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OIL CONTAMINATION OF MYTILUS GALLOPROVINCIALIS AFTER THE NASSIA ACCIDENT

NASSIA TANKER KAZASINDAN SONRA MYTILUS GALLOPROVINCIALIS'İN PETROL İLE KONTAMİNASYONU

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Abstract

Oil accumulation in mussels was investigated after the Nassia oil tanker accident on the Istanbul Strait by applying UVF and GC/MS analysis methods. Pristane and phytane ratio was used to distinguish the sources of pollution.Amounts of oil varied from 5 to 250 μg/g in three surveys. The analysis results revealed that contamination originated from the oil spilts from the tanker involved in the accident.

Introduction

Istanbul Strait has a transit traffic of 35.000 ships per annum on the average. The accidents occured 30 times in 1990, 23 in 1991, 17 in 1992, 16 in 1993 and 9 in 1994 (Ogüzülgen 1995).The tanker accidents amounted to 28 out of 205 ship accidents in total during the period of 1982-1994. The major disaster was on 13 th March 1994 when M/T Nassia carrying Russian crude oil crashed with M/V Shipbroker near the coast of Rumeli feneri çakarı on the İstanbul Strait (Fig.1). 2000 tons of crude oil was spilled causing severe pollution of the sea and also the ensuing fire contaminated the atmosphere with fumes more than 4 days.

Numerous reports appear on the effect of oil pollution on marine organisms. Mussel, which is the subject matter in this work, is a filter feeding organism. It accumulates sea pollution matter. The uptake and discharge of oil by mussel was investigated by Lee *et al.* (1972), Ahel (1984), Mironov (1991), Ehrhardt and Burns(1992).

Various methods were used for the quantitative and qualitative analysis of oil contamination. The commonly used method for the determination of oil is UV fluorospectrometry (UVF). This is based on the determination of PAH of oil in sea water, sediment or marine organisms and the extinction/emission (ex/em) of extracts were measured at 310/360 nm.

Aliphatic and aromatic fractions of oil were separated by using column chromatography (Tibbetts and Large 1988) and analysed by GC or GC/MS spectrophotometry. The problem was the differentiation of ambiguous petroleum hydrocarbon sources, while some hydrocarbons could be synthetized by marine organisms. Normal alkanes primarily with odd-numbered carbon, alkanes with 15,17,19 and 21 C atom are synthetized by marine phytoplankton and C_{21} to C_{28} n- alkanes by *Sargassum* in the highest concentration.Branched alkanes, pristane are also found in marine organisms. Cycloalkanes and cycloalkene, carotene and aromatic hydrocarbons are synthetised by marine organisms (Goldberg 1976). NAS (1975) gave some guidelines to the differentiation of petroleum and biogenous hydrocarbons as:

- Petroleum often contains homologous series of hydrocarbons with even and odd number of carbon atoms are present in nearly the same concentration.
- Petroleum contains more kinds of cycloalkane and aromatic hydrocarbons with alkyl (mono-tetra methyl) substituted.

- Petroleum contains naphteno aromatic hydrocarbons and also hetero compounds not yet reported in marine organisms.

Of the isoprenoid hydrocarbons, pristane (C_{19}) 2,6,10,14 tetramethyl pentadecane(Pr), occurs in phyto-and zooplankton,while phytane (C_{20}) 2,6,10,14 tetramethyl hexadecane (Ph) exists in much lesser amount in marine organisms (Morris and Gulkin,1975). Their ratio can distinguish the source, whether natural or due to oil pollution (Blumer 1970). The ratio can be determined by GC or GC/MS analysis.

The pollution of the sea, sediments and marine organisms of Istanbul Strait caused by Nassia tanker accident was investigated by our laboratory (Unpublished data).

This paper reports the part of the pollution studies on the aliphatic fraction of mussels.

Material

Mussel (*Mytilus galloprovincialis*) was taken from stations in Istanbul Strait by diving (Fig.1). The research was carried out from R/V Arar on the dates:
Nr 1: 21-27 April 1994,
Nr 2: 5-11 July 1994,
Nr 3: 23-28 Sept. 1994.
UV Fluorospectrophotometer (Hitachi 650-10S).
GC/MS (Hewlett Packard. HP 6890 GC System/ Mass Selective Detector).
All chemicals and solvents were HPLC grade (J.Baker).

