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Isomer Specific Analysis of Polychlorinated Naphthalenes in Pine Trees (*Pinus thunbergi* Parl.) and (*Pinus densiflora* Sieb. et Zucc) Needles Around Tokyo Bay, Japan

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Abstract

63 congeners of chloronaphthalene represented by 53 peaks fractionated and separated using two-dimensional HPLC and DB-17 capillary column were quantified using HRMS in ten samples of pine needles collected in 1999 around Tokyo Bay in Japan. Similarities and differences of chloronaphthalene concentrations and patterns between 10 sampling sites were studied using multivariate analysis. Total PCN concentrations ranged from 250 to 2100 pg/g wet weight. Except for one site, tri- and tetra-CNs highly dominated (from 54 to 80%) in CN homologue patterns of pine needles. At the easternmost site near the town of Tateyama in Chiba Prefecture the contribution from octaCN was ~20 %, while that of tri- and tetra-CNs ~42 %. Pine needles sampled from the sites around the innermost part of Tokyo Bay showed the highest load of PCNs. A multivariate analysis using the three most significant principal components explained 91% of the total variance in the measurement data. The greatest positive loading to PC1 is from the CN congeners nos. 13, 14/21/24, 15, 16, 17, 18, 19, 20, 22/23, 25, 26, 27, 28/36, 29, 30/32, 31, 33/34/37, 35, 40, 42, 43/45, 44, 47, 49, 50, 51, 52/60, 53, 57, 58, 59, 61, 62, 64, 65, 66/67, 68, 69, 71 and 72, and explains 65% variance in the data set. For PC2 the largest positive loading is from CNs nos.74 and 75, and negative load from CN nos. 38, 41, 46 and 48, which explains 17% of the variance. In case of PC3 the largest negative load is from CNs nos. 54, 56, 63, 70 and 73. A profile of the combustion process related CN congeners measured such as nos. 44, 48 and 54 didn't show any specific trend implying pollution from diffused sources of various types.

Keywords: Air pollution, PCNs, dioxin-like compounds, HRGC-HRMS analysis, vegetation, environmental pollution

Introduction

Polychlorinated naphthalenes (PCNs) are environmentally persistent, toxic and bioaccumulative compounds, which are semi-volatile [1-3]. These compounds undergo diffusion from primary and secondary sources of release into ambient air and further are long-range transported through the atmosphere and subsequently deposited on terrestrial and water surfaces worldwide. Their presence had been confirmed in ambient air over the northern

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Table 1. Details of the pine needle sampling campaign around Tokyo Bay in 1999.

Sampling site*	Pine tree species	Date of collection	Average diameter of the pine tree trunk (cm)	Sample weight (g)
A	*Pinus thunbergii* Parl.	March 14	15	16.6
F	*Pinus thunbergii* Parl.	March 16	21	12.5
G	*Pinus densiflora* Sieb. et Zucc	March 15	14	10.8
I	*Pinus thunbergii* Parl.	March 16	13	20.0
K	*Pinus thunbergii* Parl.	March 12	14	15.1
L	*Pinus thunbergii* Parl.	March 12	10	20.0
M	*Pinus thunbergii* Parl.	March 13	12	15.1
O	*Pinus thunbergii* Parl.	March 13	12	20.0
Q	*Pinus thunbergii* Parl.	March 13	13	20.3
T	*Pinus thunbergii* Parl.	March 14	13	13.6

*For location of the sampling site see Fig. 1.

hemisphere, in flue gases and fly ash production as well as effluents from municipal solid-waste incinerators and other kinds of combustion-related sources [4-10]. These compounds were manufactured since around 1910 until the 1970s and found numerous industrial applications as well as for around 20 years preceded industrial use of polychlorinated biphenyls (PCBs), which further became a main substitute to PCNs.

Through analysis of archived soil samples as well as of dated freshwater and marine sediment core samples [11-14] it has become evident that PCNs from around the 1940s become widespread man-made abiotic environment pollutants, which further tend to bioaccumulate and biomagnify in aquatic and terrestrial food webs and are accumulated in human body fluids and tissues [1, 15-22].

Until recently it was thought, that from many years only residual uses of PCNs contained in earlier manufactured materials and products remain [1]. The Halowax series are the most popular technical chloronaphthalene mixture known [23], while other industrial chemicals containing PCNs as byproducts and releasing them into the environment are technical mixtures of chlorobiphenyls (PCBs) [24, 25]. It was evidenced recently that 18 metric tons of PCNs (Halowax 1001 - like mixture) had been illegally imported in 1999-2001 from the United Kingdom to Japan for manufacture of "Neoprene FB", which is a synthetic rubber product [26].

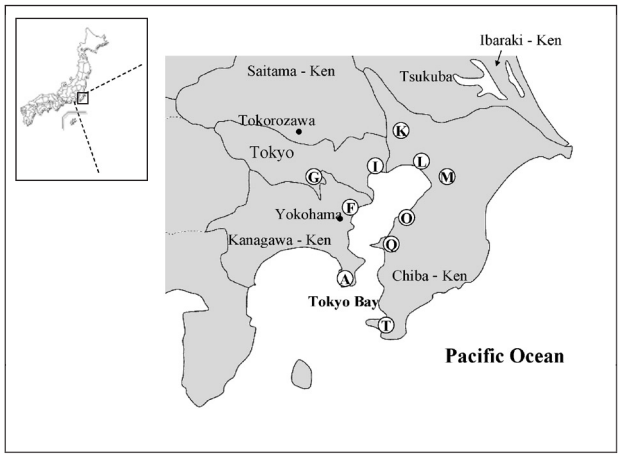


Fig. 1. Location of the sampling sites (A-T) of pine needles around Tokyo Bay, Japan.

Chloronaphthalenes are dioxin-like chemicals [27, 28] and contribute in varying rates to dioxin-like toxicity induced by polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and planar polychlorinated biphenyls (PCBs), which contaminate the environment, food and feed [29-31]. Hence, their ongoing release into the environment and migration to human and animal food resources needs to be eliminated.

Pine needles are considered a very suitable biomatrix for monitoring long-term ambient air concentrations of atmospheric pollutants such as semivolatile and persistent lipophilic compounds [31]. At the area of the Tokyo, Chiba and Kanagawa Prefectures surrounding Tokyo Bay it is possible to find many examples of industrial activity. The area around Tokyo Bay (Kanto region) is among the most urbanized and highly industrialized sites worldwide, which usually serves ambient air pollution difficulties on one side and anti-air pollution and monitoring activities on the other.

