

PCNs IN PINE NEEDLES AROUND THE TOKYO BAY, JAPAN

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Introduction

Polychlorinated naphthalenes (PCNs) have been used in numerous applications since early 1910 until 2001¹⁻³, only exception of accidental use in Japan in 2002⁴. Nevertheless, apart from the industrial use their emission sources are also technical formulations of polychlorinated biphenyls (PCBs) and thermal processes such as incineration^{5,6}. PCNs are environmentally persistent and toxic compounds, which accumulate in biota and biomagnify in marine food web^{1,7-8}. Because of semi-volatile nature PCNs undergo diffusion from primarily and secondary emissions sources and are found in ambient air, soil, wildlife and human worldwide⁹⁻¹³. Polychlorinated naphthalenes are dioxin-like chemicals and contribute in varying rates to dioxin-like effects produced by Ah-receptor inducing environmental contaminants^{14,15}.

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

The pine needle samples were collected during March 1999 at ten locations around the Tokyo Bay (Fig. 1). Details of the sampling campaign are given in Tab 1.

In brief, the samples were Soxhlet extracted using toluene and followed by 50 % dichloromethane in methanol. Chlorophyll was removed using layer of silica gel. Next, the extract was cleaned using multi-layer of silica gel and fractionated using activated basic alumina column chromatography and hexane (Fr. 1), 0.5 % dichloromethane in hexane and 1 % dichloromethane in hexane (Fr. 2) and 50 % dichloromethane in hexane (Fr. 3).

The analyte was further HPLC fractionated using a porous graphitic carbon column. The Hypercarb-HPLC column was forward eluted using 50 % dichloromethane in hexane (Fr. 2-1 or 3-1) and back flushed using toluene (Fr. 2-2 or 3-2). The Fr. 2-1 and 3-1 were spiked with ¹³C-HCB and isoctane. The effluent from Hypercarb-HPLC column was micro concentrated to 100 μ l and further subjected for additional sub-fractionation step using a pyrenyl silica column. The analyte from the PYE-HPLC column was collected each into four sub-fractions using, respectively, 10 % dichloromethane in *n*-hexane and dichloromethane – Fr. 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. Each sub-fraction was spiked with ¹³C-HCB and isoctane and further micro concentrated to 100 μ l under gentle stream of nitrogen. The sub-fractions were analyzed for tri- to octachloronaphthalene HRGC-HRMS.

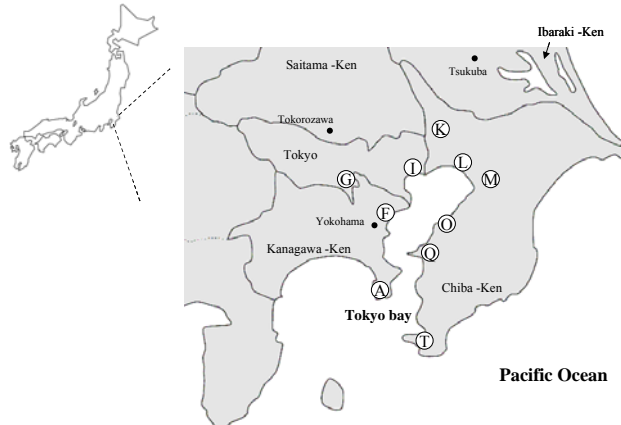


Figure 1. Location of the sampling sites around the Tokyo Bay, Japan.

Quality assurance and quality control (QA/QC) protocols included analysis of matrix spike and matrix spike duplicates, replicate samples and procedural blanks. All samples were spiked with a ^{13}C -labeled PCBs and PCDD/Fs recovery standard prior to extraction step. The detection limits of chloronaphthalene congeners were around 0.2 pg/g wet wt. and depended on the sample size and interferences. The recovery of hexachlorobenzene (syringe spike) was more than 80%.

Results and Discussion

In total 10 samples of pine needles of the same age were collected and analysed. The concentrations and to some degree also compositional pattern (fingerprint) of chloronaphthalene congeners in pine needles around the Tokyo Bay varied spatially. The total PCNs content was from 250 to 2100 pg/g wet wt. (Tab. 1). 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 wt. Mono- and di-CN were not quantified. Except of the site "T", tri- and tetrachloronaphthalene homologue groups were dominating constituents (54 to 80 %) of PCNs quantified in pine needles.

There is only one set of data published on PCN residues in plant biomass worldwide (14). In a study of pine needles collected at the western part of Kentucky and Georgia in USA by Loganathan et al. (14) the total PCNs content of 10 samples ranged from 99 to 19000 pg/g dry wt., so rather broadly reflected of various intensity impacts from the local emission sources.

