

EFFECTS OF NON-TOXIC FILAMENTOUS CYANOBACTERIA ISOLATED FROM TRI AN RESERVOIR ON *Daphnia*

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ABSTRACT

This study is aimed to examine whether the presence of non-toxic filamentous cyanobacteria can cause toxic effects on *Daphnia magna*. Six strains of *Oscillatoria perornata* were isolated from the Tri An Reservoir and cultured in our laboratory for investigation. The results revealed that all strains were negative with the *mcyA* molecular marker. The high performance liquid chromatography (HPLC) results showed that toxin was not detected in their culture products, indicating that these strains corresponded to non-toxin producing strains. However, the results of chronic assay indicated that these non-toxin producing *O. perornata* conferred toxic effects on the tested animals. The age at first reproduction of *D. magna* was delayed and the survival of *D. magna* decreased in proportional with the increase of the density of cells of *O. perornata* exposed. Significant differences in the life history responses were observed for *D. magna* exposed to *O. perornata*. These results suggested that bioactive secondary metabolites other than microcystins produced by the filamentous cyanobacteria *O. perornata* may contribute to the toxic effects on *Daphnia*. Besides cyanotoxins, other secondary metabolites must be taken into account when investigating the toxic effects of cyanobacteria.

Keywords: *Oscillatoria perornata*, chronic toxic effect, cyanobacterial crude extract, microcystin.

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INTRODUCTION

The increase of human activities along with deficient water management have led to the enhancement of eutrophication in inland waters used for recreational purposes and as drinking water sources (Pham & Utsumi, 2018). Water eutrophication characterized by high nutrient inputs (particularly nitrogen and phosphorus species) has enhanced the development of cyanobacteria in lakes and reservoirs, leading to formation of surface blooms that may accumulate as scum. The occurrence of cyanobacterial blooms (CYB) is becoming more frequent worldwide. CYBs have been reported in at least 108 countries and territories worldwide except Antarctica (Harke et al., 2016).

In freshwaters worldwide, the occurrence of CYBs are mainly caused by colonial *Microcystis* spp., filamentous *Planktothrix/Oscillatoria* spp. and *Anabaena* spp. (Figueiredo et al., 2004). These species are well-known to produce several secondary metabolite toxins such as microcystins, anatoxins and saxitoxins. However, blooms of cyanobacteria usually consist of toxic and nontoxic strains/species that cannot be distinguished by morphological observation methods because many strains of cyanobacteria appear to be identical under a microscope (Kardinaal et al., 2007; Pham et al., 2015). The detection of the presence of the *mcy* gene cluster has been widely used as means for distinguishing toxic and non-toxic genotypes in both environmental samples and axenic cultures.

In Vietnam, several studies have focused on morphological characterization and microcystin (MC) production for the identification of cyanobacteria species (Nguyen et al., 2007; Dao et al., 2010; Pham & Dang, 2019). The most commonly reported species is the colonial *Microcystis aeruginosa*, which have been reported from lake Thanh Cong, Huong River, Nui Coc, Tri An and Dau Tieng Reservoirs. Other studies have been recently reported the presence of toxins from Vietnam's waters (Dao et al., 2010; Duong et al., 2014; Pham et al., 2017).

The colonial *Microcystis* (*M. aeruginosa*, *M. botrys*, *M. wesenbergii*) and filamentous *Oscillatoria* (*O. agardhii*, *O. isothrix*, *O. rubescens*, *O. zahidii*) from freshwater in Vietnam have been reported to be toxic on aquatic plants and animals (Nguyen et al., 2007; Dang Dinh Kim et al., 2014; Dao et al., 2016).

In lake ecosystems, microcrustacean *Daphnia* is a filter-feeder, and can consumes planktonic cyanobacteria. These filter feeders are, therefore, seriously affected by the presence of toxic and nontoxic cyanobacteria presence in the water column (Dao et al., 2010). The toxic effects of cyanobacteria on *Daphnia* have been extensively investigated (Ferrão-Filho & Kozlowsky-Suzuki 2011; Herrera et al., 2015). Acute toxic effects of cyanobacteria on *Daphnia* including inhibition of filtration rate, decrease in swimming movements, and even death (Ferrão-Filho et al., 2009; Smutná et al., 2014). As for the chronic effects, decrease of fecundity and population growth rate have been reported (Dao et al., 2010; Herrera et al., 2015). However, previous studies have mainly focused on the cyanobacterium *Microcystis*, but little attention has been paid for the interaction between zooplankton and filamentous cyanobacteria.

