

Fine Sediment Contributions to Cyanobacterial Growth: Potential Threats to Drinking Water Reservoirs

by

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A thesis

presented to the University of Waterloo

in fulfillment of the

thesis requirements for the degree of

Master of Applied Science

in

Civil Engineering (Water)

Waterloo, Ontario, Canada, 2018

Authors 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.

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Abstract

In the drinking water industry, the transport and release of nutrients such as phosphorus (P) to the water column is largely overlooked in reservoir management. Phosphorus is considered the key limiting nutrient for algal and cyanobacterial growth, although micronutrients and other macronutrients like nitrogen (N) are also important in cyanobacterial growth and toxin production. Thus, an understanding of P and N dynamics in freshwater systems is essential for effective, holistic reservoir management to ensure both source water availability and quality. The importance of understanding P and N form and mobility is further underscored by their association with natural and anthropogenic landscape disturbances. These disturbances can lead to increases in erosion, sediment transport, and nutrient bioavailability.

Consequently, this thesis examined the bioavailability of P from fine sediments, and their role in cyanobacterial proliferation in two phases. Phase 1 evaluated geochemical composition, particulate P fractionation, and phosphorus mobility from fine sediments collected from two watersheds: the Elbow River watershed and the Crowsnest watershed. In the Elbow River Watershed, the Elbow River flows into the Glenmore Reservoir. Drum Creek was impacted by the 2003 Lost Creek wildfire and is located in the Crowsnest watershed. Sediment characterization revealed that bioavailable P is highest in Drum Creek, Glenmore Reservoir, and Elbow River, respectively. Batch experiments indicated that fine sediment in the Glenmore Reservoir is a source of bioavailable P to the water column. Phase 2 investigated the role of sediment-associated nutrients to cyanobacterial proliferation. A method for microcosm experiments using amended natural waters and sediments was developed and implemented. Results indicated that potential toxin-forming *M. aeruginosa* proliferation can be enhanced by fine sediment, compared to samples with no sediment. Unexpectedly, microcosms with Glenmore Reservoir sediment had significantly higher cell densities than those treated with Drum Creek sediment, and N concentrations did not have any significant effects.

The laboratory benchtop studies conducted herein demonstrate proof-of-concept that sediment-associated nutrients can lead to increases of cyanobacterial proliferation. This type of experiment and its results can be an insightful tool to bridge gaps between understanding *M. aeruginosa* proliferation from a laboratory to natural settings.

Acknowledgements

First and foremost, I would like to thank my supervisors Dr. Monica Emelko and Dr. Kirsten Müller. Both of their unwavering support, constructive feedback, and encouraging nature allowed me to grow personally and academically throughout my masters' degree. I feel lucky to have had such inspiring and influential women in STEM to mentor me.

The City of Calgary has been amazing and supportive as a partner in this work. I would like to thank Eric Camm and Ben Littlechild for their assistance on sample collection, as well as Dr. Norma Ruecker and Abe Mathais for their assistance with copyright permissions.

Thank you to Dr. Micheal Stone for his assistance in this research, advice, and support. Your insight during our discussions helped me to further understand the complexities associated with sediment geochemistry.

Further, I do not think I would have been able to complete my degree without the support of numerous mentors. I am extremely grateful for the guidance that I have received from Dr. Maria Mesquita both in and out of the lab. Maria's wealth of knowledge on cyanobacteria is incredible, and her guidance always appreciated. Dr. William Anderson (my "pseudo" supervisor), Dr. Philip Schmidt, and Alex Chik—thank you for always being an open door or phone call away. Your support and advice were always helpful, and I am extremely thankful for that.

Throughout my masters' degree, I received various help from many laboratories. I would like to thank Emily He & Kaitlyn O'Sullivan for their assistance in enumerating cells, Marianne Vandergreindt from the Ecohydrology Group, Mark Sobon & Mark Merlau from the Civil and Environmental Engineering department, Katie Thomas from Dr. Roland Hall's lab, and Heather Roshon from Canadian Phycological Culture Centre.

My friends and family have kept me grounded throughout these years. I would like to thank Thadshayini Chandrakumaran, Caitlin Watt, Frank Bevacqua, Joan Thompson and Melani-Ivy Samson, who have all been extremely supportive through the times I struggled. Completing my thesis without their help would not have been possible. Lastly, I would like to thank June, Kevin, and my mom and dad. They were always there for me no matter the hour, to deliver comfort, advice, and sometimes food. I am so thankful for having such loving and supportive family.

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Chapter 1: Introduction

1.1 Background

Algae, in particular cyanobacteria, can pose several significant threats to the provision of safe drinking water that meets both regulatory and aesthetic criteria for potable water (1,2). The biomass associated with cyanobacterial blooms can increase water turbidity, challenge coagulation/flocculation/clarification processes, clog filtration and membrane processes, and increase the chemical oxidant demand required for disinfection, thereby increasing the potential for disinfection by-product (DBP) formation (3–5). Both toxins (such as microcystin) and taste and odour compounds can be released by cyanobacterial cells prior to and during drinking water treatment (6,7). Their presence can challenge conventional water treatment processes (6,8–10) and lead to customer complaints.

The bioavailability of key nutrients is critical to cyanobacterial bloom occurrence (11,12). Phosphorus (P) is the key limiting nutrient for primary productivity and proliferation of algae in freshwater systems (13–15). Total P (TP) concentrations of $\sim 30 \mu\text{g P/L}$ are considered the threshold for eutrophication of freshwater bodies (16). Although this threshold is not predictive, cyanobacterial bloom occurrence is considered more likely when reservoir or lake water concentrations of TP are near to or exceed this level (17,18). When TP concentrations are below this threshold, nutrients are still in demand to propagate primary productivity. However, there is a balance between algae and cyanobacteria in which no particular taxa dominates to the extent that a bloom occurs. Although the drivers and dynamics of cyanobacterial bloom formation are not well understood (19,20), reducing or managing nutrient availability in drinking water reservoirs reduces the probability of their occurrence (21–23).

It is widely recognized that fine sediment is the primary vector for P transport to and within rivers (24–26). Sediment and associated nutrients (including P) can be transported downstream over long distances and subsequently deposited in reservoirs or lakes (27,28). However, in the drinking water industry, the fine sediment-associated contributions to nutrient release to the water column are largely overlooked. While P has commonly been considered the key limiting nutrient for primary productivity of algae in freshwater systems (29–32), nitrogen (N) also has been suggested as

critical to cyanobacterial bloom toxicity (33,34). Thus, an understanding of P and N dynamics is essential for reservoir management to ensure that source water quality is maintained.

The majority of available studies focused on understanding the relationship between nutrient availability and cyanobacterial proliferation have focused on lakes and reservoirs with a known history of cyanobacterial blooms (19,35–38). For example, hypereutrophic Lake Taihu in China has had recurring annual cyanobacterial blooms from spring to fall. Studies in this lake have included mesocosm studies, evaluations of P desorption from sediments, and characterization of individual cyanobacterial cells and their colony formation (35,39,40). Similarly, Lake Erie, which is located on the Canada-US border, has had recurring cyanobacterial blooms since the mid-1990s and has also been extensively studied (36,37,41). Investigations on lower nutrient (mesotrophic and oligotrophic) water bodies are uncommon. Unfortunately, these high quality source waters may be at risk due to numerous disturbance pressures (42–45). Notably, both anthropogenic disturbances (e.g., development, resource extraction) and natural disturbances (e.g., floods, hurricanes, and wildfires; which are all exacerbated by climate change) can lead to increases in erosion, sediment mobility and transport, and associated nutrient bioavailability (28,46–48). As rivers flow into downstream source water reservoirs and lakes, the deposition of fine sediment may represent a source of bioavailable P to the water column. Thus, the potential contributions of fine sediment to cyanobacterial proliferation must be considered and reflected in reservoir management, source water protection, and climate change adaptation strategies, as well as drinking water safety plan development processes.

1.2 Research Objectives

The goal of this research was to investigate the effects of nutrient availability—specifically, fine sediment-associated P and excess concentrations of N—on the proliferation of potentially toxin-forming cyanobacteria. The specific objectives of this investigation were to:

- (1) evaluate the geochemical composition, particulate P form, and P mobility of fine sediment to demonstrate its relevance to an internal bioavailable source of P in a mesotrophic-oligotrophic watershed (Elbow River to Glenmore Reservoir),
- (2) develop a laboratory-based approach for evaluating the proliferation of cyanobacteria in natural waters,

- (3) explore the role of fine sediment and associated nutrients in the proliferation of potentially toxin-forming cyanobacteria in drinking water reservoirs,
- (4) investigate the contributions of elevated N concentrations in the proliferation of potentially toxin-forming cyanobacteria in drinking water reservoirs, and
- (5) compare land disturbance (i.e. some urbanization and wildfire) on fine sediment contributions to the proliferation of potentially toxin-forming cyanobacteria.

1.3 Research Approach

To address the objectives described in Section 1.2, the overall research was conducted in two phases. Phase 1 consisted of fine sediment characterization and P adsorption/desorption (i.e. sorption) experiments to address Objectives #1 and #3. Phase 2 consisted of developing a protocol for conducting microcosm studies and conducting associated investigations of cyanobacterial proliferation in natural waters using potentially toxin-forming *M. aeruginosa* cultures to address Objectives #2, #4, and #5.

1.3.1 Phase 1

In Phase 1, key fine sediment characteristics such as the grain size distribution, geochemical composition, and particulate P speciation were evaluated. Phosphorus adsorption/desorption was evaluated to quantify the mobility of soluble reactive P from sediment to the water column. These analyses were conducted using sediments collected from mesotrophic-oligotrophic systems (Elbow River and Glenmore Reservoir). Additional sediment samples from a neighbouring watershed impacted by wildfire that had occurred approximately 8 years prior to sediment collection also were investigated (Drum Creek).

1.3.2 Phase 2

To assess the contributions of sediment-associated nutrient availability and elevated N on cyanobacterial proliferation, batch growth experiments (i.e., microcosm investigations) were conducted using reservoir water and fine sediment. Specifically, a factorial design microcosm investigation of the effects of sediment type (collected from a wildfire impacted river [Drum Creek]

and a mesotrophic-oligotrophic drinking water reservoir [Glenmore Reservoir]) and N concentrations was conducted.

1.4 Thesis Organization

Chapter 2 consists of a literature review on environmental P and sediments, drivers for cyanobacterial blooms, and implications of cyanobacterial blooms to drinking water supply and treatment. Chapter 3 details the research approach taken and includes the experimental procedures, materials and methods used, and the means for data analysis. Chapter 4 contains experimental results for the two phases of research and discussion. Chapter 5 contains the conclusions drawn from this investigation. Chapter 6 contains the implications of this research for the drinking water industry.

Chapter 2: Literature Review

A brief review of scientific literature related to sediment-associated P and its potential relationship to the proliferation of cyanobacteria in drinking water reservoirs and lakes follows. It focuses on: 1) P forms, transport, and bioavailability in the natural environment; 2) implications of cyanobacterial proliferation to reservoir management and the drinking water industry; 3) nutrient drivers of cyanobacterial bloom formation and toxicity; and 4) an overview of the current state of the science with respect to the relationship between fine sediment and proliferation of cyanobacteria. Research gaps in the literature are also identified and the relevance of the proposed research for the drinking water industry is highlighted.

2.1 Phosphorus and Sediment

2.1.1 Phosphorus Forms in Aquatic Systems

In aquatic systems, P occurs in both dissolved and particulate forms. The fraction that passes through a 0.45 μm nominal porosity filter is operationally defined as dissolved P (or soluble P), whereas the retained and larger fractions are considered particulate P (49). In aquatic environments, P forms can be further classified as organic or inorganic. Organic P is associated with organic matter (i.e., animal tissue, decaying matter), whereas inorganic P is typically of geologic origin. Both organic and inorganic P forms are either dissolved or particulate (50).

Forms of P can be further classified according to the type of analytical technique used to quantify them. These definitions include: reactive, acid hydrolysable, and organic fractions (49). Reactive P includes phosphates that respond to colorimetric tests without preliminary hydrolysis or oxidative digestion (49). This research will be primarily focused on soluble reactive P (SRP) - the portion of reactive P which is less than 45 μm . Soluble reactive P is largely a measure of orthophosphate which is considered bioavailable. Condensed (inorganic) phosphates are P compounds that contain salts and/or metals (e.g., sodium, potassium, calcium) and also are typically present as precipitates, although a small fraction is often hydrolysable, though this process is slow, making it a relatively small pool of bioavailable P (49,51). Orthophosphate is readily available to aquatic biota and is considered the primary limiting nutrient for algal and cyanobacterial growth in freshwater systems (14,52).

While most watershed assessments may involve evaluation of total P to some extent, particulate P is rarely characterized in the water supply and treatment industry, even though some particulate P forms are readily bioavailable and represent risk factors for cyanobacterial activity. Particulate P is fractionated using sequential extraction techniques to quantify non-apatite inorganic P (NAIP), apatite P (AP), and organic P (OP) (53,54). Phosphates adsorbed to non-calcium, metal hydroxide surfaces are known as NAIP and comprise the most bioavailable form of particulate P (55) — therefore, it most enables cyanobacterial and algal proliferation (56). Critically, its bioavailability largely depends on other system attributes related to the sediment and surrounding water matrix. These parameters include: ionic strength (including presence of competitor ions), temperature, redox conditions, and pH (57). Fractions of NAIP are extracted by NH_4Cl -RP (1.0M), BD-RP (0.11M, 40°C), and NaOH-RP (1.0M). Apatite P is extracted by HCL-RP and is considered to be geochemically stable; thus, it is less bioavailable. Lastly, OP is the fraction sometimes referred to as Refractory-P; it is extracted by NaOH (1.0M, 85°C). This fraction is potentially bioavailable as it can be mineralized or released with hydrolysis (58). Thus, the NAIP and OP fractions of particulate P are the most relevant to potential proliferation of cyanobacteria and eutrophication more broadly.

The geochemical fractions present in sediment can influence the solid phase concentrations of NAIP. In particular, dissolved P can bind strongly to sediment with geochemical fractions containing: Manganese (Mn), Aluminum (Al), and Iron (Fe) (30,59,60). When dissolved P binds with these fractions, they can create Fe and Al oxides and oxyhydroxides, while Mn can also form hydroxides (27,30,53,54,61). As previously discussed, the NAIP fraction is found from the sum of extracts NH_4Cl -RP (1.0M), BD-RP (0.11M, 40°C), and NaOH-RP (1.0M). In particular, extract NaOH-extractable P includes P bound to Al and metals (including Mn and Fe) in humic acids (59). Therefore, fractions of Mn, Al, and Fe may be critical to understanding how much bioavailable NAIP is present in sediment.

2.1.2 Phosphorus Mobility

External loading from rivers to receiving waters (i.e., lakes, reservoirs) has long been recognized as the primary source of bioavailable P, which is a primary driver of eutrophication (62–64). While internal loading of P in lakes from sediment to the water column has been widely examined, the

analogous expectation of internal loading of P from sediment to the water column in drinking water reservoirs has not been extensively investigated (30,65). Internal loading generally describes the release of P from bottom sediments to the water column (61). More specifically, it refers to all of the physical, chemical, and biological processes by which P is mobilized and translocated from the benthic layer to the water column (66). Partial or delayed recovery of water bodies in response to reductions in external loading of nutrients can be attributed to internal P loading (48,66–68) — these are often described as “legacy effects” (66).

The mechanisms by which contaminants and nutrients adsorb and desorb onto sediments are complex. Several factors contribute to internal loading processes, such as: oxygen concentration, iron speciation, pH, water hardness, phosphate concentration, and competitor ions in solution (24,26,66,69). Within the sediment, factors such as the depth of sediment, composition of the benthic biota community, grain size distribution, and geochemical composition can also affect internal P loading (31,70). Sediment geochemistry and physical characteristics such as grain size also can affect pore water sorption kinetics and overall P fluxes (25,71,72).

Fine sediments (<63 μm in size) are the primary vector for P delivery to and transport within aquatic systems (24,25,46). Thus, landscape disturbances that result in increased erosion and sediment transport are frequently proportional with elevated P concentrations in impacted aquatic systems (48). Wildfires represent a particularly extreme example of landscape disturbances that can result in increased delivery of sediment to receiving streams and changes to P mobility (28,46,73). This is because wildfire-associated decreases in vegetation cover on soil can cause reductions in rainfall storage, resulting in increased overland flows and erosion (74). Wildfires also can alter the density and porosity of fine sediments, and the size of flocs/aggregates that naturally form in the water column (28,71,75). Other landscape disturbances such as deforestation or agricultural development can also change the distribution and form of organic P sediment processes (76). Regardless of whether they are natural or anthropogenic, all landscape disturbances that result in increased erosion have the potential to impact both internal and external P loading to aquatic systems.

Releases of P from fine sediments to the water column may enable the proliferation of algae and cyanobacteria, which can pose potentially critical consequences for aquatic systems, especially those that serve as drinking water supplies. The primary mechanisms that control P entering the

water column include: desorption from particulate P (particularly through resuspension and increased interaction with the water column), algal and cyanobacterial biomass settling and subsequent decay, and other chemical reactions within the sediment crystalline structure (24,66,77,78). In general, the release and uptake of P onto sediment is a two-step process. When P uptake by sediment occurs: rapid surface adsorption occurs first, followed by a slower “solid-state diffusion”; the reverse occurs in P release (24). Increases in primary productivity as a result of P desorption from fine sediments have been widely documented (31,34,48,79). For example, Orihel et al. (2015) demonstrated that sediments (specifically those low in iron) can “pump” internal P, stimulating cyanobacterial blooms (31). Since a key goal of reservoir management is to provide a stable supply of source water, it follows that the potential contributions of fine sediment to the proliferation of algae and cyanobacteria must be better characterized and understood so that management strategies can be developed to mitigate the risks from such events.

Adsorption/desorption of P from fine sediment can be characterized by various isotherms, such as the Langmuir and Freundlich, as well as by determination of the equilibrium phosphate concentration (EPC_0). The EPC_0 is a measure of the potential of sediments to release or take up SRP depending on surrounding ambient SRP concentrations. Specifically, it is the value of the liquid-phase P concentration at which adsorption and desorption processes are in equilibrium. Adsorption of P to sediment is favoured when $EPC_0 <$ ambient SRP concentration; in the opposite case, P desorption is favoured. (24,28). Thus, EPC_0 informs P mobility in the water column. Experimental determination of EPC_0 is discussed in Section 3.6 and Appendix 2: EPC_0 Quality Assurance & Quality Control.

To reduce the potentially adverse impacts of P loading to downstream environments, several best management practices (BMPs) have been proposed. They include various erosion control measures, establishment of riparian buffers, construction of wetlands, and reductions in fertilizer application (64,80–83). Notably, these BMPs have achieved variable success and are undergoing continual improvement. It should be further underscored that while such BMPs are predominantly focused on reducing P transport and delivery to receiving streams, relatively fewer strategies are available for limiting P mobility within the water column.

2.2 Cyanobacteria & Cyanotoxins

Although cyanobacteria have existed and adapted through many of the earth's climates for the last 3.5 billion years (84), their proliferation and metabolic functions are not well understood (85–87). Functionally, cyanobacteria are algae as their attributes (such as their photosynthetic capabilities) are similar to eukaryotic algae. Taxonomically however, cyanobacteria constitute one of the major eubacteria. Cyanobacteria have many physiological and cellular structures similar to bacteria, such as an absence of organelles and binary fission as a means of reproduction (84). The debate on whether or not these organisms should be called algae or bacteria dates back to the 1970s and still is not completely resolved (88,89). Regardless, the accumulation of these cells, can lead to adverse consequences often referred to as a bloom. Although definitions of cyanobacterial blooms are inconsistent across the literature (1,90–92), some common descriptions of cyanobacterial blooms are provided in Table 1.

Among cyanobacteria, *Microcystis aeruginosa* (*M. aeruginosa*) is one of the most ubiquitous species of *Microcystis* spp. (93,94). *Microcystis aeruginosa* cells have the ability to control the density of their gas vesicles and inflate them, allowing for vertical movement within the water column (91,95). This adaptation gives them a competitive advantage to access light in shallow waters, and relatively nutrient rich waters close to sediment (95). Moreover, *Microcystis* spp. are preferentially not consumed by invasive zebra mussels (*Dreissena polymorpha*), which have successfully established communities in Great Britain (1824), the Netherlands (1827), the Czech Republic (1893), Sweden (1920), Italy (1973), the Great Lakes in the USA (1988), California (2008), and have also been found throughout the Great Lakes in Canada (96,97). Consequently, zebra mussel settlement also may contribute to the proliferation and potential dominance of *Microcystis* spp. in natural waters (38). *Microcystis aeruginosa* cyanobacteria are especially relevant to the drinking water industry because they are commonly present in toxic cyanobacterial blooms (98,99).

Table 1- Various definitions of algal and cyanobacterial blooms.

Algal/Cyanobacterial bloom definition	Source
Visible accumulation of algal biomass that mass occurs when chlorophyll concentrations reach approximately 20 µg/L (16).	Reynolds & Walsby (1975)
Visible accumulation of phytoplankton dominated by a single (or a few species) it is an algal or cyanobacterial bloom.	Chorus & Bartram (1999)
Accumulation of cyanobacterial cells and toxins present to be waterborne hazards to health, with toxin effects ranging from mild to fatal .	Codd et al. (2000)
More than 50% dominance of cyanobacteria of phytoplankton biomass, regardless of visible biomass.	Molot et al. (2014)
When cyanobacterial cell densities exceed one million per litre.	Sulis et al. (2014)

Cyanobacteria (such as *M. aeruginosa*) can produce toxins, most commonly referred to as cyanotoxins. Exposure to cyanotoxins by water users can pose health threats (1,100,101), thus, their removal/destruction is required during drinking water treatment when they are present. Cyanotoxins may exist within cyanobacterial cells, and are referred to as intracellular cyanotoxins, or outside of the cell and in the water matrix, as extracellular toxins (2). In addition to cyanotoxins, cyanobacteria may also produce taste and odour compounds such as geosmin and 2-methylisoborneol (MIB). Although not harmful, these compounds produce foul odours and can be detected at concentrations as low as nanograms per litre (10). Similar to cyanotoxins, taste and odour compounds may be released from the intracellular to extracellular form if cells are lysed (102). Cyanotoxins include hepatotoxins (affecting the liver) , neurotoxins (affecting the nervous system), and dermatotoxins (affecting skin) (1,11,100), which can have adverse health effects in humans and animals after both acute and long-term exposure (100,103). Symptoms of cyanotoxin exposure include gastroenteritis, liver disease, and even death in some cases, depending on dose and body weight (100,103–105). Different species of cyanobacteria may produce different types of toxins depending on their genetic composition. Toxin production by various genera of cyanobacteria is summarized in Table 2.

Table 2- Cyanotoxin production by various genera of cyanobacteria. Adapted from Paerl et al. (2014).

Toxin	Cyanobacteria genera
Aeruginosin	<i>Microcystis, Planktothrix</i>
Anatoxin-a/ homoanatoxin-a	<i>Anabaena/Dolichospermum, Aphanizomenon, Cyndrospermopsis, Lyngbya, Oscillatoria, Phormidium, Planktothrix, Raphidiopsis, Woronichinia</i>
Anatoxin-a(S)	<i>Anabaena/Dolichospermum</i>
Aplysiatoxins	<i>Lyngbya, Oscillatoria, Schizothrix</i>
beta-Methylamino-L- alanine (BMAA)	<i>Anabaena/Dolichospermum, Aphanizomenon, Calothrix, Cyndrospermopsis, Lyngbya, Microcystis, Nostoc, Nodularia, Planktothrix, Phormidium, Prochlorococcus, Scytonema, Synechococcus, Trichodesmium</i>
Cyanopeptolin	<i>Anabaena/Dolichospermum, Microcystis, Planktothrix</i>
Cyndrospermopsin	<i>Anabaena/Dolichospermum, Aphanizomenon, Cyndrospermopsis, Oscillatoria</i>
Jamaicamides	<i>Lyngbya</i>
Microcystin	<i>Anabaena/Dolichospermum, Anabaenopsis, Aphanizomenon, Cyndrospermopsis, Gloeotrichia, Hapalosiphon, Microcystis, Nostoc, Oscillatoria, Phormidium, Planktothrix, Pseudanabaena, Synechococcus, Woronichinia</i>
Nodularin	<i>Nodularia</i>
Saxitoxin	<i>Anabaena/Dolichospermum, Aphanizomenon, Cyndrospermopsis, Lyngbya, Oscillatoria, Planktothrix</i>

The cyanotoxin microcystin is widely regulated and of global concern because of its toxicity and frequent occurrence (106). Microcystin can be produced by various cyanobacteria including: *Anabaena/Dolichospermum, Cyndrospermopsis, Microcystis, Nostoc, and Oscillatoria* (Table 2) (35). Microcystin-LR it is the most frequently occurring and toxic of at least 80 variants of microcystin (93,107). Although the molecular basis of microcystin production is known, its role is still unknown. The evolutionary advantages of toxin production are generally considered as either competitive advantages or as secondary metabolites from physiological aids. Competitive advantages may include cyanotoxin production in response to grazing pressure and/or resource competition, while physiological functions may include contributions to improved cellular physiology, through benefits to homeostasis, photosynthetic efficiencies, and accelerated growth

rates (20,85–87). For example, Jang et al. (2003) found the exposure of *Microcystis* to zooplankton resulted in microcystin concentrations up to five times greater compared to controls, suggesting that *M. aeruginosa* toxin production is an induced defense mechanism (85). Other work has suggested that microcystin production is a biological function most likely tied to mitigating stress within the bacterial cell (86,87). Presumably, a better understanding of the broader ecological role of cyanotoxins may inform strategies for the management of toxic blooms.

Unfortunately, the factors governing the formation and distribution of cyanobacterial blooms and their toxin production is complex (108–110). In addition to nutrient bioavailability (Refer to Section 2.3), several environmental factors are postulated to contribute to cyanobacterial bloom formation and toxicity. These factors include temperature, precipitation patterns, salinity, water column mixing patterns, and wind speed and direction (35,110,111). Although cyanobacterial blooms occur naturally, they can be significantly affected by environmental shifts associated with anthropogenic activities and climate change. For example, elevated levels of atmospheric CO₂ that are associated with climate change also can increase the flux of carbonate into water columns, which may be used in blooms (44). Further, changes in precipitation patterns can affect water levels and introduce high loads of nutrients to streams, rivers, and lakes through surface runoff, thereby promoting bloom formation, especially in oligotrophic systems (44,45). Changes in land use and the introduction of invasive species can affect cyanobacterial bloom formation. In particular, urbanization, agriculture (e.g., cattle grazing, dairy operations), and industrial activities can increase nutrient loadings of N and P to receiving waters and exacerbate cyanobacterial growth (1,43,64,112). The mechanisms behind the cyanobacterial toxicity are also poorly understood (108–110). For example, even when cyanobacteria with toxin-producing genes are present they may not always produce cyanotoxins (2). In other words, the production of cyanotoxins can be transitory and complex (113). When cyanotoxin concentrations are high and unexpected, implementing the required processes for toxin treatment may be difficult and cost prohibitive in some cases (114,115).

2.2.1 Implications of Cyanobacteria to Drinking Water Supply and Treatment

There are many undesirable consequences associated with the formation of cyanobacterial blooms. In addition to cyanotoxin production, these include aesthetics, limitations to recreational use, as

well as implications to drinking water supply and treatment (64,116). When high concentrations of cyanobacteria enter drinking water treatment plants, conventional treatment processes may also be adversely impacted (3,117). Taste and odour compounds (geosmin and MIB) can be produced by bacterial groups including cyanobacteria (118,119). Further complications due to temporal variation (i.e. changes in retention time due to reservoir drawdown) can affect reservoir management and responses to algal and cyanobacterial growth (120). Arguably, an understanding in the production of cyanotoxins in source waters is among the most important challenges facing the drinking water industry for the prevention of direct and indirect adverse health consequences to consumers. Fortunately, well-operated conventional and advanced treatment processes are able to breakdown/remove both cyanotoxins and taste and odour compounds to some extent; however, their implementation and operation can be costly and non-ideal in some operational circumstances. The following section addresses the treatment and supply challenges, as well as regulations and guidelines associated with cyanobacterial blooms.

2.2.1.1 Removal of Cyanobacteria and their Toxins

The removal of intact cyanobacterial cells during drinking water treatment (typically during coagulation/flocculation/clarification) is commonly recognized as the preferred approach for treating source waters contaminated with cyanobacteria (1,2). This preferred approach is utilized to prevent cell lysis and release of intracellular toxins to the water matrix, which can occur using methods focused on killing cyanobacterial cells or vigorously disrupting them (e.g., sonication, copper sulfate application, etc.) (121). Cyanobacterial cell and toxin removal by common drinking water treatment processes such as oxidation, coagulation, flocculation, filtration, and other methods have been extensively investigated and are discussed below.

Conventional drinking water treatment processes comprised of coagulation, flocculation, clarification and filtration can be effective at removing intact cyanobacteria cells, although the extent of removal depends on several factors, including the species present and operational conditions affecting settling (2,113,122). In general, conventional water treatment processes become decreasingly effective with increasing cell densities, especially those more consistent with bloom conditions. The passage of cyanobacterial cells through the treatment process can lead to filter clogging and/or breakthrough (4). Dissolved air floatation (DAF) in lieu of gravity-based clarification can be especially effective at removing cyanobacteria in some cases (2). Although the

removal of cyanobacterial cells during drinking water treatment ensures that cyanotoxins are less likely to reach consumers, cyanobacteria can remain and proliferate within treatment plants. Specifically, cyanobacteria and their associated toxins can accumulate in scums and sludge within drinking water treatment plants. In one case, cell densities of 4.7×10^6 cells/mL and total microcystin concentrations of up to 10 mg/L were found in one clarifier; in that plant, total microcystin concentrations of 2.47 $\mu\text{g/L}$ were found in the final, chlorinated drinking water (113). Therefore, because final total microcystin concentrations observed may be high enough to implicate risks to end drinking water users, drinking water utilities should take care and monitor cell densities and operations within drinking water treatment plants.

Several drinking water treatment processes are able to remove or destroy microcystin. They include: oxidation, activated carbon (AC), and membrane filtration:

1. Oxidation processes in drinking water treatment plants commonly use chlorine, ozone, hydroxyl radical, chloramines, potassium permanganate, and chlorine dioxide (2). Oxidation can destroy extracellular cyanotoxins (123), although the effectiveness of the oxidants depends on the type of oxidant (107,124), cyanotoxin (102,125), contact time (126,127), DOC concentration (106,128), and pH (107,129). For example, applied oxidation using ozone, permanganate, and advanced oxidation processes can successfully remove the cyanotoxin anatoxin-A, at most typical operating conditions, whereas other oxidants like chlorine are ineffective (124). The efficacy of oxidation in destroying cyanotoxins is also related to pH. For example, chlorine has a pK_a of 7.6, and previous research has indicated that at a pH lower than 8, chlorination can effectively destroy microcystin-LR (2,9,128). Although oxidation can effectively inactivate extracellular cyanotoxins, it (especially chlorination) is not commonly practiced for this purpose because excess biomass also can increase natural organic matter concentrations, which can lead to regulated DBP formation (87,127). Further, oxidation also may lyse cyanobacterial cells, releasing intracellular toxins to the water matrix.
2. Activated carbon filtration can effectively remove some cyanotoxins, including microcystin, cylindrospermopsin and saxitoxin (2,130,131). The effectiveness of cyanotoxin removal by granular activated carbon (GAC) may depend on the type of cyanotoxin, as well as the type of activated carbon used. For example, a study conducted

by Liu (2017) found that at equilibrium, wood based carbon had the highest cylindrospermopsin removal capacity, coal based carbon had the highest microcystin-LR capacity, and coconut based carbon had the highest anatoxin-A capacity (132).

3. Membrane filtration processes including osmosis, nanofiltration, ultrafiltration eliminate various compounds including cyanotoxins based on their physical size and charge, as well as membrane characteristics (133). For example, ultrafiltration research conducted by Lee & Walker (2008) demonstrated that hydrophilic (cellulose acetate) membranes were ineffective and adsorbed little to no microcystin whereas hydrophobic membranes were able to remove ~91% of microcystin (134). In other literature, both ultrafiltration and nanofiltration were effective in removing 90% to 96% of the cyanotoxins cylindrospermopsin and microcystin (133–135).

Therefore, literature indicates that treatment processes are available for cyanotoxin removal, but challenges primarily lie in financial limitations and determining when to implement these processes when cyanotoxin concentrations are unexpectedly high.

2.2.1.2 Supply Challenges: Reservoirs and Cyanobacterial Blooms

Fluctuations in water levels and reservoir drawdown can influence the occurrence of cyanobacterial blooms. Typically, drawdown occurs in summer months, which can lead to longer retention times (due to reduced flow rates), changes to temperature stratification, and increased water matrix concentrations of nutrients may occur during this time (120,136). During this time, algal and cyanobacterial communities may be affected (136,137). Stratification of water bodies is typically common in lakes and reservoirs deeper than 7 metres, while lakes and reservoirs 5 to 7 metres may develop unpredictable vertical stratification depending on wind mixing or precipitation patterns (138). In general, increased light penetration may also allow surface waters to heat up more quickly, intensifying vertical stratification. This has been shown to extend the periodicity and range of cyanobacterial species (139). Intensified vertical stratification also can support the formation of blooms as waters cool. Some cyanobacterial species can control the density of gas vesicles within the cell, controlling vertical migration in the water column. This allows for them to benefit from the light rich waters by the surface, and nutrient rich waters in deeper waters (91,95,140). Reservoir management strategies such as vertical mixing to disrupt stratification can be effective in controlling cyanobacterial bloom formation within lakes (141). However, it should

be noted that vertical mixing is not always successful. Mixing larger volumes of water such as coastal areas or oceans are difficult to sufficiently mix (139). It appears that both reservoir drawdown and increasing water levels could promote cyanobacterial and algal growth. These seemingly contradictory findings can be attributed to the fact that there are many parameters for cyanobacterial bloom formation, and the success of management strategies depends highly on ecosystem properties such as sediment type, retention time, the quality of the inlet water, abundance of fish, and climate (120).

2.2.2 Regulations & Guidelines

Currently, only the cyanotoxin microcystin-LR is regulated in Ontario where the Drinking Water Quality Standard is 1.5 µg/L (142). The World Health Organization (WHO) has issued a 1.0 µg/L guideline for microcystin-LR (143). The United States Environmental Protection Agency (US EPA) does not regulate any cyanotoxins under the Drinking Water Protection Act, although there are 3 cyanotoxins on the candidate contaminant list and existing health advisories for microcystins and cylindrospermopsin are respectively 1.6 µg/L and 3 µg/L for adults (144). Several U.S. states have their own guidelines for cyanotoxin exposure.

In addition to cyanotoxin concentrations, cell densities are often considered. The WHO and Global Water Research Coalition (GWRC) have different warning levels (Low, Moderate, High, Very High) for guidance values of cyanobacterial cell densities for recreational and source waters, respectively (Table 3). These warning cell densities are considerably different for these water uses. The WHO's warning levels for recreational water are higher than those for drinking water. The GWRC on the other hand, has warning levels based on cyanobacterial cell densities likely to produce harmful concentrations of microcystin (138).

Table 3- WHO and GWRC guidance values on cyanobacterial cell densities for recreational and drinking water sources.

WHO (Recreational Waters)		GWRC (Sources of drinking water)	
Relative Probability of Acute Health Effects	Cyanobacteria (cells/mL)	Definition given alert level	Cyanobacteria (cells/mL)
Low	< 20,000		500- 2,000
Moderate	20,000- 100,000	Potential for toxin concentration to reach 1/2 to 1/3 of drinking water guideline for microcystins	2000- 6,500
High	100,000- 10,000,000	Potential for toxin concentration to reach drinking water guideline for microcystins	≥6,500
Very High	>10,000,000	Potential for toxin concentration to be 10× greater than drinking water guideline for microcystins	≥65,000

One of the most memorable drinking water crises associated with elevated cyanotoxin concentrations occurred on the western basin of Lake Erie. Although Lake Erie has long endured cyanobacterial blooms, in August 2014 a dramatic closure was required when elevated cyanotoxins were insufficiently removed by a drinking water treatment plant serving the City of Toledo. There, concentrations of Microcystin-LR were found to be as high as 100 µg/L (145). Over 400,000 residents of the City of Toledo were faced with a “do not drink” advisory for several days, while stores ran out of bottled water and residents fled the city (16,95).

Technologies that can effectively treat cyanotoxins during drinking water treatment have been widely investigated and are generally available. Processes typically found in a treatment plant such as filtration and oxidation can remove cyanotoxins to some extent; however, extensive treatment can be challenging, and unwanted by-products may form (2,41,129,146,147) (Refer to Section 2.2.1.1). Moreover, the unpredictable and sporadic nature of cyanobacterial blooms makes it difficult to rationalize expensive infrastructure investment in response to uncertain threats that are generally relatively short-lived; of course, those potential risks must be weighed against potential health risks and service disruptions.

2.3 Phosphorus & Nitrogen: Drivers for Cyanobacterial Blooms

The contributions of macronutrients N and P to cyanobacterial bloom formation have been extensively investigated (23,69,148–150). Despite this, the role of N and P in cyanobacterial bloom formation, toxin production, and their relative availability is not well understood. *Microcystis aeruginosa* is non-diazotrophic (i.e., unable to fix N from the atmosphere) (23,151). While ongoing source water protection and watershed management plans have widely included reduced P inputs to aquatic systems, N discharges have received considerably less attention. It has been recently suggested that N availability resulting from anthropogenic sources such as municipal wastewater discharges may promote the proliferation of non-diazotrophic *M. aeruginosa* (151). Both P and N are key nutrients to primary productivity (22,110,151), however, the dynamics of their mobility in aquatic systems differ. While they can both adsorb/desorb from fine sediment (31,58,152,153), sorption processes are key mechanisms only for P mobility, whereas N mobility is predominantly controlled by other factors such as microbial activity (24,154–156). The effects of P, N, and the dual nutrient regime on cyanobacterial proliferation and toxin formation are outlined in this section.

2.3.1.1 Phosphorus

The availability of P is considered a key factor in limiting cyanobacterial growth in freshwater environments (29). The chemical form of P is crucial to bioavailability. Soluble reactive P, usually in the form of orthophosphates (PO_4^{3-} , HPO_4^{2-} , H_2PO_4^-) is considered the most bioavailable dissolved P form (49,50). Notably, dissolved P forms are approximately five times more bioavailable than particulate forms (51,55). Reductions in P discharges to freshwaters have contributed to significant reductions in eutrophication and the occurrence of cyanobacterial blooms (29,64,157). For example, in the 1970s, Lake Erie suffered from excessive nutrient enrichment, causing severe eutrophication and cyanobacterial blooms. Once watershed management strategies were implemented (especially limits on wastewater effluent discharges) to reduce P inputs to the lake, it began to recover in the mid-1980s and served as a globally renowned case of successful restoration (64). However, since the 1990s, the health of Lake Erie has deteriorated again. The cause of this deterioration is not known; however, it has been suggested that a dual nutrient regime between N and P may be a contributing factor—this is further discussed in Section 2.3.1.3 (23,36,150,151).

2.3.1.2 Nitrogen

Historically, the impacts of N loading on cyanobacterial proliferation were largely ignored because many cyanobacteria are diazotrophic (i.e., capable of fixing N). Nonetheless, external inputs of N have been shown to enhance cyanobacterial growth (21,95,150,158). More recently, it has been demonstrated that a lack of N availability contributes to conditions that favor *M. aeruginosa* proliferation and increased toxin production (23,151,159). The form of nitrogen, similarly to P, is linked to bioavailability. For example, *M. aeruginosa* is a strong competitor for inorganic, reduced forms of N such as ammonium (110,140), which can be derived from municipal wastewater discharges; it also can desorb from sediment (58,110,160), although excess ammonium also can suppress cyanobacterial growth in some cases (161,162). Urea is a reduced N form associated with municipal wastewater discharges (23,163). A few recent investigations have shown that it can enhance cyanobacterial growth (21,150,161) and higher pigment concentrations within cells relative to other N forms (160).

2.3.1.3 Dual Nutrient Regime: Phosphorus & Nitrogen

The effect of P and N on the composition and toxicity of cyanobacteria have been extensively studied. It has been demonstrated that *M. aeruginosa* depends on P for growth, and the functionality of cells and toxin production are coupled more strongly with N limitation and environmental stressors (110). Cyanobacteria typically dominate phytoplankton communities in which N is a limiting nutrient. This appears to be the case with both diazotrophic (N fixing, such as *Anabaena/Dolichospermum*) and non-diazotrophic (non N-fixing, such as *Microcystis*) cyanobacteria, as diazotrophic cyanobacteria can utilize atmospheric N if required. Non-diazotrophic cyanobacteria are typically strong competitors for N (140). In particular, low N:P ratios (<30) enable cyanobacteria to dominate over phytoplankton communities (78,148,157). Smith (1983) reported that cyanobacterial dominance occurs at total nitrogen (TN):TP ratios less than 29:1 by weight, whereas Orihel et al. (2012) reported that a TN:TP ratio of 18:9 in Lake Taihu resulted in maximum growth of cyanobacteria (69,148). As mentioned in Section 2.3.1.2, N limited conditions have been associated with increased production of cyanotoxins such as microcystin-LR (23,151,159). Although the impacts of N:P ratios have been widely emphasized, they are not predictive of cyanobacterial proliferation or dominance. Downing et al. (2001) analyzed data from 99 lakes and concluded that N:P ratios are not strongly correlated with cyanobacterial dominance; rather, they suggested that it is more influenced by the total P, total N, or standing algae biomass

(164). Of course, several environmental factors are associated with bloom formation and toxicity, including temperature, precipitation patterns, salinity, water column mixing patterns, and wind characteristics (35,110,111). Thus, while the roles of both P and N in cyanobacterial proliferation are still being delineated, it is clear that the implications of P and N availability on cyanobacterial growth and the dual nutrient regime must be better understood to develop mitigation and reservoir management strategies.

2.4 Sediment Contributions to Cyanobacterial Proliferation

Although potential contributions of sediment-associated nutrients to cyanobacterial proliferation have been suggested, these linkages have not been incontrovertibly demonstrated or extensively investigated. The contribution of sediment-associated P to primary productivity has been widely investigated within freshwater lacustrine environments (31,34). Lehman (2011) observed that the release of SRP from sediment coupled with a decline in N coincided with an increase of diazotrophic cyanobacteria, *Aphanizomenon*, populations in an urbanized impoundment (34). Other studies have suggested that low N:P ratios in eutrophic lakes contribute to cyanobacterial bloom occurrence (31). Xie et al. (2003) used 12 mesocosms (six with, and six without sediment) to examine the effect of sediment and associated P on cyanobacterial growth in a hyper-eutrophic lake. They reported that while all of the enclosures exhibited cyanobacterial growth, increased TP and SRP levels were found in mesocosms with sediment (165). These studies suggest a link between sediment-associated P and cyanobacterial blooms. However, no studies have been conducted in natural waters using bench top mesocosms to directly measure the role of sediment in cyanobacterial proliferation.

The vast majority of previous laboratory batch experiments of cyanobacterial growth have not been conducted with natural waters (33,82). Hao et al. (2016) conducted laboratory batch experiments using a modified, P-free BG11 growth medium and concluded that sediments collected from eutrophic ponds enhanced algal growth (82). Work completed by Huang et al. (2015) utilized deionized water and sediment in batch experiments; however, these investigations only explored relative P desorption from sediment given hydrodynamic disturbances (33). While most experiments utilize nutrient-rich growth media, Huang et al. (2015) used deionized water and sediment from a highly eutrophic system and reported *M. aeruginosa* growth (33). However, the

authors did not discuss the potential implications of culturing the cyanobacteria in such a low nutrient environment (33), which would stress the organisms and even possibly lead to cell lysis. At the time of this research, the only reported batch experiments that involved high quality (in this case mesotrophic) natural waters, sediment, and potentially toxin-forming cyanobacteria were conducted by Crumb (2016). In that work, fine sediments and water collected from an engineered drinking water reservoir resulted in *M. aeruginosa* proliferation (166). This type of analysis may contribute to the development of mitigation strategies focused on managing risks of cyanobacterial bloom occurrence.

Notably, most previously reported work related to sediment contributions to algal or cyanobacterial proliferation has focused on systems already experiencing eutrophication or cyanobacterial blooms. To the author's knowledge, the potential contributions of sediment to the proliferation of cyanobacteria (such as those proposed herein) have not been reported in mesotrophic-oligotrophic systems in literature to date.

Chapter 3: Materials and Methods

3.1 General Research Approach

To address the objectives detailed in Section 1.2, two phases of research were conducted. Phase 1 consisted of fine sediment characterization and P adsorption/desorption (i.e., sorption) experiments in which the geochemical composition, particulate P form (NAIP, AP, OP) and mobility (EPC₀) of fine sediment along a downstream gradient from the forested headwater source regions. Sediments were collected from the Elbow River watershed, which is located in Alberta, Canada. In particular, suspended sediments from the Elbow River and deposited sediments from the Glenmore Reservoir were investigated. Phase 2 consisted of developing a protocol for conducting microcosm studies and associated investigations of cyanobacterial proliferation in natural waters. Specifically, bottom sediment samples from the Glenmore Reservoir and suspended solids from a wildfire impacted river (Drum Creek) were used in the microcosm experiments to investigate the potential contributions of sediment-associated nutrient (P) releases on the proliferation of potentially toxin-forming *M. aeruginosa* cultures. The potential contributions from elevated nitrate availability were also examined.

