biologia plantarum
an international journal for experimental botany

BIOLOGIA PLANTARUM (2022) 66: 67-75 DOI: 10.32615/bp.2021.076

Evaluation of the phytotoxicity of decabromodiphenyl ether (BDE-209) in Chinese cabbage

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Abstract

Decabromodiphenyl ether (BDE-209), a major component of brominated flame retardants, has been detected in considerable amounts in the soil. Given that BDE-209 is toxic, ubiquitous, and persistent, it may cause toxic effects on vegetables planted in contaminated soil. In this study, Chinese cabbage seedlings cultivated in the soil contaminated by BDE-209 (5 - 20 mg kg^{-1}) for 60 d were investigated to evaluate the phytotoxicity of BDE-209 in terms of growth, physiological responses, photosynthetic function, and antioxidant capacity. The results showed that BDE-209-induced phytotoxicity was reflected in the growth suppression, the decrease of chlorophyll content and soluble protein content, and especially in the reduced photosynthetic parameters (net photosynthetic rate and stomatal conductance). BDE-209 showed direct toxicities to plasma membranes causing their elevated permeability. In addition, BDE-209 induced the overproduction of reactive oxygen species (ROS), membrane lipid peroxidation and protein carbonylation, manifesting in the increased content of $O_2^{\bullet-}$, H_2O_2, malondialdehyde, and carbonyl. Chinese cabbage seedlings activated the antioxidant defence system (superoxide dismutase and peroxidase) to scavenge the ROS and counter-balance the oxidative stress caused by BDE-209, while the toxicity could not be effectively alleviated. Our study will provide valuable information for further understanding of the phytotoxicity of polybrominated diphenyl ethers.

Keywords: antioxidative enzymes, *Brassica rapa*, carbonyl, chlorophyll, malondialdehyde, membrane permeability, net photosynthetic rate, ROS, stomatal conductance.

Introduction

Polybrominated diphenyl ethers (PBDEs) are widely used brominated flame retardants (BFRs) with excellent thermal stability and flame retardancy, which have been used in a wide array of products, including textiles, plastics, electronic equipment, and building materials (McGrath *et al*. 2017). PBDEs consist of up to ten bromine atoms, which have 209 congeners. Penta-BDE, octa-BDE, and deca-BDE are the major commercial BFRs of PBDEs composed of a mixture of congeners (Law *et al*. 2006). Due to their toxicity, persistence, and bioaccumulation, some lower brominated congeners have been listed as persistent organic pollutants (POPs) and banned by the European

Received 31 August 2021, *last revision* 2 November 2021, *accepted* 2 December 2021.
Abbreviations: BDE-209 - decabromodiphenyl ether; BFRs - brominated flame retardants; CAT - catalase; Chl - chlorophyll; DMSO - dimethyl sulfoxide; DNPH - 2,4-dinitrophenylhydrazine; DSBs - DNA double-strand breaks; g_s - stomatal conductance; HPLC - high performance liquid chromatography; MDA - malondialdehyde; NBT - nitroblue tetrazolium; $O_2^{\bullet-}$ - superoxide anion; PAHs - polycyclic aromatic hydrocarbons; PBDEs - polybrominated diphenyl ethers; P_N - net photosynthetic rate; POD - peroxidase; POPs - persistent organic pollutants; PS II - photosystem II; ROS - reactive oxygen species; SOD - superoxide dismutase; TBA - thiobarbituric acid; TEM - transmission electron microscopy.
Acknowledgements: This work was supported by the Key Project of Natural Science Research in Colleges and Universities in Jiangsu Province (19KJA310009), the Agricultural Seed Project in Shandong Province (2019LZGC020), and the Natural Science Foundation of Shandong Province (ZR2020MC144).
Conflict of interest: The authors declare that they have no conflict of interest.

Union (Ricklund *et al*. 2008, Mohr *et al*. 2014). Deca-BDE predominantly consists of decabromodiphenyl ether (BDE-209) (≥ 97 %), and its toxicity is relatively low. However, deca-BDE can be transformed into lower brominated congeners by biotransformation or natural degradation and exhibits higher biological toxicity (Cai *et al*. 2015). Deca-BDE pollution has been detected in various environmental media and organisms (Li *et al*. 2015, Shang *et al*. 2016). It was reported that the domestic demand for deca-BDE in China is the highest in Asia, which is also widely used in other countries. Deca-BDE is not covalently bound with chemical products and can be easily released into the environment, leading to the widespread of BDE-209 in the environment (Ni *et al*. 2013, Ji *et al*. 2017). The soil content of PBDEs is as high as 2720 - 4250 μg kg^{-1} in some electronic waste recycling sites and sewage irrigated areas in Southeast China, of which BDE-209 accounts for 35 - 82 % (Leung *et al*. 2007).

