

# The chemical composition of Black Sea suspended particulate organic matter: pyrolysis-GC/MS as a complementary tool to traditional oceanographic analyses

Y. Çoban-Yıldız<sup>a,\*</sup>, G. Chiavari<sup>b</sup>, D. Fabbri<sup>b</sup>, A.F. Gaines<sup>c</sup>, G. Galletti<sup>b</sup>,  
S. Tuğrul<sup>a</sup>

<sup>a</sup> *Institute of Marine Sciences, Middle East Technical University, P.K. 28, Erdemli, İçel 33731, Turkey*

<sup>b</sup> *Dipartimento di Chimica 'G. Ciamician' e Laboratorio di Chimica Ambientale, University of Bologna, 2 via Marconi, I-48100 Ravenna, Italy*

<sup>c</sup> *Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, Scotland, UK*

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## Abstract

A “traditional” description of the abundance and chemical composition of suspended particulate organic matter (POM) in open and coastal waters of the southern Black Sea in June 1996 has been confirmed and extended by pyrolysis–gas chromatography/mass spectrometry (Py-GC/MS) analyses. Py-GC/MS provided depth profiles of the relative concentrations of twenty three marker compounds characteristic of chlorophylls (CHL), lipids, carbohydrates (CBH) and proteins produced by thermal degradation of the POM retained on the filters. No terrestrial markers, characteristic of lignin or of plant waxes, were observed. Evidence was found for considerable changes in the chemical composition of POM in the water column from the surface down to the sulphidic water layer. In surface-mixed layer, both POC:CHL-a ratios and relative abundances of CBH markers were notably high, suggesting that the suspended POM was mainly composed of detritus. The profiles of both CHL and protein markers exhibit coherent maxima at the base of the euphotic zone, coinciding with the nutricline depth in the central cyclonic eddy, where the bulk POM possessed relatively low C:N ratios. Beneath the 0.1% light depth the absence of intact phytoplankton cells and the presence of bacteria and faecal pellets was accompanied by a change in the protein composition of the POM as shown by the changes in the ratio of pyrrole:indole markers. Lipid markers increased markedly from the euphotic zone into the oxycline and remained almost constant in the suboxic waters; they then decreased in the sulphidic interface, presumably due to consumption of lipids by anaerobic bacteria. © 2000 Elsevier Science B.V. All rights reserved.

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\* Corresponding author. Fax: +90-324-5212327; E-mail: yesim@ims.metu.edu.tr

## 1. Introduction

The Black Sea, a relatively large and land-locked basin with a maximum depth of 2200 m receives large volumes of both river water, particularly in the north west (mainly Danube), and saline Mediterranean water flowing into its southwestern basin through the narrow Bosphorus Strait (Fig. 1). Therefore, a marked halocline (50–70 m thick) permanently exists below the brackish upper layer, limiting ventilation of more saline waters of the entire deep basin (Sorokin, 1983). The Black Sea is an unusual marine environment, possessing the biochemical characteristics of oxygenated, suboxic and anoxic waters throughout the year. Whereas riverine input contributes markedly to primary production in the coastal regions (Bologa et al., 1995; Cociasu et al., 1997), the major source of nutrients for the surface waters offshore is input from the nutricline, especially in winter when there is strong convective mixing (Oğuz et al., 1996). When seasonal stratification in the surface water inhibits vertical mixing, primary production is apparently sustained by the regeneration of nutrients in the euphotic zone (Vedernikov and Demidov, 1993; Oğuz et al., 1996). Primary production generally displays a major bloom in spring and a secondary peak in autumn (Sorokin,

1983; Vedernikov and Demidov, 1993); short-term summer blooms have also been reported (Hay and Honjo, 1989; Mee, 1992; Sur et al., 1996).

Suspended particulate organic matter (POM), exhibiting a relatively low sinking rate, comprises most of POM sampled by bottle casts. This material plays an important role in biomediated elemental cycling in the upper layer of the Black Sea (Karl and Knauer, 1991). Larger, rapidly sedimenting particles, which may be associated with inorganic material, can be captured only with sediment traps; their degradation in the lower layers keeps the deep basin waters anoxic (Sorokin, 1983; Karl and Knauer, 1991). In the present paper, we first discuss the variations in abundance and elemental composition (C:N:P ratio) of bulk POM in the upper layer of the Black Sea, from the oxygenated surface water down to the anoxic layer (collected in June 1996). Characteristics of the studied regions are briefly described, including simultaneous measurements of solar irradiance, primary productivity, CHL-a, inorganic nutrients, dissolved oxygen and hydrogen sulphide. This “classical” information is complemented by the use of Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS) to characterise the lipid and macromolecular composition of POM retained on filters. This technique, which can provide useful informa-

