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The uptake and translocation of PBDEs from corn roots to shoots as a result of root damage following copper exposure

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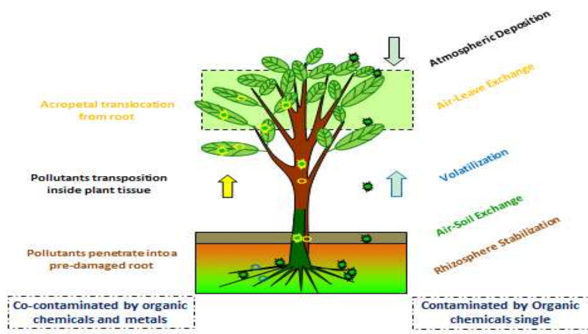

Abstract

Co-contamination by heavy metals and POPs is ubiquitous in the environment. Whether or not POPs can be taken up by plant roots and translocated upwards to the shoots is a significant concern and an area were much uncertainty still exists. This study investigated the fate of polybrominated diphenyl ethers (PBDEs) when copper (Cu) was present within the soil/water-plant system using pot and hydroponic experiments. The results showed that the presence of Cu could induce damage to root cell membranes and increase the Cu concentration in shoots and roots. Concentrations of root PBDE congeners BDE-209 and BDE-47 were enhanced when the level of Cu was increased, and the highest shoot BDE-209 and BDE-47 levels were observed with the highest rate of Cu application. In addition, positive correlations were observed between the PBDEs content of corn shoots and the electrolytic leakage of corn roots. These results indicate that within a defective root system, PBDEs can significantly penetrate plant roots and move upwards to the shoots. The potential ecological risk associated with the translocation and accumulation of POPs into plant shoots should be carefully considered in media co-contaminated with metals and POPs, whereas it is often ignored or underestimated in environmental risk assessments.

Key words: PBDEs, Cu, electrolytic leakage, passive permeation

1. Introduction

Polybrominated diphenyl ethers (PBDEs), a group of halogenated chemicals,[1, 2] can impact the safety of ecosystems and human health due to their persistence, toxicity, bioaccumulation, and long-range atmospheric transport.[3] Most previous studies regarding PBDEs have focused mainly on their physicochemical characteristics, partitioning equilibrium, toxicity and environmental fate.[4-7] Studies on the phytoremediation of PBDE-contaminated soil have also been conducted recently.[3, 8] Field surveys have shown that PBDE concentrations in plant roots were significantly correlated with soil concentrations, while PBDEs present in the shoots were mainly attributed to the deposition of PBDEs from the atmosphere onto leaf surfaces, subsequently reaching internal plant tissues through the cuticle.[9] The accumulation of PBDEs in leaves has been shown to be selective and influenced by the substitution pattern, with ortho-substituted isomers more prevalent than meta-substituted isomers.[10] However, experiments conducted in pots have provided substantiating evidence for the acropetal translocation of PBDEs in plants such as ryegrass, corn and tall fescue, in which it was also suggested that PBDE transposition in plant tissue is driven by the transpiration stream.[11-13]

The mechanism of plant uptake of trace elements has been well documented. Generally, elements are transported from the external parts of the root to the central root xylem, where material is carried to the shoot through two major pathways. In the apoplastic pathway, the presence of the lipophilic Casparian strip disrupts the apoplastic water flow and directs it across cell plasma membranes at least twice, where selective transport as well as the passive permeation of solutes occurs,[14] although the Casparian strip is only slightly permeable to ions.[15] Conversely, in the symplastic pathway, solutes can move through the cortex into the endodermis and eventually the pericycle, from which they can move into the xylem for long distance transport. With regard to the plant uptake of organic compounds, most studies have found that moderately hydrophobic organic compounds ($0.5 < \log K_{ow} < 3$) are significantly taken up by and translocated into plant tissues.[16] Compounds with $\log K_{ow} > 3$ are concentrated at the root surface and are not easily transported within plants.[16] However, the translocation of BDE-209 ($\log K_{ow} = 7.96$) from roots to shoots has been reported recently, although it is unknown if the translocation took place by the apoplastic or symplastic pathway. Substances with a large structural formula, such as metal-chelating compounds, can be taken up indiscriminately and loaded into the root xylem through breaks in the root Casparian strip.[17] Hence, it is possible that very hydrophobic PBDEs could be translocated upward to the shoots within a root system damaged by heavy metals.