According to previous studies, BDE-209 exhibits toxicity to plants and animals, posing emerging risks to humans and ecosystems *via* the food chain (Hu *et al*. 2010, Ji *et al*. 2017, Pereira *et al*. 2017, Li *et al*. 2018). PBDEs have been detected in human tissues (Cai *et al*. 2015). In recent years, the uptake, translocation, bioaccumulation, and metabolism of BDE-209 in plants were investigated by numerous studies (Huang *et al*. 2010, Chow *et al*. 2015, Bizkarguenaga *et al*. 2016, Deng *et al*. 2016, Zhang *et al*. 2021). BDE-209 can cause morphological, physiological and biochemical alterations in exposed plants (Xie *et al*. 2013, Li *et al*. 2018). Due to the higher molecular mass or lower solubility, the toxicity of BDE-209 (higher brominated PBDE) is significantly less than that of lower brominated congeners (such as BDE-47 and BDE-99) (Zhang *et al*. 2013, Farzana *et al*. 2016, Zhao *et al*. 2019). Lower plants are more sensitive to PBDE-induced toxicity than higher plants, and higher terrestrial plants are more sensitive to BDE-209 than higher aquatic plants (*e.g. Lemna minor*) (Sun *et al*. 2019 and 2020). However, a number of reports have shown that exposure to BDE-209 leads to obvious phytotoxicity to some higher terrestrial plants, such as mosses, ryegrass, rice, and mangroves (Xie *et al*. 2013, Li *et al*. 2018, Farzana *et al*. 2019, Zhao *et al*. 2019). The toxicity mechanisms of BDE-209 on plants have not been well documented at present.

The induced overproduction of reactive oxygen species (ROS) has been considered to be one of the causes of BDE-209 toxicity to plants. This toxicity is widespread in plants, including microalgae (*Heterosigma akashiwo* and *Karenia mikimotoi*), moss (*Polygonum cuspidatum*), duckweed (*Lemna minor*), ryegrass (*Lolium perenne*), rice (*Oryza sativa*), and mangroves (*Kandelia candel*) (Xie *et al*. 2013, Zhang *et al*. 2013, Farzana and Tam 2018, Li *et al*. 2018, Farzana *et al*. 2019, Sun *et al*. 2019, Zhao *et al*. 2019). At the same time, plants could prevent oxidative stress by activating different antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Li *et al*. 2018, Zhao *et al*. 2019). However, no study has been performed on the phytotoxicity of BDE-209 to vegetables until now.

Vegetables are an important food source for human beings. The toxic damage of BDE-209 to vegetables at the seedling stage can affect their productivity, nutritional value, and food safety. In this study, we used Chinese cabbage seedlings, one of the highest-yielding vegetables in East Asia, to systematically analyze the toxic effects and mechanisms of BDE-209, which will help to understand the toxicity of PBDEs on vegetables.

Materials and methods

Chemicals: BDE-209 was obtained from *CHMSRV-PM* (West Chester, USA). All solvents such as toluene, acetone, and dimethyl sulfoxide (DMSO), were of HPLC grade. Deionized water was used for all experiments. All other chemicals and reagents used in this experiment were of analytical-reagent grade.

Soil preparation: The soil (quartz sand) was naturally air-dried and passed through a 2-mm sieve, and then washed with distilled water and oven-dried at 75 ºC. Subsequently, the dried soil was autoclaved at 121 ºC for 2 h to eradicate the indigenous microorganisms. An aliquot of soil (25 kg) was individually spiked with 5 different amounts of BDE-209 dissolved in 1 000 cm^3 mixed solvent of toluene and acetone (v:v = 1:9) to reach final concentrations of 0 (solvent control), 5, 10, 15, and 20 mg kg^{-1}(soil). The amended/spiked soils were mixed thoroughly by tumbling for 2 d in the dark and then allowed to dry in a dark ventilation cabinet for 7 d with shaking for 1 h every day to evaporate toluene and acetone completely. The soils were precultivated with aeration for 14 d at room temperature in the dark.

The experiment was conducted in plastic pots with dimensions of 12 × 12 × 10 cm (length × width × height) and each pot held 1 kg of BDE-209-spiked soil. To minimize the evaporation and photolysis of BDE-209 in the amended soils, the upper 1.0 cm of each pot was covered with non-spiked soil to establish a buffer layer.

Plants and culture conditions: Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*. cv. Qingmaye) seeds were obtained from Shandong Academy of Agricultural Sciences, China. The seeds were sterilized with 3 % (v/v) H_2O_2 for 5 min, followed by thoroughly rinsing with deionized water. Then, the seeds were placed on a moistened filter paper for germination in the dark at 20 ºC. Nine germinated seeds were sown in each pot. After 5 d of emergence, the seedlings were thinned to 4 - 5 plants in order to obtain uniform plants per pot. Five replicates were prepared for each treatment.

All the pots were placed in a controlled environment chamber for 60 d at an irradiance of 200 - 240 μmol m^{-2} s^{-1}, a 14-h photoperiod, a constant temperature of 20 ± 2 ºC, and relative humidity of 50 - 60 %. The positions of the pots were randomly rearranged every 3 d. Moderate amounts of Hoagland nutrient solution were added in the pots every 3 d to maintain moisture and soil nutrients.

After a period of 60 d, the plant height of Chinese cabbage seedlings was determined from the base of

the stem to the tip of the shoot. The seedling was cut at the rhizome junction, and the fresh mass (f.m.) of the aboveground parts was measured.

