Growth Inhibition and Oxidative Stress Caused by 4,4'-dibromodiphenyl Ether (BDE-15) on a Marine Diatom Phaeodactylum Tricornutum

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Abstract

In this work, *Phaeodactylum tricornutum* was used to investigate the toxicity of 4,4'-dibromodiphenyl ether (BDE-15). Results showed that BDE-15 inhibited the photosynthetic activity and growth of *P. tricornutum* significantly with 24, 48, 72, and 96 h EC_{50} values of 1.03, 0.44, 0.41, and 0.42 mg L^{-1}, respectively, indicating that it was a highly toxic substance. Moreover, BDE-15 could cause cell deformation, and a series of physiological, biochemical, and molecular changes in the cells. Under the exposure of BDE-15, contents of chlorophyll *a* and soluble protein decreased significantly, but reactive oxygen species (ROS) were accumulated in the algal cells, which may cause or intensify the peroxidation of membrane lipids. For alleviating the toxicity of excessive ROS, activities of antioxidant enzymes increased dramatically when this diatom was exposed to BDE-15. Thus, it is concluded that the overproduction of ROS may be regarded as one of the major factors in BDE-15 toxicity.

1. Introduction

As a class of brominated flame retardants (BFRs), polybrominated diphenyl ethers (PBDEs) have been widely used in plastics, textiles, electronic circuits, building materials, household electric appliances, and other fields (Jiang et al., 2019). During the production, uses and disposal, PBDEs are released and accumulated in the environment easily because they are additives mixed into polymers and are not chemically bound to the plastic or textiles (Darnerud et al., 2001). In recent decades, PBDEs are reported to be a group of global environmental pollutants, which have been detected in various environmental matrices and human tissues (Li et al., 2016). Numerous studies have demonstrated that (1) PBDEs are one of the persistent organic pollutants (POPs) due to their lipophilic, persistent, and bio-accumulative characteristics (Wang et al., 2011); (2) PBDEs exhibit potential toxicity to fish, mammals, and even human beings, such as liver toxicity, reproductive toxicity, neurotoxicity, and others (Jiang et al., 2019); (3) the target organs of PBDEs are mainly adipose tissue nervous system thyroid and reproductive development system (Dingemans et al., 2011); and (4) low-brominated PBDEs are generally more toxic than high-brominated ones (Dingemans et al., 2011). Because of their toxicity to humans and ecosystems, the production and uses of PBDEs have been banned or phased out by the most major global entities since 2004 (Zhou et al., 2019). However, PBDEs are continuously detected in the environment due to their register of specific exemptions and stocks in in-use products (Abbasi et al., 2015). Thus, it is imperative to evaluate the potential toxicity and environmental risk of the presence of PBDEs in the environment.

As a “sink” for almost all types of pollutants, marine environment is the ultimate gathering place of PBDEs, which have been detected in seawater, sediments, and marine organisms (Jiang et al., 2019). And it was reported that PBDEs were several orders of magnitude higher in organisms than that in water because of their lipophilicity (Lee and Kim, 2015). Recently, a number of studies have focused on the influence and toxicity of PBDEs on marine organisms at physiological, biochemical, and molecular levels. For example, the formation of ROS, intracellular level of thiols, and activity and efficiency of phagocytosis in harbour seal (*Phoca vitulina*) cells were all affected when the cells were exposed to PBDEs (Frouin et

al., 2010). A marine bivalve (*Mytilus galloprovincialis*) exposed to PBDEs was observed significant DNA damage and micronuclei formation (Barón et al., 2016). Moreover, the PBDEs not only reduced motile cells proportion and swimming velocity, but also altered the swimming pattern of an unicellular marine flagellate (*Platymonas subcordiformis*), which might thereby hinder growth and survival of the alga, and subsequently threaten marine ecosystems and aquaculture industry (Zhao et al., 2019a). The PBDEs could inhibit the light-harvesting capacity, interfere with electron transport, and induce a loss of oxygen-evolving complex (OEC) activity of a marine diatom (*Phaeodactylum tricornutum*) when the diatom was exposed to 0.8 and 4 mg L^{-1} 2,2', 4,4'-tetrabromodiphenyl ether (BDE-47) (Liu et al., 2020). Consequently, PBDEs pollution in the marine environment has become an increasing public concern in recent years, and the impact of PBDEs on marine organisms still needs to be further addressed for a deeper understanding of their potential risk to the health of marine ecosystems.

Microalgae, widely distributed in marine environment, are a very diverse group of aquatic photosynthetic organisms with the ability to convert sunlight to food and energy. They play an important role in marine ecosystems as primary producers because of their key position in the trophic chain, their ability to produce high amounts of oxygen, and their participation in nutrient cycles (Tsarpali et al., 2015). Besides of the ubiquity and importance in ecosystems, microalgae are often used as an ideal biological model for (eco)-toxicological assessments due to the fact that they have a short life cycle and can respond quickly to environmental stresses (Pham et al., 2010). Among marine microalgae, diatoms are one of the most used species in marine bioassays because of their easy cultivation and significant sensitivity to metals and chemical pollutants (Wang and Zheng, 2008). Although several researchers have used diatoms to investigate the impacts of PBDEs, the data about their potential risk and toxicity to diatoms is still limited until now because PBDEs involve 209 individual congeners with various bromines substituting for hydrogen at different positions on the benzene rings (Tang et al., 2018). In addition, cells of diatoms have a range of features that make them to diverge greatly from the classical cellular structure of microalgae and higher plants (Latała et al., 2009). The toxic mechanisms of PBDEs to diatoms may be different from those of other microalgal species. Thus, more studies should be performed to further assess the toxicity of PBDEs to diatoms and to unravel the underlying toxic mechanism.

As one of the lower brominated PBDEs, 4,4'-dibromodiphenyl ether (BDE-15) has attracted considerable attention recently on its bioavailability, photooxidation kinetic, solubilization, and subacute oral toxicity (Yang et al., 2015). Additionally, BDE-15 was reported to be one of the predominant congeners present in marine environment because it has high water solubility and is easy to transfer in water environment and marine food chains (Luo et al., 2007). Therefore, in this study, BDE-15 was used as one of the representative low-brominated PBDEs. Moreover, a marine diatom (*Phaeodactylum tricornutum*) was selected as a model organism to investigate the growth and physiological responses of marine diatoms against the exposure of PBDEs because it is an important food resource for marine zooplankton and other filtering organisms in the marine food chains, and is also used as a standard marine species in bioassays on the toxicity of chemical pollutants (Wang and Zheng, 2008). The aims of this study were to evaluate the potential impacts of BDE-15 on the growth, photosynthetic activity, cellular components (i.e.,

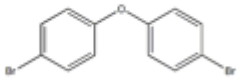
chlorophyll *a* and soluble protein), and antioxidant enzyme activity of the diatom. It is hoped that this study could provide more data and a theoretical basis upon which to unravel the toxic mechanism of PBDEs to marine diatoms and to further evaluate the safety of PBDEs in marine environment.

2. Materials And Methods

2.1. Chemical reagents

The BDE-15 with a purity of 98.9% was purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai China), and its basic physicochemical properties are presented in Table 1. A stock solution of BDE-15 (0.5 g L^{-1}) was prepared by dissolving it into dimethyl sulphoxide (DMSO), and stored at 4 ℃ for later use. All other reagents were of high analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Table 1 Physicochemical characteristics of 4,4'-dibromodiphenyl ether (BDE-15).

Parameters	
CAS number	2050-47-7
Molecular structure	
Molecular formula	$C_{12}H_8Br_2O$
Molecular weight (g mol^{-1})	327.9
Aqueous solubility at 25°C (mg L^{-1})	0.13
Melting points (°C)	57-58
Henry's law constant at 25°C	21
Octanol-water partition coefficient (K_{ow})	5.55

2.2. Marine diatom and pre-cultivation

A marine diatom (*Phaeodactylum tricornutum*) was provided by the State Key Laboratory of Marine Environmental Science (Xiamen, China), and was pre-cultivated in 500 mL Erlenmeyer flasks containing 200 mL sterile seawater with f/2 medium. The flasks were placed in a 20 ± 1 °C incubator (Jiangnan Instrument Factory, Ningbo, China), and illuminated from two sides by vertical cool white fluorescent lamps placed parallel to the flasks with a photoperiod of 12 h light/12 h dark and a light density of 40 μmol photons m^{-2} s^{-1}. All the used flasks were treated with the methods of high-temperature roasting (550°C) to eliminate the possible impacts of plastics.

