Suspended Clay Inhibits the Growth of Harmful Algal Bloom-forming Freshwater Cyanobacteria Through Physical Interactions

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Abstract

Many theories exist to predict the growth of Microcystis, one major type of toxic cyanobacteria that form harmful algal blooms. However, the impacts of suspended particles, which are ubiquitous in freshwater, on Microcystis growth have not been fully understood. Here, we show that a smectite clay can inhibit the growth of Microcystis aeruginosa, a typical toxic freshwater cyanobacterium, through physical clay-cell interactions. We grow M. aeruginosa under identical growth conditions in three nutrient solutions: one pure solution, one with a synthetic and transparent clay, and another one chemically modified by clay but with clay particles removed. Cells in pure solution and chemically-modified solution grow equally well, while cells in solutions with the physical presence of clay do not grow nor produce pigments. Microscopic imaging of clay-cell interactions suggests that the inhibition of the growth of M. aeruginosa by clay is due to the physical encapsulation of cells in clay.

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Suspended Clay Inhibits the Growth of Harmful Algal Bloom-forming Freshwater Cyanobacteria Through Physical Interactions

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Running head: Clay inhibits growth of cyanobacteria

Author contribution:
J.Q.Y. and K.T. conceived and designed the project. K.T., G.W., and S. N designed and performed the experiments. K.T. and J.Q.Y. analyzed the data and wrote the paper.

Abstract

Many theories exist to predict the growth of *Microcystis*, one major type of toxic cyanobacteria that form harmful algal blooms. However, the impacts of suspended particles, which are ubiquitous in freshwater, on *Microcystis* growth have not been fully understood. Here, we show that a smectite clay can inhibit the growth of *Microcystis aeruginosa*, a typical toxic freshwater cyanobacterium, through physical clay-cell interactions. We grow *M. aeruginosa* under identical growth conditions in three nutrient solutions: one pure solution, one with a synthetic and transparent clay, and another one chemically modified by clay but with clay particles removed. Cells in pure solution and chemically-modified solution grow equally well, while cells in solutions with the physical presence of clay do not grow nor produce pigments. Microscopic imaging of clay-cell interactions suggests that the inhibition of the growth of *M. aeruginosa* by clay is due to the physical encapsulation of cells in clay.

Key words:

Harmful Algal Blooms, *Microcystis*, clay, suspended sediment, encapsulation, cell density

Significance statement:

The United States has spent over 1 billion dollars since 2010 dealing with Harmful Algal Blooms (HABs) in rivers, lakes, bays, and drinking water systems. One of the major toxic cyanobacteria that contribute to HABs in freshwater is *Microcystis*. However, factors that control the growth of *Microcystis* have not been fully characterized. This study provides evidence and mechanistic explanation for inhibition of *Microcystis* growth by clay, which is a major component of suspended sediment ubiquitous in freshwater. The results will help predict the occurrence of HABs in aquatic environments with varying turbidity and develop strategies to prevent HABs.

Data Availability Statements:

Data will be made available in the Data Repository for University of Minnesota repository (https://doi.org/10.13020/pf7m-2f62).

Introduction

Harmful algal blooms (HABs) in aquatic ecosystems can cause serious harm to human health through a variety of exposure pathways, including consumption of contaminated water and fish (Zamyadi et al., 2013; Hardy et al., 2015), direct contact with water (Stone & Bress, 2007), and exposure to aerosolized bacteria (Cynthia Ann & Amanda Lorraine, 2021; Weirich & Miller, 2014). Additionally, HABs cause hypoxic conditions and reduce water clarity, which threaten fish and invertebrate habitats (Watson et al., 2016; Trevino-Garrison et al., 2013; Chapra et al., 2017). One major type of HAB-forming microorganism in freshwater lakes is *Microcystis*, a genus of freshwater toxin-producing cyanobacteria (Mancuso et al., 2021). *Microcystis* blooms frequently occur in the Great Lakes over the summer, which threaten local fishing economies (Gill et al., 2018) and has led to short-term "do not drink" advisories for local residents (Levy, 2017). The toxins produced during *Microcystis* blooms have been associated with a variety of negative health effects, including vomiting, throat irritation, gastrointestinal illness, and skin irritation (McHau et al., 2019; Weirich & Miller, 2014). These negative impacts of *Microcystis* blooms are becoming increasingly threatening under climate change (Michalak et al., 2013; Chapra et al., 2017; Mancuso et al., 2021), as more frequent and/or long-lasting blooms have been recorded in inland lakes due to increasing temperatures (Deng et al., 2014; Lehman et al., 2013; Hayes et al., 2020). Knowledge about factors that control the growth of *Microcystis* cells is needed to predict the occurrence and frequency of *Microcystis* blooms and develop HABs mitigation strategies.

