J. Viet. Env. **2016**, Vol. 8, No. 1, pp. 56-61
DOI: 10.13141/jve.vol8.no1.pp56-61

JOURNAL of VIETNAMESE ENVIRONMENT

Detrimental impacts of toxic *Microcystis aeruginosa* from Vietnam on life history traits of *Daphnia magna*

Ảnh hưởng tiêu cực của loài Microcystis aeruginosa có độc ở Việt Nam lên các đặc điểm vòng đời của Daphnia magna

Research article

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In this study, we tested the long-term and negative effects of microcystin-producing cyanobacterium *Microcystis aeruginosa* from Vietnam on *Daphnia magna* under the laboratory conditions. The test organisms were fed with mixtures of green alga *Scenedesmus armatus*. and toxic *M. aeruginosa* at different ratios (10% *Microcystis* + 90% *Scenedesmus*, 50% *Microcystis* + 50% *Scenedesmus*, 100% *Microcystis,* and 100% *Scenedesmus*) for over a period of 21 days. The life history traits of the organisms such as, survival, maturation, fecundity were daily recorded. Besides, the intrinsic population rate of *D. magna* in each treatment was also calculated based on the survivorship, the reproductive age and the clutch size of the animals. The results showed that survival, maturation and reproduction of the *D. magna* fed with 10, 50 and 100% *M. aeruginosa* was impaired. Additionally, the intrinsic population rate of the exposed *D. magna* was lower than that of the control. This study evidenced the adverse effects of toxic *M. aeruginosa* on both the individual and intrinsic population levels of *D. magna*. To our knowledge, this is the first report on the chronically detrimental impacts of toxic *M. aeruginosa* isolated from Vietnam on *D. magna* and contributed the scientific information on the severe influences of toxic cyanobacteria world wide.

Trong bài viết này, chúng tôi nghiên cứu ảnh hưởng xấu mãn tính của loài vi khuẩn lam Microcystis aeruginosa có khả năng sản sinh độc tố microcysin từ Việt Nam lên Daphnia magna trong điều kiện phòng thí nghiệm. Sinh vật thí nghiệm được cho ăn với hỗn hợp tảo lục Scenedesmus armatus và M. aeruginosa có độc ở các tỷ lệ khác nhau (10% Microcystis + 90% Scenedesmus, 50% Microcystis + 50% Scenedesmus, 100% Microcystis, và 100% Scenedesmus) trong thời gian 21 ngày. Các đặc điểm vòng đời của sinh vật bao gồm sức sống, sự thành thục, sức sinh sản được theo dõi hàng ngày. Bên cạnh đó, tỷ lệ phát triển quần thể của D. magna trong từng lô thí nghiệm cũng được tính toán dựa vào sức sống, tuổi sinh sản và kích cỡ sinh sản của sinh vật. Kết quả cho thấy, sức sống, tuổi thành thục và sự sinh sản của D. magna cho ăn với 10, 50 và 100% M. aeruginosa bị ảnh hưởng xấu. Bên cạnh đó, tỷ lệ phát triển quần thể của D. magna trong lô phơi nhiễm thấp hơn so với đối chứng. Nghiên cứu này chứng minh ảnh hưởng xấu của M. aeruginosa có độc lên cả hai mức độ cá thể và quần thể của D. magna. Theo hiểu biết của chúng tôi, đây là báo cáo đầu tiên về ảnh hưởng xấu mãn tính của M. aeruginosa có độc phân lập từ Việt Nam lên D. magna and đóng góp thêm thông tin khoa học cho những ảnh hưởng nghiêm trọng của vi khuẩn lam có độc trên khắp thế giới.

Keywords: life history traits, microcystins, *Daphnia magna*, *Microcystis aeruginosa*, negative effects

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http://dx.doi.org/10.13141/JVE
ISSN: 2193-6471

1. Introduction

Eutrophication is known to cause and enhance the cyanobacterial mass development. Seriously, cyanobacteria are capable of producing toxic metabolites and bioactive compounds such as microcystins (MCs), anatoxin-a, cylindrospermopsin, among others (Sinoven et al., 1999; Banker et al., 1999; Rohrlack et al., 2003) of which MCs are the most common and potent cyanobacterial toxins in freshwater bodies (Doekel et al., 2001).

