Identification of Spilled Oils in the Marine Environment by Fluorescence Fingerprints and Library Search System

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해양유출유의 형광지문에 의한 식별연구

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Multi-spectral analyses of excitation and emission fluorescence was applied to spilled crude oils in characterization of their specific fluorescence patterns which is called oil fingerprints. In the present study, oil fingerprints of 33 crude, 4 fuel and 2 other oils were analyzed to establish data base library search system. Cluster analysis showed that crude oils could be classified into two large groups according to their fluorescence characteristics. In simulated experiments, all the spilled sources was identifiable by the present library search system. In the natural environment this system could identify the exact source of weathered crude oil slicks upto 10 days. The present study shows that the fluorescence fingerprinting method with the library search system is reliable and superior to routine GC/HPLC analyses in identification of the source of weathered spilled oils in the marine environment.

해양에 유출되는 유류오염의 정확하고 신속한 식별을 기하기 위하여 국내에 반입되는 33종의 원유와 기타 6종 등 총 39종 유류의 형광특성을 연구하였다. 연구결과 각 유류는 내포되어 있는 방향족 화합물의 특성에 따라 독특한 유류지문도를 보였다. 이들에 대한 다변량 통계분석의 결과 대체로 2개의 유류군으로 대분되었다. 이들 유류가 해양에 유출된 뒤 10일이 경과되어도 물리화학적 풍화작용과 미생물에 의한 분해작용에도 불구하고 본 연구의 기법으로 오염 유류원을 식별할 수 있었다. 이에 반하여 GC에 의한 조사에서는 6일 경과된 풍화유출유의 경우 탄소수가 12 이하인 화합물의 상당부분이 소실되어 나타나 유류식별이 어려웠다. 따라서 본 연구로 해양에 유출된 유류의 정확하고 신속한 유류원 판별 식법으로 형광지문이 검증되었으며 풍화과정이 형광지문특성에 미치는 영향과 형광특성 측정을 이용한 응용방법이 논의되었다.

INTRODUCTION

In Korea, oil spill accidents are recently increasing in terms of volume (5,000 tons: '79-'85) and sometimes resulting in devastation of marine cultivation grounds (US 10 million: '79-'85).

Column chromatography of GC/HPLC has been widely used for the identification and quantitative purposes (Rasmussen, 1976; Overton et al., 1977; Clark and Jurs, 1979; Crowley et al., 1980). But it is time consuming and chromatogram peaks of low carbon number could be different from the original source as spilled oil aging in the natural marine environment. One of the problems with oil identification is that collected samples of spilled oil are sometimes aged several days after spill.

Even in the case of moderate weathering of spilled oil, it is not easy to identify with routine GC analysis unless extensive analyses are carried out such as IR (Blumer et al., 1973; Reed, 1977), HPLC, GC/MS (Reed, 1977; Atlas et al., 1981), carbon and sulfur isotope ratios (Hartman and Hammond, 1981) and determination of V/Ni ratios.

Spectrofluorometry could be an alternative method as it is fast and low cost although it lacks of specificity and information on hydrocarbon composition (Hornig, 1974; Farrington et al., 1976; U. S. Coast Guard, 1977; Mackie et al., 1978; Eastwood et al., 1978; Eastwood, 1981). Since fluorescence measurement detects mostly aromatic compounds which are more resistive to biological and chemical decomposition than aliphatic fraction of various petroleum oils, spectral analysis of fluorescence might be useful for the identification of unknown sources if proper standards are available. Application of multi-spectral analysis of excitation and emission fluorescence can give more valuable informations to characterize each oil's specific fluorescence pattern. Such patterns, called oil fingerprint, might be useful to identify unknown weathered source or to distinguish features of each sources. Recent improvement of interfacing between analog instrument and personal computer facilitates fast and efficient data aquisition and statistical interpretation.

This study is to develope a rapid and fairly convenient system for the identification of spilled oil after spill accident using multi-spectral fluorometry and computer data base library search system.

