

# Analytical pyrolysis of suspended particulate organic matter from the Black Sea water column

Yeşim Çoban-Yıldız<sup>a,\*</sup>, Daniele Fabbri<sup>b</sup>, Valentina Baravelli<sup>b</sup>, Ivano Vassura<sup>b</sup>,  
Ayşen Yılmaz<sup>c</sup>, Süleyman Tuğrul<sup>c</sup>, Elif Eker-Develi<sup>c</sup>

<sup>a</sup>BOTAS International Limited, Haydar Aliyev Marine Terminal, Golovasi Mevkii, 01944 Ceyhan, Adana, Turkey

<sup>b</sup>Laboratory of Chemistry, C.I.R.S.A., University of Bologna, Via S. Alberto 163, 48100 Ravenna, Italy

<sup>c</sup>METU-Institute of Marine Sciences, Erdemli, Turkey

Received 1 September 2003; accepted 13 March 2006

Available online 7 September 2006

## Abstract

The chemical composition of suspended particulate organic matter (SPOM) in the Black Sea water column has been characterized by pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). The results are discussed in terms of the hydro-chemical properties of the water column. Phytoplankton cultures grown in the lab also were analyzed to provide reference information for the euphotic zone SPOM. The composition of SPOM in the Black Sea displays important vertical variations dependent on specific distinct biochemical processes in the water column. Identical pyrolysis markers indicative for proteins, lipids, carbohydrates and chlorophylls were obtained from SPOM in the euphotic zone of the Black Sea and the phytoplankton cultures. Nevertheless, the relative contribution of lipids and carbohydrates to Black Sea SPOM was higher than in phytoplankton cultures. The lipid to protein ratio throughout the water column seems to determine the C/N ratio of SPOM. Below the oxycline, the relative contribution of proteins to the SPOM pool increased while the lipid content of the SPOM decreased. The protein composition changed substantially in the suboxic/anoxic transition zone, as expressed by the increase in pyrrole/indole ratio. Elemental sulfur was observed in particulates suspended at the upper anoxic zone, and the relative intensity of the S<sub>8</sub> peak varied regionally with more intense multi-peaks in SW shelf-break station, in agreement with the lateral flux of O<sub>2</sub>. Sulfur-containing organic compounds (e.g. thiophenes), including organic polysulphides (e.g. 1,2-dithiole-3-thiones) were detected in some deep anoxic samples, indicating that sulphurization of organic matter may be active in the water column.

© 2006 Elsevier Ltd. All rights reserved.

**Keywords:** Suspended particulate organic matter (SPOM); Phytoplankton; Chemical composition; Sulfur; Py-GC-MS; Black Sea

\*Corresponding author. BOTAS International Limited, Haydar Aliyev Marine Terminal, Golovasi Mevkii, 01944, Ceyhan, Adana, Turkey. Tel.: +90 322 3551724; fax: +90322 6392292.

E-mail addresses: [yesim.yildiz@botasint.com](mailto:yesim.yildiz@botasint.com), [ycobanyildiz@yahoo.com](mailto:ycobanyildiz@yahoo.com) (Y. Çoban-Yıldız), [dani.fabbri@unibo.it](mailto:dani.fabbri@unibo.it) (D. Fabbri), [ivano.vassura@unibo.it](mailto:ivano.vassura@unibo.it) (I. Vassura), [yilmaz@ims.metu.edu.tr](mailto:yilmaz@ims.metu.edu.tr) (A. Yılmaz), [tugrul@ims.metu.edu.tr](mailto:tugrul@ims.metu.edu.tr) (S. Tuğrul), [elif@ims.metu.edu.tr](mailto:elif@ims.metu.edu.tr) (E. Eker-Develi).

## 1. Introduction

Investigation of the origin and composition of particulate organic matter (SPOM) suspended in the Black Sea is of particular importance due to the unique hydro-chemical properties of this sea. Most of the SPOM in the Black Sea is suspended or

slowly sinking, and its composition should reflect the associated bio-chemical processes in each layer. Characterization of SPOM throughout the water column is therefore essential to understand these processes. Information on the composition of SPOM has generally been based on bulk parameters such as the carbon and nitrogen content, the C/N elemental ratio, and the chlorophyll concentrations (Karl and Knauer, 1991; Yılmaz et al., 1998; Çoban-Yıldız et al., 2000a; Yunev et al., 2002). Due to analytical difficulties, investigations of the molecular composition of Black Sea SPOM have been limited to studies of selected compounds (Wakeham et al., 1991; Wakeham and Beier, 1991; Wakeham, 1995; Wakeham, 1999; Wakeham et al., 2003). Despite limited existing studies (Çoban-Yıldız et al., 2000b, c), the information gap between bulk measurements and compound-specific studies on the composition of organic matter suspended in the Black Sea water column remains to be filled.

Pyrolysis—Gas Chromatography/Mass Spectrometry recently has been utilized, often supported by measurements of traditional oceanographic parameters, for chemical characterization of suspended (Saliot et al., 1984; Sicre et al., 1994; Çoban-Yıldız et al., 2000b, c) and sinking (Ishiwatary et al., 1995; Peulve et al., 1996) POM and sediments (Saliot et al., 1984; Ergin et al., 1996; Fabbri et al., 2005) from different environments. Despite the inherent difficulty of detecting the original molecular precursors due to their extensive degradation,

analytical pyrolysis has the advantage of providing useful structural information on the chemical composition of SPOM by the direct analysis of single filters, thus avoiding tedious pre-treatment procedures and the use of small samples. Each family of biomolecules (carbohydrates, proteins, lignins and so forth) produces specific compounds upon pyrolysis (e.g., Moldoveanu, 1998), which may be used as markers indicating the contribution of the family to the sample.

In this study, pyrolytic markers associated with the lipid, protein, carbohydrate and chlorophyll content of phytoplankton grown under lab conditions have been established. The recognition and monitoring of these markers from Black Sea SPOM has enabled the regional and vertical variation in the chemical composition of the SPOM to be related to the biogeochemical processes through the water column from the surface to the upper anoxic layer at a depth of 300 m.

## 2. Sampling and analytical methods

Sampling was performed systematically on board R.V. *Knorr* at each of five stations in the central Black Sea, the continental shelf and shelf-break regions of the rim current occupied during a R.V. *Knorr* cruise in May 2001 (Fig. 1).

Water from the surface to 300 m depth was collected using 10-L Niskin bottles attached to a CTD rosette sampler. All water samples were

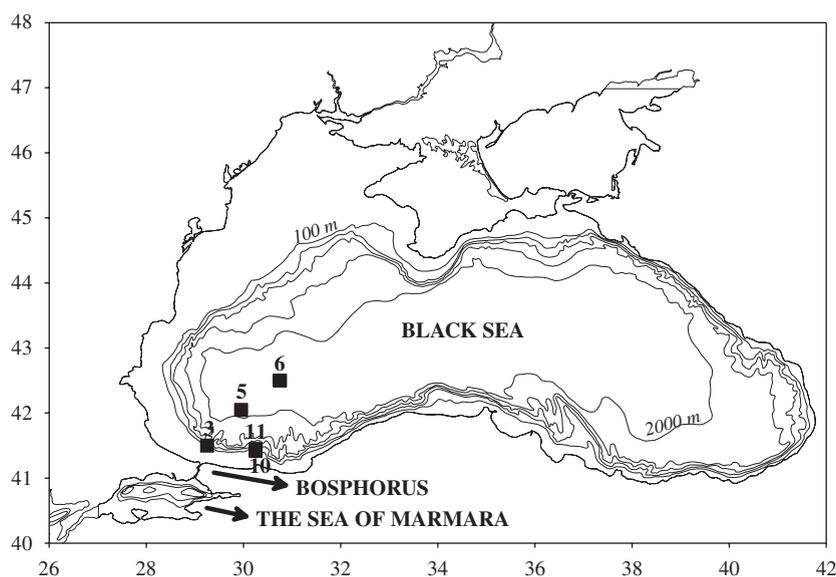


Fig. 1. Map of Stations visited (together with station numbers) during the May 2001 R.V. *Knorr* Black Sea cruise.

filtered through a 200- $\mu\text{m}$  nylon mesh to remove larger particles. Particulate samples were collected on pre-combusted GF/F filters (0.7  $\mu\text{m}$  pore size). Three to 40 L of water were filtered, depending on the location and depth of the sample, until the filters were all clogged. As the samples include particulate fraction collectable by bottle casts and the particulate matter larger than 200  $\mu\text{m}$  was removed, the samples we obtained mostly contain the suspended and slowly sinking fraction of SPOM. Reference cultures of three phytoplankton species found in the Black Sea, *Skeletonema costatum* (diatom), *Prorocentrum micans* (dinoflagellate) and *Emiliania huxleyi* (coccolithophore), were grown in F/2 medium (Guillard and Ryther, 1962; Guillard, 1975) without nutrient limitation at a constant temperature of  $20 \pm 1^\circ\text{C}$  under a regime of 12 h light ( $10\text{--}30 \mu\text{E m}^{-2} \text{s}^{-1}$ ) and 12 h dark. The cultures were harvested by filtration during the exponential growth phase. Both field and phytoplankton samples were dried and stored under vacuum until analysis.

The filters were scraped to provide samples for pyrolysis containing the maximum possible amount of SPOM and the minimum amount of glass filter. About 8 mg of the scrapings were inserted into a quartz tube holder and pyrolysed at  $700^\circ\text{C}$  for 10 s using a CDS 1000 pyroprobe platinum filament (Chemical Data System, Oxford, USA) directly connected to a Varian 3400 gas chromatograph coupled to a Varian Saturn 2000 ion trap mass spectrometer. Details of the procedures are given in Çoban-Yıldız et al. (2000b, c).

Both culture and field samples were analyzed under the same analytical conditions, and the same markers were selected for quantification. Markers generated by pyrolysis were identified by comparison of their mass spectra with those of standard compounds, literature data or by mass spectral interpretation as has been discussed previously (Saliot et al., 1984; Fabbri et al., 1996). The following 29 pyrolysis products were selected as markers for quantification from among those utilized in a previous study (Çoban-Yıldız et al., 2000b).

From carbohydrates (CBH): furaldehyde, acetyl furan, furan-2-one, 3-methyl-2-hydroxy-cyclopentene;

From proteins (PROT): pyridine, pyrrole, phenol, *m/p*-methylphenols, *o*-phenol, benzenepropanenitrile, indole;

From lipids: C10–C16 *n*-alkanes and *n*-alk-1-enes;

From chlorophyll (CHL): phytadienes (two isomers);

From aromatic hydrocarbons: toluene, styrene.

The peak areas of the markers were calculated from the mass chromatograms of ions characteristic of their mass spectra. Normalized peak areas (peak area of the marker  $\times 100$ /summed peak area of all markers) were calculated to express the relative contribution of the marker in each different sample (Fabbri et al., 1998; Çoban-Yıldız et al., 2000b, c).

