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AN ADAPTED PREPARATION METHOD FOR CLAYEY AND «POOR» POLLEN SAMPLES

by Elfi VAN OVERLOOP (*)

1. INTRODUCTION.

Up untill today, most suitable materials for pollen analysis are derived from lacustrine sediments and peat bogs. It is anyhow particularly interesting to throw a look upon other types of sediments, i. e. clayey sands, clayey loesses, clays and soils of any other origin.

It may be questioned whether pollen grains found in such sediments are reworked or not. To solve this problem, there are nowadays many suitable methods available to make at least a distinction between major periods of production of the pollen grain. In this report, the staining techniques (STANLEY, 1965) as well as the fluorescence microscopy (VAN GIJZEL, 1967) are mentioned.

As clayey samples usually contain more pollen grains, the classic preparation method (VON POST, 1916, ERDTMAN, 1935; ASSARSON & GRANLUND, 1924; FAEGRI, 1936; DUMAIT *et al.*, 1965) should be such as to extract the greatest amount of fossil grains as possible, avoiding that the clays float on the dense solution, and that pollen attached to clay minerals stay down in the dense solution as well.

Therefore, the most important stage in the preparation of a pollen sample is deflocculation.

Deflocculation is traditionally enhanced by boiling the sample in a 10% base solution (VON POST, 1916). T. VAN DER HAMMEN also uses ultrasones and it was proved that after 5 minutes only, pollen with less resistant entexines, break (oral comunication).

In 1969, BASTIN worked out a shorter laboratory procedure for the time consuming methods as established by FRENZEL. As for the clay flottation method introduced by BARTHOLOME in 1981, both are efficient but still time consuming. It is furthermore clear that the lesser steps in the laboratory preparation, the lesser material is lost or contaminated.

These arguments being in our thoughts, an efficient and time saving preparation method was looked for testing all kinds of previously known methods applied in several laboratories.

2. CRITICAL ANALYSIS OF CLASSICAL LABORATORY TREATMENT.

The several steps of classical mineral pollen sample treatment, are subject of comment with a proposal for shorter procedures at each stage.

2. 1. HCl TREATMENT.

If samples contain little $CaCO_3$ (less than 10 %), this step may be neglected when using accurate deflocculators. Excluding HCl treatment avoids coaggulation of the clay minerals. Anyhow, if the method is used, the sample should be washed thoroughly, adding a base component to the sample in order to raise the pH value above 7 again.

2. 2. THE HF TREATMENT.

HF, as it is a highly corrosive product, should be avoided for long lasting treatments. Moreover it does not dissolve all mineral components as well : clay aggregates are not dissolved but decomposed into finer elements which finally start floating on the top of the dense

(*) Kwartairgeologie - V. U. B., Pleinlaan, 2, B-1050 Brussels.
CEE-Project, Paleoclimatology - Centre for Quaternary Stratigraphy.

separation liquid. Hence, this step in the procedure is fully unapplicable for clayey sediments and should be eliminated. filtering of the residual fine clay particles is also too much time consuming and does not provide the expected results.

2. 3. THE KOH/NaOH TREATMENT.

Dealing with peat or with very dry clays, this step is useful. For all other sediments however, the KOH/NaOH treatment may be neglected and direct density separation is even more recommended. The amount of treated material is sufficiently small (gyttyas, peaty clays, clays) to deflocculate easily or does not contain disturbing amounts of vegetational remains.

2. 4. THE DENSE SOLUTION SEPARATION.

Clayey or "poor" pollen samples are generally considered not to contain pollen in sufficient abundance for statistical treatment. In order to extract as much as possible pollen and pollen species, the sample must be deflocculated and dispered at a higher level.

2. 4.1.

BASTIN (1969) proposed the use of high speed mixers for dispersion of the samples. This method works very well, but is rather expensive (the mixer, more dense solution to clean the mixer).

2. 4.2.

BARTHOLOME (1981) uses $Na_4P_2O_7$ for suspension of the sample. Indeed clay particles in suspension are filtered off a 10 mikron filter. Some pollen may also be suspended and must be recuperated from the filter as well. As was stated before, this technique is time consuming.

