GENOMICS TOOL FOR MONITORING ENGINEERED STORMWATER TREATMENT WETLANDS

by

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Abstract

In the context of this research, stormwater consists of precipitation that falls onto impervious surfaces and fails to infiltrate into the ground. Traditional stormwater management involves diverting stormwater into storm sewers followed by discharge to a watercourse. However, in Vancouver and elsewhere, there is a push from governments for a more integrated approach which makes use of low impact design (LIDs) features. For this reason, engineered wetlands, which are designed to optimize natural processes for water diversion and treatment, are becoming a more common and desirable treatment option for stormwater. However, there are barriers for the implementation of engineered wetlands and other LIDs because traditional water quality monitoring often does not provide a reliable enough validation that the wetlands are meeting water treatment objectives, thus leading to a lack of accountability for designers and operators.

In this research, a genomics-based approach was applied at an operating stormwater treatment wetland (the Lost Lagoon wetland located in Stanley Park, Vancouver British Columbia, Canada), with the goal to provide proof of concept data to inform the development of a genomics-based tool for stormwater treatment wetlands and other LIDs. In addition, a laboratory based stormwater dosing study was performed to allow for cross comparison of results. Microbial communities and functional genes with known adaptations for the contaminants found in stormwater were correlated with contaminant levels to increase the reliability and certainty of findings. Results from DNA sequencing were compared using samples extracted from the Lost Lagoon wetland and several outcomes suggested that bacteria may correlate with the performance of treatment wetlands. This was generally supported further using results from samples extracted during the stormwater dosing study. Cost estimates performed for various treatment wetland monitoring scenarios suggested that in the future, a genomics-based monitoring approach may supply more accurate treatment performance data at a lower overall cost and effort level than traditional stormwater treatment monitoring.

Proof of concept, for the application of genomics-based monitoring of stormwater treatment wetlands, was provided. It was demonstrated that genomics could supply benefits for future monitoring endeavours and that additional investigation into this field may be worthwhile.

Lay Summary

In this research, a novel treatment monitoring approach was applied at an operating stormwater treatment wetland (the Lost Lagoon wetland located in Stanley Park, Vancouver British Columbia, Canada). In addition, a laboratory based stormwater dosing study was performed to allow for comparison of results. Bacterial communities and genes with known adaptations for the contaminants found in stormwater were linked with contaminant levels to increase the reliability and certainty of treatment findings. Results were compared using samples collected from the Lost Lagoon wetland and several outcomes suggested that bacteria may correlate with the performance of treatment wetlands. This was generally supported further using results from samples collected during the stormwater dosing study. Cost estimates, performed for various treatment wetland monitoring scenarios, suggested that in the future this novel monitoring approach may supply more accurate treatment performance data at a lower overall cost and effort level than traditional stormwater treatment monitoring.

Preface

The research described in this document contains two parts of equal significance. First, a field study was conducted at the Lost Lagoon wetland in Stanley Park in Vancouver, British Columbia between June 2014 and December 2014. Second, a laboratory study was conducted using facilities at the University of British Columbia Vancouver Campus between November 2015 and April 2016. Both of these studies were student-led by the author of this document. In addition, the author was primarily responsible for the identification and design of the research program, the applications to funding agencies, the execution of both the field and laboratory studies, and the analyses of data. That being said, many individuals, including the author's primary supervisor and collaborating supervisors, contributed advice, expertise, and constructive criticism throughout the design of the research program and the analyses that were conducted by the author. Specifically, Prof. James Atwater, Dr. Susan Baldwin, Dr. Dirk Van Zyl, Dr. Bill Mohn and Chris Johnston provided direction for the two components of this research.

Additional contributions include:

- Staff from the Stanley Park Ecology Centre assisted with sample collection during the field study;
- Timothy Ma from the UBC Department of Civil Engineering conducted analyses for metals;
- Staff from Microbiome Insights performed Illumina MiSeq sequencing;
- Anastacia Kuzmin from the UBC Department of Zoology performed Illumina HiSeq sequencing; and
- Dr. Ido Hatam contributed codes and support for the analyses of Illumina MiSeq data.

Publications, Presentations and Data Deposition:

A version of the field study results in Chapter 1 and Chapter 2 has been prepared for submission.

Jessica LeNoble, James Atwater, Susan Baldwin, Chris Johnston, Ido Hatam. The application of genomics as a monitoring tool for the efficacy of engineered stormwater treatment wetlands: a case study using results from an operating stormwater treatment wetland in Stanley Park, Vancouver, British Columbia.

A version of the laboratory study in Chapter 2 has been prepared for submission:

Jessica LeNoble, James Atwater, Susan Baldwin, Chris Johnston, Ido Hatam. The application of genomics as a monitoring tool for the efficacy of engineered stormwater treatment wetlands: a proof of concept study using the results of a stormwater dosing experiment.

The outcome of this work has been presented in conferences as follows:

Jessica LeNoble, James Atwater, Chris Johnston, Maria Egerton, Susan Baldwin ad Dirk Van Zyl. Genomics Tool for Monitoring Stormwater Treatment Wetlands. Poster session at the 14th Annual Genomics Forum: Global Impact of Genomics. Genome BC. Vancouver, Canada. May 13, 2016.

Jessica LeNoble, James Atwater, Chris Johnston, Maria Egerton, Susan Baldwin ad Dirk Van Zyl. Genomics Tool for Monitoring Stormwater Treatment Wetlands. Poster session at the 3rd Annual Water and Environment Student Talks Conference: Where is Water Taking Us? University of British Columbia. Vancouver, Canada. June 7, 2016.

Upon completion of this thesis and subsequent publications, raw microbial data will be deposited in the following repositories under project name, "Genomics Tool for Engineered Stormwater Treatment Wetlands."

- NCBI
- MGRAST

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List of Abbreviations and Symbols

Symbol	*Property*
(A)RISA	(Automated) Ribosomal intergenic spacer analysis
AA	Atomic absorption
ANOSIM	Analysis of similarity
ANOVA	Analysis of variance
BCTFA	British Columbia Transportation Financing Authority
bp	Base-pair
CAMERA	Community cyberinfrastructure for advanced microbial ecology research
CEME	Civil engineering and mechanical engineering
CLPP	Community level physiological profiling
COD	Chemical oxygen demand
Cond	Conductivity
Df	Degrees of freedom
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
FAME	Fatty acid methyl ester analysis
FISH	Florescence in situ hybridization
GAAS	Genome relative abundance and average size
GPS	Global positioning system
GUSTA ME	Guide to statistical analysis in microbial ecology
ICP-OES	Inductively coupled plasma optical emission spectrometry
IMG/M	Integrated microbial genomes with microbiome samples
KEGG	Kyoto encyclopedia of genes and genomes
KWL	Kerr Wood Leidal Consulting Engineers Limited
MAFFT	Multiple alignment program for amino acid or nucleotide sequences
MeanSqs	Mean squares
MG-RAST	Metagenome rapid annotation subsystem technology
MO&G	Mineral oil and grease
NMDS	Non-metric multidimensional scaling
ORP	Oxidation-reduction potential
OTU	Operational taxonomic unit
PCB	Polychlorinated biphenyl
PCR	Polymerase chain reaction
PC-SWMM	Personal computer stormwater management model
PLFA	Phospholipid fatty acid
PVC	Polyvinyl chloride
QIIME	Quantitative insights into microbial ecology
Q-PCR	Quantitative polymerase chain reaction
RAxML	Randomized accelerated maximum likelihood
RDP	Ribosomal database project
RFLP	Restriction fragment length polymorphism
rpoB	Polymerase beta sub-unit
rRNA	Ribosomal ribonucleic acid
SCSU	Sole-carbon source utilization
SPES	Stanley Park Ecology Society
SSCP	Single strand confirmation polymorphism
STAMP	Strategies and techniques for analyzing microbial populations
SumsOfSqs	Sum of squares
TACOA	Taxonomic classification of environmental genomic fragments approach
TGGE	Temperature gradient gel electrophoresis
TOC	Total organic carbon

T-RFLP	Terminal restriction fragment length polymorphism
TSS	Total suspended solids
Turb	Turbidity
UBC	University of British Columbia
VOC	Volatile organic compound

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- Daniel Smith, laboratory and fieldwork assistance
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Dedication

This thesis is dedicated to my grade nine science teacher, Mr. Tobias Blaskovits. Without knowing it at the time, Mr. Blaskovits helped my awkward thirteen-year-old self find her niche in high school but more importantly, he was the first person to inspire my passion for environmental conservation, which has ultimately led to my pursuit of this research. Beyond this, Mr. Blaskovits connected me with the group pictured below, which includes some of my most treasured lifelong friends. Mr. Blaskovits continues to use hand-on approaches to help young students find their passion for science, engineering, and discovery. It is the teachers like Mr. Blaskovits that shape our future communities; they are deserving of our utmost appreciation and thanks.

Winning the Mind Grind in grade 9, 2007

In the photo: Mr. Tobias Blaskovitz, Edward Truong, Jessica LeNoble and Cody O'Neil photographed with CBC News Cast, Sandy Dawson and Mike Roberts

Our mind grind team in grade 12, 2010

In the photo: Edward Truong, Peter Davidson, Cody O'Neil, Connor Vandenberg, Alexa Geddes, Leanna Gruendel, and Jessica LeNoble

1. Introduction

1.1 Background

In the context of this research, stormwater consists of precipitation that falls onto impervious surfaces and fails to infiltrate into the ground. Traditional stormwater management involves diverting stormwater into storm sewers followed by discharge to a watercourse, which may or may not include prior treatment at a wastewater treatment facility. However, in Vancouver and elsewhere, there is a push by provincial and municipal governments to integrate stormwater treatment practices through the design and installation of low impact design features (British Columbia Ministry of Community, Sport, and Cultural Development, n.d.), which make use of natural processes to enhance the quality of discharged water, reduce the quantity of runoff, and recharge groundwater aquifers.

For this reason, engineered wetlands, which are designed to optimize natural processes for water diversion and treatment, are becoming a more common and desirable treatment option for stormwater. However, there are still barriers to the implementation of these wetlands as low impact design techniques for stormwater. Traditional water quality monitoring often does not provide a reliable enough validation that the wetlands are meeting water treatment objectives. Adequate pollutant removal efficiency monitoring requires continuous inflow and outflow measurements over a two-year study period (Erickson, Weiss, & Gulliver, 2013); thus, this regime is highly intensive for both resources and labour. In addition, the potential for erroneous and uncollected data is accelerated by unpredictable weather and the potential for equipment wear due to urban vandalism and routine use over an extensive study period. With diverse priorities and competition for limited resources, municipalities are unlikely to fund adequate monitoring regimes for engineered wetlands and will either choose to avoid their installation or base decision making on inadequate analyses.

As low impact design features become a greater priority, emerging analyses methods for monitoring pollutant removal efficiencies are of interest for application in the stormwater treatment sector. One such emerging analysis method for monitoring treatment effectiveness is the application of genomics, “the branch of molecular biology that is concerned with the structure, function, evolution, and mapping of genomes, or the complete set of DNA within a single cell of an organism.” (Oxford Univerisity Press, 2016) Because the toxicity of stormwater influences microbial life (Karlsson, Viklander, Scholes, & Revitt, 2010), analysis of the microbiology within engineered wetlands may compliment traditional water quality monitoring and improve the effectiveness of treatment wetlands in the future. The content in this thesis

provides data to support this claim.

1.2 Motivation

In 1999, Kerr Wood Leidal Consulting Engineers Ltd. (KWL) was commissioned by the City of Vancouver for the design and commissioning of an engineered wetland, from here forward referred to as the Lost Lagoon wetland, which would treat stormwater exiting the newly expanded Stanley Park Causeway displayed by the map in Figure 1.

Figure 1. Map of Stanley Park (City of Vancouver, 2016b) Highlighting the Lost Lagoon Wetland

At the time it was commissioned, the Lost Lagoon wetland employed many of the best engineering management practices available and, in doing so, the design received an award of excellence from The Consulting Engineers of British Columbia. However, since the wetland was installed, only limited assessment of its treatment effectiveness has been performed. Though treatment monitoring is desirable and necessary, because of reasons described in the previous section, adequate water treatment monitoring

has not been performed.

That being said, the Lost Lagoon wetland is a highly desirable site for the application of an emerging monitoring method because it was designed as an ideal treatment system with its only source of influent being stormwater diverted from the Stanley Park Causeway. There is a wealth of knowledge indicating that the toxic components of stormwater have an influence on bacteria at both the species and functional gene levels (Nies, 1999). This wealth of knowledge along with the desire to increase the use of low impact design features for stormwater treatment led to the motivation behind this research.

1.3 Objective and Study Goals

Overall, the goal of this study was to provide proof of concept data that supports or rejects developing a genomics monitoring tool for low impact design features that treat stormwater, including engineered wetlands. This goal was achieved by splitting the study's components into two chapters, with each chapter encompassing three objectives.

Chapter 1: Apply traditional water and sediment quality monitoring techniques for validation of the Lost Lagoon wetland

Using limited water and sediment sampled from the Lost Lagoon wetland:

1. Demonstrate that the Lost Lagoon wetland is meeting water quality treatment guidelines;
2. Demonstrate that the engineering best management practices employed in the design of the Lost Lagoon wetland have had some meaningful impact on the stormwater treatment efficiency; and
3. Identify knowledge gaps and opportunities for complimentary data analyses though the application of genomics.

Chapter 2: Apply genomics monitoring techniques for complimentary validation of the Lost Lagoon wetland

Using the same samples that were analysed in Chapter 1:

1. Apply genomics-based analysis methods to determine if there are shifts in the microbial communities and functional genes along the length of the Lost Lagoon wetland;
2. Determine if there is a correlation between the water and sediment quality, present over the study period, and the microbial communities and functional genes observed; and
3. Determine, through laboratory experimentation, if there are opportunities to expand and pursue genomics analyses at other stormwater treatment low impact design features.

1.4 Scope and General Research Activities

The scope of this research can be differentiated into two parts described here.

In the first part of the thesis, a field study was executed at the Lost Lagoon wetland in Stanley Park, British Columbia. The field study covered a six-month period between July, 2015 and December, 2015. Data obtained from the field study was analyzed in order to inform the conclusions of Chapter 1, where limited traditional water and sediment quality analyses were employed in an attempt to validate the Lost Lagoon wetland and to identify knowledge gaps and opportunities for complimentary analyses though the application of genomics.

In the second part of this thesis, DNA was first extracted from the field samples taken at the Lost Lagoon wetland and next sequenced, analyzed, and compared at both the bacterial species level and the functional gene level. In addition to these analyses, a laboratory study was carried out using columns of uncontaminated natural sediment sourced from a bog near Beaver Lake as highlighted in Figure 2.

Figure 2. Map of Stanley Park (City of Vancouver, 2016b) Highlighting the Beaver Lake Bog

The laboratory study ran for a four-month period between December 2015 and March 2016; however, laboratory conditions were controlled and designed to mimic the weather observed at the Lost Lagoon wetland over the period between September 2015 and December 2015. During the laboratory study period, seventeen sediment columns were repeatedly dosed with either semi-synthetic stormwater or distilled water. At one month intervals, sediment columns were sacrificed and analyzed for both the traditional water and sediment quality parameters as well as DNA. The results obtained from the field and laboratory studies were subsequently used to inform the conclusions of Chapter 2, where genomics monitoring techniques were employed in an attempt to provide complimentary validation of the Lost Lagoon wetland and to determine if there may be future opportunities to expand and pursue genomics analyses at other low impact design stormwater treatment features.

2. Chapter 1: Application of Traditional Water and Sediment Quality Monitoring Techniques for Validation of the Lost Lagoon Stormwater Treatment Wetland

2.1 Introduction and Chapter Goal

The contents of this chapter detail the background and environmental results of a field study that was undertaken at the Lost Lagoon wetland. Water and soil samples were collected from the wetland and environmental conditions were measured and analyzed. In addition, DNA was extracted and archived for future analyses in Chapter 2. Because the sampling regime was designed to optimize the collection of bacterial DNA, there were some limitations for the environmental analyses, which are further discussed later in this chapter. Most importantly, sampling of the wetland was performed over a six-month period, which is shorter than the required timespan needed to fully validate a stormwater treatment wetland.

The goal of this chapter was to demonstrate that the wetland is an ideal field site to be used for the 'proof of concept' design of a genomics-based monitoring tool for stormwater treatment wetlands. This chapter identifies common challenges that result from traditional wetland testing and also provides a lead in for opportunities to apply genomics as a method to reduce said challenges. To illustrate the need for stormwater management, background details on stormwater toxicity and treatment requirements are first provided. Next, engineered wetlands and associated best management practices are described. The Lost Lagoon wetland is then given some background and the design features are described in order to provide context for the field sampling and analysis plan. Finally, the study methodology, results, discussion and conclusions are provided.

2.2 Chapter Objectives

Based on the overall goal of this chapter, this chapter has three specific objectives.

Using water and sediment sampled from the Lost Lagoon wetland:

1. Demonstrate that the Lost Lagoon wetland is meeting or exceeding water quality treatment guidelines;
2. Demonstrate that the engineering best management practices employed in the design of the Lost Lagoon wetland have had some meaningful impact on the stormwater treatment efficiency; and
3. Identify knowledge gaps and opportunities for complimentary data analyses though the application of genomics.

2.3 Hypotheses

The Lost Lagoon wetland was designed to improve stormwater runoff quality through a variety of treatment mechanisms including filtration, sedimentation, adsorption, and biological uptake. Therefore, in order to prove that the wetland is meeting treatment guidelines and in order to begin to validate the treatment mechanisms within the wetland, two hypotheses must be true.

1. The concentrations of metals associated with stormwater decrease along the length of the wetland; and
2. The concentration of oil and grease decreases along the length of the wetland.

2.4 Literature Review

In order to provide background and context for the objectives and hypotheses stated in this chapter, a review of relevant literature was performed. First, a description of the regulatory framework for stormwater treatment in Vancouver is supplied. Next, common pollutants in stormwater are given some context, including the pollutants' origins, reasons for toxicity, expected concentration ranges, guidelines for treatment, and the expected treatment that is achievable using engineered wetlands. Barriers for implementing wetlands for stormwater treatment are described as well as a description of traditional monitoring techniques. Finally, the precedent, design components and best management practices, and past analyses of the Lost Lagoon wetland are described.

2.4.1 Regulatory Framework

In Canada, a multi-jurisdictional approach provides the authority to discharge liquid waste and different regulations and guidelines come into force depending on the source and content of the liquid waste which is to be discharged.

At the national level, there are federal regulations under Section 35(1) of the Fisheries Act (Government of Canada, 1985), which stipulate conditions for discharges to fish bearing receiving bodies. In addition, the federal Environmental Protection Act (Government of Canada, 1999) makes pollution prevention the cornerstone of national efforts to reduce toxic substances in the environment. However, these Acts do not explicitly regulate discharges of waste where the only source is stormwater. This is mainly due to the fact that management of the natural environment is largely a provincial jurisdiction in Canada and, thus, federal regulations on environmental matters are limited. Concerning stormwater, beyond the Fisheries Act and Environmental Protection Act, several federal guidelines and best management practices exist that collectively serve to provide a Canada-wide strategy for stormwater management and planning. These

guidelines are largely the result of a consensus among provincial governments reached through meetings of the Canadian Council of Ministers of the Environment.

At the provincial level, British Columbia has adopted this federal strategy through application of its guideline for managing stormwater titled, Stormwater Planning: A guidebook for BC (Stephens, Graham, & Reid, 2002) and through enforcement of the Municipal Wastewater Regulation (Government of British Columbia, 2016). Within its suite of provincial regulations, British Columbia grants the authority to permit stormwater treatment and conveyance systems to municipalities. However, because municipalities do not hold an explicit right to jurisdictional power in Canada, the province of British Columbia still directly controls liquid waste discharges by requiring all municipalities to submit and adhere to an Integrated Liquid Waste and Resource Management Plan. Said plan must first be approved by the BC Ministry of the Environment before municipalities are granted implicit rights to regulate and permit the management of liquid waste, including stormwater.

At the municipal level, by developing an Integrated Liquid Waste and Resource Management Plan, Metro Vancouver provides resources, and guidelines concerning stormwater that its fourteen member municipalities must adhere to prior to being granted local authority over stormwater management. Through applying Metro Vancouver's liquid waste management plan, the City of Vancouver developed its target specific plan, namely the Citywide Integrated Stormwater Management Plan (City of Vancouver, 2016a). Within this plan, locally relevant best management practices are supplied in an easy to apply context for developers, planners and engineers. In addition, priorities for low impact design features are placed at a high significance concerning Vancouver's sustainability goals.

Because the guidelines and regulations for treating and conveying stormwater are managed within several documents and pieces of legislation, it is easy to become lost when attempting to discern what information is most applicable. For this reason, Figure 3 has been supplied as a summary of the regulatory framework concerning stormwater management in British Columbia.

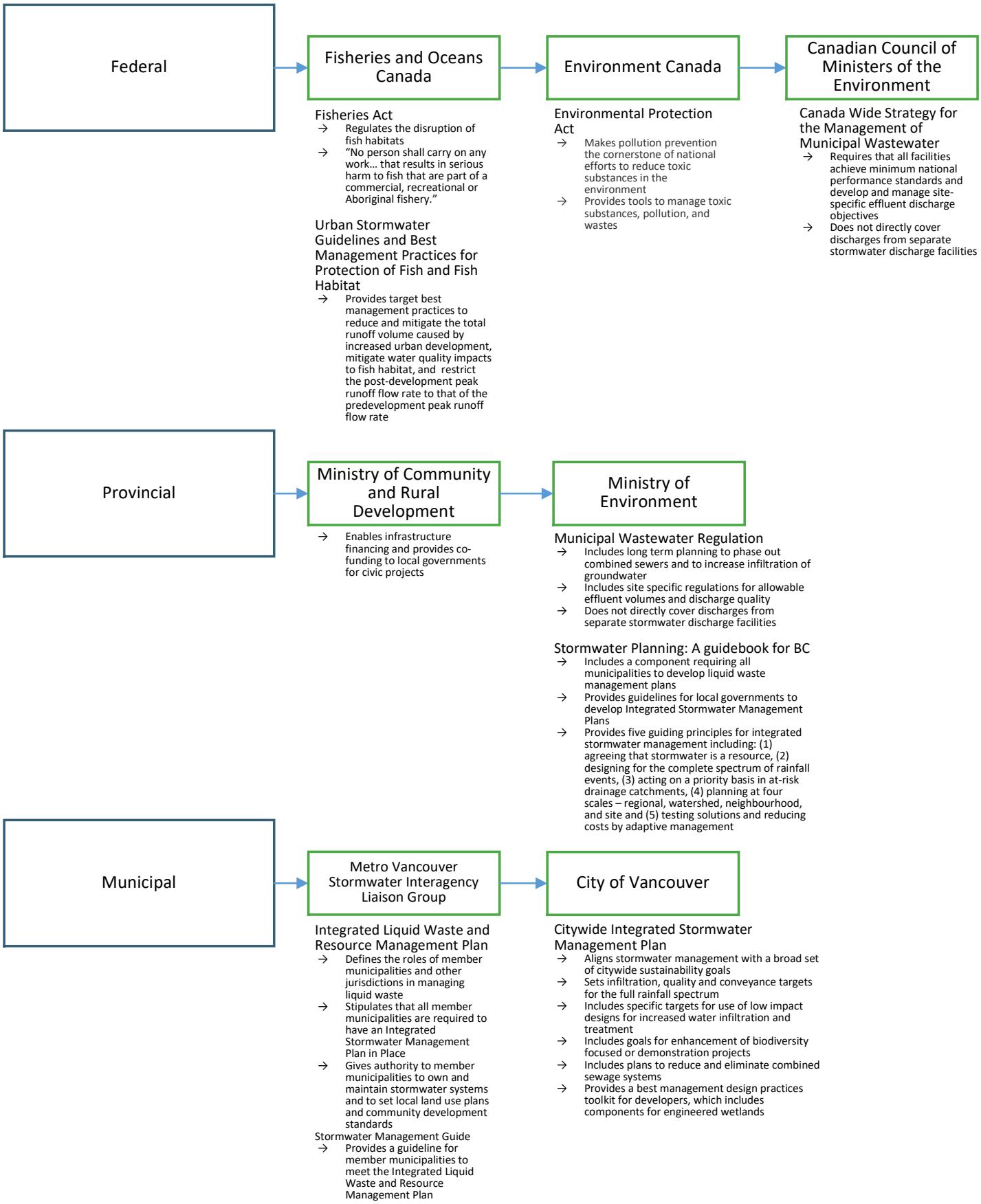

Figure 3. Visual Breakdown of the Regulatory Framework for Stormwater Management in Vancouver

2.4.2 Urban Stormwater and Accepted Treatment Efficacy of Engineered Wetlands

2.4.2.1 Description and Sources of Common Pollutants in Urban Stormwater

As urbanization increases, construction and development lead to an increase in the total impervious surface area within watersheds. Because impervious surfaces limit the ability of water to infiltrate into the ground, unmitigated urbanization can lead to an increase in runoff volumes and peak flow rates. These larger faster runoffs yield more kinetic energy, which increases the opportunity for erosion and the movement of solid particles. In addition, roadways and vehicle traffic are sources of pollutants due to combustion of fossil fuels and mechanical wear. Thus, the quality of stormwater is degraded as a number of pollutants increase in concentration. *Table 1* outlines the common pollutants of concern found in stormwater and *Table 2* outlines the sources of said pollutants.

Table 1. Description of Common Pollutants in Urban Stormwater

Pollutant	*Description*
Alkalinity	Water's capacity to neutralize acid measured as concentration of $CaCO_3$
Chloride	Concentration of dissolved Cl^-
Hardness	Dissolved calcium and magnesium, measured as $CaCO_3$
Nitrogen	Nutrient existing as particulate, dissolved, nitrate, nitrite, and ammonium
Phosphorus	Nutrient existing in numerous particulate and dissolved forms
Mineral Oil and Grease	Total concentration of hydrocarbons
Organic Carbon	Degradable organic material in total or dissolved form
pH	Function of the number of hydrogen ions in a solution
Solids	Total concentration of suspended or dissolved particulates
Temperature	Thermal property
Turbidity	Cloudiness of water, an indirect measure of particulates
Metals	Concentration of As, Ag, Al, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, V, and/or Zn in total or dissolved form

Table 2. Sources of Common Pollutants in Urban Stormwater

Pollutant	*Sources in Stormwater* [1,2]
Alkalinity	Rainwater, rocks, soil and debris
Chloride	Road de-icing rock salts,
Hardness	Rainwater, rocks, soil and debris
Nitrogen	Atmosphere, animal waste, vegetative matter and fertilizers
Phosphorus	Atmosphere, animal waste, vegetative matter and fertilizers
Mineral Oil and Grease	Atmosphere, vehicle coolants, gasoline, oils, lubricants, coal-tar based asphalt sealants
Organic Carbon	Animal waste, vegetation, oils, greases, grass clippings
pH	Rainwater, reduced buffering due to impervious surfaces
Solids	Atmosphere, pavement wear, vehicles, and road maintenance
Temperature	Changes in land use, surface cover and shading
Turbidity	Atmosphere, pavement wear, vehicles, road maintenance, vegetation
Arsenic	Atmosphere, fertilizers, animal waste, solid wastes
Silver	Diesel fuels, improper disposal of industrial wastes
Aluminum	Atmosphere, rocks, soil, and debris, vehicle exhaust, asphalt
Barium	Vehicle wear
Beryllium	Vehicle wear
Calcium	Road de-icing rock salts, grease, atmosphere, rocks, soil and debris
Cadmium	Vehicle wear, tire fillers and insecticides
Cobalt	Atmosphere, vehicle wear
Chromium	Atmosphere, vehicle wear, moving engine parts and brake linings
Copper	Soil, bearing wear, engine parts, brake linings and radiator repair
Iron	Atmosphere, soil, vehicle wear, engine parts, and road structures
Potassium	Atmosphere and fertilizers
Magnesium	Road de-icing rock salts, soil, rocks and debris, rainwater
Manganese	Atmosphere, engine parts and gasoline additives
Molybdenum	Atmosphere, vehicle wear, brake linings
Sodium	Atmosphere, road de-icing rock salts, soil, rocks and debris
Nickel	Diesel fuel, lubricating oil, bushing wear, brake linings and asphalt
Lead	Tire fillers, lubricating oil/grease, vehicle wear and radiators
Antimony	Rubber tires, enamel paints and lacquers
Vanadium	Atmosphere
Zinc	Atmosphere, tire wear, vehicle wear, soil, rocks and debris

[1] (Erickson, Weiss, & Gulliver, 2013)

[2] (British Coloumbia Ministry of the Environment, 1992)

2.4.2.2 Reasons for Toxicity for Common Pollutants in Urban Stormwater

Urban stormwater can have hydrological, chemical, biological or physical impacts on the environment; however, the greatest concern is usually biological integrity and habitat alteration (Erickson, Weiss, & Gulliver, 2013). As the concentration of certain pollutants increases in stormwater, a variety of toxic effects may become evident in the ecosystems of receiving water bodies. For this reason, untreated, unmitigated urban stormwater runoff is detrimental over time. *Table 3* outlines the specific reasons for the toxicity of the common pollutants found in urban stormwater.

Table 3. Reasons for Toxicity of Common Pollutants in Urban Stormwater

Pollutant	*Reasons for Toxicity* [1]
Alkalinity	• Low alkalinity limits the buffering capacity of receiving water to moderate changes in pH
Chloride	• High chloride concentrations indirectly affect soil properties such as swelling, porosity, water retention, and saturated hydraulic conductivity • High chloride concentrations contribute to high salinity which can be lethal for freshwater species
Hardness	• Low hardness indirectly increases toxicity as cadmium, copper, nickel and lead toxicities increase as hardness decreases
Nitrogen	• High nitrogen concentrations increase plant growth in a process called eutrophication • Eutrophication leads to reduced water clarity and the presence of blue-green algae which decomposes, reducing the oxygen content of the receiving water body
Phosphorus	• High phosphorus concentrations increase plant growth in a process called eutrophication • Eutrophication leads to reduced water clarity and the presence of blue-green algae which decomposes, reducing the oxygen content of the receiving water body
Mineral Oil and Grease	• Reduce the ability of some organisms to reproduce, negatively impact the ability of some plant species to grow, and can be lethal in high concentrations • Can accumulate in the sediment of aquatic environments, reducing oxygen content as it slowly decomposes
Organic Carbon	• Degradation consumes oxygen and impairs aquatic life
pH	• Changes in pH can be lethal for aquatic organisms • pH can indirectly influence the toxicity of other toxic compounds, including heavy metals
Solids	• High solids loadings contribute to oxygen consumption and eutrophication • High solids loadings are associated with higher concentrations of particle-bound pollutants, including heavy metals
Temperature	• Surges of elevated temperatures reduce dissolved oxygen content • Temperature can indirectly influence the toxicity of other compounds, such as ammonia
Turbidity	• High turbidity is associated with high particulate loadings and is associated with higher concentrations of particle-bound pollutants, including heavy metals
Metals	• Reduce the ability of some organisms to reproduce, negatively impact the ability of some plant species to grow, and can be lethal in high concentrations • Can bioaccumulate in the sediment of aquatic environments

[1] (Erickson, Weiss, & Gulliver, 2013)

2.4.2.3 Wetlands as an Urban Stormwater Control Measure

While the technology has improved in the last twenty years, wetlands have long been known to improve water quality (Kerr Wood Leidal Associates Ltd., 1999). Removal efficiencies for toxins associated with sediments can be as high as 90%, with average total removal efficiencies in the range of 60%-80% (Hawkins et al., 1997). The expected removal efficiencies for wetlands are comparable with other treatment options but wetlands provide the added benefit of enhanced habitats for wildlife and plants. *Table 4* outlines the expected concentration of pollutants in stormwater, the guidelines for treatment in Canada and the removal efficiency expected from engineered wetlands.

Table 4. Concentration of Common Pollutants in Urban Stormwater, Treatment Guidelines and Removal Efficiency Using Engineered Wetlands

Pollutant	*Concentration*		*Removal Efficiency [4], %*
	Stormwater [1,2]	*Guideline[3]*	
Alkalinity *(mg/L)*	8-153[1]	20	-
Chloride *(mg/L)*	0.5-75.3[1]	0.640	-
Hardness *(mg/L)*	8.2-80.3[1]	20	-
Nitrogen *(mg/L)*	0.34-20[2]	-	-19[α]
Phosphorus (*µg/L)*	64-4410[1]	-	7
Mineral Oil and Grease (*mg/L)*	5.0-63.4[1]	15	74
Organic Carbon (*mg/L)*	7.3-17.6[2]	-	31
pH	6.2-8.7[2]	6.5-9	-
Solids (*mg/L)*	44-809[1]	20% above BL*	-5[α]
Temperature	-	-	-
Turbidity	-	-	-
Arsenic (*µg/L)*	0-58[5]	5	41
Silver (*µg/L)*	3.0[5]	0.25	-
Aluminum (*µg/L)*	26-7100[1]	100	85
Barium (*µg/L)*	2-792[1]	-	34
Beryllium (*µg/L)*	-	-	-
Calcium (*µg/L)*	42-506[1]	-	67
Cadmium (*µg/L)*	0-40[5]	1	-
Cobalt (*µg/L)*	-	-	-
Chromium (*µg/L)*	0-40[5]	2	61
Copper (*µg/L)*	22-7033[5]	2	33
Iron (*µg/L)*	32-125000[1]	350	84
Potassium (*mg/L)*	5-114[1]	-	-8[α]
Magnesium (*mg/L)*	113-741[1]	-	29
Manganese (*µg/L)*	112-6910[1]	80	91
Molybdenum (*µg/L)*	-	70	-
Sodium (*mg/L)*	6.7-548[1]	-	-19[α]
Nickel (*µg/L)*	0-126[5]	25	-
Lead (*µg/L)*	73-1780[5]	3	79
Antimony (*µg/L)*		6	-
Vanadium (*µg/L)*		-	-
Zinc (*µg/L)*	5-2386[1]	7.5	71

[1] (Stime, 2014)

[2] (British Columbia Research Corporation, 1992)

[3] (CCME Guidelines for Protection of Aquatic Life, Freshwater, 2016)

[4] (Hawkins et al., 1997)

[5] (Geosyntec Consultants & Wright Water Engineers Inc., 2011)

*BL = Baseline concentration

[α]Negative values indicate that wetlands are a source of material

2.4.2.4 Barriers for Implementing Stormwater Treatment Wetlands

While the popularity of low impact design features is increasing, these systems still make up a minority of all stormwater treatment systems in British Columbia. Even with the increase of literature, which indicates the importance of low impact design features for long term urban sustainability, the cost and uncertainty behind these types of systems still remain the primary reasons that the implementation of low impact designs is challenging. Specifically, for the case of engineered wetlands, as a stormwater control measure, construction costs and long term maintenance and monitoring costs are of primary concern for land developers. Because stormwater quality is variable in nature, treatment efficacy through natural processes is challenging to monitor and validate. Proper validation of these systems often requires a two-year sampling regime, which is unlikely to be prioritized by most municipalities.

2.4.3 Traditional Water and Sediment Quality Monitoring for Validating the Efficacy of Stormwater Treatment Wetlands

2.4.3.1 Visual Inspection

Visual inspection is the first and least complex option for inspecting an engineered wetland. Visual inspection is performed by running through a pre-prepared checklist in order to see if the different components of the wetland qualitatively appear to be functioning as they were designed. The downside of visual inspection is that if there are no outward signs of malfunction, there is no guarantee that the field inspector will notice that the wetland is operating improperly. A typical visual inspection should involve review of the following wetland properties:

- History of previous visual inspections and assessments;
- Condition and extent of access to the wetland, including upstream and downstream areas;
- Condition of the inlet and outlet structures;
- Condition of each component of the wetland (i.e. forebay, low marsh, high marsh etc.);
- Condition of water –moving or stagnant as designed;
- Potential that an illicit discharge occurred;
- Signs of erosion and deposition;
- Health and condition of soil and vegetation;
- Quantity of litter and debris; and
- Stability of banks and sides of practices. (Erickson, Weiss, & Gulliver, 2013)

Taken together, assessment of these properties should indicate to a field inspector whether the wetland is being maintained properly by the owner and whether the wetland is likely functioning within its design constraints. Visual inspection gives no quantitative indication of water treatment efficacy.

2.4.3.2 Testing

Testing involves preparing a series of measurements which are taken under synthetically controlled conditions. Testing is considerably more involved than visual inspection but requires fewer resources than monitoring, which requires taking measurements during natural runoff events. Two types of testing are common when assessing stormwater treatment practices, namely capacity testing and synthetic runoff testing. Capacity testing requires taking point measurements to determine surface infiltration/filtration capacity or the remaining sediment storage available in a specific space. Synthetic runoff testing measures the overall performance of a wetland, rather than only a series of point measurements

2.4.3.2.1 *Capacity Testing*

Capacity testing using sediment retention tests can be of great value for assessing the sedimentation and thus solids removal performance of wetlands. Sediment retention tests require measurement of surface elevations using a level rod and a boat or using electronic sonar depth measurement equipment. Taken together with GPS or total station longitude and latitudes and design drawings, these measurements can provide an estimate of the retained sediment within a forebay or settling pond (Erickson, Weiss, & Gulliver, 2013). The rate and efficiency of sediment accumulation can then be estimated using predictions or measurements of the inlet water quality and the timespan that the wetland has been in operation.

Infiltration/filtration testing estimates the saturated hydraulic conductivity at specific locations within stormwater treatment systems. In the case of engineered wetlands, these measurements are less valuable because the wetlands are generally designed to inhibit infiltration and to instead convey water to a receiving water body. Infiltration/filtration capacity testing would be valuable if there is suspicion that the wetland is not functioning as designed.

2.4.3.2.2 *Synthetic Runoff Testing*

Synthetic runoff testing requires that a prescribed quantity and quality of synthetic stormwater is applied to a stormwater treatment practice during controlled conditions. In the case of engineered wetlands, theoretically, the wetland could be dosed with synthetic stormwater and the quality of water at the outlet could be measured over time. Conservative tracers such as chloride or rhodamine can be added to the synthetic stormwater in order to determine if there are dead zones or short circuiting in the wetland. The

accuracy of synthetic stormwater testing may be low because it is challenging to maintain representative and consistent suspended solids in synthetic stormwater (Erickson, Weiss, & Gulliver, 2013). This process is also limited by the amount of synthetic stormwater that can be prepared, either using a fire hydrant, water truck or other source. Synthetic stormwater testing is more practical for small stormwater systems like grit chambers and stormceptors.

2.4.3.3 Monitoring

Monitoring is the most accurate option for validating stormwater treatment systems but it is also the most time-consuming, resource intensive, and costly. Typically, monitoring is only performed when visual inspection and testing do not meet site validation goals or when stakeholders wish to use the treatment site as a demonstration of effective best management practices. Quantitatively monitoring the treatment effectiveness of engineered wetlands is achieved by collecting influent and effluent samples along each stage of the treatment system and determining the samples' pollutant concentrations through laboratory analyses. When developing a monitoring plan, it is necessary to follow standardized guidance procedures, which are described elsewhere (Erickson, Weiss, & Gulliver, 2013).

Due to the nature of weather, influent water quality and quantity is highly variable and, in order to have statistically significant analyses, repeat monitoring is generally required for all storms over a study period of fourteen to twenty-four months. Monitoring of engineered wetlands has a high potential for errors or losses in data because weather is unpredictable and the likelihood of equipment malfunctions over a long field study period increases with time.

2.4.4 Study Site: Lost Lagoon Stormwater Treatment Wetland

2.4.4.1 Precedent for Installation

In June of 1999, the Vancouver Board of Parks and Recreation commissioned KWL to prepare a stormwater management plan, which would coincide with upgrades to the Stanley Park Causeway. These upgrades were part of a larger Stanley Park Causeway rehabilitation project, which was funded under the umbrella of the British Columbia Transportation Financing Authority (BCTFA) Lions Gate Bridge project. On June 30th 1999, staff from the Park's Board and KWL held a workshop to develop recommendations for stormwater management along the causeway. The final recommendations included:

- Discharging all pavement surface runoff to Lost Lagoon;
- Treating the runoff through installation of an engineered wetland located in the northeast corner of Lost Lagoon; and

- Adding spill interceptors in two locations.

2.4.4.2 Design, Installation, Maintenance and Monitoring Regime

2.4.4.2.1 *Design*

Lost Lagoon was originally a saltwater passage between Vancouver and Stanley Park. In 1916, the eastern end of Lost Lagoon was cut off from Coal Harbour (Clifford, 1932). While there is a carp population that was seeded in the lagoon, it is recognized that, due to its artificial design, Lost Lagoon is primarily an aesthetic feature in the park and not a sensitive aquatic habitat (Kerr Wood Leidal Associates Ltd., 1999). Compared to other habitats in Stanley Park, the aquatic life in Lost Lagoon is generally tolerant to changes in salinity and water quality conditions but it was recognized during the design of the stormwater management plan in 1999 that the input of additional stormwater to Lost Lagoon should not reduce the quality of Lost Lagoon. In order to maintain the water elevation in Lost Lagoon, it is augmented by the city drinking water supply though use of a fountain. Originally, it was thought that stormwater from the causeway could supplement the inflow from the fountain but calculations proved that the stormwater inflow from the causeway would be negligible.

Before installation of the engineered wetland, the Stanley Park causeway was drained by catch basins which discharged stormwater into ditches on both sides of the road. This allowed the pollutants from the roadway to extend directly from the ditches into forested sections of Stanley Park. The new and revised drainage plan included a number of features to prevent contamination from the roadway from reaching forested areas. The causeway drainage plan had a number of provisions including:

- Two oil/water stormceptors – Stormceptor Model #3000 online with the storm sewer and located on the upper end of the causeway near the pedestrian overpass and Stormceptor Model #4000 located near the Lost Lagoon wetland system;
- A single discharge point for stormwater runoff located at the northeast corner of Lost Lagoon;
- Ditch subdrains for redirection of clean shallow groundwater directly to existing creek systems;
- A flow diversion structure at Lost Lagoon; and
- An engineered wetland including a settling forebay and flow augmentation structure.

The city drainage plan was said to ‘end’ at the discharge point of the stormceptor but the installation of engineered wetlands or ‘marshes’ was said to be required before discharging to Lost Lagoon.

The required size of the engineered wetland to be installed near Lost Lagoon was based on a design storm of 46 mm of rain in 24-hours as this was calculated to be ‘on-average’ the largest storm that would occur

within a six-month return period. The peak flow and total volume for the design storm were calculated to be 21 L/s and 1022 m^3, respectively. Comparatively, the causeway storm sewer system was designed for a 100-year return period storm. Thus, flows exiting the causeway during infrequently occurring large storms were designed to be diverted around the treatment wetland.

The final design of the Lost Lagoon wetland required construction of a berm to physically cut the wetland out of space along the side slope of Lost Lagoon. When the wetland was designed, sediments and low levels of oils, greases, nutrients, and organic matter were the primary contaminants of concern. De-icing salts were not considered to be of concern as the causeway very rarely requires de-icing. Thus, the wetland was designed to optimize removal of particulate matter through settling and removal of dissolved contaminants through adsorption on soil and bacterial processes associated with plant uptake.

The engineered wetland was designed to include several separate components for removal of various types of pollutants. Figure 4 illustrates these components. The major components include:

- A flow diversion structure, allowing flows greater than 25 L/s to bypass the wetland in order to prevent scouring and flooding;
- A sedimentation forebay, promoting settling of particles, including grit and particle-bound contaminants;
- Marsh terraces, allowing sustained contact between stormwater and soil and plant matter through extended settling, adsorption, and biological removal;
- Deep pools, contributing to biological diversity, increasing biological removal;
- Plants (e.g. *Carex* and *Scirpex*) specifically sourced to improve contaminant de-mobilization;
- An outlet structure, promoting a long residence time (2 weeks), eliminating short-circuiting and dead zones;
- Base flow inlets, helping sustain plant life during dry seasons by diverting surface watercourses if needed; and
- An augmentation structure, allowing movement of lagoon water into the forebay in the event that supplemental water is required in a drought year.

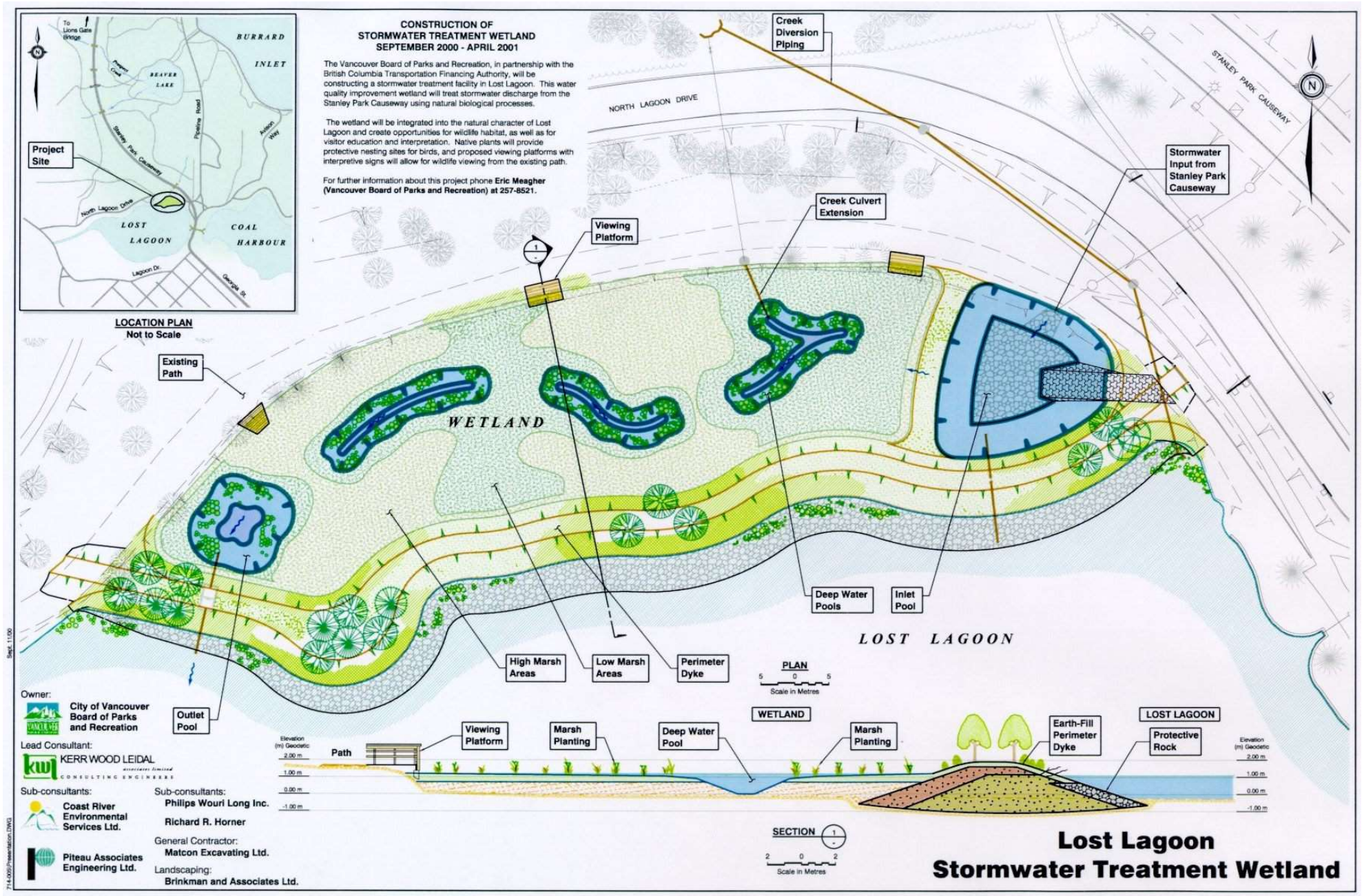

Figure 4. Illustration of the Lost Lagoon Wetland (Kerr Wood Leidal Associates Ltd., 1999)

2.4.4.2.2 *Installation*

Following its design, the Lost Lagoon wetland was constructed during the summer of 2000 and was fully commissioned for stormwater treatment in the spring of 2001. During construction, the water level in Lost Lagoon was lowered to the lowest feasible level based on environmental and aesthetic considerations. A silt curtain was set down and construction of the berm commenced first. Construction of the wetland's pools and marshes followed with subsequent construction of the access point and staging. Time was provided for expected settling and then final landscaping and planting was performed. This coincided with a monitoring and inspection plan for sediment and design quality. Figure 5 though Figure 10 are pictures, courtesy of KWL, that illustrate the installation and final wetland as commissioned in year 2001.

Figure 5. Laying of Silt Curtain

Figure 6. Construction of the Berm

Figure 7. Excavation of the Pools and Marshes

Figure 8. Vegetation Planted

Figure 9. Arial Shot Facing Northwest

Figure 10. Arial Shot Facing Southeast

2.4.4.2.3 *Maintenance and Monitoring Regime*

The BC Ministry of Transportation is responsible for the drainage sewer system, including both stormceptors and the Vancouver Board of Parks and Recreation is responsible for operating and maintaining the wetland and surrounding features.

The maintenance and monitoring regime for the wetland, as recommended by consultants at KWL includes several elements that occur during different seasons of the year and periodically. These elements are summarized in Table 5. Interestingly, there is no requirement for water or sediment quality testing, or testing of the treatment efficacy. Monitoring is performed only by visual inspection. KWL can be contacted directly for the manual on maintenance and monitoring of the Lost Lagoon wetland.

Table 5. *Elements of the Lost Lagoon Wetland Maintenance and Monitoring Regime* (Kerr Wood Leidal, 2002)

Period	*Activity*
Monthly	• Visually inspect the inlet pool, wetland marsh, inlet and outlet chambers, and Stormceptor • Record the Lost Lagoon water level at the Lagoon outlet • Check the wetland water level and record the level at the outlet flow control chamber • Remove trash • Check that people are not entering or damaging the riparian areas
Spring Maintenance (April)	• Inspect and repair observation platforms and interpretive signs • Clean out the Stormceptors • Flush the inlet flow control chamber • Flush the outlet flow control chamber • Adjust the water level in Lost Lagoon to between 0.8 m and 0.9 m • Adjust the wetland outlet weir to an elevation 1.20 m • Remove weeds and undesirable plants by hand
Summer Maintenance (July-August)	• Inspect plants for water stress • Augment inflow or irrigate if required
Fall Maintenance (October)	• Clean out the Stormceptor • Flush the inlet flow control chamber • Flush the outlet flow control chamber • Adjust the water level in Lost Lagoon to 0.6 m • Adjust the wetland outlet weir to elevation 1.15 m
Winter Maintenance (December)	• Flush the inlet flow control chamber
Annual Tasks	• Inspect wetland plants for presence, abundance and condition • Inspect bottom contours and water depths relative to plans • Inspect sediment and outlet conditions • If plant harvesting for nutrient control is desired, perform in the late summer
Periodic Tasks	• 2002, inspect plants twice per month during the summer • 2011, sediment removal
Every 5 Years	• Settlement survey • Infill/replant wetland plants

2.4.4.3 Best Management Practices Employed in the Lost Lagoon Wetland Design

When it was designed in 1999, the Lost Lagoon wetland employed many of the best management practices available to engineers. This was due to a strong desire by the City of Vancouver and the design consultants, KWL, to produce an effective and lasting treatment site in this high profile, public location. The best management practices incorporated into the wetland design for stormwater treatment included:

- Installation of two stormceptors for overflow protection;
    - The first stormceptor reduces the degree of emulsification of spilled materials with stormwater by reducing the distance that contaminants travel before capture, thus increasing capture efficiency.
- Installation of a flow diversion structure, preventing scouring, flooding and washout of the wetland;
- Inclusion of a valved outlet from the forebay to the Lost Lagoon, allowing the marsh to be bypassed during maintenance;
- Sizing the settling forebay to treat a 6-month return period design storm, allowing adequate treatment of most rainfall events that occur on the causeway;
- Sizing the wetland as a whole to have a long enough hydraulic retention time (2 weeks) to allow for adequate contaminant removal;
- Variation of the depths of terraces in the marsh system, allowing a diversity of plant and biotic life to take part in pollutant removal processes;
- Inclusion of deep pools and low-flow channels, facilitating pollutant removal mechanisms;
- Inclusion of an outlet setting pool, increasing stormwater residence time and reducing short-circuiting and under-treatment;
- Incorporation of an adjustable weir at the outlet, ensuring the water level in the marsh remains constant;
- Diversion of surface flow from nearby creeks, preventing flushing of the wetland during winter storms; and
- Inclusion of an irrigation connection along the length of the wetland berm for the case of an extreme drought, eliminating the need to add water from Lost Lagoon to the wetland as this could introduce algae and excessive sediment loads to the wetland.

The design consultants have a high interest in knowing whether these best management practices have contributed to the treatment efficacy of the wetland and this interest has been incorporated into the objectives of this study.

2.4.4.4 Previous Stormwater Quantity and Quality Data

2.4.4.4.1 *Year 2000: Drainage Area and Calculation of Design Flow*

In 1999, staff at KWL calculated the drainage area feeding into the Lost Lagoon wetland to be 2.7 hectares. This drainage area along with precipitation data from a nearby weather station in North Vancouver was used to model the 6-month design storm flow, using the PC-SWMM model (James, 2010). The calculations determined that a wetland design based on a maximum flow rate of 21 L/s would be able to treat over 92% of flow exiting the causeway on a yearly basis. Figure 11 illustrates the design hydrograph as retrieved from Kerr Wood Leidal Consulting Engineers Ltd. (1999)

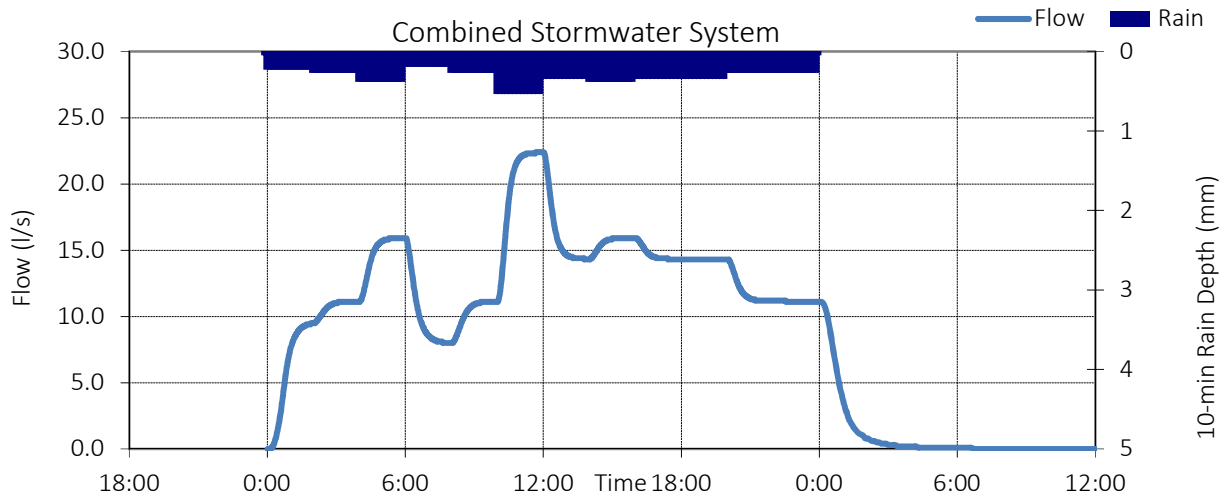

Figure 11. Design Hydrograph for Lost Lagoon Wetland (adapted from Kerr Wood Leidal, 1999)

2.4.4.4.2 *Year 2007: UBC Undergraduate Thesis*

In 2007, a group of undergraduate students, in the UBC Earth and Oceans Sciences Honors Environmental Science Program, performed an analysis on the Lost Lagoon wetland to evaluate its effectiveness so that the City of Vancouver could plan future maintenance. *Carex obnupta* and *Scirpus acutus* plant samples and sediment grab samples were collected at several locations between the wetland's inlet and outlet. The group's findings indicated that plants in the wetland had accumulated several metals associated with stormwater and that the water flowrate through the wetland contributed to higher metal uptake for plants. In addition, significant reductions in metal concentrations in the sediment were found for all metals except for arsenic. The group found that metal concentrations in the sediment were highest along the edges of the wetland, indicating that the water residence time led to an increase in the deposition of metals. Figure 12 illustrates the locations that the student group sampled for plants and sediment. Table *7* lists the mean metal concentrations for the plant and sediment samples, respectively.

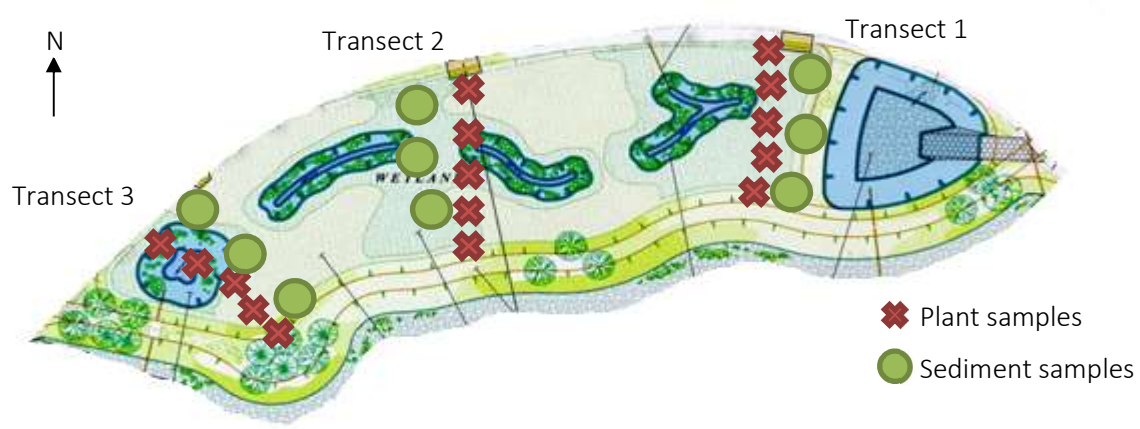

Figure 12. 2007 Sample Sites in Lost Lagoon for Plants and Sediment (adapted from Thoren et al., 2007)

Table 6. 2007 Results for Plant Specimens in Lost Lagoon Wetland (Thoren et al., 2007)

Metal	*Concentration in Carex (ppm)*				*Concentration in Scirpus (ppm)*		
	Transect 1	*Transect 2*	*Transect 3*	*Outside*	*Transect 1*	*Transect 2*	*Transect 3*
Cd	1.1	2.8	0.37	0.5	0.66	0.53	0.33
Cu	75.2	119.7	30.3	24.9	68.0	38.7	30.2
Mn	383.3	730.7	664.3	453.0	311.0	580.7	463.7
Pb	26.4	34.3	8.95	6.21	30.1	9.69	7.76
Zn	146.0	236.0	77.7	0.44	109.1	184.0	97.6

Table 7. 2007 Results for Sediment Samples in Lost Lagoon Wetland (Thoren et al., 2007)

Metal	*Mean Inlet Concentration (mg/kg dry weight)*	*Mean Outlet Concentration (mg/kg dry weight)*	*Percentage Decrease (%)*
As	3.9	2.5	36.4
Cd	0.5	0.1	73.1
Cr	28.7	20.6	28.3
Cu	66.9	23.9	64.3
Pb	27.4	5.3	80.6
Ni	26.7	21.7	18.7
Zn	132.0	57.8	56.2

Thoren et al (2007) employed a simple regression model to relate metal concentrations to the distance from the outlet.

$$y = ae^{-kx}$$

Where,

- a represents the y-intercept of the graph;
- k represents the slope or removal efficiency; and
- x represents the distance along the wetland.

Regression analysis was accompanied by R^2 and p-values, which represent the accuracy and suitability of the exponential model and the significance of the decrease, respectively. These results are summarized inTable 8.

Table 8. 2007 Regression Analysis Results for Sediment Samples in Lost Lagoon Wetland (Thoren et al., 2007)

Metal	*R^2*	*k-value*	*t-value*	*P>|t| (p-value)*
As	0.348	-0.00693	-2.63	0.0207
Cd	0.614	-0.02083	-4.55	0.0005
Cr	0.494	-0.00618	-3.56	0.0035
Cu	0.549	-0.01681	-3.97	0.0016
Pb	0.549	-0.02414	-5.18	0.0002
Zn	0.6352	-0.01314	-4.76	0.0004

Thoren et al also compared the mean, maximum, and minimum metal concentrations in the sediment to average metal concentration in the soil of Washington State. These results are summarized in Table 9.

Table 9. Comparison of 2007 Wetland Results with Sediment Data for Washington State (Thoren et al., 2007)

Metal	*Washington State (g/kg dry weight)*[1]	*Lost Lagoon Wetland Mean (mg/kg dry weight)*[2]	*Lost Lagoon Wetland Max (mg/kg dry weight)*[2]	*Lost Lagoon Wetland Min (mg/kg dry weight)*[2]
As	4.5	3.0	5.3	1.1
Cd	0.8	0.4	1.7	0.1
Cr	49.9	25.5	47.3	17.5
Cu	31	221	53.1	18.4
Pb	14	19.5	82.7	4.3
Zn	78	103.4	288.0	54.7

[1] (Washington State Department of Transportation, 2007)
[2] (Thoren et al., 2007)

Overall, the results from the 2007 assessment provide a promising reason to use the Lost Lagoon wetland as a research site for development of a genomics tool. Both plant and sediment samples indicate metals are retained within the wetland and stormwater treatment is occurring successfully. However, further evidence of these conclusions is still needed, using more recent samples and a greater depth of sampling.

2.4.4.4.3 *Year 2013: Vancouver Board of Parks and Recreation Sediment Dredging Report*

In 2013, the City of Vancouver contracted Hemmera environmental consultants to perform an in-situ investigation of the sediment quality in the Lost Lagoon wetland. This project was executed in order to confirm that the sediment would not be classified as a hazardous waste prior to dredging and disposing of the sediment in a landfill. Grab samples were taken from eight locations in the wetland forebay and the samples from five of the eight locations underwent laboratory analysis. These locations are illustrated in Figure 13. Hemmera also unsuccessfully attempted to extract core sediment samples from the wetland but further results of this attempt were not recorded in their report.

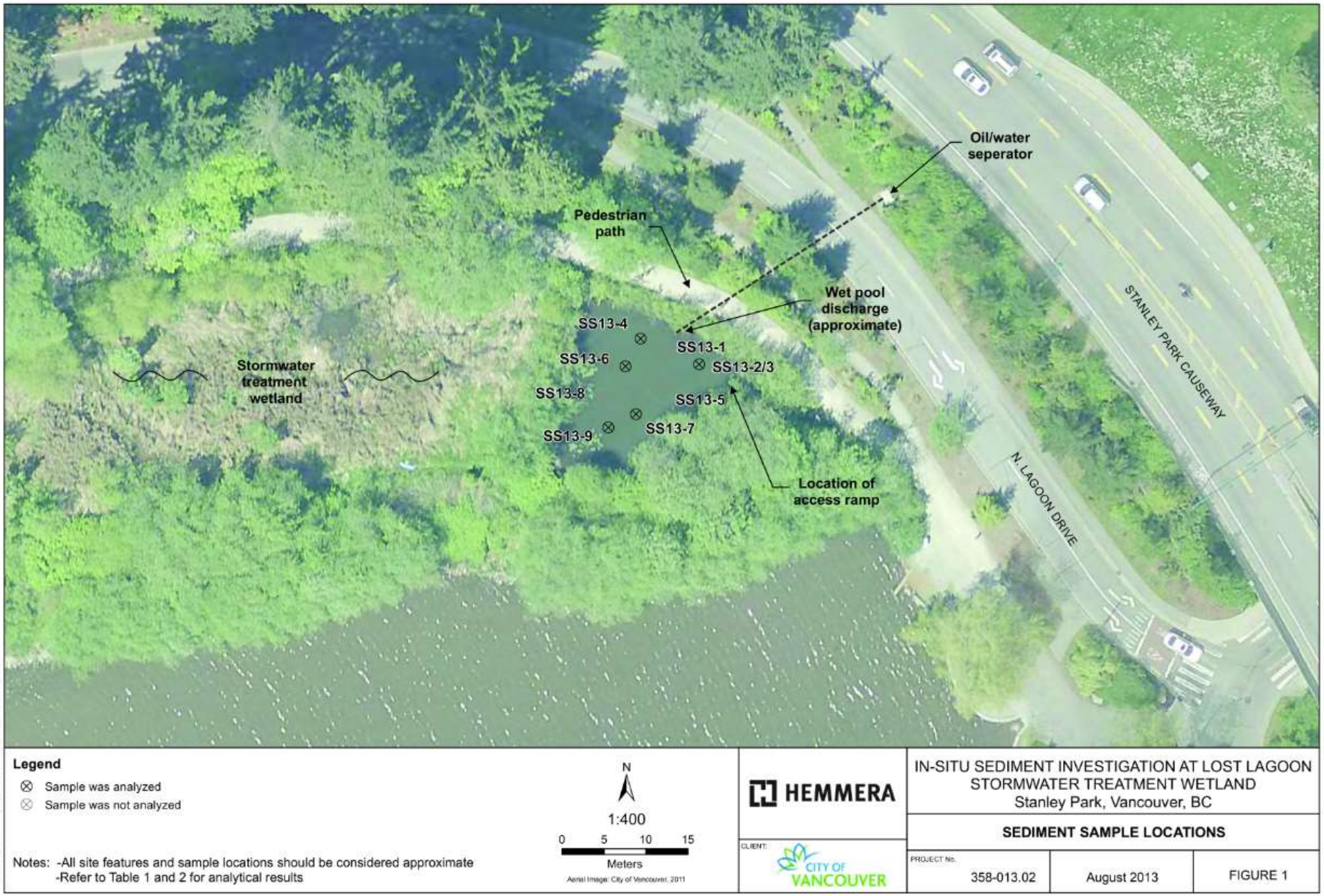

Figure 13. Locations Sampled by Hemmera During the 2013 Sediment Investigation (Hemmera, 2013)

Field observations were recorded at the time of sampling and included the following:

- "The sediment substrate at the sampling locations generally consisted of dark grey to black sand with trace silt, gravel, organics, and pine needles;
- No marine fauna was observed by Hemmera;
- A hydrogen sulfide (H_2S) odor was noted in the majority of the sediment samples collected;
- No petroleum hydrocarbon sheens were observed in the collected samples. However, a petroleum hydrocarbon-like odor was observed in two samples; and
- The moisture content measured in the sample ranged from 68.8%-72.8%." (Hemmera, 2013)

The results provided by the laboratory analysis offer a number of important observations. Each sample submitted had concentrations of one or more metal constituents above soil guidelines and these constituents primarily included antimony, chromium, copper, molybdenum, lead and zinc. In addition, all samples had concentrations of HEPH above standards. Sodium and chloride ions as well as VOCs, PCBs, chlorinated hydrocarbons, and chlorinated/non-chlorinated phenols were measured to be below the allowable levels. *Table 10* summarizes the regulatory levels and concentrations measured in the sediment of the Lost Lagoon wetland for the constituents of primary interest. These measurements indicate that a high contaminant loading was deposited and retained in the wetland forebay in the ten years prior to when the forebay was dredged.

Table 10. BC Residential Soil Standards and Metal Concentrations Measured in the Sediment of the Lost Lagoon Wetland Forebay

Metal	Regulatory Standard (mg/kg dry weight)[1]	Measured Range (mg/kg dry weight)[2]	% In Excess
Antimony	20	25-65	20-225
Chromium	100	100-140	0-40
Copper	90-150	350-650	153-620
Lead	150	160-240	7-60
Molybdenum	10	11-30	10-200
Zinc	450	600-1200	33-160

[1] (British Columbia Ministry of Water, Land and Air Protection, 2011)
[2] (Hemmera, 2013)

2.5 Methodology

The primary focus of this chapter was to demonstrate that, overall, the Lost Lagoon wetland is meeting treatment guidelines and to lay the groundwork for the microbial analyses in Chapter 2. With this goal in mind, a strategic methodology was developed for the Lost Lagoon wetland field study. Specifically, sediment quality and long term treatment trends in the wetland were of greatest concern for the environmental sampling. A detailed description of the methodology employed to answer the objectives and hypotheses listed at the beginning of this chapter is supplied here.

2.5.1 Site Visits and Sampling Regime

2.5.1.1 Field Site Survey and Conditions at the Time of the Field Study Site Visit

On April 23, 2015 at 8:00 AM, an initial field site survey was conducted to assess the conditions of the wetland. GPS coordinates and digital photographs were taken at all points of interest and locations that indicated damage to the wetland features. Figure 14 illustrates a map of the field site and GPS locations of the photos.

Figure 15 through Figure 26 illustrate some of the relevant photos from the site visit. The initial site survey indicated that all of the major elements of the wetland were intact and are being maintained. However, there were signs of beaver activity, which required further investigation with park staff members.

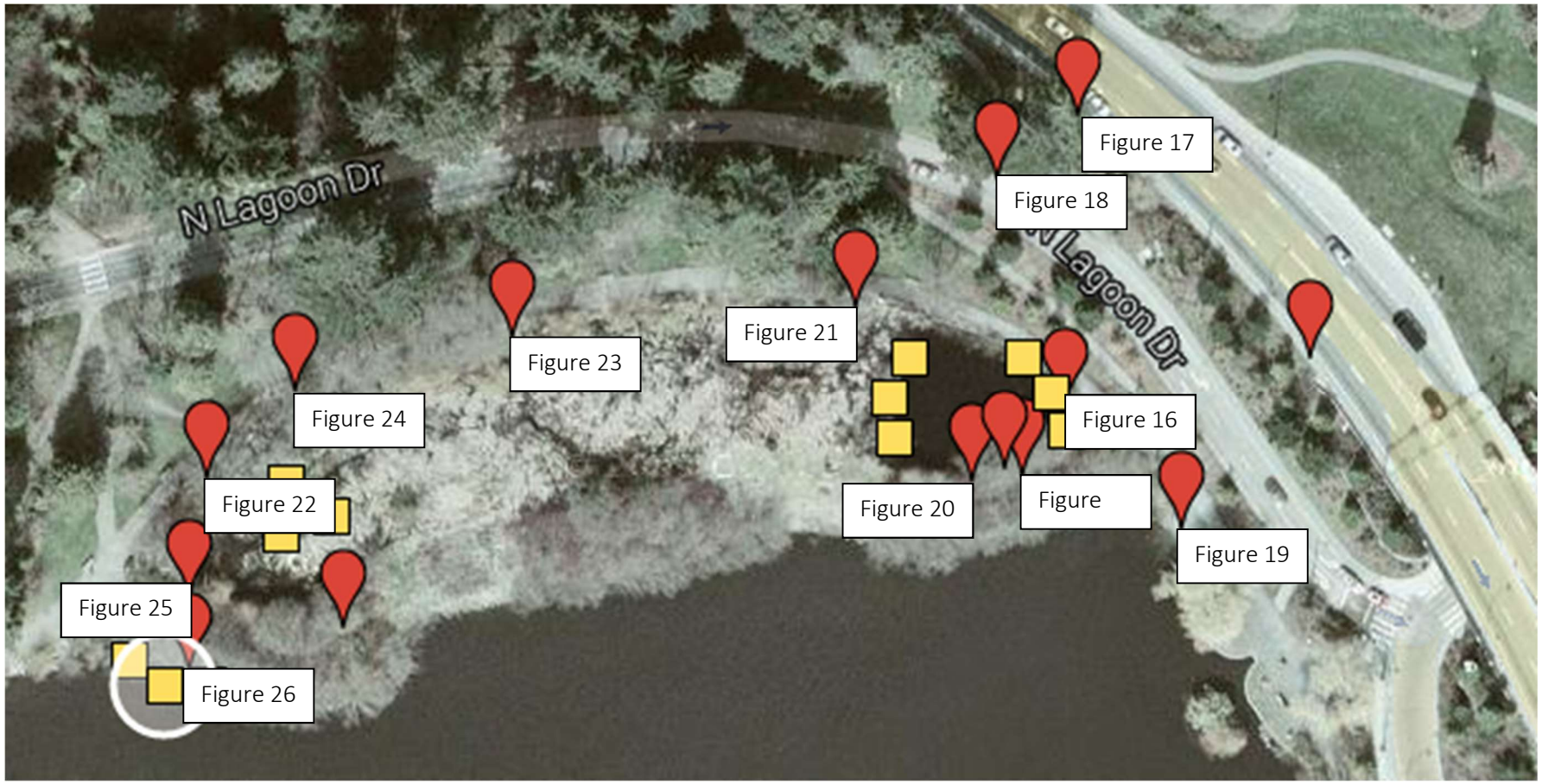

Figure 14. Survey Map of Field Site

Figure 15. Lost Lagoon

Figure 18. Access Point for the Lower Stormceptor

Figure 16. On Site Graphic of Treatment Process

Figure 19. Wetland Bypass to Lost Lagoon

Figure 17. Storm Sewer on the Stanley Park Causeway

Figure 20. Setting Forebay

Figure 21. High Marsh

Figure 24. Signs of Beaver Activity at Lost Lagoon

Figure 22. Low Marsh

Figure 25. Access Point for the Wetland Outlet Control Valve System

Figure 23. Sections of Low Marsh Showing Plant Damage and Beaver Activity

Figure 26. Outlet Point to Lost Lagoon

2.5.1.2 Sampling Locations and Dates

As sediment quality and long term treatment trends in the wetland were of greatest concern for the environmental sampling, the sampling locations and dates was optimized to obtain results that could both verify the treatment performance of the wetland and add to the microbial analyses in Chapter 2.

The hypotheses in this study require that there are differences in the sediment quality at the front and back end of the wetland. Therefore, initially a 'search sampling' methodology (Gilbert, 1987) was applied in order to divide the wetland into 6 areas for comparison as illustrated in Figure 27. These areas included:

1. The lower stormceptor;
2. The East side of the forebay, closest to the inlet pipe;
3. The centre of the forebay;
4. The West side of the forebay, furthest from the inlet pipe;
5. The settling pool closest to the outlet pipe; and
6. The exit pipe from the wetland, at the shore of Lost Lagoon.

Figure 27. Field Study Sampling Locations at the Lost Lagoon Wetland

In order to reduce the size of the comparison areas but retain statistical significance, the comparison areas were further divided into 1-m^2 plots and a 'systematic sampling' methodology (Gilbert, 1987) was applied to select study plots at equal intervals using an aligned grid. Sampling of the study plots was also performed using systematic sampling, where samples were taken from the four corners and the center of each plot.

Seven samplings of the wetland occurred between July and December of 2015. As the sample area in the wetland was relatively large, compared to the resources available to the research team, not all study plots could be sampled on a given study day. The implications of this are further discussed in the Limitations and Recommendations sections of this thesis.

Since sampling was to occur on public land in a treatment space that provides habitat for local birds and inner-city animals, great care was taken during the sampling process to reduce damage to the site. In addition, sampling plans were approved by staff at both the Vancouver Board of Parks and Recreation and at the Stanley Park Ecology Society. Documentation of approval and support for this study can be found in Appendix H.

During the sampling events, three mediums were sampled – surface sediment at the wetland floor, sediment at a depth of 10 cm below the wetland floor, and water at the soil-water interface. In some cases, inaccessibility or inoperable equipment limited the number of samples that could be taken. This is further discussed in the study's Limitations section.

Table 11 summarizes the samples, which were taken from the field study site and Figure 28 provides an overview of the field sampling process.

Table 11. Field Study Samples Taken

Site Number	Description	# Days Sampled	Dates	Sample Medias
1	Stormceptor	1	Dec 16	Water
2.1	NW Corner Forebay	3	Sept 9, Oct 21, Dec 16	Water, depth and surface sediment
2.2	N Centre Forebay	2	Nov 11, Dec 16	Water, surface sediment
2.3	NE Corner Forebay	3	Oct 6, Nov 11, Dec 16	Water, depth and surface sediment
3.1	W Centre Forebay	2	July 21, Sept 9, Oct 21	Water, depth and surface sediment
3.2	Centre Forebay	2	Oct 21, Nov 11	Water, depth and surface sediment
3.3	E Centre Forebay	2	Oct 6, Nov 11	Water, depth and surface sediment
4.1	SW Corner Forebay	3	July 21, Sept 9, Oct 21	Water, depth and surface sediment
4.2	S Centre Forebay	2	Oct 21, Nov 11	Water, depth and surface sediment
4.3	SE Corner Forebay	2	Oct 6, Nov 11	Water, depth and surface sediment
5.1	NW Settling Pond	2	Sept 22, Nov 11	Water, depth and surface sediment
5.2	Centre Settling Pond	2	Sept 22, Nov 11	Water, depth and surface sediment
5.3	SE Settling Pond	2	Sept 22, Nov 11	Water, depth and surface sediment
6.1	W Exit	3	July 21, Sept 9, Oct 21	Water, depth and surface sediment
6.2	Centre Exit	3	July 21, Sept 9, Oct 21	Water, depth and surface sediment
6.3	E Exit	3	July 21, Sept 9, Oct 21	Water, depth and surface sediment

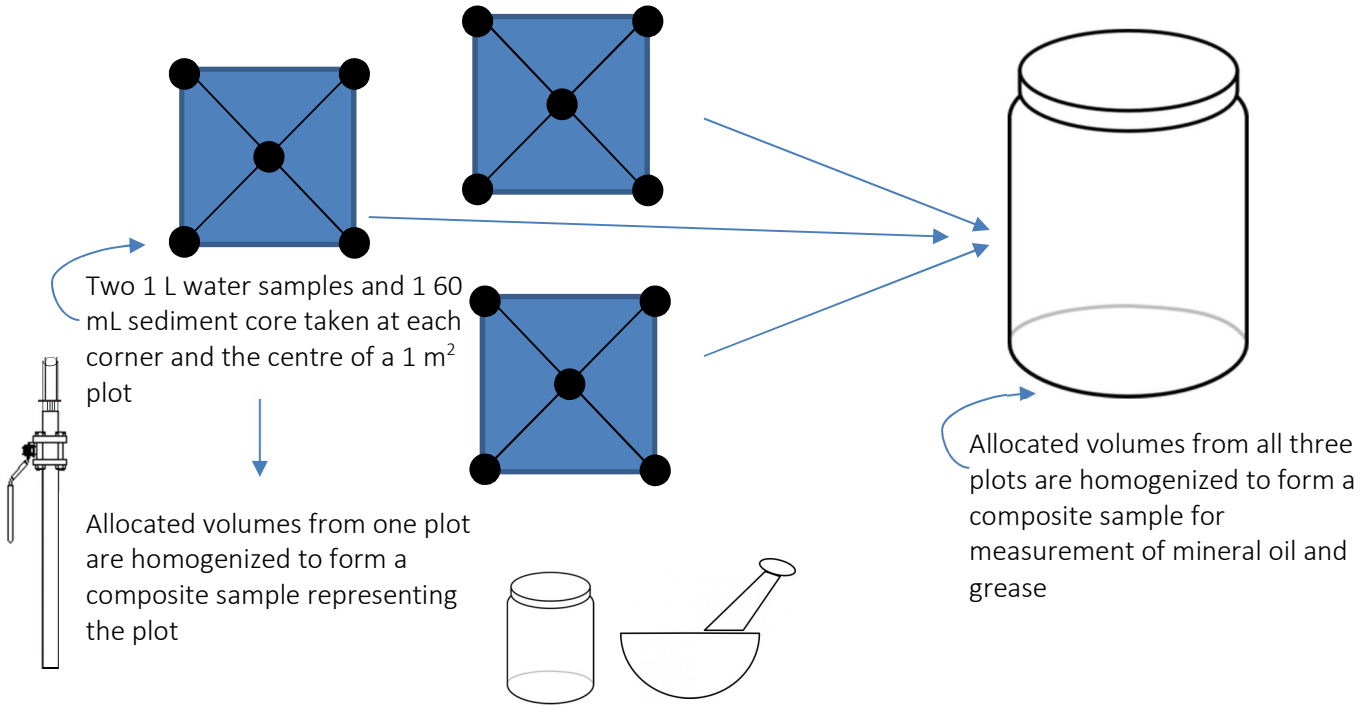

Figure 28. Overview of Field Sampling Process

2.5.1.3 Water Sampling Equipment

As illustrated in Figure 29, a syphon (Col-Parmar WZ-70607-00) and plastic tubing were used to extract two 1-L samples from each sampling location. All samples were taken while the researcher sat in a small dinghy.

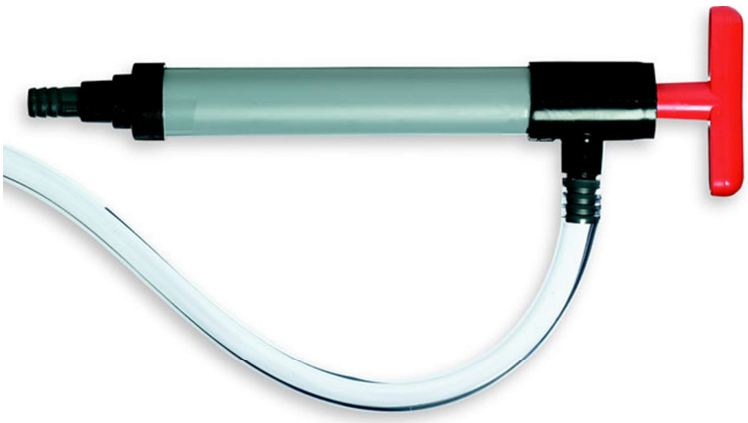

Figure 29. Image of the Water Sampling Equipment

2.5.1.4 Sediment Sampling Equipment

Sampling of sediment in the wetland represented a major challenge. Particularly in the wetland forebay, because the water depth exceeded 2 m in some locations, great care and accuracy was required to obtain a core of sediment. There was variability in the quality and consistency of the sediment with some areas being sandy soils and other areas being primarily clayey soils. In addition, sampling for microbiology and an interest in differences in the sediment at the surface of the wetland floor and below the surface of the wetland floor created additional challenges to ensure that mixing of the sediment layers did not occur during sampling.

During method development, different apparatuses were tested for their ability to extract and hold a sediment core. After several trials, a successful custom sediment core sampling was built. To build the apparatus, first a 60-mL syringe with a diameter of ¾ inches was fit at the nose end to a ball valve. The ball valve was connected to a PVC pipe to be used as a sampling rod. Next, two circular stainless steel fittings were clamped over the handle of the syringe. Two screws were driven through the metals fittings and copper rods were connected to the screws. A second 60 mL syringe was clamped to the tail end of the first syringe using stainless steel fittings and a plastic O-ring. Two stainless steel fittings were clamped over each end of the handle of the second syringe and removable screws were driven through the stainless steel clamps and screwed into the copper rods. The nose of the second syringe was sanded off so that this end of the sampler could be driven into the wetland soil using a rubber mallet. After taking a sample, the second syringe was unscrewed and unhooked from the rest of the apparatus and a new syringe was put in place. This process was repeated for each sample. Figure 30 illustrates the sediment core sampling apparatus.

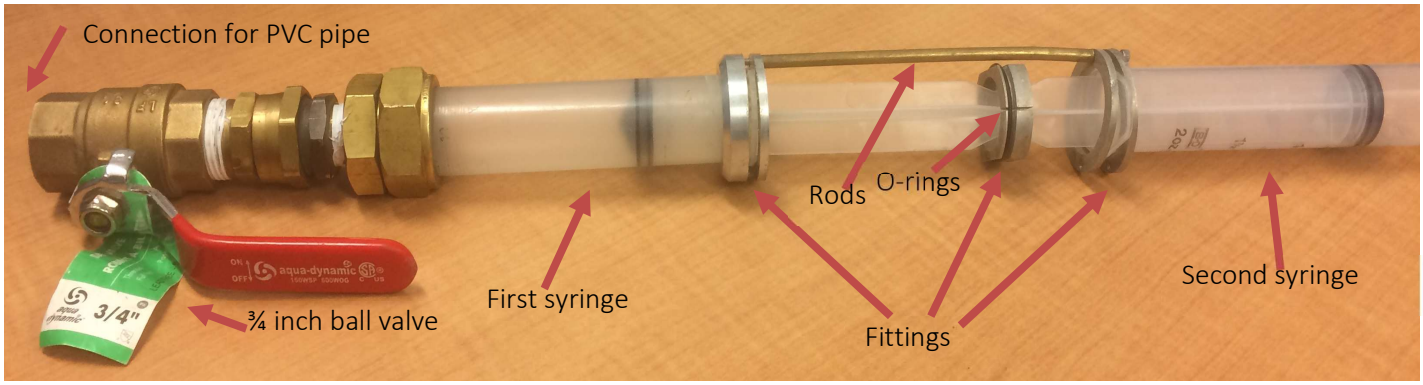

Figure 30. Photograph of the Sediment Sampler

2.5.1.5 Sample Collection, Preservation, Storage and Transport

2.5.1.5.1 *Water Samples*

Two 1 L samples were taken above the wetland floor using a syphon and plastic tubing. After a sample was taken, it was immediately poured into a clean 1 L plastic bottle and labelled. The syphon and tubing were then rinsed with distilled water and 90% ethanol solution.

The plastics bottles were brought back to shore, where a small field lab processing site was set up. 500 mL of each sample was poured into a clean wide mouthed plastic bottle and immediately tested for environmental parameters using a YSI probe. On site measurements were recorded for DO, pH, temperature, conductivity, and redox potential.

Composite water samples were prepared to represent each plot and preserved on site using the following protocols.

- Total Organic Carbon (TOC)
    - Add 25 mL from each of 5 1-L bottles
    - Add 1 drop H_3PO_4
    - Place in cooler on ice
- Chemical Oxygen Demand (COD)
    - Add 20 mL from each of 5 1-L bottles
    - Add 1 drop H_2SO_4
    - Place in cooler on ice
- Metals
    - Add 10 mL from each of 5 1-L bottles
    - Add 1 drop HNO_3
    - Place in cooler on ice
- Turbidity/Total Suspended Solids (TSS)
    - Add 100 mL from each of 5 1-L bottles
    - Place in cooler on ice
- Mineral Oil and Grease (MO&G)
    - Add 50 mL from each of 15 1-L bottles (3 sets of 5)
    - Add 2 drops H_2SO_4
    - Place in cooler on ice

Samples were also homogenized for microbial analysis. This method is described in Chapter 2.

Water samples for environmental parameters were stored on ice and transported by truck to the CEME Environmental Laboratory at UBC. The samples were stored at <4 Celsius until further processing and analysis.

2.5.1.5.2 *Sediment Samples*

During sampling, the researcher used a hard rubber mallet to drive the sampling apparatus into the sediment at the location of interest. The researcher then carefully pulled up the sampler, removed the syringe from the sampler apparatus, and wrapped both ends of the syringe in laboratory grade aluminum foil that was previously disinfected with ethanol. The syringe was immediately placed in a cooler on dry ice. For the next sampling event, the sampler was cleaned with ethanol and a new clean syringe was attached using an Allen key.

Sediment samples for were stored on dry ice and transported by truck to the CEME Environmental Laboratory at UBC. The samples were stored at <-20 Celsius until analyzed.

2.5.2 Laboratory Analysis of Water Quality Parameters

2.5.2.1 Sample Handling and Preservation

All equipment that was to come into contact with sediment was soaked in 10% bleach solution for a minimum of 24 hours prior to sample handling. All equipment was rinsed with nitric acid and then cleaned with 90% disinfectant grade ethanol between sampling.

Sediment samples remained in the plastic syringes and were frozen at <-20 Celsius until further processing. Frozen syringes were then removed from the freezer. The first 1-cm of sediment content in the five syringes which corresponded to one sample plot were cut from each sediment sample and placed in a mortar. To keep the samples frozen during processing, the mortar was placed in a stainless steel bowl that was filled with crushed dry ice. The sediment was ground and homogenized to a fine consistency using a pestle and any large rocks and sticks were pulled out prior to placing the ground sample into a disinfected plastic Ziploc bag. Samples were placed back in the freezer at <-20˚C until further processing. The same process was followed for the last 1-cm of each sediment core. By this means, both the surface sediment and sediment at a depth of 10-cm could be analyzed.

2.5.2.2 Analytical Methods

The researcher applied standard environmental laboratory tests based on equipment available in the CEME Environmental laboratory. The laboratory tests employed for each environmental parameter were:

- Environmental parameters – YSI handheld multi-parameter instrument;
- MO&G – USA EPA Method 1664 (United States Environmental Protection Agency, 1999);
- TOC – USA EPA Method 415.3 (Potter & Wimsatt, 2005);
- COD – Hach Method 8000 (Hach, 2008);
- Turbidity – USA EPA Method 180.1 (O'Del, 1993); and
- TSS – Hach Gravimetric Method 8158 (Hach, 2007)

Due to high organic content in the samples, water samples were digested for metal analysis using a custom protocol based on EPA method 3050-B (United States Environmental Protection Agency, 1996) described in Appendix A. Metals were analyzed using ICP-OES on a Varian Liberty 100/200 apparatus. Samples were analyzed in triplicate and measurements included analysis of procedural and field blanks.

2.5.3 Statistical Analyses

Analyses of the main parameters of interest, metals and mineral oil and grease, were first performed through visual assessment of the data. To compare the metal concentrations at each plot, bar graphs were prepared to illustrate the average concentrations of each metal that is associated with stormwater. Boxplots of the concentrations for each metal were used to provide a visual assessment of the symmetry of the distribution and the variability in the concentrations between the wetland entry (Site 2, 3, and 4) and the wetland exit (Site 5). Each media (water, surface sediment, and 10-cm depth sediment) was visualized individually because it is expected that that these medias will behave differently.

In order to compare the measured environmental pollutant and metal concentrations, Wilcoxon paired rank tests were performed between the results measured at the wetland entry, exit and the Lost Lagoon. The Wilcoxon rank test is the equivalent to the common paired student t-test for comparison of two means. However, the Wilcoxon rank test does not assume that the measurements are normally distributed and for this reason, the Wilcoxon rank test carries somewhat less weight. However, environmental samples tend not be normally distributed due to outliers at high concentration levels; thus, in this case, the Wilcoxon rank test is a better fit for the data.

2.6 Results and Interpretation

In this section results and interpretation are supplied for the laboratory tests. Each environmental parameter is illustrated as a bar graph by plot and then by a boxplot between the wetland entry (Sites 2, 3, and 4), wetland exit (Site 5) and Lost Lagoon (Site 6). This method of visualization allows for comparison first along the width and length of the wetland and then between the major locations at the field site. After

the visual illustration, statistical comparisons are calculated. An interpretation of the data is supplied prior to the figures and statistical calculations.

2.6.1 Turbidity, Total Suspended Solids, Chemical Oxygen Demand and Total Organic Carbon

2.6.1.1 Interpretation

Turbidity, TSS, COD, and TOC cannot be directly attributed to the stormwater entering the wetland from the roadway because sampling was performed during the autumn season and leaf matter from overhanging trees deposited directly into the wetland and contributed to the high solids content during the study period. That being said, a similar relationship for turbidity, TSS, COD, and TOC was observed between the various sites where water was sampled in the Lost Lagoon wetland. Generally, these parameters were measured to have higher averages in the wetland inlet than in the wetland outlet and also higher averages in the Lost Lagoon than in the wetland outlet. Figure 31 through Figure 38 graphically illustrate the relationship that was observed.

In *Table 12* through *Table 14*, a significant statistical difference is interpreted when $p<0.05$, or in other words, when there is at least 95% confidence that interpreting two medians as being different occurs when the two medians are truly different. No significant differences in the medians of turbidity, TSS, TOC, and/or COD were calculated between the wetland entry, wetland exit, and the Lost Lagoon. This is likely due to the high range of measurements over the sampling period caused by the contribution of organic matter over the autumn sampling season.

