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Case Review

Can analysis of a small clod of soil help to solve a murder case?

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ABSTRACT

Soil forensics utilizes extensive soil information to answer legal questions and test hypotheses. The main difficulty often is the determination of 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 vestiges (0.5 g) from a suspect's vehicle (adhered to the outside 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 could have been in contact with the edge of the Graciosa Road, approximately the place where the victim's body was located. As a result of the soil analysis and comparison, the results support the likely contact of the suspect's vehicle with the crime scene.

1. Introduction

Soil forensics utilizes an extensive range of soil information to elucidate and answer legal questions or hypotheses [1]. Although several studies have reported the use of soil vestiges to assist forensic investigations and judgements [2–5] 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, and can indicate a wide array of information about places visited [4,6,7]. If soil is wet/moist, impregnation on different surfaces and the potential of transferability and persistence of soil vestiges is increased [8,9].

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 [10,11]. This variability is important to increase the traceability of the vestige sample. However, determining the source of a soil sample may never be absolute, and a prediction of source can be useful in many instances [12–14].

There are several techniques which can provide information from vestige 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 [15–17]. 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 vestige investigations is the small amount of sample to work with (normally less than 0.5 g). To improve the strength of evidence, researchers try to produce as many quantitative variables as possible from a small soil vestige [18]. For this purpose, the chemical extraction of short range order (SRO) materials from clay fraction is recommended [19]. The SRO is more variable than crystalline phases in soil and can be different in soils occurring side-by-side in a confined area [20,21] 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 vestiges (0.5 g) sampled on a 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. The sequential extraction approach is not novelty, as it has been applied before [17,19].

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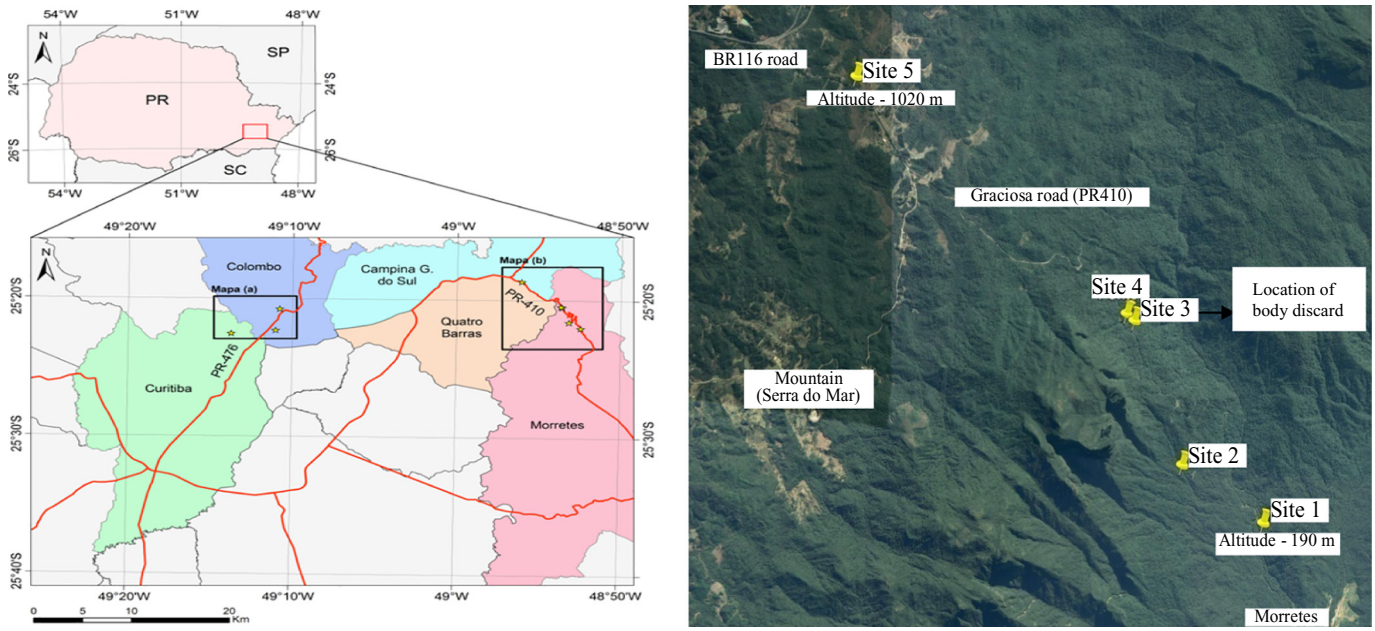

