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Sulphide Production and Management in Municipal Stormwater Retention Ponds

by

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Dedication

This thesis is dedicated to my friends and family for their continuous kindness, care, and support through this chapter of my life and into my next one.

ABSTRACT

Municipal stormwater retention ponds are a means of managing stormwater in urban settings. Due to the temporal and spatial variations involved with stormwater, numerous contaminants find their way in stormwater retention pond, creating various problems to mitigate against. The City of Edmonton owns a stormwater pond that has historically produced higher levels of hydrogen sulphide. A field study was completed in the City of Edmonton, comparing two stormwater retention ponds in terms of water quality and sediment microbial communities to understand differences in biological degradation that would encourage sulphate reduction, believed to stimulate the production of hydrogen sulphide. The field study comparison was followed by laboratory studies focused on means of suppressing sulphide production. Nitrate amendments were effective in suppressing sulphate reduction; however the addition of a carbon source stimulated greater sulphide production. Extracts from Serrano peppers were also tested as a biocide and inhibitor for sulphate reducing bacteria.

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Chapter 1. INTRODUCTION

1.1 Introduction

1.1.1 Stormwater Overview

The onset of urbanization and significant numbers of people migrating to and living in urban centres has led to increased land development and alterations. These land developments have created impervious surfaces, and has significantly changed the means that the water cycle interacts with the land. Stormwater is water that is generated from runoff due to a precipitation event (rainfall or snowmelt) that flows over land or impervious surfaces and does not infiltrate into the ground (EPA 2012). As stormwater flows over the land and impervious surfaces, such as paved streets, parking lots and building rooftops, it accumulates sediments, chemicals, such as hydrocarbons, solvents and salts, and / or other pollutants that could negatively affect water quality if released untreated (EPA 2012). Stormwater can be extremely variable in terms of both quantity and quality in a temporal manner due to its dependence on precipitation events and many other variables, such as human activity and land use. The variability of stormwater discussed results in numerous difficulties and challenges in the manner in which it is managed.

Stormwater management is a common and essential infrastructure component in urban centres and municipalities. Stormwater management infrastructure includes various components for storage and conveyance, including pipes, pump houses, stormwater retention ponds, outfalls (to natural waterways), catch basins and more recently, low impact development (LID) infrastructure.

In Alberta, municipalities are required to develop stormwater management plans that emphasize both stormwater discharge controls and, increasingly, water quality management options. An important component of stormwater management is storage for flood protection, often in the form of stormwater detention ponds, which have been constructed widely throughout Alberta – and the rest of North America – over the past four decades. Stormwater ponds temporarily store runoff that exceeds the downstream sewer conveyance capacity, and remove sediments and other pollutants carried in stormwater runoff by allowing particles to settle.

1.1.2 Sulphide

The City of Edmonton's Drainage Services manages hundreds of dry and wet stormwater retention ponds. Some stormwater ponds generate high levels of hydrogen sulphide gas (H2S) in the late winter and early spring seasons. Hydrogen sulphide is a colourless, flammable and poisonous gas with a distinct odour of rotten eggs, which at high enough concentrations will cause collapse, coma or death as it targets the human nervous system. Hydrogen sulphide gas is also a corrosive compound to iron, steel and copper. Therefore, the presence and production of hydrogen sulphide gas is both a concern for public health safety and for infrastructure maintenance. A hydrogen sulphide scrubber has been implemented downstream of the stormwater pond outlet to mitigate against this issue, however, this method requires significant resources and maintenance to operate and does not address the production of H_2S. Bioaugmentation is another technique utilized by the municipality to stimulate sludge and odour control of the ponds caused by biological activity producing hydrogen sulphide gas during the spring and summer months, which has shown variable effectiveness at this particular site of interest.

1.2 Research Objectives

One goal of the research is to analyze, characterize and understand the conditions present in two stormwater retention ponds of approximately the same age in the City of Edmonton: (1) Valencia, which produces above average levels of H_2S, and (2) Bearspaw, which exhibits little to no recorded odour issues or production of H_2S. Another goal of the research is to attempt to find a means of mitigating against the production of hydrogen sulphide gas in the pond system. The overall research goals can be described as followed:

1. Characterization and comparison of pond water and sediment quality with Valencia and Bearspaw (comparable pond with favourable quality)
2. Microbiological analysis of pond sediments in Valencia and Bearspaw
3. Understand the influence of different biostimulation factors as a means of sulphide production

4. Test a novel natural extract biocide in its ability to inhibit sulphide production in a stormwater retention pond environment

These objectives will be accomplished through a two stage study process. The first stage will be a field study. This field study includes in-field analysis of certain parameters and sample grabs for in-house and commercial lab analysis. The in-field analysis parameters include pH, conductivity, alkalinity, oxidative-reductive potential, temperature and sulfide concentrations using portable probes and the HACH field kits. The grab sample parameters for further study include sulphide concentrations, sulphate concentrations using ion chromatography (IC), total phosphorus (TP) and nitrate and nitrite concentrations using flow injection analysis, biochemical oxygen demand (BOD) using the BOD_5 test and azide modification winkler titration method, chemical oxygen demand (COD) using closed reflux colourmetric method, total organic carbon (TOC) using a TOC analyzer and dissolved metals using inductively coupled plasma mass spectrometry (ICP-MS). Microbiology will also be studied using molecular techniques such as polymerase chain reaction and quantitative real time polymerase chain reaction (PCR and qPCR respectively) to identify and quantify the microbial communities active in each pond. The second stage will be a bench scale laboratory study utilizing microenvironment configurations. These microenvironments will be created using both sediment and water samples at Valencia pond. One set of these microenvironments will be tested using a factorial design method to understand the influence of biostimulant factors. Another set of the microenvironments will be tested with doses of a natural extract biocide for its efficacy in suppressing SRB. These studies will focus on several parameters, including nitrate and nitrite concentrations using flow injection analysis, sulphate analysis using IC, sulfide analysis using ion specific probe analysis, and organics using BOD, COD and TOC analysis. The microbial community analysis will be conducted again for speciation and quantity using PCR and qPCR molecular techniques.

The significance of this research is that it will examine and characterize conditions that are conducive for hydrogen sulphide production in stormwater retention pond facilities, in which little research has been done so far. This research will also provide a possible solution to suppress and mitigate against hydrogen sulphide production during the operation of a stormwater retention pond in an economically viable form

such as biostimulation or biocidal inhibition. Overall, the studies will assist in understanding and maintaining engineered stormwater retention facilities, commonplace to many municipalities.

1.3 Research Outline

This thesis is comprised of six chapters, each contributing to the aforementioned objectives of the research. A theoretical background review of the field of study and key concepts in this area will be presented in chapter 2. The focus of chapter 3 will be on the field work component of the project. This chapter will also be broken down into methodology, experiments, and results. The laboratory scale component of the project will be presented in chapter 4 (factorial design of biostimulation) and chapter 5 (biocidal inhibition), and once again will consist of their respective methodology, experiments, and results. Finally, chapter 6 will present the final conclusions, alternative solutions, possible future work and the engineering significance of biostimulation of a stormwater retention pond engineered system.

1.4 Chapter 1 References

US EPA. 2012. Stormwater Management. Accessed online: http://www.epa.gov/oaintrnt/stormwater/ Accessed September 2012.

Chapter 2. LITERATURE REVIEW

2.1 Stormwater

Stormwater is precipitation, both rainwater and snow melt, that runs off of urban and developed surfaces (US EPA 2012). Where stormwater can be absorbed into the ground, it undergoes a natural filtration process and assists in the restoration of the groundwater system (US EPA 2012). In the instances that stormwater runs over impervious surfaces, such as pavement, roofs, and concrete surfaces, water cannot infiltrate the ground, and instead is collected in municipal drainage and sewer systems (Alberta Environment 1999).

Stormwater management has become an increasingly important issue, as reflected by the increased number of guidelines associated with its infrastructure put out by various government bodies internationally such as the Canadian Council of the Ministers of the Environment and the Environmental Protection Agency in the United States. This is due to the release of the stormwater into surface water sources or infiltrating into groundwater channels. These guidelines and parameters include water quality impacts and releases. There are also guidelines in the infrastructure and beset management practices in managing the collection and conveyance of stormwater, such as the Stormwater Management Guideline for Province of Alberta (Alberta Environment 1999). There are also groups of individuals or coalitions of groups that look at regional watershed protection and management such as the North Saskatchewan River Alliance in Edmonton, AB.

The improper management of stormwater may result in various problems and issues, including erosion, habitat destruction, flooding, changes in stream flows and hydrographs, damage to infrastructure, and water quality degradation from contamination, pollution and combined sewer overflows (US EPA 2012).

2.1.1 Stormwater Quality

Sources of pollutants that can be found in stormwater are numerous. Floatables, large material that gets incorporated in stormwater flow, including things such as cans, plastic bags, paper, and yard waste come from shopping centers, streets, parking lots, parks, and recreational areas. Sediment can come from roads, lawns, and construction sites. Nutrient sources include lawn fertilizers, detergents, automobiles

and animal wastes. Metals come from atmospheric deposition, automobiles, industrial sites, and infrastructure corrosion. Sources of organics include lawns and gardens, and parks. Oil and grease come from parking lots, roadways, gas stations and illicit dumping. Pesticides and herbicides come from lawns and gardens, parks, roadway channels and golf courses. The source of bacteria and coliform can come from lawns, septic systems, and infiltration from sanitary sewers, pet wastes, and roads. (Pazwash 2011). The following table gives an idea of the general water quality of stormwater.

Table 2-1 – Low and high ranges of contaminants found in stormwater (adapted from Makepeace et al. 1995)

Contaminant	Low Range (mg/L)	High Range (mg/L)
Total Solids	76	36200
Total Suspended solids	1	36200
Total Dissolved Solids	75.9	2792
Nitrogen (all forms)	0.07	16
Phosphorus (Total)	0.01	7.3
Dissolved Oxygen	0	14
Hardness	12	1100
Alkalinity	8	1273
Biochemical oxygen demand	1	7700
Chemical oxygen demand	7	2200
pH	4.5	8.7
Total PAH	2.40E-04	1.30E-02
Oil and Grease	0.001	110
Hydrocarbons	0.64	19.71
Total Coliforms (/100 mL)	7	1.80E+07

2.1.2 Stormwater Infrastructure

Historically, the infrastructure put in place to manage stormwater focused specifically on the collection and conveyance of the water off-site as efficiently as possible (US EPA 2012). More specifically, stormwater was an issue of quantity management and flooding prevention was prioritized (US EPA 2012). This traditional

stormwater infrastructure was comprised mostly of extensive piping networks, outfalls to surface water sources, large retention facilities, or combined sewer systems to be treated with wastewater (US EPA 2012).

Presently, and over the past several decades, stormwater management infrastructure has made a shift towards addressing water quality management and ultimately, a dual objective of solving both water quantity and water quality issues in a sustainable and holistic manner and to address issues at their source rather than after conveyance (Butler and Davies 2011 and Pazwash 2011). Various techniques have been developed and implemented to meet these demands. Low impact development (LID) is an example of these techniques that have become increasingly popular with municipalities and developers (Pazwash 2011). The main objective of LID is the restoration of the natural watershed function within urban, developed areas, utilizing small-scale treatment techniques at the source of stormwater runoff, ultimately mimicking predevelopment conditions (Pazwash 2011). Another technique that focuses on this is named wet weather green infrastructure, which includes technology that looks at the infiltration, evapotranspiration, capture and reuse of stormwater to, once again, assist in the restoration of the natural hydrological conditions of the landscape before development (Pazwash 2011).

2.1.3 Stormwater Retention Ponds

Constructed wetlands and stormwater retention ponds have become more popular over the past few decades to assist in the treatment of agricultural and urban run-off (Butler and Davies 2011). However, due to the unique design and environment that each stormwater retention pond is set in, including hydrologic and pollutant inputs, creates many issues and difficulties in predicting the performance of the stormwater retention pond (Carleton et al. 2001). Generally, the performance of stormwater retention ponds have been found to be a function of the hydraulic loading rate and the overall retention time, and are thus dependent on factors such as wet weather intensities, durations, run-off volume and stormwater retention pond design and dimensions (Carleton et al. 2001). Pollutant retention has been reported to be affected by inflow rates into the pond system, where the inflow rate can influence the

scouring of pond bottoms and re-suspend the solids that retain or adsorb metal pollutants (Carleton et al. 2001). It was found that the performance of many stormwater retention ponds or constructed wetlands performed similarly to wastewater treatment ponds in terms of nutrient removal of nitrates, ammonia and total phosphates (Carleton et al. 2001). There also appeared to be some kind of relation of pollutant removal efficiency with stormwater treatment wetland or pond surface area and the contributing watershed area into the specific stormwater treatment facility (Carleton et al. 2001).

2.2 Problems with Stormwater Retention Ponds

Similar to any piece of infrastructure, stormwater retention ponds face their own set of operational and maintenance issues.

Queen's University and the National Water Research Institute Stormwater Quality Enhancement Group studied a Kingston, Ontario's constructed pond's performance and gave recommendations of critical issues that have become apparent in their study that influence the success, failure and sustainability of stormwater quantity and quality control of stormwater retention ponds (Anderson et al. 2002). These critical issues include the initial design of the stormwater retention facility, the operation and maintenance of the pond, performance and adaptive design of the pond (Anderson et al. 2002). A common issue that was found that stormwater ponds were mainly designed for simple aesthetic or simple retention purposes and after implementation and already in operation were then considered for the purpose of water quality enhancement (Anderson et al. 2002). The initial design is often poorly designed based on best practices that have been more recently discovered and determined, such as length to width aspect ratios to retain water and minimize flow (Anderson et al. 2002). It was also pointed out that as more is understood in stormwater quality, pollutants, design, operation and management, there comes new parameters that must be considered that were not accounted for in initial designs and retrofitting becomes costly and difficult (Anderson et al. 2002).

Ice formation and the introduction of salts commonly found during winter months result in negatively impacted pond hydraulics (Semadeni-Davies 2005).

Removal efficiencies in terms of total suspended solids removal and select metals removal were lowered during the winter and early spring months (Semadeni-Davies 2005).

Algae blooms can become a problem in stormwater retention ponds, due to the high loading of organic matter in spring snowmelt and runoff and the nutrients from fertilizers that wash off from residential lawns or municipal park space (Babin et al. 1992). It is a problem of water quality and aesthetics, leading to residential complaints (Babin et al. 1992). Babin et al. (1989 and 1992) tested the effect of alum and lime addition to algae bloom and total phosphorus effects on stormwater retention ponds in Edmonton. They found that the addition of alum and lime, or with just lime were effective in reducing both algae growth and total phosphorus levels, however, regular applications were required to maintain low levels, due to influxes of nutrients into the stormwater ponds.

Odours from stormwater drainage systems are an aesthetic quality issue that arises from time to time. Often, complaints of odours from the public are addressed towards catchbasins and stormwater retention ponds, and are generally indicators of the performance of the stormwater system. A study was conducted in Korea to track and characterize offensive odourants from dry and wet stormwater catchbasins (Kabir 2010). It was found that for both wet and dry stormwater systems, the dominant odourants were ammonia and reduced sulphur compounds (Kabir 2010). The reduced sulphur compounds include hydrogen sulphide, methyl mercaptan, dimethyl sulphide and dimethyl disulphide (Kabir 2010).

2.3 Sulphide and Hydrogen Sulphide

Sulphide and hydrogen sulphide is a common source of problems that occur in municipal systems, predominantly wastewater collection and treatment facilities (ASCE 1989). Some of the more common issues that arise due to the presence of sulphide include corrosion of infrastructure and assets, odours, and health and safety (ASCE 1989).

Hydrogen sulphide is a gaseous compound that is colourless, flammable, poisonous, and odorous with the distinct smell of rotten eggs. Humans have a low

detection threshold of hydrogen sulphide at 0.00047 ppm, life threatening at 300 ppm, and immediately fatal at concentrations greater than 700 ppm (ASCE 1989). As it can be produced in sanitary sewers, it is dangerous for municipal workers that are exposed to the infrastructure (ASCE 1989).

Sulphide and hydrogen sulphide are produced through biological processes and are oxidized and reduced into different sulphur compounds in the environment through chemical and biological means (Bharathi 2008). These relationships are summarized in the sulphur cycle as discussed below (Bharathi 2008).

2.3.1 Sulphur Cycle

The sulphur cycle is one of the important cycles under the classification of the biogeochemical cycles. It specifically is the combination of oxidative and reductive processes where sulphur is transported and transformed through minerals, water systems, and biological systems. Sulphur is the fourteenth most abundant element on the planet Earth, and sulphate, its most oxidized form, is the second most abundant ion on the planet (Bharathi 2008). Sulphur is also an essential element that is a constituent of various proteins and co-factors. The sulphur cycle is represented in the following image.

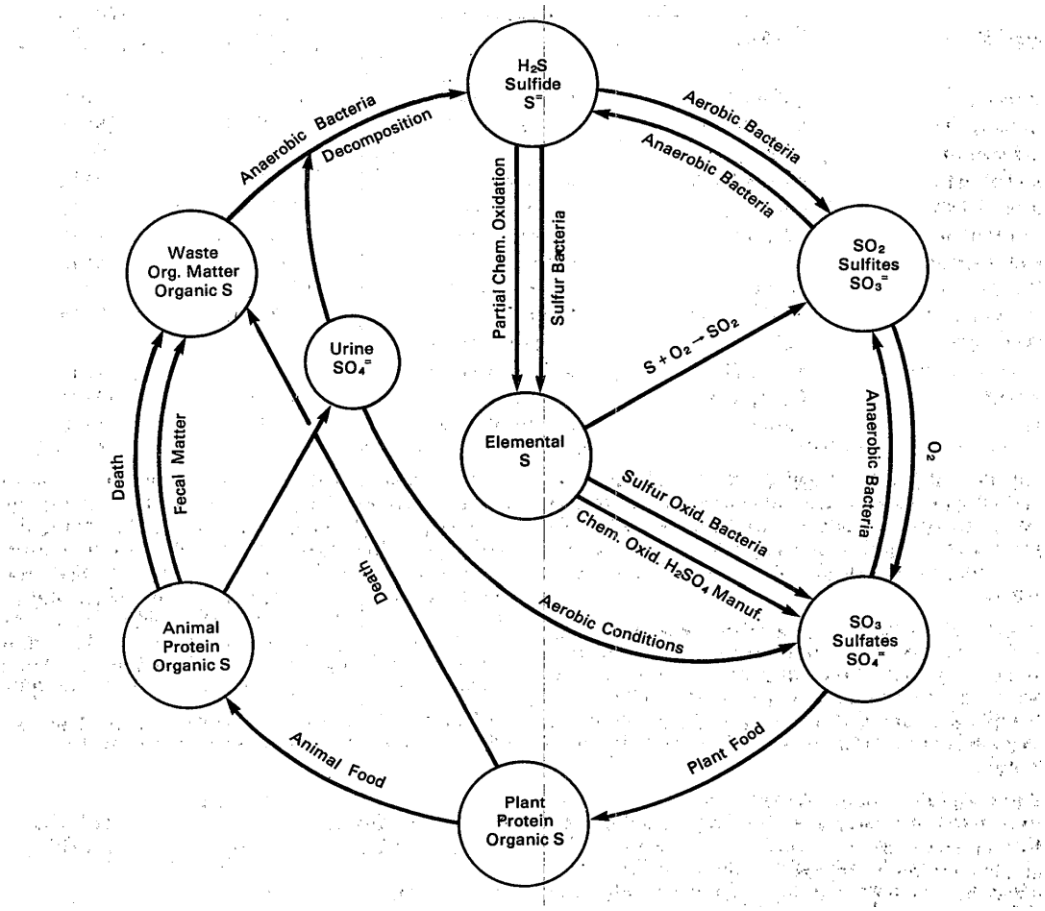

Figure 2-1 – Sulphur cycle (taken from US EPA 1985)

The sulphur cycle can be summarized in several steps:

- Reduction of sulphate (SO_4^{2-}) to sulphide (S^{2-}) (including its various intermediate ionic species)
- Oxidation of hydrogen sulphide (H_2S), sulphide (S^{2-}), and elemental sulphur (S) to sulphate (SO_4^{2-})
- Mineralization of organic sulphur to inorganic species (i.e. hydrogen sulphide (H_2S), elemental sulphur (S), and sulphide minerals)
- Incorporation of sulphide into organic compounds

Sulphur oxidation occurs through the utilization of various reduced sulphur compounds by colourless sulphur bacteria or coloured photosynthetic bacteria. These bacteria include the genus groups *Chromatium*, *Thiobacillus*, *Thiosphaera*, *Thiomicrospira*, *Thermothrix*, *Beggiatoa*, and the Archaean group *Sulfolobulus*. Some

of the reactions that occur through the activity of these microbial communities include (Bharathi 2008):

$$S_2O_3^{2-} + H_2O + 2O_2 \rightarrow SO_4^{2-} + 2H^+ = -822.6\ kJ$$

$$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+ = -798.2\ kJ$$

$$S^0 + H_2O + 1\frac{1}{2}O_2 \rightarrow SO_4^{2-} + 2H^+ = -587.1\ kJ$$

$$HS^- + \frac{1}{2}O_2 + H^+ \rightarrow S^0 + H_2O = -209.4\ kJ$$

Chemosynthetic sulphur oxidation is focused on the oxidation of H_2S, S, and $S_2O_3^{2-}$. The majority of the contributions of these reactions and activities come from reactions in thermal sea vents (Bharathi 2008).

Photosynthetic sulphur oxidation, mostly under anoxygenic conditions, converts reduced sulphur into sulphate. Some bacteria have shown their ability to oxidize reduce elemental sulphur into sulphate internally. Other bacteria oxidize sulphur compounds externally (Bharathi 2008).

Sulphate reduction within the sulphur cycle can be divided into two main categories; assimilatory and dissimilatory sulphate reduction. Under assimilatory sulphate reduction, sulphide radicals are taken into and incorporated in the biosynthetic cycle with serine to form cysteine, which is in turn transformed into other amino acids (Bharathi 2008). Assimilatory sulphate reduction does not produce sulphide ions or hydrogen sulphide (Bharathi 2008).

Dissimilatory sulphate reduction occurs where sulphate is activated by adenosine triphosphate (ATP) to form adenosine phosphosulphate, producing sulphite, and then sulphide (Bharathi 2008).

Nightingale and Mayer (2012) studied the cycling of sulphur compounds in an Albertan watershed in Canada, finding dissolution of both sulphate and sulphide compounds from soil, oxidation within bedrock, pyrite-containing shales, and anhydrite minerals via both abiotic and biotic means. Nightingale and Mayer (2012) showed, utilizing sulphur isotope analysis, that within short spatial distances, there can

be significant changes in sulphate and sulphide concentrations due to the various interactions within the environment.

2.3.2 Sulphate Reducing Bacteria

Sulphate is reduced to sulphide and hydrogen sulphide by sulphate reducing bacteria (SRB). Sulphate reduction, as part of the sulphur cycle, is an anaerobic process and is completed by biological and microbial activity.

As with anaerobic processes, sulphate reduction produces alkalinity. Chen and Wang (1999) calculated that the process of sulphate reduction releases 1.98 moles of alkalinity per mole of sulphate reduced. Thomas et al. (2009) also found that with greater availability of organic matter, in spring, large amounts of alkalinity are produced through the stimulated anaerobic process.

The sulphide that is produced from sulphate reduction activity can react with dissolved metals to form metal sulphide precipitates. Some examples of these metal sulphides include copper sulphide, zinc sulphide, lead sulphide, cadmium sulphide, and iron sulphide. Generally, these metal sulphides are stable with solubility constants ranging in the order of 10^{-19} to 10^{-45} (Hao et al. 1996).

SRB is a general classification of microorganisms that have the common functionality of reducing sulphate, however, are still divided among a variety of groups. Trophic groups include hydrogentrophs (H_2 utilizers), *Desulfovibrio* type that focus on incomplete oxidation of organic acids, *Defsulfobacter* type that work on complete oxidation of organic acids (Zavarzin 2008).

Sulphur, similar to the carbon and the nitrogen cycle, at one point or another in their respective cycles, depend on microorganisms (Ehrlich 2002). Due to the environment being studied, and the weather conditions it is exposed to, the stormwater retention ponds tend to develop anaerobic conditions. Sulphate-reducing bacteria are key components in the sulphur cycle and the geomicrobiological activity that involve sulphate compounds and thrive in these anaerobic conditions (Ehrlich 2002). Sulphate-reducing bacteria are members of the Bacteria and Archaea domain (Ehrlich 2002). Sulphate-reducing bacteria utilize various oxidized sulphur compounds and species as terminal electron acceptors during their respiration activity (Ehrlich

2002). These oxidized sulphur compounds include sulphate, elemental sulphur and thiosulphate (Ehrlich 2002). The reduction of sulphate to sulphite can be summarized in the following equations (Ehrlich 2002):

$$SO_4^{2-} + ATP \xrightarrow{ATP\ sulphurylase} APS + PP_i$$

$$PP_i + H_2O \xrightarrow{pyrophosphatase} 2P_i$$

$$APS + 2e^- \xrightarrow{APS\ reductase} SO_3^{2-} + AMP$$

In these reactions, ATP is initially activated to form adenine phosphatosulphate (APS) and pyrophosphate (PPi), and the pyrophosphate is hydrolyzed into inorganic phosphate (Pi), and it is the APS that reduces and forms into sulphite.
The reduction of sulphite to sulphide is, however, unclear and disputed between experts with many possible reactions that ultimately lead to sulphide ions involving possible intermediates such as trithionate and thiosulphate, which react to form hydrogen sulphide (Ehrlich 2002).

Although sulphate-reducing bacteria are generally considered strictly anaerobes, they have shown tolerance, albeit somewhat limited to some regards, towards the presence of oxygen (Ehrlich 2002). *Desulphovibrio desulphurican*, *D. vulgaris, D. desulphodismutans, Desulphobacterium autotrophicum, Desulpholobus propionicus* and *Desulphococcus multivorans* are all sulphate-reducing species that have been studied and found to have exhibited to some extent, the ability to utilize oxygen as a terminal electron acceptor (Ehrlich 2002). It was found that these species are only to respire microaerophilically, where dissolved oxygen concentrations are below 10 µM, but under such conditions, the microorganisms were unable to grow (Ehrlich 2002).

SRB have a wide range of compounds that can be used as electron acceptors. Most species use thiosulphate and sulphite as their electron acceptors. The SRB species that belong to the genuses *Desulfohalobium*, *Desulfofustis*, *Desulforomusa* and *Desulfospirs* have shown to survive on elemental sulphur. Rulphonates and dimethylsulphoxides have also been shown to be used as electron acceptor sources for

SRB. SRB can also use non sulphur containing compounds as electron acceptors, including nitrate, nitrite, ferric iron, arsenate, chromate, uranium, and molecular oxygen.

The list of compounds that SRB can utilize as an electron donor source for growth and activity is expansive; including hydrogen, alcohols (methanol and ethanol), acetate, lactate, propionate, butyrate and both simple and complex sugars (Liamleam and Annachhatre 2007). In general, a 0.67 chemical oxygen demand to sulphate mole ratio is the minimum requirement theoretically for achieving complete sulphate reduction (Choi and Rim 1991). Where sulphate reduction using SRB was used to treat for sulphates in wastewater, various naturally occurring and available organic carbon sources have been used as electron donors, such as sewage sludge, wood chips, animal manure, vegetation compost, sawdust, leaf mulch, mushroom compost, and whey (Dvorak et al. 1992, Hammack et al. 1994, Christensen et al. 1996, Waybrant et al. 1998). In contrast to natural organic carbon sources, synthetic carbon sources have also been used, such as lactate, propionate, pyruvate, butyrate and acetate (Okabe and Characklis 1992, Visser et al. 1993, Harada et al. 1994). By-products from fermentation processes carried out by anaerobic biodegradation activity, specifically alcohols such as methanol and ethanol, have also been used as an electron donor source (Widdel 1988).

Looking at acetate as an electron donor for SRB, it can also be used a carbon source and is generally consumed and utilized by the *Desulfotomaculum* group of bacteria. The standard free energy change of the oxidation of acetate and reduction of sulphate is as follows:

$$CH_3COO^- + SO_4^{2-} \rightarrow HS^- + 2HCO_3^-$$

$$\Delta G° = -39.5\ kJ/mol\ acetate$$

The energy produced from this above reaction creates a situation where SRB have higher affinity towards the use of this chemical compound compared to other electron donors, such as lactate, propionate, and butyrate (Rinzema and Lettinga 1988). SRB using acetate as an energy source, must initially compete with certain species of methanogens. In most cases, SRBs are out-competed for acetate due to

their lower growth rates (Yoda et al. 1987, Oude Elferink et al. 1998). However, over time, SRB can out-compete methanogens, as explained to the higher affinity SRB have towards acetate and higher utilization rates of acetate (Harada et al. 1994).

Different sulphur compounds also have inhibitory effects on SRB activity. From having the least to the most inhibitory effect of sulphur compounds on SRB activity listed is as follows; sulphate, thiosulphate, sulphite, total sulphide and hydrogen sulphide. These compounds appear in different abundances based on pH which results in different toxicities based on the environmental conditions (Lens et al. 1998). The mechanism by which sulphide causes inhibitory effects is debated using various theories (Tang et al. 2009).

Temperature can significantly affect the sulphate reduction kinetics and growth of SRB, with both mesophilic (25-35°C) and thermophilic (35-70°C) strains of SRB that exist. There have also been instances where SRB activity and efficiency was not affected at lower temperatures and if exposed to these temperatures for a long enough period of time successfully. Previous studies showed that SRB activities can be maintained at temperatures ranging from 1 to 16°C (Moosa et al. 2005, Zaluski et al. 2003, Reisinger et al. 2000, Kuyucak et al. 2006). Once allowed to acclimatize to lower temperatures, although for extended periods of time, the effect of temperature on SRB activities becomes reduced and insignificant (Tsukamoto et al. 2004, Kuyucak et al. 1994).

SRB can survive and thrive in various types of environment. In terms of pH, SRB are mostly known to survive within the range of 5-9, with reduced activity and growth in pH levels outside this range. There has also been proof that some acidophilic or acid tolerant strains of SRB can grow and function in various acidic environments, such as in acidic wastewater or in situations with acid rock drainage. It was generally found that a mixed culture of SRB shown greater tolerance for extreme conditions, such as acidic environments (Postgate 1984).

The production of sulphide ions within engineered systems by microbiological activity has created numerous problems. In energy extractives and processing industries, sulphide and hydrogen sulphide production has led to pipe corrosion, oil field plugging and increased costs to product refinement. In environments where sulphide and hydrogen sulphide is being produced, it also becomes a human health

concern, where workers and the general public are at risk for hydrogen sulphide poisoning.

SRB and sulphide are also used in engineered systems to remediate for heavy metals or radionucliotides and has become a solution towards managing acid mine drainage (Christensen et al. 1996 and Dvorak et al. 1992). Microbial fuel cells have been proposed as a possible solution to managing wastewater with higher levels of sulphur compounds (Zhao et al. 2008). The biological removal of sulphate and sulphide from wastewater in microbial fuel cell reactors would also have an added benefit of generating energy in the process whilst remediating.

2.4 Inhibition of Sulphate Reducing Bacteria

Sulphide production activity can be controlled using various means. One means of control is to limit the activity of SRB. Although SRB utilize sulphate as the electron acceptor for their growth, elevated sulphate concentrations may inhibit their growth. Al-Zuhair et al. (2008) found that at sulphate concentrations greater than 2500 g/m^3, SRB began to show inhibition and limitation in growth.

The presence of sulphide, the product of sulphate reduction, also has an inhibiting effect on SRB activity. Several theories exist in the means of sulphide inhibition. First, the precipitated metal sulphides remove the availability of essential trace metals as enzyme cofactors (Bharathi et al. 1990). Second, sulphide is absorbed into the microbial cell structure and denatures proteins via cross-linking actions (Postgate 1984). There are also conflicting theories of the sulphide inhibition being caused by undissociated H_2S or total sulphide (Moosa and Harrison 2006). O'Flaherty et al. (1998) suggested that the mechanism changes based on the pH of the environment, where pH is between 6.8 and 7.2, inhibition was associated with undissociated H_2S concentration, and where pH was greater than 7.2, inhibition was associated with total sulphides.

The presences of specific metallic ion species, such as copper and zinc, or zinc and nickel, also have shown to have cumulative toxic effects on SRB. However, if the concentration of metals decreases to a low enough level, the metallic ions could have a hormetic effect and increase the activity of SRB instead of hindering it.

Broad-spectrum biocides have been studied for use, such as glutaraldehyde, cocodiamines, diamines, and tetrakishydroxymethylphosphonium sulphate (Reinsel et al. 1996, Telang et al. 1998, Gardner and Stewart 2002, Thorestenson et al. 2002). Another method that has been used for suppression of SRB activity is the addition of nitrates and nitrites and on occasion, inoculated with a combination of nitrate reducing, sulphide oxidizing bacteria. The presence of metals also has an effect on SRB growth and activity, where high enough concentrations of metallic ions will have an inhibitory or even lethal effect on SRB. The use of nitrate amendments and biocides will be discussed further in the sections following.

2.4.1 Nitrate Inhibition

Nitrate and or nitrite amendment has become a popular means of solving issues of sulphide and hydrogen sulphide production in industrial work. For example, Eckford and Fedorak (2002), Kaster et al. (2007), Kumaraswamy et al. (2011) and Davidova et al. (2001) measured the effects of nitrate and nitrite addition in oil field production water. Their studies all showed that the addition of nitrate and or nitrite significantly reduced the presence of sulphide and hydrogen sulphide concentrations found in produced waters in the field and in the lab.

On the basis of thermodynamics, the reduction of nitrate provides more Gibbs free energy than the reduction sulphate, making nitrate a more attractive electron acceptor (Zehnder and Stumm 1988). The use of nitrate and or nitrite amendment results in the activation of NRB or nitrate reducing sulphide oxidizing bacteria (NR-SOB) (Telang et al. 1997, Thorstenson et al. 2002, Larsen et al. 2004). With the presence of nitrate and nitrite, it allows these NRB and NR-SOB to out-compete SRB for electron donors and carbon sources (Hitzman and Sperl 1994, Loveley and Chapelle 1995, Smith 2007). Eckford and Fedorak (2002) showed that inhibition of SRB activity is more effective under the stimulation of heterotrophic NRB versus the autotrophic NR-SOB. NR-SOB gain energy from the reduction of nitrates in the following reaction (Telang et al. 1997):

$$5HS^- + 2NO_3^- + 7H^+ \rightarrow 5S^0 + N_2 + 6H_2O$$

Telang et al. (1997) and Jenneman et al. (1999) found in their respective field studies, that a continuous injection of 5 mM of nitrate resulted in 50 – 100% in sulphide removal in injection and production wells.

Greene et al. (2003) stated that different concentrations of nitrite addition resulted in different inhibitory efficiencies; 2 mM gave no effect, 5 mM slowed down sulphate reduction by approximately 50%, and 10 mM resulted in complete inhibition. Although the presence of an electron acceptor that provides higher redox potential can inhibit sulphate reduction, Achtnich et al. (1995) found that this is only the case if the concentration of electron donors is limiting. Achtnich et al. (1995) had successfully inhibited the activity of SRB with the addition of nitrate and ferric iron, and the inhibition effect was found to be relieved once H_2, acetate or a mixture of potential electron donors were added.

Nitrite, through direct injection or nitrate reduction, has an extra means of SRB activity suppression. Other than encouraging out competition by NRB, the presence of nitrite acts as an inhibitor for the enzyme known as dissimilatory sulphite reductase (Dsr), the enzyme present in SRB responsible for reducing sulphite into sulphide (Wolfe et al. 1994). It has been found that Dsr has a strong affinity towards nitrite, and results in the production of ammonia from nitrite, instead of reducing sulphite into sulphide.

Indirectly, the presence of nitrate reduction intermediates, such as nitric oxide and nitrous oxide, discourage SRB activity by raising the redox potential of the environment, where sulphide generation cannot occur in environments where the redox potential is above -100 mV (Postgate 1979, Jenneman et al. 1999).
Also, under mesophilic conditions, 20 to 45°C, specific groups of SRB, known as mesophilic SRB, have periplasmic nitrite reductase enzyme that also reduces nitrite to ammonia (Pereira et al. 2000). Research has been made in analyzing nitrite reductase, finding that this enzyme is composed of two subunits and is periplasmic but loosely anchored to the cytoplasmic membrane (Moura et al. 1997). It has also been found that the enzyme, nitrite reductase, has been found in bacterial groups beyond SRB, such as Wolinella succinogenes, Sulphurospirillum deleyianum, E. coli and pathogenic bacteria (Simon et al. 2000, Bamford et al., 2002, and Poock et al. 2002). The nitrite reductase activity can vary widely among SRB species, meaning the effectiveness of

utilizing nitrate reduction and direct nitrite addition as a sulphate reduction inhibition method varies depending on the environment it is applied to (Greene et al. 2003). The electron donor concentration available to be used by SRB can also affect the nitrite reductase activity and SRB inhibition (Greene et al. 2003). Therefore, the effectiveness of utilizing nitrate reduction and or nitrite addition in mixed culture environments is variable and can be overcome.

He et al. (2010) however, proposed that other factors, including the osmotic pressure and stress, in conjunction with the nitrate and nitrite, and possible many other factors, attribute to SRB inhibition.

2.4.2 Biocides

The inhibition and control of SRB and sulphate reducing activity by bacteria have been shown to be possible with the use of biocides. Several compounds have been found to act as biocides against various bacteria, such as SRB, including glutaraldehyde, tetrakis hydroxymethyl phosphonium sulphate (THPS), quaternary ammonium compounds (QAC), bromo-nitropropanediol (BNPD) (Wen et al. 2009). It has been found that the extracts from certain plants provide a natural biocide against SRB. Oguzie et al. (2012) found extracts from the leaves of *Piper guineense* plants to be useful in the control and inhibition of SRB activity and their corrosive after effects. Oguzie et al. (2012) found that it this inhibition effect was caused by adsorption of the phytochemical compounds that disrupted the growth and essential metabolic functions of the bacteria, specifically the alkaloids, tannins and saponins from the plant extract using ethanol, petroleum spirit, methanol or water extraction techniques.

Bogan et al. (2004) found a microbial inhibitor for SRB and corrosion prevention utilizing the extracts from the *Capsicum sp.* of plants. Bogan et al. (2004) used a Soxhlet extraction technique with hexane, methylene chloride, aqueous acid and aqueous alkali on Chile de Arbol, Serrano, and Habanero peppers and found that it had successfully inhibited most plaktonic SRB species with minimal contact time. Shaban et al. (2013) found that Schiff base cationic surfactants, (E)-decyl-4-[(2-hydroxyethylamino)methyl]-N,N-dimethyl benzenaminium bromide, (E)-dodecyl-4-[(2-hydroxyethylamino)methyl]-N,N-dimethyl benzenaminium bromide, and (E)-

hexadecyl-4-[(2-hydroxyethylamino)methyl]-N,N-dimethyl benzenaminium bromide were also found to have biocidal effects on SRB. It was theorized that the mechanism for SRB growth and activity inhibition with cationic surfactants neutralize the negative charges on the bacterial cell membranes, deactivating the permeability of the outer cellular membrane and disrupting essential biological reactions.

