Legionella - A Threat to Groundwater

Pathogen Transport through Recharge Basin Media Columns

by

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ABSTRACT

This study was devised to elucidate key information concerning the potential risk posed by *Legionella* in reclaimed water. A series of biological experiments and a recharge basin soil column study were conducted to examine the survival, growth, and transport of *L. pneumophila* through engineered reclaimed water systems. A pilot-scale, column study was set up to measure *Legionella* transport in the columns under Arizona recharge basin conditions. Two columns, A and B, were packed to a depth of 122 cm with a loamy sand media collected from a recharge basin in Mesa, Arizona. The grain size distribution of Column A differed from that of Column B by the removal of fines passing the #200 sieve. The different soil profiles represented by column A and B allowed for further investigation of soil attributes which influence the microbial transport mechanism. Both clear PVC columns stand at a height of 1.83 m with an inner diameter of 6.35 cm. Sampling ports were drilled into the column at the soil depths 15, 30, 60, 92, 122 cm. Both columns were acclimated with tertiary treated waste water and set to a flow rate of approximately 1.5 m/d. The columns were used to assess the transport of a bacterial indicator, *E. coli*, in addition to assessing the study's primary pathogen of concern, *Legionella*. Approximately, 10^7 to 10^9 *E. coli* cells or 10^6 to 10^7 *Legionella* cells were spiked into the columns' head waters for each experiment. Periodically, samples were collected from each column's sampling ports, until a minimum of three pore volume passed through the columns.

The pilot-scale, column study produced novel results which demonstrated the mechanism for *Legionella* to be transported through recharge basin soil. *E. coli* was transported, through 122 cm of the media in under 6 hours, whereas, *Legionella* was

transported, through the same distance, in under 30 hours. *Legionella* has been shown to survive in low nutrient conditions for over a year. Given the novel results of this proof of concept study, a claim can be made for the transport of *Legionella* into groundwater aquifers through engineering recharge basin conditions, in Central Arizona.

DEDICATION

"37 No, despite all these things, overwhelming victory is ours through Christ, who loved us. 38 And I am convinced that nothing can ever separate us from God's love. Neither death nor life, neither angels nor demons,[b] neither our fears for today nor our worries about tomorrow not even the powers of hell can separate us from God's love. 39 No power in the sky above or in the earth below—indeed, nothing in all creation will ever be able to separate us from the love of God that is revealed in Christ Jesus our Lord."

Romans 8:37-39 (NLT)

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CHAPTER 1

INTRODUCTION AND OBJECTIVES

Motivation

Legionella are a common yet poorly understood water-bread pathogen. These bacteria are ubiquitous in water systems, where protozoan host endoparasitization and biofilm association play important roles in their life cycle. The bacteria received its name after the outbreak primarily affected persons attending an American Legion convention. Of the 182 cases, 29 people died. Current knowledge on *Legionella* ecology is severely lacking in several key areas, one of which being reclaimed water. Since *Legionella* are capable of long term survival in unheated water (Paszko-Kolva et al., 1991), the ability for *Legionella* to persist in reclaimed water systems poses a significant risk, particularly in the context of recharging reclaimed water. This risk ensures the need for further inquiry of the transport and dispersion of *Legionella* into groundwater aquifers as well as additional information concerning potential health risks due to this pathogen.

Significance. It is pertinent to point out that *Legionella* species particularly *L. pneumophila*, are responsible for more drinking water and non-recreational water-borne disease outbreaks in the United States than any other microorganism (Brunkard et al., 2011). Recently, *Legionella* was added to the EPA's candidate contaminant list 3 (CCL3) (EPA, 2009). Incidence of legionellosis has consistently and significantly risen (Hicks et al., 2011) since the discovery of the disease in 1976 (Fraser et al., 1977). Over the last several years we have studied *Legionella* survival and incidence in water resources and structures across the valley and have found high incidence rate of this bacterium in water infrastructures. (ranging from distribution systems to hot groundwater wells) (Schwake et

al., 2012). In addition, *Legionella* survival experiments performed by our group indicated that certain components in environmental water are capable of supporting the growth of this pathogen (Schwake et al., 2013).

Risk. Morbidity and epidemiological/outbreak data from public health agencies and Centers for Disease Control (CDC) are clear indicators of *Legionella* threat in our nation waters. The overwhelming epidemiological evidence led the inclusion of *Legionella* in EPA's CCL. Though this list is not binding, it prioritizes the emerging waterborne microbial threats on the national scale. This study helped to align regional focus with national priority. The incidence and prevalence data collected during this study helped in characterizing any risk in the existing water management practices in Central Arizona. Such information can be critical in good management practices and helpful in sound decision making, which ultimately translate into customer confidence.

Impact. Arizona is one of the leading states practicing groundwater recharge to meet current and future water needs; however, substantial recharge practices have their own caveat. The possibility of contaminant mobility from surface to groundwaters is presumed to increase with the high recharge rates. In addition, contaminant transport risk further multiplies under stresses caused by climate change, another significant factor in management of water resources in the valley. *Legionella* is frequently detected in reclaimed water, surface water, and treated municipal water. In a study performed in California, *Legionella* were present in reclaimed water at all five locations tested, with three of the five locations registering *L. pneumophila* in 11-40% of *Legionella*-positive samples (Palmer, Tsai, Paszko-Kolva, Mayer, & Sangermano, 1993). In a subsequent study, *L. pneumophila* was detected in 5 of the 16 sites. Surface water receiving

chlorinated effluents are reported to test positive for *Legionella* species in more than 90% of the samples tested with an average concentration of 10^3 cells per ml.

Wastewater effluent reclamation usages have become more popular during the past decade. Tertiary treated wastewater effluent is often reused for irrigation, for aquifer recharging, and for watering greenbelts. One group of organisms that is found in reclaimed water is *Legionella*. Several studies have shown that *Legionella* has a high affinity for growing in effluent reclaimed water (Jjemba, Weinrich, Cheng, Giraldo, & LeChevallier, 2010; Palmer et al., 1995; Palmer et al., 1993). These studies used predominantly PCR method for the detection and quantification of Legionella showing high concentrations of 1×10^6 cfu/L in effluent reclaimed water. *Legionella* poses a significant threat to our groundwater.

Goals of this Study

The main objective of this study is to measure *Legionella* transport through a laboratory column study. This study was developed to accomplish the following:

- To measure the distance of *Legionella* transport through recharge basin soil media in a column study
- To identify the spatial and temporal pattern of *Legionella* transport through microcosm
- To assess the presence of *Legionella* in a Central Arizona recharge basin

CHAPTER 2

LITERATURE REVIEW

Overview

Increasing populations have resulted in higher water demand ("Guidelines for the Safe Use of Wastewater, Excreta and Greywater, vol 2. Wastewater Use in Agriculture," 2006). Changing precipitation patterns have shifted where water is supplied. Public degradation of existing source waters has influenced source water dependability. For these and other reasons, freshwater is becoming increasingly scarce. (Jjemba et al., 2010). As freshwater scarcity is an ever more present issue facing the world today, reclaimed water has become an attractive water supply alternative.

Recharge Definitions. Wastewater effluent reclamation uses have become more popular during the past decade. The term “reclaimed water” is defined as effluents that have undergone a combination of physical, chemical, and biological treatments in engineered systems that utilize wastewater treatment technologies to remove suspended solids, dissolved solids, organic matter, nutrients, metals, and pathogens. Reclaimed water may contain high levels of organic and biological matter. These contents bare the potential to react with engineered disinfectants. Ideal effluent quality should be proportionate with the water quality desired for the intended use.

Transport Definitions. For the purpose of describing the documented transport mechanisms, it is helpful to define a couple key terms. The term “colloid” is assigned to particles that fall within the size range between 100 nm and 100 μm. Colloids can be both biological and non-biological in nature. The term “energy barrier” is used to refer to the repulsion between colloids and their surrounding surfaces in environmental contexts.

Groundwater Management Act. Under the Groundwater Management Act, (GMA), of 1980, five Active Management Areas (AMAs) have been created and Phoenix is among one of those AMAs. One of the founding principles of the GMA is to bring the focus areas into Safe Yield. Safe Yield is defined as the balance between groundwater withdrawal and natural and/or artificial recharge. Given the limited water resources and a growing population, Central Arizona has to excessively rely on groundwater recharge. The Central Arizona Groundwater Replenishment District (CAGRD) is required to analyze potential risk associated with recharge facilities that may be used by CAGRD for replenishment.

Impact of Project. The CAGRD strives to maintain balance between groundwater withdrawal and natural and/or artificial recharge, safe yield. The uncertainty surrounding the manner in which current and future replenishment obligations are met may translates into significant challenges in terms of the quality of groundwater recharge. The CAGRD is required to analyze potential risk associated with recharge facilities that *may* be used by CAGRD for replenishment. This study produced data to identify potential risk of *Legionella* transport and survival in aquifers under the projected water resource scenarios in the valley.

Reuse Applications. Water reuse has many applications. Treated wastewater effluent is often reused for irrigation, for aquifer recharging, and for watering greenbelts. This option is an attractive alternative in many drought-prone areas with insufficient water. Except for standard coliform testing, few studies have been performed to evaluate the microbial contents of reused water. One group of organisms that is found in reclaimed water is *Legionella*. Microbial evaluations of reclaimed water mainly focus on

evaluating the abundance of indicator bacteria such as: coliforms, *Escherichia coli*, and *enterococci* (Costán-Longares et al., 2008).

Legionella Background Information

Legionella bacteria are fastidious gram-negative aerobic bacilli (EPA, 2009). *Legionella* is commonly found exhibiting three shapes, cysis, rod, or branched. When in rod shape, *Legionella* measures at 2 μm long and 0.3 μm wide. They will only grow on buffered charcoal yeast extract agar. There are 53 species and 73 serogroups of the *Legionella* species (Lück et al., 2010). *Legionella* bacteria in water are a health risk if the bacteria are aerosolized and then inhaled. Aerosolization often occurs through air conditioning systems or in showers (Schoen & Ashbolt, 2011). Inhalation can result in a type of pneumonia known as Legionnaires disease. Approximately 80-95% of *Legionella* infections in the US are due to group 1 (EPA, 2009). *L. pneumophila* is the species responsible for 80-85% of the legionellosis outbreaks reported in the United States (Reingold et al., 1984). *Legionella longbeachae* (3.9%), and *Legionella bozemanii* (2.4%) (EPA, 2009).

History. *Legionella* was first recognized after an outbreak in Philadelphia in July, 1976 (Fraser et al., 1977). The bacteria received its name after the outbreak primarily affected persons attending an American Legion convention. Of the 182 cases, 29 people died. Early epidemiologic analysis suggested that exposure may have occurred through airborne transition in the lobby of the headquarters hotel or in the area immediately surrounding the hotel.

Engineered System Interactions. *Legionella* has been well documented for its ability to colonize engineered water systems (Brown et al., 1999; Carducci, Verani, &

Battistini, 2010). A study was performed to establish the number of interacting factors contributing to their occurrence, proliferation, and persistence. This study was documented to aid in developing new treatment technologies and/or systems that minimize or eliminate human exposure to potentially pathogenic *Legionella* (Buse, Schoen, & Ashbolt, 2012).

