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Sand fraction is not suitable for forensic investigations in subtropical soils

Vander Freitas Melo(1)*, Samara Alves Testoni(1), Lorna Anne Dawson(2) and Fábio Augusto da Silva Salvador(3)

(1) Universidade Federal do Paraná, Departamento de Solos e Engenharia Agrícola, Curitiba, Paraná, Brasil.
(2) Environmental and Biochemical Sciences Group, The James Hutton Institute, Craigiebuckler, Aberdeen AB15 8QH Scotland, United Kingdom.
(3) Setor Técnico e Científico da Polícia Federal, Curitiba, Paraná, Brasil.

ABSTRACT: Most of the forensic comparison of soils has focused on the clay and silt fractions at the expense of the coarser particles. This study aimed to test the potential of elemental and physical analyses 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. 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 (XRD) and particle size distribution (PSD) analyses were performed on the whole sand fraction. These methods did not provide clear discrimination of the sand of the soils sampled in the subtropical environment. This can be explained by the large homogenization observed in the sand fraction related to its chemical (EDS), physical (particle size distribution), morphological (SEM), and mineralogical (XRD). Under tropical and subtropical conditions, the chemical weathering processes dissolve most of the primary minerals, such as the feldspars, biotite, and Fe-bearing particles, and concentrates quartz in the sand fraction. In these environments, we recommend the prioritization of the finer soil fractions for forensic studies, both inorganic and organic.

Keywords: forensic science, soil evidence, quartz, particle size analysis, X-ray diffraction.

*** Corresponding author:**
E-mail: melovander@yahoo.com.br

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INTRODUCTION

Since 1856, when Professor Ehrenberg of Berlin analyzed sand samples found in a barrel previously filled with coins, soils have been increasingly found to be useful in criminal investigations (Siegel et al., 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 investigations (Ritz et al., 2008; Dawson and Mayes, 2014; Pirrie et al., 2017).

In a forensic investigation, the selection of the applicable methods depends on the amount, condition, and the predominant fraction of the sample available and transferred, as well as the associated costs and equipment available. Bull et al. (2006) discussed the importance of integrating different independent techniques to provide forensic rigor to an investigation. There are a wide number of analytical approaches that can be performed on soils to investigate their inorganic and organic fractions such as X-ray diffraction, spectral color, particle size analysis, elemental analysis, X-ray fluorescence, stable isotope analysis, microorganisms, plant wax compounds, and electronic macroscopy analysis (Chazottes et al., 2004; McCulloch et al., 2018; Melo et al., 2018; Prandel et al., 2018).

Most of the forensic comparisons of soils has focused on the smaller sized 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 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). In some circumstances, the sand fraction is the main fraction used in forensic case work (Bull et al., 2006; Bull and Morgan, 2006; Morgan et al., 2019). When crimes occur in sandy soils, where the amount of clay in the vestige sample is insufficient to undergo chemical and physical analyses, it is important to have prior validation of forensic protocols for the analysis of the sand fraction (Morgan et al., 2010; Carvalho et al., 2013). However, with the increasing ability to work with trace amounts of samples, this may not be such a limitation.

Normally, the sand fraction of highly weathered soils has a homogeneous mineralogy, which makes it more difficult to trace the vestige in a forensic case. Under tropical and subtropical conditions, chemical weathering dissolves out most of the primary minerals, such as the feldspars, biotite and Fe-bearing particles, and concentrates the quartz (Saye and Pye, 2004; Shudofsky et al., 2017; Chauhan et al., 2018; Melo et al., 2019). Coarser particles are normally dominated by quartz and their morphological features are focused on which in some aspects are related to transport, accumulation, and weathering processes, and are useful as they remain in and on the sand grain for a long time (Morgan and Bull, 2007; Pye et al., 2007; Farrugia et al., 2012; Batista et al., 2018; Morgan et al., 2019). Such features can provide important information regarding the nature and the provenance of the sample, depending on the analytical approach used. Mineral analysis with XRD can distinguish between different mineral phases of very similar chemistry; however, routine powder XRD is not feasible to study the sand fraction where there is a predominance of quartz. Mineral phases in concentrations below 40 g kg^{-1} are not precisely identified by XRD (Bong et al., 2012; Batista et al., 2017). Thus, investigations of the morphological aspects of quartz grains should be prioritized in tropical and subtropical soils and an important technique to verify subtle differences in the morphology of this mineral is the scanning electron microscope coupled to the energy dispersive X-ray spectrometer (SEM-EDS). Cengiz et al. (2004) compared the discrimination power of the pressed-homogenized and not homogenized forensic soil samples from Istanbul. They verified that SEM–EDS was more accurate and precise even in very small amounts of samples. The SEM-EDS can be used to determine the elemental composition of the sand particles (Nakai et al., 2014; Batista et al., 2018).

