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Factors Affecting the Interpretation of Online Phycocyanin Fluorescence to Manage Cyanobacteria in Drinking Water Sources

Liya Ma [1,*], Saber Moradinejad [1], Juan Francisco Guerra Maldonado [1], Arash Zamyadi [2], Sarah Dorner [1] and Michèle Prévost [1]

[1] Department of Civil, Geological, and Mining Engineering, Polytechnique Montréal, Montréal, QC H3C 3A7, Canada
[2] Faculty of Engineering and Information Technology, University of Melbourne, Melbourne, VIC 3010, Australias
* Correspondence: liya.ma@polymtl.ca

Table S1. Correlations between the in situ phycocyanin fluorescence probe readings and microscopic cell counts, biovolume estimation, and extracted pigment concentration.

Correlation	Experimental Result	Probe Model	Reference
Probe measured phycocyanin (μg/L) and microscopic cyanobacterial cell counts (cells/mL)	R^2 = 0.99 for laboratory cultured *Microcystis aeruginosa*; R^2 = 0.745 for field collected samples.	TriOS microFlu	[1]
	$R^2 \geq 0.9954$ for laboratory cultured 4 different unicellular species; R^2 = −0.93 and 0.73 for laboratory cultured mixed species; R^2 = 0.71 for field collected samples.	TriOS microFlu	[2]
	$R^2 \geq 0.88$ for laboratory cultured 3 different unicellular species; $R^2 \geq 0.90$ when a unicellular species is mixed with a microalga *Pseudokirchneriella subcapitata*.	Fluo-Imager Fluorometer	[3]
	R^2 =0.994 for laboratory cultured *Planktothrix agardhii*; R^2 =0.997 for laboratory cultured *Lemmermanniella* sp.; R^2 = 0.729 for field collected samples.	TriOS microFlu	[4]
Probe measured phycocyanin (RFU) and microscopic cyanobacterial cell counts (cells/mL)	R^2 = 0.96 for field collected samples.	YSI 6600	[5]
Probe measured phycocyanin (cells/mL) and microscopic cyanobacterial cell counts (cells/mL)	$R^2 > 0.993$ for laboratory cultured *Microcystis aeruginosa;* R^2 = 0.705 for field collected samples.	YSI 6600	[1]
	R^2 = 0.95 for laboratory cultured *Microcystis aeruginosa.*	Eureka Manta II	[6]
Probe measured phycocyanin (μg/L) and microscopic cyanobacterial biovolume (mm^3/L)	$R^2 > 0.85$ for laboratory cultured unicellular *Microcystis aeruginosa* and 3 filamentous species; The relationship is weak for laboratory cultured colonial strain of *Microcystis aeruginosa;* No significant relation for field collected samples.	Turner Cyclops-7, Eureka Manta II, TriLux, YSI EXO	[7]
	R^2 = 0.43 and 0.69 for field collected samples.	Turner Cyclops-7	[8]
	$R^2 \geq 0.99$ for laboratory cultured 4 different unicellular species; $R^2 \geq 0.97$ for laboratory cultured mixed species; R^2 = 0.77 for field collected samples.	TriOS microFlu	[2]
	R^2 = 0.829 for field collected samples.	TriOS microFlu	[1]
	R^2 = 0.43 and 0.63 for field collected samples.	Turner Cyclops-7	[8]
	$R^2 \geq 0.98$ for laboratory cultured 3 different unicellular species; $R^2 \geq 0.86$ when a unicellular species is mixed with a microalga *Pseudokirchneriella subcapitata;* $R^2 \geq 0.93$ when in the mixtures of two cyanobacteria species.	Fluo-Imager Fluorometer	[3]
	$R^2 \geq 0.88$ for laboratory cultured 12 species; $R^2 \geq 0.78$ for field collected samples.	Turner CyanoFluor	[9]
	Linear signal (R^2 not shown) for laboratory cultured *Planktothrix agardhii* and *Lemmermanniella* sp.	TriOS microFlu	[4]
Probe measured phycocyanin (RFU) and microscopic cyanobacterial cell biovolume (mm^3/L)	R^2 = 0.68 for field collected samples.	YSI 6600	[10]
	$R^2 \geq 0.92$ for laboratory cultured mono cell culture *Microcystis aeruginosa, Dolichospermum circinale, Cylindrospermopsis raciborskii* and *Microcystis flos-aquae.*	YSI EXO2, YSI 6600, bbe Moldanke Algaetorch, TriOS microFlu	[11]
	$R^2 \geq 0.73$ for field collected samples.	YSI EXO2	[12]
	$R^2 > 0.996$ for laboratory cultured *Microcystis aeruginosa;* R^2 = 0.87 for field collect samples.	YSI 606131, YSI 6600, TriOS microFlu, bbe Moldanke Fluoroprobe	[13]

