Rapid phytolith extraction for analysis of phytolith concentrations and assemblages during an excavation: an application at Tell es-Safi/Gath, Israel

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A rapid phytolith extraction procedure is described, that allows phytolith concentrations and morphotype assemblages to be analyzed within hours. This procedure enables the results of these analyses to be used during an archaeological excavation, in order to better understand how plants were used. The new procedure was tested using a standard phytolith extract and two experimental phytolith-sediment blends and found to be both accurate and precise. The reliability of partial slide counting was evaluated and found to be as accurate and precise as existing phytolith quantification methods. The new extraction and counting procedures were applied to an archaeological site, Tell es-Safi/Gath, Israel. The results demonstrate how information on phytolith concentrations in sediments that are available from one day to the next, can be used during an excavation to more effectively document the local features of interest and obtain better information.

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1. Introduction

Identifiable botanical remains in archaeological sites commonly include charred organic tissues, pollen and inorganic biogenic minerals such as opaline phytoliths. Phytoliths are usually preserved in large quantities in many archaeological sites worldwide and are used to study past environments, fuel types, foddering practices, diets, activity areas, and more. Phytolith analysis also provides partial information on plant taxa, as well as the parts of the plant that were used, such as stalks versus inflorescence of cereals. Phytolith analysis can thus provide key information on many aspects of a site not seen with the naked eye. Several techniques for separating the phytoliths from sediments have been published (Albert et al., 1999, 2003; Albert and Weiner, 2001; Lentfer and Boyd, 1998, 1999, 2000; Madella et al., 1998; Powers and Gilbertson, 1987; Zhao and Pearsall, 1998). All these methods are time consuming (usually requiring more than 24 h per batch of samples). Some require a battery of instruments and some use hazardous materials.

Phytolith analysis usually involves two different types of measurement: phytolith concentrations and the relative proportions of different phytolith morphotypes. A difficulty with phytolith analysis is that except for the cases in which visible phytolith-rich layers are identified in the field, all information is obtained in the laboratory with the aid of a microscope well after the excavation season is complete. Thus in the absence of a “real-time” phytolith analysis method, the sampling strategy is “blind” and important features are often either missed or mis-sampled, and their distributions are not accurately mapped. This impairs archaeological interpretation, both in the field and later when a full picture of the studied archaeological phenomenon is obtained. We have therefore developed a rapid extraction procedure that enables phytolith concentrations to be determined for 10–20 samples within hours, or both phytolith concentrations and morphotype assemblages of 4 to 6 samples in around 5 h. This makes it possible to use this information from one day to the next during an excavation. Here we present this method and illustrate its use at Tell es-Safi/Gath, Israel.

2. Materials and methods

2.1. Rapid phytolith extraction procedure

A sediment sample is sieved in order to remove the fraction larger than 0.5 mm. An accurately weighed amount of between 20
and 50 mg of this homogenized sediment is placed in a 0.5 ml conical plastic centrifuge tube (Eppendorf 0030 121.023). Fifty microliters 6 N HCl are added using an adjustable pipette (Gilson Pipetman) in order to dissolve carbonate minerals and carbonated hydroxyapatite, including small bone fragments. After the bubbling has ceased (usually a few minutes) 450 μl 2.4 g/ml sodium polytungstate solution (SPT, Na₀₆H₂[W₁₆O₄₈]-H₂O) (Sometu Ltd., Berlin) is added. The tube is vortexed for about 3 s, sonicated for ca. 10 min (using a Cole-Parmer Ultrasonic Cleaner), vortexed again and centrifuged for 10 min at 5000 rpm (Eppendorf Centrifuge 5418). At a final density of 2.26 g/ml SPT, most of the remaining minerals (mainly quartz and clay) pellet after centrifugation and the phytoliths and charred organic material remain in suspension. Note that if the sediment is rich in uncharred organic material, it can be removed by the addition of 50 μl of fresh hydrogen peroxide after the acid step. This was not necessary in the present study. If the sediment does not contain bone or calcite, then the acid step can be eliminated. If phosphate minerals more insoluble than carbonated hydroxyapatite are present then nitric acid can be added. The starting concentration of SPT will need to be adjusted in order to have a final concentration of 2.26 g/ml. We used SPT as it is non-toxic and includes sodium which disperses clays. Note that sonication may break multi-cellular phytoliths, but is necessary to disperse aggregates.