Chlorophyll (Chl) content and total soluble protein content: The Chl content of Chinese cabbage leaves was measured according to the method of Qiu *et al*. (2016). Chl was extracted with dimethyl sulfoxide and 80 % acetone (1:4 = v:v). The absorbance of the extract was recorded at 646.6 and 663.6 nm, respectively. The total soluble protein content in leaves was measured following the method of Bradford (1976), with bovine serum albumin (BSA) for calibration.

Photosynthetic parameters: The net photosynthetic rate (P_N) and stomatal conductance (g_s) were determined by a *Ciras-2* portable photosynthetic system (*Hansatech*, Hitchin, UK) under the temperature of 25 ºC, the humidity of 40 %, the irradiance of 1 000 µmol m^{-2} s^{-1}, and CO_2 concentration of 430 µmol mol^{-1}.

Plasma membrane permeability and lipid peroxidation: The plasma membrane permeability was assessed by a modified version of the protocol described by McClendon (1927) and expressed as relative conductivity [%], which was defined as original conductivity/total conductivity × 100 %.

For *in vivo* (chronic) toxicity experiment, after a 60-d exposure to BDE-209 at concentrations 0, 5, 10, 15, and 20 mg kg^{-1}(soil), the Chinese cabbage leaves were rinsed with deionized water and then put into test tubes with 20 cm^3 of deionized water. Afterwards, they were vacuumed for 10 min and left at room temperature for 30 min, and the original conductivity of the supernatant was determined. The test tubes were heated in a boiling water bath at 100 ºC for 20 min, and the total conductivity of the supernatant was measured after cooling to room temperature.

For *in vitro* (direct) toxicity experiment, the healthy leaves of Chinese cabbage seedlings cultured in Hoagland nutrient solution were cut out and rinsed with deionized water (the cultivation conditions for *in vitro* toxicity experiment were the same as in *in vivo* experiment). Afterwards, they were put into the solutions containing 0, 0.5, 1, 1.5, and 2.0 mg dm^{-3} BDE-209, respectively, and vacuumed for 10 min and left at room temperature for 60 min, allowing BDE-209 to penetrate the plant tissue. The following experimental operations were the same as the above steps.

Lipid peroxidation was determined by estimating the content of malondialdehyde (MDA) using the thiobarbituric acid (TBA) test, and the absorbance of the final TBA reactive substances was measured at 450, 532, and 600 nm, respectively (Xie *et al*. 2013).

Content of ROS ($O_2^{\bullet-}$ and H_2O_2) and protein carbonyl: The content of superoxide anion ($O_2^{\bullet-}$), a common ROS, was measured following the principles that $O_2^{\bullet-}$ oxidizes hydroxylamine hydrochloride to form nitrite which further reacts with naphthylamine and *p*-aminobenzenesulfonamide to produce a pink-coloured complex (Choudhury and Panda 2005). The production of H_2O_2 was measured according to a modified method described by Sergiev *et al*. (1997). The quantification of protein carbonyl was used as an index for oxidative damage to proteins. The level of carbonylation was assessed based on the 2,4-dinitrophenylhydrazine (DNPH) colorimetric method described by Levine *et al*. (1994).

Determination of antioxidants: For crude antioxidant enzyme extraction, fresh leaves (1.0 g) were homogenized in a mortar with 10 cm^3 ice-cold extraction solution [50 mM phosphate buffer, pH 7.8, 1 mM EDTA, 20 % (v/v) glycerol, and 1 mM dithiothreitol]. The homogenate was centrifuged at 5 000 *g* and 4 ºC for 10 min, and then re-centrifuged at 10 000 *g* and 4 ºC for 15 min. The supernatant was decanted into 1.5-cm^3 microcentrifuge tubes for the determination of enzyme activities.

The activity of superoxide dismutase (SOD) in the enzyme extract was determined based on photochemical reduction of nitroblue tetrazolium (NBT), as described by De Azevedo Neto *et al*. (2006). One unit (U) of SOD activity was defined as the quantity of enzyme required to 50 % inhibition of the NBT photoreduction rate.

The peroxidase (POD) activity was assayed using guaiacol oxidation by measuring the increase of absorbance at 470 nm (ε = 26.6 mM^{-1} cm^{-1}) for 1 min following the method of Zhang *et al*. (2007). One unit of POD activity was quantified by the amount of protein needed to produce 1 µmol tetraguaiacol in 1 min.

The catalase (CAT) activity was quantified by determining the decrease of the absorbance at 240 nm for 1 min during the decomposition of H_2O_2 (27.78 mmol cm^{-3}) (Xie *et al*. 2013). One unit of CAT activity was quantified by the amount of protein needed to decompose 1 µmol of H_2O_2 in 1 min.

Statistical analysis: All data were expressed as the means ± SDs. The statistical analyses of the data were performed using the *SPSS* software (*v. 16.0*, *SPSS Inc.*, Chicago, IL, USA). A parametric one-way analysis of variance (*ANOVA*) with Duncan's multiple-comparison test was conducted to examine the significant differences among treatments at a 95 % confidence limit ($P < 0.05$).

