Toxic Effects of 2,2′,4,4′-Tetrabromodiphenyl Ether on Chinese Cabbage

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Abstract. 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) is one of the most toxic polybrominated diphenyl ethers (PBDEs). The toxic effects of BDE-47 on Chinese cabbage seedlings were analyzed in this study. After a 30-day hydronionic exposure to BDE-47 at different concentrations (25, 50, 75, and 100 μg·L−1), the fresh weight of Chinese cabbage seedlings was significantly decreased, whereas their root:shoot ratio was increased, indicating that BDE-47 inhibited the growth of the plant, especially the underground parts. The water content, chlorophyll content, and protein content of Chinese cabbage leaves also markedly decreased with the increase of the BDE-47 concentration. In addition, BDE-47 weakened the photosynthetic capacity of the leaves, which was supported by the decreased photosynthetic parameters [net photosynthetic rate (PN), transpiration rate (ET), stomatal conductance (gS)]. Although the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in the leaves were enhanced after exposure to BDE-47, the increased malondialdehyde (MDA) content attested to the existence of membrane lipid peroxidation. The increased plasma membrane permeability and the decreased chlorophyll fluorescence parameters [the maximum quantum yield of PSII photochemistry (Fv/Fm), the electron transport rate (ETR), and the photochemical efficiency of PSII (Fv/FF)] further proved that the plasma membrane and photosynthetic membrane were damaged by BDE-47. Our study demonstrated the phytotoxicities of BDE-47 to Chinese cabbage, which can provide valuable information for understanding the toxicity of BDE-47 on vegetables.

PBDEs are excellent brominated flame retardants, widely used in various industrial products and consumer products such as plastic products, electronic appliances, building materials, and textile products. As PBDEs are not chemically bound to industrial materials, they can be easily released into the environment by volatilization, ashing, exudation, deposition, etc. (Alaee et al., 2003). Contamination by PBDEs has been detected in air, water, soil, sediment, and other environmental media (Li et al., 2018). PBDEs are lipophilic, refractory, and bioaccumulative persistent organic pollutants that can be transmitted to the higher trophic levels via the food chain (van de Merwe et al., 2011), posing a great threat to human health (Shang et al., 2016). A total of 209 homologues of PBDEs have been discovered, including tetrabromobis, pentabromobis, hexabromobis, octabromobis, decabromodiphenyl ether, etc. (Fonnum and Mariussen, 2009). At present, studies on the toxicity of PBDEs have been commonly conducted on animals (Macaulay et al., 2015), whereas the toxicological studies on plants are limited to only a few plant materials, including marine microalgae (Kallqvist et al., 2006), mangrove (Farzana et al., 2017; Farzana and Tam, 2018; Wang et al., 2014), maize (Xu et al., 2015), duckweed (Qu et al., 2018; Sun et al., 2016), rice (Chen et al., 2018; Li et al., 2018), and ryegrass (Xie et al., 2013).

The aforementioned studies show that PBDEs are toxic to most plants. The toxic effects of PBDEs on the growth and development of higher plants include the reduction of the germination rate, inhibition of root and stem elongation, leaf shedding, and significant decrease in biomass (Farzana et al., 2017; Li et al., 2018; Qu et al., 2018; Wang et al., 2014; Xu et al., 2015). The key mechanism of the phytotoxicity induced by PBDEs is the overproduction of reactive oxygen species (ROS) (Farzana et al., 2017; Qu et al., 2018; Wang et al., 2014; Xie et al., 2013; Xu et al., 2015). The excessive ROS can cause oxidative stress, including peroxidation of cellular components and enzyme inactivation, which disturbs the normal physiological metabolism of plants. The latest studies show that PBDEs can damage the membranes directly and affect the electron transport of chloroplasts and mitochondria, leading to the electron leakage and the generation of ROS (Begović et al., 2016; Pazin et al., 2015; Pereira et al., 2013; Qu et al., 2018).

