Plant uptake and phytotoxicity of decabromodiphenyl ether (BDE-209) in ryegrass (Lolium perenne L)

Xianchuan Xie, Yan Qian, Yingang Xue, Huan He and Dongyang Wei

The plant uptake and phytotoxicity of decabromodiphenyl ether (BDE-209) in ryegrass (Lolium perenne L) seedlings were investigated. Results showed that ryegrass could take up BDE-209 from the contaminated soils and most of the BDE-209 in plants is located in roots, indicating that BDE-209 has low root-to-shoot translocation. Except for about 35% inhibition of root growth and about 30% decrease of the chlorophyll b and carotenoid contents of leaves, no visual toxicity symptoms were observed in seedlings grown even at a high concentration of 100 mg kg\(^{-1}\). BDE-209 exposure significantly induced the generation of the superoxide radical (O\(_2^−\)) and malondialdehyde (MDA) in ryegrass leaves. With the increase of BDE-209 concentration, the activities of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione-S-transferase (GST) were significantly changed, and the ratio of reduced glutathione to oxidized glutathione (GSH/GSSG) was also significantly reduced. Results suggested that BDE-209 exposure could cause oxidative stress and damage, which may play an important role in the phytotoxicity of BDE-209 in ryegrass seedlings.

1 Introduction

Polybrominated diphenyl ethers (PBDEs) have been widely used as flame retardants to reduce the fire risk of plastics, carpets, electronic equipment, textiles, and building materials all over the world. Due to their toxicity, bioaccumulation, and persistence, PBDEs have been classified as persistent organic pollutants (POPs) by several organizations around the world. Because PBDEs are not chemically bound to the polymer matrices of manufactured products, they can be easily released into the environment and can migrate long distances with air or water currents. PBDEs are now ubiquitous in the world and have been detected in air, soils, sediments, water, and organisms. PBDEs have been commercialized as penta-, octa-, and deca-brominated mixtures. In China, penta-BDE and octa-BDE mixtures have no longer been produced and commercialized since 2004 due to their potentially deleterious effects on ecosystems and human health. However, the use of deca-BDE (BDE-209) is not subject to any regulatory action in China. The domestic production of deca-BDE increased from 26 000 metric tons in 2000 to approximately 41 500 metric tons in 2005, and decreased to 20 500 metric tons in 2011. BDE-209 is extremely hydrophobic (\(K_{ow} = 9.9\)) with low water solubility (<20–30 μg l\(^{-1}\) at 25 °C) and high molecular weight (959 amu). Once released into the environment, it has a strong affinity with soil particles and concentrates in soils, sewage sludges, and sediments. It was reported that the total concentration of PBDEs detected in soils from electronic waste sites in China could be as high as 97 400 ng g\(^{-1}\) dry weight and BDE-209 was the most common congener with the concentration range of 2.76–48 600 ng g\(^{-1}\) dry weight. In previous studies, accumulation and debromination of BDE-209 occurring in roots of some plants including Italian ryegrass, alfalfa, pumpkin, summer squash, maize and radish were reported. Nevertheless, the effect of PBDEs including BDE-209 on plants has not been well documented. It was demonstrated that reactive oxygen species (ROS) were one of the underlying agents inducing tissue injury after plants.
were exposed to many pollutants including organic chemicals.\textsuperscript{11,14} To avoid oxidative damage by ROS, the synthesis of antioxidative antioxidants such as ascorbic acid and glutathione can be stimulated in cells. Antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT), are frequently used to characterize the oxidative stress response produced by contamination.\textsuperscript{13–16} The parameters of antioxidant response including ROS generation and antioxidant enzyme activities are useful biomarkers for the toxic effect of pollutants. As ryegrass (Lolium perenne L) is an important fodder for domestic animals and the standard terrestrial plant for phytotoxicity in the ecological effect test guidelines of United States Environmental Protection Agency (US-EPA) (The Office of Pollution Prevention and Toxics (OPPT) 850.4150 and 4025), it was chosen as the test plant in this study. The present study investigated the plant uptake and phytotoxicity of decabromodiphenyl ether (BDE-209) in ryegrass (Lolium perenne L) seedlings.

