Acta Scientiarum
http://www.periodicos.uem.br/ojs/
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v43i1.57734

Growth of *Microcystis* strains isolated from environments with the presence and absence of submerged macrophytes in coexistence with *Ceratophyllum demersum*

Rafael Henrique de Moura-Falcão[1], Ariadne do Nascimento Moura[2] and Cihelio Alves Amorim[2,3*]

[1]Programa de Pós-Graduação em Oceanografia, Departamento de Oceanografia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil. [2]Programa de Pós-Graduação em Biodiversidade, Departamento de Biologia, Universidade Federal Rural de Pernambuco, Rua Dom Manuel de Medeiros, 52171-900, Recife, Pernambuco, Brazil. [3]Department of Biological Sciences, Middle East Technical University, Üniversiteler Mahallesi, Dumlupınar Bulvarı, Ankara, Turkey. *Author for correspondence. E-mail: alvescihelio@gmail.com

ABSTRACT. Cyanobacterial blooms can cause severe ecological and health problems in drinking water reservoirs. To alleviate this problem, allelopathically active submerged macrophytes can be used to reduce cyanobacterial growth. Accordingly, this study aimed to evaluate the sensitivity of strains of the *Microcystis aeruginosa* complex isolated from reservoirs with the presence and absence of submerged macrophytes to the allelochemicals of *Ceratophyllum demersum*. A coexistence experiment was carried out between the submerged macrophyte *C. demersum* and four *Microcystis* strains, with two treatments for each strain, one in coexistence with the submerged macrophyte (7 g L^{-1}) and control (in the absence of the macrophyte). Two strains of *M. aeruginosa* (BMIUFRPE-06 and BMIUFRPE-07) and two of *M. panniformis* (BMIUFRPE-08 and BMIUFRPE-09) were used, which were isolated from Cajueiro (with submerged macrophytes) and Tapacurá (without submerged macrophytes) reservoirs, respectively. The biomass of *Microcystis* strains from the reservoir without macrophytes (BMIUFRPE-08 and BMIUFRPE-09) was significantly inhibited in 96% (T-test: $p < 0.01$) and 74% (T-test: $p < 0.05$), when compared to the control, respectively, with lower values of growth rates (ANOVA: $p < 0.05$). The strains isolated from the reservoir with macrophytes (BMIUFRPE-06 and BMIUFRPE-07) showed a growth delay (biomass reductions of 44 and 58%, respectively) in the coexistence treatment but without significant difference from the control on the sixth day of the experiment (biomass - T-test: $p > 0.05$; growth rate - ANOVA: $p > 0.05$). These results suggest that strains isolated from environments with submerged macrophytes are less sensitive to allelochemicals of these plants, as these strains may be adapted to the coexistence with submerged macrophytes.

Keywords: adaptation; allelopathy; aquatic plants; cyanobacterial blooms; *Microcystis aeruginosa* complex.

Received on February 8, 2021.
Accepted on July 27, 2021.

Introduction

Many lakes and reservoirs may exhibit two main alternative states, one with clear water and high biomass of aquatic macrophytes, and one with turbid waters, distinguished by the high phytoplankton biomass and reduced macrophyte coverage (Scheffer, Carpenter, Foley, Folke, & Walker, 2001). In tropical waters, cyanobacterial blooms are frequent, usually due to warm climates and artificial eutrophication, which can alter the trophic interactions in the aquatic systems (Bittencourt-Oliveira, Piccin-Santos, Moura, & Aragão-Tavares, 2014; Moura, Aragão-Tavares, & Amorim, 2018; Amorim, Dantas, & Moura, 2020).

In recent years, the increase in temperature and eutrophication of water bodies has led to an increase in cyanobacterial blooms (Paerl & Otten, 2013; Burford et al., 2020), mainly those composed of the *Microcystis* genus, with records in 108 countries (all continents, except Antarctica) (Harke et al., 2016). These blooms negatively affect public supply reservoirs through the release of cyanotoxins, which compromise water quality, and reduce recreational and fishing activities (Davis & Koop, 2006). Among toxins, microcystins are the most studied and frequent in aquatic environments, which have been reported in 79 countries (Harke et al., 2016). Furthermore, there are more than 240 variants of microcystins, with different levels of toxicity (Svirčev et al., 2017), which can cause damage to aquatic macrophytes (Amorim, Ulisses, & Moura, 2017), zooplankton (Liang, Ouyang, Chen, Su, & Yang, 2017), fish (Boaru, Dragos, & Schirmer, 2006), and even humans (Carmichael et al., 2001).

As a control alternative, the introduction of submerged aquatic macrophytes has generated great results in reducing blooms in temperate and tropical regions (e.g., Dong, Yang, Li, Li, & Song, 2014; Amorim & Moura, 2020). Submerged macrophytes can act as a refuge for zooplankton, as they compete for light and nutrients and exudate allelochemicals; moreover, such mechanisms are the main action modes of these plants (Gross, 2003). Among the macrophytes, *C. demersum* L. is one of the most allelopathically active, which can release a greater amount of allelochemicals and strongly inhibit phytoplankton growth (Hilt & Gross, 2008).

Several studies have demonstrated the inhibition of cyanobacteria by the allelochemicals of aquatic macrophytes. For example, Mohamed (2017) reviewed studies showing that more than 40 aquatic macrophytes can inhibit phytoplankton through allelopathy. Most of these studies were performed with *Microcystis* spp. (e.g., Chang, Eigemann, & Hilt, 2012; Švanys, Eigemann, Grossart, & Hilt, 2016; Amorim, Moura-Falcão, Valença, Souza, & Moura, 2019). However, this genus has a wide variability in response to macrophyte allelochemicals; for example, non-microcystin-producing strains are more sensitive to the chemical compounds of these plants (Švanys et al., 2016).

Algal populations can present a wide genetic variability, which allows their adaptation to diverse environmental conditions, such as resistance to pollutants (Behra, Genoni, & Joseph, 1999) or zooplankton predation (Vanormelingen, Vyverman, De Bock, Van der Gucht, & De Meester, 2009). For *Microcystis aeruginosa* (Kützing) Kützing, some studies showed that the adaptation to different environmental pressures can be related to strain genetic variability, however, this has only been tested for microcystin producing or non-producing strains. For example, non-toxic strains are better competitors for light (Kardinäal et al., 2007), while toxic strains are favored under conditions of high temperatures and nutrients (Davis, Berry, Boyer, & Gobler, 2009).