2.3. Toxicity tests

Cells of *P. tricornutum* were pre-cultured aseptically for 7 days until the cells were induced to the exponential growth phase with a cell density of 3.4×10^7 cells mL^{-1}. After pre-cultivation, the cells were

collected by centrifugation (6000 rpm, 5 min) at 4°C, and then washed twice with sterile seawater. The collected cells were inoculated into 150 mL sterile seawater with f/2 medium at different concentrations of BDE-15 with an initial cell density of 1.9×10^6 cells mL^{-1}. According to the results of preliminary range-finding tests, concentrations of BDE-15 used in this study were 0, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 mg L^{-1}. The diatom grown on the f/2 medium and the medium with 0.1% DMSO was used as controls. The inoculation methods and cultivation conditions were described in a previous study (Deng et al., 2017a).

In the current work, three replicates were set up for each test and seven were for the controls, which were used to check the reproducibility of the data. Each flask was shaken at least three times per day and changed its position randomly for keeping the diatom grown in optimal cultivation conditions. All operations were carried out aseptically.

2.4. Algal growth and inhibition analysis

Samples were taken out every day during the 96 h of exposure, and cell numbers of *P. tricornutum* were counted using a Neubauer hemocytometer under a light microscope (Nikon, YS100, Japan). Each sample was measured at least three times. The algal cell density (*X*, cells mL^{-1}) and inhibition rate (*IR*, %) were calculated and analyzed according to a previous study (Deng et al., 2017c). Based on the concentrations of BDE-15 and the corresponding *IR*, EC_{50} values (the effect concentration of BDE-15 leading to a 50% growth inhibition of *P. tricornutum*) were calculated using the procedure of log inhibitor vs. normalize response-variable slope in GraphPad Prism® (version 5.01) (GraphPad Software Inc., San Diego, CA, USA). The detailed methods were described in a previous study (Chen et al., 2019).

2.5. Morphologic observation

Under the exposure of 0.7 mg L^{-1} BDE-15, cell morphology of *P. tricornutum* was observed by scanning electron microscope (Quanta FEG 250, FEI Ltd., Brno, Czech Republic). Specifically, cells of *P. tricornutum* were collected by centrifugation (4°C, 10000 rpm, 10 min) after 96 h of exposure, and then were washed with phosphate-buffered saline (0.02 mol L^{-1}, pH 7.4) thrice. The collected cells were fixed in 2.5% glutaraldehyde solution at 4°C for 24 h, and then were dehydrated using a graded ethanol series, followed by a graded *tert*-butyl alcohol series. At last, the cells were lyophilized and coated with gold for the final observation.

2.6. Determination of chlorophyll a content and chlorophyll fluorescence

Every 24 h, samples were taken out during the 96 h of exposure for determining the chlorophyll *a* content and chlorophyll fluorescence of *P. tricornutum*. The chlorophyll *a* was extracted with methanol, and its content was measured and calculated based on the absorbances at 750, 665, and 652 nm according to the methods described previously (Deng et al., 2016). And the chlorophyll fluorescence was analyzed by using a pulse-amplitude modulation (PAM) fluorometry (AquaPen AP-C100, Photon Systems Instruments, Drasov, Czech Republic). In brief, samples were kept in the dark for 15 min prior to measurement. The

maximum quantum efficiency of photosystem II (PSII) (F_v/F_m) and effective quantum yield of PSII (Φ_{PSII}) were determined in cuvettes (10 × 10 mm) according to a previous study (Deng et al., 2017b).

2.7. Measurements of soluble protein content and antioxidant enzyme activity

At the end of exposure, samples were taken out and centrifuged at 4 °C and 6000 rpm for 10 min to collect the cells of *P. tricornutum*. The collected cells were resuspended in 2 mL phosphate-buffered saline (0.02 mol L^{-1}, pH 7.4), and then lysed with an ultrasonic cell pulverizer (JYD-150L, Zhixin Instruments Co., Ltd., Shanghai, China) at 200 W for 10 min (using a 5-s on and 5-s off cycle) in an ice bath. After that, the cellular homogenate was centrifuged at 12000 rpm and 4 °C for 5 min to remove the cell debris, and the supernatant was used to determine soluble protein content, superoxide dismutase (SOD) and catalase (CAT) activities with diagnostic reagent kits according to the methods of manufacturer's instructions. All the kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China). Results of SOD and CAT activities were presented in the units of enzymatic activity per milligram of proteins (U mg^{-1} proteins), and content of soluble protein was expressed as micrograms per 10^6 cells (μg × 10^6 $cell^{-1}$).

2.8. Determination of reactive oxygen species (ROS) level and MDA content

Reactive oxygen species (ROS) and malondialdehyde (MDA) assay kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China). According to the methods of manufacturer's instructions, ROS level was determined using a 2,7-dichlorofuorescin diacetate (DCFH-DA) probe, and detected by a multi-mode microplate reader (Molecular Devices, LLC., San Jose, CA, USA) at the emission and excitation wavelengths of 525 and 485 nm, respectively. The relative ROS level (%) was presented in the ratios of DCFH-DA fluorescence values of the treatments to that of the controls, and calculated by the following equation:

$$\text{Relative ROS level } (\%) = \frac{\text{DCFH-DA fluorescence values of the treatments}}{\text{DCFH-DA fluorescence values of the controls}} \times 100\%$$

The MDA was extracted and determined from the supernatant liquids (above) according to the manufacturer's instructions. Results of MDA content were given as nanomoles per 10^7 cells (nmol 10^7 $cell^{-1}$).

2.9. Statistical analysis

In the current work, data were presented as the means ± standard deviation (SD) of three independent experiments in each test. And the statistical significance of the data between treatments and controls was evaluated by one-way analysis of variance (ANOVA) method followed by Duncan's multiple range tests in an SPSS software (version 18.0) for Windows (Statistical Product and Service Solutions, distributed by SPSS Inc., Chicago, IL, USA). Statistical significance was accepted at a level of $p < 0.05$.

3. Results And Discussion

3.1. Growth inhibition and morphology of the diatom

As one of the representative lower substituted PBDEs, BDE-15 was used in the current work to assess its effects on the growth of *P. tricornutum*. As illustrated in Fig. 1a, a normal growth curve was observed when *P. tricornutum* grew on the f/2 medium and the medium with 0.1% DMSO, suggesting that the cultivation conditions used in this study were very suitable for the growth of this diatom, and DMSO had no impacts on its growth. However, the growth of *P. tricornutum* was significantly inhibited by BDE-15 ($p < 0.05$), and the growth inhibition rate (*IR*) increased with the increase of BDE-15 concentrations, presenting a significant dose-response relationship. For example, the *IR* values were 12.6, 22.7, 39.7, 64.7, 86.6, and 91.8%, respectively, when *P. tricornutum* was exposed to 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 mg L^{-1} at the 96 h of exposure. Similar results were obtained by other researchers, who found that the relative growth rates of two species of marine bloom-forming microalgae (*Heterosigma akashiwo* and *Karenia mikimotoi*) decreased dramatically with increasing concentrations of decabromodiphenyl ether (BDE-209) (Zhang et al., 2013); moreover, BDE-47 had negative effects on the population growth of two diatoms (*Thalassiosira pseudonana* and *P. tricornutum*) in a time- and concentration-dependent manner (Zhao et al., 2019a; Liu et al., 2020). Based on these results, it is concluded that PBDEs posed serious potential threats to the growth of primary producers in marine ecosystems, and thus their potential environmental risks should not be overlooked due to the fact that these PBDEs were all detected in the marine environment.