2 Materials and methods

2.1 Chemicals and reagents

Decabromodiphenyl ether (BDE-209) and 2-(N-cyclohexylamino)ethanesulfonic acid of analytical grade were purchased from Sigma-Aldrich Company (Shanghai, China). Kaolin, pure quartz sand, and CaCO\textsubscript{3} of analytical grade were purchased from Nanjing Chemical Reagent Co., Ltd. Sphagnum (organic content 18–20\%) was obtained from Tref, Holland. Dichloromethane, hexane, acetone, methanol and acetonitrile of high-performance liquid chromatography (HPLC) grade were purchased from Merck Company (Shanghai, China). All the other chemicals and reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co, Ltd. (SCRC) (Shanghai, China). All aqueous solutions were prepared using reagent water from a Milli-Q Gradient system of Millipore Company (Shanghai, China).

2.2 Experimental soils

Agricultural soils (0–20 cm) used in the experiments, in which no PBDEs had been detected, were collected from paddy fields from Jiangsu Academy of Agricultural Sciences in China. The air-dried soils were sieved (40 mesh; 0.45 mm) prior to the experiments. The particle size distributions of soils were 86 g kg\textsuperscript{−1} sand, 408 g kg\textsuperscript{−1} silt, and 354 g kg\textsuperscript{−1} clay. The organic carbon content was 1.80\% and the pH was 7.73. The cation exchange capacity (CEC) was 20.60 cmol kg\textsuperscript{−1}. Spiked with n-hexane as a carrier, BDE-209 was mixed with quartz sand and then mixed evenly with soils. The spiked concentrations of BDE-209 in soils were 0, 0.1, 0.5, 1, 5, 10, 50, and 100 mg kg\textsuperscript{−1} dry weight, respectively. The soils were then amended with nutrient solution to obtain macronutrient contents of 150 mg total N, 100 mg exchangeable P\textsubscript{2}O\textsubscript{5}, and 150 mg K\textsubscript{2}O per 1000 g dry-weight. The soil was mixed thoroughly and left for 2 days to ensure n-hexane evaporation. The soils were packed into greenhouse pots (750 g dry-weight soil per pot), and then wetted to 80\% of their water-holding capacity (WHC). Thirty-two pots (4 replicates × 8 treatments) were randomly assigned to eight spiked concentrations.

2.3 Seed germination and greenhouse culture

Ryegrass (Lolium perenne L) seeds, which came from a cultivated material, were obtained from Jiangsu Academy of Agricultural Sciences, China. The surface of seeds was sterilized with 5\% (v/v) hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) for 20 min and washed with deionized water, then soaked in sterilized distilled water for 30 min. They were germinated on pledgets in an illuminated incubator at 25 \°C. The seedlings were transplanted to the greenhouse pots (10 plants per container) filled with control or spiked soils after 2 days of emergence, which was considered the start of the culture. Water was added everyday to keep the soils wet to 60 to 80\% of their WHC. The greenhouse was maintained at 20 to 25 \°C during the day and 10 to 15 \°C at night. According to the ecological effect test guidelines of United States Environmental Protection Agency (OPPTS 850.4150 and 4025), the plants were collected after a 28 day cultivation.

2.4 Analytical methods

2.4.1 Biomass production and growth of plants. After the 28 day culture, each harvested plant was washed with tap water and then divided into roots and aboveground parts. The shoot and root length and the fresh weight of the plant were determined using a ruler and balance, respectively.

2.4.2 Preparation of plant samples for analysis. After measuring the biomass production and growth of plants, all plants per container were grouped. The plant samples were prepared according to the following procedures: (1) all fresh plant leaves or roots in each container were chopped up and mixed thoroughly, and (2) a part of chopped plant leaves or roots was chosen randomly for analysis.

2.4.3 Contents of pigments. Contents of chlorophyll a and b, and total carotenoid in leaves were measured using a modified colorimetric method.\textsuperscript{17,18} With liquid nitrogen, 0.2 g fresh leaves were frozen and then homogenized in 3 ml 80\% acetone with quartz sand. The homogenate was centrifuged at 4500g for 10 min. The absorbance of the supernatant was measured at 663, 646, and 470 nm against a blank containing all the reagents except the sample.

