



Application of pyrolysis–GC/MS for the characterisation of suspended particulate organic matter in the Mediterranean Sea: a comparison with the Black Sea

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Abstract

Suspended particulate organic matter (SPOM) both from the oligotrophic Mediterranean and from the more productive Black Sea has been analysed by pyrolysis–gas chromatography/mass spectrometry (Py–GC/MS) and by such conventional techniques as elemental analysis and fluorometry. The data achieved by the two approaches generally confirmed and complemented each other. The relative concentrations of pyrolysis products (termed markers) characteristic of chlorophyll (CHL), lipid, carbohydrate (CBH), and protein (PROT) components of SPOM have been determined. The vertical distribution of lipid markers was more uniform in the Mediterranean surface waters. Their relative abundance increased rapidly in the oxycline of the Black Sea, reaching their highest levels in the suboxic zone, where the protein composition of the SPOM changed significantly. In both seas, the relative concentrations of CHL markers increased consistently within the CHL maximum zone where the CBH markers were less abundant. No lignin markers from terrestrial vegetation were detected at significant levels in the pyrograms. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Suspended particulate organic matter (SPOM) in the aquatic environments is composed of both living and non-living particles of planktonic and bacterial origin. The abundance and elemental composition (C:N:P ratio) of SPOM in surface waters, together with measurements of chlorophyll-a (chl-a) and dissolved nutrients, define the trophic status of the seas. In recent decades, analytical pyrolysis like Py–GC/MS (Saliot et al., 1984; Sicre et al., 1994; Ishiwatari et al., 1995; Klap et al., 1996; Peulvé et al., 1996) and direct temperature–resolved mass spectrometry (DT–MS; Minor et al., 1998, 2000) have been used to characterise marine particulate organic matter at the molecular level.

The land-locked Black Sea is a unique marine environment possessing the biochemical characteristics of oxygenated, suboxic and anoxic waters of the entire deep basin throughout the year. It is a relatively productive sea due to large river discharges feeding the system with nutrients. Therefore, the SPOM content of the surface waters decreases offshore. Recently, Karl and Knauer (1991) mentioned that the C:N ratio of SPOM was apparently lower than that of fast sinking POM. Hence, the majority of the rapidly sinking particles was suggested to be decomposed while sinking down to the bottom. In this redox transition zone, the low C:N ratio was suggested to be characteristic of bacterial dominance in SPOM (Bird and Karl, 1991; Coble et al., 1991; Repeta and Simpson, 1991; Çoban-Yıldız et al., 2000a).

The eastern Mediterranean Sea typifies oligotrophic seas, the nutrient inflow to its surface waters being very limited. However, in cyclonic regions of the basin, the

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SPOM content of the surface waters reaches peak values during the early spring-bloom periods because of the upwelling of relatively nutrient-rich deep waters. Thereafter, as a result of recycling in the nutrient-poor euphotic zone, the SPOM remains at background concentrations (~ 1.5 , ~ 0.15 , ~ 0.015 μM for POC, PON and PP, respectively) for most of the year (Ediger et al., 1999). However, sub-surface maxima in particulate and chl-a profiles can be observed due to the coincidence of the nutricline with the base of the euphotic zone. Regenerated production and selective nutrient uptake leads to relatively lower N:P ratios in SPOM although upwelled deep water has always high nitrate to phosphate ratio (26–28; Yılmaz and Tuğrul, 1998). During such regeneration periods, production, and thus, algal biomass, are controlled by picoplankton (Gasol et al., 1997). Consistently, in the oligotrophic Sargasso Sea, the total POC measured in the euphotic zone was composed mainly of detritus, and algal carbon was calculated to be only 10% of the POC (Li et al., 1992). Furthermore, in oligotrophic seas, at the base of the euphotic zone, the contribution of bacterial carbon may be similar to or higher than that of algal C (Li et al., 1992).

Recent studies on the abundance and elemental composition of SPOM in the Mediterranean and the Black Seas have indicated remarkable spatial and seasonal variations (Yılmaz et al., 1998; Ediger et al., 1999; Çoban-Yıldız et al., 2000b). Analysis of Black Sea SPOM by Py-GC/MS enabled the identification of markers produced from the lipid, carbohydrate, protein and chlorophyll components (Çoban-Yıldız et al., 2000a). The present study aims to characterise SPOM in the Mediterranean Sea and then to compare the relative abundances of pyrolysis markers of the Mediterranean and the more productive Black Sea waters. Conventional oceanographic parameters have also been measured simultaneously.

2. Methodology

Fig. 1 shows the stations visited in the Mediterranean in May and November 1996 and also in the Black Sea during a July'96 cruise. Seawater samples for all the biochemical parameters shown in Table 1 were collected with 5 l Niskin bottles on a rosette system. Particulate samples, collected from various depths (pre-filtered through a 200 μm pore-size mesh) were filtered through pre-combusted GF/F filters which were then kept frozen (-20°C) until analysis. The organic carbon and nitrogen (POC, PON) contents of particles were determined by the dry combustion technique (Polat and Tuğrul, 1995) using a Carlo Erba 1108 CHN analyser, with a precision of $\pm 3\%$. The filters frozen for total particulate phosphorus (PP) analysis were combusted at 500°C for 3 h the filters were then treated with 12 ml of 0.5 N HCl.

After adjusting the pH of the filtrate to 8, the total phosphorus was determined by the conventional colorimetric method (Polat and Tuğrul, 1995). Standard deviations of the replicates ranged between ± 4 and 7%. Nutrients and chlorophyll-a in seawater were measured as described in Grasshoff et al. (1983) and Holm-Hansen and Riemann (1978).

Particles were scraped from dried filters for Py-GC/MS analysis and about 6 mg of the scrapings were inserted into a quartz pyrolysis tube. The ends of the tube were plugged with pre-pyrolysed quartz wool. Five microlitres of internal standard (10 mg/l of 1,3,5-tri-*tert*-butylbenzene in methanol) was then spread evenly into the sample before pyrolysis. Two blanks were run before each analysis using the same quartz tube and quartz-wool as for the sample. Samples were pyrolysed at 700°C for 10 s using a CDS Instrument Pyroprobe 1000 at the maximum heating rate of the platinum filament pyroprobe. Separation of pyrolysis products was carried out with a DB-5MS fused-silica capillary column (30 m \times 0.25 mm, 0.25 μm film thickness, J&W Scientific, 5% phenyl-95% methylsiloxane). The column temperature was held at 50°C for 2 min, then programmed to a final temperature of 300°C at $5^\circ\text{C}/\text{min}$. Mass spectra under electron impact (70 eV) were recorded at 1 scan/s in the m/z 45–450 mass range. Complete details of the Py-GC/MS routine have been published previously (Ergin et al., 1996).

