Research article

Growth, physiological function, and antioxidant defense system responses of *Lemna minor* L. to decabromodiphenyl ether (BDE-209) induced phytotoxicity

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ABSTRACT

Polybrominated diphenyl ethers (PBDEs), represent one of the new types of persistent organic pollutants (POPs) that are currently found in ambient aquatic ecosystems. *Lemna minor* L. is a floating freshwater plant, which is widely employed for phytotoxicity studies of xenobiotic substances. For this study, we investigated the growth, physiological functions, and antioxidant capacities of *L. minor*, which were exposed to 0–20 mg L^{-1} decabromodiphenyl ether (BDE-209) for 14 days. A logistic model was suitable for describing the growth of *L. minor* when the BDE-209 concentration was in the range of from 0 to 15 mg L^{-1}. When exposed to 5 and 10 mg L^{-1} BDE-209, the growth of *L. minor* was significantly increased, where the intrinsic rate (r) and the maximum capacity of the environment (K) of *L. minor* were significantly higher than those of the control. In this case, the chlorophyll content and soluble proteins were also markedly increased. Moreover, the photosynthetic function (Fv/Fm, PI) was enhanced. However, for 15 mg L^{-1} BDE-29 treated group, the growth of *L. minor* was significantly inhibited, with decreases in chlorophyll and the soluble protein content, until the *L. minor* yellowed and expired under a concentration of 20 mg L^{-1}. Photosynthetic functions were also negatively correlated with increasing increments of BDE-209 (15 and 20 mg L^{-1}). The malondialdehyde (MDA), superoxide anion radical (O$_2^-$) content, and permeability of the plasma membranes increased with higher BDE-209 concentrations (0–20 mg L^{-1}). The superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities of *L. minor* increased when the BDE-209 concentration ranged from 0 to 10 mg L^{-1}; however, the activities of SOD and POD were decreased. Only the CAT activity remained higher in contrast to the control group under 15–20 mg L^{-1} BDE-209. These results demonstrated that 15 mg L^{-1} BDE-209 imparted high toxicity to *L. minor*, which was a consequence of the overproduction of reactive oxygen species (ROS), which conveyed oxidative damage to plant cells. This study provided a theoretical understanding of BDE-209 induced toxicity as relates to the physiology and biochemistry of higher hydrophytes.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are often used as flame retardants in myriad coatings, plastics, textiles, and other consumer products (Hu et al., 2010; Trudel et al., 2011). A variety of studies have characterized PBDEs as environmentally persistent, lipophilic, recalcitrant to degradation, and bio-enriching (de Wit, 2002; Agrell et al., 2004; Maranghi et al., 2013; Shang et al., 2016). Due to the lack of binding effects of its chemical bonds, the PBDEs that are added to products may easily enter the ambient environment by means of volatilization, exudation, and so on, to cause extensive contamination of the biosphere (Alaee, 2003; Huwe and West, 2011; Fang et al., 2015; Yang

et al., 2015; Zheng et al., 2015). Generally, PBDEs may be divided into lower PBDE congeners (tri-BDE to hepta-BDE) and higher PBDE congeners (octa-BDE to deca-BDE), according to the bromination levels (Noyes et al., 2011). Recent studies have revealed that deca-BDE (BDE-209) was in far more extensive use, accounting for ~70% of the total PBDEs (Deng et al., 2016). Therefore, specific harmful biological effects of BDE-209 on the ambient environment should be rigorously investigated and elucidated.

Numerous current studies have found that BDE-209 imparts ubiquitous toxicity to lower aquatic and terrestrial plants. It can cause acute toxic effects of "low promotion and high inhibition" on overall populations, individuals, and the cells of four species of marine microalgae (*Platymonas helgolandica*, *Karenia Mikimotoi*, *Heterosigma akashiwo*, and *Isochrysis Galbana Parke 8701*) (Jiang, 2011). Oxidative stress and the inhibition of the growth of *Lolium perenne* occurred following 28 days of BDE-209 treatment (0–100 mg kg^{-1} dry weight) (Xie et al., 2013). A study by Li et al. (2018) revealed that 500 µg L^{-1} BDE-209 might induce various phytotoxicities, including growth inhibition, and lipid peroxidation in rice (*Oryza sativa* L.) seedlings. Moreover, the lipid peroxidation product malondialdehyde (MDA) content in many terrestrial plants (*Sonchus brachyotus*, *Brassica juncea*, *Solanum nigrum*, *Houttuynia cordata*, *Ipomoea aquatica*, *Pennisetum alopecuroides*, and *Pennisetum americanum*) generally increased under BDE-209 induced stress (Liu et al., 2012; Lv et al., 2013). Considering the significant negative impacts of BDE-209 on plants, extensive investigations have been conducted on the uptake, translocation, and metabolism of BDE-209 in several plants such as rice, pumpkin, maize, radish, and aquatic phytoremediation plants (Huang et al., 2010; Wang and Frei, 2011; Chow et al., 2015; Deng et al., 2016). Interestingly, Qiu et al. (2018) reported that BDE-47 (lower PBDE congeners) at 5–20 µg L^{-1} had significant toxicity to duckweed (higher hydrophytes). However, specific knowledge related to the phytotoxicities of BDE-209 on higher hydrophytes remains scarce.