2. 4.3.

T. VAN DER HAMMEN and collaborators using ultrasoning, proved this method useful and little time consuming for which reason it is incorporated in the present methodology. For this purpose the samples in the dense solution are put for less than five minutes in a water bath in an ultrasonic apparatus (equal to the one used for the cleaning of sedimentological sieves). The ultrasones break down clay minerals which are attached to pollen grains, so that these ones become free to float.

2. 4.4.

Finally as a result of the foregoing discussion it is recommended to use for the dispersion of any pollen sample EXTRAN, a common low foaming detergent, which has a particular cleaning effect on mineral particles sticking to other materials. Only one or two drops are needed per sample, however, depending on the amount of material in the tube; if more EXTRAN is used, clay particles and some pollen may start to suspend and one should filter again, which as stated above, is not recommended. As detergents are not dissolvable in Bromoform it is suggested to use it with water dissolvable dense solutions such as Thoulet - solution and $ZnCl_2$. In the case Bromoform is used, it is suggested to use an acetolysis previous to the density separation. In this case, acetolysis is not meant as to dissolve the inner pollen grain, but simply to entirely dehydrate the sample (water molecules sometimes attach to clay minerals and lower their density, so that they easily float on a dense solution of d=2) and to deflocculate. Anyhow, as the pollen samples are not rich, the dense solution treatment should be repeated twice. However, three times seems to be a useless repetition.

2. 4.5.

A next general remark concerns stirring of centrifuged and decanted samples before addition of the following liquid. When considering effects of tyxotropy with regard to saturated very fine materials, it is useful after centrifuging and decanting, to faintly touch the residue time after time with the stirring staff, untill it becomes fluid and the stirrer sinks into the fluid. Another advantage in applying this technique, is that there is no need to use specialised stirring apparatuses (e. g. about the 100 and 55 cc centrifuge tubes).

2. 5. ACETOLYSIS.

Acetolysis is nowadays generally known not to be necessary for fossilized pollen grains. Since peat and other acid sediments do also keep the inner part of the pollen grain, acetolysis is applied on these types of samples only. Hence acetolysis is not used for all mineral samples.

2. 6. STAINING.

In the case there is a need to differentiate entexines of very different geological age (e. g. Tertiary pollen reworked into Quaternary sediments) staining is used. When fluorescence microscopy is applied, staining is not applicable.

3. SUMMARY OF THE PROPOSED PROCEDURE.

The general procedure for preparation of pollen samples other than peat may be summarised as follows : all for two cases :

3.1. Water dissolvable dense solution.	3.2. Alcohol/Aceton dissolvable dense solution.
(HCl)	(HCl)
Detergent	Acetolysis
Dense solution	Dense solution
(Acetolysis)	(Acetolysis)
(Staining)	(Staining)
Mounting	Mounting

4.BIBLIOGRAPHY.

BASTIN, B. (1964) - Essais d'analyse pollinique des loess en Belgique selon la méthode de Frenzel. *Agricultura*, vol. XII, 4.

BARTHOLOME, E. (1981) - Extraction des pollens en faible teneur dans les sédiments argileux. *Bull. Soc. belg. Géol.*, vol. 90, 4, pp. 281-286.

DUMAIT, P., MARCEAU, C., VAN CAMPO, M. (1963) - Nouvelle méthode de concentration des pollens dans les sédiments pauvres par microflottation. *C. R. Hebd. Séanc. Acad. Sc. Paris*, 156, pp. 231-233.

ERDTMAN, G. (1969) - Handbook of Palynology. An introduction to the Study of Pollen Grains and Spores. *Munksgaard*, Copenhagen.

FAEGRI, K.; IVERSEN, J. (1975) - Textbook of Pollenanalysis. *Blackwell Scient. Publ.*, Oxford.

VAN GIJZEL, P. (1977) - Die Fluoreszenz-Mikroskopie einiger Pflanzenfossilien. *Cour. Forsch.-Inst. Senckenberg*, 24, pp. 92-100.

VAN GIJZEL, P. (1967) - Palynology and fluorescence microscopy. *Rev. pal. bot. and Palyn.*, 2, pp. 49-79.