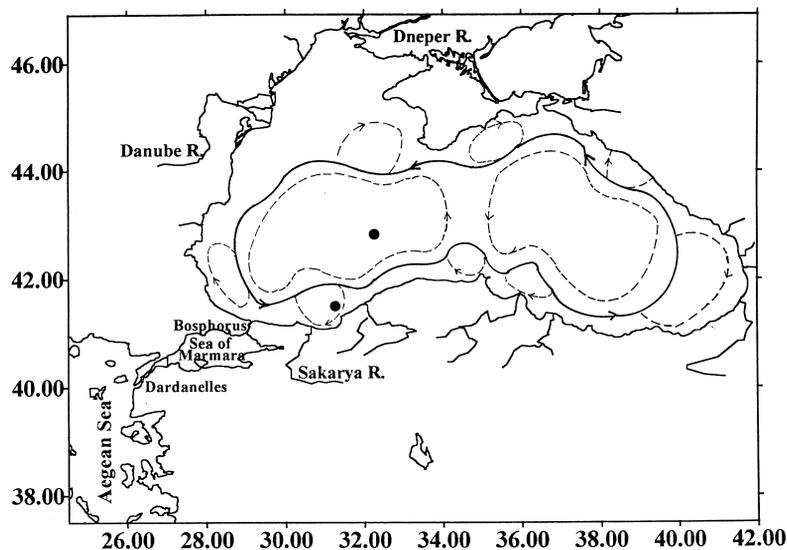


Fig. 1. Station location map and general circulation of the Black Sea. Solid line represents the cyclonic boundary current (rim current) and the dashed lines represent cyclonic and anticyclonic features. Solid dots show the location of sampling stations.

tion on the chemical composition of POM in marine environments (Saliot et al., 1984; Sicre et al., 1994; Ishiwatary et al., 1995; Klap et al., 1996; Peulvé et al., 1996), is used here to identify marker compounds characteristic of the suites of chemical structures which comprise POM. The Py-GC/MS approach has been used in our previous studies of the Black Sea sediments (Ergin et al., 1996).

## 2. Methodology

### 2.1. Characteristics of the sampling locations

Fig. 1 shows the two sampling locations selected in the western cyclone (termed as: ‘open’, total depth: 2200 m) and the Sakarya anticyclone (termed as: ‘coastal’, influenced by riverine inputs carried by cyclonic rim-current, total depth: 1500 m). Hydrographic profiles displayed in Fig. 2a–c show that the Black Sea surface water was seasonally stratified in June 1996 and 1% of the surface light intensity was reached at 35–40 m. The permanent halocline formed below the coherent temperature minimum appeared at a relatively shallow depth at the open sea location (Fig. 2b), due to the cyclonic circulation. Depth distributions of major chemical parameters displayed distinct features in the Black Sea. Fig. 3 shows that the well-oxygenated but nitrate and phosphate-poor upper layer extended down to the depth of temperature minimum both in open and coastal regions. Below, dissolved oxygen (DO) concentrations decreased so that a steep oxycline was formed between a  $\sigma_t$  of 14.5–14.8 and 15.4–15.6 where suboxic

concentrations of 20–30  $\mu\text{M}$  were reached. Deeper still, the DO declined gradually to less than 5  $\mu\text{M}$  at a depth corresponding to  $\sigma_t = 15.9$ –16.0. The nitrate and phosphate concentrations (Fig. 3b,c), increasing steadily in the oxycline, reached maximal levels within the suboxic interface. In the lower depths of the suboxic zone, the nitrate concentrations declined steadily as a result of bacterial denitrification and reached undetectable concentrations in the sulphidic waters (Sorokin, 1983; Codispoti et al., 1991). The  $\text{PO}_4$  profile exhibits a broad maximum, terminated by a marked minimum, which is more pronounced in the open station, at depths corresponding to  $\sigma_t = 15.85$ –15.9 (Fig. 3c). Then it displays a steep increase within the sulphidic water interface, reaching peak values of 5–8  $\mu\text{M}$  at a  $\sigma_t$  of 16.2–16.25, probably resulting from the dissolution of phosphate associated metal (Fe and Mn) oxides (Shaffer, 1986; Codispoti et al., 1991). The locations of characteristic chemical features (such as the oxycline, the nutricline, the nutrient maxima and the sulphidic water boundary) consistently appeared at relatively shallow depths in the cyclonic gyres of the Black Sea, deepening towards the coastal margins as noted previously by Sorokin (1983). However, they appeared at nearly identical density surfaces independent of the sampling location, as previously illustrated for the entire deep basin (Tuğrul et al., 1992; Murray et al., 1995) with only small regional differences (Baştürk et al., 1997). These findings indicate that chemical measurements performed at a single location of the open sea may be applicable to the whole deep basin of the Black Sea.

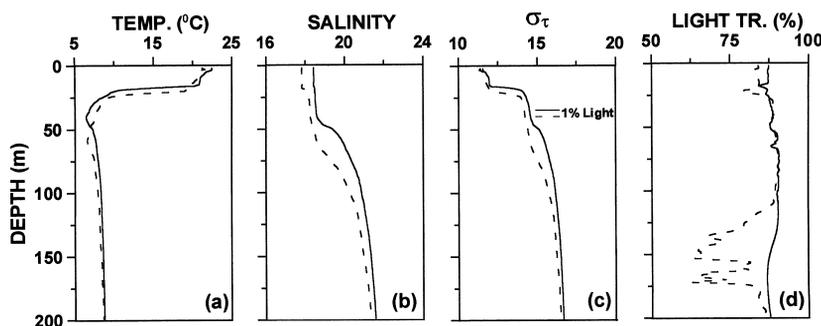


Fig. 2. Hydrographic profiles in coastal (dashed line) and open (solid line) stations: (a) temperature ( $^{\circ}\text{C}$ ), (b) salinity, (c) water density along with depth for 1% light penetration, (d) % light transmission.