Intact cell structure is necessary for maintaining normal cellular functions, while damaged cell structure will affect the cell survival and growth. In the current work, cell morphology of *P. tricornutum* was observed under the exposure of BDE-15 by scanning electron microscope in order to investigate the impacts of BDE-15 on cell morphology of this diatom. As presented in Fig. 1b, cells in the controls were regular oval with smooth surface, and a small number of extracellular polymeric substances were gathered around the cells; however, the cell surface of *P. tricornutum* was wrinkled and deformed when the diatom was exposed to 0.7 mg L^{-1} BDE-15 for 96 h, and abundant extracellular polymeric substances were observed around the cells. The cell deformation indicated that BDE-15 may intensify the membrane permeability and cause irreversible lesion on the cytomembrane and cell wall. Similar results were also reported in *P. tricornutum* when it was exposed to the fungicide azoxystrobin (Du et al., 2019). In addition, extracellular polymeric substances are often generated during the metabolism process of algal cells, which mainly include polysaccharides, proteins (i.e. enzymes and structural proteins), nucleic acids (DNA), and lipids (Xiao and Zheng, 2016). The massive accumulation of extracellular polymeric substances is a common phenomenon that occurs in algae and plants under stress conditions. This response was also observed in *P. tricornutum* and *Chlorella* sp. under the exposure of microplastics (Song et al., 2020). Thus, it is concluded that BDE-15 had physical damage to cell structure of *P. tricornutum*, which would then cause the growth inhibition of this diatom.

In addition, EC_{50} value is one of the most frequently used parameters in (eco)-toxicological studies for evaluating the toxicity of environmental pollutants to organisms. The EC_{50} values of BDE-15 against *P.*

tricornutum were calculated in the current work, and the 24, 48, 72 and 96 h EC_{50} values were 1.03, 0.44, 0.41, 0.42 mg L^{-1}, respectively, which showed that the EC_{50} values decreased first from 24 to 48 h, and then levelled off in the remaining hours of the toxicity tests. This phenomenon may result from the acclimation of the diatom to BDE-15 with the prolongation of exposure time. (Ertürk and Saçan, 2012). According to the Chinese guidelines for the hazard evaluation of new chemical substances (HJ/T 154–2004), BDE-15 could be classified as a very high toxic substance because its EC_{50} values were less than 1 mg L^{-1} after 48 h of exposure. Moreover, the lower value of EC_{50}, which is a relatively sensitive index for evaluating the toxicity of a compound, means higher toxicity. The obtained data in this study indicated that the toxicity of BDE-15 to *P. tricornutum* increased first and then levelled off with increasing the exposure time, and its maximum toxicity was achieved after 48 h of exposure. Compared with previous studies, the EC_{50} values of BDE-15 against *P. tricornutum* were higher than that of BDE-47 to *T. pseudonana* (13.53 μg L^{-1}) at 96 h of exposure (Zhao et al., 2019b), but lower than that of BDE-209 to *H. akashiwo* (22.58 mg L^{-1}) and to *K. mikimotoi* (120.8 mg L^{-1}) at 96 h of exposure (Zhang et al., 2013). Differences in the toxicity of PBDEs to microalgae may be due to the following reasons. (1) Locations and numbers of bromine substituent in the molecular structure of PBDEs are different, which determine the steric hindrance of these chemical compounds. It was reported that PBDEs with fewer bromine atoms could more easily overcome biological membranes and thereby exert greater toxic effects on organisms (Pazin et al., 2014). (2) Differences in the size and structure of algal cells and in the production of extracellular organic substances may lead to high variability in the susceptibility of algal species to chemical pollutants (Rojíčková and Marsálek, 1999). For example, cell wall of *P. tricornutum* exhibits a three-layer construction: a thin (3 nm) electron opaque layer facing the cell interior is followed by a thicker (4–6 nm), less opaque middle layer, and an outer, more opaque layer the basal part of which is approximately of the same width as the interior layer (Reimann and Volcani, 1968). Cell walls of *Scenedesmus obliquus* and other species of *Chlorococcales* also present a characteristic trilaminar structure, but they contain cellulose in the inner wall layers and insoluble, acetolysis-resistant, lipid-containing biopolymers in the outer wall layers (Voigt et al., 2014). Thus, the differences in structures and biochemical components of cell wall may cause different responses of algal cells to the exposure of PBDEs due to the fact that cell wall plays an important role in transport of materials in and out of the cells. In the future, more investigations should be performed to study the relationship between the structures of cells and toxicity of PBDEs in particular.

3.2. Changes in chlorophyll a content of P. tricornutum exposed to BDE-15

As one of the photosynthetic pigments, chlorophyll *a* is intimately involved in light harvesting, energy transfer, and light energy conversion in photosynthesis during the photoautotrophic growth and reproduction of microalgae, which is often used as an efficient parameter to evaluate the response of algal cells to environmental pollutants (Bi et al., 2012). In the current work, contents of chlorophyll *a* in *P. tricornutum* were determined daily when the diatom was exposed to BDE-15, and their changes over time are illustrated in Fig. 2. As shown in the figure, contents of chlorophyll *a* in *P. tricornutum* significantly

decreased when the exposure time and BDE-15 concentrations increased from 24 to 96 h and from 0.2 to 0.7 mg L^{-1}, respectively. For example, contents of chlorophyll *a* were only 62.1, 41.7, 41.6, and 46.7% of that in the controls when *P. tricornutum* was exposed to 0.4 mg L^{-1} BDE-15 at 24, 48, 72, and 96 h of exposure, respectively. Relative to the controls, contents of chlorophyll *a* decreased by 28.1, 40.0, 53.2, 82.5, 96.3, and 99.4% when the diatom was exposed to 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 mg L^{-1} BDE-15 at 96 h of exposure, respectively, which exhibited a significant dose-response relationship. The data were similar with that reported by (Liu et al., 2020), who found that chlorophyll *a* content in *P. tricornutum* amounted to only 79.0% and 72.8% of that in the controls, respectively, when it was exposed to 0.8 and 4 mg L^{-1} BDE-47 at 96 h of exposure. Chlorophyll content of *Lemna minor* decreased to 79.6 and 29.1% of that in the controls, respectively, when its fronds were exposed to 15 and 20 mg L^{-1} BDE-209 (Sun et al., 2019). Moreover, (Zhang et al., 2013) observed that the nucleus underwent a slight transformation in that there were some compact spots of dense chromatin, the chloroplasts were broken and their number decreased, and the structure of lamellae was indistinct when *H. akashiwo* and *K. mikimotoi* were exposed to BDE-209. The ultrastructural alterations in chloroplasts would cause a decline in the contents of chlorophyll *a* because chlorophyll and other pigments exist in the chloroplast. In addition, oxidative stress induced by reactive oxygen species (ROS) has been described as one the most plausible mechanisms of the toxicity of PBDEs to organisms (Barón et al., 2016). It is well known that chloroplast is an important apparatus responsible for the ROS production during photosynthesis in the cells (Xing et al., 2013). Normally, the ROS accumulation within chloroplasts is controlled by a complex antioxidant-scavenging system; however, excessive ROS would be produced when cells are exposed to external stimulus such as environmental pollutants, which disturb the formation of Mg-protoporphyrin IX from magnesium chelatase and magnesium (Walker and Willows, 1997). Thus, the biosynthesis pathway of chlorophyll *a* in the cells would be impeded because this process requires Mg-protoporphyrin IX. In light of the above data and discussion, it is concluded that photosynthetic efficiency of *P. tricornutum* would be impacted and its energy deficiency would also be aggravated under the exposure of BDE-15, which may be considered as one of possible toxic mechanisms/pathways of PBDEs to marine diatoms, although further exploration of the mechanisms leading to decrease of chlorophyll content induced by PBDEs is needed in the future.