2.4.4 Reactive oxygen species (ROS) intensity. The reactive oxygen species (ROS) intensity was measured according to the method of Wang et al.\textsuperscript{19} and Lynch and Thompson\textsuperscript{20} with some modification. In liquid nitrogen, 0.5 g fresh leaves were immediately frozen and then homogenized in 50 mM 2-(N-cyclohexylamino)ethanesulfonic acid buffer (pH 8.6) containing 10 mM 1,2-dihydroxybenzene-3,5-disulfonic acid (Tiron) and 0.5\% (v/v) Tween-20. The homogenate was centrifuged at 4500g for 5 min at 2 \°C. Then, a 400 \mu l aliquot of the supernatants was quickly transferred to capillary tubes (3 mm diameter), placed in liquid nitrogen, and electron paramagnetic resonance (EPR) analysis was carried out within 4 h. All operations were carried out in an incubation system with continuous N\textsubscript{2} purging. The EPR spectra of ROS were recorded on a Bruker EMX 10/12 X-band spectrometer at room temperature (25 \°C). The operation conditions were: microwave power, 20 mW; microwave frequency, 9.751 GHz; modulation frequency, 100 kHz;
modulation amplitude, 1.0 G; center field, 3476 G; sweep width, 50 G. The height of the first peak in the EPR spectra was used as an indication of ROS intensity.

2.4.5 Lipid peroxidation determination. Lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) and quantified in terms of malondialdehyde (MDA) concentration according to the described method. The amount of MDA was calculated by measuring the absorbance at 450 nm, 532 nm, and 600 nm. The non-specific absorbance at 600 nm was subtracted from the absorbance at 532 nm. The concentration of MDA was calculated by using the extinction coefficient of 1.56 \times 10^5 M^{-1} cm^{-1}.22

2.4.6 Antioxidant enzyme activities and protein content. The enzymes of ryegrass leaves were extracted according to the method of García-Limones et al. The fresh leaves were suspended in 50 mM ice-cold phosphate buffer (pH 7.5) containing 1 mM ethylenediaminnetetraacetate, 1 mM phenylmethylanesufonyl fluoride, 5 mM ascorbic acid and 4% (w/v) polyvinylpolypyrrolidone, and then homogenized for 1 min using an Ultra-Turrax T25 homogenizer at 12,000 rpm. The homogenate was centrifuged at 9000g for 30 min at 4 °C. The supernatant was collected and stored at −40 °C prior to analysis. The total protein contents of enzyme extraction were measured according to Bradford using BSA as a standard.24

Superoxide dismutase (SOD) activity was assayed by its ability to inhibit the photochemical reduction of nitroblue tetrazolium as described by Dhindsa et al. One unit of enzyme activity was defined as the amount of enzyme exhibiting 50% inhibition of the auto-oxidation rate of 2.25 mM nitroblue tetrazolium in 3 ml solution at 25 °C (U mg\textsuperscript{-1} protein).

Catalase (CAT) activity was determined with a Shimadzu UV-220 spectrophotometer by measuring the decrease of OD\textsubscript{240} nm due to H\textsubscript{2}O\textsubscript{2} decomposition.26 One unit of enzyme activity was defined as the amount of the enzyme reducing 0.01 unit of OD (U mg\textsuperscript{-1} protein).

Glutathione reductase (GR) was assayed by the method of Esterbauer and Grill with some modification. The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.8), 0.2 mM ethylenediaminnetetraacetate, 0.2 mM triphosphopyridine nucleotide reduced tetrasodium salt, 0.5 mM oxidized glutathione (GSSG), and 200 μl enzyme extract. The reaction mixture was made up to 3 ml by distilled water. The increase in absorbance at 340 nm was recorded at 25 °C. The activity was expressed as ΔA\textsubscript{340} g\textsuperscript{-1} (f.m.) s\textsuperscript{-1}.

Glutathione-S-transferase (GST) activities were assayed according to the method of Saint et al. by monitoring the changes of reduced glutathione (GSH) amount and were expressed as μmol l\textsuperscript{-1} mg\textsuperscript{-1} protein.27 All the determination assays were performed in four replicates.

2.4.7 Glutathione content. The GSH and GSSG contents were measured by the method of Hissin and Hilf.28 The fluorescence was determined at 420 nm using an excitation wavelength of 350 nm on a Hitachi fluorescence spectrophotometer F-7000.