Legionella Presence in Effluent Reclaimed Water

Several studies have shown that *Legionella* has a high affinity for growing in effluent reclaimed water. (Palmer et al., 1993), (Palmer et al., 1995), (Jjemba et al., 2010). These studies used predominantly PCR method for the detection and quantification. *Legionella* was recorded at concentrations of 1×10^6 cfu/L. Chlorination of the effluent reclaimed water did not have a substantial effect because, *Legionella* was detected in both chlorinated and non-chlorinated effluent waters (Table 2.1).

Table 2.1 *Legionella* Presence in Effluent Reclaimed Water

Location	Water (CFU/L)	Source
Treated Effluent Reclaimed Water	6×10^5	(Jjemba et al., 2010)
Chlorinated Effluent Reclaimed Water	1×10^6	(Palmer et al., 1995)

Legionella Presence in Groundwater

Legionella has been studied and detected in various soil and water samples. However, the presence and persistence of *Legionella* in groundwater is poorly documented. Few studies have assessed the presence and occurrence of *Legionella* in groundwater samples. The role of groundwater as a potential natural reservoir of *Legionella* has not yet been investigated. *Legionella* incidence in groundwater has been

sporadically reported around the world (Lieberman et al., 1994; Lye et al., 1997; Riffard et al., 2001). The presence and persistence of *Legionella spp*. in Arizona is not well understood.

Portugal. *Legionellae* were isolated from all 33 groundwater samples from a borehole over a 7-year period (Costa, Tiago, da Costa, & Veríssimo, 2005). During the same period, *Legionellae* were never recovered from a different sampled borehole. The number of L*egionellae* recovered from the positive sampled borehole samples varied between $3.0 \times 10^2$and 2.4×10^4 CFU/L (Costa et al., 2005).

United States. In a study, groundwater samples from Alabama, Florida, Idaho, Illinois, Indiana, Maryland, Michigan, Minnesota, Montana, New York, North Carolina, Ohio, Oregon, Texas, Vermont, and Washington were collected and analyzed for *Legionella*. Of the 58 groundwater samples analyzed by PCR, 5.2% were negative, 50% of samples were positive at concentrations < 44 *Legionella* cfu/mL, 31.0% of samples were positive at concentration equal to 44 *Legionella* cfu/mL, and 13.8% of samples were positive at concentrations > 44 *Legionella* cfu/mL (Lye et al., 1997). From the PCR results, 81.1% of the samples were positive for low concentrations of *Legionella*. Culture results of the 58 samples revealed that 7% of the 58 of the groundwater samples contained culturable *Legionella* cells. (Lye et al., 1997) All *Legionella* isolates were confirmed by cysteine requirement and by PCR. Interestingly, *L. pneumophila* was not detected in the groundwater samples (Lye et al., 1997).

Groundwater was sampled from two other sites in the United States. Two samples were collected from each of the 12 wells processed in this study. Of these 24 samples, 22 have tested positive for the presence of *Legionella* in the groundwater (10/12) and/or the

biofilm (9/12) samples. Concentrations of *Legionella* in water samples were 1.0×10^2 and 8.4×10^4 CFU/L (Riffard et al., 2001). In biofilm samples, concentrations of 2 to 267 CFU/cm^2 were observed (Riffard et al., 2001).

Legionella Transported through Soil

In an experiment designed under recharge conditions, a 2.4 m long, 32.5 cm diameter vertical stainless steel column was hand packed with Mohall-Laveen sandy loam soil. The soil was collected from an area northwest of Phoenix and packed to a bulk density of 1.63 g/cm^3 and a porosity of 0.38 (Cordy et al., 2004). Real-time PCR results showed that *Legionella* had been transported through the entire length of the column (Cordy et al., 2004). The author projected that *Legionella's* known ability for survival in an extreme range of environmental conditions, including thermal and chlorine disinfection (Atlas, 1999), may have aided in their survival and transport through the soil.

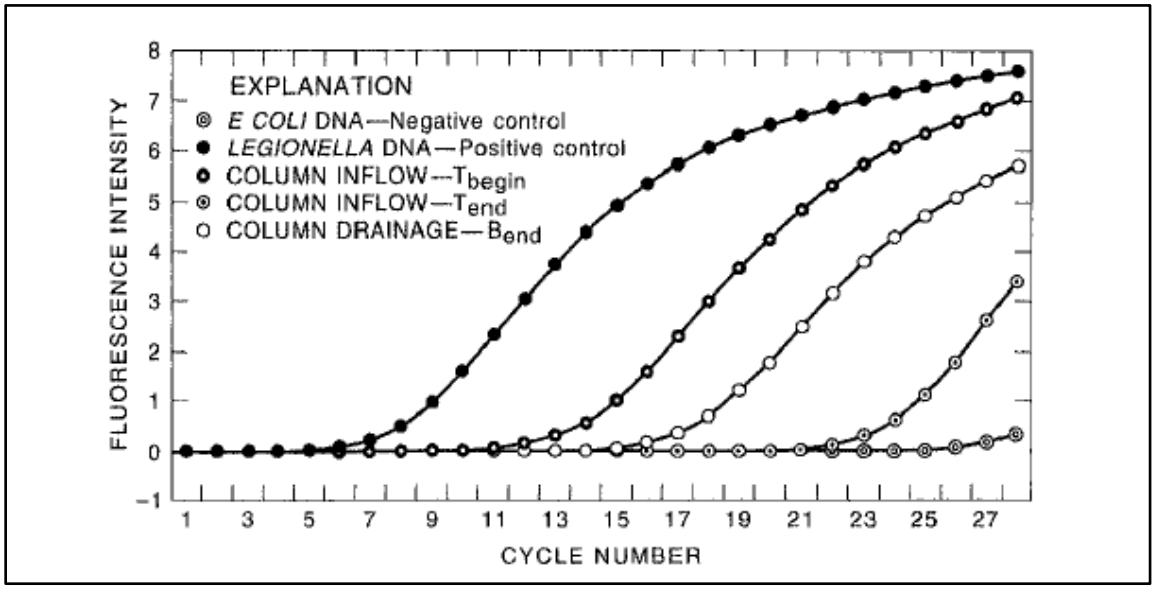

Figure 2.1 Real-Time PCR Amplification Results for *Legionella* specific primers.

Source: Reprinted from Cordy et al, 2004 (Cordy et al., 2004)

DNA amplification of column inflow (T_{begin}, T_{end}) and column drainage (B_{end}) demonstrated the presence of *Legionella* in all three samples (Figure 2.1). The author continued on to state "*Legionella* is likely to persist during typical recharge conditions and has the potential to reach groundwater." (Cordy et al., 2004).

Legionella Aerosolization Induced Risks

The potential for aerosolization through evaporation of sitting water in recharge basins is an additional concern posed by *Legionella.* As environmental engineers, it is critical that we minimize risks associated with engineered systems. Recharge basins are often designed with the dual purpose of serving as community parks and animal wildlife conservation sites. One study showed air samples, which had been collected over wastewater treatment basins, indicated that *Legionella spp*. were aerosolized during the treatment process (Palmer et al., 1995). The air sampling method used in this study obtained aerosols only 122 cm from the water surface. This distance is not far from that which may be encountered by those passing by park recharge basins. Recharge parks are visited by young and old, the risk of infection must be further researched. Frequent human interactions with these facilities may increase the risk of exposure and future outbreaks.

Selection of *E. coli* as an Indicator

In addition to the wealth of knowledge available on *E. coli*, this bacteria was selected as an indicator organism for this transport study for several reasons. Some of which are listed here:

- *E. coli* is a well-known indicator organism for fecal contamination
- *E. coli* cultures quickly and is easily worked with under laboratory conditions

- Comparisons can be made between this study and other studies' results because *E. coli* has been used repeatedly in other transport studies
- Both *Legionella* and *E. coli* are rod shaped bacteria of a similar size

E. coli, having been widely studied, was selected for its characteristics similar to those of major waterborne bacterial pathogen genera, such as *Salmonella*, *Shigella*, *Vibrio*, *Campylobacter*, and *Yersinia*, etc. (Cho, Chung, Choi, & Yoon, 2005). *Legionella*, a non-enteric pathogen, survives in biofilms and protozoa. Because of these associations, *Legionella* can endure a wide range of environmental conditions and by doing so, is commonly found in waste water (Cordy et al., 2004). *Legionella* and *E. coli* fall in the category of colloids sized between 1 and 2 μm. As will be explained later in this chapter, colloids in this size range experience less removal during transport relative to both larger and, counterintuitively, smaller organisms.

Physical, Chemical, and Biological Factors which Influence Transport

In the following sections, various factors which affect microbial transport will be discussed. Some of these factors include, but are not limited to:

- Colloid Size
- Soil Grain Size Distribution
- Soil Particle Angularity
- Porosity
- Flow rate
- pH
- Geological Settings
- Temperature

Although several of these factors are related to the colloid, such as size, and to the surrounding environmental conditions, such as temperature, many of the factors which influence transport are defined by soil characteristics. In the next few sections, these soil parameters will be evaluated in more detail.

Modeling Particle Transport in Granular Media

Microbial transport through porous media is controlled by several key mechanism processes: advection, dispersion, retention, and re-entrainment. These processes are dictated by far more complicated interactions pertaining to the porous media and the microorganism in question.

Heterogeneity of the subsurface. Both micro-scale and macro-scale physical characteristics influence advection and dispersion processes in porous media. It is important to understand the advection and dispersion properties of a soil given that the soil characterization is largely dictated by such properties. Transport is influenced by preferential flow paths (Tufenkji, 2007). Subsurface flow is initiated through pore domains and so the hydrological setting is critical to transport issues. This study focuses on colloid transport through porous media and related to the specific soil characterization, but results can also be applied more broadly to further recharge applications.

USDA Textural Classification System

Soil texture is a qualitative classification tool used to determine classes for agricultural soils based on their physical texture. Soil texture is used in both the field and laboratory settings. Textural feel is used to distinguish between classes. Sieve analysis is used to further define textural classes. Grading sieves are used to separate the relative proportions of sand, silt and clay. The soil separate terms, sand, silt, and clay, are defined by the particle size range. Soil textures are classified by the fractions of each soil separate present in the soil. Particle-size distribution (PSD) curves are used to make soil division into the soil separate classifications simple. Textural classifications are named based on the most abundant soil constituent particle size or sizes present in the sampled soil.

Therefore, sandy clay contains mostly sand and clay particles. The term "loam" is used to describe a roughly equal concentration of sand, silt, and clay.

Classification Systems. There are three main systems used to define textural classes: United States Department of Agriculture (USDA), United Soil Classification System (USCS), and American Association of State Highway and Transportation Officials (AASHO). Figure 2.2 shows the comparison between these systems.

