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原著論文

Uptake of polychlorinated dibenzo-*p*-dioxins and furans in plant leaves

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Although possible uptake pathways of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in plant leaves have been previously described, more details are remained to be solved. In this paper, we investigated the time course of concentrations of PCDDs and PCDFs in leaves and the pre-sprouting short branches including winter buds, stems and the epidermis of two kinds of deciduous trees.

We found that the concentrations of PCDDs and PCDFs in the young buds just after germination were high, then they decreased during the period of rapid growth of leaves, and finally increased again remarkably before the leaves fell. The concentration levels and the congener patterns of PCDDs and PCDFs were similar between the short branch epidermis and the young buds, while the congener patterns were quite different between the epidermis and winter buds.

The results suggest that PCDDs and PCDFs in the young buds originate from the short branch epidermis, where these chemical substances are accumulated by being transferred from the leaves before their falling. The later in concentrations of PCDDs and PCDFs in leaves before falling seems to be due to their exposure to these chemicals for an extended period of time until their fall.

〔Key Words: Dioxins, Plant leaves, Uptake pathways 〕

1 INTRODUCTION

It is important to know the concentration levels of polychlorinated-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in plants or vegetation[1),2)], since they take a role as the intial link of the food chain. Vegetative PCDD and PCDF levels can also serve as useful indicators of local air pollution[3),4)]. The accumulation pathways of persistent organic chemicals such as PCDDs and PCDFs in plant leaves have been demonstrated by many researchers[5)-7)]. Three main pathways for the uptake of these chemicals have been described: 1) root uptake and translocation to the shoot or leaf, 2) adsorption or deposition by soil particles on leaf surfaces, and 3) deposition of vapor and/or particulate phase chemicals on leaf surfaces. It is thought that the third is the dominant pathway3[3),8),9)]. However, the details of the accumulation mechanism are still unknown. We have reported the uptake mechanism of PCDDs and PCDFs using radish plant[10)] and cabbage[11)] which can be destructed any part of tissues, for example, root, stalk and leaves. In the study, whole parts of radish were analyzed separately.

To clarify the origins of PCDDs and PCDFs in tree leaves, it is necessary to investigate the distribution of PCDDs and PCDFs in the whole trees, including root, sap-conductive tissues, barks, twigs, cork, leaves and fruit, although this is quite difficult. For deciduous trees, however, the growth process of a leaf can be traced from the time of its sprouting. Hence, PCDDs and PCDFs concentration

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changes in leaves are observable in detail, including concentrations of the short branch, winter bud, outer skin and stem.

In this study, we selected two kinds of deciduous tree, *Ginkgo biloba* and *Acer*, and studied the time course of concentrations in the leaf parts.

2 MATERIALS AND METHOD

2・1 Sampling

The leaves of two kinds of trees, *Ginkgo biloba* and *Acer*, grown in the backyard of Fukuoka Institute of Health and Environmental Sciences, were collected from the beginning of April to the beginning of November, 1990. The first sampling was performed just after the germination of each tree. The leaves were then sampled around the 7th day of every month. Furthermore, the short branch was sampled from the same trees before budding in April the following year.

2・2 Apparatus

The plant leaf samples were homogenized using POLYTRON(KINEMATICA Switzerland) homogenizer. Concentrations of PCDDs and PCDFs were measured using high resolution mass spectrometer (HRMS, Finnigan MAT-90 Germany) equipped with a capillary gas chromatograph (HRGC, HP 6890 USA). The analytical columns used were an SP-2331 capillary column (0.32 mm i.d. × 30 m, 0.25 μm film thickness, Supelco USA) and an OV-17 capillary column (0.25 mm i.d. × 30 m, 0.25 μm film thickness, SGE International, Australia).

2・3 Procedure

To remove adhered particulate matter on leaves, all leaf samples were washed with tap water and then air-dried at room temperature. To estimate the growth of plant leaves, 20-50 leaves were sampled independently and their weight was measured on both a wet and dry basis.

The samples (20-40g) were cut into small pieces and homogenized with acetone using POLYTRON. The sample was filtered off, and washed with acetone. The extracts were transferred to a separatory funnel, and distilled water was added. The sample was further extracted with n-hexane, then dehydrated with anhydrous sodium sulfate, evaporated to dryness under reduced pressure, and dissolved in n-hexane and treated with concentrated sulfuric acid. PCDDs and PCDFs in the n-hexane solution were purified using silica gel and an activated carbon chromatography clean-up procedure[9]. The resulting sample was concentrated to 30 μl and analyzed by HRGC/HRMS. All samples were fortified with $^{13}C_{12}$- or $^{13}C_6$- labeled 2,3,7,8-chlorine substituted CDDs and CDFs as an internal standard before sulfuric acid treatment.

