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Effects of suspended particles on the growth of two dominant phytoplankton species of Bohai Bay, China

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ABSTRACT

Suspended particles (SP) are increasing dramatically in Bohai Bay, China and may affect the growth and composition of phytoplankton assembly. To determine the effects of SP on the growth of two dominant phytoplankton species, *Phaeodactylum tricornutum* Bohlin and *Gymnodinium* sp., we cultured and tested their growth characteristics under SP concentrations ranging from 0 g L^{-1} to 0.8 g L^{-1}. Our results show that the increase in the SP concentrations results in significant decrease in the maximum cell densities and the maximum specific growth rates of these two species. The half maximal inhibitory concentration (IC_{50}) of SP to *P. tricornutum* and *Gymnodinium* sp. were 1.07 g L^{-1} and 0.68 g L^{-1} respectively, indicating the inhibitory effect of SP on *Gymnodinium* sp. was greater than on *P. tricornutum*. These results suggest that SP inhibits the growth of the two algal species and *P. tricornutum* is more tolerant to SP than *Gymnodinium* sp.

1. Introduction

SP generates high levels of turbidity in many aquatic systems, especially in coastal regions. The particles originate from natural (e.g., river inputs, resuspension of sediment, soil erosion, etc.) and anthropogenic activities (e.g., dumping of solid waste, harbor dredging, port construction, etc.) (Guenther and Bozelli, 2004a, 2004b; Sipelgas, 2011; Guinder et al., 2009).

SP plays an important role in the growth of phytoplankton in coastal waters by controlling to a large extent the variability of the water inherent optical properties (Astoreca et al., 2012). By scattering and absorbing solar radiation, SP may attenuate the light penetration in water column and thereby interfere with phytoplankton photosynthesis (Kirk, 1985; Swift et al., 2006). It is generally observed that the scattering of light increases with the concentration of SP (Bowers et al., 2009). Experimental studies have demonstrated that light attenuation caused by SP decreases the growth rate of phytoplankton (Pierson et al., 2003; Peng et al., 2009).

SP can act as an adhesion surface to phytoplankton. Usually, the finer the particles are, the easier they adhere to phytoplankton. As reported by Walling and Moorehead (1989), approximately 10% to over 80% of the mass of the suspended sediments belongs to the clay fraction (diameter <2 μm). These small-size SPs provide a relatively large surface area for the adhesion and aggregation of phytoplankton, resulting in the sinking of algal cells (Guenther and Bozelli, 2004a).

Bohai Bay is one of the three bays forming the Bohai Sea, the innermost gulf of the Yellow Sea, in northeast China. In the surf of the development of Binhai New Area of Tianjin, along with the coastal dredging and harbor constructions, the Bohai bay is suffering an unparalleled pressure of pollution. As a result, SP in Bohai Bay rose to higher concentrations than ever, especially in the coastal regions in summer (Cui et al., 2009). According to Qiao et al. (2010), for instance, the maximum concentration of SP nearby the Caofeidian Harbor even reached up to 603.02 mg L^{-1}.

High densities of phytoplankton bloom in Bohai Bay every summer and cause red tide frequently. Few studies have been conducted to determine how SP affects the growth and composition of the dominant phytoplankton species of this area. Although some studies have investigated the effect of SP on the growth of marine phytoplankton (Guenther and Bozelli 2004a; Guinder et al., 2009), none of which evaluated the influence of SP on the two dominant species, *Phaeodactylum tricornutum* Bohlin and *Gymnodinium* sp. in Bohai Bay.

The diatom *P. tricornutum* is an important food resource for marine zooplankton and other filter organisms in the marine food chain (Wang and Zheng, 2008). The dinoflagellate *Gymnodinium* sp. is a popular red tide species which is observed in most coastal red

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tide cases (Zhao et al., 2004). The purpose of this study was to quantify the impact of different concentrations of SP on the two algal species. The Weibull dose-response models describing the relationship between SP concentrations and algal growth inhibitory rates were developed.

2. Materials and methods

2.1. Algal culture

P. tricornutum and *Gymnodinium* sp. strains were obtained from the Institute of Oceanology of the Chinese Academy of Sciences, Qingdao, China. The two strains were isolated from coastal water of Laizhou Bay of Bohai Sea. The algal cultures were maintained under axenic conditions at a temperature of 21 ± 1 °C. Illumination was provided by cool-white fluorescent lamps (Philips TLD 36W/54) and at irradiance of 260 μmol m^{-2} s^{-1} coupled with a 12:12 h dark/light cycle. The two algal strains were cultured in autoclaved (120 °C for 30 min) artificial sea water enriched with modified Guillard's f/2 medium with a salinity of 15 PSU (Guillard and Ryther, 1962). The medium was called AWG medium in this study.