Soil co-contaminated with metals and POPs is quite common and can be found in locations such as e-waste recycling sites and around smelting plants.[18] However, the risks associated with POP accumulation within plant tissue are largely ignored when conducting an environmental risk assessment, due to our limited understanding of the uptake of POPs by plants. The objective of this study was to investigate the potential fate of PBDEs within a soil/water-plant system in the presence of copper (Cu) and to determine the underlying mechanism of PBDE uptake by corn within a defective root system. The study provides new information regarding the PBDE uptake mechanism in plants and will therefore improve the environmental risk assessment of metal-POP co-contaminated environments.

2. Environmental Sections

2.1 Chemicals

Generally, in most abiotic environments, such as sediment, sewage sludge and air, the dominant PBDE congeners are BDE-209 and BDE-47. BDE-47 is also the predominant congener detected in fish, wildlife and human samples, including blood, milk and fat.[10] Hence, BDE-209 and BDE-47 were selected for investigation in this study. Standards (99% purity) of BDE209 and BDE47 were purchased from Sigma (St. Louis, MO, USA). Stock solutions of BDE 209 and BDE47 were prepared in isooctane at 1.0 mg mL^{-1}. Working solutions of BDE209 and BDE47 were prepared by gradual dilution of the stock solution with acetone. All standards and solutions of BDE47 and BDE209 were stored in amber glass vials at 4°C. Analytical reagent grade $CuSO_4 \cdot 5H_2O$ and $Cu_2(OH)_2CO_3$ were obtained from JinKe Chemicals (Shanghai, China).

2.2 Exposure to PBDEs and Cu

Corn seeds (*Zea mays* L. cv. Nongda 108) were surface sterilized with 0.5% NaClO, rinsed thoroughly with deionized water (DIW) and then germinated for 2 days.

Hydroponic experiment: Ten seedlings were placed in a pot (2.5 dm^3) containing one-half Hoagland nutrient solution and then changed to total Hoagland nutrient solution after 1 week.[17] All of the pots were placed in a greenhouse with natural light and a day/night temperature of 25-30/15-18°C. The nutrient solution was renewed every 2 days. After 2 weeks of cultivation, seedlings were pre-treated with the following different concentrations of Cu (as $CuSO_4 \cdot 5H_2O$) for 2 days in solution: 0.32 μmol L^{-1} Cu (Control), 100 μmol L^{-1} Cu (Cu100), 200 μmol L^{-1} Cu (Cu200) and 400 μmol L^{-1} Cu (Cu400). The pre-treated plants were then placed in Hoagland solution containing 0.04 mg/L BDE-209 or 0.04 mg/L BDE-47 for 3 days. Another hydroponic experiment that investigated the distribution and translocation of PBDEs in corn pre-treated with hot water is detailed in the Supporting Information (SI).

Pot experiment: Loamy soil (pH = 6.4, organic matter = 1.8%) without detectable PBDEs was air-dried, sieved through a 2-mm mesh and then blended thoroughly with BDE-209, resulting in a final BDE-209 concentration of 3 mg kg^{-1} soil.[19] Subsequently, the BDE-209-spiked soil was spiked with five concentrations of Cu (as $Cu_2(OH)_2CO_3$). Thus, five treatments (Control, Cu400, Cu800, Cu1200 and Cu1600) with three replicates each were performed, in which the concentration of Cu in each pot was 0, 400, 800, 1200 and 1600 mg kg^{-1} soil, respectively. Afterwards, the soil was covered with aluminium foil, stirred for 30 min every day, and then homogenized for 1 month at room temperature to allow the contaminants to equilibrate. Four corn seedlings were then transplanted into individual ceramic pots containing 2 kg spiked soil. During the cultivation period (60 days), deionised water (DIW) was sprayed to compensate for water loss, and the soil moisture was maintained at 60% of its water holding capacity.

2.3 Sampling

Plants were harvested at the end of cultivation. Shoots and roots were separated, washed with tap water and rinsed with DIW. A portion of the fresh roots were used to analyse electrolytic leakage. The remaining roots and all shoots were freeze-dried, measured for biomass, ground to a fine powder and stored in a freezer until later analysis.