2・4 GC/MS analysis

The HRMS was operated in the electron impact mode at 7000 to 10000 resolution. For the analysis of tetra to hexa CDDs and CDFs, a sp-2331 fused silica capillary column was used. Column temperature was held at 120 ℃ for one min., and raised to 200 ℃ at the rate of 10 ℃/min., and subsequently, to 260 ℃ at the rate of 20 ℃/min. For the analysis of hepta to octa CDDs and CDFs, column temperature was held at 130 ℃ for one min., and raised to 280 ℃ at the rate of 15 ℃/min., then held for 15 min. The concentrations of PCDDs and PCDFs in plant samples were determined by isotope dilution method.

3 RESULTS AND DISCUSSION

3・1 Growth rate of leaf of *Ginkgo biloba* and *Acer*

The time courses of leaf weights of *Ginkgo biloba* and *Acer* are shown in Figure 1. On the 11th day after germination (young buds), the leaf weight was 0.07g per sheet on a wet basis for both *Ginkgo biloba* and *Acer*. The leaf weight of *Ginkgo biloba* grew quickly till the 55th day, then almost fixed on 216th day after germination. The leaf weight of *Acer* grew quickly till the 87th day, then almost fixed on 152nd day; after that, the weight decreased to 0.57g on the 216th.

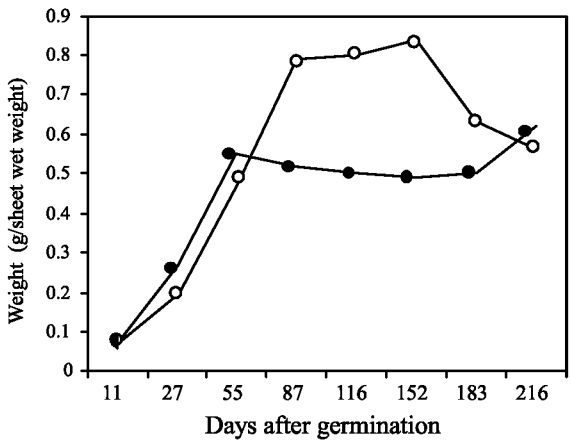

Fig. 1 Time courses of weight changes of leaves of *Ginkgo biloba* and *Acer*

● :*Ginko biloba* ○ :*Acer*

3・2 Time course of concentration of PCDDs and PCDFs in plant leaves

The time course of total concentration of PCDDs and PCDFs in the leaves of *Ginkgo biloba* and *Acer* are shown in Figures 2 and 3, respectively. On the 11th day after sprouting, the respective PCDDs and PCDFs concentrations were 370 and 220 pg/g dry in the leaves of *Ginkgo biloba* and 180 and 190 pg/g in the leaves of *Acer*. As mentioned above, the concentrations of PCDDs and PCDFs in leaves of both plants are high in the period immediately after sprouting.

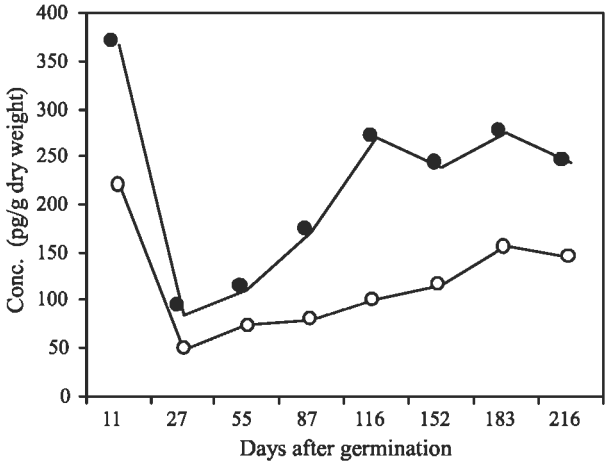

Fig. 2 Time courses of concentration of PCDDs and PCDFs in leaves of *Ginkgo biloba*.

● :Total PCDDs ○ :Total PCDFs.