The filamentous cyanobacteria *Oscillatoria* distribute widely in many lakes, rivers and reservoirs worldwide. However, little is known about their toxicity in the aquatic environment. In this study, we isolated several strains of the *Oscillatoria* from the Tri An Reservoirs and maintained in the laboratory condition. Microscopic observation was used for morphological identification. The ability to produce cyanotoxins was examined with the *mcyA* gene fragment, and toxins concentration in culture was detected by high performance liquid chromatography (HPLC). The chronic toxic effects of non-toxic strains of *Oscillatoria* spp. on the freshwater *Daphnia magna* were investigated.

MATERIALS AND METHODS

Sample collection and isolation

Cyanobacteria samples were collected from the Tri An Reservoir between February and August of 2017 using a plankton net of 25 micron mesh size. In the laboratory, samples were observed under a microscope and three dominant groups including *Microcystis*, *Oscillatoria* and *Anabaena* were identified in the samples. Living and Lugol-fixed cyanobacterial samples were used for observation. Several single cyanobacterial trichomes of *Oscillatoria* spp. from living samples were isolated by micropipetting, washed, and cultured in Z8 medium (Pham et al., 2015). These cultures were grown at 28 °C under light conditions 12 hours: 12 hours (light:dark cycle) provided by 40-W fluorescent lamps, which generated an approximate luminous intensity of 50 $\mu\text{mol photons/m}^2/\text{s}$. Cultured biomass was collected at stationary phase for microcystins analysis according to the methods reported previously by Pham et al. (2015).

DNA extraction

Total genomic DNA was extracted from the culture following the methods of Hisbergues et al. (2003) with minor modifications. Briefly, 2 mL of cultures were centrifuged and suspended in TE buffer (50 mM Tris/HCl, 40 mM EDTA, pH 8.0). An aliquot of 30 μL of 10% SDS (sodium dodecyl sulfate) and proteinase K (final concentration: 100 $\mu\text{g/mL}$ in 0.5% SDS) was then added and incubated for 60 min at 37 °C. Then, 5 M NaCl (100 μL) and CTAB/NaCl solution (10% CTAB in 0.7 M NaCl) (80 μL) were added, and the samples were incubated for 10 min at 65 °C. DNA was then extracted twice with phenol:chloroform:isoamyl alcohol (25:24:1 v/v). After centrifugation for 5 min at 6.000 $\times g$ at 4 °C, the supernatant was collected and transferred to a fresh tube. The DNA was then rinsed with 1 mL of 70% ethanol and dried under vacuum. The final DNA sample was rehydrated in 20 μL of 1 \times TE buffer (10 mM Tris and 1 mM EDTA [pH 8.0]).

PCR amplification

To detect the presence of cyanobacterial DNA, the primer pair of CYA-F/CYA-R was used to amplify a 1.200 bp fragment of the 16s rDNA gene common to all cyanobacteria (Urbach et al., 1992). Microcystin is encoded by the microcystin synthetase (*mcy*) gene cluster that consists of 10 genes (*mcyA* to *mcyJ*). To detect the toxic strains, the microcystin synthetase region *mcyA* (297 bp) in *Oscillatoria*, was amplified using the primer pair of *mcyA*-Cd1F/*mcyA*-Cd1R (Hisbergues et al. 2003). The *mcyA* gene is suitable for detection of MC-producing cells of the genera *Anabaena*, *Microcystis*, and *Oscillatoria* (Hisbergues et al. 2003). The MC-producing cyanobacterium *M. aeruginosa* NIES-102 obtained from NIES Collection (Tsukuba, Japan) was used for positive controls. For each sample, duplicate PCR reactions were conducted. PCR reactions were prepared in a volume of 20 μL containing 2 μL of 10 \times Ex-Taq Buffer, 200 μM of each dNTP, 0.5 μL of each primer (10 μM), 0.5 U of Ex-Taq polymerase and 20 ng of template DNA. Amplification was performed in a Thermal Cycler (Applied Biosystems, Foster City, California, USA) with the following condition: initial denaturation at 95 °C for 5 min, 35 cycles [94 °C/1 min, 54 °C/1 min, 72 °C/1.5 min] and a final extension step at 72 °C for 10 min. PCR products were examined on 1.5% (w/v) agarose gels stained with Safe-Red™ and photographed under UV light.

