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Potential of soil organic matter molecular chemistry determined by pyrolysis-gas chromatography/mass spectrometry for forensic investigations

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ABSTRACT

Wetlands near urban centers may be more isolated areas and can be chosen for the disposal of bodies or used as a crime scene. The predominant soils in these areas usually have a high content of organic matter (OM), classified as Histosols. Soil organic matter (SOM) is composed of many different compounds that can be identified by pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS). The study aimed to use Py-GC/MS to classify small amounts of organic soil in a forensic context. We sampled Histosols from five representative sites of Curitiba, Brazil. The molecular composition of the samples was determined by byPy-GC/MS. The factor analysis was carried out, and the factor scores showed a clear differentiation between the sites. Compounds indicative of relatively fresh plant material was separated from more recalcitrant and charred material. Py-GC/MS has the potential to be a useful tool to study the composition of SOM in Histosols to track the trace sample collected from a crime suspect.

1. Introduction

The soil can provide relevant information about the relationship between a crime scene and the evidence in a forensic investigation. Many crimes occur under circumstances where the soil can be used as evidence since it is possible in some cases to find soil attached to tools, car tires, shoe soles, and others. Usually in minimal amount but that can provide a link between a criminal and a crime scene.

Sites used for the disposal of bodies or crimes are generally uninhabited, such as river floodplains, where the commonly occurring organic soils make the work of digging a pit and burying something inside it relatively simple. Soils in such areas are often classified as histosols, which, according to the Brazilian Soil Classification System, contain > 80 g kg^{-1} of organic carbon and occur in areas of low temperatures and/or in wetlands [1]. Because they are primarily organic soils, knowing a little about the composition of the organic matter present in these soils can help in the analysis of soil traces, for example, found on the sole of a criminal's shoe and to check whether or not it coincides with the soil of the scene crime. Soil organic matter (SOM) is composed of many types of source compounds at multiple degrees of decomposition, both of which are affected by local environmental conditions [2].

Usually, small amounts of soil (< 1 g) are available to search for evidence, which creates the challenge of analyzing these samples and reach a viable result and conclusion. The SOM analytical techniques normally used, like spectroscopic techniques including carbon 13 Nuclear Magnetic Resonance (^{13}C NMR) and Fourier Transform Infrared Spectroscopy (FTIR), do not provide enough results to discriminate small amounts of soil samples due to analytical limitations and low SOM contents, usually < 5% in mineral soils [3–5]. For this reason, mineral analysis has been generally considered in forensic studies [6]. In soils with high OM content, such as Histosols, the ideal is to. prioritize the analysis of organic compounds.

Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) is a technique that enables the identification of a huge number of molecules obtained by thermal fragmentation [7]. The relative amounts of the molecules can be quantified and give a fingerprint of the composition of SOM of a sample. Py-GC/MS is highly reproducible and very useful method for the comparison of small amounts of material (1–2 mg), thereby having the potential of being an important tool for soil forensic science. This technique consists of the sudden thermal degradation of organic (macro) molecules under an inert atmosphere. The volatilized products are separated based on molecular weight and charge into a gas chromatographic column and identified with the use of mass

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Table 1
Site characteristics.

Area	Sub area	Geographical coordinates (GMS)	City/state	Description	Dominant vegetation
1	A, B, C, D	25°27′45.08″S; 49° 7′9.47″W	Piraquara/PR	H horizon > 120 cm; all subareas with a high water table	*Typha domingensis, Baccharis trimera, Phlebodium decumanum, Cyperaceae*
2	A, B, C, D	25°26′24.61″S; 49° 6′52.32″W	Piraquara/PR	Low water table	*Phlebodium decumanum, Cyperaceae, Hypolytrum pungens.*
3	A, B, C, D	25°34′17.94″S; 49°37′58.33″W	Balsa Nova/PR	H horizon > 120 cm; without a high water table, but the water came after the collection of samples.	*Cyperaceae, Typha domingensis, Bromeliaceae*
4	A, B, C, D	25°36′8.81″S; 49°40′11.22″W,	Balsa Nova/PR	H horizon > 100 cm; high water table; very obvious iron oxidation in the roots.	*Cyperaceae, Phlebodium decumanum*
5	A, B, C, D	25°35′2.32″S; 49°38′34.17″W,	Balsa Nova/PR	H horizon > 40 cm; pasture area; Low water table.	*Brachiaria, Cyperaceae*

spectrometry, by mass/charge (m/z) ratio of ions [7]. Lara-Gonzálo et al. [8] concluded that Py-GC/MS is a rapid alternative to other techniques to study SOM in soils. The authors concluded that Py-GC/MS was the best technique to identify most of the organic contaminants present at the site was studied.

Schellekens et al. [9–13] makes use of this technique many years ago and has several published works analyzing Peat and organic soils. In their work [9], several samples from deep peat in Tierra del Fuego were analyzed using Py-GC/MS to extract parameters that might be applied to the interpretation of peat molecular chemistry in terms of vegetation change, anaerobic and aerobic decomposition and fire incidence-showing that the Py-GC/MS can be a useful tool in the analysis and discrimination of soil organic matter.

