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# Behavior of Decabromodiphenyl Ether (BDE-209) in the Soil–Plant System: Uptake, Translocation, and Metabolism in Plants and Dissipation in Soil

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Deca-bromodiphenyl ether (BDE-209) is the major component of the commercial deca-BDE flame retardant. There is increasing concern over BDE-209 due to its increasing occurrence in the environment and in humans. In this study the behavior of BDE-209 in the soil–plant system was investigated. Accumulation of BDE-209 was observed in the roots and shoots of all the six plant species examined, namely ryegrass, alfalfa, pumpkin, summer squash, maize, and radish. Root uptake of BDE-209 was positively correlated with root lipid content ( $P < 0.001$ ,  $R^2 = 0.81$ ). The translocation factor (TF,  $C_{\text{shoot}}/C_{\text{root}}$ ) of BDE-209 was inversely related to its concentration in roots. Nineteen lower brominated (di- to nona-) PBDEs were detected in the soil and plant samples and five hydroxylated congeners were detected in the plant samples, indicating debromination and hydroxylation of BDE-209 in the soil–plant system. Evidence of a relatively higher proportion of penta- through di-BDE congeners in plant tissues than in the soil indicates that there is further debromination of PBDEs within plants or low brominated PBDEs are more readily taken up by plants. A significant negative correlation between the residual BDE-209 concentration in soil and the soil microbial biomass measured as the total phospholipid fatty acids (PLFAs) ( $P < 0.05$ ,  $R^2 = 0.74$ ) suggests that microbial metabolism and degradation contribute to BDE-209 dissipation in soil. These results provide important information about the behavior of BDE-209 in the soil–plant system.

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## Introduction

Polybrominated diphenyl ethers (PBDEs) are used around the world as flame retardants. Due to their high production, lipophilicity, and persistence, PBDEs have become ubiquitous contaminants in the environment and have been detected in various environmental media including air, water, and soil and sediment samples, together with samples of human and animal tissues (1). The production of PBDEs principally consists of penta-, octa-, and deca-BDE mixtures (2), and deca-BDE accounts for over 80% of the total PBDE production (3). There is increasing regulation and phasing-out of production and commercial use of penta- and octa-BDE technical mixtures due to their toxicity, bioaccumulation, and persistence. Production and use of deca-BDE, made up mostly of BDE-209, have increased in recent years (4), although this product has been banned in parts of the United States and within the European Union (5, 6). There is therefore a particular need to investigate its behavior in the environment (1).

Soils represent a major sink for organic contaminants in the terrestrial environment. In a survey of European soils (7), a total concentration of PBDEs ranging between 65 and 12 000 ng kg<sup>-1</sup> dry weight was observed in UK and Norwegian soils. Application of sewage sludges as fertilizers has been reported to increase concentrations of PBDEs in soil, with the highest increases for BDE-209 (8). Heavy discharges of PBDEs occur on sites used for the disposal or recycling of electronic wastes including computer equipment, televisions, printers, fax machines, and telephones. Research has shown that the soils in e-waste sites in Guiyu in China are polluted by PBDEs with total concentrations of 2720–4250 ng g<sup>-1</sup> dry weight in the surface soils in acid leaching sites, and BDE-209 is the dominant congener, ranging from 35 to 82% of the total PBDEs present (9).

Plant uptake of penta-BDEs from soil has been examined in a previous study (10). All the tested congeners, i.e. BDE-47, -99, and -100, were found in plant root and shoot tissues. Unfortunately this study did not include BDE-209. Although it might be presumed that the bioavailability of BDE-209 in soil will be low because of its high molecular weight (959 amu) and hydrophobicity ( $\log K_{ow} = 10$ ) (11), there is a lack of evidence to support such speculation. It therefore remains unclear whether BDE-209 can be taken up by and translocated within plants.

