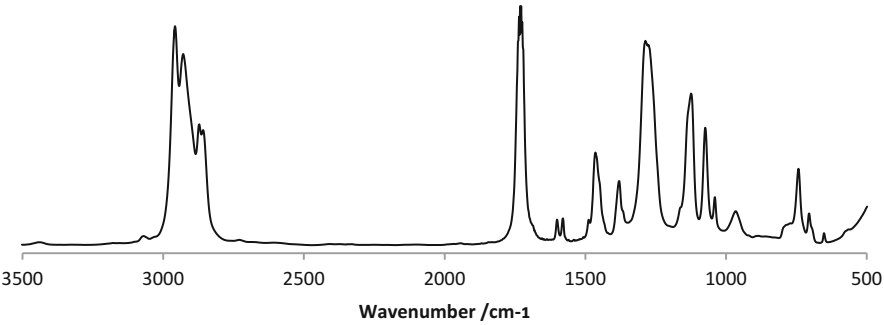


Fig. 21.1b FTIR spectra of bis(2-ethylhexyl) phthalate plasticizer in PVC

but there are bands at 955 and 1425 cm^{-1} that may be assigned to the polymer structure (Ramesh et al. 2007; Marcilla et al. 2008; Muralisrinivarasan 2012) Weak bands are also observed near 2300 cm^{-1} in the spectrum of the PVC sheeting prior to burial. These may be attributed to a phosphate ester, which are commonly used in PVC formulations as flame retardants (Berard et al. 2005). These bands are not observed in the PVC after burial.

Inspection of the spectra collected for different burial environments reveals changes to the plasticiser bands as a function of burial duration. An example spectrum is illustrated in Fig. 21.1a, showing the spectrum of PVC buried in wet soil for 12 months. The ratio of the absorbance of the 1720 cm^{-1} plasticiser band relative to the 1425 cm^{-1} PVC band decreases with burial time in each environment (Fig. 21.2). The principal plasticiser bands at 1720 and 1465 cm^{-1} have been referenced to a 1425 cm^{-1} polymer band as these bands are relatively clear of overlap with adjacent bands. However, the slopes obtained for each plot do vary according to environment and the results are summarised in Table 21.1. The slopes for the 1720/1425 plots for the wet, clay and sand environments indicate a sharper decline in plasticiser content compared to the reference and dry environments. The presence of water in the soil appears responsible for the loss of at least surface plasticiser (ATR spectroscopy samples the sample to a micrometre level), with the rate of removal more than doubled compared to the reference soil as determined from the relative slopes of the decrease in the 1720 cm^{-1} band with time. The plasticiser is not water soluble so is not being dissolved in the moisture present in the soil environment – further experimentation is required to determine the nature of the loss mechanism (e.g. enhanced microbial activity). The clay and sand environments, which also show a greater rate of reduction in the plasticiser content, are potentially capable of retaining a greater degree of moisture than the reference soil, so water is connected to the mechanism responsible for the removal of surface plasticiser. The dry environment with moisture removed shows similar behaviour to the reference soil.

There is also an indication that the 1465/1425 cm^{-1} ratios decrease with burial time and are similarly dependent on soil environment, although the changes to this ratio are less distinct than those observed for the 1720/1425 ratio. The 1465/1425