For forensic purposes, it is essential to have analytical techniques that are efficient in differentiating samples from different sites and showing the similarity between samples from the same place [14]. This study aimed to use Py-GC/MS to classify small amounts of organic soil in a forensic context.

2. Material and methods

2.1. Soil samples

The study was conducted in the Paraná State - Brazil, using Histosols from five representative sites of Curitiba metropolitan area (Piraquara and Balsa Nova cities) (Table 1 and Fig. 1). For each of the five studied sites, four samples (A, B, C and D) were collected at 0–5 cm of depth.

As the same samples were used for other studies [15], a large amount of soil was collected and processed as usual. However, for this study, only a minimum amount of soil (1 g) was used, simulating a real case of sample traces. The samples were air-dried on plastic bags, homogenized, ground and passed through a 2 mm sieve. After, it was done one partition resulting on average 150 g of each sample. The coarse particulate organic material (large roots) was separated manually. The remaining material (soil) was dried at 40 °C for 24 h. The samples were homogenized and submitted to quartering procedures until about 1 g of each sample was obtained. The standardization of a small initial aliquot was done because vestiges of soil in crime scenes are generally scarce [14].

2.2. Pyrolysis-gas chromatography/mass spectrometry

A Micro-furnace single shot PY-3030S pyrolyser coupled to a GCMS-QP2010 (Frontier Laboratories LTD.) was used. The pyrolysis time was set at 6 s, pyrolysis temperature at 600 °C ± 0.1 °C. The injection T of the GC (split 1: 20) and the GC-MS interface were set at 320 °C. The GC oven was heated from 50 to 320 °C (held 10 min) at 15 °C min^{-1}. The GC instrument was equipped with a Column UltraAlloy-5 (Frontier Laboratories LTD.), length 30 m, thickness 0.25 μm, diameter 0.25 mm with He as a carrier gas. The MS was scanning in the range of m/z 45–600.

Compounds were identified using the NIST'05 library and pyrolysis-GC/MS literature [16–18]. After the elimination of minor unidentified compounds and compounds that occurred in only one sample, 188 compounds remained and were identified. The pyrolysis products were grouped according to origin and chemical similarity into several source groups: (i) aliphatic hydrocarbons that include *n*-alkanes, *n*-alkenes and *n*-methyl ketones, (ii) aromatics and alkylbenzenes, (iii) polyaromatic hydrocarbons (PAHs) and benzofurans, (iv) methyl esters, (v) lignin phenols, (vi) N-containing compounds, (vii) phenols and catechols, and (viii) polysaccharides. Quantification of the relative contributions of pyrolysis products was based on the peak area of two characteristic ions using Masslab software. All quantifications were checked manually. The combined peak area of all quantified pyrolysis products (total peak area, TPA) was set as 100%, and the relative proportions of the pyrolysis products were expressed as a percentage of the TPA. The