It was demonstrated that the overproduction of ROS was a key mechanism in the toxicity of PBDEs (He et al., 2008; Lv et al., 2015; Wang et al., 2015). Oxidative stress induced by the overproduction of ROS may result in growth inhibition, lipid peroxidation, and the disruption of cellular structures (Lv et al., 2015; Xu et al., 2015; Qiu et al., 2018). To mitigate the damage caused by oxidative stress, antioxidant systems, which include a series of enzymes and other compounds, are activated in cells (Livingstone, 2001; Wang et al., 2015). Qiu et al. (2018) indicated that BDE-47 exposure activated the superoxide dismutase (SOD) and catalase (CAT) activities of duckweed plants. Li et al. (2018) demonstrated that the toxic stress of BDE-209 induced elevations in SOD, peroxidase (POD), and CAT activities in three species of rice seedlings, where these changes varied with species. Increased SOD and CAT activities were also found in four species of microalgae according to Meng et al. (2009). Therefore, the parameters of antioxidant responses, inclusive of MDA content, ROS production, and antioxidant enzyme activities were significant indicators of the phytotoxicity of pollutants.

The intent of this paper was to confirm the potential toxicity of BDE-209 on common duckweed *Lemna minor* L., which is a model aquatic plant for ecotoxicological research and environmental monitoring (Zezulka et al., 2013). The effects of the addition of BDE-209 on the population growth, photosynthetic physiology, lipid peroxidation levels, and ROS levels of *L. minor* were investigated under controlled laboratory conditions. The antioxidant enzymes SOD, POD, and CAT were concurrently analyzed to test the adaptation abilities of *L. minor* to BDE-209 toxicity.

2. Materials and methods

2.1. Plant culture and treatments

L. minor samples were collected from a sewage area in Qufu city,

P.R. China, located at 116° 51′ N, 35° 29′ E. The plants were cultured in Petri dishes (Ø9.5 cm) containing Steinberg solution (350 mg L^{-1} KNO$_3$, 90 mg L^{-1} KH$_2$PO$_4$, 12 mg L^{-1} K$_2$HPO$_4$, 100 mg L^{-1} MgSO$_4$·7H$_2$O, 295 mg L^{-1} Ca (NO$_3$)$_2$·4H$_2$O, 0.18 mg L^{-1} MnCl$_2$·4H$_2$O, 0.12 mg L^{-1} H$_3$BO$_3$, 0.044 mg L^{-1} Na$_2$MoO$_4$, 0.18 mg L^{-1} ZnSO$_4$·7H$_2$O, 0.76 mg L^{-1} FeCl$_3$·6H$_2$O, 1.5 mg L^{-1} Na$_2$EDTA·2H$_2$O; pH 7.0) and incubated under laboratory conditions (temperature: 23–25 °C, light/dark: 16 h/8 h, and irradiance: 80–100 µmol m^{-2} s^{-1}) (Kalčíková et al., 2017). BDE-209 (purity 99.5%, GC-MS certified), which comprises a white particulate product produced by Dr. Ehrenstorfer (Germany) was prepared for a stock solution (1 g L^{-1}) in dimethyl sulfoxide (DMSO). The 5, 10, 15, and 20 mg L^{-1} BDE-209 concentrations were realized by adding a 0.15 mL, 0.3 mL, 0.45 mL, 0.6 mL of 1 g L^{-1} stock solution to a 30 mL cultivation system, respectively. The Steinberg solution was renewed every 3 d to ensure that the concentrations of the treatment solutions were stable. The experiment proceeded for 14 days, and at the conclusion of the experiment the physiological and biochemical indices were immediately determined (Zezulka et al., 2013). Three replicates were prepared for all treatments.

2.2. Biomass assay

All *L. minor* plants in each dish were carefully removed from the Steinberg solution. These plants were then dried by filter paper for 10 min, and their fresh weight (FW) was measured `via a balance and expressed as all plants in one dish per square meter [g (FW) m^{-2}], which were fitted based on a logistic equation. The logistic equation was given by: N$_t$ = K/(1 + e$^{a-r\ t}$), where N$_t$ is the population density at different times [g (FW) m^{-2}]; t is the time (d); a is a constant, indicating the relative position of the curve to the origin; e is the natural constant 2.71828; r is the intrinsic growth rate of the population (g g^{-1} d^{-1}), and K is the maximum environmental carrying capacity (g m^{-2}). The values of K and r were calculated according to Kucharavy and Guio (2012). The growth curve of *L. minor* was plotted by means of a daily average FW with three repetitions.

2.3. Analysis of chlorophyll content and photosynthetic activity

A volume of 0.03 g *L. minor* plants per treatment was isolated via 80% acetone extraction, and the chlorophyll content was spectrophotometrically determined at 663 and 646 nm using the method of Porra (2002) with some modification, and applying the formula:

Chlorophyll content = 7.35 × A663 + 17.58 × A646

The L. *minor* plants were dark-adapted for at least 1 h prior to the determination of PSII maximal photochemistry efficiency (Fv/Fm) and performance index (PI), using a Handy PEA (Plant Efficiency Analyser; Hansatech Instrument Ltd., UK) (Kalaji et al., 2016). Ten representative individuals from each treatment were randomly selected.

