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24. *DATA REPORT:* CHARACTERIZATION OF DISTRIBUTIONS OF PHOTOSYNTHETIC PIGMENTS IN SAPROPELS FROM HOLES 966D AND 969C[1]

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ABSTRACT

The distribution of chlorins and carotenoids in 22 sapropel samples from Ocean Drilling Program Leg 160, Holes 966D and 969C, have been examined by combined liquid chromatography-mass spectrometry under atmospheric pressure ionization conditions. Up to 42 components were detected over the range of samples (including chlorophyllone *a*, pyrophaeophorbides, pyrophaeophytins, steryl chlorin esters *a* and *b*, as well as carotenes). The samples showed two basic types of pigment distribution, with no obvious relationship between these distributions and the sedimentological parameters.

INTRODUCTION

According to a definition by Kidd et al. (1978)—although not generally accepted—sapropels correspond to open-marine sediment layers more than 1 cm thick with an organic carbon content of at least 2 wt%. In the present study, the distribution of photosynthetic pigments (chlorophyll derivatives as well as carotenoids) in 22 sapropel samples from the Eastern Mediterranean Sea (Ocean Drilling Program Leg 160, Sites 966 and 969) were examined by combined reversed-phase liquid chromatography-mass spectrometry (LC-MS) under atmospheric pressure ionization (APCI) conditions, to obtain new clues about the origin and accumulation of such organic matter–rich sediments. Distributions of chlorins and carotenoids in sedimentary records are commonly used in paleoenvironmental studies to estimate the algal pigment diversity in phytoplanktonic populations, as well as to trace the fate of the organic matter during transport from the surface of the water column to the bottom sediment. Because sediments often exhibit a high pigment diversity (due mainly to the presence of numerous transformation products), chromatographic and spectrometric methods are required for pigment identification and quantification.

EXPERIMENTAL

Sample Origin and Extraction

Sediments were obtained from core sections of Holes 966D (33°48′N, 32°42′E; 940 m water depth) and 969C (33°50′N, 24°53′E; 220 m water depth), located in the Levantine Basin and on the Mediterranean Ridge, respectively. Samples originated from various depths of Hole 966D (Table 1) and from the uppermost sapropel layers of Hole 969C (Table 1). Sediment is Pleistocene (Core 160-969C-1H and Core 160-966D-2H) to Pliocene (Cores 160-966D-5H through 8H) in age.

Frozen sediment was freeze dried and the whole sample extracted in 10% aqueous acetone at 4°C for 24 hr with constant mixing. Following centrifugation, the supernatant was filtered and the extract evaporated to dryness and dissolved in 100 µL 10% aqueous acetone. The sapropels were generally dark grey to black, but the acetone extracts ranged in color from yellow to deep brown-green (Table 1).

Liquid Chromatography-Mass Spectrophotometry

Pigment analyses were carried out under high-performance liquid chromatography (HPLC) reversed-phase conditions using a C_{18} ODS 3 µm Spherisorb column (150 mm × 4.6 mm i.d.) with a C_{18} direct-connect guard column. The HPLC instrument was operated at a flow rate of 1 mL min^{-1} using a linear gradient program (Table 2) that allowed the resolution of the more polar components in total unmethylated acetone extracts without addition of an ion-pairing reagent. Fil-

Table 1. Characteristics of sapropel samples from Holes 966D and 969C.

Core, section	Depth (mbsf)	Acetone extract color	Pigment distribution
160-966D-			
2H-1	3.62-3.64	Brown-green	B
2H-1	3.64-3.66	Brown-green	B
5H-6	39.86-39.88	Brown-green	B
5H-7	41.03-41.05	Deep brown-green	A
5H-7	41.34-41.36	Yellow	B
6H-1	41.95-41.97	Brown-green	A
6H-5	48.26-48.28	Orange	B
7H-1	52.18-52.20	Orange-red	A
7H-2	52.92-52.94	Orange	A
7H-6	59.71-59.73	Orange	B
8H-2	63.02-63.04	Orange	A
160-969C-			
1H-1	0.94-0.95	Brown-green	B
1H-1	0.99-1.00	Brown-green	B
1H-1	1.20-1.22	Brown-green	B
1H-1	1.28-1.30	Brown-green	A
1H-1	1.36-1.38	Brown-green	B
1H-2	1.78-1.80	Brown-green	B
1H-2	1.82-1.84	Brown-green	A
1H-2	1.86-1.88	Orange	B
1H-2	2.26-2.28	Brown-green	B
1H-2	2.34-2.36	Brown-green	B
1H-2	2.42-2.44	Brown-green	B

Note: Vertical bars indicate samples from the same sapropel layer.