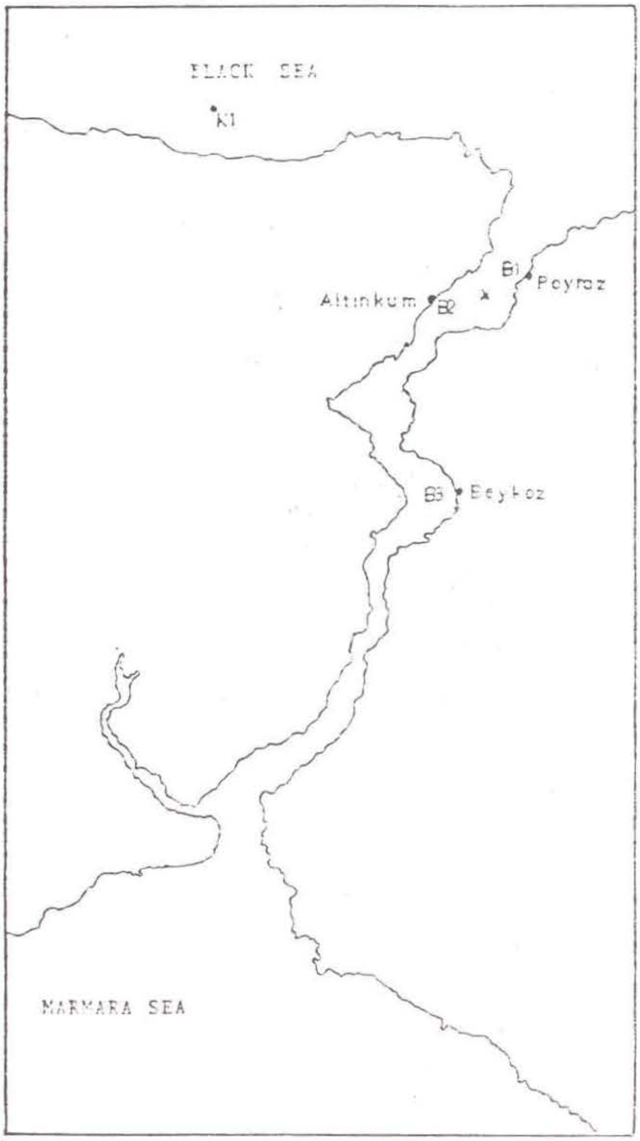
Methods

I- Extraction and saponification techniques

M-Scan standard procedure was used. 10g samples were extracted with dichloromethane (DCM) in the first survey and an isopropanol:hexane mixture (80:20) in the second and third surveys.

Fig. 1 The sampling stations in the Istanbul Strait. K1 : Karaburun, B1 : Poyraz, B2 : Altınkum, B3 : Beykoz. x : Rumeli feneri çakarı.

K1: To where Nassia tanker has been pulled after the accident.



The extract was evaporated to a small volume on a rotary evaporator, transferred to a erlanmayer and saponified with potassium hydroxide solution , then extracted with hexane. After drying with Na_2SO_4, it was filtered and concentrated by rotary evaporation then the volume was adjusted to 10 ml with hexane .5 ml of the resulting total extract was analysed by UVF and the other part was applied to silicagel column (8 g silicagel ,2x25cm column). The elution was made with pentane and DCM successively.Thus aliphatic and aromatic fractions were separated and analyzed by GC/MS spectrophotometry.

2- Analysis

2.1. UVF

A standard calibration curve was plotted by using Nassia cargo sample in concentrations of 2,5, 10 and 15 µg/ml in hexane. The ex/em was measured at 280/330 nm for distilled fuel oil, 310/360 nm for crude oil and 380/430 nm for some oxidation products of PAH_s (Ehrhardt and Burns 1992). Only 310/360 nm (ex/em) results are given in this paper. Oil concentration in mussel extract was read from the calibration curve.