3.2 Site Description

The Elbow River watershed is a forested, snow melt-dominated source water region located on the eastern slopes of the Rocky Mountains of Alberta (Figure 1, copyright permissions given in Appendix 5: Copyright Letter for Figure Permissions). Water from this river is used for a variety of municipal, agricultural, and recreational purposes. This river also provides a critical habitat for fish, and local wildlife in the river and related floodplains environments (167). It flows through the City of Calgary, into the Glenmore Reservoir (GMR) and through the associated dam. The Glenmore Reservoir is a manmade impoundment that is irregularly shaped and has a total capacity of $28.4 \times 10^6 \text{ m}^3$ (168–170). It is classified as mesotrophic-oligotrophic and has measured total P concentrations ranging from 2 $\mu\text{g/L}$ to 4 $\mu\text{g/L}$. Outside of the city limits above the Glenmore Reservoir, the healthy forested source watershed provides high-quality source water for the City of Calgary.



Figure 1- Location of the Elbow River Watershed (a) The Elbow River & Glenmore Reservoir are in Alberta, Canada, and (b) The Glenmore Reservoir is mesotrophic-oligotrophic drinking water reservoir. This photo was taken in July 2017. (c) The Elbow River originates in the eastern slopes of the Rocky Mountains and flows to the Glenmore Reservoir [Image courtesy of City of Calgary (167)], Copyright © The City of Calgary. All rights reserved. Reprinted with permission.

In 2015, suspended sediment samples were collected for approximately nine weeks (from July 3 to August 18) using Phillips Samplers (171) deployed at four sample stations in the Elbow River spanning a distance of ~103 km (Figure 1). The most upstream location is Cobble Flats (ER-CF). It is located closest to the Rocky Mountains, close to the confluence of the Little Elbow River tributary. Site ER-CF is considered to be in a forested region that is relatively unimpacted by landscape disturbances, and the land use is primarily recreational (167). The next site is 76 km downstream at Highway 22 (ER-HWY22). The surrounding area is predominantly undeveloped, however, the river does receive municipal wastewater effluent and runoff from Bragg Creek as well as agricultural land (167). The Twin Bridges site (ER-TB) is located another 19 km downstream of ER-HWY22 and land use between these two sites is primarily agricultural and residential with some cluster-type developments (167). The Weasel Head Footbridge site (ER-WFB) is located 8 km downstream of ER-TB and immediately above the inflow to Glenmore Reservoir. Thus, it receives urban runoff from stormwater outfalls serving the City of Calgary and discharging to the river (167).

The Glenmore Reservoir is divided into four distinct areas, namely Weasel Head (WH), Heritage Cove (HC), Mid Lake (ML), and Head Pond (HP) (Figure 2). Weasel Head is the sampling location closest to the Elbow River influent on the west end of the reservoir and is located near the center of the compartment. HC is in the south eastern portion of the Glenmore Reservoir. ML is the most centered location in the Glenmore Reservoir and is close to the golf club and local hospital. Lastly, HP is in the northernmost location of the Glenmore Reservoir, closest to the Glenmore dam and drinking water intake.

The reservoir acts as a sink for fine particulate matter from the Elbow River inflow. Due to landscape change and other urban impacts in the watershed, water quality has deteriorated over time due to increases in nutrients and suspended sediment (170). Consequently, reservoir classification has changed over time from oligotrophic (170), to now considered mesotrophic-oligotrophic (169). The critical forested headwater source regions of the Elbow River have not been impacted by wildfire for the past 90 years. Given the impacts of climate change on forests and the increased potential for wildfire in these regions, there is a potential threat to water quality in the reservoir from increased river inputs of sediment-associated P that would be expected as a result of severe wildfire(s) in the watershed area (28).

To evaluate the potential contributions of post-fire sediment-associated nutrients (especially P) to the growth of potentially toxin-forming cyanobacteria, additional sediment samples were collected in Drum Creek, approximately 8 years after the Lost Creek wildfire. The location, land use, hydro-climatology and impacts of the Lost Creek wildfire on Drum Creek have been described elsewhere (73). In brief, Drum Creek is located in the Castle-Crowsnest watershed, also in southwest Alberta. There, 90% of the area upstream of the sampling location burned (1064 of 1179 hectares) burned during the 2003 Lost Creek Wildfire. Increased capacity for P desorption by fine sediments within the creek has been reported (27). The insights provided by archived sediment characteristics can offer perspective and additional information for interpreting potential differences in the batch experiments.

3.3 Sample Collection

In July 2017 sediment and water samples were collected from WH, HC, ML, and HP locations in the Glenmore Reservoir (Figure 2) with a Ponar sampler (Hoskin Scientific, Burlington, Ontario). Approximately 20 L of water from each sample station in the Glenmore Reservoir was collected for use in the growth experiments described below. Each sediment sample was sectioned (top [0-2 cm], middle [2-4 cm] and bottom [13-15 cm]) and analyzed to evaluate any changes in particulate P forms as a function of depth. Thus, a total of 12 sediment samples and 4 water samples were collected. At the time of sampling, there was no visual algal growth in Glenmore Reservoir.

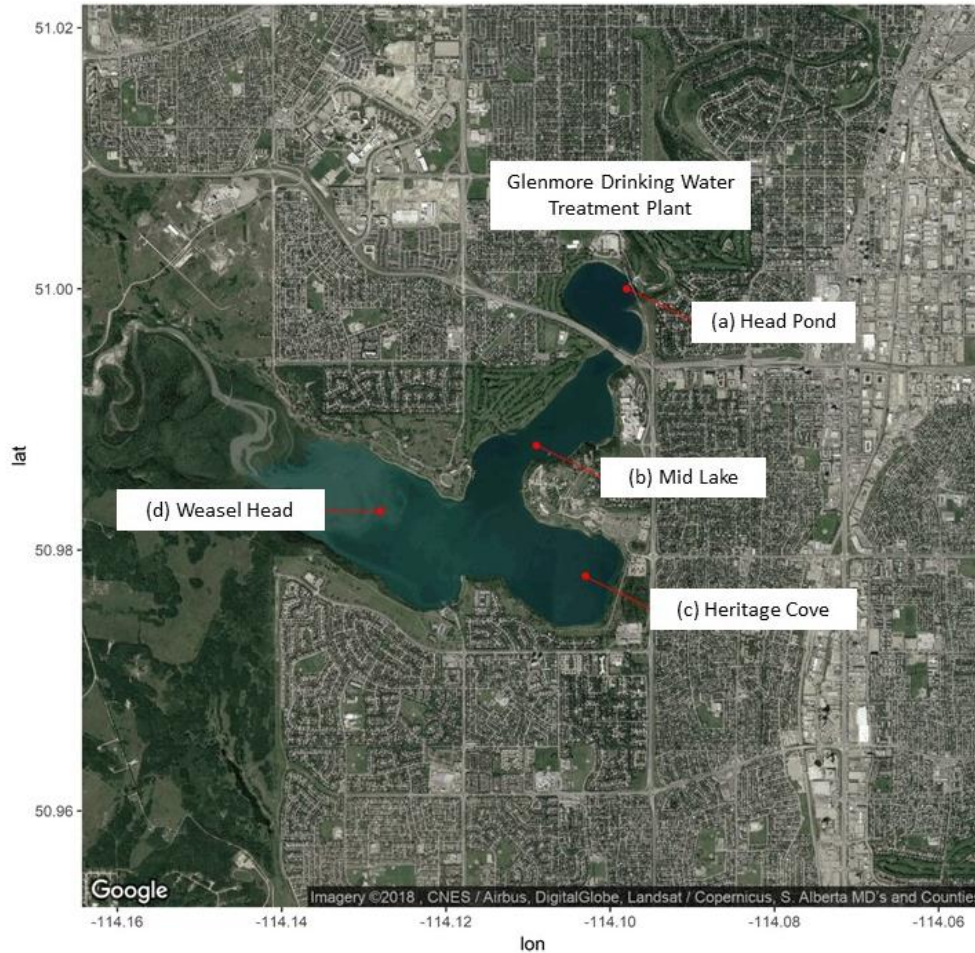


Figure 2- Sediment sampling locations in the Glenmore Reservoir in Calgary, Alberta, Canada. The four compartments from the inlet to the dam are: (a) Weasel Head, (b) Heritage Cove, (c) Mid Lake, and (d) Head Pond.

Previously collected sediment samples also were used in this investigation. In 2015, sediment samples were collected at the ER-CF, ER-HWY22, ER-TB, and ER-WFB locations using the methods described above. Philips samplers were deployed for approximately nine weeks to collect suspended sediment samples, from July 2015 to August 2015. To investigate the potential impacts of wildfire on nutrient (especially P) releases to the water column, sediments collected from Drum Creek from late May 2011 to September 2011 were used during the microcosm studies. Because the volume of archived sediment used during those experiments was insufficient for conducting additional sediment characterization analyses, the median grain size (D_{50}), specific surface area (SSA), geochemical composition, P speciation, and EPC_0 analyses conducted using sediment

collected in 2009 and 2010 were utilized—sediment composition and characteristics would not be expected to vary greatly between these sampling occasions.

3.4 Water Quality

Raw water collected from the Glenmore Reservoir was shipped to the University of Waterloo and stored at 4°C until use. The SRP concentration of all water samples was analyzed using a Technicon™ Autoanalyzer II (Seal Analytical, Mequon, WI, USA), following the ammonium molybdate/stannous chloride method (172). These same methods were used in analysis of SRP during the adsorption/desorption experiments described in Section 3.6.

Total organic carbon (TOC) and total inorganic carbon (TIC) concentrations were measured using a total carbon analyzer Model Sievers M9 TOC analyzer, from GE Analytical instruments, Colorado, USA according to Standard Method 5310 C, which is the EPA persulfate-ultraviolet method (173). Prior to use, filters were pre-washed to ensure no leaching occurred. Samples were filtered through 0.45 µm nylon Whatman filters before analyses to obtain results on the dissolved fractions of carbon.

3.5 Sediment Characterization

Physical characteristics and geochemical composition of Elbow River, Glenmore Reservoir, and Drum Creek sediments were analyzed at an accredited commercial laboratory (Activation Laboratories Ltd., Burlington, ON, Canada) according to standard methods. Analyses included grain size distribution, specific surface area, major elemental composition, and particulate P speciation. The grain size distribution, specific surface area, and the median diameter (D_{50}) were determined with a Malvern Mastersizer 2000. X-Ray fluorometry (XRF) fusion technique established by Norrish and Hutton (1969) was used to measure major element composition including: SiO_2 , Al_2O_3 , CaO , MgO , Na_2O , K_2O , Fe_2O_3 , MnO , and P_2O_5 (174). Results were reported as a percent of the total dry weight.

Particulate P forms were fractionated into three operationally defined fractions (NAIP, AP, OP) using the speciation technique described by Pettersson et al. (1988). The non-apatite inorganic P (NAIP) fraction is determined as the sum of three reactive phosphate fractions extracted by 1.0 M NH_4Cl -P, 0.11 M NaHCO_3 - $\text{Na}_2\text{S}_2\text{O}_4$, and 1.0 M NaOH. Apatite P (AP) is the particulate P fraction

extracted by 0.5 HCl and organic P (OP) is the particulate P form extracted by hot 1 M NaOH. Fractionation of particulate P forms can be used as a proxy to estimate the bioavailability of particulate P and its contributions to cyanobacterial growth in aquatic systems.

3.6 Phosphorus Sorption Experiments: Determination of EPC_0

The equilibrium phosphate concentration (EPC_0) is a measure of sediment potential to adsorb or desorb sediment-associated P to/from the water column (175,176). The EPC_0 is a measure of the ability of sediment to buffer dissolved P concentrations in the water column (Froelich, 1988). Batch experiments were conducted to determine: 1) the EPC_0 of the 12 sediment samples collected from Glenmore Reservoir and the four sediment samples collected from the Elbow River, and 2) the potential release of SRP from the sediment to the water column. Freeze dried sediment (0.25 g) was mixed with the various SRP concentrations (0, 10, 25, 50, 100 $\mu\text{g/L}$ KH_2PO_4) in autoclaved Glenmore Reservoir water (25 mL) in 50-mL polypropylene centrifuge tubes. These samples were completed in three replicates. Ambient SRP concentrations in the water collected from Glenmore Reservoir were determined by analyzing the filtrate passed through 0.45 μm nylon Whatman filters to remove particulate P.

The centrifuge tubes were gently mixed for 18 hours at room temperature on a shaker table, centrifuged at 4000 g for 5 minutes, and then filtered using a 0.45- μm nylon syringe filter. The filtrate (15mL) was placed in a scintillation vial and SRP was analyzed again to evaluate the desorption potential of the sediment. Concentrations of SRP throughout the experiment were analyzed using a Technicon TM Autoanalyzer II (Seal Analytical, Mequon, WI, USA), according to the ammonium molybdate/ stannous chloride method (172), as discussed in Section 3.4. QA/QC data are presented in Appendix 2: EPC_0 Quality Assurance & Quality Control.

3.7 *Microcystis aeruginosa*

Microcystis aeruginosa is one of the most ubiquitous species of *Microcystis* (93,94). This genus is one of the most prevalent cyanobacteria associated with blooms, particularly in North America (16,93,94). *Microcystis* is also often focused on, as several species within this genus are capable of producing the cyanotoxin microcystin (93,177). *Microcystis aeruginosa* was used in these studies because of: its potential capability to produce microcystin, its buoyancy characteristics

allowing for pipetting in the water column without drawing sediments, and its availability at the Canadian Phycological Culture Centre at the University of Waterloo.

3.7.1 Culture Conditions

Cultures of non-axenic cyanobacteria *Microcystis aeruginosa* (CPCC 300) were obtained from the Canadian Phycological Culture Centre at the University of Waterloo. They were maintained in BG11 culture medium in 500-mL glass Erlenmeyer flasks and re-cultured every month at a 1:3 ratio (i.e., 40-mL of older culture transferred into 120-mL of BG11 medium). All transfers (for re-culturing or inoculation of samples) of *M. aeruginosa* were conducted using aseptic technique inside a Class II A2 Biological Safety Cabinet (BSC) (Microzone, Canada) to reduce risk of contamination. All *M. aeruginosa* cultures and microcosms were maintained and grown in a Model E-36HO Percival growth cabinet lux (Percival Scientific Inc/ John's Scientific Inc, IA, United States), with three (3) white fluorescent bulbs with temperatures ranging between 19°C to 21°C, on a 12:12 hour light/dark cycle. A cheesecloth was placed on the top shelf to decrease light intensity, and flasks were placed on the bottom level of the growth cabinet where light was measured to be approximately 1776 lux. A modified culture grown in a 1:1 ratio of BG11 medium and autoclaved Glenmore Reservoir water collected from the HP site was maintained to acclimatize *M. aeruginosa* for growth experiments in media containing lower nutrient concentrations, such as the microcosm studies that used ambient reservoir water with some modifications (Section 4.2.3.2).

3.7.2 Experimental Conditions for *M. aeruginosa* Microcosm Experiments

All growth/microcosm experiments were conducted with sediments and ambient water collected from natural environments. Prior to use, all Glenmore Reservoir sediment and water was autoclaved, as the goal of these proof-of-concept experiments was to investigate the potential for *M. aeruginosa* growth only, and without any interaction effects of competition from other organisms.

Sediment moisture content was determined using Standard Test Method for Laboratory Determination of Water (Moisture) Content of Soil and Rock by Mass (ASTM D 2216-98). The desired dry weight was approximated from the moisture content. In treatments with sediment, cell

enumeration could be a challenge because some of the fine sediment was similar in size to *M. aeruginosa* cells and care had to be taken to ensure differentiation. Moreover, because the microcosm experiments were conducted using ambient reservoir water, cell production of sufficient concentrations of pigment for enumeration with fluorescence was initially uncertain. Cyanobacterial blooms usually occur in late summer to early fall, when low flow conditions would be expected. During periods of low flow, as rivers flow into downstream reservoirs and lakes, flows may be less turbulent (178). Thus, it is hypothesized that in the Elbow River low flow periods in the summer result in Glenmore Reservoir flows to be relatively quiescent. Consequently, the microcosm investigation conducted herein involved careful mixing at approximately 40 rpm, 1 cm above the sediment in flask microcosms. Mixing was completed with a pipette tip before sample extraction to: limit the unintentional burial of cyanobacterial cells (i.e., removing cells from the water column and causing them to become trapped in the sediment), and to collect a representative cell density within the water column.

3.7.3 Cell Quantification

Subsamples collected from the sediment-reservoir water-*M. aeruginosa* microcosms were serially diluted in sterile phosphate buffered saline (PBS) solution (pH of 7.4) to desired concentrations (approximately 2,000 cells/ mL to 10,000 cells/ mL per sample) to facilitate enumeration. Diluted cultures were stored in 1.5-mL sterile tubes (DNA LoBind Tubes, Eppendorf North America Inc., Hamburg, Germany). Subsample volumes of 10 μ L were placed on each side chamber of a Hausser Scientific Bright-Line Hemacytometer (VWR International, Mississauga, Canada) and covered with a cover slip. Cells were manually enumerated using a Zeiss Axioskop 2 microscope (Carl Zeiss Canada, Toronto, Canada) at 200 \times and 400 \times magnification, under white light and excitation wavelength of BP 546, and emission wavelength of 590 nm fluorescence. For this particular hemacytometer with 0.1 mm depth, each 1mm \times 1mm square has a volume of 10^{-4} mL. A method detection limit (MDL) was established at 10,000 cells/mL, assuming that 1 square (dimensions of 1mm \times 1mm) should have at least 1 cell. This method detection limit was calculated using the following equation:

$$MDL = \frac{1 \text{ cell}}{10^{-4} \text{ mL}} = 10,000 \text{ cells/mL}$$

Given that 5 squares were enumerated in this work, concentrations below 2,000 cells/mL are likely to lead to non-detects (10,000 cells/mL divided by 5). The gridlines of the hemacytometer and 5 squares counted are shown in Figure 3.

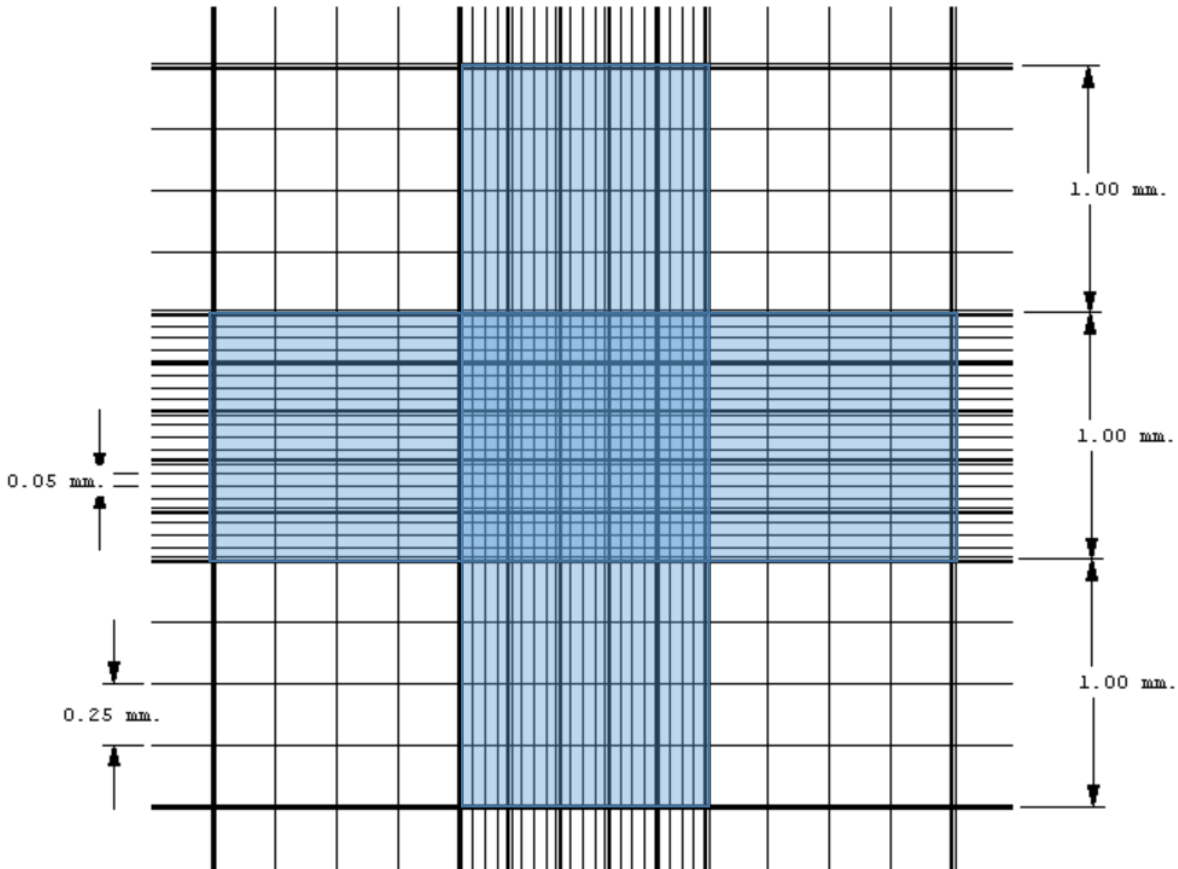


Figure 3- Hemacytometer gridlines: The five squares highlighted in blue were used to enumerate *M. aeruginosa* cells in all microcosm experiments.

3.8 Preliminary Experiments

Preliminary experiments were conducted to determine under which conditions cyanobacterial growth may occur in Glenmore Reservoir water. To the author's knowledge, because this type of experiment has not previously been conducted, it was uncertain whether cyanobacterial cells were capable of establishing populations and proliferate in natural waters. Various concentrations of inoculum, and macronutrients of phosphate, nitrate, and carbonate were utilized as a result.

3.8.1 Flask Microcosm Experiments

Flask microcosm experiments were conducted to investigate the proliferation of *M. aeruginosa* cells in suspensions containing sediment and various levels nutrients. Inocula of *M. aeruginosa* culture grown in BG11 medium were transferred to Erlenmeyer flasks containing 200 mL of autoclaved Glenmore Reservoir water collected from the HP site to yield an approximate cell density of 5×10^4 cells/mL. Initially, because it is difficult to culture *M. aeruginosa* in natural waters, nitrate, phosphate, and carbonate were added at one of three concentrations, based on the BG11 growth medium (as opposed to environmentally relevant levels). A single flask containing Glenmore Reservoir sediment and a low level of P addition was also prepared. The various treatment microcosm scenarios are summarized in Table 4.

Table 4- Preliminary flask microcosm experiments and nutrient amendments.

	Mass of sediment (g)	Stock type	Volume of Stock Added (ml)	Flask concentration (g/L)	BG11 concentration (g/L)
CO	0				
BG1	0	BG11	25		
BG2	0	BG11	50		
BG3	0	BG11	100		
N1	0	NaNO ₃	2.5	1.875	1.5
N2	0	NaNO ₃	5	3.75	1.5
N3	0	NaNO ₃	10	7.5	1.5
C1	0	Na ₂ CO ₃	0.25	0.025	0.02
C2	0	Na ₂ CO ₃	0.5	0.05	0.02
C3	0	Na ₂ CO ₃	1	0.1	0.02
P1	0	K ₂ HPO ₄	0.25	0.05	0.03
P2	0	K ₂ HPO ₄	0.5	0.1	0.03
P3	0	K ₂ HPO ₄	1	0.2	0.03
P1-SED	23.17	K ₂ HPO ₄	0.25	0.05	0.03

Cell densities were enumerated every 1 to 2 days, for 60 days. Each reported cell density was based on the average count obtained from two replicates. Based on previous investigations of *M. aeruginosa* growth rates (102,179), it was assumed that approximately 40 days would be enough time for populations to acclimatize to their environment and reach exponential growth. This acclimatization period had been observed in past studies in response to changes in nutrient

concentrations (86,180). Thus, if non-control flasks had cell densities less than those in the initial inoculation by day 41 (5×10^4 cells/mL), the experiments were discontinued. Although inoculum concentrations of around 10^4 cells/mL are commonly used for *M. aeruginosa* growth in nutrient-rich growth media and highly eutrophic natural waters (33,86,181), their proliferation in mesotrophic-oligotrophic reservoir water was initially uncertain.

3.8.2 Test Tube Microcosm Experiments

These microcosm experiments were conducted in test tubes to investigate if the presence of sediment, or singular doses of nutrients may contribute to cyanobacterial proliferation. Inocula of *M. aeruginosa* culture grown in BG11 medium were transferred to test tubes filled with 50 mL of autoclaved Glenmore Reservoir water collected from HP and inoculated with the *M. aeruginosa* culture to yield cell densities of approximately 5.0×10^5 cells/mL. This cell density was higher than that used in the flask microcosm experiments in the hope of reducing the lag time in growth. Similar to those experiments, three concentrations of nitrate, phosphate, and carbonate, as well as three amounts of sediment were investigated. When used, the nutrient amendments were based on the BG11 growth medium and decreased progressively by 50%. For example, the N3 treatment contained the same concentration of nitrate that would be found in BG11 growth medium (1.5 g/L), the N2 treatment contained half of that concentration (0.75 g/L), and the N1 treatment contained half of the N2 concentration (0.375 g/L). The various treatment scenarios are detailed in Table 5. In this experiment, cell densities were not measured. Photographs were taken approximately every week to evaluate if noticeable visual growth occurred between treatments, over a period of 4 weeks.

Table 5- Composition of test tube microcosm growth media. Microcosms contained Glenmore Reservoir water dosed with nitrate (N1, N2, N3), phosphate (P1, P2, P3), carbonate (C1, C2, C3), and sediment (A1, A2, A3). Control samples included: negative reference (CO) of only Glenmore Reservoir water, and positive control (BG11) of only BG11 growth medium.

Sample	NaNO ₃ (mg/L)	K ₂ HPO ₄ (mg/L)	Na ₂ CO ₃ (mg/L)	Dry mass of sediment added (mg)
BG11	1500	30	20	0
CO				0
N1	375			0
N2	750			0
N3	1500			0
P1		7.5		0
P2		15		0
P3		30		0
C1			5	0
C2			10	0
C3			20	0
A1				3
A2				6
A3				9

3.9 Factorial Design Microcosm Experiments

The impacts of sediment source, and nitrate amendment on *M. aeruginosa* proliferation were investigated using a factorial design experiment. Sediments were collected from two watersheds and were also impacted by different surrounding land uses. Drum Creek (DC) sediments were collected from a wildfire impacted region in the Crowsnest watershed, and Head Pond sediment (HP) were collected from a relatively urban reservoir compared to upstream waters in the Elbow River watershed. Nitrate amendments were also investigated in these experiments. Both pigment production and cell densities were evaluated in response to nitrate amendments and source of sediment.

Nitrate amendment was investigated for two reasons. First, the preliminary flask microcosm experiments presented in Section 4.2.1 suggested that N might be a limiting nutrient. This result was also supported by the dual nutrient regime of N and P that have been emphasized in recent literature (23,151,182). Second, in most cases it has been suggested that *M. aeruginosa* outcompete other organisms like green algae when adequate, but not necessarily when excess N is available (110,140). To explore this potential relationship, nitrate amendments based on the BG11 growth medium (commonly used for propagating *M. aeruginosa*) were used (183). It should be noted that concentrations of NO_3^- in Canadian lakes and rivers rarely exceed 4 mg/L (184), whereas N concentrations in this work ranged from 750 mg/L to 1500 mg/L. Therefore, concentrations in these experiments were much higher than those that would be observed in natural environments and represented excess nitrogen at levels commensurate with growth media. The experimental treatment details are provided in Table 6. A series of control and reference samples were conducted alongside the factorial design. Control/reference samples consisted of BG11, CO, P, N, HP, and DC. The BG11 microcosm was used as a positive reference as BG11 is commonly used to culture cyanobacteria. The CO (control) reference was used as a negative sample, as mesotrophic-oligotrophic waters alone were not expected to support *M. aeruginosa* growth. Reference samples of P and N were completed to determine if singular nutrient treatments of phosphorus or nitrogen alone, respectively, could support proliferation. Lastly, reference samples of HP and DC were conducted to determine if the presence of sediment alone with natural mesotrophic-oligotrophic waters could result in *M. aeruginosa* growth. Experimental reference samples, or controls are detailed in Table 7.

Table 6- Sediment types and concentrations of nutrient amendments used in the factorial design microcosm experiments.

Parameter	Levels
Sediment type	Drum Creek (DC)- approximately 8 years post-fire impacted Head Pond (HP)- reservoir sediment
Nitrate Amendment	N1- 750 mg/L of NO_3^- N2- 1500 mg/L of NO_3^-

Table 7- Experimental controls/ references were conducted along with the factorial design experiment for comparison. Microcosm CO and BG11 are considered to be a negative and positive reference, respectively. Microcosms P and N were conducted to determine if a dose of P or N along could proliferate *M. aeruginosa*. Lastly, reference microcosms HP and DC contained reservoir water and sediments from Head Pond (Glenmore Reservoir) and Drum Creek, alone to observe growth.

Sample	NaNO ₃ (mg/L)	K ₂ HPO ₄ (mg/L)	Dry mass of sediment added (g)
CO			
BG11	1500	31	
P		31	
N	1500		
HP			6
DC			6

3.9.1 Pigment Analyses

Pigment analyses were conducted at the end of the 60-day factorial design microcosm experiments following the approach of Thomas et al. (2013) (185). Each sample was filtered onto Whatman microfiber glass filters (0.7- μ m) and frozen until analysis. Pigments were extracted for 24 hours at -20°C in a solution of acetone, methanol and water (80:15:5 by volume). After the extraction, the solution was filtered again, through a 0.22- μ m polytetrafluorethylene (PTFE) syringe filter to remove large particles and other impurities. Samples were then dried under inert gas (N₂), and re-luted in 500 μ L of injection solution of acetone, ion pairing reagent, and methanol (70:25:5 by volume). This final solution was analyzed in a Waters HPLC reverse-phase system with a Symmetry C18 column (3.5 μ m), following methods modified from Leavitt et al. (1989). Prior to analyses, algal pigment standards (DHI Lab Products, Horsholm, Denmark) were used for HPLC calibration. A gradient delivery of two mobile phases was used to separate the pigment compounds: A and B, comprised of methanol and iron (90: 10 by volume), and methanol: acetone (73:27 by volume), respectively. The ion pairing reagent was comprised of 0.75 g of tetrabutylammonium acetate and 7.7 g of ammonium acetate. Geranium samples and Dye Sudan II were positioned for analyses around the first and last batch of samples processed. Sudan II was used as a standard to account for dilution and injection errors, and geranium to account for shifts in retention time of pigments during run time (185). Pigments were identified using Watters 2998 PDA detector and a

Waters 2475 Multi lambda Fluorescence detector, and by using the chromatographic mobility (186) and spectral characteristics, following information provided by Jeffrey et al. (1997).

3.9.1.1 Statistical Analyses: Factorial ANOVA

An ANOVA factorial analysis of the pigment concentrations on Day 60 was conducted. One of the assumptions of factorial ANOVA analysis is that the data are generally consistent with the normal distribution. Due to the relatively small sample size ($n=12$), it was assumed that this criterion was met. All statistical analyses were completed using R software (R Foundation for Statistical Computing, Vienna, Austria).

3.9.2 Response of Cell Density from Time and Type of Sediment

Cell densities were measured every 2 to 3 days, for a total of 21 occasions over the course of 60 days. Each reported cell density was based on the average count obtained from three replicates. Given the repeated measures approach and factorial experimental design (discussed in Section 3.9), the results were analyzed using a Mixed Factorial ANOVA.

3.9.2.1 Statistical Analysis: Mixed Factorial ANOVA

This experimental design involved three control variables (N concentration, type of sediment, and time) with an output variable of cell density (cells per millilitre). The sampling times were chosen as days 21, 39, and 60 because they are approximately equally spaced and would likely capture phases of the prokaryote growth curve (i.e., times at: end of the lag phase, growth phase, and stationary phase).

With 2 levels of nitrate concentration, 2 levels of type of sediment, and 3 levels with time, there were 12 distinct treatment options in this $2 \times 2 \times 3$ mixed factorial design. Each treatment of excess nitrate amendment and sediment was replicated in 3 flasks, for a total of 12 replicate microcosms. The microcosms included Glenmore Reservoir water initially inoculated with a target density of 5×10^6 cells/mL of *M. aeruginosa* on day 1. Analyses of cell densities considered three occasions (T1, T2, and T3) as described above. Thus, a total of 36 observations were used (Figure 4). All statistical analyses were conducted using R software (R Foundation for Statistical Computing, Vienna, Austria).

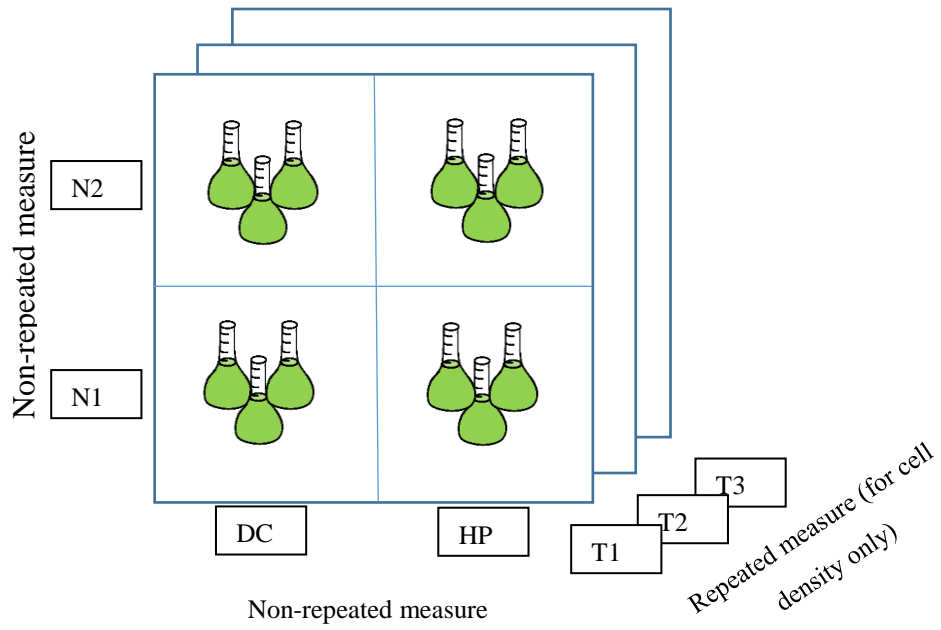


Figure 4- Conceptual diagram of mixed ANOVA experimental design. Two treatments of nitrate (N2: 1.5 g/L, N1: 0.75 g/L) and two sources of sediments (DC: wildfire impacted sediment), HP (reservoir sediment) were used in the design.

Chapter 4: Results and Discussion

4.1 Phase 1: Characterizing Particulate P Form and Mobility in the Elbow River, Glenmore Reservoir, and Drum Creek

The first phase of this research involved fine sediment characterization and P sorption experiments. Several items were evaluated in the following sections: the sediment grain size distribution and geochemical composition (Section 4.1.1), particulate P speciation (Section 4.1.2), and sorption properties (Section 4.1.3) of fine sediments collected from numerous locations in the Elbow River Watershed (Elbow River and Glenmore Reservoir) and Drum Creek.

4.1.1 Grain Size Distribution and Geochemical Composition

The study of fine sediment is critical as it is considered the primary vector for P transport in aquatic systems (26,156). The grain size distributions and geochemical composition of sediment collected in the Elbow River, Glenmore Reservoir, and Drum Creek were analyzed. The D_{50} and major element composition of the sediment in the Elbow River Watershed (upstream to downstream), as well as Drum Creek are presented in Table 8.

Spatially, the D_{50} in the Elbow River sediments decreased with distance downstream. Elbow River D_{50} ranged from 100 to 243 μm in the upper reaches (ER-CF and ER-HWY21, respectively), to 33 to 46 μm in the lower reaches (ER-TB and ER-WFB, respectively) (Table 8). This general trend of decreasing sediment grain size observed in the Elbow River and Glenmore Reservoir is consistent with previous observations. Specifically, as rivers flow downstream, most natural river bed sediments progressively become finer grained (187,188). This phenomenon is referred to as downstream fining, a fluvial process by which finer particles are preferentially transported and deposited downstream (187–189). Two main mechanisms are typically attributed to downstream fining: abrasion, where larger particles break into smaller ones, and selective deposition, which describes hydraulically driven sediment fractionation as detailed elsewhere (187–189). Larger particles generally deposit upstream, while smaller ones (i.e., fine grained sediments, typically $<63 \mu\text{m}$) travel further downstream. Thus, these data demonstrate that downstream fining in which suspended solids settle according to size and density (selective sorting) is occurring.

Table 8- D₅₀ and major element composition (% dry weight) of Elbow River, Glenmore Reservoir, and Drum Creek sediments

Sediment	Site	D ₅₀ (µm)	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃ (T)	MnO	MgO	CaO	Na ₂ O	K ₂ O	TiO ₂	P ₂ O ₅	Cr ₂ O ₃	V ₂ O ₅	LOI
Elbow River	ER-CF	243	25.73	3.57	1.42	0.028	6.84	29.56	0.22	0.8	0.18	0.09	< 0.01	0.007	31.67
	ER- HWY22	100	49.76	7.15	2.52	0.037	4.4	14.22	0.68	1.19	0.34	0.12	0.01	0.011	17.72
	ER-TB	46	49.16	6.61	2.33	0.037	4.92	15.03	0.61	1.12	0.34	0.13	< 0.01	0.009	15.09
	ER-WFB	33	51.11	6.74	2.34	0.043	4.68	14.3	0.57	1.28	0.37	0.15	< 0.01	0.009	13.42
Glenmore Reservoir	4 WH	7.23	40.03	9.28	3.51	0.05	4.03	17.33	0.44	1.69	0.46	0.15	0.01	0.02	22.97
	3 HC	3.16	40.29	11.51	4.01	0.06	3.58	16.15	0.37	2.17	0.49	0.15	0.01	0.02	21.21
	2 ML	4.04	40.49	10.62	3.78	0.06	3.82	16.76	0.38	2.00	0.48	0.15	0.01	0.02	21.69
	1 HP	4.86	42.80	11.26	4.12	0.06	3.44	14.28	0.37	1.99	0.49	0.18	0.01	0.02	20.49
Drum Creek	DC 2009	77.01	54.19	10.89	4.04	0.091	1.58	3.11	0.91	1.69	0.52	0.25	0.01	0	23.13
	DC 2010	4.63	47.82	10.42	4.32	0.091	1.29	2.97	0.72	1.6	0.46	0.24	0.01	0	28.81

The D_{50} for sediments deposited in the Glenmore Reservoir (3.16 μm to 7.23 μm) were observed to be finer than those in the Elbow River (33 μm to 243 μm). From the Elbow River inlet to the Glenmore dam, the D_{50} for WH, HC, ML, and HP sediments were observed to be 7.23 μm , 3.16 μm , 4.04 μm , and 4.86 μm , respectively (Table 8). Thus, sediment fining in Glenmore Reservoir was not perfectly observed. This could be attributed to the varied modes of sample collection and characteristics of Glenmore Reservoir. Sample collection with Ponar and Philips samplers collected sediment samples over different time frames, which allowed for temporal variation. Further, Glenmore Reservoir is an impounded reservoir, an anthropogenically formed reservoir by building a dam on a river (111), that is irregular in shape. In general, as rivers flow into lakes and reservoirs, velocity decreases and the ability to carry larger sediments also decreases (190). This trend is clear as the D_{50} values observed in the Glenmore reservoir (3.16 μm to 7.23 μm) are all smaller than those in Elbow River (33 μm to 243 μm). These results are supported by observations made by Owens et al. (2005), who observed that sediments deposited in lakes and reservoirs are predominantly fine grained (191). Thus, even though sediment fining was not perfectly observed in the Glenmore Reservoir, they were finer than those in Elbow River, as expected.

Generally, the geochemical composition of sediment collected from the Elbow River did not vary greatly, with the exception of the upper-most sampling location on the river, ER-CF. In all sample locations from the Elbow River, with the exception of ER-CF, the highest percent of element composition in the Elbow River was found to be SiO_2 (49.16% to 51.11%), LOI (13.42% to 17.72%), CaO (14.22% to 15.03%), and Al_2O_3 (6.61% to 7.15%) (Table 8). Excluding ER-CF (the most upstream sampling site), analyzed fractions were within a range of 5% of total dry weight analyzed. ER-CF had geochemical composition of sediment that was different from other sampling locations in the Elbow River. ER-CF had the highest levels of MgO, CaO, and LOI, as well as lowest levels of Al_2O_3 , Fe_2O_3 , MnO, Na_2O , K_2O , TiO_2 , P_2O_5 , and V_2O_5 . The geochemical composition of sediment at the uppermost study site in the Elbow River watershed was likely influenced by the bedrock geology and glacially deposited overburden. These trends have previously been discussed in literature, where it has been shown that the source and nature of geological materials strongly influence the geochemical composition of riverine sediment (192). The surficial materials at ER-CF consisted of colluvial deposits and may account for the observed increased concentrations of Ca and Mg compared to those of bottom sediments in the Glenmore Reservoir.

The major element composition of river sediment varied in a downstream gradient from the headwaters of the Elbow river to the Glenmore reservoir (Table 8). Other geochemical fractions not presented in Table 8 are available in Appendix 1.2: Geochemical Speciation. Glenmore Reservoir sediments were primarily composed of the same geochemical elements as Elbow River of: SiO₂ (40.03% to 42.80%), LOI (20.49% to 22.97%), CaO (14.28% to 17.33%), and Al₂O₃ (9.28% to 11.51%). Fractions of SiO₂, Al₂O₃, MgO, CaO, and Loss on Ignition (LOI) were generally at levels greater than 3% followed by Fe₂O₃, K₂O (1 to 3%), and then MnO, Na₂O, TiO₂, P₂O₅, and V₂O₅ (<1%). Levels of Al₂O₃, Fe₂O₃, MnO, TiO₂, and P₂O₅ were generally observed to have increased with distance downstream from the uppermost site in the Elbow river to the Glenmore Reservoir. This observed increase in Al, Fe, and Mn is typical of downstream increases of clay mineral content that is attributed to selective sorting of sediment in rivers (58,70). In contrast, levels of Na₂O decreased with distance downstream. The higher fractions of Al₂O₃, Fe₂O₃, and MnO may be of importance, as dissolved P can bind strongly to Fe and Al oxides and oxyhydroxides, while Mn can also form hydroxide coated surfaces, potentially indicating increased bioavailability of P in the reservoir compared to upstream locations (27,30,53,54,61). Notably, LOI is not a geochemical fraction, and is frequently used as an estimate of organic matter in sediments (193).

Between 2009 and 2010, D₅₀ values from Drum Creek sediments differed. The D₅₀ values of sediment collected from Drum Creek were 77.01 µm and 4.63 µm for 2009 and 2010, respectively. Drum Creek sediment D₅₀ may have varied due to temporal variation and time of sampling in the year. Drum Creek sediments collected by Philips samplers in 2009 occurred in the spring, when discharge was higher due to the spring freshet, relative to discharge in other seasons. Consequently, suspended sediment increased during spring melt and sediment availability from bank erosion and surface runoff was elevated. In contrast, the 2010 Drum Creek suspended sediment samples were collected during summer and fall. Thus, the particle size of suspended solids during that period were much finer due to lower flow velocities (171).

The highest geochemical fractions in Drum Creek were found to be: SiO₂ (47.82% to 54.19%), LOI (23.13% to 28.81%), Al₂O₃ (10.42% to 10.89%), and Fe₂O₃ (4.04% to 4.32%) of the total dry

weight. While these were dominant fractions, the percent composition of MnO were greater than those in Elbow River watershed. Similar to sediments from Elbow River watershed previously discussed, sediments with high fractions of Al_2O_3 and Fe_2O_3 as dissolved P can bind strongly to Fe and Al oxides and oxyhydroxides and can be released from sediment into the water column (30,79). Therefore, these geochemical composition results would indicate that Drum Creek sediments could potentially release more bioavailable P.

Overall, fine sediment characterization indicated that bioavailable P may be released from sediment into the water column. In Elbow River watershed, as D_{50} gradually decreasing downstream, fractions of Al_2O_3 , Fe_2O_3 , and MnO were gradually increasing (Table 8). The finest sediments deposited in the reservoir and had the highest Al_2O_3 , Fe, and MnO in the Elbow River watershed. Higher concentrations of metal oxides Fe_2O_3 and MnO in Drum Creek sediments might suggest more available sites for P binding in Drum Creek sediments (27,30,53,54,61). The release and bioavailability of P to the water column at such levels may indicate challenges to reservoir managers as increased P in downstream reservoirs can promote primary productivity (31,194).

4.1.2 Total Particulate P Speciation

The particulate P speciation for sediment samples collected from the Elbow River Watershed (Elbow River to Glenmore Reservoir), as well as sediment samples from Drum Creek is presented in Figure 5 and Table 9.

