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Title: Toxicity and toxin composition of *Microcystis aeruginosa* from Wangsong reservoir

Running title: Wangsong *Microcystis aeruginosa* toxicity

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Abstract

Objective

The increasing world population, resulting in increased anthropogenic water pollution, is negatively impacting the limited available water resources. In South Korea, this similarly affects the water quality of reservoirs. As water is a basic necessity for life, water quality monitoring is essential but typically does not include toxicity testing. However, as toxic bloom event frequencies are increasing, this previously neglected aspect becomes pertinent. Therefore, in the present study, the toxin composition and toxicity of a *Microcystis aeruginosa* strain isolated from a persistent bloom in lake Wangsong, South Korea, was investigated.

Methods

A combination of bioassays and chemical analysis was used for this purpose. The bioassay species included terrestrial and aquatic plants, an alga, a rotifer, a tubificid annelid, and crustaceans, representing various trophic levels.

Results

The strain was found to produce microcystin-LR, -RR, and YR, as well as β -N-methylamino-L-alanine. The bioassays indicated that the primary producers were less sensitive to the crude extract.

Conclusion

The presence or absence of a visible cyanobacterial bloom is also not an indication of the toxins that may be present in the afflicted waters, and thus does not predict exposure risk. Similarly, the presence and absence of toxins and mixtures thereof does not indicate the ecological effect. Therefore, it would be advantageous to include toxicity testing into routine water testing regimes to better understand the impact of harmful algal blooms.

Keywords:

Cyanobacteria, microcystin congeners, bioassays, toxicity

1. Introduction

Eutrophication, accepted as the main reason for the outbreak of potentially toxic cyanobacterial blooms¹, is also one of the principal driving factors for bloom formation in South Korea² where, in general, the four major rivers Han, Geum, Nakdong, and Yeongsan, are most heavily affected³⁻⁵. As they also function as potable water sources and are used for recreational purposes, the water quality is a major focus in these rivers and the lakes they collect into⁶. Typically, lake water quality and the trophic state thereof are evaluated using a variety of parameters including pH, total organic carbon, chlorophyll-a, total phosphorus, and turbidity⁷, but not toxin content or toxicity. In terms of toxin content, microcystin concentrations of 0.057 $\mu\text{g L}^{-1}$ up to 2612 $\mu\text{g L}^{-1}$ have been detected in these different river systems^{5,8}, however, to date toxicity testing seems to have been neglected. Aside from microcystins (MCs), anatoxin-a has been detected in the Daecheong reservoir⁹, yet toxin characterization data for the Wangsong lake, a major urban reservoir, is lacking.

The Wangsong reservoir, a shallow eutrophic reservoir located in Uiwang City, was built to secure a stable water resource for the area and is classified as a water supply, as a recreational feature, and is used for industrial purposes, as well as agricultural and landscape irrigation^{10,11}. The dam was also constructed as a flood control mechanism and for hydroelectric power generation. Due to ongoing expansion and housing projects, pollution of the Wangsong reservoir has steadily increased, accompanied by cyanobacterial bloom formation⁷. Hence, great attention has been paid to water quantity and quality problems of the reservoir.

Cyanotoxins constitute a threat to the health of humans in contact with contaminated waters since they have toxic effects in living organisms¹². *Microcystis aeruginosa* is the most common bloom-forming cyanobacterial species in freshwaters and has the ability to produce secondary metabolites such as the potent hepatotoxins, especially MCs¹³. To date, the dominant cyanobacterial genera which occur in the four main river systems in South Korea include

78 *Microcystis*, *Anabaena*, and *Oscillatoria*^{3,5,8,14}, with microcystin-LR, -RR, and -YR as the most
79 frequently detected MC isomers³.

80 Most of the available studies describe the toxic effects of single MCs in aquatic organisms such
81 as fish species, cladocerans, and mussels¹⁵⁻²⁰. Only a few studies include exposure of
82 phytoplankton and macrophytes to crude extracts of *M. aeruginosa*, also evaluating the
83 oxidative stress responses, which resemble a closer approach to actual environmental
84 scenarios²¹⁻²⁴. Information regarding how water quality affects primary producers will
85 furthermore shed light on how higher trophic levels will be affected.

86 The aim of the present study was to elucidate the toxin composition of the *M. aeruginosa* strain
87 isolated from the Wangsong reservoir, South Korea. Besides the toxin composition, the
88 potential toxicity was evaluated using different bioassay systems, thereby assessing the
89 potential health risk at various trophic levels.