MATERIALS AND METHODS

To establish fingerprint data base library search system, 33 crude, 4 fuel and 2 other oils were chosen in this study (Table 1). Immediately after spiking the seawater (33‰) at 10-20℃, each oil standard was extracted with methylene chloride/n-hexane. Spilled oils were collected sometimes by dipping small metal pin prewashed with n-hexane in the sea surface and later stripping off with 5 m𝑙 of n-hexane.

Table 1. List of crude fuel and other oils analyzed in this study.

Saudi Arabia:	Malaysia:
1. Arabian light	20. Tapis
2. Arabian medium	21. Bintulu
3. Arabian heavy	22. Labuan
4. Arabian (M73%+H27%)	23. Arun Condensate
Iran:	Indonesia:
5. Iranian light	24. Walio
6. Iranian heavy	25. Madura
7. Foroozan	26. Ariuna
Kuwait:	27. Attaka + Babic
8. Kuwait	Mexico:
Oman:	28. Isthmus
9. Oman	29. Isthmus + Maya
Abu Dhabi:	Equador:
10. Zakum	30. Orient
U. A. E.:	Venezuelar:
11. Murban	31. Menomata
12. Dubai	China:
Libia:	32. Dakyung
13. Brega	33. Shengli
Neutral Zone:	Fuel oils:
14. Khafji	34. Diesel oil
15. Duri	35. Bunker A
Egypt:	36. Bunker B
16. Ras Gharib	37. Bunker C
17. Belayim	Lubricant oil:
Brunei:	38. Lubricant oil
18. Champion	Waste oil:
19. Seria light	39. Bilge

Fluorescence intensities of extracts (10 ppb) were measured with Shimadzu RF-540 spectrofluorophotomater. Subsamples of extracts were saved for later GC analysis. Emission spectra from 300 to 500 nm were scanned over a range of excitation wavelength from 260 to 460 nm at 20 nm interval. Slit width of both monochromators were set at 5 nm. Scanning velocity was set at 2 nm/sec. Analog fluorescence signals (1VFS) were simultaneously transmitted to IBM/XT/AT compatible personal computer through 12 bit resolution A/D converter (PC-LabCard PCL-712) and stored in a hard disk. Multi-spectral analysis usually completed within 20 minutes. During the multi-spectral analysis, raw signal of spectra were subtracted by previousely stored background solvent spectra to eliminate any solvent impurities and scattering effects. This file of difference spectra were used to

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┌─────────────┐          ┌─────────────┐
│ Standard    │          │ Unknown     │
│ source oil  │          │ oil sample  │
└─────────────┘          └─────────────┘
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Spectrofluorometer
(Shimadzu RF 540)

Analog 1VFS

12 bit A/D converter
(PC-LabCard PCL-712)

Computer real time processing
(IBM PC/XI/AT compatible)

I/O control for scanning

Subtract solvent and | Raman scattering

Difference spectroscopy
Oil library data base system

3 dimensional contour
(Oil fingerprint map)

Multivariate
Statistical analysis

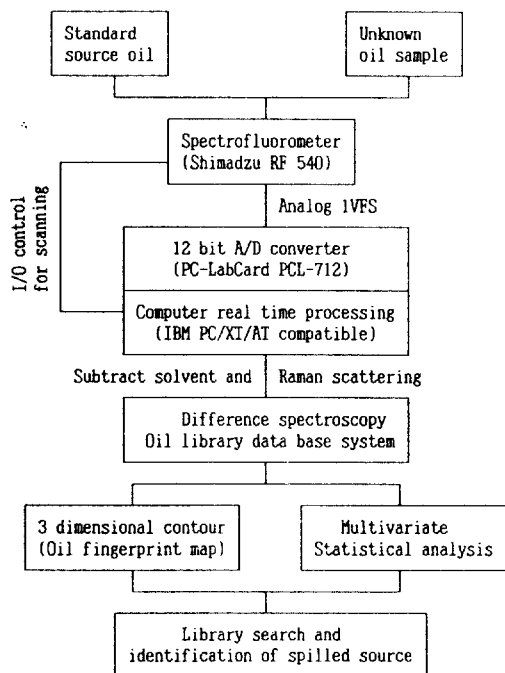Library search and
identification of spilled source

Fig. 1. A flow chart of analysis and library search identification system.

generate characteristic oil fluorescence fingerprint map by dot-matrix printer or plotter (Fig. 1).

