Physiological and ecological responses and changes of *Phaeodactylum tricornutum* under long-term stress of naphthalene

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**Abstract:** Naphthalene, as a common polycyclic aromatic hydrocarbon (PAHs), attracts broad attention due to its toxicity to human and marine organisms. This study investigate the effects of naphthalene on growth, maximum photochemical efficiency (\(F_v/F_m\)), total esterase activity and reactive oxygen species production of *Phaeodactylum tricornutum*. The study explored how algal physiological characteristics change under naphthalene’s long-term exposure and analyzed the correlation between photosynthetic efficiency with growth, total esterase activity and reactive oxygen species production, respectively. The study shows that low-concentration naphthalene promotes slightly or doesn’t affect algal growth, total esterase activity and reactive oxygen species while it promotes \(F_v/F_m\) significantly. On the other hand, high-concentration naphthalene inhibits algal growth, total esterase activity and \(F_v/F_m\) significantly, while it stimulates reactive oxygen species production and the stimulation increases with time. The study also shows that growth and \(F_v/F_m\) of *P. tricornutum* are correlative and the correlation is related with naphthalene concentration. \(F_v/F_m\) of *P. tricornutum* is significantly positively-related with total esterase activity, while is significantly negatively-related with reactive oxygen species.

1. **Introduction**  
Polycyclic aromatic hydrocarbons (PAHs), as an important component of petroleum, are raw materials for the manufacture of fuels, paints, synthetic fibers, etc., and can also be used as soil fumigants, toilet deodorants, etc\cite{1}. As a type of ubiquitous PAHs, naphthalene has many sources, wide distribution and strong toxicity and it has the same "three-effect" as other PAHs. Naphthalene has great harm to plants, aquatic animals and human bodies\cite{2}, and has been included in the blacklist of China's priority control pollutants\cite{3}. With the frequent occurrence of offshore oil spills in recent years, the widespread use of naphthalene-related products and the improper disposal of waste, human activities have caused a sharp rise in the concentration of naphthalene in the atmosphere, soil and water environment\cite{4}. Studies have shown that the content of naphthalene in water is higher, such as 0.001~10μg/L in natural water, PAHs with the most content in lake water is naphthalene\cite{5}; and the content of naphthalene in industrial wastewater can reach 1mg/L\cite{6}. Naphthalene can also migrate over long distances in the marine environment, and has high bioaccumulation, high residue, and refractory degradation\cite{7}. It can be transmitted and accumulated through the food chain, endangering marine ecosystems and human public health. Therefore, it is important to study the toxic effects of naphthalene on marine primary producers.

Marine microalgae, as primary producer of the ocean, is an irreplaceable link to maintain the balance of the marine ecosystem\cite{8}. *Phaeodactylum tricornutum* is a marine eukaryotic single-cell diatom. It is
widely distributed worldwide and has wide adaptability to salinity and temperature \[8\]. It is an important bait organism for marine economic animals and an indicator organism commonly used in marine biological toxicity tests. Regarding the toxicity of naphthalene to microalgae, it was found that the toxic effects of naphthalene on microalgae have interspecies differences. For example, when *Microcystis aeruginosa* and *Synechococcus* were exposed to 10 mg/L naphthalene respectively, the growth of the former was significantly promoted, while the latter was significantly inhibited \[9\]; Under different concentrations of naphthalene stress, the response of microalgae is different \[8\], often low concentration will induce a positive response, high concentration will induce a negative response \[8\]; In addition, the exposure time also affects the toxic effects of naphthalene on some physiological characteristics of microalgae. For example, the inhibitory effect of high concentration of naphthalene on the growth of *Synechococcus* increases significantly with time. The maximum electron transport rate of *Microcystis aeruginosa* was inhibited under naphthalene stress and returned to the control level after 7 days of exposure \[6, 9\]. This study studied the response of growth, total esterase activity, reactive oxygen species (ROS) and photosynthetic efficiency of *P. tricornutum* to long-term exposure to different concentrations of naphthalene, in order to provide a more comprehensive and in-depth understanding of the effects of naphthalene on the physiological characteristics of *P. tricornutum* and its mechanism of toxicity in both dose and time.