Data from the analysis of the particle size distribution (PSD) of sand can complement SEM-EDS analysis and provide a useful means of characterizing soils and sediments for 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) analyzed 73 soil samples from the Nirasaki district (Japan) (under temperate weather conditions). They observed that the measurements of PSD through sieving of the coarse and fine particle fraction allowed a discriminating power of 95.5 % in combination with the colour examination. However, Pye and Blott (2004) and Chazottes et al. (2004) found that the data obtained from such analysis needs to be evaluated alongside the results of other analyses such as chemical, mineralogical, and microscopic tests.

To investigate the usefulness of the sand fraction for forensic discrimination in this study, twenty soil samples were selected by sampling: 1) at two distinct geologies (claystone and limestone) and 2) at four locations under a simulated crime scene in southern Brazil. Finer sized particles (silt + clay) analyses using the same soils have already been tested and allowed a clear grouping of the samples according to their location of origin at all four sites tested, ascertaining the ability to use chemical characterization to test a link between a questioned sample and a known crime scene (Testoni et al., 2019).

Therefore, this study aimed to verify the potential of elemental and physical analyses through the use of PSD, XRD, 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.

MATERIALS AND METHODS

Description of the sampling sites

A simulated crime scene was designed in June 2017 in two neighborhoods from the urban areas in the municipalities of Curitiba (Santa Candida and Boa Vista) and Colombo (Guaraituba and Guarani), Paraná State, Brazil (Figure 1). Those two municipalities belong to the Curitiba Metropolitan Region and are separated by a distance of 2 to 4 km. Selected sites belonged to both similar and contrasting parent material (Table 1) to test for intra and inter-site comparison. Soils were all classified as Inceptsols (Soil Survey Staff, 2014), which correspond to a *Cambissolo* according to the Brazilian Soil Classification System (Santos et al., 2013). Five soil samples were collected at each location (four neighborhoods): using a quadrant with four points (top right and top left; bottom right and bottom left) at a distance of 1.5 m from one another, including a central point established as a simulated suspect sample contact location (i.e., a footprint) (Figure 2). Samples at the edge of the quadrant were collected with a sterilized metal spatula from the 0.00-0.05 m layer. Soil samples at the central position of figure 2 in each location were transferred onto the sole of a boot through a simulation of a suspect walking over a crime scene. The soil sample was oven-dried at 50 °C and stored in plastic flasks.

Sand fraction preparation and X-ray diffraction

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

The sand fraction (approximately 0.3 g) was 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 analyzed 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.

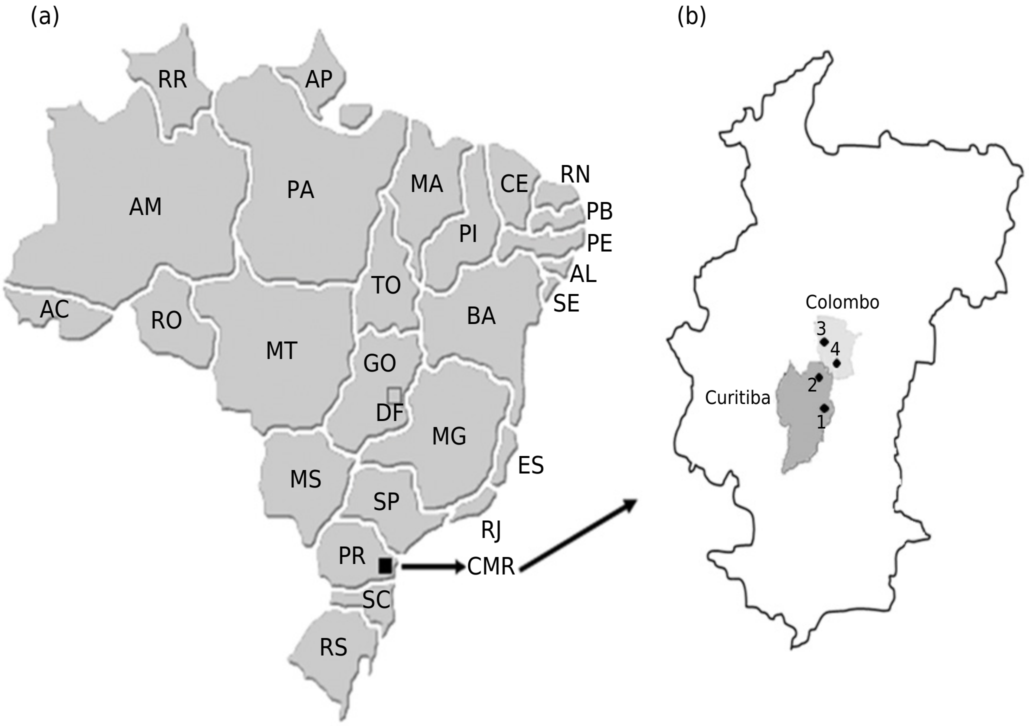


Figure 1. Map of Brazil highlighting the Paraná State (PR) (a) and the Curitiba Metropolitan Region (CMR), and Curitiba (1-Boa Vista and 2-Santa Candida) and Colombo (3-Guaraituba and 4-Guarani) (b).