Another factor that can influence the growth performance of organisms is their place of origin, as described by the ‘new weapons hypothesis’, which predicts that non-native species are efficient in controlling populations of native species when introduced into new environments (Callaway & Ridenour, 2004). However, over time, native species can adapt to coexist with exotic species as they become established in new locations (Reigosa, Sanchez-Moreiras, & Gonzalez, 1999). Accordingly, some studies have addressed the tolerance of cyanobacteria (Hilt, 2006) and planktonic green algae, *Scenedesmus obliquus* (Turpin) Kützing (Al-Shehri, 2010), to allelochemicals of submerged macrophytes over time.

Biomanipulation studies using submerged aquatic macrophytes have become frequent and have shown good results in cyanobacterial bloom control (e.g., Liu et al., 2018; Amorim & Moura, 2020). However, the previous exposure of cyanobacteria to these plants in their natural environment should be considered, as they can become adapted to the allelochemicals. Accordingly, we evaluated the sensitivity of four strains of the *M. aeruginosa* complex isolated from reservoirs with the presence and absence of submerged macrophytes to the *C. demersum* L. allelochemicals. We tested the hypothesis that strains isolated from environments without submerged plants are more sensitive to allelochemicals than those with a history of macrophyte coexistence.

Material and methods

Isolation of *Microcystis* strains and culture conditions

In the experiments, four strains of *Microcystis* were used: two *M. aeruginosa* (BMIUFRPE-06 and BMIUFRPE-07) and two *M. panniformis* Komárek et al. (BMIUFRPE-08 and BMIUFRPE-09). The strains were isolated from field samples and identified based on morphological characteristics (Komárek & Anagnostidis, 1986; Nguyen, Tanabe, Matsuura, Kaya, & Watanabe, 2012). Both species are included in the *M. aeruginosa* complex (MAC) due to their high genetic similarity (Harke et al., 2016). Although they presented different morphologies in the field, all studied strains presented the same unicellular form, with few small colonies under laboratory conditions.

The strains of *M. panniformis* were isolated from the Tapacurá reservoir, located at the municipality of São Lourenço da Mata - PE, in October 2017, and characterized by the absence of submerged macrophytes (Moura Júnior, Pott, Severi, & Zickel, 2019). The strains of *M. aeruginosa* were isolated from the Cajueiro reservoir, in the municipality of Garanhuns - PE, which has extensive stands of the submerged macrophytes *Egeria densa* Planch., *Chara* spp. and *C. demersum* (based on field observations during sampling dates), in November 2017.

Other studies have shown that these macrophytes have the potential to inhibit *M. aeruginosa* through allelopathy (e.g., Van Donk & Van de Bund, 2002; Vanderstukken, Mazzeo, Van Colen, Declerck, & Muylaert, 2011; Dong et al., 2014). Both reservoirs have similar limnological and climatic characteristics (climate type As in the Köppen classification) and annual *Microcystis* blooms (Amorim et al., 2020). Details of the limnological conditions during the sampling dates are described in Table 1 (see Amorim & Moura, 2021).

The strains were isolated using the micro-pipetting technique and kept under acclimation under the respective laboratory conditions: temperature (25°C ± 1.5), light intensity (30 µmol m-2 s-1), pH (7.5), photoperiod (12 hours), they were also homogenized three times a day. The strains were deposited at the Microalgae Culture Collection of the *Universidade Federal Rural de Pernambuco* - BMIUFRPE. Cultures of isolated cyanobacteria were maintained in flasks containing ASM1 nutrient medium (Gorham, Mclachlan, Hammer, & Kim, 1964), in a climatic chamber under controlled conditions.

Sampling and culture of the macrophyte *Ceratophyllum demersum*

Thirty days before the experiments, young and apical branches of *C. demersum* were collected in the Carpina reservoir, in the municipality of Lagoa do Carro - PE (Table 1) and transported to the laboratory. After sampling, the plants were washed several times, with a soft bristle brush and jets of distilled water to remove sediments and periphyton. The cultures were carried out in aquariums containing tap water (which remained resting for five days for chloride removal), with dimensions of 20 cm^3 and capacity of 8 L, under the same above-mentioned conditions, and with constant aeration.

Table 1. Geographical location, climate, technical characteristics, and main environmental variables in the Tapacurá, Cajueiro, and Carpina reservoirs during the sampling of phytoplankton, for the isolation of *Microcystis* strains, and the submerged macrophyte *Ceratophyllum demersum*.

	Tapacurá (October 2017)	Cajueiro (November 2017)	Carpina (August 2018)
Latitude [b]	8° 02′ 39.4” S	8° 58′ 09.2” S	7° 53′ 43.3” S
Longitude [b]	35° 11′ 52.0” W	36° 28′ 48.7” W	35° 20′ 26.2” W
Climate *sensu* Köppen [b]	As	As	As
Year of creation [a]	1978	2010	1992
Storage capacity (m^3) [a]	104.870.609	19.283.196	270.000.000
Depth (m) [c]	11.8	7.0	12.1
Water transparency (m) [b]	0.9	1.0	2.1
Light intensity (µmol m^{-2} s^{-1}) [b]	440.8	488.7	513.9
Water temperature (°C) [b]	28.4	28.1	25.8
pH [b]	8.2	8.9	8.3
Electrical conductivity (µS cm^{-1}) [b]	406.0	610.0	2723.0
Nitrite (µg L^{-1}) [b]	1.7	16.2	11.3
Nitrate (µg L^{-1}) [b]	0.5	150.9	138.3
Ammonium (µg L^{-1}) [b]	27.9	18.6	335.4
Dissolved inorganic nitrogen (µg L^{-1}) [b]	30.1	185.6	485.0
Orthophosphate (µg L^{-1}) [b]	481.4	10.0	257.2
Total dissolved phosphorus (µg L^{-1}) [b]	542.8	33.7	405.0
Total phosphorus (µg L^{-1}) [b]	834.3	75.6	562.3
Chlorophyll *a* (µg L^{-1}) [b]	21.4	6.5	3.7
Trophic state [b]	Hypereutrophic	Eutrophic	Hypereutrophic

Source: [a] *Agência Pernambucana de Águas e Clima* [APAC] (2020); [b] Amorim et al. (2020) and Amorim & Moura (2021).