2.6.1.2 Turbidity Figures

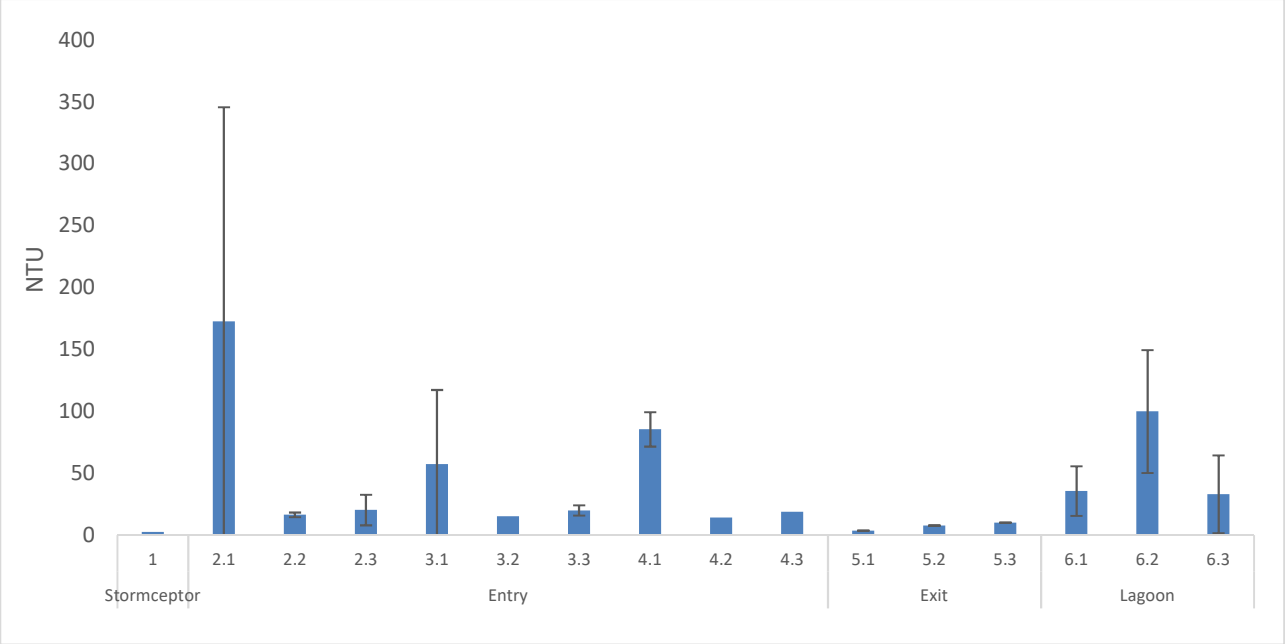

Figure 31. Barplot Comparison by Plot of Turbidity in Water Samples Collected During the Field Study

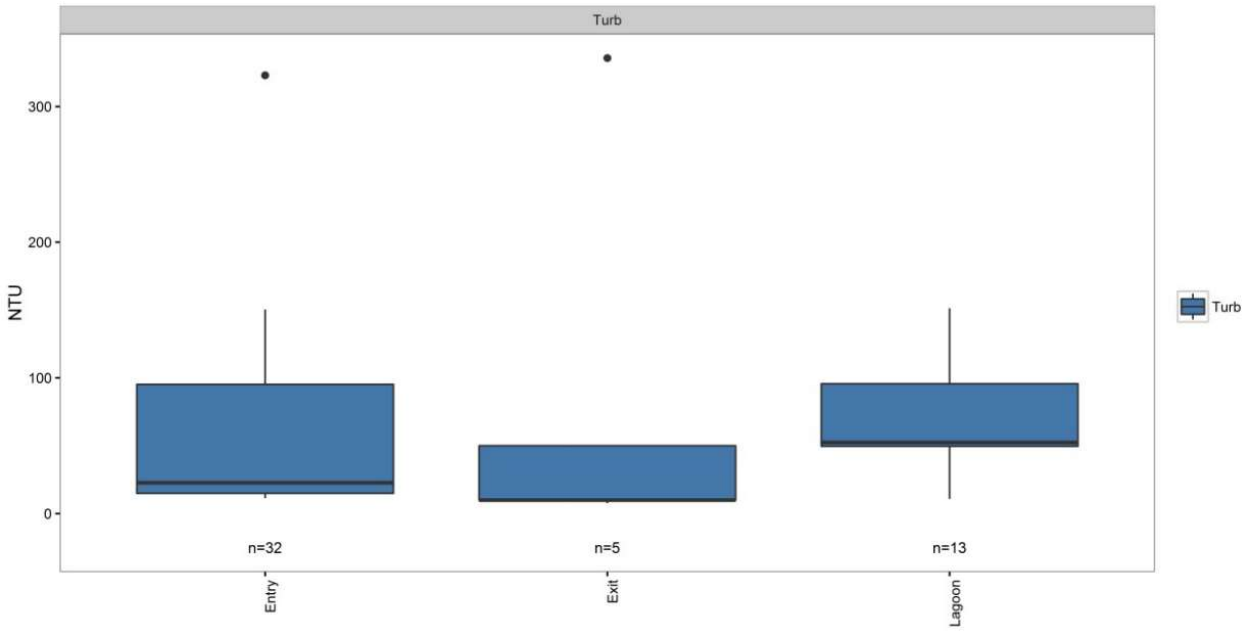

Figure 32. Boxplot Comparison of Turbidity in Water Samples Collected During the Field Study

2.6.1.3 Totals Suspended Solids Figures

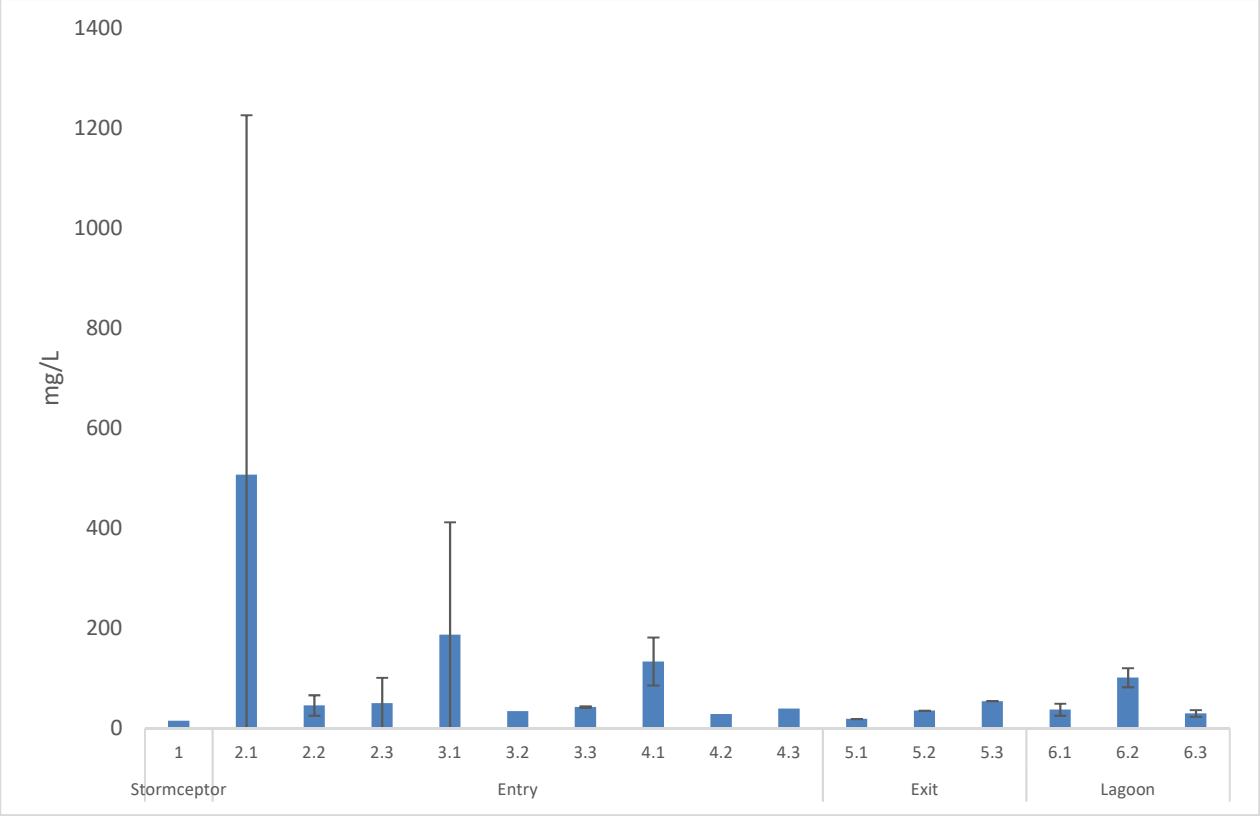

Figure 33. Barplot Comparison by Plot of TSS in Water Samples Collected During the Field Study

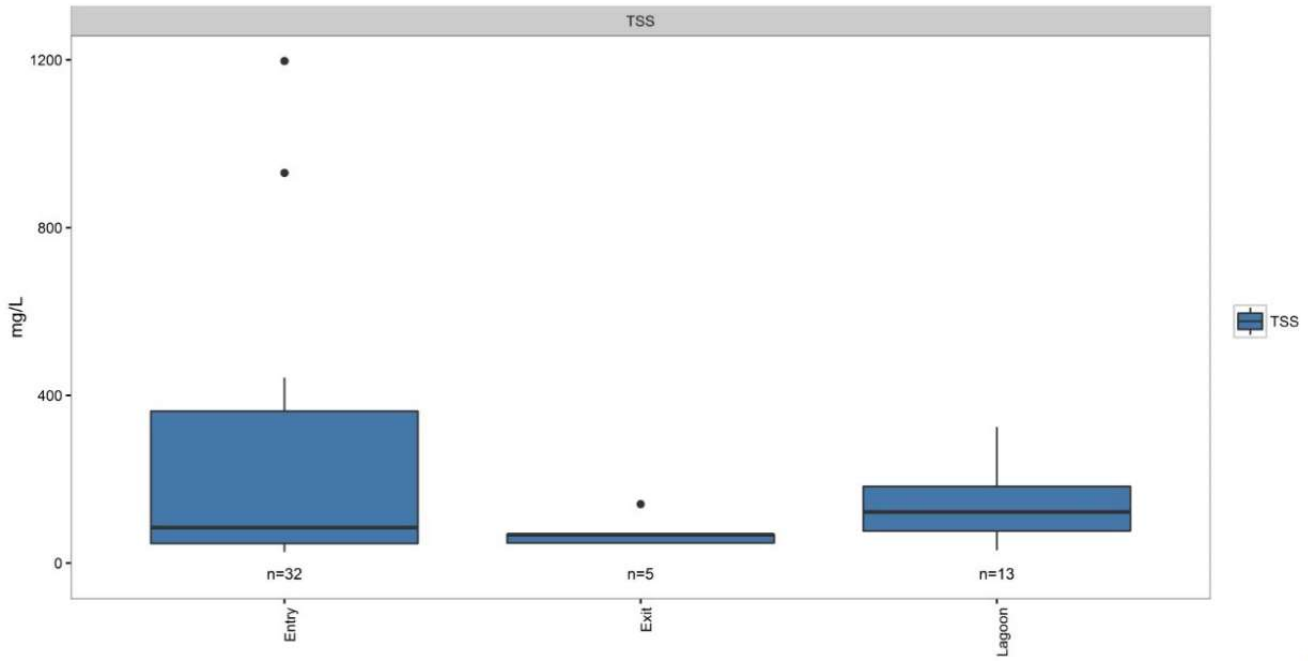

Figure 34 Boxplot Comparison of TSS in Water Samples Collected During the Field Study

2.6.1.4 Chemical Oxygen Demand Figures

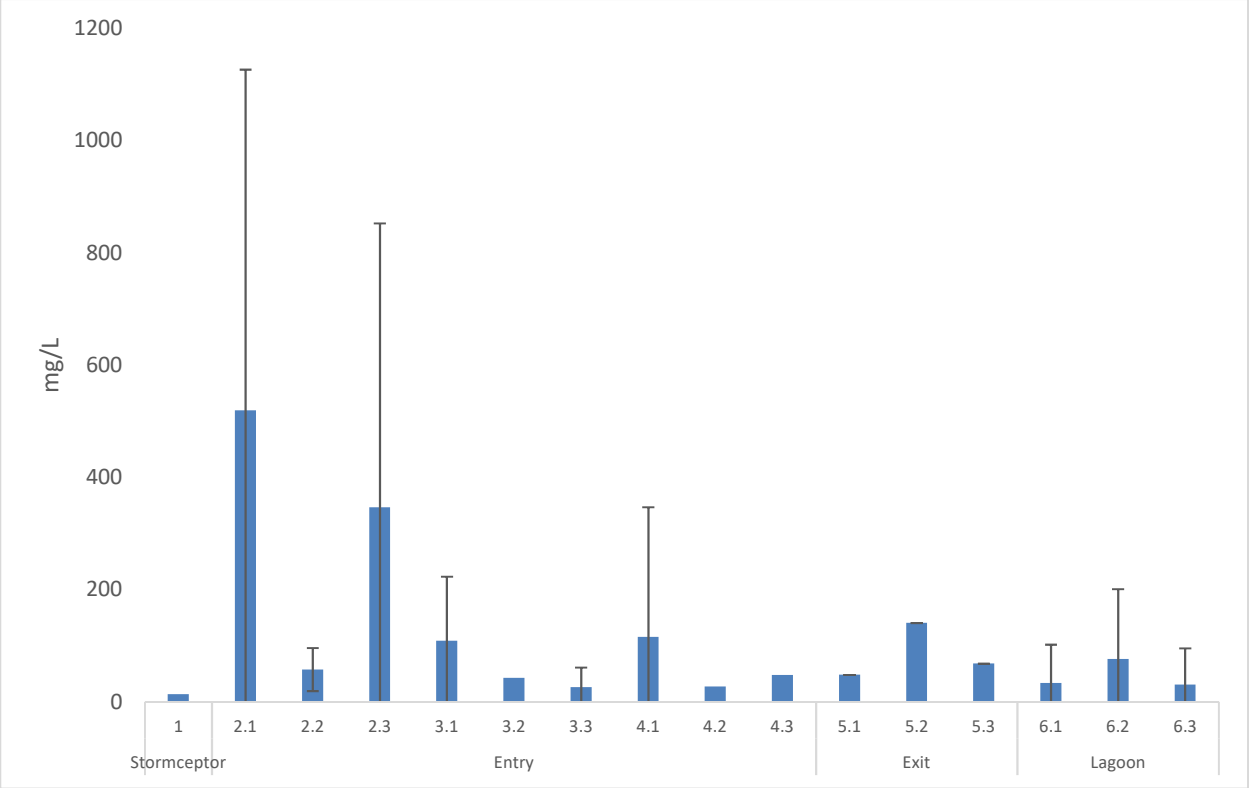

Figure 35. Barplot Comparison by Plot of COD in Water Samples Collected During the Field Study

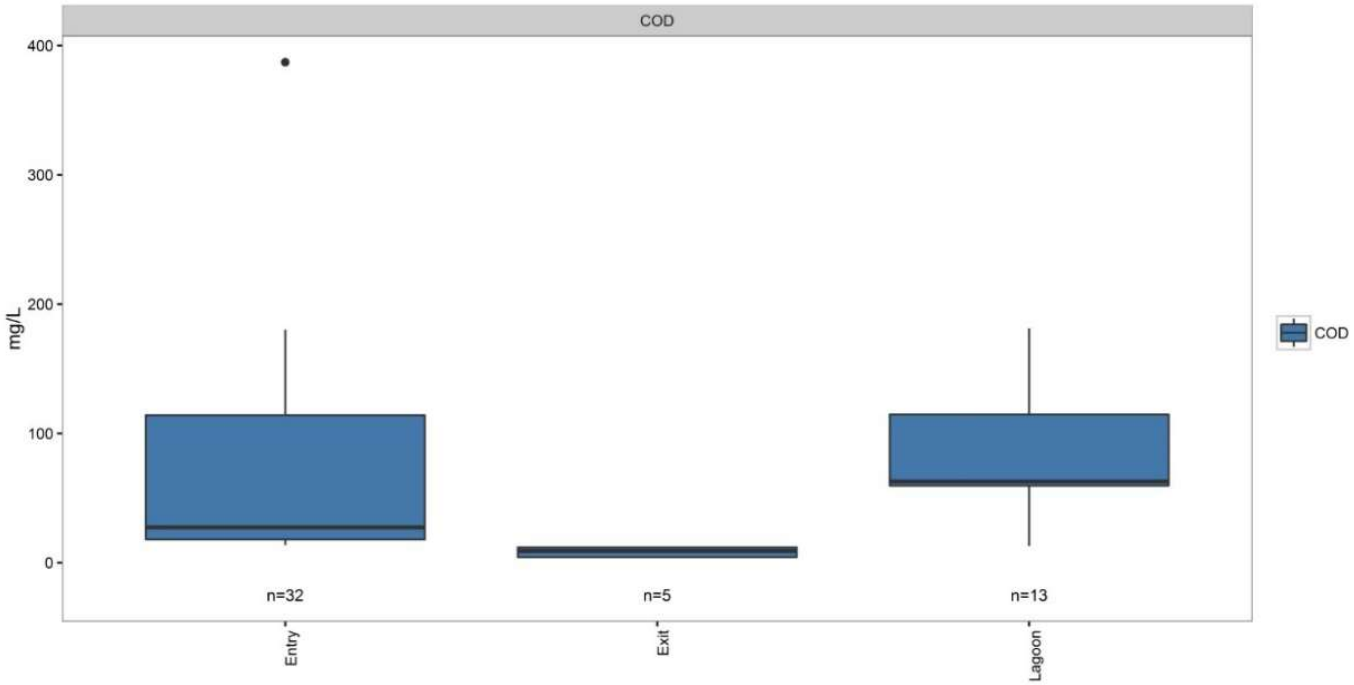

Figure 36. Boxplot Comparison of COD in Water Samples Collected During the Field Study

2.6.1.5 Total Organic Carbon Figures

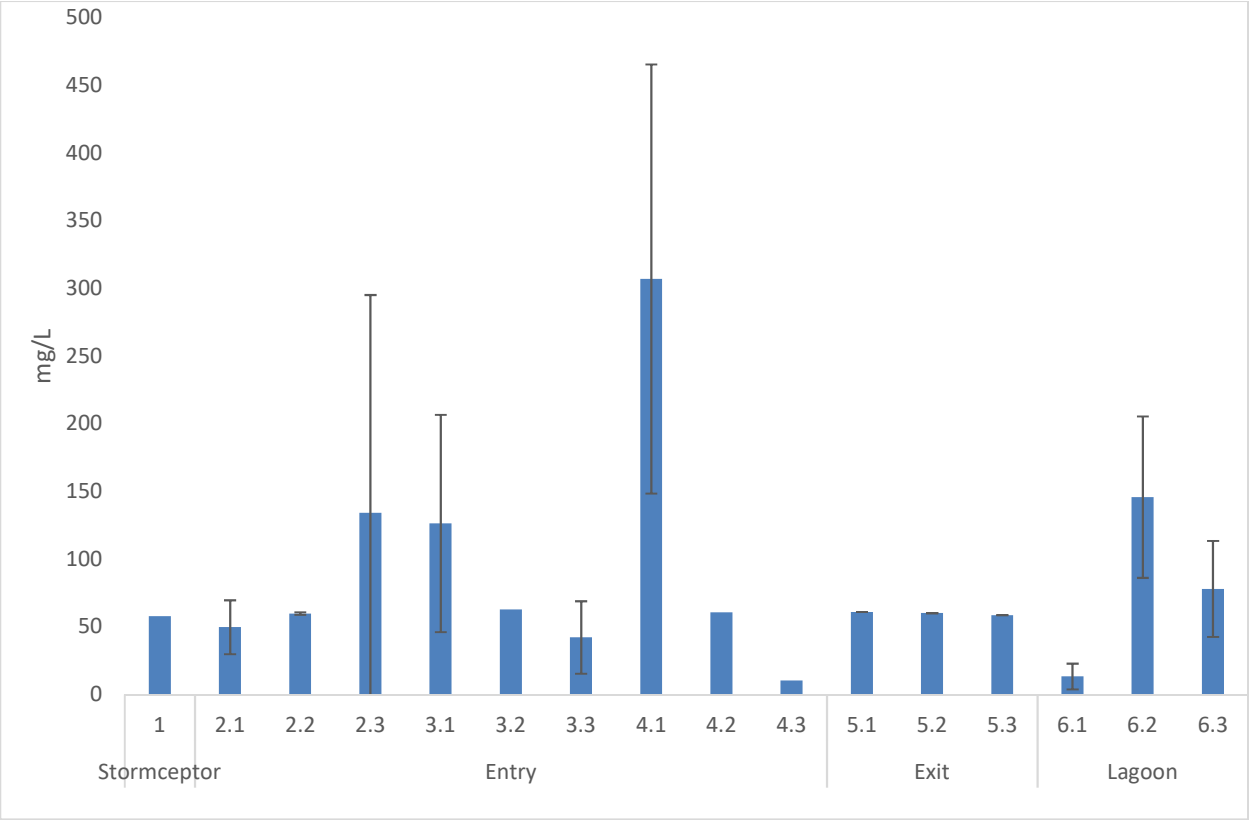

Figure 37. Barplot Comparison by Plot of TOC in Water Samples Collected During the Field Study

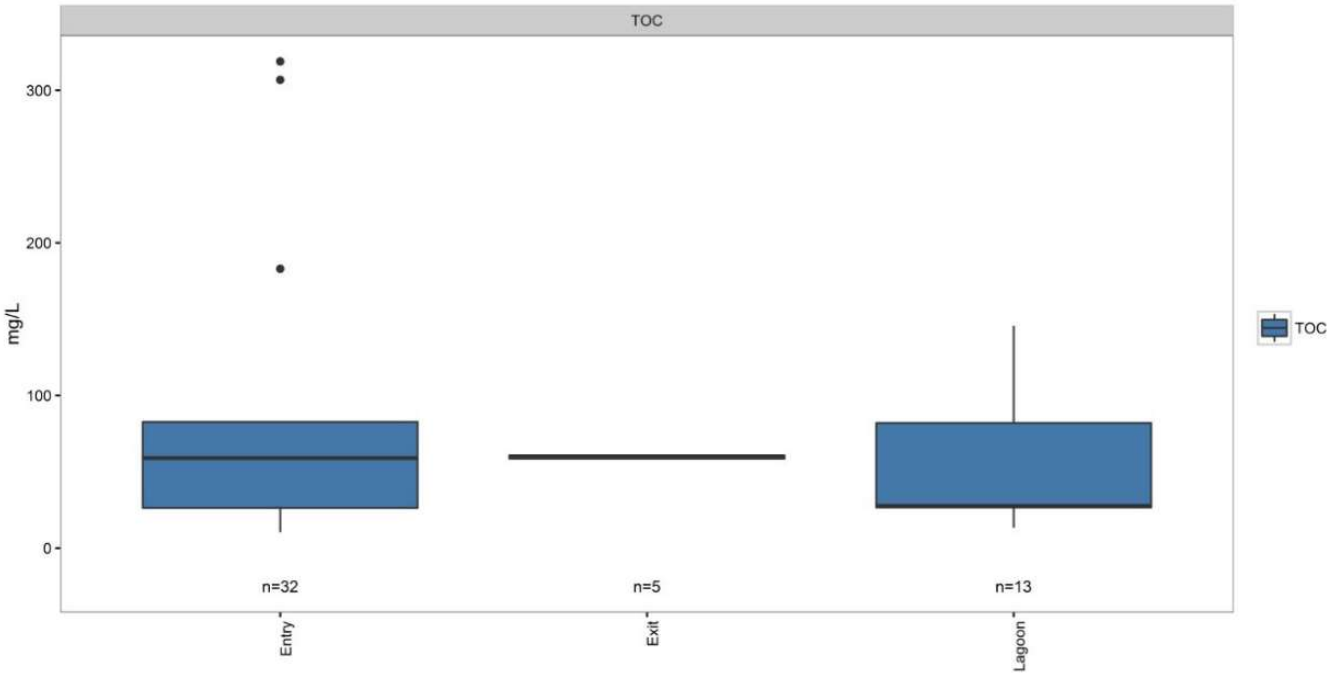

Figure 38. Boxplot Comparison of TOC in Water Samples Collected During the Field Study

2.6.1.6 Statistical Scores for Site Comparison

Table 12 through *Table 14* list the confidence levels (z-scores) computed using the Wilcoxon Paired Rank Test in the R standard package version 3.1.1, (R Core Team, 2016).

Table 12. Confidence Levels for Wilcoxon Rank Test Between Entry and Exit for Environmental Parameters

Turbidity	TSS	TOC	COD
0.170	0.076	0.193	0.386

Table 13. Confidence Levels for Wilcoxon Rank Test Between Exit and Lagoon for Environmental Parameters

Turbidity	TSS	TOC	COD
0.067	0.097	0.115	0.425

Table 14. Confidence Levels for Wilcoxon Rank Test Between Entry and Lagoon for Environmental Parameters

Turbidity	TSS	TOC	COD
0.373	0.811	0.735	0.425

2.6.2 Metals

2.6.2.1 Interpretations

Together, Figure 39 through Figure 41 illustrate the distribution of metals within the water samples obtained from the Lost Lagoon wetland. From Figure 39 and Figure 40, visually, there is a trend of decreasing metal concentrations along the length of wetland. However, Site 6.1, 6.2, and 6.3, where samples were taken from the shore of Lost Lagoon, show higher concentrations of several metals that are associated with stormwater. This could be due to additional drainage into the Lost Lagoon from neighbouring roadways including Lost Lagoon Drive and Chilco Street because it does not appear to be explained by the contribution of stormwater from the treatment wetland. Additional information is needed to explain this trend. Generally, Figure 41 also illustrates that the high concentrations of metals in some water samples at the wetland entry were no longer measured at the wetland exit.

Figure 42 and Figure 43 illustrate the distribution of metals in the surface sediment at each plot along the study site. From these graphs, there is visual evidence that the concentration of metals is lower at the back end of the wetland compared to the front end of the wetland. There is also evidence that particle setting and adsorption are contributing to the decreasing concentration of metals in the stormwater because the plot with the highest metal concentrations, Site 3.2, is in the centre of the forebay, rather than at the beginning or end of the forebay. Figure 44 illustrates the variation of metals measured in surface sediment

between the entry and exit of the Lost Lagoon wetland and Lost Lagoon. At this resolution, there is also evidence that over the entire sampling regime, there are decreasing concentrations for some of the metals commonly associated with stormwater in the surface sediment.

Figure 45 and Figure 46 illustrate the distribution of metals averaged by plot in the sediment at a depth of 10 cm below the floor of the Lost Lagoon wetland. From these graphs, there is some visual evidence that the concentration of metals is lower at the back end of the wetland compared to the front end; however, the results are less clear than with the surface sediment samples. In addition, Figure 47 illustrates the variation of metals measured in the sediment sampled at a depth of 10 cm below the wetland floor, between the entry and exit of the Lost Lagoon wetland and Lost Lagoon. The results in Figure 47 generally appear to be consistent with the results in the boxplot for surface sediment metal concentrations. Due to equipment malfunction, depth sediment samples were not obtained at Site 2.2, Site 3.2 and Site 4.2.

Due to the variability in the measurements of metal concentrations, the statistical scores in *Table 15* through *Table 17* are complex and challenging to interpret. Overall, the observations include:

Between the entry and exit of the Lost Lagoon wetland;

- Insignificant differences were calculated between the water samples measuring cobalt and copper while significant differences were calculated between the water samples for barium, manganese, nickel, and zinc. Statistical conclusions could not be calculated for cadmium, chromium, molybdenum, lead, and antimony because measurements were too close to the detection limits of the analytical method.

- Insignificant differences were calculated between the surface sediment samples for nickel and zinc while significant differences were calculated between the surface sediment samples for barium, chromium, copper manganese, and lead. Statistical conclusions could not be calculated for cadmium, cobalt, molybdenum, and antimony because measurements were too close to the detection limits of the analytical method.

- Finally, insignificant differences between depth sediment samples were calculated for barium, copper, manganese, nickel, and zinc while a significant difference was calculated for chromium. Statistical conclusions could not be calculated for cadmium, cobalt, molybdenum, lead, and antimony because measurements were too close to the detection limits of the analytical method.

Between the entry to the wetland and Lost Lagoon;

- No statistical differences were calculated.

Between the exit of the wetland and Lost Lagoon;

- Statistical differences were calculated between surface sediment samples were calculated for chromium, copper, and lead.

- No other statistical differences were calculated.

2.6.2.2 Water Samples

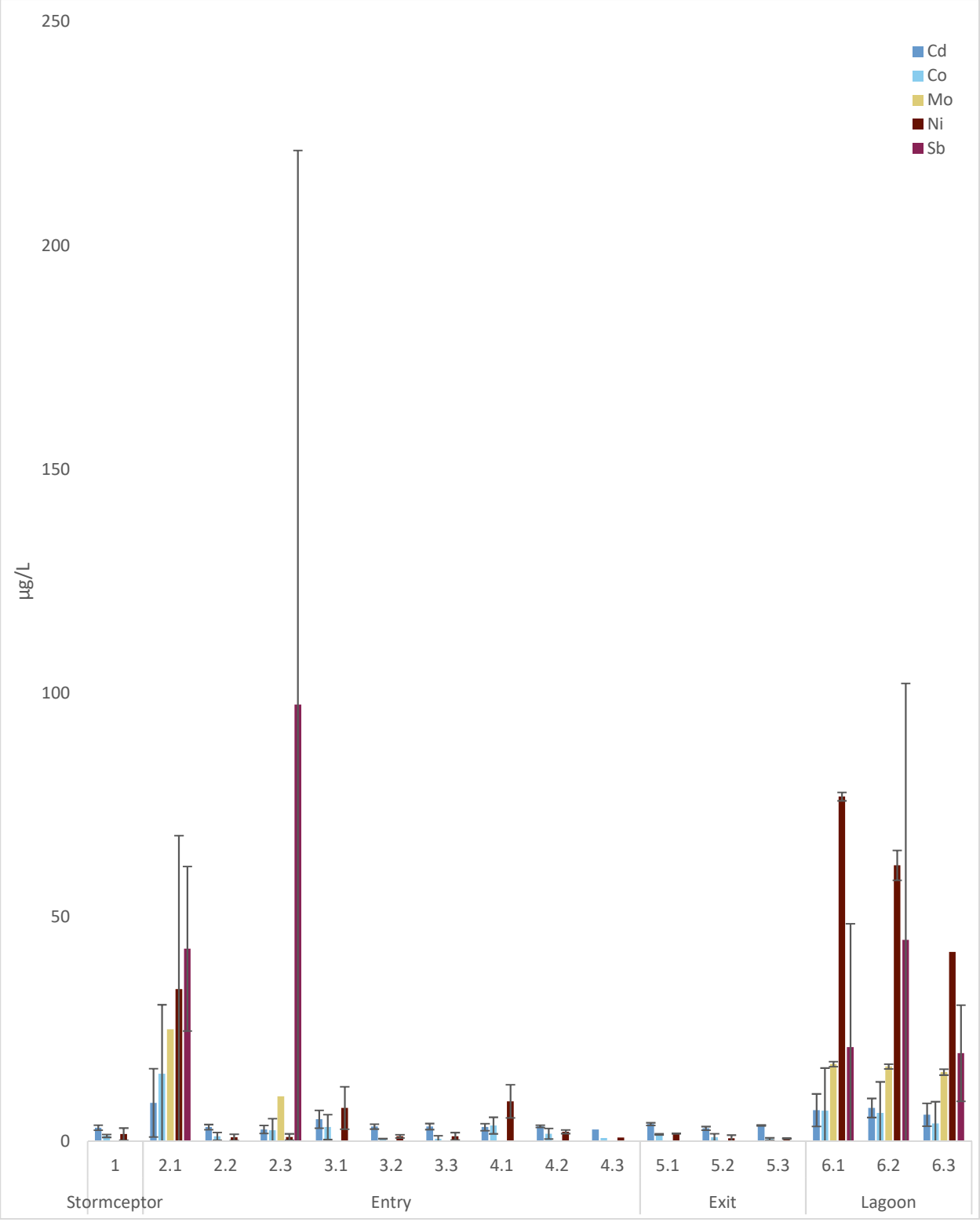

Figure 39. Barplot Comparison by Plot of Metals Associated with Stormwater in Water Samples Collected During the Field Study

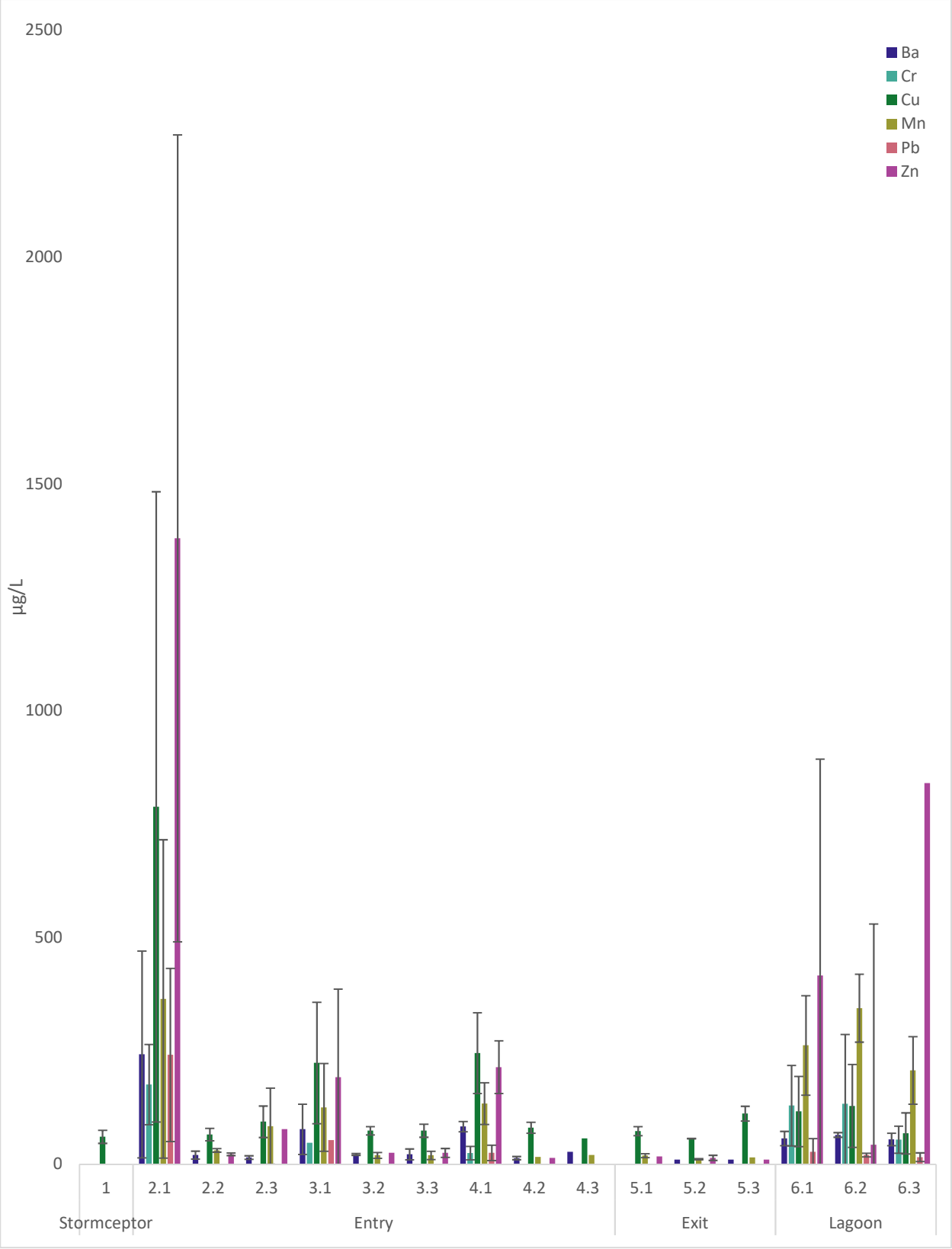

Figure 40. Barplot Comparison by Plot of Metals Associated with Stormwater in Water Samples Collected During the Field Study

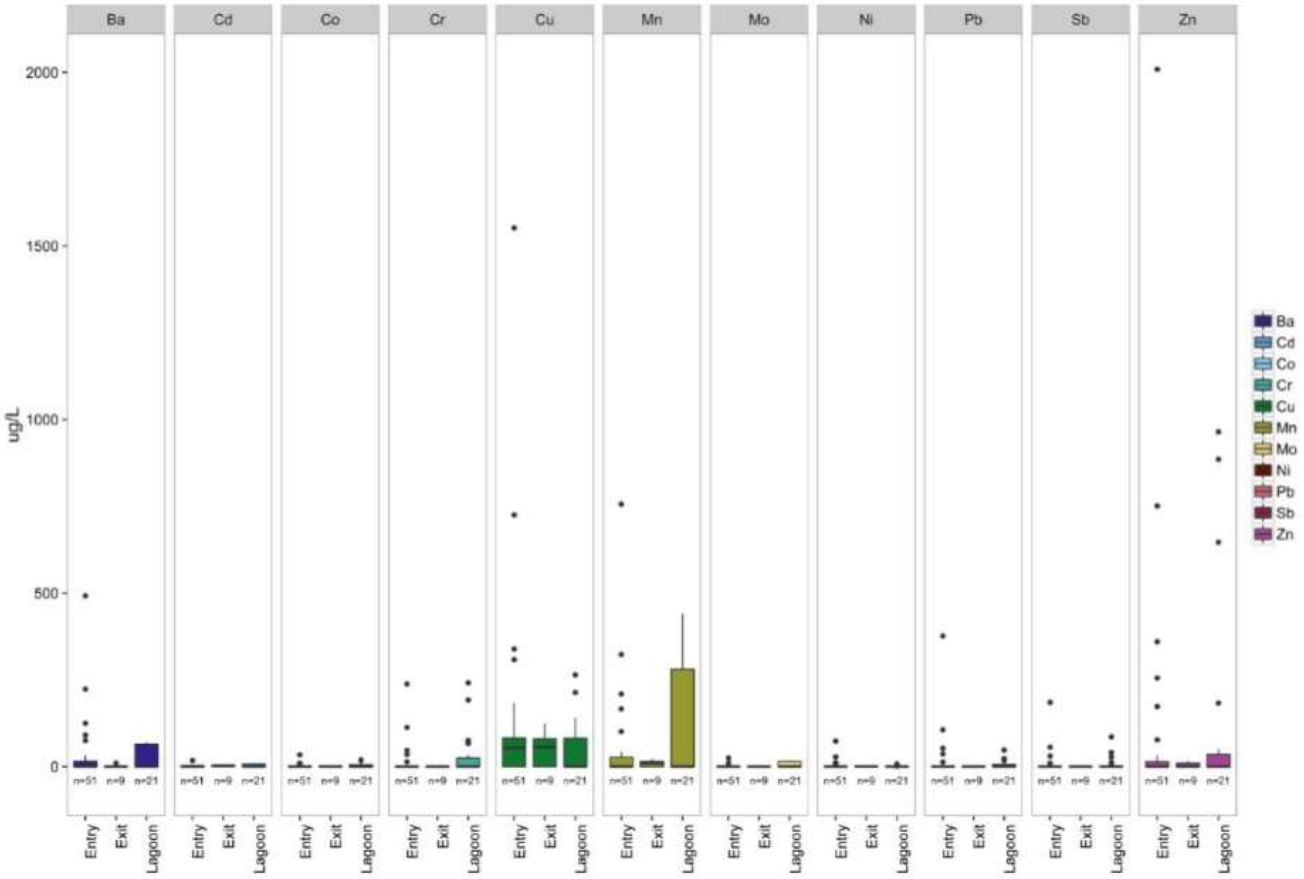

Figure 41. Boxplot Comparison of Metals Associated with Stormwater for Water Samples Collected During the Field Study

2.6.2.3 Surface Sediment Samples

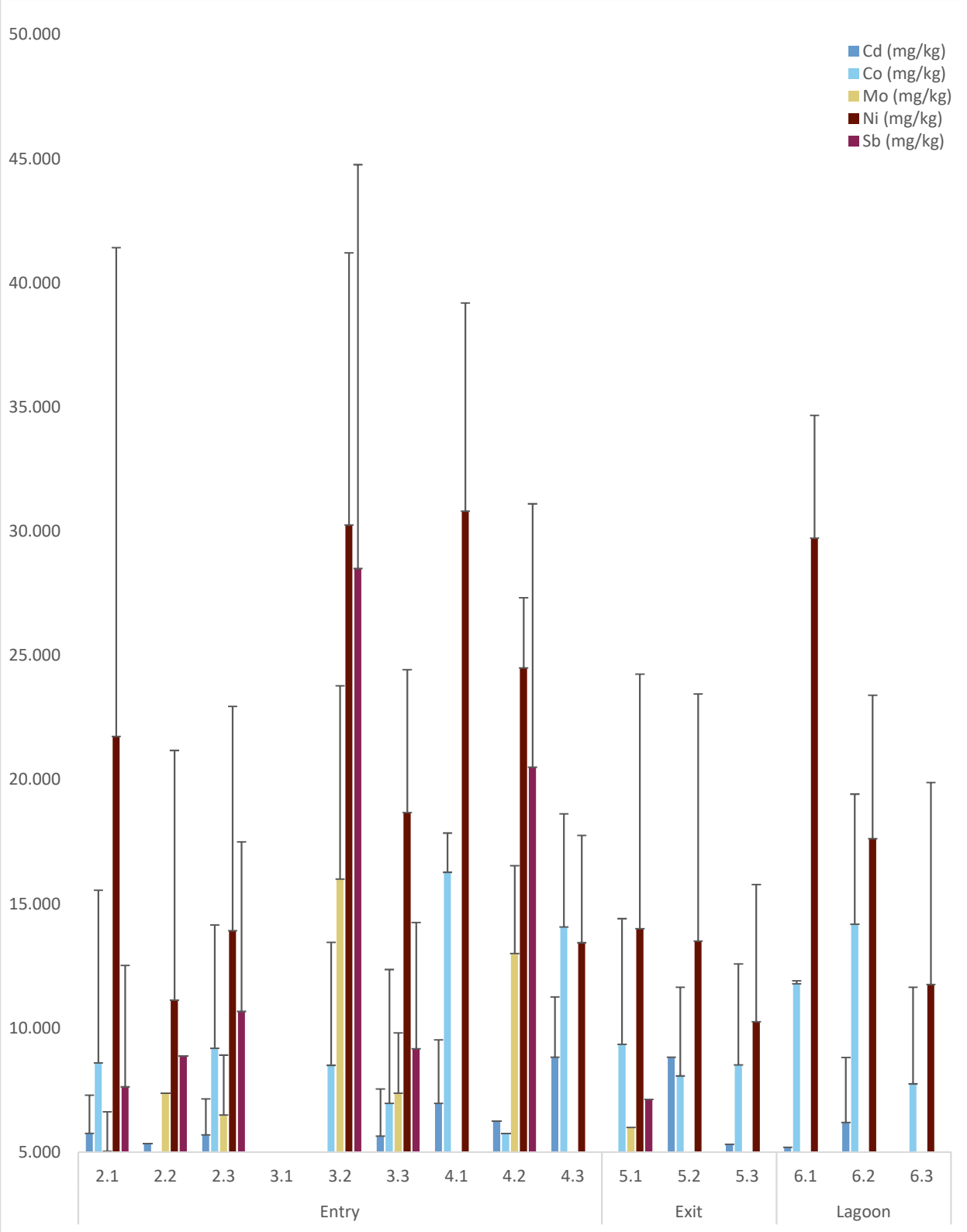

Figure 42. Barplot Comparison by Plot of Metals Associated with Stormwater in Surface Sediment Samples Collected During the Field Study

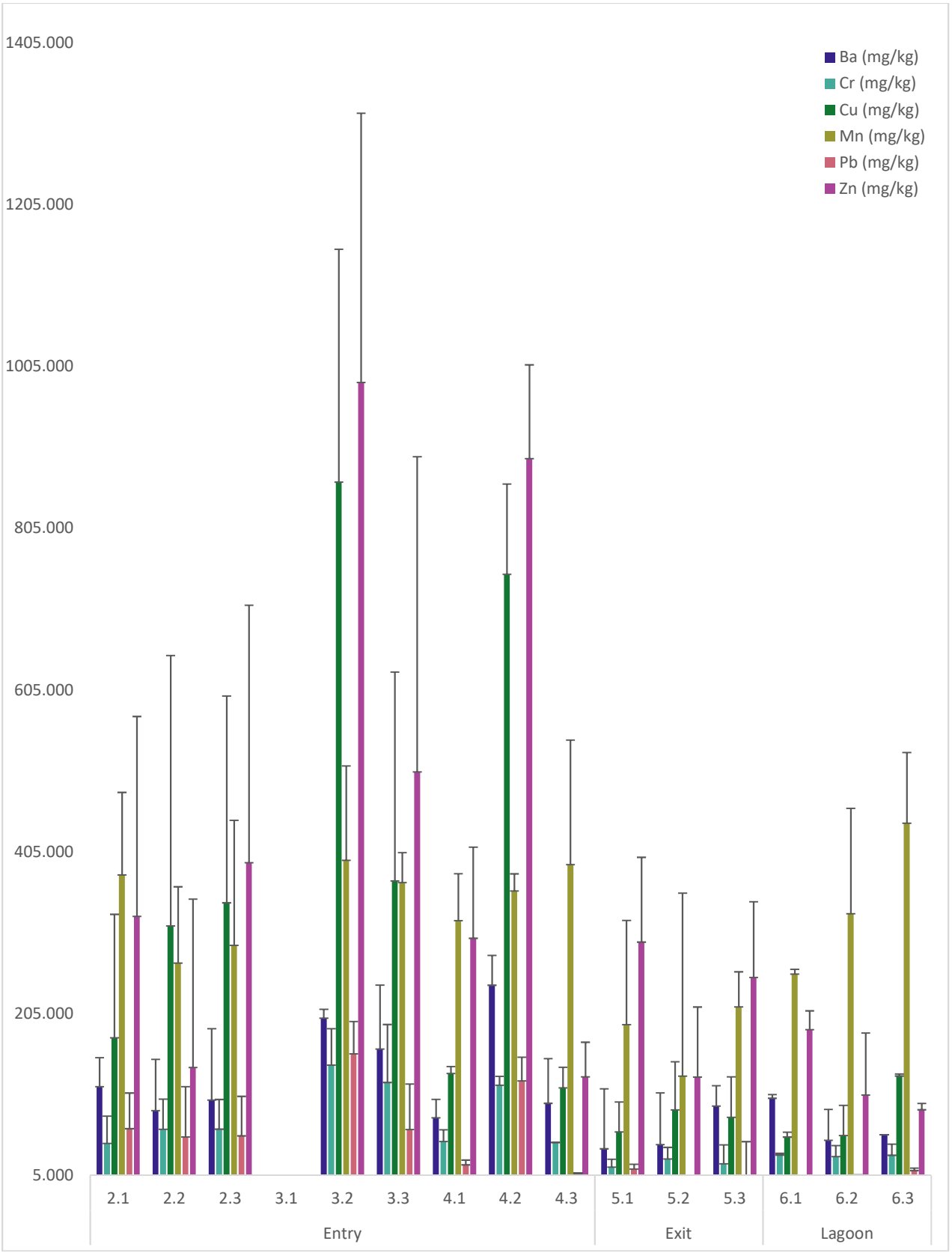

Figure 43. Barplot Comparison by Plot of Metals Associated with Stormwater in Surface Sediment Samples Collected During the Field Study

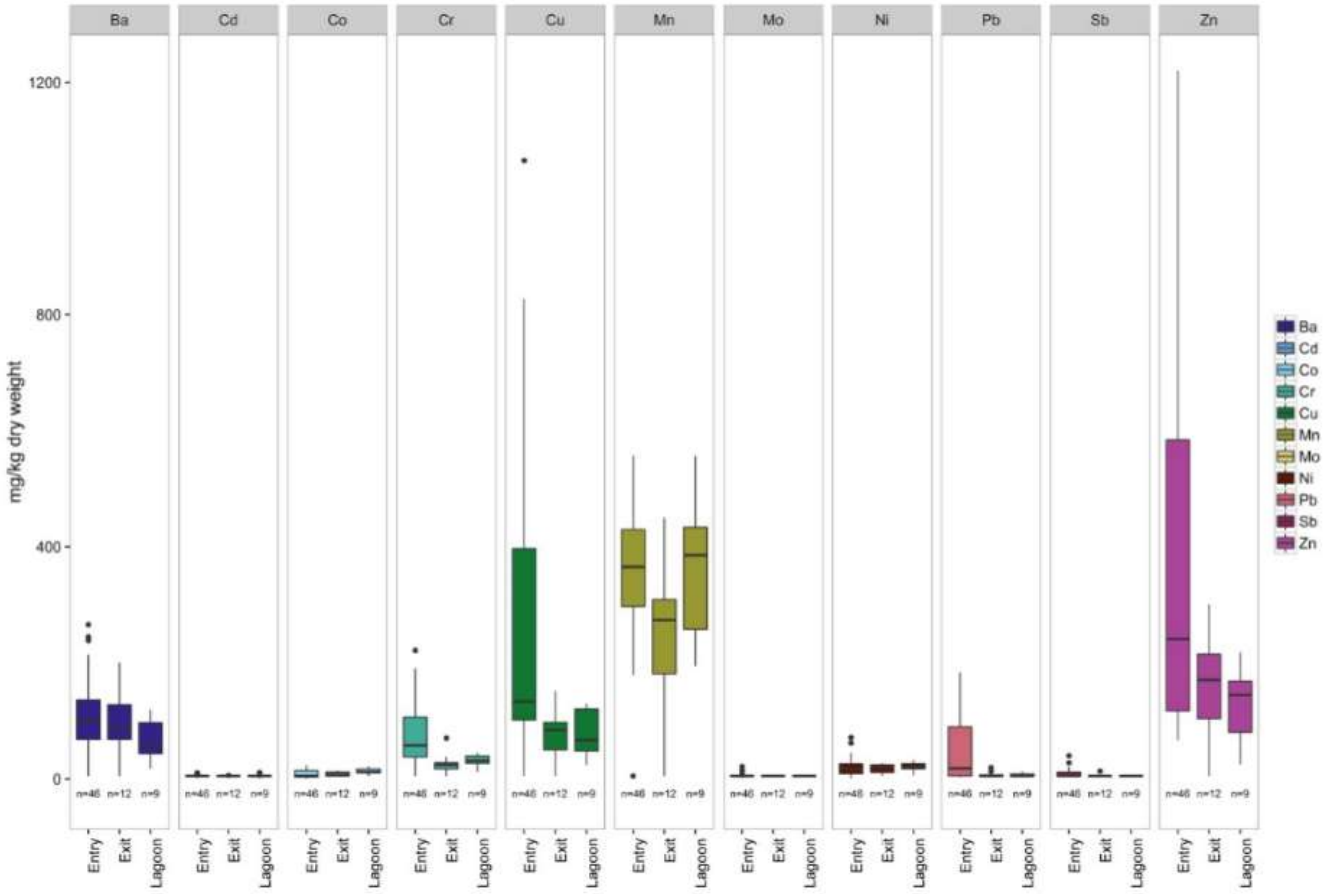

Figure 44. Boxplot Comparison by Plot of Metals Associated with Stormwater in Surface Sediment Samples Collected During the Field Study

2.6.2.4 10-cm Depth Sediment Samples

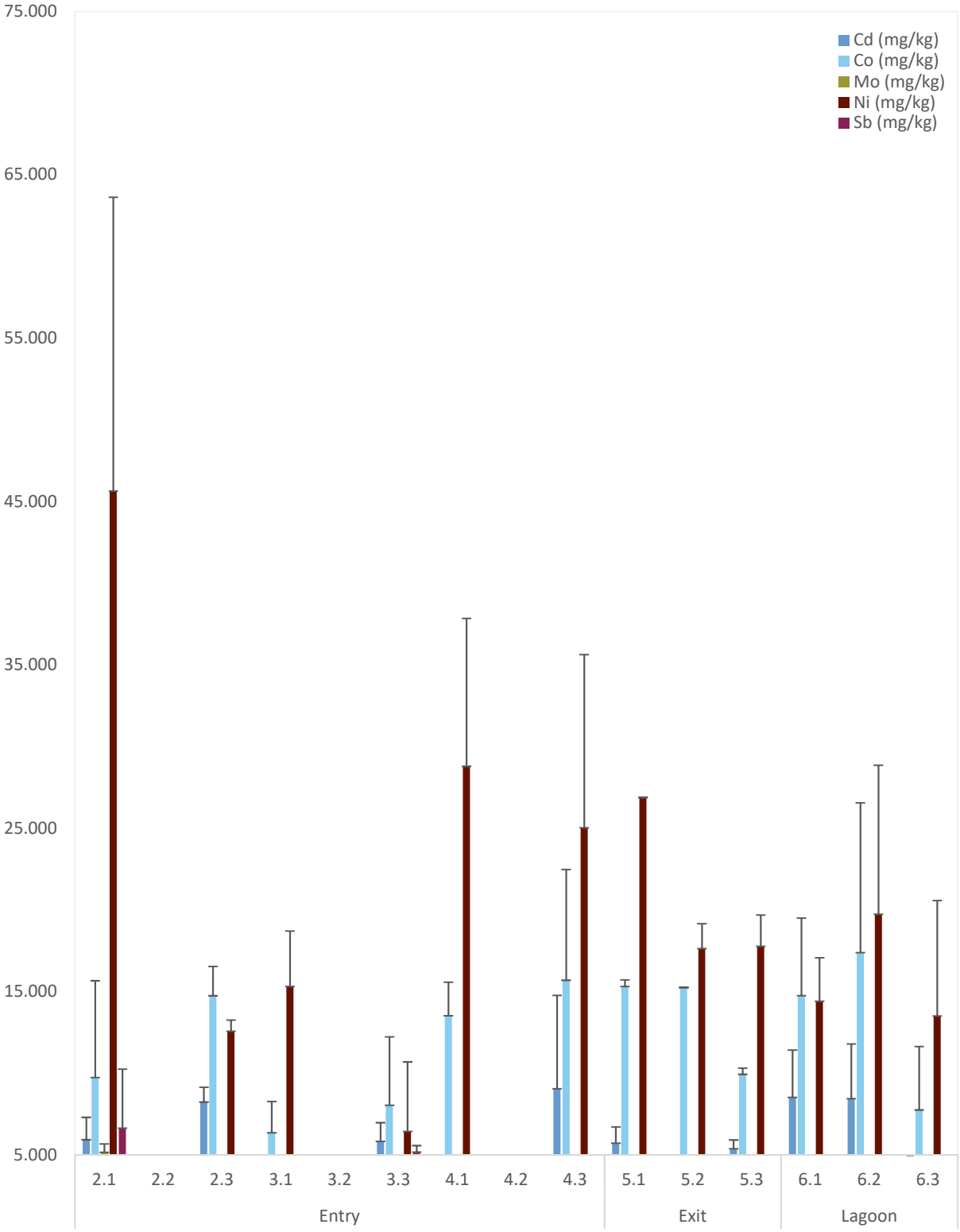

Figure 45. Barplot Comparison by Plot of Metals Associated with Stormwater in Surface Sediment Samples Collected During the Field Study

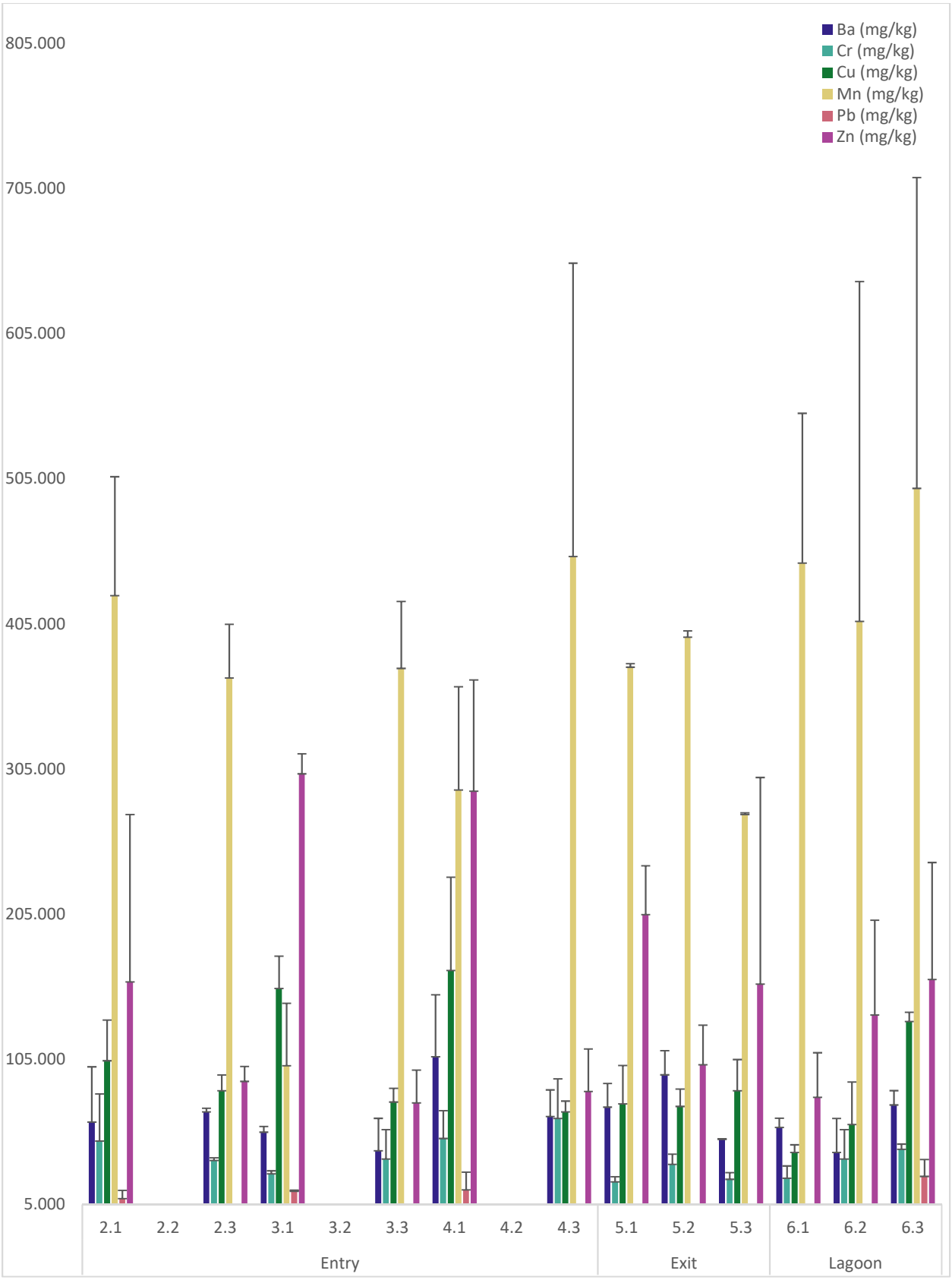

Figure 46. Barplot Comparison by Plot of Metals Associated with Stormwater in Surface Sediment Samples Collected During the Field Study

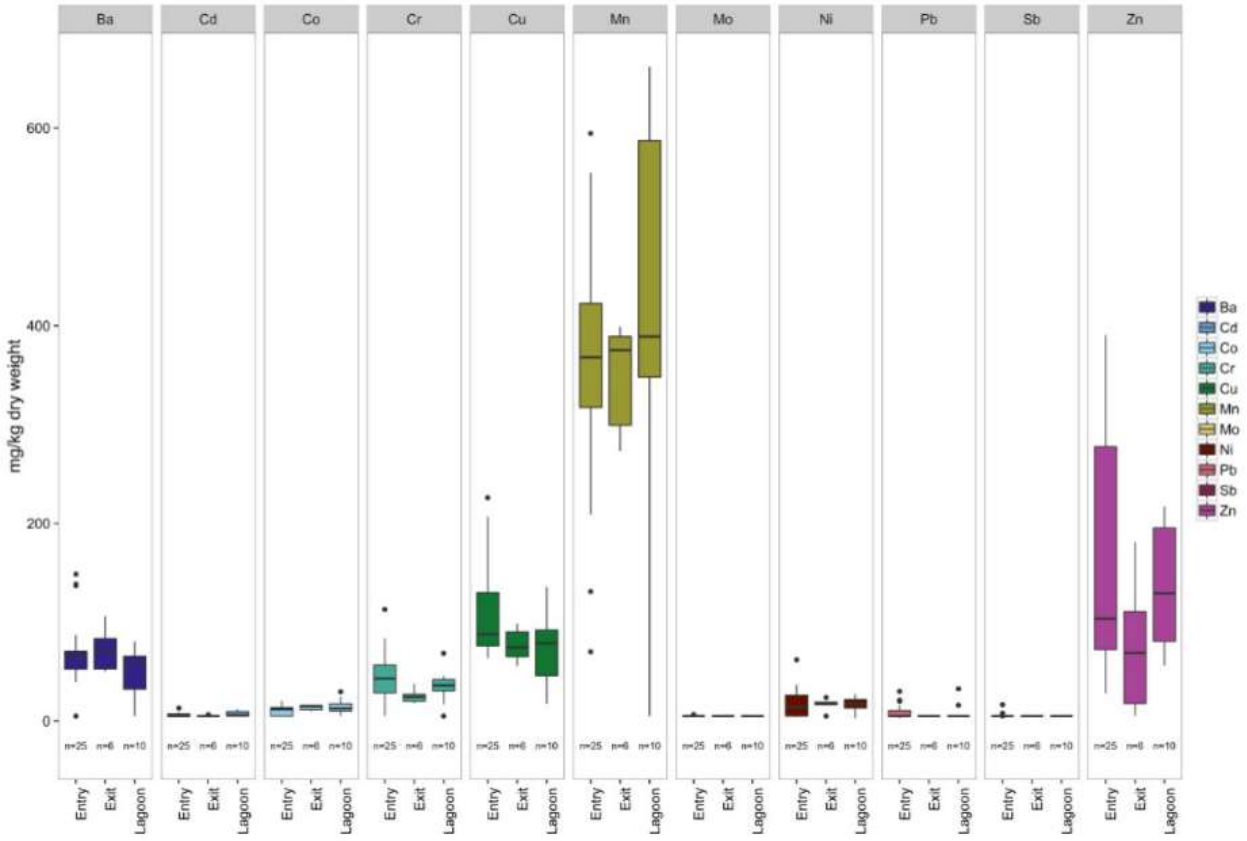

Figure 47. Boxplot Comparison of Metals Associated with Stormwater between Forebay and Exit for Samples taken at a Depth of 10 cm

2.6.2.5 Statistical Scores for Site Comparison

Table 15. Confidence Levels for Wilcoxon Paired Rank Test Between Entry and Exit for Metals

Metal	Water	Surface	Depth
Ba	0.016	0.047	0.661
Cd	-	-	-
Co	-	-	-
Cr	-	0.000	0.000
Cu	0.723	0.000	0.077
Mn	0.003	0.031	0.776
Mo	-	-	-
Ni	0.032	0.577	0.732
Pb	-	0.004	-
Sb	-	-	-
Zn	0.002	0.123	0.281

Table 16. Confidence Levels for Wilcoxon Paired Rank Test Between Exit and Lagoon for Metals

Metal	Water	Surface	Depth
Ba	0.174	0.236	0.525
Cd	-	-	-
Co	-	0.152	1
Cr	-	0.126	0.294
Cu	1	0.943	0.828
Mn	0.822	0.163	0.735
Mo	-	-	-
Ni	-	1	1
Pb	-	1	-
Sb	-	-	-
Zn	1	0.455	0.282

Table 17. Confidence Levels for Wilcoxon Paired Rank Test Between Entry and Lagoon for Metals

Metal	Water	Surface	Depth
Ba	0.372	0.075	0.525
Cd	-	-	-
Co	-	0.046	-
Cr	-	0.010	0.371
Cu	1	0.009	0.269
Mn	0.546	0.691	0.635
Mo	-	-	-
Ni	-	1	1
Pb	-	0.010	-
Sb	-	-	-
Zn	1	0.089	0.733

2.6.3 Mineral Oil and Grease

2.6.3.1 Interpretation

Figure 48 and Figure 49 illustrate the change in mineral oil and grease along the length of the wetland. Based on the date sampled, mineral oil and grease had the most variable concentration at Site 2. This was expected because the level of mineral oil and grease measured in water samples is dependent on the influent quality, which could be highly variable depending on vehicle traffic and potential vehicle leakage onto the causeway. Mineral oil and grease was measured to be below guideline levels (30-mg/L) (Canadian Council of Ministers of the Environment, 2015) at Site 4, Site 5, or Site 6. Unfortunately, only one mineral oil and grease sample was taken at Site 1 at the exit of the stormceptor, therefore the variability of mineral oil and grease entering the wetland is unknown.

Mineral oil and grease samples were only compared graphically because the sample size was too small to interpret statistical calculations.

2.6.3.2 Mineral Oil and Grease Figures

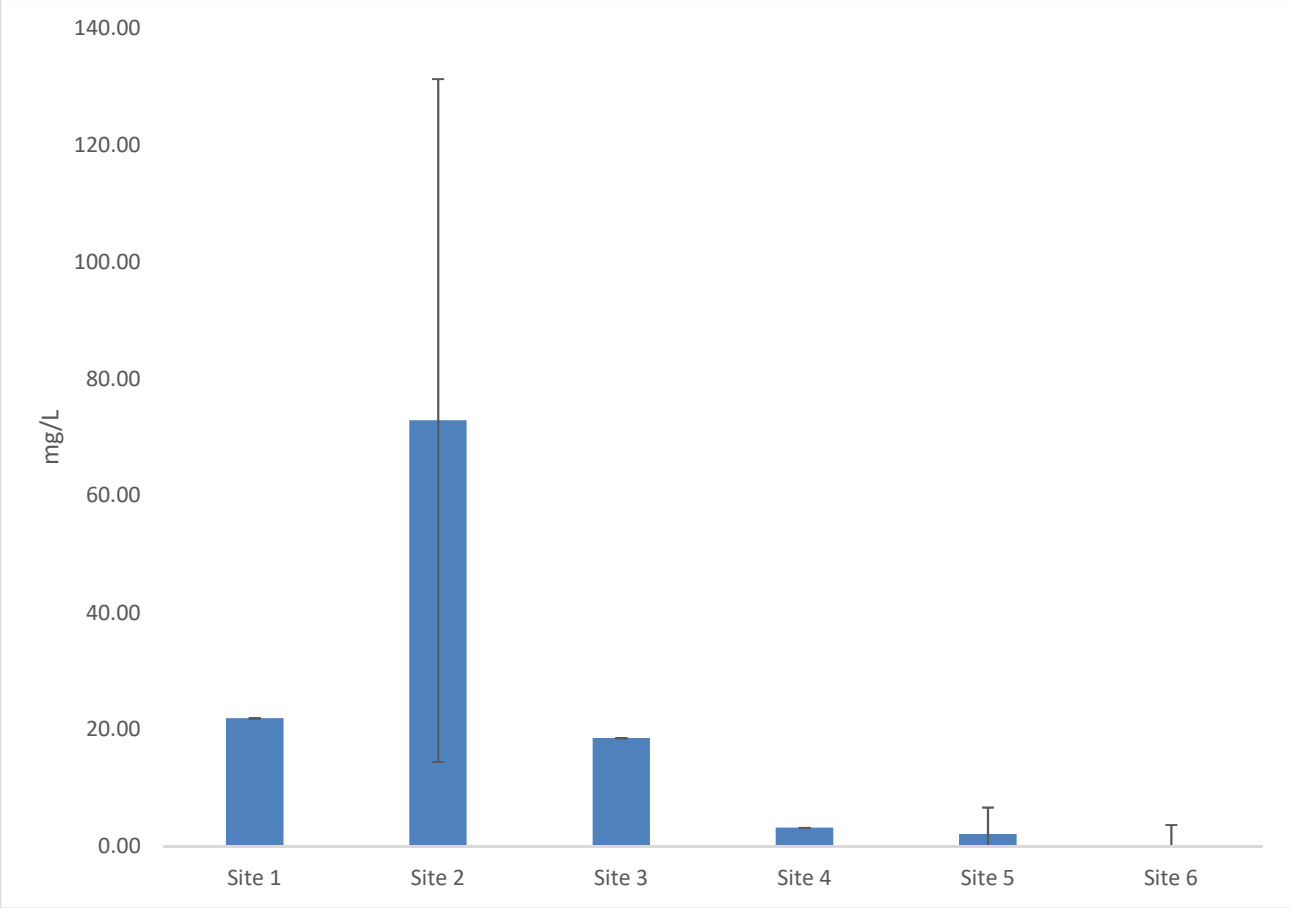

Figure 48. Comparison by Site of Total Mineral Oil and Grease in Water Samples Collected During the Field Study

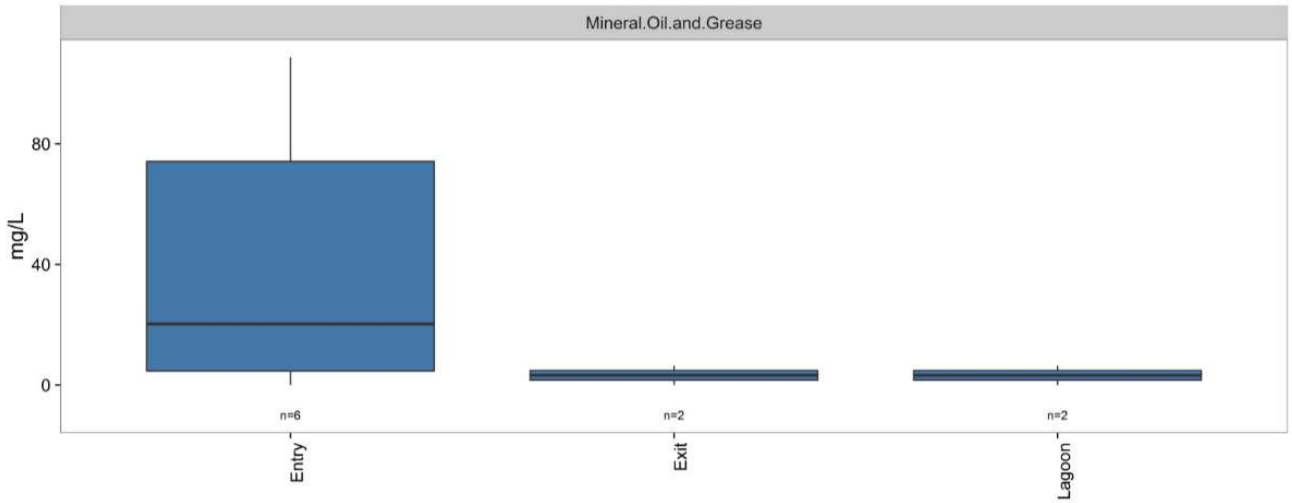

Figure 49. Boxplot Comparison of Total Mineral Oil and Grease for Water Sampled Collected During the Field Study

2.7 Discussion and Conclusion

As described previously, the goal of this study is to provide proof of concept data that supports or rejects developing a genomics-based monitoring tool for low impact design features that treat stormwater, including engineered wetlands. In this chapter, data was gathered and analyses were conducted in order to provide background information for the treatment efficacy of a functioning stormwater treatment wetland, namely the Lost Lagoon wetland in Stanley Park, Vancouver. For this, an attempt was made to answer two hypotheses and to support three objectives.

2.7.1 Chapter Hypotheses

To prove that the wetland is effectively treating stormwater and to begin to validate the treatment mechanisms within the wetland, it was previously stated that two hypotheses must be true.

1. The concentrations of metals associated with stormwater decrease along the length of the wetland; and
2. The concentration of oil and grease decreases along the length of the wetland.

Regarding metal concentrations, for the three sample types, most metal concentrations visibly decreased between samples taken near the Lost Lagoon wetland entry and exit and this was confirmed by calculating and comparing the Wilcoxon rank test parameter between population medians. The same trend was not found when comparing the wetland entry and exit to the environment in Lost Lagoon. For this chapter's purposes, this result effectively proves that the first hypothesis is true.

Mineral oil and grease more clearly decreased between the wetland entry and exit. Variable and high mineral oil and grease concentrations were measured throughout the wetland forebay while low or undetectable levels of mineral oil and grease were measured at the wetland exit and in Lost Lagoon. For the purposes of this chapter, the measured results prove that mineral oil and grease decreases along the length of the Lost Lagoon wetland.

The persistence of outliers throughout the dataset may have contributed to some of the statistical uncertainty in the results. In addition, the natural background levels of certain metals may outweigh the calculation of a difference between the wetland entry and exit, especially for metals that exist at only slightly elevated levels in stormwater, such as cobalt and antimony.

2.7.2 Chapter Objectives

In order to support the goal of this study, to provide proof of concept data that supports or rejects developing a genomics monitoring tool for low impact design features that treat stormwater, including engineered wetlands, three objectives were previously stated for this chapter:

1. Demonstrate that the Lost Lagoon wetland is meeting water quality treatment guidelines;
2. Demonstrate that the engineering best management practices employed in the design of the Lost Lagoon wetland have had some meaningful impact on the stormwater treatment efficiency; and
3. Identify knowledge gaps and opportunities for complimentary data analyses though the application of genomics.

For the first objective, comparison of the maximum pollutant concentrations (*Table 18*) in the water samples collected at the entry and exit of Lost Lagoon wetland demonstrates that the wetland is generally meeting water quality treatment guidelines. The only exception for this, is the maximum point measurement for cadmium. The maximum cadmium concentration measured at the outlet was 4 µg/L and the guideline for effluent water is 1 µg/L (Canadian Council of Ministers of the Environment, 2015). The effluent guideline is the same as the method detection limit for the ICP instrument so, using the methodology employed here, it cannot be said with confidence whether this guideline is regularly exceeded.

Table 18. Comparison of Maximum Pollutant Concentrations Measured in Water Samples at the Lost Lagoon to British Columbia Treatment Guidelines

Pollutant	*Concentration*			
	Stormwater [1]	*Inlet Maximum*	*Outlet Maximum*	*Guideline*[3]
Mineral Oil and Grease (mg/L)	5.0-63.4[1]	108	5.4	30
Organic Carbon (mg/L)	7.3-17.6	327	148	-
Solids (mg/L)	44-809[1]	1359	106	5 above BL
Turbidity, NTU	-	359	155	-
Barium (µg/L)	0.2-0.792[1]	492	10	-
Cadmium (ug/L)	0.035-2.3[1]	18	4	1
Cobalt (µg/L)		34	1	-
Chromium (µg/L)	0.01-0.13[2]	238	1	-
Copper (µg/L)	4.0-6.59[2]	1552	123	2.0
Manganese (µg/L)	0.112-6.91			
Molybdenum (µg/L)		25	20	73
Nickel (µg/L)	.002-22.6[2]	73	1	25
Lead (µg/L)	0.2-2.78[1]	376	1	3
Antimony (µg/L)		185	1	-
Zinc (µg/L)	6.5-27.5	2009	18	75

[1] (Stime, 2014)
[2] (British Columbia Research Corporation, 1992)
[3] (Canadian Council of Ministers of the Environment, 2015)

For the second objective, demonstrating that the best management practices employed in the design of the wetland have had some meaningful impact on stormwater treatment, one must review the wetland design and treatment capacity as a whole. Several different mechanisms, including sedimentation, adsorption, and plant uptake, are responsible for removing pollutants and the wetland was designed to optimize all of these mechanisms for long term stormwater treatment goals.

The stormceptors incorporated as a pre-treatment step prior to inflow to the wetland were not studied at depth during this study. However, high levels of mineral oil and grease were measured in the forebay of the wetland, indicating that use of the stormceptors as the only treatment method would not meet effluent discharge guidelines.

During the study period, there was no evidence of scouring or overflow from the wetland. In addition, sediment samples generally indicated that metal contaminant levels were higher on the front end of the wetland compared to the back end. This supports the notion that the overflow and diversion structures are beneficial to the overall treatment efficacy of the wetland.

During the initial site visit at the Lost Lagoon wetland, photographs and documentation of the state of plant species was documented. There was evidence that the plant species had adapted well to the climate within

the wetland but that additional maintenance is required to remove some invasive species, including blackberry plants. Thoren et al (2007) demonstrated that two plant species selected for the wetland design, *Carex obnupta* and *Scirpus acutus,* were effective in taking up certain metal pollutants. Therefore, continued monitoring and maintenance of the planned plant species should continue. There was also evidence of animal activity where beavers had removed trees along the berm between the wetland and Lost Lagoon. The beaver activity requires close monitoring so that the wetland outlet does not become blocked, causing backflow and damage.

Finally, the sizing of the settling forebay for a 6-month design storm was of interest during this study. In the results, there is evidence that the highest metal loading is received at the centre of the forebay. Measurements for metals taken at Location 2 (the wetland entry) and Location 3 (the centre of the forebay) were consistently higher than measurements taken at Location 4 (in the forebay, furthest from the wetland entry). However, additional analysis of flow rates and settling within the forebay would be required to properly validate this treatment stage.

For the third and final objective of this chapter, to identify knowledge gaps and opportunities for complimentary analysis through application of genomics, two statements can be made. First, while there is some evidence that the wetland is removing contaminants and meeting treatment objectives, there is still a lot of uncertainty in the results. Specifically, the results indicate that overall there is a significant decrease in contaminants along the length of the wetland but for some contaminants including cadmium, cobalt, lead, and zinc, more depth of analyses would be beneficial. Second, genomics provides an opportunity for complimentary analyses because microbial communities adapt and change due to the toxicity of pollutants. Specific species that thrive in contaminated environments will overtake other species, which due not have the same abilities. Over time, microbial communities also adapt and develop genetic tolerance mechanisms when exposed to pollutants. Analyzing species and gene differences between the microbial communities at the front and back end of the wetland would, thus, provide an additional resource to compliment uncertain pollutant treatment data.

2.7.3 Final Remarks

The work described in this chapter effectively answered both study hypotheses and provided data in support of the three objectives described here. In doing so, this chapter has laid the foundation for Chapter 2, where microbial analyses were conducted to provide proof of concept data in support of developing a genomics tool for monitoring stormwater treatment wetlands. Overall, there is evidence of effective

stormwater treatment at the chosen field study site, the Lost Lagoon wetland in Stanley Park, Vancouver, but further analyses are required to properly validate said evidence.

2.8 Limitations

Even though a wide range of techniques and several collection dates were incorporated into the environmental sampling design, single point in time measurements do not provide adequate proof that the wetland is meeting design targets. This is because the stormwater runoff entering the wetland is highly variable and the time it takes for stormwater to pass through the wetland is also variable. Therefore, one cannot directly compare water measurements taken at the front and back end of the wetland on a single date. Sediment sampling provides a clearer picture of long term treatment trends but there are still limitations because of the challenges with digesting organic rich samples prior to analysis using ICP-MS or other techniques. In contrast, there are many effective techniques to extract DNA from sediment and water samples and these are widely available from laboratory suppliers. In the future, analyzing the microbial response to contaminants may present itself as a valuable tool to validate environmental data.

In addition to the variability within the wetland, there were several limitations during this section of the research study, which contributed to uncertainty in the results. These include:

- Challenges accessing the wetland and stormceptor;
- Equipment malfunctions with the core sediment sampler;
- Budget limitations for the number of samples which could be processed;
- Limits to the number of samples which could be obtained and processed in a single day; and
- Challenges with the digestion of sediment samples.

3. Chapter 2: Application of Genomics-Based Monitoring Techniques for Complimentary Validation of the Lost Lagoon Stormwater Treatment Wetland

3.1 Introduction and Chapter Goal

The contents of this chapter expand on the results of Chapter 1 by applying genomics-based approaches to support the conclusion that the Lost Lagoon wetland is effectively treating stormwater. In addition, this chapter provides data to support the application of genomics for validation of other low impact design sites that treat stormwater. This chapter first describes the toxicity of stormwater in relation to bacteria. Next, bacterial adaptions to stormwater exposure are described with the goal of identifying potential markers for effective stormwater treatment. In support of the study methodology, potential genomics approaches are compared for application in stormwater treatment monitoring. The study methodology is described, which includes the incorporation of a laboratory based study, with the goal to illustrate the adaptability of this study's methodology for other low impact design sites. Finally, results, discussion, and conclusions are provided.

3.2 Chapter Objectives

Based on the overall goals of this research, this chapter has three specific objectives.
Using the same samples that were analysed in Chapter 1:

1. Apply genomics-based analysis methods to determine if there are shifts in the microbial communities and functional genes along the length of the Lost Lagoon wetland;
2. Determine if there is a correlation between the water and sediment quality, present over the study period, and the microbial communities and functional genes observed; and
3. Determine, through laboratory experimentation, if there are opportunities to expand and pursue genomics-based analyses at other stormwater treatment low impact design features.

3.3 Hypotheses

In order to use microbial comparisons as a monitoring parameter for stormwater treatment, one would need to observe differences in the microbial communities that exist in the presence of stormwater compared to the microbial communities that do not exist in the presence of stormwater. One would then need to meaningfully capitalize on these differences by correlating adaptation to contamination.

In order to achieve said observations and correlations, this chapter attempts to answer three hypotheses:

1. There is a shift in the composition and function of the microbial communities that exist between the entry and exit of the Lost Lagoon wetland;
2. The shift in the composition and function of the microbial communities between the entry and exit of the Lost Lagoon wetland is influenced by the decreasing concentration of contaminants along the length of the wetland;
3. There are similarities across unconnected sites in the adaptations that take place within microbial communities due to exposure to stormwater.

3.4 Literature Review

Like in Chapter 1, in order to provide background and context for the objectives and hypotheses stated in this chapter, a review of relevant literature was performed. First, a description of the toxicity of urban stormwater is provided. Next, the influence of urban stormwater contaminants on microbial communities is reviewed. After this, a summary from the literature of known microbial adaptations to stormwater is given. Finally, current methods for DNA sequencing and data analysis are discussed and compared for their advantages and disadvantages. This information advises the decisions that were made for the methodology presented in Chapter 2.

3.4.1 Toxicity of Urban Stormwater

Numerous past and current studies examine the toxicity of highway stormwater from both an environmental and human health perspective and these studies generally conclude that stormwater has some toxic elements (Gjessing et al., 1984, Mulliss, Revitt, & Shutes, 1996, Marsalek et al., 1999, Karlsson et al., 2010). Dutka et al. (1994) recommend assessment of toxicity through chronic effects testing for stormwater because, while the immediate effects due to exposure may not be severe, the prolonged effects of stormwater exposure are impactful. There are a variety of means to test toxicity including tests for cytotoxicity (cellular damage) and genotoxicity (genetic damage), which both tend to focus on toxic effects for bacteria.

Because toxicity of stormwater is influenced by the quality of said stormwater, where temporal variability and uncertainty has already been discussed, many studies tend to focus their research efforts on the toxicity of sediments in locations that have been impacted by stormwater. However, sediment sampling introduces additional uncertainties because of chemical partitioning, bioavailability, and the small sample size (Marsalek et al., 1999). Pitt, et al. (1995) identified gravity settling as the most important means of

reducing stormwater toxicity, where settling was shown experimentally to reduce stormwater toxicity by approximately 50%. However, in a review of four common toxicity testing methods for sediment and water samples, all samples were shown to include inherent uncertainties of between 10% and 50%, which limit the ability of toxicity testing to elucidate toxicity measurements (Marsalek et al., 1999).

Beyond water quality, stormwater also produces environmentally toxic effects to receiving environments due to sediment loadings and alterations to stream morphology. However, discussion of toxicity in this form is outside of the scope of this project.

While toxicity of stormwater as whole is less studied, the toxicity of specific elements within stormwater are well known. For example, chromium causes oxidative damage and inhibits sulfate membrane transport in bacteria and nickel can be highly toxic as it inhibits cell multiplication (Das, Dash, & Chakraborty, 2016). However, the toxicity of stormwater is not equal to the sum of its parts due to the interaction of pollutants including metals, natural organic matter, and hydrocarbons. Likewise, bacteria have developed complex resistance pathways, which are often correlated. The influence of stormwater on bacteria and the complexity of stormwater toxicity is further discussed in the sections that follow.

3.4.2 Influence of Urban Stormwater Contaminants on Microbial Communities

While there is a large body of literature that suggests that stormwater has toxic elements, the influence of stormwater on microbial communities, specifically bacteria, is lesser known. After an extensive review of literature, only a handful of published studies attempted to determine the influence of stormwater on the bacteria that reside within engineered wetlands or other low impact treatment systems (Nogaro et al., 2007; Hartman et al., 2008; Faulwetter et al., 2009; Karlsson et al., 2010; Truu, Juhanson, & Truu, 2009; Sun et al., 2013) Within the literature that was accessed, no study provided a dataset where bacteria were compared along the length of a stormwater treatment wetland.

Nogoro et al. (2007) examined the influence of stormwater quality on microbial characteristics. Their results showed that biogeochemical processes, including aerobic respiration, denitrification, and fermentation as well as microbial metabolism and enzymatic activities were stimulated by the presence of stormwater and the natural organic matter. Nogoro et al. (2007) also concluded that hydrocarbons and heavy metals did not have significant effect on microbial processes. However, Nogoro et al. only examined total bacteria counts, a crude index of bacteria diversity (optical density) and hydrolytic and dehydrogenase activities. The authors did not examine the bacterial community at a species or gene level, likely because the sequencing technologies were not available at the time of their study.

Hartman et al. (2008) suggested that "soil bacteria regulate wetland biogeochemical processes, yet little is known about controls over their distribution and abundance." While Hartman et al. (2008) did not specifically analyze stormwater treatment wetlands, they did perform a broad analysis of fifteen natural and restored wetlands. The analysis suggested that soil pH, land use, and restoration status greatly influenced bacterial composition and diversity but wetland type, soil carbon and nutrient concentrations had less of an impact. Land use was found to have the most significant impact on bacterial communities across all wetland sites even after accounting for wetland type and soil chemistry using pure-partial Mantel's tests. Interestingly, Hartman et al. (2008) noted that the responses of bacterial communities were dominated by a few taxa (Acidobacteria and Proteobacteria) and the authors suggested that this yields a promising result for the application of bacteria as an indicator of wetland health.

Faulwetter et al. (2009) noted that the recent application of newer molecular and genetic analysis methods has begun a "new era of treatment wetland research." In their literature review, Faulwetter et al. (2009) found that results up to 2009 confirmed the existence of microbial functional groups such as nitrifiers, denitrifiers and sulphate reducers that are responsible for pollutant removal but Faulwetter et al. also suggested that the future of this science would shift to the identification and linkage of the functional groups to the environmental factors of greatest influence. In 2009, Faulwetter et al. recognized the upcoming importance and value of microbial analysis in water treatment:

> "When we understand what controllable factors turn critical functional groups on and off we will be able to fully optimize performance for removal of a specific pollutant, or perhaps still be able to achieve the "perfect" treatment system that can satisfactorily remove virtually all pollutants from domestic wastewater, and/or other sources."

Sun et al. (2013) used 454 pyrosequencing of the 16S rRNA gene in order to investigate how estuaries responded to contaminants. While this study did not specifically address stormwater treatment wetlands, Sun et al. (2013) conclude that an abundant and pervasive core set of bacteria were largely responsible for mediating the response of the microbial community to contamination. Like Hartman et al. (2008), Sun et al. (2013) also found that the microbial community core was dominated by proteobacteria and acidobacteria. The authors observed that silt and metals together explained approximately 20% of the variation in the bacterial community and that salinity and temperature predicted approximately 11% of the microbial community. The research supported the notion that there is some functional redundancy within the bacteria of contaminated sediments but that our understanding of bacteria communities' responses and resilience to contamination is still developing.

3.4.3 Known Microbial Adaptations to Urban Stormwater Contaminants

While few studies on bacteria specifically focus on the changes of communities due to exposure to stormwater contaminants, there is a wider body of knowledge that focuses on the response of bacterial communities to metal exposure (Das, Dash, & Chakraborty, 2016). For example, one study, which compared two metal contaminated sites with an order of magnitude difference in contamination, suggested that adaptations of bacterial communities to metal exposure are subtle but significant and that the bacterial communities in freshwater sediments adapt to metal exposure without widespread changes to the bacterial population (Gillan et al. 2015). Adaptations of microbial communities may occur at either the genus/species level (e.g. *Pseudomonas fluorescens, Alcaligenes faecalis, Ochrobactrum tritici*, etc.) or at the gene level (e.g. CadB, ChrA, CopAB, etc.).

Certain species of bacteria may be able to adapt to environments with elevated metal levels, which would otherwise be toxic for other bacteria, through application of elements within their genetic systems and/or through mechanisms for maintaining their internal ecosystem (Ryan et al. 2009). In their review of bacterial adaptations, Das et al. (2016) point out that bacteria are uniquely able to adapt to all types of extreme environments due to several features including their:

- Small size;
- High surface area to volume ratio; and
- Ability to efficiently transfer genetic traits.

In addition to these features, bacteria have developed three primary methods for metal resistance including:

1. Efflux of irritant metals outside the cell by transporters;
2. Transformation of metals into less toxic forms; and
3. Bioadsorption.

Efflux requires that bacteria consume energy (ATP) to pump metal cations outside of the cell (Nies 2003). Transformation to a less toxic state requires that bacteria reduce metals to a dissimilar oxidation state. Bio-adsorption typically requires that bacteria bind metals onto their cellular surface – this typically involves formation of a biofilm, which can be highly complex and versatile (Harrison et al. 2006).

Beginning in the 1970's, numerous bacteria have been identified for their metal resistant traits. In 1999 Nies reviewed known metal resistance mechanisms to that date. In 2016, Das et al. updated the works of Nies with the goal of identifying opportunities for bioremediation. In their words, Das et al. (2016) state that "the ability of bacteria to resist toxic metals comes from a highly modified genetic system, by means

of which bacteria synthesize proteins enabling them to thrive in the presence of such elements. Bacteria survive by expressing several metal-resistant genes toward toxic metals." The relevant details from both summaries with respect to stormwater pollutants are summarized in Table 19.

Table 19. Bacterial adaptations to metals in stormwater (adapted from Nies 1999; Das et al. 2016)

Metal	Adaptation	Sources
Antimony	• ***Leishmania*** cells are able to gain resistance to arsenic and antimony by efflux.	Rosenstein et al. 1992; Sanders et al. 1997
Arsenic	• Aerobic bacteria, like ***Alcaligenes faecalis,*** are able to oxidize arsenic. • ***Leishmania*** cells are able to gain resistance to arsenic and antimony by efflux.	Laverman et al. 1995 Dey et al. 1994
Cadmium	• Resistance to cadmium in bacteria is based on cadmium efflux. • In Cyanobacteria, amplification of the **smt metallothionein** locus increases cadmium resistance and deletion of it decreases resistance. • In gram-negative bacteria, cadmium is detoxified by RND-driven systems like **Czc**, which is mainly a zinc exporter and **Ncc**, which is mainly a nickel exporter. • In gram-positive bacteria, the first example of a cadmium-exporting P-type ATPase was the **Cad-A** pump from ***S. aureus.***	Olafson et al. 1979 Gupta et al. 1992; Gupta et al. 1993; Turner et al. 1993 Thelwell et al. 1998; Nies 1995; Nies & Silver 1989b; Schmidt & Schlegel 1994 Nucifora et al. 1989; Silver et al. 1989
Chromium	• To fight chromium toxicity, microbes have developed two mechanisms of chromium resistance. The first is a method of chromate efflux from the cells, and the second method involves enzymatic reduction of toxic Cr^{6+} to less toxic Cr^{3+}. • The operon for chromium efflux if encoded in four genes, **chr-BACF** • **Chr-R** was identified as a chromate reductase gene. The general chromate transport reactions involve a family of chromate ion transporters. • Three other genes, **chr-JKL**, were later identified and proven to be involved in the chromium reduction process. • Chromium can also be reduced through bacterial excretion of enzymes but this process is lesser known. • ***Pseudomonas fluorescens*** strain LB300, was shown to reduce chromate and a broad variety of bacteria that are able to reduce chromate have since been found.	Das et al. 2016 Branco et al. 2008 Gonzalez et al. 2005 Henne et al. 2009 Batool et al. 2012; Mishra et al. 2012 Bopp & Ehrlich 1988; Cervantes & Silver 1992
Cobalt	• Resistance to cobalt in gram-negative bacteria is based on a trans-envelope efflux driven by a resistance, nodulation, cell division (RND) transporter. • Cobalt resistance seems always to be the by-product of resistance to another heavy metal, either nickel or zinc.	Liesegang et al. 1993; Schmidt & Schlegel 1994 Nies et al. 1987
Copper	• A major copper resistance mechanism in bacteria is encoded within four genes, **cop-YABZ**. Bacteria with these genes will show early copper retention followed by a metal efflux process. • Other bacteria, including ***E. Coli*** have been shown to a have a double regulatory mechanism for copper resistance, which is encoded in a sensing system controlled by the two genes, **cus-RS**, and this sensing	Odermatt et al. 1992, 1993; Wunderli-Ye & Solioz 1999; Albarracin et al. 2008 Djoko et al. 2010

	mechanism regulates metal efflux, which is controlled by four proteins cus-CFBA. • Some bacteria also have a copper efflux system where the regulatory gene, cue-R, regulates two genes, cop-A and Cue-O, which cause copper efflux. • Cso-R is another regulatory gene in bacteria, which in the presence of Cu^{+} de-represses copper resistance genes. • A *Streptococcus* strain was seen to have a copper transport operon named cop-YAZ in which cop-Y and cop-Z were established as heavy metal-binding proteins. • *Pseudomonas fluorescens* has been reported to possess a cop-RSCD operon for copper efflux. • *Helicobacter pylori* contains two separate operons for copper export and import, hpcop-AP. • *Bacillus subtilis* has another copper regulatory system, mediated and regulated by Ycn-Jk and Cso-R. Together, these genes maintain a state of copper homoeostasis. • An ATPase-driven copper efflux system is the main mechanism responsible for cytoplasmic copper removal: the multicopper oxidase Cue-O in *E.coli* and *Enetrobactin* oxidizes Cu (I) to Cu (II). • *Yersiniabactin* sequesters Cu (II) outside the bacterial cell protecting the bacteria from intracellular killing.	Djoko et al. 2010 Chang et al. 2014 Vats & Lee 2001 Hu et al. 2009 Ge & Taylor 1996 Chillappagari et al. 2009 Grass et al. 2004 Chaturvedi et al. 2012

Lead	• Lead-tolerant bacteria have been isolated, and precipitation of lead phosphate within the cells of these bacteria has been reported.
	• Several bacteria, such as Arthrobacter spp., Bacillus megaterium, Pseudomonas marginalis, Citrobacter freundii, Staphylococcus aureus, and E. coli have been found to be resistant to lead.
	  • The most studied lead efflux operon, named the pbr operon, was found to contain many structural genes, (pbr-TABCD) and one regulatory gene (pbr-R)
	  • Metal immobilization by the process of extracellular sequestration is also important for regulating metal toxicity:
	  • Lead binding by the negatively charged components of EPS has been demonstrated in *P. aeruginosa* strain CH07.
	  • *Pseudomonas marginalis* is able to resist lead through sequestration of lead in an exopolymer.
	  • Similarly, the EPS of *Paenibacillus jamilae* bioadsorbs lead
	  • There are many enzymatic activities in the bacterial EPS which assist in toxic metal transformation by chemical reaction, precipitation, or entrapment.
	  • Bioprecipitation of toxic metals to insoluble complex formation is another strategy which reduces metal bioavailability and toxicity:
	  • *Bacillus iodinium* strain GP13 and *Bacillus pumilus* strain S3 were reported to precipitate lead as lead sulfide.
		Trajanovska et al. 1997; Levinson & Mahler 1998;
		Das et al. 2016
		Borremans et al. 2001; Jarosławiecka & Piotrowska-Seget 2014
		Das et al. 2016
		De et al. 2007
		Roane 1999
		Morillo et al. 2008
		Paul 2008
		Das et al. 2016
		De et al. 2008

	• A phosphate-solubilizing bacterium, ***E. cloacae,*** was found to resist lead by immobilizing lead as a insoluble lead phosphate mineral, pyromorphite	Park et al. 2011
Nickel	• Nickel is detoxified by sequestration and/or transport. It is bound to polyphosphate in ***S. aureus***. • The best-known nickel resistance in bacteria, in ***Ralstonia sp.*** strain CH34 and related bacteria, is based on a nickel efflux pump driven by an RND transporter. • Nickel resistance in bacteria is generally mediated by efflux pumps. One such resistance mechanism has been studied in ***Cupriavidus metallidurans*** strain CH34 where it was reported that the presence of the efflux pump was encoded by the **cnr-YHXCBAT** gene system. • In ***Achromobacter xylosoxidans*** strain 31A, only one gene, **nreB**, was responsible for conferring the entire nickel resistance efflux system. • The **ncc** operon provides combined nickel, cobalt, and cadmium resistance. • Seven open reading frames (ORFs) were studied and designated **ncc-YXHCBAN**. The nucleotide sequence revealed significant similarity to the cnr and czc operons of ***Alcaligenes eutrophus*** strain CH34. • In ***E. coli***, the **rcn-A** gene encodes a membrane-bound polypeptide which had the ability to confer resistance to nickel and cobalt. • Another efflux pump was identified in ***Helicobacter pylori*** and named **czn-ABC**, for cadmium, zinc, and nickel. • In another study, the nickel/cobalt transferase gene, **NiCo-T**, from ***Staphylococcus aureus*** was amplified and established as having high resistance	Gonzalez & Jensen 1998 Nies 1999 Grass et al. 2000 Grass et al. 2005 Schmidt & Schlegel 1994 Tibazarwa et al. 2000 Rodrigue et al. 2005 Stahler et al. 2006 Zhang et al. 2007
Zinc	• Two systems are used for zinc detoxification in bacteria, P-type efflux ATPases and RND-driven transporters. • In ***E. coli*** and ***Synechocysti***, **Znt-A** and **Zia-A** are responsible for zinc efflux. Efflux pumps for cadmium resistance often also cause zinc efflux.	Beard et al. 1997; Rensing et al. 1997b Thelwell et al. 1998

3.4.4 DNA Sequencing and Data Analysis Methods

3.4.4.1 DNA Sequencing Overview

Early methods for DNA sequencing began in 1970 and were unautomated, extremely costly, and took years to complete; these methods are generally no longer in use and are described elsewhere (Chen, 1994). However, since 1995 when the first bacterial genome was sequenced (Fleischmann et al., 1995), scientific capabilities with DNA sequencing and genome-based analytics have rapidly increased. Loman et al. (2012) discuss how extremely rapid growth in this field has led to "an embarrassment of choice" between instruments and platforms and also that "vigorous competition between manufacturers has resulted in sustained technical improvements on almost all platforms." There are numerous sequencing technologies available to researchers, each offering its own set of advantages and disadvantages. There are also

numerous precursors or alternate methods for analyzing bacterial diversity and function, some of which are still commonly used and others of which are being phased out due to out-competition from emerging/modern technologies.

Selecting the right analysis method for a study depends on a number of factors including:

- The goal of the study;
- The sample media and the expected DNA quantity and quality obtainable during extraction;
- The depth and quality of data required to achieve the study goal;
- The availability of analysis technologies and institutional expertise for guidance; and
- The study timeline and budget.

Bacteria are highly concentrated in the natural environment; one gram of soil or sediment typically contains 10^{10} bacteria while one millilitre of seawater typically contains 10^{6} bacteria (Torsvik et al., 1990). Because of the massive population, comparing bacterial diversity quickly becomes extremely complex.

Bacterial diversity exists at three levels: within species (genetic), between species (species) and community (ecological) diversity (Harpole, 2010). Species diversity can be further broken down into two components – species richness and species distribution. Species richness refers to the total number of different species in the population while species distribution refers to the evenness of the different species in the population. Diversity studies can relay useful information about the stresses on an ecosystem; generally, a bacterial community that is diverse is more stable when responding to environmental stresses as it contains the genetic code for adaptability to change (Yannarell & Triplett, 2005). Diversity will change in response to stress and this can be monitored as a cause and effect relationship.

Methods for analyzing microbial diversity and abundance can be categorized into three groups: conventional (culture-based), biochemical and molecular. *Table 20* summarizes some of the most common conventional and biochemical analysis techniques.

Table 20. Common Conventional and Biochemical Techniques for Analyzing Microbial Diversity and Abundance (adapted from Fakruddin & Mannan, 2013)

Method	Description	Advantages	Disadvantages
Plate counts	• Culture bacteria on growth media followed by viable counts	• Fast • Inexpensive	• Un-culturable bacteria not detected • Bias towards fast growing bacteria
Community level physiological profiling (CLPP)/ Sole-Carbon Source Utilization (SCSU) Pattern	• Identify pure cultures of bacteria to the species level using their metabolic properties • Examine the functional capabilities of the microbial population • Compare metabolic capabilities of communities.	• Fast • Highly reproducible • Relatively inexpensive • Able to differentiate microbial communities • Generates large amount of data • Option of using bacterial, fungal plates or site specific carbon sources	• Only represents culturable fraction of community • Favours fast growing bacteria • Only represents those organisms capable of utilizing available carbon sources • Potential metabolic diversity, not *in situ* diversity • Sensitive to inoculum density
Phospholipid fatty acid (PLFA) analysis/Fatty acid methyl ester analysis (FAME)	• Use the fatty acid composition of microorganisms to aid microbial characterization • Analyze the PLFA composition of the organisms since different subsets of a community have different PLFA patterns.	• Culturing not required • Direct extraction from soil • Follow specific organisms or communities	• Can be influenced by external factors • Results can be confounded by other microorganisms

Molecular techniques, can be further divided into partial community analysis techniques and whole community analysis techniques. These techniques can also be classified as first generation, next generation, or third generation methods based on the throughput, quality and depth of information obtained.

Partial community analysis generally involves first generation PCR-based analysis techniques where DNA or RNA extracted from an environmental sample is used as a template to characterize microorganisms (Rastogi & Sani, 2011). Essentially, in partial community analysis, researchers determine the genetic signature in a sample by selecting and analyzing a specific gene that is conserved among all species such as the 16S rRNA gene or the RNA polymerase beta sub-unit (rpoB).

The disadvantage of partial community analysis is that researchers must compare to a database of known information in order to parcel out results from their samples; however, the growing databases of known species data have made these methods highly desirable in recent years. Technological advances in the throughput and depth of information that can be obtained in partial community analysis has led to the development of next generation methods including the Illumina MiSeq platform, which is a type of clone library analysis. *Table 21* summarizes common partial community analysis techniques and their advantages and disadvantages.