In this study, 42 pyrolysis products were selected as markers for semi-quantitation (Table 2). Their peak areas were calculated from the mass chromatogram using the target ions (generally being the base peak) listed in Table 2. Relative peak areas, the ratio of the peak area relative to the summed peak areas of all 42 markers, are referred to as “relative concentration” of the marker.

All POM samples were analysed in the presence of 50 ng of the internal standard (1,3,5-tri-*tert*-butylbenzene) to check the homogeneity of instrumental conditions. The relative standard deviation of the standard peak areas, determined at m/z 231, is 15% ($n=16$), and is assumed as the reproducibility of the relative concentration of each pyrolysis product. In other words, variations in relative concentrations close to or less than 15% are considered insignificant.

3. Results and discussion

3.1. Principal oceanographic properties of the studied seas

Fig. 2 shows physical and hydro-chemical properties observed for five stations. Euphotic zone, depth-averaged values of all measured parameters are compiled in Table 1.

In the more productive Black Sea, 1% light depth (which practically defines the thickness of the euphotic

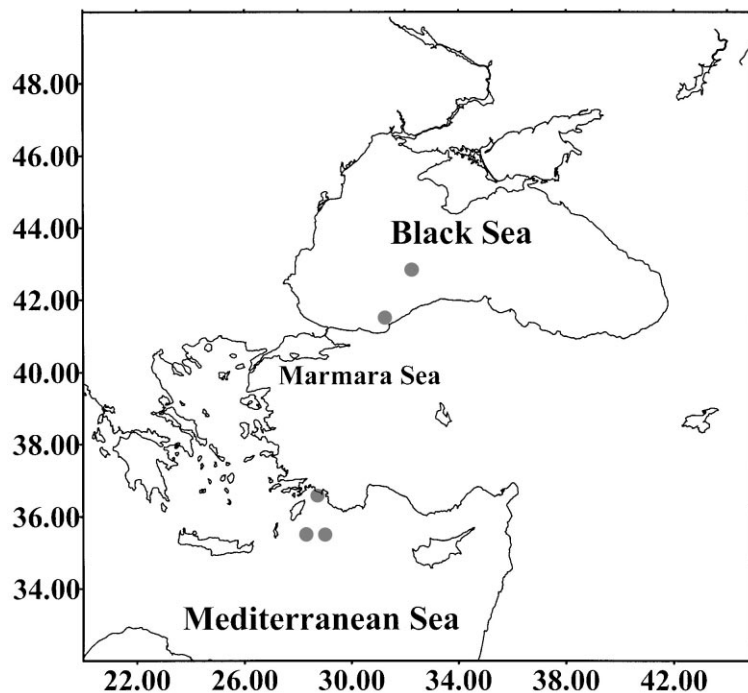


Fig. 1. Sampling stations in the Mediterranean Sea (in May and November 1996) and in the Black Sea (in June 1996).

Table 1

The average values of bio-chemical measurements for the euphotic zone of the Black Sea and the Mediterranean Sea

Parameter	July 1996 Black Sea		November 1996 Mediterranean Sea		May 1996 Mediterranean Sea
	Off-shore	Near-shore	Off-shore	Near-shore	Off-shore
EZ ^a depth (m)	40	35	77	55	60
Salinity	18.4	17.9	39.3	39.3	39.1
NO _x (μM) ^c	0.31	0.58	0.06	0.34	0.07
PO ₄ (μM) ^c	0.06	0.02	0.02	0.03	0.02
NO _x /PO ₄	4.8	30	3.2	13	3.3
POC (μM)	5.7	7.36	2.10	2.06	5.7
PON (μM)	0.55	0.69	0.23	0.25	0.74
PP (μM)	0.035	0.043	0.009	0.011	0.019
POC/PON	11	11	9	8.2	8.3
POC/PP	166	170	249	196	310
PON/PP	16	16	27	23	39
Chl-a (μg/l)	0.17	0.42	0.07	0.09	0.1
POC/chl-a (w/w)	457	253	507	345	1166
Pr.P ^b (mgCm ⁻² d ⁻²)	687	604	93	–	479
CBH (% area)	22.7	12.0	12.3	10.5	14.1
LIPID (‰)	5.9	2.9	10.7	9.0	5.7
PROTEIN (‰)	32	37.6	32.3	39.0	34.3
CHL (‰)	1.94	2.3	2.0	2.4	1.4

^a EZ, euphotic zone.

^b Pr. P, primary production.

^c Depth-averaged till nutricline, when nutricline is within the euphotic zone.

Table 2

Pyrolysis products used as markers, the m/z of the ion used in the selected ion monitoring (SIM) mode for determining the peak area, and the relative standard deviation (RSD, as percentage) of the relative peak area ($n=3$)

N	Compound	SIM	RSD
1	Toluene	91	1.3
2	Ethylbenzene	91	2.7
3	<i>m/p</i> -Xylene	91	1.7
4	Styrene	104	10.0
5	<i>n</i> -Dec-1-ene	55	8.6
6	<i>n</i> -Undec-1-ene	55	6.8
7	<i>n</i> -Dodec-1-ene	55	6.8
8	<i>n</i> -Tridec-1-ene	55	7.1
9	<i>n</i> -Tetradec-1-ene	55	8.6
10	<i>n</i> -Pentadec-1-ene	55	5.9
11	<i>n</i> -Dec-1-ane	57	9.1
12	<i>n</i> -Undec-1-ane	57	4.7
13	<i>n</i> -Dodec-1-ane	57	1.2
14	<i>n</i> -Tridec-1-ane	57	9.4
15	<i>n</i> -Tetradec-1-ane	57	7.4
16	<i>n</i> -Pentadec-1-ane	57	9.6
17	Furan-2-one	84	8.3
18	2-Hydroxy-3-methylcyclopentenone	112	9.0
19	Methylcyclopentenone	67	4.0
20	Acetylfuran	95	7.8
21	5-Methyl-2-furaldehyde	110	10.9
22	Furaldehyde	95	12.2
23	Pristene	69	6.8
24	Phytadiene-1	67	12.5
25	Phytadiene-2	67	13.6
26	<i>m/p</i> -Methylphenol	108	4.0
27	<i>o</i> -Methylphenol	107	5.6
28	Phenol	94	1.8
29	Pyridine	79	6.9
30	Pyrrrole	67	2.4
31	2-Methylpyrrrole	80	3.8
32	3-Methylpyrrrole	80	4.1
33	Benzeneacetonitrile	117	0.61
34	Benzenepropanenitrile	91	4.4
35	Indole	117	2.7
36	Methylindole	130	7.2
37	Ethylmethylpyrrrole	94	13.6
38	Trimethylpyrrrole	108	1.9
39	N-AA ^a (1)	166	7.7
40	N-AA ^a (2)	152	4.3
41	N-AA ^a (3)	166	6.5
42	N-AA ^a (4)	152	5.1