Passive air samplers were used to measure the atmospheric deposition of PBDEs onto polyurethane foam (PUF) disks (14-cm diameter, 1.2-cm thickness, and 0.035-g m^{-3} volume) during the entire cultivation period. Two samplers were hung over the ceiling of the greenhouse, and another two samplers were placed 400 m away from the greenhouse. Detailed descriptions of the set-up have been provided previously.[20]

2.4 Chemical analysis

2.4.1 PBDE analysis

Approximately 0.5 g plant samples, homogenized in 3 g anhydrous sodium sulphate or PUF discs and spiked with the surrogate standards, were extracted using hexane/acetone (3:1, V/V) for 72 h. Briefly, the fractionated extracts of plants and PUF discs were concentrated to ~0.5 ml after solvent exchange to hexane. The extracts of plants were washed with sulphuric acid and then cleaned-up using a multi-layer column containing, from bottom to top, neutral alumina (3% deactivated), neutral silica gel (3% deactivated), 50% (w/w) sulphuric acid-silica gel, and anhydrous

Na_2SO_4, with an eluent of 20 ml hexane/DCM (1:1, V/V). The PUF disc extracts were purified using the multi-layer column. After evaporating to an ~50-µl volume, ^{13}C-PCB141 was added as an internal standard before instrumental analysis.[10, 20]

BDE 47 was analysed separately using a DB5-MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). BDE-209 was analysed using a gas chromatograph/mass selective detector (Agilent GC7890A coupled with a 5975C MSD) in conjunction with a DB5-MS capillary column (15 m × 0.25 mm i.d. × 0.25 µm film thickness). The analytical details have been described previously. [20, 21]

2.4.2 Cu analysis

Plant samples were acid digested using a mixture of HNO_3 and $HClO_4$ (1:4, v/v) and analysed using inductively coupled plasma - atomic emission spectroscopy (ICP-AES).[22] An exhaustive description of the quality assurance/quality control protocol was provided in our previous study.[20] The recoveries were around 94 ± 5% for all of the metals in the plant reference materials.

2.4.3 Electrolytic leakage analysis

Approximately 0.2 g fresh roots were rinsed thoroughly with DIW to remove surface contamination and were then sectioned into 1-cm segments and placed in individual vials containing 10 ml distilled water. Samples were exposed to a vacuum at room temperature (25°C) for 3 hours. The electrical conductivity (EC) of the bathing solution (EC_1) was measured after vacuum exposure using an electrical conductivity meter (SY-2, Institute of Soil Science, Chinese Academy Sciences, Nanjing, China). Samples were then placed in a thermostatic water bath at 100°C for 15 min, and a second reading (EC_2) was determined after the solutions were cooled to room temperature. Electrolyte leakage was calculated as EL= $(EC_1/EC_2)*100$ and expressed as a percentage.

2.5 Statistical analysis

All statistical calculations, e.g., correlations and significant differences, were performed using SPSS 17.0. The statistical significance of differences and variance analysis (p <0.05) of pollutant accumulation in plants among the different treatments was performed using a one-way ANOVA and least significant difference (LSD) test.

3. Results and discussion

3.1 Corn growth and cell permeability of corn roots

Although Cu is an essential micronutrient for plants, elevated concentrations of Cu can inhibit plant growth and cause toxicity.[17] Figs. S1 and 1 show the dry matter yield of corn grown hydroponically or in soil. The growth of corn was significantly affected by Cu (Fig. 1) or hot water (Fig. S1), and when Cu and PBDEs were present together, a clear difference was apparent in the biomass between the different treatments in both soils and hydroponics. Generally, the corn biomass decreased with an increase in Cu concentration, and the prohibitive effect of Cu on roots was more pronounced than that on shoots (Fig. 1). This confirmed the previously observed growth inhibition of corn by Cu.[17, 23] When the Cu concentration in hydroponics was 400 μmol L^{-1}, the shoot and root biomass reached 79.9 and 49.7%, respectively, of the control with the BDE-209 treatment, and 72.6 and 40.0%, respectively, of the control with the BDE-47 treatment (Fig. 1 B & C). The results indicated that BDE-47 has a stronger prohibitive effect on corn growth than does BDE-209, which agrees with the results of earlier studies that the lower brominated biphenyl ethers poison organisms more intensively compared with the higher brominated biphenyl ethers at the same dosage.[24] Although the phytotoxicity of Cu in soil was much lower than that in hydroponics due to the different chemical forms and mobility of Cu,[22, 25] similar trends were also found in the experiments conducted in pots, in which 35 and 30.7% average reductions were observed in the shoot and root biomass, respectively, compared with the control (Fig. 1 A).

