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Original Research

Different Sensitivities of Unicellular and Colonial Microcystis Strains (Cyanophyceae) to Six Emergent Macrophytes

Jing Zhao[1], Xiao-min He[2], Pei-shi Sun[1], Sheng-hua Zhang[1]*

[1]Research Institute of Engineering and Technology, Yunnan University, Kunming, 650091, China
[2]Hubei Environmental Monitoring Central Station, Wuhan, 430072, China

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Abstract

The effects of six emergent macrophytes (*Typha orientalis*, *Acorus calamus*, *Oenanthe javanica*, *Scirpus validus*, *Sagittaria sagittifolia*, and *Pontederia cordata*) on the growth of two strain *Microcystis aeruginosa* were studied under co-culture conditions. And the sensitivities of unicellular and colonial Microcystis strains to six emergent macrophytes were compared using an exudation experiment. Based on laboratory experiments, *T. orientalis*, *A. calamus*, *O. javanica*, *S. validus*, *S. sagittifolia*, and *P. cordata* had strong inhibitory effects on growth of unicellular *M. aeruginosa*, while only *A. calamus* and *P. cordata* show obvious growth inhibition on colonial *M. aeruginosa*. When the biomass density was 20 g FW·L^{-1}, the growth inhibition rate of unicellular *M. aeruginosa* can exceed 90% for all of the six emergent macrophytes. When macrophytes coexisted with the colonial *M. aeruginosa*, only *A. calamus*, *P. cordata*, and *S. sagittifolia* showed the growth inhibition of algae. Maximal inhibition of Chl a growth was 75% ($p<0.05$) for *A. calamus*, 69% ($p<0.05$) for *P. cordata*, and 40% for *S. sagittifolia* at 45 g FW·L^{-1} on day 15. The results of the exudation experiment indicated that there were no significant differences between control and treatment of Chl a concentrations of colonial *M. aeruginosa* for all of the six macrophyte exudations on days 6 and 12. While after 6 d incubation in 100% and 50% macrophyte exudations (40 g FW·L^{-1}), the cell densities of unicellular *M. aeruginosa* in control were obviously higher than all those in treatment ($p < 0.05$). The maximal algal growth inhibition (89.62%) of unicellular *M. aeruginosa* was achieved in 100% exudation of *A. calamus* on day 6 ($p < 0.05$). So according to the results of exudation experiments, the unicellular *M. aeruginosa* was more sensitive than the colonial strain to six emergent macrophytes. And this different sensitivity between Microcystis species probably correlated positively with colony size.

Keywords: allelopathy, emergent macrophytes, exudation, unicellular *Microcystis aeruginosa*, colonial *Microcystis aeruginosa*

Introduction

Eutrophication of water bodies is accelerated by human activities, especially in developing countries, which has resulted in the frequent occurrence of algal blooms. Harmful algal blooms, such as cyanobacterial blooms, can cause ecological and aesthetic problems, and algal toxins can pose serious threats to animal and human health [1]. Microcystis is the most important genus responsible for the formation of water blooms and production of microcystins [2]. Therefore, suppressing the growth of Microcystis is crucial for controlling cyanobacterial blooms [3, 4].

*e-mail: zhangshenghua@yahoo.com.

Macrophytes are of great importance for the biological structure and water quality of shallow lakes [5, 6] because of improving water transparency and maintaining the clear water state by some mechanisms, such as inhibiting sediment re-suspension [7], providing structure and shelter for other organisms [8], and inhibiting algae [9, 10]. In shallow eutrophic lakes, allelopathy may be a useful strategy for macrophytes to reduce biomass of epiphytes and phytoplankton [11, 12]. Allelopathy has been described for many the aquatic macrophytes and proposed as a measure to control the growth of undesired phytoplankton, which is a threat to the aquatic ecosystem [13, 14]. Many studies exhibit that macrophytes can inhibit the growth of Microcystis aeruginosa, such as *Vallisneria spiralis* L. [15], *Stratiotes aloides* [16], *Myriophyllum spicatum* [13, 17, 18], and Potamogeton species [19]. However, these studies used unicellular *M. aeruginosa* strains. It is important to test the effects of macrophytes on problematic colonial *M. aeruginosa* because of its stronger resistance to unfavorable environmental conditions [20].

