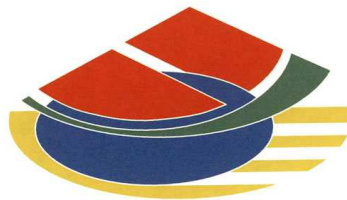


Bioaccumulation of algal toxins in aquatic systems: effects and management

Final Project Report to
The Fitzroy Basin Association
15th May, 2006



Centre for
Environmental
MANAGEMENT

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Executive summary

This report describes the main findings and outcomes of a research project partly funded by the Fitzroy Basin Association (FBA), regarding the effects of toxin-producing blue-green algal blooms. A summary of the experimental results is provided, regarding laboratory investigations undertaken with four test species and the toxic alga, *Cylindrospermopsis raciborskii*. The development of new risk prediction and management approaches are also discussed. Key outcomes of the project, including its contribution to the setting of water quality targets at Lake Elphinstone and in the wider Fitzroy Catchment.

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Project summary

This project was conducted as part of PhD studies undertaken by Susan White.

The research determined the toxic effects and potential for toxin bioaccumulation associated with toxin-producing blue-green alga, *Cylindrospermopsis raciborskii*, using environmentally relevant exposure scenarios. Short-term semi-static renewal ecotoxicity tests were conducted in the laboratory, using four freshwater test organisms, and freeze-thawed *C. raciborskii* whole cell extracts or live cultures of *C. raciborskii* containing CYN. Test organisms included species of duckweed (*Spirodela oligorrhiza*), a submerged aquatic plant (*Hydrilla verticillata*), an aquatic snail (*Melanoides tuberculata*) and tadpoles of the cane toad (*Bufo marinus*).

Timelines

Project work commenced on 31st March, 2003; the FBA provided sponsorship for the project from 15th February, 2005 onwards.

Experimental work was completed in January, 2006. The project was finalised on time, with the thesis submitted by 31st March 2006.

Summary of experimental results

The review of scientific literature (summarised above) identified deficiencies in the current knowledge base for cylindrospermopsin, especially regarding the toxic effects of this substance on aquatic organisms, and the potential for bioaccumulation. These problems were addressed by the following laboratory work.

Duckweed (*Spirodela oligorrhiza*) demonstrated variable responses to whole cell extract exposures containing 0 – 500 $\mu\text{g L}^{-1}$ of CYN. Growth stimulation and growth inhibition were recorded from the duckweed, depending on toxin exposure concentrations and length of the incubation period. The chlorophyll content of *Spirodela* was also affected by toxin exposure, with small peaks in chlorophyll *a* recorded in conjunction with exposure to 8 $\mu\text{g L}^{-1}$ CYN. Changes in frond morphology (chlorosis, necrosis) and reduced root lengths were also noted, but these effects were not consistent throughout all trials. Bioconcentration of free CYN was not detected in the tissues of *Spirodela*; small quantities of toxin recovered from the tissues probably represented toxin sorption to the cell walls.

Growth of water thyme (*Hydrilla verticillata*) was stimulated by exposure to whole cell extracts of *C. raciborskii* containing a maximum of 400 $\mu\text{g L}^{-1}$ CYN. Exposure to the test solutions appeared to promote the redistribution of plant

resources in *H. verticillata*, and the possible benefits of this, particularly with respect to increased root production, were considered. Effects on the chlorophyll content of *Hydrilla* were variable and included decreases in total chlorophyll content and changes to the chlorophyll *a:b* ratio. Bioconcentration of free toxin was not detected in the tissues of *Hydrilla*.

In trials with *Melanoides tuberculata*, exposure to whole cell extracts or live algal cultures did not significantly affect the behaviour or relative growth rates of adult snails. However, changes in the number of hatchlings released from parent snails were recorded: exposure to whole cell extracts corresponded with increased numbers of hatchlings compared with controls, whereas decreases in hatchling numbers compared with controls were recorded from treatments containing live *C. raciborskii*. Both bioconcentration and bioaccumulation occurred in the soft tissues of snails, although exposure to whole cell extracts resulted in only minor tissue contamination compared with that from live *C. raciborskii* exposures. Bioaccumulation of the analog deoxy-CYN was also recorded in the soft tissues. *M. tuberculata* did not bioconcentrate CYN in the shell.

Tadpoles of the cane toad (*Bufo marinus*) featured the most dramatic responses to the test solutions. Exposure to live *C. raciborskii* cultures resulted in up to 66% mortality of *B. marinus*, whereas all tadpoles survived exposure to whole cell extracts of comparable CYN concentrations. Decreases in the time spent swimming and relative growth rates were recorded from surviving tadpoles during both types of exposure regimes. Histological examination of *Bufo* tissues revealed tissue injuries to multiple organs, with particular severity noted in the liver, intestine, nephric ducts and gill epithelia. The extent of cellular damage was similar in whole cell extract (containing a maximum of 400 $\mu\text{g L}^{-1}$ CYN) and the live culture trials (containing a maximum of 232 $\mu\text{g L}^{-1}$), despite the unequal toxin concentrations. Bioconcentration of CYN was not evident during the whole cell extract trial, whereas exposure to live cultures resulted in maximum average tissue concentrations of 895 $\mu\text{g toxin kg}^{-1}$ fresh weight.

