A method for the combined extraction of pollen, diatoms and phytoliths from forensic soil samples

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Introduction

Biological traces in soils can be used to characterize and compare soil samples. In general a variety of biological traces, like pollen, diatoms and phytoliths, can be used. However the extraction of one type of biological trace from soil often destroys the other types (table 1). In many forensic cases the available amount of soil is so small that only one extraction method is possible. Thus a choice will be made without prior knowledge about which trace to extract. A new extraction method for small soil samples is tested in which multiple biological traces are extracted simultaneously. The method can be used to assess the abundance and quality of three biological traces and help decide which specific trace to extract further. In addition the data on the other traces can be used for comparison or exclusion.

Table 1: Characteristics of the three traces under investigation

	Substance	Density	Resistant to	Damaged by	Size (µm)
Pollen	Organic	Low (1.5)	Acid, Alkaline, Mechanical stress	Oxidants	10 - 300
Diatoms	Biosilica	Higher (2.1)	Acid (mostly), Oxidants	Alkaline, Mechanical stress	10 - 200
Phytoliths	Biosilica	Higher (2.1)	Acid, Alkaline (etching), Oxidants	Mechanical stress	5 - 500

Criteria for the ideal method

- Time efficient
- Minimal loss of traces
- Traces present can be identified and quantified
- Possibility of subsequent standard methods for extraction of single trace

Method design

A sandy soil was chosen which contained known quantities of pollen, diatoms and phytoliths. These quantities were determined by using the normal method for extracting these traces from soil and were set as 100% recovery in figure 1. The normal methods generally have a recovery of about 70% of the traces present. As internal markers glass beads were added for phytoliths and diatoms and *Lycopodium* spores for pollen. Concentrations of traces were determined by analyzing an aliquot of the extract. Each experiment was performed in duplo.

Procedure used:

- Add *Lycopodium* to 1 gram of soil, dissolve in water
- Deflocculating with sodium pyrophosphate
- Sieve with 250 and 7 micron sieve
- Heavy liquid density separation (HLDS), use sodium polytungstate (SPT) at a density of 2.3 and rinse over a 7 micron sieve
- Add glass beads
- Concentrate and analyze

Results

In table 2 an overview is given of the problems encountered after each experiment and the adjustments to the method to resolve these problems. The recovery data is combined in figure 1.

Table 2: Problems and adjustments of the experiments

	Problems	Adjustments
Experiment 1	Traces lost during sieving over 7 microns	Sieving over 7 microns removed
		Extra centrifugation steps added for rinsing (instead of sieving)
	Low recovery of traces and glass beads	HLDS using phials
Experiment 2	*Lycopodium* preferentially lost in HLDS	Dissolving *Lycopodium* pill using 10% HCl
	Increase in fragmented diatoms	HLDS using glass separator (removes some centrifugation steps)
Experiment 3	Longer extraction time	

Due to low recovery of the diatoms, the extracted sample together with the original soil sample was send to an external diatom expert to determine the concentration, recovery and quality of the diatoms present after using the new method. Results thereof are included in figure 1, experiment 3. The current version of the method is shown in figure 2 and the extracted sample in figure 3.

