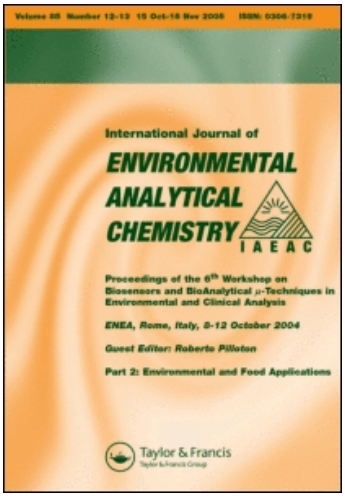

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Extractability of dioxins from soil: II. Effects of acid or alkaline pretreatment on the extractability of dioxin homologues from soil samples

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Extractability of dioxins from soil: II. Effects of acid or alkaline pretreatment on the extractability of dioxin homologues from soil samples

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The effects of sample pretreatment on the extractability of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and coplanar polychlorinated biphenyls (CoPCBs) from three suburban soil samples were evaluated. The samples were treated with 0.1 M HCl or 0.1 M NaOH and extracted by pressurized liquid extraction (PLE) with toluene. In addition, untreated soil samples were subjected to PLE with acetone. The extractability values were compared to values obtained by toluene extraction without pretreatment. Alkaline pretreatment increased the extractability of higher-chlorinated CDDs (HiCDDs), whereas acid pretreatment slightly decreased their extractability. No change in extractability was observed for higher-chlorinated CDFs under any conditions. The extractability of lower-chlorinated CDD/Fs (LoCDD/Fs) and CoPCBs was increased only by acetone extraction. PCDD/F homologue profiles in soil humic acid fractions and those in dead leaves, a major raw material of soil humus, were also determined. These results suggest that the variations in the extractability of dioxin homologues are due mainly to variations in their physical state in the soil, especially their interactions with soil humus.

Keywords: Sample pretreatment; Extractability; Dioxins; Soil; Physical state; Humus

1. Introduction

Analysis of hydrophobic organic pollutants (HOPs) in soil is difficult because the soil matrix is more complicated than other environmental media. Much attention has been focused on the interaction of HOPs with organic matter (OM) in soil. A number of reports have been published on 'unextractable residues' of xenobiotics formed by association with humus [1–4].

We previously reported that dioxins – polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like coplanar polychlorinated biphenyls (CoPCBs) – associated with precipitated humic acid (HA) cannot be easily

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extracted with organic solvents [5]. Because most of the OM in soil consists of humic substances, humus causes various problems in analysis of dioxins in soil (e.g. low reproducibility, variations in extraction efficiency depending on the extraction technique used).

Dioxins are usually extracted from soil by Soxhlet extraction with toluene [6]. In recent years, other extraction techniques such as microwave-assisted solvent extraction [7, 8], pressurized liquid extraction (PLE) [9, 10], and supercritical fluid extraction [11] have been evaluated. These advanced techniques can reduce the troublesome effects of OM on the extraction of dioxins from soil.

Pretreatment of soil samples prior to solvent extraction can increase the extractability of dioxins. Among them, acid treatment and alkaline treatment are representative strategies for removal of matrices in soil interfering with extraction of analytes. For example, acid treatment has been adopted for dioxin analysis in fly ash because acid dissolves the metal salts that reduce the extraction efficiency [12]. Saponification with methanolic alkali to release analytes trapped in humic polymers has been successfully used for extracting aromatic hydrocarbons from soil samples [13, 14]. Since dioxins occur in matrix-bound forms in soil, simple acid or alkaline pretreatment, which can strip the matrices from soil core particles, has the potential to give a higher extraction efficiency for dioxins from soil than with organic-solvent extraction only. However, pretreatment of soil samples for dioxin analysis has received little attention. Therefore, we evaluated the effects of acid or alkaline pretreatment on the extractability of various dioxin homologues from soil.

One purpose of the evaluation is to obtain information for optimization of a dioxins analytical protocol in soil, and the other purpose is to obtain information on the physical state of dioxins in soil. We were particularly interested in differences in the effects of pretreatment on the extractability of the various homologues because we expected that these differences might elucidate the physical states of the dioxins in soil. Dioxins can associate with various soil matrices which have various characters. Since the behaviour of soil matrices toward acid or alkaline pretreatment varies from matrix to matrix, dioxins associated with each of the matrices should give a specific extractability values. To obtain a high extraction efficiency for dioxins from soil, appropriate extraction conditions must be chosen on the basis of the form in which the dioxins occur in soil matrices. In a previous study, we found that dioxins on airborne particulates (APs) and dioxins associated with HA show clear differences in extractability with organic solvents [5]. These results indicate that differences in the extractabilities of dioxin homologues under various extraction conditions are due to their physical state in the soil. In this study, we made assumptions about the physical states of PCDD/F homologues in soil on the basis of their extractability by various extraction protocols.

2. Experimental

2.1 *Apparatus*

A high-volume air sampler (HV-700F, Shibata Science Technology, Tokyo) was used for sampling of the ambient atmosphere. Dioxins were extracted from soil samples by pressurized liquid extraction (PLE) performed with a Dionex ASE-200

(Dionex Corp., Sunnyvale, CA) instrument. The detailed extraction conditions have been described elsewhere [5].

PCDD/Fs and CoPCBs were analysed by high-resolution gas chromatography/mass spectrometry with an HP-6890 Plus (Agilent, Palo Alto, CA) gas chromatograph coupled to a JMS-700D mass spectrometer (JEOL, Tokyo). The analytes were determined with BPX-DXN (SGE, Austin, TX) and RH-12 ms (Inventex, Torrance, CA) capillary columns.

2.2 *Materials*

The QR-100 quartz fibre filter (QFF, 0.3 μm particle retention) and polyurethane foam plugs (PUFs, 90 mm i.d., 50 mm thick) used for collection of particle-bound or gaseous dioxins in the ambient atmosphere were obtained from Shibata.

All dioxin-analytical-grade solvents and adsorbents were purchased from either Wako Pure Chemical Industries (Osaka, Japan) or Kanto Chemicals (Tokyo). Analytical-grade hydrochloric acid and sodium hydroxide were obtained from Kanto Chemicals.