Replicate pyrolyses were performed on algal samples to estimate the precision of the computed product distributions. Percentage standard deviations of normalized peak areas were consistent with previous studies (Çoban-Yıldız et al., 2000b). In this study, percentage standard deviations ( $n = 3$ ) of the relative distribution of compound classes for *P. micans* were: 8.5% for lipids, 6.3% for proteins, 12% carbohydrates, and 7.1% for chlorophyll (phytadienes).

A portion of the GF/F filter samples were selected for quantitative analysis of elemental sulfur by HPLC analysis. These samples were subjected to extraction with 5 ml dichloromethane by sonication (30 min). After centrifugation, the supernatant was concentrated by evaporating dichloromethane by using nitrogen gas and the residue was dissolved in 2 ml acetonitrile prior to HPLC analysis. Quantification of elemental sulfur was accomplished by external standardization using calibration solutions of elemental sulfur (Aldrich, 99.998%) dissolved in acetonitrile. Solutions (20  $\mu\text{l}$ ) were injected into a Perkin Elmer 250 HPLC coupled with diode array UV-Vis detector (DAD series 200). Separation was performed with a 25 cm  $\times$  4.6 mm Restek ODS column with mobile phase consisting of 100% acetonitrile eluting at  $1 \text{ ml min}^{-1}$ . Elemental sulfur ( $\text{S}_8$ ) eluted at 8.0 min, and the corresponding peak area at 264 nm was determined for quantification. From analysis of calibration solutions the following data were obtained: precision,  $< 5\%$  as relative standard deviation; detection limit (for signal to noise (S/N) ratio  $\sim 3$ ) of  $0.020 \text{ mg l}^{-1}$ ; linearity, respectively 0.998 in the interval  $0.25 \div 10.0 \text{ mg l}^{-1}$ .

### 3. Results and discussion

#### 3.1. General characteristics of the Black Sea water column, May 2001

Hydro-dynamic and biochemical characteristics of the Black Sea water column in May 2001 were described extensively in Çoban-Yıldız et al. (2006a, b) and Yilmaz et al. (2006). The depth-

profiles of oxygen, sulfide, nitrate and ammonium for the two oceanographic locations visited in the western central basin (STA 6) and the shelf-break near the Bosphorus (STA 3) are shown in Figs. 2A and 3A. The profiles for in situ fluorescence and light transmission are presented in Figs. 2B (STA 6) and 3B (STA 3) while temperature and salinity profiles are shown in 2C (STA 6) and 3C (STA 3).

At STA 6, the water column may be regarded as a sequence of layers from the surface to 1% light depth (the surface mixed layer ~20 m), from 1% to 0.1% light depth (the thermocline, ~35 m), the oxycline (OXYC), the nitracline (NC), the denitrification zone (-NC), and the anoxic interface (~113 m). One should note that the oxycline and nitracline depths of STA 6, atypically, do not correspond to each other. At STA 3, the water column can be separated into surface—1% light depth (the surface mixed layer ~20 m), 1–0.1% light depth (the thermocline ~30 m), the nitracline (NC, which corresponds to the oxycline), the suboxic zone (-NC), and the anoxic interface.

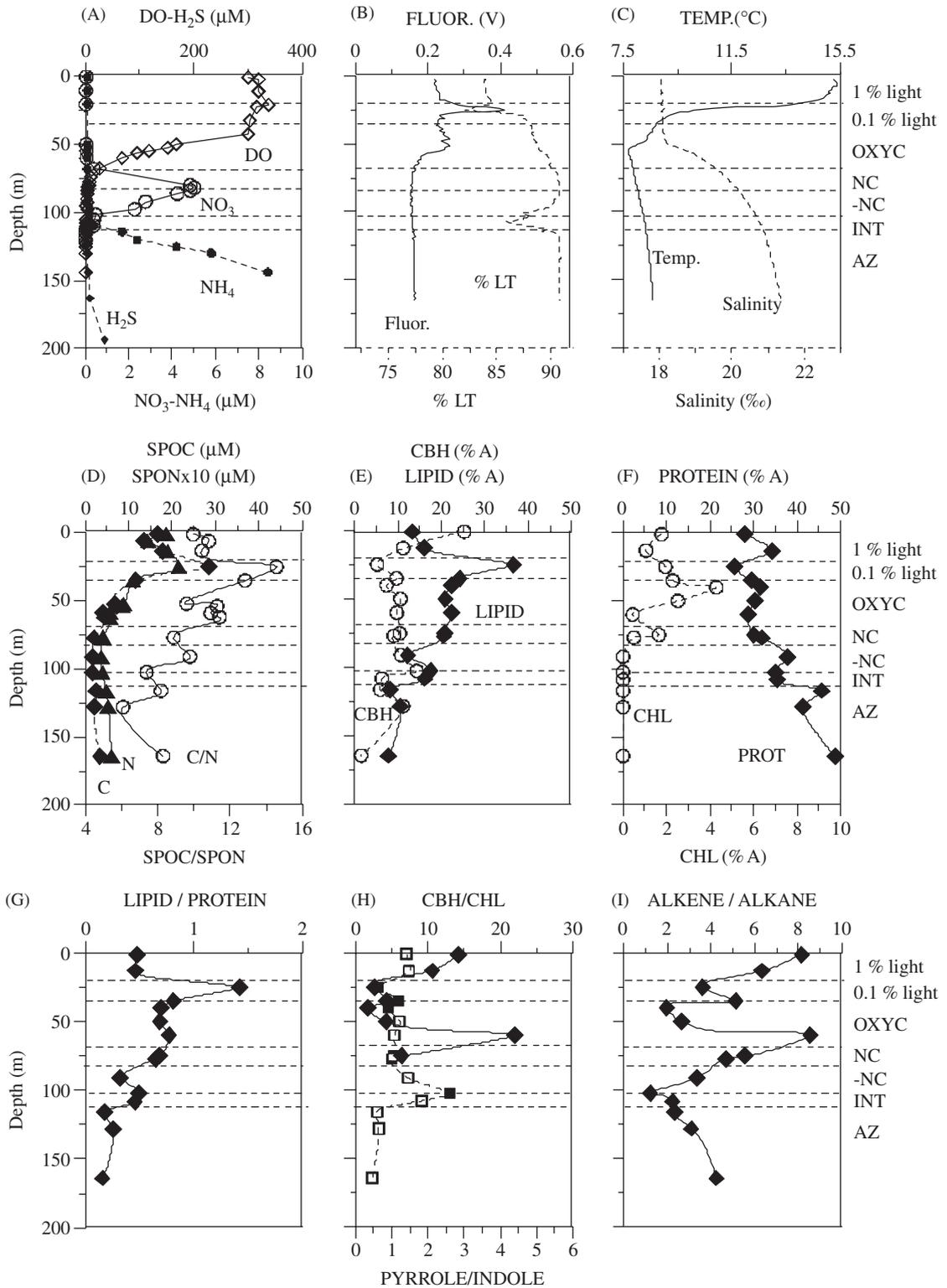
Concentrations of particulate organic carbon (POC) and nitrogen (PON) in the euphotic zone of the southwestern Black Sea in May 2001 varied regionally (Figs. 2D and 3D; Çoban-Yıldız et al., 2006a). Nevertheless, at all locations investigated, particulate concentrations were very high compared to the low chlorophyll-*a* concentrations (Fig. 2F, 3F), leading to anomalously high POC to chlorophyll-*a* ratios (Çoban-Yıldız et al., 2006a). While the C/N ratios from our phytoplankton cultures (culture mean = 8.1) were in agreement with the typical plankton-like C/N ratios (Redfield = 8.5) (i.e. Redfield et al., 1963, Hedges et al., 2002), the ratio for SPOM was higher (cruise mean = 14) (Table 1). In addition, the rates of production of both POC and PON by phytoplankton were low (Çoban-Yıldız et al., 2003; Yilmaz et al., 2006). These findings indicate that an intense spring bloom took place just before the May-2001 cruise and consumed nearly all biologically available nutrients. The dominance of dinoflagellates in the plankton population (Ediger et al., 2006) suggests the likelihood of the potential contribution of mixotrophy. The high SPOM pool in the euphotic zone of the south-western Black Sea, therefore, was composed not only of phytoplankton growing on remineralized nutrients, but also of detritus, mixotrophs and herbivorous organisms, followed by a very recent and intense phytoplankton bloom, most probably caused by a short-term pulse of nutrient-

rich surface waters from the NW shelf (Oğuz et al., 2002).

Nutrient concentrations were low at both stations from the surface to 0.1% light depth because stratification limited the vertical exchange between the euphotic zone and the nitracline, which was located below the euphotic zone (see the  $\text{NO}_3^-$  and the temperature profiles in Figs. 2A,C and 3A,C). Phytoplankton probably consumed all available nutrients as soon as they were supplied; thus, no noticeable regional variation of nutrient concentrations was observed in the euphotic zone (Çoban-Yıldız et al., 2006a). Below the 0.1% light depth nitrate concentrations were still low (Figs. 2A, 3A). The CIL was warmer than normal and depleted in nitrate compared to its concentration in previous years.

STA 6 was characterized by an unusual offset between the oxycline and the nitracline (Fig. 2A). The structure of the oxic and suboxic zone depends strongly on the ventilation of the cold intermediate layer (CIL) and the export of organic matter (Kononov and Murray, 2001). Mild winters, such as occurred in 2001, result in weak ventilation of the CIL, oxidation of SPOM exported from the euphotic zone exceeded the supply of oxygen into the oxycline and resulted in enlargement of the suboxic zone towards the surface. Nitrate concentrations, on the other hand, were still low through the oxycline, resulting in an unusual offset between the nitracline and oxycline. This is unusual because oxidation of SPOM should produce  $\text{NO}_3^-$  while  $\text{O}_2$  is being consumed. The intensity of in situ fluorescence (Fig. 2B) decreases to background values after 60 m, so assimilative uptake of nitrate in the oxycline without any net autotrophic production may be partially responsible for this shift. Unfortunately, there were no chlorophyll/phytoplankton/nitrate uptake data to support this hypothesis.

The shelf-break region off the Bosphorus Strait (STA 3) provided a good location to examine the influence of lateral transport on water column structure. The provision of modified Mediterranean water (high salinity, high temperature) carried by the Bosphorus plume to intermediate depths of the Black Sea can be traced not only by modification of the vertical profiles of DO and nitrate but also by fluctuations in temperature (Kononov et al., 2003) (Fig. 3A, C). The DO profile fluctuated below the oxycline where  $\text{H}_2\text{S}$  was still undetectable (less than  $0.1 \mu\text{M}$ ) (Fig. 3A). Therefore, the suboxic layer was



not well defined. Similar perturbations were recorded in the  $\text{NO}_3^-$  profile; loss of  $\text{NO}_3^-$  due to denitrification was partly compensated by inflow of the Bosphorus Plume as indicated by the deeper penetration of  $\text{NO}_3^-$  (Fig. 3A). The boundaries of the nitracline and oxycline coincided perfectly in the coastal region, while below the nitrate maximum, substantial concentrations of DO,  $\text{NO}_3^-$  and  $\text{NH}_4^+$  co-existed (Fig. 3A). Such overlapping of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  gradients at this shelf break station was not observed in the central region, as has been reported previously (Tebo, 1991). Mediterranean water carried along the shelf break by the Rim Current has been suggested to be the main physical process causing partial ventilation of sub-halocline waters of the Black Sea (Konovalov and Murray, 2001; Konovalov et al., 2001, 2003).