Table 2. Solvent elution program for LC-MS, and typical back-pressures at a flow rate of 1 mL min^{-1}.

Time (min)	Methanol (%)	Acetone (%)	Water (%)	Pressure (psi)
0	80	0	20	~2700
15	60	30	10	~1600
20	60	30	10	~1600
30	30	60	10	~1500
50	5	90	5	~1000
65	5	90	5	~1000
75	80	0	20	~2700

[1]Robertson, A.H.F., Emeis, K.-C., Richter, C., and Camerlenghi, A. (Eds.), 1998. *Proc. ODP, Sci. Results*, 160: College Station, TX (Ocean Drilling Program).

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tered HPLC-grade solvents (Rathburn) and distilled water were used. Before entering the ion source, the elution mixture was passed through an on-line Waters 991 photodiode array detector for determination of the absorbance (UV-VIS) spectra of components (350–700 nm) and monitoring the chromatographic separation (λ = 430 nm). A wavelength of 430 nm rather than 400 nm was chosen to allow monitoring and detection of carotenoids as well as chlorins.

LC-MS was carried out using a Waters (Watford, UK) MS 600 Silk quaternary delivery HPLC-system, fitted with a Rheodyne 7125 injection valve (20-µL calibrated loop) and a Finnigan MAT (Hemel Hempstead, UK) TSQ 700 quadruple mass spectrometer, linked by a Finnigan MAT APCI interface. The interface conditions were: vaporizer temperature 550°C, capillary temperature 300°C, corona 5 µA, sheath gas pressure 50 psi, and auxiliary gas pressure 20 psi. Mass spectra were obtained in the positive-ion mode, with scanning from m/z 400 to 1200 every 2 s.

RESULTS

Pigment Identification

The UV-VIS chromatograms showed the presence of up to 42 significant peaks over the range of sapropel samples studied (Figs. 1–4; see Table 3 for pigment identification), although only certain peaks occurred systematically in all the acetone extracts, and the relative abundance of the pigments varied from one sample to another. No bacteriochlorophyll derivative or bacterial carotenoid could be identified. Although the presence of such pigments in some of the samples cannot be excluded, their abundances were not significant. Many peaks remain unidentified, but on the basis of their absorbance and mass spectra, the components can be classified into two categories: chlorophyll-derived chlorins and carotenoids.

Chlorins

Over the 22 samples, up to 14 and 5 components derived from chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) respectively, were identified. Chlorophyllone *a* (peak 4), a Chl *a* transformation product containing an additional exocyclic ring, was present in all samples and was generally dominant (Figs. 1–4). The identification of this pigment was confirmed by comparison of the retention time and spectra with those of an authentic standard. Chlorin identities revealed also the systematic occurrence of the classical "pyro-" counterparts of phaeophorbides *a* and *b*, whereas the "pyro-" counterparts of the nonpolar phaeophytins *a* and *b* occurred only in certain samples. Eight other peaks corresponding to nonpolar chlorophyll derivatives were present in the samples containing the "pyro-" counterparts of the phaeophytins *a* and *b* and correspond to steryl chlorin esters (SCEs). Up to 10 SCE *a* components were found (peaks 37–42; including coeluting components) and, more surprisingly, up to 2 SCE *b* components were also detected (peaks 35 and 36). In the mass spectra of the SCEs, the protonated molecule indicates the presence of an esterifying alcohol on the pyrophaeophorbide nucleus and direct comparison with the spectra of pyrophaeophytin *a* and pyrophaeophytin *b* indicates that the common fragment at m/z of 535 and 549, respectively, corresponds to loss of the esterifying side-chain as a sterene.