Results and discussion

Our results showed that growth inhibition of Chinese cabbage seedlings was manifested by the reduced shoot biomass and plant height after a 60-d exposure to BDE-209 (Fig. 1). The seedlings exposed to BDE-209 at 5 mg kg^{-1}(soil) had no significant difference in the shoot biomass compared with the control group, while the fresh mass decreased greatly for BDE-209 at 10 - 20 mg kg^{-1}(soil) (Fig. 1*A*). The treatments of BDE-209 at 5 - 20 mg kg^{-1}(soil) also decreased the height of shoots (Fig. 1*B*), accompanied by the chlorosis of leaves (Fig. 2*A*). These results were in accordance with the findings of the inhibited growth of rice and *K. obovata* after exposure to

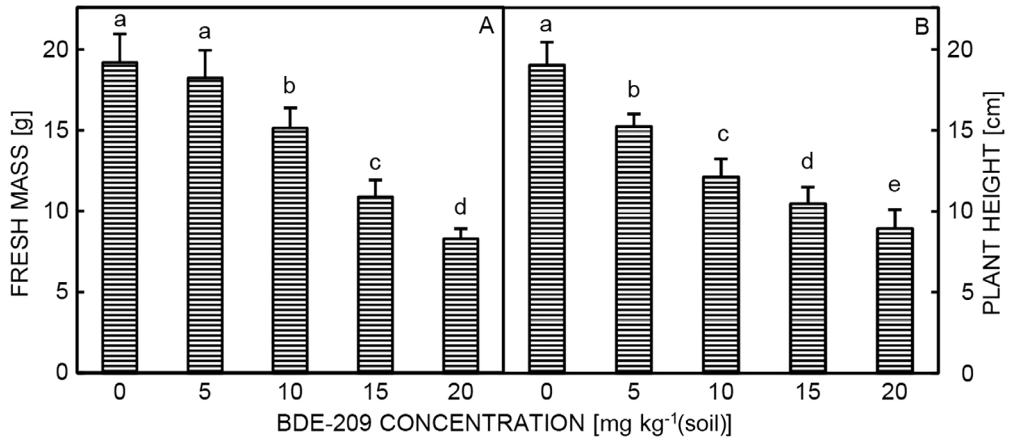

Fig. 1. The fresh mass (*A*) and plant height (*B*) of shoots of Chinese cabbage exposed to BDE-209 for 60 d. Means ± SDs of ten replicates. Different letters indicate significant differences between different treatments ($P < 0.05$).

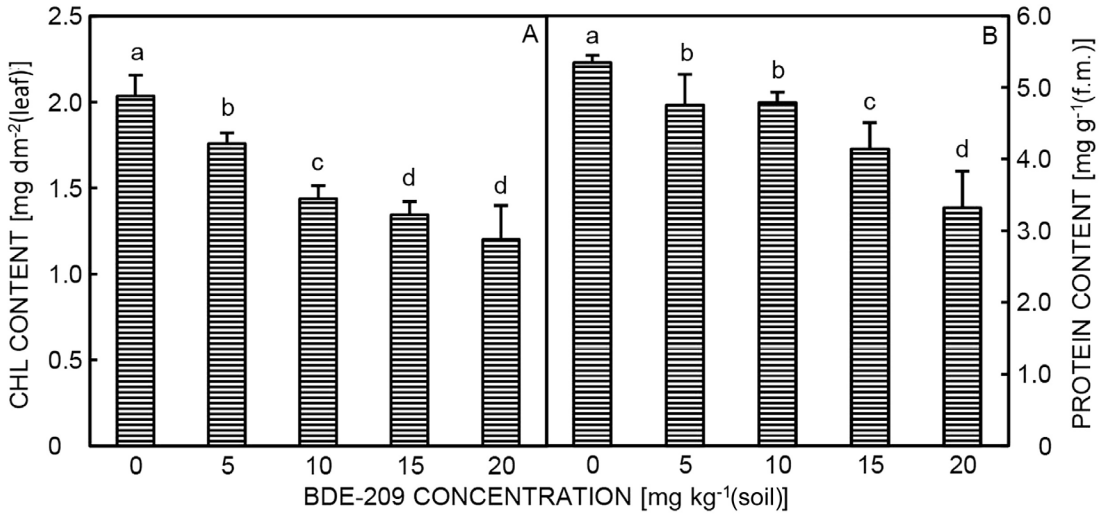

Fig. 2. The chlorophyll content (CHL [mg dm^{-2} (leaf)]; *A*) and soluble protein content (*B*) of Chinese cabbage leaves exposed to BDE-209 for 60 d. Means ± SDs of five replicates. Different letters indicate significant differences between different treatments ($P < 0.05$).

BDE-209 (Li *et al*. 2018, Farzana *et al*. 2019).

Chlorophyll is the most crucial pigment in photosynthesis, and its content can reflect the plant ageing or damage of plant tissues and organs under stress (De Azevedo Neto *et al*. 2006). In the present study, the Chl content of Chinese cabbage leaves decreased when the concentrations of BDE-209 were 5 - 20 mg kg^{-1}(soil) (Fig. 2*A*), indicating that the photosynthetic abilities of the plants were decreased and their growth was inhibited. Previous studies had shown that BDE-209 decreases the Chl content in moss (*P. cuspidatum*) (Zhao *et al*. 2019), floating freshwater plant (*L. minor*) (Sun *et al*. 2019), and mangrove plants (Farzana *et al*. 2019), which is consistent with the results in this study.