Except for submerged macrophytes, some emergent macrophytes and floating plants also can inhibit the growth of water-bloom algae. It has been reported that emergent macrophytes such as *Phragmites communis* [21], *Acorus calamus* [22], *Thalia dealbata* [23], and floating plant *Eichhornia crassipes* [24] all can effectively inhibit the growth of *M. aeruginosa*. Emergent macrophytes are widely used in water ecological restoration; therefore, it is important to find whether emergent macrophytes can be used to control algal blooms.

In the present study we investigated the effects of six emergent macrophytes on the growth of unicellular and colonial *M. aeruginosa* to determine if unicellular and colonial Microcystis strains have different sensitivities to the exudates isolated by the same macrophytes.

Materials and Methods

Macrophytes and Algae

Typha orientalis, *A. calamus*, *Oenanthe javanica*, *Scirpus validus*, *Sagittaria sagittifolia*, and *Pontederia cordata* are used in the study. All six emergent macrophytes were obtained from Yangzong Lake, Kunming, China, and all macrophytes were rooted in the lake's sediment with clear water. Before the experiment, fresh plants were rinsed carefully with distilled water to remove a few attached epiphytes and sediments without damage. Fresh weight (FW) was determined after blotting, and then each plant was placed into glass aquaria (20×20×30 cm) with 8 L of modified MIII nutrient medium [17] under 14:10 h light/dark (L/D) cycle with irradiance 47.5 μmol photons $m^{-2}·s^{-1}$ at 25±2ºC. Biomass densities of plants were 20 $g·L^{-1}$ FW. The plants were cultured 15 days before the experiment, and the culture medium in each aquarium was renewed with the freshly modified MIII medium every 3 days.

Unicellular and colonial strains of *M. aeruginosa* were used as target organisms. The unicellular *M. aeruginosa* (FACHB 905) and the colonial strains of *M. aeruginosa* (FACHB 1178) were both provided by the Freshwater Algae Culture Collection of the Institute of Hydrobiology, the Chinese Academy of Sciences. Both Unicellular and colonial strains were pre-cultivated in BG11 medium. Before the experiment, both strains were transferred into modified MIII medium [17] and maintained for 2 weeks at 25±2ºC with a 14 h:10 h light/dark cycle in an incubator with illumination at 47.5 μmol photons $m^{-2}·s^{-1}$ provided by cool-white fluorescent lamps. The algae were shaken three times every day. In the study, the tested organisms in exponential growth phase were used. The bacterial biomass in the cultures was negligible.

Coexistence Experiment

After 15 days incubating in a laboratory, the adaptive plants were taken out and put into aquaria containing 4L modified MIII medium at 5, 10, 15, and 20 g biomass $FW·L^{-1}$ for unicellular *M. aeruginosa* and of 15, 30, and 45 g $FW·L^{-1}$ for colonial *M. aeruginosa*. Unicellular *M. aeruginosa* was put into the aquaria with the initial cell density of $1×10^6$ $cells·mL^{-1}$ and the colonial strains were with the initial Chl a of 48.38±2.60 $μg·L^{-1}$. To minimize the effects of shading by macrophytes, all aquaria with the algae only were added to plastic plants. Nutrient concentrations in both treatments (control and macrophytes) were similar to the concentrations in modified MIII medium to abate the nutrient competition between macrophytes and algae. During the experiment, the concentrations of NO_3-N and o-PO_4-P were measured in the aquarium water every day, according to the standard methods described by the State Environmental Protection Administration (SEPA) of China [25] (SEPA, 2002), to measure the absorptivity of nutrients and added their decrements daily to keep the same concentrations of total nitrogen (TN) and total phosphorus (TP).

For unicellular *M. aeruginosa*, the coexistence experimental set-up consisted of 24 treatments (six emergent macrophytes with 4 biomass densities) and 1 algal control (unicellular *M. aeruginosa* with plastic plants). For the colonial *M. aeruginosa*, the coexistence experimental set-up consisted of 18 treatments (six emergent macrophytes with 3 biomass densities) and 1 algal control (colonial *M. aeruginosa* with plastic plants) and 18 macrophyte controls (six emergent macrophytes with 3 biomass densities only).