A differences and similarities in compositional pattern of chloronaphthalenes sequestered by pine needles and depending on the sampling site were further evidenced after Cluster Analysis of the data set. The fingerprint of PCNs was largely similar for the sites O and L, I and G as well as K and A. Additionally, the site Q was similar to the sites I and G. Further, the sites Q, I, G, K and A were more similar to the site M than the site F, while the sites O and L differ highly from the sites F, M, Q, I, G, K and A. Evidently, the site T outstands from the other locations examined.

A Principal Component Analysis (PCA) model with three significant factors could explain

Table 1. Details of sampling and total PCNs concentration in pine needles around the Tokyo Bay

Sampling site (*see Fig. 1)	Pine tree species	Date of collection	Average diameter of the pine tree trunk (cm)	PCNs (pg/g wet wt.)
A	<i>Pinus thunbergii</i> Parl.	March 14	15	630
F	<i>Pinus thunbergii</i> Parl.	March 16	21	710
G	<i>Pinus densiflora</i> Sieb. et Zucc	March 15	14	950
I	<i>Pinus thunbergii</i> Parl.	March 16	13	1800
K	<i>Pinus thunbergii</i> Parl.	March 12	14	1100
L	<i>Pinus thunbergii</i> Parl.	March 12	10	490
M	<i>Pinus thunbergii</i> Parl.	March 13	12	2100
O	<i>Pinus thunbergii</i> Parl.	March 13	12	570
Q	<i>Pinus thunbergii</i> Parl.	March 13	13	690
T	<i>Pinus thunbergii</i> Parl.	March 14	13	250

91 % (65 % + 17% + 9 %) of the variance of the 63 measured CNs data set.

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, which belonged to PC1, were similar for the sites L, O, Q, A, F, G and K but were largely different for the site M and much less when compared to the sites T and I (Fig. 2). The concentrations of the chloronaphthalene congeners nos. 74 and 75 as well as compounds nos. 8, 41, 46 and 48, which belonged to PC2, were similar for the sites L, O, Q, A, F, G, K and T, and differed for the site M and also the site I (Fig 2). The concentrations of chloronaphthalene congeners nos. 54, 56, 63, 70 and 73, which belonged to PC3, were similar for the sites A, L, Q, F, G, K, I and M but differed for the sites T and O (Fig. 3).

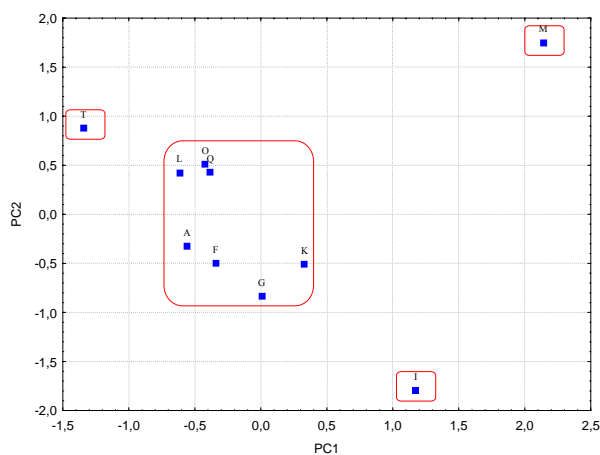


Figure 2. Distribution of the sampling sites in PC1 and PC2 space.

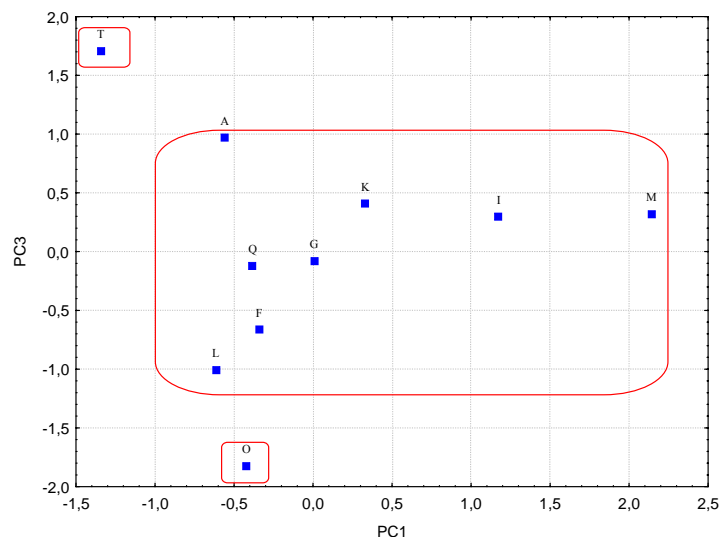


Figure 3. Distribution of the sampling sites in PC1 and PC3 space.

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