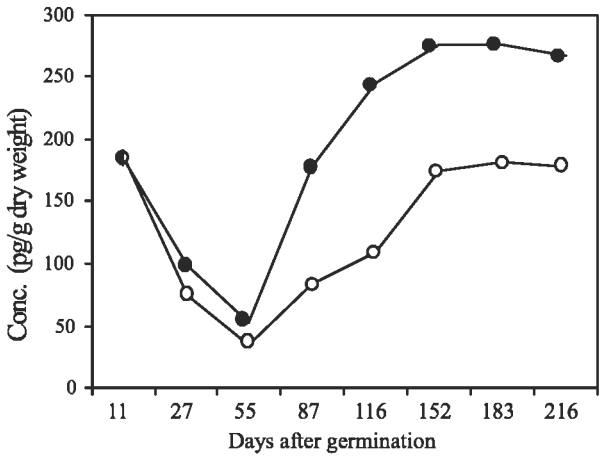

Fig. 3 Time courses of concentration of PCDDs and PCDFs in leaves of *Acer*.

● :Total PCDDs ○ :Total PCDFs

The concentrations of PCDDs and PCDFs in leaves decreased with the growth of leaves of both species until the 27th day for *Ginkgo biloba* and the 55th day for *Acer*. This was considered to be due to the dilution effect accompanying rapid growth of a leaf.

After the minimum concentrations were observed, the concentration rose gradually until the 183rd, and fell slightly on the 216th in both species. This rise can be explained by the leaves' absorption and accumulation of these chemicals from the atmosphere. In our previous study, we estimated the deposition rate of these chemicals in plant leaves to be from 0.1 to 0.5 sec/cm. The high concentration levels in plant leaves before the time of fallen leaves can be explained by the length of their exposure.

3・3 Congener patterns of PCDDs and PCDFs in the leaves of *Ginkco biloba* and *Acer*

The congener patterns of PCDDs and PCDFs in the leaves of *Ginkgo biloba* and *Acer* are shown in Figures 4,5,6 and 7 as 3-dimensional graphs. As can be seen, the congener patterns during the observation period were similar.