Soluble proteins participate in various physiological activities in cells, and their content is an important index reflecting cell activity. The result in Fig. 2*B* indicated that BDE-209 apparently reduced the soluble protein content in the leaves of Chinese cabbage seedlings, leading to a decrease in cell viability. Similar results have been reported in *L. minor*, which show greatly decreased the total soluble protein content when BDE-47 and BDE-209 concentrations were 5 - 20 μg cm^{-3} and 15 - 20 mg dm^{-3}, respectively (Qiu *et al*. 2018, Sun *et al*. 2019).

It is well known that chlorophyll and proteins are vital components of photosystems (PSs) I and II. The reduction of their content in our study indicated that the structure and function of PS II might be damaged. Another reason for the reduced PS II activity is the direct toxicity of PBDEs to the photosynthetic membranes (Qiu *et al*. 2018). PBDEs, similar to polycyclic aromatic hydrocarbons (PAHs), are lipophilic, which can directly act on the lipids of the thylakoid membranes and break the coordination bonds between chlorophyll molecules (Kreslavski *et al*. 2017). The damaged photosystem could not provide sufficient ATP and NADPH for CO_2 assimilation, and consequently reduce the P_N values of the BDE-209 treated leaves (Fig. 3). The P_N of the leaves treated with BDE-209 at 5 - 20 mg kg^{-1}(soil) was reduced to 88.4, 87.0, 73.7, and 66.8 % of the control group, respectively (Fig. 3*A*). The decrease of P_N may also be related to the damage of the dark reaction system. The BDE-209 treatments at 5 - 20 mg kg^{-1}(soil) had similar

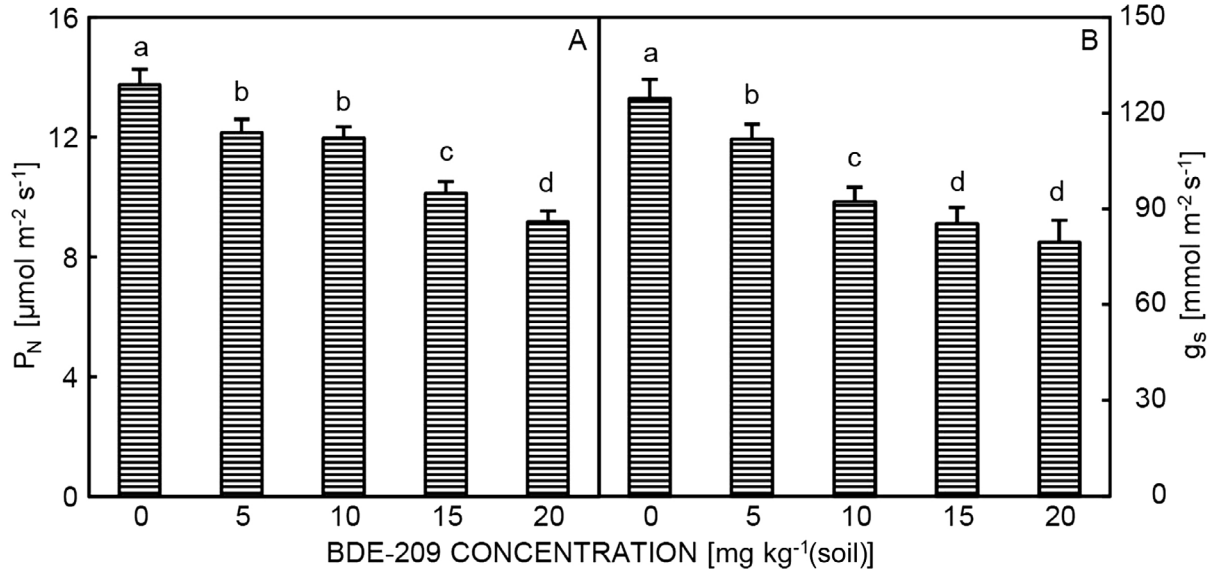

Fig. 3. Net photosynthetic rate (P_N; *A*) and stomatal conductance (g_s; *B*) of Chinese cabbage leaves exposed to BDE-209 for 60 d. The data are means ± SD of five replicates. Different letters indicate significant differences between different treatments ($P < 0.05$).

