

Simulation of the suppressive effect of zinc on cyanobacteria in paper mill wastewater

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Abstract: Harmful algal blooms (i.e. blue-green algae or cyanobacteria) and their impacts are a recurring and significant issue worldwide. Factors supporting the extensive growth of cyanobacteria include warmer temperatures, sufficient levels of reactive nitrogen and phosphorus, reduced rainfall, long hydraulic retention times within the water body (slow-moving water) and adequate sunlight. Problems associated with the growth of cyanobacteria include formation of unpleasant odours and toxins that have the potential to harm humans and wildlife that come into contact with contaminated water.

Aerated stabilization basin (ASB) systems, commonly used by the pulp and paper mill industry for treatment of wastewater, are predominantly light limited due to high colour and consequently, blooms of cyanobacteria have been reported to rarely occur in pulp and paper ASBs. However, with the relatively low colour of paper mill (PML) wastewater (without integrated pulping processes) in ASB treatment with long hydraulic retention times (HRTs), there would be risk of occurrence of cyanobacteria at bloom levels. In 2014, two occurrences of cyanobacterial blooms were observed at a PML site in South Australia and in April 2016, *Microcystis aeruginosa* occurred at bloom levels ($> 10^6$ cells/mL) in an ASB pilot plant (3 tanks of ~200 L each) operated at the PML site to simulate the full scale ASB operation. Following these incidences, it was evident that there is need for improved understanding and modelling of strategies to control cyanobacteria present in PML wastewaters.

The aim of the research reported here was to establish models that describe the dynamics of cyanobacteria presence, indicated by phycocyanin, in PML wastewaters when exposed to zinc. This was investigated based on the inhibitory effect of Zn on cyanobacteria. In this research, batch experiments were conducted using a strain of *Microcystis aeruginosa* (MA338) and *Pseudanabaena* spp. (naturally occurring) in samples collected from ASB wastewaters. Growth and inhibition of the cyanobacteria were assessed by fluorometric detection of phycocyanin (using an EXO1 sonde with EXO Total Algae PC Smart Sensor), which is a cyanobacterial specific pigment that increases linearly with cyanobacterial biomass increase.

Phycocyanin levels were determined for batch experiments where Zn was dosed at between 0.3 and 2.4 mg/L and in controls, for 7 days. At lower concentration (~0.3 mg/L), Zn was found to be supportive for the growth of MA338 based on phycocyanin data. In contrast, $Zn \geq 0.7$ mg/L was found to be effective in suppressing the cyanobacteria tested in this study. For test samples (with $Zn \geq 0.7$ mg/L), two functions [exponential peak (EP) and logistic dose response (LDR)] were fitted to % phycocyanin vs time data. From the collected data at initial and on days 1, 3, 4 and 7, the phycocyanin reduction (decay) rates at the various Zn doses ($0.7 \leq Zn \leq 2.4$ mg/L) were determined. The reduction rates ($\Delta\%Phy/\Delta time$) differ between the two models but trended consistently (EP: 52, 64, 85, 164 and LDR: 85, 249, 188, 1621 for Zn concentrations of 0.7, 1.1, 1.6 and 2.4 mg/L, respectively).

The two models broadly described the dynamics of phycocyanin levels in samples of PML wastewaters in response to Zn exposure up to 2.4 mg/L over 7 days. Both models differ in their fittings over the first few days to Zn exposure, where the EP model correctly describes an increase in phycocyanin after initial Zn exposure. However, the maximum level estimated by this approach is speculative of what is the true maximum value, and for this much finer resolution data would be needed over the early stage of batch experiments. Further investigation is needed to determine the Zn concentration with exposure time that would suppress or enhance cyanobacteria growth and the fate of Zn in PML ASB systems. Integrated modelling with phycocyanin monitoring has the potential to better implement strategies for cyanobacterial control, through rapid assessment of the responses and efficiencies of chemicals applied to inhibit cyanobacteria growth.