The mechanism for detoxifying PBDEs toxicity in plants is not well understood. PBDEs can be degraded to lower brominated homologues, hydroxylated PBDEs, and methoxylated PBDEs by debrornination, hydroxylation, and redox in organisms, respectively (Su et al., 2012; Xu et al., 2015). Lower brominated diphenyl ethers have fewer bromine atoms on the benzene ring, less steric hindrance, and greater water solubility than the higher brominated diphenyl ethers. Therefore, these molecules are more easily absorbed by cells and have greater toxicity than the higher brominated diphenyl ethers (Bragigand et al., 2006; Huang et al., 2013). Among the low-brominated diphenyl ethers, BDE-47 is one of the most toxic homologs. As far as we know, no study focused on the phytotoxicity of BDE-47 in vegetables has been conducted. Understanding the toxicity of BDE-47 on vegetables has significant implications for food safety.

Chinese cabbage (Brassica rapa L. ssp. Pekinensis) is one of the highest-yielding and the most widely cultivated vegetables in China. Hence, Chinese cabbage was used as a model plant to study the toxic effects of BDE-47 on vegetables. We systematically analyzed the toxic effects of BDE-47 on Chinese cabbage in terms of growth, physiological metabolism, photosynthetic function, and antioxidative capacity. This study provides useful information for a further understanding of the toxic effects and toxicity mechanisms of BDE-47 on vegetables.

Materials and Methods

Plant materials and culture conditions. The seeds of a Chinese cabbage cultivar (Brassica rapa L. ssp. Pekinensis; Brand
name: Qingmuyi; Purchased from Tianjin Seed Co., Ltd.) were selected and germinated in sand moistened with the Hoagland nutrient solution. All seedlings were cultured under the controlled conditions at a light intensity of 240 μmol·m⁻²·s⁻¹ provided by cool white-fluorescent lamps with a photoperiod of 14 h each day, 20 ± 2 °C, and relative humidity of 60%.

Treatments. After germination for 14 d, four uniformly germinated seedlings were transplanted into white ceramic pots (diameter: 10 cm; depth: 18 cm), which contained 2 L of Hoagland nutrient solution without (the control group) or with 10, 25, 50, 75, and 100 μg·L⁻¹ BDE-47, respectively. BDE-47 standard (purity 98.6%, gas chromatography–mass spectrometry certified) was purchased from CHMSRV-PM (Westchester, NY). The test solutions were prepared by a serial dilution of a stock solution of 1 mg·L⁻¹ BDE-47 dissolved in N,N-dimethylformamide (DMF). The no-observed-effect concentration of DMF to Chinese cabbage is 2% (v/v).

Therefore, the concentration of DMF in each test solution was adjusted to 1%. The solutions were renewed every 3 d to ensure a stable BDE-47 concentration. All seedlings of Chinese cabbage were harvested for the determination of the physiological and biochemical parameters after a 30-d exposure to BDE-47.

Measurements. The growth parameters were expressed as the total fresh weight and the root:shoot ratio. After treated with BDE-47 for 30 d, the total fresh weight of each seedling was measured, and then, the seedling was cut at the rhizome junction to measure the fresh weight of shoot (the overground parts) and the root. The root:shoot ratio was defined at root fresh weight/shoot fresh weight. The root activity of plants was expressed as the bio-oxidation amount of α-naphthylamine by using the method described by Lewis et al. (1982). The leaf water content was defined as fresh weight/dry weight.

The plasma membrane permeability of the leaves was measured with a conductivity meter and expressed as relative conductivity (%). Relative conductivity (%) was defined as primary conductivity/total conductivity × 100%. According to the method described by Bradford (1976), the total soluble protein content in leaves was quantified with bovine serum albumin as the standard. Three leaf discs were punched with a 0.9-cm diameter hole puncher and cut into 1-mm-wide filament for determination of the chlorophyll content. The chlorophyll in leaf discs was extracted with dimethyl sulfoxide and 80% acetone and then determined at 646.6 nm and 663.6 nm (Qiu et al., 2016).

The OJIP curve of the leaves was measured with a Handy PEA (Plant Efficiency Analyser; Hansatech Instrument Ltd., King’s Lynn UK). The seedlings were dark-adapted for 2 h before the measurements. The changes of the chlorophyll fluorescence parameters induced by BDE-47 were derived according to the OJIP curve and presented as radar plots. The chlorophyll fluorescence parameters include Fₘₐₓ, Fₘᵦ, Pₐᵦ, RC/CS, the ABS/CS, TRₛ, ETₛ, CS, DIₛ, PIᵦ and the DF (Kalaji et al., 2016; Stirbet and Govindjee, 2011; Strasser et al., 2000).

