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Point of Use Biosand Filters of the Rural Dominican Republic

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by,

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ABSTRACT

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The point of use biosand filter (BSF) is used globally as a drinking water treatment solution. In this research, point of use BSFs were inoculated with active biosand from the Linnwood Drinking Water Treatment plant slow sand filter beds (Milwaukee, Wisconsin) and with sands collected from point of use filters operating in the Dominican Republic. These filters were maintained with varying source waters (surface water, groundwater, or tap water to simulate chlorination encountered in the field). The microbial community of filters with varied influents and biosand inoculum were analyzed quantitatively by sequencing and qPCR. Filter efficacy and microbial community were found to be largely a function of source water and pretreatment conditions. Filters were intermittently challenged with *E. coli* as a fecal indicator bacteria and bacteriophage MS2 as a surrogate for pathogenic virus to evaluate filtration efficiency. This research suggests that the point of use BSF should not be used in conjunction with chlorinated source waters. Chlorination may inhibit biofilm colonization and allow for interstitial survival or growth of pathogenic bacteria. Based on the non-dimensional scaling analysis of genome sequencing data, the interstitial microbial community of the BSF could be grouped into four categories as a function of source waters: biosand filters in the Dominican Republic, laboratory filters maintained with tap water, groundwater, or with Lake Michigan water as influent. The microbial community within active biosand collected in the field was not retained under laboratory conditions.

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LIST OF ABBREVIATIONS

BSF: Biosand Filter

CFU: Colony Forming Units

CORAAROM: Corporación del Acueducto y Alcantrillado de la Roman

DOC: Dissolved Organic Carbon

***E. coli*:** *Escherichia coli*

EPA: United States Environmental Protection Agency

INAPA: Instituto Nacional de Aguas Potables y Alcantarillados

INDHRI: Instituto Nacional de Recursos Hidraulicos

NGO: Non-Government Organization

NMDS: Non-Metric Multidimensional Scaling

PFU: Plaque Forming Units

TNTC: Too numerous to count

WHO: World Health Organization

Chapter 1: Introduction

1.1: Water Treatment and Public Health in the Developing World

Globally, an estimated 663 million people lack access to an improved source of drinking water, while 2.4 billion lack access to improved sanitation facilities. Access to improved drinking water sources in the developing world has made significant progress in the last 15 years, climbing from 70% to 90% coverage (WHO, 2015). While Millennium Development Goals of halving the proportion of the human population without access to improved drinking water sources have been met, sanitation and hygiene have not matched these improvements. The World Health Organization's definition of an improved source includes: household connection, public standpipe, borehole, protected dug well, protected spring, or rainwater collection. This definition provides infrastructure guidelines required to meet development goals but lacks water quality guidelines. Communities receiving water from an improved source may continue to suffer from contaminated drinking water, perpetuating waterborne illness. Improved drinking water sources throughout the Dominican Republic are often contaminated with fecal coliform (Baum et al., 2014). Point of use drinking water filtration bridges the gap between improved drinking water sources and water quality required for human safety.

1.2: Drinking Water of the Rural Dominican Republic

In 2012, 24% of rural residents in the Dominican Republic lacked access to an improved drinking water source (WHO, 2012). Field surveys suggest that these estimations may be conservative as direct access to water in the home is exceptionally rare, the nearest source is often contaminated, and communities often lack a central water supply (Vásquez et al., 2012;

Witter and Carasco, 1996). Water hauling is often necessary from improved sources and may take place in storage devices contaminated with coliform from previous hauling or other sources.

Drinking water resources of the Dominican Republic are largely managed by the Instituto Nacional de Aguas Potables y Alcantarillados (INAPA), a government institution created in 1962. In addition to government institutions of the Dominican Republic, groups including USAID and Rotary Club have contributed to drinking water infrastructure development.

In La Romana Province, Corporación del Acueducto y Alcantrillado de la Romana (CORAAROM) or "La Romana Water and Sewerage Company" is responsible for local drinking water and sewerage treatment. CORAAROM was established in 1998. In conjunction with United States Agency for International Development (USAID), INAPA began plans to decentralize control over rural drinking water supply during the 1990's. This practice was intended to empower local communities with control over drinking water systems and associated skills in hygiene and system management (Lockwood, 2001). Decentralized rural systems are largely managed by unpaid volunteers who may not have the funds or communication tools that are required to implement or maintain centralized drinking water treatment or distribution systems. In many cases, drinking water distribution systems are prone to failure due to a lack of maintenance (Schweitzer, 2009). Under the decentralized drinking water supply model, INAPA created a systems management branch (INAPA-AR). Rather than fundamental construction and management position, INAPA-AR serves as advisory group over system design and assessment of NGO projects to encourage overall project sustainability. Throughout the bateyes, government institutions, NGOs and members of the press are often denied access to private property without specific permission from the Centro Romano sugarcane company.

Following the 2010 earthquake, United Nations natural disaster relief teams were sent to assist the redevelopment throughout Haiti. It is believed that a cholera outbreak originated from UN workers' latrine, which quickly spread across Haiti causing an estimated 700,000 cholera infections and 8,500 deaths between 2010 and September of 2014 (Kean, 2014). Haiti's lack of sanitation, combined with a limited number of health workers created extensive risk for continued transmission of the cholera outbreak. Antibiotics are the most effective and rapid cure for cholera; however, health professionals remain concerned for antibiotic resistance. While cholera can be treated with consistent rehydration and rest, patients without consistent access to safe drinking water may experience chronic gastroenteritis (Tauxe et al., 2011). Employing a global cost benefit analysis to cholera outbreaks, Jeuland et al., (2009) suggests that vaccinations may not be justifiable in cases where communities have pre-emptively begun treating source waters with the biosand filter (BSF). Preventative water and sanitation treatments may be more cost-effective than post-infection care for cholera and other etiologic agents for gastroenteritis.

Considering global access to safe drinking water as a function of both physical availability and socioeconomic availability, Haiti is ranked last of 174 countries analyzed (Lawrence et al., 2002). With highly mobile migrant Haitian workers frequently crossing the border from Haiti to the Dominican Republic, the need for drinking water treatment throughout the rural Dominican Republic is needed to prevent further transmission of disease and the resulting burden placed on hospitals across Hispaniola.

CHAPTER 2: BACKGROUND

2.1: BSF Operation and Efficacy

Drinking water treatment in many developing countries is an exceptional challenge as funding for installation and maintenance is difficult with a non-dependable tax base and unstable government. Due to a lack of funding and education with respect to sanitation and hygiene, centralized drinking water treatment systems are often infeasible or poorly maintained. Point of use water treatment systems is a low cost, low maintenance, and readily replaceable alternative. Of the point of use treatment systems available, BSFs have low implementation cost, significant pathogen reductions, minimal maintenance requirements, ability to treat a wide range of source waters, and strong continued use as compared to other technologies (Sobsey et al., 2008).

Point of use biosand filtration has proven a promising technology for treatment of drinking water since its first implementation in the early 1990's. The household scale BSF is gravity driven, capable of producing 20-40 liters of filtered drinking water per day. BSF efficacy is dependent upon the temporal biological maturation of the filter as pathogen reductions become increasingly efficient with aging (Elliot et al., 2011). While some studies have quantified reductions in viruses, most have focused on fecal indicator bacteria.

Several operational parameters are instrumental for microbial reductions in the BSF system including 1) hydraulic loading rate 2) residence time 3) biological maturation, 4) source water chemistry and nutrient stoichiometry, 5) sand size / sorting and 6) overall filter dimensions. Of the parameters that are critical to BSF efficacy, the microbiome is perhaps one of the least studied and understood. Studies that have investigated metagenomics of the BSF have done so in the laboratory environment but never under field conditions (Wang, 2014). The

primary goal of his research was to investigate the removal efficiency of fecal indicator bacterium *Escherichia coli* and surrogate single strand RNA bacteriophage MS2 under varying influent conditions. The variation in the microbial community within a spectrum of BSFs were characterized with genome sequencing.

Although the BSF system is a simple technology, a number of factors may affect filter efficacy. Without instruction in proper operation and maintenance, users may operate the BSF under adverse conditions. For example, excessive hydraulic loading rates do not allow for substantial residence time during which pathogens interact with the interstitial microbial community, being subjected to heterotrophic grazing and hydrolysis processes. Adding source water at a low residence time is shown to adversely affect the performance of the BSF (Elliot et al. 2008; Wang et al., 2014). In many cases, the BSF is scraped excessively to restore flow rates, leading to a loss of biomass in the schmutzdecke, and/or a removal of filtration sand causing a loss of pore volume. Moving the BSF after installation may also disturb the sand and hinder biofilm development. In cases where the BSF becomes clogged and scraping at the surface cannot restore flow it may be necessary to replace filtration media entirely.

Source water chemistry and microbiology may control BSF development, filter efficacy, taxonomy and richness of the interstitial microbial community. The complex relationship between source water chemistry and microbial ecology is little understood. Recent work has shown that the microbial ecology of slow sand filters is a function of many parameters including development time, depth, distance to surface / outflow pipe, and has established correlations with water quality parameters including ammonia, nitrate, nitrite, and orthophosphate. Key genera and evenness of the microbial community may optimize filtration efficiency to an unknown extent (Haig et al., 2015). Key genera for pathogen reductions in the point of use BSF are yet to be

identified. While many studies have focused on the metagenomics of municipal scale slow sand filters, fewer have quantified the microbial communities of the point of use BSF. There are several functional differences between the point of use and constant flow slow sand filter, including a stagnant residence period and a lack of drying / backwash treatments in point of use systems.

This research was carried out with special concern for the batey region of southern Dominican Republic where Rotary Club International has installed approximately 6,000 BSF systems in rural homes where source waters are intermittently chlorinated. Occupants of these homes are Haitian migrant workers living in homes owned by the sugarcane company. With a typical home in the bateyes housing a family of five to seven, it is reasonable to estimate that some 20-30,000 inhabitants are dependent upon the intermittently chlorinated BSF for drinking water treatment. As communication with the property owners and chlorinators of source water is difficult, it is important to develop a scientific understanding of the efficacy of intermittently chlorinating the BSF system.

Previous research conducted in the rural Dominican Republic has shown that socioeconomic disparities between Dominican and Haitian neighborhoods in the Dominican Republic extend to drinking water quality. Source water in Haitian neighborhoods are 4.25 times more likely to be contaminated with *E. coli* and 4.68 times more likely to be contaminated with coliform (Rogers-Brown et al., 2015). In this study, 88% of Haitian neighborhoods consumed source water contaminated with coliform. Due to sociopolitical and socioeconomic disparities combined with a lack of access on private land to government organizations, little is known regarding the drinking water quality, sanitation, and public health of these communities.

2.2: History and Applications of the Point of Use Biosand Filter

Throughout a series of case studies, the point of use BSF has shown consistent reductions in diarrheal disease, *E. coli* concentrations, and turbidity as compared to control groups. Skepticism remains, however, as to the validity of statistically controlled field trials in which incidences of diarrheal disease are prone to household responses with communication transcending cultural and socioeconomic boundaries between researchers and users. Placebo may likewise play a role in skewing survey results.

The occurrence of diarrheal disease across 75 households utilizing the BSF in Bonao, Dominican Republic (approximately 150 km northwest of La Romana) was shown to be 0.53 times the odds of diarrheal disease as compared to 79 control households (Stauber et al., 2009). This study, carried out in central Dominican Republic, is the only publication investigating the point of use BSF in the rural Dominican Republic.

A study investigating the impact of the Hydraid BSF in rural Honduras found a 45% decrease in diarrheal disease for children less than five years old in households using the BSF to purify drinking water. While these results were not statistically significant, households utilizing the BSF showed consistently improved drinking water quality with respect to *E. coli* concentrations and turbidity (Fabiszewski de Aceituno et al., 2012).

Throughout Guatemala, application and proper maintenance of the biosand filter showed a decline in the frequency of diarrheal disease, in conjunction with access to an improved water source and proper hygiene practices (Divelbiss et al., 2013).

A cluster randomized control study in rural Ghana found that households utilizing the point of use BSF showed a 60% reduction in incidences of diarrheal disease, on average, a 97% removal of *E. coli*, and 67% turbidity reductions. This study further investigated the role of post-

filtration water storage; when filtrate was stored, average *E. coli* removal declined from 97% to 85%, suggesting coliform growth or re-contamination within storage vessels (Stauber et al., 2012).

Similarly, an investigation of 189 households in rural Cambodia found decreased incidences of diarrheal disease in homes having plastic BSF intervention as compared to control households. However, this study suggests that diarrheal diseases may continue despite BSF intervention, extending sanitation issues beyond point of use treatment (Stauber et al., 2012).

2.3: Biofilm Development

Aquatic biofilms may form in an array of environmental conditions including the piping or distribution system, sewage system, among various natural aquatic habitats (submerged rock, sand, concrete, algae, timber, etc.). With respect to the fine sand filtration system the development of biofilm is subject to mechanical straining effects and the stratification of nutrients, dissolved oxygen and redox potential. While many studies have identified distinct shifts of microbial community composition with depth of freshwater lakes, this process is less clear within the slow sand filter.

Upon reaching a substrate, bacteria may alter gene expression from motility toward attachment. During attachment, extracellular polymeric substances (EPS) composed of carbohydrates, proteins, and humic substances are formed at the cell surface and contribute to further cell aggregation processes. During endogenous decay, EPS may form soluble microbial products (SMPs), which can be further utilized heterotrophically as electron donors. By this process, the microbial community produces a biochemical cycle with production and assimilation of SMPs resulting from metabolic activity and decay (Kang et al., 2014).

Development of biofilm is considered as three-step process:

1) Adhesion

2) Colonization

3) Detachment

The biofilm community is a function of environmental conditions including temperature, salinity, pH of feed solution, nutrient concentration and stoichiometry, flow velocity, substrate and pre-disinfection. In conjunction with adhesion and detachment, the community is in a state of constant flux. Modifying source water chemistry, pretreatment or nutrients can quickly drive quantifiable alteration of the biofilm microbial community (Boon et al., 2011).

In our special concern for chlorination throughout the bateyes, tap water is used in laboratory to simulate chlorination in the field. However, with the addition of phosphate as coagulant and chloramine disinfectant as opposed to hypochlorite in the Milwaukee municipal distribution system, some experimental differences may arise between laboratory and field condition biofilm development. Additionally, while this approach of maintaining filters subjects the microbial biome to relatively constant levels of chloramine, chlorine concentrations in the field are in flux with the intermittent addition of unknown hypochlorite concentrations.

A review of biofilm development under chlorine, chloramine, and high phosphate concentration in drinking water distribution system has been carried out (Batte et al., 2003). This analysis, in accordance with prior work, shows that Gram-negative bacteria are predominant in the chlorinated feed water biofilm community, whereas Gram-positive bacteria are predominant in the monochloramine disinfected feed water community. Chlorination reduced Alpha, Beta,

and Gammaproteobacteria gradually, to a lesser extent than other bacteria groups targeted by fluorescent in situ hybridization (FISH), eventually leading to a predominantly Proteobacteria community. The authors suggest that Proteobacteria's resistance to disinfection is enhanced by chlorine and hindered by monochloramine. In addition to disinfection investigations, the authors concluded that the addition of phosphate did not result in higher cell densities, but lead to a greater proportion of Gammaproteobacteria. In some cases, multispecies biofilms may inhibit disinfection of opportunistic pathogens (Berry et al., 2006). In a hospital hot water distribution system following on site monochloramine treatment, Betaproteobacteria relative abundance was decreased and shifted toward stronger abundances of primarily Firmicutes, Alphaproteobacteria, and Gammaproteobacteria (Baron et al., 2014). Established *E. coli* biofilms in chlorinated distribution systems may proliferate in distribution systems (Williams and Brown-Howland, 2003).

Li et al. (2010) also examined the influence of phosphate addition on the microbiome of biologically active carbon filtration systems and found that the relative abundance of Betaproteobacteria was increased significantly with the addition of phosphorus under both bench and pilot scale conditions.

Chapter 3: Materials and Methods

3.1: Hydraid Point of Use Biosand Filters

Over the course of this research, 30 HydrAid BSFs were ordered from Cascade Engineering (Michigan). These filters consist of 41 cm fine filtration sand, 5 cm separation sand,

and 7 cm underdrain gravel. The filters are gravity fed with approximately 5cm head overlying the filtration sand. The filters were installed following the manufacturer instructions:

1) Leveling the surface of the filter with shims
2) Fully saturating media during installation
3) Adding underdrain gravel
4) Adding separation gravel
5) Adding filtration sand (2 bags)
    a. In cases where an inoculum was introduced to the sand surface, the volume of inoculum was displaced from bags of provided sand volumetrically

Pore volume of the filters was estimated by adding source water used for installation in 2L increments until the media was nearly saturated, at which point a graduated cylinder was used to add water until reaching the sand surface. Therefore, pore volume estimations do not include the 5cm head overlying the sand layer, but does include the volume of the outflow pipe at the corresponding head level.

3.1.1: Characterization of Porous Media

Component	Depth (cm)	Porosity (%)
Overlying Head	5	N/A
Filtration Sand	41	35.6
Separation Gravel	5	49.9
Underdrain Gravel	7	46.1

Table 1: Filtration media depth and porosity.

Where porosity is measured as:

$$\theta = \frac{\text{Saturated Mass} - \text{Dry Mass}}{\text{Saturated Mass}} \text{ x } 100\%$$

Bulk Density:

$$\rho_b = \frac{\text{Mass of Dry 100 mL Sample (g)}}{100 \text{ cm}^3}$$

Sand Sieving:

$$\text{Uniformity Coefficient} = \frac{D_{60}}{D_{10}}$$

Average Grain Size (D_{50}) = Where (% Passing = % Collected)

	Porosity (%)	**Uniformity Coefficient (D_{60}/D_{10})**	**Average Grain Size (μm)**	**Dry Bulk Density (g/cm^3)**
HydrAid Sand	35.6	2.83	321	1.62
Good Samaritan Hospital Installation Sand	37	6.76	412	1.60

Table 2: Filtration sand characteristics.

Sieve data shows that the Hydraid filtration sand delivered with filters is superior to filtration sand obtained by the Good Samaritan Hospital (La Romana). The filtration sand is of smaller mean diameter, better sorted, lower porosity and greater density. These properties are critical for improving adsorption at pore scale. Typically, local sands available for filter installation are less ideal for filtration than homogenized quarry sand sources.

3.2: Field Results from the Dominican Republic

3.2.1 Sites

Fieldwork was carried out from November 17-20 2014 in conjunction with water and health employees from the Good Samaritan Hospital (La Romana, Dominican Republic). These workers are responsible for the installation and maintenance of filters, as well as correspondence with community water leaders (typically one woman per batey is elected as a water correspondent). These bateyes lie within the San Pedro de Macoris and La Romana basin. The principal aquifer in this region, extending from the eastern-most coast of the Dominican Republic along the southern coastal plain as far as San Cristobal, is the Quaternary Reefal Limestone aquifer system (Gilboa, 1980). While the Gilboa publication of 1980 plots groundwater vectors from the Quaternary Reefal Limestone aquifer system flowing south to the Caribbean Sea, it additionally recognizes that saltwater intrusion has created additional stress on coastal aquifers with urban centers pumping excessively to supplement the tourism industry. The Good Samaritan Hospital well, used as a backup when the city of La Romana distribution system is impeded, is experiencing elevated salinity levels as a result of saltwater intrusion. It is estimated that groundwater wells within the Quaternary Reefal Limestone aquifer system are cased between 50-150 m depth and are expected to yield 15-250 $m^3 \times hr^{-1}$ (Bilboa, 1980). While data regarding the quantity and certainly the quality of groundwater within the Dominican Republic is limited, Bilboa shows a number of wells in the region of our batey surveys cased at depths between 25-100 m. These shallow water table depths in conjunction with the high conductivity, high porosity, karstic fracture properties of a reef-limestone aquifer system suggest that the region's groundwater sources are at high risk of contamination from pathogens as well as agricultural byproducts including fertilizers, pesticides and herbicides. Throughout the bateyes,

untreated pits dug into the soil are often used as latrines. At batey Como Quiera, wastewater was observed flowing over the soil surface from a nearby latrine. Untreated wastewater latrines extending several feet below soil surface limit sorption of fecal colloids during infiltration to the water table causing high risk of fecal-oral pathogen transmission.

By correspondence with members of batey households, there are typically five to seven people in each household and BSFs were operated with a hydraulic loading rate of two to three five gallon buckets per day (approximately 40-60 $L \times d^{-1}$). Households often housed livestock including chickens, horses and dogs. Water is typically hauled from the nearby distribution pipe, carried to the home and stored until water is required, at which point a charge is added to the BSF. Storage in possibly contaminated, often uncapped devices coupled with presence of livestock in the home reflects the possibility of fecal contamination between collection and point of use. None of the households reported sicknesses related to water, and in some cases water was both filtered and boiled. For all field sites visited herein, source water was obtained from large storage tanks gravity fed to a central distribution pipe. Tanks were groundwater fed or in some cases, trucked in from another unknown source.

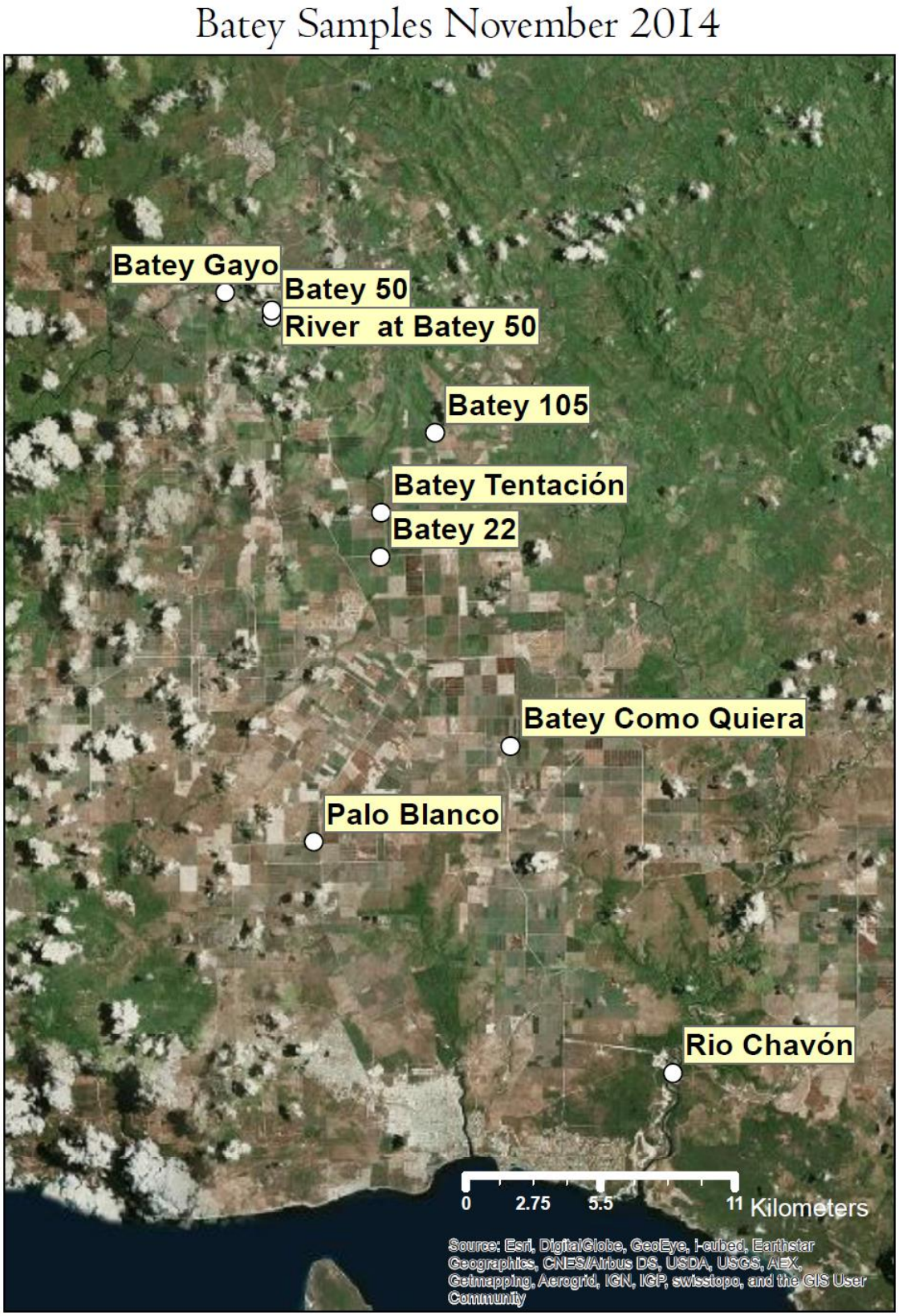


Figure 1: Sites visited during fieldwork in Southeastern Dominican Republic.

Site A: Batey Tentación (November 17, 2014)

	Approx. Value
pH	7.4
Alkalinity	200 ppm
Cl^-	0 ppm

Table 3: Water chemistry at Batey Tentación.

Figure 2: Filter "A1"

The following are coliform counts from the first batey visited (Batey Tentación) referred to as batey "A" with biosand effluent counts being labeled as A1, A2, and so on. All microbe counts herein are performed using 100mL samples through 0.45 μm filters plated upon MOD mTEC agar (with exception of Rio Chavón dilution factors):

Sample	E. coli (CFU/ 100mL)	Other	Total [CFU/100mL]
Source A	1	11	12
A1	0	13	13
A5	2	TNTC	TNTC
A6	0	2	2
A8	0	TNTC	TNTC

Table 4: *E. coli* concentrations at Batey Tentación.

The outflow pipe at Batey 22 (adjacent to the reservoir tank serving Batey 22 and Batey Tentación) was also surveyed for general chemistry parameters using test strips:

	Approx. Value
pH	7.0
Alkalinity	200-240 ppm
Cl^-	0 ppm

Table 5: Batey 22 water chemistry.

Figure 3: Outflow at Batey 22

Figure 4: Batey 22 source tank serving Batey 22 and Batey Tentación

Site B: Batey Como Quiera (November 17, 2014)

Source	Approx. Value
pH	8
Alkalinity	220 ppm
Cl^-	2-3 ppm

Table 6: Batey Como Quiera water chemistry.

Sample	*E. coli* [CFU / 100mL]	Other [CFU / 100mL]	Total [CFU/100mL]
Source B	0	31	31
B1	0	TNTC	TNTC
B2	4	37	41
B3	16	TNTC	TNTC
B4	2	TNTC	TNTC

Table 7: Batey Como Quiera *E. coli* and coliform counts.

The source water for Batey Como Quiera appears to be heavily contaminated with high coliform counts. Once again, increased *E. coli* and coliform counts in the effluent of household BSF pose substantial risk to human health. Test strips indicate the fairly recent addition of chlorine to the source water (2-3 ppm) likely as bleach (high source water pH with white/yellow, flat precipitates often showing in the sand filtration material). Filter #B4 produced several live macro-invertebrates in the effluent and was discontinued by the user due to fouling. An untreated sewage effluent sample was also taken here for DNA extraction.