The pine needles were collected in 1999 and analyzed for chloronaphthalenes to provide initial evidence on their occurrence in ambient air and potential connection to possible sources of pollution around the Tokyo Bay area in Japan.

Materials and Methods

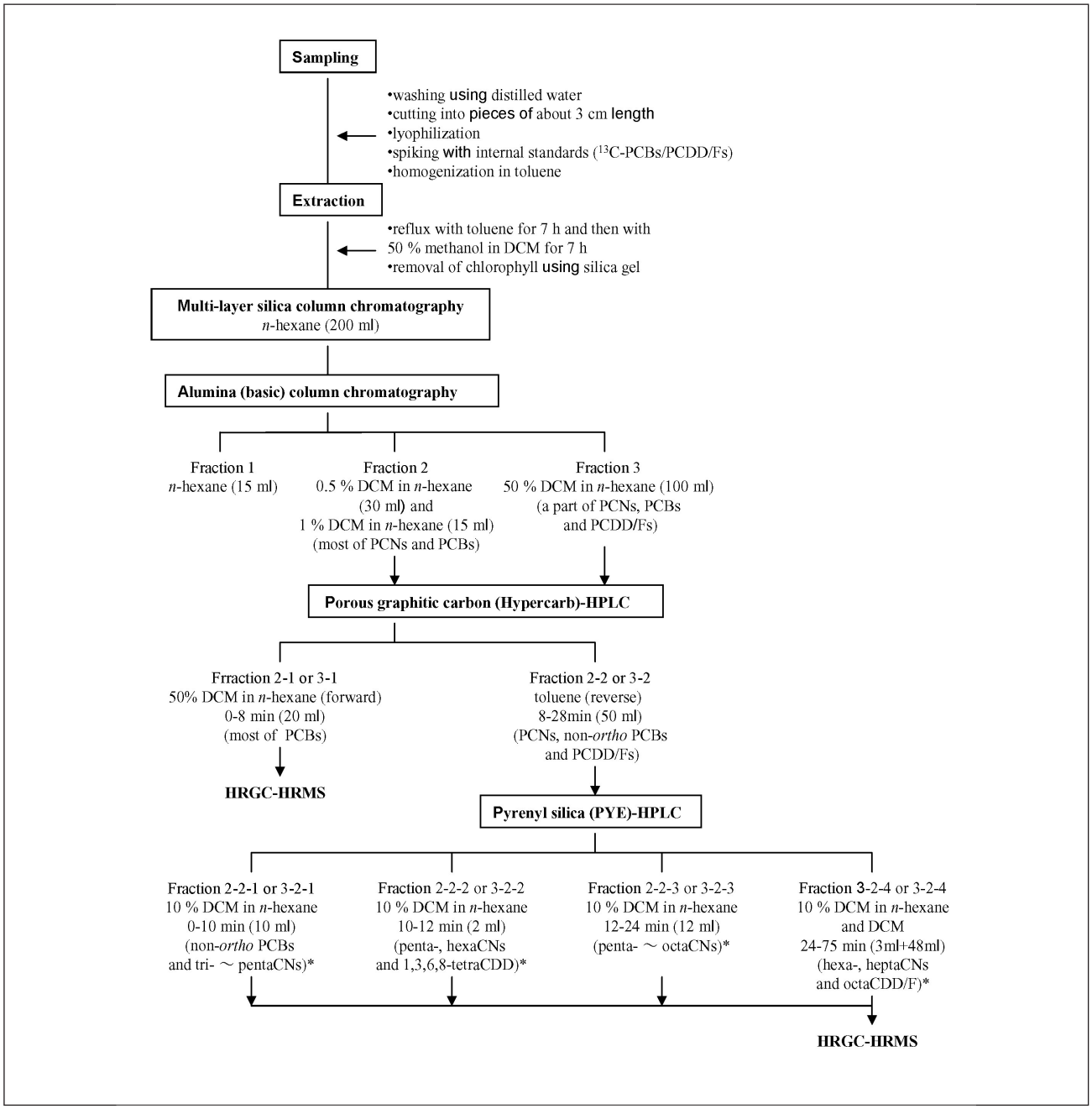
Sampling Sites

The 1-year-old pine needle samples were collected during March 1999 at ten locations around Tokyo Bay (Figure 1). Details of the sampling campaign are given in Table 1. All samples were collected at a height of approximately 1.6 m above ground level from the trees Pinus thunbergi Parl. and Pinus densiflora Sieb. et Zucc. The needles immediately after collection were wrapped in aluminum foil, packed in polyethylene bags already checked for blank background, delivered to laboratory and kept deep-frozen (-20°C) until chemical analysis.

Chemicals

Equivalent amounts (1:1:1:1) of technical chloronaphthalene formulations (Halowax 1000, 1001, 1014 and 1051) obtained from Analabs (USA) were used to prepare a mixed standard (Equi-Halowax). Particular congeners of chloronaphthalene (PCN-MXB) were from Wellington Laboratories (USA). The internal standard mixtures used were ^{13}C-labeled PCBs (EC-4937, CIL, USA), ^{13}C-labeled PCDDs/DFs (NK-LCS-P; Wellington Laboratories) and ^{13}C-HCB (Kanto Kagaku, Japan).

All solvents used were of pesticide grade (Wako Chemicals, Japan). 2% (w/w) KOH-silica gel, 44% (w/w)



Scheme 1. Schematic outline of the analytical procedure (*separation flow for particular compound is explained in detail in Table 4).

Table 2. The conditions of Hypercarb-HPLC fractionation.

Parameter	Fractionation conditions	
Model	SHIMADZU LC-10AD	
Column	Porous graphitic carbon (Hypercarb, Hypersil Co.), 100 mm × 4.6 mm i.d., 7 μm grain size	
Injector	RHEODYNE7125, sample loop: 200 μL, injection volume: 150 μL	
Flow rate	2.5 ml/min.	
Detector	UV- 254 nm	
Column temperature	Room temperature	
Eluent	50 % dichloromethane in n-hexane, 20 ml	(fraction 1, most of PCBs)
	Toluene, 50 ml (reverse flow)	(fraction 2, non-orthoPCBs, PCNs, PCDD/Fs)
	Toluene, 50 ml (reverse flow)	(washing)
	50% dichloromethane in n-hexane, 25 ml	(washing)

* Separation process was programmed from 0 to 28 minute and next up to 58 minute was column washing.

Table 3. The conditions of PYE-HPLC fractionation.

