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Oil Pollution of Marine Algae

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Petroleum hydrocarbons are important pollutants of sea and marine organisms. The origin of hydrocarbons are either biogenic (endogenic) which are synthetised by marine organisms or exogenic due to oil pollution accumulated by marine organisms. The hydrocarbons found in algae were biogenic (Clarck and Blumer 1967;Youngblood et al.1971; Rossi et al. 1978; Youngblood and Blumer 1973) or exogenic (George 1961; Farrington and Tripp 1977; Miranov et al. 1981; Knutzen and Sortland 1982; Peckol et al. 1990). Some characteristics used to distinguish the origin of hydrocarbons in the marine ecosystem were the ratios of pristane (Pr)/phytane (Ph), C 17/Pr, C18/Ph and CPI (Carbon Preferences Index) values (Clarck and Finley 1974; Gearing et al. 1976; Farrington and Tripp 1977) and also the existence of alkenes and aromatic compounds.

In this work oil pollution was investigated on the surface and inside of algae

MATERIALS AND METHODS

The algae were collected from the northern and southern ends of the Bosphorus. The sampling sites are shown in Fig. 1. The algae collected from April-Dec. 1995 were green; *Ulva lactuca* L, *Enteromorpha linza* J. Agard, brown; *Cystoseira barbata* J. Agard, red; *Ceramium rubrum* (Huds.) J. Agard, *Pterocladia capillacea* (Grev.)Thuret et Bornet.

Determination of oil pollution on the surface of the algae: 100 g wet weight alga was rinsed a few second with 3x50 ml dichloromethane (DCM), then filtrated through a filter paper. The algal residue was separated (a) and filtrate was dryed over anhydrous sodium sulphate then distilled under vacuum. The residue was taken with hexane and the volume adjusted to 10 ml and analysed by UV spectrofluorometry (UVF) and GCMS.

Determination of oil inside of algae: the algae residue as described above (a), was dried in the open air and milled, extracted with DCM in Soxhlet for 4 h. The extract was distilled under vacuum. The residue was saponified with 0.5 % KOH in methanol for 1 h, 50 ml distilled water added and transferred to a separatory funnel, shaken with 50 ml pentane. The organic phase was separated and dried over anhydrous sodium sulphate and distilled under vacuum. The residue was taken with hexane, adjusted to 10 ml and analysed by UVF and GC/MS.

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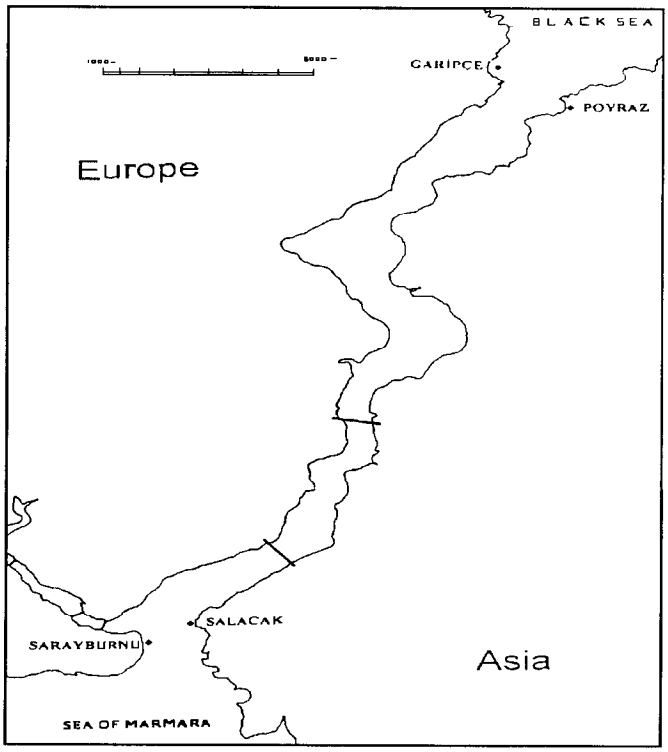

Figure 1. Sampling stations

All solvents used are HPLC grade (Lab- Scan) and controlled by GC/MS.

The amount of oil in algae was determined by using the standard curve and calculated µg/g wet weight for surface and dry weight for inside of algae. The standard curve was plotted with Russian crude oil as a reference since it was the kind of oil mainly transported via Bosphorus The concentrations of reference oil were 0.25-1.5 µg/ml in hexane. The fluorescence intensity was measured by UVF (Spectrofluorophotometer, Shimadzu RF-1501) at 310/360 nm (ex/em) (Ehrhardt et al. 1993).

