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Effect of copper on in vivo fate of BDE-209 in pumpkin

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HIGHLIGHTS

- The effect of copper ions on BDE-209 debromination was investigated.
- BDE-209 metabolism occurred preferentially in roots than in stems and leaves.
- No in vivo mineralization of BDE-209 was detected in the plants.

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ABSTRACT

A 60-day growth chamber experiments were performed to investigate the effect of Cu stress on the uptake, translocation and metabolism of decabromodiphenyl ether (BDE-209) by pumpkin. A total of nine debrominated metabolites (de-PBDEs), two hydroxylated PBDEs (OH-PBDEs) and one methoxylated PBDEs (MeO-PBDEs) were detected in the tested plants. Concentrations of the total debrominated, hydroxylated or methoxylated metabolites generally followed the order of roots > stems > leaves, and de-PBDEs > OH-PBDEs > MeO-PBDEs. These results indicate that metabolism occurred preferentially in roots than in stems and leaves. The addition of moderate dosage of Cu (50 mg/kg) resulted in increment in OH-PBDE concentrations in plant tissues, whereas higher concentrations of Cu could inhibit uptake and metabolism of BDE-209. No in vivo mineralization of BDE-209 was detected in the plants. These results provide valuable information about the behavior of BDE-209 in plant tissues under heavy metal exposure.

1. Introduction

Since the 1960s, polybrominated diphenylethers (PBDEs), a class of brominated flame retardants, have been extensively used in electronic appliances, paints, textiles and other materials to prevent fire propagation [1].

Currently, China has evolved as the world's largest electronic waste (e-waste) importer [2]. In some places of China (such as Guiyu, Taizhou), large amounts of e-waste are recycled by using primitive processes, including manual disassembly, open incineration and acid dipping [3]. These processes contribute to high releases of toxic metals (such as Cd, Hg, Cu, Ni, Pb and Zn) [4], as well as persistent organic pollutants including polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxin and dibenzofuran (PCDD/Fs), polybrominated dibenzo-p-dioxin and dibenzofuran (PBDD/Fs) and PBDEs [5,6], into the environment. It has been reported that in Guiyu, a small town in South China, the concentrations of total metals and PBDEs were in the range of 3000–28,000 and 2.9–45 μg/kg soil (dry weight, DW) respectively [7].

Phytoremediation, involving the use of higher plants and associated microorganisms for in situ treatment of soil, sediment and water, has been utilized successfully in sites contaminated by heavy metals and a variety of organic pollutants including PCBs, PCDD/Fs, etc. [8]. PBDEs in soils may also be accumulated into growing plant tissues and are possibly further metabolized in plant cells [9–16]. Plants could therefore play an important role for the transfer of PBDEs into the food chain. Thereupon, investigating the translocation and possible catabolic transformation of PBDEs in plants has important theoretical and practical significance for both food risk assessment and phytoremediation of PBDEs contamination.

Hydroxylated (OH-) and methoxylated (MeO-) PBDEs without known anthropogenic source and debromination products have been detected in plants including ryegrass (*Lolium perenne* L.) [9], alfalfa (*Medicago sativa* L.) [9], pumpkin (*Cucurbita pepo* L.) [9,16], maize (*Zea mays* L.) [9,11,14,15], and radish (*Raphanus sativas* L.) [9]. However, all previous studies focusing on the phytoextraction and phytometabolism of PBDEs did not take into consideration the

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fact that PBDE contamination is usually accompanied by the presence of high concentrations of heavy metals in e-waste recycling regions [7]. Additionally, it is unclear whether PBDEs can be mineralized by plants alone though it may be very difficult. Nevertheless, it has been reported that aromatic compounds (such as nitrobenzene, aniline and benzoic acid) could be mineralized in maize and pumpkin [17].

The aim of the present study, thereupon, is to study the effect of copper ions on translocation and metabolism of BDE-209 (3,3′,4,4′,5,5′,6,6′-decabromodiphenyl ether) in whole pumpkin plants grown in soil under aseptic condition. The possible mineralization was verified by using ^{14}C-technology.

2. Materials and methods

2.1. Chemicals

Standard of BDE-209 was obtained from Sigma (St. Louis, MO, USA). ^{14}C-labeled BDE-209 and a standard solution of PBDEs containing 27 native congeners were purchased from Wellington Laboratories (Guelph, Ontario, Canada). The specific activity of ^{14}C-labeled BDE-209 was 15 mCi/mmol. The stock standards of OH- and MeO-PBDEs were purchased from AccuStandard (New Haven, CT, USA). Surrogate standards (^{13}C-6-OH-BDE47, ^{13}C-6-MeO-BDE47, and ^{13}C-BDE99) were obtained from Wellington Laboratories (Guelph, Ontario, Canada). All solvents used were of HPLC grade. Deionized water (18.4 MΩ) was used for all experiments. All other chemicals and reagents used were of analytical-reagent grade or higher purity.