There are many theories to predict the growth of *Microcystis* that take into account factors such as salinity (Osburn et al., 2022), nutrient availability (Tatsumoto et al., 2006; You et al., 2018), and temperature (Amano et al., 2008). However, the impacts of suspended particles, which are ubiquitous in nature, on *Microcystis* growth have not been fully understood. Field investigations show that two rivers with similar nutrient conditions can have dramatically different levels of HABs due to a difference in suspended sediment concentrations (Wang, 1974). For example, the Illinois river, which has abundant nutrients and appears muddy, had no algal blooms, while its tributary Fox river, which had a similar nutrient level and agal seeding but lacks suspended sediment, had frequent HABs. This observation cannot be explained by current theories which correlate *Microcystis* growth with temperature, light intensity, and available nutrients (Mancuso 2021, Levy 2017) as well as grazing zooplanktons and turbulence (Dzialowski et al., 2011; Chan et al., 2004).

While some papers attribute the impacts of suspended sediment on algal growth to a reduction in light intensity (Dzialowski et al., 2011; Smith, 1990; Knowlton & Jones, 2000), we anticipate that the impacts of suspended fine sediment, especially clay, on algal growth is not merely through light attenuation, because there are a variety of other potential clay-cell interactions that may impair cell growth. First, clay can bond to cell surfaces through electrochemical forces, which cause them to co-aggregate (Liu et al., 2021). Secondly, clay has also been used to actively remove harmful algal blooms by binding phosphorus and causing algae cells to aggregate to sediment (Lürling & Faassen, 2012; de Magalhaes et al., 2019). Additionally, certain modified clays used in filters have combined the absorptive properties of clay with compounds that limit the photosynthetic activity of cyanobacteria, leading to cell death (Sukenik et al., 2017). Based on these previous studies, we hypothesize that clay can impact the growth of *Microcystis* cells through electrochemical or physical interactions.

Here we investigate the impacts of clay-*Microcystis* interactions on *Microcystis* growth. We grew a typical cyanobacterium that causes HABS, *Microsystis aeruginosa*, in solutions with different concentrations of clay. To eliminate the impacts of clay on light intensity, we used a transparent synthetic clay, laponite, which has similar chemical structures as the natural bentonite clay and can be classified as a smectite clay. We grew *M. aeruginosa* in nutrient solutions with different concentrations of clay in a controlled light and chemical environment and measured the cell density as a function of time. We further investigated the physical interactions between clay and algal cells by imaging them under a confocal laser scanning microscope. Based on the *M. aeruginosa* growth curves and the confocal imaging, we show that clay can physically interact with *Microcystis* cells, prevent their growth, and hinder *Microcystis* blooms.

Methods

I. Bacteria Growth

We grew *Microcystis Aeruginosa* (UTEX LB2385) in 100 mL of BG-11 solution. Specifically, a 20mL vial of the cell solution was transferred to 80 mL of BG-11 solution. The BG-11 solution consists of 16 mL of 50x-concentration BG-11 solution diluted with 784 mL DI water (Milli-Q), as well as 0.4 g of HEPES buffering agent.

We placed the flask with the cyanobacteria + nutrient solution on a shaker set at 110 rpm in an incubator set at 24 ℃. The incubator was set on a 12 hr:12 hr light:dark cycle at approximately 2000 lumens. One 4100K Cool White Color Temperature fluorescent tube light (Philips F20T12) was placed at the top of the incubator. The tube light was covered in a semi-transparent white cloth to dissipate the light, providing a consistent light intensity to the entire shaker surface. During experimentation, the light intensity reaching the flasks ranged from 1800 lumens to 2200 lumens. The pH remained between 6.5 and 8.

We kept the *M. Aeruginosa* in the growth phase by renewing its nutrient solution on a monthly basis. Specifically, we transferred 20 mL of the cyanobacteria solution from the incubated flask into a new flask with 80 mL of fresh BG-11+HEPES solution. This *M. Aeruginosa* solution in the growth phase served as the initial seeding solution for the clay-cyanobacteria culture experiments.

II. Clay-Cyanobacteria Experiment Set-Up

To investigate the impacts of clay on *Microcystis* growth, we grow *M. aeruginosa* in BG-11+HEPES solution with varying amounts of laponite RD (BYK USA Inc.), a transparent and synthetic smectite clay, and monitor the growth of the cells over time by counting the cell density using a hemocytometer. First, 10 ml *M. aeruginosa* solutions, obtained from the same cell culture in the growth phase (the 16th day of growth), were added to flasks with 90 ml three different growth solutions: one is 90 ml BG-11+ HEPES solution without clay, the second is 90 ml BG-11+ HEPES solution with 1 % (weight ratio) clay, and the third is BG-11+ HEPES solution modified by 1% clay, as shown in Table 1. The 1% clay solution was made by adding 10 grams of laponite clay to 1000mL of BG-11+HEPES solution, mixing the solution and letting it sit for two days, and

mixing again and letting it sit for another two days. This procedure allows the chemical composition between clay and the solution to reach equilibrium. The solution modified by 1% clay was made by first making the 1% clay solution and then removing the clay particles through centrifugation. Specifically, the 1% clay solution made from the procedure described above was shaken and transferred into many 50 mL centrifuge tubes and centrifuged at 3000 rpm for 30 minutes. After the clay particles settled into the bottom of the centrifuge tubes, the upper solution was then transferred to a culture flask and referred to as a clay modified solution. Visual examination of the clay modified solution using a microscope shows that there were no clay particles in the solution.