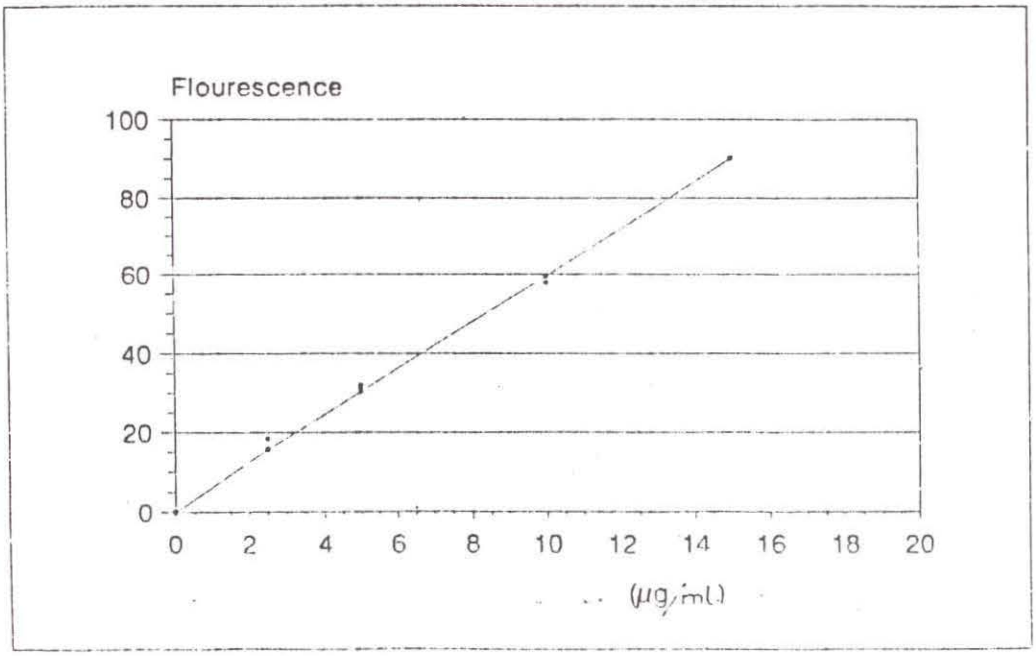
2.2. GC/MS

GC/MS analyses were run on an HP 6890 capillary gas chromatograph connected to a Hewlett Packard Mass Selective Detector(MSD) controlled by an HP Chemstation.GC/MS system equipped with 50mx200µm i.d. fused HP PONA (Methyl siloxane) coated glass capillary column.Film thickness is 0.50µm.Column temperature program is as follows; 40°C hold time 6 min, Ramp 1 is 10°C / min rate to 280°C hold time 10 min Ramp 2 is 10°C/min rate to 290°C hold time 5 min. Samples in n-hexane solution were injected in splitless mode at 300°C injektor block temperature. Helium was used for carrier gas (29.4 psi press.)

Results

The standard curve of Nassia cargo sample is shown in Fig .2.
The UVF analysis results are shown in Table 1. The amounts of oil in mussels were low in the first survey at the stations K1, B1 and B2 whereas in the second survey the levels were the highest. In the third survey oil amounts were decreased.The low amounts of oil in the first survey can be attributed to the lower extraction efficiency of hexane, than those of isopropanol: hexane mixture used in the second and third surveys. In the first survey the highest pollution was determined at B3 station.K1 station was to the west of the Black Sea entrance to the İstanbul Strait to where the tanker was pulled after the crash.The amount of pollution on the mussels taken here was as expected.When the results of B1,B2 and B3 situated in İstanbul Strait are compared the highest values for oil were found at B3 in the second survey and at B2 in the third survey.

Fig.2. The UVF standard calibration curve of Nassia crude oil.



A typical chromatogram and spectrum of aliphatic fraction of mussel taken from K1, B1, B2 and B3 stations are shown in Fig. 3-6 abc respectively. Fig. 7 shows the GC/MS chromotogram and spectrum of aliphatic fraction obtained from Nassia crude oil. Fig. 8 shows the mass spectra of pristane (Pr) and phytane (Ph) taken from HP memory.

As can be seen in these chromatograms pristane and phytane peaks are clearly visible. Phytane peaks are identical with aliphatic fraction of Nassia crude oil. The spectra of Pr and Ph obtained from mussels extracts were identical with those taken from MS memory. This can be taken as a good evidence of oil pollution, originating from the tanker Nassia.

Table.1 UVF results of mussels samples(μg/g-dry weight)

Station Number / *Survey*	*N/1*	*N/2*	*N/3*
K1	15	82	57
B1	5	15	9
B2	18	69	250
B3	60	144	53

Fig. 3 The chromatogram of aliphatic fraction of mussel in station K1 (a) and its spectrum. Pr : b1, Ph : b2.