Keywords: *Microcystis aeruginosa*, ASB, phycocyanin, dose response modelling

1. INTRODUCTION

Human exposure to cyanobacterial blooms has been associated with gastroenteritis, fever, dermatitis, irritation of the eyes, nose and throat (World Health Organization, 2015), as well as risks to liver cancer, liver damage and neurological damage (Butler *et al.*, 2009; Iwinski *et al.*, 2016). Cyanobacteria are photoautotrophic, and light is the key factor for their growth, followed by nutrients levels (Kirkwood *et al.* 2003). Kirkwood *et al.* (2003) reported that pulp and paper aerated stabilization basin (ASB) systems are severely light limited, and cyanobacterial blooms have been reported to rarely occur in pulp and paper ASBs because of the dark colour of wastewaters and mixing of waters by aerators (Mahmood & Paice 2006). However, with the discontinuation of on-site pulping and use of imported pulp only for paper production, the wastewaters of the mill studied become relatively low in colour. Further, with subsequent reduction in wastewater volumes, the hydraulic retention times (HRTs) of the established ASB became much longer and hence with sufficient nutrients and wastewater clarity, there is an increased risk of occurrence of cyanobacterial blooms (Lewis *et al.* 2018).

In 2014, cyanobacterial blooms (comprising *Pseudanabaena spp.*, *Phormidium spp.*, *Microcystis flos-aquae*, *Planktolyngbya spp.*, *Aphanocapsa spp.*) were observed in a full scale ASB system of a paper mill, where the colour of the wastewaters was low. Prior to the ASB, the wastewaters were treated by a settling clarifier for removal of suspended solids. In a pilot plant (3 tanks of ~200 L each) operated at the PML site to simulate the full scale ASB operation, *Microcystis aeruginosa* occurred at bloom levels in April 2016 ($> 10^6$ cells/mL). For drinking water resources established control methods for cyanobacteria growth include the use of stabilised hydrogen peroxide and copper (e.g. copper sulphate), and novel methods explored including ultrasonication.

The toxicities of heavy metals to cyanobacteria, such as copper (Cu), cadmium (Cd), nickel (Ni) and zinc (Zn) have been previously reported (e.g. Polyak *et al.*, 2013; Zeng and Wang, 2011). Zeng and Wang, (2011) reported the capacity of cyanobacteria to uptake Zn, Cu and Cd in BG-11 growth media. Polyak *et al.*, (2013) reported the concentrations of Cu, Ni and Zn that suppressed growth of two strains of *Microcystis aeruginosa* isolated from a lake in Russia, as well as reporting that low Zn concentrations supported their growth. Currently there is minimal information on the impact of Zn on cyanobacteria in PML wastewaters of ASB systems.

Here we report the impact of Zn on cyanobacteria based on phycocyanin detection by fluorometric probe. In this paper, we describe the modelling of phycocyanin levels under laboratory conditions conducted to determine the impact of Zn on cyanobacterial growth and decline in PML wastewater. We propose that this modelling approach integrated with in-situ phycocyanin detection enables improved understanding of Zn as a potential cyanobacterial control agent and where it occurs as a background metal in industrial wastewaters.

2. METHODOLOGY AND APPROACH

To study the effect of Zn on the growth or inhibition of cyanobacteria in PML wastewaters, batch sample experiments were conducted with Zn addition between 0.3 and 2.4 mg/L, and without Zn addition as controls. These were conducted under laboratory conditions, with temperatures ranging from 20°C to 29°C, LED lighting (18 x type 5730 LEDs producing 1125 lumens) at 12 h on/off cycles, initial pH level of 7.9 ± 0.4 , and initial dissolved oxygen (DO) level of 8.2 ± 0.3 mg/L, typical of the paper mill studied. Growth and inhibition of the cyanobacteria were assessed by fluorometric detection of phycocyanin (using an EXO1™ sonde with EXO Total Algae PC Smart Sensor). Each experiment was conducted over 7 days and measurements were taken at the start and days 1, 3, 4 and 7.

Wastewater samples were collected from a paper mill ASB (discharge of the third pond of a three-pond system) and from the inflow to the ASB (settling clarifier) having Zn concentrations of 0.058 and 0.117 mg/L, respectively, in May 2018.

To study the effects of different inhibitory concentrations of Zn, two sets of wastewaters (for experiment (Expt) #1-1: inflow; #1-2: outflow waters) were initially inoculated with *Microcystis aeruginosa* (strain MA338, sourced from the AWQC, SA) in ASM-1 (Gorham *et al.* 1964) then dosed with a range of Zn concentrations to achieve concentrations 0.3 – 2.4 mg/L. Zinc was added from a stock solution (2,580 mg/L) prepared using Analytical Grade zinc chloride (Alfa Aesar, Thermo Fisher Scientific Australia Pty Ltd) or pure Zn (99.9%). The highest level of the Zn range tested is ~50% of the 5.0 mg/L guideline limit for recreational waters under the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC, 2000).