Once library system was established, unknown oil samples could be identified through the library search system. Unknown spilled oil samples were treated with the same above procedure and their data were statistically compared by multivariate data analysis such as cluster analysis. Correlation coefficient was used as similarity index.

It usually takes about 1 minute to search the most probable source of unknown sample through the library search system. All the required softwares for controlling I/O ports of instrument, A/D interfacing, contour plot, statistical analysis and library search system were written in Turbo C language and MS fortran.

To investigate the time course variation of fingerprint due to weathering processes such as biological degradation, chemical transformation and physical fractionation in the natural marine environment, Iranian light crude oil was released into a seawater tank (100 liter) which was placed in an ambient seawater condition (33‰, 15℃). Air bub-

blers were used for the vertical mixing water column and oxygen suppley for aerobic microbial oxidation. The above identification procedure was employed at every 2 days interval and it lasted 15th day. Subsamples of extracts were saved for GC analysis. At the same sampling interval, seawater just below the sea surface was collected to investigate any change of phytoplankton community structure.

RESULTS AND DISCUSSION

Characteristic fluorescence properties of crude oils

All the sequential procedures could be accomplished within 30 minutes from instrumental fluorescence analysis, data aquisition, contouring characteristic oil fluorescence fingerprint map to computer library search. Each crude oil showed its characteristic fluorescence pattern respectivery according to the compositional variation and quantity of its aromatic fraction (Fig. 2). Heavy crude oils were apparently different from light crude oils because chemical composition of crude oils vary according to their producing regions and processes of petroleum formation. In general, hydrocarbons have been known to be the most abundant fraction accounting for 50-98% of crude oils and among them aromatic compounds occupy about 17-25% (Clark and Brown, 1977). Aromatic hydrocarbons, which is most responsible for fluorescence, contain benzene, naphthalene, biphenyls, alkylated benzene groups, and polycyclic aromatics more than three benzene rings (Clark and Brown, 1977).

