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Field Studies of Microbial Removal from Stormwater by Bioretention Cells with Fly-Ash Amendment

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Abstract: Microbial pollution in stormwater is a concern in urban areas across the U.S. and is a leading cause of water-quality impairment in the United States. This issue may be 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. Bioretention removal efficiencies have been well studied when considering nutrients and heavy metals, but field-scale treatment data are limited for microbial indicators. The primary objective of this study was to quantify microbial removal by installed bioretention cells with fly-ash amended soils. Three bioretention cells in Grove, Oklahoma were monitored over one and a half years and the removal microbial efficiency was quantified. Overall, removal rates for *E. coli*, enterococci, and coliphage were highly variable, with mean and standard deviations for removals for each site respectively: *E. coli* 87%, 35%, and 43%; enterococci 97%, 95%, and 80%; and coliphage 38%, 75%, and 32%. The site with negative removal efficiency appears to have some groundwater intrusion during storm events. Based on this relatively limited data set, these fly-ash amended bioretention cells performed 49% better than those with a sand-only filter media layer currently reported in the literature. Based on this initial field study, it appears that fly-ash amended bioretention cells may be a viable option for enhanced microbial removal from stormwater runoff.

Keywords: stormwater; bioretention; amendments; pathogen removal; filter media

1. Introduction

Stormwater is a major contributor to microbial pollution in urban areas. The use of bioretention cells is a new mechanism that has improved water quality parameters, such as nutrients and heavy metals. There is minimal research as to the impact of bioretention for bacteria and virus removal. The current literature exhibits a need for additional studies of microbial removal by bioretention. This study quantifies microbial removal by bioretention cells with and without a fly-ash amendment. Previous laboratory column studies have shown potential in increasing removal capacity of filter media when amended with iron-oxides [1,2]. The data from this study will provide a greater understanding of the benefits of bioretention with respect to microbes.

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 [3,4]. A study by Schoonover and Lockacy [3], 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 [5–7]. Irrespective of the source, pathogens in stormwater runoff are potentially harmful to humans and can degrade the water quality of receiving waters. In fact, Lehner [5] 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. Passive removal of pathogens through stormwater control devices such as bioretention cells could lead to water-quality improvements in impaired water bodies and decrease the risk to human health associated with their presence in the environment.

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. Common low impact development (LID) strategies include 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 [5,8,9]. Bioretention has been shown in recent years to be a viable best management practice (BMP) to address urban stormwater runoff. that provides multiple benefits including pollutant removal, flood reduction, aesthetic value, and animal habitat [9]. Studies of bioretention 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 [10–14]. However, there are relatively few studies currently in the literature investigating pathogen removal by bioretention compared to the removal of types of pollutants [9,15,16].

Studies 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 can vary greatly [1,17–19]. 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 [1,18,19]. Soil temperature, soil moisture, pH, sunlight, desiccation, and predation from indigenous microbial flora all have an effect on microbial survival in soils [19–21].

The primary objective of this research was 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 one 2009 study [22] stated there was no field scale data reported regarding bioretention removal ability in reference to microbial indicators.

2. Materials and Methods

2.1. Site Description

A field test using three bioretention cells in Grove, Oklahoma was conducted to determine microbial removal efficiency from stormwater runoff. These sites were designed and built with an underdrain. The sites used in this study are at 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 Oklahoma State University and built in 2007. Table 1 lists the sites selected for this study along with their size, drainage area—the area that drains into each bioretention cell [23,24]—exact location, and land cover. All sites have bioretention filter media comprised of sieved, washed local creek sand with 5% fly-ash collected from the Sooner Power Plant in Red Rock,

Oklahoma. The cells had a filter media depth of 0.85 m to 1 m. The composition of fly-ash is listed in Table 2 [24,26]. Fly-ash has been shown to adsorb heavy metals. The one element of concern is arsenic, however, the level is well below regulatory levels [25]. Sampling began during April 2014 and continued through October 2015.

Table 1. Site description, characteristics, and location of three bioretention cells used in the field study in Grove, OK, USA.

Site	Area (m^2)	Volume (m^3)	Drainage Area (Hectares)	Latitude and Longitude	Land Cover
Elm Creek Plaza (ECP)	63	128	0.62	36.579643 −94.768417	Paved
Grand Lake Association (GLA)	172	435	0.76	36.610923 −94.8033817	Paved/Turf
Grove High School (GHS)	149	161	0.26	36.5779781 −94.7555676	Paved

Table 2. Composition of fly-ash amendment in filter media layer of bioretention cells in Grove, OK, USA.

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
Other	2.40
Total	100.0

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, USA). 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 were used in conjunction with ISCO 720 flow modules in conjunction with pressure transducer level sensors to determine water depth. The depth was converted to flow with the appropriate flume and weir equations based on the specifics for the site, shown in Table 3. The Solinst level logger was utilized to measure water depth in a flume at the outlet and inlet for the determination of flow. 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. Samples were collected within 24 h of each rain event and processed in the laboratory at Oklahoma State University. Subsamples were either analyzed onsite or shipped overnight off site to remote laboratories for further analysis. Finally, samples were distributed to analysis locations.