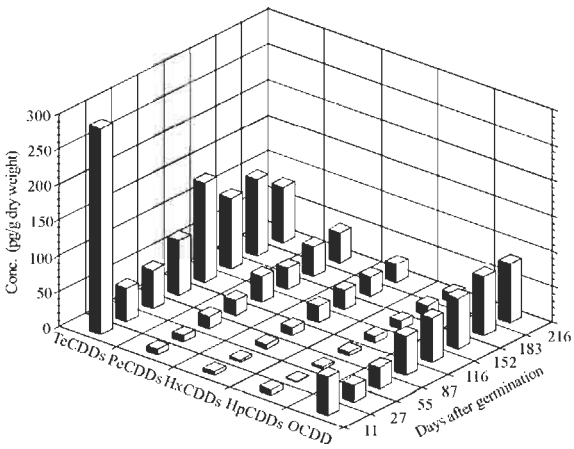

Fig. 4 Congener patterns of PCDDs in leaves of *Ginkgo biloba*

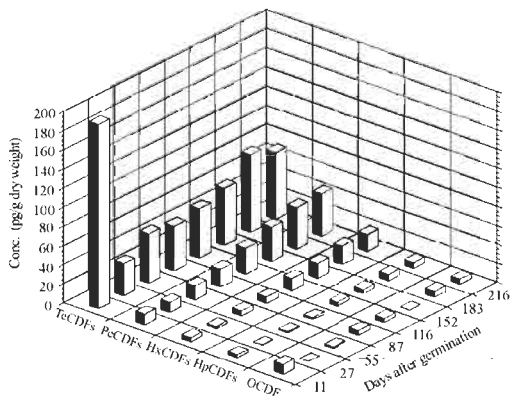

Fig. 5 Congener patterns of PCDFs in leaves of *Ginkgo biloba*

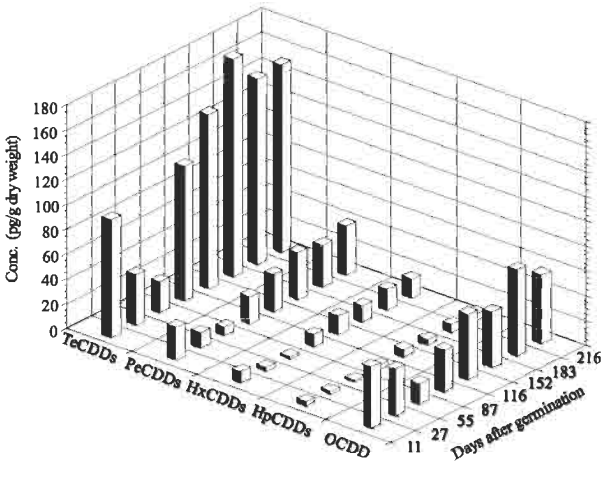

Fig. 6 Congener patterns of PCDDs in leaves of *Acer*

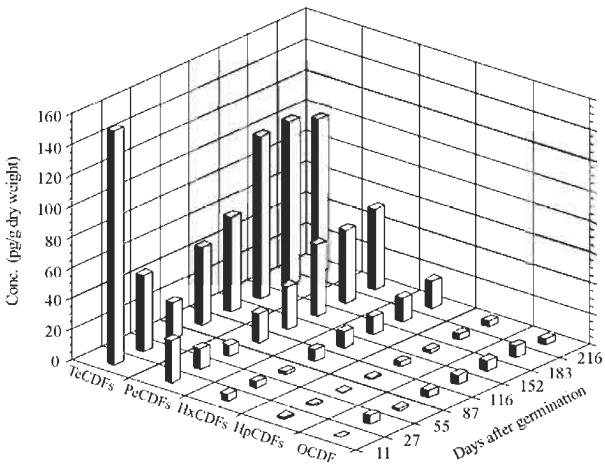

Fig. 7 Congener patterns of PCDFs in leaves of *Acer*

3・4 Concentration of PCDDs and PCDFs in segments of short branch

The short branch of *Ginkgo biloba* and *Acer* were divided into winter bud, epidermis and stem, and respective concentrations of PCDDs and PCDFs were determined. The congener patterns are shown in Figures 8, 9, 10 and 11, respectively. The concentrations of PCDDs and PCDFs in the winter buds and stems were 30 pg/g or less in both species. In contrast, the concentrations in the epidermis were 5 to 10 times higher. The concentration levels of the epidermis were very similar those of young buds, which were collected on 11th days after germination, as illustrated in Figure 4, 5, 6 and 7. On the other hand, the congener pattern of the epidermis was quite different from that of winter buds. For that matter, the congener pattern of winter buds and young buds were markedly different.

From these results, we concluded that PCDDs and PCDFs

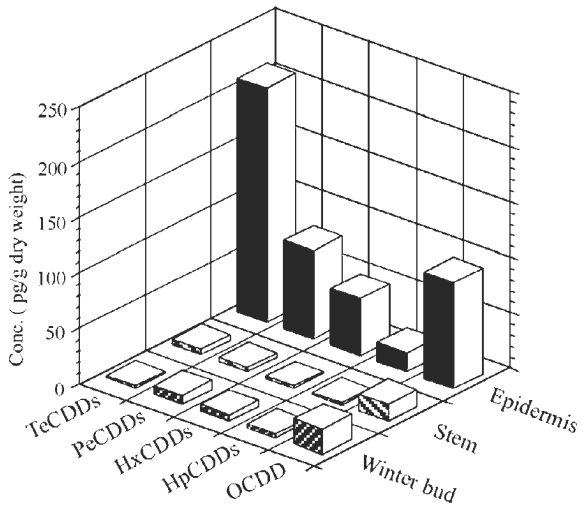

Fig. 8 Congener patterns of PCDDs in winter bud, stem and epidermis of *Ginkgo biloba*

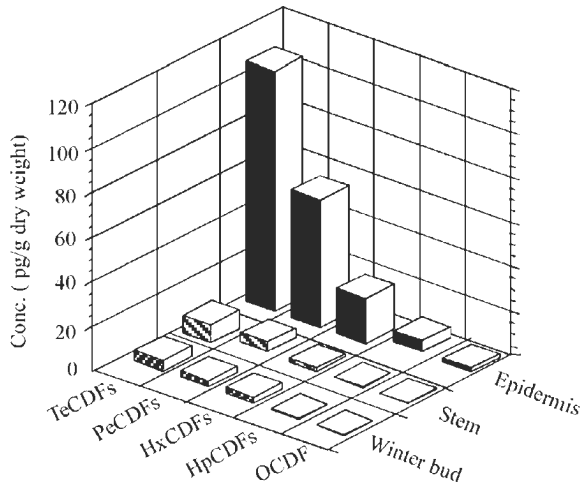

Fig. 9 Congener patterns of PCDFs in winter bud, stem and epidermis of *Ginkgo biloba*

