CHAPTER 6

THE DETERMINATION OF POLLEN AND CHARCOAL DEPOSITION RATES

Ever since the inception of Quaternary pollen analysis it
has been realised that, for both statistical and ecological
reasons, the interpretation of fluctuations in the interdependent
curves of the standard 'percentage' pollen diagram, has serious
limitations. L. von Post in his famous address of 1916 (von Post,
1918, 1967) stated that 'obviously it would have been desirable
from various points of view to use figures giving the absolute
frequencies instead of the relative measures that are indicated by
percentage figures'. Although he also discussed the total pollen
content of peat layers von Post realised that such figures would be
difficult to interpret due to differences in the rate of peat ac-
cumulation within and between bog sites. He therefore considered
it 'especially desirable to investigate the feasibility of produc-
ing comparable absolute frequency figures' (von Post, 1967).

Some authors have since published diagrams calculated on
the basis of pollen content per unit of sediment, assuming changes
in sedimentation rate to be of only minor importance at the partic-
ular site (Livingstone and Estes, 1967, Sims, 1973). However, it
is only with the widespread application of independently derived
chronologies, in particular from radiocarbon dating and other iso-
topic techniques, that the production of comparable absolute fre-
quency figures for pollen deposition, as envisaged by von Post, has
become feasible.

Due to the increased effort necessary in absolute pollen
analysis, and the scarcity of sites suitable for precise dating, it
is perhaps natural that the initial work in this area focussed on

problems that could not be adequately resolved using the existing relative pollen frequency technique alone. Thus the transition from late-glacial to early post-glacial conditions was investigated both in North America (Davis and Deevey, 1964) and the British Isles (Pennington and Bonny, 1970). Absolute pollen frequencies have also been used to reinterpret Mesolithic clearance phases of previously analysed British sites (Pennington, 1973, Sims, 1973). A number of longer diagrams from late-Pleistocene and Holocene sequences in the U.S.A. have now been published (Davis, 1969, Waddington, 1969, Maher, 1972, and Craig, 1972).

The question therefore arises as to whether it is worthwhile to attempt a systematic estimation of pollen deposition rates from a tropical lowland situation, given the current state of palynological knowledge about such areas. The only published pollen deposition rate diagram from the equatorial tropics is that of Kendall (1969) from Lake Victoria, East Africa. Livingstone (1975) comments that Kendall's results show 'an accuracy of vegetation reconstruction that has not yet been matched from any other site in Africa'. Of particular interest to the present study, the results show that an apparent increase in grass pollen in the upper levels of the core is an artefact of the relative calculation method, and in fact, whereas forest pollen decreases in absolute frequency, the deposition rate of grassland types remain comparatively stable.

Given the availability of radiocarbon chronologies from the two Markham Valley sites, it was therefore considered appropriate to attempt the estimation of pollen deposition rates in the analysed cores. A preparation technique facilitating quantitative handling of the sediment samples was therefore adopted.

The terms 'absolute' diagram or pollen frequency and 'pollen influx' should not be used without qualification. As Maher (1972) has emphasised, 'absolute' is really a misnomer: such pollen results are estimates that are always stated relative to some other quantity. In addition, the term may also be ambiguous (Davis, 1969) as it does not specify whether pollen concentrations or pollen deposition rates are involved. Nor is 'pollen influx' considered appropriate as, according to Pennington (1973), not all pollen entering a lake basin becomes permanently incorporated into the sediments.

Pollen analytical results from this study are therefore expressed:

(a)   in relative frequency terms, as percentages of a given base sum,

(b)   as concentration of grains and spores per unit volume of sediment, in this case 1 ml ('pollen concentration'), and

(c)   as a figure for pollen deposition rate (PDR) defined as number of palynomorphs deposited per cm^2 of sediment surface per year. This value is obtained by adjusting the pollen concentration for the appropriate sediment accumulation rate.

DETERMINATION OF POLLEN CONCENTRATION

The determination of the concentration of pollen and spores in a sediment sample involves a three-stage process:

(1)   the sampling of an accurately measured amount of the sediment,

(2)   the counting of a known proportion of the pollen and spores in the processed sediment, and

(3)   extrapolation from this count, within statistically stated limits, to give an estimate of the total number of pollen grains in the original sample of sediment.