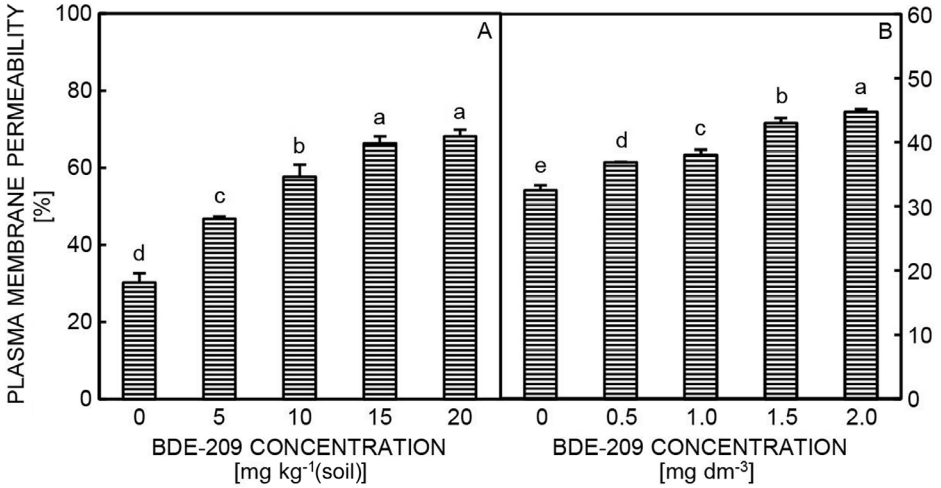

Fig. 4. Plasma membrane permeability (*A* - chronic effect, *B* - direct effect) of Chinese cabbage leaves exposed to BDE-209. For more detail, see Materials and methods. Means ± SDs of five replicates. Different letters indicate significant differences between different treatments ($P < 0.05$).

effects on the variation trend of the g_s compared with that of the P_N (Fig. 3*B*). The decrease of g_s affects the supply of CO_2 needed for photosynthesis. Hence, BDE-209 evidently affected not only the light reaction process but also the CO_2 assimilation ability of Chinese cabbage leaves.

BDE-209 increased the plasma membrane permeability of Chinese cabbage leaves (Fig. 4). The relative conductivity of the leaves treated with BDE-209 at 5, 10, 15, and 20 mg kg^{-1}(soil) increased to 1.55-, 1.91-, 2.20-, and 2.26-times of the control group, respectively (Fig. 4*A*). The increasing trend of the relative conductivity of direct (*in vitro*) toxicity experiments was similar to that of chronic (*in vivo*) toxicity experiments, indicating that BDE-209 can directly affect the stability of plasma membrane (Fig. 4*B*). A number of studies have also shown that PBDEs can easily bind to biomembranes. Therefore, in addition to damaging thylakoid membranes, PBDEs can also disrupt the structure and function of the plasma membrane and organelles (chloroplasts, mitochondria, and nuclei) membranes (Zhang *et al*. 2013, Zhao *et al*. 2017, Meng *et al*. 2018, Qiu *et al*. 2018, Sun *et al*. 2019). The ultrastructure of algae cells (*Alexandrium minutum* and *Dunaliella salina*) and the microscopic structure of *Populus tomentosa* leaf cells have been found to be visibly impaired under the PBDEs stress, as observed by transmission electron microscope (TEM) and optical microscope (Zhang *et al*. 2013, Cai *et al*. 2015, Zhao *et al*. 2017).

MDA is a degradation product of lipid peroxidation (Dazy *et al*. 2009, Farzana and Tam 2018), which can serve as a sensitive diagnostic index for oxidative injury

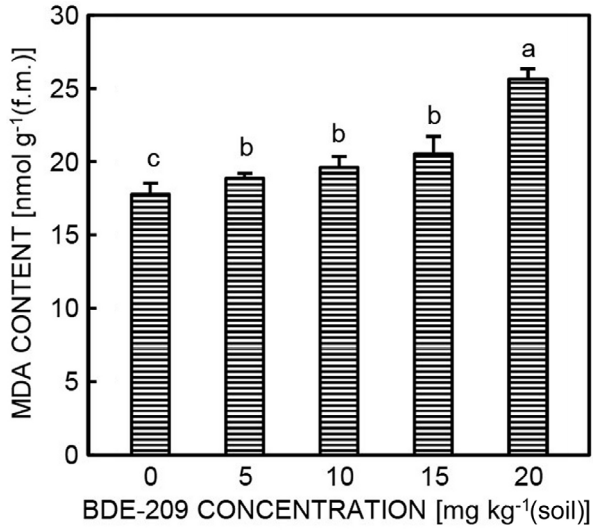

Fig. 5. Malondialdehyde (MDA) content of Chinese cabbage leaves exposed to BDE-209 for 60 d. Means ± SDs of five replicates. Different letters indicate significant differences between different treatments ($P < 0.05$).

of biomembranes in plants treated with BDE-209 (Sun *et al*. 2019, Zhao *et al*. 2019). BDE-209, at concentrations below 20 mg kg^{-1}(soil), slightly increased the lipid peroxidation in Chinese cabbage leaves, while the MDA content increased notably after being treated with BDE-209 at 20 mg kg^{-1}(soil) (Fig. 5). Oxidative damage is the secondary stress, which in turn aggravates membrane injury (Qiu *et al*. 2018). The trypan blue staining test at the cellular level revealed that BDE-209 increases the MDA content and causes visible membrane damage of roots in rice at a low concentration (100 μg dm^{-3}), which provides direct evidence for the above viewpoints (Li *et al*. 2018). Extrapolating from the above results, BDE-209 can directly damage the plasma membrane of Chinese cabbage leaves, and the biomembranes might be the primary targets of PBDEs toxicity in plant cells.

