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Adverse effects and potential accumulation of the herbicide atrazine in the cyanobacterium *Microcystis aeruginosa*

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Abstract: *Pest control in agricultural activities leads to herbicide contamination in the environment. The presence of herbicide, such as atrazine, in water systems can negatively impact aquatic organisms, especially vulnerable species, thereby disrupting ecosystem functioning. Phytoplankton, including microalgae and cyanobacteria, are primary producers in the aquatic food chain. Therefore, they hold a central position and serve as key drivers of material and energy flows in aquatic ecosystems. In this study, we tested the influence of atrazine at four different levels (0, 6, 53, 511 µg/L) on the development of Microcystis aeruginosa at two initial abundance (1.1×10^4 cells/mL, and 11.9×10^4 cells/mL) over 16 days under laboratory conditions. We also determined the total protein in dry mass and the amount of atrazine removed by M. aeruginosa at the end of the experiment. The results showed that the lowest atrazine level (6 µg/L) slightly influenced the development of M. aeruginosa, but significant growth inhibition was clearly observed from the 4th day to the end of the experimental period at higher atrazine concentrations (53 and 511 µg/L). Total protein content in cells at the low Microcystis abundance was similar between the control and atrazine exposures. At the higher Microcystis abundance, total protein increased by 32% at 53 µg atrazine/L, but decreased by 12% at 511 µg atrazine/L, compared to the control. Atrazine removal by M. aeruginosa ranged from 22% to 43%, with higher atrazine concentrations resulting in greater removal. Our study provides foundational information on the growth and total protein content of M. aeruginosa under laboratory conditions. Besides, it enhances understanding of the toxicity of atrazine to phytoplankton and the potential risks to phytoplankton grazers due to atrazine accumulation.*

Keywords: *cyanobacteria; growth; toxicity; removal*

1. Introduction

Human activities in daily life, the mining and gas industry, and industrial waste discharge activities cause environmental pollution and ecosystem degradation. Among these activities, spraying pesticides, herbicides, and fungicides in incorrect doses or specifications contributes to soil, water, and air pollution. According to the United State Environmental Protection Agency, chemicals used in agricultural land accounted for 50% of the causes of river and stream pollution (US. EPA, 2019). Pesticides indirectly enter aquatic ecosystems through various means such as spray runoff, aerial spraying, soil erosion and machine washing, causing chemical transport in soil, groundwater, surface water and air (Varjani et al., 2018). Worldwide, in many countries, the amount of pesticides consumed annually reached several hundred thousand tons reported by FAO (2023). For example, in 2021, the top pesticide consuming countries in the world were Brazil (0.72 million tons or 20% of the world total), the United States (0.46 million tons), and Indonesia (0.28 million tons). The annual application dose or the amount of pesticides used for one ha of

agricultural land has been increased over the last decade, up to seven times higher in the Trinidad and Tobago (an increase from 3.53 to 24.96 kg per ha), 3 times higher in Uruguay (from 2.58 to 7.95 kg per ha), or 2.1 times higher in Vietnam (from 2.03 to 4.28 kg per ha; FAO, 2023). Of the total pesticide production, 80% is used for insect control, 15% for herbicide control, 1.46% for fungal infection control, and 3% for other pesticide treatment (Aktar et al., 2009; Rajmohan et al., 2020). These pollutants pose toxicity risks to humans and disrupt ecosystem balance.

Herbicide is toxic to some plant species and others after short or long-term exposure (Price et al., 2015). The herbicide atrazine (2-chloro-4-ethytlamino-6-isopropylamine-1,3,5-triazine) is among the most widely used pesticides in the world (Price et al., 2015). Atrazine is commonly found in natural freshwater bodies, with concentrations reaching up to 30 μg/L in surface water and approximately 2,400 μg/L in polluted water (Santos et al., 2015; Chalifour et al., 2016; Sun et al., 2017). Phytoplankton (microalgae and cyanobacteria) are primary producers and serve as a major food source for consumers in the aquatic food web. They also play a crucial role in maintaining the balance of oxygen and carbon dioxide in aquatic ecosystems (Hoek et al., 1995). Many cyanobacterial species contribute to the enrichment of organic matter by fixing atmospheric nitrogen into ammonium ions. In phytoplankton, proteins contribute to the cell structure, and the regulation of many different processes and functions such as metabolism, catabolism, energy production, and reproduction (Martinez et al., 2020). Therefore, the impacts of herbicides including the atrazine on phytoplankton can lead to imbalances in the ecosystem.