In the incidences where SRB were found to grow within biofilm, the effectiveness of biocides decreased, and required higher concentrations to exhibit the same inhibitory strength (Wen et al. 2009, Davies 2003, and Meyer 2003). It has been shown that biofilms protect sessile bacteria from biocide effects by Denyer (1995) and Morton et al. (1998), and Stoodley et al. (1999) showed that denser biofilms resisted mass transfer due to the extracellular polymeric substances between the sessile bacterial cells. Morton et al. (1998) and Fux et al. (2005) have suggested that growing within biofilms, sessile bacteria have changes in their physiology that assists in the resistance to the effects of biocides.

The resistance of SRB and other bacteria to biocides growing within biofilms, has been found to be overcome with the addition of chelating agents. Raad and Sherertz (2001) had patented the idea and use of a chelating agent, such as ethylenediaminetetraacetic acid (EDTA), in combination with an antibacterial or biocidal compound for the treatment of microbially induced biofilm and corrosion. Wen et al. (2009) found that use of ethylenediaminedisuccinate (EDDS) had enhanced the efficacy of glutaraldehyde as a biocide against SRB that existed in biofilm environments; lowering the dosage required to show biocidal effects. The use of chelating agents has also been shown to be useful in the enhancement of biocidal effects on planktonic SRB. Wen et al. (2010) found that the use of EDTA, EDDS, and N-(2-hydroxyethyl)iminodiacetic acid (HEIDA) as chelating agents enhanced the biocidal effects of glutaraldehyde and tetrakis hydroxymethyl phosphonium sulfate (THPS) on SRB.

2.5 Chapter 2 References

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Chapter 3. FIELD STUDY COMPARISON OF WATER QUALITY AND MICROBIAL ACTIVITY IN TWO CITY OF EDMONTON STORMWATER RETENTION PONDS

3.1 Introduction

The City of Edmonton manages an extensive drainage system to convey sanitary and stormwater sewers. Focusing on stormwater infrastructure, the city manages a vast network including drainage pipes (both exclusively stormwater and inclusively combined with sanitary sewage conveyance as combined sewers), outfalls, catchbasins, pumpstations, dry and wet stormwater retention ponds, and various structures to manage the quantity and quality of stormwater.

Stormwater retention ponds are permanent structures designed in developed and urbanized areas to manage the stormwater quantity and quality. Stormwater is collected within a catchment area as it flows over impervious surfaces through catchbasins. It is then conveyed through stormwater pipes towards these stormwater retention ponds to be stored until it can be safely pumped and transferred to a water course. The stormwater is stored in the stormwater retention ponds to prevent flooding and not overwhelm the larger stormwater conveyance system downstream towards the water course. While stored, the detained stormwater allows for settling of particulate matter. These stormwater ponds are generally designed to be impermeable to infiltration of the water using various types of liners and membranes. Some stormwater retention ponds are designed as constructed wetlands, to mimic natural wetlands for aesthetic purposes, encourage ecological habitation, and to increase settling efficiency. However, stormwater is also composed of other various compounds from the overflow of these impervious surfaces, such as organic matter, dissolved metals, hydrocarbons, nutrients, and a variety of chemical compounds, and the fate of these compounds are not clearly understood.

A field study was implemented on the stormwater and bottom sediment of two City of Edmonton stormwater retention ponds: Valencia and Bearspaw. Bearspaw stormwater retention pond, in the City of Edmonton was considered by Municipal Engineers and Operators as an average to high performing pond, whereas Valencia stormwater retention pond is considered as a low performing pond and operationally problematic. Some of the problems that regularly appear at the Valencia stormwater retention pond include increased levels of hydrogen sulphide production, odorous and visual aesthetic issues, and algae blooms. Samples were obtained from these two

ponds for this comparative analysis. Valencia Pond, located in northeast Edmonton, Alberta, is the pond of interest due to the increased and unnatural levels of hydrogen sulphide gas that is produced in the late winter and early spring every year. Bearspaw Pond, located in south Edmonton, Alberta, is a pond of comparative size and age to Valencia Pond, and has not shown significant problems in operations or function. Both stormwater retention ponds are located in residential living areas with a portion of the pond open to public parks. The two ponds are also not connected within the drainage system and have no effect on one another, giving an independent comparison, however, providing challenges due to different stormwater sources.

The overall objective of this study was to evaluate the potential causes and extent of elevated H_2S emission in stormwater retention ponds. Although the present field study has a localized focus within the City of Edmonton, the odorous, corrosion, and health and safety issues of H_2S production in stormwater infrastructure is commonplace (Kabir 2010, ASCE 1989). The field study also provides understanding and knowledge in the area of sulphur compounds and the biological activity associated with these sulphur compounds in a stormwater retention pond setting that have lacked discussion or studies of in the engineering field. The goals of the field study were as follows:

- To examine and compare the general stormwater composition in the stormwater ponds
- To evaluate the potential causes of evaluated H_2S and sulphide emissions from the Valencia stormwater retention pond site
- To determine what potential means of mediation techniques can be implemented in the mitigation of or suppression of sulphide and H_2S in the stormwater retention pond

3.2 Materials and Methodology

3.2.1 Site and Sampling

The two stormwater retention ponds used in the field study are both City of Edmonton owned and managed stormwater retention ponds. Both ponds are residential municipal stormwater ponds, and are located in different parts of the city. Valencia stormwater retention pond is located in north Edmonton, with an average depth of 2.1 m, covering an approximate surface area of 28,300 m^2, and holds an estimated average volume of 55,000 m^3. Bearspaw stormwater retention pond is located in south Edmonton, with an average depth of 2.5 m, an approximate surface area of 23,300 m^2, and an estimated volume of 46,000 m^3. Figure 3-1 shows a map of the City of Edmonton, and the respective locations of Valencia and Bearspaw retention ponds in the municipality.

Water samples and sediment samples were obtained at each pond for analyses and comparison purposes. Samples were obtained at the inlet, middle, and outlet of each stormwater retention pond by means of an aluminum boat at the designated and marked off locations for consistency. Figure 3-2 and 3-3 shows the locations where samples were taken at each stormwater pond during the field study. The sampling regime began on May 31, 2012 for Valencia Pond and ended on September 27, 2012. Sampling regime for Bearspaw Pond began on June 7, 2012 and was completed on September 26, 2012. Samples were obtained with frequencies of every three to four weeks, dependent on the availability of the City of Edmonton's Drainage Operation Lake Foreman and Environmental Inspectors to provide their in-kind support. The dates of samples taken for the field study are summarized in Table 3-1 below.

Table 3-1 – Dates that samples were taken at City of Edmonton's Valencia and Bearspaw stormwater retention pond sites

Sampling Order	Valencia Pond	Bearspaw Pond
1	May 31, 2012	June 7, 2012
2	June 21, 2012	June 28, 2012
3	July 12, 2012	July 19, 2012
4	August 3, 2012	August 1, 2012
5	August 30, 2012	August 31, 2012
6	September 27, 2012	September 26, 2012

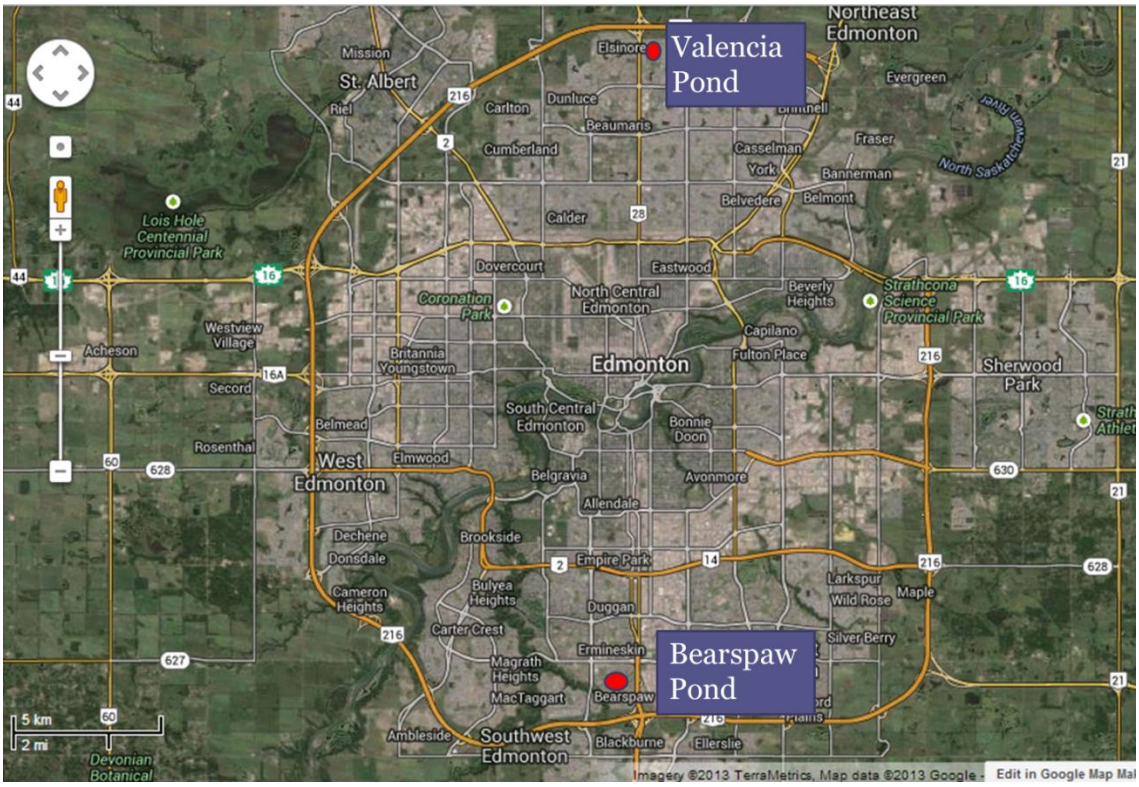

Figure 3-1 – Locations of Valencia and Bearspaw stormwater retention ponds (adapted from Google Maps 2013)

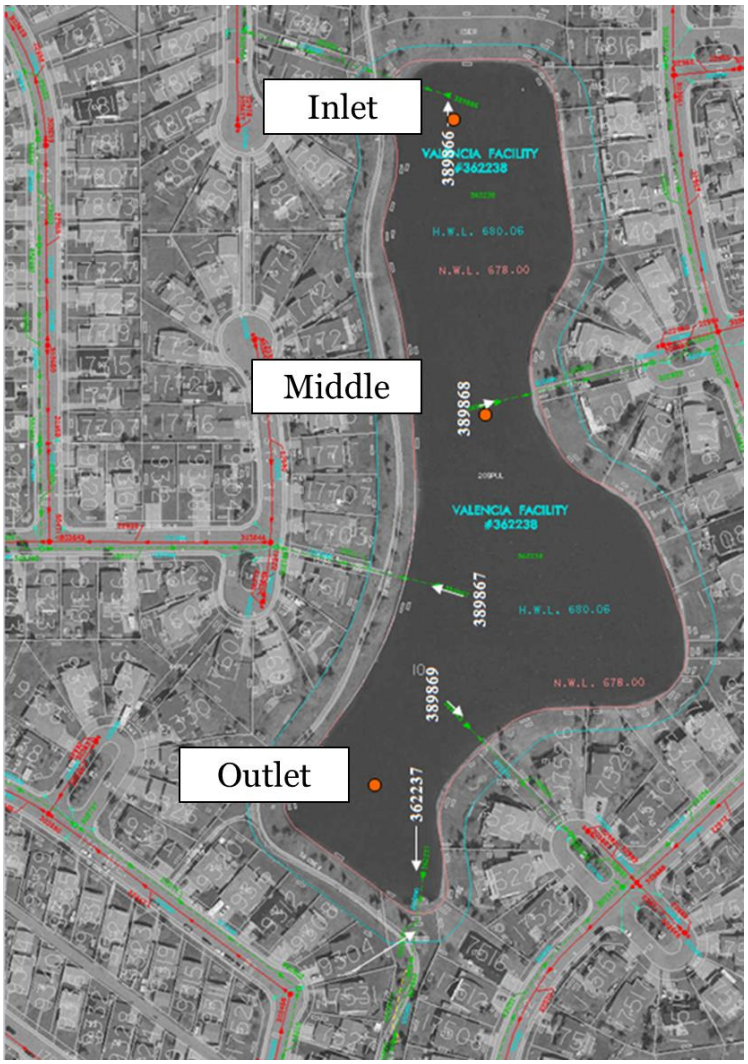

Figure 3-2 – Sampling locations of the inlet, middle and outlet at Valencia stormwater retention pond

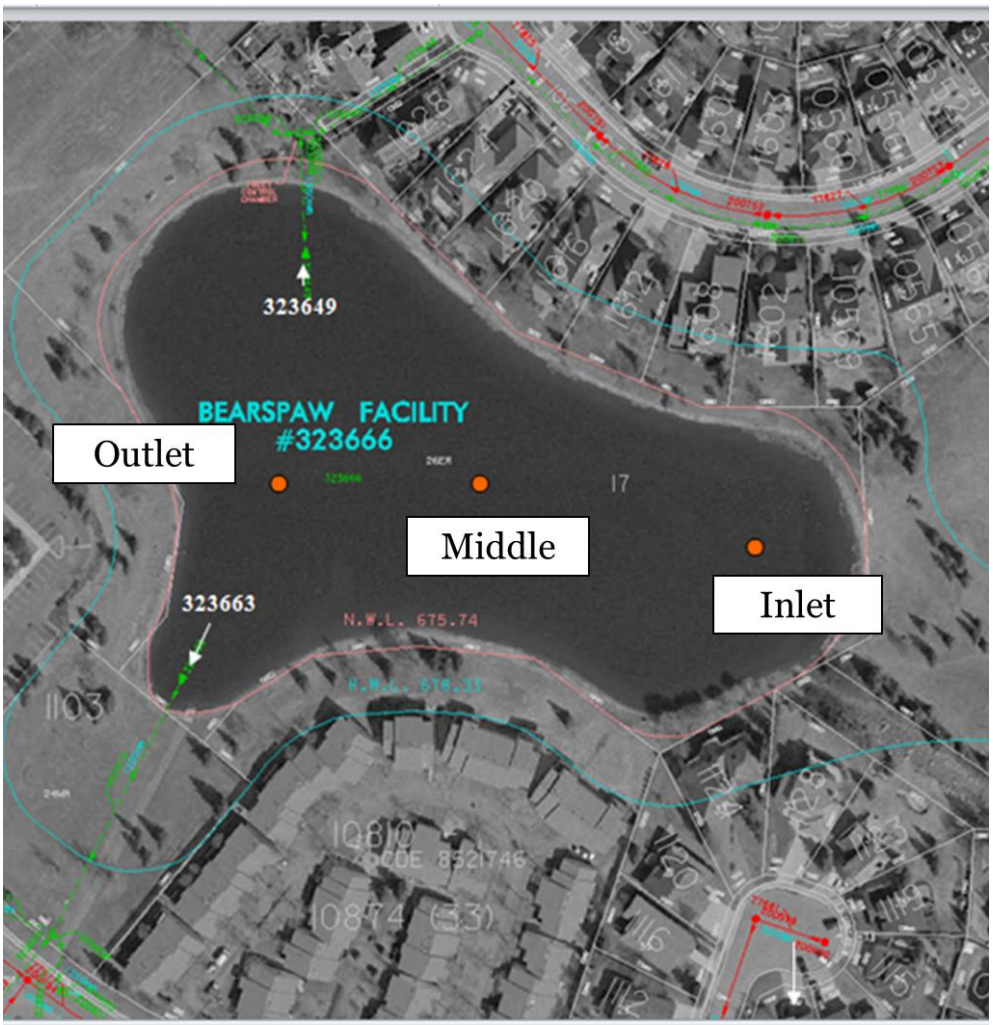

Figure 3-3 – Sampling sites of the inlet, middle, and outlet at Bearspaw stormwater retention pond

3.2.2 Water Sampling

Water samples were obtained using a device created from a 2L plastic container secured to the end of a 2.0m long water sampling rod. An Erlenmeyer flask cork was then drilled through and secured with a fitting nut and bolt, tied with 2.5m of fishing line, with the line strung through the handle of the overall apparatus. The apparatus was then weighted down with a cast iron chemistry stand base to ensure that it could reach the bottom of the pond, even with the buoyant forces acting upon it from the empty 2L liquid container. Once the apparatus was lowered to the desired depth of the lake, the cork was removed from the mouth of the 2L liquid container by

pulling on the fishing line that it was attached too. Please see Figure 3-4 below to see a picture of the water sampling apparatus created for this field study. This device and method was utilized to obtain a representable sample from the interface of the lake water and lake sediment. It was also assumed that, as the 2L liquid container was filled and being taken back up to the surface and onto the boat, there would be negligible mixing and exchange of water from the jug and the water column.

Figure 3-4 – Water sampling device used in field study

The water was then poured into the prepared sample bottles; amber glass bottles for in-house analysis and translucent plastic bottles with analyte-specific preservatives as provided by Maxxam Analytics for commercial lab analysis. The bottles were then stored in coolers filled with ice and ice packs. The samples were then delivered to Maxxam Analytics for analysis, or brought back to the in-house laboratory and stored at $4^{o}C$ until analyzed.

3.2.3 Sediment Sampling

Sediment samples were obtained using a Wildco Eckman Dredge standard grab kit sediment sampler and then stored in trace-cleaned clear glass sample jars provided by Maxxam Analytics for metals analysis at their lab, and sterile 50mL centrifuge tubes for DNA extraction and microbiological analysis at the University of Alberta. The samples were kept in coolers with ice packs until they could be delivered to their respective locations. The samples brought back to the University of Alberta were stored at 4°C until further processing could be performed.

Figure 3-5 – Eckman Dredge sediment sampler used in field study for stormwater retention pond sediment sampling

3.2.4 Field Measurements

SULPHIDE Sulphide concentrations were measured in field at time of sampling using a HACH DR/2400 portable spectrophotometer following the Standard Methods for the Examination of Water and Wastewater SM 4500-S2-D (APHA, AWWA, and WEF 1999).

ALKALINITY Alkalinity was measured using a HACH kit Digital Titrator (Model 16900) with 1.600N H2SO4 Titration Cartridges, Bromcresol Green-Methyl Red Powder Pillows, and Phenolphthalein Powder Pillows. The method of measurement used the Phenolphthalein and Total Method as described in SM 2320 B, EPA 310.2.

pH, CONDUCTIVITY, and OXIDATION-REDUCTION POTENTIAL Physical characteristics that were measured in the field include temperature, pH, conductivity, and oxidation-reduction potential (ORP). The pH and conductivity utilized the Extech ExStik EC500 pH/Conductivity/TDS/Salinity/Temperature Probe. The ORP was measured using the Oakton ORPTestr 10 Probe.

3.2.5 Water Quality Analysis

SULPHATE Sulphate concentrations were measured by both Maxxam Analytics Environmental Lab and the University of Alberta Biogeochemical Analytical Service Laboratory. Valencia samples from May 31, 2012 and Bearspaw samples from June 7, 2012 were sent to Maxxam Analytics for analysis, whilst the remaining samples were sent to the University of Alberta Biogeochemical Analytical Service Laboratory for analysis. The decision to do so was for financial reasons.

Sulphate concentrations measured by Maxxam Analytics were sent in with samples without requiring preservatives, and analyzed using an automated colourimetry method, as outlined by the EPA 375.4 Method.

Sulphate levels measured by the University of Alberta Biogeochemical Analytical Service Laboratory used the Ion Chromatography method with Dionex DX600 and Dionex ICS 2500 units with the EPA 300.1 (Modified) Method.

SULPHIDE Water samples were also measured for sulphide by Maxxam Analytics. The samples were preserved with zinc acetate and sodium hydroxide at the time of sampling before delivery to Maxxam Analytics. The method used by Maxxam Analytics was the Methylene Blue Method as outlined by Standard Methods for the Examination of Water and Wastewater SM 4500-S2-D (APHA, AWWA, and WEF 1999).

NITRATE and NITRITE and TOTAL PHOSPHORUS Nitrate and nitrite and total phosphorus concentrations were measured by both Maxxam Analytics Environmental Lab and the University of Alberta Biogeochemical Analytical Service Laboratory. Valencia samples from May 31, 2012 and Bearspaw samples from June 7, 2012 were sent to Maxxam Analytics for analysis, whilst the remaining samples were sent to the University of Alberta Biogeochemical Analytical Service Laboratory for analysis. The decision to do so was for financial reasons.

The samples sent to Maxxam Analytics did not include or require any preservatives, and were analyzed using Ion Chromatography following the Standard Methods for Water and Wastewater Analysis SM 4110-B (APHA, AWWA, and WEF 1999).

For samples sent to Maxxam Analytics for total phosphorus, they were preserved with 0.1 N sulphuric acid and analyzed using the method as outlined in the Standard Methods for Water and Wastewater Analysis SM 4500-P (APHA, AWWA, and WEF 1999).

Samples measured by the University of Alberta Biogeochemical Analytical Service Laboratory for nitrate and nitrite and total phosphorus used a Flow Injection Analysis method with a Lachat QuikChem 8500 FIA automated ion analyzer as outlined by the Standard Methods for Water and Wastewater Analysis SM 4500-NO3-I and SM 4500-P-H, respectively (APHA, AWWA, and WEF 1999).

ORGANICS: BIOCHEMICAL OXYGEN DEMAND, CHEMICAL OXYGEN DEMAND, and TOTAL ORGANIC CARBON Biochemical Oxygen Demand (BOD) was measured using the BOD-5 Test as outlined by the Standard Methods for Water and Wastewater Analysis (APHA, AWWA, and WEF 2011). The Azide-Modification Method was used to measure the dissolved oxygen concentrations at the beginning and end of the five day

test. This method was used instead of an oxygen probe because of the accuracy of Azide-Modification method. Total, carbonaceous BOD was measured for field samples, using unfiltered samples and nitrification inhibitors during the test. The first few sampling periods for both ponds required dilutions, however, over time, no dilutions were used to measure for the BOD-5 Test.

Chemical Oxygen Demand (COD) was measured using the Closed-Reflux Colorimetric Method as outlined in the Standard Methods for Water and Wastewater Analysis (APHA, AWWA, and WEF 2013). Total COD was measured instead of soluble COD as the water had low levels of suspended solids, and believed to be negligible. 2.5mL of samples were mixed with 1.5mL of digestion solution and 3.5mL of silver sulphate sulphuric acid solution and digested for 2 hours in a digester and then measured for absorbance at a wavelength of 600nm in a spectrophotometer.

Total Organic Compounds (TOC) was measured using a Shimadzu TOC-L CPH TOC Analyzer at the University of Alberta in the Geoenvironmental Engineering Analysis Lab utilizing the high temperature combustion method as described in the Standard Methods for Water and Wastewater Analysis (APHA, AWWA, and WEF 2011).

DISSOLVED METALS Dissolved metal samples were sent to Maxxam Analytics Environmental Lab for measurements at the beginning and end of the field study at each respective location; May 31, 2012 and September 27, 2012 at Valencia stormwater retention pond and June 7, 2012 and September 26, 2012 at Bearspaw stormwater retention pond. Samples were delivered to the commercial lab without preservatives or filtration due to lack of available supplies and time. Samples were then filtered in-lab and preserved with 0.1 N of nitric acid. The samples were analyzed using ICP and ICP-MS machines with the EPA 200.7 and EPA 200.8 methods.

3.2.6 Sediment Analysis

Sediments were analyzed for two parameters; total metals and microbiology. Total metals were analyzed to garner a better understanding of the sediment quality, and whether or not it provided any concerns of toxicity for its content. The sediment was also used to analyze the microbiology of the stormwater retention pond

environments because the biological activity is predominantly occurring in the sediment phase, more specifically at the interphase with the stormwater.

TOTAL METALS The analysis for total metals were completed by Maxxam Analytics Environmental Lab at the beginning and end of the field study at each respective location; May 31, 2012 and September 27, 2012 at Valencia stormwater retention pond and June 7, 2012 and September 26, 2012 at Bearspaw stormwater retention pond. Samples were delivered to the commercial lab without preservatives, and were measured using ICP-MS following the EPA 200.8 method.

3.2.7 Microbiology

DNA EXTRACTION DNA was extracted from the stormwater pond sediment samples using the MoBio PowerSOIL DNA Extraction Kit. This specific DNA extraction kit was used due to the matrix of the environmental samples extracted from. The advantage of using this specific kit is the process of removing PCR inhibitors and humic substances that interfere with molecular biology analysis. 500µL of sample were used for each DNA extraction. The DNA samples were then stored at -20°C, until used for further analysis.

QUANTITATIVE POLYMERASE CHAIN REACTION DNA samples were then analyzed and optimized for PCR conditions. Three groups of bacteria were analyzed; total bacteria, sulphate reducing bacteria, and nitrate reducing bacteria. The primer sets used to analyze for these three groups of bacteria were rpoB, dsrB and nosZ2 (Dahllof et al. 2000, Geets et al. 2006 and Muyzer et al. 1993, Henry et al. 2006). After PCR protocol optimization, the samples were further analyzed using qPCR techniques for quantification and relative distribution and comparison of the bacterial groups present in the samples. Standards for qPCR were created using the PCR products of the optimization step and purified using the Qiagen PCR Purification Kit, analyzed by Nanodrop unit, serial diluted and then stored in a -80°C freezer to maintain DNA quality. Table 3-2 summarizes the primer pairs and their respective information below.

Table 3-2 – Primer information on the rpoB, dsrB, and nosZ2 genes used in the field study of microbial community of Valencia and Bearspaw stormwater retention ponds

	Primer		
	rpoB	dsrB	nosZ2
Species	Total Bacteria	Sulphate Reducing Bacteria	Nitrate Reducing Bacteria
Target gene	RNA polymerase beta subunit	dissimilatory sulfite reductase	nitrous oxide reductase
Number of genes per organism	1	1	1
Forward Primer Sequence	rpoB1698f (5' - AACATCGGTTTGATC AAC - 3')	DSRp2060f (5'- CAACATCGTYCAYACCCAGGG - 3')	nosZ2F (5'- CGCRACGGCAASAAGGTSMS SGT-3')
Reverse Primer Sequence	rpoB2041r (5' - CGTTGCATGTTGGTACCCA T - 3')	DSR4r (5' - GTGTAGCAGTTACCGCA - 3')	nosZ2R (5'- CAKRTOGCAKSGCRTGGCAG AA-3')

The qPCR experiments were conducted using the Bio Rad I-Cycler in 96 well plates. The qPCR reactions were done in 20 µL reactions, consisting of 10 µL of Bio Rad SsoFast EvaGreen Supermix, 0.05 µL each of the forward and reverse primers of interest, 1 µL of DNA template and 8.9 µL of water. The temperature program and ramps can be reviewed in Table 3-3 below. The results of the qPCR were analyzed and managed using the Bio Rad CFX Manager 3.0 Software.

Table 3-3 – Temperature cycles, program, and ramps used for rpoB, dsrB, and nosZ2 primers for qPCR analysis

	rpoB			dsrB			nosZ2		
	Temperature (°C)	Time (minutes)	Cycles	Temperature (°C)	Time (minutes)	Cycles	Temperature (°C)	Time (minutes)	Cycles
Denature	95	3 minutes	1 x	95	3 minutes	1 x	95	3 minutes	1 x
Anneal	95	0.5 minutes	35 x	95	0.25 minutes	39 x	95	0.75 minutes	35 x
Elongation	47	1.5 minutes		62	0.5 minutes		58	0.75 minutes	
Melt Curve	65°C to 95°C (increments of 0.5°C) for 5 seconds								

3.3 Results

3.3.1 Water Quality

3.3.1.1 Sulphide and Sulphate

The first water quality parameter that will be addressed is sulphate concentration, and in conjunction, sulphide concentration. Sulphate and sulphide are discussed together due to their direct correlation to one another, where microbial sulphate reduction produces sulphide or microbial sulphur oxidation produces sulphates. Sulphide levels are important to consider as related to hydrogen sulphide. The hydrogen sulphide levels were a specific problem addressed by municipal engineers and operators at the Valencia stormwater retention pond site. Therefore a comparison of how much sulphate and sulphide concentrations differ can give a relative indication of the problem. Sulphate concentrations measured over the summer 2012 operational season for both Valencia and Bearspaw stormwater retention ponds' stormwater is displayed in Figure 3-6 below, followed by Figure 3-7 with the sulphide concentrations at each pond throughout the field study as well.

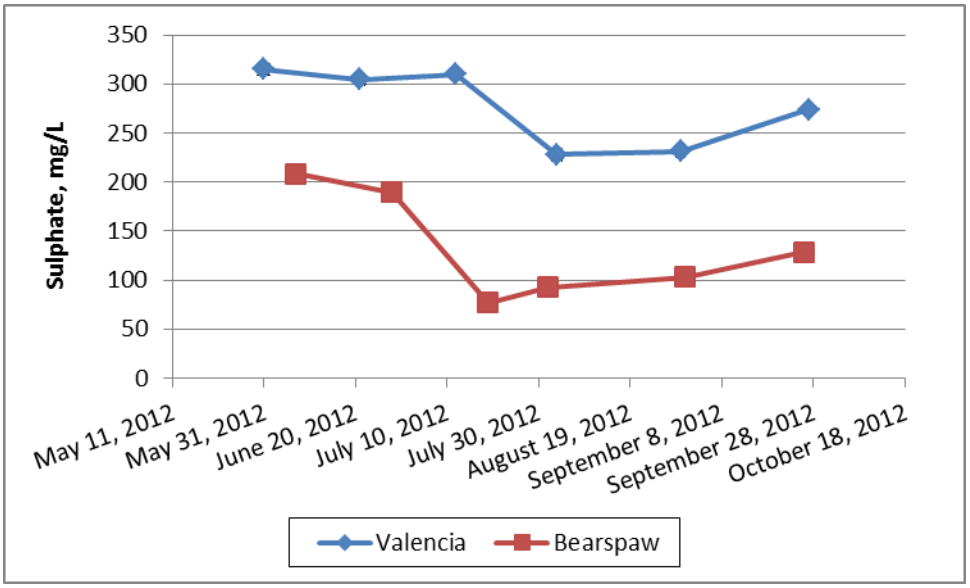

Figure 3-6 – Sulphate concentrations measured from Valencia and Bearspaw stormwater retention ponds throughout field study in 2012. Data points represent the average of water samples collected at the inlet, middle, and outlet of the stormwater pond and error bars represent plus and minus one standard deviation

The sulphate concentrations remained relatively consistent at each sampling day for Valencia stormwater pond with insignificant variability (P-values ranging from 0.07 to 0.15) with an overall decrease in concentrations throughout the field study. There were two exceptions at this site, the first instance where a decrease in concentrations of sulphate from 310mg/L to 227mg/L occurred between July 12, 2012 and August 3, 2012 (P-value = 0.00000944). The second instance where an increase of sulphate concentrations from 231mg/L to 274mg/L occurred between August 30, 2012 and September 27, 2012 (P-value = 0.0000841).

Bearspaw stormwater retention ponds' sulphate concentration varied significantly between each day of sampling throughout the summer operational season of the stormwater retention pond, having P-values ranging from 0.00000604 to 0.00975, however with an overall decrease in levels throughout the field study. The variability shown at the Bearspaw stormwater retention pond site through this field study reflects the temporal variability in water quality present when managing stormwater. However, the difference in variability found in Bearspaw versus Valencia may indicate differences in biological activity.

In both stormwater ponds, a significant decrease in sulphate concentration was found to occur in the middle of July, correlating to large rainfall data that was recorded by the City of Edmonton on July 14, 2012. This instance of large precipitation most likely diluted and flushed the total dissolved solids in both stormwater retention ponds. Both stormwater ponds also found increases in sulphate concentrations following the decrease from the rainfall event, indicating a trend of returning to a state of equilibrium of the stormwater ponds.

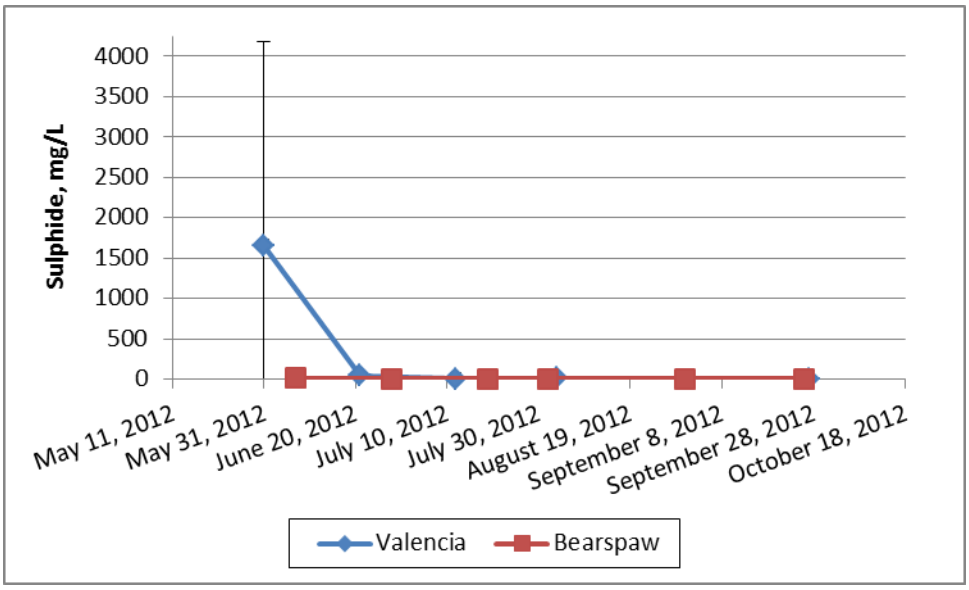

Figure 3-7 – Sulphide concentrations measured from Valencia and Bearspaw stormwater retention ponds throughout field study in 2012. Data points represent the average of water samples collected at the inlet, middle, and outlet of the stormwater pond and error bars represent plus and minus one standard deviation

The sulphide concentrations in Bearspaw stormwater stayed relatively low throughout the field study, averaging below 15µg/L. Sulphide levels did not significantly change throughout the field study, with P-values calculated at 0.08 to 0.23. The exceptions being at the beginning and end of the field study (P-values of 0.005 and 0.034 respectively), although showing more significant changes between these two sampling periods, the concentrations were at a low level that does not necessarily indicate any notable trends or reasons.

The sulphide levels at Valencia pond however, started at a higher concentration from approximately 1400 and 3400 µg/L, and began decreasing over

time to reach relatively the same levels as Bearspaw (below 25µg/L) by the end of the field study. The sulphide levels in Valencia did not significantly change during this field study, with P-values ranging from 0.114 to 0.231 on most occasions. The only determined exception, due to data variances and standard deviations, to this was between the dates of June 21, 2012 and July 12, 2012, with a P-value of 0.0151, most likely reflecting the same scenario as the sulphate concentration changes from a rainfall or increased in-flow event.

3.3.1.2 Nitrate and Nitrite

Nitrate and Nitrite was measured and analyzed due to their coupled role as electron acceptors, but also as a nutrient in the stormwater environment. Nitrate and nitrite, as an electron acceptor source for microbial activity, is higher in its oxidation-reduction potential and could be an indicator for the type of microbial community that exists in the stormwater retention pond. Nitrate and nitrite combined concentrations measured at Valencia and Bearspaw stormwater retention ponds over the summer 2012 sampling period is shown in Figure 3-8 below.

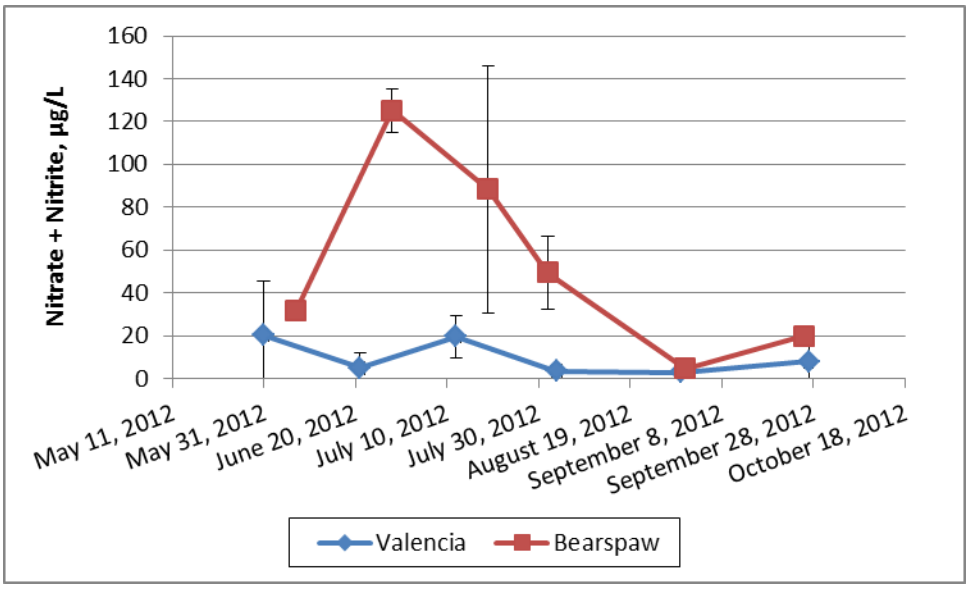

Figure 3-8 – Nitrate and nitrite concentrations measured from Valencia and Bearspaw stormwater retention ponds throughout field study in 2012. Data points represent the average of water samples collected at the inlet, middle, and outlet of the stormwater pond and error bars represent plus and minus one standard deviation

From the figure, the nitrate and nitrite concentrations in Valencia stayed around or below 40 mg/L, with most instances showing concentrations below 20 mg/L. Throughout the field study, nitrate and nitrite levels did not show any significant changes at Valencia, with P-values ranging from 0.066 to 0.27. The nitrate levels stayed well below the suggested guideline concentrations for short term periods for protection of aquatic life of 550 mg/L.

The nitrate and nitrite concentrations for Bearspaw however, showed a wider range, with values as low as 5 mg/L to levels as high as approximately 125 mg/L. In comparison to Valencia, Bearspaw showed significant changes throughout the field study, mostly between June 7, 2012 and June 28, 2012, August 1, 2012 and August 31, 2012, and August 31, 2012 and September 26, 2012 with P-values at 0.0000798, 0.0279, and 0.00541 respectively. The nitrate and nitrite concentrations stayed within short term guideline levels of 550 mg/L, however, with the exception of the August 31, 2012 sample, exceeded the long term guideline level for protection of aquatic life at 13 mg/L. This may be of initial concern, however, the stormwater retention pond was never designed to house aquatic life, and it has time to enter the stormwater system before entering and mixing with the North Saskatchewan River in Edmonton.
This variation in nitrate and nitrite concentrations at Bearspaw, in comparison with the levels in Valencia, indicate that they are being used as electron acceptors in the stormwater pond, most likely due to its abundance, availability, and the greater redox potential it provides (Sawyer et al. 2003).

3.3.1.3 Organics: Biochemical Oxygen Demand, Chemical Oxygen Demand, and Total Oxygen Demand

The organics that are present in the stormwater act as an indicator of the level of pollution in the stormwater retention ponds, and their changes in concentration act as indicators of the availability of carbon sources or electron donor for biodegradation.

BOD BOD can be defined and understood as the oxygen required by bacteria and microorganisms in their metabolism to stabilize the decomposable organic matter in the medium under aerobic conditions (Sawyer et. al, 2003). It is one of the tools that

provide a general analysis of the water's level of pollution. Figure 3-9 below depicts the measured BOD concentrations using the azide-modification method BOD-5 Test in mg/L of O_2 of Valencia and Bearspaw stormwater retention ponds during the field study.

