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Original Research

Different Sensitivities of *Selenastrum capricornutum* and Toxic Strain *Microcystis aeruginosa* to Exudates from Two Potamogeton Species

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Abstract

The sensitivities of *Selenastrum capricornutum* and the toxic strain *Microcystis aeruginosa* to exudates from *Potamogeton maackianus* and *P. malaianus* were compared using exudation experiment, and the potential allelochemicals released by these two pondweeds into surrounding water were also analyzed. The growth of *S. capricornutum* and *M. aeruginosa* was inhibited by the exudates from the two macrophytes. Compared to the control, the cell densities of *S. capricornutum* decreased by 42.7% and 61.9% in 2.5 and 5 g·L^{-1} FW *P. maackianus* treatments, and the cell densities of *S. capricornutum* also decreased by 65.8% and 73.5% in the two biomass density treatments of *P. malaianus* after three days of treatments. After 3 days' incubation in 2.5 and 5 g FW·L^{-1} *P. maackianus* exudates, the *M. aeruginosa* cell densities were higher in control than in treatment. As for *P. malaianus* treatments, the cell densities of *M. aeruginosa* were reduced by 16.5 and 65.8% of the control in 2.5 and 5 g·L^{-1} FW marophytes at the end of incubation period, respectively. The allelochemicals exuded from the macophytes, which inhibited both *S. capricornutum* and *M. aeruginosa*, belonged to lipophilic and moderately lipophilic compounds according to the bioassay results of exudate fractionations. By multiple comparison statistics, the results showed that *P. maackianus* had stronger inhibitory effects on *M. aeruginsa*, while *S. capricornutum* was more sensitive to the allelochemicals of *P. malaianus*. The different sensitivities of the two algae were probably caused by three alcohol compounds (1-methoxy-2-methyl-2-Propanol, 2-methyl-2-Hexanol, and 4-ethyl-2,6-dimethyl-4-Heptanol) through the GC-MS analysis of the most active exudate fractions.

Keywords: allelochemical, cyanobacteria, green algae, exudation, submerged macrophytes

Introduction

Submerged macrophytes play important roles in eutrophic shallow aquatic ecosystems. They improve water transparency and maintain the clear water state by some mechanisms such as inhibiting sediment re-suspension [1], providing structure and shelter for other organisms [2], and inhibiting algae [3-4]. In shallow eutrophic lakes, allelopathy may be a useful strategy for macrophytes to reduce biomass of epiphytes and phytoplankton [5-6]. It has been confirmed that many submerged macrophytes could inhibit the growth of algae through releasing allelopathic compounds. It was summed up by Mulderij [6] that allelopathic effects on phytoplankton had been studied for at least 37 submerged macrophytes species. Among these macrophytes, there were some highly active species such as *Myriophyllum spicatum*

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[7] and *Ceratophyllum demersum* [8], some moderately active species such as *Elodea* [5] and *Chara* [9], and some species that exert little or no allelopathic activity, such as *Potamogeton lucens* [10] and *P. crispus* [11].

Many studies that exhibited allelopathic effects of macrophytes on phytoplankton appear to be species-specific. The same algae species showed different sensitivities to different macrophytes, for example *Microcystis aeruginosa* could be inhibited strongly by *M. spicatum*, but showed no sensitivity to *P. crispus* [11]. Furthermore, the same kind of marophyte had different inhibitory activities on different algae, for example *Stratiotes aloides* had no inhibitory effect on Scenedesmus, but showed strong toxicity to *M. aeruginosa* [12].

At present, studies on the allelopathic activities of macrophytes have been done mostly using different bioassay systems and culture conditions for both macrophytes and phytoplankton [13]. However, studies on the comparison of the effects of submerged macrophytes on phytoplankton are scarce. As for *Potamogeton* species, although there are some reports on the natural antialgal compounds isolated from Potamogetonaceae, and such as *Potamogeton natans* [14], *P. lucens* [15], and *P. pectinatus* [16], the exudates released by *Potamogeton* species into aquatic environments are seldom studied. The species-specific activities of *P. malaianus* and *P. maackianus* on algae have not been compared yet, and the allelochemicals released by the two macrophytes were not fully known. So the main objectives of this study were to compare the allelopathic effects of *P. maackianus* and *P. malaianus* on two algae, *M. aeruginosa* and *Selenastrum capricornutum*.

Materials and Methods

Macrophytes and Algae

P. maackianus was taken from the pond of Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan, China. *P. malaianus* was obtained from Honghu Lake, Hubei province, China. Both macrophytes were rooted in lake sediment with clear water. Fresh plants were rinsed carefully with distilled water to remove the 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 [8] under 14:10 h light/dark (L/D) cycle with irradiance 47.5 μmol photons $m^{-2}s^{-1}$ at 25ºC. Biomass densities of plants were 5 g·L-1 FW. The plants were cultured 15 days before the experiment, and the culture medium in each aquarium was renewed with fresh modified MIII medium every 3 days.