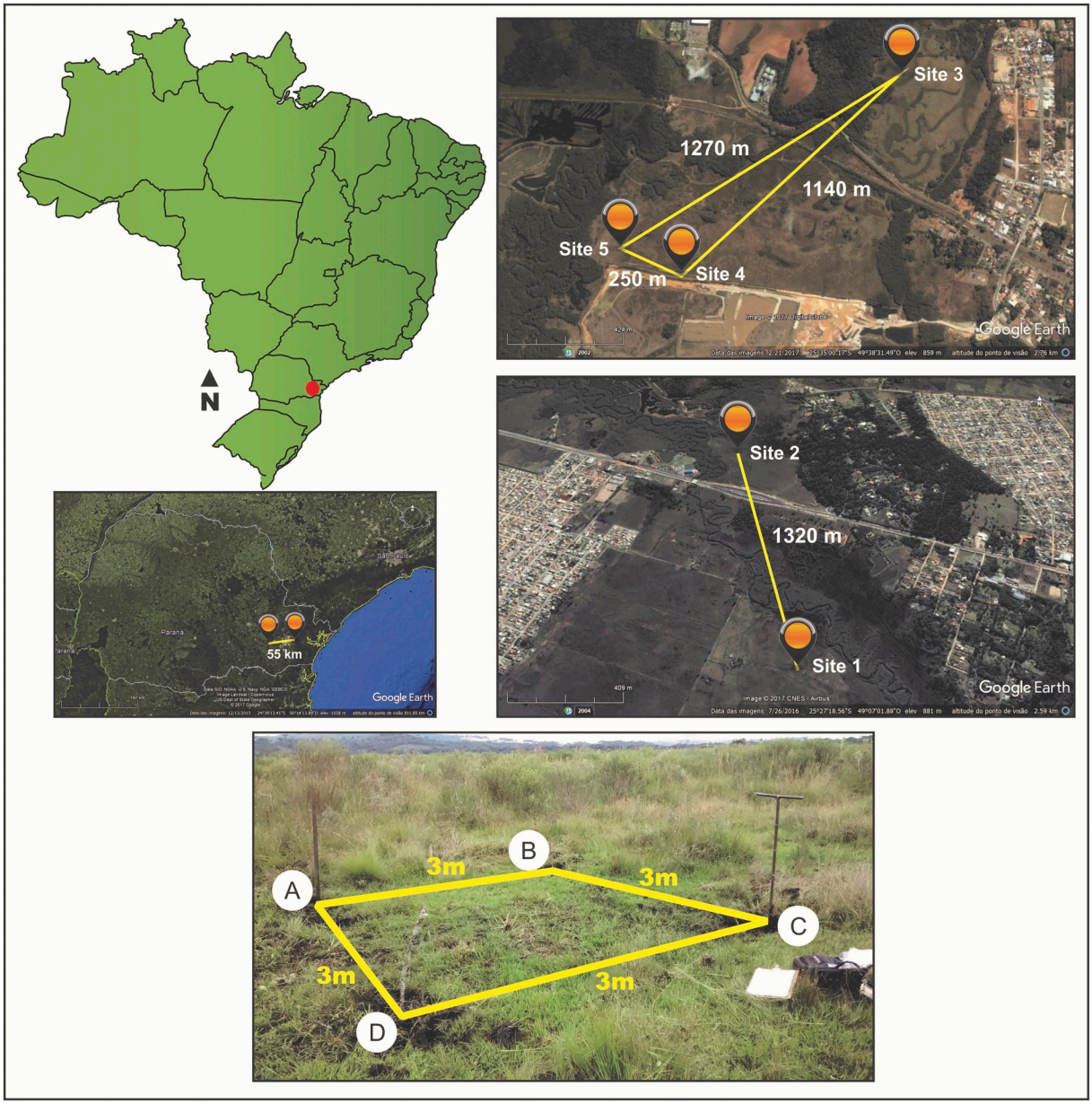

Fig. 1. Site of the Histosols areas (Piraquara and Balsa Nova cities, Paraná State, Brazil) and schematic representation of soil sampling of each study site. All collection sites were on the banks of rivers.

resulting quantification allowed comparison of the abundance of the pyrolysis products within the data set.

2.3. Statistical analysis

The data set contained 20 samples (5 sites × 4 replications) with 188 variables each. The pyrolysis data were subjected to factor analysis using Statistica (StatSoft, Tulsa OK, USA). Factor analysis was carried out to reveal the covariance of pyrolysis products. It allows the distinction of patterns of behaviour in a large number of variables. Factor analysis extracts trends from complex data sets by searching for linear correlations between variables, reducing this variation to a few factors.

3. Results and discussion

3.1. General composition of SOM pyrolysates

The 188 quantified pyrolysis products and their identification and characteristics are listed in Table 2, and the relative abundance of groups of quantified pyrolysis products for each sample is given in Table 3. Aliphatic hydrocarbons were quantified (Table 2), and most samples showed a dominance of short-chain *n*-alkanes and *n*-alkenes ($C_{10–25}$) and no predominance of typical microbial chain lengths (C_{15} and C_{17}), suggesting a significant contribution from macromolecular structures. The short chain *n*-alkanes and *n*-alkenes showed a considerable contribution for Balsa Nova sites (3, 4 and 5) compared to the Piraquara sites (1 and 2) (Table 3) in per cent. Total aromatic compounds varied between 11 and 46% (samples 1A and 1D, respectively). Aromatics in pyrolysates of SOM may originate from several sources, including lignin, carbohydrates, proteins and charcoal. The polyaromatic hydrocarbons (PAHs) compounds and most of the identified compounds were indene and naphthalene compounds. Sample 1D and 4B showed the highest amount of PAHs products in SOM pyrolysates; these compounds are usually derived from charred plant material as a result of forest or grass fires [19,20].

Sixteen different lignin-derived products were quantified. Site 1

Table 2
Quantified pyrolysis products.