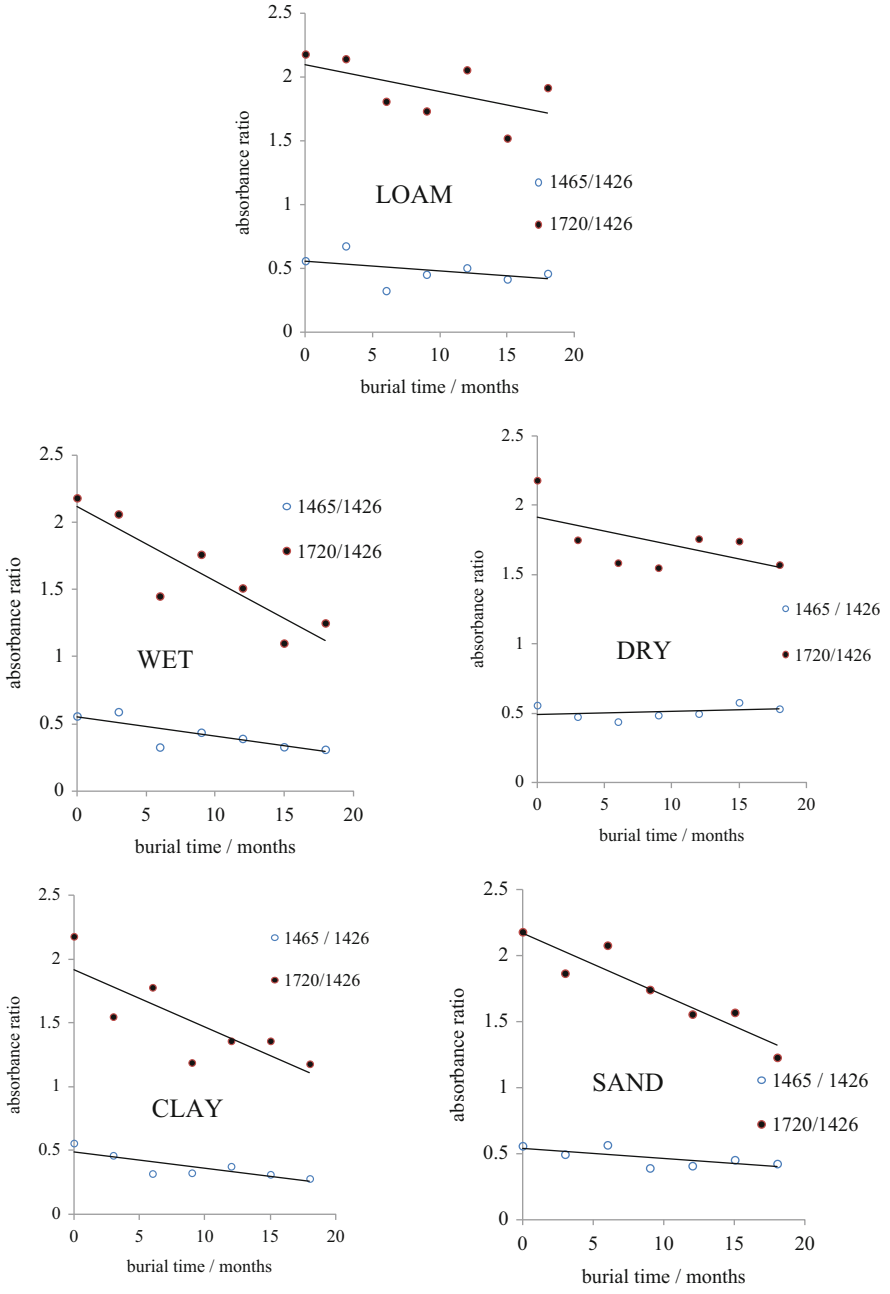


Fig. 21.2 Infrared absorbance ratios as a function of burial time for PVC specimens

Table 21.1 Slopes of absorbance ratios versus burial time plots for PVC specimens

Burial environment	1720/1426 cm^{-1} ratio	1465/1426 cm^{-1} ratio
Loam	−0.021	−0.008
Wet	−0.055	−0.014
Dry	−0.020	0.002
Clay	−0.045	−0.013
Sand	−0.047	−0.008

ratio plots are also shown in Fig. 21.2 and the slopes calculated for each plot are listed in Table 21.1. It is noted that the slopes produced by the different ratio calculations differ in each environment, but this is likely to be a consequence of the measurement of different penetration depths at the 1720 and 1495 cm^{-1} bands in the ATR spectra: there is a greater penetration depth at 1495 cm^{-1}. The observation of different slopes may provide an indication that there is a higher concentration of plasticiser at the polymer surface.

The loss of plasticiser is recognised as a PVC degradation process known to occur due to an increase in temperature (Murase et al. 1994; Jakubowicz et al. 1999). The process has been shown to be linear with time when the process is due to evaporation. It is noted that there is no indication of the other main degradation process associated with PVC, in particular, dehydrochlorination. Such a mechanism involves the loss of chlorine and the formation of C=C bonds (Singh and Sharma (2008). No changes in the 1680 cm^{-1} region due to the appearance of the C=C bond in the spectra obtained in the current study were observed so there is no indication of dehydrochlorination in these environments in the time frame studied.