Table 21. Common Partial Community Analysis Molecular Techniques for Analyzing Microbial Diversity and Abundance (adapted from Fakruddin & Mannan, 2013; Rastogi & Sani, 2011)

Method	Description	Advantages	Disadvantages
Nucleic acid re-association and hybridization	• Estimate diversity by measuring the genetic complexity of the microbial community (re-association) • Use specific probes (e.g. FISH) on extracted DNA or RNA, or *in situ* to examine and quantify known sequences (hybridization)	• Total DNA extracted • Not influenced by PCR biases • Can study DNA or RNA • Can be studied *in situ*	• Lack of sensitivity • Sequences need to be in high copy number for detection • Dependent on lysing and extraction efficiency
DNA microarrays and DNA hybridization	• Develop a microarray to elucidate function diversity of a community by identify specific target genes coding for enzymes such as nitrogenase, nitrate reductase, naphthalene dioxygenase *etc*.	• Same as nucleic acid hybridization • Thousands of genes can be analyzed • Increased specificity	• Only detect the most abundant species • Need to culture organisms • Only accurate in low diversity systems
Denaturing (DGGE) and Temperature (TGGE) Gradient Gel Electrophoresis	• Use a linear gradient of DNA denaturants (DGGE) or temperature (TGGE) to separate DNA fragments (16S or 18S rRNA) of the same length but with different base-pair sequences and differentiate the fragments based on their mobility (Mühling *et al.,* 2008)	• Large number of samples can be analyzed simultaneously • Reliable, reproducible and rapid	• PCR biases • Dependent on lysing and extraction efficiency • Sample handling can influence community • One band can represent more than one species • Detects dominant species
Single Strand Conformation Polymorphism (SSCP)	• Analyze differences in the mobility of single stranded DNA on polyacrylamide gel, resulting from the folded secondary structure of DNA, which is dependent on DNA sequences	• Same as DGGE/TGGE • No GC clamp • No gradient	• PCR biases • Some ssDNA can form more than one stable conformation
Restriction Fragment Length Polymorphism (RFLP)	• Blot electrophoresed digests from agarose gels onto membranes and hybridize with a probe prepared from cloned DNA segments of related organisms	• Detect structural changes in microbial community	• PCR biases • Banding patterns often too complex
Terminal Restriction Fragment Length Polymorphism (T-RFLP)	• Follow the same principle as RFLP except label one PCR primer with a fluorescent dye, perform PCR on the sample DNA using universal 16S rDNA primers and separate fragments by gel electrophoresis, where each unique fragment length can be counted as an OTU and the frequency of OTUs can be calculated (Liu *et al.,* 1997)	• Simpler banding patterns than RFLP • Can be automated • Large number of samples • Highly reproducible • Ability to compare differences between microbial communities	• Dependent on extraction and lysing efficiency • PCR biases • Type of *Taq* can increase variability • Choice of restriction enzymes will influence community fingerprint

Ribosomal Intergenic Spacer Analysis (RISA)/Automated Ribosomal Intergenic Spacer Analysis (ARISA)	• Detect sequence polymorphisms using silver staining in RISA or a fluorescently labeled forward primer in ARISA • Use PCR to amplify the intergenic spacer (IGS) region between the 16S and 23S ribosomal subunits , denature and separate units on a polyacrlyamide gel and differentiate between bacterial strains and species based on heterogeneity (Fisher & Triplett, 1999).	• Highly reproducible community profiles	• Requires large quantities of DNA (for RISA) • PCR biases
Quantitative polymerase chain reaction (Q-PCR)	• Use dyes or probes to measure the accumulation of amplicons in real time during each cycle of the PCR and quantify based on the exponential increase in amplicon concentration	• Rapid • Successfully used for quantification of important physiological groups	• Highly sensitive to starting template concentration • Requires microbe concentrations to be above detection limits
Clone library method (e.g. MiSeq)	• Clone and then sequence the individual gene fragments in an environmental sample (e.g. 16S rRNA genes) and compare to a known database such as GreenGenes or Silva	• Most widely used method to analyze PCR products • The 'gold standard' for preliminary microbial diversity surveys • Large availability of data for comparison • 16S rRNA gene is highly stable and conserved	• Labor intensive • Time consuming • Expensive • May not decipher the entire microbial community composition

In contrast with partial community analysis techniques, whole community analysis techniques attempt to analyze all of the genetic information extracted from a sample. The first common modern method of whole community analysis to be developed was automated Sanger sequencing (Slatko et al., 2011); however, this was a first generation technique that was costly and highly time-intensive and while it did elucidate much insight into the link between microbial function and taxonomic identity, a large body of information was still poorly understood. Next generation sequencing methods emerged in 2005 and their advent has revolutionized the scientific understanding of microbial communities and relationships (Lagares et al., 2012). Table 22 summarizes the most common techniques for whole community analysis.

Table 22. *Common Techniques for Analyzing Microbial Diversity and Abundance using Whole Community Analysis (adapted from Fakruddin & Mannan, 2013; Rastogi & Sani, 2011)*

Method	Description	Advantages	Disadvantages
Automated chain terminator (Sanger) sequencing	• Sequence whole microbial genomes using a shotgun cloning method that involves (1) extraction of DNA from pure cultures, (2) random fragmentation of obtained genomic DNA into small fragments, (3) ligation and cloning of DNA fragments into plasmid vectors, and (4) bidirectional sequencing of DNA fragments	• Small machines are available for low-throughput laboratories • Useful for some specific applications (e.g. finishing genomes)	• Costly • Time-intensive • Sequencing low number of clones captures only dominant components of the microbial communities
Metagenomics	• Investigation of the collective microbial genomes retrieved directly from environmental samples without relying on cultivation or prior knowledge of the microbial communities (Riesenfeld et al. 2004)	• Cost-effective • Higher throughput • Simpler library preparation • No cloning step • Steadily improving read lengths • Minimal hands on time	• Long run time • Short read lengths • Some methods yield high error rates or biases • Expensive reagents
Metatranscrip-tomics	• Allows monitoring of microbial gene expression profiles in natural environments by studying global transcription of genes by random sequencing of mRNA transcripts pooled from microbial communities at a particular time and place	• Suitable for measuring changes in gene expression and their regulation with changing environmental conditions	• Prokaryotic microbial mRNA transcripts are not polyA tailed, so obtaining complementary DNA is not easy.
Proteogenomics	• Deals with the large-scale study of proteins expressed by environmental microbial communities at a given point in time	• Rapid and sensitive • Protein biomarkers are more reliable and provide a clearer picture of metabolic functions than functional genes or even the corresponding mRNA transcripts of microbial communities	• New emerging technology

Concerning whole community analysis, metagenomics is the focus of this study as the goal is to develop a genomics tool for stormwater treatment applications. Therefore, those interested in further detail of other whole community analysis methods should consult elsewhere (Rastogi & Sani, 2011). Various companies supply technologies for metagenome sequencing including the Roche 454 platform (Life Sciences), the HiSeq (Illumina), and the Ion Torrent Personal Genome Machine (Thermo Fisher) (Bragg & Tyson, 2014). Each of these technologies offers advantages and disadvantages with greater advantages established with

each upcoming model (ibid). Currently, the most popular platform is the Illumina HiSeq system. Bragg and Tyson (2014) describe the Illumina sequencing protocol:

> "The Illumina sequencing protocol begins by ligating template DNA to an adaptor sequence and thence onto a glass flow cell. The template DNA is subjected to bridge amplification, whereby each template is increased to roughly 1,000 copies. By using an isothermal polymerase and 3′ inactivated fluorescent nucleotides, Illumina is able to incorporate a solitary base each cycle. Each base addition is followed by an imaging step, which reads the fluorescent label."

There are numerous models of the HiSeq platform including the HiSeq, HiSeq 2000, HiSeq 2500, and HiSeq 3000/4000. With each model upgrade, the sequencing power and efficiency increases; however, the general analysis principles remain the same.

3.4.4.2 Sequence Data Analysis Overview

Due to the complexity of data obtained, analysis of next generation sequenced data requires several steps. These are summarized as follows (Lagares et al., 2012):

1. Data filtering: Identifying and removing noisy reads based on quality scores;
2. Data trimming: Removing regions with a high likelihood of error;
3. Noise removal: Iteratively pre-clustering and deleting both chimeras and PCR artifacts;
4. Data clustering: Defining OTUs by linking sequences with a threshold for percent similarity (e.g. 97%);
5. Taxonomic assignment: Comparing with a reference alignment of known taxonomic assignments (for MiSeq) or classification based on sequence homology and composition (HiSeq);
6. Assembly of metagenomes: Finding overlaps between reads and building consensus sequences, so-called contigs, based on multiple alignments;
7. Gene annotation: Identifying metagenomic sequences using gene prediction tools;
8. Metabolic reconstruction: Using gene predictions to understand the metabolic potential of a microbial community; and
9. Comparative metagenomics: Searching for statistically significant differences between metagenomes using either taxonomic classifications or gene/metabolic annotations.

There are various software tools available to researchers for these purposes. *Table 23* summarizes some of the more common software applications and provides links to each tool's website for more information.

Table 23. Common Software Applications for Sequence Data Analysis

Application	Name	Webpage
General sequence processing	Mothur	http://www.mothur.org/
	QIIME	http://www.qiime.org/
De-noising	AmpliconNoise	http://code.google.com/p/ampliconnoise/
	DeNoiser	http://www.qiime.org/
Clustering	UCLUST	http://www.drive5.com/usearch/
	Mothur	http://www.mothur.org/
	DNACLUST	http://sourceforge.net/projects/dnaclust/
	CD-hit	http://www.bioinformatics.org/cd-hit/
Alignment	Mothur	http://www.mothur.org/
	MAFFT	http://mafft.cbrc.jp/alignment/software/
	PyNAST	http://pynast.sourceforge.net/
	RDP	http://pyro.cme.msu.edu/
	SILVA	http://www.arb-silva.de/
Phylo-genetics	RAxML	http://sco.h-its.org/exelixis/software.html
	FastTree	http://www.microbesonline.org/fasttree/
Community analysis	QIIME	http://www.qiime.org/
	Mothur	http://www.mothur.org/
	MG-RAST	http://metagenomics.anl.gov/
	R (VEGAN, GGPlot)	http://www.r-project.org/
Assembly	Newbler	http://454.com/
	Celera Assember	http://sourceforge.net/apps/mediawiki/wgs-assembler/index.php?title=Main_Page
	CLC Assembly cell	www.clcbio.com
	Meta-IDBA	http://i.cs.hku.hk/~alse/hkubrg/projects/metaidba/
	Genovo	http://cs.stanford.edu/group/genovo/
	MetaORFA	n.a.
	MetaVelvet	http://metavelvet.dna.bio.keio.ac.jp/
	Bambus 2	http://www.cbcb.umd.edu/software/bambus/
Short read gene prediction	Orphelia	http://orphelia.gobics.de/
	Metagenemark	http://exon.gatech.edu/metagenome/Prediction/
	FragGeneScan	http://omics.informatics.indiana.edu/FragGeneScan/
	MetaGeneAnnotator	http://metagene.cb.k.u-tokyo.ac.jp/
Metagenomics tools	MEGAN	http://ab.inf.uni-tuebingen.de/software/megan/welcome.html
	SOrt-ITEMS	http://metagenomics.atc.tcs.com/binning/SOrt-ITEMS/
	WebCARMA/CARMA 3	http://www.cebitec.uni-bielefeld.de/brf/carma/carma.html
	Treephyler	http://gobics.de/fabian/treephyler
	PhyloPhytiaS	http://binning.bioinf.mpi-inf.mpg.de
	TACOA	http://www.cebitec.uni-bielefeld.de/brf/tacoa/tacoa.html
	Phymm/PhymmBL	http://www.cbcb.umd.edu/software/phymm/
	Naïve Bayes classifier	http://ratite.cs.dal.ca/rita/submission
	MG-RAST	http://metagenomics.anl.gov/
	CAMERA	http://camera.calit2.net/
	IMG/M	http://img.jgi.doe.gov
	GAAS	http://sourceforge.net/projects/gaas/
	SmashCommunity	http://www.bork.embl.de/software/smash
	Meta-rep	http://www.jcvi.org/metarep
	Xipe	http://edwards.sdsu.edu/cgi-bin/xipe.cgi
	STAMP	http://kiwi.cs.dal.ca/Software/STAMP

3.5 Methodology

The primary focus of this chapter was to provide proof of concept microbial results which either support or reject further research towards a genomics-based monitoring tool for stormwater treatment wetlands and other low impact stormwater treatment sites. With this goal in mind, a two-part strategic methodology was developed. First, a field study was performed at the Lost Lagoon wetland with the collection of water and sediment samples described in Chapter 1. In support of this chapter's first two objectives and hypotheses, DNA was extracted, sequenced, analyzed and compared with environmental data. Second, a laboratory study was designed and performed, in support of this chapter's third objective and hypothesis. Like in the field study, water and sediment samples were collected over the experimental period and DNA was extracted, sequenced, analyzed and compared with environmental data.

A detailed description of the methodology employed to answer the objectives and hypotheses listed at the beginning of this chapter is supplied here.

3.5.1 Field Study Site Visits and Sampling Regime

Please consult section 2.5 for details of the field study site visits and sampling regime.

3.5.2 Column Study Preparation and Execution

A four month long, laboratory study was carried out using columns of uncontaminated natural soil sourced from a bog near Beaver Lake as highlighted in Figure 2 in the introduction to this thesis. Columns were fed either semi-synthetic stormwater or distilled water and the contaminant levels and microbial responses were measured over time.

3.5.2.1 Sourcing and Confirmation of Uncontaminated Soil

To confirm the soil quality prior to collection of uncontaminated park soil, a location that was believed to be free of stormwater contamination was sited near the Beaver Lake bog. On October 27, 2015, six samples were collected across the bog site and each sample site was marked with flag tape. The samples were packed into plastic freezer bags and were brought back to the laboratory and analyzed for metal content.

3.5.2.2 Collection of Uncontaminated Soil

After soil quality was confirmed, a soil collection day was planned for November 11, 2015. Eight large coolers were disinfected. Coolers were scrubbed with laboratory dish detergent, soaked overnight with 5% bleach solution, allowed to dry, rinsed with 1% nitric acid solution, and sprayed and wiped with 95%

ethanol. Shovels were also cleaned with dish detergent, rinsed with bleach, and sprayed with ethanol prior to soil collection.

On November 11, 2015, bog soil was collected from Stanley Park. A team of four workers shoveled soil into clean five gallon buckets and transferred the soil to the disinfected coolers. A total of eight coolers of soil were collected and transferred by truck to the UBC civil engineering department refrigerators, where they were stored at <4˚C until further processing.

3.5.2.3 Column Study Environment

In order to run the study over four months, a clean temperature controlled room was prepared. The room was emptied of shelving and the walls, ceiling, refrigeration system, and floor were scrubbed with laboratory grade dish soap and tap water. Next the surfaces were sprayed with hospital grade germicide and allowed to stand for fifteen minutes. Following germicide, the surfaces were wiped clean with paper towel and then sprayed with 10% beach solution and allowed to stand for twenty-four hours. Finally, the surfaces were given a final cleaning with 95% disinfection grade ethanol.

3.5.2.4 Pre-Study Experiment

Before the full laboratory experiment began, the column configuration was run and studied for one week on one column and the ORP, conductivity, pH, DO, and temperature were monitored and confirmed to be in the range of values measured in the Lost Lagoon wetland forebay.

3.5.2.5 Column Configuration and Set-Up

Seventeen sediment columns were analyzed over a four-month period. The sediment columns were constructed from five gallon opaque PVC buckets. Before the study began, the buckets were scrubbed with laboratory grade dish detergent and soaked in a 10% bleach-water solution for twenty-four hours. To prevent preferential flow along the smooth inside of the buckets, the inner lining of each bucket was then roughed with coarse sand paper. The buckets were again washed with laboratory grade dish detergent and soaked in bleach solution for twenty-four hours. Buckets were rinsed twice with distilled water, rinsed once with 1% nitric acid, rinsed once with distilled water, and wiped clean with 95% disinfection grade ethanol. The same cleaning process was used for the column lids.

On November 19, 2015, soil was packed into the laboratory columns. 2.5 L from each cooler was placed into a clean bucket and homogenized using a hand mixer. Large debris including sticks, rocks, leaves, roots etcetera, that had a length greater than 0.5 cm was removed. No garbage or fecal matter was observed in the soil. One litre of soil was then packed into each column using a clean rubber mallet. This process was

repeated eight times so that the height of soil packed into each column reach 15-cm. Columns were zeroed and weighed and the mass of soil added to each column was recorded. The columns were allowed to sit covered in the clean controlled room at <4˚C until further processing.

On November 25, 2015, 8-L of distilled water was added to six columns, which would serve as study controls. 5-L of distilled water was added to each of eleven columns, which would serve as the object of the study, hereafter referred to as exposed columns. A 0.5 cm hole was drilled into the lid of each column and the holes were sealed with bungs. The lids were placed on top of each column while they reached temperature equilibrium with the control room. The temperature in the control room was then increased by 2˚C on the morning of each subsequent day until the temperature of the room reached 18˚C.

3.5.2.6 Column Water Dosing Regime and Environmental Controls

3.5.2.6.1 *Stormwater Dose Quality*

Based on several resources, (Bratieres et al., 2008; Blecken et al., 2009; Lewis & Sjostrom, 2010; Zhang et al., 2015), a recipe was developed for semi-synthetic stormwater to be used as simulated urban runoff in the column study. A combination of real sediment and chemical additives was mixed with dechlorinated distilled water in order to achieve target TSS concentrations and to maintain consistent inflow, while also mimicking 'natural' conditions.

On November 27, 2015, fine sediment, which was collected from Site 2.1 in the Lost Lagoon wetland forebay, was autoclaved, centrifuged and decanted to remove water, and baked at 105˚C for 48 hours. This sediment was frozen at <-4˚C prior to use in the stormwater recipe.

Average values from literature as well as the predicted quality of sediment from Site 2.1 in the Lost Lagoon Wetland were used to set target stormwater quality and to prepare a stormwater 'recipe'. *Table 24* lists stormwater qualities found in literature and the 2013 Hemmera analyses for quality of sediment in the Lost Lagoon Wetland.

Table 24. Urban Highway Stormwater Quality from Literature and Sediment Quality Data from the Lost Lagoon Wetland in 2013

Element	Washington[1]	British Columbia[2]	Blecken et al (2009)	Dredged Sediment[3]
	µg /L	µg /L	µg/L	mg/kg
Antimony	8.7	-	-	64
Arsenic	2.6	10-130	-	7
Barium	84	-	-	205
Cadmium	2.8	-	6.7	3.5
Chromium	18	10-110	-	135
Cobalt	4.4	0.7-30	-	11
Copper	72	13-288	95	650
Lead	61	10-3775	181.5	250
Molybdenum	9.5	-	-	28
Nickel	12.9	2-126	-	46
Zinc	394	40-25500	587.3	1150
Phosphorus	500	-	-	-
Nitrogen	2800	-	-	-
TSS*	400-1200		155	-

*mg/L

[1]Washinton State (EPA), 2007

[2]British Columbia Waste Management Group, 1992

[3]Hemmera, 2013

Based on data from the state of Washington, USA, it was assumed that average highway stormwater would have a TSS concentration of approximately 800 mg/L. In order to maintain more consistent metal levels, the stormwater recipe for the laboratory column study was prepared in a semi-synthetic fashion. The stormwater was prepared using a target TSS of 400 mg/L and the remaining metal concentrations were 'topped up' using chemical additives. *Table 25* lists the target metal concentrations for the stormwater and the top up required using chemical additives.

Table 25. Target Element Concentrations for Semi-Synthetic Stormwater Recipe

Element	800 mg/L TSS	400 mg/L TSS	Target	Chemical Top Up Required
	µg/L	µg/L	µg/L	µg/L
Antimony	51.2	25.6	65	39
Arsenic	5.6	2.8	100	97
Barium	164	82	205	123
Cadmium	2.8	1.4	5	4
Chromium	108	54	100	46
Cobalt	8.8	4.4	15	11
Copper	520	260	650	390
Lead	200	100	550	450
Molybdenum	22.4	11.2	30	19
Nickel	36.8	18.4	100	82
Zinc	920	460	1100	640
Phosphorus	0	0	500	500
Nitrogen	-	-	2800	2800

Chemical additives were selected based on previous work performed by Blecken et al., (2009) and based on common availability of these additives in the laboratory.

Table 26 lists the chemical additives that were used for the stormwater recipe. Concentrated volumes of the chemical additives were prepared in separate 1-L bottles for each element and the volumes were stored in a refrigerator at <4˚C for use over the duration of the study.

Table 26. Chemical Additives Used for Semi-Synthetic Stormwater Supplementation

Element	Chemical Additive	Molecular Mass	% Element
		g/mol	g/g
Antimony	$K_2Sb_2(C_4H_2O_6)_2$	613.83	40%
Arsenic	As_2O_3	197.84	76%
Barium	$BaCl_2$	208.23	66%
Cadmium	$Cd(NO_3)_2 \bullet 4H_20$	368.45	31%
Chromium	$[Cr(H_2O)_6](NO_3)_3 \bullet 3H_2O$	535.07	10%
Cobalt	$Co(NO_3)_2 \bullet 6H_20$	291.03	20%
Copper	$CuSO_4 \bullet 5H_20$	267.70	24%
Lead	$Pb(NO_3)_2$	331.21	63%
Molybdenum	$(NH_4)_6Mo_7O_{24} \bullet 4H_20$	1,235.86	8%
Nickel	$NiCl_2 \cdot 6H_2O$	237.69	25%
Zinc	$ZnCl_2$	136.30	48%
Phosphorus	KH_2PO_4	136.09	23%
Nitrogen	NH_4NO_3	80.04	35%

On November 30, 2015, semi-synthetic stormwater was prepared to match the target concentrations listed in *Table 25*. Stormwater was mixed in disinfected 5 gallon buckets and stored in sterile glass 2-L amber bottles at <4˚C until application. Batches of semi-synthetic stormwater were prepared at two week intervals.

3.5.2.6.2 *Stormwater Dose Volume and Frequency*

To determine the stormwater dosing regime, the ratio of the top surface area sediment column to the Lost Lagoon wetland catchment area was calculated.

The watershed catchment area was calculated in Appendix E:

$$Lost\ Lagoon\ Watershed\ Catchment\ Area = 32143\ m^2$$

The column top surface area was calculated using the measured diameter:

$$Column\ Top\ Surface\ Area = \pi r^2 = \pi(0.14^2) = 0.061544\ m^2$$

The ratio of column top surface area and catchment area was calculated using:

$$Ratio = \frac{0.061544\ m^2}{32134\ m^2} = 1.91{\times}10^{-6}\frac{m^2}{m^2}$$

Next, average weather data from Environment Canada weather station 1108446, Vancouver Harbour CS, which is located 1.82 km from Stanley Park was used to determine monthly average temperature and precipitation values for the field study site over the period between August and November.

Table 27. Environment Canada Average Precipitation and Temperature Data for Vancouver Harbor (Environment Canada, 2016)

Month	Day Temperature	Night Temperature	Rain	Average Monthly Rain	Average Rain
	˚C	˚C	Days/month	mm	mm
August	23	14	10	39.5	4.0
September	20	11	11	48.2	4.4
October	14	7	20	126.8	6.3
November	10	3	23	183.4	8.0

Using the data from the Environment Canada Station and assuming that the entire catchment area drains to the Lost Lagoon wetland, the average drainage volume per storm was calculated as:

$$Drainage\ Volume\ (m^3) = \frac{Average\ Rain\ (mm){\times}Catchment\ Area(m^2)}{1000\frac{mm}{m}}$$

The ratio of the catchment area to the column area was used to scale the water volume to be added to each laboratory column during each 'precipitation' event:

$$Column\ Volume\ (mL) = Drainage\ Volume\ (m^3) \times Ratio\ (\frac{m^2}{m^2}) \times 1000 \frac{L}{m^3} \times 1000 \frac{mL}{L}$$

The frequency of precipitation events, or 'additions per week', was calculated using the average number of rain days and the days in each month:

$$Additions\ per\ week\ (n\ days) = Rain\ \left(\frac{days}{month}\right) \times \frac{7 \frac{days}{week}}{30 \frac{days}{month}}$$

Table 28. Calculated Water Addition Volumes and Frequencies for Column Study

Month	Drainage Volume	Column Volume	Additions per Week
	m^3	mL	*n days*
August	127.0	243	2
September	140.8	270	3
October	203.8	390	5
November	256.3	491	5

3 L of stormwater were initially added to each exposed column. After the initial loading of stormwater, the lids were sealed to each column and the columns were made watertight using Parafilm. Column watering was achieved using a sterilized 500 mL glass flask and a peristaltic pump. A piece of sterile tubing was connected to each side of the pump. One piece of tubing was inserted into the 500 mL glass flask and one side of the tubing was inserted through the small hole in the lid of the sample column. The glass flask was filled with the appropriate quantity of stormwater, or distilled water for the controls, and the water was fed into the column using the pump. Prior to adding volumes of water to each column, an equal volume of water in the column was removed using the pump. To avoid contamination, plastic tubing was traded out between every column watering episode. All mobile equipment was soaked overnight in a 10% bleach solution prior to use. All stationary equipment was wiped clean with 95% disinfectant grade ethanol after every watering episode.

3.5.3 Column Study Sampling Regime

As previously stated, the column study began with 17 sediment columns, six of which were fed with distilled water and eleven of which were fed with semi-synthetic stormwater. The nature of sample collection required that columns be sacrificed. On day zero, two columns that were fed with stormwater and one column that was fed with distilled water were sacrificed and analyzed. Sampling of sediment columns then occurred at four week intervals following the same procedure. Thus, ten stormwater columns and five distilled water columns were sacrificed and analyzed for the study. The remaining two columns were used

to collect daily measurements for dissolved oxygen, conductivity, pH, temperature, and redox potential using the handheld device that was described in Chapter 1.

Sample collection for the column study was generally performed in the same manner as was performed in the Lost Lagoon wetland. 1 L plastic bottles were used to collect water samples near the soil-water interface. After collection of water, a peristatic pump was used to lower the water level in the column under investigation. For soil sampling, 5 disinfected 60 mL syringes, with sanded off ends, were carefully pressed into the soil layer at the centre and at four evenly spaced points around the column's perimeter. The syringes were carefully pulled from the column, capped on both ends with aluminum foil and frozen at <-20˚C until further processing.

3.5.4 Sample Preservation, Transport, Pre-Processing, Storage and Quality Control

Water samples collected at the Lost Lagoon wetland were preserved for DNA on site using the following procedure:

- To a 100 mL glass jar, add 20 mL of sample from each of 5 1-L plastic bottles;
- Cap the glass jar and place in a plastic zip-lock bag; and
- Place the bag in a cooler on dry ice.

Water samples collected during the laboratory study were combined and preserved by the same methods that were applied in the field. This includes taking samples for environmental parameters and contaminants.

Both field and laboratory study sediment samples were prepared and preserved for laboratory testing and DNA extraction following the procedures that were applied during the field study at the Lost Lagoon Wetland. These procedures are described in Section 2.5.1.5.

Field samples collected at the Lost Lagoon wetland were transported back to UBC using the methods described in Section 2.5.1.5. Laboratory study samples did not require additional transport.

For both the field and laboratory studies, water samples that were frozen on dry ice during transport were thawed at 4˚C. 30 mL of sample water was filtered through a sterile filter paper with pore size of 0.45 µm. Prior to filtering the water sample, the filtering apparatus was soaked in a 10% bleach solution overnight. The filtering apparatus was cleaned with 95% disinfectant grade ethanol between samples.

Filter papers were rolled in on themselves, placed in individual sterile petri dishes, wrapped in aluminium foil and frozen at <-20˚C until DNA extraction.

Field and laboratory study sediment samples were pre-processed and stored according to the same procedure that is described in Chapter 1.

3.5.5 Laboratory Analysis of Water and Sediment Quality Parameters

Laboratory analysis for water and sediment quality parameters for the column samples followed the same procedures as were applied during the field study at the Lost Lagoon wetland. These procedures are described in Chapter 1.

3.5.6 Laboratory Preparation of Bacterial DNA

3.5.6.1 Sample Handling and Preservation

Prior to extracting DNA, water and soil samples from the field and lab studies were placed in sterile plastic bags, labelled and frozen at <-20˚C.

3.5.6.2 Extraction of DNA and Quality Control

All equipment used during the DNA extraction process was soaked in bleach overnight and disinfected with 95% ethanol solution prior to and during use. The extraction of DNA was performed using Mobio PowerSoil® DNA Isolation Kits, catelog number 12888-100 (Qiagen, 2016) according to the manufacturer's instructions. Soil samples were extracted without modification to the procedure. Because the contents of the water samples were filtered onto sterile 0.45µm filters, prior to extraction, the filters were cut into 2mm by 2mm squares and the squares were inserted into the bead tubes using sterile forceps. The manufacturer's DNA extraction protocol was then followed without modification.

After extraction, DNA aliquots were frozen at <-20˚C until further processing.

3.5.6.3 Quantification of DNA

DNA samples were thawed and quantified for DNA concentration using fluorimetric analysis on the Qubit® 3.0 Fluorimeter (Thermo Scientific, catalogue #Q33216)

3.5.7 Sequencing for Comparison of Microbial Community Compositions

Comparison of microbial community composition was achieved through sequencing and analysis of the 16s rRNA gene. Sequencing of the 16s rRNA gene was outsourced to Microbiome Insights, a Vancouver-based service company that has delivered microbial analyses to hundreds of both academic and industrial researchers. Prior to delivering DNA samples to Microbiome Insights, the researchers discussed with

Microbiome Insights staff to prepare a sequencing protocol that included the appropriate standards for quality control. After a satisfactory plan was established, samples were transported on dry ice to the Microbiome Insights facility, which is located approximately 300 m from the UBC chemical engineering building. Samples were frozen at <-80˚C until further processing. An electronic sample list with DNA concentrations and appropriate meta-data was also provided to Microbiome Insights. Samples were delivered in two batches. The first batch included the DNA extracts from all 185 field study samples. The second batch included DNA extracts from all 112 column study samples.

3.5.7.1 Library Preparation and Quality Control

In preparation for sequencing of the 16s rRNA gene, the following procedures were performed.

10 µM index primer aliquots were arrayed into 96-well plates as recommended by Kozich et al., (2013) as follows:

- A701 – A712 with A501 – A508
- A701 – A712 with B501 – B508
- B701 – B712 with B501 – B508
- B701 – B712 with A501 – A508

Template DNA was aliquoted into a 96-well format with blank wells included for negative control. PCR reactions were performed using ThermoFisher Phusion Hot Start II DNA Polymerase (2 U/ µL). Each sample constituted a single PCR reaction. The PCR recipe and cycling conditions are indicated in *Table 29* and Table 30.

Table 29. Recipe for PCR Used During Library Preparation Prior to 16s rRNA Gene Sequencing

PCR Mix	Volume µL/reaction	100
5x Buffer	10	1000
MgCl	1	100
Forward Primer	1	
Reverse Primer	1	
dNTP	1	100
dH_2O	33.5	3350
taq	0.5	50
template	2	
total	50	5000

Table 30. Conditions for PCR Used During Library Preparation Prior to 16s rRNA Gene Sequencing

Temperature ˚C	Time	
98	2:00	
98	0:20	30 cycles
55	0:15	
72	0:30	
72	10:00	
4	hold	

In order to validate PCR success, eleven random samples and the negative control were analyzed and validated using gel electrophoresis. PCR products were then cleaned using Agencourt Ampure XP beads with a 0.8:1 bead to sample ratio. Following cleaning, PCR products were eluted to a final volume of 20 µL.

10 µL of the clean PCR product were used for normalization using the Invitrogen SequalPrep kit, and the remaining 10 µL were stored for backup. The amplicon library was normalized as recommended by Invitrogen (1-2 ng/ µL), and 5 µL of each normalized sample was pooled into a single library per plate (ie. 4 pooled plates in a 384-sample sequencing run). Library pools were further concentrated using the DNA Clean & Concentrator kit, following the manufacturer's instructions (Zymo Research). A dilution series was performed for each of the four pooled libraries for subsequent quality control steps.

Each pool was analyzed on the Agilent Bioanalyzer using the High Sensitivity DS DNA assay in order to determine the approximate library fragment size, and to verify library integrity.

Library pools containing unintended amplicons were purified using the Qiagen QIAquick Gel Extraction kit, following the manufacturer's instructions (Qiagen).

Pooled library concentrations were then determined using the KAPA Library Quantification Kit for Illumina and following the manufacturer's instructions (Kapa Biosystems).

Library pools were diluted to 4 nM and denatured into single strands using fresh 0.2 N NaOH as recommended by Illumina. The final library loading concentration was 8 pM, with an additional PhiX spike-in of 20%.

3.5.7.2 Sequencing of the 16s rRNA Gene

The amplicon library was sequenced on the Illumina MiSeq using the MiSeq 500 Cycle V2 Reagent Kit (250 x 2).

3.5.8 Sequencing for Comparison of Microbial Functional Gene Compositions

Sequencing for comparison of microbial functional genes was achieved through metagenome sequencing. Metagenome sequencing was outsourced to the UBC Beatty NextGen Sequencing Centre. The steps towards sequencing of metagenomes are described here.

3.5.8.1 Sample Selection and Quality Control

Because meaningful results are dependent on sequence depth and quality, these parameters were of primary importance for the analysis of metagenomes. However, this also had to be balanced with the desire to maximize cost effectiveness. It was determined that a balance of sequence depth/quality and cost effectiveness could be reached when sequencing metagenomes, if six samples were sequenced per lane, using the Illumina HiSeq 2000.

To maximize the diversity of samples and to ensure redundancy was achieved, DNA extracts were pooled to form each sample. Only DNA extracts that had a concentration between 5 ng/µL and 20 ng/µL were considered for possible pooling. Prior to pooling samples, to ensure the DNA was not degraded, DNA extracts were visualized using gel electrophoresis. 12.5 µL of each of four DNA extracts were pooled to form a sample with a volume of 50 µL. Pooled samples were treated for RNA – 1 µL of RNASE A (Purelink-Introgen) was added to each pooled sample and the pooled samples were inclubated at 37 ˚C for twenty-five minutes.

To re-purfiy samples, 2 µL of 5 M NaCL was added to each 50 µL pooled sample. Samples were inverted three to five times to mix. 90 µL of cold ethanol (100%) was added to each sample and the samples were inverted three to five times to mix. Samples were centrifuged at 10,000xg for 5 minutes. The liquid was decanted and the precipitate was allowed to air dry at room temperature. The DNA was then re-suspended in 45 µL of sterile Tris, containing no EDTA (Solution 6 from the Mobio Powersoil Reagent Kit). The DNA concentration in each pooled sample was quantified a second time using the Qubit Fluorimeter 2.0.

Samples were selected based on the objectives of both field and lab studies and for the case of the field study, based on the known quality of environmental data.

A breakdown of the pooled samples is as follows:

- Lane 1
    1. Four sediment samples (2 depth, 2 surface) pooled, site 2.1 September 9, 2015
    2. Four sediment samples (2 depth, 2 surface) pooled, site 5.2, September 22, 2015
    3. Four sediment samples (2 depth, 2 surface) pooled, site 2.1, October 20 2015

4. Four sediment samples (2 depth, 2 surface) pooled, site 3.1, October 20 ,2015
5. Four sediment samples (2 depth, 2 surface) pooled, site 4.1, October 20, 2015
6. Four sediment samples (2 depth, 2 surface) pooled, site 6.2, October 20, 2015

- Lane 2
    1. Four sediment samples (2 depth, 2 surface) pooled, Column 1, December 4, 2015
    2. Four sediment samples (2 depth, 2 surface) pooled, Column 7, December 4, 2015
    3. Four sediment samples (2 depth, 2 surface) pooled, Column 8, December 4, 2015
    4. Four sediment samples (2 depth, 2 surface) pooled, Column 5, March 29, 2016
    5. Four sediment samples (2 depth, 2 surface) pooled, Column 15, March 29, 2016
    6. Four sediment samples(2 depth, 2 surface) pooled, Column 16, March 29, 2016

Pooled samples were stored at <-20˚C until they were delivered to the Beatty NextGen Sequencing Centre. Samples were placed on dry ice during transportation to the Beatty NextGen Sequencing Centre, which is located approximately 400 m from the UBC chemical engineering laboratory, where the samples were originally stored. Upon delivery, samples were stored at <-20˚C until further processing.

3.5.8.2 Library Preparation and Quality Control

Library preparation was performed following a standard Illumina protocol for the HiSeq 2000 analyzer. The TruSeq Nano DNA LT Library Prep kit was used following manufacturer's instructions with settings for the Covaris M220 sonicator and 550bp insert size. Libraries were then validated using a Qubit Fluorimeter 2.0. Libraries were then sealed and stored at -20 ˚C for less than seven days. Libraries and the PhIX control were denatured and diluted according to manufacturer's instructions. The prepared libraries and PhIX control were then combined at a ratio of 99:1.

3.5.8.3 Cluster Generation

Cluster generation was performed using the cBot 2 system (SY-312-2001) following manufacturer's instructions for preparation of reagents and consumables and for quality control.

3.5.8.4 Sequencing of Whole Bacterial Genomes

Sequencing reagents were prepared following manufacturer's instructions and the following chemistry settings:

- SBS: HiSeq SBS Kit v4;
- Index: HiSeq v4 Index; and

- PE turnaround: HiSeq PE Cluster Kit v4.

When programming the sequencing run, the SBS reagent kit was set to 250 cycles on the reagent screen. The sequencing flow cell was loaded following the manufacturer's instructions for 100 base pair, paired-end sequencing and the sequencing run was executed.

3.5.9 Analysis of Bacterial Taxa Using the 16s rRNA Gene

Analysis of bacterial taxa using the 16s rRNA gene was performed through combination of three common microbial software programs, namely USearch, Mothur, and the R package, Vegan. Initial quality filtering, bioinformatics treatment and preparation of OTU tables was performed in USearch. Taxonomic assignments and calculation of alpha diversity and community composition parameters were performed in Mothur. Statistical analyses were performed in R. Further details and justification of input parameters are described below.

3.5.9.1 Quality Filtering and Determination of Unique Sequences and Abundances

Fastq file names were returned in the formatted output from MiSeq (i.e. s1_R1_001 etc). All Fastq files were transformed to fasta files. Using USearch, sequences were truncated to 200 bp and shorter sequences were dropped so that only high quality sequences remained. An example of the code is as follows:

- USearch -fastq_filter s1_R1_001.fastq -sample s1 -relabel @ -fastq_trunclen 200 -fastaout reads1.fa

Next, all files were concatenated to a single file to be used to determine the abundance of each operational taxonomic unit (OTU):

- copy/b read*.fa reads.fa

Following this, all sequences were transformed and truncated again and base quality was accounted for by setting the fastq_maxee parameter to 1.0:

- USearch -fastq_filter s1_R1_001.fastq -sample s1 -relabel @ -fastq_trunclen 250 -fastaout filtered1.fa -fastq_maxee 1.0

Again, all files were merged to a single file to be used for OTU calling:

- copy/b filt*.fa filtered.fa

A file was prepared with only unique sequences:

- USearch -derep_fulllength filtered.fa -relabel Uniq -sizeout -fastaout uniques.fa

Unique sequences were then sorted by abundance:

- USearch -sortbysize uniques.fa -fastaout suniques.fa -minsize 1

3.5.9.2 Preparation of OTU Tables

Unique sequences were pre-clustered with a threshold of 98% similarity:

- USearch -cluster_smallmem suniques.fa -id 0.98 -maxdiffs 4 -centroids preclustered.fa

The unique pre-clustered sequences were then sorted by size:

- USearch -sortbysize preclustered.fa -fastaout preclustered.fa -minsize 1

OTUs were clustered with "-minsize 2" in order to remove singletons:

- USearch -cluster_otus preclustered.fa -minsize 2 -otus otus_preuchime.fa -relabel Otu0

Chimera removal was performed using the rdp_gold.fa database:

- USearch -uchime_ref otus_preuchime.fa -db rdp_gold.fa -strand plus -nonchimeras otus.fa

An OTU table was prepared for the samples at 97% similarity with exported formats for both Mothur and Qiime applications:

- USearch -USearch_global reads.fa -db otus.fa -strand plus -id 0.97 -otutabout willotutab1.txt -biomout willotutab.json -mothur_shared_out wsh1.shared

3.5.9.3 Taxonomic Assignments

In Mothur, OTUs were classified and taxonomy was assigned using the Silva reference database for bacteria:

- Mothur -classify.seqs (fasta=otus.fa, template=silva.bacteria.fasta, taxonomy=silva.bacteria.silva.tax)

3.5.9.4 Bioinformatics

Alpha diversity and community composition parameters and indicator species analyses were calculated using Mothur. Sequences were subsampled to 9000 sequences for the field study and 5000 sequences for the lab study. Samples that had fewer than these numbers of sequences were dropped from the dataset. The sample code is as follows:

- Mothur -count.groups(shared=current)
- Mothur -summary.single(calc=coverage-sobs-chao-invsimpson, subsample=9000)
- Mothur -rarefaction.single(shared=current, calc=sobs, freq=100)
- Mothur -indicator(shared=current, design= current, processors=4)

3.5.9.5 Statistical Analyses on Data

3.5.9.5.1 *Data Screening*

Before analyses, a number of data screening techniques were applied, using GUide to STatistical Analysis in Microbial Ecology (GUSTA ME) (Buttigieg & Ramette, 2014).

These include:

- Avoiding data dredging;

Data dredging can occur when subsets of data are used to confirm hypotheses or when hypotheses are generated after the data is observed. Data dredging was avoided by not discarding data when it did not fit the hypotheses and by testing the hypotheses on more than one dataset.

- Ensuring awareness and consideration of pseudoreplication in the study;

Pseudoreplication occurs when dependent data is assumed to be independent. For example, if three measurements of the same sample are taken, then this data is dependent. In this study, pseudo-replicates were averaged prior to hypotheses testing and prior to visualization of the data.

- Checking and correcting for missing values;

Due to the nature of analysis using both ICP for metals and DNA sequencing for bacteria, some missing values occurred in the dataset. Samples with missing values were removed from the dataset prior to hypotheses testing. This generally was performed using is.na() parameter in R.

- Screening for outliers;

Microbial outliers were screened from the dataset using the Analysis of Similarity (ANOSIM) test in R. Outlier samples were removed from the dataset prior to analyses. This is further discussed in the results section.

3.5.9.5.2 *Alpha Diversity*

In order to compare alpha diversity among the samples, four indicators were calculated using the Mothur summary.single command. These include:

- Richness, or the number of different species present in a sample, based on the Chao1 estimator;

- Coverage, or the percent of the total species present in a sample, based on the Good's coverage calculation;
- Diversity, (richness and evenness, or the relative abundances of species) based on the inverse Simpson estimator; and
- Observed OTUs based on the SOBS calculation.

To compare alpha diversity, samples were split into separate datasets for the three materials (water, surface sediment, and 10-cm depth sediment) and split into separate datasets for the field and laboratory studies. Calculations were performed based on the different sites within the wetland and based on the columns analyzed in the laboratory study. In order to illustrate the variation among the data, barplots and boxplots were prepared for the various indices. To identify if there were significant differences among the data, one-way ANOVA tests were calculated using the standard R package (R Core Team, 2016) and interpreted using a confidence of 95%. Confirmation of both positive and negative statistical results was performed using the Tukey HSD test in R.

3.5.9.5.3 *Community Composition*

To compare community composition, samples were split into separate datasets for the three materials (water, surface sediment, and 10-cm depth sediment) and split into separate datasets for the field and laboratory studies. Statistical calculations were performed based on the different locations within the wetland (stormceptor, entry, exit and Lost Lagoon) for the field study and based on the dosing of columns and the date of sample extraction for the laboratory study. Plots and statistical calculations were completed in R using the R standard package and using the vegan package. OTU tables for the various datasets were imported from Mothur into RStudio. OTU data were log transformed and dissimilarity matrices were calculated using the vegdist function in vegan with standard inputs. Two-dimensional NMDS were prepared using the metaNMDS function in vegan while setting the dissimilarity index to Bray Curtis and the maximum number of tries equal to 100. Stressplots were prepared and NMDS were only accepted if the R^2 in the stressplot was greater than 0.90 and the stress calculation was less than 0.20. Comparison between field study sites and laboratory study dosing was performed using the Adonis function in vegan with 999 permutations, the Bray Curtis dissimilarity index and the Bonferroni p-value adjustment. Fitting of environmental data was performed using the envfit statistic in vegan and 999 permutations. Statistical calculations for hypotheses tests were considered significant if the p-value was less than 0.05.

3.5.9.5.4 *Indicator Species*

The same approach for splitting the dataset was applied for the indicator species comparisons as was applied for the alpha diversity comparisons and community composition comparisons. Indicator species were calculated using the indicator() function in Mothur. Indicator species were considered statistically significant if the R statistics was greater than 80 and the p-value was less than 0.05.

3.5.10 Analysis of Bacterial Functions Using Metagenomics

Analysis of functional genes was performed using standard computational techniques. Initially, file conversion and de-multiplexing was performed using Illumina CASAVA software. Merging, assembly and quality filtering was performed using MetaVelvet. Bioinformatics treatments and preparation of functional lists were performed using MetaPathways. Additional analyses were performed using RStudio and Microsoft Excel.

3.5.10.1 File Conversion and Sequence De-Multiplexing

All sequences passing the HiSeq Q30 filter were converted by the Beatty Biodiversity centre from bcl to FastQ format with barcodes extracted using standard input to the Illumina supported software, CASAVA 1.8.2.

3.5.10.2 Read Merging, Quality Filtering, and Contig Assembly

Read merging, quality filtering and assembly of reads into contigs was performed using MetaVelvet (Namiki et al., 2011). The Kmer length was set to 31 and the minimum contig length was set to 100 bp.

3.5.10.3 Preparation of Function Lists

The MetaPathways v2.5.3 pipeline was used to perform quality control, protein prediction, clustering and similarity based annotation on sequence datasets using several bioinformatics tools as described by the authors (Konwar et al., 2014). The MetaPathways pipeline features:

1. "Open reading frame (ORF) prediction using Prodigal with BLAST or LAST annotation against the MetaCyc, RefSeq, KEGG, and COG protein databases;
2. Taxonomic analysis using MEGAN, ML-TreeMap, 16S SSU and 23S LSU rRNA homology using the Silva and GreenGenes databases; and
3. Systematic creation of Environmental Pathway/Genome Databases (ePGDBs) mapping functional information onto the MetaCyc database of metabolic Pathways." (Konwar et al., 2014).

Minimum sequence length was set to 70 bp and minimum ORF length was set to 20 bp. All other quality control indices were left as standard parameter inputs.

3.5.10.4 Analyses on Data

Analysis were performed using the Vegan package in R and using basic graphing options in Microsoft Excel. Statistical analyses were not performed on this dataset because of the small sample size and because, at the time of publication, this is an area for future work.

3.5.10.5 Review of Results

Upon completion of this project, statistical results were independently reviewed by a consultant at the UBC Applied Statistics and Data Group (ASDA). As a reference, results of this review are included in Appendix K. Some minor modifications to the description of the methodology were made to clarify outcomes of this review; however, the majority of recommendations were outside of the scope of this study and left for future follow on research.

3.6 Results and Interpretation

3.6.1 Environmental Analysis

3.6.1.1 Confirmation of Beaver Lake Bog Soil Quality

In *Table 31*, a list is provided of the averages and standard deviations for the concentrations of all metals that were measured in order to confirm the quality of the soil at the Beaver Lake Bog. This was an essential first step because this soil was to be collected and packed into the sediment columns for the future study. The metals associated with stormwater runoff were of greatest interest. Barium, cadmium, cobalt, copper, manganese, molybdenum nickel, lead, and zinc were below detection limits or near/below the concentrations of metals measured at the exit to the Lost Lagoon wetland. The only stormwater metal of concern that measured above the levels in wetland exit was antimony. The reasons for this are unclear because other metals did not observe the same trend. The observation could be due to naturally occurring higher antimony levels in the bog soil or possibly some interference on the analytical instrument, where the antimony levels are quite close to the detection limit of 10 mg/kg dry weight.

Table 31. Confirmation of Beaver Lake Bog Soil Quality

		As	Ag	Al	B	Ba	Be	Cd	Co	Cr	Cu	Fe	K	Li
		mg/kg dry weight												
		Average												
Bog 1	Surface			1051.5		23.2				20.5	31.2	1388.6	39.5	27.0
Bog 2	Surface			1821.8	49.5					43.3	35.2	3345.9	48.2	25.6
Bog 3	Surface			706.5	38.0	23.2				4.5	24.1	1028.4	52.3	25.1
Bog 4	Surface			921.0	44.7	28.5				46.7	20.5	1740.0	30.5	25.0
Bog 5	Surface			1284.3		57.0				15.4	32.4	2166.8	40.0	25.1
Bog 6	Surface			1360.3	44.3					69.5	17.7	2511.2	40.2	25.0
Site 5	Surface			9342.3	29.3	14.5				16.1	61.3	3144.4	101.5	36.3
		Standard Deviation												
Bog 1	Surface			173.3		4.5				14.3	0.6	57.6	2.5	2.4
Bog 2	Surface			217.6						19.1	3.3	585.0	5.6	0.4
Bog 3	Surface			17.3	10.1	0.8					1.5	117.3	4.5	0.1
Bog 4	Surface			159.6	2.0	6.2					3.1	66.8	5.2	0.1
Bog 5	Surface			113.5		3.5				12.8	0.6	201.4	0.0	0.2
Bog 6	Surface			249.8	4.6						0.9	522.6	4.2	0.2
Site 5	Surface			3961.9	7.5	13.4				8.8	19.4	3338.8	71.2	4.7

		Mg	Mn	Mo	Na	Ni	Pb	Sb	Se	Si	Sr	Ti	V	Zn
		mg/kg dry weight												
		Average												
Bog 1	Surface	726.9	24.4		626.5	5.8		12.9	81.4	1497.5		31.0		127.2
Bog 2	Surface	780.2			665.8	6.6		13.3	62.0	1379.7		67.3		122.6
Bog 3	Surface	302.1	20.6		528.3	4.1		14.2	78.4	1446.2		27.8		88.8
Bog 4	Surface	495.6	79.6		426.3	6.0		13.0	64.9	1601.5		33.4		94.9
Bog 5	Surface	827.8			761.5	4.3		15.3	46.5	1673.3		51.4		160.3
Bog 6	Surface	648.9	90.9		518.0	5.7		12.7	67.9	887.2		68.8		98.9
Site 5	Surface	858.5	103.3	7.0	1560.2	19.3	7.1	10.0		5286.4		184.6		253.6
		Standard Deviation												
Bog 1	Surface	199.3	9.9		222.0	2.7		0.5	22.6	743.9				10.7
Bog 2	Surface	23.4			45.0	1.3		1.4	11.6	881.5		1.7		34.3
Bog 3	Surface	14.6	4.3		70.0	2.2		2.0	20.5	612.9		1.8		16.1
Bog 4	Surface	100.9			26.0	2.8		1.5	22.4	403.0				16.1
Bog 5	Surface	19.7			149.9	0.3			12.5	800.1				
Bog 6	Surface	53.1	6.1		12.7			1.3	19.4	667.3		17.6		39.4
Site 5	Surface	1162.7	53.0		904.9	5.3	3.0			2980.2		19.2		127.2

3.6.1.2 Preliminary Study

Prior to beginning the laboratory column study, a preliminary test was performed over one week. The results are recorded in *Table 32*. Measurements were collected and recorded for DO, pH, temperature, conductivity, and ORP using a YSI probe, as described previously. The measurements were taken at the surface of the column and at the soil-water interface (below a water depth of 30-cm). Measurements were compared to see if the conditions in the column would equilibrate to similar conditions as measured at the soil water interface in the Lost Lagoon wetland forebay. DO and pH generally equilibrated to the same range as measured in the forebay. Temperature measurements were not relevant because the preliminary test was operated at room temperature (approximately 22 ˚C) and the temperature in the forebay decreased over the autumn season. Conductivity measurements in the column were lower than the average measurement in the forebay; however, the column measurements were trending towards the forebay measurements. ORP measurements in the column also trended towards the levels measured in the forebay.

Overall, the results of the preliminary study were considered adequate enough to continue moving forward with the column study.

Table 32. Measurements Recorded During Preliminary Column Test

Time	DO, mg/L	pH	Temperature, ˚C	Conductivity, µS/cm	ORP, mV
Water Surface					
Zero	8.37	6.5	16.67	53.75	322.7
36 hrs	4.69	5.71	19.21	54.97	390.2
96 hrs	5.45	5.31	20.31	57.06	378.9
192 hrs	5.35	5.48	20.45	49.95	372.6
Soil-Water Interface					
Zero	7.64	7.14	16.75	64.12	309.1
36 hrs	5.19	6.15	19.2	58.13	384.7
96 hrs	5.8	5.49	20.14	66.24	373.5
192 hrs	3.99	5.44	20.36	94.33	376.6
Wetland Forebay Soil-Water Interface					
Average	3.53	5.15	11.65	153.73	285.16
St.Dev.	1.99	1.33	3.09	55.45	126.00

3.6.1.3 Turbidity, TSS, COD and TOC

3.6.1.3.1 *Interpretation*

Turbidity, TSS, COD, and TOC were measured in the water samples of each lab study column at the time the columns were sacrificed. In Figure 50 through Figure 53, there was no observable trend in the measurements for any of these parameters with the exception that Column 1, which was sacrificed and analyzed on the first day of the column study, measured higher levels for all parameters. These higher measurements may have occurred because some organic material from the soil layer was stirred into the water layer during the initial addition of 30-L of water to the column. Unfortunately, the dataset was too small to make statistical comparisons between the columns.

In Figure 54, turbidity, TSS, TOC, and COD are visually compared between the first and last week of column samples and between the Lost Lagoon wetland entry and exit. With the exception of Column 1 having higher measurements, the field and lab study measurements for these parameters are generally within the same range. These parameters may have an influence on the microbial populations present in the water samples. Thus, in order to use the laboratory results to verify the field results, it is essential that the same range of measurements exists between the two studies.

3.6.1.3.2 *Figures*

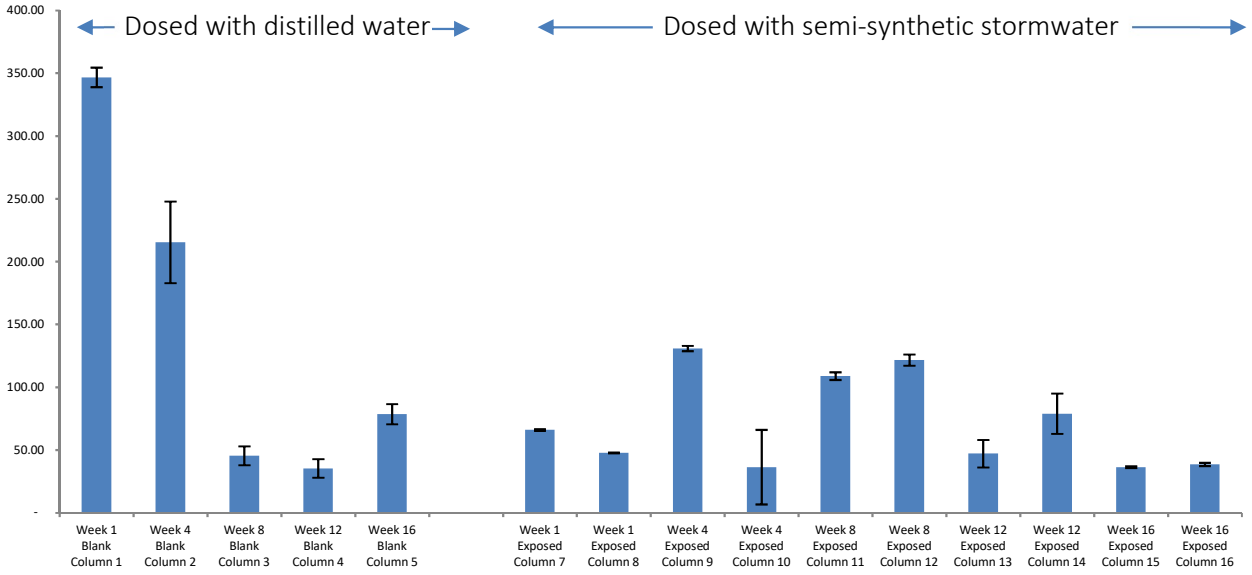

Figure 50. Barplot Comparison by Column of Turbidity in Water Samples

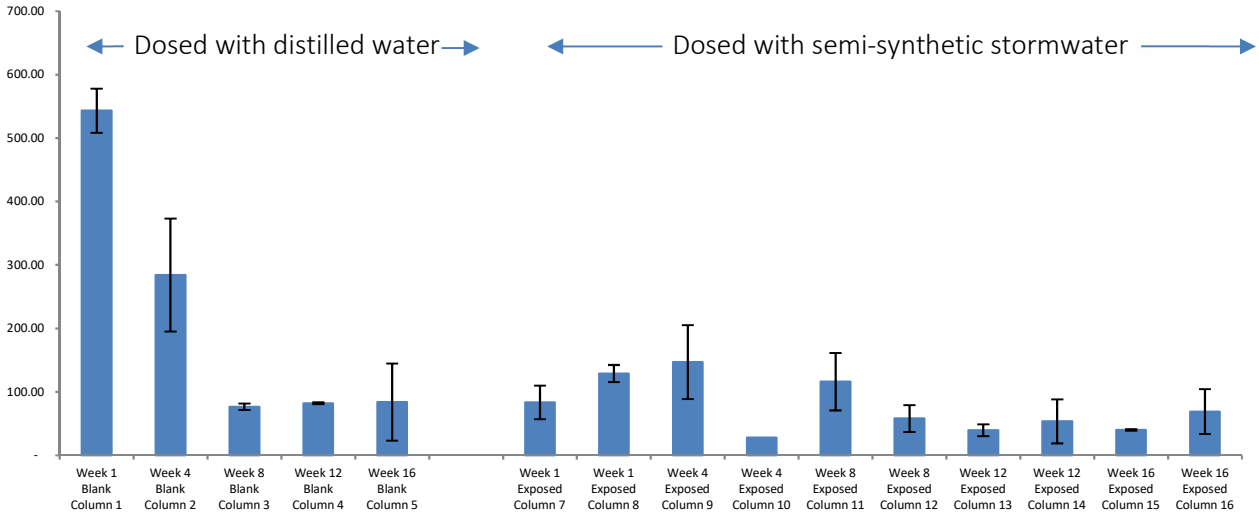

Figure 51. Barplot Comparison by Column of Total Suspended Solids in Water Samples

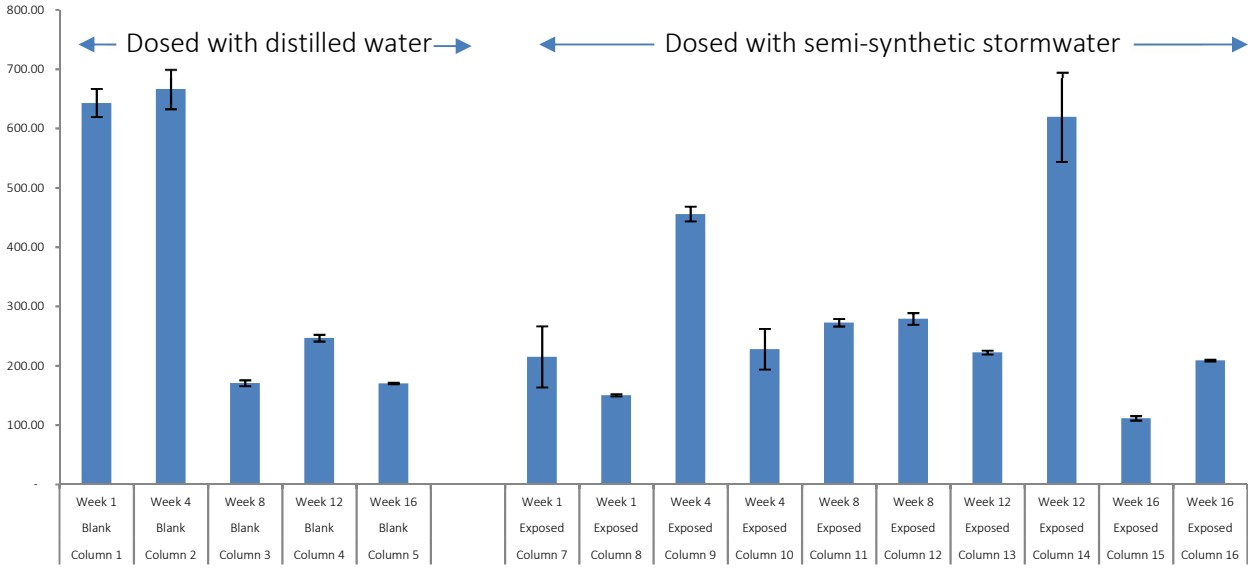

Figure 52. Comparison by Column of Chemical Oxygen Demand in Water Samples

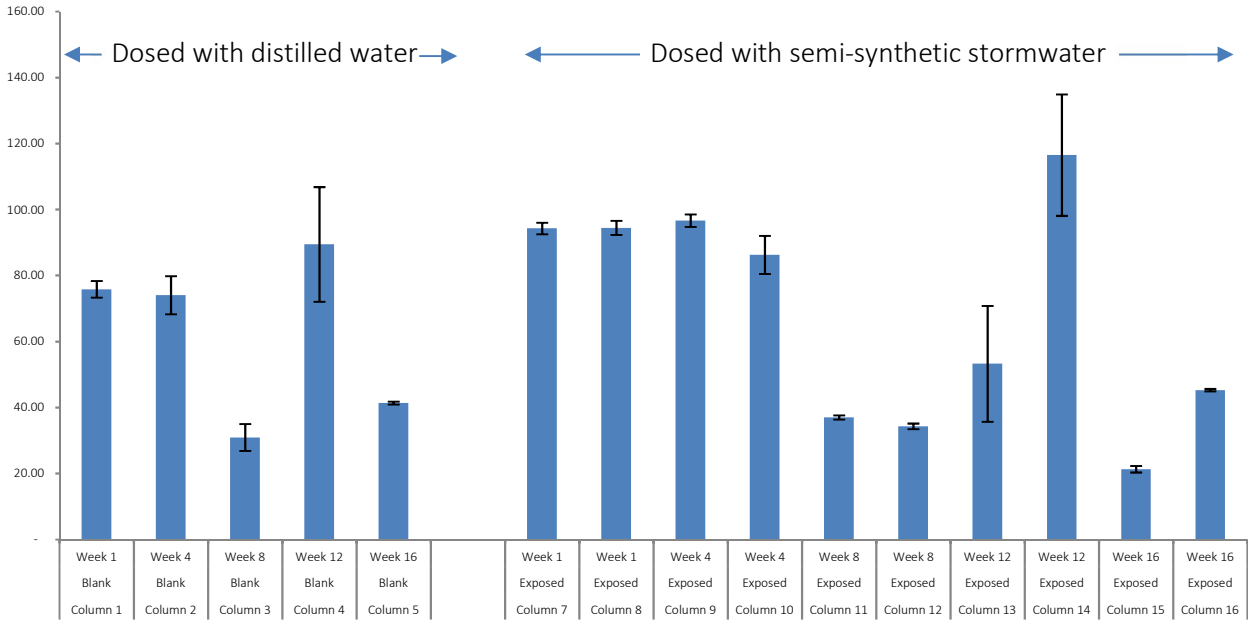

Figure 53. Comparison by Column of Total Organic Carbon in Water Samples

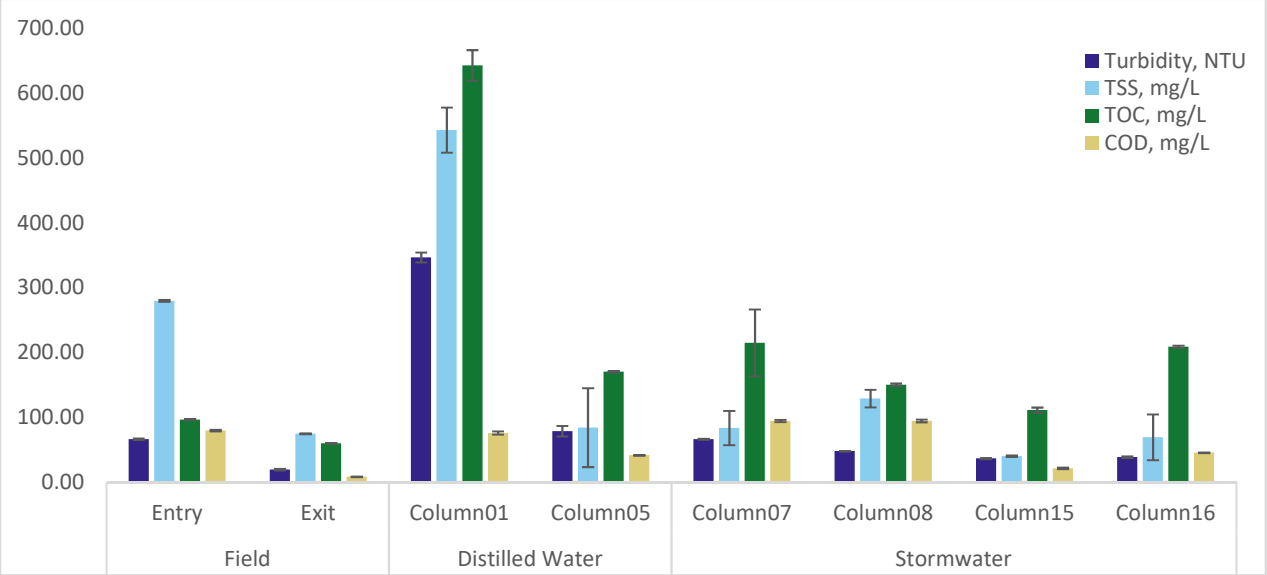

Figure 54. Comparison of Turbidity, TSS, TOC, and COD in Field and Lab Studies

3.6.1.4 Metals

3.6.1.4.1 *Interpretation*

Together, Figure 55 and Figure 56 illustrate the distribution of metals within the water samples obtained from the columns. From Figure 55 and Figure 56, visually, there is a trend over time of slightly decreasing metal concentrations in the columns that were fed distilled water and of increasing metals concentrations in the columns that were fed stormwater. This trend is most clear for molybdenum, nickel, barium, copper, manganese, and zinc. Cadmium, cobalt, and antimony measurements were near detection limits and this may account for less clarity in the results. The slight decrease in metal concentrations in the columns that were fed distilled water may be due to some partial flushing of the soil as water in the columns was exchanged with distilled water on a regularly occurring basis, following the rain patterns in Vancouver.

In Figure 57 and Figure 58, the metal concentrations, measured during week one and week sixteen of the laboratory study and at the entry and the exit of the Lost Lagoon wetland, are compared. Unfortunately, statistical tests could not be performed between the two studies because the dataset for the column study was too small in comparison to the field study. However, the metal concentrations in the water samples collected from the stormwater columns at week sixteen generally did reach the concentrations measured at the entry of the Lost Lagoon wetland and of the stormwater that was fed into them. Due to the stormwater recipe that was prepared, some metal concentrations differed, including that molybdenum and nickel concentrations were higher in the laboratory stormwater columns than in the wetland forebay

and barium and that antimony concentrations were lower in the laboratory stormwater columns than in the wetland forebay.

In Figure 59 through Figure 62, generally the same trends were observed for the surface sediment samples as were observed for the water samples. Molybdenum and nickel concentrations were also higher in the laboratory stormwater column surface sediment samples than in the wetland forebay and barium and antimony concentrations were lower in the laboratory stormwater column surface sediment samples than in the wetland forebay. Figure 63 through Figure 66 illustrate that the same trend over time was observed for the 10-cm depth samples as was observed for the water and surface sediment samples. However, metal concentrations in the depth samples taken in the column study were higher than were observed in the field study. This was generally true for all metals present in the semi-synthetic stormwater. This may have an influence on the microbial communities present in these samples.

That being said, overall, the column study achieved its goal regarding the metal concentrations, which was to mimic the concentrations in the Lost Lagoon wetland, in order to provide a dataset for microbial comparisons later on.

3.6.1.4.2 *Figures*

3.6.1.4.2.1. Water Samples

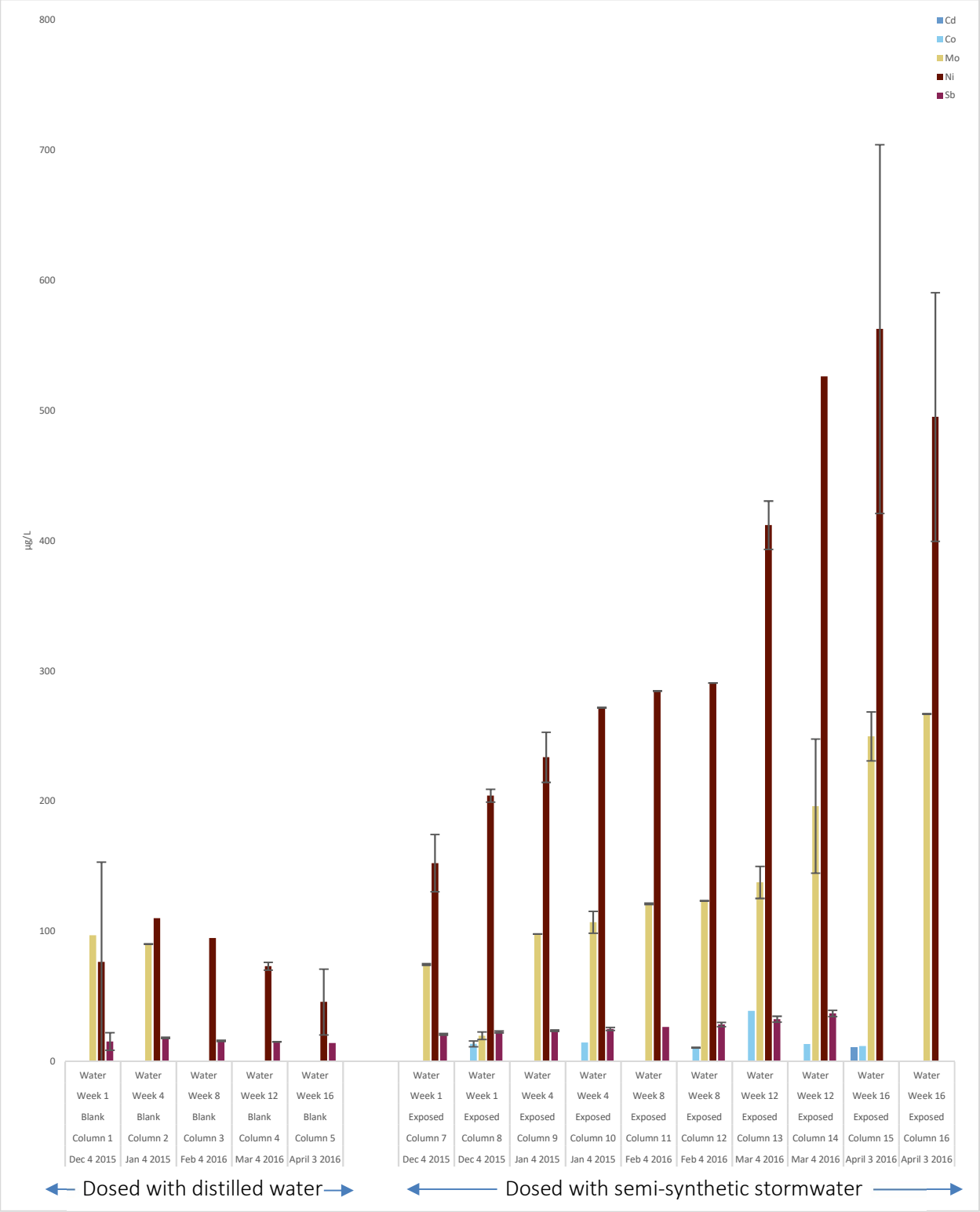

Figure 55. Time Comparison of Metals Associated with Stormwater in Column Water Samples

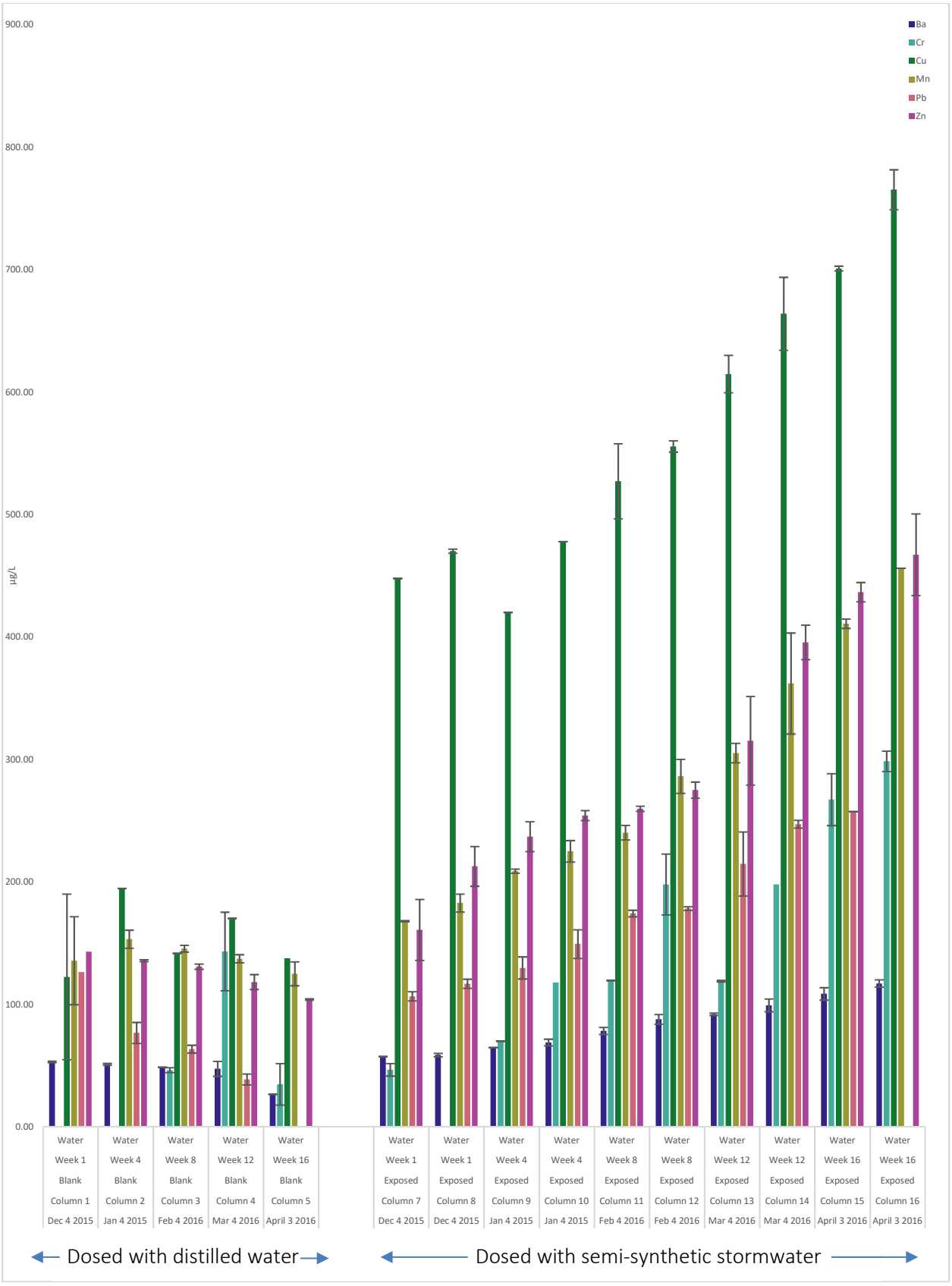

Figure 56. Time Comparison of Metals Associated with Stormwater in Column Water Samples

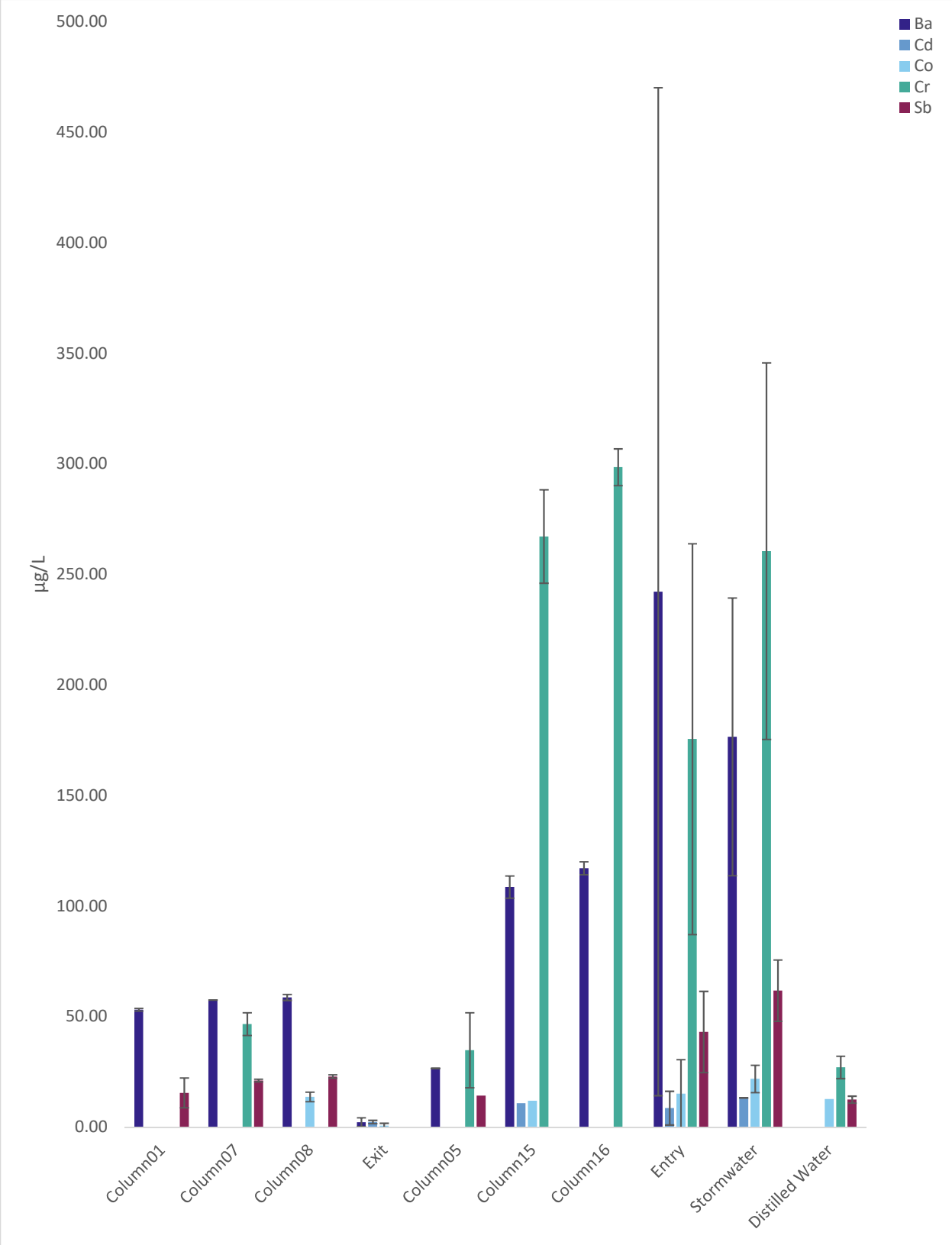

Figure 57. Comparison of Metals Associated with Stormwater in Field Study and Lab Study Water Samples

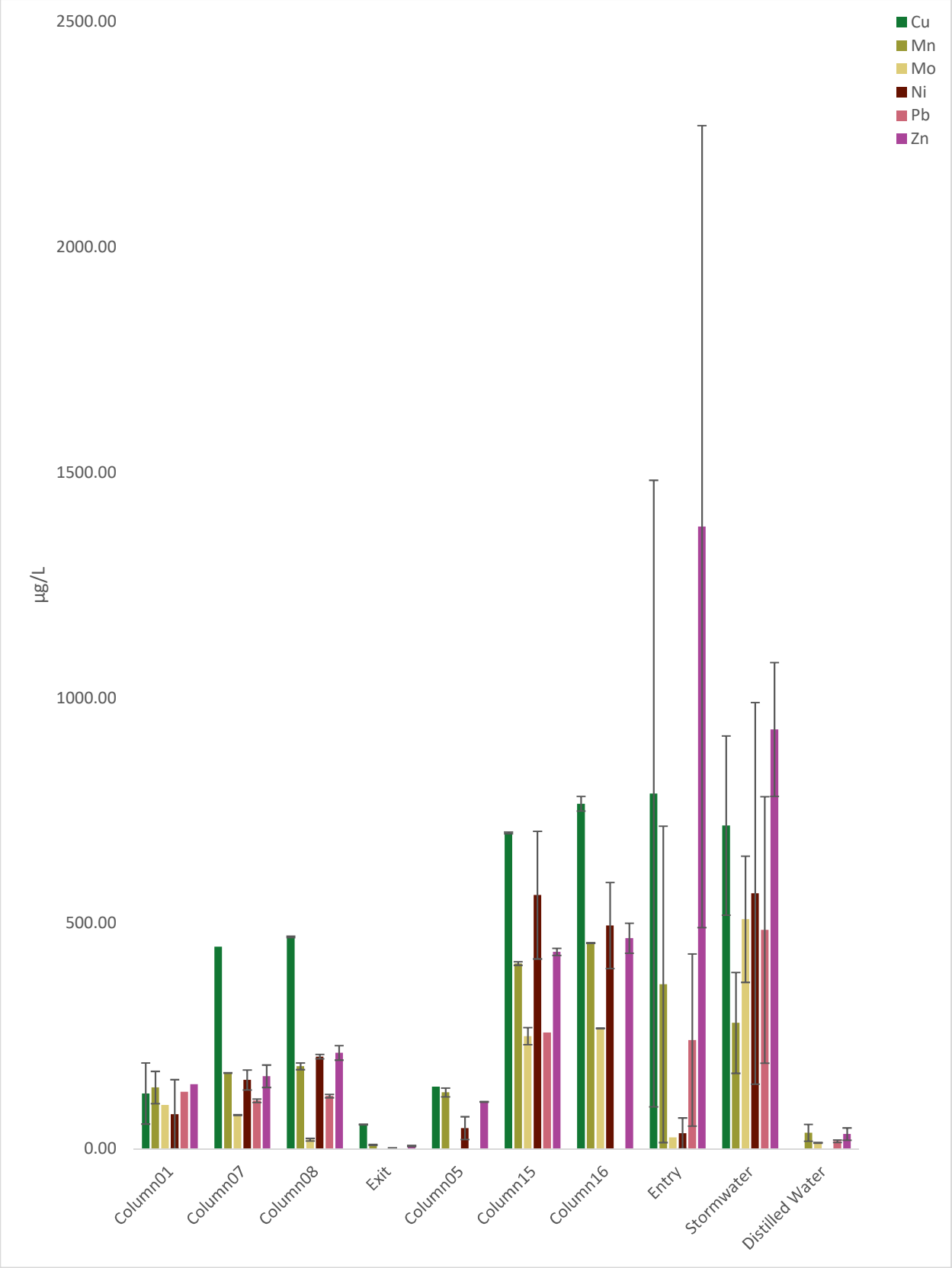

Figure 58. Comparison of Metals Associated with Stormwater in Field Study and Lab Study Water Samples

3.6.1.4.2.2. Surface Sediment Samples

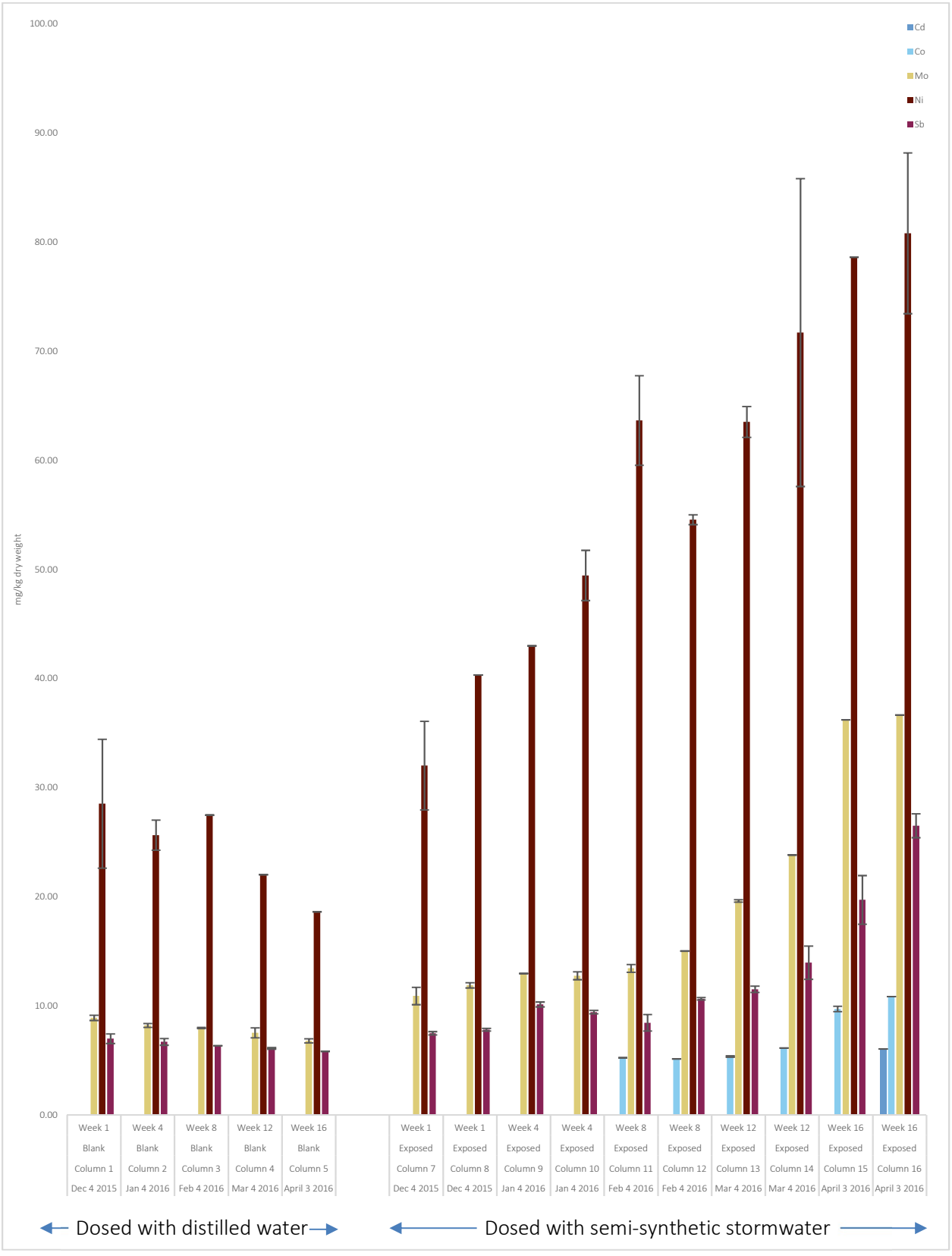

Figure 59. Barplot Comparison by Plot of Metals Associated with Stormwater in Surface Sediment

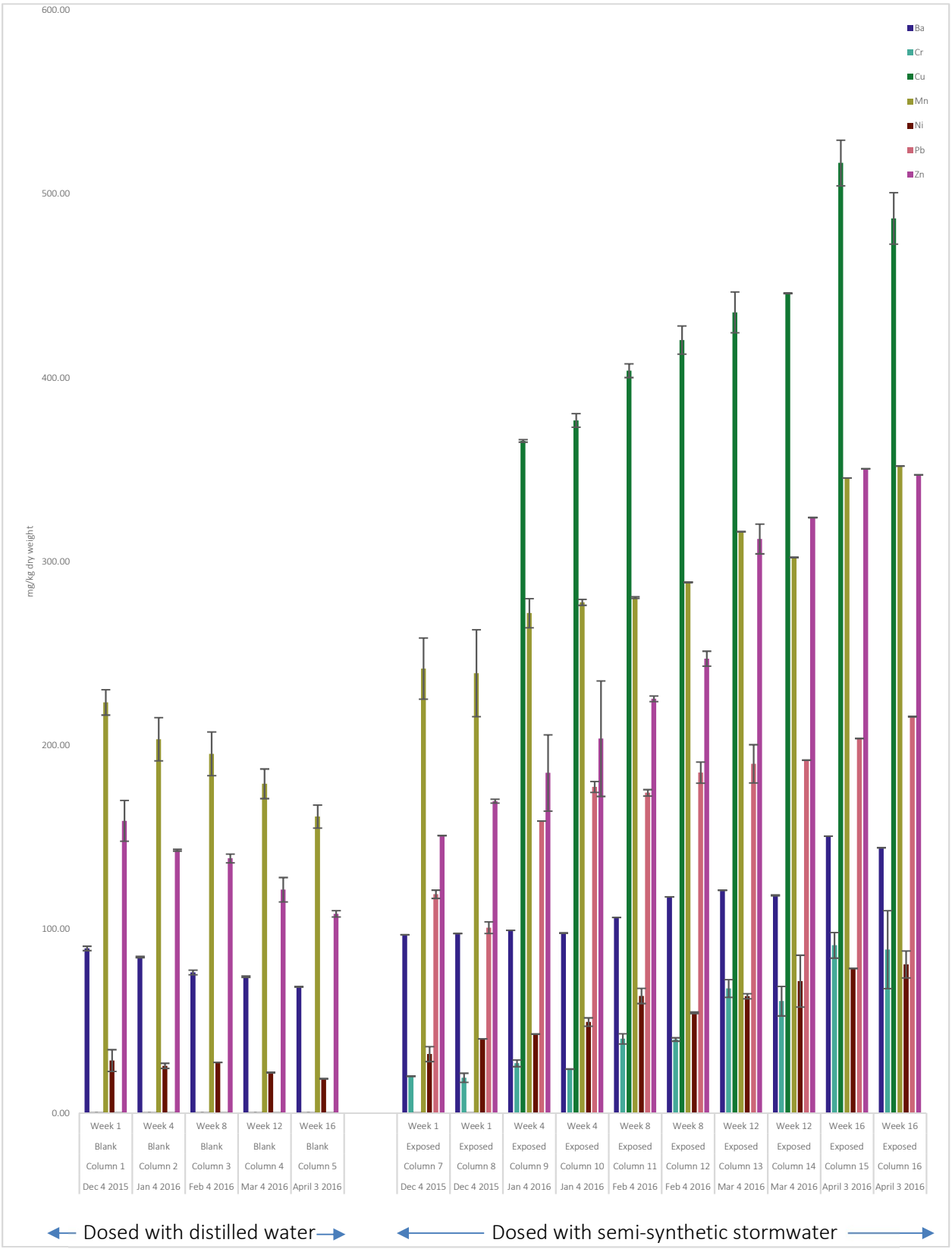

Figure 60. Barplot Comparison by Plot of Metals Associated with Stormwater in Surface Sediment

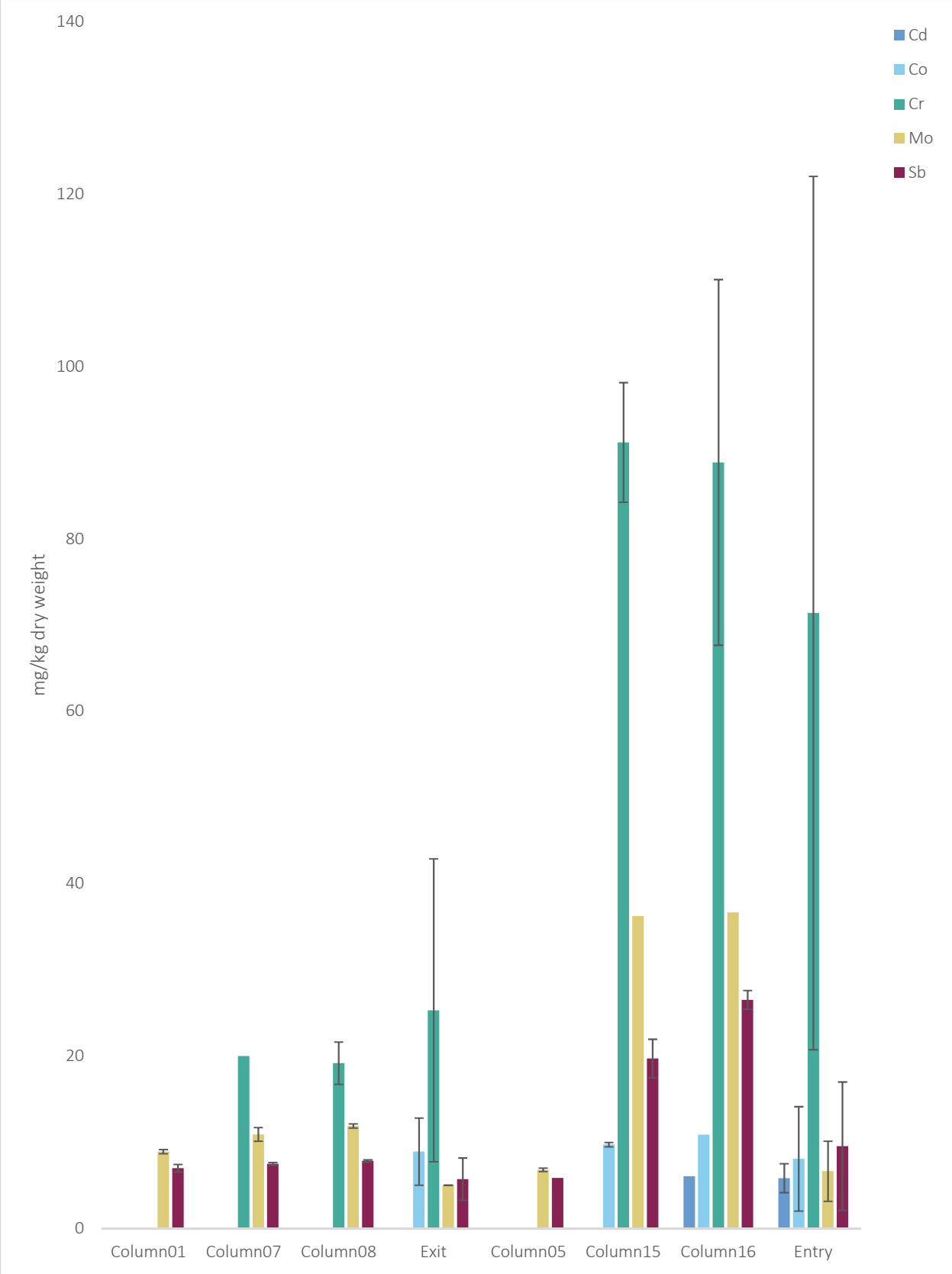

Figure 61. Comparison of Metals Associated with Stormwater in Field Study and Lab Study Surface Sediment

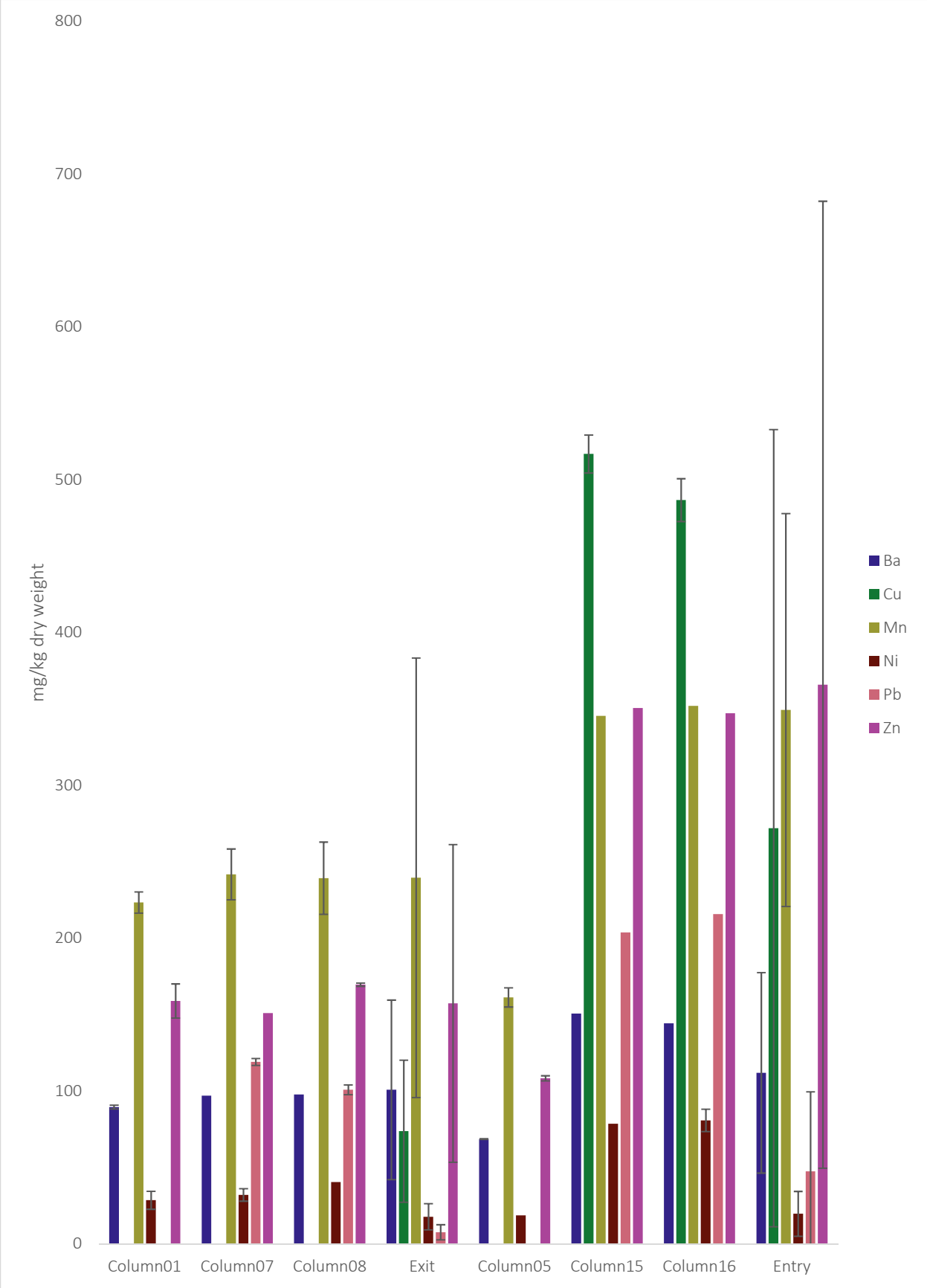

Figure 62. Comparison of Metals Associated with Stormwater in Field Study and Lab Study Surface Sediment