Table 3. Inflow, outflow, and overflow flume characteristics for three bioretention cells in Grove, OK, USA.

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

2.3. Laboratory Analysis

Flow-weighted composite samples were analyzed for nutrients (not reported here), 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 (Mettler-Toledo, LLC, Columbus, OH, USA) meter was used to measure the pH and EC of each water sample [27]. ASTM D3977–97, Method B [28] was used to measure TSS for all samples. Turbidity was measured using a Hach 2100Q Portable (Hach Company, Loveland, CO, USA)Turbidimeter [29].

All microbial analysis was completed by Dr. Dale Griffin from the USGS Microbiology Laboratory in St. Petersburg, Florida. Samples were shipped overnight, on ice, to the Florida lab. *E. coli* and enterococci analyses were completed using the Colilert and Enterolert Quanti Tray 2000 Method from IDEXX Systems [30,31]. Coliphage overlays used two milliliters volumes for each of the three replicates for all samples tested. All plates and quantitrays were incubated overnight at their respective temperatures and samples were stored overnight by refrigeration.

2.4. Statistical Analysis

In-depth examination of the data began with the statistical analysis of the percent removal for each microbe. Concentrations of microbes were measured in MPN/100 mL for *E. coli* and enterococci and PFU/100 mL for coliphage. Mean, standard deviation, and range for all data categories at each sampling location for each site based on the storm event were calculated. These data are provided in the tables and figures in the following sections. The mean was calculated at both the inlet and underdrain for each site. The underdrain microbial concentrations were compared to the United States Environmental Protection Agency (USEPA) recreational fresh water contact recommendations, 126 CFU/100 mL for *E. coli* and 35 CFU/100 mL for enterococci [32]. Microbial data were analyzed in two different ways, one by concentration change and the other by removal or trapping efficiency. Both criteria were calculated in this study.

A change of influent and effluent concentrations of individual microbes from each storm event was also calculated. The automatic samplers were calibrated to respond when runoff began, triggered samplers that provided runoff water to the sample bottles were considered a storm event. Equation (1) was utilized for all microbial indicators and coliphage concentrations. Microbes were measured as colony forming unit (CFU), most probable number (MPN), or plaque forming unit (PFU). 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), was calculated using, where,

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

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. The overall mean percent change in concentration for the microbe, $\%\Delta C_T$, is provided in Equation (2), the summation of concentration for each microbial indicator and coliphage over, n, the number of sampled storms for each site.

$$\%\Delta C_T = \left(1 - \frac{\sum_i^n O_{conc}}{\sum_i^n I_{conc}}\right) \times 100 \quad (2)$$

A microbial count balance approach was 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. The percent removal for each microbe for each individual storm event, ($\%R_1$), was calculated using Equation (3), where,

$$\%R_1 = \left(1 - \frac{C_o}{C_i}\right) \times 100, \quad (3)$$

where C_o is the count of that microbe in the outlet underdrain, and C_i is the count of that microbe from the inlet. 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).

$$\%R_T = \left(1 - \frac{\sum C_o}{\sum C_i}\right) \times 100 \quad (4)$$

Statistical tests and correlations were performed based on this field study. A two-way ANOVA (analysis of variance), Tukey one-way analysis, Mann–Whitney, and 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.

3. Results

3.1. Basic Parameters

Between May 2014 and October 2015, storms events were monitored and sampled for the bioretention cells in Grove, Oklahoma. Elm Creek Plaza (ECP) had a total of 23 storm events captured 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 for the storms that were analyzed, 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. Flow reduction, pH, EC, TSS, and turbidity were measured at each event and the results summarized in Table 4. One notable datum is the negative flow reduction values for GLA, meaning that the flow was greater at the outflow underdrain than at the inlet. This was 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 during which the sampler did not function correctly due to 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, or started too late. The data show general trends reflecting a mean increase in pH and EC and a reduction for TSS and turbidity from inlet to underdrain.

Table 4. Physical and chemical parameters 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]	−1200	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 Underdrain (NTU)	7	5	[14, 0]	4	3	[9, 1]	3	2	[5, 0]

