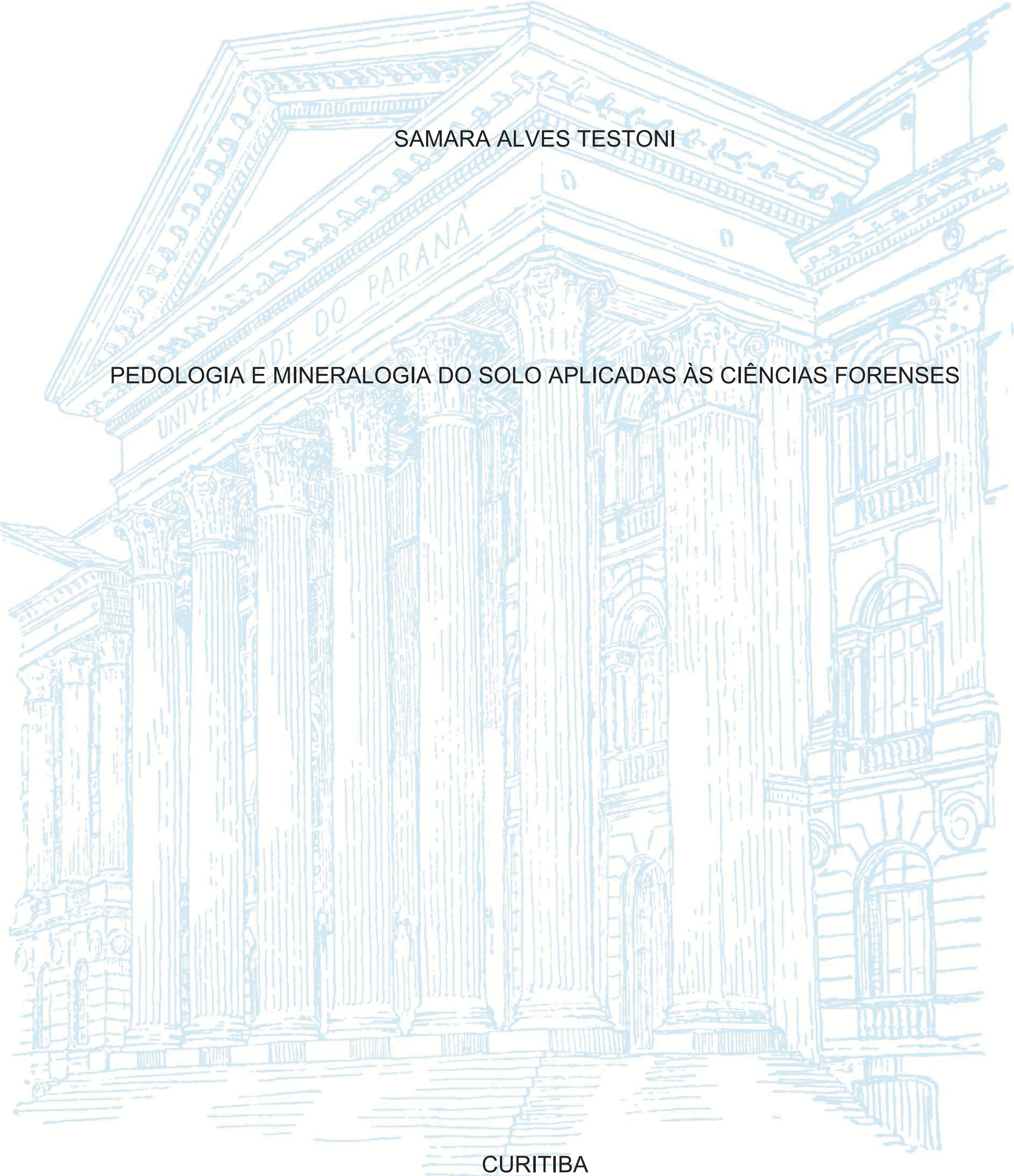
UNIVERSIDADE FEDERAL DO PARANÁ



SAMARA ALVES TESTONI

PEDOLOGIA E MINERALOGIA DO SOLO APLICADAS ÀS CIÊNCIAS FORENSES

CURITIBA

2019

SAMARA ALVES TESTONI

PEDOLOGIA E MINERALOGIA DO SOLO APLICADAS ÀS CIÊNCIAS FORENSES

Tese apresentada ao curso de Pós-Graduação em Ciência do Solo, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutora em Ciência do Solo.

Orientador: Prof. Dr. Vander de Freitas Melo

CURITIBA
2019

T345p

Testoni, Samara Alves
Pedologia e mineralogia do solo aplicadas às ciências forenses / Samara Alves Testoni. - Curitiba, 2019.
190 p.: il.

Tese (Doutorado) - Universidade Federal do Paraná. Setor de Ciências Agrárias, Programa de Pós-Graduação em Ciência do Solo.
Orientador: Vander de Freitas Melo

1. Ciências forenses. 2. Ciência do solo. 3. Argila. 4. Caulinita. 5. Mineralogia. I. Melo, Vander de Freitas (Orientador) . II. Título. III. Universidade Federal do Paraná.

CDU 631.4: 343.98

Sistema de Bibliotecas/UFPR, Biblioteca de Ciências Agrárias, Douglas Alex Jankoski – CRB 9/1167

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIENCIAS AGRARIAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO CIÊNCIA DO SOLO -
40001016014P4

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIA DO SOLO da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **SAMARA ALVES TESTONI** intitulada: **PEDOLOGIA E MINERALOGIA DO SOLO APLICADAS ÀS CIÊNCIAS FORENSES**, sob orientação do Prof. Dr. VANDER DE FREITAS MELO, que após após terem inquirido a aluna e realizado a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 06 de Setembro de 2019.

VANDER DE FREITAS MELO
Presidente da Banca Examinadora

ANTONIO CARLOS SARAIVA DA COSTA
Avaliador Externo (UNIVERSIDADE ESTADUAL DE MARINGÁ)

RODRIGO STUDART CORREA
Avaliador Externo (UNIVERSIDADE DE BRASÍLIA)

Eloana Bonfleur
ELOANA JANICE BONFLEUR
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

GIOVANA C. POGGERE
GIOVANA CLARICE POGGERE
Avaliador Externo (UNIVERSIDADE TECNOLÓGICA FEDERAL DO PARANÁ)

Com todo meu coração dedico esta tese aos meus pais José Carlos Testoni e Dulcenalva Alves Testoni pelo suporte mais verdadeiro e incondicional que já recebi. Dedico também à minha amada tia Dulcinea Alves, por me conduzir aos estudos de Agronomia.

AGRADECIMENTOS

Esta obra só foi possível pois estive amparada por uma rede de pessoas repleta de apoio financeiro, afetivo e profissional, composta por minha família, por amigos verdadeiros, por professores, servidores e profissionais magníficos, e também, por excelentes instituições.

Agradeço a Deus.

Agradeço à minha família, um verdadeiro refúgio, repleto de amor, incentivo, suporte e compreensão. Obrigada por entenderem minha ausência por longos períodos, desde minha saída de casa aos 18 anos para cursar Agronomia na Serra Catarinense, em Lages. De modo especial agradeço aos meus pais Carlos e Nalva, que apesar das dificuldades no início da vida, sempre priorizaram os estudos, o conhecimento, o investimento em livros, em cursos de informática, inglês, entre outros. O esforço de vocês valeu a pena. Agradeço à minha irmã Marcela Testoni, por utilizar da melhor maneira o seu dom em aconselhar, em ouvir e em ajudar. Sua voz doce e seu carinho foram imprescindíveis em minha estrutura emocional. Agradeço também ao meu cunhado Carlos por sua alegria contagiante e pelo modo otimista como encara a vida. Agradeço à minha tia Dulcinea, por seu amor, por inspirar conhecimento, me incentivar a estudar e a ser segura no que faço. Agradeço ao meu tio Dailso, à tia Marli, à vó Maria, à minha querida prima Fabiana e ao João, pelo carinho e amizade, e a todos de minha família. Agradeço à Ivone, por gentilmente me acolher em sua casa nos primeiros meses de Doutorado, até que eu encontrasse uma residência fixa em Curitiba.

Agradeço à segunda família que tive nesta jornada. Obrigada meu amor Wilian. Quando eu achava que não havia mais amor sincero, você apareceu. Quando eu achava que não precisava me superar, melhorar como profissional, você apareceu, me incentivando, dando suporte, e por vezes, acreditando em mim mais do que eu mesma. Seu amor é um combustível. Obrigada à minha sogrinha querida Marlene pelo carinho e pela ternura comigo.

Agradeço aos amigos verdadeiros que tive o privilégio de compartilhar a vida. Em especial à Bruna, Priscila, Mariana, Ricardo, Edvaldo, Ismael, Danilo, Lenir e muitos outros. A vida realmente fica mais leve próxima a vocês.

Agradeço à Universidade Federal do Paraná e ao Programa de Pós-Graduação em Ciência do Solo por disponibilizar à sociedade excelentes Professores e profissionais. Saibam que todo este crescimento profissional é fruto do exemplo de vocês. De modo especial agradeço ao Professor, orientador e grande amigo, Vander de Freitas Melo, pela pessoa extraordinária que é, por sua dedicação à ciência, por abraçar a causa dos Solos Forenses e me fazer acreditar que podemos conquistar muitas coisas com dedicação e disciplina. Agradeço também por seu exemplo de vida, e por me mostrar que sempre é possível evoluir sem perder as raízes e a humildade, independente de onde estivermos. Agradeço com carinho ao querido Professor Jaime Antonio de Almeida, por me descobrir em uma sala de aula repleta de alunos, 10 anos atrás, por acreditar e confiar em mim e, por gentilmente me apresentar o incrível mundo da pesquisa científica em solos. Agradeço à Técnica do Laboratório de Mineralogia do Solo, Maria Carvalho, pela excelência de seu trabalho dedicado aos alunos, por seu cuidado e paciência e por sua amizade e carinho. Agradeço também à Técnica Fabiana por me receber diversas vezes para análises no Laboratório de Biogeoquímica. Agradeço à Denise de Conti, Secretária do Programa de Pós-Graduação em Ciência do Solo, por oferecer aos alunos seu magnífico trabalho e pelo atendimento diário, feito com carinho e muita eficiência. Obrigada por sua amizade, sua torcida e por seus conselhos valiosos. Agradeço o tempo de leitura e a dedicação dos Professores Giovana Clarice Poggere, Antônio Carlos Saraiva da Costa, Rodrigo Studart Corrêa e Eloana Janice Bonfleur, por participarem como membros de banca nesta tese.

Agradeço à Polícia Federal e à Polícia Científica do Paraná por disponibilizar excelentes profissionais para este trabalho. De modo especial, agradeço ao Perito Criminal Federal Fábio Augusto da Silva Salvador, por dar asas aos meus sonhos com sua eloquência e paixão pela ciência, e também, por toda sua colaboração ao longo destes quatro anos. Agradeço aos Peritos Paulo Akira Kunii, Alexandre de Lara, Joice Malakoski, Jorge de Andrade Junqueira Neto e Edimar Cunico.

Agradeço à instituição de fomento à pesquisa Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior – CAPES, por subsidiar minha bolsa de estudos desde a iniciação científica na graduação até o término do doutorado. Agradeço também pela oportunidade única e por todo o suporte para estudar parte do doutorado no exterior.

Agradeço ao James Hutton Institute, por me receber de portas abertas, como uma profissional de solos forenses. De modo especial, dedico um agradecimento à Professora Lorna Anne Dawson, por transmitir sua experiência e conhecimentos de maneira espetacular, e por me fazer admirar ainda mais a pesquisa em solos. Agradeço à Jasmine Ross e a Jason Owen pelo tempo extremamente valioso dedicado a ensinar técnicas analíticas em solos forenses.

Muito obrigada a todos.

"Tenha bons pensamentos, porque os seus pensamentos se transformam em suas palavras.
Tenha boas palavras, porque as suas palavras se transformam em suas ações.
Tenha boas ações, porque as suas ações se transformam em seus hábitos.
Tenha bons hábitos, porque os seus hábitos se transformam em seus valores.
Tenha bons valores, porque os seus valores se transformam no seu próprio destino."

(Gandhi)

RESUMO

O solo contém propriedades que o distingue em diversos ambientes. Esta diferenciação resulta da ação simultânea de fatores (material de origem, relevo, clima, organismos e tempo) e processos (adição, perda, translocação e transformação), e lhe confere grande heterogeneidade, expressa em suas características físicas, químicas e mineralógicas. Esta diversidade de solos constitui uma importante ferramenta em investigações criminais, pois, em uma pequena escala de variabilidade espacial é possível encontrar solos com características distintas. Nesse sentido, análises físicas (distribuição do tamanho de partículas), químicas (extrações totais com ácido fluorídrico, extrações químicas sequenciais com ditionito-citrato-bicarbonato de sódio, oxalato de amônio e hidróxido de sódio), orgânicas (análise de marcadores de cera), mineralógicas (análise termogravimétrica, difração de raios-X) e microscópicas (microscopia eletrônica de varredura associada à espectroscopia por energia dispersiva) foram efetuadas para investigar crimes relacionados aos solos. Os dados obtidos foram submetidos à estatística multivariada via Análise de Componentes Principais e Similaridade de Bray-Curtis a fim de se observar as possíveis correlações entre as amostras. Buscou-se coletar amostras de solos em diferentes situações (cenas de crime reais e simuladas) e em ambientes distintos (variados materiais de origem e uso de solo) para enriquecer a matriz analítica de dados. O trabalho foi dividido em seis capítulos, sendo três em crimes simulados e três em cenas reais de crimes que produziram vestígios de solos: Cap. 1 - Avaliação de vestígios de solos forenses em cena de crime: roubo de um cofre no Brasil; Cap. 2 - Validação de Procedimento Operacional Padrão (POP) para solos forenses no Brasil: aplicação em amostragem simulada às cegas; Cap. 3 - Análise de biomarcadores de ceras de plantas no solo: aplicação em investigações forenses; Cap. 4 - Relevância da fração areia de solos subtropicais em estudos forenses; Cap. 5 - A análise de um pequeno torrão de solo pode ajudar a resolver um caso de assassinato?; e Cap. 6 - Validação de extrações químicas sequenciais no uso de vestígios de solo como evidência em investigações criminais: um caso de assassinato. Foi possível obter resultados potencialmente discriminadores, pois amostras de um mesmo local e formadas pelo mesmo material de origem puderam ser diferenciadas, bem como amostras de locais distintos. O protocolo de coleta de amostra desenvolvido na forma de Procedimento Operacional Padrão pela academia e pela Polícia Técnica Federal foi calibrado a nível de campo para ser usado na rotina da Polícia Científica em todo o Brasil. Antes desse trabalho, os vestígios de solos eram, em sua maioria, descartados em cenas de crime. Nos dois capítulos em cena real de crime contra a vida foi possível colocar o suspeito (cap. 5 - caso ainda em julgamento) e o assassino (cap. 6 - caso encerrado) na cena do crime. No capítulo 5, foi desenvolvido um protocolo de análises sequenciais mineralógicas e químicas para avaliar uma quantidade limitada de vestígios de solo (0,5 g) coletada no veículo do suspeito. Os resultados permitem concluir que o veículo do suspeito esteve em contato com a borda da Estrada da Graciosa PR (PR-410), aproximadamente no local onde o corpo da vítima foi localizado. Dentre as abordagens analíticas sugeridas, as extrações químicas sequenciais nas frações mais finas do solo revelaram-se poderosas e expressivamente úteis para análises de solos com finalidade forense em todo o Brasil e em outros países.

Palavras-chave: Ciência Forense. Vestígios de Solos. Frações Argila e Silte. Extrações Químicas Sequenciais. Minerais amorfos. Caulinita. Óxidos de Ferro.

ABSTRACT

Soil contains properties that distinguish it in many environments. This differentiation results from the simultaneous action of factors (parent material, relief, climate, organisms and time) and processes (addition, loss, translocation and transformation), and gives it great heterogeneity, expressed in its physical, chemical and mineralogical characteristics. This diversity of soils is an important tool in criminal investigations, because on a small scale of spatial variability it is possible to find soils with different characteristics. In this way, physical (particle size distribution), chemical (total extractions with hydrofluoric acid, sequential chemical extractions with sodium dithionite-citrate-bicarbonate, ammonium oxalate and sodium hydroxide), organic (analysis of wax markers), mineralogical (thermogravimetric analysis, X-ray diffraction) and microscopic (scanning electron microscopy associated with dispersive energy spectroscopy) were performed to investigate soil-related crimes. Data were submitted to multivariate statistics via Principal Component Analysis and Bray-Curtis Similarity in order to observe possible correlations among the samples. Samples were collected in different situations (real and simulated crime scenes) and in different environments (various parent materials and soil use) to enrich the analytical data matrix. The work was divided into six chapters, three in simulated crimes and three in actual crime scenes that produced soil traces: Chapter 1 - Evaluation of traces of forensic soils in crime scene: robbery of a safety deposit box in Brazil; Chapter 2 - Validation of Standard Operating Procedure (SOP) for forensic soils in Brazil: application to a simulated blind sampling; Chapter 3 - Analysis of plant wax biomarkers in soil: application in forensic investigations; Chapter 4 - Relevance of the sand fraction of subtropical soils in forensic studies; Chapter 5 - Can the analysis of a small clod of soil help to solve a murder case?; and Chapter 6 - Validation of sequential chemical extractions in the use of soil traces as evidence in criminal investigations: a murder case. Potentially discriminatory results could be obtained for samples from the same site and formed from the same parent material could be differentiated, as well as samples from different sites. The sample collection protocol developed in the form of Standard Operating Procedure by the academy and the Federal Technical Police has been calibrated at field level for use in the Scientific Police routine throughout Brazil. Prior to this work, soil traces were mostly discarded at crime scenes. In both real-life crime scene chapters it was possible to place the suspect (Chapter 5 - case still on trial) and the killer (Chapter 6 - case closed) at the crime scene. In Chapter 5, a protocol of mineralogical and chemical sequential analyzes was developed to evaluate a limited amount of soil traces (0.5 g) collected in the suspect's vehicle (adhered to the external rearview mirror and the left front fender). The results allowed us to conclude that the suspect's vehicle was in contact with the edge of Graciosa PR Road (PR-410), approximately where the victim's body was located. Among the suggested analytical approaches, sequential chemical extractions in the finer fractions of the soil proved to be powerful and expressively useful for forensic soil analysis throughout Brazil and in other countries. Thus, the scientific methods developed in the present study can help society to elucidate various crimes.

Keywords: Forensic Science. Soils Traces. Clay and Silt Fractions. Sequential Chemical Extractions. Amorphous minerals. Kaolinite. Iron oxides.

SUMMARY

1 GENERAL INTRODUCTION

Soil contains properties that can distinguish it in most varied environments. These properties are the constitute a product of the interaction among external factors (parent material, relief, climate, organisms and time) and internal processes (addition, loss, translocation and transformation) that act on it and give it great variability expressed by their physical, chemical, morphological and mineralogical characteristics. Such heterogeneity, unique to each soil, makes it a potential source of information in crime scene investigations.

The history of Soil Forensic Science, or the use of this type of material in criminal investigations, dates back over 150 years (Fitzpatrick, 2011). The first documented report of use of forensic soils was in Berlin, in a crime occurred on a Prussian railroad in April 1856 in which a barrel filled with sand was found and previously was loaded with silver coins. Professor Ehrenberg from Berlin collected sandy soils samples from several points along the railway line. Using a microscope light, he examined characteristics of sandy soil particles, such as color and shape, to compare it with barrel soil and determine where that sand samples originated. Another known case occurred later in 1891. The Australian Professor Hans Gross, considered one of the pioneers of Forensic Science - due to the use of scientific expertise to solve legal issues - used microscopic analysis of soil traces collected from a shoe, to relate a suspect to a crime. In 1904, a forensic scientist named George Popp from Frankfurt examined soil, minerals and dust removed from shoes to identify them and to use in forensic purposes (Fitzpatrick, 2011).

Using a standardised methodology for analyses of forensic soil traces as well as soil-adherent traces (plants and organisms such as bacteria, fungi etc.) collected at crime scenes and on suspects and vehicles, Dawson (2013) was able to discriminate and compare samples, in Aberdeen, Scotland. The methods commonly applied were X-rays diffraction (XRD), elemental analysis, particle size distribution (PSD), scanning electron microscopy (SEM), gas chromatography coupled to mass spectrometer (GC-MS), stable isotope analysis, Fourier transformed infrared spectroscopy (FTIR), soil colour and analyses on soil microbiology, and their results revealed great potential to be presented as evidence in a court.

In a study conducted in Australia by Woods et al. (2014), traces of soil were collected and subjected to the use of spectroscopic techniques such as visible micro spectrophotometry, total attenuated reflectance and Fourier transformed infrared spectroscopy to evaluate the colour and particle size of forensic soil traces, as well as the use of spectroscopy. X-ray fluorescence and scanning electron microscopy. In the study, it was observed that the techniques were effective in discriminating the samples, especially particle differentiation of 'forensic size' soil samples.

The synchrotron light source to thoroughly analyse the complex phases that make up the mineralogical assemblage of a soil may be a potential technique that has been used in some works. Nakai et al. (2014) produced a database of samples composed by soil sediments collected at different points in Japan to map much of the Japanese territory to assist in criminal investigations. Results showed great mineralogical variability in the sediments sampled, making it necessary to restrict the insertion of only some of the most common minerals in the soil to identify them in the database. It was decided to collect only sediments, as they have a close relationship with the parent material of the soil, thus providing important aspects.

In this way, the main purpose of forensic soil analysis is associate soil, altered rock particles or samples taken from a given point, such as shoes, clothing, tools or vehicles, with a specific location or common origin (Fitzpatrick & Raven, 2016).

Performing forensic analyses on soil traces may reveal some difficulties. Despite the heterogeneity arising from the factors and processes previously mentioned, soils can present very similar characteristics, especially when the collected samples are close together. Another difficulty lies in developing a series of variables from a tiny amount of soil sample. For this reason, much of the study of soils for forensic purposes is limited to the application of a single analytical technique, usually of physical nature, generating a limited number of variables and restricting the comparability between distinguished samples.

In this scenario it was hypothesised that soils may constitute a powerful source of information in criminal investigations, when subjected to a standardised methodology with chemical, physical and mineralogical analyses. The general objective of this study was to collect soil traces from real and simulated crime scenes, in order to assess the characteristics of each soil trace and then, to promotes the traceability of the soil samples.

2 CHAPTER I: EVALUATION OF FORENSIC SOIL TRACES FROM A CRIME SCENE: ROBBERY OF A SAFETY DEPOSIT BOX IN BRAZIL[1]

2.1 RESUMO

Vestígios de solo podem ser usados como evidência em investigações criminais devido à sua transferibilidade, grande variabilidade e persistência. Amostras de solo originadas de uma cena de crime ocorrida na Região Metropolitana de Curitiba, Brasil foram analisadas em seus atributos físicos, químicos e mineralógicos.. Todos os resultados foram analisados estatisticamente usando uma análise multivariada (PCA) para verificar o posicionamento relativo dos vestígios de solo que foram recuperados de um cofre roubado do veículo suspeito de ser utilizado no transporte do cofre e dos locais onde o cofre foi supostamente aberto e o cofre depositado. Os métodos empregados foram eficazes na discriminação entre os locais de amostragem. O solo do local utilizado na abertura do cofre poderia ser excluído como sendo o local onde o solo que foi transferido para o cofre se originou, por ser diferente em muitas características.

Palavras-chave: ciência forense; solos forenses; evidência; análises químicas do solo forense; análises mineralógicas forenses do solo; análise de componentes principais; roubo.

2.2 ABSTRACT

Soil traces can be used as evidence in criminal investigations due their transferability, great variability and persistence. Soil samples originated from a crime scene that occurred in the Curitiba Metropolitan Region, Brazil. Physical, chemical and mineralogical analyses were carried out on the soil samples. All results were statistically analysed using multivariate analysis (PCA) to verify the relative positioning of soil traces which had been recovered from a stolen safety deposit box (SDB) from a vehicle suspected of being used in the SDB transportation, and from the site allegedly used in the opening of the SDB. The methods employed were effective in discriminating between the sampling sites. The soil from the site used in the opening of the SDB could be excluded as being the location where the soil transferred to the SDB originated, as it was different in many characteristics.

Keywords: forensic science; forensic soils; evidence; forensic soil chemical analyses; forensic soil mineralogical analyses; principal components analysis; theft.

---

[1] Published on Geological Society of London, Special Publications
DOI: https://doi.org/10.1144/SP492-2019-35.

2.3 INTRODUCTION

Soils can provide excellent evidence to test for links between people, tools, vehicles and crime scenes, potentially linking a soil trace on a person or an object (shoes, clothing, shovels, vehicles etc.) to a specific location (Pye 2007; Ritz et al. 2008; Ruffell and McKinley 2008; Dawson and Hillier, 2010; Murray 2011; Fitzpatrick and Raven 2012; Fitzpatrick, 2013;). The difficulties in examining soil traces (normally less than 1 g of sample) and evaluating the results can result in a lack of value in soil as forensic evidence. However, the different external factors of soil formation such as relief, time, organisms, parent rock and climate turn soil into a material with considerable variability (Aitkenhead et al., 2014), which can help differentiate soils from different places to assist Police in criminal investigations (Fitzpatrick and Raven 2012; Fitzpatrick, 2013; Fitzpatrick et al. 2017).

Soil forensics has also been studied in laboratory experiments in recent years (Woods et al., 2014; Corrêa et al., 2017). Several workers (e.g. Fitzpatrick and Raven, 2016; Pirrie et al. 2017) have recommended that standardized methodologies using X-rays powder diffraction, elemental analysis, particle size distribution, scanning electron microscopy, soil colour and soil microbiology be used to analyse soil samples from crime scenes, suspects and vehicles in Australia and United Kingdom. Prandel et al. (2017) utilized the XRD Rietveld method for characterizing minerals in clays from the metropolitan region of Curitiba, Brazil. In both studies the results allow the discrimination of different sites and the grouping of samples collected from the same site. Melo et al. (2008) proposed a soil sequential methodology for forensic purposes, with physical (textural and spectroscopy analyses), chemical (extractions with ammonium oxalate, sodium dithionite-citrate-bicarbonate and NaOH solution) and mineralogical (thermal analyses and X-ray diffraction) analyses. From those analyses they obtained 56 quantitative variables from a reduced amount of soil sample (1 g) and verified a high potential of the sequential analyses for forensic studies. The sequential analyses were able to discriminate soil samples from sites of the same pedological unit (i.e. the same parent material and soil class) located in close neighbourhoods of Curitiba Metropolitan Region, State of Paraná State, Brazil.

The objective of the present study was to examine soil samples (c. 1.7 g) from a real crime, which had occurred in the Curitiba Metropolitan Region, Brazil, using a sequential and comprehensive protocol of physical, chemical and mineralogical analyses previously developed and validated in the same region for forensic purposes by Melo et al. (2008).

2.4 MATERIAL AND METHODS

2.4.1 CRIME SCENE AND SOIL SAMPLING

The crime scene was a closed case involving the robbery of a safety deposit box (SDB) from a bank. It occurred in December 2016, in Araucária municipality, Curitiba Metropolitan Region (CMR), State of Paraná, Brazil (Fig. 1).

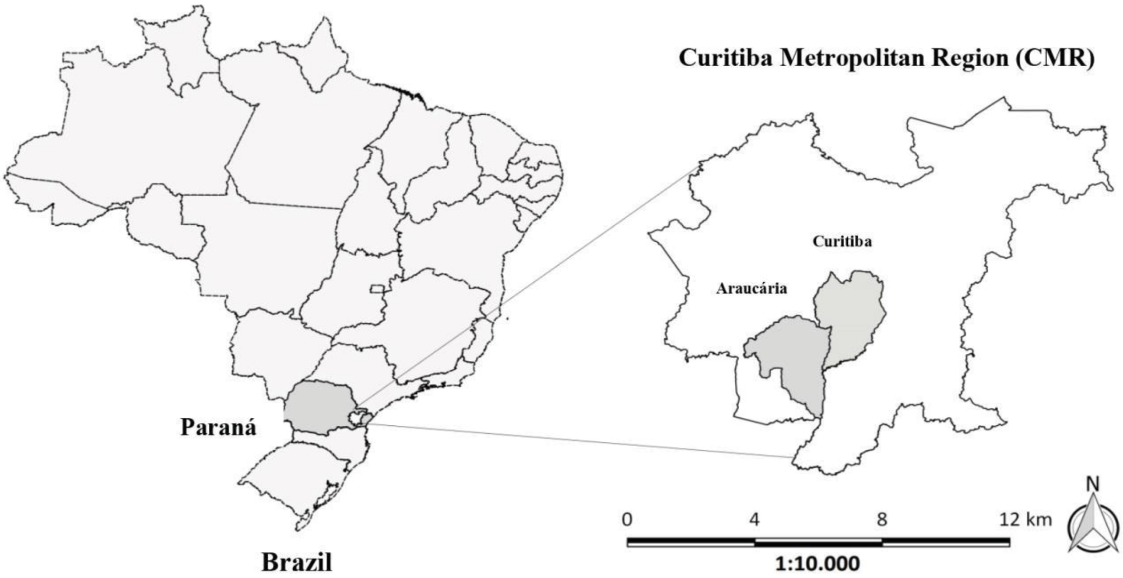


Fig. 1. Brazil map highlighting Paraná State, Curitiba Metropolitan Region (CMR) and Araucária city, where soil samples were collected.

The soil was sampled from four different reference sites in relation to the crime scene (Table 1):

Group 1 samples. After the crime, the SDB was removed to a rural area, at a distance of 13 km in the downtown area of Araucária city, where it was opened and discarded at the edge of a rural road (Fig. 2). We collected three soil samples at this location: two samples adhering to the SDB and one sample at the ground surface close to the SDB (Table 1).

Group 2 samples. The vehicle suspected of being used to transport the SDB (Fig. 3) was seized in the Federal Police parking area in Curitiba city, at a distance of 32 km from the site of the Group 1 samples. Four soil samples were collected, inside and within the tread of the tyres of the vehicle (Table 1).

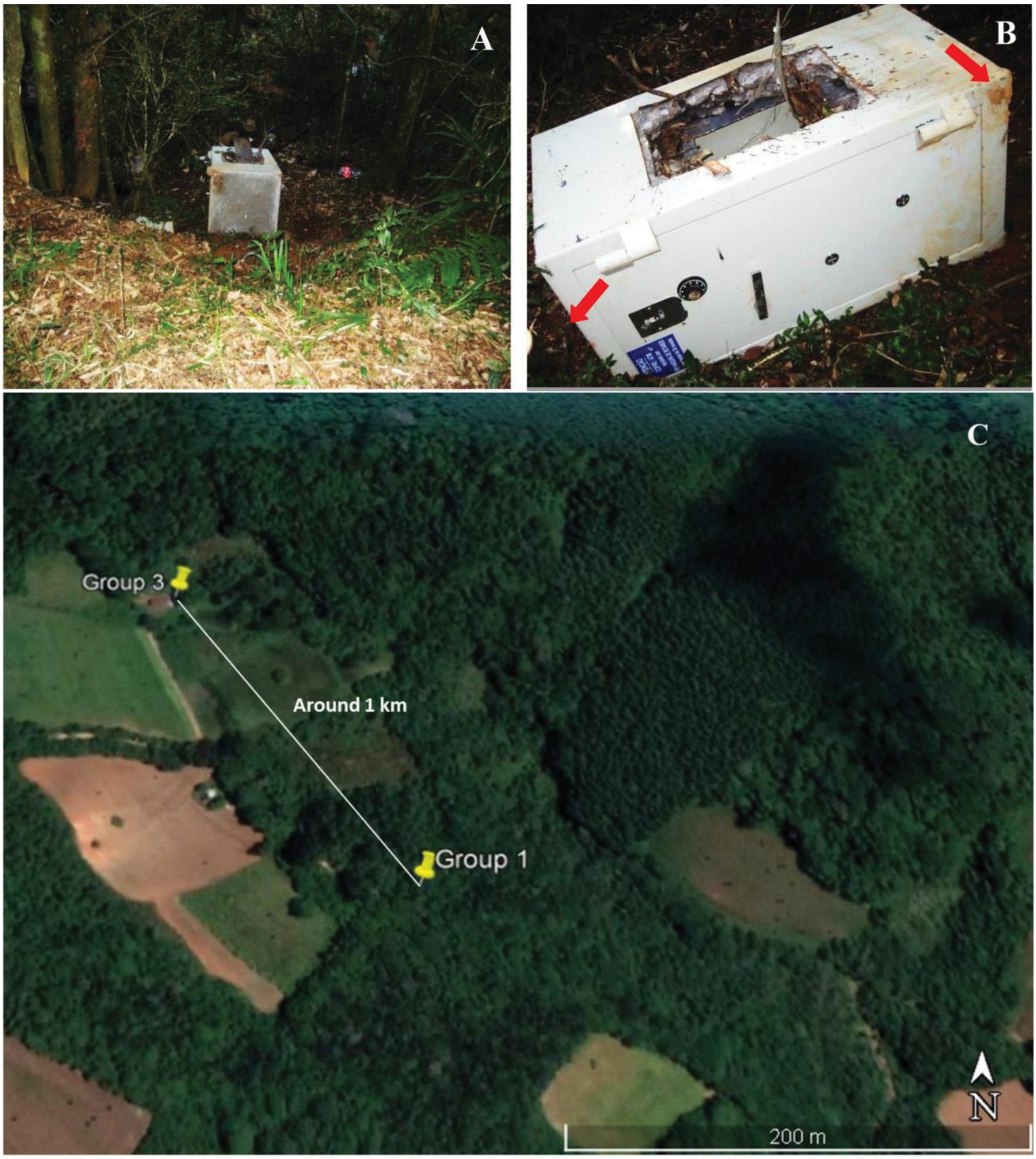


Fig. 2. Group 1 samples collection location: place of disposing of the safety deposit box (SDB) in the rural area of Araucária municipality (A) and SDB with adhered with soil traces B).

Group 3 samples. Another scene which was potentially linked to the crime is a rural property 1 km away from the site where the SDB was discarded (Group 1). According to some witnesses, on the night of the crime sounds were heard from a rural property that appeared to be related to the use of metallic objects. In this area we collected samples in different environments (Fig. 4B and Table 1): i) six samples near and in front of a house in the specific place where the SDB was allegedly opened; ii) five samples in a secondary road; and iii) three samples in one of two agriculture areas at the edges of the secondary road.

Table 1. Soil samples descriptions.

Samples	**Site description**	**UTM (22J)**		**Parent rock**
		E-W (m)	**N-S (m)**	
Group 1 - Safety deposit box (SDB) discarding site (see fig. 2a, 2b)				
1	SDB adhered soil - base	657,327 W	7,176,979 S	Unknown
2	Ground surface of disposal site	657,423	7,176,912	Granite
3	SDB adhered soil - surface	657,327	7,176,979	Unknown
Group 2 - Vehicle supposedly used to SDB transport (see fig. 3a, 3b)				
4	Inside vehicle - manual sampling	473,771	7,181,745	Unknown
5	Outside vehicle - tyres	473,771	7,181,745	Unknown
6	Inside vehicle - vacuum sampling(1)	473,771	7,181,745	Unknown
7	Inside vehicle - manual sampling	473,771	7,181,745	Unknown
Group 3 - Site supposedly used in the SDB opening (1 km from Group 1)				
8	See fig. 4a, 4b	657,294	7,177,098	Granite
9	See fig. 4a, 4b	657,299	7,177,094	Granite
10	See fig. 4a, 4b	657,290	7,177,098	Granite
11	See fig. 4a, 4b	657,283	7,177,093	Granite
12	See fig. 4a, 4b	657,290	7,177,089	Granite
13	See fig. 4a, 4b	657,290	7,177,089	Granite
14	See fig. 4a, 4b	657,290	7,177,089	Granite
15	See fig. 4a, 4b	657,290	7,177,089	Granite
16	See fig. 4a, 4b	657,279	7,177,088	Granite
17	See fig. 4a, 4b	657,279	7,177,080	Granite
18	See fig. 4a, 4b	657,290	7,177,089	Granite
19	See fig. 4a, 4b	657,300	7,177,027	Granite
20	See fig. 4a, 4b	657,310	7,177,029	Granite
21	See fig. 4a and 4b	657,312	7,177,005	Granite
Group 4 - Reference samples				
22	9.5 km from Group 1	657,214	7,177,688	Granite
23	11 km from Group 1	656,599	7,177,541	Granite
24	12 km from Group 1	657,156	7,172,798	Granite

(1) The disperse soil particles inside the vehicle were vacuum with a portable vacuum of 35 Watts.

Group 4 samples. Reference samples unconnected to the crime scene. Soil samples were collected at a distance of between 9 to 12 km away from the crime scene at the edge of the main road that linked the place where the SDB was discarded with the downtown area of Araucária city. The parent material of the reference samples was the same at the locations of Groups 1 and 3. The objective of this sampling was to check if the protocol of methodology is able to separate soil samples with the same parent material collected at the crime scene from those in distant areas (Table 1).

In the sample Groups 1 and 2 (except for the sample 2) (Table 1) a complete available sample was collected at each point and the quantity of soil traces recovered varied from 1.7 to 8.2 g. Samples 2 and 8 to 21 were collected in the 0-5 cm surface soil (around 100 g). For all samplings paint-brushes and a strong plastic trowel were used, in addition to a portable vacuum of 35 Watts for the vacuum sample from inside the vehicle (Table 1). Immediately after sampling all trace samples were stored in sterilized plastic pots within sealed evidence bags in order to avoid contamination.

Fig. 3. Group 2 samples: vehicle allegedly used to transport the safety deposit box (SDB) (a) and sampling points of the soil traces inside the vehicle (b).

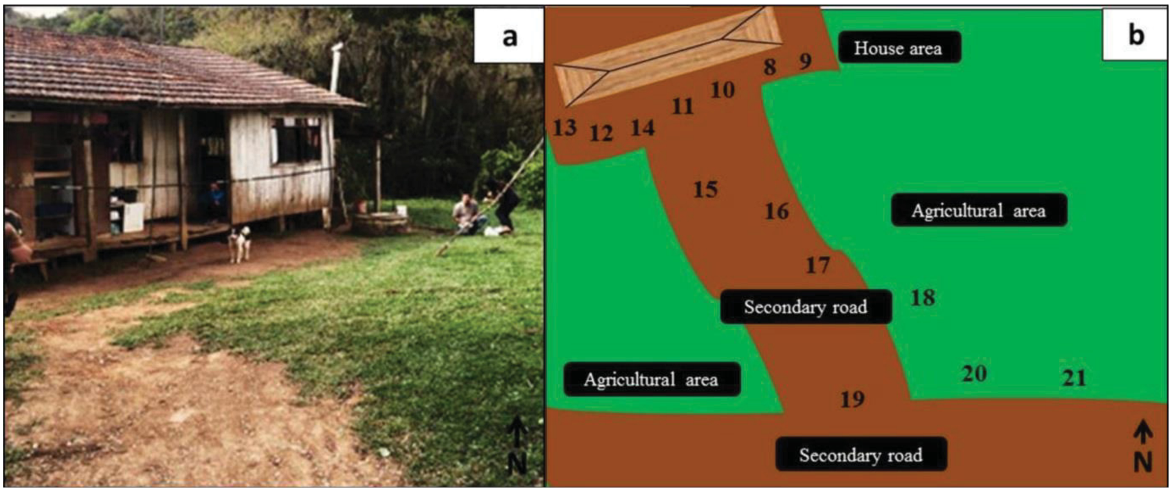


Fig. 4. Group 3 samples: rural property allegedly used to open the safety deposit box (SDB) in Araucária city (a) and relative positions of the collected samples of Group 3 on the map (no to scale) (b).

2.4.2 SOIL SAMPLE PREPARATION

Soil samples were air-dried at 40°C for 24 h, gently grinding and sieved (2mm) to remove any large stones and plant fragments for obtaining an air-dried fine earth fraction. The greater than 2 mm fraction was retained but not analysed. Sample homogenization was obtained using a plastic cone and quarterer. The partition was made to obtain 1.7 g for each sub sample. The standardization of a 1.7 g per sample for analysis was defined by the smallest quantity collected (which was found in sample 6 of Group 2 (Table 1).

2.4.3 SAMPLE DISPERSION AND SEPARATION OF SOIL FRACTIONS

1.7 g of soil was gently macerated with a rubber stick and washed with deionized water through a 0.053 mm sieve to separate the sand fraction. Suspensions containing silt, clay and organic matter particles were collected in porcelain crucibles. This step was repeated until the water after maceration was clear. The sand content was obtained after drying the sieved sand at 40°C for 24 h. The soil fractions smaller than 0.053 mm (silt, clay and organic matter) were also obtained by sample weighing after drying the suspensions collected during the sieving-maceration process.

Standardization of particle size fractions is essential to avoid misinterpretation of the results and incorrect conclusions (Fitzpatrick and Raven 2016). In addition, it is the finer particles (silt and clay) which are preferentially retained on shoes, tyres, clothes, etc (Fitzpatrick et al., 2012).

2.4.4 PROTOCOL OF ANALYSES

1.1 g of clay + silt + organic matter was weighed for physical, chemical and mineralogical analyses. We used a sequential protocol of physical, chemical and mineralogical analyses previously developed and validated in the same region

(Curitiba Metropolitan Region) for forensic purposes as described by Melo et al. (2008). The analytical methods were separated into two groups: destructive and non-destructive (Fig. 5). When possible, non-destructive analyses were performed first so that the samples could be re-used in the subsequent procedures. Another important adjustment was the reduction of the amount of sample required for the chemical (destructive) analyses. However, care was taken to keep the soil:solution ratios the same as was used in the original methods (Melo et al., 2008).

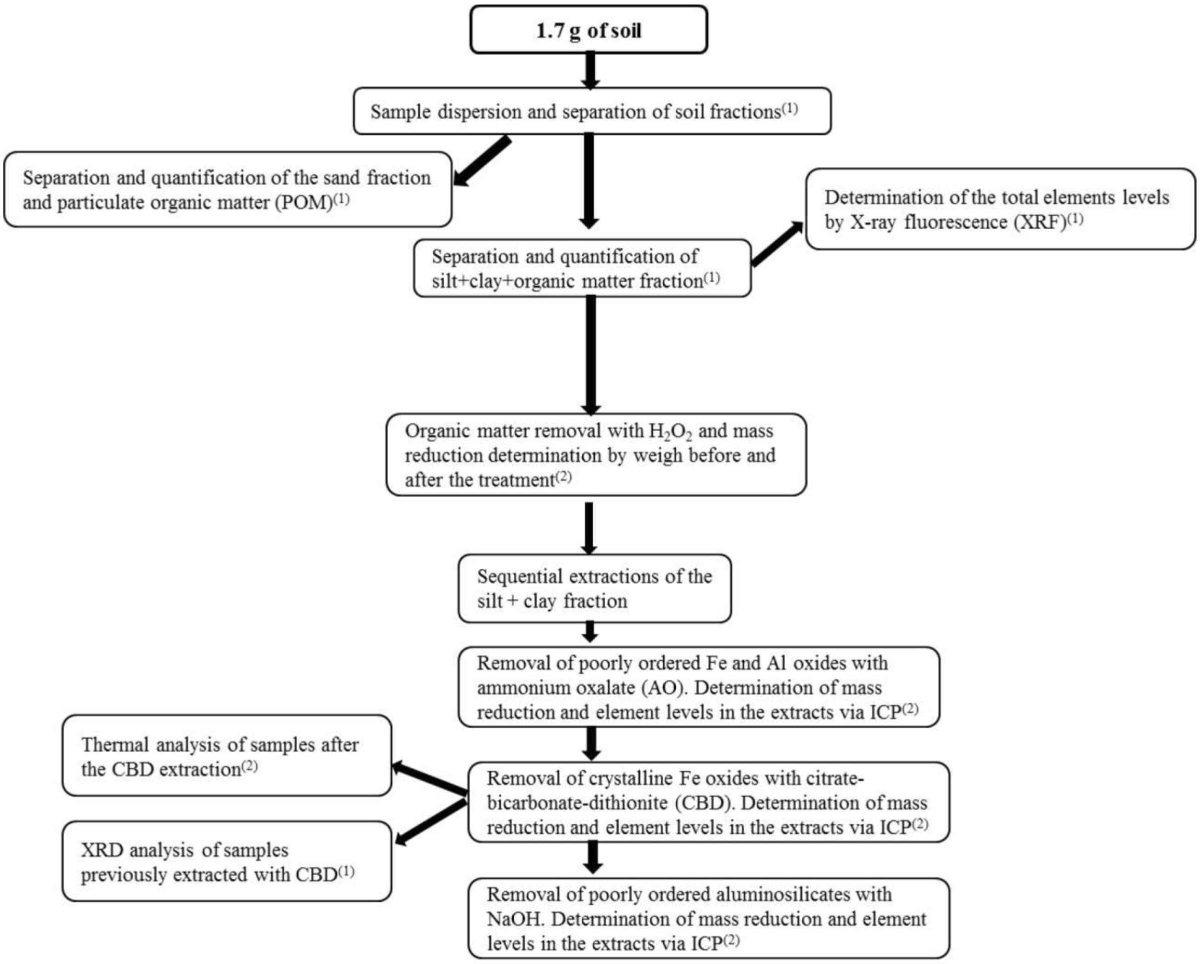


Fig. 5. Procedures for soil forensic analyses. Note: (1) Non-destructive analyses, (2) Destructive analyses. XRD: X-Ray diffraction, ICP: inductively coupled plasma spectrometry (ICP-AES). Source: Melo et al. (2008).

2.4.5 TOTAL ELEMENTS OBTAINED BY X-RAY FLUORESCENCE (XRF)

X-rays fluorescence spectroscopy is normally used to determine the type and concentration of different elements in a sample with advantages of good sensitivity, non-destructiveness The determination of the sensitivity is done through the fundamental parameter method which is based on the mathematical relationship derived from the physical properties and characteristics of the elements and efficiency of detection system.

In order to avoid shadow effect of mineral grains and diffraction of X-rays by crystals, the grain size of the sample should be as small as possible. Therefore, 0.2 g of clay + silt + organic matter was reduced to diameters lower than 0.045 mm by grinding and sieving. Subsequently, samples were analysed in an energy-dispersive X-ray fluorescence (ED-XRF) spectrometer (Fig.5), with a Rh source, for total elements determination (Si, Al, Fe, Ti, K, S, Ca, P, Mn, Zr, Cr, Sr and Zn). The spectra were obtained at 300 sec of intervals [in Na–Sc (15 kV) and Ti–U (50 kV) energy bands], in vacuum, under semi-quantitative mode. The instrument calibration was carried out with an aluminium standard sample (A250) in order to find the peak positions and the full width at half maximum of the chemical elements. The calibration was validated by using a standard sample (SUS) comprising Fe, Cr, Ni, and Mn.

2.4.6 REMOVAL OF ORGANIC MATTER

The samples analysed by X-ray fluorescence were recovered and the whole clay + silt + organic matter (1.1 g) were treated with 30% (v/v) H_2O_2 in a 70°C water bath until all the organic matter was removed (Fig. 5). After the last addition (end of the reaction) the samples were dried at 60°C for 24h and the final mass, without organic matter, was determined.

2.4.7 SEQUENTIAL CHEMICAL ANALYSES

The determination of the composition and mineral content of soils and sediments is an important means of understanding soil properties, formation, and chemical processes. The soil properties controlling moisture and chemical composition are highly dependent on soil mineralogical composition.

For all sequential extractions (ammonium oxalate, sodium citrate-dithionite-bicarbonate and NaOH) the following steps were carried out: i) all extracting solutions and volumetric flasks were filled with ultra-pure water (18.2 MΩ.cm at 25ºC –

Millipore Direct–Q System, Merck, Germany) and high purity acids were used in the analyses (Merck PA, Germany); ii) before each extraction the samples were dried for 24 hours in an oven at 40°C, ground and sieved at 0.2 mm in order to improve the efficiency of the extractions; iii) after each extraction, salt excess was removed by washing with 0.5 mol L-1 $(NH_4)_2CO_3$ and deionized water ; iv) after washing the samples were oven-dried at 40°C for 24 h, and the final mass was determined; v) extracts were obtained by centrifugation (3,000 rpm for 10 min) and all extracts were filtered in a slow filter paper (Macherey Nagel®, USA); vi) the element concentrations (Fe, Al, K, P, Mg, Zn, Mn, Cu, Pb and V) were determined by inductively coupled plasma spectrometry (ICP-AES).

2.4.7.1 EXTRACTION OF POORLY ORDERED Fe AND Al OXIDES

The acid ammonium oxalate (AAO) method is applicable to the determination of poorly crystalline forms of inorganic and organic Fe and Al from soils. It attacks most silicate minerals and goethite and hematite only slightly, but it dissolves magnetite and finely divided easily-weathered silicates such as olivine to a considerable extent (Anjos et al., 2002). Via complexation and reduction of the pH, which promote the required protonation to extract poorly ordered Fe and Al oxides, by the use of the acid ammonium oxalate (pH 3), according to the example in the following reaction:

$$Fe_2O_{3(s)} + 2H_3O+_{(aq)} \rightarrow 2Fe(OH)_2+_{(aq)} + H_2O_{(l)}$$

In this way, 0.8 g of sample with no organic matter followed all the common procedures described in the section Sequential and chemical analyses. Subsequent to the sieving at 0.2 mm, was added 20 mL of 0.2 mol l-1 pH 3.0 ammonium oxalate (AO) in the dark (McKeague and Day, 1966) in the silt + clay fraction samples in order to extract the Fe and Al poorly crystalline oxides (short-range order phases). Modifications in the method were performed to adapt the reagent proportions according to the weight of each soil sample. Samples were shaken for 2 h and then centrifuged for 10 min to separate the extract. Extracts were stored in amber flasks for the determination of the element concentrations (Fe, Al, K, P, Mg, Zn, Mn, Cu, Pb and V) by inductively coupled plasma spectrometry (ICP-AES).

2.4.7.2 EXTRACTION OF CRYSTALLINE Fe OXIDES

All samples from the AO treatment were treated with sodium citrate-bicarbonate-dithionite (CBD) in order to extract the crystalline iron oxides (McKeague, 1978) (Fig. 5). 0.7 g of sample were placed in 100 mL centrifuged tubes and extracted three times with 11.2 mL of solution of 0.3 mol L^{-1} of sodium citrate ($Na_3C_6H_5O_7.2H_2O$) + 1.4 mL of solution of 1 mol L^{-1} of sodium bicarbonate ($NaHCO_3$) + 0.28 g of sodium dithionite ($Na_2S_2O_4$). Modifications in the method were performed to adapt the reagent proportions according to the weight of each soil sample. Samples in solution were manually agitated while heated at 70°C in a hot water bath for 30 min. The extracts of the three extractions were combined for the elemental analysis.

2.4.7.3 EXTRACTION OF POORLY CRYSTALLINE ALUMINOSILICATES AND GIBBSITE

The final sequential analysis was the extraction of poorly crystalline aluminosilicates (short-range order Al-hydroxide, Al–O–Si layers and Si–O (opaline silica) resistant to previous ammonium oxalate extraction) and gibbsite by boiling 0.5 mol L^{-1} NaOH (Fig.5) (Mehra and Jackson, 1960; McKeague, 1978). 0.4 g of the residue of DCB were weighed in tubes and moistened with 2 mL of 0.5 mol L^{-1} NaOH without heating. Teflon beakers containing 15 mL of the same NaOH solution were placed in a sand bath at 200°C until the boiling point was reached; the solution was then transferred to the tube with the samples. At this point, the boiling solution remained in contact with the sample for exactly 3 minutes under constant manual agitation. Immediately after this, the samples were cooled in a bowl with cooled water and then centrifuged. Modifications in the method were performed to adapt the reagent proportions according to the weight of each soil sample.

2.4.7.4 THERMOGRAVIMETRY ANALYSIS (TGA)

0.02 g of sample extracted with DCB (Fig. 5) were heated from room temperature to 950 °C at 10 °C min s^{-1} and gas flux of 50 mL min^{-1}, under N_2 atmosphere in a Shimadzu derivatograph. Contents of kaolinite and gibbsite were estimated according to the mass reduction due to the dehydroxylation of these minerals when heated (Tan and Hajek, 1986).

2.4.7.5 X-RAYS DIFFRACTION (XRD)

0.3 g of the silt + clay samples after DCB extractions were analysed by XRD (Fig. 5). The diffraction patterns (random powder mounts) were obtained by Pananalytical X´Pert device under a speed of 0.42 °2θ s^{-1} and analysed in the 3 to 60 °2θ range. The diffractometer was equipped with a Ni filter, graphite monochromator system and used a CuKα radiation, operated at 40 kV and 40 mA. After the XRD, the samples were recovered for the subsequent analyses (Fig. 5).

2.4.8 MULTIVARIATE ANALYSES

All data were exported to a data matrix and correlated through principal component analysis (PCA) using the Statistica software (StatSoft, 2011) and Paleontological Statistics (PAST) software using Bray-Curtis similarities (Hammer and Harper, 2006). In our research the aims to apply the PCA analysis is mainly to verify which soil characterization methods indicated similarities or differences between samples. Results of these analytical procedures formed a multidimensional matrix that was submitted to PCA analyses, which consists of several statistical tools used to compress/reduce the original matrix without losing relevant information. This way, the dataset obtained from the analyses of one or more properties of a sample can be conveniently represented by a significantly lower number of factors, through an easier and effective comparison. The result of a PCA analysis can be graphically expressed as a new axis system normally referred to as principal components (PC), which are able to explain all variances present in the original data matrix. Furthermore, the multivariate approach using group-average cluster analysis, based on Bray-Curtis similarities was performed in order to evaluate general relationships between samples based on an overall set of soil differences. All data were square-root transformed before subjected to the Bray-Curtis analysis.

2.5 RESULTS AND DISCUSSION

2.5.1 ORGANIC MATTER AND MINERALOGICAL ANALYSES

The organic matter content of the silt+clay+organic matter fraction ranged from 29 to 132 g kg^{-1}, with a general mean of 70.3 g kg^{-1} (general standard deviation - SD=25.2) (Supplementary material S1). The lower content of organic matter (29 g kg^{-1}) was for sample 24 (group 4), collected in the urban area without a developed soil surface horizon, and therefore low inputs of organic matter. The higher content (132 g kg^{-1}) was for sample 2 (group 1), collected from a mature forest area (Fig. 2a). The large variation of the organic matter content is largely due to the different environments collected and consequently, to the distinct soil profiles formed.

According the thermogravimetry analysis (TGA) there was a clear predominance of kaolinite (ranging from 228.1 to 748.6 g kg^{-1}, SD=128.25), followed by small proportion of gibbsite (10.6 to 72.5 g kg^{-1}, SD=16.09) in the silt+clay fraction (Supplementary Material S1). Considering that the products of rock decomposition from chemical weathering invariably contain a higher content of combined water (hydroxyl) than the undecomposed materials, such large variations regarding the values of gibbsite and kaolinite among the soil samples could be related to the contents of H_2O or -OH which are commonly variable. Furthermore, in environments continuously under high temperatures, humidity and leaching conditions, the K-feldspars (high concentration in granite) have transformed directly to kaolinite (Melo et al., 2001).

The predominance of kaolinite in relation to gibbsite in the silt+clay fraction may be also observed in the intensity of peaks of these minerals in the XRD patterns (Supplementary material S2). The XRD pattern of the samples collected in front of the house (for example, sample 8) is highly like the sample collected at the SDB discarding location (sample 2).

2.5.2 TOTAL ELEMENT CONTENTS OBTAINED BY X-RAYS FLUORESCENCE (XRF)

The elemental analysis (by XRF) reflects the geographical and geological signature of the soil, which can be useful for establishing correlations with the original location of soil (Marumo, 2003; Ravansari and Lemke, 2018). Commonly, values for Si, K, Al, Fe, Ti, Sr, Zr and Rb can reflect lithogenetic and pedological processes in the soil system, while S, Ca and P contents may indicate anthropogenic interference.

The Si/Al ratio for all the soil samples studied ranges from 1.1 to 1.9 (Table 2). Higher values for Si/Al can be attributed to high silt content. Significant amounts of K obtained by XRF (Table 2) may be related to the K-feldspar richness of the parent material of the soil (granite). The reactions of chemical weathering and overlapping with the quartz peaks made it difficult to identify K-feldspars in the silt+clay fraction (Supplementary material S2).