Atrazine reduces the biomass of algae in rivers and streams near agricultural lands (Nödler et al., 2013), and altered structure of phytoplankton communities (Pannard et al., 2009). This herbicide could damage the reaction center of photosystem, block the electron transport on both donor and receptor sides, disrupt the absorption, transmission, and utilization of light energy, and ATP reduction in cells of phytoplankton (Weiner et al., 2004; Chalifour et al., 2016). The authors also noted that phytoplankton could absorb atrazine and smaller cells tended to absorb more atrazine from surrounding environment consequently getting more impacted by the herbicide. There were various phytoplankton species having different sensitivity and responses to a certain atrazine level (Weiner et al., 2004; Pannard et al., 2009) and different strains of the same species could have different sensitivity to the same atrazine level (Chalifour et al., 2016). The impacts of atrazine on different biomarkers of phytoplankton are not the same. For example, the photosynthesis is more sensitive than the growth rate upon exposure to atrazine (Chalifour et al., 2016). Several phytoplankton species can develop tolerance to atrazine over time (Seguin et al., 2002). The removal of atrazine by phytoplankton has been studied by several authors, but results are contradictory. Some species are capable of removing atrazine (Gonzalez-Barreiro et al., 2006; Campos et al., 2013), while others can only slightly reduce of its concentration in the water (Weiner et al., 2004; Chalifour et al., 2016). The aim of this research is to assess the growth, development, protein content, and atrazine removal potential of the toxic cyanobacterium *Microcystis aeruginosa,* isolated from Vietnam, of under laboratory conditions.

2. Materials and Methods

2.1. Organism and materials for study

The cyanobacterium *Microcystis aeruginosa,* strain TAMA2 (Pham et al., 2020) was used for present study. The *M. aeruginosa* was cultured in the Z8 medium under laboratory conditions of 27 ± 1°C, approximately 2,500 lux of light intensity, and a light: dark cycle of 12h:12h (Dao et al., 2018). In the laboratory conditions, the *M. aeruginosa* (strain TAMA2) existed in single and/or double cells, confirmed by our observation under a microscope (Olympus BX53). The Z8 medium used for the cyanobacterial cultivation was filtered through a membrane with a pore size of 0.2 μm (Millipore, UK) to ensure that the filtered medium was not contaminated with any bacteria (Vo et al., 2018). The physical characteristics of the Z8 medium including pH, hardness and alkalinity, were measured and quantified by the probe and titration (APHA, 2017), respectively. The pH of the medium ranged from 7.0 − 7.5. The average of hardness and alkalinity were 34 (mg $CaCO_3$/L) and 37 (mg $CaCO_3$/L), respectively. The atrazine (purification of 99%) was purchased from the manufacturer Merck (Germany), and used to prepare a stock solution by dissolving it into the acetone (Merck) at the concentration of 1 mg/mL. The atrazine stock solution was kept in −20 °C prior to experiment.

2.2. Experimental design, sub-sample collection and quantification

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The experiment of *M. aeruginosa* exposed atrazine was performed according to Chalifour et al. (2016) and Vo et al. (2018). For exposure, atrazine from the stock solution was spiked into the Z8 medium to make the proposed final concentrations of 0 (control), 5, 50 and 500 µg atrazine/L, and the actual concentrations of atrazine in the exposures were 6, 53, and 511 µg/L, respectively (Table 1). The atrazine concentrations used in present study were within the polluted concentration in nature, 2,400 µg/L (Dos Santos et al., 2015; Sun et al., 2017) and covered the international safety guideline value in surface water, 100 µg/L (WHO, 2017). In each treatment (control or atrazine exposure), four 100 mL-Erlenmeyer flasks (n = 4) were used, and each flask contained 50 mL solution of *M. aeruginosa*. We prepared two sets of experiments in which the first one was run with the initiate *M. aeruginosa* abundance of 11,000 cells/mL, and the second one with the initial *M. aeruginosa* abundance of 100,000 cells/mL (Table 1). We expected that the higher *M. aeruginosa* abundance on the starting day of experiment could have a higher tolerance than the lower abundance, to the same atrazine concentration, because the effects of atrazine may vary depending on the initial abundance, as interactions between atrazine and algal cells can differ based on cell volume and surface area, cell density (Tang et al., 1998)

