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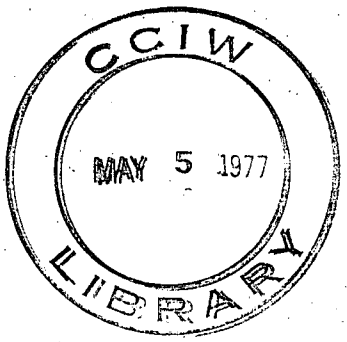

HYDRAULICS RESEARCH DIVISION

Technical Note

DATE: March, 1977 REPORT NO: 77-2

TITLE: A Method for Fossil Pollen Extraction from Sand-Rich Sediments

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REASON FOR REPORT:

This report documents a method developed at C.C.I.W. for fossil pollen analysis of coarse sediments.

CORRESPONDENCE FILE NO: -

INTRODUCTION

Fossil pollen concentration is relatively low in cores of sand-rich nearshore lacustrine sediment because pollen is hydraulically equivalent to, and tends to be associated with, the silt-clay component of the sediment. Concentration can be increased to levels suitable for pollen dating by removing the sand fraction by decantation and then applying the standard Quaternary pollen-preparation procedure (Faegri and Iversen, 1964) to the silt-clay residue. This has the additional advantage of reducing the variability in pollen frequency produced by grain-size variations within the core. This note presents a fast and inexpensive method for extracting fossil pollen from sandy sediments.

BASIS FOR DECANTATION PROCEDURE

The decantation procedure must provide for removal of the majority of the sand component of the sediment with minimal loss of the pollen. This requires that the settling velocities of the pollen types and of quartz (the major inorganic component) be known.

Pollen settling velocities are computed from data recorded by Brush and Brush (1972) with Stokes' equation:

$$V_T = \frac{d^2 g\ (S.G.-1)}{18\nu}$$

where

d = nominal diameter of pollen grain (cm)

g = acceleration due to gravity (981 cm/sec)

ν = kinematic viscosity for water at 20^{o} C (1.006×10^{-2} cm^2/sec)

S.G. = specific gravity of the pollen

Table 1 shows the computed settling velocities for Pinus and Ambrosia pollen and for 62.5-micron quartz and the settling distances associated with a 30-second fall time. Thirty seconds is sufficient to clear the upper 10.4 cm of a sediment suspension of quartz of sand size. We use a settling cylinder with a suspension column 13 cm high and with a diameter (1.6 cm) such that decantation of 20 cc is equivalent to removal of the top 10.4 cm of the suspension.

Figure 1 shows the distribution of pollen grains and quartz within an originally homogeneous suspension at time 0 and time 30 seconds. Decantation of 20 cc (80%) of the suspension after a 30-second fall time removes no quartz sand and recovers 80 per cent of the pollen minus the amount lost through the 10.4 cm interface by settling. For Pinus, this loss is equivalent to the content of 1.056 cm slice of the suspension (the 30-second fall distance) or 8.1 per cent of total Pinus. Net recovery of Pinus with the first decantation is therefore 71.9 per cent. Similarly, Ambrosia loss is the content of a 0.156 cm slice or 1.2 per cent and net recovery of the first decantation is 78.8 per cent. Two further decantations of the residue are required to increase the pollen recovery to 97.8 per cent for Pinus and 99.1 per cent for Ambrosia.

PROCEDURE

Steps in the procedure for pollen extraction are listed below and as a flow diagram in Figure 2.

1. Clean a 15 ml polypropylene, conical centrifuge tube (CANLAB 1976-C3820-15A or equivalent) and weigh to 0.0001 gm. Add approximately one gram of freeze-dried sample to the tube, weigh to 0.0001 gm and record weight of sample (Column A, Figure 3).
2. Transfer the sample to a 25 ml graduated cylinder and top to the 25 ml level with distilled water. Mix the suspension and record its temperature. Mix thoroughly for one minute by repeatedly inverting stoppered cylinder. Thirty seconds after setting cylinder down, pour 20 ml of suspension into an 100 ml centrifuge tube which has 20 ml - interval markings (CANLAB 1976 - C4039-100 or equivalent).
3. Top up the cylinder to the 25 ml level, re-suspend the residue as before and decant 20 ml into the same large centrifuge tube. Repeat this step a third time.
4. Weigh a disposable aluminum evaporating tray to 0.0001 gm. Transfer the remaining 5 ml from the cylinder to the tray and oven dry at $90^{o}C$. Weigh to 0.0001 gm and record the coarse sample weight. (Column B, Figure 3).
5. Compute the working sample weight (weight of fines) by subtracting the coarse sample weight from the original sample weight and record in Column C, Figure 3.
6. Centrifuge the 100 ml tube containing the supernatant for two minutes at 1500 rpm, decant, and wash the residue into the original 15 ml centrifuge tube with distilled water. Centrifuge for one minute at 2000 rpm and decant. This residue is the working sample.