Table 2. Total content of the elements obtained by X-rays fluorescence in the clay + silt + organic matter fractions

Sample	**Si**	**Al**	**Fe**	**Ti**	**K**	**Ca**	**P**	**Zn**	**Si/Al**
	----------------------------------g kg^{-1}-------------------------------------								
Group 1									
1	400.5	307.6	228.0	22.1	12.3	3.9	3.0	0.4	1.3
2	419.1	284.7	221.5	27.1	17.7	5.0	3.5	0.5	1.5
3	440.1	285.3	201.3	29.8	9.8	7.5	5.4	0.4	1.5
Group 2									
4	415.2	309.0	211.1	21.0	17.9	7.8	2.0	0.6	1.3
5	452.7	298.5	183.4	20.1	19.3	7.9	2.1	0.2	1.5
6	434.0	264.7	205.9	34.5	14.6	23.7	3.1	0.8	1.6
7	454.1	263.1	199.6	34.2	13.0	13.5	2.9	0.6	1.7
Group 3									
8	419.6	309.7	201.8	23.3	14.1	7.5	4.9	0.4	1.4
9	398.2	324.3	204.3	23.0	15.3	10.2	5.0	0.5	1.2
10	441.3	281.4	191.0	29.3	14.1	16.9	6.9	0.1	1.6
11	410.8	311.5	205.4	27.4	13.2	7.9	4.6	0.4	1.3
12	450.1	270.9	201.1	33.4	12.5	7.8	4.0	0.4	1.7
13	412.0	319.7	210.3	24.7	11.8	3.6	2.6	0.3	1.3
14	453.9	289.3	193.3	25.2	12.4	7.3	2.8	0.3	1.6

15	438.8	279.2	206.6	30.2	12.1	7.8	5.7	0.3	1.6
16	380.6	344.5	227.4	21.9	3.5	3.0	2.2	0.3	1.1
17	438.8	283.0	206.7	29.9	10.8	6.0	6.5	0.3	1.6
18	440.7	264.7	199.6	31.0	13.4	18.6	10.3	0.3	1.7
19	405.2	326.6	203.8	20.5	21.0	4.6	2.4	0.3	1.2
20	429.6	321.2	195.5	20.2	14.4	3.2	0.5	0.3	1.3
21	466.7	271.3	185.4	24.1	28.2	6.1	3.5	0.3	1.7
Group 4									
22	412.8	219.8	234.7	15.1	23.8	23.4	0.1	0.4	1.9
23	392.2	373.3	165.9	1.6	1.8	0.7	0.2	0.1	1.1
24	432.1	333.5	175.8	2.1	1.1	0.6	0.1	0.1	1.3

Goethite (α-FeOOH) and hematite (α-Fe_2O_3) are the main secondary mineral source of Fe in soils (Schulze, 1984; Hsu, 1989; Schwertmann and Taylor, 1989). Due to the strong overlapping effect of kaolinite and quartz peaks on goethite and hematite peaks in the XRD pattern of two representative samples from the study areas it was possible to identify only the hematite (104) peak at 0.26 nm (Supplementary material S2). The highest variation in Fe contents was verified for group 4 (165.9 to 234.7 g kg^{-1}, SD=16.71) (Table 2). The soil samples in group 4 were collected in a more extensive area (Table 1). Soil samples collected from short distances are more likely to be placed in the same group of samples using the results of the physical, chemical and mineralogical analysis, once the heterogeneity could be higher in relation to samples collected from long distances (Melo et al., 2008; Dawson and Hillier, 2010; Corrêa et al., 2017; Prandel et al., 2017).

2.5.3 SEQUENTIAL EXTRACTIONS OF THE SILT + CLAY FRACTION

The elemental content extracted by poorly ordered Al and Fe oxides (ammonium oxalate, AO), crystalline Fe oxides (dithionite-citrate-bicarbonate, DCB), gibbsite and amorphous aluminosilicates (NaOH) dissolution are presented in Tables 3, 4 and 5, respectively. The Fe and Al oxides extracted with AO (Fe_2O_3-AO and Al_2O_3-AO) varied from 1.7 to 10.3 g kg^{-1}, SD=2.70, and 1.8 to 6.9 g kg^{-1}, SD=1.28, respectively (Table 3). Such variations are to be expected because the poorly ordered minerals are formed under specific conditions of soil, which change in short distances, such as humid, organic matter profile, biological activity, pH etc (Schulze, 1984). The poorly crystalline minerals can predict important information about the environment once they are in an intermediate stage of weathering, with greater possibilities to change their content and chemical composition in short soil distances (Schwertmann and Kämpf, 1985; Barbar and Melo, 2008). Ferrihydrite is the main poorly ordered Fe oxide and is completely extracted by AO solution (Schwertmann and Taylor, 1989). The second higher content of organic matter (121 g kg^{-1}) and the highest Fe_2O_3 content extracted by ammonium oxalate (10.3 g kg^{-1}) were in sample 9 (group 3). Organic matter inhibits the crystallization of Fe oxides due the metal-organic matter complexation (Mendonça et al., 2013).

The contents of Fe-bearing crystalline oxides (mainly hematite and goethite) extracted with CBD (Fe_2O_3-CBD) ranged from 55.1 to 84.1 g kg^{-1}, SD=6.67) (Table 4). Such low contents of Fe_2O_3-CBD agree with the low contents of iron-magnesium primary minerals in the granite (17). The contents of Fe_2O_3-CBD were always higher than the contents of Fe_2O_3-AO: the ratio FeAO/FeCBD ranged from 0.03 to 0.18 (Table 4). The predominance of crystalline Fe oxides in the clay fraction of soils is reported in literature (Mehra and Jackson, 1960; Kämpf and Schwertmann, 1983; Torrent and Cabedo, 1986; Fitzpatrick, 2008; Poggere et al., 2016). The greater FeAO/FeCBD ratio for samples 8, 9 and 10 (group 3) can be attributed to higher moisture content in those soils. There was a lake close to the left side of the house in group 3, and furthermore Araucária city is located at a high altitude and in high humidity conditions. The concentration of poorly ordered Al and Fe oxides (AO) in the clay fraction is favoured by a higher humidity in the soil (Stoppe et al., 2015), maintained by landscape conditions and eventually by hydromorphic processes. The contents of Mg-AO were systematically higher than Mg-DCB contents (Tables 3 and 4) suggesting a poor relationship between Mg and the crystalline Fe-oxides.

Table 3. Iron and aluminium oxides contents and elements extracted by ammonium oxalate (AO) in the sequential analyses of the clay+ silt fraction.

Sample	**Fe_2O_3**	**Al_2O_3**	**Total**	**Weight**	**Oxide/total**		**Mg**	**Zn**	**Si**	**V**
					Fe_2O_3	**Al_2O_3**				
	----------- g kg^{-1} ---------			**%**	**--- g kg^{-1} ---**		**------------- mg kg^{-1} ------------**			
Group 1										
1	2.9	4.1	7.0	3.0	415.5	584.5	330.5	33.6	405.7	10.7
2	2.4	4.8	7.2	2.4	334.0	666.0	187.3	12.0	353.3	12.4
3	2.4	4.7	7.1	2.8	335.7	664.3	174.8	20.0	335.9	10.6
Group 2										
4	2.7	2.1	4.7	3.4	560.7	439.3	222.8	64.1	416.0	5.6

5	2.6	2.5	5.1	3.0	502.9	497.1	132.4	19.9	340.9	8.7
6	6.1	4.5	10.6	4.0	576.6	423.4	470.9	80.8	827.8	12.0
7	2.3	1.8	4.1	3.8	557.9	442.1	116.3	37.0	350.7	5.2
Group 3										
8	10.2	5.7	15.9	3.3	639.9	360.1	336.4	58.3	701.8	20.1
9	10.3	5.9	16.2	4.4	636.9	363.1	500.2	49.0	690.3	19.4
10	8.8	5.8	14.5	3.7	602.7	397.3	459.2	33.5	829.6	19.3
11	7.7	6.1	13.8	4.9	559.3	440.7	325.4	18.1	644.6	17.3
12	6.9	4.0	11.0	2.4	631.8	368.2	350.3	24.2	613.7	14.8
13	5.7	6.7	12.4	2.5	462.1	537.9	209.7	13.1	508.7	16.5
14	6.7	4.8	11.5	3.2	583.6	416.4	372.3	34.1	555.6	17.6
15	8.2	4.6	12.8	2.9	639.5	360.5	392.4	36.6	580.4	17.3
16	4.6	6.9	11.5	3.4	401.8	598.2	201.6	45.0	529.8	16.2
17	7.5	5.0	12.5	3.6	600.6	399.4	242.4	42.5	562.7	15.7
18	10.2	6.1	16.3	4.5	628.1	371.9	509.6	42.3	521.5	16.9
19	6.1	5.1	11.2	2.9	546.2	453.8	173.2	25.0	420.8	12.8
20	5.4	5.7	11.1	2.4	484.5	515.5	142.2	29.5	457.3	16.0
21	4.7	5.1	9.9	2.1	478.7	521.3	117.9	46.3	430.2	14.4
Group 4										
22	1.7	4.7	6.4	2.8	264.0	736.0	788.2	20.9	461.0	11.9
23	4.0	3.7	7.7	2.3	521.2	478.8	977.4	12.6	513.6	13.9
24	3.1	4.5	7.6	2.4	410.4	589.6	531.9	20.1	360.6	8.6

Total: sum of amorphous oxides (Fe_2O_3 + Al_2O_3). Weight loss: sample weight reduction by the AO treatment [(initial weight – final weight)/initial weight x 100]. Oxide/total: participation of Fe_2O_3 and Al_2O_3 in relation to the total (sum of oxides).

The main source of Al_2O_3 (Table 4) is the dissolution of hematite and goethite by DCB. The Al can replace part of the Fe in the hematite and goethite structure (Curi and Franzmeier, 1984; Corrêa et al., 2017). That process is favored by similarity of the ionic radii and the same coordination number between of Fe and Al (Wowk and Melo, 2005).

Table 4. Iron and aluminium oxides contents and elements extracted by sodium citrate-bicarbonate-dithionite (CBD) in the sequential treatments of the clay+ silt fraction.

Sample	**Fe_2O_3**	**Al_2O_3**	**Total**	**Weight**	**Oxide/total**		**FeO/FeD**	**Mg**	**Zn**	**Mn**	**V**
					Fe_2O_3	**Al_2O_3**					
	----------- g kg^{-1}	**----------**		**%**	**-----g kg^{-1}-----**			**------------mg kg^{-1}------------**			
Group 1											
1	73.4	12.3	85.7	15.3	856.3	143.7	0.04	88.2	41.8	333.8	87.7
2	69.8	11.9	81.7	14.6	854.0	146.0	0.03	66.6	54.7	412.3	84.0
3	65.3	9.7	75.0	13.3	870.5	129.5	0.04	66.6	53.2	374.1	68.4
Group 2											
4	68.0	11.7	79.7	14.0	852.8	147.2	0.04	97.7	78.1	283.3	82.3
5	58.4	9.6	68.0	12.8	858.3	141.7	0.04	87.7	53.3	306.9	70.0

6	60.8	9.6	70.3	15.6	863.6	136.4	0.10	97.1	95.5	372.3	67.1
7	60.9	10.6	71.5	14.9	851.1	148.9	0.04	93.5	85.2	360.5	72.5
Group 3											
8	63.9	11.8	75.7	13.5	844.4	155.6	0.16	90.2	69.5	270.6	72.1
9	63.0	11.4	74.4	14.3	847.2	152.8	0.16	80.0	61.3	436.6	71.3
10	55.1	9.7	64.8	13.4	850.7	149.3	0.16	92.8	52.6	253.4	66.2
11	66.0	11.8	77.8	14.3	848.3	151.7	0.12	75.5	42.7	482.0	74.1
12	58.2	8.7	66.9	12.2	870.6	129.4	0.12	72.1	37.9	361.9	62.5
13	76.0	16.0	92.0	16.3	826.1	173.9	0.08	102.5	39.8	232.0	90.5
14	67.2	11.4	78.6	13.4	855.1	144.9	0.10	79.7	50.1	355.9	73.0
15	61.2	9.4	70.6	12.4	866.6	133.4	0.13	70.8	46.7	368.5	64.7
16	84.8	17.3	102.1	17.8	830.6	169.4	0.05	92.2	57.0	241.3	93.9
17	64.6	10.1	74.7	13.7	864.8	135.2	0.12	50.3	52.7	512.6	70.3
18	58.1	9.7	67.8	14.3	856.8	143.2	0.18	73.9	50.4	350.4	64.3
19	72.6	12.9	85.4	15.0	849.5	150.5	0.08	70.1	44.9	331.4	83.5
20	68.3	11.6	79.9	14.7	854.4	145.6	0.08	63.6	54.1	405.6	77.0
21	60.2	8.3	68.5	11.6	879.1	120.9	0.08	72.1	49.0	341.7	65.6
Group 4											
22	64.9	6.9	71.8	13.1	903.0	97.0	0.03	189.9	44.0	516.2	111.5
23	57.6	8.0	65.6	13.1	877.6	122.4	0.07	137.6	31.5	169.7	63.0
24	61.7	9.3	71.1	12.7	868.5	131.5	0.05	95.6	64.6	819.1	71.8

Total: sum of amorphous oxides (Fe_2O_3 + Al_2O_3). Weight loss: sample weight reduction by the AO treatment [(initial weight – final weight)/initial weight x 100]. Oxide/total: participation of Fe_2O_3 and Al_2O_3 in relation to the total (sum of oxides).

The residue of AO and DCB extractions were treated with boiling 0.5 mol L^{-1} NaOH, which extracts poorly crystalline aluminosilicates (short-range order Al-hydroxide, Al–O–Si layers and Si–O (opaline silica) resistant to previous ammonium oxalate extraction) and gibbsite (Mehra and Jackson, 1960; Schwertmann and Kämpf, 1985). The most variation regarding the contents of Al_2O_3 was in group 4 (2.8 to 15.8 g kg^{-1}, SD=2.43) (Table 5).

Table 5. Iron and aluminium oxides contents and elements extracted by sodium hydroxide extraction (0.5 mol l^{-1} NaOH) in the sequential analyses of the clay+ silt fraction.

Sample	**Fe_2O_3**	**Al_2O_3**	**Total**	**Weight**	**K**	**P**
	----------------g kg^{-1} ----------------			**%**	**--- mg kg^{-1} ---**	
Group 1						
1	0.1	7.8	7.9	5.5	159.9	91.7
2	0.1	7.4	7.4	4.5	153.5	100.7
3	0.1	6.5	6.6	3.9	170.3	97.7
Group 2						
4	0.1	7.9	8.0	4.6	175.4	100.1
5	0.2	7.5	7.5	6.2	170.5	92.4
6	0.1	6.1	6.2	5.4	197.4	89.1
7	0.1	7.0	7.1	4.4	184.5	98.4

Group 3						
8	0.2	8.0	8.1	4.7	155.1	119.3
9	0.1	7.9	7.9	4.1	170.9	103.1
10	0.1	6.0	6.0	3.2	166.6	96.1
11	0.1	8.2	8.3	4.6	171.2	108.4
12	0.1	5.6	5.6	2.9	162.9	70.8
13	0.2	11.6	11.6	11.9	160.5	138.9
14	0.1	7.0	7.0	11.4	164.8	97.9
15	0.1	6.1	6.1	2.9	169.3	79.1
16	0.3	10.7	10.7	4.7	146.0	120.4
17	0.1	6.2	6.3	3.6	156.3	85.4
18	0.1	6.8	6.9	4.4	178.7	99.4
19	0.2	9.3	9.3	3.8	158.1	98.4
20	0.1	8.8	8.9	4.2	155.3	96.4
21	0.1	6.1	6.1	6.3	175.8	58.1
Group 4						
22	0.1	2.8	2.8	1.9	168.6	42.1
23	0.2	5.7	5.7	16.3	146.4	73.5
24	0.1	15.8	15.8	5.6	149.9	103.9

Total: sum of oxides (Fe_2O_3 + Al_2O_3). Weight loss: sample weight reduction by the NaOH treatment [(initial weight – final weight)/initial weight x 100].

2.5.4 GROUPING BY PRINCIPAL COMPONENT ANALYSIS (PCA) USING BRAY-CURTIS SIMILARITY

For the multivariate analyses, we included XRF, TGA and sequential extractions with AO, DCB and NaOH data for all samples. In agreement with the grouping verified on the PCA analysis (Fig. 7a) the dendrogram of Bray-Curtis clearly showed the grouping of the samples collected on the SDB and the samples collected in front of the house (samples 8, 9 and 10), presenting around 94% of similarity (Fig. 6). Such discrimination may demonstrate the efficiency of the methods applied. In Figure 7 (a, b) it is possible to verify two rotations regarding the same variables obtained, which were presented in order to indicate an independent grouping formation of the samples (1, 2 and 3 from Group 1 and 8, 9 and 10 from group 3) beside the perspective. In the first plot (Fig. 7a) the sample traces from SDB (Group 1) belong to Group 3. In both perspectives the sample 2 collected from the soil surface of the place where the SDB was discarded, remained close to traces 1 and 3. The soil residues were possibly transferred to the SDB at the time of it being discarded and at the time of its opening. The SDB was possibly released in the edge of the secondary road and rolled, accumulating soil until it halted (Fig. 2a). Results show that these two possibilities cannot be excluded.

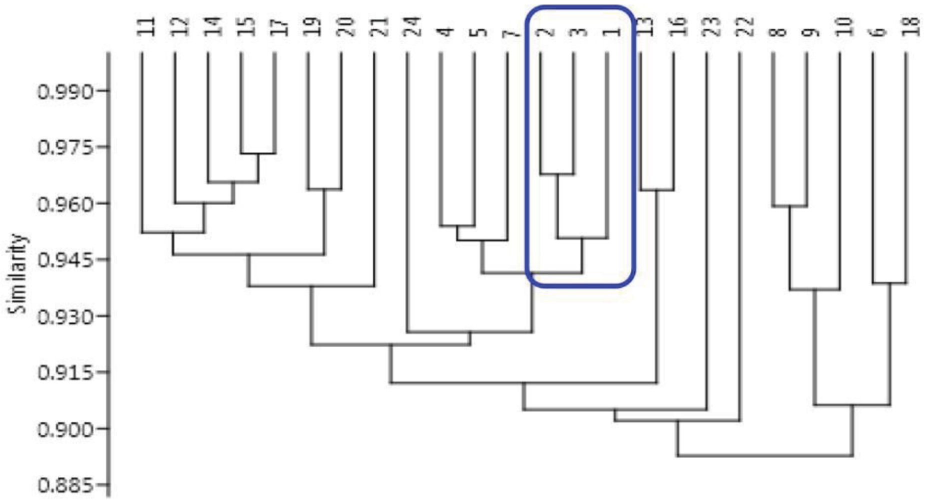


Fig. 6. Dendrogram indicating the grouping of samples 1, 2 and 3 from Group 1 - Safety deposit box (SDB) discarding site and the similarity between them (95%). The sample identification is detailed in Table 1.

Some of the samples from Group 3 (Figures 7a, 7b) were located on the secondary road close to the samples 8, 9, 18, 20 and 21 (Fig. 4). Samples 10 and 11 were also collected in front of the house (Figure 4), but the samples collected on the secondary road near to the samples 12 and 13 were dispersed and positioned in different quadrants in the PCA (Figure 7b). This reinforces the potential value of using the protocol of analyses proposed by Melo et al. 2008 (Fig. 5) to group homogeneous samples in forensic cases. On the other hand, the reference samples (Group 4) had higher dispersion in relation to the other groups of samples, which relates to the heterogeneity of the anthropic forensic soils. The sample traces collected from the questioned vehicle allegedly used to transport the SDB (Group 2) formed a relatively homogeneous group (Fig. 6). The samples recovered from the vehicle (4 and 7 - inside vehicle; 5 - outside on the tyres) formed a group with 95 % similarity. The samples adhered inside and outside the vehicle likely belong to the same place. However, the sample collected in the vehicle by vacuuming (sample 6) did not correlate with the other samples from vehicle. However, the sample collected by vacuuming inside the vehicle potentially produces a mixture of different types of traces, adhered in different locations at different times. Therefore, it is suggested that soil sampling by vacuuming should be avoided.

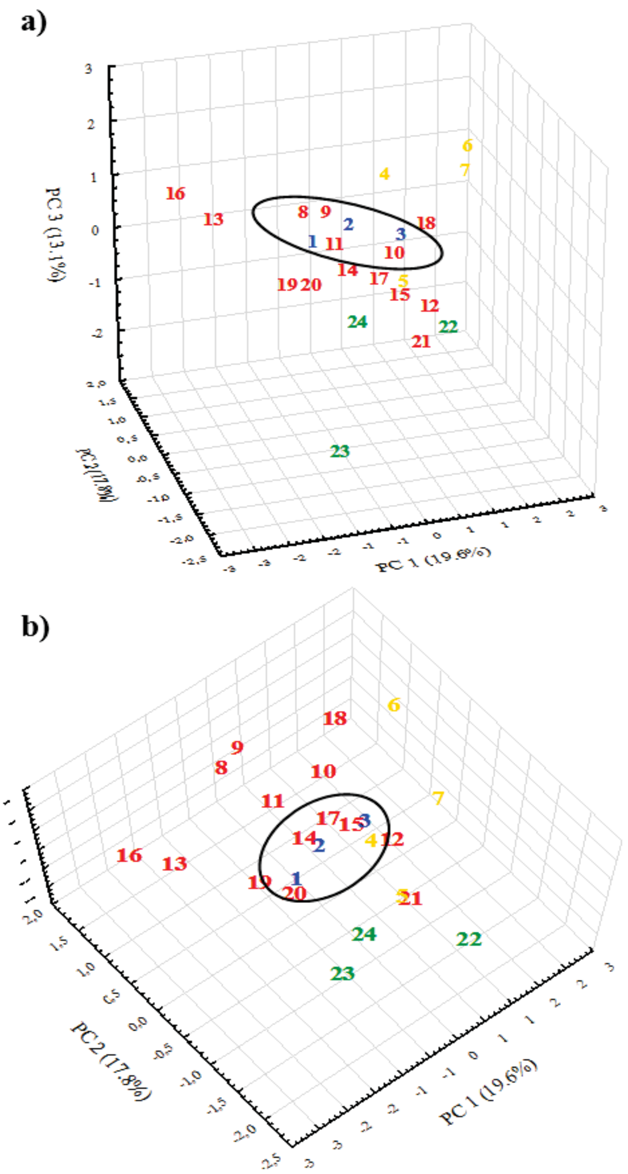


Fig. 7. Score values for the analysed samples under two different rotations (a, b). Group 1 - Safety deposit box (SDB) discarding site, Group 2 - Vehicle allegedly used to transport the SDB, Group 3 - Site allegedly used in opening the SDB, Group 4 - Reference samples. The sample identification is detailed in Table 1.

2.6 CONCLUSIONS

From only 1.7 g of soil it was possible to carry out physical, chemical and mineralogical methods (destructive and non-destructive) and to produce a large number of quantitative variables. Mineralogical data such as the XRD patterns were not enough to discriminate the samples, particularly because the proximity between the Group 1 and Group 3 (around 1 km). However, the methods employed in this study, especially the sequential extractions which provided the chemical composition of the mineralogical profile of the samples, were effective in discriminating the homogeneous sampling reference sites. From the PCA results (Figures 6 and 7), it seems that Group 1 soils (from the SDB and the site where it was found) cannot be readily distinguished from Group 3 Soils (from the vicinity of the suspect’s house). These sites are only 1km apart. Therefore, it is just as likely that the soil on the SDB came from the vicinity of the house as it is that they came from the site where the SDB was found. What the results do show is that the reference samples are more or less distinguishable from all other soils and that some of the samples from the vehicle are distinguishable from all others as well. Furthermore, it is possible to apply the analytical procedures in future case work involving soils, and depending on the context, the methodologies and techniques can also be used in the analysis of soils from other Brazilian regions or even in other countries.

2.7 REFERENCES

Aitkenhead, M. J., Coull, M. C., Dawson, L. A. (2013). Predicting Sample Source Location from Soil Analysis Using Neural Networks. *Environmental Forensics* 14, 281–292.

Anjos, M. J., Lopes, R. T., Jesus, E. F. O., Assis, J. T., Cesareo, R., Barroso, R. C., Barradas, C. A. A. (2002). Elemental concentration analysis in soil contaminated with recyclable urban garbage by tube-excited energy-dispersive X-ray fluorescence. *Radiation Physics and Chemistry* 65, 495–500.

Barbar, L. C., Melo, V. F. (2008). Variability of the Chemical and Mineralogical Characteristics of Soils of the Metropolitan Region of Curitiba (PR). *Scientia Agraria* 9, 187–197.

Corrêa, R. S., Melo, V. F., Abreu, G. G. F., Sousa, M. H., Chaker, J. A., Gomes, J. A. (2017). Soil forensics: How far can soil clay analysis distinguish between soil vestiges? *Science and Justice*, 0–1.

Curi, N., Franzmeier, D. P. (1984). Toposequence of Oxisols from the Central Plateau of Brazil. Soil *Soil Science American Journal* 48, 341–346.

Dawson, L. A., Hillier, S. (2010). Measurement of soil characteristics for forensic applications. Surface and Interface Analysis 42, 363–377.

Fitzpatrick, R. (2011). Getting the dirt’. Gazette 73, 22–23.

Fitzpatrick, R., Raven, M., Self, P. (2012). Using forensics to inspire the next generation of regolith, soil and clay scientists. *Australian Regolith and Clays Conference* Mildura, 25–28.

Fitzpatrick, R. W. (2008). *Nature, Distribution, and Origin of Soil Materials in the Forensic Comparison of Soils*. In: Tibbett, M., Carter, D. O. eds. Soil Analysis in Forensic Taphonomy: Chemical and Biological Effects of Buried Human Remains. New York: Taylor & Francis Group, 366 p.

Fitzpatrick, R.W. & Raven, M.D. (2012). How pedology and mineralogy helped solve a double murder case: Using forensics to inspire future generations of soil scientists. *Soil Horizons,* 53(5), 14-29. doi:10.2136/sh12-05-0016.

Fitzpatrick, R. W. (2013). Soil : Forensic Analysis. In: Jamieson, A., Moenssens, A. A., eds. Encyclopedia of Forensic Science. Chichester: John Wiley, 18 p.

Fitzpatrick, R.W. & Raven, M.D. (2016). Guidelines for Conducting Criminal and Environmental *Soil Forensic Investigations* (Version 10.1). Centre for Australian Forensic Soil Science, Report, CAFSS_076, 46 pp.

Fitzpatrick, RW, Raven, M.D. and Self P.G. (2017). The role of pedology and mineralogy in providing evidence for 5 crime investigations involving a wide range of earth materials. *Episodes.* 40,148-156. http://dx.doi.org/10.18814/epiiugs/2017/v40i2/017017

Hammer, Ø., Harper, D. A. T. (2006). PAST, Paleontological Statistics. Version 2.07. Reference manual. Blackwell Publishing.

Hsu, P. H. (1989). Aluminium oxides and oxyhydroxides. In: Dixon, S. B. W., ed. Minerals in Soil Environments. Madison: *Soil Science Society of America*, 331–378 p.

Tan, K. B., Hajek, I. B. (1986). Termal analysis techniques. In: Klute A., ed. Methods of Soil Analysis Parte 1: Physical and Mineralogical Methods. Madison: *American Society of Agronomy*, 151–183 p.

Kämpf, N., Schwertmann, U. (1983). Goethite and hematite in climosequence in southern Brazil and their application in classification of kaolinitic soils. *Geoderma* 29, 27–39.

Marumo, Y. (2003). Forensic Examination of Soil Evidence. *Japanese Journal of Science and Technology for Identification* 7, 95–111.

McKeague, J. A. (1978). Manual on soil sampling and methods of analysis. Ottowa: *Canadian Society of Soil Science*.

McKeague, J. A., Day, J. H. (1966). Dithionite- and Oxalate-Extractable Fe and Al as Aids in Differentiating Various Classes Of Soils. *Canadian Journal of Soil Science* 46, 13–22.

Mehra, O. P., Jackson, M. L. (1960). Iron Oxide Removal from Soils and Clays by a Dithionite-Citrate System Buffered with Sodium Bicarbonate. *Clays and Clay Minerals* 7, 317–327.

Melo, V. F., Barbar, L. C. , Zamora, P. G. P., Schaefer, C. E., Cordeiro, G. A. (2008). Chemical , physical and mineralogical characterization of soils from the Curitiba Metropolitan Region for forensic purpose. *Forensic Science International* 179, 123–134.

Melo, V. F., Fontes, M. P. F., Novais, R. F., Singh, B., Schaefer, C. E. G. R. (2001). Characteristics of iron and aluminum oxides of different soil classes. *Brazilian Journal of Soil Science* 25, 19–32.

Mendonça, T., Melo, V. F., Schaefer, C. E. G. R., Simas, F. N. B., Michel, R. F. M. (2013). Clay Mineralogy of Gellic Soils From Fildes Peninsula, Maritime Antarctica. *Soil Science Society of America Journal* 77, 1842–1851.

Murray, R.C. (2011). Evidence from the Earth: Forensic Geology and Criminal Investigation. Second edition. *Mountain Press Publishing*, Missoula, MT.

Nakai, I., Furuya, S., Bong, W., Abe, Y., Osaka, K., Matsumoto, T., Itou, M., Ohta, A., Ninomiya, T. (2014). Quantitative analysis of heavy elements and semi-quantitative evaluation of heavy mineral compositions of sediments in Japan for construction of a forensic soil database using synchrotron radiation X-ray analyses. *X-Ray Spectrometry* 43, 38–48.

Pirrie, D., Dawson, L. A., Graham, G. (2017). Predictive geolocation: Forensic soil analysis for provenance determination. *Episodes* 40, 141–147.

Poggere, G. C., Melo, V. F., Francelino, M. R., Schaefer, C. E., Simas, F. N. (2016).

Prandel, L. V., Melo, V. F., Brinatti, A. M., Saab, S. C., Salvador, F. A. S. (2017) X-ray Diffraction and Rietveld Refinement in Deferrified Clays for Forensic Science. *Journal of Forensic Sciences*, 1–7.

Ravansari, R., Lemke, L. D. (2018). Portable X-ray fluorescence trace metal measurement in organic rich soils: pXRF response as a function of organic matter fraction. *Geoderma* 319, 175–184.

Ruffell, A. & McKinley, J. (2008). *Geoforensics*. John Wiley and Sons, Ltd., Chichester.

Schulze, D. G. G. (1984). The influence of aluminum on iron oxides. VIII. Unit-cell dimensions of Al-substituted goethites and estimation of Al from them. *Clays and Clay Minerals* 32, 36–44.

Schwertmann, U., Kämpf, N. (1985). Properties of goethite and hematite in kaolinitic soils of Southern and Central Brazil. *Soil Science Society American Journal,* 139.

Schwertmann, U., Taylor, R. M. Iron oxides. In: Dixon JB, Weed SB, organizadores. Minerals in soil environments. 2o ed Madison: *Soil Science Society of America*; 1989. p. 380–427.

StatSoft I. STATISTICA (data analysis software system). 2011.

Stoppe, N., Amelung, W., Horn, R. (2015). Extracción química de oxi(hidrox)óxidos sedimentario utilizando oxalato de amonio y ditionito sódico revisado - una explicación de los procesos en sedimentos costeros. *Agro Sur* 43, 11–17.

Torrent, J., Cabedo, A. (1986). Sources of iron oxides in reddish brown soil profiles from calcarenites in Southern Spain. *Geoderma* 37, 57–66.

Woods, B., Lennard, C., Kirkbride, K. P., Robertson, J. (2014). Soil examination for a forensic trace evidence laboratory-Part 1: *Spectroscopic techniques*. Forensic Science International 245, 187–194.

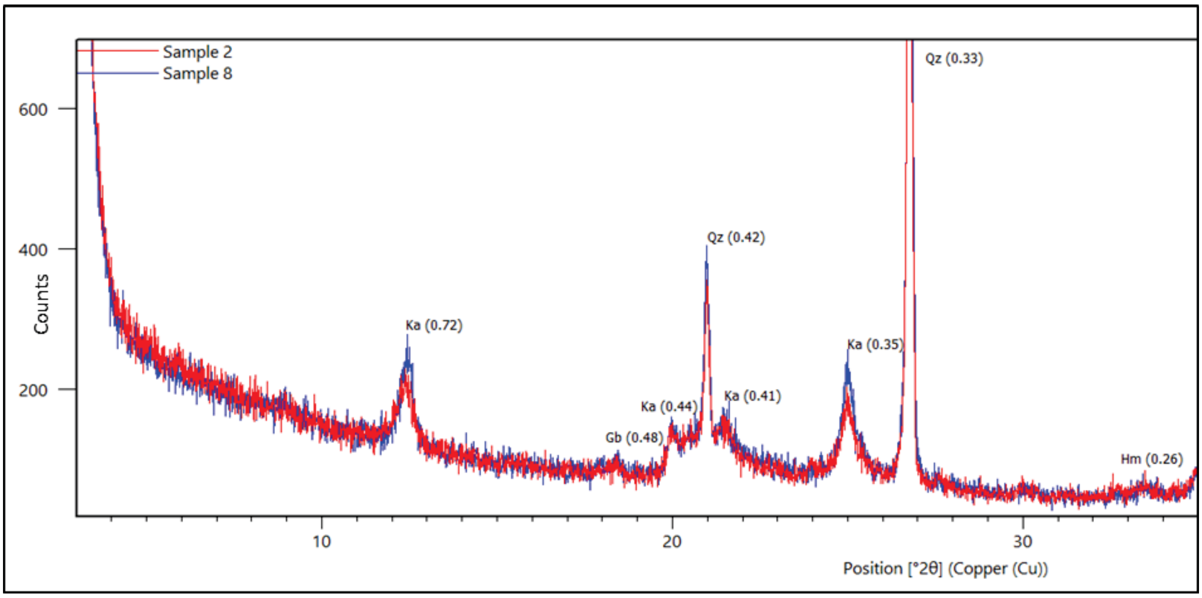
Wowk, G. I. T., Melo, V. F. (2005). Evaluation of the level of lead, in floodplain soil, from the recycling of bacteria. Brazilian *Journal of Agricultural Engineering* 9, 613–622.

Supplementary material S1. Contents of organic matter of the samples and values of gibbsite and kaolinite obtained by thermogravimetry analysis (TGA).

Sample	**Organic matter**	**Gibbsite**	**Kaolinite**
	-----------------g kg$_{-1}$	-------------------	
Group 1			
1	95	51.9	509.9
2	132	38.4	438.1
3	56	40.7	419.2
Mean	94	43.7	455.7
SD	31	5.9	39.1
Group 2			
4	81	34.5	462.7
5	76	67.5	561.6
6	73	32.9	332.3
7	71	34.8	356.0
ean	75	42.4	428.1
SD	4	14.5	91.4
Group 3			
8	94	67.1	650.8
9	121	72.5	583.6
10	74	63.8	536.8
11	71	44.7	454.5
12	63	31.3	311.4
13	72	67.1	572.1
14	71	43.2	435.5
15	65	34.1	321.6
16	50	51.3	571.9
17	62	10.6	236.9
18	98	29.0	333.4
19	48	44.0	438.8
20	43	23.8	357.7
21	41	24.3	228.1
Mean	70	43.4	430.9
SD	22	18.4	131.8
Group 4			
22	31	21.8	314.0
23	52	49.5	748.6
24	29	50.3	429.4
Mean	37	40.5	497.3
SD	10	13.2	183.8
General Mean	70.3	42.9	441.9
General SD	25.2	16.1	128.3

SD-standard deviation.

Supplementary material S2. X-ray diffraction patterns of the silt+clay fractions from sample 2, which represents a sample from Group 1 and sample 8, which is representative of a sample from Group 3.



Note: Ka – Kaolinite; Gb – Gibbsite; Qz – Quartz; Hm – Hematite. The interlayer distances in nanometres (nm) are presented in parenthesis

3 CHAPTER II: VALIDATION OF A STANDARD OPERATING PROCEDURE (SOP) FOR FORENSIC SOILS IN BRAZIL: APPLICATION TO A SIMULATED BLIND SAMPLING[2]

3.1 RESUMO

Os vestígios do solo são úteis como evidência forense devido ao seu potencial de transferência e aderência a diferentes tipos de superfícies em uma variedade de objetos ou pessoas. Este trabalho teve como objetivo testar um Procedimento Operacional Padrão (POP) para amostragem de solo forense e um protocolo analítico no Brasil entre a academia e a polícia científica por meio de uma cena de crime simulada às cegas. As amostras foram coletadas em quatro locais localizados na Região Meropolitana de Curitiba (RMC). Todos os solos foram classificados como Cambissolos e o material de origem em Curitiba é argilito, e em Colombo é calcário. Cerca de 3 g da fração silte + argila foram isoladas e analisadas por procedimento químico sequencial: i) extração de óxidos de Fe e Al pobremente ordenados com oxalato de amônio (OA); ii) extração de óxidos de Fe cristalinos com ditionito-citrato-bicarbonato de sódio (DCB); e iii) extração de aluminossilicatos e gibbsita de baixa cristalinidade com NaOH 0,5 mol L^{-1} fervente. Todos os dados foram transformados por raiz quadrada e analisados por meio de uma análise de componentes principais (ACP). A maioria das amostras foi corretamente agrupada de acordo com sua localização de origem em todos os quatro locais testados, mostrando o potencial do Procedimento Operacional Padrão (POP) em cenas de crimes. Os procedimentos de amostragem apresentados no POP foram detalhados o suficiente para permitir o trabalho policial apropriado em casos forenses em qualquer parte do Brasil. Como considerações futuras, modificações no tratamento da amostra e protocolo analítico podem ser feitas dependendo do contexto do trabalho forense, sempre buscando utilizar as melhores metodologias para o exame forense de amostras de solo. Ainda, estudos futuros podem ser alocados em áreas altamente impactadas pelo homem, como cidades ou áreas industriais. Atividades antropogênicas intensas, como o descarte de resíduos domésticos em áreas urbanas, podem reduzir potencialmente o poder de discriminação de um protocolo analítico químico proposto.

Palavras-chave: análise química sequencial, amostragem de solos, análises de solos forenses, óxidos de ferro.

3.2 ABSTRACT

Soil traces are useful as forensic evidence due to their potential to transfer and adhere to different types of surfaces on a range of objects or persons. This work aimed to test a Standard Operating Procedure (SOP) for forensic soil sampling and an analytical protocol in Brazil between the academy and the scientific police through a blind simulated crime scene scenario. Samples were collected at four sites located in the Curitiba Meropolitan Region. All soils were classified as Inceptsol and the

---

[2] Published on Revista Brasileira de Ciência do Solo, DOI:https://doi.org/10.1590/18069657rbcs20190010

parent material in Curitiba is claystone and in Colombo is limestone. Around 3 g of the silt +clay fraction was isolated and analysed by chemical sequential procedure: i) extraction of poorly ordered Fe and Al oxides with ammonium oxalate (AO); ii) extraction of crystalline Fe oxides with sodium citrate-bicarbonate-dithionite (CBD); and iii) extraction of poorly ordered aluminosilicates and gibbsite with boiling 0.5 mol L^{-1} NaOH. All data was transformed by square root and analysed using a principal component analysis (PCA). Most of the samples were correctly grouped according to their location of origin at all four sites tested, showing the potential of the Standard Operating Procedure (SOP) in a real crime scene. The sampling procedures presented in the SOP were detailed enough to allow the appropriate police work in forensic cases in any part of Brazil. As future considerations, modifications to the sample treatment and analytical protocol could be made depending on the context of the forensic work, always improving on the best methodologies for the forensic examination of soil samples. One example is that of futures studies may be areas which are highly impacted by human such as cities or industrial areas. Intense anthropogenic activities, such as domestic waste disposal in urban areas would potentially reduce the discrimination power of such a proposed chemical analytical protocol.

Keywords: sequential chemical analysis, soil sampling, forensic soils analyses, iron oxides.

3.3 INTRODUCTION

In forensic science, soil can be a powerful contact trace material as it can serve to test a link of a potential source of the soil with an item on which it is found. Its potential as evidence in crime scenes is due to its often highly individualistic nature, which results in a vast diversity and complexity, enabling it to be distinguished between soil samples (Farrugia et al., 2012; Dawson and Mayes, 2015; Finley et al., 2015; Woods et al., 2016; Demanèche et al., 2017). In addition, soil has a large probability to transfer and to retain (mainly the small fractions such as clay, silt, and associated organic matter), and it is expected that the suspect does not dispense much attention to the potential soil traces adhered in objects related to a crime (Fitzpatrick, 2013).

Soil traces can easily be visualized and collected in crime scenes, and a range of combined analytical techniques can be applied to characterize them. For a soil of unknown origin, the choice of method will depend on the aspects such as amount, condition, and the predominant fraction of the sample, as well as the equipments and time available (Marumo, 2003; Dawson and Hillier, 2010). In Brazil the agreement between the Department of Soil Science & Agricultural Engineering of the Federal

University of Parana (UFPR) and the State and Federal Scientific Police allowed substantial advance in the establishment of the chemical, physical, and mineralogical protocol of analyses to investigate soil traces (Melo et al., 2008; Prandel et al., 2017; Corrêa et al., 2018; Melo et al., 2018).

Laboratory analyses are only efficient in the characterization of the samples if the soil traces are appropriately sampled at the crime scene and subsequently follow a rigorous procedure of sampling and handling (Fitzpatrick, 2011; Fitzpatrick and Raven, 2012; Wald, 2015; Fitzpatrick and Raven, 2016). In Brazil, there is an emergent need to standardize the soil sampling and adjust the police sampling with the sampling carried out by the academic/scientific research. Until recently, soil residues in crime scenes which occurred in Brazil have been largely ignored by the criminal experts due to the unavailability of standard procedures for soil sampling, both for the object/victim and the suspect.

To improve the lack of procedures for soil sampling and to allow an efficient comparison and discrimination among the soil samples collected in forensic caseworks, a Standard Operating Procedure (SOP) was developed in Brazil. The SOP was created by Researchers of the Department of Soil Science & Agricultural Engineering from the Federal University of Paraná (UFPR) for application in the routine work of the State and Federal Police from Brazil. The final step for the wider use of the SOP by the scientific police from Brazil requires a test and calibration of the soil sampling procedures.

This work aimed to develop and test a Standard Operating Procedure (SOP) for forensic soil sampling in Brazil between the academy and the scientific police through a simulated crime scene test scenario. To verify the compatibility of the soil sampling, it was used chemical, physical, and mineralogical data from the soil samples using the sequential protocol of analyses previously developed and validated in the same region for forensic purposes by Melo et al. (2008). Those authors were able to separate soil trace samples (1.0 g) collected in areas developed under the same parent material located in different neighbourhoods, as well grouped soil samples from the same neighbourhood (samples were separated away from each other at a distance of 2.0 m) in Colombo and Curitiba, Paraná State, Brazil. The hypothesis of the present work is that the samples collected by the academy and Federal Police form homogenous groups related to their physical, chemical, and mineralogical properties when sampled from the same site (neighborhood). Such similarity of groupings certifies that the police practitioners can follow the soil sampling procedures established by the academy in the SOP and the analytical protocol developed by Melo et al. (2008) present high potential to be applied in forensic case works.

3.4 MATERIALS AND METHODS

3.4.1 SIMULATED CRIME SCENE AND SOIL TRACES SAMPLING

A mock crime scene was designed in June 2017 in two neighborhoods from the urban areas in Curitiba (Santa Candida and Boa Vista) and Colombo (Guaraituba and Guarani), Paraná State, Brazil (Figure 1). All sites belong to the Curitiba Metropolitan Region and are away from each other by a distance of 2 to 4 km. Rates of crimes are generally higher in urban and inner-city areas compared to in rural areas (Gibbs and Haldenby, 2008). The sites were located on both similar and contrasting parent material (Table 1) to test the reliability within and between comparisons. All soils were classified as Inceptisol according to U.S. Soil Taxonomy (Soil Survey Staff, 2010) and as Cambissolo Háplico according to Brazilian Soil Classification System (Santos et al., 2013).

The occurrence of a crime was simulated in each one of the four neighborhoods from both Colombo and Curitiba. Soil samples were impregnated on the sole of the boot through a simulation of a suspect walking over a crime scene (at the central position of figure 1 in each location). Soil trace samples on the sole of the boot were collected in 3 or 4 replicates according to the site of impregnation (toe and heel positions on the sole of the boot) (Figure 2). At the moment of the impregnation, the soil was wet, which allowed the impregnation of a large quantity of soil vestige on the sole of the boot (Figure 2). All procedures for sampling in unfavorable situations of little soil vestige impregnation on the tools of the suspect are presented in the SOP.

From the site of the muddy boot (central point in a frame), four points were chosen at positions around that central position, in each corner of a square with 1.5 m apart (Figure 1). The soil sampling on the sole of the boot and from the four positions around from the muddy boot central position aimed to compare the soil sampling procedures between the academy and the criminal experts, carried out on the suspect (simulated by the sole of the boot) and in the crime scene (repetitions in the frame).

Figure 1. Sampling locations within Curitiba and Colombo, State of Paraná, Brazil. Photographs taken with markers at each of the four sampling positions carried out in a frame with a central point (sole of the boot) located in four separate urban areas,

Santa Cândida - Curitiba (a), Boa Vista - Curitiba (b), Guarani - Colombo (c), Guaraituba -Colombo (d).

Table 1. Description of the sampling sites.

Sample	Position	Neighbourhood	Layer	Altitude	UTM (22J)		Bedrock
					E-W	N-S	
			——— m ———				
Site 1							
1	Frame	Santa Cândida	0.00-0.05	937	678,203 W	7,192,226 S	Claystone
2	Frame		0.00-0.05				
3	Frame		0.00-0.05				
4	Frame		0.00-0.05				
5	Heel						
6	Heel						
7	Toes						
Site 2							
8	Frame	Guarani	0.00-0.05	924	682,747 W	7,192,633 S	Limestone
9	Frame		0.00-0.05				
10	Frame		0.00-0.05				
11	Frame		0.00-0.05				
12	Heel						
13	Heel						
14	Toes						
Site 3							
15	Frame	Guaraituba	0.00-0.05	910	683,189 W	7,195,492 S	Limestone
16	Frame		0.00-0.05				
17	Frame		0.00-0.05				
18	Frame		0.00-0.05				
19	Heel						
20	Heel						
21	Toes						
Site 4							
22	Frame	Boa Vista	0.00-0.05	925	676,186 W	7,190,193 S	Claystone
23	Frame		0.00-0.05				
24	Frame		0.00-0.05				
25	Frame		0.00-0.05				
26	Heel						
27	Toes						
28	Heel						
Samples collected by Federal Police							
29	2 γ						
30	3 δ						
31	4 π						
32	5 ρ						
33	6 β						
34	7 σ						
35	8 α						
36	10 β						
37	11 σ						
38	12 ρ						
39	13 δ						
40	14 φ						
41	15 α						

42	16 π
43	17 α
44	20 ρ
45	21 γ
46	22 β
47	24 σ
48	25 π
49	28 δ
50	29 ρ
51	30 σ
52	32 β

Note: heel and toe samples are trace samples, collected at the centre of the sampling frame. Santa Cândida and Boa Vista are neighborhoods from Curitiba (state of Paraná), Guarani and Guaraituba are neighbourhoods from Colombo (Curitiba Metropolitan Region).

Figure 2. Soil residues adhered on the sole of the boot used in the simulated crime scene showing the sites of sampling in repetitions on the toes and heel areas.

The soil sampling followed all procedures established in the Standard Operating Procedure (SOP) prepared by the researchers from the Department of Soil Science & Agricultural Engineering - UFPR. This SOP will be published after this validation. Samples from each corner of the frame were collected from 0.00 to 0.05 m of depth, using a stainless steel spatula, carefully sterilizing the tool between replicate sampling. All samples from the frame and the boot were placed in sterilized plastic flasks of 30 mL (packed individually), commercialized for laboratory exams.

Researchers proceeded to soil sampling alone without the police being present and identified the samples according to the nature and site (samples from 1 to 28; Table 1). In addition, the police followed the same sampling procedure without the presence of the researchers, following only the sampling protocol from the SOP. The identification of the samples made by the experts was blind (sample 29 to 52; Table 1), which means that during the laboratory and statistical analyses the origin of the samples collected by the police was not known.

3.4.2 SOIL SAMPLE PREPARATION

Samples were dried at 40 °C, hand ground, and sieved at 2 mm to remove large stones and vegetation fragments. Sample homogenization was obtained using a plastic cone and quartered, and the partition was positioned to obtain 3 g of each sample. Even considering such a worst scenario when the amount of soil sample does not perpass 1 g of soil. In this work we decided to use 3 g of soil sample. This amount was considered in relation to the available samples both by academic and police sampling. The use of a higher amount was also possible due the environmental conditions, which presented humidity high enough to improve the adherence of the soil on the sole of the boot (Figure 2). Samples were macerated with a rubber baton and dispersed into a Na_2CO_3 0.1 g L^{-1} solution (pH 10) and sieved at 0.053 mm sieve to separate the sand fraction. Suspensions containing silt, clay, and organic matter particles were collected in porcelain capsules. This step was repeated until the water after maceration was clear. The fractions <0.053 mm (silt, clay, and organic matter) were weighed and quantified after drying. The exclusion of the sand fraction in the sequential analyses (Figure 3) was due to the reduced amount of sample available in this work. The presence of sand can reduce the sample homogenization, and it is the finer particles (silt and clay) which are preferentially adhered on shoes, tires, clothes, etc. (Fitzpatrick, 2009). Besides these aspects, we must consider that the smaller fractions, especially the colloid-size particles, are dominated by the clay minerals, humic substances, and iron, aluminum, and manganese oxi-hydroxides, which are important compounds to be considered and further investigated using sequential chemical extractions.

3.4.3 REMOVAL OF ORGANIC MATTER

Samples composed of silt+clay+organic matter were homogenized with 30% (v/v) of H_2O_2 in 70°C water bath to remove the organic matter fraction. Then, soil samples were oven-dried at 40°C for 24h.

3.4.4 CHEMICAL AND MINERALOGICAL ANALYSES

The analytical methods were separated into destructive and non-destructive (Melo et al., 2008). Samples from non-destructive analyses were re-used in the subsequent procedures.

3.4.4.1 SEQUENTIAL EXTRACTIONS

Subsamples (silt + clay) were submitted for sequential extractions according to the methodology proposed by Melo et al. (2008) (Figure 3). For all extractions (ammonium oxalate, sodium dithionite-citrate-bicarbonate, and NaOH 0.5 mol L^{-1}) the following procedures were applied : i) previously each extraction the samples were oven-dried (for 24 h) at 40 °C, grounded and sieved at 0.2 mm in order to reduce the particles size, improve the surface reactivity and increase the efficiency of the extractions; ii) subsequently each extraction, samples were washed with $(NH_4)_2CO_3$ 0.5 mol L^{-1} and deionized water, using 50 and 20 mL per washing, respectively, to remove the excess salt; iii) after washing, the samples were dried at 40 °C for 24 h, and the final mass was determined; iv) extracts were obtained by centrifugation (3,000 rpm for 10 min), and element levels (Fe, Al, As, Ba, Ca, Cd, Cr, Cu, K Mg, Mn, Ni, P, Pb, S, Si, V, Zn, and Mo) were determined by inductively coupled plasma optical spectrometry (ICP-OES).

3.4.4.1.1 EXTRACTION OF LOW CRYSTALLINITY Fe AND Al OXIDES

The first step of the sequential extractions was performed with ammonium oxalate (AO) to determine the poorly crystallized Fe, Al, and Si forms (short-range order minerals) (Figure 3) using 0.8 g of soil sample (silt + clay) and 20 mL of 0.2 mol L^{-1} ammonium oxalate at pH 3.0, in the dark (McKeague, 1978).

3.4.4.1.2 EXTRACTION OF CRYSTALLINE Fe OXIDES

Samples were recovered and the crystalline iron oxide extraction was proceeded with sodium dithionite-citrate-bicarbonate (DCB) method (Figure 3) (Mehra and Jackson, 1960). Powdered samples of 0.6 g were disposed in 100 mL tubes and subjected to the extraction three times with 9.6 mL of a solution of sodium citrate 0.3 mol L^{-1} + 1.2 mL of a solution of sodium bicarbonate 1.0 mol L^{-1} + 0.24 g of sodium dithionite. Samples in solution were manually agitated while heated at 70 °C in a water bath for 30 min.

3.4.4.1.3 EXTRACTION OF LOW CRYSTALLINITY ALUMINOSILICATES AND GIBBSITE

A mass of 0.35 g was used in the last sequential analysis for the extraction of poorly ordered aluminosilicates and gibbsite (short-range order γ-$Al(OH)_3$, Al-O-Si layers, and Si-O (opaline silica) resistant to the previous ammonium oxalate extraction) with boiling NaOH 0.5 mol L^{-1} (Figure 3) (Jackson et al., 1986; and Melo et al., 2002). Samples were disposed in tubes with 2 mL of NaOH 0.5 mol L^{-1} without heating and were homogenized and moistened with that initial solution. After that, teflon beckers containing 15 mL of the NaOH 0.5 mol L^{-1} solution were placed in a sand bath at 200 °C until it boiling; the solution was then added to the tube with the samples. The boiling solution remained in contact with the sample for three minutes under constant manual agitation. Immediately after, samples were cooled in a recipient with cool water and then centrifuged.

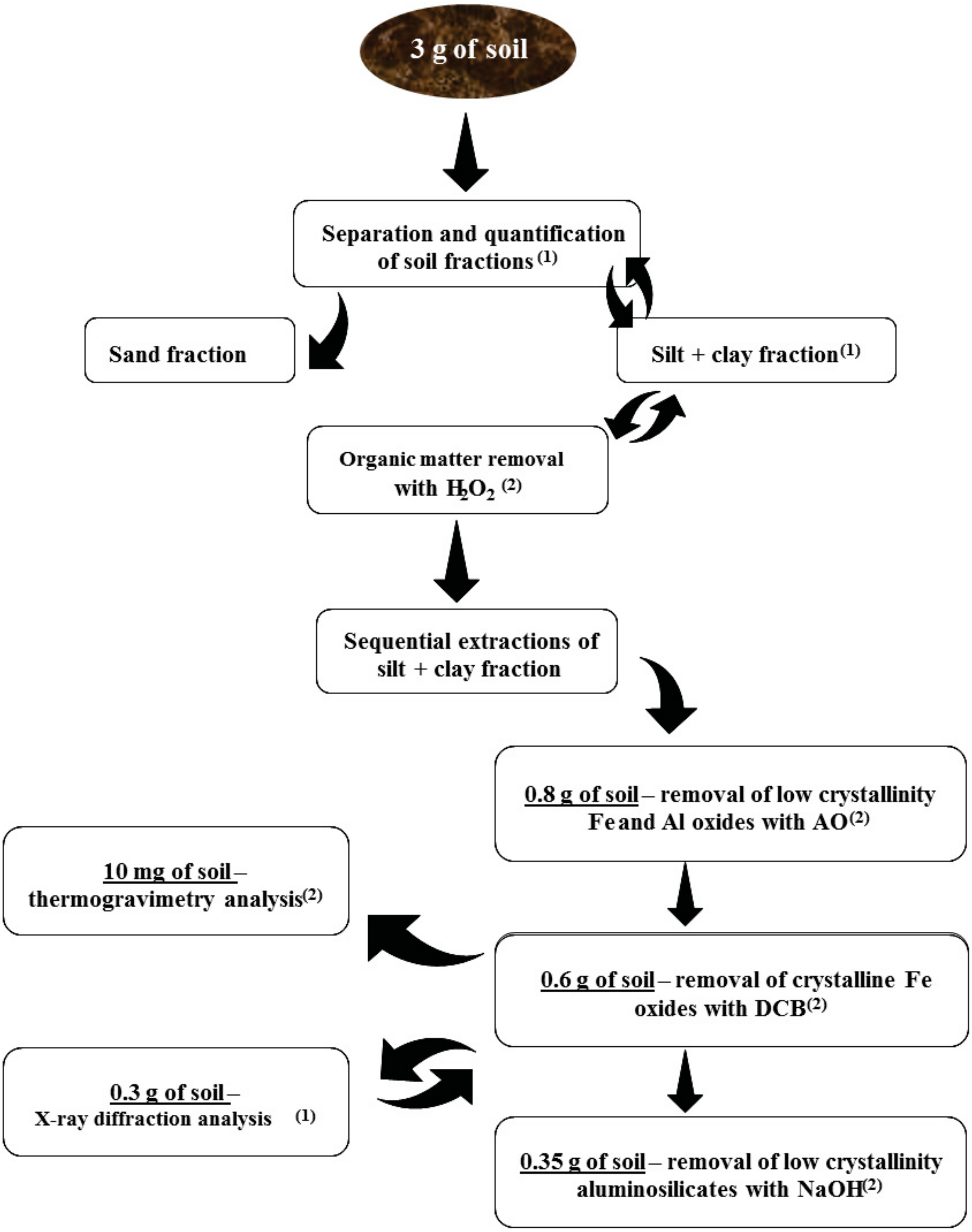


Figure 3. Scheme of analyses. Note: (1) non-destructive and (2) destructive analyses. H_2O_2: hydrogen peroxide; AO: ammonium oxalate; DCB: dithionite-citrate-bicarbonate; NaOH: sodium hydroxide.

3.4.4.2 THERMOGRAVIMETRIC ANALYSIS (TGA)

An amount of 10 mg of each sample previously extracted with DCB (Figure 3) was used to thermogravimetry analysis to obtain the TG diagrams. Samples were heated from room temperature to 950 °C at 10 °C min s^{-1} and gas flux of 50 mL min^{-1}, under N_2 atmosphere. Contents of kaolinite (Kt) and gibbsite (Gb) were obtained considering the mass loss due to the de-hydroxylization of these minerals when heated (Tan and Hajek, 1986).

3.4.4.3 SILT + CLAY MINERALOGICAL IDENTIFICATION BY X-RAY DIFFRACTION (XRD)

Approximately 0.3 g of the silt + clay samples were prepared (random powder) for XRD analysis. The diffractograms were obtained by the equipment X' $Pert^3$ Powder, using a vertical goniometer and scanning from 3° to 60° 2θ at 1° 2θ min^{-1} in accelerator mode which presents a linear array of detectors adjusted to the fast mode, for 4 min and 48 s. The diffractometer is equipped with nickel filter and Cu Kα radiation and was operated at 20 mA and 40 kV.

3.4.5 STATISTICAL ANALYSES

All results were square root transformed and then analyzed using a principal component analysis (PCA) using the Statistica software (StatSoft, 2011) and Paleontological Statistics (PAST) software testing for Bray-Curtis similarities (Hammer et al., 2001). The similarity values were then used to link each sample to a specific location, and the accuracy of classification was reached by comparing the predicted sample location to the known true sample location.

The identification of the samples from the police was only revealed after all statistical analysis was completed, and the grouping of the samples collected by the researchers and experts was unknown.

3.5 RESULTS

Contents of low crystallinity Fe and Al oxides extracted by ammonium oxalate (AO) varied from 5.7 to 24.5 g kg^{-1} and 6.1 to 17.1 g kg^{-1}, respectively (Table 2). The higher concentrations of both oxides were related to the samples formed from claystone (i.e., sites 1 and 4), and the higher variation was observed for samples collected at site 4.

Table 2. Contents of iron and aluminum oxide and elements obtained in the sequential treatments of the clay + silt fraction.