2.2. Preparation of SP stock

Stock suspensions of SP were prepared with a surface sediment (upper 5 cm layer) sample, which was collected from Haihe River Estuary in the Bohai Bay (latitude 38°59′29.97″N; latitude 117°42′53.44″E). The sediment sample was composed of clay, silt, and sand in the percentage of 38.4%, 58.1%, and 4.5%, respectively. The organic matter in the sample was removed by boiling 1 kg of sample in 2 L of 5% sodium hypochlorite solution for 1 h. After being boiled, the particles were allowed to flocculate and settle down for 24 h, and then the supernatant was discarded. The particles were then resuspended in distilled water by agitation (Kirk and Gilbert, 1990). The settlement and resuspension procedure was repeated three times and the sediment was then dried to constant weight at 105 °C. After being dried, the sediment particles were sieved through a 38 μm mesh and stored in a desiccator.

2.3. Experimental design and sample analysis

The two algal colonies were treated with different concentrations of SP using 250 mL Erlenmeyer flasks that contained 100 mL AWG medium, respectively. The treatments of SP were set as 0, 0.1, 0.2, 0.4, and 0.8 g L^{-1} respectively by adding different amount of SP stock solution. All treatments were performed in triplicate. The colonies of the two strains were transferred to the treatment medium at their exponential growth phase and the initial cell densities of all treatments were set as 5×10^5 cells mL^{-1}. During the experiment, the cultures were agitated at 100 rpm in a shaker at 21 ± 1 °C, and at irradiance of 260 μmol m^{-2} s^{-1} coupled with a dark/light cycle of 12/12 h. The experiment lasted for 9 days until the stationary growth phase of the colonies were reached. Throughout the study, 1 mL culture medium was collected from each flask every day and then was immediately fixed with acid Lugol's solution (Lovegrove, 1960). The cell densities were enumerated in random fields under microscope (CX21FS1, Olympus Corp., Tokyo, Japan) using a Neubauer hemocytometer.

2.4. Parameter calculation

The maximum specific growth rate (μ_{max}) is an important parameter in modelling algal growth under batch condition. It can be calculated using logistic function (sigmoidal equation) (Thornley et al., 2007) described as follows:

$$B_t = \frac{B_f}{1 + \frac{B_f - B_0}{B_0} \times \exp^{\frac{-4t\mu_{max}t}{B_f}}} \quad (1)$$

where B_f and B_0 are the cell densities at stationary and initial phase, respectively; t and B_t are time and the corresponding cell densities, respectively; and μ_{max} is the maximum specific growth rate, which can be obtained by nonlinear-fitting of Eq. (1) using the software Origin 8.0 (OriginLab Corp., Northampton, MA, USA).

The growth inhibitory rate (I) of phytoplankton was calculated by comparing the area under the growth curves with the control treatment using the following equations:

$$A = \frac{t_1}{2} \times (N_1 - N_0) + \frac{t_2 - t_1}{2} \times (N_1 + N_2 - 2N_0) + \cdots + \frac{t_n - t_{n-1}}{2} \times (N_{n-1} + N_{n-2} - 2N_0) \quad (2)$$

$$I = \frac{(A_c - A_t)}{A_c} \times 100\% \quad (3)$$

where A represents the area under the growth curves; A_c and A_t are the area of control and treatments, respectively; N_0 is the initial cell densities; and t_n and N_n represent the time and the corresponding cell densities, respectively.

The inhibitory effects of SP on phytoplankton can be described by the dose-response curve of contaminants, represented by the Weibull equation (Dueri et al., 2009) as follows:

$$f(x) = 1 - \exp[-\exp(\beta + \theta \log_{10} x)] \quad (4)$$

where $f(x)$ is the growth inhibitory rate; x is the concentration of SP in medium; β is the scale parameter, which is related to the growth inhibitory rate of phytoplankton approaching a stable value; and θ is the curve shape parameter. In this model, we assume that the mortality rate of phytoplankton depends on the concentration of SP. The two parameters can be obtained by nonlinear-fitting using the software Origin 8.0.

Half maximal algal inhibitory concentration (IC_{50}) was calculated using Eq. (4). IC_{50} is the SP concentration that provokes 50% of the growth inhibitory rate [$f(x)$]. Thus, IC_{50} is represented with x in Eq. (4) when $f(x)$ is 50%.

2.5. Statistic analysis

All data were analyzed using SPSS 17.0 (IBM Corp., Armonk, NY, USA). Multi-factor ANOVA tests were conducted to determine the influence of different factors. All tests were conducted at a 95% confidence interval ($\alpha = 0.05$). Multiple comparison was conducted using Duncan's test ($p < 0.05$).

3. Results

The growth of both the two algal strains was inhibited by certain concentrations of SP. The cell densities of the two algal strains decreased as the SP concentrations increased in the medium (Fig. 1). As shown in Table 1, the growth inhibitory rates that were caused by SP increased progressively as the increase of the SP concentrations. The growth inhibitory rates of *P. tricornutum* and *Gymnodinium* sp. on the 9th day, for instance, both exceeded 45% (Table 1). SP showed significant effect on the growth inhibitory rate of *P. tricornutum*, whereas it showed little effect on the maximum cell density (B_{max}) until the SP concentration reached 0.8 g L^{-1}. In contrast, SP showed significant effect on B_{max} of *Gymnodinium* sp. even at the concentration of 0.2 g L^{-1}. The effect of SP on the maximum specific growth rate (μ_{max}) of the two strains