MICROBIAL REMOVAL EFFICIENCY WITHIN BIORETENTION FILTER MEDIA IN LABORATORY AND FIELD ENVIRONMENTS

By

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Abstract:

This study will evaluate bioretention systems' ability to remove microbial pollutants in both the laboratory and field settings, while also providing essential background on urban stormwater, pollutants, treatment options, and water regulation. Increased urbanization has increased the quantity of pollutants carried by stormwater. Conventional stormwater systems assist in the mitigation of stormwater pollution but can have an adverse effect on natural hydrology. Low impact development (LID) strategies incorporate engineering designs that address pollutants at the original source while also providing some aesthetic value to the community, LIDs are multiuse best management practices (BMPs). Since unmanaged microbial pollution can result in degraded public health and the spread of disease, literature has suggested a need for quantifying microbial removal efficiencies from LID practices. There are numerous studies describing the removal efficiencies of bioretention cells for non-microbial pollutants illustrating the benefit of LID systems.

This study will quantify removal efficiencies of *E.coli*, enterococci, and coliphage in filter media with and without fly ash amended soil incorporating column experiments and field experimentation. Column experiments using soil cores from the sand layer of established bioretention cells give mean removal efficiencies of 67%, 71%, and 64% for *E.coli*, enterococci, and coliphage respectively in sand only filter media. The fly-ash amended media showed mean removal efficiencies of 64%, 83%, and 41% for *E.coli*, enterococci, and coliphage respectively. These removals do not consider other layers within the bioretention system, only the filter media layer. Additionally, the second component of this study involves field experiments from three bioretention cells sites in Grove, Oklahoma. These sites were monitored and mean removal and concentration change of microbial indicators calculated. The mean removal efficiency for each of the three sites sampled in the field study are site 1 (87%, 80%, 78%), site 2 (35%, 95%, 81%), and site 3 (43%, 97%, 46%) for *E.coli*, enterococci, and coliphage, correspondingly. Finally, the third component of this study is the development of bioretention cell design criteria that specifically targets microbial removal and destruction. This microbial removal bioretention design criteria is based on recommendations found in literature from laboratory and field studies from 2008 to current.

TABLE OF CONTENTS

Executive Summary 1

I. INTRODUCTION 3

Abstract 3
Section 1.1 – Stormwater 3
Section 1.2– Microbial Pollution 5
Section 1.3 – Stormwater Regulation 7
Section 1.4 – Stormwater Treatment 9
Section 1.5 Research Outline, Objectives, and Reasoning 10
References 14

II. LITERATURE REVIEW 17

Abstract 17
2.1 – Bioretention as a BMP for Urban Stormwater 17
2.2 – Microbe Description and Sources 19
2.2.1 – Microbe Description 19
2.2.2 Sources 22
2.3 – Transport and Removal of Bacteria and Viruses in Porous Media 23
2.3.1 – Factors Affecting Microbial Transport and Fate 24
2.3.1.a - Temperature 24
2.3.1.b – Solution Chemistry 25
2.3.1.c – Soil Moisture Content 27
2.3.1.d – Physical Filtration – Mechanical Straining 28
2.3.1.e – Adsorption and Soil Surface /Media Characteristics 29
2.3.1.f – Flow Rate Effect on Microbial Transport 31
2.3.2 – Microbial Removal by Traditional Practices 31
2.3.3 – Microbial Removal and Survival by Bottom Sediments 32
2.3.4 – Microbial Removal and Survival by Beach Sands 33
2.4 – Microbial Removal by Bioretention 34
References 37

III. MICROBIAL REMOVAL FROM SIMULATED STORMWATER BY COLUMN STUDIES USING INTACT SOIL CORES FROM THE FILTER MEDIA LAYER OF ESTABLISHED BIORETENTION CELLS IN OKLAHOMA AND ARKANSAS 43

Abstract 43
3.1 – Introduction 44
3.1.1 – Research Objective 46
3.2 – Methodology 47
3.2.1 – Sample Site Description 47
3.2.2 – Sample Collection 48
3.2.3 – Column Experiments 50
3.2.4 – Analytical Methods 52
3.2.5 – Statistical Methods and Calculations 53
3.3 – Results and Discussion 56
3.3.1 – Bioretention Cell Selection – Depth and Drainage 56
3.3.2 – Average Flowrate 57
3.3.3 – Microbial Removal 59
3.3.4 – Removal Efficiency Based on Filter Media Composition 70
3.3.4.a – Comparison by Filter Media and Microbial Removal 70
3.3.4.b – Column Particle Size Distribution and Effect on Removal and Removal Efficiency Based on Microbe 81
3.4 – Conclusion and Future Work 84
References 87

IV. MICROBIAL REMOVAL BY BIORETENTION CELLS WITH FLY-ASH AMENDMENT IN OKLAHOMA, UNITED STATES 90

Abstract 90
4.1 – Introduction 91
4.1.1 – Research Objective 93
4.2 – Methodology 94
4.2.1 – Site Description 94
4.2.2 – Sampling Methods 96
4.2.3 – Laboratory Analysis 98
4.2.4 – Statistical Analysis 98
4.3 – Results and Discussion 101
4.3.1 – Basic Parameters 101
4.3.2 – Microbial Concentrations and Removal 104
4.3.3 – Comparison of Fly-Ash Amended Bioretention Cells in Grove, Oklahoma to Sand Cells in Current Literature 111
4.4 – Conclusions and Recommendations for Future Work 112
References 115

V. BIORETENTION CELL DESIGN CRITERIA RECOMMEDATIONS FOR TAGETING MICROBIAL REMOVAL AND DESTRUCTION FROM URBAN STORMWATER RUNOFF 118

Abstract 118
5.1 – Introduction 118
5.2 – Bioretention Removal and Inactivation Factors for Targeting Microbes 122
5.3 – Cases Studies 128
5.4 – Design Criteria Recommendations 135
5.5 – Summary and Future Work 140
References 143

VI. CONCLUSIONS 148

6.1 – Overall Conclusions 148
6.2 – Lessons Learned 150
6.3 – Future Work 151
References 154

APPENDIX A – Column Study *E.coli* Removal 155

APPENDIX B – Column Study Enterococci Removal 168

APPENDIX C – Column Study Coliphage Removal 181

APPENDIX D – Column Study Particle Size Distribution 189

APPENDIX E – Field Study Raw Data 220

APPENDIX F – Regression Models and Residual Plots for Column Study 229

APPENDIX G – Pictures 236

Vita 238

LIST OF TABLES

Table Page

2.1 19
3.1 49
3.2 58
3.3 59
3.4 64
3.5 67
3.6 71
3.7 71
3.8 73
3.9 74
3.10 75
3.11 76
3.12 77
3.13 78
3.14 82
3.15 83
3.16 84
4.1 95
4.2 96
4.3 97
4.4 103
4.5 106
4.6 107
4.7 109
4.8 110
5.1 121
5.2 136

LIST OF FIGURES

Figure Page

2.1 18
2.2 20
2.3 21
2.4 22
3.1 50
3.2 52
3.3 60
3.4 79
3.5 80
3.6 80
4.1 110

Executive Summary:

This study will evaluate bioretention systems' ability to remove microbial pollutants in both the laboratory and field settings, while also providing essential background on urban stormwater, pollutants, treatment options, and water regulation. Increased urbanization has increased the quantity of pollutants carried by stormwater. Conventional stormwater systems assist in the mitigation of stormwater pollution but can have an adverse effect on natural hydrology. Low impact development (LID) strategies incorporate engineering designs that address pollutants at the original source while also providing some aesthetic value to the community, LIDs are multiuse best management practices (BMPs). Since unmanaged microbial pollution can result in degraded public health and the spread of disease, literature has suggested a need for quantifying microbial removal efficiencies from LID practices. There are numerous studies describing the removal efficiencies of bioretention cells for non-microbial pollutants illustrating the benefit of LID systems.

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removal efficiencies of 64%, 83%, and 41% for *E.coli*, enterococci, and coliphage respectively. These removals do not consider other layers within the bioretention system, only the filter media layer. Additionally, the second component of this study involves field experiments from three bioretention cells sites in Grove, Oklahoma. These sites were monitored and mean removal and concentration change of microbial indicators calculated. The mean removal efficiency for each of the three sites sampled in the field study are site 1 (87%, 80%, 78%), site 2 (35%, 95%, 81%), and site 3 (43%, 97%, 46%) for *E.coli*, enterococci, and coliphage, correspondingly. Finally, the third component of this study is the development of bioretention cell design criteria that specifically targets microbial removal and destruction. This microbial removal bioretention design criteria is based on recommendations found in literature from laboratory and field studies from 2008 to current.

Chapter 1: Introduction

Abstract

This chapter provides background on urban stormwater, pollutants, treatment options, and water regulation. Urban sprawl has increased the quantity of pollutants carried by stormwater. The mitigation of stormwater pollution is necessary and is addressed through best management practices. Drinking water and recreational water can be negatively affected by microbial and non-microbial urban pollution. Unmanaged microbial pollution can result in degraded public health and the spread of disease. Conventional systems and LID strategies have been successful at pollutant removal in urban environments. Numerous studies describing the removal efficiencies of bioretention cells for non-microbial pollutants illustrating the benefit of LID systems are now available. Removal efficiencies of microbial and non-microbial pollution for conventional systems are well documented in literature, however, published research is lacking microbial removal efficiencies from LID practices.

1.1 Stormwater

Increased runoff volumes have been conventionally addressed through a variety of natural and engineered ponds and water channeling systems working together to move water and reduce flooding. Roads, ditches, culverts, and

underground pipes serve as conveyance systems when they collect, transport, and discharge runoff into local creeks, streams, or lakes. Stormwater systems are designed to reduce peak flow runoff. The volume and duration of runoff are directly affected by urbanization. Changes brought on by urbanization alter initial design parameters and possibly render stormwater systems inaccurate, which can cause flooding or cause the failure of designed systems (Klein *et al*., 1979, Lehner *et al*., 1999). Urban development can increase impervious surfaces (i.e., rooftops, driveways, parking lots, and streets) compared to predevelopment conditions, which increases stormwater runoff volume, peak flow, and the mass of pollutants which wash off the landscape and ultimately reach receiving rivers, lakes, and streams. This runoff results in flash flooding and degraded water bodies (Klein *et al*., 1979, Lehner *et al*., 1999, Hunt *et al*., 2008).

Increased runoff volume and peak flows have resulted in more pollution reaching receiving water bodies. Lehner *et al.* (1999) noted common contaminants in stormwater include metals, organic chemicals, pathogens, nutrients, sediment, and salts. Nationally, 40% of water, segments of streams, lakes, estuaries, and rivers, which equates to over 20,000 individual water segments that do not meet water-quality standards. This impaired or partially impaired water includes 300,000 miles of rivers and approximately five-million acres of lakes, which are largely polluted by pathogens, nutrients, and sediment. A 1999 USEPA report on TMDLs said that an estimated 218 million people live

within ten miles of impaired waters. Although less than three percent of the U.S. landmass is made up of urban areas, the 2013 report from the Natural Resources Defense Council (NRDC) stated that 13% of all rivers, 18% of all lakes, and 32% of all estuaries are impaired by urban stormwater runoff.

1.2 Microbial Pollution

Urban stormwater runoff has been a major contributor to increased pathogenic contamination of receiving waters (Zhang *et al.,* 2010, USEPA, 2015). Chu *et al.* (2001), Vega *et al.* (2008), Hunt *et al.* (2008), Garbrecht *et al.* (2009), Zhang *et al.* (2010), and Park *et al.* (2012) noted the magnitude and variability of microbial pollution. In an urban environment, Lehner *et al.* (1999) states that pathogenic contamination in stormwater runoff comes in part from the fecal matter of humans, pets, and/or wildlife. Currently, there is a large body of research in the area of pollutant removal from stormwater, but gaps exist regarding the removal and destruction of microbial pollutants, or pathogens (Davis *et al.,* 2009, Roy-Poirier *et al.*, 2010, USEPA, 2015).

Pathogens in stormwater are concerning because they can degrade water quality in receiving water bodies like streams, rivers, estuaries and coastal waters and cause disease. In fact, Shuval (2013) and Quilliam *et al.* (2014) indicated that more than 120 million cases of gastrointestinal illness and 50 million cases of

respiratory illness are reported each year by people bathing in coastal waters. Degraded water quality has lead to stream, lake and coastal restrictions on drinking, recreation, and fishing waters. Increased treatment measures are necessary to decrease the risk of health impacts from drinking and recreational waters. The potential health risks due to degraded water quality can lead to beach closings, restrictions on shellfish harvesting, and increased measures for drinking water treatment.

Indicator microorganisms have been used as surrogates for pathogens in water bodies because they generally derive from the same source and have been shown to be correlated to the presence of pathogens (USEPA, 2015). These indicator microorganisms can serve as process microbial indicators, fecal indicators, or index organisms (USEPA, 2015). More specifically, total coliform are considered process microbial indicators, fecal coliform, streptococci, and *Escherichia coli* (*E. coli*) are considered fecal indicators (they infer the presence of fecal contamination, or thermotolerant coliforms) and *E. coli* and coliphage are considered index organisms or model organisms. In addition, index or model organisms could include *E. coli* as an index for *Salmonella* and F-RNA coliphage to model human enteric viruses. The USEPA report on coliphages (820-R-15-098) described coliphages as a subset of bacteriophages which are strongly linked to the fecal matter of warm – blooded animals such as humans. In November,

2008, the USEPA determined that coliphage were an eligible indicator organism in groundwater microbial monitoring.

1.3 Stormwater Regulation

As part of the remedy for human health risk brought on by water contamination, the U.S. Congress passed the 1972 Clean Water Act (CWA). The CWA (and subsequent amendments) have provided a framework to address point and non-point source (NPS) pollution in the United States. The intent of the CWA, Sections 319, 305, and 303 were to mitigate pollution. Section 305(b) required states and territories to report every two years on the quality of all waters within their borders. In order to assist in the mitigation of pollution the 303(d) list has been used when identifying bodies of water that are impaired and/or threatened, based on their designated use. Urban stormwater runoff can be both point and non-point source pollution and urban runoff that is captured in storm sewer systems is considered a point source. The Water Quality Act of 1987 required separate storm sewer systems (MS4s) to acquire National Pollutant Discharge Elimination System (NPDES) permits for addressing urban stormwater runoff from industrial discharges. The NPDES is managed by the USEPA in cooperation with state agencies. These NPDES permits set numeric limits on effluent by water quality based effluent limits (WQBELs) or waste load

allocations (WLAs). Some permits also involve management measures like USEPA approved total maximum daily loads (TMDLs).

Agricultural runoff and urban runoff are two contributors for NPS pollution and urban NPS pollution. It is generally difficult to determine the origin of NPSA pollution so it is regulated by national, state, and local entities (Gang, 2014). States are required to develop pollution controls which are management measures for impaired bodies through the TMDL process. Within each TMDL, best management practices (BMPs) are incorporated to reach the intent of the TMDL, which is compliance with USEPA water quality standards. Both traditional and non-traditional methods are utilized when addressing the pollution transport issue. Structural BMPs such as retention or detention ponds are typical, and green technology such as low impact development structures provided a unique approach.

As more research becomes available, microbial indicator measures accepted by the USEPA have improved and now better describe the possible pathogenic contamination in water bodies. The USEPA (1986) set the recommended limit for allowable concentrations of fecal coliform bacteria in primary contact water at a maximum geometric mean of 200 CFU of fecal coliforms per 100 mL. The USEPA also set the steady-state geometric mean criteria of 126 CFU of *E. coli* per 100 mL and 35 CFU of enterococci per 100 mL

for recreational water and freshwater beaches (USEPA, 2012). Management and reduction of microbial pollution in urban stormwater runoff is necessary in order to improve the water quality of receiving water bodies.

1.4 Stormwater Treatment

Stormwater has traditionally been addressed through the use of storm sewers, detention ponds and other channeling and retention practices that quickly move the excess water to a safer location. These systems primarily address volume and flooding issues. Both water quantity and quality are important factors in urban stormwater runoff, however, conventional systems do not fully address the importance of attaining water-quality standards for urban runoff. Low impact development (LID) is an effective alternative to traditional BMPs because it is an engineering approach that compliments natural settings and has a more positive impact on natural hydrology (Lehner *et al.*, 1999, USEPA, 2000).

LID focuses on controlling stormwater at its original source minimizing the impact of growing urban areas (i.e. impervious area increase) and encouraging a more natural system of microscale controls within a specific watershed (Lehner *et al.*, 1999, USEPA, 2000, USEPA, 2015). LID structures like bioretention cells, rain gardens, and swales can effectively remove pollutants while providing a cost effective and aesthetically appealing alternative to conventional BMP systems.

Regardless of the treatment structure selected, pollutants from urban runoff must undergo further removal processes—like infiltration, adsorption, biodegradation and desiccation—to reduce the flow of pollutants.

1.5 Research Outline, Objectives, and Reasoning:

The overall goal of this research is to evaluate and optimize bioretention systems' ability to remove microbial pollutants. The objective of the first paper is to quantify and compare the amount of microbial pollutants removed from the filter media layer of established bioretention cells, using intact soil cores. Established bioretention cells are defined as bioretention cells that are designed, built, and have been in place for a minimum of 24 months. It is expected that the use of intact cores from established bioretention cells will provide a direct measure of microbial removal through bioretention in an in-situ setting. The intact cores contain two soil media mixtures, sand-only and fly-ash amended, to allow for a comparison of filter media impact on the removal of pathogens in urban runoff. A "dirty" storm with high microbial concentrations and a worst-case scenario where a "clean" storm (no microbes) occurs the next day that could flush trapped microbes from the system were used in the simulations. This study used 30 intact column experiments from five different sites (one site was utilized twice, but at different locations in the cell) and five cores taken from each site. Recent

studies by Zhang *et al.* (2010) and Bradley *et al.* (2011) have shown that bioretention could attain higher microbial removal efficiencies by using soils amended with iron-oxide. Fly-ash amended soils could deliver similar results as those brought on by the iron-oxide amended soils, therefore, fifteen of the cores were collected from cells that contained fly-ash within the filter media. The other fifteen were collected from typical bioretention sand media composition, which will allow this paper to compare removal rates by media with and without fly-ash amendment in the soil cores. Paper 1 (Chapter 3) , titled "Microbial Removal from Simulated Stormwater by Column Studies Using Intact Soil Cores from the Filter Media Layer of Established Bioretention Cells in Oklahoma and Arkansas" is planned to be submitted to Water Environment Research, a research publication of the Water Environment Federation.

The second objective of this research is to quantify microbial removal by installed bioretention cells in Oklahoma. There are numerous field scale experiments for other pollution parameters such as heavy metals, phosphorous, nitrogen and suspended solids. However, full-scale field experiments specifically for microbial pollution, especially in the south-central US are less available (USEPA, 2000, Hunt *et al.*, 2006, Hunt *et al.*, 2008, Hathaway *et al.*, 2009, and LID Center INC, 2015). Three bioretention cells in one Oklahoma community were monitored for this experiment. It is hypothesized that filter media has an impact the on removal efficiency of microbial pollution and that amended soils

will more effectively capture fecal indicator bacteria (FIB) and viruses. The filter media layer of the three cells is amended with fly-ash. A secondary objective for the field scale experiment is to compare and contrast removal rates for *E. coli*, enterococci and coliphage by bioretention cells with fly-ash amendment to published results of field experiments without fly-ash. "Microbial Removal by Bioretention Cells with Fly-ash Amendment in Oklahoma, United States" is the title for the second portion of this research (Chapter 4) with planned submission to The Journal of Sustainable Water in the Built Environment, an ASCE journal.

Finally, a review of current and past research on microbial removal by bioretention is needed, since permanent removal of pollution via bioretention is the goal of this study. The design of bioretention cells has been studied to determine what is required in order to be highly efficient for various pollution parameters. However, current literature could benefit from a succinct consolidated summary of efficient techniques that target and optimize the removal of microbial contamination. Thus, the third objective of this research is to develop bioretention cell design recommendations targeting microbial removal from urban stormwater runoff. It is a reasonable assumption that bioretention can be optimized for microbial pollutants. This optimization will consider major factors and contributors for removal/trapping or movement and survival or destruction of microbes in bioretention. Along with removal and destruction mechanisms, bioretention size should be considered in order to compensate for high flowrates

during a relatively large storm event. High flow is documented to be a limitation in the ability of a bioretention cell to work properly (Yates *et al*., 1987, Seetha *et al*., 2015). In high-flow storm events, there is minimal contact time which minimizes the soils media's ability to absorb bacteria or virus. The third paper (Chapter 5) with submission to ASABE Applied Engineering in Agriculture will be titled "Bioretention Cell Design Criteria Recommendations for Targeting Microbial Removal and Destruction from Urban Stormwater Runoff".

References:

Bradley, I., Straub, A., Maraccini, P., Markazi, S., and Nguyen, T. (2011). "Iron oxide amended biosand filters for virus removal." *Water Research,* 45, 4501-4510.

Chu, Y., Jin, Y., Flury, M. and Yates, M.V. (2001). "Mechanisms of virus removal during transport in unsaturated porous media." *Water Resources Research*, 37(2), 253-263.

Davis, A., Hunt, W., Traver, R., and Clar, M. (2009). "Bioretention Technology: Overview of Current Practice and Future Needs." *J. Environ. Eng.*, 135(3), 109–117.

Dianchen Gang, D. (October 2014). "Impact Factor." *Water Environment Research*, 86(10). DOI: 10.2175/106143014X14031280668335

Garbrecht, K., Fox, G.A., Guszman, J.A., and Alexander, D. (2009). "*E. coli* Transport Through Soil Columns: Implications for Bioretention Cell Removal Efficiency." *Transactions of the ASABE,* 52(2), 481-486.

Hathaway J.M., Hunt, W.E., Wright, J.D., and Jadlocki, S.J. (2009). "Field Evaluation of Indicator Bacteria Removal by Stormwater BMPs in North Carolina." *World Environmental and Water Resources Congress*, 1-10. doi: 10.1061/41036(342)112.

Hunt, W.F. and Lord, W.G. (2006). "Urban Waterways: Bioretention Performance, Design, Construction and Maintenance." Retrieved from http://www.bae.ncsu.edu/stormwater/PublicationFiles/Bioretention2006.pdf.

Hunt, W.F., Smith, J.T., Jadlocki, S.J., Hathaway, J.M., and Eubanks, P.R. (2008). "Pollutant removal and peak flow mitigation by a bioretention cell in urban Charlotte, NC." *Journal of Environmental Engineering*, 134, 403-408.

Klein, R. (1979). "Urbanization and Stream Quality Impairment." *Water Resources Bulletin*, 15(4), 948-963.

Lehner, P., Aponte Clark, G.P., Cameron, D.M., and Frank, A.G. (June 1999). "Stormwater Strategies: Community Responses to Runoff Pollution." Natural Resources Defense Council. Retrieved August 2015 from http://www.nrdc.org/water/pollution/storm/stoinx.asp.

Low Impact Development (LID) Center, INC. “Bioretention – Pollutant Filtering.” Retrieved August 2015 from http://www.lid-stormwater.net/bio_benefits.htm.

Park, S.J., Lee, C.G., Kim, S., Chang, Y., and Yang, J. (2012). “Bacterial removal in flow-through columns packed with iron-manganese bimetallic oxide-coated sand.” *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering*, 47(10), 1364-1371. DOI:10.1080/10934529.2012.672302.

Quilliam, R., Jamieson, J., and Oliver, D. (2014). “Seaweeds and plastic debris can influence the survival of faecal indicator organisms in beach environments.” *Marine Pollution Bulletin*, 84, 201-207.

Roy-Poirier, A., Champagne, P., and Filion, Y. (2010). “Bioretention processes for phosphorus pollution control.” *Environmental Rev*, 18, 159-173.

Seetha, N., Mohan Kumar, M.S., and Hassanizadeh, M. (2015). “Modeling the co-transport of viruses and colloids in unsaturated porous media.” *Journal of Contaminant Hydrology*. http://dx.doi.org/10.1016/j.jconhyd.2015.01.002

Shuval, H. (2003). “Estimating the global burden of thalassogenic disease: human infectious disease caused by wastewater pollution of the marine environment.” *Water Health*, 1(2), 53-64.

Spalding, C. (2013). “Petition for a Determination that Stormwater Discharges from Commercial, Industrial, and Institutional Site Contribute to Water Quality Standards Violations and Require Clean Water Act Permits.” *American Rivers Conservation Law Foundation Natural Resources Defense Council.*

USEPA. (2000). Low Impact Development. Retrieved from http://www.lowimpactdevelopment.org/pubs/LID_litreview.pdf.
USEPA. (2006). “National Primary Drinking Water Regulations: Ground Water Rule: Final Rule.” *Federal Register*, 71(216).

USEPA. (2015). Office of Science and Technology, Health and Ecological Criteria Division. “Review of Coliphages as Possible Indicators of Fecal Contamination for Ambient Water Quality.” 820 – R – 15 – 098.

USEPA. (2015). Office of Water. Retrieved August 2015. "Total Maximum Daily Load (TMDL) Program and Regulations." http://www.epa.gov/ocir/hearings/testimony/106_1999_2000/062800cf.pdf

USEPA. (2015). Quality criteria for water. Retrieved May, 2015 from http://water.epa.gov/scitech/swguidance/standards/criteria/aqlife/upload/2009_01_13_criteria_goldbook.pdf. (Original work published May 1, 1986).

USEPA. (2015). "Water: best management practices: bioretention*."* Retrieved May 25 2015 from http://water.epa.gov/polwaste/swbmp/Bioretention-Rain-Gardens.cfm.

Vega, E., Lesikar B., and Pillai S. (2003). "Transport and survival of bacterial and viral tracers through submerged-flow constructed wetland and sand-filter system." *Bio Resource Technology*, 49-56.

Yates, M., Yates, S.R., Wagner, J., and Gerba, C. (1987). "Modeling virus survival and transport in the subsurface." *Journal of Contaminant Hydrology*, 1, 329-345.

Zhang, L., Seagren, E., Davis, A., and Karns, J. (2010). "The capture and destruction of Escherichia coli from simulated urban runoff using conventional bioretention media and iron oxide-coated sand." *Water Environment Research,* 82(8), 701-713.

Chapter 2: Literature Review

Abstract

This chapter examines bioretention as a best management practice (BMP) in urban environments and the mechanisms involved in transport and removal of bacteria and viruses in stormwater. It also provides background information on factors and conditions which may influence microbial transport and removal within a sandy, filter media layer of bioretention cells, including temperature, soil moisture, and porous media solution chemistry. Furthermore, it will review previous research on the transport and removal of bacteria and viruses, including studies on the survival and regrowth of microbes in a variety of soil media such as beach sand and bottom sediments. A review of the literature will provide greater understanding of the mechanisms influencing microbial removal, retention, and possible destruction within filter media.

2.1 Bioretention as a BMP for Urban Stormwater

Bioretention involves the use of plants and soils to remove pollutants from urban storm water (Garbrecht *et al.*, 2009). A typical bioretention cell includes three basic layers: mulch, top soil, and filter media, shown in *Figure 2.1*. Some may include an underdrain.

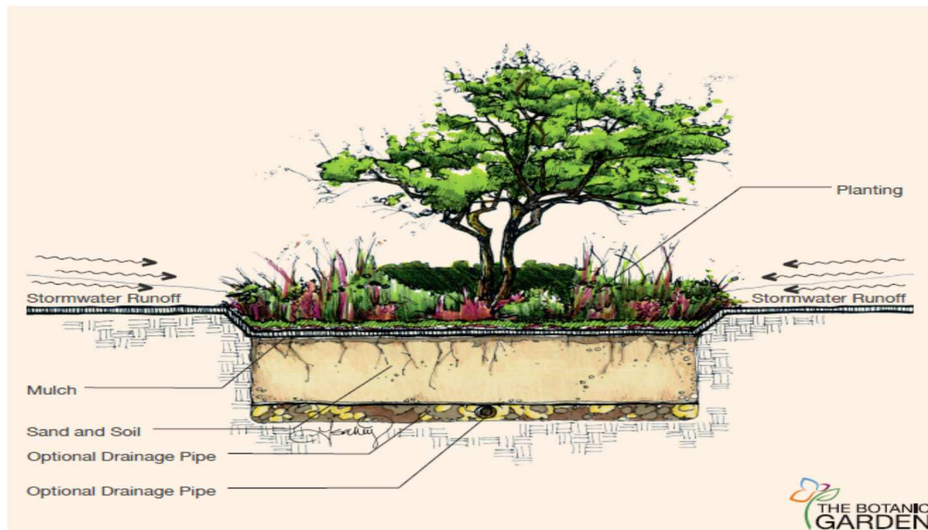

Figure 2.1 – Typical Cross – Section of a Bioretention Cell

Bioretention is well suited for urban areas since cells can generally fit into newly designed or existing landscaped areas (Hunt *et al.,* 2008, USEPA, 2015). Bioretention systems can reduce runoff volumes and peak flow effectively and incorporate removal mechanisms to reduce pollutant concentration in effluent. Previous studies have reported removal efficiencies ranging from 54% to 90% for total suspended solids (TSS), 22% to 85% for phosphorus, 55% to 80% for nitrogen (TKN – total Kjeldahl nitrogen) and 56% to 99% heavy metals shown in **Table 2.1**. Bioretention serves as a multiuse BMP as it also provides urban habitat and aesthetic value (Davis *et al.*, 2009, Roy – Poirier *et al.*, 2010, USEPA, 2015). Vega *et al.* (2003) suggests bioretention cell depth and soil media layers may have some influence on the effectiveness of these systems.

Table 2.1 – Removal Efficiencies of Bioretention Cells

Pollutant	% Removal
Total Suspended Solids (TSS)	90[6], 86[4], 54-59[2]
Total Phosphorus	70-83[5], 22-66[3], 60-80[1], 80[4], 70-85[2]
Total Kjeldahl Nitrogen (TKN)	68-80[5], 60[4], 55-65[2],
Organics	>90[6]
Metals (Cu, Zn and Pb)	93-98[5], 56-99[3], > 90[1,4],

(Davis *et al.*, 2001[1] and 2006[2], Hunt *et al.*, 2006[3], LID Center INC, 2015[4], Davis *et al*, 1998[5], USEPA, 1999[6])

2.2 Microbe Description and Sources

2.2.1 Microbe Description

E. coli, enterococci and coliphage have different size, shape and characteristics that effect their removal in porous media. E. *coli* are defined as gram negative, facultative rod-shaped bacteria that ferment lactose with production of gas and acid (Coyne *et al.,* 1994). *Figure 2.2* is an electron micrograph scanning of E. *coli.* E.*coli* are small, approximately ~ 1 × 3 μm (Jin *et al.*, 2004). E. *coli* are better indicators of fecal pollution in fresh water environments (Halliday *et al.*, 2011).

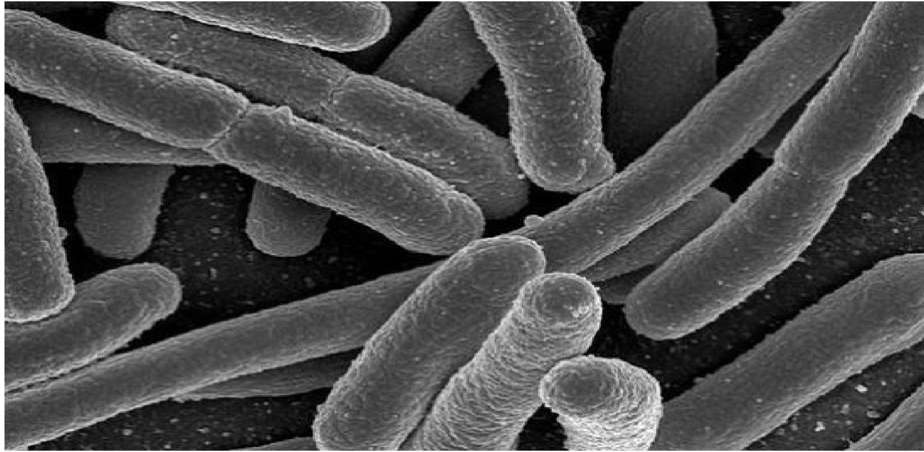

Figure 2.2 – Electron Micrograph of E. *coli* bacteria
(Source: "Escherichia Coli NIAID" by Credit: Rocky Mountain Laboratories, NIAID, NIH – NIAID – 2006)

The enterococci shown in *Figure 2.3* are gram positive, spherical or ovoid, facultative anaerobic organisms. They are 0.6 - 2.0 × 0.6 - 2.5 μm and generally appear in short chains or pairs (Enterococci.htm, 2015). They have been shown to provide a better indication of microbial contamination in brackish water conditions (Halliday *et al.*, 2011).

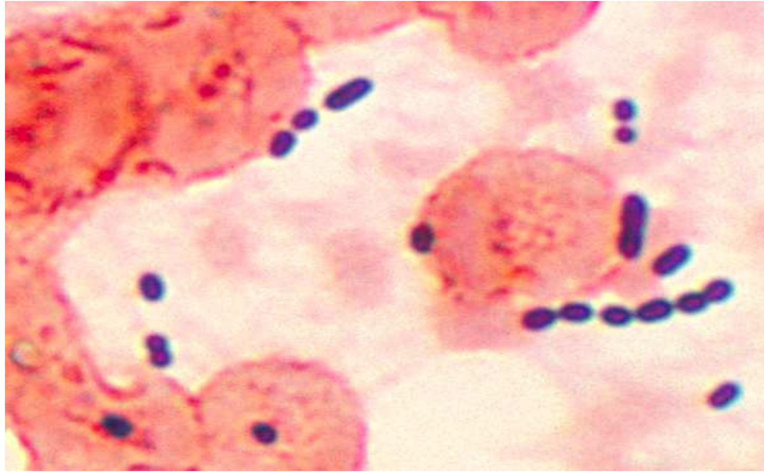

Figure 2.3 – Photomicrograph of Enterococci sp. Bacteria
(Source: CDC/Dr. Mike Miller - Centers for Disease Control and Prevention's PHIL, #2899)

Bacteriophage is a virus that infects bacterial cells. Coliphage is specific to *E.coli* bacterium infection (Clokie *et al.*, 2011, Bio Vir Laboratory, 2015). Male specific and somatic are two forms of coliphage, male specific coliphage infect through the pili while somatic coliphages infect through the cell membrane. Coliphage have multiple stuctures, i.e. octahedral head, contractile tail/sheath, long tails, and short tails (Bradley, 1963). Phages usually consist of a protien capsid which holds the genetic material, either RNA or double strand DNA (Jin *et al.*, 2002). *Figure 2.4* illustrates one of the structures of coliphage. Bacteriophage is currently an acceptable indicator of virus contamination according to the USEPA (2015).

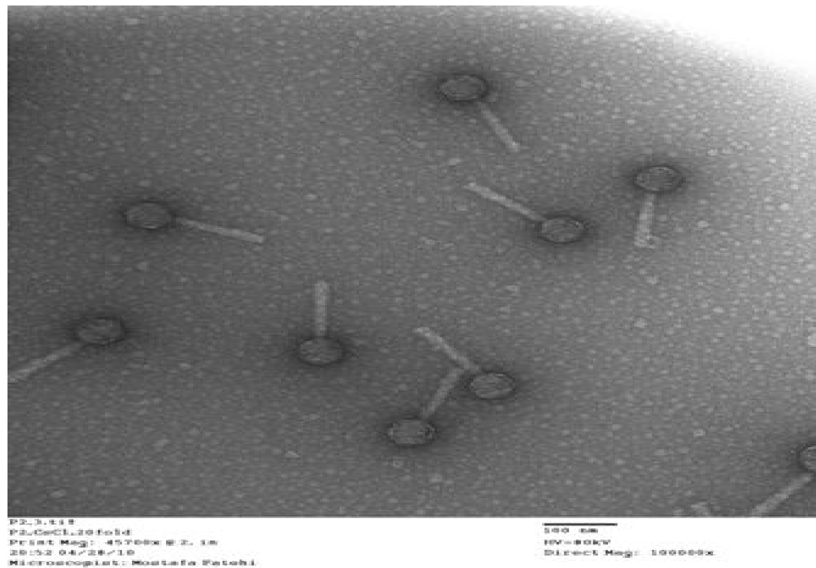

Figure 2.4 – Enterobacteria Phage- Phage That Infects *E. coli* - Using Electron Microscope
(Source: "PhageP2" by Mostafa Fatehi - Own work. Licensed under CC BY 3.0 via Commons)

2.2.2 Sources

Common sources of microbial pollution in stormwater are humans, pets, and wildlife (Lehner *et al.*, 1999). In 1999, the Center for Watershed Protection reported that dogs produced an estimated 200 grams of feces per day per dog. Other studies by the Food and Drug Administration have shown up to 340 grams of fecal matter per day was deposited for an average pet in the United States. A study in Fairfax County, Virginia estimates that approximately 11,000 pets leave 5,000 pounds of waste on the ground daily in the 20 square miles that make up the Four Mile Run watershed (NVSWCD, 2002). Another bacteria source is caused by water fowl and other wild animal populations. For example, geese can produce

between one to three pounds of waste per day and their waste has been shown to contain up to 10^4 more colony forming units of fecal coliforms than human feces (Swallow *et al.*, 2010). Pigeons are attracted to human activity since those areas are ideal locations for feeding, and their waste contributes to pathogenic pollution (USEPA, 1995, NVSWCD, 2002). Leaky septic systems, wastewater collection systems, and combined sewer systems (CSSs) are also common sources of microbial pollution in urban environments (USEPA, 2014).

Sediment in stormwater drains could also serve as reservoirs of high concentrations of microbial activity during warm and dry conditions. A field study in Michigan illustrated the potential for fecal coliform (FC) and fecal streptococcus (FS) to survive in high concentration for up to 6 days under dry weather periods (Marino *et al.*, 1991). Yakirevick *et al.* (2013) and Quilliam *et al.* (2014) suggest resuspension of stream-bottom sediment during storm events can act as another source of degradation of water quality downstream.

2.3 Transport and Removal of Bacteria and Viruses in Porous Media

Studies have shown that filtration, desiccation, thermal deactivation, and sorption, are processes that remove microbes by from water as they pass through porous media, however, the removal amount can vary greatly (Jin *et al.*, 2000, Hathaway *et al.*, 2009, Zhang *et al.*, 2010, and Park *et al.*, 2012). In an ideal treatment system, all pathogenic contamination from urban runoff would be

irreversibly removed or inactivated (killed), which essentially means that there would be no detectable contamination in effluent water. This is not realistic, therefore one of the most important factors in the design of a water treatment system that utilizes porous media for removal of pathogens is the selection of the porous filter media so as to optimize removal processes. The physical and chemical characteristics of the media will directly affect the removal and inactivation of microbial pollutants (Torkzaban *et al.*, 2006, Zhang *et al.*, 2010, Park *et al.*, 2012).