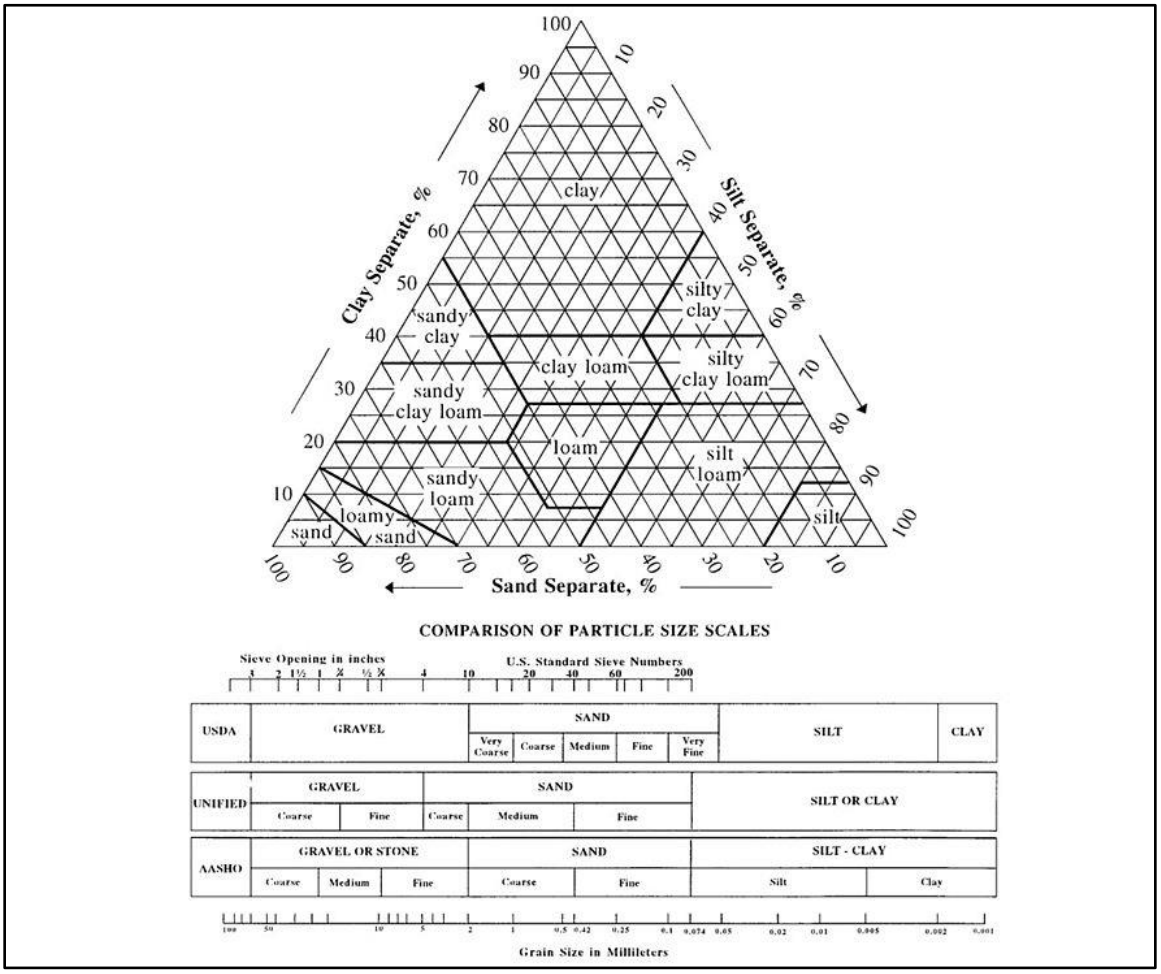

Figure 2.2 Comparison of Soil Classification Systems

Source: Soil classification. In Wikipedia, *The Free Encyclopedia*. (Wikipedia, 2014) http://en.wikipedia.org/w/index.php?title=Soil_classification&oldid=630152964

Classification System History. The first classification system, the International system, was first proposed by Albert Atterberg (1905). Atterberg selected particles with a 20 μm upper size limit for the classification of silt particles for three key reasons.

1. Particles smaller are not visible to the naked eye
2. Suspended particles could be coagulated by salts
3. Capillary rise within 24 hours was most rapid in this size range
4. Root hairs were prevented from entering pores between compacted particles

In the United States, twelve major soil texture classifications are defined by the USDA (USDA, 1987). In 1938, the USDA adopted its own system. The Food and Agriculture Organization (FAO) of the United Nations used the USDA system in 1974 for the production of the FAO United Nations Educational, Scientific and Cultural Organization World Soil Map.

Soil Conditions which Influence Flow

Microbial transport through soil is influenced by various soil conditions. The degree of soil saturation effects flow conditions. Subsurface preferential flow paths allow access to easy passageway for microorganisms to be transported to groundwater aquifers. Preferential flow paths can yield to the rapid transport of colloids.

Colloid Mobilization. Both biological and non-biological colloids can be resuspended through high flow events. Recharge basins use flooding events to discharge water into the subsurface. Each flooding cycle allows the recharge basin to fill to an excess point and then discharge for a period long enough to allow the surface to dry. These high flow events may also allow for the mobilization of formerly lodged colloids in recharge basin soils. In unsaturated regions of the soil, capillary action is stated to be

the predominant driving force that mobilizes colloids. (Rousseau et al. 2004, Wan and Tokunaga 1997, Sirivithayapakorn and Keller 2003, Saiers and Lenhart 2003, Crist et al. 2004, Zevi et al. 2005). Capillary action may bind the colloids to the air-water interface. The force of the capillary action required to bind the colloids to the air-water interface must be larger than the Derjaguin and Landau, Verwey, and Overbeek (DLVO) forces between the colloids. It is important to also recognize the interactions caused by particle hydrophobicity on colloid transport. (Gao et al. 2006, Wan and Wilson 1994).

Similarities between Biological and Non-Biological Colloids. The work of Johnson et al. (2008) demonstrated similarities between the transport behaviors of biological and non-biological colloids in environmental porous media. Similar processes control their transport behavior when in the presence of energy barriers because of common retention and re-entrainment attributes. Therefore, understanding the general transport behavior of colloids can aid in understanding pathogen transport.

Existing Filtration Theory

In classical filtration theory (CFT), two primary mechanisms are used for describing colloid deposition. CFT evaluated an idealized spherical colloid deposition on an idealized spherical collector in a clean bed of porous media.

1. Transport of colloids to porous media grain surfaces
2. Colloid-surface interactions

Colloid-surface interactions are governed by forces that may allow or prevent direct attachment of colloid with the grain surface. The system's physical properties determine the probability that the colloid will approach the grain surface. This probability is correlated to dimensionless parameters, which describe the colloid and surface, in

Equation (2) (Tufenkji and Elimelech 2004a, Nelson and Ginn 2005). Figure 2.3 shows the idealized colloid and surface, referred to as the Happel sphere-in-cell model, which is modeled in this correlation equation. (Happel 1958).

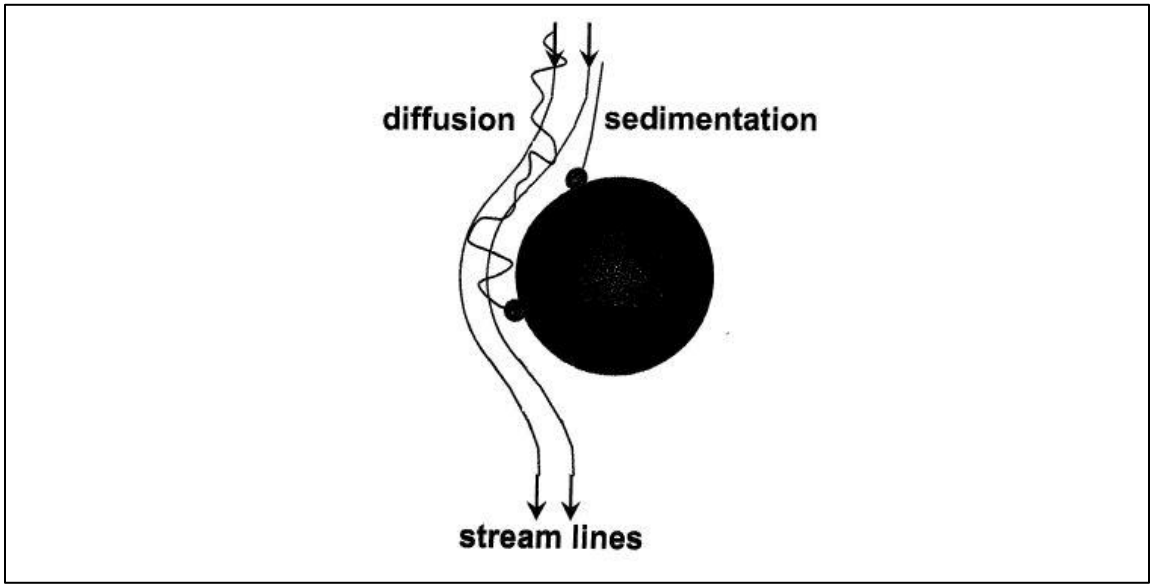

Figure 2.3 Schematic of Happel Sphere-in-Cell Unit Collector

Source: Reprinted from Abbazadegan et al., 2011, Methods to Assess GWUDI and Bank Filtration Performance, *Water Research Foundation* (Morteza Abbaszadegan, 2011)

Since bacteria can be evaluated as colloids in the intermediate size range, between 1 and 2 μm, it is important to evaluate the probability for surface collision and deposition for colloids of this size range. Depending on colloid size, different ratios of diffusion, interception, and gravitational forces dominate the filtration interactions. Intermediated sized colloids exhibit the lowest probability for surface collision and deposition. Particles that are smaller than 1 μm experience more diffusion to surfaces than colloids between 1 and 2 μm. Additionally, particles larger than 2 μm have higher interactions with straining forces. This principal is demonstrated under experimental settings in the absence of an energy barrier in Figure 2.4 (Tong & Johnson, 2006).

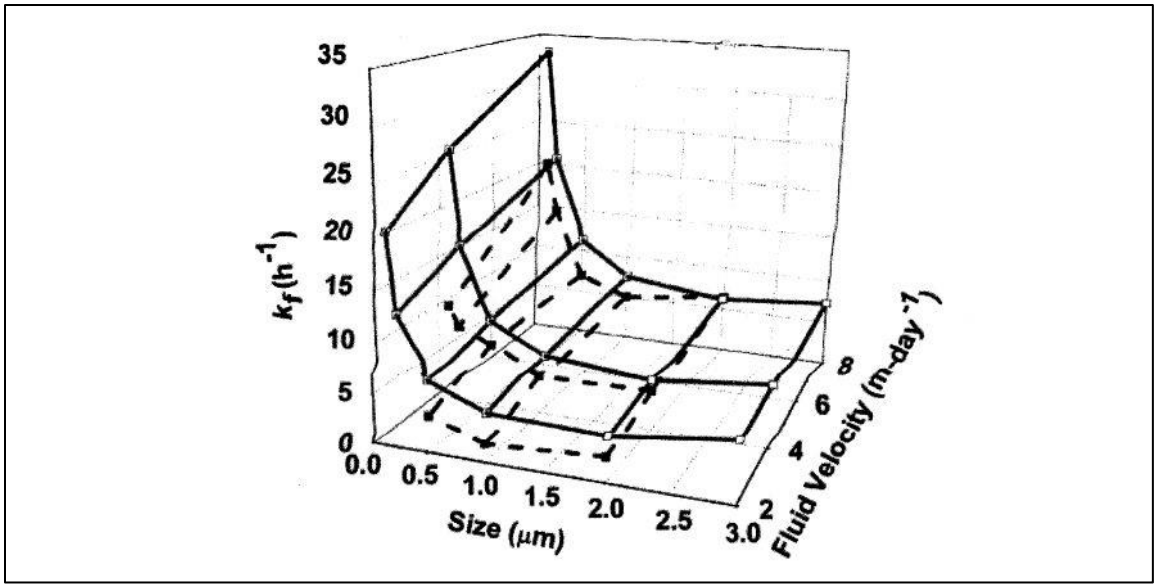

Figure 2.4 Simulated (open symbols) and Observed (closed symbols) Deposition Rate Coefficients (kf) in the Absence of an Energy Barrier to Deposition Microspheres as a Function of Fluid Velocity in Glass Beads

Source: Reprinted from Tong M., and Johnson. 2006a. Excess Colloid Retention in Porous Media as a Function of Colloid Size, Fluid Velocity, and Grain Angularity. *Environ. Sci. Technol*, 40(24):7725-7731. Copyright 2006 American Chemical Society.