The most common effects of heavy metal toxicity in plants are a reduction in seed germinability, stunted growth, leaf chlorosis, inactivation of enzymes, and inhibition of photosynthesis.[26] In addition, excess Cu can induce a number of free radical processes in proteins and lipid cell membrane components,[27, 28] resulting in destabilization of membranes and an increase in their permeability.[29] In this study, electrolytic leakage was used to monitor the permeability of root cell membranes following exposure to Cu. As Fig. 2 shows, a significant increase in electrolytic leakage was observed when the Cu level increased, and 3.5-, 4.5- and 6.8- fold enhancements were achieved with the Cu100, Cu200 and Cu400 treatments, respectively, in comparison with the control (Fig. 2). As expected, the electrolytic leakage in corn roots was also enhanced by elevating the water temperature (Fig. S2). It has been proven that Cu-induced changes in cell permeability can be attributed to non-selective conductance increases.[30] In addition, root exclusion mechanisms collapse in the presence of excessive Cu,[30, 31] thereby disrupting ion channel absorption regulation.[31] In this case, solutes would be indiscriminately taken up by the damaged roots and translocated to shoots, with unconventional regulation of ion channels.

3.2 Cu uptake and translocation within corn

In the presence of Cu and PBDEs, Cu accumulation in corn was observed (Fig. 3). Generally, the Cu contents in shoots and roots were elevated as the level of Cu applied to the soil or hydroponics increased. In the experiment conducted in pots, the shoot and root Cu concentrations ranged from 15 to 33 mg kg^{-1} and 11 to 280 mg kg^{-1}, respectively, with the highest values seen with the Cu400 treatment for both shoots and roots. In hydroponics, 42.7- and 462.6-fold average increases were observed in the Cu content of shoots and roots, respectively, compared with the control, in the BDE-209 treatments. Similarly, in the BDE-47 treatments, 44.3- and 256.9-fold average increases were observed in the shoot and root Cu concentrations, respectively, compared with the control.

Both the shoot and root Cu concentrations in corn grown hydroponically were an order of magnitude higher than those in corn grown in pots, although the initial application rate of Cu in the pots (400 - 1600 mg kg^{-1} • soil) was much higher than that in hydroponics (100 – 400 μmol L^{-1}). This difference was attributed to the chemical speciation of Cu and the physiological behaviour of corn roots. Normally, Cu is present in soil in the form of oxides, carbonates, and organic and residual matter in mineral structures, among which the water-soluble and exchangeable fractions are readily mobile and available.[22, 32] The water-soluble Cu concentration in soil in this study ranged from 0.20 - 3.83 mg kg^{-1}, which was far lower than that in hydroponics (6.4 - 25.6 mg L^{-1}). With regard to the physiological behaviour of corn roots, the extent of root damage in the pots was much less than that in hydroponics, which was validated by the increased electrolytic leakage of corn roots grown hydroponically. Although the cellular permeability of corn roots was impossible to measure accurately in plants grown in soil, the light root colour and greater root biomass and root length observed in the pot-based experiments indicated that the potential damage to corn roots was less than that in the hydroponic experiment. As expected from the preceding results of cell permeability, excessive Cu accumulated in corn roots and was then transferred by the transpiration stream to other tissues. A significantly positive correlation (R^2 = 0.99, $P < 0.05$) was also observed between Cu content and electrolytic leakage in roots in hydroponics. Despite numerous studies on metal uptake by plants indicating the presence of high- and low-affinity transporters with broad substrate specificity,[33] our study provided evidence that cell permeability also plays a significant role once ion channel regulation is disrupted.

