An inexpensive sieving method for concentrating pollen and spores from fine-grained sediments[1]

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A sieving method is described for concentrating pollen and spores from fine-grained sediments. The effectiveness of nylon screens with 5, 7, 10, and 15 μm meshes was tested in comparison with both standard digestion and sodium pyrophosphate suspension procedures.

Sieving through 7 μm mesh screens recovers more pollen than any of the other methods tested and effectively removes fines with a negligible loss of pollen (0.4%). We recommend this method for general use with fine-grained minerogenic sediments. Thus, it is possible to process and count more reliably to statistically significant sums, clay and silt-rich samples previously considered either barren of pollen or too time consuming to count.

On décrit une méthode de tamisage pour concentrer le pollen et les spores dans des sédiments fins. On a vérifié l'efficacité des tamis en nylon avec des mailles de 5, 7, 10 et 15 μm par

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comparaison avec les procédures standard de digestion et de suspension dans les pyrophosphates de sodium.

On récupère par tamisage dans les tamis avec des mailles de 7 µm plus de pollen que par les autres méthodes et on enlève les fines de façon efficace avec une perte négligeable de pollen (0.4%). Nous recommandons cette méthode pour utilisation générale avec les sédiments fins minérogéniques. Ainsi, il devient possible de traiter et de faire le décompte avec plus de confiance pour obtenir des sommes statistiquement significatives dans les échantillons riches en argile ou en silt considérés jusque là comme stériles ou comme exigeant trop de temps pour le décompte.

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Introduction

The concentration of pollen and spores from most fine-grained, mineral-rich sediments poses difficulties for Quaternary palynologists because of low pollen content. Such clastic sediments are difficult and expensive to digest chemically. This difficulty is especially frustrating because some of the more interesting paleobotanical events, such as revegetation after deglaciation, are recorded in such sediments. Palynologists generally rely on digestion methods to process these sediments (Faegri and Iversen 1975), particularly on the use of hydrofluoric acid to remove silicates. However, a substantial proportion of clays and silts often remain or a silico-fluoride precipitate is produced, forcing a compromise between the time spent counting the few grains in the preparation and the level of statistical accuracy desired. The investigator commonly decides to count to smaller, statistically less significant pollen sums.

Sieving is often used to remove coarse particles, but it has not been used routinely for recovering pollen from sediment rich in particles smaller than pollen grains. When fine-mesh metal screens became readily available, Kidson and Williams (1969) suggested this fine-screening method. However, little has been done to test its potential, probably because of the prevailing attitude that even "if sufficiently fine screens were available to take the smaller-than-pollen particles, they would be too fine to be of real help" (Faegri and Iversen 1975). That is, they felt that the mesh would have to be so small to prevent pollen loss that the screen would also trap too much of the sediment to be practical. Indeed, we have found that metal screens do clog and that they are both prohibitively expensive and fragile. Recently, our laboratories, as well as others (e.g., Heusser 1978), have begun to use, routinely, inexpensive and durable fine-mesh nylon screens for concentrating pollen grains from fine-grained sediments. No laboratory has yet demonstrated the effectiveness of this method. The purpose of this paper is to (1) describe our simple screening technique for rapidly and effectively concentrating pollen from fine-grained minerogenic sediments and (2) determine the optimal mesh size for this screening method.

The Methods

The standard method (Faegri and Iversen 1975) for chemically concentrating pollen from sediments is usually (1) hot 10% potassium hydroxide to deflocculate humified sediments or hot 10% hydrochloric acid for calcareous sediments, (2) coarse sieving (200 µm) to remove large particles, (3) hot or cold (overnight) hydrofluoric acid (40–60%) treatment to remove silicates, and (4) acetolysis to remove organics. Our method, Fig. 1, differs in two ways. First, prior to any chemical treatment, or after HCl if tablets of exotic pollen or spores are introduced, a 1 mL sample in a 50 mL centrifuge tube is washed with warm 5% sodium pyrophosphate, vigorously stirred with a wooden applicator stick, and centrifuged to remove the finest particles (see Bates *et al.* (1978) for the most recent discussion of the use of sodium pyrophosphate for disaggregating and removing clays). This step, which increases the effectiveness of the subsequent hydrofluoric acid treatment, is repeated three to five times depending on the clay content of the sample. If samples are extremely rich in clay, they may not become completely disaggregated until the second washing. During the last sodium pyrophosphate washing, the sample is decanted between two beakers to remove sand (and (or) coarse-sieved if large organics are present). Secondly, following acetolysis, the sample is resuspended in 5% sodium pyrophosphate and passed by gravity filtration through a screen with 7 µm meshes. Filtration is aided by rubbing the underside of the screen to prevent the pores from clogging. Each sample requires only 5–10 min to be sieved when using a sieve with a 7 cm diameter screen. Smaller diameter screens become clogged more quickly thus increasing the sieving time. The residue remaining on the screen is rich in pollen and free of clay and fine silt. Some coarse silt is usually present but we have never had to repeat the hydrofluoric acid treatment after sieving. The pollen-rich residue is washed from the screen surface with water back into the