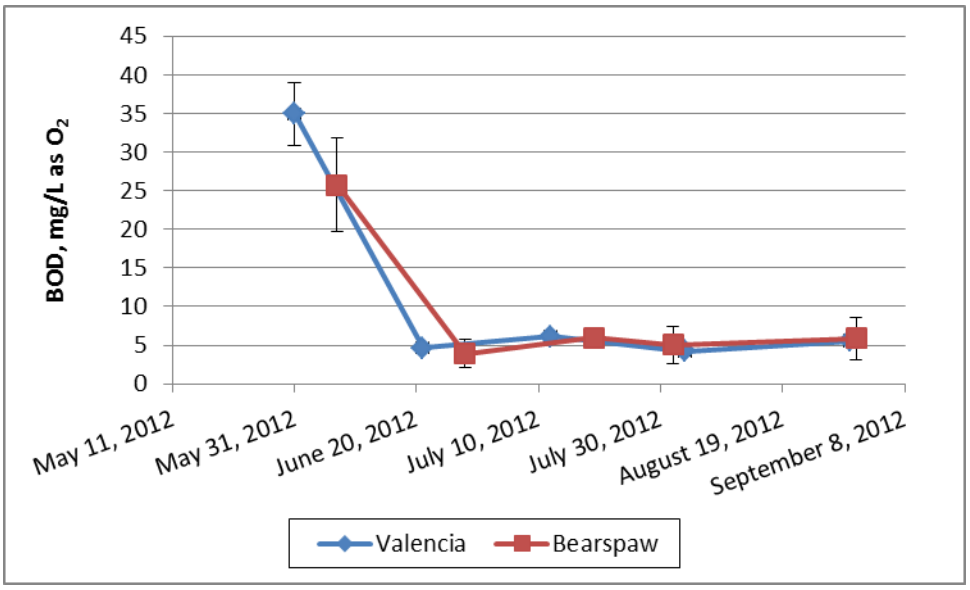

Figure 3-9 – Biochemical oxygen demand measured from Valencia and Bearspaw stormwater retention ponds throughout field study in 2012. Data points represent the average of water samples collected at the inlet, middle, and outlet of the stormwater pond and error bars represent plus and minus one standard deviation

The BOD measured at Valencia pond started at a concentration of 34.9 mg/L as O_2 on May 31, 2012. It then significantly decreased (P-value = 0.00309) to a concentration of 4.63 mg/L as O_2 by June 21, 2012. The BOD level was then measured to fluctuate between 4.26 and 6.12 mg/L as O_2 over the rest of the field study from July 12 to August 30, 2012.

The BOD measured at Bearspaw pond started at a concentration of 25.7 mg/L as O_2 on June 7, 2012 and decreased significantly (P-value = 0.00201) to a concentration of 3.90 mg/L as O_2 on June 28, 2012. The BOD levels were then measured to range between 5.07 and 5.98 mg/L as O_2 between July 19, 2012 and August 31, 2012, for the remainder of the field study.

BOD followed the same trends at both Valencia and Bearspaw stormwater retention pond sites. Both sites' initial BOD measurements were found to be the highest throughout the field study, and under the required guideline levels as outlined

by the City of Edmonton's Sewer-Use Bylaw (City of Edmonton 2013). By the second sampling date for each respective site, BOD concentrations had decreased significantly to levels below 5 mg/L as O_2.

COD COD is another indication of pollution that is commonly used in wastewater quality, and measures the oxygen required to oxidize the organic material in a media. The measurement of COD can overestimate the amount of organics as it does not take into account the biodegradability of the compounds in the media; however, it is a relatively fast test, in comparison to the five day BOD test. The measured COD concentrations of the stormwater in both Valencia and Bearspaw stormwater retention ponds can be seen in Figure 3-10, as seen below.

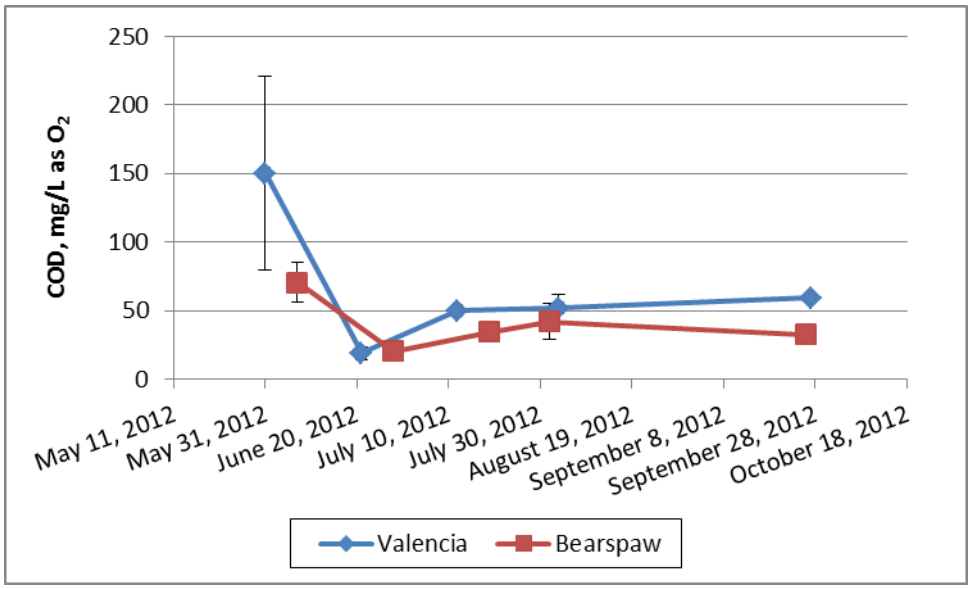

Figure 3-10 – Chemical oxygen demand measured from Valencia and Bearspaw stormwater retention ponds throughout field study in 2012. Data points represent the average of water samples collected at the inlet, middle, and outlet of the stormwater pond and error bars represent plus and minus one standard deviation

COD at the Valencia stormwater retention pond was found to start at its highest measured value at the beginning of the field study with a concentration of 150 mg/L as O_2 on May 31, 2012. The concentration then dropped significantly (P-value = 0.0422) to a level of 18.8 mg/L as O_2 measured on June 21, 2012, also found to be the lowest concentration during the field study. The COD was then found to rise up sharply (P-value = 0.000232) to a concentration of 50.0 mg/L as O_2 by the third sample

date on July 12, 2012, and remained consistent at approximately the same concentrations until the end of the field study on September 27. 2012, with a final measured value of 59.2 mg/L as O_2.

At the Bearspaw stormwater retention site, COD was measured at its highest value at the beginning of the field study with a concentration of 70.4 mg/L as O_2 on June 7, 2012. There was then a significant decrease (P-value = 0.0133) in COD concentration by June 28, 2012, to a measured value of 20.4 mg/L as O_2. The COD then rose to a concentration of 28.8 mg/L as O_2 on July 19, 2012, and remained relatively consistent, rising again to 31.3 mg/L as O_2 on August 1, 2012 and a final concentration of 32.4 mg/L as O_2 on September 26, 2012 at the end of the field study.

In general, the COD measured at both Valencia and Bearspaw stormwater retention ponds followed the same trends throughout the field study. At the beginning of the field study, both ponds were found to start with their highest respective COD readings, and by the second sampling date, had dropped to their lowest respective COD levels during the study.

TOC Total organic carbon is a general total measurement of the organic content available in a water system, both dissolved and non-dissolved. Although it cannot specify the organic compounds containing carbon in the water, it indicates a general availability of compounds that could be utilized as electron donors and or carbon sources for microbial activity. The TOC as measured throughout the 2012 field study for Valencia and Bearspaw stormwater retention ponds are represented in Figure 3-11, as shown below.

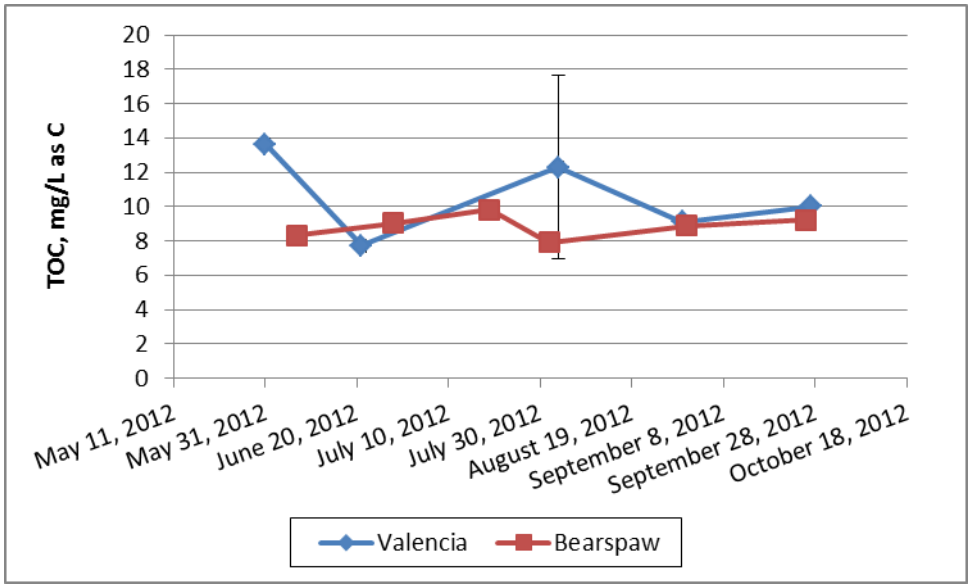

Figure 3-11 – Total organic carbon measured from Valencia and Bearspaw stormwater retention ponds throughout field study in 2012. Data points represent the average of water samples collected at the inlet, middle, and outlet of the stormwater pond and error bars represent plus and minus one standard deviation

The TOC measured at the Valencia site, started at a concentration of 13.6 mg C/L as sampled on May 31, 2012. It then decreased significantly (P-value = 0.0000003.48) to 7.72 mg/L as C on June 21, 2012, and rose to a concentration of 12.3 mg/L as C on August 3, 2012. The TOC once again dropped in concentration to 9.11 mg/L as C on August 30, 2012 and rose to a final measured value of 10.0 mg/L as C on September 27, 2012 at the end of the field study.

The TOC measurements done at Bearspaw stormwater retention pond started at a concentration of 8.33 mg/L as C on June 7, 2012. It steadily and significantly rose to 9.04 mg/L as C (P-value = 0.0379) on June 28, 2012 and again to 9.82 mg/L (P-value = 0.00848) as C on July 19, 2012. The measured TOC concentrations then dropped to 7.90 mg/L as C (P-value = 0.000131) on August 1, 2012. From there, the TOC rose to 8.88 mg/L as C on August 31, 2012 and reaching a final measured value of 9.21 mg/L as C on September 26, 2012 at the end of the field study.

Comparatively, Valencia exhibited more fluctuations and greater changes in concentrations throughout the field study between 7.7 and 13.6 mg/L as C, whereas Bearspaw had concentrations that existed between a smaller range of 8.3 and 9.8 mg/L as C.

3.3.1.4 Water Chemistry

Several water chemistry parameters were monitored and measured through the field study. This was done to understand any trends in water conditions throughout the summer operational season of the stormwater retention ponds. These parameters include total phosphorus concentrations, alkalinity, pH, ORP, and conductivity.

TOTAL PHOSPHORUS Phosphorus is a nutrient and is regulated under different guidelines. Phosphorus levels can be an indicator for eutrophication for freshwater systems as it can lead to problems such as algae blooms. The total phosphorus levels at Valencia and Bearspaw stormwater retention ponds are seen in Figure 3-12.

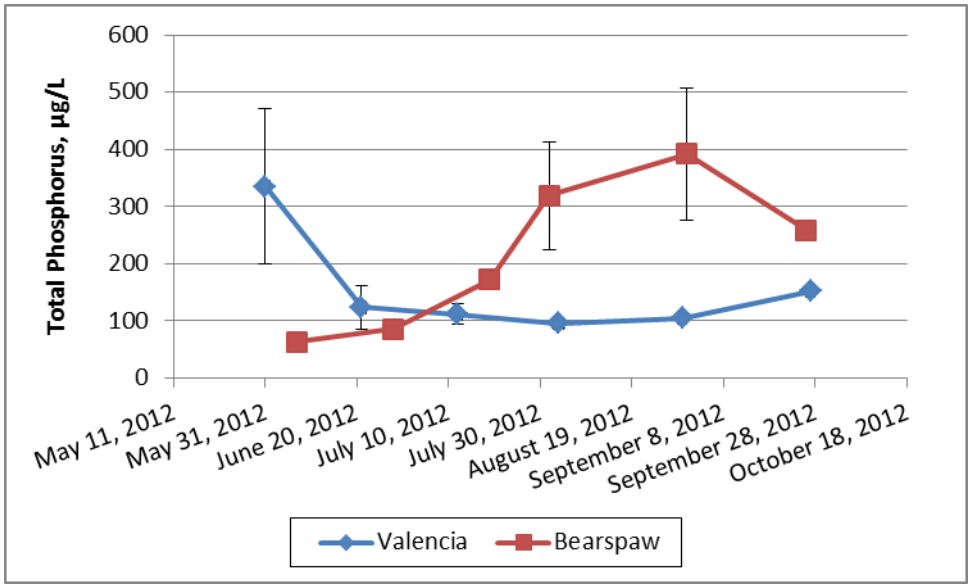

Figure 3-12 – Total phosphorus measured from Valencia and Bearspaw stormwater retention ponds throughout field study in 2012. Data points represent the average of water samples collected at the inlet, middle, and outlet of the stormwater pond and error bars represent plus and minus one standard deviation

The results as shown in the figure showed decreasing total phosphorus concentrations at Valencia, starting from approximately 340 µg/L on May 31, 2012 to about 100 µg/L on August 3, 2012. Total phosphorus levels then rose to 150 µg/L by the end of the field study on September 27, 2012. The significant changes in total phosphorus levels occurred between May 31, 2012 and June 21, 2012, with a P-value

of 0.0404, and between August 3, 2012 and September 27, 2012, with a P-value of 0.0000464. All measured total phosphorus levels have been found to be well under the City of Edmonton suggested levels of 1.0 mg/L.

The total phosphorus concentrations behaved differently at the Bearspaw location, increasing since June 7, 2012's measurements of 65 µg/L to 392 µg/L measured on August 31, 2012, before dropping down to 258 µg/L on September 26, 2012. The most significant changes in total phosphorus levels were found between June 7, 2012 and June 28, 2012 (P-value = 0.000119) and between June 28, 2012 and July 19, 2012 (P-value = 0.00000335). Once again, the measurements of total phosphorus were well under the City of Edmonton suggested levels of 1.0 mg/L.

ALKALINITY Alkalinity is a measure of the capacity of a water system to counteract and neutralize acidity. It is also a by-product of microbial activity (SOURCE). As such, the analysis of alkalinity in the stormwater ponds would show possible microbial activity trends in the respective stormwater ponds. The measured alkalinity, as measured in concentrations as $CaCO_3$, at both Valencia and Bearspaw stormwater retention ponds can be seen in Figure 3-13 as shown below.

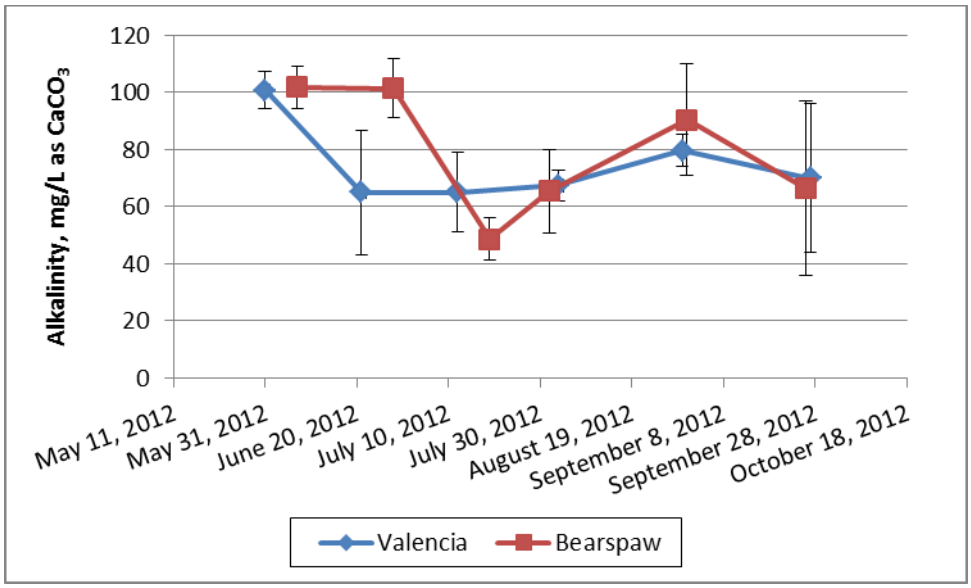

Figure 3-13 – Alkalinity measured from Valencia and Bearspaw stormwater retention ponds throughout field study in 2012. Data points represent the average of water samples collected at the inlet, middle, and outlet of the stormwater pond and error bars represent plus and minus one standard deviation

The alkalinity measured at the Valencia stormwater retention pond site started at 101 mg/L as $CaCO_3$ on May 31, 2012. It decreased significantly (P-value = 0.0265) to 65 mg/L as $CaCO_3$ on June21, 2012. The alkalinity then remained consistent for the next two sample dates; 65 mg/L as $CaCO_3$ on July 12, 2012 and 67.3 mg/L as $CaCO_3$ on August 3, 2012. It then rose quickly (P-value = 0.0277) to 79.7 mg/L as $CaCO_3$. Finally, the alkalinity came to a concentration of 70 mg/L as $CaCO_3$ by the end of the field study on September 27, 2012.

In Bearspaw stormwater retention ponds' case, the alkalinity had started at a concentration of 102 mg/L as $CaCO_3$ on June 7, 2012 and remained constant at a level of 101 mg/L as $CaCO_3$ by June 28, 2012. It had then decreased significantly (P-value =

0.00105) to 48.7 mg/L as $CaCO_3$ by the third sampling date, July 19, 2012. The alkalinity had then increased slowly to 65.3 mg/L as $CaCO_3$ on August 1, 2012 and again to 90.3 mg/L as $CaCO_3$ by August 30, 2012. Finally, the alkalinity was found to drop to a concentration of 66.3 mg/L as $CaCO_3$ at the end of the field study on September 26, 2012.

The alkalinity measured at both Valencia and Bearspaw stormwater retention pond sites exhibited the same general pattern throughout the field study. Both ponds measured their highest concentrations of alkalinity at the beginning of their field study at 101 (Valencia) and 102 (Bearspaw) mg/L as $CaCO_3$. Both situations found their alkalinity drop significantly after the initial sampling dates, slowly increasing and dropping to a lower level again by the end of their respective field study.

pH The pH of a water system is a commonly measured parameter for water quality. It can be used to describe the environment that is present and which specific groups of microorganisms are thriving. The pH of a water system can also describe the likelihood of the type of sulphur species that exists, and which ones are dominant. The pH of both Valencia and Bearspaw stormwater ponds measured over the course of the 2012 summer operation season of the stormwater ponds are depicted in Figure 3-14, as shown below.

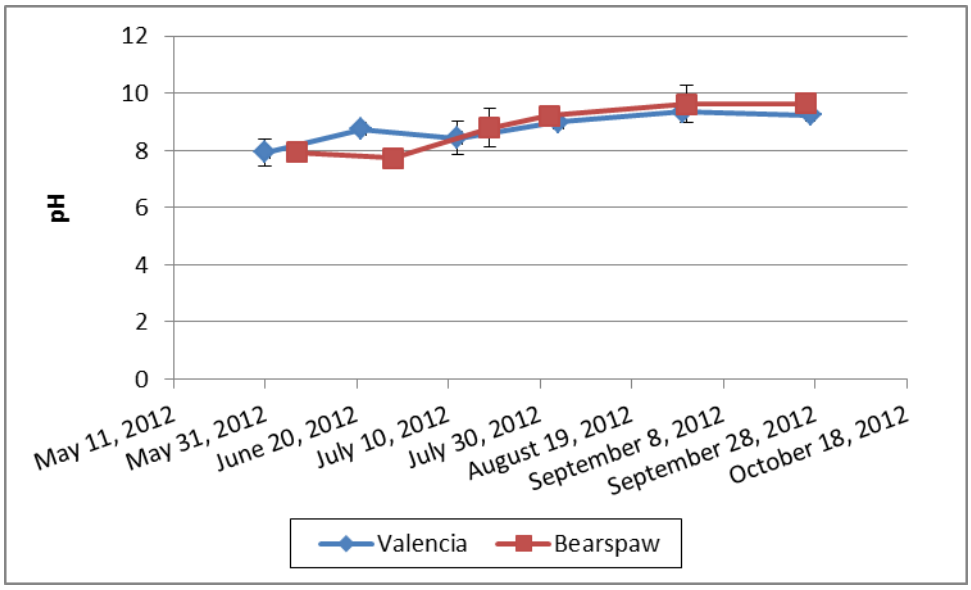

Figure 3-14 – pH measured from Valencia and Bearspaw stormwater retention ponds throughout field study in 2012. Data points represent the average of water samples collected at the inlet, middle, and outlet of the stormwater pond and error bars represent plus and minus one standard deviation

The pH measured at Valencia stormwater pond started at 7.93 on May 31, 2012 and had increased to 9.35 on August 30, 2012 and 9.24 on September 27, 2012. The significant changes in pH occurred between May 31, 2012 and June 21, 2012 from 7.93 to 8.75 (P-value = 0.0473), and between August 3, 2012 and August 30, 2012 from 8.98 to 9.35 (P-value = 0.00263). The increase between August 3 and 30, 2012 is of more significant concern as the pH was found to increase beyond the suggested pH level as outlined in the City of Edmonton's Sewer-Use Bylaw (City of Edmonton 2013). Once again, the pH reaches a level of 9.35 by the end of the field study, remaining above the guideline levels. It is not known whether or not this level remains this high as the stormwater moves through the stormwater system before it reaches the North Saskatchewan River.

For the Bearspaw stormwater retention pond, pH levels were measured to start at 7.94 (June 7, 2012) and 7.73 (June 28, 2012) and increased throughout the field study to reach 9.64 by September 26, 2012. This pH change was found to be gradual at Bearspaw, with no significant changes throughout the field study, other than the drop in pH between June 7, 2012 and June 28, 2012 (P-value = 0.00421) as mentioned

earlier. Between the samples taken on July 19 and August 1, 2012, the pH levels had increased from 8.79 to 9.21, reaching above the suggested guidelines of the City of Edmonton's Sewer-Use Bylaw.

The pH levels as measured at both Valencia and Bearspaw stormwater retention pond sites followed a trend of increasing throughout the field study. Both stormwater pond waters reach similar levels, with Bearspaw being slightly more alkaline, reaching slightly above the recommended pH level in the City of Edmonton Sewer-Use Bylaw.

ORP The oxidation-reduction potential (ORP) is another parameter commonly measured to gauge water quality, which focuses on the tendency for electrons to be transferred from the chemical species that exist in the water, and the overall likelihood of the species that exist presently would either be oxidized or reduced. The ORP is then an indicator for whether or not electron acceptors, such as sulphates and nitrates and nitrites, are likely to be further reduced in the current environment. The ORP measured at both Valencia and Bearspaw stormwater retention ponds through the field study can be seen below in Figure 3-15.

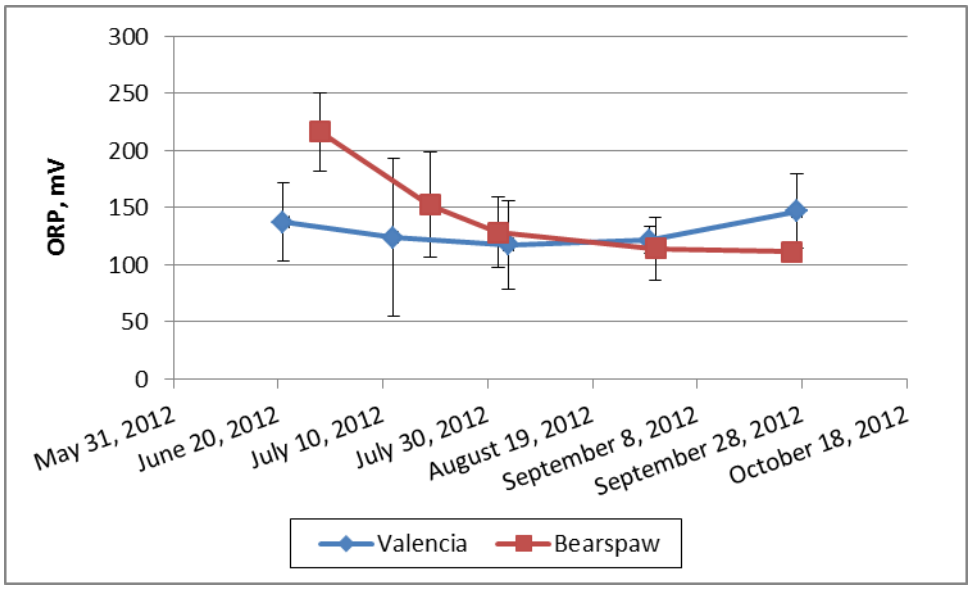

Figure 3-15 – ORP measured from Valencia and Bearspaw stormwater retention ponds throughout field study in 2012. Data points represent the average of water samples collected at the inlet, middle, and outlet of the stormwater pond and error bars represent plus and minus one standard deviation

The ORP measured at Valencia ranged from 118 to 147 mV over the duration of the field study. The measurements showed consistency throughout the field study, with no significant changes between any of the sampling dates with P-values ranging from 0.139 to 0.451 between the 5 measurements.

The ORP measured at Bearspaw started at 216 mV on June 28, 2012 and continually decreased throughout the field study, to reach 112 mV on September 26, 2012. Although continually decreasing, it was determined to be statistically insignificant changes between samples taken with P-values ranging from 0.0621 to 0.295.

Overall, both stormwater retention ponds exhibited stormwater that had higher reduction potential. This value means that the chemical species that exist in the stormwater are more likely to take electrons and form their oxidized species at the time of the study.

CONDUCTIVITY Conductivity is a general indicator of water quality, a quick measurement describing the total dissolved solids level. The conductivity measured at Valencia and Bearspaw stormwater retention pond sites over the 2012 summer operational period can be seen in Figure 3-16 below.

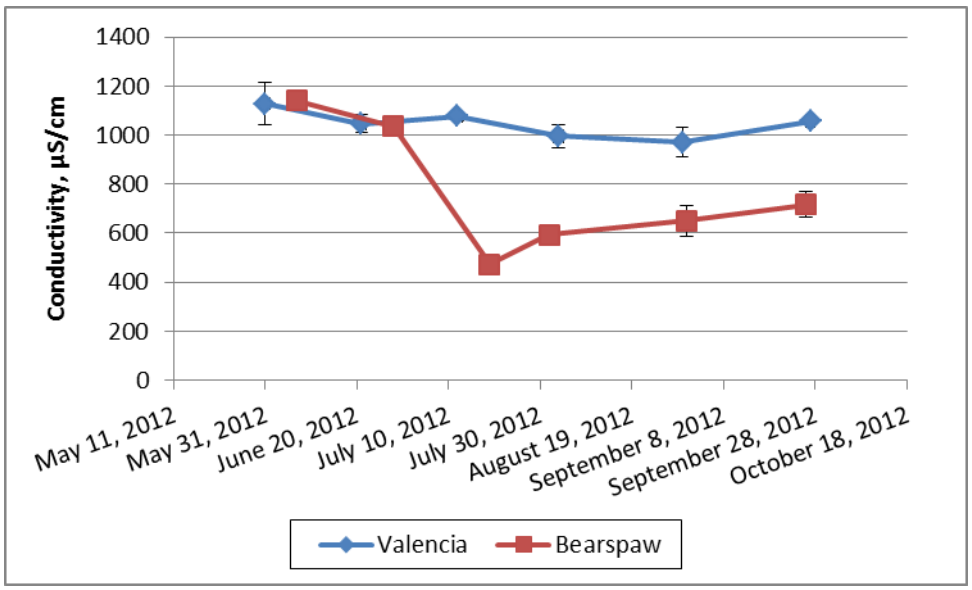

Figure 3-16 – Conductivity measured from Valencia and Bearspaw stormwater retention ponds throughout field study in 2012. Data points represent the average of water samples collected at the inlet, middle, and outlet of the stormwater pond and error bars represent plus and minus one standard deviation

For Valencia, the conductivity was measured to be between the range of 970 and 1130 µS throughout the entirety of the field study. The conductivity was determined to remain relatively consistent throughout the field study; the only exception was a significant change in levels between July 12, 2012 and August 3, 2012 (P-value = 0.0245).

In Bearspaw's case, conductivity had a greater range in measurements, from as low as 473 µS on July 19, 2012 and as high as 1140 µS on June 7, 2012. Conductivity was measured to be highest in Bearspaw at the beginning of the field study on June 7, 2012, dropping significantly over the course of two consecutive sampling sessions (the span of a month and a half) to 1037 µS on June 28, 2012 (P-value = 0.00417) and 473 µS on July 19, 2012 (P-value = 0.00000827). After this significant drop in conductivity measurements, conductivity levels were measured to increase to a measurement of 592 µS on August 1, 2012 (P-value = 0.00061) and then slowly to 717 µS by the end of the field study on September 26, 2012.

DISSOLVED METALS The dissolved metals analysis in the stormwater of both stormwater ponds were found be within limits of CCME guidelines.

3.3.2 Sediment Analysis

The sediment analysis consisted of measuring total metals and the microbiology. This was to check any other factors that could affect the sulphate reduction activity in the stormwater retention pond.

METALS The total metals analysis in the stormwater sediments of both Valencia and Bearspaw stormwater retention ponds were below CCME guidelines and will not be discussed further.

3.3.2.1 Quantification of SRB, NRB, and Total Bacteria

Total bacteria, SRB, and NRB counts were monitored throughout the summer operational period at Valencia and Bearspaw stormwater retention ponds. The qPCR results below, in Figure 3-17, assist in understanding the proportions of specific groups of bacteria in the stormwater sediment.

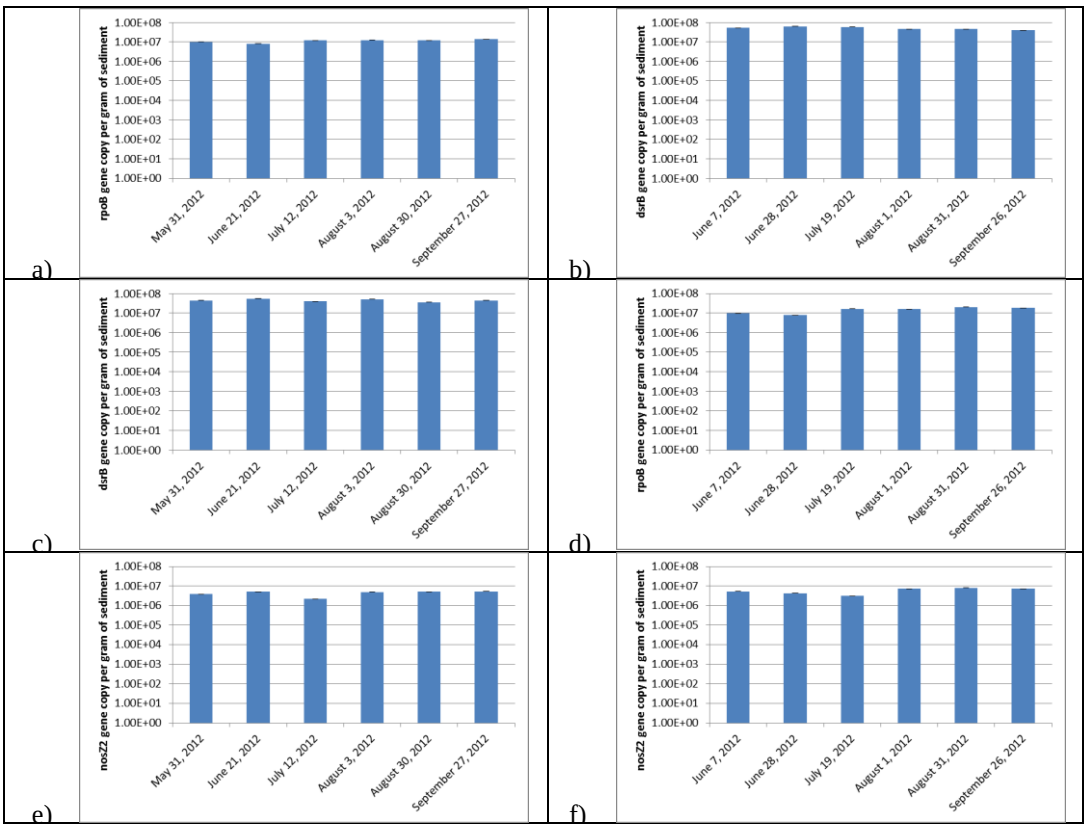

Figure 3-17 –qPCR results for a) rpoB, c) dsrB, and e) nosZ2 gene copies per gram sediment in the field study of Valencia pond and b) rpoB, d) dsrB, and f) nosZ2 gene copies per gram sediment in the field study of Bearspaw pond throughout field study in 2012. Data points represent the average of water samples collected at the inlet, middle, and outlet of the stormwater pond and error bars represent plus and minus one standard deviation

The total bacteria counts, as analyzed through the rpoB gene copy counts showed that in both Valencia and Bearspaw stormwater retention ponds, started approximately around the same count at 9.98×10^6 and 9.78×10^6gene copies per gram sediment. Both total bacteria counts also followed the same trend of increasing throughout the field study. Valencia's final count was found to be 1.36×10^7 gene copies per gram sediment. At the end of its field sampling regiment on September 27, 2012. Bearspaw's final count was found to be 1.38×10^7 gene copies per gram sediment at the end of its field sampling regiment on September 26, 2012.

The SRB counts, as analyzed with the dsrB gene copy counts, determined that SRB were not significantly different in the Valencia pond versus Bearspaw pond. Valencia dsrB counts started with an initial value of 4.35×10^7 gene counts per gram sediment on May 31, 2012, and fluctuated throughout the field study with a maximum count of 5.53×10^7 gene copies per gram sediment, on June 21, 2012 and a minimum count of 3.90×10^7 gene copies per gram sediment on July 12, 2012. A final count of 4.35×10^7 gene copies per gram sediment was found on September 27, 2012. Bearspaw pond had lower gene counts overall, however, followed the same trend as Valencia and had greater numbers of SRB than NRB. Initial counts started at 5.24×10^7 gene copies per gram sediment, and also fluctuated throughout the field study, with a maximum count at 6.34×10^7 gene copies per gram sediment. on June 28, 2012, and a minimum count at 3.96×10^7 gene copies per gram sediment, found on the final day of sampling, September 26, 2012.

The NRB counts, as represented by the nosZ2 gene copy counts, showed that overall, Bearspaw stormwater retention pond did not have significantly higher counts compared to that of those found in Valencia. The nosZ2 gene counts in Valencia began with an initial value of 3.91×10^6 per gram sediment on May 31, 2012. The gene counts, similar to dsrB counts, were found to fluctuate throughout the field study with the lowest count of 2.17×10^6 gene copies per gram sediment found on July 12, 2012, and the largest count of 5.28×10^6 gene counts per gram sediment was found on the final sample date of September 27, 2012. The nosZ2 gene counts in Bearspaw pond started with an initial count of 5.38×10^6 genes per gram sediment and fluctuated with a lowest count of 3.17×10^6 found on July 19, 2012, and a maximum count of 8.12×10^6 found on

August 31, 2012. The final sampling date on September 26, 2012 gave a measurement of 7.27×10^{6} gene copies per gram sediment.

Overall, the qPCR results were not conclusive in the significant differences in microbial populations at Valencia stormwater retention pond and Bearspaw stormwater retention pond.

3.4 Discussion

Analyzing the results of the field study comparison of Valencia and Bearspaw stormwater retention ponds found in the previous section, suggest several reasons and key differences that would explain why Valencia stormwater retention pond has an environment that encourages the production of sulphide ions.

First, there are significant differences in the availability of electron donor species found in the stormwater. As seen in Figure 3-6, throughout the entire field study, sulphate concentrations were notably higher at Valencia pond than in Bearspaw pond. The significantly higher levels of sulphate in Valencia show the abundant source of electron acceptor for SRB to utilize in their metabolism and growth. The increased availability of electron acceptor would allow for SRB to out-compete other bacteria and microbiological species in this particular environment. Al-Zuhair et al. (2008) found that growth rates of SRB increased up until concentrations greater than 2500 mg/L, SRB at that point began to show inhibition and limitation in growth, therefore higher concentrations found in Valencia would suggest higher growth rates. The possible reasons for this variation in sulphate concentrations could be due to stormwater inlet sources. Valencia, located in northeast Edmonton, is located near more industrial areas that include refineries and processing plants, where air pollution and the deposition of particulate matter contaminated with sulphate could be a source. However, air pollution alone should not be able to contribute such a significant difference in sulphate concentrations. Another possible reason for the increased levels of sulphate concentrations at Valencia could be due to its past use as an experimental site for quality control (Babin et al. 1992 and 1989). Over a period of several years in the late 1980s and early 1990s, Valencia was one of several stormwater retention ponds that had undergone experiments utilizing lime (Ca(OH)2)

and alum ($Al_2(SO_4)_3 \bullet 14H_2O$) to reduce phosphorus levels and algae growth/blooms (Babin et al. 1992 and 1989). Within the summer of 1991 alone, a dose of 150 mg/L was applied to the entire pond, and doses of 46 and 74 mg/L were applied to the shoreline. Due to the originally high pH levels in the stormwater at above 9, and to avoid creating a pH shock, only minor pH changes were made, where alum's optimal pH range is around 6 (Babin et al. 1992 and 1989). This pH factor in combination with lower temperatures in the natural open water environment would result in lower dissolution of the alum. This is suspected to have resulted in the solid alum settling and remaining in the stormwater sediment, becoming a source of sulphate pollution and electron acceptor source for SRB growth and activity. Bearspaw stormwater retention pond, in contrast to Valencia stormwater retention pond, does not have records of being utilized as an experimental site, with no applications of chemicals applied at Valencia, and significantly lower levels of sulphate. This further suggests that the alum has become a pollution source for the lake and ultimately the source of sulphide and hydrogen sulphide production so far.

The reason that the sulphide concentrations become more comparable over the sampling period in the summer is that the pond increases operation during this period with increased water flow and is more open to the atmosphere to become a more aerobic environment, less ideal for sulphate reduction and sulphide production. These sulphide results are most likely underestimated. The reason behind this is the nature of the sampling method. It was difficult to maintain anaerobic conditions for the water during the process to be stored in bottles and sent to the labs. Although the zinc hydroxide and acetate preservatives, used to raise the pH levels and fixing sulphide into solution, were used in transporting the samples to the commercial lab, the samples were still exposed to open air and oxygen when being poured into the samples bottles, most likely oxidizing the sulphides that were present.