7. Add one (or two) Lycopodium tablets (12,500 ± 500 grains each) to the working sample as a standard. Add 10 ml of 10% HCl to dissolve the tablet and to remove any calcareous materials present in the sample. Stir well with a wooden rod (applicator stick) to evenly disperse the Lycopodium pollen. Centrifuge for one minute and decant.

8. GLOVES AND GOGGLES ARE WORN IN THIS STEP!

   Carefully place 10 ml 49% (stock)HF acid into the test tube, stir with wooden rod until homogeneous and place in boiling water bath for 30 - 40 minutes. This step removes any silicates present in the sample. Centrifuge for one minute and decant into a plastic container to which 10% KOH has been added as a neutralizer.

9. Add 10 ml of 10% HCl, stir with wooden rod and place in a boiling water bath until sample breaks up into small particles (5 - 15 minutes). This step removes any silica - fluoride precipitate present. Centrifuge for one minute and decant.

10. Wash with 10 ml distilled water, centrifuge for one minute and decant.

11. Acetolysis procedure follows in which cellulose and hemi-cellulose materials are removed:

    a. Add 5 ml glacial acetic acid to the sample, mix on electric mixer (or with glass rod), centrifuge for one minute and decant.

    b. Prepare acetolysis mixture by slowly adding concentrated sulphuric acid to acetic anhydride to form a 1:9 mixture.

    c. Add 5 ml of acetolysis mixture to the sample, stir with electric mixer (or glass rod) and place in hot-water bath for 8 - 10 minutes. Samples to be stained should remain in this mixture only 1 - 3 minutes. Stir on electric mixer, centrifuge for one minute and decant.

d. Add 5 ml glacial acetic acid, stir, centrifuge for one minute and decant.

e. Wash with 10 ml distilled water, centrifuge for one minute and decant.

12.A For non-stained samples:

Prepare a slide by placing one drop of Hoyer's medium on a clean labelled slide. Add a small representative portion of the pollen concentrate with a wooden rod, mix with the medium and cover with a cover slip. Press the cover slip gently with a needle probe to remove air bubbles and disperse concentrate.

12.B For stained samples:

Add one drop of safranin straining solution to the sample and stir thoroughly with wooden rod to distribute stain. Immediately add 10 ml tertiary butyl alcohol (TBA), stir and let sit for ten minutes. Centrifuge for one minute and decant. Transfer sediment to a clean, labelled 1-dram vial with TBA. Centrifuge vial, decant and repeat if necessary to ensure removal of all the sediment.

Add eight drops of silicone oil (2000 cs viscosity) to the vial, stir with clean, two-inch glass rod and let stand overnight to evaporate TBA. Prepare a slide by stirring the pollen concentrate well, placing a small representative portion on a clean, labelled slide in the form of an X and covering with a cover slip. Press the cover slip gently with a needle probe to remove air bubbles and disperse the concentrate.

13. For both stained and unstained samples count the slide using a microscope and record information on the data sheets (Figure 3).

COMMENTS

The decantation procedure for sand removal adds about ten minutes to the time required to process each sample. The result is a cleaner sample with a higher concentration of pollen and a saving of as much as one hour per sample in time spent on microscopic analysis.

Time required for complete processing of a single sample including decantation, is approximately three hours. This can be reduced by batch processing in groups of 6 or 12 samples to 50 to 35 minutes per sample respectively.

REFERENCES

Anderson, L. E., 1954, Hoyer's solution as a rapid permanent mounting medium for bryophytes. The Bryologist, Vol 57, p 242-244.

Brush, G. A. and Brush, L. M., 1972. Transport of pollen in a sediment laden channel - a laboratory study. American Journal of Science, Vol 272, p 358-381.

Faegri, K. and Iversen, J., 1964. Textbook of pollen analysis, 2nd ed, Hofner Publishing Co. Ltd., N.Y., p 66-82.