Table 1. Sites description, locations, and sand contents of selected samples

Sample	Description(1)	Location/bedrock	Sand	UTM (22J)	
				E-W	N-S
			g kg^{-1}	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		

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

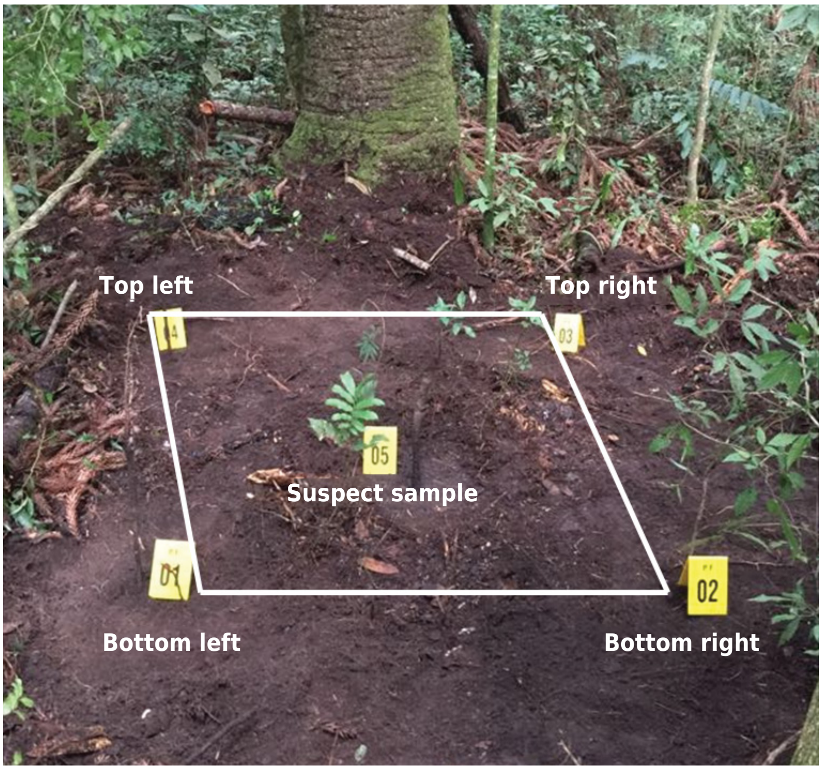


Figure 2. Design of the simulated crime scene for surface soil sampling. Source: modified from Testoni et al. (2019).

Scanning Electron Microscopy coupled to an Energy Dispersive X-ray Spectrometer

A previous separation of the sand fraction through sieving was carried out producing two subsamples: 0.25-2.00 mm (sample 1) and 0.05-0.25 mm (sample 2). These two sand size class fractions were analyzed using Scanning Electron Microscopy – Energy Dispersive X-ray analysis (SEM-EDS) with a Zeiss EVO LS10 scanning electron microscope equipped with three imaging detectors, high vacuum secondary electron, extended pressure secondary electron, and electron back-scattered. 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 of sand fraction were put on metal support and fixed on an adhesive tape placed on a stub (SEM sample holder) and subjected to several tests to define the size most suitable to provide an acceptable resolution. Comparing subsamples 1 (>0.25 mm) and 2 (<0.25 mm), it was shown that sample 2 demonstrated a higher percentage of the total analyzed elements by EDS; therefore, presenting a more detailed spectrum and containing elements which were not observed in sample 1, such as Ti, which can be related to the presence of anatase. In addition, peaks of all elements in the spectrum of sample 2 presented higher intensity, possibly related to an effect of the higher number of particles analyzed due to the smaller size of the particles in this fraction (<0.25 mm). As a consequence, only the SEM-EDS results of sample 2 (0.05-0.25 mm fraction) 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 imaged 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 from 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 carried out on individual particles. To provide a clear representation of the location, the sand sample on the sample holder was analyzed in a diffuse mode, examining all the particles in a selected area of the SEM. 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.

Particle Size Distribution (PSD)

Subsamples of the total sand fraction from all replicates (four sites × four replicates) (Table 1) were dispersed within an aqueous matrix and analyzed 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. There are, however, many factors that need to be considered when developing particle size measurements, such as the magnification (or working distance) used to analyze the particles via SEM and the size of the fractions selected.