2. Results and discussion

2.1. Culture toxin composition

The seasonal variation of *Microcystis* species in South Korean reservoirs has previously been monitored^{11,25}.

In the aqueous cell-free crude extract of the *M. aeruginosa* strain, three different microcystin congeners in total, namely MC-LR, MC-RR, and MC-YR, were detected (Fig. 1). The highest concentrations were detected for MC-LR ($176.35 \mu\text{g g}^{-1}$) followed by MC-RR ($50.27 \mu\text{g g}^{-1}$) and the lowest concentration for MC-YR ($9.25 \mu\text{g g}^{-1}$). β -N-methylamino-L-alanine (BMAA) was detected and quantified amounting to an average concentration of $0.906 \pm 0.016 \mu\text{g g}^{-1}$. In the extract, neither anatoxin-a nor cylindrospermopsin was detected by the employed quantitative analysis methods.

Figure 1: Cyanobacterial toxin composition of the *M. aeruginosa* KW strain isolated from Wangsong reservoir (South Korea). Data represent mean toxin concentration $\pm$ standard deviation ($n = 4$)

2.2. Toxicity analysis using commercial and non-commercial assays

109 The toxicity of the crude extract in various dilutions was tested using various commercially
110 available TOXKITS in combination with non-commercially available bioassays such as the
111 toxicity towards *T. tubifex* and the oxidative stress status in aquatic macrophytes.
112 Using the commercial TOXKIT bioassays (Table 1), the aqueous crude extract resulted in a
113 relatively high toxicity response using the THAMNOTOX-FTM kit with an LC₅₀ amounting to
114 0.1 µg L⁻¹ followed by the DAPHTOX pulex kit with an EC₅₀ of 1.1 µg L⁻¹ and therefore 10-
115 fold less sensitive compared to the THAMNOTOX-FTM kit. The 24-h LC₅₀ for the strain
116 obtained using the THAMNOTOX-FTM kit corresponded to previously reported toxicities for
117 *M. aeruginosa* isolated from Hungary, Germany and Brazil²⁶. The toxicity of the extract was
118 much 8.7 times higher than the previously reported toxicity of an *M. aeruginosa* extract with
119 *Daphnia pulex* (48-h LC₅₀ 9.6 µg ml⁻¹)²⁷. The ALGALTOX (EC₅₀ of 3.7 ± 1.2 µg ml⁻¹) and
120 PHYTOTOX kits (average IC₅₀ of 3.9 µg ml⁻¹) demonstrated the lowest responses with the
121 crude extract exposure, demonstrating lower sensitivities for primary producers. Previously, an
122 IC₅₀ of 3 mg ml⁻¹ was reported for *M. aeruginosa* using the Blue-green *Sinapis alba* test²⁸,
123 approximately a 1000-fold higher concentration. Using the TUBIFEX toxicity test the
124 sensitivity towards the crude extract was similar to that obtained with the DAPHTOX pulex kit,
125 interestingly as both as primary consumers.

126 **Table 1:** Determination of LC₅₀, EC₅₀ and IC₅₀ using various bioassays, commercially available ones as
 127 well as others.

Bioassay	Test organisms	Trophic level	Test outcome (LC ₅₀ , EC ₅₀ , IC ₅₀ *)	Toxicity as total MC concentration (µg MC ml ⁻¹)
THAMNOTOX-F™	<i>Thamnocephalus platyurus</i>	Primary consumer	24-h LC ₅₀	0.1 ± 0.2
ROTOTOX-F	<i>Brachionus calyciflorus</i>	Primary consumer	24-h EC ₅₀	6.5 ± 1.2
DAPHTOX pulex	<i>Daphnia pulex</i>	Primary consumer	24-h EC ₅₀	1.1 ± 0.5
TUBIFEX TOX	<i>Tubifex tubifex</i>	Detritivore	24-h EC ₅₀	1.5 ± 0.7
ALGALTOX	<i>Pseudokirchneriella subcapitata</i>	Primary producer	72-h EC ₅₀	3.7 ± 1.2
PHYTOTOX	<i>Sorghum saccharatum</i>	Primary producers	72-h IC ₅₀	3.4 ± 0.5
	<i>Sinapis alba</i>	producers	72-h IC ₅₀	4.4 ± 0.9
	<i>Lepidium sativum</i>		72-h IC ₅₀	3.9 ± 1.2

128 * LC₅₀ = lethal concentration, EC₅₀ = effect concentration, IC₅₀ = inhibitory concentration

129

130 Morphological changes monitored in three different aquatic macrophytes exposed to the bloom
 131 extract showed severe changes only in *P. perfoliatus* for which all plants became chlorotic
 132 within the exposure time of 14 days (Table 2). *C. demersum*, as well as *L. sessiliflora*, did not
 133 show any visible effects; however, in *L. sessiliflora* the leaves seemed to crinkle more than
 134 compared to the control (Table 2).

