

Toxic Effects of 2, 2', 4, 4'-tetrabromodiphenyl ether (BDE-47) on *Dicrateria inornata*

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Abstract. In this paper, we studied the toxicity of different concentrations of 2, 2', 4, 4'-tetrabromodiphenyl ether (BDE-47) on *Dicrateria inornata*. The results showed that the 96 h EC₅₀ value of BDE-47 compound was 165 µg·L⁻¹. By studying the changes of photosynthetic pigments and chlorophyll fluorescence characteristics, the toxicity mechanism of BDE-47 was studied. The results showed that BDE-47 with low concentration (25 ~ 50 µg·L⁻¹) had an induced effect on photosynthetic pigment content, and the BDE-47 with high concentration (100 ~ 200 µg·L⁻¹) had a significant inhibitory effect on photosynthetic pigment content. BDE-47 has inhibitory effect on the fluorescence characteristics of the flagellates, and the inhibitory effect increases with the increase of concentration.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are kinds of fire retardant chemical preparation. PBDEs are widely used in various kinds of products ^[1,2] like as home appliances, building materials, textiles, etc. due to the flame retardant with high efficiency, good heat resistance, wide applicable, less dosage and hydrolytic stability, etc. However, the molecular structure and chemical properties of PBDEs are very similar to polychlorinated biphenyls (PCBS), not only difficult to occur in the nature of chemical degradation, photo degradation and microbial degradation, the higher lipotropic sex makes it easy to accumulated in Marine organisms as well. With the increasing of PBDEs usage and environment the residues of PBDEs sustained growth over the past few decades, including 2, 2', 4, 4'- four bromine biphenyl ether (BDE - 47) absorption rate is greater than other PBDEs, has strong toxic effects in biology and the production and usage is banning by the Stockholm convention.

Marine microalgae with a large variety and quantity are the basis of the Marine food chain and are the main Marine primary producer play an extremely important role. The pollutants that enter the biological body often participate in the food chain cycle and eventually accumulate in the organism, and ultimately harm human health. At the same time, the toxicity of PBDEs to algal cells will also affect its growth and reproduction, resulting in insufficient bait for high-nutrition-level organisms and interfering with the balance of the entire Marine ecosystem. However, at home and abroad about the studies on environmental behaviors of PBDEs concentration in offshore sediment content changes in



time and space distribution and bioaccumulation, etc., for BDE-47 less research on the acute toxicity effect of Marine micro algae. Therefore, the study of the acute toxicity of BDE-47 on Marine microalgae has great significance for understanding the ecological effect and environmental regression.

2. The experiment methods

2.1 Microalgae culture and BDE-47 stress.

The microalgae in the exponential growth stage were inoculated in f/2 medium, and the initial cell number was $1 \times 10^5 \sim 2 \times 10^5/\text{ml}$. The mass concentration of BDE-47 is 25, 50, 100, 150 and 200 $\mu\text{g} \cdot \text{L}^{-1}$, and 3 parallel samples are set for each concentration group.

2.2 Determination of cell density.

Sample collection and determination: samples were sampled at the 0, 12, 24, 48, 72 and 96 h of the experiment, fixed with lugol reagent and counted with the microscope.

2.3 Determination of chlorophyll content.

The microalgae samples were taken 15 ml at 0, 12, 24, 48, 72 and 96 h of the experiment and 5000 RPM centrifuge for 10 min then discard the supernatant and collect in mud. With 90% acetone extraction, 721 spectrophotometer determination, and calculate the content, including g/cell number.

2.4 Determination of chlorophyll fluorescence parameters.

Get 1.5 ml algae mixture into quartz small cup at 0, 24, 48, 72, 96 h, and adaptation in dark for 15 min, to determine the chlorophyll fluorescence parameters and repeat 3 times for each sample. The initial fluorescence F_0 was measured by a weak measurement of light, and the maximum fluorescence F_m was stimulated by the saturation pulse ($4000 \mu\text{mol}/\text{m}^2 \cdot \text{s}$, with a duration of 0.8 s). PSII maximum energy conversion efficiency (F_v/F_m), PSII actual photo energy conversion efficiency (Yield), relative photosynthetic electron transfer efficiency (rETR) parameters can be read directly by the instrument.