Pₐ and gₛ were measured by a Ciras-2 portable photosynthetic system (Hansatech Instruments, Hitchin, UK). It was determined under the conditions of natural air CO₂ concentration of 430 μmol·mol⁻¹, humidity of 40%, light intensity of 1000 μmol·m⁻²·s⁻¹, and temperature of 30 °C. For the determination of antioxidant enzyme activities, fresh leaves (1.0 g) were homogenized in a mortar with 10 mL of extraction solution (pH 7.8) containing 50 mmol·L⁻¹ phosphate buffer, 1 mmol·L⁻¹ ethylenediaminetetraacetic acid, 20% (v/v) glycerol, and 1 mmol·L⁻¹ dithiothreitol in an ice bath. The homogenate was centrifuged at a rate of 5000 g, 4 °C, for 10 min and then 10,000 g, 4 °C, for 15 min. The supernatants of the crude enzyme extracts were separated in EP tubes (0.5 mL in each tube) and stored at −40 °C (Zhang et al., 2007).

The activity of SOD was assayed by the method of Tandy et al. (1989). One unit (U) of the SOD activity was defined as the amount of enzyme required for 50% inhibition of the nitro blue tetrazolium reduction to formazan. The sample absorbance was determined by a ultraviolet-visible spectrophotometer at 560 nm.

The POD activity was determined based on the oxidation of guaiacol and the increase of the absorbance at 470 nm (Zhang et al., 2007). The molar extinction coefficient of tetraguaiacol is 26.6 mm⁻¹·cm⁻¹ for the calculation of POD activity.

The CAT activity was measured by recording the decrease of the absorbance at 240 nm for 1 min, according to the method of Knoorzer et al. (1996). The molar extinction coefficient of H₂O₂ is 27.78 mm⁻¹·cm⁻¹ for the calculation of CAT activity. The degree of lipid peroxidation in plants was assessed by measuring the content of MDA following the method of Aravind and Prasad (2003).

Statistical analysis. The data were given as mean ± sd. All statistical analyses were analyzed using the statistical program package SPSS 16.0 (SPSS Inc., Chicago, IL). Analysis of variance and Duncan’s multiple range tests were used to analyze the differences among the treatments. Means were separated at a significant level of P < 0.05 by different letters.

Effects of BDE-47 on plant growth. BDE-47 treatments significantly inhibited the growth of the seedlings of Chinese cabbage. After a 30-d exposure to BDE-47 at a concentration of 25, 50, 75, and 100 μg·L⁻¹, the total fresh weight of Chinese cabbage decreased by 33.3%, 47.5%, 78.1%, and 90.7%, respectively, compared with the control group (Fig. 1A). The seedlings wilted at 100 μg·L⁻¹ BDE-47. In contrast, the root:shoot ratios of Chinese cabbage elevated significantly with the increase of BDE-47 concentration in the nutrient medium, which were 1.36, 1.79, 1.91, 2.52, and 3.90 times of the control, respectively (Fig. 1B). Therefore, BDE-47 has a significant inhibitory effect on the growth of Chinese cabbage seedlings, especially on the overground parts. BDE-47 also affected the root activity of Chinese cabbage. After a 30-d exposure to 10, 25, 50, 75, and 100 μg·L⁻¹ BDE-47, the root activity of Chinese cabbage dropped to 88.5%, 78.4%, 50.3%, 28.0%, and 26.7% of the control group, respectively (Fig. 2).

Effects of BDE-47 on physiological/toxicological aspects. The leaf water content of Chinese cabbage treated with 10 μg·L⁻¹ BDE-47 had no significant difference compared with the control, but it decreased significantly when the concentration of BDE-47 was increased to 25 to 100 μg·L⁻¹ (Fig. 3A). Compared with the control, the leaf water content of Chinese cabbage treated with 25, 50, 75, and 100 μg·L⁻¹ BDE-47 decreased by 4.81%, 7.69%, 22.95%, and 26.38%, respectively. The leaf chlorophyll content was more sensitive to the toxicity of BDE-47, which was decreased obviously even under
exposure to 10, 25, 50, 75, and 100 μg·L⁻¹ BDE-47, the soluble protein contents were reduced to 98.6%, 80.7%, 71.1%, 40.0%, and 36.1% of the control, respectively (Fig. 3C).