135 **Table 2:** Altered morphology of macrophytes exposed to cyanobacterial cell-free crude extract
136 containing MCs at a concentration of 50 µg L⁻¹ for 14 days

	<i>Ceratophyllum demersum</i>	<i>Limnophila sessiliflora</i>	<i>Potamogeton perfoliatus</i>
Control
MCs exposure (50 µg L ⁻¹)

Figure 2: Oxidative stress response monitored as changes in cellular H_2O_2 level in three submerged macrophytes: *C. demersum* (A), *L. sessiliflora* (B) and *P. perfoliatus* (C) during 14-day exposure to a cyanobacterial cell-free crude extract containing $50\ \mu g\ L^{-1}$ total MCs. Data represent average H_2O_2 content $\pm$ standard deviation ($n = 3$); * denotes statistical significance compared to the control ($p < 0.05$)

Significantly enhanced H_2O_2 levels compared to the control ($p < 0.05$; Fig. 2) were evident for *C. demersum* and *P. perfoliatus* from the onset of exposure, however, the H_2O_2 content only increased for *L. sessiliflora* after 1 hour of exposure ($p > 0.05$; Fig. 2). For *C. demersum* and *P. perfoliatus*, the H_2O_2 content increased until 24 hours of exposure, indicating that the level of

reactive oxygen species started to exceed the anti-oxidative capacity of the plants, where after the H₂O₂ decreased, hinting at recovery. However, after 14 days, the normal H₂O₂ level, as seen in the control, was not regained.

The aquatic macrophytes indeed showed adverse effects due to exposure the crude extract containing a concentration of 50 µg ml⁻¹ total MC. However, compared to the PHYTOTOX kits, for which an average IC₅₀ of 3.9 µg ml⁻¹ was achieved, the aquatic macrophytes seemed less sensitive as plant death was only observed in exposures with *P. perfoliatus* albeit the 12.8-fold higher concentration.

The results show the importance of testing toxicity at various trophic levels as the different organism displayed different sensitivities. In the present study, primary producers were found to be less sensitive to a crude extract containing MC, compared to primary consumers and detritivores such as for example the *T. platyurus*, *T. tubifex*, and *D. pulex*. In general, the strain was found to be in some cases equally toxic (as seen with *T. platyurus*) and in others more toxic (as seen with *D. pulex*) compared to blooms reported elsewhere. The study illustrates that toxicity testing is an essential test parameter that should be considered together with routine water quality evaluations.

3. Material & Methods

3.1. Cyanobacterial Strain and Crude Extract

Samples were collected from the Wangsong reservoir, South Korea, during a bloom event between July and October in 2007. The bloom consisted mainly of *M. aeruginosa* with a minor proportion of other cyanobacteria such as *Anabaena* and *Oscillatoria*. The strain, *M. aeruginosa* KW, was isolated from the bloom material and cultivated in 1 L Erlenmeyer flasks containing 500 mL of BG 11 medium²⁹ under 30-40 mmol photon m⁻² s⁻¹ with a photoperiod of 14:10 h photoperiod at 22 ± 1°C. Culture purity was evaluated microscopically using brightfield. The crude extracts were prepared as described by Romero-Oliva et al.³⁰.

3.2. Analytics of the cyanobacterial toxins

Microcystin congener (MC-LR, -RR, and -YR) determination and quantification were performed as detailed in Romero-Oliva et al.³⁰. Calibrations were linear ($R^2 = 0.999$) between 5 and 500 $\mu\text{g L}^{-1}$. Limit of detection (LOD) was set at 1 ng mL⁻¹ (signal to noise S/N > 3) and limit of quantification at 5 ng mL⁻¹ (S/N > 5) for all MCs congeners.