In a subsequent experiment, Zn (2.4 mg/L) was used to examine the effect of initial cyanobacteria level, as detected phycocyanin (#2-1: $\sim 86 \pm 2.9$ µg/L compared with #2-2: $\sim 46 \pm 4.9$ µg/L) on the effect of zinc on *M. aeruginosa* (MA338). Further, the effect of Zn (at 2.4 mg/L) on two different cyanobacteria species (#3-1: *Pseudanabaena spp.*; #3-2: MA338) were also examined. A culture of *Pseudanabaena spp.* was obtained from the ASB and grown in ASM-1 media. These cyanobacteria were chosen as these species occurred naturally at

the study site at bloom proportions ($> 10^6$ cells/mL). Source waters, initial phycocyanin and initial culture cell counts for each experiment are given in Table 1.

Table 1. Source waters, initial phycocyanin and culture cell counts for each set of experiments

No.	Source water	Culture Cyanobacteria	Purpose	Culture cell counts	Initial phycocyanin ($\mu\text{g/L}$)	Initial Zn concentration(s), (mg/L)
1-1	Inflow to ASB	MA338	Zn concentration	4.2×10^4	24.9 ± 2.8	0.3 ± 0.02 ; 2.4 ± 0.1
1-2	Pond 3 of ASB	MA338	determination	4.2×10^4	22.8 ± 2.1	0.3 ± 0.02 ; 2.4 ± 0.1
2-1	Pond 3 of ASB	MA338	Zn effect with cell	2.88×10^6	86.4 ± 2.9	2.5
2-2*	Pond 3 of ASB	MA338	number	2.88×10^6	45.6 ± 4.9	2.5 ± 0.1
3-1*	Pond 3 of ASB	<i>Pseudanabaena spp.</i>	Triplicate test with	7.61×10^6	25.7 ± 0.9	2.5 ± 0.1
3-2*	Pond 3 of ASB	MA338	Zn at 2.5 mg/L	7.50×10^6	36.1 ± 1.1	2.5 ± 0.05

* Experiments conducted as triplicates

2.1. Modelling of data

Average phycocyanin levels for samples without Zn addition (controls) were determined on days 0, 1, 3, 4 and 7 for each experiment and subsequently were used to compare those with Zn addition ($\text{Zn} \geq 0.7$ mg/L) at corresponding days. Percentage phycocyanin (%Phy) values were calculated, normalised to Day 0 values. Two exponential decay functions [exponential peak (EP, Eq. 1) and logistic dose response (LDR, Eq. 2)] equations were used to model fit the %Phy values for the various Zn doses, using TableCurve™ software. The equations were chosen based on the likelihood of these theoretically representing the release and reduction (loss) of phycocyanin with the inhibitory action of Zn on cyanobacteria (Poliak *et al.* 2011; Polyak *et al.* 2013). Coefficients of these equations were acquired by statistical analysis.

$$\%Phy = a_1 + \frac{4 \times b_1 \times \exp(-t-c_1/f_1)}{(\exp(-t-c_1/f_1) + 1)^2} \quad (1)$$

Where, t is the time from Zn dosing (day); a_1 is the minimum %Phy value (%); b_1 is the difference between the maximum and the minimum %Phy value (%); c_1 is the time (t) from dosing that is required to achieve the maximum %Phy value; f_1 is the coefficient of decay.

$$\%Phy = a_2 + \frac{b_2}{1 + (\frac{t}{c_2})^{f_2}} \quad (2)$$

Where, t is the time from Zn dosing (day); a_2 is the minimum %Phy value (%); b_2 is the difference between the maximum and the minimum %Phy value (%); c_2 is the time (t) from dosing that is required to achieve the half of the initial Phy% value; f_2 is the coefficient of decay.