Many investigations have showed that cyanobacteria and their toxins are extremely toxic to aquatic organisms, and the toxicity investigations have focused on the effects of *Microcystis* on *Daphnia* over the last few decades. For example, in the laboratory condition, the mortality of *Daphnia* increased when fed on MCs–producing cyanobacteria and purified microcystin-LR. Additionally, the effective rate strongly depended on the concentration of exposure and the sensitivity of species (Demott and Moxter, 1991; Trubetskova and Haney, 2006). Also, there have been evidences that the feeding rate was inhibited, and the growth rate and reproduction were decreased when the daphnids were priorly fed with toxic *Microcystis* (DeMott, 1999). In spite of not including any toxic impacts, the negative effects of some *Microcystis* strains on body length, fecundity (number of new born per female) and clearance rate of *Daphnia* were recorded (Lürling and Van der Grinten, 2003; Lürling, 2003).

In recent years, researchers have cared about the maternal effects of cyanobacteria and their toxins on zooplankton. If cyanobacteria and their toxins reside inside the body of *Daphnids* for a longer period of time, it can be transferred to their offspring. Guo and Xie (2006) proved the tolerance development against toxic *M. aeruginosa* of *Ceriodaphnia cornuta, Moina micriura*, but *Daphnia carinata* after exposed trans-generationally to mixture of *Scenedesmus* and toxic or non-toxic *Microcystis* for 4 weeks. Dao et al. (2010) investigated in detail chronic effects of cyanobacterial toxins, with emphasis on MCs, on *D. magna*. The low concentration of MC-LR slightly affected the growth and reproduction of parent daphnids. Survivor decreased during chronic exposure with increasing MCs concentration. Age to maturity of the offspring increased and their survival decreased after parent generation was exposed to the toxin, even if the offspring were raised in non-toxic medium. Besides, cessation of the egg/embryos was observed and malformation of neonates caused by cyanobacterial toxins was firstly recorded. In the other reports, mother *Daphnia* exposed to MCs resulted in the decrease in dry mass of its offspring even though they were raised in non-toxic medium (Ortiz-Rodriguez et al, 2012).

The occurrence of cyanobacteria in lakes and reservoirs can cause potential risks to aquatic organisms. In Vietnam, the studies of cyanobacteria have focused only on morphological taxonomy and concentration or toxin producing cyanobacteria (Shirota, 1966; Pham, 1969; Nguyen, 1983, 1997; Phung et al., 1992; Duong, 1996; Dang et al., 2000; Hummert et al., 2001). Additionally, there is little information in the literature about the negative effects of cyanobacteria from Vietnam on micro-crustaceans. Therefore, in this study, we aimed to investigate the long-term effects of cyanobacteria containing MCs isolated from Vietnam on the life history traits of *D. magna.*

2. Materials and methods

2.1 The test organisms

Daphnia magna Straus was purchased from the MicroBioTests Inc, Belgium (Fig. 1). The animal has been fed with green alga *Scenedesmus armatus* and maintained in the laboratory conditions of 22 ± 1ºC, dim light and light dark cycle of 14h:10h. *Microcystis aeruginosa* was used for exposure to *D. magna*. The cyanobacterium *M. aeruginosa* (Fig. 1) was isolated from Dau Tieng Reservoir, a drinking water supply 120 km western Hochiminh City, Vietnam. Both cyanobacterium and green alga were cultivated in Z8 medium (Kotai, 1972) with continuous aeration and under the laboratory conditions of 25 ±1ºC, light intensity of around 3000 Lux, and light dark cycle of 12h:12h.

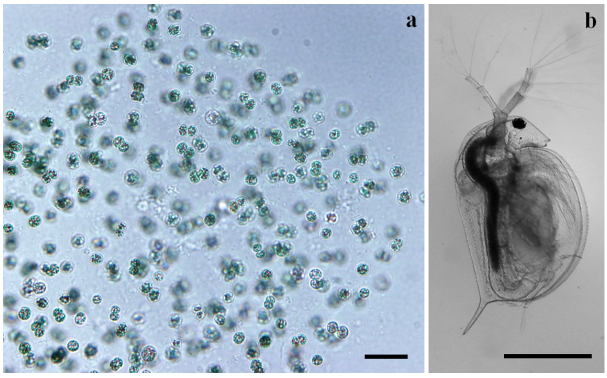

Figure 1. The organisms for the toxicity test. a, *Microcystis aeruginosa*; b, newly born *Daphnia magna*. Scale bar of a = 20 µm, and b = 300 µm.

