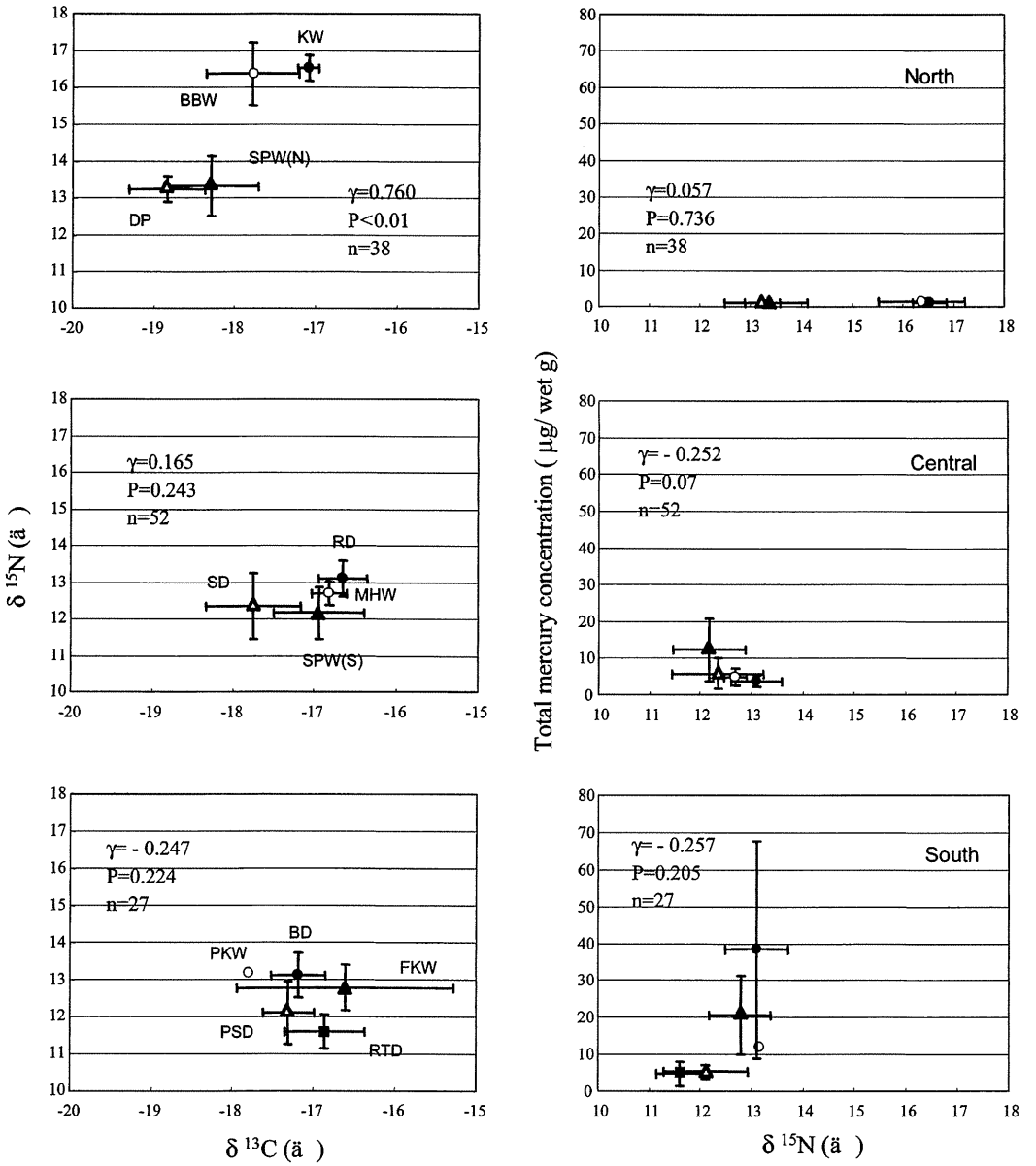

FIGURE 2. Comparison of $\delta^{13}C$, $\delta^{15}N$, and total mercury concentration in toothed whales, dolphins, and porpoises taken from northern, central, and southern Japan. KW: killer whale, BBW: Barid's beaked whale, SPW (N): short-finned pilot whale (northern form), DP: Dall's porpoise, RD: Risso's dolphin, MHW: melon-headed whale, SD: striped dolphin, SPW (S): short-finned pilot whale (southern form), BD: bottlenose dolphin, FKW: false killer whale, PKW: pygmy killer whale, PSD: pantropical spotted dolphin, RTD: rough-toothed dolphin. See Table 1.

TABLE 2. Spatial Differences in $\delta^{13}C$, $\delta^{15}N$, and Total Mercury in Baird's Beaked Whales, Short-Finned Pilot Whales, and Striped Dolphins[a]

	purchase area	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	total mercury (μg/wet g)
Baird's beaked whale	Abashiri (northern Japan), $n = 5$	-18.1 ± 0.2^{a}	$16.5 \pm 0.7^{a,b}$	0.62 ± 0.30^{a}
	Hakodate (northern Japan), $n = 6$	-18.3 ± 0.2^{a}	15.6 ± 0.4^{a}	$1.03 \pm 0.29^{a,b}$
	Ayukawa (northern Japan) and Wada (central Japan), $n = 8$	-17.2 ± 0.3^{b}	16.8 ± 0.9^{b}	1.98 ± 0.43^{b}
short-finned pilot whale	Ayukawa, northern form (northern Japan), $n = 5$	-18.3 ± 0.6^{a}	13.3 ± 0.8^{a}	1.30 ± 0.41^{a}
	Taiji, southern form (central Japan), $n = 9$	-16.8 ± 0.4^{b}	12.6 ± 0.4^{b}	$10.81 \pm 4.47^{a,b}$
	Nago, southern form (southern Japan), $n = 9$	-17.0 ± 0.8^{b}	11.7 ± 0.6^{b}	12.71 ± 11.20^{b}
striped dolphin	Taiji (central Japan), $n = 6$	-18.0 ± 0.4	12.0 ± 0.4	5.20 ± 5.27
	Nago (southern Japan), $n = 5$	-17.4 ± 0.5	12.7 ± 1.2	6.76 ± 2.22

[a] The data represent the mean ± S.D. The data were analyzed by Tukey-Kramer or Scheffe's F test. Different superscript letters indicate a significant difference ($P < 0.05$).

TABLE 3. Comparison between This Study and Previously Reported Data Regarding $\delta^{13}C$, $\delta^{15}N$, and Total Mercury

		$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	T-Hg (μg/wet g)	area	organ (type)	reference
killer whale	$n = 6$	−17.1 ± 0.1	16.5 ± 0.3	1.27 ± 0.13[b]	west North Pacific Ocean	muscle	this study and Endo et al. (*17*)
	$n = 6$	N.D.	N.D.	62.2 ± 21.9	west North Pacific Ocean	liver	Endo et al. (*17*)
	$n = 23$	−17.2 ± 1.0	17.8 ± 0.8	N.D.	east North Pacific Ocean	skin (transient)	Krahan et al. (*3*)
	$n = 13$	−17.0 ± 1.9	16.4 ± 1.7	N.D.	east North Pacific Ocean	skin (resident)	Krahan et al. (*3*)
	$n = 1$	N.D.	N.D.	88	U.K.	liver	Law et al. (*19*)
	$n = 1$	N.D.	N.D.	13.3	Japan Sea	red meat	Endo et al. (*23*)
bottlenose dolphin	$n = 10$	−17.2 ± 0.3	13.1 ± 0.6	38.3 ± 28.3	west North Pacific Ocean	red meat	this study
	$n = 2$	−18.6, −17.1	13.5, 12.6	2.57[a], 166[a]	Mediterranean Sea	muscle	Capelli et al. (*24*)
	$n = 10$	−17.7 ± 0.6	12.3 ± 0.9	5.91 ± 4.07	west North Pacific Ocean	red meat	this study
striped dolphin	$n = 27$	−17.2 ± 0.5	11.1 ± 0.6	N.D.	Mediterranean Sea	blubber	Borrell and Aguilar (*25*)
	$n = 3$	−18.3 ± 0.1	9.1 ± 0.3	28.0 ± 27.5[a]	Mediterranean Sea	muscle	Capelli et al. (*24*)
Risso's dolphin	$n = 8$	−16.7 ± 0.3	13.1 ± 0.5	3.84 ± 1.52	west North Pacific Ocean	red meat	this study
	$n = 3$	−17.2 ± 0.3	11.4 ± 0.9	91.0 ± 73.9[a]	Mediterranean Sea	muscle	Capelli et al. (*24*)

[a] Expressed by μg/g dry weight. [b] Data from Endo et al (*17*).

Hg concentrations in the Kuroshio region may be higher due to natural and anthropogenic inputs. We expected to find a positive correlation between $\partial^{15}N$ value and T-Hg concentration, as the level of Hg accumulation in biota is generally correlated with trophic level as reflected in $\partial^{15}N$ value (*18*). However, a negative correlation between the $\partial^{15}N$ value and T-Hg concentration was found in the combined samples of three areas ($\gamma = -0.238$, $n = 117$, $P < 0.01$), probably reflecting geographical variations of $\partial^{15}N$ (*6, 7, 11*) and T-Hg concentration (*12, 17*) in the seawater around Japan. In contrast, a positive correlation was found between the $\partial^{13}C$ value and T-Hg concentration in the combined samples ($\gamma = 0.219$, $n = 117$, $P < 0.05$). As mentioned above, the measurements of $\partial^{13}C$ and $\partial^{15}N$ in primary producers are necessary. The contamination levels of T-Hg in the toothed whales, dolphins, and porpoises caught off the coast of Japan appear to be determined mainly by the Hg concentration in their habitat rather than by species. In agreement with the present results, the T-Hg concentrations and $\partial^{13}C$ found in the muscle of yellowfin tuna and albacore caught off southern Japan were found to be higher than in those caught off central Japan, whereas the $\partial^{15}N$ found in the former was lower (our unpublished data).

4.2. Species and Spatial Differences in $\partial^{13}C$, $\partial^{15}N$, and Hg Contamination in the Northern Region. Table 3 summarizes the data from the present study together with that obtained from the literature regarding $\partial^{13}C$ and $\partial^{15}N$ values and T-Hg concentration in killer whales, bottlenose dolphins, striped dolphins, and Risso's dolphins.

We assumed that the killer whales stranded on the coast of Japan corresponded to the transient form because the major items found in stomach contents were an assortment of seal tissues (*21*). However, the present results for $\partial^{13}C$ and $\partial^{15}N$ in the stranded killer whales correspond closely to those in observed in the resident form (primary fish eaters) rather than the transient form (primary mammal eaters) of killer whales in the eastern North Pacific Ocean (Table 3) (*3*), and the contamination level of T-Hg in the stranded killer whales was the lowest among the 13 species studied (Table 1). As most recent studies of killer whales have been undertaken using biopsy sample of skin with blubber, contamination data for Hg are lacking. The available data for Hg listed in Table 3 were obtained from liver sample of a killer whale stranded in the United Kingdom (88 μg/wet g) (*19*) and a red meat product sold in Busan, South Korea (13.3 μg/wet g) (*23*). The T-Hg concentration in the liver is compatible with that found in the killer whales stranded in northern Japan (*17*), whereas the concentration in the red meat sample is markedly higher.

Baird's beaked whales inhabit the North Pacific Ocean and adjacent waters, where they prefer deep water, feeding on squid, octopus, and skate (*26*). The average $\partial^{15}N$ in the red meat samples from Baird's beaked whales was almost the same as that in muscle samples from killer whales (Table 1), although the trophic position of Baird's beaked whales was expected to be lower than that of killer whales. Hobson et al. (*5*) reported a higher $\partial^{15}N$ in benthic biota. The higher $\partial^{15}N$ value in the Baird's beaked whales may reflect their preference for deeper water. Around Japan, this species is found in the southern Okhotsk Sea, the eastern Sea of Japan, and off the Pacific coast (*27*) and is hunted at Abashiri, Hakodate, Ayukawa, and Wada, respectively. In agreement with the existence of separate populations, some significant differences in $\partial^{13}C$, $\partial^{15}N$, and T-Hg concentration were found among the three regional samples (Table 2, see Figure S1). The higher level of T-Hg contamination in the Pacific coast population may result from higher Hg level in the southern sea region, as we previously suggested (*12, 17*). The lower $\partial^{13}C$ in the Baird's beaked whales purchased in and around Hakodate and Abashiri may be explained by the lower $\partial^{13}C$ in the cold current regions. Takai et al. (*6*) reported the lowest level of $\partial^{13}C$ in squid caught off the Sea of Japan near the hunting area of the eastern Sea of Japan population.

Dall's porpoises are found in the North Pacific Ocean and adjacent seas. The average $\partial^{13}C$ value in Dall's porpoises was the lowest, and the average $\partial^{15}N$ was higher in our analysis among the 13 species (Table 1). In the eastern North Pacific Ocean, Dall's porpoises are a prey item of the killer whale. Interestingly, the average differences in $\partial^{13}C$ and $\partial^{15}N$ between killer whales and Dall's porpoises were 1.7 ‰ and 3.3‰, respectively, which correspond approximately to one tropic level (*9*). However, in spite of apparent differences in tropic level, the average T-Hg concentration in the red meat samples from Dall's porpoises was very similar to that in the muscle samples from killer whales (Table 1).

The average $\partial^{13}C$, $\partial^{15}N$, and T-Hg concentrations in the northern form short-finned pilot whales were similar to those in Dall's porpoises, respectively. No correlation was found

between the T-Hg concentration and $\partial^{15}N$ values from muscle samples of the four species from the northern region (Figure 2). As Hg is distributed preferentially in liver relative to muscle of mammals (*17, 20*), the hepatic concentrations of T-Hg were compared among the four species. The average T-Hg concentration in the liver from the Dall's porpoises (8.75 ± 2.54 μg/wet g, $n = 5$; our unpublished data) was markedly lower than that from the northern form of short-finned pilot whales (60.0 ± 40.1 μg/wet g, $n = 20$) (*16*), Baird's beaked whales (37.7 ± 44.1 μg/wet g, $n = 37$) (*16*), and killer whales (57.7 ± 22.5 μg/wet g, $n = 6$) (*17*). The hepatic concentrations of T-Hg in these species might not be well correlated with their $\partial^{15}N$ values.

4.3. Species and Spatial Differences in $\partial^{13}C$, $\partial^{15}N$, and Hg Contamination in the Central and Southern Regions. In the western North Pacific, off the coast of Japan, short-finned pilot whales have been morphologically, genetically, and ecologically discriminated into southern and northern forms (*28*), and these forms of whales are hunted for consumption in Nago, Taiji, Wada, and in Ayukawa, respectively. Higher levels of T-Hg contamination in the southern form pilot whales were observed (see Figure S2), probably reflecting the higher concentration of T-Hg in the Kuroshio Current region (*12, 17*). The $\partial^{13}C$ in the northern form pilot whales was significantly lower than that in the southern form pilot whales, probably reflecting lower $\partial^{13}C$ in the cold current areas. The $\partial^{15}N$ in the southern form pilot whales purchased in and around Nago was significantly lower than that in the southern form pilot whales purchased in and around Taiji and in the northern form pilot whales purchased in and around Ayukawa, probably reflecting the lower $\partial^{15}N$ in Kuroshio Current region. Little is known regarding the migration of short-finned pilot whales, and it is unclear whether the southern form short-finned pilot whales caught off Taiji and Nago are from the same population.

