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Opal phytolith extraction in oxisols

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ABSTRACT

The analysis of phytolith assemblages is an important method in studies that uses the soil as an environmental record in order to reconstruct the paleoclimatic conditions. Despite being underused in soil science, this technique is used complementary to the analysis of pollen and other microfossils, as well as to evaluate silicon reserves in the soil. Currently, there are several concentration methods for phytolith extraction from sediments, soils, and paleosols. Most were developed and applied to materials of temperate zones or hydromorphic environments. Few have been conducted in tropical soils where oxides and hydroxides of iron and aluminum, as well as organic matter that often blankets the soil matrix, are common and hamper the extraction, the observation and identification of phytoliths, compromising the morphological analysis and quantification of phytolith assemblage in soils. In this paper, three methods of pre-treatment for the removal of the coating of silt and sand particles were applied to samples of an Oxisol (Humic Hapludox) in order to compare the cleaning efficiency, integrity and number of phytoliths. The first method consisted of the oxidation of soil organic matter and of an acid hydrolysis for the removal of carbonates and oxides. In the second method, the dominant process was the reduction of iron using a combination Dithionite–Citrate–Sodium Bicarbonate. In the third method, only acetate and sodium dithionite dissolved in water was used. Overall, Method 1 was the most aggressive to phytoliths and proved less efficient and more selective in phytolith extraction. Methods 2 and 3 were similar in the pre-treatment of samples. Method 2 allows the conservation of greater variety and number of phytoliths, and smaller quantities of other particles. Method 3 is relatively inexpensive and faster because it uses fewer chemicals and centrifugation procedures.

1. Introduction

Phytoliths are silica particles formed by plants that are added to soils and they are between the most common plant remains (Piperno, 2006). The analysis of phytolith assemblages in soils is an important method for several studies, especially those aimed at reconstruction their pedoclimatic past conditions and their changing trends over time. Phytoliths can be used in place of pollen in non-hydromorphic soils (well drained, deep and sometimes old soils such as Oxisols) and buried Paleosols where the pollen grains are rare or absent (Moore et al., 1991). In addition, the analysis of phytoliths provides an alternative dataset in sediments where zooliths, pollen, sponges, and other plant residues are also present.

In the study of palaeoenvironmental significance of the umbric epipedon in Oxisol, the analyses of phytolith assemblages and stable carbon isotopes ($\delta^{13}C$ and ^{14}C) were used to interpret past pedoclimatic conditions (see Calegari et al., 2013). However, during phytolith extraction, several problems occurred due to high contents of iron and aluminum oxides (common in Oxisols) that coated the particles of the soil matrix, including the phytoliths. These problems required the need for methodological adjustments to remove the coatings prior to the phytolith extraction.

Several techniques, not always widely used, were developed for extracting phytoliths:ultrasonic bath (Parmenter and Folger, 1974; Marumo and Yanai, 1986),incineration (Powers and Gilbertson, 1987) and microwave digestion (Parr, 2002), to cite a few. However, the most widely used has been the separation of phytoliths by density, using heavy liquids (Piperno, 2006). Sodium polytung state is widely used because of its low toxicity, and ease of

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handling and recycling (Madella et al., 1998; Osterrieth et al., 2009). Prior to the density float, it is necessary to carry out pre-treatments to remove the organic matter and the carbonates. The most common approach is the acidic digestion with HCl (5% or 7%) and oxidation with H_2O_2 (33%) (Carbone, 1977; Madella et al., 1998; Piperno, 2006). The removal of organic matter and carbonate can also be achieved by burning (calcination) (Davies, 1971). However, this procedure can cause loss of water from the opal reticule, change in density and refractive index (RI) and deformations in the morphology of the morphtypes when temperatures above 500–600 °C are used for an extended period of time (Jones and Milne, 1963; Jones, and Segnit, 1969; Parr et al., 2001; Elbaum and Weiner, 2003; Parr, 2006).

