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Palynological processing of organic-rich rocks, or: How many times have you called a palyniferous sample "barren"?

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Abstract

Campanian–Maastrichtian organic-rich carbonate and marl successions in Israel contain abundant unstructured, fluffy organic matter. Samples from these sections, which were processed by standard palynological techniques, were found to be almost completely devoid of any structured palynomorphs. On the other hand, 8–12 hours of controlled bleaching by sodium hypochlorite (household bleach) yielded extremely rich and diverse palynological assemblages dominated by dinoflagellate cysts with rare terrestrial palynomorphs. It is suggested that by selectively bleaching the organic matter, dinocysts that were incorporated within the intricate fluffy organic debris were released. The fact that many samples contained high abundance of well-preserved thin-walled dinocysts suggests that use of the controlled bleaching did not "attack" the dinoflagellate cysts and did not cause a biased assemblage.

Compared to other oxidizing methods such as the "Schulze Solution", this method is much simpler, faster, safer and, thus, better for palynological study.

The proposed method enables obtaining palyniferous slides from samples that would have been considered barren if standard palynological techniques were utilized.

1. Introduction

Palynological processing of sedimentary rocks and slide preparation for microscopic study usually follow certain standard procedures that involve extraction of the organic matter from rocks by digesting the carbonates and silicates by non-oxidizing acids (HCl and HF, respectively) (e.g., Doher, 1980). In the study of organic-walled dinocysts, the extracted organic residue is usually fractionated into a coarse and fine fraction by sieving (usually through a 20–25 μm sieve). Fractionation helps in obtaining better-sorted and easier-to-study palynological slides. In organic-rich samples, better palynological slides are sometimes obtained by a short oxidation that removes the excessive organic matter (Gray, 1965). However, the oxidation may cause dissolution and complete elimination of palynomorphs from the assemblage, leaving a biased assemblage dominated by the more oxidation-resistant fossils. In addition, some of the oxidizing reagents (e.g., HNO_3) are highly dangerous and require the use of a fume-hood and other safety measures.

In a palynological study of Campanian–

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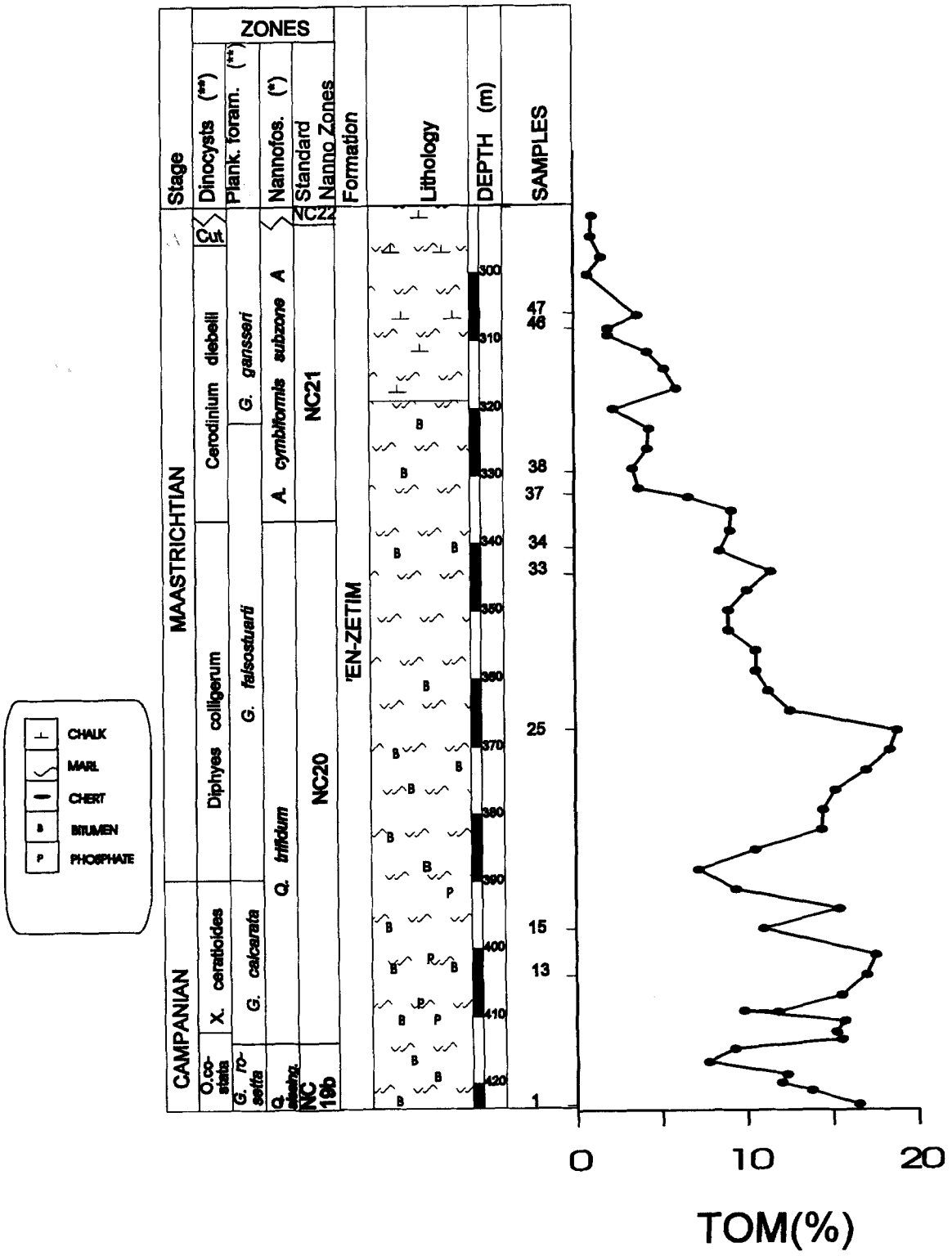