The present results for $\partial^{13}C$ and $\partial^{15}N$ in the red meat products of bottlenose dolphins, striped dolphins, and Risso's dolphins were similar to those found in the muscle of three dolphin species (*24*) and in the blubber of striped dolphins (*25*), respectively (Table 3). The T-Hg concentrations reported by Capelli et al. (*24*) were expressed in "μg/g dry weight". As the T-Hg concentrations expressed in "μg/g dry weight" are about four times higher than those expressed in "μg/g wet weight" (*21*), the present data regarding T-Hg concentrations expressed in "μg/g wet weight" are compatible with those reported by Capelli et al (*24*).

Contamination levels of T-Hg in the samples from the five species from southern Japan were markedly higher than those from northern Japan (Table 1) and tended to increase with increases in $\partial^{15}N$. According to Honda (*16*), the contamination level of T-Hg in the liver of striped dolphins caught off Taiji (central Japan) was 205 ± 139 μg/wet g ($n = 59$). Another report showed that the average T-Hg concentrations in the liver of southern form short-finned pilot whales and Risso's dolphins caught off Taiji were 230 μg/wet g ($n = 7$) and 406 μg/wet g ($n = 2$), respectively (*14*). These hepatic levels of T-Hg in the whales and dolphins from central Japan were markedly higher than those from northern species of Dall's porpoises, northern form short-finned pilot whales, Baird's beaked whales and killer whales as mentioned above, which is in agreement with the T-Hg concentrations in the red meat (muscle) samples (Table 1). Unfortunately, little is known about hepatic T-Hg concentrations in whales and dolphins in southern Japan.

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Supporting Information Available

Figures S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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Monitoring of Naturally Produced Brominated Phenoxyphenols and Phenoxyanisoles in Aquatic Plants from the Philippines

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Naturally produced brominated phenoxyphenols (OH-PBDEs) and phenoxyanisoles (MeO-PBDEs) were analyzed in aquatic plants (16 genera of green, brown, and red algae and angiosperms) collected from Luzon Island, the Philippines. Two brominated phenoxyphenols, 2′-hydroxy-2,3′,4,5′-tetrabromodiphenyl ether (2′-OH-BDE68) and 6-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (6-OH-BDE47), were detected in the phenolic fraction of extracts from most of the specimens; *Sargassum oligosystum* had the highest concentrations (101 ng/g fresh weight (fw)). The corresponding phenoxyanisole, 2′-methoxy-2,3′,4,5′-tetrabromodiphenyl ether (2′-MeO-BDE68), was most abundant in *Sargassum* aff. *bataanense* (229 ng/g fw), followed by *Padina* sp., and 6-methoxy-2,2′,4,4′-tetrabromodiphenyl ether (6-MeO-BDE47) was predominant in *Jania adhaerens* (29 ng/g fw). Hydroxy-pentaBDEs, hydroxy-methoxy-tetraBDEs, dihydroxy-tetraBDEs, dihydroxy-tetrabromobiphenyl, and hydroxy-tetrabromodibenzo-*p*-dioxins were also detected. The present study demonstrates that these aquatic plant species could be an abundant source of OH-PBDEs and MeO-PBDEs found in higher trophic organisms in the Asia–Pacific region.

KEYWORDS: Bromophenol; brominated phenoxyanisole; brominated phenoxyphenol; marine algae; *Sargassum*; *Jania*; the Philippines

INTRODUCTION

Benthic marine macroalgae in the Philippine archipelago include many endemic species and genera of red, brown, and green algae that are a major dietary source for many marine organisms (*1*). Bromophenols synthesized by the algae are important flavor components (*2*, *3*); 2,4,6-tribromophenol (2,4,6-TBP) is widely distributed in various seafood species, such as prawns, salmon, and fish dwelling in local waters (*4*). 2,4,6-TBP is the precursor of 2,4,6-tribromoanisole (2,4,6-TBA), which has been identified as a trace environmental contaminant in marine fish (*5*). It is believed that bacteria in algae could be responsible for the O-methylation of 2,4,6-TBP (*6*).

In recent years, lipophilic and bioaccumulative brominated compounds that are proposed to be of natural origin have been detected in marine biota from different sites throughout the world (*7*). One of the major congener groups is methoxylated polybrominated diphenyl ethers (MeO-PBDEs), which have been found in algae (*8*, *9*), sponges (*10*, *11*), mussels (*9*), fish (*12*–*14*), and marine mammals (*15*–*19*). Another group is dimethoxylated tetrabromobiphenyl (diMeO-BB), which has also been accumulated in fish and mammals (*14*, *17*). These compounds are likely to biomagnify within higher trophic organisms via the food chain due to their high lipophilicity (log K_{ow} > 5) (*20*). On the other hand, hydroxylated polybrominated diphenyl ethers (OH-PBDEs) such as 2′-hydroxy-2,3′,4,5′-tetrabromodiphenyl ether (2′-OH-BDE68) are produced by marine algae (*9*) and marine sponges (*21*, *22*) or their associated bacteria (symbiotic cyanobacteria) (*23*). Although OH-PBDEs have been detected in the marine food web (for example, in salmon blood) (*12*) and even in human blood (*24*), it is not clear whether they are derived from naturally produced secondary products or from metabolites of anthropogenic PBDEs.

The objective of this study was to investigate whether OH-PBDEs and their methoxylated analogues (MeO-PBDEs) are found in marine algae from shallow waters of the Philippines. In 2008, 31 specimens (16 genera) of green, brown, and red algae and angiosperms were collected from the littoral zones (four locations) and freshwaters (Taal Lake) of Luzon Island (**Figure 1**). The current survey was also undertaken to assess the source, distribution, and interspecific variation in aquatic plant brominated compounds that are found in fish and mammals from the Asia–Pacific region.

EXPERIMENTAL PROCEDURES

Sampling. In December 2008, a total of 23 marine algae (12 genera of green, red, and brown algae), 2 freshwater green algae (2 genera), and

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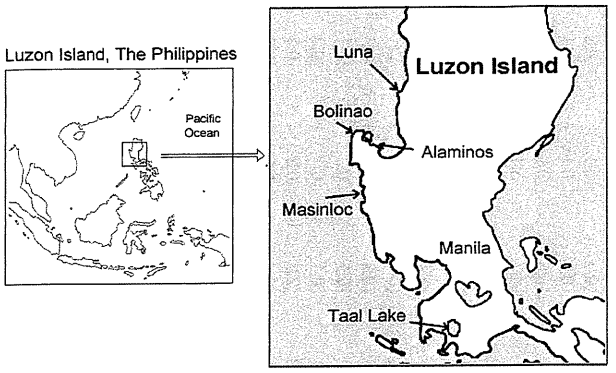

Figure 1. Sampling locations of macroalgae in the Philippines.

2 angiosperms were collected from four littoral zones (Masinloc, Bolinao, Alaminos, and Luna) and Taal Lake on Luzon Island, the Philippines (**Figure 1**). Each sample was frozen at −20 °C prior to chemical analysis.

Species Identification. Almost all of the algae were identified to genus/species level (**Table 1**) using guidebooks and some literature (*1*, *25*−*32*). The identities of critical species monitored for OH-PBDEs and MeO-PBDEs were identified as *Sargassum oligocystum* Montagne (*25*, *26*), *Sargassum* aff. *bataanense* G.C. Trono (*27*), *Halymenia durvillei* Bory de Saint-Vincent (*28*, *29*), and *Jania adhaerens* Lamouroux (*30*). None of the algae investigated have been directly used as food source.

Chemicals and Reagents. Standards of 2,4,6-TBA, 2,4,6-TBP, 2′-methoxy-2,3′,4,5′-tetrabromdiphenyl ether (2′-MeO-BDE68), 2′-hydroxy-2,3′,4,5′-tetrabromodiphenyl ether (2′-OH-BDE68), 6-methoxy-2,2′,4,4′-tetrabromodiphenyl ether (6-MeO-BDE47), and 6-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (6-OH-BDE47) were purchased from AccuStandard Inc. (USA). Dr. G. Marsh (Stockholm University) kindly provided 4′-methoxy-2,3′,4,5′,6-pentabromodiphenyl ether (4′-MeO-BDE121), 2′,6-dimethoxy-2,3′,4,5′-tetrabromodiphenyl ether (2′,6-diMeO-BDE68), and 2,2′-dimethoxy-3,3′,4,4′-tetrabromobiphenyl (2,2′-diMeO-BB80). Their corresponding dihydroxylated analogues were prepared by demethylating the dimethyoxylated analogues by boron tribromide (2 M) in dichloromethane. We used 4′-MeO-BDE121 and 6-OH-[^{13}C]BDE47 (Cambridge Isotope Laboratories, Andover, MA) as internal standards (i.s.) for the determination of neutral and phenolic compounds, respectively. The chemical structures of the target analytes are shown in **Figure 2**.

Sample Cleanup. A sample (10 g of fresh weight (fw)) was cut into pieces, and the homogenate was extracted with MeOH (50 mL) for 1 week at room temperature. The extract was concentrated, and the residue was partitioned between 0.2 M HCl and ethyl acetate (EtOAc). The EtOAc-extractable organic matter (EOM) was determined gravimetrically. A portion (20 mg) of the EOM was spiked with two internal standards, 4′-MeO-BDE121 and 6-OH-[^{13}C]BDE47 (20 ng each). We then removed the EOM by gel permeation chromatography (Bio-Beads S-X3, Bio-Rad Laboratories), eluting with dichloromethane/*n*-hexane (1:1). Brominated products in the eluate were partitioned between 1 M KOH/ethanol (7:3, v/v) and *n*-hexane. The organic phase was concentrated and purified by silica gel column chromatography (0.5 g, Wako gel S-1, Wako Pure Industries, Osaka, Japan), eluting with 12% dichloromethane in *n*-hexane (15 mL) (neutral fraction). The aqueous phase was acidified by HCl and back-extracted with *n*-hexane/diethyl ether (8:2, v/v) (phenolic fraction). A portion (5 mL) of the phenolic fraction (15 mL) was reacted with diazomethane in diethyl ether (methylated phenolic fraction). The concentrated fractions (500 μL) were subjected to gas chromatography−mass spectrometry.

Identification and Quantification. Analyses of natural organohalogens were performed using a gas chromatograph (GC, Agilent 6980N) equipped with a mass selective detector (5973i) in electron ionization and selected ion monitoring mode (EI-SIM). The GC was equipped with an HP-5MS column (30 m × 0.25 mm, 0.25 μm film thickness, J&W Scientific Inc.), and scan data were recorded in the range of *m*/*z* 200−800. Helium was used as a carrier gas at a constant flow rate of 1.0 mL/min. A 2 μL aliquot of each extract was injected in splitless mode with injector and transfer line temperatures of 250 and 280 °C, respectively. The ion source temperature was 230 °C. The GC oven program was as follows: after injection at 70 °C (1.5 min), the temperature was increased at a rate of 20 °C/min to 230 °C (2 min) and then at a rate of 4 °C/min to 280 °C (20 min). The total run time was 40 min. The ions monitored were at *m*/*z* 342 and 344 for 2,4,6-TBA, at *m*/*z* 516 and 518 for two MeO-tetraBDEs, at *m*/*z* 530 and 532 for 2,2′-diMeO-BB80 and 6-OH-[^{13}C]BDE47 (i.s.), at *m*/*z* 546 and 548 for 2,2′-diMeO-BDE68, and at *m*/*z* 594 for 4′-MeO-BDE121 (i.s.). The identification of OH- and MeO-PBDEs in samples was made by comparing the relative GC retention times (RRTs) of samples and standard references versus those of 6-OH-[^{13}C]BDE47 for the phenolic fraction and 4′-MeO-BDE121 for the neutral fraction, respectively, on an HP-5MS column.

Quality Control. Five-point calibration curves (3−500 ng/mL) were linear (r = 0.998) for each compound. A standard solution containing all of the target analytes was analyzed every day to ensure that the calibration curves remained valid (variability, relative standard deviation = 12% (n = 5)) before sample analysis. The recoveries of i.s. and target analytes were assessed by spiking with 1.0 ng of each standard through the entire extraction procedure, and the results ranged from 85 to 102% for phenolic compounds and from 88 to 97% for methoxylated compounds. The limit of quantification (LOQ) using the EI-SIM mode, which was determined using a signal-to-noise ratio of 10, ranged from 5 to 15 pg on the GC column (2.5−7.5 ng/mL) for all analytes. Solvent blanks did not contain any of the analytes under investigation, indicating no carry-over effect between GC-MS runs.

RESULTS

Table 1 shows the sampling locations of each species and the concentrations (on a fresh-weight (fw) basis) of the eight target compounds. The concentrations of 2,4,6-TBP ranged from 0.3 ng/g fw in *Hydroclathrus clathratus* to 107 ng/g fw in *Jania adhaerens*, whereas the levels of 2,4,6-TBA ranged from <0.02 ng/g fw in *Kappaphycus alvarezii* to 2.2 ng/g fw in *Caulerpa lenthillifera*. Among green algae, 2,4,6-TBP levels were lower in freshwater plants from Taal Lake than in marine algae from the littoral zones. The concentration ratios of 2,4,6-TBA/2,4,6-TBP were 0.2−1.44 for the aquatic plants and <0.01−0.28 for the marine algae.

Figure 3 shows total ion chromatograms (TIC) of the brominated compounds in the neutral and phenolic fractions of a brown alga (*Sargassum* aff. *bataanense*) and a red alga (*Jania adhaerens*) collected in Masinloc (**Figure 1**). The MeO-tetraBDEs (peaks a − d) detected in the neutral fractions of both genera were identified as 2′-MeO-BDE68, 2,2′-diMeO-BB80, 6-MeO-BDE47, and 2′,6-diMeO-BDE68, respectively (**Figure 3**). The corresponding OH-tetraBDEs in the methylated phenolic fraction (peaks a′ and c′) were identified as 2′-OH-BDE68 and 6-OH-BDE47, respectively. The mass spectrum of peak e′ in the phenolic fraction of *J. adhaerens* corresponded to dimethoxylated pentaBDE (M^+, *m*/*z* 620) (**Figure 4**), indicating the presence of OH-MeO-pentaBDE or diOH-pentaBDE (unknown structures). One of the major environmentally relevant anthropogenic compounds, BDE-47, was not present in any species investigated (LOQ, <0.02 ng/g fw).