CHAPTER 3

MATERIALS AND METHODS

The investigation of the specific selected microorganisms and their transport mechanisms was investigated in a two-part study: laboratory scale column and field studies. The column study was initiated to study the transport of *Legionella* through environmental soil mediums under laboratory conditions.

Survey of Arizona Recharge Sites

To conduct a thorough field investigation of microbial transport through reclaimed water recharge sites, Arizona recharge basin sites were surveyed to accomplish the following objectives:

1. To assess the presence of *Legionella* in a Central Arizona recharge basin
2. To summarize soil profiles in order to select a soil to be used in the pilot-scale column study

In concordance with these objectives, the following Arizona recharge sites were evaluated as potential participants in this study:

- City of Chandler, Arizona
- City of Mesa, Arizona
- City of Tucson, Arizona

These recharge sites throughout the state of Arizona were evaluated for differing degrees of participation in the field and pilot-scale studies base on the following criteria:

- Location relative to ASU
- Provided feasible sampling logistics throughout the year
- Geological profile

- Hydrogeological site conditions
- Reclaimed water loads
- Groundwater infiltration rate
- Comparability to national average

The summarized surveyed resulting characteristics of these Arizona recharge basin sites are discussed in Chapter 4. The survey resulted in the selection of City of Mesa as the site which provided the most suitable recharge basin media soil for the pilot-scale column study investigation. The City of Chandler recharge site was selected for conducting the full-scale field study for sake of feasible monitoring well, groundwater sampling logistics.

Preparation of Microbial Stocks

E. coli and *Legionella* were targeted for microbial analysis. These two bacteria were used for the transport modeling experiments in the pilot-scale column study. The primary focus of this phase of the pilot-scale column study was to model the transport of *Legionella* through the soil media under recharge conditions.

***E. coli* culture.** *E. coli* (ATCC® 25922™) strain was obtained from the American Type Culture Collection (ATCC®, Rockville, Md.). *E. coli* stocks were inoculated in tryptic soy broth (TSB) (Difco, Detroit, Mich.) and incubated in an Orbital shaker (Brunswick, Enfield, CT) at 37°C at 150 rpm for 18 hours. Bacterial cells were harvested in a 15 mL conical tube by centrifugation at $1{,}000 \times g$ for 10 minutes. The supernatant was discarded and the pellet was re-suspended in 10 mL of reclaimed water from the column feed stream. *E. coli* stock was serially diluted and samples from the dilution were analyzed to determine the concentration of the stock solution. Bacterial cell

concentrations were determined by the spread plate method on tryptic soy agar (TSA) (Difco, Detroit, Mich.) and/or Brilliance™ *E. coli/Coliform* Selective Agar (Oxoid). The *E. coli* stock was then diluted and the appropriate amount of cells were added to the head water of the columns.

***Legionella* culture.** *Legionella* (ATCC® 33153™) strain was obtained from CDC. Filter-sterilized, L-Cysteine (4%) was aseptically added to BBL™ buffered charcoal- yeast extract agar (BCYE) medium (BD, Sparks, MD or Neogen Co., Lansing, MI). The agar was then modified by adding a solution of vancomycin, polymyxin B, and cycloheximide (GVPC) then incubated for 72 hours at 37°C (Dennis, 1988; Feeley et al., 1979). The GVPC modified BCYE agar plates were used for sub-culturing. A *Legionella* colony from the BCYE was transferred into 10 mL of buffered yeast extract broth (BYE) and incubated at 37°C at 150 rpm for approximately 44 hours. Bacterial cell concentrations were established with the spread plate method. After being serially diluted, and plated onto the GVPC modified BCYE agar, the agar plates were incubated at 37°C, and subsequent *Legionella* growth was recorded 5 days later.

Sample Processing and Analytical Methods

***E. coli* detection by Spread Plate Technique.** When evaluating for *E. coli* presence in the columns and the field samples were analyzed using spread plate technique Brilliance agar media.

***Preparation of Media for* E. coli.** Additionally, Brilliance agar media (OXOID CM1046), a selective media for *E. coli*, was prepared for the detection of *E. coli* and coliform organisms, represented purple colonies for *E. coli* and red colonies for other coliforms. The first step was to boil 375 mL of DI water in a beaker. The beaker was

placed on hot plate at 100 °C until boiled. The solution was continuously mixed using a magnetic bar (200 RPM or level 6-7). Next, 10.53 g of Brilliance agar base (OXOID CM1046) was weighed and added to the boiling water. After the media had boiled, the media was thoroughly mixed, stirred and cooled. Brilliance agar media were poured into petri dishes. Each petri dish was filled with 15 mL of Brilliance agar media. The plates were cooled for several hours to let media solidify and dry, and then plates were ready to be used immediately or stored at 4 °C for later use.

Die-off, Recovery Efficiency, and Pilot-scale Column Studies. All water samples were processed within 6 hours of collection. Initially, bacterial cell concentrations were determined by the spread plate method on the nonselective media, tryptic soy agar (TSA) (Difco, Detroit, Mich.). Given the high level of bacteria present in the columns, Brilliance™ *E. coli/Coliform* Selective Agar (Oxoid) was determined to be better suited for enumerating *E. coli* for the transport experiments.

***Legionella* detection by Spread Plate Technique.** When evaluating for *Legionella* presence in the columns and the field samples were analyzed using spread plate technique modified BCYE agar media.

***Preparation of Media for* Legionella.** BCYE agar media (Becton Dickinson 212327) was prepared for the detection and enumeration of *Legionella* bacteria. The first step was to boil 500 mL of nano-pure water in a beaker. The beaker was placed on a hot plate (Thermo Scientific Cimarec™ Digital Stirring Hotplates; USA or VWR® Hot Plate/Stirrer; Radnor, PA) at 100°C until boiled. The solution was continuously mixed using a magnetic bar (200 RPM or level 6-7). Next, 19.5 g of BCYE agar base was added to the boiling water. After media was boiled and evenly mixed, pH was measured

using a calibrated probe. An initial pH of 4.5-5 and was adjusted to 6.85-7 by adding a solid powder or liquid solution of potassium hydroxide (KOH). The BCYE agar media was then autoclaved for 15 minutes at 121°C with liquid setting. After the media was autoclaved, it was cooled to 50 °C prior to adding antibiotics and 0.4 g/L of L-cysteine. The following antibiotics were added: 2.5 mL of Vancomycin solution at 1 mg/mL, 8 mL of Polymixin B solution at 1 mg/mL, 8 mL of Cyclohexamide solution (anti-fungal) at 5 mg/mL, 1.5 g of Glycine powder. Working stocks of Vancomycin, Polymixin B, and Cyclohexamide solutions were stored at 4 °C. Polymixin B is light sensitive and was protected by covering with foil. Vancomycin powder was stored at -20 °C; Polymixin B powder was stored at room temperature; and Cyclohexamide was stored at 4 °C. After the antibiotics and L-cysteine were added, the media was thoroughly mixed and poured into petri dishes. Petri dishes were filled with 20 mL of media. The plates were cooled for several hours to let media solidify and dry, and then plates were ready to be used immediately or stored at 4 °C for later use.

Die-off, Recovery Efficiency, and Pilot-scale Column Studies. All samples were processed within 6 hours of collection. Samples were subject to acid and/or heat treatments before being spread on GVPC-BCYE plates. Plates were incubated at 37°C for up to 9 days and the resulting *L. pneumophila* colonies were counted. (Costa et al., 2005).

Recharge Field Studies. All samples were processed within 24 hours of collection.

Stability of Bacterial Indicators in Elution Buffers

***E. coli* Die-Off in Elution Buffers.** Approximately 1 mL of an *E. coli* overnight culture at 10^4 CFU/mL was added to 9 mL of Column A effluent water. The spiked

culture was stored at room temperature with in the same room where both Columns A and B were kept. After 6 hours, the approximate time required for the *E. coli* to be transported through the columns, the spiked effluent column was sampled to determine the stability or potential growth of *E. coli* in that time period.

Table 3.1 Die-off Curve for *E. coli* in Column Water Elution

Time (hours)	Concentration (Average CFU/mL)
0	6.75×10^3
6	1.04×10^4

Recharge Field Site Investigations

City of Chandler, Arizona. Soil Samples were collected from recharge basin #1.

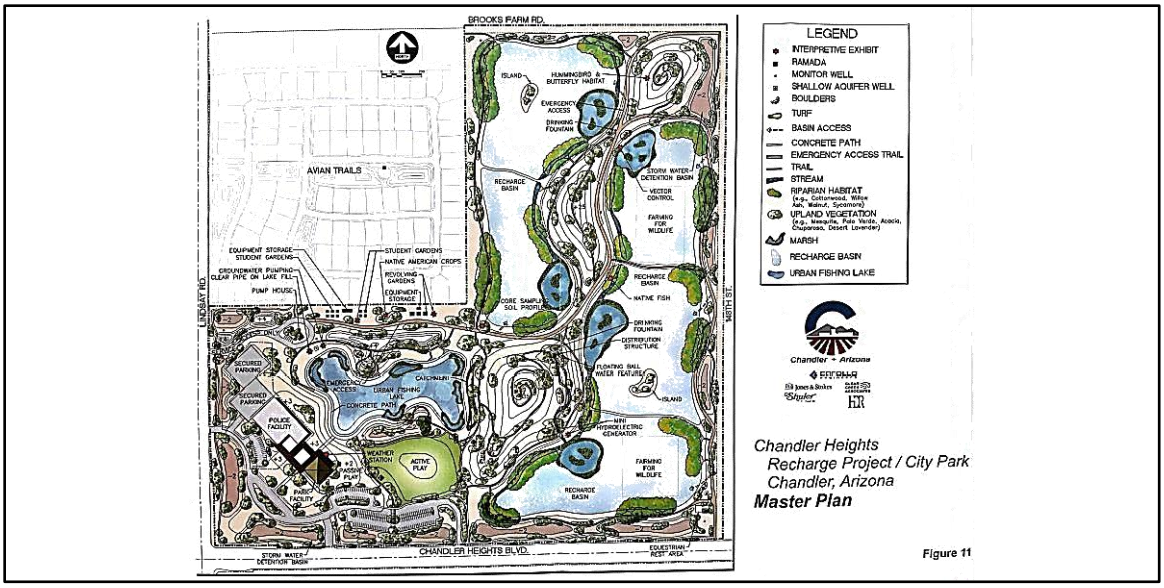

Figure 3.1 Chandler Heights Recharge Project/City Park Master Plan View

Source: Adapted from *Chandler Heights Recharge Project/City Park Design Handbook,* City of Chandler and Carollo Engineers.