M. aeruginosa (FACHB 942, kept in BG-11 medium) and *S. capricornutum* (UTEX 1648, kept in SE medium) were selected as the target algae, which were both obtained from the Freshwater Algae Collection, Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). Prior to the initiation of the exudation experiments, short-term batch cultures of these two phytoplankton were grown in 1000 ml Erlenmeyer flasks filled with 400 ml modified MIII medium and aerated permanently to provide an optimal concentration of CO_2. Light source was supplied by fluorescent tubes of one side with irradiance 47.5 μmol photons $m^{-2}s^{-1}$ at 14:10 L/D cycle. The temperature was maintained at 25ºC in an air-conditioned growth chamber. Cells in the exponential growth phase were collected from batch cultures and used as the inocula for the following exudation experiments. The culture conditions in the following exudation experiments were the same as for the algal culture unless it is mentioned.

Exudation Experiments

In these experiments, three aquaria (15×15×20 cm) with 4 L culture solutions were used for each macrophyte, two of them were filled with macrophytes at biomass of 5 $g·L^{-1}$ FW and 2.5 $g·L^{-1}$ FW, respectively, the third was filled with medium only as a control. All aquaria were incubated for 3 days under culture conditions of 14:10 h light/dark (L/D) cycle with irradiance 47.5 μmol photons $m^{-2}s^{-1}$ at 25ºC, then the incubation water was taken out and filtered through Whatman GF/F filters (pore size: 0.7 μm) to minimize influences of debris and microorganisms. The nutrient concentrations in the filtrates were kept similar to concentrations in modified MIII medium by adding nitrate and phosphate, to avoid nutrient limitation in the following experiment. Then each homogeneously mixed filtrate was divided into six 250 mL Erlenmeyer flasks containing 100 mL (two algae species with each treatment had three replicates). In the experiment, *M. aeruginosa* and *S. capricornutum* were inoculated respectively in medium with (filtrates from two *Potamogeton* sp. treatments) and without macrophytes water (filtered control). Each treatment had three replicates and the experiment repeated twice. The high initial cell densities *S. capricornutum* and *M. aeruginosa* were both $1×10^8$ cells$·L^{-1}$. The incubation lasted for 3 days, and then each algal culture was collected in glass bottles and algae cell densities calculated through cell number calculations.

Fractionation of Macrophyte Exudates

To fractionate the chemicals exudated from macrophytes on the basis of their polarity, liquid-liquid extraction (LLE) and solid phase extractions (SPE) were performed with both macrophytes.

At the completion of the macrophyte incubation in modified MIII medium at 5 $g·L^{-1}$ FW after 3 days (pure water as a GC-MS determination control), the culture medium (4 L) were filtered through Whatman GF/F filter membranes (pore size: 0.7 μm). The possible allelochemicals secreted into the culture medium by the macrophytes were extracted using the SPE and LLE methods. For SPE, the exudates were enriched by solid phrase extraction cartridges (Waters HLB, 500 mg) that were activated by CH_2Cl_2, MeOH and pure water in sequence. Afterward, the cartridge was washed with one reservoir volume of water and then dried by N_2. SPE-enriched exudates were stepwise

fractionated eluting with CH_2Cl_2 and MeOH in sequence in order to get two fractions (CH_2Cl_2 extracts and MeOH extracts) for each macrophyte, and these four extracts (CH_2Cl_2 extracts A and methanol extracts B of *P. malaianus* and CH_2Cl_2 extracts a and methanol extracts b of *P. maackianus*) were obtained after being dehydrated by anhydrous Na_2SO_4, evaporated, and then stored in -20ºC until bioassay and on the most active extract of each macrophyte were performed GC-MS analysis.

For LLE, the procedure of extraction was shown in Fig. 1. Through liquid-liquid extraction of exudates from *P. maackianus* and *P. malaianus*, 3 fractions were obtained for each plant. Hexane extracts (C), MeCOEt extracts (D), and n-butanol extracts (E) of *P. malaianus,* and hexane extracts (c), MeCOEt extracts (d), and n-butanol extracts (e) of *P. maackianus* were also dehydrated by anhydrous Na_2SO_4, evaporated, and stored at -20ºC until bioassay.