Environmental degradation of PBDEs is an important concern. PBDEs have a tendency to break down into lower brominated congeners in the environment as well as within the bodies of biota (12–14). OH-PBDEs have also been identified in samples of surface waters (15), sewage effluents (16), marine organisms (17, 18), and human blood (19). The lower brominated isomers or metabolites generated by debromination or hydroxylation may be a source of environmentally abundant PBDEs and bring additional adverse influences to bear on the environment and human health due to their different biological effects compared with their precursor PBDEs (15). However, it has not yet been demonstrated whether there is debromination or metabolism of BDE-209 in soils or plants when plant uptake occurs.

Here we report a greenhouse pot experiment conducted to explore the fate of BDE-209 in the soil–plant system. The uptake and translocation of BDE-209 by six different species of plants from BDE-209 spiked soil and factors influencing BDE-209 transportation within the plants were examined. Debrominated and OH-metabolized products of BDE-209 in plants and soil were identified. The total phospholipid

fatty acids (PLFAs) in soil were analyzed with the aim of examining the influence of soil microbial activity on BDE-209 dissipation in soil.

## Materials and Methods

**Chemicals.** Standards of BDE-209, BDE-77, PCB-30, and PCB-209 and standard solutions of OH-PBDEs (di- through penta-) were obtained from Sigma-Aldrich (Sigma-Aldrich, Inc., St. Louis, MO) and a standard solution of PBDE containing 27 native congeners was purchased from Wellington Laboratories, Inc., Guelph, Ontario, Canada. Details of the PBDE and OH-PBDE congeners are supplied in Table S1 of the Supporting Information. All solvents used, i.e., *n*-hexane, dichloromethane, toluene, chloroform, and methanol, were of HPLC grade. Distilled water was used in all of the experiments. Anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), silica gel, and alumina (100–200 mesh) used for sample cleanup were washed with hexane and heated overnight at 150 °C.

**Soil Properties and Preparation.** A loamy soil without detectable PBDEs was used in this experiment. Its selected characteristics are as follows: pH ( $\text{H}_2\text{O}$ ), 7.32; organic matter, 3.11%; cation exchange capacity, 25.0  $\text{cmol kg}^{-1}$ ;  $\text{NaHCO}_3$ -extractable P, 4.5  $\text{mg kg}^{-1}$ ; clay 23%, silt 35%, and sand 32%. The soil was air-dried, ground, passed through a 2-mm nylon sieve, and then received mineral nutrients at rates of 100  $\text{mg P (KH}_2\text{PO}_4)$ , 300  $\text{mg N (NH}_4\text{NO}_3)$ , and 200  $\text{mg K (K}_2\text{SO}_4)$   $\text{kg}^{-1}$  soil as basal fertilizers. Then an aliquot of soil (1 kg, approximately 10% of the final amount) was spiked with a solution of BDE-209 dissolved in 100 mL of a mixture of toluene and acetone (1:10 = V:V), mixed thoroughly, and placed under a fume hood for solvent evaporation for 12 h. Spiked soil was then continuously tumbled with nonspiked soil for 2 h at room temperature to ensure efficient mixing and to bring the soil to a final concentration of 5000  $\text{ng g}^{-1}$ , which was close to the higher environmental levels of BDE-209 determined in soil (9). The spiked soil was then allowed to dry in a fume hood in the dark until the toluene and acetone had volatilized completely, shaken for 30 min every day, homogenized, and incubated in the dark for 4 weeks at room temperature. Concentrations of BDE-209 and the lower brominated PBDEs in the soil were measured after incubation. The BDE-209 concentration in soil after incubation was  $4960.1 \pm 310.0 \text{ ng g}^{-1}$ . BDE-207, -206, and -208 were detected at concentrations of  $23.5 \pm 2.2$ ,  $38.2 \pm 3.0$ , and  $19.6 \pm 1.5 \text{ ng g}^{-1}$ , respectively, all less than 0.8% of BDE-209 concentration. No other PBDEs or OH-PBDEs were detected in the soil.

