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Gas Chromatographic and Capillary Gas Chromatographic/Mass Spectrometric Determination of Organic Sulfur Compounds (OSCs) in Sediment from Ports: Significance of These Compounds as an Oil Pollution Index

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Oil pollution arises in waters near petroleum industrial districts. In oily odor fish caught in the coast near petroleum industries, toluene, other aromatic hydrocarbons and olefinic hydrocarbons have been detected by the authors (Ogata et al. 1975; Ogata et al. 1976; Ogata et al. 1987; Ogata et al. 1988). The authors also reported the presence of organic sulfur compounds (OSCs), probably originating from polluting oil, in fish caught in the Seto Inland Sea after accidental oil spilling from a tank (Ishida et al. 1976; Ogata et al. 1988). Nakamura and Kashimoto (1977) also detected OSCs in the shellfish caught under similar conditions. In our previous study, OSCs detected in the oyster and mussel caught in the Seto Inland Sea are described (Ogata et al. 1983). However, the presence of OSCs and polyaromatic hydrocarbons (PAHs) in sea sediments has not been described.

In the present report, OSCs and PAHs in the sea sediments collected in several ports facing Seto Inland Sea were analyzed by a gas chromatographic/mass spectrometric (GC-MS) system.

MATERIALS AND METHODS

All the reagents used in this study were of reagent grade.

A total of three samples of sediments were collected in June 1986 at fixed sampling points of M, T and U ports in the Seto Inland Sea (Figure 5). The top layer (0-3 cm) of the sediments was placed in glass bottles. Each sediment of 50-100 grams (wet weight)

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sediment was saponified with 2N potassium hydroxide containing ethanol. Oil compounds were extracted three times with 300ml of n-hexane. The n-hexane layer was concentrated to approximately 5ml with a rotary evaporator. OSCs and PAHs were eluted from a silica gel (mesh 200, Wako Company) alumina (Brockman 90, Merk Company) (2:1) column with 200ml of n-hexane. The compounds in the concentrate were extracted with acetonitrile. The acetonitrile solution was finally concentrated to 2ml in a KD. Crude oil (0.1g) was treated similarly except for the omission of saponification procedure and the acetonitrile extract was concentrated to 10ml with KD as described in a previous report (Ogata et al. 1983).

Apparatus and analytical condition of FPD-GC and Capillary GC-MS was as follows:
Apparatus; Shimadzu GC-7AG, FPD (394nm). column; 3% silicon SE52 on chromosorb W(AW), mesh 80-100, 3mmID x 2m glass column. column temperature; 80 - 260°C, 10 °C/min. Carrier gas; N_2, 40 ml/min. H_2; 0.75 kg/cm^2. Air; 0.60 kg/cm^2.
Apparatus; Shimadzu GC-MS 9020 DF, PAC-1100 computer system. Shimadzu CBP-5 (5% phenylmethylsilicon) fused silica capillary column; 0.33 mmID x 25 m with a 0.5 µm film. Temperature; 120-250°C, programmed 4°C min^{-1}. Application on capillary column; the moving needle method. Head pressure; 100 kPa with helium gas. Sample size; 0.005ml. Injection on mass; the direct couping method. Ion source temperature; 250 °C. Electron energy; 70 eV. Acceleration voltage; 3.0KV. Scan interval; 2.2 sec.
Apparatus; JEOL HP5890/JMS-DX303, JMA-DA5100 computer system. J & W DB-5 (5 % phenylmethylsilicon) fused silica capillary column; 0.25 mmID x 30 m with a 0.25 µm film. Temperature; 40°C, 2 min. hold 40-70 °C, programmed 10°C min.$^{-1}$, 70-270 °C, programmed 3 °C min^{-1}. Application on capillary column; splittless. Head pressure; 150 kPa with helium gas. Sample size; 0.001 ml. Injection on mass; direct couping method. Ion source temperature; 250 °C. Separator temperature and injection temperature; 300°C. Electron energy; 70 eV. Acceleration voltage; 3.0 KV. Scan interval; 0.3 sec.

