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Edited by Ayşen YILMAZ

Editorial Assistance: Devrim Tezcan
Middle East Technical University,
Institute of Marine Sciences
Erdemli, İçel, Turkey

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Ayşen YILMAZ (Ed.)

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Aysen YILMAZ
Editor

Oceanography of Eastern Mediterranean and Black Sea

Similarities and Differences of Two Interconnected Basins

Macromolecular characterisation of phytoplankton and Black Sea Suspended Particulate Organic Matter (SPOM) by analytical pyrolysis

Y. Çoban-Yıldız[1], V. Baravelli[2], D. Fabbri[2], I. Vassura[2], A. Yılmaz[1], S. Tuğrul[1], E. Eker-Develi[1]

1:Institute of Marine Sciences, Middle East Technical University, Turkey
yesim@ims.metu.edu.tr; yilmaz@ims.metu.edu.tr; tugrul@ims.metu.edu.tr; elif@ims.metu.edu.tr
2: Lab. of Chemistry c/o ITIS CIRSA - University of Bologna, Italy
dfabbri@ambra.unibo.it; i.vassura@ambra.unibo.it

Abstract- Particulate organic matter suspended (SPOM) in the Black Sea water column was characterised by Pyrolysis-GC/MS and compared with pure cultures of phytoplankton grown at lab. Similar markers for lipid, protein, carbohydrate and chlorophyll were obtained in SPOM of the Black Sea euphotic zone and phytoplankton cultures, though relative contribution of lipids and carbohydrates to the Black Sea SPOM was higher. Through the water column, lipid to protein ratio seems to determine the C/N ratio of SPOM. Below the oxycline, relative contribution of proteins to SPOM pool increased while the lipid content of SPOM decreased. Protein composition changed remarkably at suboxic/anoxic transition zone where in-situ light transmission indicated particulate accumulation. Elemental sulphur was observed in particulates suspended at the upper anoxic zone, and the relative intensity of S_8 peak was regionally variable in agreement with the lateral flux of O_2.

Keywords- Suspended particulate organic matter, Black Sea, phytoplankton, lipid, protein, carbohydrate, chlorophyll, elemental sulphur

Introduction

Investigation of origin and composition of particulate organic matter suspended (SPOM) in the Black Sea has particular importance due its unique hydro-chemical properties with permanent oxic/suboxic and anoxic zones. SPOM in each layer would reflect associated bio-chemical processes, therefore characterisation of Black Sea SPOM through the water column is essential. Supported with the traditional oceanographic parameters, utility of Pyrolysis - Gas Chromatography / Mass Spectrometry for the characterisation of particulate organic matter of different marine environments (Saliot *et al.*, 1984; Sicre *et al.*, 1994; Ishiwatari *et al.*, 1995; Peulve *et al.*, 1996; Çoban-Yıldız *et al.*, 2000 a, b) has recently been reported. In this study, first, lipid, protein, carbohydrate and chlorophyll content of phytoplankton grown in lab conditions and particulate organic matter suspended in the euphotic zone of the Black Sea was compared. Then, regional and vertical variations in macromolecular composition of SPOM in the Black Sea were discussed regarding processes in the water column.

Sampling and Analytical Methods

SPOM samples were collected at 5 stations in central, continental shelf and shelf-break regions, in May 2001 aboard R/V Knorr (Fig. 1). In addition, phytoplankton cultures of three species found in the Black Sea, *Emiliana huxleyi* (coccolithophore), *Skelotenama costatum* (diatom) and *Prorocentrum micans* (dinoflagellate) were used as a reference information for field samples. Phytoplankton cultures were grown on f/2 medium (without nutrient limitation) at a constant temperature of 20 ± 1 ^{0}C under 12 hour light (10-30 µE m^{-2} s^{-1}) and 12 hour dark condition. The cultures were harvested at the end of log growth phase.