Statistical analysis

The acquired data were subsequently analyzed with chemometrics and a minimum set of elements with discriminative power to form the basic source of identification of the analyzed soil samples. 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, transformed by square root, and subjected to multivariate analysis to visualize the general relationships between samples and to ascertain the discrimination of the samples, correlating them with the relative contribution of the analytical data obtained. Clustering by Bray-Curtis and principal component analysis (PCA) were performed to retain most of the variation present in the data set provided, reducing the dimensions of a large number of variables interconnected to a few main components (PC1, PC2, and PC3) (Kumar and Sharma, 2018; Verma et al., 2019).

RESULTS

Quartz is the only common mineral to all samples examined (Figure 3). Due to the poor discrimination of the XRD in the sand fraction matrix, SEM-EDS was used to analyze the individual particles in the sand fraction (Figure 4). 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 efficient to discriminate different sites and to group the replicates at the same site, once it was established that the three types of grains (Table 2) were widely distributed in all the examined samples (Figure 4). The quartz grains were predominantly sub-rounded to sub-angular, with granular particles occasionally occurring independently of the site (Figure 4).

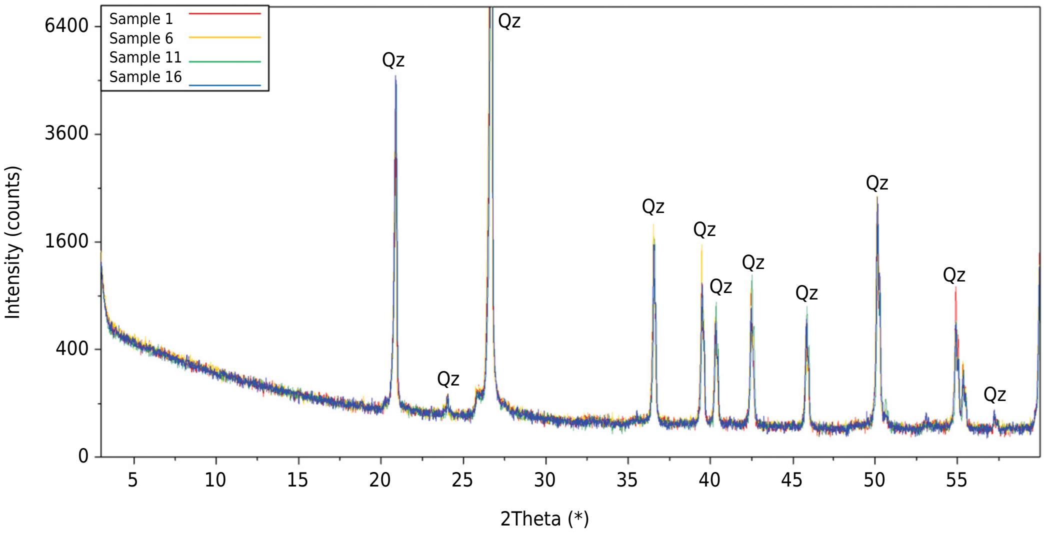


Figure 3. X-ray diffractograms of the sand fraction from four samples randomly selected in the different sites for illustrative purposes: samples 1 and 16 represent samples from Curitiba (claystone); and samples 6 and 11 represent samples from Colombo (marble) (Table 1). Qz: quartz.