Fig. 1. The M-8 section: litho- and biostratigraphy and Total Organic Matter. Stratigraphic position of samples used in the bleaching experiment is marked. *=after Eshet and Moshkovitz, 1995; **=after Hoek et al., 1996.

Maastrichtian organic-rich carbonates (Eshet et al., 1994a) from three core sections in southern Israel (Eshet et al., 1994b; Hoek et al., 1996), the standard processing techniques were found to be inadequate for palynological slide preparation: since most palynomorphs were encased within the dominant fluffy organic debris, no fossils were seen under the light microscope, even after sieving the residue and treating it in an ultrasonic bath, and the samples were considered barren at first.

The present paper describes a method that enabled obtaining palyniferous slides from fluffy, organic-rich material.

2. Materials

Fifty-three organic-rich Campanian–Maastrichtian core samples from the M-8 core hole (southern Israel) were processed and analyzed in this study. The section belongs to the Campanian–Maastrichtian 'En Zetim Formation'. It is comprised of bituminous marls and chalks, with phosphatic horizons in the lower part (Fig. 1). Organic content is high, reaching up to 25% TOM, with an average of 10% TOM (Table 1; Fig. 1). In order to test the proposed method, ten representative samples were selected for a controlled experiment that is discussed seperately. Samples and slides are stored at the Geological Survey of Israel, Jerusalem.

3. Sample processing (Fig. 2)

All organic-rich samples were treated according to the following procedure (Fig. 2):

(1) *Removal of carbonates and silicates.* Five grams of rock were crushed and digested in concentrated HCl (32%) for about 4 hours to remove the carbonate fraction. The residue was decanted and neutralized, and then treated in concentrated HF (40%) for about 8 hours to remove the silicates. At the end of this stage, an organic residue was obtained. This residue was decanted and neutralized in distilled water.

(2) *Ultrasonic treatment.* A pipette-full of organic residue was diluted in a 500 ml glass beaker and placed in an ultrasonic bath for 3–4 minutes. The ultrasonic treatment was found useful in facilitating sieving of the excessive organic matter that otherwise tends to clog the sieve.

(3) *Sieving.* The organic residue was sieved and washed thoroughly through a 15-μm nylon sieve.