Parameter	Fractionation conditions
Model	Hewlett Packard 1100 series
Column	Pyrenyl silica (PYE, Nacalai Tesque Inc.), 250 mm × 4.6 mm i.d., 5 μm grain size
Injector	RHEODYNE7725i, sample loop: 200 μl, injection volume: 50 μl
Flow rate	1.0 ml/min.
Detector	UV- 254 nm
Column temperature	Room temperature
Eluent	10 % dichloromethane in n-hexane (0-27 min)
	dichloromethane (27-90 min*)
	10 % dichloromethane in n-hexane (90-105 min)

* Separation process was programmed from 0 to 90 minute and next up to 105 minute was column washing.

Table 4. The elution pattern of PCNs and some PCBs and PCDD/Fs after PYE-HPLC column fractionation.

Fraction (sub-fraction)	Time (min.)	Compound
1 (2-2-1/3-2-1)	0-10	non-ortho PCBs
		triCN, tetraCN, 1,2,4,5,8-pentaCN
2 (2-2-2/3-2-2)	10-12	1,2,3,4,5-, 1,2,3,5,7-, 1,2,3,5,8-, 1,2,3,4,6-, 1,2,4,5,7-, 1,2,4,6,7-, 1,2,4,6,8-, 1,2,4,7,8-pentaCN
		1,2,3,4,5,8-, 1,2,4,5,7,8-hexaCN, 1,3,6,8-tetraCDD
3 (2-2-3/3-2-3)	12-24	2,3,6,7-tetraCN+, 1,2,3,4,6-, 1,2,3,5,6-, 1,2,3,5,7-, 1,2,3,6,7-*, 1,2,3,7,8-*, 1,2,4,5,6-*, 1,2,4,6,7-pentaCN
		1,2,3,4,5,7-*, 1,2,3,5,7,8-**, 1,2,4,5,6,8-hexaCN, 1,2,3,4,5,6,8-heptaCN, octaCN
4 (2-2-4/3-2-4)	24-75	1,2,3,4,5,6-**, 1,2,3,4,6,7-#,**, 1,2,3,5,6,7-#,**, 1,2,3,5,6,8-hexaCN*, 1,2,3,4,5,6,7-heptaCN*
		OCDD/F

#CN congeners separated by second fractionation using n-hexane; *CN congeners with moderate to low TEF values (< 7.0 x 10^{-4}); **CN congeners with moderate to high TEF values (> 0.9 x 10^{-3}); +2,3,6,7-tetraCN is absent in technical Halowax mixtures.

Table 5. HRGC-HRMS operating conditions set for PCNs analysis.

Parameter	Conditions set			
Instrument	JEOL JMS-700D			
Column	DB-17(J&W Scientific) 30 m length × 0.25 mm i.d., 0.25 μm film thickness			
Column temperature	70°C (1 min.), 15°C/min. to 180°C, 2°C/min. to 270°C (10 min.)			
Injection	On-column injection mode by auto injector			
Ion source	EI ion source, positive			
Source temperature	270°C			
Interface temperature	270°C			
Injector temperature	250°C			
Ionization voltage	38 eV			
Trap current	500 μA			
Accel. Voltage	10 kV			
Resolution	> 10000			
Monitored ions		M^+	$(M+2)^+$	$(M+4)^+$
	triCN	229.9457	231.9427	
	isotope ratio	100	98	
	tetraCN	263.9067	265.9038	
	isotope ratio	77	100	
	pentaCN		299.8648	301.8618
	isotope ratio		100	65
	hexaCN		333.8258	335.8229
	isotope ratio		100	81
	heptaCN		367.7868	369.7839
	isotope ratio		100	98
	octaCN		401.7479	403.7449
	isotope ratio		88	100

H_2SO_4-silica gel, 22% (w/w) H_2SO_4-silica gel, 10% (w/w) $AgNO_3$-silica gel and anhydrous sodium sulfate were of analytical grade (Wako Chemicals, Japan). Silica gel 60 (230-400 mesh) and alumina (70-230 mesh, basic) were also of analytical grade (Merck, Germany).

All the materials and chemicals used were handled as follows: the glassware was solvent-washed, dried and baked overnight at 250°C. The glass wool was Soxhlet-washed in n-hexane and further dried using vacuum aspirator. The timber filters (ADVANTEC, Japan) were baked at 300°C for 12 h, while anhydrous sodium sulfate, silica gel and alumina were baked at 500°C for 12 h.

Extraction

The pine needle samples to remove dust particles from the surface were initially washed using distilled water and next drained to remove water drops (Scheme 1). Before being lyophilized and spiked with internal standard materials consisting of ^{13}C-labeled PCBs and ^{13}C-labeled PCDDs/DFs the needles were cut into pieces of about 3 cm in length. Further, the needle samples were homogenized using 300 ml of toluene. Next, needles were initially extracted using 300 ml toluene and followed by 300 ml of 50% dichloromethane (DCM) in methanol, each extraction step proceed for 7 h in Soxhlet-extractor. Chlorophyll was removed after passage of the extract thorough a layer of silica gel. The solvents were carefully evaporated to 3 ml volume using a rotary evaporator under vacuum pressure and stream of nitrogen purged gently at 40°C.

Clean-up

Multi-layer Silica Gel Clean-up Step

The extract was pre-cleaned up by passing through several layers of silica gel packed into glass column (300

mm length ×20 mm i.d.) in descending order, as follows: silica gel (0.8 g), 2 % (w/w) KOH-silica gel (3 g), silica gel (0.8 g), 44 % (w/w) H_2SO_4-silica gel (4 g), 22 % (w/w) H_2SO_4-silica gel (4 g), silica gel (0.8 g), 10 % (w/w) $AgNO_3$-silica gel (8 g) and anhydrous sodium sulfate (5 g) loaded on the top. The column was pre-washed with n-hexane (200 ml) and analyte was eluted with the next portion of n-hexane (200 ml). The effluent was carefully concentrated to 1 ml.

Alumina Layer Clean-up and Fractionation Step

The analyte was further cleaned-up and fractionated using an activated basic alumina column chromatography. Alumina (10 g) was activated at 130°C for 12 h and packed into a glass column (300 mm length × 12 mm i.d.) with anhydrous sodium sulfate layer (2 g) on the top. The analyte was eluted with n-hexane (15 ml; fraction 1), 0.5% DCM in n-hexane (30 ml) and 1% DCM in n-hexane (15 ml; fraction 2) and 50% DCM in n-hexane (100 ml; fraction 3). All fractions were micro concentrated to 200 µl under a gentle stream of nitrogen. Alumina and solvents were lot-to-lot checked for separation efficiency using an appropriate chloronaphthalene, chlorobiphenyl, chlorodibenzo-p-dioxin and chlorodibenzofuran congener's standard solution.