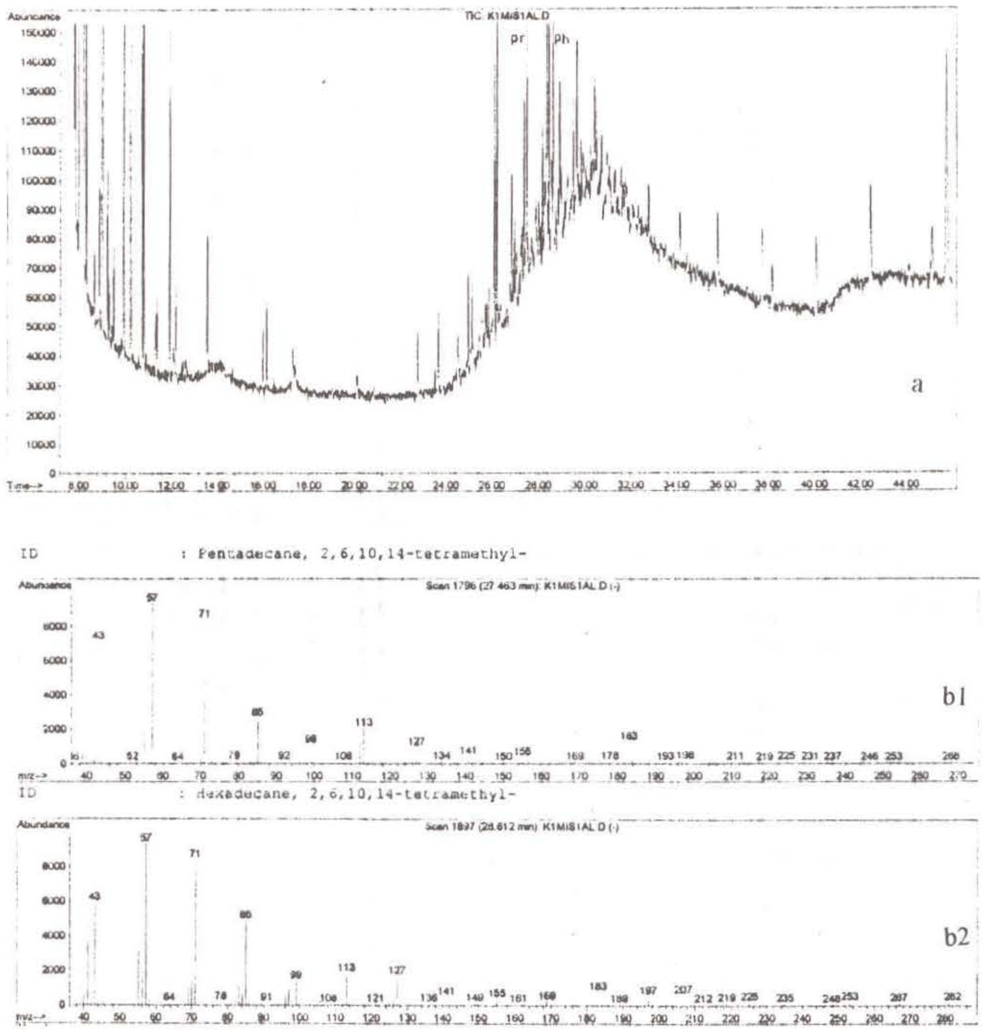


Fig. 4 The chromatogram of aliphatic fraction of mussel in station B1 (a) and its spectrum.
Pr : b1, Ph : b2.