Sample	Fe_2O_3	Al_2O_3	As	Ba	Ca	Cd	Cr	Cu	K	Mg	Mn	Ni	P	Pb	S	Si	V	Zn
	-----g kg^{-1}----		------------mg kg^{-1}------------															
Site 1																		
1	14.0	14.8	4.5	26.1	41.8	0.2	16.8	17.1	401.5	574.8	150.3	2.2	577.2	13.9	669.2	715.3	19.7	34.7
2	15.2	17.1	4.1	24.5	55.1	0.3	18.6	11.4	343.1	358.5	196.8	2.1	534.4	13.8	661.5	760.7	22.3	29.1
3	13.8	11.6	3.4	30.7	45.9	0.2	10.7	10.1	277.0	500.7	135.3	1.7	532.0	12.0	471.2	627.5	19.1	26.0
4	12.1	10.1	3.2	25.1	35.9	0.2	9.1	8.8	293.5	422.8	98.8	1.7	510.6	30.1	411.9	552.8	17.9	21.2
5	12.6	12.6	3.7	43.2	31.2	0.2	13.4	10.8	423.9	753.7	250.6	1.8	584.7	23.7	620.3	579.2	18.8	27.9
6	13.0	13.8	3.8	48.2	31.5	0.2	16.5	10.8	657.9	951.0	252.8	2.1	572.3	14.5	695.5	605.5	18.4	29.1
7	12.8	13.3	2.7	51.1	32.5	0.2	14.8	9.4	490.1	837.2	273.3	2.2	680.5	11.0	744.0	571.1	18.0	22.2
Site 2																		
8	6.7	10.2	3.8	24.4	45.0	0.1	7.6	17.6	499.3	1632.3	168.8	1.2	527.0	20.8	560.3	550.9	8.7	17.2
9	8.8	9.0	4.3	93.7	31.3	0.2	16.0	21.1	2022.7	2242.0	359.6	4.9	1376.2	17.5	1191.2	651.3	18.7	89.0
10	6.6	10.4	2.8	31.4	34.5	0.1	7.9	14.1	528.2	1584.4	200.9	1.4	525.3	20.5	578.6	618.4	9.8	12.7
11	8.6	6.1	2.8	11.6	32.5	0.1	4.5	11.4	329.7	705.1	132.2	1.5	374.3	30.6	339.8	482.4	21.8	23.4
12	6.9	10.1	1.3	26.4	32.6	0.2	8.1	11.5	521.6	1602.2	187.8	2.4	452.4	21.2	544.8	409.4	9.0	33.5
13	5.9	9.6	2.2	26.9	37.3	0.1	6.8	14.3	410.0	1437.0	178.2	1.8	378.8	28.6	499.7	544.2	8.4	15.5
14	6.1	10.0	2.9	26.5	32.5	0.1	8.3	11.0	442.5	1693.8	191.7	1.4	411.4	26.5	623.6	577.7	9.0	18.6
Site 3																		
15	6.8	11.4	1.8	13.4	29.1	0.1	8.4	13.6	374.7	1160.1	87.5	1.1	302.0	10.1	296.5	416.0	16.3	24.9
16	6.1	10.1	1.6	23.3	27.7	0.1	7.2	14.4	342.3	1113.1	150.5	1.1	357.2	30.1	432.4	520.5	8.6	13.4
17	19.0	9.1	3.1	2.1	31.3	0.3	15.3	11.4	189.0	248.0	71.4	3.9	571.7	73.8	480.1	818.3	30.9	68.9
18	6.8	12.2	3.0	11.2	32.7	0.1	13.2	15.5	412.4	1845.4	90.4	1.0	285.5	7.8	401.9	583.9	17.7	14.3
19	6.6	11.2	3.0	10.9	32.2	0.1	8.2	10.9	398.2	1377.9	97.4	1.1	315.0	13.1	298.0	510.5	15.5	19.2
20	5.7	10.0	2.0	12.5	32.4	0.1	9.3	11.3	443.4	1545.8	100.3	1.6	299.1	26.2	340.4	507.3	14.2	16.2
21	6.3	11.1	3.2	13.0	33.4	0.1	10.3	14.7	449.4	1722.9	107.9	1.2	320.7	16.1	348.0	555.0	16.7	19.0
Site 4																		
22	7.7	12.6	3.3	8.1	37.7	0.1	8.6	12.1	256.9	485.6	80.2	1.3	350.0	10.7	537.9	426.7	18.8	25.6
23	17.1	12.2	3.0	65.3	47.9	0.3	16.8	32.0	850.5	3240.5	178.2	9.3	1196.5	3.4	833.1	808.7	19.8	178.5
24	21.9	10.7	3.6	73.8	38.9	0.3	16.1	26.1	875.1	2347.8	267.8	8.3	2012.1	10.7	1348.7	1001.7	33.7	152.3
25	24.5	8.8	3.7	26.0	39.7	0.4	14.2	19.0	505.0	773.6	156.0	4.7	1677.8	20.5	682.3	1036.1	24.5	86.4
26	10.7	8.0	2.8	146.2	67.3	0.2	8.9	23.1	1065.3	2990.4	362.7	4.6	2154.8	7.9	1282.7	623.4	19.0	109.2
27	11.3	10.0	3.4	89.5	42.0	0.2	16.3	32.5	938.7	3052.9	382.8	5.5	1792.4	24.1	2297.6	678.7	19.0	116.5
28	9.1	7.5	2.5	0.0	48.1	0.1	4.2	28.6	608.1	1772.2	345.9	2.7	2432.8	2.5	679.6	605.5	13.6	119.7
Samples collected by Federal Police																		
29	14.7	15.7	3.9	33.6	37.9	0.2	17.4	13.6	355.3	618.9	184.6	2.0	581.7	10.9	656.0	683.9	23.3	26.1
30	10.7	17.2	1.9	5.9	38.3	0.1	15.1	14.4	298.3	702.7	85.6	4.0	354.4	22.5	431.6	521.3	24.4	96.4

31	6.1	9.9	1.8	24.6	30.9	0.1	7.8	11.3	480.8	1582.5	186.1	1.5	389.7	18.9	572.2	548.2	8.7	13.5
32	9.7	9.4	2.9	111.4	34.0	0.1	17.3	20.5	2405.6	2465.4	397.5	4.8	1424.3	14.1	1343.6	595.1	18.8	82.4
33	18.7	21.1	4.4	31.5	38.7	0.4	24.9	11.7	423.8	507.5	268.6	2.7	567.7	8.6	820.7	882.1	29.7	31.3
34	7.3	13.2	2.4	11.4	31.0	0.1	15.4	10.2	520.2	1692.0	89.0	1.1	292.3	13.9	428.0	424.8	17.9	18.6
35	11.0	9.8	3.2	80.3	33.4	0.2	13.3	14.1	1141.9	1430.6	353.4	2.3	1032.9	20.8	773.5	673.0	18.4	38.5
36	14.7	16.6	3.9	37.1	34.7	0.2	20.2	12.4	389.2	835.2	210.1	2.4	544.9	5.0	774.9	707.0	22.5	27.9
37	6.8	12.5	3.2	11.7	34.7	0.1	15.6	12.2	519.9	2009.4	102.3	1.4	292.6	9.6	497.6	572.8	17.8	17.0
38	6.9	10.6	1.9	27.0	37.2	0.1	8.0	15.9	474.8	1562.6	184.2	1.4	390.6	12.9	561.2	575.3	10.2	16.4
39	11.0	10.1	3.0	95.7	36.4	0.2	14.4	17.9	1364.6	3271.8	329.7	3.4	1482.1	10.3	1365.8	626.8	19.7	77.7
40	14.8	18.0	3.8	28.4	40.2	0.2	22.9	12.8	386.7	688.8	110.0	2.4	512.2	8.0	854.0	721.9	20.8	30.1
41	7.1	12.4	2.5	11.0	37.5	0.1	14.9	11.4	514.5	1908.7	101.2	1.3	287.1	9.6	478.4	576.4	18.5	19.1
42	7.9	12.1	3.5	29.8	44.3	0.1	10.4	12.2	538.7	1906.9	214.0	1.4	412.5	17.1	673.7	676.3	11.4	15.0
43	12.5	14.5	3.5	47.8	33.2	0.2	16.7	12.6	495.5	732.0	308.6	2.0	672.0	15.6	855.2	729.8	17.4	24.6
44	7.3	12.2	2.2	12.6	34.8	0.0	14.1	8.5	460.2	1924.2	111.2	1.3	293.0	11.0	432.7	592.4	18.6	28.4
45	7.9	11.1	3.3	33.0	32.8	0.1	8.6	14.1	486.0	1826.0	203.4	1.4	481.4	11.8	603.1	688.3	11.6	21.4
46	11.4	10.0	4.2	112.5	46.2	0.2	13.8	21.1	1103.4	2546.2	368.8	4.0	1734.5	8.4	1443.6	656.3	21.4	77.1
47	7.3	13.2	2.1	10.3	39.1	0.1	15.9	12.0	557.8	1971.9	99.5	1.1	300.4	10.5	473.1	570.1	19.7	28.3
48	7.0	10.0	2.9	27.3	34.4	0.1	8.7	12.4	403.0	1721.1	185.9	1.3	551.3	9.5	671.9	580.8	9.6	13.3
49	12.9	11.6	4.0	32.5	34.8	0.2	9.5	9.3	399.7	576.5	197.4	1.7	670.1	12.0	423.0	654.1	18.4	20.5
50	6.6	11.2	1.9	13.3	32.8	0.1	11.5	9.4	406.0	1791.8	92.3	1.2	257.3	11.3	374.1	654.5	17.6	13.5
51	6.9	10.9	2.6	20.9	32.2	0.1	8.7	11.2	339.7	981.7	148.7	1.3	323.0	15.9	469.6	514.7	9.4	14.4
52	10.0	8.0	2.1	155.6	34.3	0.1	10.2	22.4	1158.6	2952.5	350.0	4.0	3837.4	2.1	1508.2	535.0	17.0	117.3

Note: Iron, aluminum oxides and elemental contents extracted by ammonium oxalate (AO).

Contents of crystalline Fe_2O_3 (22.4 to 48.6 g kg^{-1}) extracted by dithionite-citrate-bicarbonate (DCB) were, approximately, the double in comparison to the AO extraction (5.7 to 24.5 g kg^{-1}) (Tables 2 and 3). In agreement with the lower contents of poor crystallinity Fe oxides verified in AO extracts, samples from site 2 and 3 showed higher contents of Fe_2O_3 extracted by DCB, ranging from 27.2 to 48.6 g kg^{-1}. As verified in the AO, the wide variation in the contents of Fe_2O_3-DCB and Al_2O_3-DCB belongs to the samples from site 4 (22.4 to 47 g kg^{-1} and 4.8 to 17.4 g kg^{-1}, respectively). Contents of Ca extracted by DCB (679.4 to 10,962.9 mg kg^{-1}) were much higher than the contents extracted by AO (27.7 to 45.9 mg kg^{-1}). The higher values were observed in the samples from site 2 and 3, formed by a calcium-rich bedrock.

The Fe extraction boiling NaOH 0.5 mol L^{-1} was very limited (Table 4). The variations in the Al_2O_3 and SiO_2 contents in the frame of site 4 was expressive (Al_2O_3: 0.7 to 12.5 g kg^{-1} and SiO_2: 0.5 to 10 g kg^{-1}). The NaOH method was efficient in K extraction, which can clearly be observed by the higher values of K-NaOH in relation to the other elements expressed by mg kg^{-1} (Table 4). Small and poorly crystalline illite layers must be extracted. The NaOH solution has very limited performance in the extraction of 2:1 aluminosilicates minerals (Melo et al., 2002). However, it is possible that exfoliated layers (very reduced mean crystal diameter - MCD (001)) and poorly crystallized illite can be extracted. The amount of 2:1 layers stacked in the "c" directionin the aluminosilicate minerals is quite variable. The process of exfoliation reduces the particle size of illite, which favors the solubilization in basic solutions (van Breemen and Buurman, 2002).

The analyses of silt + clay samples generated 48 chemical variables (Tables 2, 3, and 4) and the Principal Component Analysis (PCA) showed the first three principal components of the dataset variance accounted for 59.5 % of the variance (Figure 4). The contents of kaolinite and gibbsite by thermogravimetry analysis and identification of the mineralogical profile by XRD were removed from the data matrix due to their lowest discriminatory power among the all studied variables. The combination of the chemical variables obtained in the sequential extractions with AO, DCB, and NaOH in a 3D-PCA matrix considerably distributed the 52 samples according to their sites, even with the dispersion of the samples from site 4.

The grouping and dispersion of the samples are more visible in the analysis by Bray-Curtis clustering (Figure 5). The intragroup similarities were: blue - 94.5 %, blue without sample 1 - 96.4 %; green - 97.3 %; red - 96.9 %; purple - 93.0 %; combination of green + red - 95.1 %; combination of (green + red) + blue - 93.0 %; combination of (green + red + blue) + purple - 90.5 %. These results show that the group with the highest similarity was composed of soil samples collected in the neighborhood of Guarani in Colombo (green). On the other hand, samples collected in the Boa Vista neighborhood in Curitiba (purple) had the lowest similarities. The set of all the samples presented similarity of 90.5 %. Some samples have dispersed from their original groups: sample 11 (green) grouped with blue; samples 17 (red) and 25 (purple) grouped in a isolate group; samples 16 and 51 (red) grouped with green; samples 22 and 30 (purple) grouped with red; sample 9 (green) grouped with purple. The total percentage of samples positioned outside their original groups was 15.4 %.

Table 3. DCB Contents of iron and aluminum oxides and elements obtained in the sequential treatments of the clay + silt fraction

Sample	Fe_2O_3	Al_2O_3	As	Ba	Ca	Cd	Cr	Cu	K	Mg	Mn	P	Pb	Si	V	Zn
	------g kg^{-1}-----		----------mg kg^{-1}----------													
Site 1																
1	30.5	10.0	9.2	23.5	1080.5	0.4	9.8	1.1	938.4	77.1	67.8	949.5	14.9	2071.3	61.0	19.0
2	28.5	9.1	9.0	19.2	679.4	0.3	9.2	1.1	691.4	63.6	69.6	756.8	20.9	1837.0	58.9	15.9
3	29.9	9.3	9.7	25.3	1225.1	0.4	9.7	1.2	709.9	73.4	55.6	814.0	17.1	1861.9	62.2	16.2
4	28.4	8.9	8.3	20.4	884.6	0.3	9.3	0.6	669.8	57.0	48.7	783.0	30.3	1779.8	59.3	12.9
5	27.2	8.5	6.2	29.1	2304.3	0.3	9.2	0.9	683.6	74.8	107.4	801.9	21.3	1750.3	56.9	20.6
6	29.2	9.0	3.0	29.5	3035.0	0.3	9.5	1.3	694.7	74.0	106.0	819.9	19.5	1834.7	59.9	24.8
7	26.4	8.4	9.0	31.6	3483.5	0.3	9.4	0.7	671.2	70.9	121.2	841.6	22.9	1759.1	55.1	17.1
Site 2																
8	33.6	11.3	3.2	22.6	5357.9	0.5	8.6	0.5	746.4	101.5	118.1	930.7	14.8	1794.6	57.0	18.8
9	35.2	10.0	7.0	86.7	10962.9	0.6	19.9	1.3	1035.3	123.0	195.1	1295.6	18.7	2138.3	63.4	70.9
10	33.1	10.9	8.0	19.6	6139.7	0.5	8.4	0.4	776.4	103.3	150.3	968.7	12.1	1817.3	56.1	16.8
11	27.2	7.1	9.9	14.1	2508.9	0.4	6.3	0.8	688.9	72.3	61.6	805.8	20.5	2530.9	54.4	19.3
12	31.9	10.7	7.9	18.8	5733.0	0.5	8.5	1.0	830.5	99.6	134.1	925.5	67.8	1884.5	54.8	27.5
13	29.6	10.1	8.6	19.8	5080.6	0.4	7.6	0.5	745.6	96.8	119.7	874.6	16.7	1600.1	50.5	15.9
14	35.0	12.2	10.9	19.9	5677.9	0.6	9.6	1.3	777.2	121.9	140.4	967.2	18.3	1974.5	61.2	23.6
Site 3																
15	48.6	19.9	8.1	17.1	2534.3	0.6	19.4	0.5	801.3	119.7	60.0	1094.5	6.8	2055.6	118.4	26.9
16	35.6	11.5	6.9	18.3	2995.2	0.4	9.1	0.2	734.3	93.8	97.7	893.8	5.2	1712.5	62.8	17.2
17	35.6	9.8	9.5	6.5	269.3	0.4	12.7	0.4	746.1	73.1	32.8	891.5	20.7	2608.6	89.7	20.3
18	45.4	17.4	12.1	16.4	5682.4	0.6	18.0	0.7	769.8	143.5	62.1	974.8	8.7	1770.4	98.8	16.2
19	45.7	17.7	9.6	15.6	4886.8	0.6	17.9	0.8	801.5	123.4	59.4	1046.9	11.8	1938.7	102.1	19.5
20	42.1	16.1	5.3	15.2	4830.4	0.5	16.6	0.8	743.7	124.5	61.9	992.5	13.0	1734.5	94.0	18.0
21	47.8	18.3	14.8	20.1	6400.4	0.6	19.0	0.2	829.2	136.9	76.8	1110.4	13.4	1944.6	105.4	20.4
Site 4																
22	47.0	17.4	10.1	14.0	821.7	0.6	17.7	0.6	751.4	78.9	48.2	1014.5	8.6	1877.4	108.0	19.3
23	33.0	8.0	8.5	75.4	9183.6	0.5	12.3	0.7	841.1	154.8	141.6	1082.2	43.8	2608.2	61.6	103.4
24	24.6	4.8	6.4	60.4	8386.9	0.4	9.8	4.9	741.7	120.3	140.7	1008.8	48.0	2478.0	45.5	64.3
25	37.8	6.6	9.4	28.4	2887.6	0.4	14.6	1.3	731.4	78.2	83.7	1021.7	75.7	3152.3	73.9	36.6
26	25.2	5.9	8.7	107.8	10427.5	0.4	10.3	0.9	769.2	124.3	156.4	1165.8	18.8	1658.5	42.0	51.5
27	29.5	6.3	6.0	145.0	9103.1	0.6	11.6	1.5	849.8	239.1	248.7	1128.4	115.0	2000.8	44.6	77.2
28	22.4	5.2	6.1	207.1	9143.5	0.4	10.8	0.5	716.0	103.7	146.1	1135.8	8.4	1314.6	38.6	55.5

Samples collected by Federal Police															
29	30.3	9.6	9.8	25.3	1635.2	0.3	9.8	0.5	715.8	79.3	80.4	814.5	27.2	1856.4	62.6	16.7
30	48.2	17.3	9.4	14.0	946.1	0.6	18.0	1.3	744.8	90.0	54.5	1007.2	34.1	2139.0	107.4	30.3
31	35.2	11.6	4.7	20.5	5420.2	0.6	9.2	0.6	806.2	109.1	143.4	971.7	12.8	1924.6	59.6	19.4
32	39.4	10.5	8.8	74.8	12290.3	0.7	20.1	1.0	1110.9	145.9	229.9	1388.6	19.8	2385.6	70.4	67.8
33	25.5	8.5	6.3	22.0	1137.2	0.3	8.8	0.6	711.2	69.7	96.5	722.8	18.7	1816.3	53.5	17.8
34	48.6	18.6	9.9	18.5	2513.1	0.7	18.7	0.4	752.1	133.5	56.9	1014.6	6.3	1807.1	110.0	27.6
35	39.5	10.7	14.0	55.9	10301.7	0.6	18.7	1.2	881.6	115.0	216.8	1223.6	18.4	2326.2	74.7	50.1
36	29.1	9.0	9.9	26.4	2561.3	0.4	9.9	1.4	668.0	84.9	109.2	804.4	21.1	1849.0	60.5	20.1
37	48.8	18.9	8.3	15.7	4767.1	0.5	18.8	0.6	807.0	163.6	66.8	1062.6	6.8	1969.3	109.4	20.4
38	37.5	12.6	6.3	20.7	5941.2	0.5	9.8	0.7	761.5	119.3	137.6	975.8	14.7	2016.7	65.6	22.4
39	29.8	7.4	6.8	65.5	9884.1	0.5	10.8	1.2	830.9	190.7	184.2	1124.4	21.9	1905.7	53.2	50.7
40	32.3	10.5	10.0	25.2	1841.2	0.4	11.1	0.5	779.7	79.8	60.3	874.9	15.9	2011.9	67.5	21.6
41	47.0	18.2	10.3	15.7	4669.0	0.6	18.2	0.7	727.8	145.9	63.3	1016.3	10.3	1980.8	106.1	19.9
42	34.7	11.2	8.3	21.2	5270.9	0.5	9.0	1.0	743.6	113.3	143.9	890.0	15.4	1915.4	59.2	17.7
43	31.5	9.9	4.3	30.3	3081.8	0.5	10.9	0.7	726.9	87.2	137.9	960.9	17.7	2050.2	63.5	20.1
44	48.7	18.8	12.8	17.3	5123.6	0.5	18.6	0.6	800.9	155.2	72.7	1072.0	4.9	2015.8	108.3	25.1
45	38.0	12.8	9.7	25.9	8331.9	0.6	10.0	0.9	862.2	123.5	177.2	1028.0	17.3	2236.0	65.3	37.5
46	36.8	8.6	3.4	95.1	12470.6	0.6	14.4	1.2	987.7	126.1	228.5	1427.9	19.7	2195.0	65.7	66.8
47	48.9	19.4	14.2	16.5	4916.5	0.6	19.5	0.6	783.8	159.6	65.0	1068.8	9.6	1921.4	112.7	24.2
48	35.9	12.6	6.0	22.1	6847.2	0.6	9.6	0.7	768.5	130.2	137.5	1030.2	13.6	1861.9	62.8	21.6
49	34.0	10.8	4.4	28.2	1691.6	0.5	11.5	1.2	772.3	72.7	94.3	977.5	12.9	2055.5	67.8	21.2
50	50.6	20.3	5.5	18.5	6394.2	0.7	20.8	0.7	928.9	175.0	68.8	1177.4	8.7	2004.6	116.7	22.8
51	45.4	15.6	10.8	23.9	3330.9	0.6	12.1	0.7	993.3	107.7	110.5	1151.2	11.6	2387.2	83.5	23.1
52	24.6	5.8	4.7	91.0	10249.9	0.4	11.5	0.4	835.8	138.4	161.4	1267.1	13.2	1633.7	42.2	49.6

Note: Iron, aluminum oxides and elemental contents extracted by dithionite-citrate-bicarbonate (DCB).

3.6 DISCUSSION

Oxides extracted by AO are related to the poorly ordered Al and Fe minerals, which are widely reactive in soils due to their large specific surface area (Simas et al., 2006; Mendonça et al., 2013). Their formation and concentration are greatly influenced by environmental conditions, mainly in soils with elevated moisture and organic matter and low pH (Melo et al., 2001). The low contents of Fe_2O_3-AO indicate that the crystalline form predominates in these soils. In most samples, the Al_2O_3-AO contents were higher than the Fe_2O_3-AO contents, attributed to the dissolution of Al gels (gibbsite precursors) by the AO (Ghidin et al., 2006; Melo et al., 2008).

The predominance of Fe crystalline oxides can be explained by weathering processes. The release of Al during the DCB extraction is due to the dissolution of goethite and hematite with isomorphic substitution of Fe by Al in the mineral structure due to similar ionic charge, ionic radius, and coordination of Al and Fe (Schwertmann and Kämpf, 1985; Schwertmann and Taylor, 1989).

Contents of Si and Al extracted by boiling NaOH 0.5 mol L^{-1} represent the poorly crystalline aluminosilicates (short-range order Al-hydroxide, Al-O-Si layers and Si-O-opaline silica) and gibbsite. The highest variations of Fe_2O_3, Al_2O_3, and SiO_2 extracted by AO, DCB, and NaOH for samples collected at site 4 might be due to a considerable deposition of artifacts and waste found in that location, being a largely heterogeneous environment.

Figure 5 shows a satisfactory grouping of the samples collected by the researchers and also by the police. The proximity of the trace samples, collected from the sole of the boot in the simulated crime scene, strongly demonstrates the good application of the SOP by the police, as well as their potential for calibration of sampling. There was dispersion of only one trace sample (sample 51), which could be related to the differences commonly found in the environmental conditions. Elemental chemical results showed the similarity between the samples from the same frame and the same footprint (vestige) and were clearly able to discriminate the samples intergroup according to different neighborhoods and different parent material selected in the study. Soils formed on different bedrocks were expected to discriminate from each other than soils developed on the same parent material. Therefore, soils from different geologies may be distinguished by a wide range of techniques (Dawson and Hillier, 2010), but few techniques can discriminate soils that are similar but not identical (Dawson and Mayes, 2015; Prandel et al., 2017; Corrêa et al., 2018).

Table 4. Contents of iron, aluminium, and silicon oxide and elements obtained in the sequential treatments of the clay + silt fraction.

Sample	Fe_2O_3	SiO_2	Al_2O_3	Ba	Cr	Cu	K	Mg	Mn	Mo	P	S	V	Zn
	-----------g kg^{-1}-----------			----------------mg kg^{-1}----------------										
Site 1														
1	0.1	6.2	9.1	0.3	0.4	11.1	133.1	0.8	0.5	0.9	69.9	76.4	1.0	2.7
2	0.1	7.0	8.4	0.1	0.4	13.7	126.7	0.6	0.6	1.0	66.0	70.1	1.2	2.4
3	0.1	6.7	7.9	0.1	0.4	16.0	169.8	0.6	0.5	0.8	60.6	77.7	1.1	3.2
4	0.1	6.4	7.3	0.1	0.4	14.9	139.0	0.6	0.4	1.2	68.4	90.6	1.1	2.0
5	0.1	7.0	8.2	0.1	0.4	15.8	140.6	0.5	0.6	0.6	75.1	97.0	1.2	1.9
6	0.1	7.0	8.5	0.1	0.5	14.0	133.6	0.7	0.8	1.0	66.8	77.5	1.1	2.2
7	0.1	7.0	7.9	0.1	0.5	20.3	160.4	0.9	1.0	0.8	69.3	98.7	1.1	2.7
Site 2														
8	0.0	6.4	9.3	0.1	0.5	9.6	169.1	0.7	0.6	0.7	87.8	63.8	1.1	3.3
9	0.1	5.3	10.0	0.2	0.6	8.1	203.9	0.5	0.5	0.9	120.0	92.7	1.6	6.1
10	0.1	6.6	9.7	0.2	0.4	5.6	175.3	0.6	0.8	0.5	85.3	62.2	1.1	2.8
11	0.1	17.1	6.2	0.1	0.3	15.8	148.9	0.5	0.6	0.9	49.9	123.0	1.5	1.6
12	0.1	5.9	8.5	0.1	0.4	10.7	169.8	0.5	0.6	1.1	73.9	70.9	1.0	3.7
13	0.0	6.4	9.3	0.1	0.4	6.6	173.6	0.6	0.5	0.7	69.9	60.9	0.9	3.4
14	0.0	6.2	9.3	0.1	0.4	7.2	169.2	0.5	0.6	0.7	75.0	63.7	1.0	2.3
Site 3														
15	0.0	4.0	11.3	0.1	0.5	9.7	149.6	0.5	0.2	0.6	95.8	75.8	2.3	4.8
16	0.0	5.8	8.5	0.1	0.4	6.0	152.6	0.6	0.4	0.7	86.6	67.2	1.1	2.1
17	0.0	10.1	6.5	0.1	0.4	6.9	172.6	0.6	0.2	0.6	53.7	67.5	2.1	1.7
18	0.1	7.9	5.5	0.1	0.5	15.1	154.9	0.5	0.4	0.6	74.4	93.1	1.6	5.2
19	0.0	4.9	13.9	0.1	0.5	6.4	171.3	0.8	0.2	0.6	97.3	79.5	2.8	1.7
20	0.0	4.3	12.1	0.1	0.5	5.9	176.1	0.6	0.2	0.8	99.2	81.1	2.7	2.1
21	0.0	4.7	13.7	0.1	0.5	6.5	167.0	0.4	0.2	0.9	120.4	72.8	3.2	2.7
Site 4														
22	0.1	4.4	12.5	0.0	0.5	7.5	164.8	0.5	0.2	0.8	103.0	81.6	2.6	3.2
23	0.0	0.5	0.7	0.1	0.2	0.9	177.4	0.4	0.1	0.8	8.5	16.9	0.2	0.6
24	0.1	9.0	3.8	0.0	0.4	18.1	174.5	0.7	0.9	0.6	45.4	107.5	0.9	5.1
25	0.1	10.0	5.0	0.1	0.4	16.9	154.2	1.3	0.8	0.5	41.2	85.7	1.0	60.9
26	0.0	1.9	2.6	0.3	0.4	10.6	181.2	0.9	0.2	0.5	18.0	170.3	0.4	6.6
27	0.0	2.1	3.0	0.3	0.3	14.2	190.4	0.7	0.1	0.7	13.8	97.5	0.3	27.7
28	0.0	1.6	2.4	0.4	0.4	11.2	163.0	0.8	0.1	0.5	20.7	79.5	0.4	7.5

Samples collected by Federal Police														
29	0.1	6.9	8.2	0.0	0.6	13.1	150.9	0.7	0.7	0.5	78.3	81.9	1.1	6.0
30	0.0	4.3	10.7	0.1	0.4	6.7	139.6	0.6	0.2	0.6	86.2	83.3	2.5	2.7
31	0.1	5.9	8.7	0.1	0.4	9.5	164.9	0.7	0.6	0.7	77.2	66.6	1.1	7.1
32	0.1	5.2	10.1	0.1	0.8	11.5	207.3	0.7	0.9	0.7	127.1	110.3	1.7	15.1
33	0.1	7.5	8.7	0.0	0.6	13.8	146.1	0.7	0.8	0.6	92.8	117.6	1.3	3.6
34	0.0	3.9	12.2	0.0	0.5	6.5	146.9	0.5	0.2	0.6	121.3	88.0	3.1	22.9
35	0.1	5.5	9.4	0.1	0.6	13.3	162.4	0.9	0.8	0.5	105.2	75.3	1.2	4.9
36	0.1	6.6	7.8	0.1	0.5	16.7	162.7	0.8	0.6	0.5	79.8	110.8	1.4	2.5
37	0.0	4.3	12.9	0.0	0.4	6.1	156.6	0.6	0.2	0.7	121.3	82.4	3.1	6.3
38	0.1	5.7	8.4	0.0	0.5	11.6	152.9	0.7	0.5	1.2	92.0	100.1	0.9	3.2
39	0.0	4.2	4.8	0.1	0.3	11.3	170.8	0.9	0.3	0.9	55.6	80.4	0.4	5.1
40	0.1	5.0	6.0	0.0	0.4	13.1	99.2	0.7	0.4	0.4	53.9	75.5	0.8	2.1
41	0.0	4.4	13.4	0.0	0.4	7.1	168.0	0.4	0.2	0.6	106.0	96.2	2.8	2.1
42	0.1	6.2	9.3	0.1	0.5	6.9	167.3	0.5	0.7	0.6	77.4	73.7	1.2	3.9
43	0.1	6.2	7.3	0.1	0.5	17.9	172.3	0.6	1.2	0.8	85.3	121.1	1.2	4.0
44	0.0	4.6	13.2	0.1	0.5	7.0	158.6	0.6	0.2	0.6	105.4	83.7	2.7	9.0
45	0.1	6.6	9.6	0.0	0.5	8.8	184.2	0.4	0.7	0.4	90.7	85.1	1.0	3.4
46	0.0	4.2	4.4	0.1	0.5	10.0	183.8	0.6	0.4	0.8	72.1	73.9	0.7	3.6
47	0.0	4.5	13.7	0.1	0.5	6.0	172.6	0.4	0.2	0.8	109.4	71.2	2.9	3.3
48	0.1	5.6	8.7	0.1	0.4	8.6	180.9	0.8	0.6	1.0	83.2	85.9	1.2	3.9
49	0.1	6.6	8.1	0.1	0.6	16.8	148.3	0.5	0.7	0.6	92.0	133.0	1.2	6.0
50	0.0	4.1	12.9	0.1	0.5	7.2	175.2	0.4	0.1	0.5	109.3	79.1	2.9	6.2
51	0.0	5.7	9.2	0.1	0.3	7.7	168.0	0.5	0.4	0.5	81.3	79.8	1.0	2.7
52	0.0	1.7	2.5	0.3	0.3	8.1	208.9	0.6	0.2	0.8	16.1	82.3	0.4	2.9

Note: Iron, aluminium, silicon oxides and elemental contents extracted by NaOH 0.5 mol L^{-1}.

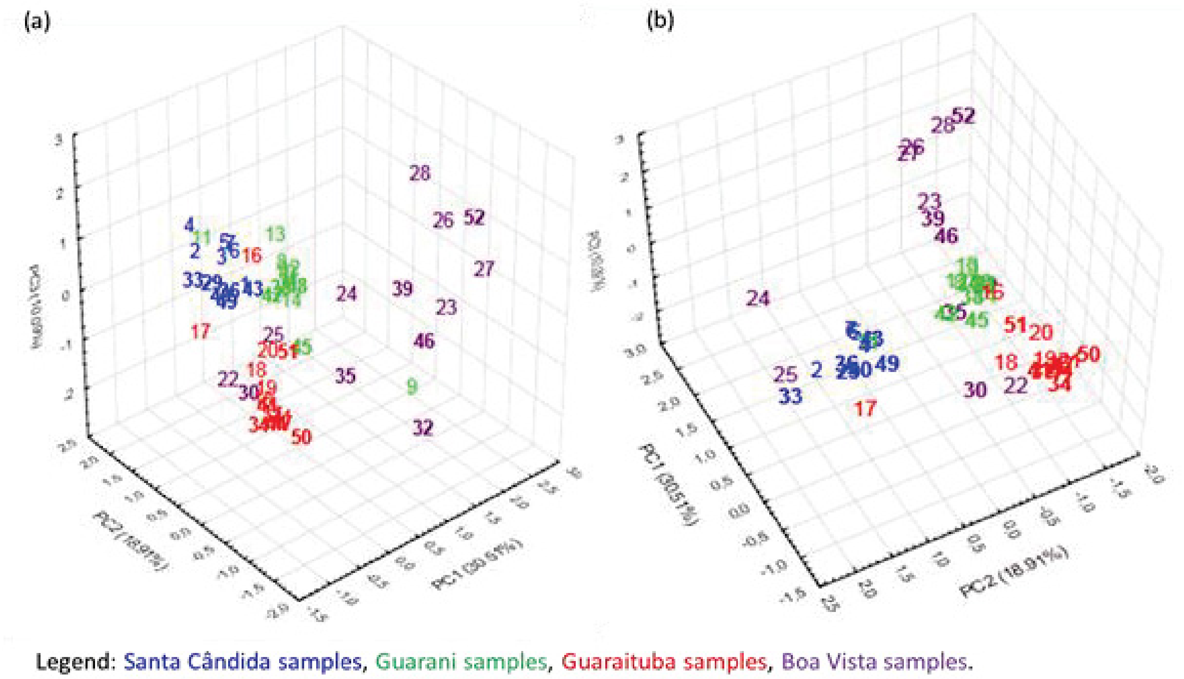


Figure 4. 3D-Principal Component Analysis (PCA) in two orientations a) and b), for all samples, distributed according the variables analysed. Blue-samples - Santa Candida; green-samples – Guarani; red-samples – Guaraituba; purple-samples - Boa Vista. Samples highlighted in dark colours were collected by Federal Police. Note: Samples from 1 to 28 were collected by the researches from UFPR; samples from 29-52 were collected by the criminal expertises from Federal Police (Table 1).

Our protocol of analyses showed a high capacity to discriminate the samples among the four sites using 48 soil chemical variables obtained from 3.0 g of soil (Figures 4 and 5). With smaller quantities of samples (around 1 g), it was also possible to apply the same protocol of analysis (Melo et al., 2008). The selected 48 variables generated from chemical analyses have shown a capacity to discriminate between samples. A similar forensic study conducted in southern Brazil on Inceptisols (*Cambissolos*) from five locations examined 56 variables on soil silt + clay and also concluded that a set composed of only 16 variables could more readily efficiently distinguish soil samples than the whole dataset (Melo et al., 2008). The 3D-PCA and the similarity analysis as well showed the potential of the SOP for soil sampling in forensic case works. A few grams of soil are usually available at crime scenes, although not from questioned samples.

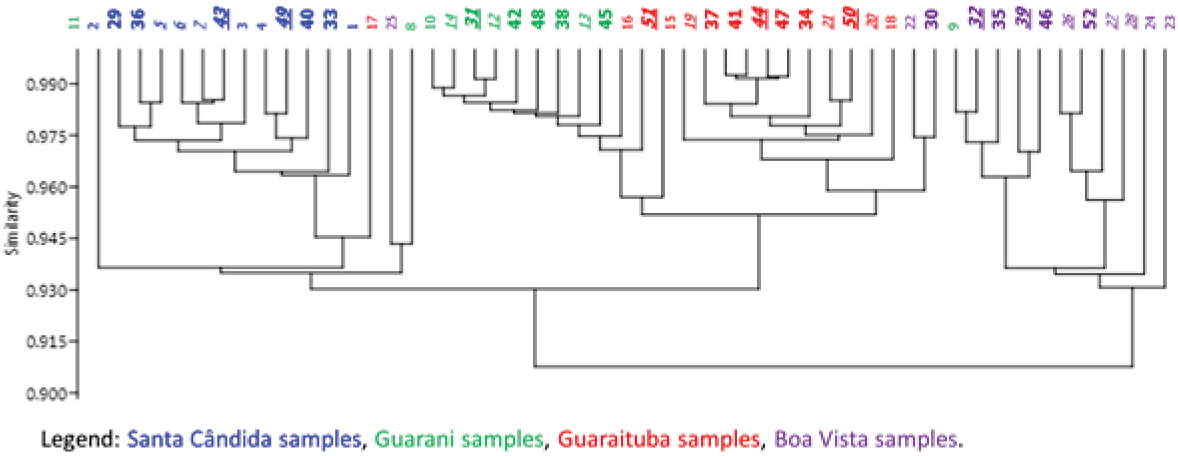


Figure 5. Diagram of Bray-Curtis similarity for all samples, distributed according the variables analysed. Colours: blue samples - Santa Candida; green samples – Guarani; red samples – Guaraituba; purple samples - Boa Vista. Samples highlighted in dark colours and in bigger font were collected by Federal Police. Note: Samples from 1 to 28 were collected by the researches from UFPR; samples from 29-52 were collected by the criminal expertises from Federal Police (Table 1).

3.7 CONCLUSIONS

Most of the samples were correctly grouped according to their location of origin at all four sites tested, showing the ability to use chemical characterization to test a link between a questioned sample and a crime scene. The researcher and the police sampling groups also found the same results, showing potential for successful use of the Standard Operating Procedure (SOP) in real crime scenes by police practitioners.

Besides the calibration of the present SOP and the potential of the sequential chemical extractions, we must consider specific conditions attributed to the analytical approach in each case work. Intense anthropic activities, such as domestic waste disposal in urban areas reduced the discrimination power of the tested chemical analytical protocol. As future considerations, modifications to the sample treatment and analytical protocol could be made depending on the context of the forensic work, always improving on the best methodologies for the forensic examination of soil samples.

3.8 REFERENCES

Corrêa RS, Melo VF, Abreu GGF, Sousa MH, Chaker JA, Gomes JA. Soil forensics: how far can soil clay analysis distinguish between soil vestiges? *Sci Justice.* 2018;58:138-44. https://doi.org/10.1016/j.scijus.2017.09.003

Dawson LA, Hillier S. Measurement of soil characteristics for forensic applications. *Surf Interface Anal*. 2010;42:363-77. https://doi.org/10.1002/sia.3315

Dawson LA, Mayes RW. Criminal and environmental soil forensics: soil as physical evidence in forensic investigations. In: Murphy BL, Morrison RD, editors. Introduction to environmental forensics.3rd ed. London*: Academic Press*; 2015. p. 457-86.

Demanèche S, Schauser L, Dawson L, Franqueville L, Simonet P. Microbial soil community analyses for forensic science: application to a blind test. *Forensic Sci Int.* 2017;270:153-8. https://doi.org/10.1016/j.forsciint.2016.12.004

Farrugia KJ, Bandey H, Dawson L, Nic Daéid N. Chemical enhancement of soil based footwear impressions on fabric. *Forensic Sci Int*. 2012;219:12-28. https://doi.org/10.1016/j.forsciint.2011.11.011

Finley SJ, Benbow ME, Javan GT. Potential applications of soil microbial ecology and next-generation sequencing in criminal investigations. *Appl Soil Ecol.* 2015;88:69-78. https://doi.org/10.1016/j.apsoil.2015.01.001

Fitzpatrick R. Getting the dirt': the value of soil in criminal investigations. Gazette. 2011;73:22-3.

Fitzpatrick RW. Soil: forensic analysis. In: Jamieson A, Moenssens A, editors. Wiley encyclopedia of forensic science. Chichester: *John Wiley & Sons*; 2009. p. 2377-88.

Fitzpatrick RW. Encyclopedia of Forensic Sciences [Internet]. 2nd ed. *Encyclopedia of Forensic Sciences*. Glen Osmond: Elsevier; 2013.

Fitzpatrick RW, Raven MD. *Guidelines for conducting criminal and environmental soil forensic investigations*. Version 7.0. Adelaide: Commonwealth Scientific and Industrial Research Organisation; 2016.

Fitzpatrick RW, Raven MD. How pedology and mineralogy helped solve a double murder case: using forensics to inspire future generations of soil scientists. *Soil Horizons*. 2012;53:14-29. https://doi.org/10.2136/sh12-05-0016

Ghidin AA, Melo VF, Lima VC, Lima JMJC. Toposseqüências de Latossolos originados de rochas basálticas no Paraná. I – Mineralogia da fração argila. *Rev Bras Cienc Solo*. 2006;30:293-306. https://doi.org/10.1590/S0100-06832006000200010

Gibbs B, Haldenby A. 2007-2008 British Crime Survey. UK; 2008.

Hammer Ø, Harper DAT, Ryan PD. PAST: *Paleontological statistics* software package for education and data analysis. Palaeontol Electron. 2001;4:4.

Jackson ML, Lim CH, Zelazny LW. Oxides, hydroxides, and aluminosilicates. In: Klute A, editor. Methods of soil analysis. Physical and mineralogical methods. 2nd ed. Madison: *American Society of Agronomy*; 1986. Pt 1. p. 101-50.

Marumo Y. Forensic examination of soil evidence. Japanese Journal of Science and Technology for Identification. 2003;7:95-111. https://doi.org/10.3408/jasti.7.95

McKeague JA. Manual on soil sampling and methods of analysis. Ottowa: *Canadian Society of Soil Science*; 1978.

Mehra OP, Jackson ML. Iron oxide removal from soils and clays by a dithionite-citrate system buffered with sodium bicarbonate. proceedings of the Seventh National *Conference on Clays and Clay Minerals*; October 20-23, 1958; Washington, DC. Oxford: Clay Clay Miner; 1960. p. 317-27.

Melo VF, Barbar LC, Zamora PGP, Schaefer CE, Cordeiro GA. Chemical , physical and mineralogical characterization of soils from the Curitiba Metropolitan Region for forensic purpose. *Forensic Sci Int.* 2008;179:123-34. https://doi.org/10.1016/j.forsciint.2008.04.028

Melo VF, Fontes MPF, Novais RF, Singh B, Schaefer CEGR. Características dos óxidos de ferro e de alumínio de diferentes classes de solos. *Rev Bras Cienc Solo*. 2001;25:19-32. https://doi.org/10.1590/S0100-06832001000100003

Melo VF, Mazzetto JML, Dieckow J, Bonfleur EJ. Factor analysis of organic soils for site discrimination in a forensic setting. *Forensic Sci Int.* 2018;290:244-50. https://doi.org/10.1016/j.forsciint.2018.07.005

Melo VF, Schaefer CEGR, Novais RF, Singh B, Fontes MPF. Potassium and magnesium in clay minerals of some Brazilian soils as indicated by a sequential extraction procedure. *Commun Soil Sci Plant*. 2002;33:2203-25. https://doi.org/10.1081/CSS-120005757

Mendonça T, Melo VF, Schaefer CEGR, Simas FNB, Michel RFM. Clay mineralogy of gelic soils from the Fildes Peninsula, Maritime Antarctica. *Soil Sci Soc Am J.* 2013;77:1842-51. https://doi.org/10.2136/sssaj2012.0135

Prandel LV, Melo VF, Brinatti AM, Saab SC, Salvador FAS. X-ray diffraction and Rietveld refinement in deferrified clays for forensic science. *J Forensic Sci.* 2017;63:251-7. https://doi.org/10.1111/1556-4029.13476

Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Oliveira JB, Coelho MR, Lumbreras JF, Cunha TJF. *Sistema brasileiro de classificação de solos*. 3. ed. Rio de Janeiro: Embrapa Solos; 2013.

Schwertmann U, Kämpf N. Properties of goethite and hematite in kaolinitic soils of Southern and Central Brazil. *Soil Sci Soc Am J*. 1985;139.

Schwertmann U, Taylor RM. Iron oxides. In: Dixon JB, Weed SB, organizadores. Minerals in soil environments. 2^{o} ed Madison: *Soil Science Society of America*; 1989. p. 380–427.

Simas FNB, Schaefer CEGR, Melo VF, Guerra MBB, Saunders M, Gilkes RJ. Clay-sized minerals in permafrost-affected soils (Cryosols) from King George Island, Antarctica. *Clay Clay Miner.* 2006;54:721-36. https://doi.org/10.1346/CCMN.2006.0540607

Statsoft Inc. Statistica - Data analysis software system. Tulsa: Statsoft Software; 2011.

Tan KB, Hajek IB, Barshad I. Termal analysis techniques. In: Klute A, editor. Methods of soil analysis. Physical and mineralogical methods. 2nd ed. Madison: *American Society of Agronomy*; 1986. Pt 1. p. 151-83.

van Breemen N, Buurman P. Soil formation. 2nd ed. Netherlandes: *Kluwer Academic Publishers;* 2002.

Wald C. The soil sleuth. Nature. 2015;520:422-4. https://doi.org/10.1038/520422a

Woods B, Lennard C, Kirkbride KP, Robertson J. 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.* 2016;262:46-55. https://doi.org/10.1016/j.forsciint.2016.02.034

4 CHAPTER III: ANALYSIS OF SOIL PLANT WAX BIOMARKERS: APPLICATION IN FORENSIC INVESTIGATIONS[3]

4.1 RESUMO

A maioria dos casos envolvendo o solo em investigações criminais tem se concentrado em análises químicas e mineralógicas, com pouco foco na matéria orgânica do solo. Este estudo discute como a natureza da matéria orgânica do solo pode constituir uma ferramenta útil para caracterizar o solo em investigações forenses. Marcadores de cera são compostos lipídicos (por exemplo, *n*-alcanos e álcoois graxos) originados em grande parte da cera superficial das plantas, que entram no solo como folhas caídas e outros detritos vegetais. A longevidade e diversidade química de marcadores de cera em solos resulta em sua caracterização tendo um grande valor na avaliação de solos para investigações forenses, pois variam de acordo com a vegetação que contribuiu para sua formação em um local específico. Avaliou-se o potencial dos compostos de cera vegetal para discriminar amostras de solo coletadas em quatro áreas urbanas no sul do Brasil com base em seus perfis de marcadores de cera de plantas. Nossos resultados demonstram o potencial forense dos biomarcadores, pois foram capazes de discriminar amostras de solo forense de acordo com sua origem urbana/geográfica, em uma escala relevante para comparação avaliativa. A abordagem do biomarcador de cera do solo, como usada em diversos casos criminais no Reino Unido, pode ser uma ferramenta valiosa para uso no Brasil. Além disso, aplicado em combinação com características inorgânicas, como perfis elementares e mineralógicos do solo, estes dados podem ser uma ferramenta analítica e evidencial particularmente poderosa e eficaz.

Palavras-chave: geociência forense; solo; marcadores de cera; cromatografia gasosa; *n*-alcanos; álcoois graxos; argilito; calcário.

4.2 ABSTRACT

Most case work involving soil in criminal investigations has focused on chemical and mineralogical analyses with little focus on soil organic matter. This study discusses how the nature of soil organic matter is a useful tool to characterise soil in forensic investigations. Wax markers are lipid compounds (e.g. *n*-alkanes and fatty alcohols) originating largely from the surface wax of plants, which enter the soil as fallen leaves and other plant litter. The longevity and chemical diversity of wax markers in soils results in their characterisation having a great value in assessing soils for forensic investigations as they vary according to the vegetation that contributed to its formation at a specific location. We assessed the potential of the plant wax compounds to discriminate soil samples collected in four urban areas in Brazil based on their plant wax marker profiles. Our results demonstrate the powerful forensic

---

[3] Submitted on Geoderma.

value of these organic biomarkers, as they are able to discriminate forensic soil samples according their urban/geographic origin at a scale relevant for forensic evaluative comparison. The soil wax biomarker approach as used in case work in the UK, could be a valuable tool for use in Brazil. Moreover, applied in combination with inorganic characteristics such as soil elemental and mineralogical profiles the soil data can be a particularly powerful and effective analytical and evidential tool.

Keywords: forensic geoscience; soil; wax markers; gas chromatography; *n*-alkanes; fatty alcohols; claystone; limestone.

4.3 INTRODUCTION

In forensic science, soil is an important form of trace evidence and can be used to test for any link between soil on a questioned item and a potential source. Since traces of soil can readily become trapped on the underside of footwear and on vehicle tyres, soil has long been considered to be a potential source of forensic information (Morgan et al., 2006; Ritz et al., 2008; Dawson and Hillier, 2010; Dawson and Mayes, 2014; Fitzpatrick and Raven, 2012; Murray et al., 2016; Corrêa et al., 2017; Prandel et al., 2017). Provided that a well-tested and robust method for the characterization of the samples is available, a potential link can be tested and established between a suspect and a crime scene. Inorganic approaches have been most generally applied in case work while organic approaches have also been used, although only routinely in some countries (e.g. New Zealand, UK, The Netherlands). Soil organic matter is complex and widely variable (Dawson, 2017) and can provide powerful information about a range of soil forming features such as the vegetation growing at the site in the past and what is currently growing. Soil organic biomarkers have until relatively recently received limited recognition in forensic soil science. The use of organic profile characteristics has been applied successfully in court several times (Dawson and Mayes, 2014; Dawson, 2017) and can characterize close proximity location by discrimination of the land-use (Mayes et al., 2008; McCulloch et al., 2017; McCulloch et al., 2018). Traditional approaches have focused on comparison of the mineralogical components and measurement and quantification of elemental profiles of soil, with complementary information of colour analysis and particulate organic matter (Pye et al., 2006; Melo et al., 2008; Dawson and Hillier, 2010; Woods et al., 2014; Corrêa et al., 2017; Prandel et al., 2017; Melo et al., 2018) although inorganic characteristics tend to vary at a much broader scale (km) while organic features vary at a smaller scale (metres).

Although the organic matter of many soils is less than 5% (w/w), it has an important impact on soil properties (Dawson, 2017). Soil organic matter can simply be described as all organic material found in soil, irrespective of origin or state of decomposition (Ritz et al., 2008; Fitzpatrick, 2008; Dawson and Mayes, 2014; Finley et al., 2015; McCulloch et al., 2017). The lipid profile of a soil is formed by the synthesis and combination of all decomposing processes on the vegetation, determined by the soil and site environment. Therefore, the relative abundance of organic compounds can vary considerably between soil samples at a short distance apart. In most soils, especially in the topsoil, the most common and most studied lipid compounds are those originating from the surface wax of plants, which enter the soil as fallen leaves and other plant litter such as seeds, flowers and stems; plant roots have low concentrations of plant wax compounds and probably make little contribution to the soil complement of these compounds in most cases (Dawson, 2017). The most common and widely studied class is the hydrocarbons, including *n*-alkanes, as well as the free and esterified long-chain fatty alcohols such as high molecular-weight, straight-chain primary alcohols, derived from natural fats and oils, and fatty acids such as carboxylic acids with a long aliphatic chain, and contain a hydrocarbon chain and a terminal carboxyl group. Lignin, the aromatic biopolymer found exclusively in vascular plants, has been used by geochemists to indicate vegetation source because of its moderate reactivity (Fitzpatrick, 2008) wide spatial and temporal distribution, and phenolic constituents have also been considered for use (Dawson and Hillier, 2010; Dawson and Mayes, 2014; Dawson, 2017).

Whilst the highest order plants contain these compounds, the relative proportions of individual compounds can differ greatly between plant species, and in some instances, between different parts of the same plant (Dove et al., 1996; Smith et al., 2001; Dove and Mayes, 2006; Mayes et al., 2008; Dawson and Hillier, 2010; Carvalho et al., 2013; Dawson and Mayes, 2014; McCulloch et al., 2018). Although they only amount to around 1% of the organic matter they are very useful markers to “fingerprint” soils for forensic investigations due to their chemical diversity, relative longevity and resistant nature, and can indicate land use or vegetation characteristic for evaluative comparison of a questioned soil sample to reference samples taken from the crime scene (Thanasoulias et al., 2002; Fitzpatrick, 2009; Fitzpatrick, 2013; Dawson and Mayes, 2014; Cheshire et al., 2017).

The plant wax components can be quantitatively analysed as individual compounds by GC (gas chromatography, which can separate and analyse specific compounds that can be vaporized without decomposition) and GCMS (gas chromatography – mass spectrometry, which combines the features of GC and mass spectrometry to identify different substances within a test sample) after solvent extraction and separation into the different compounds types by liquid chromatography, most conveniently by using solid-phase extraction columns. This ability to identify and quantify compounds with a high degree of precision and reproducibility is important for use in case work.

In Brazil some studies on the use of soils in forensic case work have been developed, however, although until now there were few approaches using soil organic matter for discrimination (Melo et al., 2018). Thus, the analysis of plant wax markers can potentially expand the horizons of forensic soil science in Brazil, and contribute to the development of this area of research. This study aimed to assess the potential of the plant wax compounds (*n*-alkanes and fatty alcohols biomarkers) to identify the provenance of soil samples collected in a forensic context from Brazil. We selected four geographically separate urban study areas formed under two different parent materials (claystone and limestone). We hypothesised that biochemical biomarkers would discriminate the locations of the study within the urban areas as well as separate the samples from at the same location, thus being of more relevance to link with specifically contact locations at a crime scene.

4.4 MATERIAL AND METHODS

4.4.1 SOIL SITES AND SAMPLING

Two areas from Curitiba and two areas from Colombo, Paraná State, in the South of Brazil with contrasting geologies were selected: Santa Candida and Boa Vista, neighbourhoods from Curitiba developed on claystone; and Guarani and Guaraituba neighbourhoods from Colombo, located at the Metropolitan Region of Curitiba with an underlying geology of limestone.

Locations with a low amount of anthropogenic influence were preferentially selected across each urban area, and at each site 4 surface soils were sampled from the corners of a quadrant 1.5 m square, to 0.5 cm depth; and 1-3 samples at the centre of the quadrant which correspond to a simulated footmark left at the crime scene, using the field sampling methodology purposed by Melo et al. (2008). A total of 52 samples collected were stored in secure individual sterile pots. Usually only the top few cm of soil is transferred to questioned items (such tools, tyres, shoes, clothes etc), mainly under conditions of high rainfall and clay-silty soils which favour the transfer and persistence of such material. However, urban soils may have their surface layer removed and another type of soil added by for example ground works, indicating a formation of allochthonous soil in these areas, and environmental conditions such as rain and bioturbation can effectively turn over the top few cm of the soil surface, altering the soil layer characteristics in relation to the entire soil profile. Soil samples were air-dried (40°C for 24 hours), sieved (2 mm) and then the fine fraction was sub-divided into portions using an eight channel quarterer to provide a set of homogenous sub-samples for the set of the subsequent analyses.

4.4.2 COLOUR SPECTROSCOPY

Soil colour was determined on a milled and dried soil sample using a spectrophotometer (Konica Minolta CM2600d) to obtain reflectance data over the 360–740 nm wavelength range. For each sample, data were averaged over triplicate measurements and converted to the CIELAB notation (Rossel et al., 2006) using SpectraMagic V3.61R2 software (Konica Minolta) to represent colour variables expressed in 3-D space as lightness-darkness (L), red-green chromacity (a*) and blue-yellow chromacity (b*).

4.4.3 PLANT WAX COMPOUNDS DETERMINATION BY GAS CHROMATOGRAPHY (GC)

The procedures for the analysis of soils for plant wax compounds commonly involve extraction, separation into different compound classes by liquid (solid phase extraction- SPE) chromatography, and analysis of individual compounds by gas chromatography (GC). For some compounds, steps to break up molecules (e.g., alkaline hydrolysis) and to form derivatives prior to GC are also required. These methods are not specific to plant wax compounds, so other lipid compounds present in the sample with similar properties can also appear in the associated GC chromatograms.