Fig. 1. Paraná State map showing the relative positions of sampling sites in Curitiba, Colombo and Graciosa Road.

2. Methodology

2.1. Case background

On May 2018 a young girl disappeared from Colombo town, 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 May 19th 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 May 9th 2018 were images recorded on the security cameras. The victim's body was found on June 8th 2018, in a forest area at the side of the Graciosa Road (State Road PR-410) (Fig. 1), approximately 50 km away from Colombo town (Fig. 1). The body was discarded at the edge of Graciosa Road (State Road PR-410) at the Sea Range (mountainous relief) approximately 30 m below the road level.

The suspect claimed to be innocent and stated that he did not drive out of the city of Colombo. The suspect attributed the soil vestiges on his vehicle to the traffic in his own city, Colombo.

2.2. 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 vestige adhering to the outside rear-view mirror (Fig. 2) and to the left front fenders (Fig. 2b) in the vehicle. Due the small amount of soil adhering, the samples from the two locations of the vehicle were recovered and combined into a single sample for subsequent analysis. The combination of the two samples collected in the car allowed the generation of a larger number of quantitative variables to make traceability more reliable. The two samples had the same morphological characteristics, such as color, texture and presence of grass leaves.

The potential impregnation of soil vestige to the outside rear-view mirror and to the left front fenders on 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 May 8th 2018 and 24 hours from May 8th 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 spot 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 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 collected in a quadrant, and in addition, two samples on tyre skid 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. 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 town 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 (in press). 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 contamination.

2.3. Sample preparation

Soil samples (reference quadrants, skid marks and vestige questioned sample) were oven-dried at 40 °C for 48 h and sieved through a 2 mm sieve in order to obtain the oven-dried fine earth fraction. A total

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 W	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	1	0-3	712,049	7,195,556	Granite/gneiss
10	(level location)	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	1	0-3	711,934	7,195,655	Granite/gneiss
22	(skid mark)	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 vestige	1		Apprehended by Civil Police		
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

of 0.94 g was available from the vestige 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. Suspensions containing silt + clay + organic matter were placed in porcelain capsules. That process was repeated until the water was limpid. Sand content of soil samples was obtained gravimetrically after the sand drying at 40 °C for 24 h. 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 vestige sample. Therefore, the same amount of this fraction was used for the other samples for subsequent

Fig. 2. Soil vestiges were sampled from the a) outside rear-view mirror and b) 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).