In situ light transmission profiles had two minima, one within the euphotic zone (at the base of the euphotic zone for STA 6 and at the surface for STA 3) and the second at the suboxic-anoxic transition layer (Figs. 2B and 3B). Below the shallow minimum, light transmission increased to the base of the euphotic zone. Its highest values were in the oxycline. As observed previously (Kempe et al., 1991), the magnitude of the second minima in light transmission was greater in the SW region (Fig. 3B). As expected, in-situ fluorescence showed an opposite trend to the light-transmission data at the surface while no fluorescence peak was detected at the light transmission minimum in the suboxic/anoxic interface (Figs. 2B and 3B).

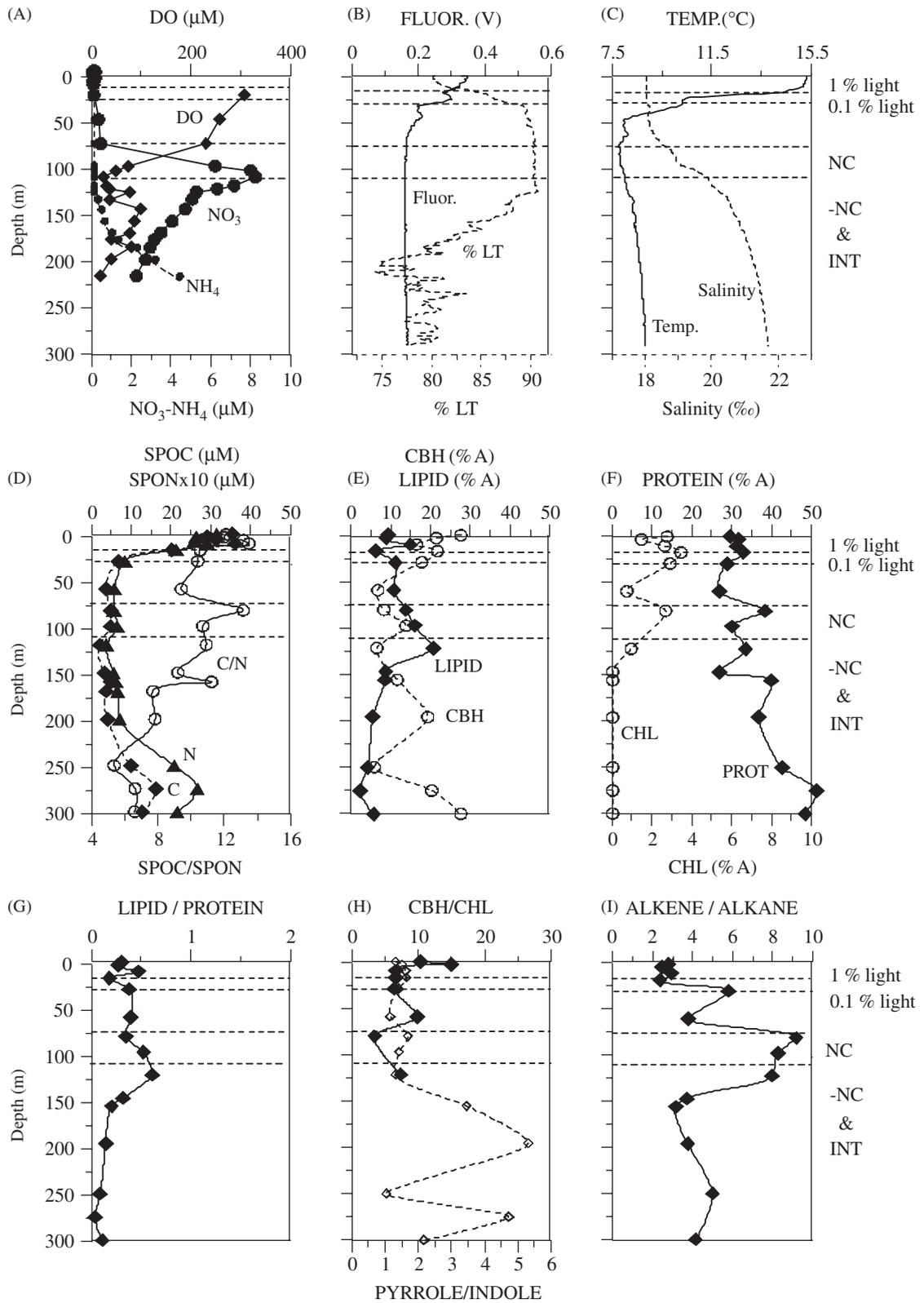
### 3.2. Elemental and chemical composition of phytoplankton cultures and Black Sea SPOM in the euphotic zone

The relative distributions of lipid, protein, carbohydrate and chlorophyll markers produced during pyrolysis of phytoplankton cultures and SPOM from the euphotic zone of the Black Sea are listed in Table 1. The chemical compositions of the three species of phytoplankton, *P. micans*, *S. costatum* and *E. huxleyi* were similar to each other (Table 1). Phytoplankton grown in good physiological condi-

tions had average protein, lipid and carbohydrate contents of 38%, 7% and 8%, respectively (Table 1). The largest inter-species variations were observed for markers of carbohydrate and chlorophyll. Recently, Hedges et al. (2002) estimated the average biochemical composition of marine plankton to be 65% protein, 19% lipid and 16% carbohydrate, on a weight basis. Their estimate was based on elemental compositions and NMR analyses coupled with model calculations assuming plankton to be composed only of protein, lipid and carbohydrate. A similar relative distribution restricted to these three groups was obtained from our pyrolysis data (72%, 13% and 15% for proteins, lipids and carbohydrates, as calculated from the mean values of Table 1). The agreement indicates that, though being semi-quantitative, pyrolysis data accurately represent natural conditions.

The mean chemical composition of the phytoplankton cultures was compared to and used as a reference for field samples. As expected, pyrolysis of phytoplankton cultures and Black Sea SPOM yielded the same markers (Fig. 4). Furthermore, the mean distribution of pyrolysis products released from SPOM collected in the euphotic layers of different sites was similar to the distribution from phytoplankton cultures (Table 1). The protein markers made the largest contribution to the bulk, followed by carbohydrates and lipids. This appears to confirm that phytoplankton make the overwhelming contribution to the chemical composition of SPOM in the euphotic zone. Nevertheless, the relative contribution of lipids and carbohydrates Black Sea particulate matter was twice that seen in the cultures, while the chlorophyll content of the SPOM was lower, yielding average protein, lipid and carbohydrate contents of 30%, 14% and 16%, respectively (Table 1). Normalizing, the bio-chemical composition to only these three groups, the protein, lipid and carbohydrate content of euphotic zone SPOM amount to 50%, 23% and 27%, respectively. As a result, the ratios of proteins to lipids were lower in the field samples while the carbohydrate/chlorophyll ratio was almost 8 times

Fig. 2. Vertical variation of: (A)  $\text{NO}_3^-$ – $\text{NH}_4^+$ , DO– $\text{H}_2\text{S}$ ; (B) fluorescence (FLUOR) and in situ light transmission (% LT); (C) temperature (TEMP) and salinity; (D) particulate organic C and N concentrations (POC, PON) and C/N molar ratio (POC/PON). Pyrolysis products grouped as: (E) carbohydrate (CBH) and lipid markers and (F) protein and chlorophyll (CHL) markers (as % area) released from SPOM. Ratios of pyrolysis products: (G) lipid/protein; (H) CBH/CHL; and (I) alkene/alkane at the central station (STA 6). Horizontal dashed lines represent the boundaries for 1% and 0.1% light depths, oxycline (OXYC), nitracline (NC), denitrification zone (-NC), suboxic/anoxic interface (INT) and anoxic zone (AZ). Note that, unusually, nitracline and oxycline correspond to different layers.



higher on average, in agreement with the higher elemental C/N ratio of Black Sea SPOM (Table 1). Phytoplankton particles have been reported to be enriched in protein and chlorophyll relative to detritus (Minor et al., 1998). The difference between the chemical ratios observed in phytoplankton and the euphotic zone SPOM, therefore, probably reflects the heterogeneous composition of the euphotic zone SPOM pool with its substantial detrital content. The total lipid content of phytoplankton has been shown to increase from 10% to 50% between the logarithmic and stationary growth phases (Brown et al., 1996), consistent with our suggestion. As lipids are known to be stored by phytoplankton under severe nutrient stress (Taguchi et al., 1987; Roessler, 1990), a high lipid to protein ratio also might be due to nitrogen limitation in the Black Sea.

Ratios of pyrrole to indole, which are specific products of amino acid pyrolysis, were used as an index of the variation of protein composition.

Pyrrole is one of the major products obtained from the pyrolysis of Hydroxyproline and Glutamine, whereas indole is a pyrolysate of Tryptophan (Chiavari and Galletti, 1992). In the euphotic zone of the Black Sea, both pyrrole and indole were present in similar proportions and the composition of the proteins in the euphotic SPOM was similar to that of the lab cultures of phytoplankton (Table 1).

Alkene/alkane chains, released from pyrolysis of particulate samples have been used as lipid markers (e.g. Ishiwatary et al., 1995; Peulve et al., 1996), and their ratio has been used to determine the freshness of organic matter (Ishiwatary et al., 1995). Pyrolysis under *hydrogen pressure* does not generate alkene/alkane doublets but only straight chain and branched alkanes, and it is suggested both alkenes and alkanes are mainly derived by pyrolysis of fatty acids and esters in phytoplankton, which would be consistent with a greater degree of branching in the fatty acid/ester chains (Çoban-Yıldız et al.,

Table 1

Distribution of pyrolysis products as means of three cultured phytoplankton species and euphotic zone averages of five Black Sea stations: lipid, protein (PROT), carbohydrate (CBH) and chlorophyll (CHL) markers, given as relative area, and ratios of pyrrole to indole and alkene to alkane areas

Sample	Lipid (%)	PROT (%)	CBH (%)	CHL (%)	Pyrr./indole	Alkene /alkane	C/N
<i>Algae</i>							
<i>E. huxleyi</i>	8.1	36	6.4	7.2	0.8	1.3	7.1
<i>P. micans</i>	7.7	37	12.5	4.3	1.2	0.4	9.3
<i>S. costatum</i>	5.0	43	4.7	2.0	0.7	2.0	8.0
Culture mean	7.0	38	7.9	4.5	0.9	1.2	8.1
CV (%)	24	9	52	59	29	67	14
<i>Station</i>							
3	10	31	22	2.6	1.5	2.7	12
5	14	29	19	2.3	1.5	5.9	14
6	15	31	18	1.4	1.5	7.3	10
10	18	31	11	0.9	1.0	6.1	13
11	11	28	10	0.6	1.1	4.4	10
Cruise mean	14	30	16	1.6	1.3	5.3	12
CV (%)	23	5	34	56	21	34	14

C to N atomic ratios were obtained by CHN elemental analyzer. All are given as mean  $\pm$  coefficient of variation CV (%). CV represents regional variation for field samples and interspecies variation for phytoplankton cultures.