Correlation	Experimental Result	Probe Model	Reference
	$R^2 = 0.77$ and 0.72 in two filed locations, but $R^2 = 0.19$ in the third field location.	YSI EXO2	[14]
	$R^2 = 0.70$ for field collected samples.	YSI EXO2	[15]
	$0.71 \leq R^2 \leq 0.81$ for laboratory cultured 4 species.	YSI EXO2	[16]
Probe measured phycocyanin (cells/mL) and microscopic cyanobacterial cell biovolume (mm^3/L)	$R^2 = 0.815$ for field collected samples.	YSI 6600	[1]
	$R^2 = 0.60$ for laboratory cultured *Microcystis aeruginosa*.	Eureka Manta II	[6]
Probe measured phycocyanin (μg/L) and laboratory extracted phycocyanin concentration (μg/L)	$R^2 > 0.70$ for field collected samples.	Turner Cyclops-7, YSI EXO	[7]
Probe measured phycocyanin (RFU) and laboratory extracted phycocyanin concentration (μg/L)	$R^2 = 0.55$, 0.93 and 0.96 for laboratory cultured 3 species.	YSI EXO2	[16]
	$R^2 = 0.54$ for mixed laboratory cultured 4 species; $R^2 = 0.84$, 0.81, 0.82 and 0.92 for laboratory cultured 4 species.	YSI EXO2	[17]
	$R^2 = 0.96$ for laboratory cultured *Microcystis aeruginosa*; $R^2 = 0.79$ for field collected samples.	YSI 6600	[18]
Probe measured phycocyanin (cells/mL) and laboratory extracted phycocyanin concentration (μg/L)	$R^2 = 0.86$ for field collected samples.	YSI 6600	[19]

Table S2. Main sources of interference associated with the in situ phycocyanin fluorescence probe.