In terms of nitrate and nitrites, as seen in Figure 3-8, nitrate levels were found to be greater in Bearspaw pond than in Valencia pond. With the higher concentrations and presence of nitrate and nitrite, this would discourage SRB activity and sulphide production due to being outcompeted or due to the inhibitory effect of nitrite on SRB (Wolfe et al. 1994) or the stimulation of NR-SOB that reduce nitrates and oxidize sulphide (Hitzman and Sperl 1994, Loveley and Chapelle 1995, Smith 2007). This may

be an indicator as to why Bearspaw pond has lower concentrations of sulphide than Valencia pond. The reason that the nitrate and nitrite levels in Bearspaw were higher than that of Valencia might be the stormwater inlet source. One of Bearspaw's inlet comes from an open water stream that covers a larger area and basin, the opportunities for nitrate sources to be fed in are greater than that of Valencia. Another possibility is the use of commercial bioaugmentation products, containing concentrated suspensions of *Nitrosomonas spp.* and *Nitrobactor spp.* bacteria and required nutrients, frequently applied to the Bearspaw stormwater retention pond (City of Edmonton 2012). The fluctuations in nitrate and nitrite levels in Bearspaw may be an indication of the nitrate reducing activity in the stormwater pond as concentrations are depleted and renewed due to microbial activity and rainfall events respectively.

The data corresponding to the organic content in the stormwater in both stormwater retention ponds, found in Figures 3-9 for BOD, 3-10 for COD, and 3-11 for TOC, suggest that the majority of organic degradation occurs in the late spring and early summer months. Both ponds showed higher concentrations of BOD and COD at the beginning of their respective field studies, and decreasing significantly after the first field samples were taken. This is due to the influx of available organics into the stormwater retention pond system from spring snowmelt (Thomas et al. 2009). This also corresponds to the decrease in sulphide concentrations displayed in Figure 3-7. COD measurements were greater than the BOD values at each measured sampling period and pond location, indicating the complex organics available in the stormwater. However, it should be noted that halide ions, especially chloride ions, can cause interference in COD measurements by reacting with the silver ions in the reagents for the test and reduce oxidizing potential (APHA, AWWA, and WEF 2011). The measured conductivity levels, discussed later, may be an indicator that there could have been interference that could skew the readings and results to be overestimated (APHA, AWWA, and WEF 2011).

Looking specifically at alkalinity, anaerobic degradation and anaerobic microbial activity has been shown to increase alkalinity; sulphate reduction producing 1.98 mol of alkalinity per mol of sulphate reduced and nitrate reduction producing 0.99 mol of alkalinity per mol of N denitrified (Chen and Wang 1999, Thomas et al. 2009).

The increase in available organic matter from spring snowmelt and lower dissolved oxygen content from winter ice cover, encouraging anaerobic degradation, as shown with sulphate and sulphide, and nitrate and nitrite results, justify the higher levels of alkalinity found at the beginning of the field study at both respective stormwater retention ponds.

The alkalinity and pH of both ponds is also affected by the presence of algae and algae blooms, where a dissolved carbon dioxide level is consumed by algae (Sawyer et al. 2003). Algae will consume CO_2 in the pond as a means to complete photosynthetic activities. This will shift the equilibrium of carbonate and bicarbonate in the water, and overall, the type of alkalinity shifts from bicarbonate to carbonate and carbonate to hydroxide (Sawyer et al. 2003). The presence of aerobic degradation can also be a source of alkalinity decrease, although, it's overall effect can be minimal. In general, the shifts in alkalinity found during this field study show shifts in the anaerobic and aerobic degradation activity during the summer operational months, these factors being affected by the availability of organic matter and electron donors or the presence of algae. Algae growth was observed at both field study locations during late July and August. This algae growth consumes carbon dioxide (CO_2) which attributes to acidity of the water. This shift in equilibrium and alkalinity causes the pH of the system to rise to levels as high as 10 and sometimes 11 (Sawyer et al. 2003).

The distinct differences in conductivity measurements are interesting in the two stormwater retention ponds over the course of the field study conducted. The consistency in higher conductivity levels in Valencia stormwater retention pond indicates high levels of total dissolved solids in the stormwater throughout the summer operational season of the stormwater pond. Valencia's conductivity levels are seemingly unaffected by factors such as rainfall or inflow events as suggested by the data presented with sulphates, or the biological activity as indicated with sulphate and sulphide, nitrate and nitrite data. In comparison, Bearspaw showed significant changes in measured conductivity throughout the field study, levelling off to a more consistent level by the end of the study. The significant changes as indicated at the Bearspaw site were consistent in the effects of rainfall events, as reflected by the sulphates, alkalinity, and total phosphorus data, or the biological activity as indicated by nitrate and nitrite levels, BOD, and COD levels. The differences as shown by these figures are

an indicator that there is a significant source for dissolved solids that is present at Valencia, that does not exist at Bearspaw.

3.5 Conclusions and Recommendations

This comprehensive field study provided a comparison of the variability found between two stormwater retention ponds within the City of Edmonton; Valencia and Bearspaw. The results of the field study also showed significant differences and trends that explained the reasons for increased sulphide and hydrogen sulphide production found in Valencia stormwater retention pond, compared to Bearspaw stormwater retention pond.

Sulphide concentrations were found to be higher in the early spring and summer months of the field study, with Valencia exhibiting significantly higher concentrations than that of Bearspaw. It was also found that the electron acceptors in both Valencia and Bearspaw stormwater retention pond varied in concentration and availability. Sulphate levels, the terminal electron acceptor for SRB that ultimately produce sulphide ions and hydrogen sulphide, were higher in Valencia stormwater pond than that of Bearspaw stormwater retention pond. On the contrary, the nitrate and nitrite concentrations, an electron acceptor with higher redox potential than that of sulphate, was found to be more abundant in Bearspaw stormwater retention pond than that of Valencia stormwater retention pond. The BOD and TOC data showed that there were greater sources of electron donors and carbon sources for biological activity at the beginning of the field study during the late spring and early summer months, reducing over the course of the field study, most likely causing a limiting factor in microbial activity.

To improve upon this study, the field sampling could begin at an earlier time to capture a wider range of changes, trends, and behaviours that encompass the earlier spring melt and influx of organic matter. It is also recommended, if possible, to look for year-round sampling and analysis to give a better understanding of seasonal variations and changes. Another suggestion is to incorporate another stormwater retention pond in the study to check if trends are strictly specific to each pond or if there are possible similarities.

Overall, the field study gave an in-depth overview of the conditions found in stormwater ponds. Results showed evidence of influential differences in water quality that would lead to varying biological activities.

3.5 Chapter 3 References

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Chapter 4. FACTORIAL DESIGNED STORMWATER RETENTION POND MESOCOSMS TESTING THE EFFECTS OF NITRATE ADDITION, ACETATE ADDITION, AND WATER MEDIUM

4.1 Introduction

The field study conducted and detailed in *Chapter 3: Field Study of Two City of Edmonton Stormwater Retention Ponds*, showed strong evidence that sulphide was being produced biogenically in the Valencia stormwater retention pond site due to the high concentrations of sulphate in the stormwater and the high numbers of sulphate reducing bacteria (SRB) in the sediment. This chapter is focused on determining what factors affect the production of sulphide ions. More specifically, a factorial design experiment was conducted, testing the effect and combination of effects of nitrate addition, acetate addition, and water medium used. Ultimately, what factor can be altered in the stormwater retention pond environment to suppress and inhibit the activity of SRB.

Nitrate was chosen as a factor to test for several reasons. First, from *Chapter 3: Field Study of Two City of Edmonton Stormwater Retention Ponds*, it was found that one particular difference between Valencia stormwater retention pond from Bearspaw stormwater retention pond, was that the former had lower concentrations of nitrate and nitrite than the latter, where the former had higher reported levels of hydrogen sulphide. Second, nitrate has a higher redox potential than sulphate on the redox potential ladder, and is a more attractive electron acceptor to use than sulphate (Sawyer et al. 2003, Achtnich et al. 1995). Third, the addition of nitrate has been found to be an indirect inhibitor against sulphide production, either producing nitrite, an SRB inhibitor, through its reduction by NRB and NR-SOB, or having NR-SOB oxidize the reduced sulphur species (Greene et al. 2003, Wolfe et al. 1994). Finally, nitrate has already been used successfully in field operations to suppress sulphide production (Jenneman et al. 1999, Telang et al. 1997). Acetate was also chosen as a factor to understand its effect on the microbial species in the stormwater sediment. It was used to determine whether the addition of an available electron donor source would further stimulate SRB, or with the addition of nitrate, would allow NRB to out-compete SRB (Achtnich et al. 1995). Water medium was tested to determine whether or not there are potentially any other factors in the water medium that are affecting SRB activity.

In this chapter, the details of the factorial design mesocosm study will be discussed. The results of the experiment give a better idea of the biological activity

that exists in the stormwater retention pond, and how the factors of adding nitrate, acetate, or changing the water medium, and any combination thereof, have on this unique environment.

4.2 Materials and Methodology

4.2.1 Field Samples

The sediment and stormwater utilized in this study was collected from the Valencia stormwater retention pond during the final sampling date at the site for the field study on September 27, 2012. The sediment was stored with a water cap in a 20L container and the stormwater was stored in 20L containers in a 4°C refrigerated room until use. Please review the Sampling section on page 12 of *Chapter 3: Field Study of Two City of Edmonton Stormwater Retention Ponds* to review details of sampling techniques.

4.2.2 Anaerobic Mineral Media

Anaerobic mineral media used to test for the optimum growth of anaerobic bacteria and cultures followed the composition as utilized by Edward and Grbić-Galić (1992).

4.2.3 Mesocosms

The design of the mesocosm was centred on simulating the conditions of the interphase of the stormwater and the sediment of the stormwater retention pond and to accommodate for the volume of samples needed for analysis.

The nitrate solution was made with a stock of 1000 mM sodium nitrate prepared the day before being applied to ensure full dissolution. The final nitrate concentration for the mesocosms was set at 10 mM for several reasons. First, industry applications in certain fields, mainly sour gas wells and other petroleum industries utilize a similar concentration or less, depending on their design and structure

(Davidova et al. 2001). Secondly, nitrate concentrations were required to stay at a reasonable level as per regulatory guidelines. Third, to add nitrate, in the form of a sodium nitrate solution, an ionic solute, may result in osmotic stress as a non-specific inhibitory mechanism, instead of focusing on the effects of nitrate itself (He et al.2010).

The acetate solution was made with a stock of 1000 mM sodium acetate prepared the day before application to ensure full dissolution.

The sediments and water media were all purged with Praxair 5.0 purity nitrogen gas through a 0.22µm filter and sterilized syringe needle for a minimum of 30 minutes before being placed in the anaerobic chamber and used to prepare the mesocosms.

Mesocosms were prepared in 2 L Pyrex bottles, containing approximately 0.8L of sediment and 1.2 L of water media, with the remaining volume in the bottle as headspace. Please review Figure 4-1 for this set up of the mesocosms.

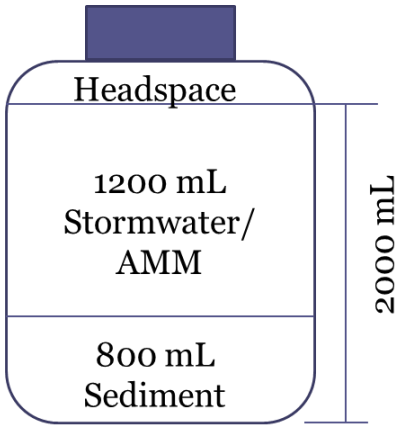

Figure 4-1 – Mesocosm set up utilizing 2000 mL pyrex bottles, each containing approximately 800 mL of stormwater sediment, 1200 mL of water media, and 275 mL of headspace

Each mesocosm was then amended with their respective chemical constituents as described by their factorial design; 0 or 10mM of Nitrate, Acetate, or both. The mesocosms were left to sit in the anaerobic chamber at room temperature for four weeks covered by opaque black garbage bags from Jan 22, 2013 to February 26, 2013. The mesocosms were also carefully and slowly mixed by inversion on a weekly basis.

At the beginning of and following the experimental period, water samples were taken to measure the initial and final concentrations of sulphate, sulphide, nitrate, nitrite, total phosphorus, biochemical oxygen demand, and chemical oxygen demand. Sediment samples were also taken at the same time to analyze for the microbial community. The overall factorial design is described in the following section.

4.2.4 Factorial Design for Testing Sulphide Production with Nitrate Addition, Acetate Addition, and Water Media

The mesocosm experiment was conducted in the fashion of a factorial design experiment (Warpole et al. 2007). The factorial design method was used to test three factors for the significance and effect on sulphide production; nitrate addition, organic carbon addition, and water media and their potential combined effects. Table 4-1, as shown below, summarizes the number of experimental conditions.

Table 4-1 – Factorial design experiment scenario break down utilizing 1 or 10 mM nitrate, 0 or 10 mM acetate, and stormwater or anaerobic mineral media

	Nitrate		Acetate		Water Media	
	Code	Actual Value (mM)	Code	Actual Value (mM)	Code	Actual Value
1	-	0	-	0	-	Stormwater
2	+	10	-	0	-	Stormwater
3	-	0	+	10	-	Stormwater
4	+	10	+	10	-	Stormwater
5	-	0	-	0	+	AMM
6	+	10	-	0	+	AMM
7	-	0	+	10	+	AMM
8	+	10	+	10	+	AMM

4.2.5 Water Chemistry Analysis

SULPHATE Sulphate levels measured by the University of Alberta Biogeochemical Analytical Service Laboratory used the Ion Chromatography method with Dionex DX600 and Dionex ICS 2500 units with the EPA 300.1 (Modified) Method (1997).

SULPHIDE Sulphide analysis was completed using an Thermo Scientific 9616BNWP silver/sulfide combination electrode as an ion specific probe. The standard test method followed the ASTM D4658 method (2009).

NITRATE, NITRITE, TOTAL PHOSPHORUS Samples were sent to the University of Alberta Biogeochemical Analytical Service Laboratory were analyzed using a Flow Injection Analysis method with a Lachat QuikChem 8500 FIA automated ion analyzer as outlined by the Standard Methods for Water and Wastewater Analysis SM 4500-NO3-I and SM 4500-P-H (APHA, AWWA, and WEF 1999).

DISSOLVED ORGANIC CARBON Dissolved Organic Compounds (DOC) samples were prepared and filtered using 0.45µm filters. DOC was measured using a Shimadzu TOC-L CPH TOC Analyzer utilizing the high temperature combustion method as described in the Standard Methods for Water and Wastewater Analysis (APHA, AWWA, and WEF 2011).

BIOCHEMICAL OXYGEN DEMAND Biochemical Oxygen Demand (BOD) was measured using the BOD-5 Test as outlined by the Standard Methods for Water and Wastewater Analysis (APHA, AWWA, and WEF 2011). The Azide-Modification Method was used to measure the dissolved oxygen concentrations at the beginning and end of the five day test. This method was used instead of an oxygen probe because of the accuracy of Azide-Modification method. Total, carbonaceous BOD was measured for field samples, using unfiltered samples and nitrification inhibitors during the test. The first few sampling periods for both ponds required dilutions, however, over time, no dilutions were used to measure for the BOD-5 Test.

CHEMICAL OXYGEN DEMAND Chemical Oxygen Demand (COD) was measured using the Closed-Reflux Colorimetric Method as outlined in the Standard Methods for Water and Wastewater Analysis (APHA, AWWA, and WEF 2011). Total COD was measured instead of soluble COD as the water had low levels of suspended solids, and believed to be negligible. 2.5mL of samples were mixed with 1.5mL of digestion solution and 3.5mL of silver sulphate sulphuric acid solution and digested for 2 hours in a digester and then measured for absorbance at a wavelength of 600nm in a spectrophotometer.

4.2.6 Microbiology

DNA EXTRACTION DNA was extracted from the mesocosm sediment samples using the MoBio PowerSOIL DNA Extraction Kit. This specific DNA extraction kit was used due to the matrix of the environmental samples. The advantage of using this specific kit is the process of removing PCR inhibitors and humic substances that interfere with molecular biology analysis. 500µL of sample were used for each DNA extraction. The DNA samples were then stored at -20°C, until used for further analysis.

qPCR DNA samples were analyzed and optimized for PCR conditions. Three groups of bacteria were analyzed; total bacteria, sulphate reducing bacteria, and nitrate reducing bacteria. The primer sets used to analyze for these three groups of bacteria were rpoB, dsrB and nosZ2 respectively. The details of the primers and conditions used can be reviewed in Table 3-2 and 3-3 in Chapter 3. The qPCR experiments were conducted using the Bio Rad I-Cycler in 96 well plates. The qPCR reactions were done in 20 µL reactions, consisting of 10 µL of Bio Rad SsoFast EvaGreen Supermix, 0.05 µL each of the forward and reverse primers of interest, 1 µL of DNA template and 8.9 µL of water. The results of the qPCR were analyzed and managed using the Bio Rad CFX Manager 3.0 Software.

4.3 Results and Discussion

The results of the factorial design mesocosms are focused on the goal of the factorial design experiments, being the factors that affect sulphide production. The first set of results will be the sulphide concentrations, along with an analysis of the factorial design experiment results, as determined through sulphide generation. Following, results of the electron donor, sulphate and its concentrations. The measured results of the nitrate and nitrite concentrations are then shown as a competing electron donor and reduced form pair to sulphate reduction, followed by dissolved organic carbon concentrations as a measurement of available electron donors and carbon source for biological activity. Total phosphorus levels, as a nutrient source follows, along with BOD and COD as further indicators of biological activity. Finally an analysis of microbiology using qPCR to quantify specific groups of bacteria is given.

4.3.1 Impact of Nitrate Addition, Acetate Addition, and Water Media on Sulphide Production

To understand the effect of each factor on the production of sulphide and the overall influence in this factorial design study, the focus will be on the sulphide concentrations before and after the experiment. The initial and final sulphide concentrations in each mesocosm can be seen in Figure 4-2 below.

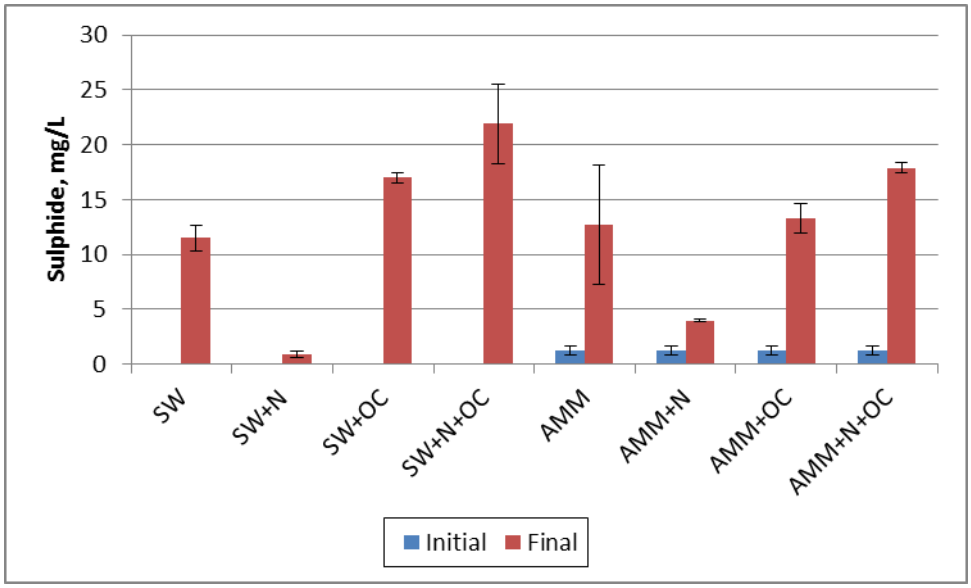

Figure 4-2 – Initial and final sulphide concentrations measured in each respective mesocosm set-up. Data points represent the average of water samples collected in duplicate mesocosms and error bars represent plus and minus one standard deviation

Where SW = Stormwater, N = Nitrate, OC = Organic Carbon/Acetate, and AMM = Anaerobic Mineral Media

The initial concentrations of the sulphide were found to be negligibly low for the mesocosms that used stormwater as its water media. The mesocosms that utilized the AMM water media had a starting concentration of 1.26 mg/L, due to the presence of FeS in the AMM. Sulphide ion concentrations were found to have increased in all mesocosms after the four week experimental period. In both water medium cases where no nitrate or organic carbon source was added, approximately 12 mg/L of sulphide was produced. Where the organic carbon, acetate, was introduced into the mesocosm, in both cases of stormwater or anaerobic mineral media water amendment, sulphide production increased to levels greater than without acetate addition. In stormwater, sulphide production reached to 17 mg/L and in anaerobic mineral media, it reached 12.5 mg/L. For the mesocosms that included 10 mM of nitrate and acetate additions, sulphide levels increased the most in both respective water media source groups. For stormwater, sulphide levels were the highest at approximately 22.5 mg/L and for anaerobic mineral media, it had reached 17 mg/L. It was originally assumed that with both nitrate addition, producing an anoxic environment and a more optimal electron source, and the added carbon source usually added to assist nitrate reducing bacteria, NRB would thrive and out-compete the SRB

and reduce sulphide production. Although the results show otherwise, the time period of four weeks for this experiment may indicate that it was too long and allowed SRB to thrive after the nitrate was consumed. Another possibility, however, is that the sediment is originally highly populated with SRB. Some of the species being facultative can survive in anoxic environments and even aerobic environments and have the ability to use nitrates as an electron donor, therefore has the potential that NRB were out-competed in the mesocosms (Ehrlich 2005).

The mesocosms that included nitrate only as an amendment both, under stormwater and anaerobic mineral media conditions produced the lowest concentrations of sulphide after the four week period. The anaerobic mineral media with nitrate addition mesocosm produced approximately 3mg/L of sulphides and the stormwater mesocosm with added nitrates produced the lowest concentration of sulphides of any mesocosm with approximately 1 mg/L. This is due to the preferred utilization of nitrate as an electron acceptor before sulphate, according to the "electron ladder" or "electron tower" concept (Metcalf and Eddy 2003). Furthermore, due to the utilization of nitrate as its electron acceptor, the formation of nitrite, nitrate's reduced and intermediate form, acts as an inhibitor to SRB (Wolfe et al. 1994).

The overall equation of sulphide generation as a result of the factorial design experiment, as shown below, summarizes the relationships of individual and combined factors that were tested in the experiment. This equation also determines the factors, or combinations of factors that would discourage sulphide generation.

$$
\begin{aligned}
&Where,\\
&N = nitrate\ factor\\
&OC = organic\ carbon\ (acetate) factor\\
&W = water\ factor\ (either\ stormwater\ or\ AMM)
\end{aligned}
$$

$$
\begin{aligned}
Sulphide\ Generation\\
&= 11.765\\
&+ \frac{1}{2}\left(-2.447\,N + 10.231\,OC - 2.129\,W + 7.210\,NOC + 0.389\,NW\right.\\
&\left. - 2.995\,OCW - 0.555\,NOCW\right)
\end{aligned}
$$

The first term, 11.765, as summarized in Table 4-2, represents the mean. This term represents the average sulphide generation in the mesocosm experimental set-up, where the 0^{th} level of factors was considered. More specifically, this would mean that if 5 mM of nitrates, 5 mM of acetates, and the water source was a 50% mix of the AMM and stormwater, the sulphide generated in the mesocosm, over the same experimental time frame, would be 11.765 mg/L. The coefficients associated with each defined variable, and combination of defined variables, represent the overall effect of that single variable or combined variable effect in the experiment. The presence of a positive sign in front of the term represents the variable or combined variable having a positive effect in terms of sulphide production. The presence of a negative sign in front of the term refers to suppression in sulphide generation. The greater the absolute value of the coefficient of the term, the greater the influence of that variable or variable combination in determining sulphide generation. For instance, the addition of organic carbon, specifically acetate, independently, has a significant overall effect on sulphide production with the coefficient of +10.231. Conversely, nitrate, independently, has a suppressing effect on sulphide generation. Finally, the combination of nitrate and organic carbon (acetate) gives an overall positive production in sulphide with a coefficient of +7.210.

Table 4-2 – Summary of factorial design results indicating coefficient/influence of each factor and their respective standard deviation

Factor[1]	Coefficient/Influence	Standard Deviation
Base	11.765	1.394
N	-2.447	0.086
OC	10.231	0.258
W	-2.129	13.251
N+OC	7.210	1.988
N+W	0.389	0.005
OC+W	-2.995	1.704
N+OC+W	-0.555	0.211

[1]Where N=Nitrate Addition, OC=Organic Carbon Addition, W=Water Media using AMM

Referencing back to and using the information from Table 4-1, the experimental set-up of the factorial design, a predicted value and overall effect can be predicted if the same conditions were applied again with specific factors being tested. More specifically, if we applied the + or – condition of the factor to the experiment; 10 or 0 mM addition of nitrate, 10 or 0 mM addition of acetate, and AMM or stormwater, respectively. With respect to this, and the objective of finding the factors that would suppress sulphide generation the most, a further analysis of the equation found is necessary.

Through the substitution of replacing the variables in the formula with a + or – 1, regarding the addition or absence of nitrate and or acetate and the type of water medium used, the overall concentration of sulphide generated in the mesocosm can be predicted. Using trial and error of each unique case and combination of possible factorial set-ups, it was found that regardless of condition, there would be some form of sulphide generation with the conditions of the experiment as is. Overall, this gives a possible solution to the sulphide problem in the stormwater retention pond via application of nitrate injection, keeping in mind to not include an organic carbon source while doing so.

The standard error can be seen in Table 4-2. Overall, these values are quite low, and give a strong degree of confidence in the results and the influence of the factors studied.

It should be noted, that although there were clear influences of factors that have been calculated and analyzed, and with low standard error values, there are limitations of these results. Firstly, the mesocosm set up, although is composed of a large amount of stormwater sediment and water media, is a small scale representation of the water and sediment interphase in the stormwater retention pond. It does not fully represent the overall system that comprises of a significant depth of stormwater sediment, approximately 1.6 to 2.1 m of stormwater above the retention pond, and the open atmosphere that the pond is exposed to and the overall size and influence of each media phase. Secondly, the source of the stormwater sediment and the stormwater for the set-up of this experiment were from the outlet of Valencia stormwater retention pond, and as seen with the data from Chapter 3: Field Study, there is variability in stormwater quality and sediment microbial communities both spatially and temporally. This means that the results of this experiment works in the case of the samples taken, however, do not necessarily reflect the universal influence of factors studied on sulphide generation for the entire stormwater retention pond, and not necessarily all stormwater retention ponds in the City of Edmonton. Thirdly, as the experiment was performed under anaerobic conditions, it does not fully represent the overall effect of the factors in a full scale. As the stormwater pond is open to the atmosphere, it could potentially be under anaerobic conditions already, due to the short water depth and water column. Fourth, the experiment was carried out over the period of four weeks, with samples and analysis of these samples taken at the beginning and at the end. Therefore, we do not have enough information to make conclusive inferences as to the influence of the factors at any point in-between these two time points.

4.3.2 Electron Acceptors: Sulphate, Nitrate and Nitrite

Electron acceptors were measured to show further detail, support, and evidence of the sulphide results and the results of the factorial design experiment. Sulphate concentrations in each mesocosm will be shown and discussed first due to its relationship as the electron acceptor for sulphate reducing bacteria and producing sulphide. Following, nitrate and nitrite will be shown and discussed to show the differences that the addition of nitrate as a factor made on the mesocosms. The initial and final concentrations of sulphate of each mesocosm can be seen in Figure 4-3 below.

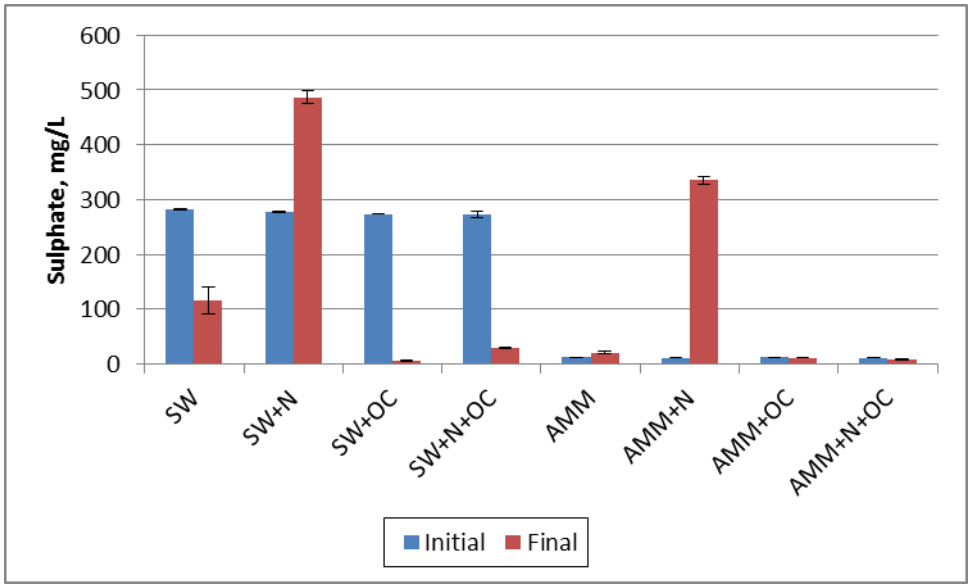

Figure 4-3 – Initial and final sulphate concentrations measured in each respective mesocosm set-up. Data points represent the average of water samples collected in duplicate mesocosms and error bars represent plus and minus one standard deviation

Where SW = Stormwater, N = Nitrate, OC = Organic Carbon/Acetate, and AMM = Anaerobic Mineral Media

The mesocosms that were created using stormwater as the water media start with significantly higher levels of sulphate at approximately 275 mg/L. The mesocosms that utilized the anaerobic mineral media as the water media instead had started with lower concentrations of sulphate with approximately 10 mg/L. In the cases where stormwater was used, sulphate concentrations dropped significantly for the mesocosms that had just stormwater, stormwater and acetate, and stormwater and

nitrate and acetate. However, in the instance where stormwater was amended with 10mM of nitrate, the sulphate concentration had increased by the final sampling, to concentrations greater than that of which was present in the initial measurement. For the mesocosms created using anaerobic mineral media, sulphate levels stayed relatively the same after the four week experimental period for those composed of just anaerobic mineral media, anaerobic mineral media and acetate, and anaerobic mineral media and nitrate and acetate. The sulphate concentration for the mesocosm composed of anaerobic mineral media water and nitrate however, increased significantly from the 10mg/L previously mentioned, to approximately 325mg/L.

For the mesocosms where there were decreases in sulphate concentrations, it indicates sulphate reduction activity and sulphide ion or reduced sulphur species production. This is further reinforced by the sulphide measurements in Figure 4-2 above.

For the mesocosms with increases in sulphate concentration however, specifically those with only nitrate addition, indicate sulphur oxidizing activity. This is where sulphide ions and other reduced sulphur species are being transformed into sulphate, the most oxidized form of sulphur (Telang et al. 1997, Larsen et al. 2004). The reasons that this could be happening in comparison to the other mesocosms is that with nitrate amendment, the system became an anoxic environment versus an anaerobic one, where both are in the absence of oxygen, the presence of nitrate promotes by-products of carbon dioxide, water, and nitrogen gas (Sawyer et al. 2003). In this case, nitrate reducing - sulphur oxidizing bacteria have an environment that allows them to thrive to oxidize sulphide and other reduced sulphur compounds into sulphate (Hitzman and Sperl 1994, Lovely and Chapelle 1995, Smith 1997).

From these results, and the sulphate results as shown in the previous section in Figure 3-6, there are two strong trends that can be concluded. The first conclusion that can be derived is that there is sulphate reduction occurring. Under anaerobic conditions, at the water and sediment interphase, at which is most likely the present condition at the stormwater retention pond, sulphate is being reduced to its various reduced species, and a significant enough portion as sulphide ions, that can react and change into hydrogen sulphide. The second conclusion that can be made is that nitrate addition can sufficiently suppress sulphide production, and does so better without the

addition of a carbon source. In actuality, the presence of excess electron donors accelerates the sulphate reduction process, similarily, Achtnich et al. (1995) found the inhibitory effect was relieved once a suitable electron donor source was introduced.

Nitrate and nitrite were measured together as an electron donor pairing. It was monitored specifically as well due to it being one of the studied factors in the mesocosms as an amendment to test whether it would have an effect or not on the sulphate reduction and sulphide generation. The initial and final nitrate and nitrite concentrations for the factorial design mesocosm experiment can be found below in Figure 4-4.

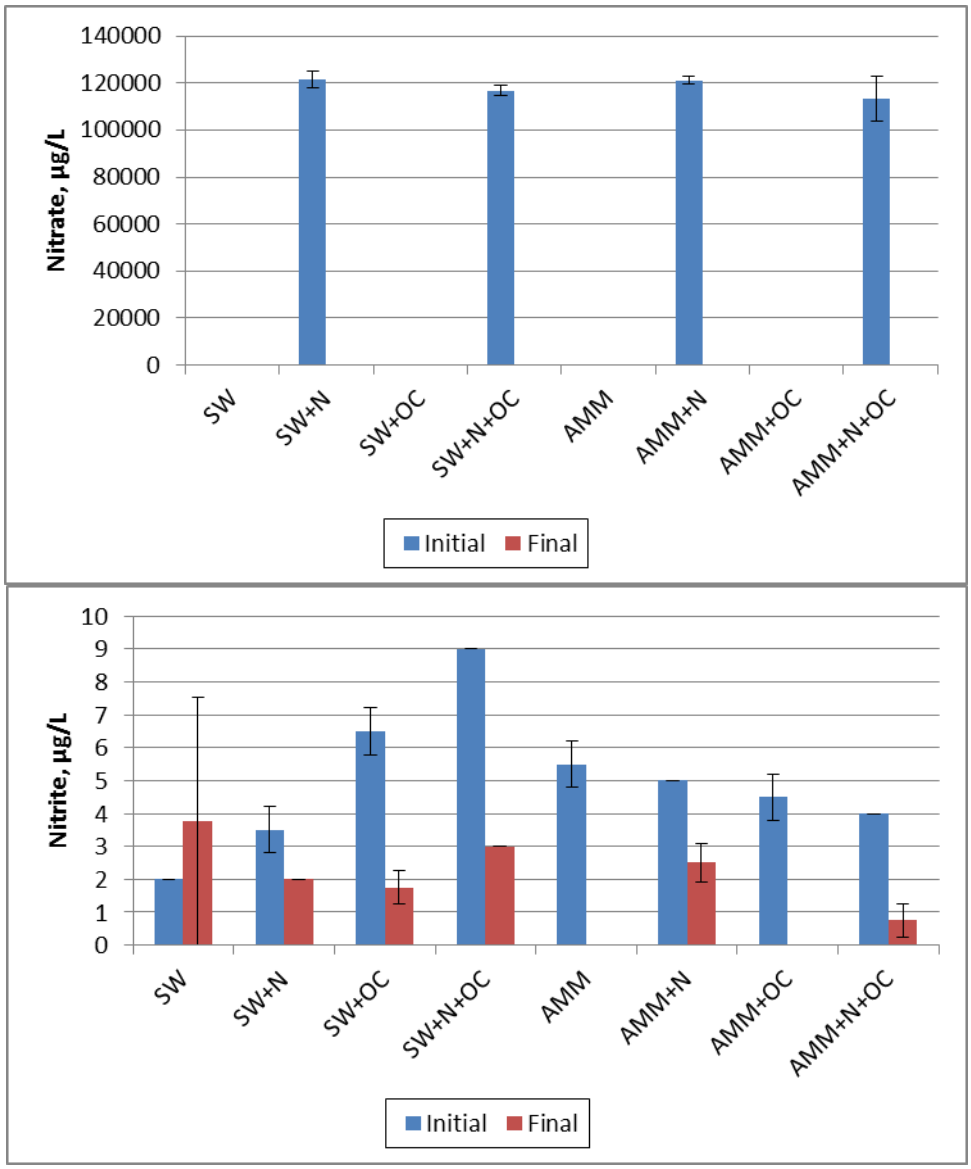

Figure 4-4 – Initial and final nitrate and nitrite concentrations measured in each respective mesocosm set-up. Data points represent the average of water samples collected in duplicate mesocosms and error bars represent plus and minus one standard deviation

Where SW = Stormwater, N = Nitrate, OC = Organic Carbon/Acetate, and AMM = Anaerobic Mineral Media

In the mesocosms where excess nitrate was artificially added, nitrate concentrations were significantly reduced in the four week experimental period. Concentrations started at approximately 125000 µg/L and were found to decrease to 5 µg/L or lower. It should be noted that there is a discrepancy in the initial measured value of nitrates, and has been investigated however with no conclusive evidence as to

why it had occurred. In the mesocosms without nitrate amendment, no notable changes and remained at near undetectable levels.

The mesocosms with organic carbon amended to them also showed decreases in overall nitrate levels. In these cases with acetate added, nitrite levels also showed reduction in concentrations, more so than the mesocosms that had nitrate addition. Whether NRB or SRB that utilized nitrate were using the organic carbon source could not be distinguished.

The mesocosms that utilized anaerobic mineral media water as its water phase appeared to show the highest reduction of nitrate and nitrite compounds, in comparison to the mesocoms that used stormwater. This may be an indication that overall, the stormwater in Valencia stormwater retention pond is not providing an environment that allows for nitrate reducing bacteria species to survive as well or outcompete sulphate reducing organisms. Another reason for this could be that SRB can be adaptive to more extreme conditions (Postgate 1984).

Considering the sulphate and sulphide data as well, the level of nitrate and nitrites that were present, without amendment, were too low to have an effect of inhibition on SRB and sulphate reducing activity.

This experiment could have been improved by including an air phase analysis to indicate the chemical composition of the headspace change over time. Another improvement to the experiment would have been to reduce the time frame that the experiment was conducted, or to have at least taken some intermittent samples, such as weekly samples. The shorter experimental time or intermittent samples would have helped assess if NRB activity was in actuality out competing with SRB activity, and also at what point or concentration of nitrates and or nitrites does this begin to change. Conversely, this could also indicate that the present environment, regardless of the addition of nitrate, is dominated by SRB and sulphate reduction activity.

4.3.3 Organics: Dissolved Organic Carbon, Biochemical Oxygen Demand, and Chemical Oxygen Demand

With the addition of acetate as an organic carbon source, the initial and final organic content found in the mesocosms were measured. This was done using three parameters; Dissolved Organic Carbon (DOC), Biochemical Oxygen Demand (BOD), and Chemical Oxygen Demand (COD).

DOC was another specific factor studied for its influence on sulphide production in the factorial design experiment through the addition or absence of amendment of 10 mM acetate. The initial and final concentrations of DOC act as an indicator of the biological activity within the mesocosms as the utilization of a carbon source as electron donors. The initial and final dissolved organic carbon content found in each respective mesocosm can be found in Figure 4-5 below.