BDE-209 interferes with the cell metabolism of plants and poses oxidative stress *via* the overproduction of ROS ($O_2^{\bullet-}$ and H_2O_2). Excessive ROS are known to be the main cause of BDE-209 phytotoxicity (Xie *et al*. 2013, Farzana *et al*. 2016, 2019, Li *et al*. 2018, Sun *et al*. 2019, Zhao *et al*. 2019). In Chinese cabbage leaves, the $O_2^{\bullet-}$ content was very sensitive to BDE-209, especially at 20 mg kg^{-1}(soil) BDE-209 concentration (Fig. 6*A*). The content of H_2O_2 was also increased in a clear dose-response relationship (Fig. 6*B*). When the seedlings were exposed to BDE-209 at concentrations of 5, 10, 15, and 20 mg kg^{-1}(soil), the content of H_2O_2 in Chinese cabbage leaves continuously increased by 17.1, 30.1, 50.7, and 78.9 % compared with the control group, respectively. The accumulation of ROS occurs in different cellular compartments, including chloroplasts, mitochondria, peroxisomes, and nuclei (Farzana *et al*. 2019). Excessive ROS can react with lipids, proteins, amino acids, and nucleic acids (Luna *et al*. 1994), leading to oxidative damage, metabolic dysfunction, and ultimately cell death (Xie *et al*. 2013, Farzana *et al*. 2016,

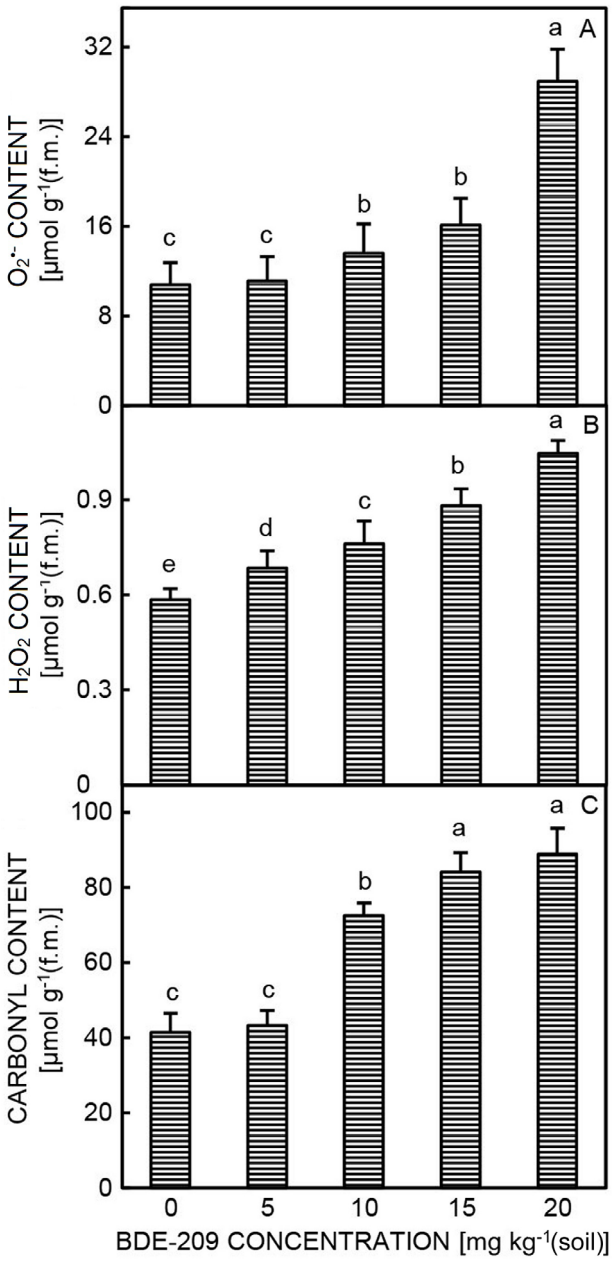

Fig. 6. The superoxide anion radical ($O_2^{\bullet-}$) content (*A*), the H_2O_2 content (*B*), and carbonyl content (*C*) of Chinese cabbage leaves exposed to BDE-209 for 60 d. Means ± SDs of five replicates. Different letters indicate significant differences between different treatments ($P < 0.05$).

Li *et al*. 2018). The decrease of the Chl content and protein content in BDE-209-treated leaves were also related to oxidative damage (Fig. 2).

Under oxidative stress, protein carbonylation is one of the most significant chemical modifications of proteins. The levels of protein carbonylation in Chinese cabbage leaves had no obvious changes after exposure to BDE-209 at 5 mg kg^{-1}(soil) compared with the control group, but the content of protein carbonyl was elevated dramatically when the exposure concentration increased to 10-20 mg kg^{-1}(soil), which were 175.16, 203.10, and 214.68 % of that in the