Experimental design

The experiments were carried out in an air-conditioned room with the same conditions described for the culture of the strains. Erlenmeyer flasks with a capacity for 1000 mL containing 500 mL of ASM1 culture medium were inoculated with the cyanobacterial strains in the exponential growth phase from the stock cultures (mean biomass of 23.5 mg L^{-1}).

Three days before starting the experiments, the plants were washed several times with distilled water, after that, they were transferred to Erlenmeyer flasks with ASM1 culture medium for acclimatization. Two treatments (control and coexistence with macrophyte) were established for each *Microcystis* strain, with four replicates for each condition. The control treatment consisted of a culture of strains in the absence of the plant, while in the coexistence treatment a branch of macrophyte (7 g L-1 of fresh weight) was added to the cyanobacterial cultures. This amount of plant was chosen because biomasses of *C.*

demersum higher than 5 g L-1 present greater allelopathic effects on cyanobacteria (Gross, Erhard, & Iványi, 2003).

Negative controls, using plastic plants, were not employed because some studies have shown that macrophytes cause no physical interference on phytoplankton and cyanobacteria (e.g., Vanderstukken et al. 2011, Vanderstukken, Declerck, Decaestecker, & Muylaert, 2014). The higher nutrient concentration in the ASM1 media excluded the possibility of nutrient limitation, while the regular homogenization of the cultures provided similar light availability to all *Microcystis* cells, preventing light limitation.

Growth of *Microcystis* and *Ceratophyllum demersum*

Aliquots of 1 mL of all experimental units were collected on days 0, 2, 4, and 6 to determine cyanobacterial cell densities, by counting the cells in a Fuchs-Rosenthal chamber (hemocytometer). The biovolume was determined according to the calculation of the geometric forms proposed by Sun and Liu (2003). The biomass of the strains was estimated by multiplying the density by the biovolume. The growth rates (μ) of the cyanobacterial strains were calculated according to Wood, Everroad, & Wingard (2005): μ (d-1) = (ln(Nt) - ln(Nt0))/t - t0, where N represents the biomass values after six days of the experiment (t) and at the initial day (t0).

The growth of branches of *C. demersum* in coexistence with *Microcystis* was analyzed. For that, branches were weighed on an analytical balance, at the beginning (day 0) and the end (day 6) of the experiment. Both were evaluated based on the fresh weight of the macrophyte.

Statistical analyses

Significant differences between the treatments for each strain during each sampling day, and in the growth of *C. demersum* (coexistence treatments) were verified through the Student's T-test (normal data) and the Mann-Whitney U test (non-normal data). A two-way ANOVA was performed to compare the growth rates (between treatments and strains) after six days of the experiment. Before the analyses of variances, data normality and homoscedasticity were verified by the Kolmogorov-Smirnov and Bartlett's tests, respectively. Statistical analyses were performed in the R program and the level of significance was adjusted to $p < 0.05$ (R Core Team, 2019).

Results

Effects of *Ceratophyllum demersum* on the growth of *Microcystis* strains

The aquatic macrophyte *C. demersum* affected the growth of *Microcystis* strains differently and depending on the origin and history of the strains. Strains isolated from the reservoir with submerged macrophytes (BMIUFRPE-06 and BMIUFRPE-07) showed a decrease in growth, beginning on the fourth day in the coexistence treatment compared to control. On the 6th day, BMIUFRPE-06 and BMIUFRPE-07 decreased biomass by 44 and 58%, respectively; however, without significant differences between the treatment with macrophytes and the control (Figure 1 A and B). In contrast, the growth of strains isolated from the reservoir without submerged macrophytes (BMIUFRPE-08 and BMIUFRPE-09) was significantly inhibited in the coexistence treatment from the fourth day until the end of the experiment, when the strains biomass was reduced by 96 and 74%, respectively (Figure 1 C and D). Both strains isolated from the reservoir without submerged plants reached lower biomasses on the sixth day than at the beginning of the experiment (Figure 1 C and D).

The strains isolated from the reservoir with macrophytes showed no differences in growth rates between the control and coexistence treatments. However, in the coexistence treatment, the BMIUFRPE-06 strain showed a lower growth rate (0.14 d^{-1}) compared to the BMIUFRPE-07 strain (0.40 d^{-1}). On the other hand, the strains isolated from the reservoir without macrophytes presented significant reductions in the growth rates when in coexistence with *C. demersum*. Lower values of growth rates in the coexistence treatment were observed for the strain BMIUFRPE-08 (-0.05 d^{-1}), followed by BMIUFRPE-09 (0.01 d^{-1}). These two strains differed in growth rates in control conditions, but not in the coexistence treatment due to the intense inhibition of growth (Figure 2).