Allelochemical Analysis

GC-MS (7890-5973, Agilent, USA) equipped with an HP 7673 autosampler (Agilent) was employed to analyze the main components in the SPE of active CH_2Cl_2 elution of these two pondweeds. Analytes were separated in an HP-5 ms capillary column (19091S-433, 0.25 mm×30 m×0.25 μm, Agilent). Oven temperature was isothermal at 280ºC. The injector operating conditions were as follows: injection volume of 1 μL; the injector temperatures were 250ºC with the splitless mode. The initial column temperature was 40ºC and programmed to increase at a rate of 10ºC min^{-1} to 270ºC and then held for 10 min. The transfer line temperature was 280ºC. Helium (purity of 99.9995%) was used as a carrier gas with a flow rate of 1 $mL \cdot min^{-1}$ at an inlet pressure of 4.93×10^4 Pa. Electronic impact (EI) ionization mode mass spectra were obtained at 70 eV and monitored on the full-scan range (m/z 50-550). Data acquisition, processing, and instrumental control were performed by Agilent WS software. Mass fragments of the components were compared to the mass fragmentation data contained in the NIST 02 library.

Bioassay

The toxicity tests of the exudate extract fractions were performed on the green alga *S. capricornutum* and cyanobacterium *M. aeruginosa*. The test on *S. capricornutum* was done using the 96-well microplate technique recommended by Environment Canada (1992) [17]. The validity of the test was controlled with a reference toxicant, potassium dichromate ($K_2Cr_2O_7$), and a 72 h IC_{50} of 0.67 $mg \cdot L^{-1}$ (0.49-0.81) was obtained with five replications of four concentrations between 0.18 and 1.07 $mg \cdot L^{-1}$. The 72 h IC_{50} value given by the Algaltoxkit FTM producer was 0.38 $mg \cdot L^{-1}$. Fractions of exudates were initially dissolved in dimethyl sulfoxide (DMSO) and then diluted in the algal culturing medium containing 1×10^6 $cells \cdot mL^{-1}$. A 96-well microplate allowed the testing of fractions with 15 replications at 30 $mg \cdot L^{-1}$. Highest DMSO level in the test wells did not exceed 0.33% (v/v).

The growth inhibition of *M. aeruginosa* was performed using the ISO 8692 method (1989) [18] with some modifications as described by Xian et al. [19]. Three mg mixture was dissolved into 30 μL DMSO in sterile 200 mL Erlenmeyer flasks and diluted with 100 mL algae medium containing 1×10^6 $cells \cdot mL^{-1}$. The algae were cultivated for 3 days at 25ºC with irradiance 47.5 μmol photons $m^{-2}s^{-1}$ at 14:10 L/D cycle. Control groups were prepared with DMSO only. Each experiment included triplicate treatments and the experiments were repeated twice. The growth inhibition percentage for specific tested substance concentrations was calculated compared to the control group through cell number calculations.

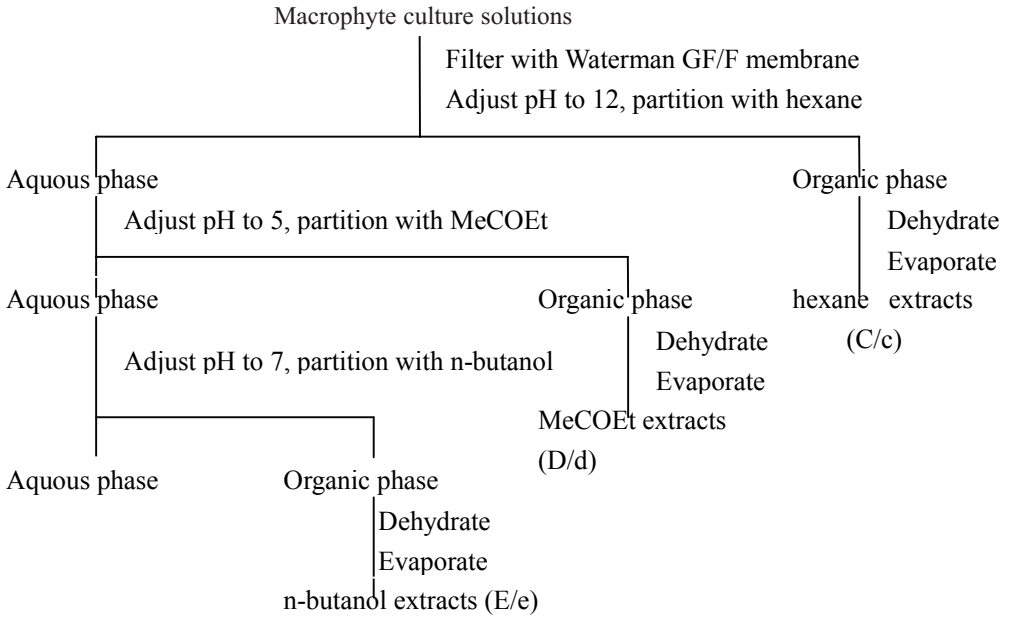

Fig. 1. The liquid-liquid extraction procedure of the exudates from two Potamogeton species.