Three replicates were made for each growth solution (Table 1). All solutions were autoclaved before usage, and the solution with clay were exposed to UV light for 30 minutes for additional sterilization. To further eliminate the impacts of light intensity due to the transparent clay on bacterial growth, we placed a flask with clay and 100 ml BG-11 solution but lacking cells on top of the flask with cells growing in the BG-11 solution. For the flasks with cells growing in BG-11 solution with 1% clay and in the solutions modified by clay, we placed flasks with pure BG-11 solution and lacking cells on top. The stacking of two flasks (Figure 1) eliminates the impacts of light attenuation due to clay on cell growth, because if light attenuation exists for the transparent clay, then the cells in pure BG-11 solution with clay solution on top will also receive less light.

Similar to the initial preparation of the bacteria solution, the experimental flasks were placed in an incubator at 24 ℃, shaken at a rate of 110 rpm, and exposed to approximately 2000 lumens of light in a 12:12 light:dark cycle.

Table 1: Setup of clay-cell culture experiments

Upper flask	Nutrient solution +1% clay	Nutrient solution	Nutrient solution
Lower flask	Nutrient solution seeded with *M. aeruginosa*	Nutrient solution + 1% clay, seeded with *M. aeruginosa*	Modified clay nutrient solution[a], seeded with *M. aeruginosa*
Quantity	3	3	3

[a] Modified clay nutrient solution was made by removing clay particles from the 1% clay solution through centrifuge and filtration.

Figure 1: Experimental set-up. *M. aeruginosa* cells were grown in three growth solutions described in Table 1 in the lower flasks. Flasks filled with solutions with and without clay were placed on top of the lower flasks to create identical light conditions for all cases. The flasks were placed on a shaker set to 110 rpm within an incubator with white fluorescent light illuminating from the top of the flasks at 12 h:12h light/dark cycle.

III. Density Measurements and Confocal Microscopic Imaging

We measured cell density three times a week. An absorbance versus wavelength graph was created from samples in each flask on the spectrophotometer. We performed a cell count under a Nikon Eclipse E400 microscope with a hemocytometer at 40x magnification. To compensate for the reduction in solution volume due to measurements and the evaporation of the bacterial solution, we added additional BG-11+HEPES solution every 4 weeks to keep the volume of solution in each flask at 100 mL. On weeks where the cyanobacterial solution was diluted with BG-11+HEPES solution, we took the cell density measurements before dilution occurred. We calculated the dilution factor for each flask by dividing the volume of the solution by 100mL. In subsequent weeks, this factor was multiplied by the cell density to compensate for the dilution effect due to the added solution.

In addition to cell density measurements, the physical interactions between cyanobacteria and clay particles were visualized using a confocal laser scanning microscope (Nikon C2 plus). Each image is around 2048 by 2048 pixels at a resolution of 0.08 um/pixel. We used a 20X objective magnification. A sequence of images was taken at 10-second intervals for 5 minutes. The laser used for excitation has a wavelength of 488 nm (FITC) and the emission wavelength is 525 nm.

Results

During the *Microcystis* clay culture experiments, we observed significant growth of *M. aeruginosa* in both the pure BG-11+HEPES solution and the modified clay solution (Flasks S and MS in Figure 3). In contrast, no significant growth was observed in the 1% clay solution (Flask C in

Figure 3). The measured *M. aeruginosa* cell density in the three different solutions over 42 days is shown in Figure 2. The initial cell density on Day 2 is between 300 and 700 cells/mm^3 for all three solutions. *M. aeruginosa* in the pure solution and modified clay solution grow to tens of thousands of cells/mm^3. Specifically, the cell density of *M. aeruginosa* in the pure solution peaked on Day 35 with a density of 18,856 cells/mm^3. The cell density within the modified clay solution also peaked on Day 35 with a density of 22,484 cells/mm^3. In contrast, on Day 35, the cell density in 1% clay solution was only 90.2 cells/mm^3. The peak density measurement for the 1% clay solution occurred on Day 21, when the measured cell density was 1,980 cells/mm^3, one order of magnitude smaller than in pure solution and in clay-modified solution.