Fig. 3. Vertical variation of: (A)  $\text{NO}_3^-$ - $\text{NH}_4^+$ , DO- $\text{H}_2\text{S}$ ; (B) fluorescence (FLUOR) and in situ light transmission (% LT); (C) temperature (TEMP) and salinity; (D) particulate organic C and N concentrations (POC, PON) and C/N molar ratio (POC/PON). Pyrolysis products grouped as: (E) carbohydrate (CBH) and lipid markers and (F) protein and chlorophyll (CHL) markers (as % area) released from SPOM. Ratios of pyrolysis products: (G) lipid/protein; (H) CBH/CHL; and (I) alkene/alkane at the shelf break station of Bosphorus (STA 3). Horizontal dashed lines represent the boundaries for 0.1% light depth, nitracline (NC), denitrification zone (-NC), suboxic/anoxic interface (INT) and anoxic zone (AZ). Note that  $\text{H}_2\text{S}$  was not detected till 211 m, and no data are available below this depth. The interface zone is based on POM parameters.

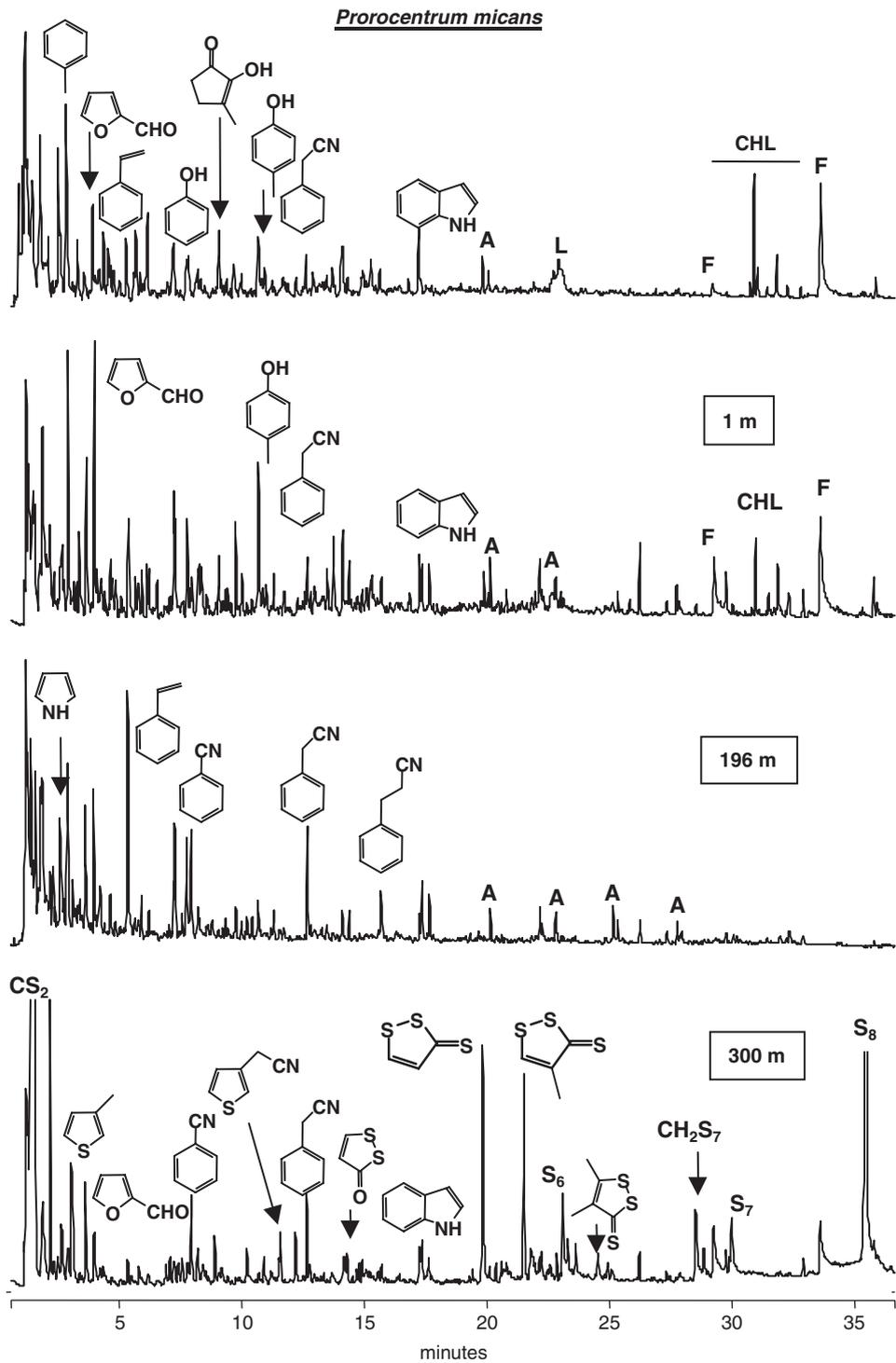


Fig. 4. MS pyrograms of filters containing a cultured algal species (*P. micans*), and suspended particulate matter collected at various depths of STA 3. A: alkene; L: levoglucosan; F: fatty acid; CHL: phytadienes.

2000a, b; Çoban-Yıldız et al., 2006a). The alkene/alkane ratios from pyrolysis of phytoplankton cultures varied from one species to another and

were lower than the ratios from Black Sea SPOM in the euphotic zone. Based on the expectation that phytoplankton samples contain pure, alive, healthy

species in exponential growth phase, the alkene/alkane ratio of pyrolysis products seems to decrease with increasing freshness of the organic matter. This suggestion is corroborated by the depth profiles of alkene/alkane ratios in Black Sea SPOM in both May-2001 (this study) and July 1996 (Çoban-Yıldız et al., 2000b). We used the alkene/alkane ratios as an index for the degree of freshness of organic matter; however, how more mature organic material might generate more alkenes under pyrolysis is uncertain.

Significant levels of lignin markers (e.g. alkylated 2-methoxyphenols) were not detected in the pyrograms. Lignin phenols have been determined in dissolved and POM collected in an estuarine environment, by thermochemolysis with tetramethylammonium hydroxide (TMAH), and decreasing concentrations were observed from riverine to coastal areas (Mannio and Harvey, 2000). Lignin is usually associated with larger particulate matter that is carried to the sea as bedload, and typically does not get transported very far off-shore (Hedges et al., 1997; Keil et al., 1998; Ergin et al., 1996). In addition, association of organic matter of terrigenous origin with mineral grains, and consequent rapid sedimentation of lignin-rich fraction has been reported (Klap et al., 1996; Wakeham, 1999). The absence of lignin in Black Sea SPOM, though it is present in certain surface sediments from the Black Sea (Ergin et al., 1996), indicates terrigenous particles to be composed of larger, rapidly sinking material (Çoban-Yıldız et al., 2000c). The absence of lignin markers in this study, even in the SPOM from the shelf station (STA 10) where the total depth was 100 m and the surface salinity was as low as 16.9‰, confirms the previous findings. The SPOM analyzed in this study was autochthonous in origin.

### 3.3. Vertical variation of SPOM composition in the Black Sea Central Station (STA 6)

The composition of SPOM varied with depth through the sequence of layers observed in the Black Sea.

#### 3.3.1. Surface mixed layer (surface—1% light depth)

The thickness of the surface mixed layer was equal to the 1% light depth, and the composition of SPOM from the euphotic zone was discussed in

Section 3.2. SPOM in the surface mixed layer of both the central and shelf break stations was characterized by higher C/N, CBH/CHL and alkene/alkane ratios compared to the phytoplankton cultures, reflecting the heterogeneous composition of the SPOM, which probably contained substantial contributions of detrital matter.

#### 3.3.2. 1–0.1% light depth (fluorescence maximum layer; thermocline)

The SPOM concentration at the central station peaked at the fluorescence maximum layer corresponding to the depths of the sharp seasonal thermocline and the minimum in situ light transmission (Fig. 2A, B and D). At the fluorescence maximum layer, both the C/N ratio and the lipid content of the SPOM reached maximum values whereas relative concentrations of protein and carbohydrate markers decreased (Fig. 2D–F). In this zone, lipids, rather than carbohydrates, made the major contribution to the high POC concentrations. Consistent with this, the pyrolysis markers generated low CBH to CHL ratios while POC to fluorometric Chl-*a* ratios were exceptionally high (>2000, w/w). Accordingly, SPOM at the fluorescence maximum depth was characterized by higher lipid to protein ratios than in the near-surface waters (Fig. 2G). The thermocline generated a physical barrier to the settling of SPOM, thereby providing suitable conditions for accumulation and re-mineralisation of slowly sinking SPOM produced within the surface mixed layer. The contribution of herbivores and/or bacteria to the SPOM accumulated at the fluorescence maximum layer has been confirmed by the observation of a peak in heterotrophic activity (Morgan et al., 2006). Though active remineralisation might have caused an increase in phytoplankton to consume the nutrients generated, there was no net primary production at the depths of the fluorescence maximum (Yılmaz et al., 2006), and this is in good agreement with the pyrolysis data.

#### 3.3.3. Oxycline

At the top of the oxycline, or at the 0.1% light depth (35–40 m), the POC and PON concentrations decreased sharply from their values at the 1% light depth. Accordingly, the POC/PON, lipid/protein and alkene/alkane ratios all decreased (Fig. 2D, G, I). The contribution of CHL markers to the bulk SPOM was substantial. One should note that increase in the % abundance of CHL markers

does not necessarily mean a quantitative increase in concentration, but emphasizes the increased relative contribution of chlorophyll to the SPOM. At 35 m, the biomass of *E. huxleyii* was unexpectedly high as were the associated pigment levels (Ediger et al., 2006). Although the reason for the increase in biomass of *E. huxleyii* is uncertain, the increase does explain the differences in chemical composition of SPOM between the fluorescence maximum and the top of the oxycline. At the depths of the fluorescence maximum the SPOM concentration was high but the composition was more heterogeneous, with a substantial contribution from detritus and herbivores (see above). At the top of the oxycline, the SPOM concentration was low but the contribution of autotrophic organisms to the bulk SPOM was higher. The increased contribution of CHL markers to the bulk was at least partly due to the increase in pigment concentration per cell caused by the decreasing light intensity. Though there is no net C production below the 1% light depth, in situ fluorescence was comparable to surface values. One reason for the nitrate deficiency at the top of the oxycline was probably the uptake of nitrogen without any net production of organic matter.

At the base of the oxycline, both the C/N ratio and the lipid content of the SPOM were lower than at the fluorescence maximum but higher than at the surface (Fig. 2D,E). In other words, the SPOM pool around the fluorescence maximum had not yet influenced the chemical composition of SPOM pool in the steep oxycline. It is known that amino acids and carbohydrates degrade much faster than lipids in the early stages of diagenesis of organic matter (Eadie and Jeffrey, 1973; Cauwet, 1978; Ittekkot et al., 1981). SPOM collected from the base of the oxycline, therefore, probably originated from organic matter produced some days earlier in the euphotic zone with lower lipid content. Despite the lower lipid to protein and C to N ratios compared to the fluorescence maximum layer, the higher CBH to CHL and alkene/alkane ratios indicate the dominance of refractory organic matter at the base of the oxycline (Fig. 2H,I).