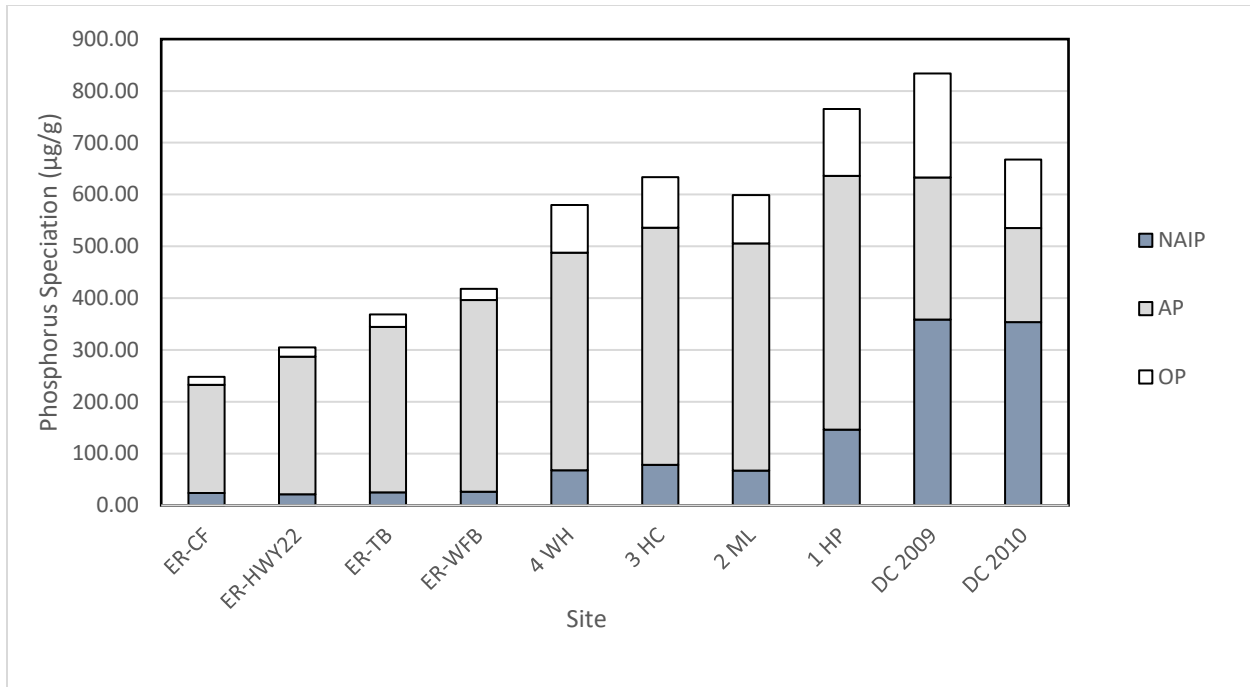


Figure 5- Phosphorus speciation of fine sediments from upstream to downstream in the Elbow River Watershed (Elbow River to Glenmore Reservoir), and the Crowsnest Watershed (Drum Creek) ($\mu\text{g/g}$).

The observed Elbow River and Glenmore Reservoir Total Particulate Phosphorus (TPP) concentrations and spatial trends were as expected—TPP concentrations generally increased downstream with decreasing grain size (as reported in Section 4.1.1). Globally, TPP concentrations may range from $<300 \mu\text{g/g}$ to $>6000 \mu\text{g/g}$ depending on surrounding land use, grain size, and other parameters (195–197). The TPP of sediment collected from the Elbow River and Glenmore Reservoir ranged from $247.81 \mu\text{g/g}$ to $304.9 \mu\text{g/g}$ in the upper reaches (ER-CF and ER-HWY21, respectively), to $368.5 \mu\text{g/g}$ to $418.1 \mu\text{g/g}$ in the lower reaches (ER-TB and ER-WFB, respectively), and $579.7 \mu\text{g/g}$ to $765.1 \mu\text{g/g}$ in the Glenmore Reservoir. These results are consistent with previously reported investigations that have demonstrated that finer particle size fractions have higher concentrations of TPP (198,199). Previous work conducted by Allin (2015) in a neighbouring watershed, also reported these trends. In that study, river sediments ($519.2 \mu\text{g/g}$ to

548.4 µg/g) had lower TPP relative to the downstream reservoir sediments (639.0 µg/g to 744.2 µg/g) (27).

Table 9- Solid phase concentrations of TPP, and fractions NAIP, AP, OP. Equilibrium Phosphate Concentrations (EPC₀) of sediments in the Elbow River Watershed (Elbow River to Glenmore Reservoir) and Crowsnest Reservoir (Drum Creek) and ambient SRP concentrations are also presented. Ambient SRP concentrations in the reservoir were less than EPC₀ concentrations, indicating that sediment is likely desorbing SRP to the water column.

Location	Sample Site	NAIP (µg/g)	AP (µg/g)	OP (µg/g)	TPP (µg/g)	EPC (µg/L)	Ambient SRP (µg/L)
Elbow River	ER-CF	23.6	209.0	15.2	247.81	34.7	
	ER-HWY22	21.3	266.0	17.6	304.9	19.4	
	ER-TB	25.4	319.0	24.1	368.5	14.0	
	ER-WFB	26.6	370.0	21.5	418.1	12.4	
Glenmore Reservoir	4 WH	67.5	420.0	92.2	579.7	8.8	2.7
	3 HC	78.4	457.7	97.7	633.8	11.6	3.4
	2 ML	67.4	438.3	93.1	598.9	10.5	3.6
	1 HP	146.4	489.3	129.3	765.1	23.7	3.1
Drum Creek	DC 2009	358.6	274.4	200.6	833.6	175.6	
	DC 2010	353.7	181.9	132.1	667.7	158.0	

Sediment NAIP concentrations also varied spatially in the Elbow River watershed. Concentrations of NAIP ranged from 21.6 µg/g to 21.3 µg/g in the upper reaches of the Elbow River (ER-CF and ER-HWY21, respectively), to 25.4 µg/g to 26.6 µg/g in the lower reaches (ER-TB and ER-WFB, respectively), and 67.4 µg/g to 146.4 µg/g in the Glenmore Reservoir (Table 9). Consistent with TPP, sediment NAIP concentrations generally increased with distance downstream. Noticeably, both TPP and NAIP were highest in the Head Pond of the reservoir and were associated with smaller particle size fractions that are typically enriched with NAIP (25). Concentrations fractions of Al₂O₃, Fe₂O₃, and MnO increased progressively downstream and these metal oxide fractions are important for the release of SRP from sediments (30,54,200). The concentration of NAIP is calculated as the sum of three extracts, namely; NH₄Cl-RP (1.0M), BD-RP (0.11M, 40°C) and NaOH-RP (1.0M) (Refer to Section 2.1). The NaOH-extractable P includes P bound to aluminum

and metals in humic acids (59). In research conducted by Smith et al. (2011), lake sediments with reactive iron highly correlated with SRP, which can subsequently cause cyanobacterial blooms (30). Therefore, the presence of these metal oxy-hydroxides in sediments could have led to progressively increasing availability of NAIP from the upper reaches of the Elbow River to the Glenmore Reservoir.

In general, Drum Creek sediments were more enriched in NAIP and OP than Elbow River watershed sediments. The concentration of NAIP in Drum Creek was noticeably greater than in the Elbow River watershed. The NAIP concentration of Drum Creek sediment was 358.6 $\mu\text{g/g}$ to 353.7 $\mu\text{g/g}$ for sediments collected in 2009 and 2010, respectively). In contrast, NAIP ranged from 21.3 $\mu\text{g/g}$ to 146.4 $\mu\text{g/g}$ in the Elbow River. Elevated levels of NAIP in sediment indicate that there is an increasing potential for the sediment associated P to be bioavailable which may be a factor in the growth of cyanobacteria (28,57,58). In Drum Creek OP levels were 200.6 $\mu\text{g/g}$ and 132.1 $\mu\text{g/g}$ in 2009 and 2010, respectively in 2009 and 2010 which is an order of magnitude greater than in the Elbow River (15.2 $\mu\text{g/g}$ to 21.5 $\mu\text{g/g}$) and Glenmore Reservoir (92.2 $\mu\text{g/g}$ to 129.3 $\mu\text{g/g}$). OP is considered potentially available for algal growth as it is hydrolysable and can be converted to inorganic P through chemical and/or biological reactions (58,201,202).

Several factors may partially explain the observed differences in the particulate P forms in Drum Creek compared to Elbow River and Glenmore Reservoir sediments. Elevated levels of NAIP for Drum Creek can potentially be attributed to the impact of wildfire on Drum Creek samples. Previous studies investigating impacts of wildfire on P bioavailability have found that it is likely that wildfires increase P availability for algal and cyanobacterial growth (28,46). Higher temperatures associated with burnt soil have been positively correlated with bioavailable P (46). Another study conducted by Allin (2015) found particulate P forms in burned and unburned sediment in the Crowsnest Pass (which includes Drum Creek) and reported a significant increase in NAIP levels in burned compared to unburned sediment (28). In this work, the TPP in sediments from the wildfire impacted region ranged from 660.6 $\mu\text{g/g}$ to 717.7 $\mu\text{g/g}$ (27,28), whereas Drum Creek sediments from this study ranged from 667.7 $\mu\text{g/g}$ to 833.6 $\mu\text{g/g}$. Thus, the TPP

fractionations observed herein were within an expected range, although they were slightly higher than those previously observed.

4.1.3 P Sorption Characteristics

The EPC₀ values observed for sediment collected in Glenmore Reservoir did not differ greatly and were consistent with the literature. The EPC₀ for sediment collected from HC, WH, and ML sampling locations in the Glenmore Reservoir ranged from 8 µg/L to 12 µg/L (Table 9). Globally, the EPC₀ reported for lakes and reservoirs ranges from <1 to 270 µg/L (Table 10). In particular, the EPC₀ for sediments collected from mesotrophic-oligotrophic water bodies range from 0.2 µg/L to 102 µg/L. Therefore, EPC₀ values reported herein are comparable to previous studies. Interestingly, the EPC₀ in HP was greatest. In general, locations closest to the dam (Head Pond) had higher EPC₀ values (23.7 µg/L) compared to other sample locations closer to the Elbow River inlet which is likely attributed to the decrease in grain size (25,70).

Table 10- EPC₀ (µg/L) ranges for lake and reservoir bed sediments with varying trophic status

Site	Trophic Status	EPC ₀ (µg/L)	Source
Lake Opeongo	Mesotrophic-oligotrophic	0.2 to 5	Cyr et al. (2009)
Oldman Reservoir	Mesotrophic-oligotrophic	64.3 to 102	Allin (2015)
Lake Taihu	Eutrophic	1 to 67	Yu et al. (2017)
Loch Leven	Eutrophic	180 to 270	Spears et al. (2007)

The EPC₀ of sediment collected from the Elbow River and Glenmore Reservoir ranged from 34.7 µg/L to 19.4 µg/L in the upper reaches (ER-CF and ER-HWY21, respectively), and 14 µg/L to 12.4 µg/L in the lower reaches (ER-TB and ER-WFB, respectively). The EPC₀ appeared to decrease with distance downstream in the Elbow River. This was the opposite of the trend observed in the Glenmore Reservoir, in which values of EPC₀ increased approaching the dam where finer particles settled. These differences may be due to the presence of larger soil aggregates and flocs that form in the water column of the Elbow River where sediment is more loosely bound. It is possible that during P mobility experiments, the shaking process may have caused the breakup of

larger particles thus producing a larger than expected EPC_0 . In the Crowsnest watershed a similar phenomenon was observed where larger EPC_0 values were occurring in the upstream river sediments compared to downstream reservoir sediments (27). These trends were attributed to differences grain size and particle morphology as well as sampling methodology (Philips vs Ponar sampling) (27).

Observed differences in the EPC_0 of Drum Creek and Glenmore Reservoir sediment were determined using batch experiments (Table 9, Figure 6). Based on long term water quality monitoring data for the Glenmore Reservoir, SRP concentrations in the water column of the reservoir typically range between 2 $\mu\text{g/L}$ and 4 $\mu\text{g/L}$. Based on the batch experiment data shown in Figure 6, bottom sediment in the Glenmore Reservoir represents an internal source of P to the water column. According to P sorption data obtained in the benchtop batch experiment, Glenmore Reservoir sediment in Head Pond can release from 2 $\mu\text{g P/g}$ sediment to 4 $\mu\text{g P/g}$ sediment. Other EPC_0 isotherms in other locations of the reservoir are presented in Appendix 1.4: Equilibrium Phosphate Concentration (EPC_0). It is important to note that EPC_0 values are likely lower than those in the reservoir. This is because P desorption from bottom sediment to the water column will increase in zones of anoxia which were observed at several sites in the Glenmore Reservoir (22,29,181).

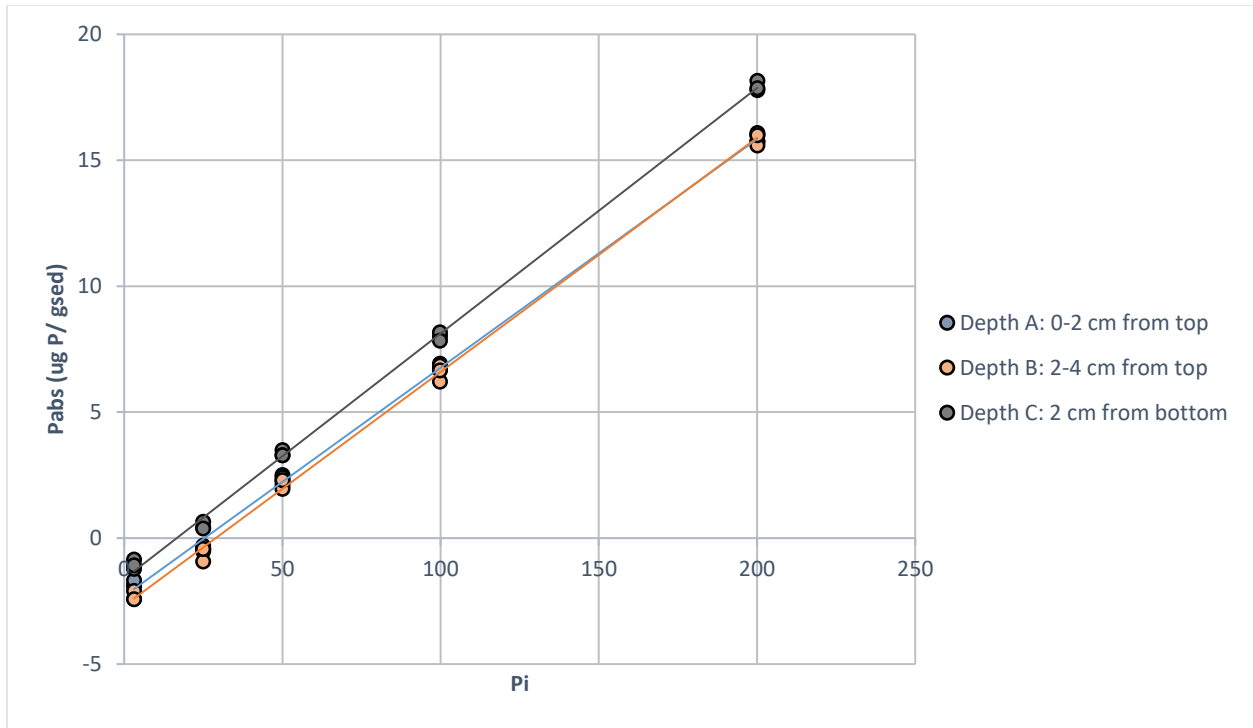


Figure 6- The sorption batch experiments results can determine the EPC_0 for various sediments. In the Head Pond, sediments collected at three different depths resulted in EPC_0 concentrations indicating a release of SRP from the sediment to the water column.

4.2 Phase 2: *M. aeruginosa* Batch Experiments

Phase 2 of this research involved developing a protocol and conducting microcosm studies to examine cyanobacterial proliferation in high quality natural waters using potentially toxin forming *M. aeruginosa* cultures. Growth experiments using various sediment types and modified reservoir water in the microcosm were completed. Results of the microcosm experiments are presented and discussed in the following sections.

4.2.1 Flask Microcosm Experiments

The cell densities observed in the experiment of samples dosed with BG11 growth medium and CO reservoir water were as expected for several reasons. *M. aeruginosa* grown in microcosms

dosed with BG11 growth medium (BG1, BG2, BG3) had cell densities ranging from 8.2×10^6 cells/mL to 3.0×10^7 cells/mL on day 59 (Figure 7). It was not a surprise that microcosms containing BG11 grew better compared to all other samples, as BG11 is a nutrient rich growth medium commonly used to cultivate *M. aeruginosa* under optimal laboratory conditions. Growth trends for BG1, BG2, and BG3, most closely resembled a prokaryote growth curve (203); with a lag phase occurring days 1 to 22, an exponential growth phase occurring days 25 to 53, and a stationary phase beginning from days 55 to the end of the experiment on day 59. The experiments did not run long enough for samples to exhibit the death phase. Other experiments utilizing BG11 growth medium have reported similar growth trends (166,204,205). The CO treatment was conducted with mesotrophic-oligotrophic water collected from Glenmore Reservoir and no nutrient amendments. In the literature, concentrations of P with $30 \mu\text{g/L}$ have been shown to promote primary productivity (16). Concentrations in Glenmore Reservoir water ranged from $2 \mu\text{g/L}$ to $4 \mu\text{g/L}$. In this case, nutrient levels essential for growth were too low in the water matrix. Therefore, the growth trends of treatments with BG11 and CO can be easily explained by nutrient availability.

Microcosms dosed with carbonate (C1, C2, C3) did not grow well throughout the experiment (Figure 7). Cell densities continually decreased in microcosms dosed with carbonate (C1, C2, C3) and were discontinued at day 41 as they were below the 5×10^4 cells/mL threshold (described in Section 3.8.1). Little work has been conducted on carbonate amendments to cyanobacterial proliferation. Most previous research has focused on changes to atmospheric CO_2 (with a focus on climate change impacts) (44). The impacts of elevated carbon concentrations are unclear. Previous work has suggested that increased atmospheric CO_2 concentrations can improve cyanobacterial growth (44,206), whereas other studies have indicated that elevated carbon concentrations can inhibit photosynthesis (207). The experiments suggest that carbonate is not a limiting nutrient for *M. aeruginosa* growth in Glenmore Reservoir waters.

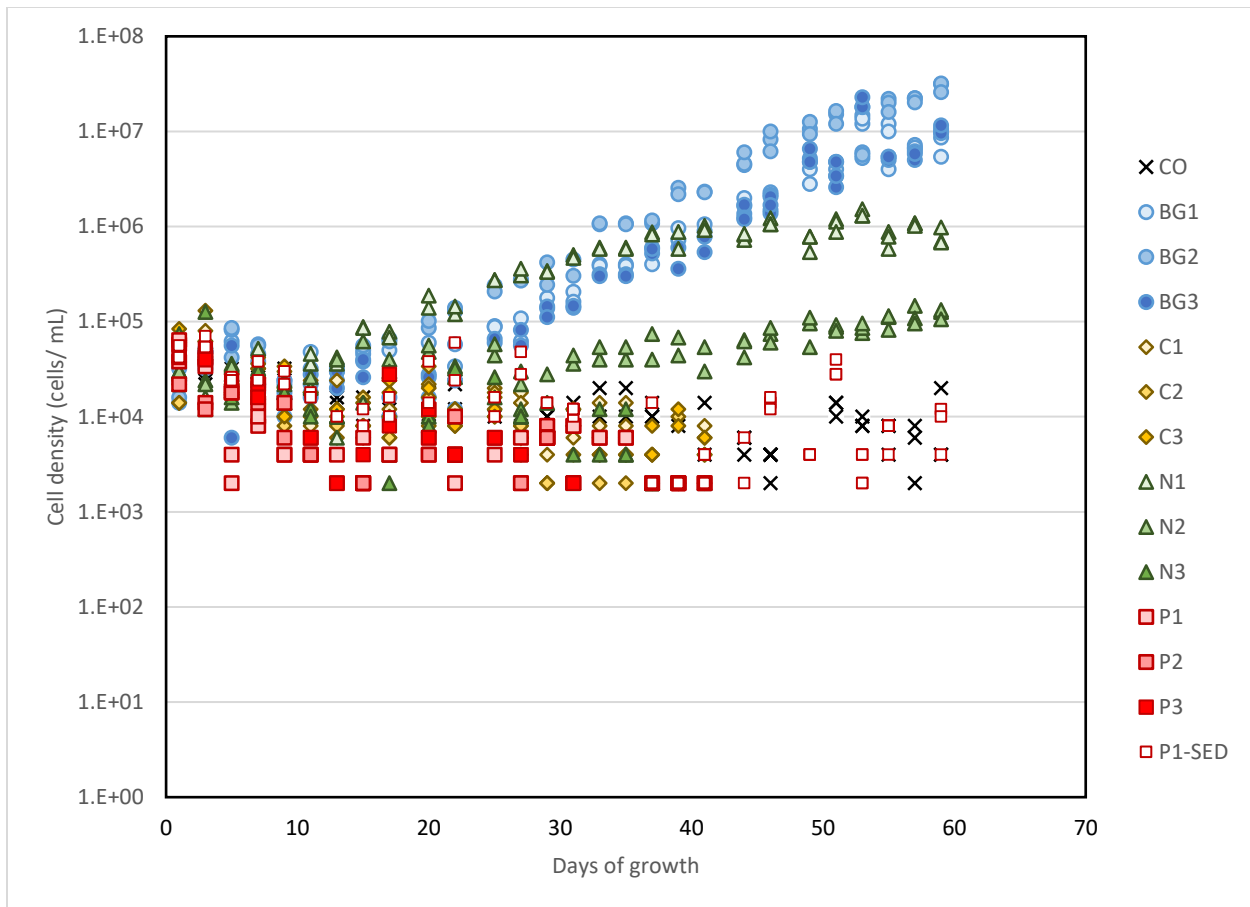


Figure 7- Cell densities of flask microcosm experiments of *M. aeruginosa* with various treatments of BG11 (BG1, BG2, BG3), carbonate (C1, C2, C3), nitrate (N1, N2, N3), phosphate (P1, P2, P3) and two controls (CO and P1-SED) measured for 60 days.

Microcosms amended with phosphate did not grow well compared to BG11 treatments (Figure 7). In flask microcosms dosed with phosphate (P1, P2, P3), cell densities progressively decreased and the experiments were subsequently discontinued at day 41 when cell densities were below the 5×10^4 cells/mL threshold (described in Section 3.8.1). The results from these microcosm experiments were unexpected because P is typically considered to be the primary limiting nutrient in freshwater bodies (12,29,78,157) and P concentrations $> 30 \mu\text{g/L}$ are understood to generally increase primary productivity (16). While SRP concentrations in Glenmore Reservoir water range

from 2 µg/L to 4 µg/L, nutrient doses for P1, P2, and P3 were 0.05 g/L, 0.1 g/L, and 0.2 g/L, respectively and were well above the 30 µg/L threshold for eutrophication (16).

The microcosm with sediment from Glenmore Reservoir and phosphate amendments supported cyanobacterial cell densities that ranged from 2×10^3 cells/mL to 3.2×10^4 cells/mL from days 41 to 59 and show that fine sediment can enhance cell growth relative to microcosms with phosphate amendment, with no sediment. Previous literature has indicated that singular nutrient amendments may not be sufficient for proliferation, whereas the synergistic contributions of P and N together can lead to growth (208). Work by Guildford & Hecky (2000) also suggests that both P and N (with specific N:P ratios), likely have a role to play in primary productivity (209). Some studies show that sediments are capable of desorbing both N (often in the form of inorganic ammonium, which is bioavailable) and P (58,110). Given that microcosms amended with only phosphate did not grow well, the data from this experiment seem to suggest that cyanobacterial growth in microcosms with sediment may have resulted from the desorption of N and P together. No analysis of dissolved species in the post experiment was conducted and future studies should determine what other factors may have contributed to growth.

In general, all singular nutrient treatments did not promote cyanobacterial growth. Previous literature has indicated that singular nutrient amendments may not be sufficient for proliferation, whereas concentrations of P and N together, or other micronutrients such as iron can lead to growth (21,23,208,210). Following the work of Guildford & Hecky (2000), Ma et al. (2015) suggest that N:P ratios have a key role in propagating cyanobacterial growth, and that limiting nutrients may change depending on the ratios of N and P (21). Singular nutrient amendments dosed at concentrations well above those commonly found in natural water systems may suppress growth (161,162), although literature regarding high doses of nutrients adversely affecting cyanobacterial growth is limited. For example, excess concentrations of ammonium have been observed to potentially suppress growth (161,162). It is possible that because the water was collected from a mesotrophic-oligotrophic reservoir, insufficient nutrient concentrations or mixtures essential for growth were not optimal in the water matrix. Therefore, the range of nutrients required for

cyanobacterial growth or specific nutrient ratios, were insufficient to support cyanobacterial proliferation (21,23,95,211).

Relative to other amendments with P and carbonate, cyanobacteria grew better in microcosms dosed with N. Cell densities increased in two of the microcosms with nitrate amendment; final cell densities on day 59 of the experiment for N1 and N2 were 7.9×10^5 cells/mL and 1.21×10^5 cells/mL, respectively (Figure 7). Clearly, N3 did not grow as well as N1 and N2 microcosms and N3 was discontinued after day 41. Previous studies have indicated that increased N can affect biomass of cyanobacteria (21,150,212). For example, Chaffin (2013) found that increased N in Lake Erie from 2002 to 2011 resulted in a linear relationship for increasing biovolume of *Microcystis* (150). It appears from these results that nitrate contributions may promote *M. aeruginosa* cell proliferation in mesotrophic-oligotrophic waters like those in the Glenmore Reservoir.

In addition to nutrient availability, the lack of replication in the experiments conducted herein could have affected the results and interpretation of the data. None of the flask microcosms were replicated and the degree of variability within and between the treatments is unknown (further elaborated on in Section 4.2.3.2). Given the results from these experiments, it appears that microcosm samples of BG1, BG2, BG3, and N1 treatments were the only treatments sufficient for cyanobacterial proliferation. Therefore, despite the lack of replicates in this experiment, the results seem to suggest that N is important for cyanobacterial growth in the Glenmore Reservoir water.

4.2.2 Test Tube Microcosm Experiments

The results from test tube microcosms dosed with (nitrate, phosphate, and carbonate) were generally expected given results from the flask microcosm experiments. Photos documenting *M. aeruginosa* cell proliferation in the test tube microcosms are presented in Appendix 3: *M. aeruginosa* Test Tube Microcosm Experiment Photographs. A photo of the microcosms taken on day 27 is presented in Figure 8. Test tube microcosms with nitrate (N1, N2, N3), phosphate (P1, P2, P3), or carbonate (C1, C2, C3) amendment exhibited little growth over the 27 day duration of the experiment. In the flask microcosm experiments previously discussed in Section 4.2.1, amendments with phosphate and carbonate grew poorly compared to nitrate amended samples

(with the exception of N3). Previous studies have indicated that singular nutrient amendments may not be sufficient for cyanobacterial proliferation (23,95,208). Therefore, the results from these experiments regarding phosphate, nitrate, and carbonate doses in growth were not surprising.

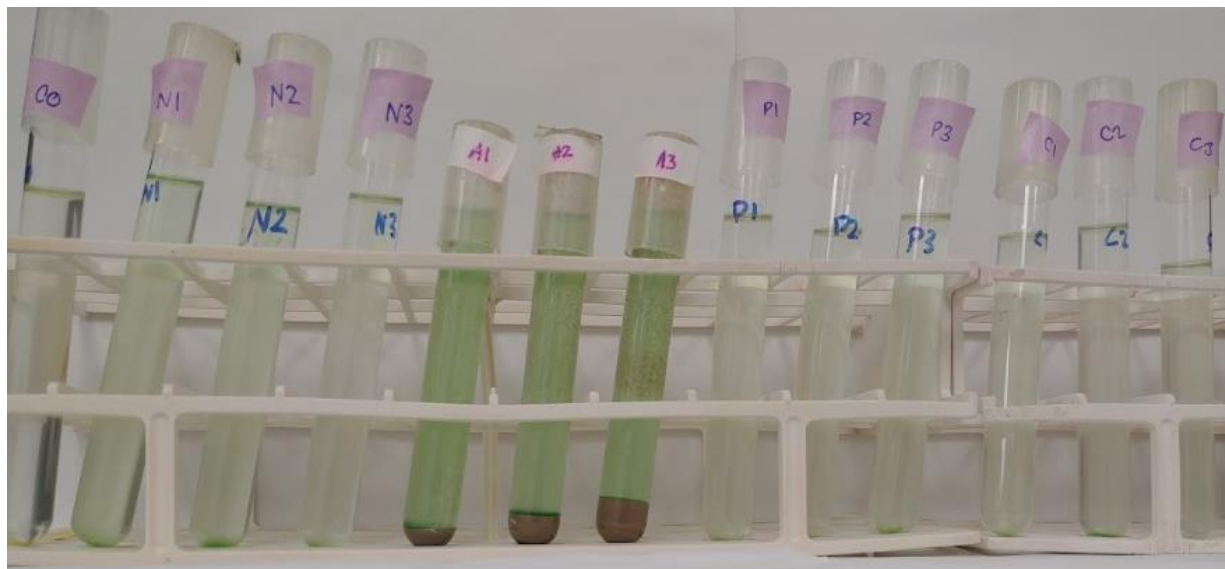


Figure 8- Day 27 of test tube microcosm experiments. Samples containing Glenmore Reservoir sediment exhibited noticeably enhanced *M. aeruginosa* proliferation.

The presence of fine sediment in the microcosms (A1, A2, A3) clearly enhanced *M. aeruginosa* cell proliferation (Figure 8). The photograph suggests that the sediment provided a sufficient mixture of macronutrients and micronutrients to support cyanobacterial growth. Within a benchtop setting, the presence of sediment proliferating cyanobacterial growth was previously been investigated by Crumb (2016). In that study, microcosms were dosed with a target cell density of 3.59×10^6 cells/mL and after 14 days of growth, cyanobacteria grew better in sediments compared to flasks only with filtered reservoir water (166). As previously discussed in Section 4.2.1, the synergistic contributions of P and N together can lead to growth (208). Guildford & Hecky (2000), along with work by Dolman et al. (2012) found that ratios of N and P can greatly affect growth (209,212). As sediments are capable of desorbing both N (often in the form of inorganic ammonium, which is bioavailable) and P, microcosms with sediment could have allowed proliferation of growth by desorption of N and P together (58,110). However, it is not possible to

confirm that growth occurred due to desorption of nutrients such as P from sediment, as concentrations of nutrients or any other dissolved constituents were not monitored throughout the experiments. Regardless, microcosms containing sediment (A1, A2, A3) were the only treatments that promoted noticeable growth and cell densities similar to those discussed in Section 4.2.1.

The cell densities observed on day 27 of this experiment were consistent with literature. Mean cell densities on day 27 of the experiment are presented in Figure 9. The mean cell density in the sediment amended microcosms was 2.16×10^6 cells/mL compared to 1.43×10^6 cells/mL, 9.38×10^5 cells/mL, and 8.42×10^5 cells/mL in the nitrate amended (N1, N2, N3), phosphate amended (P1, P2, P3), and carbonate amended (C1, C2, C3) treatments, respectively. The presence of sediment proliferating cyanobacterial growth was previously been investigated by Crumb (2016). In that study, microcosms were dosed with a target cell density of 3.59×10^6 cells/mL. Final cell densities on day 28 of those experiments had final cell densities ranging from 10^7 to 10^8 cells/mL in microcosms with sediment, and $10^{6.5}$ cells/mL to 10^7 cells/mL with microcosms without sediment (166). Cell densities reported by Crumb (2016) are much higher than those observed in the present research. The higher cell densities may be attributed to a few factors. First of all, inoculation concentrations were much greater in the Crumb (2016) experiments. Second, reservoir water used in the Crumb (2016) experiments were from a mesotrophic water source, which means the nutrient concentrations were likely higher than in the Glenmore Reservoir water (mesotrophic-oligotrophic). The reservoir ambient SRP concentration in Crumb (2016) was $32 \mu\text{g/L}$ (166), while in contrast, Glenmore Reservoir ambient SRP concentrations ranged from 2 to $4 \mu\text{g/L}$. The sediments used in Crumb (2016) also had higher EPC_0 of $82 \mu\text{g/L}$, indicating a P release of $\sim 5 \mu\text{g P/g}$ of sediment (166), which is much higher than the EPC_0 reported in the sediments used in these experiments, corresponding to a release of approximately $\sim 2 \mu\text{g P/g}$ of sediment in Head Pond (Section 4.1.3). Thus, the cell densities observed here demonstrate that growth in these experiments were comparable.

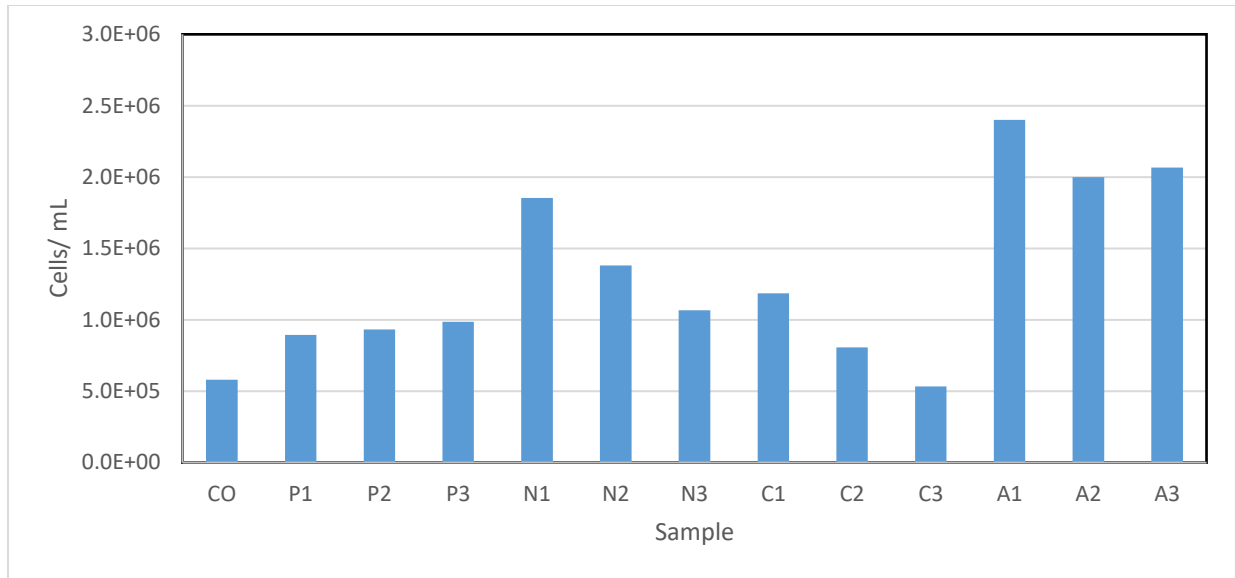


Figure 9- Cell densities of *M. aeruginosa* in test tube microcosms on day 27. Samples dosed with sediment (A1, A2, A3) had higher cell densities than other singular nutrient doses.

Differences between the various amendments may not be statistically significant because of the amendment concentrations (or in this case, mass of sediment) varied between the microcosms. Thus, replication would be required for more rigorous comparison. Increased replication was beyond the scope of this experiment. These experiments were designed to provide a simple indication of the type of amendment(s) that would enhance *M. aeruginosa* proliferation in modified mesotrophic-oligotrophic waters.

4.2.3 Factorial Design Microcosm Experiment: Investigating the Effects of Sediment Source and Nitrate Concentration

Following the test tube microcosm experiment, a factorial design microcosm experiment was designed to investigate the effects of nitrate concentration and sediment type (wildfire and anthropogenically-impacted) on *M. aeruginosa* proliferation. Pigments and cell densities in response to these parameters were evaluated. Consequently, the results from using mesotrophic-oligotrophic waters and sediment in cyanobacterial proliferation are discussed in following

Sections 4.2.3.1 and 4.2.3.2. Notably, previous benchtop experiments using cyanobacteria have never used mesotrophic-oligotrophic waters. A summary of previous works is presented in Table 11. To the author’s knowledge, no other microcosm experiments that investigated nitrate amendments and sediment source had previously been conducted. Because this work is original in utilizing natural mesotrophic-oligotrophic waters, the most relevant work will be referenced in subsequent sections.

Table 11- Summary of previous benchtop microcosm experiments using cyanobacteria.

Cyanobacteria Cultured	Growth Medium	Objective	Source
<i>M. aeruginosa</i>	Deionized water	Determine impacts of hydrodynamic disturbance on P release	Huang et al. (2015)
<i>M. aeruginosa</i>	Eutrophic-mesotrophic reservoir water	Investigate role of iron in P sequestration	Crumb (2016)
<i>M. aeruginosa</i>	P free BG11 growth medium	Evaluate P release from sediment induced by cyanobacterial blooms	Hao et al. (2016)
<i>Spirulina platensis</i>	Modified Schölsser (1982) medium (with nitrate instead of ammonium)	Explore differences in N sources to <i>S. platensis</i> proliferation	Soletto et al. (2005)
<i>M. aeruginosa</i>	Modified BG11 (use of nitrate, urea, and ammonium for N)	Photosynthetic response from various N sources and P	Peng et al. (2016)
<i>M. aeruginosa</i> and <i>M. flos-aquae</i>	Modified BG11 with 10× more carbon, and 1/50 amount of N	Explore colony formation mechanism of <i>Microcystis</i> cells	Liu et al. (2016)
<i>Cyanobium</i> sp., <i>Aphanocapsa muscicola</i> , <i>Pleurocapsa minor</i> , <i>Pseudanabaena catenata</i> , <i>Leptolyngbya boryana</i> , <i>Leptolyngbya nostocorum</i> , <i>Phormidium</i> sp., <i>Nostoc carneum</i> , and <i>Tolypothrix tenuis</i> .	Modified CHU10 medium, BG11, and Allen & Arnon medium	Investigate changes in cyanobacterial community composition to various nutrient enrichment	Loza et al. (2014)

4.2.3.1 *M. aeruginosa* Pigment Analyses

There were no obvious trends in effects of nitrate concentrations or sediment source in total pigment concentrations between the microcosms. Total pigment concentrations for samples varied from 0.77 $\mu\text{mol /L}$ to 7.77 $\mu\text{mol/L}$. The highest concentrations of detected pigment analyses are summarized in Figure 10. Chlorophyll *a* was the dominant pigment in all microcosms, which is expected given that chlorophyll *a* is related to photochemical activity of oxygen-evolving organisms, including cyanobacteria (213). The only exception to this observation was N2HP-R1, in which a fucoxanthin-like pigment was dominant. The total pigment concentration in that microcosm was more than double that in any other microcosm. Thus, it was excluded from the results because it was considered to be outside the range of natural variability and can be found in Appendix 4: *M. aeruginosa* Factorial Experiments- Supplementary Data. Overall, no clear trends in pigment production or concentration were observed.

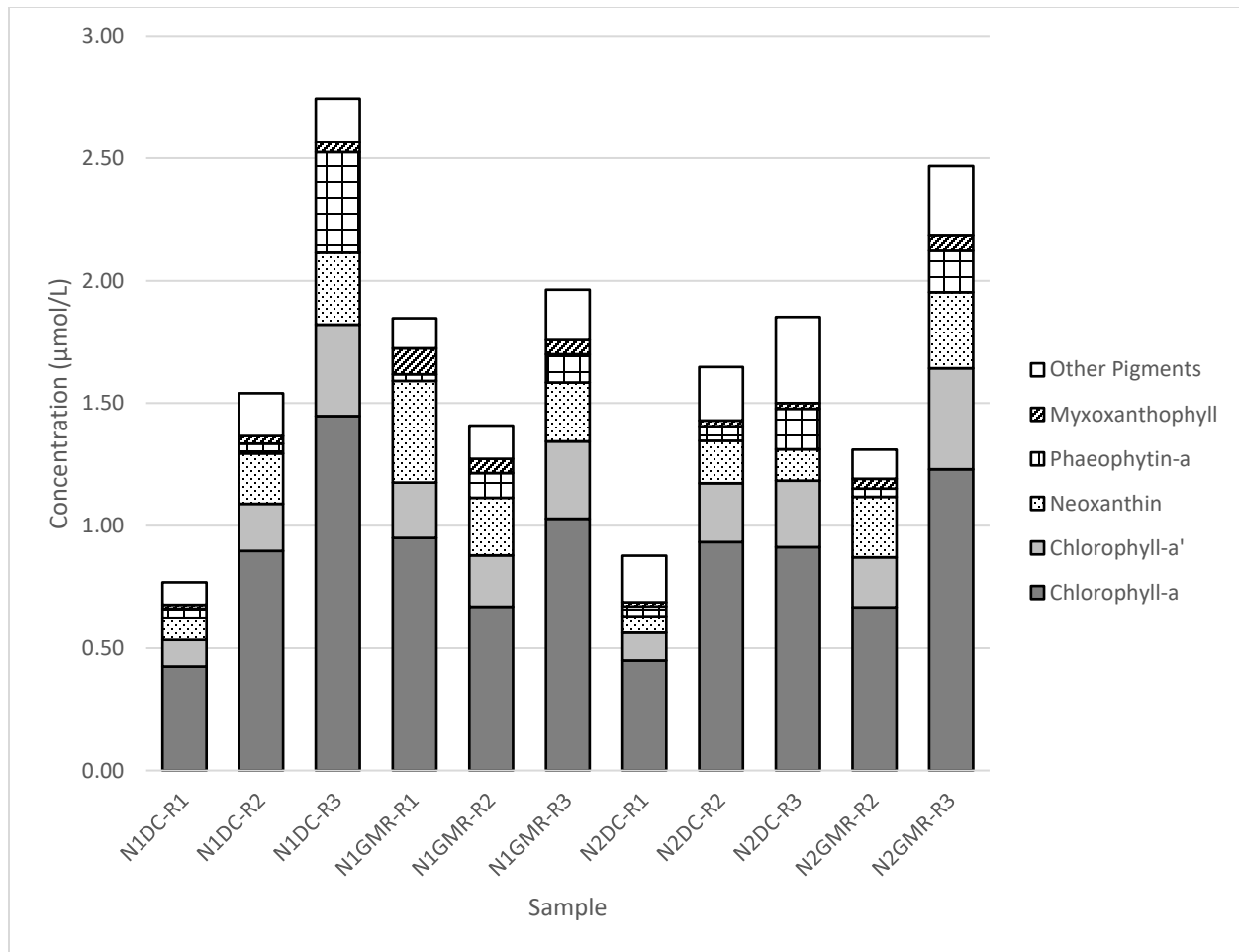


Figure 10- Dominant pigment concentrations ($\mu\text{mol/L}$) observed on Day 60 of the factorial design experiments. There were no obvious trends of pigment concentrations given various N amendments and sources of sediments. Most samples were primarily composed of chlorophyll *a*. Other pigment concentrations are available in Appendix 4.

Cyanobacteria absorb light energy through photosynthesis (1,84). Photosynthetic processes occur in the thylakoid through the use of pigments: chlorophyll *a*, chlorophyll *b*, together with carotenes or phycobilins (84,214,215). Chlorophyll *a* can be absorbed at peak wavelengths of approximately 470 nm and 680 nm. Chlorophyll *a* typically acts as the main photosynthetic pigment used to capture light energy within a certain range. Chlorophyll *b*, carotenoids, and phycobilins are accessory pigments that can extend the range of wavelengths that cyanobacteria utilize (Figure 11)

(84,216). Chlorophyll *b* has absorption peaks at approximately 450 nm and 650 nm. Carotenoids and phycobilins have peak absorbances of 460 nm and 500 nm, and 500 nm to 570 nm, respectively. The absorption spectra for chlorophyll *a*, chlorophyll *b*, carotenoids, and phycobilins are illustrated in Figure 11.

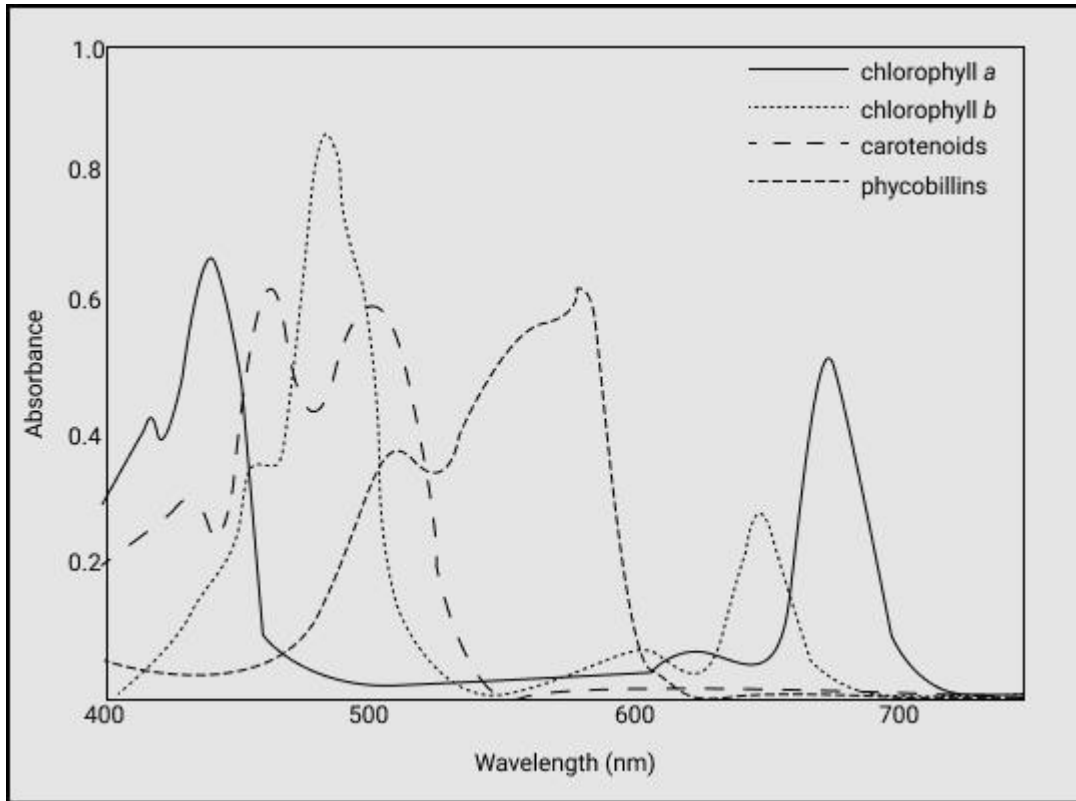


Figure 11- Absorption spectra for chlorophyll *a*, chlorophyll *b*, carotenoids, and phycobilins. Adapted from Graham & Wilcox (2000) (84) and Hemholtz Centre for Ocean Research Kiel (217).