Microcystins extraction and measurement

The MC in cyanobacteria were first extracted in 4 mL of 100% methanol (MeOH) and completed with the aid of sonication for 3 min and centrifugation at 1800 $\times g$ for 30 min. The supernatant was dried at room temperature, re-dissolved in 0.5 mL MeOH (100%) and centrifuged at 4000 $\times g$ for 5 min. The sample was then passed through a Minisart RC4 filter membrane (0.2 μm pore size, Sartorius, Germany), and kept at (-)20 °C prior analysis. To analyse the MCs content, a reverse-phase HPLC with UV-visible photodiode array (PDA) detector (Dionex

UltiMate 3000, Thermo Scientific, USA) was used. MCs were separated with a silica-based, reverse-phase C18 column (Acclaim^M 120 C18 5 μm , 4.6 \times 150 mm, USA) maintained at 40 °C. The samples were carried with a mobile phase consisting of methanol: 0.05 M phosphate buffer (pH 2.5; 50:50 v/v) at a flow rate of 0.65 mL/min. MC congeners were detected at 238 nm and identified on the basis of both their retention time and characteristic UV spectra. Three MCs (MC-LR, MC-RR, and MC-YR) purchased from Wako (Chuoku, Osaka, Japan), were used as standards. A detection limit of the HPLC system used is 0.12 $\mu\text{g/L}$.

Chronic toxicity assay

Daphnia magna from the MicroBioTests Inc, Belgium, maintained under a laboratory condition of the Institute of Tropical Biology, Ho Chi Minh City, for several months was used for the test. They were maintained in ISO medium and fed with a mixture of green algae *Chlorella* sp. and *Scenedesmus* sp., which were cultivated in COMBO medium. Both *Daphnia* and green algae were maintained in the laboratory at 25 \pm 1 °C, with a 14 h :10 h photoperiod (light: darkness).

Chronic toxicity assay was performed according to the Protocol 211 of the Organization for the Economical Cooperation and Development (OECD 2012) using 50 mL beaker cups containing 20 mL of ISO medium. Neonates of *D. magna* less than 24 hours-age were maintained individually in 50 mL beaker cups, and assigned to 3 different treatments with the density of 20 (treatment A), 50 (treatment B) and 100 (treatment C) \times 10³ cell/mL of *Oscillatoria* spp. and a control (containing only ISO medium). Densities of *Oscillatoria* spp. were determined by counting of Lugol's fixed sub-samples taken from each exposure at the starting, 6, 12, 24 and 48 hours. All samples were counted using the Sedgewick Rafter counting chamber on an inverted microscope. *Oscillatoria* densities were estimated by determining the length of 50 filaments and then dividing the total length of filaments by the length of one cell (4 μm)

(Desikachary, 1959). The control *D. magna* were fed with green algae *Chlorella* sp. and *Scenedesmus* sp. Each treatment contained 15 replicates (n = 15). Test solutions and cyanobacteria were renewed every second day. Mortality, maturation and production of live offspring were recorded daily. Each mother daphnid was checked daily for the numbers of neonates per clutch. Reproduction was calculated as total accumulated offsprings reproduced by all mother daphnids in each treatment. Fecundity was defined as the average number of offsprings in one clutch reproduced by one mother daphnid. The chronic tests lasted for 15 days.

Statistical analysis

Data on mortality, maturation and production of live offsprings were presented as the mean \pm SD. The significant difference between the exposure and control treatment was tested using one-way analysis of variance (ANOVA). When the ANOVAs were significant, the pair wise comparison using Tukey's honestly significant difference (HSD) post-hoc test was used to determine significant difference between the exposure and the control treatments. P values less than 0.05 were used for the significant difference.

RESULTS AND DISCUSSION

Isolation and morphological characteristics

In this study we aimed to investigate the toxicity of the filamentous cyanobacteria species *Oscillatoria perornata*. For this purpose, we selected and isolated several strains of this species. In total, 6 strains of *Oscillatoria perornata* have been successfully isolated from the Tri An Reservoir and maintained in cultures (Fig. 1). *Oscillatoria* is a common group of cyanobacteria in many lakes and rivers worldwide. In Vietnam, the presence of *O. perornata* has only been reported from Huong and Nhu Y Rivers (Nguyen et al., 2007), Tri An Reservoir and La Nga River (Luu & Nguyen, 2008). *Oscillatoria* is a major proportion in the phytoplankton in ponds and reservoirs in Vietnam (Pham et al., 2017).