Source	Interference and Relative Error of Phycocyanin Content	Reference
Varying cell growth phase	Maximum 125% higher phycocyanin observed during logarithmic phase comparing with the lag and stationary phases in the study of *Microcystis aeruginosa* and *Dolichospermum circinalis*.	[20]
	Phycocyanin dominates in the decay phase in the study of *Microcystis aeruginosa*.	[21]
	Decrease as *Aphanocapsa* sp. and *Sphaerospermopsis* sp. reached stationary phase, but increased for *Microcystis* sp; For *Raphidiopsis raciborskii*, the content varies throughout the growth phases.	[16]
	Microcystis aeruginosa has consistently higher phycocyanin in the exponential phase compared to stationary and/or late stationary phases; *Raphidiopsis raciborskii* shows decreases in fluorescence per unit biomass between exponential and late stationary phases; *Dolichospermum circinale* overall increases phycocyanin fluorescence per unit biomass and per unit cell, but not vary significantly between growth phases; *Aphanocapsa* sp. phycocyanin fluorescence does not vary significantly between growth phases.	[17]
Varying cyanobacterial cell size, biovolume and cellular agglomeration	Weaker relationships for colonial *Microcystis* in comparison to single-celled and filamentous species.	[7]
	Maximum 88% phycocyanin increase after the disaggregation of the colonies.	[20]
	Wide ranges of cyanobacterial biovolume, agglomeration and phycocyanin cell quota observed.	[10]
	Much weaker relationship between phycocyanin fluorescence probe readings and laboratory measured cyanobacterial biovolume when picoplanktonic sized taxa contributed > 50% of the cyanobacterial biovolume.	[14]
	93% and 226% error observed in the two strains of *Dolichospermum flos-aquae*, due to the filamentous nature and lack of probe fingerprinting.	[22]
	20–90% of error.	[23]
	Sample lysis (by sonication) had no effect on the strength of the relationships between phycocyanin and cyanobacterial biovolume for the laboratory-cultured 12 cyanobacterial species measured by handheld CyanoFluor fluorometer.	[9]
Prior light exposure	Maximum 59% underestimation during 354 $\mu mol.m^{-2}.s^{-1}$ (artificial light) compared to those in the dark.	[24]
	Maximum 25% underestimation during 1200 $\mu mol.m^{-2}.s^{-1}$ (artificial light) compared to values in grow culture.	[25]
	Prior light exposure of up to 7 h under natural light had no effect on the *Microcystis aeruginosa* phycocyanin fluorescence monitored by the YSI 606131 probe.	[13]
	No significance difference was observed when *Planktothrix agardhii* exposed to 0–1900 $\mu mol.m^{-2}.s^{-1}$ of natural light for 30 min; Three times more fluorescence was observed when *Planktothrix agardhii* exposed to 0–150 $\mu mol.m^{-2}.s^{-1}$ of artificial light for 30 min.	[4]
	An increase in the fluorescence signal following exposure to ambient light compared to dimmed ambient light.	[26]
Probe calibration algorithm	If cell concentrations are ≥ 4 times of the calibration range, error is ≥ 20%; if cell concentrations are ≤ 0.1 times of the calibration range, error is 50 to 90%.	[20]
	The calibration with cyanobacteria significantly improved the accuracy beyond 5000 cells/mL by comparison to the manufacturer's calibration for YSI 6600 probe.	[1]
Water quality		
Presence of chlorophyll-a	Maximum 200% underestimation, and maximum 405% overestimation.	[11]
	Positive correlation ($R^2 > 0.8$, $p < 0.05$).	[27]
	Maximum 600% error.	[23]
	Linear relationship between cells and chlorophyll-a, at approximately 52.4 cyanobacterial cells/μg- chlorophyll-a.	[20]
Water turbidity	Maximum 49% underestimation due to the presence of mineral turbidity.	[13]
	Turbidity >50 NTU can make probe measurement ineffective.	[28]
	The addition of mineral turbidity to the *Planktothrix agardhii* solution reduced the fluorescence intensity by 12.28% at 0.1 g/L for particle size of 0.1 mm, and 5.87% at 0.1 μg/L for a grain size of 0.316 mm.	[4]
	Lower accuracy results were observed when mineral turbidity is over 50 NTU, resulting in false positives.	[6]
	Linear relationship between cells and mineral kaolinite turbidity, at approximately 15.5 cyanobacterial cells/NTU.	[20]
Water temperature	*Microcystis aeruginosa* was spiked in Milli-Q water to reach the concentration of high (828,000 cells/mL), medium (562,000 cells/ mL), and low (181,000 cells/mL), then being assessed at the water temperature of 4, 13.8, 17, and 23.5 °C: Turner Cyclops-7 T926 and Eureka Manta II: phycocyanin fluorescence readings at 4 °C is higher from any other temperature treatments. Maximum 50% higher comparing at 23.5 °C for higher and low cell concentration; not significant different among other temperatures. Turner Cyclops-7 T927 and TriLux: no significant phycocyanin fluorescence difference with the changing of water temperature.	[7]
	Turner Cyclops-7 chlorophyll fluorometer and Turner Cyclops-7 phycocyanin fluorometer:	[29]