Most procedures for pre-treatment and extraction of phytoliths were developed and applied to soil and/or sediments of temperate or hydromorphic environments (Lentfer and Boyd, 1998; Madella et al., 1998; Parr, 2002; Osterrieth et al., 2009 among others) in which contents of iron oxides and hydroxides are lower than in tropical soils, or they are found in reduced form (Fe^{+2}) and therefore more easily removed with strong-acid chemical pre-treatments.

Rapp and Mulholland (1992) emphasized that phytoliths are susceptible to solution with extreme pH values and that the presence of iron oxides and hydroxides helps protect them from changes in edaphic and taphonomic conditions over time. However, in order to separate the phytolith from other soil components, the removal of these coatings is necessary to improve the clay dispersion during the extraction and the observation of the particles at the microscope (Madella et al., 1998).

Currently, phytolith analysis is increasingly used in soils developed under tropical conditions in Africa and South America (Kondo and Iwasa, 1981; Piperno and Becker, 1996; Alexandre et al., 1997a,b; Alexandre and Meunier, 1999; Barboni et al., 1999; Runge, 1999; Bremond et al., 2005; Borba-Roschel et al., 2006). In these regions, sand and silt often show strong coatings of Fe and Al oxides and hydroxides that jeopardize themorphological identification of phytoliths and the assemblage interpretation. However, the removal of these coatings by chemical procedures can cause dissolution, breakage and/or destruction of phytoliths, compromising the soil assemblage composition (Madella et al., 1998).

For the removal of Fe and Al oxides and hydroxides, sodium dithionite is commonly used (a strong reducer) combined with sodium citrate (a complexing agent) and sodium bicarbonate (as buffering) (Mehra and Jackson, 1960). To establish an efficient protocol of phytolith extraction in tropical soils, this study compared three different extraction techniques:

1) The Madella et al. (1998) phytolith extraction techniques using H_2O_2, HCl and sodium polytungstate but without removal of Fe/Al oxides and hydroxides;
2) The Mehra and Jackson (1960) technique to remove organic matter and Fe/Al oxides and hydroxides as a first step previous to the extraction according to Madella et al. (1998);
3) A modified approach inspired by Deb (1950) techniques for removing the organic matter and Fe/Al oxides and hydroxides where the citrate and bicarbonates are not used to avoid high pH solutions, followed by the extraction according to Madella et al. (1998).

The main objective was to determine which method would be less aggressive, more practical and more effective in removing the coatings of the soil matrix but without affecting the number and integrity of phytoliths extracted from the soil.

2. Regional setting and soil characteristics

For this comparative approach, samples were collected at 0–10 cm, 20–30 cm, 40–50 cm and 60–70 cm from a *Latossolo Vermelho-Amarelo húmico* (EMBRAPA, 2006) that corresponds to a Humic Hapludox (Soil Taxonomy, 2006). The soil is located at 21°38′04.26″ S, 45°56′22.37″ W in Machado, South Minas Gerais State, at 1115 m asl.

The geology of the area is metamorphic rocks (gneisses and migmatites) of the Guaxupé complex. The climate is moderate humid subtropical (Cwb) with hot, humid summers and cold, dry winters with an average annual rainfall of around 1500 mm. This area is characterized as a zone of ecological tension between the Sub-Perennial Tropical forest and *Cerrado* (Silva and Vidal Torrado, 1999).

The soil is clayey, with pH in water between 4.2 and 5.5 (Table 1). Content of Fe_2O_3 is ca. 8.67 g kg^{-1} and of Al_2O_3 ca. 24.71 g kg^{-1}. The soil has a kaolinitic mineralogy, with values of Ki and Kr from the top to the bottom of the trench varying from 1.36 to 1.53 and 1.17 to 1.30, respectively (Calegari, 2008).

3. Materials and methods

The samples' pre-treatment for the removal of organic matter and oxides/hydroxides of iron and aluminum started with four grams of air-dried soil: Sample 1 (0–10 cm), Sample 2 (20–30 cm), Sample 3 (40–50 cm) and Sample 4 (60–70 cm).

3.1. Removal of coatings

Method 1 Acidic digestion (Madella et al., 1998).

This method has been commonly used for archaeological studies and for paleoenvironmental reconstruction from sediments or soils of temperate, Mediterranean and subtropical regions. It consists of the removal of carbonates and partial removal of iron oxide by

Table 1
Physical and chemical characteristics of the Humic Hapludox.