Data Analysis and Statistics

All the control and treatments were replicated thrice. The homogeneity of variances of data in different groups were tested using Levene's Test. Statistical differences between the control and treatments were tested using oneway-ANOVA with SPSS software (13.0) (SPSS Inc. Chicago, IL, USA) at 95% confidence level. Subsequent multiple comparision was tested using LSD test when the ANOVA revealed significant effects.

Results

Exudation Experiment

In the exudation experiments, the investigated biomass densities of *P. malaianus* and *P. maackianus* exhibited obviously inhibitory effects on the growth of *S. capricornutum* and toxic strain *M. aeruginosa* compared to the algal controls (Fig. 2).

At the end of the experiment (3 days), the cell densities of *S. capricornutum* decreased respectively by 42.7% and 61.9% in 2.5 and 5 g·L^{-1} FW *P. maackianus* treatments compared to the control ($p<0.05$). The cell densities of *S. capricornutum* also decreased by 65.8% and 73.5% in the two biomass density treatments of *P. malaianus*. By multiple comparison statistics, the cell densities of *S. capricornutum* treated with *P. maackianus* exudates were different than those of *P. malaianus* exudate treatments ($p<0.05$), and there also exist differences between 5 g and 2.5 g biomass densities ($p<0.05$). This result indicated that the sensitivities of *S. capricornutum* were different between two *Potamogeton* spp.

After 3 days' incubation in 2.5 and 5 g FW·L^{-1} *P. maackianus* exudates, the *M. aeruginosa* cell densities were higher in control than in treatment ($p<0.05$). As for *P. malaianus* treatments, the cell densities of *M. aeruginosa* were reduced by 16.5 and 65.8% of the control in 2.5 and 5 g·L^{-1} FW marophytes at the end of the incubation period, respectively. The cell densities of toxic strain *M. aeruginosa* of two macrophyte exudate treatments were also different ($p<0.05$), and the exudates of two biomass densities exert different antialgal activities ($p<0.05$).

The results indicated that these two macrophytes had different inhibitory effects on the growth of *S. capricornutum,* and the toxic strain *M. aeruginosa*. *P. malaianus* had a stronger inhibitory activity on *S. capricornutum* growth than *P. maackianus*, while the growth of *M. aeruginosa* was affected more significant by *P. maackianus* than that by *P. malaianus*.

Fractionation of Macrophytes Exudates

CH_2Cl_2 extracts (A and a), hexane extracts (C and c) and MeCOOEt extracts (D and d) of two pondweeds showed obvious growth inhibitory activities on both *S. capricornutum* and *M. aeruginosa*. Fractions B and D have not shown any antialgal activities but a little stimulation effects on *S. capricornutum* and *M. aeruginosa*. Both fractions b and d stimulated the growth of *S. capricornutum* while inhibiting the growth of *M. aeruginosa*. This indicated that lipophilic substances and moderately lipophilic fractions in exudates from both pondweeds have high allelopathic activities.

From Fig. 3, SPE of CH_2Cl_2 elution of *P. maackianus* exhibited higher inhibitory activity on the growth of *M. aeruginosa* than that of *S. capricornutum*. On the contrary, SPE of CH_2Cl_2 elution of *P. malaianus* showed higher antialgal activity on the growth of *S. capricornutum*.

Both CH_2Cl_2 extracts (A and a) of two pondweeds possessed high allelopathic activities, the main compounds in