Source	Interference and Relative Error of Phycocyanin Content	Reference
	Whole lake water and phycocyanin extract: fluorescence signals for chlorophyll and phycocyanin are linear functions of temperature over the range 5–30 °C. Equation is derived to compensate for the error; Reversibility of temperature quench during a sequence of heating and cooling. Laboratory cultured species: fluorescence signals over the temperature range 5–30 °C are linear functions for chlorophyll in the green algae (*Scenedesmus dimorphus*), and exponential functions for phycocyanin in the cyanobacteria (*Synechococcus leopoliensis*). However, anomalous response found for other two green algae (*Scenedesmus acutus*, and *Selenastrum minutum*), where fluorescence inhibited when temperature below 20 °C.	
Presence of dissolved organic matter (DOM)	*Microcystis* sp. And *Raphidiopsis raciborskii* were added to concentrated DOM from 2 different lakes water. DOM was measured by YSI EXO2 fDOM sensor, phycocyanin was measured by YSI EXO2 phycocyanin probe. Maximum 14% underestimation when concentrated DOM was added.	[16]
	When 3 different cell species are spiked into the river water (2.65 mg/L DOC), more than an order of magnitude phycocyanin fluorescence intensity decreasing measured by fluorescence excitation-emission matrices (EEMs); while when adding extracted phycocyanin pigment, EEMs didn't change.	[30]