**Pot Experiment.** Italian ryegrass (*Lolium multiflorum* L.), alfalfa (*Medicago sativa* L. cv. Chaoren), pumpkin (*Cucurbita pepo* ssp. *Pepo* cv. Lvjinli), summer squash (*Cucurbita pepo* ssp. *Pepo* cv. Cuiyu-2), maize (*Zea mays* L. cv. Nongda 108), and radish (*Raphanus sativus* L. cv. Dahongpao) were used as the test plants. Seeds were purchased from the Chinese Academy of Agricultural Sciences, Beijing, China. They were sterilized in 10% (w/w)  $\text{H}_2\text{O}_2$  solution for 15 min, followed by thorough washing with distilled water, soaked in a 3 mM solution of  $\text{Ca(NO}_3)_2$  for 6 h in the dark, and subsequently germinated on moist filter paper in the dark.

Each pot received 600 g of spiked soil. The upper 0.5–1.0 cm of each pot was covered with nonspiked soil (65 g) to establish a buffer layer in an effort to minimize the evaporation and photolysis of BDE-209 in soil. Polyethylene bags were placed inside the pots to prevent contamination and water drainage. Ten pregerminated seeds were sown in each pot and 3 days after emergence the seedlings were thinned to 8 for ryegrass, 5 for radish and alfalfa, 2 for maize, and 1 for pumpkin and summer squash, with the aim of obtaining an approximately equivalent amount of plant biomass per pot. Nonspiked soil with plant growth and spiked soil without plant growth were set up as the BDE-209 free blank and plant-free control, respectively. Four replicate pots of each treatment were

prepared. Pots were kept in a controlled environment growth chamber for 60 d at a light intensity of  $250 \mu\text{mol m}^{-2} \text{ s}^{-1}$  provided by supplementary illumination with a photoperiod of 14 h each day, at a 25/20 °C day/night temperature regime, and a relative humidity of 70%. The pots were positioned randomly and rerandomized every two days. Distilled water was added as required to maintain moisture content at 60–70% of water holding capacity by regular weighing.

**Sample Preparation.** Plant shoots aboveground and roots belowground were harvested separately after growth for 60 d. Root samples were first carefully washed with tap water to remove any adhering soil particles. Then the root and shoot samples were thoroughly rinsed with distilled water and blotted with tissue paper. The root and shoot materials were frozen at  $-50 \text{ }^\circ\text{C}$  overnight, freeze-dried for 48 h in a lyophilizer (FD-1, Beijing Boyikang Instrument Ltd.), and weighed to determine their dry weights. Determination of root lipid content followed the same principles as employed previously in our laboratory (20). Plant biomass and lipid contents are provided in Table S2 in the Supporting Information. The dried root and shoot samples were then ground separately and stored in glass containers at  $-20 \text{ }^\circ\text{C}$  before chemical analysis. The BDE-209 free soil in the upper layer was removed and then the bulk soil was collected from each pot. The soil samples were ground and sieved ( $<0.25 \text{ mm}$ ) and then stored at  $-20 \text{ }^\circ\text{C}$  prior to determination of PBDEs and soil microbial biomass.

**Chemical Extraction and Analysis.** In brief, soil and plant samples were submitted to Soxhlet extraction with a mixture of acetone and hexane (1:1) for PBDE analysis (21). PCB-30 and PCB-209 were added as surrogate standards to the samples prior to extraction and BDE-77 was added to the final solutions as an internal standard. Sample analysis was performed with an Agilent 6890II gas chromatograph with microelectron-capture detection (GC- $\mu\text{ECD}$ ). A 15-m DB-5 HT column (0.25 mm i.d.  $\times$  0.10  $\mu\text{m}$ ) was used for the determination of BDE-209 and a HP-5MS column (30 m  $\times$  0.32 mm  $\times$  0.25  $\mu\text{m}$ ) was used for the lower brominated PBDEs. For the determination of OH-PBDEs, samples were extracted with cyclohexane and methyl *tert*-butyl ether (1:1) ultrasonically (22) and subjected to analysis with an Agilent 1200 HPLC system equipped with a diode-array detector (DAD). Details of sample extraction and analysis are provided in the Supporting Information.

