Appendix A. supplementary information

Quantification of cyanobacteria cells via a novel imaging-driven technique with an integrated fluorescence signature

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1 **Table 1 – Comparison of the most commonly used methods for cyanobacteria cell enumeration**

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Quantification	Advantages	Disadvantages
Direct Methods		
Microscopic identification and enumeration of cells with sample in counting chamber or on a filter using: 1. Bright field 2. Differential interference contrast (DIC) 3. Epifluorescence	• high resolution • quantification and identification occur simultaneously • provides information on potential toxicity of species present	• time-consuming • tedious • accuracy and reproducibility depend on analyst's experience and taxonomic expertise • may involve human error
Indirect Methods		
Microscopy combined with image acquisition and quantitative commercial image analysis software	• less time consuming than microscopy alone • adds an extra level of documentation • eases the quantification of cyanobacterial biomass when the dominant species is filamentous Minimizes human error	• errors involved specially when analyzing cells with a complex three dimensional geometry • non-target particles (debris, contaminants may cause overestimation
Biomass or biovolume determination	• provides information on relative toxin content	• involves estimation errors
Photosynthetic pigments analyses (1) chlorophyll-a analysis (2) phycocyanin analysis Spectral imaging of intracellular pigment *In-situ* or remote monitoring of pigment fluorescence	• less time consuming than microscopy • sensitive • little analyst's experience required	• may vary according to physiological state of cells • in mixed populations it gives an overestimation of cyanobacterial biomass • less specific and less precise than microscopy • no cell identification • in some scenarios may cause interference and/or false readings
Flow cytometry	• rapid • can use larger sample sizes • high statistical confidence • allows discrimination and quantification of target cells in complex samples • may be combined with staining, antibody labelling and molecular probes	• high cost equipment • requires trained personnel • no cell identification • cells need to be isolated
Molecular methods • quantitative real-time polymerase chain reaction (qPCR) • molecular probes using sandwich hybridization (SHA)	• rapid • sensitive • precise	• variability in extraction efficiencies, presence of inhibitory compounds and the design of standards for target species often compromise accuracy and reproducibility • provides no cell identification • requires trained personnel • still under development

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Sources: WHO 1999[1], Coyne et al. 2005[2], Vuorio et al. 2007[3], Australian-GWRC (2009)[4], Orozco and Medlin 2013[5], Peniuk et al. 2016[6], Health Canada 2016[7].

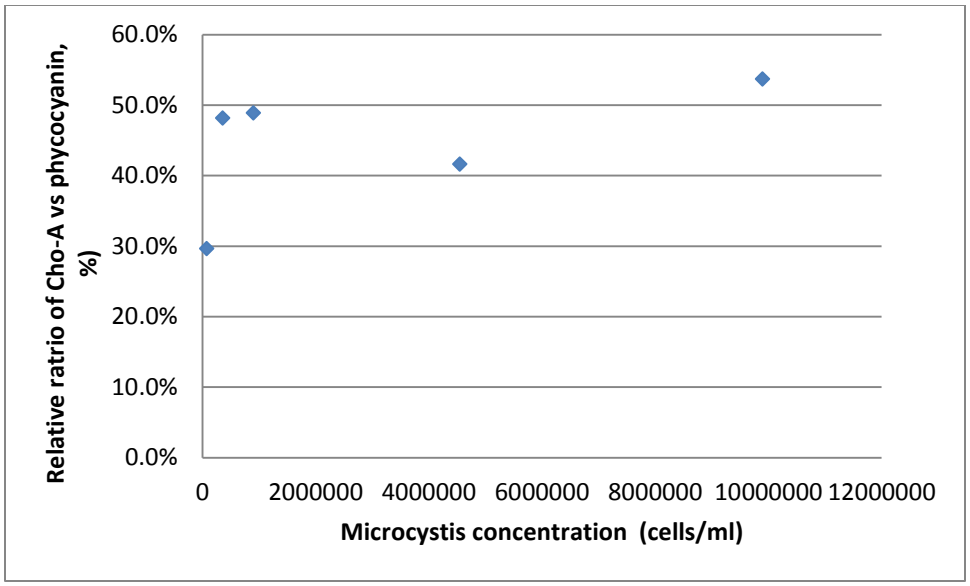

Figure S-1. Ratio of measured concentrations (mg/L) of chlorophyll-a over phycocyanin ($C_{Cho\text{-}a}/C_{Phyco}$) for *Microcystis* cells suspended in PBS