Carotenoids

Only certain of the many components apparent in the absorbance chromatograms were observed in the base peak ion chromatograms (Figs. 3B, 4B). On the basis of their absorbance spectral characteristics, most of those pigments correspond to carotenoids (Figs. 3B, 4B; Table 3). Comparison with the UV-VIS spectra and retention times of standards suggests the occurrence of zeaxanthin and canthaxanthin (peaks 12 and 17, respectively) in almost all the samples, as well as the nonspecific α- and β-carotenes (peaks 31 and 32, respectively) in some of the samples. The other carotenoids remain unidentified, because their spectral characteristics and retention time data did not match those of the standards available or those of the most typical microalgal pigments. This may suggest that most of the compounds correspond to transformation products.

Further studies (data not shown) revealed that the LC/APCI-MS conditions employed are not suitable for the identification of many carotenoids, especially the carotenes and other components whose mass spectra contained large numbers of ions with only low m/z values.

Pigment Distributions

UV-VIS and base peak chromatograms from LC/APCI-MS analysis of the sapropel acetone extracts can be divided into two basic types (A and B) on the basis of the pigment distributions (see Figs. 3 and 4 for examples and Table 3 for major pigment assignments). Both types of UV-VIS chromatograms (Figs. 3A, 4A) showed a similar pigment distribution from 15 to 35 min (peaks 1–17). However, the type B distribution exhibited up to 25 additional pigment components at longer retention times (Fig. 3A), unlike type A (Fig. 4A). Seven of the samples showed the simpler type A distribution and the other 15 exhibited the more complex type B distribution (Table 1; Figs. 1, 2). The pigment distribution over one single sapropel layer is consistent, and the type A samples originate mostly from deep sapropel layers at Site 966 (Figs. 1, 2; Table 1). In lithologic Unit I of Hole 966D, many of the organic matter–rich sediment layers are bioturbated and show geochemical evidence of partial oxidation of the organic matter. However, there does not appear to be any obvious relationship between pigment distribution and the sedimentological parameters of the sapropels (organic carbon contents were not available for most of the samples).

REFERENCES

Kidd, R.B., Cita, M.B., and Ryan, W.B.F., 1978. Stratigraphy of eastern Mediterranean sapropel sequences recovered during DSDP Leg 42A and their paleoenvironmental significance. *In* Hsü, K.J., Montadert, L., et al., *Init. Repts. DSDP,* 42 (Pt. 1): Washington (U.S. Govt. Printing Office), 421–443.