^a Defined in the text as specific markers of proteins containing two adjacent amino acids (Boon and de Leeuw, 1987).

zone) was generally reached at a depth of 35–40 m. However, as previously emphasised (Ediger et al., 1999) the euphotic zone was relatively thick (55–77 m) in the less productive NE Mediterranean (Table 1). Summer salinity profiles in Fig. 2 show the Black Sea to possess a permanent halocline at 45–70 m, which limits both particulate and dissolved nutrient transport to/from the

sulphide-bearing sub-halocline waters. In the eastern Mediterranean, the surface waters are always more saline, which significantly weakens the water column stability. Accordingly, in the Rhodes cyclone, less saline deep waters with their associated chemical properties, can rise up to the surface in winter, and then, capped by the saltier surface waters, form a new nutricline at the base of the euphotic zone till the next winter mixing. The May 1996 (Fig. 2) and March 1994 (Ediger et al., 1999) salinity profiles are examples for such a feature.

Based on the dissolved oxygen content (DO) of the water column, the Black Sea upper layer can be divided into four different zones, a well oxygenated layer at the surface, the oxycline, and suboxic and anoxic zones, whose boundaries change regionally (Fig. 2). In the NE Mediterranean, an oxygenated deep layer is separated from the well-oxygenated surface waters in the euphotic zone by a less pronounced oxycline (Fig. 2). Because of the imbalance between the supply and removal rates of nutrients, euphotic zone waters of both seas contained only low concentrations of dissolved nutrients during the sampling periods (Table 1 and Fig. 2). Nevertheless, towards the coastal margins of both seas, the nitrate content of the euphotic zone apparently increases due to nitrate-enriched riverine discharges, resulting in higher NO_3/PO_4 ratios (Table 1).

In the Black Sea, nutrient profiles display distinctly different features in the oxic/anoxic transition zone (Fig. 2). As previously discussed elsewhere (Codispoti et al., 1991; Tuğrul et al., 1992), nitrate concentrations reached their peak values at the base of the oxycline and then decreased steadily in the suboxic zone to undetectable levels in the anoxic waters. Phosphate profiles exhibited a broad peak within the suboxic zone, terminated by a minimum (which is more pronounced in the cyclone) and then increased to peak values within the suboxic/anoxic interface (Fig. 2), as a result of complicated redox-dependent processes (Codispoti et al., 1991). NO_3/PO_4 ratios were as low as 6–8 in the suboxic waters, indicating selective loss of nitrate from the redox gradient via bio-mediated (denitrification) reactions.

Layer-averaged chl-*a* concentrations in the euphotic zone were 2–6 times higher in the southern Black Sea than in the NE Mediterranean (Table 1). Moreover, chl-*a* profiles exhibited maxima of different intensities at the base of the euphotic zone, where algal cells synthesise less organic carbon per chl-*a* in the light-limited but relatively nutrient-rich waters. In addition to algal production in the surface waters, the synthesis of POM by phototrophic bacteria at very low light intensities (<0.1%) within the redox gradient zone of the Black Sea is suggested to be an important source for the SPOM pool for the upper layer (Repeta and Simpson, 1991).

Euphotic zone averaged concentrations of SPOM (Table 1) at the studied sites of the Black and the Mediterranean

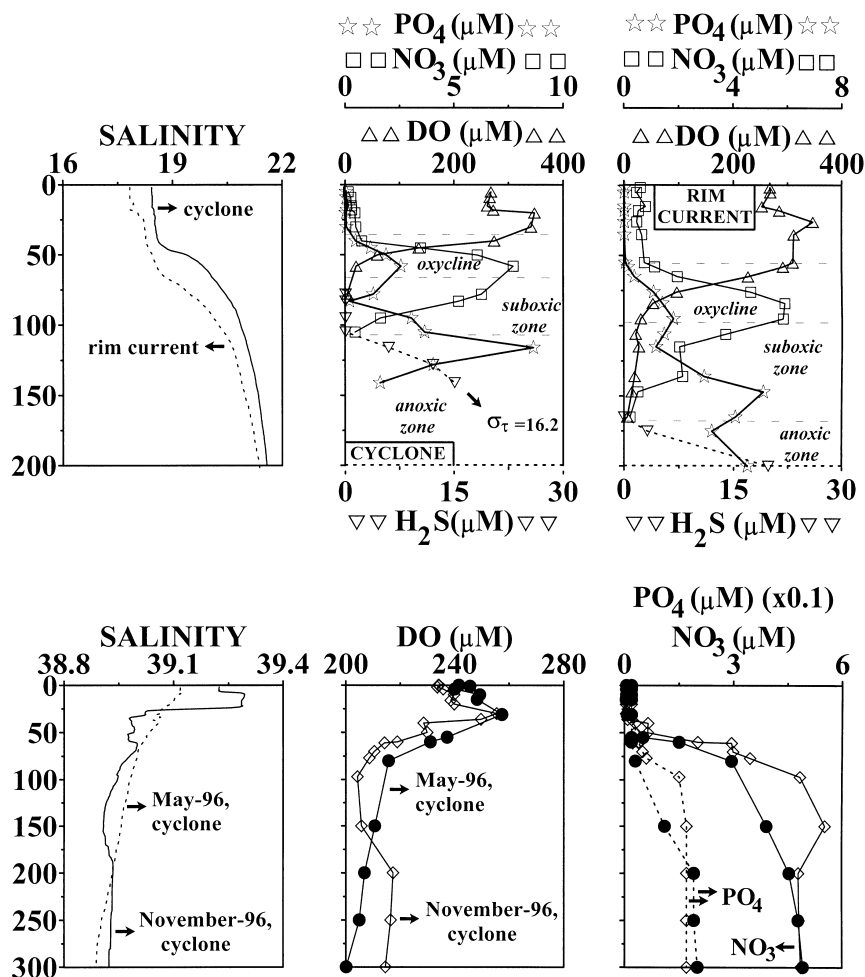


Fig. 2. Variations in the hydrochemical properties of the Black Sea (top) and the Mediterranean Sea (bottom). Labels are as indicated in the figures.