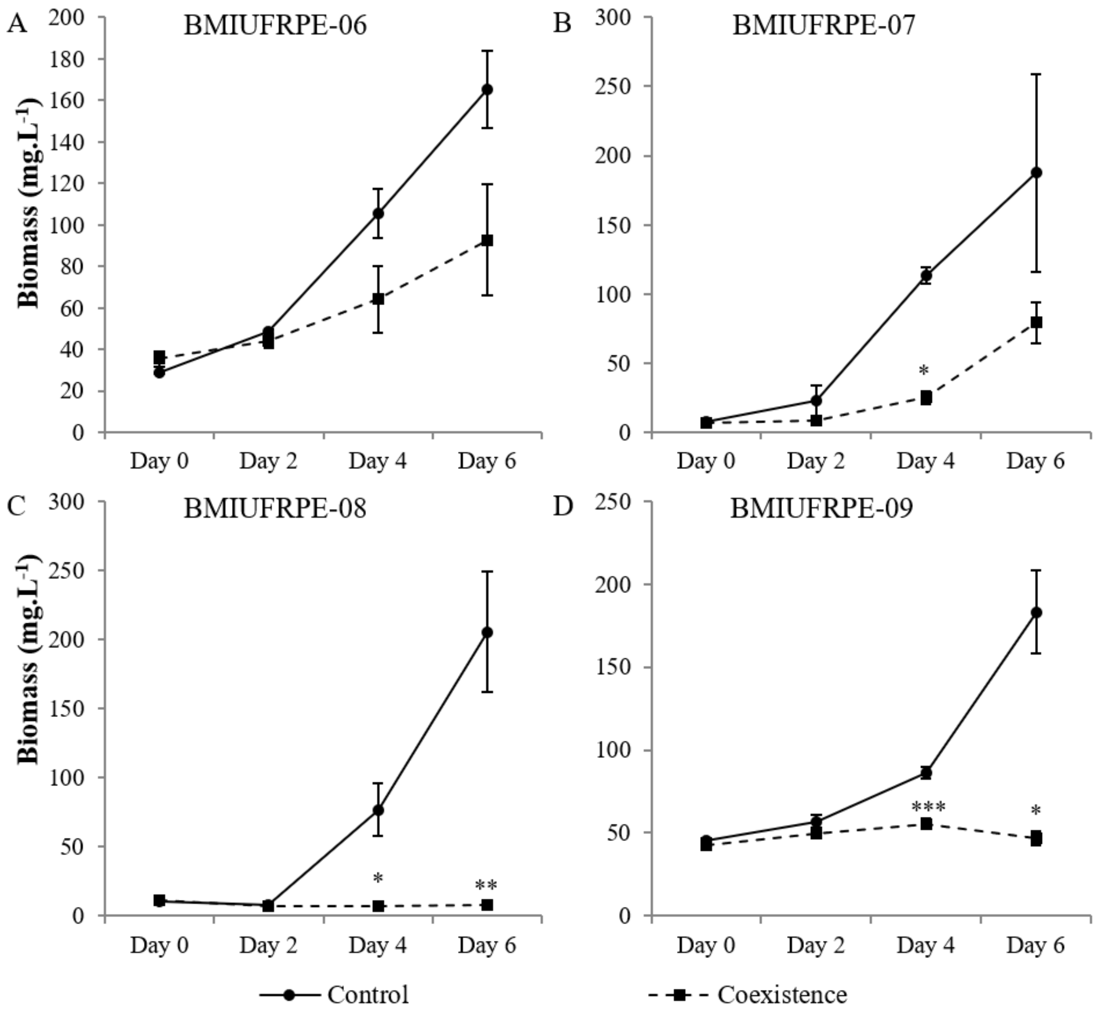

Figure 1. Effect of the aquatic macrophyte *Ceratophyllum demersum* on the growth of *Microcystis aeruginosa* strains (BMIUFRPE-06 and BMIUFRPE-07), from environments with submerged macrophytes (Cajueiro), and *Microcystis panniformis* strains (BMIUFRPE-08 and BMIUFRPE-09), from environments without submerged plants (Tapacurá). Significant differences between the coexistence and control treatments for each day are represented by asterisks (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$). The bars represent the standard deviation.

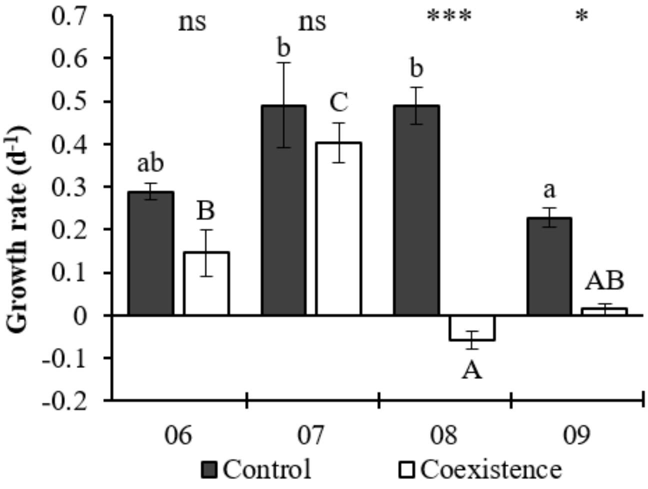

Figure 2. Effect of the aquatic macrophyte *Ceratophyllum demersum* on the growth rates of *Microcystis aeruginosa* strains (BMIUFRPE-06 and BMIUFRPE-07), from environments with submerged macrophytes (Cajueiro), and *Microcystis panniformis* strains (BMIUFRPE-08 and BMIUFRPE-09), from environments without submerged plants (Tapacurá). Asterisks indicate significant differences for each strain between control and coexistence treatments (*: $p < 0.05$; ***: $p < 0.001$). Significant differences between strains in the control and coexistence treatments are represented by different lowercase and uppercase letters, respectively.

Growth of *Ceratophyllum demersum* after coexistence with *Microcystis* strains

Based on the fresh weight of the branches of the aquatic macrophyte in the initial and final days of the experiment, we verified that *C. demersum* increased its biomass in coexistence with all *Microcystis* strains ($p < 0.05$), compared to the first day of the experiment (Figure 3). The branches showed similar biomasses in coexistence with all strains, varying from 8.2 to 9.2 g L^{-1}.

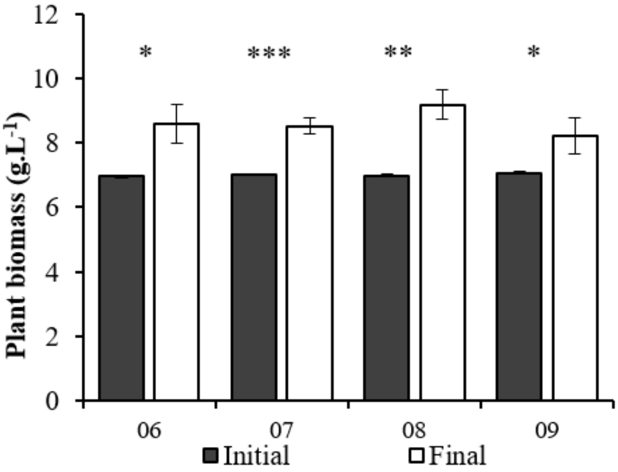

Figure 3. Fresh weight (g L^{-1}) of *Ceratophyllum demersum* in coexistence with *Microcystis aeruginosa* strains (BMIUFRPE-06 and BMIUFRPE-07), from environments with submerged macrophytes (Cajueiro), and *Microcystis panniformis* strains (BMIUFRPE-08 and BMIUFRPE-09), from environments without submerged plants (Tapacurá). Significant differences between the beginning and the end (6th day) of the experiment are represented by asterisks (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

Discussion

In this study, we evaluated the growth of *Microcystis* strains isolated from reservoirs, which differed in the presence or absence of the allelopathically active submerged macrophytes. Thus, we observed that *Microcystis* strains isolated from environments with submerged macrophytes, including *C. demersum*, were less affected by the coexistence with this plant. In contrast, *Microcystis* strains isolated from environments without submerged macrophytes were more sensitive to the allelochemicals and their growth was inhibited at the end of the experiment.