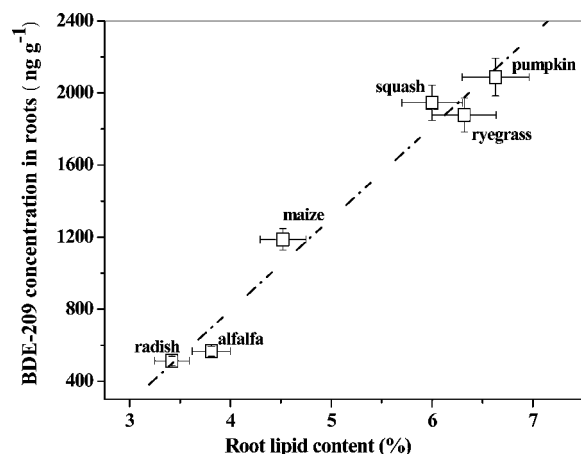
**Quality Assurance and Quality Control.** Quality control was done by regular analyses of procedural blanks, blind duplicate samples, and random injection of solvent blanks and standards. The limit of detection (LOD) of the method, a signal of 3 times the noise level, ranged from 6 to 2000  $\text{pg g}^{-1}$  for all the PBDE congeners ( $n = 6$ ). To determine potential degradation of BDE-209, anhydrous sodium sulfate (heated at 550 °C for 6 h) used as the laboratory blank was spiked with BDE-209 and processed by the same procedure of extraction, cleanup, and analysis. Only nona-BDEs (BDE-206 and -207) were detected at less than 0.02% of BDE-209 concentration. Recoveries of the surrogates of PCB-30 and PCB-209 were  $76 \pm 10\%$  and  $90 \pm 13\%$ , respectively. Recoveries of the 28 PBDE congeners ranged from 71 to 111% with relative standard deviations  $<10\%$  in three spiked laboratory blanks (PBDE congeners spiked into anhydrous sodium sulfate) and from 63 to 126% with relative standard deviations  $<15\%$  in three matrix spiked samples (PBDE congeners spiked into three blank soil samples). Details of method LODs and matrix spike recoveries for individual PBDE congeners and OH-PBDEs are provided in Table S1.

**PLFA Analysis.** Phospholipid fatty acid (PLFA) analysis is a valuable method for tracking soil microbial profiles and determination of soil microbial biomass, which play a key role in the metabolism and degradation of organic contaminants in soil. In this study the freeze-dried fresh soils

**TABLE 1. Concentration of BDE-209 in Plant Roots and Shoots, and Soils after Plant Cultivation on Dry Weight Basis (ng g<sup>-1</sup>)<sup>a</sup>**

plant species	roots	shoots	soil
radish	513.2 ± 25.7	320.4 ± 19.2 (7.6 ± 0.3)	3073.0 ± 106.1 (1.3 ± 0.1)
alfalfa	566.5 ± 28.3	490.1 ± 29.4 (5.2 ± 0.2)	4107.1 ± 228.6 (1.4 ± 0.2)
squash	1946.3 ± 97.3	225.7 ± 13.5 (9.9 ± 0.2)	3402.1 ± 180.6 (1.2 ± 0.2)
pumpkin	2088.1 ± 104.4	245.8 ± 14.7 (10.4 ± 0.2)	4393.0 ± 317.2 (1.1 ± 0.2)
maize	1187.6 ± 59.4	268.9 ± 16.1 (8.9 ± 0.2)	3612.5 ± 145.5 (1.3 ± 0.2)
ryegrass	1878.2 ± 93.9	177.9 ± 10.6 (6.4 ± 0.3)	3962.6 ± 205.7 (1.1 ± 0.2)

<sup>a</sup> Data in brackets refer to the results for non-spiked blank soil. No PBDE was detected in roots from non-spiked blank soil.