Seas were comparable with previous measurements (Krivenko et al., 1998; Fry et al., 1991; Lyutsarev and Shanin, 1996; Yilmaz et al., 1998; Ediger et al., 1999).

In May 1996, the Rhodes cyclone, POC, PON (but not PP or chl-a) concentrations and also the rate of primary production were relatively high and comparable with Black Sea summer values (Table 2 and Fig. 2). The less saline surface waters of the cyclone as compared to the summer–autumn values (Fig. 2 and Ediger et al., 1999), indicate the recent formation of a nutricline. Due to subsequent utilisation of nutrients, nitrate and phosphate concentrations were low (Fig. 2 and Table 1). Consequently, SPOM concentrations and the rate of primary production were high and comparable with those of the Black Sea (Table 1). On the other hand, in oxic environments, atmospheric input is probably the major source of biologically available (reduced) iron, necessary to maintain primary production (Measures and

Vink, 1999). Besides regeneration, the bio-available iron requirement for such high production rates could have been compensated by observed periodic (roughly once a week) Saharan dust deposition in May 1996 (Kubilay, personal communication). High production rates were also observed in March 1992, probably due to coinciding events of chimney formation (hence, the availability of excess nutrients) (Ediger and Yilmaz, 1996) and dust deposition (Kubilay et al., 2000). The calculated atmospheric bioavailable iron input during March-1992 balanced upwelled nutrients (Özsoy, 1999). In May-1996, however, very low chl-a concentrations (living biomass) in near-surface waters have led to abnormally high POC:chl-a ratios (Fig. 3). Such high ratios could have resulted from either the fast grazing of algae produced in the upper 50 m and/or the production of very specific plankton species with high living C:chl-a and POC:PP ratios. In the light-limited depths of the

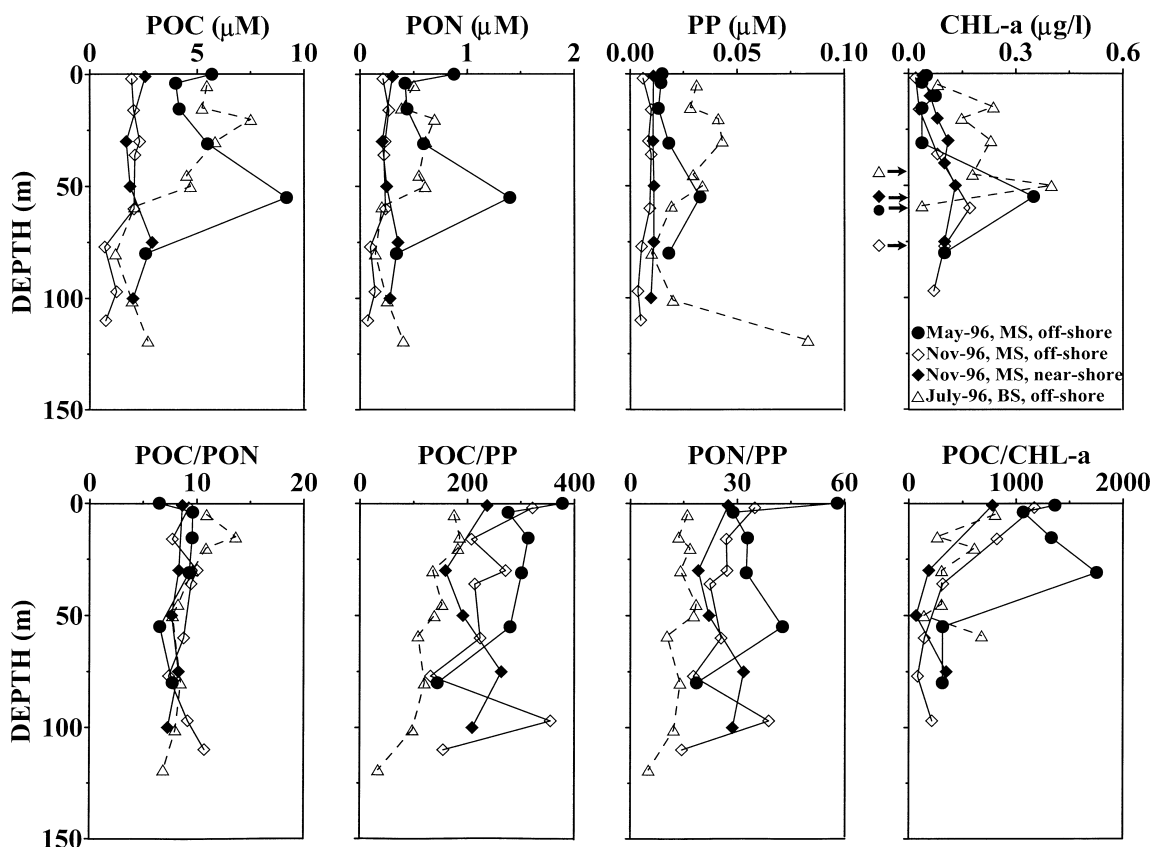


Fig. 3. Vertical profiles of suspended particulate organic matter (POM), their molar ratios, chl-a and POC/chl-a ratios for coastal and cyclonic stations in May and November 1996, Mediterranean Sea, and for cyclonic station in June 1996, Black Sea (dashed line).

euphotic zone, however, both the chl-a and SPOM displayed coherent maxima, markedly reducing the POC:chl-a ratio to 300, which was still higher than in November 1996 (Fig. 2).

In November 1996, SPOM concentrations were relatively low and almost uniformly distributed in the euphotic zones of both open and coastal stations (Fig. 3). Interestingly, even though solar light penetrated through the nutricline (Fig. 2), SPOM enrichment could not be observed within this gradient. Furthermore particulate concentrations decreased slightly (Fig. 3). Salinity and nutrient profiles in Fig. 2 indicate that, in November 1996, plankton-free but nutrient-rich deep waters lately ascended to 50 m. On the other hand, the rate and duration of algal production were not sufficient for the formation of SPOM and chl-a maxima in the light-limited waters, where the lateral and vertical movements of water masses were expected to dilute the algal biomass.