2.4.8 BDE-209 concentration in plants and soils. The shoots aboveground and roots of ryegrass, and soils were
collected separately after the 28 day culture; plant and soil samples were freeze-dried, reduced to a powder using a mill, sieved through a stainless steel 75-mesh (0.5 mm) sieve and then stored in glass containers at –20 °C prior to chemical analysis. The BDE-209 analysis in plants and soils was based on the method reported by Mai et al.28 Because of having the similar chemical–physical features with BDE-209, PCB-209 was added as surrogate standards to the samples prior to extraction.

Chemical analysis was performed with a Thermo DSQII gas chromatograph coupled with a mass spectrometer (Thermo, American) using negative chemical ionization in the selected ion monitoring mode. A Thermo TR-5ms SQC capillary column (15 m × 0.25 mm, 0.25 μm film thickness) was used for the determination of BDE-209. Helium (>99.999%) was used as the carrier gas and methane (>99.99%) as a chemical ionizer, moderating gas at a flow rate of 1.5 ml min⁻¹. The column temperature was programmed from 110 to 300 °C at a rate of 8 °C min⁻¹ (held for 20 min). Automatic injection of the 2 μl samples was conducted in the splitless mode and the split mode was turned on after 1 min.

Other detailed procedures were conducted as the reported method.30,31

2.5 Statistical analysis

Data were expressed as means ± standard deviation (SD). Statistical analysis was performed using SPSS v. 18.0. One-way analysis of variance (ANOVA) and LSD (L) test were used to test the differences between test groups and control group. Differences were considered significant if p < 0.05.

3 Results

3.1 BDE-209 concentration in plants and soils after 28 day exposure

The initial BDE-209 concentrations in soils and the BDE-209 concentrations in soils and ryegrass (mg kg⁻¹ dry weight) after 28 days of exposure are displayed in Table 1. It showed that the BDE-209 concentrations in roots and shoots of ryegrass increased as the spiked BDE-209 concentrations increased. After 28 day plant cultivation, the BDE-209 concentrations in soils were decreased by 25–38% compared with the initial concentration (Table 1).

3.2 Effects of BDE-209 on seedling growth

After ryegrass had grown in greenhouse over a 28 day period, the effects of BDE-209 on seedling growth were evaluated by examining the biomass, shoot and root lengths. It showed that the plants exposed to 0.1–100 mg kg⁻¹ of BDE-209 had no significant change in morphology, except that the root length of plant was significantly decreased at 100 mg kg⁻¹ of BDE-209 (Fig. 1) (p < 0.05).

3.3 Effects of BDE-209 on chlorophyll and carotenoid contents of seedling leaves

As indicated in Fig. 2, the chlorophyll b content in seedling leaves was significantly reduced at 100 mg kg⁻¹ of BDE-209 and the carotenoid content was significantly decreased at 50–100 mg kg⁻¹ of BDE-209 (p < 0.05), while the chlorophyll a content of all treatments had no significant change in comparison with the control.

Fig. 1 Effect of BDE-209 on biomass production and growth of ryegrass seedling (a) the picture of ryegrass seedlings after 28 day exposure; (b) dose-dependent biomass production and growth after 28 day exposure. Values are denoted as mean ± SD (n = 4). Significant differences in comparison to the control group values are tested by the LSD (L) test and denoted as *p < 0.05.

Fig. 2 Effect of BDE-209 on chlorophyll a and b, and carotenoid contents in ryegrass leaves. Values are denoted as mean ± SD (n = 4). Significant differences in comparison to the control group values are tested by the LSD (L) test and denoted as *p < 0.05, **p < 0.01.
3.4 Reactive oxygen species (ROS) induced by BDE-209

According to the previous literature, the amplitude of the EPR spectrum for tiron semiquinone is a specific and quantitative indicator of superoxide radical (O$_2^-$) production. The present study demonstrated directly that BDE-209 exposure stimulated O$_2^-$ generation in ryegrass leaves (Fig. 3). It was observed that the O$_2^-$ intensities in seedling leaves increased as the BDE-209 concentrations in soils increased, showing an obvious dose-effect relationship. The maximum O$_2^-$ intensity was observed at 50 mg kg$^{-1}$ of BDE-209, which was 3-fold higher than the control group. However, the signal intensity at 100 mg kg$^{-1}$ has dropped by about 20% as compared with that for 50 mg kg$^{-1}$ of BDE-209, although it was much higher than that of the control.