3.6.1.4.2.3. 10-cm Depth Sediment Samples

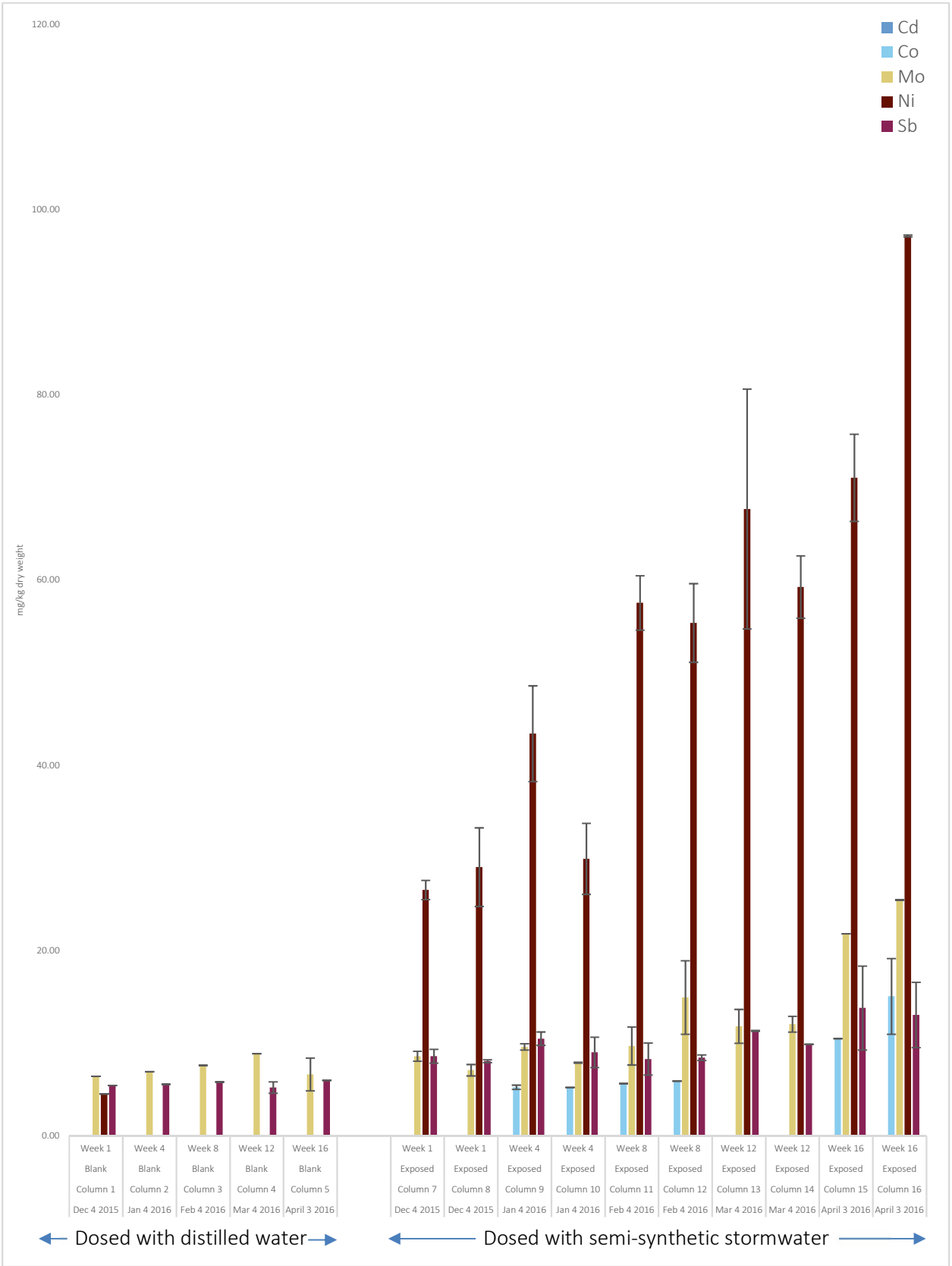

Figure 63. Barplot Time Comparison of Metals Associated with Stormwater in 10-cm Depth Sediment

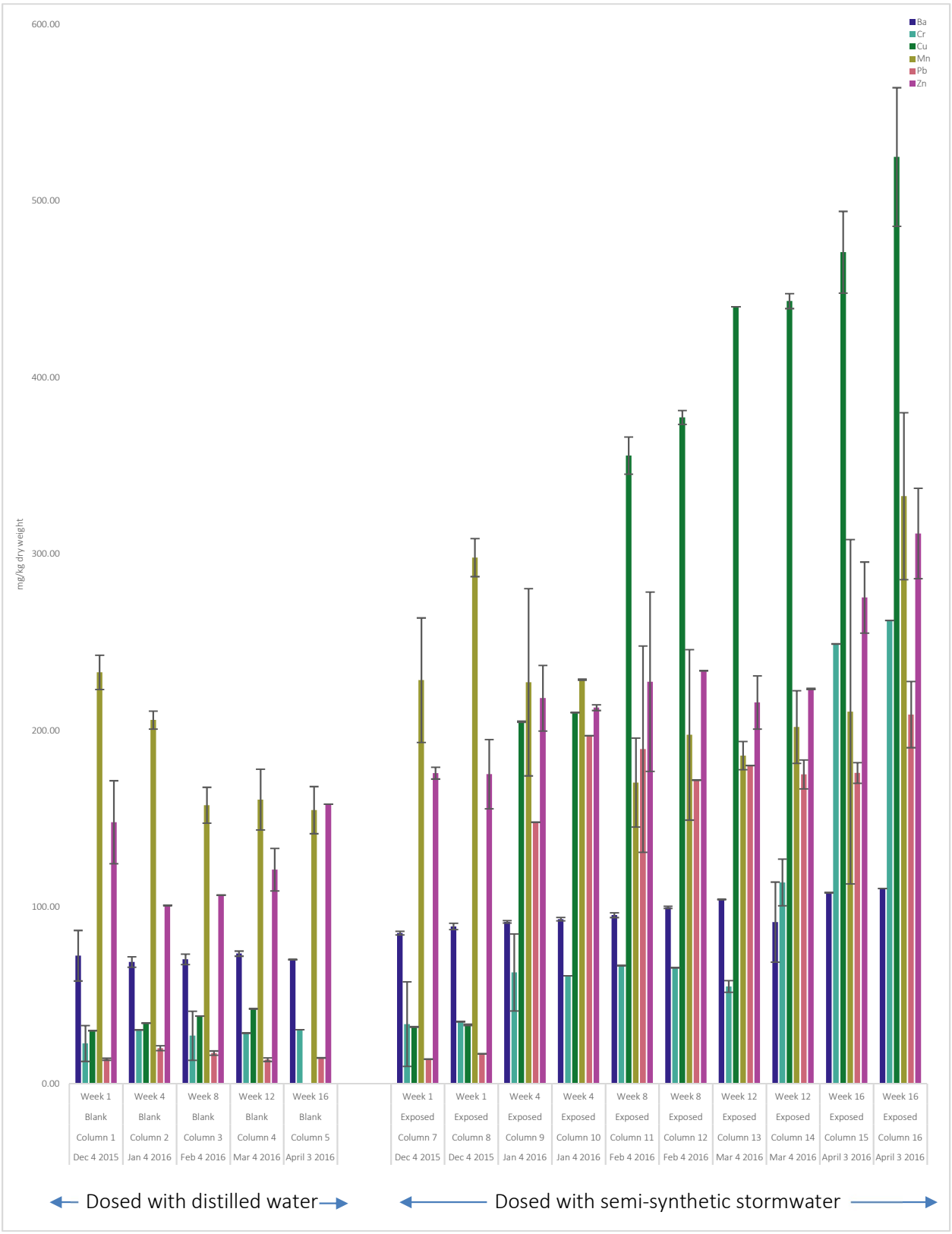

Figure 64. Barplot Time Comparison of Metals Associated with Stormwater in 10-cm Depth Sediment

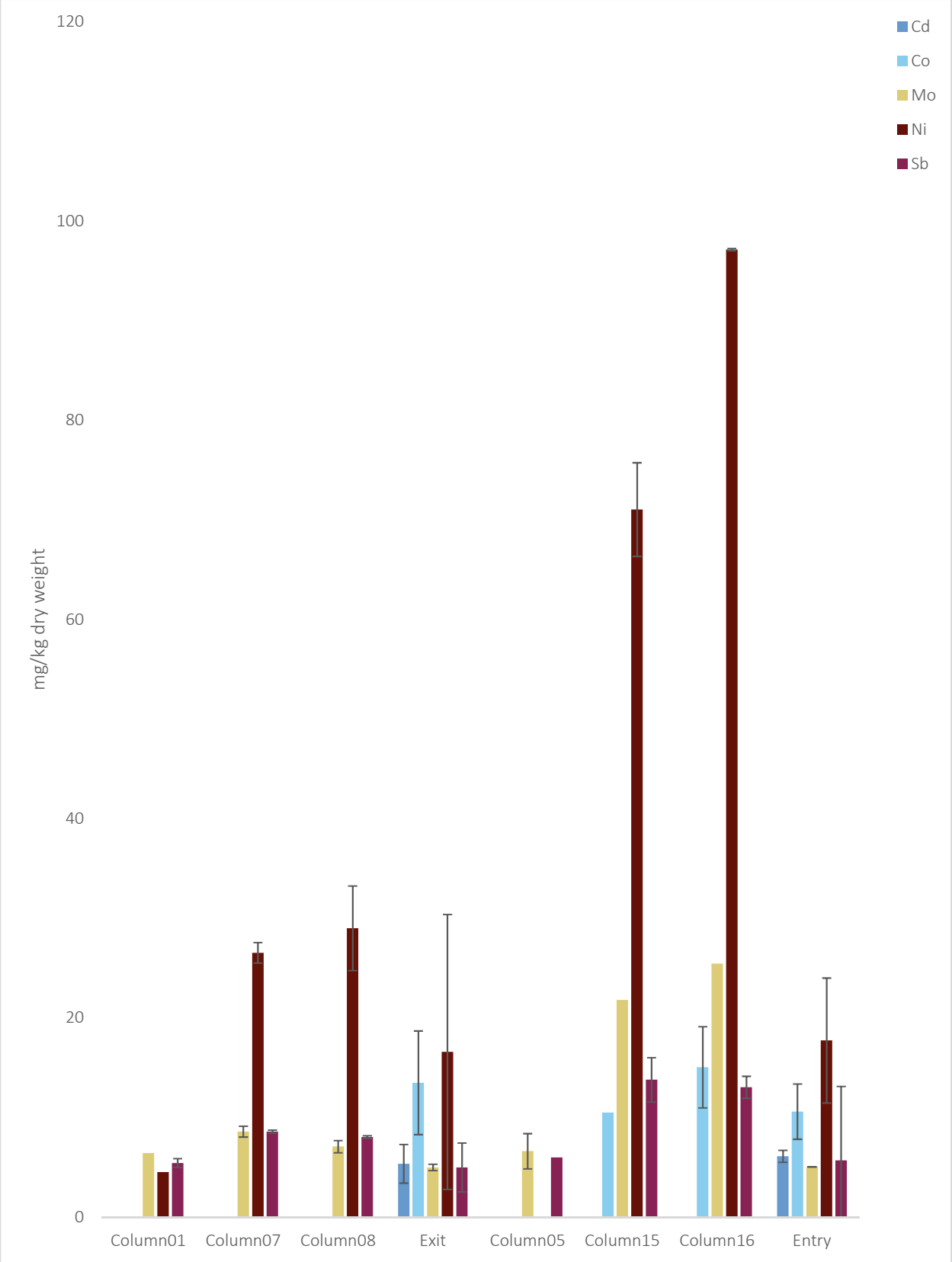

Figure 65. Comparison of Metals Associated with Stormwater in Field Study and Lab Study 10-cm Depth Sediment

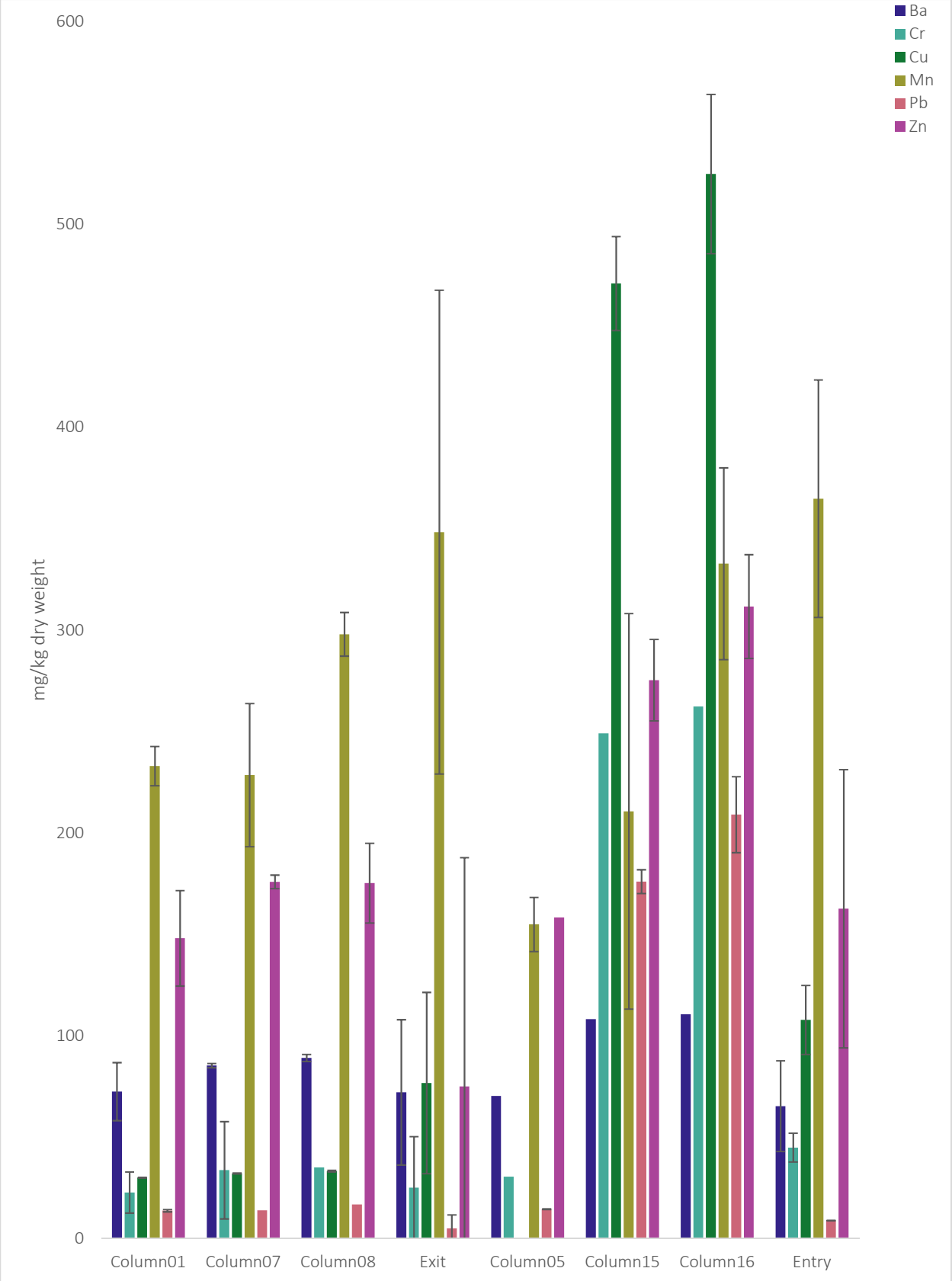

Figure 66. Comparison of Metals Associated with Stormwater in Field Study and Lab Study 10-cm Depth Sediment

3.6.2 Microbial Community Analysis

3.6.2.1 Data Quality and Screening

3.6.2.1.1 *Interpretation*

Using the count.seq command in Mothur (Schloss et al., 2009), the average sequence count for the field samples (excluding blanks) was initially calculated to be 21,888. To ensure only high quality outputs were analyzed, the minimum cutoff was set to 9000 clones and by this means two DNA extracts were eliminated from the dataset. After setting the cutoff, the new average clone count for the field samples was calculated to be 22,084 clones. Because there was a range of counts obtained using the MiSeq platform, diversity analyses were performed by randomly subsampling 9000 clones from each sample present in the dataset.

Figure 67 is a rarefaction curve for the field sample sequences, which was calculated using the rarefaction.single command and SOBS parameter in Mothur. This graph illustrates the sequence depth and cutoff for the field samples. From the figure, it is clear that there were more OTUs identified in the sediment samples (marked with black and brown lines) than in the water samples (marked with blue lines). This is expected because there is generally a higher level of microbial diversity in soil samples than in water samples when sampling in the natural environment, as was performed in this field study. Diversity analyses were performed by randomly subsampling 5000 clones from each sample present in the dataset.

Using the ANOSIM function in R (R Core Team, 2016), an assessment of outliers among pseudo-replicate field samples was performed. The boxplot output is illustrated in Figure 68. Three pseudo-replicate field samples were identified as likely to be including outliers and all three pseudo-replicates corresponded to water samples. Figure 69 is an NMDS plot, which illustrates the pseudo-replicates that contain outliers. Three outliers were identified and removed from the field study dataset and the ANOSIM calculation was performed a second time, as illustrated in Figure 70. The removal of outliers increased the R fit statistic for the water sample dataset from 0.912 to 0.974.

The same screening procedures were performed on the dataset for the sequences obtained from samples taken during the laboratory column study (Figure 71). Sequence diversity was lower in this dataset, as was expected due to the controlled conditions in the laboratory. Before screening, the average number of clones (excluding blanks) was calculated to be 7138 clones. The minimum and maximum cutoffs were set to 5000 clones and 9000 clones, respectively. A maximum cutoff was set because three DNA extracts produced an unreasonably high level of clones (more than 500% above the average). This may be the result of a laboratory handling error because the samples were consecutively located on the sequencing plate for the MiSeq platform. After setting the cutoffs, the new average number of clones was calculated to be 6353.

Using the ANOSIM function (Figure 72), four outliers were identified among pseudo-replicates in the laboratory column study dataset. These outliers are illustrated using the NMDS plot displayed in Figure 73. After removal of outliers, the R fit statistic increased from 0.985 to 0.986 for the water samples dataset, from 0.906 to 0.908 for the surface sediment sample dataset, and from 0.904 to 0.951 for the depth sediment sample dataset. The recalculated ANOSIM output is illustrated in Figure 74.

3.6.2.1.2 *Field Study*

3.6.2.1.2.1. Sequence Depth Cutoff

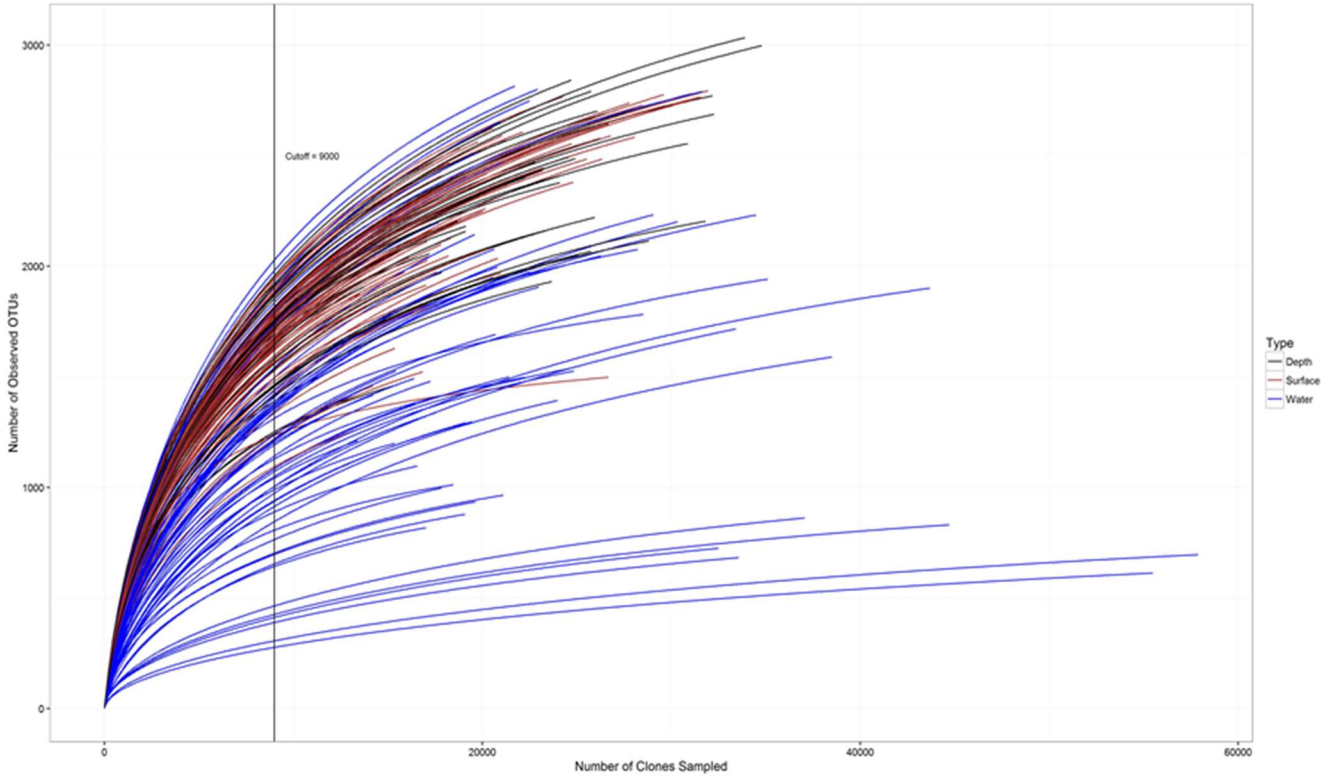

Figure 67. Rarefaction Curve Illustrating Minimum Depth Cut-off for Field Samples

3.6.2.1.2.2. Comparison of Pseudo-Replicates and Outlier Screening

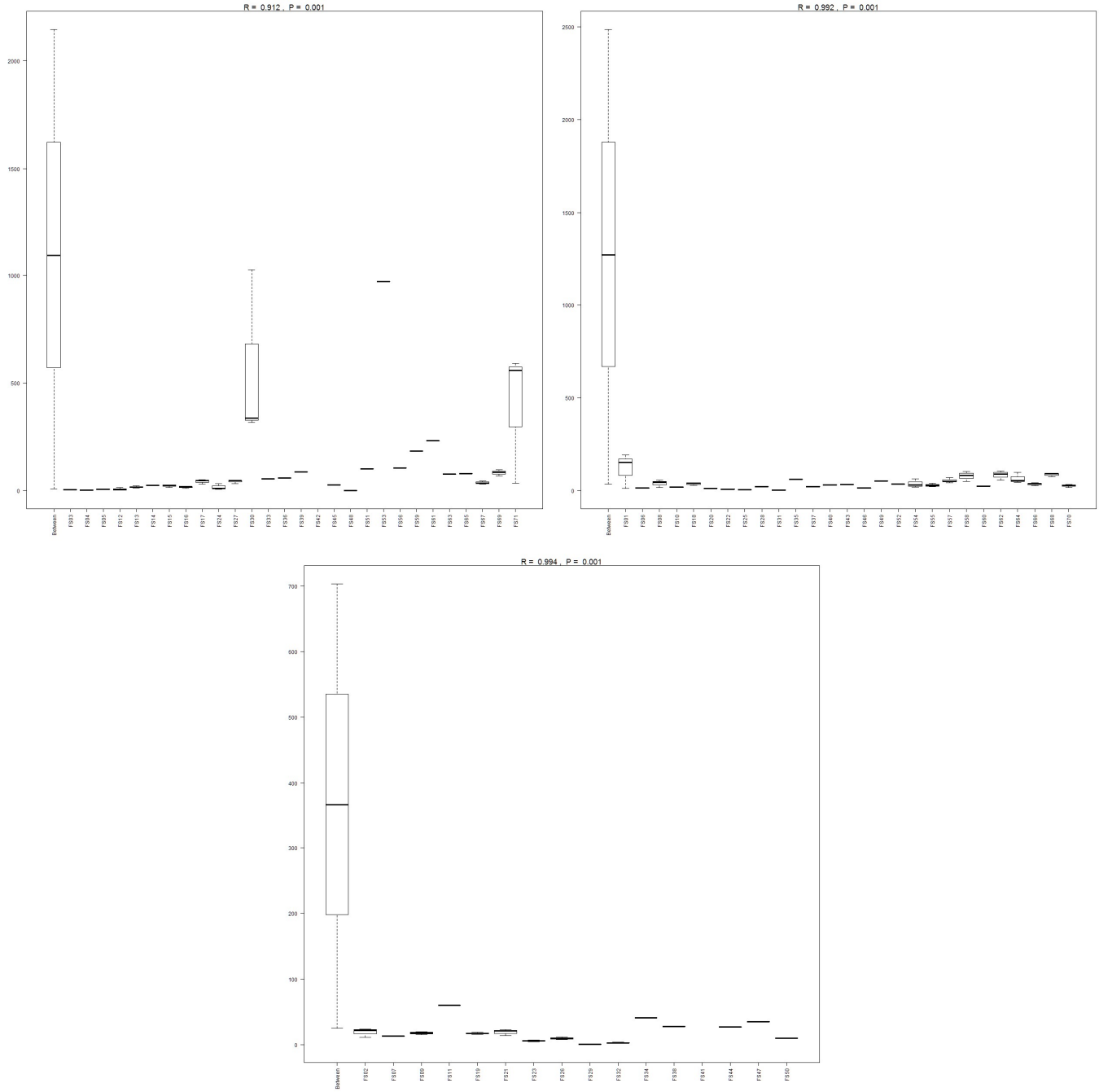

Figure 68. Anosim Boxplot Between Pseudo-Replicate Samples Prior to Outlier Screening in the Field Study (Left to right: Water, Surface Sediment, and 10-cm Depth Sediment Samples)

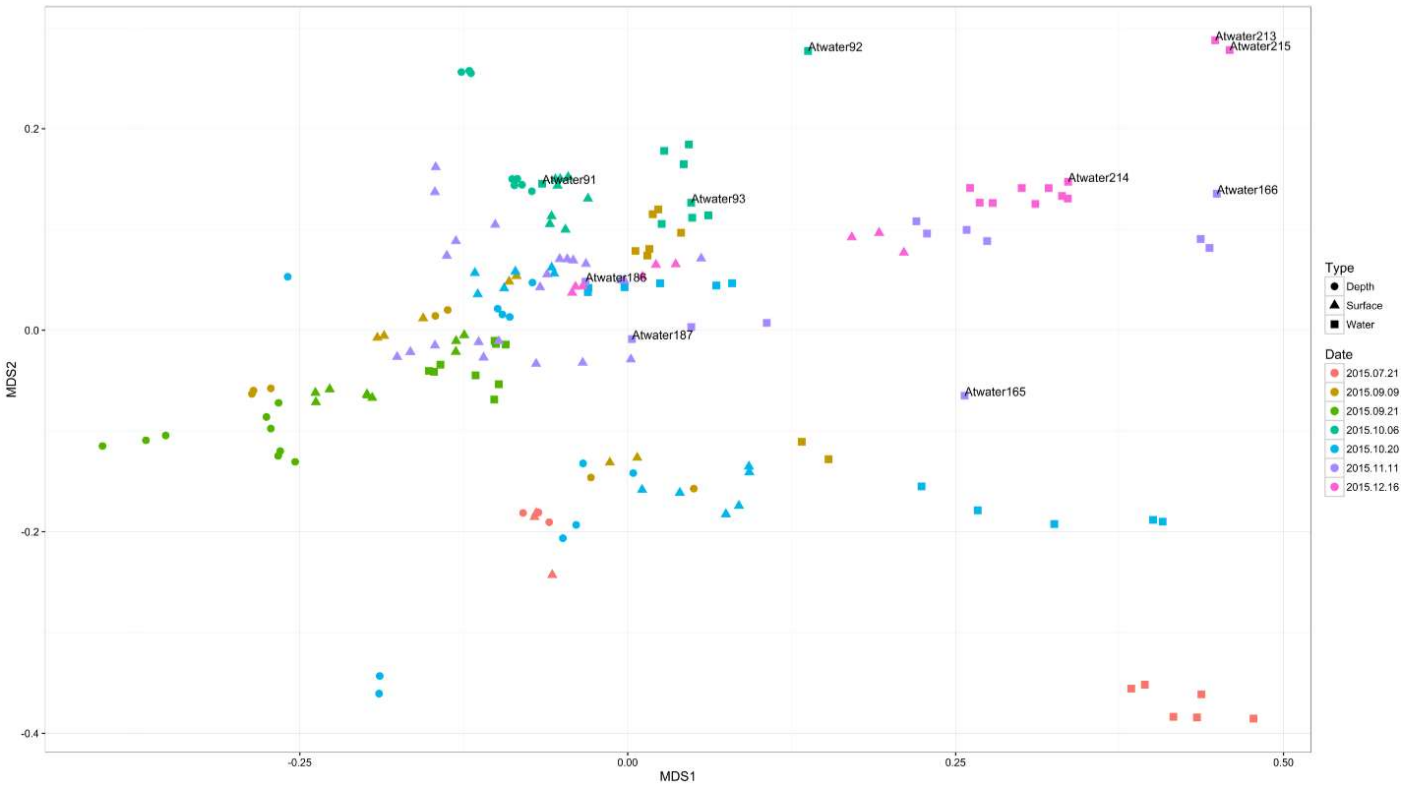

Figure 69. NMDS Plot Illustrating Suspected Outliers Among Field Samples

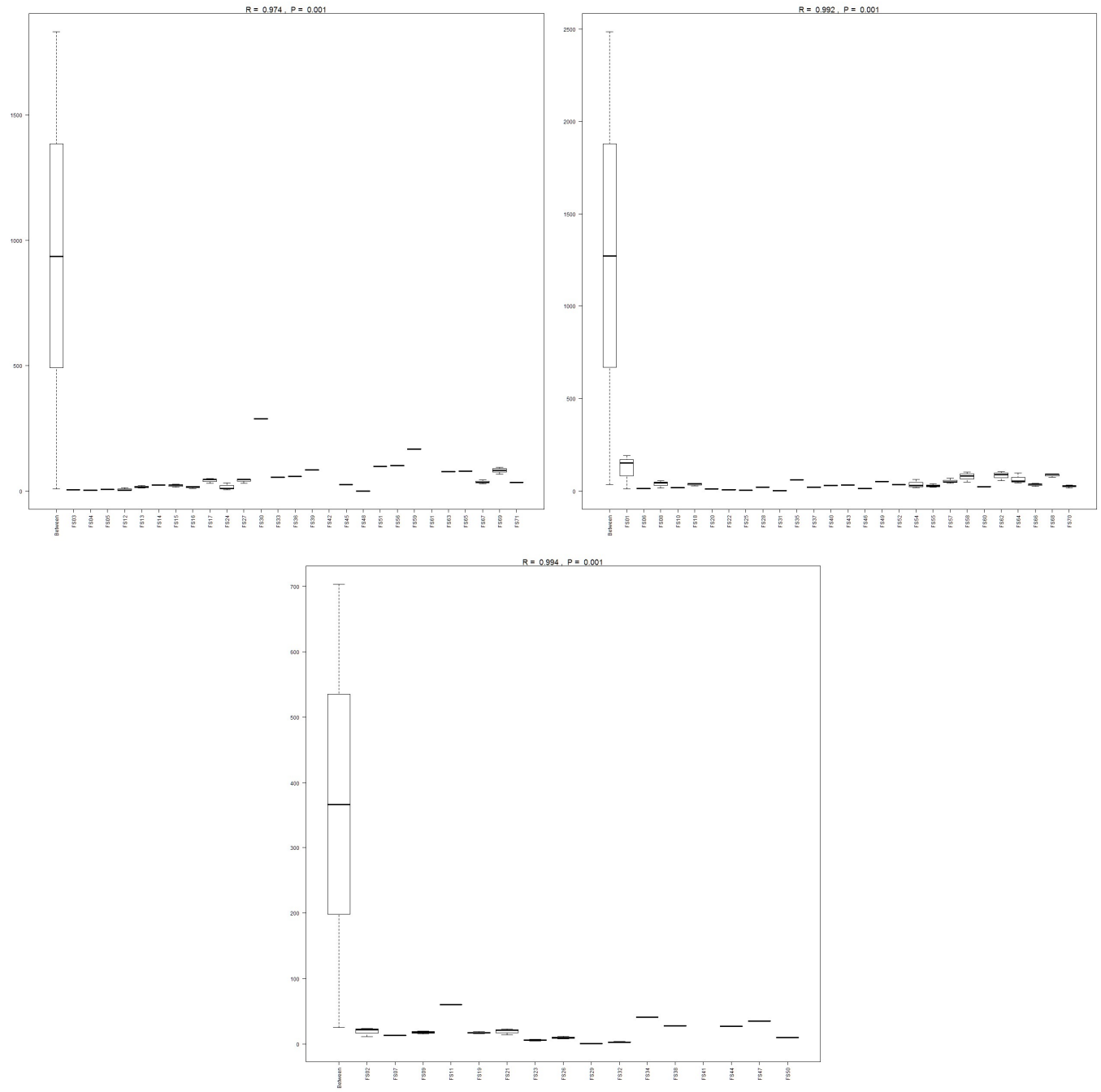

Figure 70. Anosim Boxplot Between Pseudo-Replicate Samples After Outlier Screening in the Field Study (Left to right: Water, Surface Sediment, and 10-cm Depth Sediment Samples)

3.6.2.1.3 *Laboratory Study*

3.6.2.1.3.1. Sequence Depth Cutoff

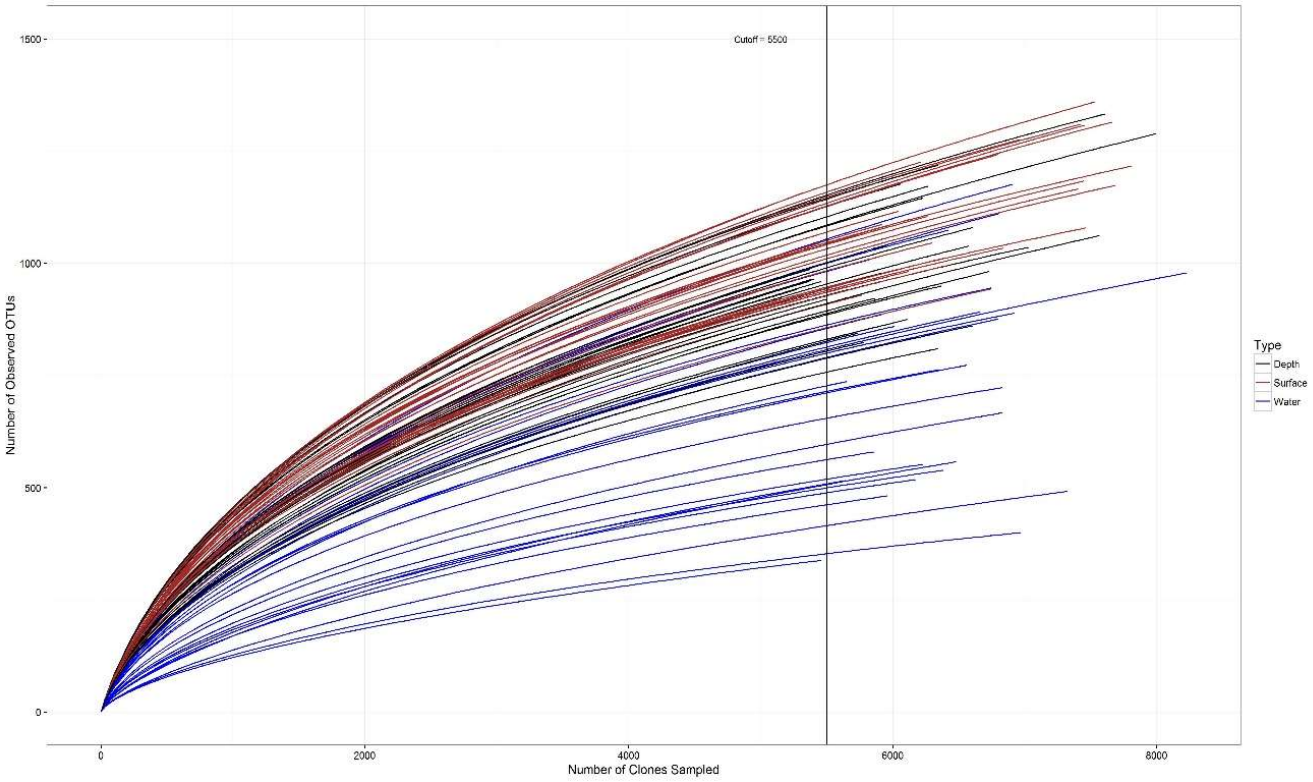

Figure 71. Rarefaction Curve Illustrating Minimum Depth Cutoff for Column Samples

3.6.2.1.3.2. Comparison of Pseudo-Replicates and Outlier Screening

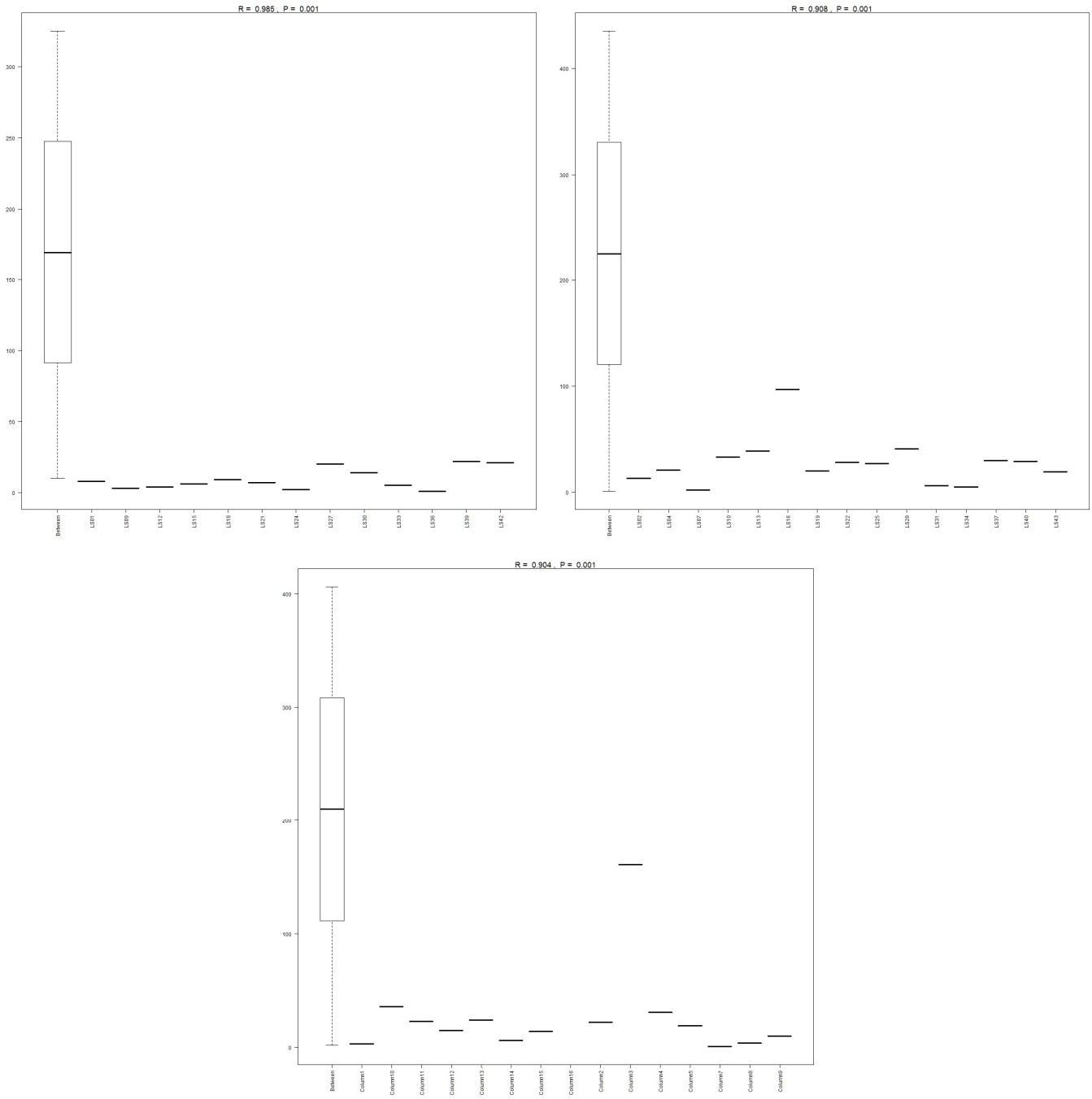

Figure 72. Anosim Boxplot Between Pseudo-Replicate Samples Prior to Outlier Screening in the Column Study (Left to right: Water, Surface Sediment, and 10-cm Depth Sediment Samples)

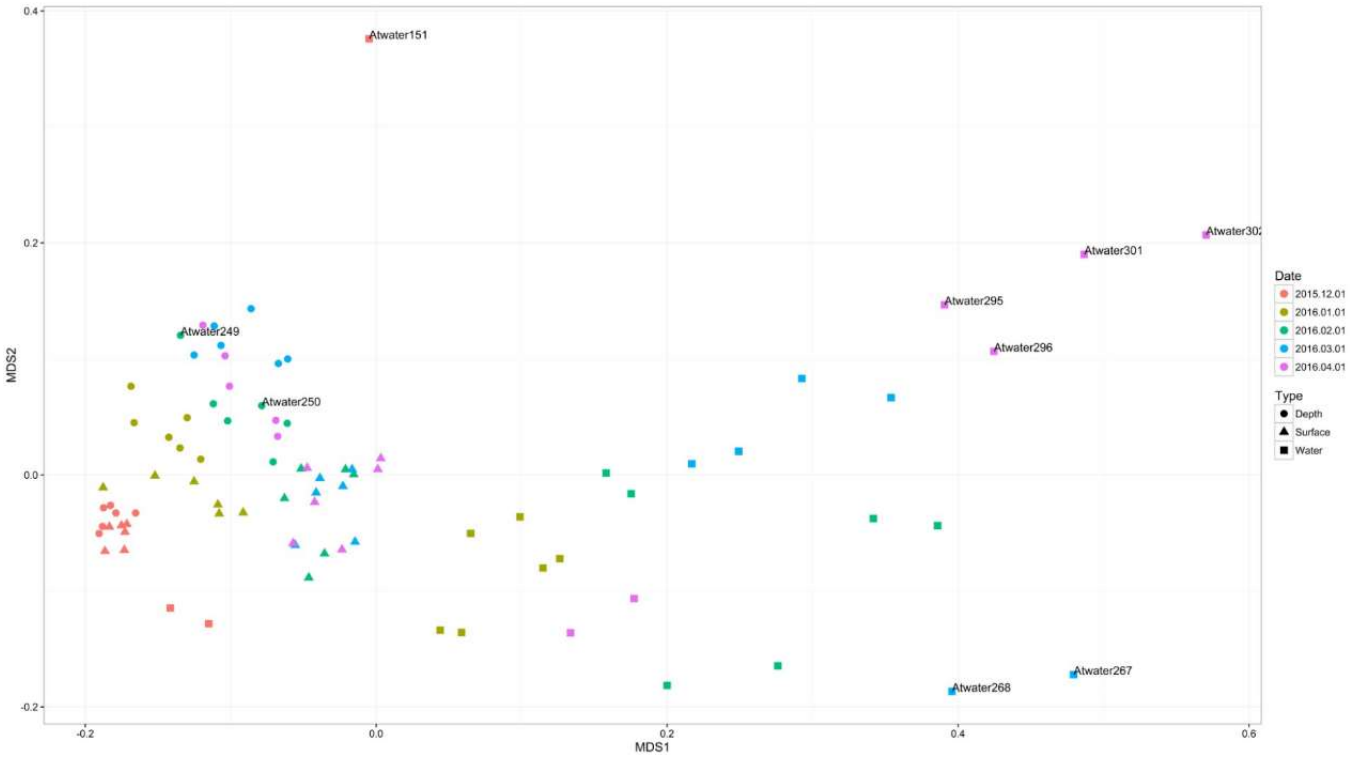

Figure 73. NMDS Plot Illustrating Suspected Outliers Among Column Samples

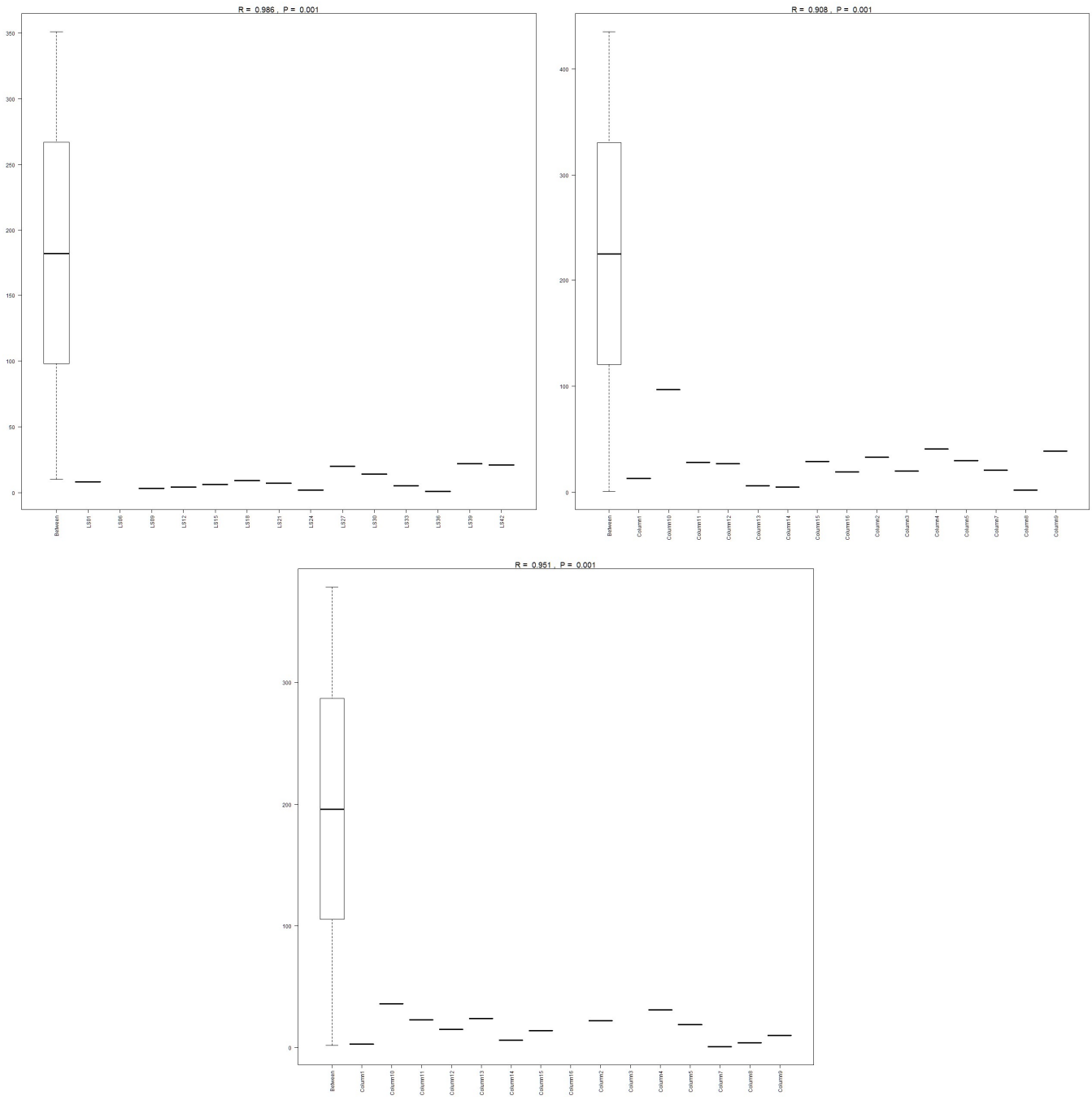

Figure 74. Anosim Boxplot Between Pseudo-Replicate Samples After Outlier Screening in the Column Study (Left to right: Water, Surface Sediment, and 10-cm Depth Sediment Samples)

3.6.2.2 Alpha Diversity

Please refer to Appendix H for the figures and tables that are referenced in this section.

In *Table 67*, one-way ANOVA comparison of the richness parameter using Chao1 indicated that there was a significant difference between the richness levels in the water samples, based on location (p-value =

0.008). Figure 109 through Figure 111 illustrate that lower richness was observed at Site 1 (stormceptor) and Site 6 (Lost Lagoon). The highest richness was observed at Site 5 (wetland exit). Alternately, in *Table 69*, comparison of coverage indicates a significant difference among locations (p-value = 0.004) with Site 1 and Site 6 having greater coverage than Site 2 through Site 5, as illustrated in Figure 112 through Figure 114. In *Table 71*, no significant difference (p-value = 0.237) was calculated for diversity among the water samples based on the Inverse Simpson index. Likewise, in *Table 72*, no significant difference (p-value = 0.130) was calculated for observed OTUs among the water samples based on SOBS. Figure 115 through Figure 120 graphically illustrate the calculations for diversity and observed OTUs.

In *Table 74*, one-way ANOVA comparison of the richness parameter using Chao1 indicated that there was a significant difference between the richness levels in the surface sediment samples, based on location (p-value = 0.011). Figure 121 through Figure 123 illustrate that lower richness was observed at Site 6 (Lost Lagoon). Alternately, in *Table 76*, comparison of coverage indicates a significant difference among locations (p-value = 0.010) with Site 6 having greater coverage than Site 2 through Site 5, as illustrated in Figure 124 through Figure 126. In *Table 78*, a significant difference (p-value = 0.003) was calculated for diversity among the surface sediment samples based on the Inverse Simpson index. However, in *Table 80*, no significant difference (p-value = 0.095) was calculated for observed OTUs among the surface sediment samples based on SOBS. Figure 127 through Figure 129 graphically illustrate the calculations for diversity and observed OTUs.

In *Table 81*, one-way ANOVA comparison of the richness parameter using Chao1 indicated that there was no significant difference between the richness levels in the 10-cm depth sediment samples, based on location (p-value = 0.727). Figure 130 through Figure 132 illustrate the similarity among sampling sites. Likewise, in *Table 82*, comparison of coverage indicated no significant difference among locations (p-value = 0.720), as illustrated in Figure 133 through Figure 135. In *Table 83*, no significant difference (p-value = 0.130) was calculated for diversity among the 10-cm depth sediment samples based on the Inverse Simpson index. In *Table 84*, no significant difference (p-value = 0.815) was calculated for observed OTUs among the 10-cm depth sediment samples based on SOBS. Figure 136 through Figure 141 graphically illustrate the calculations for diversity and observed OTUs.

In *Table 86*, using the ANOVA test, a significant difference for diversity (p-value =0.0486) was calculated between water samples taken for the column study; however, this was not confirmed with the Tukey HSD test (p-value =0.0580). No other significant differences were calculated for any of the indices. The comparisons are illustrated in *Table 85* through *Table 97* and in Figure 145 through Figure 168.

3.6.2.3 Community Composition

3.6.2.3.1 *Interpretation*

Analysis of the bacterial communities in the field samples generally indicated significant differences based on the location where the samples were collected. This significance also held true after adjusting for the date when the samples were collected.

Among the water samples taken during the field study, the hypothesis that no difference existed between field sites failed (p-value = 0.002). Further pairwise testing indicated that the Lost Lagoon had the most significantly different bacterial community from the wetland entry or forebay (p-value = 0.006) and a significantly different bacterial community from the wetland exit or settling pond (p-value = 0.03). Of importance, a significant difference was not calculated between the wetland entry and wetland exit (p-value = 0.492). In addition, no significant differences were determined between the stormceptor and any of the other sites for the bacterial communities identified in the water samples; however, this is likely due to a small sample size for the stormceptor. Figure 75 is an NMDS plot which illustrates the distance of dissimilarities among the field water samples. From this plot, there is a clear difference between the Lost Lagoon and the wetland; however, differences between the wetland entry and exit are more difficult to visually discern. Statistical outputs for the field water samples are summarized in *Table 33* and *Table 34*. In addition, using the *envfit* statistic in the vegan package in R, only nickel had a significant correlation (p-value <0.05) with the bacterial communities in the field water samples. However, nickel correlated with the Lost Lagoon, which had higher concentrations of nickel than the wetland. The lack of correlations among metal concentration and the field water samples suggest that water sampling alone would not be an adequate technique for validating a stormwater treatment wetland, such as the Lost Lagoon wetland.

Of greater significance among the bacterial community comparisons are the results between the surface sediment samples. In Figure 76, there are clear visual differences between the Lost Lagoon and the wetland forebay and wetland settling pond. These differences are supported by the statistical calculations summarized in *Table 35* and *Table 36* where all p-values are less than 0.05. In addition, using the envfit parameter, copper and chromium positively correlated with the wetland forebay samples and nickel negatively correlated with the wetland settling pond samples. The 10-cm depth sediment field samples yielded the same overall results as the surface sediment field samples. Barium concentrations also positively correlated with the wetland forebay. These results are illustrated in Figure 77 and summarized in *Table 37* and *Table 38*.

The bacterial communities, identified among the samples extracted from the laboratory column samples, yielded interesting results. These results begin to show some potential causation between the application or dosing of stormwater and the response of bacteria to said stormwater.

The NMDS plot in Figure 78 illustrates the response of bacteria in the water samples extracted over the duration of the column study. From the figure, generally, there is a departure between columns that were dosed with stormwater and between columns that were dosed with distilled water. Exposure to stormwater yielded a difference from distilled water (p-value = 0.035) even when accounting for the fact that the date that the samples were taken along the study period also yielded a significant impact (p-value = 0.003). Results positively correlated with four metals – manganese, nickel, chromium and copper. The statistical computations for the column water samples can be found in *Table 39*.

The NMDS plot in Figure 79 illustrates a similar response of bacteria in the surface sediment samples extracted over the duration of the column study as was observed with the water samples. There is significant departure between columns that were dosed with stormwater and columns that were dosed with distilled water (p-value = 0.05). Results positively correlated with copper. This held true when considering the date in which samples were extracted and computations are summarized in *Table 40*.

The NMDS plot in Figure 80 illustrates a different result for the 10-cm depth sediment samples extracted over the duration of column study. While there is some departure between the columns that were dosed with stormwater and the columns that were dosed with distilled water, there is no clear pattern with time for the progression of the bacterial communities in the columns that were dosed with distilled water. The summary of statistics for the 10-cm depth sediment samples can be found in *Table 41*.

3.6.2.3.2 *Field Study*

3.6.2.3.2.1. Water Samples

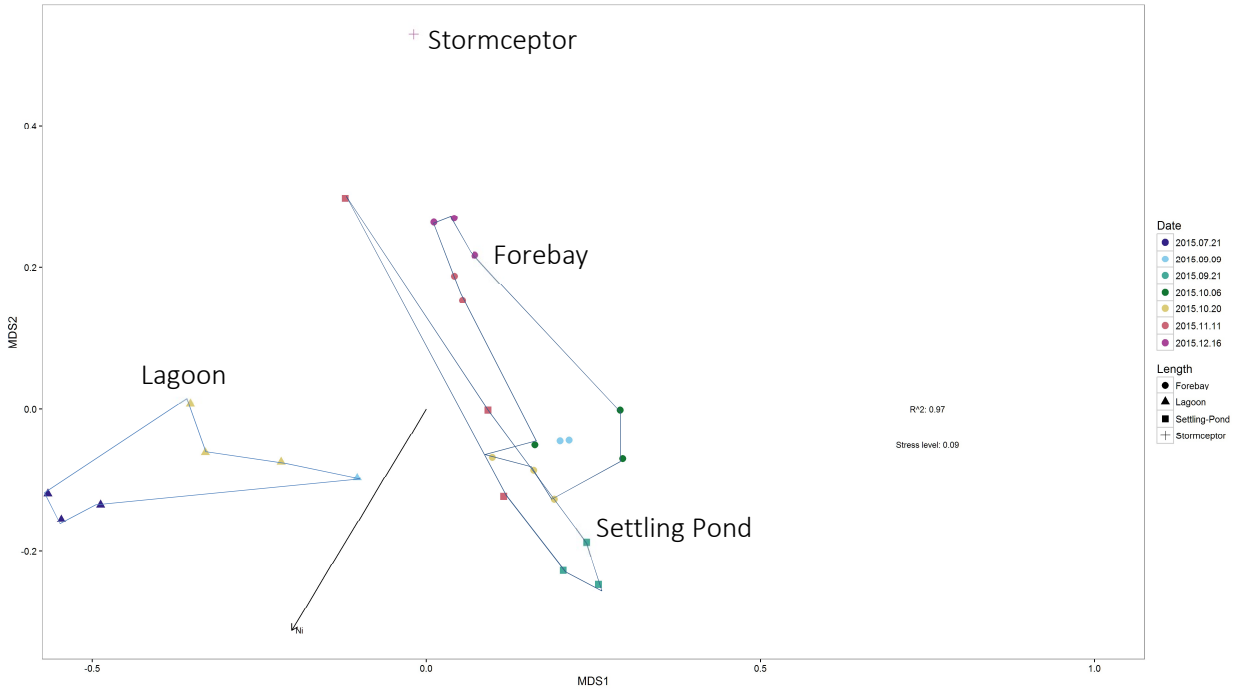

Figure 75. NMDS Plot Comparing Field Study Water Samples

Table 33. Adonis Whole Dataset Comparison of Field Study Water Samples by Location with Strata Adjustment for Date

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Length	3	2.201482	0.733827	5.13504	0.401122	0.002
Residuals	23	3.286835	0.142906	NA	0.598878	NA
Total	26	5.488317	NA	NA	1	NA

Table 34. Adonis Pairwise Comparison of Field Study Water Samples by Location

	Pairs	F.Model	R2	p.value	p.adjusted
1	Lagoon vs Forebay	18.39609	0.505441	0.001	0.006
2	Lagoon vs Settling-Pond	13.37634	0.548743	0.005	0.03
3	Lagoon vs Stormceptor	1.588809	0.209362	0.266	1
4	Forebay vs Settling-Pond	2.45167	0.126039	0.082	0.492
5	Forebay vs Stormceptor	2.783385	0.188278	0.073	0.438
6	Settling-Pond vs Stormceptor	2.92018	0.368701	0.138	0.828

3.6.2.3.2.2. Surface Sediment Samples

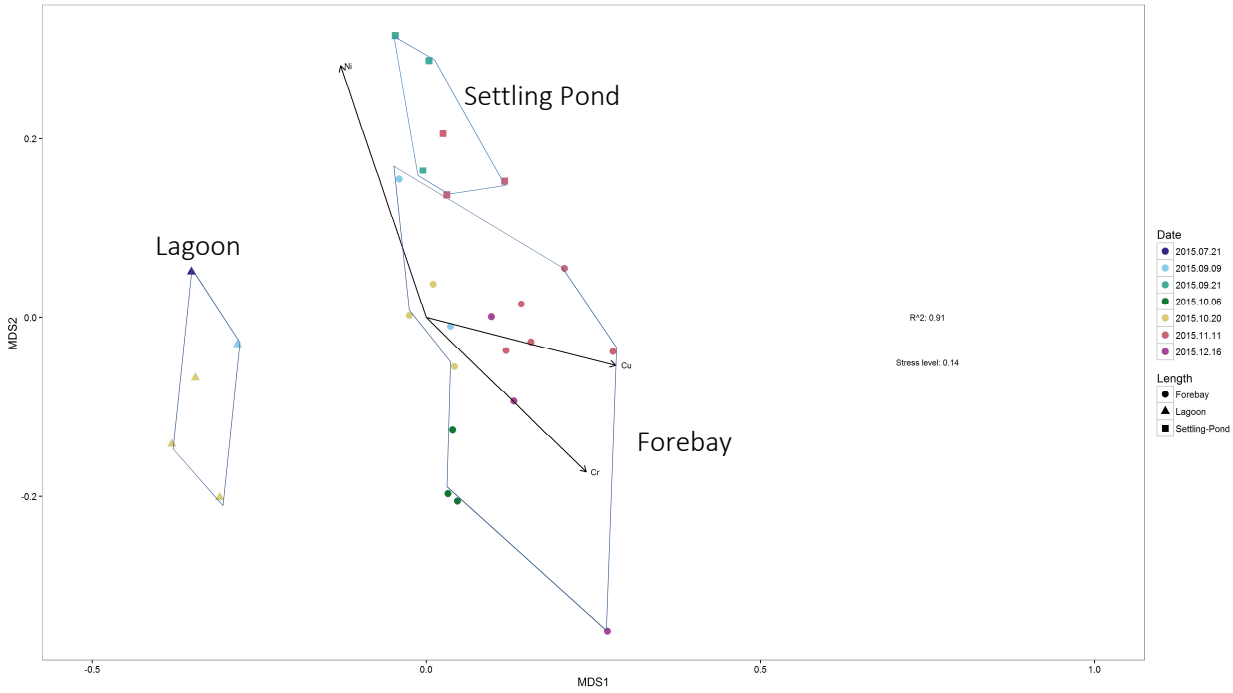

Figure 76. NMDS Plot Comparing Field Study Surface Sediment Samples

Table 35. Adonis Whole Dataset Comparison of Field Study Surface Sediment Samples by Location with Strata Adjustment for Date

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Length	2	1.215679	0.60784	6.157626	0.339121	0.002
Residuals	24	2.36912	0.098713	NA	0.660879	NA
Total	26	3.584799	NA	NA	1	NA

Table 36. Adonis Pairwise Comparison of Field Study Surface Sediment Samples by Location

	Pairs	F.Model	R2	p.value	p.adjusted
1	Lagoon vs Forebay	20.51019042	0.519111404	0.001	0.003
2	Lagoon vs Settling-Pond	22.04533539	0.710101376	0.005	0.015
3	Forebay vs Settling-Pond	6.611271881	0.248438779	0.001	0.003

3.6.2.3.2.3. 10-cm Depth Sediment Samples

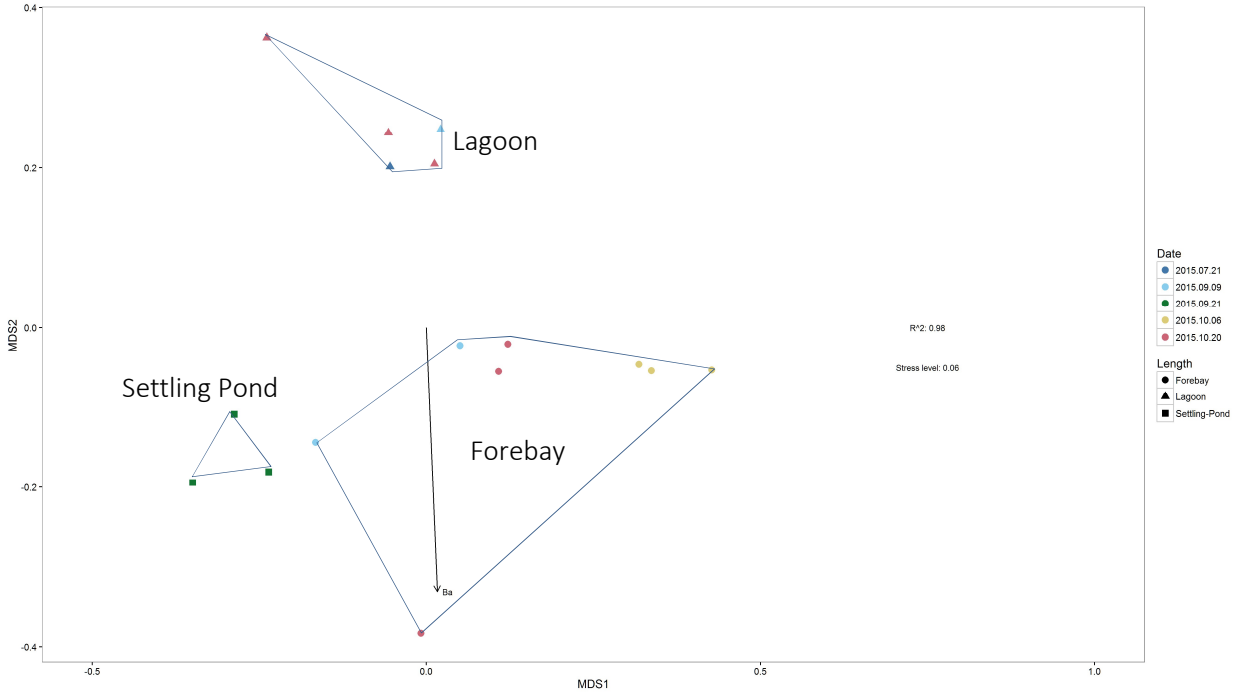

Figure 77. NMDS Plot Comparing Field Study 10-cm Depth Sediment Samples

Table 37. Adonis Whole Dataset Comparison of Field Study Depth Sediment Samples by Location with Strata Adjustment for Date

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Length	2	1.097689211	0.548844606	4.629741405	0.415979243	0.02
Residuals	13	1.541118444	0.118547573	NA	0.584020757	NA
Total	15	2.638807656	NA	NA	1	NA

Table 38. Adonis Pairwise Comparison of Field Study Depth Sediment Samples by Location

	Pairs	F.Model	R2	p.value	p.adjusted
1	Lagoon vs Forebay	6.498867792	0.371387902	0.001	0.003
2	Lagoon vs Settling-Pond	14.66670227	0.70967792	0.016	0.048
3	Forebay vs Settling-Pond	5.200414149	0.366215668	0.004	0.012

3.6.2.3.3 *Laboratory Study*

3.6.2.3.3.1. Water Samples

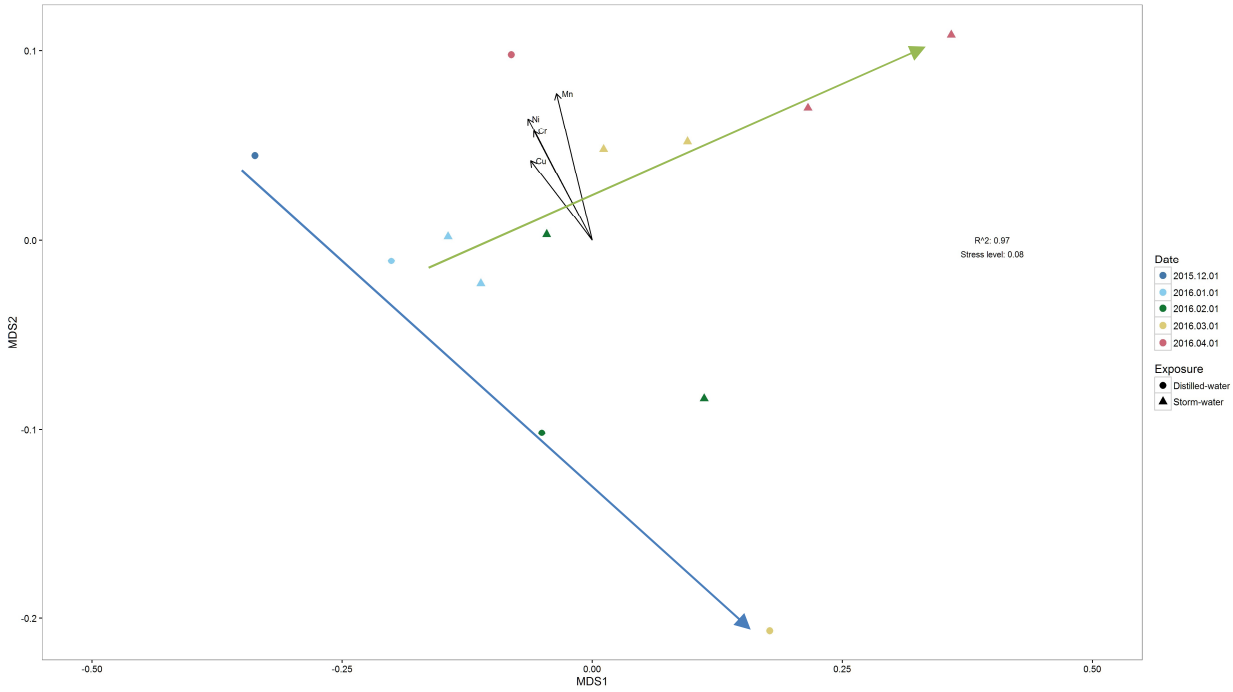

Figure 78. NMDS Plot Comparing Laboratory Study Water Sediment Samples

Table 39. Adonis Whole Dataset Comparison of Laboratory Study Water Samples by Exposure and Date

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Exposure	1	0.167920169	0.167920169	2.007923907	0.121214106	0.035
Date	4	0.631997288	0.157999322	1.889294283	0.456210748	0.003
Residuals	7	0.58540126	0.083628751	NA	0.422575147	NA
Total	12	1.385318718	NA	NA	1	NA

3.6.2.3.3.2. Surface Sediment Samples

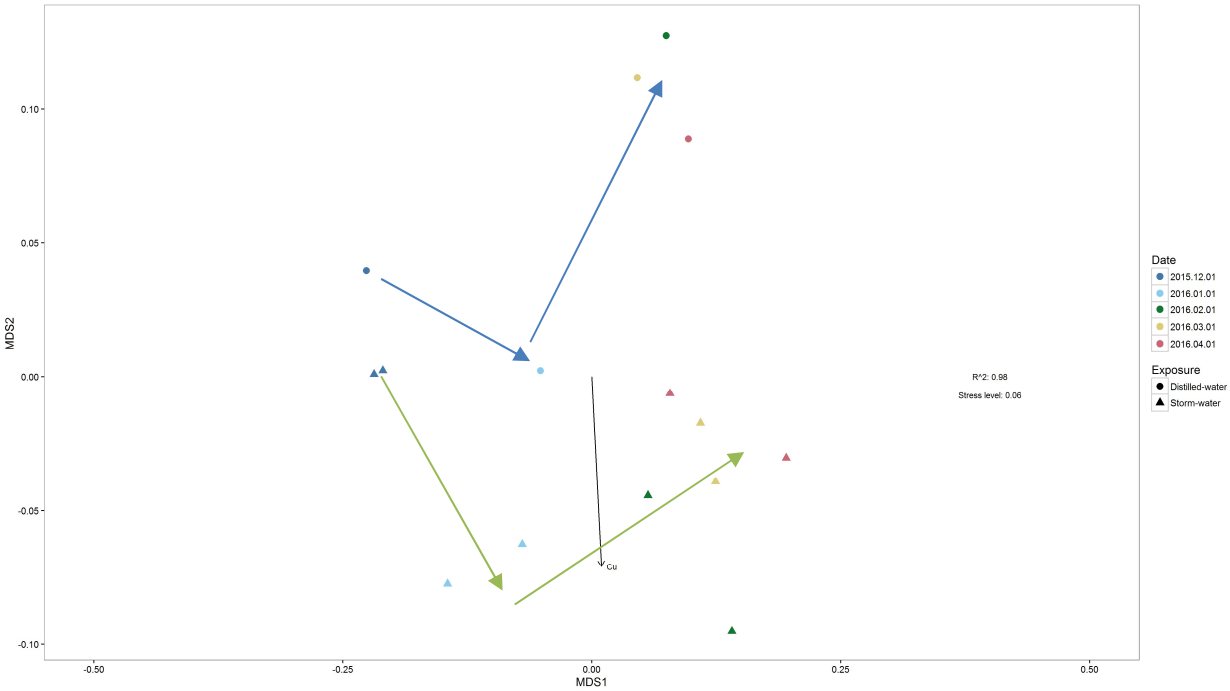

Figure 79. NMDS Plot Comparing Column Study Surface Sediment Samples

Table 40. Adonis Whole Dataset Comparison of Laboratory Study Surface Sediment Samples by Exposure and Date

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Exposure	1	0.067210634	0.067210634	1.777639956	0.085997169	0.05
Date	4	0.37405388	0.09351347	2.473318129	0.478608408	0.001
Residuals	9	0.340280217	0.037808913	NA	0.435394423	NA
Total	14	0.781544732	NA	NA	1	NA

3.6.2.3.3.3. *10-cm Depth Sediment Samples*

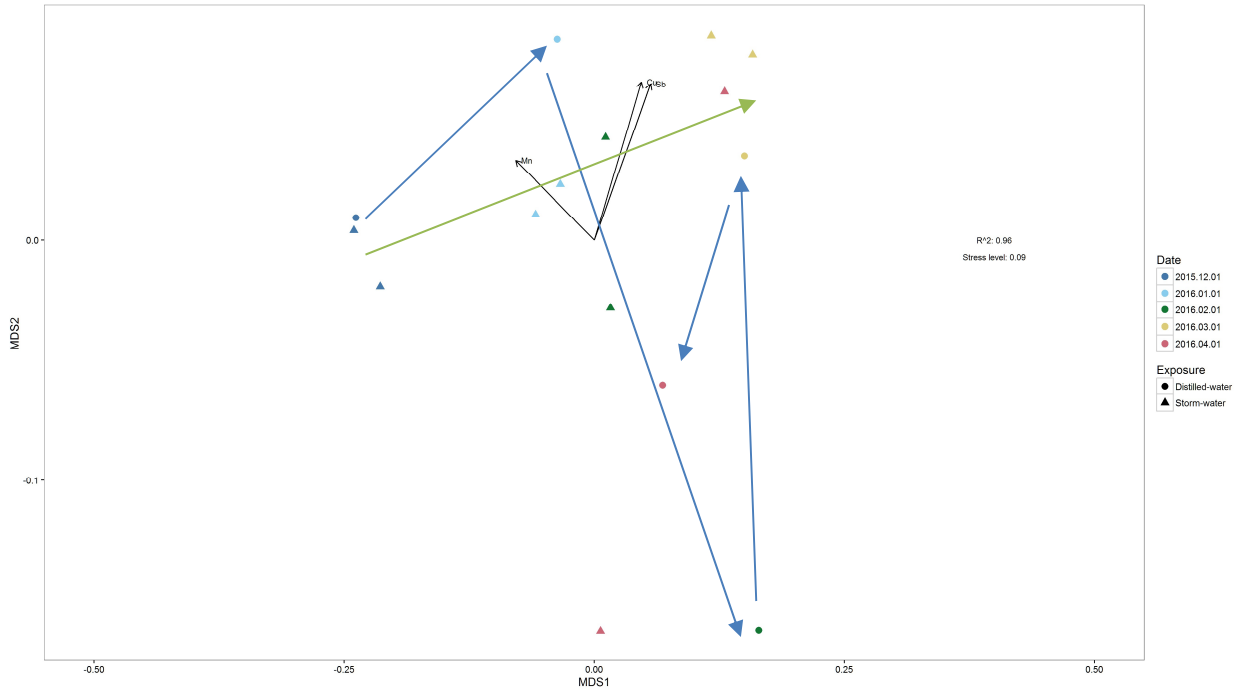

Figure 80. NMDS Plot Comparing Column Study 10-cm Depth Sediment Samples

Table 41. Adonis Whole Dataset Comparison of Laboratory Study Depth Sediment Samples by Exposure and Date

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Exposure	1	0.051649735	0.051649735	1.168781611	0.061211356	0.224
Date	4	0.394423785	0.098605946	2.231353513	0.467441217	0.001
Residuals	9	0.39771982	0.044191091	NA	0.471347427	NA
Total	14	0.843793339	NA	NA	1	NA

3.6.2.4 Indicator Species

3.6.2.4.1 *Interpretation*

Indicator species were determined using Mothur for the three different sample types extracted during both the field study and the laboratory column study. In Figure 81 through Figure 83, the top indicator species are illustrated by relative abundance for the field sites. In Figure 84 through Figure 86, the top indicator species are illustrated by relative abundance for the laboratory columns that were sampled during week 16 of the column study. While significant indicator species were determined, there are no discernable patterns at the phylum level among the identified indicator species. One discernable difference was that, generally, there were a greater number of significant indicator species identified at the sites and in the columns that were not dosed with stormwater. This might suggest that some species of bacteria are

influenced by stormwater, either negatively (extinction) or positively (adaptation). Further research and repeat testing would be required in order to deduce statistics for this hypothesis, however.

3.6.2.4.2 *Field Study*

3.6.2.4.2.1. Water Samples

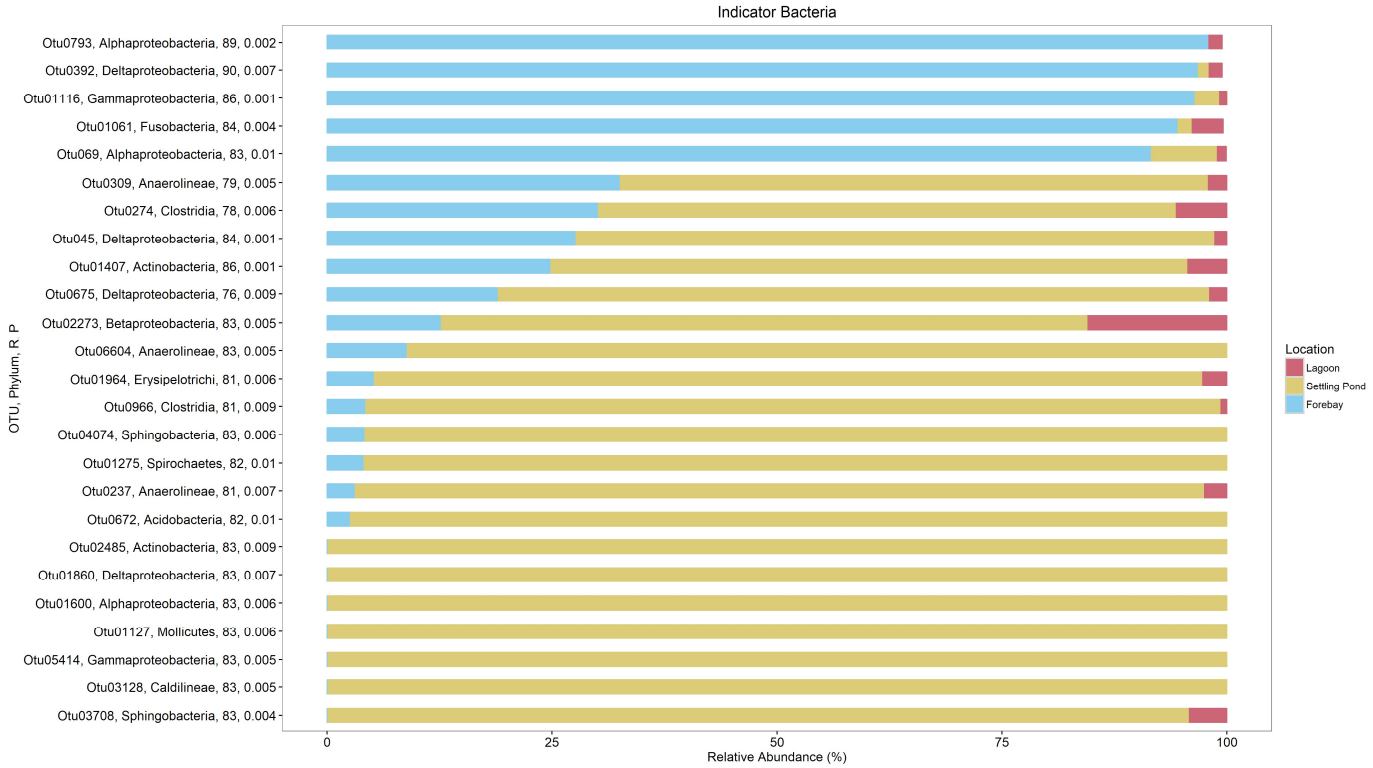

Figure 81. Indicator Species Barplot for Field Study Water Samples

3.6.2.4.2.2. Surface Sediment Samples

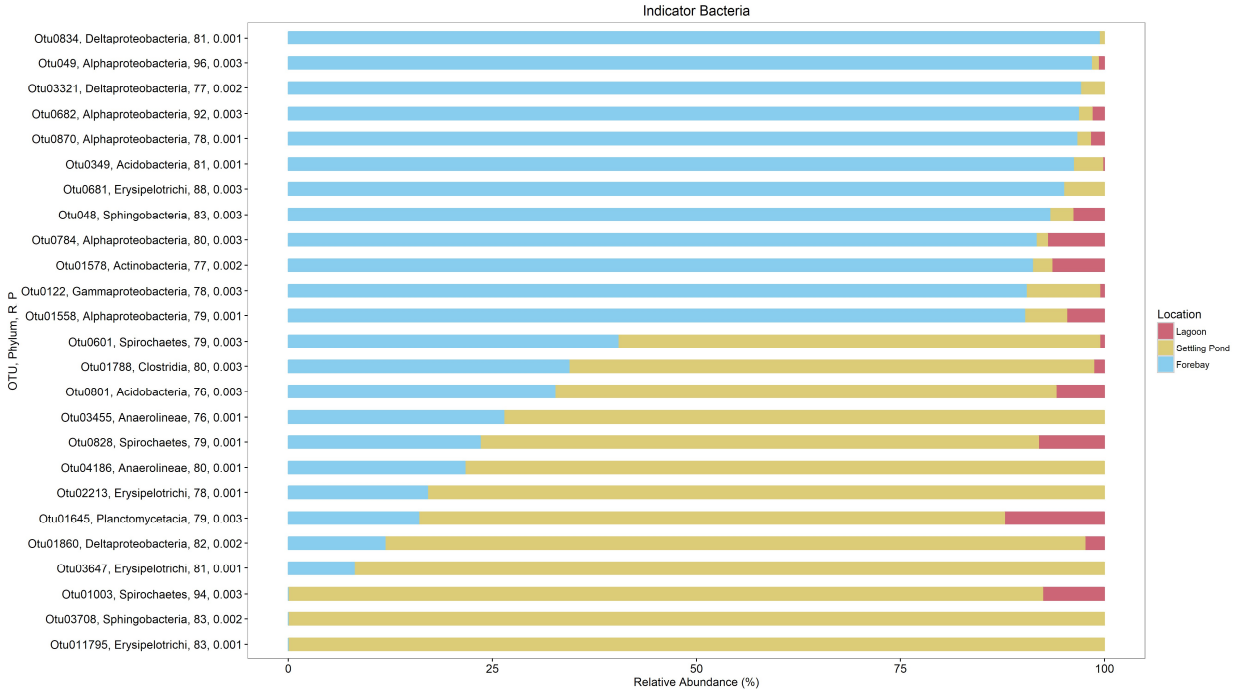

Figure 82. Indicator Species Barplot for Field Study Surface Sediment Samples

3.6.2.4.2.3. 10-cm Depth Sediment Samples

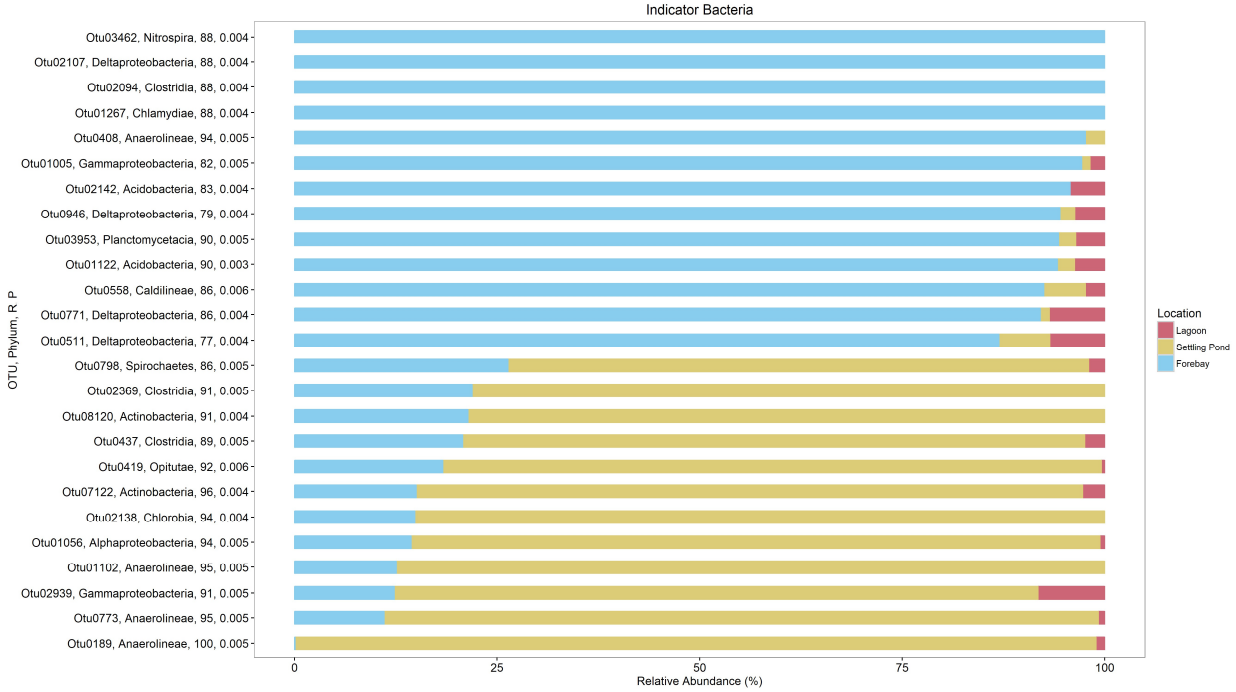

Figure 83. Indicator Species Barplot for Field Study 10-cm Depth Sediment Samples

3.6.2.4.3 *Laboratory Study*

3.6.2.4.3.1. Water Samples

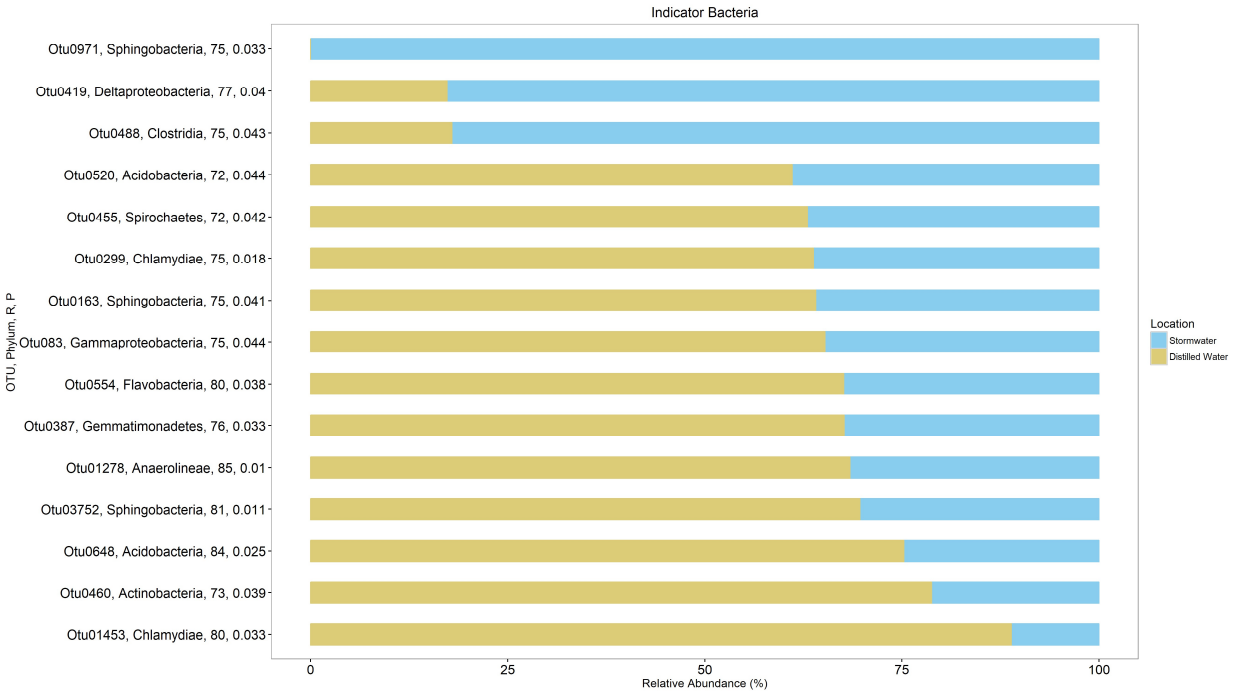

Figure 84. Indicator Species Barplot for Laboratory Study Water Sediment Samples

3.6.2.4.3.2. Surface Sediment Samples

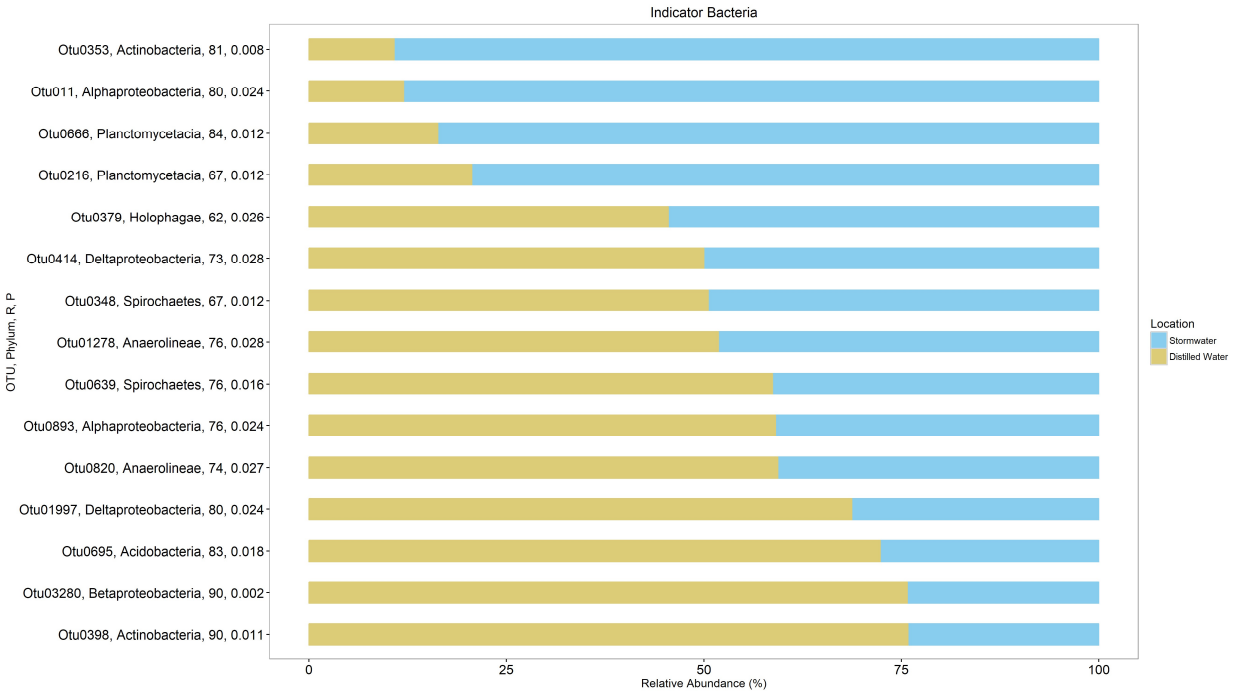

Figure 85. Indicator Species Barplot for Laboratory Study Surfaced Sediment Samples

3.6.2.4.3.3. 10-cm Depth Sediment Samples

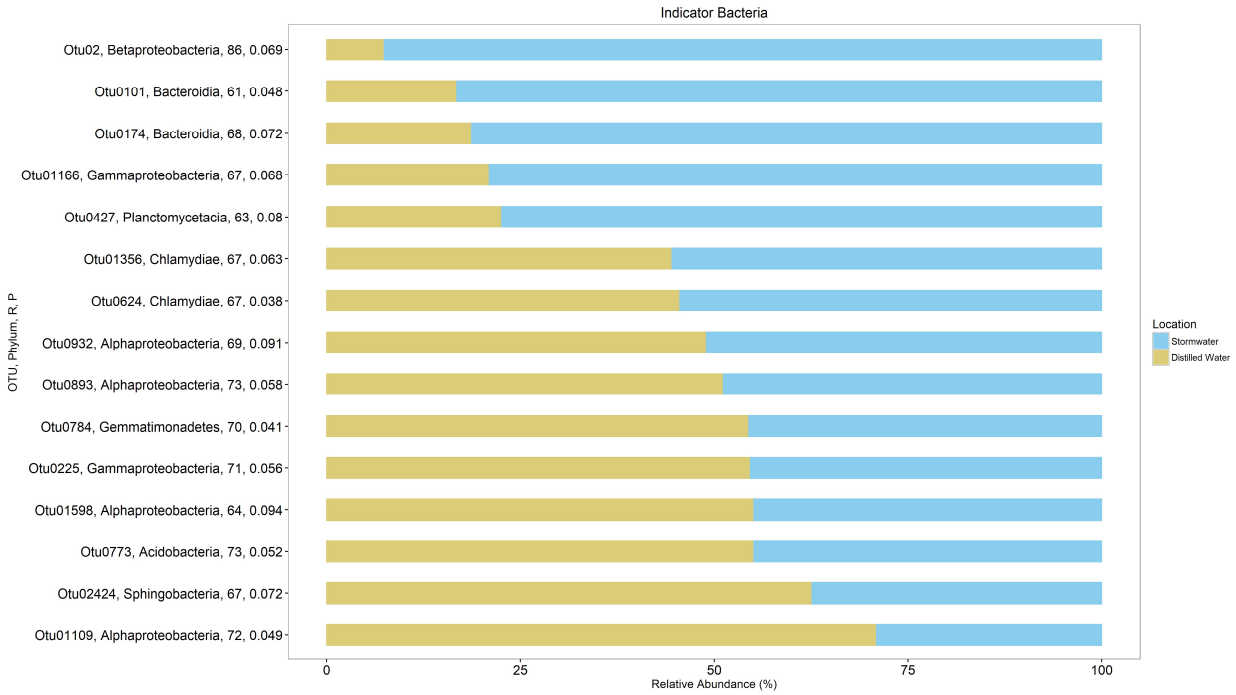

Figure 86. Indicator Species Barplot for Laboratory Study 10-cm Depth Samples

3.6.3 Microbial Functional Gene Analysis

3.6.3.1 Data Quality and Screening

Quality control removed an average of %1.80 percent of sequences and 1.79% of translated ORFs. *Table 42* and *Table 43* provide summary statistics for the quality control of sequence data.

Table 42. Summary Statistics for Sequence Data Prior to Quality Control and Screening

	Sequences (#)	Minimum Length	Average Length	Maximum Length	Total Base Pairs	Translated ORFs (amino) (#)	Minimum Length	Average Length	Maximum Length	Total Base Pairs
October Site 2	1112254	61	109	2172	122159553	1032935	18	34	523	36034670
October Site 3	874221	61	107	1606	93989112	824868	18	34	460	28107007
October Site 4	862982	61	106	11286	91791432	801450	18	33	917	27091806
October Site 5	3313614	61	103	13962	344045197	3128608	15	32	1434	102697812
October Site 6	1367387	61	104	5548	142795471	1057221	15	33	805	35373718
September Site 2	795230	61	110	6160	881879676	739436	18	35	598	26103686
Column 1	886693	61	110	2208	98099327	832115	16	35	528	29327503
Column 7	1010237	61	111	1843	99794400	949845	17	35	511	33618691
Column 8	792921	61	108	1467	86045364	731896	18	34	377	25359107
Column 5	733631	61	145	27254	107065610	704410	18	45	1114	31937114
Column 15	1068593	61	165	16241	177132959	1026190	17	50	2197	51854855
Column 16	967645	61	147	12007	142540522	927311	18	45	1237	42313345

Table 43. Summary Statistics for Sequence Data After Quality Control and Screening

	Sequences (#)	Minimum Length	Average Length	Maximum Length	Total Base Pairs	Translated ORFs (amino) (#)	Minimum Length	Average Length	Maximum Length	Total Base Pairs
October Site 2	1093496	70	110	2172	120966323	1032935	20	34	523	35458633
October Site 3	868657	70	107	1606	93627335	808769	20	34	460	27628016
October Site 4	849512	70	107	11286	90924595	784604	20	33	917	26593745
October Site 5	3309767	70	103	13962	343209220	3071139	20	32	1434	100954728
October Site 6	1284648	70	107	5548	137559094	1029315	20	33	805	34575323
September Site 2	778822	70	111	6160	87149331	724319	20	35	598	25658071
Column 1	874291	70	111	2208	97300822	813273	20	35	528	28758224
Column 7	997944	70	111	1843	111616320	929628	20	35	511	33007909
Column 8	777275	70	109	1467	85048533	715807	20	34	377	24879426
Column 5	723742	70	146	27254	106382831	693944	20	45	1114	31617064
Column 15	1045794	70	167	16241	175054974	1011977	20	50	2197	51425082
Column 16	953961	70	148	12007	141415083	914016	20	45	1237	41908807

3.6.3.2 Functional Gene Composition

3.6.3.2.1 *Interpretation*

To visualize the composition of annotated genes using the KEGG database, NMDS plots were prepared in the same fashion as was performed for visualization of bacterial communities except this time using the relative abundance of annotated genes for the samples instead of the abundance of OTUs.