There were triplicates for each treatment. A randomized block design was used to distribute the batch cultures in the incubators (25ºC, 47.5 μmol photons $m^{-2}·s^{-1}$, 14:10 h light:dark cycle) [26]. Each algal culture was collected in glass bottles. The cell numbers of unicellular *M. aeruginosa* were counted using a 0.1mL counting chamber under an anatomical microscope (×40) from day 0 to day 7 every 24 hours, and the chlorophyll a contents of colonial *M. aeruginosa* were measured from day 0 to day 15 every 3 days using the colorimetric method [27].

Exudation Experiments

In the experiment, two 4 L aquaria were used – one filled with macrophytes at biomass of 40 g FW·L^{-1} and the other filled with medium only as a control. Both aquaria were incubated for 3 days, then the incubation water was taken out to be filtered by Whatman GF/F filters (pore size: 0.7 μm). After o-PO_4-P and NO_3-N nutrients added, the nutrient concentrations in both media (control and macrophytes) were similar to concentrations in modified MIII medium, and the Erlenmeyer flasks (250 mL) were filled with 100 mL control water or macrophyte water. And a part of the exudations were diluted with equate modified MIII medium to get 50% exudation solutions for investigating the effect of concentrations on allelopathic activity of six emergent macrophytes. Thereafter, a small aliquot of dense algae, exponentially growing phytoplankton batch cultures, was added to the flasks (unicellular *M. aeruginosa* initial concentration 1×10^6 cells·mL^{-1}, the colonial strains with the initial Chl a of 48.38±2.60 μg·L^{-1}). The phytoplankton was inoculated in control as well as in exudation medium. The exudation experimental set-up consisted of 24 treatments (100% macrophyte exudations with 2 algae and 50% macrophyte exudations with 2 algae, six potamogeton species) and 2 algal controls. There were triplicates for each treatment.

The cultures were exposed at 25±2ºC with a 14 h:10 h light/dark cycle in an incubator with illumination at 47.5 μmol photons m^{-2}·s^{-1}. Each algal culture was collected in glass bottles. The cell numbers of unicellular *M. aeruginosa* were measured after 72 h incubation and 144 h incubation, and the chlorophyll a contents of colonial *M. aeruginosa* were measured after 6 and 12 days of incubation.

Statistics

All the controls and treatments were replicated thrice. Statistical differences between the control and treatments were tested using Independent-Samples T-test and One-way ANOVA with SPSS software (13.0) (SPSS, USA) at 95% confidence level. The Normality of data in different groups was tested through the Shapiro-Wilk test before statistics.

Results

The Inhibitory Activities of Six Emergent Macrophytes on the Growth of Unicellular *M. aeruginosa* under Co-Culture Conditions

The growth curves of unicellular *M. aeruginosa* in the coexistence with six emergent macrophytes are shown in Fig. 1. The cell density of algae increased much higher in the control than that in the treatments coexisting with *A. calamus* and *P. cordata* at all macrophyte biomass densities. The growth inhibitory rate of algae by *A. calamus* at 5 g FW·L^{-1} increased from 3.92% on day 1 to 24.03% on day 3, to 61.28% on day 5 and 87.31 on day 7, respectively. While the biomass density of *A. calamus* was 20g FW·L^{-1}, the inhibitory rate of unicellular *M. aeruginosa* reached 42.92% on day 3, 81.71% on day 5, and 99.51% on day 7. As for *P. cordata* (20 g FW·L^{-1}), the inhibitory rate of algae increased from 63.12% on day 3, 83.73% on day 5, and to 95.51% on day 7.

There was no obvious growth inhibition of unicellular *M. aeruginosa* that coexisted with *T. orientalis* and *O. javanica* at 5 g FW·L^{-1} biomass density. And the growth inhibitory rate of algae increased after 4 days when the macrophytes densities reached 15 and 20 g FW·L^{-1}.

When the biomass densities of *S. validus* and *S. sagittifolia* was 5 FW·L^{-1}, the growth inhibitory rate of algae was 44.93% and 74.54%. At 20 g FW·L^{-1}, the the growth inhibitory rate of algae after 7 days incubation was up to 98.17% for *S. validus* and 96.51% for *S. sagittifolia*.