Code	Name	*m/z*[a]	RT[b]	Code	Name	*m/z*	RT
	***n*-Alkanes**			N16	Indole	90 + 117	2112
10–33	C10-C33 *n*-alkanes	57 + 71	1,04-6,62	N17	Imidazole, 2-acetamido-5-methyl	69 + 97	2196
	***n*-Alkenes**			N18	1H-indole-3-ethanamine	130 + 131	2426
10:1–33:1	C10-C33 *n*-alkenes	55 + 69	1,39-6,62	N19	C2 quinoline	157	2645
	Aromatics			N20	Diketodipyrrole	93 + 186	3415
Ar1	Benzene	77 + 78	0,326	N21	Diketopiperazine derivative	70 + 154	3706
Ar2	Toluene	91 + 92	0,450	N22	Diketopiperazine derivative	70 + 194	4028
Ar3	Ethyl-benzene	91 + 106	0,642		**Polyaromatics (PAHs)**		
Ar4	Dimethyl-benzene	91 + 106	0,663	Pa1	Indene	115 + 116	1213
Ar5	Styrene	78 + 104	0,720	Pa2	Naphthalene	128	1718
Ar6	Dimethyl-benzene	91 + 106	0,727	Pa3	1H-indene,1,1-dimethyl	129 + 144	1988
Ar7	Unsaturated C3 benzene	117 + 118	0,879	Pa4	1H-indene,1,1-dimethyl	129 + 144	2002
Ar8	Propylbenzene	91 + 120	0,903	Pa5	1H-indene,1,1-dimethyl	129 + 144	2020
Ar9	Ethylmethyl-benzene	105 + 120	0,928	Pa6	1H-inden-1-one, 2,3-dihydro	104 + 132	2062
Ar10	Ethylmethyl-benzene	105 + 120	0,989	Pa7	C1 naphthalene	141 + 142	2108
Ar11	Ethylmethyl-benzene	105 + 120	1033	Pa8	C1 naphthalene	141 + 142	2168
Ar12	Ethylmethyl-benzene	105 + 120	1133	Pa9	2-ethenylnaphthalene / biphenyl	153 + 154	2396
Ar13	Unsaturated C3 benzene	117 + 118	1144	Pa10	C2 naphthalene	141 + 156	2530
Ar14	Unsaturated C3 benzene	117 + 118	1181	Pa11	C2 naphthalene	141 + 156	2541
Ar15	Acetophenone	77 + 105	1288	Pa12	C3-naphthalene	155 + 170	3060
Ar16	Benzene, 1,2,3,4-tetramethyl	119 + 134	1465	Pa13	Fluorene	165 + 166	3035
Ar17	Unsaturated - C4-benzenecompound	115 + 130	1609	Pa14	2-methyl-1-naphthalenol	129 + 158	3146
	***n*-alkybenzenes**			Pa15	C4 naphthalene	169 + 184	3483
B4-B12	C4-C12 n-alkybenzene	91 + 92	1,25-3,86	Pa16	Phenanthrene	178	3614
	Benzofurans			Pa17	2-naphthalenol	115 + 144	15,972
Bf1	C1 benzofuran	131 + 132	1423		**Phenols**		
Bf2	C1 benzofuran	131 + 132	1440	Ph1	Phenol	66 + 94	1000
Bf3	C2 benzofuran	145 + 146	1811	Ph2	2-methyl-phenol	107 + 108	1252
Bf4	Dibenzofuran	139 + 168	2841	Ph3	3/4-methyl-phenol	107 + 108	1327
	***n*-methyl ketones**			Ph4	C2 phenol	107 + 122	1588
K16-K31	C16-C31 *n*-methyl ketone	58 + 59	3,64-6,66	Ph5	C2 phenol	107 + 122	1658
	Lignin phenols			Ph6	C2 phenol	107 + 122	1756
Lg1	Guaiacol	109 + 124	1372	Ph8	Ethyl-methyl- phenol	121 + 136	1910
Lg2	4-methylguaiacol	123 + 138	1745	Ph9	4-(2-propenyl)phenol	133 + 134	2272
Lg3	4-vinylphenol	91 + 120	1851	Ph10	Catechol	64 + 110	1868
Lg4	4-ethylguaiacol	137 + 152	2051	Ph11	Methoxy-catechol	97 + 140	2076
Lg5	4-vinylphenol	91 + 120	2079		**Carbohydrates**		
Lg6	4-vinylguaiacol	135 + 150	2176	Ps1	(2H)-furan-3-one	55 + 84	0,339
Lg7	Syringol	139 + 154	2302	Ps2	(2H)-furan-3-one	55 + 84	0,496
Lg8	4-formylguaiacol	151 + 152	2472	Ps3	3-furaldehyde	95 + 96	0,572
Lg9	4-methylsyringol	153 + 168	2617	Ps4	Cyclopent-2-en-1,4-dione?	54 + 96	0,681
Lg10	4-(prop-1-enyl)guaiacol, *trans*	164	2620	Ps5	Cyclopent-1-ene-3,4-dione?	68 + 96	0,699
Lg11	4-acetylguaiacol	151 + 166	2752	Ps6	2-cyclopenten-1-one, 2-methyl	53 + 67	0,761
Lg12	4-(propan-2-one)guaiacol	137 + 180	2969	Ps7	2-acetylfuran	95 + 110	0,773
Lg14	4-acetylsyringol	181 + 196	3484	Ps8	(2H)-furan-3-one	55 + 84	0,784
Lg15	4-(propan-2-one)syringol	167 + 210	3586	Ps9	2,5-furandione, 3-methyl	68 + 98	0,807
Lg16	4-(propan-3-one)syringol	181 + 210	3731	Ps10	2,3-dihydro-5-methylfuran-2-one	55 + 98	0,815
	Methyl-ester			Ps11	5-methyl-2-furaldehyde	109 + 110	0,933
Me	Methyl-ester	74 + 87	2439	Ps12	4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one	58 + 114	1050
	N-compounds			Ps13	3-hydroxy-2-methyl-2-cyclopenten-1-one	55 + 112	1152
N1	C1 pyrrole	80 + 81	0,408	Ps14	dianhydrorhamnose	113 + 128	1197
N2	Pyridine	52 + 79	0,414	Ps15	2,3-dimethylcyclopent-2-en-1-one	67 + 110	1191
N3	Pyrrole	67	0,427	Ps16	2,5-dimethyl-4-hydroxy-3(2H)-furanone	72 + 128	1356
N4	2-ethylpyridine	66 + 93	0,538	Ps17	Maltol	71 + 126	1458
N5	C1-pyrrole	80 + 81	0,590	Ps18	1,4:3,6-dianhydro-alpha-D-glucopyranose	57 + 69	1811
N6	C1-pyrrole	80 + 81	0,608	Ps19	1,4:3,6-dianhydro-alpha-D-glucopyranose	57 + 69	1952
N7	1H-pyrrole, 2,3-dimethyl-	94 + 95	0,813	Ps20	Levogalactosan	60 + 73	2794
N8	C2 pyridine	106 + 107	0,831	Ps21	Levomannosan	60 + 73	2804
N9	1H-pyrrole, 2,3-dimethyl-	94 + 95	0,852	Ps22	Levoglucosan	60 + 73	2827
N10	Pyrrole, 4-ethyl-2-methyl	94 + 109	1000	Ps23	cyclopentanone, 2-(1-methylpropyl)-	55 + 84	2920
N11	Acetoxypyridine/3-hydroxypyridine monoacetate	95	1476		**Sterols**		
N12	1H-imidazole compound	81 + 82	1506	St1	Unidentified sterol	163 + 190	6052
N13	2,5-pyrrolidinedione	56 + 99	1532	St2	Gamma tocopherol	151 + 416	6261
N14	Methylbenzonitril	90 + 117	1551	St3	Unidentified sterol	163 + 190	6292
N15	1H-imidazole compound	121 + 136	1637	St4	Unidentified sterol	165 + 430	6394

