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Determination of Polycyclic Aromatic Hydrocarbons in Marine Samples by High-Performance Liquid Chromatography

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It has been reported that polycyclic aromatic hydrocarbons (PAHs) are widely distributed in the environment, although their concentrations are quite low (HARRISON et al. 1975, ANDELMAN et al. 1970). Some PAHs, benzo(a)pyrene, dibenz(a,h)anthracene and 3-methylcholanthrene, are carcinogenic to mammals after in vivo hydroxylation by mixed function oxidases. PAHs originate largely from smoke, soot, and exhaust gas produced by combustion and from petroleum oil spilled into the sea (MALINS 1977), so that the quantity of PAHs in the environment is broadly related to the level of contamination in a given region. Although PAHs have been determined by a TLC-fluorescence method (HORI et al. 1976, SHIRAISHI et al. 1972), a GLC method and a GC-MS method (FRETHEIM 1976), these methods suffer from complex pretreatment. On the other hand, the development of high-performance liquid chromatography (HPLC) has made it possible to analyze PAHs with good separation and high sensitivity and to simplify the pretreatment processes (DAS & THOMAS 1978, OGAN et al. 1979). In this study, ten PAHs in sediments, oyster, and wakame seaweed were determined by HPLC with a fluorescence detector (HPLC-FD). The contents and the patterns of PAHs found in sediments and marine samples may be used as an indicator of petroleum contamination in the sea.

MATERIALS AND METHODS

Sediments were collected in August 1977 and August 1978 from the Osaka Port area (Fig. 4). Six samples each of oyster and wakame seaweed were purchased in local markets. All PAH standard solutions were prepared by dissolving the chemicals in n-hexane without additional purification. All the reagents other than dimethyl sulfoxide and NaCl were of pesticide analysis grade (Wako Pure Chemical Industries, Ltd., Osaka).

A liquid chromatograph equipped with a variable-wavelength fluorescence detector was used with a 20 cm x 4.6 mm i.d. column packed with Lichrosorb RP-18 (Merck, C_{18} reversed phase) of 5-μm particle size. The mobile phase was 70% acetonitrile-30% water and was degassed before use by ultrasoinfication. Flow rate was 2.0 mL/min. Each sample (dissolved in n-hexane) was injected into the HPLC-FD machine in a volume of 200 μL. Use of n-hexane instead of acetonitrile resulted in a shorter retention time, a sharper peak, and a better separation of each PAH. The following three excitation and emission wavelength pairs were used to analyze

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ten PAHs by fluorescence detection: (a) ex 334 nm, em 384 nm for anthracene, pyrene, benz(a)anthracene and benzo(e)pyrene : (b) ex 365 nm, em 430 nm for benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene and benzo(g,h,i)perylene : (c) ex 300 nm, 393 nm for dibenz(a,h)anthracene and 3-methylcholanthrene.

The separation of PAHs from samples is illustrated in Fig. 1.

sample (10-30 g)
| saponify with 1.5 N KOH-EtOH (100-200 mL) for 2h
| add water (same volume as that of KOH-EtOH)
| extract with n-hexane (100 mL x 3)
n-hexane layer
| wash with water (100 mL x 3)
| extract with dimethyl sulfoxide (100 mL x 3)
dimethyl sulfoxide layer
| add water 300 mL, NaCl 50 g
| extract with n-hexane (100 mL x 3)
n-hexane layer
| wash with water (100 mL x 3)
| dehydrate with anhydrous Na_2SO_4
| concentrate to 5 mL
column chromatography
| upper: silica gel (Merck, art 7734) 5 g
| activation: 130 °C, 4h
| lower: alumina (Merck, art 1097) 6 g no activation
| 1st. fr. elute with n-hexane (70 mL)
| 2nd. fr. elute with 15% Et_2O in n-hexane (80 mL)
2 nd. fr.
|
HPLC-FD

Fig. 1 Separation of PAHs

Each PAH peak from a sample on a chromatogram was identified by comparison of the retention time with that of the corresponding PAH standard, and from the relative peak height under the three optimal fluorescence conditions (Fig. 2). The PAHs in oyster and wakame seaweed were identified by GC-MS. PAHs in wakame seaweed were also isolated by HPLC-FD, and the excitation and emission spectrum of each PAH was observed with a fluorescence spectrophotometer. Each peak area was calculated with an integrator.