Changes to the surface morphology of the polymer surface have been examined using SEM. A typical micrograph of the PVC prior to burial is shown in Fig. 21.3. The surface appears smooth with minimal variation in texture across the surface. A micrograph is also shown in Fig. 21.3 of the surface of PVC after burial in clay soil for 18 months. A notable change in surface texture is observed, with variation illustrated by patches if lighter regions distributed across the surface of the polymer. These regions may correlate with areas of plasticiser loss at the surface as loss of this additive is likely to result in a change in surface texture. The presence of very small bright spots is believed to be residual particles remaining from soil contact.

21.3.2 Nylon

A preliminary investigation of the effect on nylon carpet fibres of burial in wet soil has been carried out. Figure 21.4 illustrates SEM images obtained for a nylon fibre prior to burial and one exposed to wet soil for 18 months. The fibre prior to burial is smooth and even in texture. After wet burial, regions of polymer surface with an apparently rougher texture are observed to be distributed on the fibre surface. Such regions are indicative of surface modification and similar changes have been

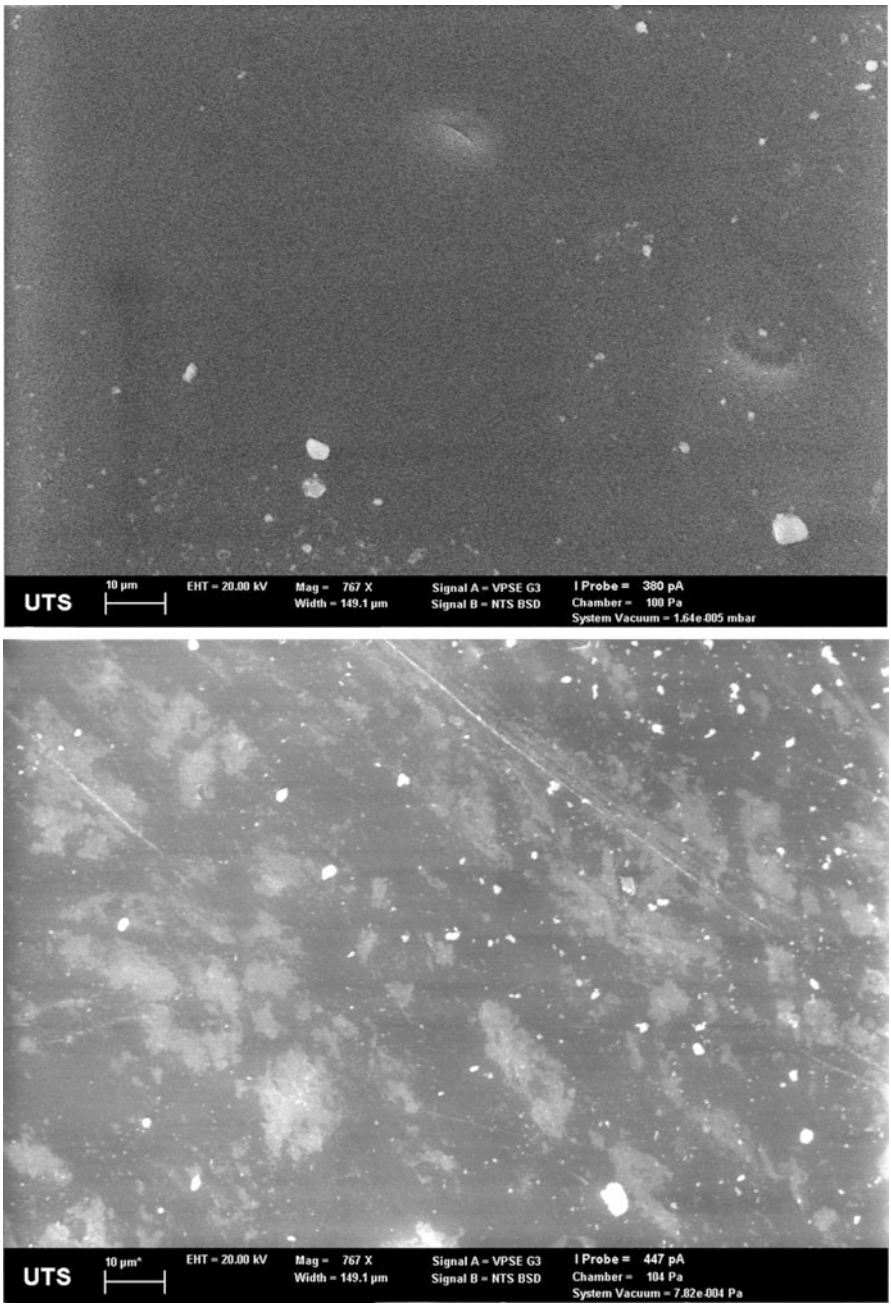


Fig. 21.3 Scanning electron micrographs (767x magnification, horizontal field width of 149 µm) of PVC fibre prior to burial (*top*) and after burial in a clay environment for 18 months (*bottom*)