Hypercarb-HPLC Sub-fractionation Step

The analtye was further HPLC sub-fractionated using a porous graphitic carbon column (100 mm length × 4.6 mm i.d., 7 µm grain size) (Hypercarb, Hypersil, USA) (32, 33) (Table 2). Prior to each fraction introduction the column was always pre-washed with 50% DCM in n-hexane (20 ml) and toluene (50 ml). Further, 150 µl aliquots of fractions 2 and 3 were injected, respectively. The remaining part of the fractions 2 and 3 was reserved and kept sealed under refrigerator condition till termination of the analysis. The Hypercarb-HPLC column was forward eluted using 50% DCM in n-hexane (20 ml; fractions 2-1 or 3-1) and back flushed using toluene (50 ml; fractions 2-2 or 3-2). Fractions 2-1 and 3-1 were spiked with ^{13}C-HCB (10 µl) and isooctane (30 µl), micro concentrated to 100 µl and HRGC-HRMS checked for each of the four mono- and di-ortho PCBs congeners containing from 4 to 7 chlorines. The remaining parts of the effluent from Hypercarb-HPLC column were micro concentrated to 100 µl and further subjected for additional sub-fractionation step using PYE-HPLC column.

PYE-HPLC Sub-fractionation Step

The analyte was further HPLC sub-fractionated to separate PCBs and dioxins (34, 35) using a pyrenyl silica column (250 mm length × 4.6 mm i.d., 5 µm grain size) (PYE, Nacalai Tesque, Japan) (Table 3). After pre-washing of the pyrenyl silica column with 27 ml of 10 % DCN in n-hexane the 50 µl aliquots of fractions 2-2 and 3-2 were injected, respectively. The remaining part of the fractions 2-2 and 3-2 was reserved and kept sealed under refrigerator condition until termination of the analysis. The analyte from the PYE-HPLC column was collected each into four sub-fractions using 10% DCM in n-hexane (27 ml) or 10% DCM in n-hexane (27 ml) and DCM (48 ml) – fractions 2-2-1, 3-2-1, 2-2-2, 3-2-2, 2-2-3, 3-2-3, 2-2-4 and 3-2-4, respectively (Scheme 1 and Table 4). Each sub-fraction was spiked with ^{13}C-HCB (10 µl) and isooctane (30 µl) and further micro concentrated to 100 µl under a gentle stream of nitrogen. The sub-fractions were analyzed for tri- to octachloronaphthalene, non-ortho PCBs and tetra- to octaCDD/Fs (Table 4) using high-resolution gas chromatography and high-resolution mass spectrometry (HRGC-HRMS).

Separation efficiency of the PYE-HPLC column was checked using standard solutions of two co-eluting pairs of hexachloronaphthalenes, respectively, 1,2,3,4,5,7- (#64) and 1,2,3,5,6,8-hexaCN (#68), and 1,2,4,5,6,8- (#71) and 1,2,4,5,7,8-hexaCN (#72) (Table 4).

HRGC-HRMS Analysis

HRGC-HRMS analysis was performed using an HP 6890 GC interfaced with a JEOL JMS-700D HRMS (Japan) at resolution R > 10000 MU (10% valley) and selected ion monitoring (SIM) mode. Each sample was analyzed using two different GC capillary columns. PCDD/Fs with 4-6 chlorines were separated and quantified using an SP-2331column (60 m length × 0.25 mm i.d. and 0.20 µm film thickness; Supelco, USA), while PCDD/Fs with 7-8 chlorines, coplanar PCBs with 4-7 chlorines and PCNs with 3-8 chlorines using a DB-17 column (30 m length × 0.25 mm i.d.; 0.25 µm film thickness; J&W Scientific). The operating conditions for the systems used are detailed in Table 5. Carrier gas (helium) flow-rate was 1.5 ml/min. Usually 1 µl of the extract was injected under split-less mode. Data acquisition of the mass spectrometer was controlled by an HP work station and data process using JEOL DioK program.

Quality Assurance/Quality Control Protocol

Quality assurance and quality (QA/QC) control protocols included analysis of matrix spike and matrix spike duplicates, replicate samples and procedural blanks. Peaks were identified by retention time compared to standards if signal to noise (S/N) ratio was > 3 and were quantified if target/qualifier ion ratios were within the 15% limit of the standard values. All samples were spiked with a ^{13}C-labeled PCB and PCDD/F recovery standard prior to extraction. Any sample with recovery below 40% was discarded. Quantification of chlorinated compounds was performed using an external standard method and quantitative standards. OctaCN peak from

Table 6. Congener-specific data of PCNs in pine needles (pg/g wet weight).

Chloronaphthalene	IUPAC No.	Site*									
		A	F	G	I	K	L	M	O	Q	T
Trichloronaphthalenes											
1,2,3-	13	24	14	30	45	40	14	45	17	14	3.4
1,2,4-/1,3,7-/1,4,6-	14/21/24	33	42	79	150	58	21	100	20	49	9.0
1,2,5-	15	9.6	7.1	21	30	21	7.4	56	5.9	14	2.1
1,2,6-	16	5.1	3.6	9.7	15	12	3.7	27	3.5	6.8	1.5
1,2,7-	17	8.8	16	17	23	16	8.2	20	12	7.5	2.6
1,2,8-	18	3.7	4.8	5.5	5.1	2.7	3.5	14	4.1	5.2	1.1
1,3,5-	19	4.8	3.9	9.8	17	9,5	2.5	21	1.9	6.4	1.0
1,3,6-	20	32	19	51	62	72	22	150	24	49	7.0
1,3,6-/1,4,5-	22/23	14	11	20	44	16	7.7	19	6.9	13	2.9
1,6,7-	25	12	21	24	30	24	11	31	17	10	3.9
2,3,6-	(26)	9.7	4.0	9.8	9.7	11	4.8	18	4.5	7.7	1.3
Tetrachloronaphthalenes											
1,2,3,4-	27	39	28	48	88	76	25	120	28	26	6.4
1,2,3,5-/1,2,5,6-	28/36	45	17	36	84	80	15	99	15	21	5.5
1,2,3,6-	29	12	38	32	43	28	17	32	22	13	3.4
1,2,3,7-/1,2,4,5-	30/32	6.0	4.7	12	18	11	4.7	27	4.3	8.2	1.9
1,2,3,8-	(31)	3.5	3.2	4,8	7.5	5.8	3.2	8.8	4.0	3.1	0.90
1,2,4,6-/1,2,4,7-/1,2,5,7-	33/34/37	57	46	87	230	82	29	260	29	71	20
1,2,4,8-	35	14	14	24	40	20	8.7	27	8.9	15	3.9
1,2,5,8-	38	47	58	55	140	53	19	42	210	34	8.2
1,2,6,7-	(39)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,6,8-	(40)	1.8	2.4	2.4	4.6	4.0	1.9	4.6	2.8	1.9	0.59
1,2,7,8-	(41)	6.0	14	19	17	7.8	5.3	8.4	7.8	5.5	1.8
1,3,5,7-	42	8.5	5.5	12	41	11	1.6	22	1.3	5.0	0.96
1,3,5,8-/1,3,6,8-	(43/45)	24	30	50	94	45	17	83	18	30	7.4
1,3,6,7-	(44)	4.2	3.8	5.0	11	6.7	3.2	8.6	3.4	2.5	0.73
1,4,5,8-	46	21	18	7.1	49	27	7.1	15	6.9	12	3.1
1,4,6,7-	47	17	17	26	72	22	9.8	73	11	23	5.8
2,3,6,7-	48	14	23	22	24	21	5.0	4.0	6.8	4.4	1.5
Pentachloronaphthalenes											
1,2,3,4,5-	49	5.3	5.5	5.2	13	10	7,2	18	6.9	6.4	1.5
1,2,3,4,6-	50	15	23	21	40	28	15	81	21	20	6.9
1,2,3,5,6-	51	10	26	22	28	23	15	78	23	16	5.8
1,2,3,5,7-/1,2,4,6,7-	52/60	22	41	34	59	47	16	170	24	25	9.3
1,2,3,5,8-	53	7.9	9.7	9.4	16	13	6.8	16	6.6	7.8	1.8
1,2,3,6,7-	54	7.7	19	17	24	14	19	18	25	16	5.6
1,2,3,6,8-	(55)	<0.10	<0.10	<0.10	<0.10	<0.10	0.13	<0.10	<0.10	0.11	<0.10