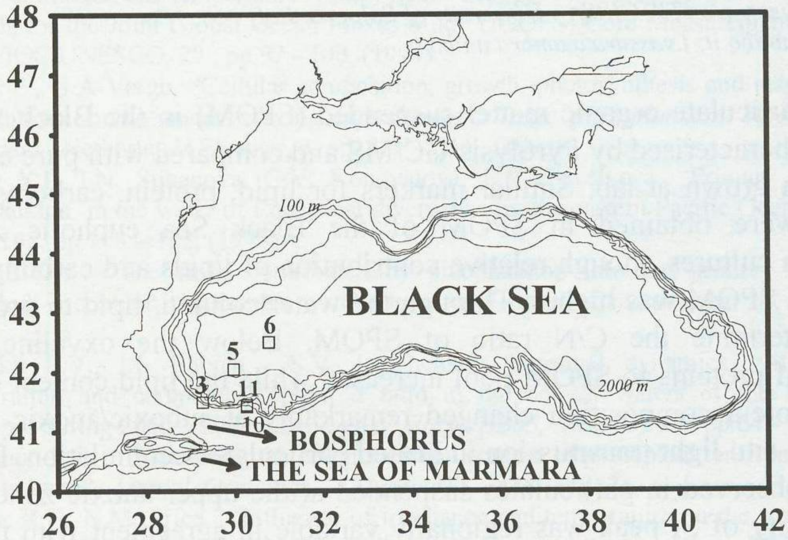

Fig. 1. Station location map for this study

Organic material is thermally decomposed in an inert atmosphere (pyrolysis) to yield volatile fragments amenable to GC/MS analysis. Normalised peak areas (peak area of the marker / summed peak area of all markers x 100) of the pyrolysis products are termed as 'relative concentration' of the marker. Both culture and field samples were analysed at the same analytical conditions and same markers were selected for quantitation for ease of comparison. These products were then classified as carbohydrate, protein, lipid and chlorophyll markers, as discussed previously (Çoban-Yıldız et al., 2000a,b). Replicate pyrolysis analyses of a single algal culture sample provide the precision of product distribution related to the analytical procedure. The coefficient of variation was less than 10 % for the four main groups.

Results and Discussion

Table 1 summarises the euphotic zone characteristics of the south-western Black Sea in May 2001. Nutrients were depleted due to seasonal stratification and limited external input to the surface layers. Concentrations of suspended particulate organic carbon, SPOC and nitrogen, SPON were very high compared to low chlorophyll-a concentrations (Table 1) and low C and N productivity by phytoplankton (Çoban-Yıldız et al., this volume). The findings strongly suggest significant contribution of detrital and partly herbivorous organic matter to the SPOM pool in the euphotic zone. Microscopic analyses have shown the dominance of dinoflagellates (Soydemir et al., 2002), and likelihood of potential contribution of mixotrophy.

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The mean distribution of pyrolysis products released from SPOM collected in the euphotic layers of different sites and phytoplankton cultures were similar to each other (Table 2). For both euphotic zone SPOM and phytoplankton cultures, protein markers had the highest contribution followed by carbohydrates and lipids. Nevertheless, relative contribution of lipids and carbohydrates to the Black Sea particulates was two folds higher than cultures, with lower chlorophyll contribution. As a result, ratio of proteins to lipids was lower in field samples while CBH:CHL ratio was almost 6 times higher, in agreement with higher C/N elemental ratio of Black Sea SPOM (Table 2). These indicate nitrogen limitation as well as heterogenous composition of suspended matter in the euphotic zone. We used ratio of pyrrole to indole, markers for different proteins (Chiavari and Galetti, 1992) as an index of the variation in protein composition and it seems that protein composition of surface suspended matter in the Black Sea is similar to lab cultures analysed. Pyrolysis products of phytoplankton have lower alkene/alkane ratio suggesting that this ratio increases with increasing contribution of decomposed organic matter. As shown by standard deviations, largest inter-species and regional variation was observed for chlorophyll and carbohydrate markers.

Table 2. Distribution of pyrolysis products as means of 3 cultured phytoplankton species and euphotic zone averages of 5 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. C to N atomic ratio was obtained by elemental analyser. All are given as mean ± standard deviation; standard deviation represents regional variation for field samples and interspecies variation for phytoplankton cultures.