The experiment was performed for 16 days under laboratory conditions and the *M. aeruginosa* was cultivated in static condition. *M. aeruginosa* abundance in each flask was monitored on the day 0, day 4, day 8, day 12 and day 16 of the experiment. On each time of sample monitoring, 1 mL of *Microcystis* solution was collected from each culture flask, transferred into a tube then fixed with one drop of the Lugol solution (Sournia, 1978) and enumerated with a Sedgewick Rafter counting chamber (Graticules Optics, UK) under an Olympus BX53 microscope at the 400 times of magnification. The cyanobacterial enumeration of each sample was completed when at least 400 cells of *M. aeruginosa* were counted (Sournia, 1978).

To confirm the atrazine concentration in the exposures, and exam the atrazine removal potential by *M. aeruginosa*, 50 mL of the testing medium from each treatment was collected on the starting day (day 0) and the final day (day 16) of the experiment. The collected medium was then filtered through a glass fiber filter (GF/A; porosity of 1.6 µm; Whatman) before being chemically analyzed. Atrazine levels were then measured using High-Performance Liquid Chromatography (HPLC) with the Alliance 2695 model from Waters Corporation (Pennsylvania, USA), set to a wavelength of 224 nm. A C18 column (4.6 × 250 mm) was used, with an injection volume of 20 µL, as described in a previous study (Luu et al., 2021). The analysis revealed that the atrazine concentrations in the atrazine treatments were 6, 53, and 511 µg/L on the starting day of the experiment (Table 1).

Tab. 1. The initial abundance of *Microcystis aeruginosa* and atrazine concentrations at the start of the experiment, and their corresponding abbreviations.

Treatments	Atrazine concentrations (µg/L)	Abbreviations
Low *M. aeruginosa* abundance (~ 11,000 cells/mL)		
Control	0	LA0
Low atrazine exposure	6	LA6
Medium atrazine exposure	53	LA53
High atrazine exposure	511	LA511
High *M. aeruginosa* abundance (~100,000 cells/mL)		
Control	0	HA0
Low atrazine exposure	6	HA6
Medium atrazine exposure	53	HA53
High atrazine exposure	511	HA511

By the end of experiment, the *M. aeruginosa* in each treatment (control or exposures; Fig. 1) were pooled before used for atrazine and nitrogen quantification. Fifty (50) mL of pooled sample was used for atrazine analysis as mentioned above, and the remaining volume was filled onto a GF/A for biomass collection. The biomass of the *M. aeruginosa* was dried (50 °C, 6 hours), then weighted and used for the total nitrogen analysis (Bridgewater et al., 2017). Total nitrogen values would be used for calculation on the total protein in *M. aeruginosa* biomass according to Lopez et al (2010).

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Fig. 1. The *M. aeruginosa* of the control and atrazine exposures on the terminated day of experiment. The 16 flasks on the left are from the lower abundance, and the 16 flasks on the right are from the higher abundance, at the starting day.

2.3. Data analysis

The growth rate (μ) of the cyanobacterium *M. aeruginosa* was calculated according to the formula of Lobban (1988): $\mu = (\ln x_2 - \ln x_1)/(t_2 - t_1)$. Where x_1, x_2 were the *M. aeruginosa* abundance at the time (day) t_1 and t_2, respectively.

Total protein (TP) in the *M. aeruginosa* biomass was calculated by the formula: $TP = TN \times 4.44$ (Lopez et al., 2010). Where, TN was the total nitrogen in the biomass of *M. aeruginosa*. The atrazine removal efficiency (RE, %) out of the testing medium was calculate with the formula: $RE (\%) = 100 \times (A0 - A16)/A0$. Where A0 and A16 were the atrazine concentrations of day 0 and day 16, respectively.

The one-way ANOVA followed by Tukey post hoc test (Sigma Plot version 15.0) was applied to examine the significant difference in the abundance and growth rate of *M. aeruginosa* in control and atrazine treatments.