Table 6. continues on next page...

1,2,3,7,8-	56	1.0	4.3	3.9	3.9	2.7	5.6	5.0	6.9	6.2	1.3
1,2,4,5,6-	57	69	11	9.2	12	11	7.1	16	8.2	10	3.9
1,2,4,5,7-	58	2.3	2.6	2.6	6.2	6.4	1.9	21	2.7	3.3	0.59
1,2,4,5,8-	59	12	9.6	16	20	23	9.7	23	11	10	4.6
1,2,4,6,8-	61	7.8	9.2	11	21	18	6.6	46	7.0	12	2.4
1,2,4,7,8-	62	11	13	12	20	17	11	25	9.0	13	3.2
Hexachloronaphthalenes											
1,2,3,4,5,6-	63	4.6	7.8	5.3	6.4	6.1	8.6	13	11	5.9	1,8
1,2,3,4,5,7-	64	1.7	2.2	3.7	3.1	2.2	3.2	22	4.9	4.3	2,7
1,2,3,4,5,8-	65	<0.15	0.86	<0.15	0.89	1.8	1.0	2.1	0.85	1.6	<0.15
1,2,3,4,6,7-/1,2,3,5,6,7-	66/67	17	29	37	40	26	27	97	42	22	14
1,2,3,5,6,8-	68	2.0	3.1	4.4	5.6	4.2	4.5	15	5.9	3.0	2.0
1,2,3,5,7,8-	69	3.7	6.1	5.6	11	6.0	8.2	25	11	8.5	4.4
1,2,3,6,7,8-	(70)	1.3	1.8	2.5	2.0	2,1	2.8	1.8	3.1	1.6	0.67
1,2,4,5,6,8-	71	1.2	1.7	1.9	3.8	3.3	2.1	13	2.8	2.9	5.1
1,2,4,5,7,8-	72	1.4	<0.15	<0.15	1.6	2.5	1.1	6.3	1.3	1.5	<0.15
Heptachloronaphthalenes											
1,2,3,4,5,6,7-	73	8.2	9.0	5.9	11	7.2	12	13	16	10	4.1
1,2,3,4,5,6,8-	74	1.7	2.7	2.3	0.85	2.9	3.8	16	5.1	5.2	9.5
Octachloronaphthalene											
1,2,3,4,5,6,7,8-	75	4.6	6.4	<0.30	5.3	9.2	12	33	11	15	51
ΣPCNs		630	710	950	1800	1100	490	2100	570	690	250

*For location of the sampling site see Fig. 1.

the blank samples slightly exceeded the assigned signal-to-noise ratio but in real samples peaks from this compound largely exceeded blank value for most of the samples. The detection limits of chloronaphthalene congeners were around 0.2 pg/g wet weight and depended on sample size and interference.

The performance of PCN analysis was successfully validated during participation in phase 1 of a recent intercalibration trial [36].

The recovery rates of tetraCDD/Fs, pentaCDD/Fs, hexaCDD/Fs, heptaCDD/Fs and octaCDD/F through the whole analytical procedure were 120±37 %, 140±66 %, 150±73 %, 125±76 % and 115±70 %, respectively, while of tetra-, penta-, hexa- and heptaCBs were 124±50 %, 125±63 %, 128±74 % and 95±45 %. The recovery of hexachlorobenzene (HCBz) (syringe spike) was more than 80%.

Multivariate Statistical Analysis

The concentration of CNs below detection limits of the analytical method used (Table 6) were completed by the value of half a detection limit, which is the standard technique. All data were autoscaled in columns. As a result, standard deviation in each column was set as 1, and mean value was set as 0.

An initial analysis of CN pattern similarity between ten sites examined was done using cluster analysis (CA) method, with single linkage (nearest neighbor) and Euclidean distance in the multidimensional space of values (sampling sites).

More accurate study of similarities between the sites, including diversity of CN congeners in each location, was done using principal component analysis (PCA). This technique may reduce multidimensional space of variables (i.e. concentration of each CN congeners) into two or three new dimensions (vectors), orthogonal to each other, which are called 'principal components' or 'PCs'. Each PC is a linear combination of well correlated original variables (concentrations), and may be interpret as a specific pattern of chloronaphtalene congeners.

Results and Discussion

Identification of CN Congeners

Equi-Halowax, a very complex mixture of chloronaphthalene congeners (37), was used to set-up separation

efficiency of HRGC (DB-17 column) and HRMS system used. Subsequent chromatograms obtained for particular chloronaphthalene homologue groups in Equi-Halowax used in this study after HRGC-HRMS with DB-17 are presented in Figures 2-4. Using the presented analytical procedure (Scheme 1) it was possible to quantify all chloronaphthalene congeners, which co-elute when using any commercially available non-polar capillary column [1, 23].