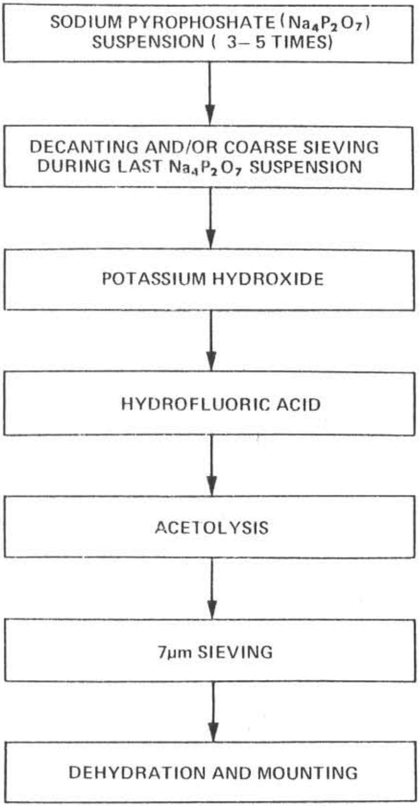

FIG. 1. Flowchart of the basic steps of our procedure. Intermediate steps such as glacial acetic and distilled water washings after acetolysis have been omitted.

centrifuge tube. The sample is centrifuged and washed with water to remove the last traces of sodium pyrophosphate. Standard methods for dehydration and mounting are then followed.

Testing the Methods

Screens with 5, 7, 10, and 15 µm meshes were tested for their efficiency in concentrating pollen in comparison with the standard method and the standard method preceded by 5% sodium pyrophosphate washings. The tests were performed on a moist, compacted, grey-black, clayey silt collected from a tundra lake in the northern Yukon Territory. A 60 mL sample (a 3 cm thick disk from a 5 cm diameter core) was homogenized by first mixing for

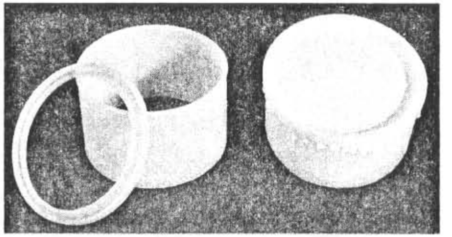

FIG. 2. Illustration of screen holder without (left) and with (right) mounted 7 µm Nitex screening. Each sieve has a screen diameter of 7.0 cm.

3 min in a Waring blender and then stirring manually for an additional 5 min. A 20 g sample of the homogenized sediment was removed for particle size analysis (ASTM Committee D-18, 1964). Eighteen 1 mL samples were taken from the remaining sediment and three of these were randomly assigned to each of the six treatments described. Tablets of *Eucalyptus* pollen were added to each sample and dissolved with 10% HCl prior to any other chemical treatment. A minimum of 400 fossil pollen grains together with the exotic *Eucalyptus* were counted per sample. In order to measure directly the pollen lost by screening, filtrates were collected and a known number of *Lycopodium* spores added to each. Because the pollen concentration of the filtrates was low, they were scanned until one-tenth of the *Lycopodium* spores had been counted.

The screens used in these tests were made from 'Nitex' brand monofilament nylon distributed by Tetko Inc. The location of the nearest supplier can be obtained from Tetko's corporate headquarters at 420 Saw Mill River Road, Elmsford, New York 10523, U.S.A. Screen holders, Fig. 2, were made from plastic containers (obtained from a kitchenware store) that had snap-lock covers. These were modified by cutting out both the bottom of the container and the inside of the cover; the screen was mounted between the modified cover and the body by means of the snap-lock. Each screen was used only once to prevent contamination, although the holder was cleaned and reused. The sieve (screen plus holder) costs less than \$1.00. The cost of the screen per sample is about \$0.40.

Results and Discussion

Figure 3 shows the results of the particle size analysis. The sediment is composed of 12.5% sand, 64.5% silt, and 23.0% clay. The fossil pollen concentration was about 5000 grains mL^{-1}.