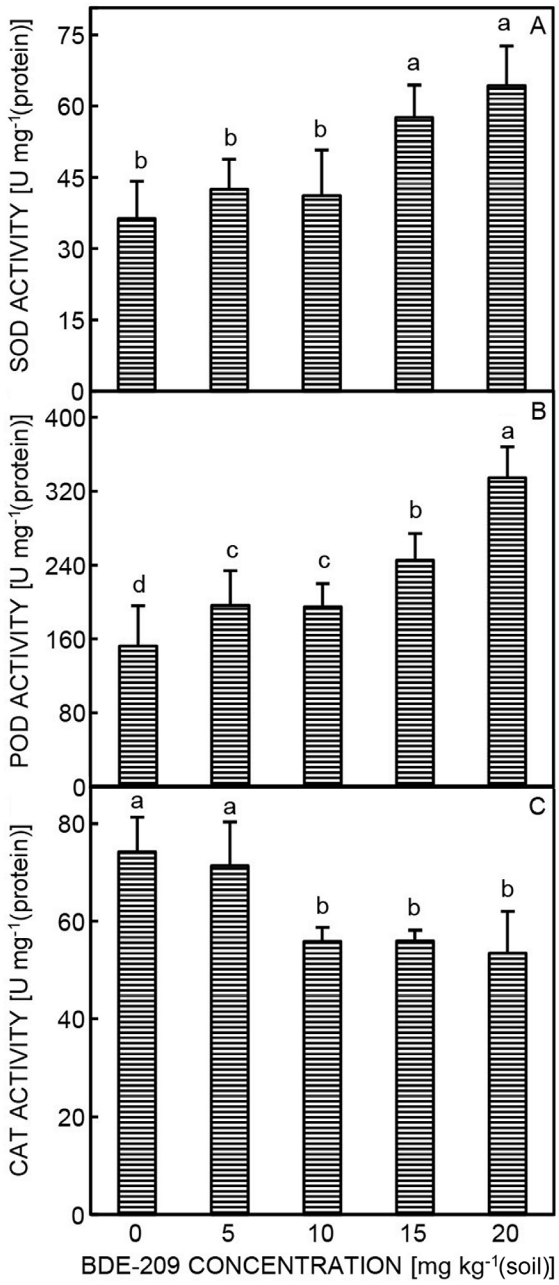

Fig. 7. The superoxide dismutase (SOD; *A*), peroxidase (POD; *B*), and catalase (CAT; *C*) activities of Chinese cabbage leaves exposed to BDE-209 for 60 d. Means ± SDs of five replicates. Different letters indicate significant differences between different treatments ($P < 0.05$).

control leaves, respectively (Fig. 6*C*). The increased content of ROS ($O_2^{\bullet-}$ and H_2O_2) (Fig. 6*A*,*B*), MDA (Fig. 5), and carbonyl (Fig. 6*C*) in the leaves of Chinese cabbage could be considered as an apparent reflection of oxidative damage caused by BDE-209 stress. Other types of PBDEs, such as BDE-47, can also cause similar oxidative damage to plants (Xu *et al*. 2015). Hence, scavenging ROS is an important mechanism to relieve the phytotoxicity of BDE-209.

Antioxidant enzymes, like SOD, POD, and CAT, could be induced to mitigate oxidative damage in plants (Farzana *et al*. 2017, Zhao *et al*. 2019). In the present study, the response model of the antioxidant enzymes (SOD, POD, and CAT) to BDE-209 toxicity in Chinese cabbage leaves was analyzed. There were no apparent variances in the SOD activity of Chinese cabbage leaves at low concentrations of BDE-209 (5 - 10 mg kg^{-1}(soil)), but the SOD activity increased conspicuously at high concentrations of BDE-209 (15 - 20 mg kg^{-1}(soil)) (Fig. 7*A*). The changes of the POD activity were similar to that of SOD, which increased slightly at low concentrations of BDE-209 but increased significantly at high concentrations of BDE-209 (Fig. 7*B*). However, the CAT activity of the BDE-209 treated leaves was not higher than that of the control group, which indicated that CAT did not play a major role in the antioxidative response of Chinese cabbage (Fig. 7*C*). Similar defensive mechanisms have also been reported in maize treated by BDE-47 and mangrove plants treated by BDE-99 and BDE-209 (Xu *et al*. 2015, Farzana *et al*. 2016, 2019).

It is generally believed that the *de novo* synthesis of the enzyme increases also its activity under stress (Deng *et al*. 2016, Sidhu *et al*. 2016). SOD catalyzes scavenging of $O_2^{\bullet-}$ to H_2O_2 and the generated H_2O_2 could be decomposed to H_2O and O_2 by POD and CAT. The effective synergy of the three antioxidant enzymes can maintain the overall defensive mechanism (Farzana and Tam 2018). In addition, POD also participates in the development, growth, morphogenesis, and differentiation in plants (Xie *et al*. 2013). Moreover, POD can help plant cells to synthesize lignin as a secondary metabolite, which acts as a physical barrier to protect tissues from the toxic effects of pollutants (Zhang *et al*. 2007).

Conclusions

The growing of Chinese cabbage seedlings in soil contaminated by BDE-209 (5 - 20 mg kg^{-1}) for 60 d indicated that the phytotoxicity of BDE-209 was chronic and accumulative. The phytotoxicity of BDE-209 was reflected by the growth suppression and the decreased photosynthesis. BDE-209 could destroy plasma membrane permeability, and caused the overproduction of ROS ($O_2^{\bullet-}$ and H_2O_2), membrane lipid peroxidation, and protein carbonylation. Chinese cabbage seedlings activated the antioxidant enzymes mainly SOD and POD to counter-balance the oxidative stress caused by BDE-209. This study improved our understanding of the phytotoxicity of BDE-209 to vegetables.

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