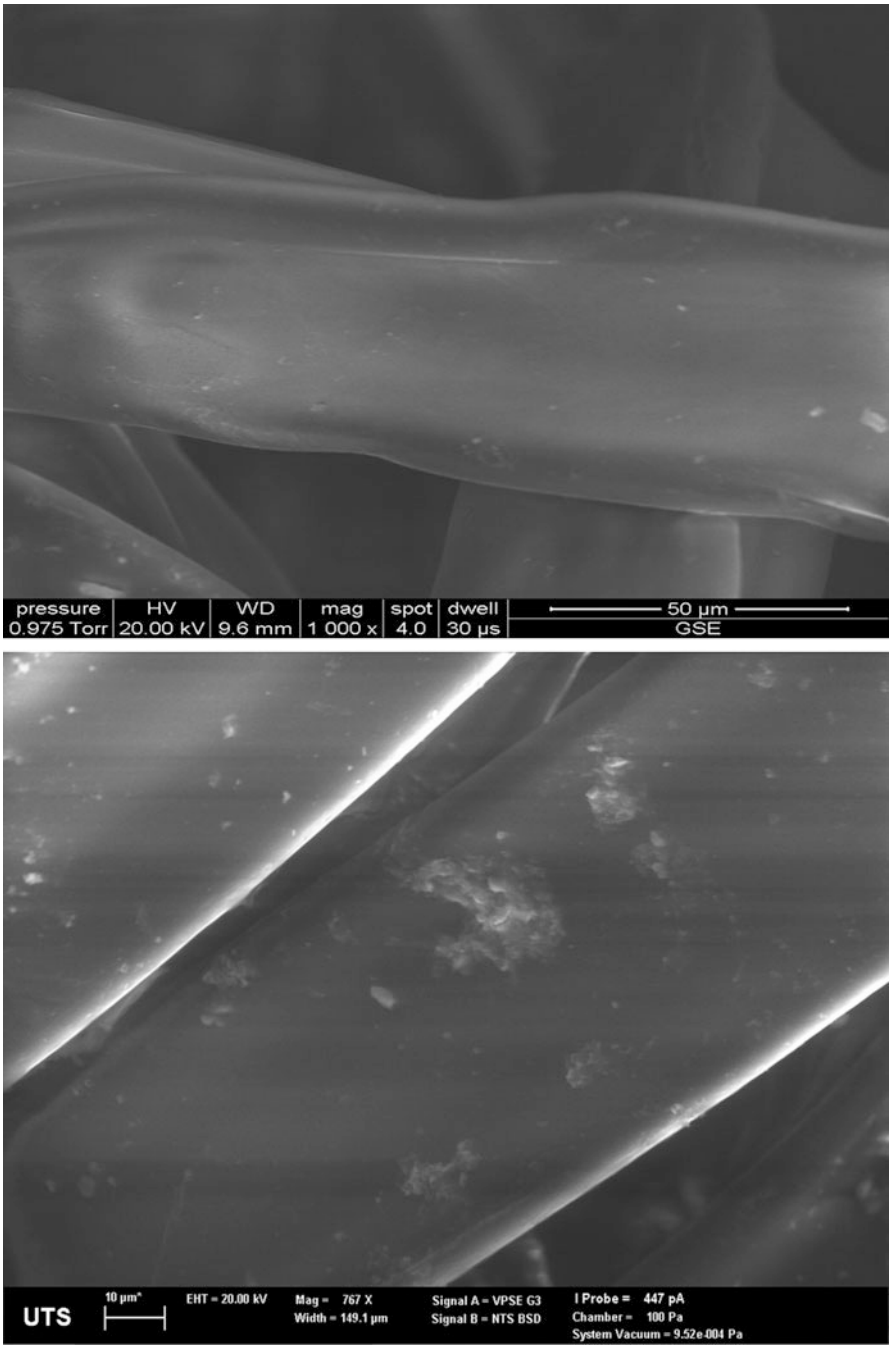


Fig. 21.4 Scanning electron micrographs of nylon fibre prior to burial (*top*; 1000x magnification, horizontal field width of 149 µm) and after burial in a wet environment for 18 months (*bottom*; 767x magnification, horizontal field width of 149 µm)

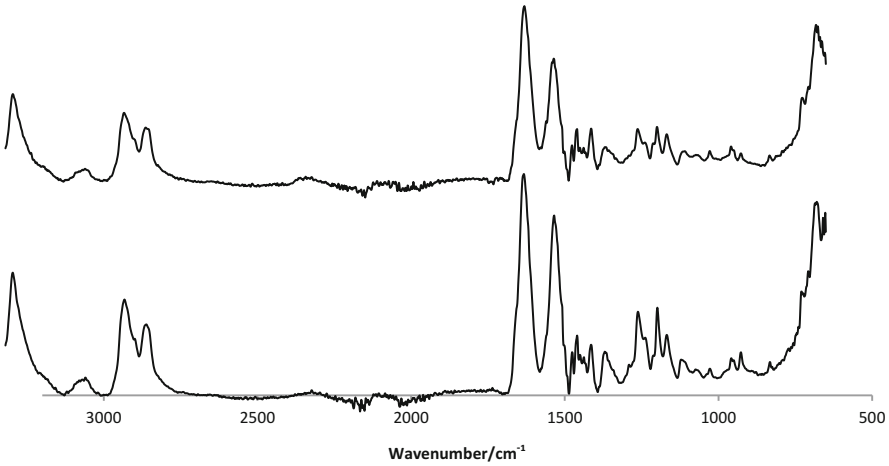


Fig. 21.5 FTIR spectra of nylon fibres prior to burial (*bottom*) and after burial in wet soil for 18 months (*top*)

reported for degraded nylon resulting from exposure to proteolytic enzymes (Parvinzadeh et al. 2009).

The FTIR spectrum of nylon after exposure to 18 months burial in wet soil, as well as the spectrum for nylon prior to burial, is illustrated in Fig. 21.5. There are some minor changes to the spectrum after exposure to wet soil. There are decreases in the intensity of bands at 1370, 1180 and 1140 cm^{-1} that are attributed to C-N-H, CH_2-NH and C-O deformations, respectively (Goncalves et al. 2007). A decrease in intensity of the combination band (N-H deformation and C-N stretching) at 3080 cm^{-1} is also observed after burial. Such spectral changes have been associated with the oxidation of the nylon structure via the amide nitrogen (Mikolajewski et al. 1964; Goncalves et al. 2007). It is also noted that the ratio of the intensities of the amide I (predominantly C = O stretching) and II (N-H bending and C-N stretching) bands at 1630 and 1535 cm^{-1}, respectively, changes after exposure to the wet soil environment. A change to this ratio has been associated to changes in the nature of the hydrogen bonding in nylon (Iwamoto and Murase 2003). It is noted that a band near 1680 cm^{-1} is not noted in the spectrum of the buried nylon. Observation of such a band is known to indicate nylon oxidation (Colin et al. 1981).