TABLE 1. Recovery of selected taxa by various treatments (grains mL^{-1})

	Cruciferae	*Betula glandulosa* type	*Salix*	*Alnus*	*Artemisia*	Gramineae	Cyperaceae	*Picea*
Standard	1070	1490	190	570	570	760	250	770
	680	850	130	380	440	490	120	220
	640	1070	140	680	310	430	140	680
Mean ($\bar{x}$)	797	1137	153	543	440	560	170	557
$Na_4P_2O_7$ washing	1030	1350	260	490	860	1030	140	720
	810	1060	150	750	470	630	170	400
	700	970	150	450	560	870	210	430
Mean ($\bar{x}$)	847	1127	187	563	630	843	173	517
7 μm mesh	920	1370	190	320	560	920	170	750
	1100	1080	190	400	740	1370	270	570
	900	1090	120	890	460	840	180	660
Mean ($\bar{x}$)	973	1180	167	537	587	1043	207	660
10 μm mesh	530	920	70	320	530	940	110	720
	480	1280	190	450	590	1170	490	690
	480	880	100	590	540	1100	400	670
Mean ($\bar{x}$)	497	1027	120	453	553	1070	333	693
15 μm mesh	80	530	60	270	490	830	180	850
	190	460	60	270	440	1000	310	530
	210	430	50	320	400	1090	290	800
Mean ($\bar{x}$)	160	473	57	287	443	973	260	727

Table 1 shows the number of pollen grains per millilitre recovered by each of the treatments for selected taxa. There are no results listed for the 5 μm mesh because rapid clogging of the meshes prevented filtration. A two-way analysis of variance of these data, following a natural log transformation, shows significant differences between treatments as a whole ($F = 17.6$ is significant at $p = 0.01$). A ranking of treatment means (at $p = 0.05$) by Duncan's new multiple range test (Sokal and Rohlf 1969) indicates that the recovery of pollen by 15 μm mesh sieving is significantly lower than all other treatments. The remaining treatments are similar except that the standard procedure recovers significantly fewer grains than sieving with 7 μm mesh screens. The interaction factor between treatments and taxa is significant, indicating that different taxa are affected in different ways by the various treatments. This is expected because the size of the pollen differs among taxa.

The total number of pollen grains recovered by the various treatments and lost by sieving (i.e., in the filtrates) is shown in Fig. 4. Sieving with 7 μm mesh recovers the greatest number of pollen grains with a negligible loss (0.4%). The mean pollen loss increases with mesh size with 5.8% lost through the 10 μm and 21.6% through the 15 μm meshes.

Tables 2 and 3 show more precisely where the differences lie between treatments. The 15 μm screen selectively loses significant numbers of small grains (Cruciferae, *Betula glandulosa* type, and *Salix*) compared with the other treatments. The 10 μm screen differs from the 7 μm, standard, and sodium pyrophosphate suspension treatments only by its significant loss of Cruciferae. The 7 μm screen does not lose significant quantities of any pollen type. The lack of significant differences in the recovery of large pollen grains such as *Picea* and Cyperaceae (Table 2) and their absence from the filtrates (Table 3) demonstrates the strength of these screens.

The standard procedure underestimates the numbers of some taxa because critical features are often hidden by attached clay or silico-fluoride particles. For example, the Gramineae values are too low for the standard treatment (Table 1) because the diagnostic annulate pore is frequently obscured. Sieving removes the clay and silico-fluoride precipitate, allowing a closer inspection of individual grains for more reliable identifications.

Sieving significantly reduces the counting time (Table 4). Sieved samples can be counted in about 20% of the time required for standard preparations. Washing with sodium pyrophosphate also decreases the counting time, but not to the same extent as sieving. Bates *et al.* (1978) have demonstrated that centrifuging with 10% sodium pyrophosphate removes a large proportion of

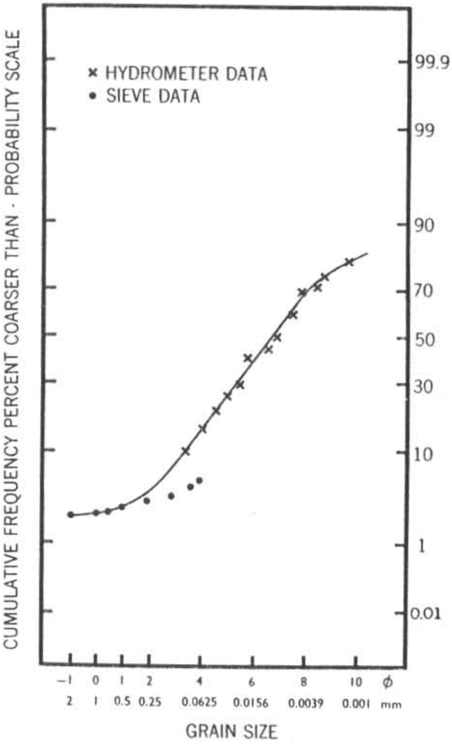

FIG. 3. Particle size analysis of the test sediment.