The sub-fractions 2-2-1 and 3-2-1 contained tri- and tetraCNs, and 1,2,4,5,8-pentaCN (Table 4). Hence, a sub-fractionation of the analyte under conditions described in this work enabled separation and quantification both of 1,2,4,5,8-pentaCN and 1,2,3,4,5-pentaCN, which tend to co-elute on traditionally used HRGC columns (Figure 2).

The sub-fractions 2-2-2 and 3-2-2 contained some of penta- and hexaCN congeners, especially 1,2,3,4,5-, 1,2,3,5,7-, 1,2,3,5,8-, 1,2,3,4,6-, 1,2,4,5,7-, 1,2,4,6,7-, 1,2,4,6,8-, 1,2,4,7,8-pentaCN, 1,2,3,4,5,8- and 1,2,4,5,7,8--hexaCN (Table 4). Hence, a sub-fractionation of the analyte enabled separation and quantification both of 1,2,4,5,6,8- and 1,2,4,5,7,8-hexaCN, which co-elute on traditionally used HRGC columns (Figure 3).

The sub-fractions 2-2-3 and 3-2-3 contained some penta- and hexaCNs, especially 1,2,3,4,6-, 1,2,3,5,6-, 1,2,3,5,7-, 1,2,3,6,7-, 1,2,3,7,8-, 1,2,4,5,6-, 1,2,4,6,7-pentaCN, 1,2,3,4,5,7-, 1,2,3,5,7,8-, 1,2,4,5,6,8-hexaCN, but also 1,2,3,4,5,6,8-heptaCN and octaCN. A sub-fractionation of the analyte enabled separation and quantification both of 1,2,3,4,5,7- and 1,2,3,5,6,8-hexaCN (Table 4), which co-elute on traditionally used HRGC columns (Figs. 3 and 4). 2,3,6,7-tetraCN, found in pine needle samples, was also a characteristic congener, which eluted in sub-fraction 3-2-3 (Table 4).

The sub-fractions 2-2-4 and 3-2-4 contained some hexaCNs, especially 1,2,3,4,5,6-, 1,2,3,4,6,7-, 1,2,3,5,6,7-, 1,2,3,5,6,8-hexaCN and 1,2,3,4,5,6,7-heptaCN. OctaCDD/ /F were also characteristic compounds contained in sub-fraction 3-2-4. Separation of 1,2,3,4,6,7- and 1,2,3,5,6,7-hexaCN (Table 4), which tend to co-elute on traditionally used HRGC columns was unsatisfactory using Hypercarb-HPLC column, what was further satisfactory solved out through alteration of the elution time at the n-hexane and PYE-HPLC fractionation step.

CNs in Pine Needles

Concentrations

In total 10 samples of pine needles of the same age from ten sites around Tokyo Bay were collected and analyzed. Chloronaphthalenes load of pine needles around

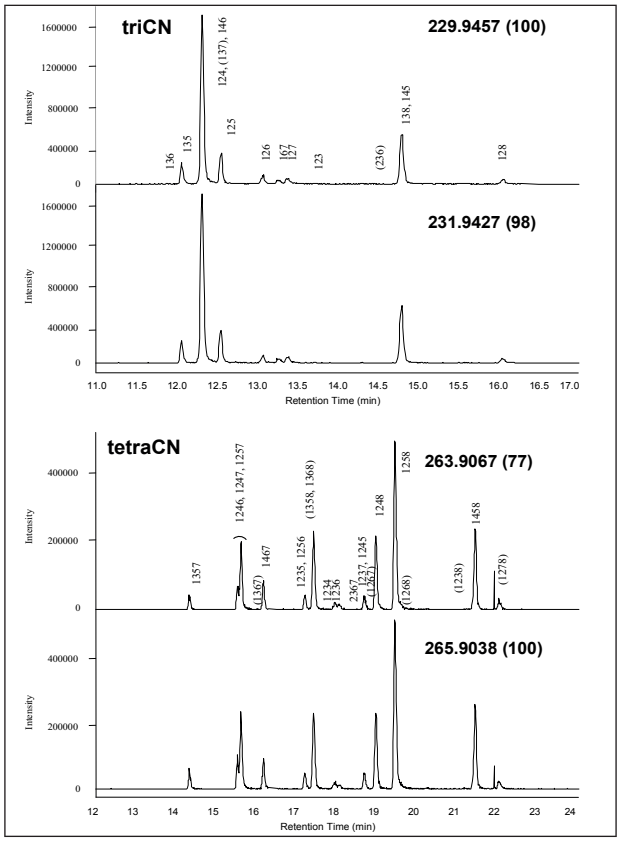


Fig. 2. SIM chromatograms of tri-CNs and tetra-CNs in Equi-Halowax on DB-17 column.

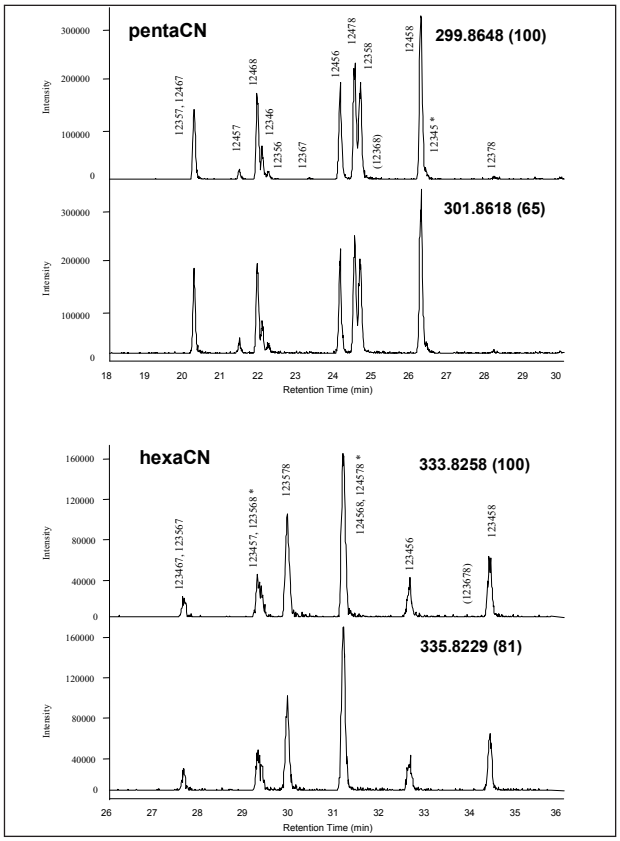


Fig. 3. SIM chromatograms of penta-CNs and hexa-CNs in Equi-Halowax on DB-17 column (*CN congeners separable when using PYE-HPLC column).