From the results of algal growth curves in coexistence experiments, it was shown that these six emergent macrophyte species can inhibit the growth of unicellular *M. aeruginosa* strongly when they coexisted in the culture system under laboratory aquatic conditions. Among them, *A. calamus* and *P. cordata* show the strongest inhibitory activities.

The Effects of Six Emergent Macrophytes on the Growth of Colonial *M. aeruginosa* under Co-Culture Conditions

The effects of six emergent macrophytes on the growth of colonial *M. aeruginosa* were shown in Fig. 2. The marked inhibitory effects of *A. calamus* and *P. cordata* on the growth of colonial *M. aeruginosa* were noted. At all three macrophyte concentrations (15, 30, and 45 g FW·L^{-1}) tested, the Chl a concentrations of colonial *M. aeruginosa* were significantly reduced during the 15-day test period ($p<0.05$). Chl a concentrations of colonial *M. aeruginosa* coexisted with 15 g FW·L^{-1} *P. cordata* and *A. calamus* on day 15 differ significantly with the control, and the growth inhibition rates reached 58% and 65%. Maximal inhibition of Chl a growth was 75% ($p<0.05$) for *A. calamus* and 69% ($p<0.05$) for *P. cordata* at 45g FW·L^{-1} on day 15.

After 15 days incubation, the Chl a concentraction of colonial *M. aeruginosa* was also lower than the control, which coexisted with *S. sagittifolia* (inhibition rate 40% at 45 g FW·L^{-1}, $p<0.05$), while there was no significant difference between the control and the treatment that coexisted with *T. orientalis* and *S. validus* ($p>0.05$) at all three biomass densities. The Chl a concentraction of colonial *M. aeruginosa* coexisted with 15 g FW·L^{-1} *O. javanica* was a little higher than the control ($p<0.05$), and there was no significant difference between the control and the treatment when biomass densities of macrophytes were 30 and 45 FW·L^{-1} ($p>0.05$).

The Effects of Six Emergent Macrophyte Exudates on the Growth of Unicellular and Colonial *M. aeruginosa*

The growth inhibition of unicellular *M. aeruginosa* in the presence of exudates from these six emergent macrophyte species are shown in Fig. 3. Compared with the con-

trol, there was no significant difference on the growth of unicellular *M. aeruginosa* after 3 days incubation with exudates ($p>0.05$). But after 6 d incubation in 100% and 50% macrophyte exudations (40 g FW·L^{-1}), the algal cell densities in control were obviously higher than all those in treatment ($p<0.05$). The maximal algal growth inhibition (89.62%) was achieved in 100% exudation of *A. calamus* on day 6 ($p<0.05$).

The effect of six macrophyte exudations on the growth of colonial *M. aeruginosa* was shown in Fig. 4. There were no significant differences of Chl a concentration of colonial *M. aeruginosa* between control and treatment for all of the six macrophyte exudations on days 6 and 12 ($p>0.05$).

The results of exudation experiments indicate that the exudates from these six emergent macrophytes can inhibit the growth of unicellular *M. aeruginosa* strongly while showing no obvious inhibition on the growth of colonial *M. aeruginosa*. The results maybe suggested that unicellular *M. aeruginosa* was more sensitive than colonial *M. aeruginosa* to the exudates isolated from the six emergent macrophytes.

Discussion

The algal growth inhibitory activities of *T. orientalis*, *A. calamus*, *O. javanica*, *S. validus*, *S. sagittifolia*, and *P. cordata* are seldom studied, although these macrophytes are