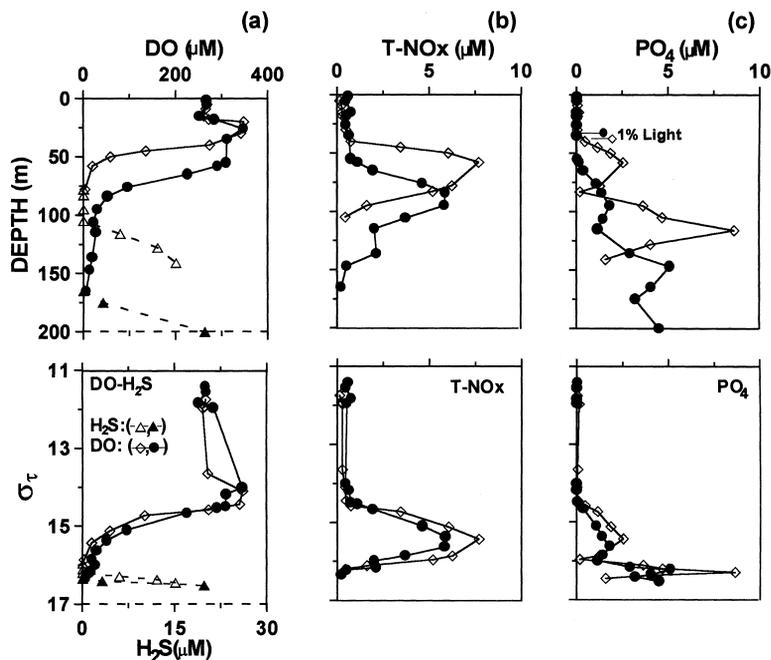


Fig. 3. Vertical distribution of (a) dissolved oxygen (DO)–hydrogen sulfide (H<sub>2</sub>S, represented with dashed line, with (▲) for coastal and (△) for open stations), (b) nitrate + nitrite (T-NO<sub>x</sub>), (c) phosphate (PO<sub>4</sub>) at the coastal (●) and open (◇) stations both as a function of depth and of water density.

## 2.2. Measurements

A Licor, Model 185 quantameter was used to measure solar irradiance and determine the thickness of the euphotic zone, corresponding to the depth where the light had 1% of its surface intensity. Water was sampled in 5-l Niskin bottles on a rosette attached to a CTD probe. The automated inorganic nutrient measurements and the spectrofluorometric chlorophyll-*a* (CHL-*a*) analyses followed the methods of Grasshoff et al. (1983) and Holm-Hansen and Riemann (1978). Suspended particles, pre-filtered through 200 μm pore-size mesh were collected under low-vacuum on pre-combusted GF/F filters (diameter: 47 mm; pore size: 0.7 μm) for organic carbon (POC), nitrogen (PON), total phosphorus (PP) and Py-GC/MS measurements. All filters were frozen (−20°C) on board, dried at 50–60°C overnight on land and stored in vacuum desiccator until they were processed. The POC and PON samples were exposed to hydrochloric acid fumes, re-dried and analysed by the dry combustion technique (Polat and

Tuğrul, 1995) using a Carlo Erba 1108 CHN analyser. The overall precision of the procedure was calculated as ±3%. The frozen PP samples were combusted at 500°C for 3 h, treated with 12 ml of 0.5 N HCl and filtered. After adjusting the pH to 8, the total phosphorus in the solution was determined colorimetrically (Polat and Tuğrul, 1995). The standard error estimated from two batches of 5-replicates was ±4–7%. Suspended particles retained on filters for Py-GC/MS (12–15 mg) were carefully scraped and about 6 mg sample was inserted into a quartz pyrolysis tube. The ends of the tube were loosely plugged with pre-pyrolysed quartz wool. Samples were pyrolysed at 700°C for 10 s at the maximum heating rate of the CDS Instrument Pyroprobe 1000 platinum filament pyrolyser. Pyrolysis products were swept by a stream of helium into a 30 m ‘J and W DB5-MS’ capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness) heated from 50 to 300°C at 5°C/min. Compounds leaving the column were detected by a Varian Saturn II ion trap mass spectrometer and mass spectra were acquired at 1 scan/s under

electron impact at 70 eV in the scan range 40–400  $m/z$ . Thermochemolysis with tetramethylammonium hydroxide (TMAH) was performed by pyrolysing the sample in the presence of 5  $\mu\text{l}$  25% aqueous TMAH. Complete details of the Py-GC/MS routine have been published previously (Ergin et al., 1996).

### 3. Results and discussion

#### 3.1. Particulate profiles

Fig. 4a–f show that the concentrations of POC, PON and PP and their molar ratios vary markedly with depth in the upper layer water column of the open and coastal Black Sea. These profiles are consistent with previous results from open waters of the Black Sea (Fry et al., 1991; Lyutsarev and Shanin, 1996; Krivenko et al., 1997; Yılmaz et al., 1998). Particulate concentrations in the surface mixed layer were higher at the coastal station than in the open

sea, presumably due to the input of nutrients by rivers (mainly the Danube River carried by along-shore rim-currents and the local effect of the Sakarya River; Fig. 1). The concentrations decreased markedly below the thermocline in the coastal region but increased at the base of the thermocline and then decreased towards the bottom of the euphotic zone in the open sea. Then they dropped to similar background levels in the oxycline and suboxic waters of both open and coastal regions (POC = 1–4  $\mu\text{M}$ ; PON = 0.1–0.3  $\mu\text{M}$ ; PP = 0.01–0.03  $\mu\text{M}$ ). Interestingly, the particulate profiles displayed an apparent increase within the anoxic interface; changes in the PP were much more pronounced and the deep PP maximum exceeded the surface concentrations, resulting in a sharp decrease in both the POC:PP and PON:PP ratios in the interface (Fig. 4e,f). Notably, suspended particles collected from the anoxic interface in the coastal region had a characteristic light-brown colour, probably due to the selective accumulation of fine particles of Mn and Fe oxides (Kempe