The plasma membrane permeability often is used as an indicator of the stability and integrity of the plasma membrane. The plasma membrane permeability of the Chinese cabbage leaves treated with 10, 25, 50, 75, and 100 μg·L⁻¹ BDE-47 were 112.1%, 115.4%, 125.3%, 136.0%, and 148.1% of the control, respectively (Fig. 4A). MDA is the end product of lipid peroxidation and often used as diagnostic index of oxidative injury in plants. After a 30-d exposure to 10, 25, 50, 75, and 100 μg·L⁻¹ BDE-47, the MDA content in the Chinese cabbage leaves increased to 1.55, 2.01, 2.15, 2.53, and 3.07 times of the control, suggesting that obvious lipid peroxidation occurred in the BDE-47–treated plants (Fig. 4B).

**Photosynthetic activity.** Photosynthesis is one of the most important and sensitive metabolic processes in plants. The BDE-47 treatments significantly affected not only the light reaction process but also the carbon assimilation ability of Chinese cabbage leaves. The chlorophyll fluorescence parameters in Fig. 5 showed that the BDE-47 treatments induced a continuous decrease in $F_{m}$ and $F_{v}/F_{m}$ and a continuous increase in $F_{o}$ and $D_{l}/CS$, which indicated that PSII in Chinese cabbage leaves was damaged after exposure to BDE-47. BDE-47 also induced a continuous decline of the amount of active PSII RCs per CS ($RC/CS$). Therefore, the $ABS/CS$, $TR_{o}/CS$, and $ET_{o}/CS$ all dropped significantly with the increase of the BDE-47 concentration, leading to the decline of the $PI$ and the $DF$ of the BDE-47–treated Chinese cabbage leaves. For example, the $P_{I_{th}}$ values of the leaves treated with 10, 25, 50, 75, and 100 μg·L⁻¹ BDE-47 were 59.9%, 50.1%, 37.5%, 15.5%, and 4.8% of the control, respectively. The results revealed that BDE-47 had obvious toxicity to the photosystem of Chinese cabbage leaves. The damaged photosystem of the BDE-47–treated leaves could not provide sufficient ATP and NADPH for carbon assimilation, which led to a decrease of the carbon assimilation capacity.

The results of $P_{n}$ showed that the CO₂ assimilation ability of Chinese cabbage leaves was inhibited by the BDE-47 treatments, which decreased to 91.94%, 91.47%, 60.57%, 36.23%, and 19.89% of the control after a 30-d exposure to 10, 25, 50, 75, and 100 μg·L⁻¹ BDE-47, respectively (Fig. 6A). The BDE-47 treatments also affected the $g_{s}$ of the leaves, which is another reason for the decline of $P_{n}$ of the BDE-47–treated leaves.

**Antioxidant defense system.** Previous studies have reported that PBDEs can induce the generation of ROS and cause remarkable oxidative damage to plants (Farzana et al., 2017; Qiu et al., 2018; Wang et al., 2014; Xie et al., 2013; Xu et al., 2015). The antioxidants play an important role in detoxifying the oxidative stress caused by PBDEs. The SOD activities of Chinese cabbage leaves were elevated continuously with the increase of the BDE-47 concentration in the nutrient medium. After a 30-d exposure to 10, 25, 50, 75, and 100 μg·L⁻¹ BDE-47, the SOD activities were 1.31, 1.58, 2.33, 4.60, and 6.52 times of that in the control, respectively (Fig. 7A). The POD activities increased continuously under 10 to 75 μg·L⁻¹ BDE-47 but dropped rapidly under 100 μg·L⁻¹ BDE-47 (Fig. 7B). The CAT activities of the BDE-47–treated leaves were all greater than that of the control group; however, CAT was less sensitive to the BDE-47 toxicity compared with SOD and POD (Fig. 7C). SOD, POD, and CAT were all involved in scavenging ROS under the stress of BDE-47.