RESULTS AND DISCUSSION

Sea sediments and crude oil were analyzed by FPD-GC using acetonitrile extracts. Gas chromatograms (GC) of 3 sediments (Figure 1B-D) contained a considerable number of the same peaks which were present in the chromatograms of the crude oil (Figure 1A). The appearance of a large number of peaks on the FPD-GC

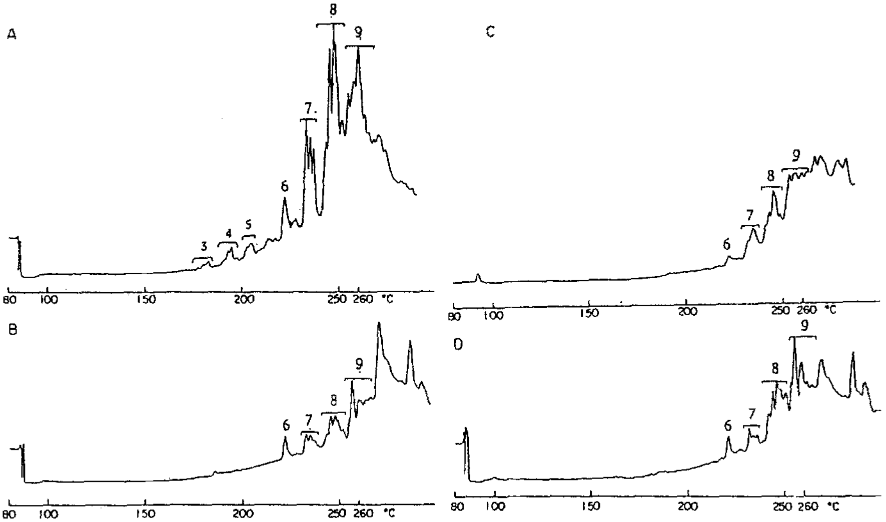

Figure 1. FPD-GC of the extract of the acetonitrile fraction obtained from (A) crude oil, (B) sediment from port M, (C) from port T and (D) from port U.
3: 2-alkylbenzothiophene, 4: 3-alkyl-benzothiophene
5: 4-alkylbenzothiophene, 6: dibenzothiophene,
7: 1-alkyl-dibenzothiophenes
8: 2-alkyl-dibenzothiophenes
9: 3-alkyl-dibenzothiophenes
note; numbers correspond to Figure 3.

of sediments, indicated that the OSCs in crude oil had moved to the sediments. Benzothiophene and alkyl-benzothiophenes of the crude oil were present in extremely small amounts in the sediments as shown in gas chromatograms of shellfish described previously (Ogata et al. 1980).

Dibenzothiophene and phenanthrene in crude oil were identified by GC-MS. Figure 2 shows the mass spectra of dibenzothiophene and phenanthrene taken from the peaks of the mass chromatograms of crude oil.

OSCs of benzothiophene, alkyl-benzothiophenes (C_1-C_4), dibenzothiophene and alkyl-dibenzothiophenes (C_1-C_4) in crude oil were presented in the mass chromatograms shown in Figure 3A. Dibenzothiophene and alkyl-dibenzothiophenes (C_1-C_4) in sediment from port U are shown in Figure 3B. Mass chromatograms of benzothiophene (M^+134), dibenzothiophene (M^+184) and of PAHs of naphtalene (M^+128), fluorene (M^+166), phenanthrene (M^+202), pyrene (M^+202), chrysene (M^+228) and benzo-α-pyrene (M^+252) in crude oil and

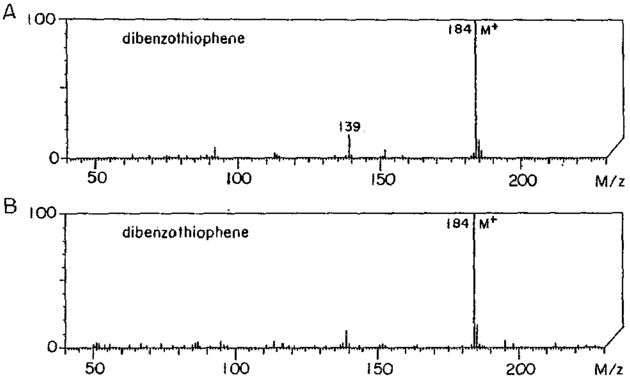

Figure 2. Mass spectra of authentic dibenzothiophene and those of in Arabian light crude oil.