Elevated neoxanthin and myxoxanthophyll pigment concentrations were significantly greater in microcosms containing Head Pond sediment compared to those with Drum Creek sediment. Samples treated with Head Pond sediment had significantly more neoxanthin ($p=0.023$) and myxoxanthophyll ($p=0.005$) pigments compared to microcosms with Drum Creek sediment. Both neoxanthin and myxoxanthophyll are carotenoids and can be produced in response to

photooxidative stress (84,215,216). These results were unexpected, as there were no changes in light variability for different treatments of sediment. The production of the neoxanthin could be due to thylakoid organization and stress (215), while myxoxanthophyll has been reported to contribute to cell wall structure and thylakoid organization of the cyanobacterium *Synechocystis* sp. (214). Assuming that the function of myxoxanthophyll is similar in *M. aeruginosa*, the presence of this pigment could further confirm that thylakoids within the cells of microcosms with Head Pond sediment were not experiencing the same environmental pressures as microcosms with Drum Creek sediment. At the time of pigment analyses, cells in the Head Pond microcosms were already in the stationary phase of growth and had higher cell densities compared to Drum Creek microcosms. Thus, it is possible that nutrient depletion was occurring in the microcosms containing Head Pond sediment. Other works have indicated that nutrient enrichment and deficiency can result in pigment and colour changes amongst phytoplankton and cyanobacteria (218,219). For example, research conducted by Collier & Grossman (1992) found that deprivation of sulfur and N, resulted in visual bleaching differences of cyanobacterium *Synechococcus* sp. cells. In this work, only concentrations of chlorophyll *a* decreased after sulfur and N deprivation (219). This is consistent with work completed by Bonilla (2005), who reported that chlorophyll *a* concentrations increased strongly in response to enrichment (218). Although there is limited literature on how other carotenoid pigments may be affected by nutrient deprivation, these works confirm that pigment chlorophyll *a* can be affected by changes in nutrient availability. Therefore, the significantly higher carotenoid pigments in Head Pond sediment than in Drum Creek sediments may be attributed to nutrient and/or thylakoid stresses.

Chlorophyll *b* concentrations in microcosms containing Drum Creek sediment were significantly higher than microcosms containing Head Pond sediment. These results were in contrast with carotenoid pigments, and microcosms with Drum Creek sediment contained significantly more chlorophyll *b* ($p=0.039$). While most microcosms with Drum Creek sediment contained chlorophyll *b*, no detectable concentrations of chlorophyll *b* were found in any of the microcosms with Head Pond sediment. Previous research has indicated that chlorophyll *b* can functionally act to substitute chlorophyll *a* in cyanobacteria (220,221). Thus, the presence of chlorophyll *b* pigment

in the Drum Creek sediment could indicate the need of cyanobacteria to access additional light spectra. In research conducted by Tandeau de Marsac (1977), growing cyanobacteria with various light sources [green (peak at approximately 525 nm), red (peak at approximately 650 nm) and white] resulted in changes to pigment production by cells (222). Although yellow light [wavelength of approximately 580 nm (223)] or effects of coloured medium were not explored by Tandeau de Marsac (1977), a yellow hue was visually observed within the Drum Creek treated sediment flasks (Figure 12) and this may have potentially interfered with accessibility to wavelengths of light. The yellow hue is also associated with the substantially higher levels of dissolved organic carbon (DOC) (224). Elevated concentrations of DOC have been documented to correlate with coloured dissolved organic matter- which can absorb substantial fractions of wavelengths adversely affecting the photosynthetic functions of algae (further discussed in Section 4.2.3.2) (207). Consequently, the elevated chlorophyll *b* concentration observed in Drum Creek may be attributed to higher DOC concentrations.

Light variability within the growth cabinet could have potentially affected the health of cyanobacterial cells. Flasks within the growth cabinet were not arranged in any order, but they were also not intentionally randomized. Thus, it is possible some flasks faced higher light intensities than others over the experimental period. With increased light intensity, concentrations of chlorophyll *a* may decrease (225). Danesi et al. (2004) found that compared to lower light intensities (2 klux), higher light intensities (5 klux) led to lesser production of chlorophyll *a* (225,226). The light intensity measured in the growth cabinet for this research was 1776 lux, or 1.78 klux, suggesting that light intensities in the growth cabinet would be sufficient for growth. Literature documenting effects of light on carotenoids have been inconsistent. While work conducted by Danesi et al. (2004) found that carotenoid concentrations may not be affected (225), research conducted by Dall'Osto et al. (2007) and Goodwin (1980), found that neoxanthin and myxoxanthophyll may be produced in response to photooxidative stress (215,216), respectively. In this work, while there were no significant differences in chlorophyll *a* [N concentrations ($p=0.742$), sediment type ($p= 0.800$), and interaction between N concentrations and sediment type ($p=0.651$)], significant differences were observed in carotenoid pigments (neoxanthin and

myxoxanthophyll) and chlorophyll *b*. Differences in carotenoid pigments and chlorophyll *b* may be explained by nutrient stresses due to high cell densities and DOC concentrations, respectively. Therefore, light variability may not have played a key role in causing significant differences observed in the pigment analyses herein.

Overall, the pigment data suggest that different environmental conditions exist in the microcosms due to differences in sediment as a function of sediment source. This would be expected, as sediments were collected from a relatively nutrient poor reservoir and a wildfire-impacted river still recovering from disturbance. While some potential explanations for the observed differences were presented above, a more in depth investigation of those ecosystem characteristics and dynamics is warranted but beyond the scope of the present study.

4.2.3.2 *M. aeruginosa* Cell Densities

All samples exhibited growth phases typically seen with prokaryotic organisms observed in batch cultures. Cell densities were significantly different between days 21, 39, and 60 ($p=0.02$). These results were expected because cell densities in batch cultures typically have the following growth phases: a lag phase, exponential growth phase, and stationary phase (203). The microcosm experiments were discontinued before the death phase could occur. The lag phase occurred from days 1 to 21, during which cell densities of all microcosms varied from 2.2×10^5 cells/mL to 2.3×10^6 cells/mL. Samples were inoculated from a culture which contained half BG11 solution and half reservoir water collected from the Head Pond region of the Glenmore Reservoir (details presented in Section 3.7.1). It is possible that the observed lag time was due to the cyanobacterial cells adapting to conditions of a lower nutrient environment (86,180). From days 24 to 42, a noticeable increase in growth rate (the exponential phase) was observed with all microcosm cell densities ranging from 7.3×10^6 cells/mL to 1.6×10^8 cells/mL. A stationary phase from days 44 to 60 (end of the experiment) was observed. The stationary phase could have occurred likely due to high density of cyanobacterial cells in the medium and/or an exhaustion of nutrients within the batch experiment as nutrients were not added after the initial inoculation. Cell densities within this time varied from 8.2×10^6 cells/mL to 9.3×10^8 cells/mL. It should be noted that cell densities counted on day 54 are likely relatively low because of a confirmed enumeration error. Previous

benchtop work conducted by Crumb (2016) inoculated samples of sediment and reservoir water with 3.59×10^6 cells/mL. In this same study, cell densities in the stationary phase ranged from 10^7 cells/mL to 10^8 cells/mL, which are within the range of cell densities in this research. Huang et al. (2015) inoculated treatments with deionized water and sediment, with an initial target density of 8×10^5 cells/mL, and growth was monitored over 21 days by measuring chlorophyll *a* (33). Although these experiments measured chlorophyll *a*, they were similar to growth patterns observed in this experiment. Both exhibited exponential and stationary phases of growth. Some variability was observed within the individual microcosms, but it is likely within the range of natural variability. Given that all treatments of the factorial experiment grew following the typical prokaryote growth curve, there is compelling evidence to support that sediment or dosed nitrate contributed to cyanobacterial proliferation. Therefore, growth phases observed in these experiments are consistent with literature.

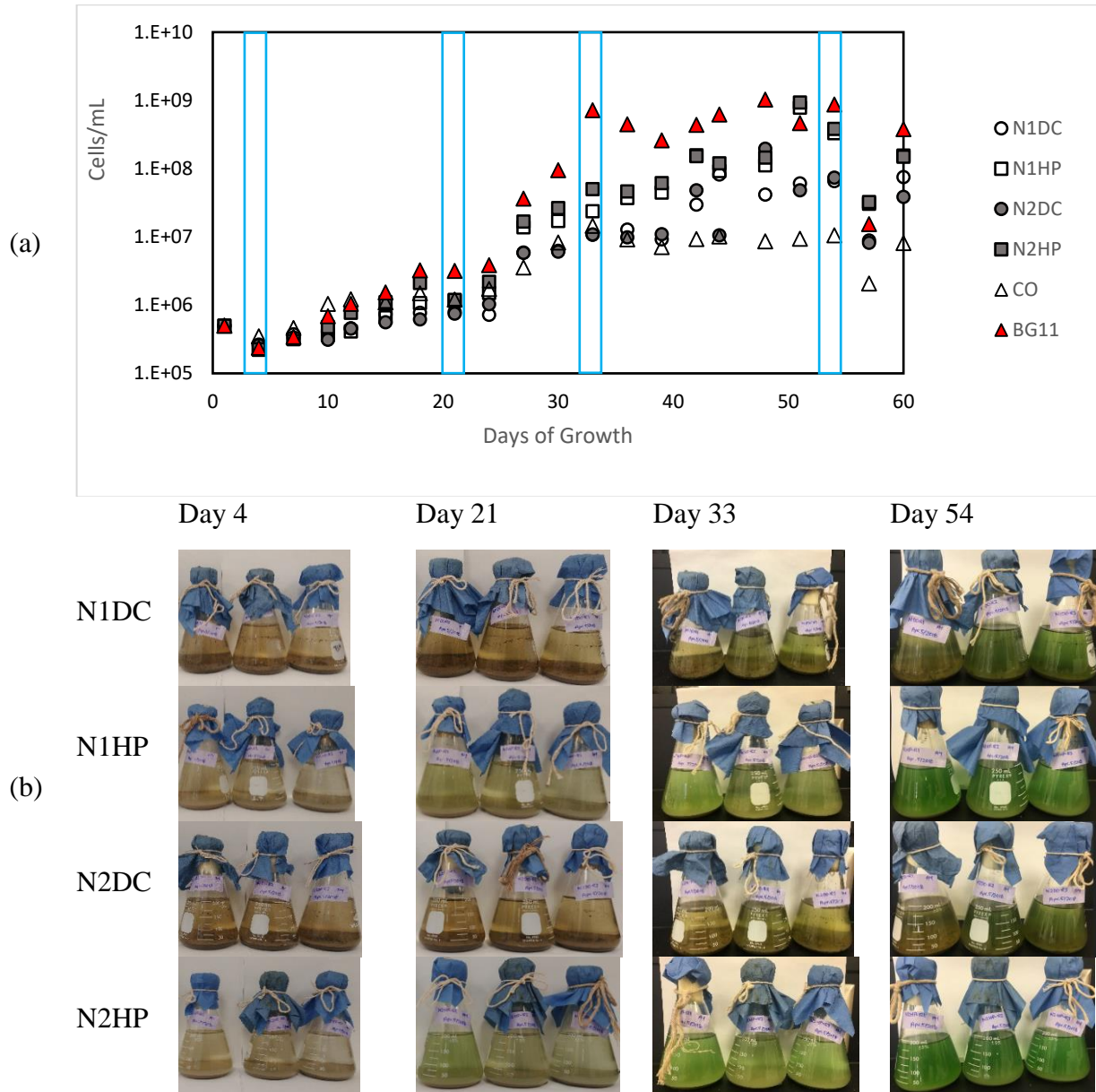


Figure 12- Photographs of factorial design microcosms illustrating cell densities (a) All cell densities of *M. aeruginosa* microcosms increased as expected over the course of 60 days (b) Sequential photos of various treatments on days 4, 21, 33, and 54 visually confirm that cell densities increased. Samples treated with DC sediment appeared to have a yellow hue compared to microcosms treated with HP sediment.

Unexpectedly, cell densities in microcosms with Head Pond sediment were significantly greater ($p=0.002$) than those grown with Drum Creek sediment. Previous studies of wildfire on primary productivity have generally agreed that post wildfire conditions lead to increases in algal and cyanobacterial biomass. Robinson et al. (1994) found that phytoplankton communities experienced the greatest changes in extensively burned catchments (227). Spencer et al. (2003) observed short term impacts of dense algal growth the first spring after a wildfire (228). In addition to potential short term impacts, algal productivity may increase in long term scenarios (five years post wildfire), as observed by Silins et al. (2014) (48). These increases in primary productivity are often attributed to increased availability of nutrients post wildfire (42,229,230). The results of Phase 1 experiments demonstrate that Drum Creek sediment desorbs more bioavailable P than Glenmore Reservoir sediment (27,28) which is contrary to what might be expected based on previous work on algal proliferation and potential nutrient availability (48). Although results from the statistical analyses were unexpected, there are several potential explanations as to why microcosms with sediment from Glenmore Reservoir had significantly higher cell densities compared to microcosms with wildfire impacted sediment from Drum Creek.

1. It is possible that P is not the driver required for cyanobacterial growth and other nutrients or environmental conditions may be limiting factors. Other nutrients such as N could potentially limit growth (160,182,210) but changes in N species were not analyzed in this experiment. In addition to N and P, other micronutrients such as iron have been documented to increase cyanobacterial and algal proliferation and play a key role in photosynthesis (210,231). Moreover, some forms of N that could potentially desorb from sediment are more bioavailable to cyanobacteria (58,110). Ammonium is the most common form of N to desorb from sediment and is considered to be more bioavailable than nitrate for cyanobacteria (160).
2. Compounds in the Drum Creek sediment may have inhibited growth. The desorption of heavy metals from sediment, which is known to increase post wildfire, could inhibit cyanobacterial growth (232–234). Previous work conducted by Burnet et al. (2009) found that treatments with copper and lead can lead to reductions in total filamentous

cyanobacteria (232). Previous studies conducted by Lu et al. (2000) and Singh & Singh (1992) also reported that mercury hindered growth of cyanobacteria *Streptomyces platensis* and *Nostoc calcicola*, respectively (233,234). Therefore, it is possible that desorption of heavy metals from wildfire impacted sediment hindered *M. aeruginosa* growth in microcosms with Drum Creek sediment.

3. The conceptual discrepancy of higher cell densities observed in microcosms with Head Pond sediment compared to microcosms with Drum Creek growth could also be attributed to elevated DOC concentrations inhibiting cyanobacterial growth (Table 12). DOC can impact cyanobacteria depending on its chemical nature and the availability of other nutrients within the system (44,207,235). Elevated concentrations of carbon could have different effects on cyanobacteria depending on their chemical nature and limiting nutrients within the system (44,207,235). In this case, it is likely that elevated concentrations of allochthonous DOC (i.e. terrestrial in origin), could have led to relatively higher light attenuation, thus, reducing photosynthetic activity and primary productivity (207).
4. Increased cell densities of cyanobacteria can potentially increase the pH (57). This increase of pH can affect the P mobility from sediment to the water column, depending on the type of sediment (57,58). Thus, it is possible that the effects of cyanobacterial growth were further perpetuating initial growth trends leading to this significant difference.

Higher levels of cyanobacterial cell densities were expected in microcosms containing post-fire sediments from Drum Creek. Accordingly, further detailed exploration is required to delineate the factors that contributed to the observed differences, but this is beyond the scope of the present investigation. This result is notable nonetheless and merits follow up to further inform wildfire-associated legacy impacts and threats to the provision of safe drinking water.

Table 12- Total Organic Carbon (TOC), Dissolved Inorganic Carbon (DIC) and Total Dissolved Carbon (TDC) at day 1 and day 60 of microcosm factorial design experiments

	<i>Day 1</i>			<i>Day 60</i>		
	DOC (µg/L)	DIC (µg/L)	TDC (µg/L)	DOC (µg/L)	DIC (µg/L)	TDC (µg/L)
N1HPR1	1.77	0.17	1.93	24.50	0.08	24.60
N1HPR2	1.77	0.17	1.93	17.63	0.10	17.73
N1HPR3	1.77	0.17	1.93	16.53	0.10	16.63
N1DCR1	1.77	0.17	1.93	42.60	0.19	42.80
N1DCR2	1.77	0.17	1.93	46.80	0.14	46.90
N1DCR3	1.77	0.17	1.93	43.83	0.13	43.93
N2HPR1	1.77	0.17	1.93	21.67	0.10	21.77
N2HPR2	1.77	0.17	1.93	17.17	0.11	17.27
N2HPR3	1.77	0.17	1.93	16.73	0.10	16.83
N2DCR1	1.77	0.17	1.93	50.67	0.42	51.07
N2DCR2	1.77	0.17	1.93	45.87	0.18	46.07
N2DCR3	1.77	0.17	1.93	51.13	0.19	51.33

Lastly, no significant differences were found between microcosms containing the various nitrate amendments or their interactions with other experimental factors. This result was unexpected as higher concentrations of N have been found to correlate sometimes with higher biovolume of cyanobacteria. Chaffin (2013) postulated that there was no linear relationship with increases of total P with *Microcystis* biovolume in Lake Erie, whereas an increase of N caused significant linear relationships with annual biovolume (150). Similarly, Ma et al. (2015) observed that N only additions induced growth, but P additions alone did not (21). The differences from those experiments may be attributed to the presence of *M. aeruginosa*, and the entire phytoplankton community (212,236), as these works investigated the potential proliferation of *M. aeruginosa* alone. There are a couple of reasons this inconsistency may have occurred. First, it is possible that ratios of nutrients between N and P were not ideal for growth, as some work indicates that the limiting nutrient depends on a certain threshold concentration (212). Further, differences may be attributed to benchtop works and growth within a growth cabinet, as it is considered within ideal

conditions. Thus, the inconsistencies between N contributions to *M. aeruginosa* and biomass are compelling, and further work to delineate reasons for these discrepancies is recommended.

Other studies have been conducted in mesocosms within the natural environment to investigate the effects of natural waters, sediment, and nitrate on cyanobacterial growth. Axler & Reuter (1996) examined the effects of nitrate amendments in mesocosms in Castle Lake, a mesotrophic-oligotrophic lake in California (237). Mesocosms with sediment in the lake were dosed with NO₃-N and NH₄-N. However, these experiments investigated and compared the preferential uptake of N form and not the biomass within the mesocosms. Therefore, results from this experiment unfortunately are not comparable. Research conducted by Xie et al. (2003) investigated the presence of sediment on proliferating cyanobacterial growth in eutrophic in-lake mesocosms. Results from Xie et al. (2003) suggested that mesocosms with and without sediment could both promote growth, but the presence of sediments could further accelerate the release of P from sediment due to changes in pH that were affected by photosynthetic activity (165). In contrast to the results of Xie et al. (2003), the benchtop microcosms in the present work did not exhibit much growth in the absence of sediment. This could potentially be attributed to the low nutrient waters (mesotrophic-oligotrophic) used compared to high nutrient hyper eutrophic waters used by Xie et al. (2003). Additionally, autoclaved sediment and water used in this research would also potentially impact growth. The direct impacts of autoclaving sediment and water are unclear. However, it is possible that growth of *M. aeruginosa* could potentially be supported through community dynamics, or populations of *M. aeruginosa* would decrease due to competition for resources. Consequently, similar research investigating natural waters, sediment, and nitrate have taken place within the environment but are not comparable.

To the author's knowledge, experiments relating the direct effects of sediment presence to natural mesotrophic-oligotrophic waters have not been conducted previously. The factorial design microcosm results presented herein are not entirely consistent with the literature. Although there are similarities in experimental design, the implementation of experiments and objectives of previous work were not the same. Investigative work for understanding cyanobacterial growth in laboratories has typically been conducted at benchtop scale in controlled environments utilizing

specific growth media (159,182,238,239). There are certainly limitations associated with these experiments because experimental conditions are not representative of natural systems. For example, autoclaved sediment and water do not allow for any microbial or phytoplankton competition in the community. Further, ideal growth conditions in growth cabinet and modified reservoir water could have allowed for unrealistic and ideal conditions which would not likely occur in nature. Despite these limitations, this type of microcosm approach using modified natural waters and sediment in a controlled laboratory environment can be a tool for better understanding the impacts of source water quality change on cyanobacterial proliferation in drinking water reservoirs. This type of analysis may be very useful in informing reservoir management, source water protection planning, and climate change adaptation and mitigation strategies. Overall, results from this research suggest that the main drivers of *M. aeruginosa* proliferation and the differences observed between the microcosms containing the various sediment types were driven by the sediment.

4.2.4 Control/ Reference Microcosms

Control/reference microcosm treatments were conducted to provide perspective on the factorial design. All treatment flasks described in Section 4.2.3, contained N and sediment amendments but control/reference microcosms were also examined. The growth curves over 60 days of *M. aeruginosa* obtained for these treatments are presented in Figure 13. These flask microcosms allowed for interpretation of the effects of singular nutrients, sediment type, or positive (BG11) and negative (CO) references.

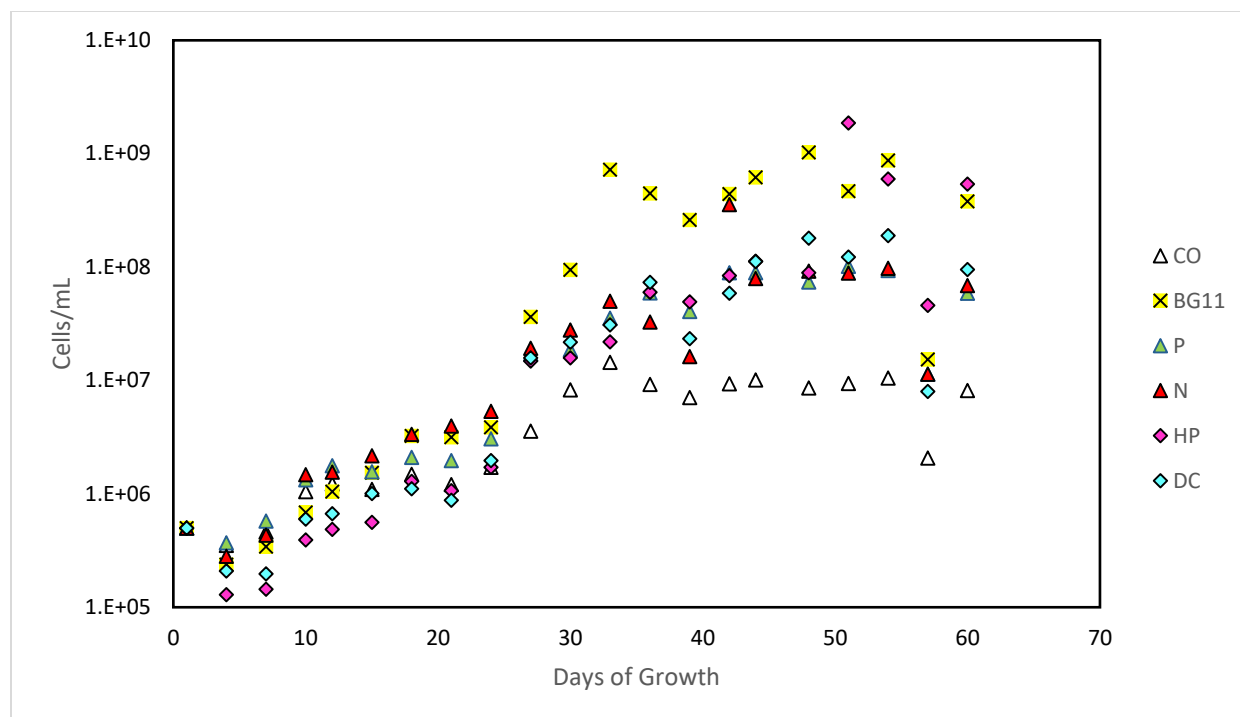


Figure 13- *M. aeruginosa* growth curves for control/reference microcosms during the factorial design microcosm experiment. These included: BG11 (BG11, positive reference), reservoir water only (CO, negative reference), Drum Creek sediment with reservoir water (DC), Head Pond sediment with reservoir water (HP), phosphate amendment (P), and nitrate amendment (N). As expected, BG11 reference microcosm had highest cell densities and CO reference microcosm had lowest cell densities amongst all other control/reference samples.

The growth for both positive reference (BG11) and negative reference (CO) were as expected. Cell densities in the BG11 control microcosm had cell densities that ranged from 1.53×10^7 cells/mL to 1.03×10^9 cells/mL from days 30 to 60. These results were not surprising as BG11 is widely used to culture *M. aeruginosa* (82,166,205,240). In contrast, CO microcosm cell densities were the lowest observed and ranged from 2.07×10^6 cells/mL to 1.45×10^7 cells/mL between days 30 to 60. CO containing Glenmore Reservoir water was relatively nutrient poor with no nutrient amendments. The effects of growing in nutrient rich medium compared to nutrient low medium have been previously discussed in Sections 4.2.1, 4.2.2, and 4.2.3. In conclusion, BG11 and CO treatments exhibited expected growth and assumed to be reliable reference samples.

The growth for treatments with N and P yielded interesting results. N and P exhibited similar growth trends to CO, with cell densities during the stationary phase that were approximately one order of magnitude higher than those in the CO microcosm. Cell densities in N and P varied from 1.13×10^7 cells/mL to 3.57×10^8 cells/mL and 1.13×10^7 cells/mL to 1.01×10^8 cells/mL from days 30 to 60, respectively. Although control samples were conducted with no replication, these microcosm results suggest that the contributions of N and P alone supported some growth, but not to the extent that BG11 could. These results are consistent with visual observations in Section 4.2.2. Therefore, N and P as singular nutrients can support some growth- but multiple nutrients (or a dual nutrient regime, as discussed in Sections 4.2.1 and 4.2.2 (21,23,95,212)) is likely what supports greatest growth in these mesotrophic-oligotrophic waters.

Microcosms of HP and DC, containing Glenmore Reservoir water and fine sediment from Head Pond and Drum Creek, respectively, were the best growing samples following BG11. Cell densities in HP and DC from days 30 to 60 were 1.6×10^7 cells/mL to 1.9×10^9 cells/mL and 8×10^6 cells/mL to 1.9×10^8 cells/mL, respectively. Notably, the cell densities are within the range of those observed in factorial experiment microcosms (discussed in 4.2.3.2). The factorial experiment microcosms also contained excess concentrations of nitrate amendment. This observation demonstrates that the *M. aeruginosa* proliferation observed in the factorial experiment microcosms containing fine sediment was predominantly driven by sediment addition rather than nitrate amendment. This conclusion is further supported by the factorial experiment microcosm data that demonstrate similar levels of *M. aeruginosa* cell proliferation irrespective of the level of nitrate amendment implemented. Additionally, these results were also consistent with visual evidence exhibited during the test tube microcosm experiments discussed in Section 4.2.2. Thus, the proliferation of samples HP and DC with sediment and natural waters alone, demonstrated that fine sediment enhances *M. aeruginosa* proliferation.

Chapter 5: Conclusions

It is widely recognized that fine sediment is the primary vector for P transport to and within rivers. When sediment is transported and subsequently deposited in downstream drinking water reservoirs, the associated contributions to nutrient (especially P) release to the water column are often overlooked. While P has commonly been considered the key limiting nutrient for primary productivity in freshwater bodies, the effects of N have been emphasized as key contributors for cyanobacterial bloom toxicity and formation. Thus, P and N dynamics are essential for reservoir management as critical drivers for cyanobacterial bloom formation and toxicity. The importance of these water quality parameters in source water protection and reservoir management strategies is further underscored by their association with landscape disturbances (e.g., floods, hurricanes, and wildfires) that are exacerbated by climate change, as well as anthropogenic disturbances (e.g., development and resource extraction), which can all lead to increases in erosion, sediment mobility and transport, and associated nutrient bioavailability.

This investigation demonstrated the direct connectivity between fine sediment and 1) nutrient releases to the water column and 2) associated potential for the proliferation of cyanobacteria. In general, this work showed that fine sediment can significantly contribute to the proliferation of toxin-forming cyanobacteria, regardless of the availability of excess nitrate. Key conclusions from this work are detailed below.

1. As rivers flow into reservoirs, changes in flow velocity cause downstream fining in which suspended solids settle according to size and density (selective sorting), such that larger particles generally deposit upstream, while smaller ones (i.e., fine grained sediments, typically $<63\ \mu\text{m}$) travel further downstream and are preferentially deposited in reservoirs. In the City of Calgary's Glenmore Reservoir, the median grain size diameter (D_{50}) of deposited sediment was $<10\ \mu\text{m}$ (averages ranging from $3.16\ \mu\text{m}$ to $7.23\ \mu\text{m}$), whereas suspended solids ranged from $243\ \mu\text{m}$ to $33\ \mu\text{m}$ in the Elbow River, at progressively downstream locations prior to entering the reservoir.

2. Fine-grained sediments that preferentially deposit in reservoirs contain relatively higher levels of P compared to the larger materials that settle upstream. In the reservoir, TPP concentrations ranged from 579.7 $\mu\text{g P/g}$ to 765.1 $\mu\text{g P/g}$ sediment, with the highest concentrations occurring at the farthest distance from the reservoir inlet. In contrast, they ranged from 247.8 $\mu\text{g P/g}$ to 418.1 $\mu\text{g P/g}$ suspended solids in the Elbow River, at progressively downstream locations prior to entering the reservoir.
3. Reservoir sediments are generally enriched with the most bioavailable particulate P form (NAIP) relative to upstream suspended solids. This is consistent with the relatively greater fractions of Al_2O_3 , Fe_2O_3 , and MnO which are associated with NAIP fractions. Here, the NAIP fraction gradually increased with downstream distance within the Elbow River and Glenmore Reservoir. These results reaffirm that smaller grain sizes (D_{50}) generally have higher fractions of NAIP, as the highest NAIP concentrations were associated with fine sediments located at the farthest distance from the reservoir inlet. The NAIP concentrations ranged from 67.5 $\mu\text{g NAIP/g}$ to 146.4 $\mu\text{g NAIP/g}$ sediment in the reservoir and 21.3 $\mu\text{g NAIP/g}$ to 26.6 $\mu\text{g NAIP/g}$ suspended solids in the river.
4. Fine-grained sediments can release P to the water column when aqueous P concentrations are below the EPC_0 —those that preferentially deposit in reservoirs are likely to release P. Trends regarding the D_{50} and EPC_0 were not clear throughout Elbow River, and this may be attributed to differences in sampling methodology. In the reservoir, the average EPC_0 at each site ranged from 8.8 $\mu\text{g P/L}$ to 23.7 $\mu\text{g P/L}$, with the highest concentrations occurring at the farthest distance from the reservoir inlet. In contrast, they ranged from 34.7 $\mu\text{g P/L}$ to 12.4 $\mu\text{g P/L}$ for suspended solids in the Elbow River, at progressively downstream locations prior to entering the reservoir.
5. While the conclusions above regarding P form and mobility as related to the grain size of riverine suspended solids and reservoir sediments are generally consistent with previously reported investigations, the specific contributions of fine sediment to potentially toxin-forming cyanobacterial growth have been suggested, but not demonstrated incontrovertibly. The laboratory benchtop studies reported herein validate that reservoir sediments can promote the proliferation of potentially toxin-forming *M. aeruginosa* cyanobacteria, even

in low nutrient, mesotrophic-oligotrophic waters—this is a first of its kind, proof-of-concept demonstration of this relationship. Further work is needed to rigorously determine the exact specific nutrient contributions from the sediment that contribute to this proliferation. Also, it should be noted that genes for toxin formation are not always expressed—an investigation of key drivers of toxin-formation gene expression was beyond the scope of this investigation.

6. The proliferation of potentially toxin-forming *M. aeruginosa* cyanobacteria was enhanced by fine sediments obtained from both a nutrient poor, mesotrophic-oligotrophic source water reservoir and a relatively nutrient rich, wildfire-impacted river. Although it has been previously reported that wildfire-derived sediments are enriched with bioavailable NAIP with relatively higher EPC₀ that contributes to greater primary productivity in impacted rivers relative to unimpacted ones, significantly higher cyanobacterial proliferation occurred in treatments containing reservoir sediment—this result was unexpected. This difference may be attributable the delivery, or lack of other key nutrients or contaminants that can affect cyanobacterial growth. Wildfire-impacted sediment may release other materials, such as heavy metals, which can inhibit cyanobacterial growth. Further explanations include: the contributions of higher DOC concentrations in microcosms containing wildfire-impacted sediment, which are known to reduce photosynthetic active irradiance (i.e., light availability needed for photosynthesis), or the impacts of other limiting nutrients or ratios of nutrients unavailable in the microcosms. Finally, it should be underscored that the wildfire-impacted sediment was collected approximately 8 years post-fire; thus, it is indicative of the legacy effects of wildfire on potential nutrient releases and cyanobacterial (and broader algal) proliferation after wildfire as opposed to immediate post-disturbance impacts (which likely would be even greater). Further investigation is needed to elucidate additional impacts of fine sediment on cyanobacterial proliferation.
7. Unexpectedly, amendments of nitrate concentrations did not significantly affect the pigment composition or cell densities of potential toxin-forming cyanobacteria, *M. aeruginosa*. In all statistical analyses, N concentrations did not contribute significantly to results. These results are inconsistent with those in literature, and may be attributed to

the quenched system of elevated N concentrations, or potentially by the unnaturally high concentrations used within this work.

8. Benchtop microcosm investigations can be conducted to investigate the proliferation of potentially toxin-forming cyanobacteria (here *M. aeruginosa*) in modified natural waters with the addition of natural reservoir (or other) sediments to investigate reservoir management, natural disturbance, and other water quality and environmental impacts on the potential proliferation of cyanobacteria. To the author's knowledge, this investigation is the first to develop and report these microcosm approaches. It should be underscored that this approach was developed in the University of Waterloo's Water Science, Technology & Policy Group's laboratories in conjunction with another individual's master's work, completed by Crumb (2016) (166). While the microcosm investigations detailed herein are by no means predictive, they are easy and inexpensive to conduct relative to other approaches (such as the use of limno-corrals). Moreover, they offer a relatively rapid means for providing insights and direction for further investigation and consideration of landscape disturbance and reservoir management impacts on source water quality and drinking water treatability.

Chapter 6: Implications and Recommendations

The proof-of-concept investigation presented herein demonstrates that reservoir sediment can significantly promote *M. aeruginosa* proliferation in low nutrient, mesotrophic-oligotrophic waters. This work emphasizes the need to evaluate and better understand the contributions of various fine sediment sources during drinking water reservoir risk management. Notably, drinking water reservoirs are typically managed to ensure water availability. When reservoirs are used as equalization basins for dampening rapid changes in water quality, the contributions of the relatively small amounts of fine sediment present within them—and the associated potential for that sediment to serve as an internal source of bioavailable P—are not typically considered. This work suggests fine sediment and its potential contributions to the proliferation of cyanobacteria and algae should be considered as part of regular reservoir management and source water protection planning in the drinking water industry.

It should also be highlighted that both anthropogenic (e.g., development, agriculture, and resource extraction) and natural (e.g., wildfire and flooding) landscape disturbances can significantly increase fine sediment availability and transport to downstream receiving waters, including drinking water reservoirs. Thus, these results have significant implications for both climate change adaptation and the management of drinking water reservoirs, especially in systems that receive high quality source water. High quality source waters are more likely to be sensitive to relatively small shifts in sediment-associated nutrient availability. Moreover, it is critical to underscore that reservoirs such as the one investigated herein may already contain sediments that can significantly enhance cyanobacterial proliferation if the system conditions (e.g., turbulence, light levels, etc.) favour their growth. Thus, an improved understanding of ecosystem dynamics is still needed. Regardless of whether or not such shifts occur due to landscape disturbance or reservoir management, the potential for fine sediment-associated proliferation of cyanobacteria should be a critical component of drinking water treatment risk management. Cyanobacterial blooms can challenge treatment infrastructure and lead to service disruptions that threaten public health.

As fine sediment characterization is not a typical component of most source water protection programs, this type of watershed characterization and associated water quality analysis may be useful for drinking water utilities in identifying both current threats to water supply and treatment and future threats associated with potential or anticipated watershed disturbances.

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Appendix 1: Sediment Laboratory Analyses Raw Data

Appendix 1.1: Grain Size Distribution



MASTERSIZER



Result Analysis Report

Sample Name:
A17-12357-1 - Average

SOP Name:

Measured:
Monday, November 06, 2017 9:39:27 AM

Sample Source & type:
Univ. of Waterloo = Bottom of Reservoir

Measured by:
analyst

Analysed:
Monday, November 06, 2017 9:39:29 AM

Sample bulk lot ref:
AY-GMR-1A

Result Source:
Averaged

Particle Name:
Sediment

Accessory Name:
Hydro 2000S (A)

Analysis model:
General purpose

Sensitivity:
Normal

Particle RI:
1.550

Absorption:
0.1

Size range:
0.020 to 2000.000 μm

Obscuration:
23.22 %

Dispersant Name:
Water

Dispersant RI:
1.330

Weighted Residual:
1.759 %

Result Emulation:
Off

Concentration:
0.0090 %Vol

Span :
2.357

Uniformity:
0.727

Result units:
Volume

Specific Surface Area:
2.61 m^2/g

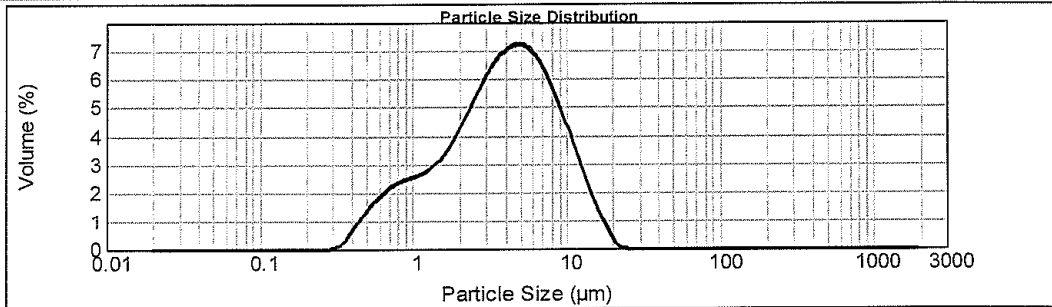
Surface Weighted Mean D[3,2]:
2.296 μm

Vol. Weighted Mean D[4,3]:
4.857 μm

d(0.1): 0.927 μm

d(0.5): 3.905 μm

d(0.9): 10.133 μm



- A17-12357-1, Monday, November 06, 2017 9:39:27 AM
- A17-12357-1, Monday, November 06, 2017 9:40:02 AM
- A17-12357-1, Monday, November 06, 2017 9:40:36 AM
- A17-12357-1 - Average, Monday, November 06, 2017 9:39:27 AM

Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %
0.020	0.00	0.183	0.00	1.675	12.68	15.334	1.96	140.341	0.00	1284.465	0.00
0.031	0.00	0.285	0.93	2.609	18.20	23.876	0.00	218.520	0.00	2000.000	0.00
0.048	0.00	0.444	4.59	4.062	20.56	37.176	0.00	340.251	0.00		
0.076	0.00	0.691	6.93	6.325	16.82	57.885	0.00	529.794	0.00		
0.118	0.00	1.076	8.46	9.848	8.87	90.131	0.00	824.925	0.00		
0.183	0.00	1.675		15.334		140.341	0.00	1284.465	0.00		

LL 06 Nov 2017

Operator notes: LL



MASTERSIZER 2000

Sample Name:
A17-12357-1 - Average

Sample Source & type:
Univ. of Waterloo = Bottom of Reservoir

Sample bulk lot ref:
AY-GMR-1A

SOP Name:

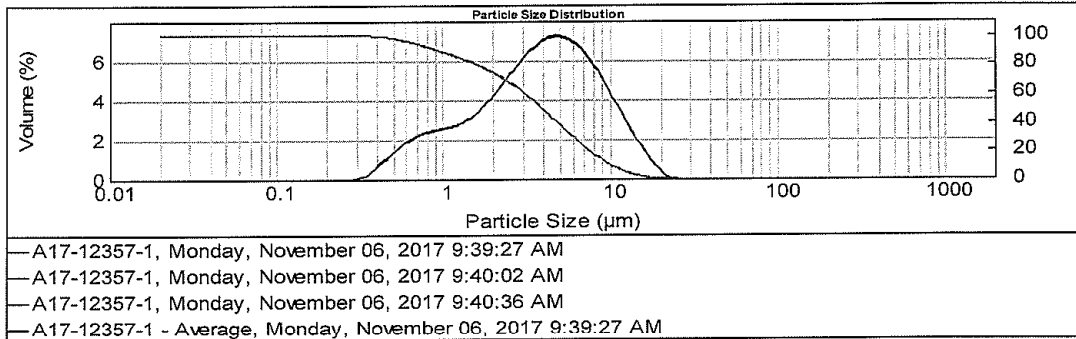
Measured by:
analyst

Measured:
Monday, November 06, 2017 9:39:27 AM

Analysed:
Monday, November 06, 2017 9:39:29 AM

Particle Name:
Sediment

Result Source: Averaged	Analysis model: General purpose	Uniformity: 0.727	Obscuration - Red: 23.22 %
Particle RI: 1.550	Absorption: 0.1	Weighted Residual: 1.759 %	Obscuration - Blue: 25.24 %
Dispersant Name: Water	Dispersant RI: 1.330	Concentration: 0.0090 %Vol	
Specific Surface Area: 2.61 m ² /g	Surface Weighted Mean D[3,2]: 2.296 µm	Vol. Weighted Mean D[4,3]: 4.857 µm	Percentage below 2.00 µm : 25.31%
Percentage below 0.10 µm : 0.00%	Percentage below 0.20 µm : 0.00%	Percentage below 0.30 µm : 0.00%	Percentage below 0.50 µm : 1.79%
D(0.10) : 0.93 µm	D(0.50) : 3.91 µm	D(0.80) : 7.58 µm	D(0.90) : 10.13 µm



Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %		
0.020	100.00	0.118	100.00	0.691	94.48	4.062	48.21	23.876	0.00	140.341	0.00	824.925	0.00		
0.031	100.00	0.183	100.00	1.076	87.55	6.325	27.65	37.176	0.00	218.520	0.00	1284.465	0.00		
0.048	100.00	0.285	100.00	1.675	79.09	9.848	10.83	57.885	0.00	340.251	0.00	2000.000	0.00		
0.076	100.00	0.444	99.07	2.609	66.41	15.334	1.96	90.131	0.00	529.794	0.00				

Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %
0.020	0.00	0.118	0.00	0.691	5.52	4.062	51.79	23.876	100.00	140.341	100.00	824.925	100.00
0.031	0.00	0.183	0.00	1.076	12.45	6.325	72.35	37.176	100.00	218.520	100.00	1284.465	100.00
0.048	0.00	0.285	0.00	1.675	20.91	9.848	89.17	57.885	100.00	340.251	100.00	2000.000	100.00
0.076	0.00	0.444	0.93	2.609	33.59	15.334	98.04	90.131	100.00	529.794	100.00		

LL 04 Nov 2017

Operator notes: LL



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Result Analysis Report

Sample Name:
A17-12357-2 - Average

Sample Source & type:
Univ. of Waterloo = Bottom of Reservoir

Sample bulk lot ref:
AY-GMR-1B

SOP Name:

Measured by:
analyst

Result Source:
Averaged

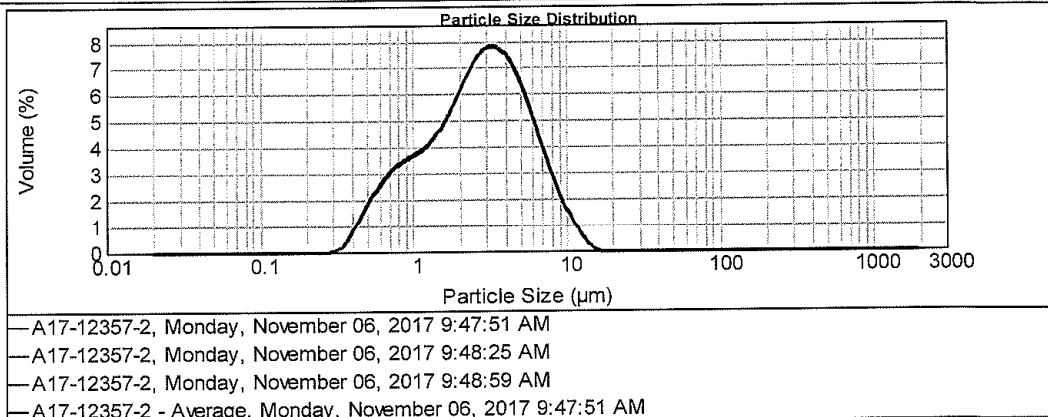
Measured:
Monday, November 06, 2017 9:47:51 AM

Analysed:
Monday, November 06, 2017 9:47:52 AM

Particle Name: Sediment	Accessory Name: Hydro 2000S (A)	Analysis model: General purpose	Sensitivity: Normal
Particle RI: 1.550	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 24.51 %
Dispersant Name: Water	Dispersant RI: 1.330	Weighted Residual: 3.251 %	Result Emulation: Off