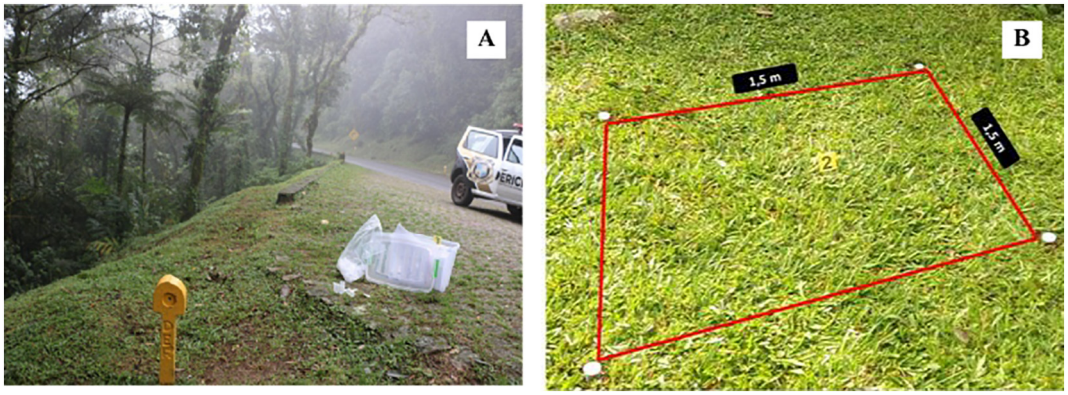

Fig. 3. a) Site 3 (victim's body deposition site) along the Graciosa Road (PR410); b) details of the soil sampling at four replicate corners of a 1.5 m quadrant.

chemical and mineralogical analyses.

2.4. Sand fraction analyses

2.4.1. X-ray diffraction

The sand fraction (approximately 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 °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. Comparison between vestige samples and the samples from Colombo town and Curitiba city 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 10 coincident peaks that were verified in samples from Sea Range and in the vestige sample from the vehicle were compared.

2.4.2. Extraction of total elements with concentrated HF and HNO_3

Sand samples that were submitted to XRD for analysis were recovered (non-destructive analysis). Samples of sand (approximately 0.3 g) were placed in a sealed teflon tube with 9 mL of concentrated HNO_3 and 3 ml of concentrated HF (EPA 3052) [22]. Tubes were subjected to microwave radiation (Mars Xpress 6, CEM) for 5.5 min to reach 180 °C, attaining a maximum pressure of 16 atm for 4.5 min digestion with constant temperature and pressure. Elements in extracts (Al, Ba, Fe, Cu, K, Mn, P, V e Zn) were subsequently obtained by optical emission spectroscopy with inductively coupled plasma (ICP-OES).