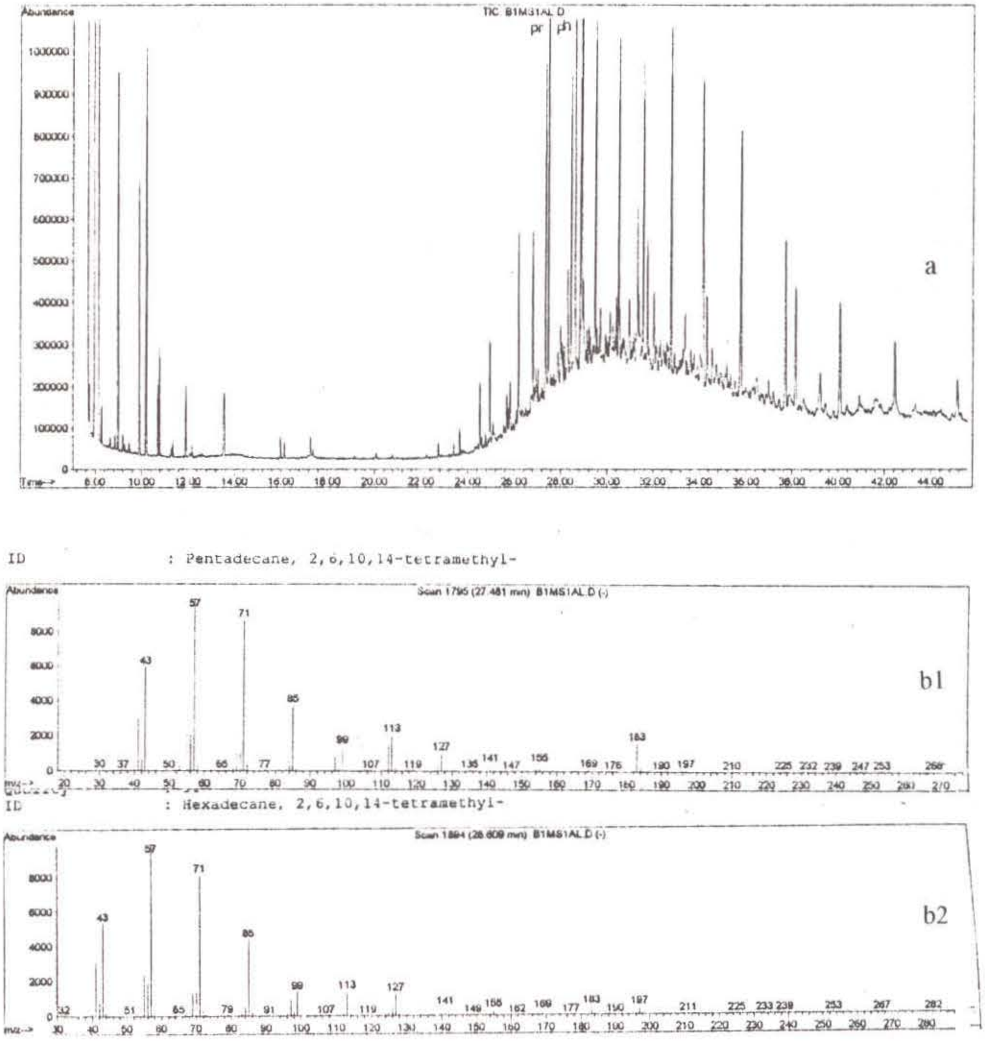


Fig. 5 The chromatogram of aliphatic fraction of mussel in station B2 (a) and its spectrum.
Pr : b1, Ph : b2.

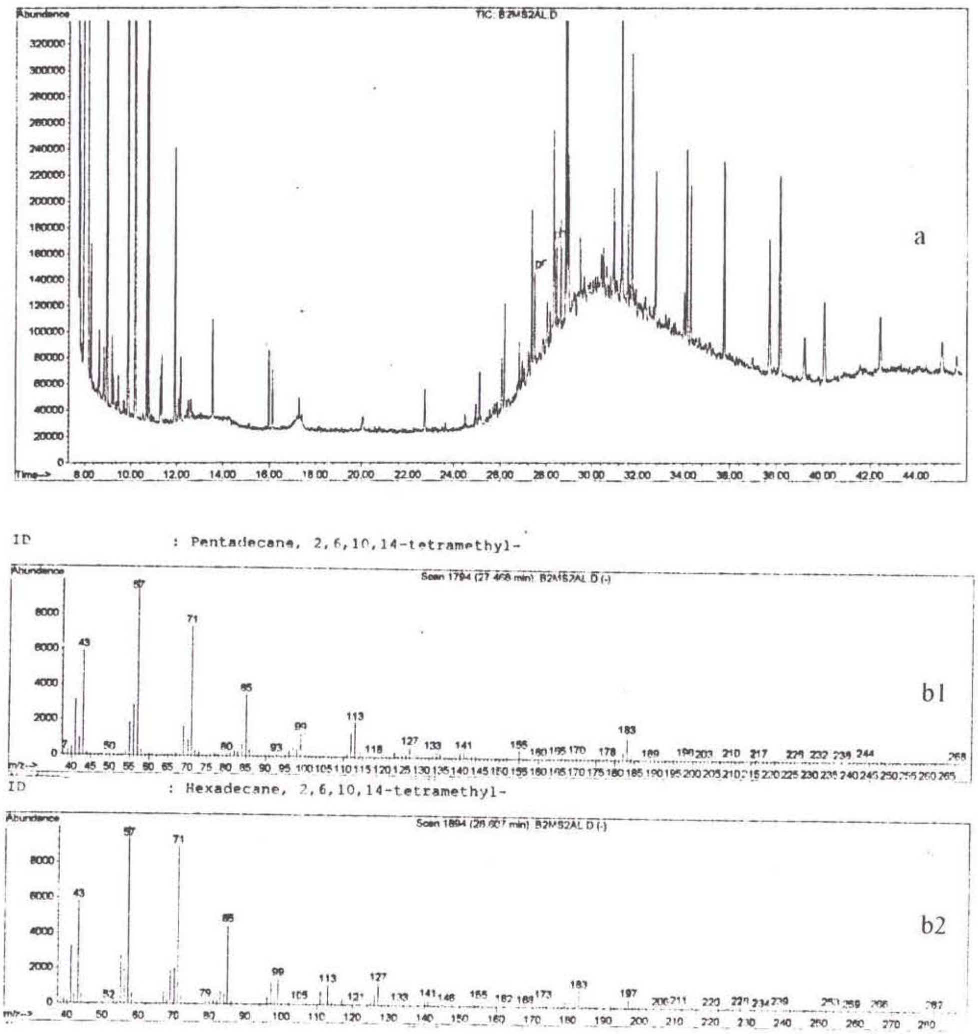


Fig. 6 The chromatogram of aliphatic fraction of mussel in station B3 (a) and its spectrum.
Pr : b1, Ph : b2.