PCDD/F and Co-PCB standards, including ^{13}C-labelled homologues, were purchased from Wellington Laboratories (Ontario, Canada) or Cambridge Isotope Laboratories Inc. (Andover, MA). A surrogate solution (SS) was prepared in acetone. This solution contained 17 ^{13}C-labelled 2,3,7,8-substituted CDD/F congeners (2,3,7,8-TeCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, 2,3,7,8-TeCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF and OCDF) and 12 ^{13}C-labelled CoPCBs (IUPAC Nos 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189), each at a concentration of 1.5 μg L^{-1}, with the exception of the ^{13}C-OCDD/F at 3.0 μg L^{-1}. A recovery standards solution (RSS) in nonane was prepared, containing ^{13}C-labelled 1,2,7,8-TeCDF, 1,2,3,4,7-PeCDD, 1,2,3,4,6,9-HxCDF, 1,2,3,4,6,8,9-HpCDF, 2,3′,4′,5-TeCB (#70), 2,2′,3,4,4′-PeCB (#85), 2,2′,3,4,4′,5′-HxCB (#138) and 2,2′,3,3′,5,5′,6-HpCB (#178), each at a concentration of 10 μg L^{-1}.

2.3 *Soil and dead leaf samples*

Three topsoil samples (S-1 to S-3) were collected in suburban Tokorozawa, Saitama, Japan (table 1). The samples were air-dried, crushed and passed through a 1-mm-mesh sieve. Prior to pretreatment, the sieved samples were dried in a desiccator with silica gel for 2 days. The PCDD/F homologue profiles of the soil samples, together with the profile of atmospheric deposition collected at Tokorozawa [15], are shown in figure 1. The similarity of these four profiles indicates that atmospheric deposition is the main source of PCDD/Fs in the soil samples.

Dead leaf samples (L-1, trident maple; L-2, Japanese cedar; L-3, cherry) were also collected at each soil-sample site. The collected leaves were washed well with distilled water, air-dried and ground with anhydrous Na_2SO_4 in a mortar.

2.4 *Analysis of soil samples*

A flow chart for the complete sample treatment protocol is shown in figure 2. We anticipated that variations in the weight, water content or particle size of the

Table 1. Characterization of the soil samples.

	S-1	S-2	S-3
Sampling site	Roadside ground	Bosk	Park
pH[a]	6.5	6.5	5.9
OC[b] ($g\,kg^{-1}$)	26.2	53.8	45.1
OC extractable with NaOH[c] ($g\,kg^{-1}$)	13.9	23.8	35.7
OC extractable with $Na_2P_4O_7$[c] ($g\,kg^{-1}$)	4.0	6.8	1.7
Unextractable OC ($g\,kg^{-1}$)	8.3	23.2	7.7
Particle size distribution (%)[d]			
125–1000 μm	74	69	78
63–125 μm	13	15	10
38–63 μm	7	10	9
< 38 μm	6	6	3
PCDDs ($pg\,g^{-1}$)[e]	990	2000	1900
PCDFs ($pg\,g^{-1}$)[e]	300	1300	860
CoPCBs ($pg\,g^{-1}$)[e]	4900	1600	440

[a]1 : 4 w/v in 0.01 M $CaCl_2$; [b]Organic carbon; [c]The soil sample was exhaustively extracted with 0.1 M NaOH and then with 0.1 M $Na_2P_4O_7$ according to a previous procedure [16]; [d]Content in sieved samples determined according to a previous procedure [17]; [e]Average quantity obtained from the untreated sample by toluene extraction.

samples might affect the extractability of the analytes. Therefore, after acid or alkaline treatment of a large amount (100 g) of each soil sample, the residues were air-dried, crushed, sieved and dried in a desiccator prior to PLE. Exactly 10 g of each pretreated sample was subjected to PLE. Because the weight of the samples was reduced by the acid or alkaline pretreatment, we measured the weight loss and water content of all the samples in advance and corrected the quantitative values of each pretreated sample accordingly. In addition, we confirmed that the water content in all samples was similar (5–8%).

The following extraction protocols were performed: (a) no pretreatment and toluene extraction (N-TE), (b) acid pretreatment and toluene extraction (Ac-TE), (c) alkaline pretreatment and toluene extraction (Al-TE), and (d) no pretreatment and acetone extraction (N-AE). In the preliminary recovery experiments using ^{13}C-labelled dioxins (by adding SS to the sample before PLE), we confirmed that no degradation of the analytes occurred during PLE with either toluene or acetone.

2.5 *Analysis of dead leaf samples*

Two grams of a well-ground dead leaf sample was subjected to PLE with acetone and continuously with toluene under the conditions described elsewhere [5]. Both extracts were combined, spiked with 0.3 mL of SS, and then subjected to clean-up and analysis as described in figure 2.

2.6 *Estimation of vapour-to-particle ratios for dioxin homologues in the ambient atmosphere*

Ambient air (1000 m^3) was drawn through a QFF and then through two PUFs using a high-volume air sampler. Dioxins were determined on APs (particle-bound phase) as described elsewhere [5]. Dioxins adsorbed by the PUFs (vapour phase) were determined by extraction with acetone and analysis following the method in figure 2.