**FIGURE 1. Correlation between concentration of BDE-209 in plant roots and root lipid content. Means and standard errors ( $n = 4$ ) on dry weight of biomass are presented.**

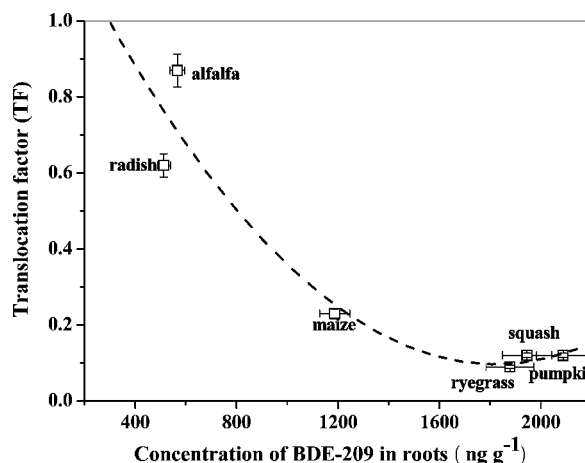
were extracted for PLFA analysis according to the method of Frostegård et al. (23). Fatty acids were analyzed on an Agilent 7890 GC-MS (5975 inert) and identified after the method of ref 24. Details of extraction and analysis of PLFAs are described in the Supporting Information.

**Data Analysis.** All of the data represent the mean and standard deviation of four replicates. The data were subjected to statistical analysis by using the SPSS version 12.0 software package. Two-way analysis of variance (ANOVA) was used to examine the significance of BDE-209 accumulation in plants and dissipation in soil as affected by plant species.

## Results and Discussion

**Uptake and Translocation of BDE-209 in Plants.** Concentrations of BDE-209 in plants and soils after plant cultivation are listed in Table 1. BDE-209 was detected in relatively high concentrations in both roots and shoots of all the six test plant species, indicating that plants have the ability to take up and then translocate and accumulate BDE-209. There were distinct differences among the plant species in the BDE-209 concentrations in their roots and shoots ( $P < 0.001$ ). Consistent with previous reports for other hydrophobic organic compounds (20, 25), concentrations of BDE-209 in roots showed a significant positive correlation with root lipid contents (Figure 1;  $P < 0.001$ ,  $R^2 = 0.81$ ), confirming the important role of plant lipids in root uptake of BDE-209 from soil.

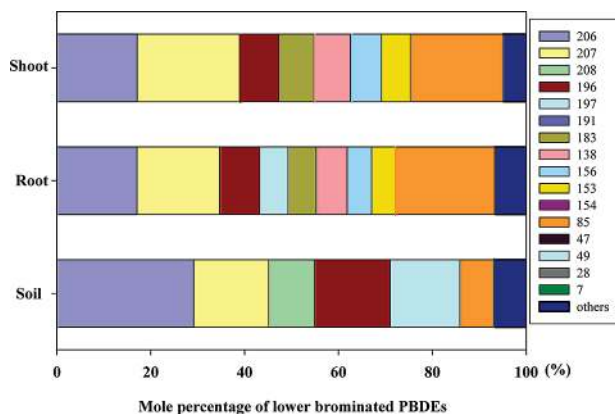
Accumulation of BDE-209 in shoots may result from a combination of uptake through the soil-to-plant pathway and foliar uptake from the air. Shoot concentrations of BDE-209 in plants growing in nonspiked soils were in the range of 5.2–10.4 ng g<sup>-1</sup> (Table 1), accounting for less than 5% of the concentrations in the plants growing in BDE-209 spiked soils. This implies that there was no appreciable contribution from foliar uptake to shoot accumulation of BDE-209 in this experiment. This observation may perhaps be ascribed to