City of Mesa, Arizona. Mesa Northwest Recharge soil samples were collected from basin #1.

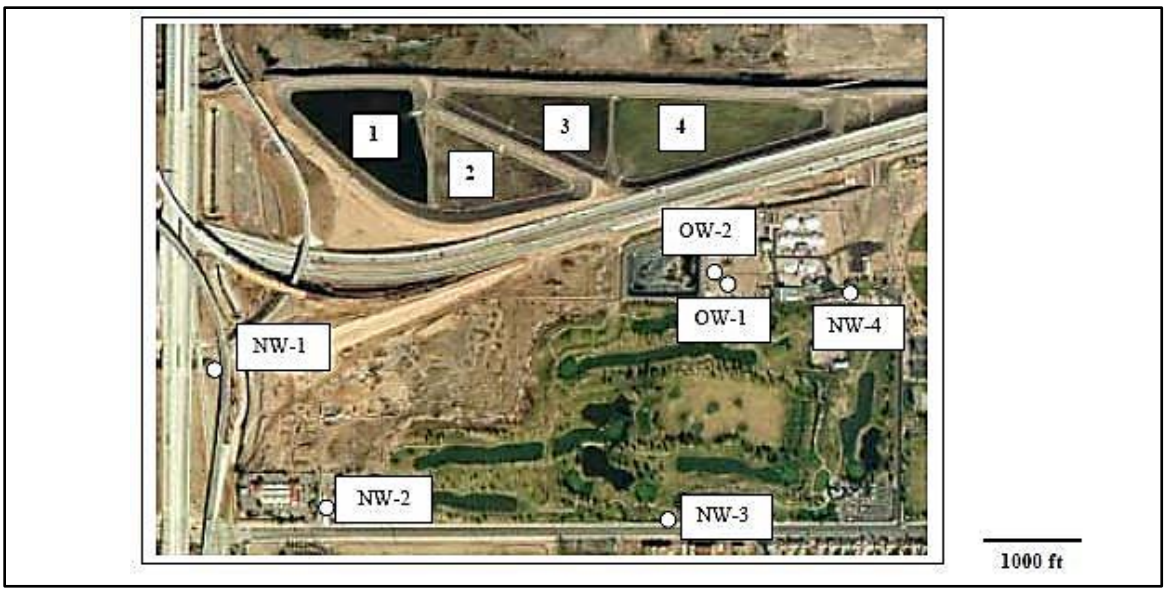

Figure 3.2 Mesa Northwest Recharge Site Map

Source: Adapted from *Soil-Aquifer Treat for Sustainable Water Reuse* by Fox et al.

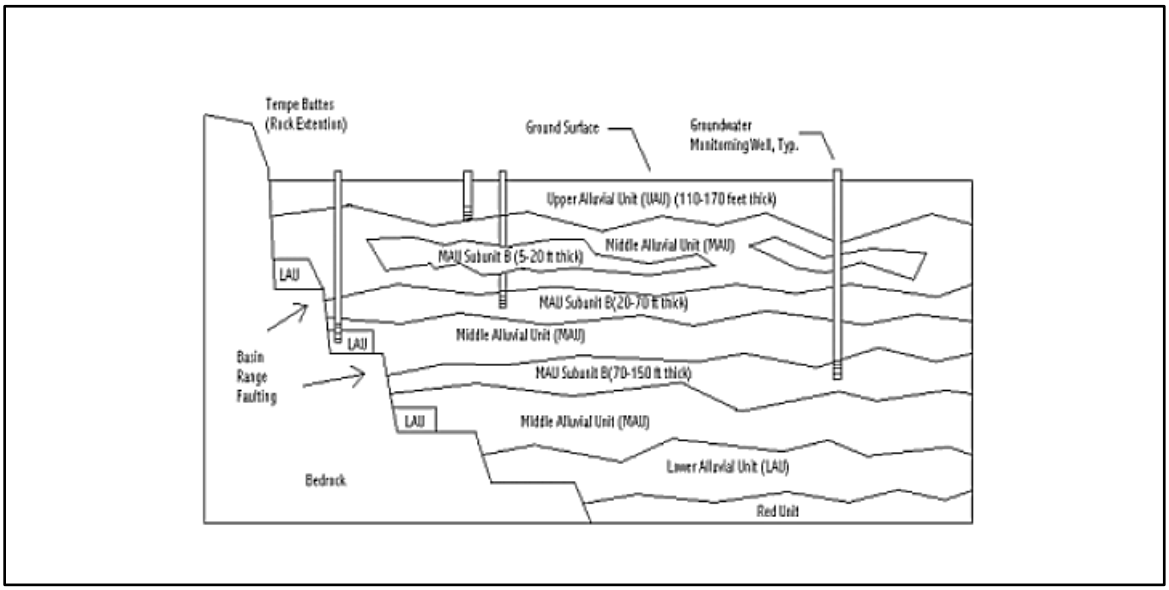

Figure 3.3 Mesa Recharge Site Geological Profile

Source: Adapted from *Soil-Aquifer Treat for Sustainable Water Reuse* by Fox et al.

City of Tucson, Arizona. Tucson Recharge Site background information.

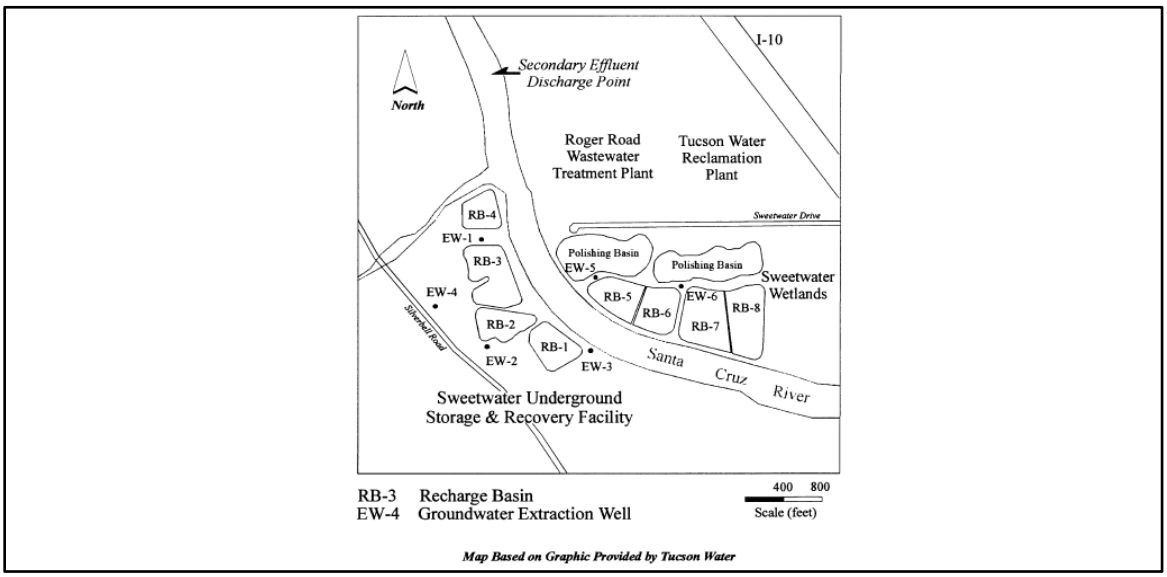

Figure 3.4 Tucson Sweetwater Recharge Site Map

Source: Adapted from *Soil-Aquifer Treat for Sustainable Water Reuse* by Fox et al.

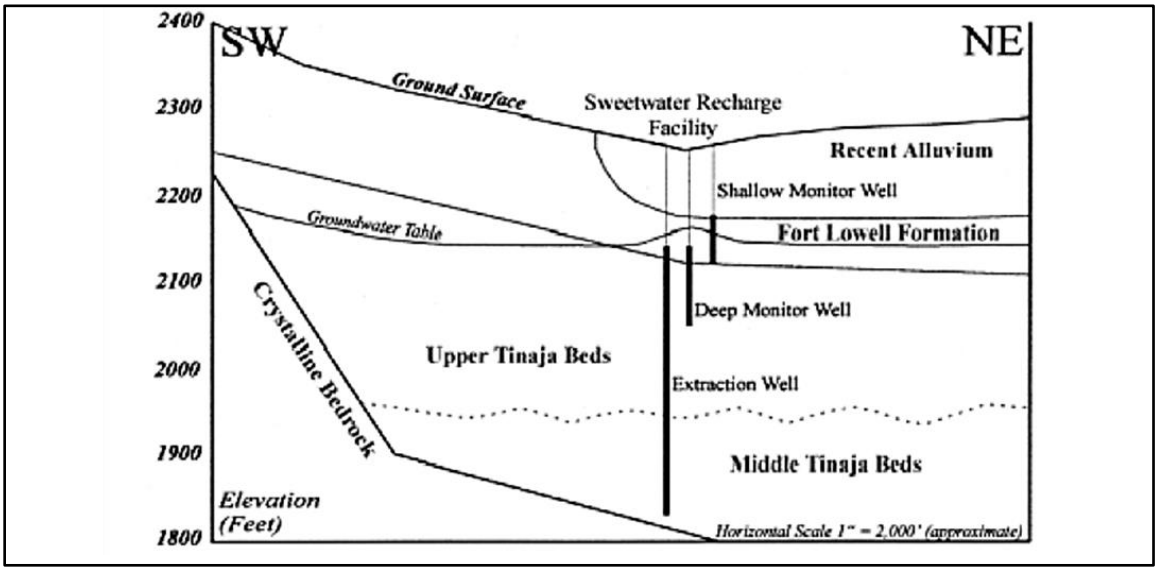

Figure 3.5 Tucson Sweetwater Recharge Site Geological Profile

Source: Adapted from *Soil-Aquifer Treat for Sustainable Water Reuse* by Fox et al.,

Pilot-Scale Investigations

In addition to the field study, a column study was initiated to evaluate the transport phenomena of the selected microorganisms under laboratory conditions. This column study was designed to perform the first domain of the study, focusing on evaluating the potential for *Legionella* to transport within reclaimed water passing through Arizona recharge basin media.

Column Soil Alternatives Analysis. Soil collected from the three recharge basin sites were sieved through the #4, #8, #16, #30, #50, #100, and #200 sieves.

The soil samples collected from the recharge basin #1 at the Northwest Water Reclamation Plant (Mesa, AZ) were used for the laboratory scale column studies.