Figure 1. Morphological appearance of *Oscillatoria perornata*. Scale bar: 10 μm

Detection of microcystin synthetase genes and quantification of microcystins with HPLC

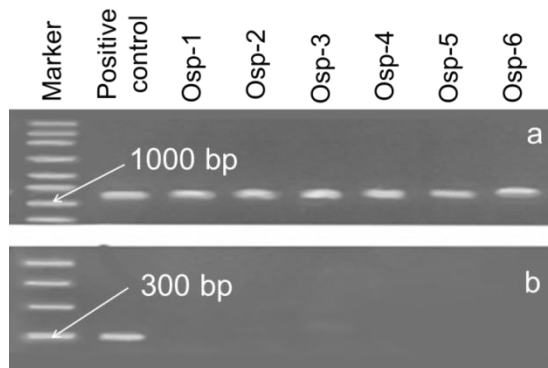


Figure 2. PCR products of agarose electrophoresis gels of (a) 16S rDNA amplification products, and (b) *mcyA* amplification products. Marker: PHY Marker; Positive control: *M. aeruginosa* NIES-102; Osp-1 to Osp-6 indicated 6 strains of *O. perornata* isolated from Tri An Reservoir

The presence of the cyanobacterial-16S rDNA fragment was examined for all strains of *O. perornata*. The results indicated that the 16S rDNA fragments are presented in all strains confirming that all strains examined were cyanobacteria (Fig. 2a). The ability to produce MCs was examined by the presence of the *mcyA* gene. The results showed that the

mcyA fragment was not amplified in all isolated strains of *O. perornata*, indicating that these strains are the non-toxin producers (Fig. 2b). The HPLC results also indicated that toxin was not detected in these cultures (Fig. 3).

Till now, little is known about the toxicity of tropical cyanobacterial microflora, especially of *Oscillatoria* species. Most of the previous studies on toxicity and MCs production of cyanobacteria from Vietnam waters were only about *Microcystis*. Only few studies have been conducted on other cyanobacteria like *Oscillatoria* or *Anabaena*. Pham et al. (2015) isolated about 70 strains of cyanobacteria from Dau Tieng Reservoir and examined their microcystin producing ability. The strains examined were mainly *Microcystis*, *Anabaena*, *Arthrospira* and *Cylindrospermopsis*, but *Oscillatoria* were not included. Dao et al. (2010) also cultured several strains of *Microcystis*, *Aphanizomenon*, *Anabaena* and *Cylindrospermopsis* from Tri An Reservoir, but *Oscillatoria* species were not included. Although many species of *Oscillatoria* were presented in the Tri An Reservoir and also in the La Nga and Dong Nai Rivers, molecular analyses as well as toxicity of those *Oscillatoria* species have never been reported. In the present study, the ability of 6 strains of *O. perornata* to synthesize microcystins was examined, but none of them has the portion of *mcyA* gene. These results are in agreement with the previous report that *Microcystis* species were the main toxin producing species in the Vietnamese waters.

Several strains of *Oscillatoria* are known to produce microcystin (Sivonen et al., 1990; Chorus & Bartram, 1999). The most frequently reported is the ability of *O. agardhii* to produce demethylated microcystins (containing d-Asp and/or dehydroalanine). Brittain et al. (2000) reported a toxic *O. tenuis*, strain E6 with the ability to produce a fully methylated microcystin (MC-LR) and a new l-homoarginine containing microcystins (MC-

LHArg). From Vietnam water the ability of some strains of *Oscillatoria* or (*Planktothrix*) isolated from a fish pond in Soc Trang Province to produce toxin has been reported (Dao et al., 2016). Many filamentous cyanobacteria from urban lakes in Mexico were reported to produce microcystins

(Pineda-Mendoza et al., 2012). Probably, the ability of filamentous cyanobacteria from Vietnam waters to produce toxic compounds was underestimation. Future research on toxin producing ability of filamentous cyanobacteria genera/species from various habitats in Vietnam is recommended.