Sediment sampling

Since the final aim is to relate pollen deposition to a volumetric measure, i.e. number of grains per unit area per unit depth (representing time), most workers have based their estimates on an initial volume of sediment (e.g. Davis, 1969, Waddington, 1969, Maher, 1972) although others (Durkee, 1964, Jørgensen, 1967) have preferred a sample of known dry weight. A volumetric sample is more difficult to collect and measure accurately than is one taken on the basis of dry weight, and a variety of techniques have been devised to produce samples of a standard size. Davis (1969) packed the sediment into a spatula of known volume. A method involving less risk of error caused by compaction is the use of a small-scale coring device to remove cylindrical samples of sediment from measured slices of the core. Such a technique has been used by Waddington (1969), and in a more mechanised form by Engstrom and Maher (1972). Alternatively the volume of the sample can be measured by displacement in an accurately calibrated measuring cylinder (Bonny, 1972, Pennington, 1973).

Once a measured sediment sample has been obtained, it may be prepared in either of two ways to produce slides in which the pollen content can be related to that of the original sample.

Quantitative preparation techniques

Marker grain method: Benninghoff (1962) outlined the now common technique of adding, before processing, a known quantity of exotic pollen grains as markers. The ratio of marker grains to fossil grains counted is then used to calculate the number of fossils in the original sediment sample. This method has been further elaborated by Matthews (1969), Waddington (1969), and Maher (1972).

Craig (1972), and Swain (1973) use polystyrene microspheres as markers rather than an exotic pollen type. Both versions of this method provide an easy means to compensate for any loss of material during preparation of the sample, if it can be assumed that the markers become evenly distributed throughout the sediment, and act in the same way as fossil sporomorphs. Not wishing to make such assumptions, Kirkland (1967) and Bonny (1972) added the exotic grains only in the later stages of slide preparation.

The 'marker grain' method requires an accurate assessment of the number of exotic grains added to the preparation. Often a homogenous suspension of the marker is made and an estimate of the pollen concentration derived by counting aliquots of this suspension on haemocytometer or ordinary microscope slides. Known fractions determined by mass (Matthews, 1969, Bonny, 1972) or volume (Waddington, 1969, Maher, 1972) are then added to the sediment preparation. A simpler method is to add an appropriate number of commercially available tablets, described by Stockmarr (1971), containing known quantities of a particular spore or pollen type as a marker. For best results the ratio of fossil grains to added marker grains should be about 2:1 (Maher, 1976).

Aliquot technique: A second established method does not involve the use of any exotic marker, but requires strict quantitative handling of the sample throughout all stages of the preparation procedure. A homogenous suspension of the processed sediment is produced and known aliquots transferred onto microscope slides. This may be done either by pipetting a measured volume (Davis, 1965, 1966), by weight (Jørgensen, 1967), or by settling the whole of the suspension onto cover-slips of known area in an evaporation

tray using the method described by Battarbee (1973) and Battarbee
and McCallan (1974). To maintain accuracy, loss of sample during
processing must be minimised, or some estimate of this loss in-
dependently obtained.

The method adopted for routine counting of slides
depends on the initial choice of preparation technique. The evap-
oration tray method produces a random distribution of grains over
the whole area of the cover-slip thus permitting analysis of only a
proportion of the slide. Any other method leads to slides in
which the pollen grains or other microfossils are not randomly dis-
tributed (Brookes and Thomas, 1967, Battarbee, 1973). Thus it is
desirable to count as much of the whole slide as possible, al-
though if marker grains have been used regularly spaced traverses
over a portion of the slide may be adequate.

Choice of method

Both the 'marker grain' and aliquot methods are capable
of producing accurate and reproducible results if all sources of
error are identified and quantified in the manner of Davis (1965)
and Maher (1972, 1976). An attempt at such precision in the deter-
mination of the pollen concentration of lake sediments and pollen
traps from the Markham Valley could not be theoretically justified.
It was considered that only large and consistent fluctuations of
pollen deposition rate would be interpretable, given the current
state of knowledge about processes of pollen dispersal and sedi-
mentation in the tropical lowland environment.

The 'marker grain' method was considered the more time-
consuming, and suitable exotic grains or microspheres were not
readily available. Use of the aliquot method therefore seemed

appropriate, if proven sufficiently accurate to reveal moderate variations in pollen concentration. Of the aliquot methods, the evaporation-tray technique of Battarbee and McCallan appears straightforward and accurate. However, the random distribution of grains on the slide is upset if any are moved to facilitate identification. As this is essential when dealing with the largely unknown pollen flora of the lowland tropics, this method was rejected in favour of a version of the volumetric aliquot technique of Davis.

