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Quantitation of Sulfur Containing Oil Compounds and Polychlorinated Biphenyls (PCB) in Marine Samples

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Oil spillage by causual boat accidents and/or discharge from boats and industrial sewages presently cause world-wide contamination of coastal sea water, beach and biota in the marine environment.

Oil contamination in the estuaries of coasts has become a serious problem not only in industrial countries, but also in non-industial ones. In the industrial areas and vicinities, offensive odours in marine products were caused. (OGATA *et al.* 1973, 1975, 1976)

For the fingerprint of oil pollution, *n*-alkanes, polyaromatic hydrocarbons are widely used in marine biota, sediments and human tissues. (BLUMER 1970, SUESS 1970, NAKANISHI *et al.* 1977)

The authors selected sulfur conaining oil compounds pollution in marine products and reported contamination of sulfur compounds in oysters from the Seto Inland Sea. (NAKAMURA *et al.* 1975) These compounds were clarified as dibenzothiophene derivatives. (NAKAMURA *et al.* 1977)

For the successive analyses of these compounds, gas chromatography on a capillary column and high pressure liquid chromatography (HPLC) technique were also reported. (WARNER 1975, NAKAMURA *et al.* 1977)

In this paper, levels of contamination of sulfur containing oil compound in shell fishes, other marine samples and sediments are determined by gas chromatography. The evaluation of Polychlorinated biphenyls(PCB) levels in the same samples are also presented.

EXPERIMENTAL

Reagents

All solvents used were PCB analysis grade available from Wako Pure Chemical Ind., Ltd.

C-class heavy oil* as an authentic reference of oil was obtainee from Mitsubishi Oil Co., Ltd. via National Institute of Health. By the use of micro-coulometer combined with gas chromatograph (GC-MCD), it was clarified that 2238 ppm of organic sulfur was contained in this heavy oil as previously reported. (MURAKAMI *et al.* 1975)

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Material and Procedure

Most of the shell fishes used were on the market in Osaka. Other samples marked (*) in Table 1 were collected directly from the estuaries in 1977.

Marine samples (Fresh wt. 50g, Sediments; dry wt. 5g) blotted into pieces were saponified with 2N KOH-ethanol for 1 hr. After saponification followed by addition of the same portion of water, this phase was extracted with *n*-hexane (100 ml) three times. Hexane extract was concentrated and chromatographed over a silica gel column (Merck Art.7734, activated at 130° for 4 hrs and 3% water addition,10g) as shown in Fig. 1. (NAKAMURA *et al.* 1977)

Gas Chromatography and Quantitation

Organic Sulfur Compounds; A Shimadzu 4B-PF gas chromatograph furnished with 394 nm optical filter (FPD-GC) was used for fingerprinting sulfur compounds in oil and marine samples. It was equipped with 2% OV-17 on Gas Chrom Q(80/100) AW-DMCS. The column oven was temperature programmed from 110° to 210° at 4°/min under conditions of gas flows; air and hydrogen 22 ml/min, nitrogen 40 ml/min.

Concentration of organic sulfur in marine samples were determined by total peak height appeared in FPD chromatograms in comparison with the peaks of sulfur containing oil compounds in authentic C-class heavy oil, in which 2238 ppm by GC-MCD analysis as described above.

PCB; Final hexane fraction shown in Fig. 1 was injected to ECD(Ni^{63})-gas chromatograph; Varian 2100 equipped with a glass column (2m x 2mm i.d.) packed with 2% OV-1 on Gas Chrom Q(100/120) AW-DMCS. Temperatures and gas flow are as follows; Det. 295°, Inj. 210°, Column oven 180° and nitrogen, 30 ml/min.

Calculation of PCB residues was made by using Kaneclor mixture (KC 300, 400, 500, 600 ; 1:1:1:1) previously reported. (UGAWA *et al.* 1974)

RESULTS AND DISCUSSION

Sulfur containing oil compounds were extracted from marine samples along with PCB. Concentration of sulfur compounds and PCB concentration in marine samples analysed are described in Table 1.

PCB are widely detected in all samples except in Turban shell (muscle).