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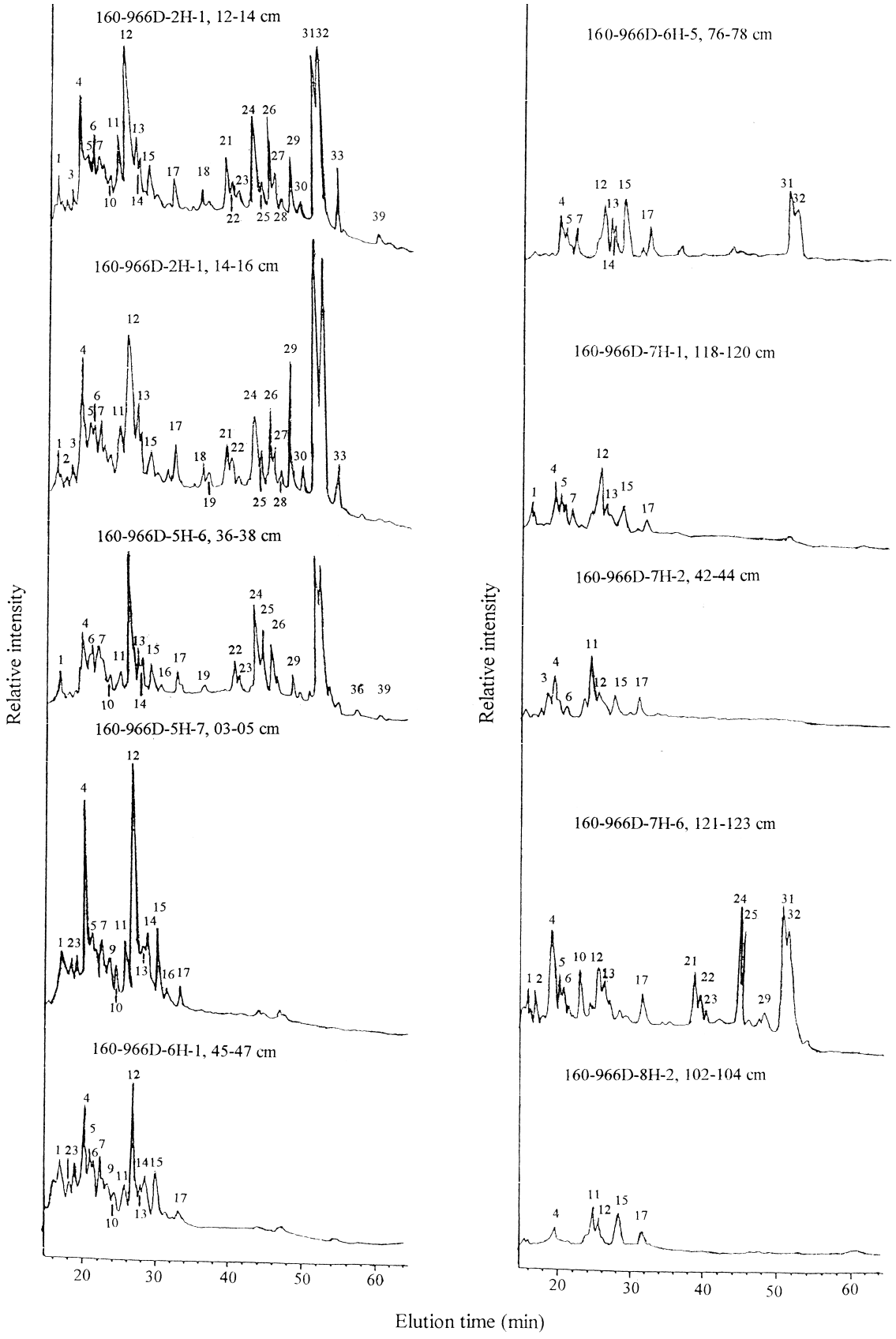

Figure 1. Partial (15–65 min) UV-VIS (λ = 430 nm) chromatograms of acetone extracts from Hole 966D samples, except Sample 160-966D-5H-7, 34–36 cm, presented in detail in Figure 4. For labeled peak identities, see Table 3.