SAMPLE	LIPID (%)	PROT (%)	CBH (%)	CHL (%)	PYRR. / INDOLE	ALKENE / ALKANE	C / N
CULTURE (n = 3)	7 ± 2	38 ± 4	8 ± 4	4 ± 3	0.9 ± 0.3	1.2 ± 0.8	8.1 ± 1.1
FIELD (n = 5)	14 ± 3	30 ± 2	16 ± 5	2 ± 1	1.3 ± 0.3	5.3 ± 1.8	12 ± 2

Fig. 2 shows the vertical variation of nutrient, DO/H_2S, particulate organic C and N concentrations and pyrolysis products released from SPOM at the central station (STA 6). It seems that high organic matter load after spring bloom caused over-consumption of DO, which resulted in rising of suboxic layer. As a result of enlarged denitrification zone, pre-formed nitracline was eroded, and oxycline was located at shallower depths than the nitracline.

The SPOM concentrations were high in the euphotic zone, peaked at the fluorescence maximum layer, which was located at the depths of sharp seasonal thermocline, and as expected, decreased with depth below the thermocline down to the suboxic zone (Fig. 2). At the depth of the SPOM peak, both C/N ratio and lipid content of SPOM also reached the peak values while protein and carbohydrate markers decreased (Fig. 2). The formation of such characteristic features within the steep thermocline, corresponding to about 1% light depth, strongly suggests the occurrence and accumulation of specific autotrophic organisms with higher lipid and lower protein content, though partial contribution from herbivorous activity could not be ruled out.

Below the fluorescence maximum layer, in the oxycline, both C/N ratio and lipid content of SPOM was lower compared to the fluorescence maximum values, though lipid content was higher than the surface. Therefore, the SPOM pool in the fluorescence maximum layer was either recently formed or self-sustained by in-situ remineralisation. Relative distribution of lipid and protein markers displayed an opposite trend below the oxycline; the protein content of SPOM increased as the lipid decreased. These features are quite different than those observed for the oxygenated open oceans, emphasising the critical role of redox dependent in-situ chemo-autotrophic and -heterotrophic processes producing SPOM in the transition zone of the Black Sea. Relative distribution of carbohydrates was quite uniform as the lipids decrease below the euphotic zone. Lipid to protein ratio was in very good agreement with the C/N ratio, suggesting that, at least for this station, C/N ratio of SPOM was mainly determined by the lipid content of bulk SPOM through the water column. Ratio of carbohydrate to chlorophyll markers decreased at the depths between 1 – 0.1 % surface light levels, reflecting shade adaptation. Alkene/alkane ratio was in perfect agreement with the CBH/CHL ratio and confirms that, in the oxycline, dominance of refractory organic matter was only confined to a narrow layer in the oxycline. At the top of the nitracline, the decrease in both CBH/CHL and alkene/alkane ratio indicate new formation of organic matter due presumably to denitrification processes in the unexpectedly enlarged suboxic zone towards the surface. Decrease in CBH/CHL ratio in this layer should be further investigated. The suboxic/anoxic interface was characterised by the lowest alkene/alkane ratio (Fig. 2) and change in protein composition (Fig. 3).

Vertical variation in protein composition is striking. At all the stations investigated, with the onset of light transmission minimum at the base of the suboxic zone, the ratio of pyrrole to indole changed (Fig. 3). This is a clear sign of a change in protein composition, likely to derive form in-situ microbial processes limited to a certain layer. The change was more pronounced for the shelf-break stations, where the negative peak in light transmission minimum was much more intense as a result of lateral intrusion of oxygenated waters from the Bosphorus region and re-suspension of surface sediments in the shelf-break region.