2.4. Determinations of plasma membrane permeability and soluble protein

Plasma membrane permeability was expressed as relative conductivity (%) and measured with a conductivity meter (Mcclendon, 1927). Briefly, 0.05 g *L. minor* plants per treatment were introduced into a 50 mL beaker that was filled with distilled water and subjected to vacuum for 30 min. Subsequently, these plants were kept at room temperature for 1 h prior to the determination of their plasma membrane permeability.

The soluble protein content was quantified using the Coomassie brilliant blue method (Bradford, 1976), with bovine albumin for calibration.

2.5. ROS and MDA content assay

The ROS was identified as the superoxide anion radical (O_2^-) according to the previous literature (Lynch and Thompson, 1984; Wang et al., 2008). The O_2^- was measured by testing the formation of nitrite from hydroxylamine, as reported by Elstner and Heupel (1976). Briefly, 0.03 g *L. minor* plants were ground with a 3 mL 65 mM phosphate buffer solution (PBS; pH 7.8) and centrifuged at 5000 g for 15 min. Following this, a 2.0 mL volume of supernatant was mixed with 0.5 mL 65 mM PBS (pH 7.8) and 0.1 mL 10 mM hydroxylamine hydrochloride, followed by incubation at 25 °C for 60 min. Afterward, 1 mL 58 mM sulfanilamide and 1 mL 7 mM α-naphthylamine were added to the mixture above and incubated at 30 °C for 40 min. The absorbance of 530 nm was measured to assay the reduction of NBT.

Thiobarbituric acid-malondialdehyde (TBA-MDA) was assayed using the technique of Aravind and Prasad (2003). Briefly, *L. minor* plants (0.05 g) were homogenized with 0.1% trichloroacetic acid (TCA) and centrifuged at 10000 g for 10 min. A 2 mL volume of supernatant was mixed with 2 mL 5% TCA containing 0.5% TBA. The absorbance of the supernatant at 450 nm, 532 nm, and 600 nm was measured, and the MDA content was calculated by the following formula:

$$MDA\ content = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$$

2.6. Antioxidant enzyme assay

L. minor plants (0.1 g) were homogenized with 10% (v/v) glycerol and 1.8 mL of 500 mM PBS (pH 7.8), containing 1 mM EDTA and 50 mM NaH_2PO_4 + Na_2HPO_4. This mixture was centrifuged at 12000 g for 20 min at 4 °C, and the supernatants were employed for enzyme assays (Zhang et al., 2007).

The SOD activity was determined by the photochemical reduction of NBT (nitroblue tetrazolium) according to Jr and Fridovich (1987). The reaction mixture consisted of 2.85 mL of 100 mM PBS, 75 µM NBT, 13 mM L-methionine, 0.1 mM EDTA, 150 µL of 45 µM riboflavin, and 50 µL of crude enzyme extract. All mixtures were irradiated at a light intensity of 40 µmol m^{-2} s^{-1} for 10 min and the absorbance at 560 nm was measured immediately following the reaction mixture, which turned from yellow to dark blue. One unit of SOD activity was determined via the 50% photochemical reduction of NBT under assay conditions (U mg^{-1} protein).

The POD activity was elucidated using the method of Maehly and Chance (1955), with ΔA470 (spectrophotometer at 470 nm), which was increased by 0.01 per minute, as an enzyme activity unit (ΔA470 min^{-1} mg^{-1} protein). This reaction mixture contained of 2.83 mL of 100 mM PBS (pH 7.8), 0.1 mM EDTA, and 25 mM guaiacol, 150 µL of 100 mM H_2O_2, and 200 µL crude enzyme extract.

The CAT activity was measured through the quantity of H_2O_2 consumed at 240 nm, which was reduced by 0.01 per minute as an enzyme unit (ΔA240 min^{-1} mg^{-1} protein) according to the method of Knörzer et al. (1996). The reaction mixture contained 1.5 mL of 100 mM PBS (pH 7.8), 200 µL of 100 mM H_2O_2, and 200 µL crude enzyme extract. This reaction was activated through the addition of H_2O_2.