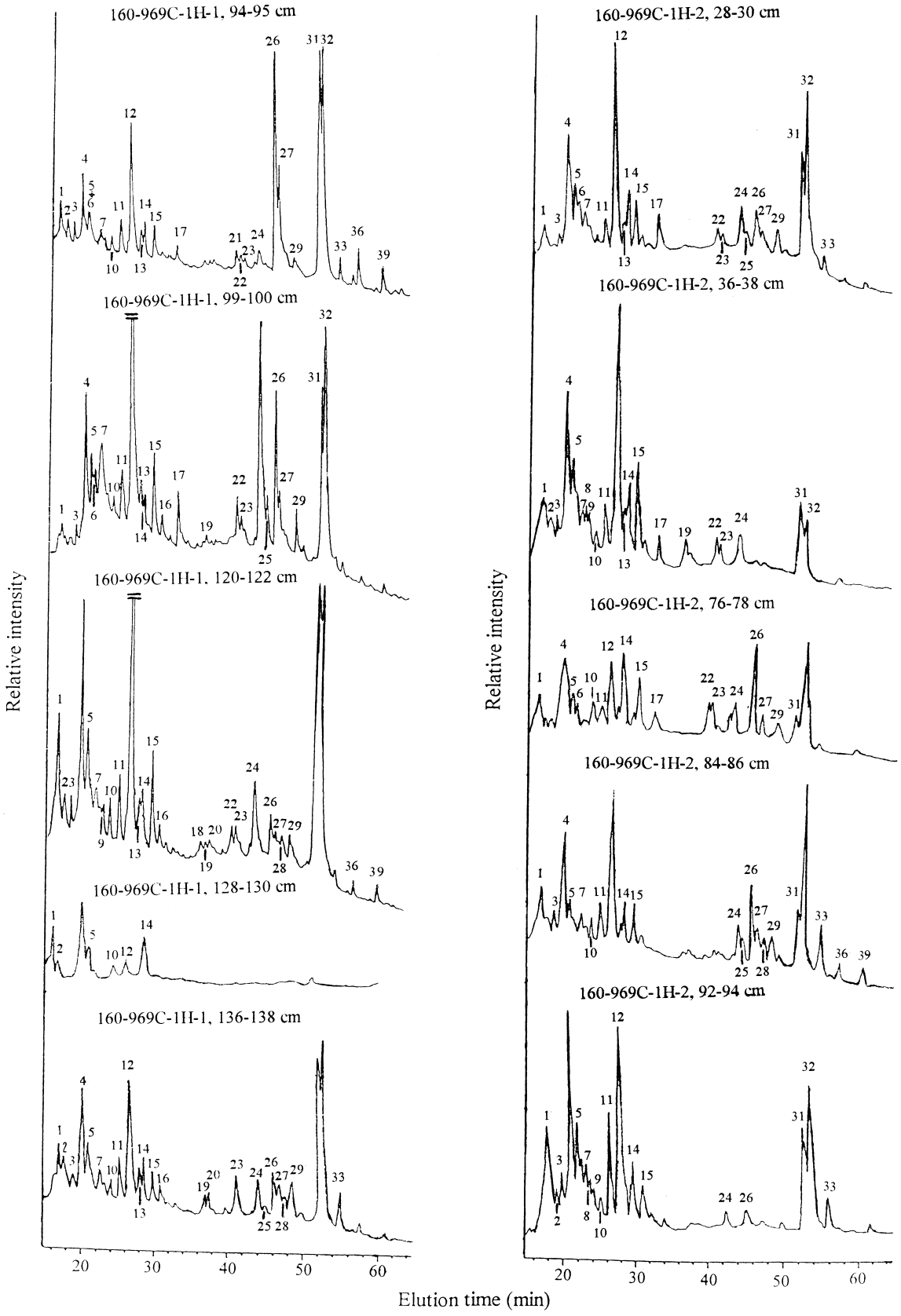

Figure 2. Partial (15–65 min) UV–VIS (λ = 430 nm) chromatograms of acetone extracts from Hole 969C samples, except Sample 160-969C-1H-2, 32–34 cm, presented in detail in Figure 3. For labeled peak identities, see Table 3.