In the present study, two groups can be divided from statistical analysis of 39 oils in the data base library. As shown in the dendrogram (Fig. 3) the first group is characterized by mostly heavier crude oils and the second group by lighter crude oils. GC analysis shows that the first group has higher carbon number fraction of hydrocarbon than the second group. It is interisting to note that the first group has maximum fluorescence emission wavelength around 380 nm with excitation around 280 nm and the second group around
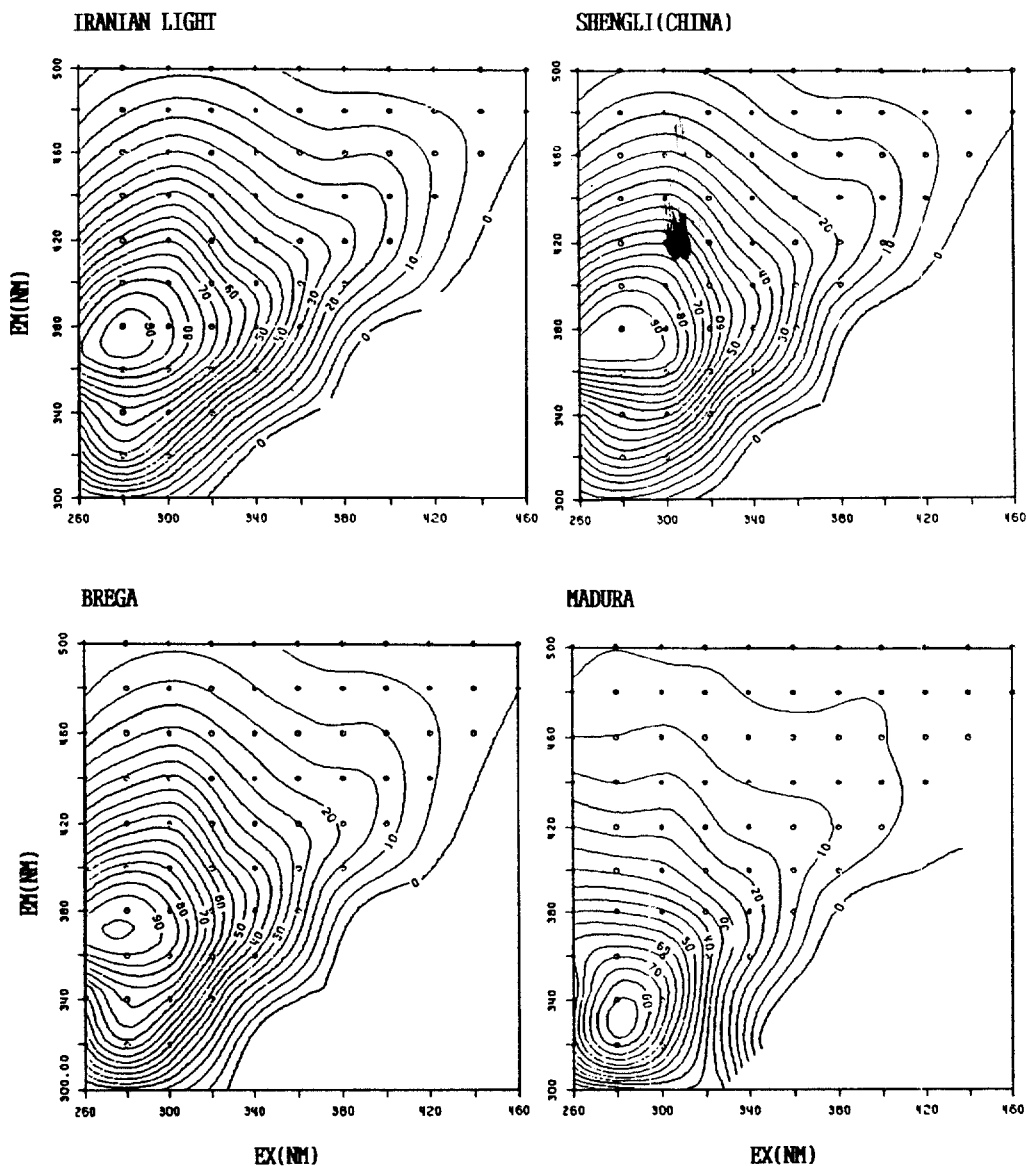
Fig. 2. Representative oil fingerprint of Iranian light, Shengli (China), Brega, and Madura crude oils showing their characteristic fluorescence properties respectively.

330 nm with excitation around 280 nm. Furthermore heavier crude oils tend to have relatively higher fluorescence distribution in the longer excitation/emission wavelengths than the second group.

It has been known that humic materials in coastal waters have background fluorescence maximum excitation at 340 nm and emission at 440 nm (Mopper and Park, 1991; Park and Lee, unpubli-

shed data). However those humic materials might not act as interference materials because they are mostly hydrophilic compounds and their fluorescence intensity is too low to affect the fluorescence pattern of spilled oil. Even though some fraction of marine humus which has maximum fluorescence excitation at 280 nm and emission at 340 nm can be extraced and concentrated from bulk seawater using hydrophobic phase column chromato-

1 Arabian light	.9989
5 Iranian light	.9982
12 Dubai	.9976
2 Arabian medium	.9993
7 Foroozan	.9989
29 Isthmus+Maya	.9983
6 Iranian heavy	.9977
36 Bunker B	.9957
16 Ras Gharib	.9986
31 Menomata	.9985
37 Bunker C	.9975
30 Orient	.9888
9 Oman	.9935
32 Dakyung	.9903
33 Shengli	.9799
3 Arabian heavy	.9992
4 Arabian(M70%+H27%)	.9985
8 Kuwait	.9995
14 Khafji	.9813
15 Duri	.9555
10 Zakum	.9982
28 Isthmus	.9953
17 Belayim	.9920
13 Brega	.9950
24 Walio	.9888
11 Murban	.9951
35 Bunker A	.7343
18 Champion	.9994
19 Seria light	.9924
22 Labuan	.9979
27 Attaka+Bablc	.9783
20 Tapis	.9893
21 Bintulu	.9991
25 Madura	.9563
23 Arun condensate	.9861
38 Lubricant oil	.9840
34 Diesel oil	.8853
26 Ariuna	.9867
39 Bilge	