Once the phytoliths are in suspension (following the first part of the procedure above), the supernatant is removed to a new 0.5 ml centrifuge tube and vortexed. An aliquot of 50 μl of the supernatant is removed and placed on a microscope slide and covered with a 24 mm × 24 mm cover-slip. Fifty microliters is the maximum volume that allows the solution to completely fill the space between the slide and the cover-slip. The transfer of the 50 μl should be carried out immediately after the vortexing to eliminate any bias due to fractionation within the tube that occurs as the phytoliths slowly float to the top. The phytolith concentration can be calculated since the total amount of phytoliths on the slide represents 10% (50 μl out of 500 μl) of the total amount of phytoliths in the initially weighed sample. Note that by transferring the phytoliths in suspension, their distribution on the slide is relatively homogeneous. In previous procedures, the phytoliths are transferred as a dry powder and then mixed with glue (usually Canada Balsam or Entellan). The phytolith distribution on the slide is then relatively heterogeneous and thus necessitates counting the whole slide or a major part of the slide. This is very time consuming.

There is no single recommended counting procedure. The method used should reflect the general phytolith concentrations, the presence of other siliceous materials such as diatoms, sponges and radiolarians (usually derived from associated weathered chalk and chert), the optimal turn-around time, and the extent to which the phytoliths are homogeneously distributed on the slide. Furthermore, it is essential to a priori define “rules” for counting, such as whether very small sized phytolith fragments are to be included, and whether multi-celled phytoliths are counted as one phytolith or as the number of individual cells within the multi-cellular structure.

A general approach we use is to count 10 fields at 200× magnification. We use this magnification for counting only, as the phytoliths can be readily identified and more phytoliths can be counted than at 400× magnification. When working on phytolith morphologies we use the standard 400× magnification in order to reveal detailed morphological characteristics. For the counting, we randomly choose two fields around the center of the slide and 8 fields closer to the periphery. If the variability of these 10 values is high (in this study, over 30%, see description in the “Results” section), then additional sets of ten fields can be counted in the same way, until an acceptable variability is obtained. The slide can also be used for analyzing the assemblage of morphotypes.

As the pH of the extraction solution is very low and the SPT crystallizes within hours once placed on the slide, the remaining supernatant can be diluted and then centrifuged (10 min at 5000 rpm). This pellets the phytoliths. The supernatant is then discarded and the pellet is re-suspended in water, centrifuged and the supernatant is removed again. This is repeated twice in order to remove all traces of the extraction solution. The sample is then air dried in the tube and can be used later for morphology analysis.

2.2. Determining reproducibility

A phytolith standard was prepared from fresh palm (Phoenix dactylifera) leaves. This genus was chosen because it is known to produce a large amount of globular echinate phytoliths (see e.g., Bamford et al., 2006). In this study we found that 67% of the leaf phytoliths of P. dactylifera are of this single morphotype, namely globular echinates (Fig. 1), based on counting and characterizing the morphologies of over 1800 phytoliths. The globular echinate morphotype is easy to identify and has no large size variations. It is thus expected that all globular echinates will behave similarly during the procedure (unlike a standard composed of phytoliths of various shapes and sizes that may behave differently during centrifugation and slide preparation). In addition, based on our experience, date palm phytoliths are extremely rare in the archaeological record in Israel. This standard can be diluted with local sediments without the potential difficulty of differentiating between globular echinates from the standard and the local sediment.

About 15 g of leaves from fresh P. dactylifera were cut into centimeter sized pieces and sonicated for ca. 30 min to remove any adhering dust. They were then burned in a furnace oven at 500 °C for 4 h. The ash was dissolved in a 3 N HCl + HNO₃ solution (1 N HCl would also be satisfactory), and the solution was removed after centrifugation (5 min at 3000 rpm) and then re-suspended in distilled water and centrifuged again. This washing procedure was repeated 3 times. The dried pellet was burned again at 550 °C for 1 h to further remove charred particles. The concentration of the globular echinates in the standard was determined by mixing a weighed amount of ca. 1.0 mg of the standard with 5 ml 2.3 g/ml SPT. Two mixtures were prepared, and in each case several slides were made by using 50 μl of the solution immediately after

Fig. 1. A globular echinate phytolith from the leaves of Phoenix dactylifera.
vortexing and sonication (as described above). All the globular echinate phytoliths on the slide were counted, and the concentration of these phytoliths in 1 g of the standard was calculated.