The ‘new weapons hypothesis’ predicts that plants are efficient at controlling populations of native species when introduced into new environments (Callaway & Ridenour, 2004). In this sense, the introduction of aquatic species in a water body promotes ecological changes through the stabilization of new species (Kumar, 2000). Thus, their development affects native communities (Vitousek, D’antonio, Loope, & Westbrooks, 1996). Therefore, the introduction of a submerged macrophyte into an environment without previous records of these plants, for example, the Tapacurá reservoir (Moura Júnior et al., 2019), can inhibit the cyanobacterial strains that are not adapted to the presence of allelochemicals. However, native species can adapt to the presence of these new individuals over time (Reigosa et al., 1999). In this case, cyanobacteria, when in coexistence with submerged macrophytes for long periods, can tolerate the presence of plant allelochemicals and thus not be strongly affected, as observed in the Cajueiro reservoir.

Studies aiming to evaluate the adaptation of phytoplankton strains to allelochemicals of aquatic macrophytes are scarce. Among them, Al-Shehri (2010) showed that a strain of *Scenedesmus obliquus*, isolated from a lake with extensive stands of *Stratiotes aloides* L., was tolerant to macrophyte allelochemicals, while a strain isolated from a lake without *S. aloides* showed reduced growth and higher oxidative stress. However, in another study, the sensitivity of *Pediastrum duplex* Meyen strains to the presence of macrophytes found in their place of origin was not confirmed. The strains isolated from environments with or without *Myriophyllum spicatum* L. presented the same sensitivity to plant allelochemicals (Eigemann, Vanormelingen, & Hilt, 2013). These contrasting results are due to the fact that green algae are less sensitive to macrophyte allelochemicals, when compared to cyanobacteria and diatoms (Körner & Nicklisch, 2002; Hilt, Ghobrial, & Gross, 2006; Hilt & Gross, 2008; Zhu, Liu, Wang, Gao, & Wu, 2010; Chang et al., 2012; Amorim et al., 2019), or because of the variable sensitivity among strains or species (Mulderij, Van Donk, & Roelofs, 2003; Al-Shehri, 2010; Amorim et al., 2019).

In our study, the presence of submerged macrophytes in the reservoirs where *Microcystis* strains were collected played an important role in their sensitivity. This result may indicate that the previous coexistence of *Microcystis* strains with submerged macrophytes during many years may provide resistance to the allelochemicals for cyanobacteria. Moreover, native species can coevolve with other coexisting species when the introduced species stabilize in the new ecosystem (Reigosa et al., 1999). However, little is known about the adaptation of *Microcystis* spp. in the presence or absence of allelopathically active macrophyte species, and whether this characteristic contributes to make it a widespread cyanobacterium in water bodies worldwide, as already reported (Harke et al., 2016).

Our results showed that the presence of allelopathically active submerged macrophytes (e.g., *Egeria densa*, *Chara* spp., and *C. demersum*) could be an important factor in the adaptation and selection of resistant strains of *Microcystis* capable of growing in the presence of the allelochemicals in the Cajueiro reservoir. The construction of this reservoir was finished in 2010 (APAC, 2020), which allowed the coexistence of strains with the submerged macrophytes for more than eight years. Considering that *Microcystis* spp. can rapidly adapt to environmental stressors through the selection of resistant cells, they can maintain their growth even under unfavorable conditions. For example, López-Rodas et al. (2007) demonstrated that sensitive strains of *M. aeruginosa* became resistant to the glyphosate in less than 60 days of exposure. Also, pre-selective mutant cells, that were occasionally found among the sensitive cultures, ensured the adaptation of the strains due to the increased growth of tolerant cells under stress conditions.

The use of submerged macrophytes to control eutrophication and cyanobacterial blooms has become frequent (Yu et al., 2016; Liu et al., 2018; Amorim & Moura, 2020). The main mechanism of action of submerged macrophytes in the inhibition of cyanobacteria is allelopathy (Hilt & Gross, 2008). Besides, submerged macrophytes, especially *C. demersum*, can control cyanobacteria even in environments with high concentrations of phosphorus (Mjelde & Faafeng, 1997), with competition for nutrients playing a lesser role compared to allelopathy (Lombardo & Cooke, 2003). This is because *C. demersum* is one of the submerged macrophytes with the greatest allelopathic potential (Hilt & Gross, 2008). Moreover, in our study, we prevented nutrient and light limitation for cyanobacterial growth through the use of a nutrient-rich culture medium and the regular homogenization of the cultures, which ensure adequate levels of nutrients and light to all *Microcystis* cells, respectively.

The high potential of *C. demersum* in the control of cyanobacteria was also demonstrated in a field experiment in a tropical reservoir in Northeastern Brazil, in which the introduction of this plant was efficient to reduce up to 85% of the total cyanobacterial biomass, in an environment with dense *Microcystis* spp. blooms and without the presence of submerged plants (Tapacurá reservoir) (Amorim & Moura, 2020). This intense reduction in the cyanobacterial bloom was attributed to allelopathy, considering that the cyanobacteria biomass was reduced by the macrophyte even after the addition of nutrients. In this case, the physicochemical parameters remained stable, there was no limitation by light or nutrients, and the zooplankton had a lesser effect on the bloom control (Amorim & Moura, 2020). This result may indicate that the introduction of a macrophyte in an environment without submerged vegetation can reduce the biomass of cyanobacteria, considering that the phytoplankton of this environment was not previously adapted to allelochemicals of this new plant, therefore corroborating the results of the present study. Nevertheless, long-term coexistence between cyanobacteria and submerged macrophytes should be considered in biomanipulation strategies with the use of submerged macrophytes since previous interactions may reduce the sensitivity of cyanobacteria to allelochemicals.

Several studies have demonstrated the adaptation of *Microcystis* spp. to environmental conditions, especially toxic or non-toxic strains, including light intensity, temperature, nutrient concentrations, and the presence of reactive oxygen species (e.g., Davis et al., 2009; Dziallas & Grossart, 2011). However, the adaptation to the macrophytes allelochemicals has not yet been tested for bloom-forming cyanobacterial strains. Therefore, this study indicates for the first time that strains of cyanobacteria from environments with a large abundance of submerged macrophytes may be less sensitive to allelochemicals.