3. RESULTS AND DISCUSSION

For both pre-ASB (clarifier) and ASB (pond 3 discharge) samples, addition of Zn at concentration of 0.3 mg/L was found to be supportive (up to day 4) for the growth of MA338. Conversely, addition of Zn at concentrations between 0.7 and 2.4 mg/L were found to suppress MA338 based on phycocyanin data (Figures 1 and 2). Coefficients of Eq. 1 and Eq. 2 for the various experiments (with $\text{Zn} \geq 0.7$ mg/L) conducted are given in Table 2. R^2 values were greater than 0.96 for both, indicating that the models well described the observed data acquired. Based on these models, the time required from dosing to achieve half of the initial Phy% value (t_{50}) and to reduce this by 90% (t_{90}) were calculated and summarised in Table 2. The phycocyanin decay rates at t_{50} are also shown in Table 2. With increases in the Zn concentrations (0.7, 1.1, 1.6, 2.4 mg/L) the modelled phycocyanin reduction (decay) rates ($\Delta\%Phy/\Delta\text{time}$) are also higher, i.e. the average decay rates for the EP model were 52, 64, 85 and 164 while that for the LDR were 85, 249, 188 and 1621, respectively.

As can be seen from Figures 1 and 2, statistical fitting of the available data to the two equations leads to markedly different decay rates, clearly indicating a need to acquire finer resolution of data between Days 1 and 3 to assess the true decay dynamics. Further, the actual increases in phycocyanin from Day 0, as a response to the Zn addition, either through cell lysis and/or in a defence response (Poliak *et al.* 2011) indicates that the LDR is less suitable for such a dose-response condition, where the response is potentially an increase followed by a decline, i.e. the LDR describes no change from an initial fitted value (average from initial and higher values) until a point of decline is reached from that value.

Table 2. Models coefficients, and t_{50} and t_{90} values

Expt #	Zn dose (mg/L)	Exponential peak model (Eq. 1 ^b)						Logistic dose response model (Eq. 2 ^c)						
		a_1 (%)	b_1 (%)	c_1 (d)	f_1	t (d) of t_{50-1} ^a	t (d) of t_{90-1}	Dec. rate ₁	a_2 (%)	b_2 (%)	c_2 (d); t_{50-2}	f_2	t (d) of t_{90-2}	Dec. rate ₂
1-1	0.7	2.7	111	0.7	0.9	2.4	4.3	-40	6.0	98	2.8	12.7	3.6	-109
	1.1	0.01	110	0.6	0.9	2.3	3.9	-40	3.0	98	2.9	17.2	3.2	-226
	1.6	2.1	133	0.6	0.5	1.8	2.9	-70	2.5	108	2.6	17.2	3.0	-176
	2.4	2.3	289	0.5	0.2	1.3	1.8	-181	2.5	116	2.0	208	2.0	-2867
1-2	0.7	0.3	105	0.5	1.0	2.7	3.8	-64	2.8	96	2.7	7.2	3.8	-61
	1.1	0.4	137	0.5	0.5	1.5	2.4	-87	0.5	103	2.6	27.8	2.9	-271
	1.6	0.5	149	0.5	0.4	1.5	2.2	-99	0.5	105	2.4	18.4	2.7	-200
	2.4	0.01	175	0.4	0.3	1.2	1.7	-147	0.01	100	1.1	16.2	1.2	-375
2-1	2.5	0.01	169	0.5	0.3	1.3	1.9	-125	0.1	100	2.1	12.9	2.5	-150
2-2	2.5	0.01	111	-0.2	0.3	0.3	0.8	-145	0.0	100	0.4	3.7	0.8	-210
3-1	2.5	0.01	132	0.4	0.3	1.1	1.7	-113	0.1	100	1.1	6.9	1.5	-158
3-2	2.5	0.01	138	0.6	0.5	1.6	2.4	-84	0.1	106	2.4	15.1	2.8	-166

^a t_{50-1} and t_{50-2} are times required from dosing to reduce half of the initial Phy% value calculated by Eq.1 and Eq.2, respectively. While, t_{90-1} and t_{90-2} are times required to reduce 90% of phycocyanin values calculated by Eq.1 and Eq.2, respectively. ^b R^2 values were greater than 0.99; ^c R^2 values were greater than 0.96