Figure 5: Macro-invertebrates in B4 effluent

Additional samples (November 17, 2014)

Samples were taken from Rio Chavón, the Good Samaritan Hospital Cistern, and the Casa de Campo tap water:

Sample	*E. coli* [CFU/100mL]	Other	Total [CFU/100mL]	*E. coli* [CFU/ 100mL]
RioChavón1mL	1	34	3500	100
RioChavón10mL	4	TNTC	TNTC	40
RioChavón100mL	18	TNTC	TNTC	18
Good Samaritan Cistern	1	TNTC	TNTC	1
Casa De Campo Tap	0	67	67	0

Table 8: *E. coli* and coliform counts for Rio Chavón, Good Samaritan Hospital Cistern, and Tap water at Casa de Campo.

Site C: Batey 105 (November 18, 2014)

For Batey 105, chlorine test strips indicated a concentration of ≈ 0 ppm.

Sample	*E. coli* [CFU / 100 mL]	Other	Total [CFU/100mL]
Source C	-	-	-
C1	4	TNTC	TNTC
C3	8	TNTC	TNTC

Table 9: *E. coli* and coliform counts at Batey 105.

Batey 50 was also visited on the 18th although the BSFs were not operating at this batey. River water (used for bathing, livestock), a nearby spring, and the source water (a leaking source tank) were plated for *E. coli* and coliform.

Sample	*E. coli* [CFU / 100 mL]	Other [CFU / 100 mL]	Total [CFU/100mL]
Batey 50 Source	0	TNTC	TNTC
Batey 50 Spring	87	TNTC	TNTC
Batey 50 River	TNTC	TNTC	TNTC

Table 10: *E. coli* and coliform counts at Batey 50.

Figure 6: Batey 50 River Sample on MOD mTEC agar

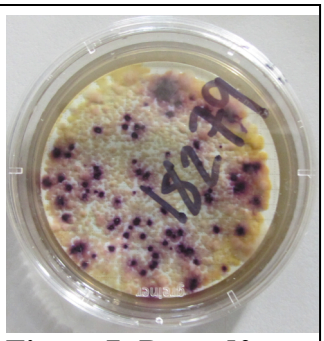


Figure 7: Batey 50 Spring sample on MOD mTEC agar.

Figure 8: River sample at Batey 50

Site D: Batey Gayo (November 18, 2014)

Chlorine strips for Batey Gayo indicated a chlorine concentration of ≈ 0 ppm.

Sample	*E. coli* [CFU / 100mL]	Other [CFU / 100 mL]	Total [CFU/100mL]
Source D	0	267	267
D1	0	TNTC	TNTC

Table 11: *E. coli* and coliform counts at Batey Gayo.

Batey Palo Blanco was surveyed November 19th for only DNA extraction and biosand collection. Biofilm growth at the surface of BSFs was exceptional here as compared to other bateyes, despite the source water having about 2 ppm Cl^-. Water levels above the biolayer were appropriate at Palo Blanco (about 2 inches), suggesting that filters are properly maintained.

3.3: Filter Seeding

3.3.1 Linnwood Drinking Water Treatment Plant (Milwaukee, WI)

Twelve filters were initially installed and seeded with 800 mL of inoculum sand from slow sand filter bed number 21 at the Linnwood Drinking Water Treatment Plant (Milwaukee,

WI). Biosand from the treatment plant was collected from the filter bed using non-sterile methodology including shoveling of the overlying anthracite layer and coring with a steel pipe. Slow sand filtration at the Milwaukee plant is precluded with flocculation, sedimentation and ozonation prior to reaching slow sand reactor beds. Seeds were stored on ice then at 4℃ and used to inoculate triplicate filters fed with influents of Lake Michigan surface water (Bradford Beach, Milwaukee, WI), dolomitic groundwater (Lapham Hall, Milwaukee, WI), and Milwaukee municipal tap water supplemented with 4 mg$\times$ L^{-1} humic acid. Seeding was accomplished by displacing HydrAid sands provided with the filter volumetrically (800mL HydrAid sands replaced with 800mL biosand sample at the surface of the filter). Initial biosand samples were stored on ice, and sequencing was performed of the initial consortia sampled from the filtration bed.

3.2.2 Dominican Republic Biosand Inoculum

Biosand samples were collected in the Dominican Republic using sterile spatulas to collect approximately 50 mL active biosand at the surface of each filter being operated in the home. Biosand samples were collected from 17 homes in four different bateyes throughout southeastern Dominican Republic. Inoculum sands were composited by bateyes in which filters appeared to be operating efficiently (Batey Tentación and Batey Gayo), and those in which filters were less effective (Batey Como Quiera and Batey 105). These designations were based on comparing *E. coli* counts of influent and effluent. In many cases, BSF effluents showed higher concentrations of *E. coli* than source water samples. Biosand samples were stored on ice during return to Milwaukee, and stored at 4 ℃ until seeding. Filters were seeded with duplicates for each sand compilation and each influent type (municipal tap water and groundwater) using

100mL of inoculum sand added to the surface of the filter, displacing stock sand provided by Hydraid. DNA from each inoculum sand compilation was extracted.

3.5 Laboratory Filter Sand DNA Sampling

DNA extractions were taken from filters on an approximately weekly basis from depths of 10.2, 20.4, and 30.6cm beneath sand surface from a three-way stop-cock attached to a ½ inch PVC sampling port. For each filter, two ½ inch holes were drilled on-center at each depth, PVC valves attached, and 3 way valve installed onto the PVC valves perpendicular to the outflow pipe. Approximately 2 mL of sand was extracted from each depth of the filter (1 mL from each side) for every sampling event, stored on ice and returned to the lab, where 1 gram samples of sand were weighed, then stored at -80℃ for DNA extraction and sequencing.

3.6 Filtration Methodology

An *E. coli* strain isolated from the berm of Bradford Beach (saturated sand) by the McLellan lab was stored in a freezer vial at -80℃. One loopful of cells was taken from the -80℃ stock and streaked upon LB plates, which were then incubated overnight at 37℃. A loopful of plated cells was transferred to 25 mL Miller LB broth in a sterile 50 mL centrifuge tube and incubated on a shaker with moderate agitation for 16 hours. Cells were then centrifuged at 4,000 RPM for 10 minutes at 4℃, supernatant poured, re-centrifuged in 10mM NaCl, supernatant poured, and vortexed to ensure separation of cells from LB broth. Centrifugation in 10mM NaCl was completed three times to ensure separation of biomass from LB broth. The resulting density of *E. coli* cells in 10 mM NaCl was quantified using UV Spectrophotometric absorbance at wavelength of 220 nm. After adjustment, a cell suspension with OD_{220} in the range of 0.65-0.7

was diluted by a factor of fifty, and 10 mL aliquots were added to each 20L filter charge for filtration. The resulting initial concentration of *E. coli* cells was on the order of $\approx 10^6$ CFU / L.

Initial *E. coli* concentrations were evaluated by adding the cell aliquot to the 20L charge, the carboy was shaken (overturned 3-4 times), and a 500 mL sample was poured from the carboy prior to adding the spiking solution to the filter. Triplicates withdrawn from the 500 mL sample were plated on mTEC agar plates and incubated at 35℃ for 2 hours, then at 44.5℃ for 22 hours following EPA method 1603 (EPA, 2009). Coliform forming units (CFU) per liter is calculated as:

$$\frac{\text{CFU}}{\text{L}} = \frac{\text{CFU}}{\text{Sample Volume (mL)}} \times \frac{1{,}000\ \text{mL}}{\text{L}}$$

Influents from the previous day are stored at room temperature with filters until the next day, such that *E. coli* reductions during the 24-hour residence time of the filter are attributed to filtration mechanisms rather than overnight survivorship.

Effluents from each BSF were collected in 13L carboys during filtration experiments. Once 13L was collected, the carboys were capped, well shaken, and a 500mL sample was poured and stored at 4℃ until plating. Effluents were plated on modified mTEC media in triplicates of 0.01mL, 0.1mL, 10 mL, or as single 100 mL plates depending on the expected concentration following EPA method 1603 (EPA, 2009). Effluent samples were stored for re-plating at 4℃.

For the introduction of bacteriophage MS2 to tap water maintained filters in the laboratory, the same three day filtration protocol was followed with influent concentrations of MS2 of approximately 10^5 plaque forming units (PFU) / L. MS2 concentrations were analyzed by double layer plate count method established by EPA method 1602 (EPA, 2001).

3.7 Enumeration of interstitial *E. coli* cells by eluent and plate count method

Upon disassembly of the BSFs, a groundwater filter seeded with biosand from the Linnwood treatment plant (Filter G1) was spiked with *E. coli* cells, which were allowed to permeate the porous matrix for 24 hours (following the filtration protocol). Sand samples were then collected from the surface, and in 10cm increments throughout the sand layer. One-gram sand samples were placed into 10 mL MOPS NaCl buffer solution, sonicated for ten minutes, briefly vortexed, and plated 9 mL of solution was plated on mTEC agar plates for *E. coli* enumeration in depth-adsorption isotherms (Liu and Li, 2008). Using this methodology, CFU / g sand is calculated as follows:

$$\frac{\text{CFU}}{\text{g Sand}} = \frac{\text{CFU}}{\text{9 mL MOPS NaCl}} \times \frac{\text{10 mL MOPS NaCl}}{\text{g Sand}}$$

The surface sample (0 cm depth) showed TNTC *E. coli* concentrations and was re-plated using 0.1 g of sand in the same methodology.

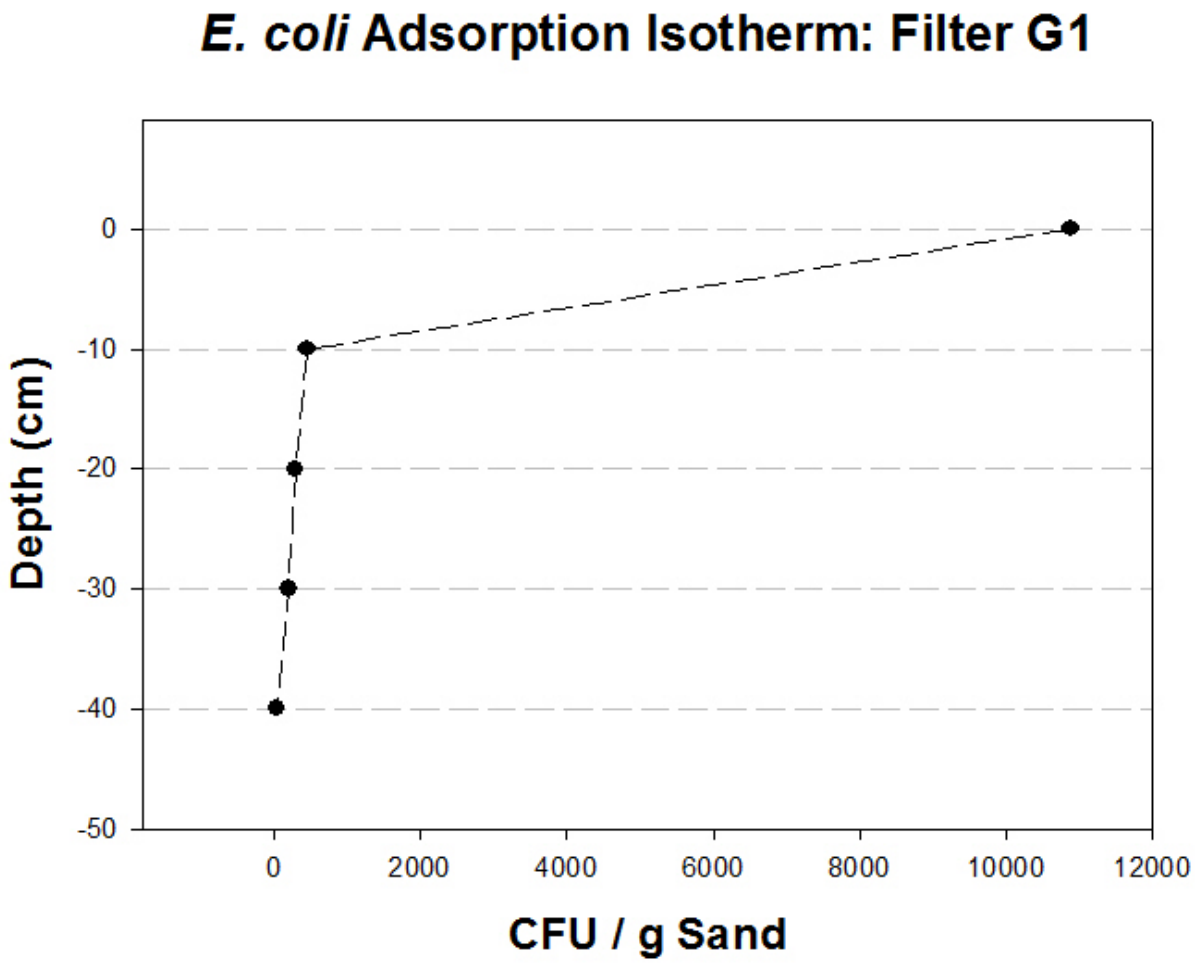


Figure 9: *E. coli* adsorption isotherm at depths from 0 to 40 cm below sand surface

Results of this analysis show that the first ten centimeters of filter depth containing the schmutzdecke is critical for the adsorption of pathogens.

3.8 Filter Installation Dates and Treatments

	Dominican Tap (AD Sand)	Dominican Tap (BC Sand)	Dominican Groundwater (AD Sand)	Dominican Groundwater (BC Sand)	Tap Water (Linnwood Sand)	Groundwater (Linnwood Sand)	Lake Michigan (Linnwood Sand)
Seed Date	1/15/2015	1/15/2015	1/28/2015	1/28/2015	10/10/2014	10/13/2014	10/10/2014
# Filters	2	2	2	2	3	3	3
Inoculum Volume (mL)	100	100	100	100	800	800	800

Treatment	20 l × d^{-1} + 4 ppm humic acid	20 l × d^{-1} + 4 ppm humic acid	20 l × d^{-1}	20 l × d^{-1}	20 l × d^{-1} + 4 ppm humic acid	20 l × d^{-1}	20 l × d^{-1}

Table 12: Filter inoculum and treatments.

3.9 Colorimetry: Nutrients and Turbidity of Source Water and Effluent

Colorimetry was performed periodically as an indication of nitrification / denitrification processes, orthophosphate assimilation and turbidity reductions. Colorimetry was performed using a Hach DR890 colorimeter with corresponding reagent packets. Turbidity is performed using an unfiltered 10 mL sample blanked with deionized water. Nutrient samples are filtered through a 0.45 µm membrane prior to analysis. Nitrate analysis is performed on a 10mL sample using a filtered sample without reagent as a blank. Orthophosphate analysis is carried out using a 25mL sample size blanked by sample without reagent.

3.10 Illumina MiSeq Sequencing and qPCR

qPCR analysis of cell density (copy numbers per gram of sand) was carried out by Dr. Jen Fisher, using a Total Bacteria Assay following Øvreås and Torsvik (1998) for DGGE end-point PCR in soils. The PCR assay amplifies the V3 region of 16S rRNA gene from positions 338 to 518 based on *E. coli* numbering. A standard curve was produced based on the *Nitrospira* 16S rRNA gene sequence. Thermoprofile for the qPCR assay was as follows:

-95 ℃ for 10:00

-40 cycles of 0:15 at 95 ℃

-0:15 at 53℃

-0:15 at 72℃

25 µl reaction volumes with 12.5 µl Power SYBR Master Mix (Life Technologies, Grand Island, NY, USA) forward and reverse primers (100 nM final concentration), 5 µl of DNA template, and molecular grade water was the balance volume. Conversion to obtain copy numbers per gram of sand was 15 where (CN/rxn x 15 = CN/g sand).

4.0 Experimental Results

4.1 Filter Flow Rates

Flow rate largely dependent upon filter source waters. While tap water and groundwater maintained filters showed little decline in flow rates with maturity, filters maintained with Lake Michigan water as influent quickly declined in flow rates and showed greater variation in flow rate among replicates.

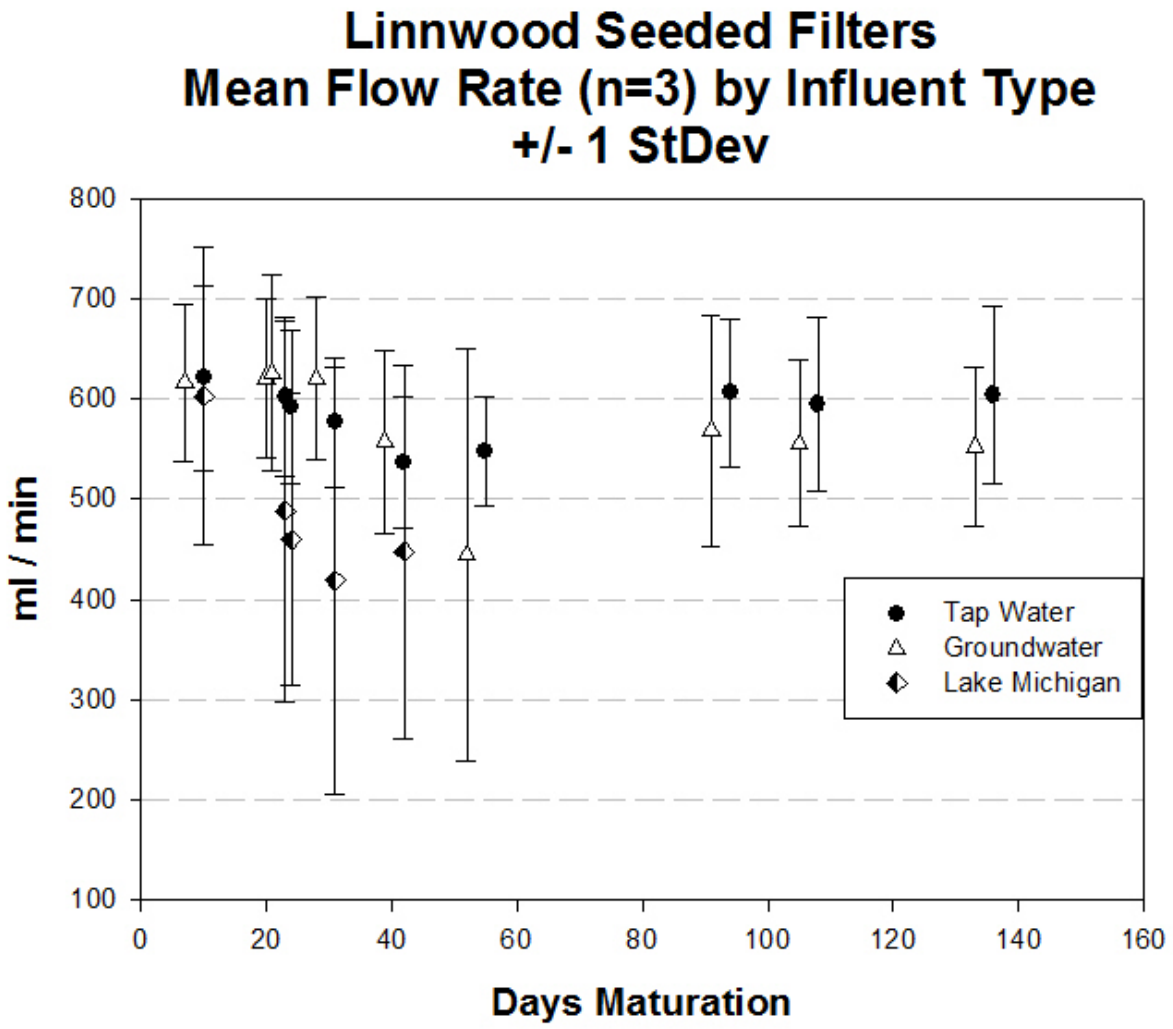


Figure 10: Mean flow rates in BSFs seeded with Linnwood Drinking Water Treatment Plant inoculum sands grouped by influent type (+/-) 1 StDev (n=3).

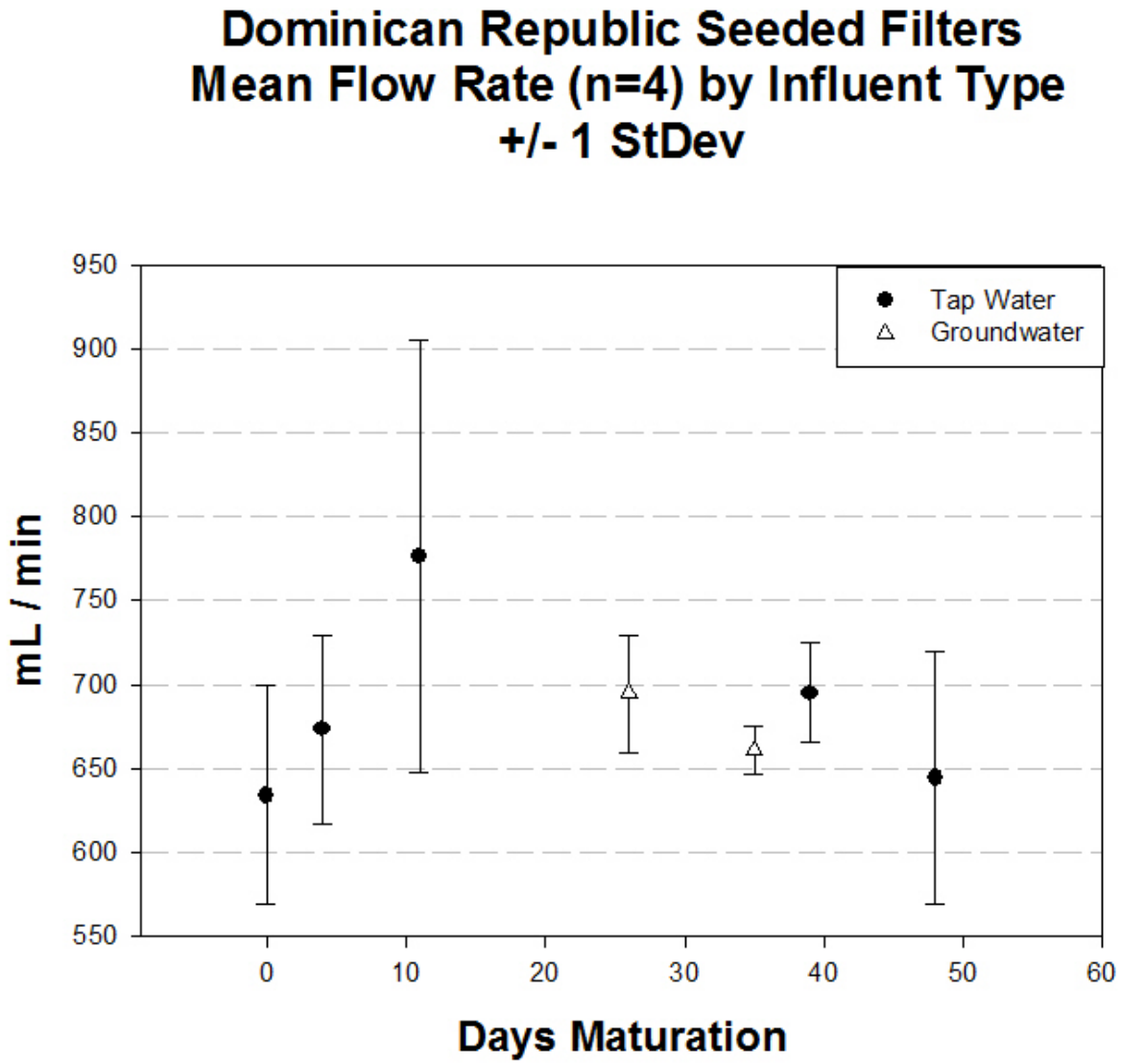


Figure 11: Mean flow rates in BSFs seeded with Dominican Republic inoculum sands grouped by influent type.

4.2 *E. coli* Reductions

4.2.1 Tap Water Maintained BSFs with Dominican Republic Inoculum Sand

For the following filtration plots, inoculum sands from the Dominican Republic are represented as triangles for bateyes A and D (or "good" inoculum) and as squares for bateyes B and C (or "bad" inoculum), with the exception of mean reductions where filters are grouped.

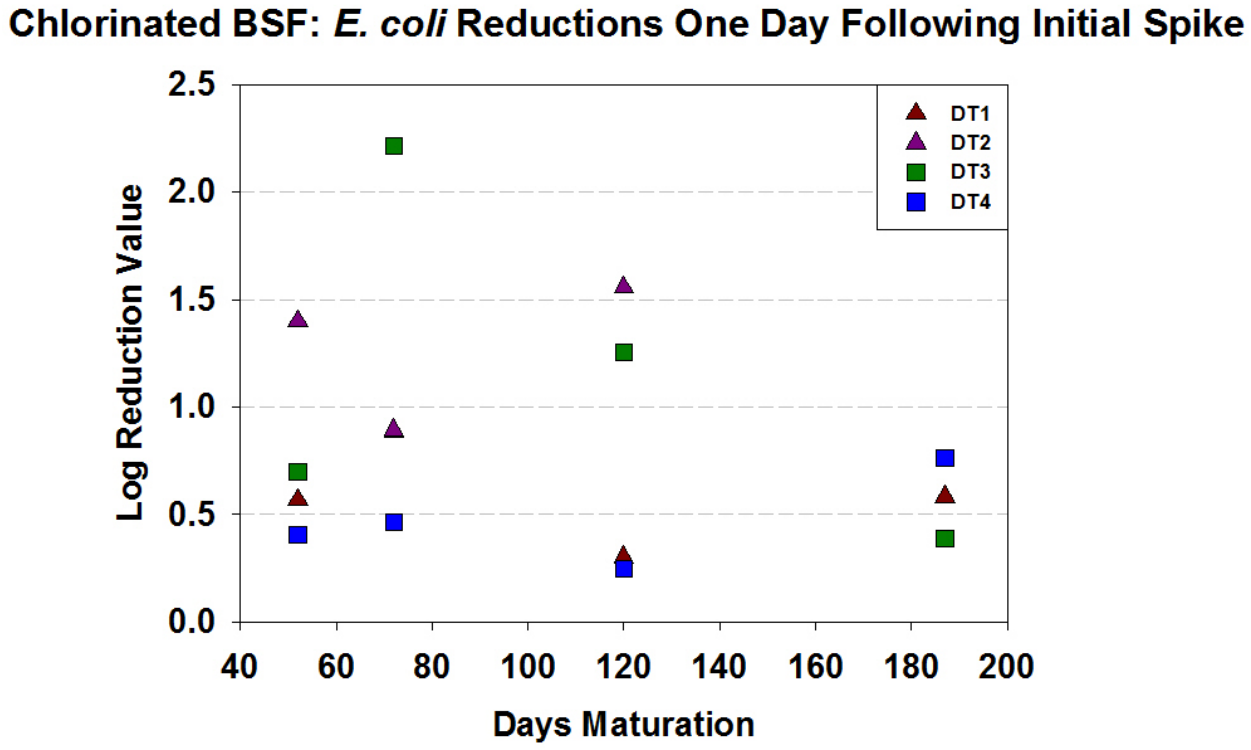


FIGURE 12: *E. coli* reductions one day after initial spiking in chlorinated BSFs seeded with inoculum sands from the Dominican Republic.