3.5 Effects of BDE-209 on malondialdehyde (MDA) contents

Fig. 4 shows that the MDA contents in leaves were significantly stimulated by 10 to 100 mg kg$^{-1}$ of BDE-209, indicating the lipid peroxidation in seeding leaves.

3.6 Antioxidant response to BDE-209 exposure

Changes of antioxidant enzyme activities and glutathione metabolism in ryegrass leaves are summarized in Table 2. The SOD activities were significantly stimulated by 1 and 5 mg kg$^{-1}$ of BDE-209 ($p < 0.05$ and $p < 0.01$), and significantly inhibited at 100 mg kg$^{-1}$ ($p < 0.05$). The CAT activities were significantly increased at 5 to 50 mg kg$^{-1}$ ($p < 0.01$, and 100 mg kg$^{-1}$ for $p < 0.05$), and the maximum value was observed at 10 mg kg$^{-1}$, which was 45% higher than that of the control. Similar to CAT, the GST and GR activities were unaffected at low BDE-209 concentrations, but they were significantly stimulated by high levels of BDE-209 ($p < 0.01$). The data showed that the GSH content was significantly decreased by 100 mg kg$^{-1}$ BDE-209, while GSSG contents were significantly stimulated by 10 to 100 mg kg$^{-1}$ of BDE-209 (10 mg kg$^{-1}$ for $p < 0.05$, and 50 to 100 mg kg$^{-1}$ for $p < 0.01$) (Table 2). The GSH/GSSG ratio, therefore, was significantly decreased when the BDE-209 concentrations were at 10 to 100 mg kg$^{-1}$ (10 mg kg$^{-1}$ for $p < 0.05$, and 50 to 100 mg kg$^{-1}$ for $p < 0.01$).
4 Discussion

The PBDEs have been emerging as a new class of global contaminants. Among 209 congeners, BDE-209 is the main congener detected in soils. Studies show that PBDEs or their metabolites may cause various adverse effects on mammals, including acute toxicity, reproductive toxicity, hepatotoxicity, neurotoxicity, carcinogenicity, etc. Nevertheless, to the best of our knowledge, studies on the toxicity of BDE-209 are mainly focused on animals at present, and little is known about the phytotoxicity of PBDEs, especially for BDE-209.

In the present study, except for little adverse effects of BDE-209 on the root growth, no visual toxicity symptoms were observed in seedlings grown even at a high concentration of 100 mg kg\textsuperscript{-1} (Fig. 1). Costa and Giordano reviewed that BDE-209 appears to have very low acute toxicity for experimental animals, such as rats, mice, rabbits and so on. Therefore, assessing morphological symptoms is insufficient to evaluate the BDE-209 phytotoxicity. To better understand the effects of BDE-209 on ryegrass, the plant uptake and some biochemical parameters have been studied here.

Due to the big molecular structure and strongly hydrophobic nature, BDE-209 was formerly supposed to have little bioavailability. In the present study, BDE-209 was detected in both roots and shoots of ryegrass, indicating that ryegrass could take up and then translocate and accumulate BDE-209 from the contaminated soils. Based on the data in Table 1, it was also observed that most of BDE-209 in plants is located in roots, indicating that BDE-209 has low root-to-shoot translocation. These results are in accordance with the observations of plants’ uptake of PBDEs in ryegrass, pumpkin, and maize which were grown directly in the contaminated soils from electronic waste recycling sites. Huang et al. reported the debromination and hydroxylolation of BDE-209 in the soil–plant system. In this study, the additional lower brominated PBDE congeners (octa-BDE and nona-BDE) were also observed in soils and ryegrass roots. The environmental effects and behavior of degradation products of BDE-209 in the soil-plant system require further study.

Recent reports have shown that PBDEs could enhance the cellular ROS level and induce oxidative stress, which was regarded as an important toxicity mechanism of PBDEs. Hu et al. suggested that BDE-209 inhibited the proliferation of Hep G2 cells by inducing apoptosis through ROS generation. Tseng et al. reported that the exposure of BDE-209 to neonate induced the generation of H\textsubscript{2}O\textsubscript{2} in the sperm of sexually mature male mice. It is well known that ROS including superoxide anion radical (O\textsuperscript{2-}), hydroxyl radical (OH\textsuperscript{-}), singlet oxygen (O\textsubscript{3}), and H\textsubscript{2}O\textsubscript{2} are free radicals with short half-lives and exist at extremely low levels in the organism; therefore directly identifying short-lived ROS in plants is very difficult. The spin trapping technique based on EPR spectroscopy is by far the most direct and reliable method to detect short-lived ROS accumulated \textit{in vivo}, and it has been applied successfully to investigate ROS generation in plants. The ROS trapped by tiron semiquinone was identified as the free radicals of O\textsuperscript{2-} according to the previous literature. The present study directly demonstrates