The changes observed to the nylon carpet fibres remain relatively minor after 18 months exposure to wet soil. Although nylon is known to absorb water after long term exposure to moisture, consideration should be given to the surface treatment of the fibres during manufacture. Nylon carpets are chemically treated to promote stain resistance as, despite this polymer's attractive physical properties for use in carpets, they are susceptible to staining. The stainblockers used are typically formaldehyde polycondensates of sulfonated, substituted phenols or naphthols (Burkinshaw and Son 2008). The presence of such agents may be responsible for inhibiting the changes to the nylon structure and the nature of pre-treatments will be considered in further studies.

21.4 Conclusions

This study has demonstrated the potential of infrared spectroscopy as a technique for characterising and monitoring the changes to polymer based materials buried in a variety of soil environments. For the time frame studied, the main mechanism for change to buried PVC is the loss of plasticiser and the rate of loss appears to be associated with nature of soil environment (i.e. wet or dry, loam, clay or sand). A more detailed statistical study is being carried out in order to test the validity of these trends. A preliminary study of nylon exposed a wet soil environment has indicated that long term burial can result in an oxidation process that can be monitored by infrared spectroscopy. The burial of nylon in a wider range of environments is also being investigated. Other analytical techniques including thermal analysis are also being employed to gain more a more complete picture of the changes to the polymers as a result of burial.

References

Anton A, Baird BR (2005) Polyamides, fibres, Kirk-Othmer encyclopedia of chemical technology. Wiley, Chichester

ASTM Standard D2124 (2011) Standard test method for analysis of components in poly(vinyl chloride) compounds using an infrared spectrophotometric technique. ASTM International, West Conshohocken

Berard MT, Daniels CA, Summers JW, Wilkes CE (2005) PVC handbook. Hanser, Munich

Burkinshaw SM, Son YA (2008) Stain resist treatments for nylon 6,6. Dyes Pigments 76:650–655

Colin G, Cooney JD, Carlsson DJ, Wiles DM (1981) Deterioration of plastic films under soil burial conditions. J Appl Polym Sci 26:509–519

Goncalves ES, Poulsen L, Ogilby PR (2007) Mechanism of the temperature-dependent degradation of polyamide 66 films exposed to water. Polym Degrad Stab 92:1977–1985

Iwamoto R, Murase H (2003) Infrared spectroscopic study of the interactions of nylon 6 with water. J Polym Sci B Polym Phys 41:1722–1729

Jakubowicz I, Yarahmadi N, Gevert T (1999) Effects of accelerated and natural ageing on plasticised poly(vinyl chloride) (PVC). Polym Degrad Stab 66:415–421

Janaway RC (1996) The decay of buried human remains and their associated materials. In: Hunter J, Roberts C, Martin A (eds) Studies in crime: an introduction to forensic archaeology. Routledge, London, pp 58–85

Janaway RC (2008) The decomposition of materials associated with buried cadavers. In: Tibbet M, Carter DO (eds) Soil analysis in forensic taphonomy: chemical and biological effects of buried human remains. CRC Press, Boca Raton, pp 153–202

Marcilla A, Garcia S, Garcia-Quesada JC (2008) Migrability of PVC plasticisers. Polym Test 27:221–233

Mikolajewski E, Swallow JE, Webb MW (1964) Wet oxidation of undrawn nylon 66 and model amides. J Appl Polym Sci 8:2067–2093

Muralisrinivarasan NS (2012) Polymer testing: new instrumental methods. Momentum Press, New York

Murase A, Sugiura M, Araga T (1994) An infrared spectroscopic study of the migration of a plasticiser in poly(vinyl chloride) resins. Polym Degrad Stab 43:415–422
Parvinzadeh M, Assefipour R, Kiumarsi A (2009) Biohydrolysis of nylon 6,6 fibre with different proteolytic enzymes. Polym Degrad Stab 94:1197–1205
Ramesh S, Leen KH, Kumutha K, Arof AK (2007) FTIR studies of PVC/PMMA blend based polymer electrolytes. Spectrochim Acta A 66:1237–1242
Singh B, Sharma N (2008) Mechanistic implications of plastic degradation. Polym Degrad Stab 93:561–584

Index

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