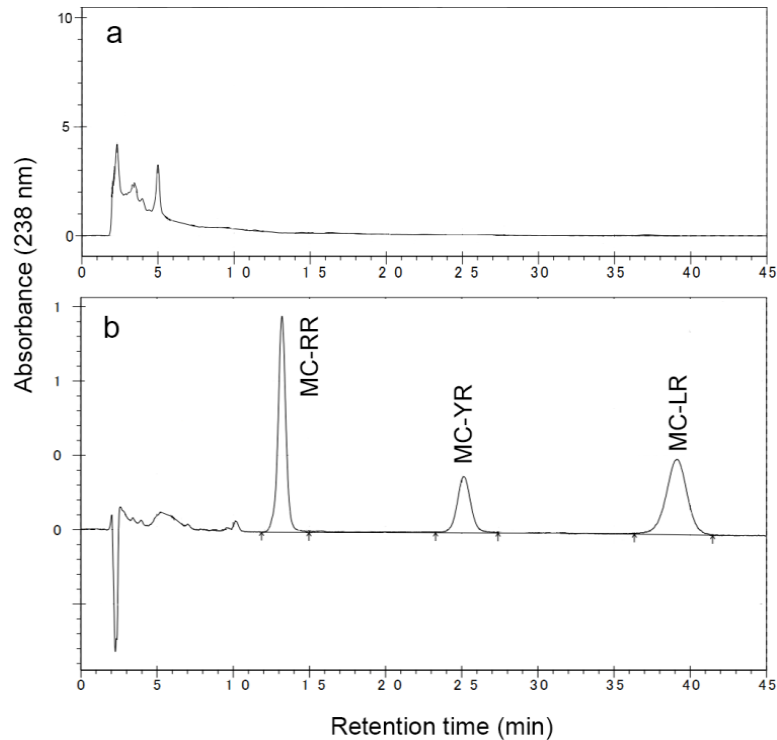


Figure 3. HPLC patterns of (a) *Oscillatoria perornata* and (b) microcystin standards

Chronic toxicity of *Oscillatoria perornata* on *Daphnia magna*

D. magna could grow well in the control condition with the mortality of less than 15% that meets well with the requirement for the chronic test. The chronic effects of *O. perornata* on the survival and reproduction of *D. magna* during 15 days of culture were shown in Figure 4. The survival of *D. magna* exposed with *O. perornata* decreased with the increase of the density of *O. perornata* in the culture. Significant differences in life history responses were observed for *D. magna* exposed to *O. perornata*. Exposure to *O. perornata* at concentration of 20×10^3 cell/mL, 50×10^3

cell/mL, and 100×10^3 cell/mL caused 33%, 48 and 54% reduction of survival, respectively, on Day 15 (Fig. 4).

The maturity age and the number of offspring per female of *D. magna* cocultured with different densities of *O. perornata* are shown in the figure 4. The growth of *D. magna* determined by the time to first reproduction was retarded by the presence of *O. perornata* (Fig. 5a) and the reproduction determined by the number offspring per female was inhibited by the presence of *O. perornata* in a dose-dependent manner (Fig. 5b). As shown in the survival curve above, many parent daphnids in the exposure treatments died before the first reproduction.

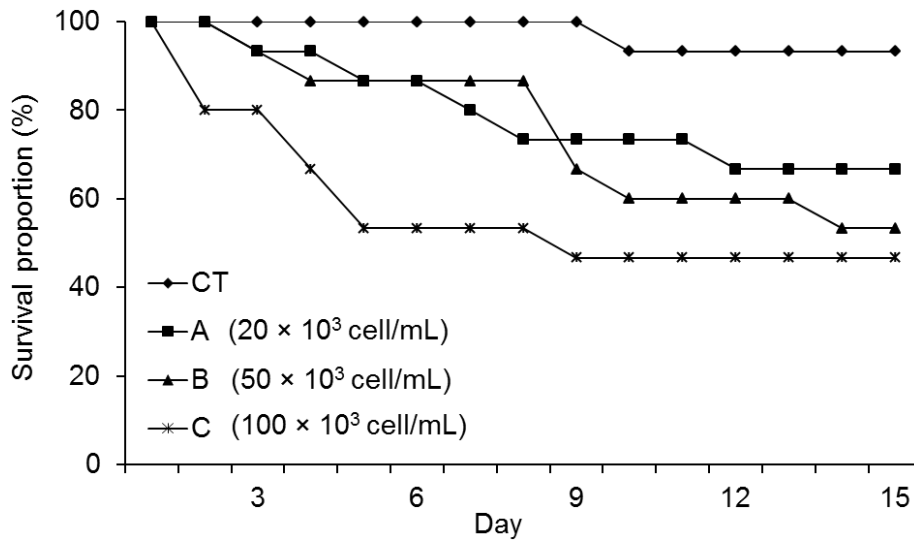


Figure 4. Chronic effects of *O. perornata* on the survival of *D. magna*
 Note: CT: contained only ISO medium.