2.2. Soil characteristics and preparation

A loamy sand soil without detectable PBDEs was used in the present work. Soil samples were air-dried, sieved through a 2-mm mesh and then sealed in plastic bags to store at 4 °C. The soil had the following basic properties: pH (1:2.5 water) 6.24; cation exchange capacity 5.38 cmol/kg; total organic carbon 3.56 g/kg, total nitrogen 1.57 g/kg, total phosphorus 0.76 g/kg; sand 58.4%, silt 21.7%, clay 19.9%; K 255 mg/kg, Ca 352 mg/kg, Mg 28 mg/kg, Cu 12 mg/kg, Pb 7.3 mg/kg, Zn 16 mg/kg, Fe 735 mg/kg, Cd 0.05 mg/kg.

To eliminate indigenous microorganisms, the soil was sterilized in an autoclave for 60 min and fertilized with sterile NPK fertilizer mixture (1 g/kg soil) containing $N:P_2O_5:K_2O = 1:0.35:0.8$ ratio.

In this study, the soil was contaminated with both ^{14}C-BDE-209 and unlabeled BDE-209. Both unlabeled and labeled BDE-209 samples were firstly dissolved in acetone, and then were added drop by drop to 25% by weight of the required quantity of soil. After acetone evaporation, the soil was blended thoroughly with the remaining 75% by weight of the required quantity of soil to bring the soil to final concentrations of 5 nCi/kg and 5000 μg/kg for unlabeled and labeled BDE-209, respectively. This concentration was close to the higher environmental levels of BDE-209 determined in some contaminated areas such as soil from electronic waste disposal sites [18]. The spiked soil was then allowed to dry in a sterile fume hood in the dark until the acetone had volatilized completely. Subsequently, the BDE-209 spiked soil was spiked with $Cu(NO_3)_2$ at varied levels. To prevent potential contamination with microorganisms during soil preparation and aging, the soil was supplemented with both cycloheximide and chloramphenicol to give final concentrations of 50 and 100 mg/kg of soil, respectively. Afterwards, the soil was covered with aluminum foil, shaken for 30 min every day, homogenized, and incubated in the dark for one month at room temperature to allow the contaminants to equilibrate. The final concentration was 4980 ± 255 μg/kg after one month incubation prior to plant cultivation. No other PBDEs, MeO- or OH-PBDEs were detected in the soil.

2.3. Experimental setup

In the present study, a plant growth chamber made of plexiglass was used for growing plants and monitoring the possible $^{14}CO_2$ evolution in the plants. The growth chamber design was modified from Chen et al. [19]. The schematic diagram of experimental set-up is shown in Figure S1 (Supporting Information).

Each chamber consisted of an upper (shoot) and a lower (root) section. The upper section had a height of 60 cm with 100 cm × 100 cm base area that gave a volume of 600 L. The lower section serving as the soil chamber consisted of a cylindrical column with 30 cm in height and 15 cm ID, respectively, resulting in a total volume of 5.3 L. To keep the roots in darkness and also to prevent photolysis of BDE-209, all root chambers and the openings between the shoot and root chambers were individually covered with black plastic films. An air inlet constructed from hard plastic tubes (PVC) with 25 mm ID was installed on the side face of the shoot chamber. The air intake tube was connected to two charcoal bottles and a bacterial filter in series. All the bottles and deionized water were autoclaved to prevent any possible introduction of exogenous microorganisms. Additionally, the entire growth chamber was sterilized by UV light and ozone.

To grow seedlings under aseptic conditions, pumpkin seeds were soaked in 98% concentrated sulfuric acid for 2 min, then in a 15% solution of hydrogen peroxide for 15 min, and finally in 1% mercury chloride for 15 min [17]. After each soak, the seeds were washed with sterile deionized water. Germination was carried out on sterile moist quartz sand at 26 ± 2 °C. One seedling having height of about 3 cm was transplanted to the soil in the growth chamber. All experiments were performed in a greenhouse with natural light and day/night temperature of 28/21 °C and humidity of 73/86%. During the experimental period, sterile deionized water was added to the root chamber by using a syringe to compensate the water losses. Soil moisture content was maintained at approximately 60% of its water holding capacity by using weighing method.

Air was continuously evacuated from the shoot chamber by using a vacuum pump at a constant flow rate of 0.3 L/min. The outlet air was passed through a series of sampling traps containing aquasol and 4 M NaOH respectively to trap CO_2 [20]. The radioactivity in the culture fluid was determined by liquid scintillation counting (LSC) using ACS II scintillation fluid (ASCII; Amersham, UK). Counts were corrected for dilution, quenching and background.

Three series of treatments with three replicates were employed: (a) plant growing in non-spiked soil; (b) control: plant growing in soil spiked with BDE-209 alone; and (c) plant growing in soil spiked with both BDE-209 and Cu.

2.4. Sample preparation

After 60 days of planting, the root, stem, and leaf of whole plant and the soil were sampled separately. The entire soil in each chamber was thoroughly homogenized, air-dried at room temperature and ground sufficiently to pass through a 100-mesh sieve. Roots were first carefully washed with tap water followed by deionized water to remove any adhering soil particles. Then shoots, leafs and roots were rinsed thoroughly with distilled water, dried with tissue paper and weighed. Afterwards, the plant sub-samples were freeze-dried (for 72 h under vacuum with a collector temperature of −50 ± 2 °C), and dry weights were recorded. Tolerance index (TI) was calculated by the following equation:

$$\mathrm{TI}(\%) = \frac{\text{phytomass in soil} + \text{metal}}{\text{phytomass in soil} - \text{metal}} \times 100. \quad (1)$$