In order to test the accuracy, precision and duration of the extraction procedure, we prepared two blends of different concentrations of the standard with a phytolith-free sediment. The sediment chosen was loess from the Negev Highlands area of southern Israel that contains practically no phytoliths (less than 100 phytoliths per g; counted using the published procedure of Albert et al., 1999). In order to obtain as homogenous a blend as possible, this sediment, composed mostly of quartz, clays and calcite, was sieved through a 0.3 mm mesh sieve and the fraction smaller than 0.3 mm was lightly ground using a mortar and pestle to produce a fine powder. A weighed aliquot of the standard was then mixed with a weighed aliquot of the powdered sediment, and was shaken (manually) in a 50 ml tube for 1 h. Two such blends were prepared, which contain 4.9% and 0.09% by weight of the palm leaves standard. The phytoliths from these blends (called 4.9% blend and 0.09% blend, see details below) were then extracted using the rapid extraction procedure described above.

2.3. Counting the whole slide as opposed to a part of the slide

We determined whether phytoliths can be counted in a portion of a slide and to what extent this procedure still produces accurate and precise results. Three sediment samples were prepared according to the rapid procedure, one from the known concentration 0.09% blend sediment, and two archaeological samples from Tell es-Safi/Gath. A single slide was prepared from each sample. Each of the three slides was counted by 4 persons, two experienced (O.K. and D.C.) and two less experienced in phytolith counting (S.W. and R.S.G.). Each person counted 4 sets of 10 fields of view at 200× magnification, as described above. The 0.09% blend sample included only globular echinate phytoliths and was thus easy to study, relative to the two archaeological samples that included both phytoliths and opal from marine microfossils (mainly sponge spicules, diatoms, radiolarians and their break-down products) due to the presence of chalk in the tell sediments. Therefore, in these samples only opaline particles which were definitely identifiable as phytoliths were counted, as some confusion may arise due to similarities between the marine microfossil debris and true plant phytoliths. This in turn implied that small opaline fragments were excluded from the count. It is therefore expected that the error between the counting persons will be smaller for the 0.09% blend counting relative to the error in counting the archaeological samples. For each person we calculated the standard deviation of the number of phytoliths counted per 10 fields. The precision for the study of the archaeological sediments should therefore correspond to the overall error between the various counting persons. We did not conduct whole-slide counting on these two samples, i.e., we did not examine the accuracy of the extraction method on the archaeological samples.

Table 1: Amount of phytoliths on slides from the two standard solutions. (a) Counted by O.K. (b) Counted by D.C.

<table>
<thead>
<tr>
<th>Slide</th>
<th>Solution 1</th>
<th>Solution 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1103 (a)</td>
<td>1373 (a)</td>
</tr>
<tr>
<td></td>
<td>1212 (b)</td>
<td>1390 (b)</td>
</tr>
<tr>
<td>2</td>
<td>1205 (a)</td>
<td>1115 (a)</td>
</tr>
<tr>
<td></td>
<td>1351 (b)</td>
<td>1337 (a)</td>
</tr>
<tr>
<td>Average (±S.D.)</td>
<td>1173 ± 61</td>
<td>1313 ± 113</td>
</tr>
<tr>
<td>Average (±S.D.) 8 measurements</td>
<td>1260 ± 120</td>
<td>1260 ± 120</td>
</tr>
</tbody>
</table>

Table 2: Amount of phytoliths on slides and calculated phytolith concentrations (millions per g) in the 4.9% blend.