![Image of Table 2: Antioxidant enzyme activities and glutathione contents in ryegrass leaves after BDE-209 exposure]

<table>
<thead>
<tr>
<th>The spiked BDE-209 concentrations</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>5</th>
<th>10</th>
<th>50</th>
<th>100</th>
</tr>
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<tbody>
<tr>
<td>SOD activity (U mg\textsuperscript{-1} protein)</td>
<td>17.9 ± 2.4</td>
<td>21.2 ± 2.6</td>
<td>21.9 ± 2.1</td>
<td>2.0 ± 0.1</td>
<td>5.6 ± 0.2</td>
<td>2.8 ± 0.2</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>CAT activity (U mg\textsuperscript{-1} protein)</td>
<td>7.3 ± 0.5</td>
<td>7.5 ± 0.6</td>
<td>6.9 ± 0.6</td>
<td>7.5 ± 0.7</td>
<td>7.6 ± 0.6</td>
<td>7.9 ± 0.6</td>
<td>6.2 ± 0.6</td>
</tr>
<tr>
<td>GST activity (U mg\textsuperscript{-1} protein)</td>
<td>3.7 ± 0.1</td>
<td>4.6 ± 0.3</td>
<td>4.5 ± 0.2</td>
<td>5.0 ± 0.3</td>
<td>6.0 ± 0.4</td>
<td>5.0 ± 0.3</td>
<td>5.5 ± 0.4</td>
</tr>
<tr>
<td>GR activity (U mg\textsuperscript{-1} protein)</td>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
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<tr>
<td>GSH/GSSG</td>
<td>3.2 ± 0.1</td>
<td>3.4 ± 0.1</td>
<td>3.4 ± 0.1</td>
<td>3.4 ± 0.1</td>
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Note: values are denoted as mean ± SD (n = 4). Significant differences in comparison to control group values are tested by the LSD (t) test and denoted as p < 0.05.
that BDE-209 exposure can significantly stimulate the generation of $\text{O}_2^-$ in ryegrass leaves (Fig. 3). It is well-known that ROS can react with proteins, lipids, and nucleic acids, consequently causing oxidative stress and damage in biological systems.41–43 In the present study, the drastic accumulation of $\text{O}_2^-$ might cause oxidative stress or damage in ryegrass leaves.

Liu et al. reported that the high ROS level may cause damage to the tissue and organelle structure of leaves and inhibit the pigment biosynthesis.13 As light-harvesting pigments, carotenoids protect chlorophyll and membrane destruction by removing ROS from the excited chlorophyll–oxygen complex.13 At a high BDE-209 level of 100 mg kg$^{-1}$, reduction of chlorophyll b and carotenoids content was observed (Fig. 2). This may be due to the high ROS load exceeding the protective capacity of carotenoids.17 These results are similar to those reported in Arabidopsis thaliana treated by phenanthrene and ryegrass grown in PAH contaminated soils.44,45

Malondialdehyde (MDA) is a primary by-product of membrane lipid peroxidation and a sensitive diagnostic indicator of oxidative stress and damage in cells.32 The increase of MDA contents in the present study suggested that oxidative damage had occurred in ryegrass leaves when the spiked BDE-209 concentrations in soils were at 10–100 mg kg$^{-1}$ of nominal concentrations, and the MDA contents were positively correlated with $\text{O}_2^-$ intensities in leaves (Fig. 4). Similar changes of MDA concentrations were also reported in mice fed with BDE-209 and the hepatic cells of rat exposed to BDE-209.33,43 Liang et al. reported that the MDA contents in mice brains were significantly induced after exposure to the dose of 0, 0.1, 40, 80 and 160 mg kg$^{-1}$ BDE-209 for 15, 30 and 60 days.44 Yao et al. also demonstrated that the MDA contents of the rat liver cell were significantly increased by 4–16 mg l$^{-1}$ of BDE-209.44 Therefore, BDE-209 exposure could induce oxidative stress and damage in cells, which may play an important role in BDE-209 toxicity.