The suppression of *M. aeruginosa* growth in the solution with clay, compared with growth in solutions without the physical presence of clay (pure solution and clay modified solution), suggests that clay slows down bacterial growth through physical interactions rather than light attenuation and chemical interactions. First, the fast growth of cells in the pure solution with a flask containing 1% clay solution on top (Figure 2) suggests that the suppression of cell growth is not due to light attenuation because if the transparent laponite clay attenuates light, the light in the pure solution would also be attenuated by the solution with clay on top. Second, the fast growth of cells in the clay modified solution suggests that the suppression of cell growth is not due to the chemical interactions, or the sorption of nutrients to clay, because if the sorption of nutrients by clay was what slows down cell growth, then the cell growth in the clay modified solution would also be slow. Based on these two reasonings, we hypothesize that the impacts of clay on the growth of *M. aeruginosa* is likely through physical interactions, i.e., forming clay-cell aggregates which have often been observed in both the field and experiments (Pan 2005, Wang 2015, Peng 2018).

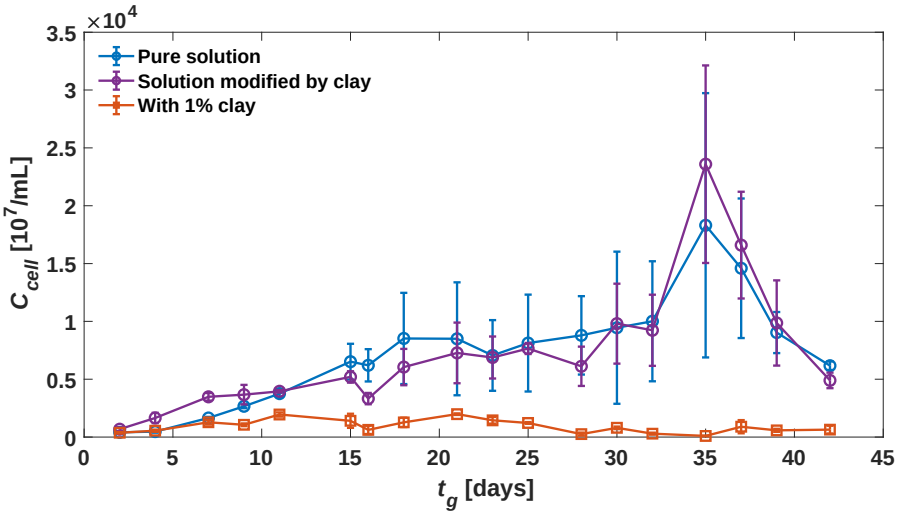

Figure 2: Growth curves of *M. aeruginosa* based on average cell density for three growth conditions: pure nutrient solution (blue symbols), modified clay nutrient solution (purple symbols), and nutrient solution with 1% clay (orange colors). The cell density of *M. aeruginosa* in both the pure solution and modified solution increased at least 30-fold over 35 days. In contrast, no consistent growth of *M. aeruginosa* was observed in the solution with 1% clay. Each point indicates the average cell density of three experimental replicates. Error bars represent the standard error of the three replicates.

In addition to measuring cell density, we also imaged the flasks containing the three solutions with growing cells during the experiments. Figure 3 shows representative images of the three bacterial solutions at 28 days and 35 days after the experiments started. On both days, the flasks with pure nutrient solution (labeled as "S") and modified clay solution (labeled as "MS") appear green, indicating that green pigment has been produced due to *M. aeruginosa* growth. In contrast, the color of the bacterial solution with 1% clay (labeled as "C"), appears yellow instead of green, further suggesting that the physical presence of clay significantly reduced the growth of *M. aeruginosa* cells and their production of pigments and also likely toxins.

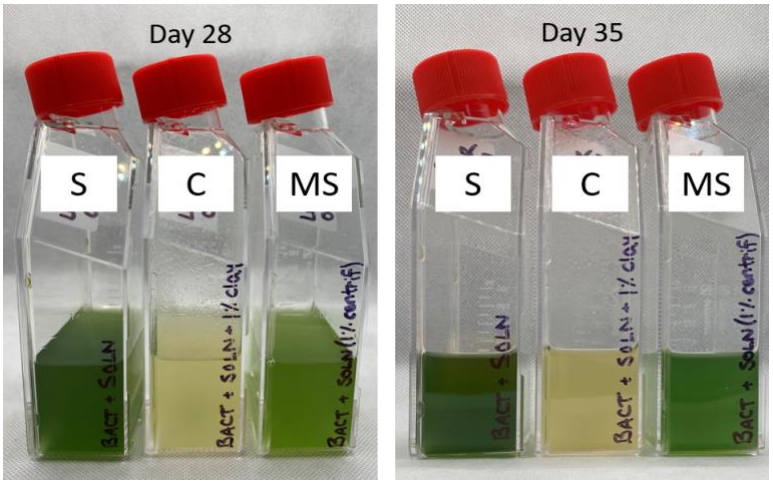

Figure 3: Photos of the lower flasks with *M. aeruginosa* growing in solutions at three different growth conditions (Table 1) at two growth times, 28 and 35 days. The green color is representative of the amount of green pigment produced by the cells for each of the three *M. aeruginosa* growth conditions: pure nutrient solution (**S**), nutrient solution with 1% clay **(C)**, and modified clay nutrient solution (**MS**).