2. Materials and methods

2.1 Experimental materials

In this experiment, *Phaeodactylum tricornutum* was donated by Yin Kedong, a research group of The School of Ocean in Sun Yat-sen University. The seawater was taken from the coastal waters of Daya Bay, Shenzhen, and was filtered through a 0.22 μm pore size mixed cellulose filter. Seed preparation and experiments f/2 medium were prepared according to the method of Guillard et al. [10].

Naphthalene reagent (Sigma-Aldrich, 184500) was dissolved in dimethyl sulfoxide to prepare a 175 g/L mother liquor, which was stored at 4 °C until use. At the time of the experiment, the f/2 medium was added at the desired experimental concentration. The reagents used in this experiment were purchased from Sigma-Aldrich.

2.2 Experimental design

Seven naphthalene concentrations groups (0.005, 0.05, 0.1, 1, 5, 10, 50 mg/L) and control groups (0 mg/L) were set in the experiment, and three parallel samples were set for each treatment group. The experimental conditions were pH 8.2, the salinity was 33 ‰, the temperature was 20±0.1 °C, the light-dark ratio L:D was 14h:10h, and the light intensity was 90±5 mol m⁻² s⁻¹. The experimental was carried out in a 500mL glass conical flask, and the volume of the algae solution was 350 mL. The flask was shaken 2-3 times a day and randomly changed their positions to eliminate the difference between the samples due to the position of the flask. The experiment lasted for 8 days. Every day the samples were analyzed on their cell optical density, total esterase activity, maximum photochemical efficiency (Fv/Fm) and reactive oxygen species (ROS). The measurement and calculation results of each indicator are expressed as mean ± standard deviation, n = 3.

2.3 Analysis method

2.3.1 Growth assay

The optical density (OD₇₅₀) of algal cells at 750 nm was measured by UV-spectrophotometer (UV-1780, SHIMADZU), and the growth rate of algae cells was calculated according to Wei’s computing method [14]. In addition, the semi-lethal concentration (EC₅₀) of naphthalene against *Phaeodactylum tricornutum* was calculated by Probit model [13].
2.3.2 Photosynthetic efficiency
The photosynthesis efficiency was characterized by the maximum photochemical efficiency $F_v/F_m$. The measurement was carried out by double modulation fluorometer (PSI, Czech Republic). 2 mL of algae solution was measured after dark adaptation for 20 min, and the time of recording the fluorescent transient was 1 s, and the result was recorded every 10 μs for the first 2 ms, and then every 1 ms. The initial fluorescence value $F_0$ is a chlorophyll fluorescence value measured at 50 μs, and the maximum fluorescence value $F_m$ is a chlorophyll fluorescence value when all reaction centers are closed. Calculate the maximum photochemical efficiency $F_v/F_m$ according to $F_0$ and $F_m$.

2.3.3 Total esterase activity
The fluorescein diacetate (FDA) hydrolysis technique is a highly efficient and sensitive technique for determining total esterase activity in cells [12]. The FDA can enter the algae cells, and the intracellular esterase can hydrolyze the FDA into a fluorescent substance [16]. The fluorescence value can be used to characterize the esterase activity of algae cells, and the specific analysis method is the same as that of Li et al. [14]. The fluorescence intensity ($E_x=485$ nm, $E_m=530$ nm, TECAN, M200PRO) was measured by a microplate reader. In order to avoid the effect of fluorescence emitted by seawater stained by FDA, the measurement results were subtracted from the background fluorescence value after seawater staining, and divided by the sample’s OD$_{750}$, the total esterase activity index was obtained [14].

2.3.4 ROS
The intracellular ROS content was determined by the method of 2,7-dichlorodi-luciferin acetoacetic acid (DCFH-DA, Sigma, D6883) fluorescent probe [15]. The DCFH-DA fluorescent dye was prepared with dimethyl sulfoxide in a mother liquor of 10mmol/L and placed at -20 °C until use. When measuring the sample, the mother liquor is configured as a working solution with secondary filtered seawater when it is needed. The sample was dyed by the working solution (final concentration of 10 μmol/L). The sample was incubated with the working solution for 120 min in a dark room at room temperature; After incubation, it was washed twice with sterile seawater, and the fluorescence intensity was measured with a microplate reader ($E_x=488$ nm, $E_m=525$ nm, TECAN, M200PRO). The measured fluorescence value was subtracted from the background fluorescence value of seawater staining, and the normalized fluorescence value was obtained by dividing OD$_{750}$ of the sample as the reactive oxygen species index [16].