3.3 PBDE accumulation in corn tissue

We investigated the accumulation of PBDEs in corn exposed to Cu or hot water (Fig. 4 & Fig. S3). In general, the BDE-209 and BDE-47 contents in shoots were enhanced as the levels of Cu applied to the soil or hydroponics increased. In the hydroponic experiment, the shoot BDE-209 and BDE-47 concentrations were in the range of 0.03 - 81.4 ng g^{-1} dry weight (DW) and 1.94 -1589 ng g^{-1} DW, respectively. Compared with the control, 26-, 102- and 2711-fold average increases for shoot BDE-209 treatments and 8.5-, 100- and 818-fold increases for shoot BDE-47 treatments were observed in the presence of Cu100, Cu200, and Cu400, respectively. Although the shoot concentrations of BDE-209 (0.93 to 10.8 ng g^{-1} DW) in soil were much lower than those in hydroponics, the variations in shoot BDE-209 concentrations corresponded well between soil and hydroponics. In addition, root BDE-209 and BDE-47 concentrations were enhanced when the Cu levels increased in hydroponics, compared with the control. A similar pattern was observed in the root BDE-209 concentrations in the pot experiment, with the highest values found in the control. The PBDE distribution in corn tissue after the hot water treatment was similar to that after the Cu treatment in the hydroponic experiment (Fig. S3).

In hydroponics, the concentration of BDE-47 in corn tissue was much higher than that of BDE-209, which indicated that BDE-47 was more likely to be taken up and translocated upwards in plant tissue than was BDE-209. Normally, semivolatiles, such as PBDEs, may volatilize from soils and later be absorbed by the waxy outer surfaces of leaves or bark.[34] The potential deposition of PBDEs from the atmosphere onto leaves was calibrated by the passive sampler. For direct soil uptake, chemicals are considered to be solubilized into soil interstitial water, after which they enter the roots and move up the xylem to the shoots of the plant.[35] Previous studies have shown that moderately hydroponic organic compounds (log Kow = 0.5-3) can easily be taken up and translocated by plants, while chemicals (log Kow > 3.0) are bound strongly to root surfaces and are difficult to transfer within the plant.[16] However, in this study, both BDE-47 (log Kow = 6.81) and BDE-209 (log Kow = 7.96) displayed the potential for transposition in corn tissue when the root system was damaged. It has been shown previously that nightshade (*Solanum nigrum*) and tobacco (*Nicotiana tabacum*) grown on undiluted biosolids (containing 334 ug kg^{-1} penta-BDE) can accumulate up to 15.4 and 76.6 ug kg^{-1} penta-BDE, respectively, with the highest levels in the stems rather than the roots or leaves.[36] It has also been demonstrated that some other compounds with a high Kow coefficient can be transferred from roots to shoots, including hepta-PCBs (log Kow ≈ 8),[37] phenanthrene (log Kow = 4.46) [38]and hexachlorocyclohexane (log Kow= 3.3).[39] The accumulation of these pollutants in shoots were in the range of <10 ng g^{-1} DW.

However, in this study, the highest concentration of shoot PBDEs in hydroponics was observed with the Cu400 treatment, in which the levels were 1589 ng g^{-1} DW for BDE-47 and 81 ng g^{-1} DW for BDE-209, respectively. Compared with the control, the largest increase in shoot PBDEs concentrations, up to 11-fold (BDE-209), were observed with the Cu1600 treatment in plants grown in soil. The much more pronounced differences in acropetal translocation of PBDEs observed in this study could be explained by the presence of Cu, which stimulated the translocation of PBDEs from roots to shoots. PBDE uptake by corn pre-treated with Cu was potentially caused by passive penetration into root cells due to enhanced electrolytic leakage in the roots, where they could then be easily transferred into the plant transpiration stream and reach other plant components. The highest root PBDE concentrations were observed in the control rather than the Cu-treated plants in both the hydroponics and pot experiments (Fig. 4). This may be attributed to the lipophilicity of PBDEs, and most of the PBDEs absorbed in the control adhered to the root outer surface, which resulted in retarded transport of organic compounds. Previous studies have demonstrated a significant positive correlation between root lipid contents and root PBDE concentrations, which confirms the important role of plant lipids in root uptake of BDE-209 from soils.[11, 40] However, no linear relationships between lipid and PBDEs contents in corn root were found in this study (data not shown). With the addition of Cu, root cell membranes collapsed, which disrupted the partition equilibrium of PBDEs between the root-water/soil interfaces. Thus, within a defective root system, the passive permeation of organic chemicals into cells would determine their fate in soil/water-plant systems rather than their physicochemical properties. Therefore, the traditional partitioning equilibrium theory of organic chemicals cannot be used on its own to evaluate the potential route of POPs within soil/water-plant systems. For example, dead rice roots (heated for 40 min at 105°C) have been observed to uptake phenanthrene and pyrene, and it was proven that the respective uptake coefficients (C_{plant}/C_{water}) of dead rice roots gradually surpassed those of fresh rice roots. The proposed explanation for this was the increased permeability of the cell membrane caused by heating the rice roots.[38] Therefore, the physiological status of the roots may have a significant effect on the movement of organic compounds within the plant system. In addition, it has been reported that imbalances among "nutrients" may lead to competitive replacement of an essential molecule within an important binding site in plant tissue with a more abundant molecule of lower affinity, resulting in a complex with impaired function.[41] This scenario appears to be common for certain heavy metals or where the external medium contains unusually high concentrations of a few "nutrients". In this study, it is possible that the passive permeation of PBDEs into corn roots was magnified by the Cu-triggered electrolytic leakage, while the permselectivity function of cell membranes was weakened in the damaged-root system.