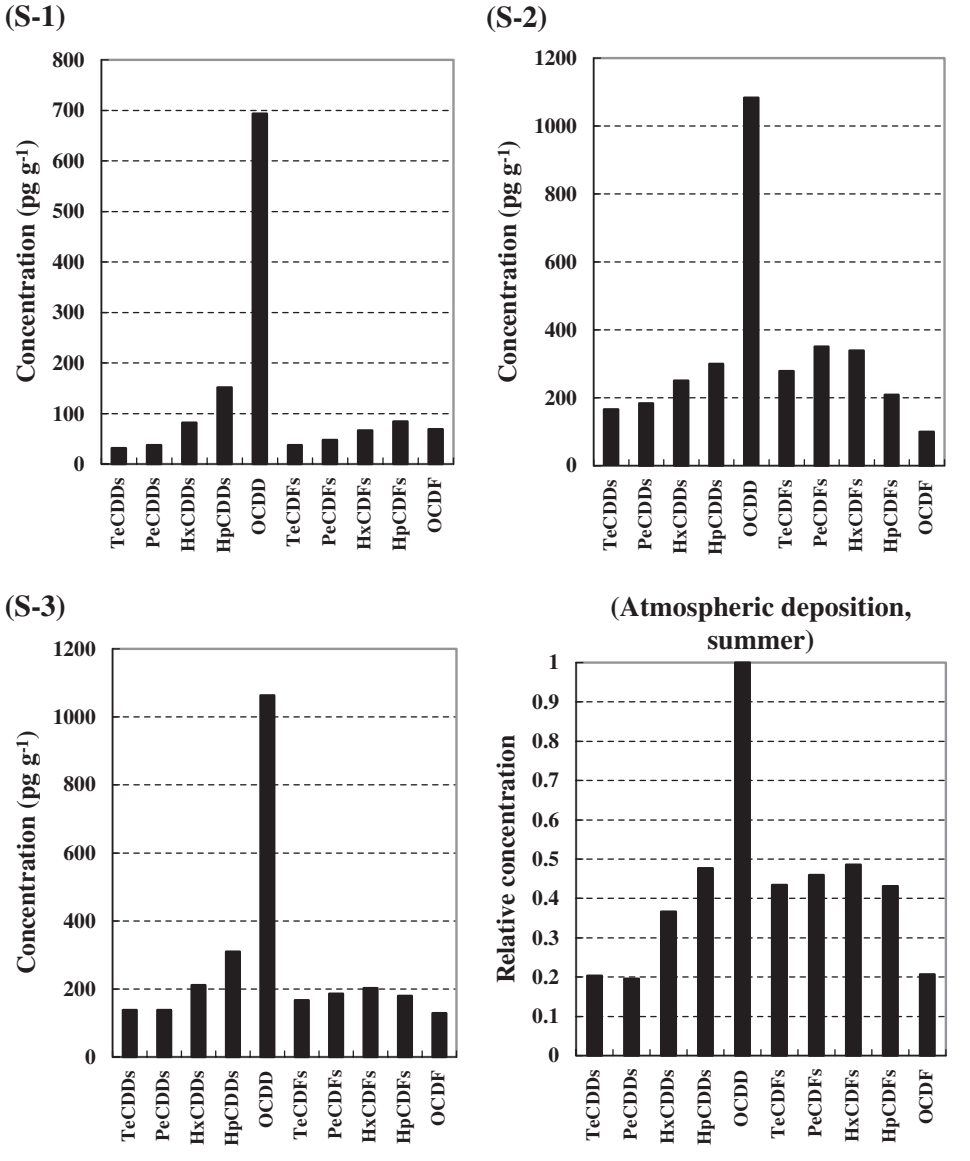

Figure 1. PCDD/F homologue profiles for three soil samples (untreated, extracted with toluene) and the atmospheric deposition profile.

Dioxins on APs and on PUFs were separately determined, and vapour-to-particle ratios were calculated for each PCDD/F homologue.

2.7 *Quantification by HRGC–HRMS*

An aliquot (1 μL) of a resulting solution was injected into a GC equipped with a BPX-DXN column for analysis of PCDD/Fs (60 m × 0.25 mm i.d.) and an RH-12 ms column for analysis of CoPCBs (60 m × 0.25 mm i.d.). The detailed conditions for

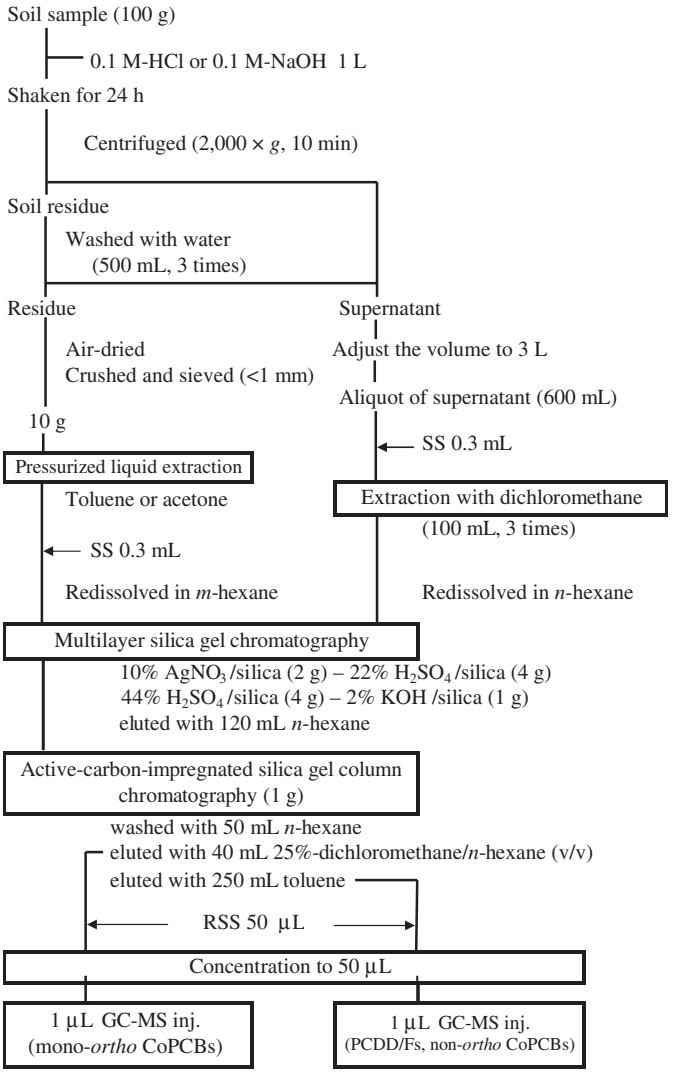

Figure 2. Analytical protocol for determination of PCDD/Fs and CoPCBs in soil samples.