Plotting filtration efficiency for each tap water maintained filter on an individual basis, there are no discernable trends in filtration efficiency among batey inoculum sand groupings.

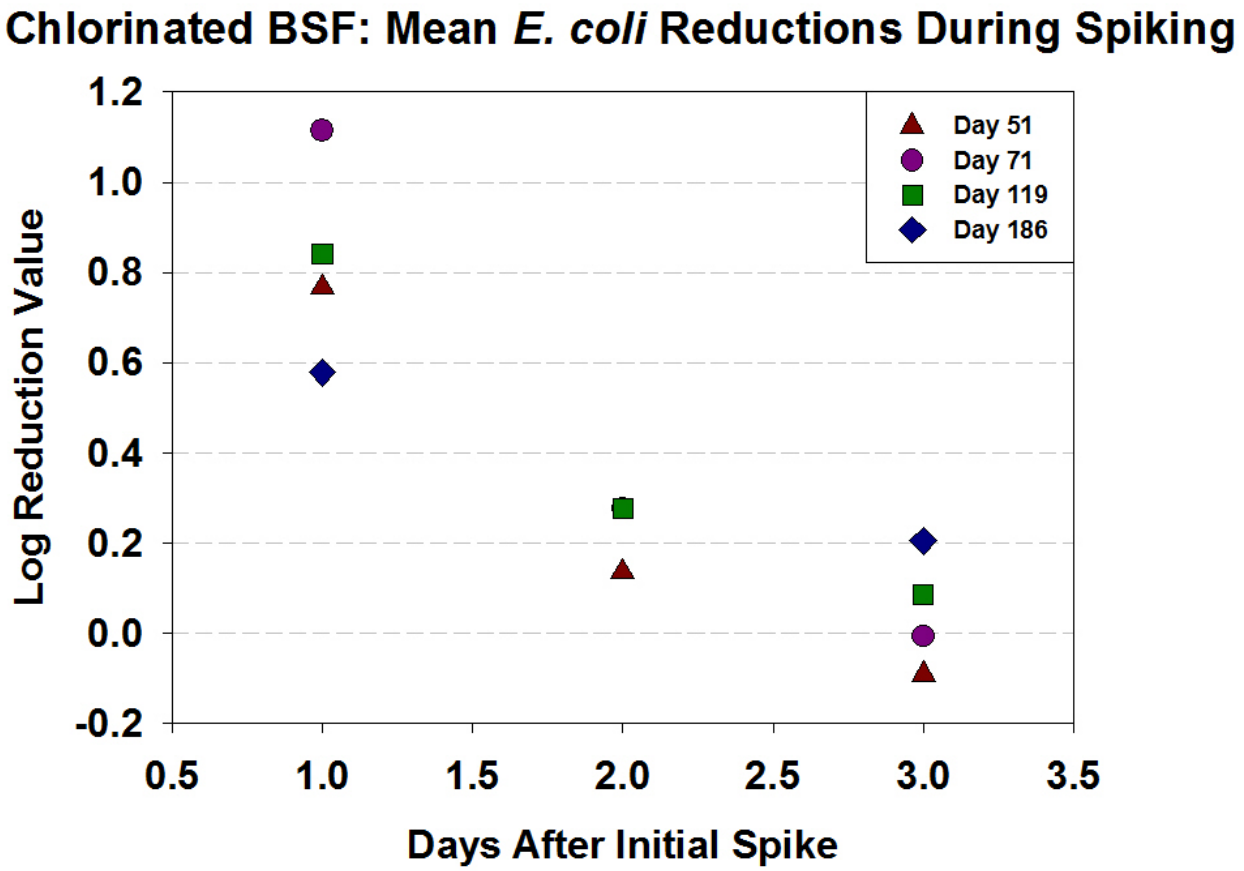


Figure 13: Mean (n=4) *E. coli* reductions during spiking in chlorinated BSFs seeded with inoculum sands from the Dominican Republic.

In a series of three day spiking events, BSFs seeded with inoculum sand from the bateyes and maintained with tap water influent showed declining filtration efficiency with continued exposure to *E. coli*. By the third day after the initial spike, reductions often become negative for tap water maintained BSFs. Furthermore, it is unclear whether any maturation trend exists in filtration efficiency.

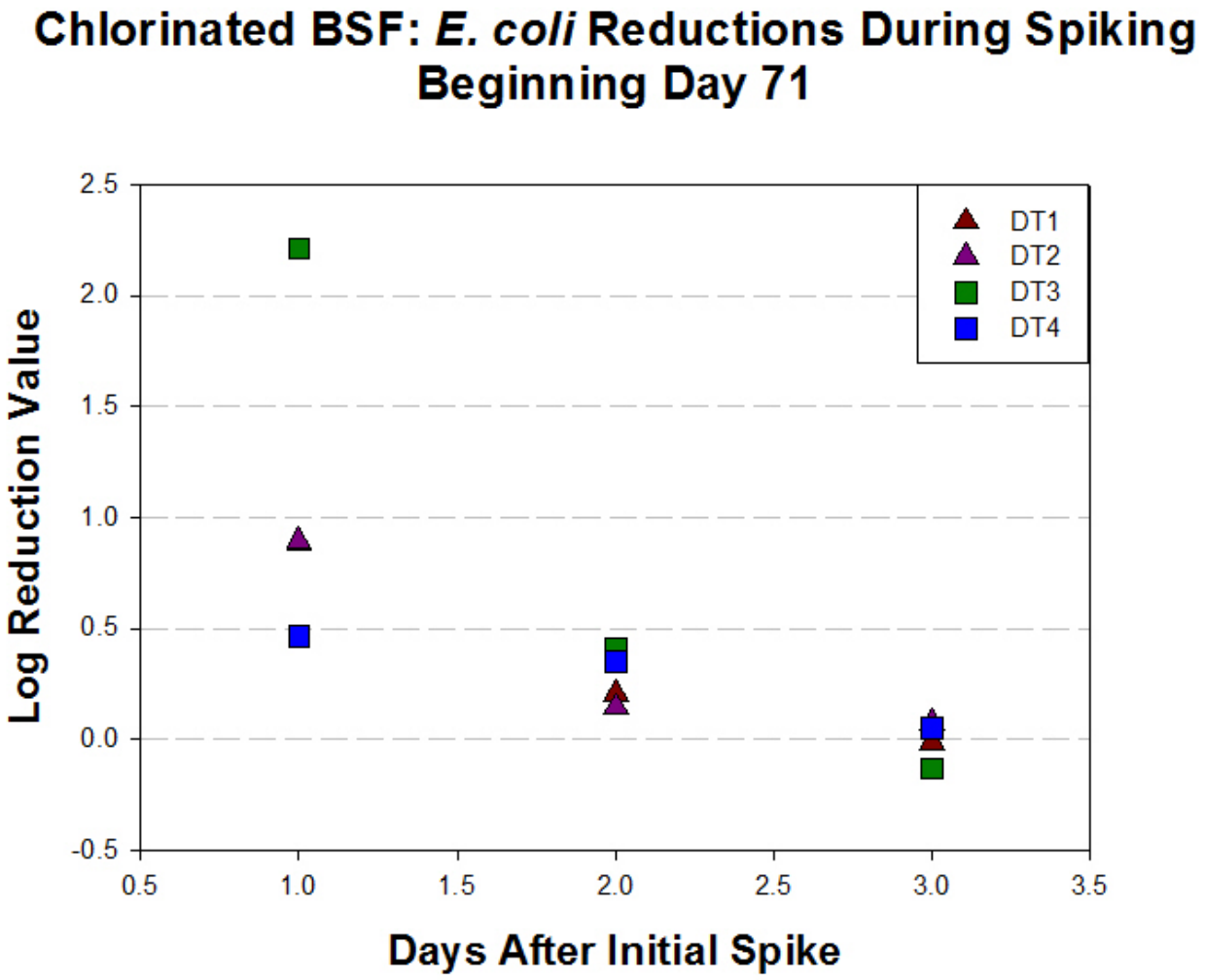


Figure 14: *E. coli* reductions during spiking beginning day 71 of filter maturity in chlorinated BSFs seeded with inoculum sands from the Dominican Republic.

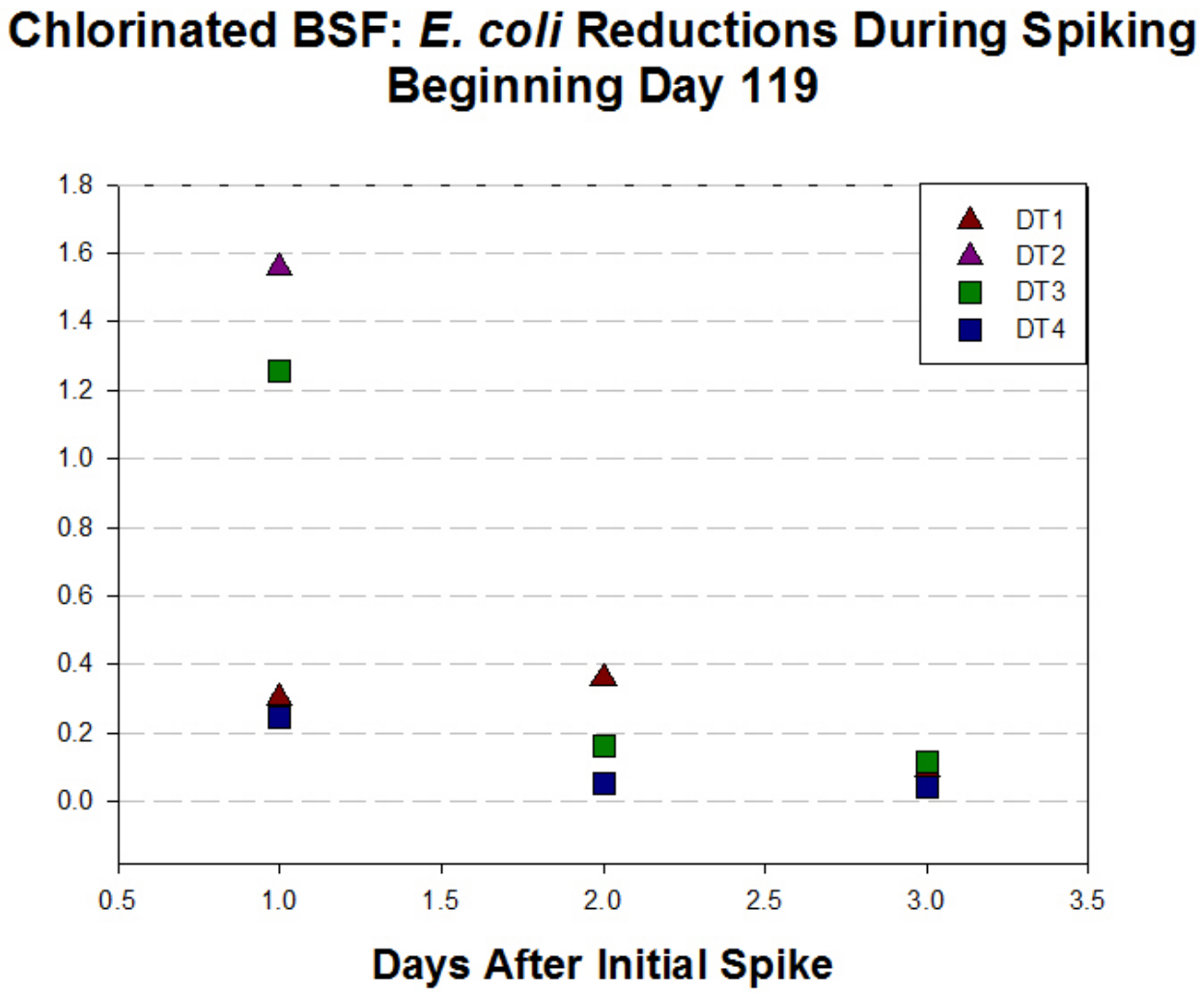


Figure 15: *E. coli* reductions during spiking beginning day 119 of filter maturity in chlorinated BSFs seeded with inoculum sands from the Dominican Republic.

Filter DT2 was discontinued at day 120 due to irreversible clogging.

One month after the final spiking period for tap maintained filters seeded with inoculum from the Dominican Republic, culturable *E. coli* continued to be found in the effluent at high concentrations for up to one month despite the filters having returned to a chlorinated influent

source following the spiking procedure.

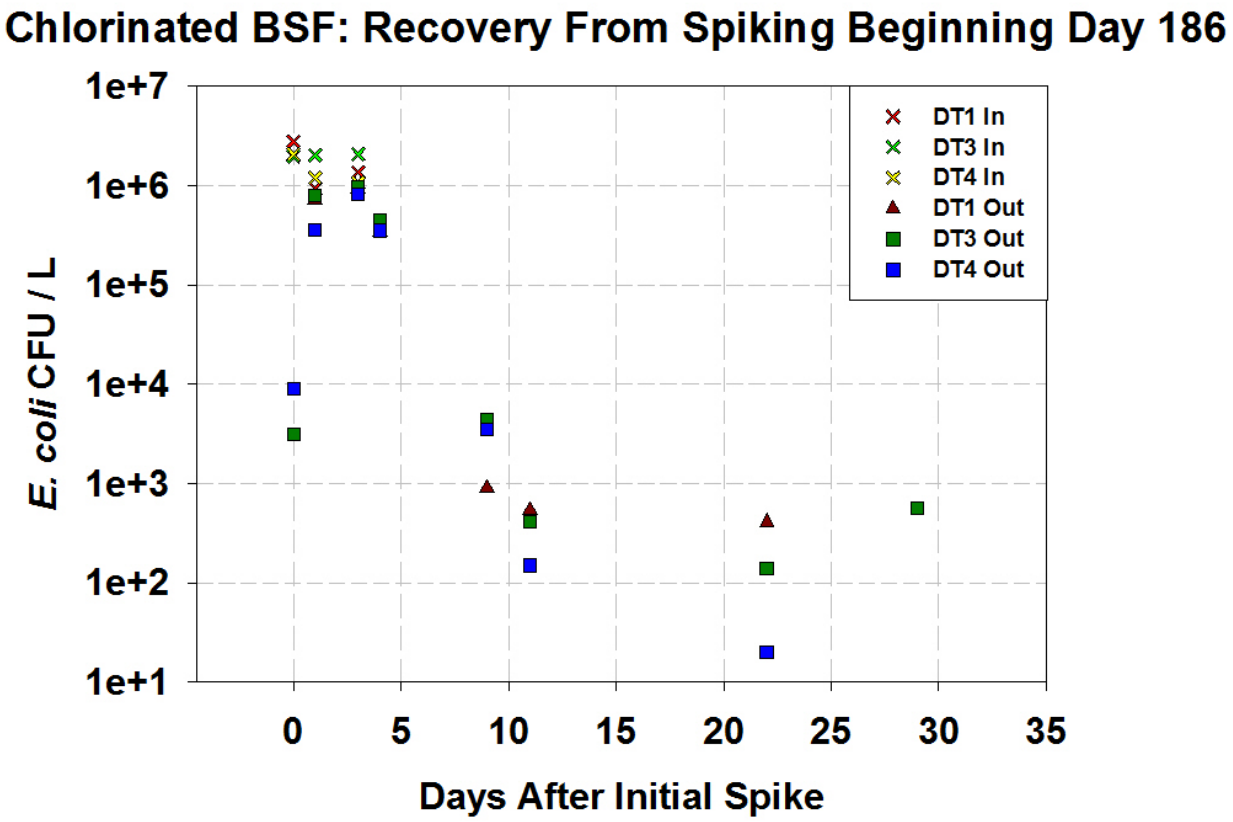


Figure 16: *E. coli* concentrations during recovery from spiking beginning day 186 of filter maturity in chlorinated BSFs seeded with inoculum sands from the Dominican Republic.

4.2.2 Groundwater Maintained BSFs with Dominican Republic Inoculum Sand

Grouping all reduction data, slight maturation trends can be found in filtration efficiency for BSFs maintained with groundwater. This maturity trend is easily recognizable when considering only *E. coli* reductions after the initial spiking event (one day after spiking).

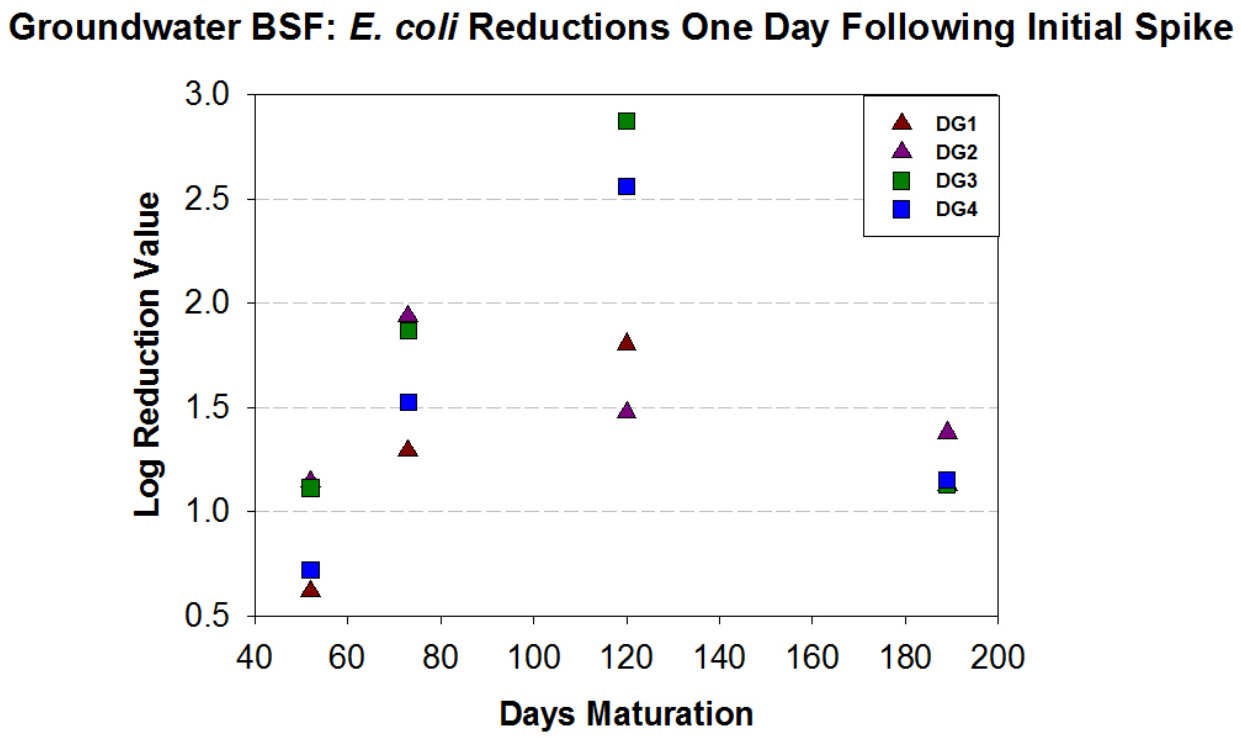


Figure 17: *E. coli* reductions one day after initial spiking in chlorinated BSFs seeded with inoculum sands from the Dominican Republic.

Taking groundwater maintained BSFs with Dominican Republic inoculum sand and grouping average reduction data from the four filters, these filters maintain filtration efficiency over the course of the three-day spiking period. In general, groundwater maintained filters are more consistent in achieving *E. coli* reductions as compared to tap water maintained BSFs. After three days of exposure, filtration efficiency commonly maintained a 1 to 2log reduction.

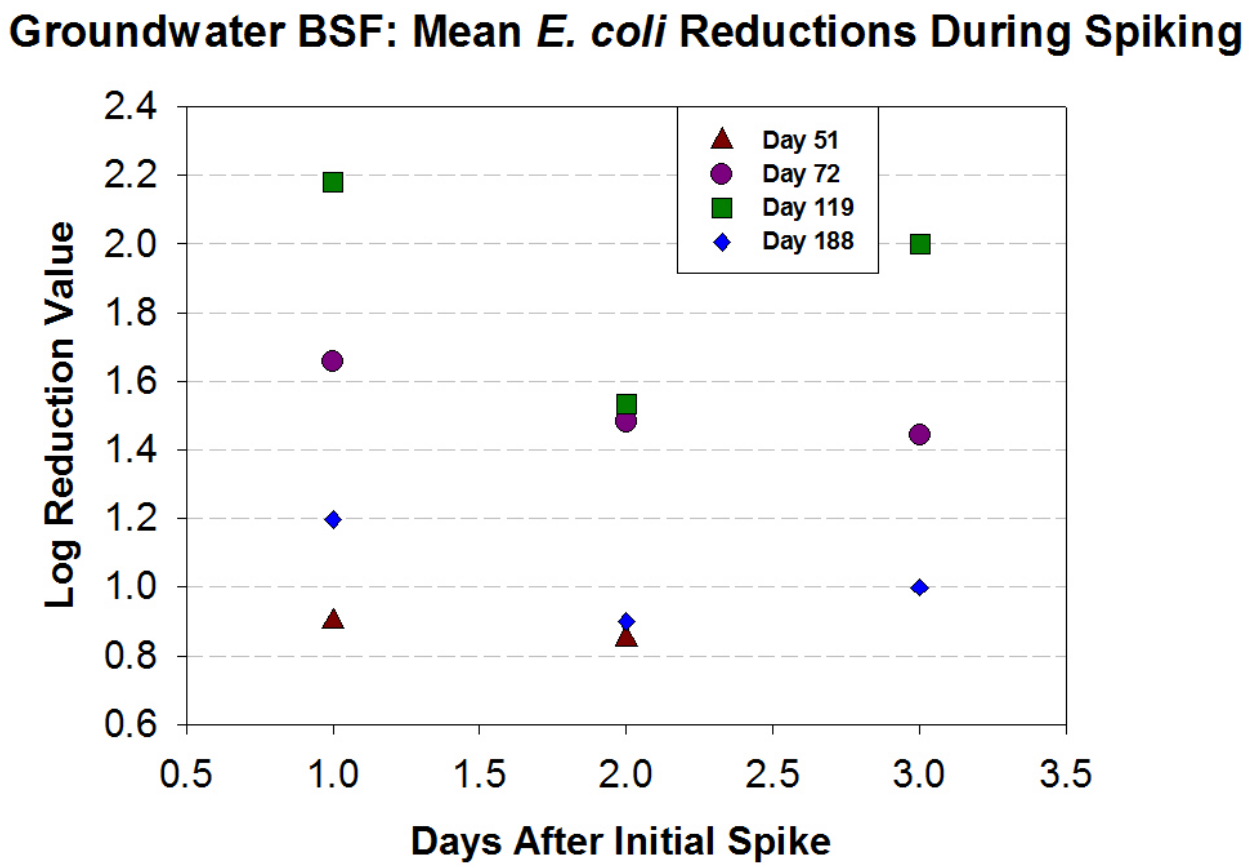


Figure 18: mean (n=4) *E. coli* reductions during spiking in groundwater maintained BSFs seeded with inoculum sands from the Dominican Republic.

While the overall *E. coli* reductions during the spiking process were more efficient than filters maintained with tap water, the groundwater maintained BSFs were slightly more gradual in recovering from spiking events.

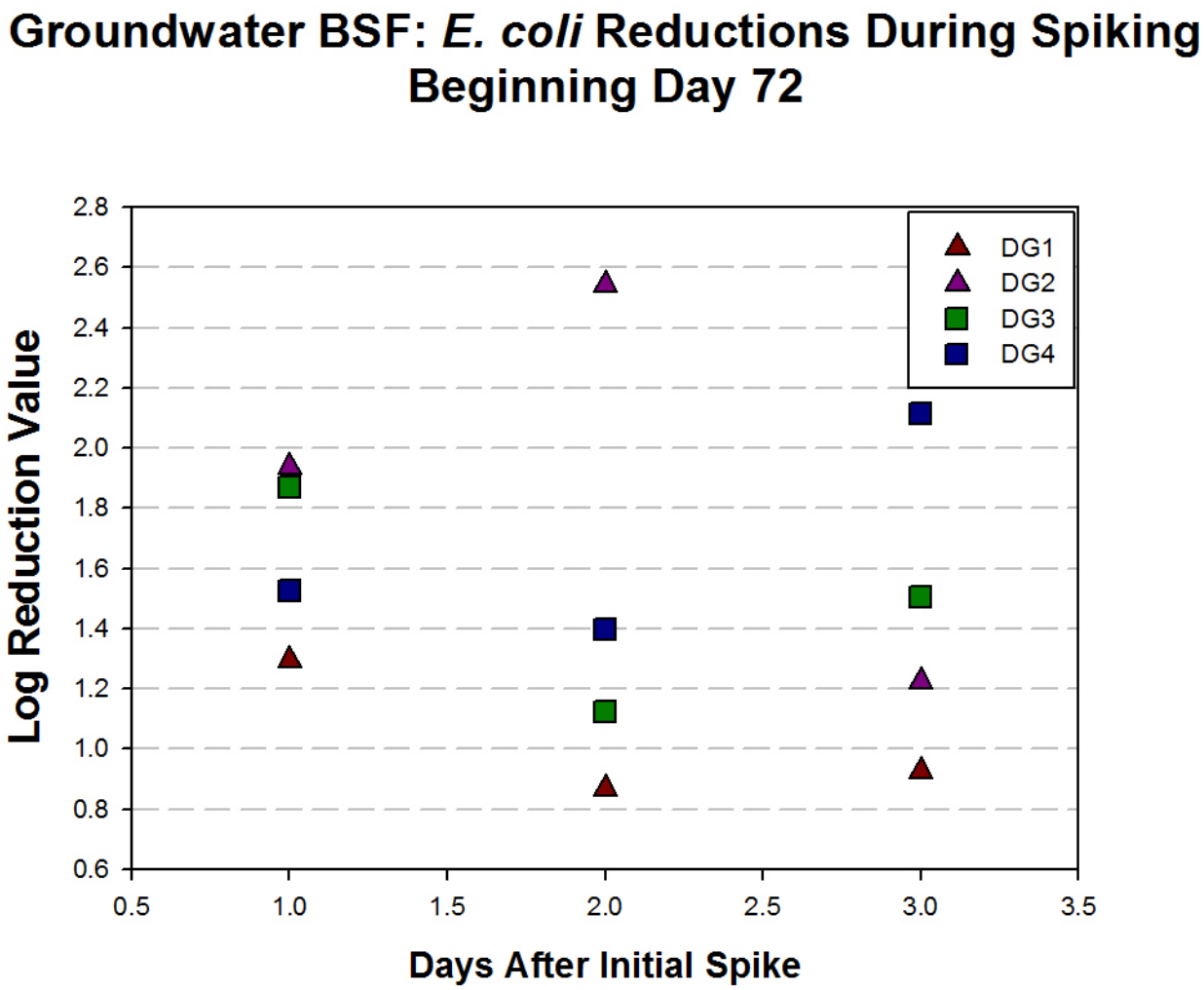


Figure 19: *E. coli* reductions during spiking beginning day 72 of filter maturity in groundwater maintained BSFs seeded with inoculum sands from the Dominican Republic.