<table>
<thead>
<tr>
<th>Slide</th>
<th>Weight (mg)</th>
<th>Phytoliths on slide</th>
<th>Phytoliths in blend (Millions per g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.7</td>
<td>1103</td>
<td>5.60</td>
</tr>
<tr>
<td>2</td>
<td>19.8</td>
<td>1335</td>
<td>6.78</td>
</tr>
<tr>
<td>3</td>
<td>18.7</td>
<td>1116</td>
<td>5.97</td>
</tr>
<tr>
<td>4</td>
<td>19.1</td>
<td>1179</td>
<td>6.17</td>
</tr>
<tr>
<td>Average (±S.D.)</td>
<td>19.0 ± 0.7</td>
<td>1131 ± 113</td>
<td>6.1 (±0.78)</td>
</tr>
<tr>
<td>Expected</td>
<td>6.2 (±0.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Amount of phytoliths on slides and calculated phytolith concentrations (per g) in the 0.09% blend.

<table>
<thead>
<tr>
<th>Slide</th>
<th>Weight (mg)</th>
<th>Phytoliths on slide</th>
<th>Phytoliths in blend (per g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54.4</td>
<td>583</td>
<td>107,200</td>
</tr>
<tr>
<td>2</td>
<td>54.5</td>
<td>544</td>
<td>99,800</td>
</tr>
<tr>
<td>3</td>
<td>50.3</td>
<td>499</td>
<td>99,200</td>
</tr>
<tr>
<td>4</td>
<td>45.3</td>
<td>536</td>
<td>118,300</td>
</tr>
<tr>
<td>Average (±S.D.)</td>
<td>51.0 ± 10.6</td>
<td>518 ± 113</td>
<td>106,100 (±10,600)</td>
</tr>
<tr>
<td>Expected</td>
<td>120,000 (±10,000)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. Accuracy and precision of the rapid extraction method, evaluated from the expected and calculated phytolith concentrations of the 4.9% (a) and 0.09% (b) blends.
Two standard-sediment blends were then prepared: the first extract represents 4.9% in the total weight of the blend. Therefore the expected phytolith concentration of globular echinates in the P. dactylifera standard is calculated to be 126 ± 12 million per gram. The uncertainty represents the standard deviation of the phytolith counting on the slides. This is about 10%.

Two standard-sediment blends were then prepared: the first was composed of 1891 mg sediment and 98 mg phytoliths (i.e., the P. dactylifera extract represents 4.9% in the total weight of the blend), and the second contained 26,300 mg sediment and 24 mg phytoliths (i.e., the P. dactylifera extract represents 0.09% of the total weight of the blend). Therefore the expected phytolith concentrations in the 4.9% and 0.09% blends were 6.2 ± 0.6 million and 120,000 ± 11,000 phytoliths per gram, respectively. The rapid extraction method was repeated four times on each sediment blend.

In processing these two blends, the procedure was adjusted by dilutions to produce slides which contain 500–1000 phytoliths; a quantity which is both large enough and reasonable to count accurately. In the case of the 4.9% blend, ca. 20 mg of the blend was weighed for each sample and processed according to the rapid extraction procedure with a further dilution step at the end as follows: the supernatant was transferred to a 15 ml tube and 4.5 ml of SPT were added. The sample was thus further diluted 10×. After vortexing, 50 µl were placed on a slide, which is expected to contain 1200 ± 120 phytoliths. In the case of the 0.09% blend 50 mg were weighed and no dilution was needed in order to produce slides with expected phytolith quantities of 600 ± 50. In each case, all the phytoliths on the slide were counted.

The results are presented in Tables 2 and 3, and Fig. 2. The results and standard deviation of the extraction and quantification of phytoliths from the 4.9% and 0.09% blends reproduce the expected value within a standard deviation. The latter includes both the error we calculated for the pure phytoliths (about 10%) and the reproducibility of the 4 slides made for each blend. The overall precision in this experiment is 12.8% and 10.0% for the 4.9% and 0.09% blends, respectively.

### 3.2. Counting phytoliths

Counting the number of phytoliths on a slide from archaeological sediments introduces a number of additional errors. These errors arise from the fact that only a part of the slide is counted in order to be able to process as many samples as possible within a short time, and from counting errors. We therefore prepared 3 slides and had 4 different persons count the same slides. Two of the counters had much experience working with phytoliths, and two did not. Fig. 3a shows the number of phytoliths counted in each of

2 h. The average counting time when 40 fields of view were counted, was less than half an hour per person per slide.