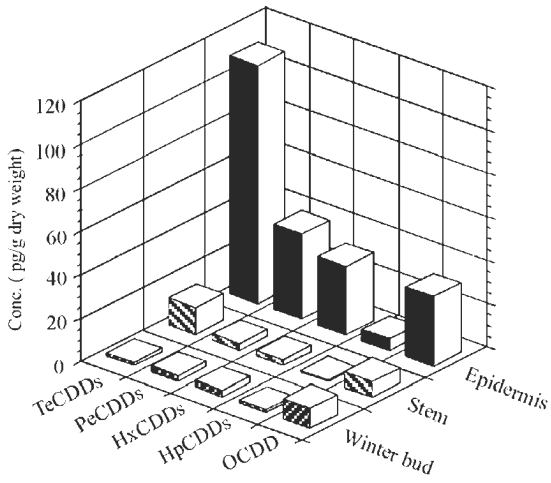

Fig. 10 Congener patterns of PCDDs in winter bud, stem and epidermis of *Acer*

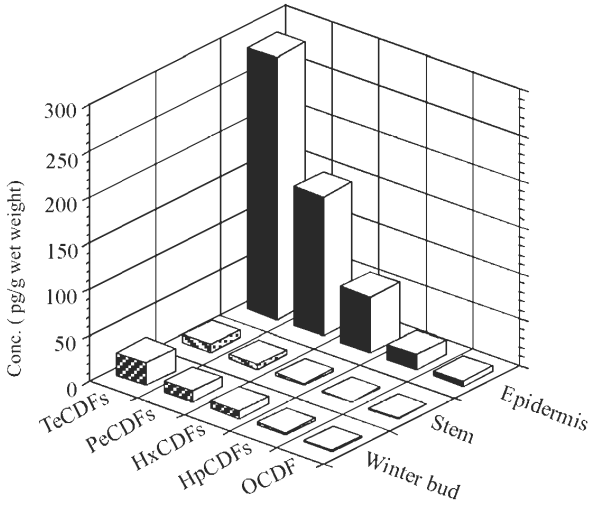

Fig. 11 Congener patterns of PCDFs in winter bud, stem and epidermis of *Acer*

present in young buds are supplied from the epidermis. If the origin of PCDDs and PCDFs detected in young buds were atmospheric deposition, the concentration would be low or hardly detectable because of the short exposure period. A total PCDDs and PCDFs concentration of 180 or more pg/g dry basis was detected in the young buds immediately after a sprouting. This suggests strongly that the PCDDs and PCDFs in young buds originate in the trees themselves, the PCDDs and PCDFs accumulated in the epidermis being translocated to sprouting buds. We assume that the PCDDs and PCDFs in the epidermis of a short branch originate in leaves before they fall.

In a previous report[13)-15)], it was supposed that translocation of PCDDs and PCDFs within plant tissues was unlikely due to high octanol/water partition coefficients (log Kow>6). In this study, our results indicate translocation of such chemicals between the short branches and young buds, and between the leaves and the short branches. The details of the mechanism of translocation are still unknown; For a possible explanation, Kew et.al[6] suggested that the sap-conductive tissues of the short branch controlling translocation within the plant.

Recent studies showed that for a wide range of plants including trees the concentrations of PCDDs and PCDFs in soil have no influence on the concentration in aerial parts of plant[16)-17)]. There are notable exceptions, however, such as plants of the genus *Cucurbita*, which are capable of taking up PCDDs and PCDFs from the soil and efficiently translocating it to the leaves and fruits. In experiments on radish plants and cabbage, we have shown that root uptake of PCDDs and PCDFs by these plants was negligible[10)-11)].

In this study, we assume that the pathways of PCDDs and PCDFs accumulation in *Ginkgo biloba* and *Acer* is a combination of atmospheric deposition on leaves and translocation to short branches.