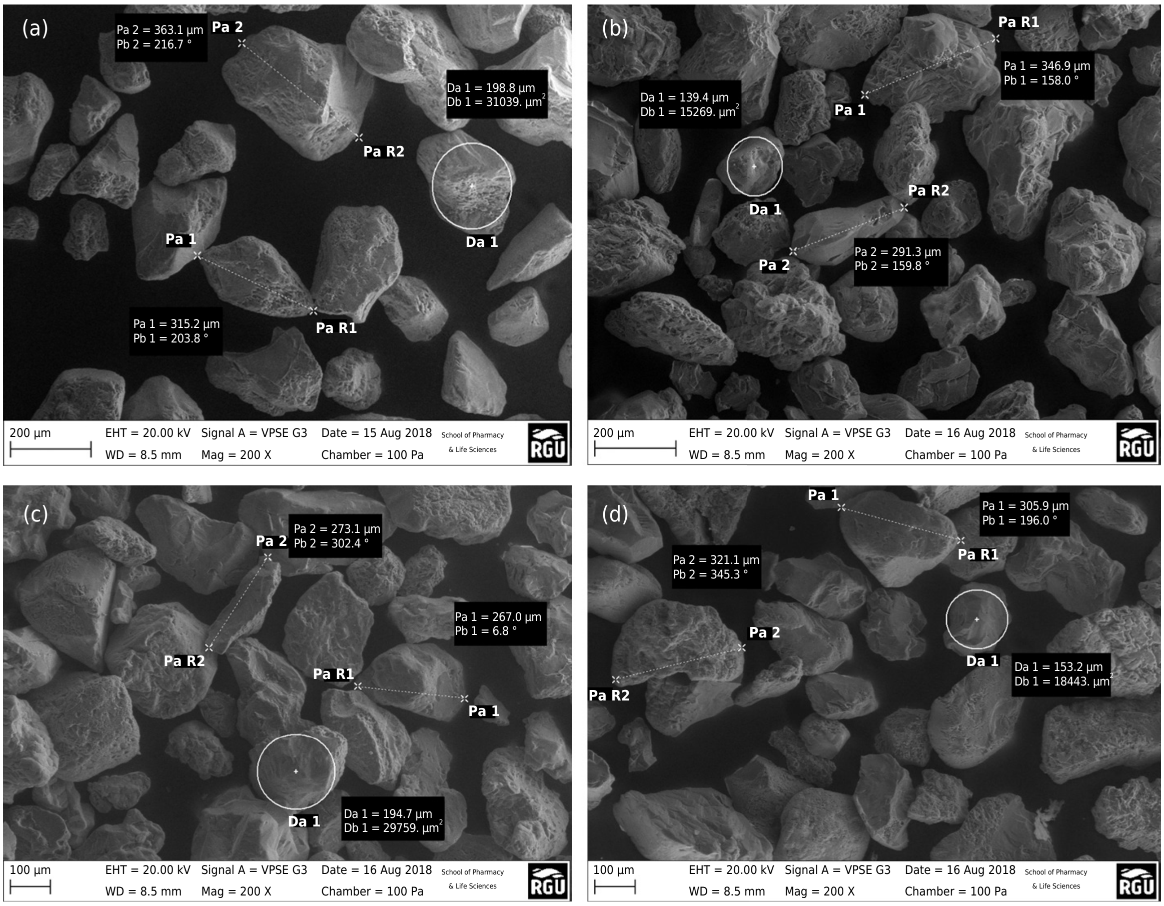
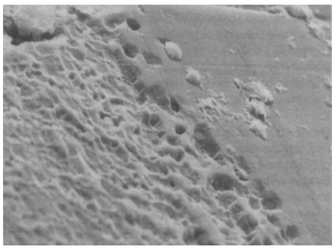
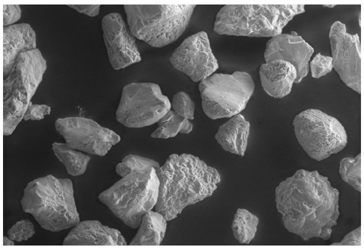
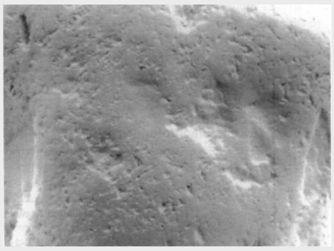
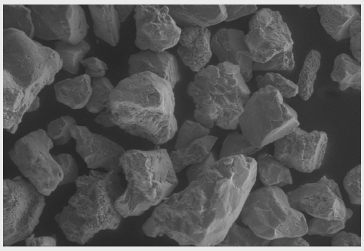
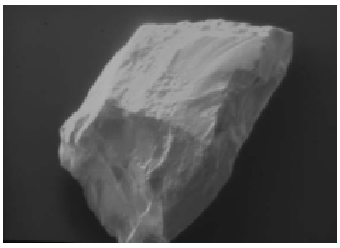
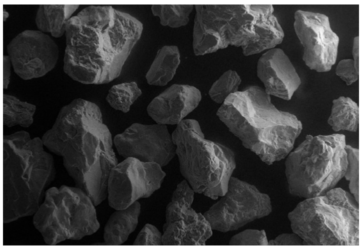


Figure 4. Representative examples of particles of the finer sand fraction (<0.25 mm) measured by SEM: a) sample 3: area 1; b) sample 7: area 2; c) sample 15: area 3; d) sample 19: area 4. Note: a complete identification of the samples is presented in table 1.

To obtain a larger range of results, the samples were examined by SEM-EDS. Analyses of isolated areas of the sand sample (SEM-EDS) allow the identification of specific elements in this fraction, such as K (Table 3), present as structural forms in the accessory minerals (e.g., residues of muscovite particles), not identified through XRD (Figure 3).

Table 2. Different quartz types according to Bull and Morgan (2006) and their correspondence with the quartz 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 from high relief with angular/ sub-angular grains and some sub-rounded additions, without no edge abrasion but later chemical smoothing		 Sample 6

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

The frequency of distribution according to the size-classes in PSD analysis showed similar curves, but with greater intra-group variation in samples in the marble domain in the Guaraituba neighborhood (Figure 5). Most of the samples were concentrated in the 0.1 to 0.2 mm size class and presented a trend in this size. The shape of the curves did not provide clear discrimination among the samples and their provenance. Boa Vista (claystone) and Guarani (marble) sites showed very similar particle size distribution profiles.

