Phosphorus Sequestration for Control of Cyanobacterial Growth in Drinking Water Reservoirs

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Engineered drinking water reservoirs are designed to facilitate particle settling for reduction of turbidity prior to conveyance to a drinking water treatment plant (DWTP). Fine cohesive sediment particles can carry significant loads of adsorbed phosphorus (P) that can desorb into the water column and intensify the growth of cyanobacteria (CB), causing problematic and potentially toxic CB blooms. In light of these reservoir sediment dynamics, strategies for mitigating rapid CB proliferation through sequestration of P were investigated.

A series of bench scale experiments were conducted to examine the impact of managing dissolved and sediment-associated P for controlling CB growth. The first phase of testing involved batch experiments with fine reservoir sediments to determine their P release characteristics and the amount of dissolved P potentially available for CB uptake. The utility of sequestering this soluble reactive P (SRP) with a common metal salt coagulant, ferric chloride ($FeCl_3$), was also investigated. These adsorption / desorption experiments showed that a dose as low as 25 mg/L was effective in precluding SRP desorption from the sediment over a relatively wide range of solution SRP concentrations. These results were critical to provide an understanding of the SRP-sediment dynamics after treatment with $FeCl_3$.

The second phase of testing involved confirmation of the importance of sediment-associated SRP on the growth of a commonly found CB, *Microcystis aeruginosa* and evaluation of the utility of $FeCl_3$ coagulation for limiting *M. aeruginosa* growth through sequestration of SRP. Standard methods for culturing / growing *M. aeruginosa* were adapted for a series of experiments, at near bloom cell counts, in the presence and absence of sediment to demonstrate the potential utility of SRP sequestration with a common coagulant used during drinking water treatment to inhibit CB growth. While the lab-scale experiments could not, and were not expected to exactly mimic reservoir behavior, they were conducted to demonstrate proof-of-concept. They were successful in doing so because *M. aeruginosa* growth was inhibited with adequate $FeCl_3$ application. Significantly lower $FeCl_3$ doses were effective when the high levels of sediment (analogous to previously deposited sediment) were removed from the system.

The results of this study have several implications for controlling the proliferation of CB through nutrient sequestration. SRP can be sequestered very effectively at doses of $FeCl_3$ typical of DWTP operations. Growth of *M. aeruginosa* can even be inhibited by sequestering P when CB cell counts are elevated to levels consistent with those that may be expected at bloom conditions; as would be expected, relatively higher $FeCl_3$ doses are then required. Further experimental work to determine the optimal dose of $FeCl_3$ at different sediment loads and lower *M. aeruginosa* starting cell populations should be considered.

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AOM	algal organic matter – extracellular organic matter excreted by algae and CB
BG11	cyanobacteria growth medium
$BG11_{50}$	modified growth medium
CB	cyanobacteria
DI	deionized water, purified filtered water
DO	dissolved oxygen
EPC_0	equilibrium phosphorus concentration
$FeCl_3$	ferric chloride hexahydrate ($FeCl_3$-$6H_2O$)
HVR	Hidden Valley Reservoir
M. aeruginosa	*Microcystis aeruginosa*
MAC	maximum acceptable concentration
MC	microcystin, a cyanotoxins
MLD	million litres per day
N	nitrogen
n	number of test replicates
P	phosphorus
Reservoir water	Hidden Valley Reservoir (HVR) water
RMOW	Regional Municipality of Waterloo
SRP	soluble reactive P
TP	total phosphorus
WQ	water quality
WRT	water retention time
WTP	water treatment plant

Chapter 1 Introduction

1.1 Background

There is a growing concern within Canada and throughout the world about the aesthetics and potential health hazards associated with cyanobacteria (CB), in both natural water bodies and engineered reservoirs used for recreation and as a source of drinking water. CB blooms can have significant economic (Howgate, 2004), ecological, and public health impacts (Paerl et al., 2001; St. Amand, 2013). In recreational waters, CB growth is often-ignored as a safety hazard until blooms form and impact recreational activities (Health Canada, 2012). CB blooms can cause fish kills and damage aquaculture operations by depleting oxygen in the water column. In addition, they can clog water treatment intakes and treatment processes such as granular media filters and membranes (Paerl & Ustach, 1982).

From a drinking water treatability perspective, some CB produce compounds that cause taste and odour (T&O) concerns (Jüttner & Watson, 2007) while other compounds are irritants (Mankiewicz et al., 2003; Metcalf & Codd, 2004; Lopez et al., 2008). More importantly, they can produce cyanotoxins, which are toxic to humans and other animals, including livestock and pets (Hitzfeld et al., 2000; O'Neil et al., 2012; Metcalf & Codd, 2004; Breu et al., 2008; Stewart et al., 2008). Conventional drinking water treatment processes are not always effective in removing these compounds to acceptable levels (Jüttner & Watson, 2007; Hoeger et al., 2005) and as such, some CB toxins pose a serious health concern in treated drinking water.

The global occurrence of toxic CB blooms is increasing (Haider et al., 2003; Loza et al., 2014; Thornton et al., 1996), and climate change is expected to further exacerbate this problem (Moore et al., 2008; Wagner & Adrian, 2009). The potential risks that toxic CB blooms pose to drinking water security were underscored in the summer of 2014 (Figure 1), when the City of Toledo issued a "Do Not Drink" advisory after unsafe levels of the CB toxin microcystin was found in treated water of drinking water plants (US EPA, 2014; Hazen & Sawyer, 2015). The advisory spanned three counties in Ohio and one in Michigan, leaving more than 400,000 people without drinking water (Michigan News, 2014). The advisory was necessary; however, because contact with most CB can cause skin irritation and rashes. Most importantly, microcystin can cause nausea, vomiting, liver damage, cancer, and even death if ingested. Microcystin has been known to kill small animals and livestock that drink contaminated water (Stewart et al., 2008; Health

Canada, 2012; Srivastava et al., 2015). Thus, an understanding of CB blooms and strategies for mitigating or preventing them is critical to global drinking water security.

Figure 1: The [cyanobacteria]-clogged waters of Lake Erie as seen from Maumee Bay State Park near Toledo, Ohio. Credit: Joshua Lott for The New York Times (2014)

The control and management of CB in surface water and treatment of cyanotoxins in drinking water supplies is critical to drinking water security. Treatment strategies for removing CB and eliminating their toxins from drinking water are costly and not always adequate because they may compromise the health of lake or reservoir ecosystems (Lopez et al., 2008; Health Canada, 2012; Antoniou et al., 2014), lyse the CB cells that would have otherwise contained the cyanotoxins (Thornton et al., 1996; Lopez et al., 2008; Ho et al., 2012), or exceed treatment process capacity (Kingston et al., 2012; US EPA, 2012a; Antoniou et al., 2014). Thus, the mitigation of CB blooms is better accomplished through preventive rather than remedial measures. Notably, the occurrence, timing, intensity, and duration of CB blooms varies from year to year because of nutrient availability, air and water temperatures, availability of sunlight, water flow conditions, and wind velocity (WHO, 1999; Paerl et al., 2001; Hoogenboezem et al., 2004; Newcombe et al., 2015). The natural variability of these factors precludes accurate prediction of CB blooms.

Nutrient availability is a critical factor contributing to the occurrence of CB blooms (Metcalf & Codd, 2004; Magrann et al., 2012). Thus, taking steps to reduce or prevent nutrient availability in drinking water supplies can reduce CB bloom occurrence and/or intensity, thereby reducing associated threats to drinking water security.

Phosphorus (P) has been identified as a critical nutrient for cellular growth and metabolism (Raven et al., 1986; CCME, 2004; Ashley et al., 2011). It is also the limiting nutrient in most fresh water systems (Schindler, 1977; Loomer & Cooke, 2011; Barlow-Busch et al., 2006). When total phosphorus concentrations are near or exceed the threshold for eutrophication (generalized as ~30 µg P/L), it can cause CB blooms (CCME, 2004; Health Canada, 2012; Dodds, 2003). In waters where P is below this threshold, nutrients are in demand; and generally a healthy balance between algae and CB populations exists, where no particular taxa dominates to the extent that they form a bloom.

Sediment governs the source, transport, fate, and mobility of P in aquatic systems (Stone & English, 1993; Stone & Droppo, 1994; Engstrom, 2005; Davies-Colley & Smith, 2001). The bioavailability of P is influenced by sediment characteristics (e.g., particle size, geochemistry) and environmental conditions (e.g., redox, temperature, competitor ions, pH) (Lijklema, 1980; Forstner, 1987; Boers, 1991; Davies-Colley & Smith, 2001; Klotz, 2014). In particular, fine sediment fractions (less than ~63 µm in size) can influence dissolved P concentrations in the water column via adsorption/desorption reactions (Stone & English, 1993; Stone & Droppo, 1994; Auer et al., 1998; Busman et al., 1997). In locations where municipally and/or agriculturally impacted river water is stored in reservoirs, the presence of fine sediment and the associated release of P into the water column has been shown to promote the growth of algae and CB (DePinto et al., 1981; Reynolds & Davies, 2001; Bowes et al., 2003; Munawar & Fitzpatrick, 2012).

Chemically induced precipitation of P can be an effective, but expensive management practice for preventing algal and CB blooms in lakes. This practice has been most commonly utilized to manage water quality for aesthetic and recreational purposes (Scherfig et al., 1973; Auer et al., 1998; Sherwood & Qualls, 2001; Zamyadi et al., 2013). Notably, drinking water suppliers are often precluded from being able to practice pre-emptive management of algae and CB blooms because of jurisdictional limitations associated with their source waters. Accordingly, they typically rely on engineered mitigation measures such as aeration, mechanical mixing, reservoir drawdown, surface skimming, ultrasound, algaecides, hypolimnetic oxygenation,

coagulation/flocculation/clarification, and artificial wetlands (Beutel, 1994; Shantz et al., 2004; Engstrom, 2005; Antoniou et al., 2014). Engineered reservoirs are often constructed for raw water storage and equalization of water quality influent to drinking water treatment plants. Surprisingly, chemical precipitation of P is not typically practiced in engineered drinking water reservoirs for the pre-emptive management of algae and CB; no studies detailing this approach are currently available in the academic literature.

1.2 Research Objectives

The overall goal of this research was to investigate P sequestration by chemical precipitation for control of CB growth in engineered drinking water reservoirs. Specifically, the utility of source water coagulation with a commonly utilized metal salt coagulant, ferric chloride ($FeCl_3$), for managing dissolved and sediment-associated P and CB growth was investigated at laboratory-scale.

Specific research objectives were to:

1) evaluate the potential for P release from fine reservoir sediments to the water column in various locations (cells) within a drinking water reservoir;
2) investigate the utility of $FeCl_3$ for sequestering soluble reactive P (SRP) in the water column to limit its availability for CB proliferation;
3) demonstrate the importance of sediment-associated P inputs for enabling the growth of CB such as *Microcystis aeruginosa* in municipally and agriculturally impacted reservoir water; and,
4) demonstrate the utility of $FeCl_3$ coagulation for limiting the growth of CB such as *M. aeruginosa* in municipally and agriculturally impacted reservoir water.

1.3 Research Approach - Overview

To address the aforementioned objectives, two types of proof-of-concept experiments were conducted at bench-scale. **Phase 1** consisted of P adsorption/desorption (i.e., sorption) experiments to address Objectives #1 and #2. **Phase 2** consisted of experiments using *M. aeruginosa* cultures to address Objectives #3 and #4.

1.3.1 Phase 1 – Sorption Studies

Sorption studies consisted of a set of controlled experiments in which the dissolved P content of reservoir water was measured and the P release capacity of the sediment (i.e., P adsorption/desorption to/from the water column) was evaluated at various aqueous P concentrations (Objective #1). The sediment-water system was then amended with various concentrations of $FeCl_3$ to evaluate its utility in preventing P desorption from Reservoir sediment (Objective #2).

1.3.2 Phase 2 – *M. aeruginosa* Growth Studies

Experiments were first conducted to demonstrate that *M. aeruginosa* could be grown in reservoir water. Then *M. aeruginosa* cell growth was evaluated in reservoir water with and without the presence of sediment to demonstrate the importance of sediment-associated P inputs for enabling its growth (Objective #3). Finally, *M. aeruginosa* cell growth was evaluated in the presence and absence of $FeCl_3$ to demonstrate the potential utility of $FeCl_3$ addition to engineered drinking water supply reservoirs to limit CB growth (Objective #4).

1.4 Thesis Organization

Chapter 2 consists of a literature review on causes of CB blooms and the data gaps on bioavailable nutrients, justification for studies and chemicals used. Chapter 3 details the experimental procedures, materials and methods used, as well as the approach for analysis of the data. Chapter 4 contains experimental results and preliminary discussion. Chapter 5 contains the conclusions drawn from these studies. Chapter 6 contains the implications this work has on the applicability of using a coagulant in a biological system to sequester P and recommendations for future investigations to further understand the role of coagulants in engineered reservoir systems, as well as new arguments and potential data gaps.

Chapter 2 Literature Review

This chapter of the thesis is a review of literature that focuses on: 1) the forms, transport and availability and implications of P in the environment; 2) increasing CB bloom occurrence, consequences and challenges to water treatment; 3) ecosystem management to mitigate the key factors that contribute to blooms; and 4) coagulants as sequestering agents for P. Research gaps in the literature are highlighted and the importance of the proposed research for the drinking water industry is discussed.

2.1 Phosphorus and Sediment in Natural Systems

2.1.1 Phosphorus

Phosphorus is present in a wide variety of chemical forms in natural waters that include both dissolved and particulate forms (APHA, 2012; Maher & Woo, 1998) and the majority of dissolved P in surface water is in the dissolved bioavailable form orthophosphate (APHA, 2012; Raven et al., 1986; CCME, 2004; US EPA, 2012b). Phosphorus is operationally defined as dissolved and particulate forms (APHA, 2012; US EPA, 2012b). The following section focuses on phosphate in natural waters because it is the most ecologically relevant and bioavailable form of P (DePinto et al., 1981; Busman et al., 1997; Barak, 1999; Reynolds & Davies, 2001; Wang et al., 2011).

2.1.1.1 Molecular Forms of Phosphate

In natural waters, phosphate can occur in both organic and inorganic forms. Both forms can be dissolved or bound to particulate matter in the water. Organic phosphates are associated with both living and dead/dying cellular material, including detritus, feces and decaying algae as a part of proteins, lipids, metabolic waste, etc. (Raven et al., 1986). The phosphate molecule in its most basic form is orthophosphate (PO_4^{3-}), but depending upon pH, it can also exist as $H_2PO_4^-$ or HPO_4^{2-} (Raven et al., 1986). Orthophosphate is also referred to as dissolved or soluble reactive P (SRP). Complex inorganic forms with several phosphate groups are called polyphosphates, or condensed phosphates (APHA, 2012; US EPA, 2012b) while other inorganic phosphate forms are associated with oxyhydroxide surfaces of particle surfaces or within the geochemical matrix of sediment (e.g. apatite and clay) (Carlson & Simpson, 1996; Engstrom, 2005; Ashley et al., 2011).

2.1.1.2 Phosphorus Forms Defined by Technique

Phosphate is operationally defined on the basis of size as P in water passing through a 0.45 µm filter. This fraction is called soluble or dissolved, while that retained on the filter are called insoluble, suspended, or particulate (APHA, 2012). The soluble fraction includes orthophosphate forms as well as P bound to colloidal materials <0.45 µm. Phosphate can also be defined by chemical reactivity. Phosphates that can be analyzed colorimetrically without being hydrolyzed or digested in advance of analysis are called "reactive phosphorus" (APHA, 2012; US EPA, 2012b). Total P (TP) includes all dissolved and particulate P forms in water (APHA, 2012).

Particulate P forms can be sequentially extracted using fractionation techniques (Stone & English, 1993; Engstrom, 2005). These techniques sequentially extract the following P forms: (1) loosely sorbed (NH_4Cl extraction); (2) reductant soluble reactive P ($NaHCO_3$*$Na_2S_2O_4$ extraction); (3) reactive P sorbed to metal oxides (NaOH extraction); (4) P bound to carbonates, apatite-P, and P released by the dissolution of oxides (HCl extraction); and (5) non-reactive organic P extractable in hot (85°C) NaOH (Stone & English, 1993). Fractions 1, 2 and 3 comprise the non-apatite inorganic P fraction (NAIP), which is predominantly bound to metal oxy-hydroxide surfaces and is the most bioavailable particulate P form because it can readily desorb from sediment into the water column. The HCL extractable form is the apatite inorganic P form (AIP) and is a calcium-phosphate mineral apatite that is related to natural weathering of geological materials (Stone & Droppo, 1994). This particulate P fraction is relatively stable and is not readily dissolved in water. Organic P extractable in hot (85°C) NaOH is referred to as organic P (OP) (Engstrom, 2005).

The physical and geochemical properties of sediment (including the various P forms that comprise it) can influence the source, transport, fate and mobility of P in aquatic and terrestrial systems (Stone & English, 1993; Stone & Droppo, 1994; Bowes et al., 2003; Weiner & Matthews, 2003). It is commonly recognized that the surface area of sediment increases with decreasing grain size (Droppo & Ongley, 1992; Wood & Armitage, 1997; MWH, 2012; Yang et al., 2013). Adsorptive properties of sediment are governed by factors such as specific surface area, porosity and the geochemical composition of sediment (MWH, 2012) and the concentration of sediment-associated nutrients and contaminants is inversely proportional to the grain size (Forstner, 1987; Stone & English, 1993; Klotz, 2014). Other environmental factors such as the ambient concentration of P in the water column, competitor ions, temperature, redox conditions and pH influence the form and mobility of P in the environment (Stone & English, 1993). Stone

& English (1993) reported that NAIP and OP fractions in sediment from two Lake Erie tributaries were inversely proportional to grain size while the AIP fraction decreased with decreasing grain size. They also demonstrated that most of the sediment transported to the Great Lakes was <63 µm, and suggested that fractions <8 µm represent a large source of potentially bioavailable P; this mechanism of P release is important as a P source for biotic uptake. Sediment <63 µm deposited in aquatic systems is termed surficial fine grain laminae (SFGL) and is an important source of P in river systems because of its large surface area (Stone & Droppo, 1994). Davies-Colley & Smith (2001) reported that suspended sediments in aquatic systems transport adsorbed pollutants such as toxic chemicals and trace elements, some of which might be beneficial for CB growth. Auer et al. (1998) investigated the bioavailability of sediment-bound P and found that soluble P was 4 to 7 times more bioavailable than particulate P. Reynolds & Davies (2001) suggested that P available for CB growth is not confined to an "analytically determined" soluble fraction, but rather to TP. In summary, it has been extensively demonstrated that both sediment composition (particle size and geochemical composition) and environmental conditions (pH, redox, temperature, competitor ions) are critical factors influencing the transport, fate and form of P in aquatic systems.

2.1.2 Importance of Sediment Transport and P Mobilization

Point-source and non-point source discharges of pollutants from predominantly industrial and urban sources can severely impact receiving water quality (Mainstone & Parr, 2002; Weiner & Matthews, 2003; Hood, 2012; GRCA, 2014). These pollutants are often sediment-associated; thus, sediment is the primary vector for these contaminants and nutrients such as P (Binkley & Brown, 1993; Biggs, 2000; Shantz et al., 2004; Emelko et al., 2015). Both natural and anthropogenic disturbances on the landscape can degrade water quality through point source releases (Table 1) such as wastewater outfalls (Feuillade & Dorioz, 1992; Mainstone & Parr, 2002; Withers & Jarvie, 2008), or indirect non-point source releases (Table 2) such as urban or agricultural run-off (Reynolds & Davies, 2001; Seeboonruang, 2012). Over the past century, land disturbances such as these have altered hydrological processes and increased erosion rates thereby accelerating the rate of eutrophication of aquatic systems (O'Neil et al., 2012). This has been exacerbated with the advent of mechanized farming practices and over-fertilization (WHO, 1999; Dunn et al., 2010; Metcalf & Codd, 2004) and to a large extent, urban development. Figure 2 demonstrates the relative contribution of sediment from different land uses. Notably, increased levels of solids and nutrients—especially P, which is limiting in freshwater systems (Schindler, 1977; Mainstone & Parr, 2002; Barlow-Busch et al., 2006; O'Neil et al., 2012)—lead to

conditions that favour primary productivity and CB proliferation (Schindler, 1977; Jarvie et al., 2002; Dodds, 2003; Dunne et al., 2005; Bowes et al., 2007; Silins et al., 2009a; Silins et al., 2009b; Ashley et al., 2011; Silins et al., 2014; Emelko et al., 2015). Moreover, these impacts are exacerbated because of changing climate (Schindler, 2001; Moore et al., 2008; Silins et al., 2009b; Wagner & Adrian, 2009; Silins et al., 2014; Emelko et al., 2015).

Table 1: Point source disturbances on aquatic systems

Point Source Pressures on Aquatic Systems	**Influenced parameters**
Municipal wastewater	Increased nutrients Increased dissolved organic carbon Other pollutants Warmer water temperature
Industrial wastewater	Increased nutrients Increased dissolved organic carbon Other pollutants Warmer water temperature
Urban storm water management (SWM) facilities	Increased sediment Increased nutrients Increased dissolved organic carbon Other pollutants

Table 2: Non-point source disturbances on aquatic systems

Indirect Pressures on Aquatic Systems	**Influenced parameters**
Agricultural practices	Increased sediment Increased nutrients Increased dissolved organic carbon Other pollutants
Urban development	Increased sediment Increased nutrients Other pollutants
Forest fires	Increased sediment Increased nutrients Increased dissolved organic carbon Other pollutants

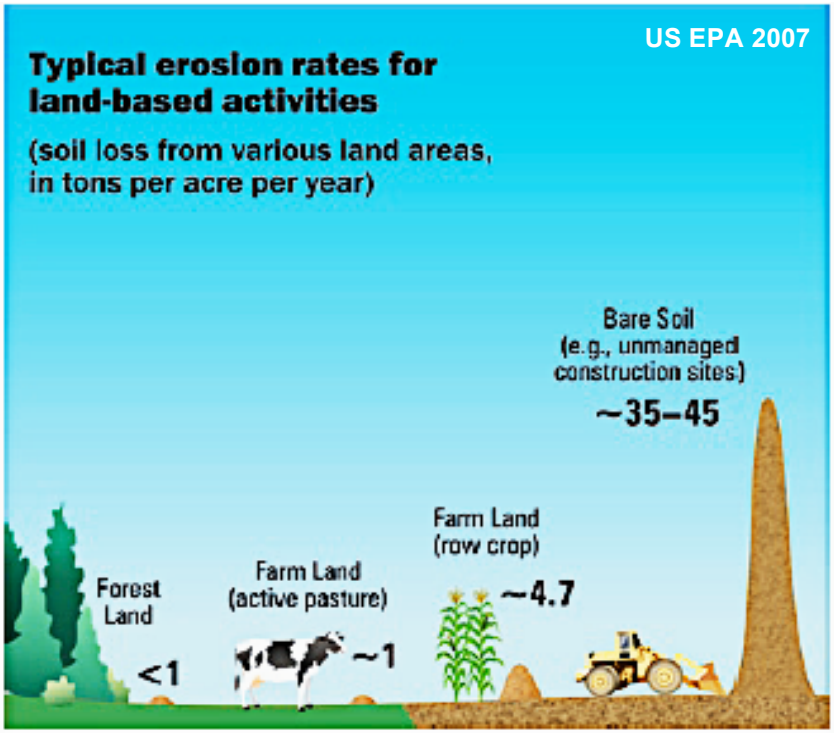


Figure 2: Erosion rates for land-based activities

Munawar & Fitzpatrick (2012) discussed non-point sources of pollution and their contributing role in eutrophication and CB blooms; notably, they suggested a target of 30 µg/L of TP (Schindler, 1974) for reducing eutrophication in the Bay of Quinte and other freshwater systems. This target has been retained as part of the International Joint Commission bi-national regulatory agreement between Canada and the United States (Lopez et al., 2008; IJC, 2013). Despite reductions of 130 kg/d (over six years) of external point source P loading into the Bay of Quinte, this waterbody is still eutrophic because non-point sources were not addressed (Munawar & Fitzpatrick, 2012). Moreover, internal loading of P from lake bottom sediment remains significant (Christie, 1968; WHO, 1999; Havens, 2008; Munawar & Fitzpatrick, 2012).

Sediment transport and fate are governed by several processes that are physical, biological, and chemical. Figure 3 provides an overview of these processes.

- Physical processes include hydrodynamics, bioturbation, and sediment porosity. Algae and CB can lower the water velocity in their immediate area and reduce advective transport of nutrients away from sediment. Molecular diffusion along a concentration gradient and advection, or bulk movement, toward the sediment surface is affected by water flow (Dodds, 2003). Adsorption/desorption of P can occur from sediment, aquatic plants and organic

matter. Bottom sediments become resuspended from wind / wave action, and from bioturbation from carp and ducks (Shantz et al., 2004).

- Biological processes include microbial action such as filtering and deposition of sediment by algae and CB; direct ingestion, incorporation, and eventual decay, sedimentation, and mineralization of P (Auer et al., 1998). Dodds (2003) explains that P uptake rates in algae and CB are influenced by population density, the need for nutrients, and their ability to come into contact and incorporate P. In other words, biomass, activity, and advective transport all vary within and between differing algae/CB populations, and this might explain why the observed P-uptake rates are often lower than expected.

- Chemical processes include pH and dissolved oxygen (DO). CB can change both the pH and the DO of the water during photosynthesis. Photosynthesis generates DO and removes CO_2 from the water making the water more acidic (Lijklema, 1980). In acidic sediments, the availability of excess protons (H^+) results in more positively charged sediment particles that can potentially adsorb more negatively charged phosphate. Adsorption of P to metals such as Fe^{3+} (as cationic FeOH species) is higher in acidic environments (Boers, 1991). Anaerobic conditions in lake sediment and pore water reduce iron-phosphorus complexes, causing disassociation of metal-phosphorus complexes that result in the release P from the sediment (Lijklema, 1980).

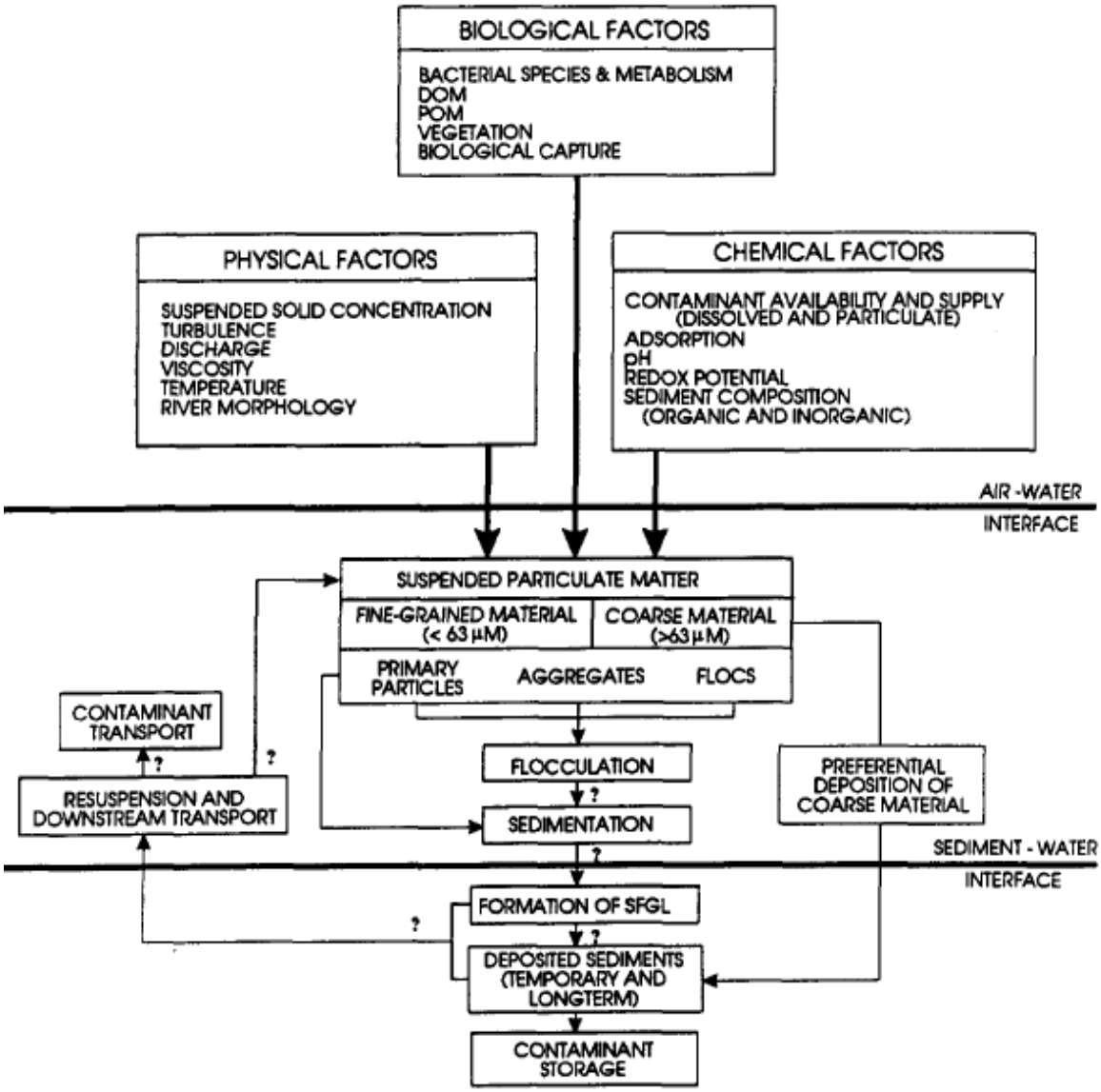


Figure 3: Conceptual overview of factors affecting sediment fate and transport dynamics. Source: Stone & Droppo (1994)

2.1.3 P Sorption Kinetics

In aquatic systems, sediment can influence P mobility through adsorption and desorption processes (Froelich, 1988; Dunne et al., 2005). Adsorption isotherms describe the relationship between equilibrium concentrations of adsorbed and dissolved P at a given temperature (Figure 4). Adsorption isotherms are unique to different combinations of sediment type and water quality. Sediment has the potential to adsorb soluble reactive P until an equilibrium has been established with the ambient P concentration or until all sorption sites are occupied (i.e.,

maximum sorption capacity, S_{max}). When the initial solution P (S_0) is low, P is desorbed from the sediment into solution (Figure 4). Adsorption only occurs when sediment P levels are higher than the ambient P levels. The equilibrium P concentration (EPC_0) is a measure of the potential of sediments to adsorb or release SRP depending on the ambient aqueous SRP concentration of aquatic systems (House & Denison, 1998; 2000). The EPC_0 is determined experimentally by plotting the measured mass of P sorbed per mass of sediment versus the initial concentration of SRP prior to contact with the sediment (Taylor & Kunishi, 1971; Froelich, 1988). Batch equilibrium experiments are used to determine the EPC_0 of various sediment types and to estimate the desorption potential of SRP from suspended sediment to the water column (House et al., 1995). Determining the EPC_0 of sediment is fundamental to understanding the kinetic control of P by sediment. By determining the EPC_0, the behaviour of the sediment in response to future P-loads can be estimated (i.e. is the sediment a source of P or a sink?). If dissolved P concentration entering the system exceeds the EPC_0, sediment will adsorb P.