The methylated phenolic fraction of *J. adhaerens* was monitored at *m*/*z* 516 (MeO-tetraBDE), *m*/*z* 546 (diMeO-tetraBDE), and *m*/*z* 530 (diMeO-BB80 and MeO-tetraBDD) (**Figure 5**). In the SIM profile at *m*/*z* 546, several peaks were identified as diMeO-tetraBDEs, indicating the presence of diOH- or OH-MeO-tetraBDEs in the algae. In the profile monitored at *m*/*z* 530, the peak (t_R = 17.6 min) was identified as 2,2′-diMeO-BB80, whereas the peaks between 21 and 25 min were tentatively identified as methoxy-tetrabromo-*p*-dioxin (MeO-tetraBDD) because the mass spectrum exhibited M^+ (*m*/*z* 526) and a characteristic fragment $[M - COCH_3]^+$ (*m*/*z* 473), indicating the presence of OH-tetraBDD (Supporting Information, Figure S1). The identity

Table 1. Sample Characterization and Levels of Brominated Compounds in Freshwater and Marine Plants Collected from Philippine Waters in 2008

			concentration (ng/g fw)									
			neutral fraction					phenolic fraction				
genus (species)	source	EOM[a] (%)	2,4,6- TBA	2′-MeO-BDE68	6-MeO-BDE47	2,2′- diMeO-BB80	2′,6- diMeO-BDE68	2,4,6-TBP	2′-OH-BDE68	6-OH-BDE47	2,2′- diOH-BB80	2′,6- diOH-BDE68
angiosperms												
Ceratophyllum sp.	Taal Lake, Tanauan Batangas	0.28	0.6	ND[b]	ND	ND	ND	0.5	ND	ND	0.1	ND
Hydrilla sp.	Taal Lake, Tanauan Batangas	0.30	0.4	ND	ND	ND	ND	1.6	0.7	0.6	0.2	0.1
green algae												
Chara sp.	Taal Lake, Tanauan Batangas	0.21	0.3	ND	0.1	ND	ND	1.5	ND	ND	0.1	ND
Cladophora sp.	Taal Lake, Tanauan Batangas	0.35	1.3	ND	ND	ND	ND	0.9	ND	ND	0.1	ND
Caulerpa lentillifera	Lucero, Bolinao, Pangasinan	0.37	2.2	1.2	1.0	0.5	0.2	30	0.7	1.2	0.6	0.4
Caulerpa taxifolia	Lucero, Bolinao, Pangasinan	0.40	0.5	2.9	1.4	1.8	0.4	20	0.8	1.5	0.2	0.3
Chaetomorpha crassa	Lucero, Bolinao, Pangasinan	0.32	0.1	0.4	0.2	0.5	0.1	7.7	0.2	0.3	0.3	0.1
Chlorodesmis sp.	Lucero, Bolinao, Pangasinan	0.73	0.1	5.9	1.7	6.4	0.8	11	1.5	0.3	0.1	0.1
brown algae												
Padina minor	Lucero, Bolinao, Pangasinan	0.45	0.2	0.7	0.2	0.9	0.1	1.1	0.3	0.2	0.2	0.1
Padina sp.	Masinloc, Zambales	0.31	0.7	44	1.4	2.4	0.5	2.5	0.9	0.5	ND	0.1
Hydroclathrus clathratus	Masinloc, Zambales	0.46	ND	2.0	0.2	0.8	0.1	0.3	0.3	0.4	0.1	0.2
Sargassum oligosystum[c]	Zacarias Island, Alaminos	0.30	ND	0.7	0.2	1.9	0.1	3.3	10.4	91	0.1	0.1
Sargassum aff. *bataanense*[d]	Masinloc, Zambales	0.28	0.4	229	3.6	2.6	30	13	3.3	10	0.2	0.9
Sargassum sp.	Masinloc, Zambales	0.38	0.1	6.1	4.2	1.4	0.4	1.7	0.2	0.3	0.1	0.3
Sargassum sp.	Masinloc, Zambales	0.15	0.3	11	0.6	1.3	1.0	3.8	0.3	0.2	0.1	0.1
Sargassum sp.	Masinloc, Zambales	0.36	ND	4.7	1.5	4.8	0.4	0.5	8.8	5.6	0.5	1.8
Sargassum sp.	Luna, La Union	0.34	0.6	0.9	0.2	0.4	0.7	6.9	0.7	4.7	5.1	4.2
Sargassum sp.	Luna, La Union	0.27	0.5	1.8	0.1	0.5	0.9	7.7	4.2	0.3	0.3	2.5
Sargassum sp.	Lucero, Bolinao, Pangasinan	0.45	ND	0.3	0.1	0.4	0.1	0.4	0.1	0.3	0.2	ND
red algae												
Gracilaria edulis	Luna, La Union	0.22	0.1	0.3	0.1	0.1	0.6	2.3	0.1	0.1	ND	1.4
Hydropuntia edulis	Lucero, Bolinao, Pangasinan	0.05	1.3	5.4	4.5	2.6	1.2	32	0.1	0.1	ND	0.1
Acanthophora specifera	Lucero, Bolinao, Pangasinan	0.22	0.4	0.5	0.2	0.7	0.2	1.9	0.3	0.3	2.2	0.2
Halymenia durvillei[e]	Lucero, Bolinao, Pangasinan	0.46	0.1	0.3	0.1	0.2	0.1	1.1	0.2	0.1	0.2	0.1
Halymenia sp.	Lucero, Bolinao, Pangasinan	0.40	0.1	0.1	0.05	ND	0.1	6.3	0.1	0.1	0.1	0.2
Ceratodictyon spongiosum	Alaminos, Pangasinan	0.27	0.7	0.9	1.2	3.7	0.3	9.3	1.9	2.3	0.2	0.1
Kappaphycus alvarezii	Alaminos, Pangasinan	0.09	ND	0.3	0.05	0.3	0.1	18	0.1	0.1	ND	ND
Kappaphycus alvarezii	Alaminos, Pangasinan	0.07	ND	0.1	0.05	0.4	ND	58	ND	ND	ND	0.1
Kappaphycus alvarezii	Masinloc, Zambales	0.10	0.1	1.3	0.1	1.5	0.2	45	0.1	0.1	0.1	0.1
Kappaphycus alvarezii	Masinloc, Zambales	0.39	ND	1.3	0.05	0.5	0.1	13	ND	0.1	ND	ND
Jania adhaerens[f]	Masinloc, Zambales	0.20	0.8	78	29	44	13	107	25	6.2	2.8	4.4
Jania sp.	Masinloc, Zambales	0.13	0.1	3.6	1.7	6.3	0.5	105	1.1	1.7	0.3	0.5

[a] EOM, extractable organic matter. [b] ND, not detected (<0.02 ng/g fw). [c] References *25* and *26*. [d] Reference *27*. [e] Reference *28* and *29*. [f] Reference *30*.

of these compounds remains to be confirmed because the appropriate standards were not available for this survey.

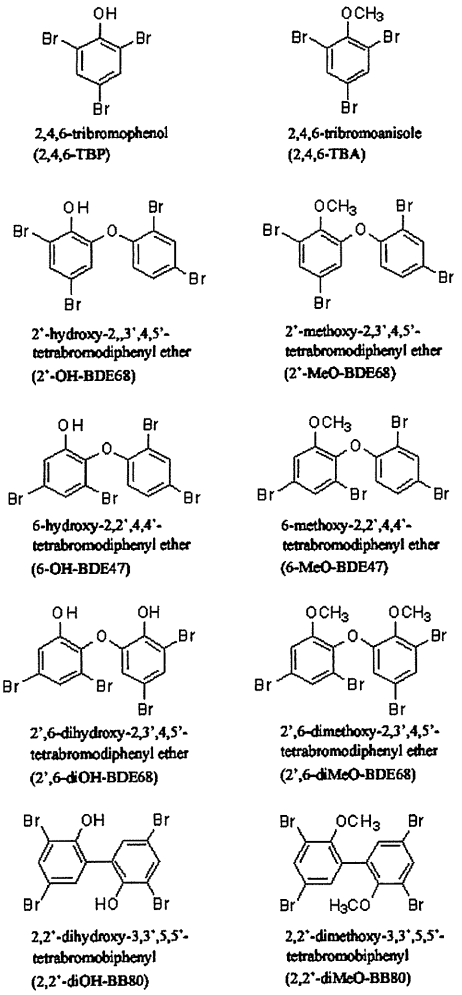

Figure 2. Chemical structures of target compounds monitored in this study.

In the neutral fraction, 2′-MeO-BDE68 and 6-MeO-BDE47 were detected in all of the samples except for the freshwater plants. The highest levels of 2′-MeO-BDE68 were detected in *Sargassum* aff. *bataanense* (229 ng/g fw), followed by *J. adhaerens* (78 ng/g fw) and *Padina* sp. (44 ng/g fw). In contrast, 6-MeO-BDE47 was predominant in *J. adhaerens* (29 ng/g fw), but its concentration was < 5 ng/g fw in the other species. The level of 2,2′-diMeO-BB80 was highest in *J. adhaerens* (44 ng/g fw), followed by *Chlorodesmis* sp. (6.4 ng/g fw), whereas 2′,6-diMeO-BDE68 was most abundant in *Sargassum* aff. *bataanense* (30 ng/g fw), followed by *J. adhaerens*.

In the phenolic fraction, 2′-OH-BDE68 and 6-OH-BDE47 were detected in all of the samples except for the freshwater algae and one red alga (*Kappaphycus alvarezii*). The highest concentrations of 2′-OH-BDE68 were observed in *J. adhaerens* (25 ng/g fw), followed by *Sargassum oligosystum* (10 ng/g fw). Concentrations of 6-OH-BDE47 were highest in *S. oligosystum* (91 ng/g fw), followed by *Sargassum* aff. *bataanense* (10 ng/g fw). The concentrations of the corresponding dihydroxylated analogues (2,2′-diOH-BB80 and 2′,6-diOH-BDE68) were < 5.1 ng/g fw in all samples. The concentrations of four analytes in *Sargassum* and *Jania* sp. are compared in Supporting Information, Figure S2.

DISCUSSION

To our knowledge, this is the first study to monitor OH- and MeO-PBDEs in aquatic plants from the Asia–Pacific region, although 2′-MeO-BDE68 has been isolated in green algae (*Cladophora fascicularis*) from Okinawa, Japan, by Kuniyoshi et al. (*8*). We found that the occurrence of OH- and MeO-PBDEs from the Philippines was species-dependent; the highest levels were observed in *Sargassum*, *Jania*, and *Padina* species from Masinloc, Zambales. The other algal species also contained smaller amounts of 2′-OH-/2′-MeO-BDE68 or 6-OH-/6-MeO-BDE47, indicating the wide distribution of these brominated products in shallow waters of the Philippines. The levels of OH- and MeO-tetraBDEs varied considerably within the genus *Sargassum*, implying that even in the same genus, particular species can produce preferably these brominated compounds. The variation may be attributed to biotic factors such as stress, grazing, water temperature, stage of the algae annual cycle. At present, it is not possible to tell whether the OH- and MeO-PBDEs

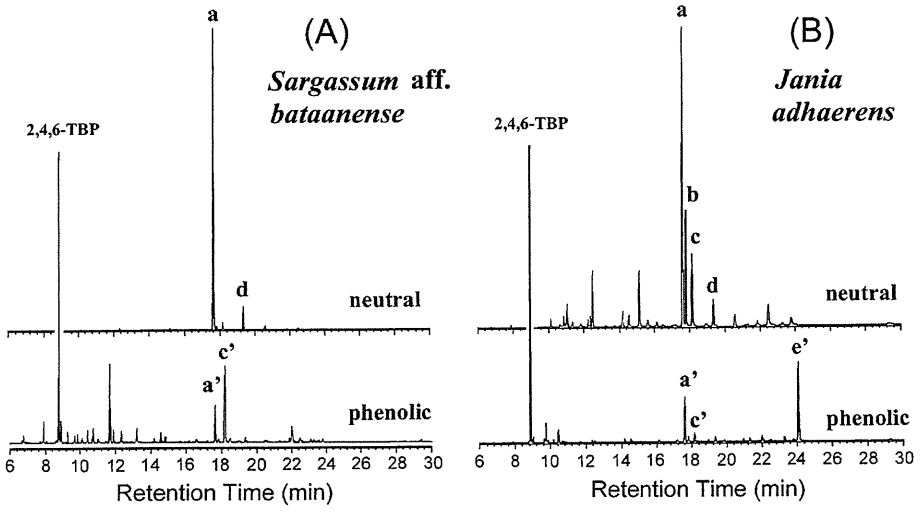

Figure 3. Total ion chromatograms of brominated compounds in neutral and methylated phenolic fractions from *Sargassum* aff. *bataanense* (**A**) and *Jania adhaerens* (**B**). In the neutral fraction: peak a, 2′-MeO-BDE68; peak b, 2,2′-diMeO-BB80; peak c, 6-MeO-BDE47; peak d, 2′,6-diMeO-BDE68. In the phenolic fraction: peak a′, 2′-OH-BDE68; peak c′, 6-OH-BDE68; peak e′, diOH-pentaBDE or OH-MeO-pentaBDE (as methylated derivatives).

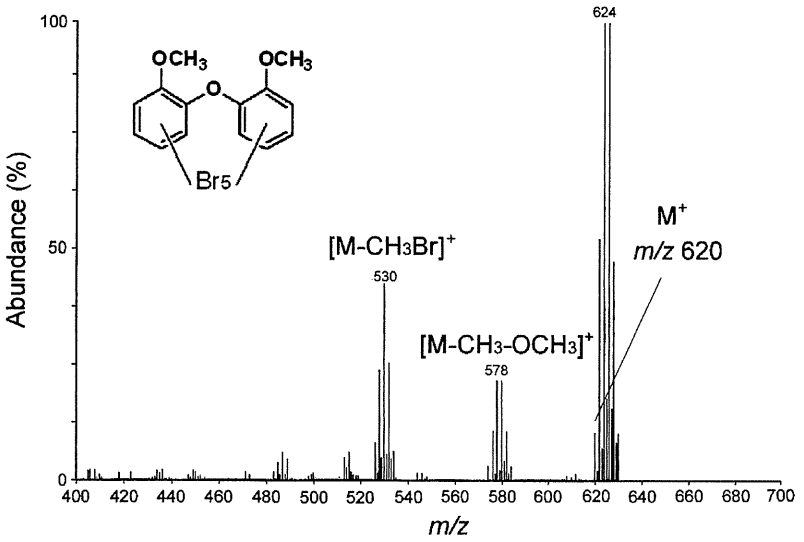

Figure 4. Mass spectrum of peak e′ in the methylated phenolic fraction of *Jania adhaerens* from **Figure 3**.

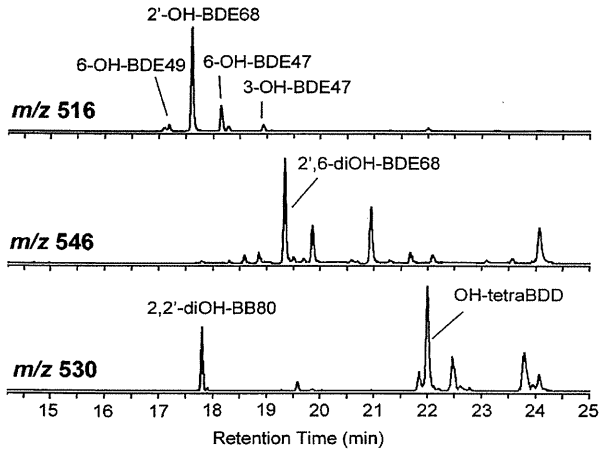

Figure 5. Selected ion monitoring of OH-tetraBDE (*m/z* 516), diOH-tetraBDE (*m/z* 546), and diOH-tetraBB/OH-tetraBDD (*m/z* 530) in the methylated phenolic fraction from *Jania adhaerens*. Some of the peaks were identified as 6-OH-BDE49 (t_R = 17.3 min), 3-OH-BDE47 (t_R = 18.8 min), 2′,6-diOH-BDE68 (t_R = 19.4 min), and OH-tetraBDD (t_R = 22.0 min).

are produced by the macroalgae themselves or by their associated symbionts (e.g., cyanobacteria) (*23*). It has been previously indicated that the producer of OH-PBDEs in the marine sponge *Dysidea herbacea* may be the symbiotic filamentous cyanobacterium *Oscillatoria spongeliae* (*23*).

The profiles of OH- and MeO-PBDEs in marine algae were similar among the five locations on Luzon Island, although the ratios and concentrations varied among species. However, the profiles of brominated compounds isolated from the Philippine algae seem to differ from those isolated in red algae from the Baltic Sea (*9*, *33*). The Philippine algae produced higher concentrations of MeO-tetraBDEs (e.g., 229 ng/g fw in *Sargassum* aff. *bataanense*) than of the corresponding OH-tetraBDEs (e.g., 30 ng/g fw in *J. adhaerens*), whereas red algae (*Ceramium tenuicorne*) from the Baltic Sea contained primarily OH-pentaBDEs (10 ng/g fw for OH-PBDEs), with smaller amounts of MeO-tetraBDEs (0.4 ng/g fw for MeO-PBDEs) (*33*). Additionally, the profiles of the Philippine algae included 2,2′-diMeO-BB80 and 2′,6-diOH/diMeO-BDE68, whereas no information about these compounds was reported for algae from the Baltic Sea. Finally, the Philippine algae contained OH-tetraBDDs and the corresponding MeO-tetraBDDs, whereas red algae from the Baltic Sea produced triBDDs and tetraBDDs (*33*,*34*). Several OH-tetraBDDs, diOH-tetraBDEs and their methoxylated analogues have been isolated from Australian marine sponges (*35*, *36*) and Palauan marine sponges (data not shown), suggesting that the profiles of hydroxylated, dihydroxylated, and methoxylated analogues are common in the Pacific.