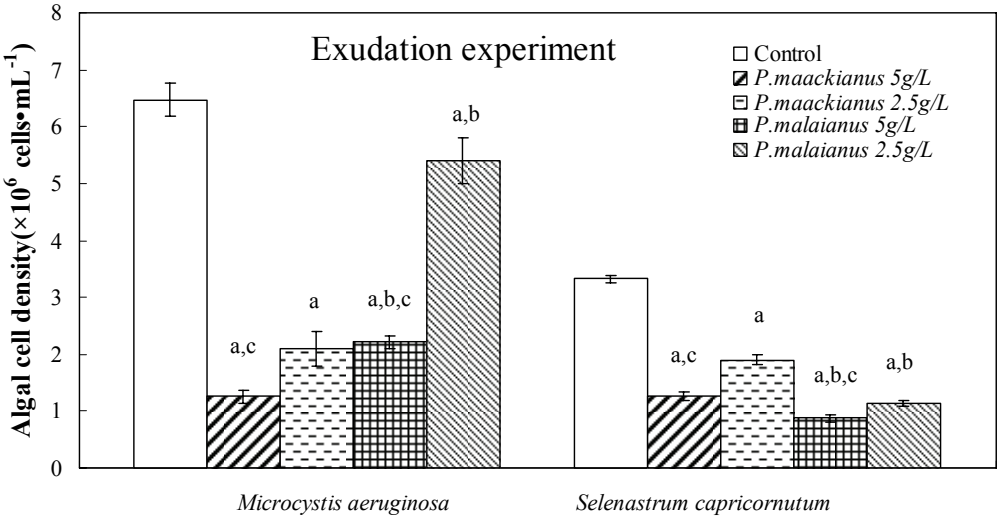

Fig. 2. Cell densities of *Microcystis aeruginosa* and *Selenastrum capricornutum* in coexistence with exudates from *Potamogeton maackianus* and *P. malaianus* after 3 days (bar=SD, n=6)
[a]$p<0.05$, compared with the control,
[b]$p<0.05$, compared with *P. maackianus* treatment,
[c]$p<0.05$, compare with 2.5g/L macrophytes.

CH_2Cl_2 extracts (A and a), which were analyzed by GC-MS, are listed in Table 1. The results indicate that many compounds exist both in exudates from *P. malaianus* and in exudates from *P. maackianus*. Some compounds, such as dibutyl phthalate, exist in the environment because of determination in pure water control. Three alcohols were determined in exudates from *P. malaianus* (A), with the most abundant being 2-Propanol, and 1-methoxy-2-methyl-. Its ion-area in spectrum of GC-MS attained 7.63%, while there was no determination in control water and exudates from *P. maackianus*.

Discussion

Our experiments showed that exudates from two *Potamogeton* species, *P. maackianus* and *P. malaianus*, reduced the growth of *M. aeruginosa* and *S. capricornutum*. The allelochemicals released by both pondweeds into surrounding water are lipophilic and moderately lipophilic compounds. The different sensitivities of *M. aeruginosa* and *S. capricornutum* to exudates from the two *Potamogeton* species were also confirmed in this paper, and the most active lipophilic compounds released by pondweeds were analyzed using GC-MS to elucidate the different antialgal activities of exudates from *P. maackianus* and *P. malaianus*.

Growth inhibition of the cyanobacterium Microcystis caused by exudates from *Potamogeton* species was already reported by Zhang et al. [20], and the antialgal activities of the aqueous extracts of *P. maackianus* and *P. malaianus* were also studied [21]; however, the differences in the sensitivities of cyanobacteria and green algae to allelopathic exudates from *Potamogeton* species and the allelochemicals released by *Potamogeton* species had not been described before. Our results expand so far the studies on comparison study of allelopathy of *Potamogeton* species toward cyanobacteria and green algae.

There are many reports on macrophyte allelopathy. As for *Potamogeton* species, there are some different allelopathic activities against phytoplankton between macrophytes belonging to Potamogetonaceae. It was reported that *P. malaianus* [22] and *P. maackianus* [20] could inhibited algal growth, while there were also some negative conclusions about *P. crispus* and *P. pectinatus* [11]. For *Potamogeton oxyphyllus*, the growth of *M. aeruginosa* was inhibited significantly, while the growth inhibition of *Aphanizomenon flos-aquae* was not observed [11]. These results could not be used to rank the pondweeds according to their allelopathic activities because the culture system and conditions were not comparable. In this paper, both of the phytoplankton were incubated in modified MIII culture solutions before experiments, and the exudation experi-