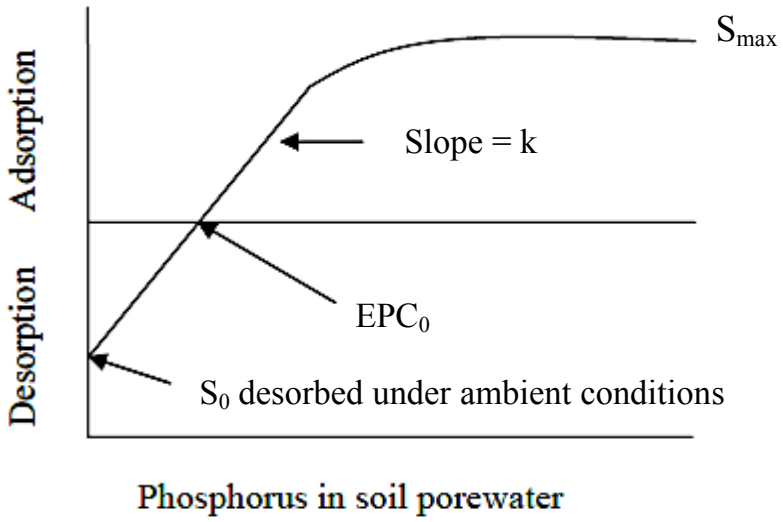


Figure 4: Sediment adsorption / desorption isotherm showing the equilibrium concentration (EPC_0). Source: Dunne et al. (2005)

2.2 Cyanobacteria and Algal Blooms: Occurrence and Challenges to Water Treatment

Due to their presence in the water column and blue-green colouring, CB are often confused with green algae (Brock & Madigan, 1991; Health Canada, 2012). In contrast to photosynthetic CB, "green algae" are simply an assemblage of small aquatic photosynthetic plants (Wehr & Sheath, 2003). In addition to developing several adaptations to nutrient limitation, CB have evolved

several other strategies to survive and reproduce in many aquatic environments. They have been found to persist in a wide variety of habitats and water depths (WHO, 1999; St. Amand, 2013), and survive in different niches with alternate light levels (Haider et al., 2003; Mankiewicz et al., 2003) and food sources (Paerl et al., 2001; Metcalf & Codd, 2004) through buoyancy regulation (Hallegraeff, 1992; Haider et al., 2003). CB are equipped with an akinete, a thick walled structure containing food reserves, which provides them with resistance to desiccation and enables their persistence in ephemeral streams or in lower water levels (WHO, 1999; Metcalf & Codd, 2004).

CB are always present in aquatic environments; however, in a healthy aquatic ecosystem, several factors regulate their abundance. Paerl et al. (2001) note that there is a fine balance between adequate irradiance and nutrient supply that determines the rate of production of phytoplankton biomass (primary production). Additional factors such as temperature, algal physiology, and competition can contribute to primary production (St. Amand, 2013).

In Canada and other temperate countries, CB blooms are observed in late summer and early fall when the dominant phytoplankton species shifts to CB (WHO, 1999; Mankiewicz et al., 2003). As the season progresses, nutrient availability becomes limited for many types of algae for two main reasons: nutrients are consumed near the water surface by phytoplankton, and nutrients associated with detritus and debris begin to settle out of the water column and become inaccessible at the surface; and CB are capable of adjusting their position in the water column to access nutrients at depth (Breu et al., 2008). *Microcystis* is a species more adapted to shorter days than other CB and this might be a key reason it is commonly found in late summer/early fall in NorthAmerica (CDWQ, 2002).

Not all CB blooms are easily recognized and some even go undetected. Some blooms are planktonic and are clearly observable at the water surface (Paerl & Ustach, 1982; Watson et al., 1997; Wehr & Sheath, 2003). They can quickly become dense surface scums when the wind blows the bloom together and concentrates the CB (WHO, 1999; CDWQ, 2002; Metcalf & Codd, 2014). However, some blooms exist as large, diffuse masses of planktonic CB below the water's surface (Watson et al., 2008; Hazen & Sawyer, 2015), or as benthic mats (Newcombe, 2009; Metcalf & Codd, 2014). In streams, highly visible, dense algae are often observed on rock substrates; however, the majority of these algae growths are green filamentous algae, *Cladophora*, or diatoms (Mason, 1988; St. Amand, 2013) and are not considered "CB blooms".

In addition to visual observation, CB blooms have been characterized in many different ways. For example, the following parameters have been used:

- chlorophyll-A (APHA, 2012; Queensland Government, 2008; Ohio EPA, 2013); and/or phycocyanin (a pigment specific to cyanobacteria only) (OECD, 2002; Wehr & Sheath, 2003; Kasinak et al., 2015)
- mass per volume (OECD, 2002; Moreno et al., 2011; Davis et al., 2015; Gerloff et al., 2015)
- biovolume (dimension/L) (Munawar et al., 1991; WHO, 1999; Queensland Government, 2008; Wood et al., 2008; Zamyadi et al., 2012b; Ohio EPA, 2013)
- growth rate / population doubling time (Paerl et al., 2001; Newcombe, 2009)
- cell counts (Hitzfeld et al., 2000; Svrcek & Smith, 2004; Wood et al., 2008)

An individual *Microcystis* cell can contain 0.2 picograms MC toxin and a *Planktothrix* cell can contain double that amount of MC per cell (WHO, 1999). Guidelines, or alert levels, are often developed based on cell counts as a more reliable indicator of bloom formation (or potential bloom formation) rather than visual observation because cell counts can provide an approximate worst case scenario of the toxicity of the water (assuming every species counted was toxic) (Hoogenboezem et al., 2004; Ahn et al., 2007). Estimates of MC content when cell counts are known for *Microcystis* or *Planktothrix* are presented in Table 3.

Table 3: Estimates of Microcystin in a sample based on cell count (WHO, 1999)

Cells/mL	**MC (µg/L) low (Microcystis) assuming 0.2 pg MC/cell**	**MC (µg/L) high (Planktothrix) assuming 0.4 pg MC/cell**
20,000	4	8
100,000	20	40
1,000,000	200	400
10,000,000	2,000	4,000
100,000,000	20,000	40,000

Accordingly, the WHO (1999) has provided guidance for assessing health risk associated with CB blooms by enumerating the number of CB cells per ml water. These include Low Probability of adverse health effect (<20,000 cells/mL); a Moderate Risk Level (20,000-100,000 cells/mL); and High and Very High Risk Levels (10^7 to 10^8 cells/mL) that are often associated with scums. The WHO does not define a specific CB concentration to indicate a CB bloom.

Alert levels for recreational waters vary depending on waterbody and location, and are generally determined based on the previous history of the water. For example, if species identification and toxin analysis has been conducted in the past in a particular waterbody, a general understanding of the potential toxicity might be known if the taxa (species assemblage) are consistent throughout the years. The following alert levels vary widely based on country, and use a far more cautionary approach, due to previously established toxicity at certain cell counts:

- S. Korea - Caution Alert Level <500 cells/mL; a Warning Alert Level is <5000 cells/mL; an Outbreak Alert Level is 10^6 cells/mL (Srivastava et al., 2015)
- International guidance - Low Alert Level is 500-2000 cells/mL; Medium Alert Level is 2000-6500 cells/mL; High Alert Level is >6500 cells/mL for an individual species or combined CB (Newcombe, 2009)
- Australia - Alert Level 1 <2000 cells/mL; Alert Level 2 is 10^6 cells/mL (Bartram et al., 1999; as cited in Ahn et al., 2007)

Canada has yet to develop alert levels based on local or national studies. Canadian researchers suggest that a CB density above of 2.5×10^6 cells/mL can constitute a bloom (Svrcek & Smith, 2004), but this does not specifically apply to any particular Canadian body of water. In Germany, a CB density greater than 10^6 cells/mL defines a CB bloom (Hitzfeld et al., 2000). Mason (1988) describes a bloom as "an aggregation of phytoplankton sufficiently dense to be readily visible". The International Joint Commission's Health Professionals Advisory Board (IJC, 2013) for the Great Lakes defines blooms as "high concentrations of algal cells that give the water a 'pea soup' appearance"

There is no formally recognised definition for a bloom (Hallegraeff, 1993; Smayda, 1997; Algae-L Forum, 2013). Many researchers avoid defining a bloom solely based on cell counts due to the variety of criteria involved. The definition of a bloom is subjective because it can vary spatially, temporally, and between species. The application of the term "bloom" also needs to take into account whether a) the species are macro- or micro- species; b) the condition is persistent or transient; c) there is a disruption in the chemistry, biology and ecology of the water; and d) whether the biomass in a surface scum will be averaged over the euphotic zone. It has been suggested that the proliferation of CB beyond a known baseline, might be more appropriate (Algae-L Forum, 2013).

Growth rates can be used as an indicator of an impending bloom (Paerl et al., 2001). For example, Newcombe (2009) reported growth rates of 0.1 to 0.4 per day, indicating that the CB populations can double in just under a week to less than 2 days, respectively. Although *Microcystis* is considered one of the slower growing phytoplankton species, they can rapidly multiply and pose significant health risks, challenge drinking water treatment, and cause problems at optimal growth conditions (Breu et al., 2008).

Blooms alter water quality in many ways that may affect drinking water treatment plant processes. Specifically, increases in turbidity from nutrients and CB biomass can reduce the efficiency of coagulation and flocculation processes and increase the chemical oxidant demand required for disinfection, which also can increase the potential for DBP formation (Aktas et al., 2012; MWH, 2012; Antoniou et al., 2014). CB can clog filters and shorten run times (MWH, 2012), which can lead to turbidity breakthrough, potentially resulting in the release of algae and CB, pathogens and other particulate matter (Pirbazari et al., 1993; APHA, 2012; WHO, 1999; Ho et al., 2006; Drikas et al., 2009; Zamyadi et al., 2012a). Cyanotoxins such as microcystin (MC) can also be released from CB cells during treatment (Jüttner & Watson, 2007; Westrick, 2008). CB blooms are often the primary cause of taste and odour compounds that are not easily removed by conventional water treatment processes (Jung et al., 2004; Skjevrak et al., 2004; Jüttner & Watson, 2007; Ho et al., 2012a).

Not all species within a CB genus are capable of producing cyanotoxins, and depending on environmental factors, toxins may or may not be actively produced (Hitzfeld et al., 2000; Watson et al., 2008). However, high cell counts likely increase the potential for toxin production. While planktonic CB are most often implicated in toxic bloom formation (WHO, 1999; Graham et al., 2008), information regarding toxic benthic blooms is lacking. For most species of CB, cyanotoxins are retained within healthy cells (WHO, 1999). When cells die and/or rupture (lyse), the toxin is released into the water (WHO, 1999; Mankiewicz et al., 2003). Cyanotoxins include potent hepatotoxins (affecting the liver) and neurotoxins (impacting the central nervous system), while others simply irritate the skin (dermatotoxins) (Hitzfeld et al., 2000; Haider et al., 2003).

Microcystin is the most significant CB toxin of concern globally is because of its toxicity and frequency of occurrence (CDWQ, 2002; Hoeger et al., 2002). *Microcystis* is one of the most prevalent genus of CB associated with blooms (Hitzfeld et al., 2000; CDWQ, 2002; Svrcek & Smith, 2004) and several species within this genus are capable of producing microcystin (WHO,

1999; Hitzfeld et al., 2000; Hoeger et al., 2005). A summary of the genera responsible for cyanotoxin production and associated health effects is presented in Table 4.

Table 4: Toxin Classification and Effects

Biochemical Group	Toxin Class	Toxin	Affected Organ	Effects	Genera
Cyclic peptides	Hepatotoxin	Microcystin	Liver	Nausea, vomiting, bleeding in the liver, cancer, death	*Anabaena, Anabaenopsis, Aphanizomenon, Aphanocapsa, Chrococcus, Hapalosiphon, Lymnothrix, Microcystis, Nostoc, Oscillatoria / Planktothrix, Pseudoanabaena, Romeria, Synechococcus, Synechocystis, Woronchinia*
		Nodularin			*Nodularia spumgena*
Alkaloids	Neurotoxin	Anatoxin (several variants with differing toxicity)	Nerve axons and synapses	Staggering, muscle twitching, respiratory distress	*Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya Oscillatoria / Planktothrix, Pseudoanabaena, Raphidiopsis*
		Saxitoxin: paralytic shellfish poison (PSP)			*Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya, Oscillatoria / Planktothrix*
		Neosaxitoxin			*Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis*
	Cytotoxin	Cylindro-spermopsin	Liver and kidneys	Bleeding	*Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya, Umezakia, Raphidiopsis*
	Dermatotoxin	Aplysia-toxin	Skin, GI tract	Swimmer's ear, dermal lesions, possible tumour promoter	*Lyngbya, Oscillatoria / Planktothrix, Schizothrix*
		Lyngbia-toxin			*Lyngbia, Oscillatoria / Planktothrix, Phormidium*
Lypopoly-saccharides (LPS)		Endotoxin	Skin, respiratory tract[1]	Irritants and allergies	All cyanobacteria
Non-protein amino acid	Neurotoxin	BMAA β -N-methyl-amino-L-alanine	Motor neurons	Gulf War Syndrome, ALS, Alzheimer's	Most cyanobacteria

Adapted from: Ellis & Korth, 1993; Anderson et al., 2002; Haider et al., 2003; Bláha et al., 2004; Hoogenboezem et al., 2004; Anderson et al., 2007; Newcombe, 2009; Oregon Public Health Department, 2012; US EPA, 2012a; Ohio EPA, 2013; Hazen & Sawyer, 2015.

[1] Respiratory tract: if potentially aerosolized by cool mist humidifiers (Anderson et al, 2002; 2007)

Although there are at least 80 variants of microcystin (Graham et al., 2008; Lopez et al., 2008; Szlag et al., 2015), the most studied form is microcystin-LR (MC-LR) because it is the most frequently occurring and the most toxic (Hitzfeld et al., 2000; Metcalf & Codd, 2004; Graham et al., 2008; Newcombe, 2009; Kingston et al., 2012). The following outlines the regulatory guidelines for cyanotoxins that pose significant human health risk:

- The Technical Support Document for Ontario Drinking Water Standards, Objectives and Guidelines (2006) in support of the Ontario Drinking-Water Quality Standards Regulation O. Reg 169/03 sets the Maximum Allowable Concentration (MAC) for MC-LR limit in drinking water at 1.5 µg/L.
- The Canadian Drinking Water Quality (CDWQ) Guidelines allows for a MAC of MC-LR in drinking water of 1.5 µg/L. Health Canada has recently circulated a new microcystin guideline (public comment period ended April 2016) and this version states that the guideline is for total microcystins (to include all of the microcystin congeners that might be present). This new guideline also suggests that authorities should inform the public during a cyanobacterial bloom, or when microcystins are detected in finished water, that an alternate suitable source of drinking water (such as bottled water) should be used to reconstitute infant formula.
- The United States have no established guidelines for cyanotoxins at this point in time; however, both CB and their cyanotoxins have been added to the US EPA Contaminant Candidate List (CCL4 - draft) with microcystins, cylindrospermopsin and anatoxin-a given the highest priority.
    - As of June 2015, the EPA has issued a 10-Day Drinking Water Health Advisory for the cyanotoxins MC-LR and cylindrospermopsin.
    - For infants and children younger than six (6) years old, they recommend levels at or below 0.3 µg/L for MC-LR and 0.7 µg/L for cylindrospermopsin.
    - For adults and children over age 6, they recommend levels at or below 1.6 µg/L for MC-LR and 3.0 µg/L for cylindrospermopsin.
- The World Health Organization (WHO, 1999) established a provisional guideline for MC-LR in drinking water of 1.0 µg/L.
    - Countries including Germany, Poland, Czech Republic, France, Spain, and New Zealand have adopted the WHO Guidelines for microcystin.

- Australia has adopted a guideline for MC-LR in drinking water of 1.3 µg/L. Furthermore, it is currently considering a 3.0 µg/L limit for anatoxin-a and saxitoxin, and a range of 1-15 µg/L for cylindrospermopsin.
- Brazil has adopted the WHO Guideline for microcystin and guidelines, similar to Australia, of 3.0 µg/L for saxitoxin, and 15 µg/L for cylindrospermopsin.
- Many countries are also considering setting guidelines for anatoxin-a, saxitoxin, and cylindrospermopsin, once more knowledge is gained.

Several policies and practices have been established to minimize the occurrence of CB blooms. Some directly target reductions in nutrient discharges, while others include restoration and enhancement of wetlands and riparian buffer zones (Binkley & Brown, 1993; MOEE, 1993) through restorative planting with native species (Mainstone & Parr, 2002; US EPA, 2007) that are capable of stabilizing the soils and metabolizing excess nutrients (Biggs, 2000; Mainstone & Parr, 2002). Decreasing of bioavailable P in freshwaters remains a primary strategy for preventing CB blooms because P is a limiting nutrient in most freshwaters (Mason, 1988; Mainstone & Parr, 2002; CCME, 2004; Ashley et al., 2011), including the Grand River (Barlow-Busch et al., 2006). P reduction in watersheds typically is approached on three (3) levels (Mason, 1988; Wehr & Sheath, 2003):

- Increase P output from a system either by selective discharge of hypolimnetic water, or overall flushing with oligotrophic source (this is not frequently recommended since it only transfers the problem downstream);
- Decrease external P loading via diversion and advanced WWTP upgrades, retention basins, wetlands, and other watershed management techniques; and
- Supress internal P loading via P-binding (coagulation), sealing lake bottoms, dredging (removal), biotic harvesting (of nutrient rich macrophytes, algae or fish), or aeration (aerobic sediment absorbs more P).

2.2.1 Phosphorus as a Key Nutrient for CB Growth

Nitrogen (N) and P are considered essential nutrients for algae and CB (Redfield, 1958; Christie, 1968; Scherfig et al., 1973). N and P levels in pristine fresh waters are generally low enough to limit CB growth. However, elevated levels can result in excessive CB growth (Schindler, 1977; Mason, 1988; Moss, 1989). Although N levels play an important role in the potential for toxicity

(Davis et al., 2015), CB growth and bloom development is more closely associated with P loads in freshwater systems (Paerl et al., 2001; Reynolds & Davies, 2001).

2.2.1.1 Phosphorus Availability

P is a key component in membranes, tissues, and proteins such as amino acids and nucleic acids (DNA, RNA) used in cell division; it is also used to make ADP and ATP, the compounds responsible for energy transfer and storage (Breu et al., 2008). Ultimately phosphorus drives reactions within living cells and is vital for metabolism (Raven et al., 1986; CCME, 2004; Ashley et al., 2011). Although P is abundant in the environment, bioavailable forms of P are not (Barak, 1999; Reynolds & Davies, 2001), and therefore P is considered the first nutrient to limit biological activity in freshwater systems (APHA, 2012; Mason, 1988; CCME, 2004; Ashley et al., 2011). The term "bioavailable P" is subjective and there is a lack of agreement on which sources of P are the most biologically available to CB (Reynolds & Davies, 2001).

Bioavailable P, or algal available P, has been frequently described using an operational definition, which is generally synonymous with ortho-P, or soluble reactive P (SRP) (Reynolds & Davies, 2001; Mainstone & Parr, 2002). Often, the terms ortho-P ($H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}) and dissolved P are interchanged, and referred to as directly available for biological uptake in plankton, algae, CB, and bacteria (DePinto et al., 1981; Auer et al., 1998; Busman et al., 1997). However, caution must be exercised when using the simpler term "dissolved" because there are many forms of dissolved P (i.e., low molecular weight OP) that would fall into this category. Thus, it has also been suggested that operational definitions are insufficient (Pierzynski, 2000), because P availability depends on numerous factors, such as the ability of CB to cleave P from otherwise inaccessible forms, or environmental factors that increase P solubility.

When dissolved-P sources are limited, CB have the capability to cleave phosphate from organic molecules via the phosphatase enzyme (Feuillade & Dorioz, 1992; Reynolds & Davies, 2001; Wehr & Sheath, 2003; Tan et al., 2012). The resulting elevated levels of phosphatase enzyme in the water can then be analytically measured and used as an indicator P-availability (Wehr & Sheath, 2003).

Notably, Reynolds & Davies (2001) discussed the conditional availability of sediment-regenerated-P. They noted that ortho-P (SRP) and/or OP liberated from sediment can either remain in solution as readily available P, or rapidly adsorb to metal oxides (NH_2Cl-P) and

hydroxides (NaOH-rP), and clay; they also can become "conditionally available" when conditions, such as pH or redox potential change (Forstner, 1987; Reynolds & Davies, 2001). They also suggest that CB utilize TP found in biomass (biomass-P, or BP), although it is unclear whether they were referring to internally stored or external sources of biomass.

Similarly, Auer et al. (1998) described total dissolved P (TDP) as the sum of SRP and dissolved organic-P (DOP), and found that TDP was most bioavailable to phytoplankton in their study, contributing 95-97% of the P used by algae. Both forms of dissolved P were accessible due to enzymatic cleavage of organic component of the P. They suggest that molecular differences such as high molecular weight or colloidal OP might be responsible for reduced availability in the other forms of P. Only a fraction of the particulate P was considered available, and particulate P became more soluble during dry-weather events, specifically the fraction associated with iron or aluminum. Auer et al. (1998) described four types of particulate P: phytoplankton-P (PhyP), zooplankton-P (ZP), "available" non-living particulate-P (ANLPP), and "unavailable" non-living particulate-P (UNLPP). This investigation underscored the importance of site-specific P-fraction bioavailability when setting nutrient targets. Breu et al. (2008) similarly commented that ortho-P, some phosphorylated sugars and phosphonates and P that can be transformed by physical (adsorption/desorption) or chemical (dissolution) or biological (enzymes) are all directly available for uptake.

Due to the multitude of P sources and their varying bioavailability, TP often is used for investigating and estimating bioavailable P. TP has been used as a surrogate for predicting biomass because most P is particle-associated, rather than in dissolved form (WHO, 1999; Reynolds & Davies, 2001; Smith et al., 2011; Bladon et al., 2014). Notably, there is little or no correlation between TP and algal biomass (DePinto et al., 1981; Watson et al., 1997; Auer et al., 1998; Hood, 2012; Chen & Taylor, 2011) in many cases; thus, SRP or a combination of water and sediment SRP are sometimes used as predictors of biomass. Several forms of P and their potential bioavailability are summarized in Table 5.

Table 5: Major forms P in solution and bioavailability

Specific Nutrient	Availability in the Environment	Reference
dissolved PO_4^{3-}	ortho-P most bioavailable	Auer et al (1998) Mainstone & Parr (2002) Reynolds & Davies (2001) Wehr & Sheath (2003)
organic-bound PO_4^{3-}	enzymatic cleavage makes P available	Auer et al (1998) Busman et al. (1997) Reynolds & Davies (2001) Wehr & Sheath (2003)
particulate-bound PO_4^{3-}	conditionally available depending on physical/chemical processes that release P	DePinto et al. (1981) House (2003) Mainstone & Parr (2002)

2.2.1.2 Nitrogen Availability

Nitrogen (N) is also an essential component of proteins, amino acids, nucleic acids and urea (Raven et al., 1986; APHA, 2012). It also plays a role in the formation of the cyanotoxin, microcystin (Stucken et al., 2014; Davis et al., 2015). Aquatic N occurs in various organic and inorganic forms. Organic N forms include amino acids, sugars, humics (Raven et al., 1986; Brock & Madigan, 1991; Breu et al., 2008; Worsfold et al., 2008). Dissolved inorganic N (DIN) forms include ammonium (NH_4^+), ammonia (NH_3), nitrite (NO_2^-), nitrate (NO_3^-), and dissolved gas, N_2 (Larsdotter, 2006; Breu et al., 2008; Worsfold et al., 2008). Although N_2 is easily lost to the atmosphere, it readily diffuses into the surface layers of water where specialized bacteria, including some species of CB, are capable of "fixing" the N_2 and converting it into a bioavailable form of N, such as NO_2^-, or NH_3, which is subsequently converted into NO_3^- (Raven et al., 1986; WHO, 1999; Paerl et al., 2001; Wehr & Sheath, 2003; Svrcek & Smith, 2004). A bacterial preference for certain types of N has been suggested; however, it is more commonly believed that large fractions DIN are potentially bioavailable due to their low molecular weight (APHA, 2012; Larsdotter, 2006; Stucken et al., 2014). Figure 5 provides an overview of operationally defined N and P forms in freshwater systems.

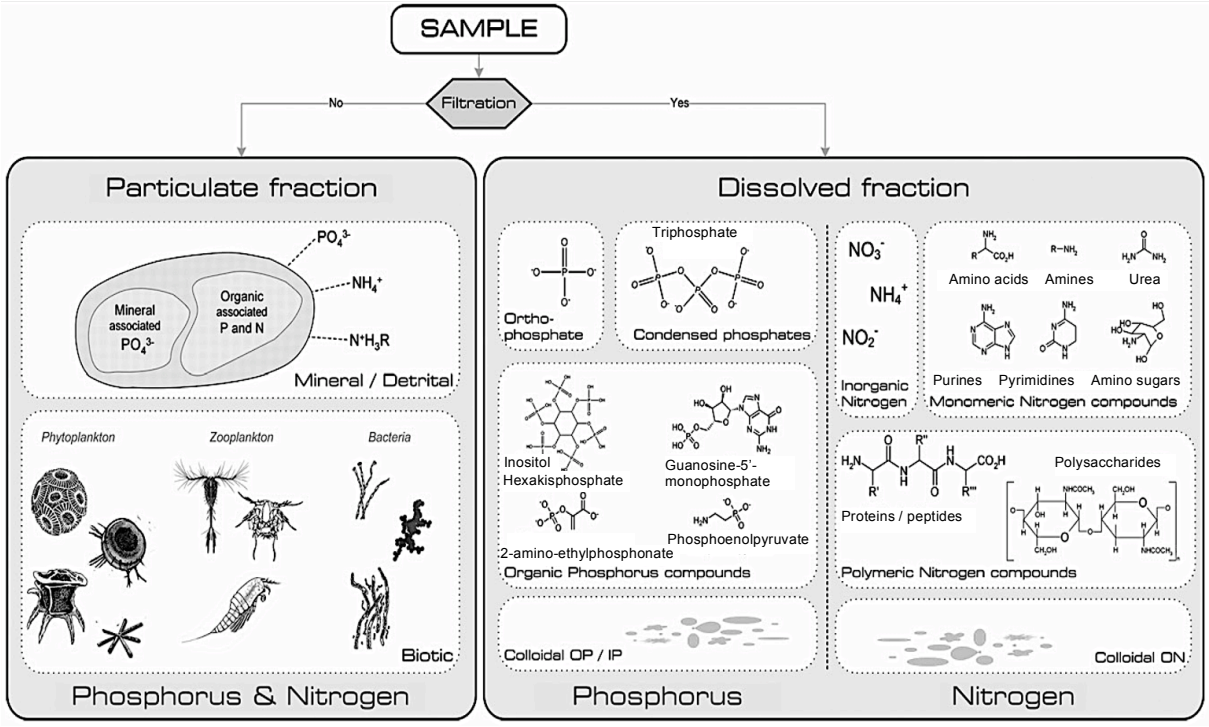


Figure 5: Schematic of typical P and N components of a water sample. Source: Worsfold et al. (2008)

N-fixers, which are typically filamentous species in *Nostocales* group such as *Anabaena* and *Aphanizomenon*, are able to dominate under low N conditions (WHO, 1999; Higgins et al., 2003; Davis et al., 2015). Once the N-fixers become established, their presence enables other CB to join the assemblage and begin to dominate in the water column (APHA, 2012; WHO, 1999; Paerl et al., 2001). The idea of N-scavenging has also been reported in the literature, and non N-fixers such as *Planktothrix* and *Microcystis* were found to dominate low N environments (Davis et al., 2015).

2.2.1.3 Nitrogen to Phosphorous (N:P) Ratio

The Redfield Ratio of 16N:1P represents the general requirement for all aquatic organisms (Redfield, 1934; Moss, 1989; Kim et al., 2007). A lower ratio (<16N:1P) from N depleted or P-enriched waters, is considered favourable for CB growth by allowing N-fixers, or N-scavenging CB to dominate (APHA, 2012; WHO, 1999; Paerl et al., 2001; Davis et al., 2015). If the N:P ratio exceeds 16:1, P is considered limiting to algal/CB growth (Mason, 1988). The ratio of nitrogen and P has been widely reported as critical for development of CB blooms in the aquatic environment. While some researchers argue that the ratio is critical to bloom formation, others

contend that overall nutrient concentration is more important (Svrcek & Smith, 2004; Wagner & Adrian, 2009). Bowes et al. (2007) suggested that at elevated nutrient levels, the ratio becomes unimportant, because no nutrient is limited. Guildford (2006) reported reservoir CB concentrations that were strongly correlated with TP levels and negatively correlated to TN:TP ratios (i.e. at decreasing ratios of <16N:1P, CB began to thrive). Havens (2008) found that TP was a better predictor of CB dominance than TN:TP ratios. Overall, all of these investigations underscore the critical importance of sediment-associated nutrient availability for CB proliferation; thus, they also reflect the opportunity to minimize CB bloom occurrence and risk to drinking water treatment by locally controlling nutrient availability in engineered reservoirs.

2.2.2 Adaptation of CB to Low P Concentrations

Given the low, or variable availability of P in some natural systems (Moss, 1989), CB have adapted and can take up and readily store P for future metabolic needs (Reynolds & Davies, 2001; Newcombe, 2009). Specifically, several CB have the ability to store excess P as polyphosphates (Thompson et al., 1994; Vahtera et al., 2007; Breu et al., 2008; Havens, 2008). This internal storage allows growth at low external P concentrations and allows CB to outcompete other phytoplankton when P levels are low (Reynolds & Davies, 2001; Mankiewicz et al., 2003). The ability to take up excessive P and store for future consumption is termed "luxury uptake" (Paerl et al., 2001; Tan et al., 2012). Notably, CB can store enough P for 2 to 4 cell divisions, equivalent to a 4 to 32 fold increase in biomass (WHO, 1999; Newcombe, 2009); as many as 20 cell divisions (Weiner & Matthews, 2003). Larsdotter (2006) summarized this capacity as follows:

- When external P levels were at 0.1 mg P/L, microalgae stored P at internal P stores of ~1 mg P/g dry weight;
- When external P levels were at 5.0 mg P/L, microalgae stored P at internal P stores of ~100 mg P/g dry weight; and
- On average, algae cells contain ~13 mg P /g dry weight.

In addition to P storage, CB have developed other physical and metabolic strategies to overcome P-limitation. CB cells adapt by changing from bright blue-green to yellow (chlorosis) and by reducing photosynthesis rates (Larsdotter, 2006; Tan et al., 2012). They also reduce the size of their cells in addition to reducing growth rates (Breu et al., 2008). Physiological changes include making P-uptake systems more efficient by producing more alkaline phosphatase enzyme,

making more uptake proteins and increasing their P uptake rate. Internal cellular processes can limit, or become independent of extracellular P levels by using alternate metabolic pathways (Breu et al., 2008; Wang et al., 2011). These diverse and numerous adaptions of CB to low levels of nutrient availability underscore potential challenges associated with bloom control; however, they also signify opportunities for management because it is also commonly recognized that CB proliferations is more likely when optimal levels of nutrients are present (Higgins et al., 2003; Wehr & Sheath, 2003; Li et al., 2009). Thus, reductions in nutrient levels should not be expected to eliminate all CB, but rather to help prevent rapid proliferation and blooms.

Water quality guidelines for preventing nuisance algae proliferation typically target TP levels of 30 µg/L or less (CCME, 2004); however, it has also been suggested that TP concentrations as low as 10 µg/L may be required to prevent blooms (WHO, 1999). Notably, extremely low SRP concentration can support CB growth in some cases. For example, Reynolds & Davies (2001) found that CB maintained a fast growth rate when external SRP was at 3 µg/L. Thus, while reducing nutrient availability can significantly reduce the probability of CB bloom occurrence, it is not guaranteed.

2.2.3 Other Water Quality Parameters Impacting CB Growth

Beyond nutrients, a number of additional water quality parameters are important to CB growth. These include: micronutrients, carbon, dissolved oxygen, salinity and alkalinity/pH. Although required in small amounts, micronutrients such as Iron (Fe) and other trace elements are critical for cellular growth and metabolism. Fe is required for photosynthesis, respiration, N-fixation, and NO_3 utilization (Paerl & Ustach, 1982; Hyenstrand et al., 2000; Paerl et al., 2001; Li et al., 2009) and can sometimes be in short supply (Reynolds & Davies, 2001; Breu et al., 2008). Other trace elements are typically not limiting (Cu, Mo, Mn, Zn, Co) and aid in N-uptake and N-fixation, as well as photosynthesis and carbon fixation (Paerl et al., 2001; CDWQ, 2002).

Carbon, in the form of dissolved inorganic carbon (CO_2, HCO_3^-, CO_3^{2-}) and dissolved organic carbon (DOC) is typically unlimited in aquatic environments due to the ongoing diffusion of CO_2 into water and prevalence of naturally occurring organic matter (Paerl & Ustach, 1982; WHO, 1999; Paerl et al., 2001). Dissolved inorganic carbon (DIC) limitation and high pH may provide competitive advantages to CB.