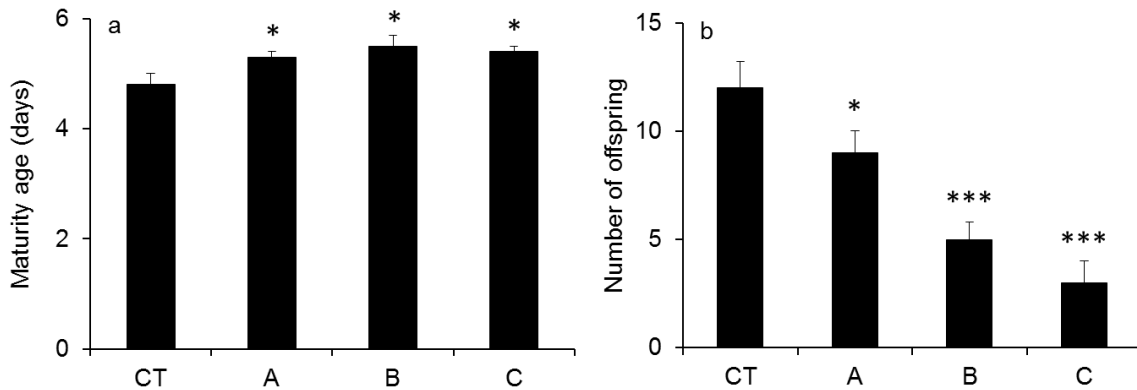


Figure 5. Maturity age (a) and number of offspring per female (b) of *D. magna* during exposure to different density of *O. perornata*. CT: control (contained only ISO medium); A: 20 × 10³ cell/mL; B: 50 × 10³ cell/mL; and C: 100 × 10³ cell/mL

Many cyanobacteria species are harmful to freshwater herbivorous grazers such as *Daphnia* (Kuster & Von-Eler, 2013). Colonial or filamentous cyanobacteria can mechanically interfere with *Daphnia*'s filtering apparatus (Shams et al., 2014). Most of cyanobacteria lack essential sterols and sufficient amounts of polyunsaturated fatty acids and therefore cyanobacteria are a nutritionally inadequate food source for cladocerans (Martin-Creuzburg, 2008). In addition, the production of some toxic secondary metabolisms may significantly reduce the fitness of *Daphnia*

(Lürling & Vander, 2003). Oberhaus et al. (2007) reported that *Daphnia* could be grazing on filamentous cyanobacteria *Planktothrix rubescens*, but they prefer to graze only small filaments. In the present study, population growth rates of *D. magna* fed with different concentration of *O. perornata* were reduced in the dose-dependent manner. The negative effects of *O. perornata* on the survival and reproduction of *D. magna* may reflect the interference of the filtering apparatus of *D. magna* and an inadequate food source of *O. perornata*.

Previous studies showed that filamentous cyanobacteria have a negative effect on *Daphnia* because of the interference of filaments with grazing on other available food sources (Kurmayer & Jüttner, 1999; Shams et al., 2014). In addition, besides the toxic microcystins, a large number of bioactive oligopeptides have been identified and reported from *Planktothrix* (*Oscillatoria*) that show the inhibitory effects on serine proteases or other bioactive potential in animal cells (Kurmayer et al., 2016). These bioactive secondary metabolites could contribute to the overall toxic effects. Similarly, Smutná et al. (2014) reported that, when *Daphnia* were exposed to different toxic and non-toxic biomass of cyanobacteria, both toxic and non-toxic biomass conferred toxic effects on the tested animals, but the effects observed in the acute and chronic assays were independent of the samples' microcystin contents. They pointed out the importance of cyanobacterial components other than microcystins, such as lipopolysaccharides, various peptides and depsipeptides, polar alkaloid metabolites or other unidentified metabolites in the overall ecotoxicity of cyanobacterial biomass. Results of the present study are consistent with previous observations that non-microcystin bioactive secondary metabolites in filamentous cyanobacteria *Oscillatoria* may contribute significantly to the toxic effects on *Daphnia*. It is strongly recommended further investigations to elucidate non-microcystin bioactive compounds in *Oscillatoria*.