2.4 Data Analysis
The experimental data were analyzed by one-way ANOVA for different naphthalene concentration treatment groups. If the variance was uniform, the LSD method was used. If the variance was not uniform, Tamhane’s T2 method is used for multiple comparison and correlation analysis. p<0.05 indicates significant difference; binary variable correlation analysis uses Pearson correlation coefficient method (IBM SPSS Statistics 20).

3. Results and analysis

3.1 Effects of naphthalene on growth
The effect of naphthalene concentration on the growth of P. tricornutum cells is shown in Figure 1a). Under low concentration of naphthalene ($\leq 0.1$ mg/L), algae cell growth showed exponential growth, and the optical density growth curve showed almost no significant difference compared with the control group ($p>0.05$). Some of these low concentrations showed a positive effect in the early stage. For the first day of exposure, 0.05 mg/L naphthalene increased the OD$_{750}$ by 12%. High concentrations of naphthalene (1 mg/L) significantly inhibited algal cell growth ($p<0.05$), and the longer the exposure time, the stronger the inhibition.
Fig. 1 Growth curves (a) and EC50 (b) of *Phaeodactylum tricornutum* under naphthaene exposure

The semi-lethal concentration (EC50) of naphthalene against *P. tricornutum* at different exposure times is shown in Figure 1(b). On the first day, the EC50 of naphthalene to *P. tricornutum* was 2.06 ± 0.1 mg/L; the EC50 decreased significantly with the increase of exposure time (*p* < 0.05); the EC50 decreased to 0.77 ± 0.01 mg/L on the seventh day of exposure. The drop was as high as 62% (*p* < 0.05).

Fig. 2 (a) Time course of *Fv/Fm* of *P. tricornutum* under naphthaene exposure; (b) The relationship between OD750 and *Fv/Fm* of *P. tricornutum* under naphthaene exposure

3.2 Effects of naphthaene on *Fv/Fm*

This study found that the effect of naphthaene on the maximum light energy conversion efficiency of *P. tricornutum* cells varied with concentration (Fig. 2(a)). When the concentration of naphthaene is low (0.005 mg/L), *Fv/Fm* is not affected by naphthaene (*p* > 0.05). With the increase of naphthaene concentration (0.05, 0.1 mg/L), naphthaene showed a significant effect on the light energy conversion efficiency *Fv/Fm* (*p* < 0.05); when the naphthaene concentration was 1 mg/L, *Fv/Fm* was significantly inhibited (*p* < 0.05); And after the 5-7th day of exposure at high concentration of naphthaene (5 mg/L), the *Fv/Fm* of *Phaeodactylum tricornutum* recovered.

The experimental results show that there is a complex correlation between the maximum light energy conversion efficiency (*Fv/Fm*) and algal cell biomass (OD750) of *P. tricornutum* under naphthaene treatment, which is a non-simple linear relationship (Fig. 2(b)). At the beginning of the experiment (Day 0), there was no significant difference in OD750 and *Fv/Fm* between the treatment groups, and there was
no significant correlation between the two indicators \((p>0.05, \text{Table 1})\). During 1-7 days, the biomass of low concentration naphthalene and control group \((0-1 \text{mg/L})\) was generally higher \((\text{OD}_{750}>0.2)\), and \(F/F_m\) and OD\(_{750}\) showed a significant negative correlation \((p<0.01)\). In the high concentration naphthalene group \((5 \text{mg/L})\), the biomass was significantly inhibited under naphthalene stress \((0.07<\text{OD}_{750}<0.2)\), and \(F/F_m\) and OD\(_{750}\) showed a significant positive correlation \((p<0.01)\).