Concentration: 0.0076 %Vol	Span : 2.160	Uniformity: 0.672	Result units: Volume
Specific Surface Area: 3.32 m ² /g	Surface Weighted Mean D[3,2]: 1.807 um	Vol. Weighted Mean D[4,3]: 3.311 um	

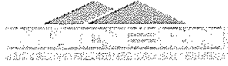
d(0.1): 0.785 um d(0.5): 2.720 um d(0.9): 6.662 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.020	0.00	0.183	0.00	1.675	18.21	15.334	0.01	140.341	0.00	1284.465	0.00
0.031	0.00	0.285	1.16	2.609	22.24	23.876	0.00	218.520	0.00	2000.000	0.00
0.046	0.00	0.444	6.22	4.062	18.23	37.176	0.00	340.251	0.00		
0.076	0.00	0.691	9.91	6.325	9.28	57.885	0.00	529.794	0.00		
0.118	0.00	1.076	12.48	9.848	2.28	90.131	0.00	824.925	0.00		
0.183	0.00	1.675		15.334		140.341		1284.465			

LL 06 Nov 2017

Operator notes: LL



MASTERSIZER 2000

Sample Name:
A17-12357-2 - Average

Sample Source & type:
Univ. of Waterloo = Bottom of Reservoir

Sample bulk lot ref:
AY-GMR-1B

SOP Name:

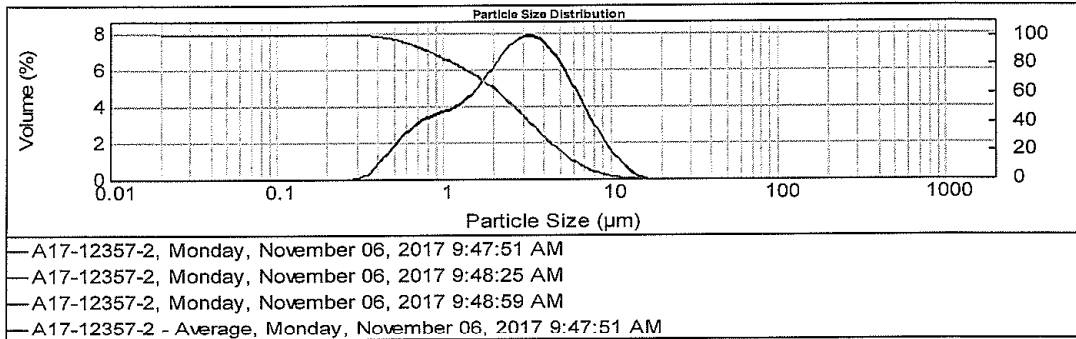
Measured by:
analyst

Measured:
Monday, November 06, 2017 9:47:51 AM

Analysed:
Monday, November 06, 2017 9:47:52 AM

Particle Name:
Sediment

Result Source: Averaged	Analysis model: General purpose	Uniformity: 0.672	Obscuration - Red: 24.51 %
Particle RI: 1.550	Absorption: 0.1	Weighted Residual: 3.251 %	Obscuration - Blue: 27.90 %
Dispersant Name: Water	Dispersant RI: 1.330	Concentration: 0.0076 %Vol	
Specific Surface Area: 3.32 m ² /g	Surface Weighted Mean D[3,2]: 1.807 µm	Vol. Weighted Mean D[4,3]: 3.311 µm	Percentage below 2.00 µm : 36.24%
Percentage below 0.10 µm : 0.00%	Percentage below 0.20 µm : 0.00%	Percentage below 0.30 µm : 0.00%	Percentage below 0.50 µm : 2.28%
Percentage below 1.00 µm : 15.51%	D(0.10) : 0.78 µm	D(0.50) : 2.72 µm	D(0.80) : 5.03 µm
			D(0.90) : 6.66 µm



Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %		
0.020	100.00	0.118	100.00	0.691	92.62	4.062	29.79	23.876	0.00	140.341	0.00	824.925	0.00		
0.031	100.00	0.183	100.00	1.076	82.72	6.325	11.57	37.176	0.00	218.520	0.00	1284.465	0.00		
0.048	100.00	0.285	100.00	1.675	70.24	9.848	2.29	57.885	0.00	340.251	0.00	2000.000	0.00		
0.076	100.00	0.444	98.84	2.609	52.03	15.334	0.01	90.131	0.00	529.794	0.00				

Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %
0.020	0.00	0.118	0.00	0.691	7.38	4.062	70.21	23.876	100.00	140.341	100.00	824.925	100.00
0.031	0.00	0.183	0.00	1.076	17.28	6.325	88.43	37.176	100.00	218.520	100.00	1284.465	100.00
0.048	0.00	0.285	0.00	1.675	29.76	9.848	97.71	57.885	100.00	340.251	100.00	2000.000	100.00
0.076	0.00	0.444	1.16	2.609	47.97	15.334	99.99	90.131	100.00	529.794	100.00		

LL 06 Nov 2017

Operator notes: LL



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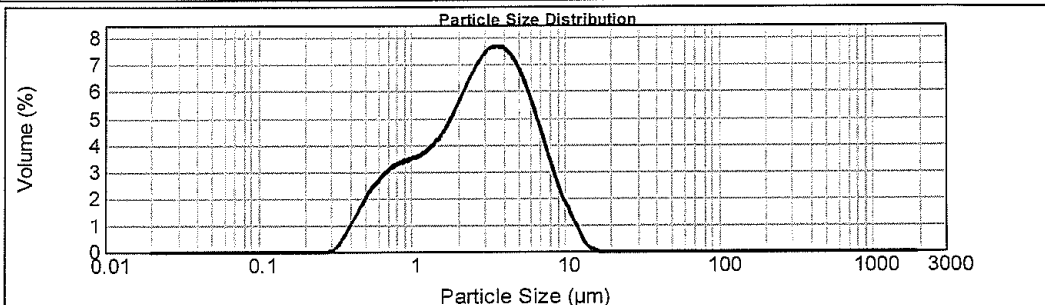
Result Analysis Report

Sample Name: A17-12357-3 - Average
Sample Source & type: Univ. of Waterloo = Bottom of Reservoir
Sample bulk lot ref: AY-GMR-1C
SOP Name:
Measured by: analyst
Result Source: Averaged
Measured: Monday, November 06, 2017 9:59:28 AM
Analysed: Monday, November 06, 2017 9:59:30 AM

Particle Name: Sediment
Particle RI: 1.550
Dispersion Name: Water
Accessory Name: Hydro 2000S (A)
Absorption: 0.1
Dispersion RI: 1.330
Analysis model: General purpose
Size range: 0.020 to 2000.000 um
Weighted Residual: 3.750 %
Sensitivity: Normal
Obscuration: 24.33 %
Result Emulation: Off

Concentration: 0.0076 %Vol
Specific Surface Area: 3.33 m²/g
Span : 2.180
Surface Weighted Mean D[3,2]: 1.802 um
Uniformity: 0.678
Vol. Weighted Mean D[4,3]: 3.442 um
Result units: Volume

d(0.1): 0.755 um d(0.5): 2.857 um d(0.9): 6.984 um

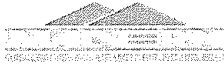


- A17-12357-3, Monday, November 06, 2017 9:59:28 AM
- A17-12357-3, Monday, November 06, 2017 10:00:03 AM
- A17-12357-3, Monday, November 06, 2017 10:00:37 AM
- A17-12357-3 - Average, Monday, November 06, 2017 9:59:28 AM

Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.020	0.00	0.183	0.00	1.675	16.56	15.334	0.02	140.341	0.00	1284.465	0.00
0.031	0.00	0.285	1.51	2.609	21.51	23.876	0.00	218.520	0.00	2000.000	0.00
0.048	0.00	0.444	6.69	4.062	19.41	37.176	0.00	340.251	0.00		
0.076	0.00	0.691	9.63	6.325	10.69	57.885	0.00	529.794	0.00		
0.118	0.00	1.076	11.44	9.848	2.55	90.131	0.00	824.925	0.00		
0.183	0.00	1.675	11.44	15.334	2.55	140.341	0.00	1284.465	0.00		

Operator notes: LL

LL 06 Nov 2017



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Sample Name:
A17-12357-3 - Average

Sample Source & type:
Univ. of Waterloo = Bottom of Reservoir

Sample bulk lot ref:
AY-GMR-1C

SOP Name:

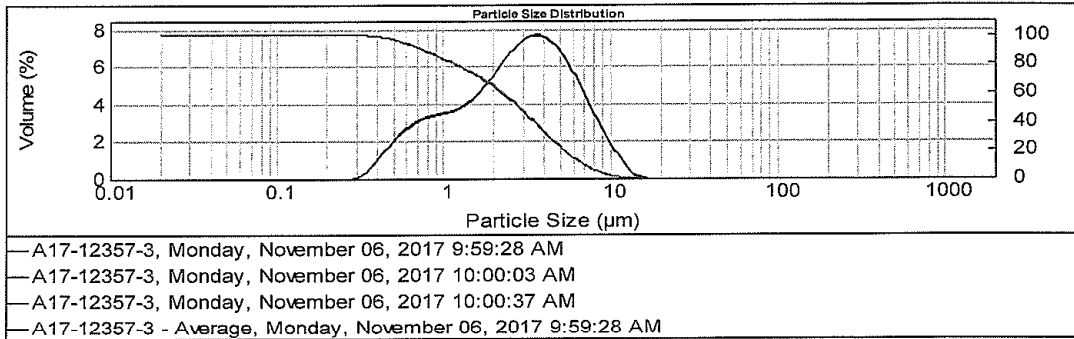
Measured by:
analyst

Measured:
Monday, November 06, 2017 9:59:28 AM

Analysed:
Monday, November 06, 2017 9:59:30 AM

Particle Name:
Sediment

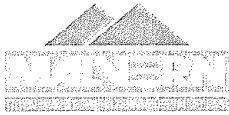
Result Source: Averaged	Analysis model: General purpose	Uniformity: 0.678	Obscuration - Red: 24.33 %
Particle RI: 1.550	Absorption: 0.1	Weighted Residual: 3.750 %	Obscuration - Blue: 27.02 %
Dispersant Name: Water	Dispersant RI: 1.330	Concentration: 0.0076 %Vol	
Specific Surface Area: 3.33 m ² /g	Surface Weighted Mean D[3,2]: 1.802 µm	Vol. Weighted Mean D[4,3]: 3.442 µm	Percentage below 2.00 µm : 35.12%
Percentage below 0.10 µm : 0.00%	Percentage below 0.20 µm : 0.00%	Percentage below 0.30 µm : 0.00%	Percentage below 0.50 µm : 2.82%
Percentage below 1.00 µm : 16.16%	D(0.10) : 0.75 µm	D(0.50) : 2.86 µm	D(0.80) : 5.32 µm
			D(0.90) : 6.98 µm



Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %
0.020	100.00	0.118	100.00	0.691	91.79	4.062	32.65	23.676	0.00	140.341	0.00	824.925	0.00
0.031	100.00	0.183	100.00	1.076	82.16	6.325	13.25	37.176	0.00	218.520	0.00	1284.465	0.00
0.048	100.00	0.285	100.00	1.675	70.73	9.848	2.56	57.885	0.00	340.251	0.00	2000.000	0.00
0.076	100.00	0.444	98.49	2.609	54.17	15.334	0.02	90.131	0.00	529.794	0.00		

Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %
0.020	0.00	0.118	0.00	0.691	8.21	4.062	67.35	23.676	100.00	140.341	100.00	824.925	100.00
0.031	0.00	0.183	0.00	1.076	17.84	6.325	86.75	37.176	100.00	218.520	100.00	1284.465	100.00
0.048	0.00	0.285	0.00	1.675	29.27	9.848	97.44	57.885	100.00	340.251	100.00	2000.000	100.00
0.076	0.00	0.444	1.51	2.609	45.83	15.334	99.98	90.131	100.00	529.794	100.00		

Operator notes: LL *LL 06 Nov 2017*



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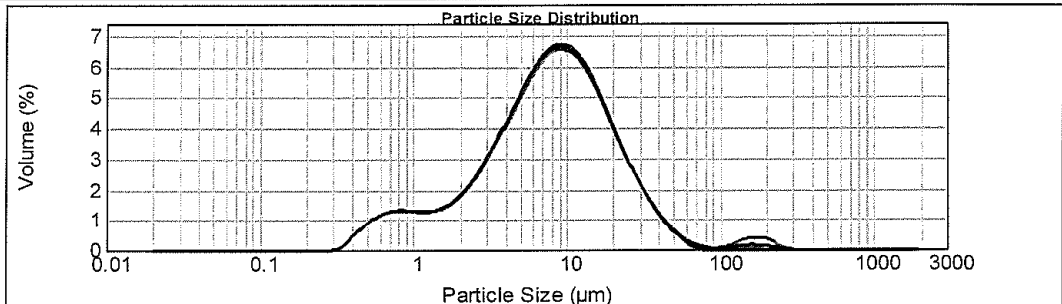
Result Analysis Report

Sample Name: A17-12357-4 - Average **SOP Name:** **Measured:** Monday, November 06, 2017 10:07:43 AM
Sample Source & type: Univ. of Waterloo = Bottom of Reservoir **Measured by:** analyst **Analysed:** Monday, November 06, 2017 10:07:44 AM
Sample bulk lot ref: AY-GMR-2A **Result Source:** Averaged

Particle Name: Sediment **Accessory Name:** Hydro 2000S (A) **Analysis model:** General purpose **Sensitivity:** Normal
Particle RI: 1.550 **Absorption:** 0.1 **Size range:** 0.020 to 2000.000 um **Obscuration:** 25.23 %
Dispersant Name: Water **Dispersant RI:** 1.330 **Weighted Residual:** 1.307 % **Result Emulation:** Off

Concentration: 0.0161 %Vol **Span :** 2.838 **Uniformity:** 0.987 **Result units:** Volume
Specific Surface Area: 1.63 m²/g **Surface Weighted Mean D[3,2]:** 3.670 um **Vol. Weighted Mean D[4,3]:** 11.704 um

d(0.1): 1.482 um d(0.5): 7.898 um d(0.9): 23.894 um



- A17-12357-4, Monday, November 06, 2017 10:07:43 AM
- A17-12357-4, Monday, November 06, 2017 10:08:17 AM
- A17-12357-4, Monday, November 06, 2017 10:08:51 AM
- A17-12357-4 - Average, Monday, November 06, 2017 10:07:43 AM

Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.020	0.00	0.183	0.00	1.675	5.51	15.334	12.54	140.341	0.39	1284.465	0.00
0.031	0.00	0.285	0.64	2.609	9.48	23.876	6.53	218.520	0.07	2000.000	0.00
0.048	0.00	0.444	2.91	4.062	14.77	37.176	2.44	340.251	0.00		
0.076	0.00	0.691	3.77	6.325	18.67	57.885	0.40	529.794	0.00		
0.118	0.00	1.076	3.81	9.848	17.89	90.131	0.17	824.925	0.00		
0.183	0.00	1.675		15.334		140.341		1284.465	0.00		

LL 06 Nov 2017

Operator notes: LL



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Sample Name:
A17-12357-4 - Average

Sample Source & type:
Univ. of Waterloo = Bottom of Reservoir

Sample bulk lot ref:
AY-GMR-2A

SOP Name:

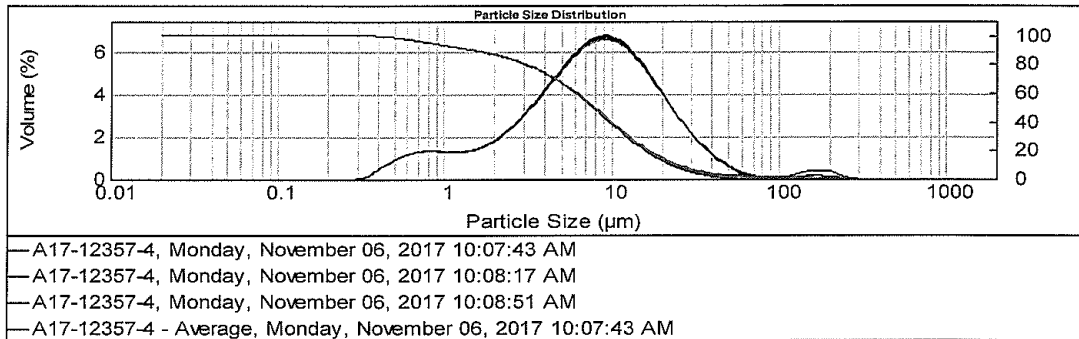
Measured by:
analyst

Measured:
Monday, November 06, 2017 10:07:43 AM

Analysed:
Monday, November 06, 2017 10:07:44 AM

Particle Name:
Sediment

Result Source: Averaged	Analysis model: General purpose	Uniformity: 0.987	Obscuration - Red: 25.23 %
Particle RI: 1.550	Absorption: 0.1	Weighted Residual: 1.307 %	Obscuration - Blue: 23.87 %
Dispersant Name: Water	Dispersant RI: 1.330	Concentration: 0.0161 %Vol	
Specific Surface Area: 1.63 m ² /g	Surface Weighted Mean D[3,2]: 3.670 µm	Vol. Weighted Mean D[4,3]: 11.704 µm	Percentage below 2.00 µm: 12.99%
Percentage below 0.10 µm: 0.00%	Percentage below 0.20 µm: 0.00%	Percentage below 0.30 µm: 0.00%	Percentage below 0.50 µm: 1.22%
Percentage below 1.00 µm: 6.70%	D(0.10): 1.48 µm	D(0.50): 7.90 µm	D(0.80): 16.52 µm
			D(0.90): 23.89 µm



Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %
0.020	100.00	0.118	100.00	0.691	96.45	4.062	73.89	23.876	10.02	140.341	0.46	824.925	0.00
0.031	100.00	0.183	100.00	1.076	92.68	6.325	59.12	37.176	3.48	218.520	0.07	1284.465	0.00
0.048	100.00	0.285	100.00	1.675	88.88	9.848	40.45	57.885	1.04	340.251	0.00	2000.000	0.00
0.076	100.00	0.444	99.36	2.609	83.37	15.334	22.55	90.131	0.84	529.794	0.00		

Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %
0.020	0.00	0.118	0.00	0.691	3.55	4.062	26.11	23.876	89.98	140.341	99.54	824.925	100.00
0.031	0.00	0.183	0.00	1.076	7.32	6.325	40.88	37.176	96.52	218.520	99.93	1284.465	100.00
0.048	0.00	0.285	0.00	1.675	11.12	9.848	59.55	57.885	98.96	340.251	100.00	2000.000	100.00
0.076	0.00	0.444	0.64	2.609	16.63	15.334	77.45	90.131	99.36	529.794	100.00		

Operator notes:

LL

LL 06 Nov 2017



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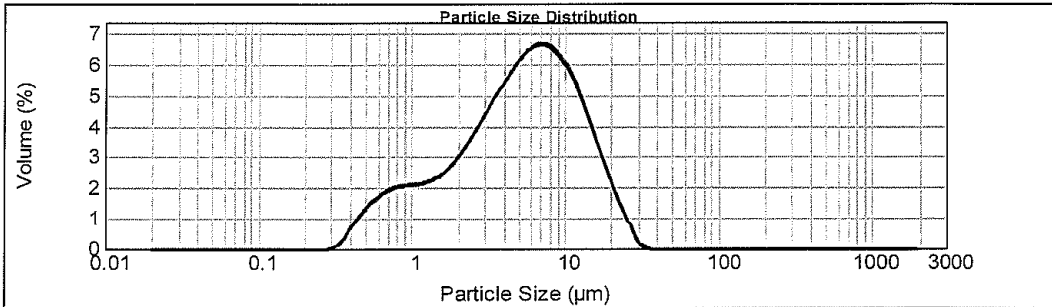
Result Analysis Report

Sample Name: A17-12357-5 - Average **SOP Name:** **Measured:** Monday, November 06, 2017 10:14:48 AM
Sample Source & type: Univ. of Waterloo = Bottom of Reservoir **Measured by:** analyst **Analysed:** Monday, November 06, 2017 10:14:49 AM
Sample bulk lot ref: AY-GMR-2B **Result Source:** Averaged

Particle Name: Sediment **Accessory Name:** Hydro 2000S (A) **Analysis model:** General purpose **Sensitivity:** Normal
Particle RI: 1.550 **Absorption:** 0.1 **Size range:** 0.020 to 2000.000 um **Obscuration:** 23.71 %
Dispersant Name: Water **Dispersant RI:** 1.330 **Weighted Residual:** 2.332 % **Result Emulation:** Off

Concentration: 0.0109 %Vol **Span :** 2.582 **Uniformity:** 0.796 **Result units:** Volume
Specific Surface Area: 2.27 m²/g **Surface Weighted Mean D[3,2]:** 2.637 um **Vol. Weighted Mean D[4,3]:** 6.714 um

d(0.1): 0.984 um d(0.5): 5.260 um d(0.9): 14.566 um

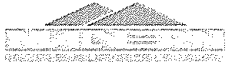


—A17-12357-5, Monday, November 06, 2017 10:14:48 AM
 —A17-12357-5, Monday, November 06, 2017 10:15:22 AM
 —A17-12357-5, Monday, November 06, 2017 10:15:56 AM
 —A17-12357-5 - Average, Monday, November 06, 2017 10:14:48 AM

Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.020	0.00	0.183	0.00	1.675	9.02	15.334	7.43	140.341	0.00	1284.465	0.00
0.031	0.00	0.285	1.00	2.609	13.34	23.876	1.22	218.520	0.00	2000.000	0.00
0.048	0.00	0.444	4.30	4.052	17.61	37.176	0.00	340.251	0.00		
0.076	0.00	0.691	5.93	6.325	18.85	57.985	0.00	529.794	0.00		
0.118	0.00	1.076	6.54	9.848	14.77	90.131	0.00	824.925	0.00		
0.183	0.00	1.675		15.334		140.341		1284.465			

LL 06 Nov 2017

Operator notes: LL



MASTERSIZER



Sample Name:
A17-12357-5 - Average

Sample Source & type:
Univ. of Waterloo = Bottom of Reservoir

Sample bulk lot ref:
AY-GMR-2B

SOP Name:

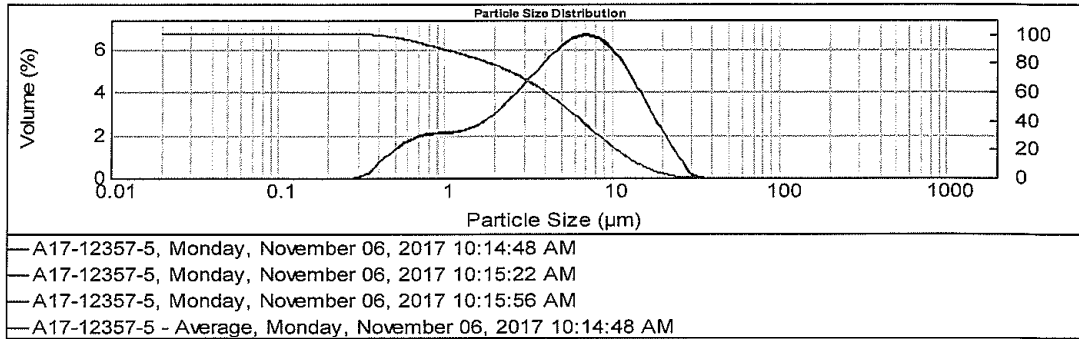
Measured by:
analyst

Measured:
Monday, November 06, 2017 10:14:48 AM

Analysed:
Monday, November 06, 2017 10:14:49 AM

Particle Name:
Sediment

Result Source: Averaged	Analysis model: General purpose	Uniformity: 0.796	Obscuration - Red: 23.71 %
Particle RI: 1.550	Absorption: 0.1	Weighted Residual: 2.332 %	Obscuration - Blue: 24.25 %
Dispersant Name: Water	Dispersant RI: 1.330	Concentration: 0.0109 %Vol	
Specific Surface Area: 2.27 m ² /g	Surface Weighted Mean D[3,2]: 2.637 um	Vol. Weighted Mean D[4,3]: 6.714 um	Percentage below 2.00 um : 20.94%
Percentage below 0.10 um : 0.00%	Percentage below 0.20 um : 0.00%	Percentage below 0.30 um : 0.00%	Percentage below 1.00 um : 10.22%
D(0.10) : 0.98 um	D(0.50) : 5.26 um	D(0.80) : 10.75 um	D(0.90) : 14.57 um



Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %
0.020	100.00	0.118	100.00	0.691	94.70	4.062	59.87	23.876	1.22	140.341	0.00	824.925	0.00
0.031	100.00	0.183	100.00	1.076	88.77	6.325	42.26	37.176	0.00	218.520	0.00	1284.465	0.00
0.048	100.00	0.285	100.00	1.675	82.23	9.848	23.41	57.885	0.00	340.251	0.00	2000.000	0.00
0.076	100.00	0.444	99.00	2.609	73.21	15.334	8.65	90.131	0.00	529.794	0.00		

Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %
0.020	0.00	0.118	0.00	0.691	5.30	4.062	40.13	23.876	98.78	140.341	100.00	824.925	100.00
0.031	0.00	0.183	0.00	1.076	11.23	6.325	57.74	37.176	100.00	218.520	100.00	1284.465	100.00
0.048	0.00	0.285	0.00	1.675	17.77	9.848	76.59	57.885	100.00	340.251	100.00	2000.000	100.00
0.076	0.00	0.444	1.00	2.609	26.79	15.334	91.35	90.131	100.00	529.794	100.00		

LL 06 Nov 2017

Operator notes:



MASTERSIZER



Result Analysis Report

Sample Name:
A17-12357-6 - Average

SOP Name:

Measured:
Monday, November 06, 2017 10:23:32 AM

Sample Source & type:
Univ. of Waterloo = Bottom of Reservoir

Measured by:
analyst

Analysed:
Monday, November 06, 2017 10:23:34 AM

Sample bulk lot ref:
AY-GMR-2C

Result Source:
Averaged

Particle Name:
Sediment

Accessory Name:
Hydro 2000S (A)

Analysis model:
General purpose

Sensitivity:
Normal

Particle RI:
1.550

Absorption:
0.1

Size range:
0.020 to 2000.000 um

Obscuration:
26.96 %

Dispersant Name:
Water

Dispersant RI:
1.330

Weighted Residual:
1.797 %

Result Emulation:
Off

Concentration:
0.0175 %Vol

Span :
2.654

Uniformity:
0.814

Result units:
Volume

Specific Surface Area:
1.64 m²/g

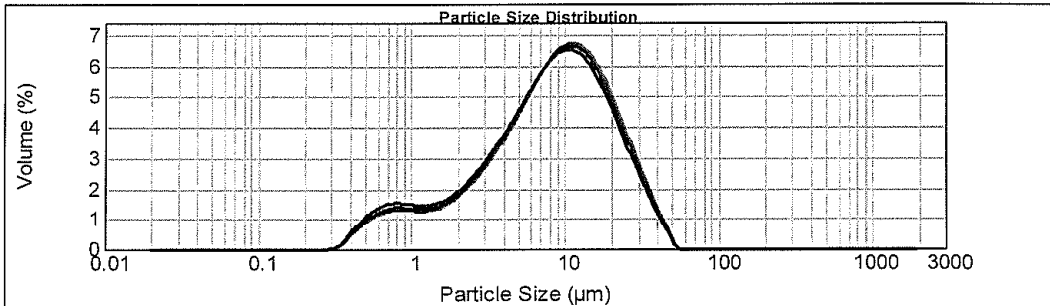
Surface Weighted Mean D[3,2]:
3.651 um

Vol. Weighted Mean D[4,3]:
10.976 um

d(0.1): 1.404 um

d(0.5): 8.518 um

d(0.9): 24.006 um

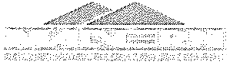


- A17-12357-6, Monday, November 06, 2017 10:23:32 AM
- A17-12357-6, Monday, November 06, 2017 10:24:07 AM
- A17-12357-6, Monday, November 06, 2017 10:24:41 AM
- A17-12357-6 - Average, Monday, November 06, 2017 10:23:32 AM

Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.020	0.00	0.183	0.00	1.675	5.41	15.334	14.90	140.341	0.00	1284.465	0.00
0.031	0.00	0.285	0.70	2.609	8.55	23.876	8.12	218.520	0.00	2000.000	0.00
0.048	0.00	0.444	3.05	4.062	13.01	37.176	2.03	340.251	0.00		
0.076	0.00	0.691	3.94	6.325	17.50	57.885	0.00	529.794	0.00		
0.118	0.00	1.076	3.96	9.848	18.85	90.131	0.00	824.925	0.00		
0.183	0.00	1.675		15.334		140.341		1284.465			

LL 06 Nov 2017

Operator notes: LL



MASTERSIZER 2000

Sample Name:
A17-12357-6 - Average

Sample Source & type:
Univ. of Waterloo = Bottom of Reservoir

Sample bulk lot ref:
AY-GMR-2C

SOP Name:

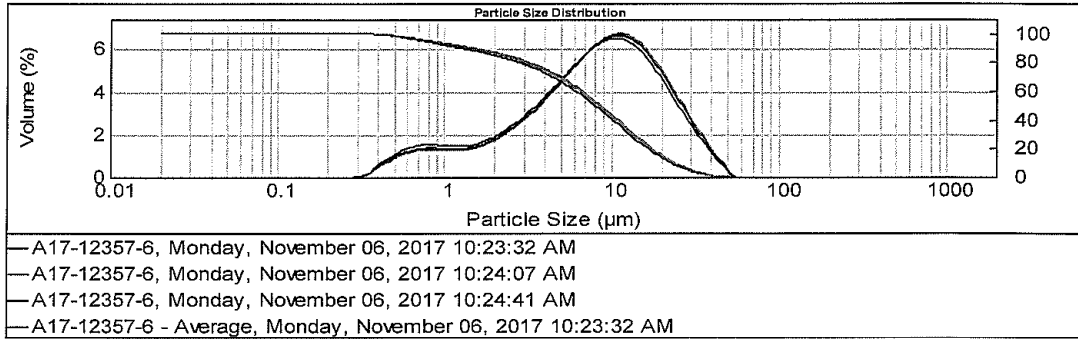
Measured by:
analyst

Measured:
Monday, November 06, 2017 10:23:32 AM

Analysed:
Monday, November 06, 2017 10:23:34 AM

Particle Name:
Sediment

Result Source: Averaged	Analysis model: General purpose	Uniformity: 0.814	Obscuration - Red: 26.96 %
Particle RI: 1.550	Absorption: 0.1	Weighted Residual: 1.797 %	Obscuration - Blue: 25.21 %
Dispersant Name: Water	Dispersant RI: 1.330	Concentration: 0.0175 %Vol	
Specific Surface Area: 1.64 m ² /g	Surface Weighted Mean D[3,2]: 3.651 um	Vol. Weighted Mean D[4,3]: 10.976 um	Percentage below 2.00 um : 13.53%
Percentage below 0.10 um : 0.00%	Percentage below 0.20 um : 0.00%	Percentage below 0.30 um : 0.00%	Percentage below 1.00 um : 7.05%
D(0.10) : 1.40 um	D(0.50) : 8.52 um	D(0.80) : 17.48 um	D(0.90) : 24.01 um



Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %
0.020	100.00	0.118	100.00	0.691	96.25	4.062	74.40	23.876	10.14	140.341	0.00	824.925	0.00
0.031	100.00	0.183	100.00	1.076	92.31	6.325	61.39	37.176	2.03	218.520	0.00	1284.465	0.00
0.048	100.00	0.285	100.00	1.675	88.36	9.848	43.89	57.885	0.00	340.251	0.00	2000.000	0.00
0.076	100.00	0.444	99.30	2.609	82.95	15.334	25.04	90.131	0.00	529.794	0.00		

Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %
0.020	0.00	0.118	0.00	0.691	3.75	4.062	25.60	23.876	89.86	140.341	100.00	824.925	100.00
0.031	0.00	0.183	0.00	1.076	7.69	6.325	38.61	37.176	97.97	218.520	100.00	1284.465	100.00
0.048	0.00	0.285	0.00	1.675	11.64	9.848	56.11	57.885	100.00	340.251	100.00	2000.000	100.00
0.076	0.00	0.444	0.70	2.609	17.05	15.334	74.96	90.131	100.00	529.794	100.00		

Operator notes:

LL

LL of Nov 2017



MASTERSIZER



Result Analysis Report

Sample Name:
A17-12357-7 - Average

SOP Name:

Measured:
Monday, November 06, 2017 10:29:23 AM

Sample Source & type:
Univ. of Waterloo = Bottom of Reservoir

Measured by:
analyst

Analysed:
Monday, November 06, 2017 10:29:24 AM

Sample bulk lot ref:
AY-GMR-3A

Result Source:
Averaged

Particle Name:
Sediment

Accessory Name:
Hydro 2000S (A)

Analysis model:
General purpose

Sensitivity:
Normal

Particle RI:
1.550

Absorption:
0.1

Size range:
0.020 to 2000.000 um

Obscuration:
25.81 %

Dispersant Name:
Water

Dispersant RI:
1.330

Weighted Residual:
2.252 %

Result Emulation:
Off

Concentration:
0.0116 %Vol

Span :
2.620

Uniformity:
0.806

Result units:
Volume

Specific Surface Area:
2.35 m²/g

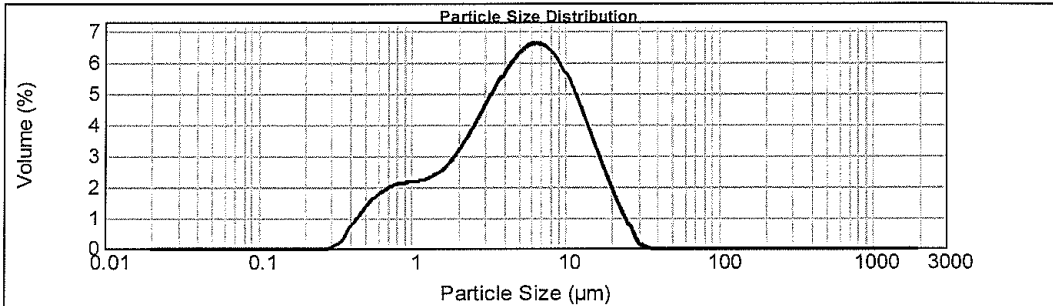
Surface Weighted Mean D[3,2]:
2.554 um

Vol. Weighted Mean D[4,3]:
6.427 um

d(0.1): 0.959 um

d(0.5): 4.977 um

d(0.9): 13.997 um

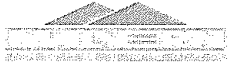


- A17-12357-7, Monday, November 06, 2017 10:29:23 AM
- A17-12357-7, Monday, November 06, 2017 10:29:57 AM
- A17-12357-7, Monday, November 06, 2017 10:30:31 AM
- A17-12357-7 - Average, Monday, November 06, 2017 10:29:23 AM

Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.020	0.00	0.183	0.00	1.675	9.53	15.334	6.69	140.341	0.00	1284.465	0.00
0.031	0.00	0.285	1.07	2.609	14.09	23.876	1.06	218.520	0.00	2000.000	0.00
0.048	0.00	0.444	4.45	4.062	18.11	37.176	0.00	340.251	0.00		
0.076	0.00	0.691	6.10	6.325	18.44	57.685	0.00	529.794	0.00		
0.118	0.00	1.076	6.79	9.848	13.65	90.131	0.00	824.925	0.00		
0.183	0.00	1.675		15.334		140.341		1284.465			

Operator notes: LL

LL 06 Nov 2017



MASTERSIZER 2000

Sample Name:
A17-12357-7 - Average

Measured:
Monday, November 06, 2017 10:29:23 AM

Sample Source & type:
Univ. of Waterloo = Bottom of Reservoir

SOP Name:

Analysed:
Monday, November 06, 2017 10:29:24 AM

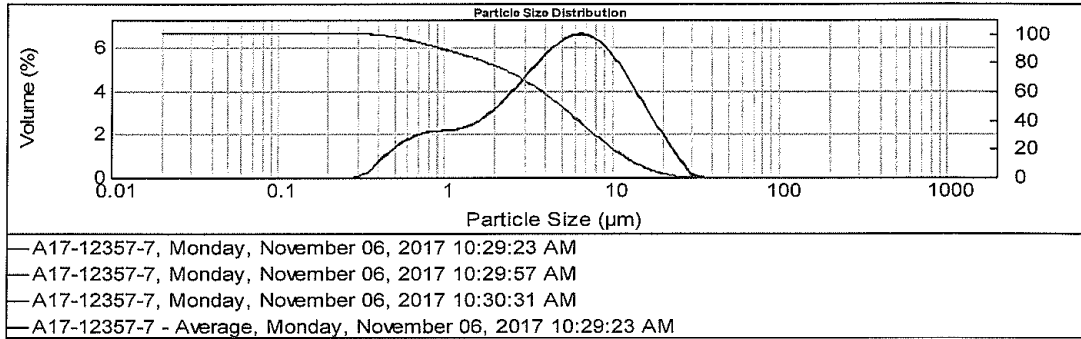
Sample bulk lot ref:
AY-GMR-3A

Measured by:
analyst

Particle Name:
Sediment

Result Source: Averaged	Analysis model: General purpose	Uniformity: 0.806	Obscuration - Red: 25.81 %
Particle RI: 1.550	Absorption: 0.1	Weighted Residual: 2.252 %	Obscuration - Blue: 26.67 %
Dispersant Name: Water	Dispersant RI: 1.330	Concentration: 0.0116 %Vol	

Specific Surface Area: 2.35 m ² /g	Surface Weighted Mean D[3,2]: 2.554 µm	Vol. Weighted Mean D[4,3]: 6.427 µm	Percentage below 2.00 µm: 21.76%
Percentage below 0.10 µm: 0.00%	Percentage below 0.20 µm: 0.00%	Percentage below 0.30 µm: 0.00%	Percentage below 1.00 µm: 10.59%
D(0.10): 0.96 µm	D(0.50): 4.98 µm	D(0.80): 10.22 µm	D(0.90): 14.00 µm



Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %
0.020	100.00	0.118	100.00	0.691	94.47	4.062	57.96	23.876	1.06	140.341	0.00	824.925	0.00
0.031	100.00	0.183	100.00	1.076	88.37	6.325	39.85	37.176	0.00	218.520	0.00	1284.465	0.00
0.048	100.00	0.285	100.00	1.675	81.58	9.848	21.41	57.885	0.00	340.251	0.00	2000.000	0.00
0.076	100.00	0.444	98.93	2.609	72.05	15.334	7.75	90.131	0.00	529.794	0.00		

Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %
0.020	0.00	0.118	0.00	0.691	5.53	4.062	42.04	23.876	98.94	140.341	100.00	824.925	100.00
0.031	0.00	0.183	0.00	1.076	11.63	6.325	60.15	37.176	100.00	218.520	100.00	1284.465	100.00
0.048	0.00	0.285	0.00	1.675	18.42	9.848	78.59	57.885	100.00	340.251	100.00	2000.000	100.00
0.076	0.00	0.444	1.07	2.609	27.95	15.334	92.25	90.131	100.00	529.794	100.00		

Operator notes:

LL

LL 06 Nov 2017



MASTERSIZER



Result Analysis Report

Sample Name:
A17-12357-8 - Average

SOP Name:

Measured:
Monday, November 06, 2017 10:36:21 AM

Sample Source & type:
Univ. of Waterloo = Bottom of Reservoir

Measured by:
analyst

Analysed:
Monday, November 06, 2017 10:36:22 AM

Sample bulk lot ref:
AY-GMR-3B

Result Source:
Averaged

Particle Name:
Sediment

Accessory Name:
Hydro 2000S (A)

Analysis model:
General purpose

Sensitivity:
Normal

Particle RI:
1.550

Absorption:
0.1

Size range:
0.020 to 2000.000 um

Obscuration:
26.00 %

Dispersant Name:
Water

Dispersant RI:
1.330

Weighted Residual:
3.073 %

Result Emulation:
Off

Concentration:
0.0092 %Vol

Span :
2.509

Uniformity:
0.78

Result units:
Volume

Specific Surface Area:
3 m²/g

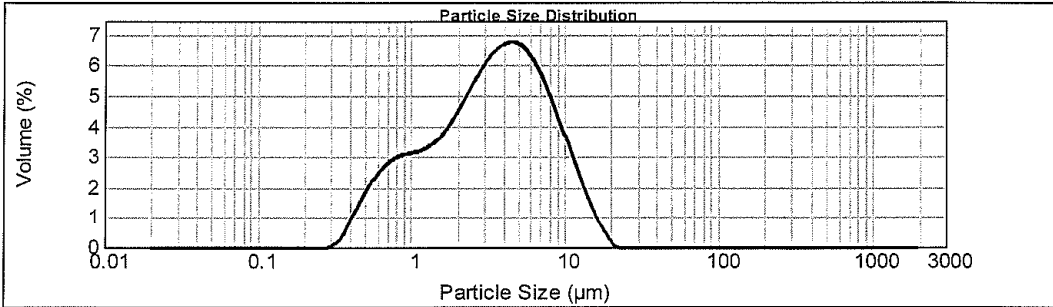
Surface Weighted Mean D[3,2]:
1.998 um

Vol. Weighted Mean D[4,3]:
4.377 um

d(0.1): 0.792 um

d(0.5): 3.422 um

d(0.9): 9.377 um

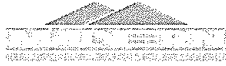


- A17-12357-8, Monday, November 06, 2017 10:36:21 AM
- A17-12357-8, Monday, November 06, 2017 10:36:55 AM
- A17-12357-8, Monday, November 06, 2017 10:37:30 AM
- A17-12357-8 - Average, Monday, November 06, 2017 10:36:21 AM

Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.020	0.00	0.183	0.00	1.675	13.43	15.334	1.34	140.341	0.00	1284.465	0.00
0.031	0.00	0.285	1.35	2.609	17.91	23.876	0.00	218.520	0.00	2000.000	0.00
0.048	0.00	0.444	6.08	4.062	19.12	37.176	0.00	340.251	0.00		
0.076	0.00	0.691	8.72	6.325	14.83	57.865	0.00	529.794	0.00		
0.118	0.00	1.076	9.86	9.848	7.37	90.131	0.00	824.925	0.00		
0.183	0.00	1.675		15.334		140.341		1284.465			

LL 06 Nov 2017

Operator notes: LL



MASTERSIZER



Sample Name:
A17-12357-8 - Average

Sample Source & type:
Univ. of Waterloo = Bottom of Reservoir

Sample bulk lot ref:
AY-GMR-3B

SOP Name:

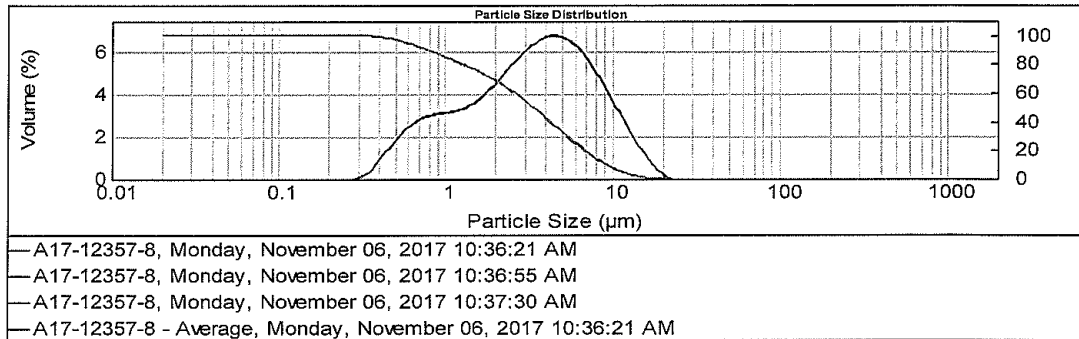
Measured by:
analyst

Measured:
Monday, November 06, 2017 10:36:21 AM

Analysed:
Monday, November 06, 2017 10:36:22 AM

Particle Name:
Sediment

Result Source: Averaged	Analysis model: General purpose	Uniformity: 0.78	Obscuration - Red: 26.00 %
Particle RI: 1.550	Absorption: 0.1	Weighted Residual: 3.073 %	Obscuration - Blue: 28.30 %
Dispersant Name: Water	Dispersant RI: 1.330	Concentration: 0.0092 %Vol	
Specific Surface Area: 3 m ² /g	Surface Weighted Mean D[3,2]: 1.998 um	Vol. Weighted Mean D[4,3]: 4.377 um	Percentage below 2.00 um : 30.81%
Percentage below 0.10 um : 0.00%	Percentage below 0.20 um : 0.00%	Percentage below 0.30 um : 0.00%	Percentage below 1.00 um : 14.65%
D(0.10) : 0.79 um	D(0.50) : 3.42 um	D(0.80) : 6.92 um	D(0.90) : 9.38 um



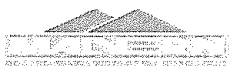
Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %
0.020	100.00	0.118	100.00	0.691	92.57	4.062	42.66	23.876	0.00	140.341	0.00	824.925	0.00
0.031	100.00	0.183	100.00	1.076	83.85	6.325	23.54	37.176	0.00	218.520	0.00	1284.465	0.00
0.048	100.00	0.285	100.00	1.675	73.99	9.848	8.71	57.885	0.00	340.251	0.00	2000.000	0.00
0.076	100.00	0.444	98.65	2.609	60.57	15.334	1.34	90.131	0.00	529.794	0.00		

Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %
0.020	0.00	0.118	0.00	0.691	7.43	4.062	57.34	23.876	100.00	140.341	100.00	824.925	100.00
0.031	0.00	0.183	0.00	1.076	16.15	6.325	76.46	37.176	100.00	218.520	100.00	1284.465	100.00
0.048	0.00	0.285	0.00	1.675	26.01	9.848	91.29	57.885	100.00	340.251	100.00	2000.000	100.00
0.076	0.00	0.444	1.35	2.609	39.43	15.334	98.65	90.131	100.00	529.794	100.00		

Operator notes:

LL

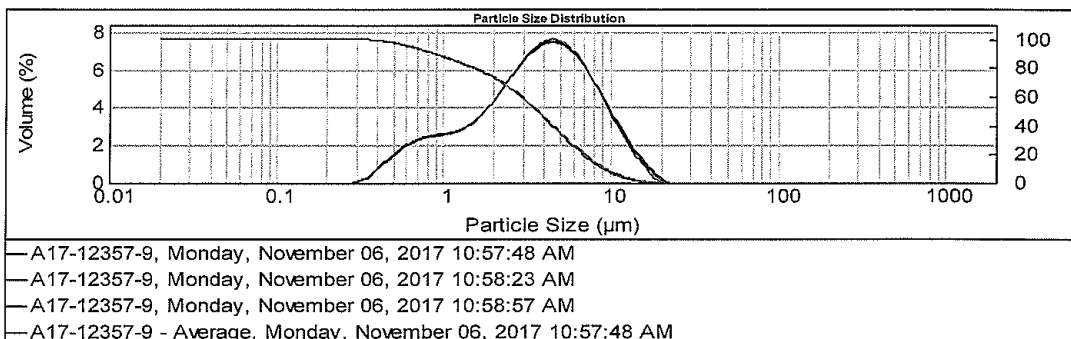
LL 06 Nov 2017



MASTERSIZER



Sample Name: A17-12357-9 - Average		Measured: Monday, November 06, 2017 10:57:48 AM	
Sample Source & type: Univ. of Waterloo = Bottom of Reservoir		Analysed: Monday, November 06, 2017 10:57:50 AM	
Sample bulk lot ref: AY-GMR-3C		Particle Name: Sediment	
SOP Name:		Measured by: analyst	
Result Source: Averaged	Analysis model: General purpose	Uniformity: 0.704	Obscuration - Red: 28.03 %
Particle Rf: 1.550	Absorption: 0.1	Weighted Residual: 2.659 %	Obscuration - Blue: 29.55 %
Dispersant Name: Water	Dispersant Rf: 1.330	Concentration: 0.0108 %Vol	
Specific Surface Area: 2.72 m ² /g	Surface Weighted Mean D[3,2]: 2.204 um	Vol. Weighted Mean D[4,3]: 4.566 um	Percentage below 2.00 um : 26.19%
Percentage below 0.10 um : 0.00%	Percentage below 0.20 um : 0.00%	Percentage below 0.30 um : 0.00%	Percentage below 0.50 um : 2.05%
Percentage below 1.00 um : 11.93%	D(0.10) : 0.89 um	D(0.50) : 3.72 um	D(0.80) : 7.05 um
			D(0.90) : 9.40 um



Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %
0.020	100.00	0.118	100.00	0.691	93.96	4.062	45.78	23.876	0.00	140.341	0.00	824.925	0.00
0.031	100.00	0.183	100.00	1.076	86.84	6.325	24.60	37.176	0.00	218.520	0.00	1284.465	0.00
0.048	100.00	0.285	100.00	1.675	78.32	9.848	8.75	57.885	0.00	340.251	0.00	2000.000	0.00
0.076	100.00	0.444	98.90	2.609	65.14	15.334	1.30	90.131	0.00	529.794	0.00		

Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %
0.020	0.00	0.118	0.00	0.691	6.04	4.062	54.22	23.876	100.00	140.341	100.00	824.925	100.00
0.031	0.00	0.183	0.00	1.076	13.16	6.325	75.40	37.176	100.00	218.520	100.00	1284.465	100.00
0.048	0.00	0.285	0.00	1.675	21.68	9.848	91.25	57.885	100.00	340.251	100.00	2000.000	100.00
0.076	0.00	0.444	1.10	2.609	34.86	15.334	98.70	90.131	100.00	529.794	100.00		

LL 06 Nov 2017

Operator notes: LL



MASTERSIZER



Result Analysis Report

Sample Name:
A17-12357-10 - Average

SOP Name:

Measured:
Monday, November 06, 2017 11:08:03 AM

Sample Source & type:
Univ. of Waterloo = Bottom of Reservoir

Measured by:
analyst

Analysed:
Monday, November 06, 2017 11:08:04 AM

Sample bulk lot ref:
AY-GMR-4A

Result Source:
Averaged

Particle Name:
Sediment

Accessory Name:
Hydro 2000S (A)

Analysis model:
General purpose

Sensitivity:
Normal

Particle RI:
1.550

Absorption:
0.1

Size range:
0.020 to 2000.000 μm

Obscuration:
26.66 %

Dispersant Name:
Water

Dispersant RI:
1.330

Weighted Residual:
1.458 %

Result Emulation:
Off

Concentration:
0.0133 %Vol

Span :
3.073

Uniformity:
0.957

Result units:
Volume

Specific Surface Area:
2.13 m^2/g

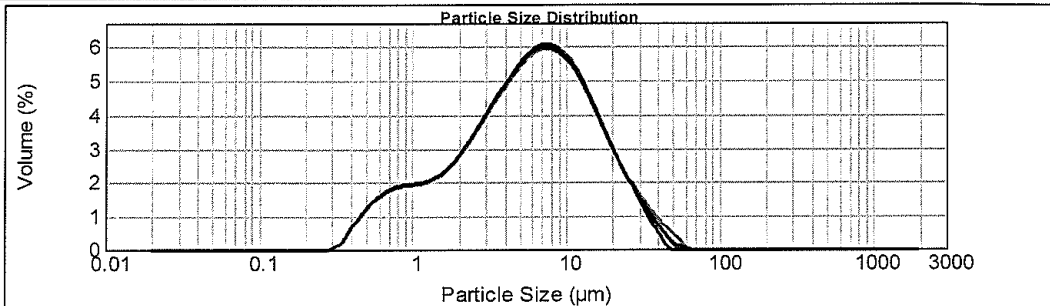
Surface Weighted Mean D[3,2]:
2.822 μm

Vol. Weighted Mean D[4,3]:
8.389 μm

d(0.1): 1.039 μm

d(0.5): 5.877 μm

d(0.9): 19.102 μm



—A17-12357-10, Monday, November 06, 2017 11:08:03 AM
 —A17-12357-10, Monday, November 06, 2017 11:08:37 AM
 —A17-12357-10, Monday, November 06, 2017 11:09:12 AM
 —A17-12357-10 - Average, Monday, November 06, 2017 11:08:03 AM

Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %
0.020	0.00	0.163	0.00	1.675	8.38	15.334	9.48	140.341	0.00	1284.465	0.00
0.031	0.00	0.285	0.98	2.609	12.13	23.876	4.72	218.520	0.00	2000.000	0.00
0.048	0.00	0.444	4.00	4.062	15.76	37.176	1.15	340.251	0.00		
0.076	0.00	0.691	5.47	6.325	17.17	57.885	0.02	529.794	0.00		
0.118	0.00	1.076	6.08	9.848	14.65	90.131	0.00	824.925	0.00		
0.183	0.00	1.675		15.334		140.341		1284.465			

Operator notes: LL

LL 06 Nov 2017



MASTERSIZER 2000

Sample Name:
A17-12357-10 - Average

Sample Source & type:
Univ. of Waterloo = Bottom of Reservoir

Sample bulk lot ref:
AY-GMR-4A

SOP Name:

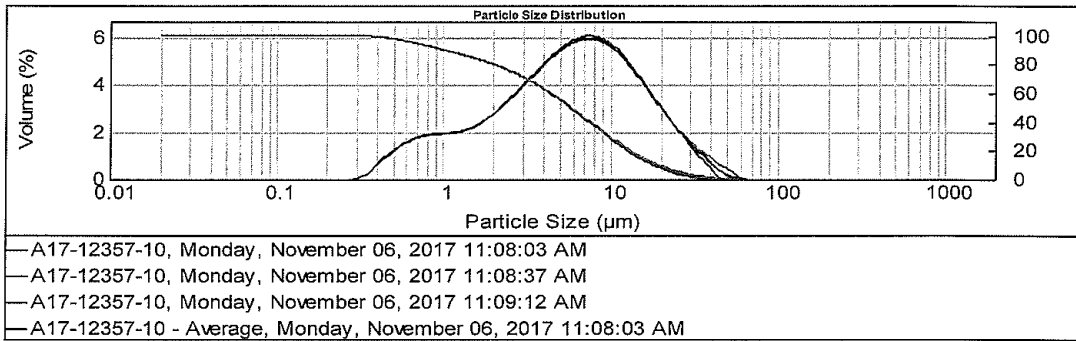
Measured by:
analyst

Measured:
Monday, November 06, 2017 11:08:03 AM

Analysed:
Monday, November 06, 2017 11:08:04 AM

Particle Name:
Sediment

Result Source: Averaged	Analysis model: General purpose	Uniformity: 0.957	Obscuration - Red: 26.66 %
Particle RI: 1.550	Absorption: 0.1	Weighted Residual: 1.458 %	Obscuration - Blue: 27.59 %
Dispersant Name: Water	Dispersant RI: 1.330	Concentration: 0.0133 %Vol	
Specific Surface Area: 2.13 m ² /g	Surface Weighted Mean D[3,2]: 2.822 µm	Vol. Weighted Mean D[4,3]: 8.389 µm	Percentage below 2.00 µm: 19.49%
Percentage below 0.10 µm: 0.00%	Percentage below 0.20 µm: 0.00%	Percentage below 0.30 µm: 0.00%	Percentage below 1.00 µm: 9.51%
D(0.10): 1.04 µm	D(0.50): 5.88 µm	D(0.80): 13.13 µm	D(0.90): 19.10 µm



Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %	Size (µm)	Vol Over %
0.023	100.00	0.118	100.00	0.691	95.03	4.062	62.96	23.876	5.89	140.341	0.00	824.925	0.00
0.031	100.00	0.183	100.00	1.076	89.56	6.325	47.20	37.176	1.18	218.520	0.00	1284.465	0.00
0.048	100.00	0.285	100.00	1.675	83.48	9.848	30.03	57.885	0.02	340.251	0.00	2000.000	0.00
0.076	100.00	0.444	99.02	2.609	75.09	15.334	15.38	90.131	0.00	529.794	0.00		

Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %
0.020	0.00	0.118	0.00	0.691	4.97	4.062	37.04	23.876	94.11	140.341	100.00	824.925	100.00
0.031	0.00	0.183	0.00	1.076	10.44	6.325	52.80	37.176	98.82	218.520	100.00	1284.465	100.00
0.048	0.00	0.285	0.00	1.675	16.52	9.848	69.97	57.885	99.98	340.251	100.00	2000.000	100.00
0.076	0.00	0.444	0.98	2.609	24.91	15.334	84.62	90.131	100.00	529.794	100.00		

Operator notes: LL LL 06 Nov 2017



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Result Analysis Report

Sample Name:
A17-12357-11 - Average

SOP Name:

Measured:
Monday, November 06, 2017 11:17:57 AM

Sample Source & type:
Univ. of Waterloo = Bottom of Reservoir

Measured by:
analyst

Analysed:
Monday, November 06, 2017 11:17:59 AM

Sample bulk lot ref:
AY-GMR-4B

Result Source:
Averaged

Particle Name:
Sediment

Accessory Name:
Hydro 2000S (A)

Analysis model:
General purpose

Sensitivity:
Normal

Particle RI:
1.550

Absorption:
0.1

Size range:
0.020 to 2000.000 um

Obscuration:
26.18 %

Dispersant Name:
Water

Dispersant RI:
1.330

Weighted Residual:
2.332 %

Result Emulation:
Off

Concentration:
0.0109 %Vol

Span :
2.429

Uniformity:
0.751

Result units:
Volume

Specific Surface Area:
2.48 m²/g

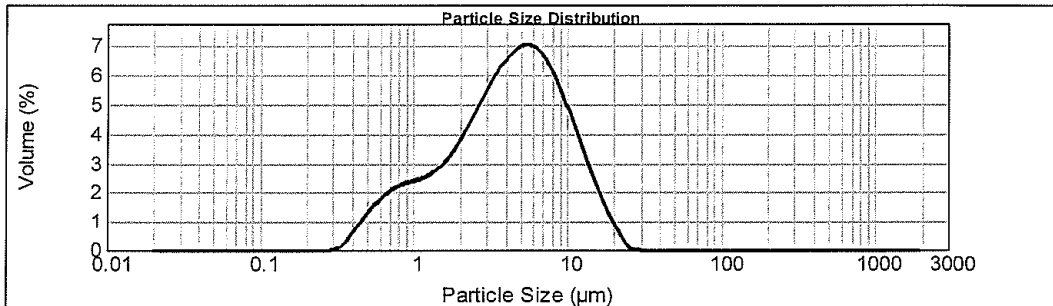
Surface Weighted Mean D[3,2]:
2.418 um

Vol. Weighted Mean D[4,3]:
5.388 um

d(0.1): 0.957 um

d(0.5): 4.284 um

d(0.9): 11.362 um

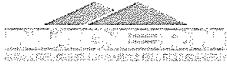


— A17-12357-11, Monday, November 06, 2017 11:17:57 AM
 — A17-12357-11, Monday, November 06, 2017 11:18:32 AM
 — A17-12357-11, Monday, November 06, 2017 11:19:06 AM
 — A17-12357-11 - Average, Monday, November 06, 2017 11:17:57 AM

Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.020	0.00	0.183	0.00	1.675	11.41	15.334	3.57	140.341	0.00	1284.465	0.00
0.031	0.00	0.285	0.88	2.609	16.61	23.876	0.09	218.520	0.00	2000.000	0.00
0.048	0.00	0.444	4.38	4.062	20.01	37.176	0.00	340.251	0.00		
0.076	0.00	0.691	6.58	6.325	17.94	57.885	0.00	529.794	0.00		
0.118	0.00	1.076	7.83	9.848	10.70	90.131	0.00	824.925	0.00		
0.183	0.00	1.675		15.334		140.341		1284.465			

11 Nov 2017

Operator notes: LL



MASTERSIZER 2000

Sample Name:
A17-12357-11 - Average

Sample Source & type:
Univ. of Waterloo = Bottom of Reservoir

Sample bulk lot ref:
AY-GMR-4B

SOP Name:

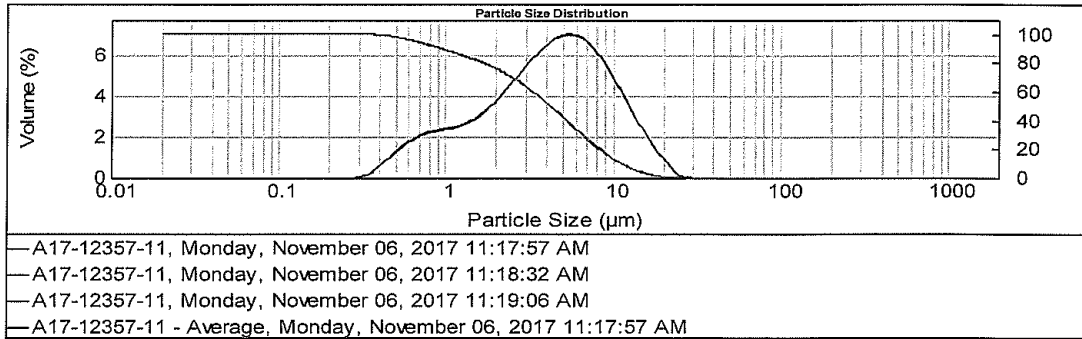
Measured by:
analyst

Measured:
Monday, November 06, 2017 11:17:57 AM

Analysed:
Monday, November 06, 2017 11:17:59 AM

Particle Name:
Sediment

Result Source: Averaged	Analysis model: General purpose	Uniformity: 0.751	Obscuration - Red: 26.18 %
Particle RI: 1.550	Absorption: 0.1	Weighted Residual: 2.332 %	Obscuration - Blue: 27.82 %
Dispersant Name: Water	Dispersant RI: 1.330	Concentration: 0.0109 %Vol	
Specific Surface Area: 2.48 m ² /g	Surface Weighted Mean D[3,2]: 2.418 um	Vol. Weighted Mean D[4,3]: 5.388 um	Percentage below 2.00 um : 23.65%
Percentage below 0.10 um : 0.00%	Percentage below 0.20 um : 0.00%	Percentage below 0.30 um : 0.00%	Percentage below 1.00 um : 10.69%
D(0.10) : 0.96 um	D(0.50) : 4.28 um	D(0.80) : 8.43 um	D(0.90) : 11.36 um



Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %
0.020	100.00	0.118	100.00	0.691	94.74	4.062	52.30	23.876	0.09	140.341	0.00	824.925	0.00
0.031	100.00	0.183	100.00	1.076	88.16	6.325	32.29	37.176	0.00	218.520	0.00	1284.465	0.00
0.048	100.00	0.285	100.00	1.675	80.33	9.848	14.36	57.885	0.00	340.251	0.00	2000.000	0.00
0.076	100.00	0.444	99.12	2.609	68.92	15.334	3.66	90.131	0.00	529.794	0.00		

Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %
0.020	0.00	0.118	0.00	0.691	5.26	4.062	47.70	23.876	99.91	140.341	100.00	824.925	100.00
0.031	0.00	0.183	0.00	1.076	11.84	6.325	67.71	37.176	100.00	218.520	100.00	1284.465	100.00
0.048	0.00	0.285	0.00	1.675	19.67	9.848	85.64	57.885	100.00	340.251	100.00	2000.000	100.00
0.076	0.00	0.444	0.88	2.609	31.08	15.334	96.34	90.131	100.00	529.794	100.00		

LL of Nov 2017

Operator notes: LL



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Result Analysis Report

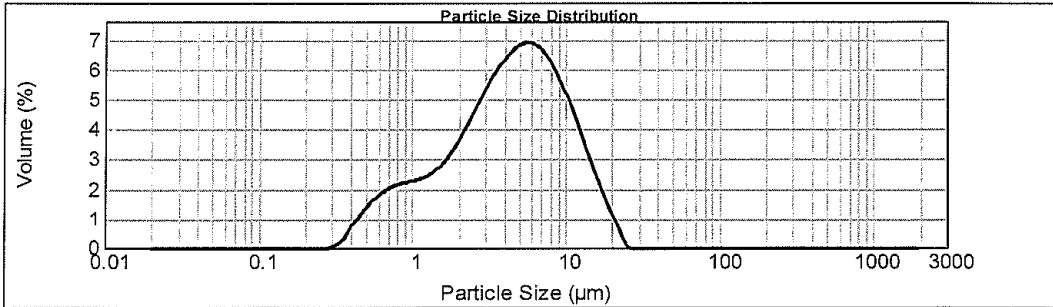
Sample Name: A17-12357-12 - Average **SOP Name:** **Measured:** Monday, November 06, 2017 11:23:21 AM
Sample Source & type: Univ. of Waterloo = Bottom of Reservoir **Measured by:** analyst **Analysed:** Monday, November 06, 2017 11:23:23 AM
Sample bulk lot ref: AY-GMR-4C **Result Source:** Averaged

Particle Name: Sediment **Accessory Name:** Hydro 2000S (A) **Analysis model:** General purpose **Sensitivity:** Normal
Particle RI: 1.550 **Absorption:** 0.1 **Size range:** 0.020 to 2000.000 um **Obscuration:** 26.77 %
Dispersant Name: Water **Dispersant RI:** 1.330 **Weighted Residual:** 2.005 % **Result Emulation:** Off

Concentration: 0.0114 %Vol **Span :** 2.479 **Uniformity:** 0.762 **Result units:** Volume

Specific Surface Area: 2.47 m²/g **Surface Weighted Mean D[3,2]:** 2.429 um **Vol. Weighted Mean D[4,3]:** 5.594 um

d(0.1): 0.946 um **d(0.5):** 4.429 um **d(0.9):** 11.925 um

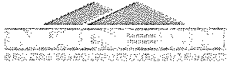


- A17-12357-12, Monday, November 06, 2017 11:23:21 AM
- A17-12357-12, Monday, November 06, 2017 11:23:56 AM
- A17-12357-12, Monday, November 06, 2017 11:24:30 AM
- A17-12357-12 - Average, Monday, November 06, 2017 11:23:21 AM

Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.020	0.00	0.183	0.00	1.675	10.94	15.334	4.31	140.341	0.00	1284.465	0.00
0.031	0.00	0.285	1.07	2.609	16.09	23.876	0.05	218.520	0.00	2000.000	0.00
0.048	0.00	0.444	4.52	4.062	19.54	37.176	0.00	340.251	0.00		
0.076	0.00	0.691	6.31	6.325	18.12	57.885	0.00	529.794	0.00		
0.118	0.00	1.076	7.40	9.848	11.64	90.131	0.00	824.925	0.00		
0.183	0.00	1.675		15.334		140.341		1284.465			

LL 06 Nov 2017

Operator notes: LL



MASTERSIZER 2000

Sample Name:
A17-12357-12 - Average

Sample Source & type:
Univ. of Waterloo = Bottom of Reservoir

Sample bulk lot ref:
AY-GMR-4C

SOP Name:

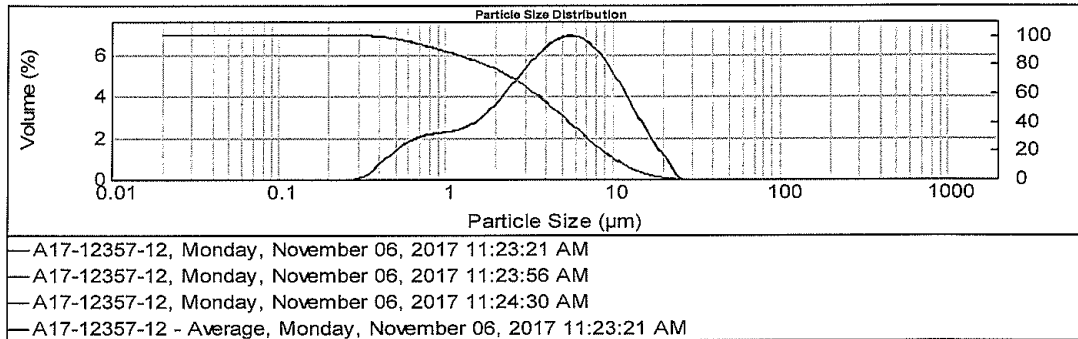
Measured by:
analyst

Measured:
Monday, November 06, 2017 11:23:21 AM

Analysed:
Monday, November 06, 2017 11:23:23 AM

Particle Name:
Sediment

Result Source: Averaged	Analysis model: General purpose	Uniformity: 0.762	Obscuration - Red: 26.77 %
Particle RI: 1.550	Absorption: 0.1	Weighted Residual: 2.005 %	Obscuration - Blue: 27.47 %
Dispersant Name: Water	Dispersant RI: 1.330	Concentration: 0.0114 %Vol	
Specific Surface Area: 2.47 m ² /g	Surface Weighted Mean D[3,2]: 2.429 um	Vol. Weighted Mean D[4,3]: 5.594 um	Percentage below 2.00 um : 23.11%
Percentage below 0.10 um : 0.00%	Percentage below 0.20 um : 0.00%	Percentage below 0.30 um : 0.00%	Percentage below 1.00 um : 10.82%
D(0.10) : 0.95 um	D(0.50) : 4.43 um	D(0.80) : 8.83 um	D(0.90) : 11.93 um



Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %	Size (um)	Vol Over %
0.020	100.00	0.118	100.00	0.691	94.40	4.062	53.66	23.876	0.05	140.341	0.00	824.925	0.00
0.031	100.00	0.183	100.00	1.076	88.09	6.325	34.12	37.176	0.00	218.520	0.00	1284.465	0.00
0.048	100.00	0.285	100.00	1.675	80.69	9.848	16.00	57.885	0.00	340.251	0.00	2000.000	0.00
0.076	100.00	0.444	98.93	2.609	69.75	15.334	4.36	90.131	0.00	529.794	0.00		

Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %	Size (um)	Vol Under %
0.020	0.00	0.118	0.00	0.691	5.60	4.062	46.34	23.876	99.95	140.341	100.00	824.925	100.00
0.031	0.00	0.183	0.00	1.076	11.91	6.325	65.88	37.176	100.00	218.520	100.00	1284.465	100.00
0.048	0.00	0.285	0.00	1.675	19.31	9.848	84.00	57.885	100.00	340.251	100.00	2000.000	100.00
0.076	0.00	0.444	1.07	2.609	30.25	15.334	95.64	90.131	100.00	529.794	100.00		

Operator notes: LL

LL 06 Nov 2017



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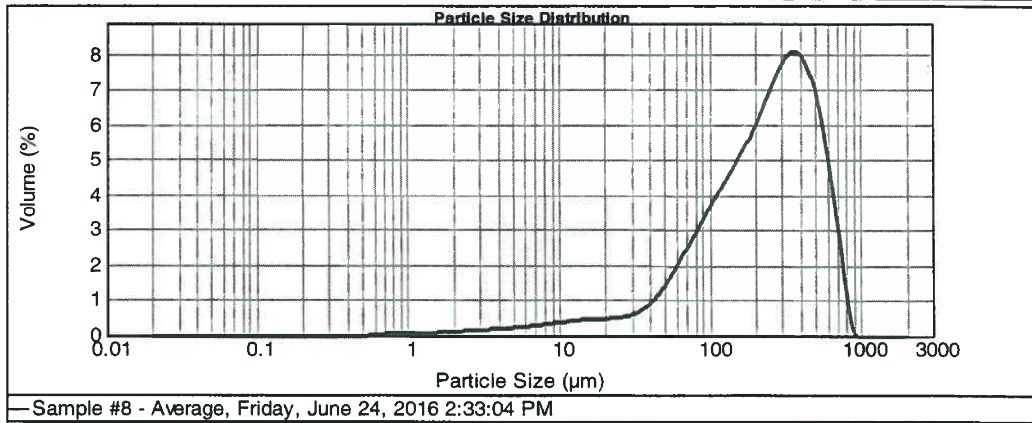
Result Analysis Report

Sample Name: Sample #8 - Average	SOP Name:	Measured: Friday, June 24, 2016 2:33:04 PM
Sample Source & type: Elbow River & Glenmore Reservoir	Measured by: Fletcher.J	Analysed: Friday, June 24, 2016 2:33:06 PM
Sample bulk lot ref: A16-05879-8 ER-CF	Result Source: Averaged	

Particle Name: Sediment	Accessory Name: Hydro 2000S (A)	Analysis model: General purpose	Sensitivity: Normal
Particle RI: 1.550	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 33.90 %
Dispersant Name: Water	Dispersant RI: 1.330	Weighted Residual: 2.015 %	Result Emulation: Off

Concentration: 0.3440 %Vol	Span : 2.030	Uniformity: 0.635	Result units: Volume
Specific Surface Area: 0.1 m ² /g	Surface Weighted Mean D[3,2]: 59.985 um	Vol. Weighted Mean D[4,3]: 274.001 um	

d(0.1): 54.224 um d(0.5): 242.548 um d(0.9): 546.682 um



— Sample #8 - Average, Friday, June 24, 2016 2:33:04 PM

Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.020	0.00	0.183	0.00	1.675	0.35	15.334	1.39	140.341	15.76	1284.465	0.00
0.031	0.00	0.285	0.00	2.609	0.49	23.876	1.76	218.520	21.07	2000.000	
0.048	0.00	0.444	0.04	4.062	0.65	37.176	3.61	340.251	22.12		
0.076	0.00	0.691	0.14	6.325	0.91	57.885	7.43	629.794	11.11		
0.118	0.00	1.076	0.19	9.848	1.21	90.131	11.59	824.925	0.18		
0.183	0.00	1.675		15.334		140.341		1284.465			

KE-24 Jun 2016

VSV 27 June 2016

Operator notes:



MASTERSIZER 2000

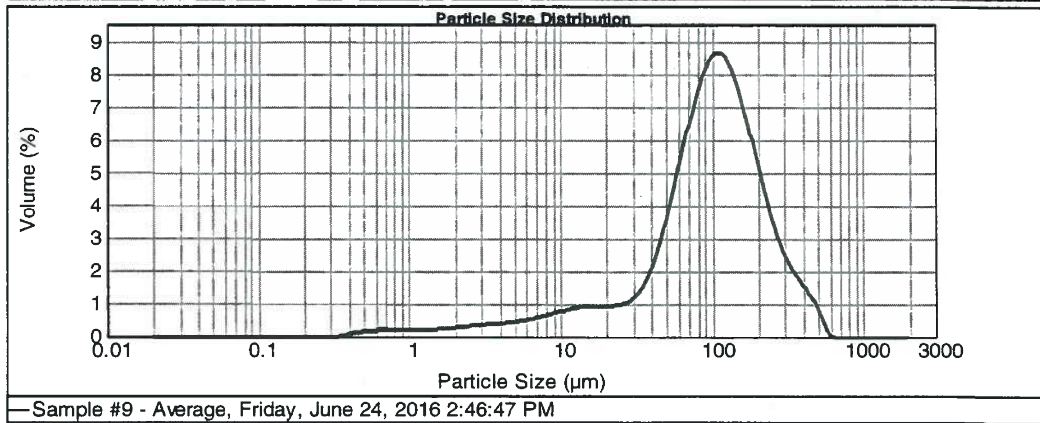
Result Analysis Report

Sample Name: Sample #9 - Average
Sample Source & type: Elbow River & Glenmore Reservoir
Sample bulk lot ref: A16-05879-9 ER-HWY21
SOP Name:
Measured by: FletcherJ
Result Source: Averaged
Measured: Friday, June 24, 2016 2:46:47 PM
Analysed: Friday, June 24, 2016 2:46:49 PM

Particle Name: Sediment
Particle Rt: 1.550
Dispersant Name: Water
Accessory Name: Hydro 2000S (A)
Absorption: 0.1
Dispersant Rt: 1.330
Analysis model: General purpose
Size range: 0.020 to 2000.000 um
Weighted Residual: 2.564 %
Sensitivity: Normal
Obscuration: 34.27 %
Result Emulation: Off

Concentration: 0.1311 %Vol
Specific Surface Area: 0.289 m²/g
Span : 2.290
Surface Weighted Mean D[3,2]: 20.727 um
Uniformity: 0.687
Vol. Weighted Mean D[4,3]: 121.770 um
Result units: Volume

d(0.1): 16.466 um d(0.5): 100.328 um d(0.9): 246.170 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.020	0.00	0.183	0.00	1.675	0.86	15.334	2.75	140.341	18.24
0.031	0.00	0.285	0.09	2.609	1.10	23.876	3.56	218.520	8.92
0.048	0.00	0.444	0.49	4.062	1.36	37.176	9.00	340.251	4.03
0.076	0.00	0.691	0.61	6.325	1.88	57.885	19.24	529.794	0.20
0.118	0.00	1.076	0.65	9.848	2.53	90.131	24.51	824.925	0.00
0.183	0.00	1.675	0.65	15.334	2.53	140.341	24.51	1284.465	0.00

KE-24Jun2016

KCV 27 June 2016

Operator notes:



MASTERSIZER



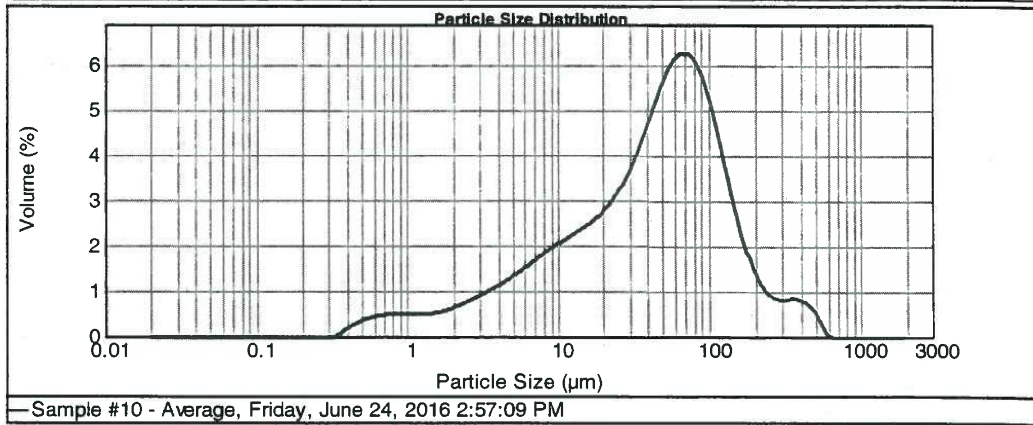
Result Analysis Report

Sample Name: Sample #10 - Average	SOP Name:	Measured: Friday, June 24, 2016 2:57:09 PM
Sample Source & type: Elbow River & Glenmore Reservoir	Measured by: FletcherJ	Analysed: Friday, June 24, 2016 2:57:10 PM
Sample bulk lot ref: A16-05879-10 ER-TB	Result Source: Averaged	

Particle Name: Sediment	Accessory Name: Hydro 2000S (A)	Analysis model: General purpose	Sensitivity: Normal
Particle RI: 1.550	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obcuration: 37.83 %
Dispersant Name: Water	Dispersant RI: 1.330	Weighted Residual: 1.361 %	Result Emulation: Off

Concentration: 0.0680 %Vol	Span : 3.016	Uniformity: 1.06	Result units: Volume
Specific Surface Area: 0.637 m ² /g	Surface Weighted Mean D[3,2]: 9.415 um	Vol. Weighted Mean D[4,3]: 67.654 um	

d(0.1): 4.643 um d(0.5): 46.417 um d(0.9): 144.638 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.020	0.00	0.183	0.00	1.675	1.91	15.334	7.81	140.341	5.86
0.031	0.00	0.285	0.25	2.609	2.77	23.876	10.60	218.520	2.56
0.048	0.00	0.444	1.13	4.082	3.91	37.176	15.32	340.251	2.07
0.076	0.00	0.691	1.43	6.325	5.21	57.885	17.82	529.794	0.13
0.118	0.00	1.076	1.46	9.848	6.45	90.131	13.32	824.925	0.00
0.183	0.00	1.675	1.46	15.334	6.45	140.341	13.32	1284.465	0.00

Ve-24 Jun 2016

V&V 27 June 2016

Operator notes:



MASTERSIZER



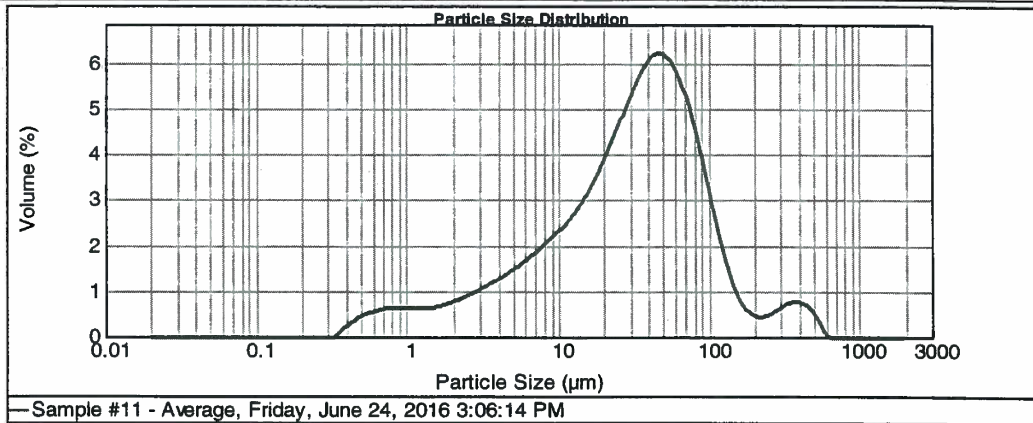
Result Analysis Report

Sample Name: Sample #11 - Average	SOP Name:	Measured: Friday, June 24, 2016 3:06:14 PM
Sample Source & type: Elbow River & Glenmore Reservoir	Measured by: Fletcher.J	Analysed: Friday, June 24, 2016 3:06:15 PM
Sample bulk lot ref: A16-05879-11 ER-WFB	Result Source: Averaged	

Particle Name: Sediment	Accessory Name: Hydro 2000S (A)	Analysis model: General purpose	Sensitivity: Normal
Particle RI: 1.550	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 34.23 %
Dispersant Name: Water	Dispersant RI: 1.330	Weighted Residual: 1.129 %	Result Emulation: Off

Concentration: 0.0499 %Vol	Span : 3.022	Uniformity: 1.17	Result units: Volume
Specific Surface Area: 0.77 m ² /g	Surface Weighted Mean D[3,2]: 7.788 um	Vol. Weighted Mean D[4,3]: 52.531 um	

d(0.1): 3.651 um d(0.5): 33.010 um d(0.9): 103.395 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.020	0.00	0.183	0.00	1.675	2.29	15.334	10.79	140.341	2.06	1284.465	0.00
0.031	0.00	0.285	0.31	2.609	3.17	23.876	15.07	218.520	1.62	2000.000	
0.048	0.00	0.444	1.43	4.062	4.32	37.176	17.73	340.251	1.98		
0.076	0.00	0.691	1.83	6.325	5.74	57.885	14.73	629.794	0.15		
0.118	0.00	1.076	1.84	9.848	7.68	80.131	7.29	824.925	0.00		
0.183	0.00	1.675		15.334		140.341		1284.465			

KE-24Jun2016

V&I 27 June 2016

Operator notes:

Appendix 1.2: Geochemical Speciation



Quality Analysis ...

Innovative Technologies

Date Submitted: 02-Nov-17
Invoice No.: A17-12357Final1
Invoice Date: 06-Feb-18
Your Reference:

University of Waterloo
200 University Ave W., Dept. of Geography
Waterloo ON N2L 3G1
Canada
ATTN: Dr. Mike Stone

CERTIFICATE OF ANALYSIS

12 Soil samples were submitted for analysis.

The following analytical package(s) were requested:

- Code 4C (11+) Whole Rock Analysis-XRF
Code S9-Particle Size (Laser) Particle Size Analysis
Code UT-1-0.5g Aqua Regia ICP/MS

REPORT A17-12357Final1

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Notes:

Assays are recommended for values above the upper limit. The Au from AR-MS is only semiquantitative. For accurate Au data, fire assay is recommended.

CERTIFIED BY:

[Handwritten signature]

ACTIVATION LABORATORIES LTD.

41 Bittern Street, Ancaster, Ontario, Canada, L9G 4V5

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Emmanuel Esemé, Ph.D.

Quality Control

Geochemical Composition of Glenmore Reservoir Sediments at Various Depths

Site	Analyte Symbol	Co ₃ O ₄	CuO	NiO	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃ (T)	MnO	MgO	CaO	Na ₂ O	K ₂ O	TiO ₂	P ₂ O ₅	Cr ₂ O ₃	V ₂ O ₅	LOI	Total
	Unit Symbol	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
	Lower Limit	0.005	0.005	0.003	0.01	0.01	0.01	0.001	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.003	
HeritageCoveA		< 0.005	0.007	< 0.003	41.36	11.6	4.18	0.06	3.48	15.24	0.36	2.11	0.5	0.16	0.01	0.024	20.73	99.8
HeritageCoveB		< 0.005	0.007	< 0.003	41.23	11.9	4.07	0.057	3.57	15.9	0.4	2.21	0.5	0.15	0.01	0.026	20.8	100.8
HeritageCoveC		< 0.005	0.006	< 0.003	38.27	11.02	3.79	0.054	3.68	17.32	0.34	2.19	0.48	0.15	0.01	0.024	22.09	99.42
WeaselHeadA		< 0.005	0.007	< 0.003	39.93	9.35	3.76	0.053	3.57	17.66	0.41	1.67	0.45	0.15	< 0.01	0.018	23.18	100.2
WeaselHeadB		< 0.005	0.006	< 0.003	40.76	9.77	3.54	0.049	3.92	17.16	0.42	1.76	0.47	0.15	0.01	0.024	22.14	100.2
WeaselHeadC		< 0.005	0.008	< 0.003	39.4	8.72	3.23	0.053	4.6	17.17	0.48	1.64	0.45	0.14	0.01	0.019	23.58	99.49
MidLakeA		< 0.005	0.007	< 0.003	41.78	10.88	3.98	0.07	3.54	15.63	0.39	1.95	0.48	0.16	< 0.01	0.025	21.04	99.93
MidLakeB		< 0.005	0.007	< 0.003	41.52	10.93	3.79	0.055	3.76	16.41	0.4	1.98	0.49	0.15	0.01	0.024	21.04	100.6
MidLakeC		< 0.005	0.005	< 0.003	38.17	10.06	3.57	0.057	4.15	18.25	0.36	2.07	0.47	0.15	0.01	0.022	22.99	100.3
HeadPondA		< 0.005	0.007	< 0.003	43.9	11.85	4.41	0.068	3.3	13.22	0.36	2.09	0.51	0.2	0.01	0.026	19.84	99.78
HeadPondB		< 0.005	0.007	< 0.003	42.55	11.15	4.01	0.056	3.53	14.94	0.37	2	0.49	0.18	0.01	0.025	20.31	99.63
HeadPondC		< 0.005	0.007	< 0.003	41.94	10.78	3.95	0.052	3.5	14.67	0.39	1.89	0.47	0.17	0.01	0.023	21.31	99.18

Appendix 1.3: Particulate Phosphorus Speciation



Date Submitted: 02-Nov-17
Invoice No.: A17-12357Final2
Invoice Date: 07-Feb-18
Your Reference:

University of Waterloo
200 University Ave W., Dept. of Geography
Waterloo ON N2L 3G1
Canada
ATTN: Dr. Mike Stone

CERTIFICATE OF ANALYSIS

12 Soil samples were submitted for analysis.

The following analytical package(s) were requested:
Code 4C (11+) Whole Rock Analysis-XRF
Code S9-Particle Size (Laser) Particle Size Analysis
Code UT-1-0.5g Aqua Regia ICP/MS

REPORT A17-12357Final2

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Notes:

Assays are recommended for values above the upper limit. The Au from AR-MS is only semi-quantitative. For accurate Au data, fire assay is recommended.

CERTIFIED BY:

[Handwritten signature]

Emmanuel Esemé, Ph.D.