Dissolved Oxygen (DO) is generated during photosynthesis but can be drastically reduced at night, or during overcast days, when respiration occurs. Respiration requires oxygen to convert chemical energy (captured during photosynthesis) into compounds required for cellular growth (Raven et al., 1986). As CB concentration/density increases, the water in and around the sediment becomes more anoxic, which in turn releases ammonia and ortho-P, stimulating internal nutrient loading and furthering the eutrophication process (Beutel, 1994).

CB require energy-intensive uptake mechanisms with specialized transport enzymes to transport P against the osmotic gradient because P is present at lower levels in the surrounding water as compared to the CB cell (Moss, 1989). At aerobic (higher DO) conditions, uptake of P from water occurs rapidly, but in low oxygen, or anoxic conditions CB use stored P (Larsdotter, 2006). The following model presented by Moss (1989) of Monod kinetics describe this uptake efficiency:

$$\mu = \frac{\mu_{max} S}{K_S + S} \qquad \text{Equation 1}$$

where:

μ is the uptake rate

μ_{max} is when enzymes are saturated to full capacity

S is the concentration of enzyme-substrate (in this case the nutrient P); and

K_S is half-saturation constant (P concentration at which half of the enzymes are fully saturated)

The tolerance for conductivity and salinity varies among CB species (Paerl et al., 2001) and alkalinity and pH are known to impact their growth. Specifically, CB prefer slightly basic water (CDWQ, 2002; Moore et al., 2008) but can tolerate a broad pH range (Wehr & Sheath, 2003). A decrease in pH can promote P release from sediment thus contributing to the growth of CB (Dodds, 2003).

2.3 Coagulants as Sequestering Agents for P

2.3.1 Coagulation

Many problematic sediment-associated contaminants such as heavy metals and P are present in the water column of aquatic systems. While not of health significance prior to treatment,

contaminants such as P, can result in conditions (i.e., CB blooms) that challenge drinking water treatment. Due to their very small size, the colloids and nano-particles that carry these contaminants require chemical coagulation prior to removal by clarification, which is typically achieved using gravitational settling processes (Duan & Gregory, 2003; MWH, 2012) and occasionally in upflow configurations such as dissolved air flotation (MWH, 2012; Newcombe et al., 2015). Coagulants are typically charged molecules (multivalent metal-salts, polymers/polyelectrolytes) that hydrolyze in water to form cationic species that are attracted to these negatively charged particles (MWH, 2012; Wyatt et al., 2012).

2.3.2 Ferric Coagulants

Ferric chloride ($FeCl_3$) is a metal coagulant commonly used to destabilize negatively charged particles, which predominate in natural waters because of the presence of NOM (MWH, 2012). It is also used as a flocculent because it is capable of binding to itself as well as forming precipitates with other contaminants (WHO, 1999; Duan & Gregory, 2003; Fritz, 2006; MWH, 2012). $FeCl_3$ can reduce the concentration of soluble P in both drinking and wastewaters (Bowes et al., 2007).

$FeCl_3$ is a robust coagulant because it effectively removes NOM and colour, works in high and low turbidity and is effective over a wide pH range and at cooler temperatures (Duan & Gregory, 2003; Engstrom, 2005; Gonzalez-Torres et al., 2014). In contrast, aluminum sulfate is sparingly soluble in pH neutral or alkaline conditions (WHO, 1999). Additionally, compared to alum-based flocs, $FeCl_3$ flocs are denser and stronger, often resulting in a faster settling rate (Duan & Gregory, 2003; Engstrom, 2005; Gonzalez-Torres et al., 2014).

Notably, well-operated $FeCl_3$ coagulation does not lyse CB cells or result in the release of cyanotoxins (Chow et al., 1998). As well, the optimal pH range for particle and organic removal using $FeCl_3$ is from 5 to 8 (Lijklema, 1980; Duan & Gregory, 2003; Caravelli et al., 2010; MWH, 2012; Newcombe et al., 2015); thus, it can be readily utilized for the treatment of most natural waters.

2.3.3 Performance of Ferric Coagulants for P Removal

Typical doses of $FeCl_3$ range from 5 to 150 mg/L depending on raw water quality and turbidity (MWH, 2012). Gonzales-Torrez (2014) used 0.01 to 1.0 mM Fe_3^+ (equivalent to 2.7 to 270.3 mg/L of $FeCl_3$-$6H_2O$, or 1.6 to 162.2 mg/L $FeCl_3$) and Chow et al. (1998) used 15 to 30 mg/L $FeCl_3$ for coagulating particles and CB rather than for P-binding; and van der Veen et al. (1987)

used a $FeCl_3$ dose of 7 mg/L to sequester P in their research. Accordingly, $FeCl_3$ dosing must be evaluated and optimized for achieving specific treatment targets such as shifting the EPC_0 of sediment to preclude P desorption in engineered source water reservoirs.

2.3.4 Use of Ferric Coagulants for CB Growth Mitigation

The concept of using iron-based coagulants to sequester P in water bodies to reduce algae and CB growth is not new. The use of $FeCl_3$ to mitigate algal blooms by fixing P in several lakes in Amsterdam was studied by van der Veen et al. (1987). They reported a large improvement in overall water quality and were able to substantially reduce the P burden in several lakes, some by over 90%, and reported heavy metal and toxic substance removal as additional benefits. Mason (1988) summarized the work of several others and said that while the removal of inorganic P was effective using alum in small ponds and lakes, there was no removal of dissolved OP. However, Mason (1988) also noted that the floc formed on the sediment acted as a blanket to supress P-release from the sediment.

$FeCl_3$ readily dissolves in water and the Fe^{3+} ion forms a relatively strong bond with phosphate (PO_4^3) to form a relatively insoluble, less-bioavailable precipitate (Snoeyink & Jenkins, 1980; Engstrom, 2005; Bowes et al., 2007). The action of phosphate binding with Fe^{3+} minerals in sediment is slightly different compared to water, as the solubility of the P-complexes are governed by pH and redox potential (Moshiri, 1993), hydrodynamics and grain size, as well as the concentration of Fe^{3+} and P (i.e., sorption potential) and other competing ions (Froelich, 1988). Froelich (1988) described two phases of adsorption/desorption as the initial rapid step (taking minutes to hours) and the secondary step where the adsorbed P diffuses into the interior of the sediment particle over a period of days to months.

Related to redox potential, sustained P-retention in the sediment is strongly governed by oxygen levels. Cook et al (1993, in Engstrom, 2005) found that long-term P-retention in the sediment was only achieved when the hypolimnion was aerated. In anoxic conditions, redox dissolution of Fe-P complexes in subsurface sediments results in diffusion of P towards the sediment surface and into solution. Redox potentials below +250 mV (Moshiri, 1993) or below +120 mV (Sherwood & Qualls, 2001) are reportedly the thresholds for releasing P. Anoxic conditions are not expected to predominate in engineered drinking water reservoirs with relatively short hydraulic detention times because water is typically oxygenated during pumping.

Surprisingly, chemical precipitation of P is not typically practiced in engineered drinking water reservoirs for the pre-emptive management of algae and CB; no studies detailing this approach are currently available in the academic literature. To our knowledge, the effectiveness of using $FeCl_3$ for P-sequestration at bench-scale or in engineered drinking water reservoirs to prevent and/or inhibit CB blooms has not been previously reported.

Chapter 3 Materials and Methods

To investigate P sequestration by chemical precipitation, bench-scale tests using source water and sediment were performed with $FeCl_3$ as the coagulant. This study focused on managing dissolved and sediment-associated P as a way to control *M. aeruginosa* growth for potential application in engineered drinking water reservoirs. The first phase of this study elucidated the importance of source water and more importantly sediment as a source (sink) for potentially bioavailable P. The second phase of this work consisted of using *M. aeruginosa* cultures to evaluate the effectiveness the P-sequestration in the presence and absence of sediment.

3.1 Water and Sediment Sources

The Regional Municipality of Waterloo (RMOW) provides drinking water to over ½ million people. The Hidden Valley Reservoir (HVR) is one of the drinking water reservoirs in Kitchener, Ontario (Figure 6). The reservoir is a linear-flow reservoir that is fed from the municipally and agriculturally impacted Grand River and supplies the Mannheim Water Treatment Plant (WTP) with a capacity of 72 million litres per day (MLD). The HVR is a large, in-line, reservoir structure with 148 million litres (ML) storage in four compartments (or cells). After being pumped into the reservoir from a low-lift pump station adjacent to the Grand River, the water flows in an under-over path in the reservoir, prior to being conveyed by the high-lift pump station to the Mannheim WTP. The water retention time (WRT) in the reservoir is approximately 2 days from inflow to outflow. During this time, some sediment settles in the reservoir cells.

3.1.1 Sediment Grain Size Characterization

Fine sediment is the primary vector of P transport in aquatic systems (Forstner, 1987; Wood & Armitage, 1997; Kaiserli et al., 2002; House, 2003). There is a gradient in particle size distribution of deposited sediment in the HVR cells; with the coarser fractions settling in Cell 1, and the finest sediments deposited in Cell 4 (Figure 7). Deposited sediment was periodically collected from three locations (south, middle, north) within each of the four HVR cells using a Ponar sediment sampler between May 2013 and September 2014. All sediment was stored at 4ºC and freeze-dried before testing to eliminate moisture content and minimize errors in weighing.

The sediment grain size distribution was analyzed on June 17, 2014 using a Mastersizer 2000 (ACT Labs, Ancaster ON; Ver. 5.54, Malvern Instruments Ltd., Malvern UK). The results within

each cell were then averaged into a single value, and are presented in Table 12 in the Results section. (Appendix B contains detailed grain size distribution results). The sediment used for sorption and CB testing in this thesis was collected from Cell 3 during September 2014, immediately prior to conducting the batch experiments.

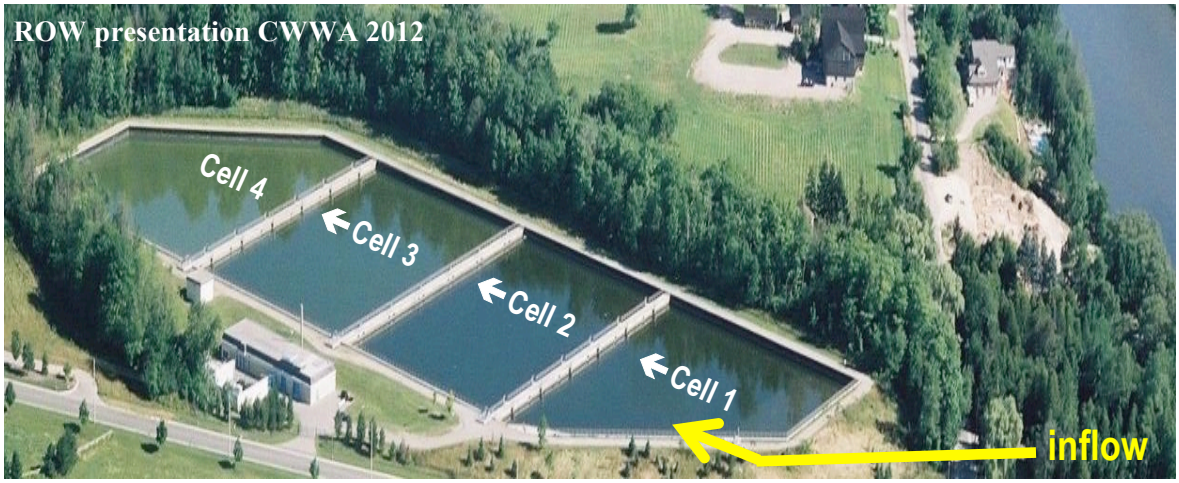


Figure 6: Hidden Valley Reservoir (HVR) location in Kitchener adjacent to the Grand River

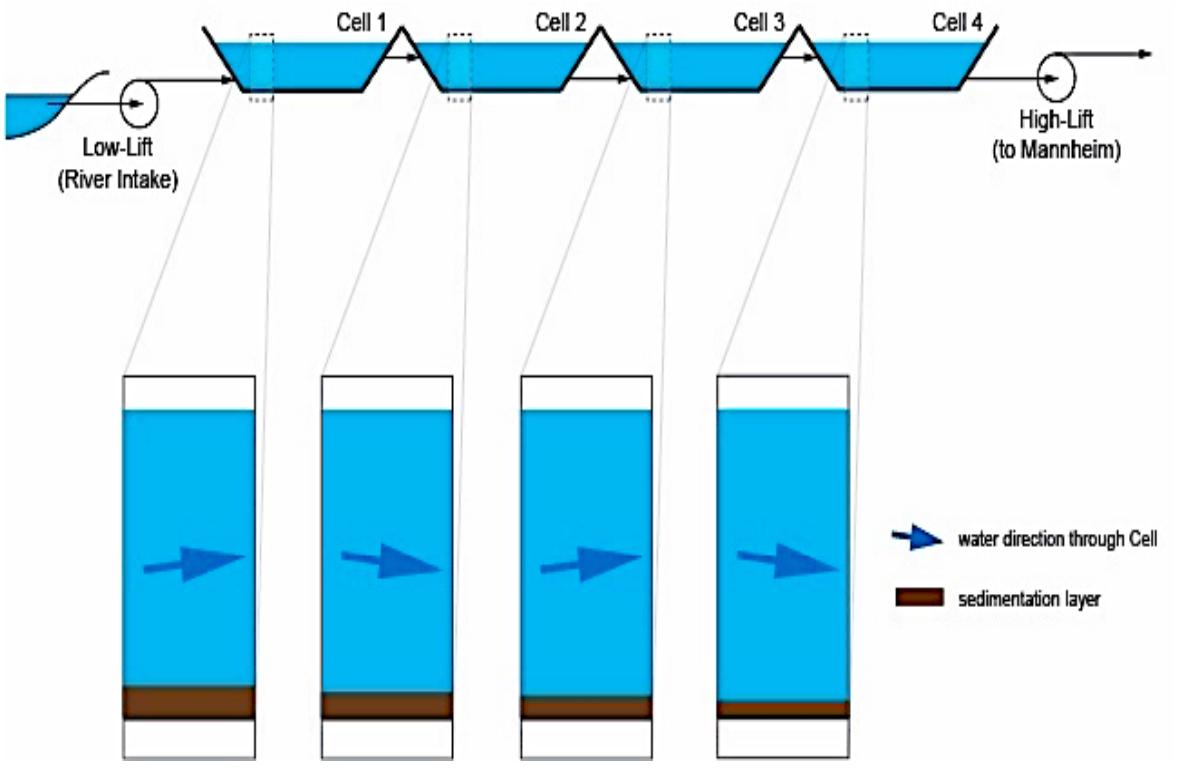


Figure 7: Hidden Valley Reservoir (HVR) configuration and sediment deposition schematic

3.1.2 Water Sources

Water from the high lift tap was used for all experiments. It was collected at various times during the year (Table 6) and filtered within a few hours of collection using 0.45 µm Whatman® 47 mm GF/F AH-934 filter and stored at 4ºC.

Table 6: Hidden Valley Reservoir water collection dates

Collection tap	Collection Date	Test	Test start date
High lift tap	14 Dec 2014	Fe[5][25][50][100] isotherm	16 Dec 2014
	26 Feb 2015	Fe[5][25][50][100] isotherm (no sediment) *M. aeruginosa* screening test (and $BG11_{50}$ medium)	26 Feb 2015 12 Mar 2015
	27 May2015	Fe[10][15][20] isotherm	27 May 2015
		M. aeruginosa growth test Fe[100][200] (no sediment)	20 July 2015*
	19 June 2015	*M. aeruginosa* growth test Fe[200][300][400] Fe[200][300][400] isotherm	6 Aug 2015 1 Oct 2015

* Water collected 27 May 2015 was acclimated on bench for two months (350 mL water: 3.5 g sediment). Sediment was filtered/removed on 6 July 2015 and supernatant used in *M. aeruginosa* Fe[100][200] 'no sed test' on 20 July 2015.

3.2 P Sorption Experiments

A series of adsorption/desorption experiments were conducted using various water types (ultrapure water, HVR water, or CB growth medium) to determine the potential of sediment to release P into the water column in HVR (Cell 3) sediment. Ideally these experiments would have been conducted using sediment from Cell 4 (i.e., finest sediment); however, sediment from Cell 4 had been removed just prior to experimentation as a part of routine maintenance at the HVR. Media preparation consisted of weighing 0.25 g of freeze-dried sediment into 50 mL centrifuge tubes in triplicate. The freeze-dried sediment was coned and quartered to minimize the variability in grain size. Depending on the specific experiment, various concentrations of P (0, 25, 50, 100, and 200 µg P/L; 25 mL per centrifuge tube) were added to ultrapure water, HVR water, or CB growth medium (BG11). pH was measured with a calibrated Orion 250A pH meter (±0.02) (Standard Method 4500-H+; APHA 1988) (Thermo Fischer Scientific, Waltham, MA) following (prior to dosing with $FeCl_3$).

During the experiments that involved coagulant addition, $FeCl_3$ was directly added into each tube at the appropriate dose. A $FeCl_3$ stock solution (5000 µg/L) was prepared to minimize the

required volume added to each tube. A ratio of 0.25 g sediment: 25 mL water was maintained. Initial sorption experiments were conducted using doses of 0, 5, 25, 50, and 100 mg/L $FeCl_3$ to be generally consistent with doses used in WTP's. After $FeCl_3$ addition, the tubes were capped and rapidly shaken for 30 seconds then placed horizontally on a shaker table (Eberbach 6000) at low speed (approx. 50 rpm) and agitated for 18 hours, to reach equilibrium (Figure F1; Appendix F). Each sample was then filtered through a Whatman™ 0.45 µm Puradisk nylon syringe filter and stored at 4ºC in acid-washed, triple-rinsed scintillation vials prior to SRP analysis.

In a subset of some experiments, $FeCl_3$ was added to additional tubes, in triplicate, after the solution had reached equilibrium (i.e., after 18 hrs of agitation). After the $FeCl_3$ addition, these tubes were rapidly shaken for 30 seconds then allowed to settle for 2-3 minutes prior to filtering and storage.

3.3 *Microcystis* Culturing

The cyanobacteria *Microcystis aeruginosa* was chosen as the study species for the following reasons:

1. *Microcystis* is one of the most prevalent genus of CB (CDWQ 2002; Hitzfeld et al. 2000; Svrcek & Smith 2004)
2. *Microcystis* species have previously been identified in the HVR during 2013 (*M. botrys*) and 2014 (*M. botrys, M. flos-aquae, M. novacekii*); and *M. aeruginosa* specifically was identified in other reservoirs in the Grand River Watershed (RMOW unpublished data).
3. *Microcystis* is considered a strong competitor for organic-bound P and is capable of storing enough P for 2 to 4 cell divisions, the equivalent to a 4 to 32 increase in biomass (WHO, 1999). This taxon could provide insight into a potential delay in response to P sequestration.
4. *Microcystis* cells are buoyant and will disperse throughout the water column to access all dissolved P. This characteristic facilitates pipetting near the surface without drawing sediment into the pipette.
5. The species is available at the Canadian Phycological Culture Centre (CPCC) at the University of Waterloo.

M. aeruginosa was grown in BG11 growth medium (Table 7) with pH adjusted to 7.5 prior to autoclaving. The culture was maintained in controlled conditions in an environmental growth chamber (Percival chamber, VWR) at temperatures 21ºC ± 2ºC with a 12hr light/12hr dark cycle

to help keep the cells in the log phase of growth. The light intensity and wavelength was maintained at 4.98–8.30 µEin/m^2/sec using cool-white fluorescent lights. The location of glass culture flasks in the growth cabinet was routinely randomized.

Table 7: Composition of BG11 liquid growth medium

Component	Stock conc. (g/L)	mL/L	Final conc. (g/L)	Element	Final element conc. (mg/L)
$NaNO_3$*	150	10	1.500	N	247.337
K_2HPO_4	30	1	0.030	P PO_4^{3-}	5.335 16.357
$MgSO_47H_2O$	75	1	0.075	Mg	7.394
$CaCl_22H_2O$	36	1	0.036	Ca	9.815
Citric Acid combined with Ferric Ammonium Citrate	6 6	1	0.012	Fe N	1.279 0.321
$Na_2EDTA2H_2O$	1	1	0.001	EDTA	0.785
Na_2CO_3	20	1	0.020	Na CO_3	4.338 11.323

*not added when growing nitrogen fixing species

Culture flasks were acid washed then triple rinsed with DI water followed by a triple rinse with ultrapure water, covered with a foam plug and bio-shield, and then sterilized by autoclaving at 121°C for 15 minutes. After use, all dishware was soaked in a 10% aqueous bleach bath for at least 24 hours to destroy any potential toxins.

To generate or refresh cultures and maintain the maximum standing crop, *M. aeruginosa* was transferred using a 1:3 ratio (40 mL culture transferred into 120 mL BG11 medium) because this genus is better maintained in a more dense population of ~2 to 5 x10^6 cells/mL (pers. comm., Heather Roshon–CPCC, 2014). The average growth rate for *M. aeruginosa* cultures was calculated as the change in cell numbers over a specific time interval (see Equation 4). At optimal conditions (i.e. fresh growth medium at ~5000 µgP/L), *M. aeruginosa*'s optimal growth rate was ~0.1/day (Figure 8); and this growth rate was within ranges reported by Newcombe (2009). Henderson et al. (2008) reported maximum *M. aeruginosa* concentrations in culture flasks (i.e., in the stationary growth phase) at $1.3\times10^7 \pm 3\times10^5$ cells/mL (log equivalent of 7.10 to 7.12 cells/mL) which are in agreement with the culture concentrations shown in Figure 8.

Although the original cultures were not axenic, all culture stocks were prepared in a Class II A2 Biological Safety Cabinet (Microzone; Canada) to minimize contamination. Cell enumeration was also conducted in a sterile environment by gently swirling the culture flask, transferring a 1-2

mL aliquot into a sterile 5 mL centrifuge vial, thoroughly mixing and then transferring a 10 µL subsample to a haemocytometer (Bright-Line, with Neubauer rulings (Hausser Scientific; PA). Counts were conducted using a Zeiss Axioskop 2 *Plus* Compound Microscope with bright field illumination (Zeiss, Oberkochen, Germany). Replicate counts obtained using a haemocytometer are considered correct if within 20-30% of each other (OECD, 2002; Environment Canada, 2007). To minimize error, samples were diluted to obtain approximately 100-250 cells per counting chamber and preserved with 1% Lugol's iodine to immobilize the cells. The volume of the counting chamber was 0.1 µL. The number of cells per millilitre was calculated by using the average of a minimum of three separate counts. The final result was expressed as number of cells per mL.

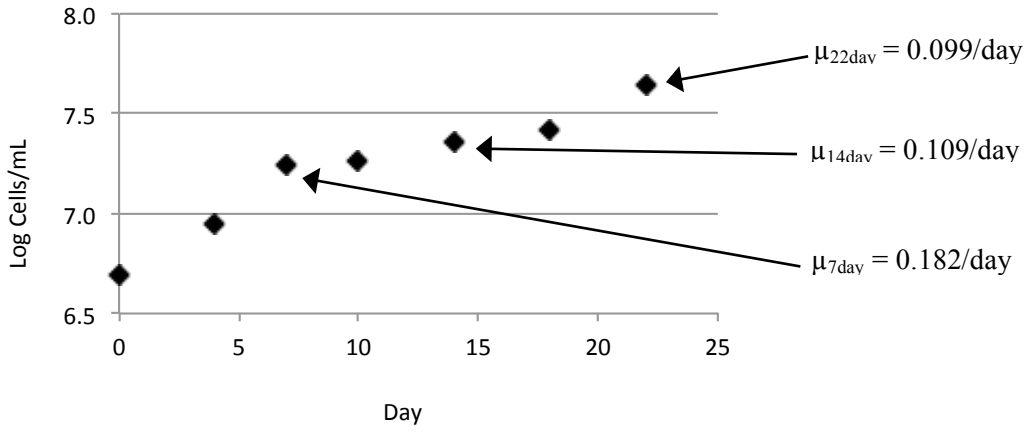


Figure 8: Growth curve of *M. aeruginosa* culture grown in BG11 medium with ~5000 µg/L P

The BG11 growth medium contains 5335 µg P/L (Table 7). To limit *M. aeruginosa* exposure to such high and environmentally atypical concentrations of P before nutrient sequestration studies, a modified BG11 growth medium containing 53 µg P/L was used. The modified growth media is hereafter referred to as $BG11_{50}$.

M. aeruginosa was transferred to the $BG11_{50}$ by a washing technique adapted from OECD (2007) and US EPA (1980). Briefly, 40 mL of a stock *M. aeruginosa* culture was centrifuged at 5000 rpm for 5 minutes and the supernatant decanted. The pellet was re-suspended in approx. 40 mL of $BG11_{50}$, centrifuged and the supernatant decanted. This step was repeated twice, and then the pellet was re-suspended in 40 mL of $BG11_{50}$, transferred to a sterile 250 mL flask and topped up with an additional 120 mL $BG11_{50}$, for a total volume of 160 mL. Typically, new cultures had an adjustment period of roughly 17 days before they recovered buoyancy, due to the effects of the centrifugation process.

3.4 *Microcystis* Growth Trends after P Sequestration

All glassware and plastics were acid washed then triple rinsed with distilled water followed by a triple rinse with ultrapure water and allowed to dry for at least 12 hours at room temperature prior to use. Sediment was added to flasks that were then covered with a foam plug and a piece of bio-shield and autoclaved at 121°C for 15 minutes. Experimental water ($BG11_{50}$ or Reservoir water) was also autoclaved prior to testing. After use, all dishware was soaked in a 10% aqueous bleach bath for at least 24 hours to destroy any potential microcystin toxin. Ultrapure water was used in preparation of all reagents. Stock solutions were stored in the dark at 4°C when not in use.

3.4.1 *Microcystis* Growth Screening Tests

A preliminary experiment was conducted to determine a) the duration (in days) before a population of *M. aeruginosa* began to naturally senesce in source waters compared to growth medium, b) the minimum volume of media required for optimal surface-air exchange in relation to flask size, c) the contribution of sediment as a nutrient source, and d) the feasibility of withdrawing small volumes for daily counting without disturbing the sediment. The experiment was carried out using Reservoir water (as a natural water source) and $BG11_{50}$ growth medium with P levels similar to those measured in Reservoir water as a control (i.e., approx. 50 µg P/L). SRP levels recorded in the HVR from 2011 to 2015 varied from approx. 3 to 84 µg/L (RMOW unpublished data); and SRP levels in reaches of the Grand River near Kitchener and the intake to the HVR varied from 5-75 µg/L over a period from 2003 to 2008 (RMOW unpublished data; Loomer & Cooke, 2011).

Two different liquid-to-volume ratios were investigated to ensure that optimal growing conditions (surface-air exchange) were utilized during the experiments. The experiment was conducted using 50 mL and 250 mL flasks with 25 mL of media and 50 mL of media, respectively. Sediment was added to a second set of flasks (0.25g sediment per 25 mL media) (Figure F4; Appendix F). The flasks containing 25 mL and 50 mL of media were each inoculated with 1 or 2 mL of *M. aeruginosa* stock culture, respectively. The stock was previously sub-cultured in $BG11_{50}$ medium for at least three weeks. Eight experimental test units were used. Test vessels were randomly placed in a growth cabinet (Percival; Iowa). Table 8 provides a summary of the experimental conditions, hereafter referred to as the "*Microcystis* Growth Screening Tests".

Literature on optimal CB inoculum concentration is limited and tends to focus more on green algae and select CB species that have different growth characteristics than *Microcystis*. The US EPA (1980) recommends an initial cell density of 10^4 for *Selenastrum* (a solitary, fast growing green algae), while Environment Canada (2007) suggests 10,000 ± 1000 cells/mL ($9\text{-}11\times10^3$) for the freshwater green algae, *Pseudokirchneriella*. The OECD (2002) recommends an initial cell density of 10^4 for *Anabaena* (a filamentous, colonial CB) and $5\times10^4\text{-}10^5$ for *Synechococcus* (solitary, rod-shaped CB). Because *M. aeruginosa* thrives in dense populations (pers. comm., Heather Roshon–CPCC, 2014), inoculum concentrations recommended for other CB species were expected to be too low for use during the present investigation. Coagulation/flocculation and trace nutrient studies, using *M. aeruginosa* specifically, reported use of higher initial concentrations ranging from 10^5 to 10^6 (Chow et al., 1998; Henderson et al., 2008; Gonzalez-Torres et al., 2014; Dang et al., 2012).

Table 8: Experimental set-up for *Microcystis* growth screening tests comparing media type and surface to volume ratios with and without sediment

Media Type	Flask Size (mL)	Media volume (mL)	Volume: Flask ratio	Sediment (g)	Inoculum (mL)
Hidden Valley Water	50	25	1:2	0.25	1
	250	50	1:5	0.50	2
	50	25	1:2	None	1
	250	50	1:5	None	2
$BG11_{50}$ Growth media	50	25	1:2	0.25	1
	250	50	1:5	0.50	2
	50	25	1:2	None	1
	250	50	1:5	None	2

Table 9 provides a summary of typical CB cell counts entering the HVR from the Low-lift tap in 2013 and 2014 (RMOW, unpublished data); and as evidenced, the total CB density in a sample was ~5×10^5 cell/mL, and the density of a single dominant species was $3\text{-}4\times10^5$ cells/mL under non-bloom conditions. Note that *Microcystis* was never a dominant species.

Table 9: Typical CB cell counts at the Hidden Valley Reservoir from the Low-lift tap

Date	Total CB density (cells/mL)	Dominant species in sample	Density of dominant species (cells/mL)
23 Oct 2013	4.28×10^5	unidentified CB	4.28×10^5
28 July 2014	3.46×10^5	*Planktolyngbia*	3.43×10^5
		unidentified CB	2.14×10^3

Table 10 provides a summary of average initial inoculum doses (in cells/mL) used in all tests in this thesis; notably, they are consistent with the average CB concentration typical of those in the Grand River. Arguably, using a slightly higher density inoculum for the slower growing *Microcystis* avoids a long lag-stage when cell density is low ($<10^4$) and avoids a potential population crash (because *Microcystis* prefer not to be diluted too heavily) while allowing for growth in the controls without depleting the nutrients (pers. comm., Heather Roshon–CPCC, 2014).

Table 10: Initial *M. aeruginosa* concentrations in inocula used in this investigation

Experiment	Initial Conc. (cells/mL)	Notes
Growth Screening Tests	3.59×10^6	Cells large and healthy.
Fe [5][25]	4.23×10^6	Variable sizes.
Fe [50][100]	1.43×10^6	All pale, variable sizes
Fe [100][200] no sediment	2.15×10^6	All pale, variable sizes
Fe [200][300][400]	1.24×10^6	Healthy, large. Slight lag until Day 4.

3.4.2 P Sequestration Experiments

To evaluate $FeCl_3$ addition to Reservoir water for achieving P-sequestration and limiting *M. aeruginosa* growth, a series of experiments was conducted using Reservoir water, Cell 3 sediment, and several $FeCl_3$ doses. Freeze-dried sediment (0.25 g) was added to 50 mL flasks in triplicate; the flasks were then autoclaved at 121°C for 15 minutes. 25 mL aliquots of autoclaved Reservoir water were added to each flask (by weight). Using the procedure detailed in Section 3.5, existing/background SRP concentrations of the Reservoir water were measured prior to each test. SRP was also measured periodically in control test units prepared and handled in exactly the same manner as the triplicate samples used for counting.

Additional experiments were conducted using $FeCl_3$ added to flasks containing only Reservoir water–and no sediment–to rule out the effects of sediment on *M. aeruginosa* growth. Prior to testing, a large batch of reservoir water and sediment were acclimated on the bench for two months using the following ratio of 350 mL water: 3.5 g sediment. Sediment was then removed by filtering through a 0.45 µm Whatman® 47 mm GF/F membrane filter. The filtrate was autoclaved at 121°C for 15 minutes. 25 mL aliquots of this 'acclimated' Reservoir water were added to each 50 mL flask (by weight) in triplicate. Methods similar to those described immediately above were used for the $FeCl_3$ amendments and *M. aeruginosa* counting.

pH was measured on a few samples with a calibrated Orion 250A pH meter to confirm that the Reservoir water was within optimal pH range for $FeCl_3$ amendment and at an acceptable level for CB growth (<8.5 to ensure availability of CO_2; APHA, 2012).