Table 1 Correlations between \(F/F_m\) and \(\text{OD}_{750}\) of \(P\). tricornutum under naphthalene exposure

| Exposure time (Day) | Exposure concentration (mg/L) | OD\(_{750}\) | Pearson correlation coefficient |
|---------------------|-------------------------------|-------------|-------------------------------|
| 0                   | All-concentrations 0-50       | \(\text{OD}_{750}<0.07\) | 0.568                         |
| 1-7                 | Low-concentrations 0-1        | \(\text{OD}_{750}>0.2\)   | -0.614*                       |
|                     | High-concentrations 5-50      | \(0.07<\text{OD}_{750}<0.2\) | 0.886*                        |

** indicates a significant correlation at the 0.01 level (both sides).

3.3 Effect of naphthalene on total esterase activity

The effect of naphthalene on the total esterase activity of \(P\). tricornutum at different concentrations is shown in Fig. 3(a). Low concentration of naphthalene \((\leq 0.1 \text{mg/L})\) inhibited its activity in the middle of the experiment (2-3d), but recovered later (5th to 7th); high concentration of naphthalene \((1 \text{mg/L})\) significantly inhibited total esterase activity of algal cells \((p<0.05)\), and increased with increasing naphthalene concentration, showed a "concentration-effect" relationship overall.

Except for the highest concentration \((50 \text{ mg/L})\), the total esterase activity of algae cells in the naphthalene concentration treatment group gradually recovered with the increase of exposure time. The medium concentration naphthalene group \((0.05-1 \text{mg/L})\) had no significant difference with the control group on the fifth day \((p>0.05)\), while the significant inhibition of the total esterase activity of \(P\). tricornutum in the higher concentration naphthalene group \((5-10 \text{ mg/L})\) continued until the fifth day (Fig. 3(a)).

Fig. 3 (a) Total esterase activity indexes, (b) Reactive oxygen species indexes of \(P\). tricornutum under naphthalene exposure

3.4 Effect of naphthalene on reactive oxygen species

The effect of naphthalene on the reactive oxygen species (ROS) of \(P\). tricornutum is shown in Fig. 3(b). The effect of naphthalene stress on ROS increased with the increasing exposure concentration and experiment time. Low-concentration naphthalene group \((1 \text{ mg/L})\) began to show stimulating effects on ROS in the late stage of the experiment (days 5 and 7) \((p<0.05)\); while the high-concentration naphthalene group \((5 \text{ mg/L})\) began to show stimulating effects on the ROS growth \((p<0.05)\) in the early stage of the experiment and presented a "concentration-effect" relationship.
3.5 Relationship between total esterase activity, ROS and Fv/Fm under naphthalene treatment

Under naphthalene treatment, the total esterase activity (FDA) of *P. tricornutum* showed a significant positive correlation with the maximum photochemical efficiency (Fv/Fm) (Day 0-3, all p<0.05, Table 2), reactive oxygen species (ROS) showed a significant negative correlation (days 1-5, all p < 0.05, Table 2). In general, naphthalene treatment affects the energy transfer and absorption of photosynthesis in algal cells, inhibits esterase activity and stimulates ROS growth.

Table 2 Correlations between Fv/Fm and total esterase activity, ROS production under nap exposure

| Day  | FDA   | ROS  |
|------|-------|------|
| Day0 | 0.782* | -0.312 |
| Day1 | 0.914**| -0.529*|
| Day2 | 0.884**| -0.529*|
| Day3 | 0.853**| -0.888**|
| Day5 | 0.675  | -0.811**|
| Day7 | 0.331  | -0.558 |

* indicates a significant correlation at the 0.05 level (both sides).
** indicates a significant correlation at the 0.01 level (both sides).

4. Discussion

4.1 Effect of naphthalene on the growth

This study shows that naphthalene as a volatile persistent organic pollutant (POPs), the low concentration of naphthalene did not affect or slightly promote the growth of *P. tricornutum* in the short term, and the high concentration of naphthalene showed significant inhibition and increased with the prolongation of exposure time. This is similar to the results of Li Weibing et al. [6], naphthalene has a dose effect on the growth inhibition of microalgae. The excitatory effect of low-dose naphthalene on the growth of *P. tricornutum*, although not obvious, also indicates that in the low concentration range, pollutants can mobilize the physiological metabolic activity of microalgae and stimulate its growth. The concentration of naphthalene in the water environment is usually low, and the excitatory effect of low concentration pollutants on the growth of microalgae may also be one of the factors triggering red tide.