RESULTS

Chromatograms of the PAH standards at the same concentration (Fig. 2) showed that each PAH could be determined quantitatively under the three optimal fluorescence conditions, even though a mixture of ten PAHs was injected. The fluorescence intensity of benzo(a)pyrene at ex 365 nm, em 430 nm was very strong, being twice those of pyrene at ex 334 nm, em 384 nm and benzo(k)fluoranthene at ex 365 nm, em 430 nm, although these compounds showed relatively strong fluorescence intensities themselves.

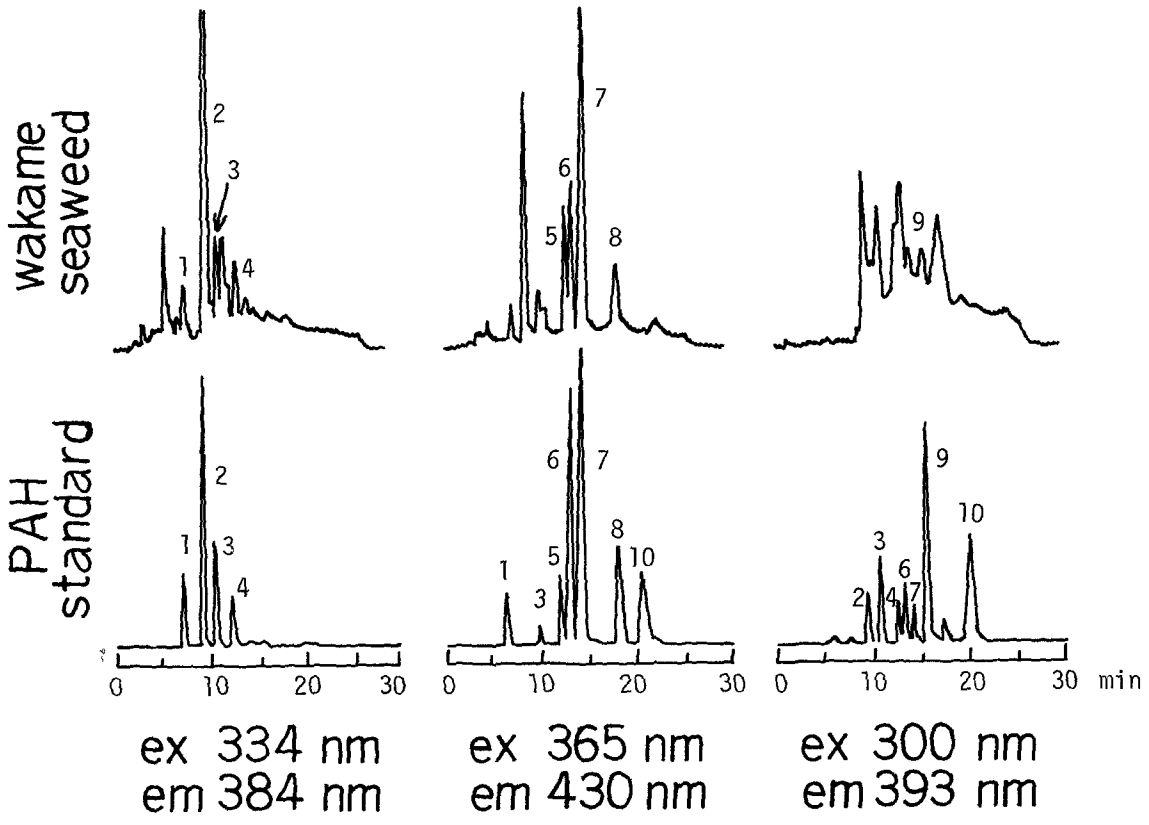

Fig. 2 HPLC-FD chromatograms of PAHs from wakame seaweed and a mixture of ten PAH standards. Peaks: 1 anthracene, 2 pyrene, 3 benz(a)anthracene, 4 benzo(e)pyrene, 5 benzo(b)fluoranthene, 6 benzo(k)fluoranthene, 7 benzo(a)pyrene, 8 benzo(g,h,i)perylene, 9 dibenz(a,h)anthracene, 10 3-methylcholanthrene.