Oyster samples indicate high concentration of sulfur compounds. Corbicula are revealed to be at the same level as oysters. One of the analysed samples of corbicula shows relatively high concentration of PCB. It would be assumed that it was caused by the contamination of industrial sewage via river.

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* This oil was designated as the same one spilled out in Dec. 1974 to the Seto Inland Sea at Mizushima district in Okayama Prefecture, Japan.

TABLE 1

Concentration of Organic Sulfur Compounds and PCB in Marine samples (ppm, whole base)

Samples	Source	Total S (Organic)	PCB
Oyster	Nagasaki	0.124	0.012
(*Crassostrea*)	Nagasaki	0.033	0.016
	Hiroshima	0.069	0.009
	Hiroshima	0.063	0.015
	Hiroshima	0.422	0.038
	Osaka	0.070	0.016
	Osaka	0.064	0.037
Short-necked Clam	—	0.012	0.003
(*Tapes*)	—	tr	0.012
	Ise.Mie	nd	0.006
	Matsusaka.Mie	0.018	0.002
Clam	—	tr	0.003
(*Meretrix*)	(Korea)	0.018	0.002
Corbicula	Shimane	0.304	0.015
(*Corbicula*)	Shimane	0.115	0.169
Turban shell, Muscle	—	nd	nd
(*Turbo*) Intestine	—	nd	0.004
Muscle	Mie	0.022	0.005
Intestine	Mie	0.009	0.005
Mud-snail(*Viviparus*)	—	0.008	0.004
Mussel(*Mytilus californianus*)	(CA, U.S.A**)	0.005	0.004
*Cellana stearnsii**	Osaka	nd	0.009
*Liolophura japonica**	Osaka		
Muscle		nd	0.019
Intestine		nd	0.011
Sea cucumber	—	nd	0.011
(*Holothuroidae*)	Mie	0.043	0.021
Sea squirt(*Pyuridae*)	Miyagi	tr	0.003

tr< 0.001 ppm, —:indistinct,

* collected directly in the estuaries of Osaka Bay,1977.

**collected at Pacific Beach in Scripps Institution,1976.

One of turban shells analysed shows that concentration of sulfur in muscle is higher than in intestine. It is not, however, the case in the other one.

In the mussel from Scripps Institution, CA and one sample of sea cucumber, sulfur compounds are also measured.

Marine Sample (Fresh wt. 50g, Dry wt. 5g)

2N KOH-EtOH (300 ml) Saponification 1 hr.&
Extraction with 100 ml *n*-Hexane (x3)

Hexane Extract

Silica Gel (Merck,Art No.7734), 130°,4hrs Act.
3% water addition (10g)
column size : 20 x 1.5 cm
n-Hexane elution : 100 ml

Hexane eluate

FPD-GC(sulfur compounds), ECD-GC(PCB)

Figure 1 Analytical procedure for sulfur containing oil compounds and PCB in marine samples.

Fig. 2 shows FPD gas chromatograms of organic sulfur compounds in corbicula (upper; 0.304 ppm), oyster (middle; 0.422 ppm) and C-class heavy oil (15 μg).These FPD gas chromatographic patterns found in both corbicula and oyster are very similar to that found in heavy oil.

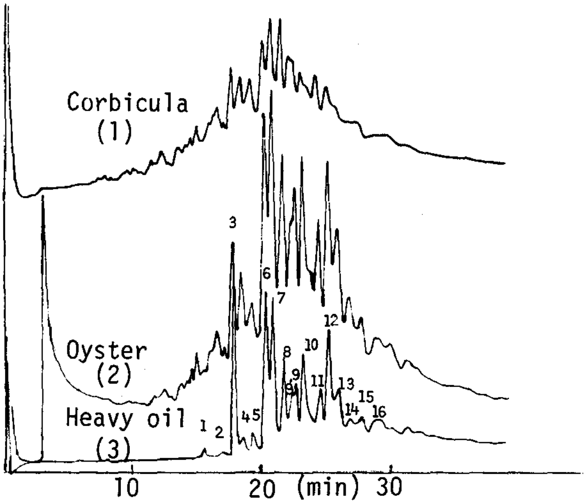

Figure 2 FPD Gas chromatograms of sulfur containing oil compounds in (1) corbicula; 0.304 ppm, (2) oyster; 0.422 ppm, (3) C-class heavy oil; 15 μg.
Gas chromatographic conditions, see text.