Despite its potential to slow or inhibit the growth of all tested *Microcystis* strains, *C. demersum* was not affected by the coexistence with cyanobacteria, showing a significant increase in its biomass in short-term exposure to all tested strains. Amorim et al. (2017) also demonstrated that the short-term exposure (three weeks) of the submerged macrophyte *Egeria densa* Planch. to toxic and non-toxic strains of *Microcystis* did not cause morphological changes or reduction in the biomass of the plant. This result was related to the efficiency of the *E. densa* antioxidant system, to prevent harmful effects of cyanobacteria. However, prolonged exposures (five weeks), both to toxic and non-toxic strains, caused a reduction in length, fresh weight, and the emission of shoots and roots in *E. densa* (Amorim et al., 2017). *Ceratophyllum demersum* also has an efficient antioxidant system that reduces the harmful effects of cyanobacteria and cyanotoxins on its physiology, in addition to absorbing and transforming cyanotoxins into non-toxic molecules (Pflugmacher, 2004). Therefore, this macrophyte can be used to control cyanobacterial blooms and cyanotoxins. In addition to this macrophyte, the other species present in Cajueiro reservoir, including *E. densa* and *Chara* spp., also exhibit the potential to inhibit cyanobacteria (Mulderij et al., 2003; Vanderstukken et al., 2011).

Conclusion

In conclusion, *Microcystis* strains were inhibited by *C. demersum* differently, i.e., strains from environments without macrophytes were strongly inhibited, while strains from environments with submerged plants were less sensitive and showed a growth delay. Thus, we confirmed the hypothesis that strains of *Microcystis* isolated from reservoirs without submerged macrophytes are more sensitive to *C. demersum* allelochemicals; however, strains from environments with extensive submerged plant stands were less affected when grown in coexistence with the plant, which demonstrates their adaptation to allelochemicals over the years of coexistence in natural habitats.

Acknowledgements

We thank to the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* -CNPq for a productivity grant to ANM (Process 305829/2019-0); and the *Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco* - FACEPE for a scholarship to RHMF.

References

Al-Shehri, A. M. (2010). Differential sensitivities of different *Scenedesmus obliquus* strains to the allelopathic activity of the macrophytes *Stratiotes aloides*. *Journal of Applied Sciences*, *10*(16), 1769-1774. DOI: http://dx.doi.org/10.3923/jas.2010.1769.1774

Amorim, C. A., & Moura, A. N. (2020). Effects of the manipulation of submerged macrophytes, large zooplankton, and nutrients on a cyanobacterial bloom: A mesocosm study in a tropical shallow reservoir. *Environmental Pollution*, *265*(B), 114997. DOI: http://dx.doi.org/10.1016/j.envpol.2020.114997

Amorim, C. A., & Moura, A. N. (2021). Ecological impacts of freshwater algal blooms on water quality, plankton biodiversity, structure, and ecosystem functioning. *Science of the Total Environment*, *758*(1), 143605. DOI: http://dx.doi.org/10.1016/j.scitotenv.2020.143605

Amorim, C. A., Dantas, E. W., & Moura, A.N. (2020). Modeling cyanobacterial blooms in tropical reservoirs: the role of physicochemical variables and trophic interactions. *Science of the Total Environment*, *744*(1), 140659. DOI: http://dx.doi.org/10.1016/j.scitotenv.2020.140659

Amorim, C.A., Moura-Falcão, R. H. D., Valença, C. R., Souza, V. R. D., & Moura, A.N. (2019). Allelopathic effects of the aquatic macrophyte *Ceratophyllum demersum* L. on phytoplankton species: contrasting effects between cyanobacteria and chlorophytes. *Acta Limnologica Brasiliensia*, *31*(1), 1-10. DOI: http://dx.doi.org/10.1590/s2179-975x1419

Amorim, C. A., Ulisses, C., & Moura, A. N. (2017). Biometric and physiological responses of *Egeria densa* Planch. cultivated with toxic and non-toxic strains of *Microcystis*. *Aquatic Toxicology*, *191*(1), 201-208. DOI: http://dx.doi.org/10.1016/j.aquatox.2017.08.012

Agência Pernambucana de Águas e Clima [APAC]. *Dados geográficos, clima, características técnicas e principais variáveis ambientais.* (2020). Retrieved from http://www.apac.pe.gov.br/

Behra, R., Genoni, G. P., & Joseph, A. L. (1999). Effect of atrazine on growth, photosynthesis, and between-strain variability in *Scenedesmus subspicatus* (Chlorophyceae). *Archives of Environmental Contamination and Toxicology*, *37*(1), 36-41. DOI: http://dx.doi.org/10.1007/s002449900487

Bittencourt-Oliveira, M. C., Piccin-Santos, V., Moura, A. N., & Aragão-Tavares, N. K. (2014). Cyanobacteria, microcystins and cylindrospermopsin in public drinking supply reservoirs of Brazil. *Anais da Academia Brasileira de Ciências*, *86*(1), 297-310. DOI: http://dx.doi.org/10.1590/0001-3765201302512.

Boaru, D. A., Dragos, N., & Schirmer, K. (2006). Microcystin-LR induced cellular effects in mammalian and fish primary hepatocyte cultures and cell lines: a comparative study. *Toxicology*, *218*(2), 134-148. DOI: http://dx.doi.org/10.1016/j.tox.2005.10.005

Burford, M. A., Carey, C. C., Hamilton, D. P., Huisman, J., Paerl, H. W., Wood, S. A., & Wulff, A. (2020). Perspective: Advancing the research agenda for improving understanding of cyanobacteria in a future of global change. *Harmful Algae, 91*(1), 101601. DOI: http://dx.doi.org/10.1016/j.hal.2019.04.004

Callaway, R. M., & Ridenour, W. M. (2004). Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment*, *2*(8), 436-443. DOI: http://dx.doi.org/10.1890/1540-9295(2004)002[0436:NWISAT]2.0.CO;2

Carmichael, W. W., Azevedo, S. M., An, J. S., Molica, R. J., Jochimsen, E. M., Lau, S., … Eaglesham, G. K. (2001). Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. *Environmental Health Perspectives*, *109*(7), 663-668. DOI: http://dx.doi.org/10.1289/ehp.01109663

Chang, X., Eigemann, F., & Hilt, S. (2012). Do macrophytes support harmful cyanobacteria? Interactions with a green alga reverse the inhibiting effects of macrophyte allelochemicals on *Microcystis aeruginosa*. *Harmful Algae*, *19*(1), 76-84. DOI: http://dx.doi.org/10.1016/j.hal.2012.06.002