2.3.1 Factors Affecting Microbial Transport and Fate

Microbial transport, removal, and survival in porous media involve a number of abiotic and biotic factors. Microbial transport can be characterized by seven factors: temperature, solution chemistry, soil moisture content, filtration and adsorption, surface and media characteristics, and flowrate. Microbial survival in soils depends on the soil temperature, soil moisture, pH, sunlight, desiccation, and predation from indigenous microbial flora (Potts, 1994, Garbrecht *et al.*, 2009, Park *et al.*, 2012).

2.3.1.a Temperature

The transport and fate of bacteria and viruses are temperature sensitive. As the temperature increases, hydrophobicity increases and adsorption to soil

increases. A study by McCaulou *et al.* (1995) showed that bacteriophage had more favorable attachment to silica beads at a temperature of 24°C versus 4°C. A similar relationship was shown for bacteria by Hendricks et *al.* (1979) described by McCaulou *et al.* (1995). Relationships between temperature and bacteria and virus inactivation rates are well documented (Yates *et al.*, 1984, Crane *et al.*, 1986, Azadpour-Keeley *et al.*, 2003). These studies show inactivation predictability based on temperature for specific virus type. Temperature is one of the leading factors in survival of viruses (Chu *et al.*, 2001)**.** Yates *et al.* (1984) developed a direct relationship between the rise in temperature and the inactivation rate of viruses, therefore it is surmised that microbial survival is increased at lower temperatures (Azadpour-Keeley *et al.*, 2003).

2.3.1.b Solution Chemistry

Soil solution chemistry is largely affected by the pH and ionic strength of porous media. The pH level and ionic strength can increase or decrease bacteria and virus transport and survival. Neutral levels in soils tend to encourage survival of virus indicators whereas both extremely high and low pH values are less suitable for bacterial survival (Zhang *et al.*, 2010). The survival rate of bacteria is in large part dependent on the ability for the bacteria to reproduce (Marino *et al.*, 1991, Shuval *et al.*, 2003, Quilliam *et al.*, 2014).

Analysis from Wan *et al.* (1994) described by Torkzaban *et al.* (2006) suggests preferential sorption of bacteria to the air water interface (AWI) was observed due to hydrophobic forces and solution ionic strength. Bacteria are known to be negatively charged microorganisms which means they need a positively charged particle to attach to in order to become immobilized or removed. Furthermore, they suggest that *E. coli* could be considered to have a weak negative charge and thus its adsorption is more likely caused by electrostatic interactions versus hydrophobic. Electrostatic interactions between bacteria and soil surface depend on the thickness of the diffuse electric double layers. Compression of the double layer—caused by increasing the ionic strength—leads to the soil retaining more bacteria because the electrostatic repulsive force is reduced (Stevik *et al.*, 2004, Zhang *et al.*, 2010).

Soil solution chemistry affects the transport and retention of viruses in porous media by media adsorption for specific virus type (Torkzaban *et al.*, 2006). The transport and removal of viruses in porous media is greatly impacted by surface charge variability in viruses (Jin *et al.*, 2000). At high pH values, experiments for both saturated and unsaturated iron-oxide coated media resulted in strong adsorption of MS2 bacteriophages. The media is believed to have strong absorption because of the presence of iron-oxide on the surface of the sand utilized in the experiment. Iron-oxide has a positive charge and MS2 has a negative charge. Bradley *et al.* (2011) found that the addition of iron-oxides did

increase virus removal by increasing adsorption in the bio-sand filter media. The upper layer of sand media remained saturated in-between operations during these experiments to assist the development of a biological active layer (Bradley *et al.*, 2011).

2.3.1.c Soil Moisture Content

Soil moisture content has a direct impact on removal and inactivation of bacteria and viruses since studies have shown unsaturated flow conditions influence virus survival (Gerba *et al.*, 1975, Jin *et al.*, 2000). Numerous studies in the 1990's suggested that virus retention is greater in unsaturated conditions compared to saturated conditions (Chu *et al.*, 2001).

Column experiments under saturated and unsaturated conditions showed that bacteria transport decreases as water saturation decreases (Gargiulo *et al.*, 2008). Since the 1970s, data supports the belief that survival of bacteria in porous media most affected by soil moisture (Gerba *et al.*, 1975, Jamieson *et al.*, 2015). Studies using manure-amended soils indicate bacteria such as *E. coli* and fecal streptococcus (FS) have increased removal in low soil-moisture conditions. These studies further indicate that survivability is increased with flooded or saturated conditions (Jamieson *et al.*, 2005).

Jin et al. (2000) states the increased removal of viruses (via sorption and/or inactivation) in unsaturated conditions is linked with the air-water interface

(AWI) as it exists only in unsaturated systems. Experiments (Jin *et al,* 2000, Chu *et al.*, 2001, Torkzaban *et al.*, 2006) indicated increased removal of two bacteriophages under unsaturated flow conditions as compared to saturated in column tests. This research illustrates that the removal of a virus in unsaturated porous media is increased via the mechanisms of sorption and/or inactivation depending on the virus type. Virus movement and survival in porous media is due to a variety of factors. In unsaturated porous media the removal of a virus is greater than that of a saturated media. Studies by Yates *et al.* (1987), Sim *et al.* (1996), Jin *et al,* (2000), Chu *et al.*, (2001), Torkzaban *et al.*, (2006) agree that this increase in removal is explained by enhanced adsorption as saturation decreases.

2.3.1.d Physical Filtration – Mechanical Straining

Straining or filtration of cells by small pores and adsorption is the main mechanism of pollutant removal for bioretention cells (Zhang *et al.*, 2010). Immobilization of bacteria through straining occurs when movement is blocked by pores that are smaller than the bacteria. Major factors that affect physical straining are bacterial size and shape and porous media particle size (Weiss *et al.*, 1995, Stevik *et al.*, 2004). Filtration is more effective in removing larger microbial cells as studies have shown filtration to be statistically proportional to bacteria size. Shape can be a factor as well since some studies have surmised that

long rod-shaped cells have greater attachment to filter media than spherical cells (vanLoosdrect *et al*, 1989). When the bacteria are greater than 5% of the mean diameter of the media particles, straining is a more significant mechanism in the bacterial removal process. Filtration contributes to removal more greatly when filter media contains a considerable amount of silt or clay. Clogging of the porous media can also affect straining (Stevik *et al.*, 2004). Additionally, the development of preferential flow paths can reduce filtration and allow for greater bacteria and virus transport through the media.

2.3.1.e Adsorption and Soil Surface /Media Characteristics

Reversible and irreversible bacterial adsorption can occur within soil media. Reversible adsorption is governed by electrostatic forces, hydrophobic interactions and van der Waals forces and is generally considered a weak interaction because bacteria can detach from soil particles and reenter the water phase. This interaction is discussed later during this paper's look at survival, regrowth, and resuspension of pathogens in media. Irreversible adsorption is the act of firm attachment to the surface of a soil particle and is considered a permanent process referred to as adhesion.

The better removal in the unsaturated media is credited to increased adsorption onto the solid-water interface (SWI), irreversible attachment to AWI and possibly attachment to solid-water-air contact lines (SWA) (Torkzaban *et al.*,

2006). The literature has conflicting views on the irreversible attachment theory but most agree that ionic strength, colloid surface properties, and pH affect the level of interfacial attachment within porous media (Yates *et al.*, 1987, Jin *et al,* 2000, Sim *et al.,* 2000, Chu *et al.*, 2001, Torkzaban *et al.*, 2006).

Virus sorption is most affected by the pH and degree of soil moisture of the soil solution (Chu *et al.*, 2001). Soil organic matter can decrease adsorption and thus support virus transport. This decreased adsorption is caused by organic matter increasing the ionic strength of the media that in turn decreases desorption of the virus particles. Further studies of pathogen transport and removal in porous media have examined both laboratory and field experiments (Zhang *et al*., 2010, Park *et al.*, 2012).

Torkzaban *et al.* (2006) states that advection (microbe transport via bulk motion of flowing fluid), dispersion (microbes spreading out by diffusion or turbulence), adsorption (movement by adhesion), and inactivation (microbe death or decay) are major factors that affect the transport and fate of microbial pollutants within the porous media layer of a bioretention cell. Furthermore, microbial transport is different from unsaturated to saturated porous media because virus sorption and inactivation are largely affected by soil moisture content and subsurface temperature variations (Sim *et al.,* 2000).

2.3.1.f Flowrate Effect on Microbial Transport.

Hydraulic factors specifically, the flowrate through the soil media has a direct impact on microbial transport and removal. Previous sand column studies have shown positive results in effectively removing bacteria and viruses with one study focusing on water flow velocity (Zhang *et* al, 2010, Park *et al.*, 2012). Park *et al.* (2012) found that bacterial removal capacity was decreased in small-scale column experiments with increasing flowrate through experimental columns. They determined the decrease in bacterial removal is most likely because higher flowrates tend to result in increased shear force at the surface of the filter media. An increase in shear force would decrease the potential removal – by fostering attachment to the sand media, and thereby reducing the bacterial removal from the media. Coffman (2008) stated the flowrate was not the limiting factor on removal but instead it is the volume of flow.

2.3.2 Microbial Removal by Traditional Practices

Sedimentation studies of stormwater detention ponds have shown that larger soil particles absorb only about 30% of the bacteria in stormwater and that approximately 50% remain unattached and are less likely to settle quickly—as they have slow settling velocities, 0.6 to 1.2 meters per day—and could stay suspended for longer periods of time (Schueler, 2000). Based on these data it would take two days for 90% of the bacteria from a typical storm event to settle

into the bottom of a traditional detention pond (Schueler, 2000). Other traditional methods for bacteria removal in urban environments include sand filtration. Sand filtration is a process where pollutant laden stormwater is passed through a layer or layers of porous media. Depending on the microbe size and shape it will be trapped within the voids of the filter media (USEPA, 2006).

2.3.3 Microbial Removal and Survival by Bottom Sediments of Streams and Beaches

Remobilization of fecal coliforms and other indicator bacteria in streambed sediments occurs during high flow events in rivers and streams. Mobile fecal coliforms cause water quality issues downstream (Schueler, 2000, Yakirevick *et al.*, 2013). Article 67 by Schueler, in the 2000 Watershed Protection Techniques states that bottom sediment is known to provide a haven for bacteria and viruses at increased concentrations when compared to water. An enlarged bacterial community in bottom sediment is credited to the large surface area available for attachment, and the non-limiting nutrient rich environment provided by the sediment (Babinchak *et al.*, 1977, Jamieson *et al.*, 2005, Quilliam *et al.*, 2014). These factors can lead to constant input of bacteria into the water column in any system. Furthermore, bottom sediment of beaches contains higher concentrations of organic nutrients when compared to the covering water, which leads to extended survival and reproduction of bacteria (Babinchak *et al.*, 1977,

Chan *et al.*, 1979). The soil characterization of the bottom sediment is key in determining the effect it could have on survival and regrowth. Fine grained media with silt and clay content distribute higher available organic nutrients than did media with larger grains and minimal clay content (Chan *et al.*, 1979). Nutrients in sediments were demonstrated to be inversely proportional to sediment particle size. Similarly, survival and regrowth of bacteria in this study indicates an analogous pattern in relation to sediment particle size (Chan *et al.*, 1979).

2.3.4 Microbial Removal and Survival by Beach Sands

Microbes in beach sands suffer inactivation effects of sunlight, temperature, and desiccation that would amplify their destruction. However, they also have been shown to increase bacteria and virus survival in certain situations (Whitman *et al.*, 2003). Bacterial survival in a beach environment is most often attributed to the existence of *Cladophora* (green alga), seaweed, underlying beach sand, and human interactions (Whitman *et al.*, 2003, Quilliam *et al.*, 2014). Bacteria survival is furthered because *Cladophora* provides ideal food and shelter. The cell wall provides a suitable attachment and nutrient-rich surface (Whitman *et al.*, 2003, Quilliam *et al.*, 2014). Seaweed surfaces are hot spots for the formation of biofilm which present a nutrient-rich environment for bacteria while providing protection from UV light and predation (Quilliam *et al.*, 2014). Since 1953, epidemiology studies have been conducted on beaches worldwide. These studies

have shown that *Enterococcus spp.* is a better indicator of health risk in marine ecosystems and that *E.coli* is more ideal for fresh water environments (Halliday *et al.*, 2011).

2.4 Microbial Removal by Bioretention

LID studies have shown bioretention can produce high removals of pollutants (USEPA – LID, 2000, Hunt *et al.*, 2006, Hunt *et al.*2008, Hathaway *et al.*, 2009). These studies include both laboratory and field experiments considering a variety of factors including filter media composition and stormwater runoff flow intensity. Dating back to 1988, the city of Austin, Texas has considered bioretention as a BMP in urban areas to remove bacteria. Coffman *et al.* (2008) summarized studies by Hunt *et al*. (2006), Davis (2007), and Rusciano *et al*. (2007) ,showing bioretention removal efficiencies from 70% to 90% for bacteria. A 2009 study in North Carolina by Hathaway *et al.* showed potential for bioretention cells to remove indicator bacteria at a rate of 89% to 92%. Of the six BMPs measured in this study, the bioretention cell was only surpassed by a wetland that lacked vegetative growth (Hathaway *et al.*, 2009). Furthermore, Zhang *et al*. (2010) measured microbial removals of 56% to 98% in column experiments with differing porous media compositions. Selected porous media composition along with depth of porous media layer affects microbial removal efficiency. The greatest removal occurred with fine sand coated with iron-oxide

(IOCS), but IOCS had greater survival than other media measured and thus could become an input source for microbial pollution during a later storm event (Zhang *et al.*, 2010).

Volume and intensity of stormwater has been referenced as a factor in bioretention microbial removal ability. Park *et al.* (2012) utilized small-scale column experiments and found with increasing the flowrate through the column the bacterial removal capacity was decreased. The decrease in bacterial removal is most likely due to higher flowrates tendency to result in increased shear force at the surface of the filter media. An increase in shear force would decrease the potential removal and thereby reduce the bacterial removal from the media. In short, the convention is that contact time is important. Meaning that in high flow events, where contact time is decreased, less removal of microbes occurs increasing the availably of microbes that can be transported into receiving waters.

Conversely, a study by Coffman *et al.* (2008) describes just the opposite of Park *et al*. (2012). In fact, Coffman states that his study does not support the belief that high flow events will decrease the removal ability of bioretention cells due to shorter contact times. Instead they find that volume, not flowrate is the limiting factor. Specifically, column experiments with different media composition blends were examined for volume using maximum design flowrate and bypass volume over specified time periods. The laboratory experiments provided removal of fecal coliform at 77% to 99% at high flowrates. These data

also showed that at lower volumes of influent the removal efficiency increased. When addressing urban stormwater runoff bioretention is extremely viable as smaller drainage areas are treated with this practice. The suggestion from this study is to increase the surface area of the bioretention cell or reduce the drainage area to invoke greater than 90% removal of microbial indicators.

Both Coffman *et al.* (2008) and Park *et al.* (2012) confirm the observation that over time, bacteria removal increases due to filter media maturation and the development of a diverse biological layer to encourage predation from indigenous microbial microflora. All of the studies described provide insight that filter media composition is of high importance.

In summary, bioretention is a LID practice commonly used in urban environments to mitigate stormwater runoff. Pets, wildlife, and human fecal matter are major contributing sources to microbial pollution in urban environments, however, bottom sediments in streams and storm drains could serve as additional sources. Transport and removal factors like, temperature, solution chemistry, soil moisture content, filtration, adsorption, soil surface or media characteristics, and flowrate alter the effectiveness of bioretention filter media. The intent of BMPs like bioretention is to permanently trap and destroy pollution from urban stormwater. Many studies have validated the use of bioretention for non-microbial pollutants and this study will provide evidence to bioretentions' removal effectiveness on microbial pollution as well.

References:

Azadpour-Keeley, A., Faulker, B.R., and Chen, J. (April 2003). "Groundwater Issue: Movement and Longevity of Viruses in the Subsurface." EPA/540/S-03/500.

Babinchak, J.A., Graikoski, J.T., Dudley, S., and Nitkowski, M.F. (1977). "Distribution of Faecal Coliforms in Bottom Sediments from the New York Bight." *Marine Pollution Bulletin,* 8, 150-153.

Bio Vir Laboratories, INC. (2015). "Bacteriophage" Retrieved May 2015 from www.biovir.com.

Bradley, D.E., (1963). "The Structure of Coliphages." *J. Gen. Microbiol.* 31, 435-445.

Bradley, I., Straub, A., Maraccini, P., Markazi, S., and Nguyen, T. (2011). "Iron oxide amended biosand filters for virus removal." *Water Research,* 45, 4501-4510.

Chan, K., Wong, S.H, and Mak, C.Y. (1979). "Effects of Bottom Sediments on the survival of Enterobacter aerogenes in seawater." *Marine Pollution Bulletin*, 10, 205-210.

Chu, Y., Jin, Y., Flury, M. and Yates, M.V. (2001). "Mechanisms of virus removal during transport in unsaturated porous media." *Water Resources Research*, 37(2), 253-263.

Coffman, L. and Ruby, M. (2008). "Bacterra by Filterra Advanced Bioretention System: Discussion of the Benefits, Mechanisms, and Efficiencies for Bacteria Removal." *Low Impact Development for Urban Ecosystem and Habitat Protection*. pp. 1-13. doi: 10.1061/41009(333)93.

Coyne, M.S. (1994). "Water Quality and Fecal Indicator Bacteria." *University of Kentucky, Department of Agronomy, Soil Science News and Views*, 15(7).

Crane, S.R., and Moore, J.A. (1986). "Modeling enteric bacterial die – off: A Review." *Water, Air, and Soil Pollution,* 27(3-4), 411-439.

Clokie, M.R.J., Millard, A.D., Letarov, A.V., and Heaphy, S. (2011). "Phages in Nature." *Bacteriophage*, 1(1), 31-45.

Davis, A.P., Shokouhian, M., Sharma, H., and Minami, C. (2001). "Laboratory Study of Biological Retention (Bioretention) for Urban Stormwater Management." *Water Environ. Res.*, 73(1), 5-14.

Davis, A.P., Shokouhian, M., Sharma, H., and Minami, C. (2006). "Water Quality Improvement through Bioretention Media: Nitrogen and Phosphorus Removal." *Water Environ. Res.*, 78(3), 284-293.

Davis, A. P. (2007). "Field performance of bioretention: Water quality." *Environmental Engineering Science*, 24(8), 1048-1064.

Davis, A., Hunt, W., Traver, R., and Clar, M. (2009). "Bioretention Technology: Overview of Current Practice and Future Needs." *J. Environ. Eng.*, 135(3), 109–117.

Enterococci size and morphology. (August 2015). Retrieved from https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/Enterococcus.htm.

Garbrecht, K., Fox, G.A., Guszman, J.A., and Alexander, D. (2009). "*E. coli* Transport through Soil Columns: Implications for Bioretention Cell Removal Efficiency." *Transactions of the ASABE,* 52(2), 481-486.

Gargiulo, G., Ustohal, P., Vereecken, H., and Klumpp, E. (2008). "Bacteria Transport and Deposition under Unsaturated Flow Conditions: The Role of Water Content and Bacteria Surface Hydrophobicity." *Vadose Zone Journal,* 7(2), 406-419.

Gerba, C.P., Wallis, C., and Melnick, J.L. (1975). "Fate of wastewater bacteria and viruses in soil." *Journal of Irrigation and Drainage Engineering*, 101, 157-174.

Halliday, Elizabeth, and Gast, Rebecca. (2011). "Bacteria in beach sands: and emerging challenge in protecting costal water quality and bather health." *Environmental Science Technology*, 45(2), 370-379.

Hathaway J.M., Hunt, W.E., Wright, J.D., and Jadlocki, S.J. (2009). "Field Evaluation of Indicator Bacteria Removal by Stormwater BMPs in North Carolina." *World Environmental and Water Resources Congress*, 1-10. doi: 10.1061/41036(342)112.

Hunt, W.F. and Lord, W.G. (2006). “Urban Waterways: Bioretention Performance, Design, Construction and Maintenance.” Retrieved from http://www.bae.ncsu.edu/stormwater/PublicationFiles/Bioretention2006.pdf.

Hunt, W.F., Smith, J.T., Jadlocki, S.J., Hathaway, J.M., and Eubanks, P.R. (2008). “Pollutant removal and peak flow mitigation by a bioretention cell in urban Charlotte, NC*.” Journal of Environmental Engineering*, 134, 403-408.

Jamieson, R.C., Gordon, R.J., Sharples, K.E.. Stratton, G.W., and Madani, A. (2002). “Movement and Persistence of Fecal Bacteria in Agricultural Soils and Subsurface Drainage Water: A Review.” Canadian Biosystems Engineering, 44(1), 1.1 – 1.9.

Jamieson, R., Joy, D. M., Lee, H., Kostaschuk, R., and Gordon, R. (2005). “Transport and deposition of sediment-associated Escherichia coli in natural streams.” *Water Research,* 39, 2665-2675.

Jin G., Jeng, H.W., Bradford H., and Englande A.J. (2004). “Comparison of E. coli, enterococci, and fecal coliform as indicators for brackish water quality assessment.” *Water Environ,* 76 (3), 245–55. doi:10.2175/106143004X141807.

Jin, Y., Chus, Y., and Li, Y. (2000). “Virus removal and transport in saturated and unsaturated sand columns.” *Journal of Contaminant Hydrology*. http://dx.doi.org/10.1016/S0169-7722(00)00084-XPotts, M. (1994). “Desiccation tolerance of prokaryotes.” *Microbiol. Mol. Biol. Rev,* 58(4), **755-805.**

Jin, Y., and Flury, M. (2002). “Fate and Transport of Viruses in Porous Media” *Advances in Agronomy*, 77, 39-101.

Lehner, P., Aponte Clark, G.P., Cameron, D.M., and Frank, A.G. (June 1999). “Stormwater Strategies: Community Responses to Runoff Pollution.” Natural Resources Defense Council. Retrieved August 2015 from http://www.nrdc.org/water/pollution/storm/stoinx.asp.

Low Impact Development (LID) Center, INC. “Bioretention – Pollutant Filtering.” Retrieved August 2015 from http://www.lid-stormwater.net/bio_benefits.htm.

Marino, R., Gannon, J. (1991). “Survival of fecal coliforms and fecal streptococci in storm drain sediment.” *Water Resources,* 25(9), 1089-1098.

McCaulou, D.R., and Bales, R.C. (1995). "Effect of Temperature – Controlled Motility on Transport of Bacteria and Microspheres through Saturated Sediment." *Water Resources Research,* 31(2).

Northern Virginia Soil & Water Conservation District. (2002). *Conservation Currents*, 23(3), http://www.gmu.edu/bios/VA/nvswcd/96-02.html.

Park, S.J., Lee, C.G., Kim, S.B., Chang, Y.Y., and Yang, J.K. (2012). "Bacterial removal in flow-through columns packed with iron-manganese bimetallic oxide-coated sand." *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering*, 47(10), 1364-1371. DOI:10.1080/10934529.2012.672302.

Quilliam, Richard, Jamieson, Julie, and Oliver, David. (2014). "Seaweeds and plastic debris can influence the survival of faecal indicator organisms in beach environments." *Marine Pollution Bulletin*, 84, 201-207.

Roy-Poirier, A., Champagne, P., and Filion, Y. (2010). "Bioretention processes for phosphorus pollution control*." Environmental Rev*, 18, 159-173.

Rusciano, G.M., and Obropta, C.C. (2007). "Bioretention Column Study: Fecal Coliform and Total Suspended Solids Reductions." *American Society of Agricultural and Biological Engineers*, 50(4).

Shuval, H. (2003). "Estimating the global burden of thalassogenic disease: human infectious disease caused by wastewater pollution of the marine environment*." Water Health*, 1(2), 53-64.

Sim, Y., and Chrysikopoulos, C.V. (1996). "One-dimensional virus transport in porous media with time-dependent inactivation rate coefficients." *Water Resources Research,* 32(8), 2607-2611.

Sim, Y., and Chrysikopoulos, V. (2000). "Virus transport in unsaturated porous media." *Water Resources Research,* 36(1), 173-179.

Schueler, T. (2000). "Microbes and Urban Watersheds: Ways to Kill 'Em: The Practice of Watershed Protection." Center for Watershed Protection, Ellicott City, MD. Pages 392-400.

Strevik, K., Aa, K., Ausland, G., and Hanssen, J.F. (2004). “Retention and Removal of Pathogenic Bacteria in Wastewater Percolating Through Porous Media: A Review.” *Water Resources*. 38(6), 1355-1367.

Swallow, M., Huffman, J., Van Why, K., and D’Angelo, G. (2010). “The Effect of Goose Management on Water Quality.” Retrieved August 2015 from https://www.aphis.usda.gov/wildlife_damage/nwdp/Publications/10pubs/Swallow%20et%20al%202010.pdf.

Torkzaban, S., Hassanizadeh, S.M., Schijven, J.F., de Bruin, H.A.M., and de Roda Husman, A.M. (2006). “Virus Transport in Saturated and Unsaturated Sand Columns.” *Vadose Zone Journal*, 5, 877-885.

USEPA. (1995). “Storm Water Discharges Potentially Addressed by Phase II of the National Pollutant Discharge Elimination System Storm Water Program.” Environmental Protection Agency, Handbook: Urban Runoff Pollution Prevention and Control Planning, EPA/625/R-93/004, EPA 833/K-94/002, 3-42. (Original work published Sept. 1993)

USEPA. (1999). Storm Water Technology Fact Sheet – Bioretention. EPA832-F-99-012. Retrieved August 2015 from http://water.epa.gov/scitech/wastetech/upload/2002_06_28_mtb_biortn.pdf.

USEPA. (2000). Low Impact Development. Retrieved from http://www.lowimpactdevelopment.org/pubs/LID_litreview.pdf.

USEPA. (2006). “Urban StormWater Preliminary Data Summary.” http://water.epa.gov/scitech/wastetech/guide/stormwater/upload/2006_10_31_guide_stormwater_usw_c.pdf.

USEPA. (2014). “Post-Construction Performance Standards and Water Quality-Based Requirements.” EPA 833-R-14-03.

USEPA. (2015). “Water: best management practices: bioretention.” Retrieved May 25 2015 from http://water.epa.gov/polwaste/swbmp/Bioretention-Rain-Gardens.cfm.

vanLoosdrecht, M., Lyklema, J., Norde, W., and Zehnder, A. (1989). “Bacterial adhesion: A physicochemical approach.” *Microbial Ecology,* 17(1), 1-15.

Vega, E., Lesikar B., and Pillai S. (2003) "Transport and survival of bacterial and viral tracers through submerged-flow constructed wetland and sand-filter system." *Bio resource Technology*, 49-56.

Wan, J.M., and Wilson, J.L. (1994). "Visualization of the role of the gas water interface on te fate and transport of colloids in porous media." *Journal of Appl. Environ. Microbio.l,* 60, 509-516.

Weiss, T.H., Mills, A.L., Hornberger, M., and Herman, J.S. (1995). "Effect of bacterial cell shape on transport of bacteria in porous media." *Environmental Science and Technology*, *29*(7).

Whitman, R. L., Shively, D. A., Pawlik, H., Nevers, M. B., and Byappanahalli, M. N. (2003). "Occurrence of *Escherichia coli* and Enterococci in *Cladophora* (Chlorophyta) in Nearshore Water and Beach Sand of Lake Michigan." *Applied and Environmental Microbiology*, 69(8), 4714–4719. http://doi.org/10.1128/AEM.69.8.4714-4719.2003.

Yakirevick, A., Pachepsky, Y., Guber, A.K., Gish, T.J., Shelton, D.R., and Cho, K.H. (2013). "Modeling transport of Escherichia coli in a creek during and after artificial high flow events: three year study and analysis." *Water Resources*, 47: 2676-2688.

Yates, M.V., and C.P. Gerba. (1984). "Factors controlling the survival of virus in groundwater." *Water Sci. Technology*. 17, 681-687.

Yates, M., Yates, S.R., Wagner, J., and Gerba, C. (1987). "Modeling virus survival and transport in the subsurface." *Journal of Contaminant Hydrology*, 1, 329-345.

Zhang, L., Seagren, E., Davis, A., and Karns, J. (2010). "The capture and destruction of Escherichia coli from simulated urban runoff using conventional bioretention media and iron oxide-coated sand." *Water Environment Research,* 82(8), 701-713.

Chapter 3: Microbial removal from simulated stormwater by column studies using intact soil cores from the filter media layer of established bioretention cells in Oklahoma and Arkansas.

Abstract

Urban storm water runoff contributes to the increased microbial pollution of water bodies and bioretention is a viable best management practice (BMP) to address urban storm water runoff. Bioretention acts as a multiuse practice providing pollutant removal capacity along with aesthetic value and animal habitat. There is a large body of research in the area of pollutant removal from stormwater, but gaps currently exist regarding removal and destruction of microbial pollutants, or pathogens. Column experiments are important to assist the understanding of transport and removal mechanisms occurring within bioretention systems. These studies are generally completed using repacked bioretention media. This study investigates microbial removal by intact, established soil cores using laboratory columns experiments. Column experiments measured removal rates between 35% and 69% with a mean removal of 55% for *E.coli* in sand-only filter media from intact, established soil cores from Oklahoma and Arkansas. Mean removals in sand-only filter media of 64% and 42% were measured for enterococci and coliphage respectively with a maximum measured removal for enterococci of 71% and coliphage of 94%. Fly-ash amended filter media produced removals of 60%, with a maximum measured removal of 99%, 83%, with a maximum measured removal of 99%, and 53%, with a maximum

measured removal of 92% for *E.coli*, enterococci, and coliphage respectively. These column studies indicate that bioretention can be a viable BMP to assist in the removal of microbial pollution from urban runoff, with greater mean removals measured in columns comprised of fly-ash amended media than the sand-only media for *E.coli* and enterococci.

3.1 Introduction

Urbanization has led to increased water quality concerns in stormwater runoff. In an urban environment, the pathogenic contamination in stormwater runoff is most generally from human, pet, and wildlife fecal matter (Lehner *et al.*, 1999). However, sediment resuspension in stormwater drains can act as another potential source of pathogenic contamination during storm events (Yakirevick *et al.*, 2013 and Quilliam *et al.*, 2014). Irrespective of the source, pathogens in stormwater runoff are concerning because they can directly degrade water quality in streams, rivers, estuaries, and coastal waters.

Urban growth and development has led to increased impervious surfaces (roof tops, driveways, parking lots, and streets) when compared to less developed areas (forests and grassland), which impedes infiltration and increases runoff during storm events (Hunt *et al*., 2008). As noted in Chapter 1, within (Lehner *et al*., 1999). If urban runoff is left unmanaged and the quantity of pollutants carried by stormwater increases, flash flooding and degraded public health by spread of

disease will also increase (Klein *et al*., 1979, Lehner *et al*., 1999, Hunt *et al*., 2008).

Traditionally urban stormwater runoff has been controlled with conventional measures (like retention/detention ponds, and pipe conveyance systems) but, conventional systems have an adverse effect on the natural hydrology of the area and addressing flooding. Because both water quantity and quality are important factors, non-conventional approaches to controlling urban stormwater runoff have been developed. Low impact development (LID) practices like rain gardens, rain barrels, swales, and bioretention systems, are effective alternatives to traditional stormwater management control measures. These practices are more harmonious with natural hydrology and address both water quantity and quality at the initial source (Lehner *et al.*, 1999, USEPA, 2000, USEPA, 2015). Furthermore, microbial contamination in stormwater runoff is measured through the use of indicator microorganisms like, *E.coli*, enterococci, and coliphage, which act as surrogates for pathogens (USEPA, 2015).

Water quality is increased when microbial and non-microbial contamination is removed or decreased from urban stormwater runoff. There are numerous mechanisms of microbial removal in bioretention systems, such as filtration, desiccation, thermal deactivation, and sorption. Previous research has shown the removals to be highly variable (Chu *et al.*, 2001, Park *et al.*, 2012). Torkzaban *et al.* (2006) and Zhang *et al.* (2010) both state that soil media

characteristics will directly affect the removal or inactivation of microbial pollutants. Studies by Zhang *et al*. (2008) showed media amended with fly-ash provided increased phosphorus removal in bioretention cells. Furthermore, Zhang *et al*. (2010) and Bradley *et al.* (2011) completed column studies that showed soil media amended with iron-oxide can provide greater microbial removal as well. These studies provide the framework for development of the intent behind this research. First, to obtain column studies with intact, established bioretention media versus laboratory packed columns. Secondly, to determine if filter media amendments like fly-ash can have substantial effect on removal of microbes, as was shown with iron-oxide.

3.1.1 Research Objective

An evaluation of bioretention systems' ability to remove microbial pollutants is the primary goal of this research. The first objective of this paper is to quantify and compare the amount of microbial pollutants removed by intact soil cores from the filter media layer of established bioretention cells. Secondly, this study will compare microbial removal rates by bioretention media with and without fly-ash amendment in the established intact soil cores detailed in the primary objective. Finally, *E. coli*, enterococci, and coliphage will be measured in this study and a comparison of removal rates for of these microbes' will be performed utilizing the same intact, established soil cores.

3.2 Methodology

3.2.1 Sample Site Description

Established bioretention cells are defined as bioretention cells that are designed, installed, and must have been in place for a minimum of 24 months**.** The use of intact, established bioretention columns provides a direct measure of microbial removal through bioretention in an in situ setting. The intact cores contain two different soil media mixtures, sand-only and fly-ash amended, to allow for a comparison of different filter media abilities to remove microbial pollutants in urban runoff. The intact cores received simulated stormwater influent, or spike, demonstrating high microbial concentrations representing a “dirty” storm along with a worst-case scenario where a “clean” storm (no microbial pollutants) occurs the following day. This subsequent clean flush could encourage movement of trapped microbes from the soil column system. Thirty intact columns from five different sites (one site was utilized twice, at different locations in the cell) and five cores per site were tested. Recent studies by Zhang *et al*. (2010) and Bradley *et al*. (2011) have shown that bioretention could attain higher microbial removal efficiencies with media amended with iron-oxide. Fly-ash amended soils could deliver similar results as those bought on by the iron-oxide amended soils. Fly-ash amendment was considered because established bioretention cells in Oklahoma were already in place with this amendment from

previous studies examining phosphorus removal efficiencies related to this amendment. Therefore, fifteen of the cores were collected from cells that contained fly-ash within the filter media layer and fifteen were collected from typical bioretention sand media composition.

3.2.2 Sample Collection

Soil cores from five bioretention cell sites located in Oklahoma and Arkansas collected for this study. Sampled bioretention cells were selected based on a design media thickness of at least 0.31 m. Five replicate cores were collected from each site except for the airport site, from which ten cores were collected. Three sites had a media mixture of sand and nominal fly-ash of 5% by weight, while the remaining two were sand-only. The Botanic Garden sites, Botanic Garden-Sand (BG – S), Botanic Garden-Fly-Ash (BG –FA), have fine Aeolian sand (Daugherty sand) from Perkins, OK. Dougherty sand is composed of 95% sand, 5% silt and clay. The sand composition in the Grove, OK sites, Grove-High School (G – HS) and Grand Lake Association (G – A), is a course to medium sieved, washed sand collected from a local creek. **Table 3.1** details the media, depth of media, location, drainage area and primary land use of each cell in this study.

Table 3.1 –Physical Information for bioretention cells sampled in Oklahoma and Arkansas. [BG-S: Botanic Garden-Sand, BG – FA: Botanic Garden- Fly-Ash, G- A: Grand Lake Association, G- HS: Grove High School, AP: Airport, AR: Arkansas]

Site	Media	Depth Media (m)	Location	Drainage Area (m^2)	Landcover
Botanic Garden (BG- S)	Sand	0.3 – 0.4	Stillwater, OK	1.29	Pavement
Botanic Garden (BG –FA)	Fly-Ash	0.3 – 0.4	Stillwater, OK	3.64	Pavement
Grand Lake Association (G- A)	Fly-Ash	0.3 – 0.4	Grove, OK	7.69	Grassland/Pavement
High School (G – HS)	Fly-Ash	0.3 – 0.4	Grove, OK	2.63	Pavement
Airport (AP and AR)	Sand	0.4 – 0.5	Fayetteville, AR	2.65	Pavement

Five soil cores were collected from each bioretention cell except for Site 1, where ten cores were collected (five at the north end and five at the south end). A Giddings soil sampler (Model GSRPS- #15 – SCS ,Giddings Machine Company Inc., Winsor, CO), a hydraulically driven soil coring implement, was used for soil core extraction using 1.22 m long, 50.8 mm diameter, clear plastic collection tubes, *Figure 3.1*. After the cores were extracted, they were capped on both ends, transported back to the lab in Stillwater, Oklahoma, and logged. All cores were processed in the lab by slicing with a hacksaw while seated securely within a custom sleeve-fitting device designed to minimize damage to the structure of the soil core while cutting. The entire set of cores used in this study had no observed roots or macro fauna features apparent within the plastic tube.

Figure 3.1 – Giddings soil core apparatus mounted on the bed of truck at the Botanic Garden in Stillwater, Oklahoma.

3.2.3 Column Experiments

Thirty column experiments were conducted with media collected from five sites in Oklahoma and Arkansas. The column test was designed to simulate two consecutive storm events, one dirty-pollutant filled event, and the subsequent event representing a relatively clean load after the microbe source had been washed away. The experimental design encompassed two days of laboratory testing. On the first day, simulated stormwater consisting of continuously stirred swine slurry diluted at a rate of 1:100 with RO water was run through the column. Four pore volumes of this diluted swine slurry passed through the columns on the initial experiment day. The swine slurry was retrieved on the Thursday afternoon preceding each new column run that began on Monday mornings. On the second

day, the column study was run with RO water for 8 to 12 pore volumes to flush any viable and detachable microbes from the cell, representing a “clean” storm. The initial experimental run also included a pre-rinse of the intact soil cores with RO water to remove any residual bacteria, since no microbes were detected in this rinse water after the first set, a pre-wash was deemed not necessary and not included in the proceeding column studies.

All laboratory column tests were conducted intact within the original plastic collection tubes. The column apparatus included a constant head reservoir, storage reservoir, pump, and overflow line. The storage reservoir contained the continuously-stirred influent or RO water depending on experiment day and was pumped through a feed line to the constant head reservoir which then entered the column. The constant head reservoir had an overflow line set at 1.52mm from the top of the column and was redirected to the storage reservoir and further recycled into the column until the desired pore volumes were captured.