GC–MS analysis and the procedures for quantification and calculation of surrogate recovery have been described elsewhere [5, 18]. The toxicity equivalency (TEQ) values of all 2,3,7,8-chlorinated congeners were calculated using 2,3,7,8-TeCDD toxicity equivalency factors (TEF) reported by the World Health Organization [19].

2.8 *Accuracy control*

Variations of HRGC–HRMS analysis and a blank (an instrumental blank and a method blank) were estimated as previously described [5].

SS was spiked with all samples (including blank tests), and their recoveries were confirmed before data processing. In this study, the recoveries from all the solid residue

samples were sufficient (78–110%), except for ^{13}C-OCDD/F (60–75%). The relatively insufficient recoveries of these result from their strong adsorption to active-carbon-impregnated silica gel.

The recoveries of ^{13}C-HpCDD/Fs and ^{13}C-OCDD/F from NaOH-supernatant of the soil sample (i.e. HA fraction) were not sufficient (52–70%) on account of their strong affinity with humic acid. Anyhow, the recoveries of all the samples were acceptable for accurate determination.

3. Results and discussion

3.1 *Effects of acid or alkaline pretreatment on extractability of dioxin homologues from soil*

We were interested in whether acid or alkaline treatment could affect the extractability of dioxins, and whether the magnitude of the effect would vary among PCDD/F homologues. The pretreatment resulted in several specific alterations to the extractability of dioxins (figure 3). A prominent feature common to all the soil samples was that the extracted quantities of higher-chlorinated DDs (HiCDDs, i.e. hepta- and octa-CDDs) were higher with Al-TE and N-AE than with N-TE. Moreover, the extracted quantities for Ac-TE were somewhat lower than those for N-TE. In addition, with respect to S-1, only N-AE gave higher extracted quantities for lower-chlorinated DD/DFs (LoCDD/Fs, i.e. TeCDD/Fs and PeCDD/Fs) and CoPCBs. It is interesting that the extractability varied for the HiCDDs but not for the higher-chlorinated DFs (HiCDFs), which have similar physicochemical properties. The results suggest that HiCDDs and HiCDFs occur in different forms in the soil.

3.2 *Distribution of dioxins in soil affecting extractabilities of specific homologues*

Information on the physical state of dioxin homologues in soil is essential for their accurate determination (i.e. optimization of an extraction protocol) in soil. Unfortunately, it is difficult (probably impossible) to isolate each soil component (humic substances, APs and mineral component) and determine dioxins in them without decomposition of the analytes. However, the differences in the extractability of each homologue will help account for their physical states in soil.

In a previous study, we found that dioxins on APs are equivalently extracted with both toluene and acetone, and that only acetone can satisfactorily recover the ^{13}C-labelled compounds from precipitated HA fraction [5]. On the basis of the data, we made assumptions about the cause of the differences in extractabilities of dioxin homologues.

First, to evaluate the detailed extractability of dioxins, we determined the quantity of each homologue obtained by a two-step extraction (at 100°C and then at 200°C) of S-1 in the four protocols. Here, we are concerned with only PCDD/Fs, and not with CoPCBs, because little difference in extractability was observed among all CoPCB congeners, and the extractability of CoPCBs was similar to that of LoCDD/Fs. Figure 4 shows the quantitative results of TeCDD/Fs and HiCDD/Fs. With respect to HiCDDs, Al-TE gave a slight increase in the extracted amount at 100°C and

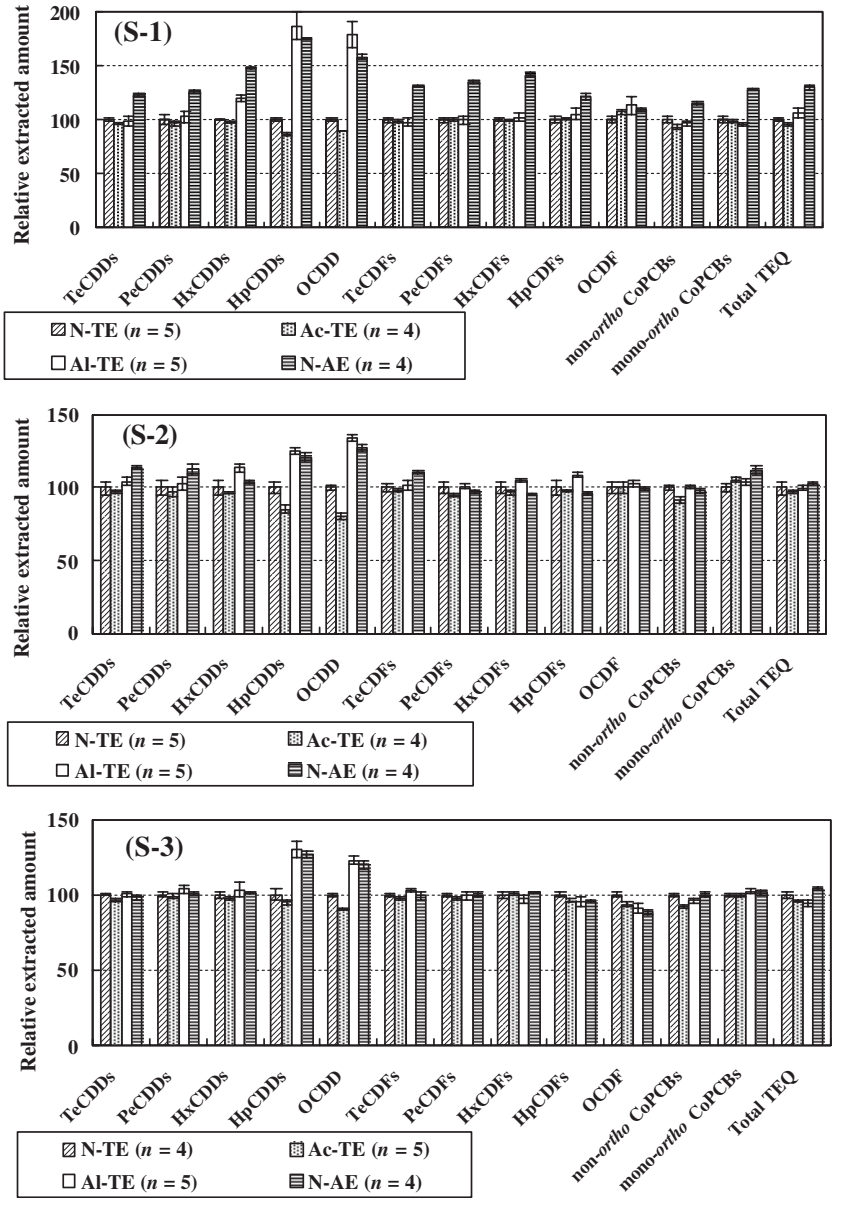

Figure 3. Comparison of extraction efficiencies of four treatment protocols, with the N-TE value set at 100%. The confidence interval at the top of each bar indicates the mean coefficient of variation for the summed quantitative values of both soil solid and filtrate.