Samples presented a weak grouping according to their sampling site of origin (Figures 6 and 7). Samples 16, 17, and 20 from the Boa Vista neighborhood (claystone) had higher intragroup similarity (group 4-93 % of similarity). The suspect sample 20 (underlined number) was positioned close to samples 16 and 17. The other samples belonging to this group had a high dispersion score: sample 18–87.5 % of similarity to group 4; sample 19–86.0 % of similarity to group 4. Even samples collected at a 1.5 m of distance from each other had intense dispersion in Santa Cândida (1-5), Guarani (6-10), and Guaraituba (11-15) neighborhoods. To group all replicates from each site, the similarity was only 88 % for the samples 1 to 5, 6 to 10, and 11 to 15; and 86 % for samples 16 to 20 (Figure 7). The suspect samples from Santa Cândida (5), Guarani (10), and Guaraituba were not found to be positioned close to samples collected at the edge of the quadrants in these neighborhoods. This false exclusion is not a helpful result in a forensic investigation.

Most of the variance of the SEM-EDS and PSD data can be explained by the first three components of the PCA analysis (60 %) (PC1 + PC2 + PC3) (Figure 7). Clusters generated by the dendrogram showed a low similarity between the samples collected from the same quadrant.

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

Sample	Elemental contents(1)					Morphology	
	Si	Al	Fe	Ti	K	Diameter(1)	Perimeter
	g kg^{-1}					µm	
1	989.3	7.8	2.9	nd	nd	175.3	226.9
2	990.2	6.0	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	971.5	6.2	22.3	nd	nd	198.8	315.2
6	937.1	62.9	nd	nd	nd	138.1	370.5
7	979.5	8.7	11.8	nd	nd	139.4	346.9
8	957.1	15.8	20.0	3.3	3.8	142.2	287.2
9	968.4	17.6	14.0	nd	nd	153.3	223.9
10	961.3	16.1	10.8	11.8	nd	180.8	265.9
11	880.1	70.7	29.8	19.4	nd	202.9	263.0
12	884.6	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	974.1	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	930.5	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	922.1	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 the diffuse model. Note: a complete identification of the samples is presented in table 1. nd: not determined, below the limit of detection 5 g kg^{-1}.

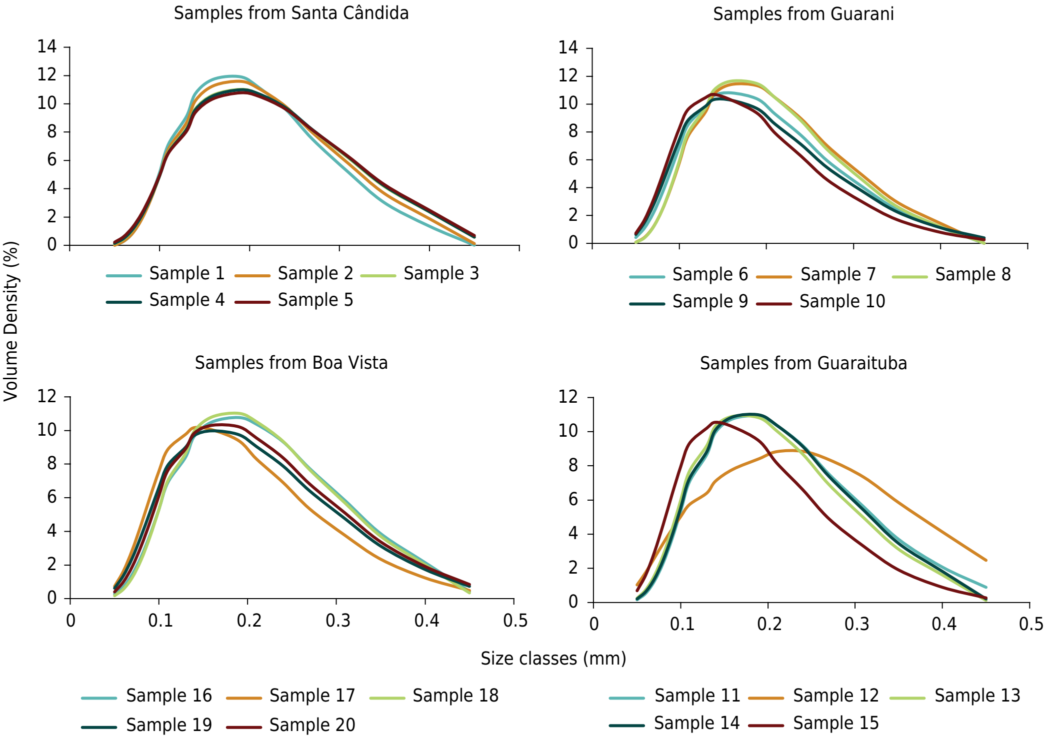


Figure 5. Frequency of distribution according to the size-classes of the sand fraction, obtained by PSD. Note: a complete identification of the samples is presented in table 1.