3. Results and discussion

3.1. Effect of atrazine on the abundance and growth rate of the Microcystis aeruginosa

Table 2 shows the *M. aeruginosa* abundance in the two experimental batches (sets) exposed to atrazine concentrations ranging from 6 to 511 μg/L. Generally, cyanobacterial abundance in the control and lower atrazine exposures (6 and 53 μg/L) strongly increased significantly over the period of incubation. However, at the highest atrazine level (511 μg/L) *M. aeruginosa* abundance steadily increased in both the LA511 and HA511 treatments over time. Despite starting with a tenfold lower abundance on day 0, after 16 days of incubation, the abundance of *M. aeruginosa* in the lower abundance treatments (LA0, LA6, LA53) was slightly higher than in the corresponding treatments with higher initial abundance (HA0, HA6, HA53; Fig. 1; Table 2).

In the low cyanobacterial abundance set, there was no significant difference of the cyanobacterial abundance between the LA0 and LA6 during over16 days of incubation, generally. However, the cyanobacterial abundance in the LA53 was much lower than in the LA0, but higher than in the LA511 (Table 2). Hence, the statistical difference among the LA0, LA53 and LA511 was atrazine-concentration dependent. In the high cyanobacterial abundance set, no significant difference was found among the HA0, HA6, and HA53 over the period time of incubation. Highest atrazine concentration caused the reduction of cyanobacterial abundance in HA511, and the statistical difference between the HA511 and the control ($p < 0.05$; Table 2).

Tab. 2. Abundance of *Microcystis aeruginosa* (cells/mL) over the 16 days of experiment. Letters (a, b, c) indicate significant differences ($p < 0.05$) as determined by one-way ANOVA followed by Tukey's multiple comparison test. Different letters denote significant differences..

Treatments	Day 0	Day 4	Day 8	Day 12	Day 16
Low *M. aeruginosa* abundance					
LA0	11,058 ± 875	19,9233 ± 9,277[a]	2,571,000 ± 113,935[a]	5,916,000 ± 246,858[a]	10,682,667 ± 200,053[a]
LA6	11,542 ± 1,501	184,400 ± 7,632[a]	2,598,333 ± 86,072[b]	6,144,000 ± 193,122[a]	10,836,000 ± 1,033,551[ab]

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LA53	11,883 ± 582	100,200 ± 6,624^{b}	1,038,500 ± 33,753^{c}	4,637,333 ± 310,286^{b}	9,141,333 ± 320,533^{b}
LA511	10,292 ± 356	13,767 ± 1,021^{c}	11,542 ± 621^{d}	19,833 ± 3,311^{c}	19,250 ± 3,681^{c}
High *M. aeruginosa* abundance					
HA0	106,433 ± 13,925	1,315,125 ± 133,076^{a}	3,435,000 ± 295,181^{a}	6,255,000 ± 272,301^{a}	7,576,667 ± 357,957^{a}
HA6	138,600 ± 5,840	1,416,667 ± 141,870^{abc}	4,273,667 ± 335,163^{ab}	6,773,333 ± 142,945^{abc}	8,440,000 ± 524,214^{a}
HA53	118,140 ± 11,950	730,625 ± 19,486^{b}	3,123,000 ± 307,877^{ac}	6,822,000 ± 455,947^{b}	8,403,333 ± 939,805^{a}
HA511	116,505 ± 8,124	118,500 ± 8,909^{c}	190,200 ± 17,085^{b}	277,875 ± 116,306^{c}	209,475 ± 42,362^{b}

In the low abundance batch, the growth rate of *M. aeruginosa* in the LA0, LA6, LA511 and LA511 ranged from 0.43-0.72, 0.43-0.69, 0.42-0.56, and 0.01-0.07 fold/day, respectively (Table 2). Compared to the control, there was a significant difference in the LA6 and LA511 from day 4 to day 16. In the high abundance batch, the growth rate of *M. aeruginosa* in the HA0, HA6, HA53, and HA511, varied from 0.2-0.63, 0.26-0.58, 0.2-0.46, and -0.02-0.11 fold/day, respectively (Table 2). Generally, the growth rate of *M. aeruginosa* in all treatments (except at the highest atrazine level, 511 µg/L) was higher in the first week of incubation and lower in the second one. Additionally, *M. aeruginosa* growth was not significantly influenced by atrazine concentrations up to 53 µg/L, but was strongly impaired by 511 µg/L (Table 3). The growth rate of *M. aeruginosa* in the lower abundance treatments at the start of the experiment (LA0, LA6, LA53) was consistently higher than that in the higher abundance treatments (HA0, HA6, HA53).