3. Results

3.1 The effect of BDE-47 stress on the growth of *Dicrateria inornata*

FIG. 1 shows the growth curve of the *Dicrateria inornata* in different concentrations of BDE-47. There was no significant effect on the growth of the *Dicrateria inornata* in 25 $\mu\text{g} \cdot \text{L}^{-1}$ and 50 $\mu\text{g} \cdot \text{L}^{-1}$ concentration of BDE-47. In addition, the growth of BDE-47 was inhibited and the cell density was significantly lower than that in the control group ($p < 0.01$).

According to the relative growth rate of microalgae, the dose-response equation was as follows: $y = 0.9889x + 0.60$, $R^2 = 0.967$, $F = 29.7$, and greater than $F_{0.05}(18.51)$, under the experimental conditions, the BDE-47 compound was $163 \mu\text{g} \cdot \text{L}^{-1}$ of the cross *Dicrateria inornata*.

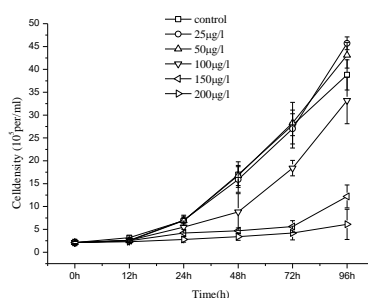


FIG. 1 shows the growth curve of the *Dicrateria inornata* in different concentrations of BDE-47

3.2 The effect of BDE-47 stress on photosynthetic pigment of the *Dicrateria inornata*

The changes of photosynthetic pigment content in the *Dicrateria inornata* were shown in FIG. 2 under different concentrations of BDE-47 stress. By the figure can be seen, within 24 h, under the stress of low concentration of BDE-47, *Dicrateria inornata* of chlorophyll a and chlorophyll c content is significantly higher than control, and carotenoid content significantly increased with the increase of concentration of BDE-47. The content of photosynthetic pigment decreased significantly under the stress of $100 \mu\text{g}\cdot\text{L}^{-1}\sim 200 \mu\text{g}\cdot\text{L}^{-1}$ concentration of BDE-47.