Anatoxin-a chromatographic detection and quantification was performed as detailed in Ha et al.³¹. Calibrations were linear ($R^2 = 0.999$) between 5 and 250 $\mu\text{g L}^{-1}$. LOD and LOQ were 1 (S/N > 3) and 5 $\mu\text{g L}^{-1}$ (S/N > 5), respectively.

BMAA was detected and quantified after derivatization using a Phenomenex EZ:Faast kit as detailed by Esterhuizen-Londt et al.³². Calibrations were linear between 0.1 and 1000 $\mu\text{g L}^{-1}$, with the limit of detection set at 100 fg on column (S/N > 3) and the limit of quantification set at 1 pg on column (S/N > 5).

Chromatographic detection and quantification of CYN were performed as detailed by Esterhuizen-Londt et al.³³. Calibrations for this method were linear ($R^2 = 0.998$) between 0.01 and 100 $\mu\text{g L}^{-1}$.

3.3. Toxicity Assays

All TOXKITS were purchased from Microbiotests, Belgium. Producer protocols were strictly followed, including verification of culture media, pH, and the quality of the controls. The dilutions of the crude extract, were prepared in the appropriate exposure media in final concentrations of 100, 20, 4, 0.8, 0.16 and 0.03 mg dw biomass mL⁻¹, i.e. 99.00, 19.80, 3.96, 0.79, 0.16, and 0.03 $\mu\text{g total MC L}^{-1}$.

THAMNOTOXKIT FTM, using the fairy shrimp *Thamnocephalus platyurus* instar II-III larvae was used for the first investigation. The test was carried out in six replicates of 30 animals each incubated with the various crude extract dilutions at 25°C in the dark for 24 h. Dead larvae were counted, and the % mortality was calculated as well as the 24 h LC₅₀ using standard methods³⁴.

200 For the ROTOXKIT F, juveniles of the rotifer *Brachionus calyciflorus* were utilized for the
201 acute 24 h toxicity test, with 30 animals per test concentration in six replicates. The plates were
202 incubated at 25°C in darkness. After 24 h, the dead animals were counted, and the % mortality,
203 as well as the LC50, was calculated³⁵.

204 For the DAPHTOXKIT pulex, *Daphnia pulex* neonates were hatched from ephippia four days
205 before the start of the tests. The test was with 50 neonates per test concentration in replicates
206 of six. Hatching was initiated in Petri dishes with 15 mL standard freshwater at 20°C under
207 continuous illumination with 8000 lux, at 25°C in darkness. After 24 h, deceased animals were
208 counted, and the % mortality, as well as the LC50, was calculated.

209 For all of the kits mentioned above, the tests were only valid with mortalities in controls being
210 less than 10%. Positive controls were performed using potassium dichromate ($K_2Cr_2O_7$) (1000
211 ppm stock solution) diluted to a series of 1.8, 1.0, 0.56, 0.32, and 0.18 mg L⁻¹.

212 TUBIFEX Toxicity TEST utilizes the oligochaete *Tubifex tubifex* for toxicity testing³⁶. The test
213 was performed in small glass beakers with 50 animals per test concentration in replicates of
214 ten. Mortality of the oligochaete was evaluated microscopically after the exposure time of 24
215 h.

216 The ALGALTOXKIT used *Selenastrum capricornutum* (renamed as *Pseudokirchneriella*
217 *subcapitata*) in a 72 h algal growth test. Optical density, as a measure of growth, was measured
218 using a spectrophotometer at 670 nm strictly according to the protocol.

219 The PHYTOTESTKIT employed seeds of three different terrestrial plants *Sorghum*
220 *saccharatum* (monocotyledon), *Lepidium sativum* and *Sinapis alba* (dicotyledons) to test for
221 toxic effects, i.e. effects on germination and early development. The tests were performed in
222 three replicates in a climate chamber for three days at 25°C in the dark. For the germination,
223 the germinated seeds were counted and values compared to those of controls as a measure of
224 toxicity.

MORPHOLOGICAL CHANGES of MACROPHYTES were determined using three different aquatic macrophytes, namely *Ceratophyllum demersum*, *Limnophila sessiliflora*, and *Potamogeton perfoliatus*. Macrophytes were exposed to the crude extract at a biomass density of 10 mg fw L⁻¹ amounting to 22.5 µg MC-LR L⁻¹, 24.7 µg -RR L⁻¹ and 2.8 µg -YR L⁻¹ (50 µg L⁻¹ in total). Morphological changes between the controls and the exposed plants were visibly assessed after 14 days.