**FIGURE 2. Relationship between the translocation factor (TF) and the concentration of BDE-209 in roots on dry weight of biomass.**

the low volatility of BDE-209 or reduced volatilization due to the boundary layer soil at the pot surface. BDE-209 was detected at a concentration of 4700.6 ± 245.1 ng g<sup>-1</sup> in the unplanted soil after the experiment and not much lower than the initial concentration, which also supports the above hypothesis of limited volatilization of BDE-209 from the soil. Therefore, shoot accumulation may reflect the characteristics of root-to-shoot translocation of BDE-209. Translocation factors ( $C_{\text{shoot}}/C_{\text{root}}$ ) for BDE-209 were calculated and plotted against BDE-209 concentrations in roots (Figure 2) and an inverse relationship was obtained. Similar inverse relationship exists between BDE-209 translocation factor and root lipid content (Figure S1), which suggests the role of root lipids in restricting translocation of BDE-209 from roots to shoots due to its high capacity to partition into root lipids. Furthermore, accumulation of BDE-209 in roots might be affected not only by root uptake from the soil but also root-to-shoot translocation. Greater translocation of BDE-209 into shoots would lead to its lower accumulation in roots.

**Metabolism of BDE-209 in the Soil–Plant System.** A total of nineteen additional lower brominated PBDE congeners (di- to nona-) were detected in the soil and plant tissues after plant harvest, confirming metabolic debromination of BDE-209 in the soil–plant system. The detailed concentrations of all the lower brominated PBDEs in soil and plant samples are provided in Tables S3–S5. The mole percentage profiles for the congeners in soil and plants for each plant species are displayed in Figure 3 for ryegrass as an example and for all the plants in Figure S2. The nona-BDE (e.g., BDE-206, -207) generally represents a high percentage of the total debrominated products, which suggests that BDE-209 is more likely to lose one bromine atom to form nona-BDE. The proportion of penta- through di-BDE congeners in plants (7.8–21.1%) was higher than in the soil samples (6.5–12.2%), suggesting that there might be further debromination of PBDEs inside plants or low brominated congeners are more

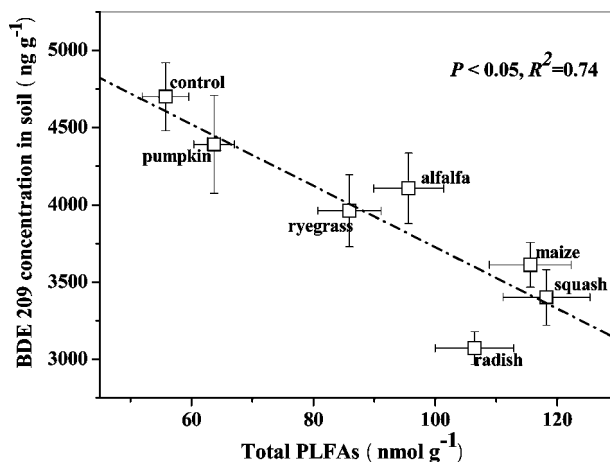


**FIGURE 3.** Mole percentage distribution profiles of lower brominated PBDEs in the soil and roots and shoots for ryegrass. The given congeners represent higher than 5% of the total lower brominated PBDE products.

readily taken up by plants. However, a detailed interpretation of debromination in soil or plants is difficult since plant accumulation of the lower brominated PBDEs reflects both the debromination of more highly brominated congeners inside plants and the direct uptake of the lower brominated PBDEs from soil (26). There is also evidence that lower brominated PBDEs are more likely to be taken up efficiently by plants (10). The OH-PBDE congeners 3'-OH-2,4-BDE-7, 2'-OH-4-BDE-7, 3'-OH-BDE-17, 3-OH-BDE-47, and 5-OH-BDE-47 were found in plant tissues (Tables S4 and S5) with 3'-OH-2,4-BDE-7 being most frequently detected. No OH-PBDE congener was detected in the soil. Congeners of OH-BDE-17, -28, -47, -49, -68, -75, -85, and -90 have been identified as metabolites in environmental samples such as surface waters (15) and sewage effluents (16) and marine organisms (18), as well as human blood (19). No report is available on OH-PBDE analysis in plant tissues, but previous studies on metabolism of PCBs in plants have demonstrated that lower chlorinated PCBs are degraded more readily than higher chlorinated ones (27).