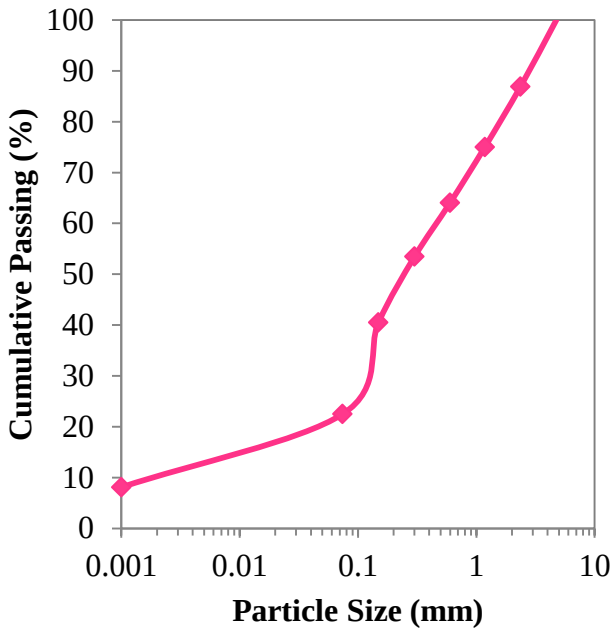

Figure 3.6 Particle Size Distribution of City of Chandler Recharge Basin Soil

Mesa Northwest Water Reclamation Plant, AZ, is a facility with shallow vadoze Zone (5-20 feet) and has multi-depth sampling capabilities below basins. Array of

shallow groundwater wells are located from 500 feet to greater than 10,000 feet from the recharge site. Using the USDA Classification system requirements, the evaluated recharge basin media fell under the Loamy Sand major textural class and subdivision.

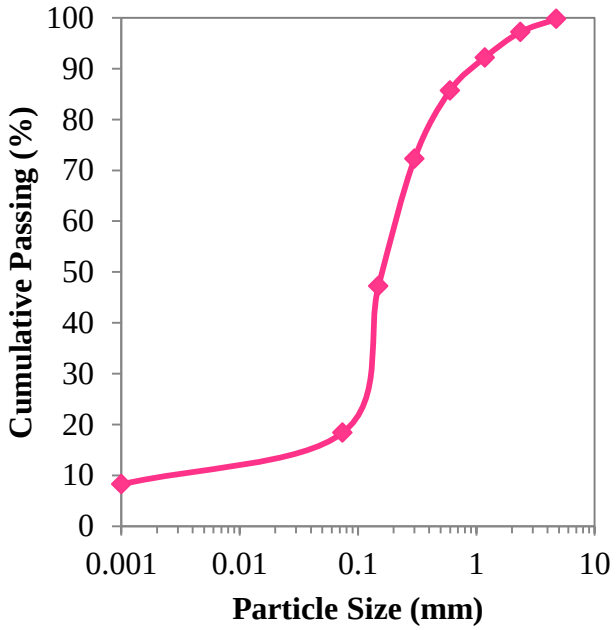

Figure 3.7 Particle Size Distribution of City of Mesa Recharge Basin Soil

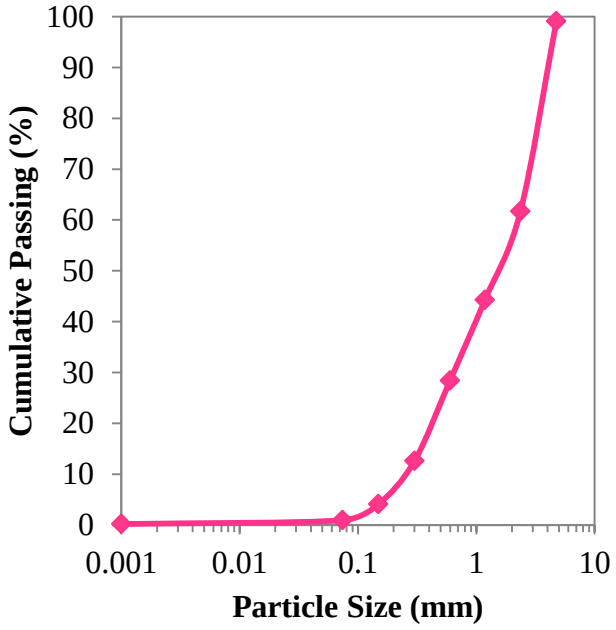

Figure 3.8 Particle Size Distribution of City of Tucson Recharge Basin Soil

The resulting particle size distribution curve was generated. Tucson soil showed highly absorbent properties. Soil sieving was performed before the soil from Tucson was properly dried. The moisture in the Tucson soil caused the soil to clump into sizes larger than the actual individual particle sizes. Therefore, the generated particle distribution curve, shown in Figure3.8, is not representative of the individual soil particles in the Tucson recharge basin, but instead, is representative of the clustered soil particles.

Soil Selection Summary. After initially evaluating the recharge basin media from the cities of Chandler, Mesa, and Tucson, the Mesa recharge basin media was selected for the column study application. The Mesa recharge basin media, loamy sand, was selected for its ability to perform as representative of average infiltration rates observed by recharge basins across Arizona. The Mesa soil was also designated as best suited for the column study application because of the workability the soil provided by exhibiting relatively low clay content.

Table 3.2 Soil Characterization

Soil Texture	Diameter Size (mm)	Percent of Media
Gravel	Above 2.00	3%
Very Coarse Sand	2.00-1.00	5%
Coarse Sand	1.00-0.50	12%
Medium Sand	0.50-0.25	20%
Fine Sand	0.25-0.10	35%
Very Fine Sand	0.10-0.05	10%
Silt	0.05-0.002	5%
Clay	Below 0.002	10%

The following diagram was used to visually identify the USDA Soil Classification. After the soils were classified, the diagram assisted in comparing the loamy sand classification to other soil classifications.

Major Textural Class - *Loamy Sand*:

1. Minimally, contain 75–85% sand
2. The percent of silt plus twice the percent of clay must not exceed 30%

Subdivision of Loamy Sand - *Loamy Sand:*

1. Contains 25% or more of very coarse, coarse, and medium sand
2. Contains less than 50% of fine and very fine sand

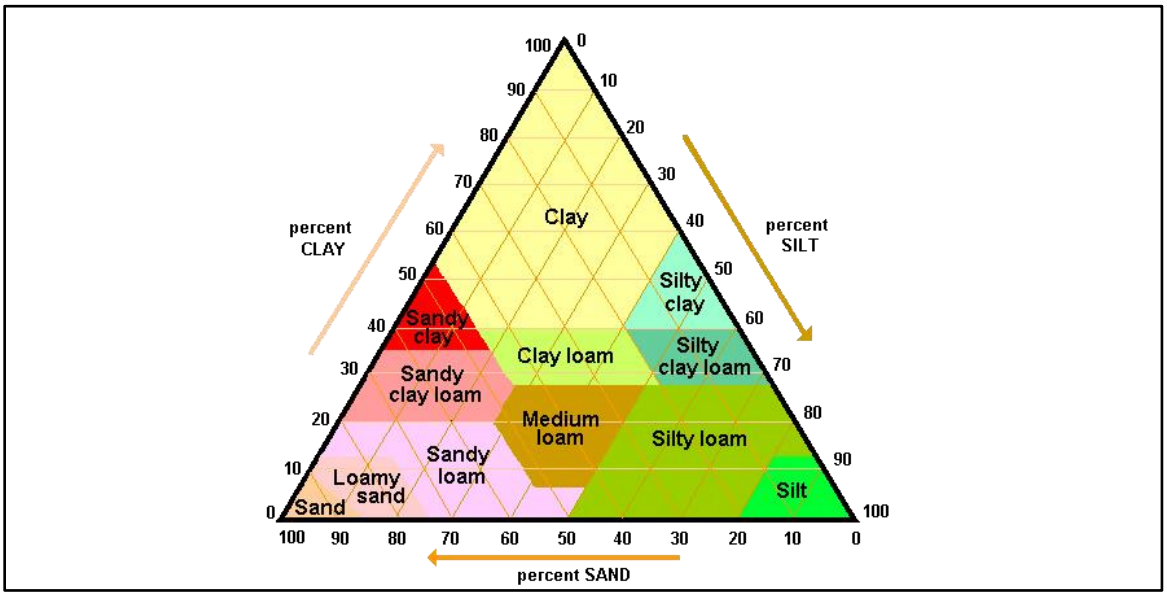

Figure 3.9 Soil Texture Triangle of the USDA System

Source: Adapted from *Tools for Ecological Land Use: Soil Texture* of the USEPA website, http://www.clu-in.org/ecotools/soilsci.cfm.

Column Construction and Acclimation. Two columns served to demonstrate the proof of concept study under conditions using two differing soil grain size distributions. The column media was distinctively selected to model the approximate average percolation rate observed by recharge basins across Arizona. The columns were packed under saturated conditions (wet packing) to a depth of four feet using a media of fine and coursed grain sands from an active Arizona recharge basin, located in Mesa. The use of active recharge basin media allowed the column study to provide understanding of the transport of *Legionella* under the influence of the native soil biological interactions.

An illustration of the laboratory-scale column utilized to evaluate the potential for *Legionella* to transport within reclaimed water passing through Arizona recharge basin media (Figure 3.10).

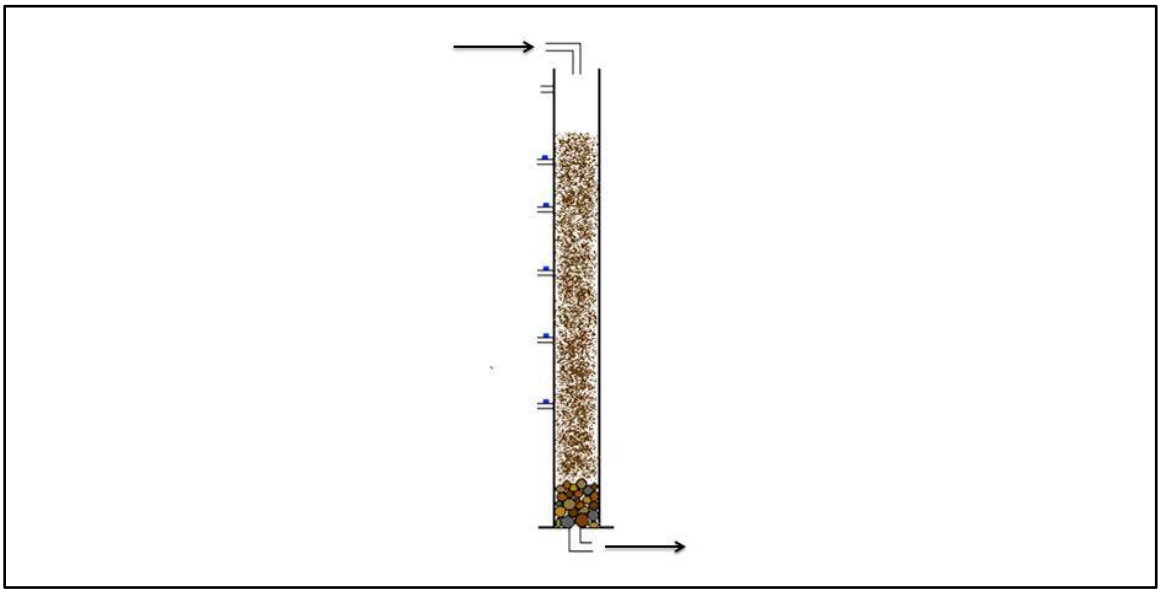

Figure 3.10 Column Apparatus Schematic

The two columns, each measured 6 feet tall with a 2.5 inch inner diameter. Sampling ports were drilled into the columns at soil depths of 6, 12, 24, 36, and 48 inches. These sampling port depths were converted to centimeters and are located at

15.24, 30.48, 60.96, 91.44, 121.92 cm. Transport study results use the rounded approximate values of 15, 30, 60, 92, 122 cm to represent Ports 1 through 5, respectively. The two separate columns were used to evaluate the transport of the selected microorganisms under two different recharge cases: reduced and high porous soil conditions. The grain size distribution of Column A differed from that of Column B by the removal of fines passing the #200 sieve.