Tab. 3. Growth rate of *Microcystis aeruginosa* (fold/day) in low and high abundance batches. Letters (a, b, c) indicate significant differences ($p < 0.05$) as determined by one-way ANOVA followed by Tukey's multiple comparison test. Different letters denote significant differences.

Treatments	Day 4	Day 8	Day 12	Day 16
Low *M. aeruginosa* abundance				
LA0	0.72 ± 0.03^{a}	0.68 ± 0.01^{a}	0.52 ± 0.01^{a}	0.43 ± 0.01^{a}
LA6	0.69 ± 0.02^{a}	0.68 ± 0.02^{a}	0.52 ± 0.01^{a}	0.43 ± 0.01^{a}
LA53	0.53 ± 0.01^{b}	0.56 ± 0.01^{a}	0.50 ± 0.01^{a}	0.42 ± 0.01^{a}
LA511	0.07 ± 0.03^{c}	0.01 ± 0.01^{b}	0.05 ± 0.01^{b}	0.04 ± 0.01^{b}
High *M. aeruginosa* abundance				
HA0	0.63 ± 0.05^{a}	0.43 ± 0.02^{a}	0.34 ± 0.01^{a}	0.20 ± 0.13^{a}
HA6	0.58 ± 0.02^{ab}	0.43 ± 0.01^{a}	0.33 ± 0.01^{b}	0.26 ± 0.01^{a}
HA53	0.46 ± 0.03^{b}	0.41 ± 0.02^{a}	0.34 ± 0.01^{ab}	0.20 ± 0.14^{a}
HA511	-0.02 ± 0.03^{c}	0.05 ± 0.01^{b}	0.11 ± 0.03^{c}	0.03 ± 0.02^{b}

Weiner et al. (2004) reported the disruption of light energy capture and transmission in plant cells by atrazine, consequently the inhibition of carbohydrate synthesis in phytoplankton (Shabana, 1987). Lockert et al. (2006) showed that atrazine affects microalgae growth. At the concentration of 100 µg/L, species of diatom, cryptomonad and euglenoid expressed the inhibition with atrazine but not significantly, while cyanobacteria was moderately inhibited and green algae was strongly inhibited by atrazine. Atrazine also impacted the growth of green algae such as *Scenedesmus quadricauda, S. obliquus,* and cyanobacteria such as *Microcystis aeruginosa, Pseudanabaena mucicola* (Chalifour et al., 2016; Le et al., 2019). Brêda-Alves et al (2021) demonstrated that atrazine affects the growth rate of cyanobacteria while Shabana (1987) found that atrazine impacts the dry mass of cyanobacteria. Our results are consistent with previous studies (Shabana, 1987; Lockert et al., 2006; Brêda-Alves et al., 2021) due to the inhibition of the atrazine on the growth rate and abundance of *M. aeruginosa*. It reflected that the atrazine level of 53 µg/L was not high enough to prevent the growth rate of *M. aeruginosa* which was supported by a study of Lockert et al (2006) conducting with other phytoplankton (e.g. diatoms, cryptomonad, euglenoid). However, at a much higher atrazine level (511 µg/L) the light uptake and transfer, and photosynthetic activity could be prevented in the exposed *M. aeruginosa*, consequently growth rate inhibition and low abundance, as reported in some other cyanobacterial species (*Aulosira fertilissima, Anabaena oryae* and *Nostoc muscorum*) by Shabana

(1987). In addition, we found that the tolerance of *M. aeruginosa* in our study was higher than several other phytoplankton species (*Isochrysis galbana, Dunaliella tertiolecta, Phaeodactylum tricornutum, Pseudokirchneriella subcapitata, Synechococcus* sp., *S. quadricauda, P. mucicola*) which was seriously inhibited at the levels of 45－91 (300) µg/L of atrazine (Wiener et al., 2004; Le et al., 2019). However, the atrazine tolerance is comparable between the cyanobacterium *M. aeruginosa* in our study and the cyanobacerium *Microcystis novacekii* in a previous one (Campos et al., 2013) at least within the 4 days of incubation.