Plant wax compounds (*n*-alkanes and fatty-alcohols) analysis was carried out according to the method detailed in (Dove and Mayes, 2006). All solvents were redistilled prior to use and all aliquots were transferred with glass tipped, calibrated, auto-pipettes. All glassware was ashed and rinsed in *n*-heptane before to use. Samples were milled, weighed (0.20 g), dried in an oven at 50°C for two hours, cooled in a desiccator, and then their moisture content was calculated. To each dry sample, internal standards of docosane (C_{22}) and tetratriacontane (C_{34}) were added in decane at a concentration of 0.03 mg g^{-1}, then n-heptacosanol (1-C_{27}-ol) was added at a concentration of 0.12 mg g^{-1} to oven-dried milled sample prior to extraction. Samples were heated in 1M ethanolic KOH (56.1 g m l^{-1}) at 90°C for 16h then the hydrocarbons were extracted in *n* -heptane, and this extract was evaporated to dryness and redissolved in heptane, purified and fractioned by loaded through silica-gel columns (Sigma Aldrich Ltd) that had been prepared with *n*-heptane. The hydrocarbons were eluted with *n*-heptane, ketones were removed from the column with 97:3 *n*-heptane:ethyl acetate, and the crude alcohol fraction was collected by washing with 80:20 *n*-heptane:ethyl acetate. The hydrocarbon and crude alcohol extracts were then evaporated to dryness. The hydrocarbon fraction was redissolved in *n*-heptane, transferred to a GC vial, evaporated to dryness, then dissolved in 20 µl dodecane prior to injection onto the GC. Each of the crude alcohol fraction were redissolved in 100 µl *n*-heptane and 30 µl was loaded onto a capped, 1 mL glass SPE column, and to which 60 µl saturated urea in ethanol had been added. The columns were heated at 70°C for 20 min and allowed to dry overnight, then the sterol/stanol fraction was recovered by elution with *n*-heptane, the urea removed by washing with water, and the fatty alcohols then eluted with *n*-heptane. Both fractions were then evaporated to dryness before to derivatisation. Acetate derivatives of the fatty alcohols were prepared by heating overnight with pyridine: acetic anhydride (5:1) at 50°C. The acetate derivative was subsequently repeatedly re-dissolved in *n*-heptane then evaporated to dryness until the residue have no pyridine or acetic anhydride smell, then dissolved in 25 µl dodecane prior to injection onto the GC (Phillips, PU4500) with a flame ionisation detector, a non-polar silica capillary column

(30m x 0.52 μm internal diameter, 0.25 μm film thickness and He carrier gas at 5 cm^3 min^{-1}) and a direct injector.

4.4.4 STATISTICAL ANALYSES

Data were exported and comparisons between samples were visualised by Multi-dimensional scaling (MDS) carried out in PAST (Paleontological Statistics, 3.20 version) (Hammer et al., 2001) and by Principal Component Analysis (PCA) based on Statistica software (StatSoft, 2011). Ford the MDS analysis, the level of confidence in the 2D representation of the multi-dimensional relationships is indicate by the MDS associated 'stress': 0.2 or less provides good representation (Hammer and Harper, 2006). The multivariate analysis was applied to assess a) if data could be used to discriminate between the specific sampled locations, b) if samples within the location could be discriminated from each other.

4.5 RESULTS AND DISCUSSION

We initially tested whether soil organic biochemical (*n*-alkane and fatty alcohol plant wax compounds) biomarkers could be used to provide forensic intelligence through indicating likely soil provenance in a blind study. This was carried out by another person providing a sample from a known location (unknown to the authors) and then subsequently compared in a double blind study. There has been some criticism of soil forensic research studies which try to demonstrate the discriminatory power of analytical methods in populations of soils that originate from clearly different soil (Morgan and Bull, 2007). The purpose of choosing different geographic areas within this study was to determine if the biochemical methods provided quantifiably consistent characteristics regardless of the soil geographical environment. Thus this study represents an assessment of analytical variability in four contrasting areas, all representative and realistic urban areas of Brazil.

Soil colour reflects the cumulative effect of the pedogenic environment and their history. The components that contribute most to soil colour are the organic matter, and geological background resulting in compounds such as iron oxides and manganese oxide contents (Sugita and Marumo, 1996). While the soil organic matter is responsible for the lightness or darkness of soil, the iron oxides generate a widely range of soil colours dependent on the state of the iron oxidation, producing soils reddish and yellowish when in the oxidized state and greyish in the reduced state. However, colour varies also with moisture content which affects soil colour by modifying the oxidation rate. Normally, colour determination is carry out on air-dried soil samples, to remove that factor of moisture. However, it is possible to develop determinations more specific, such as ashing, removing the organic matter and iron oxides (Rowe, 2005). In this way, the spectral colour gives a reflectance useful to the discrimination of forensic soils (Melo et al., 2008; Sugita and Marumo, 1996). Soil samples from Curitiba and Colombo varied widely in colour, and could not be clearly discriminated from one another. The Boa Vista neighbourhood from Curitiba discriminated from other soil groups, largely due to being located in a more heterogeneous environment under high anthropic interference and deposition of artefacts and trash. Soil from other neighbourhoods did not have consistent colour characteristics, and therefore colour analysis did not provide a strong basis for urban discrimination in the places under investigation, but can be a useful exclusionary initial step. Regarding the general dispersion (Fig. 2a) it was possible to infer that most of the samples are composed predominantly of the mineral fraction, which is iron oxides such hematite (α-Fe_2O_3) and goethite (α-FeOOH). The higher and differing quantities of organic matter in the soils from Curitiba resulted in a larger variation in the colour data which is affected by the organic content of soil (Woods et al., 2014).

In contrast to the colour results, the wax marker analysis results demonstrated their ability to discriminate between samples within urban areas. This was demonstrated through a lower variability within the different sites from n-alkane results. The use of plant wax markers in discriminating between soils/sediments from different plant communities has been demonstrated on UK soils (Mayes et al., 2008; Dawson and Hillier, 2010; McCulloch et al., 2018). The wax marker analysis results demonstrate their ability to discriminate not only between sites but also between samples within individual urban areas. This was demonstrated through a lower variability within the different sites from fatty-alcohols data (Fig. 2c), particularly to the samples from Santa Candida, Boa Vista and Guaraituba, which clearly grouped and discriminated from the other sites, in relation to the colour data (Fig. 2a). The use of plant wax markers in discriminating between soils/sediments from different plant communities has been previously demonstrated on UK soils (Mayes et al., 2008; Dawson and Hillier, 2010; Carvalho et al., 2013; McCulloch et al., 2018).

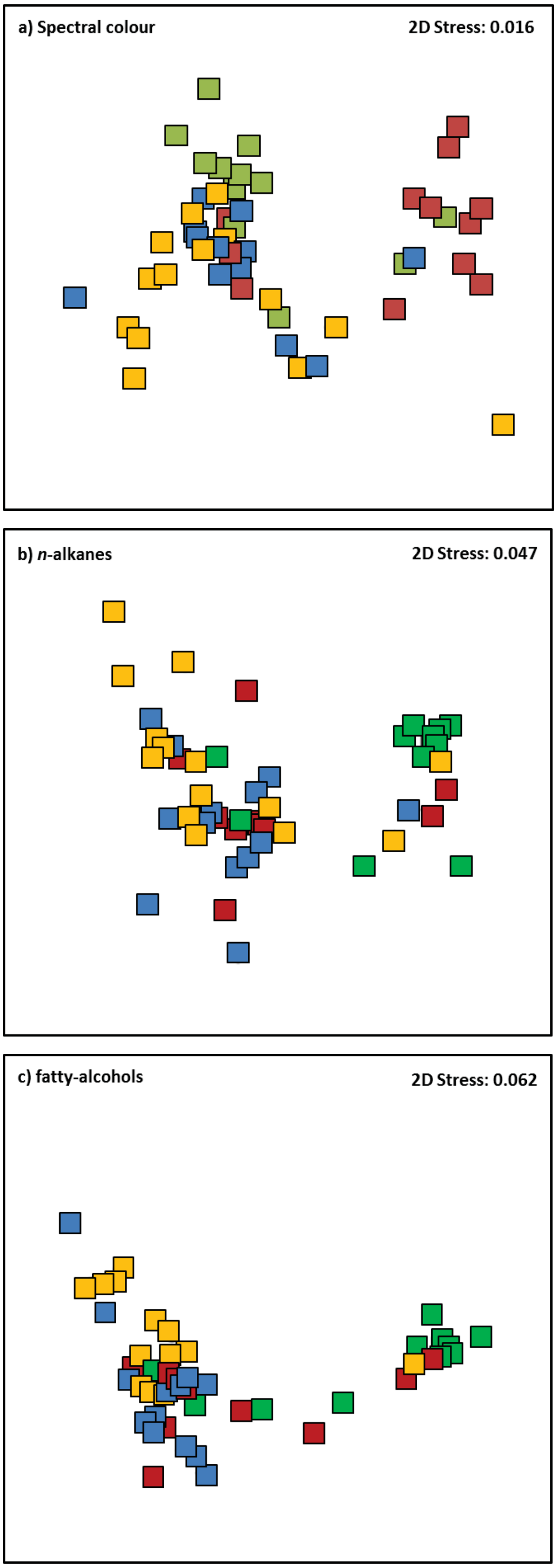


Fig. 2. Multidimensional scalling (MDS) ordination of soil samples based on analysis

of a) spectral colour; b) *n*-alkanes; c) fatty alcohols. Samples represent soils from Curitiba, located at Santa Candida (blue), and Boa Vista (orange) neighbourhoods; and soils from Colombo, located at Guarani (red) and Guaraituba (green) neighbourhoods.

Results confirmed earlier studies that *n*-alkanes with odd-numbered carbon chains were the predominant markers found in cuticular wax, especially the C_{27}, C_{29}, C_{31}, and C_{33} alkanes (Dove et al., 1996; Dawson et al., 2000; McCulloch et al., 2018). Of the analysed samples, there was a large variability within the urban area studied, which avoided clear discrimination between the city groups (Fig. 3, 5). The Guaraituba neighbourhood soils were the least variable, characterised by a small proportion of the C_{31} *n*-alkane (<4%) in both quadrants and in the central soil samples (Fig.3, 5). While this provided discrimination between the soils from Santa Candia, Boa Vista and Guarani, large variability within each neighbourhood prevented clear discrimination between the sites. Santa Candida and Guaraituba were well discriminated from other, while Guarani and Boa Vista presented higher dispersion (Fig. 3, 5).

Well resolved fatty-alcohol chromatographs were achieved for all soil samples. As with the *n*-alkanes, the soils did not discriminate clearly based on the fatty alcohol results alone. However, it was evident that within neighbourhoods variation was lower for fatty-alcohol analysis compared to *n*-alkane analysis allowing clear discrimination between sampling sites (Fig. 3). Consistent fatty-alcohol patterns were observed for Santa Candida, Guarani and Boa Vista soils, characterised by a relatively high proportion of the C_{26} and C_{28} fatty-alcohols (40-60%) (Fig. 3, 5). Similarly, all soils demonstrated consistent characteristics, with a relatively even distribution of C_{22}, C_{24}, C_{26} and C_{28} fatty-alcohols, but in different proportions. Guaraituba soils discriminated from other soils largely due to the lower proportion of the fatty-alcohols chain length (<16%) (Figure 4, 6c), and Boa Vista discriminated due to proportionally lower contribution of C_{30} and greater contributions of C_{22}, C_{24}, C_{26} and C_{28} (Fig. 3, 5).

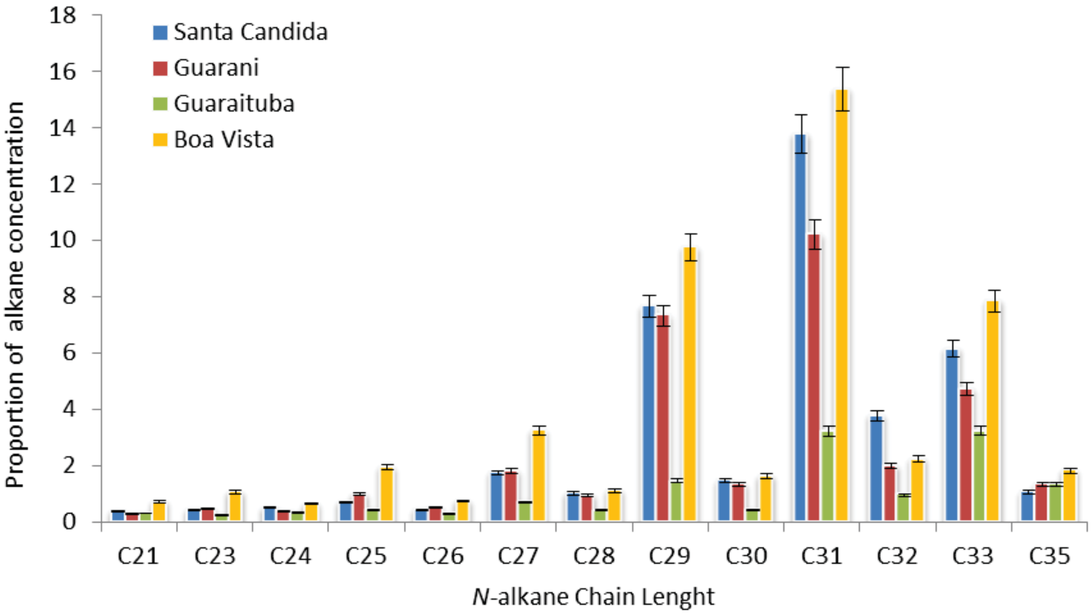


Fig. 3. Distribution of the total *n*-alkane by proportion for each sampling location.

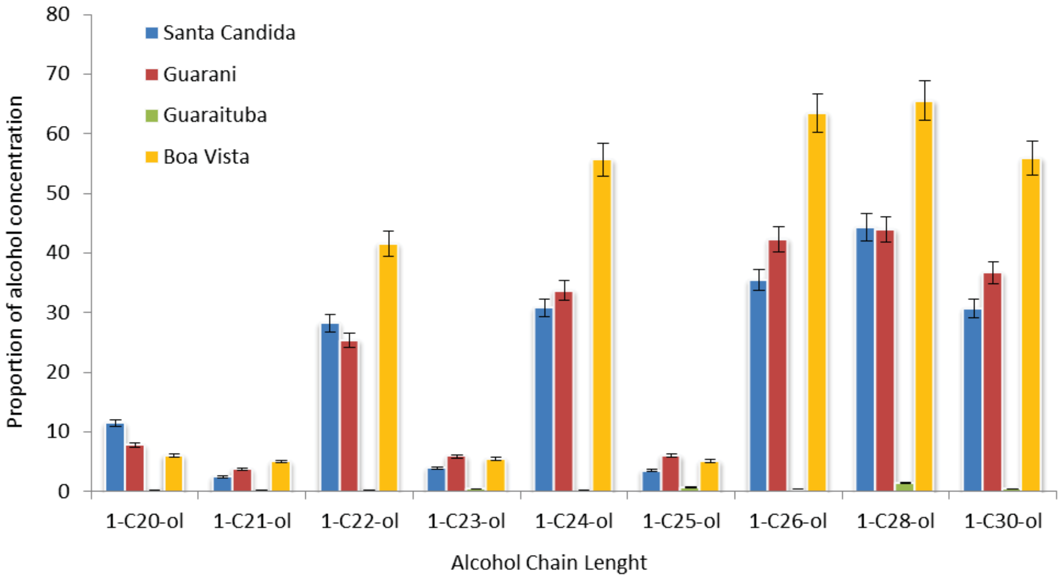


Fig. 4. Distribution of the total alcohols by proportion for each sampling location.

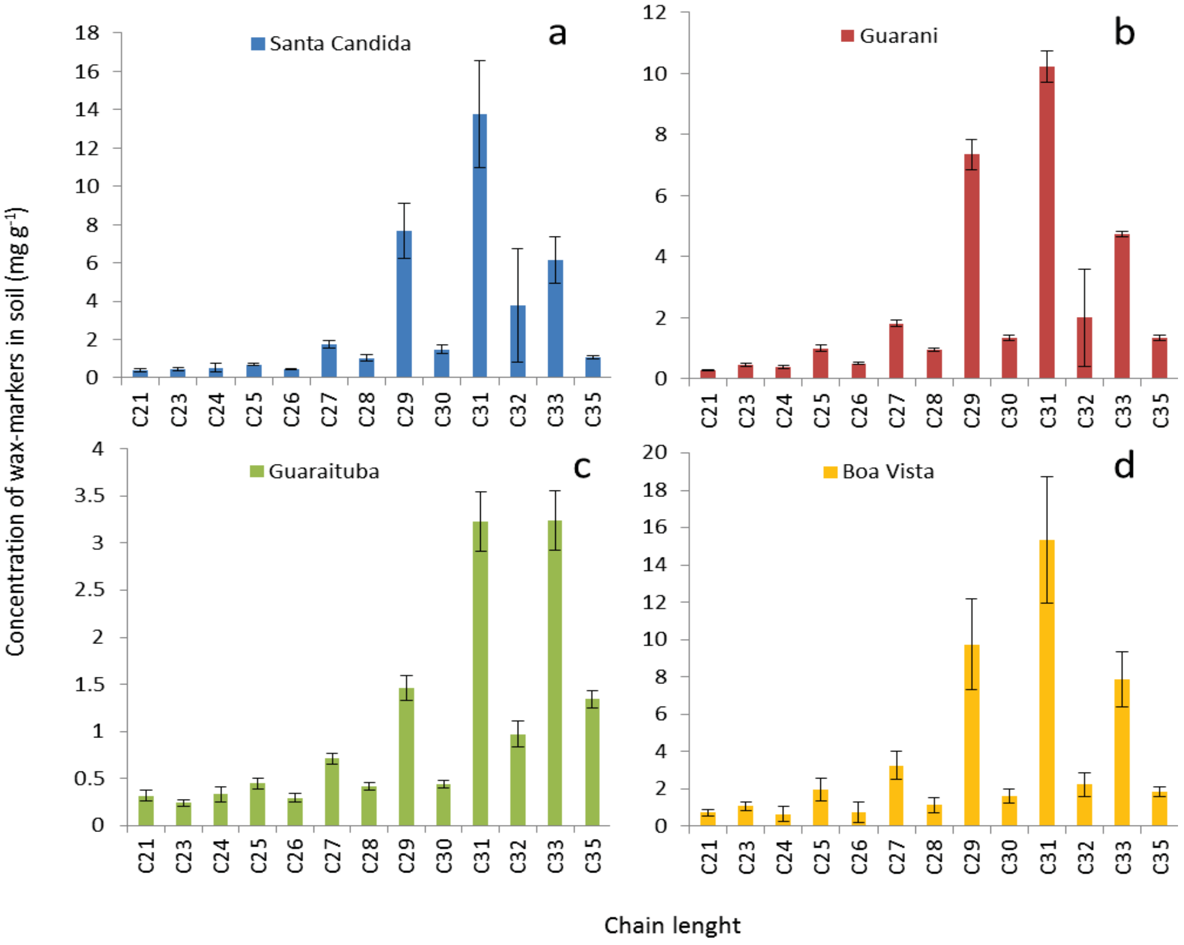


Fig. 5. GC profiles of *n*-alkanes for a) Santa Candida, b) Guarani, c) Guaraituba and d) Boa Vista neighbourhoods at Curitiba and Colombo, State of Parana, Brazil.

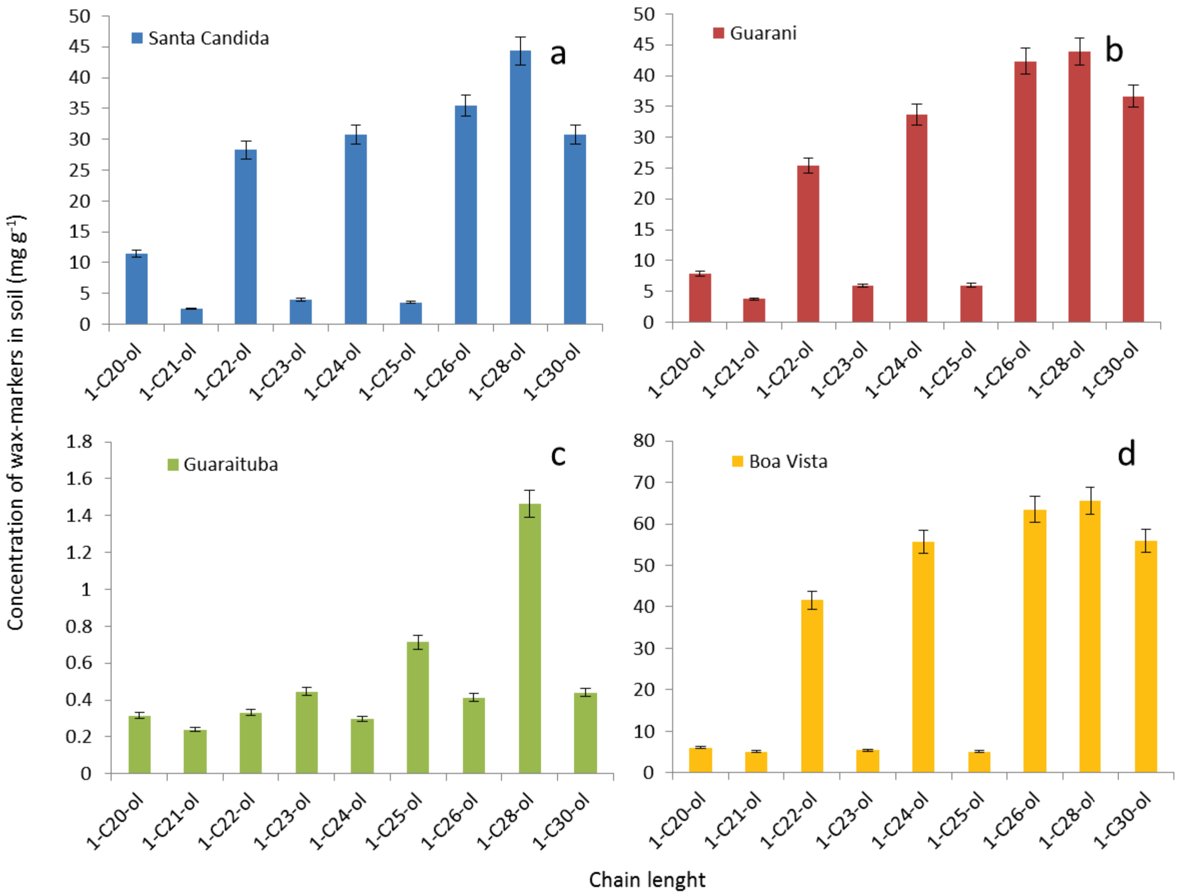


Fig. 6. GC profiles of alcohols for a) Santa Candida, b) Guarani, c) Guaraituba and d) Boa Vista neighbourhoods at Curitiba and Colombo, State of Parana, Brazil.

The wax marker profiles allowed satisfactory visual discrimination of the four sample locations from the two Curitiba and Colombo municipalities. Santa Candida and Boa Vista samples under the same parent material were similar but distinct from Guarani and Guaraituba soils formed on limestone; C_{31} was the most abundant and discriminatory marker, and C_{30} was present in a large proportion for the Santa Candida and Guarani soils, but was much reduced in the Guaraituba and Boa Vista soils. The different pattern in concentrations for the series C_{25}, C_{27}, C_{29} and C_{31} have been reported in several studies (Dawson et al., 2000; Dove et al., 1996; McCulloch et al., 2018) and for the studied soils such characteristics were also observed; however the increase appeared more linear and well distributed for the Guaraituba soils and appeared exponential for the others. Differences between the pattern of the *n*-alkane concentrations in plant cuticular wax will normally depend on an appreciation of the relative effects of plant species, plant part, and plant age (Dove et al., 1996; Smith et al., 2001; Dove and Mayes, 2006). For both *n*-alkanes and fatty alcohols, Guaraituba soils presented much lower concentrations of all compounds analysed, and it should be noted that the variability in the results at each location was considerably higher for the alcohols results, as indicated by the size of the error bars (Fig. 3, 5).

In agreement with the early data observed by MDS analysis, and also by the graphs of proportions, it was possible to verify that the samples followed the same dispersion by PCA analysis (Fig. 7). The most variance of the data analysed (spectral colour and wax biomarkers) was explained by the first principal component (52.93 %), while the first three principal components accounted around 74.2 %. Samples from Boa Vista showed the weakest grouping, largely due for the factors previously mentioned, particularly that related to anthropogenic influence in this location. Samples from Guaraituba grouped clearly, possibly because the predominant lower values of wax biomarkers observed for this location, while the other groups presented greater and similar proportions. We can also noticed that samples from the same underlying geology (blue and orange samples; red and green samples, Fig. 7) discriminated between one another. Despite of the discrimination could not be widely clear, was there a trend to intergroup differentiation according the parent materials.

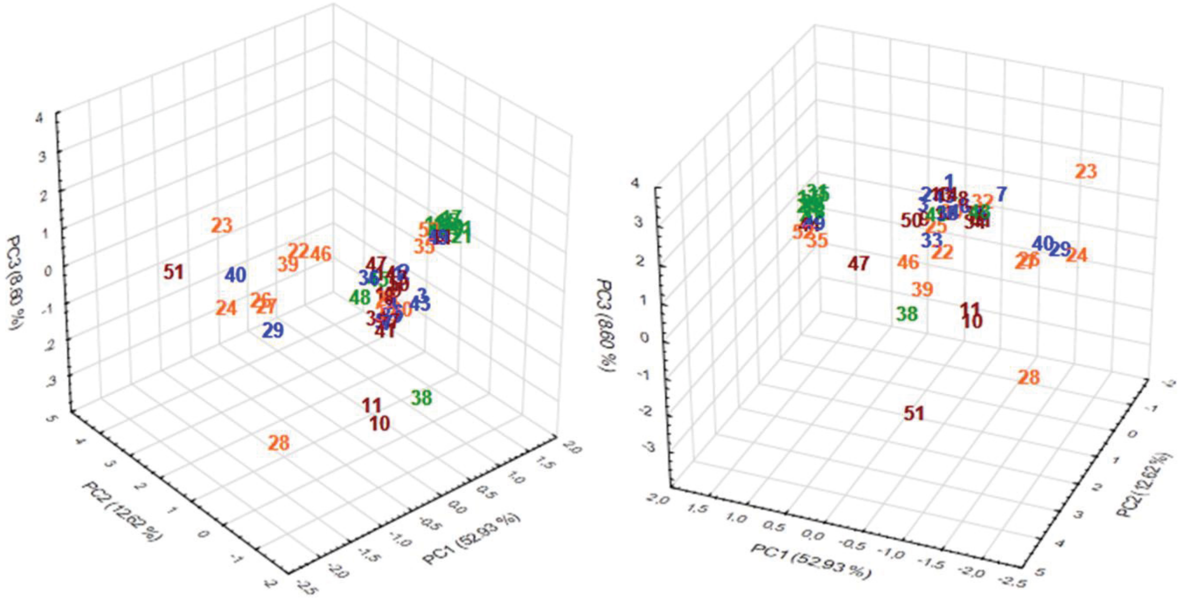


Fig. 7. Principal Component Analysis (PCA) of soil samples based on spectral colour, *n*-alkanes and fatty alcohols data. Samples represent soils from Curitiba, located at Santa Candida (blue), and Boa Vista (orange) neighbourhoods; and soils from Colombo, located at Guarani (red) and Guaraituba (green) neighbourhoods.

The high variability observed in the data is likely to be a reflection of the heterogeneous nature of the soil, and additionally we must consider the high diversity of the vegetation in subtropical climate conditions, when compared to soils from United Kingdom (Dove et al., 1996; Mayes et al., 2008; McCulloch et al., 2018; Smith et al., 2001). Besides the varied vegetation, higher temperatures contribute to elevated rates of organic matter decomposition, which is also favoured by high humidity in subtropical climates such as the studied sites. In reality more replicates would have been advantageous. However, a number of potential sources of variability in the methodology should be considered for improving the GC techniques. The sample preparation technique for the GC analysis was quite complex to learn as there were many steps in the GC method. The GC method comprised multiple concentration and reconstitution steps, using very small volumes of 20–60 ml sample. It could be difficult to achieve analytical precision and accuracy when working with smaller volumes of sample since small systematic errors have a larger relative effect on the results, when under training such as in this study. Furthermore, the large number of steps in the analytical method presented potential opportunities for small human errors, such as inadvertent, inaccurate weighing or imprecise pipetting to create variability in the resulting data. However, despite this, the concentrations of the compounds present in the samples are determined relative to known standards using the GC approach, providing a means of calibrating the chromatographic system and monitoring reproducibility.

This study has demonstrated the potential of using wax marker organic compound analysis for soils in Brazil, for forensic case work, providing much potential for the objective of providing specific information to help test for any potential link to (or exclude) a crime scene. The wax marker profiles are shown to be appropriate in site specific comparison during the evaluative stages of an enquiry as was shown in this double blind study, where areas can be readily excluded and areas of priority for search identified. Supported with appropriate knowledge of the landscape variability, this biochemical method has been shown to provide effective information and together with inorganic characteristics such as soil elemental and mineralogical information can provide cost effective analytical tools to the criminal justice system.

4.6 REFERENCES

Carvalho, Á., Ribeiro, H., Mayes, R., Guedes, A., Abreu, I., Noronha, F., Dawson, L., 2013. Organic matter characterization of sediments in two river beaches from northern Portugal for forensic application. *Forensic Science International* 233, 403–415. https://doi.org/10.1016/j.forsciint.2013.10.019

Cheshire, K., Morgan, R.M., Holmes, J., 2017. The potential for geochemical discrimination of single- and mixed-source soil samples from close proximity urban parkland locations. *Australian Journal of Forensic Sciences* 49, 161–174. https://doi.org/10.1080/00450618.2016.1144789

Corrêa, R.S., Melo, V.F., Abreu, G.G.F., Sousa, M.H., Chaker, J.A., Gomes, J.A., 2017. Soil forensics: How far can soil clay analysis distinguish between soil vestiges? *Science and Justice* 0–1. https://doi.org/10.1016/j.scijus.2017.09.003

Dawson, L.A., 2017. Soil organic characterisation in forensic case work. *Journal of International Geoscience* 40, 017–018.

Dawson, L.A., Hillier, S., 2010. Measurement of soil characteristics for forensic applications. *Surface and Interface Analysis* 42, 363–377. https://doi.org/10.1002/sia.3315

Dawson, L.A., Mayes, R.W., 2014. *Criminal and Environmental Soil Forensics: Soil as Physical Evidence in Forensic Investigations*, Third Edit. ed, Introduction to Environmental Forensics: Third Edition. Elsevier Ltd. https://doi.org/10.1016/B978-0-12-404696-2.00012-6

Dawson, L.A., Mayes, R.W., Elston, D.A., Smart, T.S., 2000. Root hydrocarbons as potential markers for determining species composition. *Plant, Cell and Environment* 23, 743–750. https://doi.org/10.1046/j.1365-3040.2000.00592.x

Dove, H., Mayes, R.W., 2006. Protocol for the analysis of n-alkanes and other plant-wax compounds and for their use as markers for quantifying the nutrient supply of large mammalian herbivores. *Nature Protocols* 1, 1680–1697. https://doi.org/10.1038/nprot.2006.225

Dove, H., Mayes, R.W., Freer, M., 1996. Effects of species, plant part, and plant age on the n-alkane concentrations in the cuticular wax of pasture plants. Australian *Journal of Agricultural Research* 47, 1333–1347. https://doi.org/10.1071/AR9961333

Finley, S.J., Benbow, M.E., Javan, G.T., 2015. Potential applications of soil microbial ecology and next-generation sequencing in criminal investigations. *Applied Soil Ecology* 88, 69–78. https://doi.org/10.1016/j.apsoil.2015.01.001

Fitzpatrick, R.W., 2013. Soil : Forensic Analysis, in: Jamieson, A., Moenssens, A.A. (Eds.), *Wiley Encyclopedia of Forensic Science*. John Wiley, Chichester, p. 18. https://doi.org/10.1002/9780470061589.fsa096.pub2.Published

Fitzpatrick, R.W., 2009. Soil: Forensic Analysis. *Wiley Encyclopedia of Forensic Science* 2377–2388. https://doi.org/10.1002/9780470061589

Fitzpatrick, R.W., 2008. Nature, Distribution, and Origin of Soil Materials in the Forensic Comparison of Soils, in: Tibbett, M., Carter, D.O. (Eds.), *Soil Analysis in Forensic Taphonomy: Chemical and Biological Effects of Buried Human Remains.* Taylor & Francis Group, New York, p. 366. https://doi.org/10.1111/j.1556-4029.2008.00886.x

Fitzpatrick, R.W., Raven, M.D., 2012. How Pedology and Mineralogy Helped Solve a Double Murder Case: Using Forensics to Inspire Future Generations of Soil Scientists*. Soil Horizons* 53, 14. https://doi.org/10.2136/sh12-05-0016

Hammer, Ø., Harper, D.A.T., 2006. PAST. *Paleontological Statistics*. Version 2.07. Reference manual. Blackwell Publishing 351. https://doi.org/10.1016/j.bcp.2008.05.025

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: *Paleontological Statistics* Software Package for Education and Data Analysis.

Mayes, R.W., Macdonald, L.M.J.., Dawson, L.A., 2008. Discrimination of domestic garden soils using plant wax compounds as markers, in: Ritz, K., Dawson, L.A., Miller, D. (Eds.), *Criminal and Environmental Soil Forensics*. Springer, London UK.

McCulloch, G., Dawson, L.A., Brewer, M.J., Morgan, R.M., 2017. The identification of markers for Geoforensic HPLC profiling at close proximity sites. *Forensic Science International* 272, 127–141. https://doi.org/10.1016/j.forsciint.2017.01.009

McCulloch, G., Dawson, L.A., Ross, J.M., Morgan, R.M., 2018. The discrimination of geoforensic trace material from close proximity locations by organic pro fi ling using HPLC and plant wax marker analysis by GC. *Forensic Science International* 288, 310–326. https://doi.org/10.1016/j.forsciint.2018.02.009

Melo, V.F., Barbar, L.C., Zamora, P.G.P., Schaefer, C.E., Cordeiro, G.A., 2008. Chemical , physical and mineralogical characterization of soils from the Curitiba

Metropolitan Region for forensic purpose. *Forensic Science International* 179, 123–134. https://doi.org/10.1016/j.forsciint.2008.04.028

Melo, V.F., Mazzetto, J.M.L., Dieckow, J., Bonfleur, E.J., 2018. Factor analysis of organic soils for site discrimination in a forensic setting. *Forensic Science International* 290, 244–250. https://doi.org/10.1016/j.forsciint.2018.07.005

Morgan, R., Bull, P., 2007. The philosophy, nature and practice of forensic sediment analysis. *Progress in Physical Geography* 31, 43–58. https://doi.org/10.1177/0309133307073881

Morgan, R.M., Wiltshire, P., Parker, A., Bull, P.A., 2006. The role of forensic geoscience in wildlife crime detection. *Forensic Science International* 162, 152–162. https://doi.org/10.1016/j.forsciint.2006.06.045

Murray, K.R., Fitzpatrick, R.W., Bottril, R., Kobus, H.J., 2016. Interpreting the transfer method of soil evidence on clothing by identifying trace soil patterns. *Forensic Science Internationa*l2 258.

Prandel, L. V., Melo, V. de F., Brinatti, A.M., Saab, S. da C., Salvador, F.A.S., 2017. X-ray Diffraction and Rietveld Refinement in Deferrified Clays for Forensic Science. *Journal of Forensic Sciences* 1–7. https://doi.org/10.1111/1556-4029.13476

Pye, K., Blott, S.J., Wray, D.S., 2006. Elemental analysis of soil samples for forensic purposes by inductively coupled plasma spectrometry - precision considerations. *Forensic Science International* 160, 178–192. https://doi.org/10.1016/j.forsciint.2005.09.012

Ritz, K., Dawson, L., Miller, D., 2008. Criminal and Environmental Soil Forensics.

Rossel, R.A.V., Minasny, B., Roudier, P., Bratney, A.B.M., 2006. Colour space models for soil science. *Geoderma* 133, 320–337.

Rowe, W.F., 2005. Forensic Application, in: Encyclopedia of Soils in the Environment.

Smith, D.., Mayes, R.W., Raats, J.G., 2001. Effect of species, plant part, and season of harvest on n-alkane concentrations in the cuticular wax of common rangeland grasses from southern Africa. *Australian Journal of Agricultural Research* 52, 875–882. https://doi.org/10.1177/09680519010070020501

StatSoft, I., 2011. STATISTICA (data analysis software system).

Sugita, R., Marumo, Y., 1996. Validity of color examination for forensic soil identification. *Forensic Science International* 83, 201–210.

https://doi.org/10.1016/S0379-0738(96)02038-5

Thanasoulias, N.C., Piliouris, E.T., Kotti, M.S.E., Evmiridis, N.P., 2002. Application of multivariate chemometrics in forensic soil discrimination based on the UV-Vis spectrum of the acid fraction of humus. *Forensic Science International* 130, 73–82. https://doi.org/10.1016/S0379-0738(02)00369-9

Woods, B., Paul Kirkbride, K., Lennard, C., Robertson, J., 2014. Soil examination for a forensic trace evidence laboratory - Part 2: Elemental analysis. *Forensic Science International* 245, 195–201. https://doi.org/10.1016/j.forsciint.2014.10.018

5 CHAPTER IV: THE RELEVANCE OF SAND FRACTION FROM SUBTROPICAL SOILS IN FORENSIC STUDIES[4]

5.1 RESUMO

A maior parte da comparação forense de solos tem se concentrado nas frações argila e silte em relação às partículas mais grosseiras. O objetivo deste estudo foi testar o potencial de análises elementares e físicas na fração areia de solos subtropicais para discriminar amostras coletadas em áreas sob diferentes materiais de origem (argilito e mármore) e em áreas com o mesmo material origem, com base em uma cena de crime simulada. A microscopia eletrônica de varredura acoplada à análise de espectrômetria de raios-X por energia dispersiva (MEV-EED) foi usada nas partículas de areia de tamanho mais fino (0,05-0,25 mm). As análises de difração de raios-X e (DRX) e distribuição de tamanho de partículas (DTP) foram realizadas em toda a fração areia. Estes métodos não forneceram uma discriminação clara das amostras de areia dos solos amostrados em áreas subtropicais. Isso pode ser explicado pela grande homogeneização observada na fração areia, relacionada às suas características químicas (EED), físicas (distribuição granulométrica), morfológicas (MEV) e mineralógicas (DRX). Em condições tropicais e subtropicais, os processos de intemperismo químico dissolvem a maioria dos minerais primários, como os feldspatos, biotita e partículas contendo Fe, e concentra quartzo na fração areia. Nesses ambientes, recomendamos a priorização de frações de solo mais finas para estudos forenses.

Palavras-chave: ciência forense; evidência do solo; quartzo; fração mais grossa; análise do tamanho de partícula; difração de raios-x.

5.2 ABSTRACT

Most of the forensic comparison of soils has focused on the clay and silt fractions at the expense of the coarser particles. The objective of this study was to test the potential of elemental and physical analyses (XRD, PSD and SEM/EDS) in the sand fraction from subtropical soils to discriminate samples collected in areas under different parent material (claystone and marble) and in areas with the same parent material at a simulated crime scene. Sites were distant from each other around 2 to 14 km. In each site were sampled four replicates in the corners of a quadrant with 1.5 m. The ideal situation of samples traceability may be grouping replicates samples in a same site and discriminate groups of samples from different sites, including those samples collected in sites with the same bedrock. Scanning electron microscopy coupled to an energy dispersive X-ray spectrometer (SEM-EDS) analysis was used on the finer sized sand particles (0.05-0.25 mm). X ray diffraction and (XRD) and particle size distribution (PSD) analyses were performed on the whole sand fraction. These methods did not provide a clear discrimination of the sand samples of the soils sampled in different subtropical areas. This can be explained by the large

---

[4] Submitted on Forensic Science International.

homogenisation observed in the sand fraction related of its chemical (EDS), physical (particle size distribution), morphological (SEM) and mineralogical (XRD) characteristics. Under tropical and subtropical conditions, the chemical weathering processes dissolves most of primary minerals, such as the feldspars, biotite and Fe-bearing particles, and concentrates quartz in the sand fraction. Under these conditions, in very sandy soils or in sandy sediments, the traceability of vestige sample was not possible using the analysis proposed in the present study.

Keywords: Quartz; Particle size analysis; X-ray diffraction; SEM-EDS.

5.3 INTRODUCTION

Since 1856, when Professor Ehrenberg of Berlin analysed sand samples found in a barrel previously filled with coins, soils have been increasingly found to be useful in criminal investigations (Fitzpatrick, 2013). The variety in the constitution, distribution and function of soils provides a combination of different characteristics that can determine its great potential for research and application in forensic studies (Ritz et al., 2008; Dawson and Mayes, 2014; Pirrie et al., 2017). In some circumstances the sand fraction from temperate regions is the main fraction used in forensic case works (Bull et al., 2006; Bull and Morgan, 2006; Morgan et al., 2018). There are no much studies under tropical and subtropical conditions testing the sand fraction in criminal investigations.

Most of the forensic comparisons of soils have focused on the smaller fractions of the soil, such as the silt and clay fractions, with less attention given to the sand fraction. Commonly, the sand fraction is removed from the bulk soil in order to improve the homogenization of the samples, and the focus is generally given to the clay fraction analyses due to its surface reactivity (Melo et al., 2008. Normally, the sand fraction of highly weathered soils is homogeneous in mineralogy, which makes it more difficult to trace the vestige in forensic case, mainly by X-ray diffraction. Under tropical and subtropical conditions, chemical weathering dissolves most of the primary minerals, such as the feldspars, biotite and Fe-bearing particles, and concentrates the quartz (Shudofsky et al., 2017; Chauhan et al., 2018). Coarser particles are dominated by quartz, particularly in their morphological features, in some aspects related to transport, accumulation and weathering processes, which remain in and on the sand grain for a long time (Pye, 2007; Farrugia et al., 2012; Morgan et al., 2018). Such features can provide important information regarding the nature and the provenance of the sample, depending on the analytical approach used.

Data from the analysis of the particle size distribution analysis (PSD) of sand can provide a useful means of characterising soils and sediments for the purpose of forensic comparisons (Pye et al., 2007). The PSD method is a relatively rapid, non-destructive and inexpensive technique, applicable to small and large samples. Sugita and Marumo (2001) analysed 73 soil samples from Nirasaki district (Japan) (under temperate weather conditions) and observed that the measurements of PSD by sieving of the coarse and fine particle fraction allowed a discriminating power of 95.5 % in combination with colour examination. Pye and Blott (2004) and Chazottes et al. (2004) found that the data obtained from PSD analysis needs to be evaluated alongside the results of other analyses such as chemical, mineralogical and microscopic tests.

Scanning electron microscope coupled to the energy dispersive X-ray spectrometrer (SEM-EDS) can show the mineral morphology and also the elemental composition of the sand particles. Cengiz et al. (2004) compared the discrimination power of the pressed-homogenized and not homogenized forensic soil samples from Istanbul and verified that SEM–EDS was more accurate and precise even in a very small amounts of samples. Some limitations of this technique has been suggested by Pirrie et al. (2017): high similarity or great homogeneity in the chemical mineral composition, which prevent a clear differentiation of the samples (similar limitation may occur in mineral analyses focused on SEM-EDS). Mineral analysis with XRD is efficient to distinguish different mineral phases in sand fraction only in younger soils developed from parent materials richer chemically. In more weathered soils from tropical and subtropical regions, the high predominance of quartz minerals prevent the clear reflection of residues from other primary minerals.

The objective of this study was to verify the potential of elemental and physical analyses through the use of PSD and SEM/EDS in the sand fraction from subtropical soils to discriminate samples collected in areas under different parent material and also in areas under the same parent material within a simulated crime scene.

5.4 MATERIAL AND METHODS

5.4.1 DESCRIPTION OF THE SAMPLING SITES

A simulated crime scene was designed in June 2017 in two neighbourhoods from the urban areas in Curitiba city (Santa Candida and Boa Vista) and in two neighbourhoods from Colombo city (Guaraituba and Guarani), Paraná State, Brazil. Those two cities belong to the Curitiba Metropolitan Region and are separated by a distance of 2 to 14 km (Fig. 1). This sampling design is important to test if the employed methods in the sand fraction were efficient in discriminating soils formed by the same climatic conditions and from similar parent materials (Table 1): claystone - Santa Candida and Boa Vista neighbourhoods and marble - Guaraituba and Guarani neighbourhoods. Soils were all classified as Inceptsols (Soil Survey Staff, 2010).

The simulated crime scene in each site is presented in Figure 2: was found a body in the quadrant location, in order to represent this site four soil samples (replicates) were collected at the corners of this quadrant (3g of soil from 0-5 cm layer). Soil samples at the central position were transferred onto the sole of a boot through a simulation of a suspect walking over a crime scene. Sample was recovered from the sole of the boot (footprint) by the use of a spatula. In an ideal situation of soil samples traceability, it is expected that the four replicates from the same site and the suspect samples from the central point of the quadrant may form a group with homogeneous characteristics regarding the sand fraction from each site.

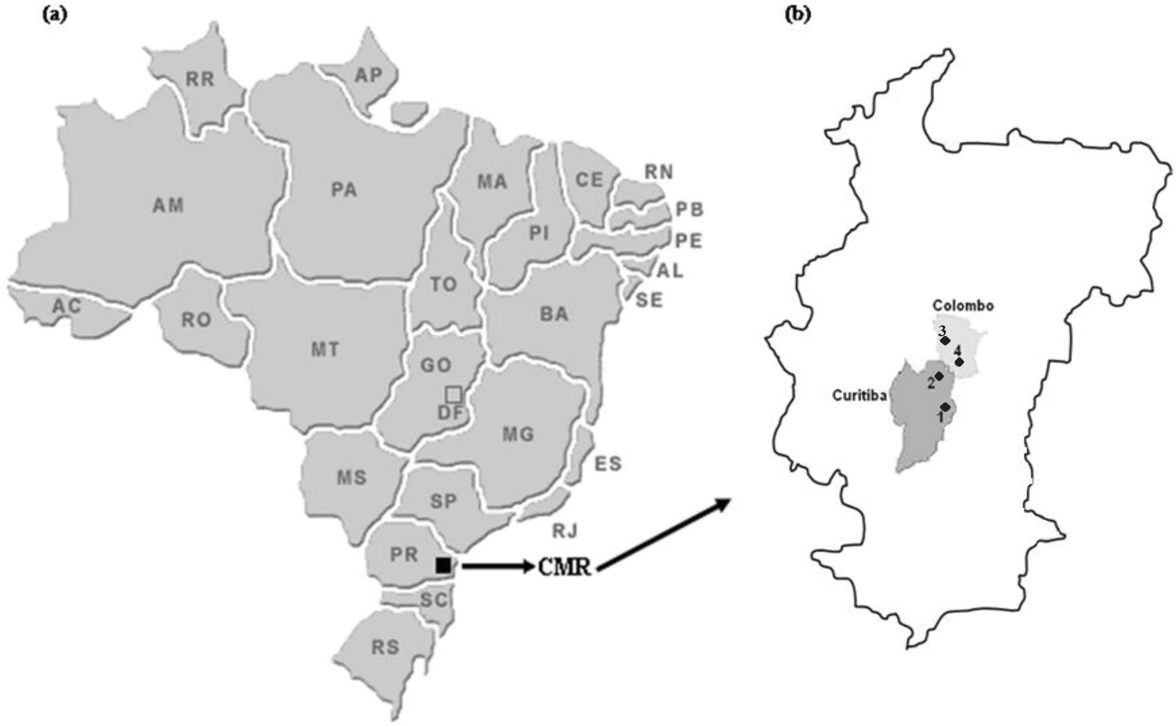


Fig 1. (a) Map of Brazil highlighting the Paraná State (PR) and (b) the Curitiba Metropolitan Region (CMR), with Curitiba city (soil sampling sites: 1-Boa Vista and 2-Santa Candida) and Colombo city (soil sample sites: 3-Guaraituba and 4-Guarani). Parent materials in Cutitiba and Colombo are claystone and marble, respectively.

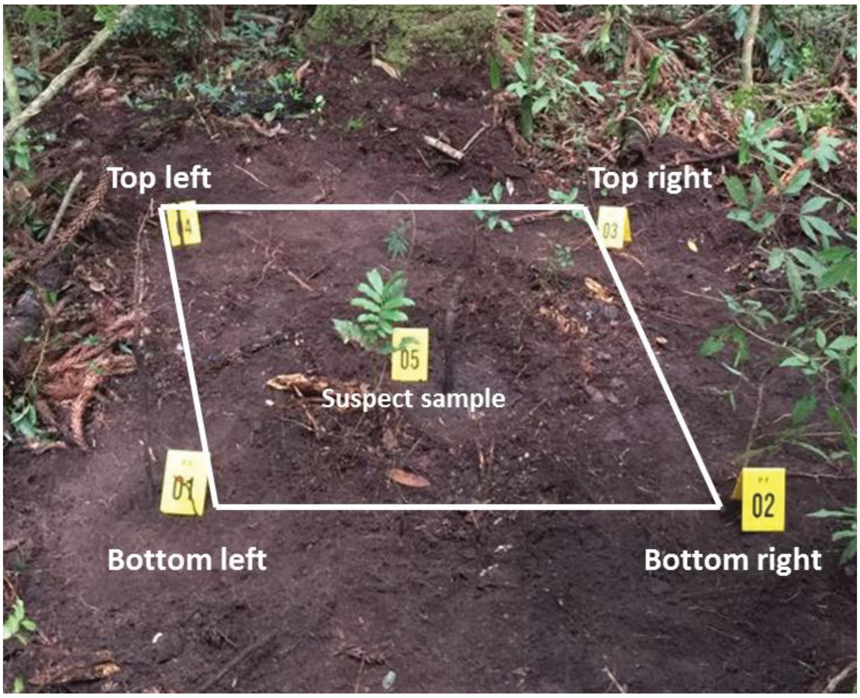


Fig. 2. Design of the simulated crime scene for surface soil sampling: samples collected at the edge of the quadrant constitute replicates of the crime scene and at the central point of the quadrant constitute the simulated suspect sample.

Table 1. Sampling sites description.

Sample	**Description***	**Location/bedrock**	**Sand g kg^{-1}**	**UTM (22J)**	
				E-W (m)	**N-S (m)**
1	Bottom right	**Santa Cândida/claystone**	15	678,203	7,192,226
2	Bottom left		17		
3	Top right		19		
4	Top left		14		
5	Suspect sample		15		
6	Bottom right	**Guarani/marble**	17	682,747	7,192,633
7	Bottom left		19		
8	Top right		16		
9	Top left		10		
10	Suspect sample		13		
11	Bottom right	**Guaraituba/marble**	30	682,747	7,192,633
12	Bottom left		34		

13	Top right		27		
14	Top left		27		
15	Suspect sample		32		
16	Bottom right	**Boa Vista/claystone**	19	676,186	7,190,193
17	Bottom left		5		
18	Top right		8		
19	Top left		11		
20	Suspect sample		8		

Note: *description of the positions in the quadrant established for the soil sampling (Figure 2). Santa Cândida and Boa Vista are neighbourhoods from Curitiba; Guarani and Guaraituba are neighbourhoods from Colombo, both are cities from Curitiba Metropolitan Region (CMR), State of Paraná, Southern Brazil.

5.4.2 SAND FRACTION PREPARATION AND X-RAY DIFFRACTION (XRD)

Organic matter was removed by treating 10 g of each soil sample with H_2O_2 (30 % v/v) in a water bath at 70 °C. Samples were dispersed with 100 mL of 0.2 mol L^{-1} of NaOH, and the sand fraction was retained in a 0.053 mm mesh sieve and dried in an oven at 50 ^{o}C for 48 hours. The dried sand sample was weighed and its content is presented in Table 1.

The sand fraction (approximately 0.3 g) was ground and sieved through a 0.2 mm sieve. The diffraction patterns (random powder samples) were obtained in the equipment Panalytical X'Pert3, under 0.42 °2θ s^{-1} speed and analysed in the range from 3 to 60 °2θ. The diffractometer was equipped with nickel filter, graphite monochromator and CuKα radiation, and it was operated at 40 kV and 40 mA.

5.4.3 SCANNING ELECTRON MICROSCOPY COUPLED TO AN ENERGY DISPERSIVE X-RAY SPECTROMETER (SEM-EDS)

A previous separation of the sand fraction by sieving was carried out: 0.25-2 mm (sample 1) and 0.05-0.25 mm (sample 2). These two sand size classes were analysed by SEM-EDS with a Zeiss EVO LS10 scanning electron microscope equipped with 3 imaging detectors, high vacuum secondary electron, extended pressure secondary electron and back scattered electron. Analytical conditions were 20kV under a working distance of 8.5 mm. The EDS system is an Oxford Instruments Inca system with X-act detector 125eV resolution detecting elements down to Be.

Subsamples 1 and 2 of sand fraction were put on a metal support and fixed on an adhesive tape placed on a stub (SEM sample holder) and subjected to several tests in order to define the size most suitable to provide an acceptance resolution. Establishing a comparison between samples 1 and 2, it was verified that sample 2 showed a higher percentage of the total analysed elements by EDS, , such as Ti, which can be related to the presence of anatase. In addition, peaks of all elements in the spectrum of the sample 2 presented greater intensity, possibly related to an effect of the higher number of particles analysed due to the smaller size of the particles. For these reasons, only the SEM-EDS results of the sample 2 (0.05-0.25 mm) were considered.

The grain morphology (measurements of the diameter and perimeter) was determined for all particles belonging to a selected area in each image sample obtained by SEM. Therefore, were considered the median values of diameter and perimeter (analysis of all particles from 0.05 to 0.25 mm) in the selected SEM area. The morphology features were also evaluated, based on the type of grains established by Bull and Morgan (2006). Their surface features were examined, with each type of grain reflecting a unique morphological pattern, shaped under different environments and chemical and physical weathering processes (Bull and Morgan, 2006): Type I - grains derived from a diagenetic sandstone exhibiting a suite of diagenetic features typically without subsequent edge abrasion; Type II - well rounded grains with subaqueous impact features (such as found after river transportation) with later chemical smoothing; Type III - grains characteristically high relief with angular/sub-angular grains with some sub-rounded additions with no edge abrasion but later chemical smoothing.

The microchemical analysis of the sand fraction by EDS was not done on individual particles. In order to provide a clear representation of the location, the sand sample on the sample holder was analysed in a diffuse mode, examining all the particles in a selected SEM area. The median composition of each sand fraction was ascertained with the limit of EDS detection adjusted to 0.5%. Both elemental compositions obtained by EDS and morphological measurements by SEM constitute an average of three areas of interest selected in each sample holder.

5.4.4 PARTICLE SIZE DISTRIBUTION (PSD) ANALYSIS

Subsamples of the total sand fraction (0.05-2 mm) were dispersed within an aqueous matrix and analysed by Mastersizer 2000 (Malvern Instruments). This equipment offers rapid sizing of particles in the range 0.02–2000 μm for a wide variety of sample types, including soils. It is possible to use this technique for sample weights of around 50 mg, although the minimum soil weight limit to produce reproducible results is strongly affected by the size distribution. Discrimination between samples is performed on the basis of the shape of the particle size curves and statistical measures of the size distributions.

5.4.5 STATISTICAL ANALYSES

Non-parametric multivariate methods were carried out in the software's Statistica (StatSoft, 2011) and PAST (Paleontological Statistics) (Hammer et al., 2001; Hammer and Harper, 2006). Values of the elemental profile obtained by EDS and PSD analyses were compiled in different data matrices and subjected to multivariate analysis of clustering by Bray-Curtis and principal component analysis (PCA) to visualise the general relationships between samples and to ascertain the discrimination of the samples, correlating them with the relative contribution of the analytical data obtained.

5.5 RESULTS AND DISCUSSION

Quartz is the only mineral common to all the samples (Fig. 3). The intensive chemical weathering promoted a mineralogical homogenisation of the sand fraction from soils developed from the two parent materials (claystone and marble). Soils developed from limestone in temperate regions present calcite and dolomite in the sand fraction (Blackburn and Taylor, 1969). Under an intensive chemical weathering the mineralogical composition of the sand fraction decreases progressively related to the parent material and with the initial environment of soil formation. Such conditions make it difficult to use X-ray diffraction (XRD) in forensic studies. Minerals with contents lower than 40 g kg^{-1} in the clay, silt and sand fractions normally cannot be precisely identified by XRD (Nakai et al., 2014). The high crystallinity of the quartz sand grains (high intensity and the reduced width at half height of the peacks) may also make the identification of other primary minerals present in the silt and sand fraction difficult (Fig. 3). The use of XRD to track soil samples under temperate conditions is much more promising in forensic cases (Ruffell and Wiltshire, 2004).

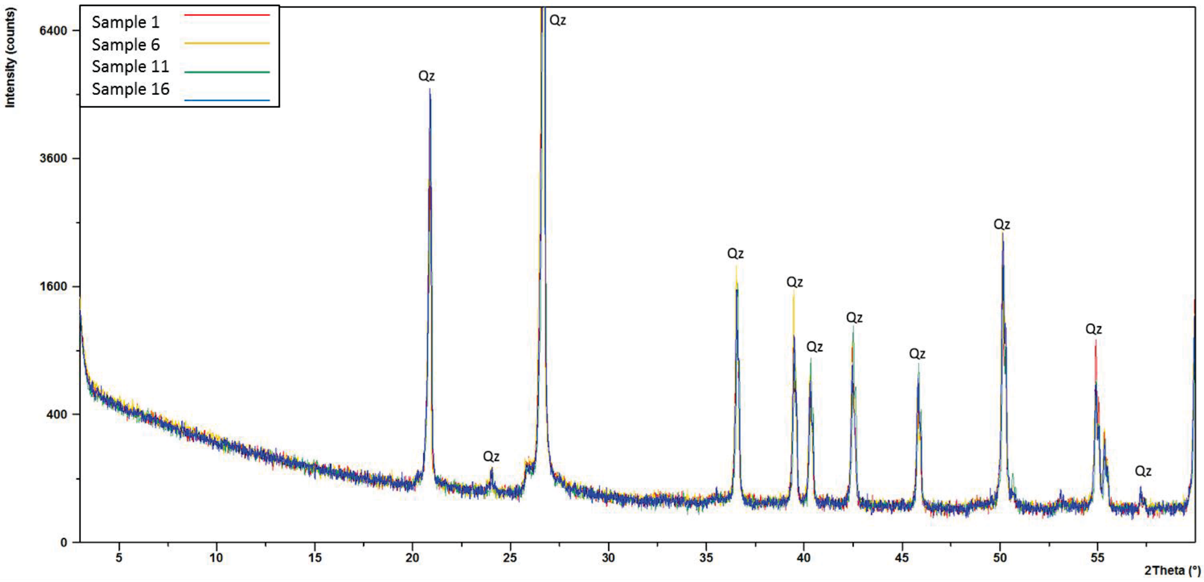
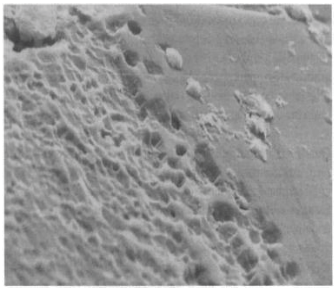
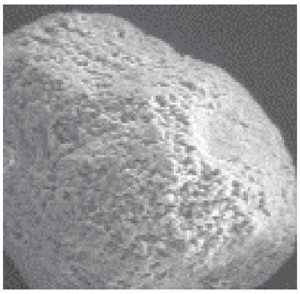
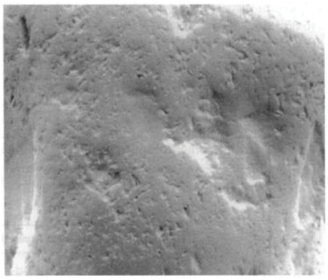
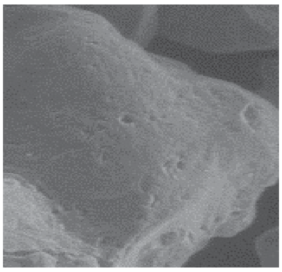
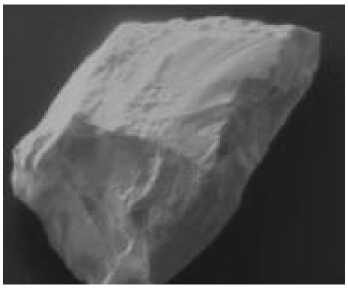
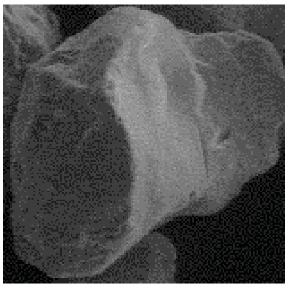


Fig. 3. X-ray diffractograms of the sand fraction from 4 samples randomly selected in the different sites for illustrative purposes: samples 1 and 16 represents samples from Curitiba (claystone); sample 6 and 11 represents samples from Colombo (marble) (Table 1). Note: Qz - quartz.