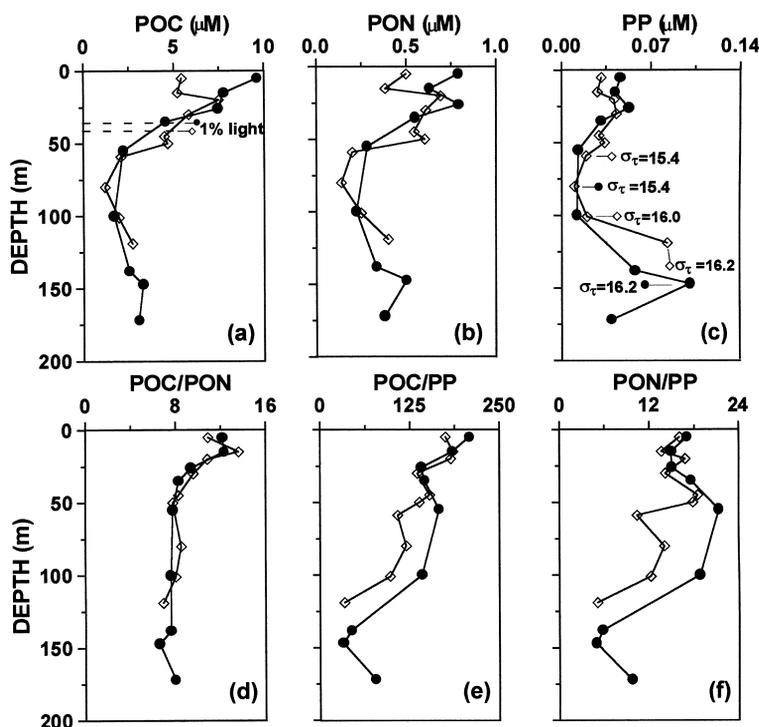


Fig. 4. Vertical profiles of suspended particulate organic matter (POM) and their molar ratios in coastal (●) and open (<math>\diamond</math>) stations: (a) POC, (b) PON, (c) PP, (d) POC/PON, (e) POC/PP, (f) PON/PP.

et al., 1991). A minimum in the light transmission in the upper anoxic waters (between  $\sigma_t = 16.1$ – $16.4$ ) of the coastal location, almost exactly coinciding with the present PP maximum and the fine particle layer (FPL) (Fig. 2d), was previously reported by Kempe et al. (1991). In contrast, the FPL was not apparent in the open sea.

The elemental composition (C:N:P ratio) of the POM down to 0.1% light depth vary with both region and depth; however, the ratios displayed similar depth profiles in the open and coastal locations (Fig. 4d–f). This indicates that the POM in the euphotic zone had similar sources. The suspended POM in the surface mixed layer possessed elevated C:N and C:P ratios but the N:P ratios of 14–17 were consistent with Redfield N:P values (16). The C:N decreased markedly below the thermocline and remained almost invariant in the suboxic waters of the open and coastal regions (Fig. 4d). The latter values are consistent with bacterial C:N values (Goldman and Dennett, 1991). However, the C:P ratio of the POM first decreased within the thermocline and then increased again in the oxycline waters of both the stations. The POC:PP ratios of the oxic/suboxic interface zone waters in coastal region were apparently higher than in the open sea; the ratio decreased consistently to minimal levels (33–34) in the anoxic interface of the open and coastal regions. The PON:PP ratio increased in the oxycline, yielding a broad maximum in the oxic/suboxic interface of the coastal station; below, the ratio displayed depth profiles with a trend very similar to the POC:PP ratios in the studied regions (see Fig. 4e,f).

### 3.2. Productivity and chlorophyll-a

Microscopic analysis of particles retained on filters indicated that in June 1996, dinoflagellates dominated the living biomass in the euphotic zone. No intact phytoplankton cells could be observed amongst the particles filtered from the suboxic waters to the anoxic zone. Fig. 5c shows the daily rate of primary production (Pr) was as high as  $25$ – $30 \text{ mg cm}^{-3} \text{ day}^{-1}$  and rather constant in the surface mixed layer of the open and coastal locations; below the 10% light depth it declined steeply and became negligible at the base of the euphotic zone. In the open station, the chlorophyll-a (CHL-a) concentration was almost invariant in the euphotic zone, then it increased and subsequently decreased to trace levels, generating a deep CHL-a maximum (DCM) at about 0.5% light depth (Fig. 5a). However, in the coastal region, the CHL-a profile displayed a relatively broad CHL-a peak between the 10% and 1% light depths. The POC:CHL-a ratio (which is a rough relative measure of the living phytoplankton in the POM; the higher the ratio the lower the biomass) was relatively low in the coastal station and decreased markedly from the surface mixed layer to the bottom of the euphotic zone (Fig. 5b). The phytoplankton cell counts (Fig. 5d) were variable in the surface mixed layer and displayed depth profiles with shapes generally similar to the CHL-a.