**BDE-209 Dissipation in Soil.** The BDE-209 concentration in the unplanted control soil was  $4700.6 \pm 245.1 \text{ ng g}^{-1}$  with a loss of about 5% at the end of the pot experiment. This limited loss could result from the combination of volatilization and adsorption to the pots, as well as microbial degradation although no plants were present. BDE-209 concentrations in the soils after plant cultivation are listed in Table 1, showing decreases of 12.1–38.5% compared with the initial concentration. The total amounts of BDE-209, lower brominated PBDEs, and OH-PBDEs accumulated in plants ranged from 1.9 nmol for alfalfa to 11.4 nmol for maize per pot, resulting in 0.06–0.36% removal of BDE-209 from the soil. Therefore dissipation of BDE-209 in soil cannot be accounted for by plant uptake alone. BDE-209 in the boundary layer soil ranged from 2.5 to 5.0 nmol (0.08–0.16% of the BDE-209 in soil). The amounts of BDE-209 adsorbed on the pots ranged from 0.18 to 0.24% of the BDE-209 in the soil. When all of these potential losses are summed up (Table S6) the mass balance on a molar basis shows that the amounts of BDE-209 after the cultivation experiment were still significantly lower compared with the unplanted control soil. We speculate that loss of BDE-209 by metabolism and degradation in soil was the major contributor to the dissipation of BDE-209 in soil. In addition, other unidentified products of PBDEs and OH-PBDEs formed might account for the mass balance deficit to some extent.

Soil microbes play a key role in the metabolism and degradation of organic contaminants in soil (28). Nineteen PLFAs were determined in the soil and the composition in detail is given in the Supporting Information. A significant



**FIGURE 4.** Correlation between residual concentration of BDE-209 in soil and soil microbial biomass measured as mole abundance of the total PLFAs.

negative correlation was obtained between the residual BDE-209 concentration in soil and soil microbial biomass measured as the total PLFAs (Figure 4,  $P < 0.05$ ,  $R^2 = 0.74$ ), supporting the hypothesis that microbial metabolism and degradation contribute to the dissipation of BDE-209 in soil.

**Environmental Relevance.** This study provides, for the first time, evidence for the uptake, accumulation, and metabolism of BDE-209 in plants. BDE-209 can be taken up by all the plant species tested and translocated from roots to shoots, implying its potential transportation and accumulation in the food chain. Transformation of BDE-209 to lower brominated isomers or metabolites in the soil–plant system may be a source of environmentally abundant PBDEs and may have implications for toxicity assessment, depending on the nature of the metabolites and their potential to cause adverse effects. Therefore, plant accumulation and metabolism may be important processes in the fate of BDE-209 in soils and may merit special attention in the evaluation of the risk posed by PBDEs in the environment.

### Acknowledgments

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### Note Added after ASAP Publication

After this paper was published ASAP December 15, 2009, a correction was made to the last paragraph before “BDE-209 Dissipation in Soil”; the corrected version was reposted December 21, 2009.

### Supporting Information Available

Extraction and analysis procedures for PBDEs and OH-PBDEs in plant and soil samples and soil PLFA analysis; relationship between translocation factor and root lipid content (Figure S1); mole percentage of lower brominated PBDEs (Figure S2); PBDE and OH-PBDE congeners involved in the present study and their LODs and recoveries (Table S1); plant biomass and lipid contents (Table S2); concentrations of PBDEs and OH-PBDEs in the soil and plant samples (Table S3–S5); mole mass balance for BDE-209 (Table S6). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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