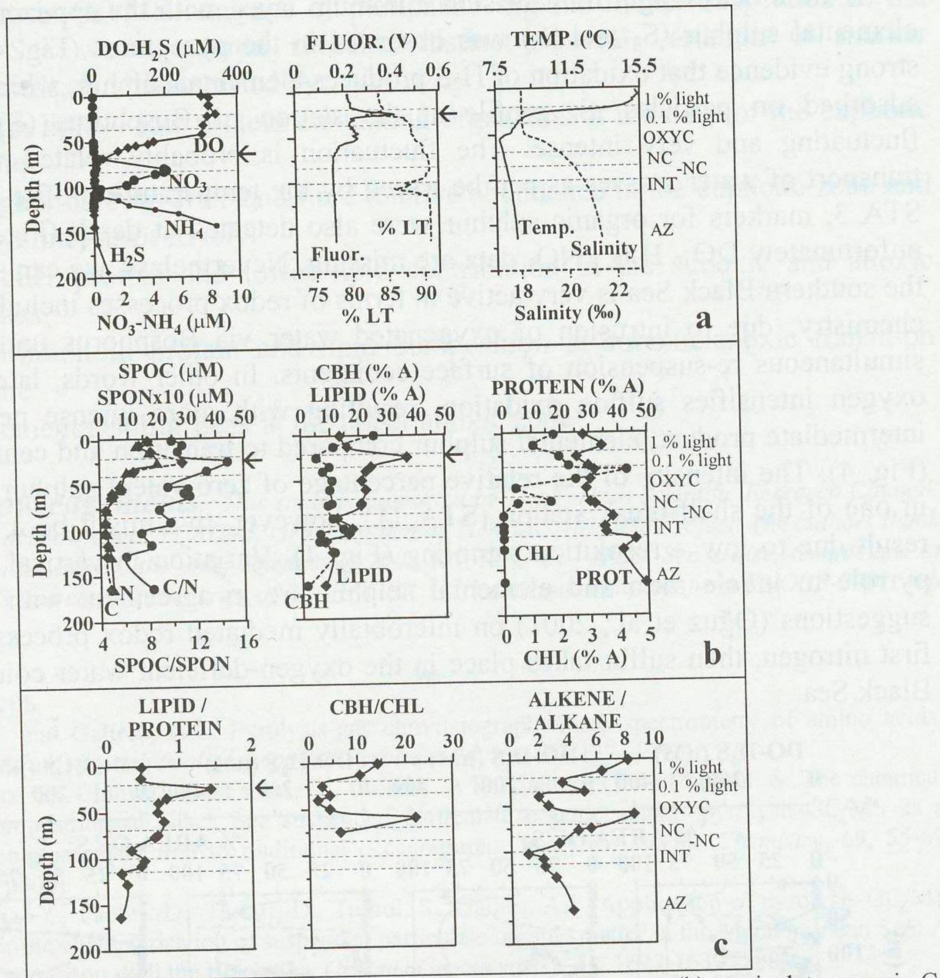

Fig. 2. Vertical variation of (a) nutrient, DO/H$_2$S and physical parameters, (b) particulate organic C and N concentrations and pyrolysis products (% area) released from SPOM at the central station (STA 6) and (c) ratio of pyrolysis products.

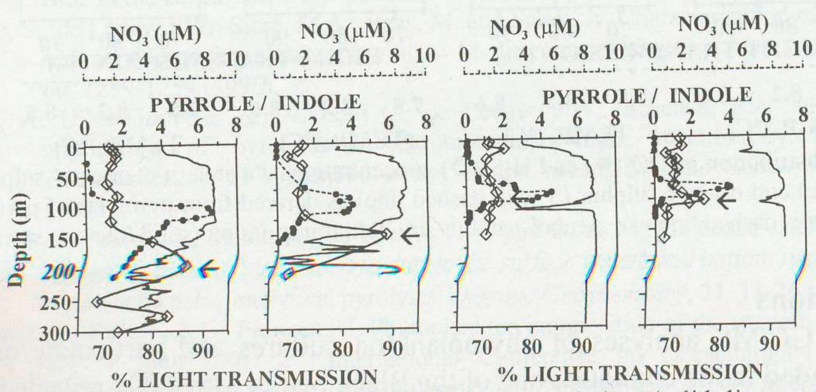

Fig. 3. Vetical distribution of NO$_3$ (●), % light transmission (solid lines w/o symbol) and pyrrole/indole ratio (◇) at different regions of the Black Sea in May 2001. Arrows show the peak in pyrrole/indole ratio.