[a] Fragment ions used for quantification.
[b] Retention time relative to phenol.

Table 3
Proportion (%) of groups of compounds for each sample.

Groups	1				2				3				4				5			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
n-Alkanes ($C_{10–25}$)	2.9	4.1	4.2	4.3	4.0	4.3	3.5	4.1	5.9	9.1	5.6	6.5	6.7	7.5	8.5	6.6	8.0	5.5	6.3	6.6
n-Alkanes ($C_{26–33}$)	0.9	1.2	1.1	0.2	1.2	1.3	1.0	1.8	1.9	3.3	2.0	2.0	2.4	2.9	2.4	2.1	3.0	2.3	2.4	2.4
n-Alkenes ($C_{10–25}$)	2.9	3.7	4.1	2.5	4.5	4.5	4.0	4.3	5.7	7.4	5.8	6.2	6.5	6.9	7.6	6.4	7.4	6.0	7.1	6.4
n-Alkenes ($C_{26–33}$)	0.7	0.7	0.8	0.1	0.7	0.6	0.7	1.3	1.3	2.1	1.0	1.3	1.3	1.4	1.2	1.2	1.8	1.1	1.4	1.2
Aromatics	10.9	13.4	13.0	45.9	19.3	25.0	15.4	15.4	15.6	17.7	14.8	14.0	17.3	30.0	17.9	16.3	19.7	13.6	15.6	14.7
n-Alkylbenzenes ($C_{4–12}$)	0.8	0.8	0.9	2.1	1.5	1.7	1.1	1.0	1.2	1.4	1.4	1.2	1.4	2.0	1.6	1.3	1.6	1.2	1.4	1.2
Benzofurans	0.4	0.5	0.5	1.0	0.7	0.9	0.6	0.7	0.5	0.7	0.5	0.6	0.7	1.3	0.6	0.6	0.6	0.5	0.5	0.4
n-Methylketones ($C_{16–31}$)	0.6	0.6	0.9	0.4	0.4	0.5	0.4	0.6	1.1	1.7	1.2	1.3	1.4	1.7	1.6	1.4	1.2	1.2	1.0	1.0
Lignins	13.3	10.7	11.9	2.3	5.5	4.0	6.5	9.0	5.3	3.1	6.2	6.6	3.7	1.7	2,3	4,5	4,0	3,6	3,2	2,9
Methylester	0.7	0.5	0.7	0.0	0.3	0.1	0.5	0.6	1.1	1.0	0.9	1.7	0.5	0.1	0.5	0.6	1.0	1.1	1.0	0.9
N-Compounds	19.0	20.4	18.0	21.4	24.7	25.8	26.2	22.1	17.0	17.4	18.3	19.4	22.8	18.0	21.4	24.3	20.8	17.3	19.0	19.9
PAHs	1.9	2.5	2.3	7.1	4.1	5.2	3.2	3.8	3.2	4.5	3.1	2.9	4.2	9.1	4.7	3.7	4.7	3.5	3.7	3.6
Phenols	12,1	11,7	9,3	8,2	13,0	18,1	13,5	15,3	8,7	10,9	8,8	9,1	11,5	14,0	11,3	11,3	10,0	8,4	6,6	8,6
Polysaccharides	33.0	29.0	32.3	4.6	20.1	7.7	23.1	19.8	31.3	19.5	30.5	27.1	19.5	3.3	18.6	19.7	16.3	34.7	30.9	30.2
Sterols	0.1	0.0	0.1	0.0	0.1	0.2	0.1	0.2	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.1	0.0	0.0