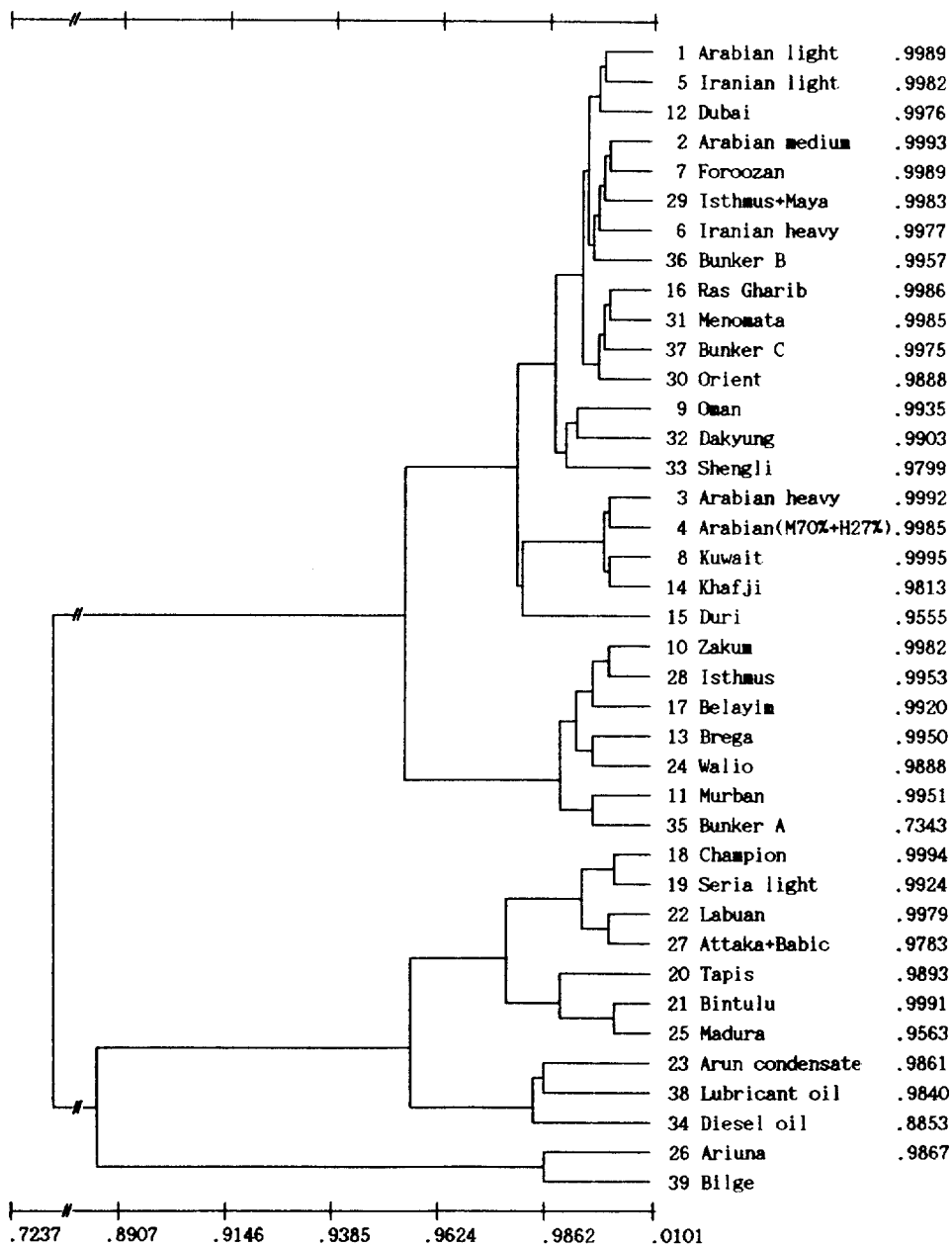.7237   .8907   .9146   .9385   .9624   .9862   .0101