2.2 Toxin analysis

The culture of *M. aeruginosa* was harvested during the exponential growth phase and filtered onto GF/A filters (Fiore, France), dried at 50ºC over night and stored at -70ºC prior to toxin determination. For MCs determination, the filters containing microbes were cut into small pieces with scissors. Extraction of MCs was conducted according to Barco et al. (2005) with minor modification. Briefly MCs were firstly extracted in 5 mL of 100% (vol/vol) aqueous methanol by shaken for 60 min followed by 2 × 60 min of extraction in 3 mL of 75% aqueous methanol. Each extraction step was followed by centrifugation (4.500 rpm, 30 min, 4°C). The supernatants of all extractions from each sample were pooled, dried at room temperature, re-dissolved in 0.5 mL MeOH (100%) and centrifuged at 8.000 rpm, 4°C for 5 minutes. The supernatant was passed through a Minisart RC 4 filter membrane (0.20 µm pore size, Sartorius Stedim Biotech, Germany), and kept at -20°C prior to reversed phase HPLC for analysis. Reverse phase HPLC (Shimadzu 10A series, Shimadzu, Kyoto, Japan) equipped with a silica based reverse phase C_{18} column (Waters SunFire^{TM} 5 µm, 3.0×250 mm, Ireland), maintained at 40°C. A 0.05 M phosphate buffer (pH 2.5) in methanol (50/50, v/v) was used as mobile phase, at a flow rate of 0.58 mL min^{-1}. MC congeners were detected by the

UV detection at 238 nm with a photodiode UV-visible array detector. Microcystin-LR, -RR and -YR purchased from Wako chemicals company (Osaka, Japan) were used as standards. The HPLC system had a detection limit of 0.01 µg L^{-1}.

2.3 Experimental setup

Fifteen neonates (< 24h old) were used for each chronic experiment (Adema, 1978) and individually raised in 50 mL beakers containing 20 mL of medium (Dao et al., 2010). In the control experiment, the *Daphnia* was fed with 100% of green alga *S. armatus* at the concentration of 1 mg C L^{-1} day^{-1} (Gustafsson et al., 2005). In exposures, *Daphnia* was fed with a mixture of *Scenedesmus* and cyanobacterium *M. aeruginosa* with total concentration of 1 mg C $L^{-1}day^{-1}$, at three different regimes (1) 10% *Scendesmus* + 90% *Microcystis*; (2) 50% *Scenedesmus* + *Microcystis*; and (3) 100% *Microcystis* (Table 1). In total, four incubations including one control and three different exposures (Table 1) were run under the temperature of 22 ± 1ºC, dim light and light dark cycle of 14h: 10h.

All medium and food were renewed every two days. The life history traits of *Daphnia* such as survival, maturity age, reproduction were daily observed during 21 days. Besides, age specific survival and clutch size were used to estimate the intrinsic rate of population increase, r, as a measure of fitness. The Euler equation (Stearns, 1992) was used to calculate r:

$$1 = \sum e^{-rx} l_x m_x$$

Where x is age (in days), l_x is the probability of surviving and m_x is the fecundity at age x

Table 1. Summary of the treatments in the toxicity test

	Scenedesmus armatus	*Microcystis aeruginosa*
Control	100%	0%
10% Ma	90%	10%
50% Ma	50%	50%
100% Ma	0%	100%

2.4 Statistical analysis

Sigmaplot version 12 was used for the data treatment. Kruskal-Wallis test was applied for calculation on statistically significant difference of the maturation of *D. magna*. P-values less than 0.05 were considered statistically significant.