Table 1
Results of a controlled bleaching experiment

Sample		0 hours		2 hours		4 hours		6 hours		8 hours		10 hours		12 hours		14 hours		16 hours		18 hours	
No.	TOM (%)	PVF	C/B	PVF	C/B	PVF	C/B	PVF	C/B	PVF	C/B	PVF	C/B	PVF	C/B	PVF	C/B	PVF	C/B	PVF	C/B
1	16.5	1.2	2.1	1.4	2.2	1.5	2.0	5.0	2.0	22.8	2.3	30.1	1.9	39.5	1.9	38.1	1.8	40.2	2.0	37.1	2.2
13	17.0	0	-	2.4	3.4	2.1	3.6	4.1	3.2	22.6	3.1	35.4	3.0	42.1	3.1	45.6	2.8	39.1	3.1	36.1	2.8
15	11.0	1.1	1.8	1.6	1.9	3.8	2.1	8.2	2.0	19.7	1.9	25.1	2.1	35.4	2.3	42.1	1.9	39.6	1.9	34.7	1.5
25	18.8	0	-	3.0	1.7	2.6	2.1	3.8	1.9	15.1	2.0	19.6	1.9	38.1	2.1	40.7	1.5	42.6	1.4	38.1	1.4
33	11.5	2.0	3.0	2.8	3.1	3.2	2.8	3.5	3.1	12.1	3.0	15.1	2.9	40.6	2.8	42.1	2.7	39.9	2.7	36.4	2.5
34	8.5	2.2	3.1	3.5	3.0	3.0	2.9	4.2	3.1	25.4	2.9	35.2	3.2	38.2	2.8	41.6	2.8	43.4	2.6	38.5	2.4
37	6.7	3.1	1.5	2.8	1.8	4.2	1.6	8.5	1.7	29.3	1.6	40.3	1.8	39.1	1.5	41.0	1.4	38.6	1.3	35.1	1.3
38	3.8	4.4	2.3	5.2	2.4	5.6	2.1	15.4	2.2	38.5	2.1	42.0	2.2	43.8	2.1	44.1	2.0	43.2	1.8	40.1	1.5
46	2.0	3.3	2.6	4.5	2.5	8.2	2.6	20.1	2.4	31.9	2.3	49.5	2.5	51.4	2.1	52.0	2.0	48.1	1.9	42.0	1.8
47	2.0	4.2	1.6	5.1	1.8	10.1	1.7	15.3	1.6	29.1	1.8	39.6	1.7	42.9	1.8	45.1	1.6	41.4	1.5	38.1	1.4

(1) No significant change in assemblage is observed until about 8 hours. (2) Increase in number of dinocysts is evident after about 8 hours, reaching optimum at 8–12 hours. (3) No significant change in number of dinocysts is evident after 12–18 bleaching hours. (4) The slight decrease in number of dinocysts after more than 12–14 bleaching hours is probably due to a too long oxidation period. (5) In organic-rich samples, a longer bleaching time is needed, compared to lower-TOM samples. (6) No significant change through time in the ratio between broken and complete dinocysts. A slight decrease after more than 14 hours is evident.