In the cyclonic region of the Black Sea, chl-a maximum was located at 50 m (less than 1% light depth), corresponding to the upper oxycline, coinciding with the permanent halocline (Figs. 2 and 3). Chl-a concentrations

then declined steeply. At the chl-maximum depth, there was no significant change in SPOM concentrations (Fig. 3) or in phytoplankton cell numbers (Uysal et al., 1998).

In the Black Sea, coastal surface waters were relatively rich in SPOM due to nutrient input via rivers (Table 1). In the open cyclonic region, particulate concentrations maximised at 20 m, then decreased continuously till to the lowest values in the suboxic zone and increased again in the suboxic/anoxic interface. The increase, which was much more pronounced for PP (Fig. 3) has previously been attributed to Fe and Mn redox chemistry and the lens of phototrophic sulphur bacteria (Wakeham and Beier, 1991).

The particulate ratios depicted in Table 1 and Fig. 3 show the upper layer SPOM composition of the Black Sea differed from that of the NE Mediterranean during the sampling periods. Interestingly, POC:PON ratios were higher in the near-surface waters of the Black Sea cyclone (Fig. 3). This may have been due to nitrogen-limited production (Yayla et al., in press) and the subsequent decomposition of SPOM. On the other hand, C:N ratios of Mediterranean SPOM, generally produced under phosphorus-limited conditions (Krom et

al., 1991; Zohary and Robarts, 1996), varied slightly in the first 100 m (Fig. 3). It appears that these ratios were very similar to the ratios of Black Sea SPOM sampled below 25 m. In the Black Sea, POC:PP and PON:PP ratios were lower and less variable in the euphotic zone. In the suboxic waters, where the chemosynthetic bacteria biomass was remarkable (Bird and Karl, 1991; Karl and Knauer, 1991) and phosphate-associated suspended particles were observed as fine structures at the anoxic interface of rim-current regions (Kempe et al., 1991), POC:PP and PON:PP ratios decreased significantly (Fig. 3). In May 1996, the SPOM from the NE Mediterranean had higher POC:PP and PON:PP but reasonable POC:PON ratios.

In May 1996, although both the chl-a and SPOM profiles displayed coherent maxima below 50 m (Fig. 3), no similar increases were recorded in the algal biomass data estimated from cell counts (Salihoğlu et al., 1997). As shown in Fig. 3, the calculated POC:chl-a ratio at chl-max layer was much above the conventional C:chl-a ratios of 40–50 (Banse, 1977) for living cells. This suggests that the relatively low rate of algal production in the light-limited zone was sufficient merely to sustain heterotrophic activities and the consequent accumulation of suspended organic particles (of both planktonic and bacterial origin) within the chl-a maximum zone. In November 1996, the lower value of SPOM was consistent with the lower production rates measured in the Mediterranean surface waters, and hence, the euphotic zone was thicker (Table 1). Interestingly, although the nutricline was within the euphotic zone (Fig. 2) and the availability of nutrients would be expected to enhance production below 50 m, particulate concentrations were almost uniform throughout the water column (Fig. 3). Salinity profiles in Fig. 2 indicate recent upwelling of phytoplankton-free deep water with lower salinity into the euphotic zone, indicating that the lack of a subsurface particulate maximum was primarily due to physical processes. The POC:PP and PON:PP ratios were high but displayed weak variations from near-shore to open cyclonic waters even though the NO_3/PO_4 ratio of the surface waters changed markedly (Table 1).

3.2. POM pyrograms

Pyrograms of the Mediterranean SPOM were similar to those obtained from Black Sea suspended particles, whose possible sources have been discussed elsewhere in detail (Çoban-Yıldız et al., 2000a). Here we assume that aliphatic hydrocarbons (*n*-alk-1-enes and *n*-alkanes) are associated with lipids, nitrogen-containing compounds and phenols to be mainly derived from proteins even though other sources are possible (chlorophyll, polyphenols), furans from carbohydrates and phytadienes from chlorophyll. No lignin phenols (i.e. 2-methoxy and 2,6-dimethoxyphenols) have been detected at significant

levels in the pyrograms; which is consistent with the findings of Hedges (1991).

Considering that all pyrograms are qualitatively similar, differences among samples have been discussed in terms of relative concentrations of selected markers. For this goal, 42 pyrolysis products (selected as markers; Table 2), were grouped into the following compound classes: Aromatic hydrocarbons (1–4), *n*-alk-1-enes (5–10), *n*-alkanes (11–16), cyclopentenones (17–19), furans (20–22), pristene (23), phytadienes (24, 25), phenols (26–28), nitrogen-containing compounds N-CC (29–36), N-CHL (37, 38), N-AA (39–42). Calculated relative concentrations of these compound classes were shown in Table 3.

Among nitrogen containing organic compounds, C3-pyrroles, namely ethylmethylpyrrole and trimethylpyrrole, are not derived from proteins but most probably from the tetrapyrrole ring of chlorophylls (Sinninghe Damsté et al., 1992), and hence are briefly termed N-CHL (Table 2). Alkylated dihydropyrrolediones and pyrrolediones (39–42 in Table 2) which are readily recognized by their ions at m/z 152, 166 and 180 in the mass spectra, are specific markers of proteins being derived from two adjacent aliphatic amino acids in peptides (Boon and de Leeuw, 1987). Since the distribution of these four compounds was similar for all samples, we have selected these as markers of the class, termed N-AA (Table 3).

The most abundant group of compounds was that of aromatic hydrocarbons (Table 3), with toluene and styrene giving the most intense peaks. The ratio of styrene to toluene was quite constant at each site, but changed spatially and seasonally. The depth-averaged ratio of styrene to toluene in November 1996 increased apparently from 0.22 ± 0.03 for coastal SPOM, to 0.32 ± 0.04 in the Rhodes cyclone. In May 1996, at Rhodes, the ratio was 0.16 ± 0.03 . Similar values were observed in the Black Sea (0.2 ± 0.06 , $n = 14$) cyclonic region. The styrene to toluene ratio can be used to assess pollution caused by plastic materials, as high values of the ratio may be indicative of the presence of styrenics (polystyrene and copolymers) (Fabbri et al., 1998).