A little below light transmission minimum layer, with the appearance of H_2S, elemental sulphur (S_8 and S_6) was observed in the pyrograms (Fig. 4). There is strong evidence that oxidation of H_2S produces elemental sulphur, which, in turn is adsorbed on particles. S_8 profile at the station off-Bosphorus (STA 3) was fluctuating and very intense. The fluctuation is probably related with lateral transport of water masses as can be traced by the temperature profile (Fig. 4). At STA 3, markers for organic sulphur were also detected at depth (Fig. 4), where, unfortunately DO - H_2S - NO_3 data are missing. Nevertheless, we can suggest that the southern Black Sea is very active in terms of redox processes including sulphur chemistry, due to intrusion of oxygenated water via Bosphorus underflow and simultaneous re-suspension of surface sediments. In other words, lateral flux of oxygen intensifies sulfide oxidation, resulting with more intense peaks of the intermediate product, elemental sulphur compared to transition and central regions (Fig. 4). The intensity of the relative percentage of zero-valent sulphur was lower in one of the shelf-break station (STA 11); however, this might be a misleading result due to low – resolution sampling (Fig. 4). Variation in vertical profiles of pyrrole to indole ratio and elemental sulphur are in agreement with the recent suggestions (Oğuz et al., 2001) on microbially mediated redox processes, which, first nitrogen, then sulfur takes place in the oxygen-deficient water column of the Black Sea.

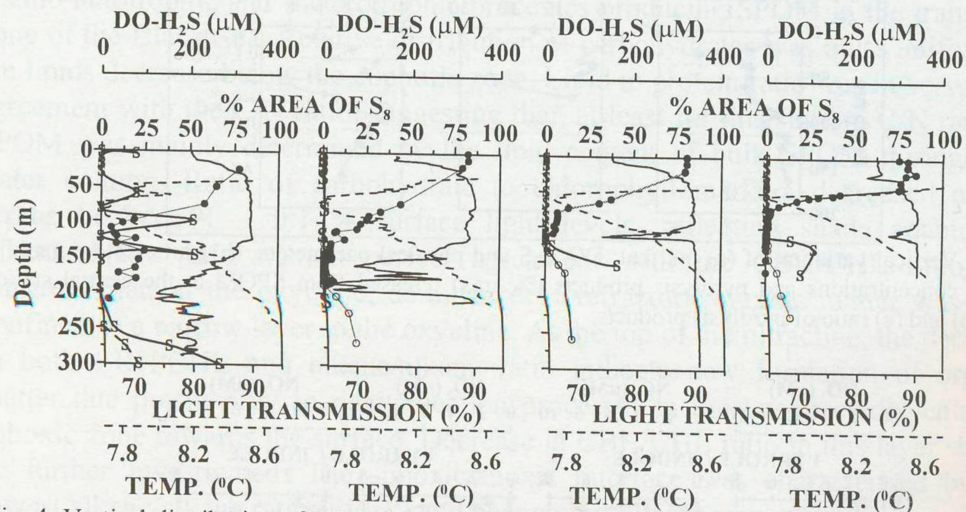

Fig. 4. Vertical distribution of DO (●) and H_2S (O) concentrations, % area of elemental sulphur, S_8 (S, with solid line) and organic sulphur (S with dashed line) as derived from pyrolysis of particulate matter, temperature (dashed line w/o symbol) and *in-situ* light transmission (solid line w/o symbol).

Conclusions

1- Pyrolysis GC/MS analyses of phytoplankton cultures and particulate organic matter suspended in the euphotic zone of the Black Sea in May 2001 revealed:

- Similar pyrolysis markers with similar distribution and
- Higher lipid and carbohydrate content of euphotic zone SPOM, in agreement with its heterogenous composition in May-2001.

2-Pyrolysis GC/MS analyses of particulate organic matter suspended in the Black Sea water column revealed distinct vertical variation of marker compounds, as characterised by:

- High lipid – low protein containing organisms at the base of the euphotic zone
- Higher carbohydrate and lipid relative abundance in the euphotic zone and oxycline, respectively.
- Higher protein and lower lipid contribution in the suboxic and anoxic zones
- Variation in protein and lipid composition at suboxic/anoxic transition zone
- Elemental sulfur peak at the upper anoxic zone

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1:Institute of Marine Sciences, Middle East Technical University, Turkey
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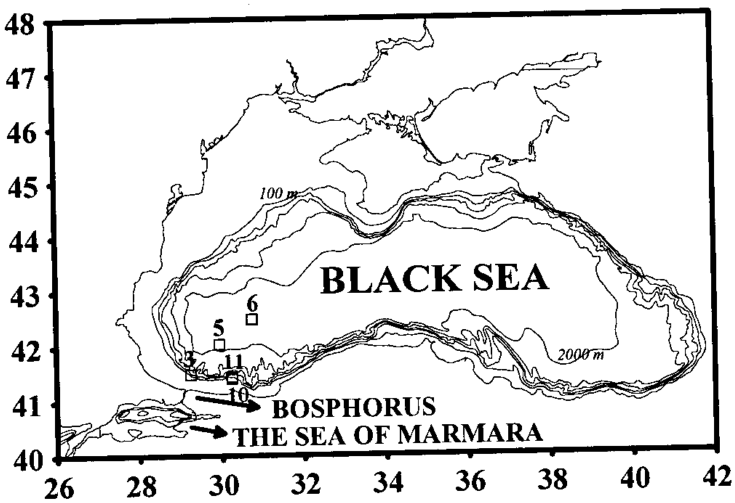