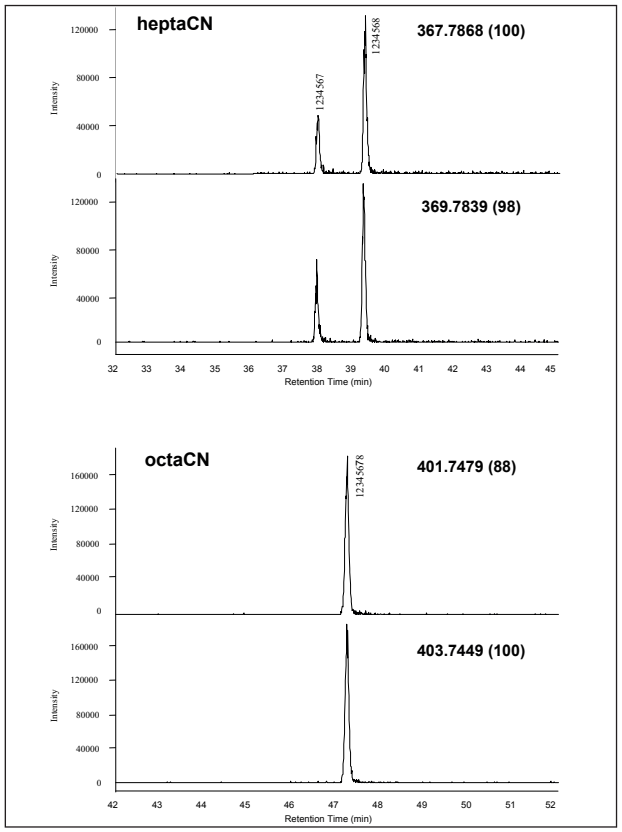


Fig. 4. SIM chromatograms of hepta-CNs and octa-CN in Equi-Halowax on DB-17 column.

Tokyo Bay apparently varied spatially and ranged in absolute concentration from 250 to 2100 pg/g wet weight (Table 8). Mono- and di-CNs were not quantified in pine needles examined. In this study apparently greater and possible source-related CN concentrations were found in pine needles collected at the site M, I, K and G, which contained, respectively, 2100, 1800, 1100 and 950 pg/g wet weight. These sites apparently are located in the heart of a densaly populated and industrialized region under current survey. Site T is the easternmost orientated site examined and somewhat distant from relatively much more densaly populated sites M, I, K and G (Figure 1). So, relatively lower load of PCNs observed in pine needles for site T seems to be principally due to its remoteness from the large-city-related and of dispersive nature local sources of environmental diffusion of PCNs, while reason for untypical for the region surveyed fingerprint of PCNs should be explained in ongoing analyses with new sets of the samples.

There is only a very limited number of data on PCN residues in plant biomass worldwide available in scientific literature. In study of ten pine needle samples collected at ten sites in the USA by Loganathan et al. [37] total PCN content ranged widely from 99 to 19000 pg/g dry matters, so apparently reflected some impact from local pollution sources.

Pattern

As shown in Figure 5 chloronaphthalene homologue group composition of pine needles is largely different for site T when compared to other locations. Tri- and tetraCNs were the predominant congeners collectively accounting for 54 to 80% of total PCNs in pine needles for nine samples from the Tokyo Bay area, Japan. A striking feature is the large contribution from octachloronaphthalene at site T, which is > 20%, while absolute concentration of PCNs of 250 pg/g wet weight for this site is lowest for the whole region investigated (Table 6).

A sample of normalized compositional pattern of CN congeners quantified in pine needles is given in Fig. 6. Apparently, congeners nos. 33/34/37, 38, 28/36 and 27 of terta-CNs and congeners nos. 14/21/24, 13 and 20 of tri-CNs are the most abundant constituents, while some relatively much more persistent CNs, e.g. such as nos. 42, 52, 60 etc. [1, 3] are minor constituents.

Differences and similarities in compositional pattern of chloronaphthalenes sequestered by pine needles and depending on the sampling site are further evidenced

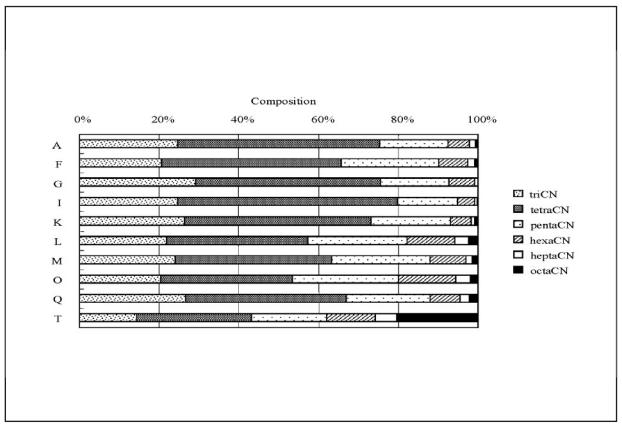


Fig. 5. Chloronaphthalene homologue group composition of pine needles around Tokyo Bay.

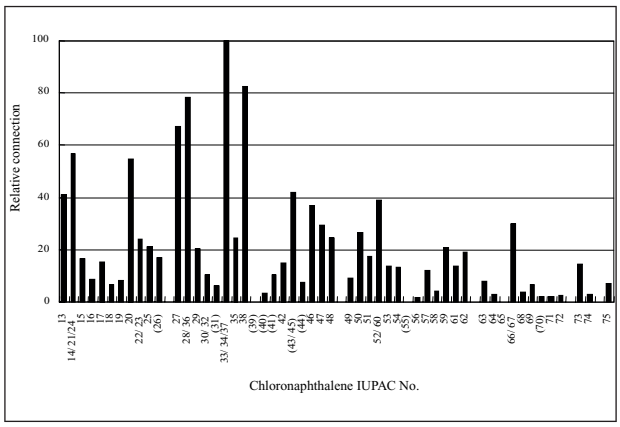


Fig. 6. Chloronaphthalene congeners composition of pine needles at site A around Tokyo Bay.

after Cluster Analysis (CA) of the data set (Fig. 7). The 'fingerprint' of PCNs is largely similar for sites Q and L, I and G as well as K and A. Additionally, site Q is similar to sites I and G. Further, sites Q, I, G, K and A are more similar to site M than site F, while sites O and L are different from sites F, M, Q, I, G, K and A. Evidently, site T at Sunosaki near Tateyama city in Chiba Prefecture, which is the easternmost orientated and somehow distant from the urbanized and industrialized area close to inner part of the bay, stands out from the other locations examined.