As previously mentioned for the Black Sea SPOM, (Çoban-Yıldız et al., 2000a) the distribution of aliphatic hydrocarbons maximised in the C10–C15 range and the relative concentration of *n*-alk-1-enes was always higher than that of *n*-alkanes. The *n*-alk-1-enes to *n*-alkanes ratio ranged from 3.2 ± 0.6 for coastal November 1996 samples, to 4.6 ± 0.6 for November 1996 and 4.4 ± 1.5 for May 1996 SPOM collected from the cyclone (Fig. 5).

Relative concentrations of both cyclopentenones and furans decreased with depth at each site (Table 3). The abundances of these two compound classes were strongly correlated ($r = 0.89$, $n = 16$ for the Mediterranean, $r = 0.94$, $n = 14$ for the Black Sea), indicating that they have a common carbohydrate source. The most abundant pyrolysis product of the series is furaldehyde,

Table 3

Estimated relative concentrations of compound classes in the water column of the Black Sea and the Mediterranean Sea

Z (depth)	1 Arom.	2 Alken.	3 Alkan.	4 C.pent.	5 Furan	6 Priste.	7 Phyta.	8 Phenol	9 N-CHL	10 N-CC	11 N-AA
<i>Black Sea, July 1996, RIM current</i>											
5	36.2	4.0	1.10	5.8	13.3	0.14	1.6	11.3	0.7	24.9	1.00
15	36.5	4.3	0.83	5.3	15.3	0.31	1.5	12.2	0.69	22.3	0.69
26	39.7	3.2	0.67	5.5	13.9	0.26	2.4	10.7	0.66	22.3	0.67
35	40.2	5.8	0.87	2.6	8.7	0.22	2.2	11.1	1.00	26.5	0.79
<i>Black Sea, July 1996, cyclone</i>											
15	39.2	6.8	1.3	5.3	17.4	0.16	0.57	9.2	0.50	19.1	0.46
20	32.7	4.9	1.0	6.1	20.5	0.17	1.2	9.8	0.60	22.4	0.59
30	37.1	3.7	0.87	6.6	19.6	0.19	1.2	9.1	0.63	20.5	0.52
40	40.5	4.2	0.79	4.6	10.8	0.12	1.7	11.0	0.74	24.6	0.75
45	41.7	5.0	0.63	3.2	6.5	0.13	3.8	10.3	0.74	27.0	1.0
50	39.6	6.9	0.95	3.9	8.9	0.18	3.7	9.0	0.66	25.6	0.60
59	47.5	11.9	1.4	3.2	7.3	0.17	0.58	6.8	0.45	20.2	0.39
86	44.6	10.0	1.3	1.6	3.5	0.13	0.28	11.6	0.83	25.6	0.33
101	48.1	9.5	2.0	1.0	3.7	0.09	0.42	9.9	0.62	24.2	0.42
119	45.3	5.6	1.5	0.43	5.1	0.1	0.12	9.6	0.41	31.6	0.29
<i>Mediterranean Sea, November 1996, coast of Dalaman</i>											
1	38.7	7.1	2.3	3.3	9.2	0.57	0.7	12.3	0.77	24.0	1.1
30	39.1	6.7	1.8	2.8	7.2	0.15	1.5	13.9	1.0	25.1	0.94
50	38.9	7.1	1.8	2.5	6.5	0.11	1.6	15.2	0.85	24.5	1.0
75	42.7	7.3	2.8	1.9	4.5	0.09	0.8	15.6	0.88	22.7	0.8
100	39.5	6.8	2.5	2.1	4.8	0.12	0.81	17.3	0.88	24.4	0.78
<i>Mediterranean Sea, November 1996, Rhodes cyclone</i>											
2	45.9	8.8	2.1	3.1	8.2	0.16	1.0	10.6	0.71	18.3	1.2
16	40.3	8.9	1.9	3.3	9.0	0.21	1.2	13.0	0.7	20.9	0.79
60	40.4	8.4	1.9	2.1	3.6	0.15	4.2	14.1	0.69	23.7	0.94
77	43.6	11.2	2.0	1.7	4.5	0.22	3.2	11.7	0.63	20.5	0.75
110	43.9	9.5	2.3	2.0	4.0	0.27	1.6	15.2	0.64	19.9	0.75
<i>Mediterranean Sea, May 1996, Rhodes cyclone</i>											
0	44.5	4.5	0.8	3.1	10.4	0.08	0.3	12.7	0.92	22.0	0.78
4	46.9	4.2	1.1	2.9	11.2	0.09	0.39	12.7	0.95	18.9	0.62
15	46.0	5.9	1.1	2.7	11.2	0.11	0.29	10.7	0.92	20.2	0.78
31	40.8	4.1	1.0	3.3	11.5	0.12	0.58	12.5	0.88	24.3	0.85
55	42.8	2.7	1.4	1.6	4.0	0.09	1.6	17.7	1.4	26.0	0.73
80	51.0	4.7	0.78	1.4	3.8	0.15	1.5	11.3	0.79	23.8	0.82

which can be considered a representative of carbohydrate (CBH) markers.

Vertical profiles of the total relative concentrations of CHL markers are shown in Fig. 4. The distribution of phytadienes was similar to that of chl-a determined by fluorescence, confirming the conclusion of Çoban-Yıldız et al. (2000) that phytadienes are good markers for the relative abundance of chlorophyll in seston. On the other hand, chlorophyll-derived pyrroles (N-CHL) were not well-correlated with phytadienes. In fact, as shown in Table 3, in coastal and cyclonic waters of the Mediterranean visited in November, the relative concentration of N-CHL was almost constant (0.86 ± 0.07 and 0.67 ± 0.4 , respectively). Exceptionally, POM sampled from the cyclonic region in May 1996, contained the highest levels of N-CHL at 55 m (1.4), consistent with

the highest chl-a concentration and lowest POC:chl-a ratio. The distribution of pristene, which has been suggested to be another pyrolysis product of chlorophyll (Ishiwatari et al., 1991, 1993), was not similar to that of chl-a, confirming previous results (Çoban-Yıldız et al., 2000a). In conclusion, phytadienes are the pyrolysis products, which best represent the vertical profile of total chlorophyll in the euphotic zone. Thus, they were considered as good CHL markers, even though their relative concentrations may be low and the reproducibility of phytadiene measurements was not as good as that of other markers (Table 2).