3.3 Effects of BDE-15 on the chlorophyll fluorescence of P. tricornutum

Among the chlorophyll fluorescence parameters, F_v/F_m and Φ_{PSII} have been recommended as a simple and rapid way to evaluate the photosynthetic activity of algae and plants, which represent the conversion efficiency of and PSII capture efficiency of primary light energy, respectively (Gao et al., 2016). Thus, values of F_v/F_m and Φ_{PSII} were measured in the current work to investigate the photosynthetic activity of *P. tricornutum* exposed to BDE-15 for 96 h, and their changes over time are presented in Fig. 3. As illustrated in Figs. 3a and 3b, values of F_v/F_m and Φ_{PSII} sustained at about 0.65 and 0.50, respectively, when *P. tricornutum* grew on the f/2 medium and the medium with 0.1% DMSO during the 96-h cultivation. Values of F_v/F_m were reported to be around 0.83 for healthy plants and somewhat lower for

algae (0.55–0.80) (Maxwell and Johnson, 2000). Thus, the data indicated that this diatom was in good physiological state and had a strong photosynthetic activity to support its autotrophic growth under the cultivation conditions used in this work. When *P. tricornutum* was exposed to 0.2, 0.3, and 0.4 mg L^{-1} BDE-15, values of F_v/F_m and Φ_{PSII} decreased within the first 24 h of exposure, and then increased slightly or levelled off in the remaining hours of the toxicity tests (Figs. 3a and 3b). The data were similar with that reported by Zhao et al. (Zhao et al., 2017), who found that values of F_v/F_m exhibited a decline within the first 24 h, and then they started to recover to values similar to the control at 120 h of exposure, when *Dunaliella salina* was exposed to BDE-47. The recovery of F_v/F_m and Φ_{PSII} values with the increase of exposure time may be attributed to the modest allocation and integration of cellular components (pigments, carbohydrates, proteins, etc.) for repairing the damage of the algal cells caused by BDE-15 or enhancing its resistance to BDE-15 (Calabrese, 2015). However, values of F_v/F_m and Φ_{PSII} decreased significantly within the first 24 h of exposure ($p < 0.05$), and then decreased gradually after 24 h when *P. tricornutum* was exposed to 0.5, 0.6, and 0.7 mg L^{-1} BDE-15 (Figs. 3a and 3b). Correspondingly, the F_v/F_m and Φ_{PSII} values were only 70.9, 42.5, 4.7% and 64.4, 36.6, 5.9% of that in the controls at 96 h of exposure, respectively. These results were consistent with a previous literature showing that the F_v/F_m values decreased with the increase of exposure time and BDE-47 concentrations, and they declined by 14, 18, and 59% relative to the controls when *Alexandrium minutum* was exposed to 0.074, 0.365, and 0.697 mg L^{-1} BDE-47, respectively, at 120 h of exposure (Zhao et al., 2019b). Generally, a decrease in F_v/F_m values indicates that the ratio of electrons generated in PSII to photons absorbed by the light-harvesting pigments decreases due to oxidation, degradation of D1 protein, or severely reduced pigment contents (Li et al., 2014). Thus, the decrease in values of F_v/F_m observed in this study may be resulted from the remarkable reduction in contents of chlorophyll (Fig. 2) and oxidative stress (Fig. 5) in the diatom cells induced by BDE-15. In addition, a marked decrease in Φ_{PSII} was observed when *P. tricornutum* was exposed to BDE-15, meaning that it had a significant inhibition on the photosynthetic efficiency of this diatom. As an index of PSII capture efficiency of primary light energy, Φ_{PSII} refers to the proportion of light quantum absorbed by PSII for photochemical reaction, which is closely related to the degree of carbon assimilation reaction (Liu et al., 2015). Based on previously published literatures, the photosynthetic toxicity mechanism of PBDEs to algal cells may be due to the fact that they could impede the formation of cell assimilatory power (NADPH and ATP), damage the process of carbon fixation and assimilation, and decrease the electron transportation ability of PSII (Zhao et al., 2017; Liu et al., 2020). Moreover, the underlying molecular mechanism of PBDEs was revealed by transcriptomic analysis, which showed that about 62 genes related to photosynthesis were differentially expressed when *P. tricornutum* was exposed to 4 mg L^{-1} BDE-47, and expressions of 58 genes involved in chlorophyll synthesis, antenna proteins, oxygen evolution, electron transport, and downstream carbon fixation were downregulated (Liu et al., 2020). Therefore, it is concluded that BDE-15 had a substantial effect on the photosynthetic activity of this diatom, which would result in a series of physiological, biochemical and molecular changes in the algal cells, and affect the algal growth eventually.

3.4. Effects of BDE-15 on soluble protein content

Soluble protein plays an important role in algae metabolism, and it can respond rapidly to a wide variety of environmental stresses by transferring the message of stressors, producing defensive and protective molecules, and degrading some unfavorable or unnecessary proteins to produce other required ones (Bajguz and Piotrowska-Niczyporuk, 2014; Kazemi-Shahandashti and Maali-Amiri, 2018). In the current work, contents of soluble protein in *P. tricornutum* were determined when it was exposed to BDE-15 at 96 h of exposure, and are shown in Fig. 4. It can be seen that there were no significant changes in the soluble protein contents when *P. tricornutum* was exposed to 0.2 and 0.3 mg L^{-1} BDE-15 relative to the controls ($p > 0.05$); however, a remarkable decrease in the soluble protein contents was observed when the diatom was exposed to 0.4, 0.5, 0.6, and 0.7 mg L^{-1} BDE-15 ($p < 0.05$). The data were similar with that reported by (Sun et al., 2019), who found that contents of soluble protein in *L. minor* fronds increased to 129.3 and 141.6% of that in the controls, respectively, when the fronds were exposed to 5 and 10 mg L^{-1} BDE-209 for 14 days, but they were reduced to 77.1 and 47.2% of that in the controls, respectively, when the fronds were treated with 15 and 20 mg L^{-1} BDE-209. Additionally, (Lv et al., 2020) observed a significant increase in contents of extracellular protein when *Chlorella* sp. was exposed to BDE-47 for 48 h; but (Qiu et al., 2018) found that BDE-47 could induce a continuous decrease of the soluble protein content in the fronds of *L. minor* with the increase of BDE-47 concentrations. Thus, it is concluded that exposure of PBDEs could cause significant changes in the contents of soluble protein in aquatic organisms according to the results obtained in this work and the published literatures, and the decrease in contents of soluble protein in the cells may be due to the following reasons. (1) Various propagating radicals and ROS would be excessively generated in the algal cells under the exposure of PBDEs, which were reported to affect the structures and compositions of protein, and hinder the synthesis of protein (Nong et al., 2021). For example, ROS would cause oxidative modifications of amino acid side chains, ROS-mediated peptide cleavage, reactions of peptides with lipids and carbohydrate oxidation products, and the formation of carbonyl derivatives of proteins (Valavanidis et al., 2006). (2) The decrease in contents of chlorophyll *a* and chlorophyll fluorescence parameters indicated that PBDEs inhibited the algal photosynthesis, which would cause the decrease in contents of soluble protein due to the fact that protein is one of the products of photosynthesis (Xiao et al., 2016). (3) The diatom metabolism would be disturbed under the exposure of BDE-15, leading to the descendance or cessation of the synthesis of protein. Therefore, contents of soluble protein in *P. tricornutum* decreased in the current work when it was exposed to BDE-15 with a concentration of ≥ 0.4 mg L^{-1}.

3.5. Effects of BDE-15 on ROS level and MDA content

Reactive oxygen species (ROS), including singlet oxygen, hydrogen peroxides, and superoxides, could alter redox-mediated cellular processes and damage cell membranes and other cellular components, resulting in toxicological stress to cells (Saleh et al., 2016). In this work, levels of ROS in *P. tricornutum* were determined when the diatom was exposed to BDE-15 for 96 h, and are shown in Fig. 5a. It can be seen that levels of ROS increased significantly with increasing the concentrations of BDE-15 from 0.2 to 0.7 mg L^{-1} ($p < 0.05$). Relative to the controls, the ROS levels were 1.99, 2.03, 2.68, 3.36, 4.24, and 5.14 times greater than that of the controls when *P. tricornutum* was exposed to 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7

mg L^{-1} BDE-15, respectively. The data were consistent with that reported by (Liu et al., 2020), who found that levels of ROS in *P. tricornutum* increased with increasing the concentrations of BDE-47, and the excessive ROS could lead to chloroplast membrane damage to aggravate the growth inhibition *via* a feedback loop; and by (Zhang et al., 2016), who observed that both BDE-47 and BDE-209 could induce the formation of ROS in the rotifer ovary, which was positively correlated with their concentrations. In addition, excessive ROS were reported to induce lipid peroxidation leading to the disruption of cell membrane, protein oxidation resulting in imbalance of enzymatic activities, nucleic acid oxidation followed by genetic mutation, and developmental toxicity (Saleh et al., 2016). Thus, the overproduction of ROS in algal cells could be regarded as one of the major factors in BDE-15 toxicity.