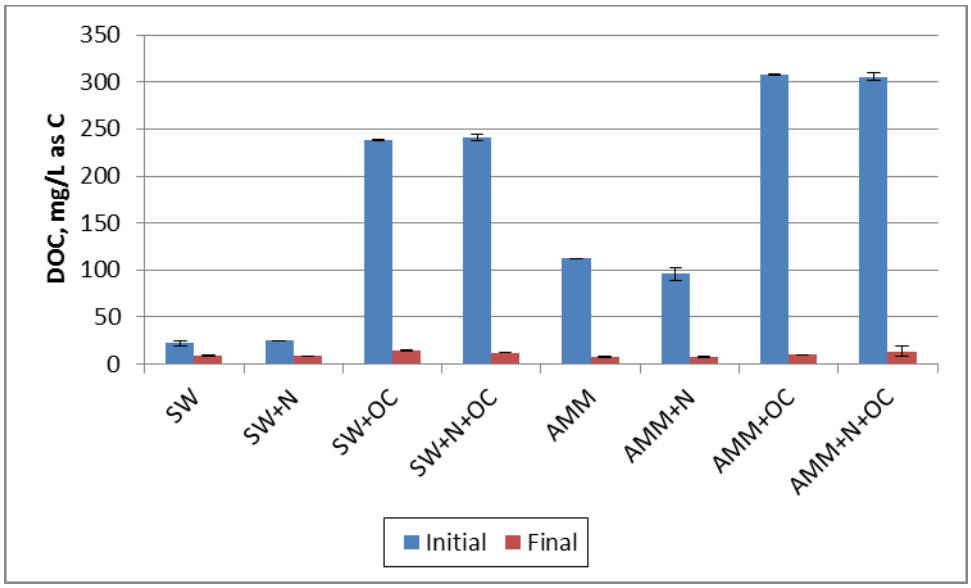

Figure 4-5 – Initial and final dissolved organic carbon concentrations measured in each respective mesocosm set-up. Data points represent the average of water samples collected in duplicate mesocosms and error bars represent plus and minus one standard deviation

Where SW = Stormwater, N = Nitrate, OC = Organic Carbon/Acetate, and AMM = Anaerobic Mineral Media

The mesocosms that utilized stormwater for its water media were found to have lower concentrations of DOC compared to their respective AMM water media counterparts. In all mesocosm environments, regardless of initial concentration of

DOC, levels dropped between 8 and 15 mg/L as C at the end of the experiment. The mesocosms that were amended with acetate addition showed the greatest consumption of DOC, with the greater the initial measured level of DOC, the greater the consumption of DOC by the end of the experiment.

Coupled with the sulphate and sulphide data, and the nitrate and nitrite data, the consumption of DOC in the mesocosms, was used for biological activity, specifically for both sulphate and nitrate reduction. To what extent which reaction the organic carbon is being utilized and coupled for is unknown.

Due to the amount of reduction in DOC in all mesocosm cases, it can be deducted that available carbon source is a limiting factor for biological activity. However, the results do not indicate if there is an ideal concentration that can be utilized for nitrate reduction, over sulphate reduction. This may be another factor to further investigate in future experiments, to what extent available carbon source is limiting the microbial activity, or even the kinetics of the utilization of the available carbon source.

BOD, as a general and common indicator of water quality, was measured as another indicator of biological activity, specifically with degradation. BOD was also another parameter that was monitored during the field study as mentioned in Chapter 3. The initial and final BOD_5 test results are shown below in Figure 4-6.

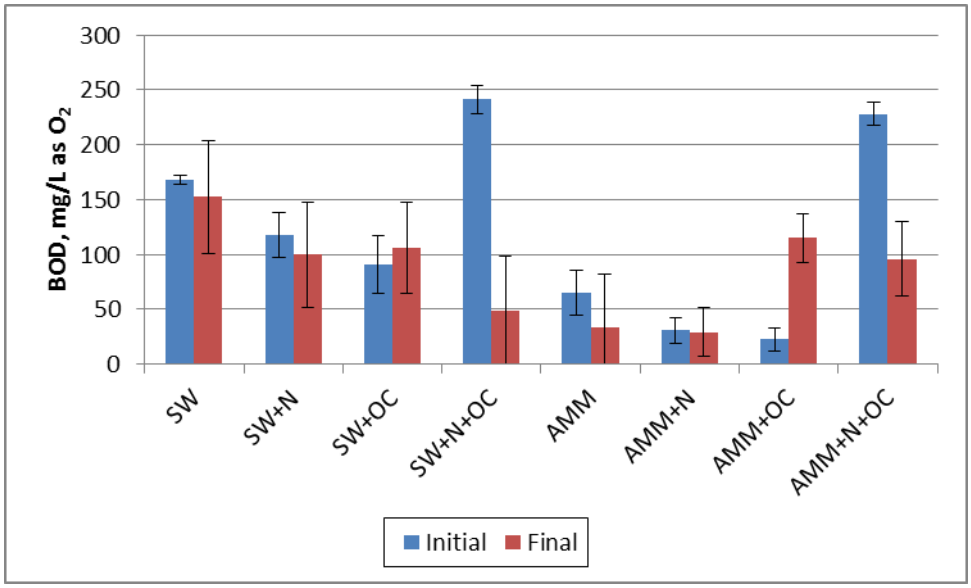

Figure 4-6 – Initial and final Biochemical oxygen demand concentrations measured in each respective mesocosm set-up. Data points represent the average of water samples collected in duplicate mesocosms and error bars represent plus and minus one standard deviation

Where SW = Stormwater, N = Nitrate, OC = Organic Carbon/Acetate, and AMM = Anaerobic Mineral Media

The BOD measured in the mesocosms that utilized stormwater as their water medium were found to have greater initial concentrations compared to the AMM counterparts.

In both water medium mesocosms, where no amendments were made, there was nitrate addition, and both nitrate and acetate addition, BOD was found to decrease by the end of the experimental period. It was also found that the mesocosms that had only nitrate added had both lower initial and final BOD concentrations than the mesocosms that had no amendments. The greatest decreases were, as can be seen in the figure above, found to be in both cases where the mesocosms were amended with both nitrate and acetate. In the mesocosms that were amended with only acetate addition both were found to have the lowest initial BOD concentration, and resulted in BOD increases at the end of the experimental period.

In conjunction with the sulphate and sulphide data, and nitrate and nitrite data, and DOC data, the BOD data indicates that the reduction of BOD is facilitated by both the nitrate reducing and sulphate reducing microorganisms. The data also indicates that the combined activity of both nitrate reducing and sulphate reducing

microorganisms provides the greatest reduction in BOD, greater than any single group of microorganisms.

COD, again another common indicator of water quality, was measured for the initial and final concentrations of each mesocosm for the factorial design experiment. COD is also used as a parameter that describes biological activity and corresponds with BOD. The initial and final measured COD concentrations are shown below in Figure 4-7.

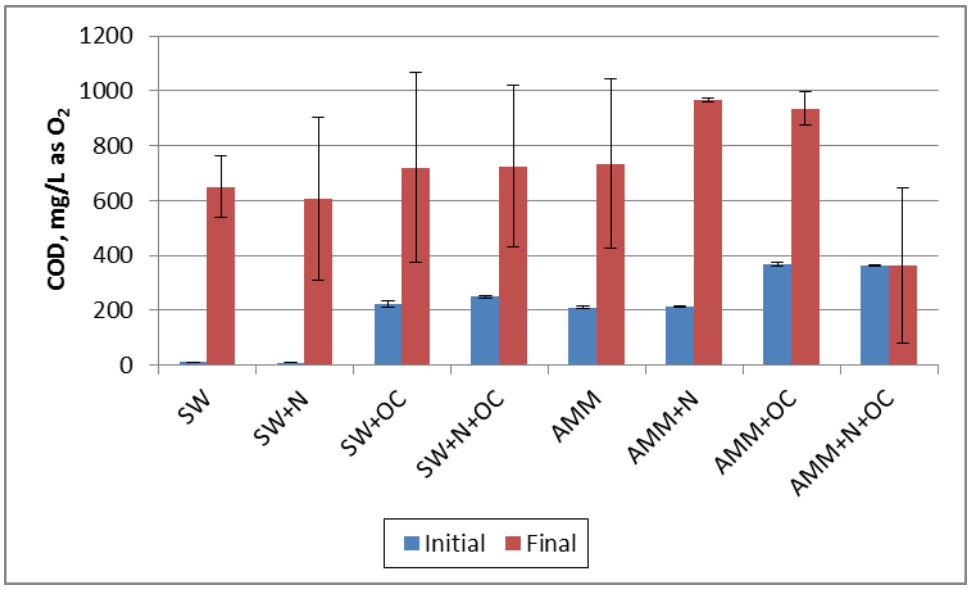

Figure 4-7 – Initial and final Chemical oxygen demand concentrations measured in each respective mesocosm set-up. Data points represent the average of water samples collected in duplicate mesocosms and error bars represent plus and minus one standard deviation

Where SW = Stormwater, N = Nitrate, OC = Organic Carbon/Acetate, and AMM = Anaerobic Mineral Media

The initial COD measured in the stormwater mesocosms were lower than their AMM counterparts. The COD levels in each mesocosm combination were found to increase in the four week experimental period.

As seen in the above figure, there are large standard deviations for a majority of the final measurements of the mesocosms, and are suspected to be due to interferences in the samples. It should be noted that halide ions, especially chloride ions, can cause interference in COD measurements by reacting with the silver ions in the reagents for the test and reduce oxidizing potential (APHA, AWWA, and WEF 2012). The measured conductivity levels may be an indicator that there could have

been interference that could skew the readings and results to be overestimated (APHA, AWWA, and WEF 2012).

It was expected that there would be a decrease in the COD in the samples due to biological activity and degradation of the available organic matter, however, that was not the case. These unexpected COD results are further opposed by the BOD and DOC results. This could be due to the measurements being total COD results, versus a measurement of soluble COD. During COD tests, the amino nitrogen species will be converted to ammonia nitrogen and organic nitrogen will be converted to nitrates (Sawyer et al. 2003). The COD test also does not distinguish organic matter for their biological assimilability, and regardless, becomes completely oxidized (Sawyer et al. 2003). Because the measurement was total COD, it would include all the organic matter that was also created through biological activity, including the organic material for cellular growth, and as previously mentioned, any of the reduced nitrogenous species that occurred throughout the experiment, as indicated by the nitrate and nitrite data would be oxidized during the process of this test. Combining all these interferences and limitations of the COD test, it would explain the high standard deviations of the results and the unexpectedly high results.

4.3.4 Total Phosphorus

Total phosphorus was measured as a nutrient for biological activity and also as a comparison of results of the field study, in an anaerobic condition as the mesocosms were put under. The initial and final concentrations of total phosphorus for each mesocosm are shown below in Figure 4-8.

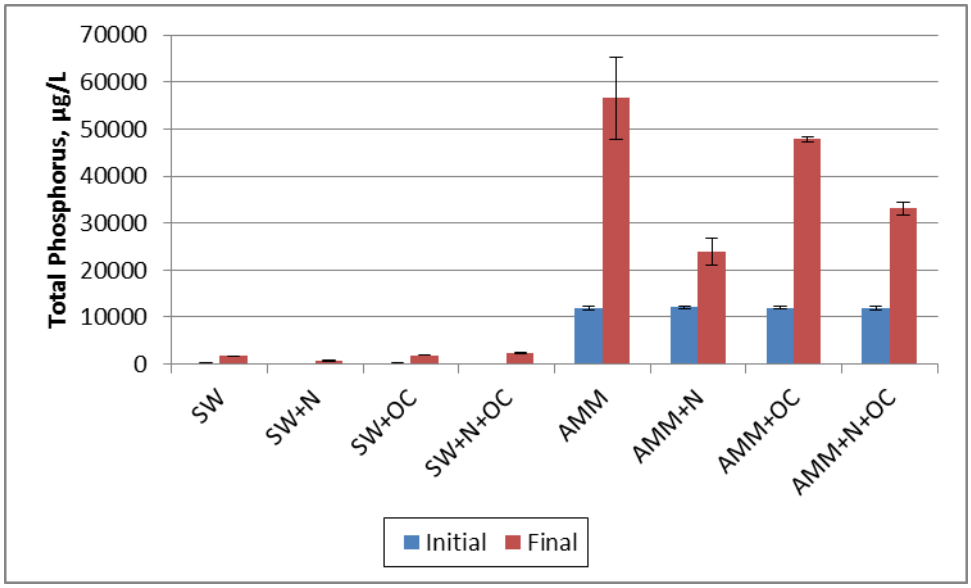

Figure 4-8 – Initial and final total phosphorus concentrations measured in each respective mesocosm set-up. Data points represent the average of water samples collected in duplicate mesocosms and error bars represent plus and minus one standard deviation

Where SW = Stormwater, N = Nitrate, OC = Organic Carbon/Acetate, and AMM = Anaerobic Mineral Media

In all mesocosms and respective scenarios, total phosphorus levels increased over the experimental time period. The mesocosms that were set up with stormwater as their water medium had lower initial concentrations of total phosphorus around 129 to 165 µg/L. These mesocosms resulted in a total phosphorus concentration of 1688 µg/L for just stormwater, 721 µg/L for stormwater with nitrate addition, 1837 µg/L for stormwater and acetate addition, and 2363 µg/L for stormwater with both nitrate and acetate addition. The mesocosms that utilized AMM as their water medium, started with higher levels of total phosphorus than their stormwater counterparts at around 11850 to 12050 µg/L. The final concentrations of total phosphorus for the AMM mesocosms, without amendment, with nitrate addition, acetate addition, and both nitrate and acetate addition, were found to be, 56535 µg/L, 23836 µg/L, 47849 µg/L, and 33078 µg/L respectively. From the results and the figure shown, the greater initial concentrations of total phosphorus, as found in the AMM mesocosms, produced higher levels of total phosphorus at the end of the experiment. In both mesocosm scenarios with only nitrate amendments, the lowest measurements of total phosphorus were found at the end of the experimental period. The other scenarios, without amendments, with acetate addition, and with both nitrate and acetate

additions all resulted in higher concentrations of total phosphorus, however, with no distinguishable patterns in either stormwater or AMM water medium mesocosms.

The increase in total phosphorus levels, present in all mesocosm cases, is possibly the activity of another group of bacteria called Phosphorus Accumulating Organisms (PAO) (Metcalf and Eddy 2003). They are characterized in wastewater treatment systems to release large amounts of phosphates under anaerobic conditions, as commonly seen in biological nutrient removal processes in wastewater treatment (Metcalf and Eddy 2003). Okabe and Characklis (1992) found that SRB can be limited by the lack of phosphorus, with a ratio of 400:1 or 800:1 carbon to phosphorus needed to grow. The effect of phosphorus release with additional carbon source would therefore lead to increased sulphate reduction activity.

4.3.5 Quantification of SRB, NRB, and Total Bacteria

The microbiology analysis of the mesocosms were completed using a quantitative molecular biology technique; qPCR. The specific groups that were analyzed were total bacteria, SRB, and NRB, utilizing the rpoB, dsrB, and nosZ2 genes respectively. The initial and final rpoB, dsrB, and nosZ2 gene copy counts per gram sediment are shown below in Figure 4-9.

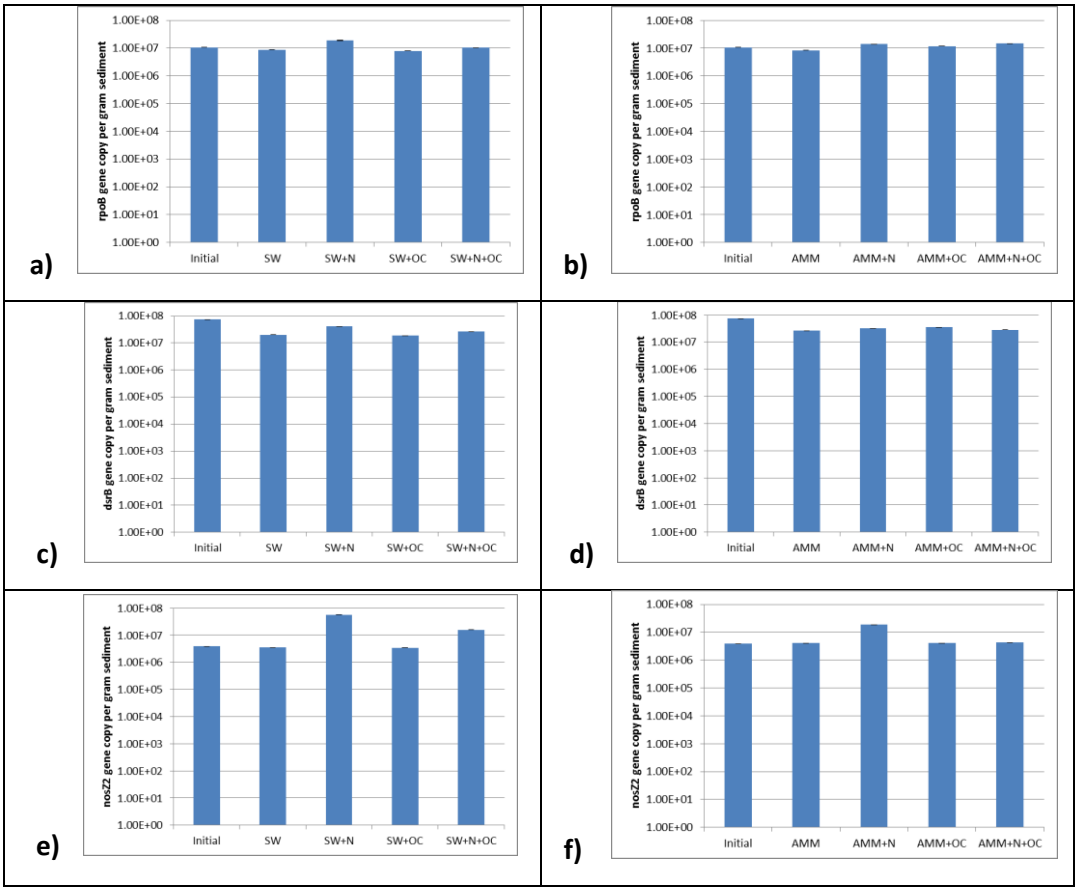

Figure 4-9 – Initial and final qPCR results for a) rpoB, c) dsrB, and e) nosZ2 gene copies per gram sediment in the mesocosms using stormwater and b) rpoB, d) dsrB, and f) nosZ2 gene copies per gram sediment in the mesocosms using AMM measured in each respective mesocosm set-up. Data points represent the average of water samples collected in duplicate mesocosms and error bars represent plus and minus one standard deviation

Where SW = Stormwater, N = Nitrate, OC = Organic Carbon/Acetate, and AMM = Anaerobic Mineral Media

The total bacteria count, according to the rpoB data shown above had initial counts of about 1.07 $\times 10^7$ gene copies per gram sediment for the mesocosms. The total bacteria count showed no change by the end of the four week experimental period in the mesocosms with rpoB measurements averaging between 7.90 $\times 10^6$ and 1.45 $\times 10^7$ gene copies per gram sediment. The lower final gene counts were found in the mesocosms that did not include any amendments, most likely due to the lack of excess electron acceptors and electron donors for microbial growth and activity. The mesocosms that included the 10mM addition of acetate were found to have the second lowest counts of rpoB gene copies. The greatest increases in rpoB gene count per gram sediment were found to occur in the mesocosms that either were amended with nitrate addition, or both nitrate and acetate addition. These results may indicate that the presence of excess organic carbon source, or electron donors, does not promote as much microbial growth as the availability of an electron acceptor that provides a greater gibbs free energy, or a combination of both.

The SRB count using the dsrB gene data began with initial counts of approximately 7.37 $\times 10^7$ gene copies per gram sediment. At the end of the experimental period, the dsrB gene counts were found to decrease in all mesocosm set-ups. The final results for dsrB gene copies per gram sediment varied from 1.88 $\times 10^7$ to 4.10 $\times 10^7$ gene copies per gram sediment, within the same magnitude of the initial count, however on the lower end of the scale. The lowest count of the dsrB gene was found in the mesocosms with only stormwater and stormwater and acetate at 1.88 $\times 10^7$ and 2.02 $\times 10^7$ gene copies per gram sediment respectively. The mesocosms with the next highest counts came from the ones with the mesocosms composed of stormwater amended with nitrate and acetate, the AMM with no amendments, and AMM with added nitrate and acetate; 2.62 $\times 10^7$, 2.65 $\times 10^7$, and 2.85 $\times 10^7$ gene copies per gram sediment respectively. Finally, the mesocosms that measured for the largest number of copies of the dsrB gene were found to be the one with AMM and nitrate addition, AMM with acetate addition, and stormwater and nitrate addition; 3.21 $\times 10^7$, 3.57 $\times 10^7$, and 4.10 $\times 10^7$ gene copies per gram sediment respectively. The mesocosms with the greatest dsrB gene count were the ones amended with nitrate. This may be an indicator that the nitrate reduction that has

occurred with sulphate reducing bacteria that have the capacity to reduce nitrate and not strict nitrate reducers.

NRB count using the nosZ2 gene for the mesocosms began with an initial count of 3.87 $\times 10^6$ gene copies per gram sediment. The NRB counts were found to decrease in the mesocosms that were composed of stormwater without amendments and stormwater with acetate with 3.50 $\times 10^6$ and 3.45 $\times 10^6$ gene copies per gram sediment respectively. There were minor increases to nosZ2 gene counts in the mesocosms that were established with AMM with no amendments, AMM with acetate, and AMM with nitrate and acetate, with 4.03 $\times 10^6$, 4.03 $\times 10^6$, and 4.32 $\times 10^6$ gene copies per gram sediment. The mesocosms that were designed with stormwater with nitrate and acetate, AMM with nitrate, and stormwater with nitrate, resulted in the highest counts of the nosZ2 gene by the end of the factorial design experiment with 1.56 $\times 10^7$, 1.85 $\times 10^7$, and 5.68 $\times 10^7$ gene copies per gram sediment respectively. The lower count of nosZ2 genes in the mesocosms design with both nitrate and acetate compared to other scenarios, describes the use of the acetate was favoured by SRB over NRB. As expected, the mesocosms that were amended with nitrates produced the highest quantity of nosZ2 genes, indicating that there were indeed nitrate reducers that grew during the process of the experiment.

Overall, the qPCR results showed that there are significant numbers of SRB in the stormwater sediment, so much so, that even with nitrate amendments, their activity overwhelms those of NRB.

4.4 Conclusions and Recommendations

The results of the factorial design mesocosm experiment produced clear results of the effects of adding nitrate, acetate, water medium, or any combination of the preceeding, have on the mimicked stormwater retention pond environment. It was found that the combination of factors that resulted in the lowest concentration of sulphides produced was stormwater medium with addition of 10 mM of nitrate. The combination of factors that resulted in the greatest concentrations of sulphides produced was stormwater medium with the addition of 10 mM of nitrate and 10 mM of acetate.

The addition of nitrate had the best means of suppressing sulphide production. It is theorized that the nitrate, being a terminal electron donor that provides greater redox potential energy, was utilized over sulphate as a terminal electron donor. Also, the addition of nitrate stimulated the activity of NR-SOB, resulting in the higher concentrations of sulphate in both cases, due to the oxidation of sulphide and other reduced sulphur species available to the bacteria. The addition of acetate, an electron donor source, resulted in the acceleration of sulphate reducing activity. Unexpectedly, the presence of additional nitrate and acetate resulted in the greatest concentration of sulphide production.

There are several means of improving the experiment and possible future work that would provide greater understanding of the interactions in the mesocosms. Most significantly, the experiment time frame should be reduced to a shorter length, or with intermittent time points to track changes within the mesocosms. Due to the length of time this experiment ran, nitrates were completely utilized, with no understanding of how long this took and whether or not there were any significant changes in water chemistry throughout. Some future work to further expand knowledge that was outside the scope of this study would include a headspace analysis would have provided greater understanding and a proper mass balance of the reduced species being produced in the mesocosms. Another future work experiment would be to assess the kinetics of the electron donors to understand the proper dosage of nitrates that would assist in affecting sulphide production.

Overall, the addition of a new electron acceptor with greater redox potential would assist in the suppression of the production of sulphide ions. The addition of an electron donor source however, would be counterproductive, and would result in greater concentrations of sulphide produced. Further tests would be needed to ensure accurate full scale remediation strategies for the stormwater retention pond.

4.5 Chapter 4 References

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Chapter 5. SERRANO PEPPER EXTRACT AS A BIOCIDE FOR SULPHATE REDUCING BACTERIA AND SUPPRESSION OF SULPHIDE PRODUCTION

5.1 Introduction

The field study conducted and detailed in *Chapter 3: Field Study of Two City of Edmonton Stormwater Retention Ponds*, concluded that sulphide was being produced biogenically in the Valencia stormwater retention pond site with high concentrations of sulphate in the stormwater and high numbers of sulphate reducing bacteria (SRB) in the sediment. This chapter is focused on the evaluation of using a plant extract as a biocide to target and inhibit SRB activity as a remediation technique for the reduction of sulphide and hydrogen sulphide production.

There have been several means of techniques studied in the suppression of sulphide production and the inhibition of SRB and their activity. Biocides have been studied for their efficacy and potential in preventing biogenic production of sulphide both in laboratory settings and in the oil and petroleum industry (Reinsel et al. 1996, Telang et al. 1998, Gardner and Stewart 2002, Thorestenson et al. 2002). Some of these compounds include, but are not limited to, glutaraldehyde, tetrakishydroxymethyl phosphonium sulphate (THPS), quaternary ammonium compounds (QAC), bromo-nitropropanediol (BNPD) (Wen et al. 2009). Cationic surfactants have also been studied to show inhibitory effects and use as a biocide against SRB (Shaban et al. 2013). The results of these studies showed that H_2S can be inhibited using these compounds, however there are concerns of their biodgredability and the potential impacts of their use in natural environments.

Plant extracts have been found, studied, and utilized as microbial biocides in different industries and systems such as water purification (Yongabi et al. 2010), pharmaceutical medicines (Balandrin et al. 1985), and extractives processing and production (Oguzie et al. 2012, Bogan et al. 2004, de Saravia and Gaylarde 1998). Bogan et al. (2004) successfully used extracts from *Capsicum sp.* plants as a microbial biocide on planktonic SRB as a means of pipe corrosion prevention. Benefits of these plant extracts include being naturally occurring compounds, biodegradability, and are effective against specific groups of microorganisms.

In the following chapter, similar to Bogan et al. (2004)'s study, a soxhlet extraction of dried Serrano peppers using hexane as a solvent was tested for its potential as a biocide against SRB and suppression of sulphide production in the

context of a stormwater retention pond environment. Using a mesocosm set-up, the changes of sulphate and sulphide concentrations and SRB cell numbers were measured over a three week period in the presence and absence of the biocide to evaluate the effect of the extract over time.

5.2 Materials and Methodology

5.2.1 Field Samples

The sediment and stormwater utilized in this study was collected from the Valencia stormwater retention pond during the final sampling date at the site for the field study on September 27, 2012. The sediment was stored with a water cap in a 20L container and the stormwater was stored in 20L containers in a 4°C refrigerated room until use.

5.2.2 Pepper Extract

Pepper extracts were tested for their use as a benign sulphide production inhibitor. The pepper extract used was produced specifically from Serrano peppers. Fresh Serrano peppers were purchased at a wholesale grocery store and prepared for extraction by removing the stems, dried at 60°C for 24 hours, ground up in a food processor, dried again at 60°C for 24 hours, ground in mortar and pestle and then stored at 4°C until used in extraction.

The extraction used was a solvent extraction process, specifically the soxhlet extraction technique (US EPA 1996). The prepared Serrano peppers were packed carefully in cellulose thimbles, weighed, and covered with glass wool to prevent overflow. The thimbles were then placed in the soxhlet extraction apparatus. 200 to 300 mL or 99% grade hexane was used as the solvent, depending on the extraction flask size used. The extraction process ran for 24 hours per thimble. The extract and solvent was then concentrated, separating and removing the hexane from the pepper extract using a rotovap.

5.2.3 Mesocosm

The mesocosms set-up consisted of 1200 mL of stormwater and 800 mL of sediment in a 2 litre Pyrex bottle. The preparations and set-up of the mesocosms used in this study follow the same procedures in the mesocosms prepared and used in Chapter 4, please review that chapter for details.

Pepper extracts were used at 0.01 or 0.05% by water volume medium in the mesocosms. Controls were created by autoclaving sediments and stormwater separately a total of three times, each with 24 hours of waiting time between, then adding sodium azide and mercuric chloride and sitting for 24 hours before combined and used. The purpose of this treatment was to kill microorganisms and eliminate any microbial activity within the mesocosms.

5.2.4 Sampling

Samples were taken from each pepper mesocosm at time 0, 1h, 3h, 6h, 12h, 24h, 48h, 72h, 168h, and 504h. First, 1.5 mL of sediment samples were acquired and stored at -20°C for DNA extraction and microbial analysis. Mesocosms were then inverted several times before water samples were taken. Water samples were measured for sulphate and sulphide concentrations, monitoring for change over time.

5.2.5 Water Chemistry

SULPHATE Sulphate levels measured by the University of Alberta Biogeochemical Analytical Service Laboratory used the Ion Chromatography method with Dionex DX600 and Dionex ICS 2500 units with the EPA 300.1 (Modified) Method (1997).

SULPHIDE Sulphide analysis was completed using an Thermo Scientific 9616BNWP silver/sulfide combination electrode as an ion specific probe. The standard test method followed the ASTM D4658 method (2009).

5.2.6 Microbiology

DNA EXTRACTION DNA was extracted from the mesocosm sediment samples using the MoBio PowerSOIL DNA Extraction Kit. This specific DNA extraction kit was used due to the matrix of the environmental samples extracted from. The advantage of using this specific kit is the process of removing PCR inhibitors and humic substances that interfere with molecular biology analysis. 500µL of sample were used for each DNA extraction. The DNA samples were then stored at -20°C, until used for further analysis.

qPCR DNA samples were analyzed and optimized for PCR conditions. Three groups of bacteria were analyzed; total bacteria, sulphate reducing bacteria, and nitrate reducing bacteria. The primer sets used to analyze for these three groups of bacteria were rpoB, dsrB and nosZ2 respectively. The details of the primers and conditions used can be reviewed in Table 3-2 and 3-3 found in Chapter 3. The qPCR experiments were conducted using the Bio Rad I-Cycler in 96 well plates. The qPCR reactions were done in 20 µL reactions, consisting of 10 µL of Bio Rad SsoFast EvaGreen Supermix, 0.05 µL each of the forward and reverse primers of interest, 1 µL of DNA template and 8.9 µL of water. The results of the qPCR were analyzed and managed using the Bio Rad CFX Manager 3.0 Software.

5.3 Results and Discussions

The following results focus on the effect of the pepper extract addition on the mesocosm over a three week period. Sulphate and sulphide concentrations were specifically measured in the mesocosm's water to check for presence of sulphate reduction. Following this analysis, results of the molecular biology work have been summarized to check on quantitative changes in microbial communities.

5.3.1 Sulphate and Sulphide

Sulphate and sulphide, as an electron donor and its reduced form, were specifically monitored during this pepper extract experiment. This was specifically monitored to look at the sulphate reducing inhibition effect that the pepper extract has and to what extent. The sulphate concentrations measured over time in the 0.01% and 0.05% pepper extract mesocosms and their respective controls are shown above in Figure 5-1.

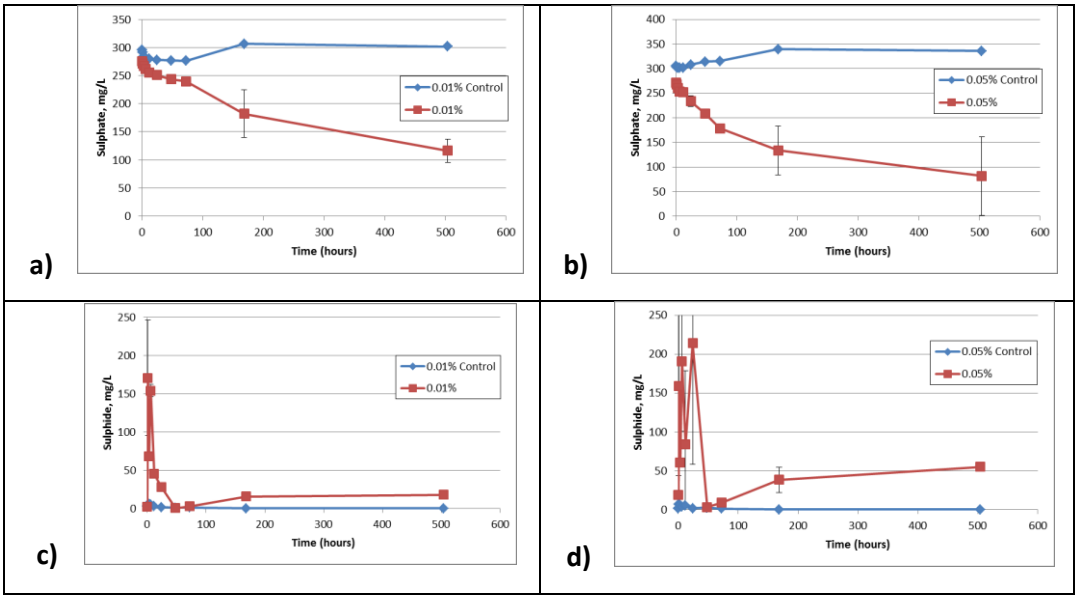

Figure 5-1 – Sulphate concentrations in the mesocosm with a) 0.01% and b) 0.05% pepper biocide and sulphide concentrations in the mesocosms with c) 0.01% and d) 0.05% pepper biocide over. Data points represent the average of water samples collected in duplicate mesocosms and error bars represent plus and minus one standard deviation

The controls for both the 0.01% and 0.05% pepper extract mesocosms both started with sulphate concentrations of 276 and 305 mg/L respectively. The 0.05% mesocosm was found to increase in sulphate concentrations over time, reaching a final concentration of 336 mg/L at 504 hours. The 0.01% mesocosm however, was found to initial decrease to a concentration of 277 mg/L at 48 and 72 hours, and then increasing to a final concentration of 302 mg/L at 504 hours.

The 0.01% and 0.05% pepper extract mesocosms showed a consistent decreasing trend throughout the three week experiment period. The 0.01% pepper extract mesocosms began with an initial sulphate measurement of 276 mg/L and reduced to a final concentration of 116 mg/L. The 0.05% pepper extract mesocosms began with a sulphate concentration of 272 mg/L and reduced to an average final concentration of 81.4 mg/L. The overall trend of the reduction of sulphate is similar in both dosages of pepper extracts, however, it was not found to be significantly different with a *p-value* of 0.231.

The sulphide measurements for the 0.01%, 0.05% and their respective control mesocosms are depicted above in Figure 5-1. The sulphide measurements for the 0.01% and 0.05% controls were found to begin at 3.28 and 1.68 mg/L respectively. The measurements for the 0.01% control mesocosm rose to a maximum concentration of 6.14 mg/L at hour 3 and decreased continuously to a concentration of 0.226 mg/L at hour 504. For the 0.05% control mesocosm, sulphide concentration rose to its highest measurement of 6.91 mg/L in the first hour and fluctuated throughout the experiment until a final concentration of 0.337 mg/L was found at hour 504.

The 0.01% pepper extract mesocosm had an initial concentration of 2.44 mg/L and jumped significantly to a concentration of 171 mg/L within the first hour of the experiment. The measurements then fluctuated to 68.0 mg/L at hour 3, 154 mg/L at hour 6, 45.3 mg/L at hour 12 and 28.1 mg/L at hour 24, before reaching its lowest sulphide concentration of 0.622 mg/L at hour 48. The sulphide levels then increased over time until a final concentration of 18.0 mg/L at hour 504.

The 0.05% pepper extract mesocosm began with a concentration of 19.1 mg/L, increased to 159 mg/L within the first hour and dropped to 60.8 mg/L by the third hour. The sulphide measurements then continued to fluctuate to 191 mg/L at hour 6, dropping to 83.7 mg/L at hour 12, jumping to its highest sulphide concentration of 214 mg/L at hour 24 before decreasing to its lowest concentration of 3.49 mg/L at hour 48. Following that, sulphide concentration increased until it reached a measurement of 55.2 mg/L at hour 504.

Both 0.01% and 0.05% pepper extract mesocosms exhibited large fluctuations in sulphide concentrations within the first 48 hours of the experiment before another increase in concentrations over time. High sulphate reduction activities were observed

for both scenarios. Along with the sulphate data, trends strongly correlate that sulphate reduction is occurring in both cases. In comparison to the control mesocosms, the production of sulphide ions appear to be mainly attributed to the biological activity.

Overall, after the initial 48 hour period of fluctuations, the pepper extracts exhibited suppression of sulphide production. These results varied with those of the original experiments done on sulphate reduction and corrosion prevention by Bogan et al. (2004), who reported that the inhibition of H2S production happened right after their experiments studied. This might be attributed to at least two reasons, (1) the complexity of the studied environment, with the presence of organics, metals, and other inorganic compounds, may reduce the effective concentrations of the pepper extracts for the SRB inihibition as there is not a lot of knowledge of the interactions or interferences between the biocide and these compounds; and (2) the concentrations of pepper extract used in the present study might be too low considering the presence of the sediment phase that had large numbers of SRB existing in it. These results may indicate that the concentrations of pepper extract used might not be enough to make a strong effect on sulphate reduction. Further studies should be performed to identify the concentration needed for pepper extracts applications in the stormwater ponds, and/or to evaluate the feasibility of adding a chelating agent to assist in the effectiveness of the biocide. It would be beneficial to consider the possible differences of sessile and biofilm SRB to the biocide versus the platonic species. This would also assist in explaining the reduced efficacy of the biocide as compared to that found by Bogan et al. (2004).

5.3.2 Quantification of SRB, NRB, and Total Bacteria

An analysis of the microbial activity and microbiology was done on the pepper mesocosms using qPCR to evaluate whether or not microbial activities can be affected by pepper extract. The use of the rpoB, dsrB, and nosZ2 genes, as mentioned previously in Chapter 3, to specifically target and quantify total bacteria, SRB, and NRB and their activity within the mesocosm. The rpoB, dsrB, and nosZ2gene counts per gram sediment for total bacteria, SRB, and NRB are shown below in Figure 5-2.