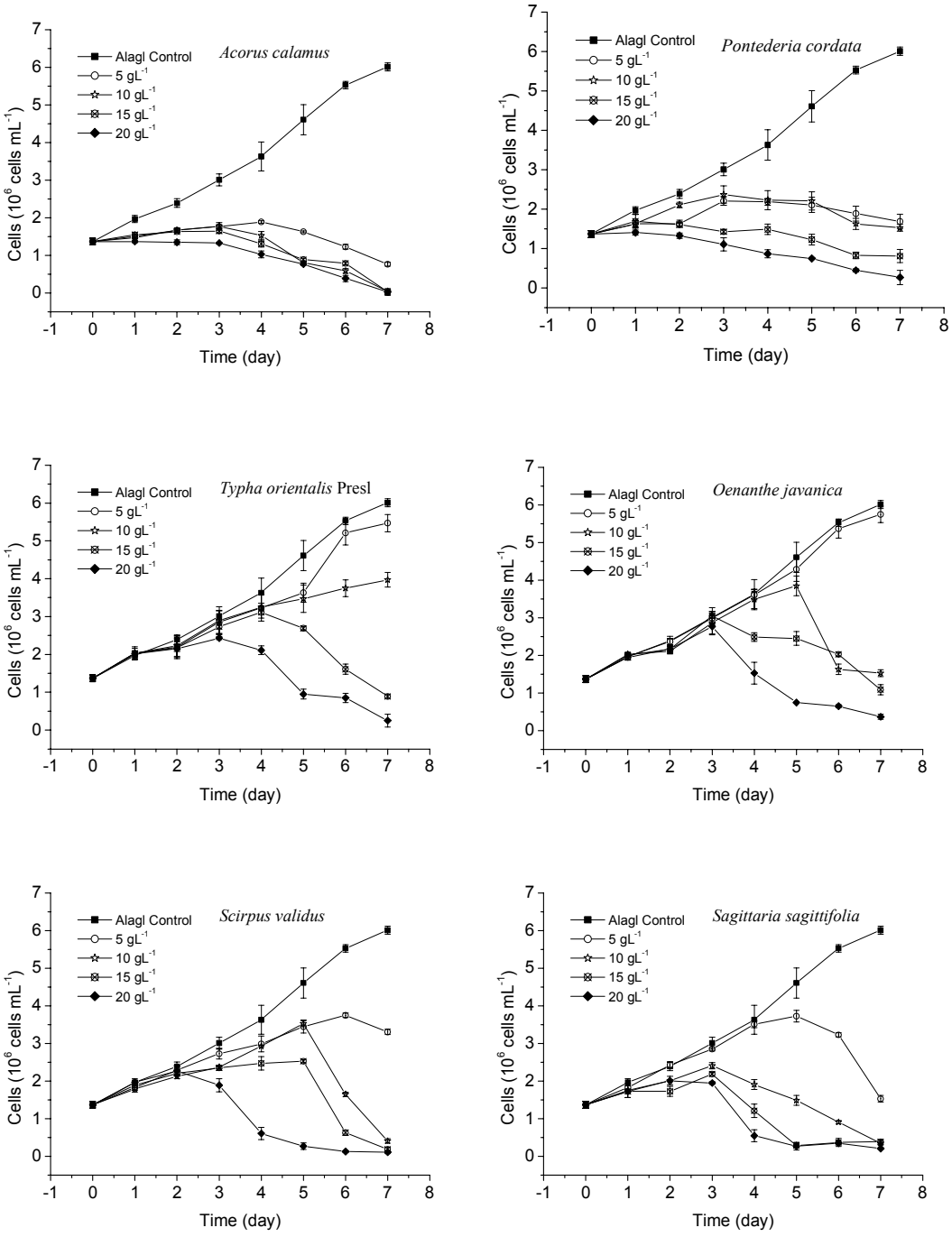

Fig. 1. The growth curves of unicellular *M. aeruginosa* coexisted with six emergent macrophyte species (*T. orientalis*, *A. calamus*, *O. javanica*, *S. validus*, *S. sagittifolia*, and *P. cordata*) (n=3, the data is the mean, and the bar is the standard deviation).

widely used in water ecological restoration, especially in wetland sewage treatment due to their luxuriant roots. Our present study found the allelopathic potential of these six emergent macrophytes on the growth inhibition of cyanobacteria. The results indicated that all these six macrophytes had strong inhibitory effects on growth of unicellular *M. aeruginosa*, while only *A. calamus* and *P. cordata* show obvious growth inhibition on colonial *M. aeruginosa*. The unicellular *M. aeruginosa* was more sensitive than colonial strains to six emergent macrophytes according to the results of exudation experiments. Many previous studies have reported inhibitory effects on only unicellular *M. aeruginosa* strains [15, 18, 19, 21], whereas growth inhibition of both unicellular and colonial forms of *M. aeruginosa* were demonstrated in this paper.