Figure 87 is an NMDS plot for the metagenomes sequenced from a subset of sediment samples collected during the field study at the Lost Lagoon wetland. From Figure 87, based on the relative distance between samples, there is some indication that samples taken at the same location but a month apart have more similar metagenomes than samples that are taken on the same date but in different locations. There is also some evidence that Site 2, Site 3, and Site 4 (wetland forebay) have greater similarity to each other than they do to Site 5 (wetland settling pond) or Site 6 (Lost Lagoon). The dataset provides some interesting proof of concept results, though there are too few data points to form definitive conclusions.

Likewise, Figure 88 is an NMDS plot for the metagenomes sequenced from sediment samples collected at the beginning and at the end of the column study. From Figure 88, there is some evidence that after sixteen

weeks, the soil columns that were dosed with stormwater formed a separate cluster from the soil column that was dosed with distilled water. As with the field study, the dataset provides some interesting proof of concept for the utility of metagenomics in monitoring stormwater treatment but more data would be needed to strengthen preliminary findings

3.6.3.2.2 *Figures*

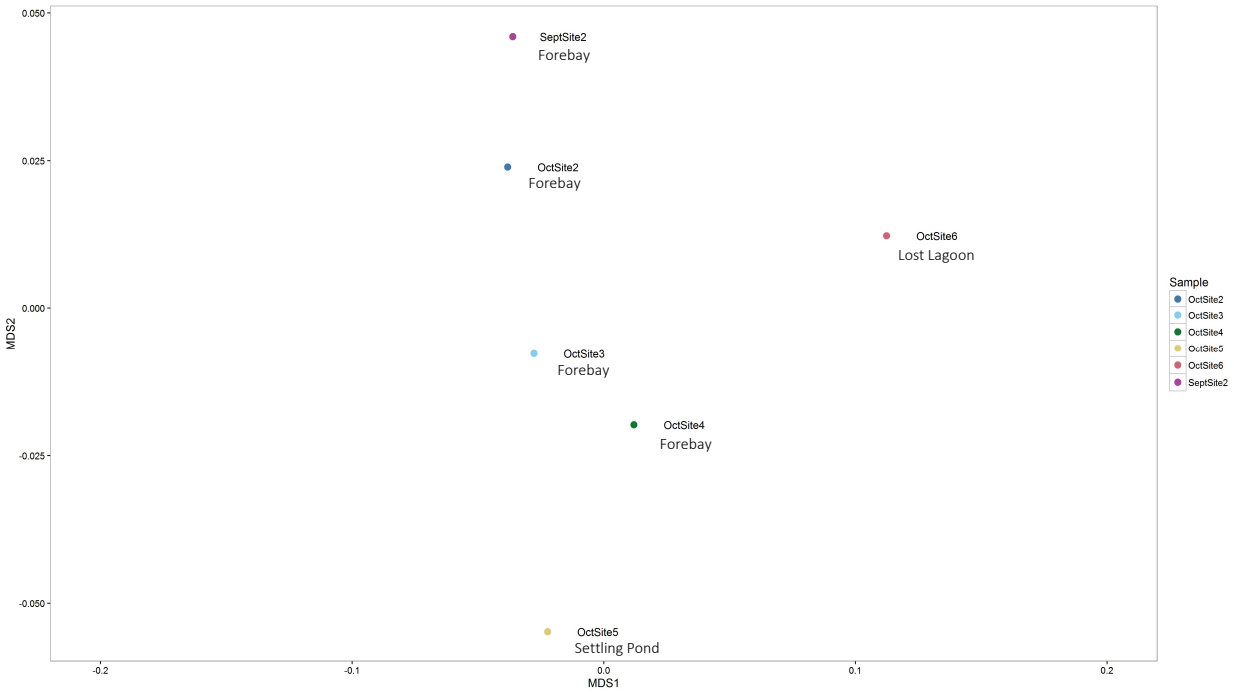

Figure 87. NMDS Plot of KEGG Annotated Genes for Field Samples

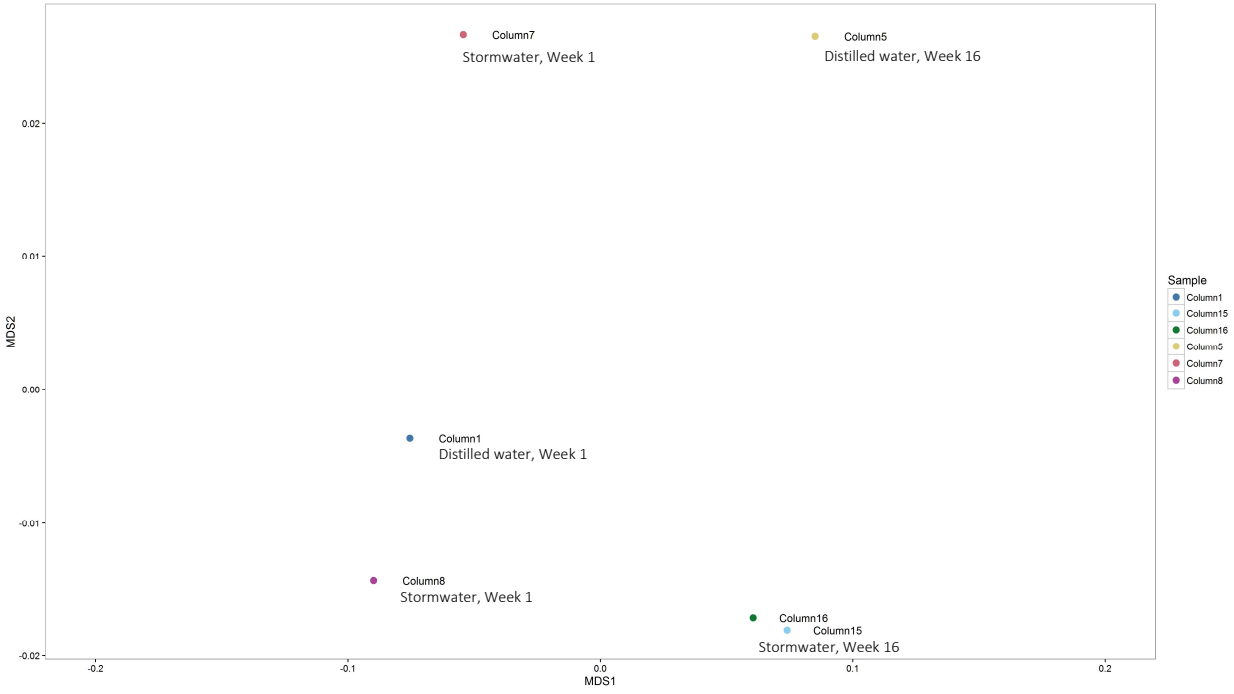

Figure 88. NMDS Plot of KEGG Annotated Genes for Column Samples

3.6.3.3 Metal Adaptation Genes

3.6.3.3.1 *Interpretation*

Genes, which are known to be partially responsible for metal adaptation, (*Table 19*) were highlighted from the dataset and plotted based on their relative abundance. It was the desire of the author to compare mechanisms for tolerance of copper, lead and zinc in this section as these three metals showed clear trends of decreasing concentrations along the length of the Lost Lagoon wetland. However, few markers for copper and no markers for lead adaptations were present in the KEGG database from 2014. The one marker that was present in the dataset for copper (CusR) did not form an identifiable trend among samples taken during the field study or among samples taken during the column study. For these reasons, comparisons were instead performed using markers for zinc, manganese/zinc/iron, and cobalt/nickel tolerances.

Figure 89 is a plot which illustrates the relative abundance of six genes that are relevant for zinc transport or resistance. The comparison is complicated because the genes do not all present the same trend. For example, the znuB gene has a downward trend between the wetland exit and entry; however, the zraP gene has an upward trend between the wetland exit and entry. The relative abundances of znuB is greater than the relative abundance of the other genes in this subset and this suggests that this gene may be more dominant in zinc transport than some of the others, though this is not conclusive. Of interest, in Figure 90, the znuB gene also has a greater relative abundance in the soil columns that were dosed with stormwater than in the soil columns that were dosed with distilled water. For this initial investigation, this suggests that znuB may be an important factor in zinc tolerance and that this could be an item for further investigation.

A similar result, as was just described, is illustrated in Figure 91 for four genes associated with manganese/zinc/iron transport. In this figure, the two genes with the highest relative abundances (troB and sitB) have higher relative abundances in the wetland entry than in the wetland exit. However unlike in the previous example for zinc, the results for the column study, illustrated in Figure 92, do not present the same result. This adds to the complexity of the observations and illustrates that there are many influential factors at play.

Finally, in Figure 93 and Figure 94, the relative abundances of the czcA sequence, which codes for cobalt and nickel resistance proteins (Gillan et al 2015), are compared for the field and column studies, respectively. CzcA expressed both a clear decrease in relative abundance along the length of the wetland and clear increase in relative abundance in soil columns that were dosed with stormwater compared to soil columns that were dosed with distilled water. Like znuB, CzcA also represents an item that may be useful for further investigation.

3.6.3.3.2 *Figures*

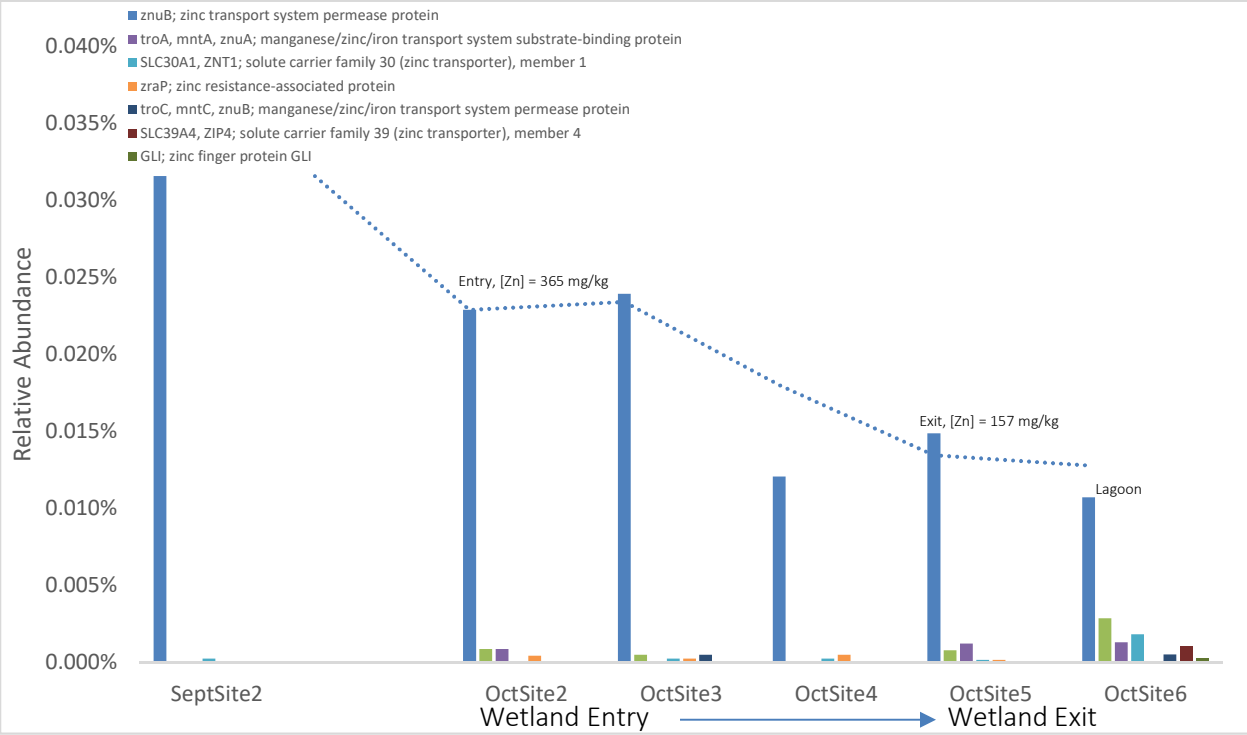

Figure 89. Relative Abundance of Genes Associated with Zinc Measured in Field Samples

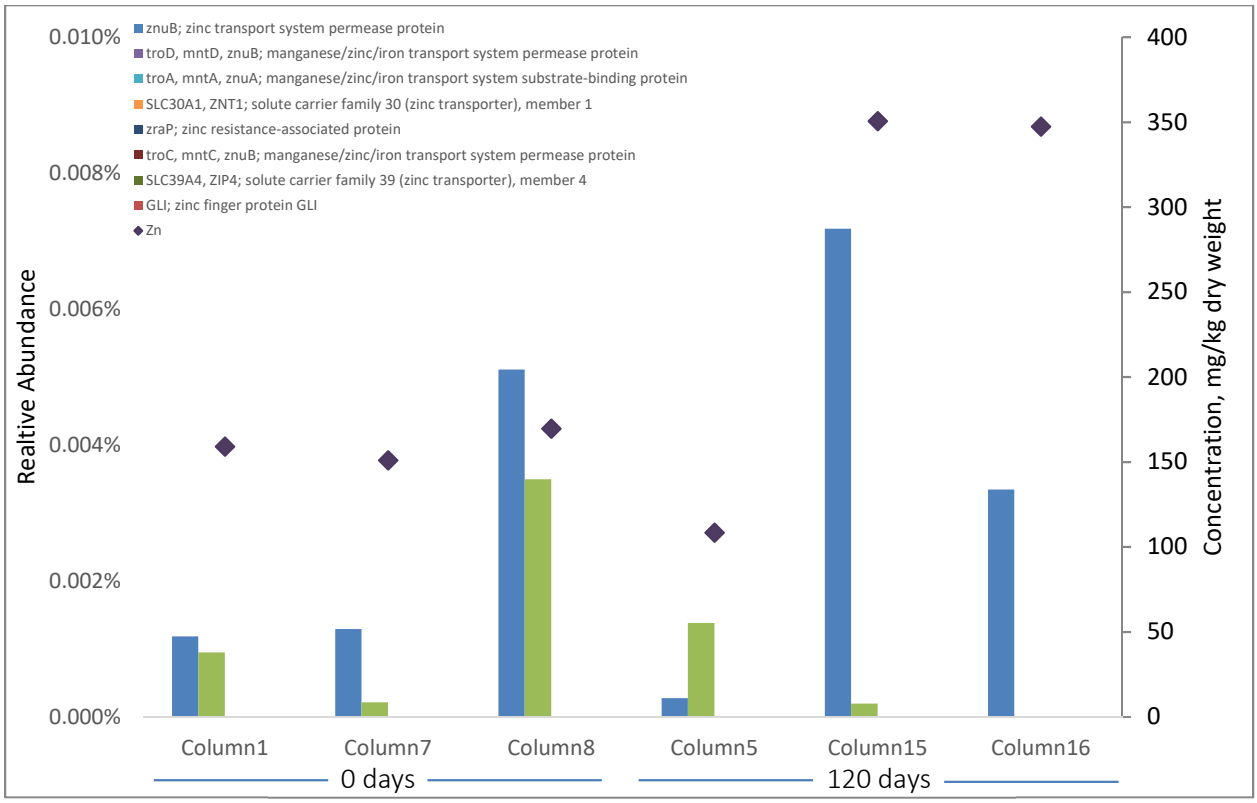

Figure 90. Relative Abundance of Genes Associated with Zinc Measured in Column Samples

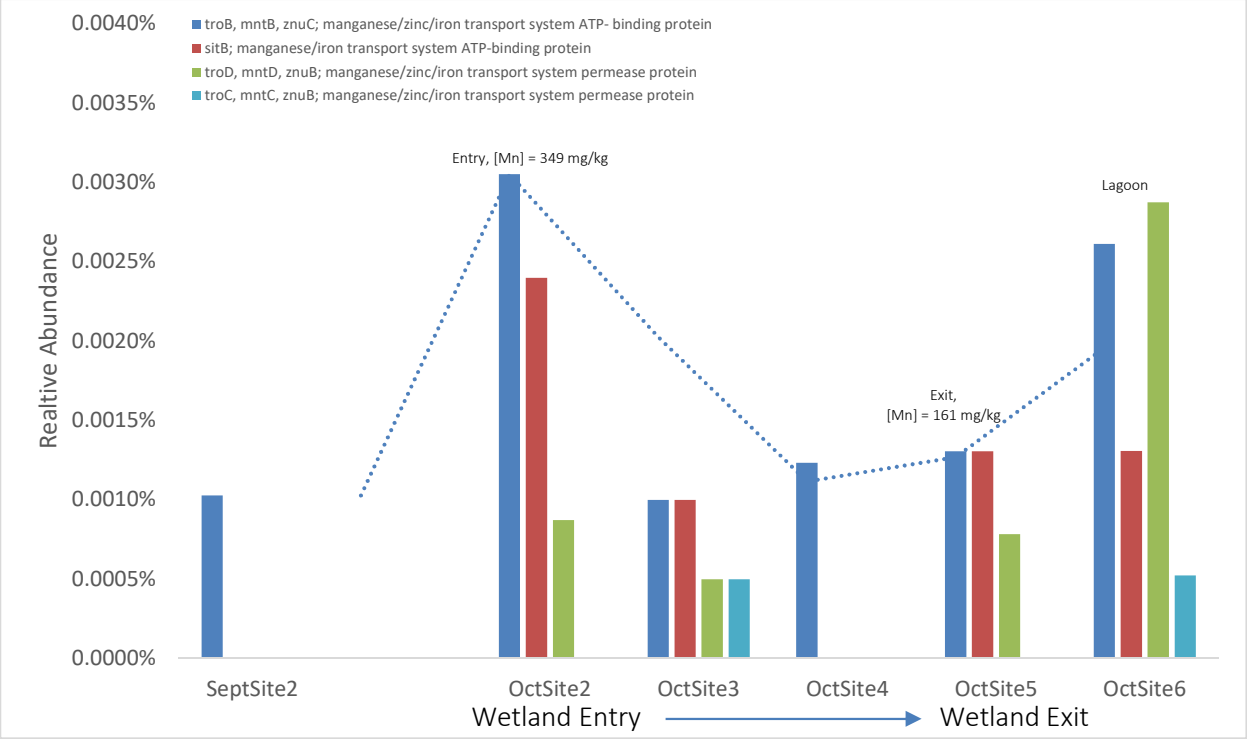

Figure 91. Relative Abundance of Functional Associated with Manganese, Zinc and Iron Measured in Field Samples

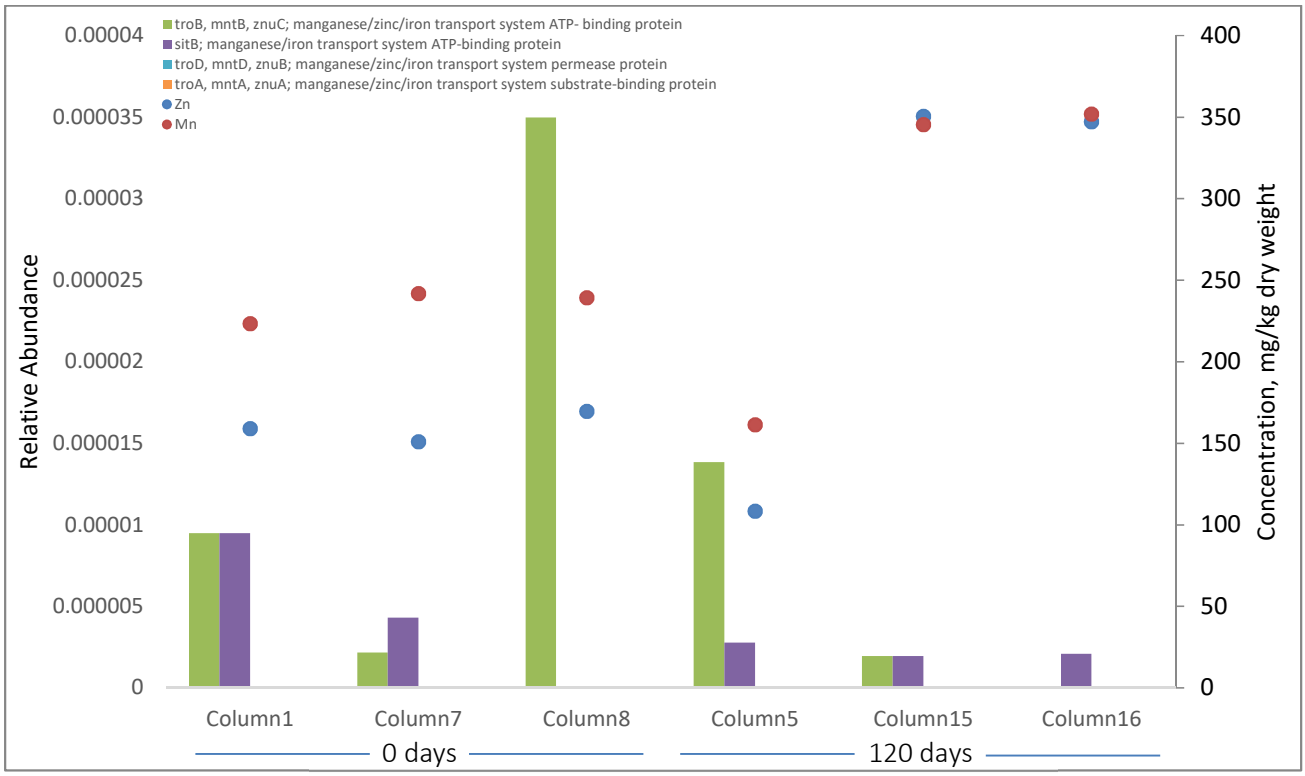

Figure 92. Relative Abundance of Genes Associated with Manganese, Zinc and Iron Measured in Column Samples

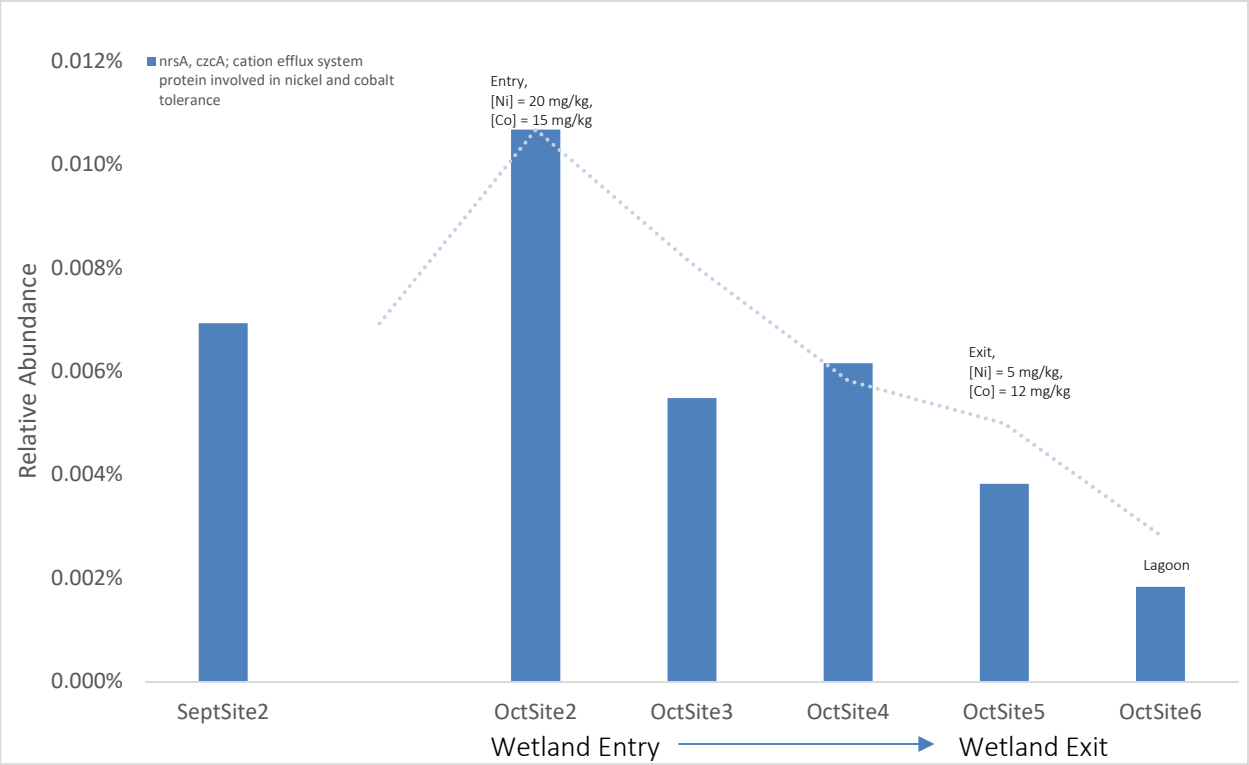

Figure 93. Relative Abundance of CzcA Tolerance Gene Measured in Field Samples

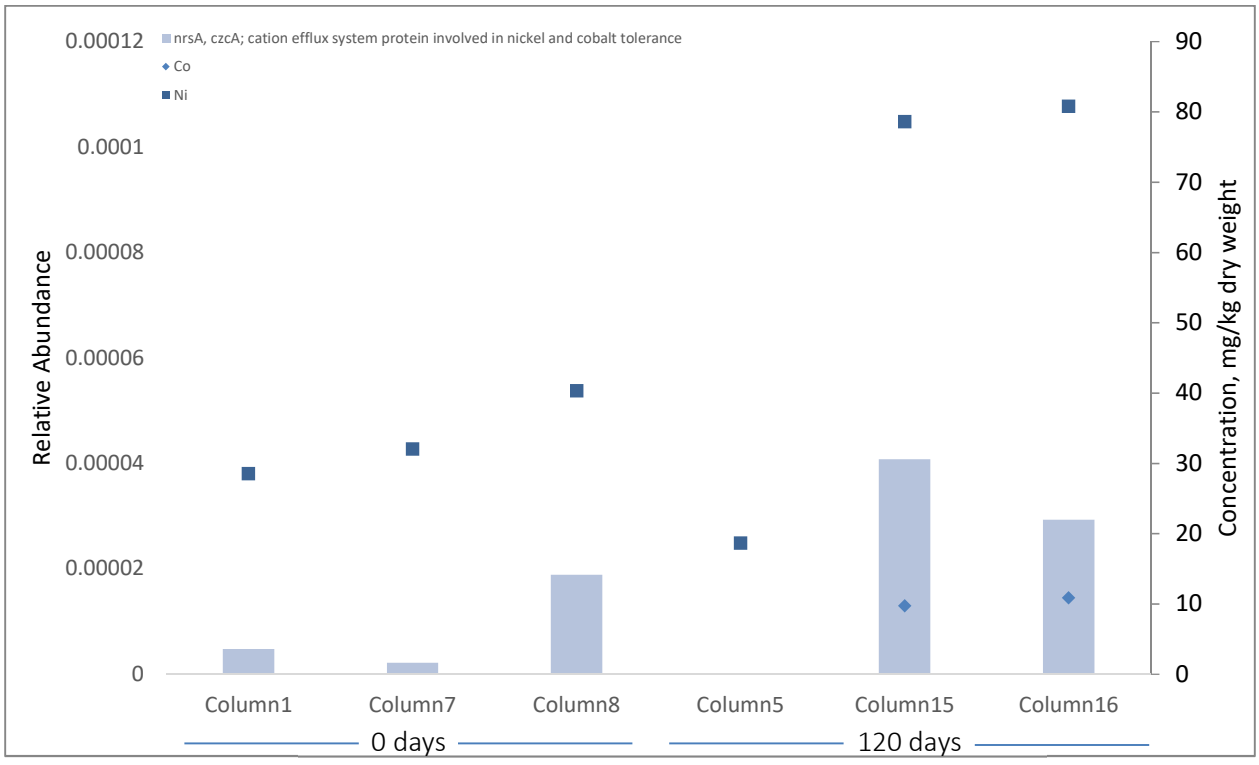

Figure 94. Relative Abundance of CzcA Tolerance Gene Measured in Column Samples

3.7 Discussion and Conclusion

While the goal of this study was to provide proof of concept data that supports or rejects developing a genomics monitoring tool for low impact design features that treat stormwater, including engineered wetlands, the goal of Chapter 2 was to expand on the results of Chapter 1 by applying genomics-based approaches to support the conclusion that the Lost Lagoon wetland is effectively treating stormwater. In addition, this chapter attempted to provide data to support the application of genomics for validation of other low impact design sites that treat stormwater. In this chapter experimentation was conducted and data was gathered and analyzed to provide proof that microbial analyses can support environmental analyses for the validation of a stormwater treatment wetland and possibly other similar systems. For this, an attempt was made to answer three hypotheses and to support three objectives.

3.7.1 Chapter Hypotheses

To provide proof of concept results for the application of genomics-based analyses as a wetland validation technique, it was previously stated that three hypotheses must be true.

1. There is a shift in the composition and function of the microbial communities that exist between the entry and exit of the Lost Lagoon wetland;
2. The shift in the composition and function of the microbial communities between the entry and exit of the Lost Lagoon wetland is influenced by the decreasing concentration of contaminants along the length of the wetland; and
3. There are similarities across unconnected sites in the adaptations that take place within microbial communities due to exposure to stormwater.

Regarding the first hypothesis, demonstrating a shift in the composition and function of microbial communities along the Lost Lagoon wetland, some important conclusions can be drawn. There was a significant difference in microbial community composition calculated between the wetland entry and exit for the surface sediment samples and for the 10-cm depth sediment samples but no significant difference was calculated for community composition between the water samples taken at the wetland entry and exit. Comparison of community diversity between the wetland entry and exit did not yield significant differences. However, a greater number of indicator species were identified at the wetland exit than at the wetland entry, suggesting that future analyses at a greater depth could focus on this element of the current study. For the proof of concept stage of analysis, the overall community composition comparisons suggest that long term trends are of greater importance for wetland validation and that further research could focus on sediment testing only.

Relating to functional genes for the first hypothesis, data was only obtained for a small subset of sediment samples collected during the field study and thus, final conclusions could not be drawn at the time of writing. Initial results suggest that there was some clustering of metagenomes based on the location where samples were collected in the wetland and that the date in which samples were collected was less important than the location in which samples were collected. These observations fit positively with the hypothesis that there is a shift in functional genes between the wetland entry and exit; however further investigation is required for validation of this hypothesis.

Regarding the second hypothesis, correlating contaminants with microbial communities and functions, additional conclusions can be drawn. Some significant correlations were determined between the wetland entry and exit. However, challenges remain where metal concentrations are only slightly higher than detection limit concentrations using ICP-OES analysis. Copper, nickel, and chromium displayed the strongest correlations with the microbial communities (p-values < 0.05) between the wetland entry and wetland exit. Likewise, some functional gene sequences that are known to code for metal tolerances had higher relative abundances in samples that were measured to have higher metal concentrations. For example, this was evident for both the znuB gene, which codes for zinc resistance and the czcA gene, which codes for nickel/cobalt resistance. That being stated, there were great complexities among the functional genes data and it is important to evaluate the dataset as a greater whole before conclusions can be drawn.

Finally, regarding the third hypothesis, determining if exposure to stormwater will shift the microbial communities at an unconnected site, unique and interesting conclusions can be drawn. For the community bacteria compositions, no significant changes in microbial diversity were determined. However, for the water samples and surface sediment samples, there was a clear departure between the microbial communities in the sediment columns that were dosed with stormwater and the microbial communities that were dosed with distilled water. This trend was not evident in the 10-cm depth sediment samples, however. As with the wetland field study, there were also a greater number of indicator species identified in sediment columns that were dosed with distilled water over sediment columns that were dosed with stormwater.

Interestingly, some similarities were present between the field and column study for functional genes. The znuB gene and the czcA gene both had higher relative abundances after sixteen weeks in the columns that were dosed with stormwater versus the column that was dosed with distilled water. However, the same result between the laboratory and field studies was not evident for genes associated with zinc/manganese/iron, thus observations are not conclusive and further exploration of the data and

experimentation is required. Metal resistance is regulated by a wide host of cellular functions and because the data output is so large, challenges arise in identifying the factors that have the greatest influence.

3.7.2 Chapter Objectives

To support the goal of this study, to provide proof of concept data that supports or rejects developing a genomics-based monitoring tool for low impact design features that treat stormwater, including engineered wetlands, three objectives were previously stated for this chapter:

1. Apply genomics-based analysis methods to determine if there are shifts in the microbial communities and functional genes along the length of the Lost Lagoon wetland;
2. Determine if there is a correlation between the water and sediment quality, present over the study period, and the microbial communities and functional genes observed; and
3. Determine, through laboratory experimentation, if there are opportunities to expand and pursue genomics analyses at other low impact design features for stormwater treatment.

Comparing the results of the hypotheses tests in this chapter serves to support the first objective, (i.e. determining if microbial shifts exist along the length of the Lost Lagoon wetland). While diversity and indicator species did not prove to be significant measures for comparison, microbial community composition presented clear shifts between the wetland entry and wetland exit, as was confirmed using common statistical techniques in microbiology, including the Adonis test in the R vegan package. Comparison of the metagenomes presented similar results and helped to confirm that there is a change in the microbial community between the entry and exit of the wetland.

For the second objective, determining if correlations exist between sample quality and bacteria, some important conclusions were drawn; however, this objective could, perhaps, be taken further with future research. Using the envfit statistic in the R vegan package, some significant correlations were calculated between metal concentrations and microbial communities but the noise present in the data presents challenges for validating conclusions. Similar results were observed for functional genes responsible for metal tolerances. Sequences that were present in greater relative abundances, such as znuB and czcA were illustrated to demonstrate some correlation with metal concentrations; however, this result was complicated by a wide array of additional sequences that may play a part in metal tolerance but are not present in a high enough quantity to be measurable or comparable.

For the third and final objective in this chapter, performing laboratory experiments to determine if there may be opportunities to perform genomics analyses at other sites, the research presented here suggests that stormwater influences bacteria and that this may be exploited for treatment monitoring purposes.

Specifically, in the column study, the departure over time, of the microbial communities in the sediment columns that were dosed with stormwater from the sediment columns that were dosed with stormwater, suggests that there is causation between stormwater contamination and the composition of microbial communities. In addition, some similar results between the field and column studies, for dominant metal resistance genes, present promise for future research.

3.7.3 Final Remarks

The work described in this chapter effectively provided data to inform the three hypotheses that were laid out in this chapter and this also supported the three objectives described here. In doing so, this chapter has provided some interesting proof of concept for the application of genomics analyses for stormwater treatment monitoring purposes, particularly for engineered wetlands. As time continues, the expansion of datasets for bacterial species and gene annotation will improve the quality of future data comparisons, only adding to the interest in this field of research. This will be particularly valuable for metals that are toxic in low concentrations and for metals that do not have strong documentation for bacterial tolerances.

3.8 Limitations

Limitations for the environmental sampling in the Lost Lagoon wetland were described in Chapter 1 and similar limitations were present throughout the methodology described in Chapter 2. The limitations experienced here were mainly due to budget constraints that limited the number of unique samples that could be sequenced and analyzed. This was true for both the community analyses and functional gene analyses. Conclusions presented in this chapter are only true for the study presented here; they are not universal for wetland treatment systems. Further research at other stormwater treatment wetlands is required to validate the application of genomics as a viable treatment monitoring method. In addition, the suggestion of causation between stormwater contamination and microbial communities demonstrated in the laboratory column study requires repetition of the column study in its entirety before conclusions can be drawn. For the proof of concept, that genomics can be used to support monitoring and validating stormwater treatment wetlands, this chapter has laid a strong foundation for future research in support of designing a more concrete monitoring tool.

4. Discussion

In Chapter 1, the results of traditional analyses techniques for monitoring and validating the efficacy of an operating stormwater treatment wetland, namely the Lost Lagoon wetland, were described. These analyses were performed to support the notion that genomics-based analyses can be applied as a tool to enhance and/or improve traditional monitoring techniques. Conclusions in Chapter 1 suggested that the uncertainty inherent to traditional monitoring for stormwater treatment may be an area where additional analyses may provide support. In Chapter 2, the results of genomics-based analyses at the Lost Lagoon wetland and a laboratory study for stormwater dosing were described. Conclusions in Chapter 2 suggest that including genomics-based analyses in stormwater treatment monitoring may provide greater certainty of treatment efficacy using a lower number of samples for analyses. Here, a cost comparison of the traditional stormwater treatment wetland validation method described by Erickson, Weiss and Gulliver (2013) is compared to the method described in Chapter 1 and Chapter 2. Theoretical costs are calculated based on a both a full validation, as well as a single compliance monitoring event, using the Lost Lagoon wetland that was analyzed in this study as an example.

4.1 Cost Comparison of Wetland Validation Techniques

4.1.1 Sample Collection

For traditional stormwater treatment validation, Erickson, Weiss and Gulliver (2013) recommend the deployment of stormwater automatic samplers at the inlet and outlet pipes of the treatment wetland. Automatic samplers would be triggered during each storm event and a field technician would be required to visit the site to collect samples each time the automatic samplers were triggered. Using the Lost Lagoon wetland as an example, there are 166 storm events in Vancouver each year (Environment Canada, 2016) and thus sampling would need to occur at this frequency.

For genomics-based treatment validation, the sampling methods described in this study would be applied. Two field technicians would be required to extract samples from the wetland at two week intervals over the rain season, which is approximately 8 months in Vancouver, thus 16 sampling events would be required. *Table 44* lists the predicted labor cost for each sampling event based on a rate of $30 per hour per technician.

Table 44. Approximate Cost Per Day for Sample Collection

Sample Collection Labor	Cost/Day
Traditional	$ 90.00
Genomics	$ 480.00

The cost of labor for sample collection using traditional methods is calculated to be:

$$166\ storms \times 2\ years \times \frac{\$90}{storm} = \$29{,}880$$

Likewise, the cost of labor for sample collection using genomics-based methods is calculated to be:

$$16\ days \times \frac{\$480}{day} = \$15{,}360$$

4.1.2 Laboratory Analyses

Laboratory analyses of environmental samples presents a major cost for validating stormwater treatment wetlands. *Table 45* lists the approximate cost per sample for a traditional wetland validation. *Table 46* lists the approximate cost per sample for bacterial community analysis and corresponding analysis of trace metals in soil. *Table 47* lists the approximate cost per sample for functional genes analyses with corresponding analysis of trace metals in water samples.

Table 45. Approximate Cost Per Sample for Traditional Stormwater Quality Analysis

Traditional Analyses	
Element	Cost[1]
pH	$ 10.00
ORP	$ 10.00
Conductivity	$ 10.00
Turbidity	$ 10.00
TSS	$ 20.00
COD	$ 30.00
TOC	$ 30.00
Oil and Grease	$ 40.00
Trace Metals Water	$ 125.00
Total	**$ 285.00**

[1]*(Canadian Association for Laboratory Accreditation, 2016)*

Table 46. Approximate Cost Per Sample for Genomics-Based Stormwater Quality Analysis Using Length Comparison of Bacterial Communities

Genomics Analyses	
Element	Cost
Trace Metals Soil	$ 150.00[1]
MiSeq	$ 75.00[2]
Total	**$ 225.00**

[1]*(Canadian Association for Laboratory Accreditation, 2016)*
[2]*(Microbiome Insights, 2016)*

Table 47. Approximate Cost Per Sample for Genomics Stormwater Quality Analysis Using Entry and Exit Comparison of Bacterial Functional Genes

Genomics Analyses	
Element	Cost
Trace Metals Water	$ 125.00[1]
HiSeq	$ 850.00[2]
Total	**$ 975.00**

[1]*(Canadian Association for Laboratory Accreditation, 2016)*
[2]*(University of British Columbia Beatty Biodiversity Sequencing Centre, 2016)*

Using the values listed in *Table 44* through *Table 47* the costs for traditional and genomics laboratory analyses are as follows.

Traditional:

$$166\ storms \times 3\ (triplicates) \times 2\ locations \times 2\ years \times \frac{\$285}{sample} = \$567{,}720$$

Genomics:

$$16\ days \times 3\ (triplicates) \times 18\ locations \times \frac{\$225}{sample} = \$194{,}400$$

$$16\ days \times 3\ (triplicates) \times 2\ locations \times \frac{\$975}{sample} = \$93{,}600$$

$$Total = \$194{,}400 + \$93{,}600 = \$288{,}000$$

4.1.3 Total Cost of Data Acquisition

By summing the cost of sample collection and laboratory analyses, an estimate for the cost of data acquisition for both a traditional and a genomics-based wetland validation is calculated.

Traditional:

$$Total = \$567{,}720 + \$29{,}880 = \$597{,}600$$

Genomics:

$$Total = \$288{,}000 + \$15{,}360 = \$303{,}360$$

Only the cost for sample collection and laboratory analyses were included in the cost estimate because these two factors were deemed to be the items of greatest significance. The cost of sampling equipment would be relatively small compared to the cost of laboratory analyses, for example. This cost estimate suggests that the genomics-based method described in this study may represent a lower cost option for data acquisition for validating stormwater treatment wetlands than traditional techniques. The cost and time for data analysis and reporting is also a major item but is not included here.

4.2 Cost Comparison of a Single Wetland Monitoring Event

The results expressed in the previous section represent total cost figures for a full wetland validation study but it is expected that this level of effort would not be expended by a municipality that is operating a stormwater treatment wetland. From an engineering perspective, monitoring tends to only be performed on one date annually or even less frequently (Chris Johnston, personal communication). This is because regulatory agencies typically do not require performance monitoring for stormwater treatment systems for road runoff, even though contaminant concentrations may be greater than effluent guidelines. Single point in time monitoring events often provide inconclusive results, which can be a barrier for the installation of engineered wetlands. This was described further in Chapter 1. Strengthening the quality of single event monitoring data may be beneficial in the face of increasing regulatory requirements and a will among municipalities to implement low impact design features, such as engineered stormwater treatment wetlands.

Thus, using the Lost Lagoon wetland as an example, if a municipality only monitored the performance of the wetland on one day, the monitoring regime would be quite limited. Based on past single point in time monitoring including that of Hemmera (2013) and Thoren et al (2007), the traditional single monitoring event would theoretically include sampling approximately nine soil and water samples from the entry and exit of the wetland followed by metal analyses performed in duplicate. This would represent a cost of:

$$\frac{\$275}{sample} \times 9\ Locations \times 2\ Sites \times 2\ (duplicate)[analyses] + \$480\ [collection]\ = \$9{,}900$$

If that same single monitoring event also included genomics-based analyses with nine locations selected for 16s bacterial community analysis and three locations selected for metagenome analysis, the cost increase would be:

$$\left(\frac{\$75}{sample} \times 9\ Locations \times 2\ Sites \times 2\ (duplicate) + \$850 \times 3\ Locations \times 2\ Sites\right)[analyses] + \$480\ [collection]\ = \$7{,}800$$

While this represents a greater cost to acquire data, the added benefit for confidence in results may be worth the expense. In addition, as methods and scientific understanding of genetic data processing increases in the future, it may one day be possible to drop the sample size or to exclude some of the metal analyses entirely. Thus, for future monitoring efforts, there is a significant financial opportunity for genomics-based methods to outcompete traditional methods for stormwater treatment monitoring, particularly for low impact design systems where microbiota influence treatment performance.

5. Conclusion

The goal of this study was to provide proof of concept data to inform the development of a genomics-based tool for monitoring stormwater treatment wetlands. In the introduction, the motivation for improving wetland monitoring techniques was described and the Lost Lagoon wetland was illustrated as an ideal location to perform a case study. In Chapter 1, background details on stormwater contaminants, stormwater treatment wetlands and the Lost Lagoon wetland were outlined. Results of traditional monitoring were prepared and conclusions illustrated that there are shortcomings present with the status quo for traditional wetland monitoring. In Chapter 2, genomics-based methods were introduced as an additional technique for monitoring stormwater treatment wetlands. Results from DNA sequencing were compared using water and sediment samples extracted from the Lost Lagoon wetland and several outcomes suggested that bacteria may correlate with the performance of treatment wetlands. This was generally supported further using results from samples extracted during a stormwater dosing study using columns of soil sourced from the Stanley Park bog in Vancouver, British Columbia.

The discussion immediately before this section provided a brief cost comparison of traditional validation and monitoring and genomics-based validation and monitoring. This cost comparison highlighted that an full wetland validation study may be less expensive using genomics and that a single point in time wetland monitoring event may be more expensive using genomics, though the improvement on data and confidence in results could be worth the cost increase. In addition to providing proof of concept data and cost analyses, this study also included method development which should serve to refine future genomics-based studies for stormwater treatment wetlands and other low impact design features. Specifically, it was found that sediment sampling provided the greatest promise when attempting to discern long-term stormwater treatment trends in both the field wetland study and in the laboratory stormwater dosing study.

Within the limits of graduate studies research, this study achieved its goal. Proof of concept, for the application of genomics-based monitoring of stormwater treatment wetlands, was provided. It was demonstrated that genomics will supply benefits for future stormwater treatment monitoring endeavours and that additional investigation into this field is worthwhile.

6. Recommendations

6.1 Follow-On Research

This study provided useful proof of concept results and preliminary conclusions. However, there are several facets which were not in the scope of this research project and could be continued with further. These include:

- Measuring the quality of stormwater that enters and exits the Lost Lagoon wetland, through installation of an automatic sampler;
- Performing a validation on the sizing of the Lost Lagoon wetland forebay based on flow velocities;
- Performing more in depth analyses of other pollutants, including petrochemicals, exiting the Stanley Park Causeway and the analyzing the impact of these other pollutants on bacteria;
- Statistically correlating indicator species with metal concentrations;
- Statistically correlating known metal resistance genes with metal concentrations;
- Sequencing a larger number of metagenomes to increase the confidence in this study's findings;
- Repeating the metagenome analyses using an updated and more widely accepted annotation tool, such as the MG-RAST server;
- Performing some additional statistical analyses, as outlined by the independent review of this project included in Appendix K;
- Repeating the field study at additional stormwater treatment wetlands of similar and different configurations and comparing the findings with the results illustrated here;
- Repeating the column study using the same controls and comparing the findings with the results illustrated here;
- Repeating the column study using modified controls and comparing the findings with the results illustrated here; and
- Modifying the approach applied here for application at other low impact design sites such as retention ponds, absorbent landscapes, and swales, among others.

Based on these facets that were outside the scope of this research project, there are several follow-on recommendations. First, at the time of publication, the twelve metagenomes, which were analyzed in this thesis, had been submitted to the MG-RAST server for gene annotation. Analysis of these results will be used to inform the articles (listed in the Preface to this thesis), which are currently in preparation and will be submitted for publication. After this analysis is confirmed and finalized, the main follow-on recommendation resulting from the present study is to repeat the sampling and analysis methodology at

additional stormwater treatment wetlands. Ideally, a follow-on study would perform the methodology recommended here at two or more additional sites – one with similar structure to that of the Lost Lagoon wetland and one with an alternate structure. Following this, results could be compared between wetlands and more significant conclusions could be drawn as to the validity in applying genomics as a monitoring tool for engineered wetlands.

6.2 Application and Improvements of Study Methodology

The research presented here provided a broad analysis of data using several sample mediums. Based on outcomes described in Chapter 1 and Chapter 2, for follow-on phase applications of this research, some optimum choices for the sampling and analyses methodology include:

- During the rainy season, collect field samples at either two or four week intervals for at least four months but preferably eight months if time and budgets permit;
- Collect and analyze samples at the inlet and outlet of each wetland instead of along the entire length of each wetland;
- Follow the environmental sampling protocols described in Chapter 1 for both sediment and water samples but analyze all samples for environmental parameters in triplicate instead of in duplicate;
- Perform DNA analyses on surface sediment samples only;
- To reduce the overall number of samples in the que for DNA sequencing, homogenize surface sediment samples across the entire wetland entry and entire wetland exit for each date sampled instead of on a 1 m plot basis for each date sampled; and
- Follow the sequencing and bioinformatics methodologies described in Chapter 2 for both 16s and metagenome analyses but also consider modifying these techniques as new improvements become available.

Taken together, these improvements should allow for a more streamlined comparison of the treatment efficacy within each wetland and between different wetlands for future applications of the tool described within this document.

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Appendix A: Acid Digestion Procedure for Water and Sediment Samples

Acid digestion of sludge and manure for metals on ICP

This method is based on United States Environmental Protection Agency method 3050B. The method is not a total digestion technique. It is a very strong acid digestion that will dissolve almost all elements that could become environmentally available. Elements bound in silicate structures are not normally dissolved by this procedure.

This procedure uses very strong acid and peroxide. These chemicals are highly corrosive and can cause severe burns. Wear a splash shield, lab coat and rubber apron and gloves when handling them.

Equipment:

- BD-46 block digester – set at 140˚C, which will give a tube temperature of 95˚C
- Digestion tubes
- Cold fingers

Reagents:

- Concentrated nitric acid
- Concentrated hydrochloric acid
- 30% hydrogen peroxide
- Aqua regia – 1:3 volume ratio of hydrochloric acid and nitric acid

Procedure:

1. Put 5 mL of sample into a digestion tube 1
2. Add 5 mL of aqua regia or use a 1:1 volume ratio of nitric acid and hydrochloric acid 2
3. Add 1 mL of 30% hydrogen peroxide 3
4. Place a cold finger on the top of the tube
5. Heat at 95˚C for two hours in the block digester
6. Cool and make the volume up to 50 mL with deionized water
7. Filter through a hardened fast filter such as Whatman 54 or equivalent
8. Transfer to the appropriate autosampler test tubes
9. Run on the AA or the ICP

Digest a blank along with the samples.

Standards should be made up in a matrix to match the samples (10% aqua regia or 1:1 volume ratio of hydrochloric acid and nitric acid). If performing trace metal analysis, use trace level concentrated acids.

If a brown gas appears (NO_2) during the digestion, then the digestion is not complete. Add more nitric acid in 1 mL increments to each tube until it disappears.]

1. If using this procedure for soils, weigh out 0.10 g of dry sample
2. A 1:1 volume mixture of nitric acid and hydrochloric acid is easier to work with
3. Do not add 30% hydrogen peroxide if there is little organics in the sample

Appendix B: Historic Water and Sediment Quality Data for the Lost Lagoon Wetland

Appendix A

NORWEST LABS

Analytical Report

Norwest Labs
#104, 19575-55 A Ave.
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Fax: (604) 514-3323

Bill to: Vancouver Park Board
Report to: Vancouver Park Board
c/o 2099 Beach Avenue
Vancouver, BC, Canada
V6G 1Z4
Attn: Eric Meagher
Sampled By:
Company:

Project ID:
Name: Stanley Park Wetland Testing
Location:
LSD:
P.O.: 4500353208
Acct. Code:

NWL Lot ID: 524772
Control Number: 314001
Date Received: Feb 09, 2007
Date Reported: Feb 20, 2007
Report Number: 966301

		NWL Number	524772-1	524772-2	524772-3	
		Sample Description	Carex - 1A	Carex - 1B	Carex - 1C	
		Matrix	Tissue	Tissue	Tissue	
Analyte		**Units**	**Results**	**Results**	**Results**	**Detection Limit**
Metals Total						
Aluminum	Total (dry weight)	ug/g	1340	1910	1500	1
Antimony	Total (dry weight)	ug/g	6.65	12.0	4.4	0.5
Arsenic	Total (dry weight)	ug/g	16.9	1.7	1.1	0.2
Barium	Total (dry weight)	ug/g	78.6	54.9	40.1	0.03
Beryllium	Total (dry weight)	ug/g	0.04	0.095	0.050	0.01
Bismuth	Total (dry weight)	ug/g	<0.5	1.2	<0.5	0.5
Cadmium	Total (dry weight)	ug/g	1.0	1.2	1.1	0.05
Calcium	Total (dry weight)	ug/g	4610	3730	3160	2
Chromium	Total (dry weight)	ug/g	9.90	15.2	14.3	0.04
Cobalt	Total (dry weight)	ug/g	1.9	4.5	1.6	0.05
Copper	Total (dry weight)	ug/g	59.1	117	49.4	0.05
Iron	Total (dry weight)	ug/g	6410	4880	4050	1
Lead	Total (dry weight)	ug/g	24.0	39.5	15.8	0.3
Lithium	Total (dry weight)	ug/g	0.64	0.70	0.94	0.1
Magnesium	Total (dry weight)	ug/g	1440	793	1160	1
Manganese	Total (dry weight)	ug/g	247	589	314	0.3
Molybdenum	Total (dry weight)	ug/g	2.7	3.7	2.1	0.05
Nickel	Total (dry weight)	ug/g	6.29	9.51	8.32	0.1
Phosphorus	Total (dry weight)	ug/g	2250	1040	1370	1
Potassium	Total (dry weight)	ug/g	10800	1500	6530	5
Selenium	Total (dry weight)	ug/g	<0.2	<0.2	<0.2	0.3
Silver	Total (dry weight)	ug/g	<0.15	<0.15	<0.15	0.2
Sodium	Total (dry weight)	ug/g	2600	177	1000	1
Strontium	Total (dry weight)	ug/g	35.0	30.4	20.5	0.02
Titanium	Total (dry weight)	ug/g	28.7	61.2	66.2	0.05
Vanadium	Total (dry weight)	ug/g	8.44	9.06	7.68	0.1
Zinc	Total (dry weight)	ug/g	121	174	143	0.1
Zirconium	Total (dry weight)	ug/g	0.61	0.73	0.3	0.05
Thallium	Total (dry weight)	ug/g	1.6	1.6	1.2	0.3

NORWEST LABS

Analytical Report

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Bill to: Vancouver Park Board
Report to: Vancouver Park Board
c/o 2099 Beach Avenue
Vancouver, BC, Canada
V6G 1Z4
Attn: Eric Meagher
Sampled By:
Company:

Project ID:
Name: Stanley Park Wetland Testing
Location:
LSD:
P.O.: 4500353208
Acct. Code:

NWL Lot ID: 524772
Control Number: 314001
Date Received: Feb 09, 2007
Date Reported: Feb 20, 2007
Report Number: 966301

		NWL Number	524772-4	524772-5	524772-6	
		Sample Description	Carex - 2A	Carex - 2B	Carex - 2C	
		Matrix	Tissue	Tissue	Tissue	
Analyte		**Units**	**Results**	**Results**	**Results**	**Detection Limit**
Metals Total						
Aluminum	Total (dry weight)	ug/g	2090	4470	2110	1
Antimony	Total (dry weight)	ug/g	9.08	13.0	2.1	0.5
Arsenic	Total (dry weight)	ug/g	3.6	2.9	3.4	0.2
Barium	Total (dry weight)	ug/g	48.8	84.4	68.0	0.03
Beryllium	Total (dry weight)	ug/g	0.12	0.20	0.080	0.01
Bismuth	Total (dry weight)	ug/g	<0.5	0.92	<0.5	0.5
Cadmium	Total (dry weight)	ug/g	2.5	4.9	1.0	0.05
Calcium	Total (dry weight)	ug/g	4120	5230	3690	2
Chromium	Total (dry weight)	ug/g	14.2	29.5	17.8	0.04
Cobalt	Total (dry weight)	ug/g	4.1	5.99	3.1	0.05
Copper	Total (dry weight)	ug/g	140	177	42.0	0.05
Iron	Total (dry weight)	ug/g	7020	10200	7010	1
Lead	Total (dry weight)	ug/g	38.3	50.7	14.0	0.3
Lithium	Total (dry weight)	ug/g	0.95	2.6	1.4	0.1
Magnesium	Total (dry weight)	ug/g	626	1640	1410	1
Manganese	Total (dry weight)	ug/g	669	784	739	0.3
Molybdenum	Total (dry weight)	ug/g	4.1	6.35	1.7	0.05
Nickel	Total (dry weight)	ug/g	7.69	18.7	8.45	0.1
Phosphorus	Total (dry weight)	ug/g	1450	2410	2610	1
Potassium	Total (dry weight)	ug/g	1120	3120	11800	5
Selenium	Total (dry weight)	ug/g	<0.25	<0.25	<0.25	0.3
Silver	Total (dry weight)	ug/g	<0.15	<0.15	<0.15	0.2
Sodium	Total (dry weight)	ug/g	254	492	1120	1
Strontium	Total (dry weight)	ug/g	29.5	39.7	24.9	0.02
Titanium	Total (dry weight)	ug/g	37.0	59.8	57.2	0.05
Vanadium	Total (dry weight)	ug/g	9.64	16.2	7.98	0.1
Zinc	Total (dry weight)	ug/g	203	386	119	0.1
Zirconium	Total (dry weight)	ug/g	<0.05	1.2	<0.05	0.05
Thallium	Total (dry weight)	ug/g	3.4	3.6	0.99	0.3

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Bill to: Vancouver Park Board
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V6G 1Z4
Attn: Eric Meagher
Sampled By:
Company:

Project ID:
Name: Stanley Park Wetland Testing
Location:
LSD:
P.O.: 4500353208
Acct. Code:

NWL Lot ID: 524772
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Date Received: Feb 09, 2007
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		NWL Number	524772-7	524772-8	524772-9	
		Sample Description	Carex - 3A	Carex - 3B	Carex - 3C	
		Matrix	Tissue	Tissue	Tissue	
Analyte		**Units**	**Results**	**Results**	**Results**	**Detection Limit**
Metals Total						
Aluminum	Total (dry weight)	ug/g	5900	644	4650	1
Antimony	Total (dry weight)	ug/g	<0.50	0.76	<0.5	0.5
Arsenic	Total (dry weight)	ug/g	3.3	1.4	4.0	0.2
Barium	Total (dry weight)	ug/g	44.2	69.3	75.5	0.03
Beryllium	Total (dry weight)	ug/g	0.11	0.02	0.12	0.01
Bismuth	Total (dry weight)	ug/g	<0.50	<0.5	<0.5	0.5
Cadmium	Total (dry weight)	ug/g	0.5	0.1	0.5	0.05
Calcium	Total (dry weight)	ug/g	4660	3830	6500	2
Chromium	Total (dry weight)	ug/g	77.5	3.58	39.4	0.04
Cobalt	Total (dry weight)	ug/g	4.6	1.1	4.4	0.05
Copper	Total (dry weight)	ug/g	27.4	14.1	49.4	0.05
Iron	Total (dry weight)	ug/g	11000	3960	10400	1
Lead	Total (dry weight)	ug/g	7.55	4.2	15.1	0.3
Lithium	Total (dry weight)	ug/g	5.00	0.53	3.6	0.1
Magnesium	Total (dry weight)	ug/g	3400	1150	2490	1
Manganese	Total (dry weight)	ug/g	288	130	555	0.3
Molybdenum	Total (dry weight)	ug/g	1.6	0.54	2.1	0.05
Nickel	Total (dry weight)	ug/g	34.3	2.7	21.4	0.1
Phosphorus	Total (dry weight)	ug/g	1240	3520	1440	1
Potassium	Total (dry weight)	ug/g	2040	12200	1160	5
Selenium	Total (dry weight)	ug/g	<0.2	<0.2	<0.25	0.3
Silver	Total (dry weight)	ug/g	<0.15	<0.15	<0.15	0.2
Sodium	Total (dry weight)	ug/g	686	2080	385	1
Strontium	Total (dry weight)	ug/g	25.8	36.0	36.2	0.02
Titanium	Total (dry weight)	ug/g	394	21.0	240	0.05
Vanadium	Total (dry weight)	ug/g	24.0	2.5	20.8	0.1
Zinc	Total (dry weight)	ug/g	77.6	33.5	122	0.1
Zirconium	Total (dry weight)	ug/g	2.0	0.2	1.9	0.05
Thallium	Total (dry weight)	ug/g	<0.25	1.5	<0.25	0.3

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Control Number: 314001
Date Received: Feb 09, 2007
Date Reported: Feb 20, 2007
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		NWL Number	524772-10	524772-11	524772-12	
		Sample Description	Scirpus - 1A	Scirpus - 1B	Scirpus - 1C	
		Matrix	Tissue	Tissue	Tissue	
Analyte		**Units**	**Results**	**Results**	**Results**	**Detection Limit**
Metals Total						
Aluminum	Total (dry weight)	ug/g	3200	4910	572	1
Antimony	Total (dry weight)	ug/g	12.8	4.8	<0.5	0.5
Arsenic	Total (dry weight)	ug/g	6.75	5.65	0.5	0.2
Barium	Total (dry weight)	ug/g	39.8	43.1	16.6	0.03
Beryllium	Total (dry weight)	ug/g	0.095	0.11	<0.01	0.01
Bismuth	Total (dry weight)	ug/g	1.1	1.0	<0.5	0.5
Cadmium	Total (dry weight)	ug/g	1.1	0.83	0.06	0.05
Calcium	Total (dry weight)	ug/g	2770	4080	1980	2
Chromium	Total (dry weight)	ug/g	24.7	36.4	7.71	0.04
Cobalt	Total (dry weight)	ug/g	1.5	2.6	0.4	0.05
Copper	Total (dry weight)	ug/g	107	85.5	11.4	0.05
Iron	Total (dry weight)	ug/g	8010	7780	1300	1
Lead	Total (dry weight)	ug/g	52.8	36.5	1.0	0.3
Lithium	Total (dry weight)	ug/g	2.0	3.6	0.64	0.1
Magnesium	Total (dry weight)	ug/g	1600	2440	1050	1
Manganese	Total (dry weight)	ug/g	220	232	481	0.3
Molybdenum	Total (dry weight)	ug/g	3.4	2.4	0.4	0.05
Nickel	Total (dry weight)	ug/g	6.80	12.6	2.8	0.1
Phosphorus	Total (dry weight)	ug/g	3360	1290	3640	1
Potassium	Total (dry weight)	ug/g	12000	3910	19900	5
Selenium	Total (dry weight)	ug/g	<0.2	<0.2	<0.2	0.3
Silver	Total (dry weight)	ug/g	<0.15	<0.15	<0.1	0.2
Sodium	Total (dry weight)	ug/g	2730	1450	3910	1
Strontium	Total (dry weight)	ug/g	21.4	29.8	14.1	0.02
Titanium	Total (dry weight)	ug/g	61.0	195	29.5	0.05
Vanadium	Total (dry weight)	ug/g	15.4	22.0	2.9	0.1
Zinc	Total (dry weight)	ug/g	160	138	29.3	0.1
Zirconium	Total (dry weight)	ug/g	0.76	0.63	0.79	0.05
Thallium	Total (dry weight)	ug/g	1.5	<0.2	1.2	0.3

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c/o 2099 Beach Avenue
Vancouver, BC, Canada
V6G 1Z4
Attn: Eric Meagher
Sampled By:
Company:

Project ID:
Name: Stanley Park Wetland Testing
Location:
LSD:
P.O.: 4500353208
Acct. Code:

NWL Lot ID: 524772
Control Number: 314001
Date Received: Feb 09, 2007
Date Reported: Feb 20, 2007
Report Number: 966301

		NWL Number	524772-13	524772-14	524772-15	
		Sample Description	Scirpus - 2A	Scirpus - 2B	Scirpus - 2C	
		Matrix	Tissue	Tissue	Tissue	
Analyte		**Units**	**Results**	**Results**	**Results**	**Detection Limit**
Metals Total						
Aluminum	Total (dry weight)	ug/g	626	2380	4140	1
Antimony	Total (dry weight)	ug/g	1.0	2.6	<0.5	0.5
Arsenic	Total (dry weight)	ug/g	1.2	1.5	2.4	0.2
Barium	Total (dry weight)	ug/g	27.6	46.0	50.0	0.03
Beryllium	Total (dry weight)	ug/g	0.02	0.075	0.080	0.01
Bismuth	Total (dry weight)	ug/g	<0.5	<0.5	<0.5	0.5
Cadmium	Total (dry weight)	ug/g	0.4	1.0	0.2	0.05
Calcium	Total (dry weight)	ug/g	4780	4000	3810	2
Chromium	Total (dry weight)	ug/g	5.22	37.2	93.4	0.04
Cobalt	Total (dry weight)	ug/g	0.89	2.6	4.1	0.05
Copper	Total (dry weight)	ug/g	27.6	61.0	27.6	0.05
Iron	Total (dry weight)	ug/g	1920	4850	9840	1
Lead	Total (dry weight)	ug/g	6.07	16.4	6.60	0.3
Lithium	Total (dry weight)	ug/g	0.52	1.8	3.6	0.1
Magnesium	Total (dry weight)	ug/g	1440	1810	2770	1
Manganese	Total (dry weight)	ug/g	468	634	640	0.3
Molybdenum	Total (dry weight)	ug/g	1.2	2.1	1.5	0.05
Nickel	Total (dry weight)	ug/g	3.6	10.7	17.8	0.1
Phosphorus	Total (dry weight)	ug/g	3440	2950	2540	1
Potassium	Total (dry weight)	ug/g	12600	7750	7250	5
Selenium	Total (dry weight)	ug/g	<0.2	<0.25	<0.25	0.3
Silver	Total (dry weight)	ug/g	<0.1	<0.15	<0.15	0.2
Sodium	Total (dry weight)	ug/g	2430	2760	1340	1
Strontium	Total (dry weight)	ug/g	27.7	28.9	23.6	0.02
Titanium	Total (dry weight)	ug/g	19.5	44.5	108	0.05
Vanadium	Total (dry weight)	ug/g	2.7	9.62	18.3	0.1
Zinc	Total (dry weight)	ug/g	314	159	80.3	0.1
Zirconium	Total (dry weight)	ug/g	0.56	0.4	0.56	0.05
Thallium	Total (dry weight)	ug/g	1.4	1.0	<0.2	0.3

NORWEST LABS

Analytical Report

Norwest Labs
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Bill to: Vancouver Park Board
Report to: Vancouver Park Board
c/o 2099 Beach Avenue
Vancouver, BC, Canada
V6G 1Z4
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		NWL Number	524772-16	524772-17	524772-18	
		Sample Description	Scirpus - 3A	Scirpus - 3B	Scirpus - 3C	
		Matrix	Tissue	Tissue	Tissue	
Analyte		**Units**	**Results**	**Results**	**Results**	**Detection Limit**
Metals Total						
Aluminum	Total (dry weight)	ug/g	365	2640	3100	1
Antimony	Total (dry weight)	ug/g	<0.5	1.8	0.80	0.5
Arsenic	Total (dry weight)	ug/g	1.5	3.7	16.9	0.2
Barium	Total (dry weight)	ug/g	19.1	97.9	60.0	0.03
Beryllium	Total (dry weight)	ug/g	<0.01	0.089	0.10	0.01
Bismuth	Total (dry weight)	ug/g	<0.5	0.58	0.50	0.5
Cadmium	Total (dry weight)	ug/g	<0.05	0.55	0.4	0.05
Calcium	Total (dry weight)	ug/g	1450	5100	4950	2
Chromium	Total (dry weight)	ug/g	6.08	26.6	23.5	0.04
Cobalt	Total (dry weight)	ug/g	0.83	3.0	5.60	0.05
Copper	Total (dry weight)	ug/g	9.38	37.8	43.3	0.05
Iron	Total (dry weight)	ug/g	1400	9750	13100	1
Lead	Total (dry weight)	ug/g	0.97	10.3	12.0	0.3
Lithium	Total (dry weight)	ug/g	0.52	1.8	2.3	0.1
Magnesium	Total (dry weight)	ug/g	1290	1650	1770	1
Manganese	Total (dry weight)	ug/g	334	309	748	0.3
Molybdenum	Total (dry weight)	ug/g	0.2	1.3	2.3	0.05
Nickel	Total (dry weight)	ug/g	4.3	12.2	15.0	0.1
Phosphorus	Total (dry weight)	ug/g	6930	2670	2490	1
Potassium	Total (dry weight)	ug/g	22400	6620	10000	5
Selenium	Total (dry weight)	ug/g	<0.2	<0.25	<0.25	0.3
Silver	Total (dry weight)	ug/g	<0.1	<0.15	<0.15	0.2
Sodium	Total (dry weight)	ug/g	1880	1360	1010	1
Strontium	Total (dry weight)	ug/g	11.1	34.5	34.3	0.02
Titanium	Total (dry weight)	ug/g	14.0	54.8	73.6	0.05
Vanadium	Total (dry weight)	ug/g	1.7	11.7	14.3	0.1
Zinc	Total (dry weight)	ug/g	32.6	106	155	0.1
Zirconium	Total (dry weight)	ug/g	0.3	0.63	0.62	0.05
Thallium	Total (dry weight)	ug/g	1.2	1.2	1.1	0.3

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NWL Lot ID: 524772
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		NWL Number	524772-19			
		Sample Description	Bird Poop			
		Matrix	Tissue			
Analyte		**Units**	**Results**	**Results**	**Results**	**Detection Limit**
Metals Total						
Aluminum	Total (dry weight)	ug/g	4050			1
Antimony	Total (dry weight)	ug/g	<0.50			0.5
Arsenic	Total (dry weight)	ug/g	0.95			0.2
Barium	Total (dry weight)	ug/g	28.3			0.03
Beryllium	Total (dry weight)	ug/g	0.060			0.01
Bismuth	Total (dry weight)	ug/g	0.60			0.5
Cadmium	Total (dry weight)	ug/g	0.1			0.05
Calcium	Total (dry weight)	ug/g	3960			2
Chromium	Total (dry weight)	ug/g	9.19			0.04
Cobalt	Total (dry weight)	ug/g	1.6			0.05
Copper	Total (dry weight)	ug/g	16.5			0.05
Iron	Total (dry weight)	ug/g	5630			1
Lead	Total (dry weight)	ug/g	3.9			0.3
Lithium	Total (dry weight)	ug/g	3.6			0.1
Magnesium	Total (dry weight)	ug/g	2370			1
Manganese	Total (dry weight)	ug/g	223			0.3
Molybdenum	Total (dry weight)	ug/g	1.5			0.05
Nickel	Total (dry weight)	ug/g	6.66			0.1
Phosphorus	Total (dry weight)	ug/g	2360			1
Potassium	Total (dry weight)	ug/g	14700			5
Selenium	Total (dry weight)	ug/g	<0.2			0.3
Silver	Total (dry weight)	ug/g	<0.15			0.2
Sodium	Total (dry weight)	ug/g	1060			1
Strontium	Total (dry weight)	ug/g	20.5			0.02
Titanium	Total (dry weight)	ug/g	196			0.05
Vanadium	Total (dry weight)	ug/g	14.5			0.1
Zinc	Total (dry weight)	ug/g	35.3			0.1
Zirconium	Total (dry weight)	ug/g	0.1			0.05
Thallium	Total (dry weight)	ug/g	<0.25			0.3

Approved by:

Walter Brandl
Operations Manager - Surrey

NORWEST LABS

Methodology and Notes

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Method of Analysis:

MethodName	Reference		Method	Date Analysis Started	Location
Metals (Total) dry weight	US EPA	*	Metals & Trace Elements by ICP-AES, 6010B	19-Feb-07	Norwest Labs Surrey

* Norwest method(s) is based on reference method

References:
US EPA — US Environmental Protection Agency Test Methods

Comments:

Please direct any inquiries regarding this report to our Client Services group.
Results relate only to samples as submitted

Appendix B. Appendix B

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Analytical Report

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Acct. Code:

NWL Lot ID: 524777
Control Number: 314038
Date Received: Feb 09, 2007
Date Reported: Feb 15, 2007
Report Number: 966305

	NWL Number	524777-1	524777-2	524777-3	
	Sample Description	Soil - 1A	Soil - 1B	Soil - 1C	
	Matrix	Soil	Soil	Soil	
Analyte	**Units**	**Results**	**Results**	**Results**	**Detection Limit**
Metals Strong Acid Digestion					
Antimony Strong Acid Extractable	ug/g	4.6	1.0	0.9	0.5
Arsenic Strong Acid Extractable	ug/g	5.3	4.3	3.8	0.2
Barium Strong Acid Extractable	ug/g	66.2	55.5	47.0	0.03
Beryllium Strong Acid Extractable	ug/g	0.24	0.16	0.17	0.01
Cadmium Strong Acid Extractable	ug/g	1.2	0.56	0.50	0.05
Chromium Strong Acid Extractable	ug/g	44.3	27.0	27.4	0.04
Cobalt Strong Acid Extractable	ug/g	5.20	5.51	5.52	0.05
Copper Strong Acid Extractable	ug/g	147	62.5	59.0	0.05
Lead Strong Acid Extractable	ug/g	71.0	24.1	24.7	0.3
Mercury Strong Acid Extractable	ug/g	0.059	0.036	0.023	0.003
Molybdenum Strong Acid Extractable	ug/g	4.3	1.8	1.8	0.05
Nickel Strong Acid Extractable	ug/g	27.4	26.6	26.7	0.1
Selenium Strong Acid Extractable	ug/g	0.6	0.8	0.8	0.3
Silver Strong Acid Extractable	ug/g	<0.2	<0.2	<0.2	0.2
Thallium Strong Acid Extractable	ug/g	<0.3	<0.3	<0.3	0.3
Tin Strong Acid Extractable	ug/g	5.4	2.0	1.8	0.2
Vanadium Strong Acid Extractable	ug/g	41.2	33.4	34.6	0.1
Zinc Strong Acid Extractable	ug/g	264	138	124	0.1
Soil Acidity					
pH 1:2 Soil:Water	pH	6.2	5.7	6.0	0.5

NORWEST LABS

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NWL Lot ID: 524777
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		NWL Number	524777-4	524777-5	524777-6	
		Sample Description	Soil - 1D	Soil - 1E	Soil - 2A	
		Matrix	Soil	Soil	Soil	
Analyte		**Units**	**Results**	**Results**	**Results**	**Detection Limit**
Metals Strong Acid Digestion						
Antimony	Strong Acid Extractable	ug/g	4.2	6.5	<0.5	0.5
Arsenic	Strong Acid Extractable	ug/g	3.7	4.1	3.2	0.2
Barium	Strong Acid Extractable	ug/g	74.2	111	45.2	0.03
Beryllium	Strong Acid Extractable	ug/g	0.19	0.30	0.14	0.01
Cadmium	Strong Acid Extractable	ug/g	0.5	1.7	0.1	0.05
Chromium	Strong Acid Extractable	ug/g	31.8	47.3	19.5	0.04
Cobalt	Strong Acid Extractable	ug/g	6.46	8.02	5.32	0.05
Copper	Strong Acid Extractable	ug/g	79.2	221	22.7	0.05
Lead	Strong Acid Extractable	ug/g	33.5	82.7	6.8	0.3
Mercury	Strong Acid Extractable	ug/g	0.041	0.079	0.022	0.003
Molybdenum	Strong Acid Extractable	ug/g	3.6	6.55	0.56	0.05
Nickel	Strong Acid Extractable	ug/g	26.8	33.6	23.3	0.1
Selenium	Strong Acid Extractable	ug/g	0.7	1.1	0.9	0.3
Silver	Strong Acid Extractable	ug/g	<0.2	<0.2	<0.2	0.2
Thallium	Strong Acid Extractable	ug/g	<0.3	<0.3	<0.3	0.3
Tin	Strong Acid Extractable	ug/g	3.9	7.7	0.4	0.2
Vanadium	Strong Acid Extractable	ug/g	37.0	46.6	33.4	0.1
Zinc	Strong Acid Extractable	ug/g	134	288	63.0	0.1
Soil Acidity						
pH	1:2 Soil:Water	pH	5.9	6.2	6.0	0.5

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NWL Lot ID: 524777
Control Number: 314038
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	NWL Number	524777-7	524777-8	524777-9	
	Sample Description	Soil - 2B	Soil - 2C	Soil - 2D	
	Matrix	Soil	Soil	Soil	
Analyte	**Units**	**Results**	**Results**	**Results**	**Detection Limit**
Metals Strong Acid Digestion					
Antimony Strong Acid Extractable	ug/g	<0.5	<0.5	<0.5	0.5
Arsenic Strong Acid Extractable	ug/g	2.2	1.7	2.6	0.2
Barium Strong Acid Extractable	ug/g	53.2	37.5	35.0	0.03
Beryllium Strong Acid Extractable	ug/g	0.14	0.13	0.13	0.01
Cadmium Strong Acid Extractable	ug/g	0.2	0.2	0.1	0.05
Chromium Strong Acid Extractable	ug/g	22.2	20.0	22.3	0.04
Cobalt Strong Acid Extractable	ug/g	6.49	5.91	5.03	0.05
Copper Strong Acid Extractable	ug/g	23.8	20.5	19.8	0.05
Lead Strong Acid Extractable	ug/g	6.9	6.0	5.4	0.3
Mercury Strong Acid Extractable	ug/g	0.022	0.024	0.023	0.003
Molybdenum Strong Acid Extractable	ug/g	1.3	0.58	0.52	0.05
Nickel Strong Acid Extractable	ug/g	24.6	25.0	24.7	0.1
Selenium Strong Acid Extractable	ug/g	0.6	<0.3	0.6	0.3
Silver Strong Acid Extractable	ug/g	<0.2	<0.2	<0.2	0.2
Thallium Strong Acid Extractable	ug/g	<0.3	<0.3	<0.3	0.3
Tin Strong Acid Extractable	ug/g	0.4	0.3	0.3	0.2
Vanadium Strong Acid Extractable	ug/g	33.9	29.3	35.8	0.1
Zinc Strong Acid Extractable	ug/g	79.2	61.3	55.3	0.1
Soil Acidity					
pH 1:2 Soil:Water	pH	5.7	6.0	6.0	0.5

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NWL Lot ID: 524777
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		NWL Number	524777-10	524777-11	524777-12	
		Sample Description	Soil - 2E	Soil - 3A	Soil - 3B	
		Matrix	Soil	Soil	Soil	
Analyte		**Units**	**Results**	**Results**	**Results**	**Detection Limit**
Metals Strong Acid Digestion						
Antimony	Strong Acid Extractable	ug/g	<0.5	<0.5	<0.5	0.5
Arsenic	Strong Acid Extractable	ug/g	2.0	2.0	1.1	0.2
Barium	Strong Acid Extractable	ug/g	36.0	36.0	36.9	0.03
Beryllium	Strong Acid Extractable	ug/g	0.12	0.13	0.15	0.01
Cadmium	Strong Acid Extractable	ug/g	0.2	0.1	0.2	0.05
Chromium	Strong Acid Extractable	ug/g	18.3	25.4	18.2	0.04
Cobalt	Strong Acid Extractable	ug/g	6.07	4.9	7.05	0.05
Copper	Strong Acid Extractable	ug/g	21.6	18.4	32.7	0.05
Lead	Strong Acid Extractable	ug/g	4.9	4.8	4.3	0.3
Mercury	Strong Acid Extractable	ug/g	0.019	0.018	0.015	0.003
Molybdenum	Strong Acid Extractable	ug/g	0.4	0.4	0.5	0.05
Nickel	Strong Acid Extractable	ug/g	20.6	23.5	19.8	0.1
Selenium	Strong Acid Extractable	ug/g	<0.3	0.7	<0.3	0.3
Silver	Strong Acid Extractable	ug/g	<0.2	<0.2	<0.2	0.2
Thallium	Strong Acid Extractable	ug/g	<0.3	<0.3	<0.3	0.3
Tin	Strong Acid Extractable	ug/g	0.2	0.2	0.4	0.2
Vanadium	Strong Acid Extractable	ug/g	36.2	33.5	41.7	0.1
Zinc	Strong Acid Extractable	ug/g	54.8	54.7	57.2	0.1
Soil Acidity						
pH	1:2 Soil:Water	pH	6.3	6.3	6.2	0.5

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	NWL Number	524777-13	524777-14	524777-15	
	Sample Description	Soil - 3C	Soil - 3D	Soil - 3E	
	Matrix	Soil	Soil	Soil	
Analyte	**Units**	**Results**	**Results**	**Results**	**Detection Limit**
Metals Strong Acid Digestion					
Antimony Strong Acid Extractable	ug/g	<0.5	<0.5	<0.5	0.5
Arsenic Strong Acid Extractable	ug/g	3.2	2.9	3.3	0.2
Barium Strong Acid Extractable	ug/g	39.2	31.6	46.2	0.03
Beryllium Strong Acid Extractable	ug/g	0.14	0.14	0.14	0.01
Cadmium Strong Acid Extractable	ug/g	0.1	0.2	0.1	0.05
Chromium Strong Acid Extractable	ug/g	17.5	19.4	22.3	0.04
Cobalt Strong Acid Extractable	ug/g	5.72	5.97	5.08	0.05
Copper Strong Acid Extractable	ug/g	23.5	24.9	19.8	0.05
Lead Strong Acid Extractable	ug/g	4.6	6.5	6.4	0.3
Mercury Strong Acid Extractable	ug/g	0.024	0.040	0.024	0.003
Molybdenum Strong Acid Extractable	ug/g	0.66	0.51	0.62	0.05
Nickel Strong Acid Extractable	ug/g	17.7	23.4	24.1	0.1
Selenium Strong Acid Extractable	ug/g	0.8	<0.3	1.0	0.3
Silver Strong Acid Extractable	ug/g	<0.2	<0.2	<0.2	0.2
Thallium Strong Acid Extractable	ug/g	<0.3	<0.3	<0.3	0.3
Tin Strong Acid Extractable	ug/g	0.2	0.3	0.3	0.2
Vanadium Strong Acid Extractable	ug/g	43.5	33.0	32.9	0.1
Zinc Strong Acid Extractable	ug/g	56.2	62.6	58.4	0.1
Soil Acidity					
pH 1:2 Soil:Water	pH	5.8	6.3	6.3	0.5

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NWL Lot ID: 524777
Control Number: 314038
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Date Reported: Feb 15, 2007
Report Number: 966305

	NWL Number	524777-16	524777-17		
	Sample Description	Soil - S-Outlet	Soil - S-Inlet		
	Matrix	Soil	Soil		
Analyte		**Units**	**Results**	**Results**	**Results**	**Detection Limit**
Metals Strong Acid Digestion						
Antimony	Strong Acid Extractable	ug/g	<0.5	5.6		0.5
Arsenic	Strong Acid Extractable	ug/g	2.2	3.3		0.2
Barium	Strong Acid Extractable	ug/g	49.5	63.0		0.03
Beryllium	Strong Acid Extractable	ug/g	0.20	0.21		0.01
Cadmium	Strong Acid Extractable	ug/g	0.73	0.53		0.05
Chromium	Strong Acid Extractable	ug/g	18.7	40.7		0.04
Cobalt	Strong Acid Extractable	ug/g	8.49	6.85		0.05
Copper	Strong Acid Extractable	ug/g	80.0	153		0.05
Lead	Strong Acid Extractable	ug/g	32.0	48.5		0.3
Mercury	Strong Acid Extractable	ug/g	0.047	0.041		0.003
Molybdenum	Strong Acid Extractable	ug/g	1.3	3.6		0.05
Nickel	Strong Acid Extractable	ug/g	18.3	19.7		0.1
Selenium	Strong Acid Extractable	ug/g	<0.3	1.6		0.3
Silver	Strong Acid Extractable	ug/g	<0.2	<0.2		0.2
Thallium	Strong Acid Extractable	ug/g	<0.3	<0.3		0.3
Tin	Strong Acid Extractable	ug/g	1.5	6.7		0.2
Vanadium	Strong Acid Extractable	ug/g	46.9	49.1		0.1
Zinc	Strong Acid Extractable	ug/g	94.1	246		0.1
Soil Acidity						
pH	1:2 Soil:Water	pH	6.6	6.5		0.5

Approved by:

Walter Brandl
Operations Manager - Surrey

NORWEST LABS

Methodology and Notes

Norwest Labs
#104, 19575-55 A Ave.
Surrey, BC. V3S 8P8
Phone: (604) 514-3322
Fax: (604) 514-3323

Bill to: Vancouver Park Board
Report to: Vancouver Park Board
c/o 2099 Beach Avenue
Vancouver, BC, Canada
V6G 1Z4
Attn: Eric Meagher
Sampled By:
Company:

Project ID:
Name: Stanley Park Wetland Testing
Location:
LSD:
P.O.: 4500353208
Acct. Code:

NWL Lot ID: 524777
Control Number: 314038
Date Received: Feb 09, 2007
Date Reported: Feb 15, 2007
Report Number: 966305

Method of Analysis:

MethodName	Reference		Method	Date Analysis Started	Location
Metals (Strong Acid Leachable) in soils	B.C.M.O.E	*	Strong Acid Leachable Metals (SALM) in Soil, V 1.0, SALM	14-Feb-07	Norwest Labs Surrey
pH and EC in Soil - 1:2 (Surrey)	McKeague	*	1:2 Soil:Water Ratio, 4.12	13-Feb-07	Norwest Labs Surrey

* Norwest method(s) is based on reference method

References:

B.C.M.O.E — B.C. Ministry of Environment
McKeague — Manual on Soil Sampling and Methods of Analysis

Comments:

HEMMERA ENVIROCHEM INC.
ATTN: James Mair
250 - 1380 Burrard Street
Vancouver BC V6Z 2H3

Date Received: 01-AUG-13
Report Date: 27-AUG-13 12:45 (MT)
Version: FINAL REV. 3

Client Phone: 604-669-0424

Certificate of Analysis

Lab Work Order #: **L1341753**
Project P.O. #: NOT SUBMITTED
Job Reference: 358-013.02
C of C Numbers: 10-340088, 10-340089
Legal Site Desc:

Comments: This report replaces the previous version and contains additional parameters.

Brent Mack
Account Manager

ALS ENVIRONMENTAL ANALYTICAL REPORT

Sample ID	L1341753-2	L1341753-3	L1341753-4	L1341753-6	L1341753-7
Description	Sediment	Sediment	Sediment	Sediment	Sediment
Sampled Date	31-JUL-13	31-JUL-13	31-JUL-13	31-JUL-13	31-JUL-13
Sampled Time					
Client ID	SS13-2	SS13-3	SS13-4	SS13-6	SS13-7
Grouping / **Analyte**					
SOIL					
Physical Tests Moisture (%)	71.4	72.8	71.8	72.8	70.1
pH (1:2 soil:water) (pH)	5.76	5.82	6.25	6.01	6.17
Saturated Paste Extractables Chloride (Cl) (mg/kg)			132	319	
% Saturation (%)			110	154	
Sodium (Na) (mg/kg)			194	103	
Metals Antimony (Sb) (mg/kg)	25.8	28.3	48.0	45.8	54.7
Arsenic (As) (mg/kg)	3.70	4.43	5.69	5.16	5.59
Barium (Ba) (mg/kg)	117	131	182	173	186
Beryllium (Be) (mg/kg)	<0.20	0.24	0.28	0.24	0.31
Cadmium (Cd) (mg/kg)	1.61	1.74	2.58	2.28	2.57
Chromium (Cr) (mg/kg)	101	112	131	135	134
Cobalt (Co) (mg/kg)	8.46	8.36	10.6	9.63	10.8
Copper (Cu) (mg/kg)	355	372	615	582	678
Lead (Pb) (mg/kg)	88.1	97.9	179	162	184
Mercury (Hg) (mg/kg)	0.085	0.085	0.150	0.128	0.154
Molybdenum (Mo) (mg/kg)	11.0	11.5	21.0	19.7	22.8
Nickel (Ni) (mg/kg)	29.4	28.7	37.5	36.3	39.3
Selenium (Se) (mg/kg)	0.98	1.18	1.11	1.07	1.30
Silver (Ag) (mg/kg)	0.39	0.34	0.58	0.57	0.73
Thallium (Tl) (mg/kg)	0.090	0.097	0.144	0.127	0.149
Tin (Sn) (mg/kg)	23.3	27.9	47.3	42.1	52.5
Uranium (U) (mg/kg)	0.473	0.500	0.702	0.648	0.723
Vanadium (V) (mg/kg)	46.2	48.3	67.6	61.0	68.4
Zinc (Zn) (mg/kg)	616	685	1010	950	1110
TCLP Extractables Benzo(a)pyrene (mg/L)					
1st Preliminary PH (pH)					
2nd Preliminary PH (pH)					
Extraction Solution Initial pH (pH)					
Final pH (pH)					
TCLP Metals Chromium (Cr)-Leachable (mg/L)					
Lead (Pb)-Leachable (mg/L)					
Volatile Organic Compounds Benzene (mg/kg)			0.038 ARL		
Bromodichloromethane (mg/kg)			<0.10		
Bromoform (mg/kg)			<0.10		
Carbon Tetrachloride (mg/kg)			<0.10		
Chlorobenzene (mg/kg)			<0.10		

* Please refer to the Reference Information section for an explanation of any qualifiers detected.

ALS ENVIRONMENTAL ANALYTICAL REPORT

	Sample ID	L1341753-9				
	Description	Sediment				
	Sampled Date	31-JUL-13				
	Sampled Time					
	Client ID	SS13-9				
Grouping	**Analyte**					
SOIL						
Physical Tests	Moisture (%)	68.8				
	pH (1:2 soil:water) (pH)	6.26				
Saturated Paste Extractables	Chloride (Cl) (mg/kg)					
	% Saturation (%)					
	Sodium (Na) (mg/kg)					
Metals	Antimony (Sb) (mg/kg)	63.9				
	Arsenic (As) (mg/kg)	6.83				
	Barium (Ba) (mg/kg)	205				
	Beryllium (Be) (mg/kg)	0.33				
	Cadmium (Cd) (mg/kg)	3.28				
	Chromium (Cr) (mg/kg)	135				
	Cobalt (Co) (mg/kg)	14.0				
	Copper (Cu) (mg/kg)	644				
	Lead (Pb) (mg/kg)	241				
	Mercury (Hg) (mg/kg)	0.183				
	Molybdenum (Mo) (mg/kg)	28.0				
	Nickel (Ni) (mg/kg)	45.9				
	Selenium (Se) (mg/kg)	0.92				
	Silver (Ag) (mg/kg)	0.76				
	Thallium (Tl) (mg/kg)	0.162				
	Tin (Sn) (mg/kg)	42.3				
	Uranium (U) (mg/kg)	0.846				
	Vanadium (V) (mg/kg)	75.5				
	Zinc (Zn) (mg/kg)	1150				
TCLP Extractables	Benzo(a)pyrene (mg/L)	<0.000050				
	1st Preliminary PH (pH)	7.13				
	2nd Preliminary PH (pH)	1.38				
	Extraction Solution Initial pH (pH)	4.95				
	Final pH (pH)	5.05				
TCLP Metals	Chromium (Cr)-Leachable (mg/L)	<0.25				
	Lead (Pb)-Leachable (mg/L)	<0.25				
Volatile Organic Compounds	Benzene (mg/kg)					
	Bromodichloromethane (mg/kg)					
	Bromoform (mg/kg)					
	Carbon Tetrachloride (mg/kg)					
	Chlorobenzene (mg/kg)					

* Please refer to the Reference Information section for an explanation of any qualifiers detected.

Reference Information

QC Samples with Qualifiers & Comments:

QC Type Description	Parameter	Qualifier	Applies to Sample Number(s)
Duplicate	Silver (Ag)	DUP-H,J	L1341753-2, -3, -4, -6, -7, -9
Laboratory Control Sample	Hexachlorocyclohexane (Total)	LCS-ND	L1341753-4
Laboratory Control Sample	Hexachloroethane	LCS-ND	L1341753-4

Qualifiers for Individual Parameters Listed:

Qualifier	Description
ABL	Approximate Result: May Be Biased Low
DLA	Detection Limit Adjusted For required dilution
DLHM	Detection Limit Adjusted: Sample has High Moisture Content
DLM	Detection Limit Adjusted For Sample Matrix Effects
DUP-H,J	Duplicate results outside ALS DQO, due to sample heterogeneity. Duplicate results and limits are expressed in terms of absolute difference.
LCS-ND	Lab Control Sample recovery was slightly outside ALS DQO. Reported non-detect results for associated samples were unaffected.
LSRA	Low surrogate recovery observed due to adsorptive material in sample (e.g. charcoal). Associated results represent solvent extractable concentrations.

Test Method References:

ALS Test Code	Matrix	Test Description	Method Reference**
CL-PASTE-COLOR-VA	Soil	Chloride in Soil (Paste) by Colourimetry	Carter-CSSS / APHA 4500-Cl E□modified)

A soil extract produced by the saturated paste extraction procedure is analyzed for chloride by ferricyanide colourimetry.

CLH-SOX-MS-VA	Soil	Chlorinated Hydrocarbons in Soil by GCMS	3510, 3610, 8121 SW-846 AND 8270 EPA

This analysis is carried out using procedures adapted from "Test Methods for Evaluating Solid Waste" SW-846, Methods 3540, 3610, 8270, published by the United States Environmental Protection Agency (EPA). The procedure uses a Soxhlet system to extract a subsample of the sediment/soilwith dichloromethane. The final extract is analysed by capillary column gas chromatography with mass spectrometric detection (GC/MS). Hexachlorocyclohexanes includes the alpha, beta, gamma, and delta isomers

CLPHEN-TMB-MS-VA	Soil	Chlorinated Phenols by Tumbler/GCMS	EPA 3570, 8270, Knapp(1979)

A subsample of the soil/sediment is rotary extracted by solvent, derivitized, and analysed by GC/MS.

EPH-TUMB-FID-VA	Soil	EPH in Solids by Tumbler and GCFID	BC MOE EPH GCFID

Analysis is in accordance with BC MOE Lab Manual method "Extractable Petroleum Hydrocarbons in Solids by GC/FID", v2.1, July 1999. Soil samples are extracted with a 1:1 mixture of hexane and acetone using a rotary extraction technique modified from EPA 3570 prior to gas chromatography with flame ionization detection (GC-FID). EPH results include Polycyclic Aromatic Hydrocarbons (PAH) and are therefore not equivalent to Light and Heavy Extractable Petroleum Hydrocarbons (LEPH/HEPH).

HG-200.2-CVAF-VA	Soil	Mercury in Soil by CVAFS	EPA 200.2/245.7

This analysis is carried out using procedures from CSR Analytical Method: "Strong Acid Leachable Metals (SALM) in Soil", BC Ministry of Environment, 26 June 2009, and procedures adapted from EPA Method 200.2. The sample is manually homogenized, dried at 60 degrees Celsius, sieved through a 2 mm (10 mesh) sieve (this sieve step is omitted for international soil samples), and a representative subsample of the dry material is weighed. The sample is then digested at 95 degrees Celsius for 2 hours by block digester using concentrated nitric and hydrochloric acids. Instrumental analysis is by atomic fluorescence spectrophotometry or atomic absorption spectrophotometry(EPA Method 245.7).

Method Limitation: This method is not a total digestion technique. It is a very strong acid digestion that is intended to dissolve those metals that may be environmentally available. By design, elements bound in silicate structures are not normally dissolved by this procedure as they are not usually mobile in the environment.

LEPH/HEPH-CALC-VA	Soil	LEPHs and HEPHs	BC MOE LABORATORY MANUAL (2005)

Light and Heavy Extractable Petroleum Hydrocarbons in Solids. These results are determined according to the British Columbia Ministry of Environment, Lands, and Parks Analytical Method for Contaminated Sites "Calculation of Light and Heavy Extractable Petroleum Hydrocarbons in Solids or Water". According to this method, LEPH and HEPH are calculated
by subtracting selected Polycyclic Aromatic Hydrocarbon results from Extractable Petroleum Hydrocarbon results. To calculate LEPH, the individual results for Naphthalene and Phenanthrene are subtracted from EPH(C10-19). To calculate HEPH, the individual results for Benz(a)anthracene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Dibenz(a,h)anthracene, Indeno(1,2,3-c,d)pyrene, and Pyrene
are subtracted from EPH(C19-32). Analysis of Extractable Petroleum Hydrocarbons adheres to all prescribed elements of the BCMELP method "Extractable Petroleum Hydrocarbons in Solids by GC/FID" (Version 2.1, July 20, 1999).

MET-200.2-CCMS-VA	Soil	Metals in Soil by CRC ICPMS	EPA 200.2/6020A

This analysis is carried out using procedures from CSR Analytical Method: "Strong Acid Leachable Metals (SALM) in Soil", BC Ministry of Environment, 26 June 2009, and procedures adapted from EPA Method 200.2. The sample is manually homogenized, dried at 60 degrees Celsius, sieved through a 2 mm (10 mesh) sieve (this sieve step is omitted for international soil samples), and a representative subsample of the dry material is weighed. The sample is then digested at 95 degrees Celsius for 2 hours by block digester using concentrated nitric and hydrochloric acids. Instrumental analysis of the digested extract is by collision cell inductively coupled plasma - mass spectrometry (modifed from EPA Method 6020A).

Reference Information

Method Limitation: This method is not a total digestion technique. It is a very strong acid digestion that is intended to dissolve those metals that may be environmentally available. By design, elements bound in silicate structures are not normally dissolved by this procedure as they are not usually mobile in the environment.