2.7. Statistics

The experimental results were expressed as mean values ± standard deviation (SD). One-way analysis of variance analysis (ANOVA) and the Duncan test were employed to analyze the differences between the control and treated groups. Means marked with different lowercase letters indicated significant differences (P < 0.05). Data logging and visualization were accomplished using Microsoft Excel 2016 and GraphPad Prism 5, respectively.
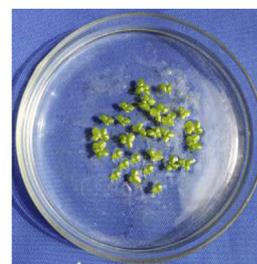
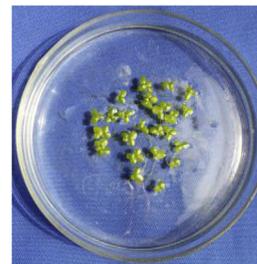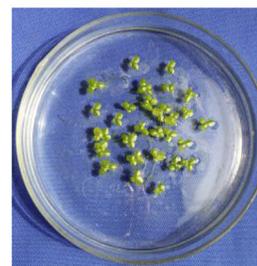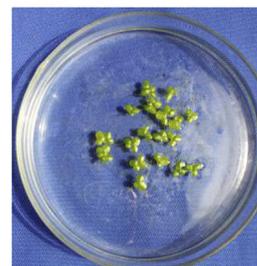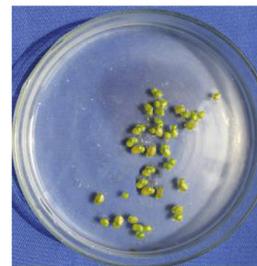Fig. 1. *L. minor* plants treated with BDE-209 at concentrations of 0, 5, 10, 15, and 20 mg L^{-1} for 14 days.

3. Results

3.1. Plant growth

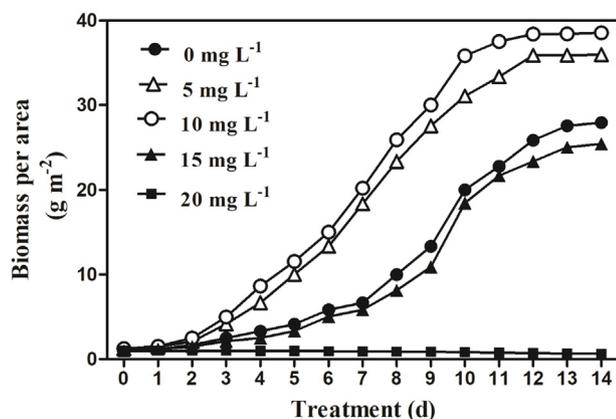Compared to the control, plant growth was enhanced at low levels

Fig. 2. Effects of BDE-209 on the biomass production of *L. minor* fronds following 14-d exposure.

of BDE-209 (5 and $10 \, mg \, L^{-1}$) exposure; however, there was an inhibitory effect on growth at high levels of BDE-209 (15 and $20 \, mg \, L^{-1}$) following 14 days. Particularly, at $20 \, mg \, L^{-1}$, the appearance of chlorotic symptoms was observed in the *L. minor* plants (Fig. 1).

The unit area biomass curves of *L. minor* revealed an "S" shape over time, when treated with 0, 5, 10, and $15 \, mg \, L^{-1}$ BDE-209 (Fig. 2), and the correlation coefficient R^2 of the curves were all higher than 0.95 (Table 1), which confirmed that the growth curves of *L. minor* met the typical logistic growth model. Further, the values of r and K were significantly increased under low BDE-209 (5 and $10 \, mg \, L^{-1}$) concentrations; however, when the BDE-209 concentration was in the range of from 10 to $15 \, mg \, L^{-1}$, both r and K were significantly decreased. When treated with $20 \, mg \, L^{-1}$ BDE-209, *L. minor* became stagnant and gradually yellowed, with a linear decline.

3.2. Chlorophyll content and photosynthetic activity

The chlorophyll content was significantly increased when treated with 5–$10 \, mg \, L^{-1}$ BDE-209, in contrast to the control group (P < 0.05); however, there was a decreasing trend with further increases in BDE-209 concentrations (15–$20 \, mg \, L^{-1}$) (Fig. 3a). Under low level (5 and $10 \, mg \, L^{-1}$) BDE-209 exposure, the chlorophyll content of *L. minor* increased to 133.02%, and 141.17% of that in the control group, respectively. However, when *L. minor* fronds were treated with 15 and $20 \, mg \, L^{-1}$ BDE-209, the chlorophyll content was reduced to 79.60%, and 29.14% of that in the control group, respectively.

Chlorophyll fluorescence technology is a sensitive probe for the detection of the degree of damage to plants caused by adverse stress (Stirbet and Govindjee, 2011). Fv/Fm and PI are two important and sensitive fluorescence parameters in chlorophyll fluorescence kinetics (Kalaji et al., 2016). The values of Fv/Fm and PI were significantly increased when treated with 5–$10 \, mg \, L^{-1}$ BDE-209, compared with the control group (P < 0.05); however, they revealed a decreasing trend when the BDE-209 concentration was in the range of from 15 to $20 \, mg \, L^{-1}$ (Fig. 3b and c). The Fv/Fm and PI of *L. minor* treated with 5, 10, 15, and $20 \, mg \, L^{-1}$ BDE-209 were 103.85%, 109.52%, 95.57%, 84.86%, and 115.65%, 133.24%, 66.35%, 13.91% of that in the control

group, respectively.