A $FeCl_3$ stock solution (5000 µg/L) was prepared to minimize the coagulant volume added to each experimental flask and to maintain the ratio of 0.25 g sediment: 25 mL water. After the $FeCl_3$ additions, the flasks were covered with a foam stopper, rapidly swirled for 30 seconds, and then allowed to settle for 18 hours on the bench top at ambient temperature (approx. 25°C). All flasks were then inoculated with *M. aeruginosa* cells using the inoculum concentrations detailed in Table 10.

The cells were added gently down the inside wall of each flask and left to acclimate on the bench top for approximately 2 hours. Cell counts were conducted on inoculation day (Day 0) to confirm the calculated initial cell concentration. Observations of cell health, fluorescence (pigment brightness) and cell size were noted; other observations such as sediment re-suspension and appearance of precipitate were recorded throughout the test period.

3.5 Assessment and Characterization of tests: P Sorption Experiments and *Microcystis* Growth after P Sequestration

Following the sorption experiments, the concentration of P adsorbed to the sediment was calculated using the following equation:

$$q = \frac{(C_0 - C_E) \times V}{M} \qquad \text{Equation 2}$$

where:

q is mass of P sorbed per mass of sediment (mg/g);

C_0 is initial concentration of P in solution (mg/L);

C_E is concentration of P in solution after equilibrium (mg/L); and

V is the volume of P aliquot (L), and M is mass of sediment (g).

Assessments of CB growth were based on guidance for freshwater algae (OECD, 1984; OECD, 2002; Environment Canada, 2007; APHA, 2012). Two different parameters were used to determine inhibition of growth: growth rate (cell counts), and dry weight (expressed as mg/L), because biomass indicators often respond differently to nutrient-limiting condition (APHA, 2012). CB cell enumeration was conducted after 0, 24, 48 hours, as well as other times,

depending on the specific test being conducted. Tests were considered valid if the Control Coefficient of Variation was ≤ 20% on Day 0 (Environment Canada, 2007). Appendix C contains detailed CB cell enumeration and test accuracy data.

Cell counts, expressed as logarithmic cell numbers/L, were used to determine % inhibition (relative to controls) and average growth rate. The % inhibition was calculated using the following equation:

$$I = \frac{R_c - R}{R_C} \qquad \text{Equation 3}$$

where:

I is the % inhibition of growth for each treatment; and

Rc and R are the mean log cell count (of 3 replicates) of the control, and of each treatment, respectively.

The average specific growth rate was calculated using the following equation:

$$\mu = \frac{ln\,(X_2/X_1)}{t2-t1} \qquad \text{Equation 4}$$

where:

X_1 is the initial raw cell count (cells/mL) and X_2 is cell count at test end; and

t is the time interval in days.

To characterize the variability in CB cell size in a test, dry mass measurements of *M. aeruginosa* were performed at the end of each test. Glass microfiber (1.2 µm pore size, 47-mm-diameter, Whatman GF/C) filters used for biomass collection were pre-weighed and dried overnight (95-105ºC) then allowed to come to room temperature in a vacuum desiccator before reweighing. Equal volumes of test media containing *M. aeruginosa* (15 mL) were filtered through Whatman™ 1.2 µm GF/C glass microfiber filters and then through Whatman™ 0.45 µm Puradisk nylon filters (General Electric, Fairfield, CT) and stored at 4ºC in scintillation vials prior to SRP analysis. (Figure F5; Appendix F).

Filter selection for biomass quantification was based on pore size and material to best retain *M. aeruginosa* cells while allowing the liquid and any fine-grained suspended particles to pass through. Complications can occur when positively charged filters react with negatively charged

bacteria surfaces (zeta potentials) (WHO, 1999; Henderson et al., 2008; Aktas et al., 2012; Cheng et al., 2015).

Microcystis species are typically 3 to 4 µm in size, but can range from 2.4 to 6.7 µm (Henderson et al., 2008; Gonzalez-Torres et al., 2014) or even as large as 9.4 µm (Komárek & Komárková, 2002). Particle size distribution results (Table 12 in Results Section 4.1.7; detailed results in Table B.1 Appendix B) indicate that for Cell 3 sediment, 1.73% of the grains are <1 µm and would likely pass through the filter pores if drawn into a pipette during the *M. aeruginosa* biomass collection at the test-end. 3.61% of the grains are <2 µm and a portion of these could become trapped on the filter paper. However, these larger grains were less likely to be disturbed and probably remained at the bottom of the test unit and were not likely drawn into a pipette when collecting liquid at the surface. Regardless, care was taken to minimize the amount of sediment drawn into pipettes at all times.

Membrane selection for CB cell enumerations has been extensively discussed. The US EPA (1980) recommended that a 0.60 µm BD Millipore® membrane filters be used for filtering algae suspensions. Jarvie et al. (2002) stated that the most common filter to use for SRP analysis is 0.45 µm cellulose-nitrate-acetate. Carlson & Simpson (1996) indicated that using glass-fibre allows more particulate material to pass through filters, resulting in more particulate matter in the soluble fraction, including small algae and bacteria. They state cellulose (Millipore) filters (0.45 µm) are the standard for P testing due to their exclusion properties, despite these membranes often containing colloidal P. They note that others use Nucleopore instead of membrane or glass fibre filters that allow for excellent size separation, and these do not contain P. Not only is pore size important for retaining the algae, it is also an important consideration with respect to dissolved P analysis. For instance, Carlson & Simpson (1996) found that using a 0.45 µm filter resulted in 30 µg/L SRP while using a smaller 0.1 µm filter resulted in 15-20 µg/L SRP for the same sample.

3.6 Soluble Reactive Phosphorus (SRP) Analysis

All samples were collected in acid washed, triple DI and ultrapure rinsed bottles. They were filtered through Whatman™ 0.45 µm Puradisk nylon membrane syringe filters (General Electric, Fairfield, CT) and stored at 4ºC until SRP analysis (Standard Method 4500 P A). SRP concentrations were measured colorimetrically using a Technicon Auto-analyzer II (Technicon Instruments Corp., Tarrytown, NY) linked to a computer running NAP™ analysis software, using

the stannous chloride–ammonium molybdate procedure (Environment Canada 1979). The method detection limit is 1 µg P/L (Stone & Droppo 1994). Ultrapure water was used to dilute samples containing >200 µg P/L and for sample blanks/washes. SRP concentrations were reported as PO_4^{3-}.

The ultrapure water was collected at 18.2 ohms (Ω) resistivity (also referred to as Type 1 water) and was used in preparation of all reagents. All chemicals, including the Phosphorus Standard (KH_2PO_4) and Ferric Chloride Hexahydrate ($FeCl_3$-$6H_20$), were of high purity (at least analytical grade). All glassware used in storing, testing and SRP analysis were acid washed and triple rinsed in both Type 2 water followed by a triple rinse in Type 1 water (ultrapure water). Stock solutions were stored in the dark at 4°C when not in use.

Prior to SRP analysis, several samples were yellowish in color; and this interfered with SRP analysis. One likely explanation for the discolouration of the effluent was due to the pigments chlorophyll-A and phycocyanin. These pigments are quite small (nm to angstrom range) (Fisher et al., 1980; Raven et al., 1986; MWH, 2012) and will pass through the 0.45 µm filters if they become extracellular (either due to senescing cyanobacteria or cell damage). Notably, Chlorophyll is rapidly degraded by sunlight (Newcombe, 2009; Robertson, 2012); consequently, the test samples were exposed to UV light to degrade these pigments prior to SRP analysis. Prior to implementing this type of modification to SRP analysis, several P standards (10, 25, 50, 100, 200 µg/L) were prepared and exposed to UV light for 1 hour; minimal P degradation was observed (i.e., mean measured values were 10, 25, 50, 102, and 203 µg/L, respectively). Thus, the all water samples were exposed to UV light for 1 hour prior to SRP analysis.

Detailed SRP data are presented in Appendix D

3.6.1 Other nutrient analysis

Nitrogen and iron content was measured in the final *M. aeruginosa* test. Samples were stored at 4ºC until analysis approx. 6 hours after collection and pH was not adjusted. All samples were measured using the portable HACH DR 1900 portable spectrophotometer (Hach; Loveland, CO). Nitrogen (as NO_3^-N) was analyzed using the Cadmium Reduction Method (Method 8171; Hach; Loveland, CO), adapted from Standard Methods (Method 3500-Al B; APHA, 2012). The method detection limit was 0.2 mg/L NO_3^-N. Nitrogen results are presented in Appendix A.

3.7 Quality Control

Five P standards (0, 25, 50, 100, 200 µg P/L) were used to generate a standard calibration curve. Samples were analyzed only when the coefficient of determination (R^2) for calibration was >0.99. To measure analytical precision during SRP analysis, seven replicates of the lowest P-standard (25 µg P/L) were analyzed randomly on several occasions and the relative standard deviation was <3%. This process was also used for random select samples and the relative standard deviation was <5%. Two reagent blanks were inserted after every three samples and after every set of standards. These data are presented in Appendix E.

For quality control purposes and method validation (i.e., to assess inter-lab variability between the RMOW and UW Labs), water from the Reservoir (Cell 4) was collected on 14 Oct 2015, split, and analyzed. The RMOW Lab uses Inductively Coupled Plasma (ICP) Spectrometry and a method modified from Standard Method 3120B (APHA, 2012) with an uncertainty relative to the concentration. At the reporting limit of 0.020 mg/L the uncertainty is ± 0.006 (RMOW lab: personal comm H. Vanderloo 2013).

3.8 Statistical Analysis

To determine if the application point of $FeCl_3$ can significantly affect the SRP levels, a statistical comparison of final SRP levels with application of $FeCl_3$ prior and after 18 hours of agitation was conducted. The hypothesis testing (one-tailed t-test) was used to quantitatively compare the final SRP values of the samples with $FeCl_3$ application prior to 18 hours of agitation—to the samples with application of $FeCl_3$ after equilibrium (18 hours of agitation).

The *p* value for this comparison was calculated and compared to a significance level of 5% as a common indication of significant effects. Three different hypothesis tests were conducted. The first and second, evaluated the significance of application point at individual $FeCl_3$ treatments (concentrations of 25 and 100 mg/L, respectively), while the third was a comparison of pooled treatments ($FeCl_3$ = 25 and 100 mg/L).

Chapter 4 Results and Discussion

4.1 Sorption Experiments

4.1.1 P Sorption in Sediment and Ultrapure Water

The P sorption characteristics of reservoir sediment (from Cell 3) equilibrated by agitation in ultrapure water for 18 hours are shown in Figure 9 (raw data are provided in Appendix D). The equilibrium phosphorus concentration (EPC_0) in this system is 104 µg P/L (note only a linear trend line could be fit to the data); thus, the sediment has the potential to desorb P into the water column at lower ambient SRP concentrations. When the overlying water contains no SRP, the maximum amount of P desorbed from the sediment is 25 µg P/g sediment in this system. The sorption characteristics (EPC_0) of sediments originating in riverine and lacustrine systems have been extensively reported and are known to range between approximately 25 and 100 µg P/L (Dunne et al., 2005; Jarvie et al., 2005; Emelko et al., 2015); differences in these values are related to differences in sediment composition and environmental conditions. The EPC_0 observed herein is consistent with previously reported values, especially those for agriculturally and municipally impacted watersheds.

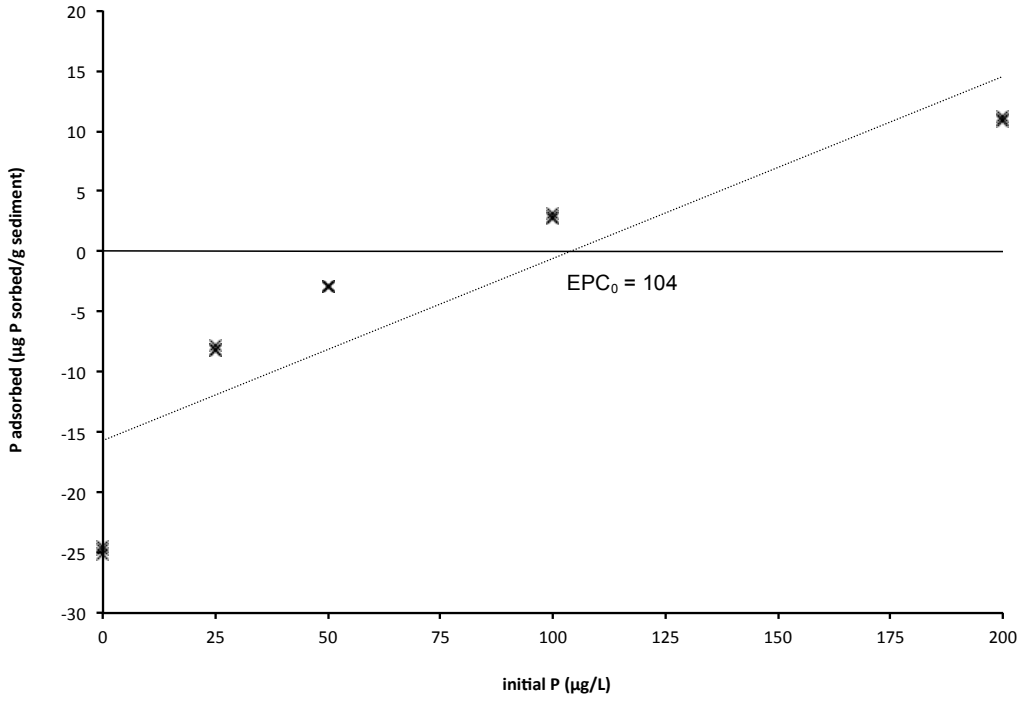


Figure 9: P sorption dynamics in ultrapure water and reservoir sediment from Cell 3. Note all replicates are plotted (n = 3)

4.1.2 P Sorption in Sediment and Reservoir Water

The P sorption characteristics of Reservoir sediment (from Cell 3) equilibrated by agitation in Reservoir water for 18 hours are shown in Figure 10 (raw data are provided in Appendix B). In this figure, the EPC_0 is 82 µg P/L, which is comparable to data presented in Figure 9. The significance of this more realistic assessment of P sorption by Reservoir sediment is that it is possible to approximate the mass of P potentially released from the sediment when SRP concentration in the water column is known. For example, an ambient SRP concentration of ~32 µg P/L was measured in the Reservoir; this corresponds to a P release of ~5 µg P/g sediment. Thus, sediment in the Reservoir can act as a significant source of P for biotic uptake.

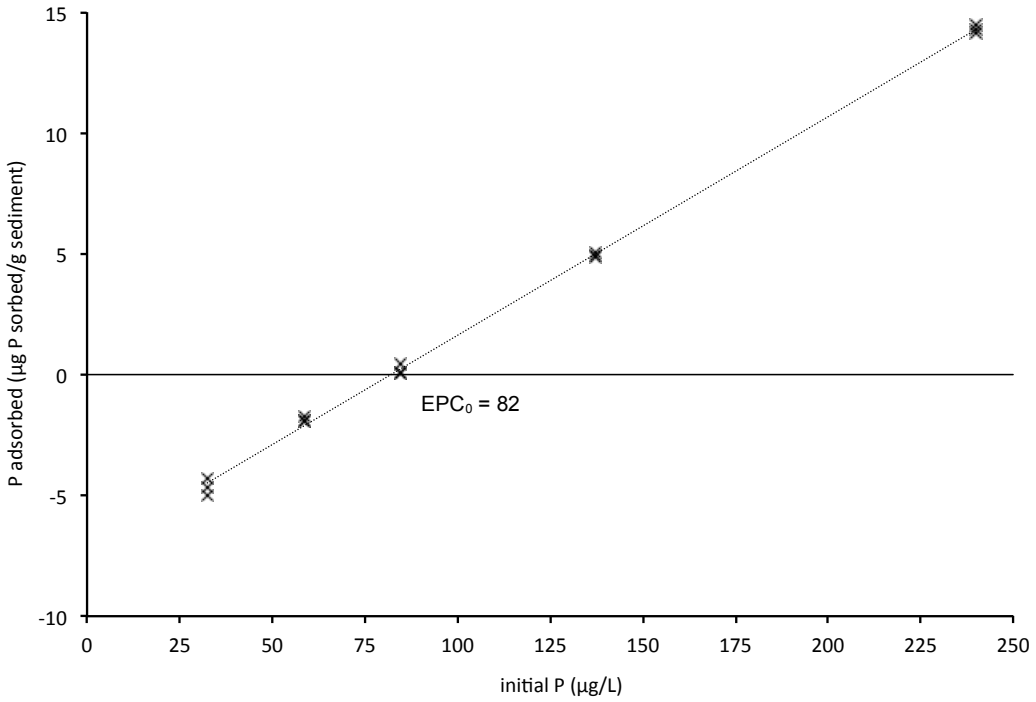


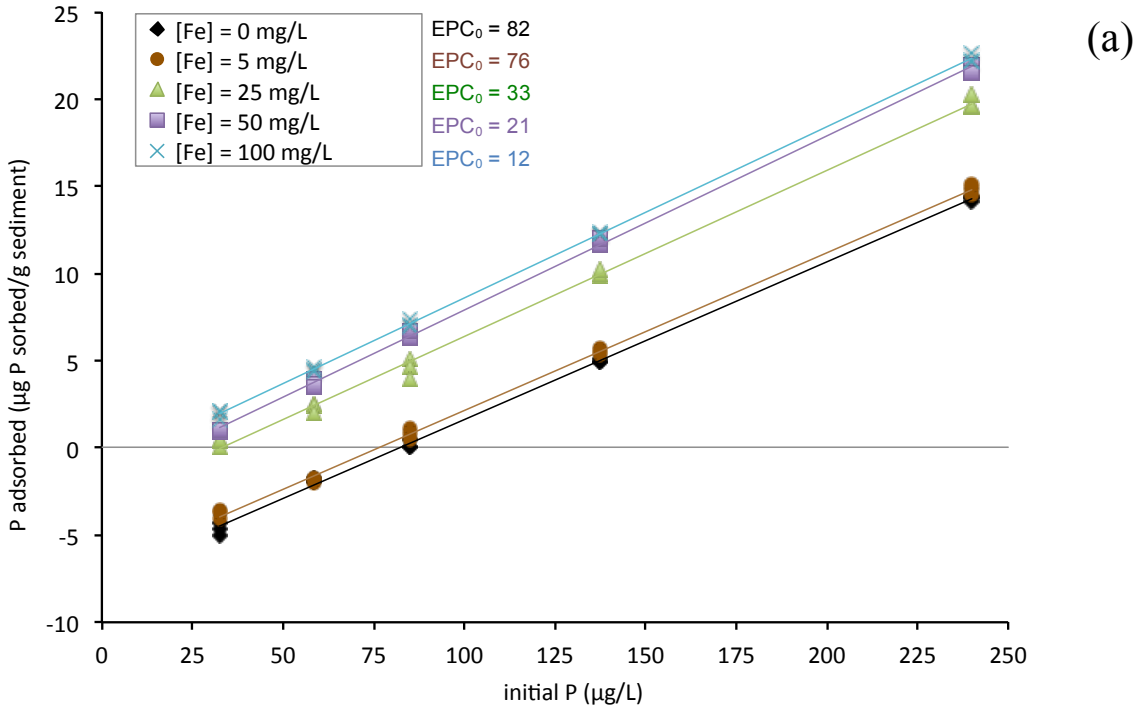
Figure 10: P sorption dynamics in Reservoir water and sediment from Cell 3. Note all replicates are plotted (n = 3)

4.1.3 P Sorption in Sediment and Reservoir Water with $FeCl_3$ Addition Prior to Agitation (Prior to Equilibrium)

To evaluate P sequestration from the water column using $FeCl_3$, the P sorption characteristics of Reservoir sediment (from Cell 3) in Reservoir water amended with $FeCl_3$ and then equilibrated by agitation for 18 hours are shown in Figure 11 (raw data are provided in Appendix B). These experiments were conducted with three separate batches of Reservoir water that contained

background SRP concentrations of ~32, ~24, and ~61 µg P/L (Figure 11a, b, and c, respectively). $FeCl_3$ addition decreased the EPC_0 in all cases, regardless of initial SRP concentration. The largest shifts in EPC_0 were observed at the highest $FeCl_3$ doses, as would be expected (Sherwood & Qualls, 2001; Duan & Gregory, 2003; Caravelli et al., 2010). For example, the EPC_0 shifted from 82 µg P/L to 33 µg P/L when $FeCl_3$ was added at a dose of 25 mg/L. Notably, the results were reproducible and generally yielded low standard deviation (Appendix D).

Sorption test 1 was conducted to identify the lowest $FeCl_3$ concentration that was likely to chemically precipitate enough SRP such that P desorption from the Reservoir sediment was precluded; as demonstrated in Figure 11a, that lowest dose of $FeCl_3$ was between 5 and 25 mg/L. Sorption test 2 was conducted to more specifically identify that lowest dose (Figure 11b). This analysis demonstrated that $FeCl_3$ doses of 10, 15, and 20 mg/L yielded similar expectations for P adsorption/desorption from the Reservoir sediment. Based on the data from Figure 11a and b, a $FeCl_3$ dose of 25 mg/L was estimated as the lowest possible dose for ensuring P sequestration in the HVR system. Sorption test 3 was conducted to evaluate potential performance benefits associated with use of high $FeCl_3$ doses. No differences in EPC_0 were observed at $FeCl_3$ greater than 200 mg/L (Figure 11c).



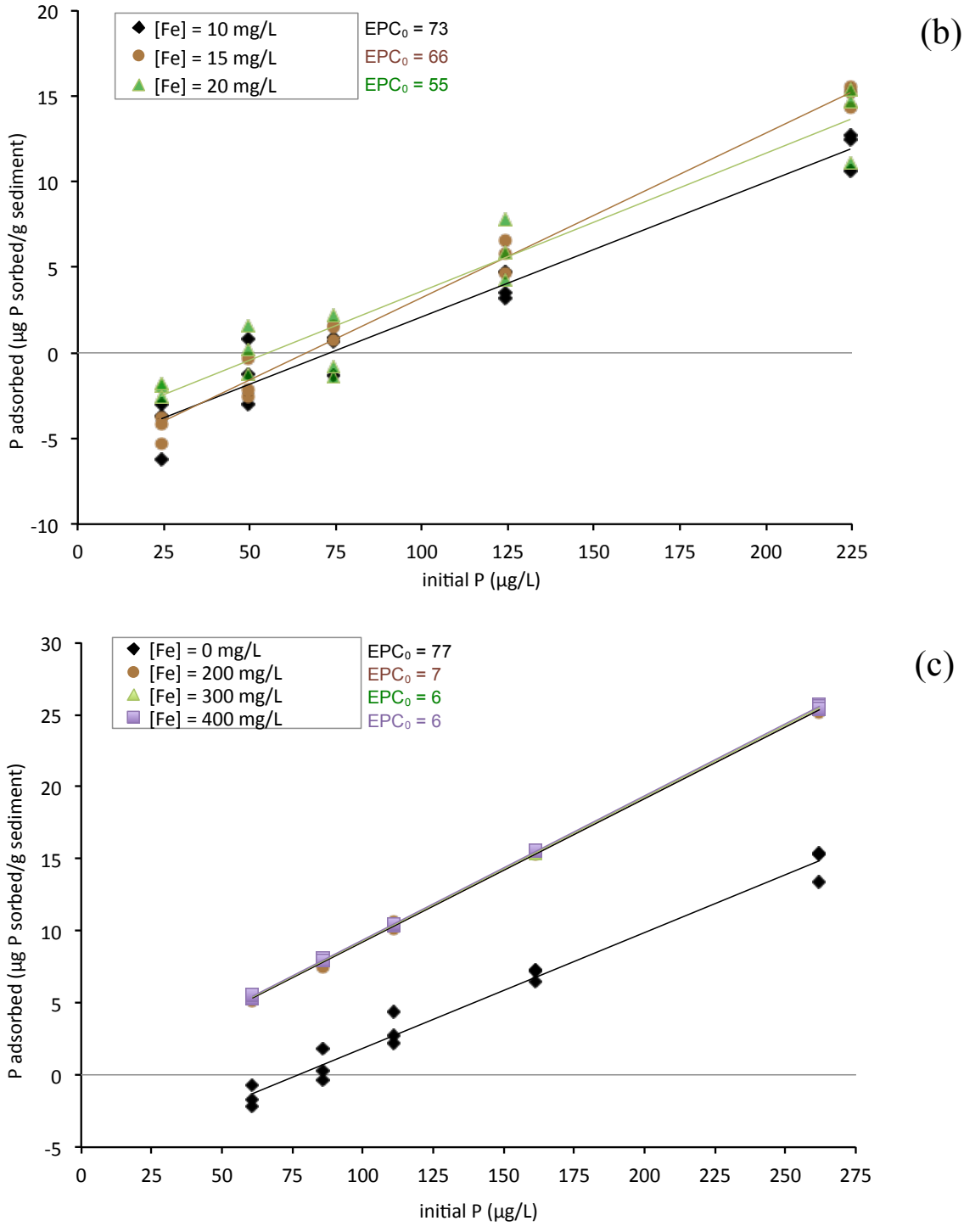


Figure 11: Impact of $FeCl_3$ on P sorption dynamics in Reservoir water and sediment from Cell 3. Note all replicates are plotted (n = 3): (a) Sorption test 1 - identification of the lowest $FeCl_3$ concentration likely to chemically precipitate enough SRP from the water column to preclude P desorption from the Reservoir sediment; (b) Sorption test 2 - detailed assessment of the lower $FeCl_3$ concentration likely to preclude desorption; (c) Sorption test 3 - evaluation of potential performance benefits associated with higher $FeCl_3$ doses

4.1.4 Comparison of $FeCl_3$ Amendments Before and After 18 hours of Agitation

To evaluate the impact of the addition point of $FeCl_3$ during the experimental protocol, additional experiments were conducted to evaluate P sequestration from the water column using $FeCl_3$. In these experiments, Reservoir sediment (from Cell 3) was equilibrated in Reservoir water by agitation for 18 hours and then $FeCl_3$ was added. This experiment variation provided minimal contact time with the sediment-water matrix to emulate a rapid mix operation. $FeCl_3$ doses of 25 and 100 mg/L were added to Reservoir water with no (0 µg P/L) and 50 µg P/L addition (and adjusted for background Reservoir water SRP levels) and evaluated in triplicate, for a total of 12 test units. After the $FeCl_3$ additions, the test units were rapidly shaken for 2 minutes then allowed to settle for an additional 15 minutes. In Figure 12, the EPC_0 results obtained using this protocol are contrasted with those previously reported for the system with 18 hours of contact (Figure 11a) with the coagulant. (Raw data are provided in Appendix D). Notably, this comparison demonstrates significantly more P sequestration (p <0.05) when $FeCl_3$ is applied after sediment equilibration in the water matrix (i.e., 18 hrs of agitation on a shaker table), regardless of applied $FeCl_3$ dose.

- p = 0.011 when comparing all SRP values when $FeCl_3$ is applied before equilibrium to all SRP values when $FeCl_3$ is applied after equilibrium
- p = 0.001 when comparing only $FeCl_3$ = 25 mg/L treatments (i.e., $FeCl_3$ = 25 mg/L is applied before equilibrium to the final SRP values after equilibrium
- p = 0.004 when comparing $FeCl_3$ = 100 mg/L treatments (i.e., $FeCl_3$ = 100 mg/L is applied before equilibrium to the final SRP values after equilibrium

This result is consistent with the literature, which recommends provision of adequate time for sediment-water matrix equilibration when evaluating P adsorption/desorption characteristics (Froelich, 1988; Reynolds & Davies, 2001; Kochevar, 2006; MWH, 2012).

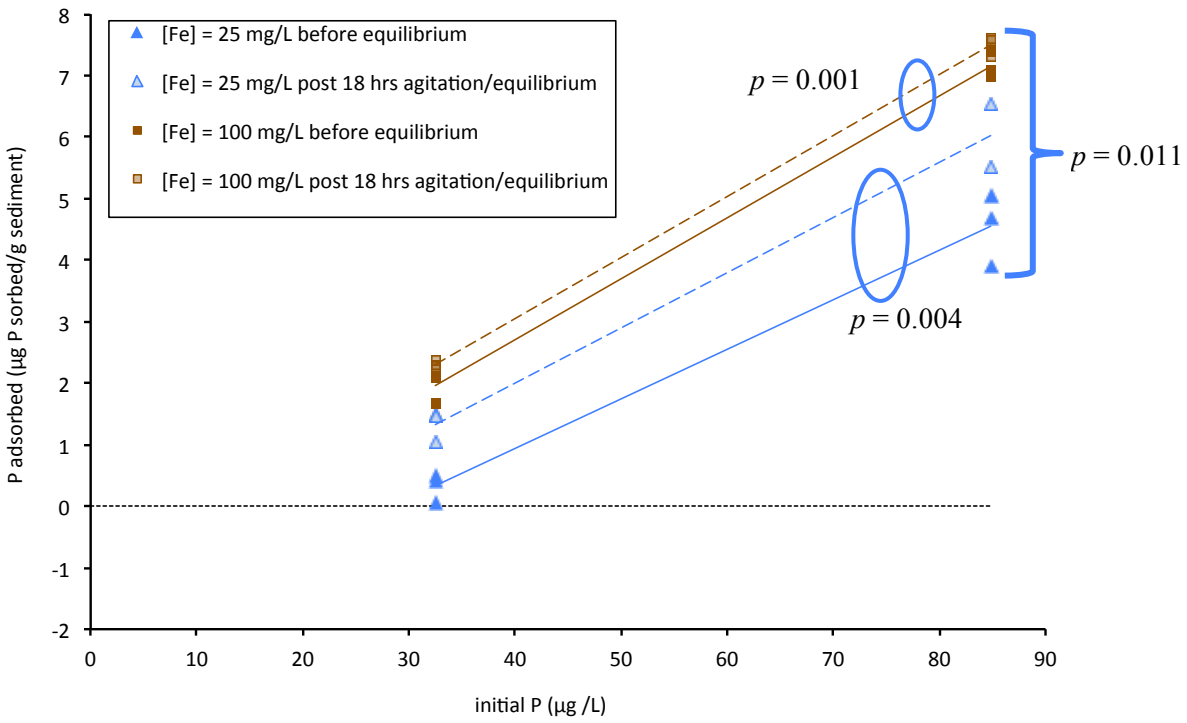


Figure 12: Comparison of impact point of $FeCl_3$ amendments: $FeCl_3$ is added to Reservoir water and sediment before and after equilibrium (18 hrs agitation). Note all replicates are plotted (n = 3). *p* values are shown for significant difference in final SRP levels within each $FeCl_3$ treatment; and for significant difference in final SRP levels when treatments are combined

4.1.5 P Precipitation in Reservoir Water with $FeCl_3$ Addition in the Absence of Sediment

To decouple the reactivity of $FeCl_3$ with dissolved P species and sediment, $FeCl_3$ also was added to P-amended Reservoir water in absence of sediment. The methods previously described in Section 4.1.3 were used, including application of the same $FeCl_3$ doses prior to 18 hours of agitation. This approach was designed to be generally representative of in-line $FeCl_3$ addition to Reservoir water immediately after removal of accumulated sediment from the Reservoir. The initial and final SRP concentrations obtained during the experiment are presented in Figure 13**,** which indicates a background SRP concentration of 29.85 µg P/L. These data clearly demonstrate that doses of at least 25 mg/L of $FeCl_3$ were required to achieve substantial reductions in SRP (i.e., SRP was reduced to between 22 and 10 µg P/L); notably, increasing the dose $FeCl_3$ of beyond 25 or 50 mg/L only had a small, incremental impact on SRP, regardless of initial SRP concentration. The average initial and final P of tests conducted with and without sediment are provided in Appendix D.