#### 3.3.4. Nitracline

In the steep nitracline, which at STA 6 marked the onset of the suboxic zone, the relative abundances of CBH, protein and lipid were similar to those of the oxycline. The ratio of CBH to CHL

markers decreased as a result of the slight increase in relative abundance of CHL markers (Fig. 2F,H).

#### 3.3.5. Denitrification zone (-NC) and the anoxic interface

In the denitrification zone, the relative distributions of lipid and protein markers displayed opposite trends; the protein content of the SPOM increased as the relative abundance of the lipids decreased (Fig. 2E,F), resulting in a decrease in the lipid/protein ratio (Fig. 2G). At the base of the denitrification layer the alkene/alkane ratio decreased to its lowest levels whereas the pyrrole to indole ratio increased to a maximum (Fig. 2I). In another words, both lipid and protein compositions changed at the denitrification zone. This change was most pronounced at the base of the suboxic zone where denitrification was expected to be most intense. These features are different from those observed in oxygenated open oceans (e.g. Tanoue, 1992; Libes, 1992), thereby emphasizing the critical role of redox-dependent, in situ chemo-autotrophic and chemo-heterotrophic processes in producing SPOM in the transition zone of the Black Sea.

SPOM from surface waters had high carbohydrate content while the relative abundance of carbohydrates in SPOM decreased below the near-surface waters and then remained almost constant till the anoxic interface (Fig. 2E). Carbohydrates are formed as for energy storage when there is lighter than needed to synthesize cell components. Towards the bottom of the euphotic zone the energy store is used up, and when there is no light it cannot be replaced. Particulate carbohydrates must be actively involved in cycling of organic matter in the surface layers as has been observed for dissolved carbohydrates in different oceans (Pakulski and Benner, 1994). Labile carbohydrate was decomposed immediately at the base of the euphotic zone and the remainder was resistant to further decomposition (Danovaro et al., 2000). Nevertheless, as a result of increased contribution of proteins at mid-depths due to in situ SPOM production by bacteria the relative concentration of carbohydrates in the Black Sea did not increase with increasing depth as observed in the open ocean (Danovaro et al., 2000).

#### 3.4. Shelf-break station off the bosporus (STA 3)

The vertical distributions of SPOM at STA 3 (especially below the CIL) were influenced by ventilation from the Bosphorus Plume.

### 3.4.1. Surface mixed layer (surface—1% light depth)

At STA 3 both the SPOM concentrations and the in situ fluorescence were higher in the surface mixed layer than at depth (Fig. 3B and D). As explained in Section 3.2, the chemical composition of SPOM was similar to that from the central region, though it possessed slightly lower lipid contents and alkene/alkane ratios.

### 3.4.2. 1–0.1% light depth (thermocline)

In contrast to STA 6, SPOM concentrations decreased sharply through the thermocline. Nevertheless, the C/N ratio of SPOM was lower than in the surface mixed layer (Fig. 3D), while the contributions of CBH, protein and CHL markers to the SPOM were similar to, or even higher than, those in the surface-mixed layer (Fig. 3E,F). This suggests that despite the sharp decrease in SPOM concentrations, the SPOM was fresh and autotrophic in origin. The thermocline did not generate a physical barrier to the settling of SPOM, possibly because the shelf break region off the Bosphorus is more dynamic than the central region.

### 3.4.3. 0.1% light depth—onset of nitracline

Below the thermocline, in the CIL, particulate concentrations remained low. While both the CBH and the protein contents of the SPOM were lower relative to the upper layers (Figs. 3E,F), POC/PON elemental ratio (Fig. 3D) and the relative abundance of lipid markers (Fig. 3E) were comparable to those in the thermocline.

### 3.4.4. Nitracline (oxycline)

The SPOM concentrations remained at low levels in the oxycline and the upper suboxic zone of STA 3 (Fig. 3D). The SPOM was composed of more refractory material, as indicated by higher POC/PON (Fig. 3D), lipid to protein (Fig. 3G) and alkene/alkane ratios (Fig. 3I).

### 3.4.5. Suboxic zone and the anoxic interface

Both the C/N (Fig. 3D) and lipid/protein (Fig. 3G) ratios of SPOM achieved their highest levels in the upper suboxic zone. Both ratios decreased through the suboxic layer suggesting that in situ formation of organic matter of bacterial origin is faster than the formation of refractory material by decomposition. In the lower suboxic zone, where, atypically, substantial concentrations of both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  co-existed (Fig. 3A), POC

and PON concentrations increased whilst their ratio decreased significantly (Fig. 3D), consistent with the broad minimum in light transmission (Fig. 3B). Below the nitrate maximum, the relative abundance of lipids declined to their lowest values and remained almost constant down to a depth of 300 m, whereas the protein content of the SPOM increased. The SPOM pool at the anoxic interface was characterized by high protein and low lipid content and the lowest C/N ratios, suggesting the dominance of bacteria. This has been confirmed by the negative nitrogen isotopic ratios detected at the interface (SPOM with  $\delta^{15}\text{N}$  values of as low as  $-8\%$ ; Çoban-Yıldız et al., 2006a), which could be considered as a chemo-autotrophic signal. Unfortunately, the method applied here (Pyrolysis-GC/MS) suffers from the disadvantage that flash pyrolysis cracks the original organic material and alters its stereochemistry. Therefore, bacterial bio-markers such as hopanes (as observed by Çoban-Yıldız et al., 2006b) and other hydrocarbons and fatty acids with stereochemistry specific to bacteria (as observed by Wakeham et al., 1991; Wakeham and Beier, 1991) were not detected due to this limitation of the analytical technique.

As opposed to its rather uniform distribution throughout the water column at the central station (Fig. 2E), the relative concentration of carbohydrate displayed large fluctuations in the shelf-break region and exhibited a maximum at the suboxic/anoxic interface (Fig. 3E). Both previous studies (Çoban-Yıldız et al., 2000b,c) and the vertical profile of percent carbohydrate at the central station strongly suggest that the carbohydrate content of the suspended organic material immediately decreased to background levels when subjected to decomposition. Therefore, the increase in the carbohydrate content of SPOM in the particle maximum formed within the suboxic/anoxic interface at the shelf-break station, suggesting either in situ carbohydrate formation or lateral transport of SPOM associated with intrusions from the Bosphorus Plume. The present results indicate that on the shelf break in the SW region, the lateral transport of water from the Bosphorus Plume modifies the depth profiles not only with respect to dissolved constituents but also the composition of SPOM. This is probably the major reason for the fluctuations in carbohydrate and chlorophyll markers through the water column of the shelf-break station (Fig. 3E,F). In the suboxic/anoxic interface, in situ chemo-synthesis was responsible for accumulation of SPOM with higher

protein content and thus lower C/N ratios. The biochemical composition of SPOM suggests that the intensity of bacterial activity in the suboxic/anoxic interface of the shelf-break station was much higher than at the central station.

### 3.5. Specific features at the suboxic/anoxic interface

#### 3.5.1. Pyrrole to indole ratio

The vertical variation of the ratio of pyrrole to indole, both markers of proteins (see Section 3.2), displayed a consistent and significant trend at the shelf break, central and transition stations (Fig. 5). The protein composition was almost uniform with

slight variations from the surface to the suboxic zone. At the base of the suboxic zone, the ratio increased at the depth of the particulate maximum (light transmission minimum) (Fig. 5). This indicates a change in protein composition, probably caused by specific in-situ microbial processes at certain depths of the anoxic transition layer. The changes were more pronounced for the shelf-break stations, where the minimum in light transmission was larger as a result of the lateral intrusion of oxygenated waters from the Bosphorus Plume and possible re-suspension of surface sediments in the shelf-break region. The depth of the maximum in the pyrrole to indole ratio appears to coincide with

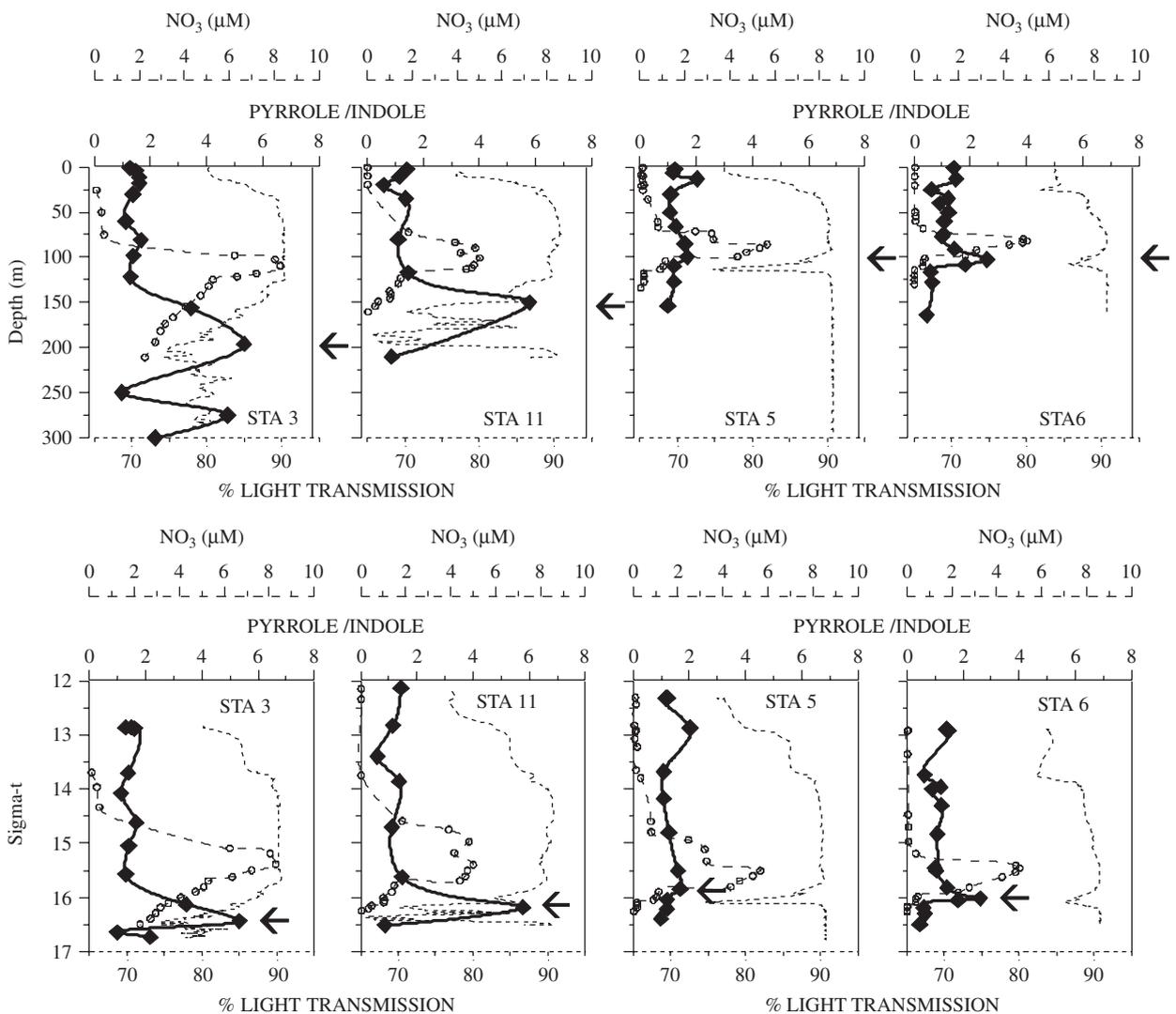


Fig. 5. Vertical distribution of  $\text{NO}_3^-$  ( $\circ$ ), % light transmission (dashed lines w/o symbol), and pyrrole/indole ratio ( $\blacklozenge$ ) at different regions of the Black Sea in May 2001. Arrows show the peak in pyrrole/indole ratio.