Development of adequate risk assessment and management approaches

A secondary aim of the thesis was to identify and address gaps in management approaches for toxin bioaccumulation and possible environmental effects associated with toxin-producing *C. raciborskii* blooms.

A predictive management strategy was developed to determine the likelihood of tissue contamination in aquatic organisms inhabiting water bodies affected by blooms. The ten-step framework for predicting bioaccumulation risk was based upon characteristics of cyanotoxin bioavailability, exposure and uptake routes during the progression of a toxic bloom. Key concepts included monitoring changes in toxin availability throughout the progression of a toxic bloom, and the prediction of bioaccumulation risks based on threshold toxin

values for selected aquatic organisms. A copy of the publication arising from this work has already been provided to the FBA.

In the final thesis chapter, the threshold values recommended in the predictive model were re-examined, using the data obtained from the laboratory trials. Current approaches for the management of blooms with respect to environmental risks were discussed, and procedures for the proper evaluation of environmental risks associated with *Cylindrospermopsis* blooms were assessed. These included adequate detection and monitoring systems, setting of environmental guideline values, and options for the control and remediation of toxic blooms. However, it was determined that a greater understanding of *Cylindrospermopsis* blooms, toxin production, and the effects of toxicity needs to be obtained before toxic blooms can be adequately managed.

Contribution to the management of Lake Elphinstone

One of the key aims of this project was to assist with the development of best practice management guidelines for reducing risks to both the environment and to human users. In particular, the FBA signalled that a key project outcome could be the development of an action plan for Lake Elphinstone. The lake is a natural water body in the far north of the Fitzroy Catchment that experiences recurring, toxin-producing blue-green algal blooms. Since *C. raciborskii* and other CYN-producing species have been reported from Lake Elphinstone, knowledge of the environmental effects and bioaccumulation potential of this cylindrospermopsin is critically important to the management of Lake Elphinstone. It should also be noted that *C. raciborskii* has been reported from a number of other water bodies within the Fitzroy Catchment, especially the Rockhampton Barrage and Awoonga Dam.

At a presentation to Nebo Shire Council, given on 24th November, 2005, the following recommendations were suggested for the future management of Lake Elphinstone.

1. Conduct ongoing monitoring of blue-green algal blooms in Lake Elphinstone, and their toxicity, when relevant.
2. Investigate ways to reduce future toxic blue-green algal blooms, and to restore the overall health of Lake Elphinstone.
3. Support further studies to investigate the bioaccumulation potential and toxicity of algal toxins in species of particular significance with respect to environmental, cultural or human-health values, within both Lake Elphinstone and the wider Fitzroy Catchment area.

The development of an action plan for the lake and the setting of appropriate water quality targets is largely managed by the Lake Elphinstone Alliance, a collaboration of key stakeholders including Nebo Shire Council, CQU, and representatives from mining and natural resource bodies. Given that the lake

has been dry for most of 2004 – 2006, there has been minimal progress towards a lake action plan or setting of water quality targets. However, baseline information arising from this research project could be important in developing these management tools. CQU is willing to participate in such initiatives if they are pursued by the Lake Elphinstone Alliance.

The Freshwater Ecology Group is also currently investigating further ways to work with Nebo Shire Council to ensure the ongoing management of Lake Elphinstone is conducted with consideration of both environmental and human health impacts.

Project reporting

Since the submission of the interim project report in January 2006, one scientific paper has been accepted for publication. This relates to the accumulation of CYN in the aquatic snail (*M. tuberculata*). A copy of this publication has been provided in Appendix A.

Three further papers are currently under consideration: these relate to experimental work conducted with the *Hydrilla*, *Melanoides* and *Bufo* tadpoles. An additional two manuscripts are in preparation; these relate to the duckweed trials and the histological work with the tadpoles. Copies of each of these additional papers can be provided to the FBA upon request, should they be accepted for publication.

Acknowledgements

We would like to take this opportunity to extend our sincere thanks to the Fitzroy Basin Association for their support of this project. We also gratefully acknowledge the contributions of other financial supporters including Nebo Shire Council, CQU's Centre for Environmental Management and the Faculty of Arts, Health and Sciences. We also thank Geoff Eaglesham, Senior Chemist at Queensland Health Scientific Services, for analyses of tissues for toxins throughout the project.

Appendix A

Copy of the publication "White, S.H., Duivenvoorden, L. J., Fabbro, L. D. & Eaglesham, G. K. (2006). Influence of intracellular toxin concentrations on cylindrospermopsin bioaccumulation in a freshwater gastropod (*Melanooides tuberculata*). *Toxicon* 47(5): 497-509.