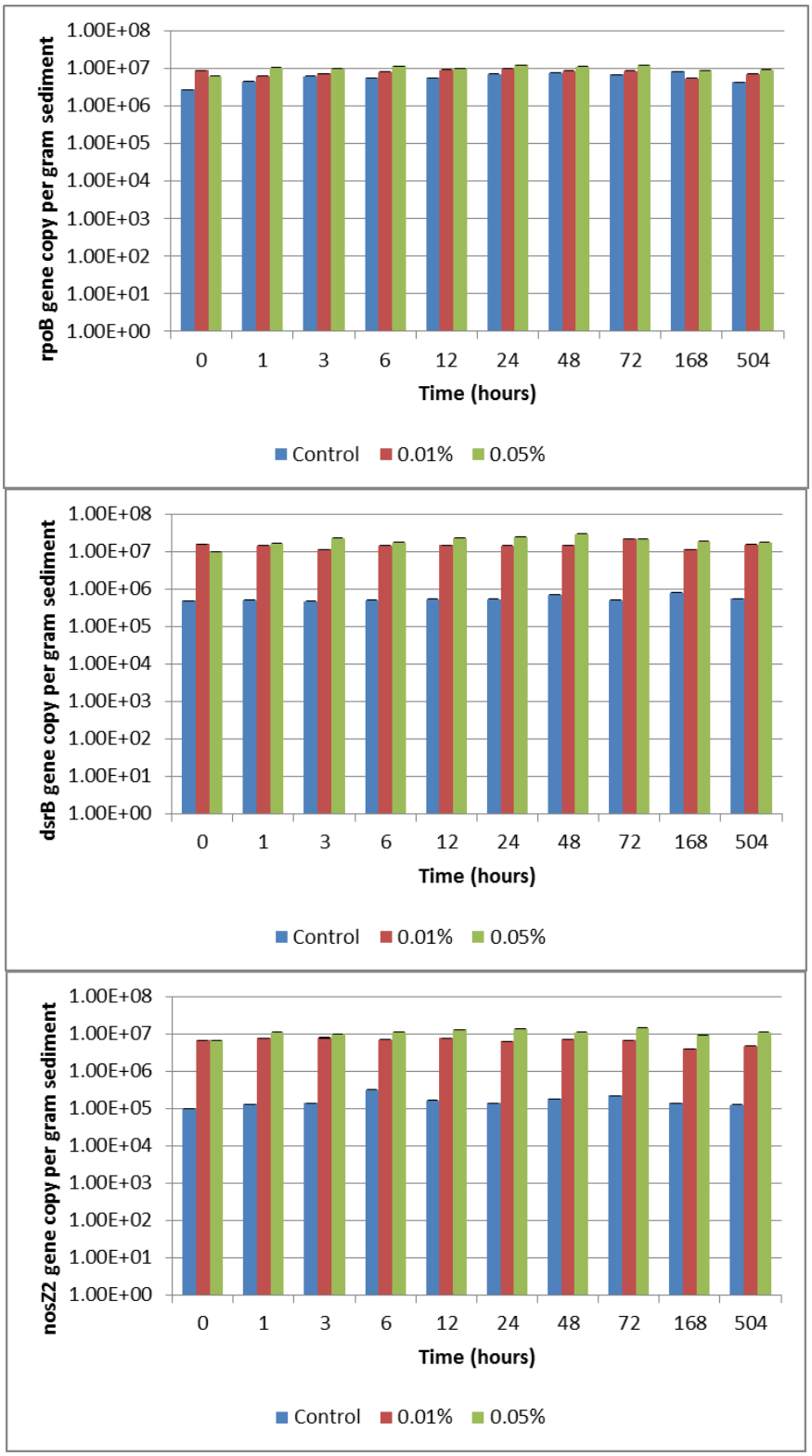

Figure 5-2 – qPCR results of the rpoB, dsrB, and nosZ2 gene present in the control, 0.01%, and 0.05% pepper extract mesocosms. Data points represent the average of water samples collected in duplicate mesocosms and error bars represent plus and minus one standard deviation

For the averaged control measurements, the initial value began at 2.72×10^6 gene copies per gram sediment. The rpoB gene copy counts then increased to a value of 4.32×10^6 gene copies per gram sediment by the first hour and fluctuated between 8.05×10^6 to 4.21×10^6 gene copies per gram sediment throughout the experimental three week period, staying within the same order of magnitude of gene counts. For the 0.01% pepper extract mesocosm, rpoB counts began at 8.80×10^6 gene copies per gram sediment and similar to the controls, had fluctuated throughout the experiment, but at higher counts, between $5.41\cdot10^6$ gene copies per gram sediment at its lowest count at hour 168 and 9.75×10^6 gene copies per gram sediment at its highest count at hour 24. For the 0.05% pepper extract mesocosm, rpoB gene counts began at 6.04×10^6 gene copies per gram sediment, and once again had risen and fluctuated throughout the experiment between 8.76×10^6 gene copies per gram sediment at its lowest count at hour 168, similar to the 0.01% mesocosm, and 1.21×10^7 gene copies per gram sediment at hour 24. The rpoB gene copy counts per gram sediment followed the same patterns in both the 0.01% and 0.05% pepper extract experiments with its lowest counts at the 168^{th} hour and its highest counts at the 24^{th} hour. This pattern follows the sulphide concentration measurements, with the highest total bacteria count attributing to the highest sulphide concentrations, and the lowest gene counts following the lowest sulphide measurements in each mesocosm.

The averaged control mesocosms, the dsrB gene count started at 4.79×10^5 gene copies per gram sediment and remained low within the 10^5 gene copies per gram sediment throughout the experiment, ranging from 4.65×10^5 to 7.90×10^5 gene copies per gram sediment. The 0.01% pepper extract mesocosm, started with dsrB gene counts of 1.57×10^7 gene copies per gram sediment, nearly two orders of magnitude greater than the control mesocosms. The dsrB gene counts in the 0.01% mesocosms remained relatively the same, ranging from 1.12×10^7 to 1.50×10^7 copies per gram sediment, until a high point of 2.19×10^7 gene copies per gram sediment was reached at hour 72 and dropped back down to 1.54×10^7 gene copies per gram sediment. For the 0.05% pepper extract mesocosm, dsrB gene copies started at 9.66×10^6 per gram sediment and increased to 1.64×10^7 gene copies per gram sediment in the first hour and fluctuated in counts up to 2.94×10^7 gene copies per gram sediment at hour 48 and

decreased to 1.77×10^7 gene copies per gram sediment by the end at hour 504. Overall, the dsrB counts in the 0.01% and 0.05% pepper extract mesocosms were nearly two orders of magnitude greater than the control indicating SRB growth and activity, even with the presence of the pepper extract.

In the case of the averaged control mesocosms, the initial measurements of nosZ2 gene count per gram sediment were found to be 1.01×10^5 gene copies per gram sediment and remained within the same order of magnitude, reaching to 3.15×10^5 gene copies per gram sediment at its highest. For the 0.01% pepper extract mesocosm, nosZ2 gene counts began at 6.61×10^6 gene copies per gram sediment and fluctuated between 6.24×10^6 and 7.77×10^6 gene copies per gram sediment until hour 72, before dropping to 3.93×10^6 and 4.65×10^6 at the end of the experiment. For the 0.05% pepper extract experiment, nosZ2 gene counts begin at 6.77×10^6 per gram sediment and increased it 1.10×10^7 gene copies per gram sediment at the first hour and fluctuated between 9.46×10^6 to $1\times44\cdot10^7$ gene copies per gram sediment for the remainder of the experimental three week period. NRB counts were found to increase to an order of magnitude and a half greater in the 0.01% mesocosms and two orders of magnitude greater in the 0.05% mesocosms compared to that of the controls.

Overall, the qPCR results did not indicate of increased total bacterial, SRB, or NRB activity with the presence of the pepper extracts within their respective mesocosms over the course of the experiment. It appeared that the NRB counts increased in the mesocosms that had higher concentrations of the added pepper extract. This may be an indication of the pepper extract as an electron donor source for the anaerobic bacteria.

5.4 Conclusions and Recommendations

The results of this study utilizing an extract from Serrano peppers using the soxhlet extraction method with hexane as a solvent showed potential for use as a biocide in a stormwater retention pond environment.

Sulphate measurements indicated that sulphate reduction was still occurring, regardless of the presence of the pepper extract.Sulphide measurements were erratic within the first 24 hours of the experiment, however, showed that at hour 48, the lowest levels of sulphide were established and increased slowly over the remainder of

the experiment. The qPCR results did not indicate and significant increases in biological activity from total bacteria, SRB, or NRB, however it did show differences in increased activity with the presence of higher concentrations of the pepper extract for NRB.

The use of this biocide varied significantly with those shown by Bogan et al. (2004), and showed that the concentrations of the pepper extract were not as effective in a complex environment composed of stormwater and sediment. The results indicate that the biocide is less effective on the sessile and biofilm SRB that thrive in the stormwater sediment than that of the planktonic SRB in Bogan et al.'s (2004) study. It is not known whether this is due to the interactions of the biocide and the sediment itself, the potential resistance to the biocide of the SRB that exist in a biofilm formation in the sediment (Denyer 1995, Meyer 2003, Morton et al. 1998, Davies 2003), or a combination of these and other factors.

Due to the complex nature of the stormwater retention pond, it is recommended that further studies be conducted using the biocide to investigate its potential in mitigating against SRB activity. Studies have already been conducted in enhancing the efficacy of biocides using chelating agents, such as ethylenediaminetetraacetic acid (EDTA) (Raad and Sherertz 2001) or ethylenediaminedisuccinate (EDDS) (Wen et al. 2009) with conclusive success.

5.5 Chapter 5 References

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CHAPTER 6: SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

6.1 Conclusions

The research conducted has added to the understanding of sulphate reduction and mitigation of sulphide production in the management of stormwater retention pond environments. Through the field study and comparative composition of water quality and microbial activity in sediments of two stormwater retention ponds, it was found that biological activity was favourable in one pond, Valencia, versus the other, Bearspaw. It was further found that different factors had different influences on sulphide production, with varying success in inhibition techniques. This research is significant in the future management of sulphide and hydrogen sulphide production in urban stormwater retention ponds in the City of Edmonton, and the other many urban centres that also rely on this infrastructure.

6.1.1 Field Study Comparison of Two Stormwater Retention Ponds in the City of Edmonton

To understand the biological activity, and more specifically the reasons and factors that lead to increased sulphate reduction activity in Valencia stormwater retention pond, a comparative analysis of water chemistry and sediment microbial activity was conducted via the means of a four month long field study of the aforementioned facility and Bearspaw stormwater retention pond. The field study showed the likely influential factors that led to greater sulphide production that occurs regularly in one stormwater retention pond over the other. The following parameters were measured: sulphide, sulphate, nitrate and nitrite, BOD, COD, TOC, alkalinity, total phosphorus, pH, conductivity, ORP, dissolved metals, total metals, and qPCR measurements of total bacteria, sulphate reducing bacteria, and nitrate reducing bacteria. The resulting conclusions based on the field study experiments are summarized as followed:

- There is a greater concentration of sulphate, the terminal electron acceptor for SRB, available in Valencia stormwater retention pond in comparison to Bearspaw stormwater retention pond. This allows for possible greater growth and activity of SRB, and produces the greater concentrations of sulphide found in Valencia.

- The nitrate and nitrite, a terminal electron acceptor for NRB that has a more favourable redox potential than that of sulphate, was greater in Bearspaw stormwater retention pond. It is suspected to also contribute in the reduced levels of sulphide found compared to Valencia stormwater retention pond.
- The majority of microbial degradation and activity within the stormwater retention pond, was suggested to occur in the beginning of the field study in the late spring and early summer months, where there were greater concentrations of BOD and COD.

The conclusions from the field study provided direction for the subsequent experimental work in the factorial design mesocosms and the use of the plant extract biocide.

6.1.2 Factorial Design Experiment Utilizing Nitrate Addition, Acetate Addition, and Water Medium

The field study indicated that one of the factors leading to the increased sulphate reduction in Valencia stormwater retention pond was the difference in availability and abundance in species of terminal electron acceptors to be utilized in biological degradation and activity. A factorial design experiment, utilizing three factors and two degrees per factor was utilized to test the influence of specific factors on the production of sulphide in the stormwater retention ponds. The factors that were tested looked at the presence of another electron acceptor, in this case, nitrate for its inhibitory effects, the presence of excess of an electron donor source, acetate, and the overall water medium, be it stormwater or anaerobic mineral media to see if another factor in the water potentially attributes to biological sulphate reduction. The experiment was designed to analyze several parameters; sulphide, sulphate, nitrate, nitrite, BOD, COD, DOC, qPCR counts of total bacteria, SRB, and NRB. The corresponding results of this experiment are as summarized in the following:

- The addition of 10 mM of nitrate resulted in the lowest production of sulphide in the mesocosms. It was also found that in the four week experimental period, the mesocosms that were amended with nitrate used up the entire available detectable amounts of nitrate with residual nitrites remaining.

- The addition of 10 mM of nitrate also resulted in increased concentrations of sulphate within the mesocosms that were only amended with nitrate. This suggests the presence and activity of nitrate reducing – sulphide oxidizing bacteria, that exist in the stormwater sediment, transforming the sulphide into sulphate while utilizing nitrate as an electron acceptor.
- The addition of 10 mM of acetate, originally theorized to assist in the biosimulation NRB to reduce the remaining concentrations found in the water, was found to encourage SRB activity instead. The presence of excess electron donors resulted in the greatest influence in sulphide production.
- The addition of both 10 mM of nitrate and 10 mM of acetate also did not confirm the suppression of biological sulphate reduction via competition with nitrate reduction over the four week experimental period. Instead, it was found to also produce sulphide within the experimental period with the complete utilization of nitrates within the mesocosms.

Nitrate addition showed to be a promising mitigation technique as long as extraneous carbon sources were not added in the stormwater retention pond.

6.1.3 Use of a Pepper Extract Biocide as Means of Inhibiting Biological Sulphate Reduction

The field study results showed clear signs of biological sulphate reduction as cause of increased levels of sulphide in the stormwater retention pond, in conjunction with increased concentrations of sulphate found in Valencia pond. The use of a pepper extract produced from the Soxhlet Extraction of Serrano peppers using hexane was tested in a mesocosms set-up similar to that of the factorial design experiment was tested for its potential efficacy in mitigating sulphide production in the stormwater retention pond. Doses of 0.01% and 0.05% of pepper extract by water volume were tested over a period of three weeks with time points of time 0, 1, 3, 6, 12, 24, 48, 72, 168, and 504 hours. The parameters analyzed were sulphate and sulphide concentrations and qPCR measurements of total bacteria, SRB, and NRB. The results of the natural extract biocide study are as follows:

- The sulphide concentrations found within the first 48 hours of the experiment in all mesocosms were found to fluctuate between high and low concentrations. After which, the concentrations levelled off to low concentrations.
- Sulphate concentrations continued to decrease through the experiment, indicating that sulphate reduction was still occurring, regardless of the presence of the biocide in the concentrations tested.

The environment of the stormwater retention pond is complex and the use of the biocide may not be effective without the presence of a biocide aid.

6.2 Recommendations

Future work as a follow up to the work completed in this thesis should be taken in two main directions; furthering scientific study and engineering management and mitigation of Valencia stormwater retention pond.
In terms of advancing scientific research and study, the following recommendations are given:

- Expand the field study and monitoring of the Valencia stormwater retention pond. It would be beneficial to either extend the field sampling and analysis of Valencia stormwater retention pond to gather data points earlier and later than the points taken in the present field study. This would also assist in possible water quality modeling studies.
- Gather data from other stormwater retention ponds beyond that of Bearspaw and Valencia stormwater retention ponds to gain better comparative understanding of any existing trends that can exist between stormwater retention ponds.
- Complete a qualitative microbial analysis of the microorganisms that thrive in the stormwater retention pond sediment to verify theories proposed in this thesis and gain better understanding of the consortium that exists in these unique environments.
- Test for optimal dosage of nitrate amendments for the suppression of sulphate reduction and sulphide generation. Tests confirmed that the addition of 10

mM of nitrate was sufficient in suppressing sulphate reduction; however, an optimized dosage has yet to be found.

- Test for optimal dosage of pepper extract to add to stormwater required to suppress sulphate reduction and sulphide generation.
- Test for supplemental compounds to the pepper extract biocide that increases its efficacy and reduces the optimal dosage of the pepper extract required to suppress sulphate reduction.

In regards to the engineering management and mitigation of sulphide and hydrogen sulphide generation in Valencia stormwater retention pond, the following recommendations are made:

- Best practice solution is to dredge Valencia stormwater retention pond. The main cause of sulphide and hydrogen sulphide in the stormwater pond site is complex and not fully understood. The removal of the complex environment favouring the increased growth and activity of sulphide ions would be the most effective in reducing sulphate reduction activity. This also opens the possibility of redesigning the stormwater retention pond to incorporate plants to become a constructed wetland that would also improve water quality overall.
- Nitrate injection can be a means of suppressing the anaerobic activity temporarily to reduce the sulphide production. This solution, however, is only temporary and is rendered ineffective in the presence of excess organic carbon. Dosage and means of injection must also be determined before being implemented.
- At this point in time and progress in research, it is not recommended to use pepper extract biocide, until further studies have been conducted with proper dosage or supplementary compounds analyzed.

The future work will add to further in-depth understanding of the environment of stormwater retention ponds and thus the future management of stormwater retention ponds in urban settings. This will result in greater efficiency and safety in operation and maintenance of stormwater infrastructure and an overall improved water quality of stormwater that is conveyed to natural surface water sources. This research

contributes to increased sustainability practices in the urban management of water resources.

APPENDIX A: RAW DATA

FIELD STUDY

Sulphate and Sulphide Concentration Measurements

Table A-1 – Sulphate concentrations (in mg/L) measured at the inlet, middle, and outlet at Valencia and Bearspaw stormwater retention ponds

Pond	Valencia			Bearspaw		
Sample Period	Inlet	Middle	Outlet	Inlet	Middle	Outlet
1	310.00	320.00	-	200.00	210.00	200.00
	310.00	320.00	-	220.00	220.00	200.00
2	312.40	301.80	301.54	189.70	189.24	186.99
	308.11	303.70	299.79	198.46	186.99	187.54
3	314.55	304.63	311.16	76.36	76.77	78.10
	313.58	305.31	309.92	76.37	77.16	77.57
4	223.22	227.68	233.08	94.55	94.48	91.62
	225.64	226.36	231.01	93.32	91.54	91.86
5	236.18	231.51	229.19	102.84	103.53	102.53
	235.09	230.50	226.77	102.05	104.15	102.69
6	277.74	274.46	270.13	130.63	128.68	127.93
	278.72	273.05	271.14	130.70	127.56	128.00

Table A-2 – Sulphide concentrations (in µg/L) measured at the inlet, middle, and outlet at Valencia and Bearspaw Stormwater retention ponds in the field and in the lab

Pond	Valencia						Bearspaw					
Sample Period	Inlet (field)	Inlet (lab)	Middle (field)	Middle (lab)	Outlet (field)	Outlet (lab)	Inlet (field)	Inlet (lab)	Middle (field)	Middle (lab)	Outlet (field)	Outlet (lab)
1	>800	380	>800	6500	>800	210	0.00	9.9	7	6.9	10	8.4
		2400		280		140		9.9		8.4		9.2
2	35	39	25	64	100	18	7	<2	13	<2	11	7.3
		84		23		32		<2		2.4		3.3
3	245	2.4	85	8.1	25	16	24	<2	18	<2	21	<2
		2.4		<2		11		<2		2.4		<2
4	32	5.7	37	4.9	453	16	25	8.1	17	2.4	22	3.3
		5.7		9		38		7.3		2.4		12
5	45		49		216		95	11	31	7.4	33	8.2
								9.9		3.3		6.6
6	46	3.3	224	5.8	44	2.5	33	<2	29	3.3	32	-
		<2		4.1		5.8		<2		<2		-

Nitrate and Nitrite Concentration Measurements

Table A-3 – Nitrate and Nitrite concentrations (in µg/L) measured at the inlet, middle, and outlet at Valencia and Bearspaw stormwater retention ponds

Pond	Valencia			Bearspaw		
Sample Period	Inlet	Middle	Outlet	Inlet	Middle	Outlet
1	29	0	-	29	33	36
	52	0	-	28	31	34
2	0	11	0	128	134	114
	2	16	0	130	134	111
3	21	28	8	19	149	89
	22	31	7	24	151	98
4	5	2	4	58	62	25
	5	3	2	58	64	31
5	2	3	3	6	2	4
	4	3	3	11	2	3
6	19	0	5	18	24	17
	19	0	4	18	27	16

ORGANICS – BIOCHEMICAL OXYGEN DEMAND, CHEMICAL OXYGEN DEMAND, and TOTAL ORGANIC CARBON

Biochemical Oxygen Demand (BOD)

Table A-4 – Biochemical oxygen demand concentrations (in mg/L of O_2) measured at the inlet, middle, and outlet at Valencia and Bearspaw stormwater retention ponds

	Valencia			Bearspaw		
Sample Period	Inlet	Middle	Outlet	Inlet	Middle	Outlet
1	33.9	34.5	40.5	22.8	23.1	26.4
1	36	27	37.5	22.5	20.4	39
2	5.4	4.8	4.5	2.7	2.76	4.8
2	4.5	3.78	4.8	2.4	3.54	7.2
3	5.4	5.76	6.09	6.05	5.25	6.15
3	7.2	6.2	-	6.05	5.6	6.75
4	3.9	5.45	3.25	5.25	3.55	2.45
4	4	4.75	4.2	10.25	5.15	3.75
5	6.25	4.5	4.7	3	7.2	9.1
5	5.4	6.15	6.5	2.65	6.5	6.8
6	-	-	-	-	-	-
6	-	-	-	-	-	-

Chemical Oxygen Demand (COD)

Table A-5 – Chemical oxygen demand concentrations (in mg/L of O_2) measured at the inlet, middle, and outlet at Valencia and Bearspaw stormwater retention ponds

Pond	Valencia			Bearspaw		
Sample	Inlet	Middle	Outlet	Inlet	Middle	Outlet
1	280	115	105	102.5	67.5	50
1	182.5	97.5	120	67.5	72.5	62.5
2	25	17.5	15	22.5	20	17.5
2	20	22.5	12.5	17.5	20	25
3	52.5	47.5	62.5	30	35	27.5
3	47.5	47.5	42.5	50		30
4	35	57.5	67.5	52.5	37.5	27.5
4	47.5	47.5	55	60	40	35
5	-	-	-	-	-	-
5	-	-	-	-	-	-
6	55	62.5	57.5	31.43	37.14	31.43
6	60	55	65	34.29	31.43	28.57

Total Organic Carbon (TOC)

Table A-7 – Total organic carbon concentrations (in mg/L of C) as non-purgable organic carbon (NPOC) and inorganic carbon (IC) measured at the inlet, middle, and outlet at Valencia and Bearspaw stormwater retention ponds

Pond	Valencia						Bearspaw					
Sample Period	Inlet		Middle		Outlet		Inlet		Middle		Outlet	
	NPOC	IC	NPOC	IC	NPOC	IC	NPOC	IC	NPOC	IC	NPOC	IC
1	13.5	23.94	13.65	20.76	13.7	18.83	8.355	28.34	7.871	27.52	8.765	27.93
2	7.6835	26.14	7.8925	26.29	7.5845	26.27	9.3345	14.495	8.9145	15.79	8.8695	15.485
3	-	-	-	-	-	-	10.022	18.93	9.5715	18.845	9.875	21.21
4	18.455	20.195	9.81	20.995	8.645	20.275	8.06	20.975	7.765	20.125	7.87	21.37
5	9.235	20.865	9.165	21	8.935	21.735	8.875	26.185	9.135	22.775	8.64	22.775
6	9.835	26.165	10.265	26.125	9.91	25.905	9.18	26.225	9.09	26.15	9.37	26.375

WATER CHEMISTRY

Total Phosphorus

Table A-8 – Total phosphorus concentrations (in µg/L) measured at the inlet, middle, and outlet at Valencia and Bearspaw stormwater retention ponds

Lake	Valencia			Bearspaw		
Sample Period	Inlet	Middle	ug/L	Inlet	Middle	Outlet
1	240	250	500	58	57	65
	250	250	520	71	65	66
2	168	107	93	87	88	84
	177	105	92	87	88	84
3	121	89	125	169	177	170
	121	89	125	169	177	170
4	102	100	86	266	248	457
	102	99	86	263	257	424
5	109	105	105	537	312	318
	108	102	103	546	322	317
6	154	150	156	247	244	279
	149	145	156	257	244	278

Alkalinity

Table A-9 – Alkalinity concentrations (in mg/L as $CaCO_3$) measured at the inlet, middle, and outlet at Valencia and Bearspaw stormwater retention ponds

Pond	Valencia			Bearspaw		
Sample Period	Inlet	Middle	Outlet	Inlet	Middle	Outlet
1	98	96	108	98	97	110
2	40	75	80	98	93	113
3	79	65	51	40	54	52
4	67	73	62	49	77	70
5	83	73	83	71	90	110
6	40	84	86	33	73	93

pH

Table A-10 – pH measured at the inlet, middle, and outlet at Valencia and Bearspaw stormwater retention ponds

Pond	Valencia			Bearspaw		
Sample Period	Inlet	Middle	Outlet	Inlet	Middle	Outlet
1	7.62	7.71	8.47	7.98	7.97	7.88
2	8.77	8.78	8.7	7.79	7.69	7.71
3	7.77	8.61	8.91	9.55	8.5	8.32
4	8.97	8.92	9.05	9.41	9.36	8.85
5	9.24	9.38	9.42	8.87	9.87	10.12
6	9.26	9.12	9.33	9.7	9.62	9.59

Conductivity

Table A-11 – Conductivity (in µS/cm) measured at the inlet, middle, and outlet at Valencia and Bearspaw stormwater retention ponds

Pond	Valencia			Bearspaw		
Sample Period	Inlet	Middle	Outlet	Inlet	Middle	Outlet
1	1218	1121	1044	1160	1138	1123
2	1088	1028	1022	1069	1036	1005
3	1096	1073	1061	500	464	455
4	957	980	1048	601	592	584
5	934	936	1040	722	609	618
6	1057	1069	1045	773	709	669

Oxidation-Reduction Potential

Table A-12 – Oxidation-reduction potential (mV) measured at the inlet, middle, and outlet at Valencia and Bearspaw stormwater retention ponds

Pond	Valencia			Bearspaw		
Sample Period	Inlet	Middle	Outlet	Inlet	Middle	Outlet
1	-	-	-	-	-	-
2	171	139	102	179	246	224
3	48	140	183	100	172	185
4	151	127	75	101	122	162
5	132	124	109	146	101	96
6	179	147	114	120	109	106

Dissolved Metals

Table A-13 – Initial dissolved metals concentrations measured at the inlet, middle, and outlet of Valencia stormwater retention pond

	UNITS	VALENCIA MID 1	VALENCIA MID 2	VALENCIA IN 1	VALENCIA IN 2
Low Level Elements					
Dissolved Cadmium (Cd)	ug/L	0.0080	0.0096	0.0058	0.0055
Elements					
Dissolved Aluminum (Al)	mg/L	0.0053	0.0078	0.0034	0.0040
Dissolved Antimony (Sb)	mg/L	<0.00060	<0.00060	<0.00060	<0.00060
Dissolved Arsenic (As)	mg/L	0.0011	0.0011	0.0010	0.00096
Dissolved Barium (Ba)	mg/L	0.040	0.040	0.036	0.035
Dissolved Beryllium (Be)	mg/L	<0.0010	<0.0010	<0.0010	<0.0010
Dissolved Boron (B)	mg/L	0.022	0.023	0.021	0.022
Dissolved Calcium (Ca)	mg/L	100	100	96	95
Dissolved Chromium (Cr)	mg/L	<0.0010	<0.0010	<0.0010	<0.0010
Dissolved Cobalt (Co)	mg/L	<0.00030	<0.00030	<0.00030	<0.00030
Dissolved Copper (Cu)	mg/L	0.00097	0.00066	0.00072	0.00095
Dissolved Iron (Fe)	mg/L	0.13	0.12	0.099	0.095
Dissolved Lead (Pb)	mg/L	<0.00020	<0.00020	<0.00020	<0.00020
Dissolved Lithium (Li)	mg/L	0.045	0.046	0.046	0.046
Dissolved Magnesium (Mg)	mg/L	35	36	35	35
Dissolved Manganese (Mn)	mg/L	0.24	0.24	0.088	0.044
Dissolved Molybdenum (Mo)	mg/L	0.00045	0.00043	0.00063	0.00062
Dissolved Nickel (Ni)	mg/L	0.0014	0.0014	0.0016	0.0016
Dissolved Phosphorus (P)	mg/L	0.20	0.21	<0.10	<0.10
Dissolved Potassium (K)	mg/L	4.7	4.7	4.7	4.7
Dissolved Selenium (Se)	mg/L	0.00035	0.00035	0.00054	0.00051
Dissolved Silicon (Si)	mg/L	1.8	1.8	1.2	1.2
Dissolved Silver (Ag)	mg/L	<0.00010	<0.00010	<0.00010	<0.00010
Dissolved Sodium (Na)	mg/L	110	110	120	120
Dissolved Strontium (Sr)	mg/L	0.62	0.62	0.61	0.61
Dissolved Sulphur (S)	mg/L	110	110	120	120
Dissolved Thallium (Tl)	mg/L	<0.00020	<0.00020	<0.00020	<0.00020
Dissolved Tin (Sn)	mg/L	<0.0010	<0.0010	<0.0010	<0.0010
Dissolved Titanium (Ti)	mg/L	<0.0010	<0.0010	<0.0010	<0.0010
Dissolved Uranium (U)	mg/L	0.0039	0.0039	0.0042	0.0042
Dissolved Vanadium (V)	mg/L	<0.0010	<0.0010	<0.0010	<0.0010
Dissolved Zinc (Zn)	mg/L	<0.0030	<0.0030	0.0039	<0.0030

Table A-14 – Final dissolved metals concentrations measured at the inlet, middle, and outlet of Valencia stormwater retention pond

	UNITS	VALENCIA IN 1	VALENCIA IN 2	VALENCIA MID 1	VALENCIA MID 2	VALENCIA OUT 1	VALENCIA OUT 2
Low Level Elements							
Dissolved Cadmium (Cd)	ug/L	<0.025	<0.025	<0.025	<0.025	0.039	<0.025
Elements							
Dissolved Aluminum (Al)	mg/L	0.0036	0.0029	0.0032	0.0040	0.0033	0.0035
Dissolved Antimony (Sb)	mg/L	<0.00060	<0.00060	<0.00060	<0.00060	<0.00060	<0.00060
Dissolved Arsenic (As)	mg/L	0.0013	0.0013	0.0012	0.0013	0.0013	0.0013
Dissolved Barium (Ba)	mg/L	0.040	0.039	0.038	0.038	0.036	0.036
Dissolved Beryllium (Be)	mg/L	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010
Dissolved Boron (B)	mg/L	0.061	0.058	0.058	0.059	0.057	0.057
Dissolved Calcium (Ca)	mg/L	81	78	77	78	76	76
Dissolved Chromium (Cr)	mg/L	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010
Dissolved Cobalt (Co)	mg/L	<0.00030	<0.00030	<0.00030	<0.00030	<0.00030	<0.00030
Dissolved Copper (Cu)	mg/L	<0.00020	<0.00020	0.00020	<0.00020	0.00022	<0.00020
Dissolved Iron (Fe)	mg/L	<0.060	<0.060	<0.060	<0.060	<0.060	<0.060
Dissolved Lead (Pb)	mg/L	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020
Dissolved Lithium (Li)	mg/L	0.047	0.047	0.046	0.046	0.045	0.046
Dissolved Magnesium (Mg)	mg/L	34	33	32	33	32	32
Dissolved Manganese (Mn)	mg/L	0.012	0.011	0.010	0.0082	<0.0040	<0.0040
Dissolved Molybdenum (Mo)	mg/L	0.00090	0.00076	0.00069	0.00074	0.00076	0.00069
Dissolved Nickel (Ni)	mg/L	0.0013	0.0013	0.0013	0.0012	0.0012	0.0012
Dissolved Phosphorus (P)	mg/L	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Dissolved Potassium (K)	mg/L	4.5	4.4	4.3	4.4	4.4	4.4
Dissolved Selenium (Se)	mg/L	0.00039	0.00042	0.00034	0.00034	0.00034	0.00036
Dissolved Silicon (Si)	mg/L	2.0	2.0	1.9	2.0	1.9	1.9
Dissolved Silver (Ag)	mg/L	<0.00010	<0.00010	<0.00010	<0.00010	<0.00010	<0.00010
Dissolved Sodium (Na)	mg/L	94	93	90	91	90	90
Dissolved Strontium (Sr)	mg/L	0.58	0.57	0.56	0.57	0.56	0.56
Dissolved Sulphur (S)	mg/L	99	100	99	100	98	98
Dissolved Thallium (Tl)	mg/L	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020
Dissolved Tin (Sn)	mg/L	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010
Dissolved Titanium (Ti)	mg/L	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010
Dissolved Uranium (U)	mg/L	0.0038	0.0038	0.0037	0.0037	0.0038	0.0037
Dissolved Vanadium (V)	mg/L	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010
Dissolved Zinc (Zn)	mg/L	<0.0030	<0.0030	<0.0030	<0.0030	<0.0030	<0.0030

Table A-15 – Initial dissolved metals concentrations measured in the initial, middle, and outlet of Bearspaw stormwater retention pond

	UNITS	BEARSPAW OUT 1	BEARSPAW OUT 2	BEARSPAW MID 1	BEARSPAW MID 2	BEARSPAW IN 1	BEARSPAW IN 2
Low Level Elements							
Dissolved Cadmium (Cd)	ug/L	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
Elements							
Dissolved Aluminum (Al)	mg/L	0.0059	0.005	0.0044	0.0047	0.011	0.0051
Dissolved Antimony (Sb)	mg/L	<0.00060	<0.00060	<0.00060	<0.00060	<0.00060	<0.00060
Dissolved Arsenic (As)	mg/L	0.00086	0.00087	0.0008	0.00076	0.00092	0.00079
Dissolved Barium (Ba)	mg/L	0.041	0.04	0.041	0.041	0.04	0.04
Dissolved Beryllium (Be)	mg/L	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010
Dissolved Boron (B)	mg/L	0.038	0.039	0.038	0.036	0.038	0.036
Dissolved Calcium (Ca)	mg/L	68	66	65	67	68	68
Dissolved Chromium (Cr)	mg/L	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010
Dissolved Cobalt (Co)	mg/L	<0.00030	<0.00030	<0.00030	<0.00030	<0.00030	<0.00030
Dissolved Copper (Cu)	mg/L	0.0079	0.008	0.0017	0.0015	0.0016	0.0013
Dissolved Iron (Fe)	mg/L	<0.060	<0.060	<0.060	<0.060	<0.060	<0.060
Dissolved Lead (Pb)	mg/L	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020
Dissolved Lithium (Li)	mg/L	0.035	0.035	0.035	0.035	0.035	0.035
Dissolved Magnesium (Mg)	mg/L	27	26	26	26	27	26
Dissolved Manganese (Mn)	mg/L	0.086	0.09	0.085	0.086	0.082	0.079
Dissolved Molybdenum (Mo)	mg/L	0.00086	0.00085	0.00098	0.00092	0.00094	0.00084
Dissolved Nickel (Ni)	mg/L	0.0011	0.0015	0.0017	0.0011	0.0019	0.0013
Dissolved Phosphorus (P)	mg/L	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Dissolved Potassium (K)	mg/L	5.5	5.5	5.3	5.3	5.4	5.4
Dissolved Selenium (Se)	mg/L	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020
Dissolved Silicon (Si)	mg/L	0.9	0.9	0.88	0.87	0.9	0.87
Dissolved Silver (Ag)	mg/L	<0.00010	<0.00010	<0.00010	<0.00010	<0.00010	<0.00010
Dissolved Sodium (Na)	mg/L	120	120	120	120	120	120
Dissolved Strontium (Sr)	mg/L	0.43	0.42	0.41	0.42	0.43	0.43
Dissolved Sulphur (S)	mg/L	65	67	68	66	65	64
Dissolved Thallium (Tl)	mg/L	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020
Dissolved Tin (Sn)	mg/L	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010
Dissolved Titanium (Ti)	mg/L	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010
Dissolved Uranium (U)	mg/L	0.0024	0.0023	0.0024	0.0023	0.0025	0.0024
Dissolved Vanadium (V)	mg/L	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010
Dissolved Zinc (Zn)	mg/L	<0.0030	<0.0030	<0.0030	<0.0030	<0.0030	<0.0030

Table A-16 – Final dissolved metals concentrations measured at the inlet, middle, and outlet of Bearspaw stormwater retention pond

	UNITS	BEARSPAW IN1	BEARSPAW IN2	BEARSPAW MID1	BEARSPAW MID2	BEARSPAW OUT1	BEARSPAW OUT2
Low Level Elements							
Dissolved Cadmium (Cd)	ug/L	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
Elements							
Dissolved Aluminum (Al)	mg/L	0.0068	0.0038	0.011	0.0047	0.041	0.0042
Dissolved Antimony (Sb)	mg/L	<0.00060	<0.00060	<0.00060	<0.00060	<0.00060	<0.00060
Dissolved Arsenic (As)	mg/L	0.0016	0.0018	0.0017	0.0017	0.0017	0.0017
Dissolved Barium (Ba)	mg/L	0.039	0.035	0.037	0.037	0.036	0.036
Dissolved Beryllium (Be)	mg/L	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010
Dissolved Boron (B)	mg/L	0.044	0.042	0.043	0.044	0.043	0.043
Dissolved Calcium (Ca)	mg/L	69	69	68	69	68	68
Dissolved Chromium (Cr)	mg/L	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010
Dissolved Cobalt (Co)	mg/L	<0.00030	<0.00030	<0.00030	<0.00030	<0.00030	<0.00030
Dissolved Copper (Cu)	mg/L	0.0016	0.00089	0.00037	0.00049	0.00038	0.00036
Dissolved Iron (Fe)	mg/L	<0.060	<0.060	<0.060	<0.060	<0.060	<0.060
Dissolved Lead (Pb)	mg/L	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020
Dissolved Lithium (Li)	mg/L	0.032	0.033	0.032	0.032	0.032	0.032
Dissolved Magnesium (Mg)	mg/L	21	21	21	21	21	21
Dissolved Manganese (Mn)	mg/L	0.0058	0.0067	0.0054	0.0064	0.0061	0.0056
Dissolved Molybdenum (Mo)	mg/L	0.00070	0.00059	0.00051	0.00055	0.00058	0.00063
Dissolved Nickel (Ni)	mg/L	0.0014	0.0013	0.0014	0.0013	0.0013	0.0012
Dissolved Phosphorus (P)	mg/L	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Dissolved Potassium (K)	mg/L	3.9	3.9	3.9	3.9	3.9	3.9
Dissolved Selenium (Se)	mg/L	<0.00020	<0.00020	0.00036	<0.00020	0.00038	<0.00020
Dissolved Silicon (Si)	mg/L	1.6	1.6	1.6	1.7	1.6	1.6
Dissolved Silver (Ag)	mg/L	<0.00010	<0.00010	<0.00010	<0.00010	<0.00010	<0.00010
Dissolved Sodium (Na)	mg/L	52	52	52	52	52	52
Dissolved Strontium (Sr)	mg/L	0.37	0.38	0.37	0.37	0.37	0.37
Dissolved Sulphur (S)	mg/L	50	51	50	51	50	50
Dissolved Thallium (Tl)	mg/L	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020
Dissolved Tin (Sn)	mg/L	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010
Dissolved Titanium (Ti)	mg/L	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010
Dissolved Uranium (U)	mg/L	0.0020	0.0020	0.0020	0.0019	0.0020	0.0020
Dissolved Vanadium (V)	mg/L	0.0010	0.0010	0.0010	<0.0010	<0.0010	0.0011
Dissolved Zinc (Zn)	mg/L	<0.0030	<0.0030	<0.0030	<0.0030	<0.0030	<0.0030

Table A-17 – Initial sediment total metals concentration measurements at the inlet, middle, and outlet of Valencia stormwater retention pond

	UNITS	VALENCIA OUT 1	VALENCIA OUT 2	VALENCIA MID 1	VALENCIA MID 2	VALENCIA IN 1	VALENCIA IN 2
Elements							
Hex. Chromium (Cr 6+)	mg/kg	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15
Total Antimony (Sb)	mg/kg	1.4	1.5	1.5	1.5	1.7	1.7
Total Arsenic (As)	mg/kg	6.7	7.1	6.7	6.3	7.8	7.9
Total Barium (Ba)	mg/kg	110	120	150	150	170	170
Total Beryllium (Be)	mg/kg	0.49	0.50	0.60	0.54	0.64	0.63
Total Cadmium (Cd)	mg/kg	0.57	0.58	0.57	0.57	0.57	0.58
Total Chromium (Cr)	mg/kg	19	21	31	31	32	32
Total Cobalt (Co)	mg/kg	8.3	8.6	7.7	7.4	8.4	8.5
Total Copper (Cu)	mg/kg	38	40	44	44	48	49
Total Lead (Pb)	mg/kg	28	30	40	45	39	39
Total Mercury (Hg)	mg/kg	0.081	0.076	0.057	0.059	0.064	0.065
Total Molybdenum (Mo)	mg/kg	3.4	3.3	2.9	3.0	3.3	3.1
Total Nickel (Ni)	mg/kg	29	30	31	30	31	32
Total Selenium (Se)	mg/kg	3.3	3.5	2.4	2.1	2.7	2.5
Total Silver (Ag)	mg/kg	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Total Thallium (Tl)	mg/kg	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30
Total Tin (Sn)	mg/kg	1.7	1.6	3.0	2.8	2.8	2.8
Total Uranium (U)	mg/kg	18	17	8.9	9.3	10	9.3
Total Vanadium (V)	mg/kg	29	31	34	31	37	36
Total Zinc (Zn)	mg/kg	170	180	250	240	260	260