Due the poor discrimination of the XRD in the sand fraction matrix, SEM-EDS was used in order to analyse the individual particles within the sand fraction. The morphology of the quartz grains was differentiated according to the degree of weathering and degree of transportation of the minerals (Table 2). The morphological analysis was not used to discriminate different sites and to group the replicates from a same site, once it was established that the three types of grains (Table 2) were widely distributed in all the samples examined (Fig. 4). The four sites were subjected to similar weathering and transportation processes, previously reported by Bull and Morgan (2006) (Table 2). The quartz sand grains were predominantly sub-rounded to sub-angular, with granular particles occurring occasionally, independently of the sampling site selected (Fig. 4). Soils collected at locations 1 (samples 1 to 5) and 4 (samples 16 to 20) from Curitiba city were developed by the weathering of claystone. Soils developed from granite/gneiss of the edge of the Sedimentary Basin from Curitiba had suffered water erosion and the sediments were transported to the interior of the Basin (Pires et al., 2007). This water transportation favored the formation of the features observed in the types I and II (Table 2), which presented predominantly rounded faces, as a result of the water smoothing and exfoliation.. Following deposition, the process of pedogenesis increases the amount of acids in the infiltrating waters, affecting the surface of the quartz sand grains (Type I). Regarding the soils from locations 2 and 3 (samples 6 to 15) were formed from the metamorphic rock weathering (marble - metamorphism of limestone) and were subjected to an intensive process of weathering, which promotes the dissolution of the carbonate minerals in the sand fraction (Fig. 3). The features formation attributed to the sand grains transportation (types I and II) at the location 2 and 3 was due to a layered erosion of the soil in an undulated relief. Soils from locations 2 and 3 were collected in the 0 to 5 cm layer in the mid-slope position of the landscape.

Table 2. Different quartz types according Bull and Morgan (2006) and their correspondence with the quart sand grain of the studied samples obtained by SEM-EDS.

Quartz grain types	**Description**	**Patterns presented by Bull and Morgan (2006)**	**Quartz sand grains observed in the present study**
Type I	Very well-rounded grain with impact features (originated by river transportation), and subsequent grain surface solution (caused by soil acids and deposition)		 **Sample 1**
Type II	Well-rounded grains with subaqueous impact features (such as found after river transportation) with later chemical		

	smoothing		**Sample 11**
Type III	Grains characteristically high relief with angular/sub-angular grains with some sub-rounded additions with no edge abrasion but later chemical smoothing		 **Sample 6**

Note: a complete identification of the samples is in Table 1.

In order to obtain a larger range of results, the samples were examined using SEM EDS by scanning and focus the energy dispersive X-ray in the diffuse mode. Analyses of isolated areas of the sand sample (SEM-EDS) allows identification of specific elements in this fraction, such as K (Table 3), which host structural forms of accessory minerals as residues of muscovite particles, not identified through XRD (Fig. 3).

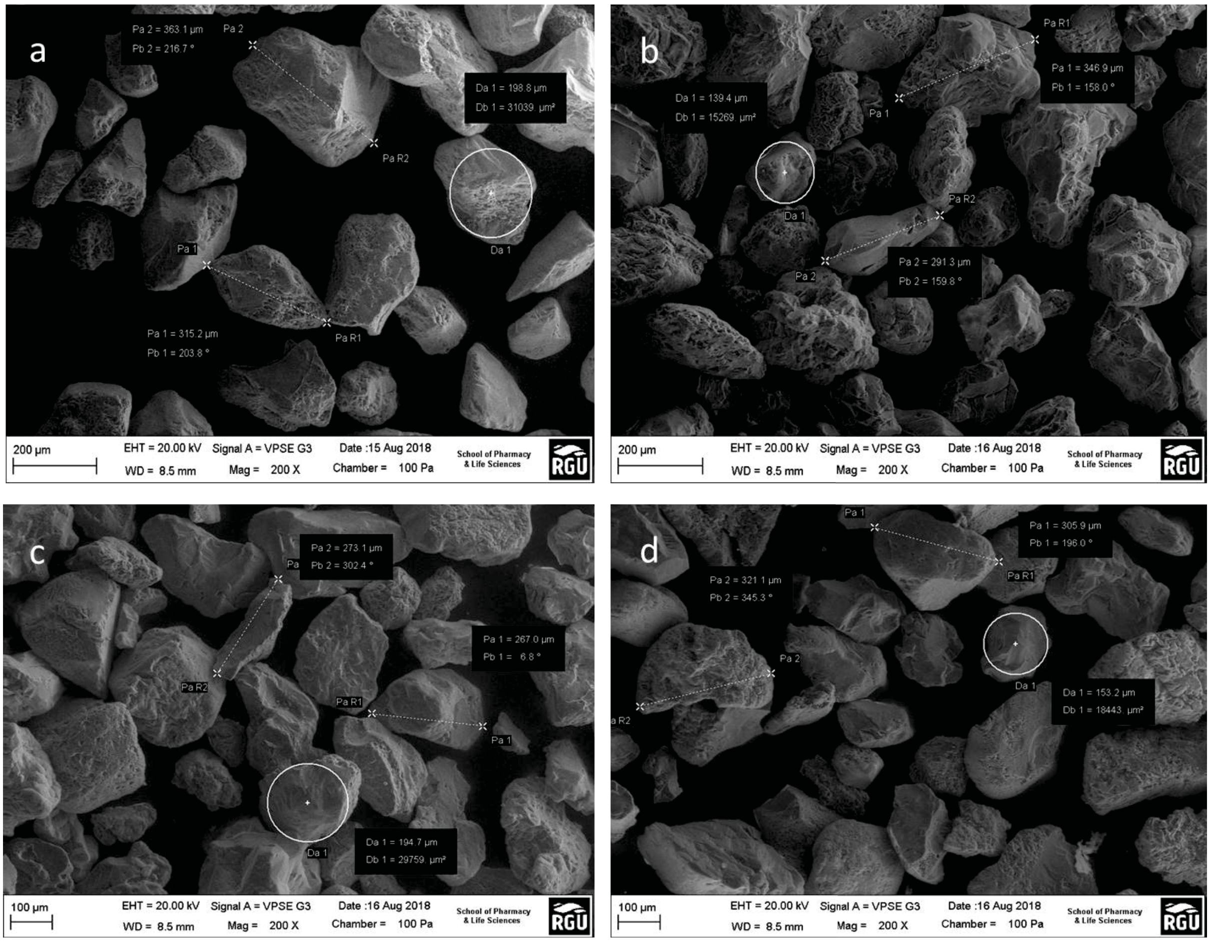


Fig. 4. Representative examples of particles of the finer sand fraction (< 0.25 mm) measured by SEM: a) sample 3 - area 1; b) sample 7 - area 2; c) sample 15 - area 3; d) sample 19 - area 4. Note: a complete identification of the samples is present in Table 1.

The widest variation in elemental distribution was observed in samples from site 4 (Boa Vista neighbourhood) (Table 4). The predominance of silica and the poor elemental diversity (Table 4) is in agreement with the XRD patterns (Fig. 3). The removal of calcite from the sand fraction of the developed marble soils was virtually complete, since no Ca was detected in samples 5 to 15 (Table 3). Calcite is very susceptible to hydrolysis reactions. The maximum Fe and Ti contents were 139.8 and 55.2 g kg^{-1}, respectively. The main sources of Fe in soils originating from sedimentary rocks (claystone) are ferruginous concretions (Zhao and Tan, 2018). Anatase and rutile mineral particles resistant to weathering are the main sources of Ti in the sand fraction of the soils (Prandel et al., 2017).

Table 3. Elemental profile and measurements from the grain morphology of the finer sand fraction (0.05-0.25 mm) obtained by SEM-EDS.

Sample	Elemental contents [1]					Morphology	
	Si	Al	Fe	Ti	K	Diameter	Perimeter
	g kg^{-1}					µm	
1	989.4	7.8	2.9	nd	nd	175.3	226.9
2	991.7	6.1	3.8	nd	nd	173.9	293.7
3	995.2	4.8	nd	nd	nd	196.4	303.3
4	993.9	6.1	nd	nd	nd	165.7	258.2
5	971.6	6.2	22.3	nd	nd	198.8	315.2
6	937.1	62.9	0.0	nd	nd	138.1	370.5
7	979.5	8.7	11.8	nd	0.0	139.4	346.9
8	957.1	15.8	20.0	3.3	3.8	142.2	287.2
9	968.5	17.6	14.0	nd	nd	153.3	223.9
10	951.4	15.9	10.7	11.6	nd	180.8	265.9
11	880.0	70.7	29.8	19.4	nd	202.9	263.0
12	884.7	6.0	54.2	55.2	nd	187.8	314.8
13	901.9	10.9	46.2	41.0	nd	171.2	261.9
14	974.0	nd	10.8	15.1	nd	187.8	266.9
15	996.8	3.2	nd	nd	nd	194.7	267.0
16	920.7	20.4	29.4	25.9	3.6	190.1	301.7
17	930.6	24.4	24.0	2.9	18.2	125.6	230.4
18	822.0	27.8	139.8	8.4	2.0	165.7	339.4
19	922.0	14.1	45.0	15.2	3.6	153.2	305.9
20	925.8	27.8	22.5	18.8	5.1	158.7	232.4

(1)Median elemental contents of all particles in diffuse model. nd – non determined, below the limit of detection 5 g kg^{-}1. Note: a complete identification of the samples is present in Table 1.

The frequency of distribution according to the size-classes in PSD analysis showed similar curves, but with greater intra-group variation among the sizes of the samples from marble in the Guaraituba neighbourhood (Fig. 5). Most of the samples were concentrated on the 0.1 to 0.2 mm size class. The shape of the curves did not provide a clear discrimination among the samples and their provenance. Boa Vista (claystone) and Guarani (marble) showed very similar particle size distribution profiles. Such lack of discrimination of soils collected from distinct geological domains makes the application of the sand particle size distribution a technique of limited use for forensic purpose (Fig. 5) and must be not used as isolated data in a forensic context (Saye and Pye, 2004).

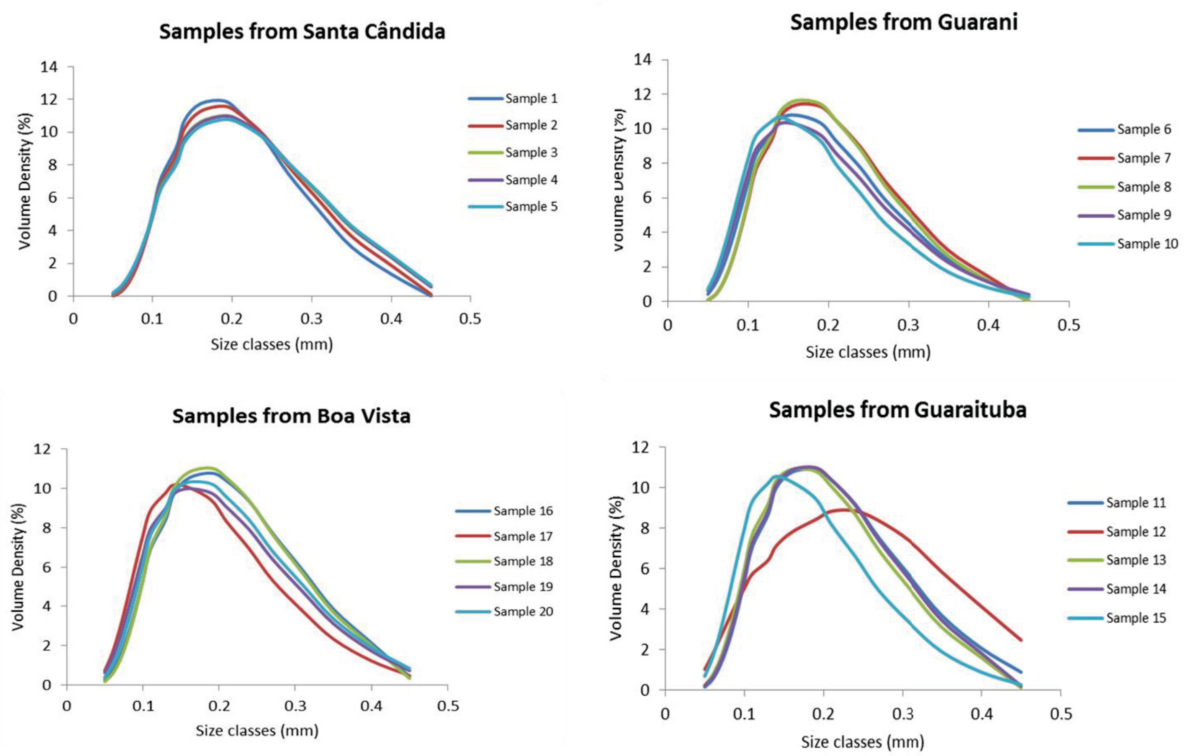


Fig. 5. Frequency of distribution according the size-classes of the sand fraction, obtained by PSD. Note: a complete identification of the samples is present in Table 1.

Samples presented a weak grouping according their sites (Fig. 6 and 7). The closest intra group similarity was observed for the Santa Candida samples, with the positioning of suspect sample (sample 20 - underlined numbers) close to samples 16 and 17 (Fig.7). Samples 16 and 17 at the same quadrant (replicates) from Santa Candida presented 95.5% of similarity. In order to group the suspect sample (sample 20), the similarity of this group reduce to 93%. To group all replicates of each site the similarity was only 87% for the samples 1 to 5, 6 to 10 and 11 to 15 and 86% for samples 16 to 20 (Fig 7). The level of grouping of the replicates samples from the same site was much lower when the finer fractions of the soils were used (Testoni et al., 2019). Data from analysis of the finer fraction of the soil also separated into clear groups of soils formed from the same parent material (two sites under claystone and two sites under marble) (Testoni et al., 2019).

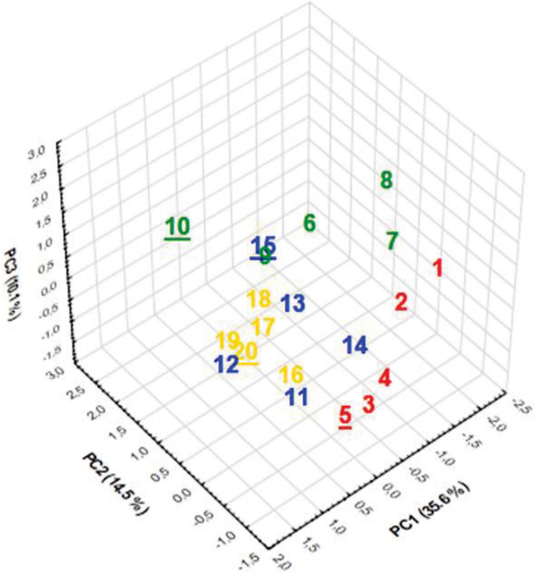


Fig. 6. Scores from the first three principal components obtained by Principal Component Analysis (PCA), showing the samples grouping according the relative contribution of the SEM-EDS and PSD data. Note: Red samples – Santa Cândida, Green samples – Guarani, Blue samples – Guaraituba, Yellow samples – Boa Vista, neighbourhoods from Curitiba and Colombo, Paraná (Table 1); samples underlined refer to the suspect sample for each location. Note: a complete identification of the samples is present in Table 1.

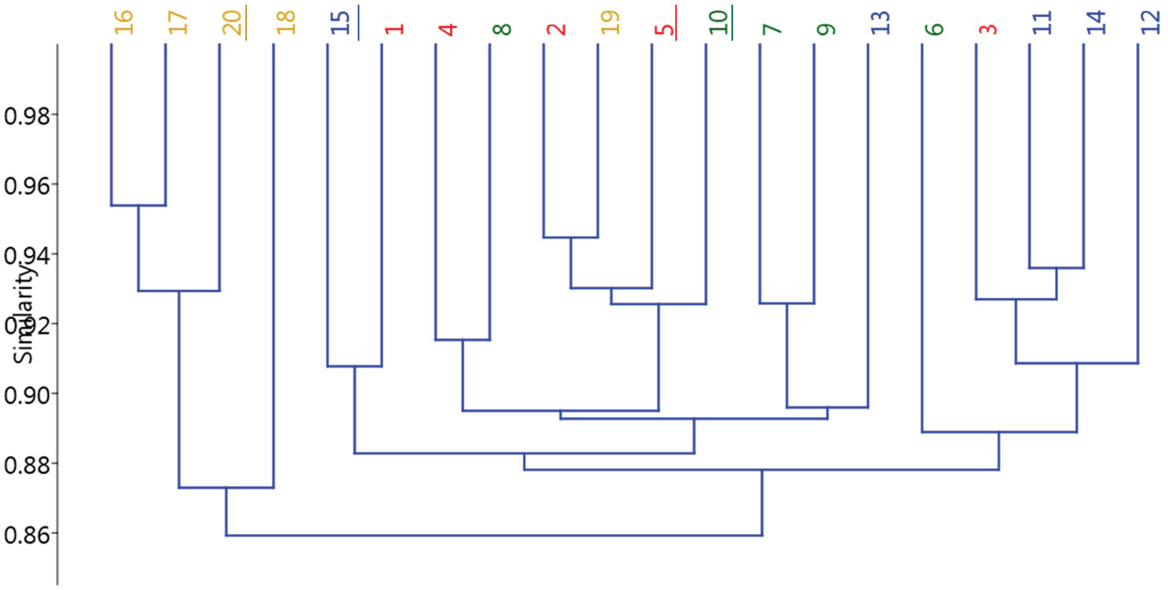


Fig. 7. Dendrogram of similarity obtained by Bray-Curtis, showing the samples similarity according the relative contribution of the SEM-EDS and PSD data. Note: Red samples – Santa Cândida, Green samples – Guarani, Blue samples – Guaraituba, Yellow samples – Boa Vista, neighbourhoods from Curitiba and Colombo, Paraná (Table 1); samples underlined refer to the suspect sample for each location.

5.6 CONCLUSIONS

The SEM-EDS, PSD and XRD methods did not provide a clear discrimination of the sand samples of the soils from different sites . This relatively poor discrimination can be explained by the large homogeneity observed within the sand fraction in relation to its chemical (elemental profile) (EDS), physical distribution (particle size distribution), morphological (shape and texture) (SEM) and mineralogical (XRD) characteristics. Our recommendation is that under tropical and subtropical conditions, the analysis of the finer soil fractions in forensic studies must be prioritised. However, for very sandy soils or in sandy sediments, where there is not enough amount of silt + clay sample, other methods of physical, chemical, and mineralogical determinations must be tested in the sand fraction.

5.7 REFERENCES

Batista AH, Melo VF, Gilkes R, Roberts M. Identification of heavy metals in crystals of sand and silt fractions of soils by scanning electron microscopy (SEM EDS/WD-EPMA). *Revista Brasileira de Ciencia do Solo.* 2018;42:1–16.

Blackburn G, Taylor RM. Limestones and Red Soils of Bermuda. *GSA Bulletin*. 1969;80: 1595-1598.

Bong WSK, Nakai I, Furuya S, Suzuki H, Abe Y, Osaka K, Matsumoto T, Itou M, Imai N, Ninomiya T. Development of heavy mineral and heavy element database of soil sediments in Japan using synchrotron radiation X-ray powder diffraction and high-energy (116 keV) X-ray fluorescence analysis: 1. Case study of Kofu and Chiba region. *Forensic science international*. 2012;220:33–49.

Brinatti AM, Mascarenhas YP, Pereira VP, Silvia C, Partiti DM, Macedo Á. Mineralogical characterization of a highly-weathered soil by the Rietveld Method pelo Método de Rietveld. *Sci Agric*. 2010;454–464.

Bull PA, Morgan RM. Sediment Fingerprints : A forensic technique using quartz sand grains. *Science & Justice*. 2006;46:107–124.

Bull PA, Parker A, Morgan RM. The forensic analysis of soils and sediment taken from the cast of a footprint. *Forensic Science International*. 2006;162:6–12.

Carvalho Á, Ribeiro H, Mayes R, Guedes A, Abreu I, Noronha F, Dawson L. Organic matter characterization of sediments in two river beaches from northern Portugal for forensic application. *Forensic Science International*. 2013;233:403–415.

Cengiz S, Karaca AC, Çakir I, Üner HB, Sevindik A. SEM-EDS analysis and discrimination of forensic soil. Forensic Science International. 2004;141:33–37.

Chauhan R, Kumar R, Sharma V. Soil forensics: A spectroscopic examination of trace evidence. *Microchemical Journal*. Elsevier B.V.; 2018;139:74–84.

Chazottes V, Brocard C, Peyrot B. Particle size analysis of soils under simulated scene of crime conditions: The interest of multivariate analyses. *Forensic Science International*. 2004;140:159–166.

Chodur NL. Caracterização de solos na região de Araucária, Contenda, PR. Universidade Federal do Paraná; 1990.

Dawson LA, Hillier S. Measurement of soil characteristics for forensic applications. *Surface and Interface Analysis*. 2010;42:363–377.

Dawson LA, Mayes RW. *Criminal and Environmental Soil Forensics*: Soil as Physical Evidence in Forensic Investigations. Introduction to Environmental Forensics: Third Edition. Elsevier Ltd; 2014.

Farrugia KJ, Bandey H, Dawson L, Nic Daéid N. Chemical enhancement of soil based footwear impressions on fabric. *Forensic Science International.* 2012;219:12–28.

Fitzpatrick RW. Encyclopedia of Forensic Sciences [Internet]. 2nd ed. *Encyclopedia of Forensic Sciences.* Glen Osmond: Elsevier; 2013.

Hammer Ø, Harper DAT. PAST. *Paleontological Statistics.* Version 2.07. Reference manual. Blackwell Publishing. 2006;351.

Hammer Ø, Harper DAT, Ryan PD. PAST: *Paleontological Statistics* Software Package for Education and Data Analysis. Palaeontologia Electronica; 2001. p. 99.

McCulloch G, Dawson LA, Ross JM, Morgan RM. The discrimination of geoforensic trace material from close proximity locations by organic pro fi ling using HPLC and plant wax marker analysis by GC. *Forensic Science International*. 2018;288:310–326.

Melo VF, Mazzetto JML, Dieckow J, Bonfleur EJ. Factor analysis of organic soils for site discrimination in a forensic setting. *Forensic Science International.* 2018;290:244–250.

Melo VF, Novais RF, Schaefer CEGR, Fontes MPF, Singh B. Mineralogia das frações areia , silte e argila de sedimentos do grupo barreiras no município de Aracruz , Estado do Espírito Santo. 2006;29–41.

Melo VF, Testoni SA, Dawson L, Lara AG De, Salvador F. Can analysis of a small clod of soil help to solve a murder case? *Science & Justice*. 2019;1–26.

Mitton RV, Melo VF. Do Aggregate Size Classes of the Subsurface Soil Horizon Have Different Chemical / Mineralogical Properties? *Brazilian Journal of Soil Science.* 2019;1–15.

Morgan RM, Bull PA. The use of grain size distribution analysis of sediments andsoils in forensic enquiry. *Science & Justice*. 2007;47:125–135.

Morgan RM, Robertson J, Lennard C, Hubbard K, Bull PA. Quartz grain surface textures of soils and sediments from Canberra, Australia: A forensic reconstruction tool. *Australian Journal of Forensic Sciences*. 2010;42:169–179.

Morgan RM, Scott KR, Ainley J, Bull PA. Journey history reconstruction from the soils and sediments on footwear: An empirical approach. *Science & Justice*. 2018;1–11.

Nakai I, Furuya S, Bong W, Abe Y, Osaka K, Matsumoto T, Itou M, Ohta A, Ninomiya T. Quantitative analysis of heavy elements and semi-quantitative evaluation of heavy mineral compositions of sediments in Japan for construction of a forensic soil database using synchrotron radiation X-ray analyses. *X-Ray Spectrometry*. 2014;43:38–48.

Olatunji OO, Oyeyiola Y, Oyediran GO. Assessment of Dithionite and Oxalate Extractable Iron and Aluminium Oxides on a Landscape on Basement Complex Soil in South-Western Nigeria. 2015;266–275.

Pires ACD, De Melo VF, Motta ACV, Lima VC. Majors soil classes of the metropolitan Region of Curitiba (PR), Brazil: II - Interaction of Pb with mineral and organic constituents. *Brazilian Archives of Biology and Technology*. 2007;50:183–192.

Pirrie D, Dawson L, Graham G. Predictive geolocation: Forensic soil analysis for provenance determination. *Episodes*. 2017;40:141–147.

Prandel L V., Melo V de F, Brinatti AM, Saab S da C, Salvador FAS. X-ray Diffraction and Rietveld Refinement in Deferrified Clays for Forensic Science. *Journal of Forensic Sciences*. 2017;1–7.

Prandel L V., Saab SC, Brinatti AM, Giarola NFB, Leite WC, Cassaro FAM. Mineralogical analysis of clays in hardsetting soil horizons, by X-ray fluorescence and X-ray diffraction using Rietveld method. *Radiation Physics and Chemistry*. Elsevier; 2014;95:65–68.

Pye K, Blott SJ. Particle size analysis of sediments, soils and related particulate materials for forensic purposes using laser granulometry. *Forensic Science International.* 2004;144:19–27.

Pye K, Blott SJ, Croft DJ, Witton SJ. Discrimination between sediment and soil samples for forensic purposes using elemental data: An investigation of particle size effects. *Forensic Science International*. 2007;167:30–42.

Ritz K, Dawson L, Miller D. Criminal and Environmental Soil Forensics. 2008.

Ruffella A, Wiltshire P. Conjunctive use of quantitative and qualitative X-ray diffraction analysis of soils and rocks for forensic analysis. *Forensic Science International.* 2004;145:13–23.

Saye SE, Pye K. Development of a coastal dune sediment database for England and Wales: forensic application. In: Pye K, Croft DJ, organizadores. Forensic Geoscience: Principles, Techniques and Application. London: *Geological Society Special Publications Society*; 2004. p. 232.

Shudofsky KN, Burden A, Vestergaard P, De Vries F. Uit het nederlands platform voor farmaceutisch onderzoek: Fractuurrisico bij patienten met COPD die intermitterend hooggedoseerde glucocorticoiden gebruiken. *Pharmaceutisch Weekblad.* 2017;152:27–30.

StatSoft I. STATISTICA (data analysis software system). 2011.

Sugita R, Marumo Y. Screening of soil evidence by a combination of simple techniques: Validity of particle size distribution. *Forensic Science International.* 2001;122:155–158.

Zhao W, Tan WF. Quantitative and structural analysis of minerals in soil clay fractions developed under different climate zones in China by XRD with Rietveld method, and its implications for pedogenesis. *Applied Clay Science.* Elsevier; 2018;162:351–361.

6 CHAPTER V: CAN ANALYSIS OF A SMALL CLOD OF SOIL HELP TO SOLVE A MURDER CASE?[5]

6.1 RESUMO

A perícia do solo utiliza informações extensas sobre o solo para responder questões legais e testar hipóteses. A principal dificuldade é a determinação de muitas variáveis diferentes a partir de uma pequena quantidade de amostra de solo coletada no suspeito. Desenvolvemos uma análise mineralógica e química sequencial para avaliar uma quantidade limitada de vestígios de solo (0,5 g) do veículo de um suspeito (aderido ao espelho retrovisor e ao pára-lama dianteiro esquerdo) envolvido em um caso de assassinato e os comparamos com as amostras de superfície encontradas no local de disposição do corpo da vítima na Estrada da Graciosa, Estado do Paraná, Brasil. Todos os resultados afirmam que o veículo do suspeito esteve em contato com a borda da Estrada da Graciosa, em torno do local onde o corpo da vítima estava localizado. Como resultado da análise e comparação do solo, produzimos a prova objetiva única de provável contato do veículo do suspeito com a cena do crime.

Palavras-chave: ciência forense; cena do crime; solos forenses; análises sequenciais; óxidos de ferro e alumínio; feldspatos potássicos.

6.2 ABSTRACT

Soil forensics utilizes extensive soil information to answer legal questions and test hypotheses. The main difficulty often is the determination of many different variables from a small amount of soil sample collected on the suspect. We developed a sequential mineralogical and chemical analyses to assess a limited quantity of soil traces (0.5 g) from a suspect's vehicle (adhered to the rear-view mirror and to the left front fender) involved in a murder case and compared them with the surface samples found at the victim's body disposal site at the Graciosa Road, Paraná State, Brazil. All results affirm that the suspect's vehicle had been in contact with the edge of the Graciosa Road, around the place where the victim's body was located. As a result of the soil analysis and comparison, we produced the unique objective proof of likely contact of the suspect's vehicle with the crime scene.

Keywords: forensic science; crime scene; soil forensics; sequential analyses; iron and aluminium oxides; K-feldspars.

6.3 INTRODUCTION

Soil forensics utilizes an extensive range of soil information to elucidate and answer legal questions or hypotheses (FITZPATRICK; RAVEN, 2012). Although

---

[5] Published on Science & Justice

DOI: https://doi.org/10.1016/j.scijus.2019.06.008

several studies have reported the use of soil traces to assist forensic investigations and judgements (CONCHERI et al., 2011; DAWSON, 2017; FITZPATRICK; RAVEN; SELF, 2014; REIDY et al., 2013) the connection between soil science and the law is relatively unexploited in many countries. Nevertheless, soil particles are often associated with items such as footwear, clothing, shovels, as well as car tyres etc., and can indicate a wide array of information about places visited (FITZPATRICK; RAVEN; SELF, 2014; MCCULLOCH et al., 2018; MURRAY et al., 2016). If soil is wet/moist, impregnation on different surfaces and the potential of transferability and persistence of the soil traces is increased (MURRAY et al., 2017; STONEY; BOWEN; STONEY, 2016).

As the soil composition is generally a reflection of the underlying geology, under particular climatic conditions, weathering and leaching processes, geographical scales, and anthropogenic activities, a wide range of different measurable soil characteristics is commonly found to help forensic studies (DAWSON; HILLIER, 2010; DAWSON; MAYES, 2014). This variability provides excellent information to test potential links among criminals with specific locations, such as a crime scene. However, determining the source of a soil sample may never be absolute, and a prediction of source can be useful in many instances (CHESHIRE; MORGAN; HOLMES, 2017; PIRRIE; DAWSON; GRAHAM, 2017; TIGHE et al., 2018).

There are several techniques which can provide information from trace samples and subsequently the examiner can interpret the different types of soils and their particular environment of formation, reflected in the elemental composition, mineralogy, and organic constituents of the soil samples (CORRÊA et al., 2017; STOPPE; AMELUNG; HORN, 2015; WOODS et al., 2014). Thus, it may be possible to develop destructive and non-destructive analysis that can detect one or more of these distinctive properties and indicate the likely source of a soil sample of interest. Despite the chosen methodology, the main difficulty in soil trace investigations is the small amount of sample to work with (normally less than 0.5 g). To improve the strength of evidence produced, researchers try to produce as many quantitative variables as possible from a small soil trace (PRANDEL et al., 2017). For this purpose, the chemical extraction of short range order (SRO) materials from the clay fraction is recommended (MELO et al., 2008). The SRO is more variable in soil and can be different in soils occurring side-by-side in a confined area (POGGERE et al., 2016; SIMAS et al., 2006) and thus is able to discriminate small locus size areas within a crime scene.

In this study we developed and applied a sequential approach of both mineralogical and chemical analyses in order to assess a limited quantity of soil traces (0.5 g) sampled on the suspect’s vehicle involved in a murder case and compared them with the surface soil samples collected at the victim’s body disposal site in Brazil.

6.4 METHODOLOGY

6.4.1 CASE BACKGROUND

On November 2018 a young girl disappeared from Colombo, Paraná State, Brazil. The disappearance was quickly treated as a murder enquiry, which was subsequently confirmed with the victim’s body being found.

The main suspect in the murder enquiry was arrested on 19 May 2018. The main evidence which was available to indicate that the suspect left a house from Colombo (Paraná State, Brazil) with the victim, during the overnight of 9 May 2018 were images recorded on the security cameras. The victim's body was found on 8 June 2018, in a forest area at the side of the Graciosa Road (State Road PR-410) (Fig. 1), approximately 50 km away from Colombo city (Fig. 1). The body was discarded at the edge of Graciosa Road (State Road PR-410) at the Sea Range (mountainous relief) around 30 m below the level of the road.

The suspect claimed to be innocent and stated that he did not drive out of the city of Colombo. The suspect attributed the soil traces on his vehicle to the traffic in his own city, Colombo. Therefore, the physical evidence derived from the soil samples including the soil trace recovered from the suspects’ vehicle supported the proposition that the soil could have come from the road adjacent to the deposition site and rejected the proposition that it could have come from the city area of Colombo (Fig. 2a, 2b).

6.4.2 SITE DESCRIPTION AND SOIL SAMPLING

A summary of characteristics of the soil sampled in this criminal investigation is presented in Table 1. There was a soil trace adhering to the rear-view mirror (Fig. 2a) and to the left front fenders (Fig. 2b) in the vehicle. Due the small amount of soil adhering, the samples from the two locations were recovered and combined into a single sample for subsequent analysis.

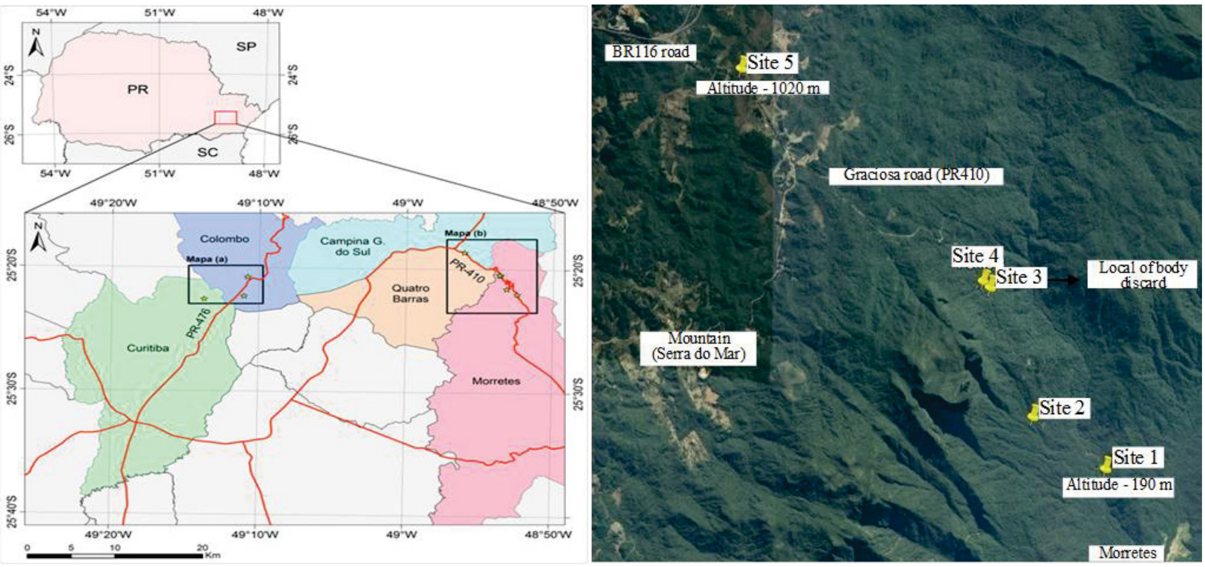


Fig. 1. Paraná State map showing the relative positions of sampling sites in Curitiba, Colombo and Graciosa Road.

Fig. 2a. Soil traces were sampled from the a) rear-view mirror and 2b) from the left front fenders at the left side of the suspect’s vehicle apprehended at the parking of the Civil Police of Colombo (State of Paraná).

Table 1. Sampling site characteristics (Graciosa Road, Curitiba, Colombo and the suspect’s vehicle).

Sample	Site	Repetition	Depth (cm)	UTM (22J)		Parent material
				E-W (m)	N-S (m)	
1	1 - Graciosa Road	1	0-3	713,912	7,192,727S	Granite/gneiss
2		2	0-3	713,912	7,192,727	Granite/gneiss
3		3	0-3	713,912	7,192,727	Granite/gneiss
4		4	0-3	713,912	7,192,727	Granite/gneiss
5	2 - Graciosa Road	1	0-3	712,725	7,193,546	Granite/gneiss
6		2	0-3	712,725	7,193,546	Granite/gneiss
7		3	0-3	712,725	7,193,546	Granite/gneiss
8		4	0-3	712,725	7,193,546	Granite/gneiss
9	3 - Graciosa Road (level location)	1	0-3	712,049	7,195,556	Granite/gneiss
10		2	0-3	712,049	7,195,556	Granite/gneiss
11		3	0-3	712,049	7,195,556	Granite/gneiss
12		4	0-3	712,049	7,195,556	Granite/gneiss
13	3 - Graciosa Road (trail to the body discarding site)	1	0-3	712,049	7,195,556	Granite/gneiss
14		2	0-3	712,049	7,195,556	Granite/gneiss
15		3	0-3	712,049	7,195,556	Granite/gneiss
16		4	0-3	712,049	7,195,556	Granite/gneiss
17	4 - Graciosa Road	1	0-3	711,934	7,195,655	Granite/gneiss
18		2	0-3	711,934	7,195,655	Granite/gneiss
19		3	0-3	711,934	7,195,655	Granite/gneiss
20		4	0-3	711,934	7,195,655	Granite/gneiss
21	4 - Graciosa Road (skidding mark)	1	0-3	711,934	7,195,655	Granite/gneiss
22		2	0-3	711,934	7,195,655	Granite/gneiss
23	5 - Graciosa Road	1	0-3	707,943	7,199,033	Granite/gneiss
24		2	0-3	707,943	7,199,033	Granite/gneiss

25		3	0-3	707,943	7,199,033	Granite/gneiss
26		4	0-3	707,943	7,199,033	Granite/gneiss
27	Vehicle with soil trace	1		**Apprehended by Civil**		
28	6- Santa Cândida (Curitiba)	1	0-3	678,203	7,192,226	Claystone
29		2	0-3	678,203	7,192,226	Claystone
30		3	0-3	678,203	7,192,226	Claystone
31		4	0-3	678,203	7,192,226	Claystone
32	7 - Guarani (Colombo)	1	0-3	682,747	7,192,633	Limestone
33		2	0-3	682,747	7,192,633	Limestone
34		3	0-3	682,747	7,192,633	Limestone
35		4	0-3	682,747	7,192,633	Limestone
36	8 - Guaraituba (Colombo)	1	0-3	683,189	7,195,492	Limestone
37		2	0-3	683,189	7,195,492	Limestone
38		3	0-3	683,189	7,195,492	Limestone
39		4	0-3	683,189	7,195,492	Limestone

The impregnation of the soil trace to the rear-view mirror and the left front fenders in the vehicle at the edge of the region from Graciosa Road was likely as a result of the continuous rainfall which had occurred in that region on the day before the victim's disappearance and likely time of contact of the vehicle with the place. The accumulated rainfall between zero hour from 8 May 2018 and 24 hours from 8 May 2018 was intense (21.2 mm).

Along the Sea Range, at the edge of the Graciosa Road (PR410) (Fig. 1), in the region where the victim's body was found (Fig. 3a), soil samples at five sites (Fig. 1) were collected at the 4 corners of a 1.5 m quadrant grid (Fig. 3b). Site 1 was located at the lower level of the Sea Range and at a lower altitude (Fig. 1); Site 2 was located at a higher level in relation to site 1 (Fig. 1); Site 3 was the place where the victim's body was found (Fig. 1 and 3a), where two sets of four samples were located. The first set of samples was collected in a quadrant at the edge of the road, in a flatter site, and the second set was positioned on the trail where the body was found; Site 4 was located at about 300 m from the location where the body was recovered (site 3) (Fig. 1). At this site, four samples were recovered in a quadrant, and in addition, two samples on tire skating marks of a vehicle (Fig. 4); Site 5 was located at the top of the Sea Range, near the federal road BR 116 (Fig. 1).

A total of 26 soil samples were sampled in the Sea Range at the edge of the Graciosa Road (PR-410). The sampling at sites 1, 2, 4 and 5, outside of the victim's body deposition site (site 3), were selected in order to obtain other points of sampling with soils formed from the same parent material (granite/gneiss) at the edge of the same road. There provided comparability of the soil sampled from the suspect's vehicle with samples from the suspected contact location (site 3) and near to the victim's deposition site (site 4). The soil samples collected at other sites at the Sea

Range help to test potential propositions from the defence, such as that the suspect’s vehicle could have been at the Graciosa Road, although at a distant position from the victim’s body deposition site. In addition, two sites in Colombo and one site in Curitiba were selected for wider soil sampling and comparability (Table 1).

All samples were collected in June 2018 and in accordance with a Standard Operating Procedure (SOP) for forensic soil sampling in Brazil, developed by the Federal University of Paraná and the Federal Police of Brazil. Surface soil samples were sampled using a stainless-steel spatula, removing any turf or gravel, where present. All samples were stored in breathable containers and allowed to air dry prior to examination and analysis. All equipment were sterilized between sampling positions to avoid any potential contamination.

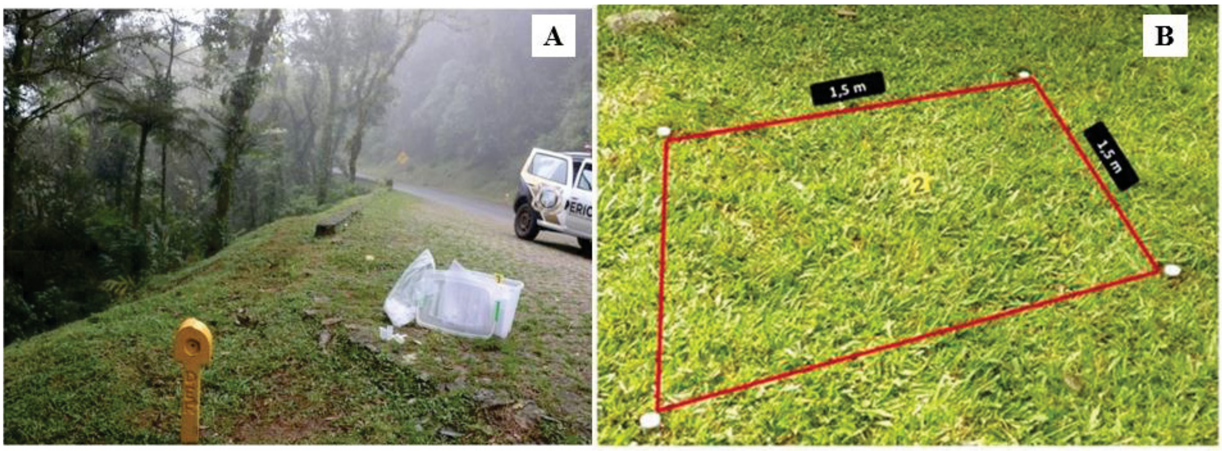


Fig.3. a) Site 3 (victim’s body deposition site) along the Graciosa Road (PR410); b) details of the soil sampling at four replicate corners of a 1.5 m quadrant.

Fig. 4.a) Details of site 4 located along to the Graciosa Road (PR410), located around 300 m distance from the victim's body deposition site; b), c) and d) muddy tyre marks on the ground surface produced during the skidding of a vehicle.

6.4.3 SAMPLE PREPARATION

Soil samples (reference quadrants, skating track and trace questioned sample) were oven-dried at 40 °C for 48 hours and sieved through a 2 mm sieve in order to obtain the oven-dried fine earth fraction. A total of 0.94 g was available from the trace sample and 1.7 g of the reference samples were ground with a rubber baton and washed with deionized water through a 0.053 mm sieve to obtain the sand fraction, thoroughly cleaned between each sample preparation. Suspensions containing silt + clay + organic matter were placed in porcelain capsules. That process was repeated until the water was limpid. The sand content was obtained gravimetrically after the sand drying at 40 °C for 24 hours. The content of soil fractions smaller than 0.053 mm (silt + clay + organic matter) was also obtained by sample weighing after drying the collected suspensions during the sieving-grinding process. It was produced 0.5 g of the silt + clay + organic matter fraction from the trace sample. Therefore, the same amount of this fraction was used for the other samples for subsequent chemical and mineralogical analyses.

6.4.4 SAND FRACTION ANALYSES

6.4.4.1 X-RAY DIFFRACTION

The sand fraction (around 0.3 g) was ground and sieved through a 0.2 mm sieve. Subsequently, the samples were analysed by X-ray diffraction (XRD). The diffraction patterns (random powder samples) were obtained in the equipment Panalytical X'Pert3, under 0.42 $°2\theta$ s^{-1} speed and analysed in the range from 3 to 60 $°2\theta$. The diffractometer was equipped with nickel filter, graphite monochromator and CuKα radiation, and was operated at 40 kV and 40 mA. Comparison between the trace samples and the samples from Colombo and Curitiba was exclusively qualitative, in particular the presence or absence of the K-feldspars peaks. In the diffraction patterns the peak heights were calculated and the areas from of 10 coincident peaks that were verified in the samples from Sea Range and in the trace sample from the vehicle were compared.

6.4.4.2 EXTRACTION OF TOTAL ELEMENTS WITH CONCENTRATED HF AND HNO_3

Sand samples that were submitted to XRD for analysis were recovered (non-destructive analysis) and placed in a sealed Teflon tube with 9 mL of HNO_3 and 3 mL of HF concentrated (USEPA, 1997). The tubes were submitted to the microwave radiation for 5.5 minutes up to180 ^{o}C. The tubes were exposed to those conditions for 4.5 min under constant temperature and pressure. The elements in the extracts (Al, Ba, Fe, Cu, K, Mn, P, V e Zn) were subsequently obtained by inductively coupled plasma spectrometry (ICP-AES).

6.4.5 ANALYSES OF THE SILT + CLAY + ORGANIC MATTER FRACTION

Due to the small amount of samples (0.5 g), it was possible to develop four sequential analyses: 1) 0.5 mol L^{-1} sodium hypochlorite; 2) 0.2 mol L^{-1} ammonium oxalate-OA; 3) sodium citrate-bicarbonate-dithionite; and 4) 0.5 mol L^{-1} NaOH.

For all sequential extractions several common steps were following: i) it was necessary reduce the amount of soil samples in relation of classic methods, but the ratio soil: solution was kept; ii) in all extractions all extracting solutions and volumetric flasks were filled with ultra-pure water (18.2 MΩ.cm at 25ºC – Millipore Direct–Q System) and high purity acids were used in the analyses (Merck PA); iii) before each extraction the samples were dried for 24 hours in an oven at 40°C, grounded and sieved at 0.2 mm in order to improve the efficiency of the extractions; iv) after each extraction, salt excess was removed by washing with 0.5 mol L^{-1} $(NH_4)_2CO_3$ and deionized water ; v) after washing the samples were oven-dried at 40°C for 24 h; vi) extracts were obtained by centrifugation (3,000 rpm for 10 min) and all extracts were filtered in a slow filter paper (Macherey Nagel®); vii) element concentrations (Fe, Al, P, Ca, Mg, Zn, Mn, Cu, Si) were determined by inductively coupled plasma spectrometry (ICP-AES).

6.4.5.1 EXTRACTION OF ORGANIC MATTER

Sample (0.5 g) were treated with 5 mL of 0.5 mol L^{-1} sodium hypochlorite in a hot bath under 90 ^{o}C for 30 min (DUARTE et al., 2012).

6.4.5.2 EXTRACTION OF POORLY ORDERED IRON AND ALUMINIUM OXIDES

Iron and aluminium oxides which were poorly crystalline were extracted using the whole sample generated after the organic matter removal (silt + clay fraction) with 7.5 mL of 0.2 mol L^{-1} ammonium oxalate at pH 3.0 (AO) (MCKEAGUE, 1978).

6.4.5.3 EXTRACTION OF CRYSTALLINE IRON OXIDES

Crystalline iron oxides present in the AO residue were extracted using the sodium citrate-bicarbonate-dithionite method (CBD) (MEHRA; JACKSON, 1960). Samples were placed in tubes of 100 mL and submitted to two extraction with 4.8 mL of 0.3 mol L^{-1} sodium citrate + 0.6 mL of 1.0 mol L^{-1} sodium bicarbonate + 0.24 g of sodium dithionite. The suspensions of the samples were manually agitated while heated at 70°C in a hot water bath for 30 min. The extracts of the two extractions were combined for the subsequent elemental analysis.

6.4.5.4 EXTRACTION OF POORLY ORDERED ALUMINOSILICATE AND GIBBSITE

The final sequential analysis was the extraction of poorly crystalline aluminosilicates (short-range order Al-hydroxide, Al–O–Si layers and Si–O (opaline silica) resistant to previous ammonium oxalate extraction) and gibbsite with boiling 0.5 mol L^{-1} NaOH (MEHRA; JACKSON, 1960; MELO et al., 2002). The CBD residue was weighed in tubes and moistened with 20 mL of 0.5 mol L^{-1} NaOH. Tubes were placed in a hot bath at 90°C for 30 min.

6.4.6 MULTIVARIATE STATISTICS

The data from the analysis of the sand fraction and the silt + clay + organic matter fraction were exported for statistical analysis by a principal component analysis (PCA) using the Statistica software (STATSOFT, 2011) and Paleontological Statistics (PAST) software using Bray-Curtis similarities (HAMMER; HARPER; RYAN, 2001). From the matrix data, three PCAs were generated: 1) sand fraction: area and height from 10 peaks by XRD of the samples from the Sea Range; 2) sand fraction: total content of the elements for all samples (Sea Range, Colombo and Curitiba); 3) silt + clay + organic matter fraction: relative percentage of the extracted elements obtained in the sequential extractions with Na hypochlorite, AO, CBD and Na hydroxide in relation to the total of each extraction (Sea Range, Colombo and Curitiba).

6.5 RESULTS AND DISCUSSION

6.5.1 QUALITATIVE RESULTS

The XRD data from the sand fraction show that the soil traces recovered from the fenders and the rear-view mirror from the suspect’s vehicle could be excluded as having come from the areas examined in Colombo and Curitiba (Fig. 1). Comparison between the XRD data of the samples was performed initially within each group of samples, in order to verify the homogeneity of the peak pattern between the samples from the same site. All groups of samples were homogenous: samples 1 to 4; samples 5 to 8; samples 9 to 12; samples 13 to 16; samples 17 to 20; samples 21 and 22; samples 23 to 26, samples 28 to 31; samples 32 to 35; and samples 36 to 39 (Table 1).

Aiming to make comparisons simpler in homogenous groups, the sample 12 from Graciosa Road region were randomly selected for represent the Sea Range (samples 1 to 26), sample 31 for Curitiba (samples 28 to 31), sample 39 for Colombo (samples 32 to 39), and a comparison between them and the trace sample (27) was realized with the diffractograms showed in Fig. 5.

It was clear from an examination of the two diffraction patterns that the sites could be clearly differentiated: 1) samples collected from Curitiba and Colombo contained only quartz in the sand fraction while samples collected from the Sea Range, at the edge of the Graciosa Road (sample 12), and the trace sample collected at the suspect’s vehicle (sample 27), contained an assemblage of quartz and K-feldspars. Under such a clear distinction it was possible to infer that the trace sample is comparable only with the samples from the Sea Range, possibly from the Graciosa Road region, near to the victim’s body deposition site. There is no compatrability of the mineralogical composition in the sand fraction from samples from around the Graciosa Road and the samples from Curitiba and Colombo. A common origin of these samples can be excluded.

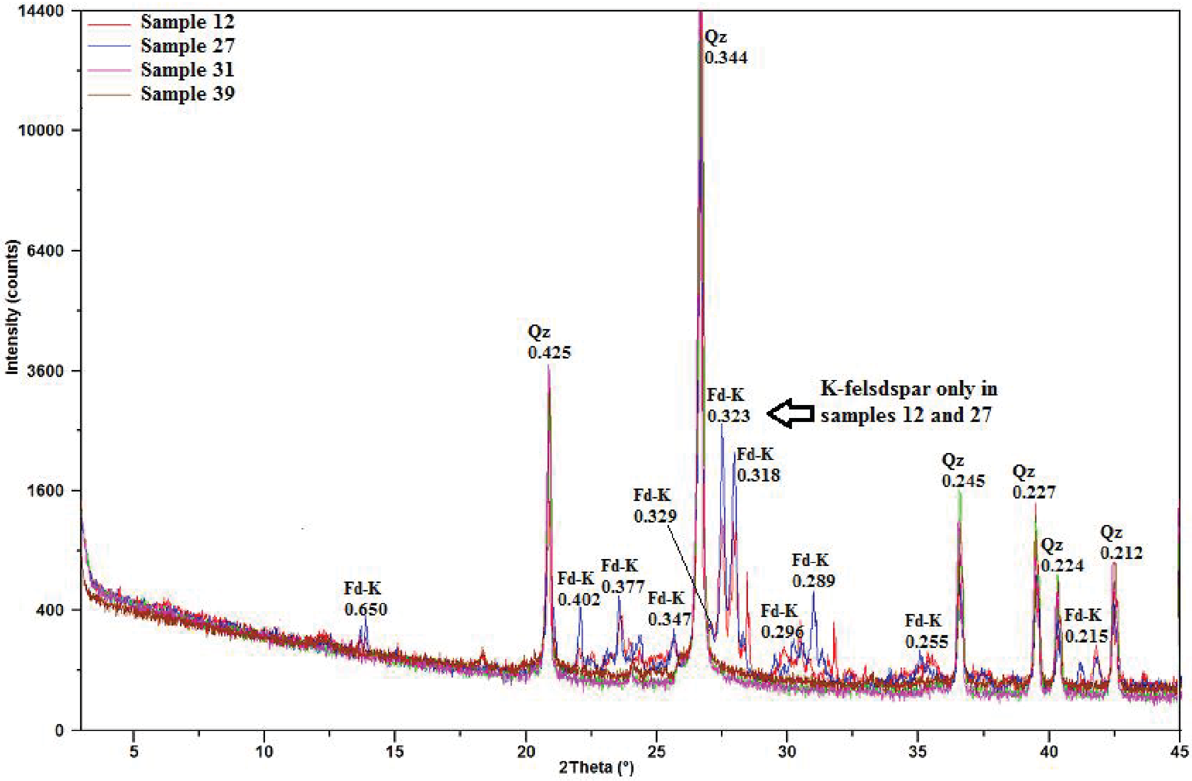


Fig. 5. X-ray diffractograms of the sand fraction from samples randomly selected in the different sites for illustrative purposes: sample 12 - represents samples from the Sea Range, at the edge of the Graciosa Road; sample 31 - represents samples from Curitiba; sample 39 - represents samples from Colombo; sample 27 - trace soil collected from the suspect's vehicle. Fd-K - K-feldspar; Qz - quartz. The interlayer distances (nm) are presented above the main peaks.

6.5.2 QUANTITATIVE RESULTS

6.5.2.1 TYRE MARKS

The muddy tyre marks on the ground surface produced during the skidding on site 4 (Fig. 4) are compatible with the tyre pattern of the suspect's vehicle (Fig. 6). The distances between the grooves of the tire coincide with the distances left at the skating site.

Fig. 6 – Pattern of tyre and marks formed through activity of skidding.

6.5.2.2 SAND FRACTION

Considering that the trace sample is comparable only with the samples collected at the edge of the Graciosa Road (Fig. 5), and all other locations can be excluded as bring the source location, the additional analyses in the X-ray diffractograms in the sand fraction of the samples collected along the Graciosa Road were tested to indicate the most probable site of transfer of the soil residue to the vehicle (site 1 to 5, Fig.1). Ten peaks were selected from the XRD of samples from Graciosa Road for calculating parameters such as height (Table 2) and area (Table 3). The higher values of height and area were obtained for P6 Qz (0.334 nm), around 26.6 °2θ (Fig. 5).

Subsequently, data were submitted to similarity analysis using a Bray-Curtis model (Fig. 7). It is possible to identify that sample 9 and 24 had grouped with the trace sample 27. The similarity of this group (9, 24 and 27) using the selected variables height and area of the peaks is 92.5%. Such a combination of samples from two distant sites (sample 9 (site 3) and 24 (site 5)) implicates that the variables analysed in the sand fraction according the X-ray diffractograms were not enough to explain which position of the Graciosa Road is compatible with the trace sample.