2.5. 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 to

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

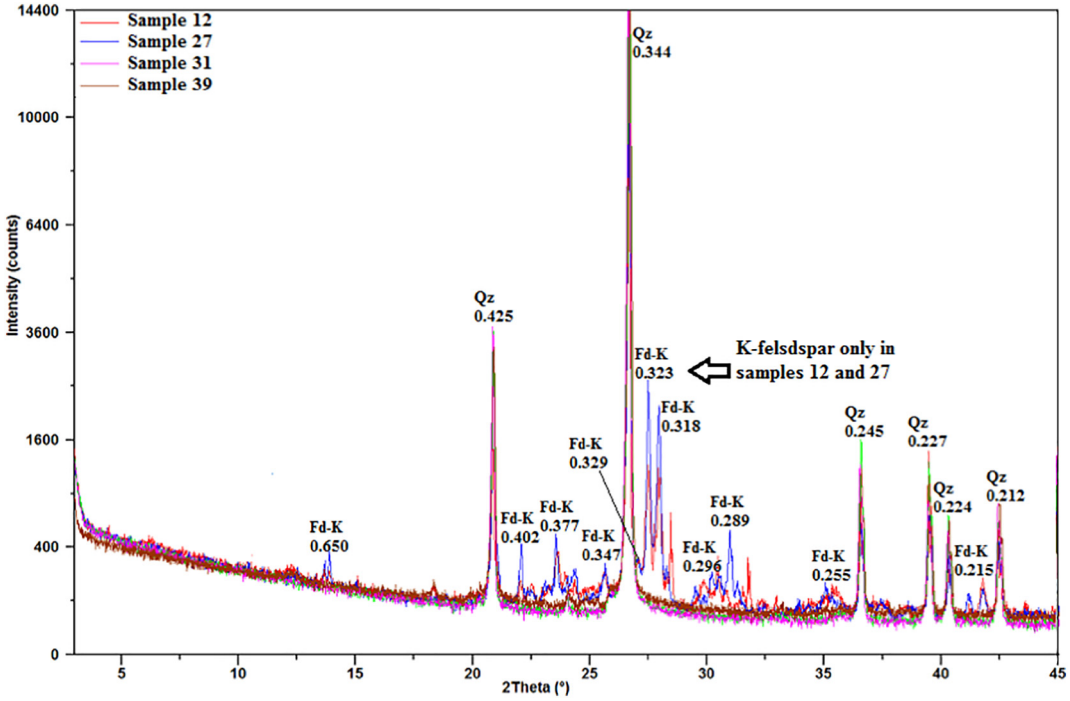

Fig. 5. X-ray diffractograms of the sand fraction from samples randomly selected in the different sites for illustrative purposes: sample 12 - represents samples from the Sea Range, at the edge of the Graciosa Road; sample 31 - represents samples from Curitiba city; sample 39 - represents samples from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

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 25°C – Millipore Direct–Q System) and high purity acids were used in the analyses (Merck PA); iii) before each extraction samples were dried for 24 h 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 samples were oven-dried at 40 °C for 24 h; vi) extracts were obtained by centrifugation (3000 rpm for 10 min) and all extracts were filtered in a slow filter paper (Macherey Nagel®); vii) elemental concentrations (Fe, Al, P, Ca, Mg, Zn, Mn, Cu, Si) were determined by optical emission spectroscopy with inductively coupled plasma (ICP-OES).

2.5.1. 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 °C for 30 min [23]. The sodium hypochlorite promotes the organic matter oxidation and carbon is lose as CO_2.

2.5.2. 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) [24].

2.5.3. Extraction of crystalline iron oxides

Crystalline iron oxides present in the AO residue were extracted using the sodium citrate-bicarbonate-dithionite method (CBD) [25]. 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. Suspensions were manually agitated while heated at 70°C in a hot water bath for 30 min. Extracts of the two extractions were combined for the subsequent elemental analysis.

2.5.4. 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 [25,26]. 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.

2.6. Multivariate statistics

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 [27] and Paleontological Statistics (PAST) software using Bray-Curtis similarities [28]. 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).

3. Results and discussion

3.1. Qualitative results

The XRD data from the sand fraction show that the soil vestiges recovered from the fenders and the outside rear-view mirror from the suspect's vehicle could be excluded as having come from the areas examined in Colombo town and Curitiba city (Fig. 1). Comparison between XRD data of samples was performed initially within each group of samples, in order to verify the homogeneity of 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

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

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	P2	P3	P4	P5	P6	P7	P8	P9	P10
	K-Fd	K-Fd	Qz	Qz	K-Fd	Qz	K-Fd	Qz	Qz	Qz
	(0.650)	(0.485)	(0.425)	(0.402)	(0.347)	(0.334)	(0.323)	(0.245)	(0.227)	(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 the sites are present in Table 1.

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 to represent Curitiba city (samples 28 to 31), sample 39 for Colombo town (samples 32 to 39), and a comparison between them and the vestige sample (27) was realized with the diffractograms showed in Fig. 5.

It was clear from the examination of the two diffraction patterns that the sites could be clearly differentiated: 1) samples collected from Curitiba city and Colombo town 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 vestige sample collected at the suspect's vehicle (sample 27), contained an assemblage of quartz and K-feldspar. Under such a clear distinction it was possible to infer that the vestige sample is comparable only to samples from the Sea Range or elsewhere, possibly from the Graciosa Road region, near to the victim's body deposition site. There is no compatibility of the mineralogical composition between 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 therefore excluded.

3.2. Quantitative results

3.2.1. 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). Distances between the grooves of the tyre coincide with the distances left at the skidding site.

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	P2	P3	P4	P5	P6	P7	P8	P9	P10
	K-Fd	K-Fd	Qz	Qz	K-Fd	Qz	K-Fd	Qz	Qz	Qz
	(0.650)	(0.485)	(0.425)	(0.402)	(0.347)	(0.334)	(0.323)	(0.245)	(0.227)	(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.