presented the highest contribution of lignins compounds, indicating that this sample has more fresh plant material. The abundance of N containing compounds varied between 17 and 26% (Table 3) and polysaccharide compounds, in some samples (1 and 4), reached up to about 30% of the total. Microbial material results in nitrogen-containing compounds and carbohydrates upon pyrolysis [21–24].

3.2. Factor analysis applied to the total pyrolysis data set

The first three factors explained > 70% of the variation. Factor 1 (F1), factor 2 (F2) and factor 3 (F3) explained 35.9%, 26.1% and 12.4% of the variance, respectively. Because each of the subsequent factors explained < 8% of the total variability, the discussion will be limited only to the first three factors. The projections of factor scores and factor loadings for the first two factors are given in Fig. 2. Almost all the Balsa Nova samples showed negative scores on both factor 1 (F1) and factor 2 (F2); while the Piraquara samples showed positive scores on F1. Balsa Nova and Piraquara samples were separated into two groups (Fig. 2b).

Factor loadings (Fig. 2a) show which compounds contribute to this separation. Compounds indicative of relatively fresh plant material showed high positive loadings on F1 and included all lignin phenols [25], catechol derived from tannin (Ph7; [26]), hemicellulose (Ps12; [27]) and cellulose (Ps22; [16]). Lignins were separated from the aliphatic compounds, which reflect the difference in degradation speed between the two groups. The intermediate loadings of *n*-alkanes and *n*-alkenes, which are pyrolysis products from cutan and suberan [28] being more resistant against degradation than lignin [25]. F1 is therefore interpreted to reflect decomposition, negative values corresponding more recalcitrant (relatively difficult to decompose) plant biopolymers (aliphatics compounds, [29]) and PAHs while relatively fresh plant material (lignins, [25]) and phenols showed positive values.

Compounds with high positive loadings on F2 included most aromatic, polyaromatic and alkylbenzene compounds, which points toward burnt material. This suggests that F2 reflects the contribution from black carbon (BC) (i.e., fire frequency) because F2 separated the aliphatics and lignins (negative loadings) from PAH products (positive loadings). Other products that showed high positive loadings on F2 are also indicative of burnt material and include benzofurans (Bf1, Bf2), pyridine (N2) and most aromatics [19].

Also, the F1–F3 projection (Fig. 3a) shows that it is possible to differentiate between the two sites. The aliphatics compounds formed a cluster with negative loadings on F1 while all lignin compounds present high positive loadings. Suggesting that same occur on the F1F2 loadings space: fresh plant material was separated from the aliphatic compounds.

By combining these observations, it is possible to divide the plot into distinct regions, which are identified in Fig. 2b and Fig. 3b. These regions can be related to the source and transformations of SOM. The interpretation of the factor analysis is summarized in Table 4.

The above considerations allow an interpretation of the samples in the graph of the factor scores (Fig. 2b and Fig. 3b) because they represent the weight of each sample in the chart of factor loadings. Samples of Piraquara and Balsa Nova plotted separately on the graph (Fig. 2b), demonstrating that Histosols differentiated between the areas. According to F1, samples of Balsa Nova plotted toward the aliphatic compounds and burnt material, while samples of Piraquara plotted the group of lignins. This suggests a higher contribution of fresh organic material in samples of Piraquara against a more degraded organic matter for samples of Balsa Nova. The Balsa Nova samples showed a large concentration of short chain n-alkanes and n-alkenes. This suggests that the Balsa Nova samples have a more decomposed OM with an influence of burnt material in relation to Piraquara, which presents samples with more fresh material, even both areas have a similar vegetation cover, with some graminoids and plants typical of wetlands, such as *Typha domingensis* and *Phlebodium decumanum* (Table 1). Py-GC/MS coupled with factor analysis has been investigated as an analytical tool for the forensic fingerprinting of soils. The results obtained in this study by Py-GC/MS show that it is possible to distinguish the analyzed sites, with organic soils, and to infer that they are different. The samples were grouped according to the similarity and quantitative chemical characteristics of OM. Py-GC/MS proved to be a reliable method to identify and quantify the organic compounds in soils.