OXIDATIVE STRESS RESPONSES of MACROPHYTE were measured in *C. demersum* in a 24 h static renewal exposure experiment. Plant material (3 g wet weight) was exposed in 100 mL medium containing the crude extract (50 ± 0.8 µg L⁻¹ total MCs, as before) in replicates of five in parallel with an unexposed control. The level of cell internal H₂O₂ as a marker for oxidative stress was colorimetrically determined according to the method of Jana and Choudhuri³⁷.

3.4.Data analyses

The TOXKIT assay effect levels were calculated using the Microtox statistical analysis software program, which calculates effect concentrations (EC1, EC10, EC20, and EC50) and associated 95% confidence intervals for 15 and 30-min exposure periods. Statistical significant differences and Pearson Correlation coefficients were calculated using Statistica software. Concentration-response curves were evaluated using Probit analysis³⁴, and the 50%-effective concentrations (LC₅₀, EC₅₀, or IC₅₀) for the respective assay. The differences and statistical significance were evaluated using ANOVA, followed by Duncan's post-hoc test. Statistical significance was considered at $p < 0.05$.

4. Conclusion

The presence or absence of a visible cyanobacterial bloom is also not an indication of the toxins that may be present in the afflicted waters and thus does not predict exposure risk. Similarly, the presence and absence of toxins and mixtures thereof do not indicate the ecological effect.

Therefore, it would be advantages to include toxicity testing into routine water testing regimes to better understand the impact of harmful algal blooms.

5. References

1. Scholz, S. N., Esterhuizen-Londt, M. & Pflugmacher, S. Rise of toxic cyanobacterial blooms in temperate freshwater lakes: causes, correlations and possible countermeasures. *Toxicological & Environmental Chemistry* **99**, 543-577 (2017).
2. Park, S. B. Algal blooms hit South Korean rivers. *Nature* doi:10.1038/nature.2012.11221 (2012).
3. Park, H. D., Kim, B., Kim, E. & Okino, T. Hepatotoxic microcystins and neurotoxic anatoxin-a in cyanobacterial blooms from Korean lakes. *Environmental Toxicology and Water Quality* **13**, 225-234 (1998).
4. Joung, S.-H., Oh, H.-M., Ko, S.-R. & Ahn, C.-Y. Correlations between environmental factors and toxic and non-toxic *Microcystis* dynamics during bloom in Daechung Reservoir, Korea. *Harmful Algae* **10**, 188-193 (2011).
5. Kim, B., Kim, H.-S., Park, H.-D., Choi, K. & Park, J.-G. Microcystin content of cyanobacterial cells in Korean reservoirs and their toxicity. *Korean Journal of Limnology* **32**, 288-294 (1999).
6. Srivastava, A., Ahn, C.-Y., Asthana, R.K., Lee, H.-G. & Oh, H.-M. Status, Alert System and Prediction of Cyanobacterial bloom in South Korea. *BioMed Research International*, Article ID 584696, <http://dx.doi.org/10.1155/2015/584696> (2015).
7. Lee, Y., *et al.* Development of a water quality index model for lakes and reservoirs. *Water Environ* **12**, S19–S28 (2014).
8. Park, H.-K., Jheong, W.-H., Kwon, O.-S. & Ryu, J.-K. Seasonal succession of toxic cyanobacteria and microcystins concentration in Paldang Reservoir,” *Algae* **15**, 29-35 (2000).