3. Results and discussion

3.1 Microcystins concentration in the *M. aeruginosa*

The results of HPLC analysis showed that the *M. aeruginosa* strain produced MC-RR with the concentration of 3733 µg g^{-1} dry weight (Fig. 2). Along with MC-LR, MC-RR is the most frequent MC variant, which posses a grave threat to both environment safety and public health (Zhang et al., 2007). This concentration of MCs was comparable with the results in previous the studies (e.g. Nguyen et al., 2007; Vasconcelos et al., 1996), in which the highest MCs concentrations were up to 4120 µg g^{-1} dry weight in culture and 1000 – 7100 µg g^{-1} dry weight in natural lakes, reservoirs and rivers. The high MCs-producing *M. aeruginosa* proposed a serious risk to local residents who daily use the water from Dau Tieng reservoir for domestic activities.

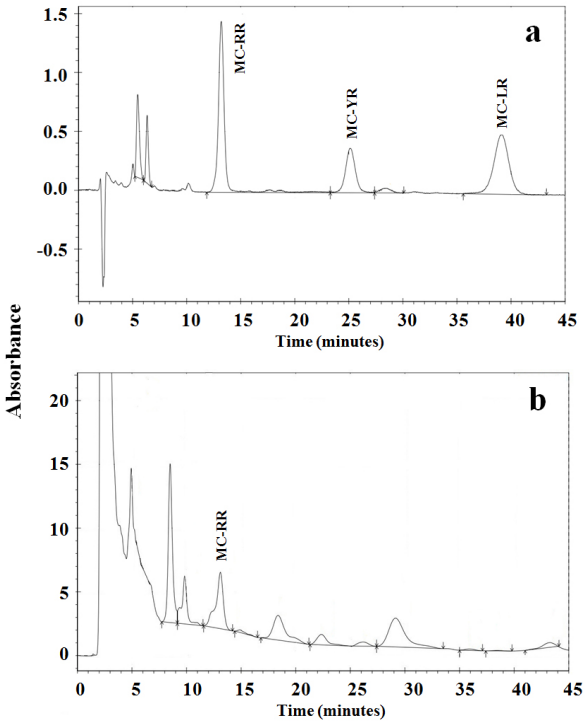

Figure 2. The HPLC chromatography of standards (a) and *Microcystis aeruginosa* (b)

3.2 Effects of *M. aeruginosa* on the survivorship of *D. magna*

In the control, all *D. magna* were well alive by the end of experiment. However, *Daphnia* started to die within approximately 1 week in all *Microcystis* treatments (Fig. 3). By the end of incubation, the *Daphnia* exposed to 10% and 50% *Microcystis* decreased their survival proportion to 47% and 33%, respectively. The survivor proportion was lowest in the treatment of 100% *Microcystis* (27%, Fig. 3) evidencing that toxic *Microcystis* had a strong impact on survival of *Daphnia* with concentration dependence. The current record was in agreement with the investigation of Dao et al. (2010), in which survivor of *D. magna* was 10% and 55% in 5 and 50 µg MC L^{-1} treatments within 2 months, respectively. With the MCs concentration of 3733 µg g^{-1} dry weight (as mentioned above) and the concentration of *M. aeruginosa* used for the test (0.1 – 1 mg C L^{-1}), the MCs concentrations in the treatments of our study were not more than 0.013 µg MC L^{-1} (100% *Microcystis* treatment). However, the survivorship of the exposed *D. magna* decreased so strong (up to 73% in 100% *Microcystis* treatment). This could be explained as (i) the cyanobacterium used in the experiment was live cells and cyanobacteria were considered to be low nutritional value for zooplankton, mainly due to the absence of essential polyunsaturated fatty acid (PUTA) and sterols (Brett and Muller-Navarra, 1997; Von Elert, 2002), and (ii) beside MCs, *Microcystis* could produce other toxic bioactive compounds which

need further investigation and chemical analysis with modern equipment (e.g. LC/MS, GC/MS) to confirm.