In the present study, we found that the growth rates of *M. aeruginosa* in the LA53 and HA53 treatments were significantly lower than those in the LA0 and HA0 treatments, respectively, on the 4th day of incubation. However, no significant differences were observed during the later stages of incubation (8th, 12th, and 16th days; Table 2). This may be due to that the *M. aeruginosa* was impacted by the atrazine on the first 4 days but its tolerance might initiate and develop afterward (Chalifour et al., 2016). Besides, it was interesting that the development (abundance and growth rate) of *M. aeruginosa* in the treatments of LAs (LA0, LA6, LA53) was higher than that of HAs (HA0, HA6, HA53; Tables 2, 3; Fig. 1) which may be explained with the nutrient availability in the two sets (batches) of experiment. There was the same nutrients (nitrate, phosphate, trace element concentrations) in low and high *M. aeruginosa* abundance at the starting experiment. In low abundance (LAs) or less total cell numbers in the same volume of medium and nutrient quota, there should be less competition for nutrients among the *M. aeruginosa* cells, hence cells in this batch could have more nutrient consequently a faster development. On the contrary, in high abundance (HAs) or more total cell numbers, the competition among the cells for nutrients should be stronger hence nutrient shortage could happen earlier, consequently growth rate was slower than the other (LAs). Our study reflected that the growth rate response of *M. aeruginosa* to atrazine could be driven by the initiate cyanobacterial abundance or nutrient availability.

3.2. Total nitrogen and protein content in the Microcystis aeruginosa

Total nitrogen and protein in the cyanobacterium *M. aeruginosa* after 16 days were showed in the Table 4. Total protein was calculated based on total nitrogen, so these two indices are directly proportional. In the low initial cyanobacterial abundance, the total protein content in LA0 (control) was 486 g protein/kg dry mass, which is within the range of the protein content in the atrazine exposures, from 480 g/kg (LA53) to 516 g/kg (LA6). The proportion of the protein content in the atrazine exposures (LA6, LA53, and LA511) compared to the control was 106%, 99% and 103%, respectively. It can be seen that the protein content in all three exposures was more a less similar to the control. In the high initiate cyanobacterial abundance, the total protein in HA0 (control) was 412 g/kg. In the atrazine exposures, the total protein ranged from 360 g/kg (HA511) to 546 g/kg (HA53). The protein content in the HA6 and HA53 was respectively 16% and 32% higher than in the HA0, while that in the HA511 was 12% lower than in the HA0 (Table 4).

Tab. 4. Total nitrogen and total protein contents in *Microcystis aeruginosa* (kg) dry mass and their proportion to the control

Treatments	Total nitrogen (g N/ kg dry mass)	Total protein (g protein/ kg dry mass)	Proportion to control (%)
Low *M. aeruginosa* abundance			
LA0	109	486	100
LA6	116	516	106
LA53	108	480	99
LA511	112	498	103
High *M. aeruginosa* abundance			
HA0	93	412	100
HA6	107	478	116
HA53	122	545	132
HA511	82	364	88

The protein content in the *M. aeruginosa* in present study is within the range of total protein in several other phytoplankton (e.g. *Scenedesmus* spp., *Arthrospira massartii, Coelastrum microsporum*) isolated in Southern Vietnam water bodies (Nguyen et al., 2019) and many phytoplankton in the world (Geada et al.,

2021). Proteins are among the primary metabolites in phytoplankton. These macro-moleculars contribute to the total energy values and fundamental function in cells of phytoplankton (Bae et al., 2021). Proteins also directly link with the biosynthesis and cell division hence development and growth in phytoplankton. The proteins in phytoplankton cells could be enhanced by the surrounding environmental conditions such as temperature, light intensity and regime, water turbulence, and nutrients (Bae et al., 2021; Geada et al., 2021). The similar total protein content in LA0, LA6, LA53 and LA511 (Table 4) revealed the no impact on atrazine (up to 511 µg/L) on the protein synthesis and accumulation in *M. aeruginosa* cells. However, yet we do not know the root of the enhance of protein in the HA6 and HA53 and reduction of protein in the HA511. This may be due to the combined effect of nutrient competition and atrazine on the *M. aeruginosa* which needs further investigations to clarify. Our study contributes the basic information on the total protein content in the *M. aeruginosa* isolated from Vietnam upon normal and atrazine exposure conditions.