TABLE 1

COMPUTED SETTLING VELOCITIES, 20° C

Particle	S.G. (wet)	Nominal Diameter (μ)	Calculated V_T (cm/sec)	30 Sec Fall Distance (cm)
Quartz	2.65	62.5 *	0.348	10.449
Pinus	1.2	57	0.0352	1.056
Ambrosia	1.3	18	0.0052	0.156

* Sand-Silt Boundary

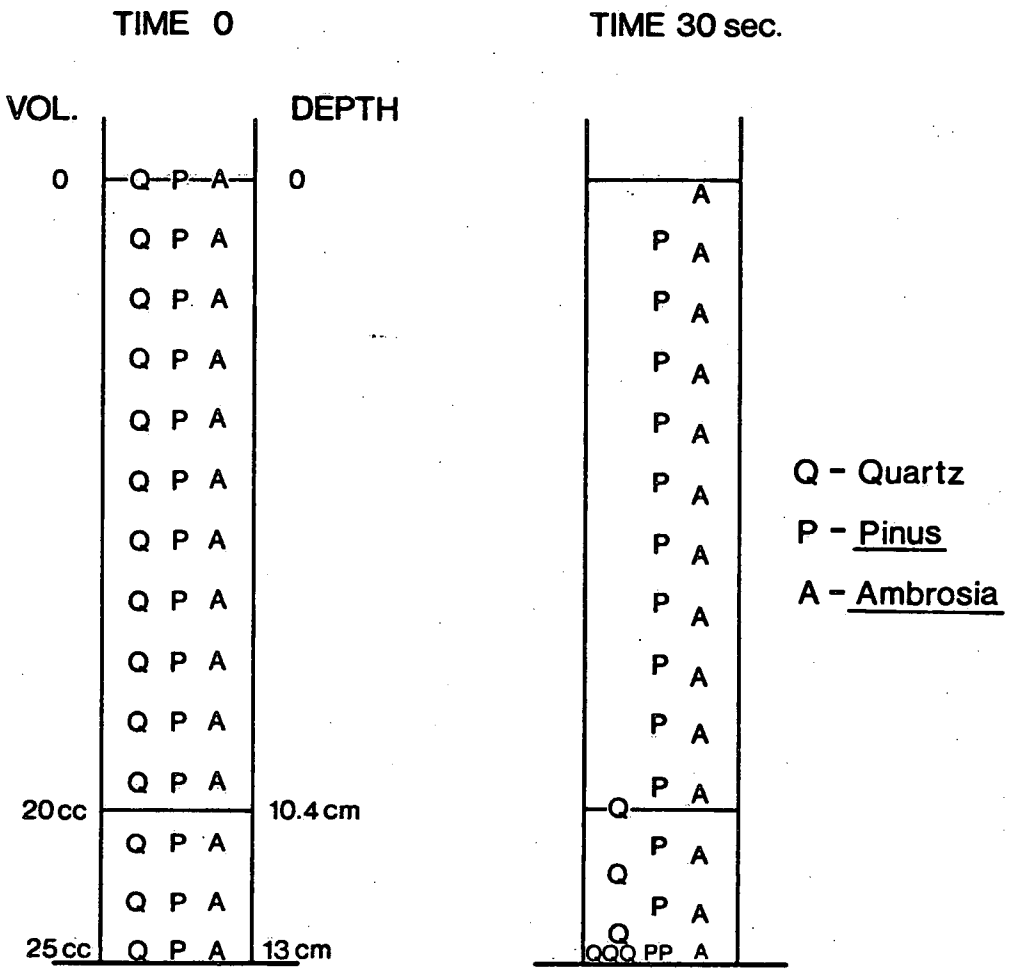

FIGURE 1. SUSPENSION DISTRIBUTION AT TIME 0 AND 30 SECONDS

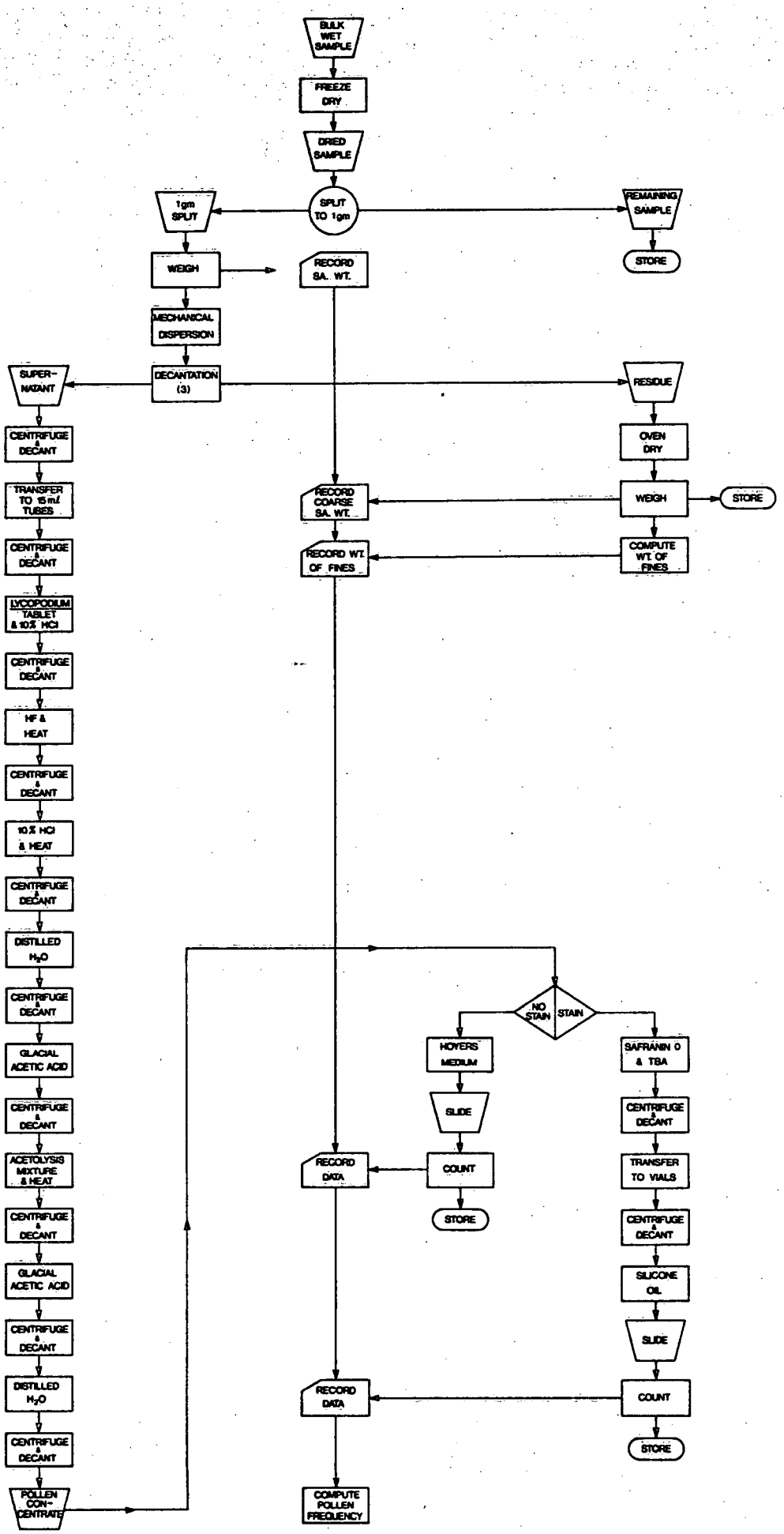

FIGURE 2. FLOW SHEET FOR POLLEN EXTRACTION FROM SAND-RICH SEDIMENTS

		A			B	C	D	E	F	G	H	I		J		K		
Sample Depth	Sa.	Original Sa.Wt. (Dry)	Wt.	Coarse Sa.	Coarse	(A-B) Fine	Total	Lyc.	Amb.	Pinus	Cast.	Amb/gm.	origSa	Pirus/	Sa. morig.	Cast/gm	Sa. orig.	Pinus Amb
(CM)	No.		Tray	+ Tray	Sa.Wt.	Sa.Wt	Lyc.	Count	Count	Count	Count	DF/A	E	DG/A	E	DH/A	E	J/I

		L		M		N												
Sample Depth (CM)	Sa.	Amb/gmfine	Wt.	Pinus/gmfine	Wt.	Cast/gmfine	Wt.	Pinus Amb.										
	No.	DF/CE		DG/CE		DH/CE		M/L										

Figure 3. Data record sheets.