Fig. 3. Result of cluster analysis of 39 oil library with correlation coefficient value.

graphy (Mopper and Park, 1991; Park and Lee, unpublished data), its fluorescence is also too low to interfere and there is little possibility for those water column humic subtances to be included in the surface oil slicks.

Library search and identification system of source of
unknown spilled oil with weathering experiment

The present study shows that results of library search are quite reproducible. Results of black box experiments of three crude oils (Iranian light Kuwait and Madura crude oils) which were intentionally released in the seawater showed good agree-

Table 2. Library search system to identify the source after weathering experiment of Iranian light crude oil. The most probable sources of weathering sample are ranked by system with R^2.

The most probable source of weathered oil		
Rank	1st day	2nd day
1	Iranian light	Iranian light
2	Arabian light	Arabian light
3	Bunker B	Bunker B
4	Dubai	Dubai
5	Iranian heavy	Iranian heavy
Rank	6th day	10th day
1	Iranian light	Iranian light
2	Arabian light	Arabian light
3	Bunker B	Bunker B
4	Dubai	Dubai
5	Iranian heavy	Iranian heavy
Rank	11th day	12th day
1	Iranian light	Iranian light
2	Arabian light	Arabian light
3	Iranian heavy	Iranian heavy
4	Bunker B	Bunker B
5	Orient	Bunker B

ment to the sources in the library data base. The exact source of each spilled oil was identified as shown in Table 2. Once the crude oils are introduced into the marine environment some characteristic parameters can be changed with the time course and degree of change in composition varies with each spilled source and environmental conditions. Weathering processes include evaporation (Jordan and Payne, 1980), dissolution (Cohen et al., 1980), emulsification (Bridie et al., 1980), sedimentation (Bassin and Ichiye, 1977), photooxidation (Zafiriou, 1977; Overton et al., 1980; Zika, 1980) and microbial biodegradation (Atlas et al., 1981).

In the present study, results of weathering experiment of Iranian light crude oil showed that oil fluorescence fingerprint library search system was very efficient to identify its source without any error until 10th day after release (Table 2). From the starting to 10th day library search system consistently suggested that the most probable source of spilled oil might be Iranian light crude oil and
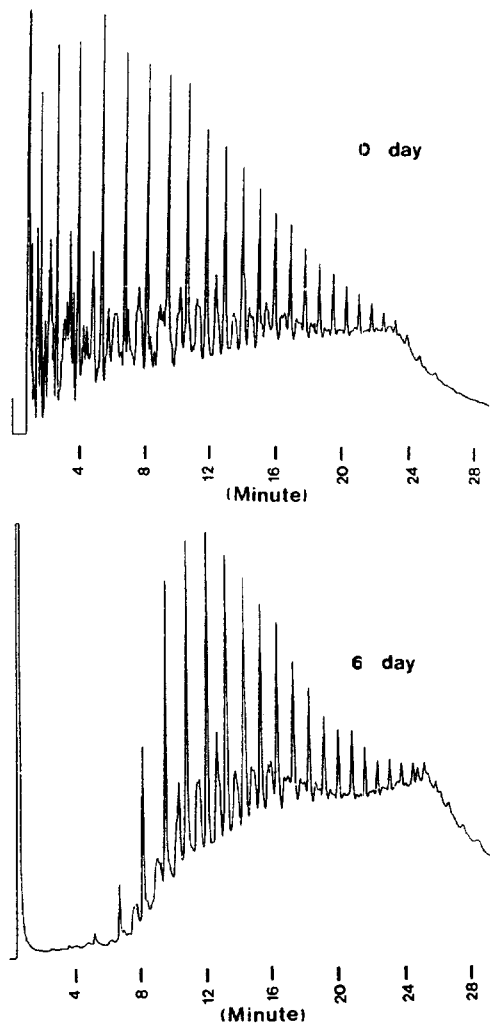