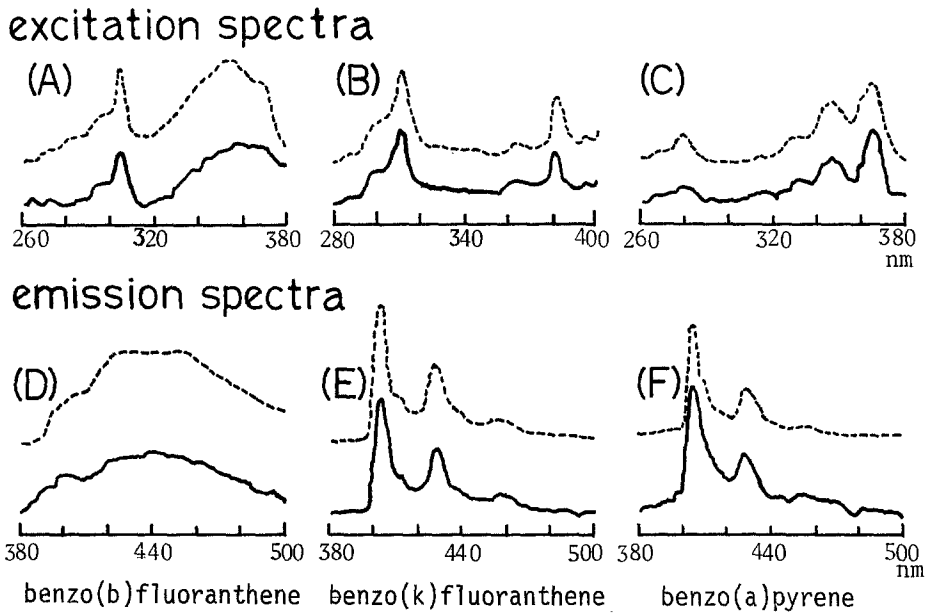

Fig. 3 Excitation and emission spectra of benzo(b)fluoranthene, benzo(k)fluoranthene and benzo(a)pyrene.
———, separated from wakame seaweed by HPLC-FD; ------, PAH standard. (A) em 440 nm, (B) em 410 nm, (C) em 430 nm, (D) ex 359 nm, (E) ex 310 nm, (F) ex 365 nm.

Chromatograms of PAHs in wakame seaweed are shown in Fig. 2. The characteristic peak of pyrene was observed in all the samples at ex 334 nm, em 384 nm and benzo(b)fluoranthene, benzo(k)fluoranthene, and benzo(a)pyrene were observed at ex 365 nm, em 430 nm.

The recoveries were between 81 and 91% when 250 ng of each PAH standard was added to 10 g of yellow fish, and the detection limit with HPLC-FD was 10 pg of benzo(a)pyrene, 20-30 pg of pyrene, benzo(k)fluoranthene and dibenz(a,h)anthracene, and 40-100 pg of the other PAHs. The calibration plots of PAHs were liner between 0 to 20 ng.

Nine PAHs in wakame seaweed, i.e., anthracene, pyrene, benz(a)anthracene, benzo(e)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(g,h,i)perylene and dibenz(a,h)anthracene, were identified from their excitation and emission spectra using a fluorescence spectrophotometer after isolation by HPLC-FD. The excitation and emission spectra of benzo(b)fluoranthene, benzo(k)fluoranthene and benzo(a)pyrene are shown in Fig. 3. The nine PAHs in oyster and wakame seaweed were also identified by GC-MS, but the isomers benzo(e)pyrene and benzo(a)pyrene, as well as benzo(b)fluoranthene and benzo(k)fluoranthene, could not be identified because they did not separate on the gas chromatograms.

PAH concentrations in the sediments (TABLE 1) had not changed

TABLE 1 Amount of PAHs in sediments collected in the Osaka Port area (ppb: on a wet basis)