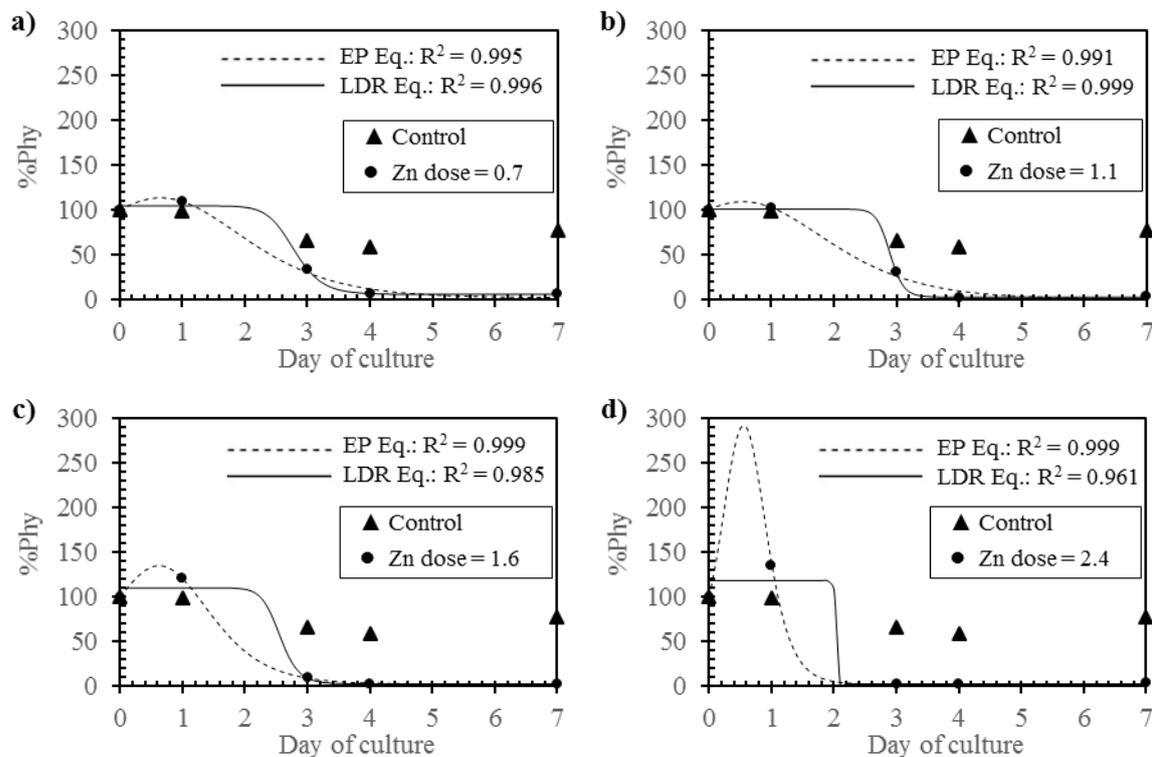


Figure 1. %Phy levels in pre-ASB waters without and with Zn subsequently added at various concentrations (a) 0.7, (b) 1.1, (c) 1.6 & (d) 2.4 mg/L Zn

In pre-ASB sampled waters (from the clarifier), Zn was found to be less effective at the tested concentrations than in ASB sampled waters (Pond 3 discharge). This may be due to the use of Zn as sacrificial anodes increasing the Zn concentration in the pre-ASB clarifier, compared to the ASB waters. Zeng *et al.* (2009) found *M. aeruginosa* acclimatized to and become resistant to Zn concentrations that would otherwise be toxic. The t_{50} for pre-ASB waters dosed with Zn was found to be generally higher at Zn concentration of 0.7 mg/L (by EP: +13%; by LDR: +4%), 1.1 mg/L (+35% & +10%), 1.6 mg/L (+17% & +8%) and at 2.4 mg/L of Zn (+8% vs +45%) than their corresponding t_{50} for ASB waters dosed with Zn. Consistent with the t_{50} , the t_{90} values followed the same trend with values higher for pre-ASB sampled waters dosed with Zn at 0.7 mg/L (by EP: +12%; by LDR: -6%), 1.1 mg/L (+38% & +9%), 1.6 mg/L (+24% & +10%) and at 2.4 mg/L of Zn (+6% vs +40%) than the corresponding ASB sampled waters. These data indicate that the impact of Zn on these cyanobacteria in PML wastewaters changes through the ASB, presumably depending on the changing characteristics of the wastewaters, the basis of which requires further investigation.