To test our hypothesis that the physical interactions between clay and the cells slow down the growth of *M. aeruginosa*, we imaged the *M. aeruginosa* cells sampled from each growth solution using a confocal laser scanning microscope. Figure 4 shows time-lapse photos of the *M. aeruginosa* taken on Day 49. Within the clay growth solution, two *M. aeruginosa* cells are visible, encapsulated in a clay particle. The cells show minimal movement at each of the three-time intervals: 0 seconds, 155 seconds, and 310 seconds. In contrast, cell movement is observed in the time lapse images taken of *M. aeruginosa* growing in both pure nutrient solution and modified clay solution. The cell densities are also notably higher in the two solutions lacking the physical presence of clay than that in the solution with clay. This observation suggests that clay and the cyanobacterial cells indeed physically interact with each other and such interactions reduce the movement and possibly replication capability of the cells, leading to the suppression of cell growth.

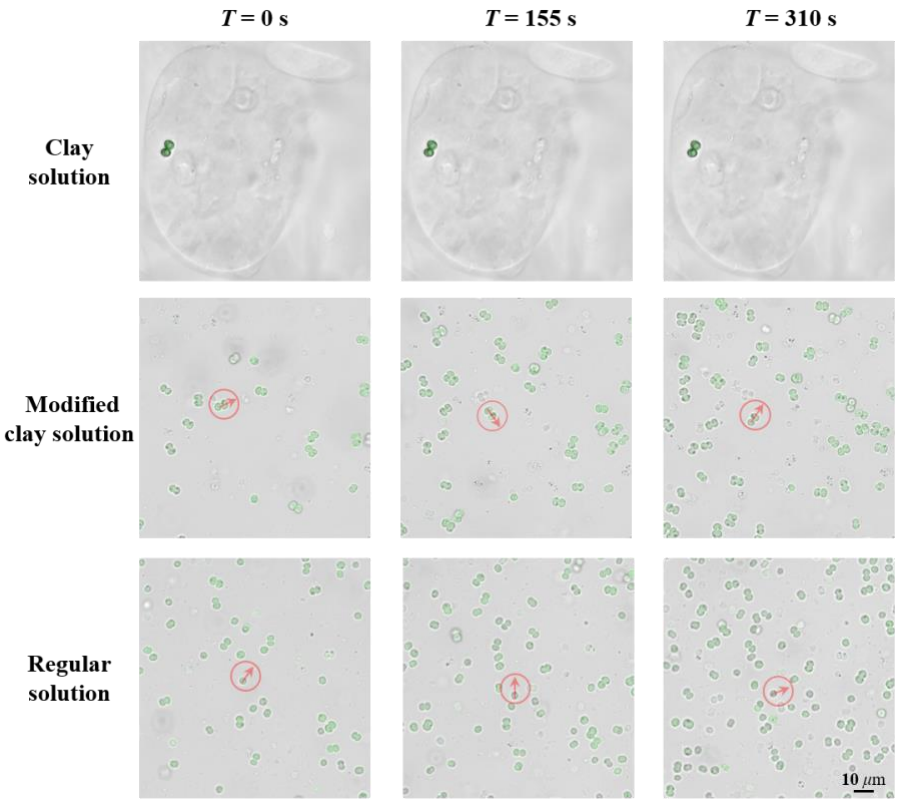

Figure 4: Time-lapse confocal images of *M. aeruginosa* solutions sampled from the lower flasks for the three different growth conditions (Table 1). $T = 0$ s represents the start of the time-lapse sequence. The number of *M. aeruginosa* cells which are green due to pigments were significantly lower in the clay solution than in the modified clay solution and the nutrient solution. The cells in the clay solution did not move during the imaging time, while the cells moved in the modified clay solution and the nutrient solution. The locations and orientations of one representative cell in the modified clay solution and the nutrient solution were indicated by the red circles and arrows, respectively.

Discussion

Our results show that the growth of *M. aeruginosa* was significantly reduced, by an order of magnitude, due to the physical presence of a transparent synthetic smectite clay (laponite). First, our bacterial-growth experiments show that *M. aeruginosa* growth is limited when grown in a nutrient solution containing 1% clay. In contrast, the cells grew fast in a flask with pure nutrient solution, which was placed under another flask of abiotic solution containing 1% clay. Therefore, the decrease in cell growth is not caused by the light attenuation by clay because the pure solution also has clay on top of it. Second, the cells grew well in the clay modified solution, which was mixed with 1% clay for 4 days but with clay filtered out afterwards. Such growth suggests that clay did not prevent cyanobacterial growth by absorbing key nutrients. Only when the *M. aeruginosa* was grown in physical contact with clay was static growth recorded. Therefore, the physical interactions between the laponite clay particle and the *M. aeruginosa* are what limit cell growth. The relationship between turbidity or clay concentration and algal bloom concentration

may not be explained by nutrient absorption, or a difference in light intensities, but rather by the physical encapsulation of the algal cells by clay.