3.4 Co-Linearity between the PBDE distribution and electrolytic leakage in corn roots

A significantly positive correlation ($R^2 = 0.810$, $p < 0.01$ for BDE-209; $R^2 = 0.842$, $p < 0.01$ for BDE-47) was found between PBDE concentrations in shoots and root electrolyte leakage in the groups pre-treated with Cu (Fig. 5), indicating that the uptake and translocation of PBDEs were strongly dependent on the breakdown of the root exclusion mechanism. Limited published data on PBDE uptake by plants following the application of Cu are available,[42, 43] with one study indicating that co-contamination with polycyclic aromatic hydrocarbons (PAHs) and heavy metals can improve the accumulation of PAHs in shoots and roots, as well as the penetration of metals or metal complexes into plant tissue.[42] It has been reported that a moderate dosage of Cu in soil can increase the concentration of OH-PBDEs in pumpkin tissues, which generally followed the order of roots > stems > leaves.[44]

It has been confirmed that Cu can passively penetrate through corn root cell membranes lacking barriers,[31] which then facilitates the penetration of PBDEs, including BDE-209 and BDE-47, into the corn roots. Excessive Cu penetration into cell membranes could be one of the explanations for the observed increases in PBDE concentrations in shoots and roots in this study.

4. Environmental implications

Previous studies have shown that the concentration of POPs in plant shoots is mainly attributed to the atmospheric deposition of POPs, with transposition from roots to shoots being negligible.[45, 46] However, it has been confirmed that high accumulation of PBDEs in roots cells and subsequent acropetal translocation to shoots within a defective corn root system can occur. This accumulation could even be underestimated, because the possible metabolism of PBDEs within plant tissues was found in some studies. Hence, it is likely that POPs enter the phytosphere through excessive uptake by metal-damaged root systems and subsequent transposition to aerial parts of the plant in sites co-contaminated with metals and

POPs.[47, 48] Thus, it is necessary to determine the underlying ecological risks when conducting risk assessments in such sites. In addition, other factors that differ widely and potentially contribute to the variability and uncertainty in the uptake of organic chemicals by plants, such as plant type and plant growth status, should be taken into consideration. Hence, studies that predict or model the potential fate of organic compounds within soil/water-plant systems should also consider biological factors, rather than rely only on the physicochemical characteristics of compounds.

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Appendix. Supplementary experiment

A supplementary experiment investigated the uptake and translocation of PBDEs in corn, within a root system pre-treated with water at different temperatures. The supplement also includes the details of the experimental set-up, figures showing the corn biomass, electrolytic leakage in corn roots, and the distribution of BDE-209 and BDE-47 in corn tissue, as well as the correlation between PBDE concentration in corn tissue and electrolytic leakage in corn roots.

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Figures legends

Figure 1 Biomass of corn tissue

Figure 2 Electrolytic leakage of corn root treated with different Cu levels

Figure 3 Copper concentrations in corn tissues influenced by different copper treatments