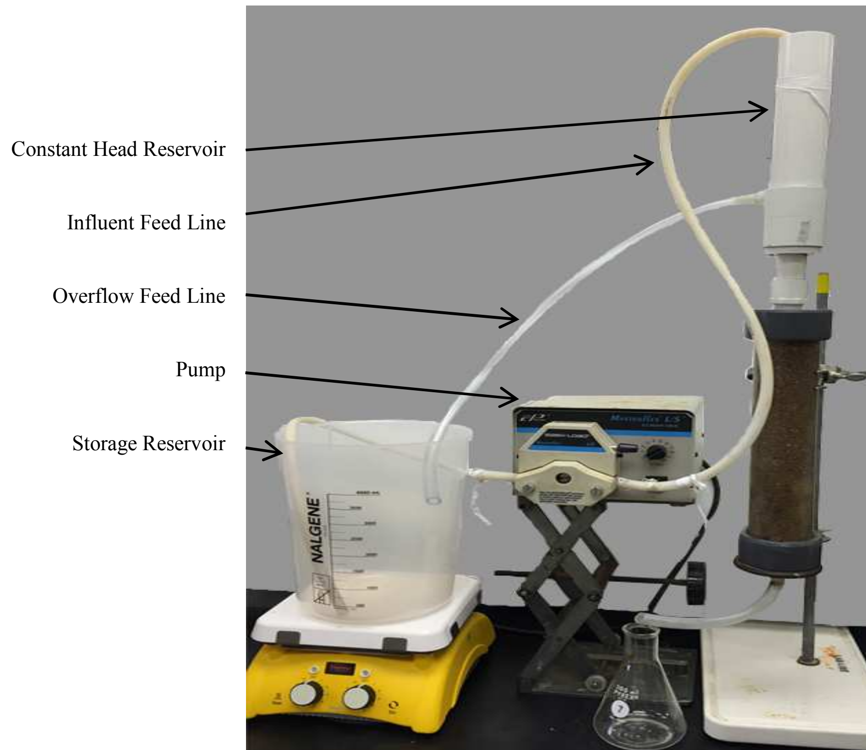

Figure 3.2 – Laboratory Setup for Experimental Column Design

3.2.4 Analytical Methods

Samples were measured for microbes, microbial measurements include *E. coli*, enterococci, and coliphage. All microbial analysis was completed by Dr. Dale Griffin from the USGS Microbiology Laboratory in St. Petersburg, Florida. Samples were shipped overnight to the Florida lab. *E.coli* and enterococci analysis is consistent with Entero and Colilert Quanti Tray 2000 Method from IDEXX Systems (IDEXX, 2013, IDEXX, 2014). The inlet sample was inoculated into sterile water for Quantitray analyses. The remaining water samples were inoculated into Quanti Tray 2000's and samples were diluted as necessary.

Coliphage overlays used two milliliters volumes by three replicated for all samples tested. All plates and quantitrays were incubated overnight at their respective temperatures and samples were stored overnight by refrigeration.

Further, microbial measurements were completed on soil samples from each destructed column after the two day experiment was complete. Particle size distribution of the porous media was completed for each of the soil columns that were destroyed after experiments were finished, using ASTM D422 (ASTM, 2009).

3.2.5 Statistical Methods and Calculations

In depth examination of the data begins with the statistical analysis of the percent removal for each microbe. Standard deviations of percent removal within each column were calculated. This data can be found in the tables and figures in the following sections.

Equation 1 uses a microbial count balance to compare the initial number of microbes in the influent to the final number of each microbe in the effluent on day 1. Microbes are measured as colony forming unit (CFU), most probable number (MPN), or plaque forming unit (PFU). This equation is utilized for all microbial indicators and coliphage concentrations. The total percent removal after Day 1, for each microbe was calculated for each of the 30 columns in this experiment. This equation represents the average flow-weighted percent removal

for one column for the entire set of pore volumes analyzed during the Day 1 slurry-spike. The percent removal for each *microbe* after day 1 ($\%R_1$), was found by Equation 1,

$$\%R_1 = (1 - \frac{C_o}{C_i}) * 100 \qquad (1),$$

where, C_o is the count of each *microbe* in the outlet from the column after day 1, and C_i is the count of microbes in the slurry-spike.

Total percent removals, $\%R_2$, based on the individual microbes (*E.*coli, enterococci, and coliphage) retained in the column after the both experimental days. Given as the count or number of microbes received in the effluent wash-out after Day 2 clean flush, was calculated by Equation 2,

$$\%R_2 = \left(1 - \frac{C_{o(Day\ 1)} + C_{o\ (Day\ 2)}}{C_i}\right) * 100 \qquad (2),$$

where, $C_{o\ (Day\ 2)}$ is the count of *microbes* that washed out from each column after Day 2, clean flush.

The mean $\%R_1$ and $\%R_2$ for each site is calculated by Equation 3.

$$\%\mathrm{R}_{mean} = (1 - \frac{\sum C_o}{\sum C_i}) * 100 \quad (3)$$

This equation is not a flow-weighted average of $\%R_1$ and $\%R_2$.

This study does not include removal that could occur in the mulch and top soil layer. It is only considering removal isolated within the porous media layer of the cell.

Confidence intervals of microbial percent removal, C.I., were calculated for each experiment to further understand the relevance of the microbial removals received. Equation 4,

$$C.I. = \left[\bar{\mathbf{x}} - t_{\alpha/2} \frac{s}{\sqrt{n}}, \bar{\mathbf{x}} + t_{\alpha/2} \frac{s}{\sqrt{n}} \right] \quad (4),$$

describes the t distribution method used to calculate confidence intervals for experiments. The sample is defined as the count of microbes for each column during each day of the experiment.

where,

$\bar{x}$ is the mean of the sample, $t_{\alpha/2}$ is the t value for the upper tail of the t distribution, s is the standard deviation of the sample, and n is the sample size, generally taken to be five, representing the five replicate columns for each site.

Further statistical analysis of microbial removal was performed using Minitab® 17. An ANOVA, Tukey one-way analysis and the Kruskal-Wallis non-

parametric test for each microbe was run for all columns and for the Botanic Garden columns.

3.3 Results and Discussion

3.3.1 Bioretention Cell Selection – Depth and Drainage

The ability to find six sites that had adequate sand media depth proved difficult. This study was designed with three sites having sand-only filter media and three sites with a fly-ash amended filter media. The fly-ash amended sites were sampled without any issue. It should be noted that both sites in Grove, OK, G – A and G – HS, had more than 0.45 m of filter media. Filter media depth was a common issue at other bioretention cell locations. A total of eight sites were sampled in order to find the five sites used in this study. The most common issue faced when sampling was that the bioretention cell design depth was not equivalent to the as-built depth. The second item that proved problematic was filter media drainage. Two sites where cores were extracted would not drain once returned to the laboratory. In both instances it was observed that the clay layer was intermixed with the sand layer and impeded the cores drainage capacity. As stated previously, five sites were used in this study not the six sites that were desired. Site five was located in Arkansas and was used twice (cores from the

north and south end of the cell) due to the discrepancies with filter depth and drainage capacity.

3.3.2 Average Flowrate

An average flowrate per column per experiment was calculated for each of the runs spike run on day 1 and the clean flush on day 2, **Table 3.2**. Although mid-run flow measurements were not collected, observation showed that the flow generally decreased during the run, likely due to initial clogging. Preferential finger-flow was also observed for the column runs with fastest average flowrates (BG-S-5, G-HS-1, G-HS-4, G-A-1, G-A-4, and AP -2). It can also be noted that additional columns from the Botanic Garden had average flowrates that would also be considered high (BG-S-1, BG-S-6, and BG-FA-6) but these columns did not experience the same observed preferential flow issues. This is possibly due to the size and characteristics of the filter media.

Table 3.2 – Total run time for laboratory column experiments in minutes and overall flowrate per column. [BG-S: Botanic Garden-Sand, BG – FA: Botanic Garden- Fly-Ash, G- A: Grand Lake Association, G- HS: Grove High School, AP: Airport, AR: Arkansas]

Column	Day 1 – Time (minutes)	Day 2 - Time (minutes)	Day 1 - Flowrate (ml/min)	Day 2 - Flowrate (ml/min)
BG - S - 1	85	39	14.1	61.5
BG - S - 2	61	140	19.7	17.1
BG - S - 3	95	209	12.6	11.5
BG - S - 5	34	156	35.3	15.4
BG - S - 6	48	58	25.0	41.4
BG - FA - 1	135	313	8.9	7.7
BG - FA - 2	383	688	3.1	3.5
BG - FA - 3	234	248	5.1	9.7
BG - FA - 4	123	300	9.8	8.0
BG - FA - 5	40	104	30.0	23.1
G - HS - 1	50	53	24.0	67.9
G - HS - 2	146	171	8.2	23.4
G - HS - 3	58	63	20.3	57.1
G - HS - 4	24	37	50.0	97.3
G - HS - 5	156	76	7.7	20.5
G - A - 1	27	25	88.9	144.0
G - A - 2	81	35	29.6	102.9
G - A - 3	62	115	38.7	28.8
G - A - 4	31	29	77.4	124.1
G- A - 5	24	56	100.0	66.7
AP - 1	251	275	6.47	25.4
AP - 2	47	50	53.3	72.0
AP - 3	59	142	57.6	25.4
AP - 5	72	166	33.3	22.0
AP - 6	159	299	15.1	12.0
AR - 1	205	300	11.7	12.0
AR - 2	108	0.0	22.2	0.0
AR - 3	54	0.0	44.4	0.0
AR - 5	0.0	0.0	0.0	0.0
AR - 6	264	110	9.1	32.7

3.3.3 Microbial Removal

Table 3.3 catalogues the slurry microbial concentrations for each individual column experiment. Representative results for Botanic Garden – Sand-Only (BG – S), column 1 are shown in *Figure 3.3*. As the diluted slurry influent enters the column on day one the concentration in the effluent increased, however, as the RO water enters the column on Day 2 the effluent concentration decreases. This observed trend was consistent throughout all columns and all sites, raw data for all experiments is available in Appendix A.

Table 3.3 –Microbial concentration of inlet swine slurry for laboratory column experiments collected from Oklahoma State University swine farm. [BG-S: Botanic Garden-Sand, BG – FA: Botanic Garden- Fly-Ash, G- A: Grand Lake Association, G- HS: Grove High School, AP: Airport, AR: Arkansas]

Site	E. coli MPN/(100 ml)	Enterococci MPN/ (100 ml)	Coliphage PFU/(100 ml)
BG – S	200,000	>24,000	9,400
BG – FA	110,000	200,000	2,600
G – A	730,000	1,700,000	29,000
G – HS	200,000	25,000	100,000
AP	37,000	1,700,000	260,000
AR	26,000	>24,000	76,000

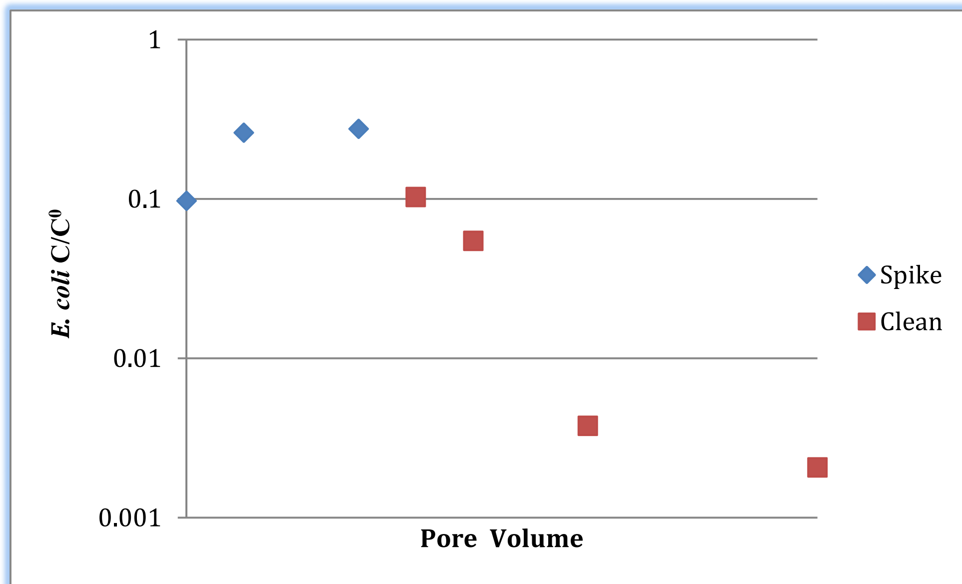

Figure 3.3 – Effluent Concentration Curves for BG – S, column 1, Botanic Garden, Stillwater, Oklahoma. [BG-S: Botanic Garden-Sand, C: final concentration, C^o: initial concentration]

A flow-weighted percent microbe removal for each of the three microbe types from each column from all sites was calculated after the initial diluted slurry-spike on Day 1 and again after the clean run on Day 2 (**Tables 3.4 – 3.5**).

Based on these data, the filter media from the sand side of the Botanic Garden bioretention cell (BG – S) yields an 80% removal, with a standard deviation of 2% across all five columns, for *E. coli* after the Day 1 spike. A standard deviation of 12% and average *E. coli* removal of 69% after the second day, clean simulation. The sand layer of BG – S had a mean removal of enterococci of 76% after the initial slurry loaded spike. Enterococci removal

reduced to 70% after day 2 clean flush, which is approximately a 6% microbial wash out or release for this site between simulated spike and clean experimental days. Coliphage had a removal of 57% after the initial spike and reduced to 51% on day 2, clean flush experiment. Further analysis of **Table 3.4** shows that BG – S, column 5 was an outlier. This column provided a similar removal after the Day 1 spike but had a 36% and 13% decrease in *E.coli*, enterococci removals respectively, after the second experiment, Day 2 clean wash. This column drained substantially faster than the other four columns from the same site, which suggest a preferential flow path or macro pore was may have occurred within the column during the Day 2 experiment. BG – S, column 5 exhibited the greatest variation in removals for all two of the indicators, coliphage maintained the same removal over both experimental days.

The Airport in Fayetteville, AR was the location of the remaining ten sand-only media columns in this study. The initial five columns were labeled AP and shown in **Table 3.4**. *E.coli,* enterococci, and coliphage yielded average removals of 44%, 73%, and 38%, respectively after the slurry-spike experiment on Day 1. These data illustrates large variation within removal or microbe trapping between columns. The variation was easily observed as **Table 3.4** shows AP, column 1 to produce a high *E.coli* removal of 82% and a low removal of 23% with AP, column 2. Similar variations are seen for enterococci and coliphage as well for this set of columns. **Table 3.4** further quantifies mean removals from the

airport after the secondary clean flush, Day 2, for all microbes previously discussed. Similar mean removal results were seen for this experimental day. There was a 64% change of *E.coli* removal from column 1 removal to column 5, 76% to 12% respectively – high variability within the columns. Enterococci provided the best removal for the AP columns, 73% on Day 1 and 71% on Day 2, approximately a 2% reduction in removals between experiments. Although some AP columns in **Table 3.4** show good removal efficiencies the mean removals are very low for *E.coli* (35%) and coliphage (32%).

The remaining five columns are labeled AR, data provided in **Table 3.4**. All ten columns are comprised of a sand-only porous media composition. The initial five columns enumerated in the previous tables were from the north end of this bioretention cell. Data for AR columns were collected from the south end of the cell and produced results that are very different than the previous five columns (AP). **Table 3.4** quantifies mean microbial removals of 77% (*E.coli*), 65% (enterococci), and 96% (coliphage) for the final five columns in this experiment, Day 1 slurry-spike. AR, column 5 did not drain thus there are no data to consider for it and therefore this experiment was actually based on the four remaining column replicates. As shown in **Table 3.4**, columns 2 and 3 failed to drain during Day 2. *E.coli* and enterococci had a 17% and 14% drop in mean removal between Day 1 and Day 2 experiments. The standard deviations are very high for *E.coli* (38%) and enterococci (47%) suggesting high variation in individual column

removals. All mean microbial removals are solely based on columns 1 and 6.Coliphage had the best removal for this experiment with 96% (Day 1) and 94% (Day 2).

Table 3.4 – Quantification of microbial removal of three indicator species using fifteen sand-only composition columns – after day 1 (slurry-spike) and day 2 (clean flush) laboratory column study. [BG-S: Botanic Garden-Sand, AP: Airport, AR: Arkansas, CI: Confidence Interval]

Site	Column	*E. coli* (% Day 1)	*E. coli* (% Day 2)	Enterococci (% Day 1)	Enterococci (% Day 2)	Coliphage (% Day 1)	Coliphage (% Day 1)
BG - S	1	77	72	81	78	64	64
BG - S	2	79	74	82	77	58	41
BG - S	3	82	72	73	65	56	43
BG - S	5	83	49	71	58	36	26
BG - S	6	80	78	76	75	72	72
BG - S	Mean (%)	80	69	76	70	57	51
BG - S	Standard Deviation	2	12	5	9	14	16
BG - S	CI (90%)	[79, 82]	[61,77]	[73, 80]	[65, 76]	[48, 66]	[40, 62]
AP	1	82	76	93	89		
AP	2	23	20	72	71	44	35
AP	3	29	20	55	54		
AP	5	27	12	67	66	33	29
AP	6	59	45	79	77		
AP	Mean (%)	44	35	73	71	38	32
AP	Standard Deviation	26	26	14	13	8	4
AP	CI (90%)	[26, 61]	[17, 52]	[63, 83]	[62, 80]	[30, 46]	[27, 36]
AR	1	91	87	86	84	96	96
AR	2	84	ND	74	ND		
AR	3	84	ND	82	ND		
AR	5	ND	ND	ND	ND		
AR	6	50	33	18	17	95	93
AR	Mean (%)	77	60	65	51	96	95
AR	Standard Deviation	18.6	39	32	47	1	2
AR	CI (90%)	[63, 92]	[19, 100]	[41, 89]	[0, 100]	[95, 96]	[93, 96]

Table 3.5 details the removal efficiencies of five soil columns from the fly-ash amended side of the bioretention cell at the Botanic Gardens, BG-FA. All five columns produced removals of 93% to 100% for *E. coli*, enterococci, and coliphage, Day 1. *E. coli* and enterococci have the highest removal across all columns. *E. coli* and enterococci have removals of 99% with a standard deviation of less than or equal to 1%. Column 5 data were corrupt for enterococci, because of analysis error, and it is not included in the percent removal calculation for BG-FA. It is important to note that we do not know the exact influent enterococci concentration, only that it is > 241,960 MPN/100ml so all enterococci removals are relative to one another. Coliphage was analyzed for two of the five columns and remained consistent in both with a flow-weighted average removal of 93%, standard deviation of 0%. **Table 3.5** also, quantifies the microbe specific removal rates from BG – FA second simulation of clean influent entered the intact soil cores column system. These data provide near perfect removal from all five columns and all microbes measured. The columns show a 0.1% decrease in mean percent removal for *E.coli* after the subsequent clean flush on Day 2. The BG - FA columns had less than a 1% by average reduction in enterococci and coliphage removal after the second day of microbial wash out occurred. Overall, BG – FA bioretention cell show between 92% and 99% removal of all three measured microbes throughout the entire experiment.

Grand Lake Association in Grove, Oklahoma, G-A, has a fly-ash amended sand layer utilized in the column experiment. **Table 3.5** quantifies the microbial removal rates of *E.coli*, enterococci, and coliphage after the spike, Day 1. The flow-weighted average percent removals are 63%, 84% and 12% for *E. coli,* enterococci, and coliphage, respectively. The standard deviation for *E. coli*, enterococci, and coliphage mean percent removals are 9%, 6% and 1% illustrating relatively low variations between individual columns. **Table 3.5** also illustrates the removal of each of the columns after the wash out from the second day. The overall *E. coli* removal after both experiment days was 60% with a standard deviation of 7%. *E. coli* removal decreased by 5% after the second day clean water ran through the system. Enterococci removal was higher than the other two microbes measured for this study. An observed 1% decrease in removal efficiency occurred between Day 1 and Day 2. The minimal decrease in removal illustrates the sand layer of the bioretention cell is still working effectively in removal of enterococci.

Table 3.5 – Quantification of microbial removal of three indicator species using fifteen fly-ash and sand-only composition columns – after day 1 (slurry-spike) and day 2 (clean flush) laboratory column study. [BG – FA: Botanic Garden – Fly-Ash, G – A: Grand Lake Association, G – HS: Grove High School, CI: Confidence Interval]

Site	Column	*E. coli* (% Day 1)	*E. coli* (% Day 2)	Enterococci (% Day 1)	Enterococci (% Day 2)	Coliphage (% Day 1)	Coliphage (% Day 1)
BG - FA	1	100	100	99	99	93	92
BG - FA	2	100	100	100	100		
BG - FA	3	100	100	99	99	93	92
BG - FA	4	100	100	100	100		
BG - FA	5	97	97	ERR	ERR		
BG - FA	Mean (%)	99	99	99	99	93	92
BG - FA	Standard Deviation	1	1	1	1	0	0
BG - FA	CI (90%)	[98, 100]	[98, 100]	[99, 100]	[100, 100]	[93, 93]	[92, 92]
G - A	1	52	49	82	81	11	4
G - A	2	77	70	92	90		
G - A	3	65	62	89	89		
G - A	4	61	60	80	80		
G - A	5	62	61	78	77	12	10
G - A	Mean (%)	63	61	84	83	12	7
G - A	Standard Deviation	9	7	6	6	1	4.3
G - A	CI (90%)	[57, 70]	[55, 66]	[80, 88]	[79, 87]	[11, 12]	[3, 12]
G - HS	1	18	2	50	31	13	5
G - HS	2	27	24	87	84		
G - HS	3	48	42	92	91		
G - HS	4	23	18	41	37		
G - HS	5	28	22	58	54	25	21
G - HS	Mean (%)	29	22	66	59	19	13
G - HS	Standard Deviation	12	15	23	27	9	11
G - HS	CI (90%)	[21, 37]	[12, 32]	[50, 81]	[41, 78]	[10, 29]	[1, 25]

An average removal of 7% was yielded after day 2, clean, coliphage removal decreased by 5% on average from Day 1 to Day 2.

A bioretention cell located in Grove, Oklahoma at the local high school is G – HS. This cell has a fly-ash amended sand layer composition. Microbe specific removal rates shown in **Table 3.5** represent removals after the initial spike. *E. coli* and removals after the first day are low, 29%. The columns showing the most *E. coli* removal were #3 and #5, even those removals were less than 50%. Data from Day 2 clean water flush were equally poor, *E.coli* returned an average removal of 22% with a standard deviation of 15% and individual column removals varying between 1% and 42%. Enterococci returned the best removal for this site with a high removal of 92% for column 3 and a low removal of 41% for column 4. The average removal across all columns corresponding to Day 1 spike for site 4 cores was 66%. Enterococci had individual column removals of 31% to 91% with an average removal of 59% (standard deviation equal to 26.8%) for the second day of experiments. Finally, coliphage had a 19% and 13% average removal with a standard deviation of 19% and 11% for Day 1 and Day 2, respectively. Column 5 had the highest removal for coliphage, 25% effective. The standard deviations for all microbes measured are less than desirable for this site across all columns.

Overall data from day 1, slurry-spike influent, showed the BG – S sand cell had removal efficiencies of approximately 78% to 82% *E. coli* removal at the 10% level of significance. Likewise on Day 1, Botanic Garden fly-ash cell

columns had a 90% confidence interval of approximately 98% to 100% for *E.coli* removal rates. The 90% level of confidence was 61% to 77 % removal after Day 2, clean flush. Notice that at 90% level of confidence the removal efficacy for *E.coli* decreases from Day 1 experiments to Day 2. The columns comprised of the Botanic Garden fly-ash media maintained the initial estimated interval of 98% to 100% at the same 90% C.I. on day 2. The other sites (G – A, G – HS, AP, and AR) had larger confidence intervals than the Botanic Garden at the 10% level of significance. Furthermore, the two sites in Grove, Oklahoma and the airport site in Fayetteville, Arkansas had large standard deviations suggesting high variability in removals and standard deviation and confidence interval calculations in **Tables 3.4 – 3.5** support this statistical observation.

The microbial removal rates, from all sites except BG, shown below are generally lower than those found in laboratory packed columns which can range between 56% to 98% (Zhang *et al.*, 2010, Coffman *et al*., 2007, Davis *et al.*, 2007, Rusciano *et al*., 2007, and Hunt *et al.*, 2006). The intact soil columns in this study have not been disinfected to remove all indigenous microbial activity, they are the exact representation of what resides in the bioretention cell at each given location.

3.3.4 Removal Efficiency Based on Filter Media Composition

3.3.4.a Comparison by Filter Media and Microbial Removal

Table 3.6 and **3.7** show removal efficiency of *E.coli*, enterococci, and coliphage by filter media composition. **Table 3.6** (sand-only) has higher removal rates for *E. coli* and coliphage after Day 1 continuous spike. **Table 3.7** (amended) illustrates higher removal rates for enterococci for the initial event of continuous swine slurry plug influent. The results change after Day 2 clean flush. **Tables 3.6 and 3.7** further indicate the amended media to provide more effective, higher continuous removal of all three pathogen indicator microbes that were measured. A side by side comparison of soil type and microbe removal provides an inconclusive answer regarding which porous media composition is more effective in removal of microbes.

Table 3.6 – Flow-weighted average microbial removal from fifteen columns with sand-only composition in the filter media layer after day 1 (slurry) and day 2 (clean). [BG-S: Botanic Garden- Sand, AP: Airport, AR: Arkansas, CI: Confidence Interval]

Site	E. coli (% Day 1)	E. coli (% Day 2)	Enterococci (% Day 1)	Enterococci (% Day 2)	Coliphage (% Day 1)	Coliphage (% Day 2)
BG – S	80	69	76	70	57	51
AP	44	35	73	71	38	32
AR	77	60	65	50	96	94
Mean (%)	67	55	71	64	64	59
Standard Deviation	20	18	6	12	29	32
CI (90%)	[49, 85]	[39, 70]	[66, 77]	[54, 74]	[38, 89]	[31, 88]

Table 3.7 – Flow-weighted average microbial removal from fifteen columns with fly and sand composition in the filter media layer after day 1 (slurry) and day 2 (clean). [BG – FA: Botanic Garden – Fly-Ash, G – A: Grand Lake Association, G-HS: Grove High School, CI: Confidence Interval]

Site	E. coli (% Day 1)	E. coli (% Day 2)	Enterococci (% Day 1)	Enterococci (% Day 2)	Coliphage (% Day 1)	Coliphage (% Day 2)
BG – FA	99	99	99	99	93	92
G – A	63	60	84	83	12	7
G – HS	29	22	66	59	19	13
Mean (%)	64	60	83	81	41	37
Standard Deviation	35	39	17	20	45	47
CI (90%)	[33, 95]	[26, 95]	[68, 98]	[63,98]	[1, 81]	[0, 79]

Previous studies have shown that iron-oxide could remove microbes more than soils without the amendment. A similar result is observed in this study when comparing sites with and without the fly-ash amended in the porous media layer. Considering data from the initial slurry plug shows *E.coli* to have a mean removals of 67% (sand-only) and 64% (fly-ash amended), this result did not support previous studies where amended soils produced higher removals. Coliphage is similar with removals of 64% (sand-only Day 1) and 41% (fly-ash amended Day 1). However, when considering the clean flush wash out from Day 2, *E.coli* removals are 55% (sand-only) and 60% (fly-ash amended), thus supporting previous column experiments with IOCS. Coliphage follows in results showing 59% removal for sand-only sites during the second day and 37% removal for the fly-ash amended sites. Enterococci was one microbe that shows high mean removals during Day 1 and 2 experiments, 71% (sand-only, Day 1) to 83% (fly-ash amended, Day 1) and 64% (sand-only, Day 2) to 81% (fly-ash amended, Day 2).

The observations stated above are further quantified and validated through statistical tests. An ANOVA, Tukey multiple comparison analysis and the Kruskal-Wallis non-parametric test for each microbe was run using site and filter media type as variables, **Table 3.8 – 3.10** illustrate the results of the ANOVA. The results showed that there are significant differences in the mean from site to site for removal of *E.coli*, enterococci, and coliphage.

Table 3.8 – Statistical analysis for *E.coli* removal in column studies using intact, established bioretention cell cores by Tukey multiple comparison for two variables: Site and Media. [BG-FA: Botanic Garden-fly-ash, G-A: Grand Lake Association, BG-S: Botanic Garden-sand only, AP: Airport, G-HS: Grove High School, AR: Arkansas]

Factor	Site Name	Sample Number	Tukey Multiple Comparison	
			Mean (%)	Grouping
Site				
	BG-FA	8	100	A
	BG-S	10	75	B
	AR	4	65	B C
	G-A	10	62	B
	AP	10	39	C D
	G-HS	10	25	D
Media				
	Fly-Ash	28	60	A
	Sand	24	58	A

*Means that share the same letter are NOT significantly different (α < 0.05) for that variable

Table 3.9 – Statistical analysis for enterococci removal in column studies using intact, established bioretention cell cores by Tukey multiple comparison for two variables: Site and Media. [BG-FA: Botanic Garden-fly-ash, G-A: Grand Lake Association, BG-S: Botanic Garden-sand only, AP: Airport, G-HS: Grove High School, AR: Arkansas]

Factor	Site Name	Sample Number	Tukey Multiple Comparison	
			Mean (%)	Grouping
Site				
	BG-FA	8	99	A
	G-A	10	84	A B
	BG-S	10	74	B C
	AP	10	72	B C
	G-HS	10	63	B C
	AR	4	51	C
Media				
	Fly-Ash	28	81	A
	Sand	24	69	B

*Means that share the same letter are NOT significantly different (α < 0.05) for that variable

Table 3.10 – Statistical analysis for coliphage removal in column studies using intact, established bioretention cell cores by Tukey multiple comparison for two variables: Site and Media. [BG-FA: Botanic Garden-fly-ash, G-A: Grand Lake Association, BG-S: Botanic Garden-sand only, AP: Airport, G-HS: Grove High School, AR: Arkansas]

Factor	Site Name	Sample Number	Tukey Multiple Comparison	
			Mean (%)	Grouping
Site				
	AR	4	95	A
	BG-FA	4	93	A
	BG-S	10	53	B
	AP	4	35	B C
	G-HS	4	16	C D
	G-A	4	9	D
Media				
	Fly-Ash	28	88	A
	Sand	24	74	B

*Means that share the same letter are NOT significantly different ($\alpha < 0.05$) for that variable

Further, when considering all the data together enterococci and coliphage showed significant differences for media type based on the ANOVA and the non-parametric test at a p – value less than 0.05, **Tables 3.11 – 3.13**. The bioretention cell at the Botanic Garden in Stillwater, Oklahoma is one unit with half the cell composition as sand-only and the other half of its composition including a fly-ash amendment. Microbial removal data from this cell can be compared for the two media types, in-situ while keeping all other variables the same. This cell was built in 2008 and is therefore, stabilized. Data from *Figures 3.4 – 3.6* indicate the cores

Table 3.11 – Statistical analysis for *E.coli* removal in column studies using intact, established bioretention cell cores by Kruskal-Wallis non-parametric test for two variables: Site and Media. [BG-FA: Botanic Garden-fly-ash, G-A: Grand Lake Association, BG-S: Botanic Garden-sand only, AP: Airport, G-HS: Grove High School, AR: Arkansas]

Factor	Site Name	Median (%)	Site Name	Median (%)	p-value*
Site	BG-FA	100	BS-S	78	< 0.001
			AR	69	0.005
			G-A	62	< 0.001
			AP	28	< 0.001
			G-HS	24	< 0.001
	BG-S	78	AR	69	0.888
			G-A	62	0.005
			AP	28	0.006
			G-HS	24	< 0.001
	AR	69	G-A	62	0.888
			AP	28	0.066
			G-HS	24	0.011
	GA	62	AP	28	0.028
			G-HS	24	< 0.001
	AP		G-HS	24	0.257
Media	Fly-Ash	61	Sand	72	0.940

*Means are significantly different at $p<0.05$

Table 3.12 – Statistical analysis for enterococci removal in column studies using intact, established bioretention cell cores by Kruskal-Wallis non-parametric test for two variables: Site and Media. [BG-FA: Botanic Garden-fly-ash, G-A: Grand Lake Association, BG-S: Botanic Garden-sand only, AP: Airport, G-HS: Grove High School, AR: Arkansas]

Factor	Site Name	Median (%)	Site Name	Median (%)	p-value*
Site	BG-FA	100	BS-S	76	< 0.001
			AR	51	0.007
			G-A	82	< 0.001
			AP	72	< 0.001
			G-HS	56	< 0.001
	BG-S	76	AR	51	1.000
			G-A	82	0.004
			AP	72	0.650
			G-HS	56	0.473
	AR	51	G-A	82	0.258
			AP	72	0.572
			G-HS	56	0.358
	GA	82	AP	72	0.021
			G-HS	82	0.140
	AP	72	G-HS	56	0.307
Media	Fly-Ash	88	Sand	74	0.004

*Means are significantly different at p<0.05

Table 3.13 – Statistical analysis for coliphage removal in column studies using intact, established bioretention cell cores by Kruskal-Wallis non-parametric test for two variables: Site and Media. [BG-FA: Botanic Garden-fly-ash, G-A: Grand Lake Association, BG-S: Botanic Garden-sand only, AP: Airport, G-HS: Grove High School, AR: Arkansas]

Factor	Site Name	Median (%)	Site Name	Median (%)	p-value*
Site	BG-FA	93	BS-S	57	0.005
			AR	94	0.386
			G-A	11	0.021
			AP	34	0.021
			G-HS	17	0.021
	BG-S	57	AR	94	0.005
			G-A	11	0.005
			AP	34	0.066
			G-HS	17	0.005
	AR	94	G-A	11	0.021
			AP	34	0.021
			G-HS	17	0.021
	GA	11	AP	34	0.021
			G-HS	17	0.149
	AP	34	G-HS	17	0.021
Media	Fly-Ash	17	Sand	57	0.030

*Means are significantly different at p<0.05

from the fly-ash side of the cell to remove microbes, over both experiments (Day 1 and Day 2), on average 31%, 29%, 41% better than the sand-only for, *E. coli*, enterococci, and coliphage, respectively. These results are similar to previous columns experiments from Zhang *et al*. (2010) and Bradley *et al.* (2011) that show increased removal potential for amended-soils verses typical bioretention sand

media layer. In all columns, over all runs the Botanic Garden site yields better removal of all measured microbes for this study.

Statistical tests were performed based on the observations listed above. An ANOVA, Tukey one-way analysis and the Kruskal-Wallis non-parametric test for each microbe was run using site and filter media type as variables for the Botanic Garden sites, BG-S and BG-FA, **Table 3.8-3.13**. Statistically, site and media type were significantly different for these two bioretention cells.

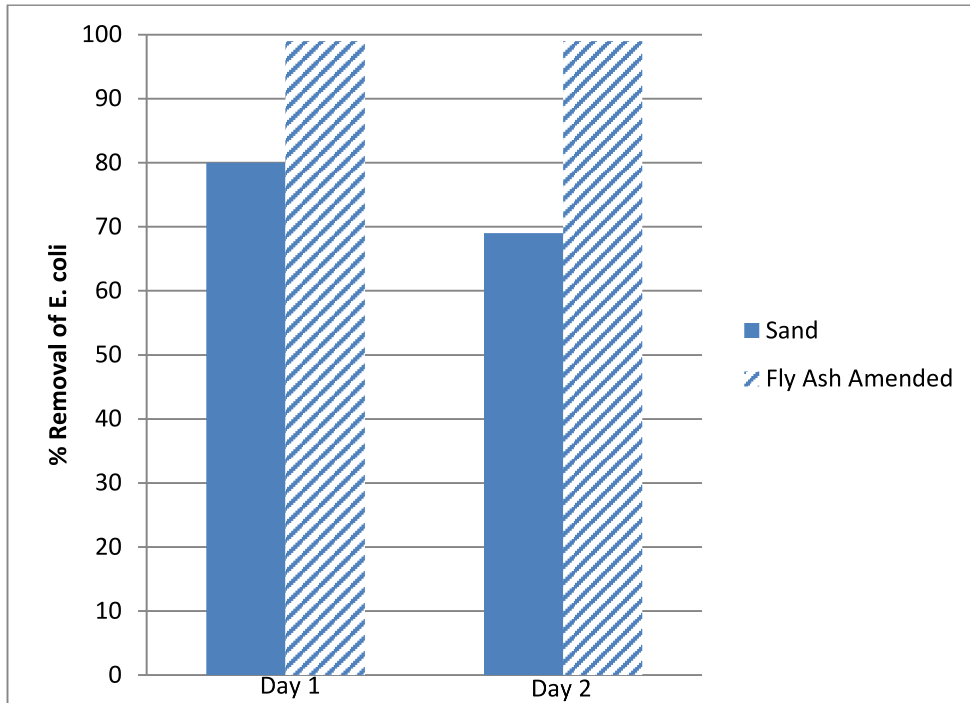

Figure 3.4 – E. coli Removal Rates for Botanic Garden – Stillwater, OK

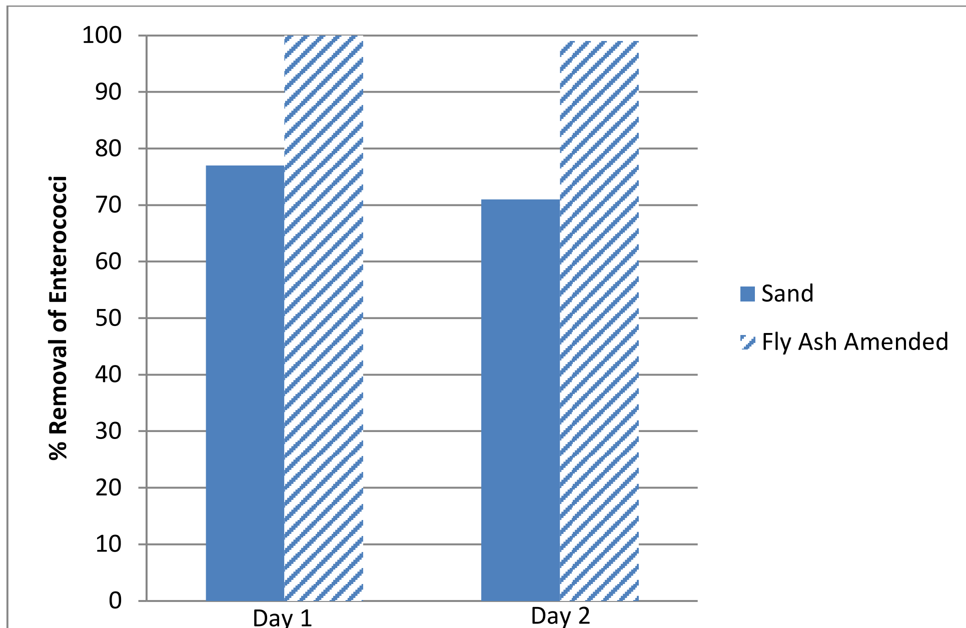

Figure 3.5 – Enterococci Removal Rates for Botanic Garden – Stillwater, OK

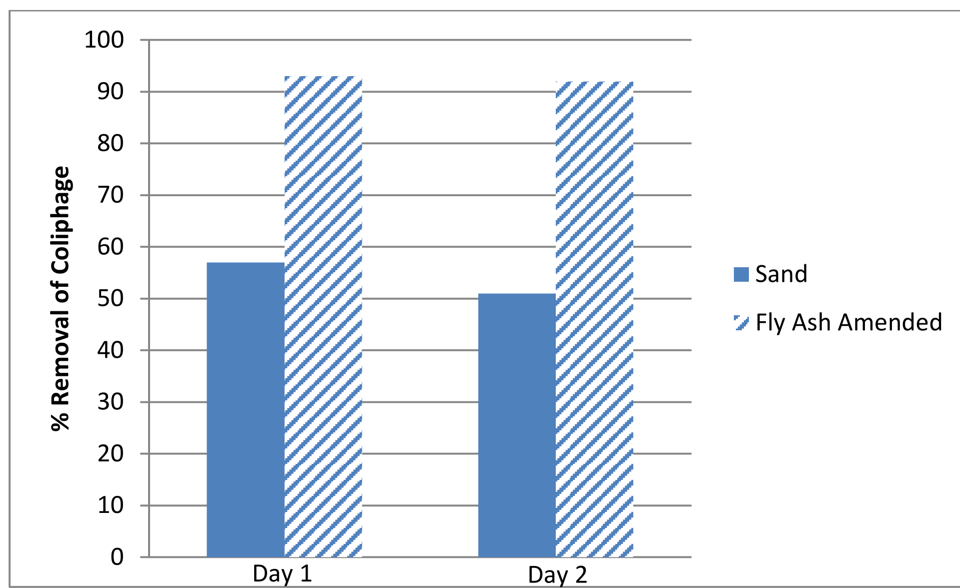

Figure 3.6 - Coliphage Removal Rates for Botanic Garden – Stillwater, OK

3.3.4.b Column Particle Size Distribution and Effect on Removal and Removal Efficiency Based on Microbe

A particle size distribution (PSD) for each column was performed using ASTM D422, both sieve and hydrometer methods. **Table 3.14** details the mean percent of course sand, medium sand, fine sand, and total sand, silt, and clay for each site. The individual PSD data for each column is located in Appendix B. Mean data show that each site contained a greater percentage of sand than silt or clay, which is expected considering that cores were extracted from the sand layer this data. It is more valuable to consider the percentages of course, medium, and fine with regard to removal (or trapping) efficiency of microbes than the percent of clay. The Botanic Garden in Stillwater, Oklahoma had the greatest amount of fine sand in its media composition with BG – S and BG – FA at 22% and 11% respectively. Previously stated, the Botanic Garden provided the greatest removals of all microbes in this laboratory column study, possibly due in part to the fine sand composition of the porous media layer. The site with the largest clay content was the airport in Fayetteville, Arkansas, some of the columns from this site failed to drain on the second day. The clay content may have been a contributor to the lack of drainage.