Column A was packed using a wet packing method on March 5, 2014. Column B was packed, using the same technique, on March 28, 2014. After being packed, both columns were attached to an assembly which used a Masterflex L/S peristaltic pump to introduce influent water to a 1 L flask. The flask was balanced at the same water level as the head water by allowing a constant drainage to pass through the flask spillway. A tubing siphon system was used to maintain the water level between the flask and the column head water level.

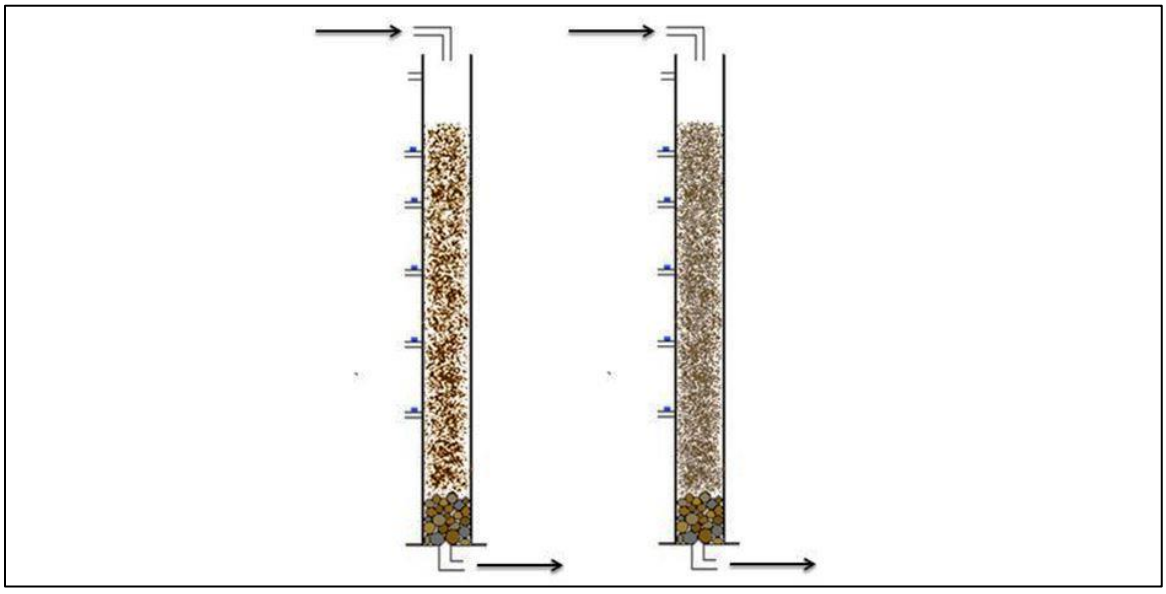

Figure 3.11 Column Schematic: Column A (left) and Column B (right)

By removing the soil fines, the percent which passed the #200 sieve, experiments in Column A demonstrated microbial transport under highly porous recharge basin soil conditions. Column B remained representative of the complete particle size distribution for the Mesa recharge basin soil, and the particles which passed the #200 sieve were left in place. Experiments conducted in Column B demonstrate microbial transport under standard conditions for USDA loamy sand recharge basin soils.

Figure 3.12 Column A (left) and B (right) Packed with Central AZ Recharge Media

Column Flow Rate. The flow rate in each column was regulated by a ball-valve located at the bottom of the column. The ball-valves required periodic adjustments to maintain the specified flow rate. In general, column flow rates were designated between the targeted range of about 1 to 4 m/day.

Table 3.3 Infiltration Rates Relative to Soil Textures

Soil Texture	Infiltration (in/hr)
Sand-coarse	1.00-8.00
Sand-very fine	0.50-3.10
Sandy loam	0.40-2.60
Loamy sand	1.65-5.00
Loam	0.08-1.00
Clay Loam	0.04-0.60
Clay	0.01-0.10

Column Feed Water Quality. The columns were initially feed with a constant stream of reclaimed water from the City of Chandler and were gradually replenished with tap water from the City of Tempe. Influent water quality data is summarized in Table 3.4.

Table 3.4 Column Feed Water Quality (Chandler Water Reclamation Facility)

	Units	Range	Average 2013	Average 2014
BOD	mg/L	2.5-180	6.0	2.5
COD	mg/L	2.0-78	30.6	17.6
TSS	mg/L	0.1-17	1.0	0.8
pH	-	7.5-8.2	8.0	7.7
Turbidity	NTU	0.3-4.3	0.5	0.6
NO_3	mg/L	0.7-4.7	2.5	2.3
Total Nitrogen	mg/L	2.5-9.0	5.2	5.5
Cl_2 Residual	mg/L	0.3-5.5	1.8	1.5

Column Transport Tests. Samples were collected from both column ports at 1, 2, 3, 6, 9, 30, 55, and 77 hours after the inoculant was added. Prior to each sample collected, a flush volume of 5 mL was discharged from each sample port.

Single Spiked Dose Experiments . The single spiked dose transport experiments were performed between March 3, 2014 and July 10, 2014. Broth cultures of *E. coli* or *Legionella* were grown for 18 hours, or for 3 days respectively. After an overnight *E. coli* culture had been given the appropriate time to grow, the *E. coli* culture was pelleted out of the broth solution using centrifugation. The pellet was resuspended and spiked into the head water of the columns. After a *Legionella* culture had been given 3 days to grow, the *Legionella* culture was approximated at about 3×10^8cfu/mL using spectroscopy. After being cultured, the *Legionella* culture was added to the head water of the columns. Approximately 10^6 to 10^9 cells of either *E. coli* or *Legionella* were added to the head water of the columns. The column sampling ports were sampled periodically for as few as 6 hours or for up to a few days.

Microbial Stocks for Column Studies and Analytical Methods. The selected microorganisms that were used in each of the column experiments and their feed concentrations is provided in Table 4.4 and 4.5. The preparation of the microbial stock solutions used in the column studies and analytical methods employed are previously described in Chapter 3. Detection limits of the analytical methods for the selected microorganisms are provided (Table 4.2).

Statistical Analysis

Microsoft Excel 2010 was used for statistical analysis and graphical presentation of data from the field and pilot-scale column studies.

CHAPTER 4

RESULTS AND DISCUSSION

Legionella survival data need to be considered in conjunction with the aquifer characteristics and specific water management practices employed in the valley. Nexus of these factors (proven to be important individually) highlights the need for studying the transport potential of *Legionella* mobility and survival under these conditions. This study investigated the occurrence of *Legionella* and the transport through aquifer under the conditions relevant to Central Arizona. Additionally, this study resulted in a better understanding of the role of groundwater as a potential natural reservoir of *Legionella* in Central Arizona. *Legionella* is among the waterborne pathogens prioritized at national level (EPA, 2009); and this study helped to align regional focus of water quality efforts with the national priorities.

Pilot-Scale Column Study

Detection Limits for Microbial Analysis during Column Studies. A 5 mL water sample was collected from each sampling ports along the length of the columns (Figure 3.11). Prior to sample collection, each sampling port was flushed by discarding the first 5 mL of water. Theoretical detection limits are not inclusive of die-off losses during sample processing. The theoretical detection limits for each analysis are summarized in Table 4.1.

Table 4.1 Detection Limits for the Microbial Analysis during Column Studies

Target Microorganism	Detection Limit	Analytical Method
E. coli	1 cell/mL	Spread Plate Method
Legionella	1 cell/mL	Spread Plate Method

Flow Rate Progression throughout Column Study. The flow rate in each column was regulated by a ball-valve located at the bottom of the column. The ball-valves required periodic adjustments to maintain the specified flow rate.

Table 4.2 Flow Rate of Columns A and B

		Flow Rate (m/day)	
	Date	A	B
	3/12/2014	3.6	-
	3/17/2014	0.9	-
adjusted	3/19/2014	1.5	-
adjusted	3/25/2014	1.5	-
	3/28/2014	1.2	-
	3/30/2014	2.0	-
	4/8/2014	1.8	1.5
adjusted	4/8/2014	1.7	1.7
	4/9/2014	1.4	1.4
	4/9/2014	1.1	0.9
adjusted	4/10/2014	2.0	2.0
	4/11/2014	1.7	2.1
	4/15/2014	1.4	1.8
	5/1/2014	1.0	1.2
	5/12/2014	0.8	1.1
adjusted	7/10/2014	2.8	1.8
adjusted	7/14/2014	2.8	2.8

Reproducibility of the Column Experiment Results. The specific column experimental details are outlined in Tables 4.3. The details for the *E. coli* and *Legionella* transport experiments are shown in Table 4.4 and Table 4.5, respectively.

Table 4.3 Experimental Conditions for Column Studies

Parameter	Column ID A	Column ID B
Soil Distribution	Particles > 200 Sieve	Entire Particle Distribution
Flow Rate (m/day)	0.8 to 3.6	0.9 to 2.8
Acclimation (days)	14	14
TSS (mg/L)	1.0	1.0
Turbidity (NTU)	0.6	0.6
Column Diameter (cm)	6.35	6.35
Soil Depth (cm)	122	122
Head Water (mL)	500	500
Porosity	0.56	0.50
One Pore Volume (PV) (mL)	2145	1930
Injection (PV)	0.25	0.25
Elution (PV)	> 3	> 3
E. coli Injected (CFU/mL)	10^3 to 10^7	10^5 to 10^7
Legionella Injected (CFU/mL)	10^4 to 10^5	10^4 to 10^5

Single Spiked Dose *E. coli* Experiments

The flow rate ranged from 1.2 to 3.6 m/day for *E.coli* transport experiments in Column A. The spiked head water concentration for the *E. coli* ranged from 5.7×10^3 to 4.5×10^7 CFU/mL. *E. coli* transported through Column B was evaluated for a flow rate of 2.8 m/day. The spiked head water concentration for the *E. coli* transport experiments through Column B ranged from 3.8×10^5 to 3.0×10^7CFU/mL. A description of each of the *E. coli* Transport Tests, including the test initiated, column flow rate, and the head water spiked concentration is summarized in Table 4.4.

Table 4.4 Single Spiked Dose *E. coli* Experimental Plan

Test	Column	Date Initiated	Flow rate (m/day)	Spiked Concentration in the Head Water (*E. coli* CFU/mL)
1	A	03-12-14	3.6	1.6×10^6
2	A	03-25-14	1.5	n/a
3	A	03-28-14	1.2	6.8×10^3
4	A	03-30-14	2.0	5.7×10^3
5	A	07-14-14	2.8	3.8×10^5
6	A	08-06-14	2.8	4.5×10^7
7	B	07-14-14	2.8	3.8×10^5
8	B	07-28-14	2.8	3.3×10^6
9	B	08-06-14	2.8	3.0×10^7

n/a: not available

Initial breakthrough and corresponding sampling times and pore volume fractions are summarized for the Port 1 and Port 5 column sampling ports, located at 15 cm and 122 cm deep in the recharge basin media. *E. coli* was transported to Port 1 at the initial sampling time for each test. These sampling times occurred between 0.07 and 0.22 pore volumes for Column A and between 0.1 and 0.19 pore volumes for Column B. Given the background of *E. coli* (usually between 0 and 30 CFU/mL), the breakthrough in Port 1 was detected by the beginning of an increasing trend of *E. coli.*

E. coli was shown to initially breakthrough Port 5 (122 cm) at sampling times between 3 and 24 hours for Column A and between 3 and 6 hours for Column B. These sampling times occurred at 0.37 and 5.31 pore volumes for Column A and between 0.57 and 1.15 pore volumes for Column B.