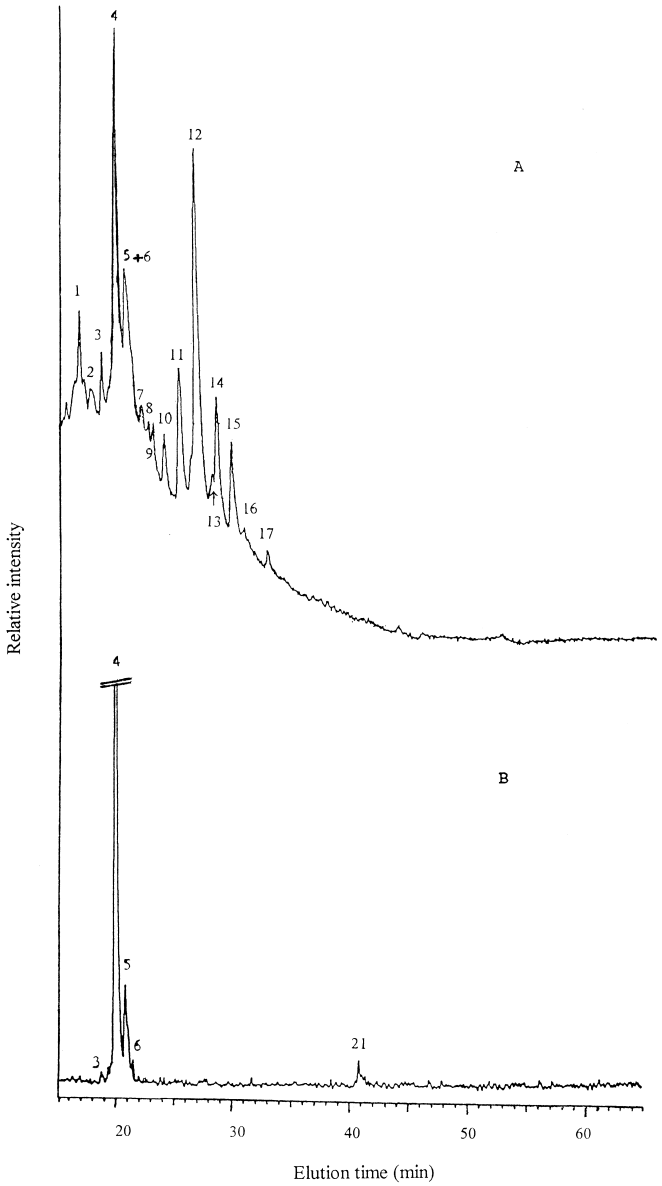

Figure 3. (**A**) Partial (15–65 min) UV-VIS (λ = 430 nm) and (**B**) base peak ion chromatograms illustrating the type A pigment distribution. Chromatograms are from the positive-ion LC/APCI-MS analysis of the acetone extract from Sample 160-969C-1H-2, 32–34 cm. Peak identities are given in Table 3.

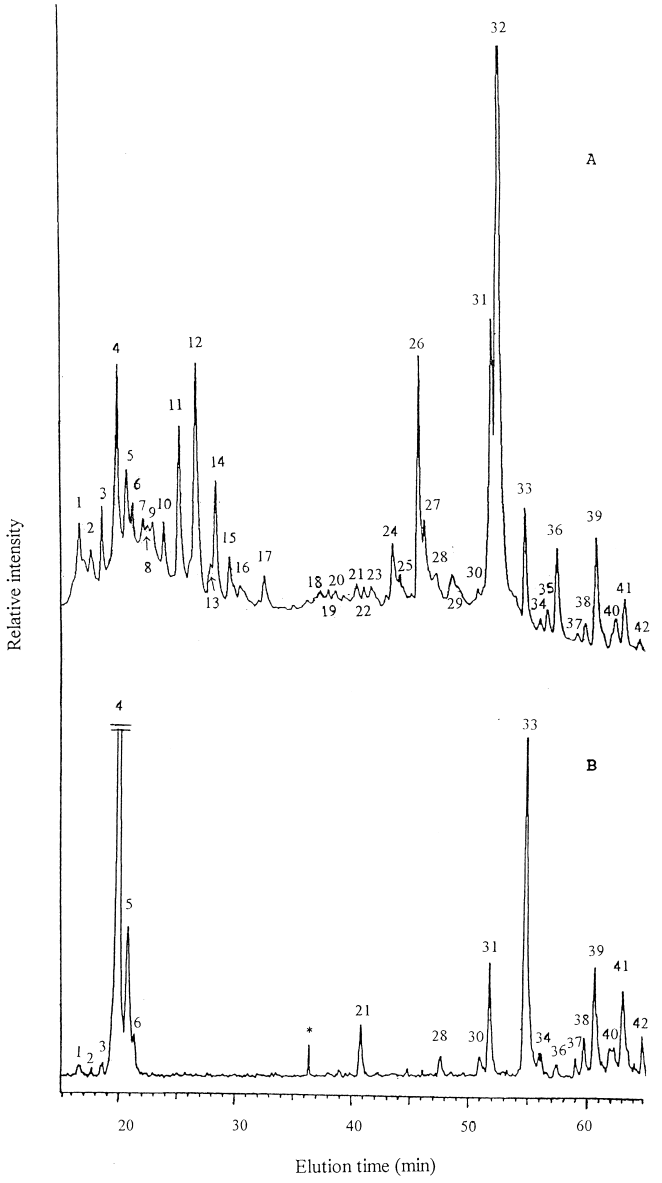

Figure 4. (**A**) Partial (15–65 min) UV–VIS (λ = 430 nm) and (**B**) base peak ion chromatograms illustrating the type B pigment distribution. Chromatograms are from the positive-ion LC/APCI-MS analysis of the acetone extract from Sample 160-966D-5H-7, 34–36 cm. Peak identities are given in Table 3. * = unknown, nonpigment impurities.