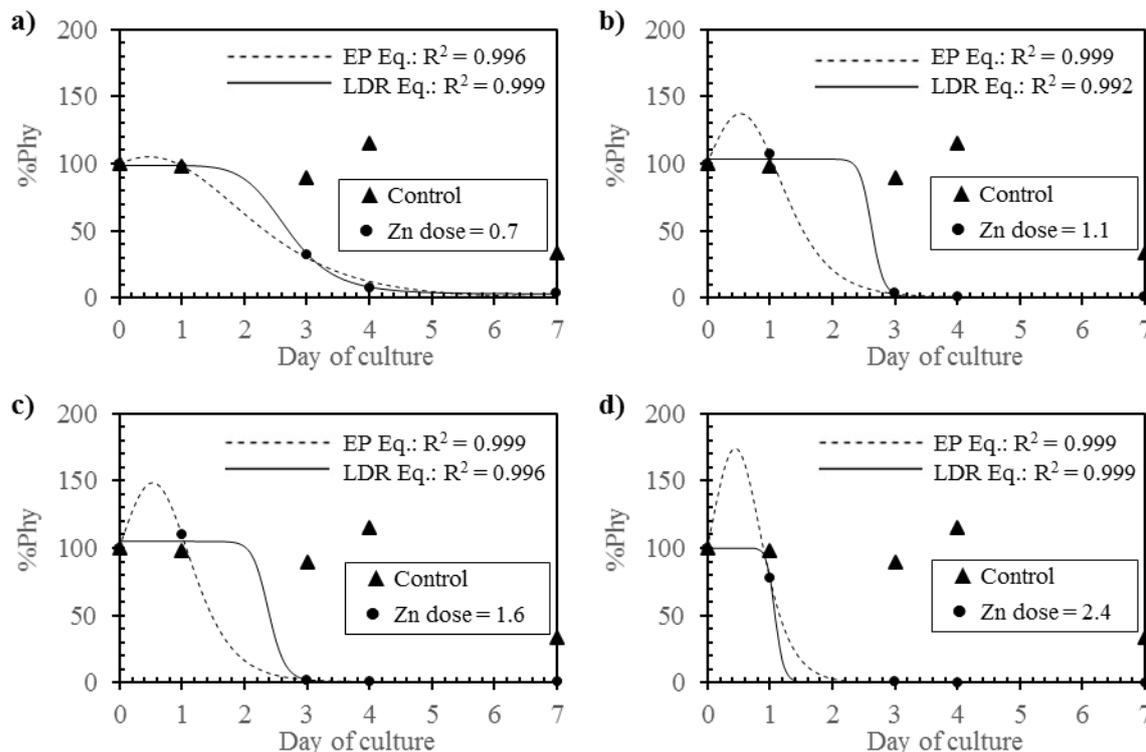


Figure 2. %Phy levels in ASB waters without and with Zn subsequently added at various concentrations (a) 0.7, (b) 1.1, (c) 1.6 & (d) 2.4 mg/L Zn

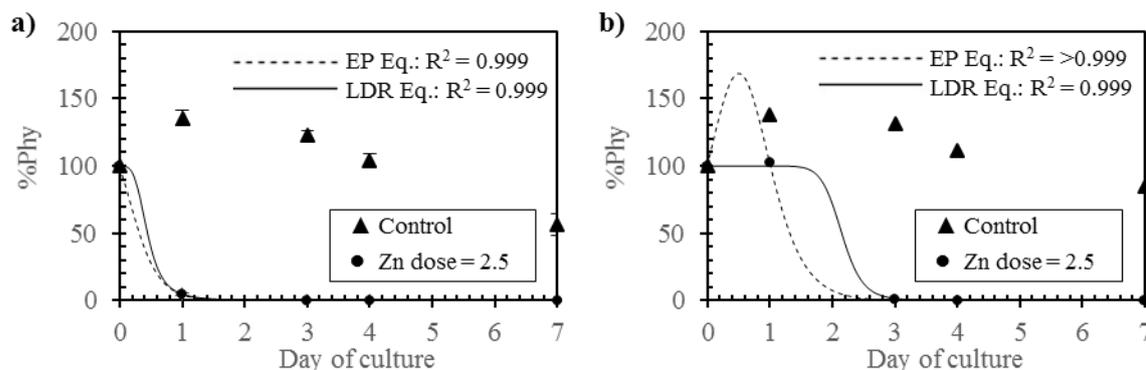


Figure 3. %Phy levels in ASB waters without and with Zn subsequently added at 2.5 mg/L spiked with initial phycocyanin at a) 46 µg/L and b) 86 µg/L

From Expt #2 conducted to investigate the effect of the initial MA338 level (based on phycocyanin levels, ~ 86 vs 46 µg/L) on the capacity of Zn (at a selected dose, of 2.5 mg/L) to inhibit this cyanobacteria (Figure 3), the decay rates were found to have an inverse correlation with the Zn:initial phycocyanin ratio, as expected. Samples inoculated with MA338 at 46 µg/L initial phycocyanin concentration showed higher decay rates (by EP: + 14%; by LDR: +29%) than their corresponding samples with 86 µg/L initial phycocyanin.