Horiz.	Depth. cm	Munsell colour	Clay g kg^{-1}	C-organic g kg^{-1}	C-total g kg^{-1}	Soil density t m^{-3}	pH (H_2O) (1:2,5)	Molecular relation	
								Ki	Kr
A	0–10	5YR 3/2	454.6	43.9	46.9	0.83	4.2	nd	nd
A2	10–60	5YR 2.5/2	498.4	35.2	45.6	0.83	4.7	nd	nd
A3	60–85	5YR 2.5/3	566.2	33.6	33.4	0.90	5.0	1.36	1.17
AB	85–120	5YR 2.5/2	539.6	22.7	29.4	0.96	5.0	nd	nd
BA	120–140	5YR 4/4	597.3	16.7	18.7	1.08	4.9	nd	nd
Bw	140–170	5YR 4/4	623.5	9.7	12.5	1.06	5.5	nd	nd
Bw2	170–210+	5YR 5/8	649.7	7.0	9.3	1.00	5.3	1.54	1.30

nd: not determined.

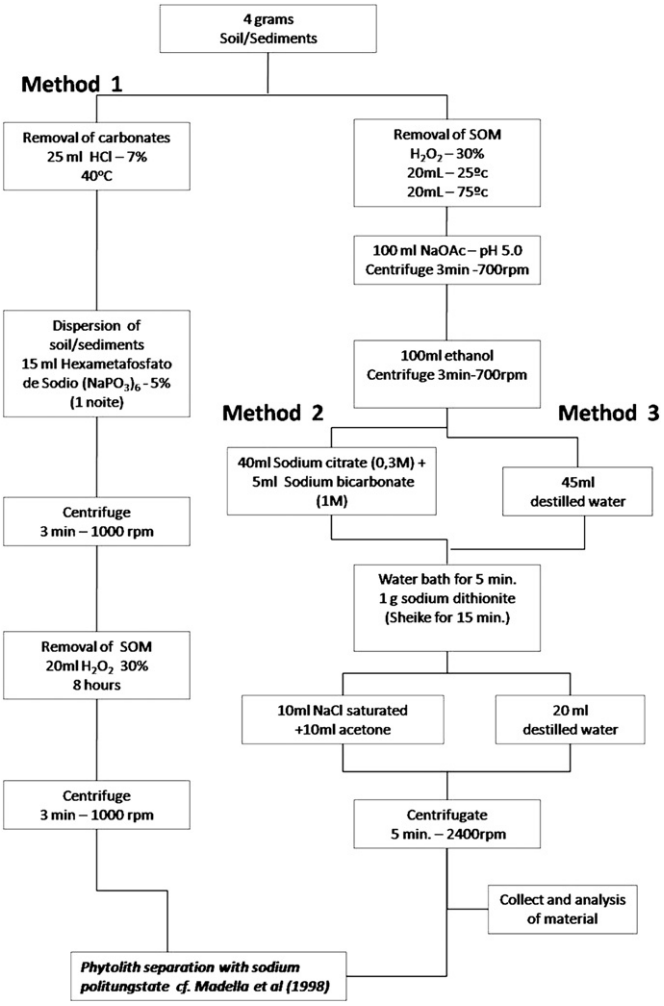

Fig. 1. Comparison of the different process stages for Methods 1, 2 and 3.

hydrolysis with strong acid (HCl) followed by an application of oxidant with H_2O_2 for the removal of the organic matter (Fig. 1).

Method 2 Citrate-dithionite-bicarbonate (Mehra and Jackson, 1960).