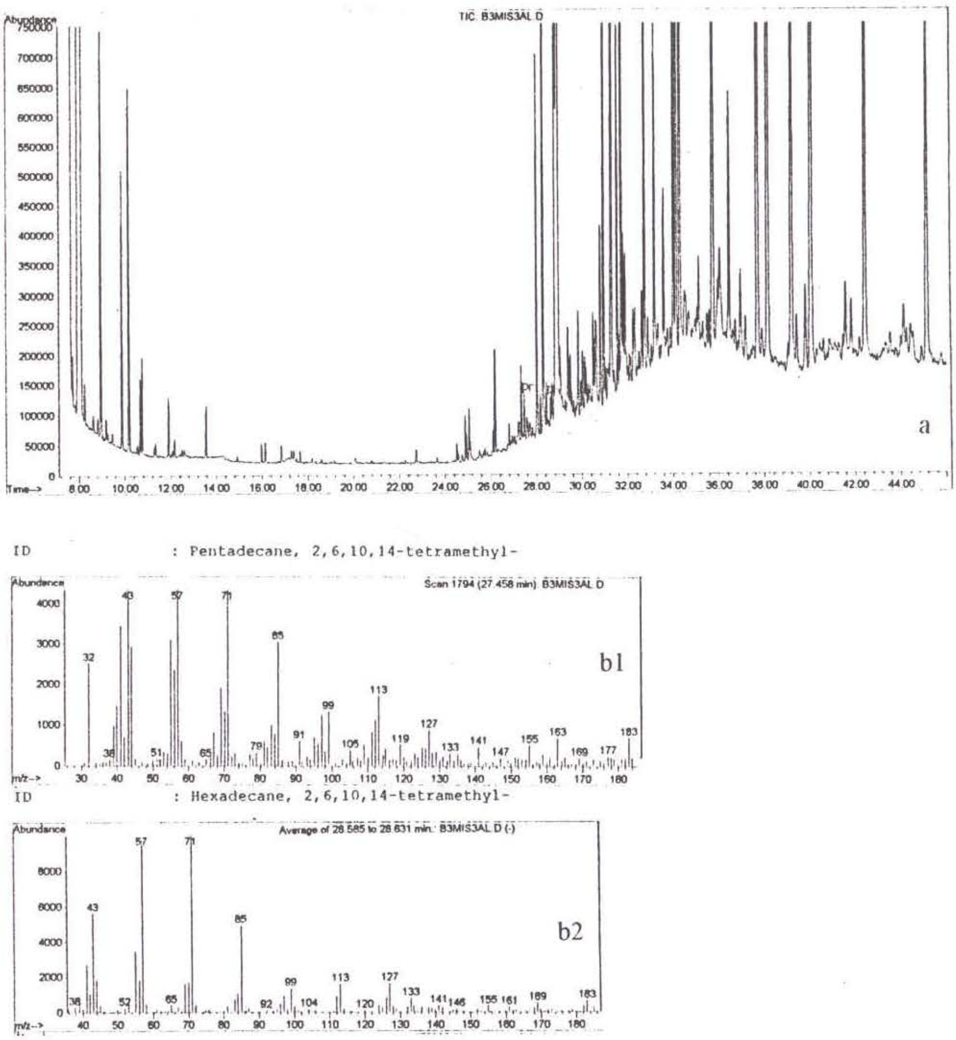


Fig. 7 The chromatogram of aliphatic fraction of Nassia crude oil (a) and its spectrum. Pr : b1, Ph : b2.