In this study, the highest production of 2′-MeO-BDE68 reached 229 ng/g fw for *Sargassum* aff. *bataanense*, which corresponded to 62 μg/g when expressed per gram of extracted organic matter (EOM). For OH-PBDEs, 6-OH-BDE47 reached 91 ng/g fw in *Sargassum oligosystum*, corresponding to 30 μg/g EOM. 2′-MeO-BDE68 and 6-MeO-BDE47 have been isolated in the marine sponge *Dysidea* sp. from the Asia–Pacific region (*36*, *37*). These products in sponge tissue (*Dysidea* sp.) may represent as much as 12% of the dry weight (*23*). Marine algae such as *Sargassum* and *Jania* sp. are more common in the Philippines' shallow waters than marine sponges (*Dysidea* sp.). As many invertebrates (e.g., thorny oyster) feed on these algae, naturally produced MeO-PBDEs could transfer and bioaccumulate in higher trophic levels via the food chain (*38*, *39*). On the other hand, OH-PBDEs are expected to be more water-soluble than MeO-PBDEs and more mobile in the environment. In fact, they have been isolated in the blood of fish and animals (*12*).

The present survey further resulted in the isolation of 2,2′-diMeO-BB80 and 2′,6-diMeO-BDE68 from algae. These compounds have also been found to accumulate in bluefin tuna (*14*), tiger sharks (*38*), and killer whales (*19*) from Japanese coastal waters. For the corresponding hydroxylated analogues, 2,2′-diOH-BB80 and 2′,6-diOH-BDE68 were detected at levels of up to 5.1 ng/g fw in *Sargassum* or *Jania* sp. As we measured both dihydroxylated products as dimethoxy derivatives by GC-MS (**Figure 4**), the precursor may be either OH-MeO- or diOH-analogues. In fact, one product isolated in *J. adhaerens* was directly (without derivatization) identified as 2′-OH-6-MeO-BDE68 (data not shown). 2,2′-diOH-BB80 has been found to be produced by the marine bacterium, *Pseudoalteromonas phenolica* sp. (*40*), whereas both 2′,6-diOH-BDE68 and 2′-OH-6-MeO-BDE68 have been also isolated in marine sponges from Indonesia (*36*, *37*).

Previous studies have discussed whether these OH-PBDEs in marine food webs originate from marine resources or from brominated flame retardants (e.g., PBDEs) through hepatic metabolism. This study strongly suggests that the OH-PBDEs identified are of natural origin because their possible precursors, such as BDE-47, were undetectable in any of the algae investigated. All of the OH- and MeO-PBDEs identified have the hydroxy/methoxy group in the ortho position(s) to the diphenyl ether oxygen and a 2-bromine or 2,4-dibromine substitution in the nonhydroxylated or nonmethoxylated phenyl ring, which is common for natural OH- and MeO-PBDEs (*37*, *41*).

In addition to OH-PBDEs, we measured 2,4,6-TBP content in algae. Similar to OH-PBDE, 2,4,6-TBP varied considerably in concentration across species (from 0.5 to 107 ng/g fw). The concentration ranges of 2,4,6-TBP observed in this study appear to be low compared to those in marine algae from Australia (up to 1900 ng/g fw) (*2*). Seasonal changes in the concentrations of these compounds may occur (*3*). Marine algae are a major source of 2,4,6-TBP in herbivorous fish and a contributing source in fish that are diverse omnivores (*4*).

The levels of 2,4,6-TBA observed in this study ranged from < 0.02 to 2.2 ng/g fw, which is lower than 2,4,6-TBP levels in most cases. The 2,4,6-TBA is likely a natural product derived from bacterial O-methylation of bromophenols (*6*) rather than a contaminant derived from man-made flame retardants (*5*). Because the ratio of 2,4,6-TBA/2,4,6-TBP varies from < 0.01 to 1.44, depending on the species, the different concentration ratios of hydroxylated and methoxylated analogues in algae may be attributed to the O-methylation ability of the algae-associated bacteria.

Many species of algae have been shown to contain bromoperoxidases (BPO), capable of brominating organic substrates in the presence of bromide and hydrogen peroxide (*42*, *43*). BPO isolated from red algae has been shown to catalyze the bromination of phenol and 2-hydroxybenzyl alcohol to 2,4,6-TBP (*44*). The condensation of 2,4,6-TBP to OH-PBDEs is presumably also catalyzed by BPO (*34*), as horseradish chloroperoxidase is known to catalyze the dimerization of chlorophenols to chlorinated dibenzo-*p*-dioxins (*45*). However, the levels of OH-PBDEs observed in this study were not correlated with those of 2,4,6-TBP (ANOVA, $p > 0.05$), indicating that the formation of OH-PBDE does not always depend on the bromophenol content as a substrate for BPO.

OH-PBDEs are bioactive against Gram-positive bacteria (*22*). Some authors have linked the survival of certain algae to the presence of volatile organohalogens in interalgal competition as a defense against bacterial and fungal infections (*2*, *46*, *47*). Thus, it is possible that OH-PBDE analogues may contribute to the chemical defenses of the algae. On the other hand, OH-PBDEs such as 6-OH-BDE47 have been shown to have higher transthyretin binding properties than PBDEs and to disrupt thyroid hormone homeostasis (*41*). Furthermore, OH-PBDEs possess neurotoxic properties of environmental significance (*48*). The OH-tetraBDDs isolated from *J. adhaerens* in this study may be the same as OH-tetraBDD congeners from Australian *Dysidea dendyi* that have been reported to be cytotoxic against mouse Ehrlich carcinoma cells (*35*). Because human populations have probably been exposed to these materials through seafood consumption over a long period of time, the exposure may have historical as well as current health implications.

In conclusion, the present study demonstrates the widespread occurrence of OH- and MeO-PBDE analogues in marine and freshwater algae and in angiosperms from the Philippines and suggests a possible source of such compounds accumulated in fish and higher trophic organisms in the Asia–Pacific region. Our results show that it is important to characterize the origin and occurrence of OH-PBDEs as well as the details of their toxic properties.

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Supporting Information Available: Mass spectrum of MeO-tetraBDD in the methylated phenolic fraction from *Jania adhaerens* (Figure S1) and comparison of the concentrations of four brominated compounds abundant in two algal species (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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多次元ガスクロマトグラフー飛行時間型質量分析計による水酸化PCBの測定に関する検討

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Measurement of Hydroxy PCB by a Comprehensive Multi Dimensional Gas Chromatogragh-time-of-flight Mass Spectrometer

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Summary

We developed for accurate measuring method of mono-hydroxyl polychlorinated biphenyls (HO-PCBs) which has 839 of ideal congeners by coupling comprehensive gas-chromatograph (GC×GC) and high resolution time-of flight mass spectrometer (HRTOFMS). One hundred and nineteen of HO-PCB standard isomers were measured to determine the retention time on the two-dimensional chromatograms of GC×GC-HRTOFMS and then the method was applied to river sediment samples.

Under optimized condition of the GC×GC-HRTOFMS equipped a 5%-phenyl/methyl silicorn (as 1st column) and a 50%-phenyl/methyl silicorn capillary column (as 1st column), 111 peaks were obtained on the two-dimensional chromatogram. Only 5% of peak separation was improved against generic a GC.

One hundred and fifty two peaks derived from HO-PCBs in river sediment were detected by the GC×GC-HRTOFMS although 125 peaks detected by single GC mode. Methyl derivatized HO-PCB was, however, not identified in major peaks on the chromatograms of the GC × GC-HRTOFMS because of inefficient sensitivity of the detector and foreign substances.

To more utilize this method, it is necessary to improve sensitivity and dynamic range of detector on a TOFMS and develop a new software allows easy quantification and identification on data of a GC×GC-HRTOFMS.

Key words: GC×GC, HRTOFMS, simultaneous analysis, 2D-GC, 2D-chromatogram

1. はじめに

環境を広く汚染しているPCBは[1-3]，様々な生物から検出されており[4-8]，もちろんヒトも例外ではない。我が国や台湾における「油症事件」患者ばかりではなく[9-12]，一般人もPCBによって汚染されている[13,14]。生体中のPCBは，水酸化PCBに代謝され，体外に排出される[15-17]が，その過程で，毒性を発現することが知られている[18-19]。また，脳神経細胞の発達抑制[20]，女性ホルモンや甲状腺ホルモンなどの攪乱作用[21-23]など，水酸化PCBそのものの毒性についても報告されている。

水酸化PCBには，モノ水酸化体だけでも831種の異性体があるが，その毒性は，異性体により大きく異なることが示唆されてい

る[21-23]。また，大気[24]，降雨[25]，湖水[25]や底質[26, 27]，土壌[28]などの環境試料からも水酸化PCBは検出されるが，場所により異性体組成が異なるといった報告もある[25, 28]。環境中の水酸化PCBは，PCBの光化学反応[29]によって生成するという報告の他，パルプ工場排水から化学的生成が疑われる異性体が検出されている[30]。

しかしながら，一般的なガスクロマトグラフ（GC）による分析では，多数ある水酸化PCB異性体を分離するためには，能力が不足しているといえる。現在，環境分析で主流の5％－フェニル／メチルシリコーン系のキャピラリーカラムを用いたとしても，底質などから検出できる水酸化PCBのメトキシ誘導体のピーク数は，未同定のものも含め200本程度である[27, 28]。しかも，入手できる標準品の種類も少ないため，未同定の異性体が多数を占めている[26-28, 31]。従って，水酸化PCBの毒性や生体中動態，環境中挙動やその発生源に関する研究の進展のためには，その異性体別の分析法の確立が重要な課題であるといえる。

近年，分析化学の分野では多次元ガスクロマトグラフ（GC × GC）が普及してきている。これは，GCより分離能力が十から数十倍高いことが特徴で，石油化学[32, 33]，香料や食品化学[34, 35]の分野では，数千物質の化学物質の分離定性に威力を発揮している。環境分野への応用も始まっており，大気粒子中の炭化水素や多環芳香族化合物（PAHs）の定性[36]や環境試料中のダイオキシン類の分離定量[37]にも応用されつつある。

そこで，本研究では，GC × GCの分離能力を活用した水酸化PCBの異性体測定法を開発し，環境試料の測定により，その有効性の確認を行った。

2. 方法

2. 1 標準試薬

本研究には，Table 1に示す水酸化PCBとメトキシPCBを使用した。内訳は，奥村から譲渡された59種，アキュスタンダード社から購入した41種，ウェリントン社から購入した11種の水酸化PCBと7種の^{13}Cラベルされた水酸化PCB，31種のメトキシPCBと^{13}Cラベルされた6種のメトキシPCBであった。

2. 2 誘導体化

一般的なGC/MS測定で使用される微極性GCカラム（5％－フェニル／メチルシリコーン系）を用いる場合，水酸化PCBを誘導体化することで，数倍から千倍程度の感度が得られることが報告されている[38]。また，エチル化，トリメチルシリル（TMS）化[39]など，様々な誘導体化のうち，本研究では，報告数が多く，比較的安定なメチル化を採用した。さらに，メチル化法にも，ジアゾメタン法，硫酸ジメチル法などがあるが，異性体による誘導体化率による差が小さい[40]とされるトリメチルシリル－ジアゾメタン（TMS-DAM）法を採用した。