a marked increase in the amount at 200°C. In addition, with respect to TeCDD/Fs and HiCDDs, the extracted amount at 100°C with acetone was 1.5–2-fold higher than that by other toluene extraction protocols. In contrast, most of the HiCDFs were extracted at 100°C by any protocol.

A small amount of HiCDFs was detected in the second extraction fraction at 200°C, indicating that their extraction from the sample was almost completed. Thus, HiCDFs

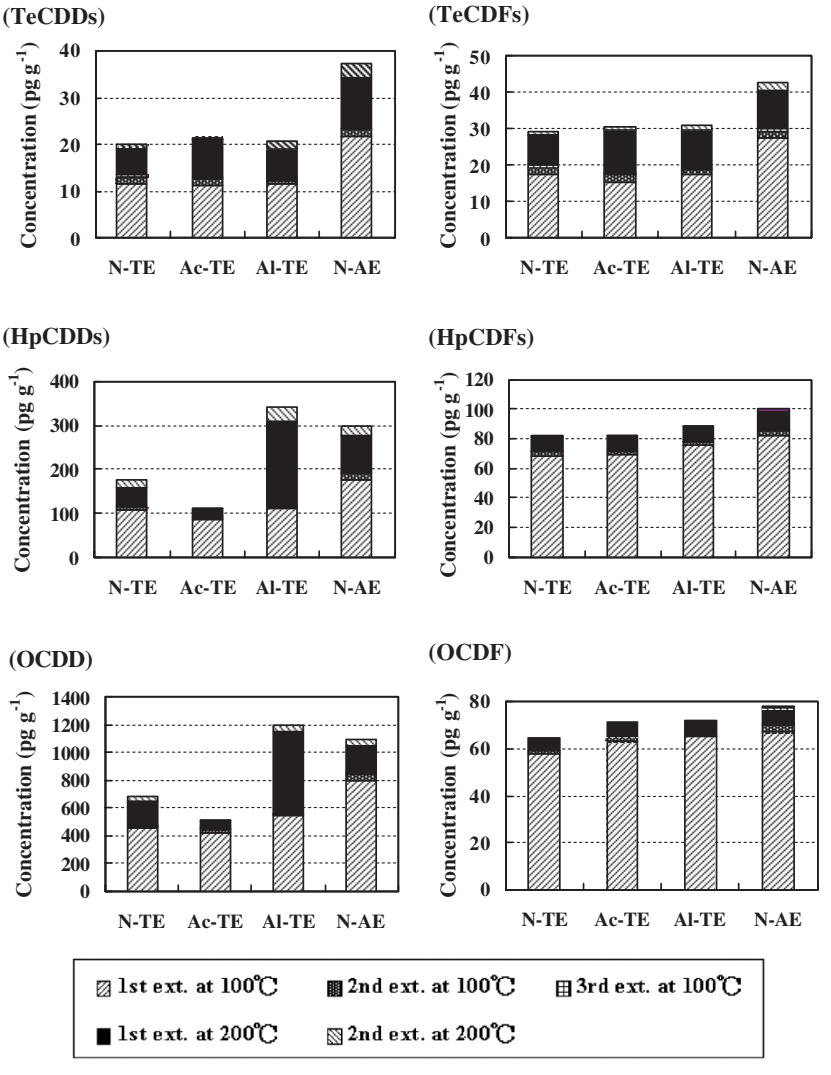

Figure 4. TeCDD/F, HpCDD/F, and OCDD/F quantities obtained by extraction of S-1 three times at 100°C and twice at 200°C.

probably occur in an easily extractable form. We have concluded that native dioxins on APs can be easily extracted [5]. Therefore, it seems reasonable to suppose that most part of HiCDFs would occur on the APs detached from interfering matrices (probably humus), i.e. on the freely deposited APs in soil.

On the other hand, it is likely that the marked increase in extracted amount of HiCDDs by Al-TE was attributed to removal of NaOH-extractable humic substances from the soil particles. That is, even the mild alkaline treatment (shaking at room temperature) which dissolves some of the humus markedly increased the accessibility of still-sequestered dioxins to toluene under the astringent extraction conditions (extraction at 200°C).

On the basis of the data in figure 4, we hypothesized that there are particles occluded in soil humus and that the particles have a higher HiCDDs to HiCDFs ratio compared to that in APs. Moreover, it is clear that the physical state, which is attributed to the variation of extractability, of LoCDD/Fs is different from that of HiCDDs, because no change in extractability was observed for LoCDD/Fs by alkaline treatment. We have found that acetone can extract humic-bound dioxins more effectively than toluene [5]. On the basis of the finding, we set up a second hypothesis that the LoCDD/Fs fraction, which is attributed to the increase in the extracted amount of these by acetone extraction, would occur in a humic-bound form.

To evaluate these hypotheses, we must consider the pathways of soil contamination with dioxins. With respect to general (sub)urban soils, we consider atmospheric deposition to be the main source of dioxin contamination. One of the other possible ways of soil pollution is through plant organs, a raw material of soil humus. That is, humification of dioxin-containing plant components (leaves, branches or seeds) will produce humus that contains dioxins [20]. In that case, information on dioxin profiles in the plant components will be a clue to elucidate their physical state in soil. Therefore, we determined dioxin profiles in dead leaves, which are raw materials of soil humus (figure 5).