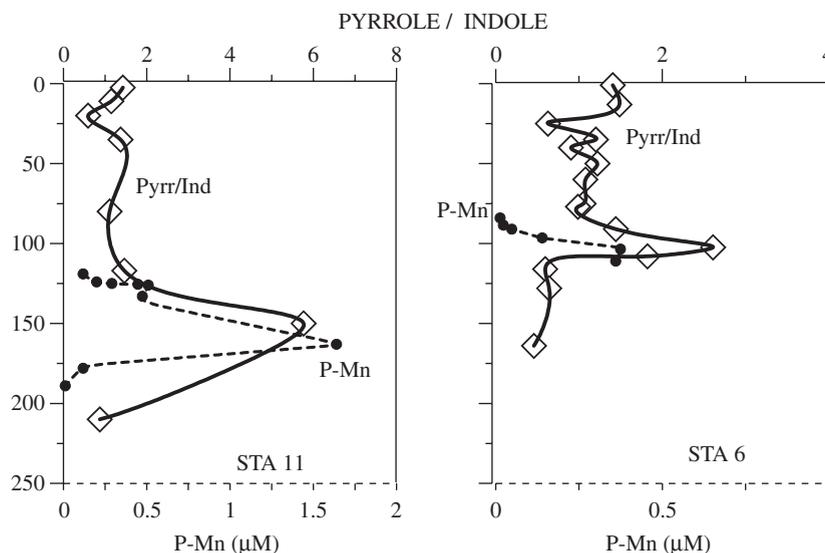


Fig. 6. Vertical distribution of pyrrole/indole ratio ( $\diamond$ ) and Particulate Manganese (P-Mn; dashed lined with symbol ( $\bullet$ )) at the shelf break station off Sakarya (STA 11) and central station (STA 6). Particulate Manganese data were obtained from KNORR-2001 web site at: [www.ocean.washington.edu/cruises/Knorr2001](http://www.ocean.washington.edu/cruises/Knorr2001); data of Prof. J.W. Murray.

the depth of the maximum in particulate manganese (Fig. 6; Data of J.W. Murray/K. Stewart; from the web site at: [www.ocean.washington.edu/cruises/Knorr2001](http://www.ocean.washington.edu/cruises/Knorr2001)).

### 3.6. Elemental sulfur and sulfur-containing organic compounds (S-CC)

$\text{H}_2\text{S}$  and elemental sulfur ( $\text{S}_8$  and  $\text{S}_6$ ) were observed in the pyrograms of SPOM just below the depth of the particle maximum (Figs. 4 and 7). The presence of elemental sulfur was confirmed by HPLC-UV analyses (Fig. 7). Elemental sulfur has previously been reported just below the sulfide interface (Jorgensen et al., 1991; Luther et al., 1991). There is strong evidence that oxidation of  $\text{H}_2\text{S}$  produces elemental sulfur, which, in turn is adsorbed on particles. Elemental sulfur concentrations increased from the central Black Sea towards the SW shelf (Fig. 7). The lateral flux of oxygen associated with the Bosphorus Plume appears to intensify sulfide oxidation, resulting in increased concentrations of elemental sulfur (Fig. 7). The  $\text{S}_8$  profile closest to the Bosphorus (STA 3) had large fluctuations and the intense maxima. The fluctuation is probably related to the lateral transport of water masses as can be traced by the temperature profile (Fig. 3C) (Konovalov et al., 2003). Konovalov and Murray (2001) proposed that as much as

50% of the upward flux of sulfide was oxidized by oxygen injected by the Bosphorus Plume.

Pyrolysis of suspended matter collected at the deepest layers of STA 3 (275 and 300 m) produced pyrograms characteristic of presence of sulfur-containing organic compounds (S-CCs). S-CCs were detectable at 250 m, and they became major pyrolysis products at 275 and 300 m (Figs. 4 and 7). Saiz-Jimenez (1995) noted that Curie-point Py-GC-MS of humic acids in the presence of sulfur yielded alkylthiophenes and other sulfur derivatives, which were not detected in the absence of sulfur. However, elemental sulfur occurs in several samples at other locations but no S-CCs were detected. We therefore exclude the possibility that S-CCs are formed as secondary pyrolysis products from the reaction of sulfur with organic matter. The S-CCs depicted in the 300-m sample (Fig. 4) were tentatively identified by matching experimental mass spectra with those listed in the NIST92 database. The only exception was the intense peak eluting at 19.8 min, which was tentatively identified as 1,2-dithiole-3-thione according to its mass spectrum reported in Fig. 8, together with the mass spectrum of a pyrolysis product identified as 4-methyl-1,2-dithiole-3-thione (Pedersen and Moller, 1972). In fact, the relative abundance of the ( $\text{M}^{+2}$ ) ion at  $m/z$  136 is in agreement with the occurrence of three S atoms, while the ions at  $m/z$  58 and 69 are characteristic of

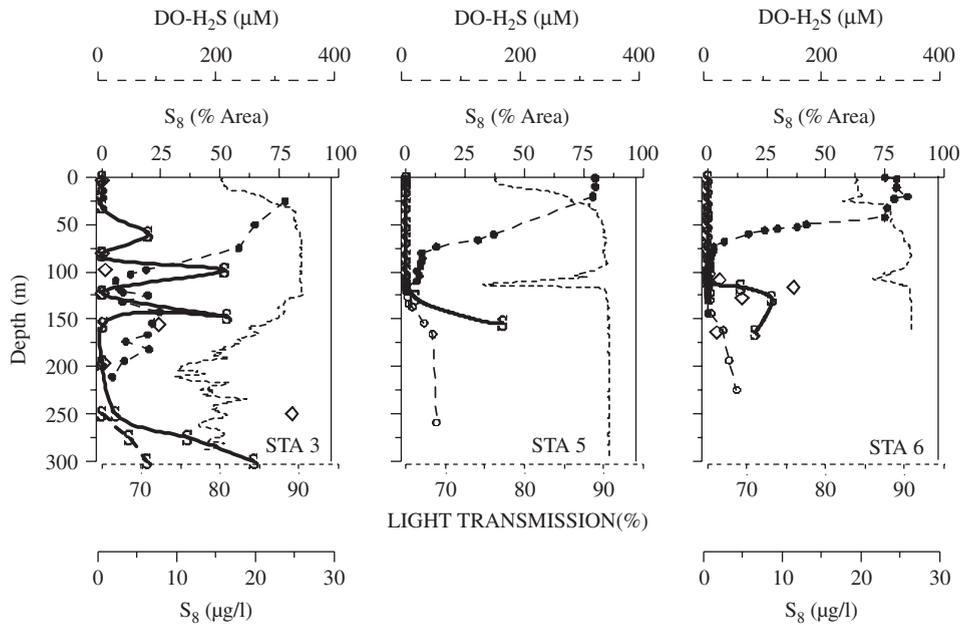


Fig. 7. Vertical distribution of DO (●) and H<sub>2</sub>S (○) concentrations, % area of elemental sulfur, S<sub>8</sub> (S with solid line) and organic sulfur (S with dashed line; observed at 250–300 m of STA 3) as derived from pyrolysis of particulate matter, concentrations of elemental sulfur (◇, without line) measured by HPLC and in situ light transmission (dashed line w/o symbol) at different regions of the Black Sea. Note there are no H<sub>2</sub>S data for STA 3 and no HPLC-S<sub>8</sub> analysis for STA 5.

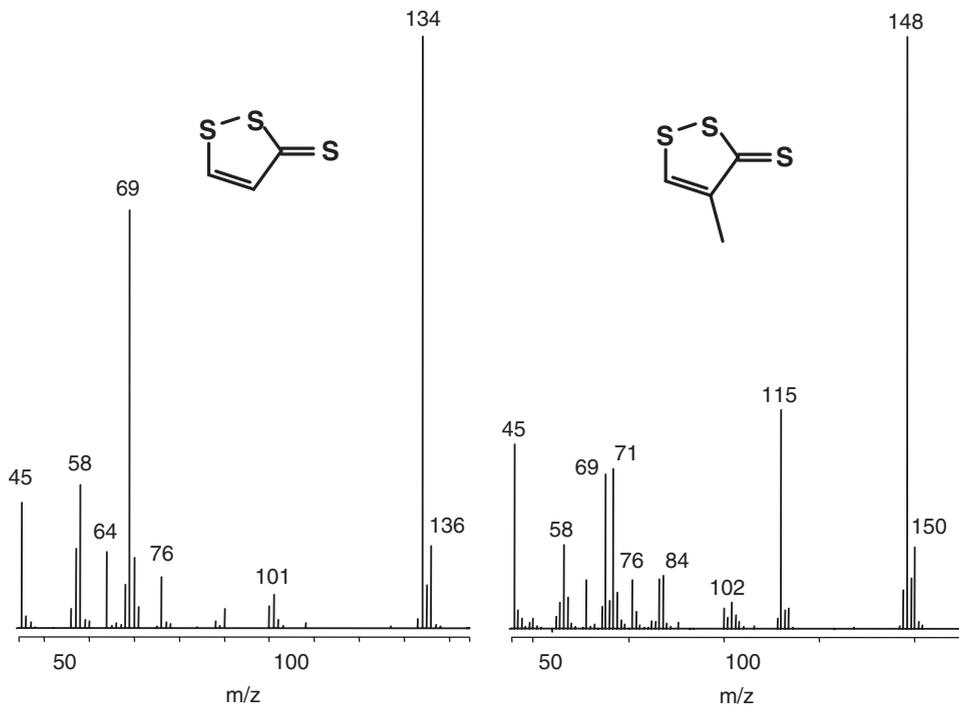


Fig. 8. Mass spectra and proposed structural assignments of the two main peaks eluting at: left 19.8 min, and right 21.5 min in the pyrogram of SPOM collected at 300 m depth at STA 3 (see Fig. 4).