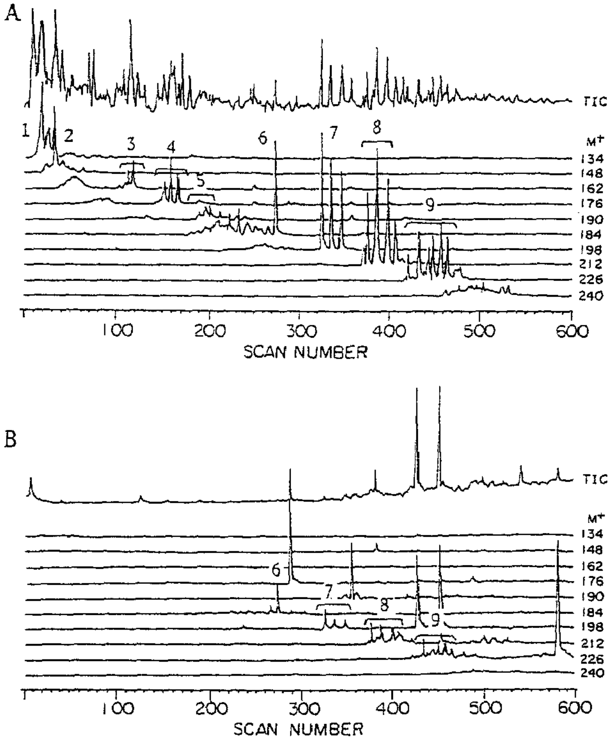

Figure 3. Mass chromatograms (EI) of dibenzothiophene
1: benzothiophene, 2: 1-alkyl-benzothiophene
3: 2-alkyl-benzothiophene 4: 3-alkyl-benzothiophene
5: 4-alkyl-benzothiophene 6: dibenzothiophene
7: 1-alkyl-dibenzothiophenes
8: 2-alkyl-dibenzothiophene
9: 3-alkyl-dibenzothiophenes
in Arabian light crude oil (A) and sediment from port U (B)

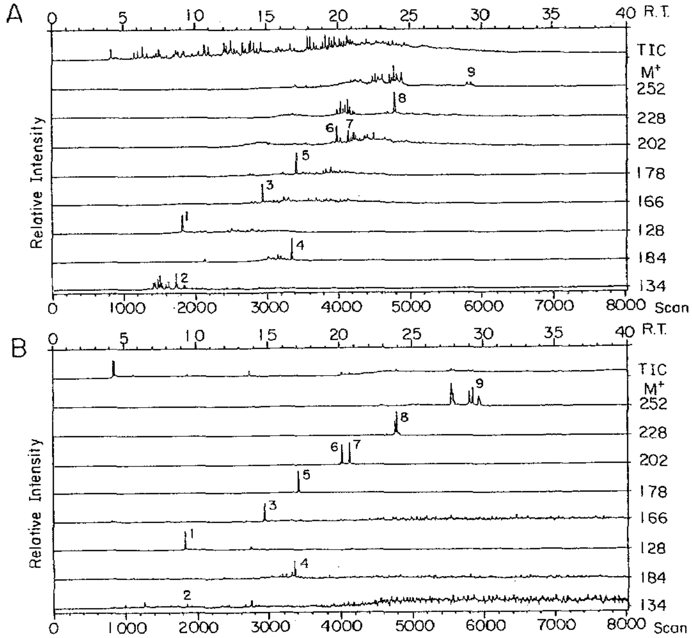

Figure 4. Mass chromatograms (EI) of naphtalene (1); benzothiophene (2); fluorene (3); dibenzothiophene (4); phenanthrene (5); fluoranthene (6); pyrene (7); chrysene (8); and benzo-α-pyrene (9), in Arabian light crude oil (B) and in the sediment and from port U (D).

of sediments from U ports are shown in Figures 4A-B. The results indicated the presence of benzothiophene, dibenzothiophene and 6 kinds of PAHs. Concentrations of OSCs and PAHs in the crude oil and sediments were determined by a selective ion monitor method.