Fig. 4. GC chromatograms of Iranian light crude oil from weathering experiment. GAS chromatography analytical condition: Column: Stainless steel (2 m×3 mm dia.) Silicon OV-1, Chromosorb-W; Detector: FID; Temperature program: 80~300°C (9.5°C/min).

the second to be Arabian light crude oil. However, this system could not differentiate Iranian light crude oil from Arabian light crude oil after 12th day.

Comparison with gas chromatography

GC chromatogram from the subsample of the same condition showed that physical evaporation and/or biodegradation of lower carbon number

Table 3. Compositional variation of phytoplankton population during weathering experiment.

1st day	605 cells/ml	19 species occurred	
	Microflagellates (20 m)		30.0%
	Paralia sulcata		19.9%
	Chlorella vulgaris		9.9%
2nd day	595 cells/ml	16 species occurred	
	Microflagellates (20 m)		64.4
	Paralia sulcata		5.9
6th day	960 cells/ml	8 species occurred	
	Microflagellates (20 m)		85.4%
	Paralia sulcata		8.3%
	(protozoa occurred: 50 cells/ml)		
9th day	833 cells/ml	3 species occurred	
	Microflagellates (20 m)		94.9%
	(protozoa occurred: 50 cells/ml)		

hydrocarbons, mostly short aliphatic hydrocarbon chains, occurred even after 2nd day (Fig. 4). GC chromatogram of 6th day was quite different from that of starting day. Those lost fraction of low carbon number less than 12 could be attributed to physical evaporation and partly to biological degradation. To identify the source of weathered oil with GC, it is mandatory to simulate the natural condition in the laboratory and compare weathered oil with possible weathered source. Present study clearly shows why it is so difficult to identify the source of spilled oil with GC when the spilled sample is collected several days after an oil spill accident.

Impact of biological response on the fluorescence characteristics of spilled oil

Since most of marine bacterial assemblage responsible for the biodegradation of hydrocarbon can utilize aliphatic hydrocarbons rather than condensed polycyclic aromatic hydrocarbons with more than five benzene rings which are more resistent to microbial oxidation (Cripps and Watkinson, 1978; Hopper, 1978). If there is any significant utilization of hydrocarbon by marine bacteria, there might be any reflection in community structure of smaller plankton due to changes in food chain and food web.

In the present study, species composition of phytoplankton began to change after 5th day of experiment. At the first day phytoplankton community was stable with diverse species composition (19 species) with most dominent population to be flagellates (38.0%), the second to be paralia sulcata (19.8%) and the third to be Chlorella vulgaris (9.9 %). At the second day, species composition was nearly the same with the first day with slight decrease in species occurrence (16 species). But after 6th day, species occurrence reduced into 8 species and at 9th day, it occurred only 3 species mostly dominated by smaller (less than 20 m) heterotrophic microflagellates (Table 3).

It is remarkable that heterotrophic microflagellates increased from 38.0% at the first day, 66.4% at the second day, 85.4% at 6th day and to 94.9% at the 9th day. Interestingly protozoa appeared in a large mumber in 6th and 9th day's sample. Therefore it can be indirectly deduced that spilled crude oil promoted growth rate of marine bacteria which might be responsible for those biodegradable aliphatic portion and in turn prosperity of bacterial population might have induced rapid increase of smaller heterotrophic flagellates and protozoa grazing on them. This study shows that fluorescence characteristics of crude oil are not so much affected by biological degradation up to more than 10 days. It implies that fluorophore of crude oil is mostly composed of aromatic fraction.