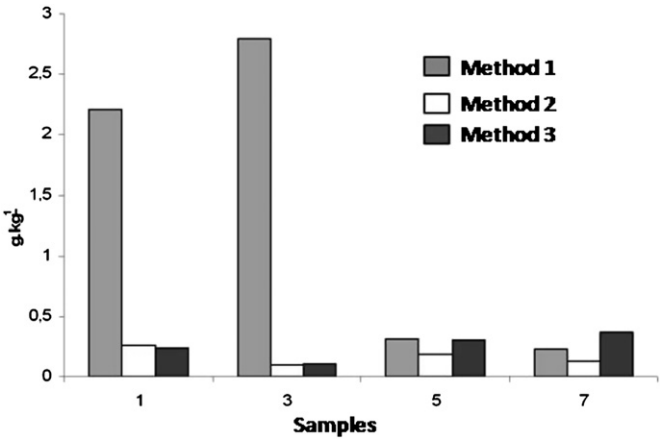

Fig. 2. Amount of final residues extracted (sodium polytungstate at 2.35 g cm^{-3}) from each method.

Table 2
Coefficient of variation (CV) and arithmetic mean of the phytolith assemblage.

		Method 1		Method 2		Method 3	
		CV	Mean	CV	Mean	CV	Mean
Poaceae	Panicoideae	116	2	74	4	68	3
	Pooideae	91	12	66	9	89	5
	Chloridoideae	110	5	91	4	53	4
	Elongate	82	14	45	16	85	18
	Bulliform	47	31	64	18	56	18
Dicotiledoneae	Globular	108	6	86	12	68	9
Araucariaceae	Globular echinate	131	2	41	14	82	8
Arecaceae	Crater-shaped	165	1	82	2	133	7

This method was proposed in soil analysis by Mehra and Jackson (1960) for the removal of organic matter with H_2O_2 and solubilization of free iron with a mixture of dithionite, citrate and sodium bicarbonate (Fig. 1). Reduction is the predominant process.

Method 3 Acetate–Sodium dithionite.

This is an adaptation of the method of Deb (1950) that uses sodium acetate buffered at pH 5, sodium dithionite dissolved in water for the solubility of free iron (see Fig. 1 for solution concentration).

3.2. Concentration of phytoliths

The concentration and subsequent extraction of phytoliths were performed gravimetrically using a solution of sodium polytungstate ($Na_6(H_2W_{12}O_{40})H_2O$) with density 2.35 g cm^{-3} as in Madella et al. (1998) and Osterrieth et al. (2009).

3.3. Classification and counting of phytoliths

The test slides were prepared as liquid mounting with immersion oil and three horizontal transects were selected in each slide (top, middle and bottom). Phytoliths were counted three times for each transect in each sample as suggested by Carnelli (2002). The observations were performed on a Zeiss microscope (400

Table 3
Mean of phytoliths counted per slide, phytolith proportion in respect to the extracted silica fraction (final residue) and estimated grams of phytoliths per kg of soil.

	Mean of phytoliths in the final residue	% on the slide	g kg^{-1}
Sample 1			
Method 1	1587795a	5.7a	2a
Method 2	190967a	20a	0.3a
Method 3	551578a	9a	1a
Sample 2			
Method 1	4874431a	6b	1a
Method 2	113946a	16a	0.2a
Method 3	593390a	10ab	0.2a
Sample 3			
Method 1	3943911a	6b	0.3ab
Method 2	121302a	13a	0.2b
Method 3	379644a	7ab	0.5a
Sample 4			
Method 1	246821a	10a	0.3a
Method 2	64653a	12a	0.1a
Method 3	218509a	3b	0.2a

Mean values followed by the same letter do not differ in the Tukey test ($p < 0.05$).

Table 4
Identified mean number of phytoliths from the 3-counts performed in each extraction method.

	Poaceae					Dicotiledoneae	Araucariaceae	Arecaceae
	Panicoideae	Pooideae	Chloridoideae	Elongate	Bulliform	Globular	Globular echinate	Crater shaped
Sample 1								
Method 1	4a	21a	4a	0a	10a	7a	4a	0a
Method 2	6a	7a	1a	18a	23a	14a	5a	2a
Method 3	3a	5a	3a	13a	20a	8a	11a	7a
Sample 2								
Method 1	3a	6a	4a	31a	31a	6a	3b	1a
Method 2	3a	6a	3a	18a	9a	8a	22a	1a
Method 3	2a	5a	3a	20a	19a	7a	11ab	7a
Sample 3								
Method 1	2a	19a	8a	8a	26a	4a	2b	5a
Method 2	4a	12a	6a	15a	15a	13a	16a	2a
Method 3	2a	5a	4a	19a	15a	10a	7ab	9a
Sample 4								
Method 1	0a	1a	5a	17a	58a	7a	1a	0a
Method 2	5a	4a	6a	14a	25a	11a	12a	2a
Method 3	3a	6a	5a	18a	18a	10a	4 a	7a