CONCLUSION

Results of the present study indicated that the species *O. perornata* isolated from the Tri An Reservoir were non-toxic strains. However, the living biomass of the non-toxic *O. perornata* caused significantly chronic toxic effects on *D. magna*. Higher densities have generated greater toxic effects on the test animals, suggesting that other second metabolites than cyanotoxins are likely to be responsible for the adverse effects. The mechanisms of toxicity of these unknown compounds remains to be determined. Other toxic and unknown compounds must be taken

into account when investigating the toxic effects of cyanobacteria.

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REFERENCES

- Brittain S., Mohamed Z. A., Wang J., Lehmann V. K. B., Carmichael W. W., Rinehart K. L., 2000. Isolation and characterization of microcystins from a River Nile strain of *Oscillatoria tenuis* Agardh ex Gomont. *Toxicon*, 38(12): 1759–1771.
- Chorus I., Bartram J., 1999. Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management, Published on behalf of WHO, Spon Press, London.
- Dao T. S., Cronberg G., Nimptsch J., Do-Hong L. -C., Wiegand C., 2010. Toxic cyanobacteria from Tri An Reservoir, Vietnam. *Nova Hedwigia*, 90(3-4): 433–448.
- Dao T. S., Tran P. T., Nguyen T. T. L., Nguyen T. S., Bui B. T., 2016. First report on toxicity of the cyanobacterium *Planktothrix rubescens* isolated from a fish pond in Soc Trang province. *Journal of Biology*, 38(1): 115–123. (in Vietnamese with English summary).
- Dang Dinh Kim, Duong Thi Thuy, Nguyen Thi Thu Lien, Dao Thanh Son, Le Thi Phuong Quynh, Do Hong Lan Chi, 2014. Toxic cyanobacteria in freshwater. Publishing House of Natural Science and Technology, 325 pp. (in Vietnamese).
- Desikachary T. V., 1959. Cyanophyta. Indian council of agriculture research, New Delhi.
- Duong T. T., Jähnichen S., Le T., Ho C., Hoang T., Nguyen T., Vu T., Dang, D. K., 2014. The occurrence of cyanobacteria and microcystins in the Hoan Kiem Lake and the Nui Coc reservoir (North Vietnam). *Environ. Earth Sci.*, 71(5): 2419–2427.