The elemental data showed that the samples from Graciosa Road and the questioned sample (sample 27) presented greater amounts of K (9133 to 21341 mg kg^{-1} and 16934 mg kg^{-1}, respectively) in relation to the samples from Curitiba and Colombo, which presented lower amounts of K (146 to 235 mg kg^{-1} and 80 to 167 mg kg^{-1}, respectively) (Table 4). Similar results were verified for the contents of Al, which presented high amounts for the samples from Graciosa Road (6222 to 11895 mg kg^{-1}) and for the trace sample (8511 mg kg^{-1}) in comparison to the samples from Curitiba (2056 to 3342 mg kg^{-1}) and Colombo (1795 to 3635 mg kg^{-1}). Increased contents of K an Al were justified by the predominant presence of the K-feldspars ($KAlSi_3O_8$) in soils formed under granite rocks, which was also confirmed by the common peaks of this mineral in those soils, previously identified by XRD analysis (Fig. 5).

A second grouping of samples was performed based on the content of the total elements in the sand fraction (Table 4). The data from Table 4 were also analysed using a Bray Curtis similarity analysis in order to test the grouping of the samples (Fig. 8). The group containing the questioned sample, which presents a similarity of greater than 95% was formed by the samples 27, 3 and 33. In the same way for the XRD parameters, the contents of the total elements in the sand fraction were not also suitable to grouping the samples from the Graciosa Road. This placed the sample collected in the inferior level of the Sea Range (sample 3) in the same group as the sample collected at the superior level of the Sea Range (sample 23) (Fig. 1).

Table 2. Values of height (intensity) from 10 peaks selected (P1 to P10) in the samples collected at the edge of the Graciosa Road.

Sample	P1 K-Fd (0.650)	P2 K-Fd (0.485)	P3 Qz (0.425)	P4 Qz (0.402)	P5 K-Fd (0.347)	P6 Qz (0.334)	P7 K-Fd (0.323)	P8 Qz (0.245)	P9 Qz (0.227)	P10 Qz (0.224)
1	51	63	2191	90	82	9497	988	763	638	873
2	35	91	2334	108	71	13199	384	1645	726	420
3	93	60	2740	147	109	15416	642	539	374	252
4	16	77	2475	87	81	14346	379	656	680	514
5	17	79	2147	110	96	15729	140	1820	730	660
6	40	74	2153	44	82	14915	637	1386	961	520
7	40	117	1925	74	58	17673	466	574	591	298
8	27	116	2778	30	61	11635	961	793	1091	385
9	186	32	2048	106	52	10099	3447	834	635	293
10	73	29	2340	64	128	6929	796	935	399	615
11	29	53	1403	30	48	12587	1337	861	356	278
12	65	39	1128	61	69	9426	776	707	1316	219
13	67	44	2210	224	118	14481	1078	1361	623	381
14	82	50	1619	57	95	12455	709	628	655	331

15	33	51	1467	47	45	6386	474	585	249	184
16	82	33	1277	241	78	10352	755	537	523	517
17	44	21	1190	104	86	7475	1501	866	390	243
18	31	39	761	36	51	3412	201	932	206	230
19	36	18	1788	216	102	7110	1352	390	383	254
20	186	13	561	498	217	9254	2007	363	258	200
21	25	11	1279	62	52	11395	442	405	388	307
22	45	19	1227	51	59	7457	216	448	600	241
23	74	33	2217	292	92	10156	564	858	561	271
24	84	34	2177	57	57	11779	2690	898	702	353
25	25	13	2121	68	40	14039	247	1177	735	406
26	44	26	2466	16	51	17239	94	930	841	539
27	79	8	2357	281	106	9831	1966	415	643	332

K-Fd-: K-feldspar; Qz:quartz. Numbers in parenthesis indicate the interlayered distance of the mineral (nm). Details about de sampling sites are present in Table 1.

Table 3. Values of area (intensity versus °2θ) from 10 peaks selected (P1 to P10) in the samples collected at the edge of the Graciosa Road.

Sample	**P1 K-Fd (0.650)**	**P2 K-Fd (0.485)**	**P3 Qz (0.425)**	**P4 Qz (0.402)**	**P5 K-Fd (0.347)**	**P6 Qz (0.334)**	**P7 K-Fd (0.323)**	**P8 Qz (0.245)**	**P9 Qz (0.227)**	**P10 Qz (0.224)**
1	7.2	9.1	294.2	10.1	46.6	1172.1	179.0	94.8	72.1	87.0
2	10.6	13.8	274.2	14.0	37.6	1639.7	78.2	173.9	78.7	55.5
3	7.9	7.1	319.7	18.2	47.6	1700.0	178.5	65.8	44.5	32.5
4	8.9	8.9	285.4	11.6	41.0	1614.5	87.1	85.6	72.1	60.5
5	11.1	14.8	290.4	12.0	37.3	1869.6	44.9	195.7	77.6	71.4
6	3.9	9.8	266.8	6.3	33.9	1694.9	72.7	145.5	89.7	58.6
7	6.7	20.1	348.1	10.0	53.3	2056.9	91.8	75.3	82.1	46.7
8	13.3	16.6	357.1	13.6	39.4	1426.9	165.2	106.1	113.0	45.9
9	17.2	6.0	247.2	15.0	61.2	1069.3	492.9	92.3	71.0	34.5
10	13.7	4.5	318.3	16.3	31.6	876.2	172.7	80.9	38.2	66.0
11	3.2	11.4	200.2	8.6	59.6	1307.7	172.2	77.1	37.2	34.6
12	5.9	9.2	167.2	13.4	94.4	984.5	148.7	76.7	97.3	29.2
13	13.2	2.6	237.4	22.7	64.6	1382.6	200.7	124.0	73.6	38.1
14	7.3	13.6	223.0	14.8	46.2	1341.1	156.4	62.4	67.3	36.9
15	22.1	5.4	181.7	10.0	60.5	931.8	88.3	59.3	31.0	24.0
16	8.5	4.5	192.3	21.0	63.2	1215.0	140.2	59.5	58.4	52.1
17	37.7	2.5	147.5	14.6	28.2	778.9	266.6	85.0	43.8	34.6
18	16.3	3.7	101.8	9.3	37.2	460.1	59.9	78.4	22.6	24.8
19	5.1	6.2	190.4	21.1	47.0	854.2	171.7	44.4	38.9	34.1
20	18.3	1.7	133.6	38.3	64.3	1032.4	283.7	41.1	30.9	21.6
21	6.7	23.9	184.5	23.3	24.2	1409.6	89.6	70.4	46.9	39.7
22	10.6	2.5	170.1	7.3	23.5	870.9	61.6	61.2	64.9	37.9
23	8.8	1.9	319.5	24.2	29.1	1141.2	181.2	97.3	62.0	38.7
24	6.9	2.2	296.0	26.4	76.6	1389.8	336.6	89.2	70.9	48.2

25	9.9	0.5	364.9	16.5	36.4	1764.1	50.6	146.6	98.9	48.2
26	5.1	2.4	247.2	51.9	25.8	2001.2	46.5	113.8	75.4	68.7
27	11.42	8.72	284.98	37.58	49.63	1132.86	331.73	65.17	76.36	34.77

K-Fd - K-feldspar; Qz - quartz. Numbers in parenthesis indicate the interlayered distance of the mineral (nm). Details about the sampling sites are present in Table 1.

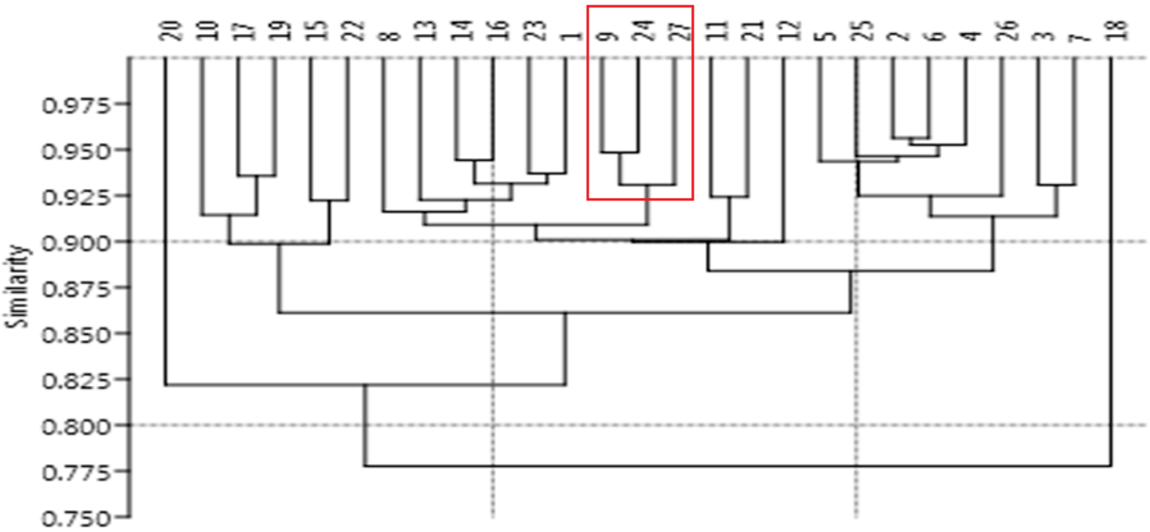


Fig. 7. Dendrogram of similarity by Bray-Curtis of the samples collected at the edge of the Graciosa Road, including the Questioned sample and considering the variables height and area from 10 peaks selected in the X-ray diffractograms of the sand fraction. Identification of the samples is in Table.

Table 4. Total content of elements (mg kg^{-1}) extracted of the sand fraction.

Sample	Al	Ba	Cu	Fe	K	Mg	P	V	Zn
1	7833	493	38	19399	12019	749	1032	53	79
2	8285	465	50	16101	10714	734	877	51	86
3	8387	525	48	14924	16896	971	1292	58	103
4	7663	488	41	16529	12818	797	943	54	90
5	7898	328	32	12539	12426	795	1811	60	99
6	8670	299	32	17088	9133	728	1450	80	66
7	8098	372	55	18432	12001	956	1743	84	84
8	7959	372	38	13577	13098	723	1864	63	65
9	8027	580	66	19149	15075	982	1500	133	122
10	9076	921	87	34749	19191	1607	1786	292	173
11	8109	712	113	65534	16619	2208	1764	494	260
12	6222	742	107	31124	15441	1470	2423	258	182
13	7476	674	76	23830	15940	1125	2142	190	134
14	8681	635	97	63663	15528	1837	1470	321	198
15	10221	757	113	55954	15461	1869	1710	350	230
16	8931	580	106	34996	14488	1185	2046	190	131

17	9890	819	101	38689	16848	2632	2471	329	215
18	8746	762	339	91380	14888	2083	2926	1042	184
19	11895	814	111	40692	16114	1991	2008	328	193
20	11718	712	99	25375	21341	1626	1877	217	144
21	7678	848	132	28359	13105	2297	1240	200	146
22	9115	816	120	37801	16694	2297	2193	292	190
23	10660	214	74	14636	16649	672	1113	46	113
24	10086	121	78	11388	15257	452	1367	31	154
25	7990	172	74	7494	11802	417	612	17	76
26	8935	139	84	8838	4327	375	640	29	57
27	8511	357	75	13032	16934	917	1229	48	108
28	2056	62	65	9115	206	774	146	42	35
29	3342	67	32	13554	210	620	235	76	42
30	2120	63	39	11410	133	549	166	50	19
31	2900	54	28	19552	144	603	180	84	23
32	2335	67	51	10859	319	447	104	43	25
33	2088	45	29	10955	233	465	127	43	23
34	3635	73	57	14919	427	641	167	58	43
35	3272	55	23	11300	383	505	152	41	23
36	2149	38	52	16193	163	1615	104	93	54
37	1795	31	51	7518	103	683	91	56	31
38	1812	31	53	11449	118	1153	80	80	40
39	2005	54	55	14486	257	1452	107	82	59

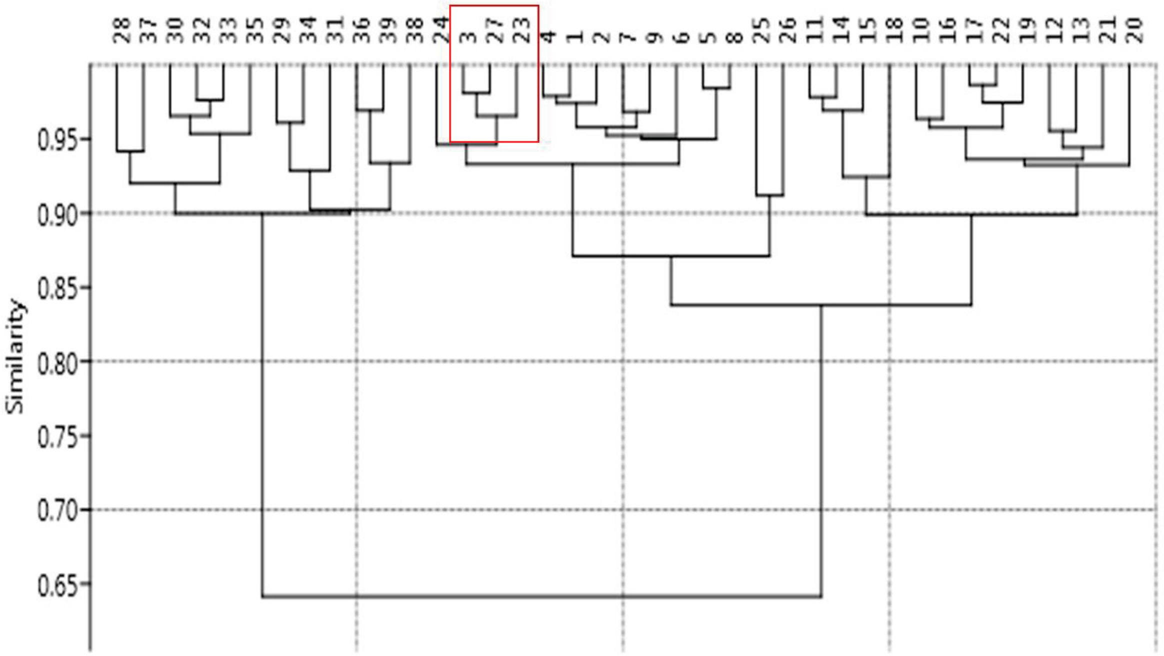


Fig. 8. Dendrogram of similarity by Bray-Curtis considering the content of total elements of the sand fraction as variables.

6.5.2.3 SILT + CLAY + ORGANIC MATTER FRACTION

The data from the sequential analyses on silt + clay + organic matter fraction are presented in Table 5 and the PCA analysis is present in Fig. 9. As expected, there was a reduction in the mass of the samples with the sequential extractions. The initial amount of sample containing silt + clay + organic matter was around 0.5 g and, before the last sequential extraction (NaOH) the amount was around 0.3 g (Table 6).

Samples 17, 19, 20, 21, 22 and 27 formed a group with high homogeneity in the dispersion of the samples obtained by the PCA 3D (Fig. 9), confirmed by the high similarity value between the sample in the dendrogram (Fig. 10). The use of multivariate statistical analysis techniques for discriminating forensic chemical data has been widely used of a number of reports (CORRÊA et al., 2017; MELO et al., 2008; PRANDEL et al., 2017; REIDY et al., 2013).

There were 8 groups formed with high similarity between samples from the same groups and lower similarity between samples from different groups (Fig. 10). In a general way, the formation and separation of groups follow the sites of sampling at the Sea Range (Graciosa Road), Curitiba and Colombo. Group 1 (similarity intragroup of 95%) - samples collected in the site 1 of the Sea Range (samples 1, 3 and 4) and samples collected in the site 2 at the Sea Range (samples 5, 6, 7 and 8 - all repetitions have located in the same group). These two sites of sampling are located at the inferior level of the Sea Range (Fig. 2), that is, the silt + clay + organic matter fraction from soils of these sites presented similar chemical characteristics; Group 2 (similarity intragroup of 96%) - samples collected in at flatter part (suitable to parking vehicles) of the victim’s body discarding site (samples 9, 10, 11 and 12 - all repetitions positioned in a same group). Site 3 (bodies’ disposal) was paved with a narrow strip until the trail ended in a very compacted soil with extremely low change of residues transferring onto the suspect’s vehicle;

Group 3 (similarity intragroup of 97%) - samples collected on the trail where the body was deposited (samples 13 and 14). The two remaining samples (15 and 16) of this group dispersed along to the dendrogram. Such behaviour was expected, since these samples were not collected in a quadrant (the pattern used for other samplings), but, in a sequence along the route and the steep trail near the victim’s body deposition site. Such differences in the altitude positions were likely responsible for the dispersion of samples 15 and 16; Group 4 (similarity intragroup of 97%) -

samples collected around 300 m away from the victim’s body discarding site (samples 17, 19, 20, 22 and 27). Samples 17, 19 and 20 belong to the corners of the quadrant of sampling (replicates) from this site. Sample 20 and 21 were collected on the position of the tyre tracks left by the vehicle’s (Fig. 4). In Group 4, there is the greatest similarity between the questioned sample with sample 20 (Fig. 10). In addition to the similarity verified between the pattern of the tyres from the suspect’s vehicle and the tracks left in the site of the skating (Fig. 6), there is corroboration regarding the side of soil impregnation at the vehicle: the soil trace on the fenders and on the rear-view mirror was located at the passenger side of the vehicle, and the tracks left through likely a car having skidded was also produced by a tyre on the right side of a vehicle. Based on the results, was hypothesized and proposed the follow sequence of events as being the most likely scenario for having taken place on the day of the victim’s disappearance: the suspect first looking for a site to dispose of the victim’s body, then he stopped before in the site 4 (he was driving from the Federal Road BR 116 to Morretes). Prior to the occasion of the disappearance of the victim there was a period of rainfall and site 4 is not paved (grass-covered). The suspect’s vehicle started to skid at that site, where there was an opportunity for adherence of the soil residue (sample 27). The suspect fearing for loss of traction of the vehicle, left site 4 and 300 m ahead at the Graciosa Road he found suitable conditions to park his vehicle car and discarding the victim’s body (site 3); Group 5 (similarity intragroup of 97%) - samples collected at the higher level in the Sea Range, near to the junction with the Federal Road BR 116 (samples 23, 24 and 26); Group 6 (similarity intragroup of 97%) - samples collected in the Santa Cândida neighbourhood from Curitiba (samples 28, 30 and 31); Group 7 (similarity intragroup of 97%) - samples collected in Guarani neighbourhood from Colombo (samples 33, 34 and 35); Group 8 (similarity intragroup of 97%) - samples collected in Guaraituba neighbourhood from Colombo (samples 36, 37 and 38).

The protocol of chemical analyses used in the silt + clay + organic matter fraction was efficient in separating the group of samples collected at the different sites, formed under the same parent material and soil type. There was a clear separation of the four Groups from the Sea Range (formed on granite-gneiss) and of the two Groups from Colombo (formed on limestone).

Table 5. Elemental concentrations (mg kg^{-1}) extracted by Na hypochlorite (NaClO), ammonium oxalate (AO), citrate-bicarbonate-dithionite (CBD) and Na hydroxide (NaOH).

Sample	NaClO			AO						CBD						NaOH		
	Ca	**Mg**	**P**	**Al**	**Cu**	**Fe**	**Mn**	**P**	**Si**	**Al**	**Fe**	**Mn**	**P**	**Si**	**Zn**	**Al**	**P**	**Si**
1	175	59	46	3361	23	3709	225	321	401	3549	17975	123	352	995	33	20047	145	9511
2	289	128	47	3786	29	3603	211	670	471	3623	18952	122	452	993	51	24063	179	9748
3	232	55	47	3059	44	5435	253	529	332	2763	15762	130	362	896	48	16116	148	8772
4	322	73	64	2980	24	5025	254	388	362	2791	14759	146	341	936	41	17580	158	8499
5	250	89	58	4507	29	6517	165	596	380	4104	17295	102	434	968	35	21127	214	9390
6	289	87	55	4363	23	6155	149	456	358	4779	19213	110	430	923	38	24139	232	10106
7	325	76	49	4037	50	6047	158	504	348	4497	18768	108	448	958	46	22307	217	8555
8	315	67	48	3820	34	6157	166	445	359	3836	17488	114	399	888	42	20105	220	9766
9	75	22	56	3229	15	4260	182	541	459	2862	20131	91	751	1187	20	13021	249	11144
10	58	17	42	3103	18	4206	200	534	431	3536	23479	121	975	1102	22	15341	289	10573
11	43	9	43	2869	14	3393	226	535	478	3854	25964	145	1167	1244	25	16788	302	9690
12	48	11	36	3599	21	3325	321	408	494	3981	25647	213	966	1218	25	17101	286	10243
13	286	117	61	4017	24	4478	347	838	620	3212	22506	211	876	1322	30	12666	251	10248
14	297	121	36	3671	79	5081	399	542	586	3879	26140	234	944	1387	40	14543	257	10613
15	449	234	54	3622	30	4722	349	473	611	3602	25689	224	934	1316	40	14494	272	11808
16	159	112	63	3643	45	4877	332	748	478	3292	24024	208	869	1032	31	13695	259	9580
17	340	116	62	2862	50	6661	402	461	438	1629	15782	231	322	1345	34	6189	132	10685
18	279	85	49	4181	42	6252	541	460	528	2546	21599	353	630	1534	24	10202	170	11700
19	246	107	70	4186	23	6800	630	652	538	1960	16098	206	467	1433	30	7388	192	11600
20	374	135	70	3143	26	5846	432	604	519	1308	11303	133	269	1226	22	5489	132	9792
21	285	105	67	3005	35	7343	367	487	496	1575	14015	215	325	1358	28	6002	137	11013
22	289	105	53	3234	22	6175	691	505	581	1511	15151	196	336	1446	27	4702	114	9477
23	340	195	43	2361	40	2496	269	230	385	1956	14094	136	228	1088	52	9959	107	8869
24	231	157	42	2163	21	3062	256	173	359	1739	12824	126	201	1005	66	9818	81	8523
25	277	125	50	2768	43	1756	437	251	345	1318	11441	144	179	964	42	7324	86	7924

26	216	120	41	2531	27	1633	197	133	329	2335	14427	128	209	779	29	12958	98	8378
27	277	140	70	1889	40	3488	216	301	569	1079	9199	140	183	1021	41	3101	53	4968
28	425	183	29	6244	13	6768	220	180	508	3997	15467	65	190	1147	14	8483	127	16084
29	206	99	51	6369	21	6881	128	158	580	4459	16028	48	194	1297	16	8831	124	16975
30	414	164	30	6015	15	6972	202	169	490	3820	15165	53	176	1114	13	8404	126	17201
31	329	137	26	7023	16	7675	261	162	577	4081	16320	62	182	1263	18	9001	130	17656
32	447	234	42	4375	19	3499	143	152	534	4068	15705	62	216	1059	12	9754	122	13248
33	584	323	39	4231	16	3632	188	160	535	4121	15431	72	216	1004	12	8981	120	11964
34	717	371	38	4365	27	3995	221	162	616	3992	16654	95	223	1009	14	9666	140	12873
35	566	289	35	4827	18	3862	218	153	683	4149	16371	97	221	1141	13	9880	134	13404
36	227	118	21	5457	19	3937	79	82	454	6257	20667	37	248	837	10	12950	134	9840
37	254	145	22	5976	14	4276	69	81	476	6683	22270	38	256	878	10	13289	142	10703
38	300	143	25	5482	16	3748	66	83	463	6750	21817	38	278	1051	11	13107	143	10631
39	416	228	25	5329	16	3792	86	82	550	6507	22069	41	268	1009	11	11731	129	9128

Table 6. Initial mass (g) defined by the amount of trace sample from the vehicle and by the reduction of mass in each sequential analysis.

Sample	NaClO	AO	CBD	NaOH
1	0.509	0.431	0.387	0.297
2	0.506	0.431	0.396	0.292
3	0.505	0.434	0.391	0.296
4	0.507	0.431	0.401	0.296
5	0.508	0.421	0.381	0.294
6	0.506	0.421	0.369	0.294
7	0.502	0.417	0.379	0.292
8	0.509	0.421	0.384	0.296
9	0.502	0.425	0.403	0.299
10	0.508	0.443	0.411	0.289
11	0.508	0.447	0.421	0.289
12	0.507	0.439	0.407	0.293
13	0.503	0.415	0.387	0.296
14	0.507	0.421	0.388	0.297
15	0.508	0.411	0.381	0.293
16	0.504	0.412	0.381	0.295
17	0.504	0.424	0.396	0.294
18	0.506	0.431	0.399	0.288
19	0.509	0.413	0.381	0.295
20	0.505	0.425	0.398	0.295
21	0.507	0.427	0.393	0.293
22	0.502	0.417	0.386	0.298
23	0.509	0.42	0.394	0.302
24	0.501	0.428	0.402	0.299
25	0.503	0.434	0.407	0.298
26	0.501	0.431	0.407	0.298
27	0.473	0.419	0.397	0.293
28	0.503	0.416	0.371	0.301
29	0.501	0.406	0.362	0.295
30	0.507	0.414	0.369	0.301
31	0.504	0.396	0.341	0.296
32	0.506	0.437	0.398	0.294
33	0.503	0.426	0.395	0.294
34	0.501	0.424	0.384	0.296
35	0.504	0.424	0.381	0.296
36	0.501	0.439	0.398	0.29
37	0.508	0.439	0.395	0.291
38	0.508	0.439	0.393	0.298
39	0.502	0.432	0.387	0.293

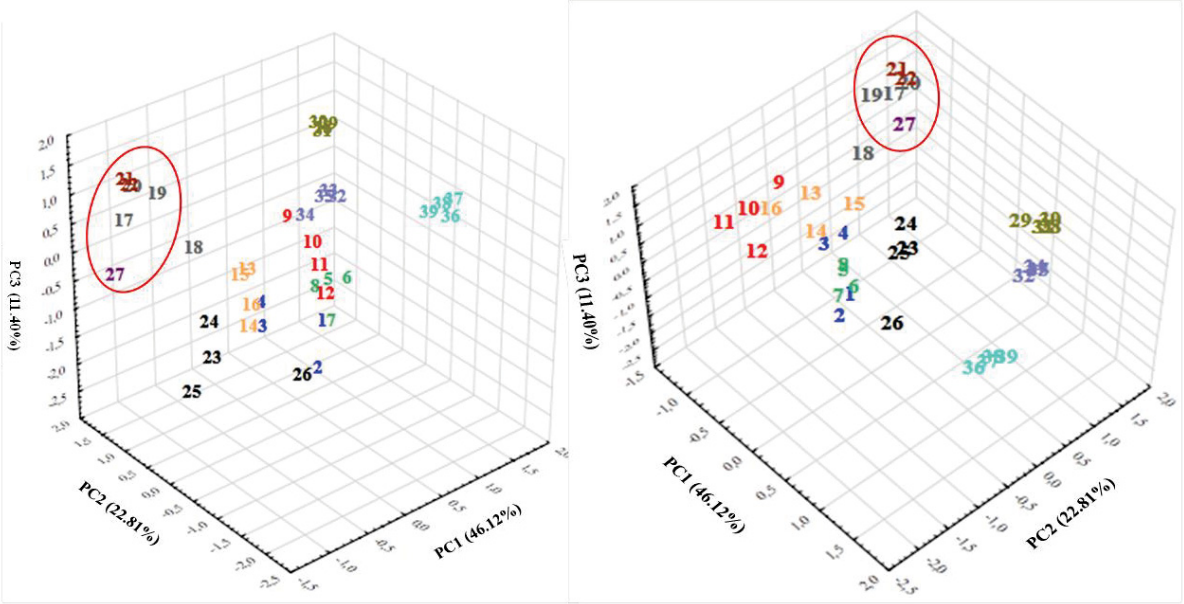


Fig. 9. Scores from the first three principal components of the samples analysed, considering the relative contribution of the total elements extracted in the sequential analyses by NaClO, ammonium oxalate, citrate-bicarbonate-dithionite and NaOH in the silt + clay + organic matter fraction.

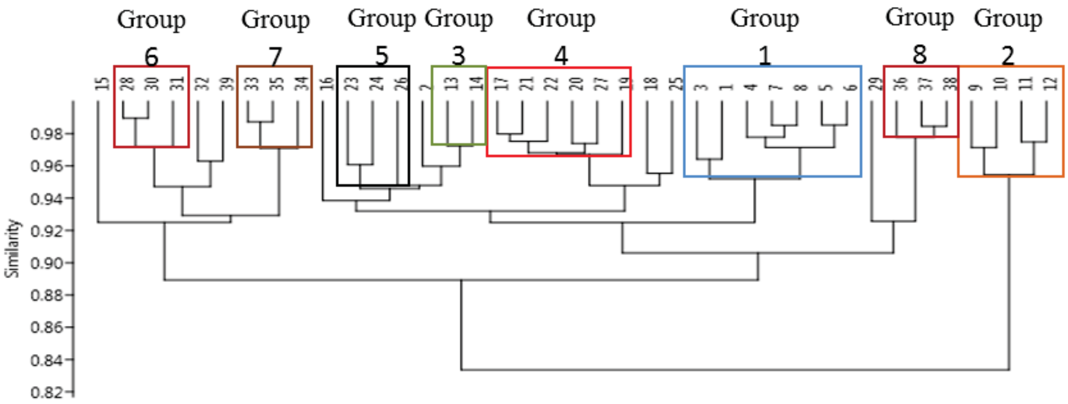


Fig. 10. Dendrogram of the results of the sample analysis, considering the relative contribution of the total elements extracted in the sequential analyses by Na hypochlorite, ammonium oxalate, citrate-bicarbonate-dithionite and Na hydroxide in the silt + clay + organic matter fraction.

6.6 CONCLUSIONS

A great number of quantitative variables (22) were obtained from a small trace amount of silt + clay + organic matter sample (0.5 g). A sequential chemical analysis is recommended when there is a limited amount of soil vestige available.

The data from the analysis of the finer fraction of the soil (silt + clay + organic matter) separated into clear groups of soils formed from the same parent material but which were collected from different sites. Data from the chemical and mineralogical analysis of the sand fraction showed that the soil trace could be excluded as having originated from Curitiba (claystone) and Colombo (limestone) city areas.

All the results of the soil analyses confirm the hypothesis that the soil recovered from the suspect’s vehicle, apprehended by the Civil Police of Colombo (State of Paraná), had originated from a common source as the soil which was located at the edge of the Graciosa Road (PR410), which was located adjacent to the site where the victim’s body was discarded. Through the examinations and multiple analyses of the soil trace recovered from the suspects’ vehicle, in a context of other relevant locations, including the alibi location in the suspect’s home city, it was possible to produce physical evidence of likely contact on the suspect vehicle with material near to the crime scene. This supported the proposition that the vehicle had been positioned at the road way adjacent to where the victim’s body was found.

6.7 REFERENCES

Cheshire, K.; Morgan, R. M.; Holmes, J. The potential for geochemical discrimination of single- and mixed-source soil samples from close proximity urban parkland locations. *Australian Journal of Forensic Sciences*, v. 49, n. 2, p. 161–174, 2017.

Concheri, G. et al. Chemical elemental distribution and soil DNA fingerprints provide the critical evidence in murder case investigation. *PLoS ONE,* v. 6, n. 6, p. 4–8, 2011.

Corrêa, R. S. et al. Soil forensics: How far can soil clay analysis distinguish between soil vestiges? *Science and Justice*, n. March, p. 0–1, 2017.

Dawson, L. A. Soil organic characterisation in forensic case work. *Journal of International Geoscience*, v. 40, n. 2, p. 017–018, 2017.

Dawson, L. A.; Hillier, S. Measurement of soil characteristics for forensic applications. *Surface and Interface Analysis*, v. 42, n. 5, p. 363–377, 2010.

Dawson, L. A.; Mayes, R. W. *Criminal and Environmental Soil Forensics:* Soil as Physical Evidence in Forensic Investigations. Third Edit ed. [s.l.] Elsevier Ltd, 2014.

Duarte, A. P. et al. European Journal of Soil Biology Changes in the forms of lead and manganese in soils by passage through the gut of the tropical endogeic earthworm (*Pontoscolex corethrurus). European Journal of Soil Biology*, v. 53, p. 32–39, 2012.

Fitzpatrick, R.; Raven, M.; Self, P. Clay mineralogy as significant evidence in 4 murder investigations involving a wide range of earth materials from Perth , Adelaide , Melbourne and Sydney. *Australian Clay Minerals Society Conference*, n. February, p. 23–26, 2014.

Fitzpatrick, R. W.; Raven, M. D. Centre for Australian Forensic Soil Science Guidelines for Conducting Criminal and Environmental Soil Forensic Investigations : Version 7 . 0. v. 76, 2012.

Hammer, Ø.; Harper, D. A. T.; Ryan, P. D. Past: *Paleontological Statistics* Software Package for Education and Data AnalysisPalaeontologia Electronica, , 2001.

Mcculloch, G. et al. The discrimination of geoforensic trace material from close proximity locations by organic pro fi ling using HPLC and plant wax marker analysis by GC. *Forensic Science International*, v. 288, p. 310–326, 2018.

Mckeague, J. A. Manual on soil sampling and methods of analysis. Ottowa: *Canadian Society of Soil Science*, 1978.

Mehra, O. P.; Jackson, M. L. Iron Oxide Removal from Soils and Clays by a Dithionite-Citrate System Buffered with Sodium Bicarbonate. *Clays and Clay Minerals*, v. 7, n. 1, p. 317–327, 1960.

Melo, V. F. et al. Chemical , physical and mineralogical characterization of soils from the Curitiba Metropolitan Region for forensic purpose. *Forensic Science International,* v. 179, p. 123–134, 2008.

Melo, V. F. F. et al. Potassium and magnesium in clay minerals of some Brazilian soils as indicated by a sequential extraction procedure. Commun. *Soil Sci. Plant. Anal*, v. 33, n. 13&14, p. 2203–2225, 2002.

Murray, K. R. et al. Interpreting the transfer method of soil evidence on clothing by identifying trace soil patterns. *Forensic Science International2*, v. 258, n. 88–

100, 2016.

Murray, K. R. et al. Patterns produced when soil is transferred to bras by placing and dragging actions : The application of digital photography and image processing to support visible observations. *Forensic Science International*, v. 276, p. 24–40, 2017.

Pirrie, D.; Dawson, L.; Graham, G. Predictive geolocation: Forensic soil analysis for provenance determination. *Episodes*, v. 40, n. 2, p. 141–147, 2017.

Poggere, G. C. et al. Characterization of products of the early stages of pedogenesis in ornithogenic soil from Maritime Antarctica. *European Journal of Soil Science*, v. 67, n. 1, p. 70–78, 2016.

Prandel, L. V. et al. X-ray Diffraction and Rietveld Refinement in Deferrified Clays for Forensic Science. *Journal of Forensic Sciences*, p. 1–7, 2017.

Reidy, L. et al. Elemental fingerprinting of soils using ICP-MS and multivariate statistics: A study for and by forensic chemistry majors. *Forensic Science International*, v. 233, n. 1–3, 2013.

Simas, F. N. B. et al. Clay-sized minerals in permafrost-affected soils (Cryosols) from King George Island, Antarctica. *Clays and Clay Minerals*, v. 54, n. 6, p. 721–736, 2006.

Statsoft, I. Statistica (data analysis software system), 2011. Disponível em: <www.statsoft.com.>

Stoney, D. A.; Bowen, A. M.; Stoney, P. L. Loss and replacement of small particles on the contact surfaces of footwear during successive exposures. *Forensic Science International,* v. 269, p. 78–88, 2016.

Stoppe, N.; Amelung, W.; Horn, R. Extracción química de oxi(hidrox)óxidos sedimentario utilizando oxalato de amonio y ditionito sódico revisado - una explicación de los procesos en sedimentos costeros. *Agro Sur,* v. 43, n. 2, p. 11–17, 2015.

Tighe, M. et al. Georeferenced soil provenancing with digital signatures. *Scientific Reports*, v. 8, n. 1, p. 1–9, 2018.

Usepa, U. S. E. P. A. Method 3052 – Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices. 1997.

Woods, B. et al. Soil examination for a forensic trace evidence laboratory - Part 2: Elemental analysis. *Forensic Science International*, v. 245, p. 195–201, 2014.

7 CHAPTER VI: VALIDATION OF CHEMICAL SEQUENTIAL EXTRACTIONS IN THE USE OF SOIL VESTIGES AS EVIDENCE IN CRIMINAL INVESTIGATIONS: A MURDER CASE[6]

7.1 RESUMO

O uso de solos em investigações forenses pode ser uma valiosa fonte de informação. Um caso de homicídio ocorreu no Estado do Paraná, no sul do Brasil, e foi concluído por meio dos tribunais. Uma pá com vestígios de solo aderidos foi apreendida na casa do assassino confesso usada para enterrar partes do corpo da vítima. O torso da vítima foi encontrado enterrado entre 0 a 0,40 m de profundidade do solo em uma área agrícola e as pernas da vítima foram encontradas a 10,6 km do local onde o tronco foi encontrado, também enterrado entre 0 a 0,40 m de profundidade, em uma área sob vegetação nativa (floresta). Essas condições foram consideradas ideais para testar uma análise química sequencial utilizada no Brasil: primeiramente - 0,2 mol L^{-1} de oxalato de amônio a pH 3,0; em segundo, ditionito-citrato-bicarbonato de sódio e, em terceiro lugar, 0,5 mol L^{-1} de NaOH. Foi possível elaborar os eventos ocorridos e a validação química de extrações sequenciais foi realizada com sucesso. O solo do horizonte A do local do enterro do tronco apresentou-se muito similar ao volume de solo recuperado da pá. O local onde as pernas estavam enterradas contribuiu para uma baixa aderência do vestígio de solo na pá. O longo período de armazenamento da pá (três anos) não reduziu o poder da análise química sequencial para rastrear na amostra vestígios, mesmo com a interferência externa das atividades agrícolas. Análises químicas sequenciais devem ser priorizadas em vez de extrações isoladas únicas.

Palavras-chave: ciência forense, evidência do solo, caso fechado, rochas graníticas, óxidos de ferro.

7.2 ABSTRACT

The use of soils in forensic investigations can be a valuable source of information. A case of homicide occurred in the State of Paraná, south Brazil and had been concluded through the courts. A spade with soil traces adhering was seized at the confessed killer's house used to bury parts of the victim's body. The torso of the victim was found buried into 0-0.40 m depth of soil in an agricultural area and the victim’s legs were found 10.6 km away from the place where the torso was found, also buried at 0-0.40 m depth, in an area under native vegetation (forest). These conditions were considered ideal to test a sequential chemical analysis used in Brazil: firstly- 0.2 mol L^{-1} pH 3.0 ammonium oxalate; secondly- dithionite-citrate-bicarbonate and thirdly- 0.5 mol L^{-1} NaOH. It was possible to work out the events of what happened and the chemical sequential extractions validation was performed successfully. The A horizon soil from the burial location of the torso was found to be very similar to the bulk of the soil which was recovered from the spade. The location where the legs were buried contributed to a low adherence of soil trace on the spade.

---

[6] Submitted on Criminology & Criminal Justice.

The long period of time that of spade had been stored (three years) did not reduce the power of sequential chemical analysis to track back the trace sample, even with the external interference of agricultural activities. Sequential chemical analyses should be prioritised instead of single isolated extractions.

Keywords: forensic science, soil evidence, closed case, granitic rocks, iron oxides.

7.3 INTRODUCTION

Several types of evidence have been extensively used to elucidate what has happened at crime scenes, such as DNA, hair, paint, glass, and plant and soil traces. Although it has been only recently more deeply investigated (Concheri et al., 2011; Dawson, 2017; Reidy et al., 2013), the use of soil to assist criminal investigations dates back around 150 years ago (Fitzpatrick et al., 2014). The use of soils in forensic investigations can be a valuable source of information because given their variable nature, it is possible to predict the origin of soil traces adherent to a wide range of objects and people, including shoes, tires, clothes, hair, etc (Farrugia et al., 2012; Finley et al., 2015; Woods et al., 2016; Demanèche et al., 2017). Soil variability derives from its location in the landscape, geological parent material, predominant local climate, anthropic action, living organisms in the soil and time (Fontana et al., 2017; Ramos et al., 2017). Each of these factors in turn defines the intensity of weathering, which will result in unique soil types. Once adhered to the different types of surface, traceability can be created upon the questioned sample.

Chemical extractions have been frequently used in forensic studies: total extraction and analysis by ICP-OES/AES/MS (Pye et al., 2007; Concheri et al., 2011; Reidy et al., 2013); organic extractions (Carvalho et al., 2013; Dawson et al., 2017; Melo et al., 2018). All these chemical methods were applied in an isolated matrix of the soil. The advantage of a chemical sequential analysis is to dissolve mineral phases in a pre-established sequence, where the separation and the characterization of each mineral phase improves the quality of the soil analysis (Melo et al., 2002).

Some work in Brazil has concentrated on sequential chemical extraction of amorphous materials and crystalline minerals from the clay fraction in forensic cases (Melo et al., 2008; Corrêa et al., 2017; Prandel et al., 2017; Melo et al., 2019; Testoni et al., 2019a; Testoni et al., 2019b). The sequence of analyses adopted mixed methodologies for extraction of short range order materials (SOR) and crystalline Fe oxides (Simas et al., 2006; Mendonça et al., 2013; Poggere et al., 2016, with modifications): i) 0.2 mol L^{-1} pH 3.0 ammonium oxalate (AO) - amorphous Fe and Al oxides, allophane and imogolite; ii) sodium dithionite-citrate-bicarbonate (DCB) - crystalline Fe oxides (hematite and goethite); iii) 0.5 mol L^{-1} NaOH - Al-hydroxide, Al-O-Si layers and Si-O (opaline silica) resistant to previous ammonium oxalate extraction. The main modification used in the present study in relation to the method proposed by Poggere et al. (2016) was the inclusion of the extraction step with DCB. Sodium dithionite, as a strong reducing agent, solubilizes hematite and goethite from the clay fraction of the soil (Mehra & Jackson, 1960).

There is a distribution of the elements in different phases and forms in the soil (Correa et al., 2008; Pereira et al., 2010; Medeiros et al., 2013). Elements in the soluble fraction forming outer-sphere complexes with the negative and positive charges of mineral and organic colloids should not be used in forensic science (Melo et al., 2008), as they are the most available and mobile forms of the elements. Local variations in soil management (such as fertilization and liming) and heavy rainfall change the chemical characteristics of the soil. If there is one of these management interferences on the soil surface after the suspect's presence at the crime scene, the trace sample may lose its comparability and hence traceability. To avoid these transitory variations, Melo et al. (2008), Prandel et al (2017), Melo et al. (2019), Testoni et al. (2019a) and Testoni et al. (2019b) used more aggressive extraction methods to analyze different mineral phases. The extraction of SOR by AO and NaOH allows access to the elements with intermediate mobility and solubility in relation to soluble and exchangeable and crystalline forms (Poggere et al., 2016; Melo et al., 2016; Testoni et al., 2019b). This perspective is essential to discriminate soils which are highly similar pedologically (i.e. the same soil class and parent material) but separated by short distances (i.e. tens to hundreds of meters). Under a situation of great similarity, the best form to ascertain local chemical changes is the assessing of low crystallinity materials (Oliveira et al., 2014). The inclusion of DCB (crystalline minerals) in sequential extraction was due to the great importance of pedogenetic Fe oxides (hematite and goethite) in the differentiation of pedogenetic and geochemical environments (Muggler, 1998; Inda Jr, 2002). It is important to emphasize that the potential of soil sample discrimination with these extractions is greatly increased with the use of the sequential protocol for improve the separation of the soil mineral phases.

Melo et al. (2008) proposed significant changes in traditional analytical methods to adapt the reduced amount of soil traces. These authors worked with only 1.0 g of soil and used only the silt+clay fraction in the sequential analyses. The sand fraction compromises the homogenization of a small amount of sample (1.0 g) and has mainly primary minerals (quartz), which present low geochemical response to environmental variations (Melo et al., 2008). From this first work of methodological definitions, other authors have successfully used sequential chemical extractions in simulated forensic cases (Corrêa et al., 2017; Prandel et al., 2017; Melo et al., 2019; Testoni et al., 2019a; Testoni et al., 2019b). Recently, there has been the possibility of applying the sequential analysis protocol to a real case of murder (feminicide) in the State of Paraná, Brazil (Melo et al., 2019). Sequential mineralogical and chemical analyses as listed above were used to assess a limited amount of soil traces (0.5 g) recovered forensically from a suspect's vehicle (adhering to the outside rear view mirror and to the left front fender) were applied. All results suggest that the suspect's vehicle could have been in contact with the edge of the Graciosa Road (State of Paraná, Brazil), close to the place where the victim's body was located. Soil was the only objective evidence of a link to the crime and the suspect will subsequently go to the popular jury for the feminicide. However, there was no confession of the suspect in this feminicide case.

Another closed case of homicide occurred in the State of Paraná, south Brazil. Nevertheless, in this case the suspect confessed to the police. A spade with soil traces was seized at the suspect’s house. Under this context, as a first hypothesis, the application of the protocol of sequential analysis to soil samples collected from the spade will necessarily place the killer at the crime scene (validation of the method). The crime occurred in 2016 and the spade with adherent soil traces was made available for analyses by the Scientific Police in 2019. Provided that soil samples are dry (room temperature), long storage time of soil traces will not compromise the quality of forensic analyses. Part of the victim's body was buried in an agricultural area, therefore subject to the application of fertilizers and lime in the soil over the period of burial. It is hypothesised that the management of agricultural soils will not affect the soil solid phase composition and the quality of sequential analyses.

7.4 MATERIAL AND METHODS

7.4.1 CASE BACKGROUND AND SOIL SAMPLING

In 2016, a man was murdered, his body was quartered, and parts of his body were buried in a rural area at Colombo municipality, Curitiba Metropolitan Region (CMR), State of Paraná, Brazil (Figure 1a). The victim's ex-wife confessed to the crime and indicated where the parts of the body were buried, being subsequently arrested. The torso was found buried in 0-0.40 m of soil depth in an agricultural area and the legs were found 10.6 km away from the place where the torso was found, also buried 0-0.40 m, in an area under native vegetation (forest).

The CMR is composed by three main cities (Figure 1a): Curitiba (capital of the state of Paraná), Araucária (location where the parts of the body were buried), Colombo and São José dos Pinhais. The predominant soil class in these four cities is an Inceptsol (Soil Survey Staff, 2010), with variation of parent materials: Curitiba municipality - claystone; Araucária municipality - granite/gneiss, Colombo municipality - limestone, São José dos Pinhais municipality - granite/gneiss. Therefore, soil sampling in Curitiba and Colombo municipalites aimed to validate the discriminatory power of the sequential analyses of soil samples collected from different parent materials (Melo et al., 2008; Prandel et al., 2017, Melo et al., 2019; Testoni et al., 2019b). The inclusion of soils from São José dos Pinhais municipality aimed to test sequential analysis to discriminate soils from the same pedological unit (Inceptsol) and parent material (granite/gneiss) in relation of crime scene. From this scenario, the design of sampling locations is shown in Figure 1.

Location 1 – (Araucária municipality) where the torso was buried (coordinates -25°33'36''S, -49°28'31''W);
Location 2 – (Araucária municipality) references samples of the torso, placed 1.8 km near from the location 1 (coordinates -25°33'43"S, -49°28'12"W). Soils from both locations 1 and 2 are Inceptsols, formed by granite/gneiss and are under the same land use (agricultural area). Even with the great similarity and proximity among soils, the sequential analysis should be able to discriminate the locations 1 and 2;
Location 3 – (Araucária municipality) where the legs were buried, placed 8.8 km from the location 2 (coordinates -25°31'28''S, -49°25'39''W);

Location 4 – (Araucária municipality) reference samples of the legs, placed 0.95 km near from the location 3 (coordinates -25°31’46”S, -49°25’42”W). Locations 3 and 4 – Inceptsols from granite/gneiss under native vegetation (forest).

Location 5 – (Curitiba municipality) reference samples formed from claystone, distant by 43.5 km from the location 4 (coordinates - 25°22’20.55’’S, - 49°11’23.97’’W);

Location 6 – (Colombo municipality) reference samples, formed from limestone, distant by 25.5 km from the location 5 (coordinates - 25°33’37.56’’S, - 49°12’30.54’’W);

Location 7 – (São José dos Pinhais municipality) reference samples formed from granite/gneiss, distant by 15.5 km from the location 6, and by 20.8 km from the location 1 (coordinates -25°32’45.02’’S, -49°19’52.15’’W).

The sampling procedure was carried out using a Standard Operating Procedure (SOP) for forensic soils developed in Brazil (Testoni et al., 2019). At each location 4 replicates of soil samples were sampled from the corners of a quadrant (distant by 1.5 m from each other), in A and B horizons (0-0.1 cm and 0.1-0.4 cm, respectively) (Figure 2). The separation in two horizons aimed to simulate the crime conditions, where the spade used to bury the parts of the body had contact with both A and B horizons (0-0.4 m). The sampling separation allowed for an assessment to be made regarding which horizon the spade was more in contact with. Sampling equipment was carefully sterilized between sampling positions to avoid any potential cross contamination between positions.

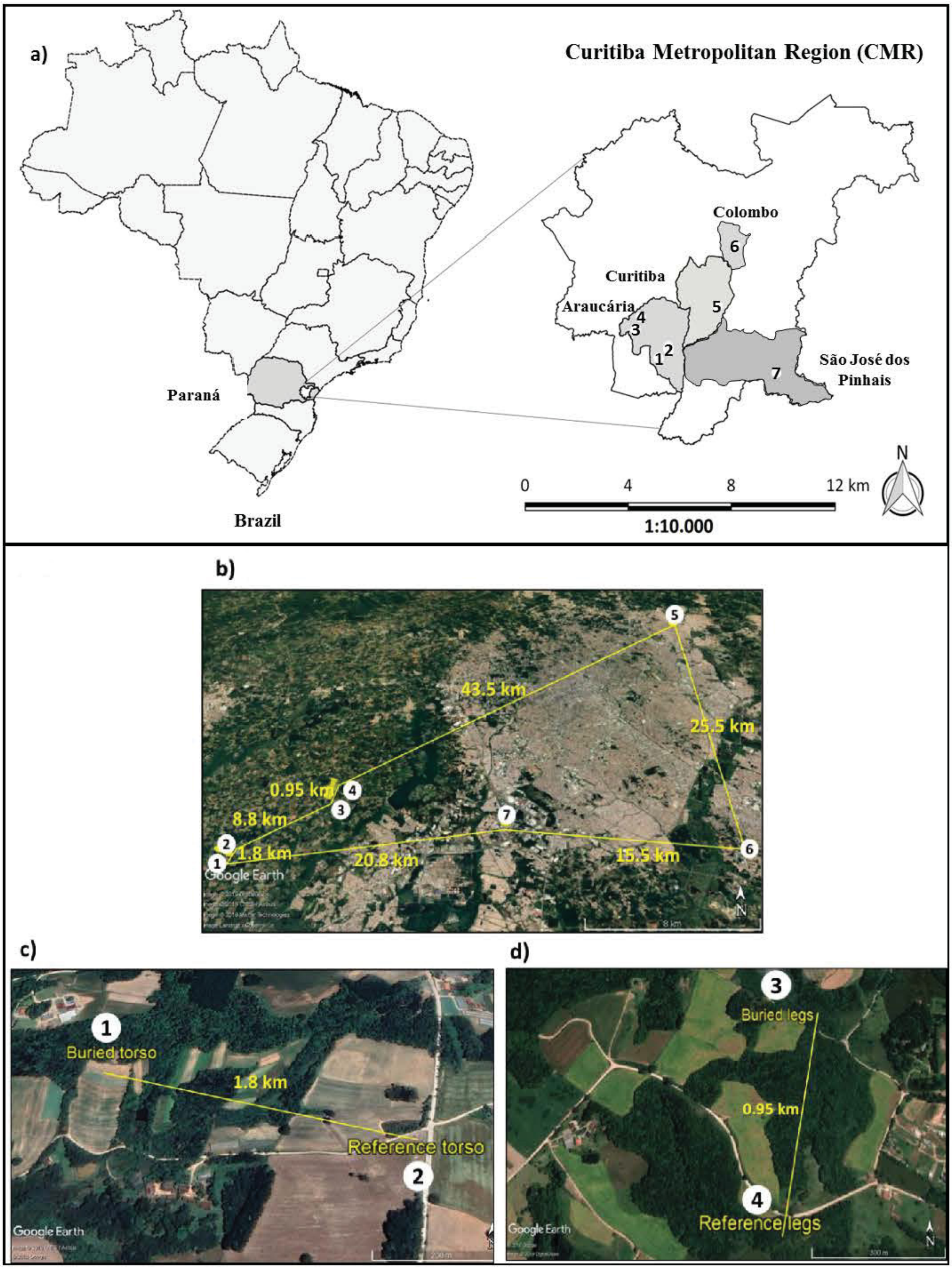


Figure 1. a) Map of Brazil showing State of Paraná and sites where the samples were collected (Araucária municipality, Colombo municipality, Curitiba municipality and São José dos Pinhais municipality); b) sampling locations and their relative distances: 1) Location 1 (torso samples - Araucária municipality); 2) Location 2 (reference torso samples - Araucária municipality); 3) Location 3 (legs samples -

Araucária municipality); 4) Location 4 (reference legs samples - Araucária municipality); 5) Location 5 (claystone samples - Curitiba municipality); 6) Location 6 (limestone samples - Colombo municipality); 7) Location 7 (granite samples - São José dos Pinhais municipality); c) position of the location 1 in relation to the location 2; d) position of the location 3 in relation to the location 4.

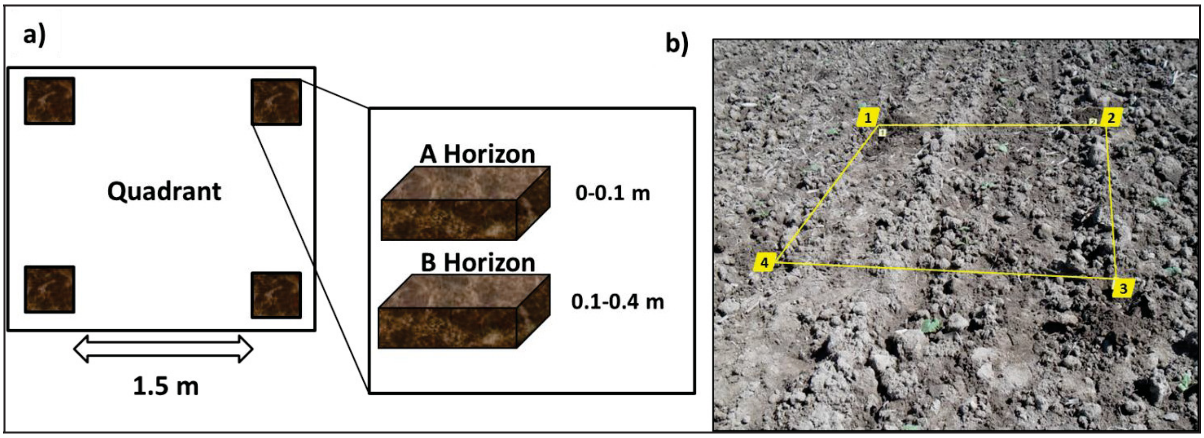


Figure 2. Sampling procedure was accomplished in accordance with a Standard Operating Procedure (SOP) for forensic soils in Brazil (Testoni et al., 2019a): a) at each location 4 replicates of soil samples were sampled from the corners of a quadrant (distant by 1.5 m from each other), in A and B horizons (0-0.1 cm and 0.1-0.4 cm, respectively); b) example of soil sampling in an agricultural area (locations 1 and 2).

Three years after the case was closed due to the confession and arrest of the murderer, the Scientific Police of Paraná State made available the spade used to bury parts of the body for analysis of the soil traces. Samples adhering to the spade were collected both from the front and from the rear of the tool, at several positions (Figure 3). Samples from both front and rear of the spade showed great homogeneity of colour. Six subsamples (replicates) were collected from the spade. The smallest subsample of the spade determined the amount of soil sample for all collections (3 g).