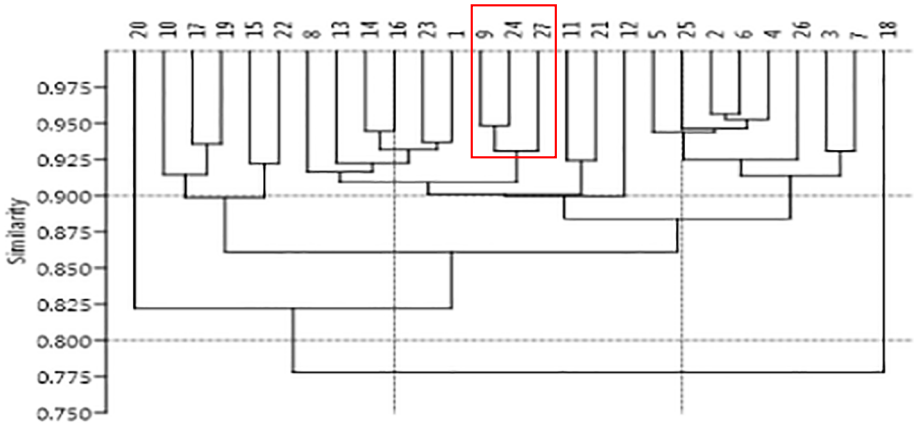

Fig. 7. Dendrogram of similarity by Bray-Curtis of the samples collected at the edge of the Graciosa Road, including the Questioned sample and considering the variables height and area from 10 peaks selected in the X-ray diffractograms of the sand fraction. Identification of the samples is in Table.

3.2.2. Sand fraction

Considering that the vestige sample is comparable only with the samples collected at the edge of the Graciosa Road (Fig. 5), and all other sampled locations can be excluded as bring the source location, the additional analyses in the X-ray diffractograms in the sand fraction of samples collected along the Graciosa Road were tested to indicate the most probable site of transfer of the soil vestige 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 was possible to identify that sample 9 and 24 had grouped with the vestige 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 vestige sample.

The elemental data showed that the samples from Graciosa Road and the questioned sample (sample 27) presented greater concentrations of K (4327 to 21341 mg kg^{-1} and 16934 mg kg^{-1}, respectively) in relation to the samples from Curitiba city and Colombo town, which presented lower concentrations of K (133 to 219 mg kg^{-1} and 103 to 427 mg kg^{-1}, respectively) (Table 4). Similar results were verified for the contents of Al, which presented high concentrations in soil samples from Graciosa Road (6222 to 11895 mg kg^{-1}) and for the vestige sample

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

(8511 mg kg^{-1}) in comparison to the samples from Curitiba city (2056 to 3342 mg kg^{-1}) and Colombo town (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 group of samples was formed based on the content of the total elements in the sand fraction (Table 4). 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 group 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 on the superior level of the Sea Range (sample 23) (Fig. 1).

3.2.3. Silt + clay + organic matter fraction

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 approximately 0.5 g and, before the last sequential extraction (NaOH) the amount was approximately 0.3 g (Table 6).

Samples 17, 19, 20, 21, 22 and 27 formed a group with high homogeneity (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 in a number of reports [3,17–19].