Different algae species have different tolerance to naphthalene. For example, the same exposure to 0.01 mg/L naphthalene has no significant effect on the growth of *P. tricornutum*, but can significantly inhibit the growth of *Synechococcus* sp. and promote the patina Microcystis aeruginosa grows [10]. The concentration of naphthalene in natural water is 0.001~10μg/L; in industrial wastewater, it can reach 1mg/L [6]. Naphthalene in these environments may inhibit the growth of certain algae, such as the above-mentioned *Synechococcus*; For *P. tricornutum* and Microcystis aeruginosa, it may not inhibit or even promote growth. There are few researches on the influence of naphthalene on the growth mechanism of different algae. Studies have found that this may be related to the different metabolic pathways of naphthalene to microalgae. For example, Cerniglia et al. also found that the tolerance of cyanobacteria (*A. quadruplicatum*) to naphthalene may be attributed to the ability to metabolize naphthalene to 1-phenol [18]. The response mechanism of microalgae to naphthalene and its interspecies differences remain to be further studied.

As the exposure time increased, the semi-lethal concentration (EC50) of naphthalene to *P. tricornutum* gradually decreased, and the chronic toxicity effect became more and more significant. This is similar to the trend of EC50 in the study of *P. tricornutum* (168 hours, EC50=0.2669~19.1849mg/L) exposed to naphthalene by Li Yanmei et al. [12]. The difference is that the EC50 of the study for 1 day of naphthalene exposure is 19.2 mg / L, which is quite different from the study of 2.06 mg / L; but when the exposure time is extended (>2 days), the two studies have similar EC50 values, this may be because the microalgae in this study is in the early stage of the logarithmic growth phase and the initial cell concentration is low.

4.2 Effect of naphthalene on the maximum light energy conversion efficiency Fv/Fm

The results of this study indicate that the photosynthetic system of *P. tricornutum* is weakly tolerant to naphthalene. Except that the lowest concentration had no significant effect (0.005mg/L), the low concentration of naphthalene (0.05-0.1 mg/L) significantly promoted the Fv/Fm of *P. tricornutum*,
indicating that in the low concentration range, naphthalene can stimulate the activity of PSII reaction center of *P. tricornutum*, improve the conversion efficiency of light energy, and make the captured light energy more used in photochemical reactions, alleviating the stress of pollutants on photosynthetic system; At high concentrations, naphthalene significantly inhibited \(F_v/F_m\). Studies have shown that naphthalene, as a highly toxic pollutant, can enter the interior of algae cells and bind to the outer membrane of chloroplasts and thylakoid membranes; low concentrations of PAHs can stimulate microalgae to synthesize more chlorophyll and enhance photosynthesis efficiency; High concentrations of PAHs can damage the reaction center of algal cell photosystem II (PS II) and impede electron transfer in photosynthesis, thereby inhibiting the original reaction of photosynthesis, affecting the utilization efficiency and conversion efficiency of algal cell photosynthetic system, which is reflected in reducing photosynthetic efficiency and inhibiting growth. The study also found that \(F_v/F_m\) of *P. tricornutum* could gradually recover from naphthalene stress with the prolongation of exposure time, but the growth was still significantly inhibited. As a volatile POPs, naphthalene showed a "accumulation effect" on the growth toxicity and a "weakening effect" on \(F_v/F_m\). Combined with the above, the low-concentration polycyclic aromatic hydrocarbons may promote the growth of microalgae as a red tide triggering factor. The gradual recovery of microalgae \(F_v/F_m\) under long-term high concentration stress should also be paid attention to. The acute and chronic toxic effects of naphthalene on microalgae are worthy of further investigation.

### 4.3 Effect of naphthalene on total esterase activity

The study found that the cellular activity of algal cells. In this study, the activity of naphthalene on the cells of *P. tricornutum* showed that there was no significant effect or promotion in low concentration, and high concentration inhibition. The study found that when plants are exposed to pollutants such as polycyclic aromatic hydrocarbons, in the early stage plants show the rapid absorption of pollutants, which produces a certain stress response, and even stimulates the “excitatory effect” in a short period of time. Later, it is showed as the accumulation and dispersion of pollutants in plants. When naphthalene is in water, part of it can adsorb to the surface of microalgae cells, reducing the osmotic adjustment ability of cell membrane, leading to leakage of K+ and H+, thereby reducing algal cell activity, disrupting the balance of algal cells’ internal environment; A part of naphthalene can enter the interior of algal cells, and bind to the membrane of chloroplasts, thylakoids and other organelles, causing damage. These processes can also affect enzyme synthesis and ion transport in algal cells.