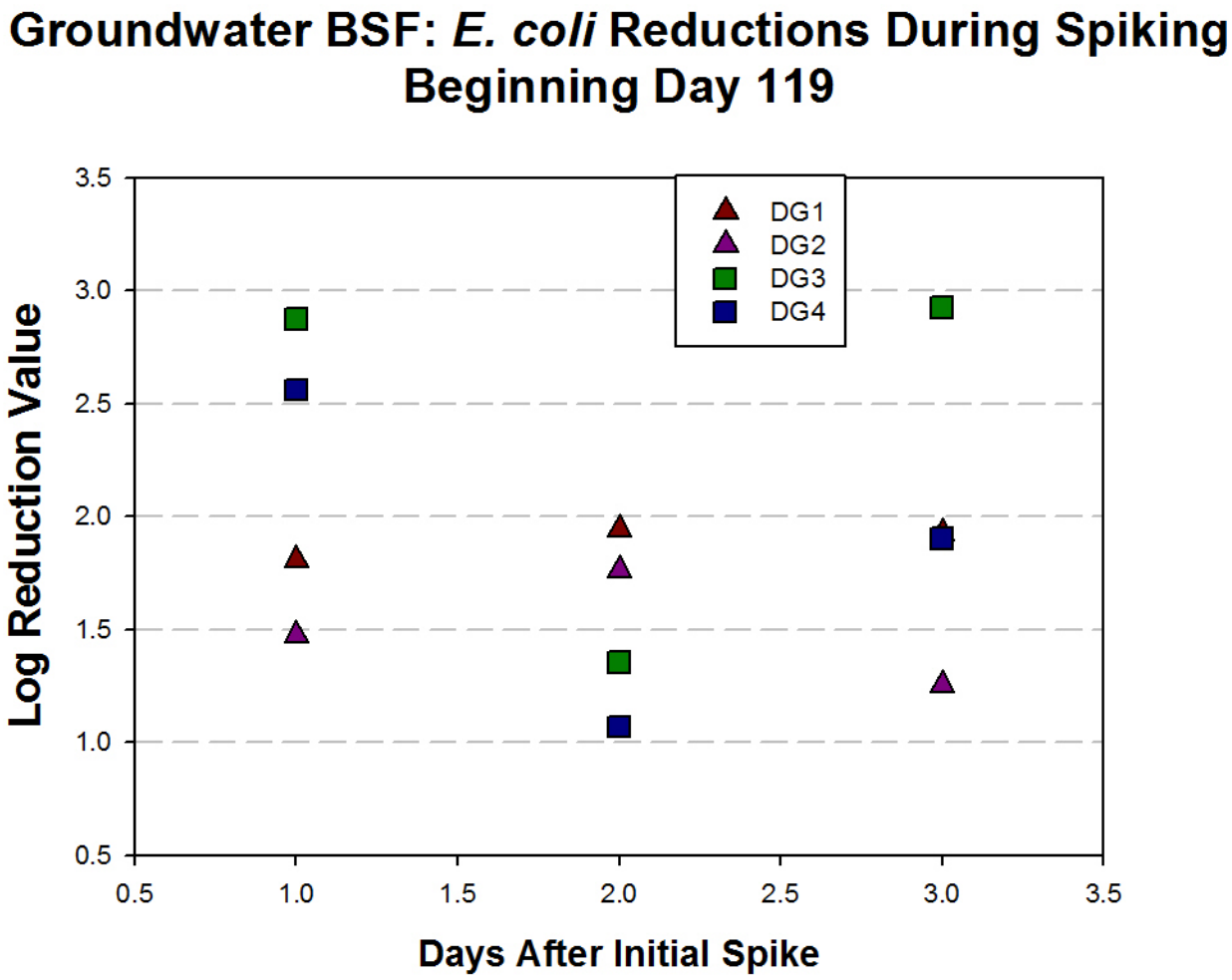


Figure 20: *E. coli* reductions during spiking beginning day 119 of filter maturity in groundwater maintained BSFs seeded with inoculum sands from the Dominican Republic.

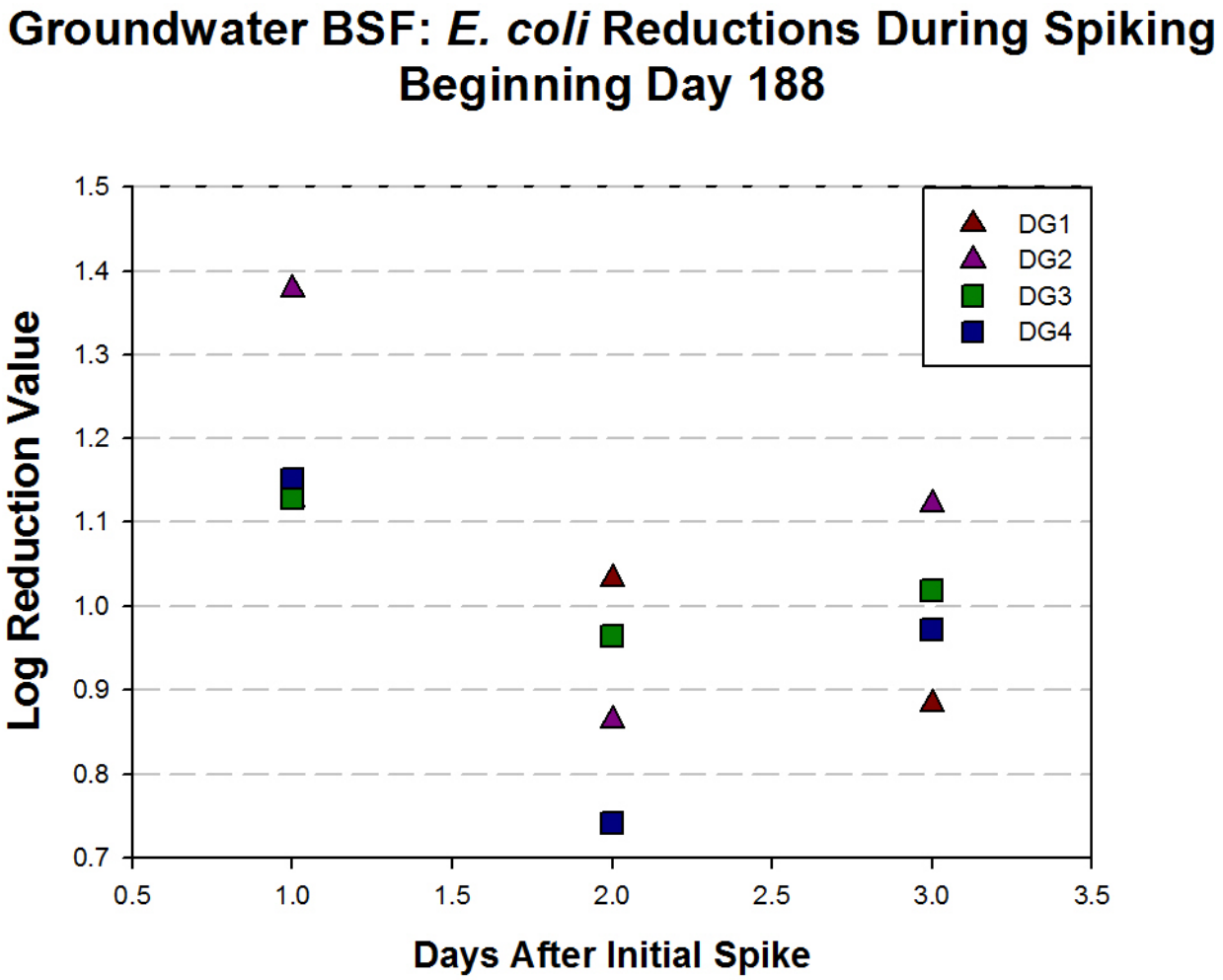


Figure 21: *E. coli* reductions during spiking beginning day 188 of filter maturity in groundwater maintained BSFs seeded with inoculum sands from the Dominican Republic.

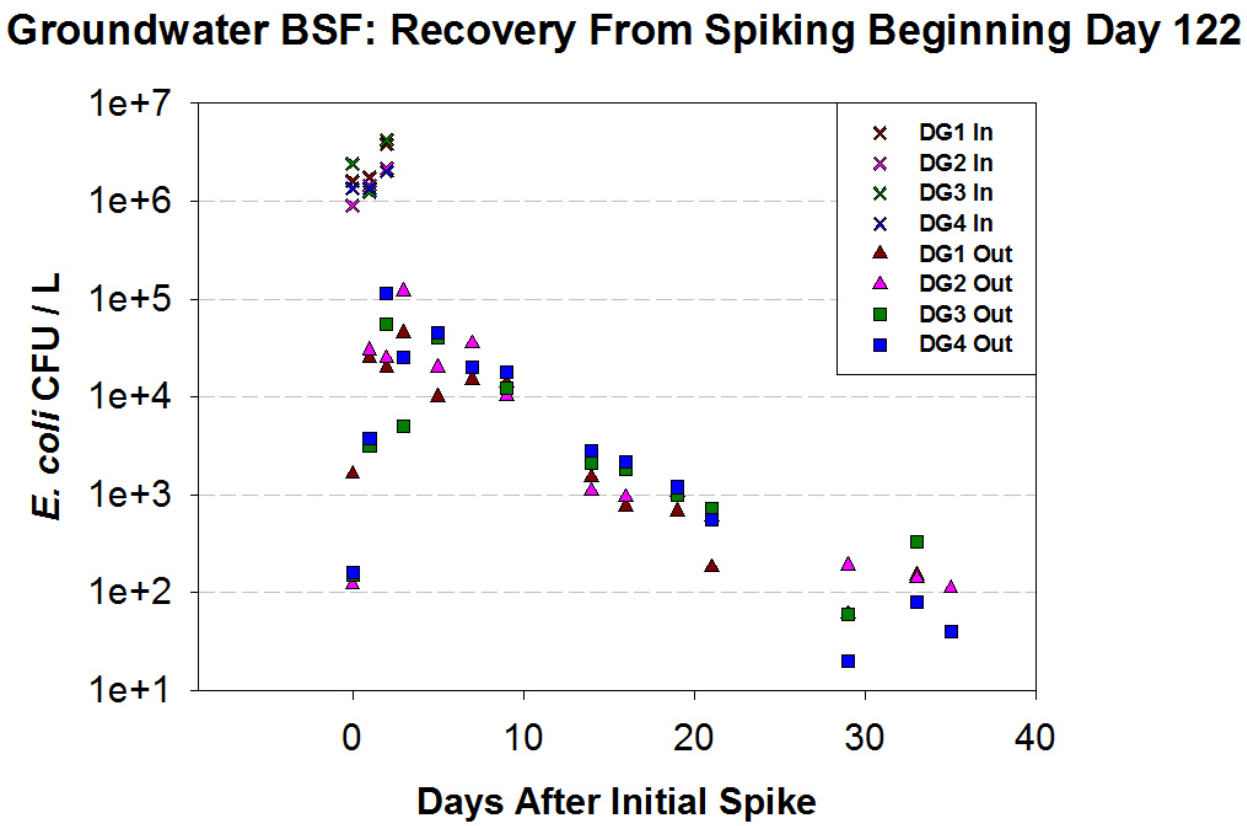


Figure 22: *E. coli* concentrations during recovery from spiking beginning day 122 of filter maturity in groundwater maintained BSFs seeded with inoculum sands from the Dominican Republic.

4.1.3 Linnwood Treatment Plant Seeded Tap Water Maintained BSF

Filters seeded with Linnwood Drinking Water Treatment Plant inoculum sand were spiked with *E. coli* at days 134 and 200 of filter maturation for groundwater maintained filters, and days 137 and 203 for filters maintained with tap water.

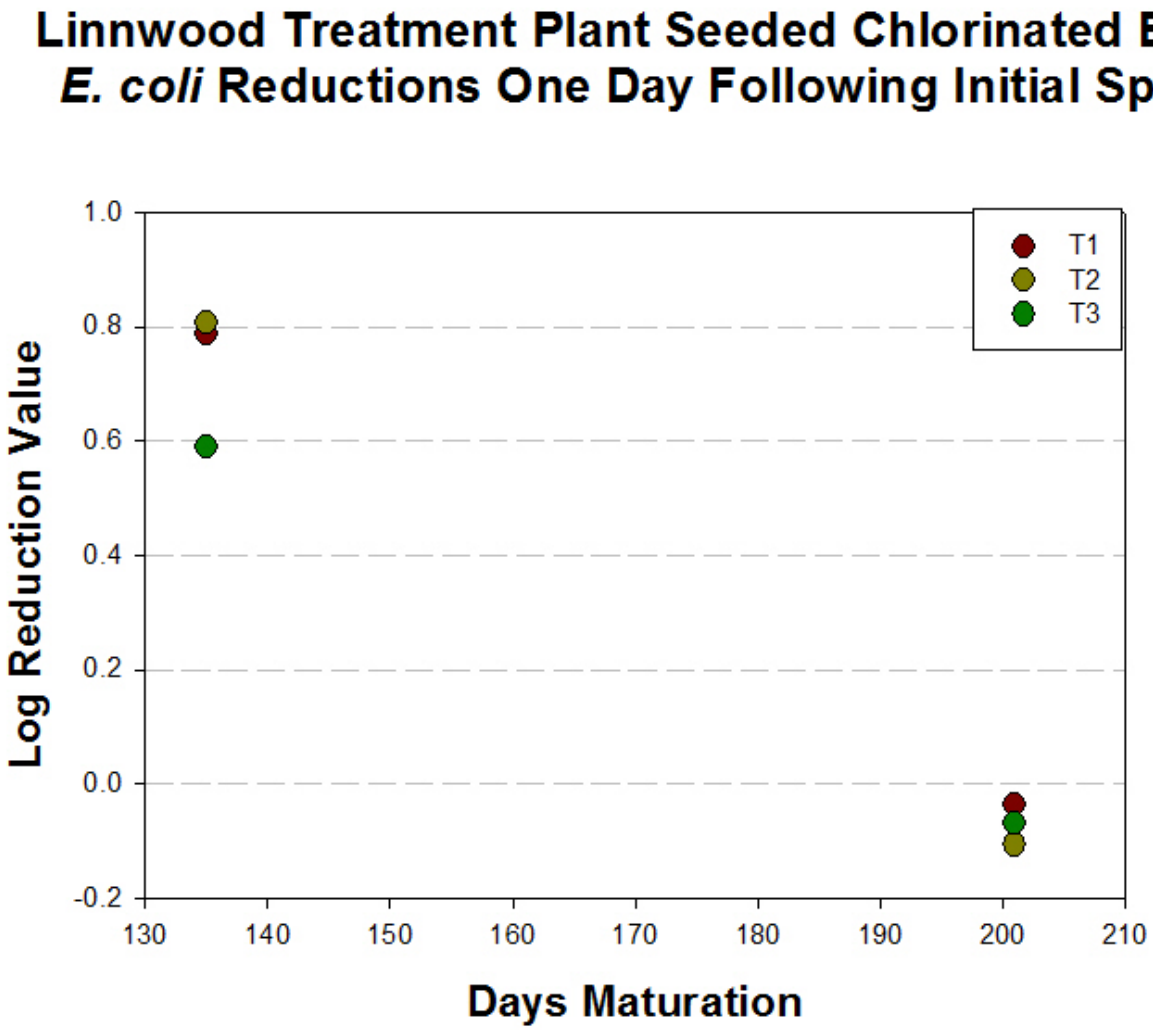


Figure 23: *E. coli* reductions one day after initial spiking in chlorinated BSFs seeded with inoculum sands from the Linnwood drinking water treatment plant.

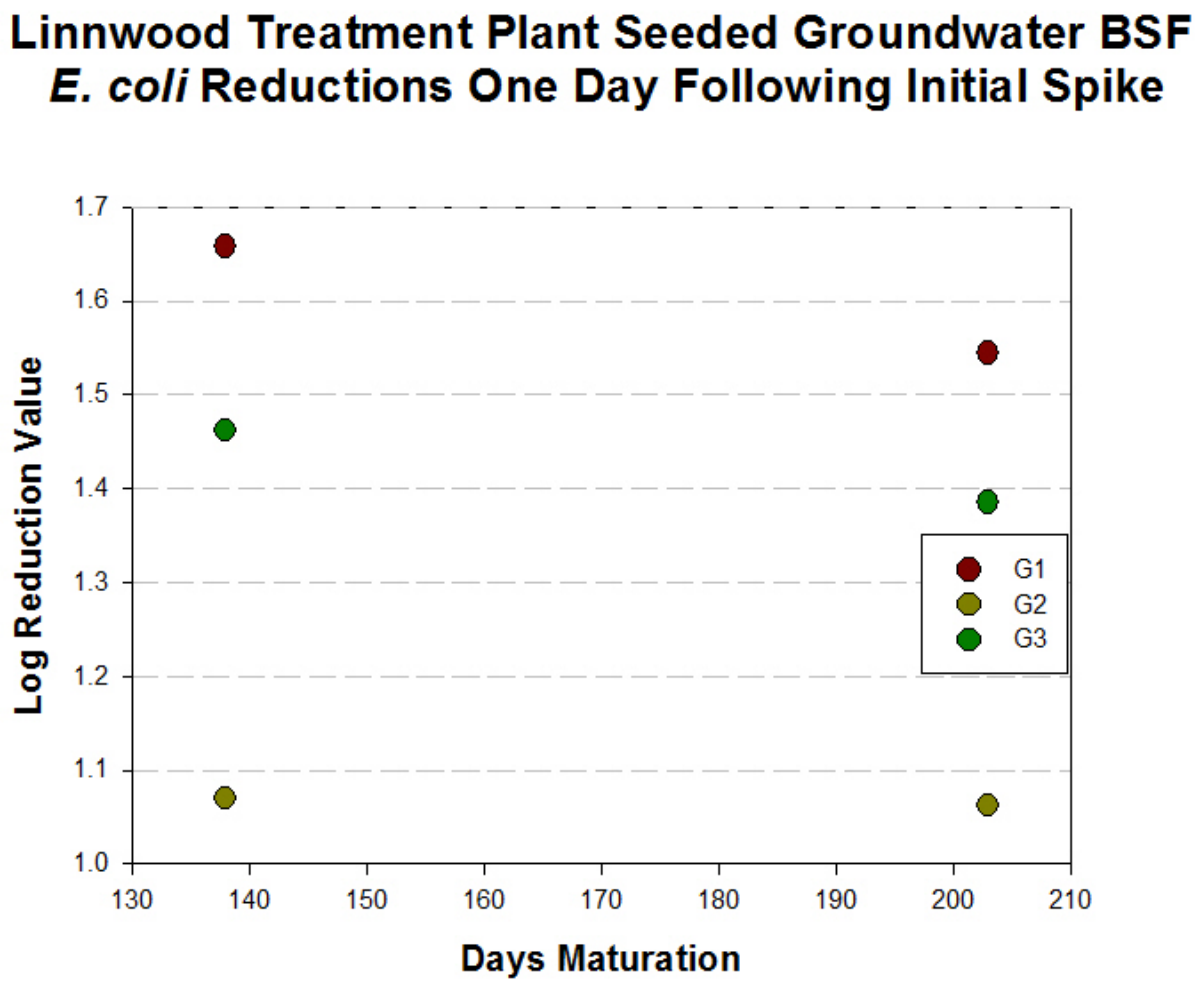


Figure 24: *E. coli* reductions one day after initial spiking in groundwater maintained BSFs seeded with inoculum sands from the Linnwood drinking water treatment plant.

As experienced with tap water maintained filters seeded with inoculum sand from the Dominican Republic, tap water maintained filters seeded with inoculum sand from the Linnwood Drinking Water Treatment Plant quickly lost affinity for *E. coli* reductions during the spiking period.

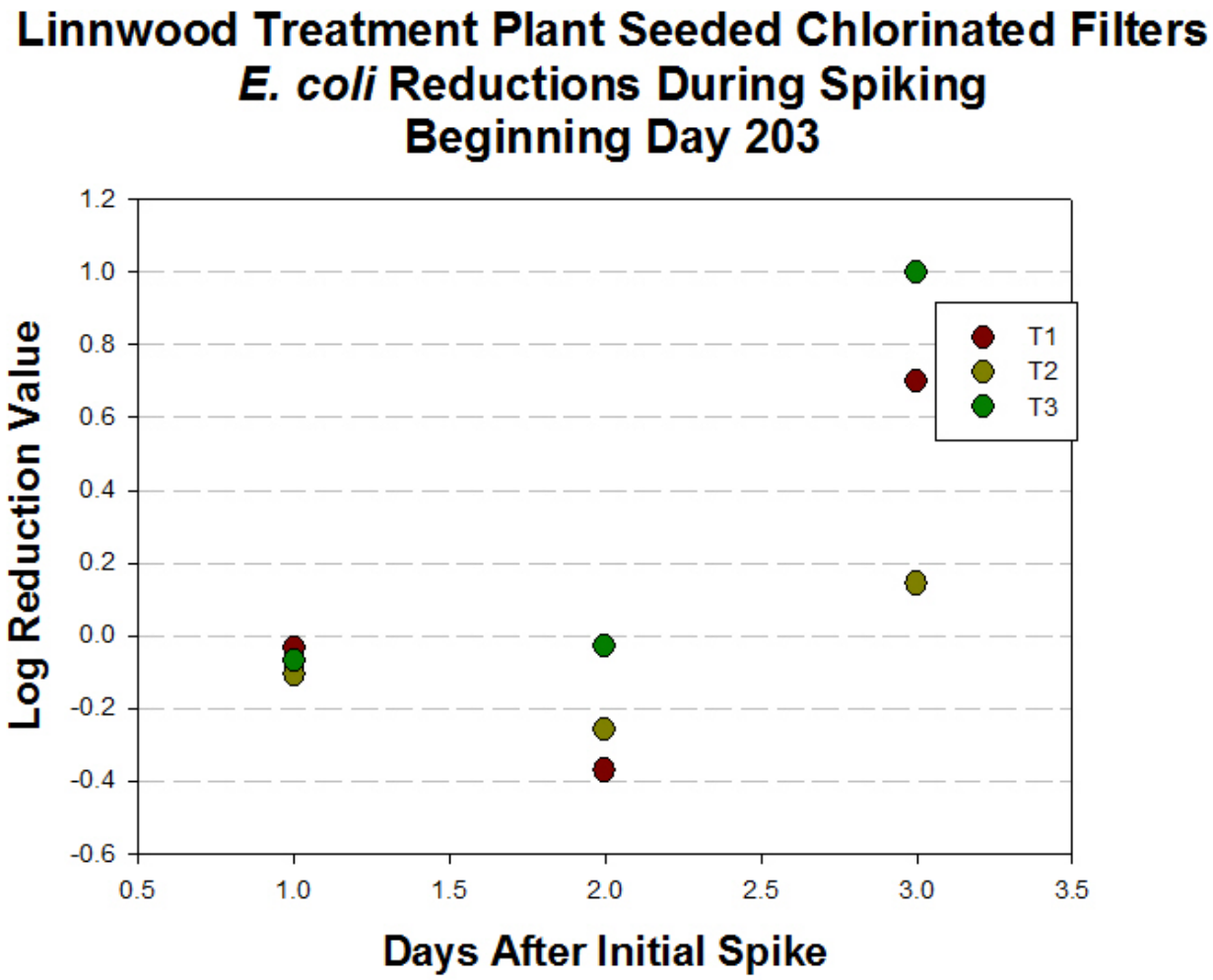


Figure 25: mean (n=3) *E. coli* reductions during spiking starting day 200 of filter maturity in chlorinated BSFs seeded with inoculum sands from the Linnwood drinking water treatment plant.

Similar to groundwater maintained BSFs seeded with inoculum from the Dominican Republic, Linnwood treatment plant seeded groundwater BSFs maintained filtration efficiency throughout the three-day spiking period, plateauing to approximately 1log reduction after three days exposure to *E. coli*.

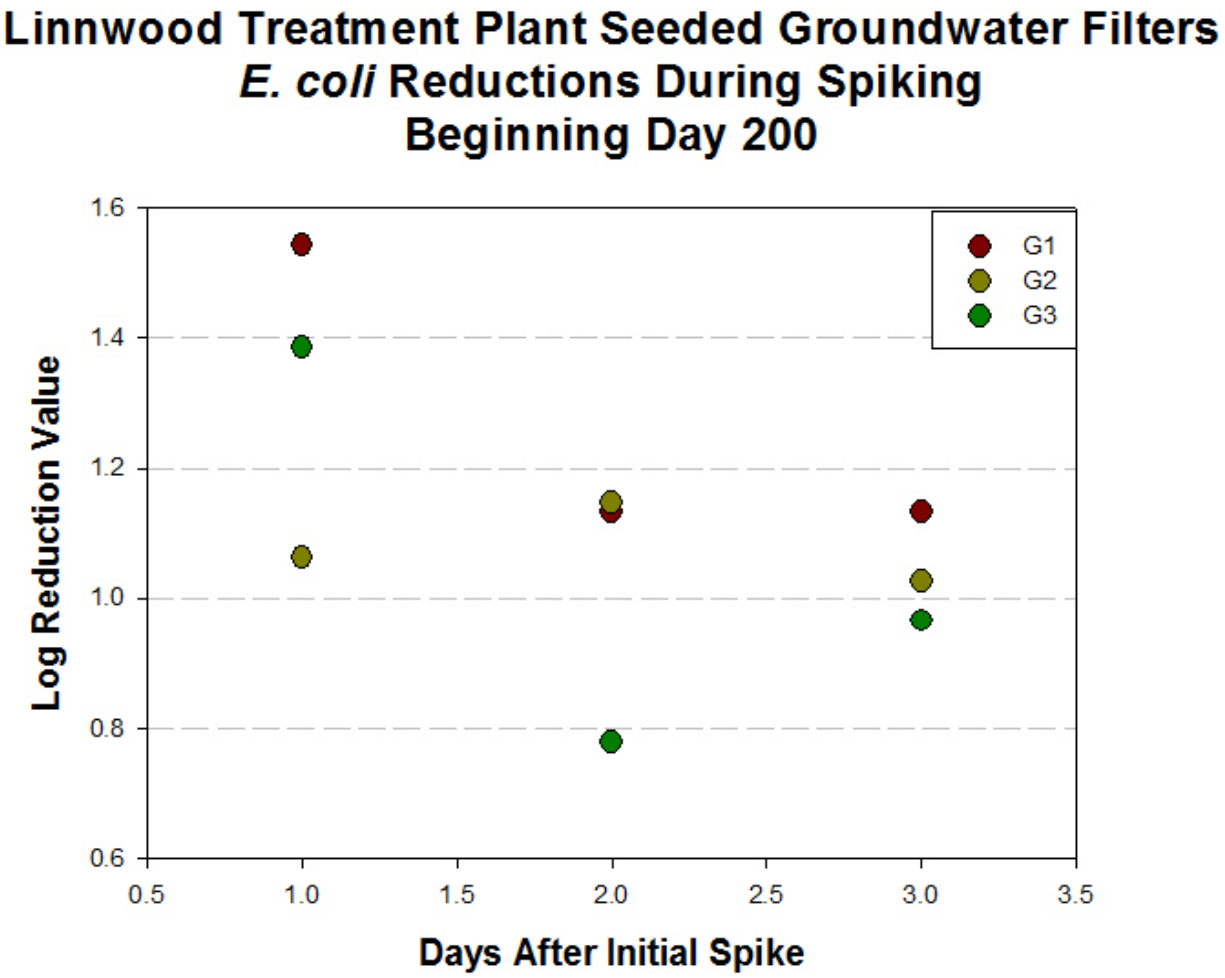


Figure 26: mean (n=3) *E. coli* reductions during spiking starting day 200 of filter maturity in groundwater maintained BSFs seeded with inoculum sands from the Linnwood drinking water treatment plant.