In a previous study in the same area [15], we used the same samples to determine the total elemental content and perform physical fractionation of the soil (> 53 mm and < 53 mm). For both soil size fractions, total C and N contents were determined, and the elements adsorbed to organic matter was determined only for the < 53 mm size fraction. Chemometric multivariate analyses were conducted for the entire data set, where the first three factors explained > 77% of the variation. It was determined that Ca, Ba, and Mg adsorbed to organic matter, and total Ba, Ca, K, Mg, Mo, and C contents were most important in sample groupings. As expected in forensic science, the five sites were efficiently distinguishable from each other, and the four replicates collected at the same individual site were grouped. Therefore, the molecular analysis of

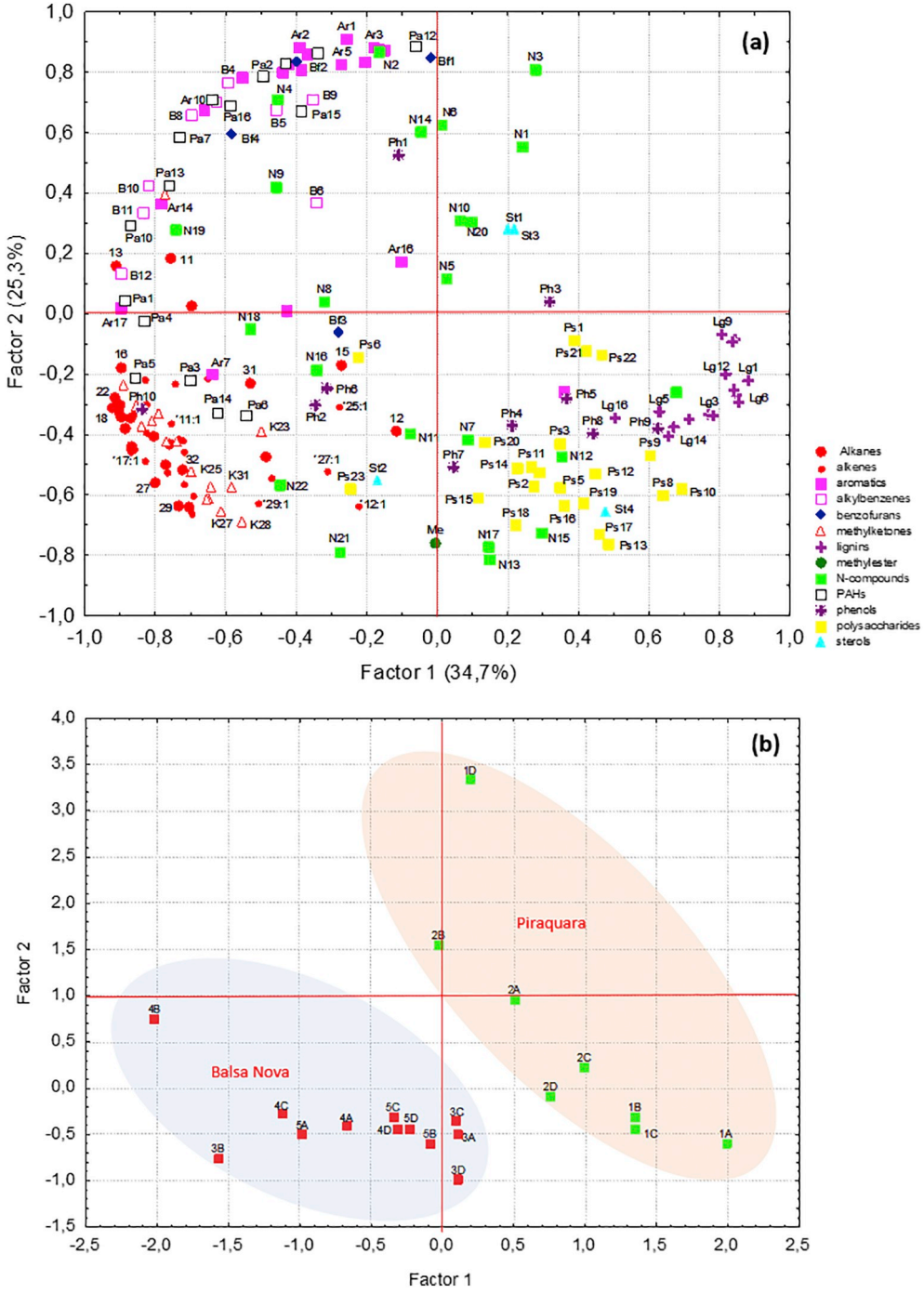

Fig. 2. (a) Projection of the factor loadings of the pyrolysis products in F1-F2 space. (b) Projection of the factor scores in F1-F2 space.

the OM by Py-GC/MS corroborated with the results obtained in this previous study.OM analysis is essential for the characterization of soils with low mineral content and high OM content, since from site comparison to a sample trace of a crime scene. Therefore, OM can help to define a specific locus of a crime, excluding alibi locations at another site, and even the crime location within this locus.