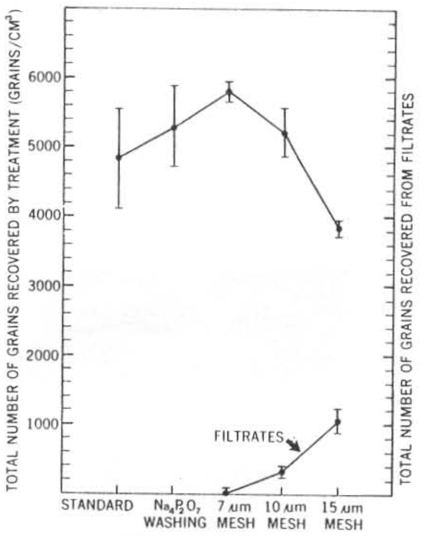

FIG. 4. Graph of recovery and loss (filtrates) of pollen by treatment. Bars indicate one standard error about the mean.

TABLE 2. Ranking of treatment* means by Duncan's multiple range test for each taxon

Taxon	Treatments				
Cruciferae	7	N	S	10	15
Betula glandulosa type	7	N	S	10	15
Salix	N	7	S	10	15
Alnus	N	S	7	10	15
Artemisia	N	7	10	15	S
Gramineae	10	7	15	N	S
Cyperaceae	10	15	7	N	S
Picea	15	10	7	N	S

NOTES: Lines join treatments with means that do not differ significantly at $p = 0.05$. Treatment means decrease progressively from left to right. The pollen taxa are arranged in order of increasing size downward.
*S = standard preparations; N = sodium pyrophosphate ($Na_4P_2O_7$) suspensions; 7, 10, and 15 represent sieving treatments with screens of 7, 10, and 15 µm meshes respectively.

clays. In our experiments, samples washed with 5% sodium pyrophosphate but not sieved required almost twice as much time to count as sieved samples. The decreased counting time for sieved samples is due to the higher pollen concentration in these preparations so that fewer slides need to be scanned. For example, 15–20 slides of the standard preparations had to be scanned to reach a minimum of 400 pollen grains compared to 3–4 slides for samples sieved with 7 µm mesh screens. Thus, it is far easier to count sieved samples to statistically significant pollen sums.

The 7 µm mesh screen is the most efficient size for this sediment in which small pollen grains are abundant. The larger mesh sizes, particularly 10 µm mesh, may be effective where pollen is larger, e.g., in Holocene sediment from temperate latitudes.

We have used this method successfully on a variety of fine-grained sediments, including marine and freshwater (deep modern lakes) clays, late-glacial clays, and clayey sediments of subarctic lakes. Although we have not tested the effectiveness of the method with fine-grained organic sediments, we believe it may be successfully applied to such sediments, especially to coals.

Conclusion

Screens of 5 µm mesh cannot be used for concentrating pollen because rapid clogging of the

TABLE 3. Pollen content of filtrates

	Cruciferae	*Betula glandulosa* type	*Salix*	*Alnus*	*Artemisia*	Gramineae	Cyperaceae	*Picea*
7 μm	10	—	—	—	—	—	—	—
	10	—	—	10	—	—	—	—
	30	10	—	—	—	—	—	—
Mean ($\bar{x}$)	17	3	—	3	—	—	—	—
10 μm	180	60	20	20	10	—	—	—
	150	30	20	20	—	—	—	—
	220	120	40	50	—	20	—	—
Mean ($\bar{x}$)	183	70	27	30	3	7	—	—
15 μm	300	540	100	230	150	10	—	—
	340	360	70	190	80	10	—	—
	320	240	70	110	10	—	—	—
Mean ($\bar{x}$)	320	380	80	177	80	7	—	—

TABLE 4. Time in minutes to count 100 grains for various treatments

	Standard	$Na_4P_2O_7$	Mesh size		
			7 μm	10 μm	15 μm
	490	150	90	90	100
	510	190	90	60	110
	380	150	80	70	70
Mean ($\bar{x}$)	460	163	87	73	93

NOTE: The ranking of treatment means by Duncan's multiple range test ($p = 0.05$) is shown by the line at the bottom of the table which joins means that do not differ significantly.

meshes prevents gravity filtration. Screens of 10 and 15 μm selectively lose significant quantities of pollen and cannot be recommended for use on sediment containing small pollen grains. Sieving in combination with sodium pyrophosphate reduces the clay content of samples more effectively than centrifuging with sodium pyrophosphate without sieving.

Our method using 7 μm mesh screens recovers more pollen than all other treatments tested and effectively removes fines with a negligible loss of pollen (0.4%). We recommend 7 μm mesh screens for general use with fine-grained minerogenic sediments. The counting time compared to standard preparations is reduced by a factor of five. The removal of clay and fine silt particles by sieving allows closer scrutiny of individual grains permitting more reliable identifications. Thus, it is possible to process efficiently clayey and silty samples, and to count them more quickly and reliably to statistically significant pollen sums; previously, such samples were often considered barren of pollen or too time consuming to count.

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