The volumetric preparation technique

Before sampling the sediment cores their dimensions were checked for any shrinkage due to water loss since collection. In most cases shrinkage was minimal. Selected cores were sampled for pollen at 10 cm or 20 cm intervals and around marked stratigraphic boundaries. For each sample a horizontal slice 10 mm thick was cut from the core with a sharp knife and transferred to a small petrie dish. A cylindrical portion was then excised using an accurately made glass tube with an internal diameter of 11.28 mm. This produced a sample of about 1 ml that was transferred to a weighed glass vial, sealed and weighed. The remainder of the slice was used for the determination of water and organic carbon content.

The measured sediment was then processed using, in the main, standard palynological preparation techniques (Faegri and Iversen, 1964) summarised in Table 6.1. No estimation was made for loss of sample during preparation, although major spillage was noted and the sample not used for estimation of pollen concentration. All samples required sieving to produce countable slides. Large volumes of water were used to ensure maximum recovery of pollen

TABLE 6.1. *Summary of preparation techniques*

Treatment	Time	Purpose	Samples treated
Cold 10% HCl	10 mins	Removal of $CaCO_3$	Calcareous samples only (all samples from YAN 2, YANMC 1)
Hot 5% KOH or NaOH	20 mins	Removal of humic acids and deflocculation	All samples
Sieving through *c.* 150 µm terylene mesh	–	Removal of coarse organic and inorganic particles	All samples
Cold 40% hydrofluoric acid	12 to 18 hours	Removal of moderate quantities of silica	Most fossil samples
$ZnBr_2$ heavy-liquid separation	–	Separation of organic matter from large amounts of mineral sediments	Highly inorganic sediments only (most samples from YAN 2, YANMC 1)
Acetylation ('Acetolysis') technique (Erdtman, 1952)	3 mins	Removal of excessive cellulose	All samples
Ultra-sonic bath	10 secs or less	Break-up of large particles	Excessively clumped samples only
Dehydration in ethanol series	–	Removal of water prior to mounting in silicone oil	All samples

and spores from the sieve. The heavy liquid separation procedure was also a potential source of error due to retention of pollen, and this was used on all sediment samples from Yanamugi cores.

After dehydration in ethanol, the sample was suspended in a measured volume (10 ml or 15 ml depending on the quantity of sediment remaining) of tertiary-butyl-alcohol (TBA). A small magnetically operated stirring rod or 'flea' was introduced and the suspension stirred automatically until homogenous. Working within a laminar flow cabinet,aliquots of 0.02 ml taken using an 'Eppendorf' automatic micropipette with disposable plastic tips were transferred onto a drop of silicone oil (Wacker AK 2000) on a warmed microscope slide, and the TBA evaporated. Sufficient aliquots were added to produce a slide of suitable density for pollen analysis. In practise this ranged from one to seventeen aliquots (0.02 to 0.34 ml) with most slides containing the material from between 0.1 to 0.2 ml of TBA. When all TBA had evaporated, a 38 x 22 mm cover-slip was positioned and sealed with clear lacquer.

Samples were analysed using a Carl Zeiss (Oberkochen) photo-microscope, employing a magnification of X 160 or X 400 for routine scanning of the slide. Identification of well known types was made at X 400 whilst a X 100 planapochromatic oil-immersion objective was used for final identification of problem grains and investigation of unknowns at a magnification of X 1600.

Only a proportion of each slide was counted. Traverses across the narrow axis of the slide were made, usually at 1 mm intervals, along the whole width of the cover-slip. For a few slides, mainly the very dense pollen trap content preparations, the interval was increased to 2 mm or even more, but traverses were always regularly spaced over the whole slide area. With an

interpupillary distance of 70 mm, the proportion of the slide

scanned with the various combinations of magnification and traverse

interval was measured as:

	Traverse Interval		
Proportion counted	1 mm	2 mm	4 mm
At X 160	0.87	0.435	0.2175
At X 400	0.35	0.175	0.0875

The pollen content of each preparation was thus estimated

by calculating:

$$\text{concentration} = T \times \frac{V_p}{V_s \times C}$$

Where T = total number of pollen and spores counted per
      sample

V_p = Volume of preparation suspension

V_s = Volume of TBA transferred to slides

  C = Proportion of each slide counted.

As the volume of sediment taken was in all cases 1 ml this formula

represents the concentration of sporomorphs per ml.