4.1.5 *E. coli* Survivorship in Source Waters

In order to understand the effect of chlorinated source water on bacteria survivorship, a 20L segment of source waters were spiked with *E. coli* and plated on MOD mTEC agar in 0.1 mL increments following EPA method 1603. The treatments were 20L tap water with 4 mg/L humic acid, 20L deionized water with 4 mg/L humic acid, and dolomitic groundwater.

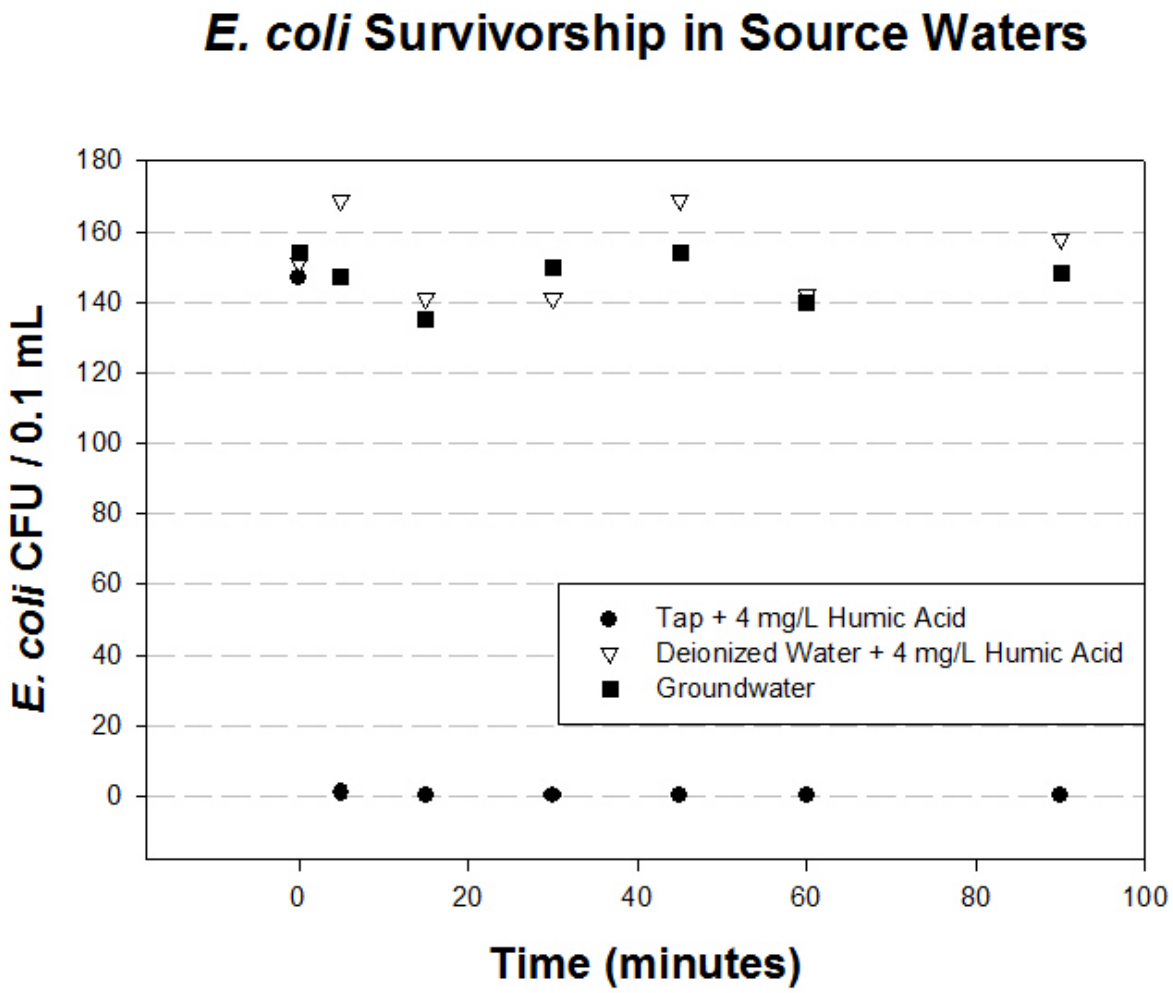


Figure 27: Culturable *E. coli* survivorship in 20L carboys with varying source waters: tap water with 4 mg/L humic acid, deionized water with 4 mg/L humic acid, and groundwater.

While deionized water and groundwater maintained survivorship over the course of 90 minutes, chlorinated municipal tap water survivorship had reached 0.6% survivorship after five minutes and 0% survivorship after 15 minutes. For filters maintained with tap water, chlorination may contribute to culturable *E. coli* counts in the effluent once the filters are returned to tap water after spiking (immediately following each three day spiking period).

4.2 Bacteriophage MS2 Reductions in Chlorinated BSFs

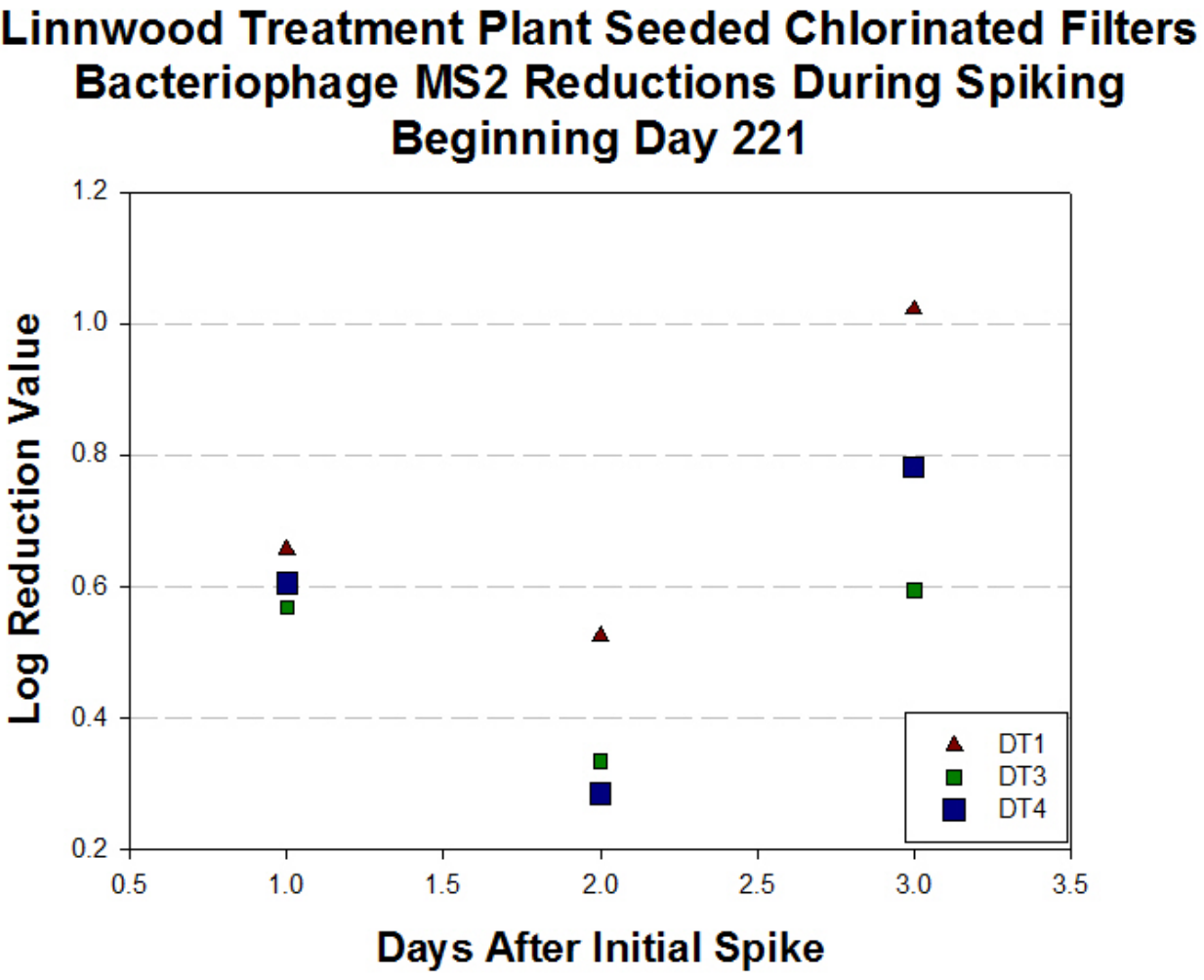


Figure 28: Bacteriophage MS2 reductions during spiking in chlorinated BSFs seeded with inoculum sands from the Dominican Republic. DT1 = "good" (AD) inoculum, DT3 and DT4 = "bad" (BC) inoculum.

Bacteriophage reductions in the fully mature tap water maintained BSF ranged from a minimum of 0.285 log reduction (48%) for filter DT4 on day two after spiking, to a maximum of 1.02 log reduction (90.48%) for filter DT1 on day three after spiking.

4.4 Source Water and Effluent Chemistry

4.4.1 Lapham Hall Tap Water and Groundwater

General water chemistry parameters for tap water and groundwater used to maintain filters taken January 16, 2015:

Parameter	Groundwater	Tap Water
pH	7.30	7.65
Turbidity	8	1
PO_4 (mg/L)	0.00	1.97
NO_3 (mg/L)	1.4	1.8

Table 13: General water chemistry of groundwater and municipal tap source waters.

4.4.2 Bradford Beach, Lake Michigan BSF

One unseeded BSF was maintained with source water from the south end of Bradford Beach. Source water and effluent were analyzed for pH, dissolved NO_3, dissolved orthophosphate, turbidity, and A_{254}. The filter was periodically spiked with high concentration ($\approx 10^6$ CFU×L^{-1}) *E. coli* and the effluent evaluated for filtration efficiency. While sand samples

were not taken for sequencing, this filter allows for a baseline representation of filter development when maintained with surface water, the assimilation of nutrients, and the overall dynamics of organic matter in the BSF system.

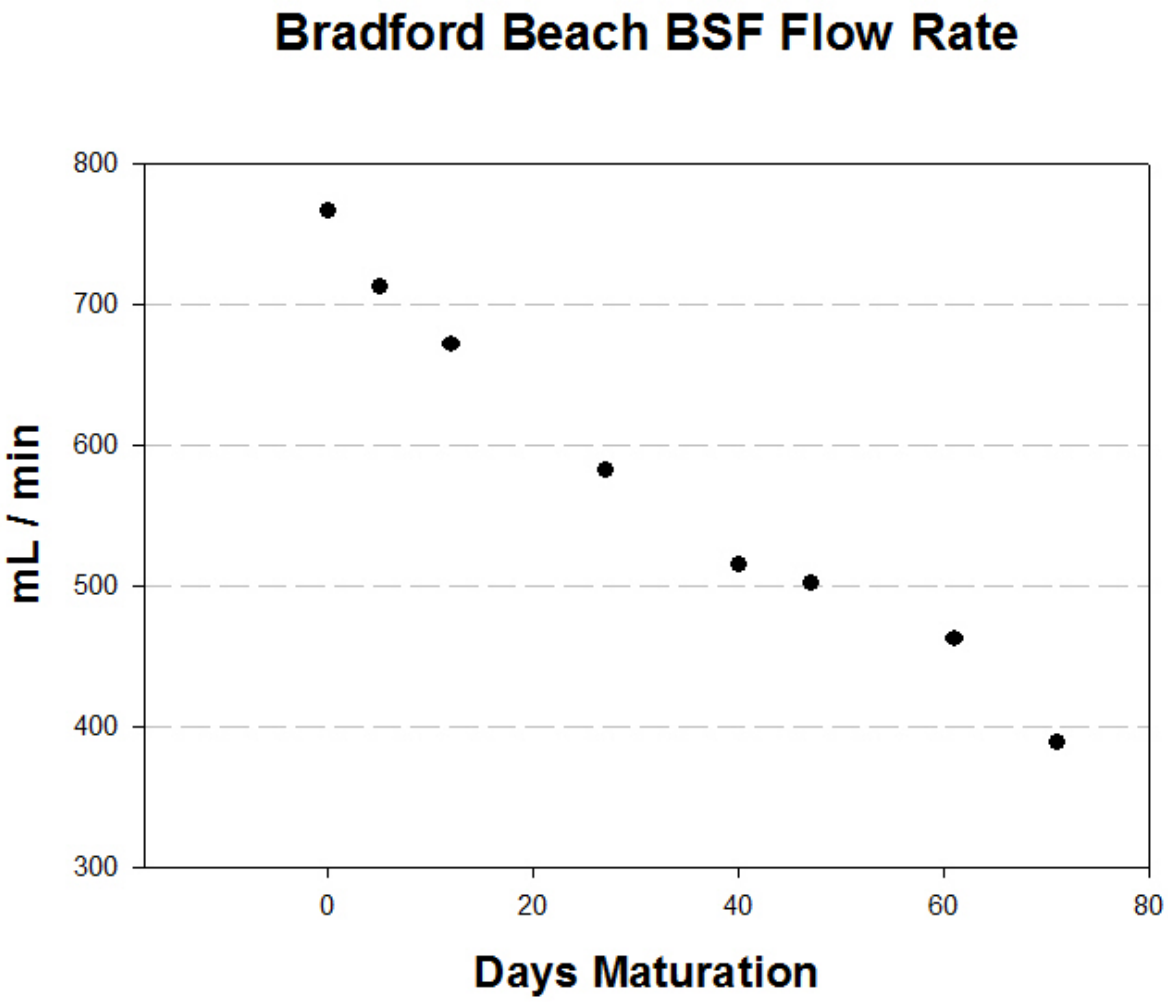


Figure 29: Flow rate of BSF maintained with Lake Michigan water during maturation.

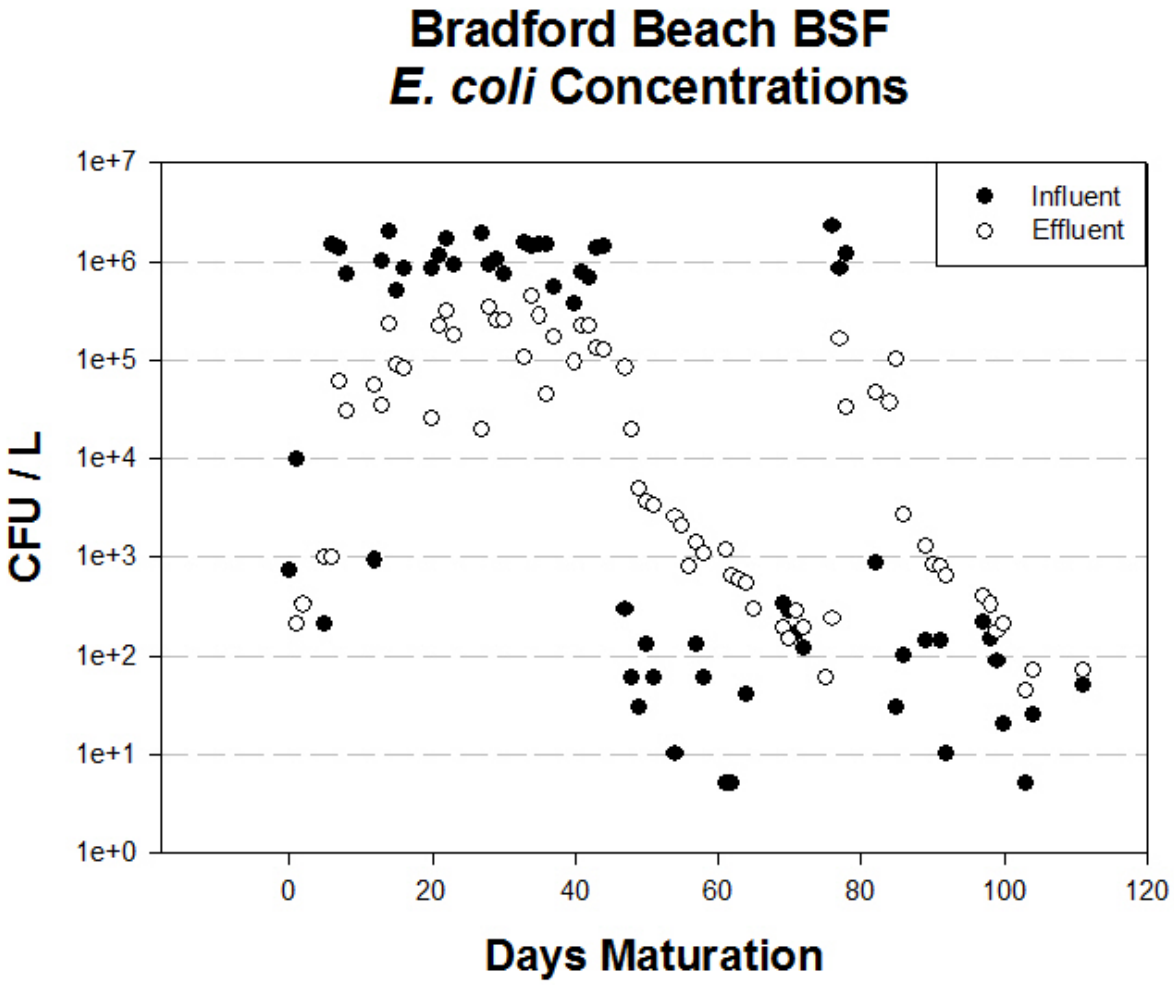


Figure 30: Influent and effluent *E. coli* concentrations in BSF maintained with Lake Michigan water. High concentration spiking occurred during days 6-44 and days 76-78 of filter maturity.

During the first 44 days of filter maturity in which the BSF was spiked with *E. coli*, filtration efficiency varied from 0.22 – 1.65 log10 reduction. During days 76-78, filtration efficiency grouped more closely in the range of 1.15-1.40 log10 reduction.

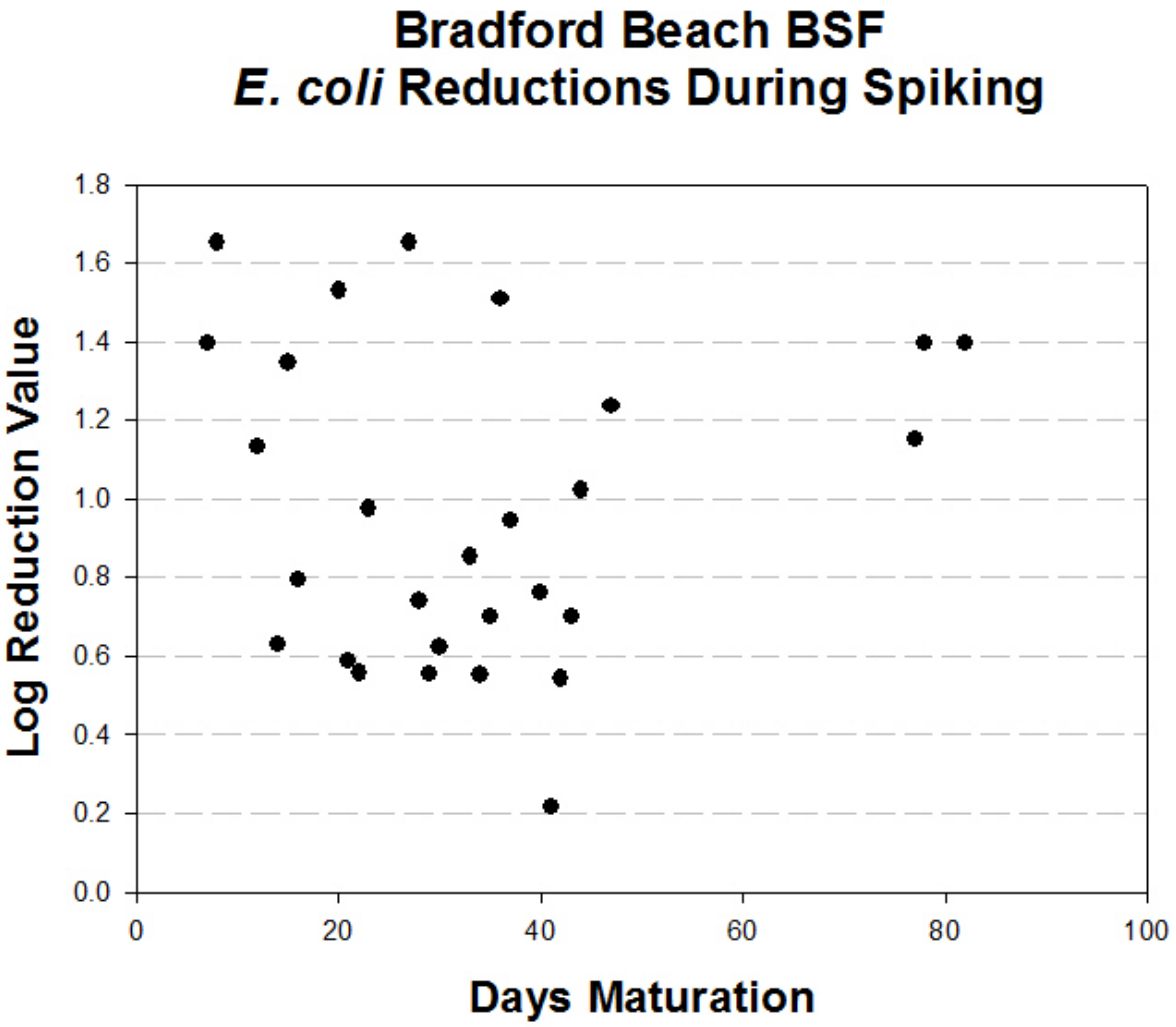


Figure 31: *E. coli* log reduction values during spiking in BSF maintained with Lake Michigan water. High concentration spiking occurred during days 6-44 and days 76-78 of filter maturity.

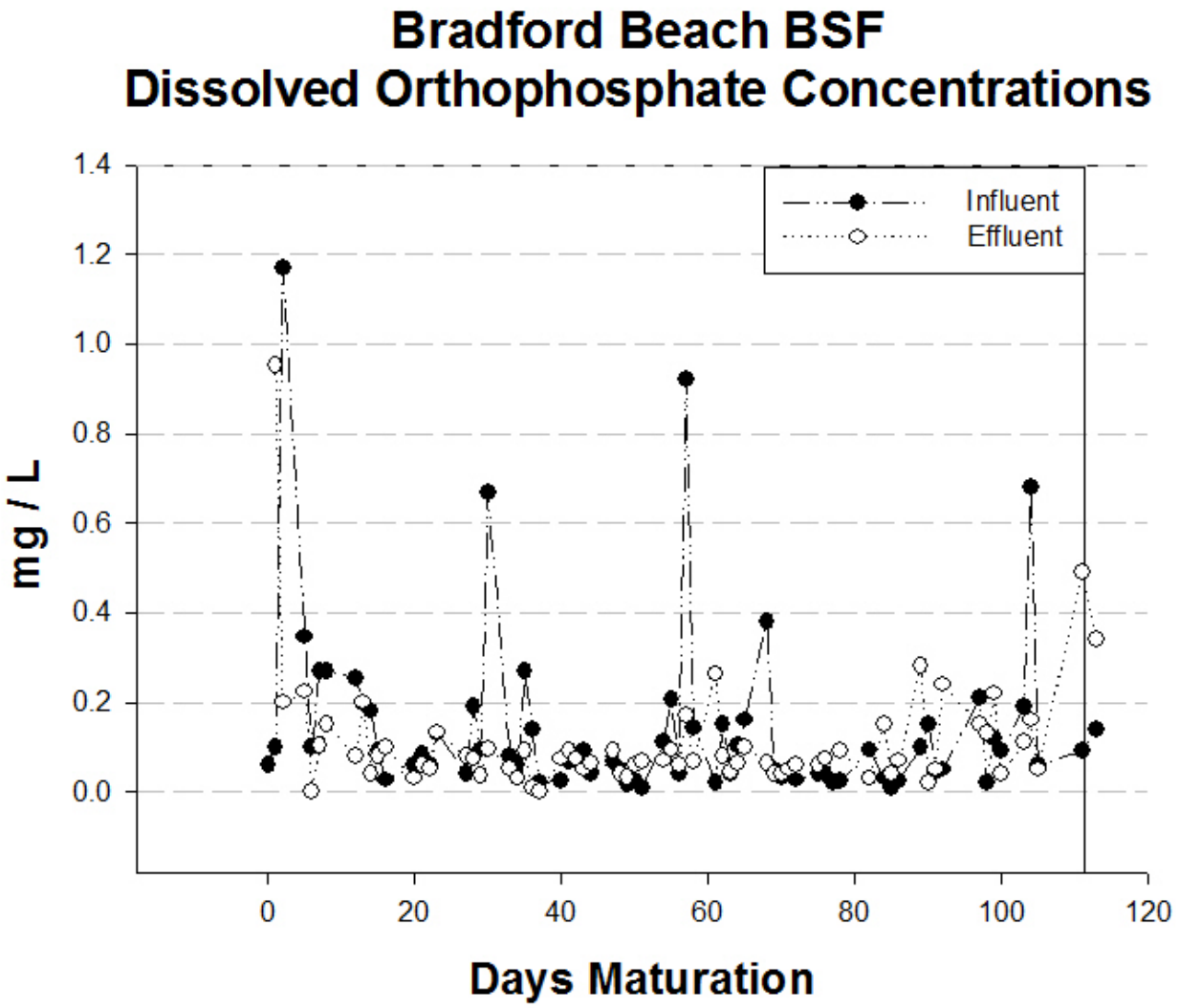


Figure 32: Bradford Beach BSF dissolved orthophosphate concentrations.