In this study sediments from M, T and U port (Figure 5) were taken for analysis. Concentrations of OSCs and PAHs in crude oil and sediments from M, T and U ports are shown in Table 1. The results revealed the presence of dibenzothiophene in sediment indicating that sediments in the ports are polluted with dibenzothiophene containing oil. Similary, PAHs were identified in the crude oil as well as in the sediments. High concentrations of dibenzothiophene, phenanthrene and fluorene are detected in the crude oil. On the other hand, phenanthrene, fluoranthene, pyrene, chrysene and benzo-α-pyrene are present at high concentrations in the sediments.

In this report dibenzothiophene and its methyl derivatives were detected in the sediment obtained from ports facing Seto Inland Sea. And benzothiophene and its methyl derivatives were very present in small amount in the sediments. Ogata et al (1980) reported that the main OSCs transferred from oil to shellfish caught in the sea were dibenzothiophene and its methyl derivatives and these compounds were also found in oil polluted sea. Therefore, dibenzothiophene and its methyl derivatives in OSCs will be suitable compounds for monitoring oil pollution in the sea.

Dibenzothiophene levels in water from ports were in descending order of U, M and T and its levels in the mussels were also in descending order of M, U and T (Kira et al. 1983). In this report, we collected samples of sediment from the same three ports which were measured by Kira et al.(1983). Dibenzothiophene levels in the sediment from ports were in descending order of U, M and T. Order of dibenzothiophene concentration in the sediment were similar to the order of its concentrations in the water and mussel.

The authors previously reported that both dibenzothiophene and PAHs were present in shellfish (Ogata et al. 1983). In this report, PAHs were detected also in sediments. All the PAHs present in the crude oil were present in the sediments. PAHs levels in the sediment were in descending order of U, M and T. The amounts of higher molecular weight PAHs in the sediment were relatively greater as compared with the those of lower molecular weight compounds. Larsen et al.(1983) reported that sixteen kinds of PAHs were found in Casco bay and that high molecular weight compounds had tended to accumulate in sediment. Obana et al.(1981) reported that the amount of PAHs in sediment collected in Osaka port was determined by HPLC. The amount of PAHs which they found was less than that determined by us. PAHs were found in the descending order of pyrene, benzo-α-pyrene and anthracene, which compare to our present results. However, the presence of benzothiophene and dibenzothiophene are not described in their reports.

Dibenzothiophene and most PAH levels in the sediment for U were highest in spite of it being the smallest port. Mouth of U port is very narrow, water in the port is a closed environment. When oil was spilled out in a port, compounds with relatively high molecular weight may not evaporate or diffuse out of the port but sorb to the mud under the port. A small fishery port which had no sources for pollution showed highest concentrations of dibenzothiophene and PAHs, suggesting that the main source of this contamination in sediment was due to oil spilled from