3.3. Soluble protein content and plasma membrane permeability

Under low levels (5 and $10 \, mg \, L^{-1}$) of BDE-209, the soluble protein content of *L. minor* fronds increased to 129.32% and 141.65.3% of that in the control group, respectively, after 14 days. However, when the fronds were treated with 15 and $20 \, mg \, L^{-1}$ BDE-209 for 14 days, the soluble protein content was reduced to 77.13% and 47.23% of that in the control group, respectively (Fig. 4a). An increase in plasma membrane permeability was observed in the *L. minor* fronds following 14-d exposure to BDE-209, and the maximum plasma membrane permeability was measured under $20 \, mg \, L^{-1}$ BDE-209, which was almost two folds higher than the control (Fig. 4b).

3.4. Redox balance and antioxidant enzymes

There were significant increases in the $O_2^{\cdot-}$ and MDA content with respect to the BDE-209 concentrations tested (Fig. 5). The $O_2^{\cdot-}$ and MDA content of *L. minor* fronds treated with 5, 10, 15, and $20 \, mg \, L^{-1}$ BDE-209 were 1.14, 1.20, 1.31, 1.62, and 1.17, 1.27, 1.33, 1.42 times that of the control, respectively.

Under low (5 and $10 \, mg \, L^{-1}$) BDE-209 levels, the SOD, POD, and CAT activities were 29.86%, 21.37%, 64,71%, and 50.41%, 49.57%, 50.00% higher than that of the control, respectively. However, under high (15 and $20 \, mg \, L^{-1}$) BDE-209 levels, the SOD and POD activities were reduced to 78.53%, 79.49% and 59.69%, 60.97% of the control group, respectively. The CAT activity treated with $15 \, mg \, L^{-1}$ and $20 \, mg \, L^{-1}$ BDE-209 was lower in contrast to that under $10 \, mg \, L^{-1}$ BDE-209; however, it was still higher than that in the control group.

4. Discussion

4.1. Effects of BDE-209 on growth

The biomass of *L. minor* fronds is considered as an indicator of natural stressors, chemicals, or contaminants (Mazur et al., 2016; Qiu et al., 2018). The effects of BDE-209 on the growth of *L. minor* plants followed a dose-response with stimulation at $5 \, mg \, L^{-1}$ and $10 \, mg \, L^{-1}$ and inhibition at higher levels (Figs. 1 and 2), which was termed as hormesis (Calabrese and Blain, 2009). One explanation for this stimulation was that some organic pollutants (BDE-209 in this research) might have served as a carbon source for the growth of organisms (Ren et al., 2016). Growth inhibition at higher BDE-209 exposure levels might have been due to the action of the contaminants, which likely reduced the uptake of nutrients (Xu et al., 2015). Our results differed from $5 \, \mu g \, L^{-1}$ BDE-47, which significantly inhibited the growth of *L. minor* fronds (Sun et al., 2016; Qiu et al., 2018), and the biomass of ryegrass and rice, which decreased under BDE-209 treatment ($100 \, mg \, kg^{-1}$ dry weight and $100 \, \mu g \, L^{-1}$, respectively (Xie et al., 2013; Li et al., 2018)). This indicated that: 1. The phytotoxicity of higher PBDE congeners was relatively less than lower brominated PBDE congeners (Bragigand et al., 2006; Mhadhbi et al., 2012). 2. Higher aquatic plants might be less sensitive and more resistant to BDE-209 than higher terrestrial plants.

Table 1

The Logistic growth model of *L. minor* under different BDE-209 concentrations, where the initial plant number = 10. Mean ± SD are shown.

Treatment	Logistic equation	r	K	R^2
$0 \, mg \, L^{-1}$	$N_t = 27.92/(1 + e^{3.61-0.51t})$	0.51 ± 0.03c	27.92 ± 1.05b	0.96
$5 \, mg \, L^{-1}$	$N_t = 35.96/(1 + e^{4.26-0.72t})$	0.72 ± 0.02b	35.96 ± 1.25a	0.95
$10 \, mg \, L^{-1}$	$N_t = 38.54/(1 + e^{4.55-0.76t})$	0.76 ± 0.02a	38.54 ± 1.50a	0.99
$15 \, mg \, L^{-1}$	$N_t = 25.42/(1 + e^{3.22-0.42t})$	0.42 ± 0.03d	25.42 ± 1.32c	0.95