Phenols were abundant pyrolysis products and, given the absence of lignin phenols their origin is autochthonous (protein and/or non-proteinaceous algal phenols, as suggested by van Heemst et al., 1999). Their

distribution with depth was quite uniform (14.8 ± 1.9 , 12.9 ± 1.8 , 12.9 ± 2.4 in the near- and off-shore waters in November and the off-shore waters in May 1996, respectively), as is seen in Table 3.

Nitrogen-containing compounds (N-CC) are generally associated with proteins (or aminoacids), proteins being the principal source of organic nitrogen in seawater. However, precursors other than proteins (such as melanoidin-type precursors) have been suggested to produce N-CCs upon pyrolysis (Peulvé et al., 1996). N-AA were found to be specific markers for proteins, at least for their aliphatic amino acidic components. As seen in Table 3, in the euphotic zone of the Mediterranean Sea, relative concentrations of N-AA were almost constant with site and depth (Table 3; total mean value $0.85 \pm 16\%$). The relative concentrations of N-CC were also quite uniform (total mean value 22 ± 2.2). Among the different pyrolysis products included in this class, indole displayed the greatest variation in its relative concentration (total mean value 3.9 ± 0.9), while pyrrole exhibited less variable values (3.7 ± 0.4). Methylpyrroles and pyridine exhibited relative standard deviations close to 15%. The pyrrole/indole ratio was variable with depth (Fig. 5), mainly due to variation in indole abundance (Table 3). In the Rhodes cyclonic region in May, the vertical distribution of the pyrrole/indole ratio displayed a minimum at 55 m (Fig. 5), where the relative concentration of protein markers (mainly indole) reached its peak value, as POC:PON ratio decreased slightly (Fig. 3). Actually, the pyrrole/indole ratio decreased at the chl-max depths at other stations as well, but only slightly. In the cyclonic region of the Black Sea, the pyrrole/indole ratio increased within the sub-oxic zone (Fig. 5), as reported previously (Çoban-Yıldız et al.,

2000a). The pyrrole/indole ratios of the Mediterranean Sea SPOM (total mean value 1.0 ± 0.3) were much lower than those of the open Black Sea, as seen in Fig. 5.

3.3. Comparison of relative abundances of markers

Although the same markers were observed in the SPOM of the Black and the Mediterranean Seas, their relative abundances were different. On the other hand, vertical distributions of the relative concentrations of markers generally displayed similar features in the euphotic zone, which is much thicker in the Mediterranean Sea (Fig. 4). This similarity is principally the result of similar abiotic and biotic factors controlling the occurrence and removal of SPOM in the oxygenated upper waters. However, the present method is not capable of distinguishing biological markers of bacterial origin, especially in the suboxic waters of the Black Sea, where bacteria are abundant and the POC:PON ratios (Fig. 3) are slightly lower than in the euphotic zone (Çoban-Yıldız et al., 2000a).

In both the Black and the Mediterranean Seas, relative abundances of CBH markers were higher and lipid markers were lower in surface waters. CBH markers decreased markedly near the base of the euphotic zone and the relative abundances of both protein and CHL markers increased (Fig. 4). Such molecular distributions confirm our previous discussion on the increased enzyme activity in plankton, producing more chlorophyll per cell in order to harvest light more efficiently in the light-limited zone. In the suboxic-anoxic transition zone of the Black Sea, the total relative abundance of protein markers increased, probably due to POM synthesis by anaerobic bacteria in this layer (Çoban-Yıldız et al., 2000a).

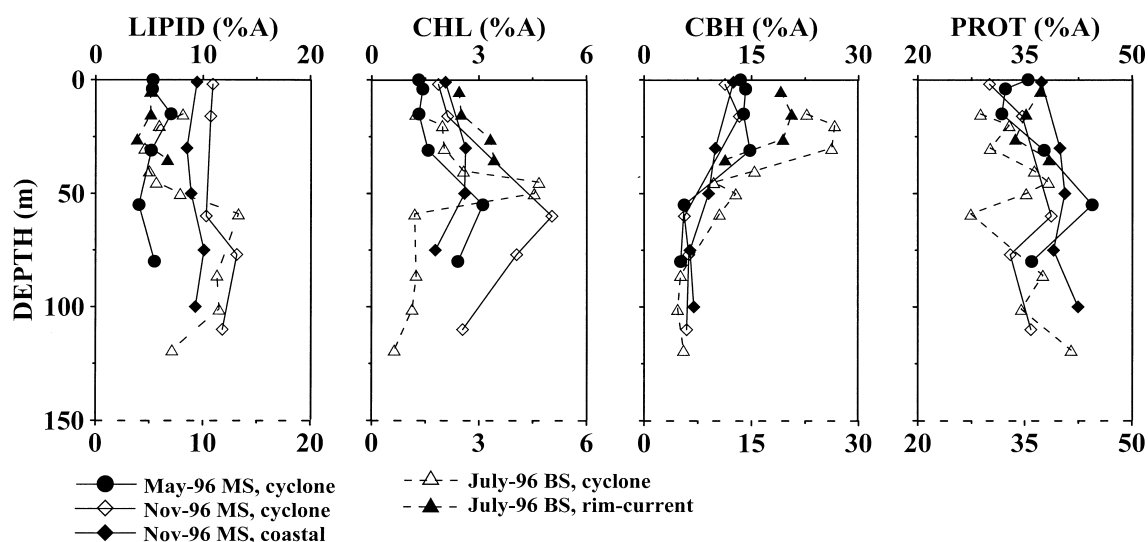


Fig. 4. Vertical profiles of pyrolysis markers for lipid, chlorophyll (CHL), carbohydrate (CBH), protein (PROT) as relative concentrations in Mediterranean Sea (MS; upper, solid scale) and Black Sea (BS; lower, dashed scale).

The total relative concentrations of lipid markers were almost uniform throughout the euphotic zone of the Mediterranean Sea (Fig. 4). Below 1% light depth, these concentrations increased only slightly whereas this change was much more pronounced in the open Black Sea. The sharp increase of lipid and decrease of CBH markers below the euphotic zone (in the oxycline and in the suboxic zone) of the Black Sea indicates enhanced bacterial decomposition of organic material, as suggested by Sicre et al. (1994). The much less pronounced increase of lipids in the Mediterranean could be the result of the relatively weak stratification, which permits larger particles to sink faster through the water column and thus limits the bacterial activity in the light-limited zone. Therefore, sinking POM is expected to be less degraded in the upper layer of the Mediterranean Sea. Coccoliths and many diatom species (Kjørboe et al., 1990; Riebesell, 1991) tend to aggregate especially when there is nutrient depletion (Kjørboe et al., 1990). Therefore, the dominance of *Emiliana huxleyi* in May and diatoms in November 1996 (Salihoğlu et al., 1997) might have also caused faster sinking of particulates in nutrient-depleted Mediterranean waters. On the other hand, in the Black Sea in July 1996, dinoflagellates (Uysal et al., 1998), with much lower aggregation potential (Riebesell, 1991) were dominant.