GCMS analysis was made in hexane extract by HP 6890 capillary GC connected to a Hewlett Packard Mass Selective Detector (MSD) controlled by HP ChemStation. Operating conditions were: 50 mx0.20mm fused HP PONA, methyl siloxane, glass capillary column, oven temperature programme: 110-290°C at 10°C/min, at 10°C/min, 290°C at 10 min, split injector temperature 250°C the carrier was gas helium, flow rate 1.2 ml/min.

The aliphatic and polyaromatic compounds were analysed by GC/MS. Identification of each compound in algal extract was made by comparing the retention times and its spectrum with that taken from HP memory and also with the EPA standard (PM-6 10).

Table 1. The amount of oil in algae (µg/g, wet weight).

Station	Month Year	*U. lactuca*		*E. linza*		*C. barbata*		*C. rubrum*		*P. capillacea*	
		Surface	Inner part	Surface	Inner part	Surface	Inner part	Surface	Inner part	Surface	Inner part
GARİPÇE	4/95	0.42	6.50	0.04	3.58	19.59	-	38.09	196.76	4.97	21.70
	5/95	0.14	0.62	0.09	35.34	2.88	8.08	0.02	3.46	7.73	15.01
	6/95	-	-	0.04	30.67	0.25	69.37	0.46	0.49	-	-
	12/95	-	-	0.98	23.92	2.65	91.11	-	-	6.89	30.39
POYRAZ	4/95	2.72	15.82	2.33	5.40	2.06	11.97	0.94	24.61	6.41	29.00
	5/95	0.05	4.15	0.57	106.6	5.21	16.03	0.38	32.24	5.64	21.68
	6/95	-	-	0.33	14.47	0.26	17.87	1.24	1.35	-	-
	12/95	-	-	0.16	8.39	0.71	2.22	-	-	0.48	4.92
SARAYBURNU	4/95	3.15	-	0.18	5.48	x	x	0.91	7.59	0.41	150.00
	5/95	2.44	12.78	0.28	3.10	x	x	-	-	5.72	98.90
	12/95	1.67	35.84	-	-	x	x	-	-	-	-
SALACAK	4/95	0.10	2.42	0.17	11.14	0.17	4.54	x	x	x	x
	5/95	3.33	22.75	3.27	6.66	4.70	9.19	x	x	x	x
	12/95	0.27	7.81	-	-	-	-	x	x	x	x

- Not determined, x: the alga was not found in this area..

The ratios of C17/Pr, Cl 8/Ph, Pr/Ph and CPI ($2x(n\text{-}C_{27}+n\text{-}C_{29})/n\text{-}C_{26}+n\text{-}C_{28}$) values were calculated from their peak areas on the GC/MS chromatogram.

RESULTS AND DISCUSSION

The amounts of oil on the surface and inner part of the algae are shown in Table 1 .The oil pollution in the algae of Bosphorus varied in the northern entrance: surface 0.02-38.09 µg/g and inner part 0.49-196.76 µg/g, in the southern exit: surface 0.17-5.72 µg/g and inner part 0.42-150.00 µg/g. Thus the level of pollution in algae (except *U. lactuca)* was higher in the northern entrance than in the southern exit of Bosphorus.The highest pollution of Bosphorus algae was found in *C. rubrum* at Garipçe and in *P. capillacea* at Sarayburnu. The rank of oil pollution in algae was changed according to collection site. After the Nassia Tanker accident in the northern entrance of Bosphorus on 14 March 1994 the oil pollution of *U. lactuca* was found to be 175.5 µg/g (wet weight) at Beykoz near the place of the accident. The oil contamination of *U. lactuca* showed that accumulation was high following the accident and reduced a year later(Güven et al. unpublished data)..

The oil pollution in 1995 varied in seawater 1.97-5.53 µg/L at the entrance and 3.00-19.20 µg/L at the exit (Güven et al. 1996). These findings showed that the oil amount on the surface and inner part of algae were higher than in the surrounding seawater.