A best subsets regression model was performed on all 30 columns using *E.coli* removal, enterococci removal, and coliphage removal as the response and % course, % medium, % fine, % sand, % silt, % clay, flowrate and media type as variables. The regression showed that at a $p < 0.05$, *E.coli* removal was

significantly affected by % medium sand, % fine sand, and media type. When considering enterococci removal the significant variables were media type and % medium sand, and % clay. Finally, media type, flowrate, % clay, and % medium sand were the variables that were significant for coliphage removal. A similar regression analysis was performed for only the ten columns from the Botanic Garden in Stillwater, Oklahoma. Media type was significant ($p < 0.05$) for all microbes in this regression. Flowrate was not a significant factor for any microbe in these ten columns though particle size had varied affects. Specifically, coliphage was affected by % silt and media type, while *E.coli* and enterococci where only affected significantly by media type. Regression equations, **Table 3.15**, and residual plots are located in Appendix F.

Table 3.14 – Mean particle size distribution for each site (BG – S, BG – FA, G – A, G – HS, AP, and AR) used in the laboratory column experiments.

Site	% Course Sand	% Medium Sand	% Fine Sand	% Sand	% Silt	% Clay
BG - S	5	69	22	96	3	1
BG - FA	19	54	11	95	4	2
G - A	42	45	5	92	5	3
G - HS	23	51	8	89	8	3
AP	12	53	9	74	19	7
AR	14	53	11	78	17	5

Table 3.15 – Multiple regression relationships for microbial removal from bioretention media column experiments. [All: All columns used (30 columns), BG: Only Botanic Garden columns used (10 columns)]

Pathogen	Sample	Regression Equation*	R^2	Root Mean Square Error
E.coli (MPN)	All	*Ecoli* = -0.540 + 0.1898 Media Type + 0.01273 % Medium Sand + 0.01154 % Fine Sand	0.56	0.20
	BG Only	0.4920 + 0.2540 Media Type	0.77	0.07
Enterococci (MPN)	All	-0.477 + 0.2934 Media Type + 0.01256 % Medium Sand + 0.0285 % Clay	0.29	0.18
	BG Only	0.4770 + 0.2590 Media Type	0.86	0.06
Coliphage (PFU)	All	-1.146 + 0.236 Media Type + 0.02168 % Medium Sand + 0.0738 % Clay + 0.00416 Flowrate	0.65	0.20
	BG Only	0.2808 + 0.3589 Media Type – 0.03589 % Silt	0.85	0.10

*Media type: 1=sand, 2=fly-ash

Table 3.16 lists the microbe that had the greatest and least removal from each site. In four of the six column experiments enterococci had the greatest removal, this is likely due its size and characteristics. In five of six column experiments coliphage has the least removal. Column AR is the only outlier. It is reasonable to consider this experiment an anomaly because these columns did not drain on the second day and thus the data are incomplete.

Table 3.16 – Greatest and least removal by site (BG – S, BG – FA, G – A, G – HS, AP, and AR) per laboratory column experiment.

Site	Greatest % Removal	Least % Removal
BG - S	E. coli	Coliphage
BG - FA	Enterococci	Coliphage
G - A	Enterococci	Coliphage
G - HS	Enterococci	Coliphage
AP	Enterococci	Coliphage
AR	Coliphage	Enterococci

3.4 Conclusion and Future Work

This study successfully quantified microbial removals of *E.coli*, enterococci, and coliphage by intact columns from established bioretention cells in Oklahoma and Arkansas. Total removals ranged from 35% to 69% for *E.coli,* 50% to 70%, enterococci, and 32% to 94%, coliphage for sand-only filter media columns. Fly-ash amended columns ranged from 22% to 99%, *E.coli,* 59% to 99%, enterococci, and 7% to 92%, coliphage removals after the second day wash-out occurred. When considering all sites and all data enterococci removal was significantly different ($\alpha < 0.05$) for site and filter media type. *E.coli* and coliphage were significantly different ($\alpha < 0.05$) for site but not for media type and day had no significance at all for any of the microbes measured.

This study also compared microbial removal rates and discussed possible reasons specific microbes' attained better removal than others. The results support

the expectation that bioretention is a viable BMP for urban stormwater runoff. Furthermore, the results suggest that fly-ash amended soils can assist in microbial removal in addition to its ability to increase phosphorous removal. This observation was validated with the bioretention cell in Stillwater, Oklahoma. Statistical analyses (ANOVA with $\alpha < 0.05$) showed that site and filter media type were significantly different for removal efficiencies of all three microbes. The PSD provides valuable insight to sand selection: although a fine sand could cause pores to clog over time, the sites with the greater percentages of fine sand compared to medium or coarse grain sand yielded more removal, i.e. greater trapping of microbes.

A regression analysis of all columns in this study showed that media type, % medium sand, and % find sand were the variables that affected *E.coli* removal. Enterococci removal regression equation was made up of media type, % medium sand, and % clay, while coliphage removal variables were % medium sand, % clay, and flowrate. When considering only the Botanic Garden in Stillwater, Oklahoma flowrate was no longer significant for any microbe and media type became significant for all microbes. The media type showed the largest affect for *E.coli* and enterococci removal but % silt had some significance on coliphage removal for this bioretention cell. All regressions were for a $p < 0.05$.

A complement to this study would include additional experiments with intact, established soil cores with other soil amendments in the porous media layer of the bioretention cell. An exhaustive analysis of typical amendments used in removal of other urban runoff pollutants would be beneficial. More research experiments could identify an amendment that would optimize numerous pollutants. Also, these experiments show that particle size has some impact on removal although the effect is varied for different microbes. Overall, this study provided important data for industry on quantification of microbial removal rates based on a worst-case simulated rainfall event followed by an additional clean rainfall event. These data will assist in design and construction of bioretention cells that are targeting microbial removal used in urban environments.

References:

ASTM D422. (2009). Standard Test Method for Particle Size Analysis of Soils.

Bradley, I., Straub, A., Maraccini, P., Markazi, S., and Nguyen, T. (2011). "Iron oxide amended biosand filters for virus removal." *Water Research,* 45, 4501-4510.

Chu, Y., Jin, Y., Flury, M. and Yates, M.V. (2001). "Mechanisms of virus removal during transport in unsaturated porous media." *Water Resources Research*, 37(2), 253-263.

Coffman, L. and Ruby, M. (2008). "Bacterra by Filterra Advanced Bioretention System: Discussion of the Benefits, Mechanisms, and Efficiencies for Bacteria Removal." *Low Impact Development for Urban Ecosystem and Habitat Protection*. pp. 1-13. doi: 10.1061/41009(333)93.

Davis, A. P. (2007). "Field performance of bioretention: Water quality." *Environmental Engineering Science*, 24(8), 1048-1064.

Davis, A., Hunt, W., Traver, R., and Clar, M. (2009). "Bioretention Technology: Overview of Current Practice and Future Needs." *J. Environ. Eng.*, 135(3), 109–117.

Gavlak,R., Horneck, D., Miller, R.O., and Kotuby-Amacher, J. (2003). "Soil, Plant and Water Reference Methods for the Western Region", 2nd ed. WCC-103 Publication, Colorado State University, Ft. Collins, Colorado, USA, 56-57 and 62-63.

HACH. (2013). 2100Q and 2100Qis Basic User Manual. 2nd Edition.

Hunt, W.F. and Lord, W.G. (2006). "Urban Waterways: Bioretention Performance, Design, Construction and Maintenance." Retrieved from http://www.bae.ncsu.edu/stormwater/PublicationFiles/Bioretention2006.pdf.

Hunt, W.F., Smith, J.T., Jadlocki, S.J., Hathaway, J.M., and Eubanks, P.R. (2008). "Pollutant removal and peak flow mitigation by a bioretention cell in urban Charlotte, NC." *Journal of Environmental Engineering*, 134, 403-408.

IDEXX. (2014). IDEXX Enterolert Test Method for the Detection of Enterococci in Water.

IDEXX. (2013). IDEXX Colilert Test Method for the Detection of Total Coliforms and *E.coli* in Water.

Klein, R. (1979). "Urbanization and Stream Quality Impairment." *Water Resources Bulletin*, 15(4), 948-963.

Lehner, P., Aponte Clark, G.P., Cameron, D.M., and Frank, A.G. (June 1999). "Stormwater Strategies: Community Responses to Runoff Pollution." Natural Resources Defense Council. Retrieved August 2015 from http://www.nrdc.org/water/pollution/storm/stoinx.asp.

Method 4500-PF. (2005). Standard Methods for the Examination of Water and Wastewater. 21th Edition, 4-155 - 4-157.

Park, S.J., Lee, C.G., Kim, S., Chang, Y., and Yang, J. (2012). "Bacterial removal in flow-through columns packed with iron-manganese bimetallic oxide-coated sand." *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering*, 47(10), 1364-1371. DOI:10.1080/10934529.2012.672302.

Quilliam, R., Jamieson, J., and Oliver, D. (2014). "Seaweeds and plastic debris can influence the survival of faecal indicator organisms in beach environments." *Marine Pollution Bulletin*, 84, 201-207.

Roy-Poirier, A., Champagne, P., and Filion, Y. (2010). "Bioretention processes for phosphorus pollution control." *Environmental Rev*, 18, 159-173.

Rusciano, G.M., and Obropta, C.C. (2007). "Bioretention Column Study: Fecal Coliform and Total Suspended Solids Reductions." *American Society of Agricultural and Biological Engineers*, 50(4).

SevenMulti™. (2012). pH and EC meter Operating Instructions.

Torkzaban, S., Hassanizadeh, S.M., Schijven, J.F., de Bruin, H.A.M., and de Roda Husman, A.M. (2006). "Virus Transport in Saturated and Unsaturated Sand Columns." *Vadose Zone Journal*, 5, 877-885.

USEPA. (2000). Low Impact Development. Retrieved from http://www.lowimpactdevelopment.org/pubs/LID_litreview.pdf.

USEPA. (2012). "Recreational Water Quality Criteria." Office of Water Quality, 820-F-12-058.

USEPA. (2015). "Water: best management practices: bioretention*."* Retrieved May 25 2015 from http://water.epa.gov/polwaste/swbmp/Bioretention-Rain-Gardens.cfm.

Yakirevick, A., Pachepsky, Y., Guber, A.K., Gish, T.J., Shelton, D.R., and Cho, K.H. (2013). "Modeling transport of Escherichia coli in a creek during and after artificial high flow events: three year study and analysis." *Water Resources*, 47: 2676-2688.

Zhang, L., Seagren, E., Davis, A., and Karns, J. (2010). "The capture and destruction of Escherichia coli from simulated urban runoff using conventional bioretention media and iron oxide-coated sand." *Water Environment Research,* 82(8), 701-713.

Zhang, W., Brown, G.O., Storm, D.E., and Zhang, H. (2008). "Fly ash amended sand as filter media in bioretention cells to improve phosphorous removal." *Water Environ Res.*, 80(6), 507-516.

Chapter 4: Microbial removal by bioretention cells with fly-ash amendment in Oklahoma, United States.

Abstract

Stormwater in urban areas is a leading cause of microbial water quality impairment in the United States. This issue is addressed through the use of best management practices (BMPs) and target limits for pathogenic indicator species. Bioretention is a commonly used low impact development strategy that addresses this growing pollution problem at the source in a microscale setting. Bioretention removal efficiencies are well studied when considering nutrients and heavy metals, but data are limited in field scale studies for microbial indicators. Three bioretention cells in Grove, Oklahoma were monitored over one and a half years and the removal microbial efficiency was quantified. The maximum and minimum removal over all sites and all storm events were, *E.coli* [100, -3690], enterococci [100, -227], and coliphage [100, -94], noting the negative is indicative of an increase in concentration, not a removal. Further, previous laboratory column studies have shown potential in increasing removal capacity of filter media when amended with iron-oxides (Zhang *et al.*, 2010 and Bradley *et al.*, 2011). The cells in Grove, Oklahoma all contain filter media amended with fly-ash, a coal waste product. This study further compares removals from field scale bioretention cells with and without the fly-ash amended. Based on a limited data

set, fly-ash amended bioretention cells perform 49% better than those with sand-only filter media layer.

4.1 Introduction

Non-point source pollution from rainfall runoff is a growing concern in urban environments. Urbanization leads to increased impervious surfaces (roof tops, driveways, parking lots, and streets) which leads to an increase in pollutant transport, including pathogens, to receiving water bodies (Schoonover and Lockacy, 2006, Line *et al.*, 2008). A study by Schoonover and Lockacy (2006), based on 18 watersheds in Georgia, showed that watersheds with 24% or more impervious area released more fecal coliform when compared to watersheds with 5% or less impervious area. Humans, pets, and wildlife are the most typical sources of pathogenic pollution in urban stormwater runoff but sediment resuspension from stormwater drains can also serve as a potential source (Lehner *et al*., 1999, Yakirevick *et al.*, 2013, and Quilliam *et al.*, 2014). Irrespective of the source, pathogens in stormwater runoff are potentially harmful to humans and can degrade water quality of receiving waters. In fact, Lehner (1999) noted that urban stormwater runoff has impaired 13% of all rivers, 18% of all lakes, and 32% of all estuaries. Urban runoff contains both microbial and non-microbial sources of pollution that when left untreated can negatively affect drinking and recreational waters.

In 1987 the Clean Water Act (CWA) was amended to address the Pollutant Discharge Elimination System (NPDES) permit program and further mitigate non-point source pollution in surface waters. The 303(d) list provided a way of identifying impaired water bodies in need of management measures using total maximum daily loads (TMDLs). Within each TMDL, best management practices (BMPs) are utilized to comply with water-quality standards and reach the intent of the TMDL. Low impact development (LID) strategies include the BMPs bioretention, rain gardens, and swales. They are becoming more commonly used as stormwater control measures in urban settings. The concept of LID practices is to mimic predevelopment hydrology in post development conditions and address stormwater runoff quantity and quality at the initial source (Lehner *et al*., 1999, USEPA, 2000, USEPA, 2015).

Bioretention provides multiple benefits including pollutant removal, flood reduction, aesthetic value, and animal habitat (USEPA, 2015). Bioretention studies both in field and laboratory settings are well documented for the removal of pollutants such as, sediment, nutrients, and heavy metals. Previous studies have reported removal efficiencies for bioretention ranging from 54% to 90% for total suspended solids (TSS), 22% to 85% for phosphorous, 55% to 80% for nitrogen (TKN – total Kjeldahl nitrogen), and 56% to 99% for heavy metals (USEPA, 1999, Davis *et al*., 2001, Davis *et al.*, 2006, Hunt *et al.*, 2006, and LID INC, 2015). The literature currently has minimal documentation regarding removal and

destruction of microbial pollutants, or pathogens (Davis *et al.,* 2009, USEPA, 2015, Roy-Poirier *et al.*, 2010).

Studies by Jin *et al.* (2000), Hathaway *et al.* (2009), Zhang *et al.* (2010), and Park *et al.* (2012) have shown that microbes are removed from water passing through porous media, through a variety of processes including filtration, desiccation, thermal deactivation, and sorption, but the removal amount varies greatly. Microbial fate in filter media involves a number of factors and mechanisms including temperature, solution chemistry, soil moisture, filtration, adsorption, surface and media characteristics and flowrate. Transport of microbes in filter media have been shown to be impacted by factors including soil moisture, adsorption, filtration, and flowrate (Hathaway *et al.*, 2009, Zhang *et al.*, 2010, Park *et al.*, 2012). Soil temperature, soil moisture, pH, sunlight, desiccation, and predation from indigenous microbial flora all have an effect on microbial survival in soils (Potts, 1994, Garbrecht *et al.*, 2009, Park *et al.*, 2012).

4.1.1 Research Objective

The primary purpose of this research is to quantify microbial removal by installed bioretention cells with fly-ash amended media in Oklahoma. There are numerous field scale experiments for other pollution parameters but Hunt *et al.* (2008) stated there was no field scale data reported regarding bioretention removal ability in reference to microbial indicators. It is further the intent of this

research to show the impact of filter media on microbial removal. Recent studies by Zhang *et al.* (2010) and Bradley *et al.* (2011) have shown that bioretention could attain higher microbial removal efficiencies with soils amended with iron-oxide. The installed bioretention cells utilized in this study are amended with fly-ash. Fly-ash is a waste by product of burning coal. Fly-ash amended soils could deliver similar results as those brought on by the iron-oxide amended soils. This study will compare its measured results with published results from other field studies that do not utilize fly-ash in the filter media.

4.2 Methodology

4.2.1 Site Description

A field test using three bioretention cells in Grove, Oklahoma was conducted to determine microbial removal efficiency. These sites were selected because they all were designed and build with an underdrain, which provides this study with the ability to determine removal percentages based on an inlet and outlet (underdrain) for each storm event. Sites were selected and used in this study are Elm Creek Plaza (site 1), Grand Lake Association (site 2), and Grove High School (site 3). All three cells were designed by the Department of Biosystems and Agricultural Engineering at OSU and built in 2007. **Table 4.1** lists the sites selected for this study along with their size, drainage area, and land use. All sites

are comprised of sieved, washed local creek sand with 5% fly-ash filter media and a filter media depth of 0.85m to 1 m. Fly-ash was collected from the Sooner Power Plant in Red Rock, Oklahoma and its composition is listed in **Table 4.2** (Zhang *et al.*, 2008). Sampling began during April, 2014 and continued through October, 2015.

Table 4.1 –Site description, characteristics, and location of three bioretention cells used in the field study from Grove, OK

Site	Area (m^2)	Volume (m^3)	Drainage Area (m^2)	Latitude and Longitude	Landcover
Elm Creek Plaza (ECP)	63	128	0.62	36.579643 -94.768417	Paved
Grand Lake Association (GLA)	172	435	1.9	36.610923 -94.8033817	Paved/Turf
Grove High School (GHS)	149	161	0.65	36.5779781 -94.7555676	Paved

Table 4.2 –Composition of fly-ash amendment in filter media layer of bioretention cells in Grove, Oklahoma.

Composition	Content (%)
SiO_2	38.1
Al_2O_3	18.4
Fe_2O_3	5.93
MnO	0.02
MgO	5.43
CaO	22.9
Na_2O	1.82
K_2O	0.56
Ti_2O	1.39
P_2O_5	1.37
BaO	0.69
Cr_2O_3	0.01
SrO	0.30
Loss on ignition	0.69
Total	97.6

4.2.2 Sampling Methods

Three samplers per site were installed to gather data for the three bioretention sites in Grove, Oklahoma. The influent, effluent, and overflow were sampled by refrigerated ISCO - Avalanche automatic samplers (ISCO, INC, Lincoln, NE). Flow-weighted composite sampling was utilized at each sampling location. The samplers at the inlet, underdrain, and overflow each stored 14-bottle kits that were acid washed prior to being installed and used for sample collection. The automatic samplers were programmed to a storage temperature of less than 4°C. The samplers are used in conjunction with ISCO 720 flow modules that measure water depth. The flow module converts the flow incorporating the flume

specifics for the site, shown in **Table 4.3**. The Solinst level logger was utilized to measure water depth in each cell near the outlet and inlet. A calibrated ISCO 674 rain gauge was also connected to each installed automatic sampler at each bioretention cell to record rainfall. Samplers were set up in the spring of 2014 for all locations, sampling began in May, 2014 and continued through October 2015.

Table 4.3 – Inflow, outflow, and overflow flume characteristics for three bioretention cells in Grove, OK.

Site	Flume Characteristics		
	Inflow	Outflow	Overflow
Elm Creek Plaza (ECP)	0.3 m H flume	Palmer Bowlus flumes	Rectangular Concrete Weir
Grand Lake Association (GLA)	0.46 m H flume	Palmer Bowlus flumes	Rectangular Concrete Weir
Grove High School (GHS)	0.46 m H flume	Palmer Bowlus flumes	Rectangular Concrete Weir

Samples were collected within 24 hours after each rain event and processed in the laboratory at Oklahoma State University. Subsamples were either analyzed onsite or shipped overnight on site to remote laboratories for further analysis. Finally, samples were distributed to analysis locations.

4.2.3 Laboratory Analysis

Flow-weighted composite samples were analyzed for nutrients, pH, electric conductivity (EC), total suspended solids (TSS), turbidity, *E. coli*, enterococci, and coliphage to determine the event mean concentrations (EMC) for each storm event. The depth during each storm event was also measured and converted to a flowrate. The Mettler Toledo SevenMulti meter was used to measure the pH and EC of each water sample (SevenMulti™, 2012). ASTM D3977 – 97, Method B was used to measure TSS for all samples. Turbidity was measured using a Hach 2100Q Portable Turbidimeter (HACH, 2013).

All microbial analysis was completed by Dr. Dale Griffin from the USGS Microbiology Laboratory in St. Petersburg, Florida. Samples were shipped overnight to the Florida lab. *E.coli* and enterococci analyses were completed using the Colilert and Enterolert Quanti Tray 2000 Method from IDEXX Systems (IDEXX,2014, IDEXX, 2013). Coliphage overlays used two milliliters volumes by three replicated for all samples tested. All plates and quantitrays were incubated overnight at their respective temperatures and samples were stored overnight by refrigeration.

4.2.4 Statistical Analysis

In depth examination of the data begins with the statistical analysis of the percent removal for each microbe. Mean, standard deviation, and range for all

data categories at each sampling location for each site based on storm event were calculated. These data are provided in the tables and figures in the following sections. The mean is calculated at both the inlet and underdrain for each site. The underdrain microbial concentrations were compared to the USEPA recreational fresh water contact recommendations, 126 CFU/100 ml for *E.coli* and 35 CFU/100 ml for enterococci (USEPA, 2012). Microbial data are analyzed in two different ways, one by concentration change and the other by removal or trapping efficiency. Both criteria are calculated in this study.

A change of influent and effluent concentrations of individual microbes from each storm event is also calculated. Equation 1 is utilized for all microbial indicators and coliphage concentrations. The change in concentration for each microbe was calculated for each sampled storm event between May, 2014 and October, 2015. This equation represents the percent change in concentration for one storm event (at a given site. The percent concentration reduction for each microbe after storm event 1 (%ΔC **)**, is calculated using,

where,

$$\%\Delta C = \left(1 - \frac{O_{conc}}{I_{conc}}\right) * 100 \qquad (1),$$

O_{conc} is the outlet concentration of the microbe from the underdrain, and I_{conc} is the inlet concentration of the microbe during the storm from the inlet.

Concentrations of microbes are measured in MPN/100 ml for *E.coli* and enterococci and PFU/100 ml for coliphage. The overall mean percent change in concentration for microbe, $\%\Delta C_T$, is given in Equation 2, the summation of concentration for each microbial indicator and coliphage over, n, the number of sampled storms for each site.

$$\%\Delta C_T = (1 - \frac{\sum_i^n O_{conc}}{\sum_i^n I_{conc}}) * 100 \qquad (2)$$

A microbial count balance approach is used to compare the initial count in the influent to the final count of each microbe in the effluent from the underdrain in each bioretention cell. Microbe are measured as colony forming unit (CFU), most probable number (MPN), or plaque forming unit (PFU). This equation is utilized for all microbial indicators and coliphage. The percent removal for each microbe was calculated for each storm event sampled that had paired samples for the inlet and underdrain. This equation represents the percent removal for one storm event (at a given site. The percent removal for each microbe after storm event ($\%R_1$**)**, is calculated using Equation 3,

where,

$$\%R_1 = (1 - \frac{C_o}{C_i}) * 100 \qquad (3),$$

where, C_o is the count of each microbe in the outlet, underdrain and C_i is the count of microbes from the inlet. The count of microbes is measured in MPN or PFU depending on the specific type.

The mean $\%R_T$ for each site is calculated using Equation 4. The number of storm events varied for each cell, Elm Creek plaza (n = 23), Lake Association (n=14), and Grove High School (n=16).

$$\%R_T = (1 - \frac{\sum C_o}{\sum C_i}) * 100 \quad (4),$$

Statistical tests and correlations were performed based for this field study. An ANOVA, Tukey one-way analysis and the Kruskal-Wallis non-parametric test for each microbe was run using site, influent, and effluent as variables. A multiple comparison by microbe type was also run using the Kruskal-Wallis non-parametric test.

4.3 Results and Discussion

4.3.1 Basic Parameters

Between May, 2014 and October, 2015 storms events were monitored for the bioretention cells in Grove, Oklahoma. Elm Creek Plaza (ECP) had a total of 23 storm events capture with 20 events with paired data from the inlet and outlet.

Twelve of the fourteen captured storm events were paired for the Grand Lake Association (GLA) cell and the high school (GHS) cell had six of the sixteen storm events with paired data. The mean rainfall was 26.4 mm, 33.0 mm, and 22.8 mm for ECP, GLA, and GHS respectively. The rainfall ranged from 0 cm to 97.2 mm. GLA and GHS each had one overflow event during the sampling period. The raw data depicting the overflow is included in Appendix C. Flow reduction, pH, EC, TSS, and turbidity were measured at each event and the results summarized in **Table 4.4**. One notable datum is the negative flow reduction values for GLA illustrating the flow increased at the outflow underdrain. This is due to an increase in the groundwater table, GLA is very close in proximity to Grand Lake. Furthermore, it is important to understand the relevance of the percent storm sampled at the inlet and underdrain. In most cases greater than 70% of the storm was captured in both locations, however there are some events that the sampler did not function correctly, mechanical failure or battery power failure. Also, the sampler can only capture based on the way it is programmed and in some cases samplers missed part of the event, shut off too early, started too late.

Table 4.4 Summary of basic water-quality measurements for storm events from May, 2014 to October, 2015 for three bioretention cells in Grove, Oklahoma.

Site	Elm Creek Plaza (ECP)			Grand Lake Association (GLA)			Grove High School (GHS)		
	Mean	Standard Deviation	Range [high, low]	Mean	Standard Deviation	Range [high, low]	Mean	Standard Deviation	Range [high, low]
Flow Reduction (%)	73	12	[91, 47]	-1,200	3329	[80, -12,639]	8	86	[69, -220]
Storm Sampled (% Inlet)	94	6	[100, 91]	110	34	[172, 82]	96	4	[100, 84]
Storm Sampled (%Underdrain)	84	21	[100, 40]	84	22	[100, 54]	85	16	[98, 51]
pH (Inlet)	6.8	0.8	[3.7, 7.6]	7.1	0.3	[7.4, 6.2]	6.8	0.7	[8.5, 5.5]
pH (Underdrain)	7.7	0.2	[7.1, 7.9]	7.9	0.2	[8.3, 7.5]	7.6	0.2	[7.8, 7.3]
Electric Conductivity (EC) Inlet (µmhos/cm)	74	26	[159, 43]	95	24	[146, 67]	160	238	[805, 37]
Electric Conductivity (EC) Underdrain (µmhos/cm)	210	37	[305, 148]	330	87	[393, 61.6]	175	29	[240, 138]
Total Suspended Solids (TSS) Inlet (mg/L)	117	73	[251, 23]	84	105	[337, 12]	78	74	[258, 0]
Total Suspended Solids (TSS) Underdrain (mg/L)	44	27	[87, 0]	27	28	[80, 0]	37	32	[90, 0]
Turbidity Inlet (NTU)	67	52	150, 0]	9	4	[15, 3]	17	16	[46, 0]
Turbidity	7	5	[14, 0]	4	3	[9, 1]	3	2	[5, 0]

The data shows general trends reflecting a mean increase in pH and EC and a reduction for TSS and turbidity from inlet to underdrain.

4.3.2 Microbial Concentrations and Removal

Table 4.5, which includes all collected data, both paired and unpaired, shows the mean, standard deviation (s.d.), the range (maximum, minimum) for each of the microbial indicators measured in this study, *E.coli*, enterococci, and coliphage. The mean *E.coli* input concentration at GLA was substantially larger (4859 MPN/100 ml) when compared to either ECP (1591 MPN/100 ml) or GHS (1791 MPN/100 ml). This trend is also shown for enterococci, one possible explanation is that GLA is three times the watershed drainage area size of the other two sites and contains grassed areas versus only paved areas. A higher density of microbial pollution sources may be contained within the watershed. However, most of the inlet values are high and the standard deviations and broad ranges illustrate high variability within this data set.

The mean *E.coli* removal efficiency is 87% for ECP, 35% for GLA, and 43% for GHS, the standard deviations for GLA and GHS are very high suggesting high variability in individual removals. Conversely, the standard deviation is relatively small for ECP. ECP and GLA showed a reduction in concentration from inlet to outlet but GHS showed an increase (-8% change). Even with this apparent increase in concentration at the underdrain for GHS all three bioretention cells

met USEPA criterion for *E.coli* for recreation water (126 CFU/ 100 ml) five times, 22%, 36%, and 31% of storms for ECP, GLA, and GHS correspondingly (USEPA, 2012). For enterococci, GHS has the highest removal efficiency at 97%, GLA was measured at 95% and ECP showed 80% removal ability. The standard deviations were relatively low (47, 24, and 25 respectively). The change in concentration was favorable for GLA (98%, 4 s.d.) and GHS (78%, 93 s.d.). ELP measured a 33% (80 s.d.) decrease in enterococci concentration over the duration of the sampling period. The USEPA recreation water criterion for enterococci is 35 CFU/100ml, this limit was met only once for ECP and GLA and twice for GHS, equivalent to 4%, 7%, and 13% respectively (USEPA, 2012). Coliphage concentrations were reduced from the inlet to the underdrain outlet by 38% for ECP, 75% for GLA, and 32% for GHS, illustrating bioretention is viable to inhibit the mobility of viruses. Furthermore, removal rates of coliphage for the three cells were 78%, 81%, and 46% respectively.

The paired storm event data, shown in **Table 4.6** creates a complete assessment of each storm measured, by analyzing data from the inlet and outlet and calculating statistical measurements thereafter. The mean concentration change (or reduction) increased or maintained when considering paired events for all microbial indicators. The mean removal efficiency increased for all microbial indicators. Also, the percentage of each site to meet the USEPA recreational water *E.coli* criterion was increased 30%, 42%, and 33% for ECP, GLA, and GHS

Table 4.5 Statistics of inlet and underdrain microbial concentrations from sampled storm events from May, 2014 to October, 2015 for the three monitored bioretention cells in Grove, Oklahoma.

Site	Elm Creek Plaza (ECP)			Grand Lake Association (GLA)			Grove High School (GHS)		
	Mean	Standard Deviation	Range [high, low]	Mean	Standard Deviation	Range [high, low]	Mean	Standard Deviation	Range [high, low]
E.coli Inlet (MPN/100 ml)	1,600	1,940	[6,900, 10]	4,900	7,700	[26,000, 104]	1,800	4,700	[18,000, <DL]
E.coli Underdrain (MPN/100 ml)	810	1,200	[3,700, <DL]	310	380	[1,300, <DL]	2,000	3,000	[9,200, 104]
Underdrain Met *E.coli* Recreation Limit (126/100 ml)	5/23			5/14			5/16		
Enterococci Inlet (MPN/100 ml)	3,130	4,200	[20,000, 67]	15,000	10,000	[24,000, 52]	3,400	6,300	[1,400, 40]
Enterococci Underdrain (MPN/100 ml)	2,100	3,600	[16,000, <DL]	350	440	[1,300, < 40]	800	1,700	[5,800, 20]
Underdrain Met Enterococci Recreation Limit (35/100 ml)	1/23			1/14			2/16		
Coliphage Inlet (PFU/100 ml)	14	22	[67, <DL]	7	11	[33, <DL]	5	10	[17, 0]
Coliphage Underdrain (PFU/100 ml)	9	23	[100, <DL]	2	5	[17, <DL]	4	10	[<DL]

DL = Detection Limit

Table 4.6 Microbial analysis from paired storm events from the inlet and underdrain of three bioretention cells in Grove, Oklahoma from May, 2014 to October, 2015.

Site	**Elm Creek Plaza (ECP) n=20**	**Grand Lake Association (GLA) n=12**	**Grove High School (GHS) n=6**
E.coli Change in Concentration inlet to underdrain (%)	51	94	22
E.coli Mass Removal inlet to underdrain (%)	91	39	58
Did not meet *E.coli* limit on underdrain sample	**14/20**	**7/12**	**4/6**
Enterococci Change in Concentration inlet to underdrain (%)	30	98	-9
Enterococci Mass Removal inlet to underdrain (%)	81	95	20
Did not meet Enterococci limit on underdrain sample	**19/20**	**11/12**	**5/6**
Coliphage Change in Concentration inlet to underdrain (%)	25	75	100
Coliphage Mass Removal inlet to underdrain (%)	78	81	100

accordingly, a 5% mean increase over all sites. A similar observation is seen regarding USEPA recreational water enterococci criterion. Paired event data met enterococci criterion 5% (ECP), 8% (GLA), and 16% (GHS). A two-way ANOVA was run for the three bioretention cells in Grove, Oklahoma using microbe as the response variable. Type, inlet and underdrain and site were used as the factors, **Table 4.7**. Enterococcus was the only microbe that was significant for this comparison, shown in *Figure 4.1*. Furthermore, paired t-tests and Mann-Whitney statistical comparisons were run for each microbe to determine if there is a statistical difference between the inflow (inlet) and outflow (underdrain) concentrations for the three bioretention cells in Grove, Oklahoma, **Table 4.8**. The paired t-test showed *E.coli* enterococci to be significantly different between the inlet and the underdrain. Coliphage was not significant. Similarly, the non-parametric, Mann-Whitney test was run for the three sites and three microbes provided the same results, inlet and outlet concentrations were significantly different for *E.coli* and enterococci but not coliphage.

Table 4.7 Two-way ANOVA results for three bioretention cells in Grove, Oklahoma using three microbes, enterococci, *E.coli*, and coliphage as response variables and type (inlet, underdrain) and site (ECP: Elm Creek Plaza, GLA: Grand Lake Association, GHS: Grove High School) as factors.

Response Variable	Factor		p-value	Mean	Tukey's Multiple Comparison
Enterococci (MPN)	Type	Inlet	<0.001	6700	A
		Underdrain		1200	B
	Site	1	<0.001	8200	A
		3		2500	B
		2		1200	B
	Media Type*Site Interaction		<0.001	N/A	N/A
E coli (MPN)	Type	Inlet	<0.001	3600	N/A
		Underdrain		1400	N/A
	Site	2	0.199	3400	N/A
		1		2800	N/A
		3		1300	N/A
Coliphage (PFU)	Type	Inlet	0.495	7	N/A
		Underdrain		4	N/A
	Site	3	0.199	10	N/A
		1		4	N/A
		2		1	N/A

*Means with the same letter are NOT significantly different ($\alpha < 0.05$) for that variable

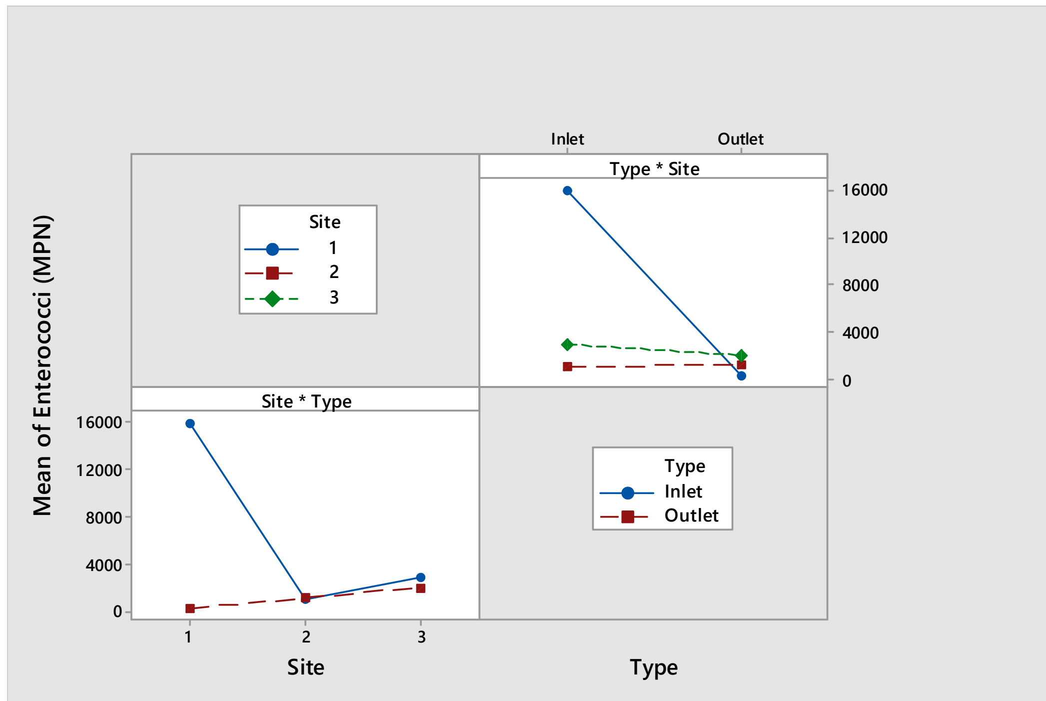

Figure 4.1 Interaction plots for enterococci for three bioretention cells in Grove, Oklahoma.[Site 1 = GLA: Grand Lake Association, Site 2 =GHS: Grove High School, Site 3 = ECP: Elm Creek Plaza, Type = Inlet and Outlet, Inlet = inflow from the inlet, Outlet = outflow from the underdrain]

Table 4.8 Statistical comparison between inflow and outflow concentrations of *E.coli*, enterococci, and coliphage for three bioretention cells in Grove, Oklahoma.