Mean values followed by the same letter do not differ in the Tukey test ($p < 0.05$).

magnification). At least 245 identifiable phytoliths were counted (named according to ICPN, Madella et al., 2005) in all slides except for the slide of sample 1 from method 1 in which it was possible to count only 38 phytoliths, even after observing the entire slide surface. The phytolith assemblages are expressed in terms of percentage of the sum of morphtypes identified in each slide per sample.

Four groups of morphtypes were formed based on the standard morphology of the different plant material: (a) Poaceae (Twiss et al., 1969; Tieszen et al., 1979; Twiss, 1992), (b) Arecaceae (Palm trees) (Kondo and Iwasa, 1981; Alexandre et al., 1997b; Alexandre and Meunier, 1999; Barboni et al., 1999; Piperno, 2006), (c) Dicotiledoneae (Runge, 1999), and (d) Araucariaceae (Parr and Watson, 2007).

3.4. Statistical analysis

The assemblages were assessed using the Shapiro–Wilk distribution test and the Turkey test ($p < 0.05$) for variance and mean comparison (software Statistical Analyses System Institute, 2005) to analyze the statistical differences between the samples of the same profile and between the procedures. Thus, the average values of each morphotype and the number of phytoliths were compared between samples (1, 2, 3 and 4) and between procedures (methods 1, 2 and 3).

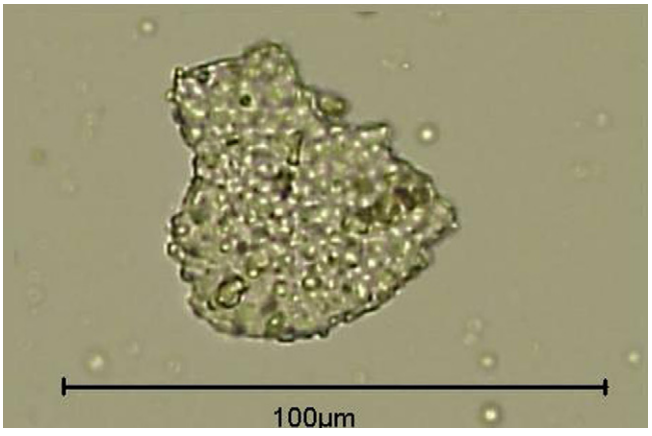

Fig. 3. Corrosion on a *Bulliform* phytolith extracted using Method 1 (sample 1/ 0–10 cm).

4. Results and discussion

The results show that, regardless of the method, the number of phytoliths extracted decreased with depth. This is an effect of taphonomic processes and the vertical and lateral translocation by water (Alexandre and Meunier, 1999; Runge, 1999).

The tested pre-treatment techniques applied in the phytolith extraction influence the recovered assemblages in relation to the pH of the used solutions (HCl, DCB and Dithionite diluted in water). Method 1 recovered 20–30 times more material in the acid insoluble fraction, composed of phytoliths, diatoms, quartz and other silicates. However, after centrifugation with Na polytungstate, the final residue was the one with the least phytoliths (Fig. 2), most of them strongly affected by dissolution. This is the result of the strong effect of an acid solution applied in sediments with little or no carbonates that can buffer the pH. Also, the high amount of non-phytolith material that was recovered indicates that the dispersive effect of Method 1 was not enough to specifically eliminate the clay fraction of the materials.

Absolute and proportional numbers of the counted phytoliths assemblages, when tested with the Shapiro–Wilk test, showed normal distribution. Method 1 showed the highest coefficient of variation in the percentages of identified morpohtypes (Table 2) and overall the average number of phytoliths per $g\,kg^{-1}$ of soil differed only for Method 1. However, inefficiency in the dispersion in Method 1caused a higher presence of other silica materials (e.g. diatoms, silicates, etc.) in the final residue, and consequently an overestimate in the amount of phytoliths extracted (and therefore in the amount per $g\,kg^{-1}$) as the final residue is supposedly mostly composed of plant opal silica.