Sugiyama *et al.* reported a predominance of LoCDD/Fs over HiCDD/Fs in dead leaves [20]. As shown in figure 4, all the dead leaves collected at the three soil sample sites also exhibited characteristic homologue profiles, apparently different from those of the APs, in which lower-chlorinated homologues were abundant. Figure 6 shows the gas–particle partition profiles for PCDD/F homologues in the ambient atmosphere collected in the city of Tokorozawa. The greater volatility of LoCDD/Fs as shown in figure 6 provides a plausible explanation for this observation. That is, one can safely state that lower-chlorinated homologues are abundant in leaves because these homologues are incorporated in gaseous form.

Another noticeable feature common to all the leaves is that HiCDDs were more abundant than HiCDFs. The OCDD/OCDF ratios in the dead leaves (11.2–30.6) were clearly higher than the ratios for APs (1.7–2.3) [5] and atmospheric deposition (4.8, see figure 1). Although not clear, the higher OCDD/OCDF ratios in the dead leaves would be caused by a lower stability of OCDF compared to OCDD [21, 22]. Since nearly all the HiCDDs exist in the particle-bound phase in the atmosphere (figure 6), the dominant HiCDDs in the leaves have not been incorporated in gaseous form, unlike LoCDD/Fs. Leaves are known to deposit minute APs in their stomata by gas exchange [23]. Accordingly, the dominant HiCDDs in the leaves will occur on the particles deposited in stomata.

The data in figure 5 support the hypotheses stated before. When the leaves containing appreciable quantities of LoCDD/Fs are converted to humus, the lower-chlorinated homologues, which have been incorporated in gaseous form, would be strongly sorbed on the humus. Although loss of LoCDD/Fs by vaporization and biodegradation during humification is not negligible [20, 24, 25], the LoCDD/Fs content (ratio to HiCDD/Fs) in humus originating from the leaves should still be higher that that in APs. On the other hand, the minute particles which have been adsorbed in stomata on the leaves can be occluded within the humus by humification, and therefore are more affected by humus than freely deposited APs. On the occluded particles, HiCDFs, which are relatively unstable compared to HiCDDs, would be subject to degradation during humification.

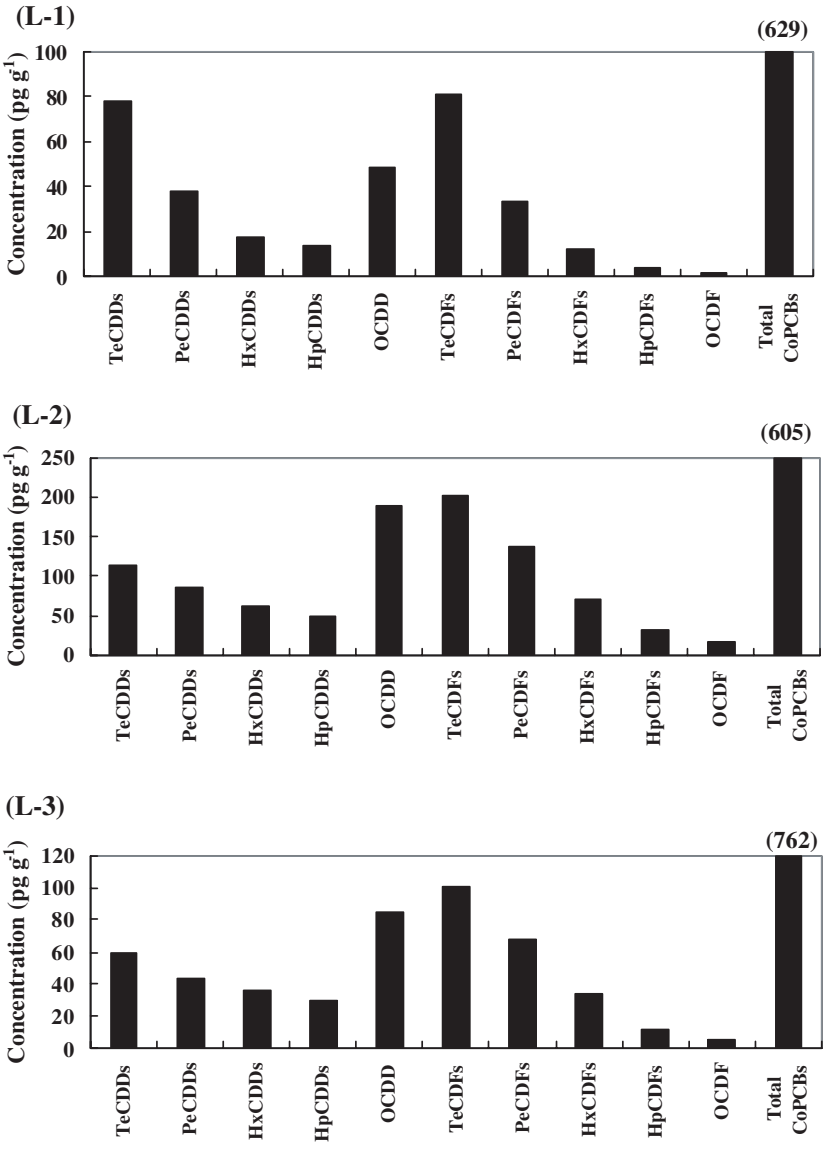

Figure 5. Dioxin concentration in three dead leaf samples.