Table A-18 – Final sediment total metals concentrations measured at the inlet, middle, and outlet of Valencia stormwater retention pond

	UNITS	VALENCIA IN	VALENCIA MID	VALENCIA OUT
Elements				
Soluble (Hot water) Boron (B)	mg/kg	1.3 (1)	1.7 (1)	1.8 (1)
Hex. Chromium (Cr 6+)	mg/kg	<0.15	<0.15	<0.15
Total Antimony (Sb)	mg/kg	1.6	1.1	<1.0
Total Arsenic (As)	mg/kg	7.7	6.4	5.7
Total Barium (Ba)	mg/kg	190	140	130
Total Beryllium (Be)	mg/kg	0.79	0.70	0.57
Total Cadmium (Cd)	mg/kg	0.51	0.75	0.51
Total Chromium (Cr)	mg/kg	34	32	20
Total Cobalt (Co)	mg/kg	8.5	8.0	7.8
Total Copper (Cu)	mg/kg	45	43	32
Total Lead (Pb)	mg/kg	33	57	30
Total Mercury (Hg)	mg/kg	0.061	0.064	0.055
Total Molybdenum (Mo)	mg/kg	2.4	2.8	1.7
Total Nickel (Ni)	mg/kg	33	31	26
Total Selenium (Se)	mg/kg	2.1	2.8	2.1
Total Silver (Ag)	mg/kg	<1.0	<1.0	<1.0
Total Thallium (Tl)	mg/kg	<0.30	<0.30	<0.30
Total Tin (Sn)	mg/kg	2.4	2.3	1.2
Total Uranium (U)	mg/kg	6.4	11	8.8
Total Vanadium (V)	mg/kg	42	34	28
Total Zinc (Zn)	mg/kg	230	230	130

Table A-19 – Initial sediment total metals concentrations measured at the inlet, middle, and outlet of Bearspaw stormwater retention pond

	UNITS	BEARSPAW OUT 1	BEARSPAW OUT 2	BEARSPAW MID 1	BEARSPAW MID 2	BEARSPAW IN 1	BEARSPAW IN 2
Elements							
Soluble (Hot water) Boron (B)	mg/kg	<10 (1)	<10 (1)	<10 (1)	<10 (1)	<10 (1)	<10 (1)
Hex. Chromium (Cr 6+)	mg/kg	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15 (2)
Total Antimony (Sb)	mg/kg	1.3	1.4	1.5	1.5	1.8	1.7
Total Arsenic (As)	mg/kg	8.0	8.6	8.2	8.4	8.1	8.4
Total Barium (Ba)	mg/kg	210	210	210	210	180	170
Total Beryllium (Be)	mg/kg	0.72	0.77	0.76	0.76	0.63	0.65
Total Cadmium (Cd)	mg/kg	0.52	0.56	0.52	0.52	0.53	0.53
Total Chromium (Cr)	mg/kg	26	27	29	29	31	31
Total Cobalt (Co)	mg/kg	10	11	11	11	11	11
Total Copper (Cu)	mg/kg	97	97	71	73	57	62
Total Lead (Pb)	mg/kg	23	24	25	26	29	28
Total Mercury (Hg)	mg/kg	0.094	0.092	0.089	0.089	0.085	0.089
Total Molybdenum (Mo)	mg/kg	2.0	2.4	3.0	3.0	2.5	2.7
Total Nickel (Ni)	mg/kg	32	32	33	33	32	33
Total Selenium (Se)	mg/kg	1.3	1.4	1.3	1.3	1.0	1.1
Total Silver (Ag)	mg/kg	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Total Thallium (Tl)	mg/kg	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30
Total Tin (Sn)	mg/kg	1.4	1.5	1.9	1.9	3.0	2.9
Total Uranium (U)	mg/kg	6.4	7.0	6.8	6.8	4.2	5.0
Total Vanadium (V)	mg/kg	40	43	42	41	38	39
Total Zinc (Zn)	mg/kg	160	170	190	190	240	240

Table A-20 – Final sediment total metals concentrations measured at the inlet, middle, and outlet of Bearspaw stormwater retention pond

	UNITS	BEARSPAW IN	BEARSPAW MID	BEARSPAW OUT
Elements				
Soluble (Hot water) Boron (B)	mg/kg	1.7 (1)	1.4 (1)	1.3 (1)
Hex. Chromium (Cr 6+)	mg/kg	<0.15	<0.15	<0.15
Total Antimony (Sb)	mg/kg	1.4	1.6	2.0
Total Arsenic (As)	mg/kg	8.2	9.0	8.7
Total Barium (Ba)	mg/kg	230	210	180
Total Beryllium (Be)	mg/kg	0.85	0.83	0.65
Total Cadmium (Cd)	mg/kg	0.57	0.63	0.56
Total Chromium (Cr)	mg/kg	32	35	34
Total Cobalt (Co)	mg/kg	11	11	11
Total Copper (Cu)	mg/kg	100	72	58
Total Lead (Pb)	mg/kg	25	34	30
Total Mercury (Hg)	mg/kg	0.070	0.068	0.050
Total Molybdenum (Mo)	mg/kg	1.8	2.9	2.9
Total Nickel (Ni)	mg/kg	33	36	34
Total Selenium (Se)	mg/kg	1.4	1.3	1.1
Total Silver (Ag)	mg/kg	<1.0	<1.0	<1.0
Total Thallium (Tl)	mg/kg	<0.30	<0.30	<0.30
Total Tin (Sn)	mg/kg	1.6	2.0	2.9
Total Uranium (U)	mg/kg	5.4	6.0	4.7
Total Vanadium (V)	mg/kg	49	49	44
Total Zinc (Zn)	mg/kg	190	220	260

MOLECULAR BIOLOGY – QUANTITATIVE POLYMERASE CHAIN REACTION (qPCR)

Table A-21 – qPCR gene counts per gram sediment for Total Bacteria (rpoB gene), SRB (dsrB), NRB (nosZ2), measured at the inlet, middle and outlet at Valencia stormwater retention pond in triplicate

Sample Period	Location	Total Bacteria			SRB			NRB		
1	Inlet	1.44E+04	2.35E+04	1.57E+04	6.32E+04	7.26E+04	6.87E+04	9.27E+03	7.73E+01	8.03E+03
	Middle	4.27E+04	3.12E+04	4.18E+04	1.25E+05	1.22E+05	1.27E+05	1.77E+04	6.03E+02	1.65E+04
	Outlet	1.97E+04	1.82E+04	2.09E+04	1.42E+05	1.42E+05	1.32E+05	1.75E+04	5.65E+03	1.41E+04
2	Inlet	2.49E+04	2.75E+04	2.60E+04	1.23E+05	1.22E+05	1.64E+05	2.25E+04	7.06E+02	1.70E+04
	Middle	4.67E+04	5.53E+04	4.61E+03	1.28E+05	1.31E+05	1.26E+05	9.89E+03	1.37E+04	3.91E+03
	Outlet	1.22E+04	1.60E+04	1.95E+04	1.79E+05	1.33E+05	1.59E+05	1.81E+04	1.51E+04	1.49E+04
3	Inlet	4.71E+03	5.95E+03	7.03E+03	9.97E+03	1.03E+04	1.34E+04	2.07E+03	1.41E+03	1.87E+03
	Middle	6.76E+04	1.06E+05	8.40E+04	1.56E+05	1.87E+05	1.47E+05	1.06E+04	3.77E+03	1.04E+04
	Outlet	1.48E+04	1.77E+04	1.05E+04	1.81E+05	1.10E+05	7.73E+04	5.88E+03	5.71E+03	7.81E+03
4	Inlet	3.58E+04	7.10E+04	4.51E+04	1.11E+05	1.07E+05	1.18E+05	9.74E+03	7.54E+03	1.01E+04
	Middle	4.59E+04	4.24E+04	4.45E+04	1.18E+05	1.06E+05	9.94E+04	6.28E+03	1.54E+04	1.23E+04
	Outlet	2.29E+04	3.80E+04	4.65E+04	1.67E+05	1.61E+05	1.84E+05	1.51E+04	1.65E+04	1.85E+04
5	Inlet	3.26E+04	3.89E+04	4.06E+04	7.33E+04	9.36E+04	8.73E+04	1.10E+04	1.05E+04	1.34E+04
	Middle	4.27E+04	5.88E+04	5.86E+04	9.97E+04	8.67E+04	5.29E+04	1.63E+04	1.45E+04	1.20E+04
	Outlet	2.26E+04	2.62E+04	2.78E+04	1.03E+05	1.27E+05	9.32E+04	1.81E+04	1.16E+04	1.09E+04
6	Inlet	5.63E+04	5.66E+04	5.03E+04	1.28E+05	1.48E+05	7.95E+04	1.90E+04	2.03E+04	1.90E+04
	Middle	5.20E+04	5.73E+04	3.87E+04	1.03E+05	1.27E+05	1.18E+05	1.34E+04	9.94E+03	1.19E+04
	Outlet	2.60E+04	2.75E+04	2.62E+04	9.41E+04	1.06E+05	9.26E+04	1.50E+04	4.33E+03	7.71E+03

Table A-22 – qPCR gene counts per gram sediment for Total Bacteria (rpoB gene), SRB (dsrB), NRB (nosZ2), measured at the inlet, middle and outlet at Bearspaw stormwater retention pond in triplicate

Sample Period	Location	Total Bacteria			SRB			NRB		
1	Inlet	1.26E+04	1.26E+04	1.96E+04	1.31E+05	1.37E+05	1.34E+05	6.28E+03	1.89E+04	1.18E+03
	Middle	1.53E+04	1.66E+04	1.54E+04	1.07E+05	1.14E+05	1.19E+05	6.16E+03	7.45E+03	1.36E+04
	Outlet	3.80E+04	4.10E+04	5.25E+04	1.49E+05	1.50E+05	1.56E+05	2.32E+04	2.24E+04	2.38E+04
2	Inlet	4.46E+03	2.23E+04	1.77E+04	1.28E+05	5.74E+04	8.88E+04	4.34E+03	8.24E+03	7.08E+02
	Middle	3.93E+04	4.34E+04	2.09E+04	1.87E+05	2.17E+05	1.17E+05	8.20E+03	1.67E+04	2.05E+04
	Outlet	3.58E+02	3.19E+04	2.00E+02	2.40E+05	2.02E+05	2.15E+05	6.38E+01	2.20E+04	1.80E+04
3	Inlet	3.20E+04	4.29E+04	4.64E+04	2.68E+05	1.89E+05	1.45E+05	1.12E+04	1.21E+04	5.92E+03
	Middle	3.84E+04	2.11E+04	4.71E+04	6.92E+04	6.06E+04	1.79E+05	1.47E+04	4.02E+02	1.28E+04
	Outlet	4.28E+04	4.14E+04	6.79E+04	1.34E+05	N/A	N/A	4.03E+02	3.02E+02	1.46E+04
4	Inlet	2.32E+04	2.50E+04	2.41E+04	7.30E+04	1.15E+05	9.97E+04	1.38E+04	1.90E+04	1.32E+04
	Middle	5.56E+04	5.81E+04	3.93E+04	1.32E+05	1.39E+05	1.39E+05	1.63E+04	2.26E+04	2.21E+04
	Outlet	3.85E+04	5.15E+04	4.13E+04	1.01E+05	1.14E+05	1.33E+05	2.18E+04	1.92E+04	2.01E+04
5	Inlet	2.78E+04	3.80E+04	3.39E+04	5.93E+04	9.24E+04	9.79E+04	1.06E+04	1.82E+04	1.14E+04
	Middle	4.05E+04	4.35E+04	4.71E+04	1.28E+05	1.41E+05	1.29E+05	1.92E+04	2.05E+04	1.71E+04
	Outlet	5.63E+04	1.03E+05	7.26E+04	1.30E+05	1.23E+05	1.31E+05	2.59E+04	3.76E+04	2.52E+04
6	Inlet	3.09E+04	3.60E+04	3.35E+04	7.40E+04	9.50E+04	9.60E+04	1.60E+04	1.10E+04	1.62E+04
	Middle	4.27E+04	4.09E+04	4.00E+04	1.19E+05	8.07E+04	1.05E+05	1.96E+04	1.55E+04	1.39E+04
	Outlet	5.75E+04	7.47E+04	6.12E+04	1.51E+05	1.01E+05	8.52E+04	2.59E+04	2.39E+04	2.43E+04

FACTORIAL DESIGN MESOCOSM STUDY

Sulphide Measurements

Table A-23 – Initial sulphide measurements in mesocosms based on water media using the HACH kit an ion-specific sulphide probe in triplicate

Sample	HACH (µg/L)	Probe (mV)	[S2-] (mg/L)
SW1	20	-592.4	1.65E-05
SW2	23	-627.7	9.82E-05
SW3	27	-629.8	1.09E-04
AMM1	630	-805	0.760
AMM2	685	-818	1.47
AMM3	696	-819	1.54

Table A-24 – Final sulphide measurements in mesocosms for each mesocosm scenario using the HACH kit an ion-specific sulphide probe scenario in duplicates

Sample	HACH (µg/L)	Probe (mV)	[S2-] (mg/L)
1A	2432	-852.4	10.7
1B	704	-854.8	12.4
2A	761	-815.2	1.12
2B	591	-807.6	0.709
3A	673	-860.4	17.0
3B	711	-859.7	17.0
4A	867	-866.1	24.0
4B	586	-862.2	19.0
5A	618	-853.9	12.0
5B	629	-856.5	14.0
6A	6088	-835.9	4.00
6B	6656	-836.3	4.00
7A	685	-857.1	14.2
7B	485	-854.8	12.4
8A	659	-860.6	17.5
8B	638	-861.2	18.2

Table A-25 – Factorial design mesocosm results and influence factors

Run	A	B	C	AB	AC	BC	ABC	y		
1	-	-	-	+	+	+	-	10.683	12.353	
2	+	-	-	-	-	+	+	1.124	0.709	
3	-	+	-	-	+	-	+	17.338	16.619	
4	+	+	-	+	-	-	-	24.482	19.334	
5	-	-	+	+	-	-	+	10.442	12.436	
6	+	-	+	-	+	-	-	2.679	2.775	
7	-	+	+	-	-	+	-	12.943	11.097	
8	+	+	+	+	+	+	+	16.293	16.942	

ybar			ni	vi	di	di^2	Si2	
11.518	11.7655625	Mean	2	1	1.67	2.7889	1.39445	1.39445
0.9165	-2.446625	A	2	1	-0.415	0.172225	0.0861125	0.0861125
16.9785	10.230875	B	2	1	-0.719	0.516961	0.2584805	0.2584805
21.908	-2.129375	C	2	1	-5.148	26.501904	13.250952	13.250952
11.439	7.210125	AB	2	1	1.994	3.976036	1.988018	1.988018
2.727	0.389375	AC	2	1	0.096	0.009216	0.004608	0.004608
12.02	-2.995125	BC	2	1	-1.846	3.407716	1.703858	1.703858
16.6175	-0.555375	ABC	2	1	0.649	0.421201	0.2106005	0.2106005

Sulphate

Table A-26 – Initial sulphate concentrations in each mesocosm scenario scenario in duplicates

Mesocosm	Concentration (mg/L)
1	282.19
1	282.11
2	276.04
2	278.94
3	273.46
3	273.63
4	269.42
4	276.81
5	12.09
5	11.92
6	11.56
6	11.76
7	12.06
7	12.34
8	11.55
8	11.77

Table A-27 – Final sulphate concentrations in each mesocosm scenario scenario in duplicates

Mesocosm	Concentration (mg/L)
1A	136.72
1A	137.11
1B	93.55
1B	93.77
2A	473.86
2A	477.95
2B	497.84
2B	494.29
3A	6.28
3A	5.87
3B	7.45
3B	7.13
4A	27.37
4A	28.02
4B	29.41
4B	29.178
5A	23.54
5A	23.84
5B	18.53
5B	18.99
6A	341.44
6A	341.79
6B	330.71
6B	328.87
7A	11.2
7A	11.2
7B	11.09
7B	10.98
8A	8.09
8A	8.62
8B	10.49
8B	9.98

Nitrate and Nitrite

Table A-28 – Initial nitrate and nitrite, nitrate, and nitrite concentrations for each mesocosm scenario in duplicates

Mesocosm	Nitrate+Nitrite (µg/L)	Nitrite (µg/L)	Nitrate (µg/L)
1	3	2	1
1	3	2	1
2	124000	4	123996
2	119200	3	119197
3	27	7	20
3	26	6	20
4	118400	9	118391
4	115200	9	115191
5	8	6	2
5	5	5	0
6	120000	5	119995
6	122400	5	122395
7	8	5	3
7	7	4	3
8	106400	4	106396
8	120000	4	119996

Table A-29 – Final nitrate and nitrite, nitrate, and nitrite concentrations for each mesocosm scenario in duplicates

Mesocosm	Nitrate+Nitrite (µg/L)	Nitrite (µg/L)	Nitrate (µg/L)
1A	7	3	4
1A	8	3	5
1B	8	0	8
1B	7	9	0
2A	4	2	2
2A	3	2	1
2B	6	2	4
2B	7	2	5
3A	0	2	0
3A	0	2	0
3B	0	2	0
3B	0	1	0
4A	0	3	0
4A	0	3	0
4B	0	3	0
4B	0	3	0
5A	2	0	2
5A	0	0	0
5B	0	0	0
5B	0	0	0
6A	2	2	0
6A	3	2	1
6B	2	3	-1
6B	2	3	-1
7A	0	0	0
7A	0	0	0
7B	0	0	0
7B	0	0	0
8A	0	1	-1
8A	0	1	-1
8B	0	1	-1
8B	0	0	0

*The values with 0, indicate below method detection limits

*The values with -1, indicate errors and discrepancies in nitrate and nitrite balance by the laboratory equipment

Dissolved Organic Carbon (DOC)

Table A-30 – Initial dissolved organic carbon concentrations for each mesocosm scenario including non-purgable organic carbon (NPOC) and inorganic carbon (IC) in duplicates

Mesocosm	NPOC (mg C/L)	IC (mg C/L)
1	20.55	27.2
1	24.03	27.77
2	24.88	27.63
2	24.66	27.81
3	238.2	27.96
3	237.7	27.93
4	239.1	27.48
4	243.6	27.71
5	112.3	264.6
5	112.3	266.5
6	100.5	234.3
6	91.27	213.3
7	307.1	212
7	307.8	212.6
8	308.4	213.6
8	303.2	210.2

Table A-31 – Final dissolved organic carbon concentrations for each mesocosm scenario including non-purgable organic carbon (NPOC) and inorganic carbon (IC) in duplicates

Mesocosm	NPOC (mg C/L)	IC (mg C/L)
1A	9.884	60.38
1B	8.875	59.49
2A	8.642	65.01
2B	8.499	60.72
3A	14.3	124.2
3B	15.13	137.4
4A	12.61	157
4B	12.07	149.6
5A	7.59	157.9
5B	8.522	174.4
6A	7.576	173.8
6B	7.917	192.3
7A	9.809	216.6
7B	10.25	229.2
8A	9.966	249.1
8B	17.32	278.1

Biochemical Oxygen Demand (BOD)

Table A-32 – Initial biochemical oxygen demand (in mg/L O2) results measured in each mesocosm scenario in duplicate

BOD-0				BOD-5				
Sample	Initial (mL)	Final (mL)	Total (mL)	Sample	Initial (mL)	Final (mL)	Total (mL)	BOD (mg/L)
1	0	13.75	13.75	1	0	8.05	8.05	171
	13.75	26.8	13.05		8.05	15.6	7.55	165
2	26.8	39.75	12.95	2	15.6	24.15	8.55	132
	0	11.45	11.45		24.15	32.15	8	103.5
3	11.45	21.85	10.4	3	32.15	38.9	6.75	109.5
	21.85	32.85	11		38.9	47.5	8.6	72
4	32.85	43.8	10.95	4	0	2.6	2.6	250.5
	0	10.55	10.55		2.6	5.4	2.8	232.5
5	10.55	19.6	9.05	5	5.4	12.75	7.35	51
	19.6	28.7	9.1		12.75	19.2	6.45	79.5
6	28.7	36.35	7.65	6	19.2	26.1	6.9	22.5
	36.35	44.6	8.25		26.1	33.05	6.95	39
7	0.05	7.9	7.85	7	33.05	40.4	7.35	15
	7.9	16.8	8.9		40.4	48.3	7.9	30
8	16.8	24.4	7.6	8	48.3	48.55	0.25	220.5
	24.4	32.5	8.1		48.55	48.8	0.25	235.5
Blank	32.5	40.1	7.6	Blank	16.6	25.2	8.6	-1
	40.1	50.3	10.2		25.2	34	8.8	1.4

Table A-33 – Final biochemical oxygen demand (in mg/L O2) results measured in each mesocosm scenario in duplicate

BOD-0				BOD-5				
Sample	Initial (mL)	Final (mL)	Total (mL)	Sample	Initial (mL)	Final (mL)	Total (mL)	BOD (mg/L)
1A	0	7.4	7.4	1A	0	1.1	1.1	189
1B	7.4	13.85	6.45	1B	1.1	3.7	2.6	115.5
2A	13.85	20.7	6.85	2A	3.7	8.35	4.65	66
2B	20.7	28.2	7.5	2B	8.34	11.4	3.06	133.2
3A	28.2	34.3	6.1	3A	11.4	14.95	3.55	76.5
3B	34.3	40.55	6.25	3B	14.95	16.7	1.75	135
4A	40.55	46.6	6.05	4A	16.7	22.3	5.6	13.5
4B	0.25	3.4	3.15	4B	22.3	22.65	0.35	84
5A	3.4	9	5.6	5A	22.65	28.25	5.6	-5.3E-14
5B	9	13.8	4.8	5B	28.25	30.8	2.55	67.5
6A	13.8	18.9	5.1	6A	30.8	34.4	3.6	45
6B	18.9	23.7	4.8	6B	34.4	38.75	4.35	13.5
7A	23.7	28.45	4.75	7A	38.75	40.2	1.45	99
7B	28.45	34.3	5.85	7B	40.2	41.7	1.5	130.5
8A	34.3	41.4	7.1	8A	41.7	44.8	3.1	120
8B	41.4	48.9	7.5	8B	44.8	49.9	5.1	72
Blank	22.5	32.15	9.65	Blank	28.5	37.9	9.4	0.25
Blank	32.15	41.5	9.35	Blank	37.9	48.1	10.2	-0.85

Chemical Oxygen Demand (COD)

Table A-34 – Initial total chemical oxygen demand (in mg/L of O_2) of each mesocosm scenario in duplicate

Mesocosm	Absorbence	Absorbence	Concentration (mg/L)	Concentration (mg/L)
1	0.013	0.013	11.4	11.4
2	0.01	0.011	8.64	9.55
3	0.256	0.236	232	214
4	0.278	0.272	252	247
5	0.228	0.234	207	212
6	0.235	0.234	213	212
7	0.408	0.397	370	360
8	0.399	0.4	362	363

Table A-35 – Final total chemical oxygen demand (in mg/L of O_2) of each mesocosm scenario in duplicate

Sample	Absorbence	Concentration (mg/L)
1A	0.374	570
1B	0.471	732
2A	0.522	817
2B	0.271	398
3A	0.317	475
3B	0.611	965
4A	0.343	518
4B	0.592	933
5A	0.342	517
5B	0.603	952
6A	0.616	973
6B	0.609	962
7A	0.619	978
7B	0.567	892
8A	0.37	563
8B	0.13	163

Total Phosphorus

Table A-36 – Initial total phosphorus concentrations for each mesocosm scenario in duplicate

Mesocosm	Concentration (µg/L)
1	153
1	176
2	131
2	135
3	161
3	168
4	129
4	128
5	11700
5	12200
6	11900
6	12200
7	12200
7	11800
8	12200
8	11500

Table A-37 – Final total phosphorus concentrations for each mesocosm scenario in duplicate

Mesocosm	Concentration (µg/L)
1A	1,680
1A	1,700
1B	1,690
1B	1,680
2A	743
2A	746
2B	894
2B	500
3A	1,810
3A	1,830
3B	1,858
3B	1,848
4A	2,333
4A	2,363
4B	2,414
4B	2,343
5A	64,438
5A	63,933
5B	48,783
5B	48,985
6A	26,462
6A	26,260
6B	21,311
6B	21,311
7A	47,369
7A	47,369
7B	48,278
7B	48,379
8A	34,441
8A	34,239
8B	31,815
8B	31,815

qPCR

Table A-38 – qPCR gene counts per gram sediment for Total Bacteria (rpoB gene), SRB (dsrB), NRB (nosZ2), measured in each mesocosm scenario in triplicate

Mesocosm	Total Bacteria			SRB			NRB		
Initial 1	2.89E+04	2.92E+04	3.20E+04	9.77E+04	1.21E+05	1.38E+05	1.33E+04	1.20E+04	1.20E+04
Initial 2	2.84E+04	3.06E+04	3.01E+04	1.39E+05	1.49E+05	1.90E+05	1.33E+04	1.17E+04	1.45E+04
Initial 3	3.31E+04	1.27E+04	2.05E+04	1.98E+05	3.49E+05	3.03E+05	3.68E+03	3.81E+03	4.12E+03
1A	1.85E+04	1.87E+04	1.97E+04	5.85E+04	3.92E+04	5.86E+04	8.82E+03	9.18E+03	1.07E+04
1B	2.33E+04	2.53E+04	2.73E+04	4.71E+04	5.61E+04	4.86E+04	8.68E+03	9.28E+03	6.61E+03
2A	4.40E+04	4.75E+04	2.50E+04	8.94E+04	9.83E+04	9.35E+04	1.44E+05	1.17E+05	1.37E+05
2B	5.56E+04	6.29E+04	5.36E+04	1.14E+05	1.27E+05	1.02E+05	1.66E+05	1.39E+05	1.63E+05
3A	1.84E+04	1.74E+04	2.15E+04	3.66E+04	4.01E+04	3.34E+04	9.29E+03	6.21E+03	8.54E+03
3B	2.13E+04	2.11E+04	2.07E+04	6.24E+04	6.19E+04	5.22E+04	1.03E+04	9.63E+03	8.69E+03
4A	2.04E+04	2.04E+04	1.56E+04	4.35E+04	3.82E+04	4.23E+04	2.65E+04	2.17E+04	2.72E+04
4B	3.40E+04	3.21E+04	3.44E+04	8.86E+04	9.81E+04	8.79E+04	5.05E+04	5.64E+04	5.53E+04
5A	3.11E+04	2.47E+04	2.77E+04	1.02E+05	9.29E+04	1.05E+05	1.36E+04	1.13E+04	1.44E+04
5B	1.08E+04	1.58E+04	1.72E+04	3.26E+04	3.33E+04	3.75E+04	6.91E+03	8.27E+03	6.97E+03
6A	3.31E+04	3.53E+04	3.32E+04	1.06E+05	9.23E+04	9.38E+04	5.07E+04	4.69E+04	5.57E+04
6B	3.97E+04	3.77E+04	3.52E+04	5.58E+04	5.68E+04	8.54E+04	4.57E+04	3.86E+04	4.37E+04
7A	3.29E+04	3.11E+04	3.32E+04	9.85E+04	1.17E+05	1.01E+05	9.92E+03	1.18E+04	1.07E+04
7B	2.92E+04	2.80E+04	2.49E+04	8.58E+04	7.65E+04	6.54E+04	1.05E+04	1.06E+04	8.02E+03
8A	4.41E+04	4.63E+04	5.02E+04	1.00E+05	1.17E+05	1.14E+05	1.37E+04	1.37E+04	1.15E+04
8B	2.03E+04	2.34E+04	N/A	3.21E+04	3.54E+04	3.14E+04	8.79E+03	8.96E+03	9.15E+03

PEPPER BIOCIDE MESOCOSM EXPERIMENT

Sulphate

Table A-39 – Sulphate concentrations (in mg/L) measured in each pepper biocide mesocosm and their respective controls

Time (hours)	0.01% A	0.01% B	0.01% Control	0.05% A	0.05% B	0.05% Control
0	274.87	277.09	295.87	274.37	274.53	305.29
0	274.89	276.98	295.09	264.48	273.43	304.55
1	268.59	273.18	291.51	269.85	266.78	302.86
1	268.06	274.92	290.28	268.26	267.40	302.65
3	265.41	269.07	284.71	259.63	260.43	301.45
3	265.63	268.09	282.41	259.64	259.98	301.94
6	259.50	261.26	282.19	252.35	252.52	301.24
6	260.03	264.32	281.34	252.27	253.51	301.08
12	252.97	259.06	280.18	253.94	248.05	300.20
12	250.54	259.96	280.00	254.92	248.81	303.16
24	248.51	252.23	278.13	242.86	224.92	305.98
24	249.33	253.39	278.08	242.42	223.18	310.14
48	241.97	245.69	276.76	212.71	204.00	311.78
48	241.97	244.90	276.61	212.31	202.38	316.21
72	236.02	242.00	276.58	184.84	172.90	312.94
72	237.48	242.64	276.48	184.32	171.53	317.26
168	145.91	218.45	306.78	90.75	176.45	337.65
168	145.12	219.00	305.62	90.78	176.41	340.45
504	99.08	132.20	301.51	12.21	150.29	336.73
504	97.95	135.91	302.32	11.92	151.35	335.15

Table A-40 – Sulphide concentrations (in mg/L) measured in each pepper biocide mesocosm and their respective controls

Time (hours)	0.01% A	0.01% B	0.01% Control	0.05% A	0.05% B	0.05% Control
0	2.757964	2.114445	3.282651	1.252877	36.85847	1.678758
1	223.7721	117.5079	3.832068	90.81793	227.4047	6.909797
3	71.32956	64.76028	6.14128	57.8568	63.72579	6.371782
6	159.567	148.4132	5.145757	254.5386	127.3604	3.946706
12	46.17914	44.357	3.380854	16.60965	150.8224	5.539279
24	25.86312	30.38195	1.847538	104.1396	324.0826	1.654198
48	0.476761	0.766669	1.187324	3.087045	3.898955	2.40884
72	2.871258	3.162519	1.448706	7.855325	10.41238	1.48109
168	16.34433	15.57351	0.448866	26.92555	50.05105	0.256381
504	16.85442	19.24514	0.226193	56.43906	53.99796	0.336745

qPCR

Table A-41 – qPCR gene counts per gram sediment for Total Bacteria (rpoB gene), SRB (dsrB), NRB (nosZ2), measured in the 0.01% Pepper biocide mesocosm scenario in triplicate

Time (hours)	Total			SRB			NRB		
0	2.41E+04	2.54E+04	2.29E+04	3.82E+04	4.08E+04	4.79E+04	1.63E+04	1.93E+04	1.80E+04
0	2.14E+04	2.00E+04	2.04E+04	3.76E+04	3.94E+04	3.48E+04	1.48E+04	1.56E+04	1.67E+04
1	1.63E+04	2.30E+04	1.79E+04	3.16E+04	3.61E+04	3.05E+04	1.94E+04	1.96E+04	2.15E+04
1	1.43E+02	1.57E+04	2.08E+04	3.36E+04	5.08E+04	3.83E+04	1.68E+04	1.98E+04	1.77E+04
3	1.77E+04	2.00E+04	1.90E+04	2.62E+04	3.23E+04	3.18E+04	1.79E+04	1.63E+04	1.76E+04
3	1.77E+04	1.77E+04	1.74E+04	2.55E+04	2.87E+04	2.61E+04	2.28E+04	2.11E+04	2.29E+04
6	1.93E+04	2.02E+04	1.87E+04	2.94E+04	3.52E+04	3.14E+04	1.64E+04	2.01E+04	2.13E+04
6	2.20E+04	1.93E+04	2.17E+04	3.60E+04	4.54E+04	3.78E+04	1.79E+04	1.41E+04	1.71E+04
12	2.32E+04	2.18E+04	2.28E+04	4.15E+04	4.83E+04	3.28E+04	2.01E+04	1.72E+04	1.92E+04
12	2.48E+04	2.31E+04	2.26E+04	3.70E+04	3.65E+04	3.19E+04	1.99E+04	2.05E+04	1.90E+04
24	1.86E+04	2.36E+04	2.00E+04	2.91E+04	4.08E+04	3.12E+04	1.75E+04	1.93E+04	1.70E+04
24	3.40E+04	2.44E+04	2.81E+04	3.38E+04	4.58E+04	3.94E+04	2.28E+04	1.79E+04	6.41E+02
48	2.01E+04	2.13E+04	2.30E+04	3.35E+04	3.36E+04	3.61E+04	1.66E+04	1.82E+04	1.74E+04
48	1.91E+04	2.39E+04	2.26E+04	3.14E+04	4.39E+04	4.37E+04	1.85E+04	1.91E+04	2.07E+04
72	2.10E+04	2.23E+04	2.35E+04	4.56E+04	5.15E+04	4.13E+04	2.03E+04	1.80E+04	1.53E+04
72	2.13E+04	2.24E+04	1.91E+04	8.07E+04	6.13E+04	5.38E+04	1.42E+04	1.85E+04	1.48E+04
168	2.16E+04	1.15E+04	2.00E+04	4.50E+04	3.98E+04	2.94E+04	1.08E+04	1.13E+04	9.75E+03
168	9.88E+03	9.67E+03	9.79E+03	1.96E+04	2.07E+04	1.56E+04	9.89E+03	9.63E+03	8.62E+03
504	2.02E+04	1.70E+04	1.73E+04	4.29E+04	4.83E+04	3.66E+04	1.37E+04	1.31E+04	1.33E+04
504	1.78E+04	1.60E+04	1.73E+04	4.36E+04	3.37E+04	2.93E+04	5.65E+03	1.39E+04	1.12E+04

Table A-42 – qPCR gene counts per gram sediment for Total Bacteria (rpoB gene), SRB (dsrB), NRB (nosZ2), measured in the 0.05% Pepper biocide mesocosm scenario in triplicate

time (hours)	Total			SRB			NRB		
0	7.06E+03	6.80E+03	9.65E+03	9.75E+03	1.28E+04	1.20E+04	1.14E+04	1.05E+04	1.11E+04
0	2.15E+04	2.51E+04	2.19E+04	3.24E+04	3.84E+04	4.19E+04	2.26E+04	2.40E+04	2.37E+04
1	2.26E+04	2.18E+04	2.21E+04	2.84E+04	2.88E+04	2.80E+04	2.71E+04	2.35E+04	2.51E+04
1	2.72E+04	3.13E+04	3.41E+04	5.37E+04	5.83E+04	5.33E+04	2.79E+04	3.28E+04	3.11E+04
3	2.48E+04	2.73E+04	3.50E+04	5.69E+04	6.97E+04	5.85E+04	2.66E+04	3.34E+04	3.35E+04
3	6.32E+03	2.80E+04	2.58E+04	5.65E+04	6.14E+04	4.72E+04	1.98E+04	2.06E+04	2.01E+04
6	2.39E+04	2.57E+04	2.89E+04	5.08E+04	4.83E+04	6.99E+04	2.86E+04	3.38E+04	3.17E+04
6	3.64E+04	2.43E+04	3.60E+04	2.24E+04	2.83E+04	4.44E+04	2.43E+04	2.06E+04	3.00E+04
12	2.37E+04	2.28E+04	2.32E+04	6.05E+04	5.21E+04	2.14E+04	3.19E+04	2.99E+04	3.13E+04
12	2.72E+04	2.43E+04	2.91E+04	7.52E+04	6.34E+04	7.06E+04	3.41E+04	3.40E+04	3.50E+04
24	2.75E+04	2.77E+04	2.47E+04	5.66E+04	8.56E+04	6.79E+04	3.00E+04	3.29E+04	3.06E+04
24	3.95E+04	3.15E+04	3.34E+04	5.33E+04	5.70E+04	4.61E+04	3.54E+04	3.84E+04	3.89E+04
48	2.92E+04	3.00E+04	2.46E+04	6.98E+04	9.16E+04	7.48E+04	3.34E+04	2.60E+04	3.93E+04
48	2.88E+04	2.95E+04	2.43E+04	7.75E+04	6.85E+04	6.59E+04	2.80E+04	1.82E+04	2.31E+04
72	3.24E+04	3.12E+04	2.89E+04	7.44E+04	8.29E+04	7.57E+04	4.26E+04	4.06E+04	3.70E+04
72	3.04E+04	2.78E+04	3.19E+04	3.48E+04	2.76E+04	3.03E+04	3.22E+04	3.40E+04	3.38E+04
168	2.07E+04	2.25E+04	2.42E+04	7.03E+04	2.73E+04	2.69E+04	2.84E+04	2.79E+04	2.29E+04
168	2.24E+04	2.63E+04	1.75E+04	6.03E+04	4.81E+04	4.79E+04	1.82E+04	3.02E+04	1.66E+04
504	2.53E+04	2.34E+04	2.14E+04	4.95E+04	4.22E+04	4.99E+04	2.84E+04	2.72E+04	2.62E+04
504	2.70E+04	1.76E+04	2.27E+04	4.81E+04	3.90E+04	4.06E+04	3.16E+04	2.86E+04	2.64E+04

Table A-43 – qPCR gene counts per gram sediment for Total Bacteria (rpoB gene), SRB (dsrB), NRB (nosZ2), measured in the 0.01% and 0.05% control Pepper biocide mesocosm scenario in triplicate

time (hours)	Total			SRB			NRB		
0	6.12E+03	8.22E+03	5.33E+03	1.95E+03	1.91E+03	1.71E+03	2.72E+02	2.39E+02	2.70E+02
0	1.01E+04	6.60E+03	5.00E+03	5.60E+02	5.76E+02	6.00E+02	2.41E+02	3.42E+02	1.71E+02
1	8.28E+03	6.97E+03	8.23E+03	1.66E+03	1.62E+03	1.54E+03	2.87E+02	3.03E+02	2.80E+02
1	1.52E+04	1.42E+04	1.30E+04	9.11E+02	9.89E+02	8.22E+02	2.95E+02	2.97E+02	4.94E+02
3	8.20E+03	8.47E+03	8.77E+03	1.56E+03	1.85E+03	1.60E+03	3.19E+02	3.67E+02	3.11E+02
3	1.86E+04	2.33E+04	2.56E+04	2.77E+02	1.09E+03	7.10E+02	3.88E+02	3.74E+02	3.25E+02
6	9.24E+03	8.85E+03	8.76E+03	1.81E+03	2.13E+03	1.29E+03	3.09E+02	3.08E+02	1.89E+03
6	2.24E+04	9.90E+03	2.56E+04	7.59E+02	6.85E+02	9.69E+02	1.54E+03	3.79E+02	3.65E+02
12	1.54E+04	8.77E+03	8.72E+03	1.88E+03	1.74E+03	2.15E+03	4.25E+02	2.71E+02	4.03E+02
12	1.66E+04	1.64E+04	1.88E+04	6.96E+02	9.73E+02	8.30E+02	3.87E+02	4.18E+02	5.98E+02
24	5.06E+03	9.08E+03	8.22E+03	1.54E+03	1.63E+03	1.45E+03	2.61E+02	2.92E+02	3.15E+02
24	1.97E+04	3.27E+04	3.01E+04	1.17E+03	1.12E+03	1.17E+03	4.27E+02	4.08E+02	4.37E+02
48	1.22E+04	1.19E+04	1.14E+04	2.18E+03	2.62E+03	2.50E+03	3.69E+02	4.56E+02	4.32E+02
48	2.80E+04	2.43E+04	2.67E+04	6.87E+02	1.30E+03	1.16E+03	4.24E+02	5.27E+02	5.52E+02
72	1.70E+04	1.09E+04	9.70E+03	1.65E+03	1.63E+03	1.46E+03	1.13E+03	8.21E+02	4.42E+02
72	2.13E+04	2.00E+04	2.26E+04	8.76E+02	1.13E+03	9.06E+02	2.67E+02	3.52E+02	2.96E+02
168	1.30E+04	1.39E+04	1.36E+04	1.68E+03	1.76E+03	1.53E+03	2.94E+02	2.80E+02	3.12E+02
168	2.42E+04	2.58E+04	3.22E+04	1.52E+03	1.67E+03	3.87E+03	3.91E+02	4.11E+02	4.11E+02
504	8.92E+03	8.12E+03	1.03E+04	1.67E+03	1.88E+03	1.70E+03	1.95E+02	2.81E+02	2.82E+02
504	2.57E+04	3.92E+02	N/A	1.22E+03	1.16E+03	8.79E+02	3.56E+02	4.53E+02	3.57E+02