Assessment of accuracy: The percentage of the total suspension

analysed ranged from 0.01% to 0.35% for pollen traps, and 0.19% to

3.65% for sediment cores. 'Typical' values were 0.03% for traps

and 0.5% to 1.75% for cores. These figures are of the same order

as those used by Davis (1965), although the method used varied from

Davis' in several ways. The most significant were use of smaller

volumes of suspension and aliquot, and counting of only a prop-

ortion of each slide. As all of these factors could lead to

greater inaccuracy, the precision of the adopted technique was

tested.

Three tablets of *Lycopodium clavatum*, batch 212761

(Stockmarr, 1971) were dissolved in 10% HCl and the spores suspen-

ded in 10 ml of TBA. Maher (1976) has calibrated the spore con-

centration of this batch at 12 489 per tablet, with a standard

deviation (S.D.) of 491. The concentration of the suspension was

thus 37 467 with the standard deviation calculated as

$$S.D._n = \sqrt{n} \times S.D._1$$

Where $S.D._n$ = standard deviation of n tablets

$S.D._1$ = standard deviation of one tablet

n = number of tablets

although Maher states that this may tend to underestimate the true

value for standard deviation.

The total number of spores used represents a typical

value for pollen concentration per ml in the sediment cores

studied.

Four series of volumetric aliquot slides were then pro-

duced from this suspension, representing the mid range of actual

volumes counted from the sediment preparations. The slides were

analysed at a magnification of X 160, and a traverse interval of

1 mm. For each series of counts, the arithmetic mean $(\bar{x})$, standard

deviation (S.D.), variance $(S.D.^2)$ and coefficient of variation (V)

were calculated (Table 6.2).

As expected, the mean number of spores counted per slide

was directly proportional to the number of aliquots per slide. The

correlation coefficient (r) between these two variables is greater

than +0.99. The coefficient of variation generally decreases with

increased number of spores, indicating greater precision for

larger counts. The exception is the most concentrated series (0.2 ml per slide) where the higher value for V may be due to excessive clumping of the large number of spores on the slide.

The variance of a Poisson distributed population approximates the mean (Gregory, 1968). Calculations of variance for the *Lycopodium* counts give values around and below the mean, suggesting a distribution varying between 'homogeneous' and Poisson (Kendall, 1969). In order to test the fit of the counts to a Poisson distribution, the method used by Davis (1965) was employed. For Poisson distributed data the product of the formula

$$\frac{\Sigma (x - \bar{x})^2}{\bar{x}}$$

is approximately chi^2 distributed (Cochran, 1954). Chi^2 was therefore calculated using this formula (Table 6.2). Deviation from a Poisson fit was not significant for any series of slides.

Table 6.3 compares the results obtained by counting the aliquot series with those expected from the spore content of the tablets determined with an electronic particle counter (Maher, 1976). The counts are consistently larger than the expected values, the percentage difference increasing with size of aliquot. This pattern of divergence is not easy to explain, but as all values were well within 10% of those predicted, they were considered acceptable within the context of the study.

Estimation of confidence limits: Accepting the Poisson fit of the data, it may be assumed that the number of pollen and spores counted (T) is equivalent to the mean number for a given aliquot size, and therefore to the variance. The 95% confidence interval

TABLE 6.2. *Results of replicate counts on slides containing*
different volumes of Lycopodium clavatum *suspension*

Vol. of slide (ml)	No. of slides counted	Range of counts	$\bar{x}$	$S.D.^2$	S.D.	V (%)	chi^2	d.f.
0.02	6	57- 73	65.33	26.23	5.12	7.84	2.41	5
0.08	6	256-293	275.00	146.33	12.10	4.40	3.19	5
0.14	6	460-515	481.50	345.25	18.58	3.86	4.30	5
0.20	5	661-732	704.80	808.76	28.44	4.04	4.80	4

TABLE 6.3. *Comparison of results obtained by counting slides*
containing different volumes of Lycopodium clavatum
suspension with those expected from the given
content of the spore tablets

% of suspension counted	$\bar{x}$ no. counted	$\bar{x}$ no. expected	% difference of count from expected
0.174	65.33	65.19	+ 0.21
0.696	275.00	260.77	+ 5.46
1.218	481.50	456.36	+ 5.51
1.740	704.80	651.94	+ 8.11

for a count can therefore be given as approximately twice the square root of the count. Thus the formula for the estimation of pollen concentration, with 95% confidence limits, becomes

$$T \times \frac{V_p}{V_s \times C} \pm 2\sqrt{T} \times \frac{V_p}{V_s \times C}$$

This formula provides an overestimate of the confidence limit if the grains are more homogenously distributed, and an underestimate if the occurrence of grains is more variable than in a Poisson distribution. Precision may be increased by counting a larger number of grains by increasing the proportion of the total suspension analysed.