### 3.3. Py-GC / MS analysis of POM

Py-GC/MS analysis of the POM filtered from the Black Sea yields at least 100 volatile pyrolysis prod-

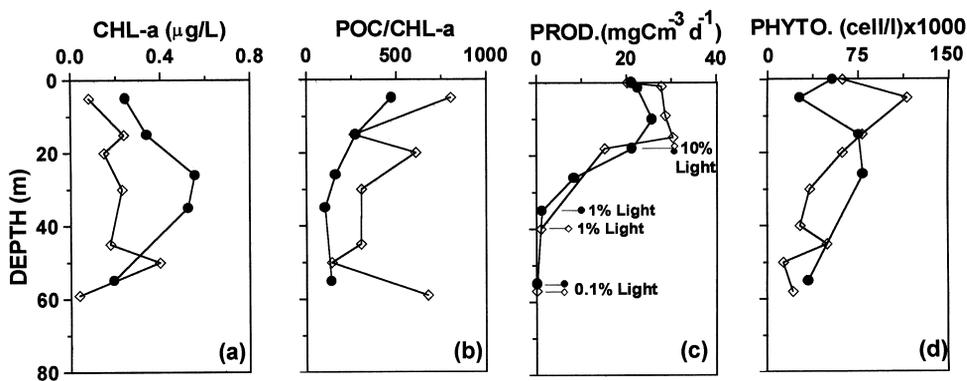


Fig. 5. Vertical profiles of (a) chlorophyll-a, (b) POC to chlorophyll-a ratio (w/w), (c) primary production, (d) phytoplankton cell counts in coastal (●) and open (◇) stations.

ucts (typical pyrogram shown in Fig. 6). Examination of the corresponding mass spectra indicates that, consistent with the observations of Wakeham et al. (1991), most products are derived from lipids, chlorophyll, carbohydrates and proteins (see Fig. 6 caption). None of the lignin monomers abundant in pyrograms obtained from surficial sediments from

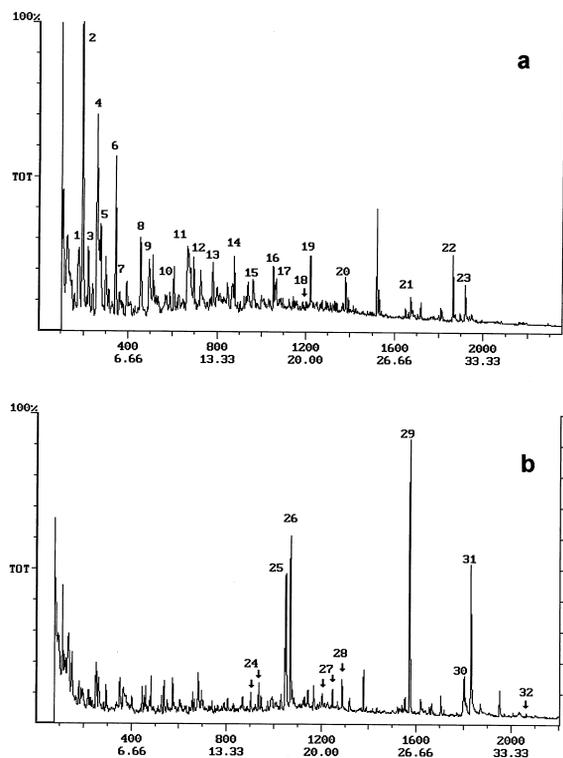


Fig. 6. Total ion chromatograms obtained from (a) Py-GC/MS and (b) TMAH thermochemolysis of Black Sea POM collected at the central basin site (30 m water depth). Suggested origin of pyrolysis products: l: lipid, chl: chlorophyll-a, c: carbohydrate, p: protein markers. Peak number 1: pyrrole (p), 2: toluene (?), 3: furan-2-one (c), 4: furaldehyde (c), 5: 3-methyl pyrrole (p), 6: styrene (?), 7: methylcyclopentenone (c), 8: methylfuraldehyde (c), 9: phenol (p), 10: 2-hydroxy-3-methyl-2-cyclopenten-1-one (c), 11: m/p phenol (p), 12: undecene (l), 13: phenylacetonitrile (p), 14: dodecene (l), 15: benzenepropanenitrile (p), 16: tridecene (l), 17: indole (p), 18: methyl indole (p), 19: tetradecene (l), 20: pentadecene (l), 21: pristene (chl), 22: phytadiene1 (chl), 23: phytadiene2 (chl) 24: methylated pentonic acids (c), 25: 1,2,4-trimethoxybenzene (c), 26: dehydrated, partially methylated deoxyaldonic acid (c), 27: methylated deoxyhexonic acids (c), 28: dodecanoic acid methyl ester (l), 29: tetradecanoic acid methyl ester (l), 30: hexadecanoic acid methyl ester (l) 31: hexadecanoic acid methyl ester (l) 32: octadecanoic acid methyl ester (l).

the southern slope of the Black Sea (Fig. 3 in Ergin et al., 1996) were observed in the pyrograms of the POM filtered from the water column, even from the coastal location. Twenty-three compounds were selected as markers and the intensity (peak area) of each marker compound was measured by mass chromatography using a characteristic ion — either the base peak or the molecular ion — as quantitation ion. The contribution of each marker to the total intensity of all 23 markers was calculated and expressed as a percentage. This has been termed the “relative concentration” of the marker (Figs. 7 and 8) and has been used in other applied pyrolysis studies (Fabbri et al., 1998). Pyrolysis yields can be assumed constant in all samples under same experimental conditions and relative concentrations may therefore be used for comparing variation in the relative concentrations (abundances) of compound classes in the filtered POM with depth. Relative concentrations of pyrolysis markers for chlorophyll, lipids, carbohydrates and protein are shown as a function of depth in Figs. 7 and 8. The relative standard deviation inherent in repeated Py-GC/MS analyses is about 10% (Fabbri et al., 1998).