MET-TCLP-ICP-VA Soil Metals by ICPOES (TCLP) EPA 1311/6010B

This analysis is carried out in accordance with the extraction procedure outlined in "Test Methods for Evaluating Solid Waste - Physical/Chemical Methods Volume 1C" SW-846 EPA Method 1311, published by the United States Environmental Protection Agency (EPA). In summary, the sample is extracted at a 20:1 liquid to solids ratio for 16 to 20 hours using either extraction fluid #1 (glacial acetic acid, water and sodium hydroxide) or extraction fluid #2 (glacial acetic acid), depending on the pH of the original sample. The extract is then filtered through a 0.6 to 0.8 micron glass fibre filter and analysed using inductively coupled plasma - optical emission spectrophotometry (EPA Method 6010B).

MOISTURE-VA Soil Moisture content ASTM D2974-00 Method A

This analysis is carried out gravimetrically by drying the sample at 105 C for a minimum of six hours.

PAH-TCLP-SF-MS-VA Soil PAH's IN TCLP LEACHATE EPA 3510/8270 LIQ-LIQ GCMS

The sample is extracted at a 20:1 liquid to solids ratio for 16 to 20 hours using either extraction fluid #1 (acetic acid, water and sodium hydroxide) or extraction fluid #2 (acetic acid and water) depending on the pH of the original sample. The extract is filtered and then extracted with dichloromethane. The extract is solvent exchanged to toluene prior to analysis by capillary column gas chromatography with mass spectrometric detection (GC/MS). Because the two isomers cannot be readily chromatographically separated, benzo(j)fluoranthene is reported as part of the benzo(b)fluoranthene parameter.

PAH-TMB-H/A-MS-VA Soil PAH - Rotary Extraction (Hexane/Acetone) EPA 3570/8270

This analysis is carried out using procedures adapted from "Test Methods for Evaluating Solid Waste" SW-846, Methods 3545 & 8270, published by the United States Environmental Protection Agency (EPA). The procedure uses a mechanical shaking technique to extract a subsample of the sediment/soil with a 1:1 mixture of hexane and acetone. The extract is then solvent exchanged to toluene. The final extract is analysed by capillary column gas chromatography with mass spectrometric detection (GC/MS). Surrogate recoveries may not be reported in cases where interferences from the sample matrix prevent accurate quantitation. Because the two isomers cannot be readily chromatographically separated, benzo(j)fluoranthene is reported as part of the benzo(b)fluoranthene parameter.

PCB-SE-ECD-VA Soil PCB by Extraction with GCECD EPA8082, 3630

This analysis is carried out using procedures adapted from "Test Methods for Evaluating Solid Waste" SW-846, Methods 3500, 3620, 3630, 3660, 3665 & 8082, published by the United States Environmental Protection Agency (EPA). The procedure involves a solid-liquid extraction of a subsample of the sediment/soil using a mixture of hexane and acetone. Water is added to the extract and the resulting hexane extract undergoes one or more of the following clean-up procedures (if required): florisil clean-up, silica gel clean-up, sulphur clean-up and/or sulphuric acid clean-up. The final extract is analysed by capillary column gas chromatography with electron capture detection (GC/ECD).

PCB-SUM-CALC-VA Soil Total PCBs in soil CALCULATION

Calculation of Total PCB. Total PCB is the sum of the concentrations of PCB aroclors 1016, 1221, 1232, 1242, 1248, 1254, 1260, 1262, and 1268. Results below detection limit (DL) are treated as zero. The Total PCB detection limit is equal to the highest of the aroclor detection limits used in the sum.

PH-1:2-VA Soil pH in Soil (1:2 Soil:Water Extraction) BC WLAP METHOD: PH, ELECTROMETRIC, SOIL

This analysis is carried out in accordance with procedures described in the pH, Electrometric in Soil and Sediment method - Section B Physical/Inorganic and Misc. Constituents, BC Environmental Laboratory Manual 2007. The procedure involves mixing the dried (at <60□C) and sieved (No. 10 / 2mm) sample with deionized/distilled water at a 1:2 ratio of sediment to water. The pH of the solution is then measured using a standard pH probe.

PHEN-TMB-MS-VA Soil Phenolics by Tumbler/GC-MS EPA 3570 & 8270, Knapp (1979)

A subsample of the soil/sediment is rotary extracted by solvent, derivitized, and analysed by GC/MS.

PHTHALATE-ED Soil Phthalates EPA 3540/8270-GC/MS

SAR-CALC-MGKG-ICP-VA Soil SAR in Soil (Paste) by ICPOES Carter-CSSS / EPA 6010B (modified)

A soil extract produced by the saturated paste extraction procedure is analyzed for Sodium, Calcium, and Magnesium by ICPOES. Sodium Adsorption Ratio (SAR) is calculated as per "Soil Sampling and Methods of Analysis" by M. Carter.

SAT-PCNT-VA Soil Saturation Percentage Carter-CSSS

Saturation Percentage (SP) is the total volume of water present in a saturated paste (in mL) divided by the dry weight of the sample (in grams), expressed as a percentage, as described in "Soil Sampling and Methods of Analysis" by M. Carter.

VOC-HSMS-VA Soil VOCs in soil by Headspace GCMS EPA8260B, 5021, 5035, BC MOE

The soil methanol extract is added to water and reagents, then heated in a sealed vial to equilibrium. The headspace from the vial is transferred into a gas chromatograph. Target compound concentrations are measured using mass spectrometry detection.

VOC7-L-HSMS-VA Soil VOCs in soil by Headspace GCMS EPA8260B, 5021, 5035, BC MOE

The soil methanol extract is added to water and reagents, then heated in a sealed vial to equilibrium. The headspace from the vial is transferred into a gas chromatograph. Target compound concentrations are measured using mass spectrometry detection.

VOC7/VOC-SURR-MS-VA Soil VOC7 and/or VOC Surrogates for Soils EPA METHODS 8260B & 524.2

Reference Information

XYLENES-CALC-VA	Soil	Sum of Xylene Isomer Concentrations	EPA 8260B & 524.2

Calculation of Total Xylenes

Total Xylenes is the sum of the concentrations of the ortho, meta, and para Xylene isomers. Results below detection limit (DL) are treated as zero. The DL for Total Xylenes is set to a value no less than the square root of the sum of the squares of the DLs of the individual Xylenes.

** ALS test methods may incorporate modifications from specified reference methods to improve performance.

The last two letters of the above test code(s) indicate the laboratory that performed analytical analysis for that test. Refer to the list below:

Laboratory Definition Code	Laboratory Location
ED	ALS ENVIRONMENTAL - EDMONTON, ALBERTA, CANADA
VA	ALS ENVIRONMENTAL - VANCOUVER, BRITISH COLUMBIA, CANADA

Chain of Custody Numbers:

10-340088 10-340089

GLOSSARY OF REPORT TERMS

Surrogate - A compound that is similar in behaviour to target analyte(s), but that does not occur naturally in environmental samples. For applicable tests, surrogates are added to samples prior to analysis as a check on recovery.
mg/kg - milligrams per kilogram based on dry weight of sample.
mg/kg wwt - milligrams per kilogram based on wet weight of sample.
mg/kg lwt - milligrams per kilogram based on lipid-adjusted weight of sample.
mg/L - milligrams per litre.
< - Less than.
D.L. - The reported Detection Limit, also known as the Limit of Reporting (LOR).
N/A - Result not available. Refer to qualifier code and definition for explanation.

Test results reported relate only to the samples as received by the laboratory.
UNLESS OTHERWISE STATED, ALL SAMPLES WERE RECEIVED IN ACCEPTABLE CONDITION.
Analytical results in unsigned test reports with the DRAFT watermark are subject to change, pending final QC review.

Appendix C: Rainfall Records During Lost Lagoon Wetland Site Visits

Blue stars indicate the dates when sampling was performed at the Lost Lagoon wetland.

Data was retrieved from the Vancouver Harbour Weather Station (Environment Canada, 2016)

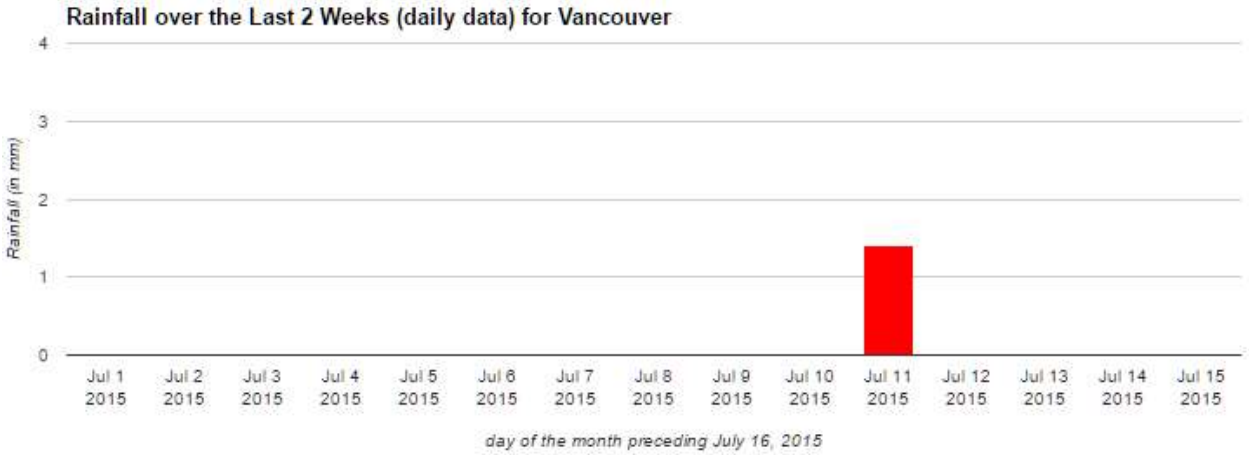


Figure 95. Rainfall Recorded for Downtown Vancouver Between July 1 and July 15, 2015

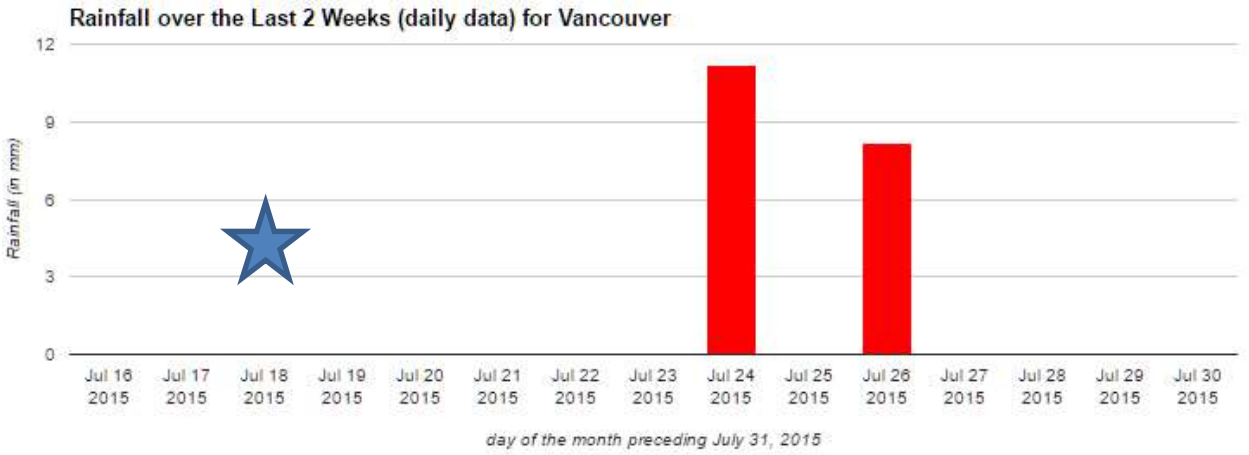


Figure 96. Rainfall Recorded for Downtown Vancouver Between July 15 and July 30, 2015

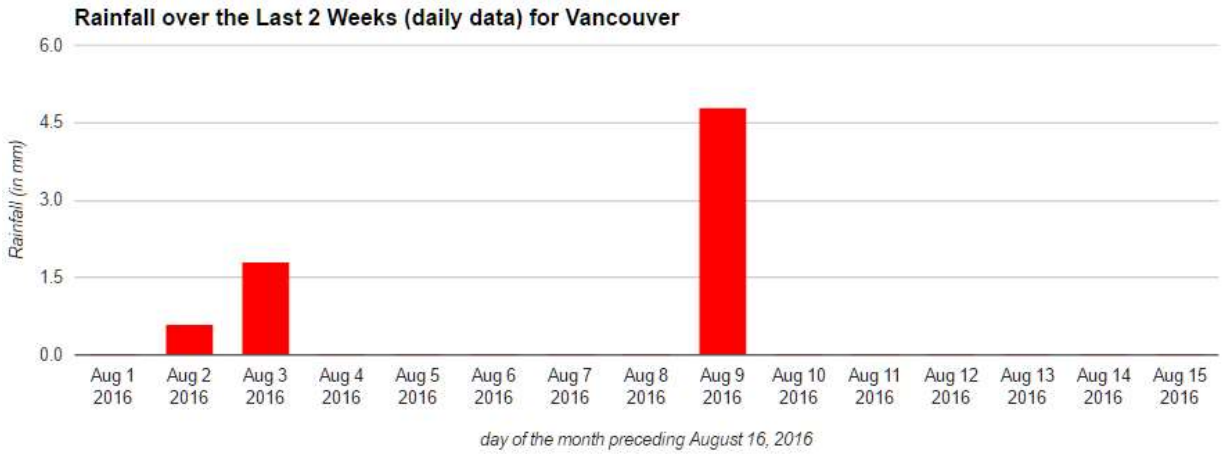


Figure 97. Rainfall Recorded for Downtown Vancouver between August 1 and August 15

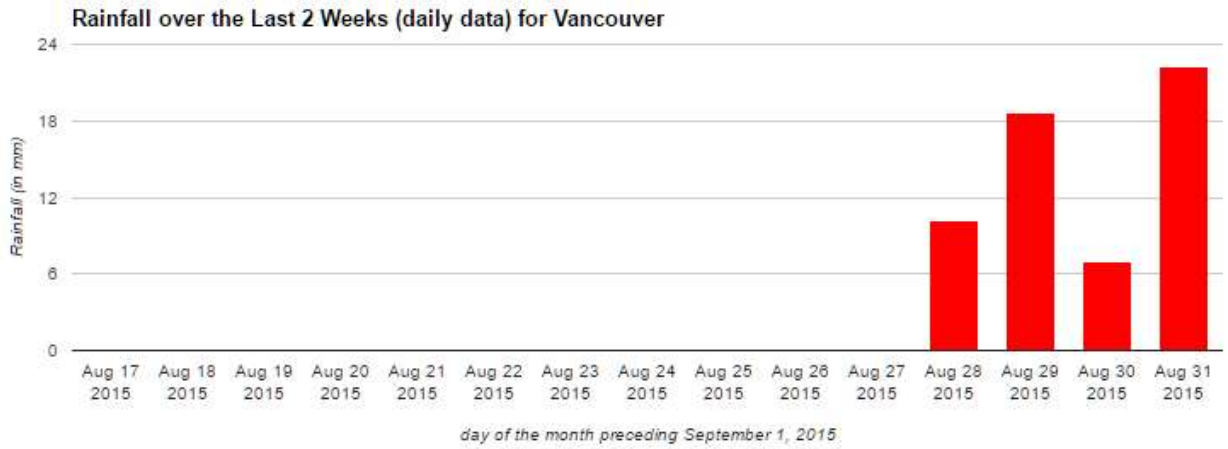


Figure 98. Rainfall Recorded for Downtown Vancouver Between August 17 and August 31, 2015

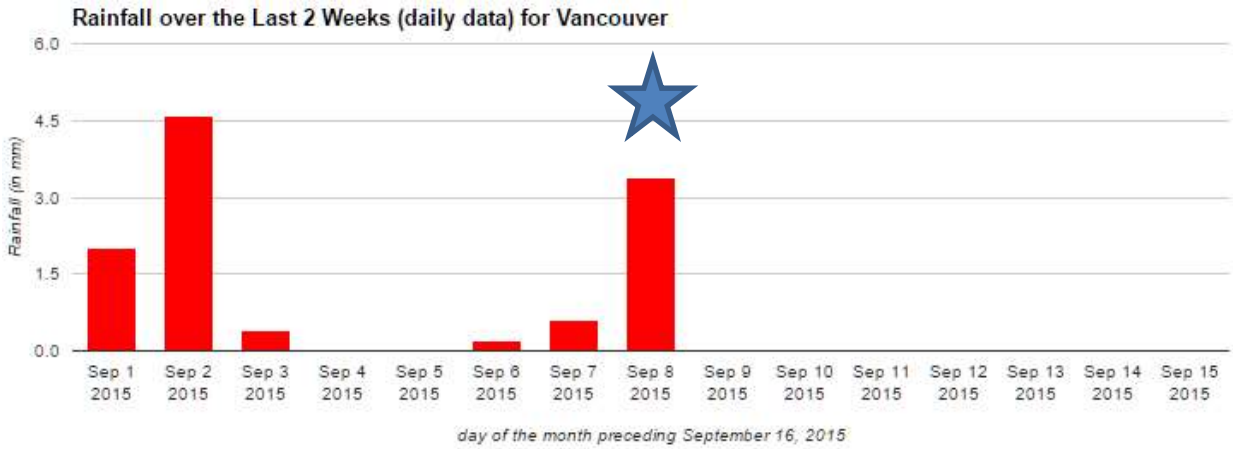


Figure 99. Rainfall Recorded for Downtown Vancouver Between September 1 and September 15, 2015

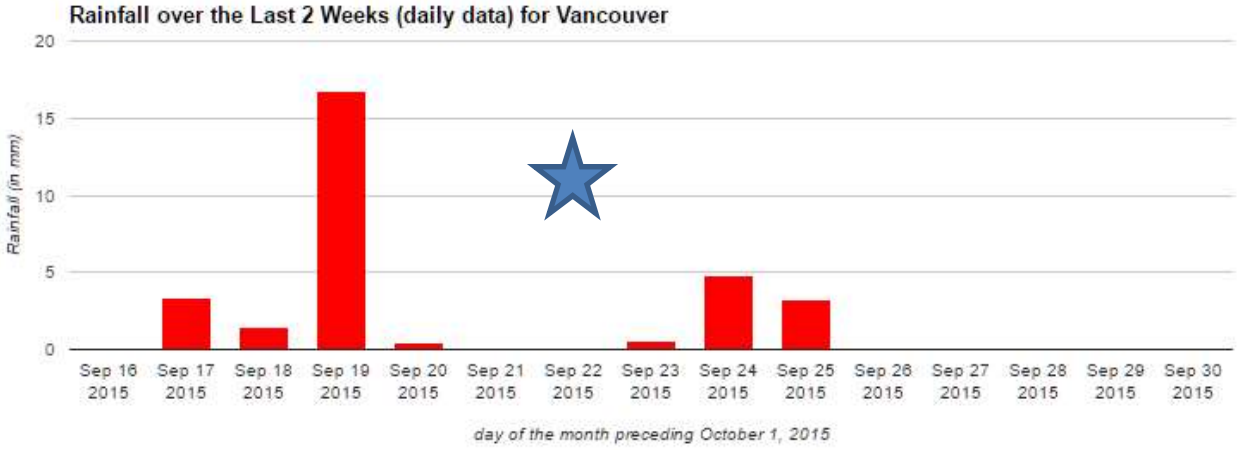


Figure 100. Rainfall Recorded for Downtown Vancouver Between September 16 and September 30, 2015

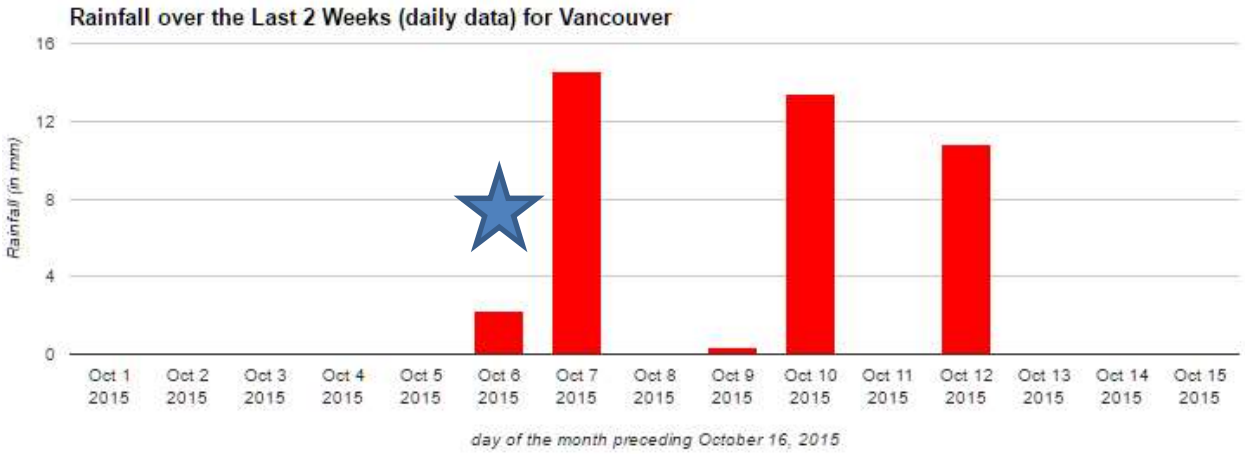


Figure 101. Rainfall Recorded for Downtown Vancouver Between October 1 and October 15, 2015

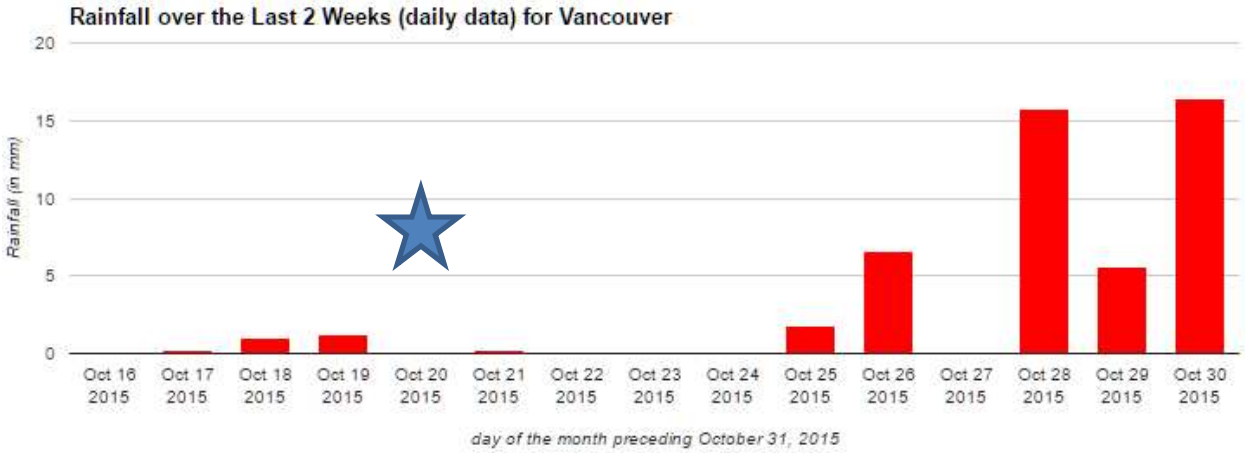


Figure 102. Rainfall Recorded for Downtown Vancouver Between October 16 and October 30, 2015

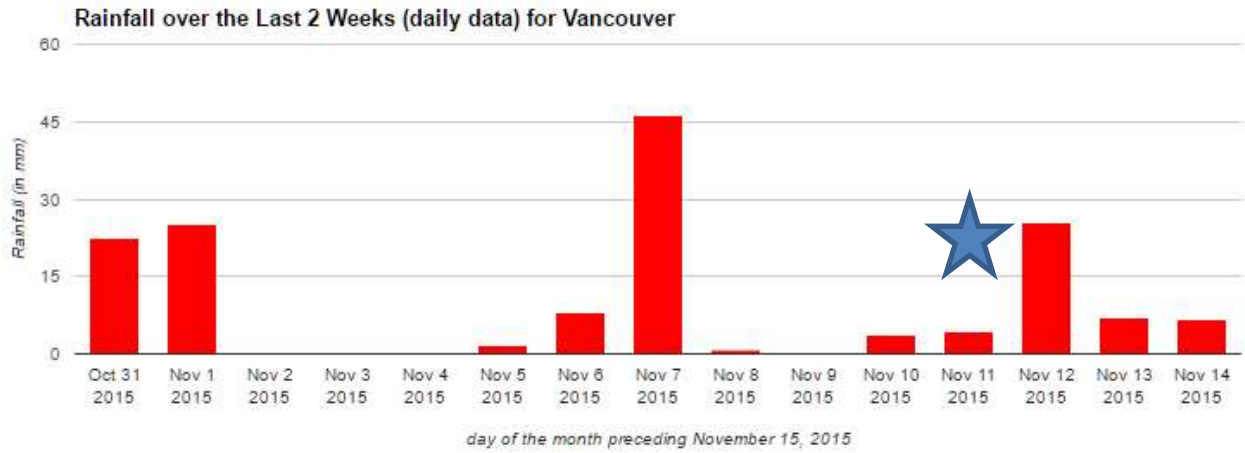


Figure 103. Rainfall Recorded for Downtown Vancouver Between October 31 and November 14, 2015

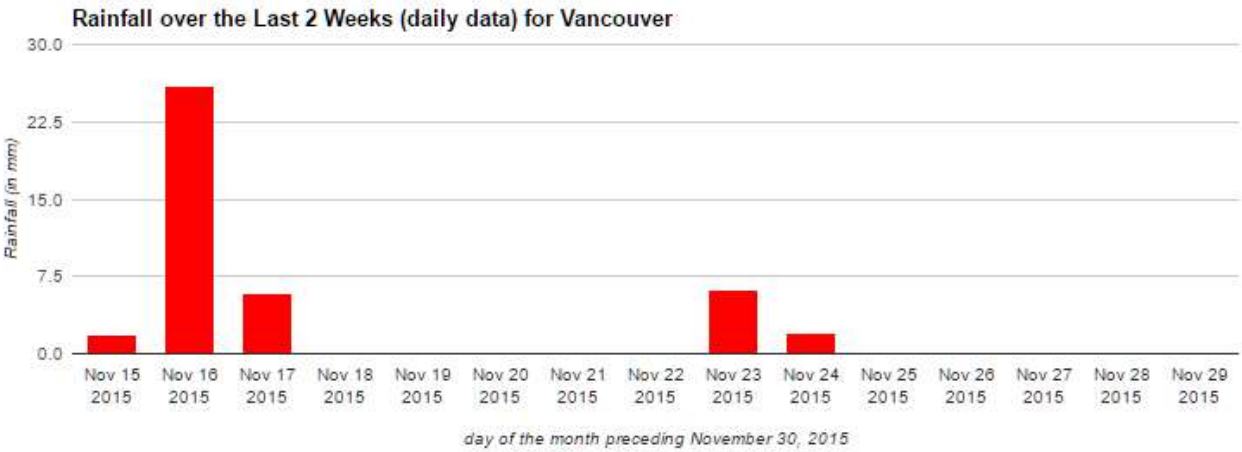


Figure 104. Rainfall Recorded for Downtown Vancouver Between November 15 and November 29, 2015

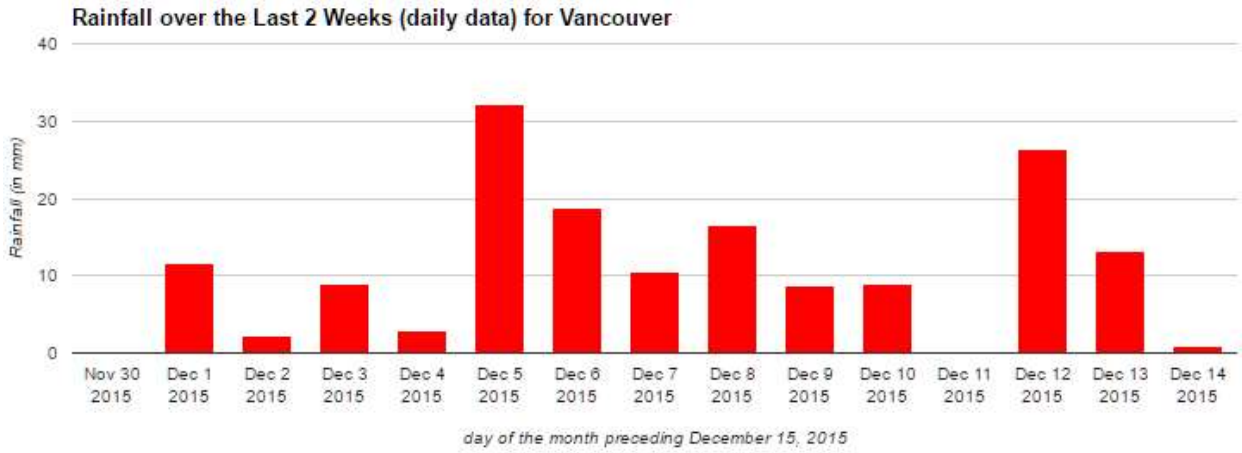


Figure 105. Rainfall Recorded for Downtown Vancouver Between November 30 and December 14, 2015

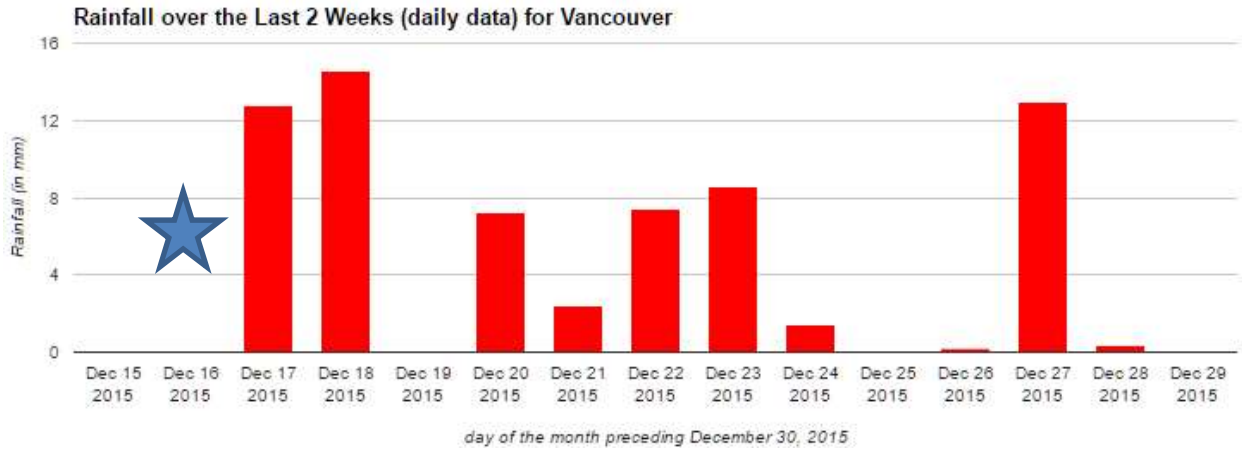


Figure 106. Rainfall Recorded for Downtown Vancouver Between December 15 and December 29, 2015

Appendix D: Temperature Records During Lost Lagoon Wetland Site Visits

Bold fonts indicate the dates when sampling was performed at the Lost Lagoon wetland.

Data was retrieved from the Vancouver Harbour Weather Station (Environment Canada, 2016)

Table 48. Environment Canada Temperature Records for July 1, 2015 through August 31, 2015

Date	Maximum	Mean	Minimum	Date	Maximum	Mean	Minimum
Jul 1 2015	23.9 °C	20.6 °C	17.2 °C	Aug 1 2015	24.3 °C	20.6 °C	16.8 °C
Jul 2 2015	24.6 °C	21.2 °C	17.8 °C	Aug 2 2015	23.9 °C	20.7 °C	17.5 °C
Jul 3 2015	24.8 °C	20.7 °C	16.5 °C	Aug 3 2015	23.2 °C	18.8 °C	14.4 °C
Jul 4 2015	24.6 °C	20.7 °C	16.7 °C	Aug 4 2015	22.5 °C	19.2 °C	15.9 °C
Jul 5 2015	25.6 °C	20.9 °C	16.2 °C	Aug 5 2015	19.3 °C	16.9 °C	14.4 °C
Jul 6 2015	27.1 °C	21.8 °C	16.5 °C	Aug 6 2015	22.4 °C	18.4 °C	14.4 °C
Jul 7 2015	22.7 °C	18.6 °C	14.4 °C	Aug 7 2015	23.2 °C	18.4 °C	13.6 °C
Jul 8 2015	23.8 °C	19.5 °C	15.2 °C	Aug 8 2015	21.8 °C	18.7 °C	15.6 °C
Jul 9 2015	26.0 °C	20.4 °C	14.7 °C	Aug 9 2015	23.4 °C	19.6 °C	15.8 °C
Jul 10 2015	22.9 °C	19.5 °C	16.0 °C	Aug 10 2015	22.6 °C	18.5 °C	14.4 °C
Jul 11 2015	18.7 °C	17.3 °C	15.9 °C	Aug 11 2015	24.1 °C	19.4 °C	14.6 °C
Jul 12 2015	24.2 °C	20.1 °C	16.0 °C	Aug 12 2015	26.8 °C	22.0 °C	17.1 °C
Jul 13 2015	21.8 °C	19.3 °C	16.7 °C	Aug 13 2015	24.3 °C	20.1 °C	15.8 °C
Jul 14 2015	22.3 °C	18.1 °C	13.8 °C	Aug 14 2015	18.8 °C	16.9 °C	15.0 °C
Jul 15 2015	23.1 °C	18.5 °C	13.8 °C	Aug 15 2015	20.6 °C	17.6 °C	14.5 °C
Jul 16 2015	21.9 °C	18.9 °C	15.9 °C	Aug 16 2015	22.2 °C	18.4 °C	14.5 °C
Jul 17 2015	22.9 °C	18.2 °C	13.4 °C	Aug 17 2015	21.8 °C	17.0 °C	12.2 °C
Jul 18 2015	**26.4 °C**	**21.5 °C**	**16.5 °C**	Aug 18 2015	22.8 °C	18.4 °C	14.0 °C
Jul 19 2015	27.3 °C	22.6 °C	17.8 °C	Aug 19 2015	24.6 °C	20.6 °C	16.5 °C
Jul 20 2015	24.3 °C	20.4 °C	16.5 °C	Aug 20 2015	22.4 °C	18.9 °C	15.3 °C
Jul 21 2015	22.7 °C	19.5 °C	16.2 °C	Aug 21 2015	21.0 °C	17.7 °C	14.3 °C
Jul 22 2015	22.5 °C	18.2 °C	13.8 °C	Aug 22 2015	24.0 °C	17.3 °C	10.6 °C
Jul 23 2015	23.4 °C	17.7 °C	12.0 °C	Aug 23 2015	22.7 °C	17.2 °C	11.7 °C
Jul 24 2015	18.1 °C	16.2 °C	14.2 °C	Aug 24 2015	22.8 °C	17.9 °C	12.9 °C
Jul 25 2015	21.8 °C	17.9 °C	14.0 °C	Aug 25 2015	20.2 °C	16.0 °C	11.7 °C
Jul 26 2015	18.3 °C	16.1 °C	13.9 °C	Aug 26 2015	21.8 °C	16.8 °C	11.7 °C
Jul 27 2015	21.4 °C	17.3 °C	13.1 °C	Aug 27 2015	25.4 °C	20.1 °C	14.7 °C
Jul 28 2015	22.4 °C	18.1 °C	13.7 °C	Aug 28 2015	21.5 °C	18.3 °C	15.0 °C
Jul 29 2015	24.2 °C	19.5 °C	14.7 °C	Aug 29 2015	21.4 °C	18.3 °C	15.1 °C
Jul 30 2015	26.4 °C	20.2 °C	14.0 °C	Aug 30 2015	20.2 °C	16.9 °C	13.6 °C
				Aug 31 2015	16.5 °C	15.4 °C	14.2 °C

Table 49. Environment Canada Temperature Records for September 1, 2015 through October 31, 2015

Date	Maximum	Mean	Minimum	Date	Maximum	Mean	Minimum
Sep 1 2015	16.2 °C	15.0 °C	13.7 °C	Oct 1 2015	15.0 °C	11.5 °C	8.0 °C
Sep 2 2015	17.0 °C	13.4 °C	9.8 °C	Oct 2 2015	14.6 °C	11.9 °C	9.1 °C
Sep 3 2015	16.4 °C	13.3 °C	10.2 °C	Oct 3 2015	15.9 °C	11.4 °C	6.8 °C
Sep 4 2015	16.9 °C	12.3 °C	7.6 °C	Oct 4 2015	16.9 °C	11.5 °C	6.1 °C
Sep 5 2015	18.8 °C	13.6 °C	8.4 °C	Oct 5 2015	16.4 °C	11.4 °C	6.3 °C
Sep 6 2015	16.7 °C	14.3 °C	11.9 °C	**Oct 6 2015**	**17.7 °C**	**12.8 °C**	**7.8 °C**
Sep 7 2015	20.3 °C	15.9 °C	11.5 °C	Oct 7 2015	15.4 °C	14.3 °C	13.1 °C
Sep 8 2015	**19.8 °C**	**16.8 °C**	**13.8 °C**	Oct 8 2015	17.2 °C	15.2 °C	13.1 °C
Sep 9 2015	22.7 °C	18.5 °C	14.3 °C	Oct 9 2015	18.8 °C	15.3 °C	11.8 °C
Sep 10 2015	20.2 °C	16.2 °C	12.1 °C	Oct 10 2015	17.8 °C	15.8 °C	13.8 °C
Sep 11 2015	20.1 °C	16.8 °C	13.5 °C	Oct 11 2015	15.6 °C	12.1 °C	8.6 °C
Sep 12 2015	20.6 °C	17.7 °C	14.8 °C	Oct 12 2015	13.2 °C	12.2 °C	11.1 °C
Sep 13 2015	17.6 °C	15.7 °C	13.8 °C	Oct 13 2015	14.5 °C	11.4 °C	8.2 °C
Sep 14 2015	15.7 °C	12.1 °C	8.5 °C	Oct 14 2015	13.1 °C	9.7 °C	6.2 °C
Sep 15 2015	17.1 °C	13.7 °C	10.3 °C	Oct 15 2015	14.6 °C	10.3 °C	6.0 °C
Sep 16 2015	19.2 °C	14.6 °C	9.9 °C	Oct 16 2015	15.4 °C	10.1 °C	4.7 °C
Sep 17 2015	18.1 °C	15.3 °C	12.4 °C	Oct 17 2015	16.5 °C	13.4 °C	10.2 °C
Sep 18 2015	16.8 °C	14.6 °C	12.4 °C	Oct 18 2015	14.3 °C	13.4 °C	12.4 °C
Sep 19 2015	17.1 °C	15.5 °C	13.9 °C	Oct 19 2015	14.5 °C	12.6 °C	10.6 °C
Sep 20 2015	20.6 °C	16.7 °C	12.8 °C	**Oct 20 2015**	**14.4 °C**	**11.6 °C**	**8.7 °C**
Sep 21 2015	15.9 °C	12.2 °C	8.5 °C	Oct 21 2015	13.8 °C	10.0 °C	6.2 °C
Sep 22 2015	**16.8 °C**	**12.0 °C**	**7.2 °C**	Oct 22 2015	13.0 °C	10.0 °C	6.9 °C
Sep 23 2015	18.2 °C	12.1 °C	6.0 °C	Oct 23 2015	12.5 °C	8.9 °C	5.2 °C
Sep 24 2015	17.5 °C	14.2 °C	10.8 °C	Oct 24 2015	12.2 °C	8.2 °C	4.1 °C
Sep 25 2015	15.6 °C	12.4 °C	9.2 °C	Oct 25 2015	13.5 °C	11.0 °C	8.4 °C
Sep 26 2015	15.5 °C	11.4 °C	7.2 °C	Oct 26 2015	14.5 °C	11.8 °C	9.0 °C
Sep 27 2015	14.9 °C	10.5 °C	6.1 °C	Oct 27 2015	14.0 °C	9.8 °C	5.5 °C
Sep 28 2015	15.7 °C	10.8 °C	5.9 °C	Oct 28 2015	12.0 °C	10.6 °C	9.2 °C
Sep 29 2015	16.6 °C	11.8 °C	7.0 °C	Oct 29 2015	15.4 °C	12.7 °C	10.0 °C
Sep 30 2015	17.1 °C	12.1 °C	7.1 °C	Oct 30 2015	13.6 °C	11.9 °C	10.2 °C
				Oct 31 2015	15.8 °C	12.5 °C	9.2 °C

Table 50. Environment Canada Temperature Records for November 1, 2015 through December 31, 2015

Date	Maximum	Mean	Minimum	Date	Maximum	Mean	Minimum
Nov 1 2015	11.6 °C	9.4 °C	7.1 °C	Dec 1 2015	10.8 °C	8.0 °C	5.2 °C
Nov 2 2015	10.8 °C	7.7 °C	4.6 °C	Dec 2 2015	11.0 °C	8.9 °C	6.8 °C
Nov 3 2015	10.1 °C	6.2 °C	2.3 °C	Dec 3 2015	13.9 °C	10.5 °C	7.1 °C
Nov 4 2015	8.7 °C	5.5 °C	2.2 °C	Dec 4 2015	10.4 °C	7.4 °C	4.4 °C
Nov 5 2015	10.9 °C	7.8 °C	4.6 °C	Dec 5 2015	9.4 °C	8.2 °C	6.9 °C
Nov 6 2015	10.6 °C	7.9 °C	5.2 °C	Dec 6 2015	10.7 °C	9.1 °C	7.4 °C
Nov 7 2015	11.1 °C	9.4 °C	7.7 °C	Dec 7 2015	10.3 °C	9.4 °C	8.5 °C
Nov 8 2015	10.3 °C	7.9 °C	5.4 °C	Dec 8 2015	13.8 °C	11.0 °C	8.1 °C
Nov 9 2015	9.6 °C	6.1 °C	2.5 °C	Dec 9 2015	10.6 °C	8.6 °C	6.5 °C
Nov 10 2015	8.9 °C	5.0 °C	1.0 °C	Dec 10 2015	10.8 °C	8.3 °C	5.8 °C
Nov 11 2015	**10.2 °C**	**7.1 °C**	**4.0 °C**	Dec 11 2015	11.3 °C	7.0 °C	2.6 °C
Nov 12 2015	9.2 °C	6.2 °C	3.2 °C	Dec 12 2015	8.7 °C	5.1 °C	1.5 °C
Nov 13 2015	12.2 °C	10.1 °C	8.0 °C	Dec 13 2015	8.6 °C	7.3 °C	5.9 °C
Nov 14 2015	8.9 °C	7.7 °C	6.4 °C	Dec 14 2015	6.7 °C	4.3 °C	1.8 °C
Nov 15 2015	8.1 °C	7.0 °C	5.8 °C	Dec 15 2015	5.3 °C	3.6 °C	1.8 °C
Nov 16 2015	6.9 °C	3.3 °C	-0.3 °C	**Dec 16 2015**	**5.3 °C**	**2.7 °C**	**0.1 °C**
Nov 17 2015	13.1 °C	9.3 °C	5.5 °C	Dec 17 2015	3.4 °C	2.1 °C	0.8 °C
Nov 18 2015	7.5 °C	5.5 °C	3.4 °C	Dec 18 2015	8.5 °C	5.7 °C	2.9 °C
Nov 19 2015	6.7 °C	2.7 °C	-1.4 °C	Dec 19 2015	7.3 °C	5.4 °C	3.5 °C
Nov 20 2015	6.1 °C	1.5 °C	-3.1 °C	Dec 20 2015	7.6 °C	5.0 °C	2.3 °C
Nov 21 2015	6.2 °C	1.4 °C	-3.4 °C	Dec 21 2015	5.9 °C	4.0 °C	2.0 °C
Nov 22 2015	6.9 °C	2.7 °C	-1.6 °C	Dec 22 2015	4.9 °C	2.4 °C	-0.1 °C
Nov 23 2015	5.5 °C	4.3 °C	3.0 °C	Dec 23 2015	6.1 °C	3.6 °C	1.1 °C
Nov 24 2015	8.6 °C	3.2 °C	-2.2 °C	Dec 24 2015	5.0 °C	3.6 °C	2.1 °C
Nov 25 2015	6.3 °C	1.2 °C	-4.0 °C	Dec 25 2015	5.1 °C	2.2 °C	-0.7 °C
Nov 26 2015	6.4 °C	1.4 °C	-3.7 °C	Dec 26 2015	3.8 °C	1.9 °C	-0.1 °C
Nov 27 2015	6.3 °C	1.2 °C	-4.0 °C	Dec 27 2015	5.4 °C	3.3 °C	1.2 °C
Nov 28 2015	5.2 °C	0.3 °C	-4.7 °C	Dec 28 2015	4.6 °C	2.1 °C	-0.4 °C
Nov 29 2015	4.3 °C	-0.1 °C	-4.5 °C	Dec 29 2015	5.6 °C	1.9 °C	-1.9 °C
Nov 30 2015	7.5 °C	1.1 °C	-5.3 °C	Dec 30 2015	2.6 °C	-1.0 °C	-4.5 °C
				Dec 31 2015	2.7 °C	-1.4 °C	-5.4 °C

Appendix E: Delineation of the Lost Lagoon Wetland Watershed

The ArcGIS online watershed area calculation tool was used for the calculation of the Lost Lagoon wetland watershed. Below is an illustration of the output.

Figure 107. Delineation of Lost Lagoon Wetland Watershed Using ArcGIS Online Tool (2016)

Appendix F: Raw Measurements for the Lost Lagoon Wetland Field Study

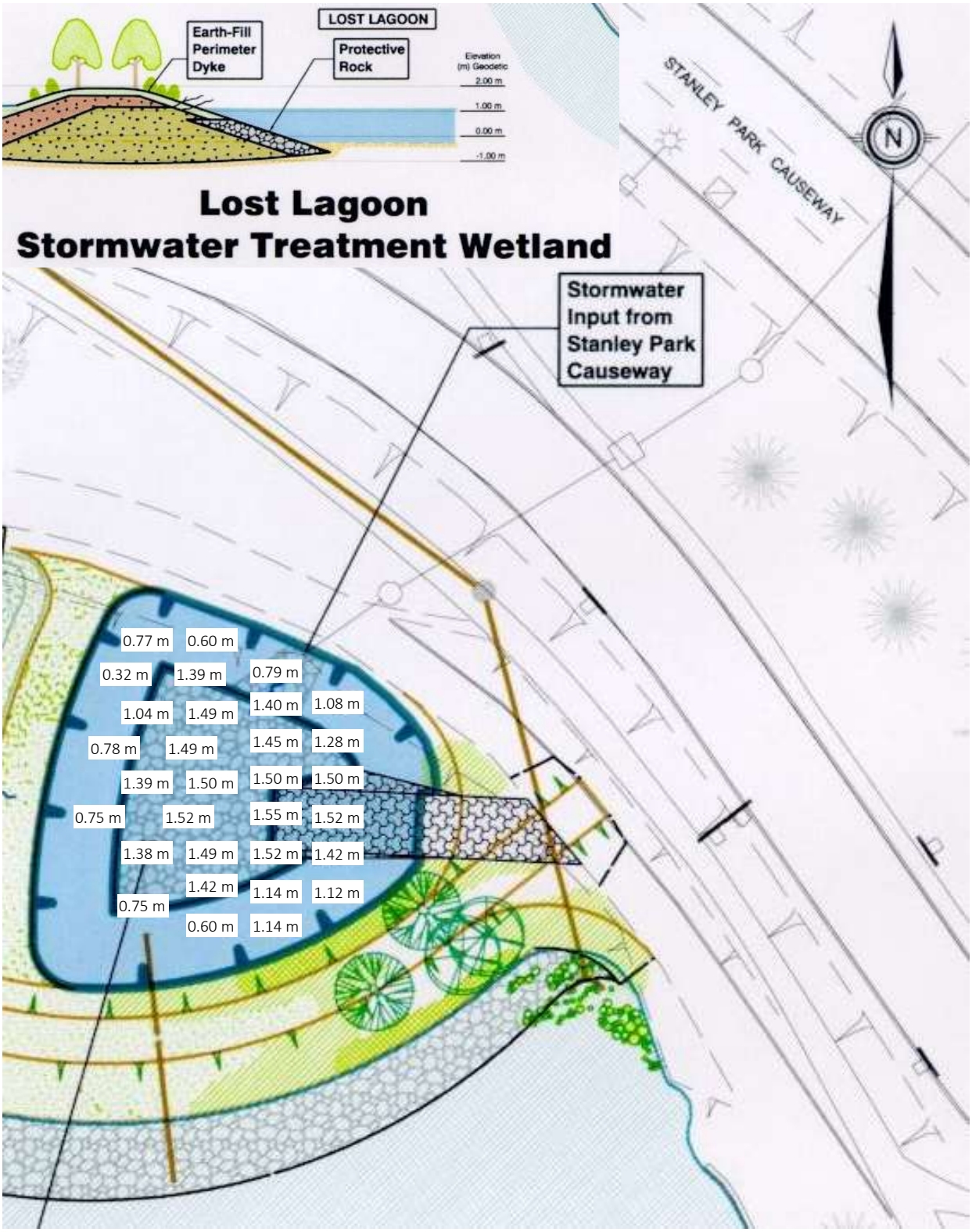


Figure 108. Depth Profile Measurements Taken in the Lost Lagoon Wetland Forebay

Table 51. In Situ Recordings of Dissolved Oxygen, pH and Temperature in the Lost Lagoon Wetland Forebay at the Water Surface and Water Floor

		Oct 16 2015		
Depth	Site	DO	pH	Temp
		mg/L		Celsius
Surface	1.2	0.94	5.2-5.3	12.5-12.7
Floor	1.2	0.85		
Surface	1.1	1.04		
Floor	1.1	0.98		
Surface	1.3	0.77		
Floor	1.3	0.19		
Surface	2.1	1		
Floor	2.1	0.85		
Surface	2.2	0.8		
Floor	2.2	0.67		
Surface	2.3	0.43		
Floor	2.3	0.34		

Table 52. Temperature, DO, pH, Conductivity, ORP - Raw Data - Field Samples

Date	Site	Temp	DO	pH	Cond	ORP
21-Jul	6.1	18.66	2.37	6.09	51.23529	-6.6
21-Jul	6.1	18.97	6.25	6.28	50.47059	23.6
21-Jul	6.1	20.17	9.02	6.38	271.4706	8.7
21-Jul	6.2	19.37	4.93	7.09		94
21-Jul	6.2	19.75	4.93	6.57		59.5
21-Jul	6.2	18.82	5.97	6.2	73.41176	10.9
21-Jul	6.3	20.36	8.81	6.42	52.76471	33
21-Jul	6.3	20.17	8.63	6.42	86.41176	11.1
21-Jul	6.3	20.53	9.92	6.49	61.17647	24.1
08-Sep	2.1	15.4		8.35	58.11765	219.5
08-Sep	2.1	15.32		7.89	57.35294	208.8
08-Sep	2.1	15.52		7.65	58.88235	202.4
08-Sep	4.1	15.44		7.17	58.11765	252.9
08-Sep	4.1	15.6		7.17	58.88235	206.5
08-Sep	4.1	15.61		7.13	58.88235	162.5
08-Sep	6.2	15.07		7.01	55.82353	214.9
08-Sep	6.2	16.08		7.07	35.17647	196.1
08-Sep	6.2	16.18		7.14	91.76471	186.6
22-Sep	5.1	15.3	4.5	6.66	58.11765	39
22-Sep	5.1	15	2.5	6.27	58.88235	20
22-Sep	5.1	15.4	1.5	6	61.17647	45
22-Sep	5.2	18.5	3.5	6.5	58.88235	
22-Sep	5.2	17	3.5	6.68	58.88235	
22-Sep	5.2	16.6	1	6.57	35.17647	
22-Sep	5.3	16.95	2.4	6.5	60.41176	21
22-Sep	5.3	17	1.6	6.72	63.47059	32
22-Sep	5.3	16.5	1.2	6.64	52	38
06-Oct	2.3	12.71	0.13	5.81	247.2	447
06-Oct	2.3	12.61	0.15	5.47	171.7	421.6
06-Oct	2.3	12.62	0.16	5.41	164	404.1
06-Oct	3.3	14.78	4.94	5.66	201.5	557.6
06-Oct	3.3	14.97	2.34	5.6	190.1	500.7
06-Oct	3.3	14.96	1.75	5.55	178.3	481.8
06-Oct	4.3	15.62	1.52	5.29	216.9	622.4
06-Oct	4.3	15.63	0.27	4.95	217.8	576.1
06-Oct	4.3	15.55	1.14	5.63	324.8	613.6
20-Oct	2.1	13.29	1.88	5.55	160	335
20-Oct	2.1	13.23	2.8	5.52	98	218
20-Oct	2.1	13.35	1.3	5.4	170	171
20-Oct	3.1	13.68	1.7	5.35	180	320
20-Oct	3.1	13.78	1.7	5.4	179	314
20-Oct	3.1	13.8	2	5.4	179	313
20-Oct	4.1	15.55	1.6	5.3	190	313
20-Oct	4.1	15.92	2		188	335
20-Oct	4.1	15.94	2.1	5.4	189	321
20-Oct	6.1	18.46	5.4	5.4	24	228
20-Oct	6.1	17.57	5.4	5.4	15	219
20-Oct	6.1	17.86	5.8	6.6	23	232
20-Oct	6.2	19.57	6.6	5.7	24.8	198.6
20-Oct	6.2	18.37	6.7	5.65	19.6	136
20-Oct	6.2	18.41	5.9	5.9	16.98	146
20-Oct	6.3	17.84	7.02	5.92	21.53	199.5
20-Oct	6.3	18.3	7.81	6.03	29.45	215.3
20-Oct	6.3	18.14	7.79	6.17	20.05	230.5
11-Nov	2.2	9.71	5.59	4.23	111.2	202.1
11-Nov	2.3	9.75	5.5	4.4	112.2	182.5
11-Nov	3.1	9.62	5.7	4.03	109.9	148.3
11-Nov	3.2	9.72	5.61	3.15	134.5	195.5
11-Nov	3.3	9.73	5.44	2.8	155.2	177.5
11-Nov	4.2	9.76	5.61	3.65	119.5	216.2
11-Nov	5.1	8.65	5.25	2.78	132.5	171
11-Nov	5.2	8.48	5.32	6.79	133.7	114.3
11-Nov	5.3	8.37	5.31	2.75	144.5	121.8
16-Dec	1.1	8.47	10	5.15	375.5	90
16-Dec	2.1	7.81	1.84	5.87	235.7	297
16-Dec	2.2	7.3	4.53	5.69	169.7	340
16-Dec	2.3	7.66	3.35	6	200.3	249.5

Table 53. Turbidity - Raw Data - Field Samples

Date	Site	Turbidity, NTU	Date	Site	Turbidity, NTU
21-Jul	6.1	50.0	06-Oct	Lab Blank	0.40
21-Jul	6.1	48.5	06-Oct	Lab Blank	0.29
21-Jul	6.1	50.4	06-Oct	Lab Blank	0.38
21-Jul	6.2	94.0	20-Oct	6.1	22
21-Jul	6.2	100.0	20-Oct	6.1	22.15
21-Jul	6.2	93.0	20-Oct	6.1	19.7
21-Jul	6.3	54.7	20-Oct	6.2	58.1
21-Jul	6.3	53.6	20-Oct	6.2	57.3
21-Jul	6.3	57.2	20-Oct	6.2	41.65
21-Jul	Field Blank	0.4	20-Oct	6.3	11.75
21-Jul	Field Blank	0.3	20-Oct	6.3	9.44
21-Jul	Field Blank	0.4	20-Oct	6.3	10.945
08-Sep	2.1	143	20-Oct	Lab Blank	0.645
08-Sep	2.1	161	20-Oct	Lab Blank	0.47
08-Sep	2.1	147	11-Nov	2.2	15.2
08-Sep	4.1	91.7	11-Nov	2.2	14.8
08-Sep	4.1	104	11-Nov	2.2	14.7
08-Sep	4.1	89.9	11-Nov	2.3	15.3
08-Sep	6.2	144	11-Nov	2.3	13.7
08-Sep	6.2	155	11-Nov	2.3	13.2
08-Sep	6.2	155	11-Nov	3.1	13
08-Sep	Field Blank	0.30	11-Nov	3.1	17.3
08-Sep	Lab Blank	0.20	11-Nov	Lab blank	0.12
08-Sep	Trip Blank	0.12	11-Nov	Lab blank	0.18
06-Oct	2.3	32.9	16-Dec	1	1.9
06-Oct	2.3	28	16-Dec	1	2.6
06-Oct	2.3	42	16-Dec	1	2.8
06-Oct	3.3	20.1	16-Dec	2.1	11.3
06-Oct	3.3	22.4	16-Dec	2.1	11.1
06-Oct	3.3	25.6	16-Dec	2.1	11.4
06-Oct	4.3	17.8	16-Dec	2.2	16.6
06-Oct	4.3	20.5	16-Dec	2.2	16.6
06-Oct	4.3	17.9	16-Dec	2.2	19.5
06-Oct	Field Blank	0.16	16-Dec	2.3	12
06-Oct	Field Blank	0.19	16-Dec	2.3	12.2
06-Oct	Field Blank	0.13	16-Dec	2.3	11.7

Table 54. Chemical Oxygen Demand – Raw Data – Field Samples

Date	Site	COD, mg/L	Date	Site	COD, mg/L
21-Jul	Field Blank	10	06-Oct	3.3	42
21-Jul	Field Blank	-	06-Oct	3.3	59
21-Jul	Field Blank	-	06-Oct	3.3	51
21-Jul	Lab Blank	0	06-Oct	4.3	36
21-Jul	Trip Blank	6	06-Oct	4.3	79
21-Jul	6.2	232	06-Oct	4.3	28
21-Jul	6.2	172	20-Oct	2.1	1160
21-Jul	6.2	144	20-Oct	2.1	1256
21-Jul	6.1	116	20-Oct	2.1	1176
21-Jul	6.1	144	20-Oct	3.1	167
21-Jul	6.1	-	20-Oct	3.1	189.5
21-Jul	6.3	115	20-Oct	3.1	212
21-Jul	6.3	129	20-Oct	4.1	131.5
21-Jul	6.3	-	20-Oct	4.1	107
08-Sep	Field Blank	12	20-Oct	4.1	108
08-Sep	Field Blank	18	20-Oct	6.1	21
08-Sep	Field Blank	8	20-Oct	6.1	34
08-Sep	Lab Blank	0	20-Oct	6.1	46
08-Sep	Lab Blank	-	20-Oct	6.2	102
08-Sep	Lab Blank	4	20-Oct	6.2	72
08-Sep	Trip Blank	10	20-Oct	6.2	55
08-Sep	Trip Blank	12	20-Oct	6.3	32
08-Sep	Trip Blank	4	20-Oct	6.3	24
08-Sep	2.1	382	20-Oct	6.3	35
08-Sep	2.1	324	20-Oct	Lab Blank	-
08-Sep	2.1	300	20-Oct	Lab Blank	-
08-Sep	4.1	459	11-Nov	3.1	29
08-Sep	4.1	433	11-Nov	3.1	26
08-Sep	4.1	435	11-Nov	3.1	-
08-Sep	6.2	277	11-Nov	4.2	18
08-Sep	6.2	344	11-Nov	4.2	36
08-Sep	6.2	351	11-Nov	4.2	-
06-Oct	Field Blank	30	11-Nov	3.2	39
06-Oct	Field Blank	43	11-Nov	3.2	46
06-Oct	Field Blank	10	11-Nov	3.2	-
06-Oct	Lab Blank	0	11-Nov	2.2	74
06-Oct	Lab Blank	-	11-Nov	2.2	95
06-Oct	Lab Blank	4	11-Nov	2.2	-
06-Oct	Trip Blank	-	11-Nov	3.3	1
06-Oct	Trip Blank	-	11-Nov	3.3	1
06-Oct	Trip Blank	-	11-Nov	3.3	-
06-Oct	2.3	28	11-Nov	2.3	37
06-Oct	2.3	81	11-Nov	2.3	96
06-Oct	2.3	20	11-Nov	2.3	-
11-Nov	5.1	55	11-Nov	Lab blank	-
11-Nov	5.1	41	16-Dec	1	8
11-Nov	5.1	-	16-Dec	1	19
11-Nov	5.2	157	16-Dec	1	13
11-Nov	5.2	124	16-Dec	2.1	28
11-Nov	5.2	-	16-Dec	2.1	24
11-Nov	5.3	67	16-Dec	2.1	26
11-Nov	5.3	69	16-Dec	2.2	30
11-Nov	5.3	-	16-Dec	2.2	22
11-Nov	Lab blank	-7	16-Dec	2.2	39
11-Nov	Lab blank	-	16-Dec	2.3	936
			16-Dec	2.3	925

Table 55. Total Suspended Solids - Raw Data - Field Samples

Date	Site	TSS, mg/L	Date	Site	TSS, mg/L
21-Jul	Field Blank	0.0	06-Oct	3.3	41
21-Jul	Field Blank	-	06-Oct	3.3	33
21-Jul	Field Blank	-	06-Oct	3.3	52
21-Jul	lab blank	0.0	06-Oct	4.3	41
21-Jul	trip blank	0.0	06-Oct	4.3	33
21-Jul	6.2	66.0	06-Oct	4.3	45
21-Jul	6.2	67.0	20-Oct	2.1	1306
21-Jul	6.2		20-Oct	2.1	1359
21-Jul	6.1	10.0	20-Oct	2.1	-
21-Jul	6.1	-	20-Oct	3.1	327
21-Jul	6.1	31.0	20-Oct	3.1	366
21-Jul	6.3	37.0	20-Oct	3.1	-
21-Jul	6.3	40.0	20-Oct	4.1	157
21-Jul	6.3	42.0	20-Oct	4.1	111
08-Sep	Field Blank	-	20-Oct	4.1	-
08-Sep	Field Blank	-	20-Oct	6.1	37
08-Sep	Field Blank	5	20-Oct	6.1	38
08-Sep	Lab Blank	-	20-Oct	6.1	-
08-Sep	Lab Blank	1	20-Oct	6.2	97
08-Sep	Lab Blank	-	20-Oct	6.2	106
08-Sep	Trip Blank	0	20-Oct	6.2	-
08-Sep	Trip Blank	4	20-Oct	6.3	28
08-Sep	Trip Blank	5	20-Oct	6.3	32
08-Sep	2.1	179	20-Oct	6.3	-
08-Sep	2.1	174	20-Oct	Lab Blank	7
08-Sep	2.1	170	20-Oct	Lab Blank	5
08-Sep	4.1	59	11-Nov	3.1	33
08-Sep	4.1	70	11-Nov	3.1	23
08-Sep	4.1	70	11-Nov	3.1	-
08-Sep	6.2	103	11-Nov	4.2	14
08-Sep	6.2	88	11-Nov	4.2	44
08-Sep	6.2	100	11-Nov	4.2	-
06-Oct	Field Blank		11-Nov	3.2	25
06-Oct	Field Blank		11-Nov	3.2	44
06-Oct	Field Blank	3	11-Nov	3.2	-
06-Oct	Lab Blank	-	11-Nov	2.2	58
06-Oct	Lab Blank	-	11-Nov	2.2	63
06-Oct	Lab Blank	-	11-Nov	2.2	-
06-Oct	Trip Blank	-	11-Nov	3.3	53
06-Oct	Trip Blank	-	11-Nov	3.3	35
06-Oct	Trip Blank	-	11-Nov	3.3	-
06-Oct	2.3	98	11-Nov	2.3	34
06-Oct	2.3	115	11-Nov	2.3	38
06-Oct	2.3	109	11-Nov	2.3	-
11-Nov	5.1	21	16-Dec	1	13
11-Nov	5.1	17	16-Dec	1	18
11-Nov	5.1	-	16-Dec	1	-
11-Nov	5.2	34	16-Dec	2.1	12
11-Nov	5.2	37	16-Dec	2.1	18
11-Nov	5.2	-	16-Dec	2.1	-
11-Nov	5.3	50	16-Dec	2.2	38
11-Nov	5.3	59	16-Dec	2.2	25
11-Nov	5.3	-	16-Dec	2.2	-
11-Nov	Lab blank	12	16-Dec	2.3	12
11-Nov	Lab blank	11	16-Dec	2.3	6
11-Nov	Lab blank	-	16-Dec	2.3	-

Table 56. Total Organic Carbon - Raw Data - Field

Date	Site	TSS, mg/L	Date	Site	TSS, mg/L
21-Jul	Field Blank		06-Oct	3.3	24.807
21-Jul	Field Blank		06-Oct	3.3	22.6145
21-Jul	Field Blank	0.10725	06-Oct	3.3	21.8105
21-Jul	lab blank	-0.00215	06-Oct	4.3	11.6075
21-Jul	trip blank	6.856	06-Oct	4.3	9.913
21-Jul	6.2	28.916	06-Oct	4.3	9.401
21-Jul	6.2	24.202	20-Oct	2.1	43.26475
21-Jul	6.2	-	20-Oct	2.1	83.9025
21-Jul	6.1	29.178	20-Oct	2.1	-
21-Jul	6.1	24.128	20-Oct	3.1	186.997
21-Jul	6.1	-	20-Oct	3.1	182.698
21-Jul	6.3	29.034	20-Oct	3.1	179.5035
21-Jul	6.3	26.392	20-Oct	4.1	310.2305
21-Jul	6.3	-	20-Oct	4.1	307.4545
08-Sep	Field Blank	1.9295	20-Oct	4.1	302.842
08-Sep	Field Blank	2.2115	20-Oct	6.1	13.917
08-Sep	Field Blank	2.175	20-Oct	6.1	12.955
08-Sep	Lab Blank	0.4428	20-Oct	6.1	12.823
08-Sep	Lab Blank	0.14175	20-Oct	6.2	148.074
08-Sep	Lab Blank	-	20-Oct	6.2	146.6555
08-Sep	Trip Blank	1.3615	20-Oct	6.2	142.55
08-Sep	Trip Blank	1.32	20-Oct	6.3	77.5135
08-Sep	Trip Blank	1.125	20-Oct	6.3	77.843
08-Sep	2.1	25.5425	20-Oct	6.3	78.4525
08-Sep	2.1	26.5955	20-Oct	Lab Blank	0.2432
08-Sep	2.1	28.1905	20-Oct	Lab Blank	0.20775
08-Sep	4.1	82.574	11-Nov	3.1	70.9973
08-Sep	4.1	87.999	11-Nov	3.1	68.1439275
08-Sep	4.1	77.686	11-Nov	3.1	-
08-Sep	6.2	84.679	11-Nov	4.2	56.177737
08-Sep	6.2	79.916	11-Nov	4.2	65.0074645
08-Sep	6.2	81.5505	11-Nov	4.2	-
06-Oct	Field Blank	26.5485	11-Nov	3.2	63.35835
06-Oct	Field Blank	22.378	11-Nov	3.2	61.956378
06-Oct	Field Blank	19.2495	11-Nov	3.2	-
06-Oct	Lab Blank	-	11-Nov	2.2	59.9747445
06-Oct	Lab Blank	-	11-Nov	2.2	60.850977
06-Oct	Lab Blank	-	11-Nov	2.2	-
06-Oct	Trip Blank	-	11-Nov	3.3	60.8105355
06-Oct	Trip Blank	-	11-Nov	3.3	61.174509
06-Oct	Trip Blank	-	11-Nov	3.3	-
06-Oct	2.3	28.792	11-Nov	2.3	59.4984335
06-Oct	2.3	24.4865	11-Nov	2.3	58.307656
06-Oct	2.3	21.214	11-Nov	2.3	-
11-Nov	5.1	60.275809	16-Dec	1	58.5098635
11-Nov	5.1	61.4755735	16-Dec	1	56.914671
11-Nov	5.1	-	16-Dec	1	-
11-Nov	5.2	60.2892895	16-Dec	2.1	57.840332
11-Nov	5.2	59.916329	16-Dec	2.1	59.017629
11-Nov	5.2	-	16-Dec	2.1	-
11-Nov	5.3	59.619758	16-Dec	2.2	59.520901
11-Nov	5.3	57.4583845	16-Dec	2.2	58.5188505
11-Nov	5.3	-	16-Dec	2.2	-
11-Nov	Lab blank	0.536478965	16-Dec	2.3	327.1762285
11-Nov	Lab blank	0.54721843	16-Dec	2.3	310.5952135
11-Nov	Lab blank	-	16-Dec	2.3	-

Table 57. Metals - Raw Data - Field Samples - Water

Date	Site	As	Ag	Al	B	Ba	Be	Ca	Cd	Co	Cr	Cu	Fe	K	Li	Mg	Mn	Mo	Na	Ni	Pb	Sb	Se	Si	Sr	Ti	Tl	V	Zn
		(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
2015-07-21	6.2		0.011	1.779	0.316	0.065	0.006	92.262	0.009	0.018	0.241	0.060	6.809	2.741	0.026	45.074	0.313	0.016	84.324	0.175	0.023	0.085		10.151	0.397	3.054		0.014	0.886
2015-07-21	6.2		0.010	2.349	0.449	0.069	0.004	131.814	0.009	0.007	0.024	0.264	5.846	2.882	0.026	48.191	0.270	0.017	88.307		0.017	0.004		10.481	0.436	0.067		0.015	2.603
2015-07-21	6.2																												
2015-07-21	6.1		0.010	1.607	0.319	0.072	0.005	55.025	0.011	0.019	0.192	0.214	8.607	3.086	0.027	47.381	0.397	0.018	86.301	0.297	0.048	0.040		10.759	0.411	1.890		0.018	0.965
2015-07-21	6.1		0.010	1.630	0.323	0.069	0.004	111.492	0.009	0.007	0.066	0.071	6.062	2.939	0.026	46.823	0.295	0.017	86.864	0.004	0.006	0.002		10.129	0.427	0.059	0.012	0.015	0.646
2015-07-21	6.1																												
2015-07-21	6.3		0.010	1.772	0.293	0.064	0.004	50.790	0.008	0.010	0.075	0.062	6.329	2.516	0.025	43.376	0.280	0.015	81.869	0.083	0.022	0.027		10.280	0.366	0.570	0.012	0.018	0.183
2015-07-21	6.3		0.010	2.332	0.348	0.068	0.004	99.471	0.008	0.005	0.033	0.128	6.022	2.525	0.025	45.163	0.261	0.016	83.932		0.009	0.012		10.417	0.402	0.074		0.013	1.498
2015-07-21	6.3																												
2015-09-08	2.1			14.720	0.076	0.223		15.710	0.007	0.010	0.113	0.725	17.890	0.394	0.016	5.881	0.323		10.870	0.028	0.106	0.030		23.170	0.111	0.654		0.050	0.751
2015-09-08	2.1																												
2015-09-08	2.1																												
2015-09-08	4.1			5.310	0.073	0.091		9.494	0.004	0.005	0.035	0.308	7.526	0.284		2.600	0.166		6.777	0.012	0.037			9.706	0.063	0.220		0.024	0.255
2015-09-08	4.1																												
2015-09-08	4.1																												
2015-09-08	6.2			7.460	0.150	0.069		27.220	0.009	0.005		0.141	16.360	1.373	0.019	22.450	0.440			0.007				15.450	0.231	0.313		0.037	0.035
2015-09-08	6.2																												
2015-09-08	6.2																												
2015-10-06	2.3			0.104	0.056							0.144	7.322	0.020	0.050	0.269	0.209	0.010	182.921				0.179	15.414					0.077
2015-10-06	2.3																												
2015-10-06	2.3																												
2015-10-06	3.3			0.960	0.107	0.032		8.922	0.003	0.000		0.062	1.450	0.239		1.548	0.026		15.380	0.002				5.599	0.058	0.031		0.012	0.033
2015-10-06	3.3																												
2015-10-06	3.3																												
2015-10-06	4.3			0.869	0.083	0.027		8.327	0.003	0.001		0.057	1.340	0.230		1.432	0.020		20.320	0.001				5.267	0.052	0.024		0.011	
2015-10-06	4.3																												
2015-10-06	4.3																												
2015-10-20	2.1			40.970	0.080	0.492		27.340	0.018	0.034	0.238	1.552	52.440	0.619	0.038	14.760	0.756	0.025	12.900	0.073	0.376	0.056		52.950	0.231	1.850		0.127	2.009
2015-10-20	2.1																												
2015-10-20	2.1																												
2015-10-20	3.1			8.321	0.059	0.125		14.150	0.007	0.006	0.047	0.339	11.190	0.412	0.011	4.152	0.209		10.070	0.012	0.053			15.250	0.095	0.384		0.034	0.360
2015-10-20	3.1																												
2015-10-20	3.1																												
2015-10-20	4.1			3.545	0.073	0.075		10.890	0.003	0.002	0.014	0.182	4.890	0.231		2.422	0.101		7.332	0.006	0.013			8.898	0.071	0.136		0.020	0.173
2015-10-20	4.1																												
2015-10-20	4.1																												
2015-10-20	6.1			0.985	0.210	0.043		36.160	0.004	0.001		0.090	2.333	2.136	0.013	37.290	0.178			0.003				4.306	0.351	0.014		0.019	0.027
2015-10-20	6.1																												
2015-10-20	6.1																												
2015-10-20	6.2			2.884	0.210	0.058		38.330	0.005	0.002		0.082	7.598	2.103	0.016	36.090	0.301			0.003				7.951	0.349	0.090		0.024	0.051
2015-10-20	6.2																												
2015-10-20	6.2																												
2015-10-20	6.3			0.801	0.200	0.043		36.070	0.004	0.000		0.041	1.593	2.077	0.013	36.580	0.143			0.002				4.089	0.346	0.014		0.019	
2015-10-20	6.3																												
2015-10-20	6.3																												
2015-11-11	3.1			2.033	0.130	0.030		6.787	0.004	0.001		0.114	2.197	0.167		1.518	0.043		5.295	0.004				7.537	0.048	0.092		0.012	0.024
2015-11-11	3.1			1.939	0.081	0.028		6.754	0.003	0.001		0.101	2.053	0.158		1.468	0.040		5.148	0.003				7.030	0.047	0.066		0.010	0.022
2015-11-11	3.1																												
2015-11-11	4.2			1.083	0.068	0.011		3.782	0.003	0.001		0.072	1.052	0.093		0.754			2.082	0.002				4.880	0.024	0.023			0.014
2015-11-11	4.2			1.219	0.042	0.016		4.383	0.004	0.003		0.089	1.260	0.096		0.872	0.016		2.243	0.002				5.616	0.028	0.031			
2015-11-11	4.2																												
2015-11-11	3.2			5.834	0.075	0.015		6.160	0.004	0.002		0.093	1.840	0.150		1.320	0.023		4.536	0.002				7.171	0.041	0.058		0.010	0.012
2015-11-11	3.2			1.986	0.053	0.012		5.281	0.003	0.001		0.080	1.671	0.134		1.147	0.014		3.740	0.001				6.176	0.035	0.049			
2015-11-11	3.2																												
2015-11-11	2.2			1.485	0.045	0.026		5.971	0.003	0.002		0.065	1.544	0.135		1.214	0.028		3.857	0.001				6.326	0.040	0.042			0.024
2015-11-11	2.2			1.661	0.108	0.029		6.846	0.004	0.002		0.085	1.741	0.146		1.350	0.036		4.521	0.001				7.173	0.046	0.051		0.010	0.020
2015-11-11	2.2																												
2015-11-11	3.3			1.435	0.065	0.012		4.321	0.004	0.001		0.090	1.533	0.110		0.948	0.013		2.543	0.000				4.557	0.028	0.044			0.020
2015-11-11	3.3			1.386	0.042	0.011		4.281	0.003	0.000		0.081	1.457	0.110		0.937			2.527	0.000				4.608	0.027	0.044			0.014
2015-11-11	3.3																												
2015-11-11	2.3			1.346	0.144	0.015		4.538	0.003	0.002		0.073	1.423	0.127		0.990	0.018		3.255	0.000				5.248	0.030	0.044			
2015-11-11	2.3			1.470	0.088	0.020		5.129	0.003	0.000		0.075	1.506	0.134		1.087	0.028		3.652	0.002				6.061	0.034	0.046		0.010	
2015-11-11	2.3																												
2015-11-11	5.1			0.473	0.080			4.760	0.004	0.002		0.080	0.744	0.112		0.704	0.022		2.494	0.002				4.555	0.028				
2015-11-11	5.1			0.714	0.045			4.600	0.004	0.001		0.066	0.658	0.106		0.646	0.016		2.284	0.002				4.161	0.025				0.017
2015-11-11	5.1																												
2015-11-11	5.2			0.718	0.082			4.350	0.003	0.001		0.057	1.175	0.114		0.745	0.010		2.541	0.000				3.674	0.026				0.010
2015-11-11	5.2			0.806	0.062	0.010		4.594	0.003	0.000		0.056	1.368	0.116		0.803	0.012		2.623	0.001				3.936	0.029			0.010	0.018
2015-11-11	5.2																												
2015-11-11	5.3			0.744	0.073	0.010		5.703	0.003	0.000		0.123	1.216	0.144		0.967			3.516	0.001				4.310	0.035				0.010
2015-11-11	5.3			0.723	0.043			5.407	0.004	0.001		0.100	1.184	0.137		0.937	0.015		3.319	0.001				4.271	0.033				
2015-11-11	5.3																												
2015-12-16	1.1			0.412	0.123			5.120	0.003	0.002		0.057	0.154	0.114		0.806			3.998	0.003				4.224	0.037				
2015-12-16	1.1			0.712	0.098			3.380	0.003	0.001		0.048	0.198	0.095		0.539			2.479	0.000				2.668	0.023				
2015-12-16	1.1			0.390	0.169			5.084	0.002	0.001		0.076	0.187	0.115		0.796			3.807	0.002				3.945	0.036				
2015-12-16	2.1			0.391	0.070	0.012		5.366	0.003	0.002		0.093	0.282	0.134		0.800	0.012		8.377	0.001				3.771	0.036	0.116			
2015-12-16	2.1			0.829	0.062	0.011		5.237	0.000	0.001		0.080	0.394	0.121		0.835	0.017		8.188	0.001				4.312	0.034				
2015-12-16	2.1																												
2015-12-16	2.2			0.510	0.057	0.014		6.160	0.003	0.000		0.054	0.284	0.130		0.944	0.030		8.029	0.002				4.502	0.041				
2015-12-16	2.2			0.688	0.046	0.011		5.746	0.003	0.000		0.058	0.410	0.125		0.943	0.028		7.073	0.000				4.487	0.038				
2015-12-16	2.2																												
2015-12-16	2.3			0.436	0.064	0.011		5.641	0.003	0.006		0.055	1.153	0.132		0.871	0.017		7.695	0.002		0.185		5.114	0.037	2.840		0.010	
2015-12-16	2.3			0.542	0.044	0.013		5.888	0.001	0.002		0.071	0.390	0.128		0.928	0.022		8.089	0.000				4.784	0.039				
2015-12-16	2.3																												

Table 58. Metals - Raw Data - Field Samples - Sediment

Date	Site	mg/kg dry	As	Ag	Al	B	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	Li	Mg	Mn	Mo	Na	Ni	Pb	Sb	Si	Sr	Ti	V	Zn
21-Jul	6.2	Surface		8.5	17,670.00	31	119.5	12,970.00	-	16.72	42.5	120.5	24,690.00	141.5	22.5	7,415.00	385.5	-	1,177.50	21.56	9	-	5,455.00	69.5	2,532.50	86	218
21-Jul	6.2	Surface							-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21-Jul	6.2	Depth			14,290.00	25.5	31	13,040.00	5.57	15.55	42.5	93	21,445.00	119	19	7,220.00	340	-	1,216.00	19.41	-	-	4,382.00	52	2,121.50	77.5	173.5
21-Jul	6.2	Depth	23.5		18,940.00	22.5	36	20,635.00	6.34	14.24	35	73.5	24,510.00	107.5	19.5	7,485.00	404	-	1,559.50	14.97	-	-	4,329.50	81	2,034.00	96.5	155.5
08-Sep	2.1	Surface			25,180.00	99	136.5	-	6.93	-	74.62	224.17	29,066.02	221	40.49	10,104.30	480.84	-	2,414.50	6.6	33.51	10.27	690	-	3,014.16	-	305.2
08-Sep	2.1	Surface			15,795.00	73.5	96	-	-	-	35.15	185.17	19,420.45	163.5	34.46	4,922.02	365.49	-	1,392.00	4.22	25.25	10.65	693.5	-	1,570.76	-	262.14
08-Sep	2.1	Surface			20,955.00	95	128	-	-	-	69.84	259.43	33,321.79	217.5	41.81	8,993.04	438.81	-	1,612.50	6.45	39.74	17.56	906.5	-	2,463.90	-	306
08-Sep	2.1	Depth			21,710.00	97.5	56.5	-	-	-	-	83.35	22,380.85	206	39.3	5,571.59	511.5	-	-	-	-	-	908	-	1,611.23	-	27.97
08-Sep	2.1	Depth			19,915.00	84	87	-	8.26	-	33.04	74.83	25,256.23	163.5	37.76	7,803.26	461.64	-	1,491.50	5.98	-	-	802	-	2,470.66	-	74.22
08-Sep	2.1	Depth			18,680.00	88	66	-	5.68	-	45.56	84.88	24,530.92	151.5	38.49	7,180.33	482.88	-	1,670.50	4.56	-	-	996	-	2,576.90	-	57.58
08-Sep	2.1	Surface		9.5	11,440.00	27.5	87	16,780.00	-	16.22	49	262.5	16,900.00	183.5	17	5,410.00	301.5	-	1,279.50	28.84	34.5	-	8,645.00	42.5	1,366.50	58.5	676
08-Sep	2.1	Surface	8		21,835.00	48.5	148	29,900.00	5.32	23.76	101.5	408	24,075.00	238	21.5	7,015.00	361	5.5	1,613.50	45.6	72.5	14.5	14,025.00	78	2,195.00	77.5	863.5
08-Sep	2.1	Depth		9	14,875.00	26.5	137	11,810.00	5.18	16.2	81	108.5	20,275.00	103	14.5	5,925.00	336.5	-	1,430.00	20.05	10.5	-	5,720.00	62	2,006.00	55.5	288
08-Sep	2.1	Depth	17.5		21,295.00	17.5	70.5	19,150.00	8.31	20	50.5	130	29,000.00	133	18.5	8,360.00	421	-	1,999.00	28.66	15.5	-	5,735.00	77.5	2,523.00	117.5	368.5
08-Sep	4.1	Surface	14		13,350.00	23	115	7,995.00	7.47	15.04	47	135.5	17,605.00	161.5	12	6,405.00	276	-	1,093.00	38.73	26.5	-	7,970.00	55	1,322.00	58.5	379.5
08-Sep	4.1	Surface	23.5		13,015.00	43.5	116.5	13,835.00	-	16.49	39	125	18,070.00	161	12.5	6,110.00	284	-	1,417.00	37.08	17	-	9,635.00	58	1,402.00	61	404
08-Sep	4.1	Depth	23.5		16,905.00	22	70	13,860.00	-	12.13	28.5	226	20,165.00	140	15	5,815.00	328.5	-	1,309.50	15.9	-	-	6,360.00	56.5	1,943.00	65	308.5
08-Sep	4.1	Depth	14		16,905.00	18.5	70	11,905.00	-	16.38	73.5	83	21,135.00	135	16	6,935.00	368	-	1,275.00	29.63	-	-	6,930.00	63.5	2,206.00	69	233
08-Sep	6.2	Surface	26		20,830.00	38.5	60.5	19,780.00	-	17.31	45	67	25,295.00	168	21	7,995.00	433.5	-	1,397.00	27.3	-	-	5,540.00	70	2,788.50	80.5	145
08-Sep	6.2	Surface	11		18,055.00	26	43	17,565.00	6.15	17.87	37.5	71	23,900.00	148.5	24.5	7,520.00	395.5	-	1,473.00	20.28	-	-	5,690.00	73	2,357.00	80.5	162.5
08-Sep	6.2	Depth	17		27,145.00	25	67.5	19,615.00	12.06	29.61	37	89.5	37,895.00	177.5	36	13,485.00	662	-	1,650.50	27.36	-	-	5,860.00	88	4,022.00	138	202.5
08-Sep	6.2	Depth			24,565.00	27	63	18,395.00	11.71	24.84	34.5	84	33,715.00	163	29	11,455.00	609.5	-	1,575.00	26.32	-	-	7,320.00	79.5	3,397.50	112.5	209.5
22-Sep	5.1	Surface	18		7,890.00	27.5	64.5	6,790.00	-	12.94	23	64	14,890.00	111.5	8.5	3,993.50	213	-	884	27.4	13	-	4,318.50	35.5	924.5	38	235.5
22-Sep	5.1	Surface	18		9,475.00	54	76.5	7,400.00	-	14.45	24	88	17,090.00	123.5	9.5	4,566.00	253	-	1,058.00	34.93	19.5	-	5,835.00	41.5	1,009.50	41.5	286
22-Sep	5.1	Depth	23		14,725.00	24	83.5	10,865.00	6.42	15.59	23	55.5	19,260.00	153	15.5	7,655.00	373.5	-	1,352.00	30.02	-	-	4,882.50	53	1,698.50	61	228.5
22-Sep	5.1	Depth	32.5		12,875.00	35.5	60.5	13,685.00	-	15.04	18	93	19,010.00	112	15.5	6,750.00	377	-	1,160.50	23.75	-	-	4,665.00	55	1,519.50	56.5	181
22-Sep	5.2	Surface	16.5		11,110.00	24.5		8,955.00	-	10.64	27	84.5	16,650.00	137	10	4,201.00	-	-	1,162.50	24.06	-	-	7,835.00	64	1,042.00	44	136.5
22-Sep	5.2	Surface	57.5		18,930.00	22	119	-	-	11.64	38	99	18,270.00	153	12	4,976.50	414.5	-	1,960.50	19.93	-	-	-	102.5	1,186.00	54.5	156
22-Sep	5.2	Depth	62		16,985.00	10	82.5	11,285.00	-	15.25	27.5	64	23,970.00	243	20	6,975.00	393	-	1,318.50	18.71	-	-	7,955.00	71	2,091.00	88.5	120.5
22-Sep	5.2	Depth	10		21,800.00	15.5	106	-	-	15.25	37.5	81	24,240.00	246	19	7,295.00	399	-	1,125.50	16.55	-	-	3,923.00	60.5	1,841.00	88	82
22-Sep	5.3	Surface	8.5		11,240.00	38	72	11,725.00	-	12.01	24.5	97	17,430.00	116.5	11.5	6,190.00	319	-	1,258.00	24.67	-	-	6,005.00	48.5	1,459.00	49	167
22-Sep	5.3	Surface	17.5		11,765.00	26	69.5	-	6.27	12.05	-	84	17,560.00	117	11.5	5,160.00	304.5	-	955	24.63	-	-	4,778.50	50	1,342.00	51.5	174.5
22-Sep	5.3	Depth	24		10,665.00	14	50	9,885.00	-	10.2	19	68	15,720.00	133.5	11.5	5,600.00	273	-	982	16.41	-	-	3,421.50	44	1,374.00	50	56
22-Sep	5.3	Depth			10,645.00	14	50	-	5.75	9.66	25.5	98.5	15,900.00	135	12	5,665.00	274.5	-	1,033.00	19.13	-	-	3,420.00	46.5	1,381.50	50.5	257.5
06-Oct	3.3	Surface			20,915.00	127.5	92	-	-	-	221.46	90.38	24,170.12	197	37.7	6,844.33	429.34	-	2,596.00	10.33	-	4.16	622.5	-	2,121.70	-	66.52
06-Oct	3.3	Surface			18,340.00	126.5	77.5	-	-	-	183.42	101.75	21,619.81	161	36.61	5,934.29	389.74	-	1,785.00	13.94	-	5.79	850	-	2,247.64	-	88.26
06-Oct	3.3	Surface		9.5	16,735.00	98	92.5	-	-	-	190.43	121.03	21,330.53	180	35.81	5,474.86	365.12	-	1,583.00	9.98	6.2	11.7	756	-	2,100.79	-	126.3
06-Oct	3.3	Depth			23,475.00	95.5	61	-	-	-	30.33	67.69	20,449.75	141	35.29	4,860.95	317.38	-	2,066.00	4.45	-	5.88	927	-	1,868.59	-	52.53
06-Oct	3.3	Depth			17,125.00	79	58	-	-	-	67.62	64.73	21,881.17	151.5	38.31	6,614.01	384.57	-	1,613.00	4.77	-	-	674	-	1,889.85	-	54.02
06-Oct	3.3	Depth			20,105.00	102.5	-	-	-	-	44.03	75.69	24,140.04	169	36.87	6,670.85	411.66	-	2,135.00	3.96	-	-	904.5	-	2,176.34	-	72.24
06-Oct	Lab Blank	Water			0.06	0.06	-	-	-	-	0.07	0.09	0.55	0.01	0.05	0.01	0.01	-	0.49	0.04	-	-	1.18	-	-	-	0.1
06-Oct	Lab Blank	Water			0.09	0.03	-	-	-	-	0.03	0.08	0.49	0.01	0.05	0.03	-	-	0.4	0.18	-	-	1.17	-	-	-	0.02
06-Oct	Lab Blank	Water			0.03	0.06	-	-	-	-	0.07	0.05	0.88	0.01	0.05	0.01	-	-	0.48	0.23	-	-	1.12	-	0.6	-	-
06-Oct	2.3	Surface			15,905.00	13	76	13,040.00	5.58	15.27	52	235	21,955.00	129	18	7,665.00	361	-	1,212.50	15.54	18.5	-	4,610.50	57.5	2,198.00	69	191
06-Oct	2.3	Surface	14.5		17,055.00	17	66.5	12,645.00	8.61	15.32	48	160	25,720.00	114	20	8,330.00	397.5	-	1,210.50	15.48	11	-	3,250.00	57	2,357.50	79	164
06-Oct	2.3	Depth	17		18,010.00	22	67	15,000.00	7.59	13.47	34	91	22,095.00	125.5	20.5	7,785.00	341.5	-	1,249.00	12.1	-	-	2,740.00	56	2,557.00	89.5	82.5
06-Oct	2.3	Depth			16,785.00	19	70.5	12,490.00	8.88	16.01	36.5	75.5	24,520.00	149.5	21.5	8,320.00	394	-	1,008.00	13.05	-	-	3,873.00	55	2,686.00	77	97
06-Oct	3.3	Surface	21.5		15,820.00	14.5	54.5	11,710.00	6.47	13.88	37.5	111.5	23,030.00	121.5	18	8,270.00	386	-	1,074.50	17.79	-	-	2,732.50	38.5	2,369.50	70.5	126.5
06-Oct	3.3	Surface			17,035.00		88.5	11,840.00	10.05	14.53	54.5	119.5	24,535.00	195	20.5	8,665.00	389.5	-	1,418.00	18.47	-	-	3,818.50	57.5	2,181.50	75	118
06-Oct	3.3	Depth	9		13,425.00	15.5	46	11,190.00	7.05	11.9	21.5	87	19,335.00	-	15.5	-	336	-	1,026.50	14.02	-	-	3,046.00	-	1,945.50	55.5	94
06-Oct	3.3	Depth	11		16,550.00	12.5	39.5	14,585.00	7.11	13.3	18	82.5	22,685.00	84	20	7,655.00	422.5	-	1,079.50	-	-	-	3,147.50	69.5	2,040.50	54	102
06-Oct	4.3	Surface			12,465.00	10	55	9,565.00	7.11	10.85	45.5	95	20,235.00	104	14	5,605.00	280	-	1,161.00	10.38	-	-	2,914.00	45	1,934.50	71	96.5
06-Oct	4.3	Surface	7		20,030.00	15.5	133	15,565.00	10.54	17.29	45.5	131	29,390.00	141	21.5	8,980.00	498	-	1,227.00	16.49	10	-	2,877.50	69.5	2,982.00	92.5	157
06-Oct	4.3	Depth		235.5	25,135.00	9	78.5	17,765.00	13.09	20.49	83.5	74	34,770.00	164	29	13,485.00	594.5	-	1,213.50	32.52	-	-	2,656.50	56.5	3,117.50	110	103.5
06-Oct	4.3	Depth			12,830.00	12.5	52.5	-	-	10.9	45	63.5	19,490.00	140	16.5	6,945.00	308.5	-	978.5	17.53	-	-	1,534.50	33	1,711.00	56.5	62
20-Oct	2.1	Surface			16,290.00	91.5	109.5	-	-	-	72.42	103.64	20,787.04	178.5	34.27	6,583.38	389.41	-	1,511.00	7.47	-	8.89	810.5	-	-	-	103.33
20-Oct	2.1	Surface			21,595.00	90.5	86	-	-	-	57.6	97.75	26,678.58	179	38.23	8,275.44	528.4	-	2,242.00	13.03	8.04	-	829.5	-	2,266.05	-	112.76
20-Oct	2.1	Surface			21,965.00	128	83	-	-	-	77.08	115.49	26,678.92	181.5	35.57	7,786.50	556.96	-	1,856.50	71.29	17.93	7.1	-	-	2,208.50	-	147.47
20-Oct	2.1	Depth			14,285.00	93.5	68	-	-	-	-	90.01	24,849.56	135.5	35.84	6,737.06	554.73	6.6	990.5	271.05	5.17	5.24	758.5	-	1,740.22	-	68.53
20-Oct	2.1	Depth			13,675.00	126	69	-	-	7.42	61.9	87.8	16,599.00	131	33.37	4,252.57	298.16	-	1,641.00	25.99	2.96	7.85	1,025.50	-	1,610.90	-	116.02
20-Oct	2.1	Depth			17,900.00	81	-	-	-	-	112.98	102.02	24,923.04	328	38.59	7,024.91	442.85	-	1,648.50	61.96	8.77	6.82	984.5	-	2,577.53	-	139.15
20-Oct	2.1	Surface	7		19,450.00	19	123	10,525.00	9.01	15.54	52.5	250	26,570.00	159	19.5	9,695.00	441	-	1,386.00	27.6	50	5.5	9,855.00	58.5	2,039.00	78	391
20-Oct	2.1	Surface			20,470.00	16.5	104.5	13,820.00	10.05	18.42	37.5	193.5	28,050.00	196	20	10,455.00	501.5	-	1,654.50	25.01	15	-	2,260.50	46	2,648.00	81.5	169
20-Oct	2.1	Depth	22.5		13,305.00	14	53.5	10,000.00	-	12.16	50.5	109.5	18,835.00	112	15.5	6,325.00	348.5	-	1,069.00	12.3	10.5	16.5	3,322.50	54.5	1,853.00	62.5	166
20-Oct	2.1	Depth	20		17,350.00	10	-	11,630.00	6.83	16.51	41	169	23,800.00	184	21.5	8,350.00	388	-	1,353.50	20.82	21	-	4,542.00	50.5	2,254.00	78	277.5
20-Oct	3.1	Surface	11		65.5	14	-	648.5	-	-	-	23	65	30	-	87	-	-	622.5	0.76	-	-	434.5	-	-	-	78
20-Oct	3.1	Surface	17		71	12	-	280.5	-	-	-	26	75	29.5	-	61.5	-	-	482	3.9	-	-	578.5	-	8.5	-	73
20-Oct	3.1	Depth	8.5		5,800.00	28.5	57.5	5,465.00	-	7.71	24.5	169.5	8,055.00	70	-	2,695.00	131	-	836	12.91	14.5	-	6,280.00	31.5	499	21.5	311.5
20-Oct	3.1	Depth	16.5		3,721.00	40	52	5,295.00	-	-	27.5	138	4,547.00	61	-	1,231.00	70	-	838.5	17.71	13.5	-	5,675.00	28.5	238.5	11.5	292
20-Oct	4.1	Surface			19,475.00	27.5	-	14,080.00	10.39	18.42	34	140.5	26,925.00	163.5	24.5	8,910.00	402.5	-	1,090.00	25.76	13.5	-	6,215.00	54.5	2,050.50	75	241
20-Oct	4.1	Surface	55		14,065.00	26.5	68	11,450.00	-	15.13	67	122.5	18,690.00	112.5	15	6,810.00	317	-	984	21.68	14.5	-	4,253.00	35	1,866.50	73	167.5
20-Oct	4.1	Depth	10	63.5	10,440.00	32.5	148.5	8,460.00	-	11.96	43	149.5	13,505.00	126.5	11.5	4,815.00	209	-	935.5	32.96	20	-	7,430.00	46	1,173.50	48	227
20-Oct	4.1	Depth	23		12,735.00	27.5	138.5	10,405.00	-	13.61	56.5	206.5	15,275.00	155	13	6,455.00	257	-	1,067.00	36.65	30	-	8,670.00	52.5	1,139.00	48.5	390.5
20-Oct	6.1	Surface	15.5		10,825.00	33.5	103.5	8,415.00	5.4	11.86	31.5	56.5	14,110.00	143.5	11.5	4,674.50	249.5	-	859	33.22	-	-	7,915.00	48.5	1,152.00	52	201.5
20-Oct	6.1	Surface			11,710.00	21	97	8,510.00	-	11.7	28.5	48	14,895.00	137.5	12	5,510.00	258	-	991.5	26.22	-	-	7,520.00	56	1,368.00	52.5	168.5
20-Oct	6.1	Depth	21		13,460.00	22	53.5	9,345.00	6.48	11.38	17	37	20,360.00	143	25	6,440.00	374	-	944	12.53	-	-	1,998.00	53	1,731.50	59.5	57
20-Oct	6.1	Depth	23.5		15,825.00	19.5	62.5	11,755.00	10.57	18.11	29	44.5	27,805.00	205	28	8,470.00	520	-	1,086.00	16.29	-	-	3,681.00	50	2,754.50	80.5	100.5
20-Oct	6.2	Surface	12.5		23,890.00	25	43.5	8,675.00	11.03	21.04	27	38	30,970.00	200.5	64	14,880.00	556.5	-	887.5	20.41	-	-	5,000.00	52	2,165.00	102.5	69.5
20-Oct	6.2	Surface	34		8,075.00	21.5	18	5,725.00	-	7.14	12	23.5	12,005.00	107	21.5	4,555.50	194.5	-	685	11.19	-	-	3,954.50	18	1,068.50	38	25
20-Oct	6.2	Depth	116.5		13,105.00	36	27.5	8,990.00	-	10.08	68.5	49	19,390.00	116.5	16	7,455.00	351	-	929	22.49	-	-	3,618.50	32	1,402.00	55.5	74
20-Oct	6.2	Depth	24		78	21	-	1,741.50	-	-	-	17.5	65	30	-	69.5	-	-	516.5	2.74	-	-	400.5	-	-	-	56
20-Oct	6.3	Surface			29,000.00	34	51	14,150.00	-	10.5	39.5	126	34,240.00	122.5	66	13,370.00	502	-	1,996.50	17.5	8.5	-	4,153.00	85	1,941.00	98	80.5
20-Oct	6.3	Surface		7	22,840.00	31.5	58.5	13,550.00	-	-	20	129.5	24,995.00	179	62	8,080.00	378.5	-	1,428.50	6	13	-	-	60.5	1,817.00	83.5	91.5
20-Oct	6.3	Depth			24,450.00	27.5	80.5	19,200.00	-	10.5	45.5	135.5	33,850.00	181.5	62	11,035.00	650	-	-	18.5	16	-	6,560.00	95.5	2,849.00	101	217
20-Oct	6.3	Depth		7.5	17,730.00	34.5	66.5	9,765.00	-	-	40.5	126.5	22,150.00	-	50	6,895.00	347	-	1,738.00	8.5	32.5	-	7,040.00	66	1,972.00	65	103
11-Nov	2.1	Surface			23,315.00	29.5	-	14,010.00	-	10	35	115.5	32,675.00	180	52.5	10,280.00	527	-	1,498.50	10.5	33.5	-	7,810.00	85	2,693.00	98	98.5
11-Nov	2.1	Surface		7.5	19,500.00		64.5	10,395.00	-	7.5	112.5	682	23,860.00	174.5	45	6,440.00	350.5	11.5	1,443.50	24	127	21	4,351.50	75	1,571.00	63.5	790.5
11-Nov	3.2	Surface		7.5		26.5	191.5	9,390.00	-	-	109.5	658.5	22,925.00	189	43.5	6,280.00	312	10.5	1,817.50	22.5	127	17	10,885.00	84.5	1,193.50	58.5	750
11-Nov	3.2	Surface			20,140.00	22	207	12,740.00	-	12	173	1,065.50	32,280.00	213.5	48.5	8,515.00	477	21.5	2,788.00	38	183.5	40	19,725.00	98	2,295.50	91	1,220.50
11-Nov	4.2	Surface		-	25,595.00	23	266	10,310.00	-	6.5	124	827	25,795.00	257	44.5	6,950.00	371.5	15.5	1,962.50	26.5	142.5	28	7,825.00	-	1,741.00	69	973
11-Nov	4.2	Surface		7.5	20,325.00	18	214	12,515.00	-	-	108.5	669	23,220.00	220.5	-	6,600.00	341.5	10.5	2,059.50	22.5	101	13	11,555.00	79	1,657.00	63	809
11-Nov	2.2	Surface		7.5	19,665.00	24	177	5,820.00	-	-	58.5	399.5	12,775.00	192	43.5	3,391.50	179	-	2,188.50	8.5	59	-	12,160.00	79	972	31.5	-
11-Nov	2.2	Surface		10.5	9,885.00	25	97	10,605.00	6.38	-	113.5	750	24,575.00	115.5	40.5	6,665.00	371	14.5	1,495.00	26	138	20.5	7,265.00	46	1,467.50	-	443
11-Nov	3.3	Surface		7.5	20,500.00	32	238.5	11,545.00	-	5.5	112	710.5	24,770.00	239.5	44.5	6,815.00	377	11	2,948.00	26	140	18	18,215.00	90	-	63.5	883
11-Nov	3.3	Surface		7	21,645.00	22.5	243.5	10,585.00	-	-	92	547.5	21,310.00	239	45	5,475.00	307	8.5	3,050.50	20.5	95.5	6	20,535.00	99	1,465.50	63	921.5
11-Nov	2.3	Surface		8	16,645.00	28.5	190	-	-	-	-	-	-	179	41.5	-	-	-	2,570.50	-	-	-	16,940.00	89.5	1,164.00	49.5	737.5
11-Nov	2.3	Surface		13		12.5	-	9,505.00	-	5.5	87	621.5	20,200.00	25	32	5,435.00	297	8.5	-	-	96	12	-	-	-	-	-
11-Nov	5.1	Surface		8.5	15,995.00	32	-	-	-	-	-	-	-	168	41.5	-	294.5	-	2,283.50	26	-	13.5	12,930.00	78	1,388.00	52	-
11-Nov	5.1	Surface		8.5	18,165.00	42	-	3,524.50	-	-	8	77.5	5,485.00	189.5	41.5	719.5	-	9	2,890.50	20	13.5	-	-	91	1,171.50	49.5	208.5
11-Nov	5.2	Surface		12	2,672.00	45.5	43	487.5	-	-	-	8	41.5	51.5	33	-	86	-	1,118.00	-	-	-	3,788.50	26	93	6	208.5
11-Nov	5.2	Surface		13.5		12	-	-	-	-	31	152	-	25.5	32	47.5	-	-	641	-	-	-	-	-	-	-	-
11-Nov	5.3	Surface		9	8,925.00	15	123	6,825.00	-	-	27	120.5	-	92.5	35	2,484.00	-	-	1,033.50	17.5	-	-	6,610.00	62.5	456	33	-
11-Nov	5.3	Surface		10	7,625.00	29	98	8,080.00	-	-	20.5	-	13,325.00	82	34.5	1,890.00	224	-	1,394.50	13.5	-	-	8,380.00	50	184.5	19.5	300.5
16-Dec	2.1	Surface		9	9,880.00	25	132	7,145.00	-	-	10.5	112	17,590.00	87	35	2,386.00	270	-	1,753.00	20	134	-	10,320.00	63	321	27.5	320.5
16-Dec	2.1	Surface		9.5	8,115.00	20.5	115.5	9,960.00	-	-	-	98	16,245.00	71.5	34.5	1,854.50	242	-	1,430.50	16	121	-	7,800.00	57.5	290.5	24	295.5
16-Dec	2.2	Surface		7.5	14,265.00	18	60.5	-	-	-	50.5	65	22,455.00	119.5	40	5,070.00	323	-	1,675.00	-	8	-	-	71	1,534.00	53.5	-
16-Dec	2.2	Surface		9	8,575.00	12	-	6,710.00	-	-	25	39	15,790.00	88	36	2,926.50	196.5	-	1,266.50	-	-	-	3,851.00	38	741.5	24.5	100.5
16-Dec	2.3	Surface		9	14,040.00	15.5	-	7,525.00	-	-	67.5	390	17,375.00	161	40.5	4,717.00	245.5	-	1,828.50	13	72.5	16	10,430.00	58	1,185.00	41	492
16-Dec	2.3	Surface		6	23,745.00	21	245	12,640.00	-	9	112.5	639.5	28,825.00	255.5	48	8,055.00	430	10.5	2,496.50	29.5	119.5	21	14,440.00	95	1,892.00	76.5	759.5