9. Joung, S.-H., *et al.* Water quality and cyanobacterial anatoxin—a concentration in Daechung reservoir. *Korean Journal of Limnology* **35**, 257-265 (2002).
10. Cho, D.-H., *et al.* Characteristics of Water Quality in Wangsong Reservoir and Its Inflow Streams. *Journal of Korean Society of Water and Wastewater* **26**, 201-208 (2012).
11. Jung, S., *et al.* The effect of phosphorus removal from sewage on the plankton community in a hypertrophic reservoir. *Journal of Ecology and Environment* **40**, 1-9 (2016).
12. Carmichael, W.W. A review. Cyanobacteria secondary metabolites- the cyanotoxins. *J. Appl. Bacteriology* **72**, 445-459 (1992).
13. Omid, A., Esterhuizen-Londt, M. & Pflugmacher, S. Still challenging: the ecological function of the cyanobacterial toxin microcystin – What we know so far. *Toxin Reviews* <http://dx.doi.org/10.1080/15569543.2017.1326059> (2017).
14. Kim, S.-G., *et al.* Determination of Cyanobacterial diversity during algal blooms in Daechung Reservoir, Korea, on the basis of cpcBA intergenic spacer region analysis. *Applied and Environmental Microbiology* **72**, 3252-3258 (2006).
15. Amé, M., *et al.* Microcystin-LR, -RR, -YR and -LA in water samples and fishes from a shallow lake in Argentina. *Harmful Algae* **9**, 66-73 (2010).
16. Chen, J. & Xie, P. Microcystin accumulation in freshwater bivalves from lake Taihu, China, and the potential risk to human consumption. *Environmental Toxicol. Chem.* **26**, 1066-1073 (2007).
17. Chislock, M.F., Doster, E., Zitomer, R.A. & Wilson, A.E. 2013. Eutrophication: causes, consequences, and controls in aquatic ecosystems. *Nature Education Knowledge* **4**, 10.

18. El Ghazali, E., *et al.* Effects of the microcystin profile of a cyanobacterial bloom on growth and toxin accumulation in common carp *Cyprinus carpio* larvae. *J. Fish Biol.* **76**, 1415-1430 (2010).
19. Herrera, N., Echeverri, L. & Ferrão-Filho, S. Effects of phytoplankton extracts containing the toxin microcystin-LR on the survival and reproduction of cladocerans. *Toxicon* **95**, 38-45 (2015).
20. Li, X.-Y., Chung, I.-K., Kim, J.-I. & Lee, J.-A. Subchronic oral toxicity of microcystin in common carp (*Cyprinus carpio* L.) exposed to *Microcystis* under laboratory conditions. *Toxicon* **44**, 821-827 (2004).
21. Pflugmacher, S., Amé, M., Wiegand, C. & Steinberg, C. Cyanobacterial toxins and endotoxins their origin and their ecophysiological effects in aquatic organisms. *Wasser Boden* **53**, 15-20 (1999).
22. Pflugmacher, S., *et al.* Uptake, effects, and metabolism of cyanobacterial toxins in the emergent reed plant *Phragmites australis* (Cav.) Trin. Ex. Steud. *Environ. Toxicol. Chem.* **20**, 846-852 (2001).
23. Pflugmacher, S. Possible allelopathic effects of cyanotoxins, with reference to microcystin-LR, in aquatic ecosystems. *Environ. Toxicol.* **17**, 407-413 (2002).
24. Babica, P., Bláha, L. & Marsalek, B. Exploring the natural role of microcystins – A review of effects on photoautotrophic organisms. *J. Phycology* **42**, 9-20 (2006).
25. Oh, H.-M., Lee, S. J., Kim, J.-H., Kim, H.-S. & Yoon, B.-D. Seasonal variation and indirect monitoring of microcystin concentration in Daechung Reservoir, Korea. *Appl. Environ. Microbiol.* **67**, 1484-1489 (2001).
26. Törökné, A. K., *et al.* Water quality monitoring by Thamnotoxkit F™ including cyanobacterial blooms. *Water Science and Technology* **42**, 381-385 (2000).

27. DeMott, W. R., Zhang, Q.-X. & Carmichael W. W. Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and three species of *Daphnia* *Limnol. Oceanogr.* **36**, 1346-1357 (1991).
28. Kós, P., Gorzó, G., Surányi, G. & Borbély, G. Simple and efficient method for isolation and measurement of cyanobacterial hepatotoxins by plant tests (*Sinapis alba* L.). *Anal Biochem.* **225**, 49-53 (1995).
29. Stanier, R. Y., Kunisawa, R., Mandel, M. & Cohen-Bazire, G. Purification and properties of unicellular blue-green algae (Order Chroococcales). *Bacteriol. Rev.* **35**, 171-205 (1971).
30. Romero-Oliva, C., Contardo-Jara, V. & Pflugmacher, S. Time dependent uptake, bioaccumulation and biotransformation of cell free crude extract microcystins from Lake Amatitlán, Guatemala by *Ceratophyllum demersum*, *Egeria densa* and *Hydrilla verticillata*. *Toxicon* **105**, 62-73 (2015).
31. Ha, M. H., Contardo-Jara, V. & Pflugmacher, S. Uptake of the cyanobacterial neurotoxin, anatoxin-a, and alterations in oxidative stress in the submerged aquatic plant *Ceratophyllum demersum*. *Ecotoxicol Environ Saf.* **101**, 205-12 (2014).
32. Esterhuizen-Londt, M., Downing, S. & Downing, T. G. Improved sensitivity using liquid chromatography mass spectrometry (LC-MS) for detection of propyl chloroformate derivatised β -N-methylamino-L-alanine (BMAA) in cyanobacteria. *Water SA* **37**, 133-138 (2011).
33. Esterhuizen-Londt, M., Kühn, S. & Pflugmacher, S. Development and validation of an in-house quantitative analysis method for cylindrospermopsin using HILIC liquid chromatography tandem mass spectrometry: Quantification demonstrated in four aquatic organisms. *Environmental Toxicology and Chemistry* **34**, 2878-83 (2015).
34. US EPA Methods for measuring the acute toxicity of effluents to freshwater and marine organisms. Toxdat. Multimethod program (binomial, moving average and probit). 3.ed.