Table 3. Significant components in acetone extracts from sapropel samples identified by LC-MS analysis.

Peak	t_R (min)	λ_{max} (nm)	$[M+H]^{+}$*	Prominent ions†	Assignment**
1	16.7	413-435-653	549	529-503-475	Pyrophaeophorbide *b*
2	17.8	413-435-653	549	529-503-575	Pyrophaeophorbide *b* isomer
3‡	18.6	450-480-505	—		Unidentified
		406-500-535-603-663	535	517-507-491	Pyrophaeophorbide *a*
4	19.9	411-506-535-611-668	533	515-505-489	Chlorophyllone *a*
5	20.9	411-506-535-611-668	533	515-505-489	Chlorophyllone *a* epimer
6‡	21.4	400-503-535-615-670	549	521	Unknown Chl *a* derivative
		467-495-522	565		Unidentified
7	22.1	480	—		Carotenoid
8	22.4	420-445-470	—		Carotenoid
9	23.0	422-448-473	—		Carotenoid
10	23.8	420-449-479	—		Carotenoid
11	25.2	422-448-473	—		Carotenoid
12	26.5	426-451-477	—		Zeaxanthin ?
13	27.8	560	—		Unidentified
14	28.3	423-450-475	—		Carotenoid
15	29.6	421-445-473	—		Carotenoid
16	30.4	418-441-472	—		Carotenoid
17	32.5	475	—		Canthaxanthin?
18	36.7	(355)-413-(442)	—		Unidentified
19	37.7	(355)-413-(422)	—		Unidentified
20	38.1	(355)-413-(422)	—		Unidentified
21‡	40.7	420-445-475	—		Carotenoid
		467-495-525	538		Unidentified
22	41.2	420-447-475	—		Carotenoid
23	41.7	415-442-472	—		Carotenoid
24	43.5	485	551		Unidentified
25	44.0	408-507-535-610-669	—		Chlorin
26	45.6	427-453-481	—		Carotenoid
27	46.1	429-475-500	—		Lycopene derivative ?
28‡	47.5	409-505-535-608-665	887	609-559	Phaeophytin *a* allomer
		390	536		Unidentified
29	48.5	425-442-474	—		Carotenoid
30	51.0	410-505-535-610-667	871	593	Phaeophytin *a*
31‡	51.8	413-435-653	827	549	Pyrophaeophytin *b*
		422-446-472	—		α-carotene
32	52.3	422-450-474	—		β-carotene
33	54.8	410-505-535-610-667	813	535	Pyrophaeophytin *a*
34	56.0	410-505-535-610-667	813	535	Pyrophaeophytin *a* isomer
35	56.5	413-435-653	915	549	Steryl chlorin ester *b*
36	57.3	413-435-653	929	549	Steryl chlorin ester *b*
37	59.0	410-505-535-610-667	887	535	Steryl chlorin ester *a*
38	59.8	410-505-535-610-667	901	535	Steryl chlorin ester *a*
39‡	60.5	410-505-535-610-667	915, 901	535	Steryl chlorin esters *a*
40‡	62.2	410-505-535-610-667	915, 929	535	Steryl chlorin esters *a*
41	63.3	410-505-535-610-667	903	535	Steryl chlorin ester *a*
42‡	65.0	410-505-535-610-667	903, 945, 931	535	Steryl chlorin esters *a*

Notes: * = protonated molecule. † = other ions in mass spectrum. ** = based on UV-VIS and mass spectral interpretation. ‡ = coelution. — = not observed.

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