Davis, R., & Koop, K. (2006). Eutrophication in Australian rivers, reservoirs and estuaries – a southern hemisphere perspective on the science and its implications. *Hydrobiologia, 559*(1), 23-76. DOI: http://dx.doi.org/10.1007/s10750-005-4429-2

Davis, T. W., Berry, D. L., Boyer, G. L., & Gobler, C. J. (2009). The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. *Harmful Algae*, *8*(5), 715-725. DOI: http://dx.doi.org/10.1016/j.hal.2009.02.004

Dong, J., Yang, K., Li, S., Li, G., & Song, L. (2014). Submerged vegetation removal promotes shift of dominant phytoplankton functional groups in a eutrophic lake. *Journal of environmental Sciences*, *26*(8), 1699-1707. DOI: http://dx.doi.org/10.1016/j.jes.2014.06.010

Dziallas, C., & Grossart, H.-P. (2011). Increasing oxygen radicals and water temperature select for toxic *Microcystis* sp. *PLoS One*, *6*(9), e25569. DOI: http://dx.doi.org/10.1371/journal.pone.0025569

Eigemann, F., Vanormelingen, P., & Hilt, S. (2013). Sensitivity of the green alga *Pediastrum duplex* Meyen to allelochemicals is strain-specific and not related to co-occurrence with allelopathic macrophytes. *PloS One*, *8*(10), e78463. DOI: http://dx.doi.org/10.1371/journal.pone.0078463

Gorham, P. R., Mclachlan, J., Hammer, U. T., & Kim, W. K. (1964). Isolation and culture of toxic strains of *Anabaena flos-aquae* (Lyngb.) de Bréb. *Verhandlungen des Internationalen Verein Limnologie, 15*(2), 796-804. DOI: https://doi.org/10.1080/03680770.1962.11895606

Gross, E. M., Erhard, D., & Iványi, E. (2003). Allelopathic activity of *Ceratophyllum demersum* L. and *Najas marina* ssp. *intermedia* (Wolfgang) Casper. *Hydrobiologia, 506*(1), 583-589. DOI: http://dx.doi.org/10.1023/B:HYDR.0000008539.32622.91

Gross, E. M. (2003). Allelopathy of aquatic autotrophs. *Critical Reviews in Plant Sciences*, *22*(3-4), 313-339. DOI: http://dx.doi.org/10.1080/713610859

Harke, M. J., Steffen, M. M., Gobler, C. J., Otten, T. G., Wilhelm, S. W., Wood, S. A., & Paerl, H. W. (2016). A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium*, Microcystis* spp. *Harmful Algae, 54*(1), 4-20. DOI: http://dx.doi.org/10.1016/j.hal.2015.12.007

Hilt, S., & Gross, E. M. (2008). Can allelopathically active submerged macrophytes stabilize clear water states in shallow lakes? *Basic and Applied Ecology*, *9*(4), 422-432. DOI: http://dx.doi.org/10.1016/j.baae.2007.04.003

Hilt, S. (2006). Allelopathic inhibition of epiphytes by submerged macrophytes. *Aquatic Botany*, *85*(3), 252-256. DOI: http://dx.doi.org/10.1016/j.aquabot.2006.05.004

Hilt, S., Ghobrial, M. G. N., & Gross, E. M. (2006). In situ allelopathic potential of *Myriophyllum verticillatum* (Haloragaceae) against selected phytoplankton species. *Journal of Phycology, 42*(6), 1189-1198. DOI: http://dx.doi.org/10.1111/j.1529-8817.2006.00286.x

Kardinäal, W. E. A., Tonk, L., Janse, I., Hol, S., Slot, P., Huisman, J., & Visser, P. M. (2007). Competition for light between toxic and nontoxic strains of the harmful cyanobacterium *Microcystis*. *Applied and Environmental Microbiology*, *73*(9), 2939-2946. DOI: http://dx.doi.org/10.1128/AEM.02892-06

Komárek, J., & Anagnostidis, K. (1986). Modern approach to the classification system of cyanophytes. 2-Chroococcales. *Archiv fur Hydrobiologie*, *73*(2), 157-226.

Körner, S., & Nicklisch, A. (2002). Allelopathic growth inhibition of selected phytoplankton species by submerged macrophytes. *Journal of phycology, 38*(5), 862-871. DOI: http://dx.doi.org/10.1046/j.1529-8817.2002.t01-1-02001.x

Kumar, A. B. (2000). Exotic fishes and freshwater fish diversity. *Zoos' Print Journal*, *15*(11), 363-367. DOI: http://dx.doi.org/10.11609/JoTT.ZPJ.15.11.363-7

Liang, Y., Ouyang, K., Chen, X., Su, Y., & Yang, J. (2017). Life strategy and grazing intensity responses of *Brachionus calyciflorus* fed on diferente concetrations of microcystin-producing and microcystin-free *Microcystis aeruginosa*. *Scientific Reports, 7*(1), 43127. DOI: http://dx.doi.org/10.1038/srep43127

Liu, Z., Hu, J., Zhong, P., Zhang, X., Ning, J., Larsen, S.E., … Jeppesen, E. (2018). Successful restoration of a tropical shallow eutrophic lake: Strong bottom-up but weak top-down effects recorded. *Water Research, 146*(1), 88-97. DOI: http://dx.doi.org/10.1016/j.watres.2018.09.007

Lombardo, P., & Cooke, G.D. (2003). *Ceratophyllum demersum* - Phosphorus interactions in nutrient enriched aquaria. *Hydrobiologia, 497*(1-3), 79-90. DOI: http://dx.doi.org/10.1023/A:1025461604808

López-Rodas, V., Flores-Moya, A., Maneiro, E., Perdigones, N., Marva, F., García, M. E., & Costas, E. (2007). Resistance to glyphosate in the cyanobacterium *Microcystis aeruginosa* as result of pre-selective mutations. *Evolutionary Ecology, 21*(4), 535-547. DOI: http://dx.doi.org/10.1007/s10682-006-9134-8

Mjelde, M., & Faafeng, B. (1997). *Ceratophyllum demersum* hampers phytoplankton development in some small Norwegian lakes over a wide range of phosphorus concentrations and geographical latitude. *Freshwater Biology*, *37*(2), 355-365. DOI: http://dx.doi.org/j.1365-2427.1997.00159.x