348 Cincinnati: Environmental Monitoring and Support Laboratory, U. S. Environmental
349 Protection Agency, EPA/600/4-85/013 (1985).

350 35. ASTM Standard Guide for Acute Toxicity Test with the Rotifer *Brachionus*. Method
351 E1440–91 Reapproved 1998 (1998).

352 36. Kyselková I., & Maršálek, B. Using of *Daphnia magna*, *Artemia salina* and *Tubifex*
353 *tubifex* for cyanobacterial microcystins detection. *Biologia* **55**, 637-643 (2000).

354 37. Jana, S. & Choudhuri, M. A. Glycolate metabolism of three submerged aquatic
355 angiosperms during ageing. *Aquat. Bot.* **12**, 345–354 (1982).

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Figure titles:

Figure 1: Cyanobacterial toxin composition of the *M. aeruginosa* KW strain isolated from Wangsong reservoir (South Korea). Data represent mean toxin concentration $\pm$ standard deviation (n = 4)

Figure 2: Oxidative stress response monitored as changes in cellular H₂O₂ level in three submerged macrophytes: *C. demersum* (A), *L. sessiliflora* (B) and *P. perfoliatus* (C) during 14-day exposure to cyanobacterial cell-free crude extract containing 50 $\mu\text{g L}^{-1}$ total MCs. Data represent average H₂O₂ content $\pm$ standard deviation (n = 3); * denotes statistical significance compared to the control (p > 0.05)

377 **Table 1:** LC₅₀, EC₅₀ and IC₅₀ of extract using various bioassays

Bioassay	Test organisms	Trophic level	Test outcome (LC ₅₀ , EC ₅₀ , IC ₅₀ *)	Toxicity as total MC concentration (µg MC ml ⁻¹)
THAMNOTOX-F™	<i>Thamnocephalus platyurus</i>	Primary consumer	24-h LC ₅₀	0.1 ± 0.2
ROTOTOX-F	<i>Brachionus calyciflorus</i>	Primary consumer	24-h EC ₅₀	6.5 ± 1.2
DAPHTOX pulex	<i>Daphnia pulex</i>	Primary consumer	24-h EC ₅₀	1.1 ± 0.5
TUBIFEX TOX	<i>Tubifex tubifex</i>	Detritivore	24-h EC ₅₀	1.5 ± 0.7
ALGALTOX	<i>Pseudokirchneriella subcapitata</i>	Primary producer	72-h EC ₅₀	3.7 ± 1.2
PHYTOTOX	<i>Sorghum saccharatum</i>	Primary producers	72-h IC ₅₀	3.4 ± 0.5
	<i>Sinapis alba</i>	producers	72-h IC ₅₀	4.4 ± 0.9
	<i>Lepidium sativum</i>		72-h IC ₅₀	3.9 ± 1.2

378 * LC₅₀ = lethal concentration, EC₅₀ = effect concentration, IC₅₀ = inhibitory concentration

380 **Table 2:** Altered morphology of macrophytes exposed to cyanobacterial cell-free crude extract
381 containing MCs at a concentration of 50 µg L⁻¹ for 14 days

	<i>Ceratophyllum demersum</i>	<i>Limnophila sessiliflora</i>	<i>Potamogeton perfoliatus</i>
Control
MCs exposure (50 µg L ⁻¹)