Appendix G: Raw Measurements for the Laboratory Column Test

Table 59. Determination of the Water Content in Beaver Lake Bog Soil

Dec 6 2015						
Sample	Dish	Mi	Mf	Change	Change %	Water Content
	g	g	g	g	%	g/g
1	0.9957	14.839	3.3151	10.5282	71%	0.71
2	0.9896	15.829	3.5311	11.3083	71%	0.71
3	1.0012	15.6708	3.4155	11.2541	72%	0.72
Average	-	-	-	-	71.4%	0.714
St Dev	-	-	-	-	0.4%	0.004

Table 60. Raw Data Recorded for the 2-Week Preliminary Column Study

Nov 8, 2015										
30 cm sediment, 30 cm water										
	Depth					Surface				
Time	DO	pH	Temp	Cond	ORP	DO	pH	Temp	Cond	ORP
Zero	7.64	7.14	16.75	64.12	309.1	8.37	6.5	16.67	53.75	322.7
36 hrs	5.19	6.15	19.2	58.13	384.7	4.69	5.71	19.21	54.97	390.2
96 hrs	5.8	5.49	20.14	66.24	373.5	5.45	5.31	20.31	57.06	378.9
192 hrs	3.99	5.44	20.36	94.33	376.6	5.35	5.48	20.45	49.95	372.6

Table 61. Mass of Soil Added to Each Column for the Column Study

Nov 30, 2015	
Column	Mass (kg)
1	8.18
2	8.16
3	8.36
4	8.6
5	8.46
6	8.82
7	8.36
8	8.9
9	9.24
10	9.84
11	8.38
12	7.6
13	8.46
14	8.56
15	8.82
16	9.08
Average	8.61375
St Dev	0.49597

Table 62. Temperature, DO, pH, Conductivity, ORP - Raw Data – Column Log

Date	Temp	DO	pH	ORP	Cond	Temp	DO	pH	ORP	Cond
	Distilled Water Column					Stormwater Column				
01-Dec	17.46	2.3	6.12	328.1	34.31	17.36	2.85	5.7	345.6	60.55
02-Dec	17.64	4.93	4.62	303.3	36.75	16.68	3.76	4.72	295.8	53.15
06-Dec	17.61	4.7	4.75	330.6	48.25	17.71	1.44	4.54	270	61.99
09-Dec	17.81	5.61	4.9	303.4	55.74	17.39	3.4	4.99	312.4	79.52
13-Dec	17.21	3.11	4.91	292.9	67.55	17.61	1.1	4.92	358.4	77.26
16-Dec	17.81	5	4.86	310.1	75.34	17.14	4.98	4.92	350.2	81.22
19-Dec	17.16	1.96	4.96	332.1	74.37	17.59	2.61	5.39	281.1	89.74
23-Dec	17.1	2.57	4.88	285.7	70.04	17.46	1.75	5.24	288.6	90.15
26-Dec	17.58	0.43	5.14	323.7	82.82	17.75	0.31	5.09	299.15	83.58
30-Dec	17.38	0.63	5.26	289.5	86.94	17.63	0.53	5.18	287.3	93.75
02-Jan	17.56	0.41	5.14	285.5	91.66	17.45	0.12	5.4	278.3	88.26
04-Jan	17.62	0.41	5.15	278.9	88.62	17.13	0.57	5.34	284.2	94.3
06-Jan	16.99	1.5	5.38	385.2	81.29	16.85	0.86	4.95	237.7	81.72
08-Jan	16.17	0.54	4	228	100.2	15.84	1.66	4.42	233.2	103.8
11-Jan	16.16	1.33	4.38	210.7	78.1	15.59	0.72	4.23	223	106.3
13-Jan	16.23	0.78	4.5	215.1	89.3	16.01	0.99	4.7	215	103.5
15-Jan	15.94	1.3	4.23	225.6	78.03	15.98	0.93	4.36	260.2	100.3
18-Jan	16.03	0.93	3.68	256.1	128.1	16.03	0.93	3.68	256.1	128.1
20-Jan	16.55	0.57	3.97	223.3	90.74	15.37	1.64	3.77	226.9	111.8
22-Jan	16	0.18	4.11	192.5	118.4	16.01	0.44	4.07	207.9	123.3
25-Jan	16	1.12	4.52	158.4	112.4	16	0.91	4.31	179.4	112.1
27-Jan	16.01	1.17	4.27	203.4	101.9	15.78	1.17	4.27	182.3	111.5
29-Jan	15.35	0.27	4.98	255.1	111.4	15.76	0.2	4.7	234.4	93.1
01-Feb	15.21	0.45	5.03	231.5	111.9	15.61	0.4335	4.5	229	97
03-Feb	16.01	1.09	4.9	226.1	112.9	14.74	1.87	4.62	245.3	103
04-Feb	13.1	0.29	4.89	231.1	111.5	13.1	0.67	4.67	240.5	104.1
05-Feb	11.72	1.77	4.76	269.8	92.74	11.6	0.71	5	244.5	78.94
08-Feb	11.08	1.23	4.82	209.7	110.1	11.1	0.54	4.98	201.4	87.67
09-Feb	11.07	0.28	5.31	186.2	98.7	10.7	0.21	5.07	191.4	90.36
10-Feb	11	1.3	5.1	184.2	98.4	10.79	0.12	5.05	190.17	90.91
11-Feb										
12-Feb										
15-Feb						11.58	0.9	5.11	311.7	109.3
16-Feb	11.48	0.4	4.88	256.6	127	11.42	0.44	5.13	288.5	98.8
17-Feb	11.41	0.35	4.85	254.3	132.5	9.65	0.76	5.44	301.4	99.9
18-Feb	11.39	0.33	4.85	250.1	134.9	9.78	0.36	5.39	295.7	109.1
19-Feb	10.41	0.34	5.3	270.1	110.8	10.12	0.76	5.4	290.1	110.1
21-Feb	10.35	0.21	5.28	256.2	113	11.1	0.75	5.32	293.2	101.1
23-Feb	10.38	0.81	5.28	261.2	109.7	9.8	0.3	5.4	290.3	144.1
24-Feb	10.3	0.86	5.42	260.6	90.81	10.48	1.02	5.24	282.1	96.99
25-Feb	10.41	0.64	5.16	308.1	100.8	9.78	0.95	5.42	257.2	75.83
26-Feb						10.37	1.96	5.58	352.3	73.93
28-Feb	7.69	1.06	5.45	366	80.38	6.83	0.23	5.23	334.7	71.47
01-Mar	10.51	0.58	5.43	311.8	77.32	10.12	2.84	5.45		66.99
02-Mar	10.64	0.55	5.39	307.5	82.21	9.79	0.57	5.31	341.8	75.88
03-Mar	10.44	0.21	5.5	299.2	74.25	10.47	0.14	5.52	317.1	55.84
04-Mar	6.58	1.26	5.4	322	82.76	6.7	3.23	5.8	372.1	51.97
06-Mar	7.58	2	5.33	331.1	71.04	5.16	0.62	5.63	258.1	45.83
08-Mar	6.92	0.51	5.75	285.2	76.12	5.06	0.24	5.61	252.1	50.62
09-Mar	5.91	0.57	5.67	324.1	55.8	5.08	0.5	5.83	324.1	47.12
10-Mar	6.55	2.32	5.9	345	62.79	5.99	1.9	5.6	339	43.12
11-Mar	6.44	3.13	5.49	334.7	59.49	5.53	1.87	5.73	298.1	45
13-Mar	6.45	1.24	5.6	321	58.13	5.51	1.1	5.69	295.1	46.1
15-Mar	6.43	1.16	5.48	321.1	59.5	5.49	0.97	5.69	281.1	47.1
16-Mar	6.41	1	5.5	320	59.01	5.98	0.95	5.7	282.1	47.1
17-Mar	5.9	0.7	5.39	325.5	60.31	6.1	0.87	5.69	281.1	48.5
18-Mar	4.58	2.66	5.92	331.4	54.37	5.58	3.04	5.89	319.1	51.95
20-Mar	6.47	3.34	5.95	297.8	67.6	6.6	4.1	5.95	245.5	47.54
22-Mar	6.13	3.16	5.83	257	61.16	5.74	2.03	5.85	269.9	46.85
23-Mar	5.94	1.31	5.76	245.3	53.92	5.99	2.26	5.99	263.1	45.63
24-Mar	6.63	1.76	5.83	233.4	50.07	5.93	1.09	5.81	244.3	40.91
25-Mar	6.66	1.94	5.82	233	50.37	6.13	1.14	5.83	225.2	43.48
28-Mar	5.88	0.86	5.87	267.1	67	6.02	0.6	5.9	279.1	51.52

Table 63. Temperature, DO, pH, Conductivity, ORP - Raw Data – Column Log

Column	Date	Temp	DO	pH	ORP	Cond
Distilled Water Columns						
1	Dec 4 2015	17.75	0.99	4.52	312.9	83.2
2	Jan 3 2016	17.62	0.41	5.15	278.9	88.62
3	Feb 3 2016	15.21	0.63	5.49	85.11	25.11
4	Mar 3 2016	10.4	0.67	5.68	37.25	331
5	April 1 2016	5.82	0.98	6.23	31.47	266.5
Stormwater Columns						
7	Dec 4 2015	17.66	2.16	4.51	330.7	86.43
8	Dec 4 2015	17.63	1.11	4.48	322.4	92.27
9	Jan 3 2016	17.13	0.86	4.95	284.3	94.3
10	Jan 3 2016	17.01	0.97	4.46	297.4	92.1
11	Feb 3 2016	14.91	0.97	5.01	230.1	82.1
12	Feb 3 2016	14.50	0.64	4.93	235.1	83.2
13	Mar 3 2016	10.5	1.69	5.93	345.1	50.59
14	Mar 3 2016	10.41	1.31	5.84	345.7	33.93
15	April 1 2016	6.21	0.40	6.23	263.1	31.01
16	April 1 2016	6.44	1.31	6.23	266.5	31.17

Table 64. Turbidity, TSS, COD, TOC - Raw Data - Column Study

Date	Site	Type	Turbidity NTU	TSS mg/L	COD mg/L	TOC mg/L
Dec 4 2015	Column 1	Water	338.00	576.00	670.00	74.03
Dec 4 2015	Column 1	Water	353.00	506.67	633.00	77.58
Dec 4 2015	Column 1	Water	349.00	546.67	626.00	-
Jan 4 2015	Column 2	Water	192.33	347.00	642.50	69.92
Jan 4 2015	Column 2	Water	238.33	221.00	690.50	78.09
Jan 4 2015	Column 2	Water	-	-	-	-
Feb 4 2016	Column 3	Water	40.00	73.00	167.00	33.77
Feb 4 2016	Column 3	Water	50.67	80.00	174.00	28.00
Mar 4 2016	Column 3	Water	-	-	-	-
Mar 4 2016	Column 4	Water	43.67	81.00	250.50	69.41
Mar 4 2016	Column 4	Water	32.67	83.00	242.50	98.22
April 3 2016	Column 4	Water	29.67	-	-	100.66
April 3 2016	Column 5	Water	72.83	41.00	169.33	41.05
April 3 2016	Column 5	Water	84.17	127.00	171.00	41.64
April 3 2016	Column 5	Water	-	-	-	-
Dec 4 2015	Column 7	Water	66.70	63.33	273.67	93.03
Dec 4 2015	Column 7	Water	65.90	73.33	193.00	95.49
Dec 4 2015	Column 7	Water	65.30	113.33	177.67	-
Dec 4 2015	Column 8	Water	48.10	136.67	149.67	92.92
Dec 4 2015	Column 8	Water	47.80	136.67	148.33	95.92
Dec 4 2015	Column 8	Water	47.30	113.33	152.33	-
Jan 4 2015	Column 9	Water	132.33	97.00	464.50	95.31
Jan 4 2015	Column 9	Water	129.33	133.00	447.00	97.99
Jan 4 2015	Column 9	Water	-	211.00	-	-
Jan 4 2015	Column 10	Water	57.33	28.00	203.50	82.18
Jan 4 2015	Column 10	Water	15.33	-	252.00	90.36
Jan 4 2015	Column 10	Water	-	-	-	-
Feb 4 2016	Column 11	Water	106.67	148.00	277.00	36.54
Feb 4 2016	Column 11	Water	111.00	84.00	268.00	37.42
Feb 4 2016	Column 11	Water	-	-	-	-
Feb 4 2016	Column 12	Water	118.33	43.00	272.00	33.67
Feb 4 2016	Column 12	Water	124.67	73.00	286.00	34.88
Feb 4 2016	Column 12	Water	-	-	-	-
Mar 4 2016	Column 13	Water	55.33	46.00	220.00	40.80
Mar 4 2016	Column 13	Water	51.33	33.00	224.50	65.65
Mar 4 2016	Column 13	Water	34.67	-	-	-
Mar 4 2016	Column 14	Water	89.00	29.00	673.50	103.46
Mar 4 2016	Column 14	Water	87.33	78.00	566.00	129.50
Mar 4 2016	Column 14	Water	60.33	-	-	-
April 3 2016	Column 15	Water	35.73	41.00	114.00	20.59
April 3 2016	Column 15	Water	36.93	39.00	108.67	22.00
April 3 2016	Column 15	Water	-	-	-	-
April 3 2016	Column 16	Water	37.60	44.00	207.67	45.50
April 3 2016	Column 16	Water	39.40	94.00	210.00	44.99
April 3 2016	Column 16	Water	-	-	-	-
Dec 4 2015	Stormwater Week 1	Water	39.50	169.00	-	54.70
Dec 4 2015	Stormwater Week 1	Water	53.00	162.00	-	54.96
Dec 4 2015	Stormwater Week 1	Water	50.20	201.00	-	-
Jan 4 2015	Stormwater Week 4	Water	22.33	64.00	178.50	84.16
Jan 4 2015	Stormwater Week 4	Water	20.67	70.00	195.50	84.32
Jan 4 2015	Stormwater Week 4	Water	-	-	-	-
Feb 4 2016	Stormwater Week 8	Water	25.33	83.00	108.00	5.57
Feb 4 2016	Stormwater Week 8	Water	28.33	85.00	119.00	5.45
Feb 4 2016	Stormwater Week 8	Water	-	-	-	-
Mar 4 2016	Stormwater Week 12	Water	6.00	406.00	134.50	48.75
Mar 4 2016	Stormwater Week 12	Water	6.00	188.00	143.50	59.87
Mar 4 2016	Stormwater Week 12	Water	4.00	-	-	-
April 3 2016	Stormwater Week 16	Water	91.57	267.00	234.67	3.18
April 3 2016	Stormwater Week 16	Water	92.57	1,491.00	191.00	3.05
April 3 2016	Stormwater Week 16	Water	-	-	-	-
Dec 4 2015	Blank Week 1	Water	0.19	-	-	0.58
Dec 4 2015	Blank Week 1	Water	1.64	1.00	-	0.56
Dec 4 2015	Blank Week 1	Water	1.58	2.00	-	-
Jan 4 2015	Blank Week 4	Water	0.24	-	3.00	0.56
Jan 4 2015	Blank Week 4	Water	0.13	-	10.00	0.55
Jan 4 2015	Blank Week 4	Water	-	-	-	-
Feb 4 2016	Blank Week 8	Water	1.30	1.00	11.00	0.92
Feb 4 2016	Blank Week 8	Water	1.00	-	2.00	1.10
Feb 4 2016	Blank Week 8	Water	-	-	-	-
Mar 4 2016	Blank Week 12	Water	-	-	-	1.04
Mar 4 2016	Blank Week 12	Water	-	3.00	-	0.95
Mar 4 2016	Blank Week 12	Water	-	-	-	-
April 3 2016	Blank Week 16	Water	0.14	-	-	0.53
April 3 2016	Blank Week 16	Water	0.13	2.00	-	0.81
April 3 2016	Blank Week 16	Water				

Table 65. Metals - Raw Data - Column Study - Water

Column	As	Al	B	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	Pb	Sb	Se	Si	Sr	Ti	V	Zn
	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Column 1		0.96	0.08	0.05					0.07	1.34	0.12		0.11			0.02	0.15	0.01	0.03		0.02		0.01	
Column 1		0.91	0.08	0.05					0.17		0.15	1.02	0.16	0.10	8.79	0.13	0.10	0.02	0.05	3.39	0.04	0.04	0.01	0.14
Column 2		0.89	0.07	0.05	5.75						0.15	1.02	0.16		8.58	0.11	0.08	0.02	0.05	3.25	0.04	0.04	0.01	0.14
Column 2		0.88	0.07	0.05	5.67				0.19	2.09	0.14	1.01	0.15	0.09	8.52	0.11	0.07	0.02	0.04		0.03	0.04	0.01	0.14
Column 3		0.78	0.07	0.05	5.39			0.04		2.09	0.14	1.01	0.15	0.09	8.29	0.10	0.07	0.02	0.04	3.25	0.03	0.03	0.01	0.13
Column 3		0.76	0.07	0.05	11.75			0.05	0.14	1.96	0.13	0.97	0.14	0.09	8.15	0.09	0.06	0.02	0.04		0.03	0.03	0.01	0.13
Column 4	0.03	0.72	0.07	0.05	10.47			0.12	0.17	1.74	0.13	0.95	0.14	0.08	7.68	0.07	0.04	0.02	0.04	3.24	0.03	0.03	0.01	0.12
Column 4		0.71	0.07	0.05	8.76			0.17		1.72	0.13	0.93	0.14		7.52	0.07	0.04	0.02	0.04	3.17	0.03	0.03	0.01	0.12
Column 4	0.01	0.57	0.06	0.04	10.22				0.17	1.63	0.13	0.92	0.13		7.28	0.07	0.03	0.02	0.04	3.05	0.03	0.01	0.01	0.11
Column 5		0.52	0.06	0.03	11.33			0.02	0.14	1.56	0.13	0.89	0.13	0.08	7.27	0.06	0.02	0.01	0.04	3.02	0.02	0.01	0.01	0.10
Column 5		0.46	0.05	0.03	10.48			0.05	0.13	1.40	0.13	0.65	0.12	0.08	7.21	0.03	0.02	0.01	0.04	2.81	0.02	0.01	0.01	0.10
Column 7		1.04	0.08	0.06	4.85			0.04		2.58	0.15	1.06	0.17	0.08	13.41	0.14	0.10	0.02	0.05	2.30	0.04	0.04	0.01	0.14
Column 7		1.13	0.08	0.06	5.40			0.05	0.45	2.59	0.16	1.12	0.17	0.07	14.87	0.17	0.11	0.02	0.05	2.03	0.04	0.04	0.01	0.18
Column 8	0.02	1.20	0.09	0.06	3.67		0.01	0.06	0.47	2.63	0.16	1.13	0.18	0.02	14.93	0.20	0.11	0.02	0.05	3.46	0.04	0.04	0.01	0.20
Column 8	0.06	1.21	0.10	0.06	3.48		0.02		0.47	2.75	0.17	1.17	0.19	0.02	16.31	0.21	0.12	0.02	0.05	3.46	0.04	0.04	0.01	0.22
Column 9	0.01	1.28	0.10	0.06	15.44			0.07	0.42	2.81	0.18	1.17	0.21	0.10	16.54	0.22	0.12	0.02	0.05	3.57	0.04	0.05	0.02	0.23
Column 9		1.31	0.10	0.06	14.43			0.07		2.88	0.18	1.20	0.21	0.10	17.04	0.25	0.14	0.02	0.05	3.80	0.05	0.05	0.02	0.25
Column 10		1.35	0.11	0.07	9.90					2.92	0.18	1.20	0.22	0.10	17.81	0.27	0.14	0.02	0.05	3.80	0.05	0.05	0.02	0.25
Column 10		1.59	0.12	0.07	12.36		0.01	0.12	0.48	2.96	0.22	1.23	0.23	0.11	18.88		0.16	0.03	0.05	3.81	0.05	0.06	0.02	0.26
Column 11		1.93	0.12	0.08	7.77				0.51	3.57	0.25	1.27	0.24	0.12	19.90		0.17	0.03	0.05	3.91	0.06	0.06	0.02	0.26
Column 11		2.00	0.12	0.08	8.25			0.12	0.55		0.26	1.27	0.24	0.12	20.16	0.28	0.18	0.03	0.06		0.06	0.07	0.02	0.26
Column 12	0.02	2.05	0.13	0.08	5.64		0.01	0.18	0.55		0.26	1.29	0.28		20.57	0.29	0.18	0.03	0.06	3.98	0.07	0.07	0.02	0.27
Column 12		2.06	0.13	0.09	6.69		0.01	0.22	0.56	3.57	0.26	1.31	0.30	0.12	21.15		0.18	0.03	0.06	4.10	0.07	0.08	0.02	0.28
Column 13		2.06	0.14	0.09	4.90			0.12	0.60	3.85	0.27	1.32	0.30		21.87	0.40	0.18	0.03	0.06	4.57	0.07	0.09	0.02	0.29
Column 13	0.02	2.07	0.17	0.09	4.47			0.12	0.61	3.87	0.27	1.34	0.31	0.13	22.20		0.23	0.03	0.06	4.63	0.07	0.09	0.02	0.30
Column 13		2.14	0.21	0.09	5.30		0.04		0.63	4.06	0.28	1.42	0.31	0.15	22.82	0.43	0.23	0.03	0.06	4.74	0.07	0.09	0.02	0.36
Column 14	0.04	2.16	0.21	0.09	7.76		0.01		0.64	5.91	0.28	1.56	0.32		24.06		0.24	0.04	0.06	4.83	0.08	0.09	0.02	0.38
Column 14		2.24	0.22	0.10	8.06			0.18	0.65	6.01	0.29	1.63	0.37	0.16	24.10	0.53	0.25	0.04	0.07	4.88	0.08	0.09	0.02	0.40
Column 14	0.02	2.32	0.25	0.10	8.29			0.22	0.70	6.46	0.29	1.71	0.40	0.23	24.20		0.25	0.04	0.07	4.90	0.09	0.10	0.02	0.40
Column 15		2.57	0.25	0.10	5.92	0.01	0.01	0.25	0.70	6.52	0.31	1.76	0.41	0.24	25.66	0.66	0.26		0.07	5.25	0.09	0.10	0.03	0.43
Column 15	0.08	2.61	0.25	0.11	5.72			0.28	0.70	7.06	0.36	1.76	0.41	0.26	25.87	0.46			0.07	5.32	0.09	0.11	0.03	0.44
Column 16	0.11	2.86	0.29	0.12	5.68			0.29	0.75	8.90	0.37	1.80	0.46	0.27	27.81	0.56			0.07	5.35	0.11	0.55	0.03	0.44
Column 16	0.06	2.87	0.33	0.12	5.68			0.30	0.78	10.04	0.38	2.05	0.46	0.27		0.43			0.07	5.84	0.11		0.03	0.49
Stormwater Week 1		4.57	0.27	0.18	3.44	0.01	0.03		0.77	12.16	0.07	1.32	0.35	0.40	18.25	1.07	0.29	0.05	0.04	8.04	0.03	0.22	0.02	0.82
Stormwater Week 1		4.04	0.17	0.20	3.16	0.01	0.03		0.82	13.19	0.06	1.18	0.34	0.42	16.98	1.20	0.28	0.07	0.05	7.67	0.02	0.18	0.03	0.88
Stormwater Week 4		2.12	0.14	0.10	2.64		0.03		0.56	10.91	0.05	0.74	0.48	0.56	15.13		0.32	0.06	0.05	4.86	0.02	0.11	0.02	0.72
Stormwater Week 4		1.36	0.21	0.10	2.49		0.02		0.48	4.71	0.04	0.52	0.23	0.52	13.23	0.90	0.33	0.05	0.05	4.09	0.01	0.07	0.01	0.74
Stormwater Week 8	0.03	2.25	0.05	0.12	2.36		0.02	0.22	0.51	3.16	0.05	0.77	0.11	0.32	14.02	0.41	0.21	0.05	0.05	4.40	0.01	0.11	0.01	1.01
Stormwater Week 8	0.04	3.52	0.07	0.14	3.98		0.02	0.11	0.59	3.86	0.06	1.17	0.21	0.38	20.24	0.22	0.25	0.04	0.05	7.20	0.02	0.18	0.02	1.11
Stormwater Week 12		7.82	0.05	0.24	4.06		0.01	0.29	0.91	8.36	0.10	2.24	0.14	0.67	15.99	0.25	0.85	0.06	0.04	14.01	0.04	0.38	0.03	1.02
Stormwater Week 12		9.34	0.05	0.28	5.00		0.01	0.28	1.11	9.85	0.12	2.79	0.18	0.75	10.98	0.36	0.99	0.06	0.07	16.55	0.04	0.43	0.03	1.14
Stormwater Week 16	0.04	4.03	0.10	0.21	3.64	0.01		0.13	0.71	4.37	0.06	1.27	0.39	0.54	5.24	0.15	0.67	0.05	0.05	7.72	0.02	0.19	0.02	0.93
Stormwater Week 16	0.09		0.12			0.02	0.02	0.53			0.28		0.37			0.22		0.11	0.05		0.11	1.24	0.07	
Blank Week 1		0.11			0.05					2.19		0.02	0.10	0.01					0.05	1.03			0.01	0.04
Blank Week 1		0.09	0.10			0.01				3.40		0.02	0.14	0.01	1.08		0.02		0.05	0.90			0.01	0.04
Blank Week 4			0.10						0.11			0.03	0.09	0.01	1.26		0.01	0.02	0.05	1.09			0.01	0.04
Blank Week 4	0.01	0.13			0.13				0.11	2.48		0.03	0.17	0.01			0.01	0.01	0.08	1.10		0.01	0.01	0.04
Blank Week 8		0.12	0.09		0.20		0.02					0.09	0.07	0.03	1.52		0.05	0.02	0.05	1.22			0.02	0.07
Blank Week 8	0.01	0.09	0.08		0.14			0.02		0.43	0.02	0.08	0.02			0.03	0.05		0.06					0.04
Blank Week 12	0.02	0.06	0.07				0.01		0.06			0.01	0.08	0.01	0.71		0.02	0.01	0.06	0.55			0.01	0.02
Blank Week 12	0.01	0.07	0.08					0.03	0.03	0.17			0.02		0.60	0.08			0.06	0.63				0.02
Blank Week 16		0.05	0.04		0.54			0.07	0.04	0.39		0.12			0.84	0.03		0.01	0.04	0.52				0.04
Blank Week 16		0.06	0.04		0.72			0.14	0.05	0.58		0.15	0.01		1.05	0.08		0.01	0.06	0.70				0.05

Table 66. Metals – Raw Data - Column Study - Sediment

Date	Column	Sediment	Al (mg/kg)	B (mg/kg)	Ba (mg/kg)	Ca (mg/kg)	Cd (mg/kg)	Co (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	K (mg/kg)	Mg (mg/kg)	Mn (mg/kg)	Mo (mg/kg)	Na (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Sb (mg/kg)	Zn (mg/kg)
Dec 4 2015	Column 1	Surface	1525.62	29.74	-	14590.41	-	-	-	-	2932.54	23.95	886.21	228.22	9.08	-	34.57	-	7.31	151.05
Dec 4 2015	Column 1	Surface	-	29.68	88.67	14299.08	-	-	-	-	2864.73	23.37	885.27	-	8.98	2680.83	28.24	-	7.15	166.81
Dec 4 2015	Column 1	Surface	1525.62	27.47	90.37	13325.11	-	-	-	-	2745.01	22.65	884.75	218.48	8.63	2504.64	22.77	-	6.48	-
Jan 4 2016	Column 2	Surface	-	27.40	84.42	13222.67	-	-	-	-	2727.06	21.48	914.01	211.59	8.41	2485.42	24.18	-	6.57	-
Jan 4 2016	Column 2	Surface	1437.94	26.28	85.09	12623.69	-	-	-	-	-	20.32	852.33	-	8.13	2483.48	25.80	-	7.05	142.55
Jan 4 2016	Column 2	Surface	1465.42	-	85.05	11702.02	-	-	-	-	2636.59	21.37	850.52	194.98	8.07	2362.90	26.93	-	6.48	143.28
Feb 4 2016	Column 3	Surface	1343.70	-	-	11661.47	-	-	-	-	2717.72	19.12	676.61	190.73	8.01	2083.92	-	-	-	-
Feb 4 2016	Column 3	Surface	1285.75	23.42	77.35	11482.28	-	-	-	-	2597.35	19.84	814.80	208.91	7.97	2249.87	-	-	6.34	140.13
Feb 4 2016	Column 3	Surface	1209.56	24.64	75.52	11256.94	-	-	-	-	2589.79	18.66	737.67	186.52	7.92	2027.08	27.47	-	6.36	136.80
Mar 4 2016	Column 4	Surface	1177.52	22.92	74.54	10875.86	-	-	-	-	2596.36	18.45	736.13	188.08	7.87	2055.73	-	-	6.05	128.40
Mar 4 2016	Column 4	Surface	1174.63	-	73.89	9722.96	-	-	-	-	2552.69	18.55	699.34	172.57	7.68	-	-	-	6.15	120.76
Mar 4 2016	Column 4	Surface	-	-	73.89	7393.53	-	-	-	-	2581.85	-	838.04	176.59	7.01	2020.08	22.01	-	-	115.20
April 3 2016	Column 5	Surface	1050.22	22.71	68.77	5644.48	-	-	-	-	2446.49	18.05	657.20	158.37	6.92	1908.03	18.62	-	5.83	109.51
April 3 2016	Column 5	Surface	1068.21	22.73	68.48	5040.16	-	-	-	-	2026.57	17.64	587.10	156.94	6.66	1811.39	-	-	-	107.11
April 3 2016	Column 5	Surface	-	-	-	-	-	-	-	-	2439.99	18.65	-	168.44	-	-	-	-	-	-
Dec 4 2015	Column 7	Surface	1628.57	41.40	96.94	15328.71	-	-	-	-	-	25.08	902.52	230.01	10.33	2916.05	34.88	120.57	7.37	150.94
Dec 4 2015	Column 7	Surface	1635.84	38.40	-	15414.99	-	-	20.00	-	-	25.02	861.50	253.50	11.45	3038.51	29.14	117.38	7.59	-
Dec 4 2015	Column 8	Surface	1646.69	41.40	97.69	15520.93	-	-	20.89	-	-	25.14	959.89	255.97	11.71	3130.09	40.32	103.04	7.72	168.85
Dec 4 2015	Column 8	Surface	1685.12	43.77	-	15676.16	-	-	17.42	-	-	26.67	1016.23	222.53	12.05	4021.57	-	98.56	7.90	170.36
Jan 4 2016	Column 9	Surface	1881.78	50.37	-	17966.27	-	-	28.24	365.15	-	25.79	999.79	266.29	-	3172.10	42.99	158.78	10.28	170.30
Jan 4 2016	Column 9	Surface	1829.04	49.95	99.35	17073.15	-	-	25.71	366.09	3027.24	27.78	1024.05	277.49	12.96	3695.47	-	-	9.97	199.66
Jan 4 2016	Column 10	Surface	1841.17	49.27	97.86	16794.02	-	-	23.86	374.11	3109.95	30.31	1053.04	278.90	13.01	-	47.81	179.46	9.53	181.42
Jan 4 2016	Column 10	Surface	-	48.48	-	16483.31	-	-	-	379.35	2956.01	29.88	1035.02	276.64	12.50	3512.95	51.07	175.27	9.32	225.84
Feb 4 2016	Column 11	Surface	1903.22	52.40	106.39	21546.60	-	5.24	38.37	401.18	3333.85	29.86	1084.48	280.07	13.69	3407.08	60.74	172.90	8.98	224.26
Feb 4 2016	Column 11	Surface	1991.74	53.96	-	21554.99	-	-	42.30	406.44	3250.65	30.36	1117.57	280.71	13.18	3580.71	66.54	175.39	7.90	226.43
Feb 4 2016	Column 12	Surface	2039.44	58.10	117.56	22840.07	-	5.14	40.63	415.07	3401.45	30.75	1167.95	288.74	15.02	3812.54	54.23	181.09	10.57	244.25
Feb 4 2016	Column 12	Surface	-	64.51	-	23296.70	-	-	39.33	425.91	3439.57	31.55	1124.76	-	-	-	54.86	189.25	10.72	250.02
Mar 4 2016	Column 13	Surface	2596.64	68.51	-	23780.81	-	5.30	71.16	427.70	3622.37	31.01	-	316.20	19.54	4600.69	62.51	182.55	11.31	318.02
Mar 4 2016	Column 13	Surface	2366.85	71.64	121.18	24041.01	-	5.39	64.35	443.40	3582.91	33.92	1260.08	-	19.70	3338.50	64.51	197.31	11.72	306.55
Mar 4 2016	Column 14	Surface	2457.86	80.76	118.17	25040.65	-	6.13	66.50	445.91	3581.56	42.19	1297.79	-	-	5160.07	61.72	-	12.87	323.88
Mar 4 2016	Column 14	Surface	2326.62	81.03	118.55	27966.34	-	-	55.14	-	-	37.26	1221.63	302.30	23.83	4721.10	81.67	191.93	15.03	323.97
April 3 2016	Column 15	Surface	-	91.09	150.59	29033.97	-	9.89	96.09	508.04	4488.20	35.57	1307.80	-	-	5561.68	78.61	-	18.14	-
April 3 2016	Column 15	Surface	3375.88	91.16	-	31383.00	-	9.54	86.27	525.60	4044.50	48.56	-	345.46	36.21	5731.93	-	203.67	21.29	350.51
April 3 2016	Column 16	Surface	3492.38	104.75	144.31	37912.45	-	10.85	103.87	476.74	4004.22	49.11	1335.00	-	36.64	-	86.01	215.66	25.73	347.20
April 3 2016	Column 16	Surface	3221.18	109.84	-	38138.42	6.04	-	73.87	496.54	4760.73	-	-	351.95	-	-	75.59	-	27.28	-
Dec 4 2015	Column 1	Depth	76.74	31283.32	-	29.85	2886.52	32.63	1182.59	223.90	-	4469.80	-	14.42	-	167.37	76.74	31283.32	-	29.85
Dec 4 2015	Column 1	Depth	84.12	9723.96	29.88	30.08	2616.00	28.19	861.27	231.85	6.44	4077.63	-	13.35	-	154.86	84.12	9723.96	29.88	30.08
Dec 4 2015	Column 1	Depth	56.48	9537.64	15.54	30.08	2605.59	28.95	856.29	243.18	-	-	-	13.46	5.44	121.86	56.48	9537.64	15.54	30.08
Dec 4 2015	Column 7	Depth	84.52	11237.87	16.71	31.93	2664.30	52.08	1012.19	203.56	8.98	3800.08	25.81	13.84	8.06	173.50	84.52	11237.87	16.71	31.93
Dec 4 2015	Column 7	Depth	85.99	19133.53	50.64	32.22	2924.00	36.88	1123.22	253.45	8.21	4079.25	27.26	-	9.12	178.23	85.99	19133.53	50.64	32.22
Dec 4 2015	Column 8	Depth	87.77	11885.02	35.09	32.99	2853.95	31.21	930.30	290.34	7.52	3891.88	26.00	-	8.17	161.36	87.77	11885.02	35.09	32.99
Dec 4 2015	Column 8	Depth	90.26	19253.47	-	33.56	2741.65	33.02	1076.51	305.57	6.65	-	32.00	16.83	7.95	189.16	90.26	19253.47	-	33.56
Jan 4 2016	Column 2	Depth	65.41	15159.82	-	34.18	3372.13	39.29	1229.41	209.29	-	4735.34	-	-	-	-	65.41	15159.82	-	34.18
Jan 4 2016	Column 2	Depth	70.90	12687.10	30.41	34.34	3064.78	34.72	1093.03	200.08	-	4523.90	-	20.99	5.56	-	70.90	12687.10	30.41	34.34
Jan 4 2016	Column 2	Depth	70.24	26653.80	-	-	3735.21	45.35	1447.49	208.33	6.93	-	-	19.04	-	100.94	70.24	26653.80	-	-
Jan 4 2016	Column 9	Depth	91.16	19781.10	47.52	204.77	2650.28	29.97	968.22	189.83	9.36	3071.60	39.75	-	9.98	205.17	91.16	19781.10	47.52	204.77
Jan 4 2016	Column 9	Depth	92.13	15873.45	78.37	205.28	3101.98	34.59	1115.48	264.87	9.83	2786.38	47.06	148.08	11.00	231.44	92.13	15873.45	78.37	205.28
Jan 4 2016	Column 10	Depth	92.49	30073.50	61.07	210.16	3036.34	30.18	1202.91	228.57	7.87	2662.73	32.61	-	7.86	211.81	92.49	30073.50	61.07	210.16
Jan 4 2016	Column 10	Depth	93.80	8946.96	-	-	2741.56	29.87	930.86	229.00	7.94	2610.63	27.19	197.09	10.17	214.07	93.80	8946.96	-	-
Feb 4 2016	Column 3	Depth	-	15605.74	-	-	3882.58	39.97	1012.65	165.28	-	2440.04	-	15.99	5.81	-	-	15605.74	-	-
Feb 4 2016	Column 3	Depth	68.27	17003.28	17.28	38.22	2173.38	29.20	842.79	161.58	7.61	2845.20	-	17.39	-	106.76	68.27	17003.28	17.28	38.22
Feb 4 2016	Column 3	Depth	72.43	25651.09	36.95	-	2385.57	35.12	993.52	146.12	-	-	-	18.38	-	-	72.43	25651.09	36.95	-
Feb 4 2016	Column 11	Depth	94.43	10163.88	66.91	348.27	2103.55	21.56	597.96	152.69	8.25	2368.84	55.45	148.08	7.06	191.69	94.43	10163.88	66.91	348.27
Feb 4 2016	Column 11	Depth	96.34	21657.76	-	363.16	2820.72	30.61	1020.27	188.34	11.15	2848.89	59.60	230.74	9.51	263.52	96.34	21657.76	-	363.16
Feb 4 2016	Column 12	Depth	99.37	24755.68	-	374.56	2909.65	29.24	1178.99	231.63	17.74	-	58.36	171.84	8.22	233.89	99.37	24755.68	-	374.56
Feb 4 2016	Column 12	Depth	100.23	28784.73	65.59	380.11	2135.98	25.41	979.79	163.39	12.13	2709.21	52.35	-	8.64	-	100.23	28784.73	65.59	380.11
Mar 4 2016	Column 4	Depth	72.52	13749.12	-	-	2908.03	28.20	857.94	179.26	-	1735.02	-	-	5.66	115.43	72.52	13749.12	-	-
Mar 4 2016	Column 4	Depth	73.07	10596.40	28.63	42.41	2418.43	25.55	682.21	145.23	8.88	1482.77	-	12.83	4.78	134.95	73.07	10596.40	28.63	42.41
Mar 4 2016	Column 4	Depth	75.25	16010.01	-	-	2436.11	27.31	772.57	158.08	-	1777.67	-	14.25	-	113.12	75.25	16010.01	-	-
Mar 4 2016	Column 13	Depth	104.30	7927.01	52.68	-	3579.86	25.97	811.93	191.42	13.11	1742.78	58.50	180.10	11.37	226.52	104.30	7927.01	52.68	-
Mar 4 2016	Column 13	Depth	-	13392.54	57.38	440.07	2760.56	25.03	817.05	180.16	10.53	1665.96	76.81	-	11.30	205.17	-	13392.54	57.38	440.07
Mar 4 2016	Column 14	Depth	107.52	11349.75	104.61	440.25	5191.54	26.11	807.29	216.55	11.45	1531.56	61.62	169.35	9.88	223.35	107.52	11349.75	104.61	440.25
Mar 4 2016	Column 14	Depth	75.46	9602.39	123.26	446.26	3353.59	20.61	750.49	187.39	12.66	1412.92	56.85	180.93	-	223.77	75.46	9602.39	123.26	446.26
April 3 2016	Column 5	Depth	-	8288.39	-	-	3175.62	22.49	633.81	165.01	-	1753.51	-	14.65	5.99	158.31	-	8288.39	-	-
April 3 2016	Column 5	Depth	70.24	14576.27	30.53	-	2261.22	24.49	749.78	159.93	7.88	1329.04	-	-	-	-	70.24	14576.27	30.53	-
April 3 2016	Column 5	Depth	-	6064.44	-	-	1870.92	19.81	578.97	139.76	5.38	1210.18	4.53	14.42	-	-	-	6064.44	-	-
April 3 2016	Column 15	Depth	-	12179.55	249.06	454.56	2052.15	22.56	665.16	141.68	-	1121.18	74.35	171.85	17.00	289.55	-	12179.55	249.06	454.56
April 3 2016	Column 15	Depth	108.21	6715.62	-	487.31	7096.38	19.25	588.96	279.62	21.81	1451.15	67.71	180.14	10.59	261.12	108.21	6715.62	-	487.31
April 3 2016	Column 16	Depth	110.52	15609.73	262.29	497.15	8616.85	26.34	771.92	299.29	25.46	1834.52	97.05	222.25	10.56	293.51	110.52	15609.73	262.29	497.15
April 3 2016	Column 16	Depth	-	11408.66	-	552.65	-	26.04	552.99	366.18	-	1622.96	97.20	195.77	15.53	329.71	-	11408.66	-	552.65

Appendix H: Alpha Diversity

Field Study

Water Samples

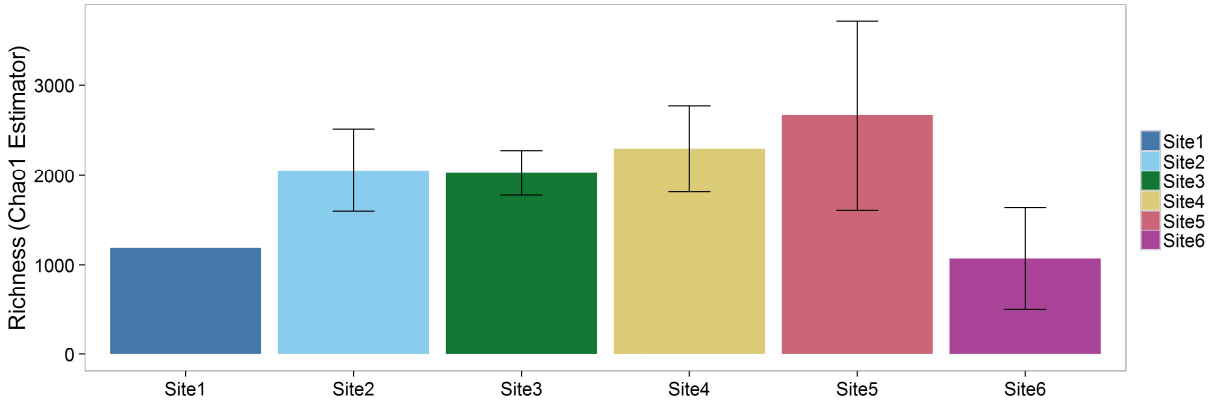


Figure 109. Barplot Between Field Site Water Samples for Richness Based on the Chao1 Estimator

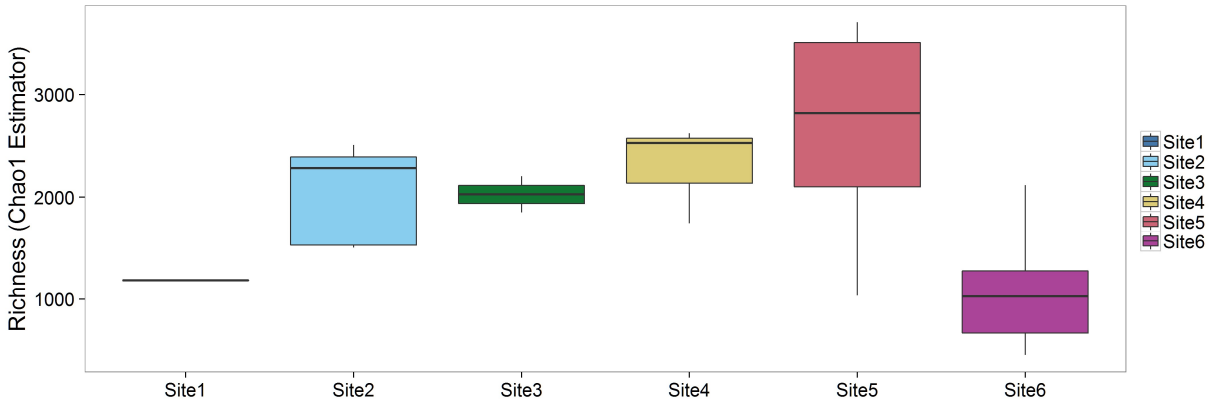


Figure 110. Boxplot Between Field Site Water Samples for Richness Based on the Chao1 Estimator

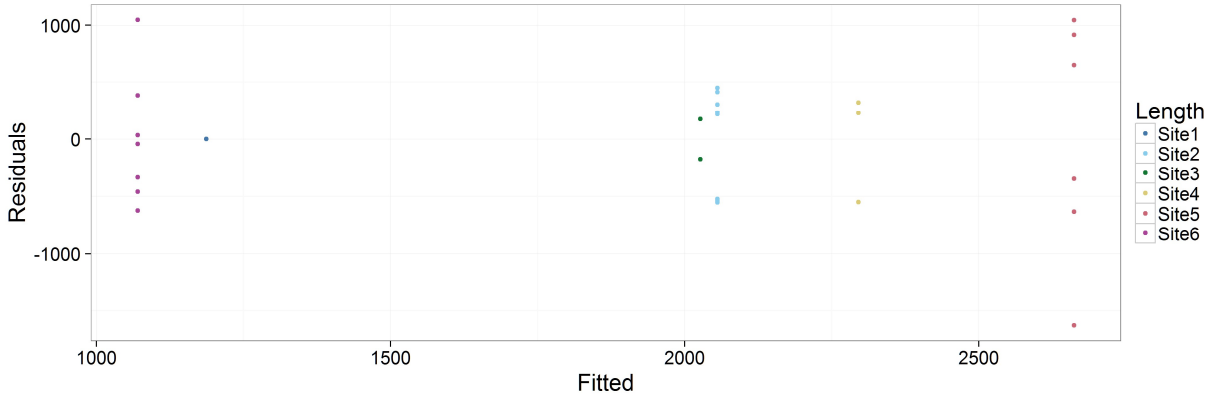


Figure 111. ANOVA Residuals Between Field Site Water Samples for Richness Based on the Chao1 Estimator

Table 67. One Way ANOVA Test Result for Richness Comparison of Water Samples by Field Site Based on Chao1 Estimator

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Length	5	9484682.454	1896936.491	4.225	0.008
Residuals	21	9429328.86	449015.66	NA	NA

Table 68. Tukey HSD Test Result for Diversity Comparison of Water Samples by Field Site Based on Chao1 Estimator

Comparison	p-value
Site2-Site1	0.821324
Site3-Site1	0.904872
Site4-Site1	0.70785
Site5-Site1	0.355519
Site6-Site1	0.999981
Site3-Site2	1
Site4-Site2	0.994339
Site5-Site2	0.561553
Site6-Site2	0.089305
Site4-Site3	0.997624
Site5-Site3	0.849941
Site6-Site3	0.498426
Site5-Site4	0.969094
Site6-Site4	0.128793
Site6-Site5	0.004016

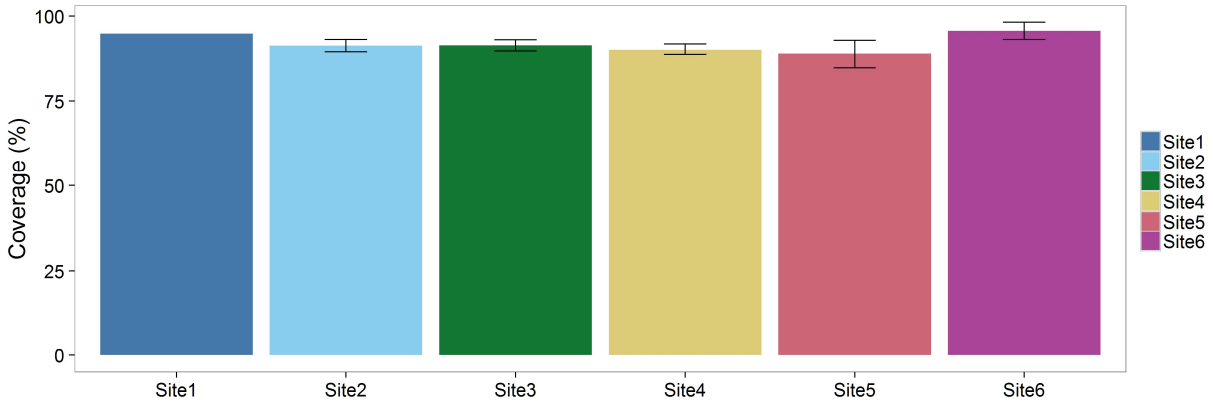


Figure 112. Barplot Between Field Site Water Samples for Coverage Based on Good's Coverage

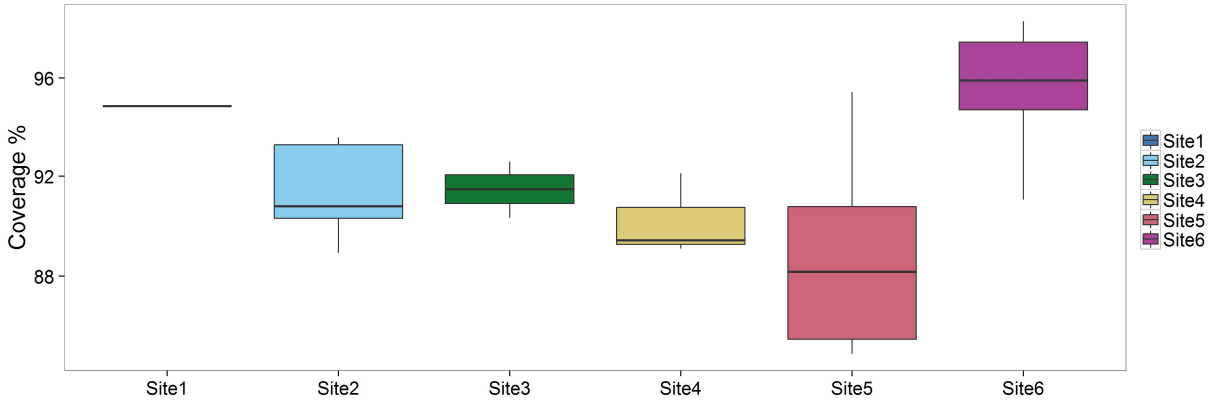


Figure 113. Boxplot Between Field Site Water Samples for Coverage Based on Good's Coverage

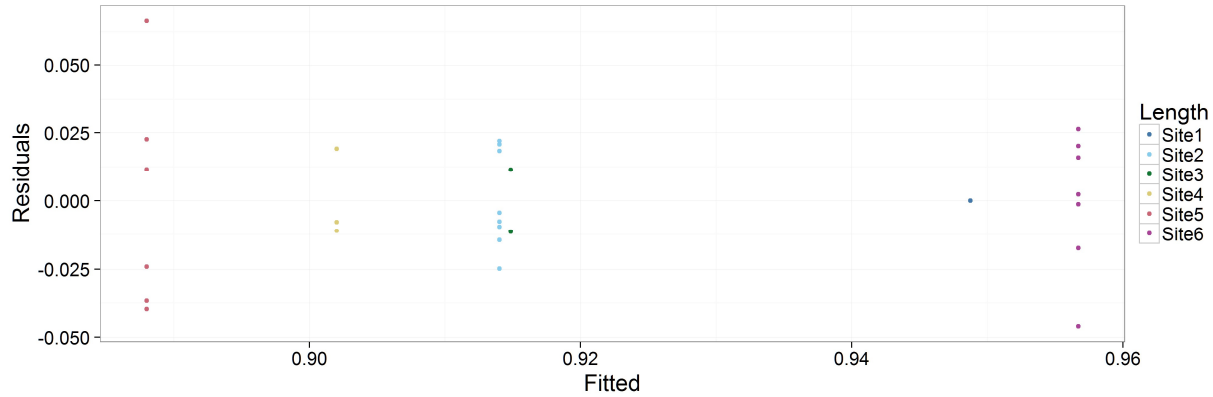


Figure 114. ANOVA Residuals Between Field Site Water Samples for Coverage Based on Good's Coverage

Table 69. One Way ANOVA Test Result for Coverage Comparison of Water Samples by Field Site Based on Good's Coverage

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Length	5	0.018	0.004	4.820	0.004
Residuals	21	0.015	0.001	NA	NA

Table 70. Tukey HSD Test Result for Coverage Comparison of Water Samples by Field Site Based on Good's Coverage

Comparison	p-value
Site2-Site1	0.827992639
Site3-Site1	0.905485326
Site4-Site1	0.671668950
Site5-Site1	0.336667924
Site6-Site1	0.999753717
Site3-Site2	0.999999986
Site4-Site2	0.985021091
Site5-Site2	0.499809440
Site6-Site2	0.059521831
Site4-Site3	0.994793003
Site5-Site3	0.825560531
Site6-Site3	0.414424802
Site5-Site4	0.975734200
Site6-Site4	0.075627941
Site6-Site5	0.002070515

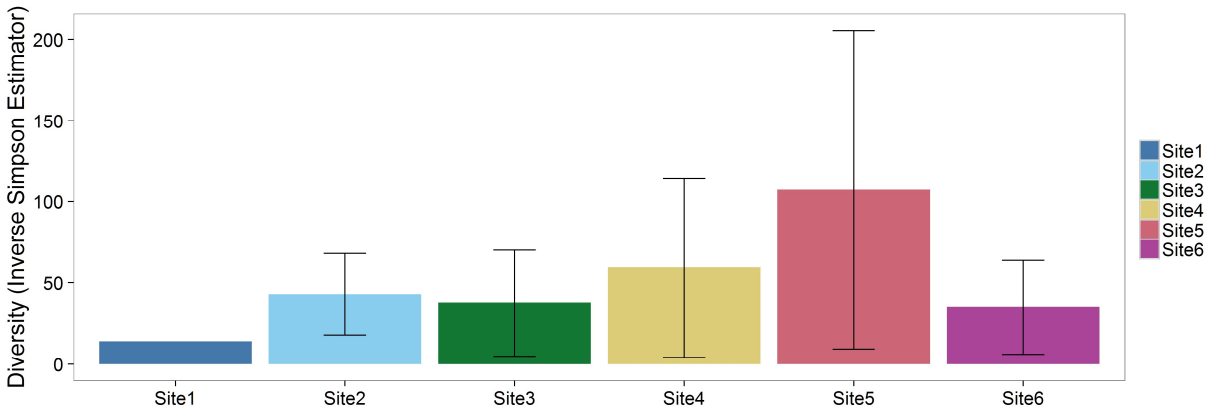


Figure 115. Barplot Between Field Site Water Samples for Diversity Based on the Inverse Simpson Estimator

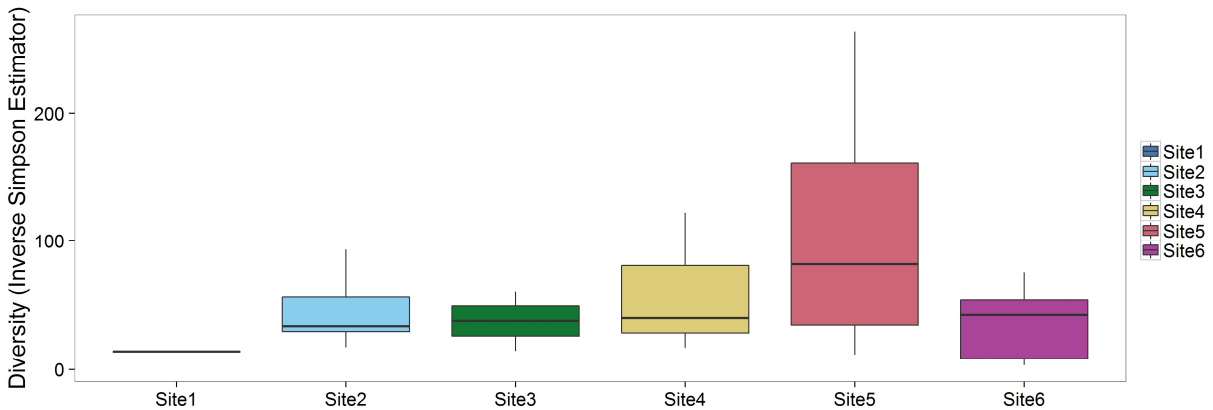


Figure 116. Boxplot Between Field Site Water Samples for Diversity Based on the Inverse Simpson Estimator

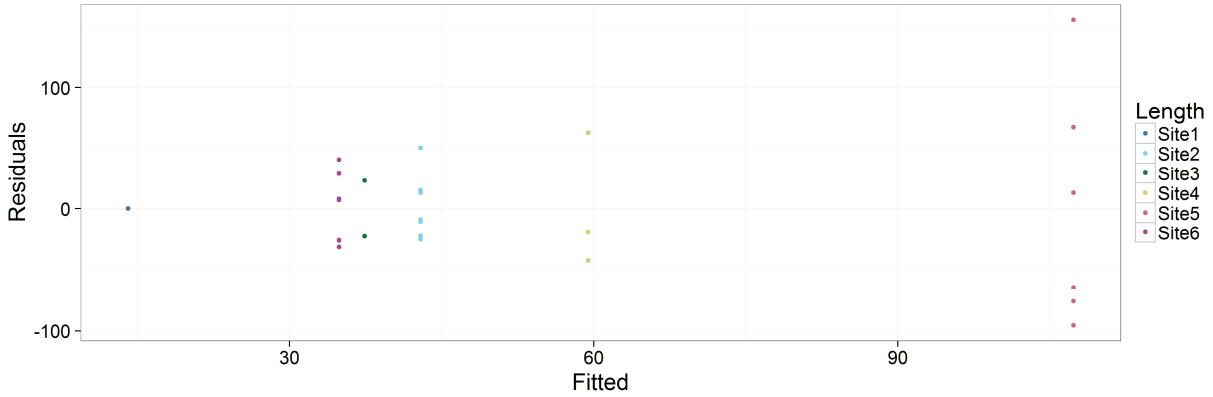


Figure 117. ANOVA Residuals Between Field Site Water Samples for Diversity Based on the Inverse Simpson Estimator

Table 71. One Way ANOVA Test Result for Diversity Comparison of Water Samples by Field Site Based on the Inverse Simpson Estimator

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Length	5	22762.53	4552.507	1.484	0.237
Residuals	21	64442.52	3068.692	NA	NA

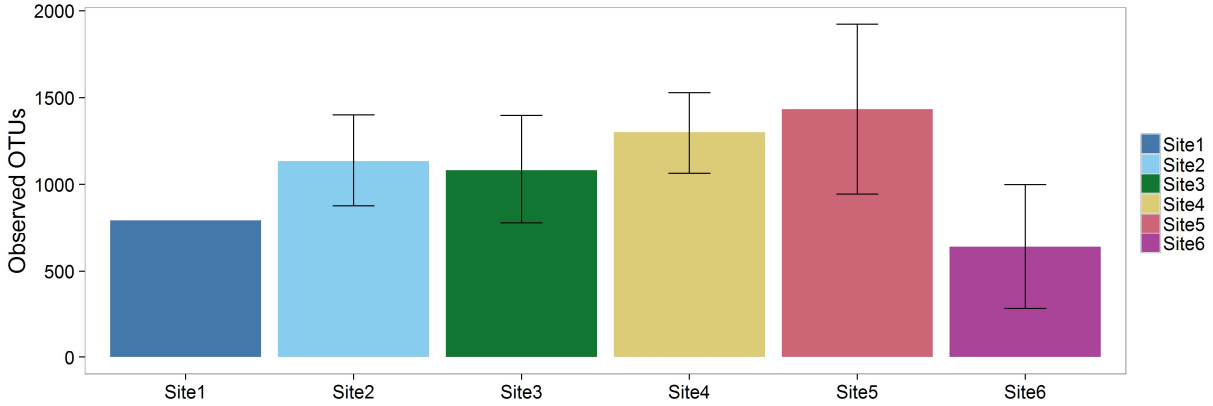


Figure 118. Barplot Between Field Site Water Samples for Observed OTUs Based on the SOBS Calculation

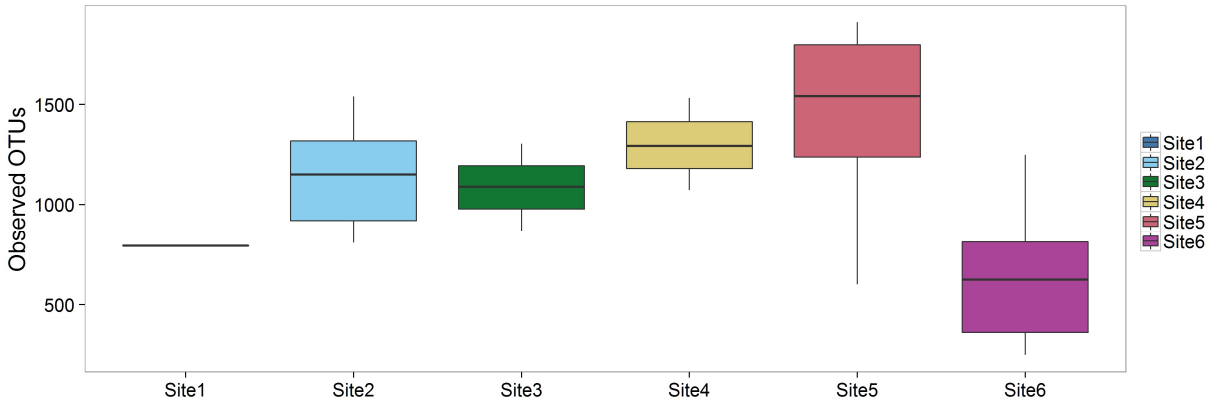


Figure 119. Boxplot Between Field Site Water Samples for Observed OTUs Based on the SOBS Calculation

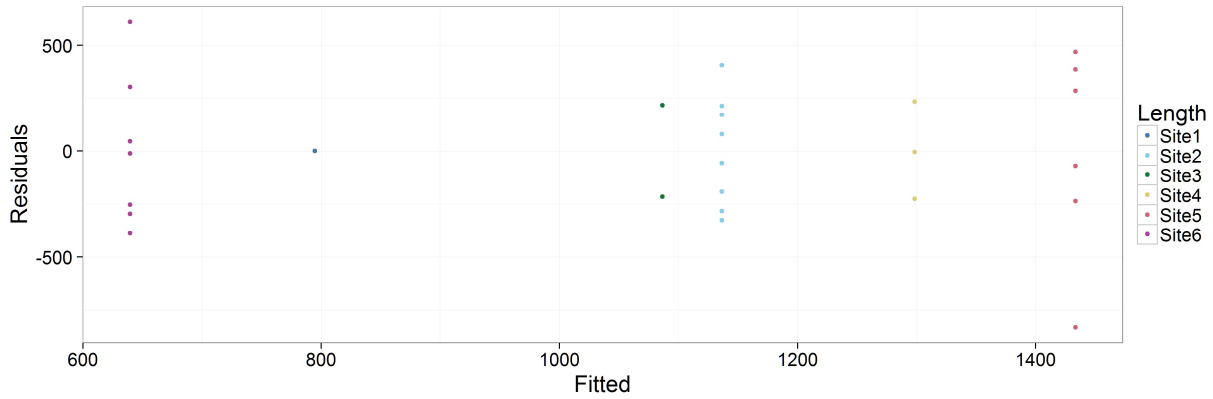


Figure 120. ANOVA Residuals Between Field Site Water Samples for Observed OTUs Based on the SOBS Calculation

Table 72. One Way ANOVA Test Result for Observed OTUs Comparison of Water Samples by Field Site Based on SOBS Calculation

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Length	5	2356581	471316.3	3.752	0.014
Residuals	21	2638105	125624.1	NA	NA

Table 73. Tukey HSD Test Result for Observed OTUs Comparison of Water Samples by Field Site Based on SOBS Calculation

Comparison	p-value
Site2-Site1	0.93985
Site3-Site1	0.983178
Site4-Site1	0.817223
Site5-Site1	0.56575
Site6-Site1	0.998299
Site3-Site2	0.999971
Site4-Site2	0.9829
Site5-Site2	0.637026
Site6-Site2	0.115232
Site4-Site3	0.985022
Site5-Site3	0.832528
Site6-Site3	0.624077
Site5-Site4	0.993807
Site6-Site4	0.118609
Site6-Site5	0.007

Surface Sediment Samples

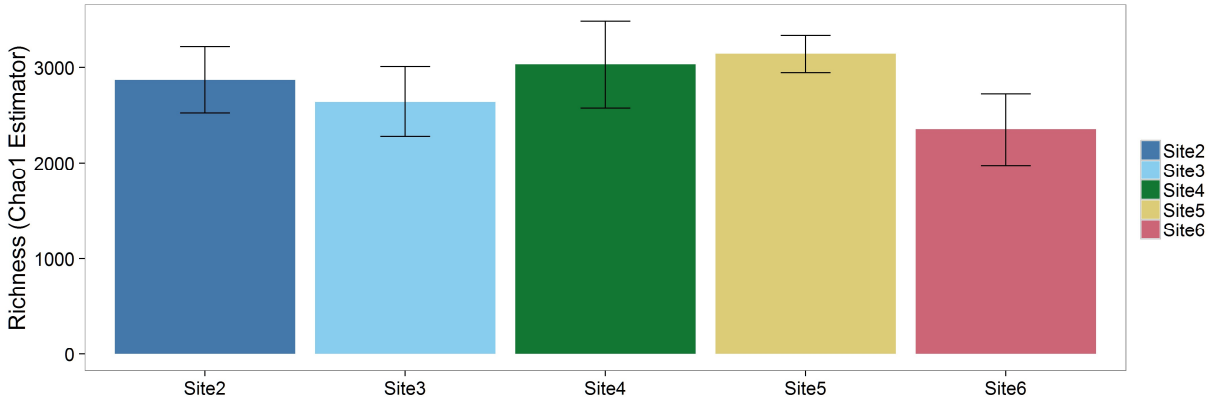


Figure 121. Barplot Between Field Site Surface Sediment Samples for Richness Based on the Chao1 Estimator

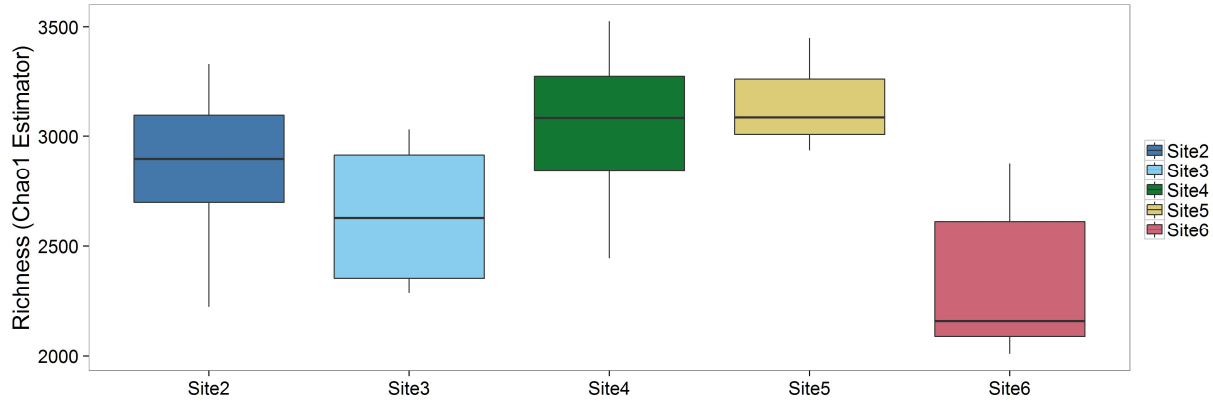


Figure 122. Boxplot Between Field Site Surface Sediment Samples for Richness Based on the Chao1 Estimator

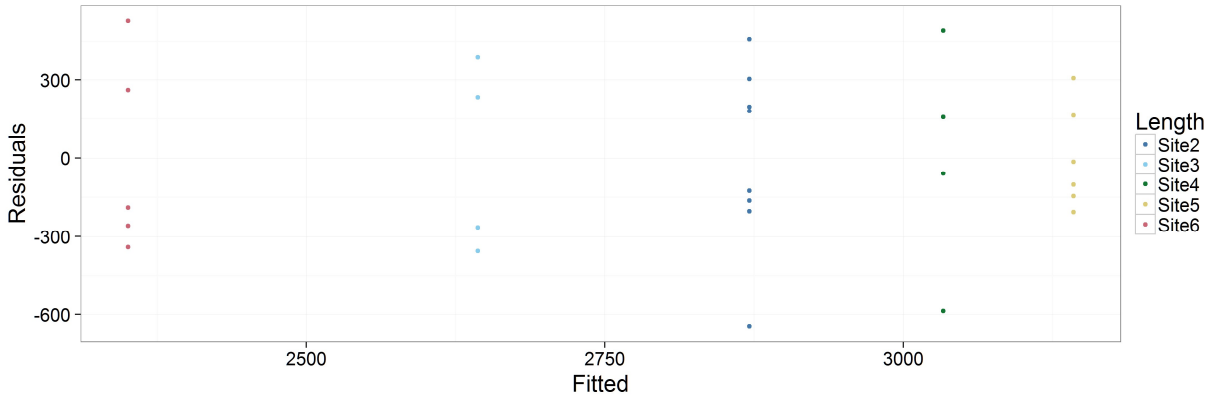


Figure 123. ANOVA Residuals Between Field Site Surface Sediment Samples for Richness Based on the Chao1 Estimator

Table 74. One Way ANOVA Test Result for Diversity Comparison of Surface Sediment Samples by Field Site Based on Chao1 Estimator

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Length	4	2052628	513157	4.261	0.011
Residuals	22	2649487	120431.2	NA	NA

Table 75. Tukey HSD Test Result for Diversity Comparison of Surface Sediment Samples by Field Site Based on Chao1 Estimator

Comparison	p-value
Site3-Site2	0.819704
Site4-Site2	0.938196
Site5-Site2	0.604435
Site6-Site2	0.098849
Site4-Site3	0.519824
Site5-Site3	0.20691
Site6-Site3	0.717862
Site5-Site4	0.987759
Site6-Site4	0.053663
Site6-Site5	0.008423

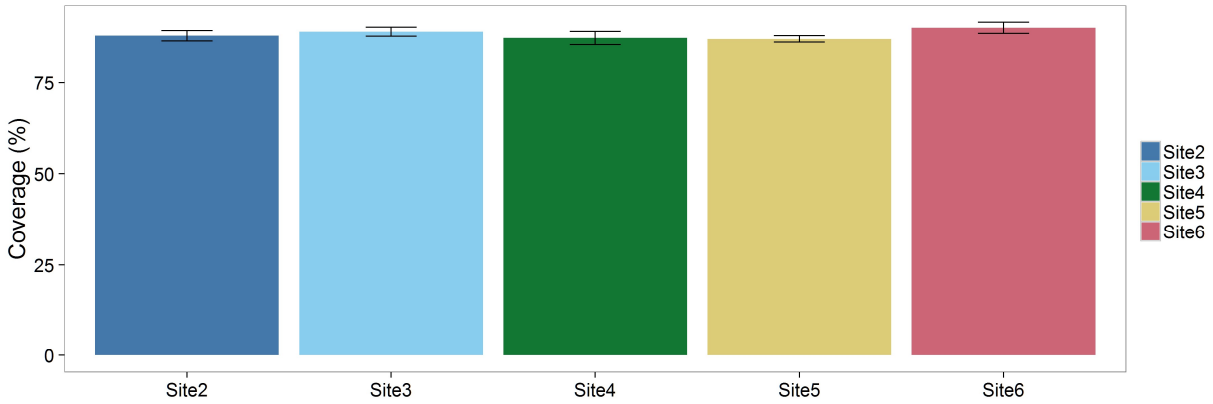


Figure 124. Barplot Between Field Site Surface Sediment Samples for Coverage Based on Good's Coverage

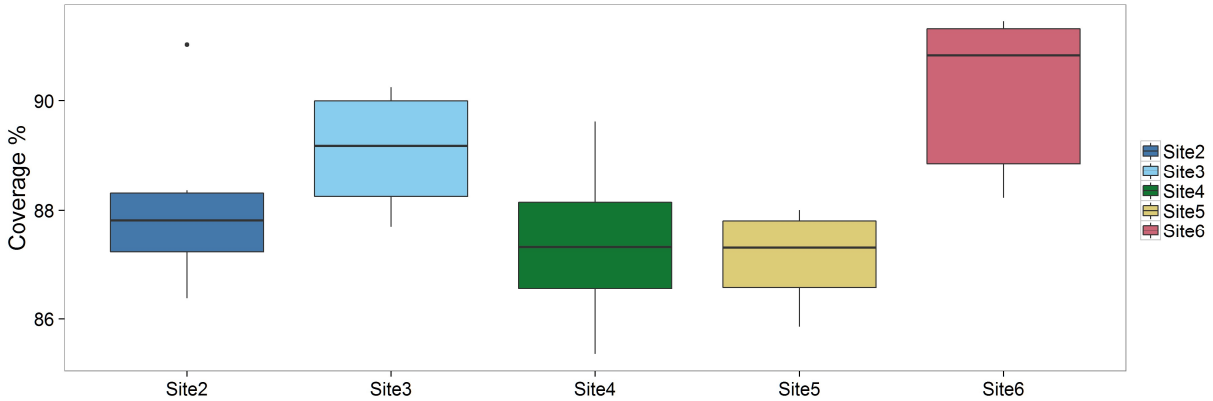


Figure 125. Boxplot Between Field Site Surface Sediment Samples for Coverage Based on Good's Coverage

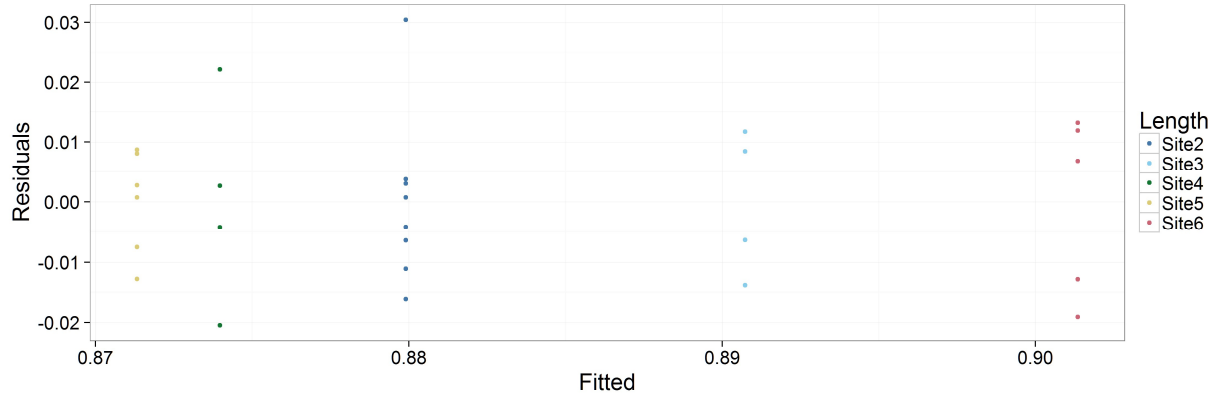


Figure 126. ANOVA Residuals Between Field Site Surface Sediment Samples for Coverage Based on Good's Coverage

Table 76. One Way ANOVA Test Result for Coverage Comparison of Surface Sediment Samples by Field Site Based on Good's Coverage

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Length	4	0.003	0.001	4.302	0.010
Residuals	22	0.004	0.000	NA	NA

Table 77. Tukey HSD Test Result for Coverage Comparison of Surface Sediment Samples by Field Site Based on Good's Coverage

Comparison	p-value
Site3-Site2	0.688732
Site4-Site2	0.950255
Site5-Site2	0.764651
Site6-Site2	0.072843
Site4-Site3	0.423353
Site5-Site3	0.207471
Site6-Site3	0.766981
Site5-Site4	0.998012
Site6-Site4	0.044596
Site6-Site5	0.01057

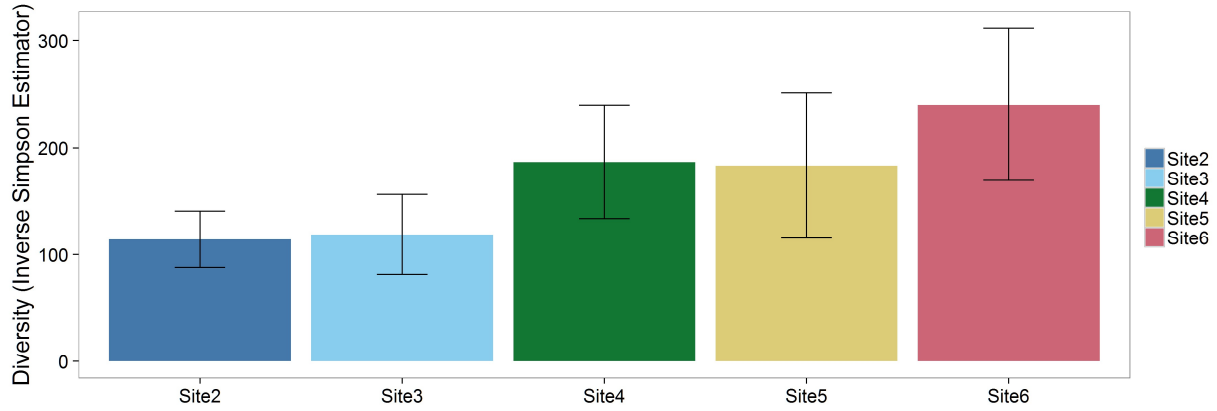


Figure 127. Barplot Between Field Site Surface Sediment Samples for Diversity Based on the Inverse Simpson Estimator

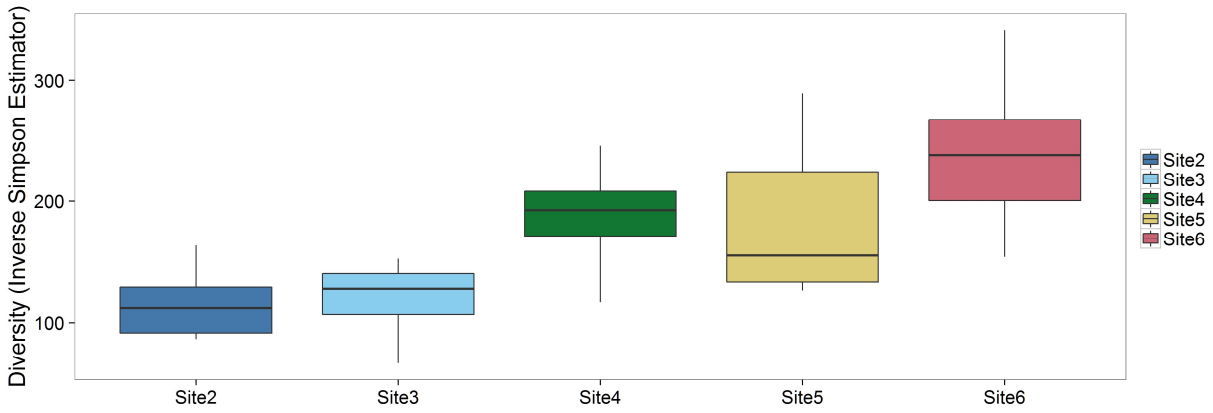


Figure 128. Boxplot Between Field Site Surface Sediment Samples for Diversity Based on the Inverse Simpson Estimator

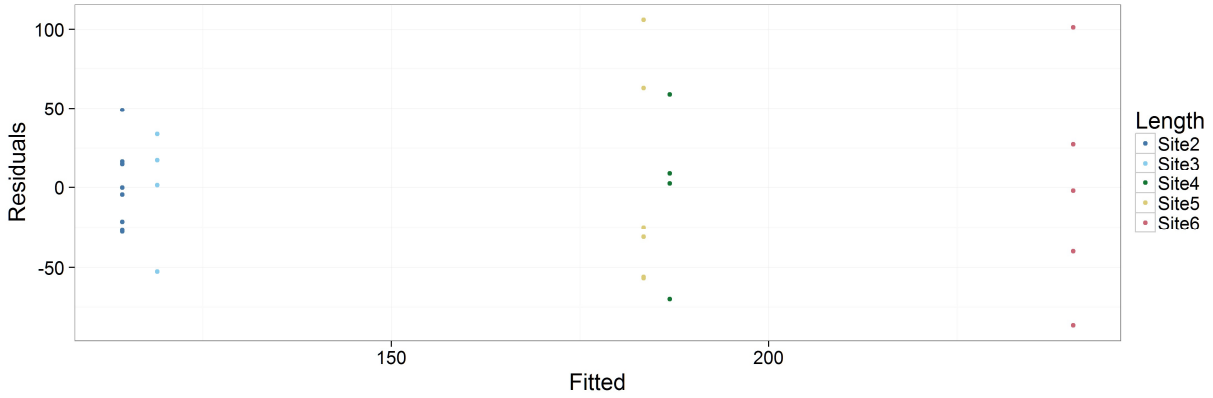


Figure 129. ANOVA Residuals Between Field Site Surface Sediment Samples for Diversity Based on the Inverse Simpson Estimator

Table 78. One Way ANOVA Test Result for Diversity Comparison of Surface Sediment Samples by Field Site Based on the Inverse Simpson Estimator

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Length	4	61359.32	15339.83	5.558	0.003
Residuals	22	60716.58	2759.844	NA	NA

Table 79. Tukey HSD Test Result for Diversity Comparison of Surface Sediment Samples by Field Site Based on the Inverse Simpson Estimator

Comparison	p-value
Site3-Site2	0.999893
Site4-Site2	0.197192
Site5-Site2	0.142988
Site6-Site2	0.003011
Site4-Site3	0.383686
Site5-Site3	0.346143
Site6-Site3	0.017658
Site5-Site4	0.999974
Site6-Site4	0.562339
Site6-Site5	0.404104

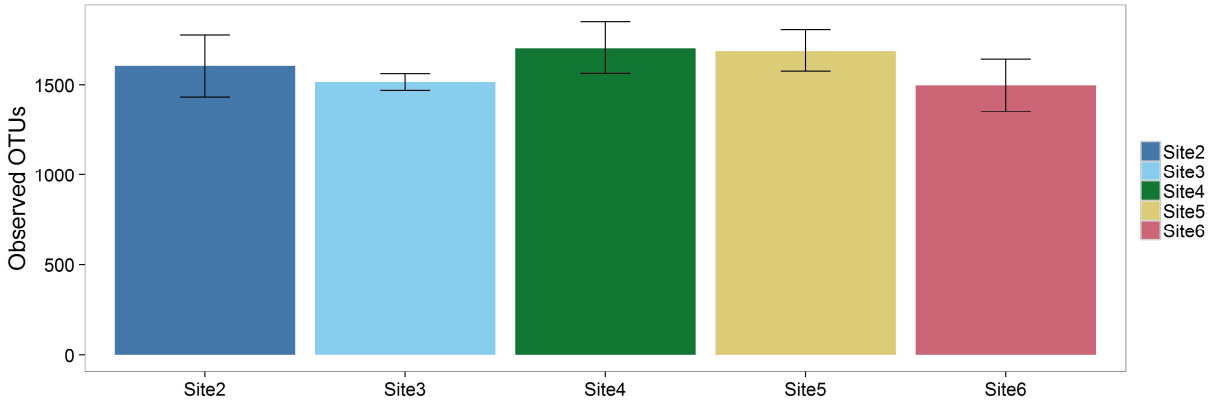


Figure 130. Barplot Between Field Site Surface Sediment Samples for Observed OTUs Based on the SOBS Calculation

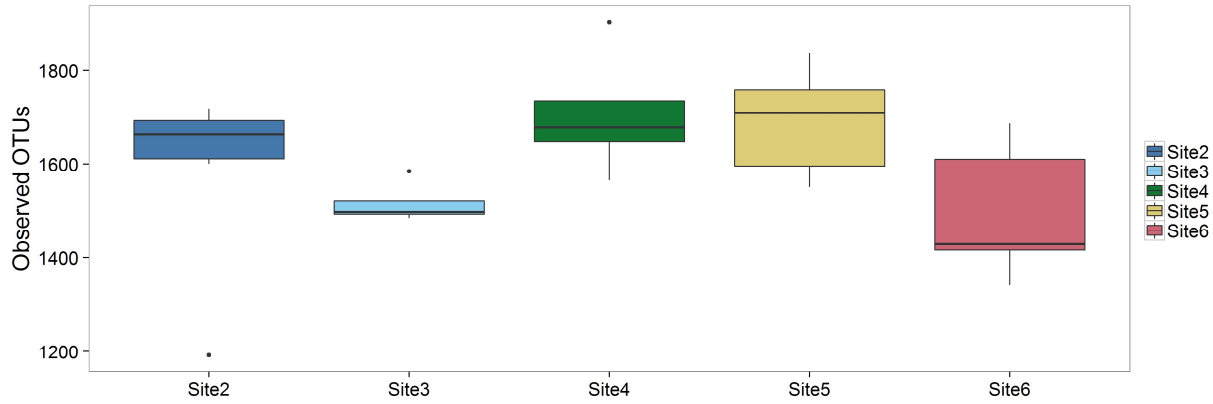


Figure 131. Boxplot Between Field Site Surface Sediment Samples for Observed OTUs Based on the SOBS Calculation

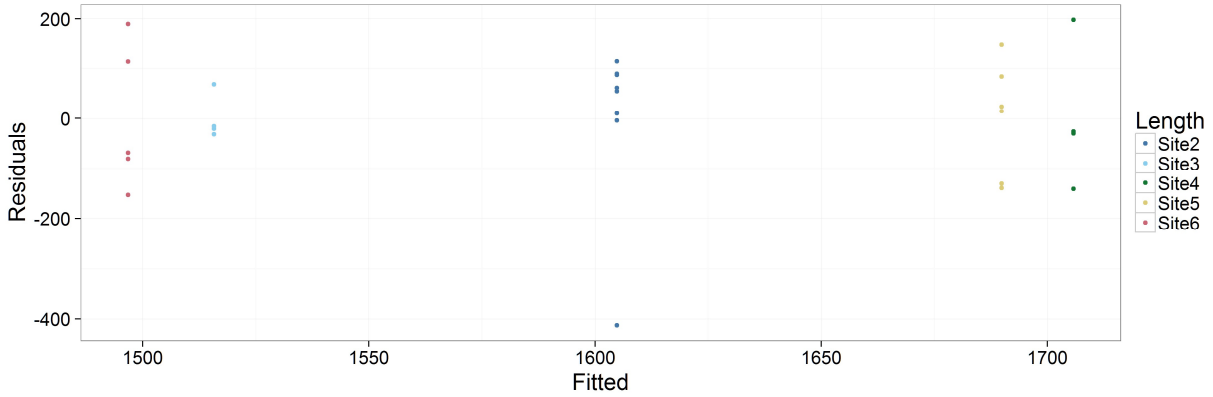


Figure 132. ANOVA Residuals Between Field Site Surface Sediment Samples for Observed OTUs Based on the SOBS Calculation

Table 80. One Way ANOVA Test Result for Observed OTUs Comparison of Surface Sediment Samples by Field Site Based on SOBS Calculation

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Length	4	174258.734	43564.683	2.264	0.0948
Residuals	22	423382.085	19244.640	NA	NA

10-cm Depth Sediment Samples

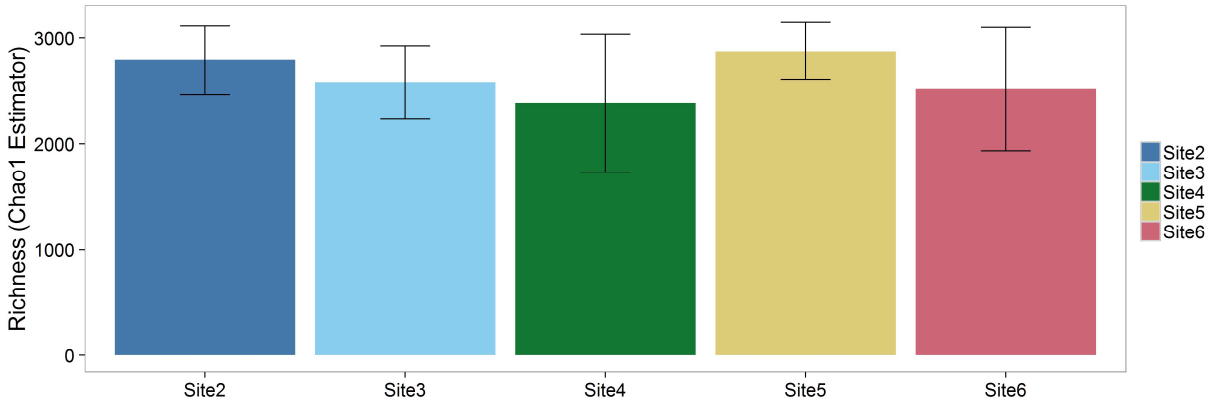


Figure 133. Barplot Between Field Site 10-cm Depth Sediment Samples for Richness Based on the Chao1 Estimator