Further, the t_{50} values for samples at 46 µg/L initial phycocyanin were found to be lower (by EP: -77%; by LDR: -81%) than their corresponding values for samples at 86 µg/L initial phycocyanin. The t_{90} values followed the same trend, being lower for samples with 46 µg/L initial phycocyanin (by EP: -58%; by LDR: -68%) compared to their corresponding values for samples with 86 µg/L initial phycocyanin. This demonstrates that the impact of Zn is a function of the amount of Zn exposed to MA338 biomass.

From Expt #3 conducted to compare the effect of Zn (at 2.5 mg/L) on the two different cyanobacterial species (*Pseudanabaena spp.* and MA338), it was found that Zn similarly impacted both species based on loss of phycocyanin by Day 3 (Figure 4). Although the EP model was fitted to the *Pseudanabaena spp.* data, then assuming a maximum phycocyanin response level greater than at Day 0, the acquired data at Day 1 indicates no increase and further that the LDR function is a better representation of that data (Figure 4a). This is in

contrast to data of MA338, where phycocyanin levels consistently increased from Day 0 to Day 1, and the EP model is the better of the two models. The dynamics of phycocyanin levels in the controls varied, and with both declining to low percentages of original levels by Day 7. Samples inoculated with *Pseudanabaena spp.* and dosed with Zn had lower t_{50} values (EP: -45%; LDR: -118%) than corresponding samples inoculated with the MA338. The t_{90} values for samples inoculated with *Pseudanabaena spp.* are lower (EP: -41% and LDR: -87%) compared with corresponding values for samples inoculated with MA338. Further investigation is needed to determine if phycocyanin dynamics of cyanobacteria upon exposure to Zn is specie specific and if so, which model function provides the best description for a particular cyanobacterium.

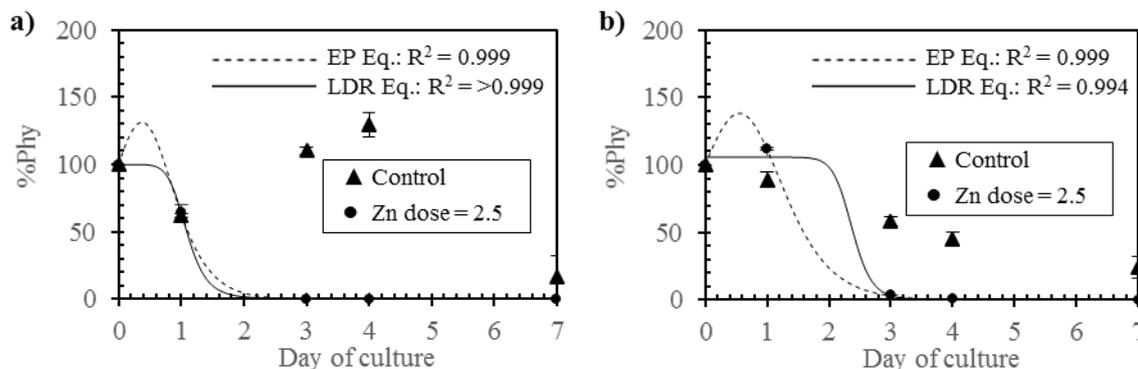


Figure 4. %Phy levels in ASB waters with Zn subsequently added at 2.5 mg/L spiked with a) *Pseudanabaena spp.* and b) MA338 species

The EP model correctly described increase in phycocyanin after initial Zn exposure to MA338, but the maximum level fitted based on data from days 1, 3, 4 and 7 is speculative of what would be the actual value, i.e. the actual maximum value and occurrence time would require high-resolution data from the start of the experiment to ~ 24 hr of Zn exposure. The actual maximum phycocyanin level and when this occurs may be incidental in terms of the effect of Zn on the timeframe for the loss of phycocyanin to a minimum level but may be significant in terms of a toxicity response to an exposure time of a specific Zn concentration. Here, for MA338, the initial response that is evident is phycocyanin increase, presumably from cell lysis and/or as a defence response to Zn presence at stressor levels (Poliak *et al.* 2011).