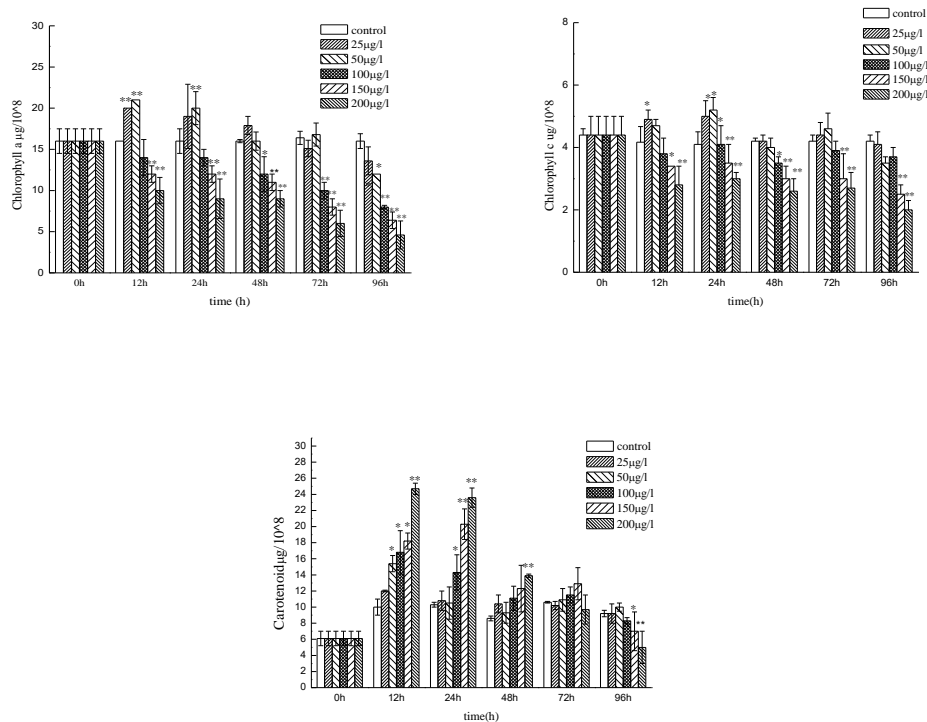


FIG. 2 The effect of BDE-47 stress on photosynthetic pigment of the *Dicrateria inornata* (Compared to the control group, *: $0.01 < p < 0.05$; **: $p < 0.01$)

3.3 The effect of BDE-47 stress on the fluorescence parameters of *Dicrateria inornata*

The changes of chlorophyll fluorescence parameters of chlorophyll in different concentrations of BDE-47 stress were shown in FIG. 3. The figure shows that including $25 \mu\text{g}\cdot\text{L}^{-1}$ and $50 \mu\text{g}\cdot\text{L}^{-1}$ concentration of BDE-47 the F_v/F_m , F_v/F_o , Y_{ield} , r_{ERT} of *Dicrateria inornata* had no significant effect, and high concentration ($100 \mu\text{g}\cdot\text{L}^{-1} \sim 200 \mu\text{g}\cdot\text{L}^{-1}$) of BDE-47 is influential to *Dicrateria inornata* fluorescence parameters, significantly lower than the control group.

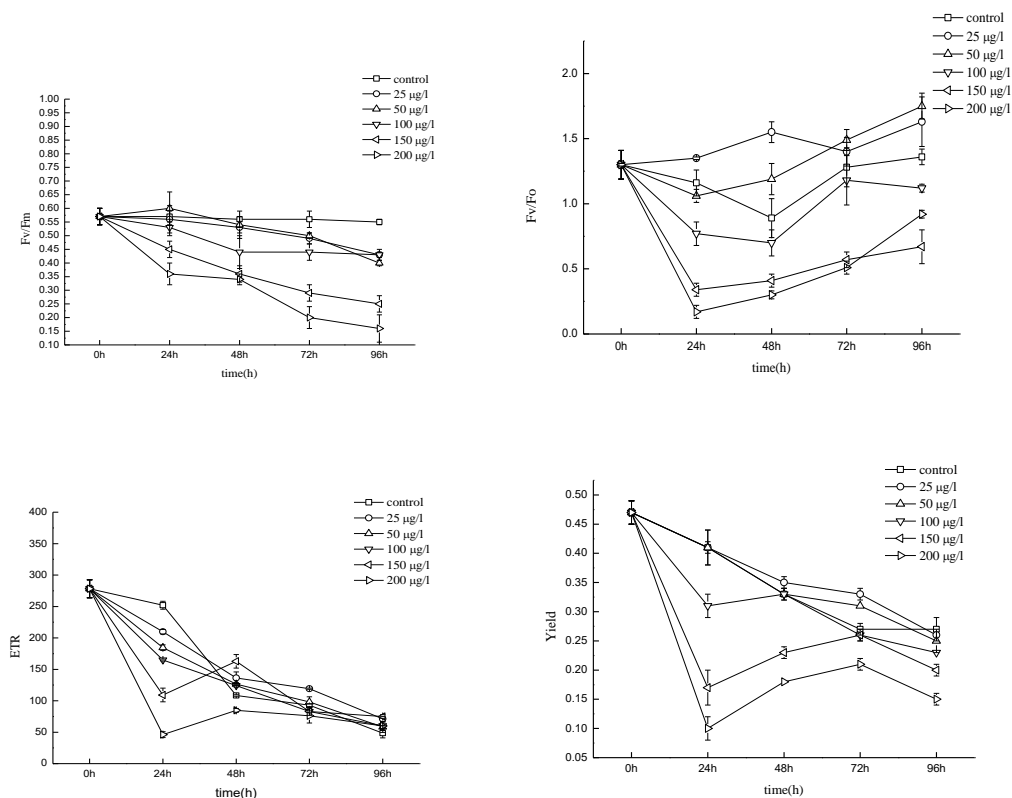


FIG. 3 The effect of BDE-47 stress on the fluorescence parameters of *Dicrateria inornata*