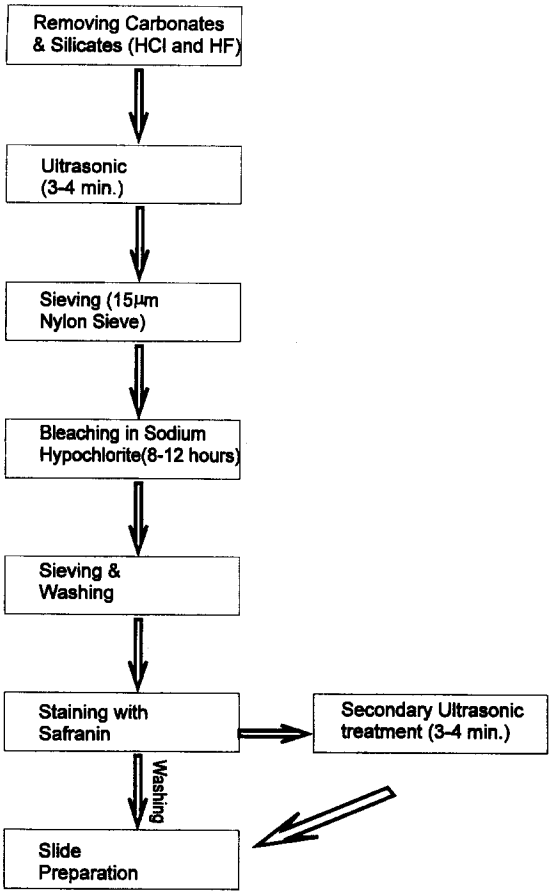


Fig. 2. Sample preparation procedures in the proposed bleaching method.

(4) *Bleaching*. The fluffy nature of the unstructured organic residue and the high organic content made it impossible to observe palynomorphs that were incorporated in the thick organic debris. Therefore, there was a need to apply a controlled and mild oxidation in order to selectively remove as much of the fluffy organic matter as possible, without affecting the structured palynomorphs. In palynological preparations, oxidation is usually done by oxidizing reagents such as Schulze Solution—a solution of $KClO_3$ in nitric acid (HNO_3), or hydrogene peroxide (H_2O_2). These methods and additional useful reagents were described by Brown (1960), Gray (1965), Fægri and Iversen (1964) and Doher (1980), among others.

In order to compare these oxidizing methods to the proposed bleaching method, selected samples were tested by both Schulze Solution and by hydrogen peroxide. Schultze Solution was found too destructive; it completely destroyed most of the palynomorphs after less than 3 minutes. Hydrogen peroxide, on the other hand, was ineffective: it had no apparent effect on the residue even after a long period of 12 hours. This is probably due to the high organic content of the samples.

Therefore, the sieved residue was mixed with 250 ml of a sodium hypochlorite solution. In this study, a commercial brand of 10% solution of sodium hypochlorite called "Economica", used as a household bleach, was utilized to selectively oxidize the excess fluffy organic debris. This solution is simple to use: it is not dangerous in contact and does not require using a fume hood. In the past, sodium hypochlorite was suggested as a useful oxidizing reagent by Erdtman and Erdtman (1933) and Gray (1965), but no details were provided as to the required period of oxidation and the problems of processing samples of such a high organic content such as in this study.

In order to avoid digestion of palynomorphs by over-bleaching, the residue was sieved and washed thoroughly every two hours to test changes in the state of the fluffy organic matter. Since sodium hypochlorite gradually loses its effect during the oxidation process, the "Economica" was decanted after 10 hours and fresh solution was added to maintain the bleaching process.

In most samples, a period of 8–12 hours was enough to remove most of the fluffy organic debris and obtain a rich and diverse assemblage, with no apparent damage to the palynomorphs (Table 1). However, very rich assemblages were obtained even after 18 hours of bleaching. The reliability of the dinocyst assemblages recovered by the selective bleaching is demonstrated by the fact that in the present study some assemblages, dominated by an overwhelming abundance of thin-walled and delicate peridinioid cysts, were obtained even after more than 10 hours of bleaching (e.g., Plate II, 2, 4)

In order to examine the rate of organic matter digestion by sodium hypochlorite, and the rate of "exposure" of palynomorphs from the fluffy organic matter, a controlled experiment was conducted on ten selected samples of different organic content (Table 1). Equal amounts of residue from each sample were treated in sodium hypochlorite for a period of 18 hours. Every 2 hours, the residue was washed and then studied under a microscope, estimating palynomorph abundance (counting the number of observed palynomorphs per visual field—PVF in Table 1 and Fig. 3) and preservation (calculating the ratio between complete and etched, or broken fossils—C/B ratio in Table 1 and Fig. 3). The results suggest the following:—Six to eight hours is the minimum time-period required for etching the fluffy organic debris and exposing palynomorphs for microscopic study. In most samples, the amount of palynomorphs observed in the assemblage began to increase drastically after six hours, reaching an optimum at 8–12 hours. Bleaching the residue for more than 12 hours did not yield in a significantly richer assemblage. Although rich and diverse assemblages were recovered even after 18 hours of bleaching, some decrease in palynomorph abundance was observed usually after more than 16 hours. Since this decrease occurred after more than 16 hours in all of the tested samples, we suggest that it is due to the removal of cysts by a too long bleaching. As shown in Table 1, samples with a high organic content (e.g., sample 1) require a longer bleaching time to obtain the optimal assemblage, compared to samples with a lower organic content (e.g., sample 47). The C/B ratio can be used to check the effect of digestion on palynomorphs. No sig-