Sources of error: Counts of sub-fossil grains from core samples were examined for excessive variance, indicating possible divergence from Poisson distribution. Of eleven samples with counts from two or three slides of equal volume, one-third showed excessive variance above the mean. This may indicate a non-Poisson distribution, although the number of replicate slides and counts is low.

The experimental results suggest that homogenisation of the suspension and aliquot pipetting can be achieved with accuracy. Similarly the counting technique, at least where a large proportion of the slide has been scanned, seems reliable. Excessive small-scale clumping on the slide, either through high pollen concentration or large amounts of debris, causes greater variability in the count. The effect of this can be reduced by counting the whole slide, or reducing the concentration of material.

Any deficiency in the estimate of pollen concentration per unit volume of sediment is therefore more likely to be the result of other factors. Although none was quantitatively assessed, and is not therefore encompassed by the stated confidence limits, the main sources of error are thought to be:

(a) Inaccuracy in sediment sampling. Replicates were not taken, but inspection of the samples suggests that, in reasonably fine homogenous organic or mineral sediments, the error was within $\pm$ 5%. For coarser material, especially poorly humified and fibrous organic deposits, the method was less accurate.

(b) Loss of material during preparation. The extent of this depends on the processing techniques used. Raine (1974) estimates loss of material during preparation to be low, although he did not use the heavy-liquid separation technique. As no estimate of small losses was made, all values for pollen concentration must be regarded as minimum figures. However, as similar methods were used on all samples within the same core, these results should be consistent, even if not strictly comparable with results from other cores or pollen traps.

The technique adopted for the estimation of pollen concentration is a flexible one able to handle large variation in the pollen content of the sediment and at the same time allow full manipulation of grains for identification and recording. The method is not greatly time-consuming and yet appears sufficiently reliable to disclose moderate changes in pollen concentration. The

confidence limits calculated provide a useful indication of the probable variation, although they may represent an underestimate for some samples due to the possible sources of error mentioned.

DETERMINATION OF CHARCOAL CONCENTRATION

Carbonised plant fragments are frequently encountered in lake sediments, and their occurrence has been associated (e.g. Waddington, 1969, Swain, 1973) with natural or artificial fires within the catchment. Charcoal particles were noticed during initial sediment analyses from Lake Wanum, and, in view of the role of fire in the maintenance of present day grassland, some index of their past abundance was considered desirable.

Such carbonised fragments usually survive routine pollen analytical preparation treatments, and it is possible to record the occurrence of both charcoal and pollen from the same slide. If insufficient particles are found, or if greater precision is sought, separate slides may be prepared by treatment with hot concentrated nitric acid. However, Swain (1973) notes that both procedures produce closely similar results. In this study, all charcoal counts were made on the slides quantitatively prepared for pollen analysis.

Carbonised particles from Lake Wanum core LW II were tallied by their 'apparent surface area' as measured by an eyepiece micrometer. Three size classes were distinguished: 25 - 600 μm^2, 600 - 3 000 μm^2 and 3 000 - 6 000 μm^2. Particles smaller than 25 μm^2 were ignored, these being difficult to distinguish from mineral grains. Examination of the larger particles retained during sieving of samples revealed a general correlation between the abundance of the fragments in the sieve, and that of the smaller ones on the slide.

Estimates for charcoal concentration and deposition rate were derived in a similar manner to those for palynomorphs. However, as fewer transects were usually counted per slide, the carbonised particle results are probably less accurate. These estimates may be expressed in various ways. Although Waddington (1969) and Swain (1973) counted a large number of size classes, both subsequently summed the class totals to produce a single 'charcoal index', expressed as area of particles cm^{-2} yr^{-1}. This procedure was initially adopted for the Lake Wanum samples. However it was clear that the three size classes did not always show parallel trends. More information is retained if each size class is shown individually as the number of carbonised particles cm^{-2} yr^{-1}, as in Fig. 8.1.

A cruder index of charcoal abundance was used for the sediments of Yanamugi, where more rigorous sample treatment had been used. For each sample, numbers of carbonised fragments were counted (by J. Guppy) along two transects of a slide. The concentration of particles was calculated, and the results expressed as a percentage of the maximum concentration, occurring in the sample from 670 cm, (Fig. 8.6). This rapid method appears adequate to reveal major fluctuations in the carbonised particle content of the sediments, and is perhaps more appropriate where the chronology to derive annual deposition rates is lacking.