There were 8 groups formed with high similarity between soil samples from the same groups and lower similarity between samples from different groups (Fig. 10). Roughly, groups were formed and separated according the sites of sampling at the Sea Range (Graciosa Road), Curitiba city and Colombo town. Group 1 (similarity intragroup of 95%) - samples collected from site 1 at Sea Range (samples 1, 3 and 4) and samples collected from 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 on 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 chance 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 approximately 300 m away from

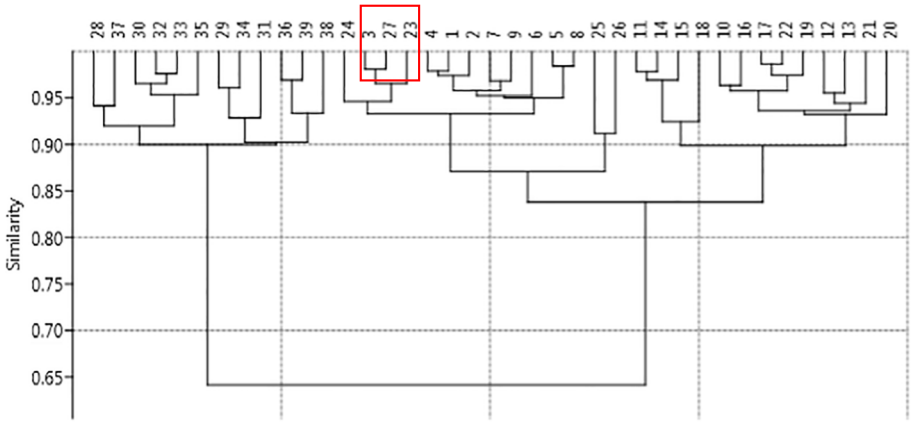

Fig. 8. Dendrogram of similarity by Bray-Curtis considering the content of total elements of the sand fraction as variables.

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

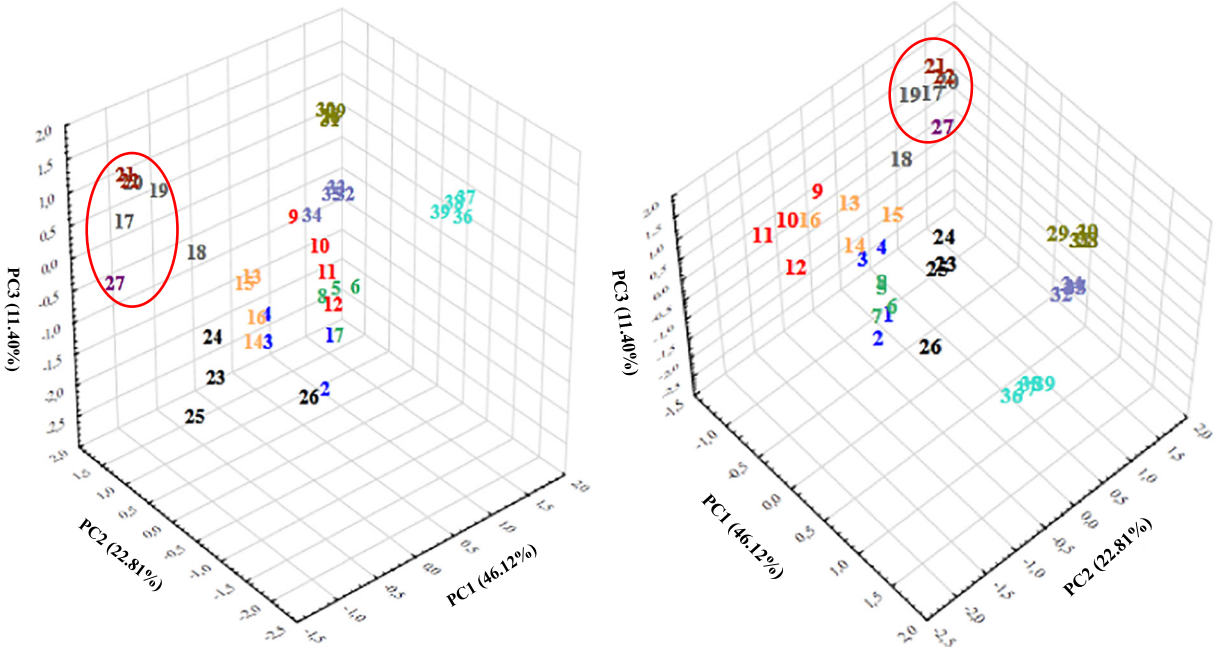

Fig. 9. Scores from the first three principal components of the samples analysed, considering the relative contribution of the total elements extracted in the sequential analyses by NaClO, ammonium oxalate, citrate-bicarbonate-dithionite and NaOH in the silt + clay + organic matter fraction.