In figure 4, acetone extraction gave a higher extracted amount of HiCDDs at 100°C than toluene extraction and gave a total amount equivalent to that by Al-TE. These higher extractabilities would be due to the high permeability of acetone into polar solid humic substances and thus the high accessibility to the sequestered particles. In contrast, the extracted amount of HiCDDs for Ac-TE was somewhat lower than that for N-TE (figures 3 and 4). Jones and Tiller have proposed that humic macromolecules adopt a more compact conformation upon acidification [26]. It may be that the decrease was the result of the inaccessibility of the particles sequestered in the compact HA conformation to toluene. Although somewhat unclear because

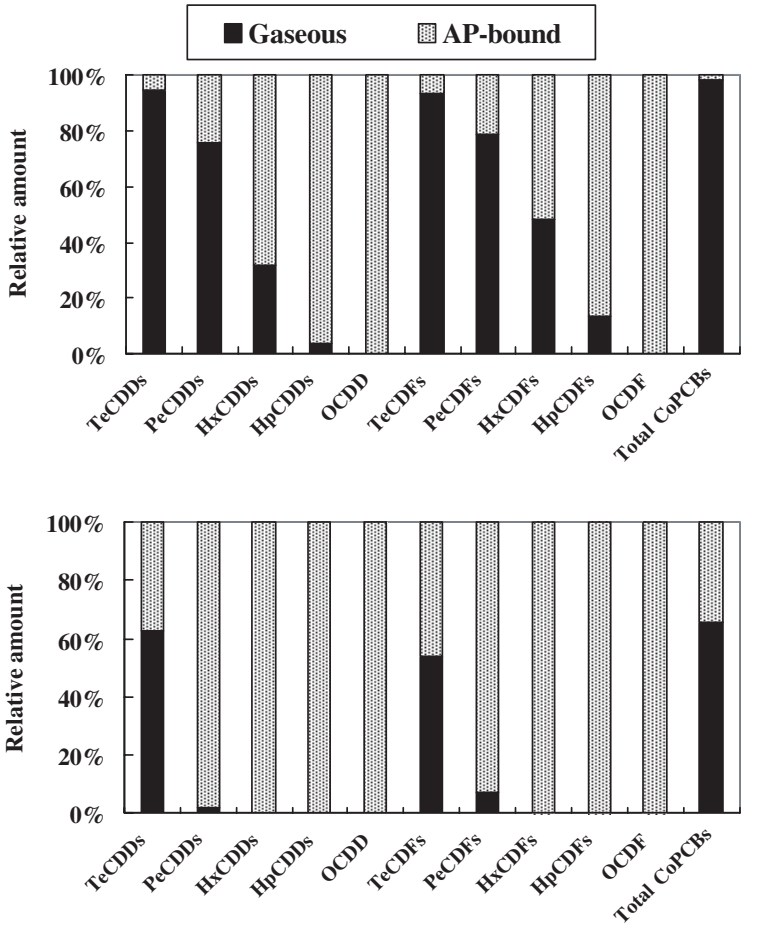

Figure 6. Gas–particle partition profiles of PCDD/F homologues in the ambient atmosphere collected in summer (top) and winter (bottom).

we cannot separate freely deposited APs and humus-bound APs from soil, these results suggest that HiCDDs-rich particles occluded in humus are attributed to the variation of extractability of HiCDDs.

Next, in order to evaluate humic-bound LoCDD/Fs, we determined dioxins in the NaOH-extractable HA fraction of the soils. Table 2 shows the PCDD/Fs homologue profiles (expressed as each homologue to octachloro-congener ratios) in the three soil samples and the supernatant obtained by NaOH treatment of them (for comparison, no analyte was detected in the supernatant obtained by HCl treatment). In the table, since the exact quantity of dioxins in soil cannot be estimated, the quantity obtained by N-AE, which gave the highest extraction efficiency, was regarded as the total amount in the soil samples.

We expected the HA fraction to exhibit a PCDD/F homologue profile in which LoCDD/Fs are dominant, as well as that in the dead leaves, whereas, as shown in table 2, the apparent dominance of LoCDD/Fs was not observed for all samples. The LoCDD/Fs to OCDD/F ratios in the NaOH-extractable HA fraction were

Table 2. Homologue profiles of PCDD/Fs in whole soil and humic acid fraction.

	Whole soil sample		Humic acid fraction	
	Concentration ($pg\,g^{-1}$)[a]	Ratio to octacongener[b]	Amount (pg)[c]	Ratio of octacongener[b]
(S-1)				
TeCDDs	39	0.035	14	0.041
PeCDDs	47	0.043	18	0.053
HxCDDs	120	0.11	47	0.14
HpCDDs	270	0.25	67	0.20
OCDD	1100	1	340	1
TeCDFs	49	0.65	21	0.60
PeCDFs	64	0.84	28	0.80
HxCDFs	94	1.2	39	1.1
HpCDFs	100	1.3	43	1.2
OCDF	76	1	35	1
(S-2)				
TeCDFs	190	0.14	140	0.25
PeCDFs	210	0.15	130	0.23
HxCDDs	260	0.19	160	0.29
HpCDDs	360	0.26	140	0.25
OCDD	1400	1	560	1
TeCDFs	310	3.1	250	4.3
PeCDFs	340	3.4	320	5.5
HxCDFs	320	3.2	300	5.2
HpCDFs	200	2.0	150	2.6
OCDF	100	1	58	1
(S-3)				
TeCDDs	140	0.11	140	0.25
PeCDDs	140	0.11	130	0.23
HxCDDs	210	0.16	180	0.32
HpCDDs	390	0.30	200	0.35
OCDD	1300	1	570	1
TeCDFs	170	1.5	200	2.5
PeCDFs	190	1.7	220	2.8
HxCDFs	210	1.9	200	2.5
HpCDFs	170	1.5	140	1.8
OCDF	110	1	80	1

[a]Average quantity obtained from the untreated sample by acetone extraction; [b]Calculated for both PCDDs and PCDFs; [c]Average quantity in the NaOH filtrate of a 10 g soil sample ($n = 3$).

almost equivalent to those in whole soil for S-1, whereas the ratios of the HA fraction were somewhat higher than those of whole soil for S-2 and S-3, from which small increases in extractability of LoCDD/Fs were observed, even with acetone. The lower LoCDD/Fs to OCDD/F ratios in the NaOH-extractable HA fraction may be due to their loss during humification or higher transferability of HiCDD/Fs than LoCDD/Fs from APs to humic substances, as reported by Osako *et al.* [27].

Decreases in soil organic matter, determined on the basis of loss on 600°C ignition, by the mild alkaline treatment of S-1, S-2 and S-3 were 20%, 12% and 20%, respectively. Since only a part of humic substance was dissolved in the NaOH supernatant, the results in table 2 are not a great aid to clarity.