Table 2 shows the n-C 17/Pr, n-C18/Ph, Pr/Ph ratios and CPI values. These guidelines were used to differentiate the origin of hydrocarbons in algae. High n-C17/Pr and low C18/Ph ratios indicated biologic origin (Gearing et al. 1976). No phytane was detected in any alga sample (Clarck and Blumer 1967). The origin of phytane was exogenic. n-C17/Pr ratio was found to be 3.1 for *Fucus vesiculosus* (Clarck and Finley 1974). We found the ratios of n-C17/Pr and n-C18/Ph varied in examined algae as 0.80-1.32 and 0.89-1.43 respectively. We detected pristane and large amount of phytane in on surface and inner parts of algae and Pr/Ph ratio was lower than 1 in all algae tested. These findings indicated oil pollution.

CPI value describes the nature of the $n\text{-}C_{20}\text{-}n\text{-}C_{30}$ range. It is defined by dividing the sum of the concentration of odd-carbon numbered n-alkanes by the sum of even-carbon numbered n-alkanes. This value was found in Chlorophyceae 1.0, in Rhodophyceae 0.4-1.3, in Phaeophyceae 0.7-1.5 (Clarck and Blumer 1967), 1.7 for *Fucus vesiculosus* (Clarck and Finley 1974), 24.2 unpolluted and 1.83 polluted area for *Macrocystis pyrifera* (Rossi et al. 1978). CPI value in algae tested in this work varied as 0.51-1.01 . The range of CPI value below 2 indicates a petrogenic oil pollution.

Table 2. C17/Pr, C18/Ph, Pr/Ph and CPI values of algae.

ALGAE	C17/Pr	C18/Ph	Pr/Ph	CPI
Ulva lactuca	0.80	0.89	0.85	0.51
Enteromorpha linza	1.32	1.34	0.95	0.73
Cystoseira barbata	0.99	1.41	0.87	0.69
Ceramium rubrum	1.02	1.39	0.87	1.01
Pterocladia capillacea	1.29	1.43	0.72	0.79

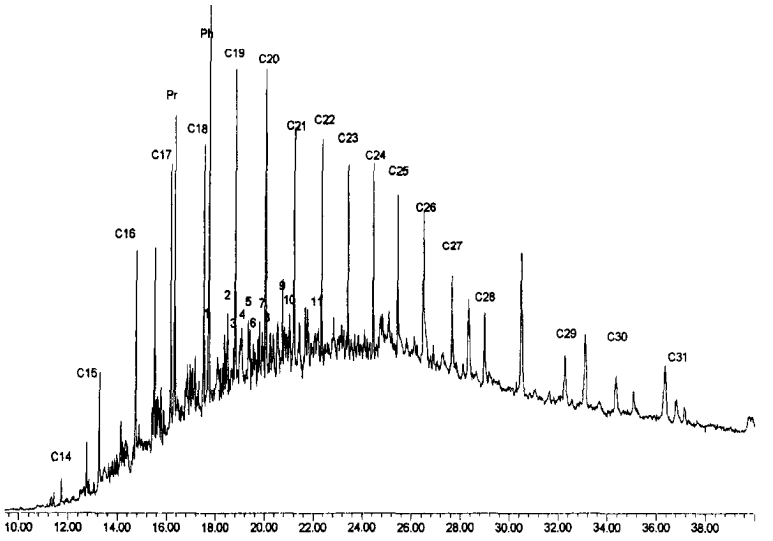

Figure 2. GC/MS chromatogram in surface of *U. lactuca*. The compounds correspondings to the peak numbers are shown in the text.

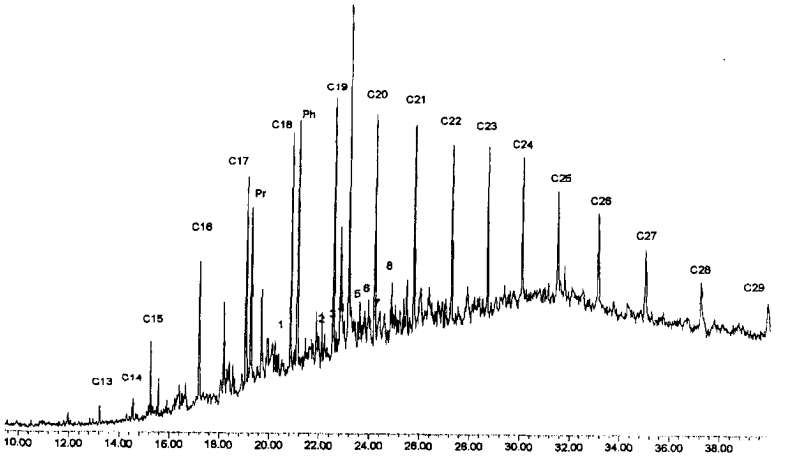

Figure 3. GC/MS chromatogram in inner part of *C. rubrum*. The compounds correspondings to the peak numbers are shown in the text.