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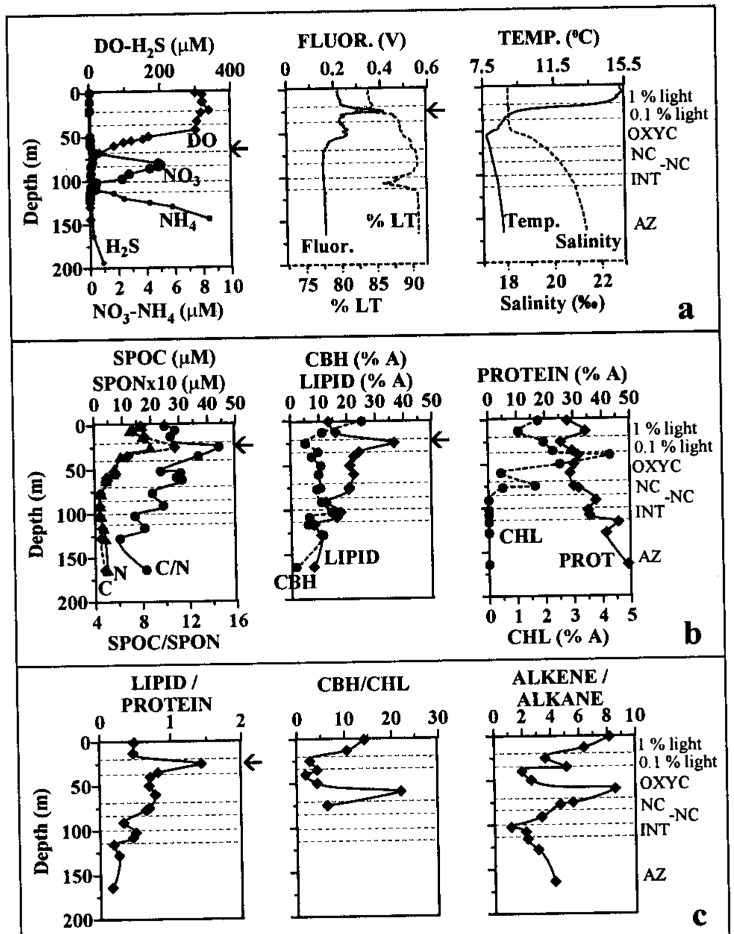

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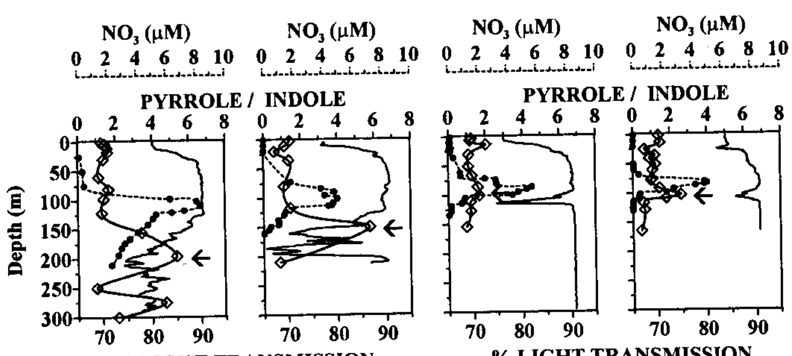

Fig. 3. Vetical distribution of NO_3 (●), % light transmission (solid lines w/o symbol) and pyrrole/indole ratio (◇) at different regions of the Black Sea in May 2001. Arrows show the peak in pyrrole/indole ratio.