It is likely that the low-chlorinated homologues associate with various humic fractions (e.g. NaOH-extractable HA, NaOH-unextractable HA, or humin), and that the extractabilities of the homologues by solvent extraction vary depending on the property of the associated fraction. For example, we found in a previous report that

the recoveries of dioxins from metal-free HA precipitate are apparently higher than those from metal-bound HA precipitate [5], suggesting that dioxins associated with 'free HA' (i.e. HA not in association with mineral particles) are easily extracted. As shown in table 1, S-3 has more NaOH-extractable OC (79% of total OC) than S-1 and S-2 (53% and 44%, respectively). With respect to S-3, most part of humus-bound LoCDD/Fs may occur in the 'free HA' fraction and may be sufficiently extracted with toluene. On the other hand, it may be that, for S-1, a certain amount of LoCDD/Fs occurs in the NaOH-unextractable humic fractions (i.e. humic substances in strong association with mineral particles) and cannot be extracted with toluene. The higher LoCDD/Fs and CoPCBs extractabilities obtained only with N-AE for S-1 would be, as well as HiCDDs, due to the high permeability of acetone into the mineral-associated humic substances in the soil. In any case, we have only limited information on the extractability of LoCDD/Fs from humic fractions. There is room for further investigation on the distribution of these in humus.

In conclusion, the differences in extractabilities of specific homologues yielded information about the distribution of dioxins in the soil; these are demonstrated in figure 7 and summarized as follows. Most of the PCDD/Fs and CoPCBs, and almost all of the HiCDFs, probably occur on the freely deposited APs detached from humus in soil. They can be extracted with toluene in satisfactory quantities regardless of whether or not the samples were pretreated.

A fraction of LoCDD/Fs and CoPCBs in soil would be strongly sorbed on the humus. Their extractabilities would vary depending on the property of the associated humic fraction.

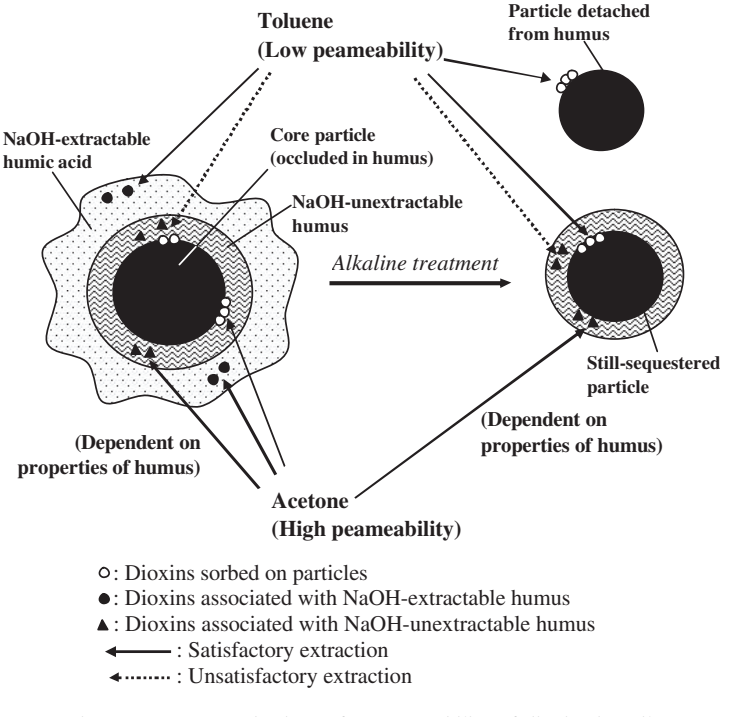

Figure 7. Proposed scheme for extractability of dioxins in soil.

There would be some particles occluded in humus, and the particles would be abundant in HiCDDs. The HiCDDs can be extracted with toluene when the surface humus is removed by alkaline treatment. In addition, acetone can extract these, regardless of whether or not the samples are pretreated, due to its high permeability in humus.

It is suggested that quantitative results of dioxins in soil are liable to vary with extraction protocol. In the analysis of humus-rich soil, alkaline pretreatment and acetone extraction were shown to be effective for satisfactory extraction of dioxins. In particular, the total toxicity equivalency value of S-1 obtained by N-AE was apparently higher than that obtained by N-TE, which resulted from a higher extraction efficiency of 2,3,7,8-LoCDD/F congeners. However, it should be noted that some PCDF homologues are subject to decomposition by acetone extraction at high temperatures [28].

In this study, we established the hypothesis that the variation in extractability of dioxin homologues is attributed to the difference in their physical states in soil, and tested the hypothesis on the basis of the PCDD/F homologue profiles in the dead leaves, soil humic acid fraction, and ambient air. Under the present circumstances, we cannot satisfactorily verify the hypothesis because we cannot fractionate each component (freely deposited APs, APs occluded within humus and humus fraction) and determine dioxins in them separately. However, on the basis of the obtained data, we can be fairly certain that the extractability of LoCDD/Fs, CoPCBs and HiCDDs, which depended on the pretreatment protocol and the extracting solvent, is influenced by soil humus.

Alkaline treatment will certainly diminish the effect of HA by dissolving it. However, even alkaline treatment cannot diminish the effect of humin, which is unextractable humus. The soil samples used in this study contained some humin, a humus fraction that is not extractable with either NaOH or $Na_2P_4O_7$ (17–43% of the total OC in our three soil samples). It has been reported that humin is extremely stable because it strongly binds with inorganic components, and that extracting analytes sequestered in it may be impossible without decomposition of the analytes [29]. Xie *et al*. found that the lipid fraction in humin is the dominant sink for hydrophobic xenobiotics [30]. If gaseous LoCDD/Fs incorporated into plants are associated with cuticular waxes, they may diffuse into humin lipids during humification and become unextractable.

Researchers have proposed various explanations for PCDD/F mass imbalances and homologue-profile differences between source-emission estimates and environmental deposition rates. The formation of 'unextractable dioxin residues' may be another explanation.

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