In a study which investigated the effects of Cu, Ni and Zn on two strains of *M. aeruginosa* (CALU 972 and 973) sourced from the Karelian lakes, Russia, Polyak *et al.* (2013) reported that addition of low Zn concentrations (25–100 $\mu\text{g/L}$) to a culture medium induced growth in both strains. This is in contrast to Cu and Ni, where no concentration at or above 25 $\mu\text{g/L}$ induced biomass growth was found (Polyak *et al.* 2013). Cyanobacteria culture medium ASM-1, (Gorham *et al.* 1964) has about 0.04 $\mu\text{g/L}$ Cu as a trace element. Polyak *et al.* (2013) reported Cu having the highest suppression to biomass growth of both strains tested, followed by Ni and then Zn (i.e. 50 $\mu\text{g/L}$ Cu, 225 $\mu\text{g/L}$ Ni and 250 $\mu\text{g/L}$ Zn).

In our study, phycocyanin detection was used as an indicator of biomass growth, as well as of cell stress and lysis, and subsequent phycocyanin reduction. High percentage loss of phycocyanin was also empirically correlated with observed settling of cyanobacteria into a sludge layer, in batch test jars. Zeng *et al.* (2009) studied the acclimation of a strain of *Microcystis aeruginosa* (FACHB-905, [MA905] sourced from Lake Dianchi, China) to Zn (at up to 3.0×10^{-8} M $[\text{Zn}^{2+}]$) over 1 to 15 days. They found that intra-Zn concentrations in cells that were acclimated for different durations, were significantly greater than the controls, but reduced to about control levels after 5 days of recovery. Zeng *et al.* (2009) found that the MA905 became more tolerant of metal toxicity following acclimation using the highest Zn concentration tested (3.0×10^{-8} M $[\text{Zn}^{2+}]$). However, they found that the cyanobacterium became very sensitive following 1 day of recovery, while the tolerance of the acclimated-recovered cyanobacterial cells was comparable to the controls following 5 days of recovery. Their findings demonstrated potential responses by *M. aeruginosa* to Zn exposure where this is at non-toxic levels, including acclimation and efflux. In our study Zn concentrations tested were much higher than those assessed by Zeng *et al.* (2009), and in the toxic range. The effect of Zn on cyanobacteria is highly dependent on concentration ranging from it being an essential metal, a stressor and to being toxic. Application of Zn for suppression of cyanobacteria would need to ensure concentrations are applied and maintained at levels that are toxic, and avoidance of levels that support growth.

4. CONCLUSIONS

We propose that this modelling approach integrated with in-situ phycocyanin detection enables improved understanding of Zn as a potential cyanobacterial control agent as well as understanding its importance where it occurs as an apparent background metal in industrial wastewaters. Although both model equations used in this study broadly simulated the dynamics of phycocyanin in samples of PML wastewaters in response to Zn exposure between 0.7 and 2.4 mg/L, further investigation is needed at particular times of exposure. Initial assessment involved data collections on days 1, 3, 4 and 7 with maximum effect (based on complete loss of phycocyanin) evident by day 4. However, the results obtained so far show that higher resolution data is needed to understand better the response of cyanobacteria to Zn from initial exposures (within 1 day). Further, determination is needed of the effective period of cyanobacteria exposure time to Zn at specific concentration to cause a toxicity response (phycocyanin loss) i.e. integration of Zn concentration over exposure time. The magnitude of the peak in phycocyanin levels is speculative based on low resolution data available and where the phycocyanin levels exceed the initial levels. To address this, phycocyanin levels would need to be monitored at higher resolution but this presents logistical challenges in batch experiments. Any consideration of the use of Zn as a cyanobacteria control agent needs to account for the potential of low concentrations of Zn inducing growth. Low level ambient Zn in the PML wastewaters, with low colour and adequate nutrients might explain in part, the basis for the occurrences of cyanobacterial blooms in the PML wastewaters.

ACKNOWLEDGMENTS

We thank Mr Malcolm Abbott for sample collection and analyses, Mr Graham Burch and Mr Josef Marzouk for project direction and Ms Sandy Dickson and Mr Cédric Dajnak for support in laboratory investigations.

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