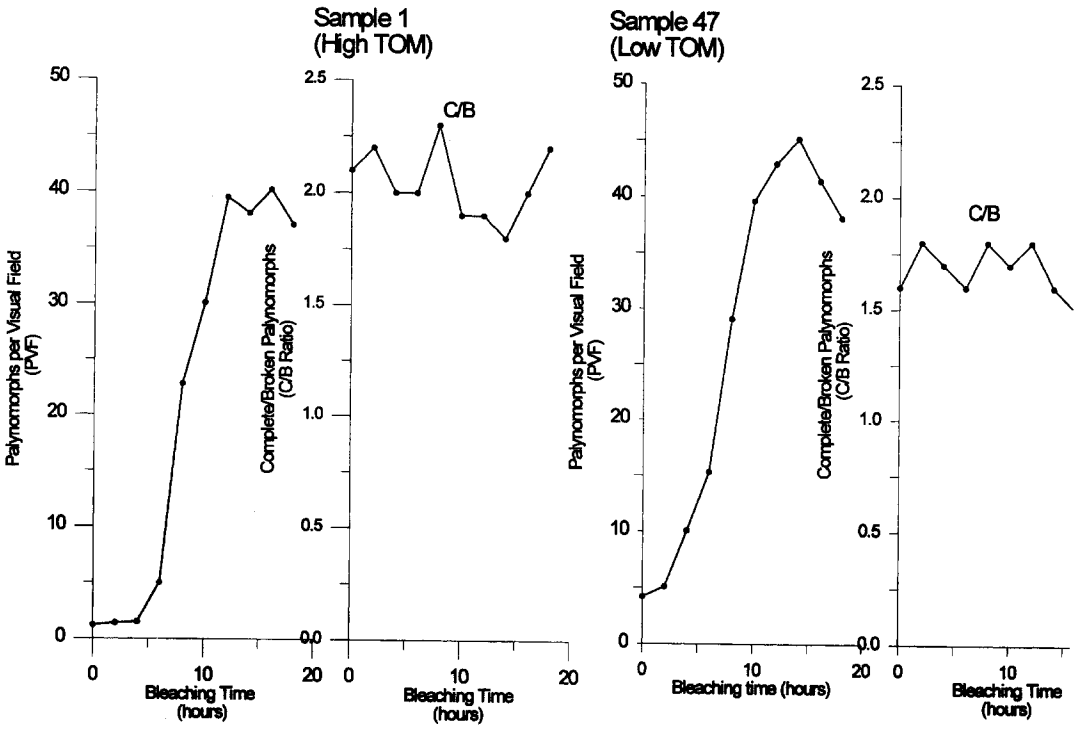