	No.1[a]		No.2[a]		No.3[a]	
	1977	1978	1977	1978	1977	1978
anthracene	88	56	32	15	38	21
pyrene	630	580	240	190	240	150
benz(a)anthracene	230	110	49	38	48	44
benzo(e)pyrene	650	460	290	180	250	160
benzo(b)fluoranthene	580	640	230	290	270	220
benzo(k)fluoranthene	250	260	110	110	100	96
benzo(a)pyrene	320	300	140	130	140	100
benzo(g,h,i)perylene	270	520	86	130	90	69
dibenz(a,h)anthracene	40	-[b]	30	-[b]	19	-[b]

a) See the sampling location in Fig. 4.
b) Not determined

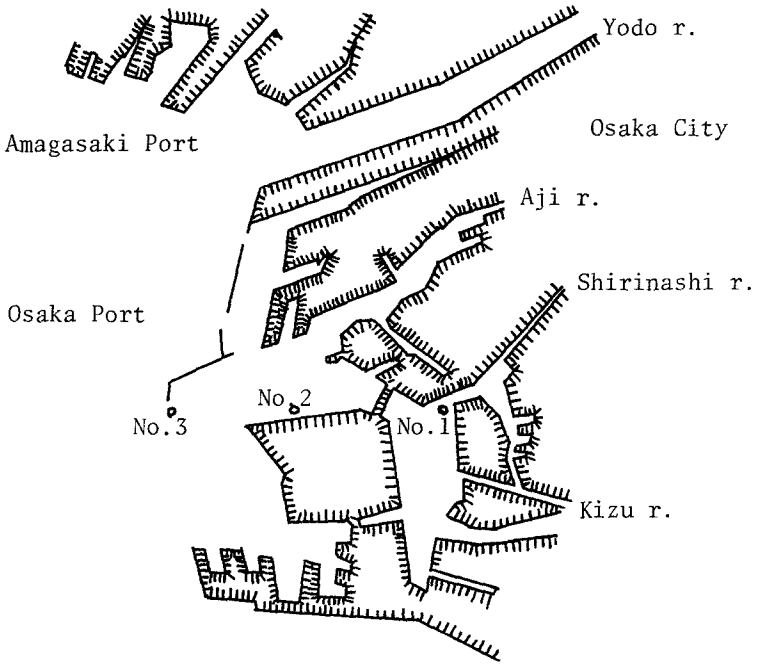

Fig. 4 Location of sediment sampling sites in the Osaka Port area.
Locations: No. 1 (E 135°26', N 38°20')
No. 2 (E 135°25', N 38°20')
No. 3 (E 135°24', N 38°20')

TABLE 2 Amount of PAHs in oyster (ppb: om a wet basis)

	Sampling location					
	Fuchisaki[a]	Saka[a]	Oshika[b]	Teno[a]	Akitsu[a]	Akitsu[a]
anthracene	N.D.[c]	4.2	N.D.[c]	N.D.[c]	1.6	1.5
pyrene	7.0	52	38	19	20	16
benz(a)anthracene	1.5	10	2.4	3.3	3.2	3.1
benzo(e)pyrene	2.8	32	6.4	5.7	11	7.1
benzo(b)fluoranthene	3.0	20	4.8	4.5	7.2	4.5
benzo(k)fluoranthene	0.7	5.1	1.3	N.D.[d]	0.7	0.6
benzo(a)pyrene	0.3	2.6	1.0	0.6	1.3	1.0
benzo(g,h,i)perylene	0.5	2.7	0.6	N.D.[e]	2.8	1.6
dibenz(a,h)anthracene	N.D.[f]	N.D.[f]	N.D.[f]	N.D.[f]	N.D.[f]	N.D.[f]

a) Hiroshima prefecture
b) Miyagi prefecture
c) N.D. $<$ 0.25 ppb
d) N.D. $<$ 0.06 ppb
e) N.D. $<$ 0.20 ppb
f) N.D. $<$ 0.06 ppb

TABLE 3 Amount of PAHs in wakame seaweed (ppb: on a dry basis)

	Sampling location					
	Shimabara Nagasaki pref.	Utatsu Miyagi pref.	Tokura Miyagi pref.	Omoto Iwate pref.	Kunisaki Ōita pref.	Yura Hyōgo pref.
anthracene	2.7	2.4	2.5	1.6	1.7	12
pyrene	13	12	20	12	41	260
benz(a)anthracene	3.4	1.6	2.7	2.1	1.6	69
benzo(e)pyrene	2.3	2.1	0.9	2.1	15	410
benzo(b)fluoranthene	4.3	1.9	2.8	3.4	24	68
benzo(k)fluoranthene	2.2	0.9	1.2	1.7	12	120
benzo(a)pyrene	2.0	0.8	0.6	1.1	9.0	81
benzo(g,h,i)perylene	2.0	0.8	0.6	1.6	11	130
dibenz(a,h)anthracene	0.5	0.1	N.D.[a]	0.1	0.3	10
3-methylcholanthrene	N.D.[b]	N.D.[b]	N.D.[b]	N.D.[b]	N.D.[b]	N.D.[b]