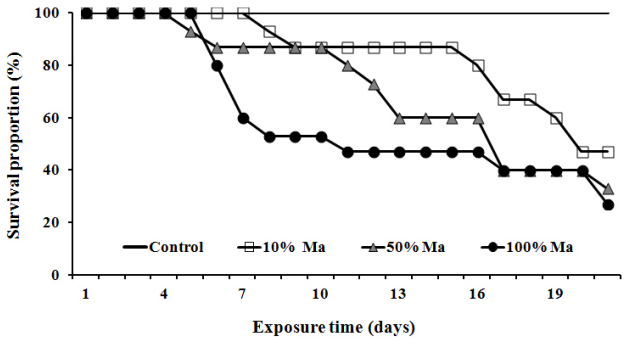

Figure 3. Survival of *D. magna* from control and exposures during 21 days of incubation. Abbreviation as in Table 1.

3.3 Effects of *M. aeruginosa* on the maturation of *D. magna*

Daphnia raised in control reached its maturity at the age of around 6 days old. However, the animals fed with toxic *M. aeruginosa* significantly delayed their maturation to the ages from 10 – 11 days (Fig. 4). Seriously, some organisms in the *Microcystis* treatments were not able to reach their maturation although they were alive during 21 days of experiment. The negative effect of MCs on maturation of the tested organisms in this study was similar to the investigation of Dao et al. (2010).

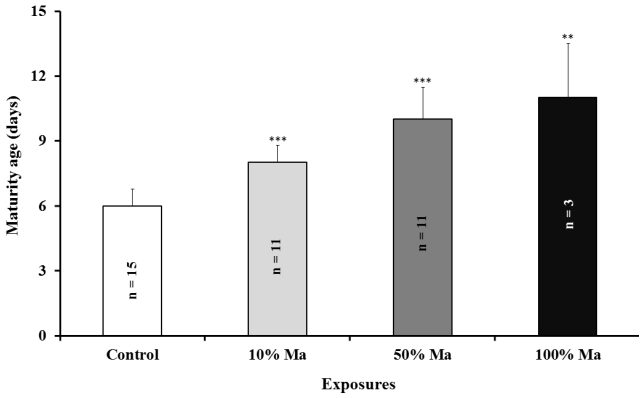

Figure 4. Maturation of *D. magna* (mean value ± SD of n as indicated in the columns) from control and exposures during 21 days of incubation. Asterisks indicate significant difference by Kruskal-Wallis test (, p < 0.01; ***, p < 0.001). Abbreviation as in Table 1.**

Some studies reported that cyanobacteria do not have enough nutrient and energy for development and maturation of the animals, mainly due to the absence of essential polyunsaturated fatty acids (PUFA) and sterols (Bret and Muller-Navarra, 1997; Von Elert, 2002). Therefore, the mal-nutrient effect should be the root for the maturation postponement of *D. magna* in 100% *Microcystis* treatment of our study. However, as green alga *Scenedesmus* is a good food for *Daphnia*, the delayed maturation in 10% and 50% *Microcystis* treatments could be partly because of the mal-nutrient and partly because of toxic compounds in the *Microcystis* cells (e.g. MC-RR, and other bioactive compounds) affecting the animal physiology. Additionally, during the experiment, we observed the smaller body size of the *Microcystis* exposed *Daphnia* compared to the control. Green (1956) and Ebert (1991) reported that smaller *Daphnia* took more instars to mature consequently late maturation than the larger *Daphnia.*

3.4 Effects of *M. aeruginosa* on the reproduction of *D. magna*

In the control, the clutch size of mother *D. magna* was around 10 offspring. However, the clutch size of mother *D. magna* was decreased by in *Microcystis* treatments. The average number of offspring per clutch in both 10% and 50% *Microcystis* treatments were approximately 5 individuals whereas that in 100% *Microcystis* treatment was only 1 individual (Fig. 5).

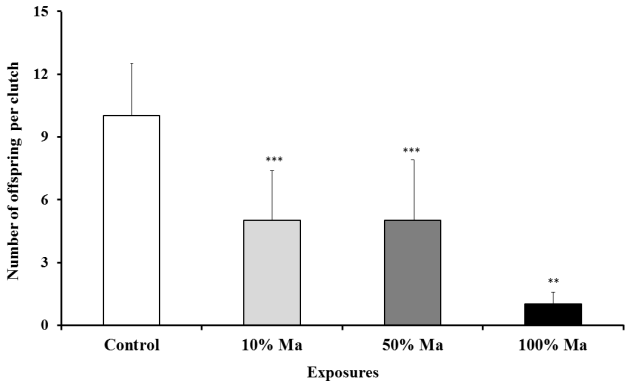

Figure 5. Fecundity of *D. magna* (mean value ± SD of n as indicated in the columns) from control and exposures during 3 weeks of incubation. Asterisks indicate significant difference by Kruskal-Wallis test (, p < 0.01; ***, p < 0.001). Abbreviation as in Table 1.**