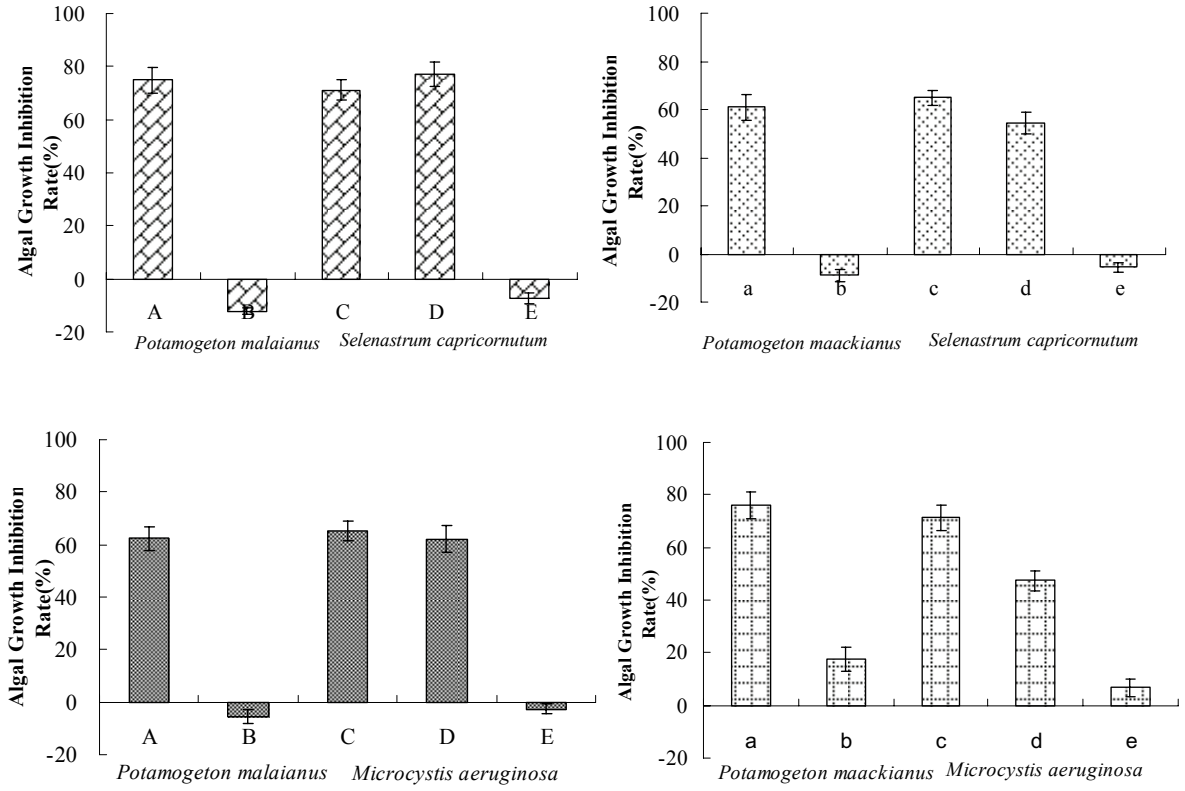

Fig. 3. Algal growth inhibition rates of the fractions extracted from the exudation of *P. maackianus* and *P. malaianus* (bar=SD, for *S. capricornutum* n=30, for *M. aeruginosa* n=6.

A, B – SPE of CH_2Cl_2 and MeOH extracts of *P. malaianus*

a, b – SPE of CH_2Cl_2 and MeOH extracts of *P. maackianus*

C, D, E – LLE of hexane extracts, MeCOOEt extracts and n-butanol extracts of *P. malaianus*

c, d, e – LLE of hexane extracts, MeCOOEt extracts and n-butanol extracts of *P. maackianus*

Table 1. The main compounds contained in the most active CH_2Cl_2 extracts of exudates from *Potamogeton maackianus* and *P. malaianus* using the GC-MS method.

Retention time	MS Identification	Culture solution Control (Area %)	*P. maackianus* (Area %)	*P. malaianus* (Area %)
10.0062	Benzothiazole	/	4.370	6.936
12.8280	Tetradecane	0.631	0.759	0.582
14.6723	Butylated Hydroxytoluene	0.469	0.483	0.424
14.7945	2,5,8,11-Tetraoxatetradecan-13-ol, 4,7,10-trimethyl-	/	4.549	5.381
14.8722	2-Propanol, 1-[2-(2-methoxy-1-methylethoxy)-1-methylethoxy]-	/	8.655	10.761
14.9499	2-Propanol, 1-methoxy-2-methyl-	/	/	7.626
15.0054	2-Hexanol, 2-methyl-	/	/	1.613
15.1054	4-Heptanol, 4-ethyl-2,6-dimethyl-	/	/	2.654
15.1833	2-Butenedioic acid, 2-methoxy-, dimethyl ester	/	1.360	5.080
15.8721	Hexadecane	0.741	5.624	4.073
16.0610	Benzothiazole, 2-(methylthio)-	/	0.613	0.560
16.1276	Cedrol	/	0.602	0.729
17.4386	Heneicosane	0.216	1.043	0.603
17.6941	2,6-Diisopropylnaphthalene	/	0.657	0.565
18.0163	Heptacosane	1.546	1.769	1.428
18.5273	1-Nonadecene	/	1.558	0.996
18.6050	Octadecane	2.310	7.690	5.300
19.5605	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	/	2.867	2.929
20.7270	Dibutyl phthalate	1.579	1.692	1.252
21.0270	1-Octadecene	1.192	1.320	0.576
21.0936	Eicosane	2.467	6.472	3.498
23.3711	Docosane	3.420	4.798	2.650
23.9600	Benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester	/	5.408	3.866
25.4709	Tetracosane	1.182	2.569	1.594
27.4039	Eicosane, 9-octyl-	/	/	1.868
32.8921	(+-)-5-(1-Acetoxy-1-methylethyl)-2-methyl-2-cyclohexen-1-one semicarbazone	/	0.632	1.050

ments were conducted under the same conditions. So the allelopathic activites of two pondweeds could be compared.