Figure 4 Distribution of PBDEs in corn tissue

Figure 5 Co-linearity between PBDEs in shoot and electrolytic leakage of root

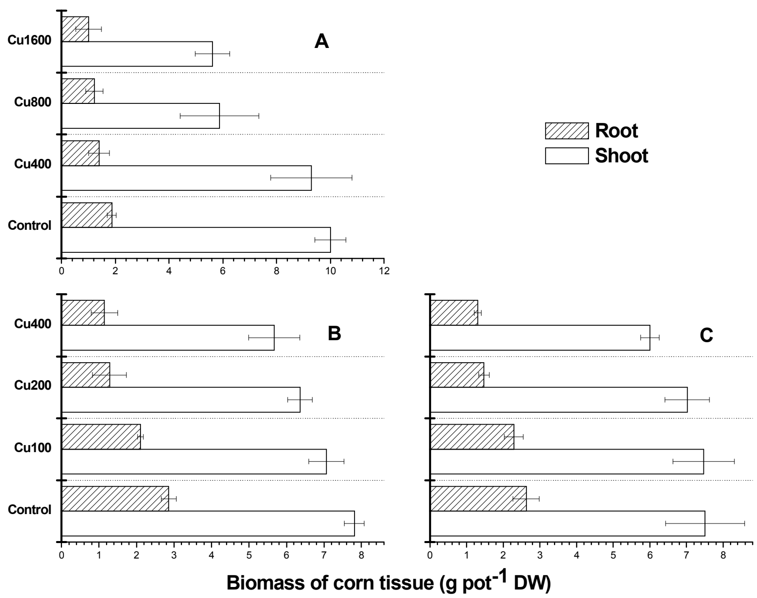

Figure 1 Biomass of corn tissue (A: pot experiment; B: Hydroponics contaminated by BDE47; C: Hydroponics contaminated by BDE209). Error bar show standard error of the mean (n=3).

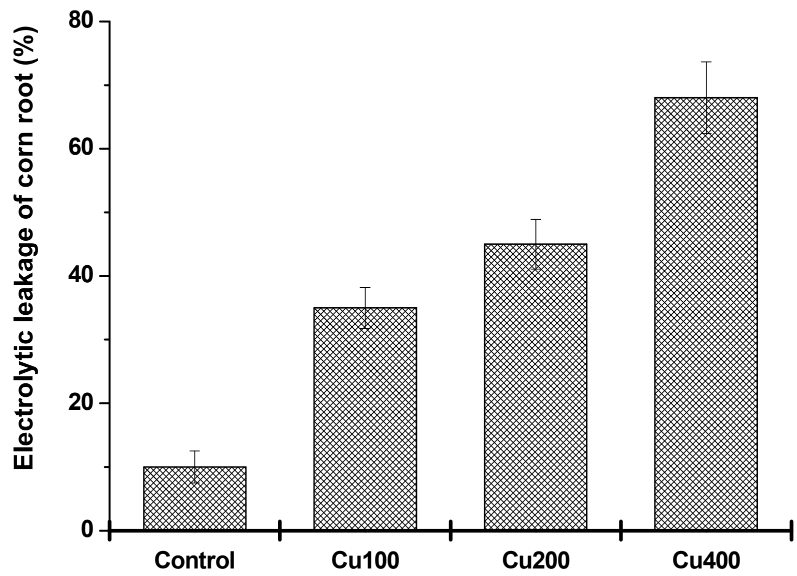

Figure 2 Electrolytic leakage of corn root treated with different Cu levels. Error bar show standard error of the mean (n=3).

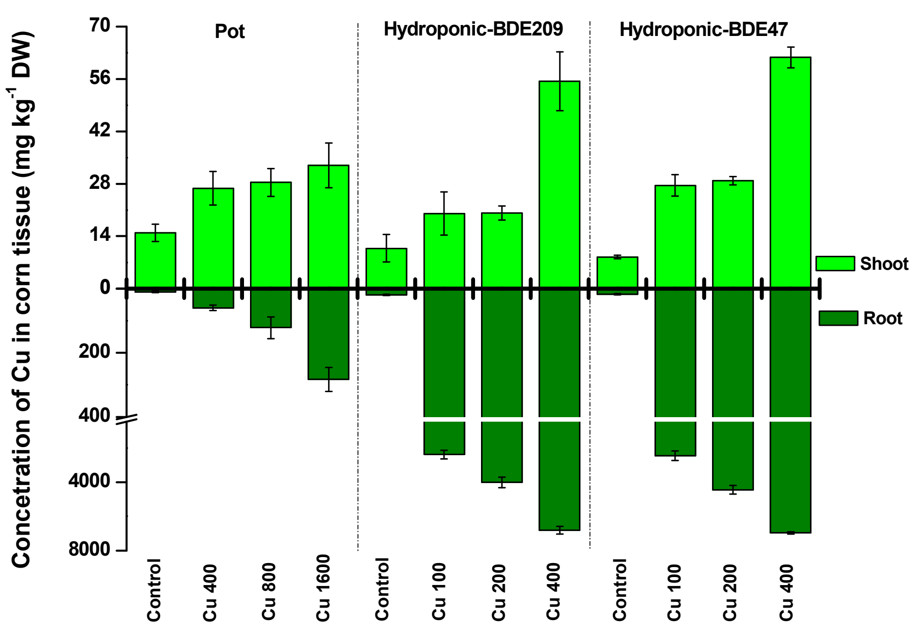

Figure 3 Copper concentrations in corn tissues influenced by different copper treatments. Pot represents corn grown on soil contaminated by BDE-209; Hydroponic-209 represents corn grown