誘導体化は，GC測定用バイアル（200 μL不活化ガラスインサー

Table 1 List of standards of hydroxy and methoxy PCBs used for experiments

No. of chlorine	Chemical name	code	supplyer	name based on IUPAC
mono-Cl	2-chlorobiphenyl-4-ol	HPCB-1002S	AccuStandards	4-HO-CB1
	5-chlorobiphenyl-2-ol	HPCB-1001S	AccuStandards	5-HO-CB1
	3-chlorobiphenyl-4-ol	HPCB-1003S	AccuStandards	4-HO-CB2
	4'-chlorobiphenyl-4-ol	HPCB-1004S	AccuStandards	4'-HO-CB3
di-Cl	2',3'-dichlorobiphenyl-2-ol	HPCB-2005S	AccuStandards	2'-HO-CB5
	2',5'-dichlorobiphenyl-2-ol	HPCB-2001S	AccuStandards	2'-HO-CB9
	2',5'-dichlorobiphenyl-3-ol	HPCB-2002S	AccuStandards	3'-HO-CB9
	2',5'-dichlorobiphenyl-4-ol	HPCB-2003S	AccuStandards	4'-HO-CB9
	3',4'-dichlorobiphenyl-2-ol	HPCB-2006S	AccuStandards	2'-HO-CB12
	3',4'-dichlorobiphenyl-4-ol [$^{13}C_{12}$]	M4H12	Wellington	4'-HO-CB12 [^{13}C]
	3,5-dichlorobiphenyl-4-ol	HPCB-2004S	AccuStandards	4-HO-CB14
tri-Cl	2,2',5'-trichlorobiphenyl-3-ol	B007	Dr. Okumura	3'-HO-CB18
	2,2',5'-trichlorobiphenyl-4-ol	HPCB-3004S	AccuStandards	4'-HO-CB18
	2',3,3'-trichlorobiphenyl-2-ol	B039	Dr. Okumura	2'-HO-CB20
	2',3,3'-trichlorobiphenyl-4-ol	B008	Dr. Okumura	4'-HO-CB20
	2',3,4'-trichlorobiphenyl-2-ol	B049	Dr. Okumura	2'-HO-CB25
	2,3',4-trichlorobiphenyl-3-ol	B009	Dr. Okumura	3-HO-CB25
	2',3,4'-trichlorobiphenyl-4-ol	B011	Dr. Okumura	4'-HO-CB25
	3',4,6-trichlorobiphenyl-3-ol	B046	Dr. Okumura	5-HO-CB25
	3',4,6-trichlorobiphenyl-2-ol	B010	Dr. Okumura	6-HO-CB25
	2',3,5'-trichlorobiphenyl-4-ol	HPCB-3005S	AccuStandards	4'-HO-CB26
	2,3',5-trichlorobiphenyl-4-ol	B012	Dr. Okumura	4-HO-CB26
	3,3',6-trichlorobiphenyl-2-ol	B013	Dr. Okumura	6-HO-CB26
	2',5,5'-trichlorobiphenyl-2-ol	HPCB-3002S	AccuStandards	6'-HO-CB26
	2',5,5'-trichlorobiphenyl-2-ol	B014	Dr. Okumura	6'-HO-CB26
	2,4,4'-trichlorobiphenyl-3-ol	B015	Dr. Okumura	3-HO-CB28
	2',4',5'-trichlorobiphenyl-4-ol [$^{13}C_{12}$]	M4H29	Wellington	4'-HO-CB29 [^{13}C]
	2',4',6'-trichlorobiphenyl-2-ol	HPCB-3001S	AccuStandards	2'-HO-CB30
	2',4',6'-trichlorobiphenyl-3-ol	HPCB-3003S	AccuStandards	3'-HO-CB30
	2',4',6'-trichlorobiphenyl-4-ol	HPCB-3006S	AccuStandards	4'-HO-CB30
	2',4,5'-trichlorobiphenyl-3-ol	B082	Dr. Okumura	3'-HO-CB31
	2,4',5-trichlorobiphenyl-4-ol	B016	Dr. Okumura	4-HO-CB31
	3,4',6-trichlorobiphenyl-2-ol	B017	Dr. Okumura	6-HO-CB31
	2,3',4'-trichlorobiphenyl-4-ol	B040	Dr. Okumura	4'-HO-CB33
	3',4',6-trichlorobiphenyl-3-ol	B084	Dr. Okumura	5'-HO-CB33
	3',5',6-trichlorobiphenyl-3-ol	B085	Dr. Okumura	5'-HO-CB34
	3,3',4'-trichlorobiphenyl-2-ol	B100	Dr. Okumura	2'-HO-CB35
	3,3',4'-trichlorobiphenyl-4-ol	B089	Dr. Okumura	4'-HO-CB35
	3',4,4'-trichlorobiphenyl-3-ol	B097	Dr. Okumura	3'-HO-CB35
	3',4',5-trichlorobiphenyl-2-ol	B083	Dr. Okumura	6'-HO-CB35
	3,3',5-trichlorobiphenyl-2-ol	B090	Dr. Okumura	2'-HO-CB36
	3,3',5-trichlorobiphenyl-2-ol	B098	Dr. Okumura	2-HO-CB36
	3,3',5'-trichlorobiphenyl-4-ol	B099	Dr. Okumura	4'-HO-CB36
	3',5,5'-trichlorobiphenyl-2-ol	B091	Dr. Okumura	6'-HO-CB36
	3',4,5'-trichlorobiphenyl-2-ol	B088	Dr. Okumura	2'-HO-CB39
	3,4',5-trichlorobiphenyl-2-ol	B045	Dr. Okumura	2-HO-CB39
tetra-Cl	2,2',3,5-tetrachlorobiphenyl-4-ol	B042	Dr. Okumura	4'-HO-CB44
	2,2',3,6-tetrachlorobiphenyl-3-ol	B105	Dr. Okumura	2'-HO-CB46
	2',4,5',6-tetrachlorobiphenyl-2-ol	B036	Dr. Okumura	6-HO-CB49
	2,2',4',6'-tetrachlorobiphenyl-4-ol	HPCB-4006S	AccuStandards	4'-HO-CB50
	2,2',5',6-tetrachlorobiphenyl-3-ol	B115	Dr. Okumura	3'-HO-CB53
	2,2',6,6'-tetrachlorobiphenyl-4-ol	4H54	Wellington	4-HO-CB54
	2',3,3',4-tetrachlorobiphenyl-2-ol	B044	Dr. Okumura	2'-HO-CB56
	2',3,3',5-tetrachlorobiphenyl-2-ol	B041	Dr. Okumura	2'-HO-CB58
	3',5,5',6-tetrachlorobiphenyl-2-ol	B102	Dr. Okumura	6-HO-CB58
	2',4,4',5-tetrachlorobiphenyl-2-ol	B114	Dr. Okumura	6'-HO-CB60
	2',3',4',5-tetrachlorobiphenyl-2-ol	HPCB-4001S	AccuStandards	2'-HO-CB61
	2',3',4',5-tetrachlorobiphenyl-3-ol	HPCB-4004S	AccuStandards	3'-HO-CB61
	2',3',4',5-tetrachlorobiphenyl-4-ol	HPCB-4007S	AccuStandards	4'-HO-CB61
	2',3',4',5'-tetrachlorobiphenyl-4-ol [$^{13}C_{12}$]	M4H61	Wellington	4'-HO-CB61 [^{13}C]
	2',3',5',6'-tetrachlorobiphenyl-2-ol	HPCB-4002S	AccuStandards	2'-HO-CB65
	2',3',5',6'-tetrachlorobiphenyl-3-ol	HPCB-4005S	AccuStandards	3'-HO-CB65
	2',3',5',6'-tetrachlorobiphenyl-4-ol	HPCB-4010S	AccuStandards	4'-HO-CB65
	2,3',4,4'-tetrachlorobiphenyl-3-ol	B116	Dr. Okumura	3-HO-CB66
	3',4,4',6-tetrachlorobiphenyl-3-ol	B123	Dr. Okumura	5-HO-CB66
	2',3,4',5-tetrachlorobiphenyl-2-ol	B111	Dr. Okumura	2'-HO-CB68
	2,3',4,5'-tetrachlorobiphenyl-3-ol	B112	Dr. Okumura	3-HO-CB68
	3',4,5',6-tetrachlorobiphenyl-3-ol	B113	Dr. Okumura	5-HO-CB68
	3',4,5',6-tetrachlorobiphenyl-2-ol	B120	Dr. Okumura	6-HO-CB68
	2',3,4',6'-tetrachlorobiphenyl-4-ol	HPCB-4008S	AccuStandards	4'-HO-CB69
	2',4',5,6'-tetrachlorobiphenyl-2-ol	HPCB-4003S	AccuStandards	6'-HO-CB69
	2,3',4',5-tetrachlorobiphenyl-4-ol	B121	Dr. Okumura	4-HO-CB70
	3,3',4',6-tetrachlorobiphenyl-2-ol	B122	Dr. Okumura	6-HO-CB70
	2',3,5,5'-tetrachlorobiphenyl-2-ol	B101	Dr. Okumura	2'-HO-CB72
	2',3,5,5'-tetrachlorobiphenyl-4-ol	HPCB-4009S	AccuStandards	4'-HO-CB72
	2,3',5,5'-tetrachlorobiphenyl-4-ol	B119	Dr. Okumura	4-HO-CB72
	3,3',4',5-tetrachlorobiphenyl-2-ol	B038	Dr. Okumura	2'-HO-CB79
	3,3',4',5-tetrachlorobiphenyl-2-ol	B104	Dr. Okumura	2'-HO-CB79
	3,3',4',5-tetrachlorobiphenyl-4-ol	B103	Dr. Okumura	4'-HO-CB79
	3,3',5,5'-tetrachlorobiphenyl-2-ol	B117	Dr. Okumura	2-HO-CB80
penta-Cl	2',3,3',5,6-pentachlorobiphenyl-2-ol	B125	Dr. Okumura	6-HO-CB83
	2,2',3',4',5'-pentachlorobiphenyl-4-ol	HPCB-5003S	AccuStandards	4'-HO-CB86
	2,2',3',5',6'-pentachlorobiphenyl-4-ol	HPCB-5004S	AccuStandards	4'-HO-CB93
	2,2',3,4',5'-pentachloro-4-methoxybiphenyl	4PM97	Wellington	4'-MeO-CB97
	2,2',3',4,6-pentachlorobiphenyl-3-ol	B037	Dr. Okumura	3-HO-CB98
	2,2',4',5,5'-pentachlorobiphenyl-3-ol	HPCB-5008S	AccuStandards	3'-HO-CB101
	2,2',4',5,5'-pentachlorobiphenyl-4-ol	HPCB-5009S	AccuStandards	4'-HO-CB101
	2',3,4',5',6-pentachlorobiphenyl-2-ol	HPCB-5010S	AccuStandards	6'-HO-CB101
	2,2',4',6,6'-pentachlorobiphenyl-4-ol	4H104	Wellington	4'-HO-CB104
	2',3,3',4',5'-pentachlorobiphenyl-4-ol	HPCB-5005S	AccuStandards	4'-HO-CB106
	2',3',4',5,5'-pentachlorobiphenyl-2-ol	HPCB-5001S	AccuStandards	6'-HO-CB106
	2,3,3',4',5-pentachlorobiphenyl-4-ol	4H107	Wellington	4-HO-CB107
	3,3',4',5,6-pentachlorobiphenyl-2-ol	B048	Dr. Okumura	6-HO-CB107
	2',3,3',4',5-pentachlorobiphenyl-4-ol	4H108 (95%)	Wellington	4'-HO-CB108
	2,3',4',5,6-pentachlorobiphenyl-3-ol	B126	Dr. Okumura	5-HO-CB110
	3,3',5,5',6-pentachlorobiphenyl-2-ol	B043	Dr. Okumura	6-HO-CB111
	2',3,3',5',6'-pentachlorobiphenyl-4-ol	HPCB-5006S	AccuStandards	4'-HO-CB112
	2',3',5,5',6'-pentachlorobiphenyl-2-ol	HPCB-5002S	AccuStandards	6'-HO-CB112
	2,3,4,4',5-pentachloro-2'-methoxybiphenyl	2PM114	Wellington	2'-MeO-CB114
	2,3',5,5',6-pentachlorobiphenyl-3-ol	B127	Dr. Okumura	5-HO-CB113
	2,3',4,4',5-pentachlorobiphenyl-3-ol	3H118	Wellington	3-HO-CB118
	2,3',4,4',6-pentachlorobiphenyl-3-ol	B050	Dr. Okumura	3-HO-CB119
	2,3',4,5,5'-pentachloro-4'-methoxybiphenyl	4PM120	Wellington	4'-MeO-CB120
	2',3,4',5,5'-pentachlorobiphenyl-4-ol [$^{13}C_{12}$]	M4H120	Wellington	4'-HO-CB120 [^{13}C]
	2',3,4',5,6'-pentachlorobiphenyl-2-ol	B124	Dr. Okumura	2'-HO-CB121
	2,3',4,5',6-pentachlorobiphenyl-3-ol	B047	Dr. Okumura	3-HO-CB121
	2',3,4',5,6'-pentachlorobiphenyl-4-ol	HPCB-5007S	AccuStandards	4'-HO-CB121
	3,3',4,5,5'-pentachloro-4'-methoxybiphenyl	4PM127	Wellington	4'-MeO-CB127
hexa-Cl	2,2',3,3',4',5-hexachlorobiphenyl-4-ol	4H130	Wellington	4'-HO-CB130
	2,2',3,3',5,6-hexachloro-4-methoxybiphenyl	4M134	Wellington	4-MeO-CB134
	2,2',3',4,4',5-hexachlorobiphenyl-3-ol	3H138	Wellington	3'-HO-CB138
	2,2',3,4',5,5'-hexachlorobiphenyl-4-ol	4H146	Wellington	4-HO-CB146
	2,2',4,4',6,6'-hexachloro-3,3'-dimethoxybiphenyl	33PDM155	Wellington	3,3'-2MeO-CB155
	2',3,3',4',5,5'-hexachlorobiphenyl-4-ol	HPCB-6001S	AccuStandards	4'-HO-CB159
	2',3,3',4',5,5'-hexachlorobiphenyl-4-ol [$^{13}C_{12}$]	M4H159	Wellington	4'-HO-CB159 [^{13}C]
	2,3,3',4',5,5'-hexachloro-4-methoxybiphenyl	4M162	Wellington	4-MeO-CB162
	2,3,3',4',5,6-hexachloro-4-methoxybiphenyl	4M163	Wellington	4-MeO-CB163
	2',3,3',5,5',6'-hexachlorobiphenyl-4-ol	HPCB-6002S	AccuStandards	4'-HO-CB165
	2,2',3,3',4',5,5'-heptachlorobiphenyl-4-ol	4H172	Wellington	4'-HO-CB172
	2,2',3,3',4',5,5'-heptachlorobiphenyl-4-ol[$^{13}C_{12}$]	M4H172	Wellington	4'-HO-CB172 [^{13}C]
	2,2',3,3',4',5,6-heptachloro-4-methoxybiphenyl	4M177	Wellington	4-MeO-CB177
	2,2',3,3',5,5',6-heptachloro-4-methoxybiphenyl	4M178	Wellington	4-MeO-CB178
	2,2',3',4',4',5,5'-heptachlorobiphenyl-3-ol	3H180	Wellington	3'-HO-CB180
	2,2',3,4,4',5,6'-heptachloro-3'-methoxybiphenyl	3PM182	Wellington	3'-MeO-CB182
	2,2',3',4',4',5,6'-heptachloro-3-methoxybiphenyl	3PM183	Wellington	3'-MeO-CB183
	2,2',3,4,4',6,6'-heptachloro-3'-methoxybiphenyl	3PM184	Wellington	3'-MeO-CB184
	2,2',3,4',5,5',6-heptachlorobiphenyl-4-ol	4H187	Wellington	4-HO-CB187
	2,2',3,4',5,5',6-heptachlorobiphenyl-4-ol [$^{13}C_{12}$]	M4H187	Wellington	4-HO-CB187 [^{13}C]
	2,3,3',4',5,5',6-heptachloro-4-methoxybiphenyl	4M193	Wellington	4-MeO-CB193
octa-Cl	2,2',3,3',4',5,5',6-octachloro-4-methoxybiphenyl	4PM198(4PM199)	Wellington	4'-MeO-CB199
	2,2',3,3',4,5,5',6-octachloro-4'-methoxybiphenyl	4PM198	Wellington	4'-MeO-CB198
	2,2',3,3',4,5,6,6'-octachloro-4'-methoxybiphenyl	4PM200	Wellington	4'-MeO-CB200
	2,2',3,3',4,5',6,6'-octachloro-4'-methoxybiphenyl	4PM201	Wellington	4'-MeO-CB201
	2,2',3,3',5,5',6,6'-octachloro-4-methoxybiphenyl	4M202	Wellington	4-MeO-CB202
	2,2',3,3',5,5',6,6'-octachloro-4,4'-dimethoxybiphenyl	44PDM202	Wellington	4,4'-2MeO-CB202
	2,2',3,4,4',5,5',6-octachloro-3'-methoxybiphenyl	3PM203	Wellington	3'-MeO-CB203
nona-Cl	2,2',3,3',4,5,5',6,6'-nonachloro-4'-methoxybiphenyl	4PM208	Wellington	4'-MeO-CB208

ト容器入り2 mLバイアル）中で行った。100 ng/μLの標準試薬5 μL（ノナン溶液）に，メタノール20 μl，トルエン70 μL，トリメチルジアゾメタン（TMS-DAM）を10 μL添加し，室温で20分以上放置し，メチル誘導体化を行った。各試料それぞれに内標準物質として^{13}Cラベル化されたメトキシPCBを添加して分析試料とした。

2. 3 底質試料

本研究には，先山らの方法[26]によって精製後，誘導体化（メチル化）された都市河川底質試料液を使用した。分析フロー図をFig. 1に示す。風乾した5-20 gの底質に，表2. 6. 2に示す^{13}Cラベル水酸化PCBsを各10 ng添加し，アセトニトリルで振とう抽出した後，抽出液を遠心分離により分取後，ヘキサンで振とう洗浄した。次に精製水を加え水酸化PCBをヘキサン層に転溶，濃縮後，フロリジルカートリッジカラム（Sep-Pak Plus Florisil，ウォーターズ社製）で精製した。0. 5 %-ジエチルエーテル／ヘキサン溶液6 mLで夾雑物を溶出させた後，50 %-アセトン／メタノール6 mLで水酸化PCBを回収した。水酸化PCB画分を乾固寸前まで濃縮後，硫酸ジメチルに3 M-水酸化カリウム／エタノール溶液を加えて，メチル誘導体化した。その溶液を約70 ℃で1時間加熱した後，精製水とヘキサンを加え，振とう抽出を行い，フロリジルカートリッジカラムにより再度精製した。ヘキサン2 mLにより夾雑物を溶出・除去した後，5 %-ジエチルエーテル／ヘキサン6 mLでメトキシPCB（水酸化PCBの誘導体化物）を溶出・回収した。このメトキシPCB画分を濃縮し，試料液とした。