### 3.1. Accuracy and precision of the extraction method

The average number of globular echinates on the slides of the standard when counting the whole slide is 1260 ± 120 (Table 1). This average represents the preparation and counting of whole slides conducted separately by two people. The standard deviation therefore represents both analytical and counting errors. The phytolith concentration of globular echinates in the P. dactylifera standard is calculated to be 126 ± 12 million per gram. The uncertainty represents the standard deviation of the phytolith counting on the slides. This is about 10%.

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the 40 fields of archaeological sample TS-8181 by all four persons, while Fig. 3b shows the average number of phytoliths per field and standard deviations for each of the 4 sets of 10 fields. For each person, the results do not differ much between the 4 sets of 10 fields and the average and standard deviation of all 40 fields. Thus for each person there is no difference if 10 or 40 fields are counted. Furthermore, three of the four persons produced comparable results. One person consistently counted more phytoliths than the other three, demonstrating the need for well defined counting guidelines. Guidelines were therefore better defined prior to counting archaeological sample TS-5572, namely to only count opaline particles that can definitively be identified as having been derived from a phytolith. This eliminated counting many small fragments. The multicellular structures or articulated phytoliths (Madella et al., 2005) were counted as a single phytolith.

Fig. 4 show the results for sample TS-5572 in a similar manner as presented for the archaeological sample above. As in the former sample, there is not much difference if each person counted 10 or 40 fields. However, TS-5572 demonstrates the importance of experience, as the two experienced persons produced better results than the less experienced persons. There are fewer differences between the sets of fields counted by the experienced persons, and they both generally had the same results. However, as we have emphasized earlier, we cannot conclude from this experiment whether the two experienced or the less experienced persons reached more accurate results. For both archaeological samples the overall error was around 30% (calculated as the average of the errors of the 4 counting persons in the two archaeological samples).

3.3. Archaeological demonstration of the usefulness of the rapid extraction method with respect to phytolith concentrations

The archaeological demonstration was carried out at Tell es-Safi/Gath, located in the Shephelah (Judean foothills), Israel (Fig. 5). The Iron Age strata in Area A include evidence of a major destruction of the site in the mid- to late 9th century BCE (Stratum A3; Maier, 2004, 2008). One 5 × 5 m square (87B; Fig. 5) was excavated using the décapage technique, namely removing layers of sediment 5 cm thick, following features of the almost horizontal stratigraphy. The excavation started at 175.80 m above sea level (a.s.l.), several tens of centimeters above the presumed floor on which the destruction occurred. The square was cleaned to create a horizontal level, and its eastern part was divided into a grid of 15 sub-squares of 1 m² (Fig. 6a). Steps around the periphery were left unexcavated for later sampling of blocks for micromorphology. All macroscopic finds (e.g., bone fragments, charred material, etc.) and sediment samples were registered on a three-dimensional (xyz) grid. Sediment samples were collected for analysis along the excavation and analyzed on a daily basis. The initial sampling strategy was to collect representative samples from the center of each excavated sub-square. The phytolith analyses were then carried out in a temporary laboratory that was set up in a room in the boarding area of the excavation team, ca. 10 km from the site. The phytolith concentrations obtained each day enabled a change in the sampling strategy to be made before excavation commenced the next day. Thus features revealed by the presence of high or low phytolith concentrations, could be further sampled and in this way the feature could be better defined. Fig. 6b shows the phytolith concentrations measured using the initial systematic sampling strategy in the center of each sub-square (i.e., roughly evenly spaced samples) extracted from the sediments at an elevation of 175.70 m a.s.l. Two samples with relatively high concentrations indicated the possible presence of a phytolith concentration in the southeast corner where an assemblage of ceramic vessels was also exposed, and one sample contained high phytolith concentrations in the northern part of the square (Fig. 6b). Based on these results, the sampling strategy was changed the next day, and more samples were taken from the same level before excavation proceeded, in order to verify and better delineate these observations. These additional samples indeed verified the presence of both high concentrations (small font Fig. 6b), and highlighted the association of one of these concentrations with the ceramic vessel assemblage, and the other phytolith concentration with a stone assemblage. Further excavation verified these trends, as phytolith concentrations remained relatively high near these two localities.
The fact that this information was available during the excavation enabled us to adjust the excavation methods and sampling strategy on a daily basis. In general, the ability to conduct various analyses both in the field itself, and/or in a temporary laboratory while the excavation is in progress, enables the results (even if preliminary) of these analyses to be incorporated into the planning of excavation procedures, priorities and strategies, in “real time”. This approach can rectify a common problem encountered in the post-exavation laboratory analysis of archaeological finds, namely that all too often additional sampling and/or changing the excavation strategy are necessary in order to better document interesting observations, but is no longer possible.