TABLE 2

Organic Sulfur Compounds in heavy oil

Peak No.	Compounds
1	$C_{12}H_8S$ (dibenzothiophene)
2	—
3 - 5	$C_{13}H_{10}S$
6 - 8	$C_{14}H_{12}S$
9'	—
9-11	$C_{15}H_{14}S$
12-14	$C_{16}H_{16}S$
15,16	$C_{17}H_{18}S$

Dibenzothiophene derivatives corresponding to each number in the chromatogrm were earlier determined as listed in Table 2. (NAKAMURA *et al.* 1977)

Fig. 3 shows FPD gas chromatograms of organic culfur compounds in turban shell (muscle; lower and intestine; upper). Concentration of sulfur compounds in muscle is higher than in intestine, however, predominant peaks in intestine are neither found in muscle nor in heavy oil. These peaks, therefore, are believed to be original compounds in intestine or contaminants and would be further investigated.

Fig. 4 shows FPD gas chromatograms of oyster and neighbouring sediment revealed 0.07 ppm and 0.016 ppm respectively.

It should be possible to say that sulfur compound in marine organisms, especially oyster and corbicula are bioaccumulated via reflux of oil spilled out and/or discharge.

GIAM *et al.* (1977) reported the use of overall distribution of hydrocarbon for fingerprints of oil.
In addition, these fingerprints of sulfur containing oil compounds also provide the useful parameter of oil pollution in the marine environment. (OGATA *et al.* 1977)

Nevertheless, neither in shell fishes nor in fishes as well as sediments, environmental assessment of these heterocyclic compounds such as dibenzothiophene derivatives is still certain.

It is, therefore, necessary for further investigation on the evaluation of oil components such as *n*-alkanes, polyaromatic hydrocarbons, heavy metals (V, Ni, Cu) usually found in marine samples including PCB and other chlorinated compounds to be studied by the systematical project like " The Mussel Watch " proposed by GOLDBERG (1975).

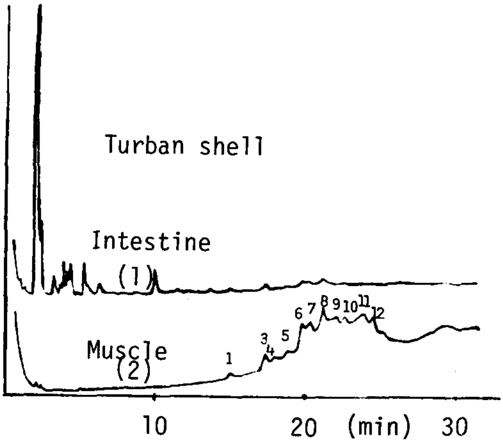

Figure 3 FPD Gas chromatograms of sulfur containing oil compounds in turban shell: (1) Intestine; 0.009 ppm, (2) Muscle; 0.022 ppm.

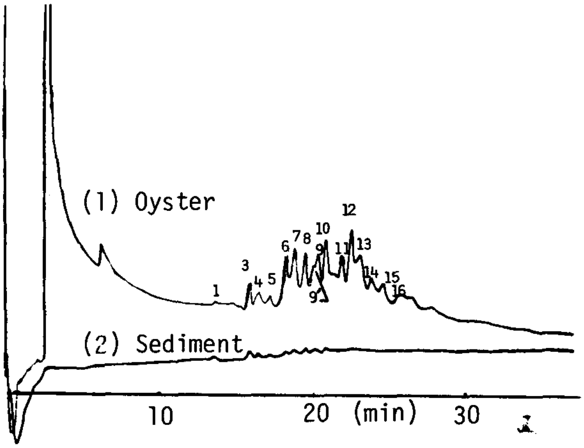

Figure 4 FPD Gas chromatograms of sulfur containing oil compounds in (1) oyster; 0.070 ppm (2) neighbouring sediment; 0.016 ppm.

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