4. Discussions

This study results show that under the experimental conditions BDE-47 compounds to *Dicrateria inornata* EC₅₀ values of 96 to 163 (including g L⁻¹, according to the guidelines for the new chemical hazard evaluation criteria of BDE-47 to *Dicrateria inornata* belongs to high toxic substances (EC₅₀ < 1.0 mg/L).

The low concentration of BDE-47 causes the growth, photosynthesis, and higher photosynthetic pigments called toxic excitation effect^[3]. Low concentrations of pollutants can promote the growth of microalgae, possibly because: (1) microalgae can directly use pollutants as a nutrient source for growth. For example the low concentration of alpha-naphthol can be used as the growth carbon source of *Chlorella*; it can be absorbed promoting the growth of microalgae^[4]. (2) the pollutants of micro algae poison and micro algae degradation of pollutants can exist at the same time, the two process of pollutants in low concentration when the dominant degradation process, and thus overall performance degradation, and degradation products can also be used as a nutritional powerhouses promoting growth of micro algae^[5]. The high concentration of pollutants caused irreversible damage to the growth of microalgae. Xie yonghong^[6] et al. found that when the mass concentration of TBT was 2.0 µg·L⁻¹, the growth of the *Chaetoceros socialis* was completely inhibited. Analysis showed that high concentration of pollutants can poison microalgae cells, mainly lies in its attack micro algae cell membrane, causes the other toxins in the environment smoothly into the algal cells, thereby increasing poison of micro algae.

Photosynthetic pigments play a key role in photosynthesis of plants, and their content changes are often related to physiological activities of leaves, adaptation of plants to environment^[7]. The research results show that the low concentration of BDE-47 on algal photosynthetic pigment has a significant role in promoting, while the influence of the high concentration of pollutants in the embodied in inhibition of micro algae photosynthetic pigment synthesis and to promote the degradation of

photosynthetic pigment. Similar laws exist in previous studies on the toxicity of organic pollutants. The different concentrations of NaHSO₃ on saline *Dunaliella salina* cells growth and photosynthetic pigment content, the influence of NaHSO₃ can significantly promote the growth of saline *Dunaliella salina* cells, and increase the chlorophyll a (Chla) and b (Chlb) content and total chlorophyll (chlorophyll, carotenoid content (Car); The promoting effect increased with the increase of concentration, and the lower concentration ($< 0.10 \text{ mM} \cdot \text{L}^{-1}$) was better^[8]. Yan Cuiwei^[9] etc. found that under the low concentration ($5 \text{ mg} \cdot \text{L}^{-1}$) or less multi-effect azole stress the content of chlorophyll-a of *Pavlova viridis* Tseng rised, while high concentration ($15 \text{ mg} \cdot \text{L}^{-1}$) or higher stress could reduce its content, and with the increase of the concentration of pollutants, the content of chlorophyll-a has a tendency to quickly reduce. Research and analysis, the cause of the above phenomenon lies in the high concentration of pollutants in this experiment set micro algae cells produce certain simulative effect; promote the synthesis of photosynthetic pigment, causing micro algae biomass increase.