In light of the above analysis, lipid peroxidation is one of the primary manifestations of excessive ROS in organisms under the exposure of PBDEs. Malondialdehyde (MDA) is a cytotoxic product of lipid peroxidation and an indicator of ROS production and consequent tissue damage (Wang et al., 2011). In the current work, contents of MDA in *P. tricornutum* were measured when it was exposed to different concentrations of BDE-15 after 96 h, and are shown in Fig. 5b. It can be seen that the MDA contents did not change significantly when *P. tricornutum* was exposed to low BDE-15 concentrations (0.2, 0.3, and 0.4 mg L^{-1}), relative to the controls ($p > 0.05$), but they were significantly increased with the increase of BDE-15 concentrations from 0.5 to 0.7 mg L^{-1} ($p < 0.05$). The increased MDA contents suggested that excessive ROS would be generated in the cells of *P. tricornutum* when it was exposed to BDE-15 because contents of MDA in *P. tricornutum* were reported to be positively correlated with the ROS levels under the exposure of PBDEs according to Pearson's correlation analysis (Liu et al., 2020). The excessive ROS would cause lipid peroxidation and a shortage of metabolic energy *via* oxidation of unsaturated fatty acids on biofilms and consuming some additional metabolic energy, respectively, which was supported by the findings drawn from the impacts of BDE-15 exposure on photosynthetic activity of this diatom (Figs. 2 and 3). In addition, these data were in agreement with that reported by (Qiu et al., 2018) and (Sun et al., 2019), who found that contents of MDA in the fronds of *L. minor* were 1.10, 1.20, 1.25, 1.34 times and 1.17, 1.27, 1.33, 1.42 times greater than that of the controls, respectively, when the fronds were exposed to 5, 10, 15, 20 μg L^{-1} BDE-47, and 5, 10, 15, 20 mg L^{-1} BDE- 209. In cells, MDA was reported to cause cross-linking of proteins, nucleic acids, and other biological molecules, which coupled with the effects of ROS and antioxidant enzymes would lead to the breakage in single and double strands, base modifications, fragmentation of deoxyribose (Bacanlı et al., 2014). Therefore, it is concluded that excessive ROS were accumulated in the cells of *P. tricornutum* under the exposure of BDE-15, resulting in a visible increase in MDA content, which may lead to growth inhibition, decrease of photosynthetic activity, and changes in chemical compositions of this diatom.

3.6. Effects of BDE-15 on the activities of antioxidant enzymes

It is a well-known phenomenon that external pollutants could provoke an oxidative burden on algal cells either directly or indirectly by triggering the overproduction of ROS. The excessive ROS would be removed by intracellular antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and

peroxidase (POD), to prevent oxidative damage, and then protect cells from the adverse effects of ROS (Wang et al., 2017). In this work, oxidative stress of antioxidant defense system in the cells of *P. tricornutum* caused by BDE-15 was determined by using SOD and CAT as biomarkers, and their activities were measured when *P. tricornutum* was exposed to different concentrations of BDE-15 at 96 h of exposure. As shown in Fig. 6, activities of SOD and CAT increased with increasing the concentrations of BDE-15 from 0.2 to 0.5 mg L^{-1}, and then decreased when the concentrations increased from 0.6 to 0.7 mg L^{-1}. And the maximum SOD and CAT activities of 5.55 and 3.29 U mg^{-1} proteins were obtained when *P. tricornutum* was exposed to 0.5 mg L^{-1} BDE-15, respectively. The data were in line with that reported by (Sun et al., 2019), who found that the SOD, POD, and CAT activities of *L. minor* increased when the BDE-209 concentrations increased from 0 to 10 mg L^{-1}, and then decreased with a further increase in the BDE-209 concentrations. Moreover, (Qiu et al., 2018) observed that the POD and CAT activities exhibited a parabolic trend with the increase of BDE-47 concentrations from 0 to 20 μg L^{-1} when *L. minor* was exposed to BDE-47. As we know, SOD is responsible for the transformation of potentially toxic superoxide anion radicals into H_2O_2, and CAT is capable of efficient H_2O_2 decomposition in algal cells (Tripathi et al., 2006). The co-ordinated function of SOD-CAT redox system plays a significant role in ROS scavenging. Thus, BDE-15 caused a great enhancement of SOD and CAT activities in the cells of *P. tricornutum* relative to the controls, which was accounted for an evidence for overproduction of ROS under the exposure of BDE-15, and considered as a protective strategy against the potential increase of ROS production and an adjustment in response to the oxidative conditions. However, a significant decrease in SOD and CAT activities was observed when *P. tricornutum* was exposed to 0.6 and 0.7 mg L^{-1} BDE-15, suggesting that activities of the two enzymes were suppressed because their scavenging capacity was exceeded by the production rate of ROS. And furtherly, the excessive ROS caused a decrease in photosynthetic activity (Figs. 2 and 3) and soluble protein content (Fig. 4), and a visible increase in MDA content (Fig. 5b) when *P. tricornutum* was exposed to BDE-15. Therefore, the overproduction of ROS could be regarded as one of the primary toxicity mechanisms of PBDEs to marine diatoms.

4. Conclusion

Based on these results, several key conclusions were yielded and listed as follows:

(1) Growth of *P. tricornutum* was significantly inhibited by BDE-15, and its maximum toxicity was achieved after 48 h of exposure.

(2) BDE-15 had toxic effects on cell structure and photosynthetic activity of this diatom, and caused a series of physiological, biochemical, and molecular changes in the cells.

(3) Metabolism process of *P. tricornutum* was disturbed by BDE-15, leading to the decreases in chlorophyll *a* and soluble protein contents.

(4) Under the exposure of BDE-15, excessive ROS were accumulated in the algal cells, which may cause or intensify the peroxidation of membrane lipids. The overproduction of ROS in algal cells may be

regarded as one of the major factors in BDE-15 toxicity.

(5) The increase in activities of antioxidant enzymes could be considered as a protective strategy against the potential toxicity of excessive ROS and an adjustment in response to the oxidative conditions; however, high concentrations of BDE-15 would disturb the algal antioxidant defense system balance and lead to an irreversible damage.

Declarations

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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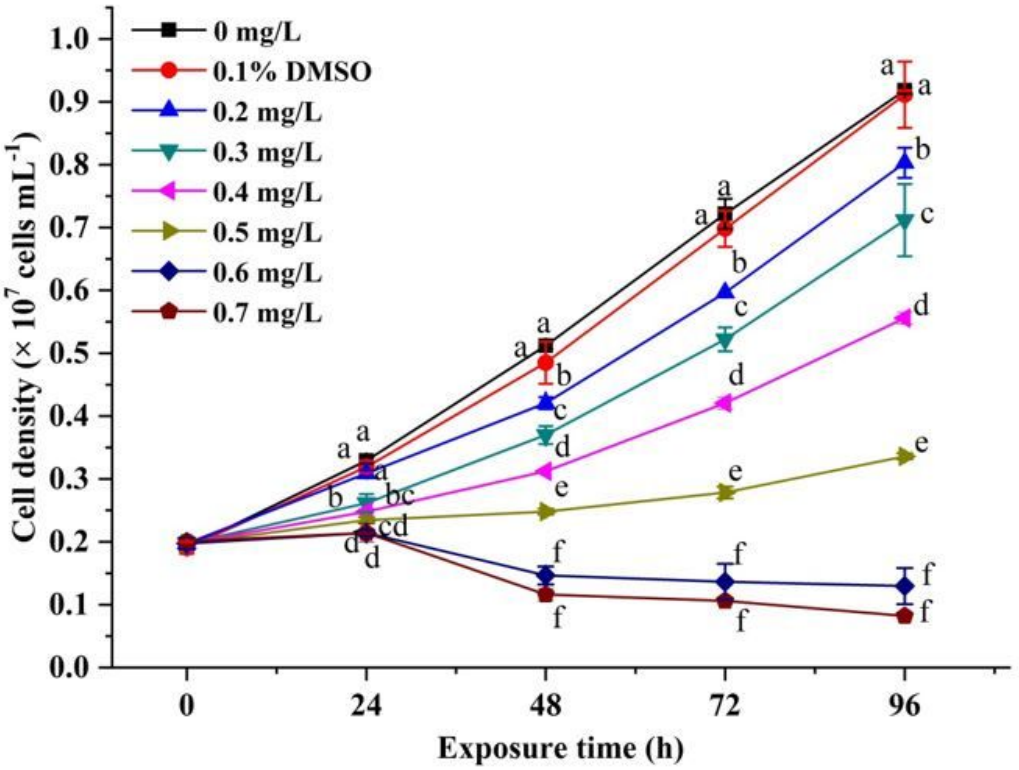
Figures