1,2-dithiole-3-thione (Egsgaard and Carlsen, 1991). In particular, the intense peak at  $m/z$  69 is explained by the direct loss of the  $\text{HS}_2$  radical from the molecular ion, a typical fragmentation of 1,2-dithiole-3-thiones with no substituents at the 5-position (Pedersen and Moller, 1972). The homologous series of alkylated thiophenes, from methyl to undecyl, also was identified in the pyrograms. These compounds previously have been found in the pyrolysates of sedimentary organic matter and are indicative of the sulphurization of organic matter occurring in sediments (Schouten et al., 1994). Natural sulphurization involves intra- and intermolecular reactions of bio-chemicals with reduced sulfur species (Sinninghe Damste and de Leeuw, 1990; Wakeham et al., 1995). Reaction of reduced sulfur with active forms of iron is thought to be a faster process (Sinninghe Damste and de Leeuw, 1990), though both iron sulfides and organic sulfur can form simultaneously at high concentrations (Brüchert and Pratt, 1996).

It has been shown that sulfur incorporation into functionalized lipids can occur in very young sediments, even at the sediment–water interface (Wakeham et al., 1995). The results reported here suggest that sulphurization might start within the water column, when favorable conditions are met. As polysulphides are believed to be involved in the sulphurization process, the co-occurrence of elemental sulfur and sulphides is a fundamental requisite (Schouten et al., 1994). Unfortunately, data on sulfide and polysulphide concentrations are not available below 211 m at STA 3. Nonetheless the identification in the pyrograms of 1,2-dithiole-3-thione and its alkylated derivatives supports the involvement of polysulphides. These organic polysulphides might be the pyrolytic fragments of precursors with an alkyl chain larger than methyl, possibly derived from lipids. A compound containing the 1,2-dithiole-3-thione ring (namely 5-methyl-4-(4,8,12-trimethyltridecyl)-1,2-dithiole-3-thione) has been identified among the products formed from the sulphurization reaction of phytadienes simulating natural conditions (de Graaf et al., 1992). These findings support the view that cyclic organic polysulphidated compounds might be intermediates formed at an early stage of sulphurization.

The absence of lignin markers, which have been found in surface sediments in the same region (Ergin et al., 1996), the fairly negative nitrogen isotopic ratio (Çoban-Yıldız et al., 2006a) and the lower C/N ratio of SPOM (Fig. 3D), taken together, strongly

suggest that in situ processes like bacterial production in the water column dominate the composition of SPOM.

The variation in the vertical profiles of the pyrrole to indole ratio and the observation of elemental and organic sulfur are consistent with recent discussions (e.g. Jorgensen et al., 1991; Murray et al., 1995) the sequence of microbially mediated redox processes. Lateral transport seems to intensify these processes through enhancing redox reactions due to the intrusion of oxygenated, nitrate containing water from the Bosphorus Plume.

#### 4. Conclusions

Py-GC/MS analysis of marine SPOM can be used to identify the main components of its chemical composition and to provide a general approach to determine how bio-chemical processes through the water column influence the composition of organic matter. This approach has been applied to SPOM in the Black Sea. Phytoplankton cultures were analyzed for comparison. Py-GC/MS SPOM in the euphotic zone of the Black Sea in May 2001 revealed the same markers as those generated by pyrolysis of pure phytoplankton cultures. Quantification of these markers permitted calculation of the relative abundance of lipids, carbohydrates, chlorophyll and proteins in the SPOM from selected depths and locations in the Black Sea. These results were used to identify the source of organic carbon and to define the C/N ratio in the fluorescence maximum of station 6. The higher lipid/protein and C/N ratios and carbohydrate contents of euphotic zone SPOM compared to phytoplankton cultures are in agreement with the heterogeneity of the euphotic zone and/or the nutrient limitation of productivity in May 2001. The bio-chemical composition of POM suspended in the water column of the Black Sea revealed distinct and specific vertical variations that were different from those observed in the oxygenated open ocean. Particulate carbohydrates are actively involved in re-cycling of organic matter throughout the euphotic zone but relatively little (and refractory) carbohydrates remain below the euphotic zone. The lipid to protein ratio appears to determine the C/N ratio of SPOM throughout the water column. SPOM at the depths of the nitrate-maximum was characterized by a higher lipid relative abundance, indicating the dominance of refractory organic matter. In the suboxic and anoxic zones, the SPOM had a different composition

with higher protein and lower lipid content. Variations in both lipid and protein compositions, as determined by the peak in pyrrole/indole and decrease in alkene/alkane ratios at the base of the suboxic zone (particulate maximum), reflected the dominance of particles produced by in situ processes. Mid-water SPOM production by chemoautotrophic and -heterotrophic bacterial activities influences the biochemical composition of SPOM at the suboxic/anoxic interface.

At the shelf-break in the SW Black Sea intrusion of oxygenated, nitrate-rich waters of Mediterranean origin modify the redox processes occurring at intermediate depths, thus yielding a distinct spatial variation in mid-water SPOM concentration and composition. This variation is mainly expressed by the increase in the relative abundance of protein and pyrrole/indole ratio, the decrease in the C/N ratio and the intensity of the elemental sulfur peak. The detection of organic sulfur compounds at the shelf-break station off the Bosphorus Strait suggests the possibility of sulphurization in the water column.

### Acknowledgements

This study was supported by The Turkish Scientific Research Council, TÜBİTAK (YDABAG—101 Y020). The authors thank Prof. James W. Murray, the chief scientist of 2001 R.V. *Knorr*—Black Sea cruise. CTD, in situ fluorescence and light transmission data were obtained from the KNORR-2001 web site at: [www.ocean.washington.edu/cruises/Knorr2001](http://www.ocean.washington.edu/cruises/Knorr2001). The particulate manganese data of Prof. Murray were obtained from the same site. We are grateful to Professor Stuart Wakeham and an anonymous reviewer for their constructive contributions and to Dr. Alec Gaines for his constant encouragement. The authors also thank the technicians of METU-IMS and the captain and crew of R.V. *Knorr* for their help.

### References

- Brown, M.R., Dunstan, G.A., Norwood, S.J., Miller, K.A., 1996. Effects of harvest stage and light on the biochemical composition of the diatom *Thalassiosira pseudonana*. *Journal of Phycology* 32, 64–73.
- Brüchert, V., Pratt, L.M., 1996. Contemporaneous early diagenetic formation of organic and inorganic sulfur in estuarine sediments from St. Andrew Bay, Florida, USA. *Geochimica et Cosmochimica Acta* 60, 2325–2332.
- Cauwet, G., 1978. Organic chemistry of sea water particulates: concepts and developments. *Oceanologica Acta* 1 (1), 99–105.
- Chiavari, G., Galletti, G.C., 1992. Pyrolysis-gas chromatography/mass spectrometry of amino acids. *Journal of Analytical and Applied Pyrolysis* 24, 123–137.
- Çoban-Yıldız, Y., Tuğrul, S., Ediger, D., Yılmaz, A., Polat, S.Ç., 2000a. A comparative study on the abundance and elemental composition of SPOM in three interconnected basins: the Black, the Marmara and the Mediterranean Seas. *Mediterranean Marine Science* 1 (1), 51–63.
- Çoban-Yıldız, Y., Fabbri, D., Tartari, D., Tuğrul, S., Gaines, A.F., 2000b. Application of pyrolysis-GC/MS for the characterisation of suspended particulate organic matter in the Mediterranean Sea: a comparison with the Black Sea. *Organic Geochemistry* 31, 1627–1639.
- Çoban-Yıldız, Y., Chiavari, G., Fabbri, D., Gaines, A.F., Galletti, G., Tuğrul, S., 2000c. The chemical composition of Black Sea suspended particulate organic matter: pyrolysis-GC/MS as a complementary tool to traditional oceanographic analyses. *Marine Chemistry* 69, 55–67.
- Çoban-Yıldız, Y., McCarthy, J., Nevins, J.L., Yılmaz, A., 2003. Nitrogen cycling in the off-shore waters of the southern Black Sea. Presented at Second International on the Oceanography of the Eastern Mediterranean and Black Sea, 14–18 October 2002, Ankara, Turkey. In: Yılmaz, A. (Ed.), *Oceanography of the Eastern Mediterranean and Black Sea-Similarities and Differences of Two Interconnected Basins*. TUBITAK Publishers, Ankara, Turkey, pp. 609–614.
- Çoban-Yıldız, Y., Altabet, M., Tuğrul, S., Yılmaz, A., 2006a. Carbon and Nitrogen isotopic ratios of suspended particulate organic matter in the Black Sea water column. *Deep-Sea Research Part II, Special Issue (this issue)* [doi:10.1016/j.dsr2.2006.03.021].
- Çoban-Yıldız, Y., Gaines, A.F., Keating, P., Love, G.D., Mc Loughlin, D., Snape, C.E., 2006b. Hydrocarbon biomarkers generated by the hydrogenation of organic particulates suspended in the eastern Mediterranean and Black Seas. *Organic Geochemistry*.
- Danovaro, R., Dell'Anno, A., Pusceddu, A., Marralle, D., Croce, N.D., Fabiano, M., Tselepidis, A., 2000. Biochemical composition of pico-, nano- and micro-particulate organic matter and bacterioplankton biomass in the oligotrophic Cretan Sea (NE Mediterranean). *Progress in Oceanography* 46, 279–310.
- Eadie, B.J., Jeffrey, L.M., 1973.  $\delta^{13}\text{C}$  analyses of oceanic particulate organic matter. *Marine Chemistry* 42, 1265–1269.
- Ediger, D., Soydemir, N., Kideys, A.E., 2006. Estimation of phytoplankton biomass using HPLC pigment analysis in the southwestern Black Sea (this issue).
- Egsgaard, H., Carlsen, L., 1991. Thermally induced sulphur scrambling in 1,2-dithiole-3-thione. A study of  $^{34}\text{S}$ -isotopomers. *Journal of Chemical Research (S)* 226–227.
- Ergin, M., Gaines, A., Galletti, G.C., Chiavari, G., Fabbri, D., Yücesoy-Eryılmaz, F., 1996. Early diagenesis of organic matter in marine sediments: characterisation and source assessment. *Applied Geochemistry* 11, 711–720.
- Fabbri, D., Chiavari, G., Galletti, G.C., 1996. Characterisation of soil humin by pyrolysis (/methylation)—gas chromatography/mass spectrometry: structural relationships with humic acids. *Journal of Analytical and Applied Pyrolysis* 37, 161–172.
- Fabbri, D., Mongardi, M., Montanari, L., Galletti, G.C., Chiavari, G., Scotti, R., 1998. Comparison between CP/MAS  $^{13}\text{C}$ -NMR and Pyrolysis-GC/MS in the structural