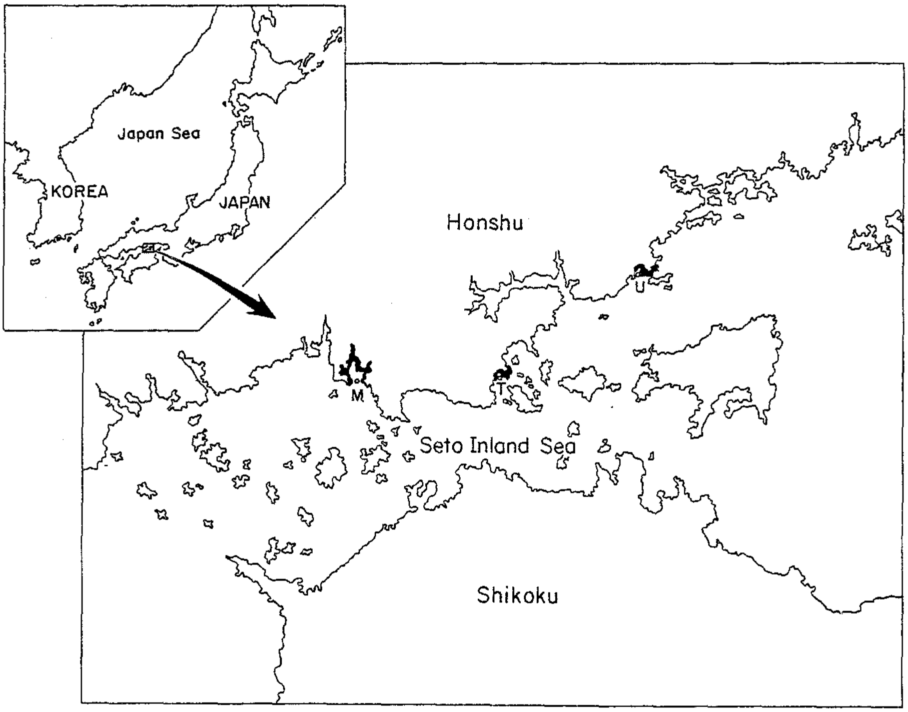

Figure 5. Ports M, T and U where sediment was sampled.

Table 1. Concentration of OSCs and PAHs in Arabian light crude oil and in the sediment from 3 ports (U, M, T) in Seto Inland Sea (µg/g: on a wet basis)

Compound	crude oil	U	M	T
Benzothiophene(BT)	8.92	0.007	0.009	---*
Dibenzothiophene(DBT)	215.70	0.062	0.043	0.037
Naphtalene	85.80	0.120	0.160	---*
Fluorene	30.70	0.054	0.069	---*
Phenanthrene	155.60	1.160	0.980	1.196
Anthracene	2.54	0.020	0.141	---*
Fluoranthene	2.75	1.790	1.020	0.756
Pyrene	8.95	1.560	0.834	0.690
Chrysene	20.18	1.140	0.636	1.263
Benzo-α-pyrene	7.811	1.250	0.688	---*
BT + DBT		0.069	0.052	
PAHs		7.094	4.528	

* not calculated

boats.

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REFERENCES

Ishida T, Imanaka M, Matsunaga K, Ikeda M, Ogata M, Okazaki T, Ueno S, Kawahara H (1976) The hygienic studies on topped oil (II) The analytical method of topped oil components (organosulfur compounds) in marine products and its application. Ann Hyg Lab Okayama Prefecture 23:27-30

Kira S, Izumi T, Ogata M (1983) Detection of dibenzothiophene in mussel, mytilus edulis, as a marker of pollution by organosulfur compounds in a marine environment. Bull Environ Contam Toxicol 31:518-525

Larsen PF, Gadbois DF, Johnson AC, Doggett LF (1983) Distribution of polycyclic aromatic hydrocarbons in the surficial sediments of Casco Bay, Maine Bull Environ Contam Toxicol 30:530-535

Nakamura K, Kashimoto K (1977) Contamination of organic sulfur compounds in marine products. J Food Hyg Soc Japan 18:252-259

Obana H, Hori S, Kashimoto T (1981) Determination of polycyclic aromatic hydrocarbons in marine samples by high-performance liquid chromatography. Bull Environ Contam Toxicol 26:613-620

Ogata M, Miyake Y (1973) Compound from floating petroleum accumulating in fish. Water Res 7:1493-1504.

Ogata M, Ogura T (1976) Petroleum components and objectionable malodorous substances in fish flesh polluted by boiler fuel oil. Water Res 10:407-412

Ogata M, Miyake Y (1980) Gas chromatography combined with mass spectrometry for the identification of organic sulfur compounds in shelfish and fish. J Chromatogr Sci 18:594-605

Ogata M, Fujisawa K (1983) Capillary GC/MS determination of organic sulfur compounds detected in oyster and mussel caught in the sea as an oil pollution index. J Chromatogr Sci 21:420-424

Ogata M, Miyake Y, Fujisawa K (1987) Oil smell and oil components in fish flesh reared in seawater containing heavy oil. Oil and Chem Pollution 3: 329-341

Ogata M, Fujisawa K (1988) Oil-tainted water and fish. Water Quality Bulletin 13:56-60

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