The allelopathy of macrophytes presents species-specific effects on the algae. It was found that Cyanophyta was more sensitive to phenolic extracts of *M. spicatum* than Chlorophyta [23]. Körner and Nicklisch [8] found that members of the Oscillatoriales and *M. aeruginosa* were more sensitive to the allelopathy of *M. spicatum* than the cyanobacterium *A. flosaquae*, the diatom *Stephanodiscus minutulus*, and the green alga *Scenedesmus armatus*. Mulderij et al. [9] investigated allelopathic effects of a mixture of *Chara globularis* var. *globularis* and *C. contraria* var. *contraria* on three different green algae. The results indicated allelopathic effects of *Chara* on the growth of the green algae *S. capricornutum* and *Chlorella minutissima*, whereas *Scenedesmus obliquus* seemed not affected. Even within cyanobacteria, the toxic strain was more sensitive to Stratiote exudates than the non-toxic one [24]. So to some degree, the sensitivities of phytoplankton to allelochemicals released by macrophytes are different. Some research results show that cyanobacteria are more sensitive than green algae [10, 12]. In this study, the hypothesis was confirmed that *Potamogeton* species could show different antialgal activities by exudating different compounds, the allelochemicals of *P. maackianus* were more toxic to

M. aeruginosa than those of *P. malaianus*, and the green alga *S. capricornutum* was more sensitive to compounds released by *P. malaianus* than those of *P. maackianus*.

The question of whether the potential allelochemicals are released by the macrophytes is often ignored [25]. Only a few macrophytes have been reported to release active compounds into surrounding water, e.g. *M. spicatum*, *Najas marina*, *C. demersum*, and *S. aloides* [7, 8, 24, 26, 27]. For almost all *Potamogeton* species, the released active compounds have not yet been confirmed and analyzed. Gross et al. [27] demonstrated that both *C. demersum* and *N. marina* intermedia exuded allelopathically active compounds into the surrounding medium by SPE analysis of their incubation water. In this paper, the bioassay results of fractions that were enriched and fractionated by LLE and SPE showed that the allelochemicals of *P. maackianus* and *P. malaianus* belonged to the lipophilic and moderately lipophilic compounds. The results were different with those about the allelochemical exudates from *M. spicatum*, in which the main allelochemicals were phenolic compounds [7]. So the results showed that the allelochemicals of these two pondweeds could be exuded into surrounding water to inhibit algal growth, and these allelochemicals could be extracted from the incubation water of *P. malaianus* and *P. maackianus*. However, the dose of a single chemical to show significant inhibitory effect was much higher than those of exudates. So algal growth inhibition might be caused by co-action.

The bioassay results indicated that CH_2Cl_2 elution (A and a) of both pondweeds had high allelopathic activities, so the allelochemicals inhibiting algae belonged to the lipophilic substances and lipophilic fractions. The GC-MS analysis of CH_2Cl_2 elution showed that there were three alcohols determined only in exudates from *P. malaianus*, although there were many compounds existing in both exudates from two pondweeds. So the different sensitivities between *M. aeruginosa* and *S. capricornutum* were probably caused by these three alcohol compounds. The ranking of algal sensitivities to macrophytes would differ with the macrophytes because of the different exudated allelochemicals.

Our results demonstrated that allelochemicals exudated from *P. maackianus* and *P. malaianus* could inhibit the growth of *M. aeruginosa* and *S. capricornutum*, and the sensitivities of the phytoplankton differed with the species of macrophytes because of the different released active compounds. Further studies are needed to identify the main active compounds, including in exudations toxic to each alga, and make sure that the difference in sensitivities to two pondweeds between *M. aeruginosa* and *S. capricornutum* are caused by three alcohol compounds.