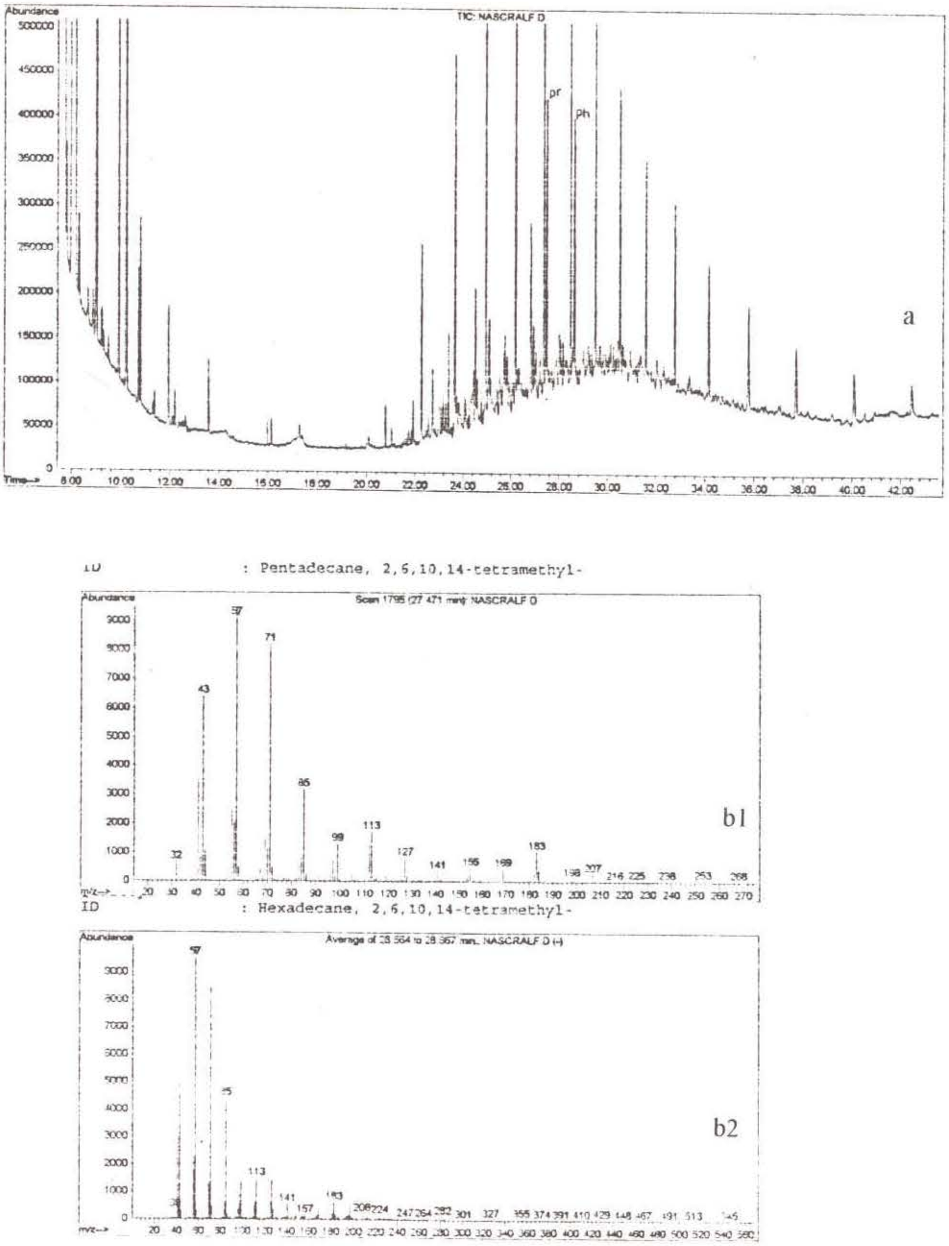
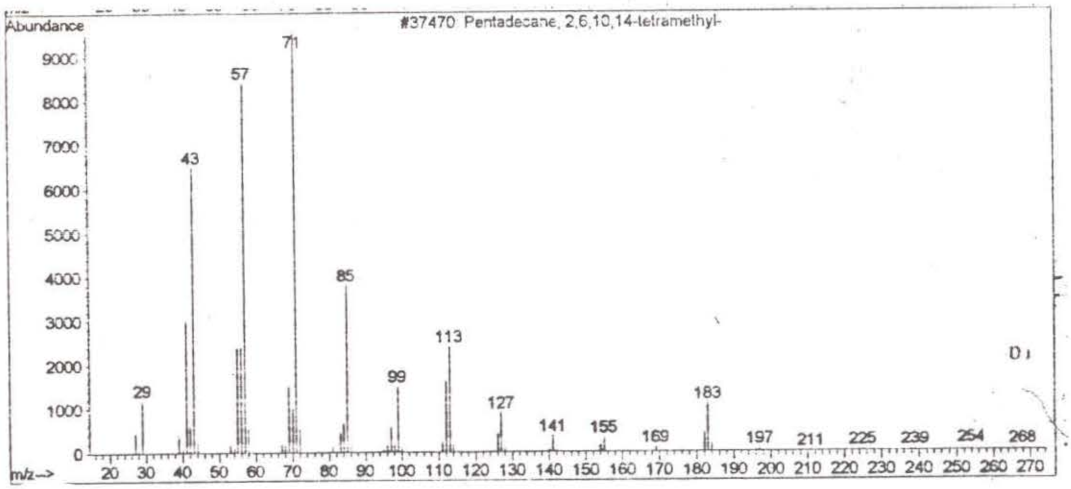
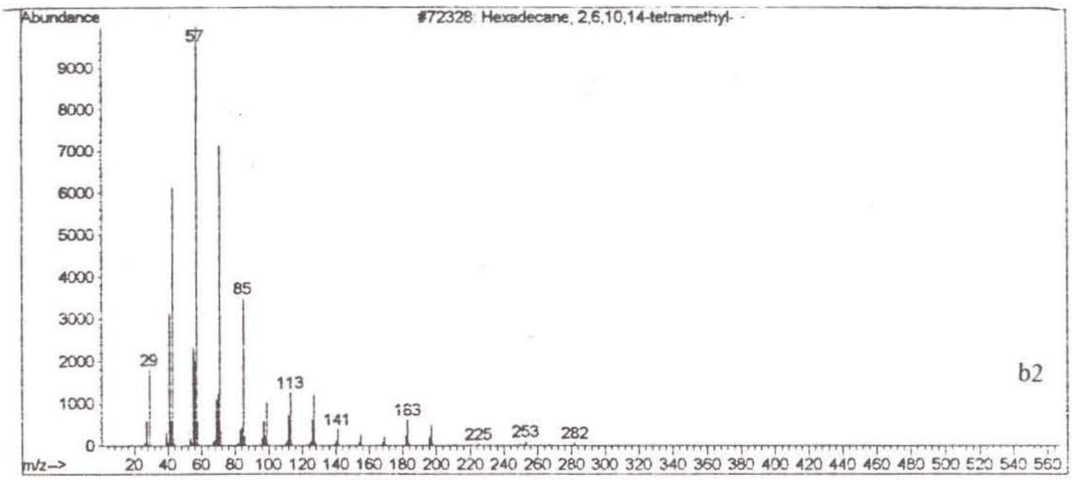