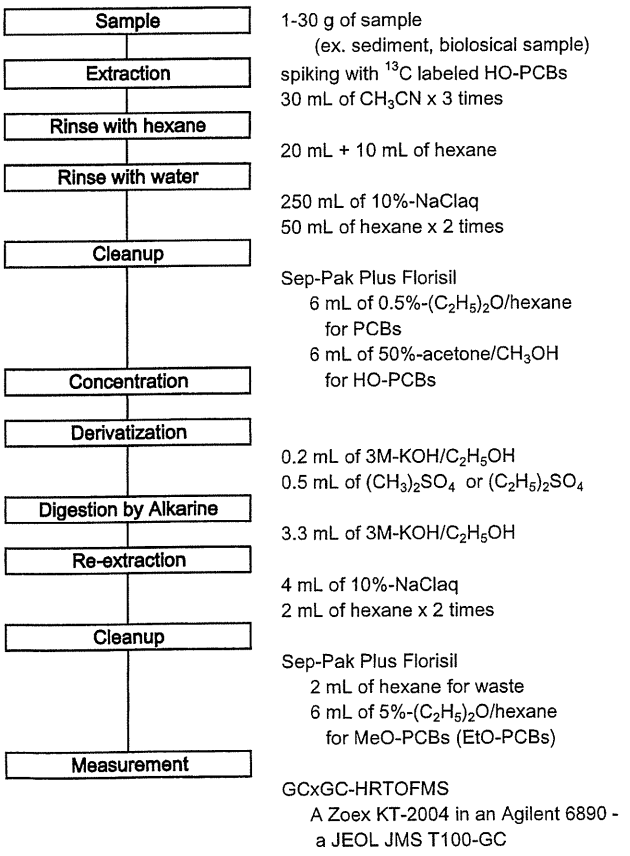


Fig. 1 Flow chart of analytical method for HO-PCBs in environmental samples

2. 4 測定装置

測定装置には，Zoex KT2004 GC × GC（ゲステル社）を装着した6890 GC（アジレントテクノロジー社製）およびJMS-T100 GC（日本電子社製）を用いた。試料導入は，スプリットレス方式により行った。使用したGCキャピラリーカラムは，他物質への汎用性を考慮し，報告数の多いカラムの組み合わせ[36, 37, 41, 42]として，第一カラムをInertCap 5 MS/Sil（長さ60 m，内径0. 25 mm，膜厚0. 1 μm；ジーエルサイエンス社製，5 %-フェニル／メチルシリコーン），第二カラムをInertCap 17 MS/Sil（長さ1. 5 m，内径0. 075 mm，膜厚0. 1 μm；ジーエルサイエンス社製，50 %-フェニル／メチルシリコーン）の組み合わせ，第一カラムがHT8（長さ60 m，内径0. 25 mm，膜厚0. 25 mm；SGE社製）と第二カラムがInertCap 17 MS/Sil（長さ1 m，内径0. 1 mm，膜厚0. 1 mm；ジーエルサイエンス社製）の組み合わせ，一次元目GCカラムがDB-5，（長さ60 m，内径0. 25 mm膜厚0. 25 um；アジレントテクノロジー社製）と第二カラムがInertCap 17 MS/Sil（長さ1 m，内径0. 1 mm，膜厚0. 1 mm；ジーエルサイエンス社製）について検討した。第二カラムにおけるキャリアーガス線速度；130 cm/秒，モジュレーション時間；3～4秒，リリース時間；0. 3秒，オーブンプログラム；100 ℃（1分保持）180 ℃まで20 ℃／分昇温270 ℃まで2 ℃／分昇温300 ℃まで5 ℃／分昇温（9分保持），MSイオン化電圧；5 V，イオン化電流；600 μA，MCP電圧；2500～2700 V，質量範囲；35-600 m/z，データ記録間隔；0. 04秒で測定を行った。試料導入温度は，230～350 ℃とした。

このうち，オーブンプログラム条件は，既報におけるダイオキシン，PCB測定の最適値とした。

データ処理は，JMS-T100 GCによる測定データをデータマネージャ（日本電子社製）でAIAフォーマット（.cdf）に変換後，MASSTransit（Palisade社製）によりテキストファイルに変換し，MS-Excel 2003および2007（マイクロソフト社製）上で動作する自作のマクロプログラムにより定性と定量を行った。

3. 結果と考察

3. 1 誘導体化について

本研究では，水酸化PCBの誘導体化法として報告例が多いメチル化のうち，異性体による誘導体化率による差が小さい[40]トリメチルシリルジアゾメタン（TMS-DAM）によるメチル化を採用した。水酸化PCB標準品に対する最適誘導体化条件の検討の結果，メチル化試薬の割合や反応時間により，誘導体化率や生成物に違いがあることが分かった。メチル化時にメタノールを添加しなかった場合（標準品5 μL（ノナン溶液）に，トルエン70 μL，トリメチルジアゾメタン（TMS-DAM）を10 μL添加）は，誘導体化試薬添加後の放置時間が長い場合，メトキシPCBよりもトリメチルシリルPCB（TMS-PCB）の生成が優勢になった（Fig. 2）。しかし，メタノール20 μLを添加することによりTMS-PCBの生成はほとんどみられなくなった。

3. 2 メトキシPCB標準品によるGC × GC分離条件の検討

35種類のメトキシPCB異性体標準品の混合試料（ウェリントン社製）を用いて，GCカラムの選択とGC条件の最適化を図った。はじめに，PCBの異性体分析に用いられる一般的なGCカラムであるHT-8（SGE社製）を第一カラムとして検討した。第二カラムは中極性の50 %-フェニル／メチルシリコーン系カラムであるInertCap 17 MS/Sil（ジーエルサイエンス社製）を用いた。その測定条件をTable 2に示す。測定の結果，同族体別に計

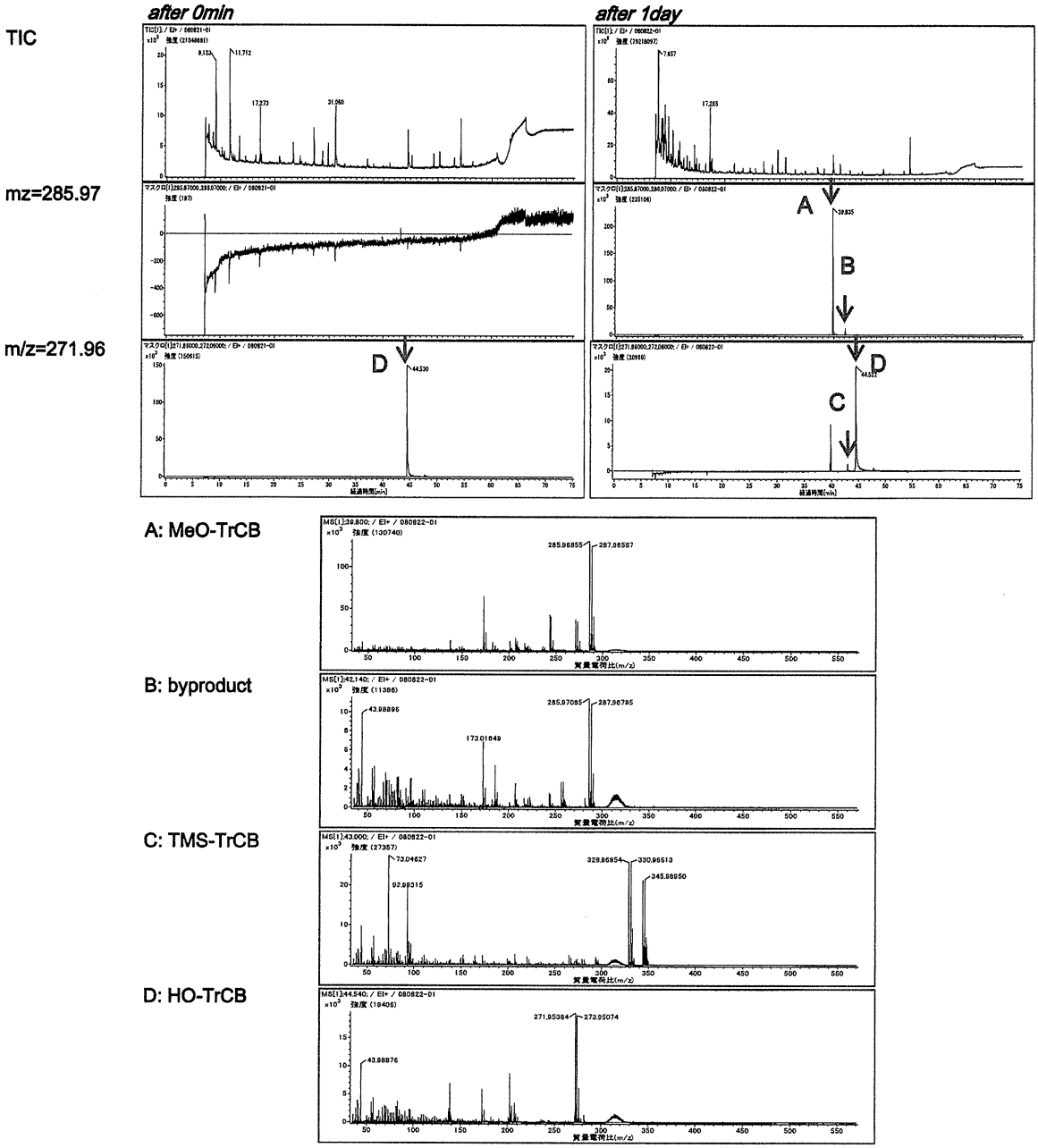


Fig. 2　Total ion and selected ion chromatograms and mass spectra of major peaks after derivatization of HO-PCB standards by TMS-DAT
This is an example of derivatization of 4 -HO-2' ,3' ,4' -TrCB

30本のピークがGC × GCクロマトグラム上に確認できた。GC条件を変えつつ検討したが，それ以上のピーク分離は確認できなかった。例えばメトキシPCBの五塩化同族体の標準品には8異性体が含まれていたが，GC × GCクロマトグラム上に確認できたピーク数は5本であった。また，メトキシPCBの六塩化同族体標準品の異性体数7に対しピーク数は6本であった。次に，POPsなどの有機微量環境汚染物質の測定に一般的に用いられている微極性カラム（5％-フェニル／メチルシリコーン系）であるDB-5 ms（アジレントテクノロジー社製）を第一カラムとして，異性体分離を調べた。測定条件をTable 3に示す。検討の結果，35種の異性体を32本のピークに分離できることを確認した。分離したピークの数は，HT-8に比べDB-5の方が多くなった。8種の異性体を含むメトキシPCBの五塩化同族体標準品では，7本のピークが確認でき（HT-8では5本），7種のメトキシPCBの六塩化異性体は全て分離することができた（HT-8では6本のピーク）。ただし，Fig. 3及び4に示すように，HT-8とDB-5を単独で使用した（通常のGCの）場合には，HT-8で分離可能だがDB-5では分離しなくなる異性体もあった。従って，今回検討したメトキシPCB異性体については，DB-5とInertCap17 ms/Silの組み合わせが分離に適しているといえる。

3. 3　水酸化PCB標準品によるGC × GC保持時間の確認

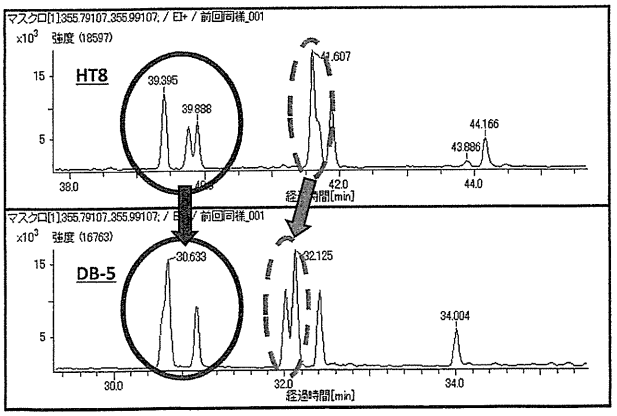
126種の一～九塩化水酸化PCB異性体の個々の標準品をGC × GC-HRTOFMSにより測定し，各異性体のGC × GC保持時間を確

Table 2 Parameters of a GC × GC–HRTOFMS for measurement of MeO–PCBs (A)

GC×GC	
GC×GC:	Agilent 6890N equipped Zoex KT2004
GC columns:	1st: SGE HT8, 60 m, 0.25 mmI.D, film thickness 0.25 μm 2nd: GL Science InertCap17MS/SIL, 1 m, 0.1 mmI.D, film thickness 0.1 μm
column for the modurator:	1.5m, 0.1mmI.D.
oven program:	100 °C (1 min) → 20 °C/min → 180 °C → 3 °C/min → 320 °C (hold)
injector temp:	280 °C
injection mode:	Splitless, constant flow
initial head pressure:	427 kPa (100 °C)
linier velosity on out side of column:	170 cm/s
moduration period:	5 sec
hot jet gas blow time:	0.3 sec
MS	
MS:	JEOL JMS-T100GC
m/z range:	300-500
MCP voltage:	2500 V
ionizatio mode:	EI+, 70 eV, 300 μA
data sampring period:	0.04 sec (25 Hz)

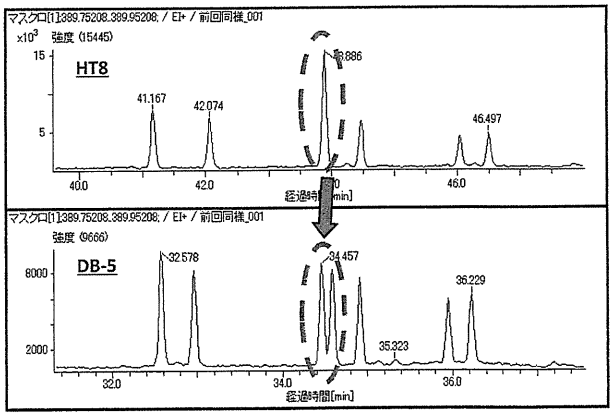
Table 3 Parameters of a GC × GC–HRTOFMS for measurement of MeO–PCBs (B)

GC×GC	
GC×GC:	Agilent 6890N equipped Zoex KT2004
GC columns:	1st: Agilent DB-5ms, 60 m, 0.25 mmI.D, film thickness 0.25 μm 2nd: GL Science InertCap17MS/SIL, 1 m, 0.1 mmI.D, film thickness 0.1 μm
column for the modurator:	1.5 m, 0.1 mmI.D.
oven program:	120 °C (1 min) → 20 °C/min → 180 °C → 2 °C/min → 320 °C (hold)
injector temp:	280 °C
injection mode:	Splitless, constant flow
initial head pressure:	643 kPa (120 °C)
linier velosity on out side of column:	170 cm/s
moduration period:	3 sec
hot jet gas blow time:	0.35 sec
MS	
MS:	JEOL JMS-T100GC
m/z range:	300-500
MCP voltage:	2500 V
ionizatio mode:	EI+, 35 eV, 300 μA
data sampring period:	0.04 sec (25 Hz)



m/z 355.89107±0.025 Da (-Cl×5, -OH×1)

Fig. 3 Comparison of peak separation of methoxylated HO-PeCB isomers on HT-8 and DB-5 GC columns



m/z 389.85208±0.025 Da (-Cl×6, -OH×1)

Fig. 4 Comparison of peak separation of methoxylated HO-HxCB isomers on HT-8 and DB-5 GC columns