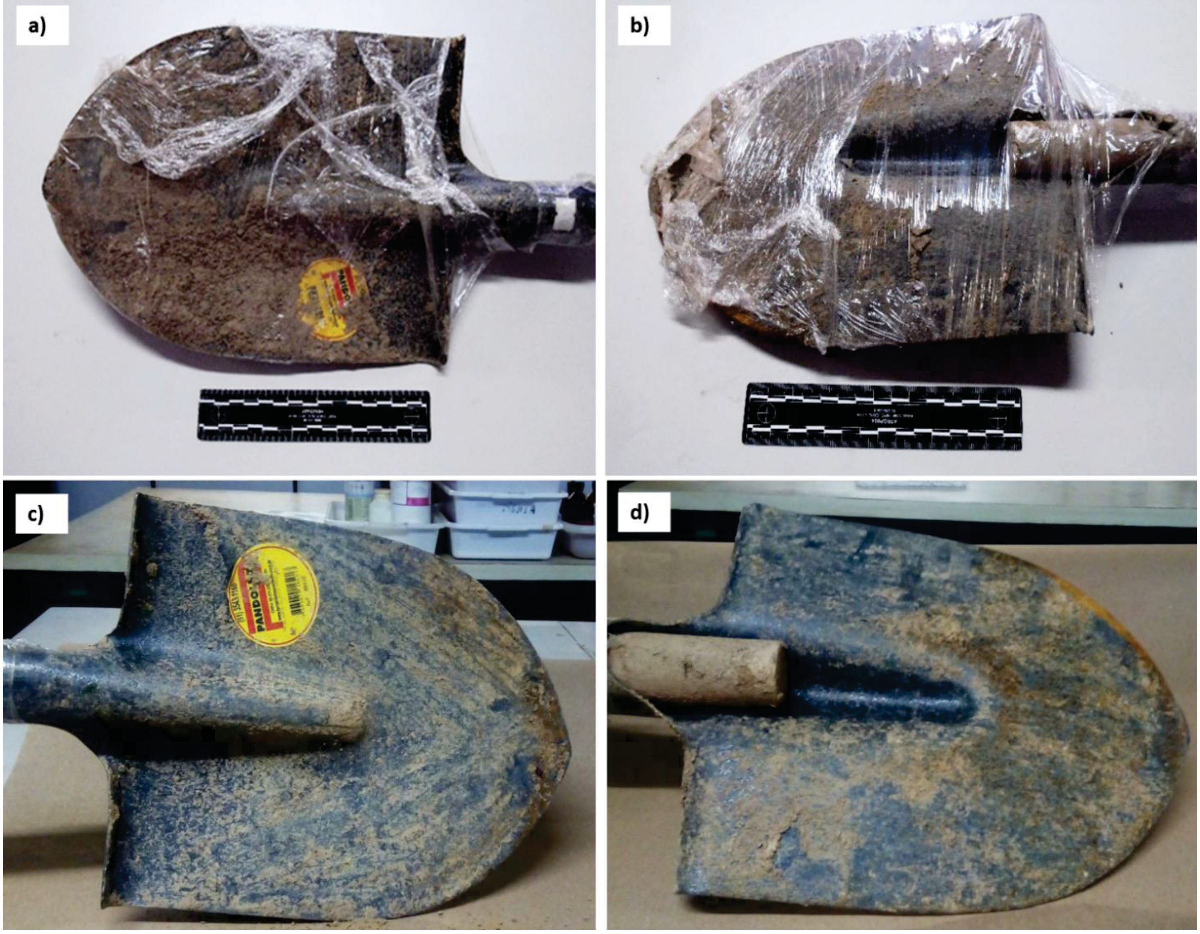


Figure 3. General aspect of the spade used to bury the parts of the body: before (a and b) and after (c and d) of the soil traces sampling. The centre of the sampling places is shown the figures a and b (1 to 6).

7.4.2 SAMPLES PREPARATION AND SOIL CHEMICAL ANALYSES

Soil samples were oven-dried (40°C, for 24h) and sieved (2 mm). A sequence of soil analytical procedures was carried out on the sampled soils, following the procedures developed by Melo et al. (2008) (Figure 4). The amount of dried soil (3 g) for all samples used in this study was defined by the available amount in the location 5, where the trace samples on the spade were collected (Figure 2). Three grams of soil was subjected to the organic matter removal with hydrogen peroxide (H_2O_2) 30% (v/v) in water bath under 70 °C. The soil was dispersed via maceration with a rubber stick in the presence of pH 10 deionized water (1 g of Na_2CO_3 in 10 L of H_2O). The sand fraction was retained in a 0.053 mm mesh. Fractions smaller than 0.053 mm (silt+clay) were grounded and sieved at 0.2 mm mesh. The most reactive fraction to the partial chemical extractions is the clay fraction. However, due to the reduced amount of soil traces (3.0 g), the separation of the silt fraction was unviable. The finer particles (silt and clay) are preferentially retained on shoes, tyres, clothes etc (Fitzpatrick and Raven, 2012). This soil trace amount may yet be more reduced in the sequential extraction. One gram of soil trace has been used in several works with mock crime scenes (Melo et al., 2008; Prandel et al., 2017, Melo et al., 2019; Testoni et al., 2019b). Otherwise, in the application in a real criminal case, Melo et al. (2019) sampled only 0.5 g of trace sample, but applied the full procedure described in Figure 4. Therefore, this work followed the same procedures of previous works which applied the sequential analyses in the silt+clay fraction (Melo et al., 2008; Prandel et al., 2017, Melo et al., 2019; Testoni et al., 2019b).

0.8 g of silt+clay fraction was placed in centrifuge tubes covered with aluminium foil. After addition of 20 mL of 0.2 mol L^{-1} ammonium oxalate pH 3 solution (AO) (Jackson et al., 1986), the tubes were agitated for 2 hours. The suspension was centrifuged at 5000 rpm and the supernatant collected to determine element contents.

The AO residue was treated with sodium dithionite-citrate-bicarbonate (DCB) method (Mehra and Jackson, 1960). Samples of 0.65 g were placed in 100 mL tubes and subjected to the extraction three times with 10.4 mL of solution of sodium citrate 0.3 mol L^{-1} + 1.3 mL of a solution of sodium bicarbonate 1.0 mol L^{-1} + 0.26 g of sodium dithionite. Samples in solution were manually agitated while heated at 70 °C in a water bath for a period of 30 min.

The last sequential step was the NaOH extraction. Around 0.40 g of DCB residue was placed in tubes with 2 mL of NaOH 0.5 mol L^{-1} without heating and homogenized with the initial solution. Then, teflon beckers containing 15 mL of the NaOH 0.5 mol L^{-1} solution were placed in a sand bath at 200 °C until boiling; the solution was then added to the tube with the samples. The boiling solution remained in contact with the sample for three minutes under constant manual agitation. Immediately after, samples were cooled in a recipient with cool water and then centrifuged.

For all sequential extractions common steps were accomplished: i) the extracting solutions and volumetric flasks were filled with ultra-pure water (18.2 MΩ.cm at 25ºC – Millipore Direct–Q System) and high purity acids were used in the analyses (Merck PA); iii) before each extraction the samples were dried for 24 hours in an oven at 40°C, grounded and sieved at 0.2 mm in order to improve the efficiency of the extractions; iv) after each extraction, salt excess was removed by washing with 0.5 mol L^{-1} $(NH_4)_2CO_3$ and ultrapure water ; v) after washing the samples were oven-dried at 40°C for 24 h; vi) extracts were obtained by centrifugation (3,000 rpm for 10 min) and all extracts were filtered in a slow filter paper (Macherey Nagel®); vii) element concentrations were determined by optical emission spectrometry inductively coupled with plasma (Varian-720S) (ICP-OES). The elements were selected with values higher than the limit of detection (LD) in the ICP-OES for all samples and extraction methods: Al (LD – 0.01 mg L^{-1}), Fe (LD – 0.009 mg L^{-1}), Mg (LD – 0.006 mg L^{-1}), Zn (LD – 0.003 mg L^{-1}), Cu (LD – 0.01 mg L^{-1}), Mn (LD – 0.006 mg L^{-1}) and Pb (LD – 0.003 mg L^{-1}).

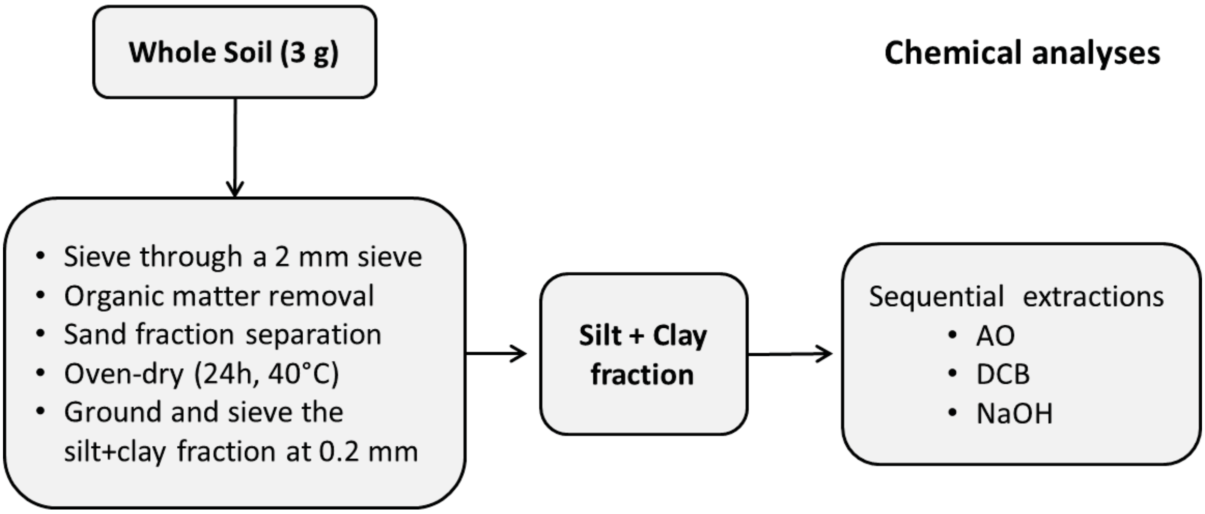


Figure 4. Scheme of physical and chemical procedures applied to the soil samples in this study. Note: AO - ammonium oxalate, DCB - dithionite-citrate-bicarbonate.

7.4.3 MULTIVARIATE STATISTICS

The application of multivariate statistics for soil data is useful and sometimes crucial to elucidate data in criminal cases. This type of statistical approach offers a clear view of grouping among the samples and among the groups of samples as well as separation, i.e. inter and intragroup. In addition, it is possible to verify the similarities and the dissimilarities and discrimination in a set of soil samples based on the analytical data obtained.

Data obtained from the silt + clay fraction were exported from a single matrix for statistical analysis by principal component analysis (PCA) using the softwares

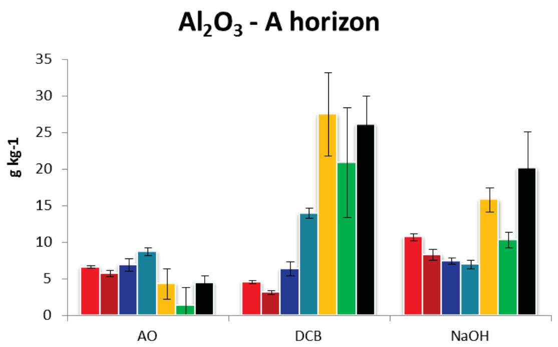
Statistica (Statsoft, 2011) and Paleontological Statistics (PAST), by the application of clustering by Bray-Curtis similarities (Hammer et al., 2001). From the matrix data, four PCAs were generated: PCA 1) elemental contents extracted from AO, DCB and NaOH extractions for the A horizon; PCA 2) an alternative view of the PCA 1; PCA 3) elemental contents extracted from AO, DCB and NaOH extractions for the B horizon; PCA 4) an alternative view of the PCA 3. The analysis of clustering by Bray-Curtis has been successfully used in other works (Melo et al., 2019; Testoni et al., 2019a), and presents the groups (clusters) and the index of maximum similarity among the groups of samples, particularly those with share common characteristics.

7.5 RESULTS AND DISCUSSION

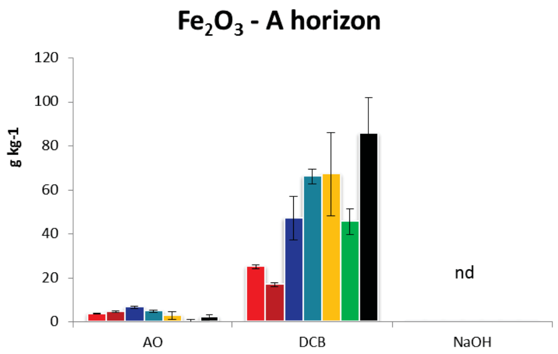
Data obtained by sequential chemical extraction with AO (ammonium oxalate), DCB (dithionite citrate-bicarbonate) and 0.5 mol L-1 NaOH were compiled in a matrix separated by horizons, which originated graphics present in Figures 5 and 6. The elemental profiles (contents of Al_2O_3, Fe_2O_3, Mg, Zn, Cu, Mn and Pb) of the samples from A horizon showed a very similar pattern compared to the samples from B horizon, with low variations.

The highest Fe_2O_3 contents were obtained by DCB extraction, which justifies the positive inflection of the oxide content curves in Figures 5 and 6. Only AO and DCB extractions can extract Fe minerals (Melo et al., 2002). One of the advantages of sequential extraction was the possibility of isolating these two mineral phases. If DCB was applied in the sample without previous treatment, all the forms of pedogenetic Fe oxides (ferryhydrite + hematite + goethite) would be extracted, reducing the power of the soil chemical characterisation. This behavior follow the nature of sequential and selective extractions: AO - dissolution of allophans and imogolites (short range order material-SRO with Si/Al molar ratio greater than 2:1; and amorphous Fe minerals (ferrihydrite, fougerite and schwertmannite), Mn and Al oxides (Singh and Gilkes, 1991; Jackson et al., 1986); DCB - crystalline Fe oxides (hematite and goethite) (Mehra & Jackson, 1960); NaOH - Al-hydroxide, Al–O–Si layers resistant to previous AO extraction and Si–O (opaline silica) (Poggere et al., 2016). The greater extraction of Fe_2O_3 by DCB in relation to AO is due to the predominance of crystalline forms of Fe (hematite and goethite) over Fe-SRO (eg. ferryhidrite) (Kämpf et al., 1988, Fontes and Weed, 1991).

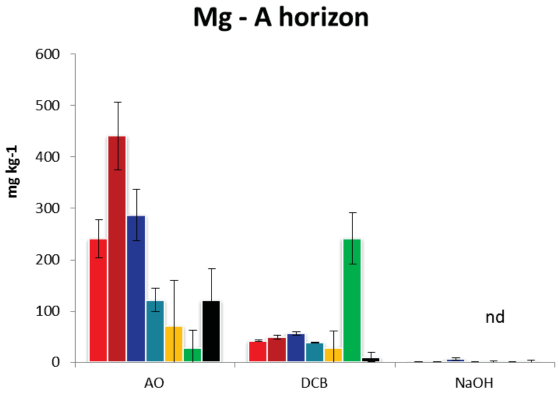
The highest Fe_2O_3-DCB contents were in the soils of external municipalities (used as references locations - Curitiba, Colombo and São José dos Pinhais) to the burial locations of the victim's body parts (Araucária municipality). The formation of two distinct large groups clarified the forensic separation of the samples used as reference samples. Sampling of reference soils, for example from basalt region, would further favour this discrimination, as Fe_2O_3-DCB contents of basalt soils are usually greater than 180 g kg^{-1} (Ghidin et al., 2006). However, these soils present low interest at this crime scene as they do not occur in the Curitiba metropolitan region (CMR - Figure 1a). The granite/gneiss from São José dos Pinhais municipality presents higher levels of primary iron-magnesian minerals in the sand fraction (Melo et al., 2008; Prandel et al., 2017). The weathering of these minerals released more Fe from hematite and mainly goethite formation in the clay fraction (highest positive peak of Fe_2O_3-DCB contents for the samples from São José dos Pinhais municipality).



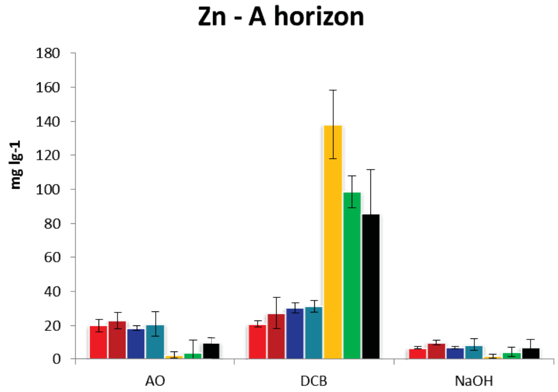
Al_2O_3 - A horizon



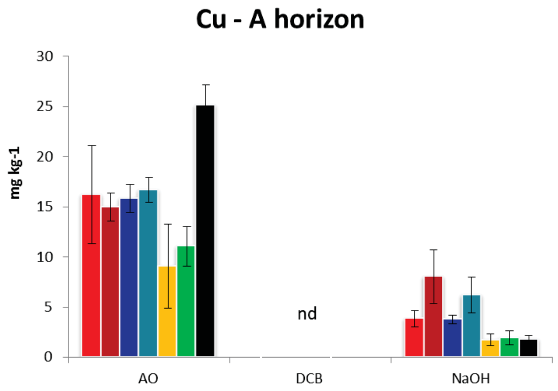
Fe_2O_3 - A horizon



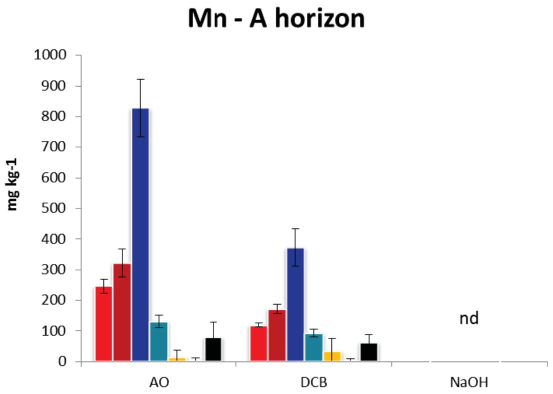
Mg - A horizon



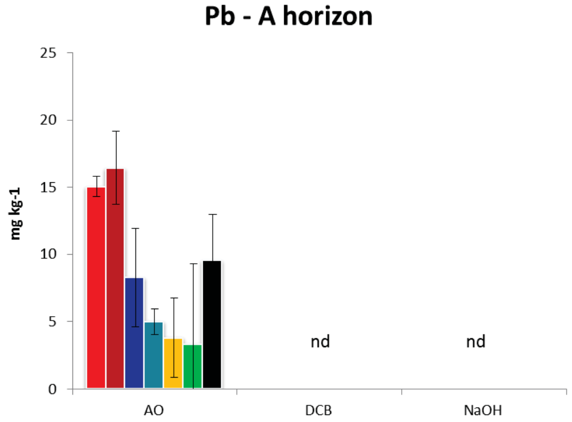
Zn - A horizon



Cu - A horizon



Mn - A horizon



Pb - A horizon

🟥 Torso 🟥 Ref torso 🟦 Legs 🟦 Ref legs 🟨 Claystone 🟩 Limestone ⬛ Granite

Note: nd – non detected.

Figure 5. Average contents (means of four samples at each quadrant) and standard deviations of oxides and elements obtained by chemical extractions with AO (ammonium oxalate), DCB (dithionite-citrate-bicarbonate) and NaOH 0.5 mol L^{-1} in the A horizon for the sampled locations. Ref-reference; Torso, Ref torso; Legs and Ref legs samples belong to Araucária city; Claystone samples belong to Curitiba; Limestone samples belong to Colombo; Granite samples belong to São José dos Pinhais; cities from Metropolitan Region of Curitiba (MRC), State of Paraná, south Brazil.

Note: nd – non detected.

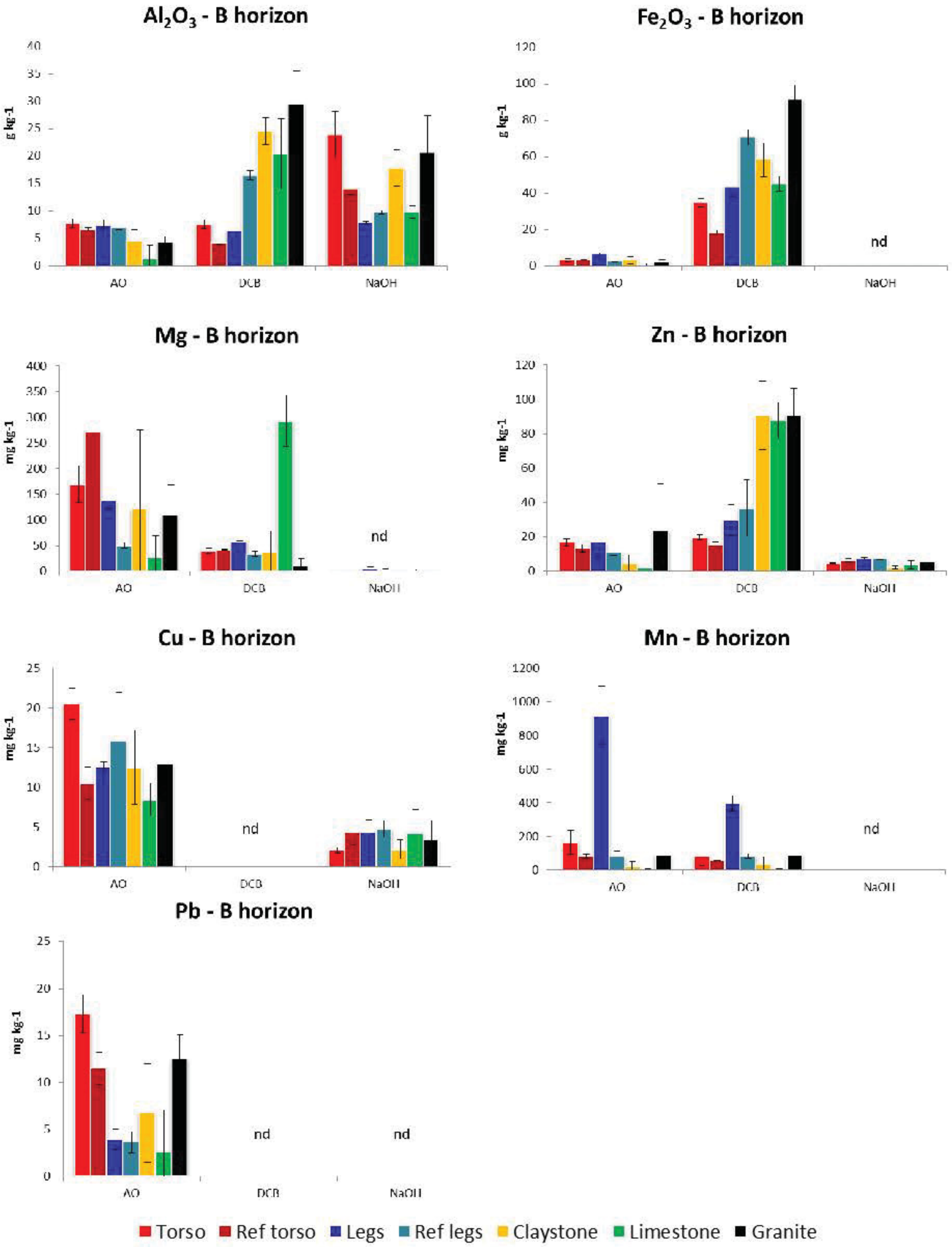


Figure 6. Average contents (four samples at each quadrant) and standard deviations of oxides and elements obtained by chemical extractions with AO (ammonium oxalate), DCB (dithionite-citrate-bicarbonate) and NaOH 0.5 mol L^{-1} in the B horizon for the sampled locations. Ref-reference; Torso, Ref torso; Legs and Ref legs

samples belong to Araucária city; Claystone samples belong to Curitiba; Limestone samples belong to Colombo; Granite samples belong to São José dos Pinhais; cities from Metropolitan Region of Curitiba (MRC), State of Paraná, south Brazil.
Note: nd – non detected.

Once DCB does not extract Al_2O_3 in an isolated phase, the solubilisation of crystalline Fe oxides was responsible for the release of this oxide. The incorporation of Al into the hematite and goethite structure is verified by isomorphic substitution (IS) of Fe^{3+} by Al^{3+}, both in octahedral coordination. This IS is favoured by the small difference between the ionic radii of Fe^{3+} (0.067 nm) and Al^{3+} (0.057 nm). The highest correlation coefficient ($p < 0.01$) between the elements extracted by the DCB was observed between Fe and Al, indicating similar sources for both elements (Table 1). The IS level is very dependent on environmental conditions (which improve forensic studies) during the genesis of these minerals (such as soil acidity level), and the theoretical upper limit of IS in goethite and hematite is 0.33 mol mol^{-1} and 0.17 mol mol^{-1}, respectively (Schwertmann et al., 1979; Schwertmann, 1985). Hematite and goethite of the A horizon from the place where the torso was buried and its reference presented lower IS levels (smaller Al_2O_3-DCB contents), which also contributed to the forensic separation of this environment.

Table 1. Pearson correlations among the elements obtained by AO (ammonium oxalate), DCB (sodium dithionite-citrate-bicarbonate) and NaOH 0.5 mol L^{-1} extractions for all samples of this study.

			AO extraction			
	Cu	**Fe**	**Mg**	**Mn**	**Pb**	**Zn**
Al	0.43**	0.77**	0.46**	0.45**	0.40**	0.49**
Cu		^{ns}	^{ns}	^{ns}	0.29*	^{ns}
Fe			0.63**	0.77**	0.27*	0.36**
Mg				0.43**	0.63**	0.39**
Mn					^{ns}	0.33**
Pb						0.43**
			DCB extraction			
	Cu	**Fe**	**Mg**	**Mn**	**Pb**	**Zn**
Al	^{ns}	0.80**	^{ns}	0.54**	^{ns}	0.76**
Cu		^{ns}	^{ns}	^{ns}	^{ns}	0.53**
Fe			^{ns}	^{ns}	^{ns}	^{ns}
Mg				^{ns}	^{ns}	-0.42**
Mn					^{ns}	^{ns}

Pb						^{ns}
			NaOH extraction			
	Cu	**Fe**	**Mg**	**Mn**	**Pb**	**Zn**
Al	-0.47^{**}	^{ns}	-0.37^{**}	^{ns}	^{ns}	-0.31^{*}
Cu		^{ns}	^{ns}	^{ns}	^{ns}	0.60^{**}
Fe			^{ns}	^{ns}	^{ns}	^{ns}
Mg				^{ns}	^{ns}	0.28^{*}
Mn					^{ns}	^{ns}
Pb						^{ns}

Note: * and **: significant at 5 and 1 % of probability, respectively, ^{ns}: not significant.

AO extracted more amorphous Al oxides than amorphous Fe oxides (Figures 5 and 6). This behaviour has also been verified by other authors (Mendonça et al., 2013; Pogere et al., 2016). However, samples that had more amorphous Al oxides also had higher contents of amorphous Fe oxides ($r=0.77$, $p<0.01$) (Table 1). The conditions that favour the formation and stabilisation of amorphous Fe and Al oxides are higher organic matter contents and lower redox potential conditions (excess of water) (Borer and Hug, 2014).

Al_2O_3-NaOH contents were important only to isolate soil samples collected at Curitiba municipality (claystone) and São José dos Pinhais municipality (granite/gneiss), reference locations in the set of A horizon samples (Figure 5). The behaviour for B horizon samples was even more random (Figure 6), with greater variation of Al_2O_3-NaOH and, consequently, with low power of forensic samples discrimination.

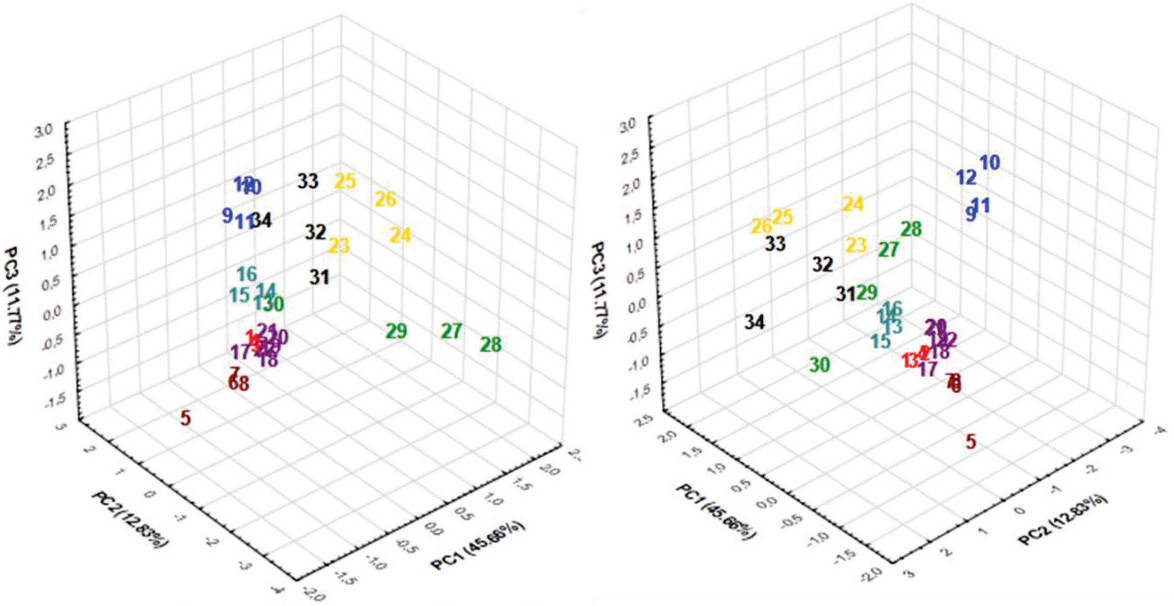
In general, for both A and B horizons, the main source of Mn was the amorphous oxides extracted by AO (Figures 5 and 6). Another possibility is the association between Fe and Mn in the same crystal of amorphous oxides. The correlation coefficient between Fe-AO and Mn-AO contents was high, positive and significant ($r = 0.77$, $p <0.01$) (Table 1). The difference of the ionic radii between Fe^{3+} (0.067 nm) and Mn^{4+} (0.052 nm) is only 22%. Under room temperature and pressure conditions the theoretical limit of ionic radius difference to enable isomorphic substitution is 35% (Zanardo and Marques, 2009). Mn-AO contents were high for the A horizon samples of the legs burial location and reference torso location, being lower than only the legs burial location. In this way, Mn-AO contents isolated these samples from the others.

The other elements (Mg, Cu, Zn and Pb) are not responsible to form mineral phases but are associated with the colloidal fraction of the soil by IS or by specific adsorption to the surface reactive groups of minerals. The highest Mg-DCB contents in the A and B horizons from Colombo municipality soils are consistent with the carbonate material (Figures 5 and 6). These sedimentary rocks are formed by calcium (calcite), Mg (magnesite) and Ca-Mg (dolomite) carbonates (Bigarella and Salamuni, 1962). The Zn-DCB contents separated the reference samples group from the A and B horizons from Curitiba municipality, Colombo municipality and São José dos Pinhais municipality from the other samples belonging to the crime scene region (Araucária municipality). Higher Mg-AO contents of A horizon also separated the locations where the victim's torso and legs were buried (Figure 5). The higher contents of Pb in the A horizon of the agriculture area (torso and reference torso) (Figure 5) is compatible with long time addition of fertilizers in soil, mainly phosphate based ones (García-Rodeja et al., 2004; Valle et al., 2016).

The sequential chemical extractions carried out with ammonium oxalate (AO), dithionite-citrate-bicarbonate (DCB) and NaOH 0.5 mol L^{-1} (Figure 4) were clearly efficient in separate samples from the sampling locations by PCA (Figures 7 and 8). A tendency of greater clustering was observed for the set of soil samples collected in superficial soil layers (A horizon). Even with the organic matter removal previously the sequential extractions, the effect of this colloidal fraction was registered in the chemical characteristics of the secondary minerals from the different sampling locations. The organic matter favours the formation and stabilisation of SRO minerals in the clay fraction (Schöning et al., 2005; Hanke et al., 2015). Depending on the amount and on the nature of the humidified organic compounds, it is possible to verify specific chemical aspects of the organic-mineral interaction in the soil (Zhang et al., 2012; Borer and Hug, 2014; Pašalić et al., 2017).

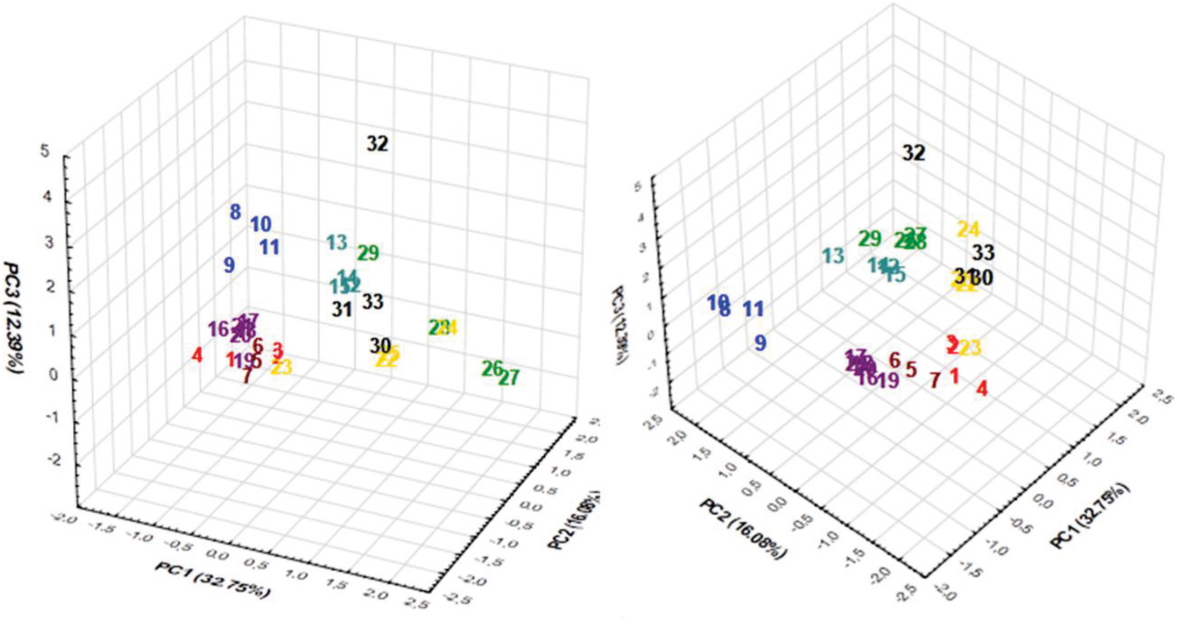
The cluster analysis allows a comparison of the samples collected at the same location (replicates) (Figures 9 and 10). In the A horizon was verified the following decreasing sequence of similarity among the sample groups (Figure 9): i) with 98% of similarity it was observed a grouping of all six replicates collected on the spade. This shows the great similarity of the soil mass adhered on the spade during the burial of the victim’s parts. The four replicates collected at the torso burial location presented 97% similarity. At 97% similarity there was also a clustering of all reference samples of the place where the legs were recovered from and two samples of the burial location where the legs were recovered from, and the limestone and granite reference locations. It was expected to group all replicates collected at the same place with a high index of similarity. However, even if replicates are collected at close distance (1.5 m from each other), horizontal differences in the chemical characteristics of the soil samples may occur; ii) the best grouping was for the samples collected on the spade with the samples collected at the burial location of the torso (95% of similarity). As the murderer had confessed to having used the same spade to bury all the victim's parts, the clustering data show that there was a preferential adherence of the A horizon sample during the torso burial. This was the most important association of this work. At a similarity index of 95% there was also a grouping of all reference samples of the location where the legs were recovered from and the grouping of three replicates of the samples collected at the location of legs burial; iii) the large group of spade plus torso burial location + torso reference samples showed 85% of similarity (the torso reference samples were different in 15% in relation to the torso burial samples). Even though these two sampling sites were positioned at close horizontal distance and due to the great similarity of the two environments (agricultural soils under the same management and formed from the same parent material) (Figure 1), data produced by chemical extractions were able to identify greater similarity of replicates (intragroup similarity - similarity of the isolated groups of the torso and reference torso equal to 97 and 95%, respectively); iv) with the similarity index of 73%, there was the insertion of the samples from the legs burial location in the previous group (spade + torso + reference torso + legs). This data confirms the preferential adherence of soil on the spade at the torso burial location; v) the similarity index of all samples reduced to 57%.

For the lower B horizon the formation of the groups was more dispersed and with lower similarity indexes (Figure 10): i) the group of all the samples of the torso burial location presented lower similarity (92%) compared to the A horizon (97%); ii) the first major composition of two groups occurred only at the 83% similarity index (torso + reference torso). The second important group (torso + legs) showed similarity of only 80%; iii) at only 77% of similarity index the spade was placed at the burial location (large group legs + torso + reference torso + spade); iv) the sample groups of the different parent materials (São José dos Pinhais municipality - granite/gneiss, Curitiba municipality - claystone and Colombo municipality - limestone) showed only 87% of intragroup similarity.



Legend: torso, reference torso, legs, reference legs, spade, claystone, limestone, granite.

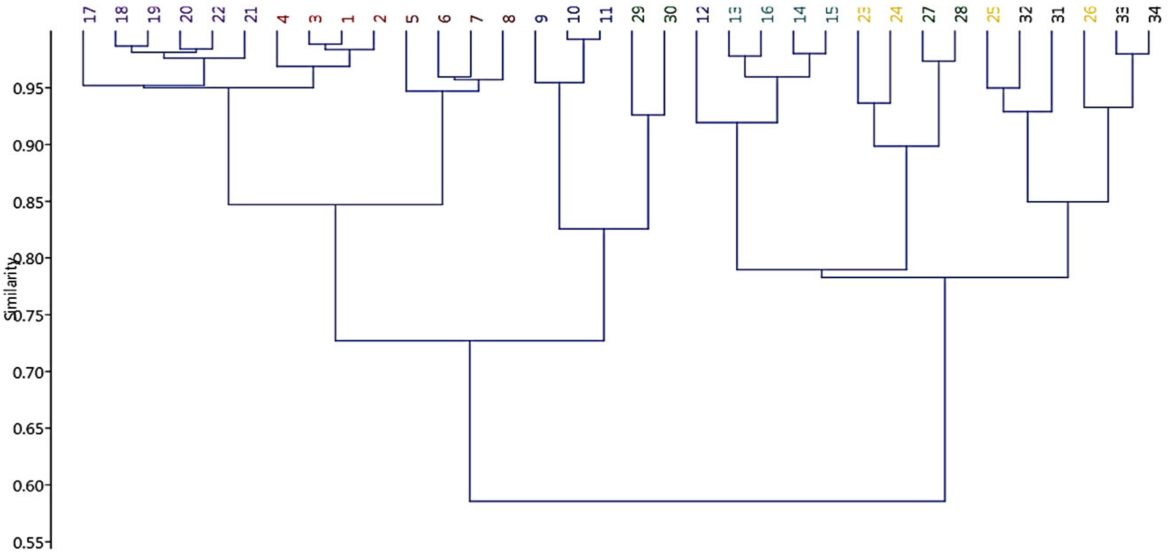
Figure 7. Different perspectives of the same grouping by Principal Component Analysis (PCA). Data matrix corresponds to the sequential extractions with AO (ammonium oxalate), DCB (dithionite-citrate-bicarbonate) and NaOH 0.5 mol L^{-1} obtained in the *A horizon* for all samples of this study (including spade samples). Ref – reference.



Legend: torso, reference torso, legs, reference legs, spade, claystone, limestone, granite.

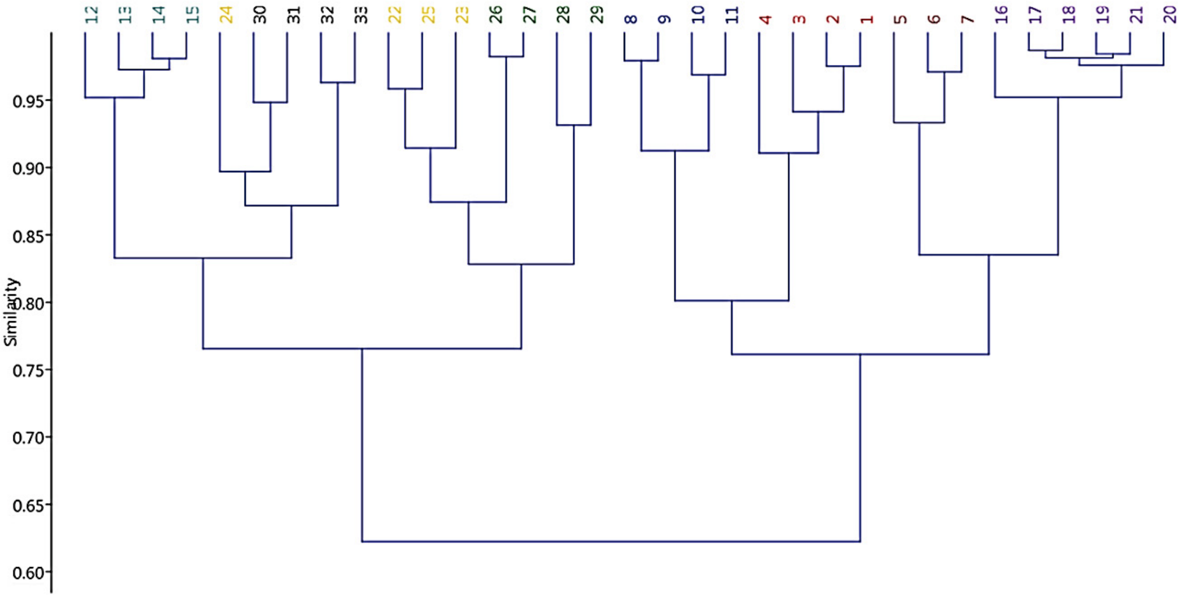
Figure 8. Different perspectives of the same grouping by Principal Component Analysis (PCA). Data matrix corresponds to the sequential extractions with AO

(ammonium oxalate), DCB (dithionite-citrate-bicarbonate) and NaOH 0.5 mol L^{-1} obtained in the *B horizon* for all samples of this study (including spade samples). Ref – reference.



Legend: torso, reference torso, legs, reference legs, spade, claystone, limestone, granite.

Figure 9. Clustering by Bray-Curtis. Data matrix corresponds to the sequential extractions with AO (ammonium oxalate), DCB (dithionite-citrate-bicarbonate) and NaOH 0.5 mol L^{-1} obtained in the *A horizon* for all samples of this study (including spade samples). Ref – reference.



Legend: torso, reference torso, legs, reference legs, spade, claystone, limestone, granite.

Figure 10. Clustering by Bray-Curtis. Data matrix corresponds to the sequential extractions with AO (ammonium oxalate), DCB (dithionite-citrate-bicarbonate) and NaOH 0.5 mol L^{-1} obtained in the *B horizon* for all samples of this study (including spade samples). Ref – reference.

7.6 CONCLUSIONS

Based on the initial hypothesis of the work, is possible to conclude that: i) the validation of the chemical sequential extractions was performed successfully, after the crime events were validated: the A horizon at the burial torso site had adhered preferentially to the spade. The site where the legs were buried contributed with less trace soil to the spade; ii) despite the fact that the spade had been stored for three years it did not reduce the power of sequential chemical analysis to track back the trace sample location of origin; iii) even under external interference of the agricultural activities for three years it was still possible to link the crime scene with the questioned item (spade).

Therefore, both the analytical procedures presented in this study and the forensic soil sampling defined by Testoni et al. (2019b) presented a promising perspective for soils collected at crime scenes in Brazil and worldwide. The sequential chemical analysis should be considered and prioritised instead of isolated extractions.

7.7 REFERENCES

Batista AH, Melo VF, Rate AW, Uhlman A, Gilkes R. More aggressive sequential extraction procedure to access stable forms of Pb and As in clay minerals of soils. *Applied Clay Science* Volume 147, October 2017, Pages 44-53.

Batista AH, Melo VF, Gilkes R, Roberts M. Identification of heavy metals in crystals of sand and silt fractions of soils by scanning electron microscopy (SEM EDS/WD-EPMA). *Revista Brasileira de Ciencia do Solo*. 2018;42:1–16.

Bigarella J, Salamuni R. Caracteres texturais dos sedimentos da Bacia de Curitiba. *Bol da UFPR*. 1962;7:1–164.

Borer P, Hug SJ. Photo-redox reactions of dicarboxylates and a-hydroxydicarboxylates at the surface of Fe(III) (hydr)oxides followed with in situ ATR-FTIR spectroscopy. *J. Colloid Interface Sci*. 2014; 416: 44–53

Carvalho A, Ribeiro H, Mayes R, Guedes A, Abreu I, Noronha F, Dawson L. Organic matter characterization of sediments in two river beaches from northern Portugal for forensic application. *Forensic Science International*, 2013 vol: 233 (1-3) pp: 403-415

Chazottes V, Brocard C, Peyrot B. Particle size analysis of soils under simulated scene of crime conditions: The interest of multivariate analyses. *Forensic Science International.* 2004;140:159–166.

Cheshire K, Morgan RM, Holmes J, The potential for geochemical discrimination of single- and mixed-source soil samples from close proximity urban parkland locations, *Aust. J. Forensic Sci*. 49 (2017) 161–174.

Concheri G, Bertoldi D, Polone E, Otto S, Larcher R, Squartini A. Chemical elemental distribution and soil DNA fingerprints provide the critical evidence in murder case investigation, *PLoS One* 6 (2011) 4–8.

Correa M, Ker J, Barrón V, Fontes M, Torrent J, Curi N. Caracterização de óxidos de ferro de solos do ambiente tabuleiros costeiros. *Revista Brasileira de Ciencia do Solo*, 2008 vol: 32 (3) pp: 1017-1031

Corrêa RS, Melo VF, Abreu GGF, Sousa MH, Chaker JA, Gomes JA. Soil forensics: how far can soil clay analysis distinguish between soil vestiges? *Sci Justice.* 2018;58:138-44. https://doi.org/10.1016/j.scijus.2017.09.003

Dawson LA, Mayes RW. *Criminal and environmental soil forensics*: soil as physical evidence in forensic investigations. In: Murphy BL, Morrison RD, editors. Introduction to environmental forensics.3rd ed. London: Academic Press; 2015. p. 457-86.

Dawson LA. Soil organic characterisation in forensic case work*, J. Int. Geosci*. 40 (2017) 017–018.

Fitzpatrick RW, Raven MD. How pedology and mineralogy helped solve a double murder case: using forensics to inspire future generations of soil scientists. *Soil Horizons*. 2012;53:14-29. https://doi.org/10.2136/sh12-05-0016.

Demanèche S, Schauser L, Dawson L, Franqueville L, Simonet P. Microbial soil community analyses for forensic science: application to a blind test. *Forensic Sci Int.* 2017;270:153-8. https://doi.org/10.1016/j.forsciint.2016.12.004

Farrugia KJ, Bandey H, Dawson L, Nic Daéid N. Chemical enhancement of soil based footwear impressions on fabric. *Forensic Science International*. 2012;219:12–28.

Finley SJ, Benbow ME, Javan GT. Potential applications of soil microbial ecology and next-generation sequencing in criminal investigations. *Appl Soil Ecol.* 2015;88:69-78. https://doi.org/10.1016/j.apsoil.2015.01.001

Fitzpatrick, M. Raven, P. Self, Clay mineralogy as significant evidence in 4 murder investigations involving a wide range of earth materials from Perth, Adelaide, Melbourne and Sydney, *Aust. Clay Miner. Soc. Conf*. (2014) 23–26.

Fontana A, Chagas CS, Donagemma GK, Menezes AR, Calderano Filho B. Soils developed on geomorphic surfaces in the mountain region of the State of Rio de Janeiro. *Rev Bras Cienc Solo*. 2017;41:e0160574.

Fontes, M.P.F. & Weed, S.B. Iron oxides in selected Brazilian Oxisols: I. Mineralogy. Soil Sci. *Soc. Am. J*., 55:1143-1149, 1991.

García-Rodeja E, Nóvoa J, Pontevedra X, Martínez-Cortizas A, Buurman P. Aluminium fractionation of European volcanic soils by selective dissolution techniques. *Catena*, 2004 vol: 56 pp: 155-183.

Ghidin AA, Melo VF, Lima VC, Lima JMJC. Toposseqüências De Latossolos Originados De Rochas Basálticas No Paraná . I - Mineralogia. *Rev Bras Cienc do Solo*. 2006;30:293–306.

Hammer Ø, Harper DAT, Ryan PD. PAST: *Paleontological statistics* software package for education and data analysis. Palaeontol Electron. 2001;4:4.

Hanke D. Gênese, interação organo-mineral e estabilidade de agregados de solos desenvolvidos de basalto. 2012. 101p. Dissertação (Mestrado em Ciência do Solo) – Universidade Federal do Paraná, Curitiba, 2012.

Horrocks M Coulson S Walsh K. Foresic palynology: variation in the pollen content of soil surface samples. *Journal of Forensic Sciences*. 1998 vol: 43 pp: 320-323.

Jackson ML, Lim CH, Zelazny LW. Oxides, hydroxides, and aluminosilicates. In: Klute A, editor. Methods of soil analysis. Physical and mineralogical methods. 2nd ed. Madison: *American Society of Agronomy*; 1986. Pt 1. p. 101-50.

Kämpf, N.; Klant, E & Schneider, P. Óxidos de ferro em Latossolos do Brasil sudeste e sul. In: Reunião De Classificação, Correlação De Solos E Interpretação De Aptidão Agrícola, 3, Rio de Janeiro. 1988. Anais. Rio de Janeiro, Empresa

Brasileira de Pesquisa Agropecuária, Serviço Nacional de Levantamento e Classificação do Solo, 1988a. p.153-183.

McKeague JA. Manual on soil sampling and methods of analysis. Ottowa: *Canadian Society of Soil Science*; 1978.

Medeiros PSC, Nascimento PC, Inda AV, Silva DS. Classificação e Caracterização de solos graníticos em topossequência na região Sul do Brasil. *Ciência Rural*, Santa Maria, v. 43, n. 7, p. 1210-1217, 2013.

Mehra OP, Jackson ML. Iron oxide removal from soils and clays by a dithionite-citrate system buffered with sodium bicarbonate. proceedings of the Seventh National Conference on Clays and Clay Minerals; October 20-23, 1958; Washington, DC. Oxford: *Clay Clay Miner*; 1960. p. 317-27.

Melo VF, Schaefer CEGR, Novais RF, Singh B, Fontes MPF. Potassium and magnesium in clay minerals of some Brazilian soils as indicated by a sequential extraction procedure. *Commun Soil Sci Plant*. 2002;33:2203-25. https://doi.org/10.1081/CSS-120005757

Melo VF, Barbar LC, Zamora PGP, Schaefer CE, Cordeiro GA. Chemical , physical and mineralogical characterization of soils from the Curitiba Metropolitan Region for forensic purpose. *Forensic Sci Int*. 2008;179:123-34.

Melo VF, Batista AH, Gilkes RJ, Rate AW. Relationship between heavy metals and minerals extracted from soil clay by standard and novel acid extraction procedures. *Environmental monitoring and assessment* 2016 v.188 no.12

Melo VF, Testoni SA, Dawson LA, Lara AG, Salvador FAS. Can analysis of a small clod of soil help to solve a murder case?. *Science & Justice*. https://doi.org/10.1016/j.scijus.2019.06.008

Melo VF, Mazzetto JML, Dieckow J, Bon EJ. Factor analysis of organic soils for site discrimination in a forensic setting. *Forensic Science International.* 2018;290:244–250.

Mendonça T, Melo VF, Schaefer CEGR, Simas FNB, Michel RFM. Clay mineralogy of gelic soils from the Fildes Peninsula, Maritime Antarctica. *Soil Sci Soc Am J.* 2013;77:1842-51. https://doi.org/10.2136/sssaj2012.0135.

Morgan RM, Scott KR, Ainley J, Bull PA. Journey history reconstruction from the soils and sediments on footwear: An empirical approach. *Science & Justice.* Elsevier; 2018;1–11.

Muggler CC. Plygenetic Oxisoil on tertiary surfaces, Minas Gerais, Brazil: soil genesis and landscape development. Wageningen, Wageningen Agricultural University, 1998.

186p. (Tese de Doutorado).

Oliveira Jr JC, Melo VF, Souza LCP, Rocha HO. Terrain attributes and spatial distribution of soil mineralogical attributes. *Geoderma*, 2014, 213, 214-225.

Pašalić H, Aquino AJA, Tunega D, Haberhauer G, Gerzabek MH, Lischka H. Cation–π interactions in competition with cation microhydration: a theoretical study of alkali metal cation–pyrene complexes. *Journal of Molecular Modeling.* 2017; 23:131.

Pereira T, Ker J, Schaefer C, de Barros N, Neves J, Almeida C. Gênese de latossolos e cambissolos desenvolvidos de rochas pelíticas do Grupo Bambuí - Minas Gerais. *Revista Brasileira de Ciencia do Solo*, 2010 vol: 34 (4) pp: 1283-1295

Pirrie D, Dawson L, Graham G. Predictive geolocation: Forensic soil analysis for provenance determination. *Episodes.* 2017;40:141–147.

Poggere GC, Melo VF, Francelino MR, Schaefer CE, Simas FN. Characterization of products of the early stages of pedogenesis in ornithogenic soil from Maritime Antarctica. *European Journal of Soil Science*, January 2016,67 , 70–78.

Poggere GC, Inda AV, Barrón V, Curi N. Maghemite quantification and magnetic signature of Brazilian soils with contrasting parent materials. *Applied Clay Science*, 2018, 161.

Prandel LV, Melo VF, Brinatti AM, Saab SC, Salvador FAS. X-ray diffraction and Rietveld refinement in deferrified clays for forensic science. *J Forensic Sci.* 2017;63:251-7. https://doi.org/10.1111/1556-4029.13476

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

Ramos PV, Dalmolin RSD, Marques Júnior J, Siqueira DS, Almeida JA, Moura-Bueno JM. Magnetic Susceptibility of Soil to Differentiate Soil Environments in Southern Brazil. *Rev Bras Cienc Solo*. 2017;41:e0160189.

Reidy, K. Bu, M. Godfrey, J.V. Cizdziel, Elemental fingerprinting of soils using ICP-MS and multivariate statistics: a study for and by forensic chemistry majors, *Forensic Sci. Int.* 233 (2013), https://doi.org/10.1016/j.forsciint.2013.08.019.

Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Oliveira JB, Coelho MR, Lumbreras JF, Cunha TJF. Sistema brasileiro de classificação de solos. 3. ed. Rio de Janeiro: *Embrapa Solos*; 2013.

Schwertmann U, Fitzpatrick RW, Taylor RM, Lewis DG. the Influence of Aluminum on Iron Oxides. Part Ii. Preparation and Properties of a1-Substituted Hematites. *Clays Clay Miner*. 1979;27:105–112.

Schwertmann U, Kämpf N. Properties of goethite and hematite in kaolinitic soils of Southern and Central Brazil. *Soil Sci Soc Am J*. 1985;139.

Simas FNB, Schaefer CEGR, Melo VF, Guerra MBB, Saunders M, Gilkes RJ. Clay-sized minerals in permafrost-affected soils (cryosols) from King George Island, Antarctica. *Clays and Clay Minerals* , Vol. 54, No. 6, 721–736, 2006.

Singh, B. & Gilkes, R.J. Concentration of iron oxides from soil clays by 5 M NaOH treatment: The complete removal of sodalite and kaolin. *Clay Miner*., 26:463-472, 1991.

Statsoft Inc. Statistica - Data analysis software system. Tulsa: Statsoft Software; 2011.

Testoni SA, Melo VF, Dawson LA, Salvador FAS, Prandel LV. Evaluation of forensic soil traces from a crime scene: robbery of a safety deposit box in Brazil. *Geological Society, Special Publications*. 2019a DOI: https://doi.org/10.1144/SP492-2019-35

Testoni SA, Melo VF, Dawson LA, Salvador FAS, Kunii PA. Validation of a Standard Operating Procedure (SOP) for forensic soils investigation in Brazil. *Rev Bras Cienc Solo*. 2019b;43:e0190010.

Tighe M, Forster N, Guppy C, Savage D, Grave P, Young IM, Georeferenced soil provenancing with digital signatures, *Sci. Rep.* 8 (2018) 1–9.

Uitdehaag S, Wiarda W, Donders T, Kuiper I. Forensic Comparison of Soil Samples Using Nondestructive Elemental Analysis. *Journal of Forensic Sciences*. 2017;62:861–868.

Valle L, Rodrigues S, Ramos S, Pereira H, Amaral D, Siqueira J, Guilherme L. Beneficial use of a by-product from the phosphate fertilizer industry in tropical soils: Effects on soil properties and maize and soybean growth. *Journal of Cleaner Production*, 2016 vol: 112 pp: 113-120

Zanardo, A.: Marques Junior, J. Conceitos Básicos Em Mineralogia. In: Alleoni, L.R.F.; Melo, V.F. (Eds) *Química E Mineralogia Do Solo*. Viçosa,-Mg, Sbcs, 2009, V.1, P.73-150.

Zhang L, Luo L, Zhang S. Integrated investigations on the adsorption mechanisms of fulvic and humic acids on three clay minerals. *Colloids Surf.* 2012; 406: 84–90.

Schöning I, Knicker H, Kögel-knabner I. Intimate association between O/N-alkyl carbon and iron oxides in clay fractions of forest soils. Org. Geochem., 2005; 36:1378-1390.

Woods B, Lennard C, Kirkbride KP, Robertson J. 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.* 2016;262:46-55. https://doi.org/10.1016/j.forsciint.2016.02.034

8 GENERAL CONCLUSION

From a variety of perspectives, this work has highlighted the undoubted potential of soils for use in forensic purposes. These perspectives varied from simulated and real crime scenes, as well as under different parent material, types of land use (preserved, urban and agricultural), amount of sample, condition and storage time of varying samples.

The unique nature of soils allows us to differentiate them in the most diverse environments, under different climatic conditions, relief, landscape and anthropic interference. The methodology selected is directly dependent on a number of factors, which derive from the context of each crime scene and the amount of soil sample.

In our work we tested different analysis techniques in all soil fractions (sand, silt, clay and organic matter). We clearly found that the finer soil fractions (clay and silt) respond better to the analytical approaches, depending on the particularities of each soil fraction. However, when there are cases in which only coarse fractions are present, satisfactory results can still be obtained. Regarding the adoption of the ideal methodology, despite being subjected to screening according to the criminal context, we observed in both simulated and real scenes that chemical sequential extractions of the different mineralogical soil phases were clearly efficient in forensic soil under subtropical conditions. This efficiency was strongly evidenced by the high discriminatory power and grouping of samples according to their place of origin, which allowed a clear traceability of soil traces.

10 REFERENCES

Aitkenhead, M. J., Coull, M. C., Dawson, L. A. (2013). Predicting Sample Source Location from Soil Analysis Using Neural Networks. *Environmental Forensics* 14, 281–292.

Anjos, M. J., Lopes, R. T., Jesus, E. F. O., Assis, J. T., Cesareo, R., Barroso, R. C., Barradas, C. A. A. (2002). Elemental concentration analysis in soil contaminated with recyclable urban garbage by tube-excited energy-dispersive X-ray fluorescence. *Radiation Physics and Chemistry* 65, 495–500.

Barbar, L. C., Melo, V. F. (2008). Variability of the Chemical and Mineralogical Characteristics of Soils of the Metropolitan Region of Curitiba (PR). *Scientia Agraria* 9, 187–197.