Quality Control

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Particulate Phosphorus Fraction of Glenmore Reservoir Sediments at Various Depths

Analyte Symbol	P	P	P	P	P
Unit Symbol	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$
Lower Limit	0.02	0.02	0.02	0.02	0.02
Method Code	HCL- RP	BD-RP	NH4CL- RP	NaOH- RP	Refract ory-P
HeritageCoveA	468	34.1	< 10	51.7	100
HeritageCoveB	467	27.0	< 10	44.7	93.1
HeritageCoveC	438	19.5	< 10	43.2	100
WeaselHeadA	409	21.4	< 10	61.6	125
WeaselHeadB	445	10.5	< 10	31.1	79.6
WeaselHeadC	406	18.5	< 10	44.5	72.0
MidLakeA	434	14.3	< 10	47.2	116
MidLakeB	450	13.7	< 10	32.4	90.5
MidLakeC	431	30.1	< 10	49.5	72.9
HeadPondA	469	29.0	< 10	174	176
HeadPondB	514	34.4	< 10	70.0	105
HeadPondC	485	32.8	< 10	84.1	107

Appendix 1.4: Equilibrium Phosphate Concentration (EPC_0)

Glenmore Reservoir EPC₀ Data

Legend

DEPTH	
A	0 to 2 cm from top
B	2 to 4 cm from top
C	0 to 2 cm from bottom
REPLICATION	
R1	Replicate 1
R2	Replicate 2
R3	Replicate 3

Location	Depth	Conc	SedMass	StdVol	ActConc	P _{ug_L}	P _{abs}	P _{abs} (Calc)/ Q _e
HC	A	3.4	0.25	25.044	3.400	9.372	-0.598	-0.597
HC	A	3.4	0.249	25.012	3.400	13.334	-0.998	-0.997
HC	A	3.4	0.249	25.012	3.400	5.929	-0.254	-0.254
HC	A	3.4	0.249	25.012	3.400	8.891	-0.552	-0.551
HC	A	3.4	0.25	25.002	3.400	10.062	-0.666	-0.666
HC	A	25	0.25	25.026	25.185	11.980	1.322	1.321
HC	A	25	0.25	25.015	25.185	14.064	1.113	1.112
HC	A	25	0.249	25.012	25.185	9.631	1.562	1.562
HC	A	50	0.251	25.015	50.098	15.139	3.484	3.482
HC	A	50	0.251	25.021	50.098	16.885	3.311	3.308
HC	A	50	0.249	25.005	50.098	16.158	3.408	3.408
HC	A	100	0.25	25.013	100.024	12.775	8.729	8.725
HC	A	100	0.249	25.025	100.024	10.752	8.972	8.963
HC	A	100	0.251	25.010	100.024	8.471	9.122	9.119
HC	A	200	0.25	25.015	200.116	19.084	18.114	18.103
HC	A	200	0.249	25.002	200.116	19.335	18.152	18.151
HC	A	200	0.25	25.011	200.116	17.687	18.251	18.243
HC	B	3.4	0.25	25.052	3.400	13.611	-1.023	-1.021
HC	B	3.4	0.25	25.052	3.400	7.786	-0.440	-0.439
HC	B	3.4	0.25	25.052	3.400	9.713	-0.633	-0.631
HC	B	3.4	0.249	25.009	3.400	7.980	-0.460	-0.460
HC	B	3.4	0.25	25.002	3.400	13.193	-0.979	-0.979
HC	B	3.4	0.25	25.002	3.400	6.835	-0.344	-0.344
HC	B	3.4	0.25	25.002	3.400	9.575	-0.618	-0.618
HC	B	25	0.249	24.996	25.185	13.786	1.144	1.144

HC	B	25	0.249	25.007	25.185	11.154	1.409	1.409
HC	B	25	0.251	25.040	25.185	17.066	0.810	0.809
HC	B	50	0.249	25.010	50.098	17.080	3.316	3.315
HC	B	50	0.249	25.010	50.098	20.146	3.008	3.007
HC	B	50	0.249	25.010	50.098	19.712	3.052	3.051
HC	B	50	0.25	24.991	50.098	15.823	3.426	3.428
HC	B	50	0.25	25.111	50.098	20.516	2.971	2.958
HC	B	100	0.249	25.020	100.024	11.222	8.923	8.916
HC	B	100	0.25	25.030	100.024	10.177	8.996	8.985
HC	B	100	0.25	25.071	100.024	10.942	8.934	8.908
HC	B	200	0.251	25.025	200.116	22.811	17.678	17.660
HC	B	200	0.25	25.023	200.116	17.781	18.250	18.234
HC	B	200	0.25	25.107	200.116	21.751	17.913	17.837
HC	C	3.4	0.25	25.005	3.400	14.491	-1.109	-1.109
HC	C	3.4	0.25	25.005	3.400	11.029	-0.763	-0.763
HC	C	3.4	0.25	25.005	3.400	8.757	-0.536	-0.536
HC	C	3.4	0.249	25.016	3.400	5.672	-0.228	-0.228
HC	C	3.4	0.251	25.069	3.400	8.519	-0.511	-0.510
HC	C	25	0.251	25.018	25.185	12.351	1.279	1.278
HC	C	25	0.25	25.009	25.185	15.302	0.989	0.988
HC	C	25	0.25	25.026	25.185	13.685	1.151	1.150
HC	C	50	0.25	25.003	50.098	14.095	3.601	3.600
HC	C	50	0.25	25.010	50.098	20.087	3.002	3.001
HC	C	50	0.251	25.010	50.098	15.346	3.463	3.461
HC	C	100	0.25	25.043	100.024	9.362	9.082	9.066
HC	C	100	0.249	25.013	100.024	10.857	8.957	8.953
HC	C	100	0.25	24.953	100.024	10.087	8.977	8.994
HC	C	200	0.25	25.003	200.116	24.457	17.568	17.566
HC	C	200	0.25	25.003	200.116	24.023	17.611	17.609
HC	C	200	0.25	25.003	200.116	15.599	18.454	18.452
HC	C	200	0.249	24.998	200.116	19.591	18.124	18.125
HC	C	200	0.25	25.023	200.116	17.596	18.269	18.252
WH	A	2.7	0.25	24.997	2.700	7.416	-0.472	-0.472
WH	A	2.7	0.25	24.997	2.700	7.450	-0.475	-0.475
WH	A	2.7	0.25	24.997	2.700	9.603	-0.690	-0.690
WH	A	2.7	0.25	24.999	2.700	5.069	-0.237	-0.237
WH	A	2.7	0.249	25.000	2.700	7.239	-0.456	-0.456
WH	A	25	0.25	25.017	25.048	10.290	1.477	1.476
WH	A	25	0.249	25.002	25.048	5.954	1.917	1.917
WH	A	25	0.25	25.009	25.048	9.793	1.526	1.525
WH	A	50	0.251	25.009	49.954	12.357	3.746	3.745
WH	A	50	0.25	25.017	49.954	7.575	4.241	4.238
WH	A	50	0.25	25.012	49.954	12.729	3.724	3.723
WH	A	100	0.25	25.019	99.957	6.208	9.382	9.375

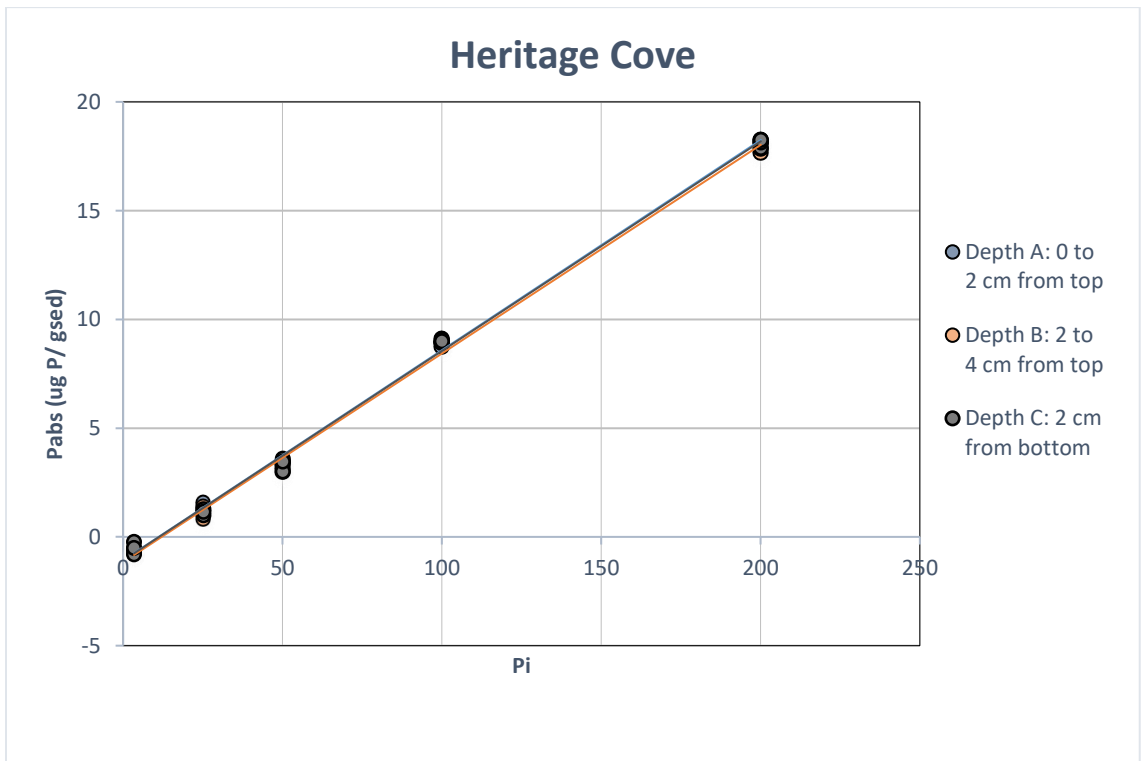
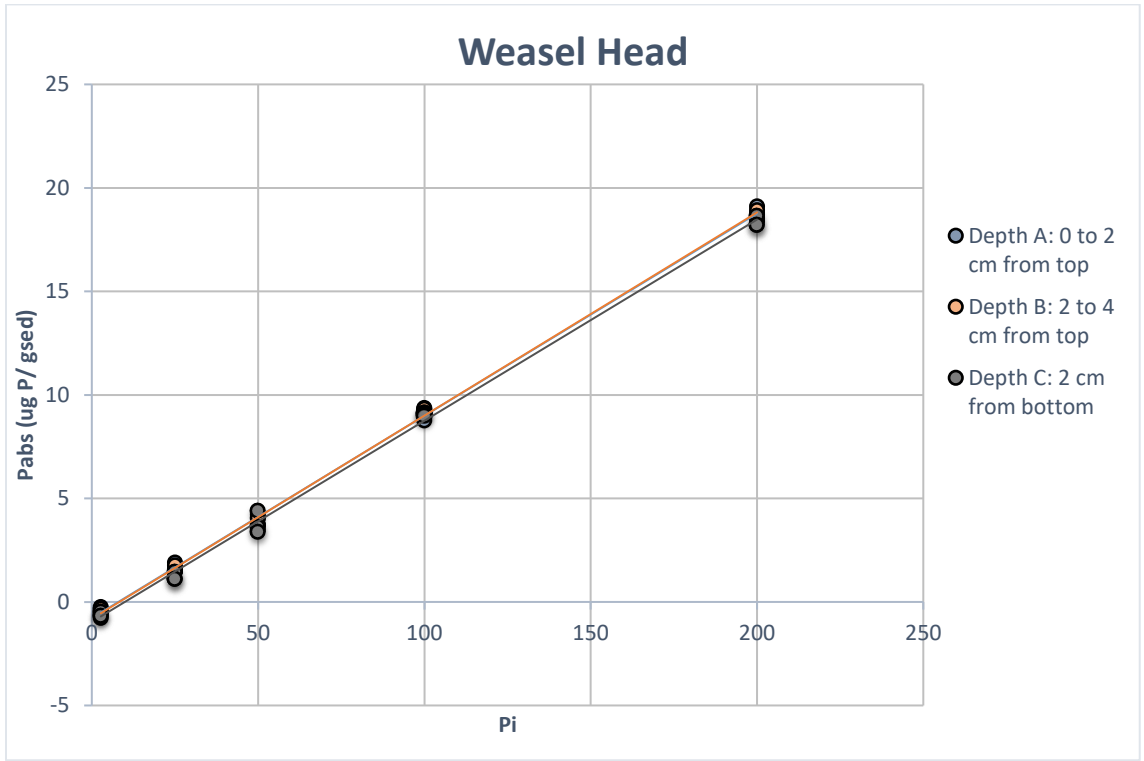
WH	A	100	0.251	25.063	99.957	6.753	9.307	9.283
WH	A	100	0.25	25.023	99.957	12.286	8.775	8.767
WH	A	200	0.249	24.994	200.033	16.729	18.400	18.404
WH	A	200	0.25	24.999	200.033	8.841	19.118	19.119
WH	A	200	0.25	25.028	200.033	12.946	18.730	18.709
WH	B	2.7	0.25	24.999	2.700	6.220	-0.352	-0.352
WH	B	2.7	0.25	25.006	2.700	10.344	-0.765	-0.764
WH	B	2.7	0.249	24.995	2.700	6.044	-0.336	-0.336
WH	B	25	0.249	25.007	25.048	9.000	1.612	1.611
WH	B	25	0.251	25.011	25.048	10.386	1.461	1.460
WH	B	25	0.251	25.011	25.048	10.852	1.415	1.414
WH	B	25	0.251	25.011	25.048	8.573	1.642	1.641
WH	B	25	0.25	24.949	25.048	7.257	1.775	1.779
WH	B	50	0.251	24.993	49.954	9.521	4.026	4.027
WH	B	50	0.249	25.019	49.954	15.102	3.502	3.499
WH	B	50	0.25	25.020	49.954	8.983	4.100	4.097
WH	B	100	0.25	25.019	99.957	8.256	9.177	9.170
WH	B	100	0.25	25.022	99.957	6.589	9.345	9.337
WH	B	100	0.251	25.009	99.957	7.931	9.169	9.166
WH	B	200	0.25	25.002	200.033	12.163	18.789	18.787
WH	B	200	0.251	25.011	200.033	13.816	18.556	18.548
WH	B	200	0.25	25.023	200.033	10.714	18.949	18.932
WH	C	2.7	0.249	25.051	2.700	9.094	-0.643	-0.642
WH	C	2.7	0.25	24.997	2.700	7.491	-0.479	-0.479
WH	C	2.7	0.251	25.025	2.700	9.496	-0.678	-0.677
WH	C	25	0.249	25.029	25.048	13.691	1.142	1.140
WH	C	25	0.251	25.018	25.048	10.539	1.446	1.445
WH	C	25	0.251	25.008	25.048	13.742	1.126	1.126
WH	C	50	0.25	25.010	49.954	13.544	3.643	3.641
WH	C	50	0.251	25.005	49.954	5.784	4.400	4.399
WH	C	50	0.25	25.031	49.954	15.978	3.402	3.398
WH	C	100	0.249	24.999	99.957	8.791	9.153	9.153
WH	C	100	0.251	25.001	99.957	8.629	9.097	9.096
WH	C	100	0.25	25.002	99.957	10.232	8.973	8.973
WH	C	200	0.25	25.030	200.033	17.929	18.232	18.210
WH	C	200	0.251	24.993	200.033	12.994	18.624	18.629
WH	C	200	0.25	25.169	200.033	17.642	18.362	18.239
ML	A	3.6	0.249	25.011	3.600	7.056	-0.347	-0.347
ML	A	3.6	0.25	25.004	3.600	10.420	-0.682	-0.682
ML	A	3.6	0.25	25.034	3.600	7.880	-0.429	-0.428
ML	A	25	0.25	25.020	25.025	9.686	1.535	1.534
ML	A	25	0.251	25.031	25.025	14.254	1.074	1.073
ML	A	25	0.251	25.054	25.025	9.650	1.535	1.531
ML	A	50	0.249	24.997	49.977	7.674	4.247	4.247

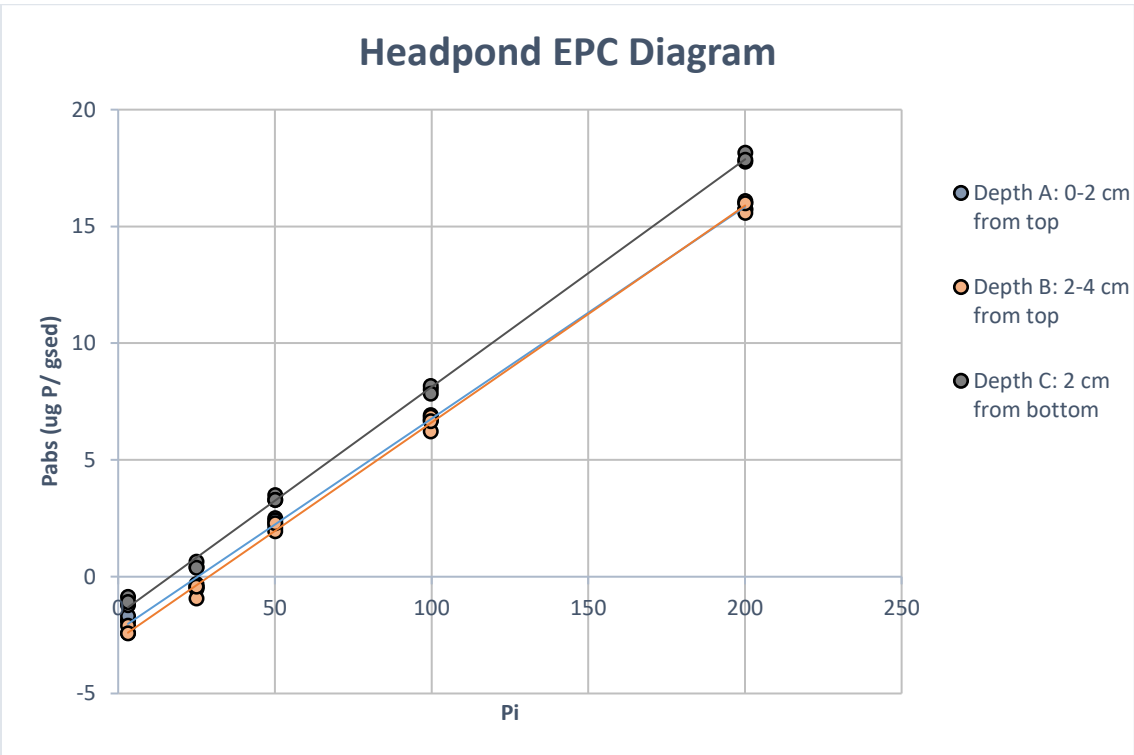
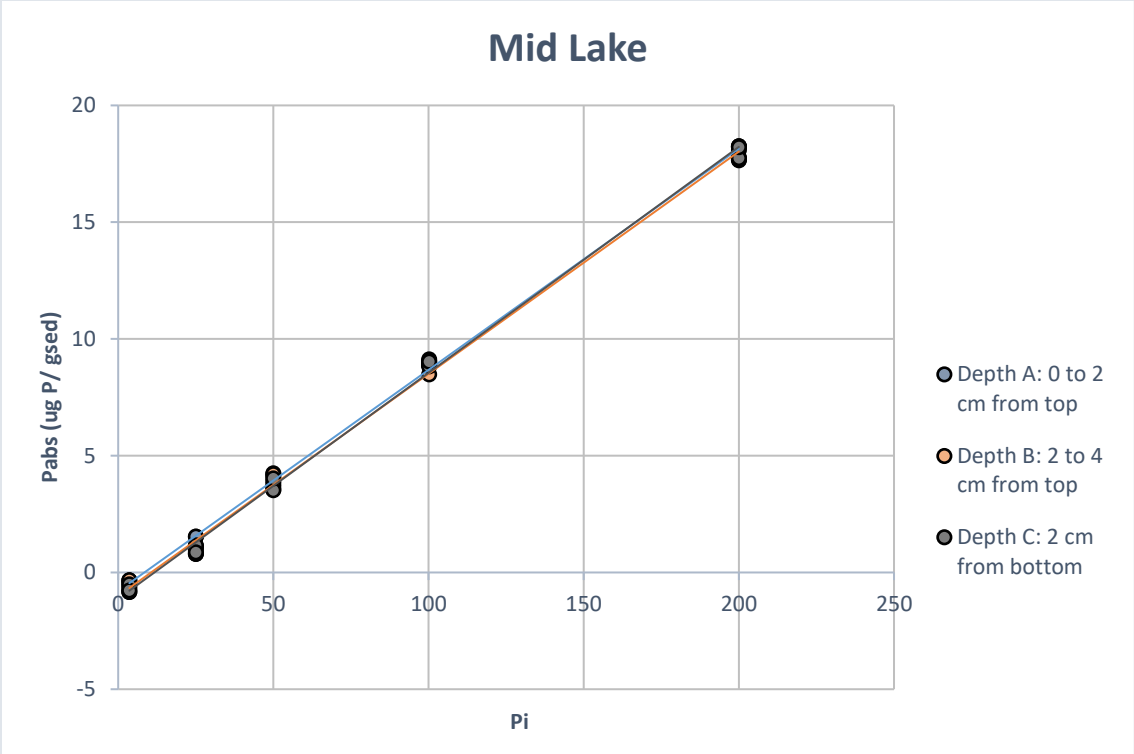
ML	A	50	0.25	25.001	49.977	14.430	3.555	3.555
ML	A	50	0.25	25.004	49.977	8.743	4.124	4.123
ML	A	100	0.25	25.000	100.220	8.928	9.129	9.129
ML	A	100	0.25	25.016	100.220	9.591	9.069	9.063
ML	A	100	0.25	25.005	100.220	11.397	8.884	8.882
ML	A	200	0.249	25.028	199.988	20.379	18.053	18.033
ML	A	200	0.249	25.028	199.988	20.398	18.051	18.031
ML	A	200	0.249	25.028	199.988	18.752	18.217	18.196
ML	A	200	0.25	25.022	199.988	24.116	17.603	17.587
ML	A	200	0.25	25.022	199.988	23.545	17.660	17.644
ML	A	200	0.25	25.022	199.988	22.659	17.749	17.733
ML	A	200	0.25	25.019	199.988	17.532	18.260	18.246
ML	B	3.6	0.251	25.093	3.600	9.321	-0.572	-0.570
ML	B	3.6	0.25	25.014	3.600	6.866	-0.327	-0.327
ML	B	3.6	0.25	25.018	3.600	13.466	-0.987	-0.987
ML	B	3.6	0.25	25.018	3.600	10.796	-0.720	-0.720
ML	B	3.6	0.25	25.018	3.600	11.748	-0.815	-0.815
ML	B	25	0.25	24.991	25.025	15.479	0.954	0.955
ML	B	25	0.25	25.014	25.025	13.710	1.132	1.131
ML	B	25	0.249	25.002	25.025	17.058	0.800	0.800
ML	B	50	0.249	25.958	49.977	13.381	3.815	3.674
ML	B	50	0.251	25.002	49.977	7.697	4.211	4.211
ML	B	50	0.25	24.995	49.977	10.799	3.917	3.918
ML	B	100	0.25	24.999	100.220	11.292	8.892	8.893
ML	B	100	0.251	25.013	100.220	9.800	9.011	9.006
ML	B	100	0.25	25.002	100.220	16.566	8.366	8.365
ML	B	100	0.25	25.002	100.220	17.156	8.307	8.306
ML	B	100	0.25	25.002	100.220	12.624	8.760	8.760
ML	B	200	0.251	25.001	199.988	22.485	17.680	17.680
ML	B	200	0.25	25.003	199.988	17.812	18.220	18.218
ML	B	200	0.25	25.003	199.988	22.244	17.777	17.774
ML	C	3.6	0.25	25.006	3.600	8.619	-0.502	-0.502
ML	C	3.6	0.25	24.996	3.600	12.005	-0.840	-0.841
ML	C	3.6	0.251	25.011	3.600	14.942	-1.130	-1.130
ML	C	3.6	0.251	25.011	3.600	11.439	-0.781	-0.781
ML	C	3.6	0.251	25.011	3.600	7.615	-0.400	-0.400
ML	C	25	0.25	25.015	25.025	13.365	1.167	1.166
ML	C	25	0.249	25.019	25.025	17.045	0.802	0.801
ML	C	25	0.25	25.009	25.025	15.118	0.991	0.991
ML	C	25	0.25	25.009	25.025	18.813	0.621	0.621
ML	C	25	0.25	25.009	25.025	15.286	0.974	0.974
ML	C	50	0.251	25.015	49.977	12.677	3.717	3.715
ML	C	50	0.251	25.015	49.977	9.690	4.015	4.013
ML	C	50	0.251	25.015	49.977	13.081	3.677	3.675

ML	C	50	0.25	25.029	49.977	15.999	3.402	3.398
ML	C	50	0.25	25.029	49.977	11.649	3.837	3.833
ML	C	50	0.25	25.029	49.977	16.636	3.338	3.334
ML	C	50	0.251	25.034	49.977	9.513	4.036	4.030
ML	C	100	0.25	25.002	100.220	11.216	8.901	8.900
ML	C	100	0.251	25.016	100.220	11.669	8.826	8.820
ML	C	100	0.25	25.020	100.220	9.981	9.031	9.024
ML	C	200	0.25	25.006	199.988	17.476	18.256	18.251
ML	C	200	0.251	25.013	199.988	21.778	17.759	17.750
ML	C	200	0.25	25.018	199.988	17.819	18.230	18.217
HP	A	3.1	0.249	25.012	3.100	21.860	-1.884	-1.884
HP	A	3.1	0.25	25.001	3.100	21.883	-1.878	-1.878
HP	A	3.1	0.25	24.994	3.100	19.885	-1.678	-1.679
HP	A	25	0.25	25.036	24.956	30.623	-0.567	-0.567
HP	A	25	0.25	25.036	24.956	31.389	-0.644	-0.643
HP	A	25	0.25	25.036	24.956	26.694	-0.174	-0.174
HP	A	25	0.25	25.025	24.956	28.018	-0.306	-0.306
HP	A	25	0.25	25.018	24.956	30.114	-0.516	-0.516
HP	A	50	0.251	24.995	50.061	24.877	2.508	2.508
HP	A	50	0.25	25.027	50.061	25.899	2.419	2.416
HP	A	50	0.25	25.001	50.061	27.425	2.264	2.264
HP	A	100	0.25	24.996	99.770	32.163	6.760	6.761
HP	A	100	0.25	25.017	99.770	30.577	6.924	6.919
HP	A	100	0.25	25.024	99.770	32.932	6.690	6.684
HP	A	200	0.249	24.995	200.098	43.825	15.687	15.690
HP	A	200	0.249	24.995	200.098	42.268	15.843	15.846
HP	A	200	0.249	24.995	200.098	43.511	15.718	15.722
HP	A	200	0.251	25.003	200.098	39.363	16.011	16.009
HP	A	200	0.25	25.022	200.098	42.707	15.753	15.739
HP	B	3.1	0.25	25.073	3.100	27.171	-2.414	-2.407
HP	B	3.1	0.249	25.007	3.100	23.935	-2.092	-2.092
HP	B	3.1	0.25	25.042	3.100	27.352	-2.429	-2.425
HP	B	25	0.251	25.015	24.956	29.223	-0.425	-0.425
HP	B	25	0.25	25.011	24.956	34.227	-0.927	-0.927
HP	B	25	0.249	25.000	24.956	29.205	-0.427	-0.427
HP	B	50	0.25	24.994	50.061	29.142	2.091	2.092
HP	B	50	0.25	24.999	50.061	30.535	1.953	1.953
HP	B	50	0.25	24.999	50.061	27.119	2.294	2.294
HP	B	100	0.25	25.005	99.770	31.249	6.853	6.852
HP	B	100	0.25	25.005	99.770	31.521	6.826	6.825
HP	B	100	0.25	25.005	99.770	30.928	6.886	6.884
HP	B	100	0.25	25.027	99.770	38.675	6.116	6.110
HP	B	100	0.25	25.027	99.770	37.794	6.204	6.198
HP	B	100	0.25	25.027	99.770	36.310	6.353	6.346

HP	B	100	0.25	25.018	99.770	33.205	6.661	6.657
HP	B	200	0.25	25.027	200.098	39.227	16.104	16.087
HP	B	200	0.25	25.002	200.098	44.268	15.584	15.583
HP	B	200	0.25	24.994	200.098	40.110	15.995	15.999
HP	C	3.1	0.251	25.034	3.100	11.681	-0.856	-0.855
HP	C	3.1	0.25	25.013	3.100	15.291	-1.220	-1.219
HP	C	3.1	0.25	25.153	3.100	16.752	-1.374	-1.365
HP	C	3.1	0.25	25.153	3.100	11.548	-0.850	-0.845
HP	C	3.1	0.25	25.153	3.100	13.445	-1.041	-1.035
HP	C	25	0.25	25.018	24.956	20.695	0.426	0.426
HP	C	25	0.25	25.032	24.956	18.444	0.652	0.651
HP	C	25	0.25	24.990	24.956	21.139	0.382	0.382
HP	C	50	0.251	25.024	50.061	14.981	3.497	3.494
HP	C	50	0.25	24.996	50.061	18.019	3.204	3.204
HP	C	50	0.25	24.996	50.061	18.401	3.165	3.166
HP	C	50	0.25	24.996	50.061	14.840	3.522	3.522
HP	C	50	0.25	25.027	50.061	17.125	3.297	3.294
HP	C	100	0.25	25.015	99.770	19.572	8.025	8.020
HP	C	100	0.25	25.100	99.770	18.038	8.206	8.173
HP	C	100	0.25	25.001	99.770	21.409	7.836	7.836
HP	C	200	0.249	25.006	200.098	23.005	17.785	17.780
HP	C	200	0.251	25.020	200.098	17.774	18.174	18.160
HP	C	200	0.25	25.006	200.098	21.479	17.866	17.862

EPC₀ Isotherms for Glenmore Reservoir





Elbow River EPC₀ Data

Sample.	P_STD_conc	P_abs	P_STD_act	P_abs_sd
CobbleFlats	0	-1.030	0.000	0.212
CobbleFlats	25	1.599	25.072	0.486
CobbleFlats	50	4.317	50.143	0.109
CobbleFlats	100	8.936	100.232	0.069
CobbleFlats	200	18.267	200.492	0.068
ERCF	0	-2.469	0.000	0.266
ERCF	25	-0.516	25.072	0.657
ERCF	50	1.316	50.143	0.247
ERCF	100	3.958	100.232	0.245
ERCF	200	9.714	200.492	0.015
ERTB	0	-1.450	0.000	0.313
ERTB	25	0.914	25.072	0.540
ERTB	50	3.227	50.143	0.178
ERTB	100	7.388	100.232	0.194
ERTB	200	15.338	200.492	0.126
ERWFB	0	-1.274	0.000	0.132
ERWFB	25	0.917	25.072	0.678
ERWFB	50	3.203	50.143	0.283
ERWFB	100	7.185	100.232	0.240
ERWFB	200	14.457	200.492	0.296
GR31	0	-1.004	0.000	0.244
GR31	25	1.618	25.072	0.645
GR31	50	4.163	50.143	0.055
GR31	100	8.950	100.232	0.274
GR31	200	18.501	200.492	0.114
GRS19	0	-0.924	0.000	0.299
GRS19	25	1.582	25.072	0.558
GRS19	50	4.114	50.143	0.166
GRS19	100	8.834	100.232	0.087
GRS19	200	18.054	200.492	0.203
GRS2	0	-2.000	0.000	0.113
GRS2	25	0.658	25.072	1.028
GRS2	50	3.383	50.143	0.093
GRS2	100	8.046	100.232	0.067
GRS2	200	17.064	200.492	0.061
GRS25	0	-1.073	0.000	0.225
GRS25	25	1.583	25.072	0.553
GRS25	50	4.183	50.143	0.098
GRS25	100	9.167	100.232	0.118

GRS25	200	18.350	200.492	0.134
GRS37	0	-1.034	0.000	0.220
GRS37	25	1.722	25.072	0.695
GRS37	50	4.229	50.143	0.123
GRS37	100	9.100	100.232	0.303
GRS37	200	18.790	200.492	0.114
GRS4	0	-1.238	0.000	0.088
GRS4	25	1.508	25.072	0.645
GRS4	50	4.181	50.143	0.140
GRS4	100	8.990	100.232	0.096
GRS4	200	18.477	200.492	0.015
GRS48ADJ	0	-0.906	0.000	0.171
GRS48ADJ	25	1.778	25.072	0.510
GRS48ADJ	50	4.591	50.143	0.054
GRS48ADJ	100	9.386	100.232	0.053
GRS48ADJ	200	19.112	200.492	0.213
HWY22	0	-1.876	0.000	0.577
HWY22	25	0.425	25.070	0.749
HWY22	50	2.866	50.143	0.314
HWY22	100	6.473	100.237	0.754
HWY22	200	14.287	200.493	0.397
TwinBridge	0	-1.225	0.000	0.757
TwinBridge	25	0.927	25.072	0.558
TwinBridge	50	3.388	50.143	0.244
TwinBridge	100	7.414	100.232	0.624
TwinBridge	200	16.407	200.492	0.054
WFB	0	-1.619	0.000	0.218
WFB	25	0.716	25.068	0.506
WFB	50	3.248	50.142	0.223
WFB	100	7.521	100.232	0.082
WFB	200	13.859	200.493	3.597

Appendix 2: EPC₀ Quality Assurance & Quality Control

Glenmore Reservoir samples

Sorption samples

Samples were freeze dried, ground and weighed out to 0.25 grams in polypropylene centrifuge tubes. Equilibrium experiments were performed by mixing weighed out samples with 25ml of ambient P in reservoir water, 25, 50, 100, 200 $\mu\text{g/L}$ KH_2PO_4 . Triplicate samples were done for each sample and concentration. Samples were shaken for 20 hours at room temperature of 24 ± 1 $^\circ\text{C}$. Then samples were centrifuged at 4000G for 5 minutes, and the supernatant was filtered with 0.45 μm syringe filters.

The mass of inorganic P adsorbed or desorbed was determined using the following equation:

$$P_{\text{ads}} = [(P_{\text{initial}} - P_{\text{final}}) * 0.025\text{L}] * \text{wt}_{\text{sed}}^{-1}$$

Glenmore Reservoir

Triplicate samples (separately weighed out samples) for Glenmore reservoir had an average standard deviation of 0.20 $\mu\text{g P/g}_{\text{sed}}$ (median: 0.20 $\mu\text{g P/g}_{\text{sed}}$).

AA Run

The following QA/QC is for colorimeter analysis of soluble reactive phosphorus.

Samples were run on two AA2 channels using a Stannous Chloride and Ammonium Molybdate method.

Quality Cups and Drifts

Quality control cups for P concentrations of 25 $\mu\text{g/L}$ were placed evenly throughout the runs and in triplicate.

Table 13- Quality cup results by channel for Glenmore Reservoir QA QC Samples

P Standard calculated (µg/L)	N	Channel 1		Channel 2	
		Quality cup average (µg/L)	Quality cup sd	Quality cup average (µg/L)	Quality cup sd
25.03 (02/11)	3	26.0	0.7	24.9	3.3
25.07 (06/11)	3	26.7	1.3	26.9	3.5
Null (02/11)	18	0.4	2.4	1.0	1.9
Null (06/11)	17	1.7	1.4	2.2	2.5

Drifts (200 µg/L) were placed throughout the run, for channel 1 drifts came back as 99.6 and 201.1 µg/L with no variation for 02/11 and 06/11 respectively, while channel 2 measured the drift at 97.7 and 199.6 µg/L with no variation for 02/11 and 06/11 respectively. The standard concentration that was used for drifts was calculated to be 100.1 and 201.1 µg/L (using weights of P intermediate solution).

GRELB

10% of Glenmore Reservoir samples were run in triplicate for both runs.

The average standard deviation of samples run in triplicate for both dates was 2.14 µg/L (median: 2.02 µg/L), therefore this is within the method detection limit of 5 µg/L.

Elbow River samples

Sorption samples

Samples were freeze dried, ground and weighed out to 0.25 grams in polypropylene centrifuge tubes. Equilibrium experiments were performed by mixing weighed out samples with 25ml of 0, 25, 50, 100, 200 $\mu\text{g/L}$ KH_2PO_4 . Triplicate samples were done for each sample and concentration. Samples were shaken for 20 hours at room temperature of 24 ± 1 $^\circ\text{C}$. Then samples were centrifuged at 4000G for 5 minutes, and the supernatant was filtered with 0.45 μm syringe filters.

The mass of inorganic P adsorbed or desorbed was determined using the following equation:

$$P_{\text{ads}} = [(P_{\text{initial}} - P_{\text{final}}) * 0.025\text{L}] * \text{wt}_{\text{sed}}^{-1}$$

Elbow River

Triplicate samples (separately weighed out samples) for Glenmore reservoir and Elbow river had an average standard deviation of 0.37 $\mu\text{g P/ g}_{\text{sed}}$ (median: 0.22 $\mu\text{g P/ g}_{\text{sed}}$).

Note that for WFB at 200 $\mu\text{g P/L}$ two of the triplicate values came back around 44 $\mu\text{g/L}$, other sample came back at 104 $\mu\text{g/L}$, this could be a human error, or it could be that there was a rock or something that was taking up substantial weight with limited sorption capacity.

**See metadata file for data file descriptions

AA Run

The following QA/QC is for colorimeter analysis of soluble reactive phosphorus.

Samples were run on two AA2 channels using a Stannous Chloride and Ammonium Molybdate method.

Quality Cups and Drifts

Quality control cups for P concentrations of 250, 200, and 100 $\mu\text{g/L}$ were run in triplicate, while 50 $\mu\text{g/L}$ was run in duplicate. Duplicates and triplicated were evenly spaced throughout the run.

Table 14- Quality cup results by channel for Elbow River QA QC Samples

P Standard calculated (µg/L)	N	Channel 1		Channel 2	
		Quality cup average (µg/L)	Quality cup sd	Quality cup average (µg/L)	Quality cup sd
50.25	2	49.42	2.53	50.58	0.09
100.55	3	106.94	6.77	107.42	6.22
201.60	3	199.59	9.15	196.07	7.01
251.55	3	248.28	1.93	247.79	7.90
Null	27	-0.12	3.25	3.65	10.99

Drifts were placed throughout the run, for channel 1 drifts came back as 250.18 µg/L with no variation, while channel 2 measured the drift at 250.13 µg/L, again with no variation. The standard concentration that was used for drifts was calculated to be 251.55 µg/L (using weights of P intermediate solution).

AA sample triplicates

Note that each sample was weighed out in triplicate for each P concentration.

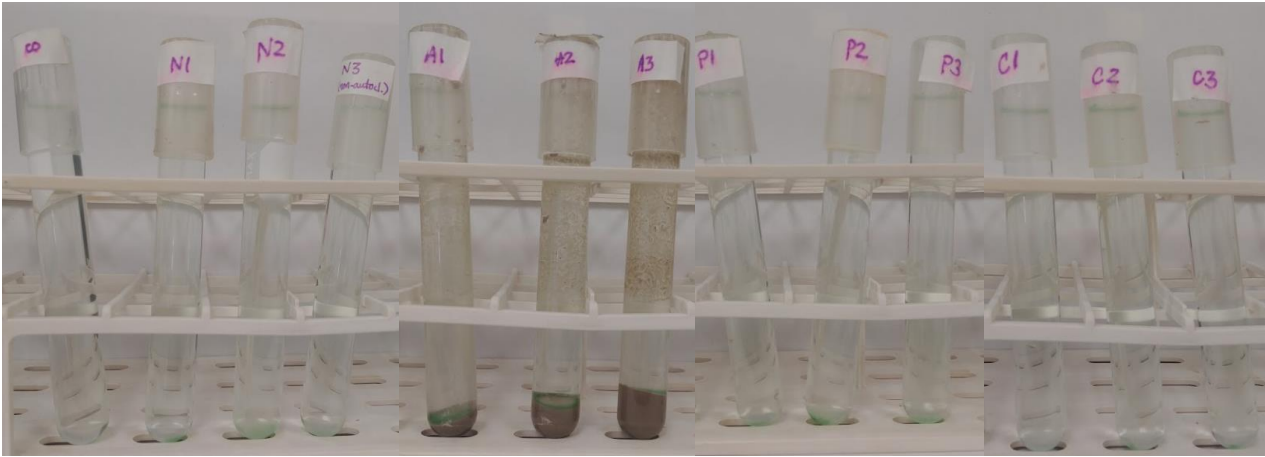
GRELB

10% of GRELB samples (23 samples) were run in triplicate

The average standard deviation of samples run in triplicate was 3.24 µg/L (median: 2.37 µg/L), therefore this is within the method detection limit of 5 µg/L.

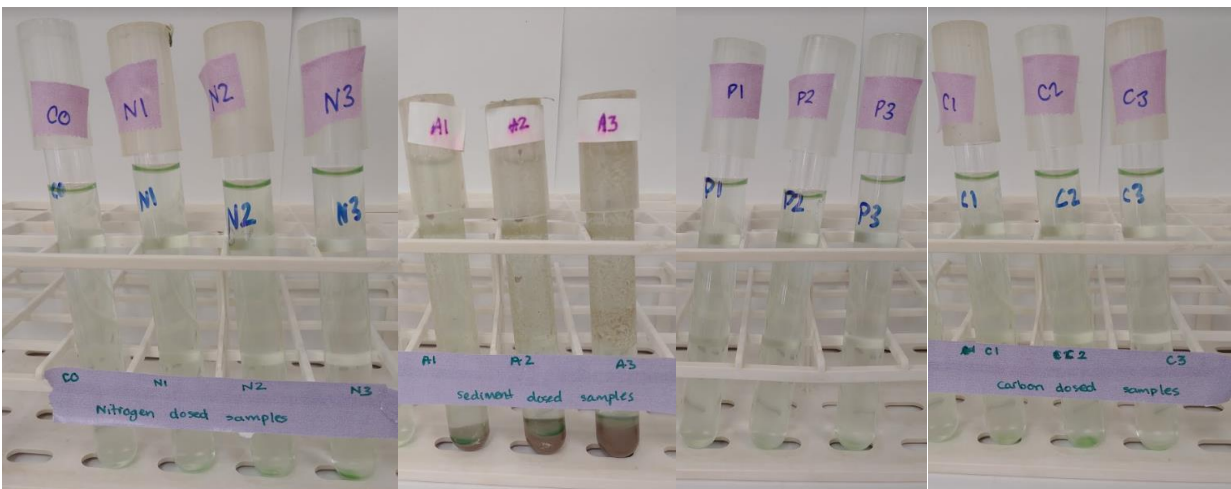
**Appendix 3: *M. aeruginosa* Test Tube Microcosm
Experiment Photographs**

MARCH 23RD: Day 1



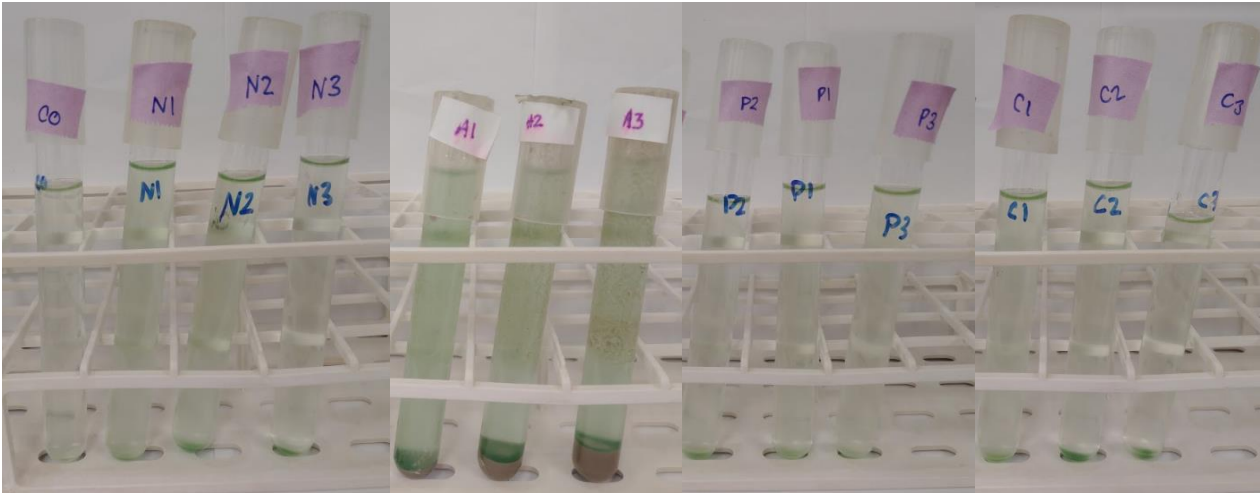
CO	N1	N2	N3	A1	A2	A3	P1	P2	P3	C1	C2	C3
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MARCH 31ST: Day 8



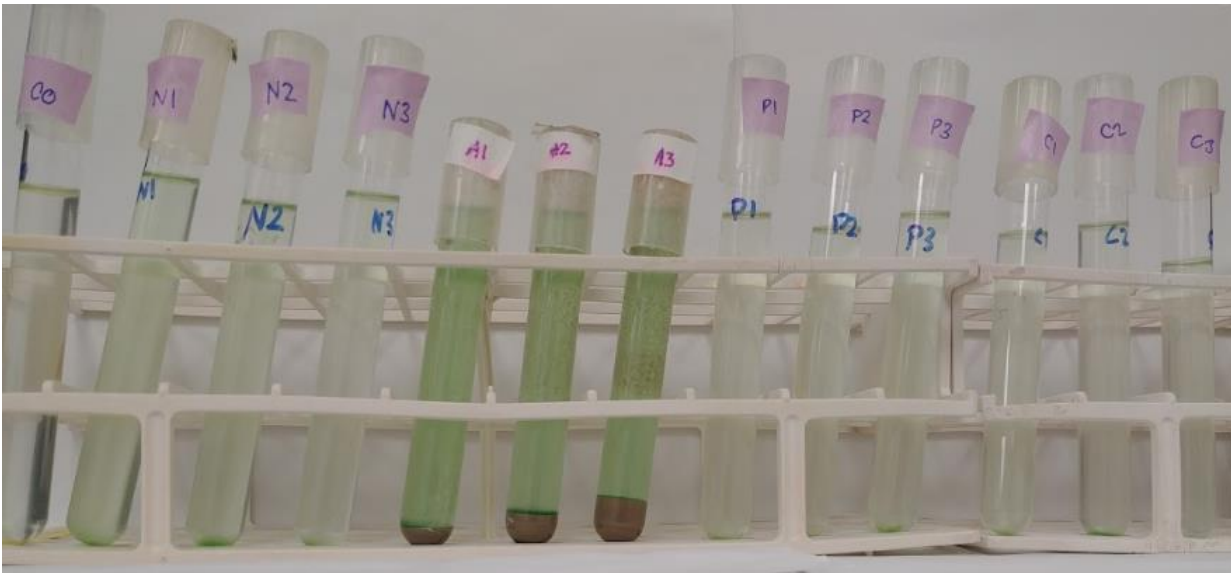
CO	N1	N2	N3	A1	A2	A3	P1	P2	P3	C1	C2	C3
----	----	----	----	----	----	----	----	----	----	----	----	----

APRIL 11TH: Day 19



CO	N1	N2	N3	A1	A2	A3	P1	P2	P3	C1	C2	C3
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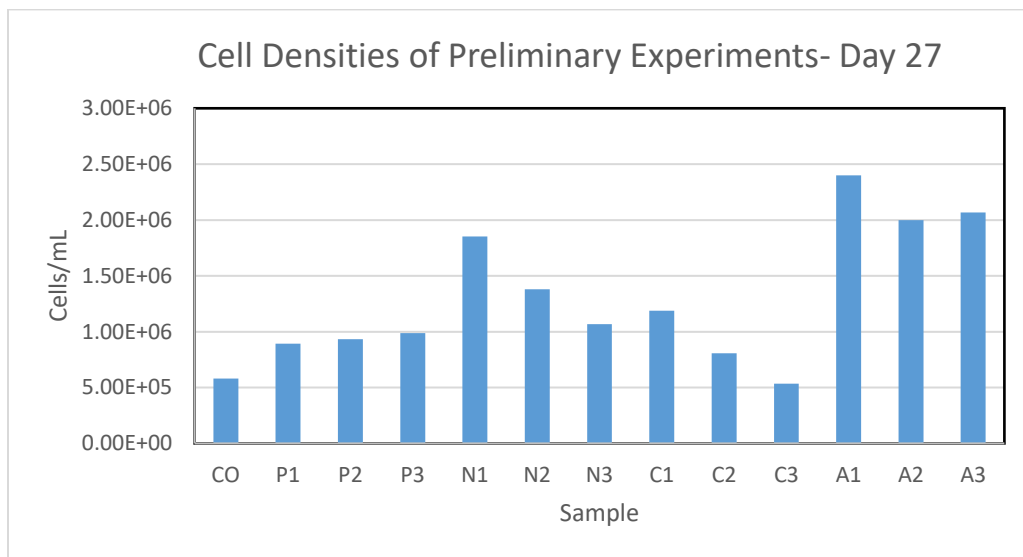
APRIL 19TH: Day 27