Mohamed, Z. A. (2017). Macrophytes-cyanobacteria allelopathic interactions and their implications for water resources management – a review. *Limnologica-Ecology and Management of Inland Waters, 63*(1), 122-132. DOI: http://dx.doi.org/10.1016/j.limno.2017.02.006

Moura, A. N., Aragão-Tavares, N. K. C., & Amorim, C. A. (2018). Cyanobacterial blooms in freshwaters bodies in a semiarid region, northeastern Brazil: a review. *Journal of Limnology*, *77*(2), 179-188. DOI: http://dx.doi.org/10.4081/jlimnol.2017.1646

Moura Júnior, E. G., Pott, A., Severi, W., & Zickel, C. S. (2019). Response of aquatic macrophyte biomass to limnological changes under water level fluctuation in tropical reservoirs. *Brazilian Journal of Biology*, *79*(1), 120-126. DOI: http://dx.doi.org/10.1590/1519-6984.179656

Mulderij, G., Van Donk, E., & Roelofs, J. G. M. (2003). Differential sensitivity of green algae to allelopathic substances from *Chara*. *Hydrobiologia, 491*(1), 261-271. DOI: http://dx.doi.org/10.1023/A:1024483704903

Nguyen, A. L. V., Tanabe, Y., Matsuura, H., Kaya, K., & Watanabe, M. M. (2012). Morphological, biochemical and phylogenetic assessments of water-bloom-forming tropical morphospecies of *Microcystis* (Chroococcales, Cyanobacteria). *Phycological research*, *60*(3), 208-222. DOI: http://dx.doi.org/10.1111/j.1440-1835.2012.00650.x

Paerl, H. W., & Otten, T. G. (2013). Harmful cyanobacterial blooms: causes, consequences, and controls. *Microbial Ecology, 65*(4), 995-1010. DOI: http://dx.doi.org/10.1007/s00248-012-0159-y

Pflugmacher, S. (2004). Promotion of oxidative stress in the aquatic macrophyte *Ceratophyllum demersum* during biotransformation of the cyanobacterial toxin microcystin-LR. *Aquatic Toxicology, 70*(3), 169-178. DOI: http://dx.doi.org/10.1016/j.aquatox.2004.06.010

R Core Team. *R: A language and environment for statistical computing*. Vienna, AT: R Foundation for Statistical Computing.

Reigosa, M. J., Sanchez-Moreiras, A., & Gonzalez, L. (1999). Ecophysiological approach in allelopathy. *Critical Reviews in Plant Sciences, 18*(1), 577-608. DOI: http://dx.doi.org/10.1080/07352689991309405

Scheffer, M., Carpenter, S., Foley, J.A., Folke, C., & Walker, B. (2001). Catastrophic shifts in ecosystems. *Nature*, *413*(6856), 591-596. DOI: http://dx.doi.org/10.1038/35098000

Sun, J., & Liu, D. (2003). Geometric models for calculating cell biovolume and surface area for phytoplankton. *Journal of Plankton Research*, *25*(11), 1331-1346. DOI: http://dx.doi.org/10.1093/plankt/fbg096

Švanys, A., Eigemann, F., Grossart, H. P., & Hilt, S. (2016). Microcystins do not necessarily lower the sensitivity of *Microcystis aeruginosa* to tannic acid. *FEMS Microbiology Letters, 363*(2), 1-4. DOI: http://dx.doi.org/10.1093/femsle/fnv227

Svirčev, Z., Drobac, D., Tokodi, N., Mijović, B., Codd, G. A., & Meriluoto, J. (2017). Toxicology of microcystins with reference to cases of human intoxications and epidemiological investigations of exposures to cyanobacteria and cyanotoxins. *Archives of Toxicology, 91*(1), 621-650. DOI: http://dx.doi.org/10.1007/s00204-016-1921-6

Van Donk, E., & Van De Bund, W. J. (2002). Impact of submerged macrophytes including charophytes on phyto- and zooplankton communities: allelopathy versus other mechanisms. *Aquatic Botany*, 72(3-4), 261-274. DOI: http://dx.doi.org/10.1016/S0304-3770(01)00205-4

Vanderstukken, M., Declerck, S.A., Decaestecker, E., & Muylaert, K. (2014). Long-term allelopathic control of phytoplankton by the submerged macrophyte *Elodea nuttallii*. *Freshwater Biology*, *59*(5), 930-941. DOI: http://dx.doi.org/10.1111/fwb.12316

Vanderstukken, M., Mazzeo, N., Van Colen, W., Declerck, S.A.J., & Muylaert, K. (2011). Biological control of phytoplankton by the subtropical submerged macrophytes *Egeria densa* and *Potamogeton illinoensis*: a mesocosm study. *Freshwater Biology, 56*(9), 1837-1849. DOI: http://dx.doi.org/10.1111/j.1365-2427.2011.02624.x

Vanormelingen, P., Vyverman, W., De Bock, D., Van Der Gucht, K., & De Meester, L. (2009). Local genetic adaptation to grazing pressure of the green alga *Desmodesmus armatus* in a strongly connected pond system. *Limnology and Oceanography, 54*(2), 503-511. DOI: http://dx.doi.org/10.4319/lo.2009.54.2.0503

Vitousek, P. M., D'antonio, C. M., Loope, L. L., & Westbrooks, R. (1996). Biological invasions as global environmental change. *American Scientist*, *84*(5), 468-478.

Wood, A. M., Everroad, R. C., & Wingard, L. M. (2005). Measuring growth rates in microalgal cultures. In R. A. Andersen (Ed.), *Algal culturing techniques* (p. 269-285). Amsterdam, NL: Academic Press; Phycological Society of America.

Yu, J., Liu, Z., Li, K., Chen, F., Guan, B., Hu, Y., ... Jeppesen, E. (2016). Restoration of shallow lakes in subtropical and tropical China: response of nutrients and water clarity to biomanipulation by fish removal and submerged plant transplantation. *Water*, *8*(10), 1-13. DOI: http://dx.doi.org/10.3390/w8100438

Zhu, J., Liu, B., Wang, J., Gao, Y., & Wu, Z. (2010). Study on the mechanism of allelophatic influence on cyanobacteria and chlorophytes by submerged macrophyte (*Myriophyllum spicatum*) and its secretion**.** *Aquatic Toxicology, 98*(2), 196-203. DOI: http://dx.doi.org/10.1016/j.aquatox.2010.02.011