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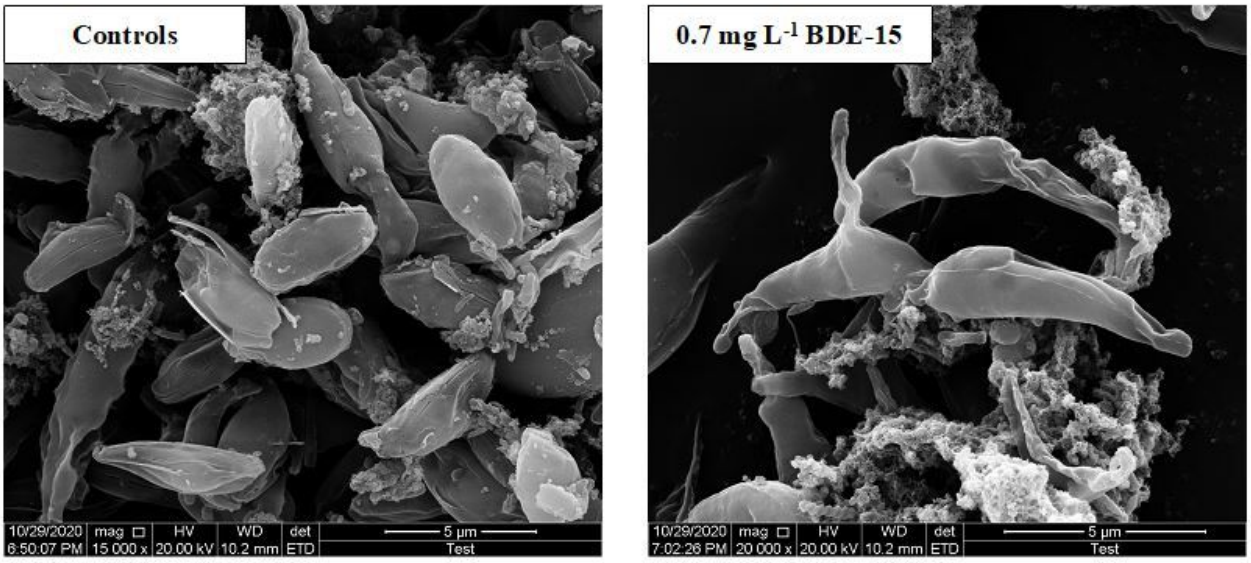


b

Figure 1

(a) Growth curves of Phaeodactylum tricornutum under different BDE-15 concentrations during 96 h of exposure. Each point represents the mean of 3 biological replicates; and error bars represent the standard deviation (SD). Different letters indicate statistically significant differences at $p < 0.05$ between the treatments and controls as determined by Duncan's multiple range tests. (b) The scanning electron microscope images of P. tricornutum grown in the controls and exposed to 0.7 mg L-1 BDE-15 for 96 h.

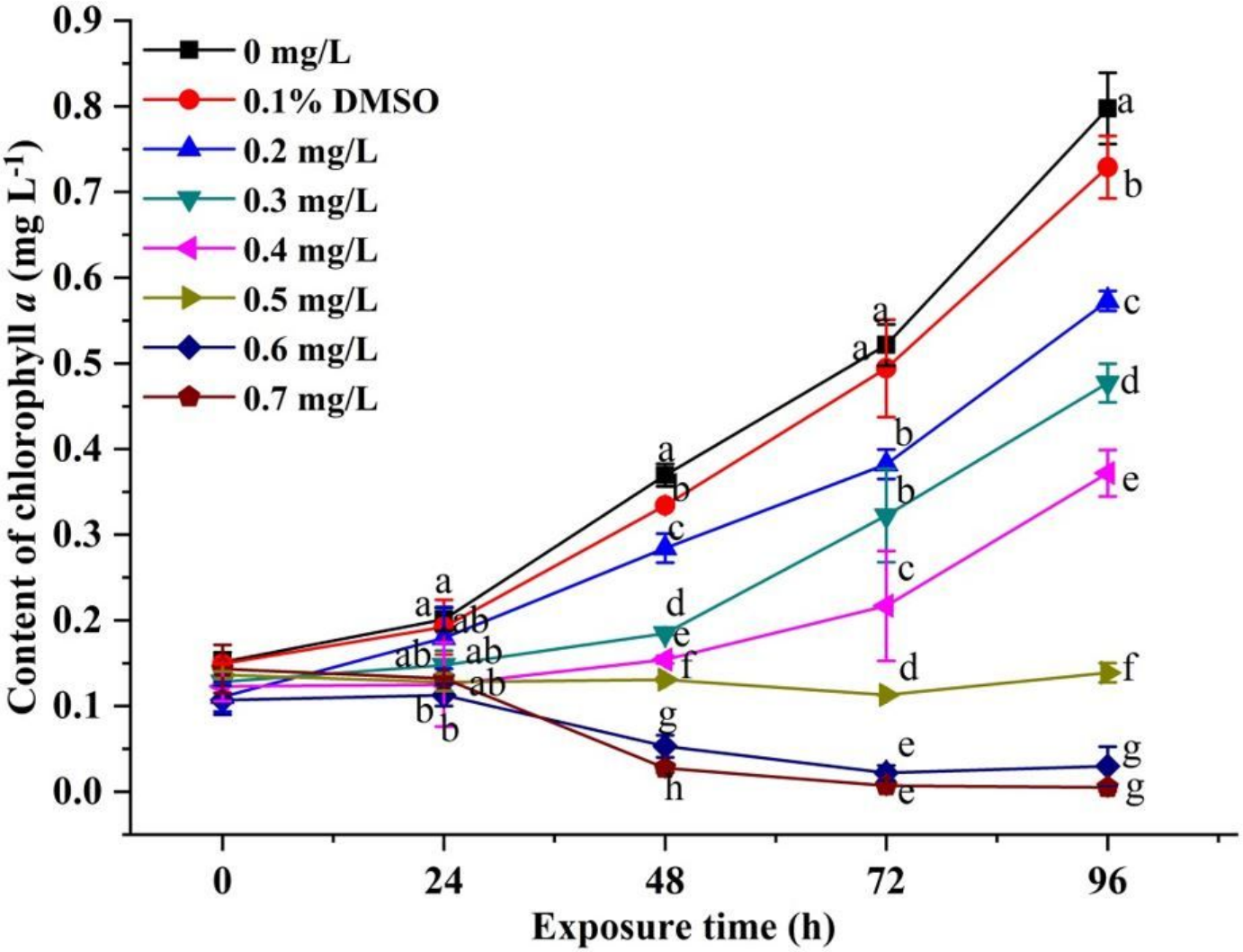


Figure 2

Variations in the contents of chlorophyll a in Phaeodactylum tricornutum when it was exposed to different concentrations of BDE-15 for 96 h. Each point represents the mean of 3 biological replicates; and error bars represent the standard deviation (SD). Different letters indicate statistically significant differences at $p < 0.05$ between the treatments and controls as determined by Duncan's multiple range tests.