The Principal Component Analysis (PCA) model including the three most significant PCs explains 91% of the total variance in the measurement data. The greatest positive loading to PC1 is from the CN congeners nos. 13, 14/21/24, 15, 16, 17, 18, 19, 20, 22/23, 25, 26, 27, 28/36, 29, 30/32, 31, 33/34/37, 35, 40, 42, 43/45, 44, 47, 49, 50, 51, 52/60, 53, 57, 58, 59, 61, 62, 64, 65, 66/67, 68, 69, 71 and 72, and explains 65% variance in the data set. For PC2 the largest positive loading is from CNs nos.74 and 75, and negative load from CNs nos. 38, 41, 46 and 48, which explains 17% of the variance. In the case of PC3 the largest negative load is from CNs nos. 54, 56, 63, 70 and 73 (Fig. 8).

The concentrations of chloronaphthalene congeners nos. 13, 14/21/24, 15, 16, 17, 18, 19, 20, 22/23, 25, 26, 27, 28/36, 29, 30/32, 31, 33/34/37, 35, 40, 42, 43/45, 44, 47, 49, 50, 51, 52/60, 53, 57, 58, 59, 61, 62, 64, 65, 66/67, 68, 69, 71 and 72 belonging to PC1 are similar for sites L, O, Q, A, F, G and K, while they are highly different for site T and much less when compared to sites T and I.

The concentrations of the chloronaphthalene congeners nos. 74 and 75 as well as 38, 41, 46 and 48 belonging to PC2 are similar for sites L, O, Q, A, F, G, K and T, and differ for site M and also site I.

The concentrations of chloronaphthalene congeners nos. 54, 56, 63, 70 and 73 belonging to PC3 are similar for sites A, L, Q, F, G, K, I and M but differ for sites T and O (Fig. 9).

Some CN congeners and especially chloronaphthalene nos. 29 (1,2,3,6-tetraCN), 39 (1,2,6,7-tetraCN), 44 (1,3,6,7-tetraCN), 48 (2,3,6,7-tetraCN) and less nos. 66 (1,2,3,4,6,7-hexa-CN) and 67 (1,2,3,5,6,7-hexa-CN) are

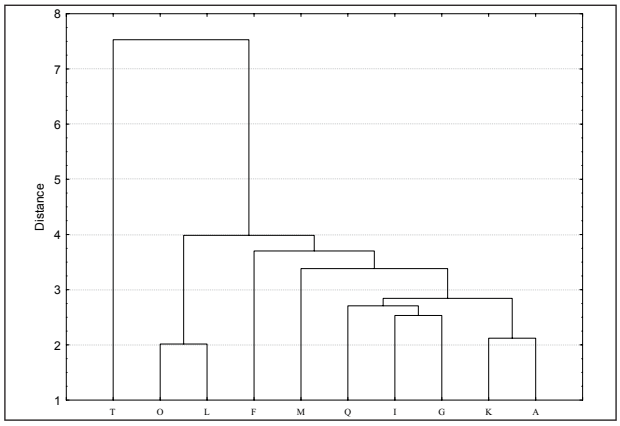


Fig. 7. Cluster analysis data of PCNs of pine needles around Tokyo Bay.

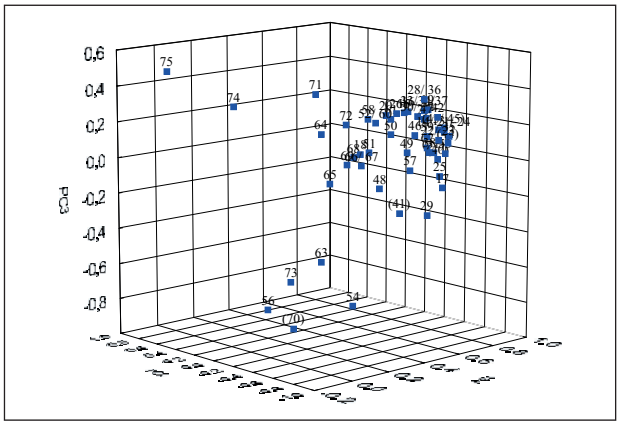


Fig. 9. The score plot of the sampling sites in the space of the three first principal components.

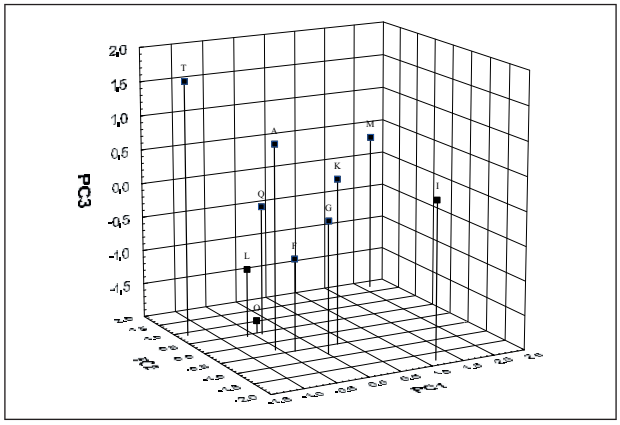


Fig. 8. Plot of loadings from PCA based on chloronaphthalene congeners compositional pattern.

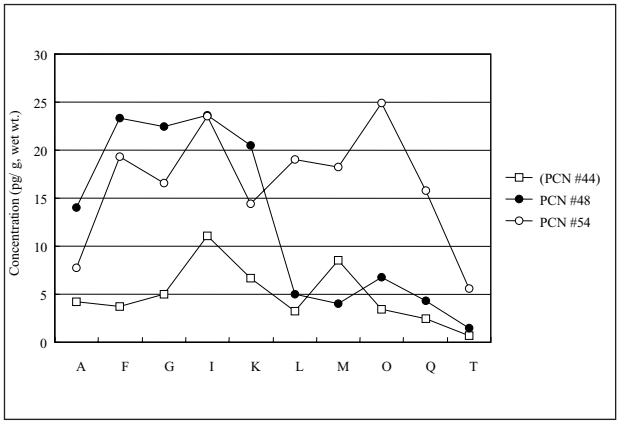


Fig. 10. Profile of selected "thermal processes related" congeners of chloronaphthalene in pine needles around Tokyo Bay.

enriched in ambient air due to combustion process (Stoker type incinerators) and can serve as combustion markers (38). As shown in Fig. 10 chloronaphthalenes nos. 44, 48 and 54 showed different trend in pine needles from ten sampling sites, which largely implies on diffused ambient air pollution by PCNs originating from sources of various type both due to combustion processes as well as vaporization from products and wastes contaminated with mixtures of technical PCNs and PCBs.

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