Our result explains previous field observations that the Illinois river, which has abundant nutrients and appears muddy, had no algal blooms, while its tributary Fox river, which had a similar nutrient level and agal seeding but lacks suspended sediment, had frequent HABs (Wang, 1974). The mechanisms of cell-clay interactions revealed in this study will improve future predictions of the occurrence of HABs in aquatic environments with varying turbidity and development of strategies to prevent HABs. Specifically, our results demonstrate that smectite clay can slow down the growth of *Microcystis* cells through physical encapsulation, suggesting that clay can potentially be used to reduce the growth of harmful algal cells and the development of HABs.

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https://doi.org/10.1016/j.watres.2013.02.040

Metadata template[1] for datasets of *L&O-Letters* articles

Table 1. Description of the data supporting inhibition of the growth of harmful algal bloom-forming freshwater cyanobacteria by clay

Title of dataset	*Data supporting Inhibition of the Growth of Harmful Algal Bloom-forming Freshwater Cyanobacteria by Clay*
URL of dataset	*https://conservancy.umn.edu/handle/11299/226612*
Abstract	*This dataset contains cell density measurements and confocal images of Microcystis aeruginosa. The raw data from hand counting cells under a microscope with a hemocytometer is included, as well as the calculated cell density based on the hemocytometer measurements. The bacteria cells were grown in one of three growth conditions. Each growth condition was simulated in three different flasks. For each date, the cell densities of identical growth conditions were averaged. Cell densities for each date and growth condition were subsequently plotted with error bars to determine any trends. Additionally, data on the environmental conditions of cell growth are included on dates when the measurements were taken. The physical interactions between cyanobacteria and clay particles were visualized using a confocal laser scanning microscope (Nikon C2 plus). Each image is around 2048 by 2048 pixels at a resolution of 0.08 um/pixel. We used a 20X objective magnification. A sequence of images was taken at 10-second intervals for 5 minutes. The laser used for excitation has a wavelength of 488 nm (FITC) and the emission wavelength is 525 nm.*
Keywords	*Harmful Algal Blooms, Microcystis, clay, laponite, suspended sediment, encapsulation, cell density, Saint Anthony Falls laboratory, confocal microscopy images*
Lead author for the dataset	*Judy Yang*
Title and position of lead author	*Assistant professor and principal investigator*
Organization and address of lead author	*Address of the lead author during the collection of the data, as well as current address if it is different*
Email address of lead author	*judyyang@umn.edu*
Additional authors or contributors to the dataset	*Katherine R Tomaska (tomas098@umn.edu), Guanju Wei (wei00235@umn.edu)*
Organization associated with the data	*Saint Anthony Falls Laboratory, University of Minnesota*
Funding	*This study was supported by JQ Yang's startup funds and University of Minnesota's MnDRIVE Environment seed grant.*
License	*CC0 1.0 Universal*
Geographic location – verbal description	*Text description of the region of study (can include government entities as in county, province, state, country, etc) as well as finer-scaled information*
Geographic coverage bounding coordinates	*Saint Anthony Falls Laboratory, University of Minnesota*
Time frame - Begin date	*09/29/2021*
Time frame - End date	*11/08/2021*
General study design	*Four types of laboratory measurements were collected and each measurement is described below.*

[1] *This document liberally borrows from a similar document provided by the Environmental Data Initiative*