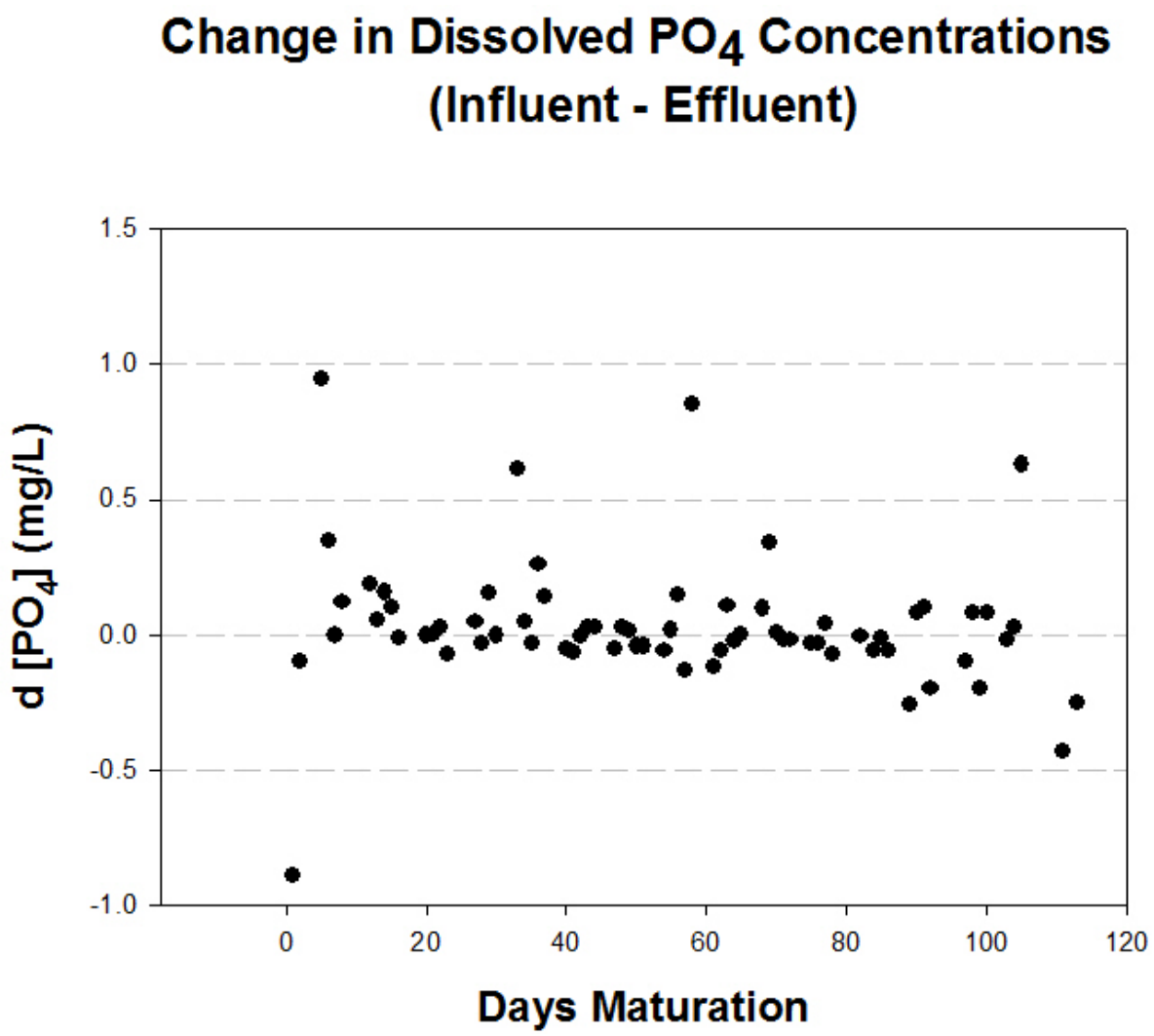


Figure 33: Orthophosphate difference (Influent – Effluent) in BSF maintained with Lake Michigan water in mg/L.

Accumulation of dissolved orthophosphate to the filtration media or assimilation by microbes is the overall trend found in the difference between influent and effluent concentrations. The highly negative value on day one of filter maturation may be due to the washout of silt from filtration media interfering with absorbance measurements.

By integration of the above orthophosphate data, total accumulation of orthophosphate within the Bradford Beach BSF can be calculated. After 110 days of maturation, the BSF had accumulated approximately 47 mg orthophosphate where:

$$\text{Total Accumulated } PO_4(\text{mg}) = \sum_{0}^{n} [\text{d}[PO_4] \left(\frac{\text{mg}}{\text{L}}\right) \times 20\text{L}]$$

Where n = total number of 20 L charges added to the filter.

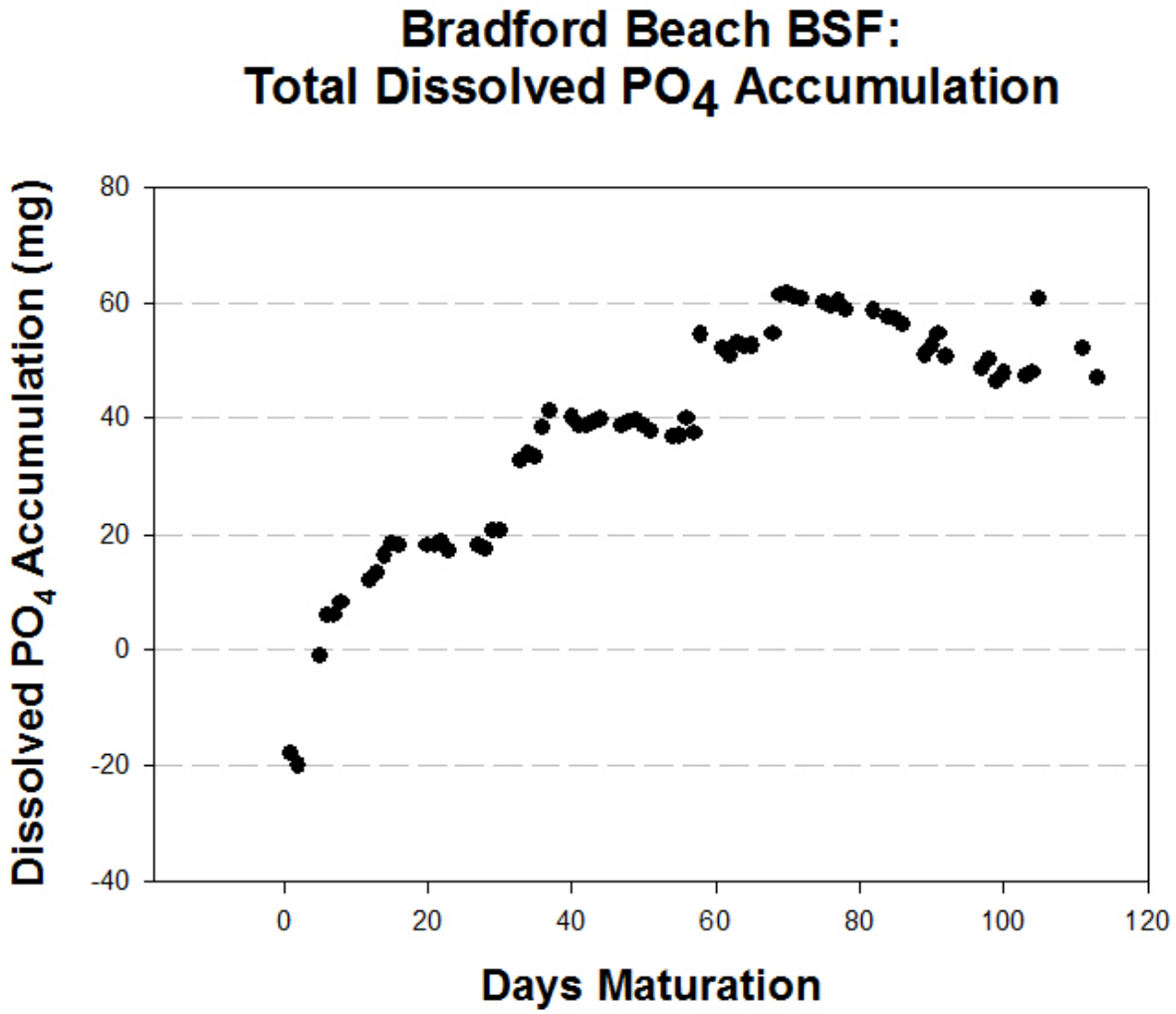


Figure 34: Total accumulation of dissolved orthophosphate in BSF maintained with Lake Michigan water.

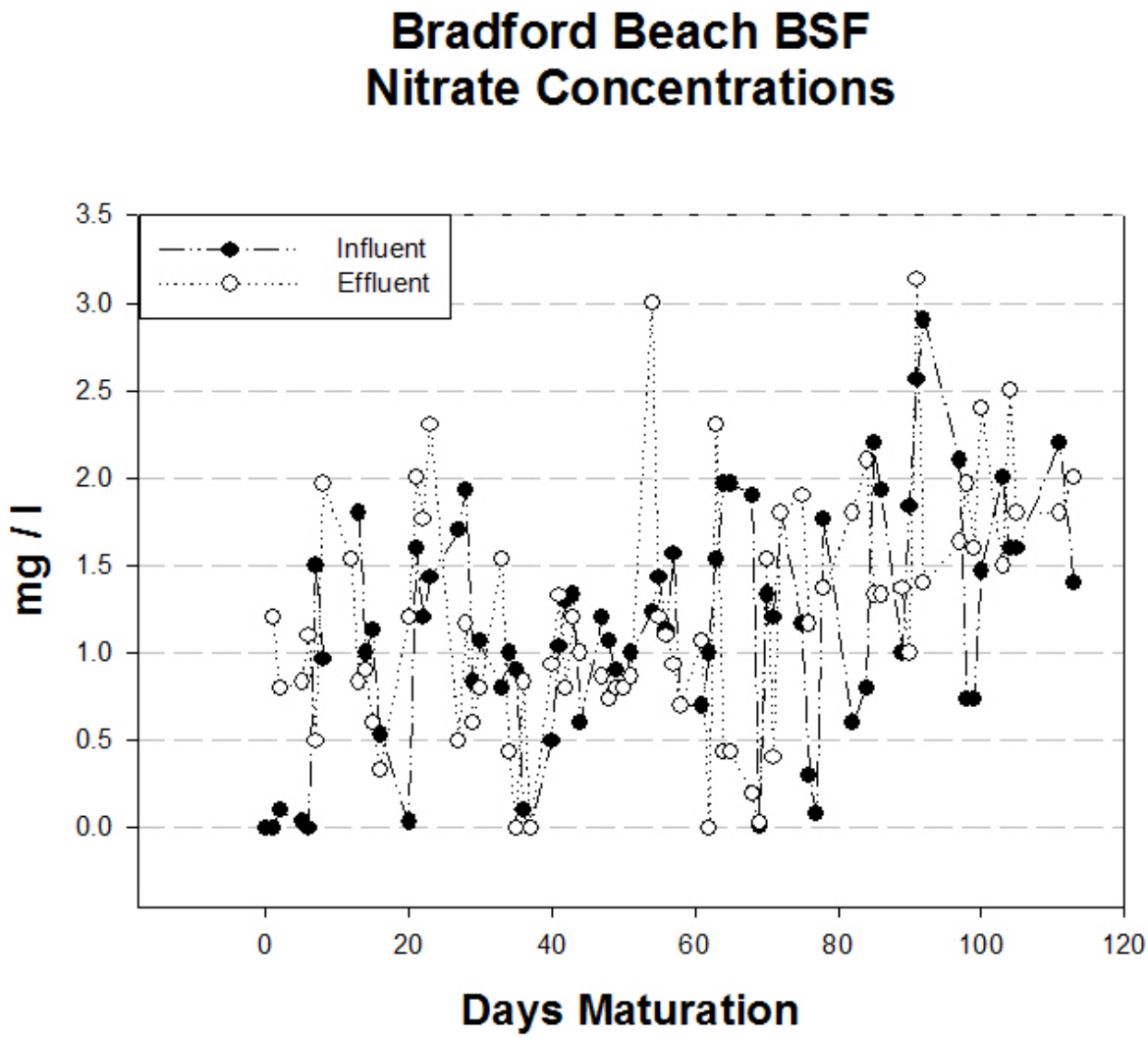


Figure 35: Bradford Beach BSF nitrate concentrations.

While turbidity of Bradford Beach fluctuates throughout the summer months, the BSF produced effluent with a maximum of 7 NTUs.

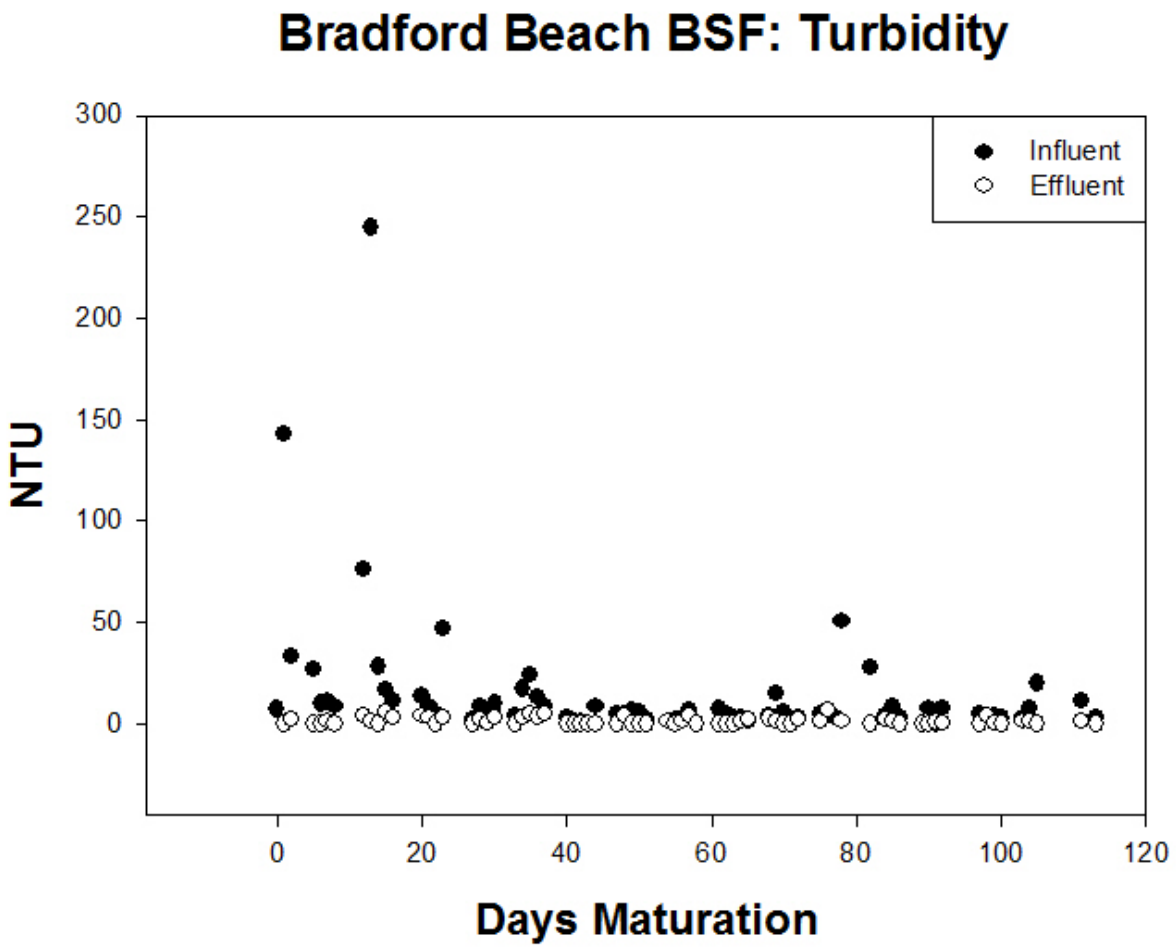


Figure 36: Source water and effluent turbidity for BSF maintained with Lake Michigan water.

Overall, trends for pH show that the influent has a higher pH than the effluent of the Bradford Beach BSF. This suggests that with microbial respiration, CO_2 levels increase driving acidification of filter effluent.

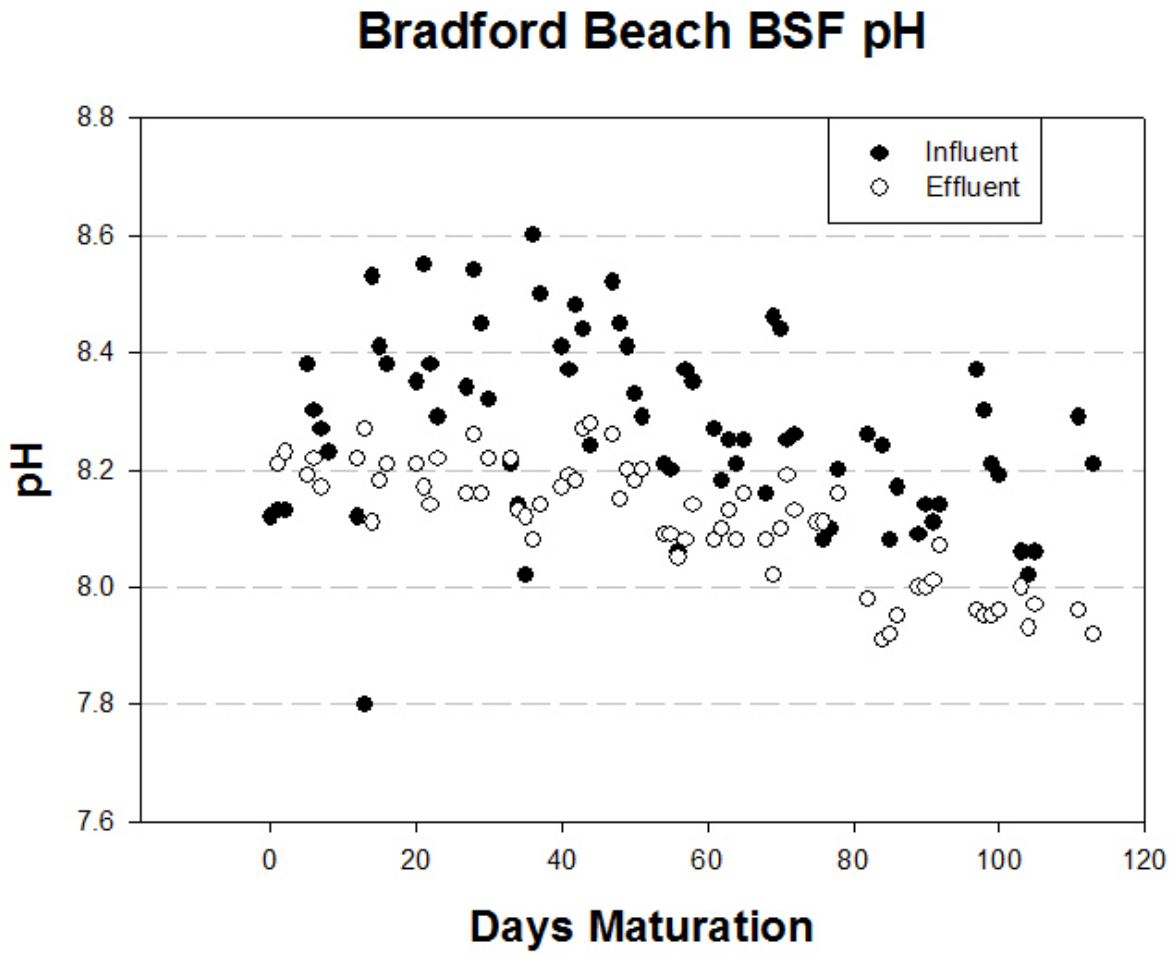


Figure 37: pH of influent and effluent of BSF maintained with Lake Michigan water.

Absorbance at 254 nm wavelength was used as an indicator of organic matter concentrations for both Bradford Beach source water and BSF effluent. Fluctuations in organic matter concentrations between the influent and effluent were strongest in the first 50 days of filter maturation, eventually leveling out to near equivalence.

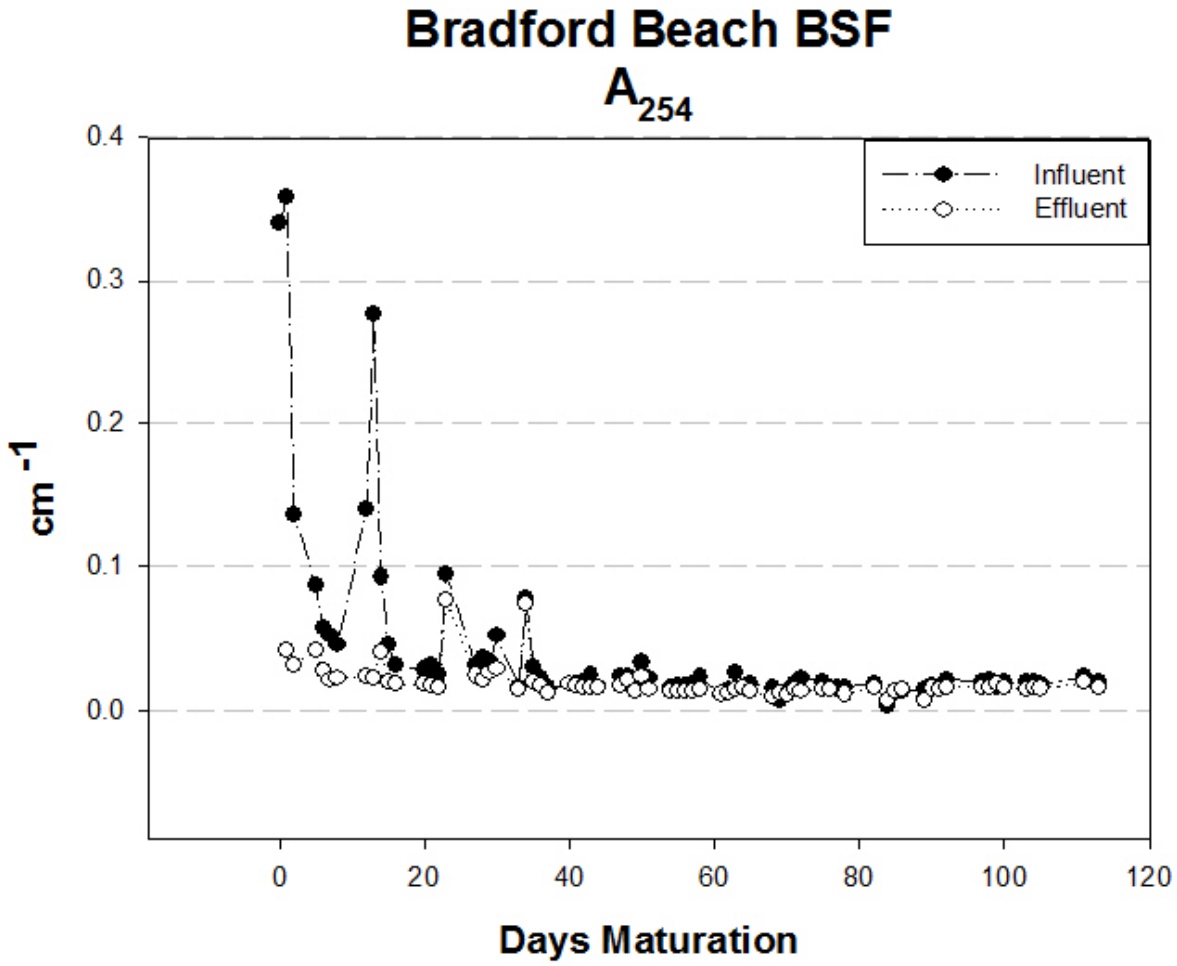


Figure 38: Absorbance at 254 nm of influent and effluent of the Bradford Beach BSF.

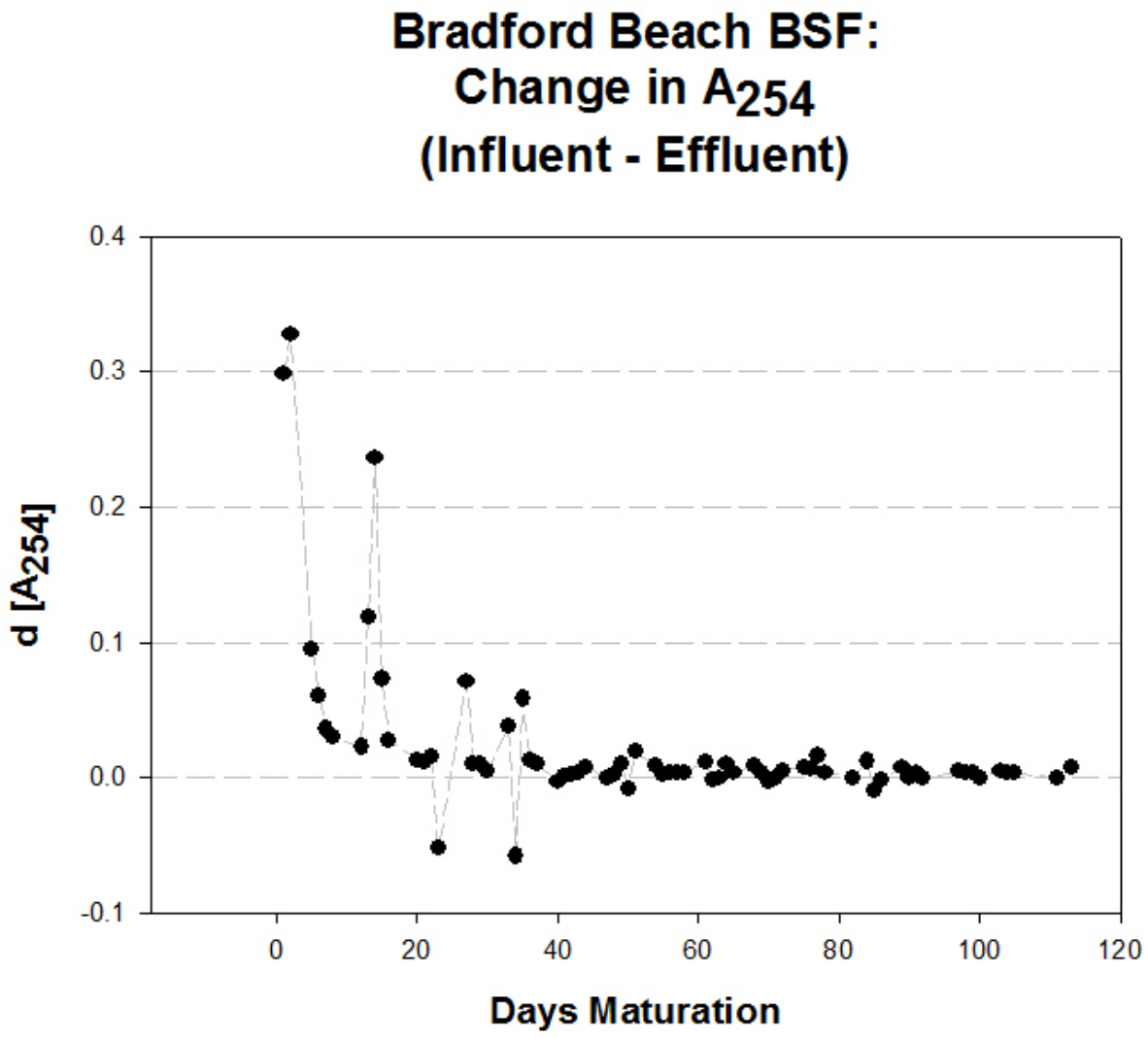


Figure 39: Approximation of dissolved organic matter concentrations for influent and effluent of BSF maintained with Lake Michigan water.