At the same time, because total esterase activity is a manifestation of a variety of enzyme activities, including electron transport, chlorophyll-related esterase activity. In this study, \(F_v/F_m\) showed a significant positive correlation with total esterase activity, indicating that naphthalene entered the interior of algae cells during the early stage of the exposure, and combined with chloroplast outer membrane and thylakoid membrane, in addition to directly affecting electron transport and energy conversion, it also affects photosynthesis and inhibits algae’s growth by reducing the esterase activity of *P. tricornutum*. This correlation between \(F_v/F_m\) and total esterase activity is consistent with previous studies. In the later stage of the experimental, although the \(F_v/F_m\) and total esterase activities were all restored, the degree of recovery was not synchronized. Studies have shown that esterases have non-energy-dependent metabolic activity characteristics, that is, dead cells still show this metabolic activity. When naphthalene increases the exposure time with *P. tricornutum*, whether the in conformity of its growth, \(F_v/F_m\) and esterase activities is related to the non-energy-dependent metabolic activity of esterase, or whether the microalgae photosynthetic system may increase the tolerance to naphthalene during exposure, and the mechanism remains to be further studied.

### 4.4 Effect of naphthalene on ROS production of *P. tricornutum*

Naphthalene is lipophilic and tends to accumulate on the surface of the algae cell lipid membrane in a water environment, and can cause damage to it, constituting oxidative stress on algal cells. In the present study, low concentrations of naphthalene (1mg/L) did not significantly stimulate ROS.
production in *P. tricornutum* at the early stage of exposure (Fig. 3(b)). The reason may be that microalgae induces an increase in antioxidant enzyme activity in the algae and activates its own antioxidant defense line [31]. Studies have found that microalgae form an antioxidant mechanism under the induction of low concentration of PAHs, and intracellular protective enzyme activities such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxide Enzyme (GSH) enhances the removal of excess superoxide ions in cells and promotes the synthesis of proteins with defense and repair functions [9, 19]. When the concentration of naphthalene was 5 mg/L, the ROS production of *P. tricornutum* was continuously stimulated during the experiment, which inhibited the growth of algae cells. On the one hand, high concentration of naphthalene may stimulate the accumulation of peroxides (ROS, MDA, etc.) in algae cells, causing damage to cells [32]; on the other hand, it may be a decrease in the content and activity of protective enzymes [8].

As a photosynthetic organism, microalgae inevitably produce ROS during aerobic metabolism. Photosynthetic electrons are transmitted in the chloroplast and O$_2$ is produced simultaneously. When O$_2$ and electrons meet, a series of reactive oxygen species are produced, which is the source of active oxygen species [16]; However, under pollutant or environmental stress, photosynthetic efficiency is impaired, and a large number of electrons and O$_2$ accumulate inside the chloroplast, producing a large amount of ROS. Excessive ROS accumulated in the thylakoids, and when the production of reactive oxygen species exceeds the ability of scavenging and tolerating, it will produce oxidative stress on PSII of microalgae [14], causing PS II inactivation and light inhibition, and thus affecting the normal physiological function photosynthetic organisms [33].

5. Conclusion

(1) From the dose dimension, low concentration of naphthalene has no significant or promoting effect on growth, maximum photochemical efficiency and total esterase activity of *P. tricornutum*, while high concentration of naphthalene has obvious inhibition; There was no significant change in ROS accumulation under low-concentration naphthalene stress, and it increased under high concentration of naphthalene stress. This indicates that *P. tricornutum* has weak tolerance to naphthalene.

(2) From the time dimension, the inhibition of the maximum photochemical efficiency and total esterase activity of high concentration naphthalene on *P. tricornutum* recovered with time, but the recovery was not synchronized; The inhibition of the growth of *P. tricornutum* and the promotion of ROS production by high concentration of naphthalene increased with time. This may be because naphthalene has different effects on the growth and physiological indicators of *P. tricornutum*.