As noted previously, antioxidant enzymes including SOD, CAT, GST, and GR are important parts of antioxidative defense because they can prevent oxidative damage by removing ROS.19,23 Results in the present study showed that CAT, GST and GR activities were all stimulated by varying degrees after the 28 day exposure of BDE-209. In order to protect the plant cell, the biosynthesis of CAT, GST, and GR enzymes in leaves was significantly stimulated to remove the ROS which was induced by BDE-209. A similar response was reported in earthworms exposed to BDE-209.46 In the present study, SOD activities were significantly increased at 1 and 5 mg kg$^{-1}$ of BDE-209, and significantly inhibited at 100 mg kg$^{-1}$ of BDE-209 (Table 2). It is well-known that SOD, which is the first line of antioxidative defense, can catalyze the conversion of $\text{O}_2^-$ to $\text{H}_2\text{O}_2$ in leaf cells.18 The increase of SOD activities at low concentrations of BDE209 in this study may be due to the stimulation of SOD generation in leaf cells for preventing oxidative damage. A similar symptom occurred in phenanthrene treated leaves.13,14 The inhibition of SOD activity at high concentrations of BDE-209 may be due to oxidative damage induced by excess ROS.14 CAT activities in leaves were significantly stimulated by 5–100 mg kg$^{-1}$ of BDE-209 after 28 day exposure, and the highest CAT activity, which occurred at 50 mg kg$^{-1}$ of BDE-209, was 1.6 times that of the control group. The increase in activity indicates that CAT plays an important protective role in the anti-oxidative process of seedling leaves after BDE-209 exposure.12,13

Glutathione is a tripeptide containing thiol and is also an important antioxidant in cells.37 Reduced glutathione (GSH) can scavenge excessive free radicals. GSH conjugates with electrophilic intermediates in detoxification reactions or directly reacts with ROS through the oxidation of GSH to GSSG. In the glutathione pathway to remove ROS, GR is responsible for the reduction of GSSG to GSH.27,28,46 Various responses of glutathione metabolism have been observed after exposure to PBDEs.41,45,48–50 In the present results, GSH contents were significantly depressed at high concentrations of 100 mg kg$^{-1}$ (Table 2), indicating GSH depletion.45 The GSSG accumulation at high BDE-209 concentrations (10–100 mg kg$^{-1}$) might contribute to the excessive oxidation of GSH. The observed stimulation of GST and GR activities possibly resulted from the increased glutathione metabolism in order to relieve the oxidative stress in cells. The GSH/GSSG ratio, the maintenance of which is essential for the normal cell, is considered as a reliable biomarker of oxidative stress in cells.46 In the present study, the depletion of GSH and accumulation of GSSG resulted in the decreased GSH/GSSG ratio at 10 to 100 mg kg$^{-1}$ (Table 2), indicating that severe oxidative stress occurred in ryegrass leaves. Similar findings were reported on the rats exposed to BDE-99 and the PBDE-dosed birds.46,49 Bellés et al. reported that the increase of the GSSG/GSH ratio occurred in brain tissues of adult male rats which were fed with a single dose of 0.6 and 1.2 mg kg$^{-1}$ BDE-99 body weight for 45 days.49 Fernie et al. observed the increased hepatic GSSG level and GSSG/GSH ratio in American kestrels which were exposed to the mixture of BDE-47, -99, -100, and -153.50 Zhao et al. also reported that there was no significant change of GSH contents in fish after BDE-209 exposure for 13 days, while the GSSG level and GSSG/GSH ratio were not reported.50

5 Conclusion

The present study reported the plant uptake of BDE-209 with low root-to-shoot translocation and the potential effects of BDE-209 on ryegrass seeding. The EPR spectra demonstrated that ROS generation was induced by BDE-209 in ryegrass leaves. The antioxidant parameters, including antioxidant enzymes and glutathione, clearly revealed that oxidative stress occurred in ryegrass seedlings after BDE-209 exposure. Further investigation on the specific mechanisms of ROS induction is necessary to understand the cause of phytotoxicity induced by PBDEs. The results of the present study showed that all tested antioxidant enzymes and MDA seem to be good biomarkers to assess the ROS toxicity of PBDEs in soils towards plants.

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