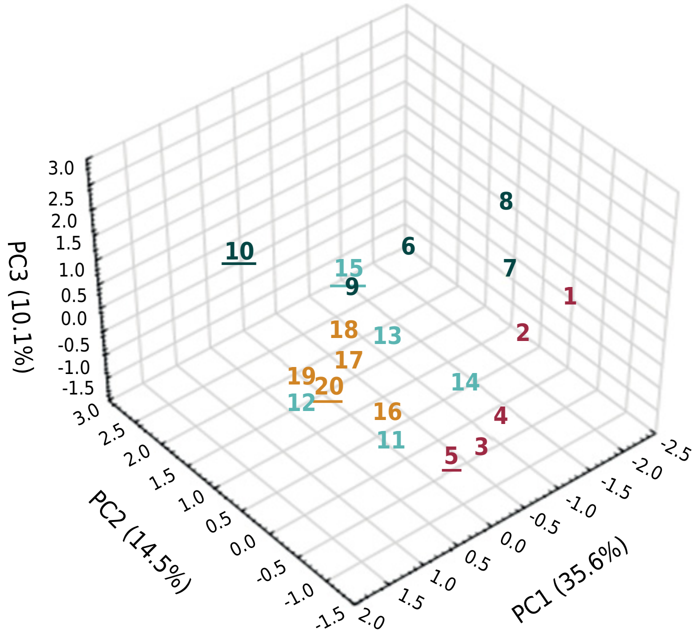


Figure 6. Scores from the first three principal components obtained by Principal Component Analysis (PCA), showing the grouping of the samples according to the relative contribution of the SEM-EDS and PSD data. Note: Red samples – Santa Cândida, Green samples – Guarani, Blue samples – Guaraituba, Yellow samples – Boa Vista, neighborhoods from Curitiba and Colombo, Paraná (Table 1); samples underlined refer to the suspect.

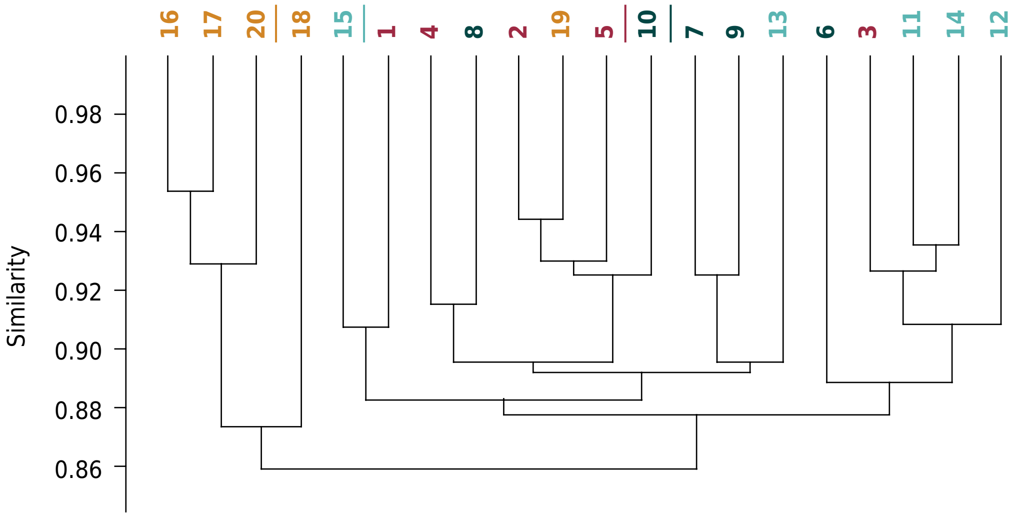


Figure 7. Dendrogram of similarity obtained by Bray-Curtis, showing the similarity of the sample according to the relative contribution of the SEM-EDS and PSD data. Note: Red samples – Santa Cândida, Green samples – Guarani, Blue samples – Guaraituba, Yellow samples – Boa Vista, neighborhoods from Curitiba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

DISCUSSION

The intensive chemical weathering promoted a mineralogical homogenization under subtropical conditions of the sand fraction of the 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; Ruffell and Wiltshire, 2004). Under 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 (subtropical and tropical conditions) make it difficult to use only X-ray diffraction in forensic studies. The residue of minerals with contents lower than 40 g kg^{-1} in the clay, silt, and sand fractions normally cannot be precisely identified by XRD (Bong et al., 2012; Nakai et al., 2014). The soil samples were collected from the 0.00–0.05 m layer, which also favored the sand particles from weathering. This superficial layer is more important to produce soil vestige in a suspect of crime as it is the most frequently contacted. The high crystallinity of the quartz grains (high intensity and the reduced width at half height) also made the identification of other primary minerals present in the sand fraction more difficult (Figure 3). The use of XRD to track soil samples under temperate conditions can be more useful in forensic cases (Ruffell and Wiltshire, 2004).