4 CONCLUSIONS

To elucidate the pathway of accumulation of PCDDs and PCDFs in tree leaves, we followed the time course of PCDDs and PCDFs levels in two kinds of deciduous tree, namely, *Ginkgo biloba* and *Acer*. Relatively high levels of PCDDs and PCDFs were detected in young buds just after germination. From days 27th to 55th of sprouting, the PCDDs and PCDFs levels decreased, but rose again thereafter. This time course of PCDDs and PCDFs levels seems to reflect the translocation of dioxins from the epidermis in the short branches to the buds, dilution due to rapid growth of leaves, and deposition of PCDDs and PCDFs originating from the air. Since young buds have been exposed to the air for only a short period of time, most of the PCDDs and PCDFs detected in young buds are not considered to have originated from the air directly. To evaluate the validity of this view, we divided the pre-sprouting short branches into winter buds, stems and the epidermis, and analyzed the concentrations and congener patterns of PCDDs and PCDFs in each segment. This revealed that PCDDs and PCDFs detected in young buds originated from the epidermis. It has been thought that PCDDs and PCDFs do not accumulate through the roots from soil. Therefore, we considered that the PCDDs/PCDFs found in the epidermis were translocated to it from leaves before they fell.

REFERENCES

1) E.Bacci, D.Calamari, C. Gaggi and M.Vighi: Environ. Sci. Technol., 24, 885 (1990).
2) L-O. Kjeller, K.C.Jonse, A.E.Johnston and C. Rappe: Environ. Sci. Technol., 25, 1619 (1991).
3) A.Reischl, M.Reissinger, H.Thoma and O.Hutzinger: Chemosphere, 18, 561 (1989).
4) T.Tojo, N.Seike, M.Matsuda M.Kawamoto and T.Wakimoto: Journal of Environ. Chem., 10, 13 (2000) (in Japanese).

5) K.W.Pausch, M.S.Mclachlan and G.Umlauf: Environ. Sci. Technol., 29, 1090 (1995).
6) G.A.Kew, J.L.Schaum, P.White and T.T.Evans: Chemosphere, 18, 1313 (1989).
7) S.Safe, K.W.Brown, K.C.Dooelly, C.S.Anderson, K.V. Marklewcz, M.S.McLachlan, A.Reischl and O.Hutzinger: Environ. Sci. Technol., 26, 394(1992).
8) T.Matsueda, H.Hirakawa, T.Iida, Y.Kurokawa and R. Suda: The summary of 32nd annual meeting of the Japan Society for Atomospheric Pollution, 476 (1991) (in Japanese).
9) T.Matsueda: thesis, (in Japanese) (1997).
10) M.Nakamura, T.Matsueda, Y.Kurokawa, S.Takada, and K.Fukamachi:Organohalogen Compounds, 20, 103 (1994).
11) M.Nakamura, R.Suda, T.Matsueda, Y.Kurokawa, S.Takada, and K.Fukamachi: Organohalogen Compounds, 24, 497 (1995).
12) L.Lamparski and T.J.Nestrick: Anal. Chem.,52, 2045 (1980).
13) A.Reischl, M.Reissinger, H.Thoma and O.Hutzinger: Chemosphere, 19, 467(1989).
14) G.G Briggs, R.H. Bromilow, A.A. Evans: Pestic Sci, 13, 495 (1982).
15) E. Bacci, C.Gaggi: Environcontam. Toxicol., 35, 673 (1985).
16) A.Hülster,H.Marschner:Chemosphere, 27, 439(1993).
17) J.F.Müller, A.Hülster, O. Päpke, M.Ball,and H.Marschner:Chemosphere, 27, 195 (1993).

ダイオキシン関連物質の樹木の葉への濃縮

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〔キーワード: ダイオキシン類, 植物, 濃縮 〕

樹木の葉への PCDDs と PCDFs の蓄積のメカニズムを明らかにするため，2 種類の落葉樹（イチョウとアメリカフウ）について,PCDDs と PCDFs 濃度の経時変化を調べた．発芽直後の新芽に比較的高濃度の PCDDs 及び PCDFs が検出された．イチョウは出芽後 27 日まで,アメリカフウは 55 日まで濃度の減少が見られたが，その後,濃度の上昇が観察された.この濃度変化は短枝の外皮から芽へのダイオキシンの移動とその後の葉の急成長による希釈及び大気からの沈着によるものと考えられた.

新芽の大気への暴露期間が短いため，新芽中のダイオキシンは大気由来とは考えられない．この見解の妥当性を評価するために,短枝部位の冬芽，茎及び表皮に分割して，各々の部分の PCDDs 及び PCDFs の濃度と同属体パターンを解析した．その結果，新芽に含まれる PCDDs 及び PCDFs の由来は表皮からの移行と推定された．土壌中の PCDDs 及び PCDFs は根を経由して葉に濃縮されないと考えられていることから，表皮に含まれる PCDDs 及び PCDFs は葉に蓄積されたものが落葉前に移行したものと考えられた.