CO	N1	N2	N3	A1	A2	A3	P1	P2	P3	C1	C2	C3
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Samples and Cell Densities on Day 27

Sample	CO	P1	P2	P3	N1	N2	N3	C1	C2	C3	A1	A2	A3
Cell Density	5.80E+05	8.93E+05	9.33E+05	9.87E+05	1.85E+06	1.38E+06	1.07E+06	1.19E+06	8.07E+05	5.33E+05	2.40E+06	2.00E+06	2.07E+06
Average			9.38E+05			1.43E+06			8.42E+05			2.16E+06	



**Appendix 4: *M. aeruginosa* Factorial Experiments-
Supplementary Data**

Appendix 4.1: Pigment Analyses

Pigment Concentrations of Samples from Factorial Design

Sample	Chlorophyllide-a	Neoxanthin	Fucoxanthin-like	Myxoxanthophyll	Prasincoxanthin-like	Alloxanthin	Zeaxanthin/Lutein
N1DC-R1	0.00	90.36	3.04	15.24	25.38	1.61	18.78
N1DC-R2	0.00	204.49	5.96	31.58	30.91	2.40	37.83
N1DC-R3	0.00	292.90	5.95	42.71	26.36	5.00	53.02
N1HP-R1	0.00	415.76	4.56	105.51	0.00	2.48	57.40
N1HP-R2	0.00	236.24	3.32	57.93	0.00	1.96	39.14
N1HP-R3	0.81	240.52	26.41	57.41	35.85	3.64	38.93
N2DC-R1	0.00	67.26	9.43	16.54	76.50	0.00	26.98
N2DC-R2	0.00	173.53	9.27	22.42	34.72	2.13	37.63
N2DC-R3	0.00	127.71	10.88	23.80	80.15	5.04	44.73
N2HP-R1	0.00	510.85	5877.00	90.14	0.00	5.09	54.46
N2HP-R2	0.00	247.03	0.00	39.72	0.00	3.58	35.06
N2HP-R3	0.00	310.57	37.22	63.92	97.87	6.27	46.36
CO	0.00	38.82	0.00	9.55	0.00	0.48	5.21
BG11	0.00	74.78	0.00	55.13	0.00	6.39	168.24
[P]	0.00	56.35	3.03	15.85	0.00	0.19	8.78
[N]	0.00	80.79	0.00	31.07	0.00	2.23	8.78
DC	0.00	312.16	12.28	53.60	0.00	7.16	65.99
HP	1.44	407.52	4.29	91.25	0.00	3.88	51.23

Average Values

	Chlorophyllide-a	Neoxanthin	Fucoxanthin-like	Myxoxanthophyll	Prasincoxanthin-like	Alloxanthin	Zeaxanthin/Lutein
N1DC	0.00	195.91	4.99	29.84	27.55	3.00	36.54
N1HP	0.27	297.51	11.43	73.62	11.95	2.69	45.15
N2DC	0.00	122.84	9.86	20.92	63.79	2.39	36.45
N2HP	0.00	356.15	1971.41	64.59	32.62	4.98	45.29

Pigment Concentrations of Samples from Factorial Design (continued)

Sample	Violaxanthin	Canthaxanthin	Chlorophyll-b	Chlorophyll-a	Chlorophyll-a'	Echinenone	Phaeophytin-b
N1DC-R1	1.45	13.87	3.34	424.24	109.08	0.50	5.10
N1DC-R2	6.25	29.35	17.74	896.52	193.38	1.03	13.65
N1DC-R3	7.82	33.26	0.00	1447.95	373.89	2.84	0.00
N1HP-R1	9.83	30.23	0.00	949.56	226.10	1.23	0.00
N1HP-R2	8.94	22.37	0.00	669.31	208.88	1.60	5.84
N1HP-R3	5.65	29.93	0.00	1027.91	316.76	2.73	24.28
N2DC-R1	6.49	18.25	15.69	448.63	114.40	0.00	14.07
N2DC-R2	10.34	25.27	5.12	932.59	240.42	1.81	42.46
N2DC-R3	21.94	39.09	35.63	912.53	270.94	1.23	40.61
N2HP-R1	9.17	41.40	0.00	826.74	260.96	2.24	10.08
N2HP-R2	3.37	35.38	0.00	666.13	204.51	0.00	21.65
N2HP-R3	13.40	41.54	0.00	1230.17	412.63	7.09	0.00
CO	0.00	7.10	0.00	46.01	27.97	0.18	0.00
BG11	10.69	52.72	0.00	1536.63	440.42	6.78	0.00
[P]	0.87	5.57	0.00	96.08	49.04	0.14	0.00
[N]	0.92	13.95	0.00	223.12	69.52	32.15	0.00
DC	12.85	29.31	26.92	21.79	22.29	2.56	33.68
HP	5.78	53.79	0.00	1274.46	383.04	3.27	0.00

Average Values

	Violaxanthin	Canthaxanthin	Chlorophyll-b	Chlorophyll-a	Chlorophyll-a'	Echinenone	Phaeophytin-b
N1DC	5.17	25.49	7.03	922.91	225.45	1.46	6.25
N1HP	8.14	27.51	0.00	882.26	250.58	1.85	10.04
N2DC	12.93	27.54	18.81	764.58	208.59	1.01	32.38
N2HP	8.64	39.44	0.00	907.68	292.70	3.11	10.58

Pigment Concentrations of Samples from Factorial Design (continued)

Sample	Phaeophytin-a	alpha Carotene	Chlorophyll-d	beta Carotene
N1DC-R1	37.84	0.00	3.27	14.82
N1DC-R2	40.00	0.86	5.34	22.95
N1DC-R3	409.67	0.00	9.90	32.03
N1HP-R1	26.99	0.00	8.60	9.44
N1HP-R2	99.94	0.55	8.86	44.43
N1HP-R3	114.79	0.00	0.00	37.97
N2DC-R1	40.30	0.00	0.00	22.68
N2DC-R2	60.21	1.74	4.05	44.45
N2DC-R3	166.16	0.51	12.13	59.35
N2HP-R1	55.25	0.00	17.53	9.30
N2HP-R2	33.48	0.00	4.67	15.61
N2HP-R3	169.64	0.00	3.96	27.36
CO	8.36	0.00	0.42	11.87
BG11	35.42	0.73	0.00	37.67
[P]	32.12	0.61	0.00	15.52
[N]	5.99	1.05	0.00	21.74
DC	203.59	2.29	10.58	60.96
HP	98.74	0.00	8.49	13.77

Average Values				
	Phaeophytin-a	alpha Carotene	Chlorophyll-d	beta Carotene
N1DC	162.50	0.29	6.17	23.27
N1HP	80.57	0.18	5.82	30.61
N2DC	88.89	0.75	5.40	42.16
N2HP	86.12	0.00	8.72	17.42

Appendix 4.2: Cell Densities

Factorial Design Samples- *M. aeruginosa* cells/mL

DAY	N1DC-R1	N1DC-R2	N1DC-R3	N1HP-R1	N1HP-R2	N1HP-R3	N2DC-R1	N2DC-R2	N2DC-R3	N2HP-R1	N2HP-R2	N2HP-R3
1	5.00E+05	5.00E+05	5.00E+05	5.00E+05	5.00E+05	5.00E+05	5.00E+05	5.00E+05	5.00E+05	5.00E+05	5.00E+05	5.00E+05
4	2.64E+05	3.36E+05	2.58E+05	2.10E+05	2.58E+05	2.52E+05	3.42E+05	1.96E+05	2.90E+05	2.06E+05	2.74E+05	1.80E+05
4	2.54E+05	2.82E+05	2.38E+05	2.14E+05	1.80E+05	2.36E+05	2.60E+05	1.76E+05	2.56E+05	2.20E+05	2.00E+05	2.86E+05
4	3.04E+05	2.90E+05	1.56E+05	1.96E+05	1.52E+05	3.00E+05	3.34E+05	2.80E+05	2.00E+05	2.16E+05	1.60E+05	2.92E+05
7	3.86E+05	4.38E+05	3.42E+05	3.90E+05	2.96E+05	3.74E+05	3.40E+05	3.70E+05	2.80E+05	5.00E+05	3.44E+05	3.66E+05
7	3.96E+05	3.26E+05	3.80E+05	3.72E+05	1.90E+05	3.76E+05	3.36E+05	3.34E+05	3.42E+05	3.72E+05	1.16E+05	3.48E+05
7	3.56E+05	3.06E+05	4.38E+05	3.14E+05	2.24E+05	3.06E+05	3.38E+05	3.54E+05	3.36E+05	3.88E+05	2.60E+05	1.90E+05
10	1.80E+05	6.00E+05	3.20E+05	3.40E+05	3.20E+05	3.80E+05	2.40E+05	2.80E+05	3.00E+05	3.40E+05	4.60E+05	4.60E+05
10	3.80E+05	3.80E+05	5.40E+05	2.80E+05	2.00E+05	4.60E+05	2.80E+05	4.00E+05	3.60E+05	5.40E+05	5.20E+05	5.40E+05
10	4.20E+05	6.00E+05	2.80E+05	5.40E+05	3.00E+05	4.60E+05	2.60E+05	3.00E+05	3.80E+05	4.40E+05	5.20E+05	4.80E+05
12	4.00E+05	4.80E+05	4.40E+05	6.20E+05	3.80E+05	5.40E+05	5.60E+05	3.40E+05	4.20E+05	9.60E+05	4.00E+05	4.60E+05
12	3.20E+05	5.20E+05	4.40E+05	4.60E+05	2.60E+05	4.40E+05	4.60E+05	4.80E+05	6.20E+05	1.12E+06	7.80E+05	4.80E+05
12	4.20E+05	5.60E+05	5.60E+05	5.00E+05	2.80E+05	2.40E+05	4.00E+05	3.60E+05	4.60E+05	1.36E+06	8.00E+05	5.60E+05
15	6.60E+05	8.80E+05	7.80E+05	7.80E+05	6.00E+05	5.80E+05	7.00E+05	2.60E+05	5.80E+05	1.40E+06	8.00E+05	1.20E+06
15	3.40E+05	8.20E+05	9.40E+05	9.20E+05	5.80E+05	5.40E+05	6.00E+05	4.20E+05	6.40E+05	1.02E+06	1.04E+06	1.00E+06
15	8.40E+05	8.20E+05	5.60E+05	9.60E+05	5.80E+05	4.20E+05	3.80E+05	7.80E+05	7.00E+05	1.46E+06	9.20E+05	1.12E+06
18	5.40E+05	7.40E+05	9.20E+05	1.80E+06	9.20E+05	8.80E+05	4.00E+05	6.20E+05	4.40E+05	2.00E+06	1.20E+06	9.08E+06
18	5.60E+05	8.00E+05	8.60E+05	1.20E+06	9.40E+05	1.16E+06	7.00E+05	5.60E+05	7.00E+05	1.62E+06	9.60E+05	1.00E+06
18	5.00E+05	9.60E+05	1.10E+06	7.00E+05	1.16E+06	8.60E+05	6.60E+05	5.80E+05	9.20E+05	1.50E+06	8.20E+05	9.20E+05
21	6.00E+05	8.20E+05	1.06E+06	1.42E+06	1.34E+06	9.00E+05	5.20E+05	7.00E+05	1.04E+06	1.46E+06	4.40E+05	1.48E+06
21	4.20E+05	1.04E+06	9.60E+05	1.34E+06	1.32E+06	9.00E+05	5.60E+05	6.40E+05	8.60E+05	2.18E+06	5.40E+05	1.14E+06
21	4.20E+05	7.80E+05	1.06E+06	1.46E+06	1.26E+06	5.40E+05	5.80E+05	9.20E+05	9.40E+05	1.44E+06	6.80E+05	1.34E+06
24	1.20E+05	7.80E+05	1.18E+06	2.44E+06	1.64E+06	1.10E+06	7.00E+05	8.00E+05	1.16E+06	3.50E+06	1.48E+06	1.54E+06
24	1.00E+05	1.04E+06	1.28E+06	2.36E+06	9.80E+05	1.08E+06	9.00E+05	1.10E+06	1.50E+06	3.64E+06	1.20E+06	1.80E+06
24	8.00E+04	5.80E+05	1.36E+06	1.96E+06	9.40E+05	1.04E+06	1.04E+06	9.00E+05	1.18E+06	2.84E+06	1.56E+06	2.28E+06
27	9.00E+05	4.36E+06	1.26E+07	2.30E+07	1.06E+07	8.00E+06	2.10E+06	8.20E+06	8.80E+06	2.64E+07	1.00E+07	1.32E+07
27	9.60E+05	4.12E+06	1.28E+07	2.36E+07	9.60E+06	1.08E+07	2.48E+06	8.40E+06	5.00E+06	2.76E+07	1.12E+07	1.22E+07
27	9.60E+05	4.24E+06	1.20E+07	2.20E+07	8.20E+06	8.60E+06	2.40E+06	6.80E+06	8.40E+06	2.74E+07	8.40E+06	1.46E+07
30	2.00E+06	6.20E+06	8.80E+06	2.94E+07	1.32E+07	1.12E+07	2.42E+06	7.60E+06	1.04E+07	5.40E+07	3.40E+06	1.34E+07
30	2.28E+06	7.00E+06	7.80E+06	2.80E+07	1.24E+07	1.02E+07	2.68E+06	7.80E+06	7.60E+06	6.00E+07	1.06E+07	2.34E+07
30	2.06E+06	8.20E+06	1.20E+07	2.86E+07	1.16E+07	9.40E+06	2.82E+06	6.40E+06	7.20E+06	4.80E+07	1.14E+07	1.46E+07
33	3.48E+06	1.02E+07	1.18E+07	3.22E+07	1.38E+07	1.78E+07	2.98E+06	1.26E+07	1.90E+07	1.18E+08	2.38E+07	1.82E+07
33	3.44E+06	1.02E+07	1.62E+07	3.04E+07	2.10E+07	1.76E+07	3.22E+06	1.04E+07	2.06E+07	1.42E+08	1.72E+07	2.26E+07

33	2.68E+06	1.70E+07	2.22E+07	4.10E+07	2.04E+07	1.98E+07	2.78E+06	1.06E+07	1.56E+07	6.40E+07	1.90E+07	2.54E+07
36	7.20E+06	1.34E+07	1.86E+07	6.60E+07	2.62E+07	1.92E+07	3.80E+06	1.02E+07	1.26E+07	9.40E+07	1.60E+07	2.82E+07
36	8.60E+06	1.46E+07	1.72E+07	7.80E+07	1.12E+07	2.06E+07	6.40E+06	1.32E+07	1.10E+07	1.18E+08	1.48E+07	2.54E+07
36	6.80E+06	1.42E+07	1.44E+07	8.00E+07	1.80E+07	1.36E+07	5.60E+06	1.36E+07	1.20E+07	8.20E+07	1.74E+07	2.20E+07
39	7.40E+06	8.80E+06	1.36E+07	9.40E+07	2.34E+07	1.04E+07	7.00E+06	1.38E+07	1.10E+07	1.50E+08	8.00E+06	3.02E+07
39	5.20E+06	9.40E+06	1.02E+07	8.80E+07	1.84E+07	1.38E+07	5.80E+06	1.40E+07	1.40E+07	1.52E+08	1.44E+07	2.02E+07
39	5.80E+06	9.00E+06	1.38E+07	1.14E+08	2.04E+07	1.74E+07	5.60E+06	1.56E+07	1.16E+07	1.38E+08	1.54E+07	2.64E+07
42	6.20E+06	1.62E+07	1.84E+07	1.62E+08	7.40E+07	2.76E+08	1.00E+07	1.22E+08	1.78E+07	1.50E+08	2.70E+08	5.60E+07
42	5.40E+06	1.42E+07	1.98E+07	1.42E+08	3.80E+07	2.52E+08	6.60E+06	1.22E+08	1.54E+07	1.12E+08	2.44E+08	7.60E+07
42	5.20E+06	1.58E+08	2.32E+07	1.12E+08	6.40E+07	2.90E+08	1.04E+07	1.16E+08	1.46E+07	1.26E+08	2.56E+08	8.20E+07
44	6.40E+06	1.98E+08	6.40E+07	1.46E+08	4.00E+07	5.80E+07	8.80E+06	1.24E+07	6.20E+06	1.54E+08	1.24E+08	6.40E+07
44	9.80E+06	2.20E+08	4.60E+07	1.64E+08	6.80E+07	7.40E+07	6.80E+06	1.68E+07	7.00E+06	1.44E+08	1.38E+08	3.80E+07
44	1.00E+07	1.40E+08	4.80E+07	1.48E+08	6.00E+07	7.40E+07	1.02E+07	2.04E+07	6.00E+06	1.80E+08	1.72E+08	6.80E+07
48	1.22E+07	3.40E+07	5.80E+07	1.34E+08	6.80E+07	8.60E+07	1.14E+07	2.74E+08	2.94E+08	1.36E+08	7.20E+07	2.26E+08
48	1.20E+07	7.40E+07	7.00E+07	1.66E+08	9.20E+07	9.00E+07	1.20E+07	3.04E+08	2.82E+08	2.12E+08	9.60E+07	1.28E+08
48	1.02E+07	4.00E+07	6.40E+07	1.94E+08	7.60E+07	1.04E+08	1.38E+07	2.84E+08	2.90E+08	1.82E+08	8.40E+07	1.78E+08
51	9.00E+06	5.40E+07	9.80E+07	2.38E+09	1.04E+08	1.24E+08	1.30E+07	7.40E+07	5.00E+07	2.18E+09	1.42E+08	1.44E+08
51	9.20E+06	1.16E+08	9.80E+07	2.36E+09	8.20E+07	1.06E+08	1.20E+07	6.00E+07	9.00E+07	2.50E+09	1.44E+08	1.44E+08
51	6.40E+06	7.40E+07	8.20E+07	2.24E+09	9.40E+07	1.18E+08	1.22E+07	3.80E+07	8.40E+07	2.84E+09	1.20E+08	1.50E+08
54	1.26E+07	6.60E+07	1.20E+08	7.00E+08	1.88E+08	1.76E+08	1.20E+07	1.04E+08	1.18E+08	9.80E+08	1.40E+08	1.80E+08
54	1.44E+07	6.20E+07	1.04E+08	6.80E+08	1.70E+08	1.16E+08	1.02E+07	1.08E+08	9.80E+07	7.80E+08	1.06E+08	1.78E+08
54	1.34E+07	8.60E+07	1.16E+08	6.80E+08	1.36E+08	1.46E+08	1.54E+07	1.00E+08	1.00E+08	7.40E+08	1.28E+08	2.14E+08
57	8.00E+06	1.40E+07	8.00E+06	2.00E+07	1.00E+07	1.00E+07	2.00E+06	1.60E+07	4.00E+06	6.00E+07	1.20E+07	6.00E+06
57	6.00E+06	1.00E+07	4.00E+06	1.20E+08	1.00E+07	1.40E+07	4.00E+06	1.20E+07	1.00E+07	8.00E+07	1.60E+07	1.20E+07
57	4.00E+06	8.00E+06	1.80E+07	6.00E+07	1.20E+07	2.00E+07	2.00E+06	1.20E+07	1.20E+07	6.00E+07	0.00E+00	1.40E+07
60	1.18E+07	8.00E+07	8.00E+07	3.20E+08	8.00E+07	8.80E+07	1.22E+07	5.20E+07	5.00E+07	2.20E+08	5.80E+07	1.38E+08
60	1.46E+08	2.80E+07	8.20E+07	3.00E+08	4.60E+07	7.40E+07	1.34E+07	6.40E+07	4.80E+07	2.60E+08	1.12E+08	1.12E+08
60	1.30E+08	4.80E+07	7.20E+07	3.60E+08	6.40E+07	7.20E+07	1.02E+07	4.00E+07	5.60E+07	2.00E+08	1.04E+08	1.46E+08

Control Samples- *M. aeruginosa* cells/mL

DAY	CO	BG11	[P]	[N]	DC	HP
1	5.00E+05	5.00E+05	5.00E+05	5.00E+05	5.00E+05	5.00E+05
4	3.56E+05	2.60E+05	3.78E+05	2.70E+05	2.48E+05	1.20E+05
4	3.18E+05	2.54E+05	2.90E+05	2.52E+05	1.78E+05	1.52E+05
4	3.78E+05	1.98E+05	4.42E+05	3.20E+05	2.00E+05	1.16E+05
7	3.88E+05	4.88E+05	5.18E+05	4.70E+05	1.60E+05	1.56E+05
7	5.84E+05	2.88E+05	7.00E+05	2.40E+05	2.56E+05	1.48E+05
7	4.12E+05	2.48E+05	5.08E+05	5.90E+05	1.76E+05	1.28E+05
10	1.10E+06	7.80E+05	1.40E+06	1.54E+06	3.80E+05	4.20E+05
10	1.02E+06	6.20E+05	1.70E+06	1.94E+06	7.80E+05	4.40E+05
10	1.02E+06	6.60E+05	9.00E+05	9.40E+05	6.40E+05	3.20E+05
12	1.26E+06	1.32E+06	1.62E+06	1.90E+06	8.40E+05	5.80E+05
12	1.24E+06	7.80E+05	1.92E+06	1.34E+06	7.00E+05	4.20E+05
12	1.16E+06	1.04E+06	1.78E+06	1.42E+06	4.60E+05	4.60E+05
15	1.14E+06	1.52E+06	1.50E+06	2.18E+06	9.80E+05	6.00E+05
15	1.10E+06	1.58E+06	1.42E+06	1.74E+06	9.80E+05	5.60E+05
15	1.04E+06	1.50E+06	1.78E+06	2.56E+06	1.06E+06	5.20E+05
18	1.38E+06	2.64E+06	2.14E+06	3.16E+06	9.60E+05	1.32E+06
18	1.30E+06	3.58E+06	1.96E+06	3.42E+06	1.06E+06	1.86E+06
18	1.78E+06	3.50E+06	2.20E+06	3.48E+06	1.30E+06	6.80E+05
21	1.28E+06	3.54E+06	1.42E+06	3.98E+06	7.80E+05	1.08E+06
21	1.40E+06	3.32E+06	2.40E+06	4.52E+06	8.40E+05	1.12E+06
21	9.40E+05	2.64E+06	2.06E+06	3.38E+06	1.02E+06	1.00E+06
24	1.40E+06	4.00E+06	2.00E+06	5.00E+06	1.50E+06	1.46E+06
24	1.70E+06	3.20E+06	3.80E+06	5.40E+06	2.60E+06	1.82E+06
24	2.06E+06	4.40E+06	3.40E+06	5.60E+06	1.78E+06	1.88E+06
27	3.02E+06	3.62E+07	1.72E+07	1.64E+07	1.66E+07	1.46E+07
27	4.24E+06	3.66E+07	1.82E+07	2.20E+07	1.54E+07	1.32E+07
27	3.44E+06	3.60E+07	1.60E+07	1.92E+07	1.56E+07	1.66E+07
30	7.60E+06	8.60E+07	1.22E+07	2.90E+07	2.24E+07	1.42E+07
30	9.00E+06	1.04E+08	2.10E+07	2.62E+07	2.10E+07	1.76E+07
30	8.20E+06	9.40E+07	2.26E+07	2.86E+07	2.18E+07	1.58E+07

33	1.44E+07	6.36E+08	3.48E+07	5.42E+07	2.94E+07	2.22E+07
33	1.28E+07	8.12E+08	3.68E+07	4.44E+07	3.16E+07	2.20E+07
33	1.62E+07	7.16E+08	3.50E+07	5.12E+07	3.20E+07	2.14E+07
36	8.80E+06	3.80E+08	5.60E+07	3.38E+07	7.80E+07	6.80E+07
36	8.80E+06	5.00E+08	6.40E+07	3.06E+07	7.20E+07	5.20E+07
36	1.00E+07	4.60E+08	5.80E+07	3.40E+07	7.00E+07	6.00E+07
39	5.40E+06	1.80E+08	4.40E+07	1.56E+07	2.60E+07	4.40E+07
39	6.40E+06	3.00E+08	3.80E+07	1.72E+07	2.40E+07	5.40E+07
39	9.40E+06	3.00E+08	4.00E+07	1.60E+07	2.00E+07	5.00E+07
42	7.20E+06	3.20E+08	1.06E+08	3.60E+08	6.80E+07	8.80E+07
42	8.80E+06	5.40E+08	8.80E+07	3.58E+08	5.20E+07	8.00E+07
42	1.20E+07	4.60E+08	7.40E+07	3.52E+08	5.60E+07	8.40E+07
44	9.00E+06	5.60E+08	1.02E+08	7.60E+07	1.30E+08	9.80E+07
44	1.20E+07	8.40E+08	6.00E+07	1.04E+08	8.80E+07	1.30E+08
44	9.40E+06	4.60E+08	1.06E+08	5.80E+07	1.18E+08	1.10E+08
48	8.00E+06	9.20E+08	5.80E+07	7.80E+07	1.74E+08	8.00E+07
48	8.40E+06	1.12E+09	8.80E+07	1.04E+08	1.76E+08	8.80E+07
48	9.40E+06	1.04E+09	7.60E+07	9.40E+07	1.90E+08	1.00E+08
51	9.20E+06	5.00E+08	8.60E+07	7.60E+07	1.16E+08	1.90E+09
51	7.60E+06	4.80E+08	9.80E+07	1.06E+08	1.40E+08	1.82E+09
51	1.14E+07	4.20E+08	1.20E+08	8.40E+07	1.12E+08	1.86E+09
54	8.40E+06	6.40E+08	1.28E+08	9.00E+07	1.70E+08	4.60E+08
54	1.12E+07	1.12E+09	5.40E+07	7.80E+07	2.22E+08	6.20E+08
54	1.20E+07	8.60E+08	9.80E+07	1.24E+08	1.78E+08	7.20E+08
57	2.00E+06	2.40E+07	1.20E+07	1.00E+07	8.00E+06	4.40E+07
57	2.00E+06	6.00E+06	1.20E+07	1.20E+07	6.00E+06	4.20E+07
57	2.20E+06	1.60E+07	1.00E+07	1.20E+07	1.00E+07	5.20E+07
60	9.40E+06	2.00E+08	6.80E+07	7.20E+07	1.04E+08	4.80E+08
60	5.60E+06	3.20E+08	6.00E+07	5.00E+07	8.00E+07	4.40E+08
60	9.40E+06	6.20E+08	4.80E+07	8.40E+07	1.02E+08	7.00E+08

Appendix 4.3: Photographs

Day 4: APRIL 8TH

Control Samples



CO	NCONTROL	PCONTROL	DCCONTROL	HPCONTROL	BG11CONTROL
----	----------	----------	-----------	-----------	-------------

Factorial Experiment Samples



CO	N2HP-R1	N2HP-R2	N2HP-R3
----	---------	---------	---------



CO N2DC-R1 N2DC-R2 N2DC-



CO N1HP-R1 N1HP-R2 N1HP-R3



CO N1DC-R1 N1DC-R2 N1DC -

Day 12: APRIL 16

Control Samples

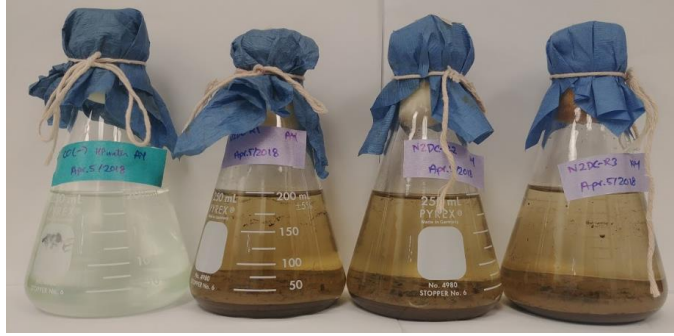


CO	NCONTROL	PCONTROL	DCCONTROL	HPCONTROL	BG11CONTROL
----	----------	----------	-----------	-----------	-------------

Factorial Experiment Samples



CO	N2HP-R1	N2HP-R2	N2HP-R3
----	---------	---------	---------



CO N2DC-R1 N2DC-R2 N2DC-



CO N1HP-R1 N1HP-R2 N1HP-R3



CO N1DC-R1 N1DC-R2 N1DC -

Day 18: APRIL 22ND

Control Samples



CO	NCONTROL	PCONTROL	DCCONTROL	HPCONTROL	BG11CONTROL
----	----------	----------	-----------	-----------	-------------

Factorial Experiment Samples



CO	N2HP-R1	N2HP-R2	N2HP-R3
----	---------	---------	---------



CO N2DC-R1 N2DC-R2 N2DC-



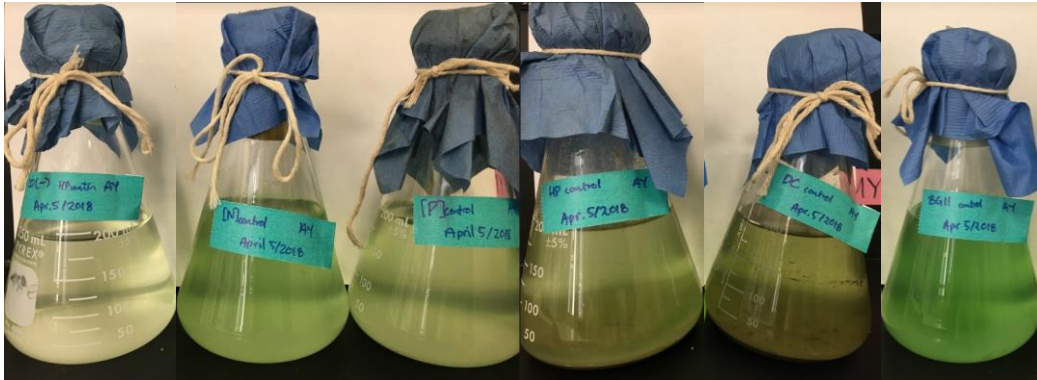
CO N1HP-R1 N1HP-R2 N1HP-R3



CO N1DC-R1 N1DC -R2 N1DC -

Day 26: APRIL 30TH

Control Samples



CO	NCONTROL	PCONTROL	DCCONTROL	HPCONTROL	BG11CONTROL
----	----------	----------	-----------	-----------	-------------

Factorial Experiment Samples



N2HP-R1	N2HP-R2	N2HP-R3
---------	---------	---------



N2DC-R1 N2DC-R2 N2DC-R3



N1HP-R1 N1HP-R2 N1HP-R3



N1DC-R1 N1DC -R2 N1DC -R3

Day 30: MAY 4

Control Samples

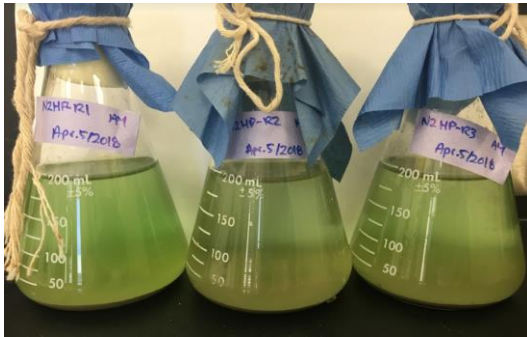


CO	NCONTROL	PCONTROL	DCCONTROL	HPCONTROL	BG11CONTROL
----	----------	----------	-----------	-----------	-------------

Factorial Experiment Samples



N2HP-R1	N2HP-R2	N2HP-R3
---------	---------	---------



N2DC-R1 N2DC-R2 N2DC-R3



N1HP-R1 N1HP-R2 N1HP-R3



N1HP-R1 N1HP-R2 N1HP-R3

Day 35: MAY 11TH

Control Samples



CO NCONTROL PCONTROL DCCONTROL HPCONTROL BG11CONTROL

Factorial Experiment Samples



N2HP-R1 N2HP-R2 N2HP-R3



N2DC-R1 N2DC-R2 N2DC-R3



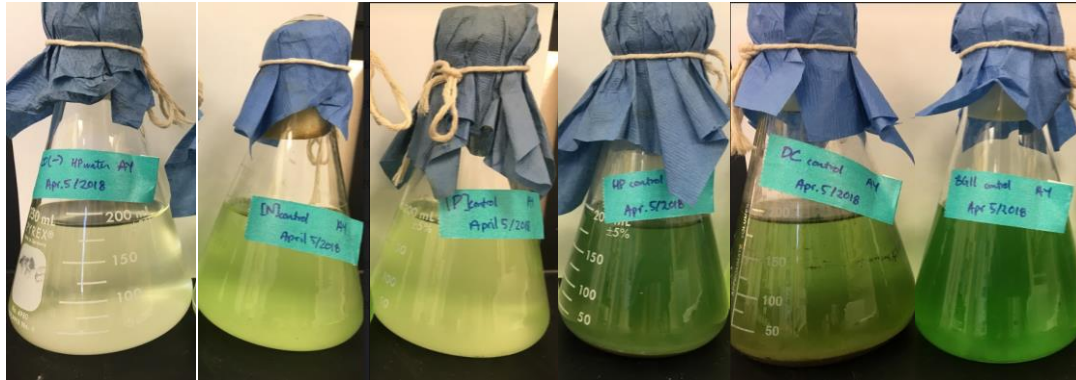
N1HP-R1 N1HP-R2 N1HP-R3



N1DC-R1 N1DC -R2 N1DC -R3

Day 44: MAY 18TH

Control Samples



CO	NCONTROL	PCONTROL	DCCONTROL	HPCONTROL	BG11CONTROL
----	----------	----------	-----------	-----------	-------------

Factorial Experiment Samples



N2HP-R1	N2HP-R2	N2HP-R3
---------	---------	---------



N2DC-R1 N2DC-R2 N2DC-R3



N1HP-R1 N1HP-R2 N1HP-R3



N1DC-R1 N1DC -R2 N1DC -R3

Day 51: MAY 25TH

Control Samples



CO	NCONTROL	PCONTROL	DCCONTROL	HPCONTROL	BG11CONTROL
----	----------	----------	-----------	-----------	-------------

Factorial Experiment Samples



N2HP-R1	N2HP-R2	N2HP-R3
---------	---------	---------



N2DC-R1 N2DC-R2 N2DC-R3



N1HP-R1 N1HP-R2 N1HP-R3



N1DC-R1 N1DC -R2 N1DC -R3

Day 57: MAY 31ST

Control Samples

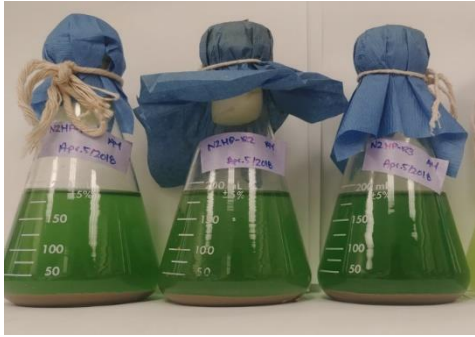


CO	NCONTROL	PCONTROL	DCCONTROL	HPCONTROL	BG11CONTROL
----	----------	----------	-----------	-----------	-------------

Factorial Experiment Samples



N2HP-R1	N2HP-R2	N2HP-R3
---------	---------	---------



N2DC-R1 N2DC-R2 N2DC-R3



N1HP-R1 N1HP-R2 N1HP-R3



N1DC-R1 N1DC -R2 N1DC -R3

Day 60: JUNE 30TH

Control Samples

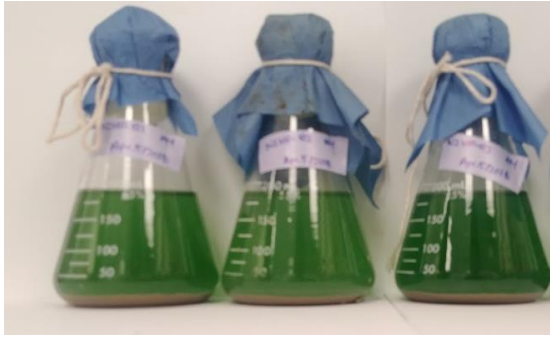


CO	NCONTROL	PCONTROL	DCCONTROL	HPCONTROL	BG11CONTROL
----	----------	----------	-----------	-----------	-------------

Factorial Experiment Samples



N2HP-R1	N2HP-R2	N2HP-R3
---------	---------	---------



N2DC-R1 N2DC-R2 N2DC-R3



N1HP-R1 N1HP-R2 N1HP-R3



N1DC-R1 N1DC -R2 N1DC -R3

Appendix 4.4: Factorial Design Experiment Variability Between Flasks

Factorial Design Experiment Variabilities in Treatments

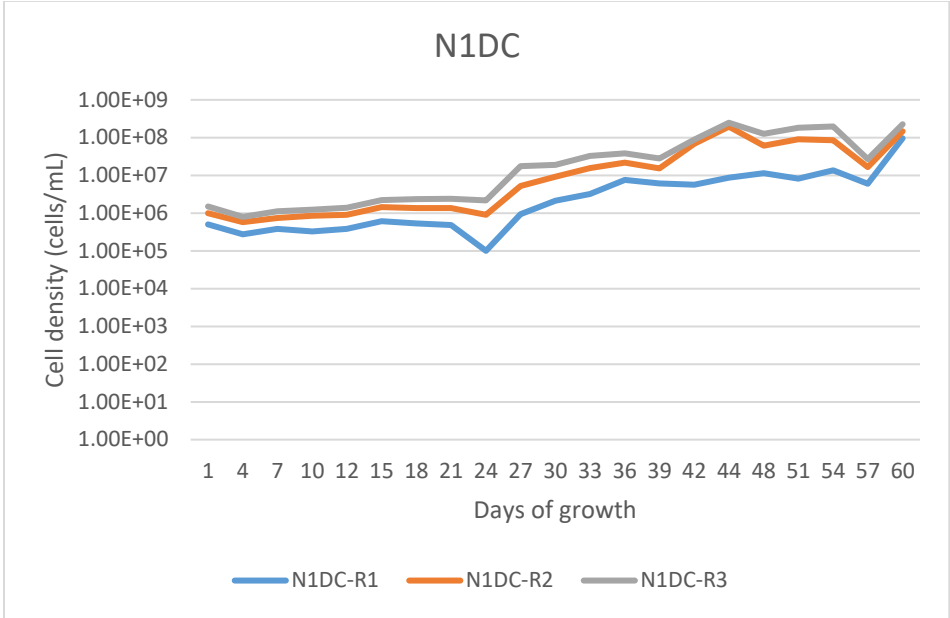


Figure 14- Factorial Design Experiment Variability Between Flasks in Treatment N1DC

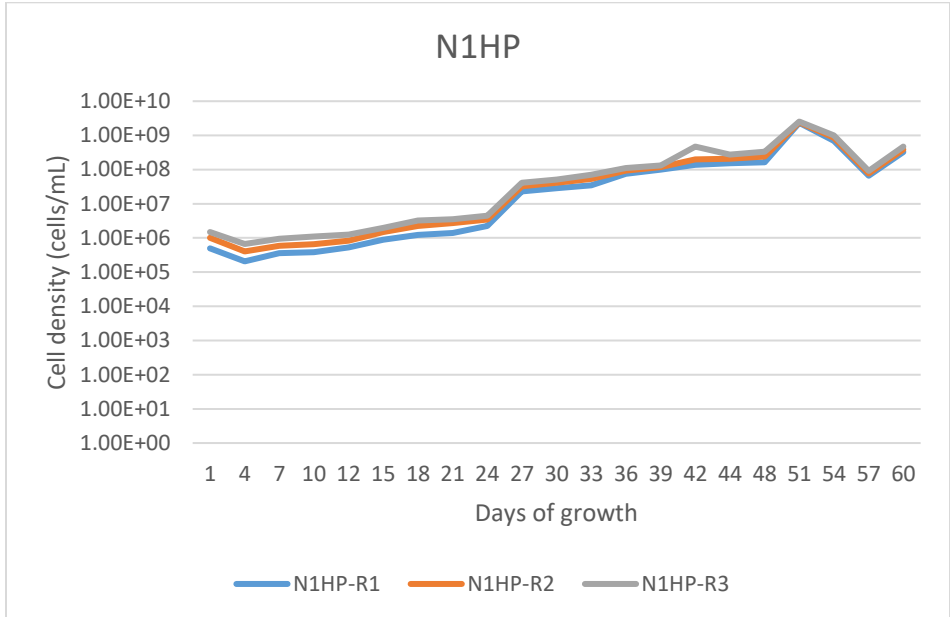


Figure 15- Factorial Design Experiment Variability Between Flasks in Treatment N1HP

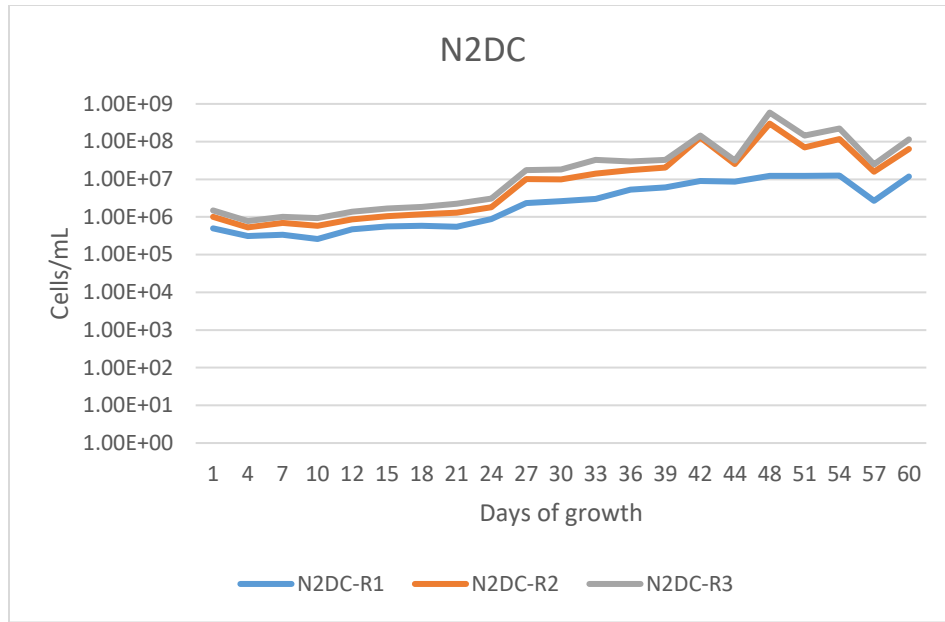


Figure 16- Factorial Design Experiment Variability Between Flasks in Treatment N2DC

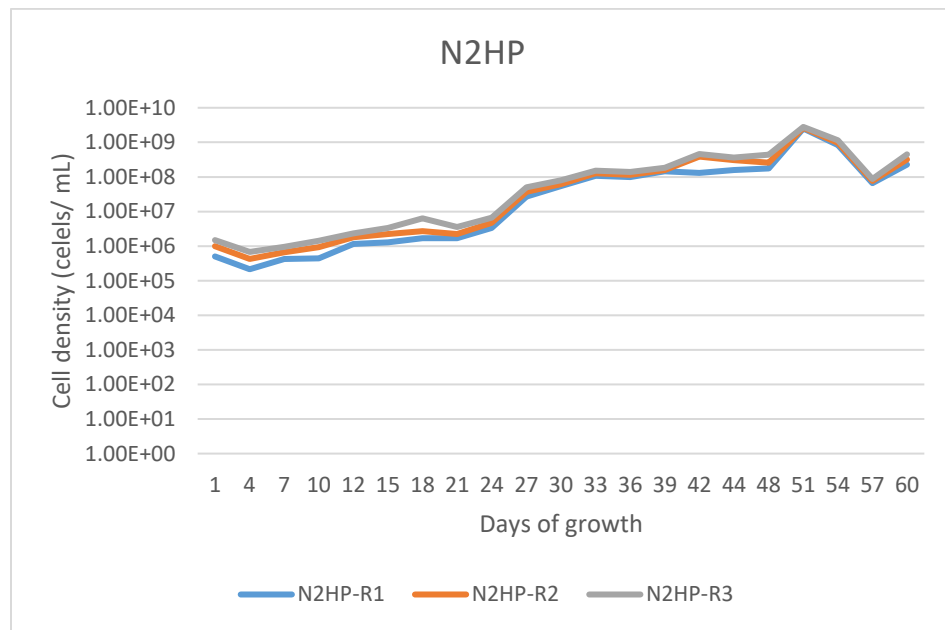


Figure 17- Factorial Design Experiment Variability Between Flasks in Treatment N2HP

Appendix 5: Copyright Letter for Figure Permissions



2018 December 11

Our file: CRR_AGR_15735

Amy Yang, Master of Applied Science Candidate (Civil Engineering - Water)
c/o Waterloo University, Faculty of Engineering
Department of Civil and Environmental Engineering
200 University Ave W
Waterloo ON N2L 3G1

Dear Ms. Yang:

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1. Glenmore Reservoir Bathymetry Map - Attachment 1 hereto
2. Elbow River Watershed Sampling Sites Map - Attachment 2 hereto (collectively referred to as the "Works")

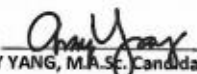
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Dated December 11, 2018 at Waterloo, Ontario, Canada.

Sincerely,


Lisa J. Sierra
Manager, Innovation, Data & External Access
Corporate Analytics & Innovation
T 403.268.4715 | F 403.268.3638 | Mail code #8026
Floor #6, Calgary Municipal Building, 800 Macleod Tr. S.E.

per: 
AMY YANG, M.A.Sc. Candidate