Since algal growth might be inhibited due to competition for nutrients, light and/or allelopathy, the results of algal inhibition could only be attributed to allelopathy from macrophytes after these interfering factors have been ruled out. In this experiment, light was irradiated from one side of the aquarium and all aquaria were exposed to 47.5 μmol photons $m^{-2} \cdot s^{-1}$ light to minimize the effects of light. In terms of nutrients, the universal methods in allelopathy and algae toxicology research are to keep the nutrients at a high level that could meet the need of algae growth. It was indicated that it couldn't influence algae growth when PO_4^{3-}-P con-

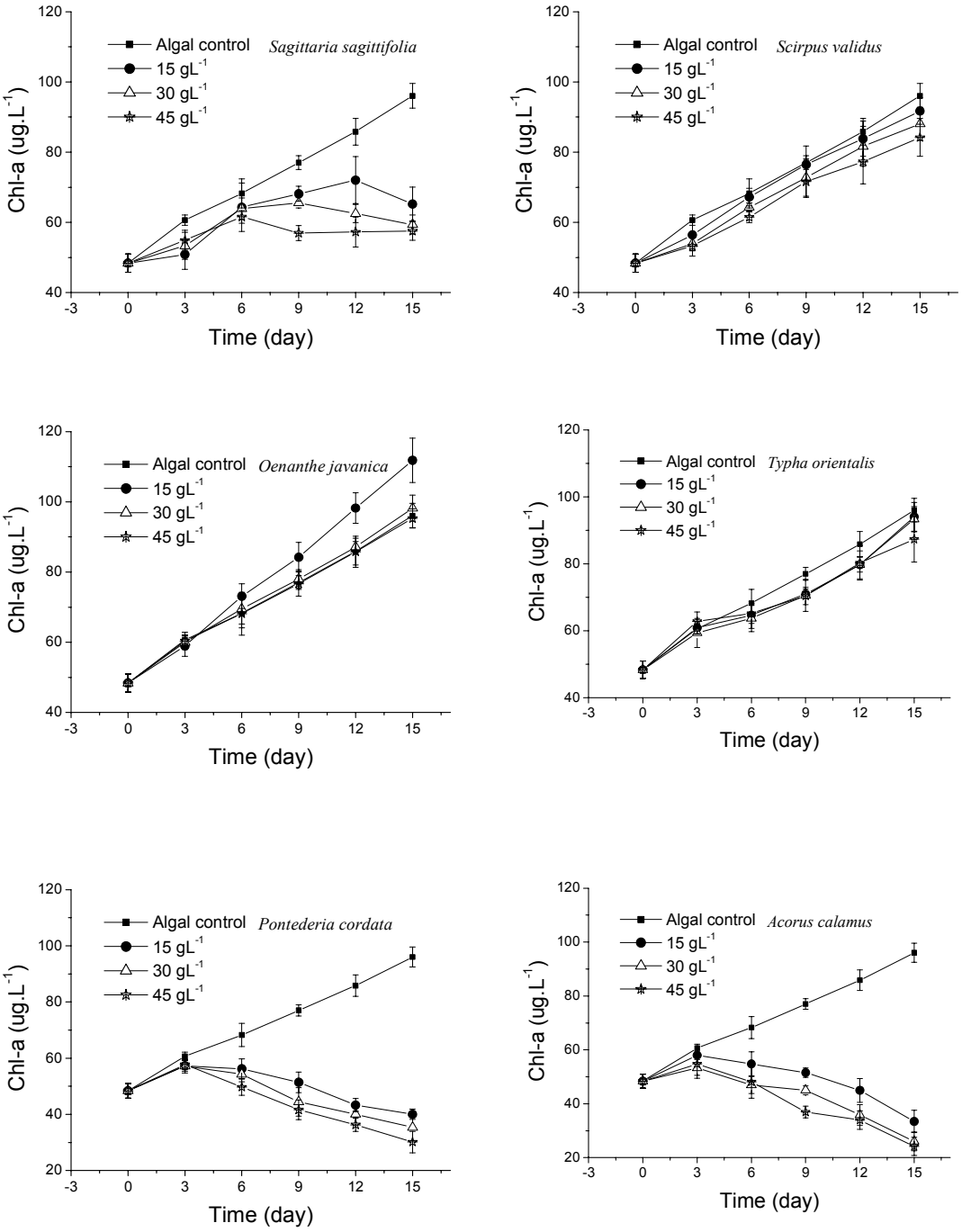

Fig. 2. The Chl a concentration of colonial *M. aeruginosa* coexisted with six emergent macrophyte species (*T. orientalis*, *A. calamus*, *O. javanica*, *S. validus*, *S. sagittifolia,* and *P. cordata*) (n=3, the data is the mean, and the bar is the standard deviation).