Using humic acid in deionized water as a standard, a calibration curve was produced to develop a general relationship between A_{254} and organic matter concentrations.

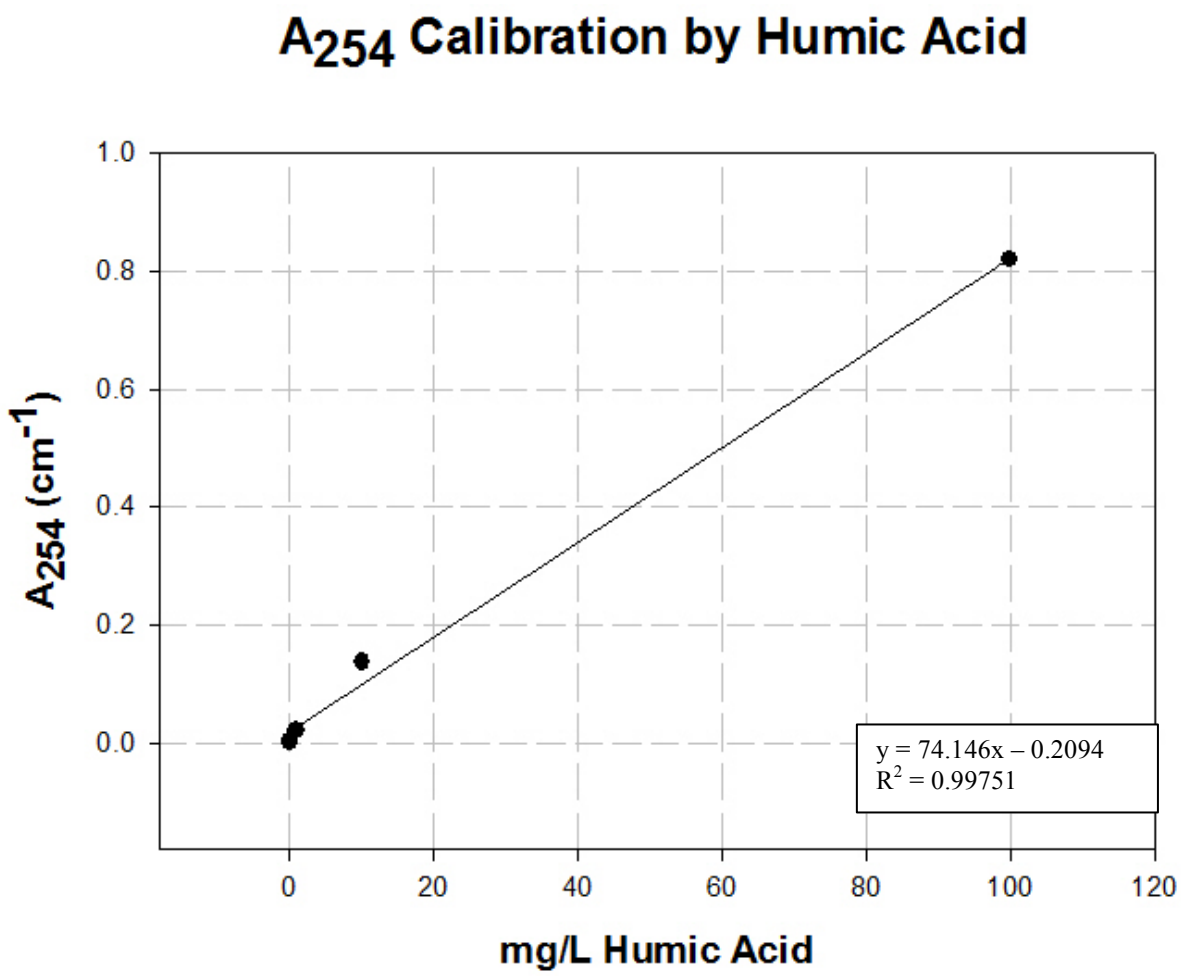


Figure 40: Calibration curve for Absorbance at 254 nanometers versus humic acid concentration in deionized water.

At day 77 of filter maturity, *E. coli* reductions in the Bradford Beach BSF were examined over the course of one pore volume. This data, similar to prior research, shows deviations in the log reduction values for *E. coli* throughout the filtration process. Grab samples taken from BSF effluent vary in *E. coli* concentrations depending on the location where the water parcel was stagnant during the 24-hour residence period (Elliot et al., 2008).

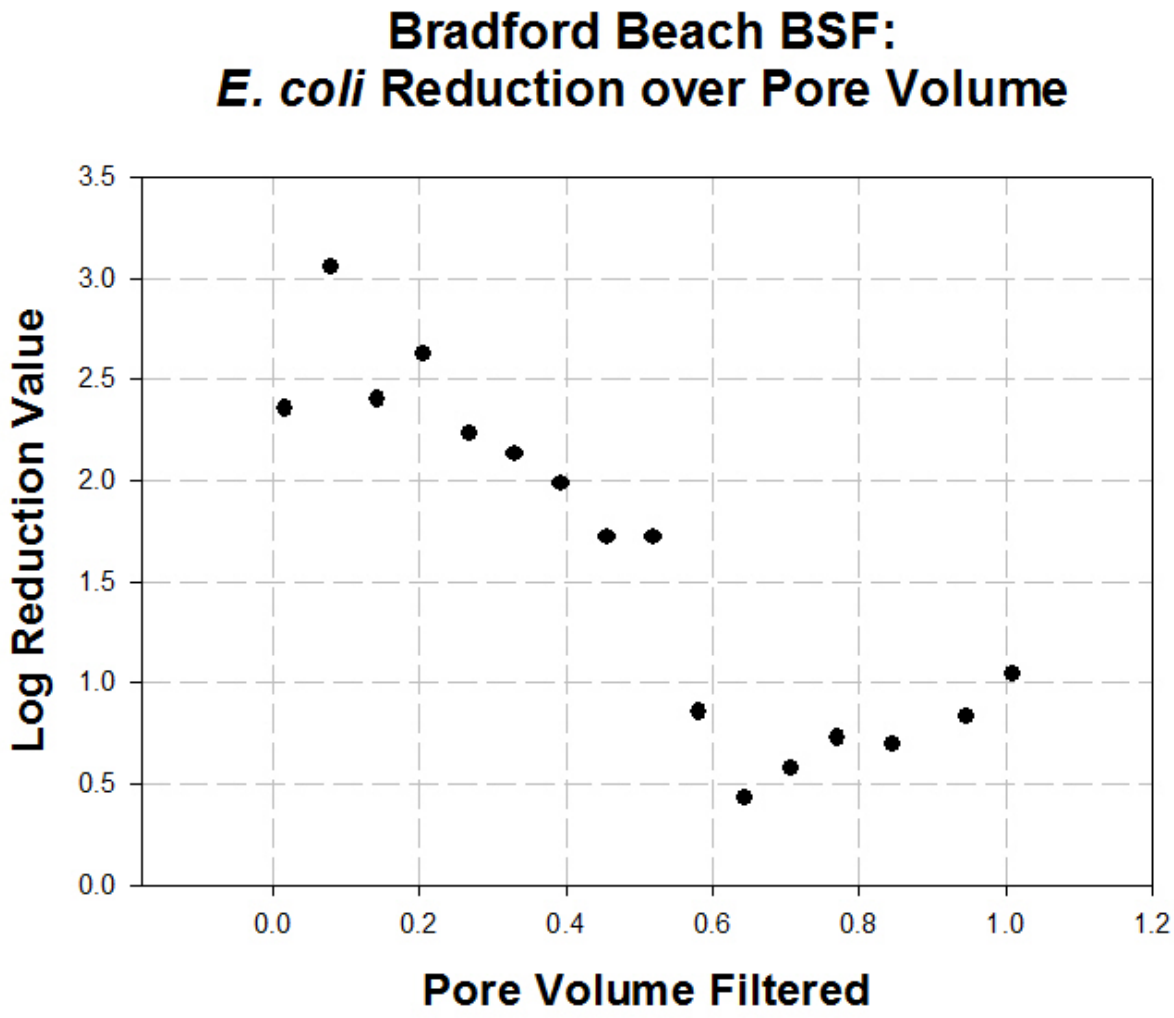


Figure 41: Reduction of *E. coli* over 20L in BSF maintained with Lake Michigan water.

5.0 Microbiome of the BSF

5.1 Illumina MiSeq 16S rRNA Sequences

Sequencing results were grouped by 10 major phyla, 25 most abundant families, and 25 most abundant taxa. Horn index for multi-dimensional scaling was applied to sequencing data in order to group the microbiome of filters in the laboratory and in the field by community similarity. The Horn index shows distinct differences in the microbiome of filters operating in

the Dominican Republic and the laboratory, and significant differences between filters maintained with Lake Michigan water versus groundwater and tap water.

Sample ID	Type	Location	Depth	Treatment
Linnwood filter bed	inoculum	Milwaukee	0.5 m	Lake water
BateyA- biosand filter1	inoculum	Batey, DR	surface	Chlorinated water
BateyA-biosand filter5	inoculum	Batey, DR	surface	Chlorinated water
BateyB-biosand filter3	inoculum	Batey, DR	surface	Chlorinated water
BateyB-biosand filter4	inoculum	Batey, DR	surface	Chlorinated water
BateyC-biosand filter1	inoculum	Batey, DR	surface	Chlorinated water
BateyC-biosand filter2	inoculum	Batey, DR	surface	Chlorinated water
BateyC-biosand filter3	inoculum	Batey, DR	surface	Chlorinated water
BateyC-biosand filter4	inoculum	Batey, DR	surface	Chlorinated water
BateyD-biosand filter1	inoculum	Batey, DR	surface	Chlorinated water
BateyD-biosand filter2	inoculum	Batey, DR	surface	Chlorinated water
BateyD-biosand filter4	inoculum	Batey, DR	surface	Chlorinated water
Rio Chavon	source water	La Romana, DR	N/A	-
Hospital Cistern	municipal water	La Romana, DR	N/A	Municipal water
BSF L1-A a	experiment	UWM	A	Lake water
BSF G1-A a	experiment	UWM	A	Groundwater
BSF G-A	experiment	UWM	A	Groundwater
BSF G-B	experiment	UWM	B	Groundwater
BSF G-C	experiment	UWM	C	Groundwater
BSF L-A	experiment	UWM	A	Lake water
BSF L-B	experiment	UWM	B	Lake water
BSF L-C	experiment	UWM	C	Lake water

BSF DTBC2 a	experiment	UWM	A	Tap water
BSF DGBC1 b	experiment	UWM	B	Groundwater
BSF DGBC2 b	experiment	UWM	B	Groundwater
BSF DTAD1 b	experiment	UWM	B	Tap water
BSF DTAD1 c	experiment	UWM	C	Tap water
BSF DTAD2 a	experiment	UWM	A	Tap water
BSF DGAD2 a	experiment	UWM	A	Groundwater
BSF DGAD2 B	experiment	UWM	B	Groundwater
BSF DGAD2 c	experiment	UWM	C	Groundwater

Table 14: Biosand filter samples sequenced by Illumina MiSeq.

Depths A, B, and C refer to ports installed at 10.2, 20.4, and 30.6 cm below sand surface, respectively.

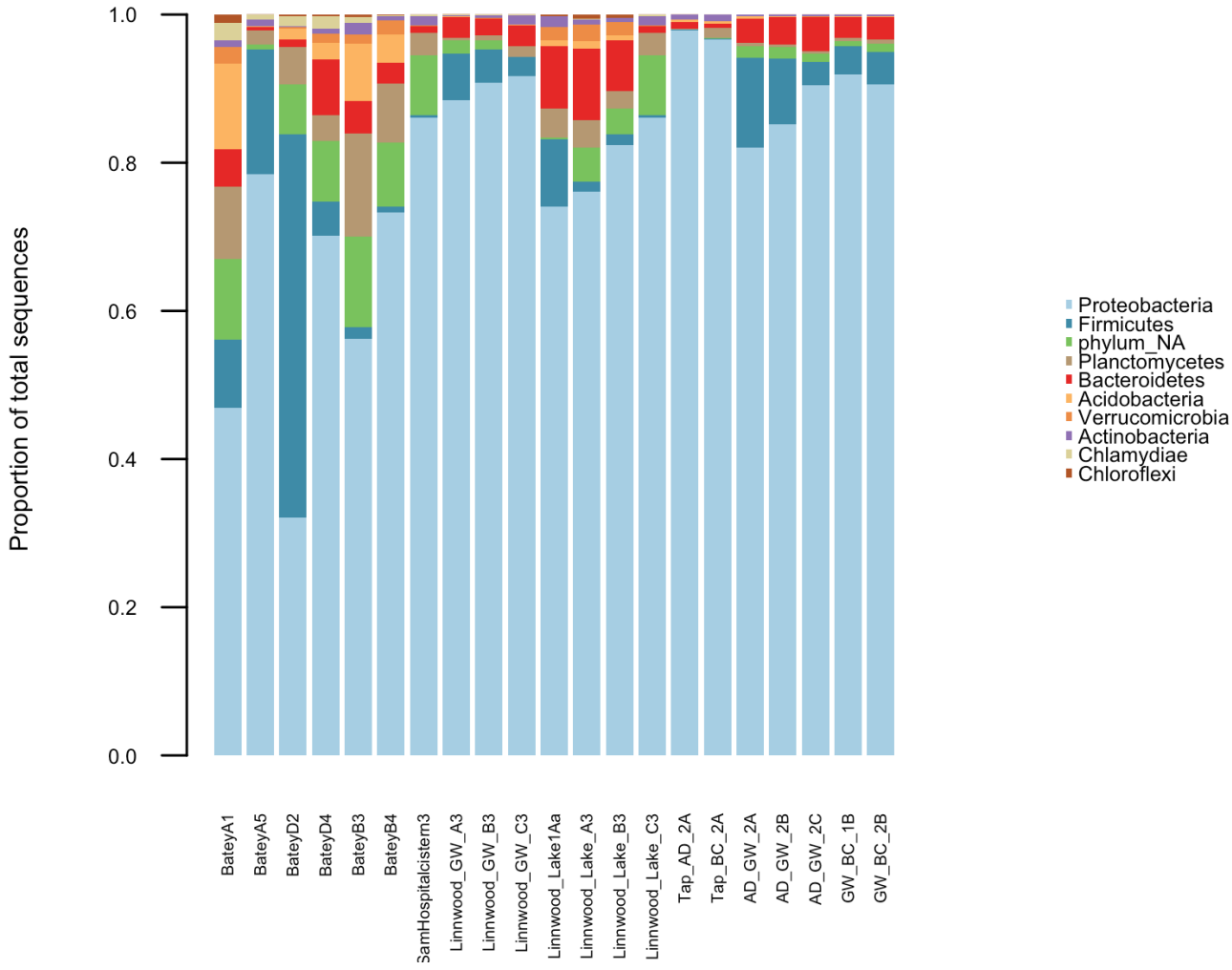


Figure 42: 10 most abundant phyla.

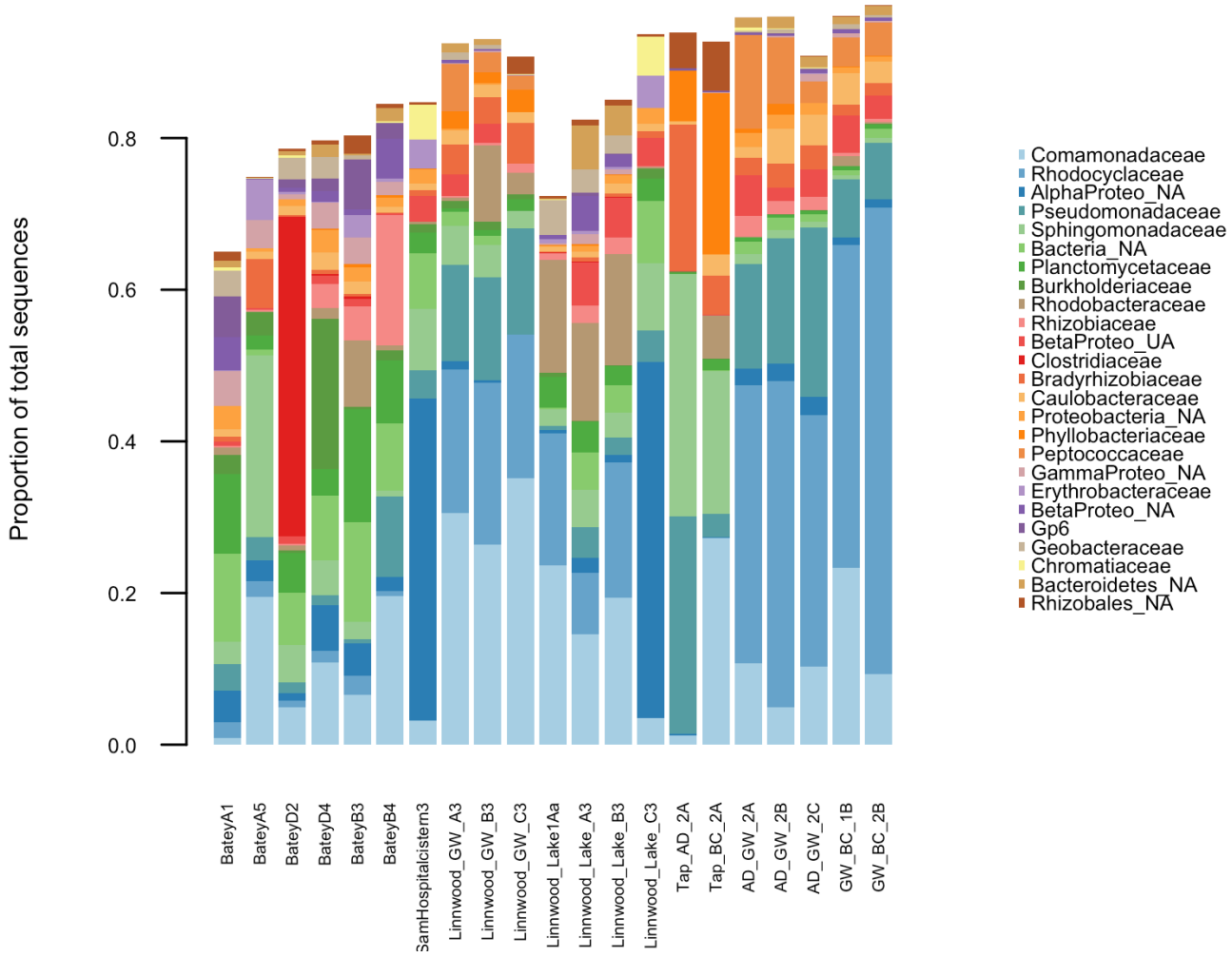


Figure 43: 25 most abundant families.

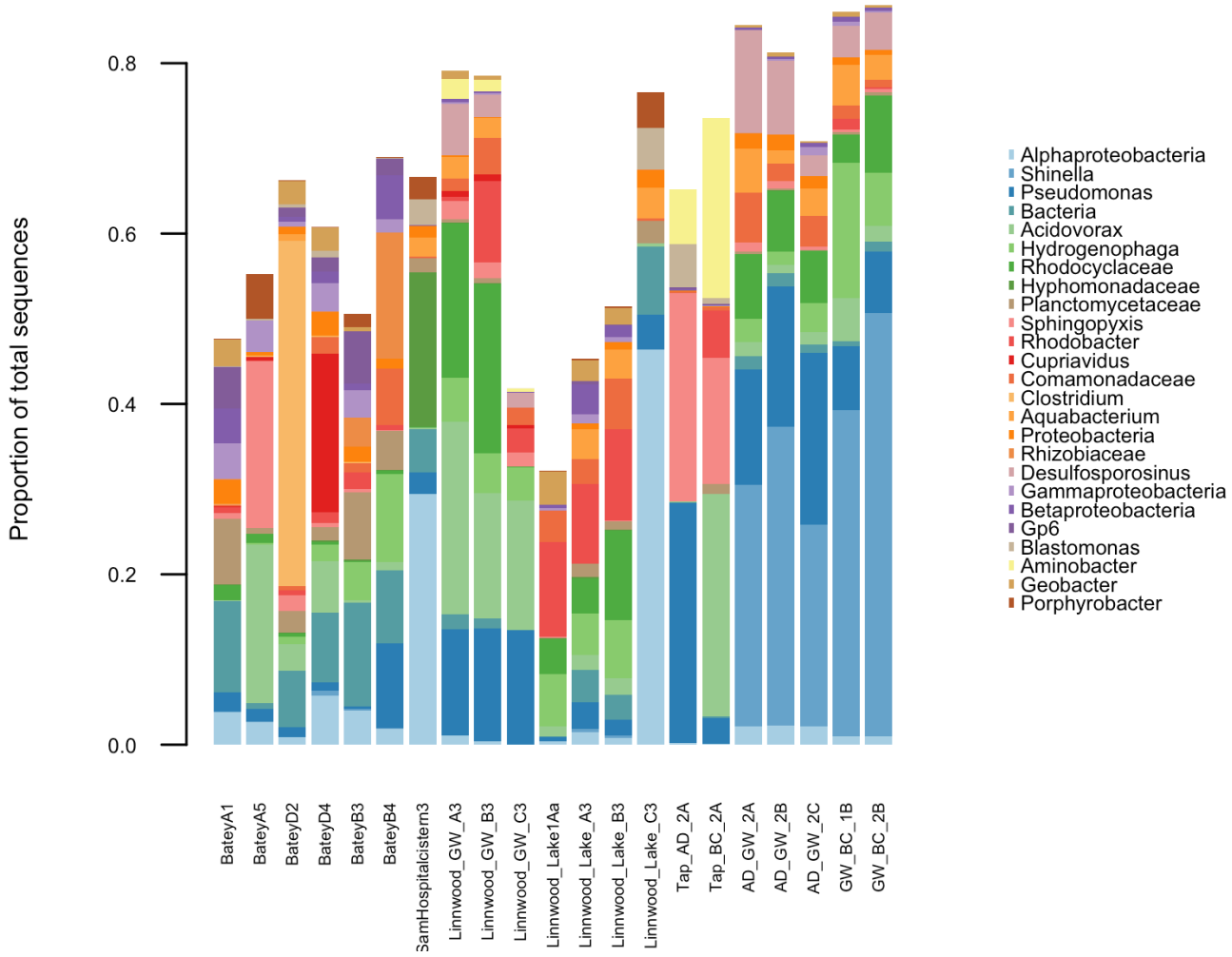
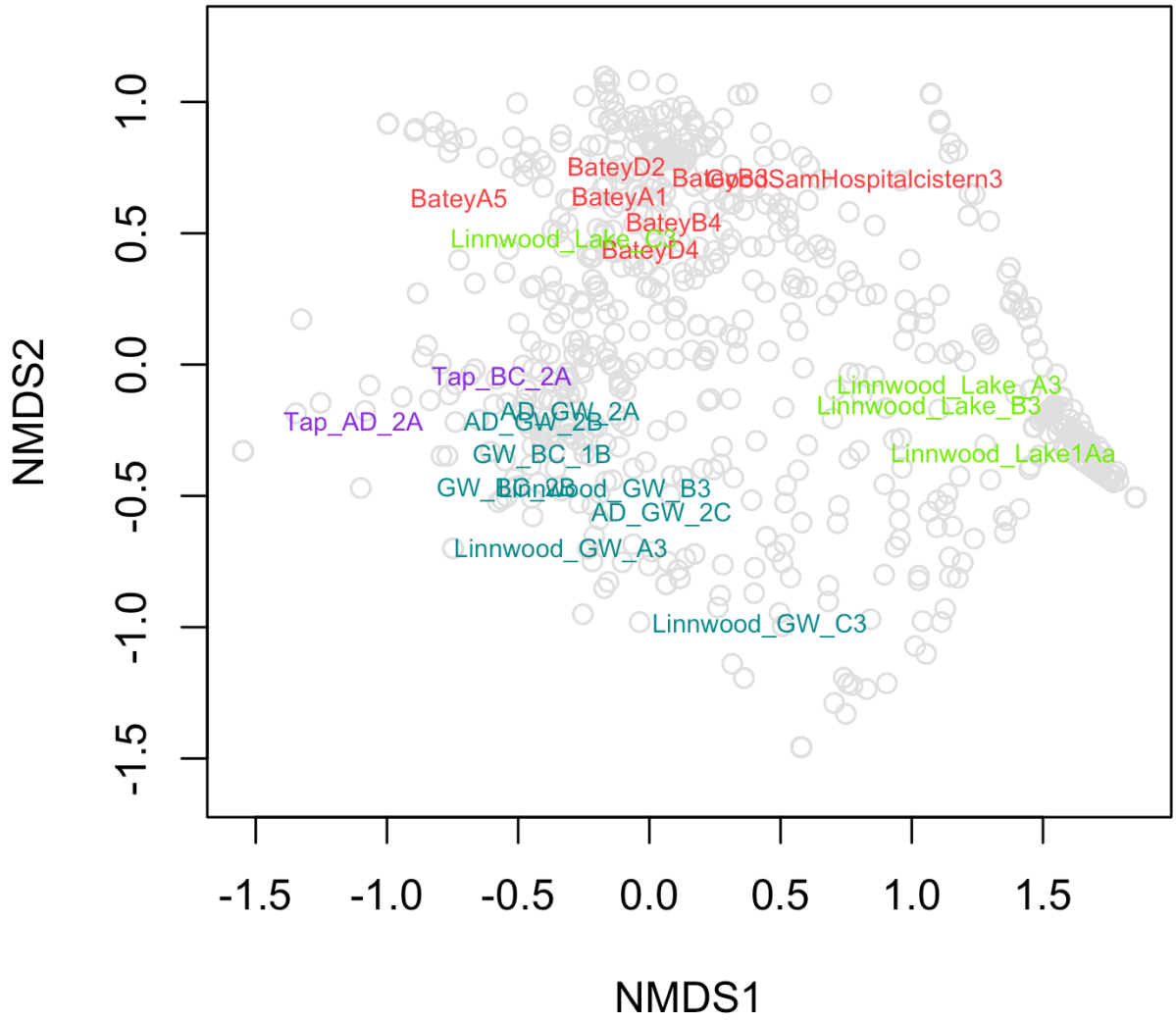


Figure 44: 25 overall most abundant taxa.