·Different lowercase letters indicate a significant difference between BDE-47 treatments at P = 0.05.
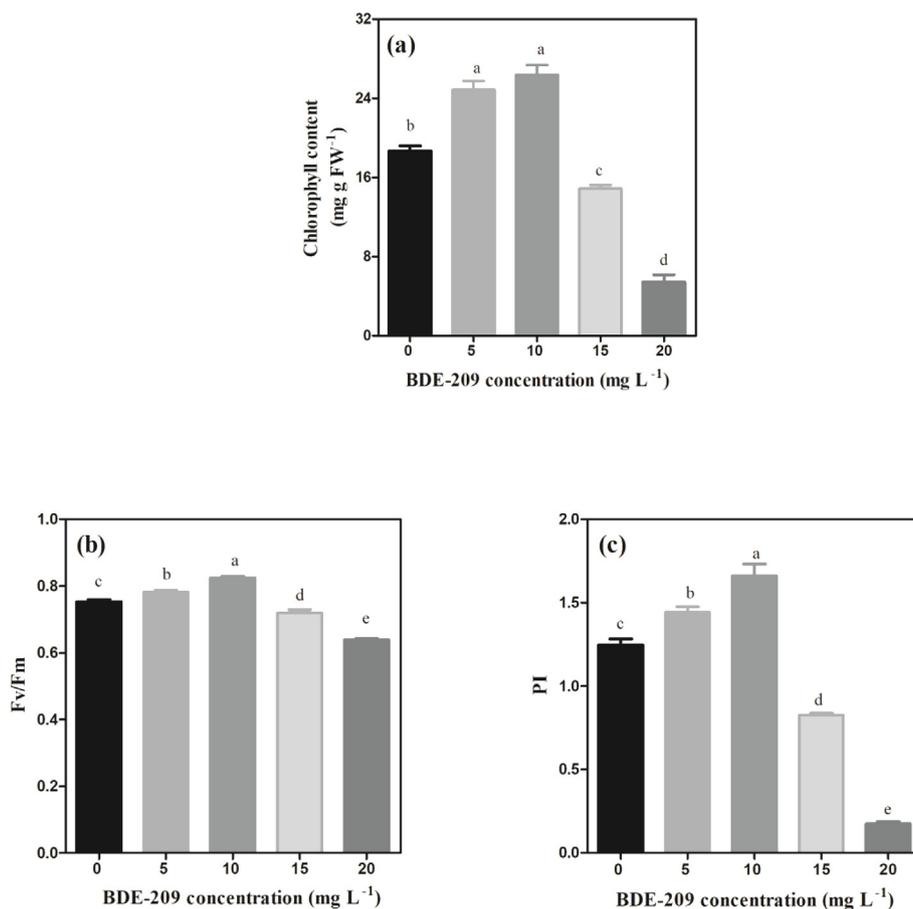
Fig. 3. Effects of BDE-209 on chlorophyll content (a) and photosynthetic activity (b and c) of *L. minor* fronds following 14-d exposure. Values are expressed as mean ± SD (n = 3).
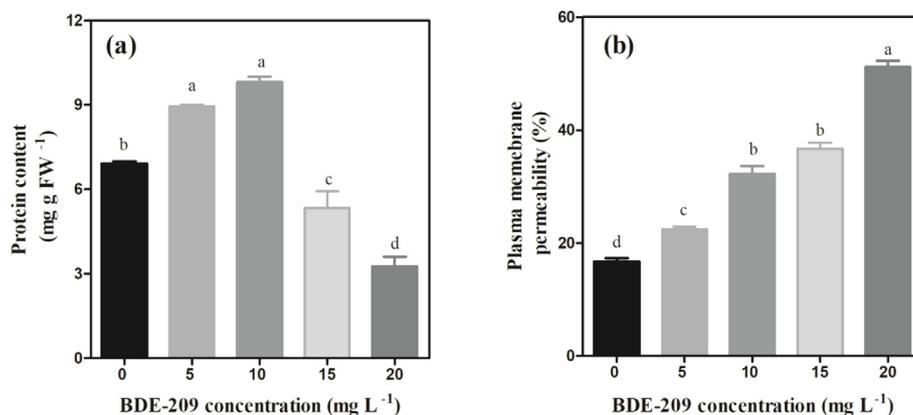

Fig. 4. Effects of BDE-209 on protein content (a) and plasma membrane permeability (b) of *L. minor* fronds following 14-d exposure. Values are expressed as mean ± SD (n = 3).

Interestingly, for our study, r was more sensitive to BDE-209 than K in the logistic model (Table 1; Fig. 2), which suggested that r was a more suitable indicator to reflect the phytotoxicity of BDE-209 on *L. minor* than K. According to the "r/K selection theory" (Pianka, 1970), we also inferred that the "behaviors" of *L. minor* populations adopted a more "r" like strategy at initial growth, as more resources were allocated to plant growth when the *L. minor* plants were at low density, while at high densities, their "behaviors" are more akin to the "K" strategy, as there was a reduction in the allocation of reproductive resources toward the maintenance of a constant biomass.