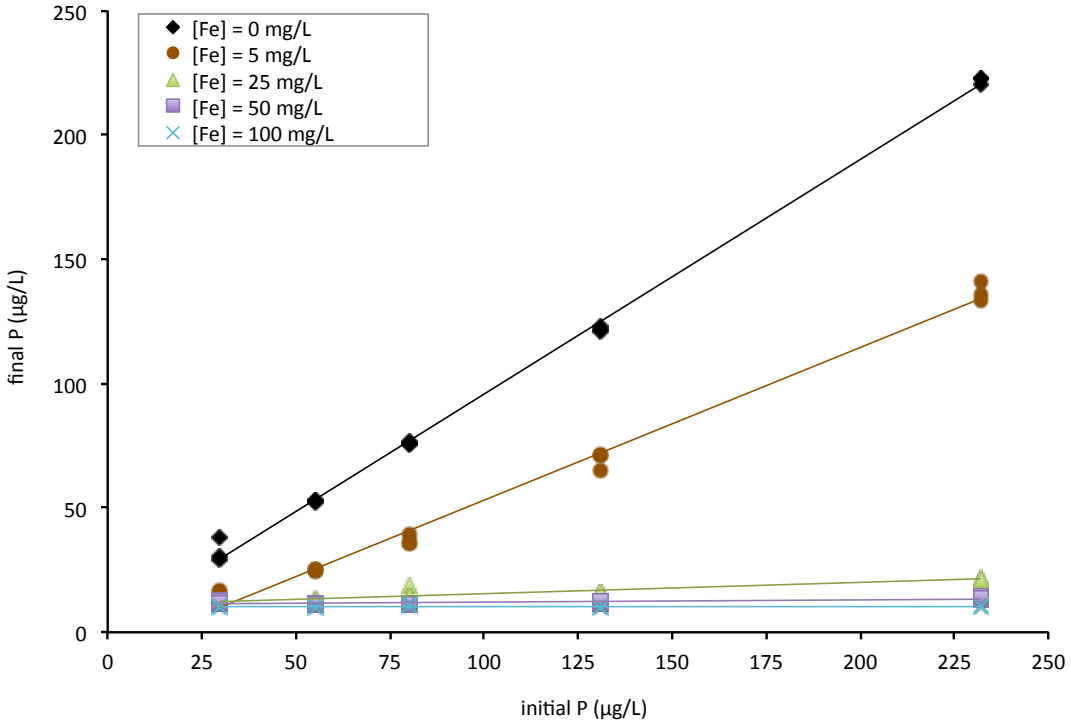


Figure 13: Impact of lower doses of $FeCl_3$ on reservoir water in the absence of sediment. Note all replicates are plotted (n = 3)

The initial/background SRP concentrations during the experiments were similar because the Reservoir water samples were collected during the same season. SRP removal during these experiments (i.e., SRP removal via sequestration with ferric hydroxide species compared to SRP removal using both sequestration and sediment adsorption) is summarized in Table 11, which indicates when the greatest amount of SRP removal was achieved. Three observations were made based on these data:

1. P removal efficacy ([P] removed: [$FeCl_3$] dosed) declined with increases in $FeCl_3$ dose above 25 mg/L, even at low initial SRP concentrations (Figure 13)
2. In general, less P was removed from the water column when sediment was present (because when sediment is present, the $FeCl_3$ has to react with it as well as the SRP), and
3. In absence of sediment, SRP could not entirely be removed from the system; it could only be decreased to a minimum concentration (~15 µg/L), regardless of $FeCl_3$ dose. This likely reflects the equilibrium of the P removal mechanism (sorption of P to iron hydroxide complex and/or precipitation).

Table 11: Comparison of final SRP levels when $FeCl_3$ is added to reservoir water and sediment vs. reservoir water without sediment

Avg.[1] Initial P (µg/L)	$FeCl_3$ (mg/L) 0	5	25	50	100	Avg.[1] Initial P (µg/L)	$FeCl_3$ (mg/L) 0	5	25	50	100
	Avg. Final P (µg/L) with sediment						Avg. Final P (µg/L) with no sediment				
32[2]	81	70	14	23	6	30[2]	32	15	12	12	10
59	77	77	17	21	7	55	53	25	13	11	10
85	83	77	20	19	8	80	76	37	16	11	11
137	86	81	21	18	11	131	122	69	16	12	10
240	99	88	27	21	14	232	222	137	22	14	10

[1] Average of replicates (n = 3)
[2] Background Reservoir water levels

4.1.6 Year-to-year Trends: Reservoir Sediment collected in 2013 and 2014

Sediment collected and analyzed in 2013 was compared to sediment collected in 2014 in an attempt to characterize annual differences between P sorption characteristics (Figure 14). Maximum P desorption from sediment was ~10 µg P/g sediment in 2013, whereas it was ~25 µg P/g sediment in 2014. The final SRP concentrations were similar at initial SRP concentrations ≥25 µg/L. These data indicated that the sediment EPC_0 was 108 µg P/L in 2013 and 104 µg P/L in 2014. This behaviour is an important consideration for water treatment operators interested in knowing if $FeCl_3$ application in the reservoir would require re-assessment likely on an annual basis.

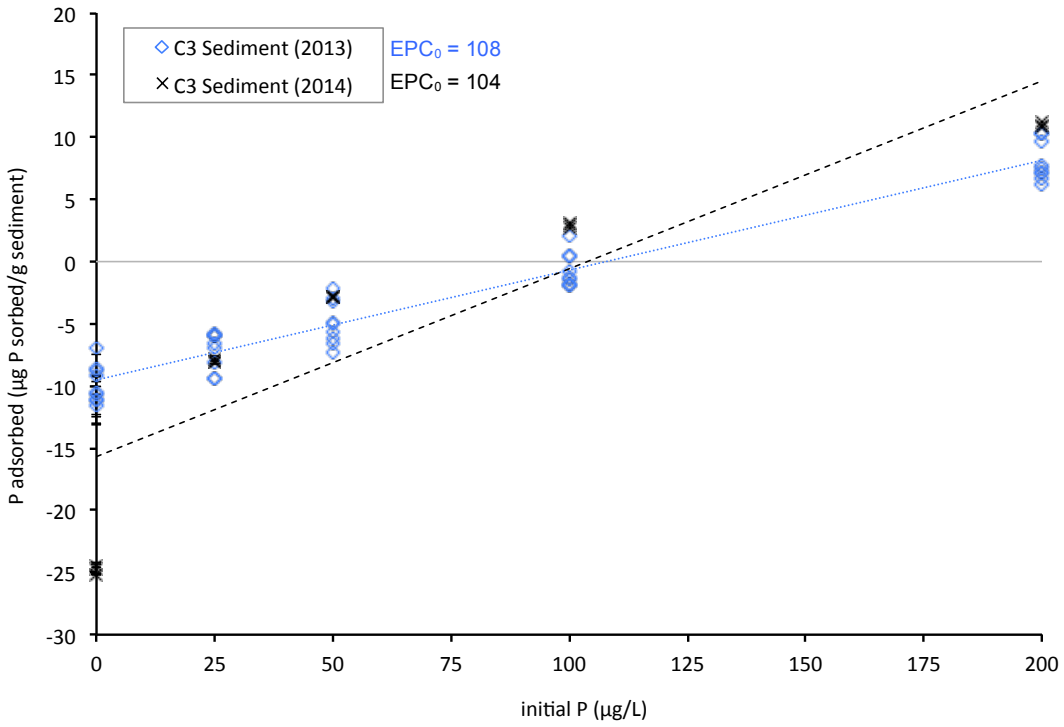


Figure 14: P sorption dynamics in Reservoir water and sediment collected from Cell 3 in 2013 and 2014. Note all replicates are plotted (n = 9 in 2013; n = 3 in 2014)

4.1.7 Sediment Grain Size and Geochemistry in Reservoir Cells

Three sediment samples were collected in each of the four cells in May 2013; as well, samples were collected from Cell 1 in the summer and fall. The grain size distributions and geochemical composition of sediment collected in the Reservoir Cells (1, 2, 3 and 4) were analyzed to determine if sediment (on a per gram basis) has a different P carrying capacity depending on location (reservoir Cell) within the Reservoir (Table B1; Appendix B). The P adsorption/desorption characteristics of the sediment within each cell were also evaluated in ultrapure water (Figure 15). These data demonstrate that the potential for P release progressively increased within the Reservoir as water flowed from Cell 1 to 4. While the P sorption characteristics of sediment in Cells 1 and 2 were somewhat similar (EPC_0 of 47 and 35 µg P/g sediment, respectively), the EPC_0 was considerably higher in Cell 3 (108 µg P/g sediment) and Cell 4 (407 µg P/g sediment).

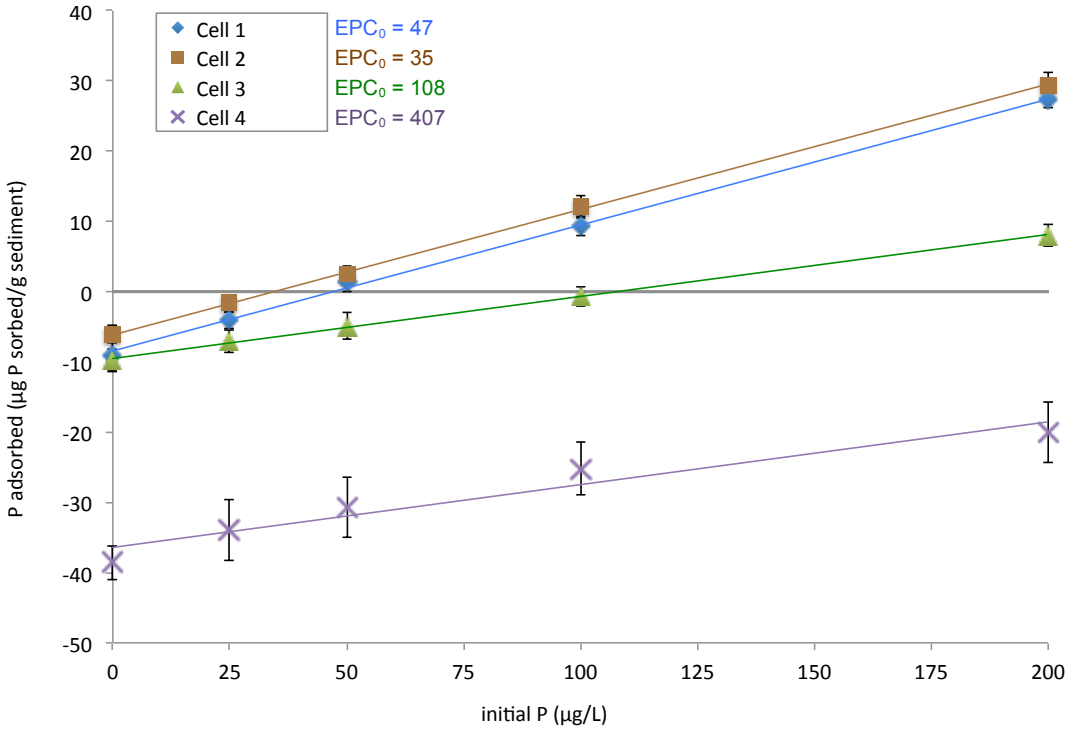


Figure 15: P sorption dynamics in ultrapure water and sediment collected from 4 different Reservoir compartments in May 2013. Note treatment means are plotted (n = 9) ±1 SD

The grain size characteristics (d_{10}, d_{50}, d_{90}, and specific surface area) of deposited sediment in each of the Reservoir cells are summarized in Table 12. These data indicate that the size fractions of the deposited sediment in the Reservoir cells were generally similar in that they were fine grained and predominantly <100 µm in size. Thus, grain size characteristics alone could not adequately explain why the P release potential of deposited sediment within the Reservoir progressively increased from Cell 1 to 4 (Figure 15).

Table 12: Average particle size distribution from Reservoir cell

	Percentage of grains at or below a specific diameter (µm) = D_x				Specific surface area
Reservoir Cell	10%	50%	80%	90%	m^2/g
Cell 1	5.03	24.81	65.31	122.56	0.622
Cell 2	4.73	21.14	52.32	92.82	0.670
Cell 3	5.14	23.12	53.96	87.71	0.624
Cell 4	5.16	25.74	63.52	111.42	0.603

The major element composition and mineralogy of the deposited sediment in each of the Reservoir cells are summarized in Table 13, Table 14, and Table 15. In general, these data indicate that the sediment in Cell 3 and Cell 4 had similar levels of iron (Fe_2O_3), manganese (MnO), and aluminum (Al_2O_3) that were higher than those in the sediment from Cells 1 and 2 (Table 13; Table 15). Additionally, the sediment from Cell 4 had substantially higher amounts of chlorite relative to the sediment from the other Reservoir cells (Table 14). Chlorites are known to contain Fe, Mn, and Al in their lattice (Brittanica, 2016). Notably, these elements form P-sorbing metal oxy-hydroxides on sediment surfaces (Froelich, 1988; Reynolds & Davies, 2001; Sherwood & Qualls, 2001; Withers & Jarvie, 2008; Worsfold et al., 2008). While not incontrovertible, these data suggest that preferential settling of fine sediments containing metal oxy-hydroxides resulted in the progressively increasing P release potential of deposited sediment within the Reservoir, from Cell 1 to 4 (Figure 15).

Table 13: Mean major element concentration (%) ± SD (n=3)

Cell	1 Summer		1 Fall		2		3		4	
Elements	Avg %	±SD	Avg %	±SD	Avg %	±SD	Avg %	±SD	Avg %	±SD
SiO_2	40.7	0.5	41.7	0.1	37.6	0.1	38.7	0.3	40.4	0.2
Al_2O_3	8.9	0.1	9.1	0.1	8.8	0.0	9.5	0.1	10.1	0.1
Fe_2O_3(T)	4.40	0.05	4.40	0.14	4.43	0.03	5.15	0.07	5.15	0.02
MnO	0.168	0.05	0.143	0.00	0.166	0.01	0.235	0.02	0.224	0.01
MgO	3.33	0.05	3.31	0.07	3.14	0.02	3.08	0.01	3.04	0.02
CaO	14.4	0.05	13.8	0.33	16.0	0.08	14.5	0.14	13.4	0.09
K_2O	1.79	0.05	1.87	0.01	1.71	0.03	1.88	0.05	2.06	0.04
LOI	25.1	0.05	24.0	0.26	27.0	0.12	25.8	0.11	24.4	0.04
Total	100.4	0.05	100.0	0.49	100.4	0.15	100.5	0.35	100.3	0.29
C-Organic (calc)	3.43	0.05	3.17	0.20	3.85	0.12	3.95	0.10	3.69	0.06

Table 14: Mean mineral concentration (%) ± SD (n=3)

		Calcite		Dolomite		Quartz		Plagioclase		Microcline	
Cell	N	Avg %	±SD	Avg %	±SD	Avg %	±SD	Avg %	±SD	Avg %	±SD
1(S)	3	19.0	0.3	14.8	2.6	21.2	1.7	8.6	0.6	4.5	1.2
1(F)	3	18.6	1.5	12.4	1.7	22.8	1.0	9.8	0.9	5.5	0.6
2	3	22.1	0.9	11.0	2.2	17.2	0.3	8.0	0.8	4.0	0.3
3	3	20.7	0.9	11.1	1.5	17.0	0.6	7.1	0.6	4.0	1.0
4	3	19.8	1.5	10.6	1.7	19.7	0.9	8.0	1.6	5.4	0.4

Table 14 cont'd: Mean mineral concentration (%) ± SD (n=3)

		Muscovite		Chlorite		Amphibole		Amorphous	
Cell	N	Avg %	±SD	Avg %	±SD	Avg %	±SD	Avg %	±SD
1(S)	3	8.4	0.4	1.5	0.3	1.7	0.6	20.3	1.7
1(F)	3	9.3	0.7	1.8	0.2	1.5	0.3	18.3	3.0
2	3	8.7	0.7	1.7	0.1	1.7	0.3	26.2	2.9
3	3	9.3	1.3	1.7	0.1	1.4	N/A	28.5	2.5
4	3	11.5	1.7	2.3	0.2	2.1	N/A	22.0	3.5

Table 15: Mean metal concentration (%) ± SD (n=3)

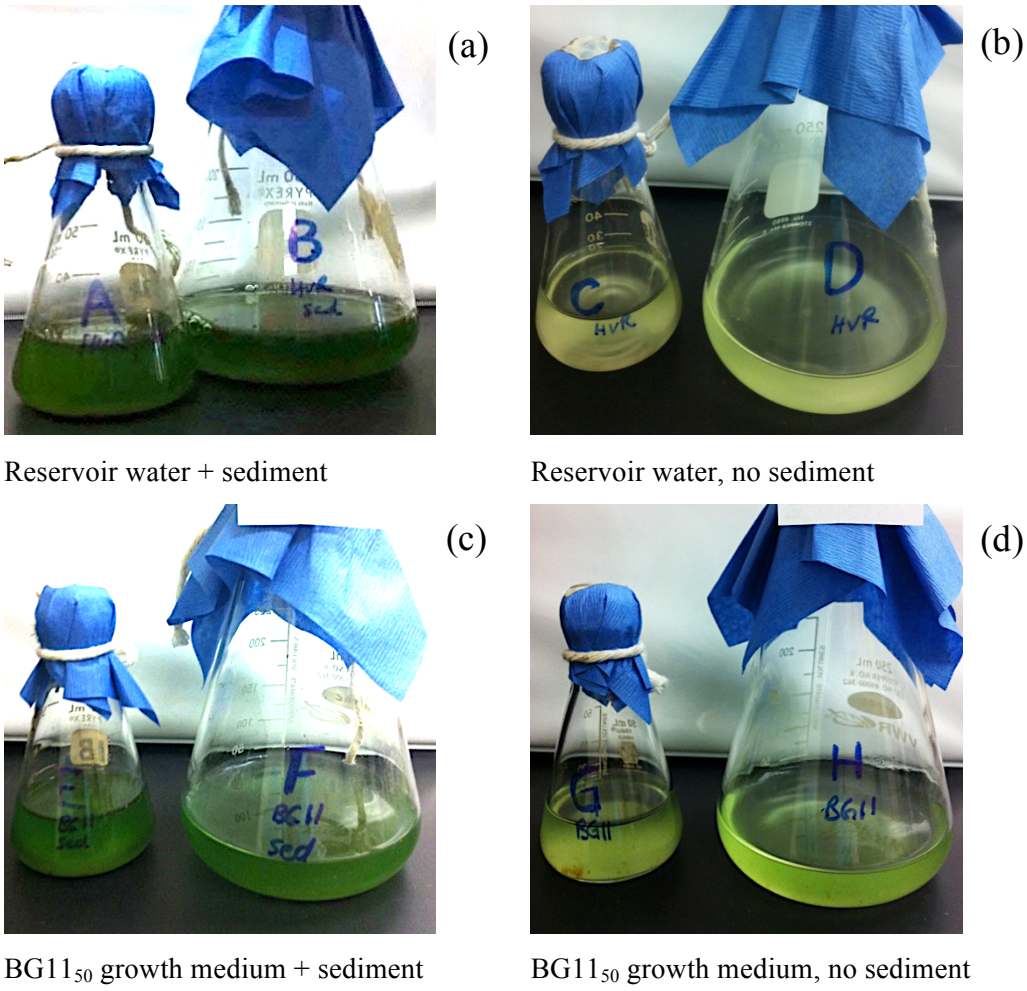
	Cell	1 (Summer)		1 (Fall)		2		3		4	
Metals	Unit	Avg %	±SD	Avg %	±SD	Avg %	±SD	Avg %	±SD	Avg %	±SD
Li	ppm	20.3	2.3	22.8	0.6	22.4	1.3	25.6	0.5	27.7	0.8
B	ppm	26.0	3.5	0.6	0.6	0.5	1.5	30.0	1.0	30.7	1.5
Al	%	2.05	0.2	0.6	0.1	0.5	0.1	2.47	0.0	2.63	0.1
Cr	ppm	33.7	2.1	33.7	1.5	34.7	1.5	38.0	1.0	38.7	0.6
Mn	ppm	1240	36.1	1043	49.3	1233	50.3	1740	144.2	1697	35.1
Fe	%	2.77	0.1	2.73	0.1	2.76	0.1	3.26	0.1	3.34	0.0
Co	ppm	9.4	0.4	9.5	0.2	9.7	0.2	11.2	0.2	11.6	0.1
Ni	ppm	23.6	0.8	23.9	0.5	24.4	0.4	26.9	0.5	27.8	0.3
Cu	ppm	33.3	1.4	31.2	0.7	35.8	0.6	34.2	0.4	32.5	0.2
Zn	ppm	153	5.9	151	2.5	170	2.1	181	3.8	176	1.7
As	ppm	4.60	0.1	4.37	0.2	4.27	0.1	5.03	0.2	5.40	0.1
Ag	ppm	0.118	0.0	0.118	0.0	0.138	0.0	0.143	0.0	0.135	0.0
Ba	ppm	139	8.5	136	4.2	145	1.5	167	3.1	169	3.5
Cd	ppb	0.530	0.0	0.543	0.0	0.553	0.0	0.557	0.0	0.533	0.0
Au	ppm	3.37	0.7	0.73	0.8	2.63	0.7	3.93	1.4	1.53	0.6
Pb	ppm	24.6	1.6	26.2	0.3	28.2	0.5	31.8	1.0	30.6	0.4
Hg	ppb	15.0	8.7	21.7	14.4	25.0	18.0	26.7	5.8	26.7	5.8

4.2 *Microcystis* Growth Trends after P Sequestration

4.2.1 *Microcystis* Growth Screening Tests

A preliminary experiment was conducted to understand effect of sediment and surface: volume ratio on the dynamics of CB growth in both Reservoir water and $BG11_{50}$ growth medium (Figure 16). No difference in *M. aeruginosa* growth between flasks with and without sediment were observed the first 8 days for both Reservoir water and $BG11_{50}$ medium (i.e., cell counts were not only within the same order of magnitude, but within 12%) (Table C2; Appendix C). However, from Day 14 onwards, all flasks with sediment had higher cell counts than those without sediment—this is evident in both Reservoir water (Figure 17) and $BGll_{50}$ growth medium

(Figure 18), but more so in Reservoir water. The initial SRP concentration in the Reservoir water was 24.4 µg P/L and ~50 µg P/L in $BGll_{50}$ growth medium; the additional P introduced with the *M. aeruginosa* inoculum only increased background SRP concentrations to ~25 µ P/L in the Reservoir waters. Background SRP concentrations were effectively unchanged in the $BG11_{50}$ growth medium, because the introduced inoculum contained the same concentration of SRP as the fresh growth medium (i.e. 50 µg P/L) (Appendix C).



Reservoir water + sediment

Reservoir water, no sediment

$BG11_{50}$ growth medium + sediment

$BG11_{50}$ growth medium, no sediment

Figure 16: *M. aeruginsoa* growth on Day 14 in Reservoir water with (a) and without sediment (b), and in $BGll_{50}$ growth medium with (c) and without (d) sediment. Note the two flask sizes (50mL and 250 mL) will have different surface: volume ratios

Consistent with the observed changes in *M. aeruginosa* growth between flasks with and without sediment around Day 14, the stationary growth phase of *M. aeruginosa* began earlier—between Day 8 and Day 14—in flasks without sediment, whereas it began approximately 1-3 weeks later, on Day 21 in Reservoir water and on Day 35 in $BG11_{50}$ growth medium. These observations are

consistent with nutrient limitation (Scherfig et al., 1973; Hallegraeff, 1993; Leão et al., 2009; Dang et al., 2012; Edwards et al., 2012; Whitton, 2012) in absence of the sediment. Notably, *M. aeruginosa* growth did not appear to vary between the surface:volume ratios (i.e., different flask sizes) investigated, regardless of suspension fluid. It should be noted that while these experiments confirm that SRP desorbed from Reservoir sediment can enhance the proliferation of *M. aeruginosa* in Reservoir water and other media, the intention of these experiments was not to mimic the onset of a bloom.

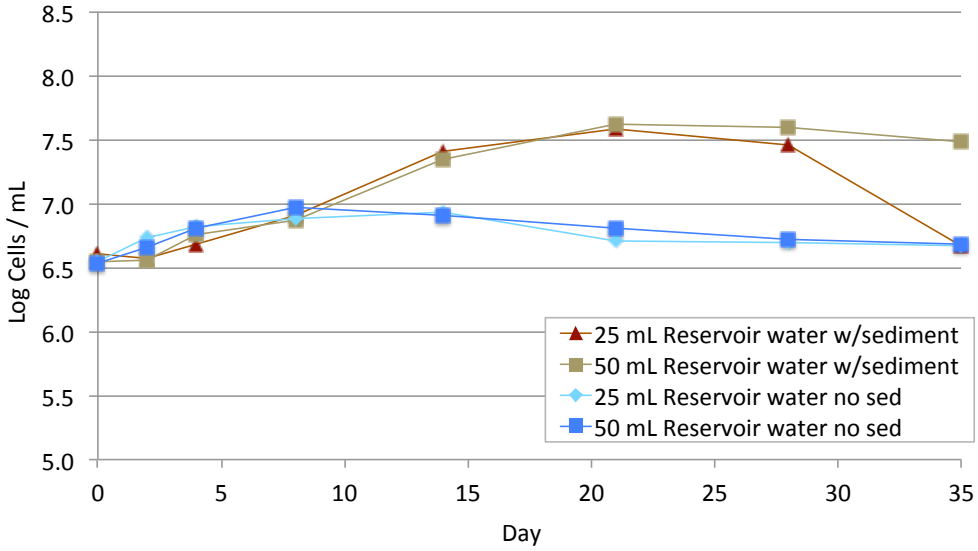


Figure 17: Comparison of *M. aeruginosa* growth in Reservoir water with and without sediment in 25 and 50 mL of solution

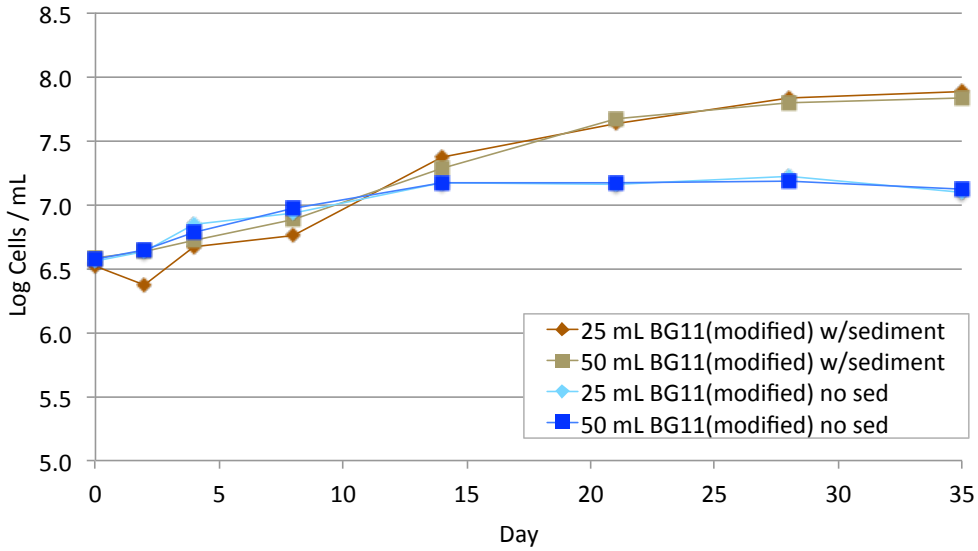


Figure 18: Comparison of *M. aeruginosa* growth in $BG11_{50}$ medium with and without sediment in 25 and 50 mL of solution

Notably, the final SRP in solution was highest in flasks that contained sediment. It was also lower than the initial SRP in all treatments except for the system with Reservoir water containing sediment. The decreases in SRP were attributable to CB uptake. In the system with Reservoir water and sediment, increases in SRP were attributable to P desorption from the sediment, as had been demonstrated previously in sorption tests with sediment. Specifically, P desorbed from the sediment in both ultrapure (Figure 9) and Reservoir water (Figure 10) at solution SRP concentrations less than 104 and 82 µg P/L, respectively. During the present experiment, the initial SRP concentration in Reservoir water (with the additional SRP from inoculum) was ~25 µg P/L and the final SRP concentrations were 498 and 428 µg P/L in the respective 25 mL and 50 mL of Reservoir water and sediment flasks, thereby indicating substantial release of P to the water column from the Reservoir sediment. Although *M. aeruginosa* growth was declining toward the end of the experiments, the primary source of the increased concentrations of solution SRP was from desorption from the Reservoir sediment, rather than CB death because the pairs of flasks had similar final SRP concentrations, but somewhat different cell counts (Figure 19).

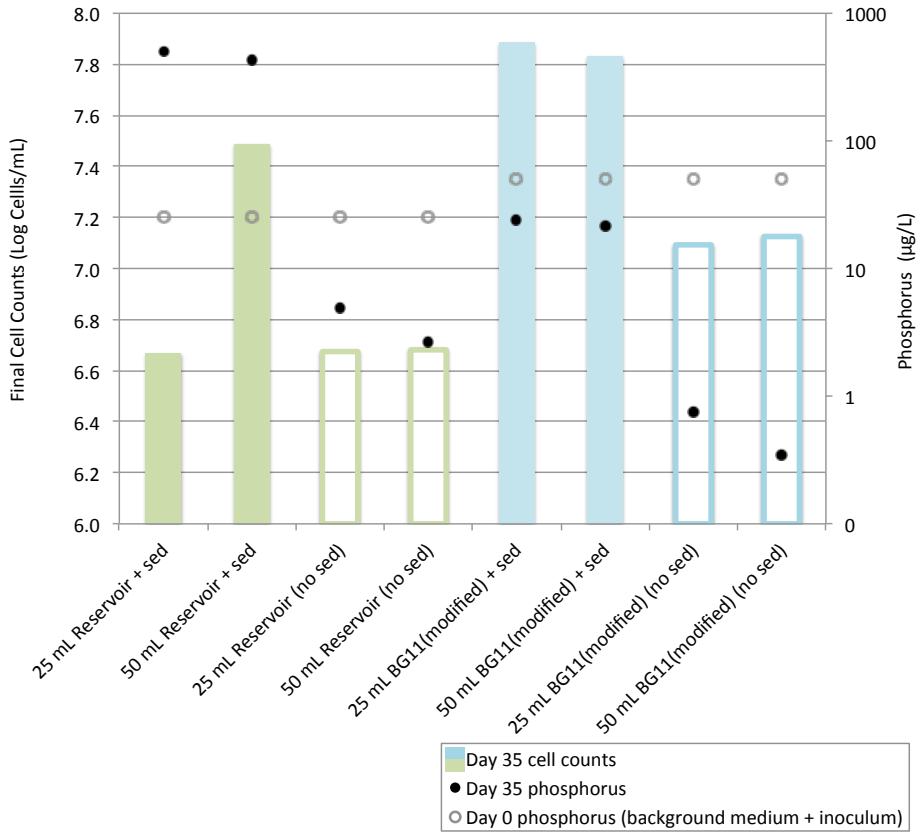


Figure 19: Relationship between final *M. aeruginosa* cell counts and final SRP levels in Reservoir water with and without sediment, and $BG11_{50}$ growth medium with and without sediment on Day 35

4.2.2 Addition of Ferric Chloride to Sediment and Reservoir Water

To investigate the impacts of P limitation on CB growth, proof-of-concept experiments were conducted. In these experiments, *M. aeruginosa* were grown in Reservoir water containing sediment from Cell 3 in the Reservoir; several doses of $FeCl_3$ were applied and *M. aeruginosa* growth was evaluated. It must be noted that these experiments were conducted with higher initial concentrations of *M. aeruginosa* cells (~10^6 cells/mL), which may be considered a bloom (Hitzfeld et al., 2000; Svrcek & Smith, 2004)–or a moderate risk level requiring diligence (WHO, 1999). Thus, the experiments were not representative of a situation in which $FeCl_3$ application in an engineered drinking water supply reservoir might prevent a CB bloom, but rather, they were representative of a challenging scenario in which a high concentration of CB entering from the river might be treated.

The experiments were conducted at higher cell densities for several reasons: 1) experimental limitations associated with culturing and growing *M. aeruginosa* (i.e. need for higher cell concentrations); 2) the reduced experiment time from competition for nutrients; and 3) historical data showing elevated cell counts entering the Reservoir (~5 x 10^5) (Table 9).

M. aeruginosa cell growth at bloom conditions in reservoir water containing sediment from Reservoir Cell 3 was investigated. $FeCl_3$ was added at concentrations of 200, 300, and 400 mg/L. As demonstrated in Figure 20 (and Table 16), although the $FeCl_3$ amendments on Day 0 reduced the initial SRP concentrations from ~52 µg P/L to ~7, ~3, and ~2 µg P/L, respectively, *M. aeruginosa* cell growth continued, potentially enabled by internal stores of P (Thompson et al., 1994; Reynolds & Davies, 2001; Vahtera et al., 2007; Breu et al., 2008; Havens, 2008).