Acknowledgements

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References

1. SCHEFFER M., HOSPER S.H., MEIJER M.L., MOSS B., JEPPESED E. Alternative equilibria in shallow lakes. Trends in Ecol. and Evolu. **8**, 275, **1993**.
2. LAURIDSEN T.L., BUENK I. Diel changes in the horizontal distribution of zooplankton in the littoral zone of two shallow eutrophic lakes. Archiv. fur Hydrobiol. **137**, 161, **1996**.
3. VAN DONK E., VAN DE BUND W.J. Impact of submerged macrophytes including charophytes on phyto- and zooplankton communities: allelopathy versus other mechanisms. Aqua. Bot. **72**, 261, **2002**.
4. GROSS E.M. Allelopathy of aquatic autotrophs. Crit. Rev. Plant Sci. **22**, 313, **2003**.
5. ERHARD D., GROSS E.M. Allelopathic activity of *Elodea canadensis* and *Elodea nuttallii* against epiphytes and phytoplankton. Aqua. Bot. **85**, 203, **2006**.
6. MULDERIJ G. Chemical warfare in freshwater – Allelopathic effetcs of macrophytes on phytoplankton. Ph.D. thesis, Netherlands Institute of Ecology, The Netherlands, **2006**.
7. GROSS E.M., MEYER H., SCHILLING G. Release and ecological impact of algicidal hydrolysable polyphenols in *Myriophyllum spicatum*. Phytochemistry **41**, (1), 133, **1996**.
8. KÖRNER S., NICKLISH A. Allelopathic growth inhibition of selected phytoplankton species by submerged macrophytes. J. Phycol. **38**, 862, **2002**.
9. MULDERIJ G., VAN DONK E., ROELOFS J.G.M. Differential sensitivity of green algae to allelopathic substances from *Chara*. Hydrobiol. **491**, 261, **2003**.
10. JASSER I. The influence of macrophytes on a phytoplankton community in experimental conditions. Hydrobiol. **306**, 21, **1995**.
11. NAKAI S., INOUE Y., HOSOMI M., MURAKAMI A. Growth inhibition of blue-green algae by allelopathic effects of macrophytes. Wat. Sci. Tech. **39**, 47, **1999**.
12. MULDERIJ G., MAU B., VAN DONK E., GROSS E.M. Allelopathic activity of *Stratiotes aloides* on phytoplankton - towards identification of allelopathic substances. Hydrobiol. **584**, 89, **2007**.
13. HILT S., GROSS E.M. Can allelopathically active submerged macrophytes stabilize clear-water states in shallow lakes? Basic. Appl. Ecol. **9**, 422, **2008**.
14. DELLAGRECA M., FIORENTINO A., ISIDORI M., MONACO P., TEMUSSI F., ZAREELLI A. Antialgal furano-diterpenes from *Potamogeton natans* L. Phytochemistry **58**, 299, **2001**.
15. WARIDEL P., WOLFENDER J.L., LACHAVANNE J.B., HOSTETTMANN K. ent-Labdane glycosides from the aquatic plant *Potamogeton lucens* and analytical evaluation of the lipophilic extract constituents of various *Potamogeton* species. Phytochemistry **65**, 945, **2004**.
16. WARIDEL P., WOLFENDER J.L., LACHAVANNE J.B., HOSTETTMANN K. ent-Labdane diterpenes from the aquatic plant *Potamogeton pectinatus*. Phytochemistry **64**, 1309, **2003**.

17. Environment Canada. Biological Test Method: Growth Inhibition Test Using the Freshwater Alga *Selenastrum capricornutum* Environmental Protection Series 1/RM/25, **1992**.
18. ISO 8692 – Water quality – Fresh water algal growth inhibition test with *Scenedesmus subspicatus* and *Selenastrum capricornutum*.
19. XIAN Q., CHEN H., LIU H., ZOU H., YIN D. Isolation and identification of antialgal compounds from the leaves of *Vallisneria spiralis* L. by activity-guided fractionation. Environ. Sci. Pollut. Res. **13**, 233, **2006**.
20. ZHANG S.H., CHENG S.P., WANG H.Q., HE F., WU Z.B. Allelopathic interactions between the *Potamogeton* spp and toxic cyanobacteria (*Microcystis aeruginosa*). Allelopathy J. **23**, 379, **2009**.
21. WANG H.Q., CHENG S.P., ZHANG S.H., HE F., LIANG W., ZHANG L.P., HU C.Y., GE F.J., WU Z.B. Chemical composition in aqueous extracts of *Potamogeton malaianus* and *Potamogeton maackianus* and their allelopathic effects on *Microcystis aeruginosa*. Pol. J. Environ. Stud. **19**, (1), 213, **2010**.
22. WU Z.B., DENG P., WU X.H., LUO S., GAO Y.N. Allelopathic effects of the submerged macrophyte *Potamogeton malaianus* on *Scenedesmus obliquus*. Hydrobiol. **592**, (1), 465, **2007**.
23. PLANAS D., SARHAN F., DUBE L., GODMAIRE H., CADIEUX C. Ecological significance of phenolic compounds of *Myriophyllum spicatum*. Verh. Internat. Verein. Limnol. **21**, 1492, **1981**.
24. MULDERIJ G., MOOIJ W.M., SMOLDERS A.J.P., VON DONK E. Allelopathic inhibition of phytoplankton by exudates from *Stratiotes aloides*. Aqua. Bot. **82**, 284, **2005**.
25. WILLIS R.J. The historical bases of the concept of allelopathy. J. History Biol. **18**, 71, **1985**.
26. NAKAI S., INOUE Y., HOSOMI M., MURAKAMI A. *Myriophyllum spicatum* - released allelopathic polyphenols inhibiting growth of blue-green algae *Microcystis aeruginosa*. Water Res. **34**, 3026, **2000**.
27. GROSS E.M., ERHARD D., IV´ANYI E. Allelopathic activity of *Ceratophyllum demersum* L. and *Najas marina* ssp. intermedia (Wolfgang) Casper. Hydrobiol. **506-509**, 583, **2003**.