Pathogen	paired t test	Mann-Whitney
	p value*	p value*
E. coli	0.026	0.026
Enterococci	0.001	< 0.001
Coliphage	0.478	0.166

*inflow and outflow concentrations are significantly different at $p < 0.05$

4.3.3 Comparison of Fly-Ash Amended Bioretention Cells in Grove, Oklahoma to Sand Cells in Current Literature

A basic comparison of performance between three bioretention cells amended with fly-ash and three bioretention cells with sand filter media composition was performed. The three cells in Grove, OK with fly-ash amended media had removals of 91%, 58%, and 39% for *E.coli* with an average removal of 63% during monitoring. Sand-only media cells in Charlotte, NC and Wilmington, NC monitored by Hathaway *et al.* (2009) had *E.coli* removals of 92%, 70%, and -119% and an average removal of 14%. The mean cell depth for the fly-ash amended and sand-only cells was 0.8 m and 0.7 m, respectively.

Although no statistical tests were completed because of the small sample set (only three fly-ash amended and three sand-only media cells), it appears that bioretention cells with fly-ash amended media demonstrate a similar removal performance for mean *E.coli* when compared to three cells in North Carolina, with both types of media exhibiting high variability of removal. The design characteristics of each of the six cells are not uniform, and therefore some variation in removal is undoubtedly due to the design differences, i.e. filter media depth and cell size. Furthermore, these comparisons are based solely on *E.coli* as the indicator species. Chapter 3 of this study shows that enterococci had a greater variance than *E.coli* between sand-only filter media to fly-ash amended media, giving a 17% increase of removal with fly-ash amended soils and only 5%

increase of removal was shown for *E.coli*. While, this is an interesting observation, it is recognized that the data in both media compositions are limited for full-scale bioretention cells.

4.4 Conclusions and Recommendations for Future Work

This study provides additional field data for researchers addressing microbial stormwater pollution through the use of bioretention control measures in urban environments. There is conclusive evidence that bioretention cells with fly-ash amendment do remove indicator bacteria and viruses. Furthermore, this study illustrates the variability of indicator removal and concentration change from influent to effluent. Mean removal for the three bioretention sites in Grove, Oklahoma monitored by this study were 63% (*E.coli*), 65% (enterococci), and 67% (coliphage) based on paired data. As these bioretention cells outlet into receiving water bodies, these concentration changes and microbial removal efficiencies may not be sufficient reduction and removal for the watersheds, since in most storm events the criteria was not met for indicator bacteria. On the other hand, depending on the receiving waters' ability to assimilate the influx of microbial contamination these bioretention cells could be acceptable in their current state. In any case, the three cells sampled in Grove, Oklahoma show microbial indicator removal and concentration reduction capability.

The use of amended filter media for increased bacterial removal efficiency was shown in laboratory results from Zhang *et al.* (2010) and Bradley *et al.* (2011). The observation that amended media produces a greater removal than not amended media was further corroborated with this study and Hathaway *et al.* (2009) bioretention data. Though this data set is somewhat limited, three cells with less than 30 storms sampled per cell, it does provide some evidence that further exploration of amended filter media in bioretention cells could be useful for increased indicator bacteria removal efficiencies. An area of further concern is meeting the USEPA recreation criteria for *E.coli* (126 CFU/ 100 ml) and enterococci (35 CFU/100 ml) for effluent exiting bioretention cells in urban settings. The sand composition and the amended filter media bioretention cells met the USEPA limit for either indicator species less than 65% of the time over all storm events captured. This criteria is set to protect against human health impacts, thus a higher percentage is preferred. Despite the increase in data available from field studies using bioretention as microbial indicator removal BMP, the removals are highly variable. Also, enterococci has not been measure in all studies to date, therefore comparing filter media effects on enterococci removal is difficult. Conceivably the most important need in future bioretention field studies considering microbial removal and inactivation with regard to the size, depth and filter media composition of each monitored bioretention cells. These are all factors that would benefit more research in the field setting to

determine their individual or coupled effect on the performance ability in the realm of microbial removal and increased public health in urban areas.

References:

ASTM D3977 – 97. (2013). Standard Test Methods for Determining Sediment Concentration in Water Samples, Method B.

Bradley, I., Straub, A., Maraccini, P., Markazi, S., and Nguyen, T. (2011). "Iron oxide amended biosand filters for virus removal." *Water Research,* 45, 4501-4510.

Davis, A.P., Shokouhian, M., Sharma, H., and Minami, C. (2001). "Laboratory Study of Biological Retention (Bioretention) for Urban Stormwater Management." *Water Environ. Res.*, 73(1), 5-14.

Davis, A.P., Shokouhian, M., Sharma, H., and Minami, C. (2006). "Water Quality Improvement through Bioretention Media: Nitrogen and Phosphorus Removal." *Water Environ. Res.*, 78(3), 284-293.

Davis, A., Hunt, W., Traver, R., and Clar, M. (2009). "Bioretention Technology: Overview of Current Practice and Future Needs." *J. Environ. Eng.*, 135(3), 109–117.

Garbrecht, K., Fox, G.A., Guszman, J.A., and Alexander, D. (2009). "*E. coli* Transport Through Soil Columns: Implications for Bioretention Cell Removal Efficiency." *Transactions of the ASABE,* 52(2), 481-486.

Gavlak ,R., Horneck, D., Miller, R.O., and Kotuby-Amacher, J. (2003). "Soil, Plant and Water Reference Methods for the Western Region", 2nd ed. WCC-103 Publication, Colorado State University, Ft. Collins, Colorado, USA, 56-57 and 62-63.

HACH. (2013). 2100Q and 2100Qis Basic User Manual. 2nd Edition.

Hathaway, J.M., Hunt, W.E., Wright, J.D., and Jadlocki, S.J. (2009). "Field Evaluation of Indicator Bacteria Removal by Stormwater BMPs in North Carolina." *World Environmental and Water Resources Congress*, 1-10. doi: 10.1061/41036(342)112.

Hunt, W.F. and Lord, W.G. (2006). "Urban Waterways: Bioretention Performance, Design, Construction and Maintenance." Retrieved from http://www.bae.ncsu.edu/stormwater/PublicationFiles/Bioretention2006.pdf.

Hunt, W.F., Smith, J.T., Jadlocki, S.J., Hathaway, J.M., and Eubanks, P.R. (2008). "Pollutant removal and peak flow mitigation by a bioretention cell in urban Charlotte, NC." *Journal of Environmental Engineering*, 134, 403-408.

IDEXX. (2014). IDEXX Enterolert Test Method for the Detection of Enterococci in Water.

IDEXX. (2013). IDEXX Colilert Test Method for the Detection of Total Coliforms and *E.coli* in Water.

Jin, Y., Chus, Y., and Li, Y. (2000). "Virus removal and transport in saturated and unsaturated sand columns." *Journal of Contaminant Hydrology*. http://dx.doi.org/10.1016/S0169-7722(00)00084-XPotts, M. (1994). "Desiccation tolerance of prokaryotes." *Microbiol. Mol. Biol. Rev,* 58(4), 755-805.

Lehner, P., Aponte Clark, G.P., Cameron, D.M., and Frank, A.G. (June 1999). "Stormwater Strategies: Community Responses to Runoff Pollution." Natural Resources Defense Council. Retrieved August 2015 from http://www.nrdc.org/water/pollution/storm/stoinx.asp.

Line, D.E., White, N.M., Kirby-Smith, W.W., and Potts, J.D. (2008). "Fecal coliform export from four coastal North Carolina areas." *Journal of the American Water Resources Association*, 44(3), 606-617.

Low Impact Development (LID) Center, INC. "Bioretention – Pollutant Filtering." Retrieved August 2015 from http://www.lid-stormwater.net/bio_benefits.htm.

Method 4500-PF. (2005). Standard Methods for the Examination of Water and Wastewater. 21th Edition, 4-155 - 4-157.

Park, S.J., Lee, C.G., Kim, S.B., Chang, Y.Y., and Yang, J.K. (2012). "Bacterial removal in flow-through columns packed with iron-manganese bimetallic oxide-coated sand." *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering*, 47(10), 1364-1371. DOI:10.1080/10934529.2012.672302.

Potts, M. (1994). "Desiccation tolerance of prokaryotes." *Microbiol. Mol. Biol. Rev*, 58(4), 755-805.

Quilliam, R., Jamieson, J., and Oliver, D. (2014). "Seaweeds and plastic debris can influence the survival of faecal indicator organisms in beach environments." *Marine Pollution Bulletin*, 84, 201-207.

Roy-Poirier, A., Champagne, P., and Filion, Y. (2010). "Bioretention processes for phosphorus pollution control." *Environmental Rev*, 18, 159-173.

SevenMulti^{TM}. (2012). pH and EC meter Operating Instructions.

Schoonover, J.E. and B.G. Lockacy. (2006)."Land cover impacts on stream nutrients and fecal coliform in the lower Piedmont of West Georgia." *Journal of Hydrology*, 331, 371-382.

USEPA. (1999). "Storm Water Technology Fact Sheet – Bioretention." EPA832-F-99-012. Retrieved August 2015 from http://water.epa.gov/scitech/wastetech/upload/2002_06_28_mtb_biortn.pdf

USEPA. (2000). Low Impact Development. Retrieved from http://www.lowimpactdevelopment.org/pubs/LID_litreview.pdf.

USEPA. (2012). "Recreational Water Quality Criteria." Office of Water Quality, 820-F-12-058.

USEPA. (2015). "Water: best management practices: bioretention." Retrieved May 25 2015 from http://water.epa.gov/polwaste/swbmp/Bioretention-Rain-Gardens.cfm.

Yakirevick, A., Pachepsky, Y., Guber, A.K., Gish, T.J., Shelton, D.R., and Cho, K.H. (2013). "Modeling transport of Escherichia coli in a creek during and after artificial high flow events: three year study and analysis." *Water Resources*, 47: 2676-2688.

Zhang, L., Seagren, E., Davis, A., and Karns, J. (2010). "The capture and destruction of Escherichia coli from simulated urban runoff using conventional bioretention media and iron oxide-coated sand." *Water Environment Research,* 82(8), 701-713.

Chapter 5: Bioretention cell design criteria recommendations for targeting microbial removal and destruction from urban stormwater runoff.

Abstract

Bioretention cells have been studied to determine what is required in order to be efficient for nutrients and heavy metal removal. However, current literature lacks a succinct, consolidated summary of efficient techniques to target and optimize the removal of microbial contamination. It is reasonable that bioretention can be optimized for microbial pollutants. This optimization will consider major factors and contributors for removal/trapping or movement and survival or destruction of microbes in bioretention. The goal of this paper is to optimize bioretention design for the reduction of runoff volume and peak flow while specifically targeting the removal of microbes in the effluent based on the available literature published for microbes and bioretention. Three factors are considered in designing bioretention for microbial removal and inactivation, filter media size, cell size, and filter media composition. These design factors have been shown to have the greatest impact on removal by filtration and adsorption and inactivation by desiccation and predation.

5.1 Introduction

Microbial removal efficiency by bioretention best management practices will be explored and optimized in this study. Urbanization can increase

stormwater runoff which serves as a transport mechanism for pollutants to enter local creeks, streams, lakes and other receiving water bodies. When microbial urban pollution is increased urban runoff leads to decreasing water quality that can negatively affect drinking and recreational waters. Furthermore, urbanization, which increases the percentage of in impervious surface in an area, peak flow, runoff volume, time to peak, and duration of runoff. As a result this urbanization, stormwater systems may no longer be capable of handling the larger runoff discharges, causing increased flooding, and failure of stormwater systems (Klein *et al.*, 1979 and Lehner *et al.*, 1999). Another concern is increased delivery of pollutants to receiving waters. The low impact development (LID) practice of bioretention is a commonly used best management practice (BMP) in urban stormwater management that has been experiencing growing interest for microbial removal from stormwater runoff (Garbrecht *et al.*, 2009, Hathaway *et al.*, 2009). This practice was formally introduced in the early 1990's by Prince George's County, Maryland (Liu *et al*, 2014). Specific design guidance for targeting microbes with bioretention cells is currently lacking in the literature (Hunt *et al.*, 2008).

Bioretention involves the use of plants and soils to removal pollutants from urban stormwater runoff (Garbrecht *et al.*, 2009). Bioretention is well suited for urban areas since cells can fit into newly designed or existing landscaping areas (Hunt *et al.*, 2008, USEPA, 2015). The ability of these systems to remove

pollutants is well documented in literature. Removal efficiencies for bioretention ranging from 54% to 90% for total suspended solids (TSS), 22% to 85% for phosphorous, 55% to 80% for nitrogen (TKN – total Kjeldahl nitrogen), and 56% to 99% for heavy metals have been reported in previous studies (USEPA, 1999, Davis *et al*., 2001, Davis *et al.*, 2006, Hunt *et al.*, 2006, and LID INC, 2015). Hunt *et al*. (2008) stated that prior to 2008 no data were reported in literature regarding the removal ability of bioretention in reference to pathogens or indicator organisms, which are organisms like, *E coli*, enterococci, and coliphage that are used to indicate the possible presence of pathogens.

Microorganisms are a grouping of many different organisms, such as bacteria, viruses, and protozoa. **Table 5.1** further describes microbial type, description, and size characteristics, including bacteria, protozoa, and viruses. Microbial contamination in urban stormwater is measured by indicator organisms and water quality criteria recommendation of these indicator organisms are set by federal, state, and local government entities. Enterococci, and *E.coli* are common bacteria indicators considered in surface waters and are more readily used to identify surface water pollution in urban environments. *E.coli* are used to indicate fecal pollution in fresh water while enterococci are more commonly used when considering brackish water (Halliday *et al.*, 2011). According to the USEPA, bacteriophage is also an accepted indicator of virus contamination (2015).

Table 5.1 – The type, size, and description of three basic microbial indicator species.

Type	Size	Description
Bacteria	~ 1 × 3 μm - *E.coli* 0.6 - 2.0 × 0.6 - 2.5 μm - Enterococci	Single celled organism, no nuclear membrane
Protozoa	~ < 50μm 8 – 14 μm – Giardia 4 – 6 μm - Cryptosporidium	single celled organism, enclosed in nuclear membrane
Viruses	20 to 400 nm	Infects an organism, either DNA or RNA protein coated

Concern about microbial degradation of stormwater across the United States and abroad has heightened as urbanization increases. Common sources of microbial pollution in urban stormwater runoff are humans, pets, and wildlife (Lehner *et al.*, 1999). Studies by Jin *et al.* (2000), Hathaway *et al.* (2009), Zhang *et al.* (2010), and Park *et al.* (2012) have shown that microbes are removed from water passing through porous media, such as exists in bioretention cells, through a variety of processes including filtration, desiccation, thermal deactivation, and sorption, but the removal amount varies greatly. Ideal treatment would mean all pathogenic contamination from urban runoff would be irreversibly removed or inactivated (killed), meaning there would be no detectable contamination in effluent water. However this is not realistic with today's available technologies.

Therefore, one of the most important factors in the design of a water treatment system that use porous media for removal of pathogens is the selection of the media that optimizes removal processes. The physical and chemical characteristics of the media directly affect the removal and inactivation of microbial pollutants (Torkzaban *et al.*, 2006, Zhang *et al.*, 2010, and Park *et al.*, 2012). The literature has differing views on the effect of relatively large storm events and the need to address high flowrates in reference to microbial removal in filter media (Coffman *et al.*, 2008, Park *et al.*, 2012).

Microbial transport, removal, and inactivation by bioretention are the foci of this study. The primary objective of this study is to develop bioretention cells design recommendations targeting pathogen removal from urban stormwater runoff. Recommendations will be formed from current and previous published literature studies utilizing both laboratory column studies and field scale bioretention studies, including current work from Chapters 3 and 4.

5.2 Bioretention Removal and Inactivation Factors for Targeting Microbes

Microbial fate in bioretention media is a function of a number of factors and mechanisms. Soil moisture, adsorption, filtration, and flowrate have been shown to have a large impact on the transport of microbes in filter media (Hathaway *et al.*, 2009, Zhang *et al.*, 2010, Park *et al.*, 2012). Potts (1994),

Garbrecht *et al.* (2009), and Park *et al.* (2012) agree that survival of microbes in soil depends on numerous factors including soil temperature, soil moisture, pH, sunlight, desiccation, and predation from indigenous microbial flora. Desiccation and predation of natural microbial flora may have the greatest effect on survivability of microbes (Coffman *et al.*, 2008, Zhang *et al.*, 2010, Clark and Pitt, 2012, and Park *et al.*, 2012). Soil moisture directly impacts adsorption, filtration and desiccation in filter media. Soil moisture, adsorption, filtration, flowrate, desiccation, and predation are the transport and survival factors that will be addressed further, survival factors are directly related to the transport factors.

Soil moisture content has a direct impact on removal and inactivation of bacteria and viruses. Since the 1970s data has shown that microbial survival in porous media is greatly affected by soil moisture (Gerba *et al.*, 1975, Jamieson *et al.*, 2005). Several mechanisms may be present during lower moisture content that enhance microbe adsorption to soil particles. Studies have indicated unsaturated flow conditions influence both virus and bacteria removal and survival. Specifically, column studies by Gargiulo *et al.* (2008) and Chu *et al.* (2001) show results of greater retention of microbial indicators in unsaturated conditions. One common assumption is that when solution chemistry is optimal for adsorption, soil moisture works in harmony with the mechanism of adsorption that is as saturation decreases adsorption of microbes' increases (Sim *et al.*, 1996, Yates *et al.*, 1987, Jin *et al.*, 2000, Chu *et al.*, 2001, Strevik *et al.*, 2004, and Torkzaban *et*

al., 2006). Torkzaban *et al.* (2006) describes a linear function relating adsorption coefficients with water content, further noting that there are some cases where a nonlinear function may be more appropriate. In the nonlinear case, absorption coefficients are a function of absorbed virus concentration at the air-water interface (AWI) versus moisture content. Chu *et al.* (2001) concludes that effect of soil moisture content on virus adsorption is dependent more on the species and surface properties of the filter media. Strevik *et al.* (2004) also surmised unsaturated conditions greatly decrease the survival of microbes. Zhao *et al.* (2008) states that inactivation occurs more rapidly with decreasing soil water content and this has been proven through many batch and column experiments. Desiccation is also a factor in microbial survival, as soils dry out microbes will die off. The rate of die off varies for individual types of microbe.

Reversible and irreversible microbial adsorption can occur within soil filter media. Reversible adsorption is generally considered a weak interaction because bacteria can detach from soil particles and reenter the water phase, however, reversible adsorption is governed by electrostatic forces, hydrophobic interactions and van der Waals forces (Chu *et al.*, 2001 and Zhang *et al.*, 2010). Irreversible adsorption is the act of firm attachment to the surface of a soil particle and is considered a permanent process referred to as adhesion (Chu *et al.*, 2001). Ionic strength, colloid surface properties, and pH are factors that affect the level of interfacial attachment within porous media, although researchers do not agree

on the validity of irreversible attachment theory (Yates *et al.*, 1987, Jin *et al,* 2000, Sim *et al.,* 2000, Chu *et al.*, 2001, Torkzaban *et al.*, 2006). Optimal solution chemistry- an increase in ionic strength with neutral pH- supports the mechanism of adsorption. Torkzaban *et al.* (2006) experiments showed that adsorption due to diffusion within zones of immobilized water will encourage increased adsorption at lower moisture contents.

Filtration of microbial cells by small pores and adsorption are the main mechanisms of microbial pollutant removal for bioretention cells (Zhang *et al.*, 2010). However, filtration is not a governing removal mechanism for viruses due to their size. Immobilization of bacteria through physical filtration occurs when movement is blocked by pores that are smaller than the bacteria. Weiss *et al.* (1995) and Strevik *et al.* (2004) state that physical straining is largely affected by bacterial size and shape and porous media particle size. Filtration by straining at narrow pores is more effective in removing larger microbial cells, like protozoan cysts, and studies have shown filtration to be statistically proportional to microbial size (Tufenkji *et al.*, 2004, Grebel *et al.*, 2013). vanLoosdrect *et al* (1989) noted that long rod-shaped cells have greater attachment to filter media than spherical cells, suggesting that shape of bacteria can be a factor related to filter media removal ability. Strevik *et al.* (2004) stated that straining is a more significant mechanism in the bacterial removal process when bacteria are greater than 5% of the mean diameter of the filter media particles. Filtration contributes

to removal more greatly when filter media contains a considerable amount of silt or clay, which decreases average pore size in the media. Clogging of the filter media can also affect straining by reducing the infiltration rate of the media (Strevik *et al.*, 2004).

The development of preferential flow paths can both reduce filtration and enhance bacteria and virus transport through the media. Also, amended media can have an impact on removal of microbes. Organic matter (OM) from compost has been used as an amendment in bioretention cells to date. Removal of pollutants can be increased within OM by adsorption and it is sometimes used as a pre-filtering mechanism (VA DEQ, 2011). However, research has shown that OM can negatively impact the removal capacity of other pollutants in urban stormwater runoff. Zhang *et al.* (2010) showed that iron-oxide coated sands could produce greater removal of *E.coli*.

The flowrate through the soil media has been shown to have a direct impact on microbial transport and removal, although results have been mixed. Coffman *et al.* (2008) conducted column and field studies that differ from convention regarding the necessity of extended contact times to remove microbes efficiently. Specifically, in column experiments, he found that removal was not altered due to flowrate, showing similar removals at both high and low flowrates. Instead the data showed increased removal rates were directly related to the volumes of water entering the bioretention media. In short, the observation by

Coffman *et al.* (2008) was that if the volume of water could be contained in the cell or column then the media would be able to remove the microbes. Sand column studies by Zhang *et* al. (2010) and Park *et al.* (2012) have shown positive results in effectively removing bacteria and viruses with Park *et al.* (2012) study focusing on water flow velocity. Their results differed from Coffman *et al.* (2008) and were in agreement with convention that shorter contact times during high flow events will decrease the ability of the filter media to remove microbes. Therefore high flowrates decrease removal capacity. Because volume and flowrate are related, all three studies provide insight that bioretention cells must address the quantity of water along with the quality. Coffman *et al.* (2008) recommends that the bioretention surface area be doubled, with respect to the 3% to 8% size based on watershed area suggested by Hunt and White (2001) or the watershed drainage area be decreased by half to account for increased volume of runoff.

Desiccation and predation by natural microbial flora contribute directly to the inactivation or death of microbes in filter media (Coffman *et al.*, 2008, Zhang *et al.*, 2010, Clark and Pitt, 2012, and Park *et al.*, 2012). Desiccation is increased in media with higher infiltration rates which allows the media to drain between storm events. Furthermore, sunlight aids in the drying out of media and therefore can be a positive influence for increased desiccation of microbes. Predation by protozoa and other bacterial predators assist with inactivation of microbes in

bioretention media similarly to sand infiltration systems (Strevik *et al.*, 1998 and Zhang *et al.*, 2010). Further, it is noted that while predation can enhance *E.coli* inactivation it may also encourage growth of other bacteria (Zhang *et al.*, 2010). Bradley *et al.* (2011) suggested creating an active biological layer in the top layer of the bioretention cell to enhance inactivation by predation. Literature suggests that over time, the cell will mature and develop a diverse biological layer to encourage predation from indigenous microorganisms, which will enhance microbial removal (Park *et al.,* 2012, Zhang *et al.,* 2010, Zhao *et al.*, 2008 and Coffman *et al.,* 2008).

5.3 Case Studies

Many laboratory column experiments examining the removal of indicator bacteria using sand filter media have been published. For example, Rusciano *et al.* (2007) showed microbial indicator organism fecal coliform removals of 96% over a 9-month testing period. The influent was diluted manure slurry which was used to simulate worst case stormwater runoff. Prior to 2008 no data from bioretention field-scale studies were reported in the literature regarding the removal ability of bioretention in reference to pathogens or indicator organisms according to Hunt *et al.* (2008). Eight representative cases studies from 2008 to 2012 with published data on microbial removal by bioretention are discussed. The case studies include both laboratory and field experiments. These case studies agree that bioretention

is a viable management practice to reduce bacteria transport in urban environments, but have varied findings on removal efficiency and primary removal characteristics.

A bioretention cell was monitored for nutrients, metals, and microbial indicator organisms in Charlotte, NC, only the results from the microbial indicators organisms measured are discussed here (Hunt *et al.*, 2008). The bioretention cell had a 0.4 ha watershed area that was primarily impervious, and a 0.02 ha surface area with a loamy sand filter media with a depth of 1.2 m. This site incorporated an underdrain and was designed to pond between 152 mm and 304 mm of water and drain within 24 hours to allow the cell to dry out between storm events. Results showed 71% and 69% reductions for fecal coliform (FC) and *E.coli* from the influent to the effluent. Hunt *et al.* (2008) states that sunlight and dry conditions are imperative to achieve microbial destruction and inactivation. The Hunt *et al.* (2008) bioretention cells are also cited by Clary *et al.* (2008) where the major observation for bioretention cells for the removal of bacteria is filter media and soil moisture as primary removal and destruction mechanism for microbial indicators.

Hathaway *et al.* (2009) evaluated multiple BMPs in Charlotte, NC for indicator bacteria. The bioretention cell described above from the Hunt *et al.* (2008) study was one of the BMPs evaluated in this study. Removal efficiencies were calculated based on 19 events for FC and 14 events for *E.coli* also measured.

This evaluation found 89% to 92% removal of indicator organisms for the sampling period, of all the BMPs monitored only a wetland was comparable with 98% and 96% removals for FC and *E.coli*. Hathaway *et al.* (2009) notes that bioretention provides other measures that aid in the destruction of microbes. First, by design, bioretention uses filter media to physically filter the indicator organisms from the influent. Secondly, compared to other evaluated BMPs, bioretention is designed to dry out between storm events and thus promotes microbial destruction via desiccation.

More studies by Hathaway *et al.* (2009b) and (2011) describe the BMPs evaluated in the previous study with four additional control measures in Wilmington, NC. Within those additional BMPs, one was a bioretention cell with a smaller (0.14 Ha, impervious) watershed area. This bioretention cell is a two sided cell, one side built at half the depth of the other side, 0.3 m and 0.6 m respectively. The shallow side of the cell received more runoff due to the grading of the impervious watershed area and thus is wetter (saturated) than the deeper side of the cell. This deeper site of the bioretention cell showed removal efficacies for FC and *E.coli* of 60% and 80% for the deep side of the cell based on nine events. The shallow side had increases in indicator organisms from the influent to the effluent. The researchers believe this is due to the design features of the shallow cell that negatively impacted the cells ability to remove microbes, whereby there was an increase in indicator bacteria. The shallow cell,

approximately 0.3 m filter media depth resulted with unsatisfactory microbial removals. It could be suggested that no less than 0.6 m should be used to design bioretention cells targeting microbial removal. Further, it was noted that if an optimal environment is developed, some BMPs, including bioretention cells, could serve as sources of indictor organisms.

Coffman *et al.* (2008) investigated the impact of high flowrates on microbial removal efficiency in column studies using maximum design flowrate and bypass volume over specified time periods. They found that volume not flowrate is the limiting factor. Column experiments with different media composition blends were examined for volume using maximum design flowrate and bypass volume over specific time periods. Fecal coliform removal of 77% to 99% was measured at high flowrates during column experiments. These data also showed the removal efficiency increased with lower volumes of influent. The study suggested that addressing urban stormwater runoff by bioretention is extremely viable as smaller drainage areas are treated with this practice. The recommendation from this study was to increase the surface area of the bioretention cell or reduce the drainage area to invoke greater than 90% removal of microbial indicators. Coffman *et al.* (2008) advocates for a particular filter media blend as optimal, but this blend is proprietary. However, this study also provides insight that filter media composition is of high importance.

Small-scale column experiments found that by increasing the flowrate through the column, the bacterial removal capacity was decreased (Park *et al.*, 2012). Conversely to Coffman *et al.* (2008), they hypothesized the decrease in bacterial removal is most likely because higher flowrates tend to result in increased shear force at the surface of the filter media. An increase in shear force would decrease the potential bacterial removal from the media. In summary, contact time is important and therefore if there is a high flow event and contact time is decreased less removal of microbes and greater availably of microbes to be transported into receiving waters is expected. Additionally, high flow is documented to be an issue in the ability for a bioretention cell to operate properly according to Seetha *et al.* (2015). Coffman *et al.* (2008) and Park *et al.* (2012) agree that over time, bacteria removal increases due to filter media maturation and the development of a diverse biological layer to encourage predation from indigenous microbial microflora. Similar to Coffman *et al.* (2008), this study also demonstrates that filter media composition is an important factor for efficient microbial removal from stormwater treated by bioretention cells.

Zhang *et al.* (2010) compared bacterial removal using filter media composition both amended and non-amended. These experiments showed the importance to media particle size when quantifying infiltration rates. Column experiments were conducted with synthetic, simulated urban runoff for a common storm, equivalent to 4 mm/hr with a return period of less than one year. Columns

were comprised of conventional bioretention media (CBM) or iron- oxide coated sand (IOCS). Sand, Silica Mystic White II pool filter sand, soil, with a composition of 63% sand, 18% silt, and 19% clay and mulch was combined at 5:3:2 volume ratio to make the CBM used in this study. IOCS columns had a higher bacterial removal (99%) when compared to CBM columns (82%) which is likely caused by the increased surface roughness and more positively charged iron-oxide in the system. These two factors increase the electrostatic adsorption between the bacteria and soil media (Zhang *et al.*, 2010 and Strevik *et al.*, 2004). Removal was higher with IOCS media versus CBM, conversely die off was faster with CBM than IOCS media. Zhang *et al.* (2010) stated the change in die off, 99% within a week versus 52% respectively, was due to predation by indigenous microorganism populations that exist in the CBM but not in the IOCS. Bradley *et al.* (2011) also used IOCS with similar results to those found in the Zhang experiments.

Clark and Pitt (2012) assess method to targeting stormwater treatment measure design for specific pollutants. When considering physical filtration of bioretention filter media, the authors state that removal is a function of filter media grain size, porosity of the media, and pollutant size and shape. That study describes a need for filter media with smaller pores for pollutants like bacteria and viruses. They further state that indicator microbe's surface charge can assist in filtration and adsorption in the filter media. Similar to Coffman *et al.* (2008),

Zhang *et al.* (2010), Clark and Pitt (2012), and Park *et al.* (2012), they suggest that removal does not equate inactivation, noting permanent destruction of microbes to be of vital importance. Natural predation is likely a major contributor to permanent destruction of microbes however, it is not the only mechanism involved since sunlight works as well.

Hunt *et al.* (2012) summarized current research on bioretention design for targeted pollutant removal, including bacterial indicator organisms. That study states that filtration and adsorption are the primary mechanisms for removal of microbes within bioretention filter media. Initially, Hunt *et al.* (2012) cites previous laboratory column experiments by Zhang *et al.* (2010, 2011) described above as evidence that *E.coli* removal will increase with time. That result was also found in the case studies by Coffman *et al.* (2008) and Park *et al.* (2012). Hunt *et al.* (2012) further identifies 0.6 m as a minimum filter media depth for bioretention cells that are designed to target microbial removal. Also, filter media should have an infiltration rate of 0.007 to 0.014 mm/s that will reduce the likelihood of bacteria survival on the surface that could become a source. Predation is considered to be the main mechanism of inactivation of microbes, although desiccation should not be ignored. Finally, a brief discussion of vegetation is presented. Initial findings from Hathaway *et al.* (2009) and Hunt *et al.* (2008) do not suggest moderate vegetation will hinder removal and inactivation. It should be noted that excess vegetation could assist in the creation

of sources for microbial contamination as the foliage will prevent sunlight that aids in drying the top of the bioretention cell, and creates animal habitat that would lead to fecal deposits.

5.4 Design Criteria Recommendations

By designing bioretention cells with removal and destruction processes in mind, engineers and designers can provide cost-effective stormwater treatment to help solve locally relevant water quality issues. Soil moisture is a consistent factor in all case studies, filter media composition and depth are also identified as the major factors that target microbial removal through the mechanisms of filtration and absorption based on the case studies presented in this research. Design consideration for volume, including cell size and drainage area were also presented by more than one case study as an avenue of addressing increased removal and decreasing microbial transport during high flow events. Overall, unsaturated conditions, natural predation, and sunlight were consistently considered the greatest factors for microbial inactivation.

Table 5.2 shows the recommended criteria for each of the factors that target microbial removal discussed in the bioretention media case studies, it also provides a description of the benefit each factor offers to enhance microbial removal and ranges of increase for the stated benefits.

Table 5.2 – Bioretention design criteria for optimization of microbial removal of pollution in urban environments.

Factor	Optimal Criteria	Benefit	Reference
Filter Media Size	Fine Sands	Enhanced microbial removal via straining, and increased infiltration Rate (0.007 to 0.014 mm/s)	6, 9, 10, 12
Cell Size	Filter Media Depth, Greater Surface Area	Enhanced runoff detention, increased potential to effectively filter high volume events Depth (0.6 m to 1.2 m)and 6% to 16% of the surface area to account for overflow	1, 2, 3, 5, 6, 8, 11,13
Filter Media Composition	Amended Soils	Enhanced microbial removal via absorption with mean increase for removal by fly-ash of *E.coli* (5% to 31%), Enterococci (17% to 29%), Coliphage (0% to 15%)	4, 6, 7, 12, 13

Marino *et al.*, 1991[1], Whitman *et al.*, 2003[2], Coffman *et al.*, 2008[3], Zhao *et al.*, 2008[4], Hathaway *et al.*, 2009[5], Zhang *et al.*, 2010[6], Bradley *et al.*, 2011[7], Hathaway *et al.*, 2011[8], Clark and Pitt, 2012[9], Hunt *et al.*, 2012[10], Park *et al.*, 2012[11], Chapter 3[12], and Chapter 4[13].

Soil moisture was a common thread throughout every case study and previous column study research that was not considering only bioretention, but porous media. The first factor that can address soil moisture is media size. Zhang

et al. (2010) and Clark and Pitt (2012) both state that particle size is a limiting factor for the removal of microbes, due to physical straining of microbes within the pores of the filter media. Further the particle size affects the filter media infiltration rate. Chapter 3 from this research shows that fine sands are the optimal for maximum reduction of microbes. Filter media should be optimized by considering infiltration rates given above.

Cell size is important when considering high-flow events. Desired ponding depth for bioretention cells is usually between 152 mm and 304 mm. The minimum filter media depth discovered by Hathaway *et al.* (2009) is 0.6 m. However, based on a comparison in Chapter 4 between the Hathaway *et al.* (2009) bioretention cells and three cells in Grove, OK, the cell that performed the best had 1.2 m depth at 91% removal of *E.coli* and the second performed at 90% with a filter media depth of 0.85 m. Chapter 3 in this research noted that multiple bioretention sites were sampled for soil cores and they were unusable because the depth of filter media was less than 304 mm and/or they would not drain. Marino *et al.* (1991) and Whitman *et al.* (2003) agree that high flow must be addressed as it can affect the ability for a bioretention cell to work as designed. Therefore, another consideration with cell size is the surface area of the cell. The area of the cell is generally calculated to be 3% to 8% of the watershed drainage area for sandy soils (Hunt and White, 2001). However, based on Coffman *et al.* (2008) and Park *et al.* (2012) if the cell is sized larger than the minimum it could assist in

increased mitigation of stormwater volume and therefore increase the removal potential during high flow events.

The final component suggested to optimize microbial removal is amended filter media. Zhang *et al.* (2010) and Bradley *et al.* (2011) both showed increased removal in iron-oxide coated sands. The enhanced removal is due to adsorption. Also, Zhao *et al.* (2008) showed soil coated in Al-oxide enhanced both removal and inactivation of microbe because of irreversible sorption caused by strong electrostatic interactions. This finding was further verified in Chapters 3 and 4 of this research using fly-ash amended sands. In all cases, these amendments led to increased capture of microbes. In Chapter 3, a side by side comparison of filter media from a paired cell was analyzed and the fly-ash amended soil removed more than 31%, 29%, and 41% of *E.coli*, enterococci, and coliphage respectively when compared to the sand-only filter media. Statistically, filter media type was found to be significant ($\alpha < 0.05$) for all microbes for the bioretention cell in Stillwater, Oklahoma. Furthermore, the column study in Chapter 3 provide increased removals of *E.coli* (5% to 31%), Enterococci (17% to 29%), Coliphage (0% to 15%), respectively for soil amended with fly-ash. Data from Chapter 3 indicated that *E.coli* removal is most related to the variables of media type, percent medium sand, and percent fine sand. Enterococci removal involves media type, percent medium sand, and percent clay, coliphage is similar to enterococci with the addition of flowrate as a component in the regression

equation. Chapter 4, compared field scale bioretention cells with fly-ash to those in Hathaway *et al.* (2009) with sand-only filter media. Fly-ash amended performed better by 39% for *E.coli* removal. The sand-only cells had other factors that may have caused the difference in *E.coli* removal, specifically the cell depth.

Finally, Zhang *et al.* (2010), Coffman *et al.* (2008) and Park *et al.* (2012), Clark and Pitt (2012) all agree that removal does not equate inactivation noting permeant destruction of microbes to be of vital importance. However, desiccation and predation are major contributors to permeant destruction of microbes. Sunlight availability and unsaturated conditions are necessary to foster an environment for desiccation. An underdrain can further assist infiltration and enhance drainage of the cell. A paired cell in North Carolina described by Hathaway *et al.* (2009) actually increased in microbial concentration from the influent to the effluent due to saturated conditions. Saturated conditions that extend past the 12 to 24 hour ponding criteria can negatively impact the removal ability of bioretention and create an ideal microcosm for survival and regrowth of microbes. The installation of an underdrain could prove very beneficial in reducing the risk of creating an optimal microbial haven by aiding the dry out of the cell more completely between storm events. However, depending on the site specifications an underdrain may not provide added benefit. In some situations, provided the runoff captured in the bioretention cells will not travel to the groundwater it might be acceptable to maintain saturated conditions. If the

microbes cannot exit the cell then they will not cause harm, or potentially cause harm. Meaning, capture could be enough in some situations.