Batista AH, Melo VF, Gilkes R, Roberts M. Identification of heavy metals in crystals of sand and silt fractions of soils by scanning electron microscopy (SEM EDS/WD-EPMA). *Revista Brasileira de Ciencia do Solo*. 2018;42:1–16.

Batista AH, Melo VF, Rate AW, Uhlman A, Gilkes R. More aggressive sequential extraction procedure to access stable forms of Pb and As in clay minerals of soils. *Applied Clay Science* Volume 147, October 2017, Pages 44-53.

Bigarella J, Salamuni R. Caracteres texturais dos sedimentos da Bacia de Curitiba. *Bol da UFPR*. 1962;7:1–164.

Blackburn G, Taylor RM. Limestones and Red Soils of Bermuda. *GSA Bulletin*. 1969;80: 1595-1598.

Bong WSK, Nakai I, Furuya S, Suzuki H, Abe Y, Osaka K, Matsumoto T, Itou M, Imai N, Ninomiya T. Development of heavy mineral and heavy element database of soil sediments in Japan using synchrotron radiation X-ray powder diffraction and high-energy (116 keV) X-ray fluorescence analysis: 1. Case study of Kofu and Chiba region. *Forensic science international*. 2012;220:33–49.

Borer P, Hug SJ. Photo-redox reactions of dicarboxylates and a-hydroxydicarboxylates at the surface of Fe(III) (hydr)oxides followed with in situ ATR-FTIR spectroscopy. *J. Colloid Interface Sci*. 2014; 416: 44–53

Brinatti AM, Mascarenhas YP, Pereira VP, Silvia C, Partiti DM, Macedo Á. Mineralogical characterization of a highly-weathered soil by the Rietveld Method pelo Método de Rietveld. *Sci Agric*. 2010;454–464.

Bull PA, Morgan RM. Sediment Fingerprints : A forensic technique using quartz sand grains. *Science & Justice*. 2006;46:107–124.

Bull PA, Parker A, Morgan RM. The forensic analysis of soils and sediment taken from the cast of a footprint. *Forensic Science International*. 2006;162:6–12.

Carvalho, Á., Ribeiro, H., Mayes, R., Guedes, A., Abreu, I., Noronha, F., Dawson, L., 2013. Organic matter characterization of sediments in two river beaches from northern Portugal for forensic application. *Forensic Science International* 233, 403–415. https://doi.org/10.1016/j.forsciint.2013.10.019

Cengiz S, Karaca AC, Çakir I, Üner HB, Sevindik A. SEM-EDS analysis and discrimination of forensic soil. *Forensic Science International*. 2004;141:33–37.

Chauhan R, Kumar R, Sharma V. Soil forensics: A spectroscopic examination of trace evidence. *Microchemical Journal*. Elsevier B.V.; 2018;139:74–84.

Chazottes V, Brocard C, Peyrot B. Particle size analysis of soils under simulated scene of crime conditions: The interest of multivariate analyses. *Forensic Science International.* 2004;140:159–166.

Cheshire, K., Morgan, R.M., Holmes, J., 2017. The potential for geochemical discrimination of single- and mixed-source soil samples from close proximity urban parkland locations. *Australian Journal of Forensic Sciences* 49, 161–174. https://doi.org/10.1080/00450618.2016.1144789

Chodur NL. Caracterização de solos na região de Araucária, Contenda, PR. Universidade Federal do Paraná; 1990.

Concheri, G. et al. Chemical elemental distribution and soil DNA fingerprints provide the critical evidence in murder case investigation. *PLoS ONE*, v. 6, n. 6, p. 4–8, 2011.

Correa M, Ker J, Barrón V, Fontes M, Torrent J, Curi N. Caracterização de óxidos de ferro de solos do ambiente tabuleiros costeiros. *Revista Brasileira de Ciencia do Solo*, 2008 vol: 32 (3) pp: 1017-1031

Corrêa RS, Melo VF, Abreu GGF, Sousa MH, Chaker JA, Gomes JA. Soil forensics: how far can soil clay analysis distinguish between soil vestiges? *Sci Justice.* 2018;58:138-44. https://doi.org/10.1016/j.scijus.2017.09.003

Dawson LA, Hillier S. Measurement of soil characteristics for forensic applications. Surf Interface Anal. 2010;42:363-77. https://doi.org/10.1002/sia.3315

Dawson LA, Mayes RW. *Criminal and environmental soil forensics*: soil as physical evidence in forensic investigations. In: Murphy BL, Morrison RD, editors.

Introduction to environmental forensics.3rd ed. London: *Academic Press*; 2015. p. 457-86.

Dawson, L. A., Hillier, S. (2010). Measurement of soil characteristics for forensic applications. *Surface and Interface Analysis* 42, 363–377.

Dawson, L.A., 2017. Soil organic characterisation in forensic case work. Journal of International *Geoscience* 40, 017–018.

Dawson, L.A., Mayes, R.W., Elston, D.A., Smart, T.S., 2000. Root hydrocarbons as potential markers for determining species composition. *Plant, Cell and Environment* 23, 743–750. https://doi.org/10.1046/j.1365-3040.2000.00592.x

Demanèche S, Schauser L, Dawson L, Franqueville L, Simonet P. Microbial soil community analyses for forensic science: application to a blind test. *Forensic Sci Int.* 2017;270:153-8. https://doi.org/10.1016/j.forsciint.2016.12.004

Dove, H., Mayes, R.W., 2006. Protocol for the analysis of n-alkanes and other plant-wax compounds and for their use as markers for quantifying the nutrient supply of large mammalian herbivores. *Nature Protocols* 1, 1680–1697. https://doi.org/10.1038/nprot.2006.225

Dove, H., Mayes, R.W., Freer, M., 1996. Effects of species, plant part, and plant age on the n-alkane concentrations in the cuticular wax of pasture plants. *Australian Journal of Agricultural Research* 47, 1333–1347. https://doi.org/10.1071/AR9961333

Duarte, A. P. et al. European Journal of Soil Biology Changes in the forms of lead and manganese in soils by passage through the gut of the tropical endogeic earthworm (Pontoscolex corethrurus). *European Journal of Soil Biology*, v. 53, p. 32–39, 2012.

Farrugia KJ, Bandey H, Dawson L, Nic Daéid N. Chemical enhancement of soil based footwear impressions on fabric. *Forensic Sci Int*. 2012;219:12-28. https://doi.org/10.1016/j.forsciint.2011.11.011

Finley SJ, Benbow ME, Javan GT. Potential applications of soil microbial ecology and next-generation sequencing in criminal investigations. *Appl Soil Ecol.* 2015;88:69-78. https://doi.org/10.1016/j.apsoil.2015.01.001Curi,

Franzmeier, D. P. (1984). Toposequence of Oxisols from the Central Plateau of Brazil. *Soil Soil Science American Journal* 48, 341–346.

Fitzpatrick, R. (2011). Getting the dirt'. Gazette 73, 22–23.

Fitzpatrick, R., Raven, M., Self, P. (2012). Using forensics to inspire the next generation of regolith, soil and clay scientists. *Australian Regolith and Clays Conference* Mildura, 25–28.

Fitzpatrick, R. W. (2008). *Nature, Distribution, and Origin of Soil Materials in the Forensic Comparison of Soils*. In: Tibbett, M., Carter, D. O. eds. Soil Analysis in Forensic Taphonomy: Chemical and Biological Effects of Buried Human Remains. New York: Taylor & Francis Group, 366 p.

Fitzpatrick, R.W. & Raven, M.D. (2012). How pedology and mineralogy helped solve a double murder case: Using forensics to inspire future generations of soil scientists. *Soil Horizons*, 53(5), 14-29. doi:10.2136/sh12-05-0016.

Fitzpatrick, R. W. (2013). Soil : Forensic Analysis. In: Jamieson, A., Moenssens, A. A., eds. *Encyclopedia of Forensic Science*. Chichester: John Wiley, 18 p.

Fitzpatrick, R.W. & Raven, M.D. (2016). Guidelines for Conducting Criminal and Environmental Soil Forensic Investigations (Version 10.1). *Centre for Australian Forensic Soil Science*, Report, CAFSS_076, 46 pp.

Fitzpatrick, RW, Raven, M.D. and Self P.G. (2017). The role of pedology and mineralogy in providing evidence for 5 crime investigations involving a wide range of earth materials. *Episodes.* 40,148-156. http://dx.doi.org/10.18814/epiiugs/2017/v40i2/017017

Fontana A, Chagas CS, Donagemma GK, Menezes AR, Calderano Filho B. Soils developed on geomorphic surfaces in the mountain region of the State of Rio de Janeiro. *Rev Bras Cienc Solo*. 2017;41:e0160574.

Fontes, M.P.F. & Weed, S.B. Iron oxides in selected Brazilian Oxisols: I. Mineralogy. *Soil Sci. Soc. Am. J.*, 55:1143-1149, 1991.

García-Rodeja E, Nóvoa J, Pontevedra X, Martínez-Cortizas A, Buurman P. Aluminium fractionation of European volcanic soils by selective dissolution techniques. *Catena*, 2004 vol: 56 pp: 155-183.

Ghidin AA, Melo VF, Lima VC, Lima JMJC. Toposseqüências de Latossolos originados de rochas basálticas no Paraná. I – Mineralogia da fração argila. *Rev Bras Cienc Solo*. 2006;30:293-306. https://doi.org/10.1590/S0100-06832006000200010

Hammer, Ø., Harper, D. A. T. (2006). PAST, *Paleontological Statistics*. Version 2.07. Reference manual. Blackwell Publishing.

Hanke D. Gênese, interação organo-mineral e estabilidade de agregados de solos desenvolvidos de basalto. 2012. 101p. Dissertação (Mestrado em Ciência do Solo) – Universidade Federal do Paraná, Curitiba, 2012.

Horrocks M Coulson S Walsh K. Foresic palynology: variation in the pollen content of soil surface samples. *Journal of Forensic Sciences.* 1998 vol: 43 pp: 320-323.

Gibbs B, Haldenby A. 2007-2008 British Crime Survey. UK; 2008.

Hammer Ø, Harper DAT, Ryan PD. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol Electron*. 2001;4:4.

Hsu, P. H. (1989). Aluminium oxides and oxyhydroxides. In: Dixon, S. B. W., ed. Minerals in Soil Environments. Madison: *Soil Science Society of America*, 331–378 p.

Jackson ML, Lim CH, Zelazny LW. Oxides, hydroxides, and aluminosilicates. In: Klute A, editor. Methods of soil analysis. Physical and mineralogical methods. 2nd ed. Madison: *American Society of Agronomy*; 1986. Pt 1. p. 101-50.

Kämpf, N., Schwertmann, U. (1983). Goethite and hematite in climosequence in southern Brazil and their application in classification of kaolinitic soils. *Geoderma* 29, 27–39.

Kämpf, N.; Klant, E & Schneider, P. Óxidos de ferro em Latossolos do Brasil sudeste e sul. In: Reunião De Classificação, Correlação De Solos E Interpretação De Aptidão Agrícola, 3, Rio de Janeiro. 1988. Anais. Rio de Janeiro, Empresa Brasileira de Pesquisa Agropecuária, *Serviço Nacional de Levantamento e Classificação do Solo*, 1988a. p.153-183.

Marumo, Y. (2003). Forensic Examination of Soil Evidence. *Japanese Journal of Science and Technology for Identification* 7, 95–111.

Mayes, R.W., Macdonald, L.M.J.., Dawson, L.A., 2008. Discrimination of domestic garden soils using plant wax compounds as markers, in: Ritz, K., Dawson, L.A., Miller, D. (Eds.), *Criminal and Environmental Soil Forensics*. Springer, London UK.

McCulloch, G., Dawson, L.A., Brewer, M.J., Morgan, R.M., 2017. The identification of markers for Geoforensic HPLC profiling at close proximity sites. *Forensic Science International* 272, 127–141.

McCulloch, G., Dawson, L.A., Ross, J.M., Morgan, R.M., 2018. The discrimination of geoforensic trace material from close proximity locations by organic profiling using HPLC and plant wax marker analysis by GC. *Forensic Science International* 288, 310–326. https://doi.org/10.1016/j.forsciint.2018.02.009

McKeague, J. A. (1978). Manual on soil sampling and methods of analysis. Ottowa: *Canadian Society of Soil Science*.

McKeague, J. A., Day, J. H. (1966). Dithionite- and Oxalate-Extractable Fe and Al as Aids in Differentiating Various Classes Of Soils. *Canadian Journal of Soil Science* 46, 13–22.

Medeiros PSC, Nascimento PC, Inda AV, Silva DS. Classificação e Caracterização de solos graníticos em topossequência na região Sul do Brasil. *Ciência Rural,* Santa Maria, v. 43, n. 7, p. 1210-1217, 2013.

Mehra, O. P., Jackson, M. L. (1960). Iron Oxide Removal from Soils and Clays by a Dithionite-Citrate System Buffered with Sodium Bicarbonate. *Clays and Clay Minerals* 7, 317–327.

Melo, V. F., Barbar, L. C. , Zamora, P. G. P., Schaefer, C. E., Cordeiro, G. A. (2008). Chemical , physical and mineralogical characterization of soils from the Curitiba Metropolitan Region for forensic purpose. *Forensic Science International* 179, 123–134.

Melo, V. F., Fontes, M. P. F., Novais, R. F., Singh, B., Schaefer, C. E. G. R. (2001). Characteristics of iron and aluminum oxides of different soil classes. *Brazilian Journal of Soil Science* 25, 19–32.

Melo VF, Mazzetto JML, Dieckow J, Bonfleur EJ. Factor analysis of organic soils for site discrimination in a forensic setting. *Forensic Sci Int.* 2018;290:244-50. https://doi.org/10.1016/j.forsciint.2018.07.005

Melo VF, Schaefer CEGR, Novais RF, Singh B, Fontes MPF. Potassium and magnesium in clay minerals of some Brazilian soils as indicated by a sequential extraction procedure. *Commun Soil Sci Plant.* 2002;33:2203-25. https://doi.org/10.1081/CSS-120005757

Melo VF, Testoni SA, Dawson L, Lara AG De, Salvador F. Can analysis of a small clod of soil help to solve a murder case? *Science & Justice*. 2019;1–26.

Mendonça, T., Melo, V. F., Schaefer, C. E. G. R., Simas, F. N. B., Michel, R. F. M. (2013). Clay Mineralogy of Gellic Soils From Fildes Peninsula, Maritime Antarctica. *Soil Science Society of America Journal* 77, 1842–1851.

Mitton RV, Melo VF. Do Aggregate Size Classes of the Subsurface Soil Horizon Have Different Chemical / Mineralogical Properties? *Brazilian Journal of Soil Science.* 2019;1–15.

Morgan RM, Bull PA. The use of grain size distribution analysis of sediments andsoils in forensic enquiry. *Science & Justice*. 2007;47:125–135.

Morgan, R., Bull, P., 2007. The philosophy, nature and practice of forensic sediment analysis. *Progress in Physical Geography* 31, 43–58. https://doi.org/10.1177/0309133307073881

Morgan RM, Robertson J, Lennard C, Hubbard K, Bull PA. Quartz grain surface textures of soils and sediments from Canberra, Australia: A forensic reconstruction tool. *Australian Journal of Forensic Sciences*. 2010;42:169–179.

Morgan RM, Scott KR, Ainley J, Bull PA. Journey history reconstruction from the soils and sediments on footwear: An empirical approach. *Science & Justice*. 2018;1–11.

Morgan, R.M., Wiltshire, P., Parker, A., Bull, P.A., 2006. The role of forensic geoscience in wildlife crime detection. *Forensic Science International* 162, 152–162. https://doi.org/10.1016/j.forsciint.2006.06.045

Muggler CC. Plygenetic Oxisoil on tertiary surfaces, Minas Gerais, Brazil: soil genesis and landscape development. Wageningen, Wageningen Agricultural University, 1998. 186p. (Tese de Doutorado).

Murray, K.R., Fitzpatrick, R.W., Bottril, R., Kobus, H.J., 2016. Interpreting the transfer method of soil evidence on clothing by identifying trace soil patterns. *Forensic Science Internationa*l2 258.

Murray, R.C. (2011). Evidence from the Earth: Forensic Geology and Criminal Investigation. Second edition. *Mountain Press Publishing*, Missoula, MT.

Murray, K. R. et al. Patterns produced when soil is transferred to bras by placing and dragging actions : The application of digital photography and image processing to support visible observations. *Forensic Science International*, v. 276, p. 24–40, 2017.

Nakai, I., Furuya, S., Bong, W., Abe, Y., Osaka, K., Matsumoto, T., Itou, M., Ohta, A., Ninomiya, T. (2014). Quantitative analysis of heavy elements and semi-quantitative evaluation of heavy mineral compositions of sediments in Japan for construction of a forensic soil database using synchrotron radiation X-ray analyses. *X-Ray Spectrometry* 43, 38–48.

Olatunji OO, Oyeyiola Y, Oyediran GO. Assessment of Dithionite and Oxalate Extractable Iron and Aluminium Oxides on a Landscape on Basement Complex Soil in South-Western Nigeria. 2015;266–275.

Oliveira Jr JC, Melo VF, Souza LCP, Rocha HO. Terrain attributes and spatial distribution of soil mineralogical attributes. *Geoderma*, 2014, 213, 214-225.

Pašalić H, Aquino AJA, Tunega D, Haberhauer G, Gerzabek MH, Lischka H. Cation–π interactions in competition with cation microhydration: a theoretical study of alkali metal cation–pyrene complexes. *Journal of Molecular Modeling*. 2017; 23:131.

Pereira T, Ker J, Schaefer C, de Barros N, Neves J, Almeida C. Gênese de latossolos e cambissolos desenvolvidos de rochas pelíticas do Grupo Bambuí - Minas Gerais. *Revista Brasileira de Ciencia do Solo*, 2010 vol: 34 (4) pp: 1283-1295

Pires ACD, De Melo VF, Motta ACV, Lima VC. Majors soil classes of the metropolitan Region of Curitiba (PR), Brazil: II - Interaction of Pb with mineral and organic constituents. *Brazilian Archives of Biology and Technology*. 2007;50:183–192.

Pirrie, D., Dawson, L. A., Graham, G. (2017). Predictive geolocation: Forensic soil analysis for provenance determination. *Episodes* 40, 141–147.

Poggere GC, Melo VF, Francelino MR, Schaefer CE, Simas FN. Characterization of products of the early stages of pedogenesis in ornithogenic soil from Maritime Antarctica. *European Journal of Soil Science*, January 2016,67 , 70–78.

Poggere GC, Inda AV, Barrón V, Curi N. Maghemite quantification and magnetic signature of Brazilian soils with contrasting parent materials. *Applied Clay Science,* 2018, 161.

Prandel L V., Saab SC, Brinatti AM, Giarola NFB, Leite WC, Cassaro FAM. Mineralogical analysis of clays in hardsetting soil horizons, by X-ray fluorescence and X-ray diffraction using Rietveld method. *Radiation Physics and Chemistry.* Elsevier; 2014;95:65–68.

Prandel, L. V., Melo, V. F., Brinatti, A. M., Saab, S. C., Salvador, F. A. S. (2017) X-ray Diffraction and Rietveld Refinement in Deferrified Clays for Forensic Science. *Journal of Forensic Sciences*, 1–7.

Pye, K., Blott, S.J., Wray, D.S., 2006. Elemental analysis of soil samples for forensic purposes by inductively coupled plasma spectrometry - precision considerations. *Forensic Science International* 160, 178–192. https://doi.org/10.1016/j.forsciint.2005.09.012

Pye K, Blott SJ. Particle size analysis of sediments, soils and related particulate materials for forensic purposes using laser granulometry. *Forensic Science International.* 2004;144:19–27.

Pye K, Blott SJ, Croft DJ, Witton SJ. Discrimination between sediment and soil samples for forensic purposes using elemental data: An investigation of particle size effects. *Forensic Science International*. 2007;167:30–42.

Ramos PV, Dalmolin RSD, Marques Júnior J, Siqueira DS, Almeida JA, Moura-Bueno JM. Magnetic Susceptibility of Soil to Differentiate Soil Environments in Southern Brazil. *Rev Bras Cienc Solo.* 2017;41:e0160189.

Ravansari, R., Lemke, L. D. (2018). Portable X-ray fluorescence trace metal measurement in organic rich soils: pXRF response as a function of organic matter fraction. *Geoderma* 319, 175–184.

Reidy, L. et al. Elemental fingerprinting of soils using ICP-MS and multivariate statistics: A study for and by forensic chemistry majors. *Forensic Science International,* v. 233, n. 1–3, 2013.

Ritz, K., Dawson, L., Miller, D., 2008. Criminal and Environmental Soil Forensics.

Rossel, R.A.V., Minasny, B., Roudier, P., Bratney, A.B.M., 2006. Colour space models for soil science. *Geoderma* 133, 320–337.

Rowe, W.F., 2005. Forensic Application, in: Encyclopedia of Soils in the Environment.

Ruffell, A. & McKinley, J. (2008). Geoforensics. John Wiley and Sons, Ltd., Chichester.

Ruffella A, Wiltshire P. Conjunctive use of quantitative and qualitative X-ray diffraction analysis of soils and rocks for forensic analysis. *Forensic Science International.* 2004;145:13–23.

Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Oliveira JB, Coelho MR, Lumbreras JF, Cunha TJF. Sistema brasileiro de classificação de solos. 3. ed. Rio de Janeiro: Embrapa Solos; 2013.

Saye SE, Pye K. Development of a coastal dune sediment database for England and Wales: forensic application. In: Pye K, Croft DJ, organizadores. Forensic Geoscience: Principles, Techniques and Application. London: *Geological Society Special Publications Society;* 2004. p. 232.

Shudofsky KN, Burden A, Vestergaard P, De Vries F. Uit het nederlands platform voor farmaceutisch onderzoek: Fractuurrisico bij patienten met COPD die intermitterend hooggedoseerde glucocorticoiden gebruiken. *Pharmaceutisch Weekblad.* 2017;152:27–30.

Schulze, D. G. G. (1984). The influence of aluminum on iron oxides. VIII. Unit-cell dimensions of Al-substituted goethites and estimation of Al from them. *Clays and Clay Minerals* 32, 36–44.

Schwertmann, U., Kämpf, N. (1985). Properties of goethite and hematite in kaolinitic soils of Southern and Central Brazil. *Soil Science Society American Journal,* 139.

Schwertmann, U., Taylor, R. M. Iron oxides. In: Dixon JB, Weed SB, organizadores. Minerals in soil environments. 2o ed Madison: Soil Science Society of America; 1989. p. 380–427.

Schöning I, Knicker H, Kögel-knabner I. Intimate association between O/N-alkyl carbon and iron oxides in clay fractions of forest soils. *Org. Geochem.*, 2005; 36:1378-1390.

Simas FNB, Schaefer CEGR, Melo VF, Guerra MBB, Saunders M, Gilkes RJ. Clay-sized minerals in permafrost-affected soils (Cryosols) from King George Island, Antarctica. *Clay Clay Miner*. 2006;54:721-36. https://doi.org/10.1346/CCMN.2006.0540607

Singh, B. & Gilkes, R.J. Concentration of iron oxides from soil clays by 5 M NaOH treatment: The complete removal of sodalite and kaolin. *Clay Miner.*, 26:463-472, 1991.

Smith, D., Mayes, R.W., Raats, J.G., 2001. Effect of species, plant part, and season of harvest on n-alkane concentrations in the cuticular wax of common rangeland grasses from southern Africa. *Australian Journal of Agricultural Research* 52, 875–882. https://doi.org/10.1177/096805190100700205 01

StatSoft I. STATISTICA (data analysis software system). 2011.

Stoney, D. A.; Bowen, A. M.; Stoney, P. L. Loss and replacement of small particles on the contact surfaces of footwear during successive exposures. *Forensic Science International,* v. 269, p. 78–88, 2016

Stoppe, N., Amelung, W., Horn, R. (2015). Extracción química de oxi(hidrox)óxidos sedimentario utilizando oxalato de amonio y ditionito sódico revisado - una explicación de los procesos en sedimentos costeros. *Agro Sur* 43, 11–17.

Sugita, R., Marumo, Y., 1996. Validity of color examination for forensic soil identification. *Forensic Science International* 83, 201–210. https://doi.org/10.1016/S0379-0738(96)02038-5

Tan KB, Hajek IB, Barshad I. Termal analysis techniques. In: Klute A, editor. Methods of soil analysis. Physical and mineralogical methods. 2nd ed. Madison: *American Society of Agronomy*; 1986. Pt 1. p. 151-83.

Testoni SA, Melo VF, Dawson LA, Salvador FAS, Prandel LV. Evaluation of forensic soil traces from a crime scene: robbery of a safety deposit box in Brazil. *Geological Society, Special Publications*. 2019a DOI: https://doi.org/10.1144/SP492-2019-35

Testoni SA, Melo VF, Dawson LA, Salvador FAS, Kunii PA. Validation of a Standard Operating Procedure (SOP) for forensic soils investigation in Brazil. *Rev Bras Cienc Solo.* 2019b;43:e0190010. https://doi.org/10.1590/18069657rbcs20190010

Thanasoulias, N.C., Piliouris, E.T., Kotti, M.S.E., Evmiridis, N.P., 2002. Application of multivariate chemometrics in forensic soil discrimination based on the UV-Vis spectrum of the acid fraction of humus. *Forensic Science International* 130, 73–82. https://doi.org/10.1016/S0379-0738(02)00369-9

Tighe, M. et al. Georeferenced soil provenancing with digital signatures. *Scientific Reports,* v. 8, n. 1, p. 1–9, 2018.

Torrent, J., Cabedo, A. (1986). Sources of iron oxides in reddish brown soil profiles from calcarenites in Southern Spain. *Geoderma* 37, 57–66.

Uitdehaag S, Wiarda W, Donders T, Kuiper I. Forensic Comparison of Soil Samples Using Nondestructive Elemental Analysis. Journal of Forensic Sciences. 2017;62:861–868.

USEPA, Method 3052 – Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices. 1997.

Valle L, Rodrigues S, Ramos S, Pereira H, Amaral D, Siqueira J, Guilherme L. Beneficial use of a by-product from the phosphate fertilizer industry in tropical soils: Effects on soil properties and maize and soybean growth. *Journal of Cleaner Production*, 2016 vol: 112 pp: 113-120

Van Breemen N, Buurman P. Soil formation. 2nd ed. Netherlandes: *Kluwer Academic Publishers;* 2002.

Wald C. The soil sleuth. *Nature*. 2015;520:422-4. https://doi.org/10.1038/520422a

Woods, B., Lennard, C., Kirkbride, K. P., Robertson, J. (2014). Soil examination for a forensic trace evidence laboratory-Part 1: Spectroscopic techniques. *Forensic Science International* 245, 187–194.

Woods B, Lennard C, Kirkbride KP, Robertson J. 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*. 2016;262:46-55. https://doi.org/10.1016/j.forsciint.2016.02.034

Wowk, G. I. T., Melo, V. F. (2005). Evaluation of the level of lead, in floodplain soil, from the recycling of bacteria. *Brazilian Journal of Agricultural Engineering* 9, 613–622.

Zanardo, A.: Marques Junior, J. Conceitos Básicos Em Mineralogia. In: Alleoni, L.R.F.; Melo, V.F. (Eds) Química E Mineralogia Do Solo. Viçosa,-Mg, Sbcs, 2009, V.1, P.73-150.

Zhang L, Luo L, Zhang S. Integrated investigations on the adsorption mechanisms of fulvic and humic acids on three clay minerals. *Colloids Surf.* 2012; 406: 84–90.

Zhao W, Tan WF. Quantitative and structural analysis of minerals in soil clay fractions developed under different climate zones in China by XRD with Rietveld method, and its implications for pedogenesis. *Applied Clay Science*. Elsevier; 2018;162:351–361.

APÊNDIX 1 – PROCEDIMENTO OPERACIONAL PADRÃO (POP) PARA COLETA DE VESTÍGIOS DE SOLO EM LOCAL DE CRIME.

Ministério da Justiça
Secretaria Nacional de Segurança Pública

PROCEDIMENTO OPERACIONAL PADRÃO (POP)
PERÍCIA CRIMINAL

SOLOS FORENSES
COLETA DE VESTÍGIOS E MICROVESTÍGIOS DE SOLO EM LOCAL DE CRIME

FINALIDADE

O principal objetivo nas análises de solos forenses é associar o solo coletado em um local de crime com vestígios coletados em sapatos, roupas, ferramentas, veículos, etc. do suspeito. As partículas coloidais do solo (principalmente argila) apresentam alta área superficial específica e cargas elétricas de superfície, o que lhes confere capacidade de adesão no estado úmido ou molhado a outras superfícies. Neste âmbito, orientar o profissional de perícia na coleta de vestígios e microvestígios de solo em locais de crime para futura realização de análises químicas, físicas e mineralógicas, bem como destinação de alíquotas para exames entomológicos, palinológicos e de metagenômica, é fundamental para que a associação mencionada se estabeleça. O procedimento de coleta de vestígios e microvestígios de solo é uma fase de primordial importância na cadeia de custódia, que deve ser mantida e documentada durante toda a investigação, chegando íntegra até o fim do processo criminal. Consequentemente, todo o processo de coleta, bem como a armazenagem segura das amostras, a caracterização do solo e os métodos analíticos, requerem procedimentos rigorosos, que têm como finalidade tornar o material coletado um elemento probatório e finalmente, assumir validade indubitável no âmbito da justiça.

PÚBLICO ALVO:

Profissionais da perícia criminal.

1. ABREVIATURAS E SIGLAS

Não se aplica.

2. RESULTADOS ESPERADOS

Padronização dos procedimentos de coleta de vestígios e microvestígios de solo em locais de crime para fim de análises das ciências forenses do solo. Para que esta padronização seja efetiva, devem-se considerar os seguintes aspectos relativos aos procedimentos de coleta: em uma cena de crime, as amostras de solo podem ser classificadas por duas formas, amostras questionadas e amostras conhecidas. As amostras questionadas têm origem desconhecida ou contestada e frequentemente pertencem à vítima ou suspeito. As amostras de origem conhecida podem assumir três tipos, conforme sua associação com o material sob investigação: i) amostra de solo controle – geralmente provém de locais de cena do crime; (ii) amostra de solo referência – pode abranger as amostras mantidas em museus ou arquivos destinados a amostras de solo (soloteca) e material geológico, amostras coletadas em locais ligados à vítima, amostras oriundas de locais ou áreas encontrados em mapas de solos ou geológicos, que podem ser usados para ajudar a fazer comparações entre solos; e (iii) amostras de solo álibi – geralmente provenientes de locais sugeridos pelo suspeito ou ligadas a um suspeito.

Para que os resultados esperados sejam alcançados, deve-se considerar que as comparações entre as amostras questionadas com uma ou mais amostras de origem conhecida são normalmente baseadas em várias propriedades pedológicas, físicas, mineralógicas, biológicas e químicas, que visam avaliar a significância das semelhanças e diferenças observadas para se chegar a uma conclusão sobre uma possível associação.

A comparação avaliativa do solo através de vestígios de evidência em comparação com outro, ou em comparação com um local conhecido, pode servir como evidência nos tribunais.

3. MATERIAL

3.1 Identificação e registro do local do crime

Fita para demilitação do local do crime

Canetas esferográficas

Formulário para rotulação da cena. Aqui deve incluir espaço para esquematizar a cena de coleta de amostras de solo, apresentando detalhes da área, como utilização atual do solo (agrícola, urbana, etc.), cobertura vegetal, cor do solo (importante identificar áreas com cores diferentes de solo), presença de lençol freático aflorante, entre outros importantes.

Câmera fotográfica para registros próximos (por exemplo, de características específicas e gerais) e registros em perspectiva, obtidos a uma certa distância, para fornecer informações sobre o contexto ambiental. As fotografias devem ser rotuladas com o nome do caso, a coleta de pessoas, a data e o horário. As fotos devem estar em sintonia com o desenho/esquema da cena da cena de coleta das amostras de solo.

Aparelho de GPS

Escalas para fotos

Modernamente, scanners 3D e drones

Marcadores de vestígio / posições de coleta

3.2 Coleta e armazenamento das amostras

Canetas de tinta permanente

Luvas de látex descartáveis e limpas. Não utilizar pó de talco nas luvas pois há risco de a camada de silicato do talco contaminar a amostra de solo

Etiquetas para identificação dos locais selecionados para coleta de vestígios e etiquetas para identificação de registro inequívoco do vestígio, contendo data, hora, local, nome do responsável pela coleta, que devem ser colocadas nos recipientes contendo os vestígios de solo coletados

Espátulas de aço inoxidável ou de plástico

Pinceis de diferentes tamanhos (um para cada de tipo de suporte)

Pinças

Facas

Escova pequena

Colher

Embalagens plásticas (potes universais) lacráveis com etiquetas de identificação

Swabs estéreis embalados individualmente, secos e umedecidos com água destilada, ambos para coleta em superfícies (pele, tecido, sola de sapato, ferramentas, etc) contendo microvestígios de solo (um para cada suporte)

Fitas adesivas

Rolinhos para limpeza de roupas

Aspirador portátil com filtros descartáveis

Máscara facial

4. PROCEDIMENTOS

4.1 Ações preliminares

A obtenção de informações gerais antes da amostragem do solo será benéfica para situar a coleta em um contexto mais amplo e integrado. Estas informações, quando passíveis de obtenção, incluem características do caso, levantamento do solo da área, informação de levantamento geológico, mapas apropriados (topográficos e rodoviários) e a localização exata do local de coleta, incluindo coordenadas geográficas (GPS) e mapa esquemático de rota com os acessos para se chegar ao local de coleta.

Como ações preliminares à coleta, sugerem-se:

Conferência de todos os materiais necessários à coleta e garantia de sua validade e manutenção (checklist)

Avaliar as condições climáticas para cada estratégia de coleta (caso chova, os vestígios de solo no local do crime podem ser removidos ou alterados)

Proceder de modo a evitar a contaminação com outros materiais, bem como com outros tipos de solo

A coleta e a embalagem do suporte/objeto/amostra com solo devem ser feitas de modo a não prejudicar outras análises, tais como papiloscópicas, balísticas ou de genética forense. Projéteis que contenham resíduos de solo devem ser coletados sem a utilização de pinça, de forma a preservar suas marcas individualizadoras. Nesse caso, pincéis são mais apropriados para a coleta.

4.2 Coleta de vestígios de solo

Esta seção apresenta resumidamente os procedimentos gerais a serem efetuados para que as amostras de solo coletadas tornem-se apropriadas para os objetivos específicos da investigação forense do solo. Na seção Anexos são apresentados os esquemas de coleta em função da natureza de geração dos vestígios de solo.

O processo de coleta e armazenamento das amostras de solo necessita fundalmentalmente obedecer alguns cuidados, para que todo o material coletado seja aproveitado posteriormente. Estes cuidados envolvem o uso constante de ferramentas limpas e não porosas, como ferramentas feitas de aço inoxidável (para amostras de solos mais duros) ou ferramentas plásticas (para amostras de solo mais macios). As amostras de solo devem ser armazenadas em frascos ou saquinhos de polietileno. Ainda, grandes pedaços de solo devem ser mantidos em um único pedaço, e se possível, deve ser armazenado em pote de tamanho adequado ou em plástico bolha devidamente lacrado.

IMPORTANTE: quando vestígios de solo forem encontrados, a coleta deve ser feita: i) com um swab úmido – indicado para coleta de microvestígio de solo, como pequenas quantidades de solo localizadas em orifícios nasais e auriculares de vítimas; ii) pincel, espátula ou escova pequena – para coleta onde os vestígios aparecem em maiores quantidades (acima de 0,05 g), como em solados de sapato e rodados de veículos. Um detalhe muito importante no caso de superfícies que geram maiores quantidades de vestígios de solos é nunca misturar amostras de solos de cores diferentes. Caso tenha impregnado outro material ao solo (fragmento vegetal, pedaço de vidro, fio de cabelo, etc.), identificar e colocar esses resíduos em frascos separados. Na sala de recepção e armazenagem das amostras de solo, os frascos/saquinhos devem ser abertos e as amostras deixadas para secar ao ar. Essa sala deve ser fechada e não sujeita a correntes de vento para evitar a contaminação das amostras. Após secagem, os frascos/saquinhos devem ser fechados e selados com fita de prova serrilhada indicadora de violação.

Vestígios de solo puro

- Identificar os pontos de coleta, que podem ser rodagens de veículos, tapetes internos de veículos, ferramentas, roupas, calçados e outros. Avaliar marcas deixadas por rodagens de veículos, bem como por pegadas de pessoas e animais. Amostras de solo contidas debaixo de um veículo devem ser coletadas, bem como da frente e traseira do veículo coletadas separadamente. Tomar o cuidado de examinar outras áreas, tais como arcos de rodas, rodas interiores, poços de pára-lamas e outras áreas de captação debaixo do veículo para coleta de possíveis amostras de solo (Figura 1 a 4). Documentar a coleta das diferentes localizações das amostras. Ainda, devem ser consideradas amostras dos filmes de pó dos veículos, que consiste em uma área de aproximadamente 10 cm a 20 cm, localizada acima do fundo exterior das portas do veículo e é onde o solo é lançado para cima do veículo a partir dos pneus (Figura 5 e 6). Este filme de pó pode ser amostrado usando um swab ou escova, pincel ou espátula, conforme a quantidade de vestígio (ver orientações anteriores).

Figuras 1 e 2. Locais de comum aderência de vestígios de solo em veículos: arcos de rodas, rodas interiores, poços de pára-lamas e outras áreas de captação debaixo do veículo para coleta de possíveis amostras de solo.

Figuras 3 e 4. Locais de comum aderência de vestígios de solo em veículos: arcos de rodas, rodas interiores, poços de pára-lamas e outras áreas de captação debaixo do veículo para coleta de possíveis amostras de solo.

Figuras 5 e 6. Locais de comum aderência de vestígios de solo em veículos: arcos de rodas, rodas interiores, poços de pára-lamas e outras áreas de captação debaixo do veículo para coleta de possíveis amostras de solo.

Vestígios de solo associado

Vestígios de solo associado são fluidos biológicos ou minerais, tais como sêmen, sangue, urina e outros tipos de produtos químicos, como gasolina, álcool, óleos, que podem estar impregnados nas amostras de solo. Sua classificação e análises dependem do contexto ambiental e dos fins criminalísticos a que se objetivam, pois exames de DNA, ópticos ou físico-químicos (espectroscopia, MEV, absorção atômica etc.) podem ser necessários e concomitantes.

Havendo condições apropriadas, após uma possível remoção do material impregnante, os vestígios de solo associados deverão ser secos à temperatura ambiente e ao abrigo da luz solar, em condições que evitem contaminações, conforme descrito anteriormente.

Vestígios de solo associados que não podem ser inicialmente secos para preservação do material biológico associado devem ser congelados. Quando as condições permitirem, o material associado deve ser descongelado e após sua separação o solo deve ser seco a temperatura ambiente.

5. PONTOS CRÍTICOS

Proceder à coleta dos vestígios de solo logo após a ocorrência do crime/evento, pois os eventos climáticos, e outros fatores, podem alterar as verdadeiras características do material.

Identificar previamente os pontos de coleta e os frascos onde serão armazenados os vestígios, pois, à primeira vista, os vestígios possuem muita semelhança entre si.

Diferentemente dos materiais biológicos, amostras de solo têm normalmente pouco potencial infectante. Entretanto, por ser na maioria das vezes impregnado com substâncias desconhecidas, a priori, recomenda-se o emprego de Equipamentos de Proteção Individual (EPI) adequados ao manuseio desse material. Espátulas e objetos semelhantes usados na coleta não devem ser necessariamente descartados, mas sim higienizados entre uma coleta e outra. Recomenda-se a lavagem e condicionamento adequado. Pincéis, swabs e objetos assemelhados não devem ser reutilizados.

6. ESTRUTURA BÁSICA DO LAUDO

Não se aplica.

7. REFERÊNCIAS

Brasil. Secretaria Nacional de Segurança Pública. **Procedimento operacional padrão: perícia criminal / Secretaria Nacional de Segurança Pública**. Brasília: Ministério da Justiça, 2013. 242 p.

Fitzpatrick, R. W. & Raven, M. D. **Guidelines for Conducting Criminal and Environmental Soil Forensic Investigations: Version 10.1**. Centre for Australian Forensic Soil Science. Australian. 2016.

8. GLOSSÁRIO

SWAB: chumaço de algodão, ou outro material absorvente, preso a uma extremidade adequadamente esterilizada e que se emprega ou para aplicação de medicamento, ou para coleta, por atrição, de material destinado a estudos.

9. ANEXOS

9.1. Procedimentos para coleta de amostras de solo em locais de crime

Situação 1 - vítima ou objeto de delito encontrados em área com afloramento de solo

a) coletar amostra de solo na vítima ou no objeto do delito (por exemplo, cofre descartado após arrombamento), ilustrado em verde na Figura 7. Caso a quantidade de amostra de solo seja muito reduzida, conforme discriminado anteriormente (< 0,05 g) (ver item 4.2), usar o swab úmido para coleta. Na situação onde há grande dispersão de resíduo de solo na vítima ou no objeto, mesmo onde a cor das amostras de solo seja similar, coletar separadamente amostras de cada local na vítima ou no objeto (frascos separados).

b) realizar uma grade de amostragem no local, a partir do ponto central da ocorrência criminal (vítima ou objeto), com coleta padronizada em espaços regulares e em mesma quantidade de amostra (Figura 2). Nos pontos do entorno (4 a 10 pontos, de acordo com a análise do perito no momento do exame) deve-se coletar cerca de 40 g (1 colher) de amostra de solo da camada superficial (0 a 3 cm). Se os pontos mais próximos ao centro do delito estiverem totalmente cobertos por vegetação (situação que não produz vestígios de solos no suspeito), procurar pontos mais distantes, de preferência no possível caminhamento do suspeito na fuga do local do delito, onde o solo aflora para coleta das amostras.

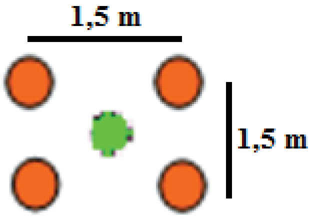


Figura 7 - Esquema de coleta de amostra regulares (pontos em laranja) na volta do ponto central do delito (ponto em verde). Como exemplo, foi escolhida uma situação, de acordo com a análise do perito no momento do exame, com coleta de apenas quatro pontos no entorno do delito. As amostras regulares devem ser espaçadas umas das outras em 1,5 m. Colocar todas as amostras em frascos separados. Caso sejam coletadas 10 amostras regulares em um crime de acordo com a análise do perito no momento do exame, manter a distância de 1,5 m e distribuir os pontos em malha na volta do centro do delito.

Situação 2 - coleta de vestígio de solo no suspeito do crime: solado de sapato, rodado de veículo, interior e exterior de veículo, etc.

a) Como exemplo de coleta será usado o solado de um sapato (situação vale para as outras superfícies). Um detalhe muito importante no caso de superfícies que geram maiores quantidades de vestígios de solos é nunca misturar amostras de solos de cores diferentes. Se em um sapato tiver solo de cores escura, vermelha e amarela em diferentes posições do solado, coletar três amostras-vestígios em diferentes recipientes. Caso a amostra de um mesmo local do sapato apresente mais de uma cor (sobreposição de amostras diferentes) coletar o conjunto da amostra em um único recipiente, mas tomando-se o cuidado de não desagregar a amostra. No laboratório, após secagem da amostra, o analista irá separar as amostras sobrepostas por classe de cor. Caso a quantidade de amostra de solo seja muito reduzida, conforme discriminado anteriormente (< 0,05 g), usar o swab úmido para coleta. Na situação onde há grande dispersão de resíduo de solo, mesmo onde as cores das amostras de solo sejam similares ao longo do solado, coletar pelo menos quatro repetições (frascos separados) (Figura 8).

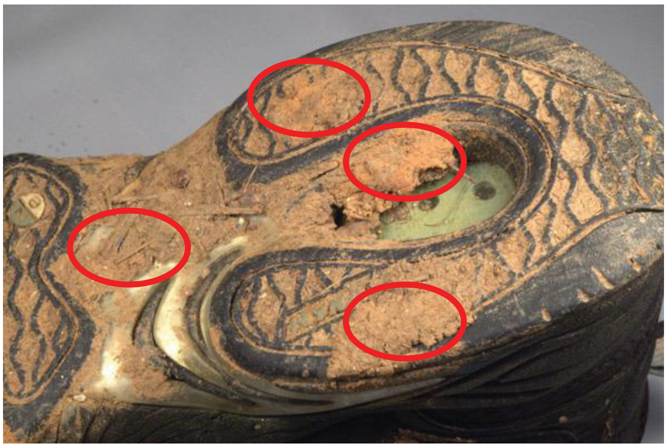
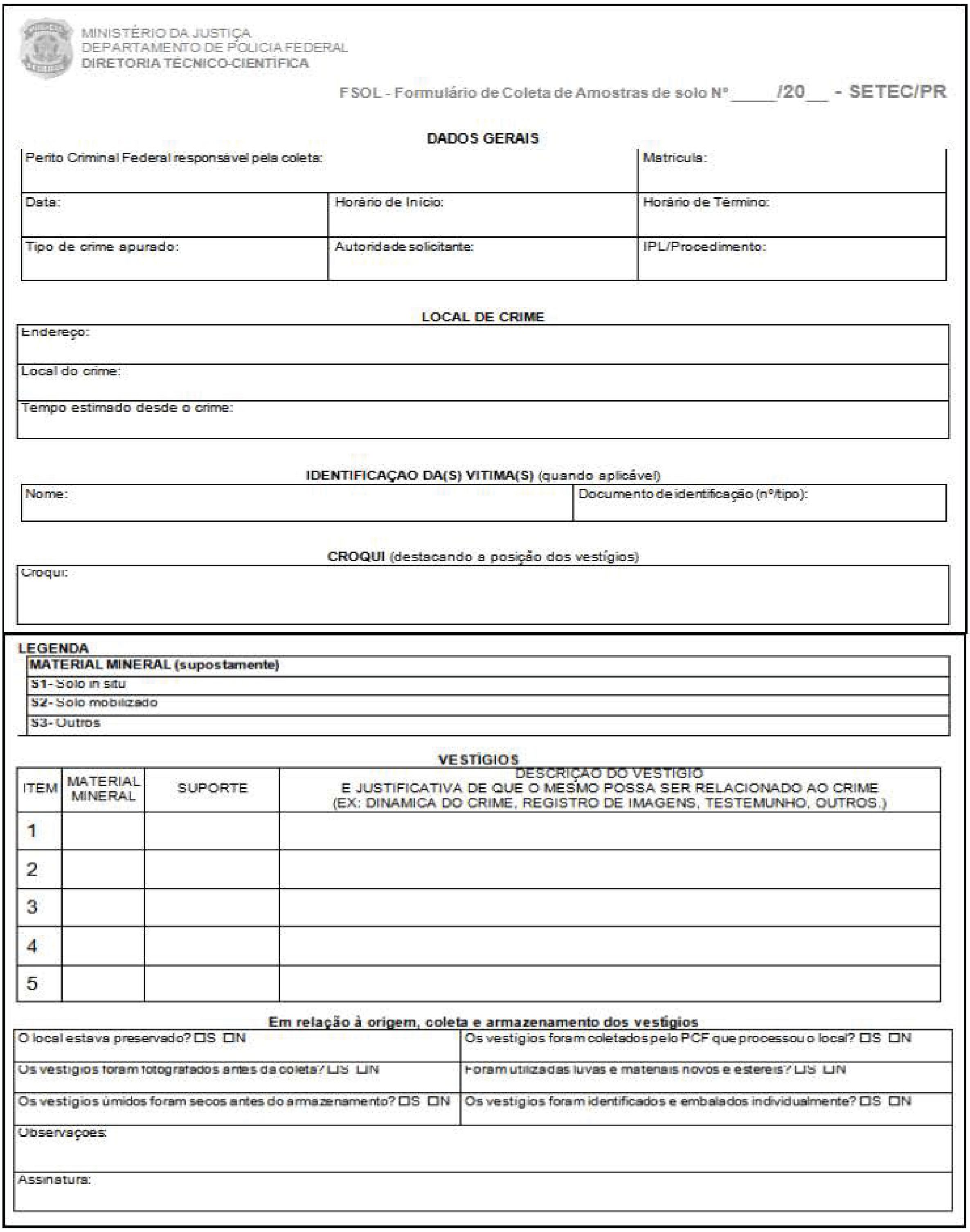


Figura 8 - Demonstração de coleta de quatro repetições de vestígios de solo (círculos em vermelho) em superfície onde ocorreu intensa impregnação de solo.

9.2. Formulário pericial de coleta de vestígios de solo



MINISTÉRIO DA JUSTIÇA
DEPARTAMENTO DE POLICIA FEDERAL
DIRETORIA TÉCNICO-CIENTIFICA

FSOL - Formulário de Coleta de Amostras de solo Nº_____/20__ - SETEC/PR

DADOS GERAIS

Perito Criminal Federal responsável pela coleta:		Matricula:
Data:	Horário de Inicio:	Horário de Término:
Tipo de crime apurado:	Autoridade solicitante:	IPL/Procedimento:

LOCAL DE CRIME

Endereço:
Local do crime:
Tempo estimado desde o crime:

IDENTIFICAÇAO DA(S) VITIMA(S) (quando aplicável)

Nome:	Documento de identificação (nº/tipo):

CROQUI (destacando a posição dos vestígios)

Croqui:

LEGENDA

MATERIAL MINERAL (supostamente)
S1- Solo in situ
S2- Solo mobilizado
S3- Outros

VESTIGIOS

ITEM	MATERIAL MINERAL	SUPORTE	DESCRIÇAO DO VESTIGIO E JUSTIFICATIVA DE QUE O MESMO POSSA SER RELACIONADO AO CRIME (EX: DINAMICA DO CRIME, REGISTRO DE IMAGENS, TESTEMUNHO, OUTROS.)
1			
2			
3			
4			
5			

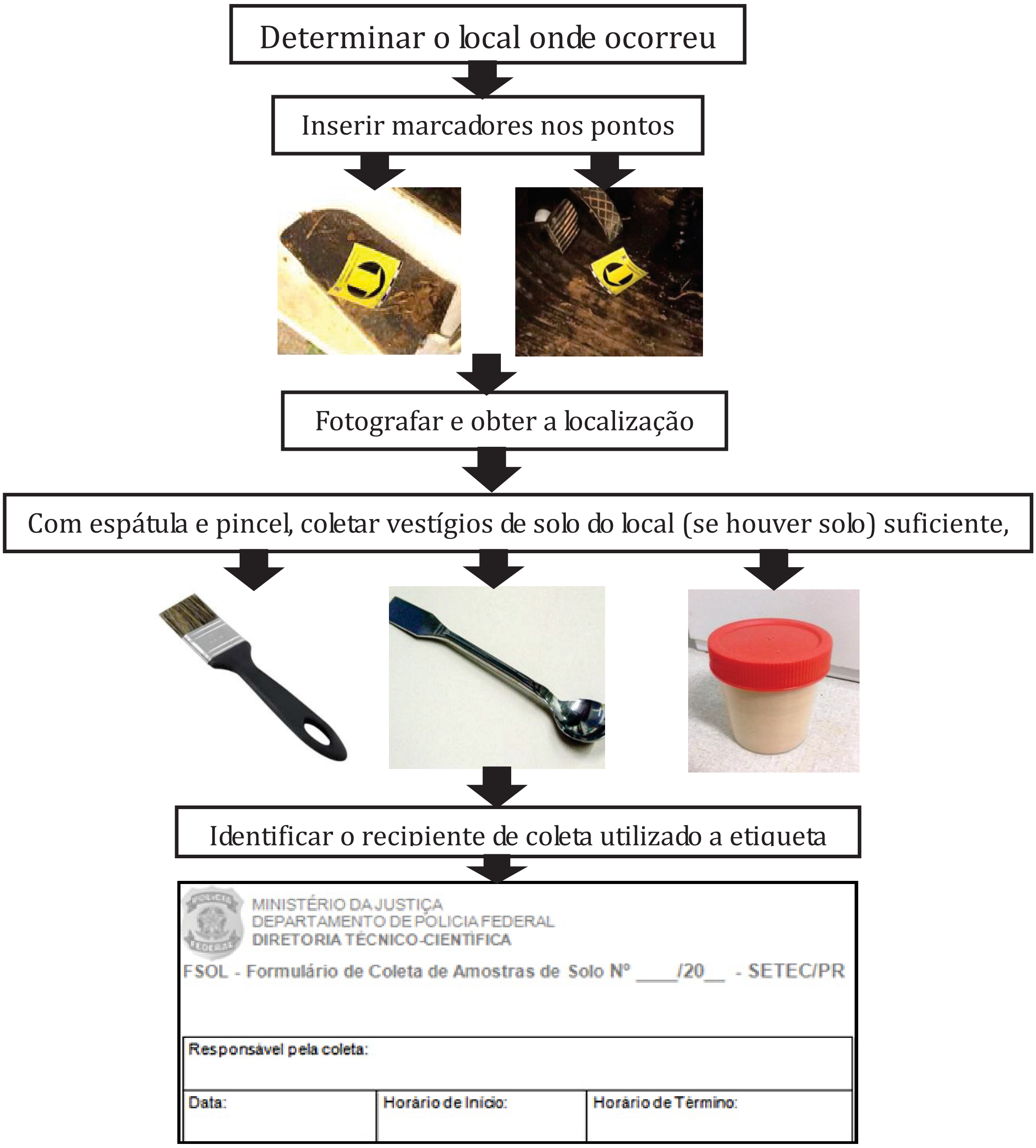
Em relação à origem, coleta e armazenamento dos vestígios

O local estava preservado? ☐S ☐N	Os vestigios foram coletados pelo PCF que processou o local? ☐S ☐N
Os vestigios foram fotografados antes da coleta? ☐S ☐N	Foram utilizadas luvas e materiais novos e estereis? ☐S ☐N
Os vestigios úmidos foram secos antes do armazenamento? ☐S ☐N	Os vestigios foram identificados e embalados individualmente? ☐S ☐N

Observações:
Assinatura:

Figura 9. Formulário para coleta de amostras de solo.

9.3. Procedimentos para coleta de vestígios de solo em locais de crime



MINISTÉRIO DA JUSTIÇA
DEPARTAMENTO DE POLICIA FEDERAL
DIRETORIA TÉCNICO-CIENTÍFICA

FSOL - Formulário de Coleta de Amostras de Solo Nº ____/20__ - SETEC/PR

Responsável pela coleta:		
Data:	Horário de Início:	Horário de Término:

Figura 10. Procedimentos para coleta de vestígios de solo.

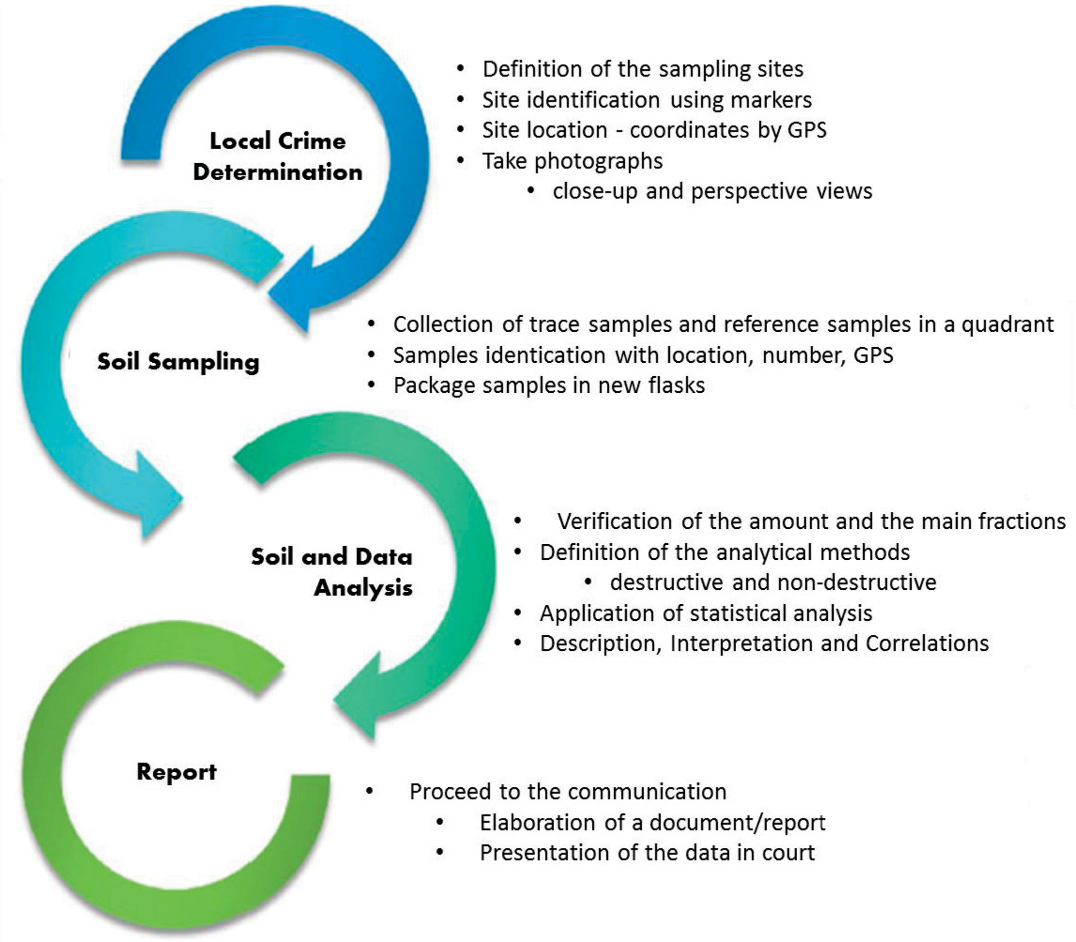


Figura 11. Procedimentos requeridos em casos forenses envolvendo vestígios de solo.