The four sites were subjected to similar weathering and transportation processes, previously reported by Bull and Morgan (2006) (Table 2). The distinct physical appearance of particles of sand grain may be largely affected by three main processes: i) the origin of the grain and how it was removed from its parent rock; ii) the transportation mechanism of the grain away from the parent rock to where it now lies; iii) the history of the grain subsequent to deposition in the soil or other sediment where it now rests. Soils collected at sites 1 (samples 1 to 5) and 4 (samples 16 to 20) were developed by the weathering of fine-grained sedimentary rocks. Soils developed from granite/gneiss of the edge of the Sedimentary Basin of the Curitiba had suffered water erosion and the sediments were transported to the interior of the Basin to form the claystone (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. In such conditions, the sand fraction was subjected to a double temporal process of weathering (first in the soils at the edge and second within the Basin), conditioning the formation of the types I, II, and III. Following deposition, claystone develops into soils by the natural input of organic matter, especially in subtropical conditions. This process of pedogenesis increases the content of acids or bases in the infiltrating waters, affecting the surface of the quartz sand grains (Type I) (Bull and Morgan, 2006).

Regarding the soils from sites 2 and 3 (samples 6 to 15), they were formed from the metamorphic rock weathering (marble) and subjected to an intensive process of weathering, which promotes the dissolution of the carbonate minerals in the sand fraction (Figure 3). The features formation attributed to the sand grains transportation (types I and II) at sites 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 0.00-0.05 m layer in the mid-slope position of the landscape.

The widest variation in elemental distribution was observed in samples from site 4 (Boa Vista neighborhood) (Table 4). The predominance of silica and the low elemental diversity is in agreement with the XRD patterns of the sand fractions (Figure 3). Quartz is a mineral particularly resistant to mechanical breakdown during the erosion of rocks and the subsequent movement of sediment in water, wind, or gravity. This resistance is enhanced by the ability of quartz to resist chemical attacks by atmospheric agents, soil acids, and percolating waters (Bull and Morgan, 2006). Quartz is resistant to chemical weathering due to its stacking of Si tetrahedra in a hexagonal system, with the Si-O bond presenting a strong covalent character (Mitton and Melo, 2019). The removal of calcite from the sand fraction of the developed soils was virtually complete since Ca was not 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 (Olatunji et al., 2015; 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 (Brinatti et al., 2010; Prandel et al., 2018).

The poor discrimination of soils collected from distinct geological domains using PSD analysis limits its application in forensic investigations (Saye and Pye, 2004). The natural conditions at the time of soil sampling such as moisture (as a reference or contact trace), the transferability, the pre-treatment of the soil prior to sieving and the form of sieving (wet or dry) may also influence the resultant particle distribution (Dawson and Hillier, 2010).

The level of grouping of the replicate samples (Figures 6 and 7) was lower when the finer fractions of the soils were used. Testoni et al. (2019) applied sequential chemical analyses to assess a limited quantity of clay and silt fraction and obtained the grouping of the same samples (1 to 5, 6 to 10, 11 to 15, and 16 to 20) with similarity level higher than 95 %. 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) but collected from different sites (Testoni et al., 2019).

CONCLUSIONS

The scanning electron microscopy coupled to an energy dispersive X-ray spectrometer (SEM-EDS), particle size distribution (PSD), and X-ray diffraction (XRD) methods did not provide clear discrimination of the sand samples of the subtropical soils investigated. This relatively poor discrimination can be explained by the large homogeneity observed within the sand fraction in relation to its chemical profile (elements) (EDS), physical distribution (particle size distribution), morphology (shape and texture) (SEM), and mineral profile (XRD). Our recommendation is that under tropical and subtropical conditions, the analysis of the finer soil fractions in forensic studies must be prioritized over an investigation of the coarser sized particle fraction. In addition, the organic characterization of the fine fraction would potentially allow an improved discrimination and both the inorganic and organic components can be investigated together. Further analysis of the sand fraction could also be tested and added to the range of methods used in the present study to test and potentially extend the power of grouping samples collected at the same site and of separating samples collected at different sites, regardless of the soil parent material in such subtropical environments.

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