The variability observed in the number of phytoliths $g\,kg^{-1}$ (Table 4) shows that Method 2 and 3 were more efficient in phytolith deflocculation and extraction for tropical soils of this nature. The phytolith assemblage obtained by Method 2 was the most diverse and composed of Poaceae, Arecaceae, Araucariaceae and Dicotiledoneae.

The absolute and proportional numbers of phytoliths on each slide are presented in Table 3 and show that:

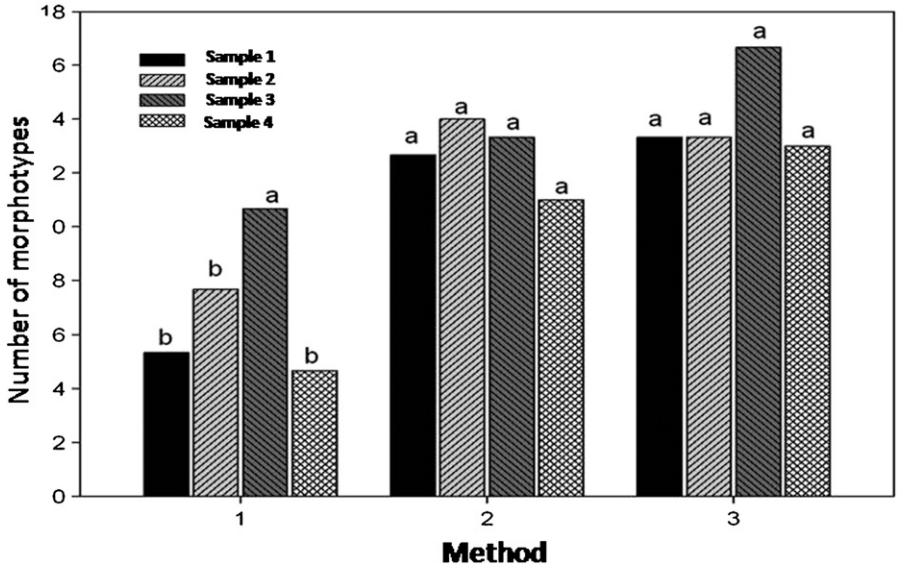

Fig. 4. Variability of morphotypes as a comparison between the different extraction methods. Mean values followed by the same letter do not differ in the Tukey test ($p < 0.05$).

a) The total number of phytoliths contained in the final residue (light fraction) showed little statistical variability;
b) The percentage of phytoliths on the slides differs between the methods in samples 2, 3 and 4;
c) The content of phytoliths in the soil ($g\,kg^{-1}$) decreases with depth and showed little variability between methods, with the exception of sample 3 that has a significant difference between Method 1 and 2.