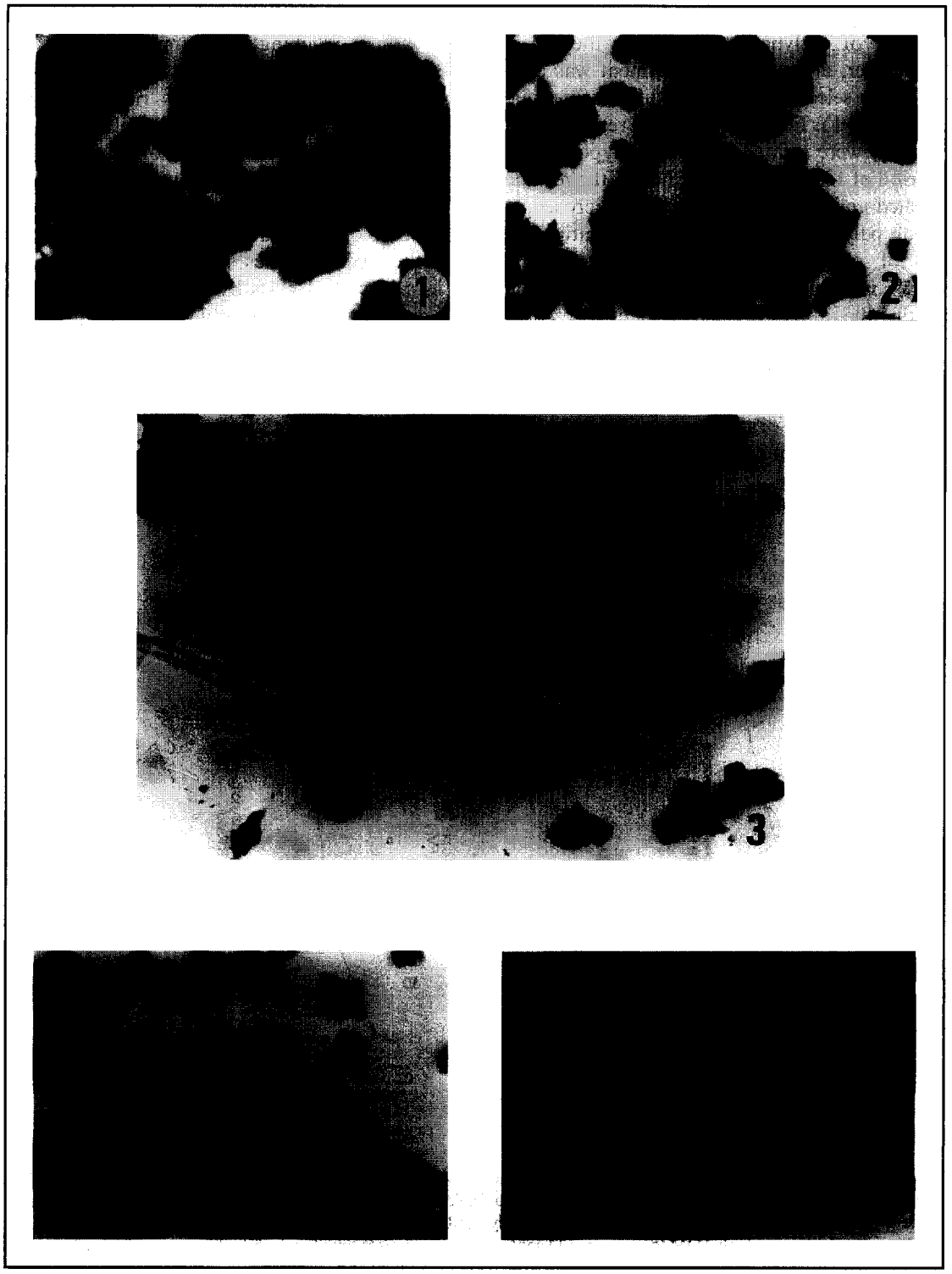