(3) Under naphthalene stress, the total esterase activity of *P. tricornutum* was positively correlated with photosynthesis; ROS production was negatively correlated with photosynthesis; $F_d/F_m$ of *P. tricornutum* was coupled with growth phase and correlated with naphthalene concentration.

6. Outlook

At present, the study on the toxicity of naphthalene to marine microalgae mainly focuses on its effects on growth, physiology, photosynthetic pigment content or maximum photon yield [8, 9]. However, the effects of naphthalene on the photosynthesis of marine microalgae (including PSII structure, electron transport, energy transfer, etc.) are still rare. Considering the effects of naphthalene, especially the low concentration of naphthalene on the physiological responses of microalgae and its impact on the balance of marine ecosystems, this aspect should be taken seriously.

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References

[1] Jiang J. (2010) The toxic effects of naphthalene on Scenedesmus obliquus and Microcystis aeruginosa [D], Northeast Normal University. (in Chinese)

[2] Liu J W, Lin F K. (2002) Effects of PAHs(naphthalene) Pollution on the Physiological Index of Hydrophyte[J]. Journal of East China University of science and technology (Natural science), 28(5):520-524. (in Chinese)

[3] Zhou W M, Fu D Q, Sun Z G. (1990) Water priority control pollutant blacklist [J]. Environmental Monitoring in China, 4:3-5. (in Chinese)

[4] Luo L. J. (2013) Degradation of high molecular weight polycyclic aromatic hydrocarbons by Selenastrum capricornutum [D], Sun Yat-sen University. (in Chinese)

[5] Li J, Zhang G, Qi S H. (2003) Distribution Characteristic And Seasonal Change of Bioavailable Polycyclic Aromatic Hydrocarbons (Pahs) In Luhu Water, Guangzhou City [J]. Chongqing Environmental Science, 25(1): 150-155. (in Chinese)

[6] Li W B, Xiao N W, Gao J X, et al. (2013) Effects of Naphthalene Stress on the Physiological Characteristics of Ceratophyllum demersum [J]. Research of Environmental Sciences, 26(4): 425-431. (in Chinese)

[7] Wang X C. (2014) Effects of polycyclic aromatic hydrocarbons on growth and physiological characteristics of Microcystis aeruginosa. [D], Nanjing Agricultural University. (in Chinese)

[8] Li Y M, Zeng W L, Yu Q, et al. (2012) Toxic effects of naphthalene on the growth of Phaeodactylum tricornutum and relevant biochemical indexes[J]. China Environmental Science, 32(01): 150-155. (in Chinese)

[9] Tan X, Dai K W, Duan Z P, et al. (2018) Comparasion of the effects of naphthalene on the growth and chlorophyll fluorescence of Microcystis aeruginosa and Synechococcus sp. [J]. Journal of Hohai University (Natural Sciences), 46(2):115-121. (in Chinese)

[10] Guillard R R, Ryther J H. (1962) Studies of marine planktonic diatoms, Cyclotella nana hustedt, and detonula confervacea(cleve)gran [J]. Can J Microbiol, 8(2): 229.

[11] Wei P J, Jiang Y L. (2018) Effects of Heavy Metals Cd²⁺, Cu²⁺ and Zn²⁺ on Growth and Lipid Accumulation of Conticribra weissflogii [J]. Periodical of Ocean University of China, 48(7):50-57. (in Chinese)

[12] Battin T J. (1997) Assessment of fluorescein diacetate hydrolysis as a measure of total esterase activity in natural stream sediment biofilms [J]. Science of the Total Environment, 198(1): 51-60.

[13] Liang Z, Ge F, Zeng H, et al. (2013) Influence of cetyltrimethyl ammonium bromide on nutrient uptake and cell responses of Chlorella vulgaris [J]. Aquatic Toxicology, 138–139(2): 81-87.

[14] Li M, Jiang Y, Chuang C-Y, et al. (2019) Recovery of Alexandrium tamarense under chronic exposure of TiO2 nanoparticles and possible mechanisms [J]. Aquatic toxicology, 208(98-108).

[15] Yoshida J, Ishibashi T, Nishio M. (2003) Antiproliferative effect of Ca 2+ channel blockers on human epidermoid carcinoma A431 cells [J]. European Journal of Pharmacology, 472(1–2): 23-31.