3.2. Microbial Concentrations and Removal

Table 5, which includes all collected data, both paired and unpaired, shows the mean, standard deviation (s.d.), and 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 was also shown for enterococci; one possible explanation is that GLA 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 was 87% for ECP, 35% for GLA, and 43% for GHS. The standard deviations for removal efficiency at GLA and GHS are very high suggesting high variability in individual removals. Conversely, the standard deviation is relatively small for ECP. This may be due to watershed uniformity of the sites. ECP is a more uniform watershed, almost entirely impermeable, where the other two are a mix of parking lots and grassed areas. ECP and GLA showed a reduction in concentration from inlet to outlet but GHS showed an increase (−8% change). This increase is possibly due to the burrowing animals that live near and inside the bioretention cell at this site. 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), six, five, and two times, respectively for ECP, GLA, and GHS [28]. For enterococci, GHS had the highest removal efficiency at 97%, GLA was calculated at 95%, and ECP showed 80% removal ability. The standard deviations of mass removal were relatively low (47, 24, and 25, respectively). The reduction in concentration was favorable for GLA (98%, 4 s.d.) and GHS (78%, 93 s.d.). ELP had 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/100 mL, this limit was met only once for ECP and GLA, and twice for GHS, equivalent to 4%, 7%, and 13%, respectively [28]. Coliphage concentrations were reduced from the inlet to the underdrain outlet by 38% for ECP, 75% for GLA, and 32% for GHS, illustrating that bioretention is viable for the inhibition of 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 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 to 30%, 42%, and 33% for ECP, GLA, and GHS, respectively, a 5% mean increase over all sites. A similar observation can be made regarding the USEPA recreational water enterococci criterion. Paired event data met the enterococci criterion 5% (ECP), 8% (GLA), and 16% (GHS) of the time. 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, see Table 7. Enterococcus was the only microbe that was significant for this comparison, shown in Figure 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, see Table 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 that 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 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)	1600	1940	[6900, 10]	4900	7700	[26,000, 104]	1800	4700	[18,000, <DL]
E. coli Underdrain (MPN/100 mL)	810	1200	[3700, <DL]	310	380	[1300, <DL]	2000	3000	[9200, 104]
Underdrain Met *E. coli* Recreation Limit (126/100 mL)	5/23			5/14			5/16		
Enterococci Inlet (MPN/100 mL)	3130	4200	[20,000, 67]	15,000	10,000	[24,000, 52]	3400	6300	[1400, 40]
Enterococci Underdrain (MPN/100 mL)	2100	3600	[16,000, <DL]	350	440	[1300, <40]	800	1700	[5800, 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]

Table 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

Table 7. Two-way ANOVA (analysis of variance) 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.

Table 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 $p < 0.05$.

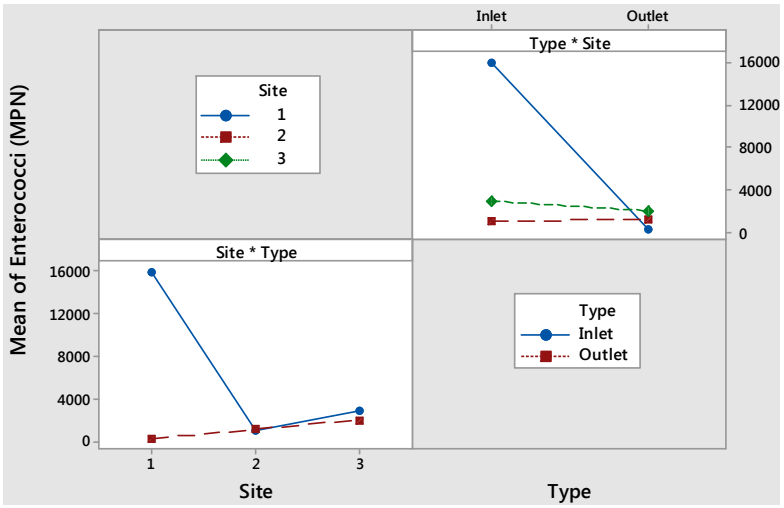

Figure 1. Interaction plots for enterococci for three bioretention cells in Grove, Oklahoma (Site 1 = GLA: Grand Lake Association, Site 2 = GHS: Grove High School, Site 3 = ECP: Elm Creek Plaza, Type = Inlet and Outlet, Inlet = inflow from the inlet, Outlet = outflow from the underdrain).

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, USA and Wilmington, NC, USA, monitored by Hathaway [19] 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. While this is an interesting observation, it is recognized that the data in both media compositions are limited for full-scale bioretention cells.

3.4. Conclusions and Recommendations for Future Work

A growing concern across the United States is microbial pollution of stormwater. Minimal data is available on the effectiveness of bioretention cells to aid designers and regulators in determining if bioretention cells would be appropriate technology for microbial pollution treatment. The use of fly-ash has been recommended in Oklahoma for phosphorous removal. Thus, this project tested the expanded use of fly-ash for microbial removal. These methods are expected to be transferable to other locations and bioretention cells.

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 discharge into receiving water bodies, these concentration changes and microbial removal efficiencies may not be sufficient levels of reduction and removal for the watersheds, since in most storm events the criteria was not met for the 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 the laboratory results. The observation that amended media produces greater removal than non-amended media was further corroborated with additional 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. These criteria are set to protect against human health impacts, thus a higher percentage is preferred. Despite the recent increase in data available from field studies using bioretention for microbial indicator removal, 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 is to consider microbial removal and inactivation with regard to the size, depth and filter media composition of each monitored bioretention cell. These are all factors that would benefit more research in the field setting to determine their individual or coupled effects on performance ability in the realm of microbial removal and increased public health in urban areas.

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