Notably, the reductions in SRP were well below the water quality guidelines of 30 µg/L (of TP) that are generally believed to prohibit growth of nuisance CB (CCME, 2004). Although substantial reductions in cell growth were not observed upon $FeCl_3$ addition, it is worth noting that cell growth was generally and consistently lower at higher applied $FeCl_3$ concentrations (Figure 20), thereby suggesting that some inhibition of *M. aeruginosa* cell growth was achieved**.** These results offer promise for preventing *M. aeruginosa* cell growth from reaching bloom conditions by decreasing P availability.

At the end of the experimental time period, cell counts were similar between treatments and controls; despite this, Day 18 dry weight biomass decreased as $FeCl_3$ dose increased (Figure 22b).

The decrease in biomass was not linearly related to cell counts (Figure 22a), and it was observed that cell size was generally smaller in the treatments, although quite variable (Figure 21). While this decrease in mass in the $FeCl_3$ treatments might be attributable to reduced cell size, variability in cell size measurements (Table 17) and temporal variability in cell size precluded a conclusive assessment.

The average specific growth rates (Table 16) for the control were well within the range observed in the culture stock grown in unmodified medium (Figure 8); and these controls were considered to be growing "normally."

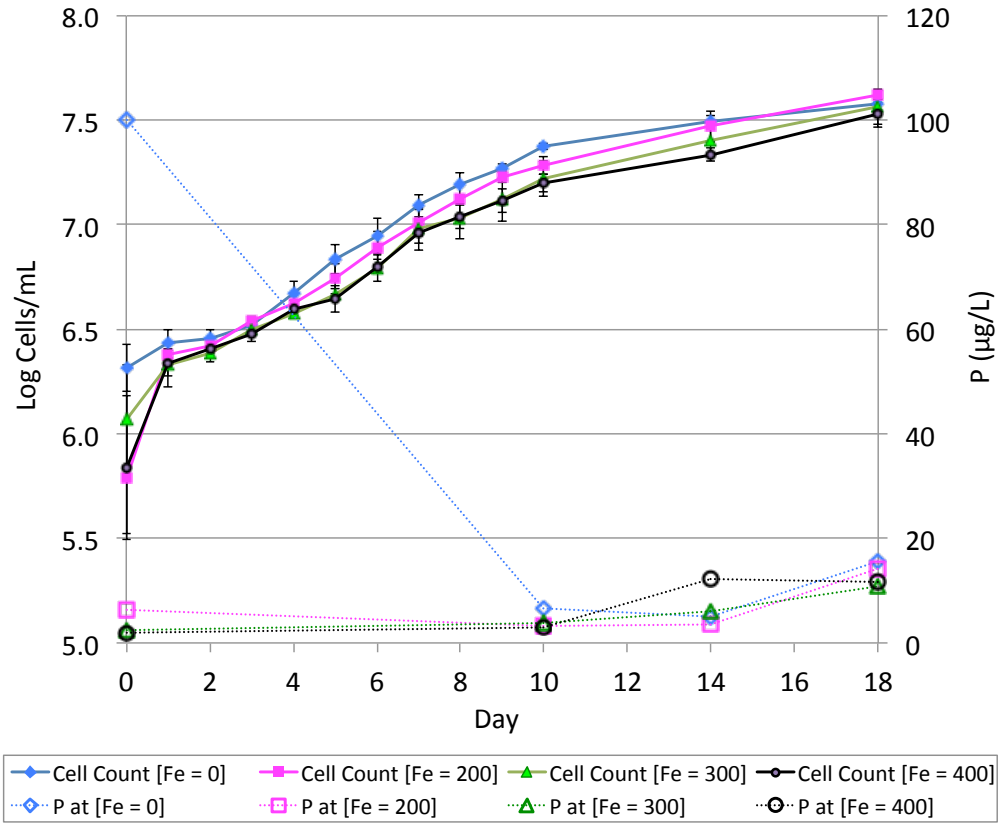


Figure 20: Relationship between *M. aeruginosa* growth and SRP levels in Reservoir water with sediment as a function of higher doses of $FeCl_3$. Note treatment means ± 1 SD (n = 3) for cell growth are plotted on the primary Y-axis; SRP levels are plotted on the secondary Y-axis

Table 16: SRP concentrations and average growth rates of *M. aeruginosa* in response to higher doses of $FeCl_3$ in Reservoir water with sediment

$FeCl_3$ (mg/L)	P (µg/L)[1]				Average growth rate (µ)	Average growth rate (µ)
	Day 0[2]	Day 10	Day 14	Day 18	Day 7	Day 14
0	100.00	6.54	5.03	15.63	0.254	0.193
200	6.21	3.23	3.65	14.19	0.384	0.268
300	2.44	3.67	5.98	10.82	0.286	0.212
400	1.95	2.95	12.30	11.57	0.340	0.232

[1] Background Reservoir water SRP = 52.20 µg/L (prior to sediment addition)
[2] Day 0 SRP values collected 18 hours after sediment additon and $FeCl_3$ amendments, and prior to inoculum (Reservoir water had 18 hours contact time with sediment)

Table 17: *M. aeruginosa* cell size comparison after 14 days exposure to higher $FeCl_3$ doses. Reported values are the mean of 3 or 4 cell measurements per treatment

$FeCl_3$ (mg/L)	Diameter (µm)
0	5.14
200	5.68
300	4.97
400	5.26

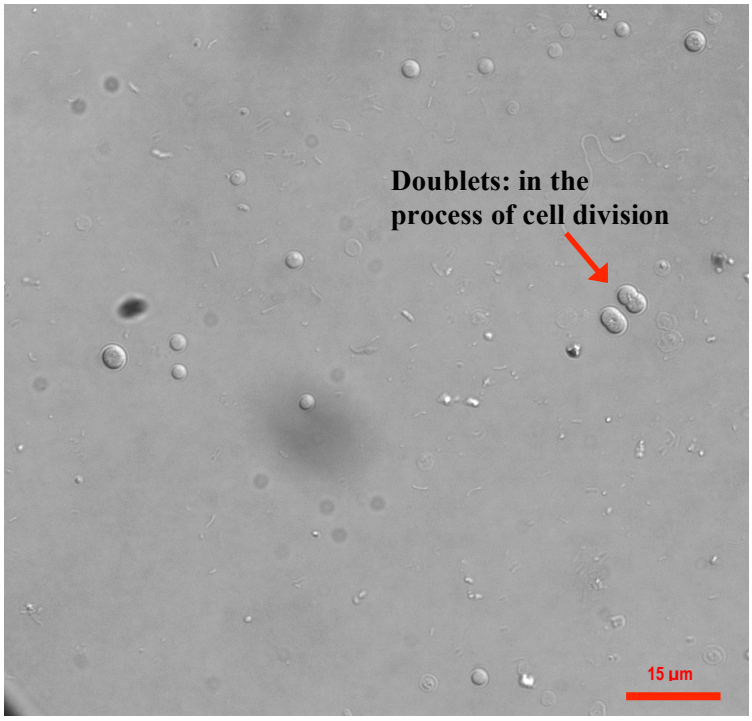


Figure 21: Photo of variable cell sizes of *M. aeruginosa* in $FeCl_3$ (400 mg/L) under an optical microscope at high magnification (x400). Photo taken on Aug 25, 2015

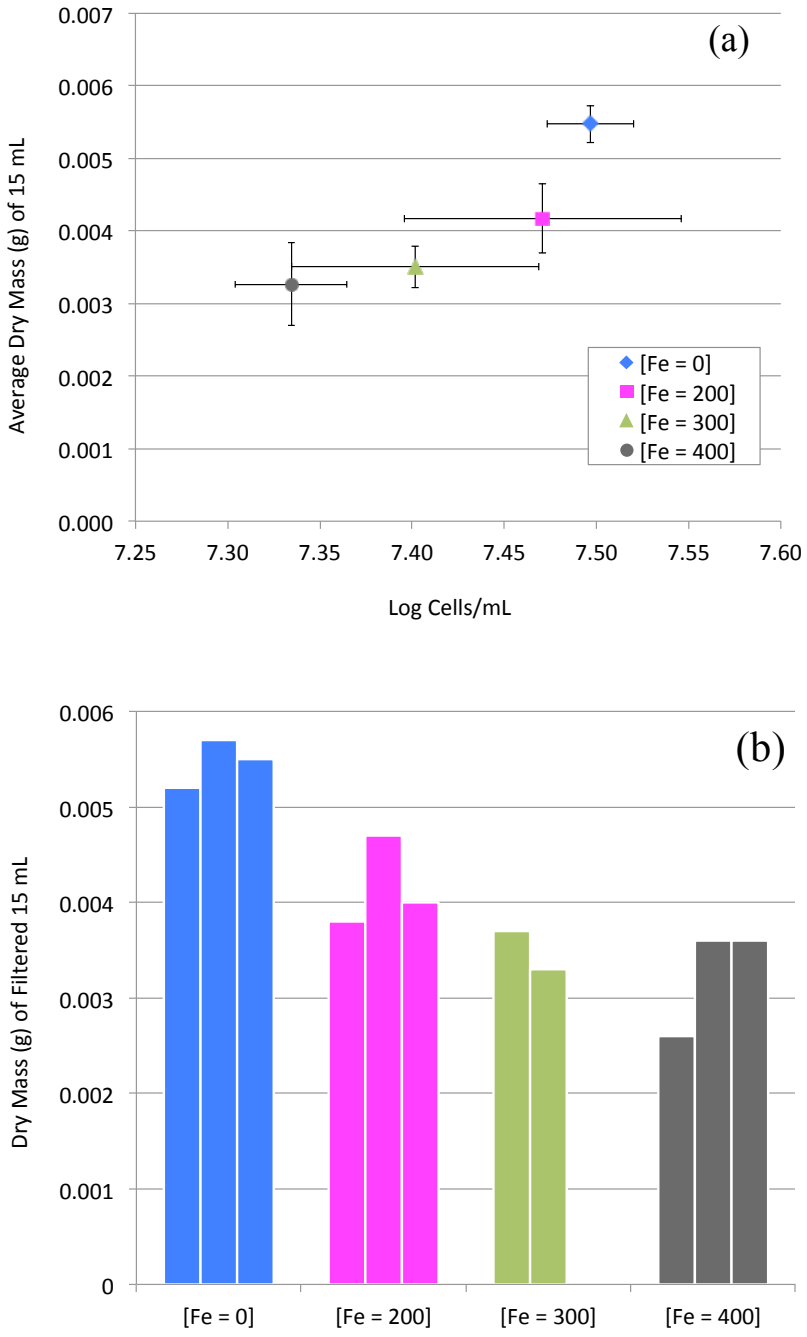


Figure 22: Dry mass comparisons of 15 mL of filtered *M. aeruginosa* at higher doses of $FeCl_3$ in Reservoir water and sediment: a) treatment averages (n = 3) ± 1 SD; b) individual replicate dry masses. Note that negative dry masses cannot be plotted and two values were plotted for $FeCl_3$ = 300 mg/L

4.2.3 Addition of Ferric Chloride to Reservoir Water in the Absence of Sediment

M. aeruginosa cell growth at bloom conditions in reservoir water in absence of sediment also was investigated. $FeCl_3$ was added at concentrations of 100 and 200 mg/L. As demonstrated in Figure 23 (and Table 18), both $FeCl_3$ amendments on Day 0 reduced the initial SRP concentrations from ~74 µg P/L to ~1 µg P/L. Notably, $FeCl_3$ addition significantly decreased *M. aeruginosa* cell growth relative to the controls (Figure 23).

Because there was no sediment in this experiment (and no $FeCl_3$ in the controls), *M. aeruginosa* uptake was solely responsible for the large decrease in SRP in the control. Analogously, the lack of substantial increase in cell concentration in the treatments suggested that $FeCl_3$ coagulation can be useful for limiting the growth of CB such as *M. aeruginosa* in municipally and agriculturally impacted reservoir water.

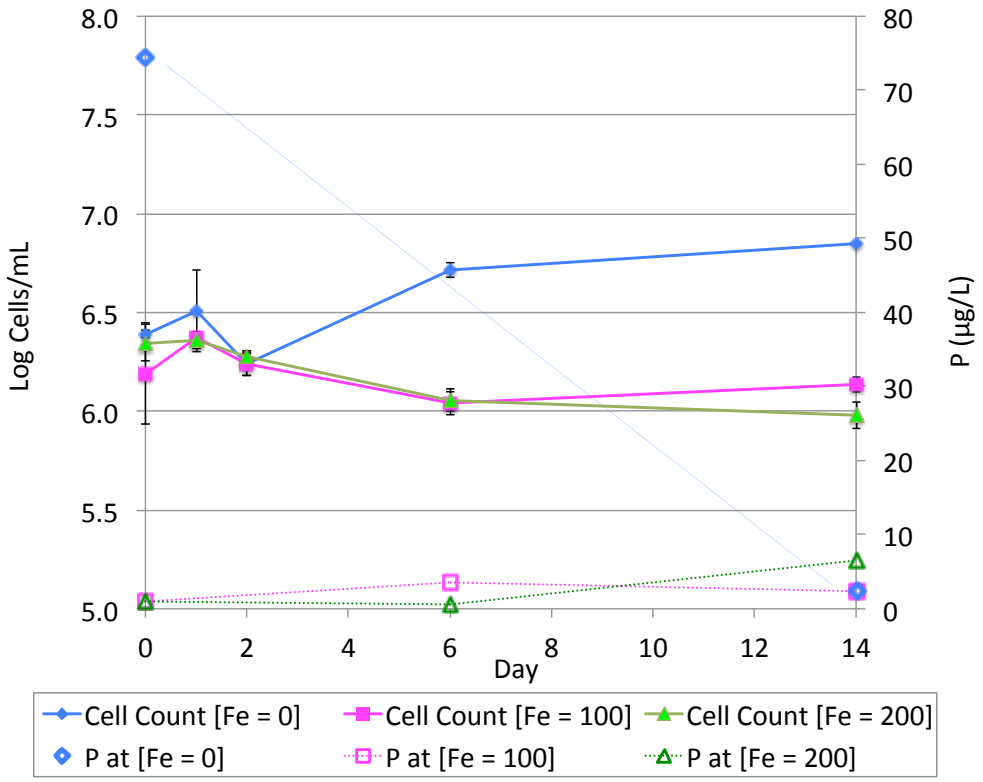


Figure 23: Relationship between *M. aeruginosa* growth and SRP levels in Reservoir water (in the absence of sediment) as a function of moderate doses of $FeCl_3$. Note treatment means ± 1 SD (n = 3) for cell growth are plotted on the he primary Y-axis; SRP levels are plotted on the secondary Y-axis

The average specific growth rate (Table 18) on Day 6 for the control was well within the range observed in the culture stock grown in unmodified medium (Figure 8) for the same time period. The average specific growth rate for the controls on Day 14 was 0.075/day; this rate was slightly lower than the culture stock growth rate for Day 14 (0.109/day); however, the controls still appeared healthy.

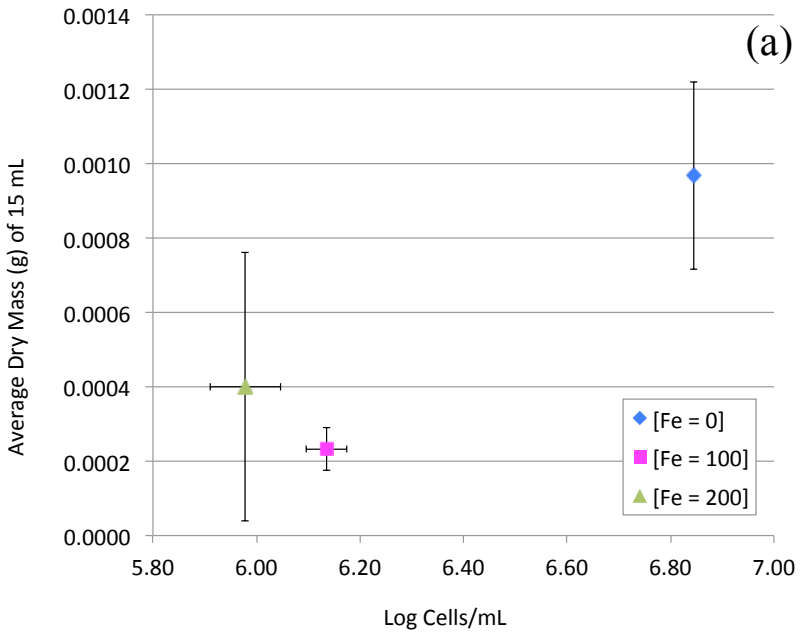
Table 18: SRP concentrations and average growth rates of *M. aeruginosa* in response to moderate doses of $FeCl_3$ in Reservoir water (in the absence of sediment)

$FeCl_3$ (mg/L)	P (µg/L)[1]			Average growth rate (µ)	Average growth rate (µ)
	Day 0[2]	Day 6	Day 14	Day 6	Day 14
0	74.40	Not sampled	2.37	0.125	0.075
100	0.99	3.58	2.34	-0.074	-0.017
200	1.07	0.51	6.51	-0.114	-0.061

[1] Background Reservoir water (SRP = 105.80 µg/L) mixed with sediment and acclimated for 2 months on the benchtop. Sediment was then removed. The resulting P was 74.40 µg/L

[2] Day 0 SRP values collected 18 hours after $FeCl_3$ amendments, and prior to inoculum (Reservoir water had 18 hours contact time with sediment)

Consistent with the experiments conducted with sediment, *M. aeruginosa* cell sizes were variable, but relatively small in the treatments. Dry weight biomass decreased as $FeCl_3$ dose increased (Figure 24b). The decrease in bionass was not linearly related to cell counts (Figure 24a), and it was observed that cell size was generally smaller in the treatments, although quite variable. While this decrease in mass in the $FeCl_3$ treatments might be attributable to reduced cell size, variability in cell size measurements and temporal variability in cell size precluded a conclusive assessment.



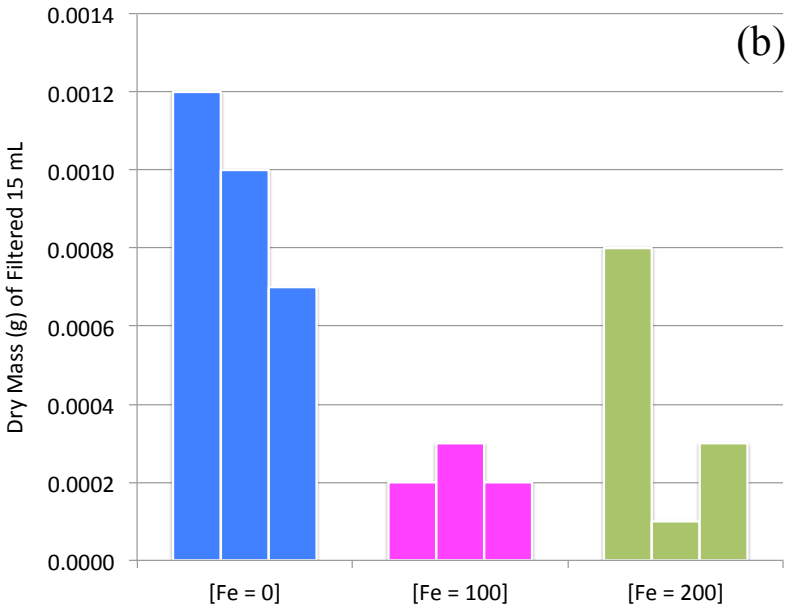


Figure 24: Dry mass comparisons of 15 mL of filtered *M. aeruginosa* at moderate doses of $FeCl_3$ in Reservoir water in the absence of sediment: a) treatment averages (n = 3) ± 1 SD; b) individual replicate dry masses

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Chapter 5 Conclusions

This research was conducted to understand the sediment-bound P loads in an engineered drinking water reservoir and the importance of SRP released from fine sediment in enabling *M. aeruginosa* growth; and to evaluate the utility of $FeCl_3$ in sequestering the SRP to limit it's availability in the water column and to limit the SRP such that *M. aeruginosa* does not proliferate. Key conclusions of this research are listed below.

5.1 Sorption experiments

- Sediment is partitioned in multi-cell engineered drinking water supply reservoirs. Sediment chlorite levels were highest in Cell 4 (i.e., the cell furthest from the reservoir inlet). These P-sorbing metal oxy-hydroxides are capable of releasing large amounts of P.
- Internal loading and subsequent release of P can be significant in engineered drinking water supply reservoirs.
- P release from engineered drinking water supply reservoir sediment could be minimized by $FeCl_3$ addition at doses typical of DWTP's (i.e. ~25 mg/L).
- P loading in the study Reservoir does not appear to substantially vary from year to year.
- $FeCl_3$ application is more effective for reducing SRP in absence of sediment, as would be expected. Thus, removing sediment from all Reservoir cells annually may maximize P sequestration by application of $FeCl_3$ within engineered drinking water supply reservoirs.
- The timing of $FeCl_3$ application is important (i.e., the dosing and mixing protocol matters during bench-scale evaluation). $FeCl_3$ efficiency declined when applied prior to agitation and equilibration of sediment within the water matrix.
- Chemical precipitation of phosphorus appeared to be the main mechanism of P sequestration in the engineered drinking water supply reservoir, as would be expected.

5.2 Controlling Growth of *M. aeruginosa*

- *M. aeruginosa* growth experiments in Reservoir water confirm that CB growth can be inhibited when P is sequestered; particularly when previously deposited sediment has been removed from the system. The starting inoculum concentration used for these experiments is near or at bloom concentrations and represents the ability for $FeCl_3$ treatments to control growth under challenging conditions. Further work should be completed to examine non-

bloom inoculum concentrations and optimization of the $FeCl_3$ concentrations required for sequestration of P.

- Nutrient limitation for *M. aeruginosa* cells during the moderate and higher doses of $FeCl_3$ in sediment tests demonstrated that cell size (biomass) was negatively impacted. Additional work should be carried out to quantify cell size under nutrient-limited conditions
- Sequestration of P does not necessarily inhibit *M. aeruginosa* growth immediately. More research is needed to investigate if internal P stores within cells are sufficient for blooms to occur. The biomass and cell size data suggest that this is unlikely.

Chapter 6 Implications and Recommendations

The results of these *M. aeruginosa* growth experiments coupled with the sorption studies have implications for optimizing the use of a coagulant in drinking water supply reservoirs to control CB growth. The following recommendations are based on findings in this current study:

- Consideration must be given to when and where the coagulant is applied to ensure that rapid mixing allows for binding and precipitation of P.
- $FeCl_3$ addition requires a feed system, storage and monitoring; and increased sedimentation will require more frequent sediment removal. It is likely that these benefits, in combination with the likely reductions in in plant coagulant dose requirements, would outweigh the costs associated with the potential risk of CB blooms and potential toxin release.
- The removal of sediment from a drinking water supply reservoir (e.g., annually) increases the probability that P sequestration with $FeCl_3$ (or other coagulants) will be effective at low coagulant doses.
- CB initially/primarily utilize dissolved P, but can revert to other sources of P. CB are less likely to bloom at such conditions. Nonetheless, when attempting to control CB growth in reservoirs, a reduction in TP levels should be targeted as well.
- Predicting CB density based on current P levels is problematic in that there is often a delay in growth in relation to P levels. In a reservoir, monitoring P levels in continually flowing water will not capture spikes in P that CB can rapidly consume prior to monitoring and/or analytical detection.
- Cell counts only partially describe CB growth behaviour. While cell counts remained consistent for $FeCl_3$ treatments, biomass decreased and is likely associated with observations of decreased cell size.
- The impact of biomass should be investigated to determine if there are mitigated or increased health risks when *M. aeruginosa* employ compensation strategies (i.e. does toxicity decrease or increase when cells are smaller because of nutrient limitation).

Several considerations and suggestions for future research include:

- The reservoir contains approx. 1920 m^3 of sediment in total, and a bulk water volume of 148000 m^3; this ratio is equivalent to 0.09 g dry sediment per 25 mL of water. (Appendix B). The experiments were conducted using a ratio of 0.25 g sediment per 25 mL of water, so the

sorption experiments and *M. aeruginosa* assays were conducted using higher sediment loads that would be found in the reservoir. Future testing should involve more realistic sediment-water ratios.

- More rapid and/or robust techniques for biomass quantification should be evaluated. The use of fluorescent probes is an option for determining biomass in addition to cell numbers.
- Dissolved P levels need to be reduced below the threshold at which CB experience P-limitation, and this threshold is site specific; this may be less than 30 µg TP/L, and some suggest less than 10 µg/L to prevent blooms (WHO, 1999). Accordingly, additional work should examine the need to drop P levels below this threshold.
- Tests with lower inoculum concentrations should be conducted to determine if there is any significant difference in effectiveness of $FeCl_3$ on CB growth.

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Appendix A

Water Quality Parameters During Testing

The following tables provide a summary of the various nutrient levels and water quality parameters on select dates during the *M. aeruginosa* test at high $FeCl_3$ doses.

N and P ratios are an important consideration when determining nutrient levels. Based on the results in Table A1, N is not considered a limiting factor.

Table A1: N and P concentrations and ratios during *M. aeruginosa* test at high $FeCl_3$ doses

Nutrient	$FeCl_3$ (mg/L)	Day 0	Day 10	Day 14	Day 18
PO_4 (µg/L)	0	100.00	6.54	5.03	15.63
	200	6.21	3.23	3.65	14.19
	300	2.44	3.67	5.98	10.82
	400	1.95	2.95	12.30	11.57
P (µmol/L)	0	1.05	0.07	0.05	0.16
	200	0.07	0.03	0.04	0.15
	300	0.03	0.04	0.06	0.11
	400	0.02	0.03	0.13	0.12
NO_3^{2-}-N (mg/L)	0	2.90	3.40	0.20	0.47
	200	3.40	2.90	1.80	0.10
	300	2.70	3.70	2.65	0.63
	400	3.00	3.00	5.60	0.90
N (µmol/L)	0	207.00	242.68	14.28	33.31
	200	242.68	207.00	128.48	7.14
	300	192.72	264.10	189.15	45.21
	400	214.13	214.13	399.71	64.24
N: P ratio	0	197	3524	270	202
	200	3711	6086	3343	48
	300	7501	6834	3004	397
	400	10429	6894	3086	527

Table A2: DO concentrations during *M. aeruginosa* test at high $FeCl_3$ doses

Day	$FeCl_3$ (mg/L)	Solution DO (mg/L)	Sediment interface DO (mg/L)	pH
0*	0	5.95	-	7.15
	200	6.49	-	6.92
	300	6.60	-	6.87
	400	7.43	-	6.55
4	0	8.01	8.01	-
	200	7.93	7.13	-
	300	7.77	8.22	-
	400	7.67	7.17	-
5	0	7.81	7.81	-
	200	7.51	7.32	-
	300	8.09	7.37	-
	400	7.58	7.55	-

*Day 0: DO and pH levels measured 18 hrs after $FeCl_3$ application but before inoculation

Table A3: Initial pH values of *M. aeruginosa* tests

Media type	$FeCl_3$ (mg/L)	pH*	Avg. pH
Reservoir water and sediment	0, 5, 25	7.36	7.36
Reservoir water and sediment	0, 50, 100	7.65	7.60
		7.53	
		7.62	
Reservoir water and sediment	0, 200, 300, 400	7.74	7.74
Reservoir water	0, 100, 200	7.20	7.10
		7.00	
		7.10	

*pH obtained after sediment and water were mixed but prior to $FeCl_3$ application and inoculum

Appendix B

Grain Size and Distribution Results and Reservoir Sediment Calculations

Table B1: Mastersizer particle size distribution results from 2014

Reservoir Cell	S = south. F = far	Percentage of grains below a given diameter					
		< 0.1 µm	< 0.2 µm	< 0.3 µm	< 0.5 µm	< 1 µm	< 2 µm
1	1 a S	0	0	0	0.16	1.48	3.08
	1 b S	0	0	0	0.17	1.49	3.14
	1 c S	0	0	0	0.19	1.73	3.75
	1 a F	0	0	0	0.21	2.04	4.66
	1 b F	0	0	0	0.19	1.73	3.75
	1 c F	0	0	0	0.22	1.96	4.30
	avg	0	0	0	0.19	1.74	3.78
2	2a	0	0	0	0.22	1.95	4.15
	2b	0	0	0	0.19	1.76	3.72
	2c	0	0	0	0.30	2.01	4.09
	avg	0	0	0	0.24	1.91	3.99
3	3a	0	0	0	0.17	1.53	3.13
	3b	0	0	0	0.2	1.79	3.75
	3c	0	0	0	0.21	1.88	3.96
	avg	0	0	0	0.19	1.73	3.61
4	4a	0	0	0	0.19	1.7	3.61
	4b	0	0	0	0.22	1.83	3.84
	4c	0	0	0	0.18	1.63	3.43
	avg	0	0	0	0.20	1.72	3.63

Reservoir Cell	S = south. F = far	Percentage of grains at a specific diameter (diameter at which X % of a sample is comprised)				Specific surface area
		10%	50%	80%	90%	m^2/g
1	1 a S	5.95	27.86	70.80	125.67	0.543
	1 b S	5.95	29.62	80.77	190.06	0.535
	1 c S	5.01	24.24	58.61	94.83	0.621
	1 a F	3.95	19.99	53.43	94.28	0.732
	1 b F	4.92	24.10	62.96	109.67	0.624
	1 c F	4.39	23.06	65.29	120.83	0.674
	avg	5.03	24.81	65.31	122.56	0.622
2	2a	4.50	19.95	48.70	84.21	0.694
	2b	4.93	21.26	53.33	98.19	0.646
	2c	4.76	22.21	54.93	96.05	0.670
	avg	4.73	21.14	52.32	92.82	0.670
3	3a	5.74	25.11	58.75	98.14	0.569
	3b	4.96	22.66	53.03	85.82	0.638
	3c	4.71	21.6	50.1	79.16	0.665
	avg	5.14	23.12	53.96	87.71	0.624
4	4a	5.11	25.48	63.23	112.76	0.604
	4b	4.97	25.5	63.14	110.67	0.621
	4c	5.41	26.24	64.19	110.82	0.583
	avg	5.16	25.74	63.52	111.42	0.603

Table B2: Reservoir sediment calculations

	beaker mass (g)	wet mass (g)	dry mass (g)	net wet wt (g)	net dry wt (g)	Wet: dry ratio
beaker A	30.76	43.09	33.79	12.33	3.03	4.07
beaker B	31.52	42.00	34.04	10.48	2.52	4.16
			avg (g)	11.41	2.78	4.11
			avg (kg)	0.0114	0.0028	

HVR volume sediment present		HVR volume water present (m^3)	
Cell	Vol (m^3)		
1	456		
2	504		
3	504		
4	456		
Total	1920	Total	148000

50mL beakers packed with 10 mL sediment and 0.5 mm standing water

- 10ml = 0.01 L = 0.00001 m^3

Density of experiment wet sediment

- density (avg net wet wt (kg) / 0.00001m^3 = 1140.5 kg/m^3
- 1140.5 kg/m^3 = g/L = mg/mL

Sediment – volume fraction in HVR to bulk water

- 148000 m^3/1920 m^3 = 0.013 m^3/ m^3
- 0.013 m^3 = L/L = mL/mL

For experimental equivalency to HVR (using 12 x 50 mL flasks), required amounts are:

- 600 mL HVR water (12 x 25mL)
- 7.78 mL wet sediment (600 mL x 0.013 mL/mL sediment-volume fraction)
- 8877.41 mg wet sediment (7.78 mL wet sediment x 1140.5 mg/mL density)
- 2160.00 mg dry sediment (8877.41 mg wet / 4.11 ratio wet: dry)
- 2.16 g dry sediment

Based on isotherm/sorption experiments, required amounts are:

- 25 mL HVR water
- 0.32 mL wet sediment
- 369.89 mg wet sediment
- 90.00 mg dry sediment
- 0.09 g dry sediment
    - 0.25 g of sediment were used in testing, therefore sorption tests were conducted under 2.7 x more sediment than natural systems.