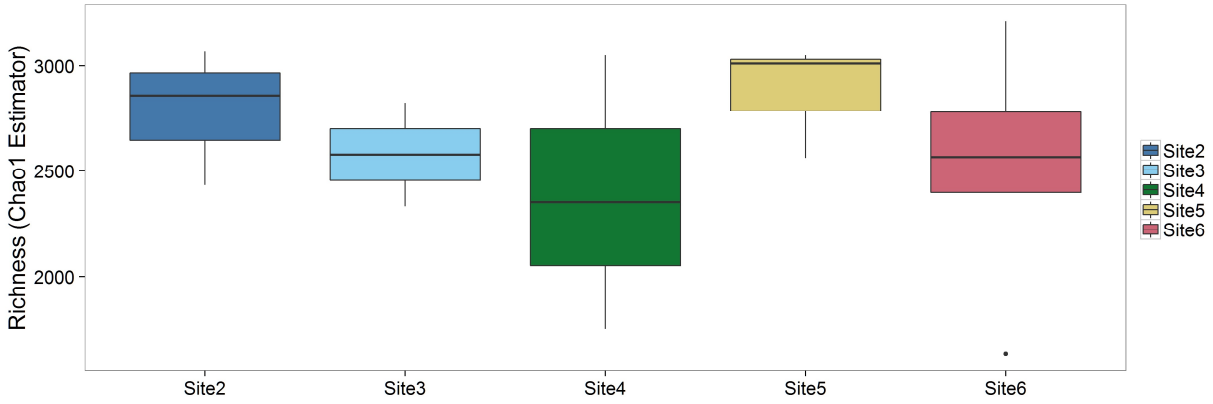


Figure 134. Boxplot Between Field Site 10-cm Depth Sediment Samples for Richness Based on the Chao1 Estimator

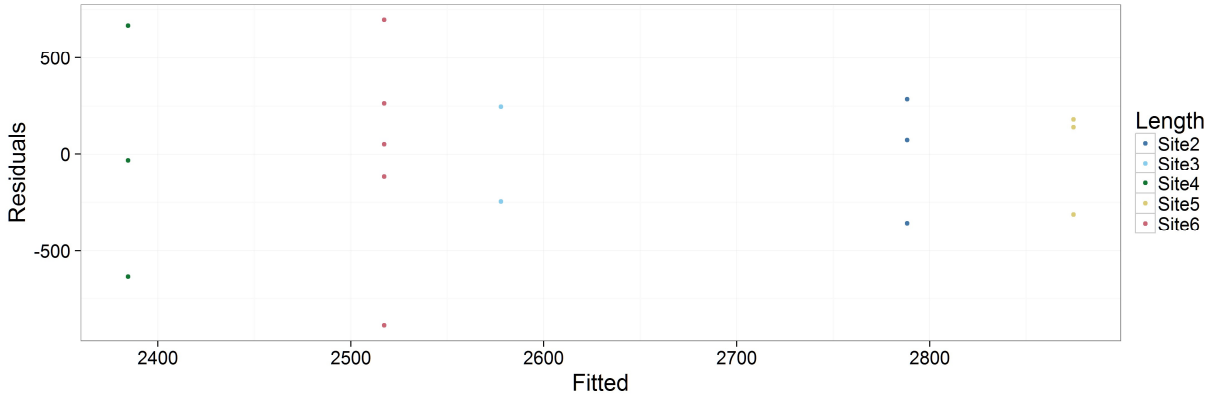


Figure 135. ANOVA Residuals Between Field Site 10-cm Depth Sediment Samples for Richness Based on the Chao1 Estimator

Table 81. One Way ANOVA Test Result for Richness Comparison of 10-cm Depth Sediment Samples by Field Site Based on Chao1 Estimator

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Length	4	501734.553	125433.638	0.514	0.727
Residuals	11	2685664.111	244151.283	NA	NA

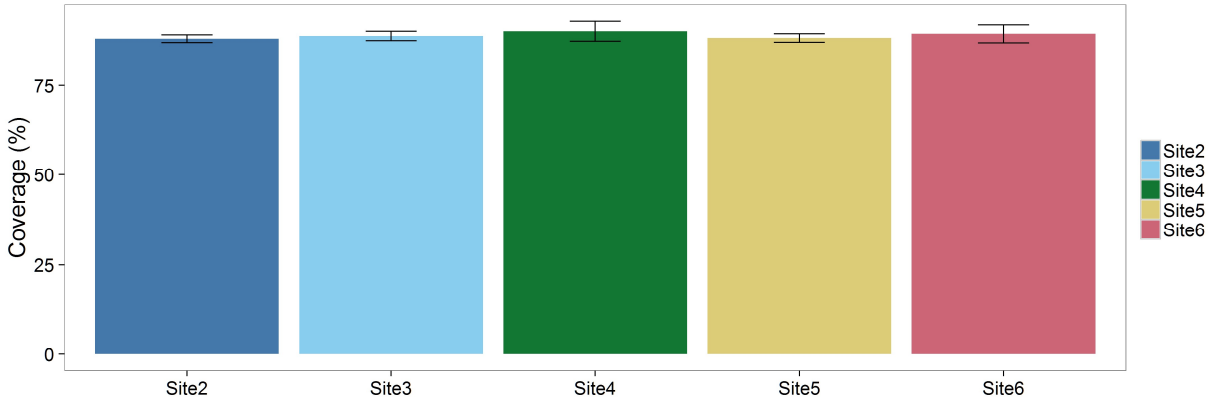


Figure 136. Barplot Between Field Site 10-cm Depth Sediment Samples for Coverage Based on Good's Coverage

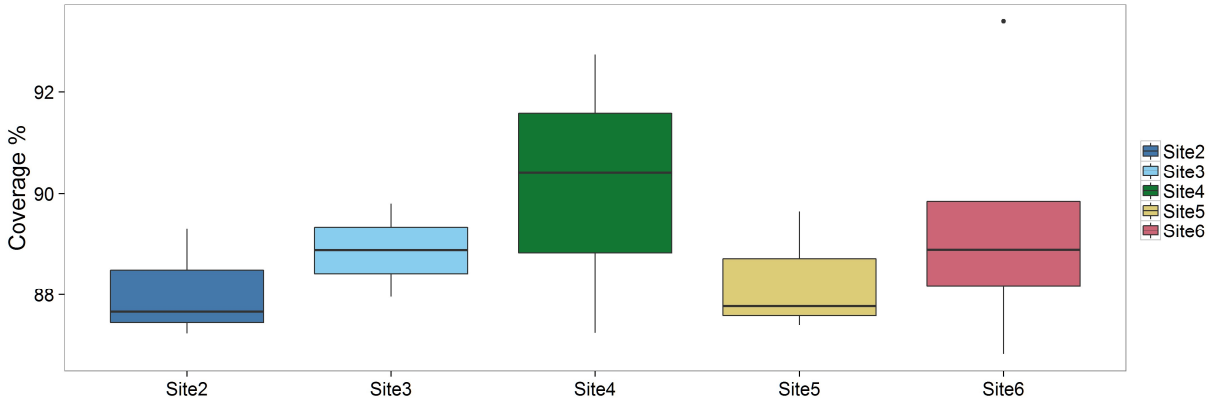


Figure 137. Boxplot Between Field Site 10-cm Depth Sediment Samples for Coverage Based on Good's Coverage

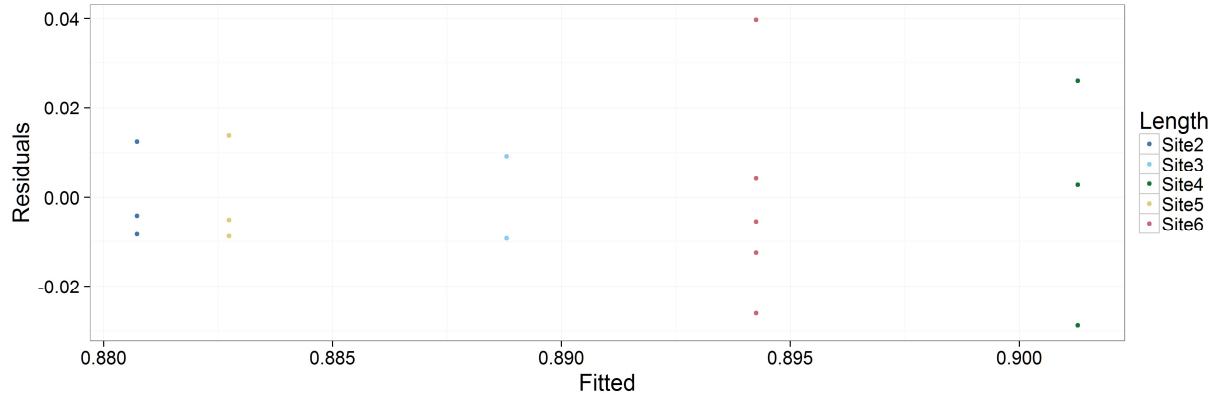


Figure 138. ANOVA Residuals Between Field Site 10-cm Depth Sediment Samples for Coverage Based on Good's Coverage

Table 82. One Way ANOVA Test Result for Diversity Comparison of 10-cm Depth Sediment Samples by Field Site Based on Good's Coverage

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Length	4	0.001	0.000	0.524	0.720
Residuals	11	0.005	0.000	NA	NA

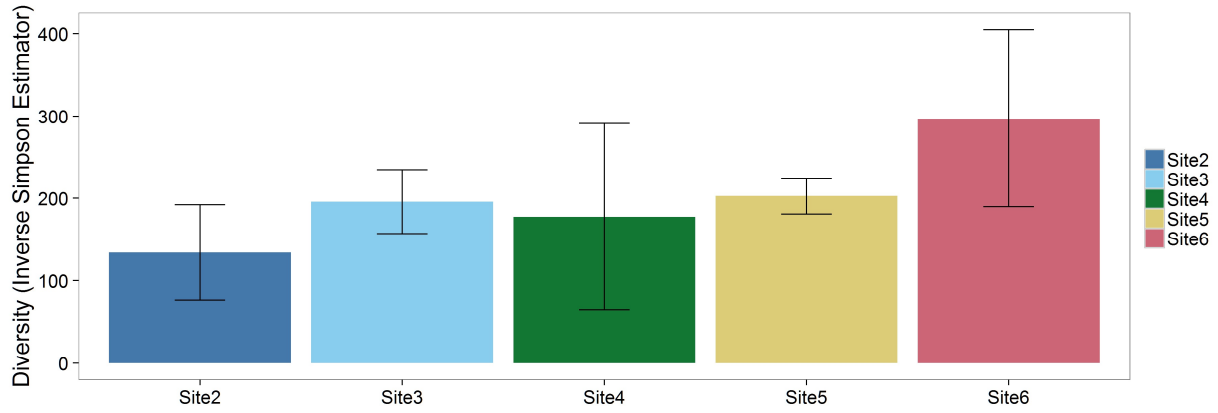


Figure 139. Barplot Between Field Site 10-cm Depth Sediment Samples for Diversity Based on the Inverse Simpson Estimator

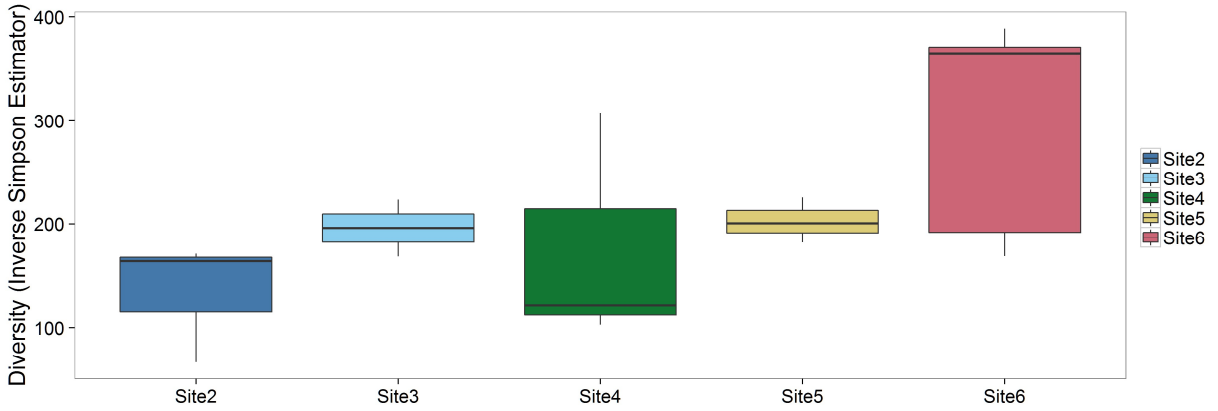


Figure 140. Boxplot Between Field Site 10-cm Depth Sediment Samples for Diversity Based on the Inverse Simpson Estimator

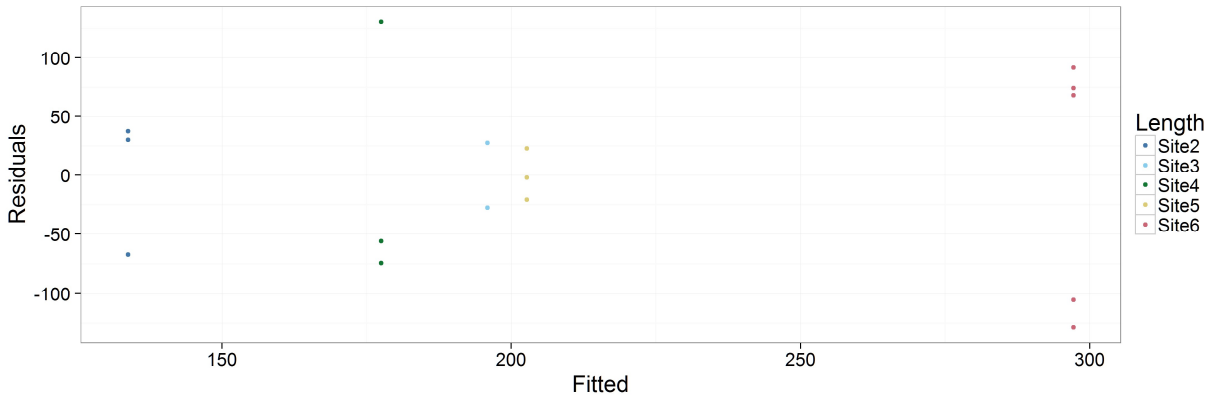


Figure 141. ANOVA Residuals Between Field Site 10-cm Depth Sediment Samples for Diversity Based on the Inverse Simpson Estimator

Table 83. One Way ANOVA Test Result for Diversity Comparison of 10-cm Depth Sediment Samples by Field Site Based on the Inverse Simpson Estimator

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Length	4	58934.719	14733.680	1.995	0.165
Residuals	11	81253.934	7386.721	NA	NA

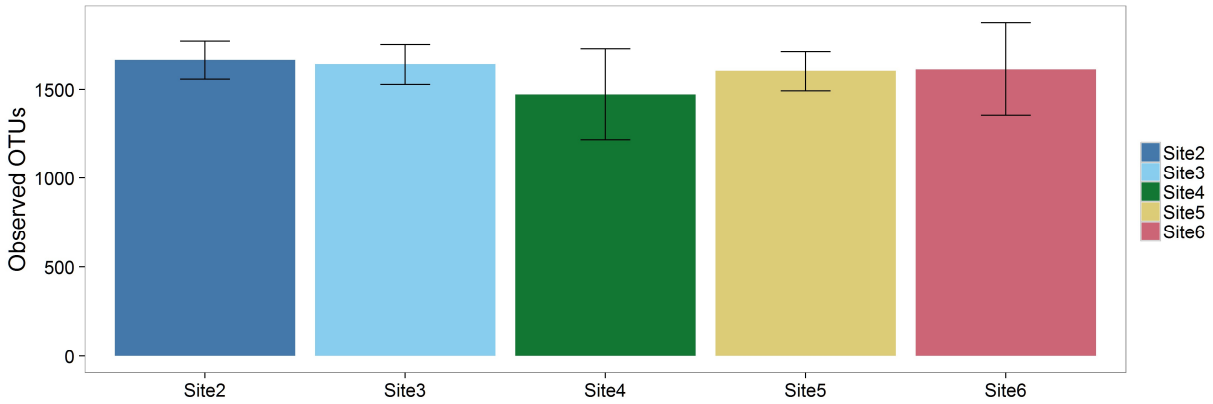


Figure 142. Barplot Between Field Site 10-cm Depth Sediment Samples for Observed OTUs Based on the SOBS Calculation

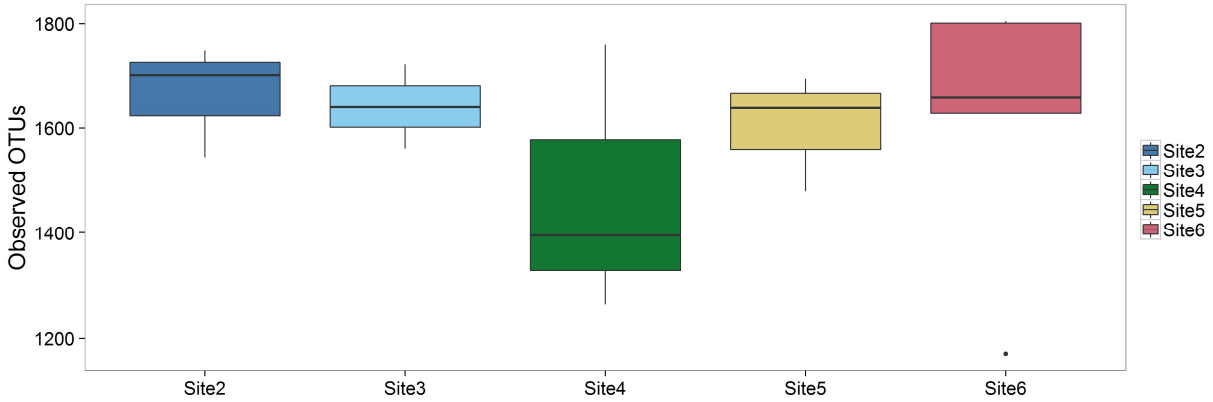


Figure 143. Boxplot Between Field Site 10-cm Depth Sediment Samples for Observed OTUs Based on the SOBS Calculation

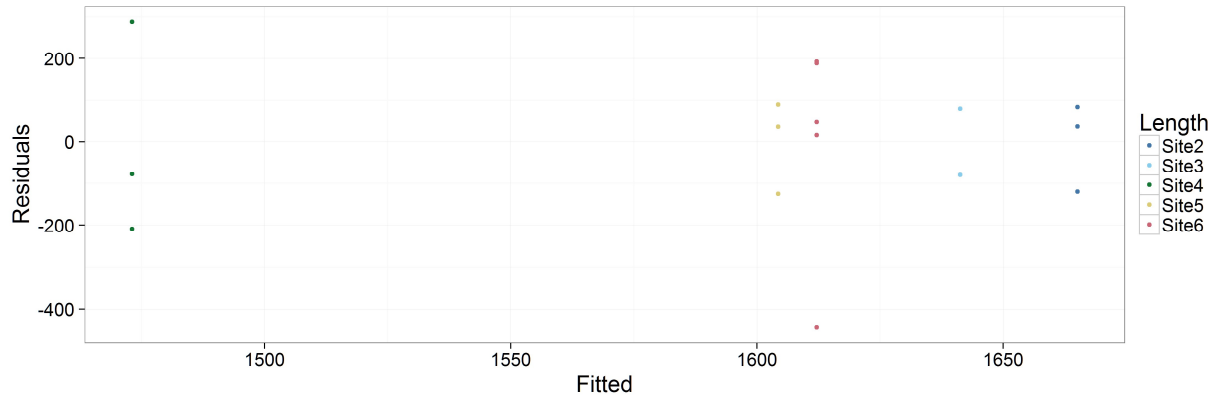


Figure 144. ANOVA Residuals Between Field Site 10-cm Depth Sediment Samples for Observed OTUs Based on the SOBS Calculation

Table 84. One Way ANOVA Test Result for Diversity Comparison of 10-cm Depth Sediment Samples by Field Site Based on SOBS Calculation

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Length	4	65168.126	16292.032	0.385	0.815
Residuals	11	464907.031	42264.276	NA	NA

Laboratory Study

Water Samples

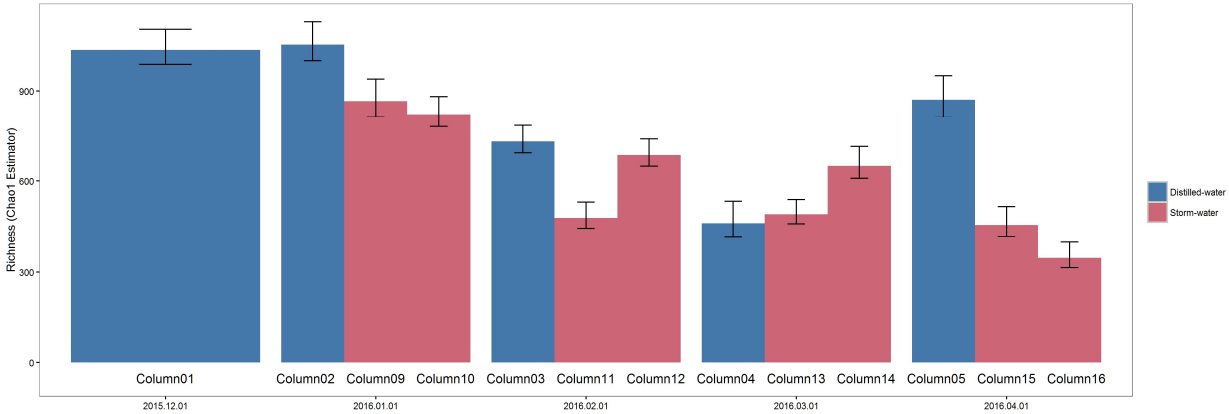


Figure 145. Barplot Between Column Samples for Richness Based on the Chao1 Estimator

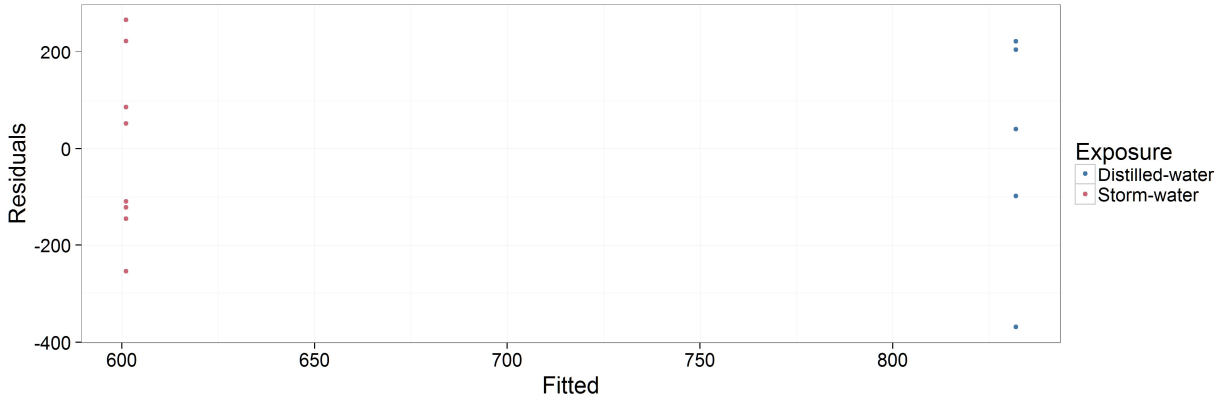


Figure 146. ANOVA Residuals Between Column Water Samples for Richness Based on the Chao1 Estimator

Table 85. One Way ANOVA Test Result for Richness Comparison of Water Samples by Column Based on the Chao1 Estimator

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Exposure	1	164052.902	164052.902	3.743	0.079
Residuals	11	482161.543	43832.868	NA	NA

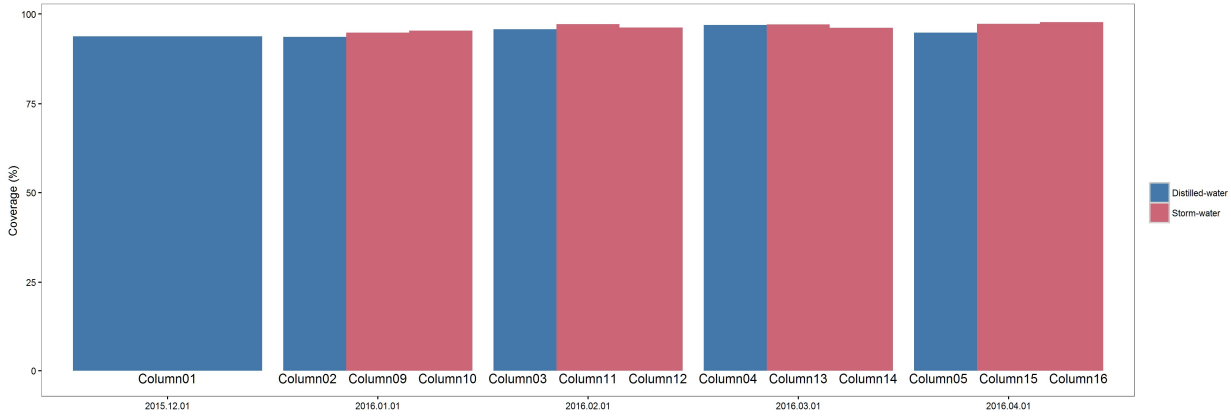


Figure 147. Barplot Between Column Water Samples for Coverage Based on the Good's Coverage

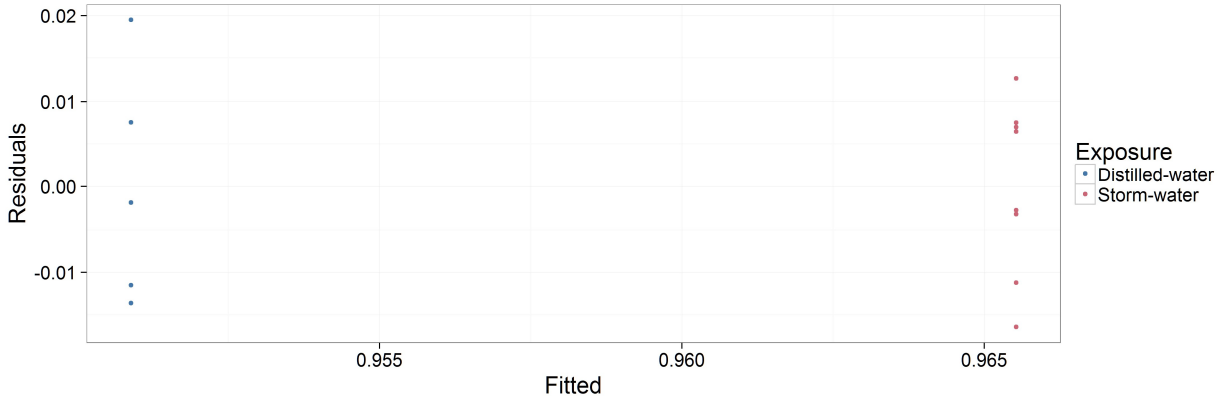


Figure 148. ANOVA Residuals Between Column Water Samples for Observed OTUs Based on the Good's Coverage

Table 86. One Way ANOVA Test Result for Coverage Comparison of Water Samples by Column Based on the Good's Coverage

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Exposure	1	0.001	0.001	4.912	0.0486
Residuals	11	0.001	0.000	NA	NA

Table 87. Tukey HSD Test Result for Coverage Comparison of Water Samples by Column Based on the Good's Coverage

Comparison	p-value
Distilled-Storm	0.0486

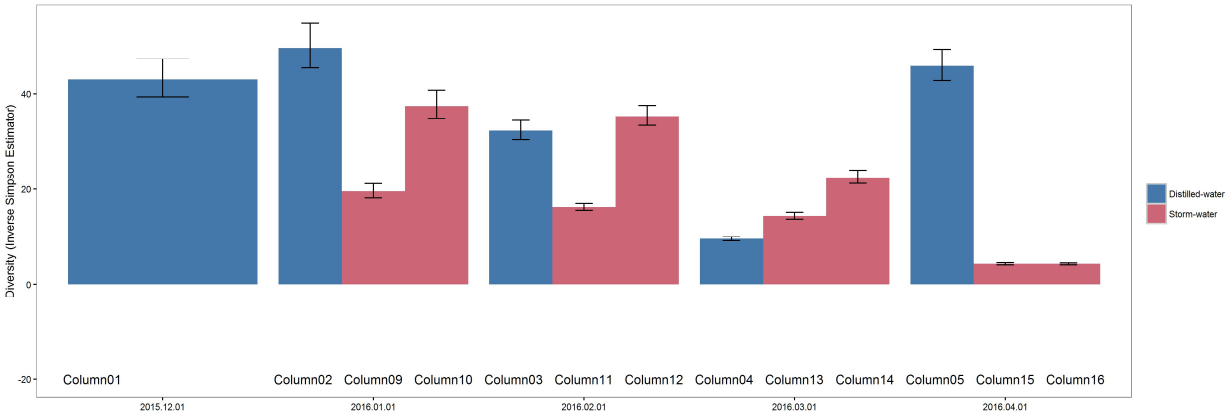


Figure 149. Barplot Between Column Water Samples for Diversity Based on the Inverse Simpson Estimator

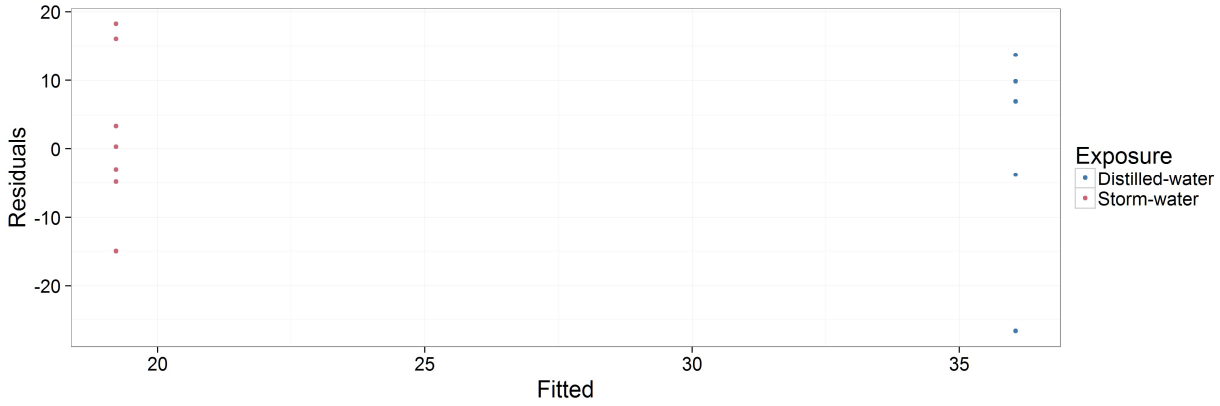


Figure 150. ANOVA Residuals Between Column Water Samples for Observed OTUs Based on the Inverse Simpson Estimator

Table 88. One Way ANOVA Test Result for Diversity Comparison of Water Samples by Column Based on the Inverse Simpson Estimator

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Exposure	1	871.026	871.027	4.478	0.0580
Residuals	11	2139.510	194.501	NA	NA

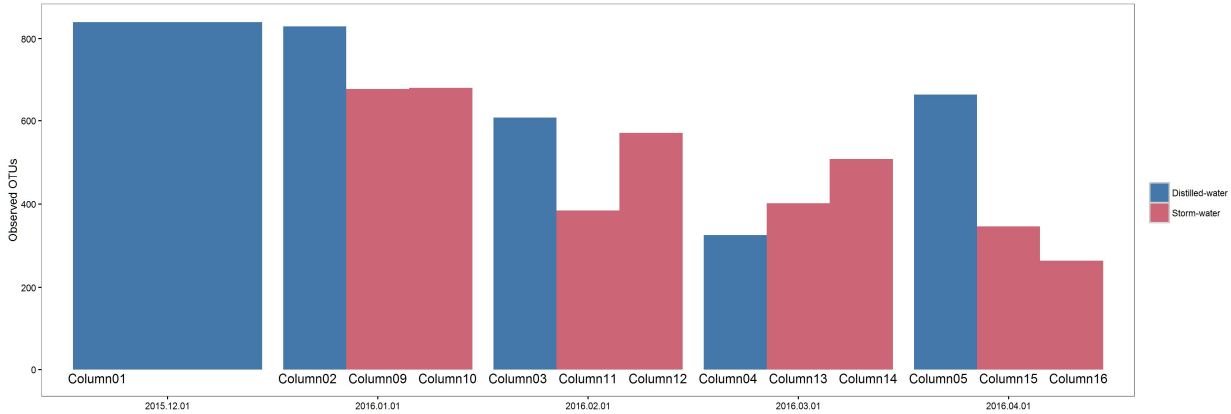


Figure 151. Barplot Between Column Water Samples for Observed OTUs Based on the SOBS Calculation

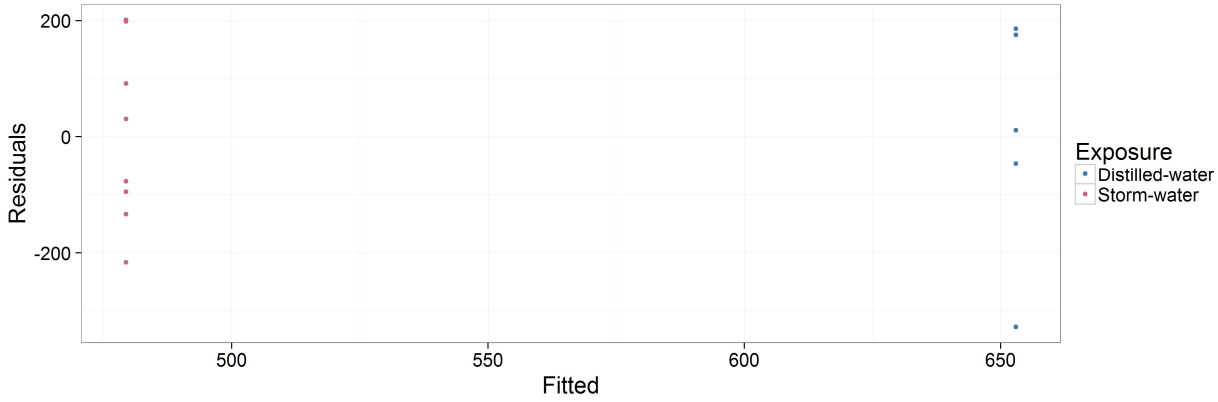


Figure 152. ANOVA Residuals Between Column Water Samples for Observed OTUs Based on the SOBS Calculation

Table 89. One Way ANOVA Test Result for Diversity Comparison of Water Samples by Column Based on SOBS Calculation

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Exposure	1	92725.930	92725.930	2.972	0.113
Residuals	11	343157.497	31196.136	NA	NA

Surface Sediment Samples

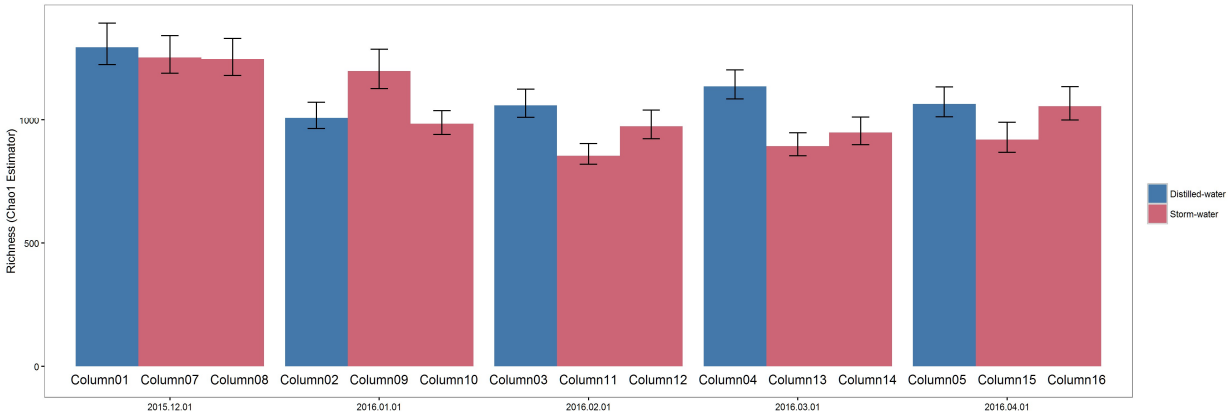


Figure 153. Barplot Between Column Surface Sediment Samples for Richness Based on the Chao1 Estimator

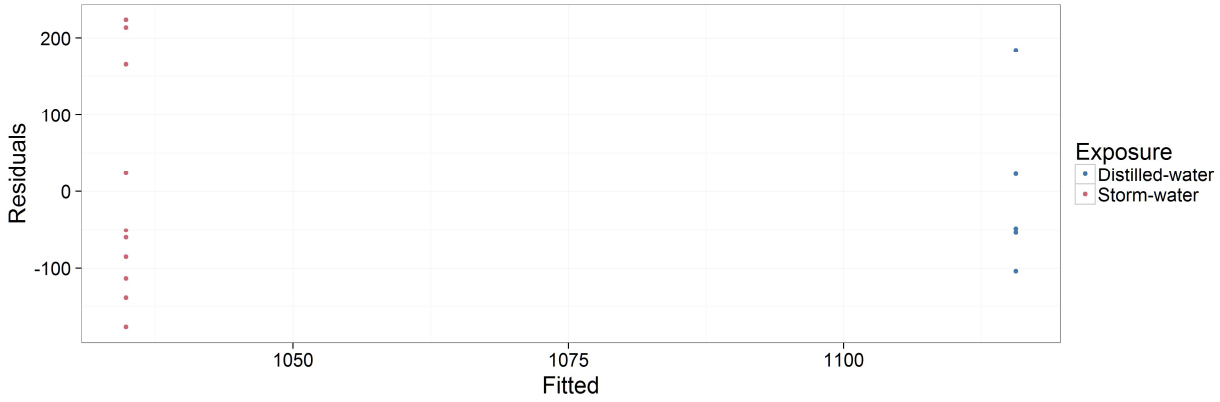


Figure 154. ANOVA Residuals Between Column Surface Sediment Samples for Observed OTUs Based on the Chao1 Estimator

Table 90. One Way ANOVA Test Result for Diversity Comparison of Surface Sediment Samples by Column Based on the Chao1 Estimator

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Exposure	1	21786.842	21786.842	1.130	0.307
Residuals	13	250705.703	19285.054	NA	NA

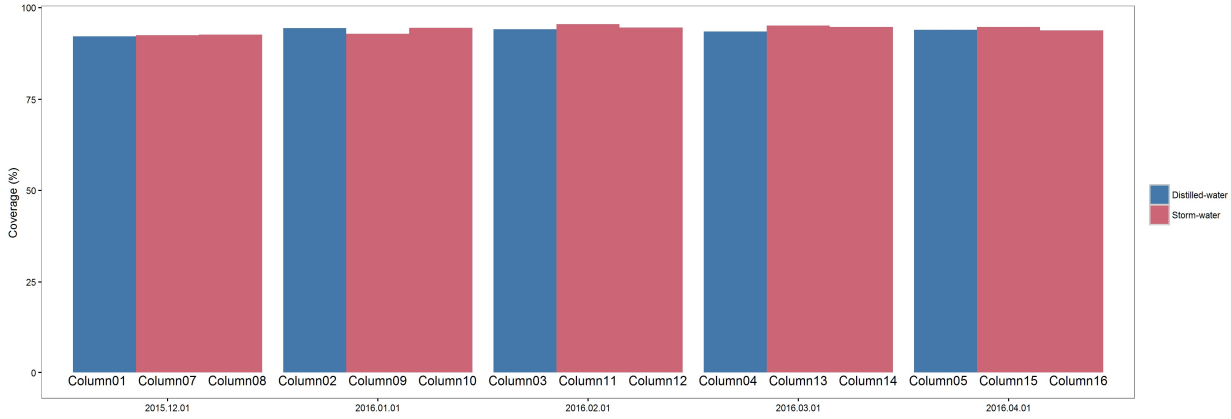


Figure 155. Barplot Between Column Surface Sediment Samples for Coverage Based on the Good's Coverage

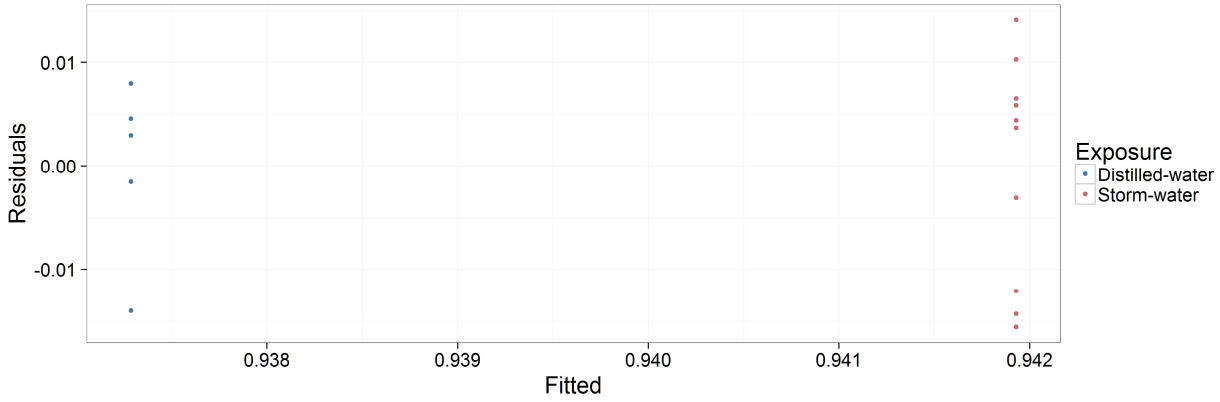


Figure 156. ANOVA Residuals Between Column Surface Sediment Samples for Observed OTUs Based on the Good's Coverage

Table 91. One Way ANOVA Test Result for Diversity Comparison of Surface Sediment Samples by Column Based on the Good's Coverage

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Exposure	1	7.19E-05	7.19E-05	0.713	0.414
Residuals	13	0.001	0.000	NA	NA

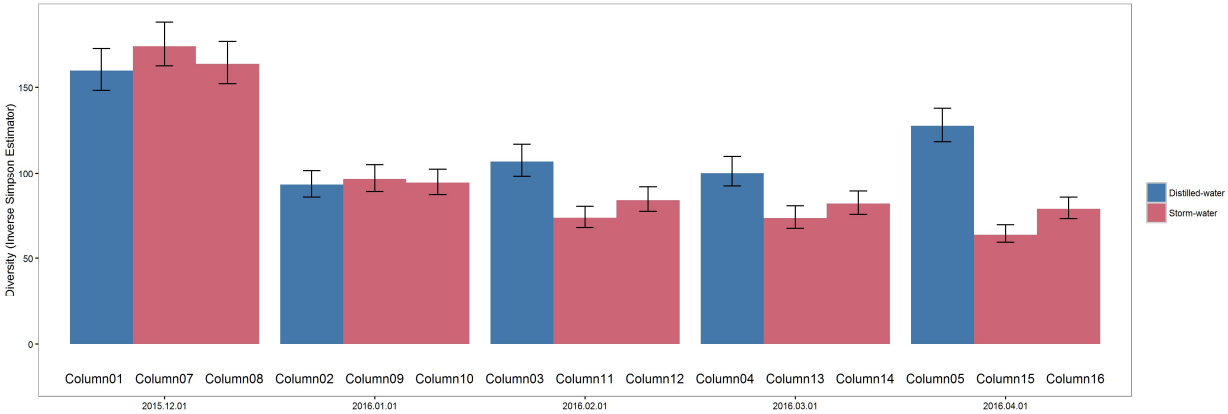


Figure 157. Barplot Between Column Surface Sediment Samples for Diversity Based on the Inverse Simpson Estimator

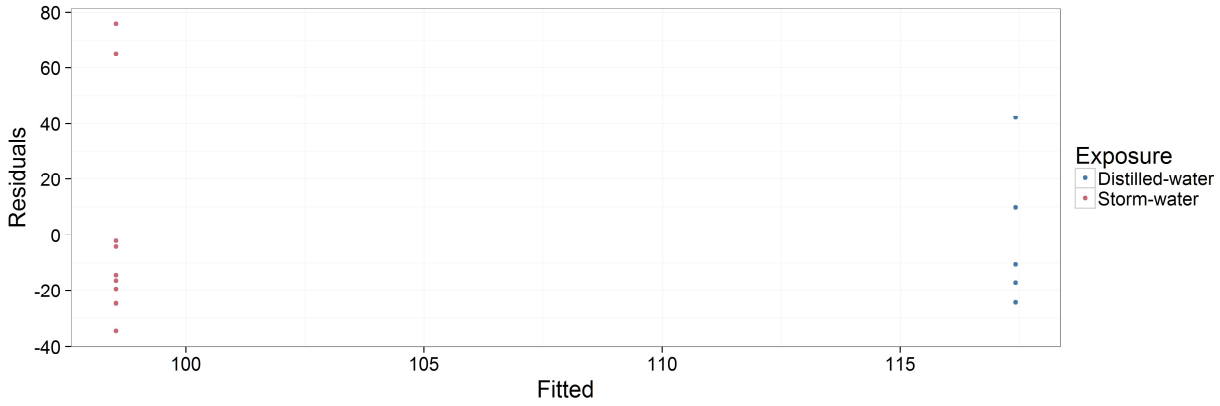


Figure 158. ANOVA Residuals Between Column Surface Sediment Samples for Observed OTUs Based on the Inverse Simpson Estimator

Table 92. One Way ANOVA Test Result for Diversity Comparison of Surface Sediment Samples by Column Based on the Inverse Simpson Estimator

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Exposure	1	1189.593	1189.593	0.955	0.346
Residuals	13	16194.774	1245.752	NA	NA

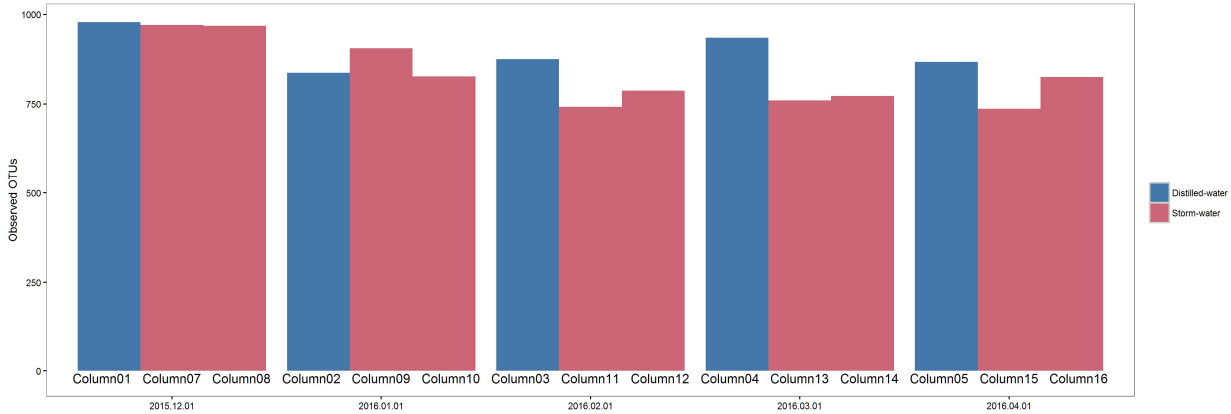


Figure 159. Barplot Between Column Surface Sediment Samples for Observed OTUs Based on the SOBS Calculation

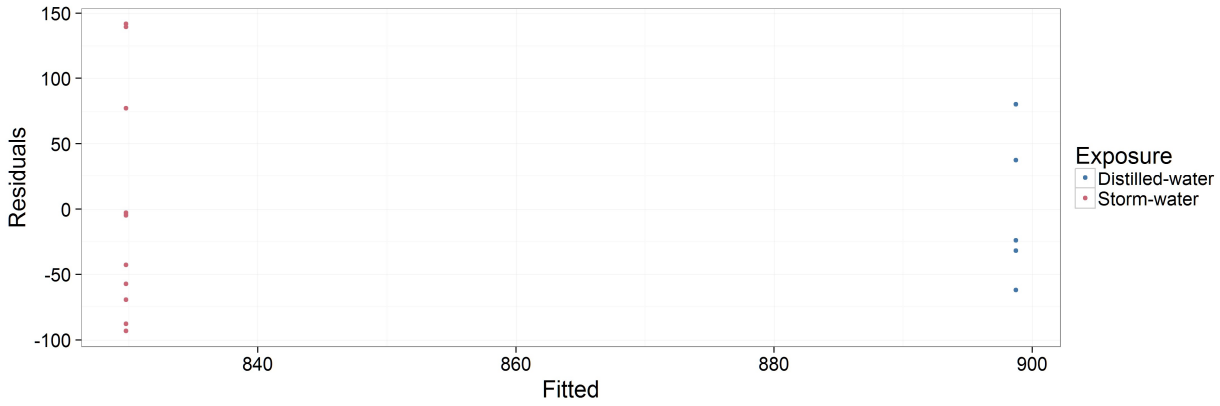


Figure 160. ANOVA Residuals Between Column Surface Sediment Samples for Observed OTUs Based on the SOBS Calculation

Table 93. One Way ANOVA Test Result for Diversity Comparison of Surface Sediment Samples by Column Based on SOBS Calculation

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Exposure	1	15856.479	15856.479	2.414	0.144
Residuals	13	85388.725	6568.363	NA	NA

10-cm Depth Samples

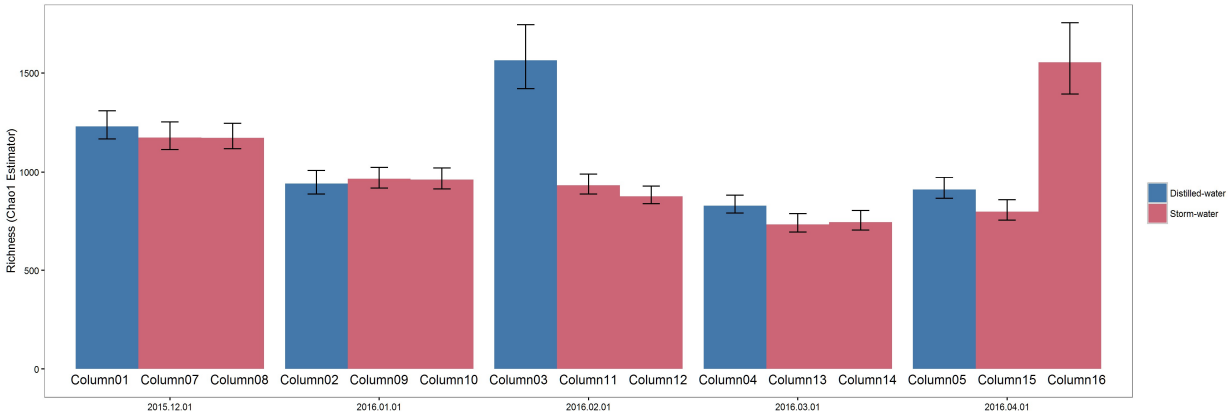


Figure 161. Barplot Between Column 10-cm Depth Sediment Samples for Richness Based on the Chao1 Estimator

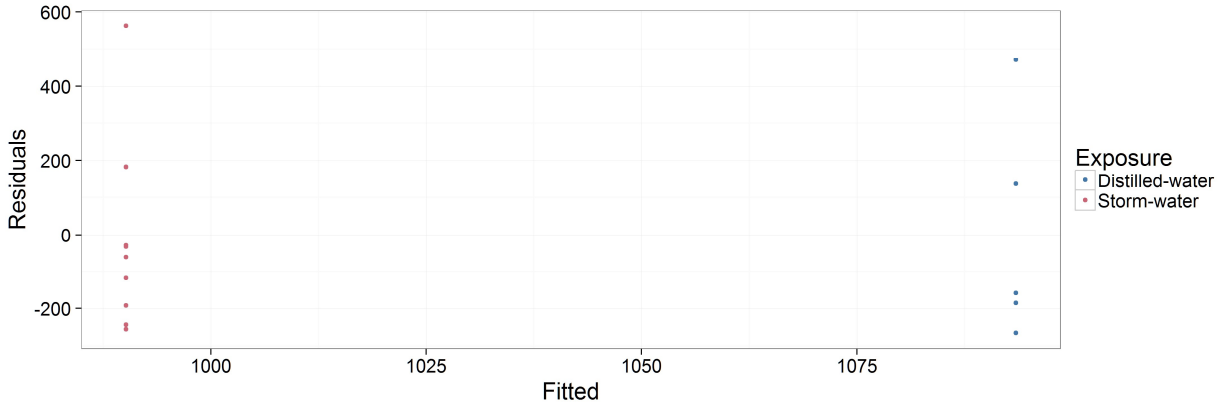


Figure 162. ANOVA Residuals Between Column 10-cm Depth Sediment Samples for Observed OTUs Based on the Chao1 Estimator

Table 94. One Way ANOVA Test Result for Diversity Comparison of 10-cm Depth Sediment Samples by Column Based on Chao1 Estimator

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Exposure	1	35596.312	35596.312	0.496	0.494
Residuals	13	932234.181	71710.322	NA	NA

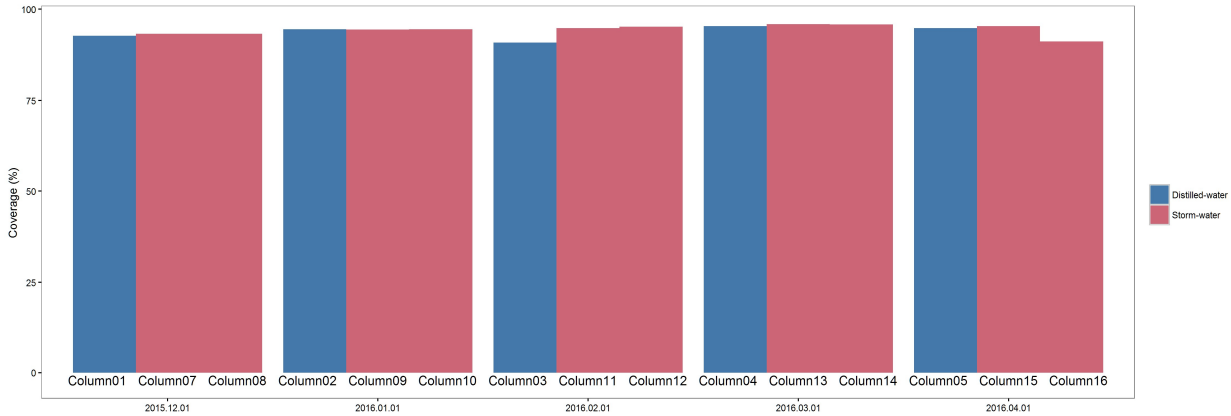


Figure 163. Barplot Between Column 10-cm Depth Sediment Samples for Coverage Based on the Good's Coverage

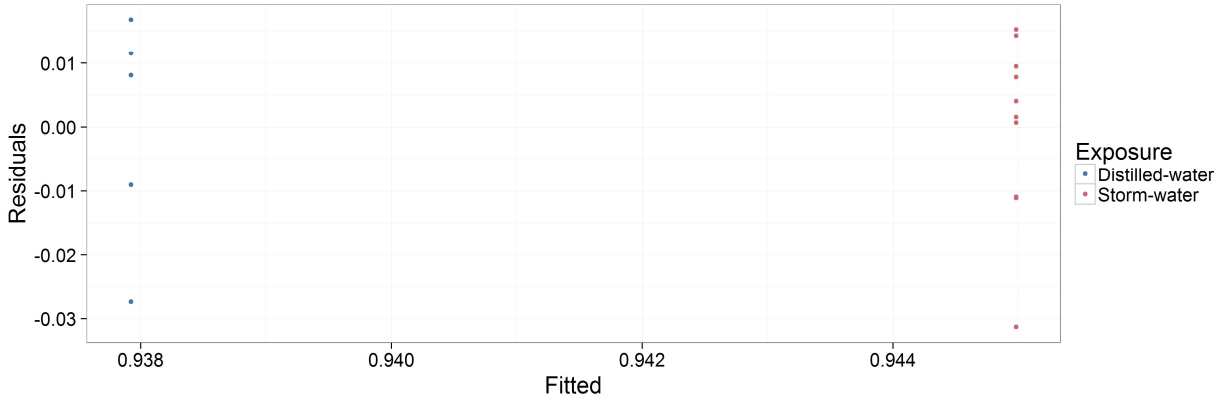


Figure 164. ANOVA Residuals Between Column 10-cm Depth Sediment Samples for Observed OTUs Based on the Good's Coverage

Table 95. One Way ANOVA Test Result for Diversity Comparison of 10-cm Depth Sediment Samples by Column Based on Good's Coverage

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Exposure	1	0.000	0.000	0.687	0.422
Residuals	13	0.003	0.000	NA	NA

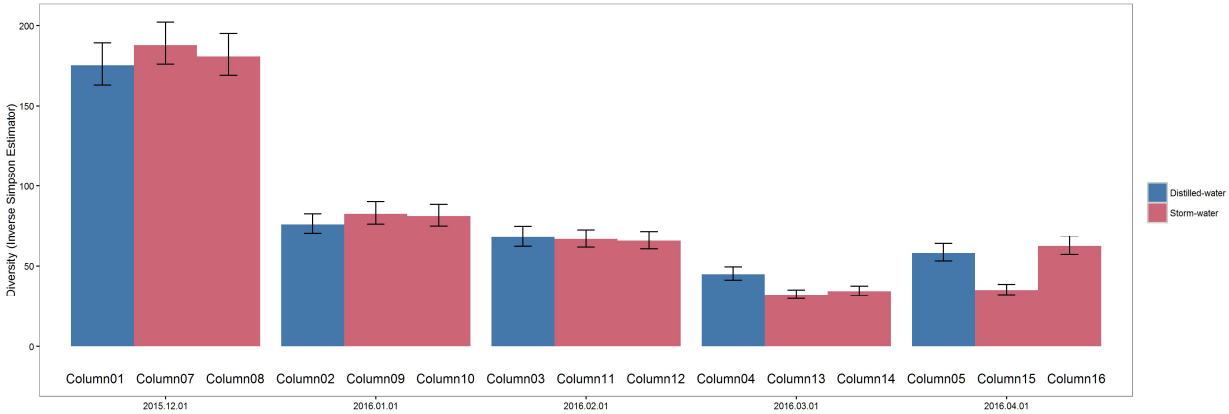


Figure 165. Barplot Between Column 10-cm Depth Sediment Samples for Diversity Based on the Inverse Simpson Estimator

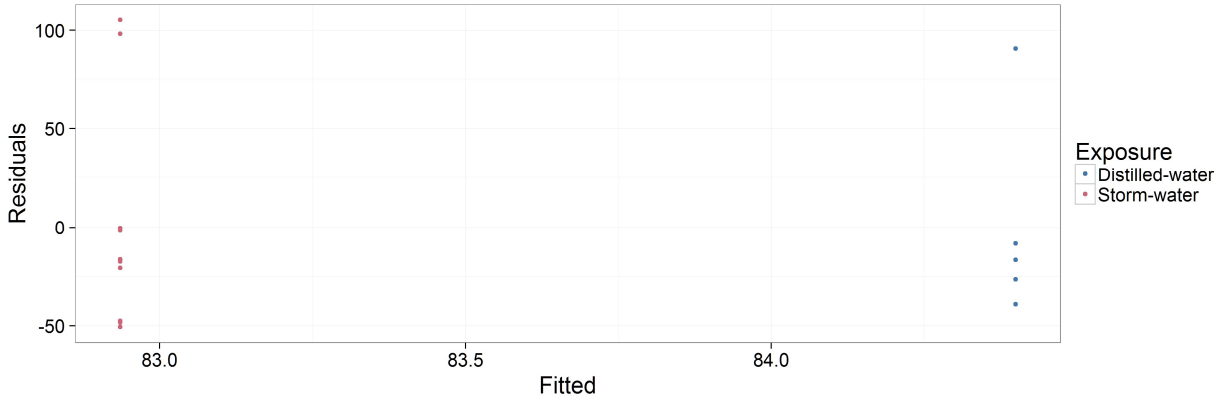


Figure 166. ANOVA Residuals Between Column 10-cm Depth Sediment Samples for Observed OTUs Based on the Inverse Simpson Estimator

Table 96. One Way ANOVA Test Result for Diversity Comparison of 10-cm Depth Sediment Samples by Column Based on the Inverse Simpson Estimator

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Exposure	1	7.152	7.152	0.002	0.962
Residuals	13	39758.351	3058.335	NA	NA

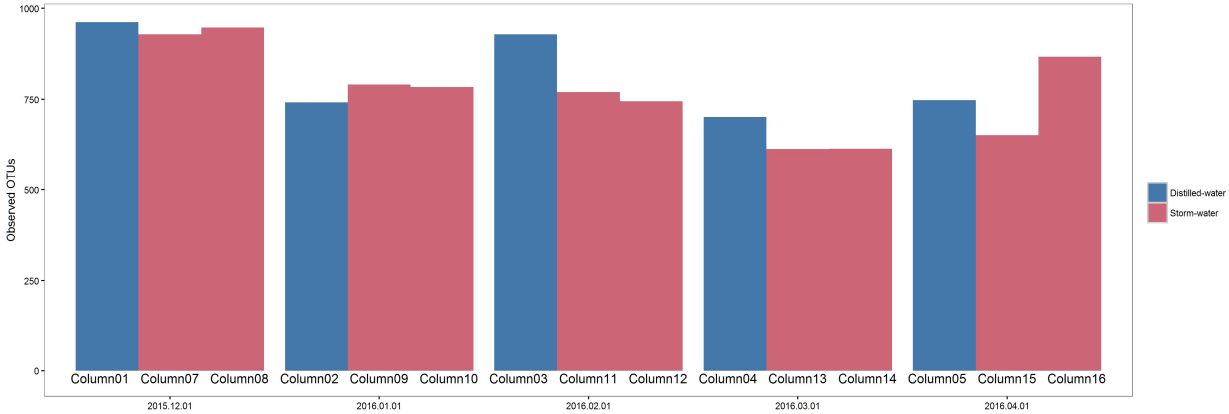


Figure 167. Barplot Between Column 10-cm Depth Sediment Samples for Observed OTUs Based on the SOBS Calculation

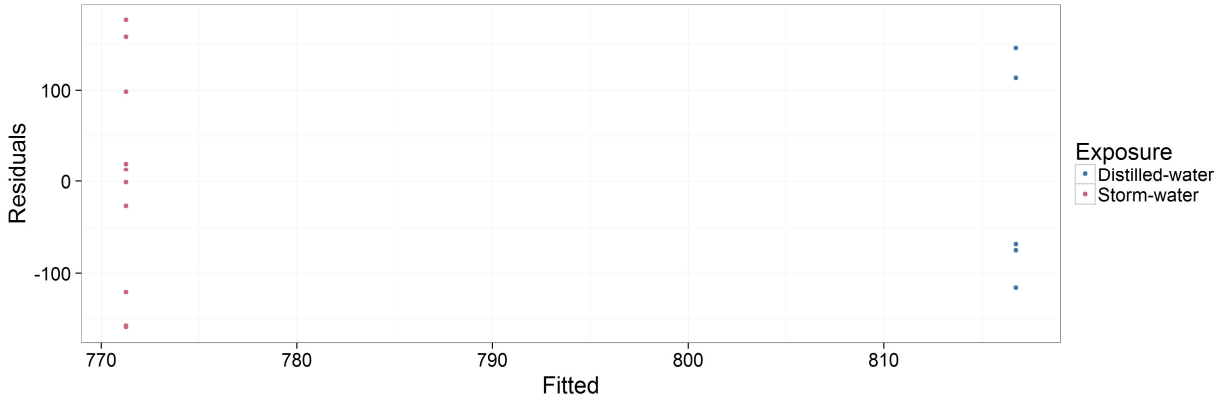


Figure 168. ANOVA Residuals Between Column 10-cm Depth Sediment Samples for Observed OTUs Based on the SOBS Calculation

Table 97. One Way ANOVA Test Result for Diversity Comparison of 10-cm Depth Sediment Samples by Column Based on SOBS Calculation

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Exposure	1	6898.043	6898.0429	0.473	0.504
Residuals	13	189753.330	14596.410	NA	NA

Appendix I: Letters of Permission and Support for the Research Project

a place of mind
THE UNIVERSITY OF BRITISH COLUMBIA

Department of Civil Engineering
2002 - 6250 Applied Science Lane
Vancouver, BC Canada V6T 1Z4

Phone 604 822 2637
Fax 604 822 6901
info@civil.ubc.ca

Dear Genome BC

As the academic project leader for this project, I fully support the proposed research, to develop a biomarker tool for monitoring and validating stormwater treatment wetlands. I also support the proposed collaboration between UBC and Kerr Wood Leidal Consulting Engineers Ltd. The results from this collaboration will benefit UBC and will provide research opportunities for students in the Pollution Control and Waste Management group at UBC. For these reasons, I agree that UBC will provide in-kind funding to support the analysis of environmental samples for metals. Based on estimates provided by Paula Parkinson, the manager of the UBC Civil-Environmental lab, where the lab supplies cost is $20/sample (without overhead), UBC will provide in-kind support at a value of $4000, for the analysis of 200 samples for metals.

Sincerely,

Prof. James Atwater

Associate Professor
jatwater@civil.ubc.ca
T: 604.822.4694
F: 604.822.6901
CEME - Room 2004C
Civil and Mechanical Engineering Building
University of British Columbia
6250 Applied Science Lane
Vancouver BC V6T 1Z4
Canada

a place of mind
THE UNIVERSITY OF BRITISH COLUMBIA

Department of Civil Engineering
2002 – 6250 Applied Science Lane
Vancouver, BC Canada V6T 1Z4

Phone 604 822 2637
Fax 604 822 6901
info@civil.ubc.ca

Dear Genome BC,

The UBC Department of Civil Engineering is dedicated to world-class research and supports partnerships with industry which aim to innovate new technologies. For these reasons, on Prof. James Atwater's advice, the UBC Department of Civil Engineering supports the collaboration between UBC and Kerr Wood Leidal Consulting Engineers Ltd. for the development of a biomarker tool to monitor and validate stormwater treatment wetlands. This collaboration is further supported by our department through an in-kind contribution valued at $4,000, for analysis of metals in soil and water samples using our facilities. In return for the in-kind contribution, UBC expects to grow its reputation in academia through the opportunity to publish research results and to support current graduate students within our department.

Sincerely,

Dr. Perry Adebar

adebar@civil.ubc.ca
T: 604.822.6820
F: 604.822.7006
CEME – Room 2002E
Civil and Mechanical Engineering Building
University of British Columbia
6250 Applied Science Lane
Vancouver BC V6T 1Z4
Canada

STANLEY PARK
ECOLOGY SOCIETY

Connecting People with Nature

Stanley Park Ecology Society
PO Box 5167
Vancouver BC V6B 4B2

Telephone 604 257 6908
Facsimile 604 257 8378
www.stanleyparkecology.ca

Dear Genome BC,

Stanley Park Ecology Society (SPES) is a non-profit organization founded in 1988 that works alongside the Vancouver Board of Parks and Recreation to promote stewardship and conservation in Stanley Park. Our mission is to promote awareness of and respect for the natural world and to play a leadership role in the stewardship of Stanley Park through collaborative initiatives in education, research and conservation.

SPES is supporting collaboration with Kerr Wood Leidal (KWL) Consulting Engineers Ltd. and the University of British Columbia (UBC) for the development of a biomarker tool to monitor and validate the microbial uptake of metals at storm water treatment sites. The proposed project is beneficial for our goals and mission as it will provide greater insight into the key microbiological activities within the Lost Lagoon wetland and may highlight potential concerns for the establishment of an additional wetland inside Stanley Park. SPES has an interest in this project's outcomes because the location for analysis is the Lost Lagoon storm water treatment wetland in Stanley Park. Because Stanley Park is a Canadian National Historic Site, Lost Lagoon has significant historical importance and environmental benefits for British Columbia and Canada.

In exchange for the ability to influence this project's outcomes and to publish the results of this project in our annual publications, SPES has agreed to be a co-applicant for this Genome BC LIPP application and to support this project, as a user-partner, through an in-kind contribution valued at $2,000. This in-kind contribution includes performing site maintenance at the Lost Lagoon wetland and assisting with site sampling and public relations and outreach for the project, particularly due to the fact that the sampling site is in an area near high pedestrian traffic.

SPES looks forward to the results of this project and is interested in the development of a biomarker tool for validating and monitoring wetlands. For this reason, we hope that Genome BC will accept and fund this proposed project.

Sincerely,

June Pretzer
Conservation Project Manager

KERR WOOD LEIDAL
consulting engineers

Greater Vancouver
200 - 4185A Still Creek Drive
Burnaby, BC V5C 6G9
T 604 294 2088
F 604 294 2090

Aug 27, 2015,

Genome British Columbia
400 – 575 West 8th Ave.
Vancouver, BC V5Z 0C4

Re: Support of Genome BC User Partnership Program

Dear Genome BC,

KWL is committed to developing a genomics tool that can be used to monitor and validate stormwater treatment systems. KWL is playing a significant role in the development of this tool through cash and in-kind support as well as through consulting and leadership support throughout the course of the project. KWL has committed $12,000 in cash to be paid towards a stipend for graduate student, Jessica LeNoble, who will act as the project manager for the sampling and analysis plan. In addition, KWL will supply incremental in-kind support for this project at a value of $2,000, which will cover printing and office supplies, software licenses, and resources for information sharing.

KWL has been active in the development of this tool from the beginning of the brainstorming phase to the refinement of a practical genomics application. To ensure useful results are obtained KWL will remain engaged throughout the research and development activities. If results suggest follow on research and development is required, KWL is committed to exploring options for the further development of this biomarker tool.

KWL will benefit from this project because a greater understanding of the biological activity taking place in treatment wetlands will allow us to modify our best practices in order to produce even more effective treatment systems in the future. The development of a database with biomarkers for bacteria that contribute to the successful uptake of metals from wetland treatment systems will provide a monitoring and validation tool that will have great benefits for engineered wetlands operating in British Columbia.

KWL is in full support of this funding application for the Genome BC User Partnership Program.

Sincerely,
KERR WOOD LEIDAL ASSOCIATES LTD.

Chris Johnston, P.Eng.
Vice President, Stormwater
Kerr Wood Leidal

September 14, 2015

Dear Genome BC,

The Vancouver Board of Parks and Recreation (VBPR), Stanley Park Department, supports this application to the Genome BC User Partnership Program for funding to research and develop a biomarker tool with the aim to monitor and validate stormwater treatment wetlands.

The mission of the VBPR is to provide, preserve, and advocate for parks and recreation to benefit all people, communities, and the environment. This mission is in line with using best practices to manage water treatment. As such, the VBPR contracted Kerr Wood Leidal Consulting Engineers Ltd. in 1999 to design the Lost Lagoon stormwater treatment, which treats stormwater exiting the Stanley Park Causeway. The VBPR also manages the upkeep and maintenance of the Lost Lagoon wetland. This includes having contracted and overseen the dredging of the sediment basin in the wetland in 2013.

Because the site of interest for the proposed project is on property managed by the VBPR, the VBPR has an interest in the data which will result from this analysis. This project is also in line with our Strategic Plan's mandate to be a "leader in greening" or "to demonstrate leading green and horticultural practices and [to] preserve, protect and create green space."

Wetlands in our parks provide habitat for plants and animals and give citizens and tourists the opportunity to connect with nature. We believe this project may have benefits to the City of Vancouver as it may provide a better understanding of treatment wetlands and allow for an expansion of these types of green practices in the future.

Sincerely,

Brian Quinn

Manager of Park Operations
Vancouver Board of Parks and Recreation
Office: 604-257-8521
Email: brian.quinn@vancouver.ca

CITY OF VANCOUVER

mailing address
Vancouver Board of Parks and Recreation
2099 Beach Avenue
Vancouver, British Columbia
Canada V6G 1Z4

tel 311 (within Vancouver)
tel 604.873.7000 (outside Vancouver)
fax 604.257.8427
website vancouverparks.ca

The University of British Columbia

Biohazard Approval Certificate

PROTOCOL NUMBER: **B16-0012**

INVESTIGATOR OR COURSE DIRECTOR: **James W. Atwater**

DEPARTMENT: **Civil Engineering**

Type of Biological Material	Host Range	Biological Material	Species/Source/Common Name	Other Information	Building	Room Stored	Room Used	Containment Level
Environmental Samples	Not Applicable	Soil	Indigenous	Lost Lagoon Wetland Stanley Park Vancouver, BC Canada	Chemical and Biological Engineering Building	522	522	1

PROJECT OR COURSE TITLE: **Wetland Monitoring Tool**

APPROVAL DATE: **February 12, 2016** START DATE: **January 1, 2015**

APPROVED CONTAINMENT LEVEL: **CL1**

FUNDING TITLE: **DNA-Based Tool for Monitoring and Validating Stormwater Treatment Wetlands**
FUNDING AGENCY: **Genome British Columbia**

UNFUNDED TITLE: **N/A**

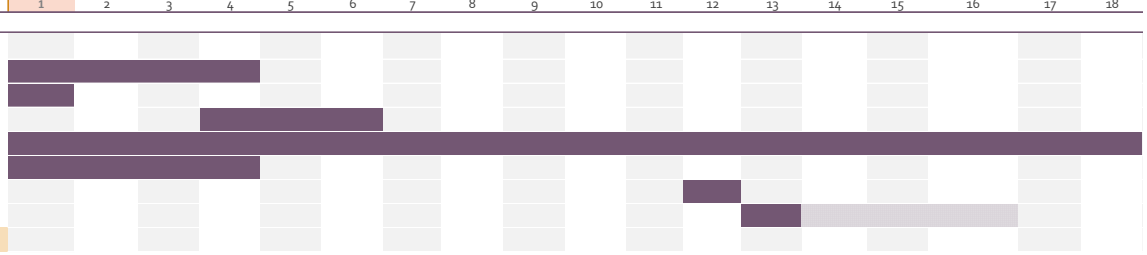
The Principal Investigator/Course Director is responsible for ensuring that all research or course work involving biological hazards is conducted in accordance with the University of British Columbia Policies and Procedures, Biosafety Practices and Public Health Agency of Canada guidelines.

This certificate is valid for one year from the above start or approval date (whichever is later) provided there are no changes. Annual review is required.

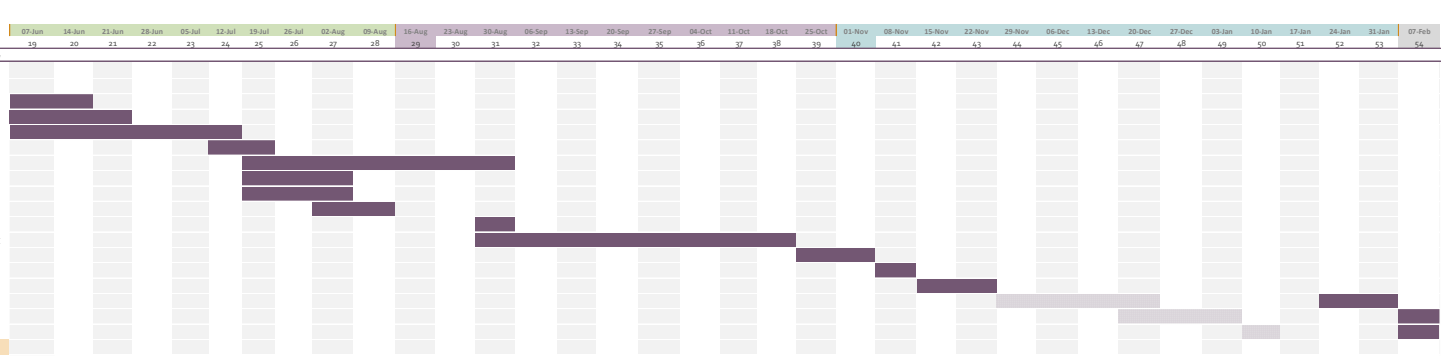
Appendix J: Project Management – Timeline and Budget

Plan Actual % Complete Actual (postponed)

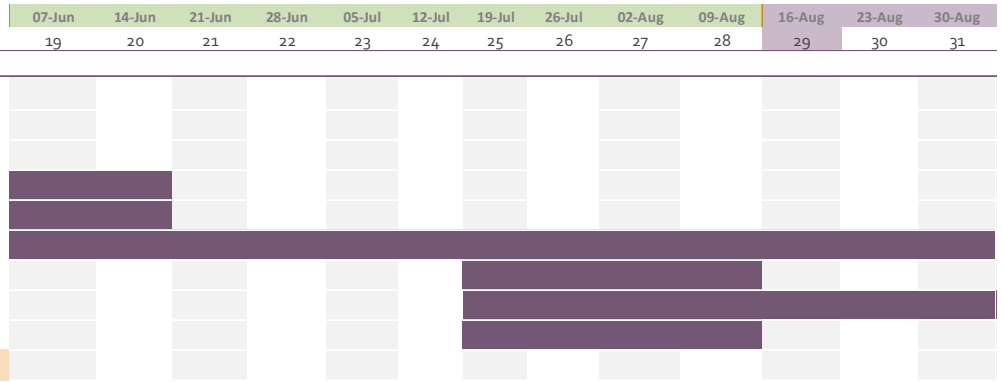
ACTIVITY	PLAN START WEEK	PLAN DURATION WEEK(S)	ACTUAL START WEEK	ACTUAL DURATION WEEK(S)	PERCENT COMPLETE	Responsibility
NSERC IPS Proposal						
Establish KWL's user need	1	4	1	4	100%	CJ/PL
Determine project timeline	1	1	1	1	100%	JA/JL
Assemble project team	4	3	4	3	100%	JA/JL
Train graduate student on lab methods	1	18	1	18	100%	JA/JL
Establish funding/partner relationship	1	4	1	4	100%	JA/CJ
Submit proposal to NSERC	12	1	12	1	100%	JA
NSERC review	13	4	13	1	100%	
Milestone: Project Accepted by NSERC						



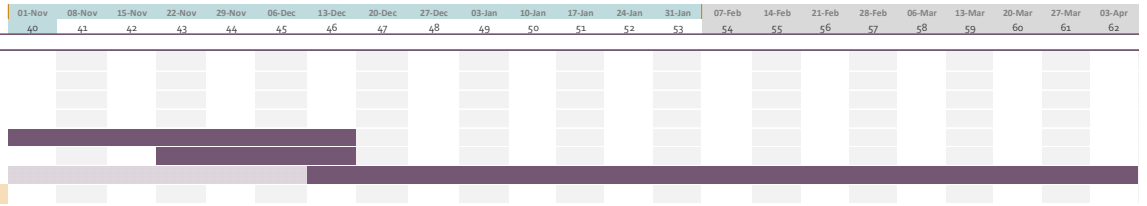
ACTIVITY	PLAN START WEEK	PLAN DURATION WEEK(S)	ACTUAL START WEEK	ACTUAL DURATION WEEK(S)	PERCENT COMPLETE	Responsibility
NSERC IPS Proposal						
Genome BC Proposal						
Confirm KWL's user need	19	2	19	2	100%	CJ/PL/JL
Finalize goals, objectives, milestones	19	3	19	3	100%	CJ/JA
Gather project collaborators and support	19	6	19	6	100%	JL
Establish SPES' user need	24	2	24	2	100%	JP/JL
Calculate detailed budget	25	7	25	7	100%	JL
Manage risks	25	3	25	3	100%	JL
Establish project management structure	25	3	25	3	100%	JL
Review proposal	27	2	27	2	100%	JA/SB/CJ
Submit proposal to Genome BC	31	1	31	1	100%	JA/SB/CJ
Genome BC review	31	8	31	8	100%	Genome BC
Modify proposal based on Genome BC re	39	2	39	2	100%	JA/SB/CJ
Resubmit proposal to Genome BC	41	1	41	1	100%	JA/SB/CJ
Genome BC resubmission review	42	1	42	2	100%	Genome BC
Prepare for Genome BC project launch	44	4	52	2	100%	All
Genome BC project launch meeting	47	3	54	1	100%	JA/SB/CJ
Genome BC project launch	50	1	54	1	100%	JA/SB/CJ
Milestone: Project Accepted by Genome BC						



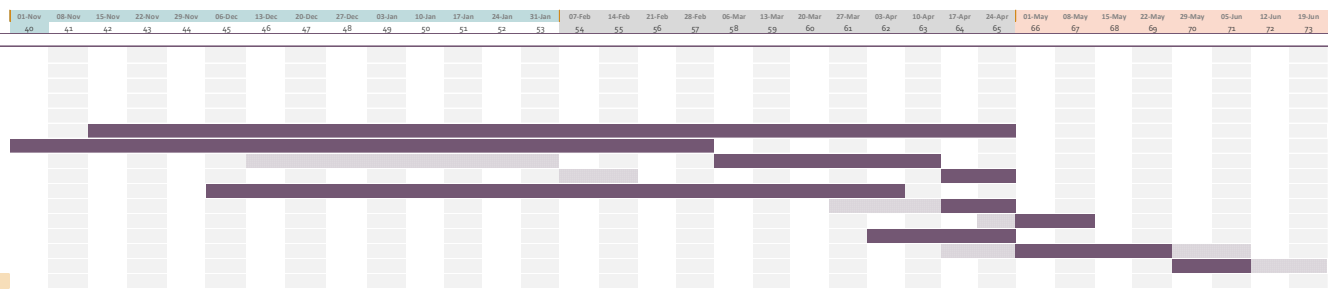
ACTIVITY	PLAN START WEEK	PLAN DURATION WEEK(S)	ACTUAL START WEEK	ACTUAL DURATION WEEK(S)	PERCENT COMPLETE	Responsibility
NSERC IPS Proposal						
Genome BC Proposal						
Method Development						
Select research site	19	2	19	2	100%	CJ
Obtain site and lab access	19	2	19	2	100%	JL
Define research plan	19	13	19	13	100%	JL
Determine site characteristics	25	4	25	4	100%	JL
Assemble lab and field equipment	25	7	25	7	100%	JL
Validate lab methods	25	4	25	4	100%	JL
Milestone: Methods and Research Plan Developed and Finalized						

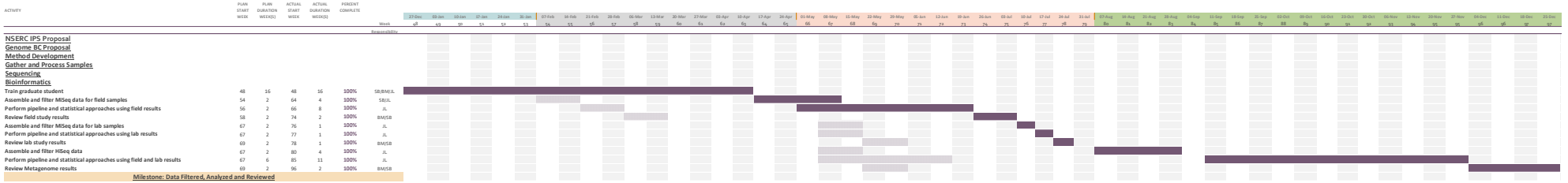


ACTIVITY	START WEEK	DURATION WEEK(S)	START WEEK	DURATION WEEK(S)	COMPLETE	Responsibility
NSERC IPS Proposal						
Genome BC Proposal						
Method Development						
Gather and Process Samples						
Extract and process field samples	25	20	25	22	100%	JL
Build lab study setup	39	1	43	4	100%	DV/JL
Extract and process lab study samples	39	16	46	17	100%	JL
Milestone: Field and Lab Samples Analyzed for Environmental Parameters and Extracted/Preserved for DNA Analysis						



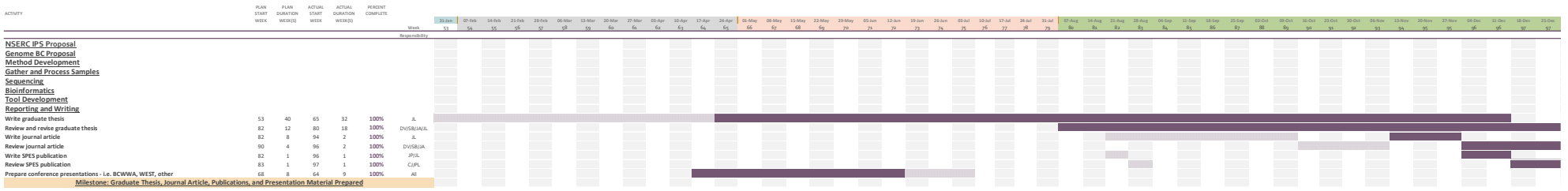
ACTIVITY	START WEEK	DURATION WEEK(S)	START WEEK	DURATION WEEK(S)	COMPLETE	Responsibility
NSERC IPS Proposal						
Genome BC Proposal						
Method Development						
Gather and Process Samples						
Sequencing						
Train graduate student	42	12	42	24	100%	SB/JL
Prepare field study samples for MiSeq	40	12	40	18	100%	JL
MiSeq field study samples	46	8	58	6	100%	JL
Review field study results	54	2	64	2	100%	BM/SB
Prepare lab study samples for MiSeq	45	16	45	18	100%	JL
MiSeq lab study samples	61	4	64	2	100%	JL
Review lab study results	65	2	66	2	100%	BM/SB
Prepare samples for HiSeq	62	2	62	4	100%	JL
HiSeq samples	64	8	66	4	100%	JL
Review results	72	2	70	2	100%	BM/SB
Milestone: MiSeq and HiSeq Data Received and Reviewed						





ACTIVITY	PLAN START WEEK	PLAN DURATION WEEK(S)	ACTUAL START WEEK	ACTUAL DURATION WEEK(S)	PERCENT COMPLETE	Responsibility
NSERC IPS Proposal						
Genome BC Proposal						
Method Development						
Gather and Process Samples						
Sequencing						
Bioinformatics						
Train graduate student	48	16	48	16	100%	SB/BM/JL
Assemble and filter MiSeq data for field samples	54	2	64	4	100%	SB/JL
Perform pipeline and statistical approaches using field results	56	2	66	8	100%	JL
Review field study results	58	2	74	2	100%	BM/SB
Assemble and filter MiSeq data for lab samples	67	2	76	1	100%	JL
Perform pipeline and statistical approaches using lab results	67	2	77	1	100%	JL
Review lab study results	69	2	78	1	100%	BM/SB
Assemble and filter HiSeq data	67	2	80	4	100%	JL
Perform pipeline and statistical approaches using field and lab results	67	6	85	11	100%	JL
Review Metagenome results	69	2	96	2	100%	BM/SB
Milestone: Data Filtered, Analyzed and Reviewed						

ACTIVITY	PLAN START WEEK	PLAN DURATION WEEK(S)	ACTUAL START WEEK	ACTUAL DURATION WEEK(S)	PERCENT COMPLETE	Responsibility
NSERC IPS Proposal						
Genome BC Proposal						
Method Development						
Gather and Process Samples						
Sequencing						
Bioinformatics						
Tool Development						
Compare field and lab study results	78	2	94	2	100%	JL
Compare study results with literature	78	2	94	2	100%	JL
Review results, observations, and conclusions	78	2	94	2	100%	SB/JA
Discuss commercialization opportunities	80	1	94	2	100%	JA/JL
Discuss limitations and shortcomings	80	1	94	2	100%	JA/JL
Write methodology for follow up	83	2	94	2	100%	JA/CJ/JL
Milestone: Observations Drawn and Suggestions Given for Follow Up						



ACTIVITY	PLAN START WEEK	PLAN DURATION WEEK(S)	ACTUAL START WEEK	ACTUAL DURATION WEEK(S)	PERCENT COMPLETE	Responsibility
NSERC IPS Proposal						
Genome BC Proposal						
Method Development						
Gather and Process Samples						
Sequencing						
Bioinformatics						
Tool Development						
Reporting and Writing						
Write graduate thesis	53	40	65	32	100%	JL
Review and revise graduate thesis	82	12	80	18	100%	DV/SB/JA/JL
Write journal article	82	8	94	2	100%	JL
Review journal article	90	4	96	2	100%	DV/SB/JA
Write SPES publication	82	1	96	1	100%	JP/JL
Review SPES publication	83	1	97	1	100%	CJ/JL
Prepare conference presentations - i.e. BCWWA, WEST, other	68	8	64	9	100%	All
Milestone: Graduate Thesis, Journal Article, Publications, and Presentation Material Prepared						

ACTIVITY	PLAN START WEEK	PLAN DURATION WEEK(S)	ACTUAL START WEEK	ACTUAL DURATION WEEK(S)	PERCENT COMPLETE	Responsibility
						Week
						21-Aug 82
						28-Aug 83
						04-Sep 84
						11-Sep 85
						18-Sep 86
						25-Sep 87
						02-Oct 88
						09-Oct 89
						16-Oct 90
						23-Oct 91
						30-Oct 92
						06-Nov 93
						13-Nov 94
						20-Nov 95
						27-Nov 95
						04-Dec 96
						11-Dec 96
						18-Dec 97
						25-Dec 97
NSERC IPS Proposal						
Genome BC Proposal						
Method Development						
Gather and Process Samples						
Sequencing						
Bioinformatics						
Tool Development						
Reporting and Writing						
Sharing and Publishing						
Present User Partners with results and recommend follow up	82	8	96	2	100%	All
Submit article to relevant journals for publishing	92	4	96	2	100%	
Publish graduate thesis in UBC CiRcle	92	4	96	2	100%	All
Include publication in SPES annual report	88	8	96	2	100%	
Add raw data to repositories	88	8	96	2	100%	
Milestone: Written Material Submitted for Publishing						
Milestone: Data Available to Advise Follow on Phases						

Table 98. Project Budget and Finances

Item	Budgeted	Spent	Difference	Description
1	400.00	165.00	235.00	Sampling disposables
2	10.00	20.00	-10.00	Renting sediment samplers
3	80.00	80.49	-0.49	Supplies for sampling equipment
4	110.00	0.00	110.00	Supplies for column test
Subtotal	600.00	265.49	334.51	Field and lab study execution
5	10200.00	1431.87	8768.13	DNA Preparation
6	900.00	829.00	71.00	DNA Consumables
8	8400.00	4000.00	4400.00	Environmental lab
9	3150.00	14033.25	-10883.3	MiSeq
10	6250.00	8929.66	-2679.66	HiSeq
Subtotal	28900.00	29223.78	-323.78	Sequencing and preparation
11	10000.00	10000.00	-	Graduate student stipend
12	12000.00	12000.00	-	Graduate student stipend
13	2500.00	2500.00	-	Bioinformatics
14	2000.00	2000.00	-	Printing, publishing
Total	54000.00	53989.27	10.73	

Appendix K: Independent Statistical Review by UBC Applied Statistics and Data Science Group

Upon completion of this thesis, an independent review was conducted by the University of British Columbia Applied Statistics and Data Science Group. The following is the report on statistical limitations and potential opportunities for future exploration.

Revision of Statistical Methods for the master thesis: Genomics tool for monitoring and validating engineered stormwater treatment wetlands

Biljana J Stojkova
Applied Statistics and Data Science Group
Department of statistics
University of British Columbia

June 13, 2017

Abstract

This report provides a revision to the statistical methods used in the thesis. The thesis work under review performs an exploratory statistical analysis in order to provide a 'proof of concept' that the wetland treatment does work when it comes to purifying the storm-water. In general, the project fulfills its objective of providing an exhaustive exploratory statistical analysis supported by appropriate statistical methods. The statistical hypotheses are established to match the scientific question, a sound sampling design is used to collect the data, appropriate statistical tests are used to prove that the hypotheses hold, and an abundant graphics assists with deeper understanding of the data space. The use of the specific statistical tests is well explained and justified throughout the thesis, and the results are clearly presented. The reminder of this report will focus on limitations and guidelines for future work on these project's data.

Limitations and guidelines for future continuation of the project

Addressing the limitations for the future continuation of this project:

- Sampling design is done in two phases, first the 'search sampling' is used to detect the hot spots of contaminated areas, and then 'systematic sampling' is applied to sample from grids. The validity of the 'search sampling' depends on the accuracy of prior info on where or when to begin the search or on the accuracy of measurement to guide the search. If this assumption is violated, the resulting estimates might be biased. In order to justify that the 'search sampling' is appropriately used here, the section 2.5.1.2 needs some explanation of what kind of prior knowledge was available in order to satisfy the assumption of the 'search sampling'. Gilbert 1987 [3], Chapter 10, provides statistical methods on how to find hot spots of high contamination given that the target hot spot is circular or elliptical. Therefore, if the methods by Gilbert 1987 [3], Chapter 10 were used in this thesis work, then it should be mentioned in section 2.5.1.2 along with explanation and justification of whether the target hot spot is circular or elliptical.

- As regards the 'systematic sampling', an aligned grid of equidistant lines has been used to determine the population of the sampling units. Sampling on the aligned grid might introduce bias in the estimates as a result of unsuspected periodicities over the space or some other environmental factors ([3]). To reduce the introduced bias one can use unaligned sampling grid. This is simply a reminder of the possibility that bias might be introduced when sampling on aligned grid. A brief mentioning in the section 2.5.1.2 that aligned grid has been used could clarify sampling design.

- Systematic sampling occurs when samples are taken at regularly spaced intervals over time or space. Initial time or location are chosen at random, and then the remaining samples are collected at regular intervals over time or space. Again, section section 2.5.1.2 would be more clear by including an explanation of how the initial random sample was chosen and if samples were obtained at same locations but different times.

- In section 3.5.9.5.1 checking and correcting for missing values, samples with missing values were removed from the dataset prior to hypothesis testing. Some explanation and references about dealing with missing data are needed here. Some of the missing data techniques do remove the samples with missing data, however, one has to check if the data are missing at random, or learn the distribution of missing data (see for example [1]). After that, decision can be made on how the missing data problem can be handled. Could be that the DNA sequencing technology makes random errors? This needs some explanation, before the missing data are removed.

Some of the deletion methods for missing data are list-wise deletion, and pairwise deletion, and these are their disadvantages:

  - Removing the missing data reduces statistical power.
  - The estimates might be biased if the data are not missing completely at random.
  - Does not use all information.

- In section 3.5.9.5.1, when screening for outliers, the outliers are removed from the dataset without learning the distribution of the measurements with and without outliers. Can we learn how the outliers affect the results? At least obtaining results with and without outliers would show how the outliers affect the results (see for example [2]).

- In section 3.5.9.5.1, one-way ANOVA was used to compare diversity of the bacterial communities among different sites, and Tukey test was run only for the sites where significant differences have been identified. Why not perform Tukey test for all sites, but only for those sites where ANOVA detected significant differences? At least findings should be stated in terms of whether the results from the one-way ANOVA were confirmed or not.

- In Section 3.6.1.4.1, a decreasing trend in the contaminants, such as molybdenum, nickel, barium, copper manganese and zinc, is observed by visual inspection of the graphs, which is the obvious thing to do for exploration purposes. However, if more formal proof is needed to estimate the trend, some modeling should be considered. For instance, time series models or regression model similar to that in section 2.4.4.4.2 could be used here.

- There are repeated measures in time, that have not been used. Although comparisons are made at different time points, the correlations between repeated measures are not taken into account. Having in mind that this was a pilot project, exploratory analysis is the first step towards learning what is hidden in the data. However, in the continuation of this project, the data can be modeled so that all the available information could be used. For instance, mixed model could use all available information, while taking into account the correlations between the repeated measures in Chapter 2. One could model the in-site and between-site variability in how toxicity in the storm-water affects composition and function of the microbial communities.

Bibliography

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Appendix L: Reflections on the Work

Here, I (Jessica LeNoble), present a personal reflection on the work that was performed over the duration of this research project and for the preparation of this thesis. This narrative was inspired by my reading of a past graduate student's thesis, who studied under my supervisor.

During my graduate degree, I learned that there is a great deal of education that takes place beyond the classroom and beyond the design of a research plan and the achievement of one's intended (or unintended) results and conclusions. Some examples include:

- I learned to adapt: when I arrived at UBC, my original research goals involved testing a very different set of hypotheses for the mining sector. At the time of this research, the finances and desire for a student-led project did not exist within the local mining community but there was desire for a similar project to be conducted for stormwater treatment systems. I struggled at first but was ultimately able to design a project that met my desire to learn a new skill in genomics and to experiment with metal-contaminated sites, which was the area for which I was most passionate.
- I learned to think quickly: with field work, there is really no end to the unexpected troubles one can encounter. Especially while working in a public park, thinking quickly or creatively is an essential trait that can only be acquired through experience. My experiences in the wetland will not be forgotten. Highlights include, blowing up my research vessel (dinghy) without a pump (i.e. with my mouth), fending off wild animals (racoons) who were enticed by my tin foil, fixing equipment with packing tape, and working around the general public who walk the gravel path that boarders Lost Lagoon.
- Finally, I learned to appreciate the research community: while at UBC, I experienced an incredible level of kindness and guidance. Everyone I worked with wanted to see me succeed with my project and I observed a fantastic level of passion for pollution control and conservation among my colleagues and supervisors.

I have taken the opportunity to share my experiences with others through presentations in several forums. First, while studying at UBC, I worked for a program called eng·cite where I shared my experience of studying and researching in engineering with hundreds of girls between the age of 8 and 18, encouraging them to consider entering a technical discipline. I think back to the privilege that I experienced at a young age where I was exposed to the environmental field by a teacher in grade 9 and I hope that this outreach may have the same effect for someone else. I also presented my research as a narrative on my experiences in graduate studies at two conferences – the Water and Environment Student Talks Conference and at the young professionals' reception during the British Columbia Water and Waste Association Annual

Conference. In both presentations, rather than focusing on my research outcomes, I focused on the three learning outcomes that I have listed above. I believe that while our research conclusions shape our presentations and publications, it is ultimately our research experiences that shape our futures. It was my goal with these presentations to inspire others to pursue research in the area where they are most passionate so that they too may produce positive changes in the fields of their interests.

I have been incredibly fortunate to work with an excellent team and to have contributed a useful resource to fields of growing importance, namely pollution control, waste management, stormwater treatment, microbiology, and environmental conservation.