The aliphatic hydrocarbons identified in algae were: C13: tridecane, C 14: tetradecane, C 15: pentadecane, C 16: hexadecane, C 17: heptadecane, C 18: octadecane, C 19: nonadecane, C20: eicosane, C21: heneicosane, C22: docosane, C23: tricosane, C24: tetracosane, C25: pentacosane, C26: hexacosane, C27: heptacosane, C28: octacosane, C29: nonacosane, C30: triacontane, C31: hentriacontane. Predominant n-alkanes were found as C_{25} in *Macrocystis pyrifera* (Rossi et al. 1978) and C_{15} in *Ulva rigida,* C_{19} in *Enteromorpha intestinalis* (Miranov et al. 1981) from polluted, C_{15} in *Macrocystis pyriferu* (Rossi et al. 1978), C_{17} in *Ulva rigida* (Miranov et al. 1981) from unpolluted area. In this work 20 aliphatic(C_{14}-C_{31}) were detected in algae.In our finding however in surface and inner part of *C. rubrum* at Salacak C_{19} and in surface of *U. lactuca* at Salacak C_{19}, at Sarayburnu C_{17} and at Anadolu Feneri C_{29} were predominant It was thus concluded that dominance of alkane number in algae varied with the place of collection. As an example two GC/MS chromatograms are given to show aliphatic and polyaromatic compounds in algae i.e. Figure 2 surface of *U. lactuca* and Figure 3 inner part of *C. rubrum.*

Unresolved complex mixture (UCM) was observed in all chromatograms of algae and indicated recent petroleum pollution.

Information of polyaromatic hydrocarbons (PAHs) in algae is scanty. Knutzen and Sortland (1982) studied the occurence of PAHs and found 11-28 PAH compounds including l-7 carcinogenic substances in 3 algae collected from the coast of Norway. The PAH compounds found in surface and inner part of algae are listed through the retention time order in Table 3. The numbers given in the paranthesis in the list of PAH compounds correspond to the peak numbers on the chromatograms in Figure 2 and 3.

Table 3. PAH compounds in algae. a: *Ulva lactuca,* b: *Enteromorpha linza, c: Cystoseira barbata,* d: *Ceramium rubrum,* e: *Pterocladia capillacea.* - not found

Compounds	surface	inner part
Anthracene (1)	a b c d e	a b c d e
1-methyl dibenzothiophene	a b c e	-
3-methyl dibenzothiophene (2)	a c e	a d e
4-methyl dibenzothiophene	b	-
5-methyl dibenzothiophene (3)	a c e	-
1-methyl, anthracene (4)	a	-
2-methyl anthracene (5)	a b c d e	a d e
9-methyl anthracene (6)	a d e	c d
1,7-dimethyl dibenzothiophene (7)	a e	a d e
2,7-dimethyl dibenzothiophene (8)	a e	a c d e
4-methyl naphto[2,1-b] thiophene (9)	b c	-
4,9-dimethyl naphto [2,3-b] thiophene	a b e	a c d e
2,5 dimethyl phenanthrene (10)	a c e	a c d e
3,6 dimethyl phenanthrene(11)	a c e	-

Especially anthracene and 2-methyl anthracene were found in many algae samples.

As it is seen in the table3 and the figures 2 and 3, PAH compounds are higher on surface than inside of algae. No correlation could be established between the algae species and the oil content. Specific aromatic compounds in algae could not be related to species but rather they changed with location.

The possibility was considered that rinsing with DCM might have extracted the hydrocarbons from inside the algae. The absence of peaks of aliphatic and polyaromatic hydrocarbons in chromatograms of some surface extracts proved that rinsing instantaneously with DCM did not extract hydrocarbons from inside algae.

In this work oil pollution was determined first time separately on surface and inside of algae; in addition 14 PAHs contaminating in surface and 8 PAHs inner part of algae are identified.

These findings support the importance of using algae as an indicator of seawater pollution.

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