### 3.4. Chlorophyll markers

Phytadienes, which are clearly observed in the pyrograms, serve as markers of chlorophyll (CHL) since they are generated by the pyrolysis of the phytol side chain esterified to the tetrapyrrole ring (van de Meent et al., 1980; Ishiwatary et al., 1991). Previous workers have ascribed the origin of pristene to zooplankton and to tocopherols (Blumer et al., 1964; Goosens et al., 1984) and its abundance may be a reflection of precursor other than chlorophyll. Comparison of Fig. 5b and Fig. 7a reveals the closeness of the relationship between the vertical variation of the POC:CHL-a ratio and of the relative concentration of phytadienes (which is a measure of the chlorophyll per particle pyrolysed). The highest relative concentrations of phytadienes were determined at the depth of the POC:CHL-a minimum coinciding with the deep CHL-a maximum. In the oxycline, below the 0.1% light depth, relative concentrations of phytadienes declined steeply and dropped to low levels at the depth of the nitrate maximum ( $\sigma_t = 15.4$ , Fig. 3b), where the POC:CHL-a ratio exceeded 700.

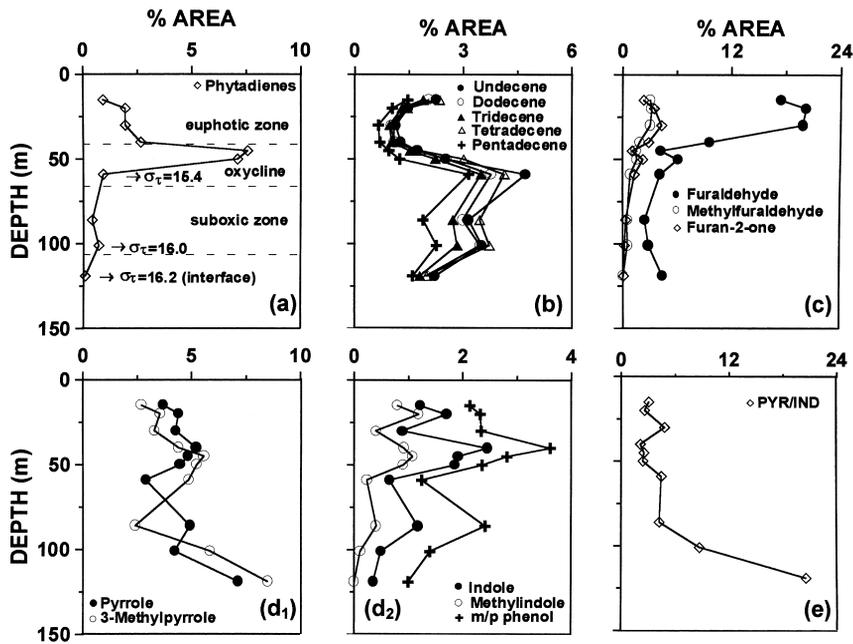


Fig. 7. Vertical profiles of pyrolysis markers for (a) chlorophyll, (b) lipid, (c) carbohydrate, (d) protein, (e) pyrrole to indole ratio as relative concentrations in open station.

In the suboxic zone below the nitrate maximum, the phytadienes were nearly invariant and their concen-

trations became undetectable within the sulphidic water interface ( $\sigma_t = 16.2$ ).

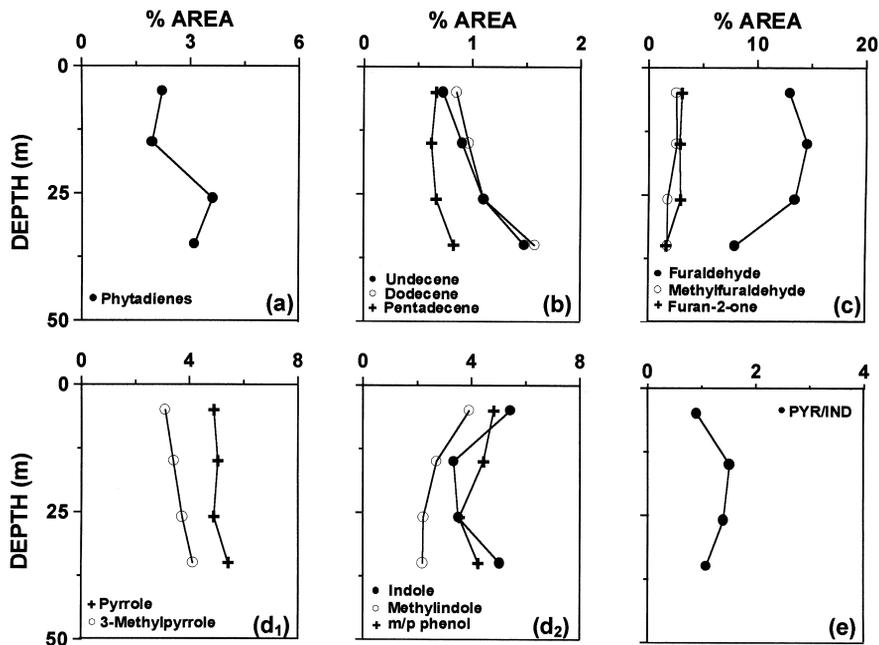


Fig. 8. Vertical profiles of pyrolysis markers for (a) chlorophyll, (b) lipid, (c) carbohydrate, (d) protein, (e) pyrrole to indole ratio as relative concentrations in coastal station.