**Discussion**

Only a few phytotoxicity studies of PBDEs have been carried out on higher plants at present, and none has been reported in vegetables. The toxic effects of BDE-47 on Chinese cabbage were investigated in this study. Although there were no significant changes in the biomass (Fig. 1), leaf water content (Fig. 3A), and leaf protein content (Fig. 3C) of Chinese cabbage after a 30-d exposure to 10 μg·L⁻¹ BDE-47, the obvious toxic effects of 10 μg·L⁻¹ BDE-47 were found on root activity (Fig. 2), chlorophyll content (Fig. 3B), biomembrane (Fig. 4), and photosynthesis (Fig. 5 and Fig. 6) of Chinese cabbage leaves. High levels of BDE-47 (25–100 μg·L⁻¹) in the nutrient medium showed obvious toxic effects on both the growth and the metabolism of Chinese cabbage, and the Chinese cabbage seedlings wilted at 100 μg·L⁻¹ BDE-47. Similar toxic effects of BDE-47 also have been reported in other higher plants. After a 14-d exposure to 5 to 15 μg·L⁻¹ BDE-47, duckweed plants were significantly suppressed and died at 20 μg·L⁻¹ BDE-47 (Qiu et al., 2018; Sun et al., 2016). The germination rate and growth of maize were markedly inhibited by 15 μg·L⁻¹ BDE-47 (Xu et al., 2015). The tolerance of different rice varieties to 100 to 500 μg·L⁻¹ BDE-47 toxicity was different, mainly because of the different contents of amino acids and organic acids that contributed to scavenging the ROS in each rice variety (Chen et al., 2018). *Kandelia obovata* had the best resistance to BDE-47 toxicity among these plants, which can survive 5 μg·L⁻¹ BDE-47 for 56 d (Wang et al., 2014). These studies and our results suggest that Chinese cabbage (Qingmaye) was a very sensitive plant to the toxicity of BDE-47.

Our study showed that the toxic effects of BDE-47 on Chinese cabbage included the following four aspects. First, the BDE-47 treatments affected the water metabolism of Chinese cabbage, resulting in a decrease of the leaf water content (Fig. 3A). The decrease of leaf water content would cause the stoma
weed and phenomena have been reported in duckweed and Kandelia obovata (Qiu et al., 2018). As BDE-47 is bound up with the formation of ROS, Chinese cabbage can increase the activities of SOD, POD, and CAT to scavenge ROS under BDE-47 stress (Fig. 7). However, our study showed that the elevated antioxidant activity was not a key mechanism to relieve the BDE-47 toxicity, because the metabolites of PBDEs such as lower brominated congeners, hydroxylated congeners, and methoxylated congeners are more stable in vivo and still exert adverse influences on organisms (Huang et al., 2013; Qiu et al., 2007; Su et al., 2012; Wan et al., 2010). For instance, BDE-47 can be debrominated, hydroxylated, and redox degraded to lower brominated homologue congeners, hydroxytetrabromodiphenyl ethers and methoxylated dibromodiphenyl ethers, respectively, which are more biohazardous than BDE-47 (Su et al., 2012; Xu et al., 2015). So far, metabolic pathways for completely degrading PBDEs have not been discovered. Some plants such as maize and Kandelia obovata have strong resistance to BDE-47 (Wang et al., 2014; Xu et al., 2015), but their resistance mechanism is still unclear.

**Conclusion**

Our results showed that Chinese cabbage (Qingmaye) was a sensitive vegetable to the toxicity of BDE-47. Exposure to 10 to 100 µg L⁻¹ BDE-47 for 30 d caused a prominent toxic effect on Chinese cabbage seedlings, including growth inhibition, metabolic interference, biomembrane disruption, and photosynthesis reduction. Although the antioxidant enzyme system was activated under the stress of BDE-47 in Chinese cabbage, the BDE-47 toxicity could not be effectively relieved. Therefore, Chinese cabbage (Qingmaye) can be used as a material for the in-depth research of
BDE-47 phytotoxicity. Since physiological changes induced by BDE-47 might affect the yield and quality of Chinese cabbage, our results can also provide useful reference for Chinese cabbage cultivation in PBDE-polluted soil.

Fig. 6. Net photosynthetic rate (Pn) (A) and stomatal conductance (gs) (B) of Chinese cabbage leaves exposed to BDE-47 for 30 d. The bars are means ± s (n = 5).

**Fig. 7.** The superoxide dismutase (SOD) (A), peroxidase (POD) (B), and catalase (CAT) (C) activities of Chinese cabbage leaves exposed to BDE-47 for 30 d. The bars are means ± s (n = 5).

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