[16] Wang M L, Jiang YL. (2018) Effects of Manganese on the Growth and Fluorescence Induction Kinetics of Conticribra weissflogii [J]. Environmental Science, 39(12):5514-5522. (in Chinese)

[17] Cerniglia C E, Gibson D T, Baalen C V. (1979) Algal oxidation of aromatic hydrocarbons: formation of 1-naphthol from naphthalene by Agmenellum quadruplicatum, strain PR-6 [J]. Biochemical & Biophysical Research Communications, 88(1): 50-58.

[18] Kreslavski V D, Lankin A V, Vasilyeva G K, et al. (2014) Effects of polyaromatic hydrocarbons on photosystem II activity in pea leaves [J]. Plant Physiol Biochem, 81(135-142).
[19] Zhu X Z, Kong H L, Gao Y Z, et al. (2012) Low concentrations of polycyclic aromatic hydrocarbons promote the growth of Microcystis aeruginosa [J]. J Hazard Mater, 237(371-375).

[20] Pokora W, Tukaj Z. (2010) The combined effect of anthracene and cadmium on photosynthetic activity of three Desmodesmus (Chlorophyta) species [J]. Ecotoxicology & Environmental Safety, 73(6): 1207-1213.

[21] Lankin A V, Kreslavski V D, Khudyakova A Y, et al. (2014) Effect of naphthalene on photosystem 2 photochemical activity of pea plants [J]. Biochem-Moscow, 79(11): 1216-1225.

[22] Liang Y, Jin Y M, Tian C Y. (2008) Effects of phosphorus restriction and supplement on the chlorophyll fluorescent parameters of Chlorella sp.[J]. South China fisheries science, 4(4):1-7. (in Chinese)

[23] Gilbert F, Galgani F, Cadiou Y. (1992) Rapid assessment of metabolic activity in marine microalgae: application in ecotoxicological tests and evaluation of water quality [J]. Marine Biology, 112(2): 199-205.

[24] Liu Y, Zou L, Liu L, et al. (2013) Characterization of Microbial Activities in Marine Mudflat Sediment Using FDA Hydrolase Analysis[J]. Environmental Science, 34(10):3818-3824. (in Chinese)

[25] Kvesitadze E, SadunishviliT, Kvesitadze G. (2009) Mechanisms of organic contaminants uptake and degradation in plants [J]. World Academy of Science Engineering & Technology, 6860(55): 202-216.

[26] Wang Y, Tang X, Li Y, et al. (2002) Stimulation effect of anthracene on marine microalgae growth [J]. The journal of applied ecology, 13(3): 343-346.

[27] Tezel U, Pierson J A, Pavlostathis S G. (2006) Fate and effect of quaternary ammonium compounds on a mixed methanogenic culture [J]. Water Res, 40(19): 3660-3668.

[28] Alison R T, Colin B, Glen L W. (2012) Proton channels in algae: reasons to be excited [J]. Trends in Plant Science, 17(11): 675-684.

[29] Gibbin E M, Putnam H M, Davy S K, et al. (2014) Intracellular pH and its response to CO2-driven seawater acidification in symbiotic versus non-symbiotic coral cells [J]. Journal of Experimental Biology, 217(Pt 11): 1963.

[30] Novo D, Perlmutter N G, Hunt R H, et al. (2015) Accurate flow cytometric membrane potential measurement in bacteria using diethylxacarbocyanine and a ratiometric technique [J]. Cytometry, 35(1): 55-63.

[31] Wolfe-simon F, Grzebyk D, Schofield O, et al. (2010) The role and evolution of superoxide dismutases in algae [J]. J Phycol, 41(3): 453-465.

[32] Kong Q, Zhu L, Shen X. (2010) The toxicity of naphthalene to marine Chlorella vulgaris under different nutrient conditions [J]. J Hazard Mater, 178(1): 282-286.

[33] Chuian-Fu K, Tung-Ming H, Zong-Xian H, et al. (2005) Characterization of Fe/Mn-superoxide dismutase from diatom Thalassiosira weissflogii: cloning, expression, and property [J]. Journal of Agricultural & Food Chemistry, 53(5): 1470-1474.