APPENDIX B: CALIBRATION CURVES

Chemical Oxygen Demand

Table B-1 – Example standard curve used for calibrating COD measurements from Valencia Pond Sample tests done on May 31, 2012

Std	Absorbence
0	0
125	0.054
250	0.107
500	0.207

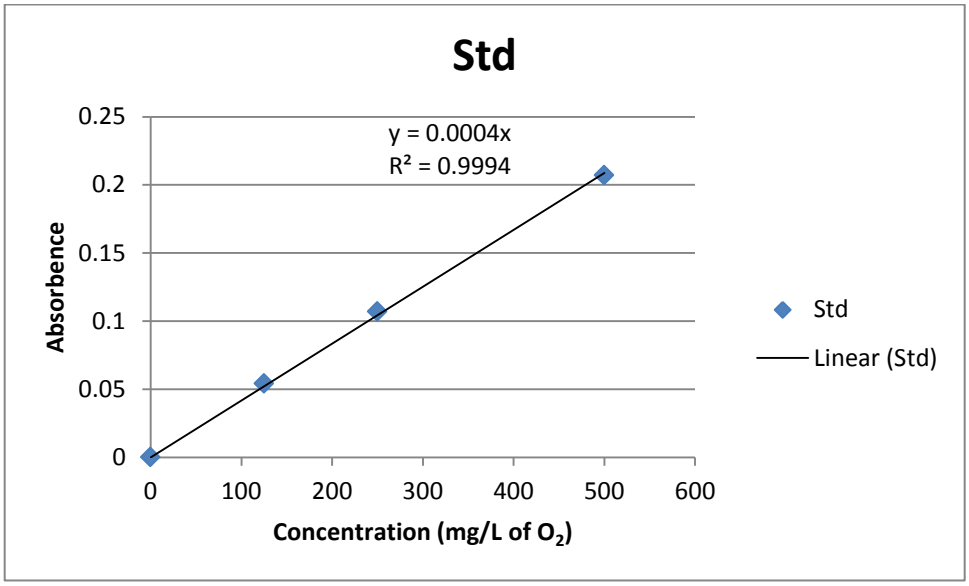

Figure B-1 – Example calibration curve used for calculating COD concentrations in samples, taken from Valencia Pond Sample tests done on May 31, 2012

qPCR

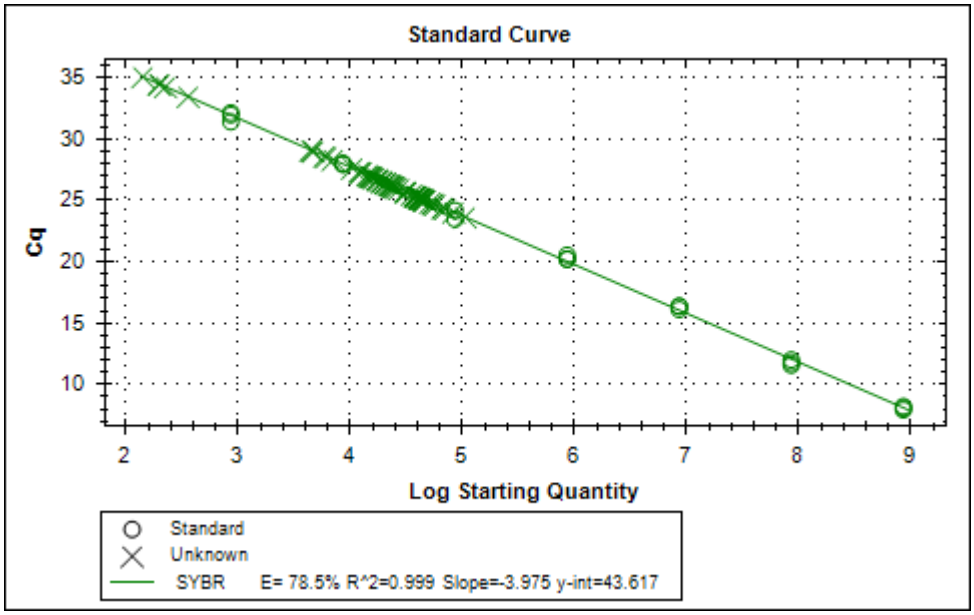

Figure B-2 – Field samples first standard curve for qPCR rpoB gene count analysis

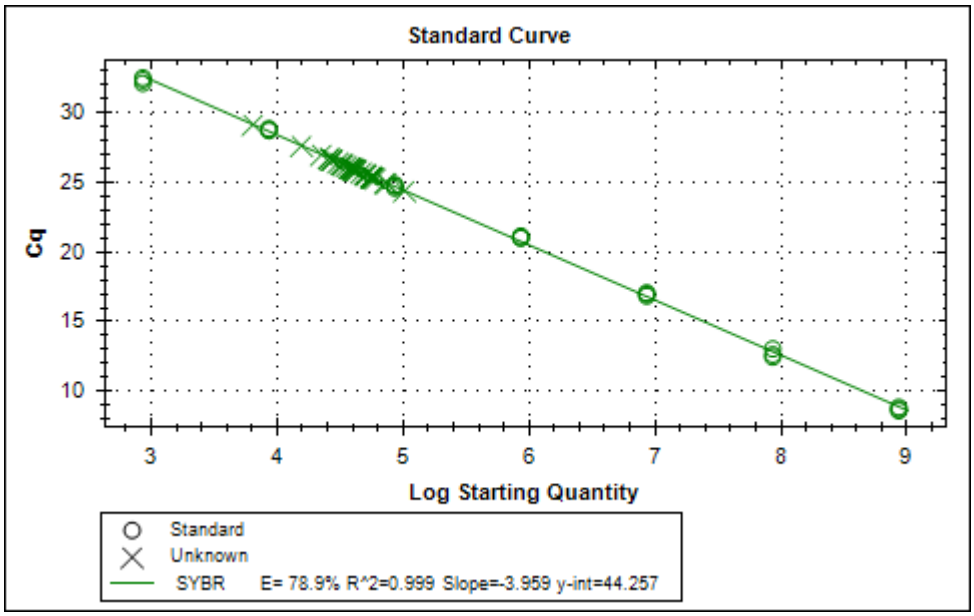

Figure B-3 - Field samples second standard curve for qPCR rpoB gene count analysis

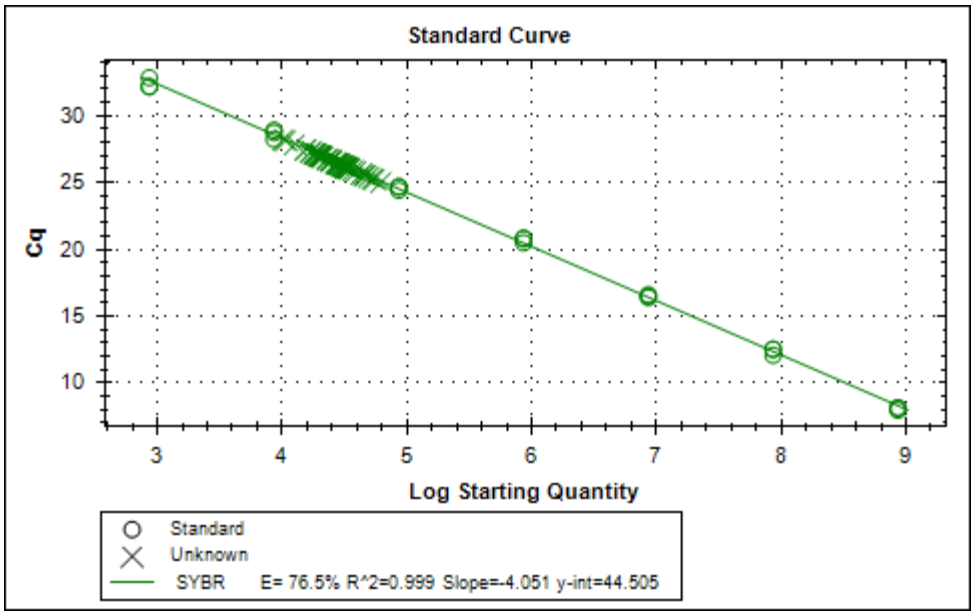

Figure B-4 - Mesocosm samples standard curve for qPCR rpoB gene count analysis

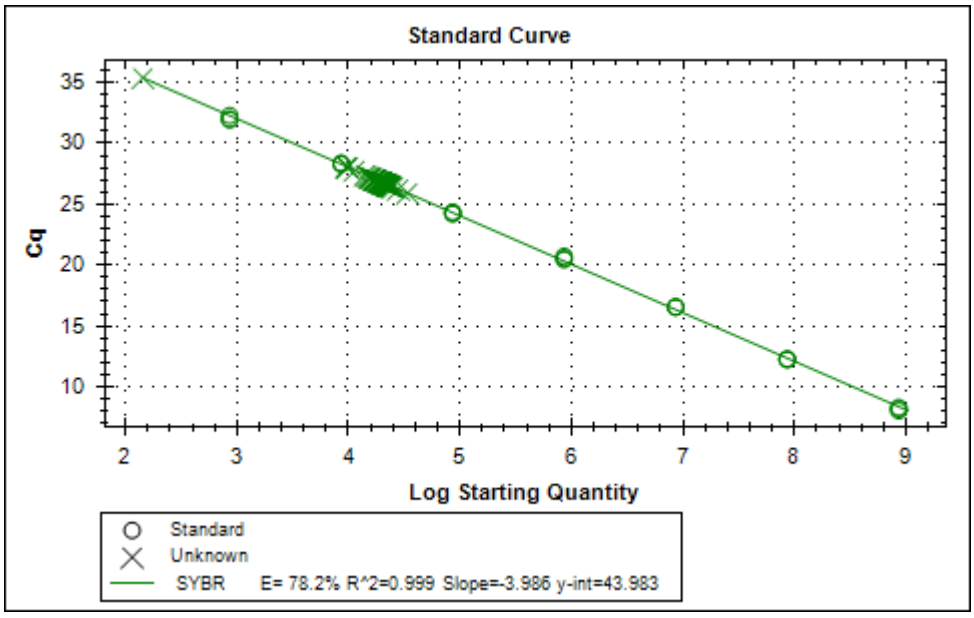

Figure B-5 – 0.01% Pepper biocide mesocosms samples standard curve for qPCR rpoB gene count analysis

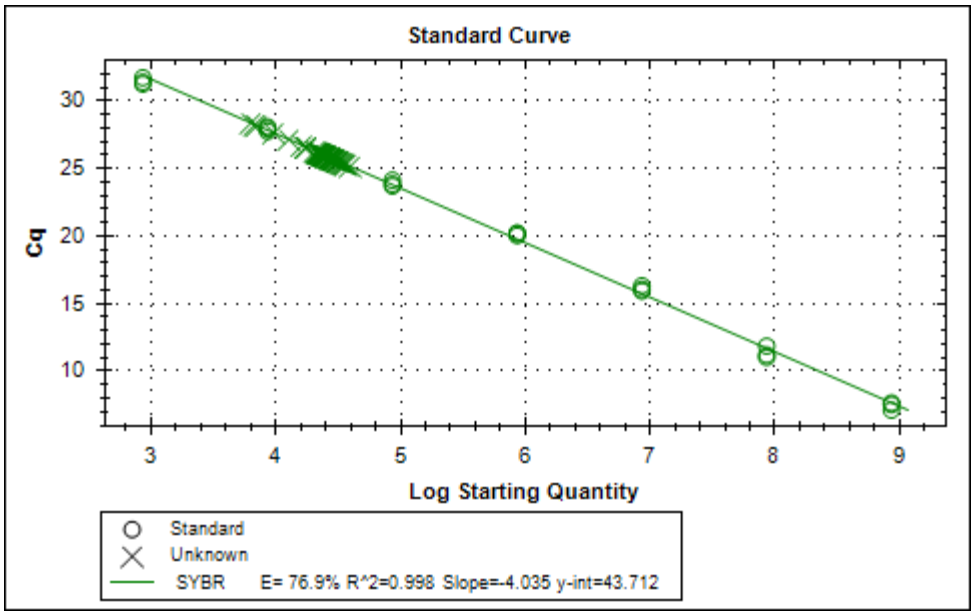

Figure B-6 – 0.05% Pepper biocide mesocosms samples standard curve for qPCR rpoB gene count analysis

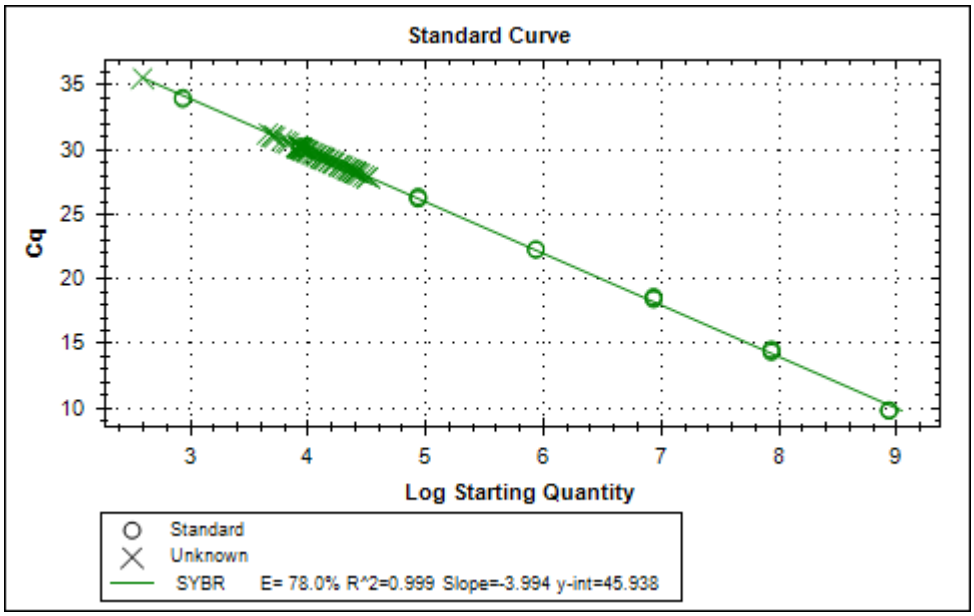

Figure B-7 – Pepper biocide control mesocosms samples standard curve for qPCR rpoB gene count analysis

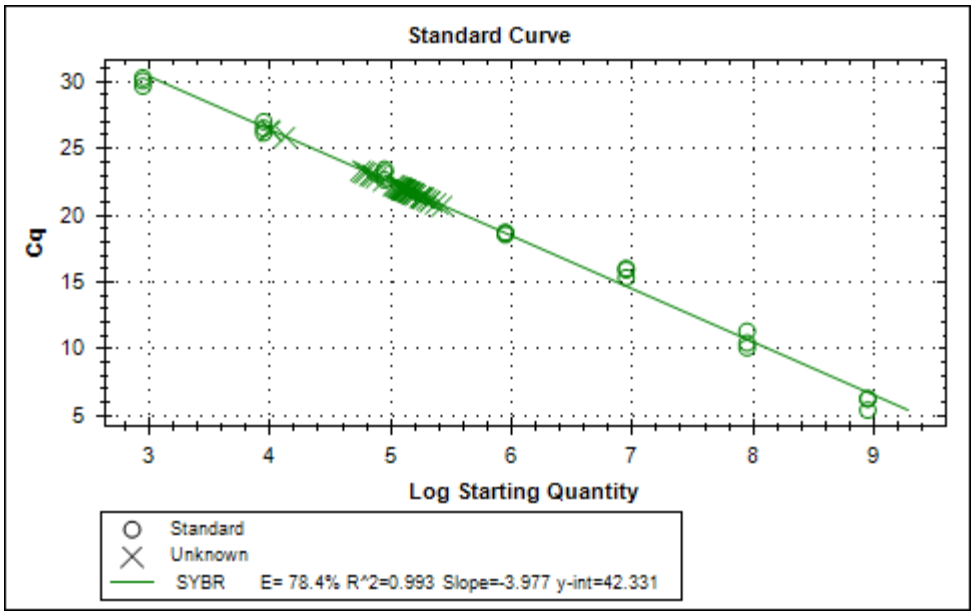

Figure B-8 – Field samples first standard curve for qPCR dsrB gene count analysis

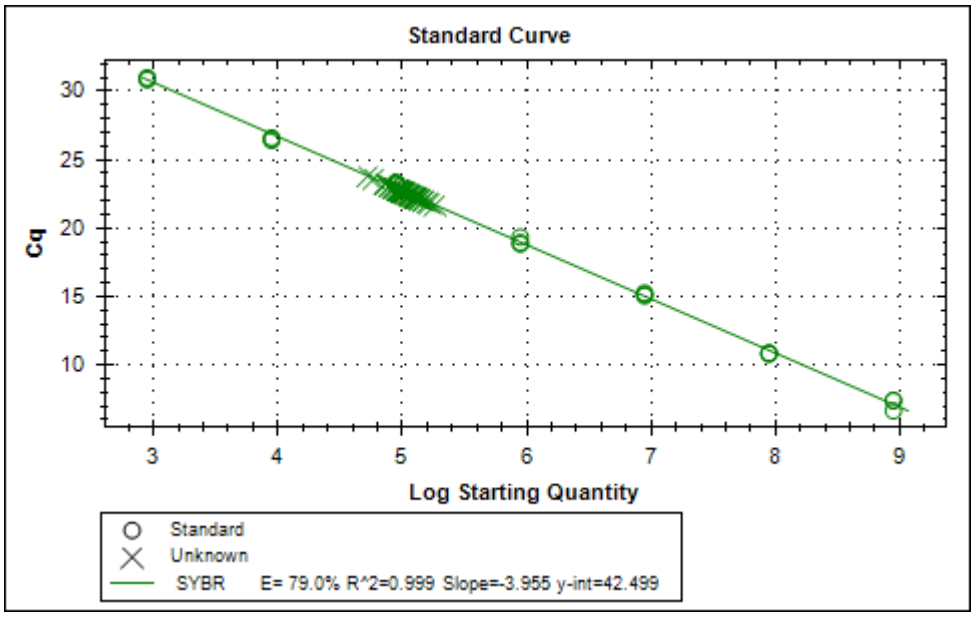

Figure B-9 – Field samples second standard curve for qPCR dsrB gene count analysis

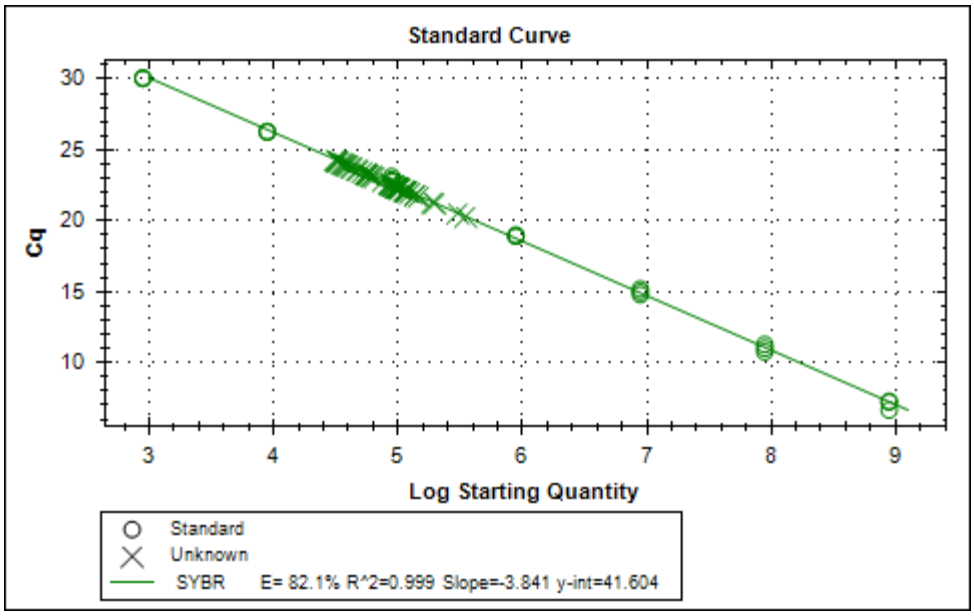

Figure B-10 – Mesocosm samples standard curve for qPCR dsrB gene count analysis

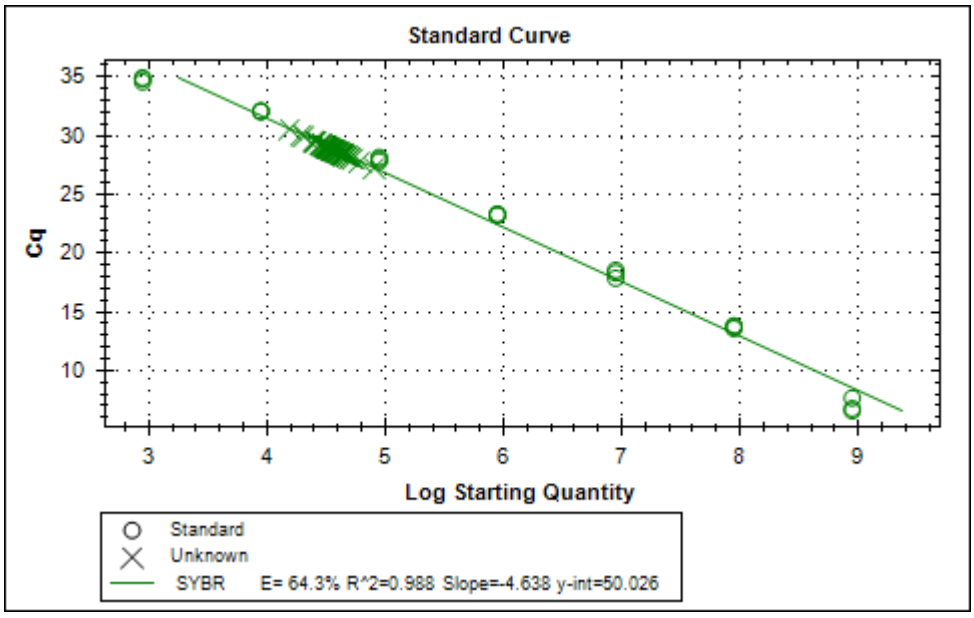

Figure B-11 – 0.01% Pepper biocide mesocosms samples standard curve for qPCR dsrB gene count analysis

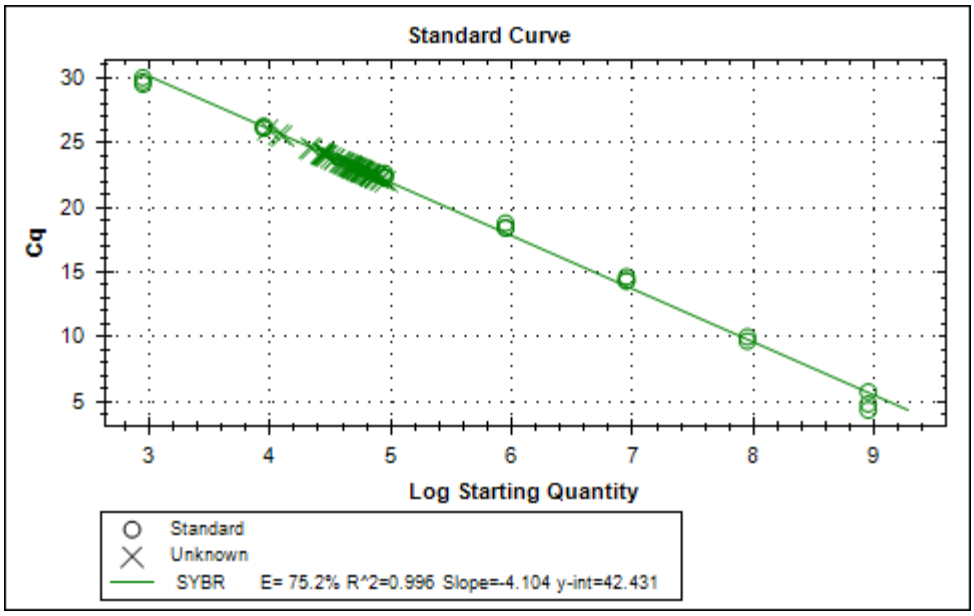

Figure B-13 – 0.05% Pepper biocide mesocosms samples standard curve for qPCR dsrB gene count analysis

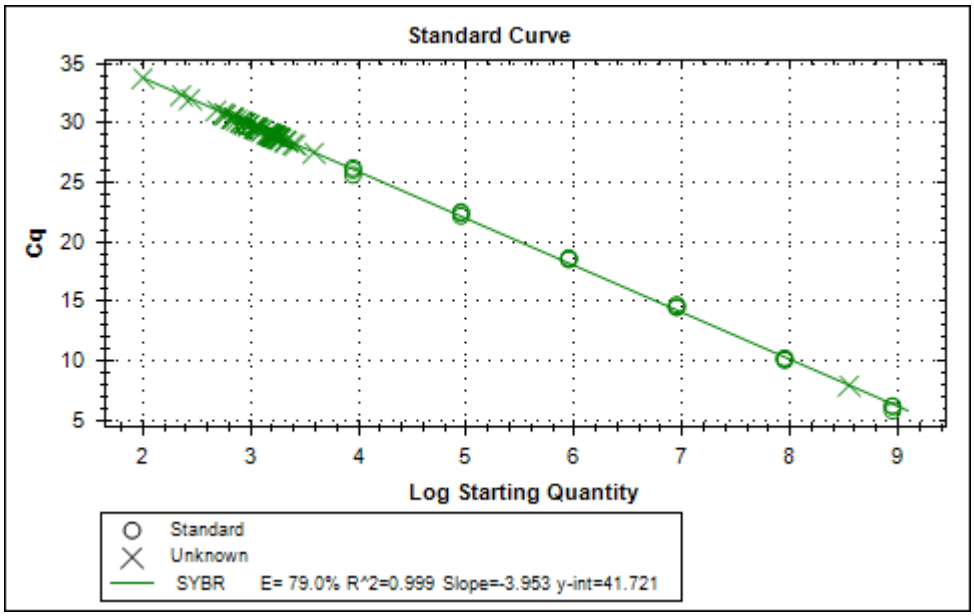

Figure B-14 – Pepper biocide control mesocosms samples standard curve for qPCR dsrB gene count analysis

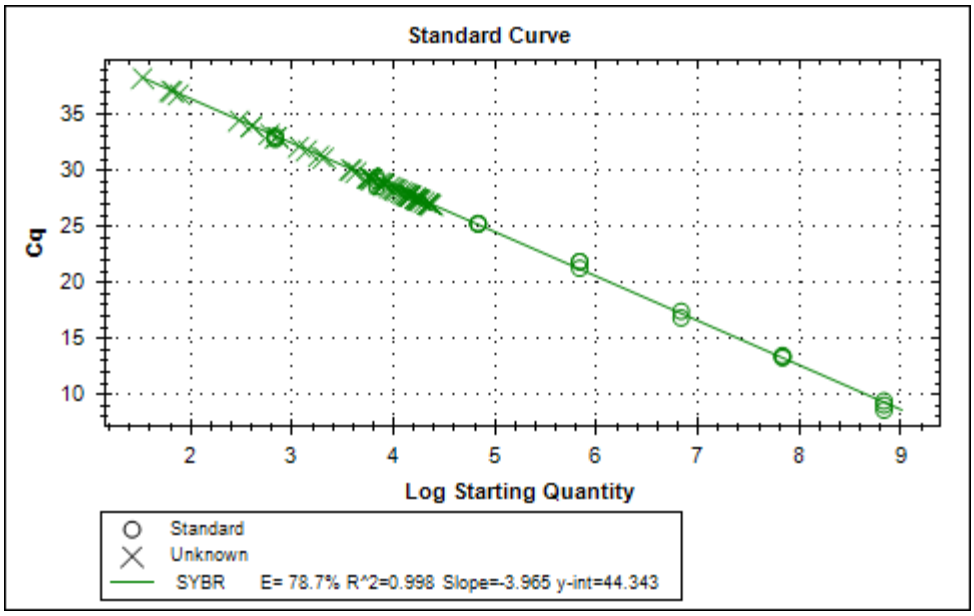

Figure B-15 – Field samples first standard curve for qPCR nosZ2 gene count analysis

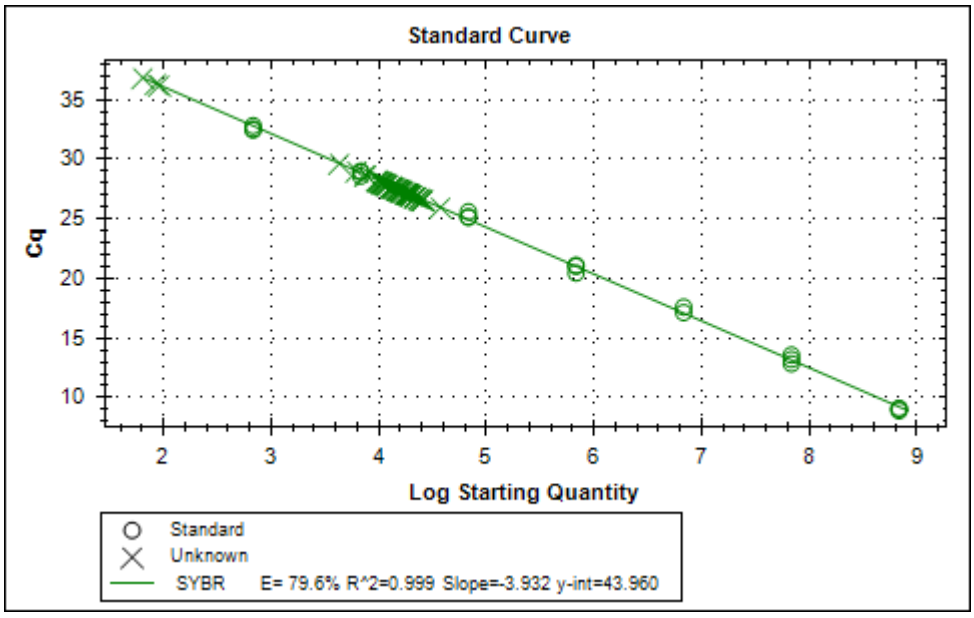

Figure B-16 – Field samples second standard curve for qPCR nosZ2 gene count analysis

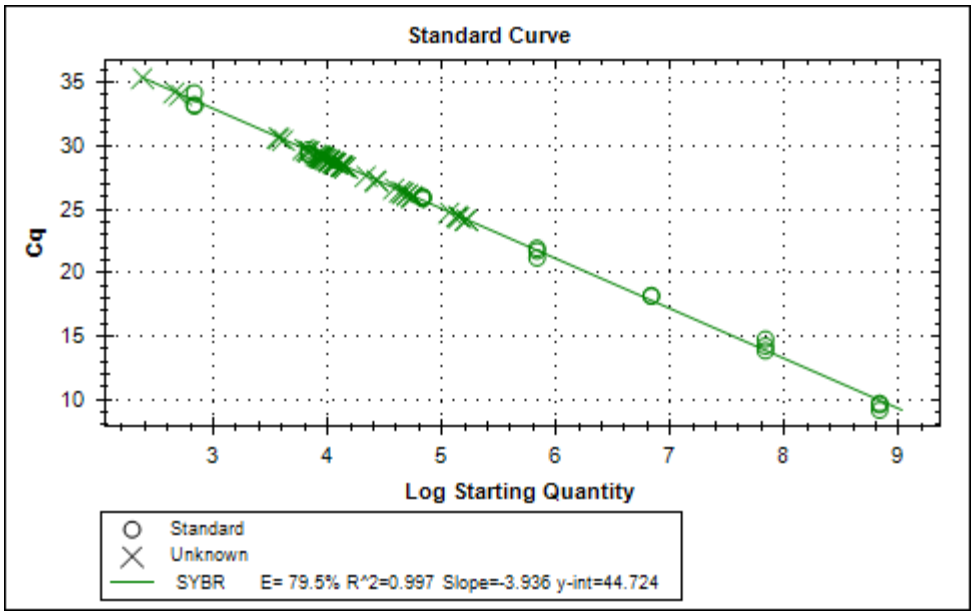

Figure B-17 – Mesocosm samples standard curve for qPCR nosZ2 gene count analysis

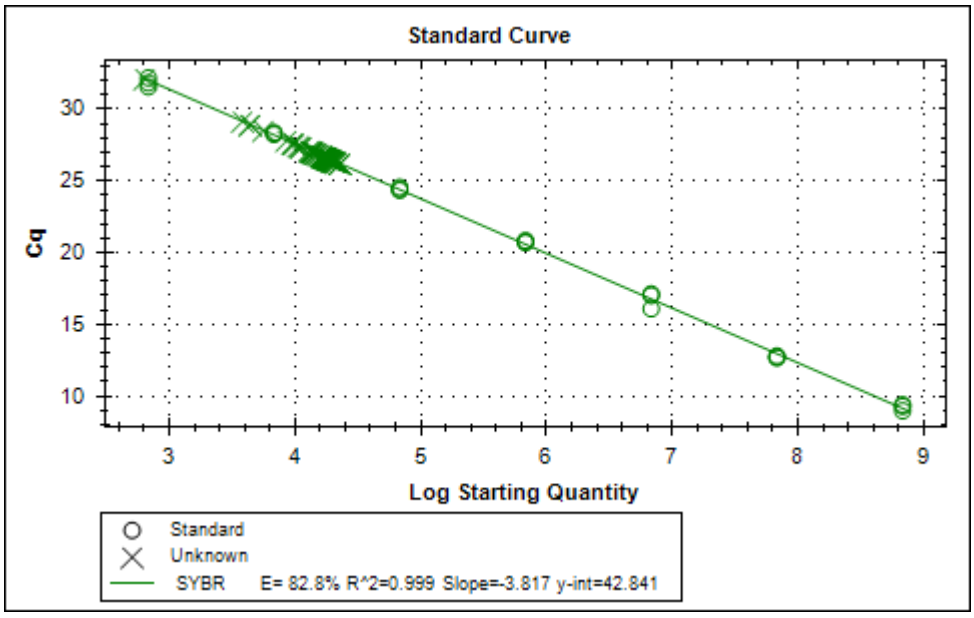

Figure B-18 – 0.01% Pepper biocide mesocosms samples standard curve for qPCR nosZ2 gene count analysis

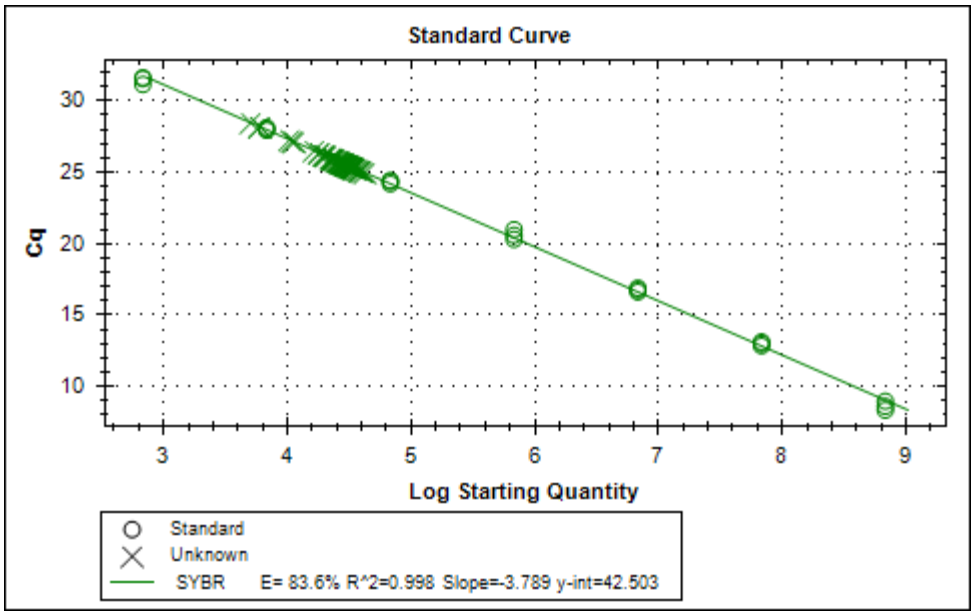

Figure B-19 – 0.05% Pepper biocide mesocosms samples standard curve for qPCR nosZ2 gene count analysis

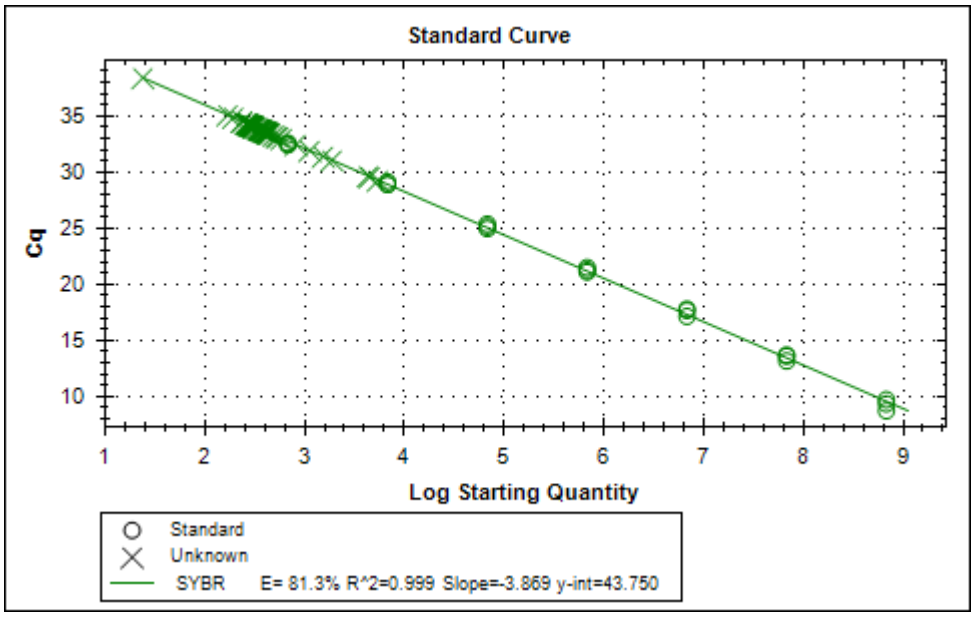

Figure B-20 – Pepper biocide control mesocosms samples standard curve for qPCR nosZ2 gene count analysis