centration was above 0.6 mg·L^{-1} and NO_3^--N concentration was above 2.3 mg·L^{-1} [28]. In this experiment, the nutrient concentrations were never limiting for the growth of phytoplankton because nitrogen and phosphorus concentrations remained about the concentrations of 1.55 mg·L^{-1} o-PO_4 and 7 mg·L^{-1} NO_3-N through adding their decrements daily. And the initial algal density was chosen to make sure the algae were healthy and sensitive.

Allelopathic growth inhibition of algae by aquatic plants has been studied for many macrophytes [6, 9, 11, 13, 17]. Among them many studies exhibit that allelopathic effects of macrophytes on phytoplankton appear to be species-specific. Mulderij et al. [16] showed that the sensitivity of cyanobacteria to Stratiotes water was not higher than that of other phytoplankton strains, and within cyanobacteria, the toxic strain was more sensitive than the non-toxic one. Mulderij et al. [26] investigated allelopathic effects of a mixture of *Chara globularis* var. *globularis* and *Chara contraria* var. *contraria* on three different green algae. The results indicated allelopathic effects of Chara on the growth of the green algae *Selenastrum capricornutum* and *Chlorella minutissima*, whereas *Scenedesmus obliquus* seemed not affected. Körner and Nichlisch [17] found that members of the Oscillatoriales and *M. aeruginosa* were more sensitive to the allelopathy of *M. spicatum* than the cyanobacterium *Aphamzomenon flos-aquae*, the diatom *Stephanodiscus minutulus*, and the green alga *Scenedesmus armatus*. Planas et al. [29] found that Cyanophyta were more sensitive to phenolic extracts of *M. spicatum* than chlorophyta. The results of Nakai [13] indicated that in subsequent initial addition assays using *Potamogeton oxyphyllus*, the growth of *M. aeruginosa* was inhibited significantly while the growth inhibition of *A. flos-aquae* was not observed. *S. capricornutum* and *M. aeruginosa* have different sensitivities to exudates from two Potamogeton species [30]. In our study, the exudates from six emergent macrophytes can inhibit the growth of unicellular *M. aeruginosa* after 6 days incubation, while there are no differences

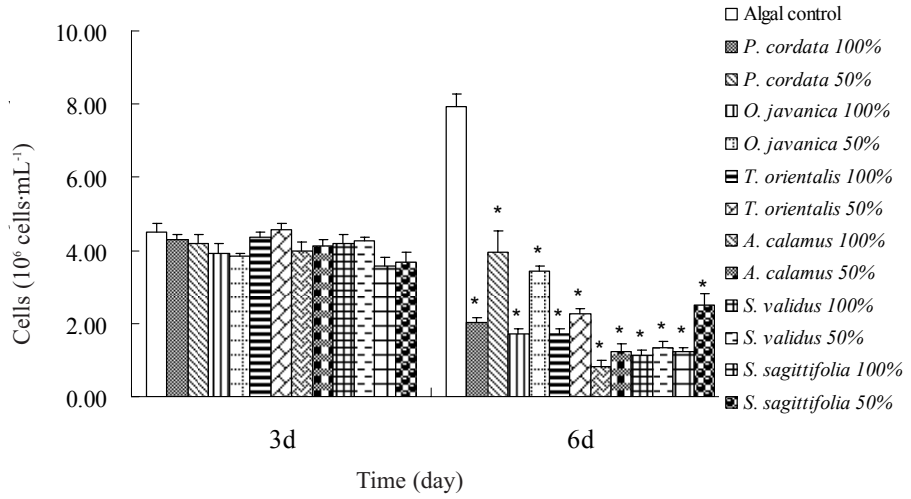

Fig. 3. Cell densities of unicellular *M. aeruginosa* in coexistence with exudates from six emergent macrophyte species *T. orientalis, A. calamus, O. javanica, S. validus, S. sagittifolia,* and *P. cordata*) after 3 and 6 days incubation (bar = SD, n=3, * p<0.05, compared with the control).