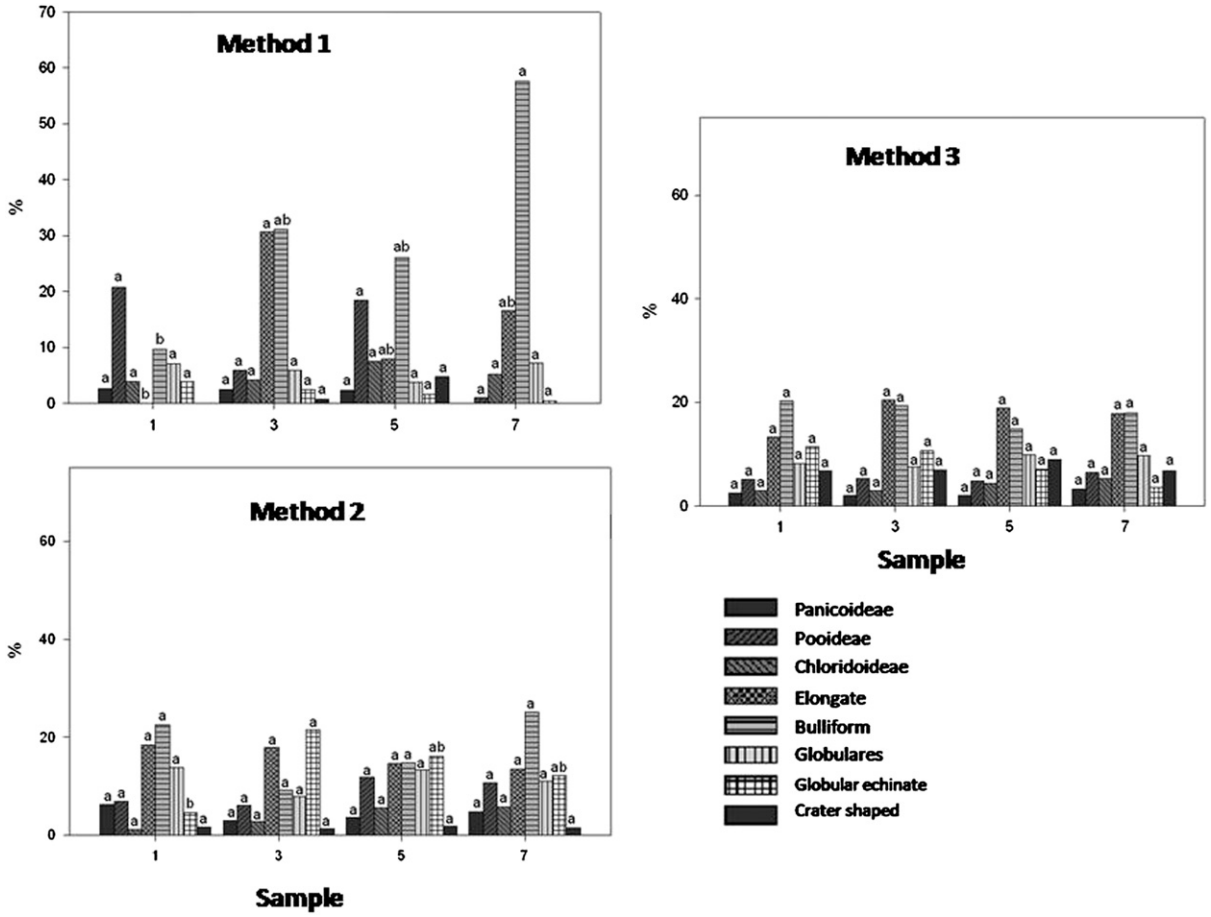

Fig. 5. Comparison of the different processed samples using the same extraction method. Mean values followed by same lowercase letter do not differ in the Tukey test ($p < 0.05$).