認し，相対保持時間を算出した。使用した水酸化 PCB 標準品，誘導体化条件，GC × GC-HRTOFMS の測定条件をそれぞれ Table 4 及び 5 に示した。水酸化 PCB の標準品は，2. 6. 2. 2 の誘導体化法に従って誘導体化し，そのうちの 1 ～ 2 μL を測定した。個々の異性体の一次元および二次元クロマトグラム上の保持時間を Table 6 に示す。本研究に使用した Zoex KT-2004 と JMS-T100 GC は，ともに Agilent 6890 GC のスタート信号に同期するように設定したが，6890 GC と JMS-T100 GC の信号同期には数秒の範囲内でバラツキがあることが明らかになった（この現象は，メーカーも確認済みだが，2009 年 3 月現在では対応できていない）。この測定開始時間のバラツキは，通常の GC での測定においては問題にならないレベルであるが，モジュレーション間隔（二次元目 GC 測定時間）が数秒の GC × GC では，大きな問題となる。そこで，我々は，塩素置換数が同じ水酸化 PCB（メトキシ PCB）異性体に同一の ^{13}C ラベルされたメトキシ PCB を内部標準（IS）として添加し，その IS を基準にし，各異性体の GC × GC 保持時間を補正した。また，各同族体のうち GC × GC 保持時間が最も短い（最も早く溶出する）異性体の保持時間を 1 とした場合の，相対保持時間も異性体毎に計算した。また，同族体間の保持時間の補正は，複数の同族体の混合標準品（MPCB-MXA ～ MXI）を測定し，その保持時間と合わせることにより行った。Fig. 5 に，測定した全てのメトキシ PCB 異性体の保持時間を二次元散布図として示す。図より，一次元目 GC の保持時間が長い異性体ほど二次元目 GC の保持時間も長い傾向にあることが分かる。しかし，二次元目 GC における保持時間は異性体により大きく異なっており，今回の測定条件(モジュレーション間隔 :4 秒）では，1 回のモジュレーションで全ての異性体が溶出しきれず，2 回目以降のモジュレーションに溶出する現象（ターンオーバー）が観察された。今回は特に一次元目 GC における異性体分離に重点を置き，モジュレーション間隔を短くしたことが影響したと考えられる。水酸基（メトキシ基）の置換位置と二次元目 GC 保持時間の間に何らかの関係が見いだせることを期待したが，今回の GC カラムの組み合わせでは明確な分離特性は発見できなかった。

今回の GC × GC 測定条件における異性体分離数（ピーク数）を同族体毎に Table 7 に示す。比較のため，同一試料を通常の GC により同一条件（モジュレーションのみ OFF）で測定した場合の分離ピーク数（80 % 谷分離）と比較すると，通常の GC で分離できなかった成分（異性体）：四塩化同族体 ; 5，五塩化同族体 ; 2，六塩化同族体 ; 2，七塩化同族体 ; 2 が GC × GC により分離することが確認できた。また，人体や他の生物から高濃度に検出される水酸化 PCB をメトキシ誘導体化した 2,3,3',4',5-pentachloro-4-methoxy biphenyl,2,2',3 ,4',5,5'-hexachloro-4-methoxybiphenyl,2 ,2',3' 4,4',5-hexachloro-3-methoxybiphenyl,2,2',3,4',5,5',6-heptachloro-4-methoxybiphenyl,2,2',3,3',4,5 ,5'-heptachloro-4'-methoxybiphenyl,2,2',3,4',5,5' 6-heptachloro-4-methoxybiphneyl,2,2',3 ,3',4 ,5,5'-heptachloro-4'-methoxybiphenyl については，全て分離することを確認した（Fig. 6)。特に 2 ,2',3 ,3',4,5, 5'-heptachloro-4'-methoxybiphenyl と 2 ,2',3 ,4',5,5' 6 -hepta-chloro-4-methoxy biphneyl は，通常の GC では分離されていなかった。

Table 4　Conditions of derivatizing for hydroxy PCBs by TMS-DAM

After combing the reagents, they were kept at room temperature (about 20 C) for 30 mins or more over

Derivatizing reagent
Trimethylsilyldiazomethane(TMS-DAM) (10v/v% in Hexane)

Composition of reagents, solvents and sample solution.	Amount
Standard (Nonane or Isooctane)	1-5μl
IS (MMPCB-MXA) PCB-MeO　(Nonane)	10μl
Methanol	20μl
Toluene	70μl
TMS-DAM	10μl

Table 5　Informations and Parameters of a GC × GC-HRTOFMS for measurement of MeO-PCBs in river sedement samples

GC×GC	
GC×GC:	Agilent 6890N equipped Zoex KT2004
GC columns:	1st: GL Science InertCap5MS/SIL, 60 m, 0.25 mmI.D, film thickness 0.25 μm 2nd: GL Science InertCap17MS/SIL, 1 m, 0.1 mmI.D, film thickness 0.1 μm
column for the modurator:	1.5 m, 0.1 mmI.D.
oven program:	100 °C (1 min) → 20 °C/min → 150 °C → 2 °C/min → 250 °C → 6 °C/min → 300 °C (hold)
injector temp:	280 °C
injection mode:	Splitless, constant flow
initial head pressure:	643 kPa (120 °C)
linier velosity on out side of column:	170 cm/s
moduration period:	4 sec
hot jet gas blow time:	0.3 sec
MS	
MS:	JEOL JMS-T100GC
m/z range:	35-550
MCP voltage:	2500 V
ionizatio mode:	EI+, 35 eV, 600 μA
data sampring period:	0.04 sec (25 Hz)

Table 6 Retention times of methoxy PCBs on GC × GC

	ID	compound name	1st RT(min)	2nd RT(sec)
MoCB-OMe	1001S	5-Chlorobiphenyl-2-methoxybiphenyl	20.60	2.708
	1002S	2-Chloro-4-methoxybiphenyl	21.27	2.908
	1003S	3-Chloro-4-methoxybiphenyl	24.73	0.058
	1004S	4'-Chloro-4-methoxybiphenyl	25.20	3.820
DiCB-OMe	2001S	2',5'-Dicrloro-2-methoxybiphenyl	23.60	3.773
	2005S	2',3'-Dichloro-2-methoxybiphenyl	24.87	0.480
	2002S	2',5'-Dichloro-3-methoxybiphenyl	26.93	3.930
	2004S	3,5-Dichloro-4-methoxybiphenyl	27.87	3.692
	2003S	2',5'-Dichloro-4-methoxybiphenyl	28.47	0.160
	2006S	3',4'-Dichloro-2-methoxybiphenyl	28.47	0.320
TrCB-OMe	3001S	2',4',6'-Trichloro-2-methoxybiphenyl	26.00	3.849
	B049	2',3,4'-Trichloro-2-methoxybiphneyl	27.60	3.700
	B010	3',4,6-trichloro-2-methoxybiphenyl	27.80	3.770
	B039	2,3,3'-Trichloro-2-methoxybiphenyl	28.73	0.562
	B017	3,4',6-trichloro-2-methoxybiphenyl	28.80	3.970
	B090	3,3',5'-Trichloro-2-methoxybiphenyl	29.20	3.462
	3003S	2',4',6'-Trichloro-3-methoxybiphenyl	29.33	0.007
	B098	3,3',5-trichloro-2-methoxybihenyl	29.73	3.492
	3002S	2',5,5'-Trichloro-2-methoxybiphenyl	30.53	0.524
	B045	3,4',5-Trichloro-2-methoxybiphenyl	30.60	3.540
	3006S	2',4',6'-Trichloro-4-methoxybiphenyl	30.67	0.324
	3004S	2,2',5'-Trichloro-4-methoxybiphenyl	31.53	0.722
	B013	3,3',6-Trichloro-2-methoxybiphneyl	31.73	0.404
	B007	2,2',5'-trichloro-3-methoxybiphenyl	31.87	1.517
	B009	2,3',4-Trichloro-3-methoxybiphenyl	31.93	0.445
	B015	2,4,4'-trichloro-3-methoxybiphneyl	32.60	0.562
	B085	3',5',6-Trichloro-3-methoxybiphenyl	33.20	3.969
	B088	3',4,5'-Trichloro-2-methoxybiphenyl	33.27	0.405
	B091	3',5,5'-Trichloro-2-methoxybiphenyl	33.53	0.205
	B046	3',4,6-Trichloro-3-methoxybiphenyl	34.20	0.882
	B012	2,3',5-Trichloro-4-methoxybiphenyl	35.00	1.039
	B084	3',4',6-Trichloro-3-methoxybiphenyl	35.53	0.802
	B016	2,4',5-Trichloro-4-methoxybiphenyl	35.60	1.437
	B011	2',3,4'-trichloro-4-methoxybiphneyl	35.67	1.398
	3005S	2',3,5'-Trichloro-4-methoxybiphenyl	35.67	1.397
	B040	2,3',4'-trichloro-4-methoxybiphenlyl	36.27	1.280
	B008	2',3,3'-Trichloro-4-methoxybiphenyl	37.00	2.389
	B099	3,3',5'-Trichloro-4-methoxybiphenyl	38.47	1.157
	B097	3',4,4'-trichloro-3-methoxybiphenyl	39.40	1.791
^{13}C-TrCB-OMe	M4M29	2,4,5-Trichloro-4'-methoxy[$^{13}C_{12}$]biphenyl	34.87	0.722
TeCB-OMe	B101	2',3,5,5'-Tetrachloro-2-methoxybiphenyl	32.53	0.343
	4003S	2',4',5,6'-Tetrachloro-2-methoxybiphenyl	32.87	2.278
	4002S	2',3',5',6'-Tetrachloro-2-methoxybiphenyl	33.07	2.080
	B111	2',3,4,5'-tetrachloro-2-methoxybiphneyl	33.07	0.137
	B036	2',4,5',6-Tetrachloro-2-methoxybiphenyl	33.33	1.766
	B041	2',3,3',5-tetrachloro-2-methoxybiphenyl	34.00	1.248
	4006S	2,2',4',6-Tetrachloro-4-methoxybiphenyl	34.07	1.566
	B117	3,3',5,5'-Tetrachloro-2-methoxybiphenyl	34.47	0.026
	B114	2',4,4',5-tetarchloro-2-methoxybiphenyl	35.20	1.605
	B115	2,2',5',6-tetrachloro-3-methoxybiphenyl	35.67	2.676
	B120	3',4,5',6-Tetrachloro-2-methoxybiphenyl	35.73	1.405
	B044	2',3,3',4'-Tetarchloro-2-methoxybiphenyl	36.13	2.201
	4005S	2',3',5',6'-Tetrachloro-3-methoxybiphenyl	36.73	2.120
	B112	2,3',4,5'-Tetrachloro-3-methoxybiphneyl	37.00	0.971
	4001S	2',3',4',5'-Tetrachloro-2-methoxybiphenyl	37.07	2.241
	B038	3,3',4',5-tetrachloro-2-methoxybiphenyl	37.13	0.970
	4009S	2',3,5,5'-Tetrachloro-4-methoxybiphenyl	37.13	1.010
	B105	2,2',3,6-Tetrachloro-3-methoxybiphneyl	37.13	3.548
	4008S	2',3,4',6'-Tetrachloro-4-methoxybiphenyl	37.60	2.238
	B102	3',5,5',6-Tetrachloro-2-methoxybiphneyl	37.73	1.923
	4010S	2',3',5',6'-Tetarachloro-4-methoxybiphenyl	38.33	2.281
	B113	3',4,5',6-Tetrachloro-3-methoxybiphenyl	39.13	1.368
	B116	2,3',4,4'-tetrachloro-3-methoxybiphneyl	39.47	2.003
	4004S	2',3',4',5'-Tetrachloro-3-methoxybiphenyl	40.73	2.000
	B123	3',4,4',6-Tetrachloro-3-methoxybiphneyl	41.67	2.161
	4007S	2',3',4',5'-Tetrachloro-4-methoxybiphenyl	42.47	2.478
	B121	2,3',4',5-Tetrachloro-4-methoxybiphenyl	42.47	2.756
	4PM79	3,3',4',5-Tetrachloro-4-methoxybiphenyl	42.67	1.765
^{13}C-TeCB-OMe	M4M61	2,3,4,5-Tetrachloro-4'-methoxy[$^{13}C_{12}$]biphenyl	42.47	2.478
PeCB-OMe	B124	2',3,4',5,6'-Penatchloro-2-methoxybiphenyl	35.87	0.690
	5010S	2',3,4',5',6-Pentachloro-2-methoxybiphenyl	37.40	1.327
	B043	3,3',5,5',6-Pentachloro-2-methoxybiphneyl	38.73	0.890
	B037	2,2',3',4,6-Pentachloro-3-methoxybiphenyl	38.80	2.161
	B125	2',3,3',5,6-Pentachloro-2-methoxybiphenyl	38.80	2.160
	5007S	2',3,3',5',6'-Penatchloro-4-methoxybiphenyl	39.33	1.168
	B047	2,3',4,5',6-Pentachloro-3-methoxybiphenyl	39.47	0.930
	5002S	2',3',5,5',6'-Penatachloro-2-methoxybiphenyl	40.33	2.517
	B048	3,3',4',5,6-Pentachloro-2-methoxybiphenyl	41.73	1.802
	5004S	2,2',3',5',6'-Pentachloro-4-methoxybiphenyl	41.80	2.755
	B050	2,3',4,4',6-Pentachloro-3-methoxybiphenyl	42.07	1.762
	4PM120	2,3',4,5,5'-Penatchloro-4'-methoxybiphenyl	44.07	1.485
	4PM101	2,2',4,5,5'-Pentachloro-4'-methoxybiphenyl	44.20	2.558
	5009S	2,2',4',5,5'-Penatchloro-4-methoxybiphenyl	44.27	2.398
	2PM114	2,3,4,4',5-Penatchloro-2'-methoxybiphenyl	44.67	2.635
	5001S	2',3',4',5,5'-Penatchloro-2-methoxybiphenyl	44.73	2.557
	5006S	2',3,3',5',6'-Pentachloro-4-methoxybiphenyl	45.60	3.430
	5003S	2,2',3',4',5-pentachloro-4-biphenyl	45.60	2.754
	3M118	2,3',4,4',5-Pentachloro-3-methoxybiphenyl	46.00	2.280
	4PM108	2,3,3',4,5'-Penatchloro-4'-methoxybiphenyl	46.13	2.635
	4M107	2,3,3',4',5-Pentachloro-4-methoxybiphenyl	46.27	2.398
	4PM97	2,2',3,4',5'-Pentachloro-4-methoxybiphneyl	46.60	3.551
	4PM127	3,3',4,5,5'-Pentachloro-4'-methoxybiphenyl	49.07	2.040
	5005S	2',3,3',4',5'-Penatchloro-4-methoxybiphenyl	50.00	3.628
HxCB-OMe	4M134	2,2',3,3',5,6-Hexachloro-4-methoxybiphenyl	46.33	3.310
	6002S	2',3,3',5,5',6'-Hexachloro-4-methoxybiphenyl	46.73	2.360
	4M146	2,2',3,4',5,5'-Hexachloro-4-methoxybiphenyl	47.20	2.278
	3PM138	2,2',3'4,4',5-Hexachloro-3-methoxybiphenyl	49.07	3.229
	4PM130	2,2',3,3',4',5-Hexachloro-4-methoxybiphenyl	49.27	3.390
	4M163	2,3,3',4',5,6-Hexachloro-4-methoxybiphenyl	49.80	3.272
	6001S	2',3,3',4',5,5'-Hepatchloro-4-methoxybiphenyl	51.47	2.715
	4PM159	2,3,3',4,5,5'-Hexachloro-4'-methoxybiphneyl	51.53	2.715
	4M162	2,3,3',4',5,5'-Hexachloro-4-methoxybiphenyl	51.93	2.715
HpCB-OMe	3PM184	2,2',3,4,4',6,6'-Hepatchloro-3'-methoxybiphenyl	46.80	1.962
	4M178	2,2',3,3',5,5',6-Heptachloro-4-methoxybiphenyl	50.07	2.638
	3PM183	2,2',3',4,4',5,6'-Heptachloro-3-methoxybiphneyl	50.27	2.755
	3PM182	2,2',3,4,4',5,6'-Heptachloro-3'-methoxybiphneyl	50.27	2.478
	4M177	2,2',3,3',4',5,6-Heptachloro-4-methoxybiphenyl	53.00	4.693
	3PM180	2,2',3,4,4',5,5'-Heptachloro-3',methoxybiphenyl	54.20	2.360
	4PM172	2,2',3,3',4,5,5'-Heptachloro-4'-methoxybiphenyl	54.33	2.320
	4PM187	2,2',3,4',5,5'6-Heptachloro-4-methoxybiphneyl	54.33	2.359
	4M193	2,3,3',4',5,5',6-Heptachloro-4-methoxybiphenyl	54.87	2.003
^{13}C-HpCB-OMe	M4M187	2,2',3,4',5,5',6-Heptachloro-4-methoxy[$^{13}C_{12}$]biphenyl	50.67	2.835
	M4M172	2,2',3,3',4,5,5'-Heptachloro-4'-methoxy[$^{13}C_{12}$]biphenyl	54.33	2.400
OCB-OMe	4M202	2,2',3,3',5,5',6,6'-Octachloro-4-methoxybiphenyl	52.80	3.710
	4PM201	2,2',3,3',4,5',6,6'-Octachloro-4'-methoxybiphenyl	53.40	3.273
	3PM203	2,2',3,4,4',5,5',6-Octachloro-3'-methoxybiphenyl	56.33	1.090
	4PM198	2,2',3,3',4,5,5',6-Octachloro-4'-methoxybiphenyl	56.47	1.170
	4PM199	2,2',3,3',4',5,5'6-Octachloro-4-methoxybiphenyl	56.67	1.138
	4PM200	2,2',3,3',4,5,6,6'-Octachloro-4'-methoxybiphenyl	57.27	1.368
NoCB-OMe	4PM208	2,2',3,3',4,5,5',6,6'-Nonachloro-4'-methoxybiphenyl	58.13	0.385