The Kruskal-Wallis test again indicated the significant effects between the *Microcystis* treatments and control (p < 0.01, Fig. 5). In addition, during three weeks of experiment, the total accumulative offspring in the control were highest, 637 offspring. However, that in the *Microcystis* treatments decreased considerably. In the exposures to 10%, 50% and 100% *Microcystis* treatments, the total offspring were 97, 83 and 4, respectively (Table 2).

Table 2. Accumulative neonates of *D. magna* after three weeks of incubation. Abbreviation as in Table 1

	Control	10% Ma	50% Ma	100% Ma
Total offspring	637	97	83	4

The record in our study revealed negative effects of *M. aeruginosa* on *Daphnia* reproduction which are in line with previous investigations (Lürling and Van der Grinten, 2003; Dao et al., 2010). This result showed the seriously effects of MCs on population development for the next generations. Therefore, population of *D. magna* may be strongly reduced in case of cyanobacterial bloom lasting for a long time, consequently aquatic ecosystem may be unbalance, which needs further *in situ* investigation.

3.5 Effects of *M. aeruginosa* on the intrinsic population rate of *D. magna*

As mentioned above, the *Microcystis* strongly affected on survivor, maturity and reproduction of the animals, consequently effects on the intrinsic population rate. *Daphnia* in all *Microcystis* treatments had significantly lower intrinsic rate of population than the control. The intrinsic population rate of *Daphnia* in the control was 0.295 whereas those in 10%, 50% and 100% *Microcystis* treatments were 0.127, 0.041, 0.003, respectively (Fig. 4). This result is in line with results of previous investigations reporting the negative effects of *Microcystis* on fitness of *D. magna* (de Bernardi and Guisanni, 1990; Gustafson et al., 2005).

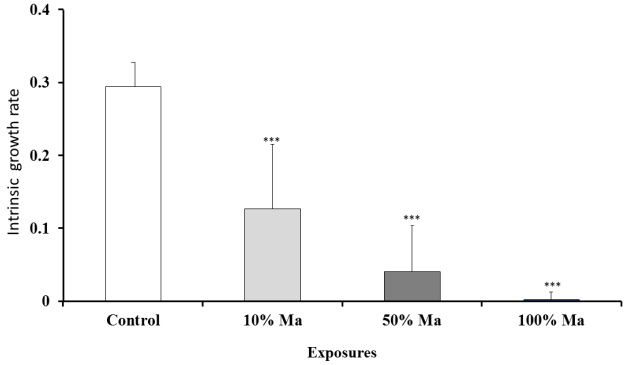

Figure 5. The intrinsic growth rate of *D. magna* (mean value ± SD of n as indicated in the columns) from control and exposures during 3 weeks of incubation. Asterisks indicate significant difference by Kruskal-Wallis test (, p < 0.01; ***, p < 0.001). Abbreviation as in Table 1.**

4. Conclusions

The *M. aeruginosa* strain from the Dau Tieng Reservoir produced a high MCs concentration. Our study proved the negative effects of living cells of toxic *M. aeruginosa* on life history traits of *D. magna* including survival reduction, maturation delay, reproduction inhibition and intrinsic population rate reduction. To our knowledge, this is the first report on the chronically detrimental impacts of *M. aeruginosa* strain isolated from Vietnam on *D. magna*. The MCs or some bioactive compounds in cyanobacteria posed a serious risk to *D. magna* in particular and to aquatic organisms in general. Therefore, more attention to the presence, distribution in nature and impacts of cyanobacterial blooms on aquatic organisms should be paid to protect the aquatic environment quality and ecosystem balance.

5. Acknowledgement

This research is funded by Hochiminh City University of Technology – VNU-HCM and CARE-RESCIF initiative under grant number Tc-MTTN-2016-04, and Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106-NN.04-2014.69.

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