In the Black Sea, the apparent increase in the relative abundance of alkenes (Fig. 4) in the oxycline (between 40 and 70 m in Fig. 3), which coincides with the nutricline, may be an indicator of early diagenesis of autochthonously produced POM due to selective degradation of aminoacids and carbohydrates (Ishiwatari et al., 1995). Most probably, bacteria, responsible for decomposition and chemo-autotrophic production of POM, contribute to such a pronounced increase in

the relative abundance of lipids in the steep oxycline of the Black Sea. Unfortunately we failed to determine bacterial markers to confirm this suggestion, probably due to low sample size (Çoban-Yıldız et al., 2000a).

In May 1996, in correspondence of a peak in POC, PON and PP at 55 m of the Mediterranean cyclonic water, pyrolysis data show a strong decrease in the relative concentrations of *n*-alk-1-enes and furans, and an increase in the relative concentration of phenols, N-CC, N-CHL and phytadienes, all addressing to increased enzyme activities of phytoplankton. Observed decrease in pyrrole/indole, furaldehyde/indole ratios (Fig. 5) and increase in phenol relative abundance (Table 3) might indicate dominance of amino acids, tryptophan and tyrosine (Chiavari and Galletti, 1992; Klap et al., 1996) in this layer.

The correlation between the results of Py-GC/MS analyses with the elemental C, N and fluorometric chl-*a* measurements in suspended matter was estimated by linear regression. In the Rhodes cyclone of the Mediterranean Sea in May 1996, 87% of the increase in the POC:PON elemental ratio was accounted for by an increase in the ratio of CBH:PROT relative areas. In November 1996, in the cyclone, POM data showed no close relationship between these ratios, whereas 80% of the increase in POC:PON was accompanied with the increase in CBH:PROT ratio for coastal station. On the other hand, more than 95% of the increase in POC:chl-*a* (w/w) was found to be related with the increase in the ratio of CBH:CHL pyrolysis markers for all stations. Therefore, the lack of correlation between POC:PON vs CBH:PROT for the Rhodes cyclone in November 1996 can be attributed mainly to dominance of nitrogen-enriched POM produced by bacteria or microzooplankton, as confirmed by the higher lipid relative abundance (Fig. 4).

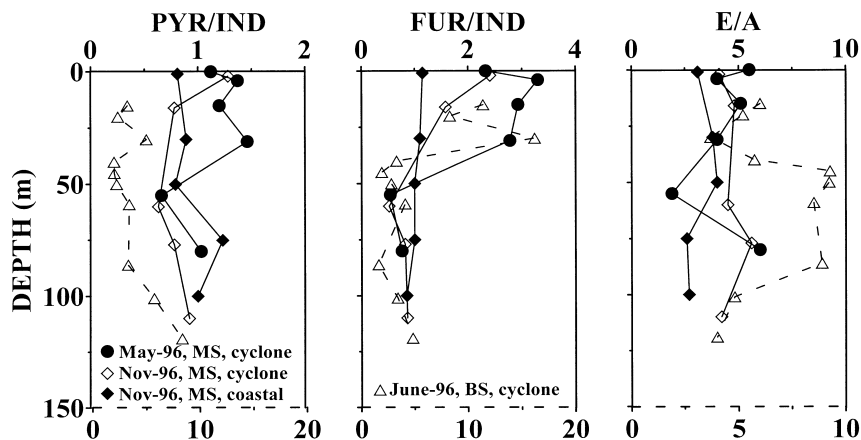


Fig. 5. Vertical profiles of pyrrole/indole (PYR/IND), furaldehyde/indole (FUR/IND) and alkene/alkane (E/A) ratios in the Mediterranean Sea (upper, solid scale) and in the Black Sea (lower, dashed scale).

4. Conclusions

The chemical composition of SPOM in the eastern Mediterranean and the Black Sea has been determined both by analysis of elemental C:N:P ratios and by Py-GC/MS. Such data, being complemented by physical, biological and hydrochemical observations, permit one to compare the very different marine environments of the oligotrophic Mediterranean and the more productive Black Sea.

In 1996, relatively lower and constant C:N ratios were determined in SPOM from the thicker euphotic zone of the Mediterranean Sea. Therefore, in the summer, the more productive surface waters of the Black Sea possessed relatively carbohydrate-enriched SPOM. However, below the 1% light depth, both the abundance and the C:N ratios of SPOM were similar in both seas, irrespective of the thickness of their euphotic zones and their algal production rates. Analyses of SPOM by Py-GC/MS techniques yielded similar pyrolysis products in both seas. However, the total relative abundance of lipid markers was greater and more uniform in the euphotic zone of the Mediterranean Sea. In the surface layers of the Mediterranean Sea, the greater abundance of protein markers was consistent with the possession of lower POC:PON ratios. The total relative concentrations of CHL markers increased consistently within the chlorophyll maximum zones of both seas, where the CBH markers became less abundant and the POC/chl-a ratio dropped to minimal levels. In the stratified Black Sea, SPOM appears to be produced and decomposed by in-situ processes in each layer, while weaker stratification in the Mediterranean Sea limits bacterial activity. Consequently, although different protein compositions were observed in oxic and suboxic/anoxic zones of the Black Sea, protein compositions were less variable throughout the water column of the Mediterranean Sea. Although no clear bacterial markers could be observed from the pyrograms, we suggest a significant fraction of the SPOM, especially in the aphotic layers of the Black Sea to be of bacterial origin. Absence of lignin markers of terrestrial origin in both seas indicates that larger, rapidly sinking particles do not significantly contribute to the chemical composition of SPOM.

Changes in the ratios of CBH:PROT and CBH:CHL markers were generally closely correlated with changes in POC:PON and POC:chl-a ratios. Therefore, the relative abundances of markers produced by Py-GC/MS can be used consistently and successfully to determine the macromolecular chemical composition of SPOM.

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