Furthermore, careful consideration to plant selection is necessary as they will have some impact on survival because they could limit the amount of sunlight available. Additionally, vegetation can be an instigator of increased bacteria deposits by water fowl or other animal inhabitants. Hathaway *et al.* (2009) and Hunt *et al.* (2008) both state that moderate vegetation is acceptable and will not hinder removal or inactivation of microbes. Park *et al.* (2012), Zhang *et al.* (2010), Zhao *et al.* (2008) and Coffman *et al.* (2008) all suggest that microbial removal will increase over time as the cell matures and develops a diverse biological layer to encourage predation from indigenous microorganisms. Bradley *et al.* (2011) suggested creating an active biological layer in the top layer of the bioretention cell to enhance inactivation more quickly.

5.5 Summary and Future Work

Filter media size, cell size (depth and surface area), and filter media composition are the three components strongly recommended for enhanced removal of microbes for bioretention. Filter media size will have more effect on bacteria and protozoa while filter media composition will likely enhance bacteria and virus removal by adsorption. Finally, the depth and surface area will allow all microbes' greater ability to effectively filter through the bioretention cell and be

removed by filtration and/or adsorption mechanisms. Survival reduction could be addressed through the addition of an underdrain since all microbes survive better in moist environments and the underdrain could assist in unsaturated conditions for the cell. There is still more to learn about the science behind microbial removal and inactivation in filter media, specifically in bioretention cells used in urban environments to reduce the release of pathogens into receiving waters. There are three areas that should be addressed to further understand the interworking of the microbial removal by bioretention. 1) Further analysis of competition/antagonism and predation on microbial survivability. Additional experiments considering each of these factors individually to determine their respective effect. 2) Soil media composition data are minimal, more experiments comparing side by side studies of filter media with and without amendments both in the laboratory and field setting are needed. 3) Systematic laboratory column experiments coupled with field testing addressing filter media size, to further understand optimal size not just soil classification that will produce the infiltration rate recommended.

The factors listed in **Table 5.2** were selected based on overlapping data from multiple literature sources. It is important to understand there are undoubtedly additional interactions between these and other factors that are still not well understood. This recommendation is not an attempt at a one size fits all criteria. Meaning, if you are optimizing for more than just microbes you will need

to consider the impact these selected, suggested criteria could have on other pollutant removal or increases. One example is the need for desaturation by increased infiltration rates or an underdrain, if the watershed area and soils you are designing lack the ability to either infiltrate the groundwater or overflow the bioretention cell, removal may be enough. That particular design need may not include limiting survival of microbes which is better addressed in unsaturated conditions.

The recommendations given in this study provides further design guidance that will target microbial removal for bioretention. Urban stormwater runoff must be addressed through the use of BMPs and bioretention is viable as it addresses stormwater at the source, in smaller quantities. More research in filter media depth, size, composition and the effects of indigenous microbial communities are necessary to develop a more detailed understanding of the mechanisms that enhance performance for bioretention. Overall, this recommendation will assist in construction of bioretention practices that necessitate the mitigation of microbes, however, as discussed in Chapter 3, quality control must be considered as the design is not always what is built and that will affect the actual performance of bioretention.

References:

Bradley, I., Straub, A., Maraccini, P., Markazi, S., and Nguyen, T. (2011). “Iron oxide amended biosand filters for virus removal.” *Water Research,* 45, 4501-4510.

Chu, Y., Jin, Y., Flury, M. and Yates, M.V. (2001). “Mechanisms of virus removal during transport in unsaturated porous media.” *Water Resources Research*, 37(2), 253-263.

Clark, S.E., and Pitt, R. (2012). “Targeting treatment technologies to address specific stormwater pollutants and numeric discharge limits.” *Water Research*, 46, 6715-6730.

Clary, J., Jones, J., Urbonas, B., Quigley, M., Strecker, E., and Wagner, T. (2008). “Can Stormwater BMPs Remove Bacteria?” New Findings from the International Stormwater BMP Database. *Stormwater Magazine*. 9(3).

Coffman, L. and Ruby, M. (2008). “Bacterra by Filterra Advanced BioretentionSystem: Discussion of the Benefits, Mechanisms, and Efficiencies for Bacteria Removal.” *Low Impact Development for Urban Ecosystem and Habitat Protection*. pp. 1-13. doi: 10.1061/41009(333)93

Davis, A.P., Shokouhian, M., Sharma, H.,and Minami, C. (2001). “Laboratory Study of Biological Retention (Bioretention) for Urban Stormwater Management.” *Water Environ. Res.*, 73(1), 5-14.

Davis, A.P., Shokouhian, M., Sharma, H., and Minami, C. (2006). “Water Quality Improvement through Bioretention Media: Nitrogen and Phosphorus Removal.” *Water Environ. Res.*, 78(3), 284-293.

Davis, A., Hunt, W., Traver, R., and Clar, M. (2009). “Bioretention Technology: Overview of Current Practice and Future Needs.” *J. Environ. Eng.*, 135(3), 109–117.

Garbrecht, K., Fox, G.A., Guszman, J.A., and Alexander, D. (2009). “*E. coli* Transport Through Soil Columns: Implications for Bioretention Cell Removal Efficiency.” *Transactions of the ASABE,* 52(2), 481-486.

Gargiulo, G., Ustohal, P., Vereecken, H., and Klumpp, E. (2008). “Bacteria Transport and Deposition under Unsaturated Flow Conditions: The Role of Water Content and Bacteria Surface Hydrophobicity.” *Vadose Zone Journal,* 7(2), 406-419.

Gerba, C.P., Wallis, C., and Melnick, J.L. (1975). "Fate of wastewater bacteria and viruses in soil." *Journal of Irrigation and Drainage Engineering*, 101, 157-174.

Gerbel, J.E., Mohanty, S.K., Torkelson, A.A., Boehm, A.B., Higgins, C.P., Maxwell, R.M., Nelson, K.L., and Sedlak, D.L. (2013). "Engineered Infiltration Systems for Urban Stormwater Reclamation." *Environmental Engineering Science,* 30(8), 437-457.

Halliday, E., and Gast, R. (2011). "Bacteria in beach sands: and emerging challenge in protecting costal water quality and bather health*." Environmental Science Technology*, 45(2), 370-379.

Hathaway, J.M., Hunt, W.E., Wright, J.D., and Jadlocki, S.J. (2009). "Field Evaluation of Indicator Bacteria Removal by Stormwater BMPs in North Carolina." *World Environmental and Water Resources Congress*, 1-10. doi: 10.1061/41036(342)112.

Hathaway, J.M., Hunt, W.F., and Jadlocki, S.J. (2009)."Indicator Bacteria Removal in Storm-Water Best Management Practices in Charlotte, North Carolina." *Journal of Environmental Engineering*, 135, 1275-1285.

Hathaway, J., Hunt, W., Graves, A., and Wright, J. (2011). "Field Evaluation of Bioretention Indicator Bacteria Sequestration in Wilmington, North Carolina." *J. Environ. Eng.*, 137(12), 1103–1113.

Hunt, W.F. and White, N. (2001). "Designing Rain Gardens (Bioretention Areas). *Urban Waterways*, AG-588-3. Raleigh, NC: North Carolina Cooperative Extension Service.

Hunt, W.F. and Lord, W.G. (2006). "Urban Waterways: Bioretention Performance, Design, Construction and Maintenance." Retrieved from http://www.bae.ncsu.edu/stormwater/PublicationFiles/Bioretention2006.pdf.

Hunt, W.F., Smith, J.T., Jadlocki, S.J., Hathaway, J.M., and Eubanks, P.R. (2008). "Pollutant removal and peak flow mitigation by a bioretention cell in urban Charlotte, NC*." Journal of Environmental Engineering*, 134, 403-408.

Hunt, W.F., Davis, A.P., and Traver, R.G. (2012). "Meeting Hydrologic and Water Quality Goals through Targeted Bioretention Design." *Journal of Environmental Engineering*, 138, 689-707.

Jamieson, R., Joy, D. M., Lee, H., Kostaschuk, R., and Gordon, R. (2005). "Transport and deposition of sediment-associated Escherichia coli in natural streams." *Water Research,* 39, 2665-2675.

Jin, Y., Chus, Y., and Li, Y. (2000). "Virus removal and transport in saturated and unsaturated sand columns." *Journal of Contaminant Hydrology*. http://dx.doi.org/10.1016/S0169-7722(00)00084-X

Klein, R. (1979). "Urbanization and Stream Quality Impairment." *Water Resources Bulletin*, 15(4), 948-964.

Lehner, P., Aponte Clark, G.P., Cameron, D.M., and Frank, A.G. (June 1999). "Stormwater Strategies: Community Responses to Runoff Pollution." Natural Resources Defense Council. Retrieved August 2015 from http://www.nrdc.org/water/pollution/storm/stoinx.asp.

Low Impact Development (LID) Center, INC. "Bioretention – Pollutant Filtering." Retrieved August 2015 from http://www.lid-stormwater.net/bio_benefits.htm.

Lui, J., Sample, D.J., Bell, C., and Guan, Y. (2014). "Review and Research Needs of Bioretention Used for the Treatment of Stormwater." *Water*, 6, 1069-1099.

Marino, R., Gannon, J. (1991). "Survival of fecal coliforms and fecal streptococci in storm drain sediment." *Water Resources,* 25(9), 1089-1098.

Park, S.J., Lee, C.G., Kim, S.B., Chang, Y.Y., and Yang, J.K. (2012). "Bacterial removal in flow-through columns packed with iron-manganese bimetallic oxide-coated sand." *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering*, 47(10), 1364-1371. DOI:10.1080/10934529.2012.672302.

Potts, M. (1994). "Desiccation tolerance of prokaryotes." *Microbiol. Mol. Biol. Rev*, 58(4), 755-805.

USEPA. (1999). Storm Water Technology Fact Sheet – Bioretention. EPA832-F-99-012. Retrieved August 2015 from http://water.epa.gov/scitech/wastetech/upload/2002_06_28_mtb_biortn.pdf
USEPA. (2015). "Water: best management practices: bioretention*."* Retrieved May 25 2015 from http://water.epa.gov/polwaste/swbmp/Bioretention-Rain-Gardens.cfm.

Rusciano, G.M., and Obropta, C.C. (2007). "Bioretention Column Study: Fecal Coliform and Total Suspended Solids Reductions." *American Society of Agricultural and Biological Engineers*, 50(4).

Sim, Y., and Chrysikopoulos, C.V. (1996). "One-dimensional virus transport in porous media with time-dependent inactivation rate coefficients." *Water Resources Research,* 32(8), 2607-2611.

Sim, Y. Chrysikopoulos, V. (2000). "Virus transport in unsaturated porous media." *Water Resources Research,* 36(1), 173-179.

Strevik, K., Hanssen, J.F., Jenssen, P.D. (1998). "A comparison between DAPI direct count (DDC) and the most probable number (MPN) to quantify protozoa in infiltration systems. *Journal of Microbiol.Methods,* 33(1), 13-21.

Strevik, K., Aa, K., Ausland, G., and Hanssen, J.F. (2004). "Retention and Removal of Pathogenic Bacteria in Wastewater Percolating Through Porous Media: A Review." *Water Resources*. 38(6), 1355-1367.

Seetha, N., Kumar, M.S.M., and Hassanizadeh, S.M. (2015). "Modeling the co-transport of viruses and colloids in unsaturated porous media." *J. Contam. Hydrol*,.181, 82-101.

Torkzaban, S., Hassanizadeh, S.M., Schijven, J.F., de Bruin, H.A.M., and de Roda Husman, A.M. (2006). "Virus Transport in Saturated and Unsaturated Sand Columns." *Vadose Zone Journal*, 5, 877-885.

Tufenkji, N., Miller, G.F., Ryan, J.N., Harvey, R.W., and Elimelech, M. (2004). "Transport of *Cryptosporidium* oocysts in porous media: Role of straining and physicocemical filtration." *Environ. Sci. Technol.*, 38, 5932-5938.

vanLoosdrecht, M., Lyklema, J., Norde, W., and Zehnder, A. (1989). "Bacterial adhesion: A physicochemical approach." *Microbial Ecology,* 17(1), 1-15.

VA DEQ, Virginia DEQ Stormwater Design Specification No. 9. (2011). "Bioretention" received August, 2015, http://www.vwrrc.vt.edu/swc/NonPBMPSpecsMarch11/VASWMBMPSpec9BIORETENTION.html.

Weiss, T.H., Mills, A.L., Hornberger, M., and Herman, J.S. (1995). “Effect ofbacterial cell shape on transport of bacteria in porous media.” *Environmental Science and Technology*, *29*(7).

Whitman, R. L., Shively, D. A., Pawlik, H., Nevers, M. B., and Byappanahalli, M. N. (2003). “Occurrence of *Escherichia coli* and Enterococci in *Cladophora* (Chlorophyta) in Nearshore Water and Beach Sand of Lake Michigan.” *Applied and Environmental Microbiology*, 69(8), 4714–4719. http://doi.org/10.1128/AEM.69.8.4714-4719.2003.

Yates, M., Yates, S.R., Wagner, J., and Gerba, C. (1987). “Modeling virus survival and transport in the subsurface.” *Journal of Contaminant Hydrology*, 1, 329-345.

Zhang, L., Seagren, E., Davis, A., and Karns, J. (2010). “The capture and destruction of Escherichia coli from simulated urban runoff using conventional bioretention media and iron oxide-coated sand.” *Water Environment Research,* 82(8), 701-713.

Zhang, L., Seagren, E., Davis, A., and Karns, J. (2011). “Long-term sustainability of Escherichia coli removal in conventional bioretention media.” *J. Environ. Eng.,* 137(8), 669-677.

Zhao, B., Zhang, H., Zhang, J., and Jin, Y. (2008). “Virus adsorption and inactivation in soil as influenced by autochthonous microorganisms and water content.” *Soil Biology & Biochemistry*, 40, 649-659.

Chapter 6: Overall conclusions for bioretention laboratory and field studies including bioretention design criteria to target microbial pollutants.

6.1 Overall Conclusions

A commonly used LID practice in urban environments is the bioretention cell. Pollution for urban stormwater runoff is a result of numerous sources including pets, wildlife, humans, and bottom sediments in streams and stormwater drains. The increase of pollution has become a growing concern due to an influx of urbanization. The growth in cities has reduced the ability for pollutants to naturally filter due to a vast increase of impervious surfaces. There is a plethora of published data characterizing the removal efficiency of bioretention and other BMPs when considering pollution with the exception of microbes. This research was devoted to measure microbial removal rates of bioretention cells in both laboratory and field settings in an attempt to develop a microbial removal design criteria for these types of control measures.

A laboratory study using 30 intact columns obtained from established bioretention cells in Oklahoma and Arkansas quantified and compared removal rates of *E.coli*, enterococci, and coliphage. These removals were described by two different criteria. The first was based on influent, Day 1 - diluted manure slurry influent and Day 2 - clean flush influent. Average removals measured after the initial diluted slurry experiment day was 65% for *E.coli*, 77% for enterococci, and 53% for coliphage for all experiments. *E.coli* removal of 51%, enterococci

removal of 72%, and coliphage removal of 48% was found after the second day experiments. The sampled bioretention cells had either a sand-only or fly-ash amended filter media providing this study the ability to measure removals based on media type as well. Studies by Zhang *et al.* (2010) and Bradley *et al.* (2011) showed amended media could provide greater microbial removal than those not amended. Data did not support greater inactivation with amended soils, however. The column experiments were designed to have fifteen columns with sand-only filter media and fifteen with fly-ash amended media. Results from this study showed that both site and filter media type were significantly different ($\alpha < 0.05$) for all three microbes removal efficiencies when considering the Botanic Garden site only. Furthermore, only site was significant ($\alpha < 0.05$) for all microbes when considering all 30 cores. In short, results from this laboratory column experiment confirm that bioretention will remove microbes up to 99%. Additionally, media type, site, and the particle size of the filter media do have a significant effect on removal of microbes. The extent of variable effect depends on the specific microbe. Meaning, *E.coli* and enterococci had media type and % medium sand in common but differed in the other variables that were important in their regression equations, Appendix F.

The field study utilized three of the bioretention cells sampled and used in the column study described previously. These cells were all located in Grove, Oklahoma all having the fly-ash amendment in the filter media layer of the cell.

This study quantified the removal capacity of bioretention with fly-ash amended soils. Measured results are conclusive that filter media containing the fly-ash amendment successfully remove microbes. The mean removal for the Grove, Oklahoma sites was 63% for *E.coli*, 65% for enterococci, and 67% for coliphage.

Literature and results from both column and field studies described above provide insight as to which factors most enhance the removal and inactivation of microbes from urban stormwater runoff by bioretention. Filter media size, type, and size of the bioretention cell (both depth and surface area) are three overlapping factors that undoubtedly impact the removal of microbial pollution. Regression equations show that filter media type is a common thread in all three microbes' removal. The regression analyses were different depending on microbes when considering filter media size. Finally, depth of bioretention was shown to be a limiting factor by Hathaway *et al.* (2011) and Coffman *et al.* (2008) stated that surface area affects the removal capacity of bioretention.

6.2 Lessons Learned

The first observation was found when sampling established bioretention cells in Oklahoma for use in the laboratory experiment. A common finding was the design depth and media consistency was not the same as the as-built depth and media consistency. This proved to be a problem as cells were not deep enough to core and media would not drain because there was excessive clay content within the filter media layer. Also, there is conflicting views in literature on the effect of

flowrate on removal efficiency of bioretention. In the column study the flowrate was not measured for each pore volume. This was an oversight on the part of the researcher. The overall column flowrate was measured however, but the regression equations showed this flowrate was not significant to removal efficiency. It would be interesting to know whether the pore volume flowrate would have different results. Another limiting factor in this research occurred within the field study. The lack of sand-only bioretention data to compare to the fly-ash amended bioretention data in reference to microbial removal was noted.

6.3 Future Work

This research has merely breached the surface of understanding the removal relationships between microbes in urban stormwater runoff and bioretention. Additional laboratory experiments utilizing intact soil cores from established bioretention cells optimizing both media size and soil amendments would be highly beneficial. This may not be possible based on current established bioretention cells available for sampling. Another option would be packed columns with the variable being soil amendments and the control the media size, and vice versa. An exhaustive analysis of amendments and media size could lead to optimization of bioretention for more than only microbial removal.

Both sand-only and fly-ash amended bioretention cells met the USEPA recreation criteria for *E.coli* and enterococci less than 65% of the time based on

captured storms on the sampled cells. Although, there is difference between water that outlets into receiving water that can further assimilate the influx of microbial pollution and those that cannot, it should be the goal of control measures like bioretention to have high removal efficiency while also striving to achieve USEPA criteria. This goal will only have a positive impact on human health as related to microbial contamination in urban environments. Complementary experiments in a laboratory setting and field setting of the same intact, established filter media from bioretention would be ideal to better define the individual and coupled effect of factors like filter media size and type.

The major removal processes occurring in bioretention is physical filtration and adsorption. Media size is a limiting factor for the removal of microbes according to Zhang *et al.* (2010) and Clark and Pitt (2012). The microbe size likely has an impact on the affect filter media plays in removal, larger microbes like bacteria and protozoa for example will have greater removal from filtration. Filter media composition, meaning amended or not will also have an effect on microbial removal. Amended media in bioretention is shown to enhance *E.coli* removal by 5% to 31%, enterococci removal by 17% to 29%, and coliphage removal up to 15% over sand-only filter media based on data from column and field studies in this research. Regression equations found in Appendix F show interesting relationships between % fine sand, % medium sand, and % clay based on target microbe. These relationships need to be validated and further understood

through additional experiments and both in the field and laboratory. In summary, this entire study has found that filter media size and type are factors that observably and statistically have significant impact on removal of microbial pollution. These factors need to be better defined and understood as interactions are still not well understood. Also, there is still a debate on whether or not flowrate truly affect the ability for bioretention to removal pollution (Coffman *et al.*, 2008). Regardless, experiments have shown that cell depth has an impact as cells with less than 0.6 m actually served as a source for pollution versus removal (Hathaway *et al.*, 2011). Furthermore, numerous researchers suggest that removal of microbes will increase as bioretention matures. Finally, this study would be remiss if it did not note the impact of plant selection and sunlight availability on the removal of microbes as well. Though the filter media layer is of great importance there is no question that the surface of the cells is also a location to assist in removal or inactivation of microbial pollution.

This study provides recommendations on ways to target microbial removal with bioretention. It is feasible to consider that through additional laboratory and field experiments bioretention could be optimized to enhance the removal of microbial pollution along with other pollutants. As most watersheds are not merely affected by only one pollutant a coupled or grouped optimization is a future goal for bioretention removal.

References:

Bradley, I., Straub, A., Maraccini, P., Markazi, S., and Nguyen, T. (2011). "Iron oxide amended biosand filters for virus removal." *Water Research,* 45, 4501-4510.

Clark, S.E., and Pitt, R. (2012). "Targeting treatment technologies to address specific stormwater pollutants and numeric discharge limits." *Water Research*, 46, 6715-6730.

Coffman, L. and Ruby, M. (2008). "Bacterra by Filterra Advanced BioretentionSystem: Discussion of the Benefits, Mechanisms, and Efficiencies for Bacteria Removal." *Low Impact Development for Urban Ecosystem and Habitat Protection*. pp. 1-13. doi: 10.1061/41009(333)93

Hathaway, J., Hunt, W., Graves, A., and Wright, J. (2011). "Field Evaluation of Bioretention Indicator Bacteria Sequestration in Wilmington, North Carolina." *J. Environ. Eng.*, 137(12), 1103–1113.

Zhang, L., Seagren, E., Davis, A., and Karns, J. (2010). "The capture and destruction of Escherichia coli from simulated urban runoff using conventional bioretention media and iron oxide-coated sand." *Water Environment Research,* 82(8), 701-713.

APPENDIX A

Table A1 – *E.coli* column data, columns 1 to 3, for Botanic Garden (BG) – sand-only bioretention cell in Stillwater, OK.

Column	Treatment	*E.coli* (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration *E.coli* (MPN/100 ml)	Flow-Weighted Average % Removal	*E. coli* Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
BG S-1-1	spike	19350	1	300	0.90	198630	77.5	1846395	5003	6.5
BG S-1-2	spike	51720	2	600	0.74	198630				
BG S-1-4	spike	54750	4	1200	0.72	198630				
BG S-1-1	clean	20460	1	300		0				
BG S-1-2	clean	10860	2	600		0				
BG S-1-4	clean	750	4	1200		0				
BG S-1-8	clean	410	8	2400		0				
BG S-2-1	spike	11120	1	300	0.94	198630	79	1881975	5435	6.9
BG S-2-2	spike	57940	2	600	0.71	198630				
BG S-2-4	spike	46110	4	1200	0.77	198630				
BG S-2-1	clean	32550	1	300		0				
BG S-2-2	clean	3500	2	600		0				
BG S-2-4	clean	1460	4	1200		0				
BG S-2-8	clean	520	8	2400		0				
BG S-3-1	spike	13760	1	300	0.93	198630	82.3	1961670	10048	12.3
BG S-3-2	spike	43520	2	600	0.78	198630				
BG S-3-4	spike	41060	4	1200	0.79	198630				
BG S-3-1	clean	38730	1	300		0				
BG S-3-2	clean	15150	2	600		0				
BG S-3-4	clean	4410	4	1200		0				
BG S-3-8	clean	2280	8	2400		0				

Table A2 – *E.coli* column data, columns 5 and 6, for Botanic Garden (BG) – sand-only bioretention cell in Stillwater, OK.

Column	Treatment	*E.coli* (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration *E.coli* (MPN/100 ml)	Flow-Weighted Average % Removal	*E. coli* Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
BG S-5-1	spike	34480	1	300	0.83	198630	82.7	19700115	33866	41.3
BG S-5-2	spike	34410	2	600	0.83	198630				
BG S-5-4	spike	34480	4	1200	0.83	198630				
BG S-5-1	clean	129970	1	300		0				
BG S-5-2	clean	19180	2	600		0				
BG S-5-4	clean	34480	4	1200		0				
BG S-5-8	clean	3500	8	2400		0				
BG S-6-1	spike	14970	1	300	0.92	198630	79.6	1896435	4131	2.6
BG S-6-2	spike	54750	2	600	0.72	198630				
BG S-6-4	spike	43520	4	1200	0.78	198630				
BG S-6-1	clean	12670	1	300		0				
BG S-6-2	clean	2260	2	600		0				
BG S-6-4	clean	310	4	1200		0				

Table A3 – *E.coli* column data, columns 1 to 3, for Airport (AP) – sand-only bioretention cell in Fayetteville, AR.

Column	Treatment	*E.coli* (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration *E.coli* (MPN/100 ml)	Flow-Weighted Average % Removal	*E. coli* Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
AP - 1 - 1	spike	14600	1	300	0.60	36800	81.9	723600	1567	7.8
AP - 1 - 2	spike	8500	2	600	0.77	36800				
AP - 1 - 4	spike	5200	4	1200	0.86	36800				
AP - 1 - 8	spike	4100	8	2400	0.89	0				
AP - 1 - 1	clean	11000	1	300		0				
AP - 1 - 2	clean	5200	2	600		0				
AP - 1 - 4	clean	0	4	1200		0				
AP - 1 - 8	clean	0	8	2400		0				
AP - 1 -12	clean	0	12	3600		0				
AP - 2 - 1	spike	28500	1	300	0.23	36800	23.3	206000	1104	12.9
AP - 2 - 2	spike	29400	2	600	0.20	36800				
AP - 2 - 4	spike	23800	4	1200	0.35	36800				
AP - 2 - 8	spike	32700	8	2400	0.11	0				
AP- 2 - 1	clean	4100	1	300		0				
AP - 2 - 2	clean	3100	2	600		0				
AP- 2 - 4	clean	0	4	1200		0				
AP - 2 - 8	clean	1000	8	2400		0				
AP - 2 -12	clean	0	12	3600		0				
AP - 3 - 1	spike	29200	1	300	0.21	36800	28.6	252300	3017	28.7
AP - 3 - 2	spike	47300	2	600	0.29	36800				
AP - 3 - 4	spike	24300	4	1200	0.34	36800				
AP - 3 - 8	spike	27500	8	2400	0.25	0				
AP- 3 - 1	clean	13400	1	300		0				
AP - 3 - 2	clean	5200	2	600		0				
AP - 3 - 4	clean	3000	4	1200		0				
AP - 3 - 8	clean	0	8	2400		0				
AP - 3 -12	clean	3000	12	3600		0				

Table A4 – *E.coli* column data, columns 5 and 6, for Airport (AP) – sand-only bioretention cell in Fayetteville, AR.

Column	Treatment	*E.coli* (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration *E.coli* (MPN/100 ml)	Flow-Weighted Average % Removal	*E. coli* Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
AP - 5 - 1	spike	37900	1	300	0.03	36800	26.6	234750	5313	54.3
AP - 5 - 2	spike	38400	2	600	0.04	36800				
AP - 5 - 4	spike	54800	4	1200	0.49	36800				
AP- 5 - 8	spike	28500	8	2400	0.23	0				
AP - 5 - 1	clean	27500	1	300		0				
AP - 5 - 2	clean	3000	2	600		0				
AP - 5 - 4	clean	3100	4	1200		0				
AP- 5 - 8	clean	4100	8	2400		0				
AP- 5 - 12	clean	2000	12	3600		0				
AP - 6 - 1	spike	10900	1	300	0.70	36800	58.6	517350	5050	23.4
AP - 6 - 2	spike	18100	2	600	0.51	36800				
AP - 6 - 4	spike	15800	4	1200	0.57	36800				
AP- 6 - 8	spike	14600	8	2400	0.60	0				
AP - 6 - 1	clean	12200	1	300		0				
AP - 6 - 2	clean	8600	2	600		0				
AP - 6 - 4	clean	7500	4	1200		0				
AP- 6 - 8	clean	2000	8	2400		0				
AP- 6 - 12	clean	2000	12	3600		0				

Table A5 – *E.coli* column data, columns 1 to 3, for Airport (AR) – sand-only bioretention cell in Fayetteville, AR. ND = No Drain.

Column	Treatment	*E.coli* (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration *E.coli* (MPN/100 ml)	Flow-Weighted Average % Removal	*E. coli* Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
AR - 1 - 1	spike	13400	1	300	0.95	261300	91.3	5727300	6763	4.3
AR - 1 - 2	spike	25900	2	600	0.90	261300				
AR - 1 - 4	spike	20100	4	1200	0.92	261300				
AR - 1 - 8	spike	27500	8	2400	0.89	0				
AR - 1 - 1	clean	25900	1	300		0				
AR - 1 - 2	clean	4100	2	600		0				
AR - 1 - 4	clean	0	4	1200		0				
AR - 1 - 8	clean	8600	8	2400		0				
AR - 1 -12	clean	5200	12	3600		0				
AR - 2 - 1	spike	32700	1	300	0.87	261300	84.3	5284950		
AR - 2 - 2	spike	56300	2	600	0.78	261300				
AR - 2 - 4	spike	69700	4	1200	0.73	261300				
AR - 2 - 8	spike	1000	8	2400	1.00	0				
AR- 2 - 1	clean	ND	1	300		0				
AR - 2 - 2	clean	ND	2	600		0				
AR- 2 - 4	clean	ND	4	1200		0				
AR - 2 - 8	clean	ND	8	2400		0				
AR - 2 -12	clean	ND	12	3600		0				
AR - 3 - 1	spike	139600	1	300	0.47	261300	84.2	5281950		
AR - 3 - 2	spike	43200	2	600	0.83	261300				
AR - 3 - 4	spike	26200	4	1200	0.90	261300				
AR - 3 - 8	spike	18700	8	2400	0.93	0				
AR- 3 - 1	clean	ND	1	300		0				
AR - 3 - 2	clean	ND	2	600		0				
AR - 3 - 4	clean	ND	4	1200		0				
AR - 3 - 8	clean	ND	8	2400		0				
AR - 3 -12	clean	ND	12	3600		0				

Table A6 – *E.coli* column data, column 6 for Airport (AR) – sand-only bioretention cell in Fayetteville, AR, column 5 did not drain for the experiment.

Column	Treatment	*E.coli* (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration *E.coli* (MPN/100 ml)	Flow-Weighted Average % Removal	*E. coli* Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
AR-6-1	spike	110600	1	300	0.58	261300	50.0	3135900	44392	34.0
AR-6-2	spike	146700	2	600	0.44	261300				
AR-6-4	spike	error	4	1200		261300				
AR-6-8	spike	123600	8	2400	0.53	0				
AR-6-1	clean	2000	1	300		0				
AR-6-2	clean	3100	2	600		0				

Table A7 – *E.coli c*olumn data, columns 1 to 3, for Botanic Garden (BG) – fly-ash amended bioretention cell in Stillwater, OK.

Column	Treatment	*E.coli* (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration *E.coli* (MPN/100 ml)	Flow-Weighted Average % Removal	*E. coli* Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
BG FA-1-1	spike	100	1	300	0.99	1119000	99.9	13415640	571	0.0
BG FA-1-2	spike	520	2	600	0.99	1119000				
BG FA-1-4	spike	2160	4	1200	0.99	1119000				
BG FA-1-1	clean	2590	1	300		0				
BG FA-1-2	clean	920	2	600		0				
BG FA-1-4	clean	200	4	1200		0				
BG FA-1-8	clean	0	8	2400		0				
BG FA-2-1	spike	0	1	300	1.00	1119000	100	13428000	13	0.0
BG FA-2-2	spike	0	2	600	1.00	1119000				
BG FA-2-4	spike	0	4	1200	1.00	1119000				
BG FA-2-1	clean	100	1	300		0				
BG FA-2-2	clean	0	2	600		0				
BG FA-2-4	clean	0	4	1200		0				
BG FA-2-7	clean	0	7	2100		0				
BG FA-3-1	spike	300	1	300	0.99	1119000	99.9	13418865	1551	0.2
BG FA-3-2	spike	630	2	600	0.99	1119000				
BG FA-3-4	spike	1200	4	1200	0.99	1119000				
BG FA-3-1	clean	6760	1	300		0				
BG FA-3-2	clean	1990	2	600		0				
BG FA-3-4	clean	630	4	1200		0				
BG FA-3-8	clean	310	8	2400		0				

Table A8 – *E.coli c*olumn data, columns 4 and 5, for Botanic Garden (BG) – fly-ash amended bioretention cell in Stillwater, Oklahoma.

Column	Treatment	*E.coli* (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration *E.coli* (MPN/100 ml)	Flow-Weighted Average % Removal	*E. coli* Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
BG FA-4-1	spike	100	1	300	0.99	1119000	99.9	13420725	450	0.0
BG FA-4-2	spike	200	2	600	0.99	1119000				
BG FA-4-4	spike	1350	4	1200	0.99	1119000				
BG FA-4-1	clean	100	1	300		0				
BG FA-4-2	clean	100	2	600		0				
BG FA-4-4	clean	200	4	1200		0				
BG FA-4-8	clean	1100	8	2400		0				
BG FA-5-1	spike	14390	1	300	0.98	198630	96.9	13021185	1647	0.3
BG FA-5-2	spike	29090	2	600	0.97	198630				
BG FA-5-4	spike	51720	4	1200	0.95	198630				
BG FA-5-1	clean	3790	1	300		0				
BG FA-5-2	clean	1830	2	600		0				
BG FA-5-4	clean	1090	4	1200		0				
BG FA-5-8	clean	1350	8	2400		0				

Table A9 – *E.coli* column data, columns 1 to 3, for Grand Lake Association (G-A) – fly-ash amended bioretention cell in Grove, OK.

Column	Treatment	*E.coli* (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration *E.coli* (MPN/100 ml)	Flow-Weighted Average % Removal	*E. coli* Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
G - A - 1 - 1	spike	116200	1	300	0.84	727000	51.8	9045000	13896	5.5
G - A - 1 - 2	spike	344800	2	600	0.53	727000				
G - A - 1 - 4	spike	435200	4	1200	0.40	727000				
G - A - 1 - 8	spike	344800	8	2400	0.53	0				
G - A - 1 - 1	clean	108100	1	300		0				
G -A - 1 - 2	clean	6300	2	600		0				
G - A - 1 - 4	clean	13400	4	1200		0				
G - A - 1 - 8	clean	2000	8	2400		0				
G - A -1 -12	clean	0	12	3600		0				
G - A - 2 - 1	spike	65000	1	300	0.91	727000	77.2	13465650	55588	9.9
G - A - 2 - 2	spike	185000	2	600	0.75	727000				
G - A - 2 - 4	spike	178900	4	1200	0.75	727000				
G - A - 2 - 8	spike	179300	8	2400	0.75	727000				
G - A - 2 - 1	clean	46400	1	300		0				
G -A - 2 - 2	clean	83300	2	600		0				
G - A - 2 - 4	clean	58100	4	1200		0				
G - A - 2 - 8	clean	50400	8	2400		0				
G - A -2 -12	clean	47300	12	3600		0				
G - A - 3 - 1	spike	214300	1	300	0.71	727000	64.7	11287500	17846	3.8
G - A - 3 - 2	spike	218700	2	600	0.70	727000				
G - A - 3 - 4	spike	261300	4	1200	0.64	727000				
G - A - 3 - 8	spike	290900	8	2400	0.60	727000				
G - A - 3 - 1	clean	50400	1	300		0				
G - A - 3 - 2	clean	47300	2	600		0				
G - A - 3 - 4	clean	28100	4	1200		0				
G - A - 3 - 8	clean	1000	8	2400		0				
G - A - 3-12	clean	2000	12	3600		0				

Table A10 – *E.coli* column data, columns 4 and 5, for Grand Lake Association (G-A) – fly-ash amended bioretention cell in Grove, OK.

Column	Treatment	*E.coli* (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration *E.coli* (MPN/100 ml)	Flow-Weighted Average % Removal	*E. coli* Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
G - A - 4 - 1	spike	44600	1	300	0.94	727000	61.3	10697100	6763	1.5
G - A - 4 - 2	spike	344800	2	600	0.53	727000				
G - A - 4 - 4	spike	275500	4	1200	0.62	727000				
G - A - 4 - 8	spike	344800	8	2400	0.53	727000				
G - A - 4 - 1	clean	19500	1	300		0				
G - A - 4 - 2	clean	7400	2	600		0				
G - A - 4 - 4	clean	4100	4	1200		0				
G - A - 4 - 8	clean	8500	8	2400		0				
G - A - 4 -12	clean	0	12	3600		0				
G - A - 5 - 1	spike	290900	1	300	0.60	727000	62.1	10826475	6071	1.0
G - A - 5 - 2	spike	325500	2	600	0.55	727000				
G - A - 5 - 4	spike	461100	4	1200	0.37	727000				
G - A - 5 - 8	spike	17890	8	2400	0.98	727000				
G - A - 5 - 1	clean	25900	1	300		0				
G - A - 5 - 2	clean	20900	2	600		0				
G - A - 5 - 4	clean	5200	4	1200		0				
G - A - 5 - 8	clean	0	8	2400		0				
G - A - 5 -12	clean	0	12	3600		0				

Table A11 – *E.coli* column data, columns 1 to 3, for Grove High School (G-HS) – fly-ash amended bioretention cell in Grove, OK.

Column	Treatment	*E.coli* (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration *E.coli* (MPN/100 ml)	Flow-Weighted Average % Removal	*E. coli* Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
G - HS - 1 - 1	spike	155310	1	300	0.22	198630	18.4	877980	22461	92.0
G - HS - 1 - 2	spike	104620	2	600	0.47	198630				
G - HS - 1 - 4	spike	198630	4	1200	0.00	198630				
G - HS - 1 - 8	spike	241960	8	2400	0.23	198630				
G - HS - 1 - 1	clean	173290	1	300		0				
G - HS - 1 - 2	clean	24890	2	600		0				
G - HS - 1 - 4	clean	11870	4	1200		0				
G - HS - 1 - 8	clean	3840	8	2400		0				
G - HS - 1 -12	clean	3010	12	3600		0				
G - HS - 2 - 1	spike	68670	1	300	0.65	198630	26.8	1278870	5939	11.1
G - HS - 2 - 2	spike	129970	2	600	0.35	198630				
G - HS - 2 - 4	spike	241960	4	1200	0.22	198630				
G - HS - 2 - 8	spike	173290	8	2400	0.13	198630				
G - HS - 2 - 1	clean	20460	1	300		0				
G - HS - 2 - 2	clean	9340	2	600		0				
G - HS - 2 - 4	clean	5200	4	1200		0				
G - HS - 2 - 8	clean	4280	8	2400		0				
G - HS - 2 -12	clean	970	12	3600		0				
G - HS - 3 - 1	spike	54570	1	300	0.73	198630	48.4	2305095	12449	12.9
G - HS - 3 - 2	spike	98040	2	600	0.51	198630				
G - HS - 3 - 4	spike	98040	4	1200	0.51	198630				
G - HS - 3 - 8	spike	129970	8	2400	0.35	198630				
G - HS - 3 - 1	clean	92080	1	300		0				
G - HS - 3 - 2	clean	18720	2	600		0				
G - HS - 3 - 4	clean	4870	4	1200		0				
G - HS - 3 - 8	clean	2920	8	2400		0				
G - HS - 3 -12	clean	740	12	3600		0				

Table A12 – *E.coli* column data, columns 4 and 5, for Grove High School (G-HS) – fly-ash amended bioretention cell in Grove, Oklahoma.