NMDS2
1.0
0.5
0.0
-0.5
-1.0
-1.5
BateyD2
BateyA5
BateyA1
BateyB4
Linnwood_Lake_C3
BateyD4
Hospitalcistern3
Tap_BC_2A
Tap_AD_2A
AD_GW_2A
AD_GW_2B
GW_BC_1B
AD_GW_2C
Linnwood_GW_A3
Linnwood_GW_C3
Linnwood_Lake_A3
Linnwood_Lake_B3
Linnwood_Lake1Aa
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
NMDS1

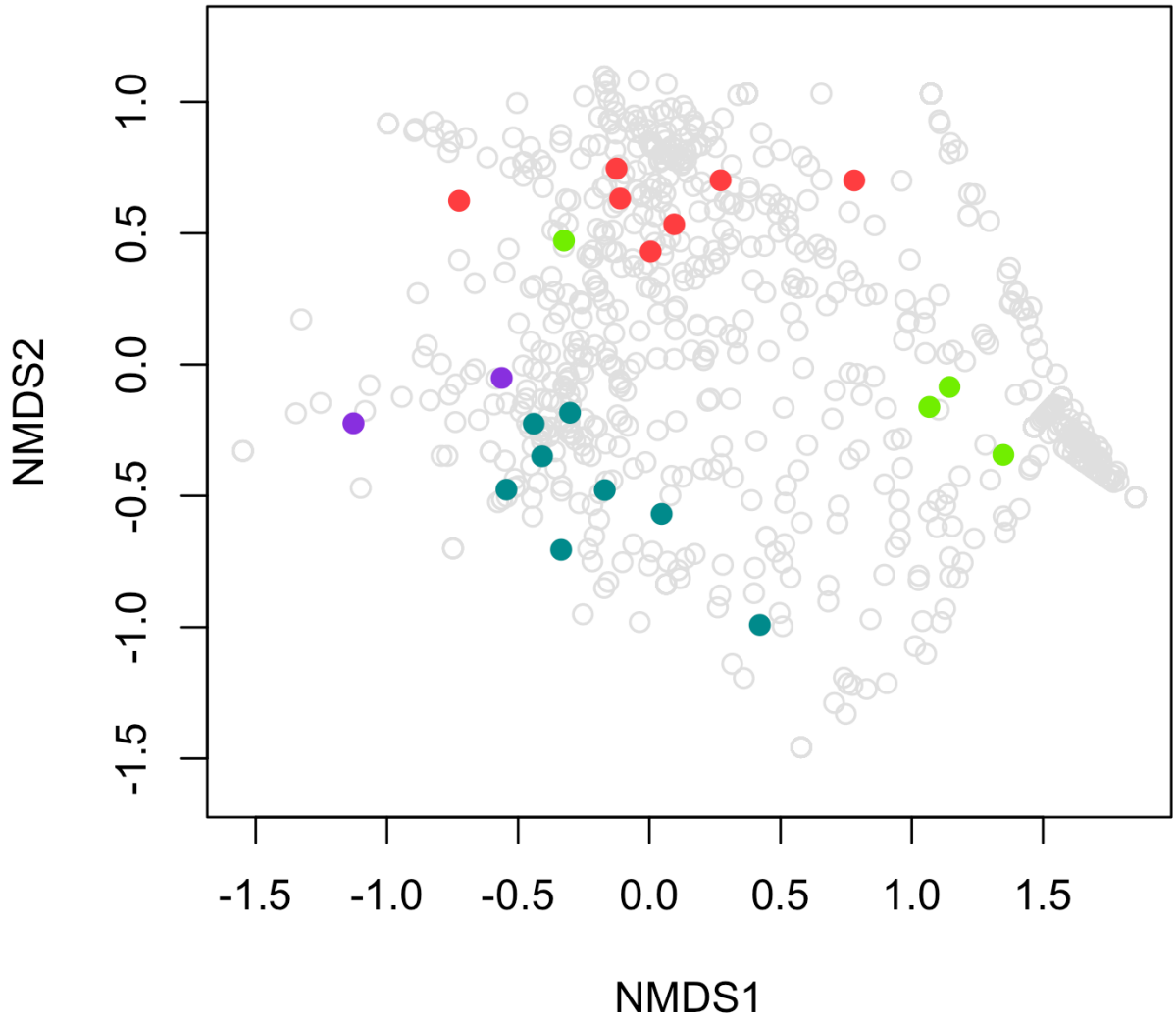


FIGURE 45: NMDS ordination of sequencing data by Horn Index group by influent water.

In accordance with prior research, Proteobacteria were most abundant members of the microbial community (Wang et al., 2014; Haig et al., 2015; Liao et al., 2013). Filters maintained in the laboratory with tap water showed highest relative abundance of Proteobacteria overall. For laboratory filters maintained with tap or groundwater, Proteobacteria accounted for >80% of total sequences. Proteobacteria are Gram (-), primarily aerobic or facultative anaerobes. Alpha and Betaproteobacteria are the most common classes encountered in the epilimnion of freshwater lakes (Newton et al., 2011).

Alphaproteobacteria families included Sphingomonodaceae, Rhodobacteraceae, and Rhizobiaceae. Sphingomonodaceae appear to be exceptionally dominant members of the microbial community under chlorinated conditions: both in tap water maintained filters in the laboratory and filters operating in the Dominican Republic. Sphingomonodaceae are ubiquitous in the environment and have been isolated from freshwater samples, marine environments, contaminated soils, and deep aquifers (Jogler et al., 2011). Sphingomonodaceae have been demonstrated as increasingly dominant members of community composition under chlorinated conditions rather than chloraminated conditions (Hwang et al., 2012). Sphingomonodaceae have been correlated with slow sand filter removal efficiency (Haig et al., 2015) and may form biofilms under chlorinated conditions (Hong et al., 2010). The *Shinella* genus of the Rhizobiaceae family accounted for a large proportion of the microbial community in laboratory filters maintained with groundwater in the laboratory. The presence of Rhizobiaceae in groundwater maintained filters may reflect a lack of ammonia as some free-living species of Rhizobiaceae are capable of dissimilatory nitrate reduction to ammonia (Poehlein et al., 2016).

Families within the Betaproteobacteria class included Comamonadaceae, Rhodocyclaceae, and Burkholderiaceae. Comamonadaceae, followed by Rhodocyclaceae, were

the most abundant families overall. Comamonodaceae are aerobic, mobile via flagella, and curve or rod-shaped. Comamonodaceae have been isolated from freshwater, soil, groundwater, and activated sludge (Rosenberg et al., 2014). Rhodocyclaceae are aerobic or denitrifying, rod-shaped, and typically prefer oligotrophic conditions. The Burholderiaceae genus contains pathogens of both plants and animals. Burkholderiaceae are rod-shaped, motile, obligate aerobes. Rhodocyclaceae were increasingly dominant in laboratory filters maintained with groundwater and seeded with inoculum from the Dominican Republic. In filters maintained with groundwater and seeded with biosand from the Linnwood treatment plant, Rhodocyclaceae were less dominant members of the microbial community while the relative abundance of Comamonodaceae increased.

Among Gammaproteobacteria, the predominant families were Pseudomonodaceae, Bradyrhizobiaceae, Caulobacteraceae, Phyllobacteriaceae, Erythrobacteraceae, and Chromatiaceae. The *Pseudomonas* genus of the Pseudomonodaceae was shown as the third most abundant taxa overall. The *Pseudomonas* genus includes a number of opportunistic pathogens, such as enterotoxigenic *P. aeruginosa*.

Firmicutes were the second most abundant phylum encountered and comprise a greater proportion of the microbial community in filters maintained with groundwater rather than tap water. The abundance of Firmicutes in these filters may be explained by the stress responses to chlorination or a lack of nutrients. Firmicutes are Gram positive and capable of producing endospores under stress and desiccation. Firmicutes were more dominant in field conditions as compared to laboratory conditions maintained with tap water. Firmicutes may proliferate under free chlorine disinfection (Gomez-Alvarez et al., 2012). Unidentifiable phyla comprised the greatest relative abundance of microbial communities of BSF in the field followed by lake water

maintained filters. Clostridiaceae was the most abundant family of Firmicutes (ranked number 12 in overall family abundance), followed by the Peptococcaceae family (ranked number 17 in overall family abundance). Clostridiaceae include the spore-forming *Clostridium* species, which may be highly resistant to disinfection (Hwang et al., 2012). *Clostridium* have been identified as a major component of groundwater samples (Maamar et al., 2015). Peptococcaceae are chemoorganotrophic, non-spore forming, anaerobic, spherical cocci.

Planctomycetes were the third most abundant phylum, comprising a greater overall proportion of community abundance under field conditions and in filters maintained with surface water. Previous research has shown an increase in growth of Planctomycetes during algae blooms in marine environments, which may be attributed to Planctomycetaceae's ability to degrade algal polymers (Pizzetti et al., 2011). Planctomycetes have been isolated from a variety of freshwater, brackish, marine, and soil environments ranging from oligotrophic to eutrophic. Planctomycetes have also been isolated from macroalgae biofilms. Recently, interest has grown in Planctomycetes due to a number of characteristics typically found in only eukaryotic cells including membrane-bounded cell compartments and the ability to carry out endocytosis (Lage et al, 2013). Planctomycetes have uniquely compartmentalized cells enclosed in a single or double bilayer. The paryphoplasm contains no ribosomes and lies between the intracytoplasmic membrane (an internal membrane) and the cytoplasmic membrane. The inner pirellulosome region contains ribosomes and the nucleoid, enclosed by the intracytoplasmic membrane. Freshwater Planctomycetes reproduce by budding structures (Fuerst and Sagulenko, 2011). In many Planctomycete genera, cell growth buds in non-cellular stalk structures from a central space (Fuerst, 1995). Until recently, it was believed that Planctomycetes lacked peptidoglycan in the cell wall entirely. However, peptidoglycan has been isolated from the Planctomycete cell

wall and the Planctomycete genome contains genes required for peptidoglycan synthesis (Jeske et al., 2015).

Bacteroidetes phylum (or *Cytophaga-Flexibacter-Bacteriodes* group) is the fourth most abundant phylum encountered overall. This phylum is highly diverse, comprised of Gram negative, rod shaped bacteria, which have been isolated from freshwater, marine, soil, skin, and gut environments. Bacteroidetes, together with Firmicutes, are important members of the human gastrointestinal tract and may account for over 98% of the total overall gut microbiota of mammals detected by rRNA sequencing. The Bacteroides group occupies a wide range habitats and biological niches, acting as polymeric organic matter degraders both in the environment and gastrointestinal tract. While gastrointestinal Bacteroidetes consist primarily of the Bacteriodia class, Flavobacteria, Cytophagia, and Sphingobacteria classes are most common in environmental habitats (Thomas et al., 2011). Similarly to their role in the mammalian gastrointestinal tract, environmental Bacteroidetes degrade a diverse range of complex polysaccharides and proteins. Some members of the *Bacteriodes* genus including *B. fragilis* are opportunistic pathogens of anaerobic infections upon escaping the gastrointestinal tract and host robust antibiotic resistance mechanisms (Wexler, 2007).

Acidobacteria were the fifth most abundant phylum, found in BSFs operating in the field and in surface water maintained BSFs to a greater extent than laboratory BSFs maintained with groundwater or tap water. Acidobacteria have been isolated from freshwater lakes and sediments, soils and (Newton et al., 2011). Although they are found ubiquitously in the environment, the functional role of Acidobacteria has not been heavily studied.

Verrucomicrobia were the sixth most abundant phylum. Verrucomicrobia are Gram (-), heterotrophic, and non-motile. While typically accounting for a minor proportion of the freshwater microbial community, Verrucomicrobia make major contributions to polysaccharide hydrolysis (Martinez-Garcia et al., 2012).

Actinobacteria followed the Verrucomicrobia phylum in abundance. Actinobacteria may proliferate in freshwater lakes (Newton et al., 2007). Actinobacteria are Gram positive, aerobic, and noted for high G and C content in their DNA.

The Chlamydiae phylum was encountered primarily within filtration sands of BSFs operating in the bateyes. The Chlamydiae phylum is comprised of obligate intracellular pathogens of eukaryotes, having a two part lifecycle as infectious particle form or intracytoplasmic reproductive form (Baron, 1996).

5.2 Interstitial Biomass by qPCR Copy Numbers

A total bacteria assay was performed by the McLellan lab following Øvreås and Torsvik (1998) DGGE end-point PCR for soils. 16s rRNA copy numbers were typically higher at shallow sand depths of the BSF. Inoculum type and influent water also showed changes number of copy numbers. The Dominican Republic “good” inoculum showed higher copy numbers than Dominican Republic “bad” inoculum for filters maintained with both tap water and groundwater. Filters maintained with lake water and seeded with inoculum sands from the Linnwood treatment plant showed highest overall copy numbers.

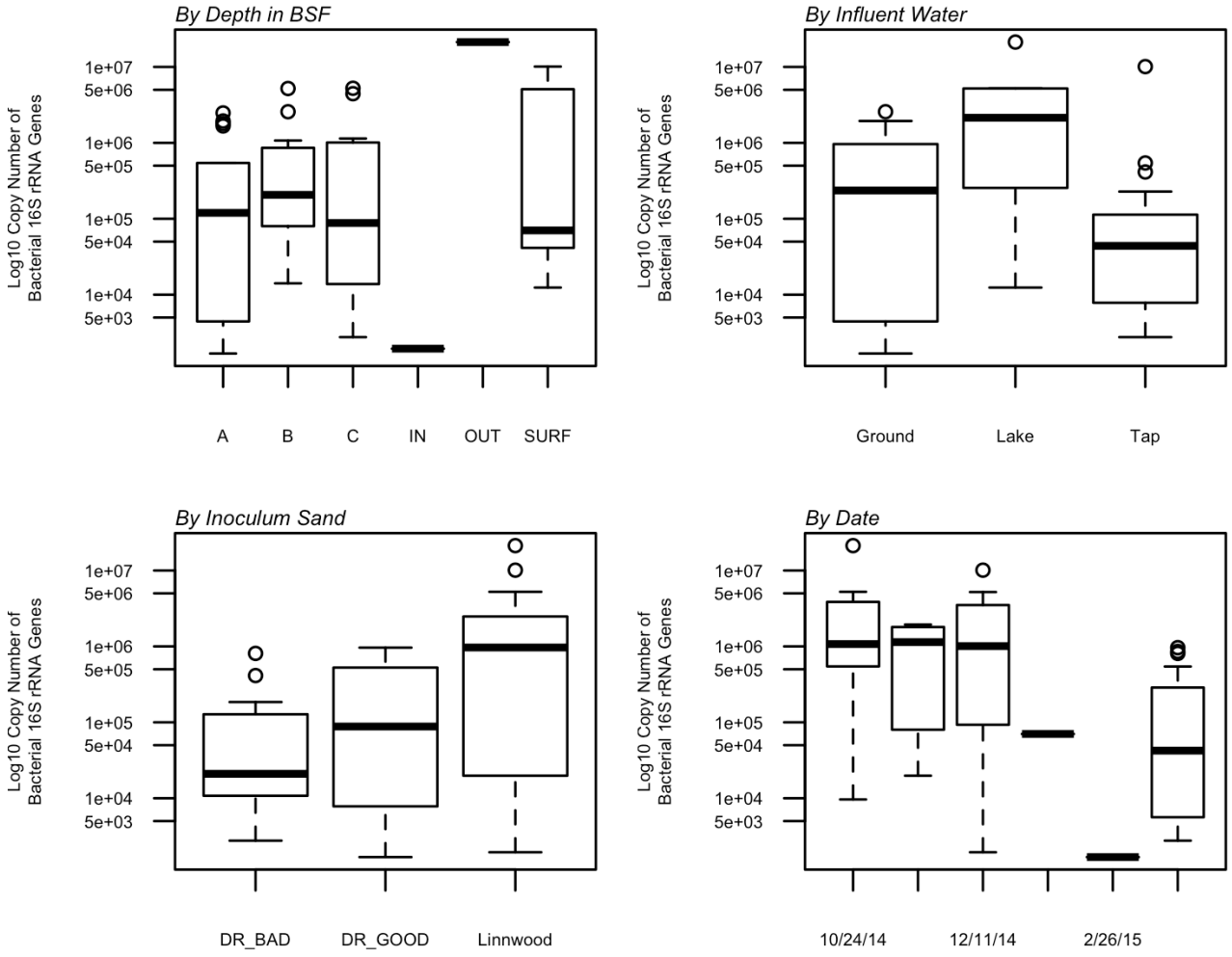


Figure 46: qPCR copy numbers for filters grouped by depth, influent water, sand inoculum, and over time.

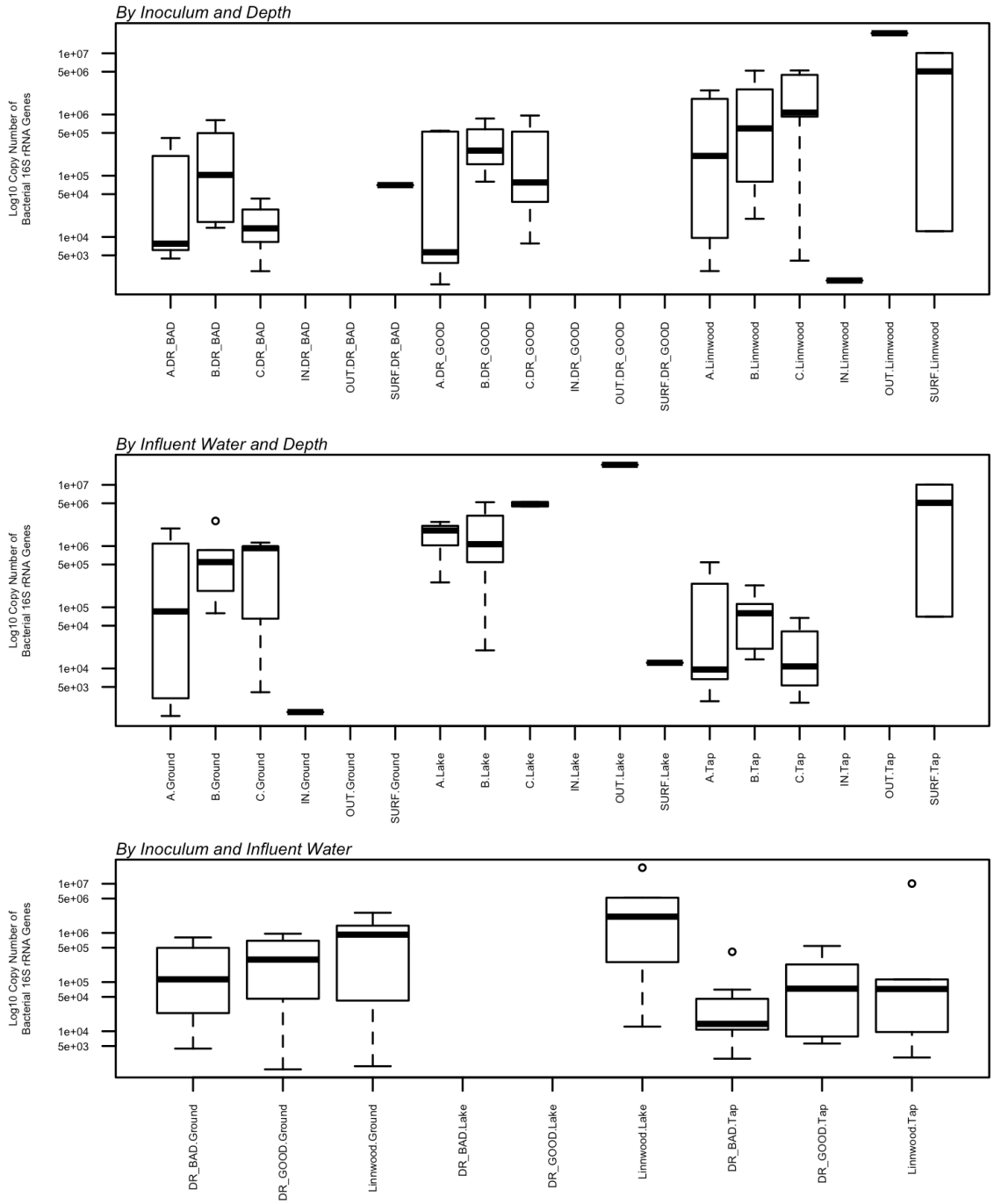


Figure 47: Interstitial and source water biomass by qPCR copy numbers.

Overall, the number of 16s rRNA copy numbers was a function of both source water and inoculum. Filters maintained with lake water show a greater number of copy numbers (by at least one order of magnitude) at all filter depths. Groundwater maintained filters show a greater number of copy numbers than tap water maintained filters. Filters seeded with a "good" (AD) inoculum had a greater number of copy numbers than "bad" (BC) inoculated filters under both chlorinated (tap water) and un-chlorinated conditions. Filters seeded with inoculum sands from the Linnwood Drinking Water Treatment Plant produced a greater number of 16S rRNA copy numbers than both "good" and "bad" inoculum sands from the Dominican Republic. It appears that this inoculum sand from the slow sand filtration beds was more robust than sands encountered in the point of use biosand filter.

6.0 Conclusions

In scenarios where source waters may be intermittently chlorinated, the point of use BSF is not recommended for use. In such a case, low biomass interstitial microbial community may not provide sufficient sorption or microbial competition to effectively remove pathogens from source waters with repeated exposure. Additionally, the research suggests that the BSF may serve as a source of pathogens under chlorinated conditions for several weeks following exposure to highly contaminated source water. While groundwater maintained filters continue to show pathogens in the effluent long after exposure to high concentration source water, chlorinated filters fail to provide any significant pathogen reductions after only three days exposure to pathogens. Additionally, bacteriophage filtration shows different patterns than *E. coli* filtration under chlorinated conditions. While *E. coli* shows a strong initial reduction at day one of the three day spiking window, bacteriophage MS2 shows a small ($\approx 0.6\log$) initial reduction which remains fairly consistent over the three day spiking window.

This research highlights shortcomings of water policy and communication throughout the bateyes. The BSFs funded by Rotary Club and installed by the Good Samaritan Hospital, although proving ineffective in the removal of pathogens from source waters, have provided a fundamental groundwork for water treatment and sanitation. This is a critical first step in improving sanitation conditions, and has been successful in establishing water correspondents for each village responsible for correspondence with the Good Samaritan Hospital. Drinking water treatment is most dependable as a multilevel process in which the failure of one component is reinforced by the resilience of another. Chlorination, while effective for the removal of pathogens, is unlikely to be a consistent process throughout the bateyes. Chlorination in conjunction with the BSF has proven ineffective. Abiotic filtration systems including the ceramic filter will remain unhindered when chlorination is present, and continue to provide treatment when chlorination is absent from source waters. Low turbidity source waters as encountered throughout the bateyes are ideal for continued operation of ceramic filters.

Based on NMDS ordination, this research shows that the microbial community of the BSF is a function of source water characteristics (chemistry, biomass, and pre-treatment) and to a lesser extent is affected by introduction of a biologically active biosand inoculum at the filter surface. To what extent the biosand inoculum community changes over time or contributes to filter maturation remains unclear. In general, NMDS ordination clearly groups the microbiome of filters in four distinct categories dependent upon source water: BSF maintained with surface water, groundwater, tap water, and finally BSF microbiome encountered throughout the bateyes.

Using qPCR copy numbers as a proxy, interstitial biomass is most robust under surface water source, followed by groundwater source, and finally chlorinated source water. qPCR results suggest that inoculums obtained from the Dominican Republic which appeared to be

performing “well” showed greater biomass (one order of magnitude greater copy numbers) after being maintained under chlorinated conditions than inoculum designated as “poor” performing in the field. “Well” performing inoculums sands may have better adapted to chlorinated source water conditions, maintaining resilience after inoculation to laboratory BSFs.

To more accurately maintain the microbiome of BSFs encountered in the field, source water chemistry must be carefully monitored to prevent shifting of inoculum community from field conditions. Further, to simulate field scale chlorination, dosing with $NaHClO_4$ may appropriate rather than maintaining with municipal water supply as monochloramine disinfected waters contain an excess of ammonia.

While controlled laboratory conditions tend to provide predictable behavior and filtration efficiency, the lack of education and intricacies of communication in developing countries transcend difficulties in sanitation and hygiene. The unpreventable pretreatment by chlorine prior to exposure to the BSF by the sugarcane company, the over-scraping of filter surfaces leading to a loss of filtration media, excessive hydraulic loading of filters up to 100 liters per day, and storage of food products at the surface of the filter encountered during field work raises further precautions for the successful design of point of use filtration systems.

7.0 Future Work

In order to determine the viability of inoculating fresh filters with active inoculum sands, additional factors must be considered, including a threshold volume of inoculum or proportion relative to filter pore volume, and community scale comparison of source waters against interstitial biofilm. While these results suggest that the overall community composition of inoculum sands are not maintained in the filter over time, a more robust inoculum sand may

provide an improved substrate for cell attachment and biofilm formation, or the community may shift as a reflection of the community structure of influent waters. The extent to which the microbial community of an inoculated filter deviates from the microbial community of the inoculum sand is largely a function of water chemistry parameters, including pretreatment, trophic status, pH and DOC.

To produce an inoculum resistant to chlorination, some bacterial taxa are of interest for chlorine resistance and prevalence in freshwater biofilms. These bacteria groups include the Sphingomonodaceae family, which is found in this study to comprise a major proportion of the bacterial community composition in chlorinated filters. Sphingomonodaceae have been isolated from chlorinated drinking water distribution system biofilms in many cases. Some species of the *Mycobacterium* genus (within theActinobacteria phylum) are likewise resistant to disinfection due to cell wall complexity, and have been isolated from drinking water distribution system biofilms (Liu et al., 2012). Mycobacterium may play a critical role in disinfected biofilm formation through the production of EPS (Gomez-Alvarez et al., 2012). However, the Mycobacterium genus contains many pathogenic species including agents for leprosy and tuberculosis. Strains of interest for biofilm development under chlorinated conditions create challenges to preventing the proliferation of potentially pathogenic agents.

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APPENDIX

Appendix A: Growth Media and Chemical Preparation

LB Broth: for plating *E. coli* from -80℃

-Autoclave 15 minutes at 15 PSI

TSB + Streptomycin / Ampicillin: broth for overnight *E. coli* inoculation of C3000 (F_{AMP}) Host Strain

-To 900 mL H_2O add 30g TSB

-Dilute to 1L

-Autoclave 15mins at 15 PSI

-Add 10 ML Streptomycin / Ampicillin Stock

Streptomycin / Ampicillin Stock:

-Dissolve 0.15 g Ampicillin Sodium salt and 0.15 g Streptomycin Sulfate in 100 mL Water

-Filter through sterile 0.22 μm membrane

LB Agar: for overnight *E. coli* inoculation of "Berm" strain

-Autoclave 15 minutes at 15 PSI

Modified mTEC Agar: for enumeration of *E. coli*

-Autoclave 15 minutes at 15 PSI

0.7% Triptic Soy Agarose

To 250 mL deionized water:

-Add 7.5g Bacto Triptic Soy Broth

-Add 1.75g Agar

-Autoclave 15 minutes at 15 PSI

Phosphate Buffered Saline

To 800 mL deionized water:

-8g NaCl

-0.2g KCl

-1.44g Na_2HPO_4

-0.24g KH_2PO_4

-Adjust pH to 7.4 with HCl and dilute to 1L

-Autoclave 15 minutes at 15 PSI

Appendix B: Microbial Stock Cultures

***E. coli* C3000 (F_{AMP}): Host for Bacteriophage MS2**

-ATCC # 15597

Bacteriophage MS2

-ATCC # 15597-B1

"Berm" *E. coli*:

-*E. coli* strain isolated by McLellan lab from berm region of Bradford Beach, Milwaukee, WI.

-Stored at -80℃ until inoculation