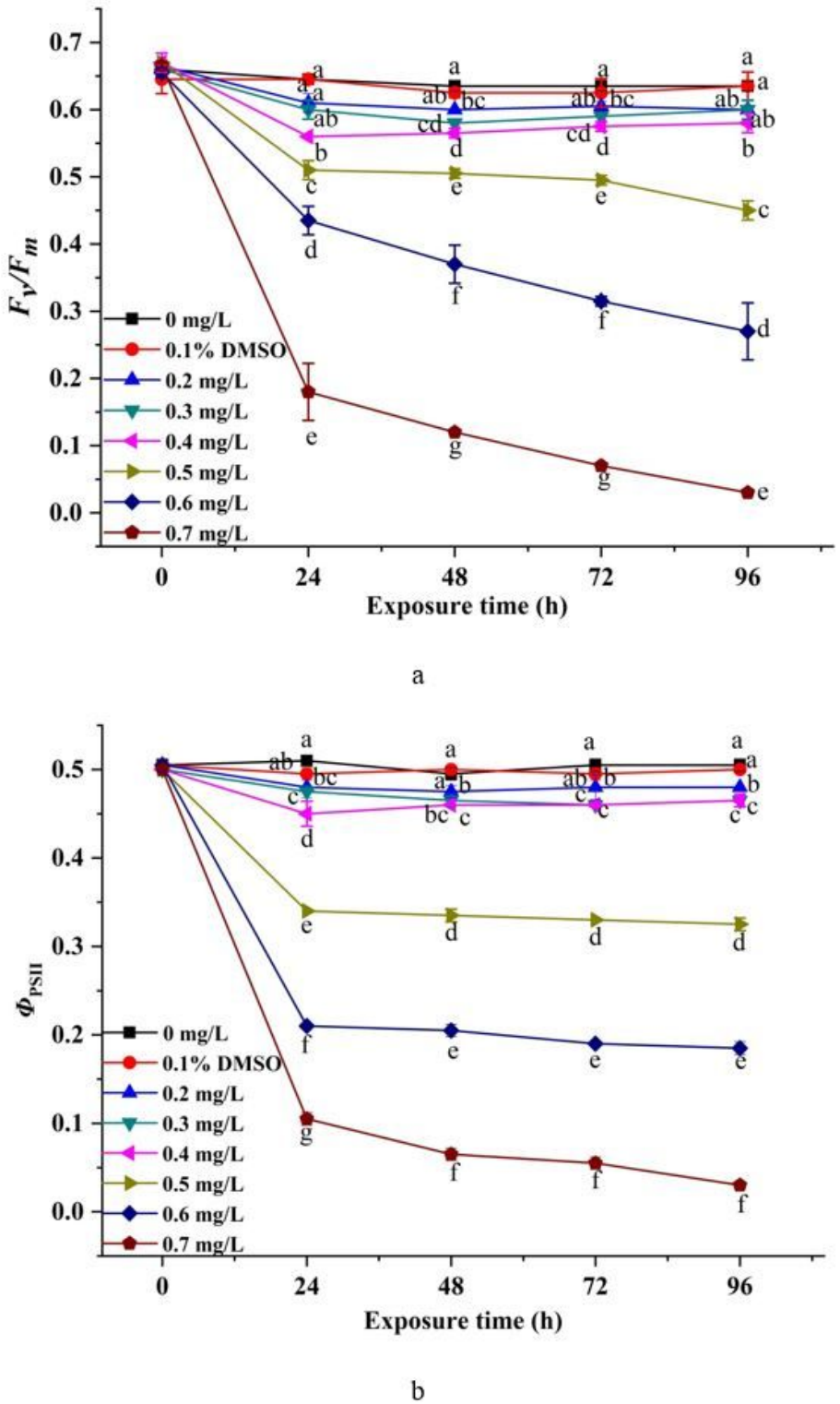


Figure 3

Changes in the maximum quantum efficiency of photosystem II (PSII) (Fv/Fm) (a) and effective quantum yield of PSII (ΦPSII) (b) of Phaeodactylum tricornutum when it was exposed to different concentrations of BDE-15 for 96 h. Each point represents the mean of 3 biological replicates; and error bars represent the standard deviation (SD). Different letters indicate statistically significant differences at $p < 0.05$ between the treatments and controls as determined by Duncan's multiple range tests.

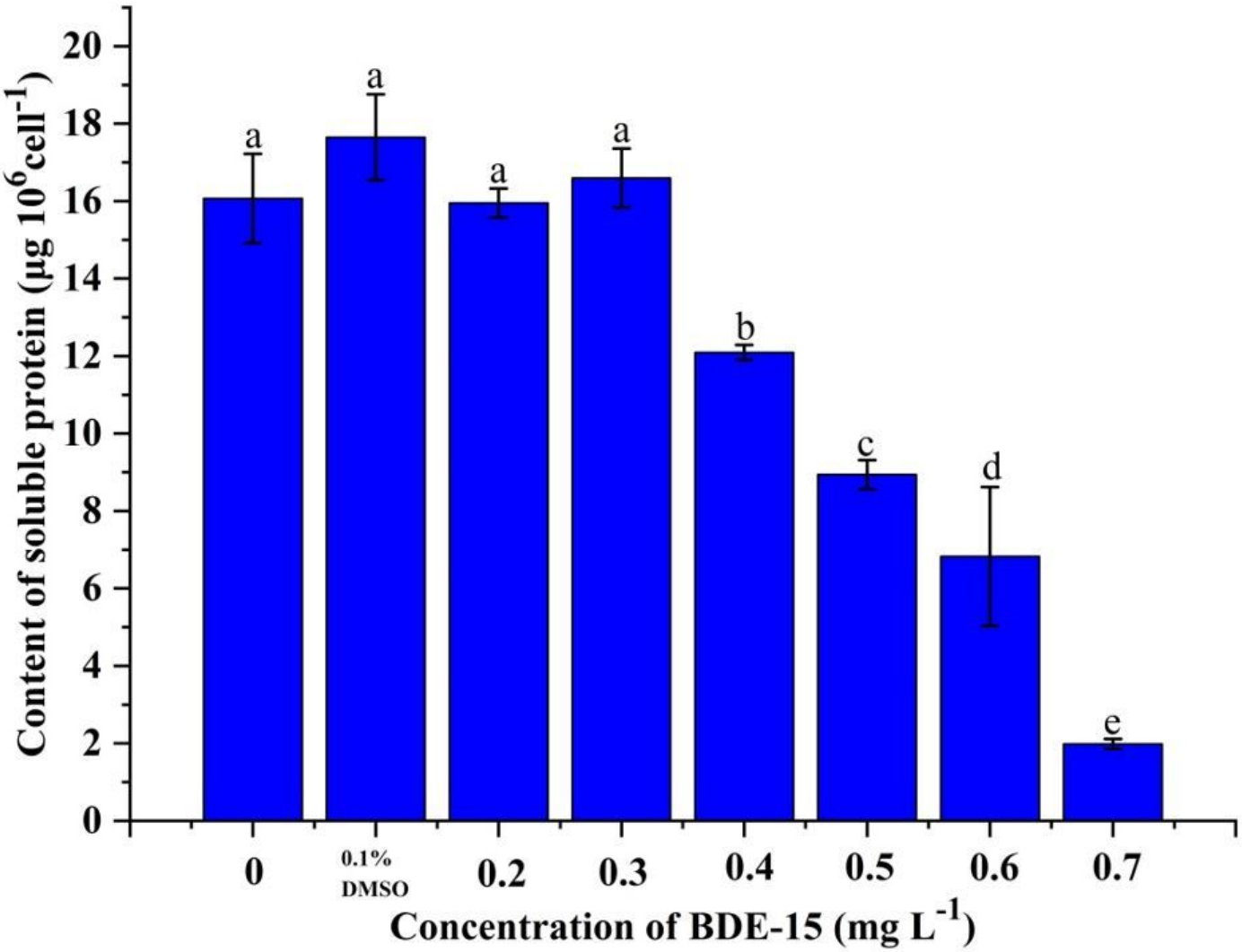
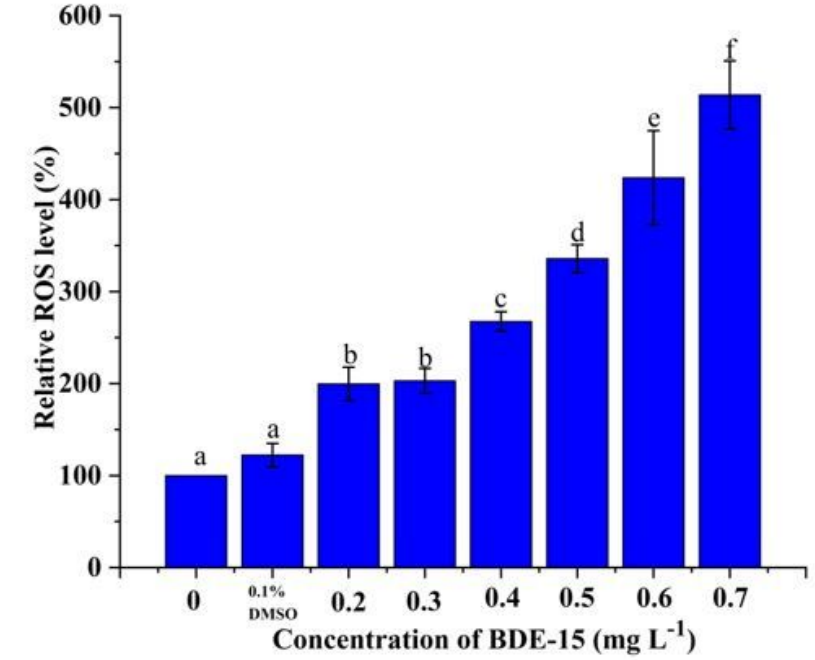
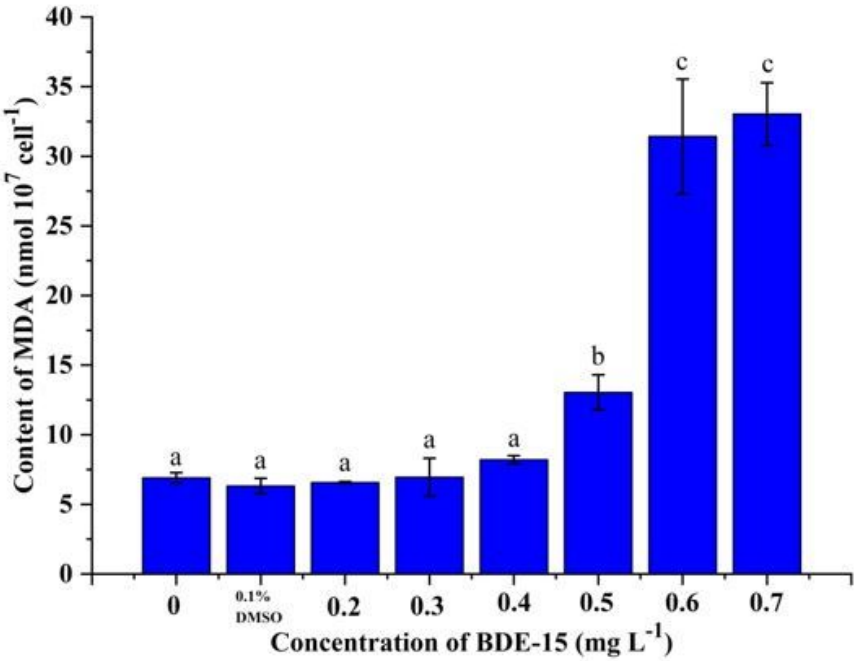