- Ferrão-Filho A. D. S., Kozłowsky-Suzuki B., 2011. Cyanotoxins: Bioaccumulation and effects on aquatic animals. *Mar. Drugs*, 9(12): 2729–2772.
- Ferrão-Filho A. D. S., Soares M. C. S., Magalhães D. F. V., Azevedo S. M. F. O., 2009. Biomonitoring of cyanotoxins in two tropical reservoirs by cladoceran toxicity bioassays. *Ecotoxicol. Environ. Saf.*, 72(2): 479–489.
- Figueiredo D. R., Azeiteiro U. M., Esteves S. M., Goncalves F. J., Pereira M. J., 2004. Microcystin-producing blooms - a serious global public health issue. *Ecotoxicol. Environ. Saf.*, 59(2): 151–163.
- 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: 4–20.
- Herrera N. A., Echeverri L.F., Ferrão-Filho A. S., 2015. Effects of phytoplankton extracts containing the toxin microcystin-LR on the survival and reproduction of cladocerans. *Toxicon*, 95: 38–45.
- Hisbergues M., Christiansen G., Rouhiainen L., Sivonen K., Börner T., 2003. PCR-based identification of microcystin-producing genotypes of different cyanobacterial genera. *Arch. Microbiol.*, 180(6): 402–410.
- Kardinaal W. E. A., Janse I., Agterveld M. K. V., Meima M., Snoek J., Mur L. R., Huisman J., Zwart G., Visser P. M., 2007. *Microcystis* genotype succession in relation to microcystin concentrations in freshwater lakes. *Aquat. Microb. Ecol.*, 48(1): 1–12.
- Kurmayer R., Juttner F., 1999. Strategies for the co-existence of zooplankton with the toxic cyanobacterium *Planktothrix rubescens* in Lake Zurich. *J. Plankton Res.*, 21: 659–683.
- Kurmayer R., Deng L., Entfellner E., 2016. Role of toxic and bioactive secondary metabolites in colonization and bloom formation by filamentous cyanobacteria *Planktothrix*. *Harmful Algae*, 54: 69–86.
- Kuster C. J., Von-Eler E., 2013. Interspecific differences between *D. pulex* and *D. magna* in tolerance to cyanobacteria with protease inhibitors. *PLoS One*, 8(5): e62658.
- Luu T. T. N., Nguyen T. T., 2008. Planktonic cyanobacteria species composition and seasonal change in La Nga river. *Journal of Science and Development*, 11(07). (in Vietnamese).
- Lüring M., Vander G. E., 2003. Life-history characteristics of *Daphnia* exposed to dissolved microcystin-LR and to the cyanobacterium *Microcystis aeruginosa* with and without microcystins. *Environ. Toxicol. Chem.*, 22: 1281–1287.
- Martin-Creuzburg D., Von-Elert E., Hoffmann K. H., 2008. Nutritional constraints at the cyanobacteria-*Daphnia magna* interface: The role of sterols. *Limnol. Oceanogr.*, 53: 456–468.
- Nguyen L. T. T., Cronberg G., Larsen J., Moestrup Ø., 2007. Planktic cyanobacteria from freshwater localities in Thua Thien-Hue Province, Vietnam I. Morphology and distribution. *Nova Hedwigia*, 85: 1–34.
- Oberhaus L., Gélinas M., Pinel-Alloul B., Ghadouani A., Humbert J. F., 2007. Grazing of two toxic *Planktothrix* species by *Daphnia pulex*: potential for bloom control and transfer of microcystins. *J. Plankton Res.*, 29(10): 827–838.
- OECD, 2012. OECD guideline for the testing of chemicals, number 2011, *Daphnia magna* Reproduction Test.
- Pham T. L., Dang T. N., 2019. Microcystins in freshwater ecosystems: Occurrence, distribution, and current treatment approaches, in Water and wastewater treatment technologies, edited by Bui X. T., Chiemchaisri C., Fujioka T., Varjani S. pp. 15–36, Springer Singapore, Singapore.

- Pham T. L., Utsumi M., 2018. An overview of the accumulation of microcystins in aquatic ecosystems. *J. Environ. Manage.*, 213: 520–529.
- Pham T. L., Dao T. S., Tran N. D., Nimptsch J., Wiegand C., Motoo U., 2017. Influence of environmental factors on cyanobacterial biomass and microcystin concentration in the Dau Tieng Reservoir, a tropical eutrophic water body in Vietnam. *Ann. Limnol-Int. J. Lim.*, 53: 89–100.
- Pham T. L., Dao T. S., Shimizu K., Lan-Chi D. H., Utsumi M., 2015. Isolation and characterization of microcystin-producing cyanobacteria from Dau Tieng Reservoir, Vietnam. *Nova Hedwigia*, 101(1–2): 3–20.
- Pineda-Mendoza R. M., Olvera-Ramírez R., Martínez-Jerónimo F., 2012. Microcystins produced by filamentous cyanobacteria in urban lakes. A case study in Mexico City. *Hidrobiológica*, 22(3): 290–298.
- Shams S., Cerasino L., Salmaso N., Dietrich D. R., 2014. Experimental models of microcystin accumulation in *Daphnia magna* grazing on *Planktothrix rubescens*: Implications for water management. *Aquat. Toxicol.*, 148 (Supplement C): 9–15.
- Sivonen K., 1990. Effects of light, temperature, nitrate, orthophosphate, and bacteria on growth of and hepatotoxin production by *Oscillatoria agardhii* strains. *Apply. Environ. Microbiol.*, 56: 2658–2666.
- Smutná M., Babica P., Jarque S., Hilscherová K., Maršálek B., Haeba M., Bláha L., 2014. Acute, chronic and reproductive toxicity of complex cyanobacterial blooms in *Daphnia magna* and the role of microcystins. *Toxicon*, 79(0): 11–18.
- Urbach E., Robertson D. L., Chisholm S. W., 1992. Multiple evolutionary origins of prochlorophytes within the cyanobacterial radiation. *Nature*, 355(6357): 267–270.