Column	Treatment	*E.coli* (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration *E.coli* (MPN/100 ml)	Flow-Weighted Average % Removal	*E. coli* Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
G - HS - 4 - 1	spike	155310	1	300	0.22	198630	23.1	1102530	9873	21.5
G - HS - 4 - 2	spike	141360	2	600	0.29	198630				
G - HS - 4 - 4	spike	155310	4	1200	0.22	198630				
G - HS - 4 - 8	spike	241960	8	2400	0.22	198630				
G - HS - 4 - 1	clean	92080	1	300		0				
G - HS - 4 - 2	clean	6570	2	600		0				
G - HS - 4 - 4	clean	2980	4	1200		0				
G - HS - 4 - 8	clean	1210	8	2400		0				
G - HS - 4 -12	clean	1080	12	3600		0				
G - HS - 5 - 1	spike	68670	1	300	0.65	198630	27.6	1316790	10408	18.9
G - HS - 5 - 2	spike	129970	2	600	0.35	198630				
G - HS - 5 - 4	spike	129970	4	1200	0.35	198630				
G - HS - 5 - 8	spike	198630	8	2400	0.00	198630				
G - HS - 5 - 1	clean	77010	1	300		0				
G - HS - 5 - 2	clean	9870	2	600		0				
G - HS - 5 - 4	clean	6500	4	1200		0				
G - HS - 5 - 8	clean	2430	8	2400		0				
G - HS - 5 -12	clean	1320	12	3600		0				

APPENDIX B

Table B1 –Enterococci column data, columns 1 to 3, for Botanic Garden (BG) – sand-only bioretention cell in Stillwater, OK.

Column	Treatment	enterococci (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration enterococci (MPN/100 ml)	Flow-Weighted Average % Removal	enterococci Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
BG S-1-1	spike	7480	1	300	0.98	300000	80.6	2902470	4108	3.4
BG S-1-2	spike	57940	2	600	0.81	300000				
BG S-1-4	spike	92080	4	1200	0.69	300000				
BG S-1-1	clean	29870	1	300		0				
BG S-1-2	clean	1210	2	600		0				
BG S-1-4	clean	310	4	1200		0				
BG S-1-8	clean	100	8	2400		0				
BG S-2-1	spike	24000	1	300	0.92	300000	81.8	2943090	7483	6.0
BG S-2-2	spike	61310	2	600	0.80	300000				
BG S-2-4	spike	68670	4	1200	0.77	300000				
BG S-2-1	clean	54750	1	300		0				
BG S-2-2	clean	1870	2	600		0				
BG S-2-4	clean	520	4	1200		0				
BG S-2-8	clean	300	8	2400		0				
BG S-3-1	spike	30760	1	300	0.90	300000	72.6	2613840	11574	10.6
BG S-3-2	spike	68670	2	600	0.77	300000				
BG S-3-4	spike	129970	4	1200	0.57	300000				
BG S-3-1	clean	46110	1	300		0				
BG S-3-2	clean	11450	2	600		0				
BG S-3-4	clean	6970	4	1200		0				
BG S-3-8	clean	3360	8	2400		0				

Table B2 – Enterococci *c*olumn data, columns 5 and 6, for Botanic Garden (BG) – sand-only bioretention cell in Stillwater, OK.

Column	Treatment	enterococci (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration enterococci (MPN/100 ml)	Flow-Weighted Average % Removal	enterococci Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
BG S-5-1	spike	61310	1	300	0.80	300000	71.1	2560530	19983	19.0
BG S-5-2	spike	98040	2	600	0.67	300000				
BG S-5-4	spike	92080	4	1200	0.69	300000				
BG S-5-1	clean	92080	1	300		0				
BG S-5-2	clean	8550	2	600		0				
BG S-5-4	clean	17220	4	1200		0				
BG S-5-8	clean	1320	8	2400		0				
BG S-6-1	spike	20980	1	300	0.93	300000	75.9	2732820	3119	1.4
BG S-6-2	spike	86640	2	600	0.71	300000				
BG S-6-4	spike	92080	4	1200	0.69	300000				
BG S-6-1	clean	9850	1	300		0				
BG S-6-2	clean	1340	2	600		0				
BG S-6-4	clean	410	4	1200		0				

Table B3 – Enterococci *c*olumn data, columns 1 to 3, for Airport (AP) – sand-only bioretention cell in Fayetteville, AR.

Column	Treatment	enterococci (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration enterococci (MPN/100 ml)	Flow-Weighted Average % Removal	enterococci Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
AP - 1 - 1	spike	11800	1	300	0.99	1732900	92.9	38652450	40800	3.8
AP - 1 - 2	spike	48200	2	600	0.97	1732900				
AP - 1 - 4	spike	103900	4	1200	0.94	1732900				
AP - 1 - 8	spike	233300	8	2400	0.87	0				
AP - 1 - 1	clean	39900	1	300		0				
AP - 1 - 2	clean	41700	2	600		0				
AP - 1 - 4	clean	0	4	1200		0				
AP - 1 - 8	clean	0	8	2400		0				
AP - 1 -12	clean	0	12	3600		0				
AP - 2 - 1	spike	365400	1	300	0.79	1732900	71.5	29739600	16675	1.3
AP - 2 - 2	spike	648800	2	600	0.62	1732900				
AP - 2 - 4	spike	816400	4	1200	0.53	1732900				
AP - 2 - 8	spike	64880	8	2400	0.96	0				
AP- 2 - 1	clean	68300	1	300		0				
AP - 2 - 2	clean	45200	2	600		0				
AP- 2 - 4	clean	12200	4	1200		0				
AP - 2 - 8	clean	5200	8	2400		0				
AP - 2 -12	clean	2000	12	3600		0				
AP - 3 - 1	spike	344800	1	300	0.80	1732900	54.9	22840050	16671	1.8
AP - 3 - 2	spike	461100	2	600	0.73	1732900				
AP - 3 - 4	spike	920800	4	1200	0.47	1732900				
AP - 3 - 8	spike	980400	8	2400	0.43	0				
AP- 3 - 1	clean	48100	1	300		0				
AP - 3 - 2	clean	21600	2	600		0				
AP - 3 - 4	clean	21600	4	1200		0				
AP - 3 - 8	clean	6300	8	2400		0				
AP - 3 -12	clean	13200	12	3600		0				

Table B4 – Enterococci column data, columns 5 and 6, for Airport (AP) – sand-only bioretention cell in Fayetteville, AR.

Column	Treatment	enterococci (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration enterococci (MPN/100 ml)	Flow-Weighted Average % Removal	enterococci Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
AP - 5 - 1	spike	579400	1	300	0.67	1732900	66.5	27681450	16946	1.5
AP - 5 - 2	spike	325500	2	600	0.81	1732900				
AP - 5 - 4	spike	648800	4	1200	0.63	1732900				
AP- 5 - 8	spike	648800	8	2400	0.63	0				
AP - 5 - 1	clean	98300	1	300		0				
AP - 5 - 2	clean	15600	2	600		0				
AP - 5 - 4	clean	13800	4	1200		0				
AP- 5 - 8	clean	4100	8	2400		0				
AP- 5 - 12	clean	10900	12	3600		0				
AP - 6 - 1	spike	116900	1	300	0.94	1732900	78.9	32843850	42196	3.1
AP - 6 - 2	spike	248900	2	600	0.86	1732900				
AP - 6 - 4	spike	325500	4	1200	0.81	1732900				
AP- 6 - 8	spike	579400	8	2400	0.67	0				
AP - 6 - 1	clean	139100	1	300		0				
AP - 6 - 2	clean	40800	2	600		0				
AP - 6 - 4	clean	35500	4	1200		0				
AP- 6 - 8	clean	27500	8	2400		0				
AP- 6 - 12	clean	37900	12	3600		0				

Table B5 – Enterococci column data, columns 1 to 3, for Airport (AR) – sand-only bioretention cell in Fayetteville, AR.

Column	Treatment	enterococci (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration enterococci (MPN/100 ml)	Flow-Weighted Average % Removal	enterococci Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
AR - 1 - 1	spike	3100	1	300	0.99	2419600	85.3	49723650	33975	2.5
AR - 1 - 2	spike	30700	2	600	0.99	2419600				
AR - 1 - 4	spike	143700	4	1200	0.94	2419600				
AR - 1 - 8	spike	920800	8	2400	0.62	0				
AR - 1 - 1	clean	248900	1	300		0				
AR - 1 - 2	clean	15800	2	600		0				
AR - 1 - 4	clean	5200	4	1200		0				
AR - 1 - 8	clean	20600	8	2400		0				
AR - 1 -12	clean	13400	12	3600		0				
AR - 2 - 1	spike	410600	1	300	0.83	2419600	73.6	42749400		
AR - 2 - 2	spike	517200	2	600	0.79	2419600				
AR - 2 - 4	spike	1299700	4	1200	0.46	2419600				
AR - 2 - 8	spike	8600	8	2400	0.99	0				
AR- 2 - 1	clean	ND	1	300		0				
AR - 2 - 2	clean	ND	2	600		0				
AR- 2 - 4	clean	ND	4	1200		0				
AR - 2 - 8	clean	ND	8	2400		0				
AR - 2 -12	clean	ND	12	3600		0				
AR - 3 - 1	spike	770100	1	300	0.68	2419600	81.7	47425050		
AR - 3 - 2	spike	727000	2	600	0.70	2419600				
AR - 3 - 4	spike	435200	4	1200	0.82	2419600				
AR - 3 - 8	spike	152900	8	2400	0.94	0				
AR- 3 - 1	clean	ND	1	300		0				
AR - 3 - 2	clean	ND	2	600		0				
AR - 3 - 4	clean	ND	4	1200		0				
AR - 3 - 8	clean	ND	8	2400		0				
AR - 3 -12	clean	ND	12	3600		0				

ND = No Drain, the column did not drain.

Table B6 – Enterococci column data, column 6 for Airport (AR) – sand-only bioretention cell in Fayetteville, AR.

Column	Treatment	enterococci (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration enterococci (MPN/100 ml)	Flow-Weighted Average % Removal	enterococci Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
AR-6-1	spike	1553100	1	300	0.36	2419600	17.9	10398000	12350	2.9
AR-6-2	spike	1553100	2	600	0.36	2419600				
AR-6-4	spike	error	4	1200		2419600				
AR-6-8	spike	2419600	8	2400	0.00	0				
AR-6-1	clean	21600	1	300		0				
AR-6-2	clean	3100	2	600		0				

Table B7 – Enterococci column data, columns 1 to 3, for Botanic Garden (BG) – fly-ash amended bioretention cell in Stillwater, OK.

Column	Treatment	enterococci (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration enterococci (MPN/100 ml)	Flow-Weighted Average % Removal	enterococci Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
BG FA-1-1	spike	100	1	300	0.99	172800	98.8	2048775	179	0.2
BG FA-1-2	spike	410	2	600	0.99	172800				
BG FA-1-4	spike	5040	4	1200	0.97	172800				
BG FA-1-1	clean	730	1	300		0				
BG FA-1-2	clean	100	2	600		0				
BG FA-1-4	clean	100	4	1200		0				
BG FA-1-8	clean	100	8	2400		0				
BG FA-2-1	spike	0	1	300	1.00	172800	100	2073600	0.0	0.0
BG FA-2-2	spike	0	2	600	1.00	172800				
BG FA-2-4	spike	0	4	1200	1.00	172800				
BG FA-2-1	clean	0	1	300		0				
BG FA-2-2	clean	0	2	600		0				
BG FA-2-4	clean	0	4	1200		0				
BG FA-2-7	clean	0	7	2100		0				
BG FA-3-1	spike	310	1	300	0.99	172800	98.9	2050530	31	0.0
BG FA-3-2	spike	1560	2	600	0.99	172800				
BG FA-3-4	spike	3360	4	1200	0.98	172800				
BG FA-3-1	clean	100	1	300		0				
BG FA-3-2	clean	100	2	600		0				
BG FA-3-4	clean	0	4	1200		0				
BG FA-3-8	clean	0	8	2400		0				

Table B8 – Enterococci column data, columns 4 to 5, for Botanic Garden (BG) – fly-ash amended bioretention cell in Stillwater, OK.

Column	Treatment	enterococci (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration enterococci (MPN/100 ml)	Flow-Weighted Average % Removal	enterococci Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
BG FA-4-1	spike	200	1	300	0.99	172800	99.8	2070210	0.0	0.0
BG FA-4-2	spike	100	2	600	0.99	172800				
BG FA-4-4	spike	520	4	1200	0.99	172800				
BG FA-4-1	clean	0	1	300		0				
BG FA-4-2	clean	0	2	600		0				
BG FA-4-4	clean	0	4	1200		0				
BG FA-4-8	clean	0	8	2400		0				
BG FA-5-1	spike	129970	1	300	0.25	172800			652	
BG FA-5-2	spike	Error	2	600	Error	172800				
BG FA-5-4	spike	Error	4	1200	Error	172800				
BG FA-5-1	clean	3360	1	300		0				
BG FA-5-2	clean	520	2	600		0				
BG FA-5-4	clean	100	4	1200		0				
BG FA-5-8	clean	310	8	2400		0				

Table B9 – Enterococci column data, columns 1 to 3, for Grand Lake Association (G-A) – fly-ash amended bioretention cell in Grove, OK.

Column	Treatment	enterococci (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration enterococci (MPN/100 ml)	Flow-Weighted Average % Removal	enterococci Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
G - A - 1 - 1	spike	65700	1	300	0.96	1732900	81.7	33957300	11271	1.2
G - A - 1 - 2	spike	307600	2	600	0.82	1732900				
G - A - 1 - 4	spike	416000	4	1200	0.76	1732900				
G - A - 1 - 8	spike	307600	8	2400	0.82	1732900				
G - A - 1 - 1	clean	38400	1	300		0				
G -A - 1 - 2	clean	12300	2	600		0				
G - A - 1 - 4	clean	20300	4	1200		0				
G - A - 1 - 8	clean	3000	8	2400		0				
G - A -1 -12	clean	2000	12	3600		0				
G - A - 2 - 1	spike	16100	1	300	0.99	1732900	92.2	38344650	38213	2.4
G - A - 2 - 2	spike	59400	2	600	0.97	1732900				
G - A - 2 - 4	spike	95900	4	1200	0.94	1732900				
G - A - 2 - 8	spike	275500	8	2400	0.84	1732900				
G - A - 2 - 1	clean	12000	1	300		0				
G -A - 2 - 2	clean	50400	2	600		0				
G - A - 2 - 4	clean	43500	4	1200		0				
G - A - 2 - 8	clean		8	2400		0				
G - A -2 -12	clean		12	3600		0				
G - A - 3 - 1	spike	195600	1	300	0.89	1732900	89.1	37073700	11158	0.7
G - A - 3 - 2	spike	209800	2	600	0.88	1732900				
G - A - 3 - 4	spike	193500	4	1200	0.89	1732900				
G - A - 3 - 8	spike	165800	8	2400	0.90	1732900				
G - A - 3 - 1	clean	17100	1	300		0				
G - A - 3 - 2	clean	17300	2	600		0				
G - A - 3 - 4	clean	24300	4	1200		0				
G - A - 3 - 8	clean	3100	8	2400		0				
G - A - 3-12	clean	2000	12	3600		0				

Table B10 – Enterococci column data, columns 4 and 5, for Grand Lake Association (G-A) – fly-ash amended bioretention cell in Grove, OK.

Column	Treatment	enterococci (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration enterococci (MPN/100 ml)	Flow-Weighted Average % Removal	enterococci Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
G - A - 4 - 1	spike	18500	1	300	0.99	727000	80.0	33280650	6488	0.5
G - A - 4 - 2	spike	198900	2	600	0.89	727000				
G - A - 4 - 4	spike	410600	4	1200	0.76	727000				
G - A - 4 - 8	spike	488400	8	2400	0.72	727000				
G - A - 4 - 1	clean	13400	1	300		0				
G - A - 4 - 2	clean	6300	2	600		0				
G - A - 4 - 4	clean	4100	4	1200		0				
G - A - 4 - 8	clean	8600	8	2400		0				
G - A - 4 -12	clean	2000	12	3600		0				
G - A - 5 - 1	spike	105000	1	300	0.94	727000	77.6	32272050	5267	0.4
G - A - 5 - 2	spike	260300	2	600	0.85	727000				
G - A - 5 - 4	spike	387300	4	1200	0.78	727000				
G - A - 5 - 8	spike	579400	8	2400	0.67	727000				
G - A - 5 - 1	clean	15800	1	300		0				
G - A - 5 - 2	clean	24600	2	600		0				
G - A - 5 - 4	clean	2000	4	1200		0				
G - A - 5 - 8	clean	1000	8	2400		0				
G - A - 5 -12	clean	0	12	3600		0				

Table B11 – Enterococci column data, columns 1 to 3, for Grove High School (G-HS) – fly-ash amended bioretention cell in Grove, OK.

Column	Treatment	enterococci (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration enterococci (MPN/100 ml)	Flow-Weighted Average % Removal	enterococci Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
G - HS - 1 - 1	spike	15760	1	300	0.36	24810	50.4	300165	3145	37.7
G - HS - 1 - 2	spike	28510	2	600	0.15	24810				
G - HS - 1 - 4	spike	43520	4	1200	0.75	24810				
G - HS - 1 - 8	spike	36540	8	2400	0.47	24810				
G - HS - 1 - 1	clean	28510	1	300		0				
G - HS - 1 - 2	clean	2180	2	600		0				
G - HS - 1 - 4	clean	1100	4	1200		0				
G - HS - 1 - 8	clean	410	8	2400		0				
G - HS - 1 -12	clean	410	12	3600		0				
G - HS - 2 - 1	spike	13760	1	300	0.46	24810	87.4	520680	960	4.4
G - HS - 2 - 2	spike	27550	2	600	0.11	24810				
G - HS - 2 - 4	spike	41060	4	1200	0.65	24810				
G - HS - 2 - 8	spike	68670	8	2400	1.76	24810				
G - HS - 2 - 1	clean	8390	1	300		0				
G - HS - 2 - 2	clean	730	2	600		0				
G - HS - 2 - 4	clean	310	4	1200		0				
G - HS - 2 - 8	clean	200	8	2400		0				
G - HS - 2 -12	clean	100	12	3600		0				
G - HS - 3 - 1	spike	11450	1	300	0.54	24810	92.2	548700	317	1.4
G - HS - 3 - 2	spike	11980	2	600	0.52	24810				
G - HS - 3 - 4	spike	27550	4	1200	0.11	24810				
G - HS - 3 - 8	spike	81640	8	2400	2.29	24810				
G - HS - 3 - 1	clean	1730	1	300		0				
G - HS - 3 - 2	clean	750	2	600		0				
G - HS - 3 - 4	clean	100	4	1200		0				
G - HS - 3 - 8	clean	100	8	2400		0				
G - HS - 3 -12	clean	100	12	3600		0				

Table B12 – Enterococci column data, columns 4 and 5, for Grove High School (G-HS) – fly-ash amended bioretention cell in Grove, OK.

Column	Treatment	enterococci (MPN/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration enterococci (MPN/100 ml)	Flow-Weighted Average % Removal	enterococci Retained by Column (MPN)	Flow-Weighted Average Concentration in Clean (MPN)	% Retained that Washed Out after Clean Flush
G - HS - 4 - 1	spike	34480	1	300	0.39	24810	41.3	245970	958	9.4
G - HS - 4 - 2	spike	29090	2	600	0.17	24810				
G - HS - 4 - 4	spike	77010	4	1200	2.10	24810				
G - HS - 4 - 8	spike	39680	8	2400	0.60	24810				
G - HS - 4 - 1	clean	7710	1	300		0				
G - HS - 4 - 2	clean	840	2	600		0				
G - HS - 4 - 4	clean	410	4	1200		0				
G - HS - 4 - 8	clean	200	8	2400		0				
G - HS - 4 -12	clean	200	12	3600		0				
G - HS - 5 - 1	spike	12460	1	300	0.50	24810	57.8	344400	978	6.8
G - HS - 5 - 2	spike	18660	2	600	0.25	24810				
G - HS - 5 - 4	spike	46110	4	1200	0.89	24810				
G - HS - 5 - 8	spike	36540	8	2400	0.47	24810				
G - HS - 5 - 1	clean	7710	1	300		0				
G - HS - 5 - 2	clean	520	2	600		0				
G - HS - 5 - 4	clean	410	4	1200		0				
G - HS - 5 - 8	clean	310	8	2400		0				
G - HS - 5 -12	clean	310	12	3600		0				

APPENDIX C

Table C1 – Coliphage column data, columns 1 to 3, for Botanic Garden (BG) – sand-only bioretention cell in Stillwater, OK.

Column	Treatment	coliphage (PFU/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration coliphage (PFU/100 ml)	Flow-Weighted Average % Removal	coliphage Retained by Column (PFU)	Flow-Weighted Average Concentration in Clean (PFU)	% Retained that Washed Out after Clean Flush
BG S-1-1	spike	3367	1	300	0.64	9350	63.8	71575	0.00	0.00
BG S-1-2	spike	6783	2	600	0.27	9350				
BG S-1-4	spike	0	4	1200	1.00	9350				
BG S-1-1	clean	0	1	300		0				
BG S-1-2	clean	0	2	600		0				
BG S-1-4	clean	0	4	1200		0				
BG S-1-8	clean	0	8	2400		0				
BG S-2-1	spike	1517	1	300	0.84	9350	58.1	65200	795	29.3
BG S-2-2	spike	4733	2	600	0.49	9350				
BG S-2-4	spike	4700	4	1200	0.50	9350				
BG S-2-1	clean	3317	1	300		0				
BG S-2-2	clean	783	2	600		0				
BG S-2-4	clean	400	4	1200		0				
BG S-2-8	clean	267	8	2400		0				
BG S-3-1	spike	1767	1	300	0.81	9350	55.6	62426	578	22.2
BG S-3-2	spike	4533	2	600	0.52	9350				
BG S-3-4	spike	5350	4	1200	0.43	9350				
BG S-3-1	clean	2717	1	300		0				
BG S-3-2	clean	1217	2	600		0				
BG S-3-4	clean	0	4	1200		0				
BG S-3-8	clean	33	8	2400		0				

Table C2 – Coliphage column data, columns 5 and 6, for Botanic Garden (BG) – sand-only bioretention cell in Stillwater, Ok.

Column	Treatment	coliphage (PFU/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration coliphage (PFU/100 ml)	Flow-Weighted Average % Removal	coliphage Retained by Column (PFU)	Flow-Weighted Average Concentration in Clean (PFU)	% Retained that Washed Out after Clean Flush
BG S-5-1	spike	3483	1	300	0.63	9350	35.9	40250	0.00	0.00
BG S-5-2	spike	6350	2	600	0.32	9350				
BG S-5-4	spike	7317	4	1200	0.22	9350				
BG S-5-1	clean	0	1	300		0				
BG S-5-2	clean	0	2	600		0				
BG S-5-4	clean	0	4	1200		0				
BG S-5-8	clean	0	8	2400		0				
BG S-6-1	spike	667	1	300	0.93	9350	72.4	81251	0.00	0.00
BG S-6-2	spike	3883	2	600	0.58	9350				
BG S-6-4	spike	2550	4	1200	0.72	9350				
BG S-6-1	clean	0	1	300		0				
BG S-6-2	clean	0	2	600		0				
BG S-6-4	clean	0	4	1200		0				

Table C3 – Coliphage column data, columns 2 and 5, for Airport (AP) – sand-only bioretention cell in Fayetteville, AR.

Column	Treatment	coliphage (PFU/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration coliphage (PFU/100 ml)	Flow-Weighted Average % Removal	coliphage Retained by Column (PFU)	Flow-Weighted Average Concentration in Clean (PFU)	% Retained that Washed Out after Clean Flush
AP - 2 - 1	spike	100333	1	300	0.61	256333	43.5	2675998	22250	20.0
AP - 2 - 2	spike	133000	2	600	0.48	256333				
AP - 2 - 4	spike	124333	4	1200	0.51	256333				
AP - 2 - 8	spike	194333	8	2400	0.24	0				
AP- 2 - 1	clean	49000	1	300		0				
AP - 2 - 2	clean	23000	2	600		0				
AP- 2 - 4	clean	3667	4	1200		0				
AP - 2 - 8	clean	1000	8	2400		0				
AP - 2 -12	clean	0	12	3600		0				
AP - 5 - 1	spike	144000	1	300	0.44	256333	32.9	2021987	10445	12.4
AP - 5 - 2	spike	133000	2	600	0.48	256333				
AP - 5 - 4	spike	179667	4	1200	0.30	256333				
AP- 5 - 8	spike	197667	8	2400	0.23	0				
AP - 5 - 1	clean	29333	1	300		0				
AP - 5 - 2	clean	20667	2	600		0				
AP - 5 - 4	clean	8667	4	1200		0				
AP- 5 - 8	clean	8667	8	2400		0				
AP- 5 - 12	clean	0	12	3600		0				

Table C4 – Coliphage column data, columns 1 and 6, for Airport (AR) – sand-only bioretention cell in Fayetteville, AR.

Column	Treatment	coliphage (PFU/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration coliphage (PFU/100 ml)	Flow-Weighted Average % Removal	coliphage Retained by Column (PFU)	Flow-Weighted Average Concentration in Clean (PFU)	% Retained that Washed Out after Clean Flush
AR-1-1	spike	1667	1	300	0.98	76333	96.4	1766487	153	0.31
AR-1-2	spike	5333	2	600	0.93	76333				
AR-1-4	spike	2667	4	1200	0.97	76333				
AR-1-8	spike	1667	8	2400	0.98	0				
AR-1-1	clean	667	1	300		0				
AR-1-2	clean	333	2	600		0				
AR-1-4	clean	0	4	1200		0				
AR-1-8	clean	0	8	2400		0				
AR-1-12	clean	333	12	3600		0				
AR-6-1	spike	333	1	300	0.99	76333	94.5	1731993	1334	1.84
AR-6-2	spike	3000	2	600	0.96	76333				
AR-6-4	spike	error	4	1200		76333				
AR-6-8	spike	6000	8	2400	0.92	0				
AR-6-1	clean	2667	1	300		0				
AR-6-2	clean	0	2	600		0				

Table C5 – Coliphage column data, columns 1 and 3, for Botanic Garden (BG) – fly-ash amended bioretention cell in Stillwater, OK.

Column	Treatment	coliphage (PFU/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration coliphage (PFU/100 ml)	Flow-Weighted Average % Removal	coliphage Retained by Column (PFU)	Flow-Weighted Average Concentration in Clean (PFU)	% Retained that Washed Out after Clean Flush
BG FA-1-1	spike	17	1	300	0.99	2633	93.4	29520	21	1.69
BG FA-1-2	spike	67	2	600	0.97	2633				
BG FA-1-4	spike	383	4	1200	0.85	2633				
BG FA-1-1	clean	16.7	1	300		0				
BG FA-1-2	clean	0	2	600		0				
BG FA-1-4	clean	50	4	1200		0				
BG FA-1-8	clean	0	8	2400		0				
BG FA-3-1	spike	0	1	300	1.00	2633	93.3	29495	18	1.45
BG FA-3-2	spike	50	2	600	0.98	2633				
BG FA-3-4	spike	417	4	1200	0.84	2633				
BG FA-3-1	clean	17	1	300		0				
BG FA-3-2	clean	50	2	600		0				
BG FA-3-4	clean	17	4	1200		0				
BG FA-3-8	clean	0	8	2400		0				

Table C6 – Coliphage column data, columns 1 and 5, for Grand Lake Association (G-A) – fly-ash amended bioretention cell in Grove, OK.

Column	Treatment	coliphage (PFU/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration coliphage (PFU/100 ml)	Flow-Weighted Average % Removal	coliphage Retained by Column (PFU)	Flow-Weighted Average Concentration in Clean (PFU)	% Retained that Washed Out after Clean Flush
G - A - 1 - 1	spike	19000	1	300	0.34	29000	11.0	76650	1333	62.6
G - A - 1 - 2	spike	32300	2	600	0.11	29000				
G - A - 1 - 4	spike	31700	4	1200	0.09	29000				
G - A - 1 - 8	spike	30000	8	2400	0.03	29000				
G - A - 1 - 1	clean	12000	1	300		0				
G -A - 1 - 2	clean	2000	2	600		0				
G - A - 1 - 4	clean	333	4	1200		0				
G - A - 1 - 8	clean	0	8	2400		0				
G - A -1 -12	clean	0	12	3600		0				
G - A - 5 - 1	spike	16300	1	300	0.44	29000	12.0	83550	528	15.2
G - A - 5 - 2	spike	26300	2	600	0.09	29000				
G - A - 5 - 4	spike	25300	4	1200	0.13	29000				
G - A - 5 - 8	spike	29000	8	2400	0.00	29000				
G - A - 5 - 1	clean	1667	1	300		0				
G - A - 5 - 2	clean	1000	2	600		0				
G - A - 5 - 4	clean	333	4	1200		0				
G - A - 5 - 8	clean	333	8	2400		0				
G - A - 5 -12	clean	333	12	3600		0				

Table C7 – Coliphage column data, columns 1 and 5, for Grove High School (G-HS) – fly-ash amended bioretention cell in Grove, OK.

Column	Treatment	coliphage (PFU/100 ml)	Pore Volume	Volume (ml)	% Removal	Initial concentration coliphage (PFU/100 ml)	Flow-Weighted Average % Removal	coliphage Retained by Column (PFU)	Flow-Weighted Average Concentration in Clean (PFU)	% Retained that Washed Out after Clean Flush
G - HS - 1 - 1	spike	107000	1	300	0.04	103333	12.9	319007	5306	59.9
G - HS - 1 - 2	spike	115333	2	600	0.12	103333				
G - HS - 1 - 4	spike	111000	4	1200	0.07	103333				
G - HS - 1 - 8	spike	128000	8	2400	0.24	103333				
G - HS - 1 - 1	clean	36762	1	300		0				
G - HS - 1 - 2	clean	6095	2	600		0				
G - HS - 1 - 4	clean	3143	4	1200		0				
G - HS - 1 - 8	clean	1429	8	2400		0				
G - HS - 1 -12	clean	952	12	3600		0				
G - HS - 5 - 1	spike	55000	1	300	0.47	103333	25.4	630999	4488	17.1
G - HS - 5 - 2	spike	130333	2	600	0.26	103333				
G - HS - 5 - 4	spike	126333	4	1200	0.22	103333				
G - HS - 5 - 8	spike	82333	8	2400	0.20	103333				
G - HS - 5 - 1	clean	29333	1	300		0				
G - HS - 5 - 2	clean	5524	2	600		0				
G - HS - 5 - 4	clean	3143	4	1200		0				
G - HS - 5 - 8	clean	1048	8	2400		0				
G - HS - 5 -12	clean	1048	12	3600		0				

APPENDIX D

Table D1- Particle Size Distribution for Column 1 at the Botanic Garden (BG –S) in Stillwater, OK.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	93.61	100.00	pebble/sand boundary
No. 10	2.000	76.05	81.24	
No. 40	0.425	14.65	15.65	
No. 200	0.075	1.46	1.56	sand/silt boundary
Hydrometer	0.003	0.92	0.98	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	17.56	18.76
% MEDIUM SAND	61.40	65.59
% FINE SAND	13.19	14.09
% SAND	98.5	98.4
% SILT	0.5	0.6
% CLAY	0.9	1.0
Texture	sand	sand

Table D2- Particle Size Distribution for Column 2 at the Botanic Garden (BG –S) in Stillwater, OK.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	100.00	100.00	pebble/sand boundary
No. 10	2.000	98.01	98.01	
No. 40	0.425	33.75	33.75	
No. 200	0.075	3.05	3.05	sand/silt boundary
Hydrometer	0.003	1.21	1.21	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	1.99	1.99
% MEDIUM SAND	64.26	64.26
% FINE SAND	30.70	30.70
% SAND	97.0	97.0
% SILT	1.8	1.8
% CLAY	1.2	1.2
Texture	sand	sand

Table D3- Particle Size Distribution for Column 3 at the Botanic Garden (BG –S) in Stillwater, OK.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	98.62	100.00	pebble/sand boundary
No. 10	2.000	95.64	96.98	
No. 40	0.425	21.59	21.89	
No. 200	0.075	1.68	1.70	sand/silt boundary
Hydrometer	0.003	0.00	0.00	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	2.98	3.02
% MEDIUM SAND	74.05	75.09
% FINE SAND	19.91	20.19
% SAND	98.3	98.3
% SILT	1.7	1.7
% CLAY	0.0	0.0
Texture	sand	sand

Table D4- Particle Size Distribution for Column 5 at the Botanic Garden (BG –S) in Stillwater, OK.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	100.00	100.00	pebble/sand boundary
No. 10	2.000	97.89	97.89	
No. 40	0.425	34.02	34.02	
No. 200	0.075	10.61	10.61	sand/silt boundary
Hydrometer	0.003	1.51	1.51	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	2.11	2.11
% MEDIUM SAND	63.87	63.87
% FINE SAND	23.41	23.41
% SAND	89.4	89.4
% SILT	9.1	9.1
% CLAY	1.5	1.5
Texture	sand	sand

Table D5- Particle Size Distribution for Column 6 at the Botanic Garden (BG –S) in Stillwater, OK.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	99.86	100.00	pebble/sand boundary
No. 10	2.000	99.68	99.82	
No. 40	0.425	21.38	21.41	
No. 200	0.075	1.80	1.80	sand/silt boundary
Hydrometer	0.003	0.00	0.00	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	0.18	0.18
% MEDIUM SAND	78.30	78.41
% FINE SAND	19.58	19.61
% SAND	98.2	98.2
% SILT	1.8	1.8
% CLAY	0.0	0.0
Texture	sand	sand

Table D6- Particle Size Distribution for Column 1 at the Airport (AP) in Fayetteville, AR.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	96.81	100.00	pebble/sand boundary
No. 10	2.000	83.12	85.85	
No. 40	0.425	31.52	32.56	
No. 200	0.075	17.77	18.35	sand/silt boundary
Hydrometer	0.003	4.78	4.94	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	13.70	14.15
% MEDIUM SAND	51.60	53.30
% FINE SAND	13.75	14.21
% SAND	82.2	81.6
% SILT	13.0	13.4
% CLAY	4.8	4.9
Texture	loamy sand	loamy sand

Table D7- Particle Size Distribution for Column 2 at the Airport (AP) in Fayetteville, AR.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	94.04	100.00	pebble/sand boundary
No. 10	2.000	83.56	88.86	
No. 40	0.425	29.23	31.08	
No. 200	0.075	24.24	25.77	sand/silt boundary
Hydrometer	0.003	5.05	5.37	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	10.48	11.14
% MEDIUM SAND	54.34	57.78
% FINE SAND	4.99	5.30
% SAND	75.8	74.2
% SILT	19.2	20.4
% CLAY	5.0	5.4
Texture	loamy sand	sandy loam

Table D8- Particle Size Distribution for Column 3 at the Airport (AP) in Fayetteville, AR.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	96.82	100.00	pebble/sand boundary
No. 10	2.000	83.12	85.85	
No. 40	0.425	35.89	37.07	
No. 200	0.075	25.92	26.77	sand/silt boundary
Hydrometer	0.003	8.98	9.28	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	13.70	14.15
% MEDIUM SAND	47.23	48.78
% FINE SAND	9.97	10.30
% SAND	74.1	73.2
% SILT	16.9	17.5
% CLAY	9.0	9.3
Texture	sandy loam	sandy loam

Table D9- Particle Size Distribution for Column 5 at the Airport (AP) in Fayetteville, AR.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	97.82	100.00	pebble/sand boundary
No. 10	2.000	88.15	90.11	
No. 40	0.425	34.78	35.55	
No. 200	0.075	28.01	28.64	sand/silt boundary
Hydrometer	0.003	6.29	6.43	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	9.67	9.89
% MEDIUM SAND	53.37	54.56
% FINE SAND	6.77	6.92
% SAND	72.0	71.4
% SILT	21.7	22.2
% CLAY	6.3	6.4
Texture	sandy loam	sandy loam

Table D10- Particle Size Distribution for Column 6 at the Airport (AP) in Fayetteville, AR.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	95.25	100.00	pebble/sand boundary
No. 10	2.000	83.31	87.47	
No. 40	0.425	33.21	34.86	
No. 200	0.075	27.80	29.19	sand/silt boundary
Hydrometer	0.003	6.37	6.68	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	11.94	12.53
% MEDIUM SAND	50.10	52.60
% FINE SAND	5.41	5.68
% SAND	72.2	70.8
% SILT	21.4	22.5
% CLAY	6.4	6.7
Texture	sandy loam	sandy loam

Table D11- Particle Size Distribution for Column 1 at the Airport (AR) in Fayetteville, AR.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	95.79	100.00	pebble/sand boundary
No. 10	2.000	83.86	87.54	
No. 40	0.425	27.88	29.11	
No. 200	0.075	21.26	22.20	sand/silt boundary
Hydrometer	0.003	7.70	8.04	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	11.93	12.46
% MEDIUM SAND	55.97	58.43
% FINE SAND	6.62	6.91
% SAND	78.7	77.8
% SILT	13.6	14.2
% CLAY	7.7	8.0
Texture	loamy sand	sandy loam

Table D12- Particle Size Distribution for Column 2 at the Airport (AR) in Fayetteville, AR.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	96.00	100.00	pebble/sand boundary
No. 10	2.000	87.32	90.96	
No. 40	0.425	38.51	40.11	
No. 200	0.075	26.68	27.79	sand/silt boundary
Hydrometer	0.003	8.63	8.99	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	8.68	9.04
% MEDIUM SAND	48.81	50.84
% FINE SAND	11.83	12.32
% SAND	73.3	72.2
% SILT	18.0	18.8
% CLAY	8.6	9.0
Texture	sandy loam	sandy loam

Table D13- Particle Size Distribution for Column 3 at the Airport (AR) in Fayetteville, AR.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	89.29	100.00	pebble/sand boundary
No. 10	2.000	66.86	74.88	
No. 40	0.425	19.60	21.95	
No. 200	0.075	11.43	12.81	sand/silt boundary
Hydrometer	0.003	3.61	4.04	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	22.43	25.12
% MEDIUM SAND	47.26	52.94
% FINE SAND	8.16	9.14
% SAND	88.6	87.2
% SILT	7.8	8.8
% CLAY	3.6	4.0
Texture	sand	sand