	A. Filename: Hemocytometer_Data_Final.xlsx *Short description: This file contains the data collected from hemocytometer measurements, as well as data regarding the environmental conditions of growth for the cyanobacteria (including light intensity and pH). The excel spreadsheet is broken down into different columns based on category. The first three columns include the day #, date, and flask number. In total, 9 flasks were used to grow the bacteria. These flasks were separated into three different growth conditions. The next four columns are a record on environmental conditions including light intensity and pH. The next set of columns are a record of dates when a growth solution was added to the flasks. The additional growth solution resulted in a dilution factor that was applied when calculating the overall cell density in each flask. The next column is the average cell count per box. This column represents the average number of cells counted in each 0.004 mm^3 square of the hemocytometer for a given sample. The raw data used to compute this average is located in the last columns, under the "Detailed Hemocytometer count" tab. Finally, the values in the "Average Cell Density" column were calculated by dividing the average cell count per square by the volume of the square (0.004 mm^3) and multiplying by the dilution factor.* *B. Filename: Code_Cell_counting_final.m* *Short description: This file contains the MatLab code that was used to process the data within the file Hemocytometer_Data.xlsx. For each date, the MatLab code averages the cell densities of flasks with identical growth conditions. The standard error of the mean for each group of 3 flasks was used to create error bars for the data. The average cell density per treatment type, as well as the error bars associated with these averages, are plotted together. This plot offers a comparison of average cell growth across the three different treatment conditions.* *C. Filename: Data Deposit_Confocal images (1.66 GB).zip* *Short description: The confocal imaging shows the physical interactions between cyanobacteria and clay particles. The data set includes 3 folders: 6#_clay solution: Raw data of clay solution experiments 7#_modified clay solution: Raw data of modified clay solution 8#_regular solution: Raw data of regular solution Each folder contains a Nikon confocal microscopy file (.nd2) and twenty output images.* *2. Relationship between files: The average cell density in the Hemocytometer_Data.xlsx sheet is read by the MatLab Code file. Error bar calculations based on the data are also calculated in the MatLab code.* *The confocal imaging is separate from the hemocytometer data. It serves as a visual representation of the quantitative cell density results counted under the microscope.*
Methods description	*1. Description of methods used for collection/generation of data:* *This experiment studied the impacts of three different growth media scenarios on M. aeruginosa growth. Nine flasks were used to grow the bacteria. Therefore, there were 3 flasks for each growth media scenario. The three scenarios included a control with M. aeruginosa grown in BG-11 solution, a scenario where M. aeruginosa was grown ini BG-11 solution with 1% laponite*

	added, and a scenario where M. aeruginosa was grown in a solution modified by laponite and centrifuged. *The experimental flasks were placed in an incubator at 24 ℃, shaken at a rate of 110 rpm, and exposed to approximately 2000 lumens of light in a 12:12 light:dark cycle.* *We measured cell density three times a week. We performed a cell count under a Nikon Eclipse E400 microscope with a hemocytometer at 40x magnification. To compensate for the reduction in solution volume due to measurements and the evaporation of the bacterial solution, we added additional BG-11+HEPES solution twice during the experiment to keep the volume of solution in each flask at 100 mL. On weeks where the cyanobacterial solution was diluted with BG-11/hepes solution, the cell density measurements were taken before dilution occurs. We calculated the dilution factor for each flask by dividing the volume of the solution by 100mL. In subsequent weeks, this factor was multiplied by the cell density to compensate for the dilution effect due to the added solution. Additionally, the pH of each bacteria solution, as well as the light intensity within the incubator, was recorded at least once a week to ensure steady environmental conditions. The pH was estimated with pH paper.* *In addition to cell density measurements, the physical interactions between cyanobacteria and clay particles were visualized using a confocal laser scanning microscope (Nikon C2 plus). Each image is around 2048 by 2048 pixels at a resolution of 0.08 um/pixel. We used a 20X objective magnification. A sequence of images was taken at 10-second intervals for 5 minutes. The laser used for excitation has a wavelength of 488 nm (FITC) and the emission wavelength is 525 nm.* *2. Methods for processing the data:* *The raw data includes the hemocytometer data. The number of cells in each 0.004 mm^3 volume square was recorded in a spreadsheet. The overall average cell density of each sample was calculated by dividing the average cell count per square by the volume of the square (0.004 mm^3) and multiplying by the dilution factor. The calculated cell densities for identical growth scenarios were averaged on each date within the Matlab code. The three growth scenarios were plotted together with error bars on MatLab to identify any trends. The error bars were calculated as the standard error of the mean.* *3. Instrument- or software-specific information needed to interpret the data:* *The .nd2 files of the confocal images can be opened by NIS software, which can be downloaded here:* *https://www.microscope.healthcare.nikon.com/en_EU/products/software/nis-elements/viewer* *4. Standards and calibration information, if appropriate:* *5. Environmental/experimental conditions: The experimental flasks were placed in an incubator at 24 ℃, shaken at a rate of 110 rpm, and exposed to approximately 2000 lumens of light in a 12:12 light:dark cycle.*