Next steps on to make SOM a tool to be used in forensic investigations are to establish sampling and analytical protocols since the amount of sample available for forensic purposes is usually small. To properly access the use of SOM for forensic purposes, more studies are necessary to compare the soil sampled at the site with trace samples from tires or boots and compare the results. The use of biomarkers compounds and plant (not done in this study due to the lack of plant samples) would be ideal, giving a more accurate comparison between the samples.

4. Conclusions

The SOM composition in the Balsa Nova and Piraquara Histosols studied was different. Although both sites have essentially organic soils,

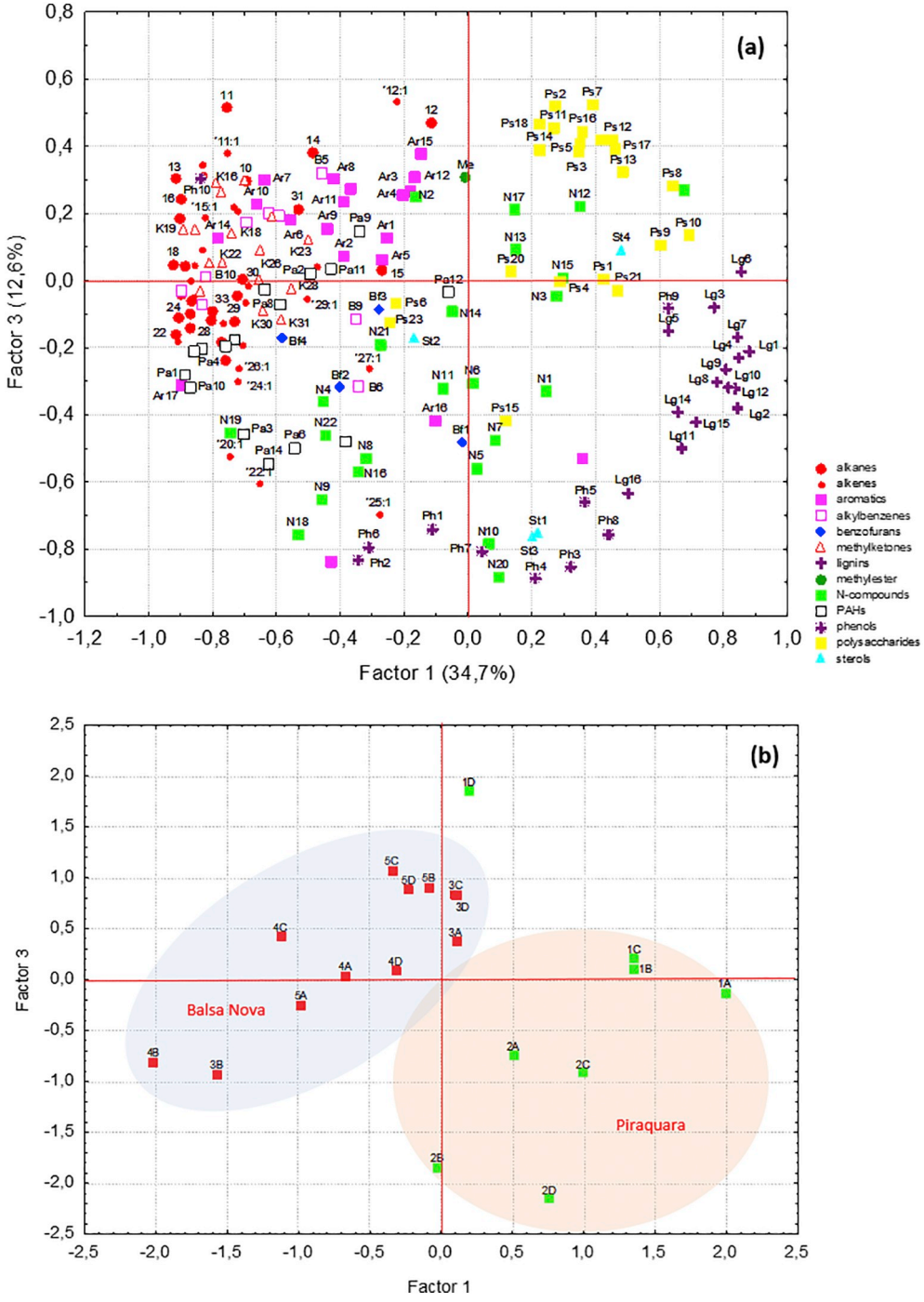

Fig. 3. (a) Projection of the factor scores in F1-F3 space; (b) Projection of the factor loadings of the pyrolysis products in F1-F3 space.

Table 4
Summary of interpretation of loadings factor analysis.

	Positive	Negative
F1	Fresh plant material	Charred and recalcitrant material
F2	Charred material	Fresh plant and recalcitrant material

this OM is different from one location to another and may even have a specific composition which allows comparisons with a trace sample and to be compatible or not. Therefore Py-GC/MS has the potential to identify the location of a sample from Histosols for forensic purposes.

Declaration of Competing Interest

None

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