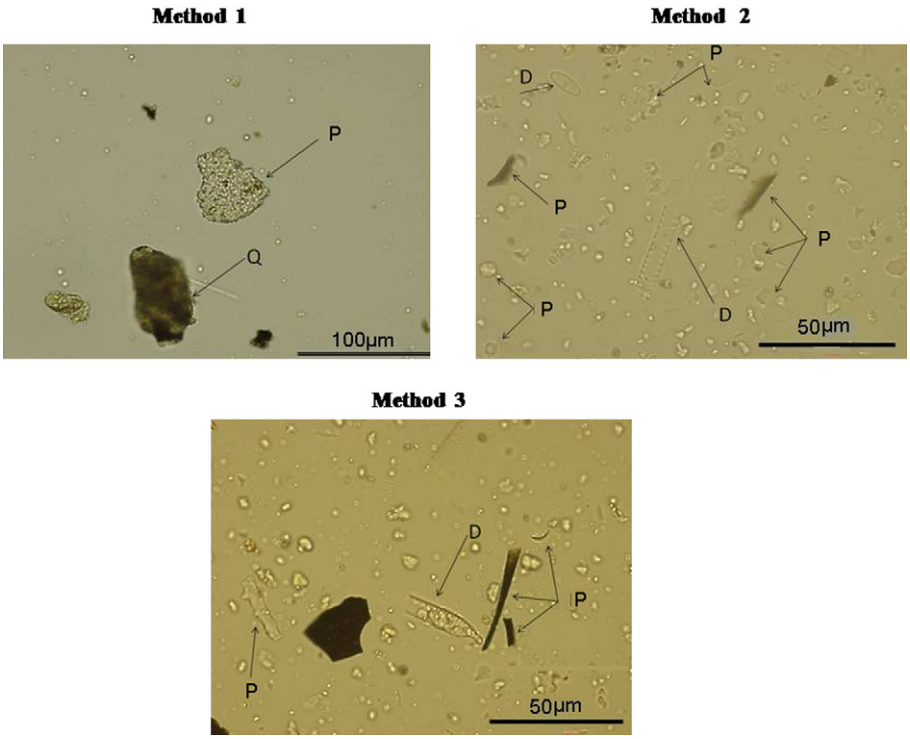

Fig. 6. Microscopy view of the final residue as mounted on slide resulting from the three different extraction methods. P: Phytoliths, D: Diatoms.

The lower proportion and less morphotype variety obtained by Method 1 seem to result from the higher solubility of silica triggered by extreme acidity (pH < 2) during the pre-treatment with HCl. Most of the phytoliths in the samples treated with Method 1 showed signs of corrosion. On the other hand, this method produced excellent results in samples from archaeological sites and natural soils from temperate and subtropical regions (Osterrieth et al., 2009). The fundamental difference is that the samples used in this work are devoid of carbonates and the HCl promoted avery strong acidic hydrolysis (pH < 2), which, besides dissolving the iron oxides and hydroxides, caused the partial dissolution and corrosion of the mineral particles (quartz) and especially of biogenic opal. This effect was felt more strongly in phytoliths with low surface-to-volume ratio (SA:V), such as globular forms (Table 4). The correlation between dissolution and SA:V was studied by Bartoli and Wilding (1980) and Drees et al. (1989) and according to these authors, the dissolution rate of silicate minerals is directly proportional to the size of the specific surface. The almost constant higher presence of bulliforms in Method 1 assemblages could be related to the specific morphology of this phytolith type that allows identification even when heavily taphonomised (Figs. 3and 4).

The only statistically significant morphotype count differences are the ones between Method 1 and 2 in samples 2 and 3. This is the *Globular echinate* morphotype produced by Arecaceae (Table 4). Furthermore, Method 2 assemblage has morphotypes representing different diameter classes while for Method 3 larger diameters predominated. This is possibly showing again the effect of higher opal silica dissolution, due to low pH, in Method 1 and, to a much lesser degree, of Method 3.Method 2 and 3 have very similar phytolith average values and they are more efficient in cleaning the opal bodies.

The comparison between the same samples processed with the different methods showed statistical difference in Method 1 for the morphotype *Bulliform* between samples 1 and 2 and for *Elongate* between samples 1 and 4. In Method 2,a difference was observed only between samples 1 and 2 for *Globular echinate* morphotypes produced by Arecaceae. Method 3 showed no significant difference between samples (Fig. 5).

From observation carried out at the microscope during the counting, the samples treated with Method 2 and 3showed phytoliths in which the organic matter and “free iron” coatings were better removed than in Method 1 (with Method 2 slightly better than Method 3) (Fig. 6). Also, corrosion marks (such as surface pitting) increased with sample depth. This occurrence is considered normal given the longer exposure for the lower assemblages to soil taphonomic processes (Rapp and Mulholland, 1992). The exception was Method 1, where all silica bodies (phytoliths as well as diatoms) had clear sign of dissolution/corrosion in all samples, probably as a result of the low pH (below 2) during the pre-treatment with HCl.

5. Conclusion

From the comparison of the three phytolith extraction techniques presented in this work, it is concluded that:

- Method 1 was the most aggressive on phytoliths due to extremely acidic environment produced by using HCl in a low-carbonate soil.
- Methods 2 and 3 showed no relevant differences in the removal of the coating with the advantage of Method 3 being relatively cheaper and faster, because it requires fewer chemicals and centrifugation steps.
- Method 2 was the most effective in terms of clay dispersion and showed the highest diversity and numbers of phytoliths as well as less impurities, probably better representing the original vegetation input.

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