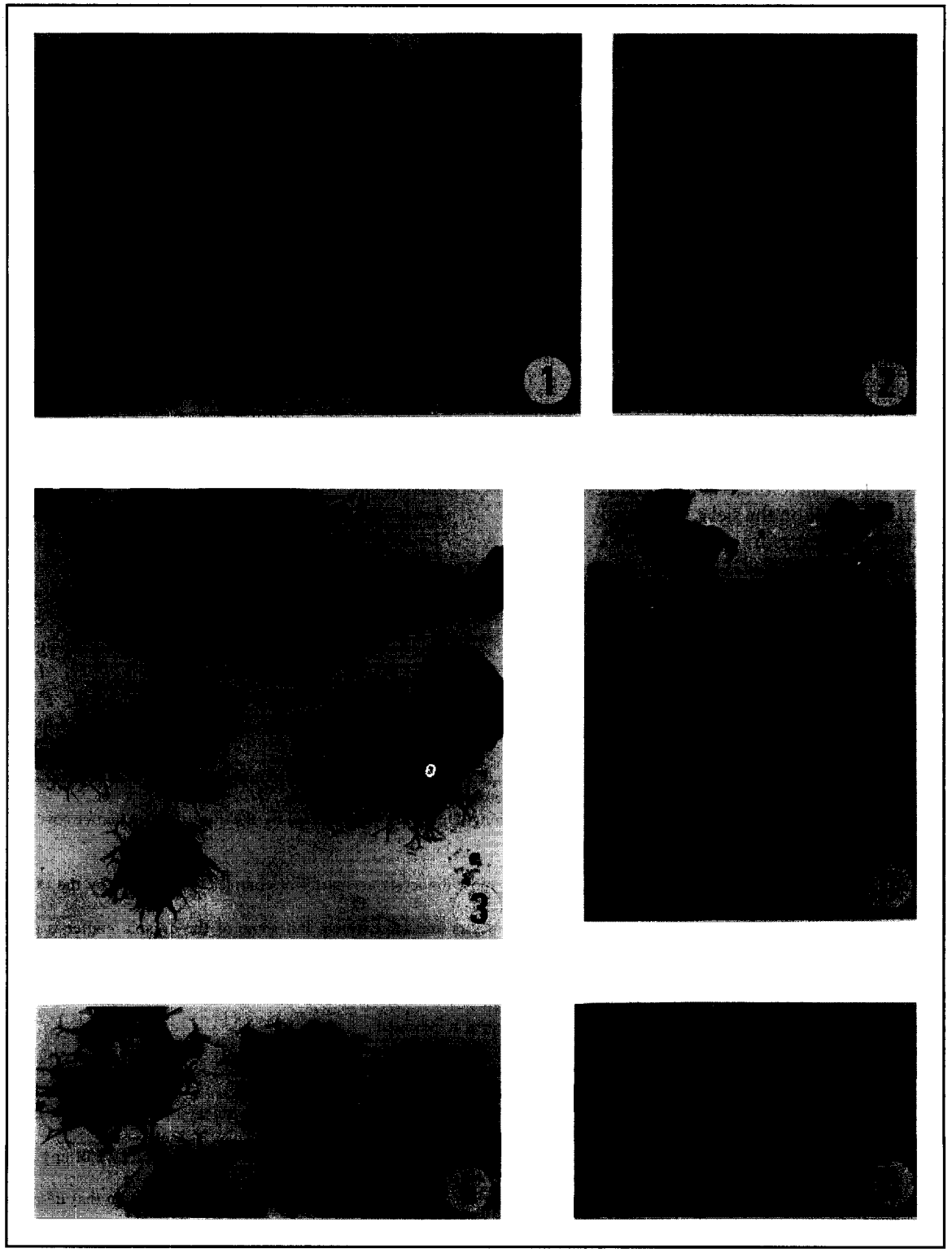
Fig. 3. Changes in the preservation (Complete/Broken palynomorphs = *C/B* ratio) and abundance (Palynomorphs per Visual Field = *PVF*) during 18 hours of bleaching of selected samples (refer to Table 1). Note: (1) Except for after 16–18 hours, preservation does not change considerably. (2) Abundance increases continuously until it begins to decrease after 16–18 hours, probably due to too long bleaching. (3) Samples of high TOM (e.g., sample 1) content require a longer time for the bleaching to produce rich assemblages, compared to a shorter period required by samples with a lower organic content (e.g., sample 47).