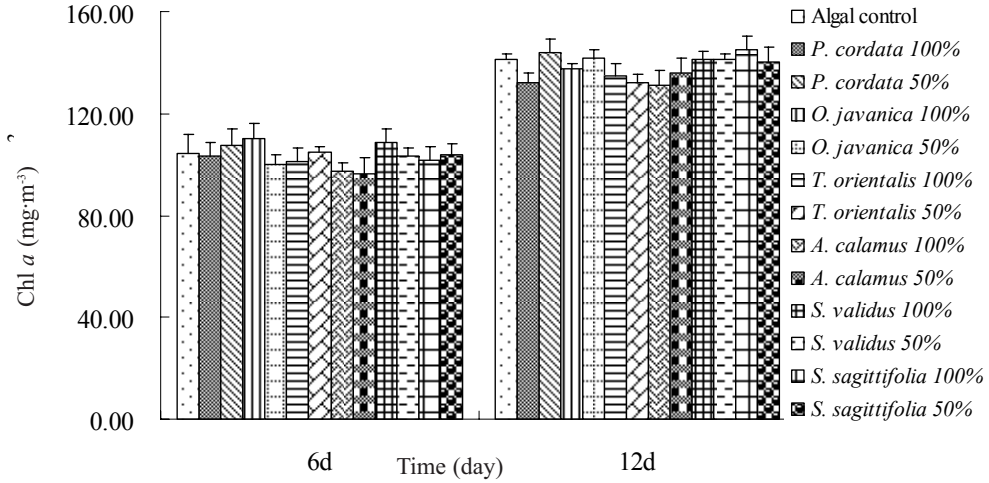

Fig. 4. The Chl a concentration of colonial *M. aeruginosa* in coexistence with exudates from six emergent macrophyte species (*T. orientalis, A. calamus, O. javanica, S. validus, S. sagittifolia*, and *P. cordata*) after 6 and 12 days incubation (bar = SD, n=3, *p<0.05, compared with the control).

between the colonial *M. aeruginosa* control and the treatment. That is to say the unicellular *M. aeruginosa* is more sensitive than the colonial strain. This result is consistent with the results of Park et al. [31], who has reported that growth inhibition of unicellular *M. aeruginosa* was much higher than that of colonial *M. aeruginosa* when treated with rice hull crude extract. In the coexistence experiments, the growth of colonial *M. aeruginosa* was also inhibited by *A. calamus* and *P. cordata*, but inhibition was much lower than that observed with the unicellular strain. The colonial Microcystis strains were identified by their strong resistance to Cu stress and higher affinity for inorganic carbon [32, 33]. And the colonial strains endure stress better than the unicellular strains [34]. So the reason for different sensitivities of unicellular and colonial Microcystis strains (Cyanophyceae) to six emergent macrophytes may be the different resistant ability to the exudates from the same macrophytes. And colonial Microcystis strains have a better ability to resist the allelopathic activity of macrophytes than unicellular Microcystis strains. Based on these results, we may conclude that the ability of Microcystis species to resist allelochemical stress correlates positively with colony size.

The present study helps to reduce the large gap in our knowledge about the allelopathic inhibition of these six emergent macrophytes on unicellular and colonial Microcystis strains (Cyanophyceae), and the different sensitivities of unicellular and colonial Microcystis strains to six emergent macrophytes. Our conclusions, however, are based on laboratory experiments showing that *T. orientalis*, *A. calamus*, *O. javanica*, *S. validus*, *S. sagittifolia*, and *P. cordata* had strong inhibitory effects on growth of unicellular *M. aeruginosa*, while only *A. calamus* and *P. cordata* show obvious growth inhibition on colonial *M. aeruginosa*. The unicellular *M. aeruginosa* was more sensitive than the colonial strain to six emergent macrophytes according to the results of exudation experiments. And this different sensitivity between Microcystis species was probably to correlate positively with colony size.

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