- characterization of humins and humic acids of soil and sediments. *Fresenius' Journal of Analytical Chemistry* 362, 299–306.
- Fabbri, D., Sangiorgi, F., Vassura, I., 2005. Pyrolysis-GC/MS to trace terrigenous organic matter in marine sediments: a comparison between pyrolytic and lipid markers in the Adriatic Sea. *Analytica Chimica Acta* 530 (2), 253–261.
- de Graaf, W., Sinnighe Damsté, J.S., de Leeuw, J.W., 1992. Laboratory simulation of natural sulphurisation: I. Formation of monomeric and oligomeric isoprenoid polysulphides by low-temperature reactions of inorganic polysulphides with phytol and phytadienes. *Geochimica et Cosmochimica Acta* 56, 4321–4328.
- Guillard, R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L., Chanley, M.H. (Eds.), *Culture of Marine Invertebrate Animals*. Plenum Press, NY, USA, pp. 26–60.
- Guillard, R.R.L., Ryther, J.H., 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. *Canadian Journal of Microbiology* 8, 229–239.
- Hedges, J.J., Keil, R.G., Benner, R., 1997. What happens to terrestrial organic matter in the ocean? *Organic Geochemistry* 27 (5/6), 195–212.
- Hedges, J.I., Baldock, J.A., Gelinas, Y., Lee, C., Peterson, M.L., Wakeham, S.G., 2002. The biochemical and elemental compositions of marine plankton: a NMR perspective. *Marine Chemistry* 78, 47–63.
- Ishiwatary, R., Yamamoto, S., Handa, N., 1995. Characterisation of sinking particles in the ocean by pyrolysis-gas chromatography/mass spectrometry. *Journal of Analytical and Applied Pyrolysis* 32, 75–89.
- Ittekkot, V., Brockmann, U., Michaelis, W., Degens, E., 1981. Dissolved free and combined carbohydrates during a phytoplankton bloom in the North Sea. *Marine Ecology Progress Series* 4, 299–305.
- Jorgensen, B.B., Fossing, H., Wirsén, C.O., Jannasch, H.W., 1991. Sulfide oxidation in the anoxic Black Sea chemocline. *Deep-Sea Research Part II* 38, S1083–S1103.
- Karl, D.M., Knauer, G.A., 1991. Microbial production and particle flux in the upper 350 m of the Black Sea. *Deep-Sea Research Part II* 38, S438–S447.
- Keil, R.G., Tsamakis, E., Giddings, J., Hedges, J.J., 1998. Biochemical distributions (amino acids, neutral sugars and lignin phenols) among size-classes of modern marine sediments from the Washington coast. *Geochimica et Cosmochimica Acta* 62 (8), 1347–1361.
- Kempe, S., Diercks, A.R., Lieberzeit, G., Prange, A., 1991. Geochemical and structural aspects of the pycnocline in the Black Sea (*R.V. Knorr* 134-8 Leg 1, 1988). In: Murray, J.W., Izdar, E. (Eds.), *Black Sea Oceanography*. NATO ASI Series C, 351. Kluwer Academic Publishers, Netherlands, pp. 89–110.
- Klap, V.A., Boon, J.J., Hemminga, M.A., von Soelen, J., 1996. Assessment of the molecular composition of particulate organic matter exchanged between the Saefinghe salt marsh (southwestern Netherlands) and the adjacent water system. *Marine Chemistry* 54, 221–243.
- Konovalov, S.K., Murray, J.W., 2001. Variations in the chemistry of the Black Sea on a time scale of decades (1960–1995). *Journal of Marine Systems* 31, 217–243.
- Konovalov, S.K., Ivanov, L.I., Samodurov, A.S., 2001. Fluxes and budget of sulphide and ammonia in the Black Sea anoxic layer. *Journal of Marine Systems* 31, 203–216.
- Konovalov, S.K., Luther, G.W., Friederich, G.E., Nuzzio, D.B., Tebo, B.M., Murray, J.W., Oguz, T., Glazer, B., Trouwborst, R.E., Clemet, B., Murray, K.J., Romanov, A.S., 2003. Lateral injection of oxygen with the Bosphorus plume—fingers of oxidizing potential in the Black Sea. *Limnology and Oceanography* 48 (6), 2369–2376.
- Libes, S.M., 1992. *An Introduction to Marine Biogeochemistry*. Wiley, Canada, (734pp).
- Luther, G.W., Church, T.M., Powell, D., 1991. Sulfur speciation and sulfide oxidation in the water column of the Black Sea. *Deep-Sea Research Part II* 38 (Suppl. 2), S1121–S1137.
- Mannio, A., Harvey, R.H., 2000. Terrigenous dissolved organic matter along an estuarine gradient and its flux to the coastal ocean. *Organic Geochemistry* 31, 1611–1625.
- Minor, E.C., Eglinton, T.I., Olson, R., Boon, J.J., 1998. The compositional heterogeneity of particulate organic matter from the surface ocean: an investigation using flow cytometry and DT-MS. *Organic Geochemistry* 29 (5–7), 1561–1582.
- Morgan, J.A., Quinby, H.L., Ducklow, H.W., 2006. Bacterial abundance and production in the Western Black Sea. *Deep Sea Research Part II, Special Issue*, this issue [doi:10.1016/j.dsr2.2006.03.023].
- Moldoveanu, S.C., 1998. Analytical pyrolysis of natural organic polymers, In: *Techniques and Instrumentation in Analytical Chemistry*, vol. 20, Elsevier, Amsterdam.
- Murray, J.W., Codispoti, L.A., Friederich, G.E., 1995. Oxidation-reduction environments: the suboxic zone in the Black Sea. In: Huang, C.P., O'Melia, C.R., Morgan, J.J. (Eds.), *Aquatic Chemistry*. American Chemical Society, Washington, DC, pp. 157–176.
- Oğuz, T., Deshpande, A.G., Malanotte-Rizzoli, P., 2002. The role of mesoscale processes controlling biological variability in the Black Sea coastal waters: inferences from SeaWiFS-derived surface chlorophyll field. *Continental Shelf Research* 22, 1477–1492.
- Pakulski, J.D., Benner, R., 1994. Abundance and distribution of carbohydrates in the ocean. *Limnology and Oceanography* 39 (4), 930–940.
- Pedersen, C.Th., Møller, J., 1972. Mass spectrometric studies of methyl- and phenyl-substituted 1,2-dithiole-3-thiones and 1,2-dithiole-3-ones. *Acta Chemica Scandinavica* 26, 250–256.
- Peulve, S., de Leeuw, J.W., Sicre, M.A., Baas, M., Saliot, A., 1996. Characterization of organic matter in sediment traps from the northwestern Mediterranean Sea. *Geochimica et Cosmochimica Acta* 60, 1239–1259.
- Redfield, A.C., Ketchum, B.H., Richards, F.A., 1963. The influence of organisms on the composition of sea water. In: Hill, M.N. (Ed.), *The Sea*, vol. 2. Interscience, New York, pp. 26–77.
- Roessler, P.G., 1990. Environmental control of glycerolipid metabolism in microalgae: commercial implications and future research directions. *Journal of Phycology* 26, 393–399.
- Saiz-Jimenez, C., 1995. Reactivity of the aliphatic humic moiety in analytical pyrolysis. *Organic Geochemistry* 23, 955–961.
- Saliot, A., Ulloa-Guevera, A., Viets, T.C., de Leeuw, J.W., Schenck, P.A., Boon, J.J., 1984. The application of pyrolysis-gas chromatography-mass spectrometry to the chemical characterization of suspended matter in the ocean. *Organic Geochemistry* 6, 295–304.

- Schouten, S., de Graaf, W., Sinninghe Damste, J.S., van Driel, G., de Leeuw, J.W., 1994. Laboratory simulation of natural sulphurization: II. Reaction of multi-functionalized lipids with inorganic polysulphides at low temperatures. In: Telnaes, N., van Graas, G., Øygaard, K. (Eds.), *Advances in Organic Geochemistry, Organic Geochemistry*, vol. 22, pp. 825–834.
- Sicre, M.A., Peulve, S., Saliot, A., de Leeuw, J.W., Baas, M., 1994. Molecular characterization of the organic fraction of suspended matter in the surface waters and bottom nepheloid layer of the Rhone delta using analytical pyrolysis. *Organic Geochemistry* 21, 11–26.
- Sinninghe Damste, J.S., de Leeuw, J.W., 1990. Analysis, structure and geochemical significance of organically-bound sulphur in the geosphere: state of the art and future research. In: Durand, B., Behar, F. (Eds.), *Advances in Organic Geochemistry 1989, part II, Organic Geochemistry 16*. Pergamon Press, Oxford, pp. 1077–1101.
- Taguchi, S., Hirata, J.A., Laws, E.A., 1987. Silicate deficiency and lipid synthesis of marine diatoms. *Journal of Phycology* 23, 260–267.
- Tanoue, E., 1992. Occurrence and characterization of particulate proteins in the Pacific Ocean. *Deep-Sea Research* 39 (5), 743–761.
- Tebo, B.M., 1991. Manganese (II) oxidation in the suboxic zone of the Black Sea. *Deep-Sea Research Part II* 38, S883–S906.
- Wakeham, S.G., 1995. Lipid biomarkers for heterotrophic alteration of suspended particulate organic matter in oxygenated and anoxic water columns of the ocean. *Deep-Sea Research I* 42 (10), 1749–1771.
- Wakeham, S.G., 1999. Monocarboxylic, dicarboxylic and hydroxy acids released by sequential treatments of suspended particles and sediments of the Black Sea. *Organic Geochemistry* 30, 1059–1074.
- Wakeham, S.F., Beier, J.A., Clifford, C.H., 1991. Organic matter sources in the Black Sea as inferred from hydrocarbon distributions. In: Murray, J.W., Izdar, E. (Eds.), *Black Sea Oceanography*. NATO Adv. Studies Inst., Kluwer Academic Publishers, Dordrecht, pp. 319–341.
- Wakeham, S.G., Beier, J.A., 1991. Fatty acid and sterol biomarkers as indicators of particulate matter source and alteration processes in the Black Sea. *Deep-Sea Research* 38, S943–S968.
- Wakeham, S.G., Sinninghe Damste, J.S., Kohnen, M.E.L., de Leeuw, J.W., 1995. Organic sulfur compounds formed during early diagenesis in Black Sea sediments. *Geochimica et Cosmochimica Acta* 59 (3), 521–533.
- Wakeham, S.G., Lewis, C.M., Hopmans, E.C., Schouten, S., Sinninghe Damsté, J.S., 2003. Archaea mediate anaerobic oxidation of methane in deep euxinic waters of the Black Sea. *Geochimica et Cosmochimica Acta* 67 (7), 1359–1374.
- Yılmaz, A., Tuğrul, S., Polat, Ç., Ediger, D., Çoban, Y., 1998. On the production, elemental composition (C,N,P) and distribution of photosynthetic organic matter in the southern Black Sea. *Hydrobiologia* 363, 141–156.
- Yılmaz, A., Çoban-Yıldız, Y., Telli-Karakoç, F., E., Bologa, A., 2006. Surface and midwater sources of organic carbon by photo- and chemo-autotrophic production in the Black Sea. *Deep Sea Research Part II, Special Issue (this issue)* [doi:10.1016/j.dsr2.2006.03.015].
- Yunev, O.A., Vedernikov, V.I., Baştürk, Ö., Yılmaz, A., Kıdeys, A.E., Moncheva, S., Kononov, S., 2002. Long-term variations of surface chlorophyll-a and primary production in the open Black Sea. *Marine Ecology Progress Series* 230, 11–28.