Appendix C

Microcystis aeruginosa Inocula, Cell Counts, Masses and Precision

Equation C1 describes the calculations to determine the initial concentration of P in the *M. aeruginosa* screening tests, taking into account the various sources of P including the inoculum, background water, and corresponding volumes of each:

$$[P_{test}]V_{test} = [P_{innoc}]V_{innoc} + [P_{HVR}]V_{HVR} \quad \text{Equation C1}$$

Where:

P_{test} = concentration of P in the test unit immediately after inoculation
V_{test} = volume of test water (including inoculum and Reservoir water starting volume)
P_{innoc} = concentration of P in the inoculum culture
V_{innoc} = volume of inoculum added to the Reservoir water starting volume
$P_{Reservoir}$ = concentration of P in the raw Reservoir water
$V_{Reservoir}$ = volume of raw Reservoir water starting volume for the test unit

Table C1: Concentration of P introduced with inoculum shows the initial concentration of P in the *M. aeruginosa* Screening tests. Based on these results, it was assumed that approx. 0.05 µg/L of P was introduced in each test vessel for the remainder of this study.

Table C1: Concentration of P introduced with inoculum

P_{test} (µg/L)	$P_{innoc\ culture}$ (µg/L)	V_{innoc} (L)	$P_{Reservoir\ water}$ (µg/L)	$V_{Reservoir\ water}$ (L)	V_{test} (L)
25.38	50.00	0.001	24.40	0.025	0.026
50.00	50.00	0.001	50.00	0.025	0.026

Mass P_{innoc} (µg/L)	Mass $P_{Reservoir\ water}$ (µg/L)	% P introduced
0.05	0.61	3.88
0.05	1.25	0.00

Table C2: *M. aeruginosa* growth screening test cell count data

Test	Day 0	Day 2	Day 4	Day 8	Day 14	Day 21	Day 28	Day 35
			Average of three (3) haemocytometer counts (cells/mL)					
25 mL Reservoir water w/sediment	4.01E+06	3.73E+06	4.83E+06	8.15E+06	2.54E+07	3.86E+07	2.87E+07	4.67E+06
50 mL Reservoir water w/sediment	3.50E+06	3.65E+06	5.74E+06	7.45E+06	2.24E+07	4.15E+07	3.99E+07	3.07E+07
25 mL Reservoir water no sed	3.46E+06	5.33E+06	6.56E+06	7.73E+06	8.55E+06	5.12E+06	4.99E+06	4.73E+06
50 mL Reservoir water no sed	3.38E+06	4.50E+06	6.49E+06	9.35E+06	8.10E+06	6.49E+06	5.29E+06	4.80E+06
25 mL BG11(modified) w/sediment	3.28E+06	2.36E+06	4.63E+06	5.67E+06	2.38E+07	4.34E+07	6.85E+07	7.68E+07
50 mL BG11(modified) w/sediment	3.79E+06	4.25E+06	5.29E+06	7.60E+06	1.91E+07	4.64E+07	6.15E+07	6.78E+07
25 mL BG11(modified) no sed	3.59E+06	4.25E+06	6.93E+06	8.60E+06	1.46E+07	1.43E+07	1.65E+07	1.24E+07
50 mL BG11(modified) no sed	3.74E+06	4.43E+06	6.08E+06	9.47E+06	1.47E+07	1.47E+07	1.52E+07	1.33E+07
				Log cells (cells/mL)				
25 mL Reservoir water w/sediment	6.60	6.57	6.68	6.91	7.41	7.59	7.46	6.67
50 mL Reservoir water w/sediment	6.54	6.56	6.76	6.87	7.35	7.62	7.60	7.49
25 mL Reservoir water no sed	6.54	6.73	6.82	6.89	6.93	6.71	6.70	6.67
50 mL Reservoir water no sed	6.53	6.65	6.81	6.97	6.91	6.81	6.72	6.68
25 mL BG11(modified) w/sediment	6.52	6.37	6.67	6.75	7.38	7.64	7.84	7.89
50 mL BG11(modified) w/sediment	6.58	6.63	6.72	6.88	7.28	7.67	7.79	7.83
25 mL BG11(modified) no sed	6.56	6.63	6.84	6.93	7.17	7.15	7.22	7.09
50 mL BG11(modified) no sed	6.57	6.65	6.78	6.98	7.17	7.17	7.18	7.12
% difference reservoir water (sediment vs. non sediment)	1	2	2	1	7	12	12	11
% difference BG11(modified) (sediment vs. non sediment)	1	4	3	3	3	7	8	10

All analysis was conducted using the mean cell count of three (3) replicates in log cells/mL.

Equation C2 describes the % difference in growth between flasks of similar solution with and without sediment in the screening tests (note only 2 flasks per treatment)

$$\%\ difference = \left(\frac{\max - min}{max}\right) * 100 \qquad \text{Equation C2}$$

Where:

max = log cell count on a given day (use highest cell count)
min = log cell count on a given day (use lowest cell count)

Equation C3 describes the calculations to determine the yield at the end of a test:

$$yield = initial - final \qquad \text{Equation C3}$$

Where:

yield = change in population density at the end of a test,
initial = mean cell count on Day 0 after inoculation; and,
final = mean cell count at end of test.

The average specific growth rate was calculated using the following equation:

$$\mu = \frac{ln\,(X_2/X_1)}{t2-t1} \qquad \text{Equation 4}$$

Where:

X_1 is the initial raw cell count (cells/mL) and X_2 is cell count at test end; and
t is the time interval in days.

The coefficient of variation represents the variability within a data set, or within the triplicate cell counts, and was calculated using the following equation:

$$\%CV = \left(\frac{StDev}{Avg}\right) \times 100 \qquad \text{Equation 5}$$

Where:

CV is the % coefficient of variation (or relative standard deviation, RSD)
StDev is the standard variation around the mean; and,
Avg is the mean of triplicate log-cell counts.

Cell counts, expressed as logarithmic cell numbers/L, were used to determine % inhibition of control, and average growth rate. The % inhibition of control was calculated using the following equation:

$$I = \left(\frac{R_c - R}{R_C}\right) \times 100 \qquad \text{Equation 6}$$

Where:

I is the % inhibition of growth for each treatment; and
Rc and R are the mean cell count (log counts) of the control, and of each treatment, respectively.

Table C3: Cell count data and precision at higher doses of $FeCl_3$

$FeCl_3$ = 0 mg/L (Control)													
Rep	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 14	Day 18
Average of three (3) haemocytometer counts (cells/mL)													
1	1.54E+06	2.26E+06	2.63E+06	3.06E+06	4.47E+06	5.73E+06	7.67E+06	1.08E+07	1.48E+07	1.92E+07	2.31E+07	3.31E+07	3.51E+07
2	2.28E+06	2.92E+06	3.17E+06	3.63E+06	5.49E+06	7.99E+06	1.10E+07	1.37E+07	1.81E+07	1.78E+07	2.45E+07	3.15E+07	3.94E+07
3	2.51E+06	3.02E+06	2.77E+06	3.19E+06	4.27E+06	6.97E+06	8.21E+06	1.29E+07	1.42E+07	1.89E+07	2.32E+07	2.97E+07	3.86E+07
Log cells (cells/mL)													
1	6.19	6.35	6.42	6.49	6.65	6.76	6.88	7.03	7.17	7.28	7.36	7.52	7.55
2	6.36	6.47	6.50	6.56	6.74	6.90	7.04	7.14	7.26	7.25	7.39	7.50	7.60
3	6.40	6.48	6.44	6.50	6.63	6.84	6.91	7.11	7.15	7.28	7.36	7.47	7.59
Avg	6.31	6.43	6.45	6.52	6.67	6.83	6.95	7.09	7.19	7.27	7.37	7.50	7.58
St Dev	0.11	0.07	0.04	0.04	0.06	0.07	0.08	0.05	0.06	0.02	0.01	0.02	0.03
%CV	1.77	1.07	0.65	0.59	0.87	1.06	1.20	0.76	0.78	0.24	0.20	0.31	0.35
												Yield	-1.26
												Average specific growth rate (Day 7)	0.254
												Average specific growth rate (Day 14)	0.193

$FeCl_3$ = 200 mg/L													
Rep	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 14	Day 18
Average of three (3) haemocytometer counts (cells/mL)													
1	5.40E+05	2.35E+06	2.51E+06	3.32E+06	3.69E+06	4.60E+06	6.85E+06	8.98E+06	1.27E+07	1.69E+07	1.76E+07	2.95E+07	3.97E+07
2	1.20E+06	2.31E+06	2.50E+06	3.65E+06	4.67E+06	6.38E+06	9.64E+06	1.20E+07	1.39E+07	1.82E+07	2.14E+07	3.52E+07	4.31E+07
3	3.60E+05	2.57E+06	2.90E+06	3.39E+06	4.34E+06	5.74E+06	7.01E+06	9.86E+06	1.30E+07	1.60E+07	1.90E+07	2.49E+07	4.27E+07
Log cells (cells/mL)													
1	5.73	6.37	6.40	6.52	6.57	6.66	6.84	6.95	7.10	7.23	7.25	7.47	7.60
2	6.08	6.36	6.40	6.56	6.67	6.80	6.98	7.08	7.14	7.26	7.33	7.55	7.63
3	5.56	6.41	6.46	6.53	6.64	6.76	6.85	6.99	7.11	7.20	7.28	7.40	7.63
Avg	5.79	6.38	6.42	6.54	6.62	6.74	6.89	7.01	7.12	7.23	7.29	7.47	7.62
St Dev	0.27	0.02	0.04	0.02	0.05	0.07	0.08	0.06	0.02	0.03	0.04	0.08	0.02
%CV	4.60	0.38	0.56	0.33	0.79	1.07	1.20	0.90	0.29	0.39	0.59	1.00	0.26
%inhib	8.32	0.80	0.53	-0.33	0.74	1.35	0.84	1.18	1.02	0.53	1.19	0.35	-0.60
%control	91.68	99.20	99.47	100.33	99.26	98.65	99.16	98.82	98.98	99.47	98.81	99.65	100.60

Yield	-1.83
Average specific growth rate (Day 7)	0.384
Average specific growth rate (Day 14)	0.268

$FeCl_3$ = 300 mg/L													
Rep	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 14	Day 18
Average of three (3) haemocytometer counts (cells/mL)													
1	6.30E+05	1.61E+06	2.23E+06	3.18E+06	3.64E+06	4.90E+06	7.27E+06	1.19E+07	1.41E+07	1.67E+07	2.01E+07	2.95E+07	4.42E+07
2	2.11E+06	2.47E+06	2.37E+06	3.01E+06	3.78E+06	4.30E+06	5.56E+06	7.47E+06	9.43E+06	1.03E+07	1.37E+07	2.51E+07	3.75E+07
3	1.20E+06	2.42E+06	2.66E+06	3.23E+06	3.88E+06	4.65E+06	5.83E+06	9.86E+06	9.48E+06	1.36E+07	1.64E+07	2.16E+07	3.00E+07
Log cells (cells/mL)													
1	5.80	6.21	6.35	6.50	6.56	6.69	6.86	7.08	7.15	7.22	7.30	7.47	7.65
2	6.32	6.39	6.38	6.48	6.58	6.63	6.75	6.87	6.97	7.01	7.14	7.40	7.57
3	6.08	6.38	6.42	6.51	6.59	6.67	6.77	6.99	6.98	7.13	7.22	7.34	7.48
Avg	6.07	6.33	6.38	6.50	6.58	6.66	6.79	6.98	7.03	7.12	7.22	7.40	7.57
St Dev	0.26	0.11	0.04	0.02	0.01	0.03	0.06	0.10	0.10	0.11	0.08	0.07	0.08
%CV	4.33	1.67	0.60	0.25	0.21	0.42	0.92	1.46	1.44	1.49	1.16	0.91	1.12
%inhib	3.91	1.64	1.11	0.30	1.47	2.50	2.25	1.57	2.22	2.02	2.09	1.27	0.13
%control	96.09	98.36	98.89	99.70	98.53	97.50	97.75	98.43	97.78	97.98	97.91	98.73	99.87

Yield	-1.50
Average specific growth rate (Day 7)	0.286
Average specific growth rate (Day 14)	0.212

$FeCl_3$ = 400 mg/L													
Rep	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 14	Day 18
Average of three (3) haemocytometer counts (cells/mL)													
1	6.55E+05	1.94E+06	2.35E+06	2.86E+06	3.72E+06	3.75E+06	6.83E+06	1.03E+07	1.25E+07	1.51E+07	1.55E+07	2.30E+07	3.94E+07
2	3.20E+05	2.09E+06	2.47E+06	2.84E+06	4.39E+06	4.68E+06	6.39E+06	8.85E+06	9.81E+06	1.20E+07	1.75E+07	2.18E+07	3.12E+07
3	1.56E+06	2.54E+06	2.90E+06	3.27E+06	3.72E+06	4.95E+06	5.87E+06	8.24E+06	1.05E+07	1.22E+07	1.44E+07	2.00E+07	3.10E+07
Log cells (cells/mL)													
1	5.82	6.29	6.37	6.46	6.57	6.57	6.83	7.01	7.10	7.18	7.19	7.36	7.60
2	5.51	6.32	6.39	6.45	6.64	6.67	6.81	6.95	6.99	7.08	7.24	7.34	7.49
3	6.19	6.40	6.46	6.51	6.57	6.69	6.77	6.92	7.02	7.09	7.16	7.30	7.49
Avg	5.84	6.34	6.41	6.47	6.59	6.65	6.80	6.96	7.04	7.11	7.20	7.33	7.53
St Dev	0.34	0.06	0.05	0.03	0.04	0.06	0.03	0.05	0.06	0.06	0.04	0.03	0.06
%CV	5.90	0.95	0.74	0.54	0.64	0.96	0.49	0.70	0.79	0.78	0.60	0.41	0.79
%inhib	7.54	1.49	0.70	0.64	1.19	2.75	2.07	1.89	2.19	2.15	2.38	2.17	0.64
%control	92.46	98.51	99.30	99.36	98.81	97.25	97.93	98.11	97.81	97.85	97.62	97.83	99.36

Yield	-1.69
Average specific growth rate (Day 7)	0.340
Average specific growth rate (Day 14)	0.232

Table C4: Cell count data and precision at moderate doses of $FeCl_3$ in the absence of sediment

$FeCl_3$ = 0 mg/L (Control)					
Rep	Day 0	Day 1	Day 2	Day 6	Day 14
Average of three (3) haemocytometer counts (cells/mL)					
1	2.55E+06	5.61E+06	1.94E+06	5.05E+06	6.95E+06
2	2.49E+06	2.45E+06	1.50E+06	4.85E+06	6.91E+06
3	2.35E+06	2.45E+06	1.77E+06	5.73E+06	7.12E+06
Log cells (cells/mL)					
1	6.41	6.75	6.29	6.70	6.84
2	6.40	6.39	6.18	6.69	6.84
3	6.37	6.39	6.25	6.76	6.85
Avg	6.39	6.51	6.24	6.72	6.84
St Dev	0.02	0.21	0.06	0.04	0.01
%CV	0.29	3.19	0.89	0.56	0.10
				Yield	-0.45
			Average specific growth rate (Day 6)		0.125
			Average specific growth rate (Day 14)		0.075

$FeCl_3$ = 100 mg/L					
Rep	Day 0	Day 1	Day 2	Day 6	Day 14
Average of three (3) haemocytometer counts (cells/mL)					
1	2.69E+06	2.27E+06	1.76E+06	9.40E+05	1.26E+06
2	1.66E+06	2.51E+06	1.50E+06	1.19E+06	1.35E+06
3	8.30E+05	2.37E+06	1.99E+06	1.20E+06	1.51E+06
Log cells (cells/mL)					
1	6.43	6.36	6.24	5.97	6.10
2	6.22	6.40	6.18	6.07	6.13
3	5.92	6.37	6.30	6.08	6.18
Avg	6.19	6.38	6.24	6.04	6.14
St Dev	0.26	0.02	0.06	0.06	0.04
%CV	4.15	0.35	0.97	0.98	0.64
%inhib	3.15	2.03	-0.05	10.04	10.36
%control	96.85	97.97	100.05	89.96	89.64
				Yield	0.05
				Average specific growth rate (Day 6)	-0.074
				Average specific growth rate (Day 14)	-0.017

$FeCl_3$ = 200 mg/L					
Rep	Day 0	Day 1	Day 2	Day 6	Day 14
Average of three (3) haemocytometer counts (cells/mL)					
1	1.74E+06	2.43E+06	2.02E+06	1.09E+06	8.20E+05
2	2.47E+06	2.05E+06	1.87E+06	1.31E+06	9.40E+05
3	2.55E+06	2.41E+06	1.77E+06	1.03E+06	1.12E+06
Log cells (cells/mL)					
1	6.24	6.39	6.31	6.04	5.91
2	6.39	6.31	6.27	6.12	5.97
3	6.41	6.38	6.25	6.01	6.05
Avg	6.35	6.36	6.27	6.05	5.98
St Dev	0.09	0.04	0.03	0.06	0.07
%CV	1.45	0.66	0.46	0.92	1.14
%inhib	0.70	2.30	-0.60	9.84	12.65
%control	99.30	97.70	100.60	90.16	87.35
				Yield	0.37
				Average specific growth rate (Day 6)	-0.114
				Average specific growth rate (Day 14)	-0.061

Table C5: Dry mass data using higher doses of $FeCl_3$

Rep	Filter + boat (g)	Filter + boat + 15 mL algae dry wt (g)	algae dry wt (g)	Avg dry wt (g)	StDev
$FeCl_3$ = 0					
1	1.1096	1.1148	0.0052		
2	1.1057	1.1114	0.0057	0.0055	0.0003
3	1.1018	1.1073	0.0055		
FeCl3 = 200					
1	1.1082	1.1120	0.0038		
2	1.1009	1.1056	0.0047	0.0042	0.0005
3	1.1204	1.1244	0.0040		
FeCl3 = 300					
1	1.0969	1.1006	0.0037		
2	1.0988	1.1021	0.0033	0.0035	0.0003
3	1.1046	1.0985			
FeCl3 = 400					
1	1.1066	1.1092	0.0026		
2	1.0932	1.0968	0.0036	0.0033	0.0006
3	1.1024	1.1060	0.0036		

Table C6: Dry mass data at moderate doses of $FeCl_3$ in the absence of sediment

Rep	Filter + boat (g)	Filter + boat + 15 mL algae dry wt (g)	algae dry wt (g)	Avg dry wt (g)	StDev
		$FeCl_3$ = 0			
1	1.1008	1.1020	0.0012		
2	1.0978	1.0988	0.0010	0.0010	0.0003
3	1.0743	1.0750	0.0007		
		FeCl3 = 100			
1	1.1165	1.1167	0.0002		
2	1.0999	1.1002	0.0003	0.0002	0.0001
3	2.0804	2.0806	0.0002		
		FeCl3 = 200			
1	1.0865	1.0873	0.0008		
2	1.1072	1.1073	0.0001	0.0004	0.0004
3	1.0960	1.0963	0.0003		

Appendix D

SRP Results
in ultrapure water and reservoir water

Table D1: SRP values from sorption test using sediment and ultrapure water

Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
0	1	0.249	20.021	307.00	4.36	-24.68
0	2	0.252	19.982	308.00		-24.42
0	3	0.250	19.969	315.00		-25.16
25	1	0.249	20.000	122.00	2.31	-7.79
25	2	0.249	20.001	126.00		-8.11
25	3	0.249	19.989	126.00		-8.11
50	1	0.250	20.006	86.10	0.15	-2.89
50	2	0.250	20.029	85.90		-2.88
50	3	0.251	20.003	86.20		-2.88
100	1	0.251	20.009	63.50	1.80	2.91
100	2	0.253	20.010	65.40		2.74
100	3	0.249	20.032	61.80		3.07
200	1	0.249	19.995	64.70	2.46	10.86
200	2	0.249	20.004	63.90		10.93
200	3	0.250	19.996	60.10		11.19

Table D2: SRP values from sorption tests using low to moderate doses of $FeCl_3$ added to sediment and reservoir water prior to agitation

$FeCl_3$ = 0 mg/L						
Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
32.48	1	0.270	24.999	82.70	2.32	-4.65
32.48	2	0.265	24.842	78.20		-4.29
32.48	3	0.244	24.977	81.40		-5.01
58.64	1	0.250	24.997	78.00	1.51	-1.94
58.64	2	0.250	24.997	77.60		-1.90
58.64	3	0.243	24.995	75.20		-1.70
84.83	1	0.247	24.958	80.30	2.25	0.46
84.83	2	0.249	24.982	84.30		0.05
84.83	3	0.244	24.973	84.10		0.07
137.28	1	0.259	25.052	86.40	1.90	4.92
137.28	2	0.252	24.999	88.10		4.88
137.28	3	0.262	24.987	84.30		5.05
239.98	1	0.246	25.043	101.00	1.88	14.15
239.98	2	0.247	24.983	98.60		14.30
239.98	3	0.247	24.994	97.30		14.44

$FeCl_3$ = 5 mg/L						
Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
32.48	1	0.251	25.024	68.2	2.20	-3.56
32.48	2	0.249	25.023	72.6		-4.03
32.48	3	0.255	24.991	70.3		-3.71
58.64	1	0.249	25.010	76.1	1.27	-1.75
58.64	2	0.254	25.019	78.60		-1.97
58.64	3	0.253	24.982	77.00		-1.81
84.83	1	0.250	24.989	73.40	3.40	1.14
84.83	2	0.249	24.987	76.60		0.83
84.83	3	0.257	24.987	80.20		0.45
137.28	1	0.251	25.050	80.40	0.86	5.68
137.28	2	0.257	24.990	82.1		5.37
137.28	3	0.253	25.005	81		5.56
239.98	1	0.258	25.008	86.7	1.49	14.86
239.98	2	0.260	24.986	89.5		14.46
239.98	3	0.249	24.998	89		15.16

$FeCl_3$ = 10 mg/L						
Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
24.40	1	0.252	24.996	87.60	17.25	-6.27
24.40	2	0.251	24.996	54.50		-3.00
24.40	3	0.256	25.027	62.60		-3.73
49.40	1	0.254	25.017	80.00	19.27	-3.01
49.40	2	0.254	24.999	62.10		-1.25
49.40	3	0.251	24.998	41.50		0.79
74.40	1	0.253	25.005	65.40	11.91	0.89
74.40	2	0.248	25.013	87.20		-1.29
74.40	3	0.254	25.017	68.00		0.63
124.40	1	0.251	24.999	92.50	7.91	3.18
124.40	2	0.258	24.998	88.30		3.50
124.40	3	0.252	25.056	77.20		4.69
224.40	1	0.249	25.018	119.00	11.87	10.59
224.40	2	0.250	25.001	97.30		12.71
224.40	3	0.250	25.004	99.80		12.46

$FeCl_3$ = 15 mg/L						
Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
24.40	1	0.251	24.994	77.50	7.73	-5.29
24.40	2	0.251	25.008	62.60		-3.81
24.40	3	0.251	25.002	66.50		-4.19
49.40	1	0.252	25.024	75.00	12.00	-2.54
49.40	2	0.250	25.013	71.00		-2.16
49.40	3	0.250	24.999	52.50		-0.31
74.40	1	0.255	25.004	58.50	4.52	1.56
74.40	2	0.252	24.999	59.30		1.50
74.40	3	0.250	24.998	66.70		0.77
124.40	1	0.253	25.195	58.30	9.38	6.58
124.40	2	0.255	25.006	77.00		4.65
124.40	3	0.251	25.000	66.30		5.79
224.40	1	0.250	25.000	72.40	7.63	15.20
224.40	2	0.251	25.000	81.10		14.27
224.40	3	0.255	25.015	65.90		15.55

$FeCl_3$ = 20 mg/L						
Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
24.40	1	0.252	25.001	44.20	3.98	-1.96
24.40	2	0.250	25.005	49.80		-2.54
24.40	3	0.252	25.013	42.10		-1.76
49.40	1	0.252	25.004	62.30	14.46	-1.28
49.40	2	0.258	25.022	33.40		1.55
49.40	3	0.252	25.063	47.10		0.23
74.40	1	0.252	25.003	88.30	19.08	-1.38
74.40	2	0.251	25.025	52.70		2.16
74.40	3	0.255	25.012	82.40		-0.79
124.40	1	0.251	25.000	46.20	17.27	7.79
124.40	2	0.255	25.000	80.70		4.28
124.40	3	0.255	25.023	64.90		5.84
224.40	1	0.254	25.010	75.50	23.23	14.66
224.40	2	0.255	25.012	67.30		15.41
224.40	3	0.255	25.003	111.00		11.12

$FeCl_3$ = 25 mg/L						
Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
32.48	1	0.254	24.952	31.90	2.47	0.06
32.48	2	0.266	25.022	27.90		0.43
32.48	3	0.249	24.991	27.40		0.51
58.64	1	0.249	25.002	33.30	2.91	2.54
58.64	2	0.251	25.039	34.10		2.45
58.64	3	0.252	24.987	38.70		1.98
84.83	1	0.249	25.057	34.60	5.27	5.05
84.83	2	0.261	25.003	35.90		4.69
84.83	3	0.259	25.010	44.30		3.91
137.28	1	0.254	25.035	37.00	1.88	9.88
137.28	2	0.260	24.991	33.90		9.94
137.28	3	0.244	25.062	37.30		10.27
239.98	1	0.252	25.079	43.80	3.01	19.52
239.98	2	0.254	25.005	40.40		19.65
239.98	3	0.250	25.011	37.80		20.23

$FeCl_3$ = 50 mg/L						
Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
32.48	1	0.253	24.982	23.40	0.53	0.90
32.48	2	0.251	25.007	23.20		0.92
32.48	3	0.253	24.995	22.40		1.00
58.64	1	0.250	24.982	19.30	1.94	3.93
58.64	2	0.249	25.003	21.90		3.69
58.64	3	0.252	25.006	23.10		3.53
84.83	1	0.251	24.996	17.40	1.68	6.71
84.83	2	0.252	25.006	20.60		6.37
84.83	3	0.249	25.001	18.10		6.70
137.28	1	0.252	24.978	16.00	2.06	12.02
137.28	2	0.251	25.028	20.10		11.68
137.28	3	0.248	25.015	18.40		11.99
239.98	1	0.255	24.994	18.80	3.73	21.68
239.98	2	0.252	24.995	19.10		21.91
239.98	3	0.249	25.004	25.40		21.55

$FeCl_3$ = 100 mg/L						
Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
32.48	1	0.249	25.004	11.40	2.38	2.12
32.48	2	0.247	25.019	11.80		2.10
32.48	3	0.251	25.012	15.70		1.67
58.64	1	0.247	25.017	12.60	1.66	4.66
58.64	2	0.265	25.004	11.20		4.48
58.64	3	0.251	25.031	14.50		4.40
84.83	1	0.252	25.009	14.60	2.20	6.97
84.83	2	0.249	24.992	14.20		7.09
84.83	3	0.251	24.989	10.60		7.39
137.28	1	0.248	25.012	15.90	1.48	12.24
137.28	2	0.249	25.027	15.00		12.29
137.28	3	0.252	25.010	13.00		12.33
239.98	1	0.256	25.002	13.00	0.23	22.17
239.98	2	0.251	24.984	12.60		22.63
239.98	3	0.256	24.991	12.60		22.20

Table D3: SRP values from sorption tests using higher doses of $FeCl_3$ added to sediment and reservoir water prior to agitation

$FeCl_3$ = 0 mg/L						
Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
60.90	1	0.251	25.007	83.50	4.39	-1.71
60.90	2	0.248	24.998	74.80		-0.75
60.90	3	0.250	25.000	78.10		-2.12
86.03	1	0.249	25.007	68.30	10.72	-0.34
86.03	2	0.251	24.998	82.10		1.86
86.03	3	0.251	24.994	89.40		0.28
111.20	1	0.250	24.998	67.40	11.14	4.38
111.20	2	0.251	25.001	83.20		2.79
111.20	3	0.250	25.001	88.90		2.23
161.51	1	0.251	24.996	88.60	4.16	7.26
161.51	2	0.249	25.000	89.70		7.21
161.51	3	0.251	25.006	96.30		6.50
262.10	1	0.249	25.004	110.00	11.02	15.27
262.10	2	0.250	25.003	108.00		15.41
262.10	3	0.250	24.997	128.00		13.41

$FeCl_3$ = 200 mg/L						
Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
60.90	1	0.252	25.000	9.31	1.42	5.12
60.90	2	0.250	25.003	6.54		5.44
60.90	3	0.250	24.998	7.38		5.35
86.03	1	0.250	24.997	6.54	2.50	7.95
86.03	2	0.250	24.995	10.80		7.52
86.03	3	0.249	25.000	6.40		8.00
111.20	1	0.249	25.005	7.12	2.03	10.45
111.20	2	0.250	25.003	4.67		10.65
111.20	3	0.252	24.996	8.69		10.17
161.51	1	0.250	25.002	7.24	0.29	15.43
161.51	2	0.252	25.001	6.96		15.33
161.51	3	0.250	25.012	6.66		15.49
262.10	1	0.252	24.999	8.57	0.71	25.15
262.10	2	0.251	24.993	7.23		25.38
262.10	3	0.251	24.997	7.52		25.35

$FeCl_3$ = 300 mg/L						
Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
60.90	1	0.249	25.010	7.79	0.87	5.33
60.90	2	0.249	25.008	6.22		5.49
60.90	3	0.250	25.006	6.35		5.46
86.03	1	0.250	25.019	6.80	0.28	7.93
86.03	2	0.249	24.994	6.92		7.94
86.03	3	0.249	25.005	7.33		7.90
111.20	1	0.250	24.998	7.29	0.54	10.39
111.20	2	0.249	24.996	7.32		10.43
111.20	3	0.250	24.997	6.37		10.48
161.51	1	0.250	25.006	6.83	0.57	15.47
161.51	2	0.251	24.998	5.97		15.49
161.51	3	0.250	24.997	7.04		15.45
262.10	1	0.250	25.008	7.43	0.61	25.47
262.10	2	0.252	25.002	6.21		25.39
262.10	3	0.250	25.006	6.78		25.54

$FeCl_3$ = 400 mg/L						
Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
60.90	1	0.252	24.995	5.75	1.08	5.47
60.90	2	0.250	25.006	7.54		5.34
60.90	3	0.250	25.003	5.59		5.53
86.03	1	0.250	25.001	6.91	1.35	7.91
86.03	2	0.250	24.993	4.56		8.14
86.03	3	0.249	25.005	6.90		7.95
111.20	1	0.251	25.007	6.57	0.90	10.42
111.20	2	0.253	24.998	4.78		10.51
111.20	3	0.251	24.997	5.67		10.51
161.51	1	0.251	24.997	5.23	0.50	15.56
161.51	2	0.249	25.011	6.01		15.62
161.51	3	0.249	25.002	6.15		15.60
262.10	1	0.250	24.997	5.39	0.61	25.67
262.10	2	0.252	24.997	4.22		25.58
262.10	3	0.253	24.998	5.11		25.39

Table D4: SRP values from sorption tests using moderate doses of $FeCl_3$ added to sediment and reservoir water after agitation

Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
$FeCl_3$ = 25 mg/L						
32.48	1	0.253	24.973	17.30	2.42	1.50
32.48	2	0.262	24.978	17.30		1.45
32.48	3	0.266	25.011	21.50		1.03
84.83	1	0.252	25.003	19.00	1.91	6.53
84.83	2	0.262	22.907	21.70		5.52
$FeCl_3$ = 100 mg/L						
32.48	1	0.251	24.922	9.51	0.36	2.28
32.48	2	0.250	24.964	8.87		2.36
32.48	3	0.253	24.981	9.46		2.27
84.83	1	0.248	24.984	9.35	0.43	7.60
84.83	2	0.250	25.089	9.37		7.57
84.83	3	0.254	24.882	10.10		7.32

Table D5: SRP values from sorption tests using lower doses of $FeCl_3$ added to sediment and BG11 growth medium prior to agitation

$FeCl_3$ = 0 mg/L					
Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	µg P adsorbed / g sediment
5335.00	1	0.261	24.990		
5335.00	2	0.259	25.004		
5335.00	3	0.249	25.009		
5359.77	1	0.249	24.977		
5359.77	2	0.245	25.004		
5359.77	3	0.247	25.004		
5384.70	1	0.251	24.992		
5384.70	2	0.250	25.003	Out of range	n/a
5384.70	3	0.250	24.991		
5434.53	1	0.252	24.986		
5434.53	2	0.258	24.995		
5434.53	3	0.250	25.015		
5533.79	1	0.256	25.003		
5533.79	2	0.256	24.997		
5533.79	3	0.247	24.985		