4.2. Effects of BDE-209 on physiological function

The photosynthesis and soluble protein content of plant organisms are important indicators of their capacity to metabolize, and are known to reflect the degree of external environmental stress (Sun et al., 2016). In the present study, photosynthesis and the soluble protein content revealed a "promoted under low level and inhibited under high level" trend (Figs. 3 and 4a). Herein, we suggest that plants increased their photosynthesis and protein synthesis capacities to resist this stress when they were subjected to certain levels of contaminant poisoning; however, when the damage to plant exceeded their control, the soluble
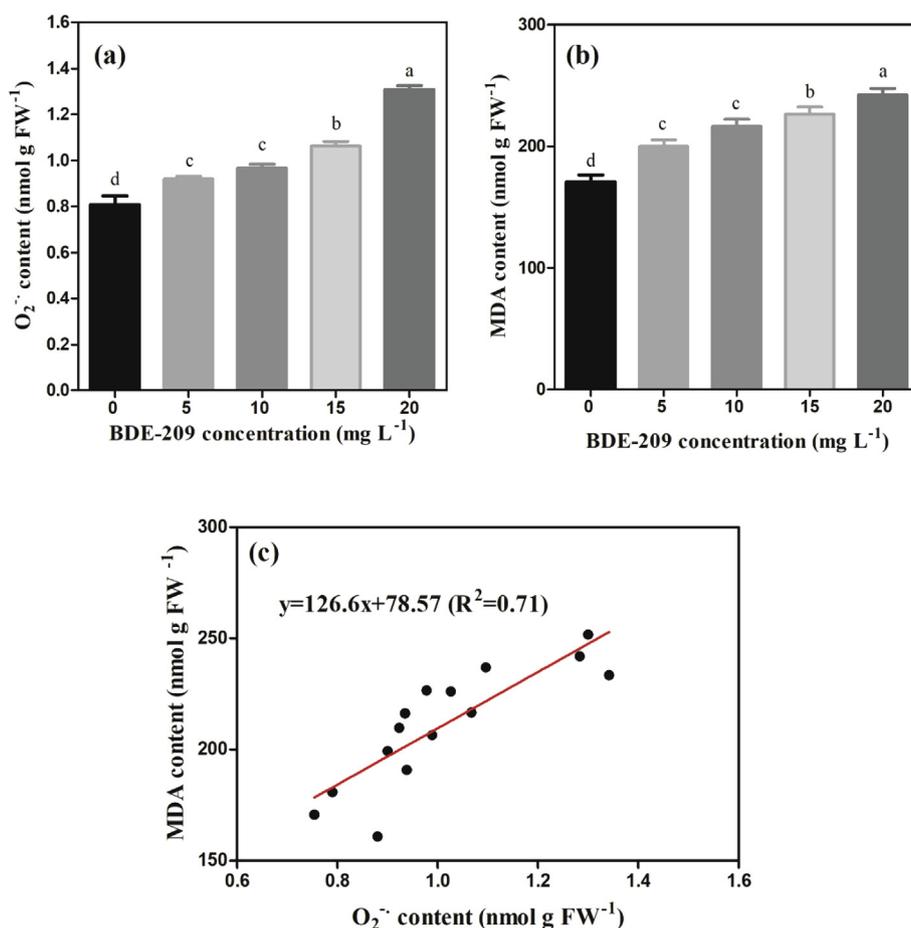
Fig. 5. $O_2^{\cdot-}$ content (a), MDA content (b) and positive correlations between $O_2^{\cdot-}$ content and MDA content of *L. minor* fronds under different concentrations of BDE-209 for 14 days. Values are expressed as mean $\pm$ SD (n = 3).

protein content of the plant decreased. Xie et al. (2013) demonstrated that 0–100 mg kg^{-1} of the BDE-209 dry weight had no obvious effect on the chlorophyll content of ryegrass, in contrast to a control. This might have been due to only a portion of the BDE-209 being vertically translocated from the soil to the fronds (Huang et al., 2010; Xie et al., 2013).

Previous studies revealed that PBDEs might affect both the mitochondrial and plasma membranes (Pereira et al., 2013, 2014). The increases of plasma membrane permeability in the present study suggested that BDE-209 could do harm to cells. This was consistent with the results of Zhao et al. (2017), who suggested that under the PBDEs stress, the cellular structures of *Alexandrium minutum* and *Dunaliella salina* were found to be significantly impaired, as observed by transmission electron microscopy. The mechanism of BDE-209 on plasma membranes was likely due to its being lipophilic; thus, it could bind easily to biomembranes (Kreslavski et al., 2017; Qiu et al., 2018).

4.3. Effects of BDE-209 on antioxidant defense system

As discussed above, the overproduction of ROS (regarded as one of the primary toxicity mechanisms of PBDEs) (Hu et al., 2007; Jin et al., 2010; Yan et al., 2011; Wang et al., 2014) under BDE-209 exposure indicated that *L. minor* cells were experiencing oxidative stress. This fact was also confirmed by monitoring the increased generation of MDA (Fig. 5b and c). Meanwhile, the antioxidant enzyme system (e.g., SOD, POD, and CAT) could be activated to scavenge excess ROS and convert them to harmless substances in vivo, thus playing a detoxification role (Ye and Tam, 2007; Ye et al., 2010; Ke et al., 2011; Song et al., 2011). POD proceeds as a ROS remover, whereas together, SOD and CAT can

modify ROS to H_2O and O_2 toward the mitigation of oxidative stress (Tarrahi et al., 2018).

The SOD and POD activities of *L. minor* displayed primarily biphasic responses with increased BDE-209 concentrations (Fig. 6a and b). A similar phenomenon occurred in BDE-47-treated fronds (Sun et al., 2016). This indicated that low-level BDE-209 contamination (0–10 mg L^{-1}) activated antioxidant enzyme activity in vivo to scavenge ROS. However, the activities of SOD and POD were inhibited under high-level (10–20 mg L^{-1}) BDE-209 exposure, which may have been due to the denaturation of enzymes via excess ROS (Yin et al., 2010; Gill et al., 2015). Interestingly, a noticeable increase in CAT activity was observed under different BDE-209 levels compared to the control group (Fig. 6c). This might have been due to these antioxidative enzymes being located at different cell sites, which may have possessed different tolerances for BDE-209 (Mittler, 2002).