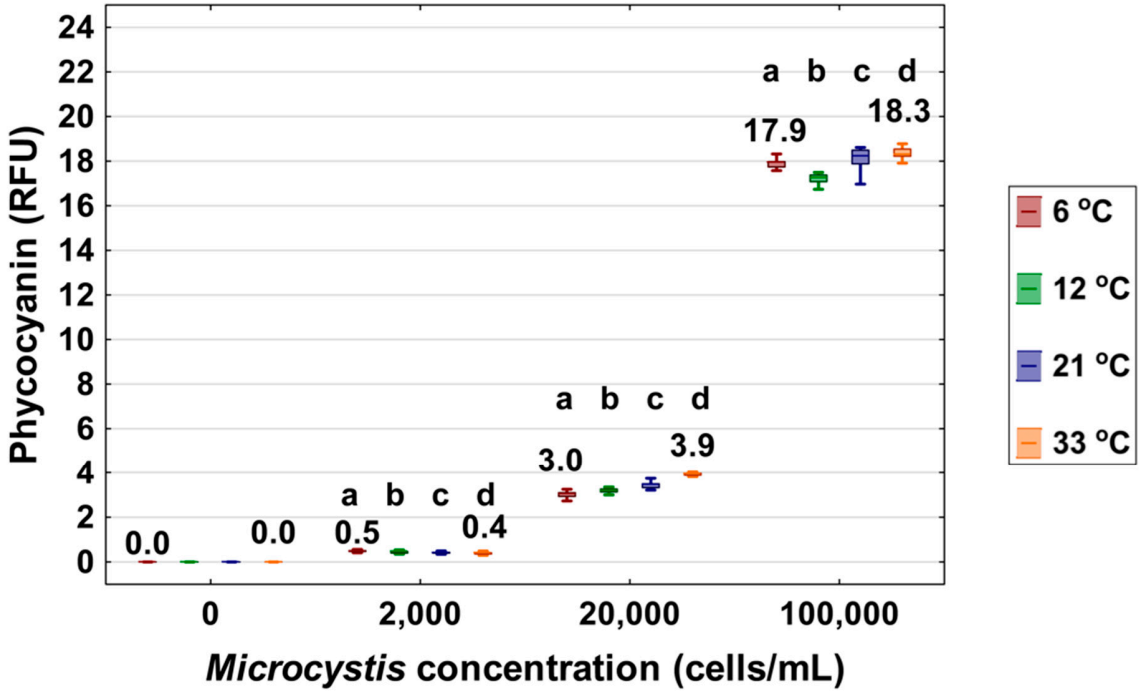

Figure S1. Phycocyanin readings (RFU) with the YSI EXO2 probe of the 0, 2000, 20,000, and 100,000 cells/mL of *Microcystis aeruginosa* strain CPCC 300 suspended in 6, 12, 21, 33 °C of ultrapure water. The probe measured each of the sample at a 250 ms interval for 10 min. The temperature interference was removed by adjusting the raw data to a reference temperature of 21 °C using Equation (2). The bottom and top of each box represent the 25th–75th percentiles, respectively. The whiskers represent the minimum and maximum values. The line within each box corresponds to the median value. Different letters represent significant differences between treatment comparisons (Kruskal–Wallis followed by Dunn's post hoc test, $p \leq 0.05$)

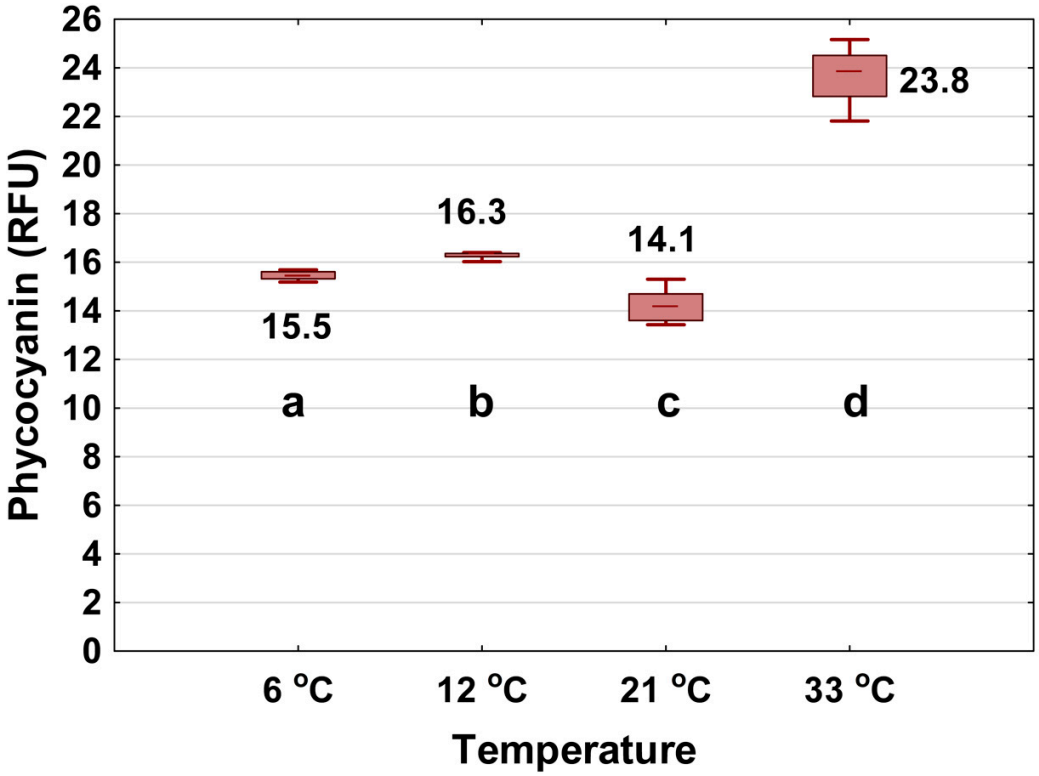

Figure S2. Phycocyanin readings (RFU) using the YSI EXO2 probe of 1 mg/L of extracted phycocyanin pigment in the 6, 12, 21, 33 °C of ultrapure water. All samples were tested over the period of 10 min. The probe measured each of the samples at a 250 ms interval for 10 min. The temperature interference was removed by adjusting the raw data to a reference temperature of 21 °C using Equation (2). The bottom and top of each box represent the 25th–75th percentiles, respectively. The whiskers represent the minimum and maximum values. The line within each box corresponds to the median value. Different letters represent significant differences between treatment comparisons (Kruskal–Wallis followed by Dunn's post hoc test, $p \leq 0.05$).

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