Table D14- Particle Size Distribution for Column 5 at the Airport (AR) in Fayetteville, AR.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	95.84	100.00	pebble/sand boundary
No. 10	2.000	84.23	87.88	
No. 40	0.425	33.69	35.16	
No. 200	0.075	23.90	24.94	sand/silt boundary
Hydrometer	0.003	0.00	0.00	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	11.61	12.12
% MEDIUM SAND	50.54	52.73
% FINE SAND	9.79	10.22
% SAND	76.1	75.1
% SILT	23.9	24.9
% CLAY	0.0	0.0
Texture	loamy sand	loamy sand

Table D15- Particle Size Distribution for Column 6 at the Airport (AR) in Fayetteville, AR.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	97.39	100.00	pebble/sand boundary
No. 10	2.000	86.91	89.23	
No. 40	0.425	36.03	36.99	
No. 200	0.075	22.27	22.86	sand/silt boundary
Hydrometer	0.003	5.15	5.28	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	10.49	10.77
% MEDIUM SAND	50.87	52.23
% FINE SAND	13.76	14.13
% SAND	77.7	77.1
% SILT	17.1	17.6
% CLAY	5.1	5.3
Texture	loamy sand	loamy sand

Table D16- Particle Size Distribution for Column 1 at the Botanic Garden (BG-FA) in Stillwater, OK.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	97.83	100.00	pebble/sand boundary
No. 10	2.000	94.61	96.71	
No. 40	0.425	25.66	26.23	
No. 200	0.075	2.86	2.92	sand/silt boundary
Hydrometer	0.003	1.58	1.62	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	3.22	3.29
% MEDIUM SAND	68.95	70.48
% FINE SAND	22.80	23.31
% SAND	97.1	97.1
% SILT	1.3	1.3
% CLAY	1.6	1.6
Texture	sand	sand

Table D17- Particle Size Distribution for Column 2 at the Botanic Garden (BG-FA) in Stillwater, OK.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	99.99	100.00	pebble/sand boundary
No. 10	2.000	96.66	96.67	
No. 40	0.425	31.17	31.17	
No. 200	0.075	4.06	4.06	sand/silt boundary
Hydrometer	0.003	2.27	2.27	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	3.33	3.33
% MEDIUM SAND	65.49	65.50
% FINE SAND	27.11	27.11
% SAND	95.9	95.9
% SILT	1.8	1.8
% CLAY	2.3	2.3
Texture	sand	sand

Table D18- Particle Size Distribution for Column 3 at the Botanic Garden (BG-FA) in Stillwater, OK.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	98.64	100.00	pebble/sand boundary
No. 10	2.000	94.06	95.36	
No. 40	0.425	33.09	33.54	
No. 200	0.075	4.05	4.11	sand/silt boundary
Hydrometer	0.003	1.32	1.33	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	4.58	4.64
% MEDIUM SAND	60.97	61.81
% FINE SAND	29.03	29.43
% SAND	95.9	95.9
% SILT	2.7	2.8
% CLAY	1.3	1.3
Texture	sand	sand

Table D19- Particle Size Distribution for Column 4 at the Botanic Garden (BG-FA) in Stillwater, OK.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	98.84	100.00	pebble/sand boundary
No. 10	2.000	95.37	96.49	
No. 40	0.425	35.95	36.37	
No. 200	0.075	10.85	10.98	sand/silt boundary
Hydrometer	0.003	1.33	1.35	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	3.47	3.51
% MEDIUM SAND	59.42	60.12
% FINE SAND	25.10	25.39
% SAND	89.1	89.0
% SILT	9.5	9.6
% CLAY	1.3	1.3
Texture	sand	sand

Table D20- Particle Size Distribution for Column 5 at the Botanic Garden (BG-FA) in Stillwater, OK.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	97.29	100.00	pebble/sand boundary
No. 10	2.000	93.79	96.41	
No. 40	0.425	17.68	18.17	
No. 200	0.075	2.58	2.65	sand/silt boundary
Hydrometer	0.003	0.82	0.85	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	3.50	3.59
% MEDIUM SAND	76.11	78.23
% FINE SAND	15.10	15.52
% SAND	97.4	97.3
% SILT	1.8	1.8
% CLAY	0.8	0.8
Texture	sand	sand

Table D21- Particle Size Distribution for Column 1 at the Grand Lake Association (G-A) in Grove, OK.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	97.19	100.00	pebble/sand boundary
No. 10	2.000	57.22	58.87	
No. 40	0.425	11.97	12.32	
No. 200	0.075	6.64	6.84	sand/silt boundary
Hydrometer	0.003	2.25	2.31	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	39.98	41.13
% MEDIUM SAND	45.24	46.55
% FINE SAND	5.33	5.48
% SAND	93.4	93.2
% SILT	4.4	4.5
% CLAY	2.2	2.3
Texture	sand	sand

Table D22- Particle Size Distribution for Column 2 at the Grand Lake Association (G-A) in Grove, OK.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	96.84	100.00	pebble/sand boundary
No. 10	2.000	52.92	54.65	
No. 40	0.425	11.16	11.52	
No. 200	0.075	7.93	8.19	sand/silt boundary
Hydrometer	0.003	2.86	2.95	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	43.92	45.35
% MEDIUM SAND	41.76	43.12
% FINE SAND	3.23	3.34
% SAND	92.1	91.8
% SILT	5.1	5.2
% CLAY	2.9	2.9
Texture	sand	sand

Table D23- Particle Size Distribution for Column 3 at the Grand Lake Association (G-A) in Grove, OK.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	98.56	100.00	pebble/sand boundary
No. 10	2.000	54.38	55.18	
No. 40	0.425	12.58	12.77	
No. 200	0.075	8.05	8.17	sand/silt boundary
Hydrometer	0.003	2.21	2.24	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	44.18	44.82
% MEDIUM SAND	41.80	42.41
% FINE SAND	4.53	4.60
% SAND	91.9	91.8
% SILT	5.8	5.9
% CLAY	2.2	2.2
Texture	sand	sand

Table D24- Particle Size Distribution for Column 4 at the Grand Lake Association (G-A) in Grove, OK.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	95.19	100.00	pebble/sand boundary
No. 10	2.000	57.74	60.66	
No. 40	0.425	17.18	18.05	
No. 200	0.075	10.91	11.47	sand/silt boundary
Hydrometer	0.003	6.09	6.39	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	37.45	39.34
% MEDIUM SAND	40.56	42.61
% FINE SAND	6.27	6.58
% SAND	89.1	88.5
% SILT	4.8	5.1
% CLAY	6.1	6.4
Texture	sand	sand

Table D25- Particle Size Distribution for Column 5 at the Grand Lake Association (G-A) in Grove, OK.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	97.15	100.00	pebble/sand boundary
No. 10	2.000	59.61	61.35	
No. 40	0.425	12.59	12.96	
No. 200	0.075	7.26	7.47	sand/silt boundary
Hydrometer	0.003	2.34	2.40	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	37.55	38.65
% MEDIUM SAND	47.02	48.40
% FINE SAND	5.33	5.48
% SAND	92.7	92.5
% SILT	4.9	5.1
% CLAY	2.3	2.4
Texture	sand	sand

Table D26- Particle Size Distribution for Column 1 at the Grove High School (G-HS) in Grove, OK.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	97.14	100.00	pebble/sand boundary
No. 10	2.000	53.36	54.93	
No. 40	0.425	8.72	8.98	
No. 200	0.075	4.54	4.67	sand/silt boundary
Hydrometer	0.003	1.69	1.74	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	43.79	45.07
% MEDIUM SAND	44.64	45.95
% FINE SAND	4.18	4.30
% SAND	95.5	95.3
% SILT	2.8	2.9
% CLAY	1.7	1.7
Texture	sand	sand

Table D27- Particle Size Distribution for Column 2 at the Grove High School (G-HS) in Grove, OK.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	99.04	100.00	pebble/sand boundary
No. 10	2.000	57.22	57.78	
No. 40	0.425	25.49	25.73	
No. 200	0.075	20.76	20.96	sand/silt boundary
Hydrometer	0.003	5.04	5.09	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	41.82	42.22
% MEDIUM SAND	31.73	32.04
% FINE SAND	4.73	4.78
% SAND	79.2	79.0
% SILT	15.7	15.9
% CLAY	5.0	5.1
Texture	loamy sand	loamy sand

Table D28- Particle Size Distribution for Column 3 at the Grove High School (G-HS) in Grove, OK.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	97.17	100.00	pebble/sand boundary
No. 10	2.000	60.65	62.42	
No. 40	0.425	15.55	16.01	
No. 200	0.075	8.37	8.61	sand/silt boundary
Hydrometer	0.003	5.41	5.56	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	36.51	37.58
% MEDIUM SAND	45.10	46.42
% FINE SAND	7.18	7.39
% SAND	91.6	91.4
% SILT	3.0	3.1
% CLAY	5.4	5.6
Texture	sand	sand

Table D29- Particle Size Distribution for Column 4 at the Grove High School (G-HS) in Grove, OK.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	97.74	100.00	pebble/sand boundary
No. 10	2.000	59.00	60.37	
No. 40	0.425	14.82	15.17	
No. 200	0.075	8.75	8.96	sand/silt boundary
Hydrometer	0.003	2.32	2.37	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	38.73	39.63
% MEDIUM SAND	44.18	45.20
% FINE SAND	6.07	6.21
% SAND	91.2	91.0
% SILT	6.4	6.6
% CLAY	2.3	2.4
Texture	sand	sand

Table D30- Particle Size Distribution for Column 5 at the Grove High School (G-HS) in Grove, OK.

Sieve	Diameter (mm)	Percent Passing with Pebbles	Percent Passing without Pebbles	
No. 4	4.750	92.87	100.00	pebble/sand boundary
No. 10	2.000	54.24	58.40	
No. 40	0.425	19.50	21.00	
No. 200	0.075	13.41	14.44	sand/silt boundary
Hydrometer	0.003	1.47	1.59	silt/clay boundary

Distribution Details	Percent Passing with Pebbles	Percent Passing without Pebbles
% COURSE SAND	38.63	41.60
% MEDIUM SAND	34.74	37.40
% FINE SAND	6.10	6.56
% SAND	86.6	85.6
% SILT	11.9	12.8
% CLAY	1.5	1.6
Texture	sand	loamy sand

APPENDIX E

Table E1 – *E.coli* data for storms collected at Elm Creek Plaza (ECP) in Grove, OK.

Date	Rain (in)	Flow Reduction	*E. coli* *Inlet* (MPN/100 ml)	*Underdrain* (MPN/100 ml)	*Change in Concentration inlet to under*	*Removal inlet to under*	*Met Recreation Limit* (126 / 100 ml)
5-Jun-14	0.75	75%	429	210	51%	87%	No
9-Jun-14	0.67	77%	4352	3681	15%	79%	No
23-Jul-14	0.67	76%	256	189	26%	81%	No
7-Aug-14	0.56	83%	10	10	0%	81%	Yes
2-Oct-14	1.17	47%	NA	NA			
10-Oct-14	3.83	54%	4611	391	92%	98%	No
23-Oct-14	0.31	68%	556	172	69%	89%	No
26-Mar-15	1.40	75%	20	62	-210%	63%	Yes
1-Apr-15	0.28	89%	20	62	-210%	66%	Yes
2-Apr-15	0.51	78%	62	62	0%	78%	Yes
8-May-15	0.29	91%	4068	472	88%	100%	No
20-May-15	0.44	83%	1476	580	61%	93%	No
29-May-15	0.75	87%	6867	537	92%	99%	No
18-Jun-15	2.07	90%	4494	3448	23%	92%	No
8-Jul-15	1.96	59%	2576	2652	-3%	58%	No
22-Jul-15	0.39	69%	124	340	-174%	14%	No
6-Aug-15	0.43	70%	864	484	44%	83%	No
19-Aug-15	2.03	66%	884	1984	-124%	23%	No
8-Sep-15	2.25	55%	208	0	100%	100%	Yes

Table E2 – Enterococci data for storms collected at Elm Creek Plaza (ECP) in Grove, OK.

Date	Rain (in)	Flow Reduction	Enterococci *Inlet* (MPN/100 ml)	*Underdrain* (MPN/100 ml)	*Change in Concentration inlet to under*	*Removal inlet to under*	*Met Recreation Limit* (35 / 100 ml)
5-Jun-14	0.75	75%					
9-Jun-14	0.67	77%	6867	6488	6%	76%	No
23-Jul-14	0.67	76%	602	1146	-90%	52%	No
7-Aug-14	0.56	83%	602	1137	-89%	64%	No
2-Oct-14	1.17	47%	67	0	100%	100%	Yes
10-Oct-14	3.83	54%	19863	15531	22%	82%	No
23-Oct-14	0.31	68%	2792	242	91%	97%	No
26-Mar-15	1.40	75%	1112	262	76%	97%	No
1-Apr-15	0.28	89%	394	398	-1%	89%	No
2-Apr-15	0.51	78%	1720	1112	35%	86%	No
8-May-15	0.29	91%	3940	836	79%	99%	No
20-May-15	0.44	83%	3120	296	91%	98%	No
29-May-15	0.75	87%	4611	3448	25%	90%	No
18-Jun-15	2.07	90%	3340	4352	-30%	86%	No
8-Jul-15	1.96	59%	4184	3292	21%	68%	No
22-Jul-15	0.39	69%	2084	248	88%	96%	No
6-Aug-15	0.43	70%	592	340	43%	82%	No
19-Aug-15	2.03	66%	164	536	-227%	-13%	No
8-Sep-15	2.25	55%	252	40	84%	93%	No

Table E3 – Coliphage data for storms collected at Elm Creek Plaza (ECP) in Grove, OK.

Date	Rain (in)	Flow Reduction	Coliphage *Inlet* (PFU/100 ml)	*Underdrain* (PFU/100 ml)	*Change in Concentration inlet to under*	*Removal inlet to under*
5-Jun-14	0.75	75%				
9-Jun-14	0.67	77%		0		
23-Jul-14	0.67	76%		100		
7-Aug-14	0.56	83%		0		
2-Oct-14	1.17	47%				
10-Oct-14	3.83	54%	17	33	-94%	54%
23-Oct-14	0.31	68%	17	0	100%	100%
26-Mar-15	1.40	75%		0		
1-Apr-15	0.28	89%		0		
2-Apr-15	0.51	78%		0		
8-May-15	0.29	91%	50	0	100%	100%
20-May-15	0.44	83%		0		
29-May-15	0.75	87%	33	0		
18-Jun-15	2.07	90%	67	17	75%	97%
8-Jul-15	1.96	59%	17	0		
22-Jul-15	0.39	69%		0		
6-Aug-15	0.43	70%		0		
19-Aug-15	2.03	66%		17		
8-Sep-15	2.25	55%		0		

Table E4 – *E.coli* data for storms collected at Grand Lake Association (GLA) in Grove, OK.

Date	Rain (in)	Flow Reduction	*E. coli* *Inlet* (MPN/100 ml)	*Underdrain* (MPN/100 ml)	*Change in Concentration inlet to under*	*Removal inlet to under*	*Met Recreation Limit* (126 / 100 ml)
5-Jun-04	0.98	-170%					
23-Aug-15	0.00	80%	15531	226.00	99%	100%	No
2-Sep-14	0.00	-196%	839	1301.00	-55%	-192%	No
18-Sep-14	0.87	-12639%	5794	776.00	87%	-1747%	No
10-Oct-14	3.64	11%	327	288.00	12%	48%	No
4-Nov-14	1.42	-1153%	346	82.00	76%	-238%	Yes
26-Mar-15	1.15	-149%	104	40.00	62%	-1%	Yes
1-Apr-15	0.69	-267%	558	20.00	96%	95%	Yes
2-Apr-15	0.86	-277%	25994	292.00	99%	95%	No
18-Jun-15	1.62	-265%	5510	369.00	93%	75%	No
8-Jul-15	2.51	62%	1476	0.00	100%	100%	Yes
8-Sep-15	1.69	62%	1536.00	0.00	100%	100%	Yes

Table E5 – Enterococci data for storms collected at Grand Lake Association (GLA) in Grove, OK.

			Enterococci				
Date	**Rain** (in)	**Flow Reduction**	*Inlet* (MPN/100 ml)	*Underdrain* (MPN/100 ml)	*Change in Concentration inlet to under*	*Removal inlet to under*	*Met Recreation Limit* (35 / 100 ml)
5-Jun-04	0.98	-170%					
23-Aug-15	0.00	80%	12033	85.00	99%	100%	No
2-Sep-14	0.00	-196%	24196	1301.00	95%	90%	No
18-Sep-14	0.87	-12639%	24196	146.00	99%	17%	No
10-Oct-14	3.64	11%	24196	1187.00	95%	97%	No
4-Nov-14	1.42	-1153%	13734	82.00	99%	91%	No
26-Mar-15	1.15	-149%	852	124.00	85%	62%	No
1-Apr-15	0.69	-267%	1326	62.00	95%	93%	No
2-Apr-15	0.86	-277%	31062	518.00	98%	93%	No
18-Jun-15	1.62	-265%	12262	173.00	99%	95%	No
8-Jul-15	2.51	62%	18444	<40	100%	100%	Yes
8-Sep-15	1.69	62%	14436.00	40.00	100%	100%	No

Table E6 – Coliphage data for storms collected at Grand Lake Association (GLA) in Grove, OK.

Date	Rain (in)	Flow Reduction	Coliphage *Inlet* (PFU/100 ml)	*Underdrain* (PFU/100 ml)	*Change in Concentration inlet to under*	*Removal inlet to under*
5-Jun-04	0.98	-170%				
23-Aug-15	0.00	80%	0	0		
2-Sep-14	0.00	-196%	0	17.000		
18-Sep-14	0.87	-12639%	0	0		
10-Oct-14	3.64	11%	17	0	100%	
4-Nov-14	1.42	-1153%	33	0	100%	100%
26-Mar-15	1.15	-149%	0	0		
1-Apr-15	0.69	-267%	0	0		
2-Apr-15	0.86	-277%	0	0		
18-Jun-15	1.62	-265%	0	0		
8-Jul-15	2.51	62%	17	0	100%	100%
8-Sep-15	1.69	62%	0.00	0		

Table E7 – *E.coli* data for storms collected at Grove High School (GHS) in Grove, OK.

Date	Rain (in)	Flow Reduction	*E. coli* *Inlet* (MPN/100 ml)	*Underdrain* (MPN/100 ml)	*Change in Concentration inlet to under*	*Mass Removal inlet to under*	*Met Recreation Limit* (126 / 100 ml)
1-Apr-15	0.43	2%	0	126			Yes
2-Apr-15	0.71	-31%	126	104	17%	-8%	No
8-May-15	0.31	-220%	1712	388	77%	23%	No
20-May-15	0.07	-35%	40	1516	-3690%	-4915%	No
29-May-15	0.00	69%	18416	6510	65%	90%	No
18-Jun-15	1.43	52%	2668	9208	-245%	-70%	No

Table E8 – Enterococci data for storms collected at Grove High School (GHS) in Grove, OK.

Date	Rain (in)	Flow Reduction	**Enterococci** *Inlet* (MPN/100 ml)	*Underdrain* (MPN/100 ml)	*Change in Concentration inlet to under*	*Removal inlet to under*	*Met Recreation Limit* (35 / 100 ml)
1-Apr-15	0.43	2%	40.00	20.000	50%	75%	Yes
2-Apr-15	0.71	-31%	1352.00	320.00	76%	69%	No
8-May-15	0.31	-220%	1400.00	584	58%	-42%	No
20-May-15	0.07	-35%	424.00	248	42%	23%	No
29-May-15	0.00	69%	1019.00	474	53%	87%	No
18-Jun-15	1.43	52%	2562.00	5794	-126%	-11%	No

Table E9 – Coliphage data for storms collected at Grove High School (GHS) in Grove, OK.

Date	Rain (in)	Flow Reduction	Coliphage *Inlet* (PFU/100 ml)	*Underdrain* (PFU/100 ml)	*Change in Concentration inlet to under*	*Removal inlet to under*
1-Apr-15	0.43	2%	0	0		
2-Apr-15	0.71	-31%	0	0		
8-May-15	0.31	-220%	17	0	100%	100%
20-May-15	0.07	-35%	0	0		
29-May-15	0.00	69%	0	0		
18-Jun-15	1.43	52%	0	0		

APPENDIX F

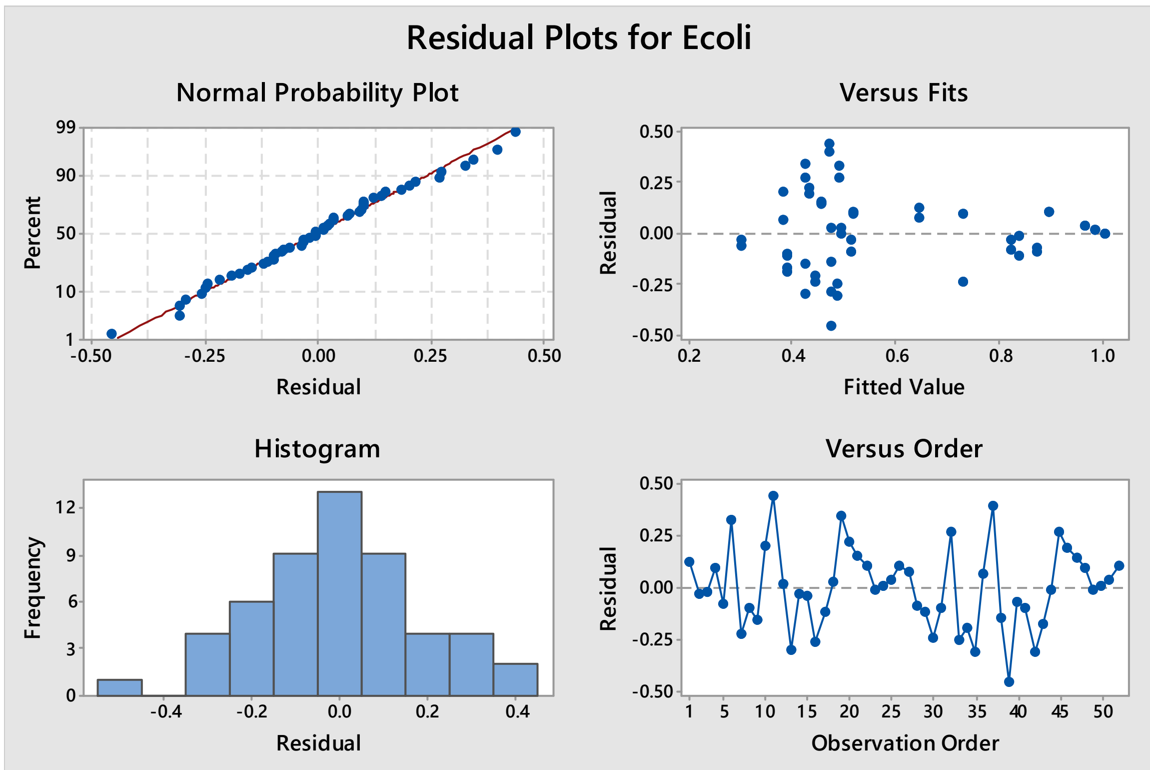

Figure F1 – Residual plots for E.coli removal for all cores in the laboratory column study, Chapter 3.

(Regression Equation: Ecoli = -0.540 + 0.1898 Media Type + 0.01273 % Medium Sand + 0.01154 % Fine Sand)

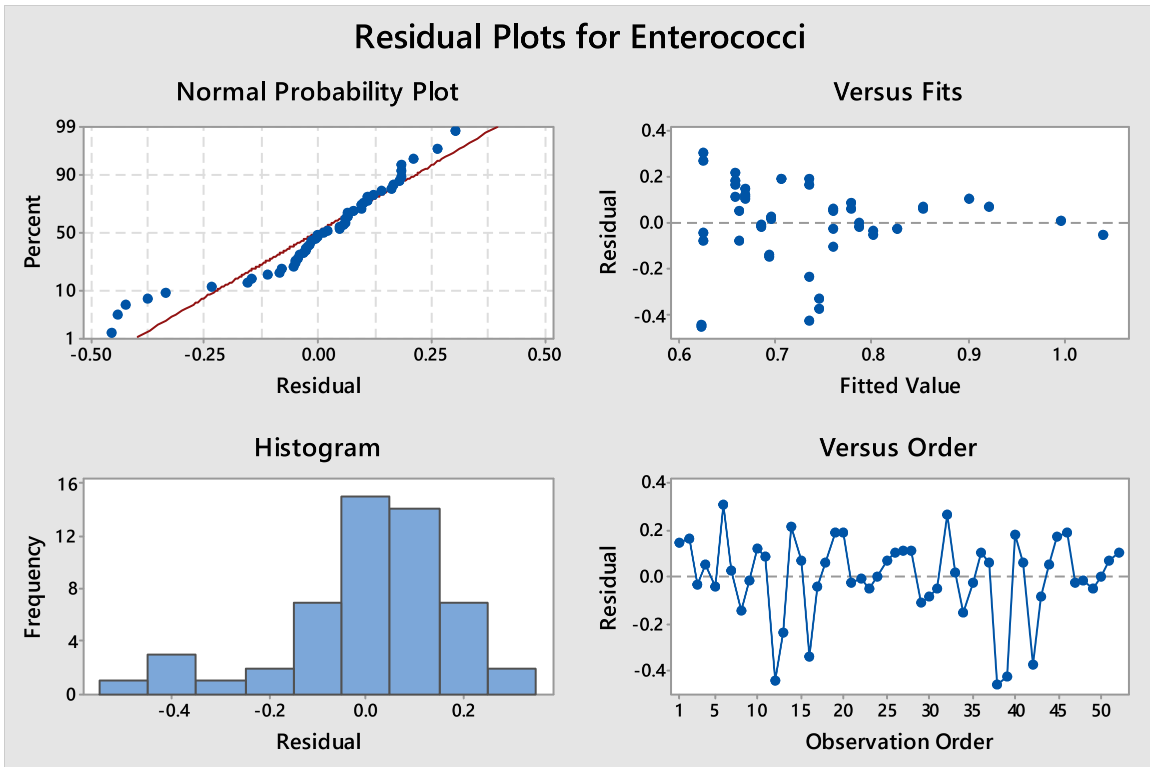

Figure F2 – Residual plots for enterococci removal for all cores in the laboratory column study, Chapter 3.

(Regression Equation: Enterococci = -0.477 + 0.2934 Media Type + 0.01256 % Medium Sand + 0.0285 % Clay)

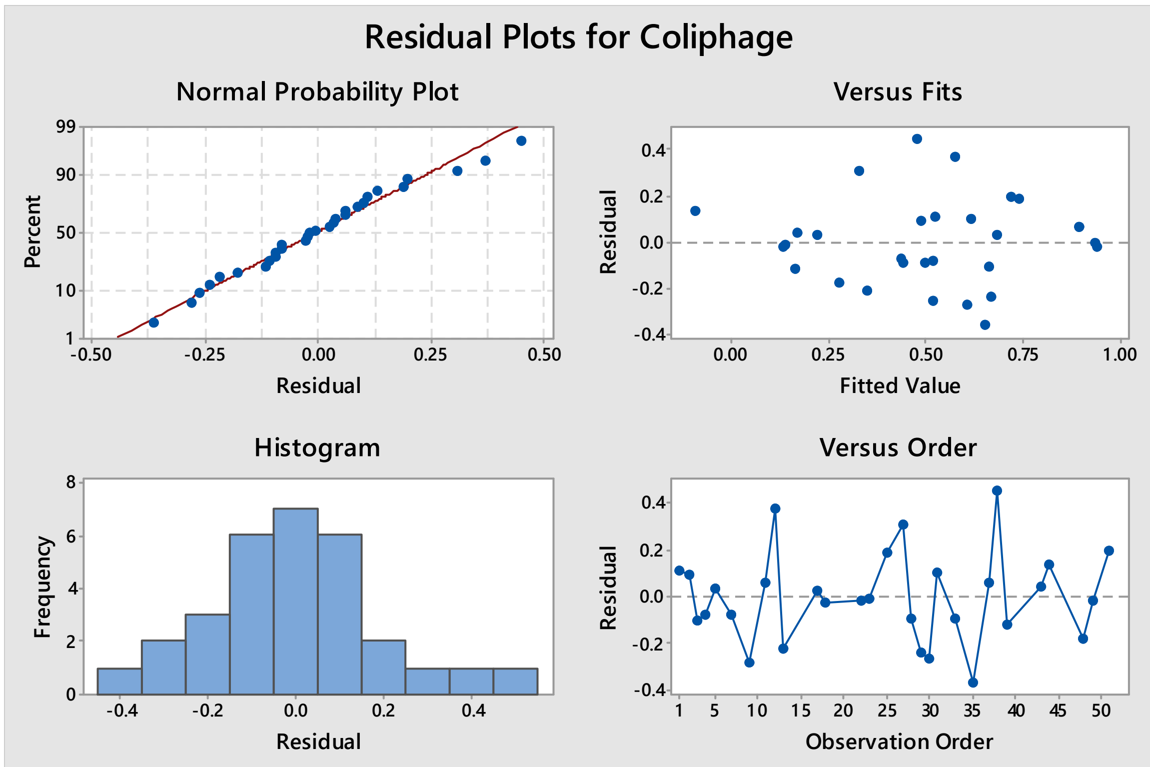

Figure F3 – Residual plots for coliphage removal for all cores in the laboratory column study, Chapter 3.

(Regression Equation: Coliphage = -1.146 + 0.02168 % Medium Sand + 0.0738 % Clay - 0.00416 Flowrate + 0.236 Media Type)

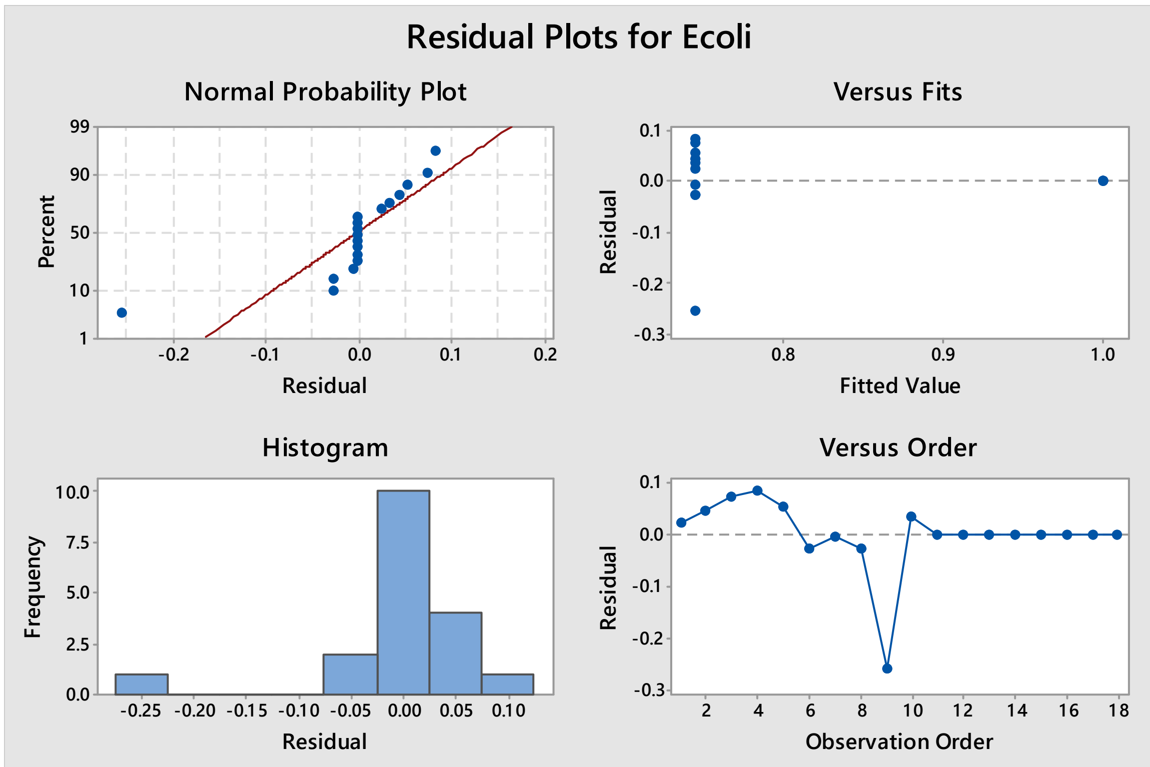

Figure F4 – Residual plots for E.coli removal for 10 cores from the Botanic Garden in Stillwater, Oklahoma in the laboratory column study, Chapter 3.
(Regression Equation: Ecoli = 0.4920 + 0.2540 Media Type)

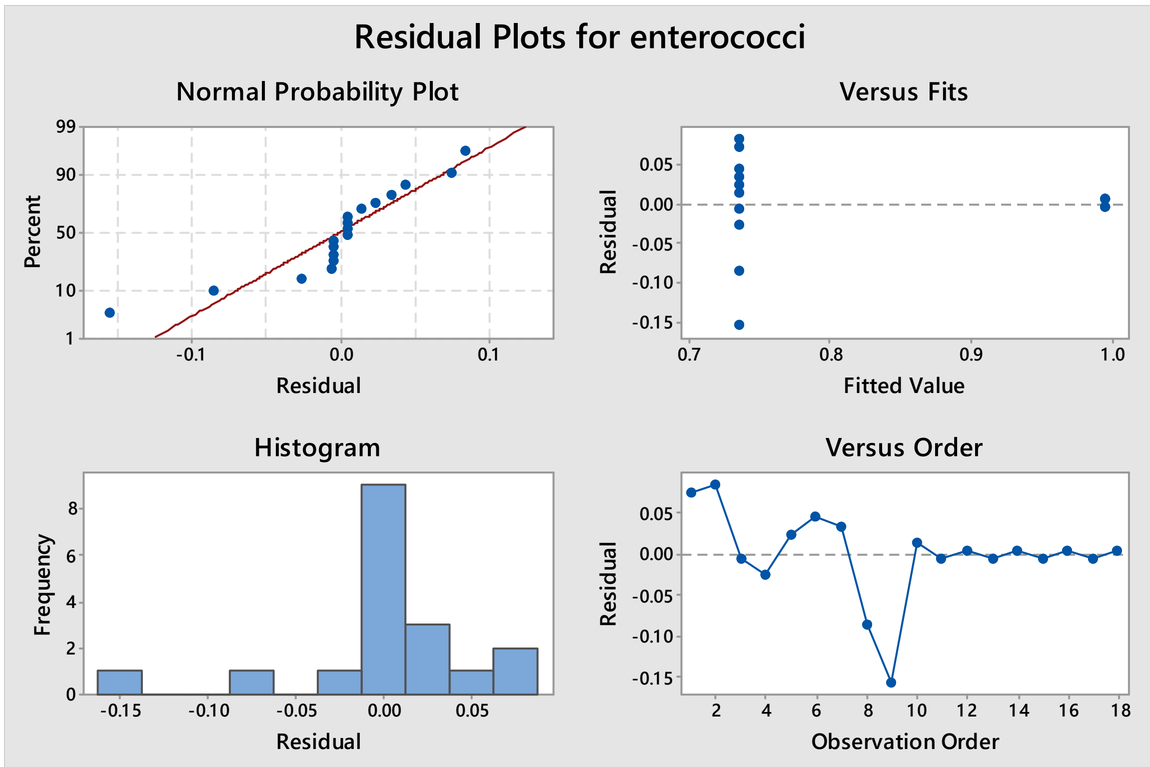

Figure F5 – Residual plots for enterococci removal for 10 cores from the Botanic Garden in Stillwater, Oklahoma in the laboratory column study, Chapter 3.
(Regression Equation: enterococci = 0.4770 + 0.2590 Media Type)

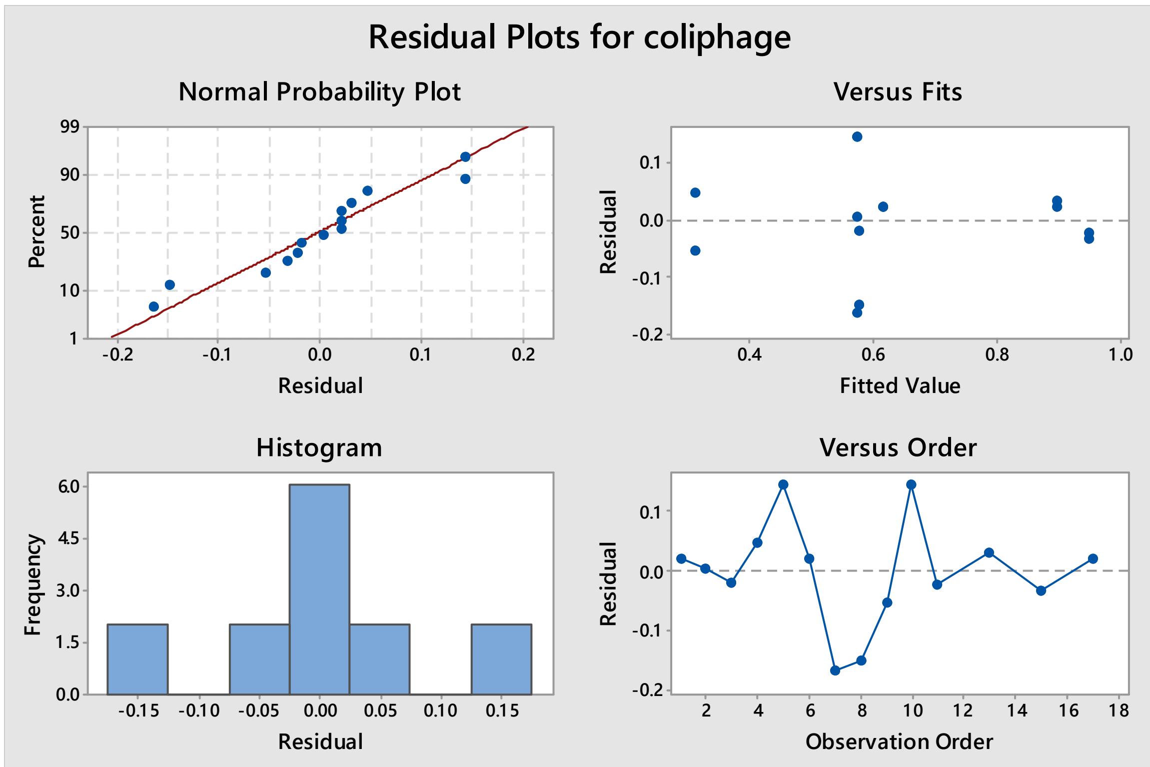

Figure F6 – Residual plots for coliphage removal for 10 cores from the Botanic Garden in Stillwater, Oklahoma in the laboratory column study, Chapter 3.
(Regression Equation: coliphage = 0.2808 + 0.3589 Media Type - 0.03589 % Silt)

APPENDIX G

Figure G1 – Laboratory assistance by Grace during the column experiments.

Figure G2 – Emma unloading soil cores used during the column experiments.

Figure G3 –Good help is hard to come by, Jason changing the tire in Arkansas

VITA

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