### 3.5. Lipid markers

All particulate samples generated virtually identical distributions of *n*-alk-1-ene/*n*-alkane doublets on pyrolysis. The doublets spanned a carbon number range extending to C19 and maximized at C10–C15. The alkanes were present at much lower intensities than the alkenes. POM in the euphotic zone consisted at least partly of phytoplankton, and the *n*-alkenes/*n*-alkanes present in samples of these depths are thought to be derived from lipid constituents of phytoplankton containing long methylene chains in their structure. Among them fatty acids and corresponding derivatives (salts, triglycerides), waxes (Saiz-Jimenez, 1995; Hartgers et al., 1995; McKinney et al., 1996), as well as non-saponifiable aliphatic biopolymers (algaenans) recognised in certain phytoplankton species (Gelin et al., 1999) upon pyrolysis may yield *n*-alkanes/*n*-alk-1-enes. When subjected to thermochemolysis in the presence of tetramethyl ammonium hydroxide (TMAH), fatty acid derivatives yield the corresponding methyl esters without significant degradation (Dworzanski et al., 1990). When simple esters are pyrolysed in the presence of TMAH they are converted into corresponding methyl esters without the formation of alkenes (Challinor, 1994). For the present samples, it has been found that, whereas only low concentrations of fatty acids were observed under normal Py-GC/MS conditions, in the presence of TMAH, the proportion of alkenes decreased sharply and methyl esters of fatty acids became prominent (Fig. 6b). This implies that fatty acid derivatives are an important source of the alkenes detected by Py-GC/MS (Hatcher and Clifford, 1994). There was no evidence of higher molecular weight or hydroxylated FAMES characteristic of terrestrial vegetation (del Rio and Hatcher, 1998). The depth profiles in Fig. 7b show the relative concentrations of lipids to decrease slowly from the surface mixed layer to the lower euphotic zone of the open sea while an opposite trend is apparent in the coastal water samples (Fig. 8b). In the open sea, the relative concentrations of lipids show a second increase in the oxycline, reaching maximum intensities at the oxic to suboxic transition zone ( $\sigma_t = 15.4\text{--}15.6$ ). In this layer, since the decrease in the POC concentration was less pronounced than in the total CHL-a (Fig. 4a and Fig. 5a) the POC:CHL-a ratio increased

dramatically (Fig. 5b), consistent with the depth profiles of CHL markers (Fig. 7a). In the suboxic waters, the composition of the alkenes remained unchanged but their relative concentration appeared to slowly decrease in the sulphidic waters below the suboxic zone. The absence of lipid markers characteristics of bacterial alteration of POM does not necessarily mean that bacterial reprocessing is unimportant. Rather the bacteria may biosynthesise lipids similar in composition to that of the particles they are degrading (Wakeham, 1995). We failed to unambiguously determine the presence in the pyrograms of 1,2,3,4-tetramethylbenzene, which has been proposed as a pyrolysis marker for photosynthetic green sulphur bacteria (Hartgers et al., 1994).

### 3.6. Carbohydrate markers

The observed furans are typical pyrolysis products of carbohydrates, monosaccharides and polysaccharides (e.g., cellulose, starch, galactans) (Simmonds et al., 1969; Shafidazeh, 1982; van der Kaaden et al., 1983; Helleur et al., 1985). Similar to results from a Py-GC/MS study of Mediterranean sinking particles (Peulvé et al., 1996), levoglucosan, a typical pyrolysis product of polysaccharides, though not of biomass (Fraga et al., 1991), was not detected. However, the presence of carbohydrates was confirmed by TMAH thermochemolysis of POM (Fig. 6) which resulted in the production of 1,2,4-trimethoxybenzene and methylated deoxyaldonic acids, characteristic markers for mono- and polysaccharides (Fabbri and Helleur, 1999). Ishiwatary et al. (1995) found pyrolysis yields of furaldehyde to correlate with the total carbohydrate content of oceanic particles. Hence, the relative concentration of furans observed here may be assumed to represent the relative contribution of carbohydrates in our samples. The relative concentrations of furans indicate carbohydrate concentrations remain approximately constant within the euphotic zone at both stations but relative concentrations of all carbohydrate markers decreased markedly to minimal levels immediately below the euphotic zone (Fig. 7c) where the CHL marker displayed a coherent maximum (Fig. 7a). Thus, for example, in the cyclonic region, the relative furaldehyde concentration diminished by a factor of about four when the suboxic zone was reached at 60 m, while the lipid

markers consistently displayed opposite trends. At the bottom of the euphotic zone, the comparative decrease of carbohydrate results in lower C:N ratios (Fig. 4d), close to the Redfield value (Goldman, 1986).

### 3.7. Protein markers

Figs. 7d and 8d show the relative abundances of protein markers in the POM filtered from the Black Sea water column. In the euphotic zone where the POM contains a measurable quantity of phytoplankton (Fig. 5d), especially of dinoflagellates in the summer of 1996 (Uysal et al., 1998), most of the protein will occur as enzymes. The pyrolytic production of pyrroles, indoles and aromatic nitriles has been shown to indicate the presence of proteins or amino acids (Tsuge and Matsubara, 1985; Ishiwatary et al., 1995), though pyrolysis of other materials, such as melanoidin-like macromolecules, may also contribute (Peulvé et al., 1996). C3–C4 pyrroles also observed in the chromatograms may drive from chlorophyll and other tetrapyrroles (Sinninghe Damsté et al., 1992); however, their relative concentrations are too low for pigments to have been significant precursors of pyrrole and methylpyrroles. There being no clear lignin pyrolysis markers observable in the POM; phenol and *p*-methyl phenol probably arise from proteins (tyrosine) or from algal polyphenols (van Heemst et al., 1996). The presence of proteins appears to be confirmed by the identification in the pyrograms of low levels of alkylated dihydro-pyrrolediones and pyrrolidinediones (characteristic ions at  $m/z$  152, 166 and 180), specific markers of two adjacent aliphatic amino acids in (poly) peptides (Boon and de Leeuw, 1987), which have been used previously as qualitative representatives of the content of proteins in marine POM (Peulvé et al., 1996). The protein markers fall into two groups (Fig. 7d); depending on whether the relative concentrations increase (pyrrole, methyl pyrrole and the aromatic nitriles) or decrease (indole, methyl indole and the phenols) with depth. Most of them displayed a well defined maximum near the depth of the DCM formed just below the euphotic zone in the cyclonic open sea (Figs. 7d and 5a). This coincides with where the POC:PON ratio and the relative abundances of the CBH markers apparently