hydroponically contaminated by BDE-209; Hydroponic-47 represents hydroponically contaminated by BDE-47. Error bar show standard error of the mean (n=3).

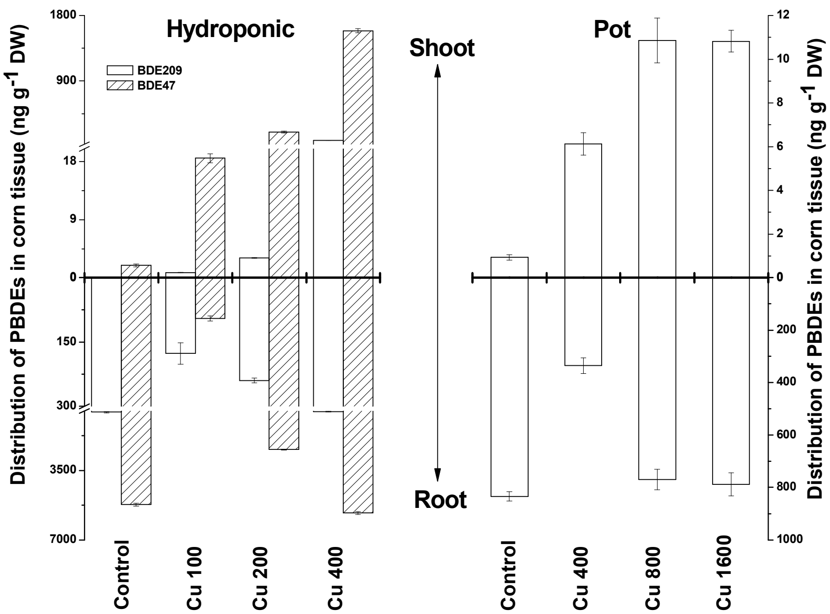

Figure 4 Shoot (upper) and root (lower) PBDEs concentration after 60-day growth. Error bar show standard error of the mean (n=3).

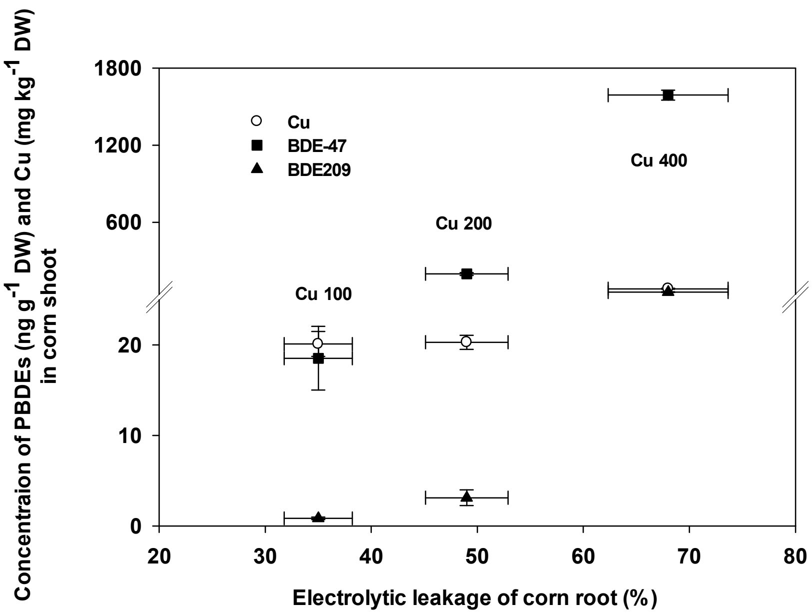

Figure 5 Co-linearity between PBDEs in shoot and electrolytic leakage of root. Error bar show standard error of the mean (n=3).