Figure 4

Effects of BDE-15 on the contents of soluble protein in Phaeodactylum tricornutum when it was exposed to different concentrations of BDE-15 at the 96 h of exposure. Each column represents the mean of 3 biological replicates; and error bars represent the standard deviation (SD). Different letters above the columns indicate statistically significant differences at $p < 0.05$ between the treatments and controls as determined by Duncan's multiple range tests.



a



b

Figure 5

Effects of BDE-15 on the relative ROS levels (a) and malondialdehyde (MDA) contents (b) of Phaeodactylum tricornutum when it was exposed to different concentrations of BDE-15 at the 96 h of exposure. Each column represents the mean of 3 biological replicates; and error bars represent the standard deviation (SD). Different letters above the columns indicate statistically significant differences at $p < 0.05$ between the treatments and controls as determined by Duncan's multiple range tests.

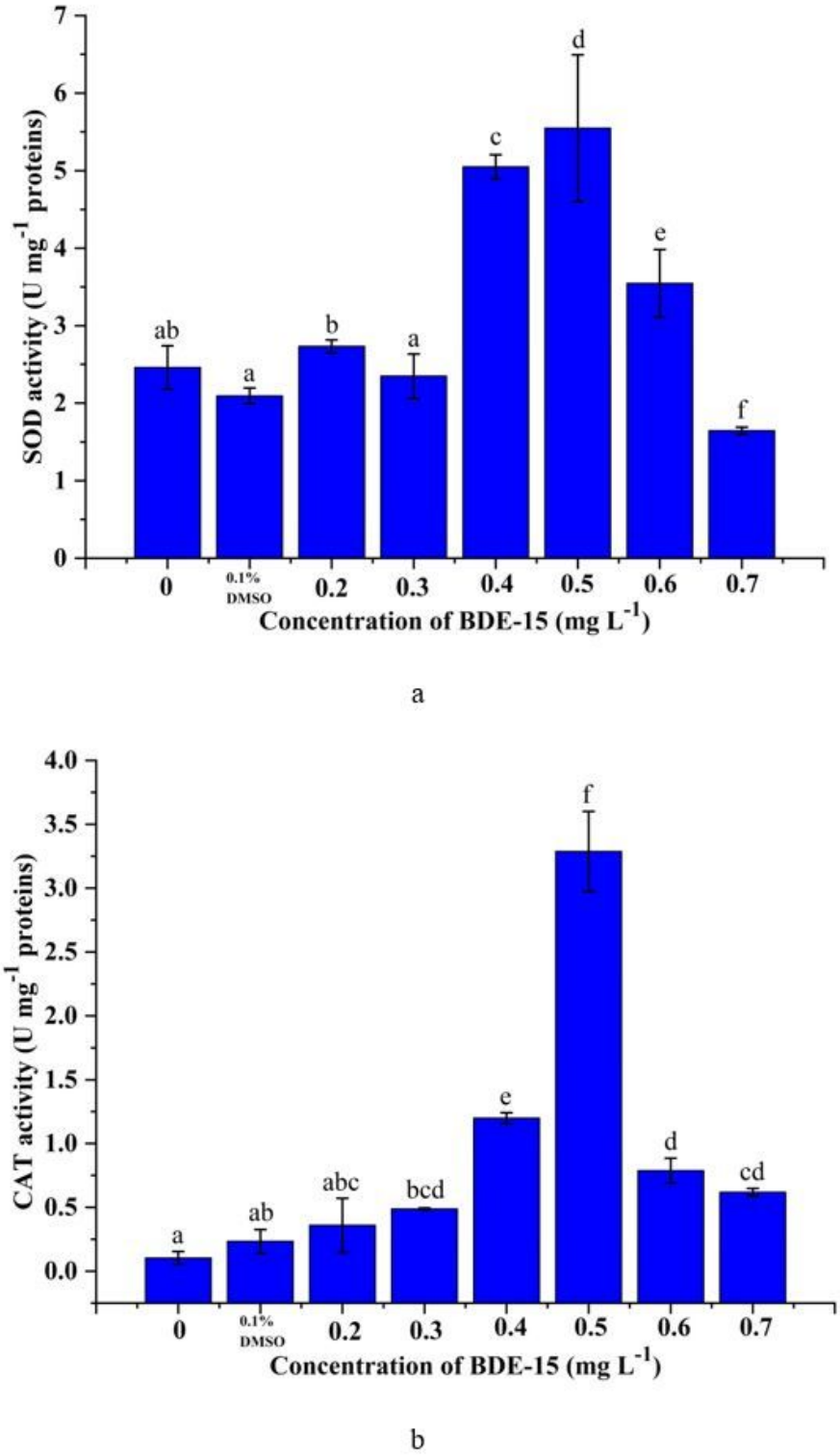


Figure 6

Effects of BDE-15 on the activities of superoxide dismutase (SOD) (a) and catalase (CAT) (b) in P. tricornutum when it was exposed to different concentrations of BDE-15 at the 96 h of exposure. Each column represents the mean of 3 biological replicates; and error bars represent the standard deviation (SD). Different letters above the columns indicate statistically significant differences at $p < 0.05$ between the treatments and controls as determined by Duncan's multiple range tests.