decreased (Figs. 4a and 7c). Pyrroles and indoles display different depth-profiles below the euphotic zone, suggesting that, at such depths, they have different sources. The suspended POM in the water column below the euphotic zone is presumably composed of aggregated bacteria, small fecal pellets and material adsorbed on inorganic particles. Their protein composition may differ from that of the euphotic zone POM. Despite the changes in their relative concentrations with depth, pyrrole to indole ratio was almost invariant throughout the euphotic zone, suggesting the protein composition of phytoplankton to be independent of the mode of growth limitation. The ratio increases slightly in the thermocline (a steep density gradient zone) where heterotrophic bacteria may have accumulated selectively.

## 4. General discussion and conclusions

The distribution and chemical composition of POM in marine environments is determined by various interacting biotic and abiotic factors in the water column. The permanently stratified upper layer of the Black Sea forms a good natural laboratory for understanding the relative importance of such factors on the abundance and chemical composition of bulk POM in different water layers of distinct biochemical characteristics. In the well oxygenated surface mixed layer, extending down to nearly 10% light depth, the suspended POM possessed relatively high POC:PON and C:CHL-*a* ratios at both open and coastal stations in June 1996. In the nutrient-poor surface mixed layer of the studied sites the PON:PP ratio was similar to the typical Redfield N:P value of 16, indicating similar sources sustain the POM pool (composed of mainly detrital and partly living algal biomass). Elevated relative abundances of CBH pyrolytic markers and lower relative concentrations of protein markers in the POM from the surface mixed layer, compared to the deeper layers, confirm this conclusion (carbonaceous rich, detritus-dominated POM pool in the upper depths of the euphotic zone) derived from the classical analyses. Vostokov and Vedernikov (1988) had previously reached the same conclusion based on biological data obtained in the summer. The POC:PON ratio decreased apparently below the steep thermocline (at about 10% light

depth) down to about 0.5–1% light depth, very consistent with the coherent decline in the relative abundance of CBH pyrolysis markers. When the 0.5% light depth was reached in the upper nutricline of the open station, the CHL and protein markers both reached peak values, despite the decrease in the relative abundances of CBH markers. At this depth the POC:PON and POC:CHL-a ratios consistently dropped to the lowest levels, indicating the increasing contribution of algal biomass to the POM pool (having less carbohydrates) in the light-shaded nutricline zone. Interestingly, the N:P ratios (14–18) of POM produced under nutrient-limited summer conditions remained relatively constant in the euphotic zone even though the carbohydrate and algal biomass components varied markedly with depth. Though the coastal surface waters possessed a higher nitrate to phosphate ratio than in the open seas, the N:P ratio of POM did not vary with region. In the light-limited region of the euphotic zone there was a limited abundance of carbohydrates (probably in the form of starch) but increased proportions of proteins in the POM, especially at the open station. This influenced the relative abundances of protein, CBH and CHL markers while the PON:PP ratio was affected only slightly. Evolution of phytoplankton in the light-limited zone has selected those cells with increased chlorophyll concentrations, though it is not yet clear whether this involves larger or more numerous chloroplasts or greater numbers of chlorophyll molecules per chloroplast.

In the oxic-to-anoxic transition zone profound changes occur in the chemical properties of the water column. Not unexpectedly, chemo-autotrophic production occurs in addition to heterotrophic bacterial activities (Bird and Karl, 1991; Karl and Knauer, 1991). Interestingly the POM samples from the suboxic zone and sulphidic water interface of the open station contained low relative concentrations of carbohydrate markers, high relative abundances of lipid markers (Fig. 7b,c), and different protein composition (pyrrole:indole ratio). Pyrograms of the lipid markers show that lipid composition remains almost unchanged in the suboxic zone. The decrease in the relative concentration of lipid markers at the anoxic interface is presumably due to lipid consumption by anaerobic bacteria. Bird and Karl (1991) estimated that bacterial biomass from the oxycline to the anoxic

interface amounts to about 1  $\mu\text{M}$  of organic carbon, corresponding to nearly 40% of POC filtered from this layer. Thus, the bacterial synthesis of organic matter and bacterial biomass should increase within the suboxic/anoxic transition zone (Bird and Karl, 1991; Coble et al., 1991; Repeta and Simpson, 1991). Although no clear bacterial markers could be observed from the pyrograms, we suggest a significant fraction of the measured POM in this layer is bacterial in origin, and is mixed with partially decayed sinking particles with relatively high C:N ratios (Karl and Knauer, 1991). This inference is supported by the work of Wakeham and Beier (1991) who found seston retained on GF/F filters to be of bacterial origin.

The absence of lignin and lipid markers from terrestrial vegetation demonstrates that, consistent with the observations of Wakeham et al. (1991), large, rapidly sinking biogenic particles collectable in sediment traps do not significantly contribute to the chemical composition of POM.

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