Application of spectrum deconvolution analysis of spilled oil

Mostly bilge oil is composed of various waste oils of fuel and lubrication oils. When two or more different oils are mixed up and spilled in the ocean it is very difficult to seperate and identify them. In the present study spectrum deconvolution technique is applied to resolve two mixed oil sources. To demonstrate the capability of the present method, bilge oil which is known as the waste oil mixture in the ship was analyzed accor-

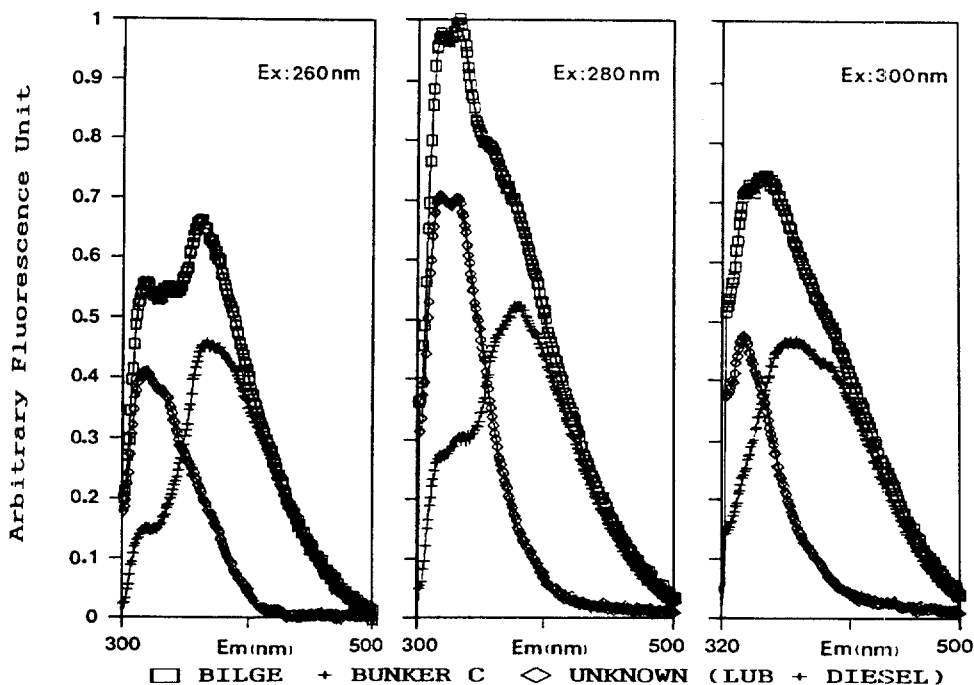## Deconvolution Analysis of Mixed Oil

Fig. 5. Result of deconvolution analysis of bilge oil. Original spectra of bilge (□) were subtracted by bunker C(+). Resulted spectra (◇) were identified by library search system.

ding to the previously mentioned method. Then spectrum of Bunker C oil, assuming as the major fuel source included in the bilge was subtracted with the least fitting method. Resulted spectrum from the subtraction was identified by library search system. It suggested that the most possible source might be lubrication oil and the next to be diesel oil (Fig. 5). In fact lubrication oil can be commonly found in the bilge and mixture fuel of diesel with Bunker C is generally used in ship. Therefore at least two mixed oil can be seperated and identified by conjugated method of spectrum analysis and library search system.

The present study showed that the oil fluorescence fingerprint method with the library search system is efficient, reliable and superior to routine GC/HPLC analysis in identification of the source of weathered spilled oils in the marine environment. From the fluorescence fingerprint map of various crude oils, two characteristic maximum

fluorescence peaks at excitation of 280 nm and emissions of 330 nm and 380 nm were found. Recently remote sensing devices of airplanes and satellites are under development in monitoring marine oil spills (O'Neil et al., 1980; Klemas, 1980; Massin, 1984). Currently the most promising remote sensing device is known to be the laser-induced UV fluorescence sensor with high speed and sensitive multi-diode array, since this remote sensing system can discriminate oil slicks in the sea surface. Therefore informations on fluorescence characteristics of various oil sources from the present study are fundamental and indispensible in developing a better remote sensing system using laser-induced fluorescence technique in monitoring and identification of oil spills in the ocean.

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