Fig. 8 The spectrum Pr : b1, Ph : b2 taken from HP memory.





Discussion

Mussels are used in this work to measure the pollution effect of Nassia Tanker accident.The highest pollution was determined at station B3 followed by B2, whereas Bl showed the least polluted. In the first survey the oil amounts in the mussels were found low in all the tests because hexane was found insufficient as the extraction solvent,whereas oil content in sea water in the first survey was higher than in the second and third surveys at the same stations (Unpublished data).

Ahel (1984) reported that the oil concentration of *Mytilus galloprovincialis* in Rijeka Bay was around 1.5-19 µg/g (fresh weight). Aromatic hydrocarbons in mussels from different regions of the Black Sea were in western Crimea 14-238 µg/g, in Karadağ trace and Danube region 350 µg/g, Kerchensky Strait 16 µg/g (Shechekaturina 1991). The oil amount found in mussels at Istanbul Strait after Nassia tanker accident was higher than that reported at Rijeka Bay. Our results were also higher than those reported for the mussels of the Black Sea (except Danube).

GC/MS analysis on Pr/Ph ratio proved that oil pollution increased in the mussels near the location of the accident.

Özet

İstanbul Boğazında meydana gelen Nassia tanker kazası sonrası yapılan çalışmalarda değişik istasyondan alınan su, sediment ve deniz canlıları üzerinde yığışan petrol kirliliği tayini enstitümüzde araştırılmıştır. Petrol kirliliği miktarı midyenin total ekstraktında PAH, UVF analizi ile tayin edilmiştir. Bu petrol kirliliğinin Nassia tanker kazasından oluştuğu GC/MS analizi ile saptanmıştır. Bu yayında midye üzerinde yığışan petrol kirliliğine ait yalnız alifatik fraksiyon ele alınmıştır. Bu analiz pristane/phytane oranına dayandırılmıştır. Bu çalışmada ayrıca ekstraksiyonda kullanılan solvanın ekstraksiyondaki rolü de belirtilmiştir. Kaza sonrası midyelerde yığışan petrolün miktarı değişik tarihlerde yapılan incelemeler sonunda bulunan bulgulara göre belirtilmiştir. Nassia tanker kazasından sonra midyelerde yığışan petrol artığı miktarının Karadenizde başka araştırıcılar tarafından yapılan çalışmalarda tespit edilen miktardan (Tuna hariç) yüksek olduğu saptanmıştır.

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