In this experiment, under the stress of different concentrations of BDE-47, Fv/Fm values of *Dicrateria inornata* increased with the extension of the stress time, and the increase of high concentration was significantly lower than that of low concentration. In the experiment, the concentration of BDE-47 was damaged in the PSII reaction center. Fv/Fo represents the light energy transfer ability from Chla/b protein complex LHCP to PSII, which is often used to measure the potential activity of PSII.ETR can reflect the transfer efficiency of high-energy electrons through the electron transport chain between PSII and PSI^[10]. In this experiment, Fv/Fo and ETR values decreased with the increase of BDE-47 concentration, and the stress time had little effect. It is shown that BDE-47 can suppress the initial light energy conversion and transmission efficiency, and the efficiency of transmission can be reduced significantly.

The fluorescence parameters Fv/Fm, Fv/Fo, Yield and rETR were significantly reduced under the stress of BDE-47. The decrease of Fv/Fm and Fv/Fo indicates that BDE-47 has caused damage to the PSII reaction center of the *Dicrateria inornata*, which hinders the photosynthetic electron transfer process. The decrease of Yield indicates that the formation of algal cell homogenization is prevented, and the fixation and assimilation of carbon are affected. The decline of rETR showed that the electron transfer efficiency of the *Dicrateria inornata* was decreased.

4. Conclusions

Low concentration of BDE-47 could promote the growth of micro algae and increase the content of photosynthetic pigment, whereas high concentrations of BDE-47 can cause irreversible damage to the *Dicrateria inornata*, which can significantly reduce the photosynthetic pigment and seriously affect the photosynthesis of microalgae.

Acknowledgments

The authors acknowledge funding from: National Key R&D Plan (No.2016YFC1400801, No. 2016YFC1400802, No. 2016YFC1400803, No. 2016YFC1400804 and No.2017YFC1403700)

References:

- [1] Johnson-Restrepo B, Kannan K. *An assessment of sources and pathways of human exposure to polybrominated diphenyl ethers in the United States* [J]. Chemosphere, 2009, 76(4): 542-548.
- [2] Jakobsson K, Thuresson K, Rylander L, et al. *Exposure to polybrominated diphenyl ethers and tetrabromobisphenol A among computer technicians* [J]. Chemosphere, 2002, 46(5): 709-716
- [3] Stebbing A R D. *Hormesis-The stimulation of growth by low levels of inhibitors* [J]. Science of the Total Environment, 1982, 22: 213-234.
- [4] Peng J I, Yan G A, Shen G X, et al. *The Effects of a-Naphthol on the Growth and the Antioxidase Activities of Chlorella Vulgaris* [J]. Wuhan University Journal (Natural Science Edition), 2001, (4): 449-452

- [5] Shen G X, Yan G A, Peng J L, et al. *Study on ecotoxicology for pesticides to algae II: toxic mechanism and accumulation, degradation* [J]. Progress in Environmental Science, 1999, 7(6): 131-140
- [6] Xie Y H, Su R G, Zhang L X, et al. *The toxic effects of chloride tributyltin on the predominant marine algae in China coastal water* [J]. Ecology and Environmental Sciences, 2011, 20(6-7): 1075-1080
- [7] Liu B Y, Zhou P J, Li J J, et al. *Effects of γ -HCH on the Growth and Photosystem and Lipid Peroxidation of *Scenedesmus Obliquus* kütz*[J]. Journal of Agro-Environment Science, 2006, 25(1): 204-207
- [8] Cheng J F, Hu F H, et al. *Effects of NaHSO₃ on the growth and contents of photosynthetic pigments in *Dunaliella salina** [J]. Journal of Tropical Oceanography, 2010, 29(3):65-70
- [9] Yan C W, Xiao Y H, Zhu Y S, et al. Effects of PP₃₃₃ on Growth and Activities of Antioxidative Enzymes of Two Marine Microalgae [J]. Periodical of Ocean University of China, 2008, 38(2): 291-296
- [10] Su X, Hu D Q, Lin Z F, et al. Effect of air pollution on the chlorophyll fluorescence characters of two afforestation plants in Guangzhou [J]. Acta Phytocologica Sinica, 2002, 26(5): 599-604