Table 6
Initial mass (g) defined by the amount of vestige 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

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). The sample 18 is more closely grouped with sample 25 instead of samples 17, 19 and 20. There was an enrichment of Fe in parent material of soil of the point 18 (Fe-AO = 21599 mg kg^{-1}), which caused the distancing of this sample in relation to the other samples from group 4 (17, 19 and 20). On the other hand, sample 18 presented practically the same contents of Ca-NaClO, P-NaClO and Cu-AO in relation to sample 25. 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 skidding (Fig. 6), there is corroboration regarding the side of soil impregnation at the vehicle: the soil vestige on the fenders and on the outside rear-view mirror was located at the left side passenger in 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; 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 city (samples 28, 30 and 31); Group 7 (similarity intragroup of 97%) - samples collected in Guarani neighbourhood from Colombo town (samples 33, 34 and 35); Group 8 (similarity intragroup of 97%) - samples collected in Guaraituba neighbourhood from Colombo (samples 36, 37 and 38).

Based on the results, it 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 town). 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 vehicle traction, left site 4 and 300 m ahead at the Graciosa Road he found suitable conditions to park his vehicle car and discard the victim's body (site 3).

4. Conclusions

A considerable number of quantitative variables (22) were obtained from a small vestige 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.

Data from 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 chemical and mineralogical analysis of the sand fraction showed that the soil vestige could be excluded as having originated from Curitiba city (claystone) and Colombo town (limestone) 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

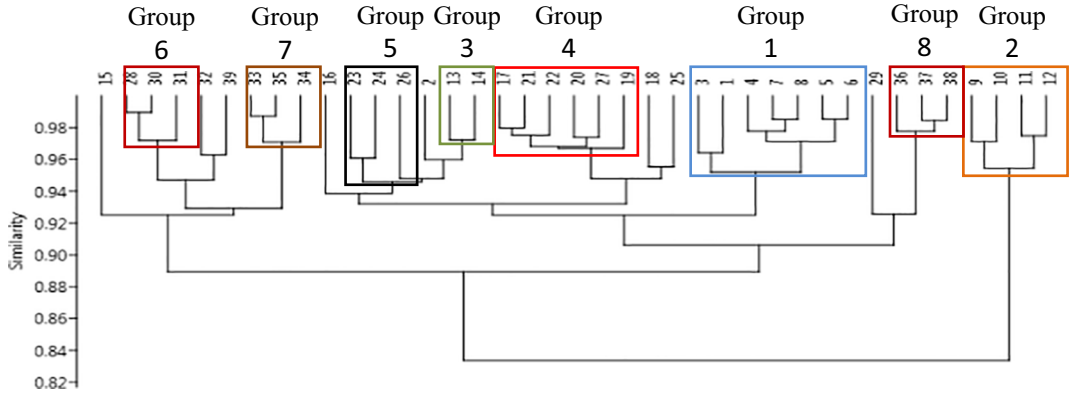

Fig. 10. Dendrogram of the results of the sample analysis, considering the relative contribution of the total elements extracted in the sequential analyses by Na hypochlorite, ammonium oxalate, citrate-bicarbonate-dithionite and Na hydroxide in the silt + clay + organic matter fraction.

soil vestige 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 material evidence of a probable contact of the suspect vehicle with soil material near to the crime scene. This supported the proposition that the vehicle had been positioned at the roadway adjacent to where the victim's body was found.

Declaration of competing interests

There is no conflict of interest.

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