As previously noted, the dissolved concentration of BDE-209 in ambient aquatic systems was found to be only 2.59 ng L^{-1} (Yang et al., 2015). However, 5–20 mg L^{-1} of BDE-209 was employed for laboratory experiments to study its short-term phytotoxicity. The experimental concentrations of the BDE-209 contaminant, established in the laboratory to produce toxic effects in organisms over the short term, were often far higher than the actual concentrations found in the natural environment (Breitholtz et al., 2008). However, the degradative biological effects that were likely to occur in nature under these higher concentrations may guide more stringent water purification and environmental remediation practices in the future.
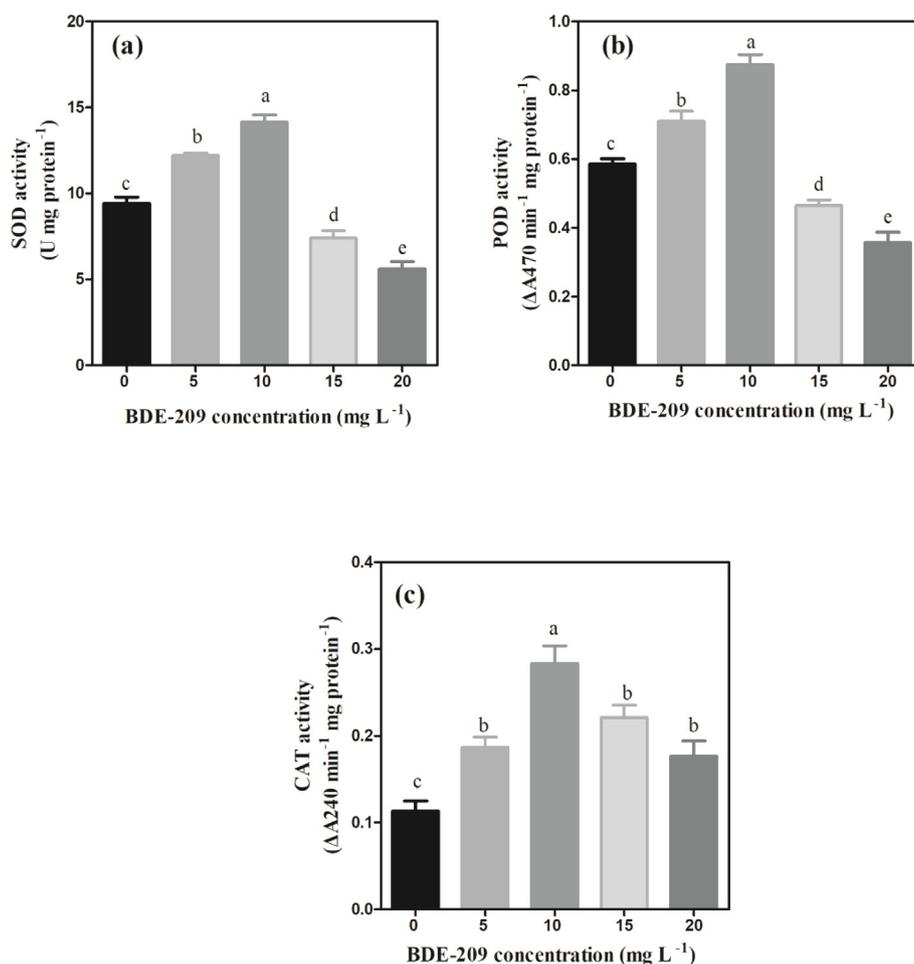
Fig. 6. Effects of BDE-209 on SOD (a), POD (b) and CAT (c) activities of *L. minor* fronds following 14-d exposure. Values are expressed as mean ± SD (n = 3).

5. Conclusion

In conclusion, the exposure of *L. minor* plants to 15–20 mg L^{-1} of BDE-209 induced significant phytotoxicity, which caused reduced biomass, lower photosynthetic function, the inhibition of chlorophyll and protein synthesis, increased plasma membrane permeability, and a disabled antioxidant enzyme system, which imparted oxidative stress and peroxide damage to cells. The kinetics of this inhibition might have involved the overproduction of ROS initiated by BDE-209. Low (0–10 mg L^{-1}) BDE-209 exposure levels imparted stimulatory effects on *L. minor* plants, which was consistent with (Calabrese and Blain, 2009), who argued that numerous xenobiotics may have unexpected hormesis effects on plants at low concentrations. These findings culminated in the development of a viable monitoring strategy for high-levels of BDE-209 in *L. minor* plants in the ambient aquatic environment.

Contributions

Yuan Sun and Peng Sun designed and performed the experiments. Yuan Sun analyzed the data, interpreted the results, and drafted the manuscript. Cuiting Wang performed the experiments and revised the manuscript. Jiahui Liao, Juanping Ni, Tianan Zhang, and Runsong Wang performed the experiments. Honghua Ruan designed the experiments, revised the manuscript, and supervised the project.

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