A little below light transmission minimum layer, with the appearance of H_2S, elemental sulphur (S_8 and S_6) was observed in the pyrograms (Fig. 4). There is strong evidence that oxidation of H_2S produces elemental sulphur, which, in turn is adsorbed on particles. S_8 profile at the station off-Bosphorus (STA 3) was fluctuating and very intense. The fluctuation is probably related with lateral transport of water masses as can be traced by the temperature profile (Fig. 4). At STA 3, markers for organic sulphur were also detected at depth (Fig. 4), where, unfortunately DO - H_2S - NO_3 data are missing. Nevertheless, we can suggest that the southern Black Sea is very active in terms of redox processes including sulphur chemistry, due to intrusion of oxygenated water via Bosphorus underflow and simultaneous re-suspension of surface sediments. In other words, lateral flux of oxygen intensifies sulfide oxidation, resulting with more intense peaks of the intermediate product, elemental sulphur compared to transition and central regions (Fig. 4). The intensity of the relative percentage of zero-valent sulphur was lower in one of the shelf-break station (STA 11); however, this might be a misleading result due to low – resolution sampling (Fig. 4). Variation in vertical profiles of pyrrole to indole ratio and elemental sulphur are in agreement with the recent suggestions (Oğuz et al., 2001) on microbially mediated redox processes, which, first nitrogen, then sulfur takes place in the oxygen-deficient water column of the Black Sea.

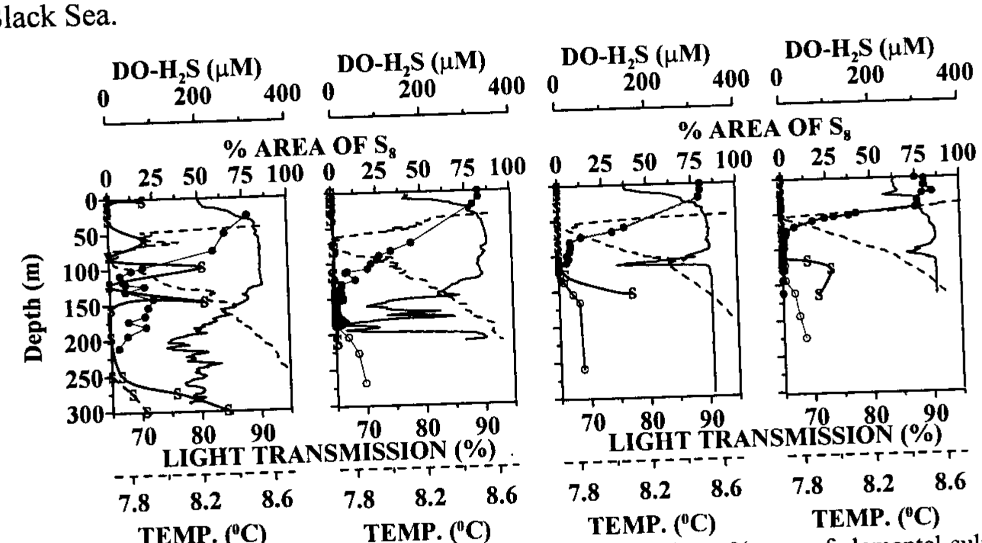

Fig. 4. Vertical distribution of DO (●) and H_2S (O) concentrations, % area of elemental sulphur, S_8 (S, with solid line) and organic sulphur (S with dashed line) as derived from pyrolysis of particulate matter, temperature (dashed line w/o symbol) and *in-situ* light transmission (solid line w/o symbol).

Conclusions

1- Pyrolysis GC/MS analyses of phytoplankton cultures and particulate organic matter suspended in the euphotic zone of the Black Sea in May 2001 revealed:

- Similar pyrolysis markers with similar distribution and
- Higher lipid and carbohydrate content of euphotic zone SPOM, in agreement with its heterogenous composition in May-2001.

2-Pyrolysis GC/MS analyses of particulate organic matter suspended in the Black Sea water column revealed distinct vertical variation of marker compounds, as characterised by:

- High lipid – low protein containing organisms at the base of the euphotic zone
- Higher carbohydrate and lipid relative abundance in the euphotic zone and oxycline, respectively.
- Higher protein and lower lipid contribution in the suboxic and anoxic zones
- Variation in protein and lipid composition at suboxic/anoxic transition zone
- Elemental sulfur peak at the upper anoxic zone

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