4. Discussion

The rapid extraction method is capable of producing accurate and precise phytolith concentration measurements. The sample preparation time is reduced from ca. 24 h to ca. 30 min per sample. With minimal additional effort, the remaining sample can be washed and stored dry for later analysis of morphotype proportions, or this can be done immediately while the sample is still in the extraction solution. We also show that counting time can be significantly reduced by counting only a small portion of the slide area. We emphasize that the number of phytoliths counted is large enough statistically to obtain reproducibility comparable to that of the more labor intensive procedures. We in fact use the rapid technique for determining phytolith concentrations routinely and not only in the field.

The precision of Albert et al.’s (1999) extraction method is also ca. 30% (Katz et al., 2007; Shahack-Gross et al., 2003). Here we showed that comparable precision can be achieved in a very short time, even when some of the most challenging samples are counted, namely those that contain other archaeologically irrelevant biogenic opaline fossils, and many unidentifiable fragments of opal. The precision will probably increase if more homogeneous samples are counted, based on the results shown using the artificial blends.
of phytoliths and sediment. One reason for the improvement is that the rapid technique includes only one weighing step and no transfers of dry powder – both of which are sources of error. Furthermore, the fact that the dry powder does not have to be mixed with glue for mounting on the slide, eliminates another serious problem, namely that the distribution of phytoliths on the slide is inhomogeneous, and varies from slide to slide. This in turn necessitates counting many more fields of view. We did note that when 50 microliters of the extraction solution are placed on a slide, the periphery of the slide tends to contain more phytoliths than the center. We thus recommend counting central and peripheral areas in a systematic manner.

The new method presented here was optimized for sediments from Tell es-Safi/Gath, and can probably be used in other Mediterranean open air sites. Soils and sediments in other parts of the world are different and the procedure has to be modified accordingly. The final SPT concentration may have to be adjusted for removing the presence of minerals other than the clay and quartz that are common in our samples. The supernatant may also include organic and/or charred materials in addition to phytoliths. Therefore, in cases where the sediment is composed of high quantities of organic or charred materials, these may interfere with the identification of the phytoliths. The uncharred organic material at least can be removed by the addition of a small amount of fresh hydrogen peroxide into the extraction solution. Thus, when applying this method in a new site, one should consider the possibility of additional processing of the sample to remove organic and charred materials. Note however that if a certain volume of hydrogen peroxide is added to this one-step procedure the density of the SPT to be added should be re-calculated so that the total final density of all solutions within the test tube will be ca. 2.2 g/ml for a successful separation of phytoliths from clay and quartz. In addition, it is recommended that the accuracy and precision of this extraction method be reported for each site, as well as the guidelines chosen for counting.

The SPT tends to crystallize on the slide, making it impossible to count phytoliths after several hours. Slides should therefore be examined a short time after preparation. Moreover, as the extraction solution is acidic (pH = 1), the phytoliths will completely dissolve after several days, and the supernatant should not be kept for more than several hours. If the phytoliths extracted from the sediment are needed for further analysis, they should be removed from the solution (see Method above).

5. Conclusions

We show that the rapid extraction and counting method does not produce less accurate and precise results compared to existing methods. This method enables the analysis of phytolith concentrations in 15 to 30 sediment samples in 4 to 6 h. Thus a set of samples can be analyzed from one day to the next, and the data obtained can be used to re-sample and/or expand the sampling density before excavation begins the next day. In addition, the rapid turn-over of results can be used in the field in order to adjust the sampling strategy, as shown in the archaeological application in this study. Moreover, the method can be used for pre-screening of samples suitable for morphological analysis, and thus reduce the number of samples processed for this time consuming task. If necessary, morphotype analysis, as well as phytolith concentrations, can be carried out on a few samples in an afternoon/evening.

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