	6. Describe any quality-assurance procedures performed on the data: We conducted experiments using three replicate samples to improve data quality. *7. People involved with sample collection, processing, analysis and/or submission:* *Katherine Tomaska (sample collection, processing, analysis, submission)* *Judy Yang (MatLab code for processing, analysis, submission)* *William (for the confocal imaging, processing, submission)*
Laboratory, field, or other analytical methods	*1. Number of variables: 10* *2. Number of cases/rows: 180* *3. Missing data codes:* *Code/symbol Definition* *Code/symbol Definition* *4. Variable List* *A. Name: Day #* *Description: Number of days since beginning of experiment (when the stock cyanobacteria was added to corresponding nutrient solutions)* *B. Name: Date* *Description: Date of data collection* *C. Name: Flask #* *Description: Flask number (1-9) that corresponds to the type of solution* *Flasks 1 - Stock cyanobacteria solution (not included in analysis)* *Flasks 2,5,8 - Nutrient Solution* *Flasks 3,6,9 - Nutrient Solution with 1% Clay* *Flasks 4,7,10 - Clay-Modified Nutrient Solution* *D. Name: Light Intensity Lower* *Description: Light intensity (in lux) at the bottom of the shaker* *E. Name: Light Intensity Middle* *Description: Light intensity (in lux) on top of the first row of flasks on the shaker* *F. Name: Light Intensity Upper* *Description: Light intensity (in lux) on top of the second row of flasks on the shaker* *G. Name: pH* *Description: Approximate pH as measured from pH paper strips* *H. Name: Volume* *Description: Volume of the solution in the flask, if applicable* *I. Name: Volume Added* *Description: Volume of BG-11 solution added to the original flask to bring the total volume back up to 100mL, if applicable* *J. Name: Dilution Factor* *Description: Dilution factor to be applied to the average cell density. Calculated by multiplying the previous day's conversion factor by (volume added +volume)/ (volume)* *K. Name: Average Cell Count per Box* *Description: Average value of all cells under the "detailed hemocytometer count" for a given row.* *L. Name: Average Cell Density (cells/mm^3)* *Description: Average cell density of the solution. Calculated by dividing the average cell count by the size of the box (0.004mm^3), and multiplying by the dilution factor.*

	M. Name: Detailed Hemocytometer Count *Description: For each row, each box under the detailed hemocytometer count label represents the number of cells counted in one 0.004 mm^3 square.*
Taxonomic species or groups	*The microbe used in this study, Microcystis aeruginosa, is a prokaryotic principally photosynthetic organisms*
Quality control	*We conducted experiments using three replicate samples to improve data quality.*
Additional information	*No*

Table 2. Data dictionary: description of the variables (i.e., columns) in EACH dataset.

Dataset filename: *Hemocytometer_Data_Final.xlsx*

Dataset description: *This file contains the data collected from hemocytometer measurements, as well as data regarding the environmental conditions of growth for the cyanobacteria (including light intensity and pH). The excel spreadsheet is broken down into different columns based on category. The first three columns include the day #, date, and flask number. In total, 9 flasks were used to grow the bacteria. These flasks were separated into three different growth conditions. The next four columns are a record on environmental conditions including light intensity and pH. The next set of columns are a record of dates when a growth solution was added to the flasks. The additional growth solution resulted in a dilution factor that was applied when calculating the overall cell density in each flask. The next column is the average cell count per box. This column represents the average number of cells counted in each 0.004 mm^3 square of the hemocytometer for a given sample. The raw data used to compute this average is located in the last columns, under the "Detailed Hemocytometer count" tab. Finally, the values in the "Average Cell Density" column were calculated by dividing the average cell count per square by the volume of the square (0.004 mm^3) and multiplying by the dilution factor.*

Column name	Description	Units	Code explanation	Data format	Missing data code
Day #	*Number of days since beginning of experiment (when the stock cyanobacteria was added to corresponding nutrient solutions)*	*day*	*NA*	*Number in excel sheet*	*NA*
Date	*Date of data collection*	*mm/dd/yy*	*NA*	*Number in excel sheet*	*NA*
Flask #	*Flask number (1-9) that corresponds to the type of solution* *Flasks 1 - Stock cyanobacteria solution (not*	*Flask #*	*NA*	*Number in excel sheet*	*NA*

	included in analysis) Flasks 2,5,8 - Nutrient Solution Flasks 3,6,9 - Nutrient Solution with 1% Clay Flasks 4,7,10 - Clay-Modified Nutrient Solution				
Light Intensity Lower	*Light intensity (in lux) at the bottom of the shaker*	*number*	*NA*	*Number in excel sheet*	*NA*
pH	*Approximate pH as measured from pH paper strips*	*number*	*NA*	*Number in excel sheet*	*NA*
Volume	*Volume of the solution in the flask, if applicable*	*number*	*NA*	*Number in excel sheet*	*NA*
Volume Added	*Volume of BG-11 solution added to the original flask to bring the total volume back up to 100mL, if applicable*	*number*	*NA*	*Number in excel sheet*	*NA*
Dilution Factor	*Dilution factor to be applied to the average cell density. Calculated by multiplying the previous day's conversion factor by (volume added +volume)/ (volume)*	*number*	*NA*	*Number in excel sheet*	*NA*
Average Cell Count per Box	*Average value of all cells under the "detailed hemocytometer count" for a given row.*	*number*	*NA*	*Number in excel sheet*	*NA*
Average Cell Density	*Average cell density of the solution. Calculated by dividing the average cell count by the size of the box (0.004mm^3),*	*cells/mm^3*	*NA*	*Number in excel sheet*	*NA*

	and multiplying by the dilution factor.				
Detailed Hemocytometer Count	*For each row, each box under the detailed hemocytometer count label represents the number of cells counted in one 0.004 mm^3 square.*	*number*	*NA*	*Number in excel sheet*	*NA*