3.3. Atrazine removal by Microcystis aeruginosa

The atrazine removal efficiency after 16 days of experiment is showed in the Table 5. Because the degradation of atrazine in testing medium, if happened, was negligible (Chalifour et al., 2016), the reduction of the atrazine in the medium could be attributed to the *M. aeruginosa*. Generally, at the same atrazine of the starting day, low and high *M. aeruginosa* had similar amount of atrazine reduction and removal efficiency at the terminated day of experiment (Table 5). Although the lowest initiate atrazine level (6 µg/L in LA6, and HA6) resulted in the highest atrazine removal efficiency (42—43%), the amount of atrazine reduced by *M. aeruginosa* was the lowest (0.13 µg atrazine) after 16 days of incubation. This could be due to the different atrazine availability in the testing medium. The more atrazine in surrounding environment, the more absorption and/ or uptake of this chemical onto surface/ into cells of *M. aeruginosa* (Weiner et al., 2004).

Tab. 5. Atrazine removal by *Microcystis aeruginosa*

Treatments	Atrazine concentration (µg/L)		Total reduction of atrazine over 16 day (µg)	Atrazine removal efficiency (%)
	Day 0	Day 16		
Low *M. aeruginosa* abundance				
LA6	6.0	3.5	0.13	42
LA53	53.0	38.6	0.72	27
LA511	511.0	383.4	6.38	25
High *M. aeruginosa* abundance				
HA6	6.0	3.4	0.13	43
HA53	53.0	38.9	0.71	27
HA511	511.0	396.9	5.71	22

Our study is controversial to previous studies (Weiner et al., 2004; Chalifour et al., 2016) in which the authors found almost no atrazine removal or the atrazine removal not more than 2% of the initial concentration. However, the atrazine removal by *M. aeruginosa* in present study is in line with another investigation of Campos et al (2013). The authors used *Microcystis novacekii* for their study and the cyanobacterium could have an atrazine uptake of 27.2%. Gonzalez-Barreiro et al (2006) reported that the phytoplankton *Synechococcus elongatus* and *Chlorella vulgaris* could remove 73% and 83% of triazine (a similar chlorinated herbicide with atrazine) in testing medium, respectively. It is important to note that different species or even strains could have different sensitive or tolerant capacity to the same toxicant (Chalifour et al., 2016). Cell size of phytoplankton species or surface to volume ratio is also an important physical characteristic closely link to the response of the cell to atrazine (Weiner et al., 2004). Besides, medium and cultivating conditions for phytoplankton and lipid composition in phytoplankton species are driving factors for the tolerance to atrazine (Chalifour et al., 2016). The removal of atrazine from the testing medium or its bioconcentration in the cells of *M. aeruginosa* poses a potential risk to animals in aquatic environment. *M. aeruginosa* can be consumed or filtered by various animals, such as zooplankton, shrimp

larvae, planktivorous fishes, and bivalves. As a result,atrazine could be transferred to higher trophic level in the food web of aquatic ecosystems.

4. Conclusion

In conclusion, this study provides insights into the effects of the herbicide atrazine on microalgae. *M. aeruginosa* could growth normally at a low atrazine concentration of 6 µg/L. The effects of atrazine on the cyanobacterium became evident at higher concentration (53 µg/L) and inhibitory at 511 µg/L. The growth rate of *M. aeruginosa* was faster with lower initial abundance at the start of experiment, likely due to the nutrient availability in the testing medium. The total protein content in *M. aeruginosa* was similar between the control and atrazine exposures at low initial cyanobacterial abundance. However, it varied considerably at high initial abundance, increasing in treatments with 6 and 53 µg/L of atrazine and decreasing in the 511 µg/L treatment. We also observed that atrazine removal by *M. aeruginosa* was concentration-dependent. This highlights the bioconcentration of atrazine in the cyanobacterium, which poses potential health risks to aquatic ecosystems.

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Conflicts of interest

The authors declare no conflict of interest.

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