PLATE I



(for description see p. 108)

PLATE II



(for description see p. 108)

nificant change in the ratio was seen through time, suggesting that the bleaching process does not cause a considerable change in the assemblage composition. Table 1 and Fig. 3 show a slight decrease in the C/B ratio after more than 14 hours, indicating the increasing etching effect of bleaching on the completeness of cysts.

(5) *Sieving and washing.* After enough of the organic debris was removed, the oxidation process was terminated and the residue was washed and sieved with distilled water through a 15-μm nylon sieve. Since the mild sodium hypochlorite does not harm the sieve, the washing and sieving could be conducted directly, without prior dilution.

(6) *Staining.* After bleaching, most of the originally light-yellow palynomorphs, especially the delicate-walled dinoflagellate cysts, became translucent and were not easily observable under the microscope without the aid of an interference contrast light. Therefore, the organic residue was stained, while still on the sieve, by directly applying 5–10 drops of concentrated Safranin O for about 2 minutes. The residue was then washed thoroughly with distilled water, until all excess Safranin was removed. In too heavily stained palynomorphs, a secondary 2-minute ultrasonic treatment, followed by washing and sieving, was found helpful.

(7) *Slide preparation.* The stained residue was removed to a bottle and mounted on a slide in Canada Balsam

4. Palynological assemblages

The procedure utilized in the present study led to the recovery of well-preserved, very rich and diverse palynological assemblages from most of the samples (Plates I, II). Average abundance is about 40 palynomorphs per visual field (Table 1). Dinoflagellate cysts are the most dominant palynomorphs. Land-derived palynomorphs (pollen, spore, woody-tracheids) are extremely rare, reaching less than 1% of the assemblage (Hoek et al., 1996). The dinocyst assemblages are usually dominated by *Spiniferites* and *Achomosphaera*, associated with *Hystrichosphaeridium*, *Kenleyia*, *Cannosphaeropsis*, *Adnatosphaeridium*, *Exochosphaeridium*, *Cyclonephelium*, *Glaphyrocysta*, *Cordosphaeridium*, *Fibrocysta*, *Hystrichokolpoma*, *Florentinia* and *Oligosphaeridium*. Peridinioid cysts are usually less frequent, but in some intervals representing peaks of higher paleoproductivity, they become the dominant taxa (Eshet et al., 1994a). Peridinioid cysts include the genera *Andalusiella*, *Cerodinium*, *Chatangiella*, *Isabelidinium*, *Manumiella*, *Palaeocystodinium*, *Palaeoperidinium*, *Senegalinium* and *Trithyrodinium*.

PLATE I. (see p. 106) All magnifications are ×250, unless otherwise indicated.

1–2. Fluffy organic matter, typical of all samples, before bleaching. Dinocysts are obscured by the thick organic matter cover.

1. ×500.

2. ×250.

3. Assemblage after too long (18 hours) bleaching. Note that most dinocysts are partly or completely destroyed by the excessive oxidation.

4. Fluffy organic debris after 4 hours of bleaching. Most dinocysts are still covered, but some of the organic matter is already dissolved, exposing dinocysts, as seen in the lower right side.

5. Organic debris after 6 hours of bleaching. Fluffy particles are only slightly removed, but some dinocysts begin to "show up" as seen in the upper central part.

PLATE II. (see p. 107) All magnifications are ×500, unless otherwise indicated.

1–6. Dinocyst assemblages after almost all fluffy organic debris has been removed by bleaching.

1. After 10 hours of bleaching. Assemblage dominated by *Spiniferites–Achomosphaera* complex and *Exochosphaeridium bifidum*. Note *Palambages morulosae* in upper right. Note that dinocysts are not harmed by the bleaching.

2, 6. Dinocyst assemblage after 12 hours of bleaching, revealing abundant and unharmed dinocysts. Dominating cysts are *Trithyrodinium* spp. and *Chytroeisphaeridia baetica*. ×250.

3, 5. Dinocyst assemblages, dominated by *spiniferites* complex, after 12 hours. Note good preservation and the lack of any sign of cyst digestion by the bleaching process.

4. Assemblage after 14 hours of bleaching, dominated by *Trithyrodinium* spp. and *Chytroeisphaeridia baetica*. Note that despite the long bleaching period, most of the delicate peridinioid cysts are still intact.

5. Summary

Bleaching the fluffy organic debris in organic-rich carbonates by sodium hypochlorite has been found useful in obtaining palyniferous slides. All other preparation techniques were found inadequate for processing this material. The proposed method is simple, fast and inexpensive.

A bleaching period of 8–12 hours was found most effective, although palynomorphs were still not severely digested even after more than 18 hours.

After about 16–18 bleaching hours, both number of palynomorphs per visual field and the ratio between complete and broken palynomorphs show a slight decrease. This decrease is the result of excessive bleaching that either digested or etched palynomorphs in the assemblage.

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