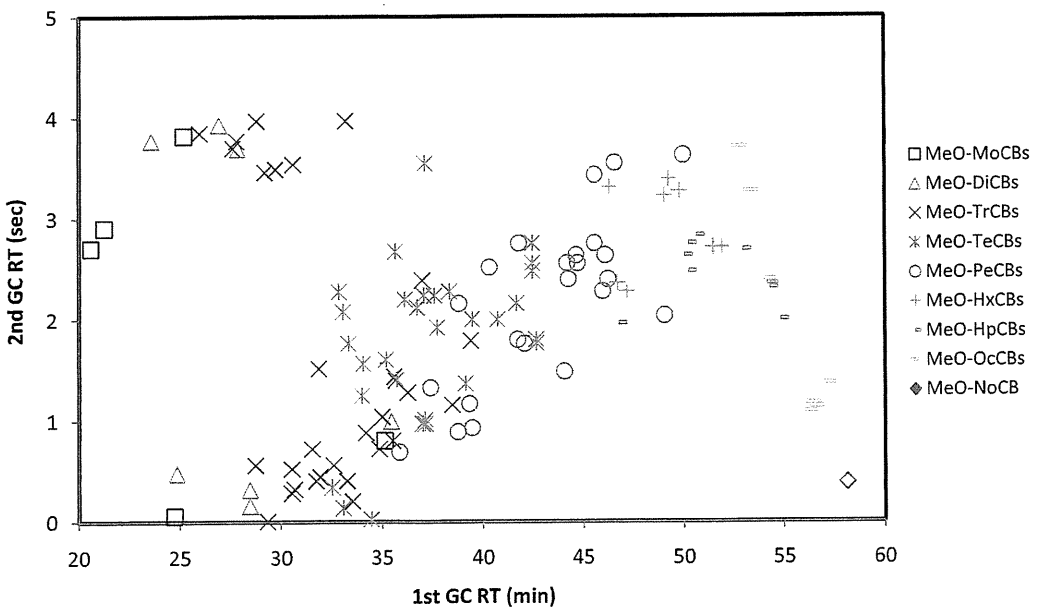


Fig. 5 2D-mapping of retention times of MeO-PCB isomers on a GC × GC

3. 4　河川底質試料中の水酸化 PCB の測定

開発した測定法の検証のため，河川底質を対象とし，実測を行った。試料は大都市を流れる河川とその流域運河の底質を風乾したものを用いた。試料は，先山らの方法[24] に順じ，水酸化 PCB 測定のための精製を行ったのち，誘導体化（メチル化）を行った（2. 3 参照）。その精製試料液を GC × GC-HRTOFMS により測定して得られた代表的な三次元トータルイオンクロマトグラム（3 D-TIC）に標準試料測定により確認した水酸化 PCB（メトキシ PCB）の保持時間を重ね合わせたものを Fig. 7 に示す。水酸化 PCB 用に精製を行った試料液からも多数のピークがクロマトグラム上に現れていることが分かる。しかも，GC × GC により通常の GC では分離不能なピーク（成分）が分離していることが分かる。このことは，水酸化 PCB の異性体分離に有効なばかりではなく，共雑成分との分離にも効果があることを示していると言える。主だったピークと標準試料の保持時間位置はほとんど重なりがなく，大きなピークのほとんどが，水酸化 PCB（メトキシ PCB）由来のものではないことが示唆された。しかしながら，検討に用いた標準試薬の数は 837 種あるモノ水酸化 PCB 異性体のうちの約 18 % しかカバーしておらず，この結果のみで判断するのは尚早であるといえる。

Table 7　Number of isorated peaks on a GC × GC, standard isomers used for this experiment and ideal number of HO-PCB isomers

MeO-PCB	isolated peak no.	STD isomer no.	ideal isomer no.
Monochloro	4	4	19
Dichloro	6	6	64
Trichloro	29	30	136
Tetrachloro	26	30	199
Pentachloro	23	24	199
Hexachloro	8	9	136
Heptachloro	8	9	64
Octachloro	6	6	19
Nonachloro	1	1	3
total	111	119	839

Fig. 8 は，同じ底質試料の一～九塩化モノ水酸化 PCB（モノメトキシ PCB）に相当する質量部分を抜き出し，それらを 1 つに合成した RIC（Reconstructed Ion Chromatogram）である。図から分かるように，測定した試料からは二～八塩化モノ水酸化 PCB（モノメトキシ PCB）と予想されるピークが検出されている。検出されたピークの数を通常の GC によるものと比較したものを Table 8 にまとめた。二～六塩素化体において，GC × GC によるピーク検出数（分離数）は，通常の GC のものを上回っており，総数では，GC × GC で 151 本，通常の GC で 125 本であった。このことより，通常の GC と比較して，GC × GC では約 20 % のピーク分離が向上したといえる。この場合でも，入手した水酸化 PCB 標準品の RT と一致するものはほとんどなく，大部分の異性体の同定はできなかった。HRTOFMS 測定より得られた質量スペクトルの解析から，主なピークのほとんどがメトキシ PCB のものではないことが，確認できた（Fig. 9）（一部のピークはメトキシ塩素化ジフェニルエーテルのものであることが確認できた）。しかし，遙かに多数のマイナーなピークが出現しており，その中に水酸化 PCB（メトキシ PCB）が含まれる可能性は否定できない。検出装置の感度向上に加え，現在の手作業による確認手法では，膨大なピークの物質の同定はほぼ不可能であり，何らかの解析手法あるいは検出法を開発する必要があると考えられた。

4. まとめ

理論異性体数が 839 あるモノ水酸化 PCB の精密な分析を可能に

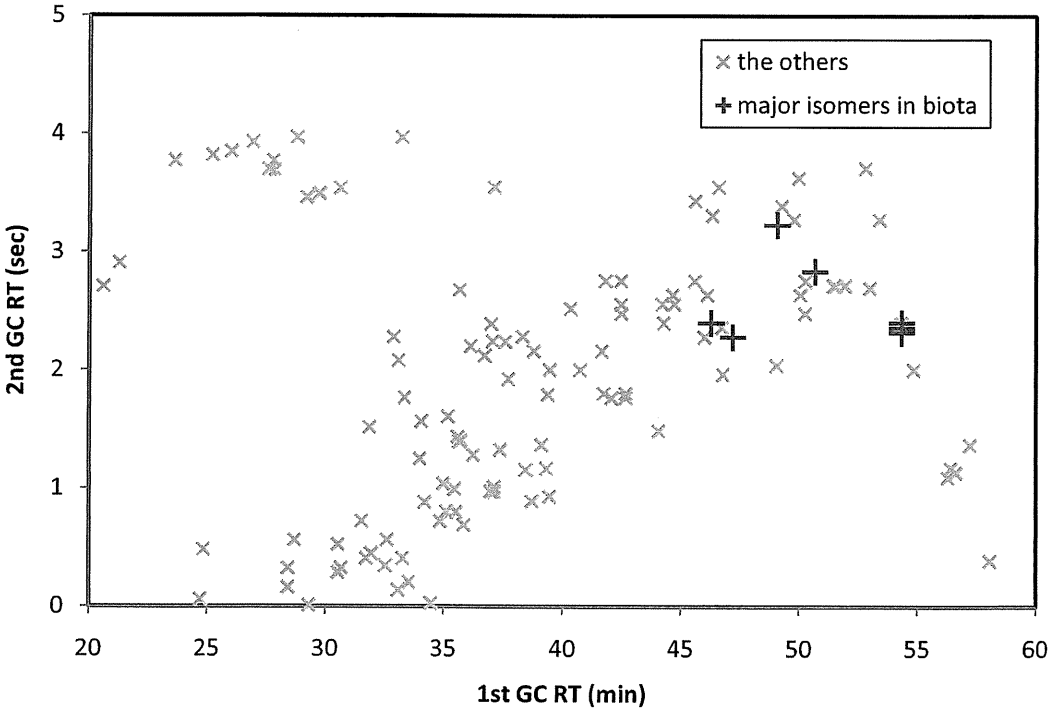


Fig. 6　2D-mapping of major isomers and the others of MeO-PCB in biological samples on a GC × GC

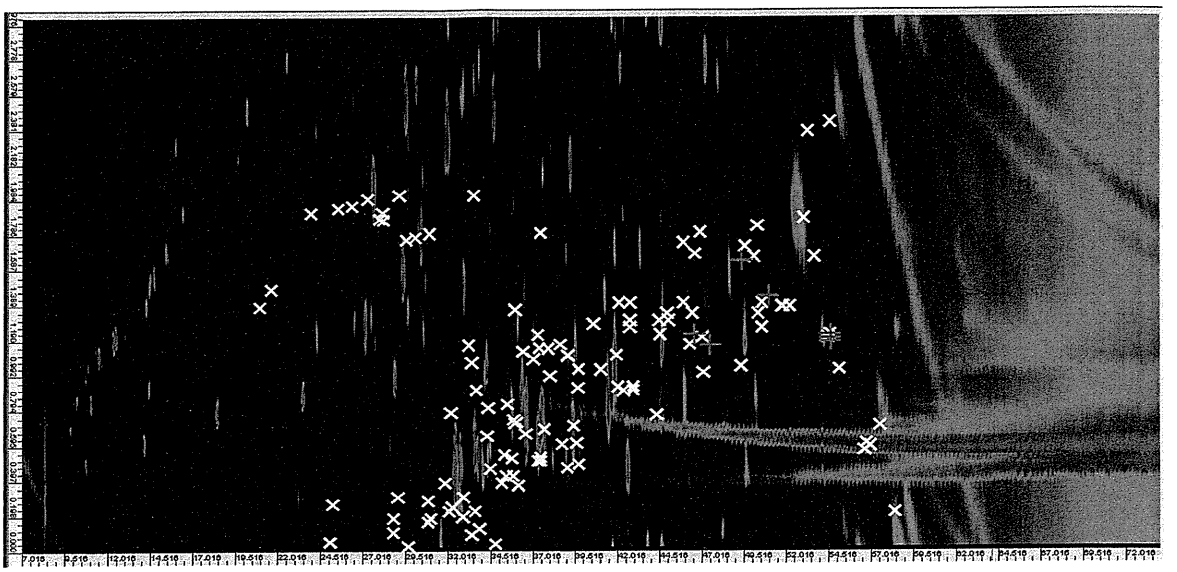


Fig. 7 2D-map of MeO-PCB isomers over layed on 2D-total ion chromatogram of urban river sedeiment measured by a GC×GC-HRTOFMS

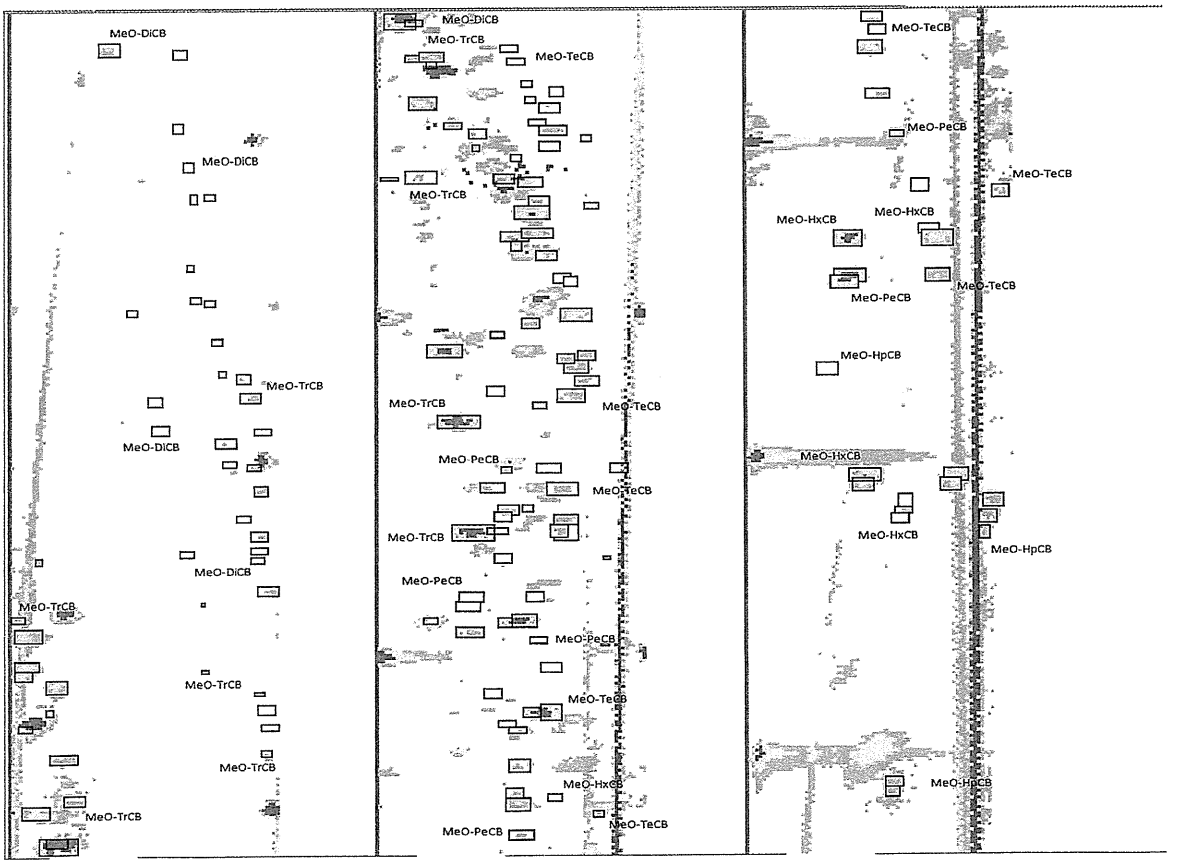


Fig. 8 2D-map of MeO-PCB isomers over layed on 2D-recomposed ion chromatogram (for MeO-PCBs) of urban river sedeiment measured by a GC×GC-HRTOFMS