a) N.D. $<$ 0.06 ppb
b) N.D. $<$ 0.1 ppb

markedly between 1977 and 1978. Pyrene, benzo(e)pyrene and benzo-(b)fluoranthene concentrations ranged from 580 to 650 ppb at area No.1. In contrast to the rather high levels of PAHs in sediments, the surface water at the three locations in 1978 contained pyrene at a level of 0.5 to 0.7 ppt and a trace of benzo(a)pyrene, but no other PAH.

PAH contents in oyster and wakame seaweed are listed in TABLE 2 and 3, respectively. Pyrene was the major constituent of PAHs in both oyster and wakame seaweed (the exceptionally high value of 260 ppb observed in the wakame seaweed sample taken at Yura is discussed later).

DISCUSSION

HPLC-FD showed better separation of PAHs and higher sensitivities than other methods (SHIRAISHI et al. 1972, FRETHEIM et al. 1976, OGAN et al. 1979). For instance, HPLC-FD with a reversed phase column made it possible to separate benzo(b)fluoranthene, benzo(k)fluoranthene and benzo(a)pyrene, which was difficult by previously reported method (MAEDA et al. 1975), and thus each component of PAHs can be collected and identified separately. This is desirable, because each PAH has a different effect on human health. Separation and cleanup of PAHs in this study were also much simpler than in the previous method (SHIRAISHI et al. 1972). Characteristic chromatograms of PAHs were obtained by using a fluorescence detector with the three wavelength pairs which was suitable to enhance the detection limit of pyrene, benzo(a)pyrene and dibenz(a,h)anthracene. They give the typical chromatograms showing noteworthy contamination, although the three pairs are different from OGAN's (1979). The extraction with dimethyl sulfoxide and the chromatographic cleanup can be omitted when the content of interfering compounds is relatively small as in the case of oyster or wakame seaweed.

HARRISON et al. (1975) compared benzo(a)pyrene concentrations in sediments from Italy (100-3000 ppb, on a dry basis), an industrialized area, with those from Greenland (5 ppb), a nonindustrialized area. The benzo(a)pyrene concentrations in sediments from Osaka Port (TABLE 1) were similar to the concentrations in sediments from Italy. Although the PAH concentrations in the sediments from Area No. 1 were higher than the others, this difference was consistent with the results of analysis of organic sulfur compounds and PCBs in the same samples by NAKAMURA et al. (1979). The amounts of these contaminants in Area No. 3, located approximately 5 km distant from the estuary, suggest that they will become diluted in the sea environment. IBE et al. reported the amounts of benzo(a)pyrene, n-paraffin and iso-paraffin in sediments from Tokyo Bay. They showed that the sediments, which had contained high benzo(a)pyrene (3 ppb), had the high n-paraffin (0.2 ppm) and iso-paraffin (0.03 ppm), however the sediments contained low levels of benzo(a)pyrene (1 ppb) had also low n-paraffin (Trace) and iso-paraffin (trace). These suggest that PAHs in sediments were related to petroleum contamination.

Benzo(a)pyrene concentrations in oyster are similar to the values previously reported by MAEDA et al. (0.8-56 ppb), but these values might be artificially high because the separation of benzo(a)pyrene from benzo(k)fluoranthene was difficult and the emission spectra of two compounds were very similar (Fig. 3). SHIRAISHI et al. (1979) pointed out that benzo(a)pyrene concentration was accelerated 20% higher in the presence of equal amount of benzo(k)fluoranthene by TLC-fluorescence method. PAH levels in wakame seaweed from Yura (TABLE 3) were much higher than in the others. Concerning the composition of PAHs, moreover, pyrene content in the seaweed from Yura was lower than that in seaweed from the other areas and proportion of each PAH was similar to that in sediments from Osaka Port. It was considered that this wakame seaweed had been heavily contaminated by petroleum oil. The results indicate that oyster and wakame seaweed usually contain PAHs at ppb levels. On the other hand, the detection of much higher PAHs in shellfish can be considered to be an indicator of environmental pollution, especially by petroleum oil.

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