$FeCl_3$ = 5 mg/L					
Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	µg P adsorbed / g sediment
5335.00	1	0.249	25.009		
5335.00	2	0.258	25.004		
5335.00	3	0.252	25.017		
5359.77	1	0.247	25.009		
5359.77	2	0.246	25.010		
5359.77	3	0.277	25.002		
5384.70	1	0.254	24.984		
5384.70	2	0.252	25.002	Out of range	n/a
5384.70	3	0.247	25.000		
5434.53	1	0.246	24.991		
5434.53	2	0.255	25.003		
5434.53	3	0.252	25.012		
5533.79	1	0.259	25.004		
5533.79	2	0.244	24.995		
5533.79	3	0.268	24.992		

$FeCl_3$ = 25 mg/L						
Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
5335.00	1	0.260	24.998			
5335.00	2	0.248	24.988			
5335.00	3	0.248	24.988	Out of range		n/a
5359.77	1	0.258	24.985			
5359.77	2	0.240	25.004			
5359.77	3	0.262	25.023	1110.00		405.89
5384.70	1	0.242	25.003	1080.00	78.10	444.75
5384.70	2	0.287	25.011	1070.00		376.01
5384.70	3	0.242	24.989	940.00		458.96
5434.53	1	0.240	24.991	904.00	57.85	471.76
5434.53	2	0.241	24.981	982.00		461.53
5434.53	3	0.249	24.998	869.00		458.35
5533.79	1	0.243	25.006	1250.00	158.15	440.83
5533.79	2	0.250	25.017	936.00		460.09
5533.79	3	0.269	24.008	1060.00		399.28

$FeCl_3$ = 50 mg/L						
Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
5335.00	1	0.253	24.988	470.00	4.00	480.50
5335.00	2	0.243	25.010	478.00		499.89
5335.00	3	0.243	24.996	474.00		500.02
5359.77	1	0.243	24.986	519.00	55.43	497.74
5359.77	2	0.249	24.987	432.00		494.50
5359.77	3	0.243	24.989	416.00		508.39
5384.70	1	0.246	24.985	523.00	55.37	493.78
5384.70	2	0.241	24.985	440.00		512.63
5384.70	3	0.245	24.997	418.00		506.75
5434.53	1	0.250	25.020	442.00	85.08	499.65
5434.53	2	0.249	25.006	454.00		500.17
5434.53	3	0.249	25.009	595.00		486.07
5533.79	1	0.244	24.993	683.00	118.08	496.87
5533.79	2	0.248	24.997	525.00		504.86
5533.79	3	0.250	24.988	452.00		507.94

$FeCl_3$ = 100 mg/L						
Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
5335.00	1	0.248	24.973	101.00	14.57	527.05
5335.00	2	0.251	24.998	129.00		518.48
5335.00	3	0.264	25.000	108.00		494.98
5359.77	1	0.246	24.994	110.00	4.51	533.39
5359.77	2	0.250	24.998	114.00		524.53
5359.77	3	0.251	24.999	105.00		523.36
5384.70	1	0.249	25.006	118.00	6.66	528.91
5384.70	2	0.244	24.995	117.00		539.62
5384.70	3	0.250	25.009	129.00		525.76
5434.53	1	0.261	25.000	128.00	67.86	508.29
5434.53	2	0.245	25.058	244.00		530.87
5434.53	3	0.248	24.981	125.00		534.83
5533.79	1	0.250	24.999	118.00	5.13	541.56
5533.79	2	0.248	24.984	115.00		545.90
5533.79	3	0.255	25.003	125.00		530.34

Table D6: SRP values from sorption tests using lower doses of $FeCl_3$ added to sediment and BG11 growth medium after agitation

Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
$FeCl_3$ = 5 mg/L						
5335.00	1	0.249	24.985			
5335.00	2	0.260	24.990			
5335.00	3	0.252	25.020	Out of range		n/a
5384.70	1	0.249	25.001			
5384.70	2	0.252	24.994			
5384.70	3	0.267	25.004			
$FeCl_3$ = 25 mg/L						
5335.00	1	0.246	24.982	981.00	128.42	442.16
5335.00	2	0.258	24.982	871.00		432.25
5335.00	3	0.273	25.006	725.00		422.26
5384.70	1	0.248	24.993	930.00	76.10	448.94
5384.70	2	0.260	25.005	871.00		434.10
5335.00	1	0.249	25.019	779.00		462.77
$FeCl_3$ = 50 mg/L						
5335.00	1	0.247	25.004	389.00	87.73	500.69
5335.00	2	0.266	25.012	273.00		475.98
5335.00	3	0.261	24.995	217.00		490.13
5384.70	1	0.254	24.999	226.00	13.32	507.73
5384.70	2	0.251	25.001	202.00		516.23
5384.70	3	0.251	24.987	204.00		515.74

Table D7: SRP values from sequestration (precipitation) tests using low to moderate doses of $FeCl_3$ added to reservoir water in the absence of sediment

Initial SRP (µg/L)	Rep	$FeCl_3$ = 0 mg/L			$FeCl_3$ = 5 mg/L		
		Mass standard (g)	Final SRP (µg/L)	Std Dev	Mass standard (g)	Final SRP (µg/L)	Std Dev
29.85	1	24.873	38.10	4.97	24.986	17.00	2.16
29.85	2	25.003	29.00		24.989	12.80	
29.85	3	25.007	30.10		24.993	15.80	
55.13	1	25.009	53.00	0.31	24.997	24.40	0.29
55.13	2	24.980	52.40		24.992	24.90	
55.13	3	25.008	52.80		25.008	24.90	
80.34	1	24.992	75.40	0.66	24.997	39.10	1.85
80.34	2	24.993	76.20		25.000	36.00	
80.34	3	24.990	76.70		24.991	35.80	
130.83	1	25.008	121.00	1.00	25.003	71.30	3.52
130.83	2	24.987	122.00		24.990	71.10	
130.83	3	24.992	123.00		25.009	65.10	
231.86	1	24.990	220.00	1.73	24.995	141.00	4.04
231.86	2	24.993	223.00		25.011	136.00	
231.86	3	25.000	223.00		24.995	133.00	

Initial SRP (µg/L)	Rep	$FeCl_3$ = 25 mg/L			$FeCl_3$ = 50 mg/L		
		Mass standard (g)	Final SRP (µg/L)	Std Dev	Mass standard (g)	Final SRP (µg/L)	Std Dev
29.85	1	25.014	12.50	0.47	24.980	12.70	0.35
29.85	2	24.980	11.60		25.002	12.40	
29.85	3	24.991	11.80		24.996	12.00	
55.13	1	24.988	13.70	0.38	24.995	11.70	0.47
55.13	2	24.982	13.10		24.986	11.50	
55.13	3	25.000	13.00		24.996	10.80	
80.34	1	25.006	18.80	2.57	25.003	11.90	0.44
80.34	2	24.981	14.40		25.003	11.10	
80.34	3	25.003	14.30		25.000	11.20	
130.83	1	24.985	16.50	0.31	25.006	12.50	0.26
130.83	2	24.988	16.30		25.002	12.00	
130.83	3	24.998	15.90		24.994	12.10	
231.86	1	25.009	22.30	0.62	25.005	14.10	0.46
231.86	2	24.996	21.10		25.002	13.20	
231.86	3	24.990	21.40		25.007	13.50	

$FeCl_3$ = 100 mg/L				
Initial SRP (µg/L)	Rep	Mass standard (g)	Final SRP (µg/L)	Std Dev
29.85	1	24.985	10.40	0.32
29.85	2	24.996	9.85	
29.85	3	24.998	10.40	
55.13	1	24.993	10.90	0.71
55.13	2	24.998	9.91	
55.13	3	25.004	9.52	
80.34	1	24.991	11.40	0.56
80.34	2	25.016	11.00	
80.34	3	24.992	10.30	
130.83	1	25.009	10.30	0.21
130.83	2	24.998	9.89	
130.83	3	24.998	10.00	
231.86	1	25.031	10.40	0.21
231.86	2	24.991	10.50	
231.86	3	24.991	10.10	

Table D8: SRP levels during *M. aeruginosa* growth tests at varying $FeCl_3$ doses

Test	Day 0	Day 6	Day 10	Day 14	Day 18	Day 21	Day 22	Day 35	Day 39
	SRP (µg/L)								
Reservoir water + sed	25.38							498.00, 428.00	
Reservoir water (no sed)	25.38							4.86, 2.67	
$BG11_{50}$ + sed	50.00[1]							24.00, 21.71	
$BG11_{50}$ (no sed)	50.00[1]							0.74, 0.35	
$FeCl_3$ [0]	100.00		6.54	5.03	15.63				
[200]	6.21		3.23	3.65	14.19				
[300]	2.44		3.67	5.98	10.82				
[400]	1.95		2.95	12.30	11.57				
$FeCl_3$ [0] (no sed)	74.40	n/a		2.37					
[100]	0.99	3.58		2.34					
[200]	1.07	0.51		6.51					

[1] Estimated value

Appendix E

Quality Control and Statistical Analysis

Table E1: Quality control and precision of lowest P-standard during SRP analysis

P-standard ID no.	22 Aug 15	29 Aug 15	30 Aug 15
	Measured Value (µg/L)		
25a	24.60	23.50	23.20
25b	24.70	23.60	24.20
25c	25.20	22.70	24.50
25d	24.90	22.60	24.40
25e	24.90	23.10	23.50
25f	23.70	22.90	24.30
25g	23.40	22.50	24.70
Average	24.49	22.99	24.11
StDev	0.67	0.43	0.55
% CV	2.74	1.89	2.29

Table E2: Quality control and precision of random replicate samples during SRP analysis

Date	Sample	Measured Value (µg/L)						% CV
14 Dec 2014	107j	30.9	32.48					3.53
	WA	32.8	31.9	33.2	32.5	33.6	30.9	2.99
1 Mar 2015	285j	29.6	30.0					0.95
	286j	30.1	30.9					1.85
26 June 2015	1m1	45.70	44.54	43.20				2.81
29 Aug 2015	420	100.00	98.2					1.28
	421	15.7	14.7					4.65
14 Oct 2015	507	59.9	61.9					2.32

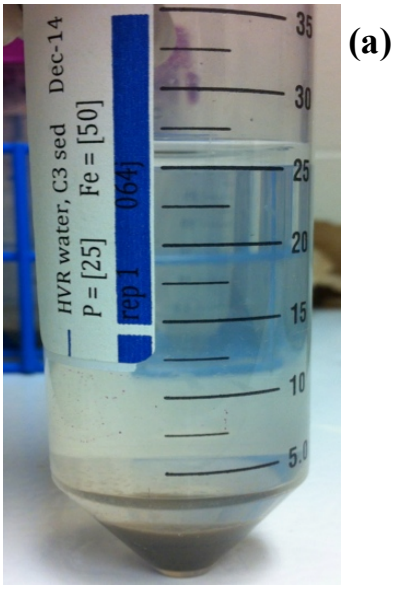
Table E3: T-tests - comparison of final SRP values for $FeCl_3$ amendments before vs. after 18 hours of agitations

Initial SRP (µg/L)	$FeCl_3$ (mg/L)	rep	Final SRP (µg/L)		Treatment comparisons
			$FeCl_3$ added prior to agitation	$FeCl_3$ added after 18 hrs agitation	
32.48	Fe = [25]	1	31.9	17.3	p = 0.001 (✓ significant difference)
32.48	Fe = [25]	2	27.9	17.3	
32.48	Fe = [25]	3	27.4	21.5	
84.83	Fe = [25]	1	34.6	19.0	
84.83	Fe = [25]	2	35.9	21.7	
84.83	Fe = [25]	3	44.3	-	
32.48	Fe = [100]	1	11.4	9.51	p = 0.004 (✓ significant difference)
32.48	Fe = [100]	2	11.8	8.87	
32.48	Fe = [100]	3	15.7	9.46	
84.83	Fe = [100]	1	14.6	9.35	
84.83	Fe = [100]	2	14.2	9.37	
84.83	Fe = [100]	3	10.6	10.1	
Comparison of all final SRP values (before vs. after)			p = 0.011 (✓ significant difference)		

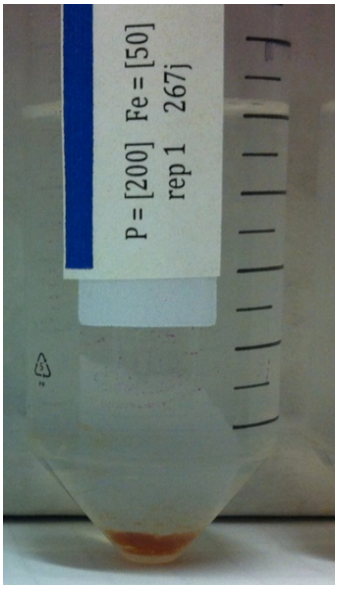
Appendix F

Photos of experiments

Figure F1: Shaker table apparatus and test units



(a)

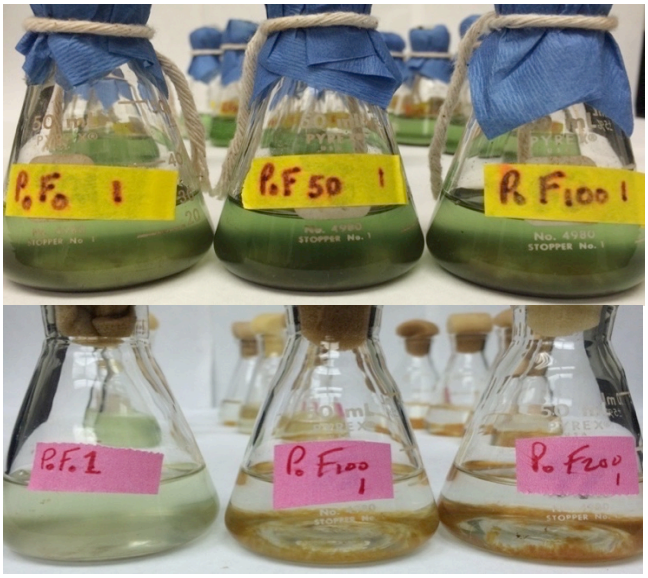


(b)

Reservoir Water + sediment
P = 25µg/L. $FeCl_3$ = 50 mg/L

Reservoir Water (no sediment)
P = 200 µg/L. $FeCl_3$ = 50 mg/L

Figure F2: Photos of floc and precipitate formation in sorption tests with (a) and without (b) sediment. Precipitation is more obscured in tests with (a) sediment



(a)

M. aeruginosa in Reservoir water + sediment

P_0 (background SRP control) = 105.80 µg/L

$FeCl_3$ = 0, 50, 100 mg/L

(b)

M. aeruginosa in Reservoir water (no sediment)

P_0 (control) = 105.80 µg/L

$FeCl_3$ = 0, 100, 200 mg/L

Figure F3: Photos of floc and precipitate formation in *M. aeruginosa* tests with (a) and without (b) sediment. Precipitation is more obscured in tests with (a) sediment

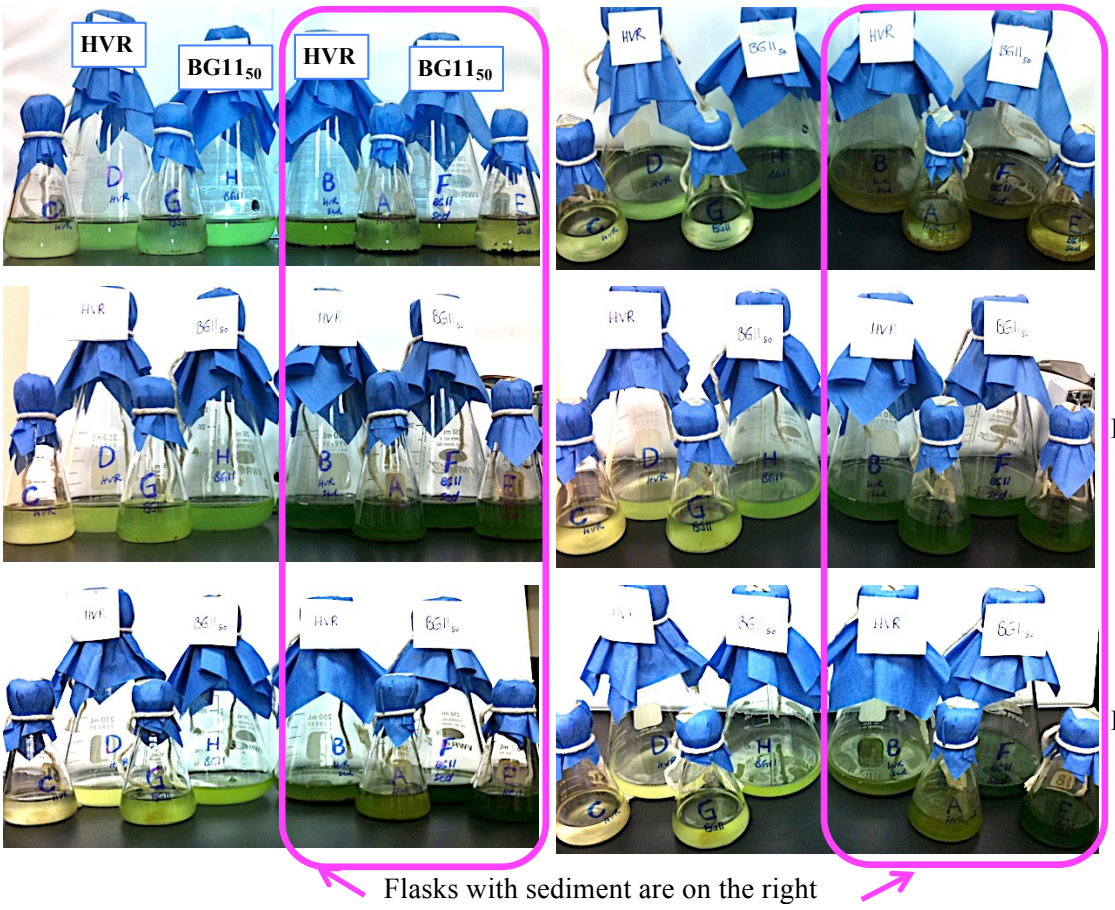
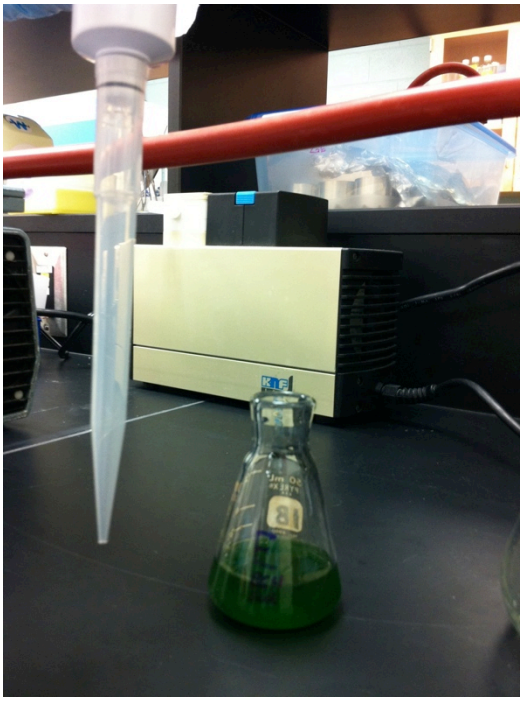
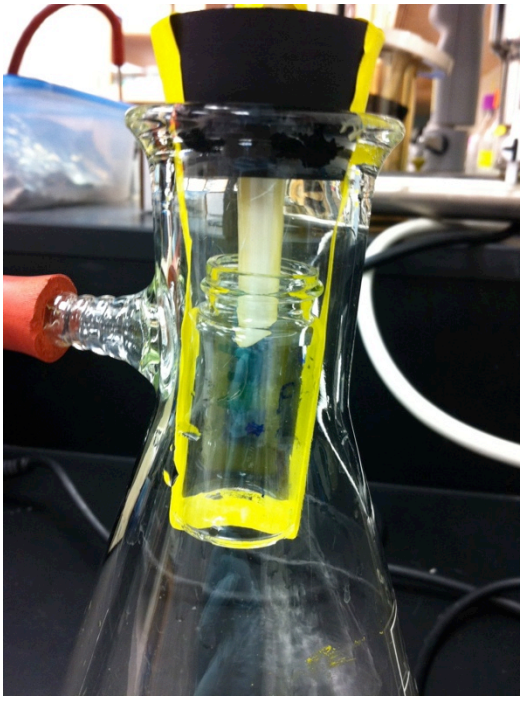


Figure F4: Photos of Screening Tests on different dates. Flasks with sediment are darker green indicating more CB growth

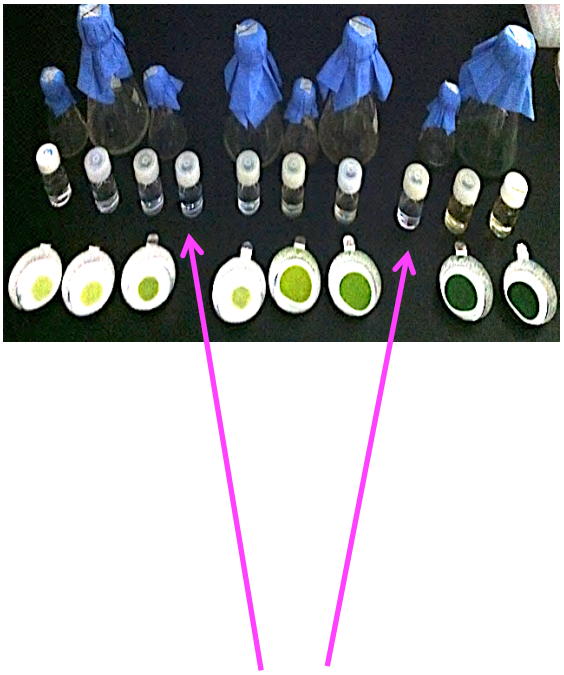


Collection of 15 mL near surface to avoid uptake of sediment

Pre filter (1.2 µm) to trap *M. aeruginosa* for dry mass



Effluent retained and re-filtered (0.45 µm) into clean vial for SRP analysis



Filtered wet samples and 2 filter blanks (method blanks)

Figure F5: Photos of *M. aeruginosa* test process and filter apparatus

Appendix G

SRP Results in BG11 growth medium

Sorption experiments were conducted using BG11 growth media, Reservoir sediment, and varying $FeCl_3$ doses and P- standards to determine if there were additional ionic interactions from the growth media that would affect the ability of $FeCl_3$ to sequester the P from solution (as preparation for the *M. aeruginosa* testing).

As shown in Figure G1, the X-axis reflects the initial background P-levels in the (unmodified) BG11 growth medium, in addition to the P-amendments. The initial P concentrations were extremely high in the growth medium (>5000 µg P/L to enable CB growth) and consequently the sediment only adsorbed P from the water column – no desorption occurred; and no horizontal equilibrium line was plotted.

$FeCl_3$ doses lower than 25 mg/L (i.e. 0, 5 mg/L) were unable to sequester enough P from the water column to reduce the final P to levels that could be measured on the autoanalyzer; these samples were labelled 'out of range'. At 25 mg/L $FeCl_3$, the first treatment with no added P (i.e., P standard = 0 µg P/L) was also out of range; and these triplicate data points were not plotted on Figure G1.

Despite a 6-fold dilution of the 'out of range' samples, the values remained out of range. The autoanalyzer is capable of reading up to 200 µ/L P and it was determined the final P in solution was over 1200 µg P/L (i.e., 6X dilution x 200 max detection is ≥ 1200 µg P/L).

A comparison of initial vs. final solution SRP is shown in Figure G2. $FeCl_3$ doses of 100 mg/L are capable of reducing initial P levels in excess of 5500 µg P/L to approx. 100 µg/L. Lower $FeCl_3$ doses of 25 mg/L are capable of reducing initial P levels in excess of 5500 µg/L to approx. 1000 µg/L. The exception was the first treatment with no added P-standard (adjusted to background P of 5335 µg P /L).

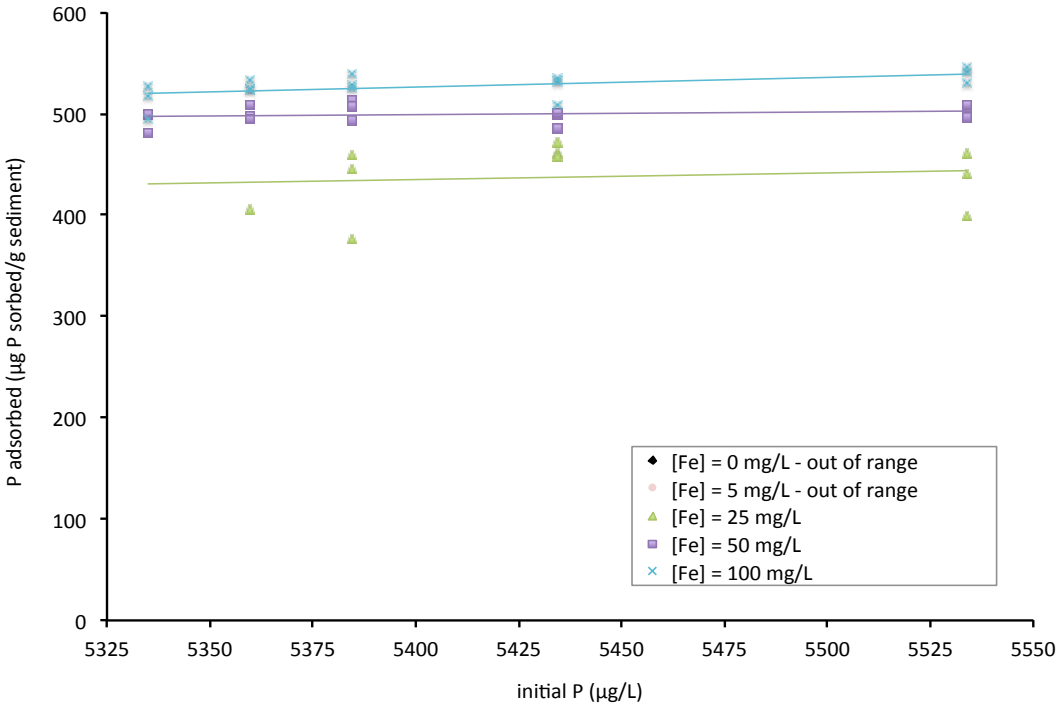


Figure G1: Impact of $FeCl_3$ on P sorption dynamics in BG11 growth medium and sediment from Cell 3. Note all replicates are plotted (N = 3) where feasible (i.e., within detection range)

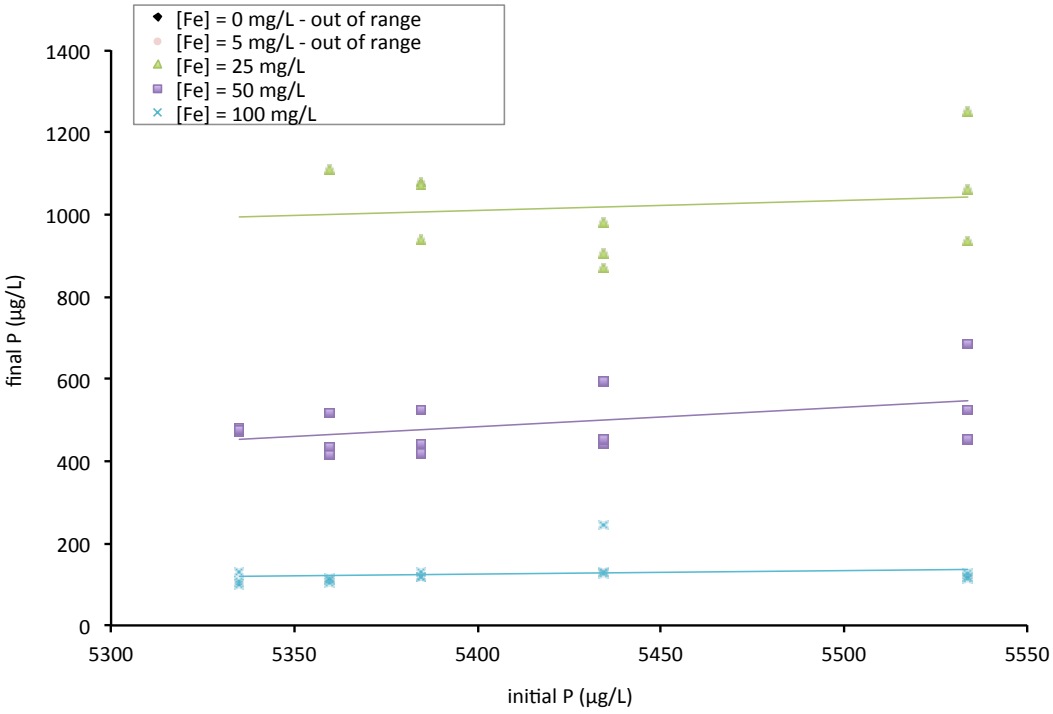


Figure G2: Impact of $FeCl_3$ on the initial and final solution levels of SRP in BG11 growth medium and sediment

Table G1: SRP values from sorption tests using lower doses of $FeCl_3$ added to sediment and BG11 growth medium prior to agitation. Note that SRP results for doses of $FeCl_3$ lower than 25 mg/L were out of range

$FeCl_3$ = 25 mg/L						
Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
5335.00	1	0.260	24.998			
5335.00	2	0.248	24.988			
5335.00	3	0.248	24.988			
5359.77	1	0.258	24.985			
5359.77	2	0.240	25.004			
5359.77	3	0.262	25.023	1110.00		405.89
5384.70	1	0.242	25.003	1080.00	78.10	444.75
5384.70	2	0.287	25.011	1070.00		376.01
5384.70	3	0.242	24.989	940.00		458.96
5434.53	1	0.240	24.991	904.00	57.85	471.76
5434.53	2	0.241	24.981	982.00		461.53
5434.53	3	0.249	24.998	869.00		458.35
5533.79	1	0.243	25.006	1250.00	158.15	440.83
5533.79	2	0.250	25.017	936.00		460.09
5533.79	3	0.269	24.008	1060.00		399.28

$FeCl_3$ = 50 mg/L						
Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
5335.00	1	0.253	24.988	470.00	4.00	480.50
5335.00	2	0.243	25.010	478.00		499.89
5335.00	3	0.243	24.996	474.00		500.02
5359.77	1	0.243	24.986	519.00	55.43	497.74
5359.77	2	0.249	24.987	432.00		494.50
5359.77	3	0.243	24.989	416.00		508.39
5384.70	1	0.246	24.985	523.00	55.37	493.78
5384.70	2	0.241	24.985	440.00		512.63
5384.70	3	0.245	24.997	418.00		506.75
5434.53	1	0.250	25.020	442.00	85.08	499.65
5434.53	2	0.249	25.006	454.00		500.17
5434.53	3	0.249	25.009	595.00		486.07
5533.79	1	0.244	24.993	683.00	118.08	496.87
5533.79	2	0.248	24.997	525.00		504.86
5533.79	3	0.250	24.988	452.00		507.94

$FeCl_3$ = 100 mg/L						
Initial SRP (µg/L)	Rep	Mass sediment (g)	Mass standard (g)	Final SRP (µg/L)	Std Dev	µg P adsorbed / g sediment
5335.00	1	0.248	24.973	101.00	14.57	527.05
5335.00	2	0.251	24.998	129.00		518.48
5335.00	3	0.264	25.000	108.00		494.98
5359.77	1	0.246	24.994	110.00	4.51	533.39
5359.77	2	0.250	24.998	114.00		524.53
5359.77	3	0.251	24.999	105.00		523.36
5384.70	1	0.249	25.006	118.00	6.66	528.91
5384.70	2	0.244	24.995	117.00		539.62
5384.70	3	0.250	25.009	129.00		525.76
5434.53	1	0.261	25.000	128.00	67.86	508.29
5434.53	2	0.245	25.058	244.00		530.87
5434.53	3	0.248	24.981	125.00		534.83
5533.79	1	0.250	24.999	118.00	5.13	541.56
5533.79	2	0.248	24.984	115.00		545.90
5533.79	3	0.255	25.003	125.00		530.34

Glossary

Benthic	dwelling on or near the bottom or on a substrate
Control	test vessel not amended with P or $FeCl_3$
Eutrophic	polluted; nutrient enriched; turbid due to presence of algae; highly primary producer productivity
Hydrolysis	proton transfer, hydrogen atom; or breaking a bond
Inoculum	CB cells collected from a liquid stock culture and transferred to a new medium, resulting in a lower cell concentration
Phytoplankton	small, suspended, photosynthetic plants and cyanobacteria
Redox	electron transfer, loss or gain