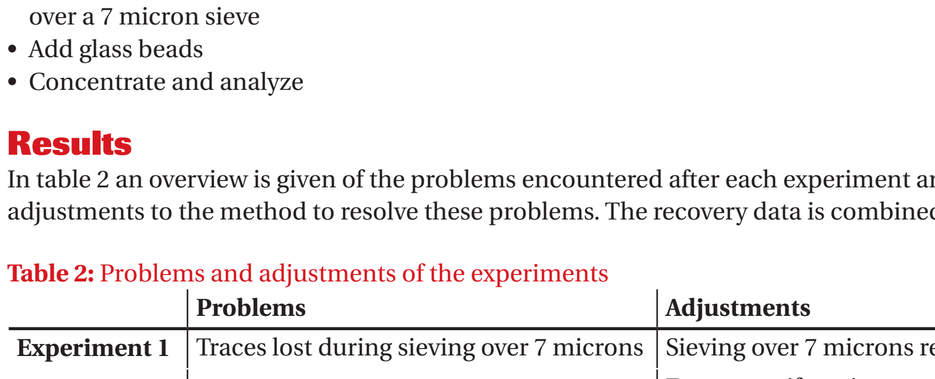

Figure 1: Recovery of traces during development of extraction method

Current status of project

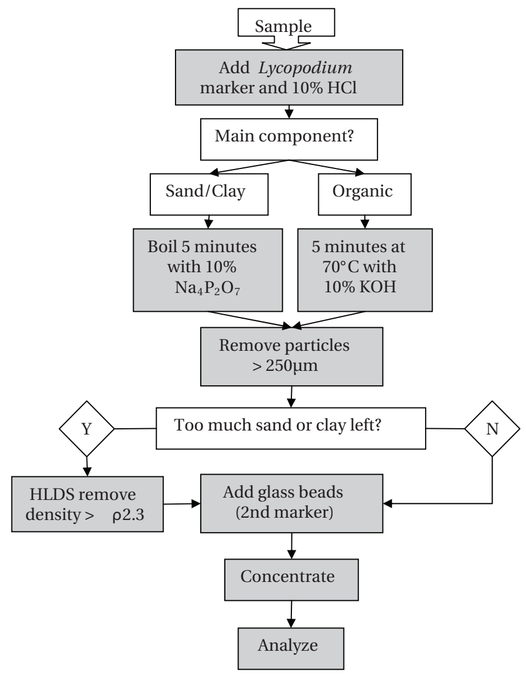

Figure 2: Flowchart of current status of extraction method

Discussion

- The recovery of the internal markers is a percentage of the amount of markers added to the sample and will be below 100% due to loss. Using standard procedures a recovery of 70%-90% is generally accepted. In samples with unknown quantities of traces the markers can be used to detect preferential losses.
- The pollen recovery is higher compared to the normal method, but some pollen features can not be distinguished. This is probably due to the mild treatments used in the new method.
- The diatom recovery is low. The sand particles present in the sample probably damage the diatoms during centrifugation. In samples with less sand particles the recovery is probably higher. This will be tested in the future.
- The recovery of the phytoliths varies, probably caused by variation in the soil sample. In experiment 2 the high amount of phytoliths probably consists of a part that can be dissolved in acid (as in exp. 3) and a part that is smaller than the pore width of 7 microns (as in exp. 1).
- The current version of the method has a time consuming HLDS, but most of that time is spend waiting for the sample to settle and can be used for other purposes.
- The quantative loss of traces can be compensated for by additional analyses as long as the loss of traces is not selective in any way.
- As shown in figure 3 the traces can be identified using a light microscope and quantified using the internal markers
- The end result of the method is a concentrated sample in alcohol and water. After analysis this can still be further extracted using the normal procedures (or shortened versions thereof)

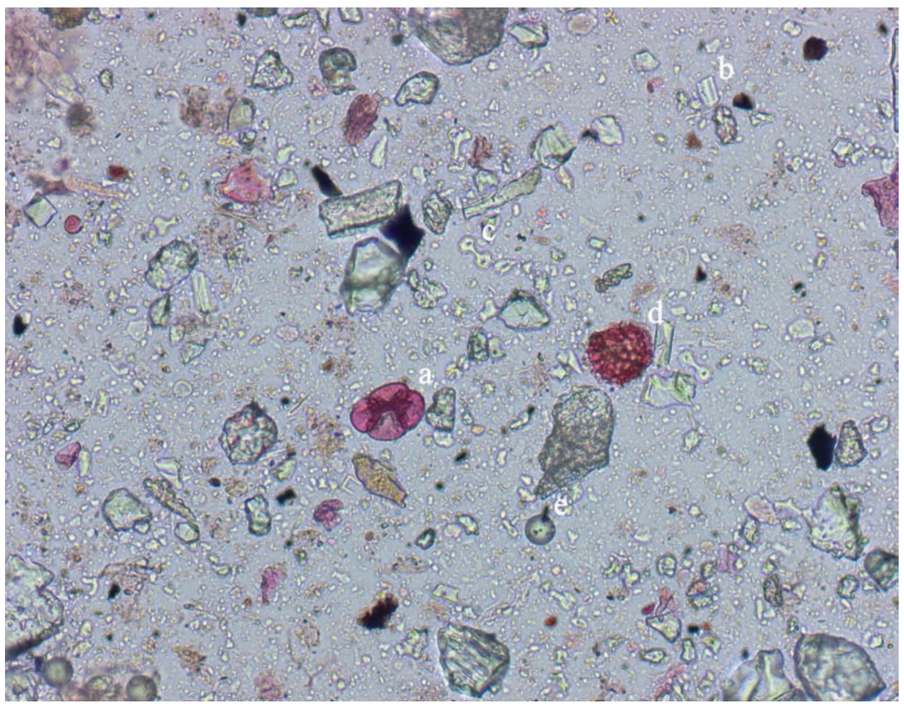

Figure 3: Microscopic view of soil sample. Sample has been coloured with safranin after extraction using method from experiment 3. Pollen (a), Diatom (b), Phytolith (c), Lycopodium (d), Glass bead (e)

Future plans

The next step is testing the method on different soil types. A selection of clays is under investigation to find a suitable sample with sufficient amounts of the three biological traces. After optimizing the method for clay samples an organic rich soil sample can be tested. Then a final calibration of the method for all three general soil types (sand, clay, organic) is necessary to combine the three soil types in either the same or different pathways.

Notes

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