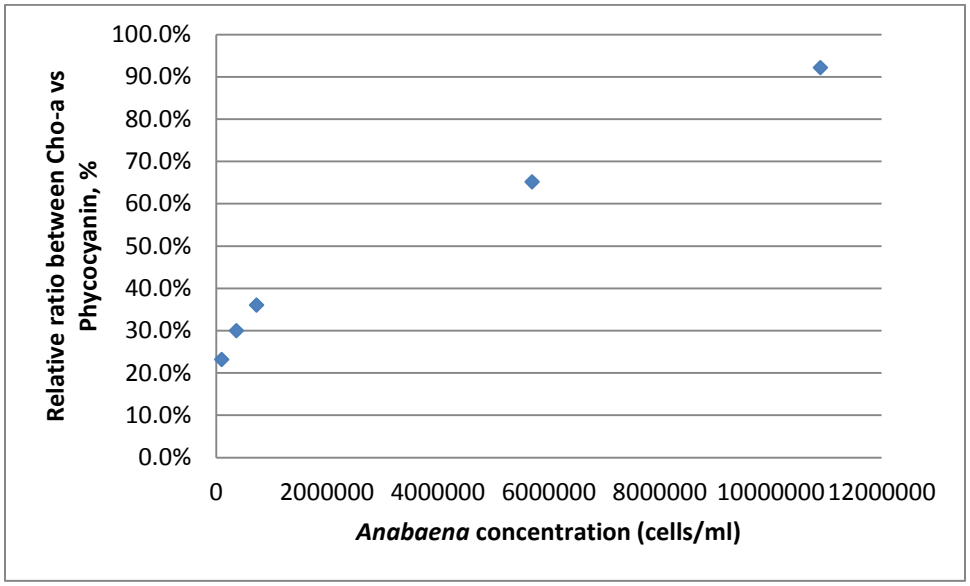

Figure S-2. Ratio of measured concentrations (mg/L) of chlorophyll-a over phycocyanin ($C_{Cho\text{-}a}/C_{Phyco}$) for *Anabaena* cells suspended in PBS

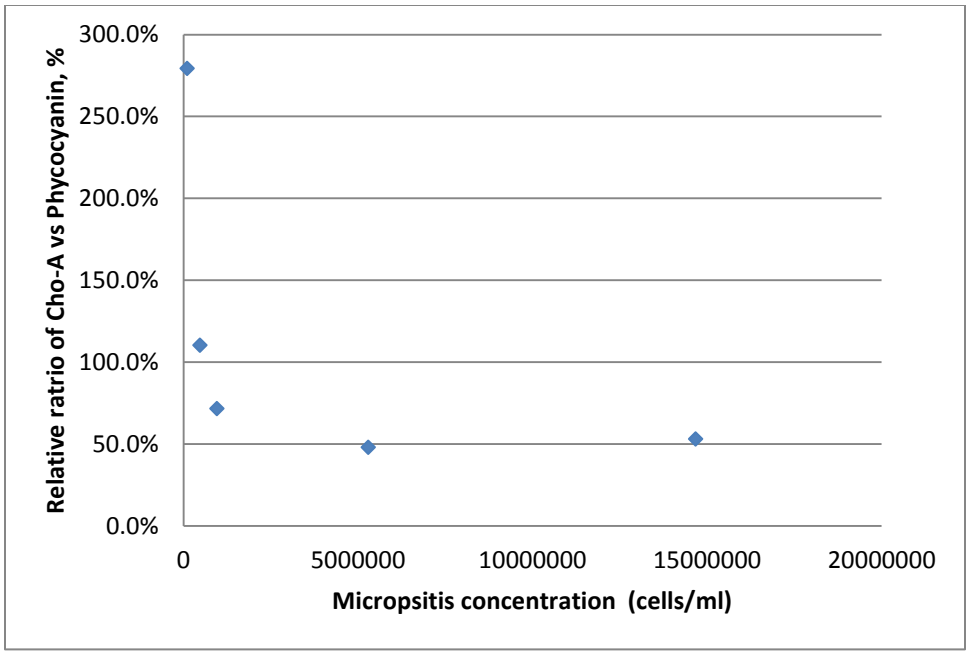

Figure S-3. Ratio of measured concentrations (mg/L) of chlorophyll-a over phycocyanin ($C_{Cho\text{-}a}/C_{Phyco}$) for *Microcystis* cells suspended in lake water

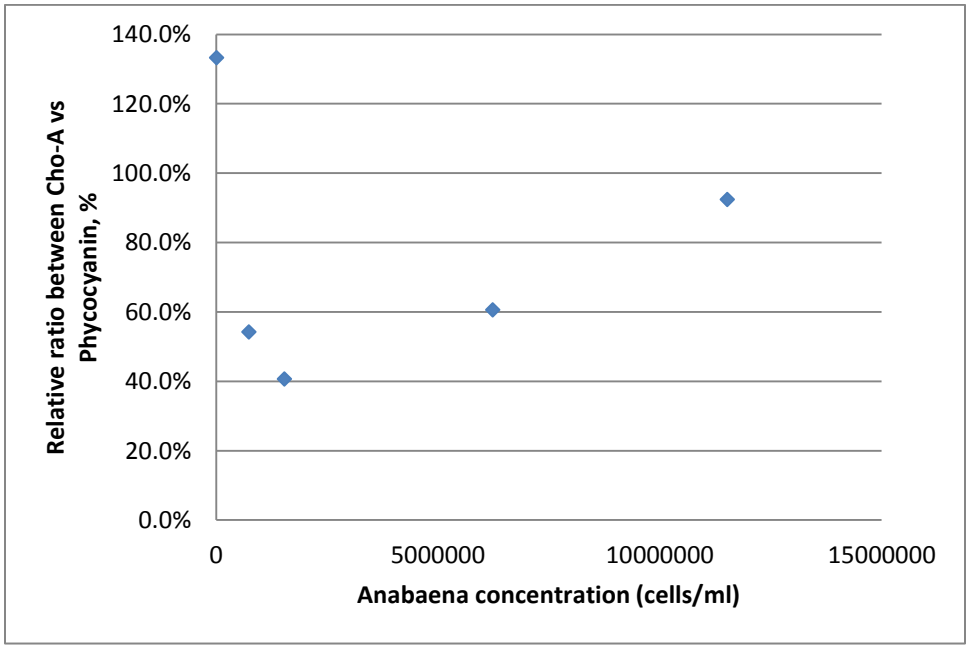

Figure S-4. The normalized portion of measured concentrations (mg/L) of chlorophyll-a over phycocyanin ($C_{Cho\text{-}a}/C_{Phyco}$) for *Anabaena* cells suspended in lake water

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