Of these, the most information can be gained by evaluating the breakthrough pore volumes observed during Tests 3 and 4. During these tests, *E. coli* was consistently monitored in Port 5 and it was not until the 0.52 and 0.37 pore volumes that *E. coli* breakthrough was observed.

Table 4.5 Single Spiked Dose *E. coli* Breakthrough Summary

Test	Column	Flow rate (m/day)	Port 1 (15 cm) (hours)	Port 1 (15 cm) (PV)	Port 5 (122 cm) (hours)	Port 5 (122 cm) (PV)
1	A	3.6	1*	0.22	24*	5.31
2	A	1.5	n/a	n/a	n/a	n/a
3	A	1.2	1*	0.07	7	0.52
4	A	2.0	1*	0.12	3	0.37
5	A	2.8	1*	0.17	6*	1.03
6	A	2.8	0.5*	0.08	3*	0.52
7	B	2.8	1*	0.19	6*	1.15
8	B	2.8	1*	0.19	6*	1.15
9	B	2.8	0.5*	0.10	3*	0.57

PV: pore volume fraction
n/a: not available
*The first sample collected

Single Spiked Dose *E. coli* Experiments in Column A

***E. coli* Transport Test 1.** Figure 4.1 shows the sampled concentrations, recorded as C/Co, as a function of the time after the column head water was spiked. The column flow rate was set to 3.6 m/day when the *E. coli* culture was spiked into the head water of Column A. Samples were collected from Ports 1 through 5 at 1, 2, 3, and 24 hours after the column was spiked. Samples were analyzed using culture based technique and data are presented in Figure 4.1

The figure shows that Port 1 and Port 2 were monitored for 3 hours and then Ports 1 through 5 were sampled 24 hours after spiking the column head water. The concentration of *E. coli* was initially highest in the influent but after 24 hours passed, the influent concentration was less than the concentration all throughout the column. By the column had run for one hour, Port 1 reach its breakthrough peak concentration. The concentration in Port 1 continued to decline for all the future samples. The concentration in Port 2 increase for the first 3 hours after the column was spiked. However, by 24 hours after spiking the column, the concentration decreased. After 24 hours, the concentration in throughout the column was highest in the top ports and lower in the bottom ports.

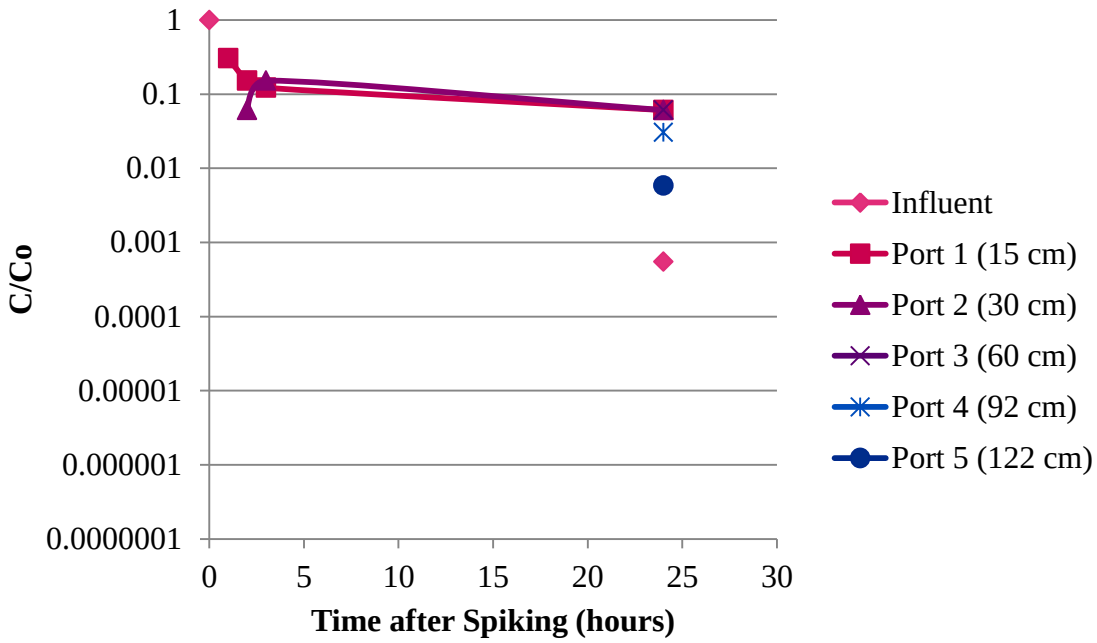

Figure 4.1 *E. coli* Transport through Recharge Media- Column A Test 1 (Time)

Figure 4.2 displays the same concentration (C/Co) trends; however, this graph is shown as a function of pore volumes after the column was spiked. After 3 pore volumes, the concentration in throughout the column was highest in the top ports and lower in the bottom ports.

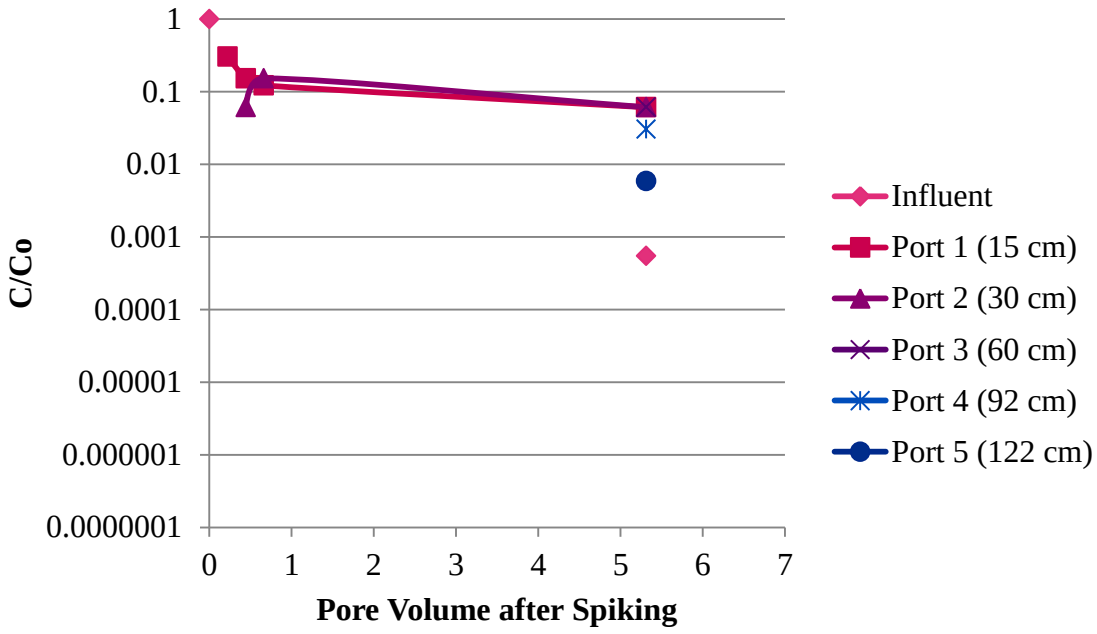

Figure 4.2 *E. coli* Transport through Recharge Media– Column A Test 1 (PV)

The following picture (Figure 4.3) shows the observed concentration of *E. coli* throughout column. From left to right, the sample plates are displayed as Influent, Port 1, 2, 3, 4, and 5 for the 24 hour of sampling after the column head water was spiked.

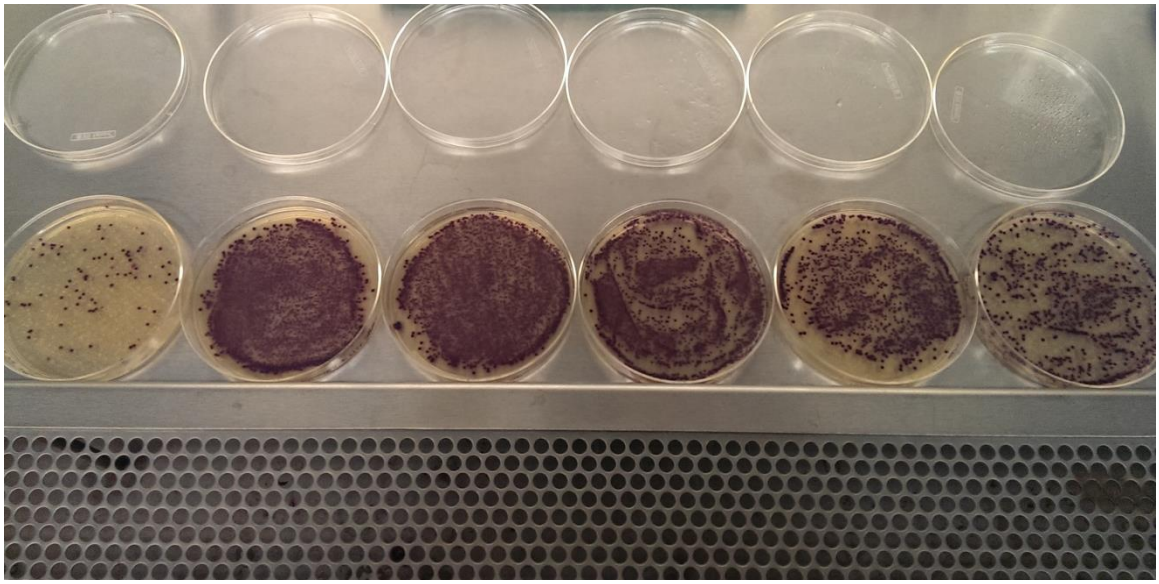

Figure 4.3 *E. coli* Transport– Column A Test 1 (24 Hours after Spiking) Influent, Port 1 (15 cm), Port 2 (30 cm), Port 3 (60 cm), Port 4 (92 cm), and Port 5 (122 cm)

The concentration in Ports 1, 2, and 3 are too numerous to count, however they show a decreasing concentration as from top to bottom of the column.

***E. coli* Transport Test 2.** Ten µL of the resuspended *E. coli* culture was spiked into the head water of Column A. The column flow rate was set to 1.5 m/day. All samples were analyzed using culture based technique.

Unfortunately, this column did not yield transport data, potentially due to the spiked volume of 10 µL. With such a small spiked volume, there is a chance that *E. coli* culture was not mixed well, causing an even lower spiked concentration than originally calculated. Additionally, the column water may have possessed a residual of chlorine because none of the *E. coli* survived in the spiked head water.

***E. coli* Transport Test 3.** For this test, 1 mL of the proper dilution was spiked to the head water of Column A. The column flow rate was set to 1.2 m/day. Samples were collected from Ports 1 through 5 at 1, 2, 3, 5 and 7 hours after the column was spiked. Samples were analyzed using culture based technique and data are presented in Figure 4.4 and 4.5.

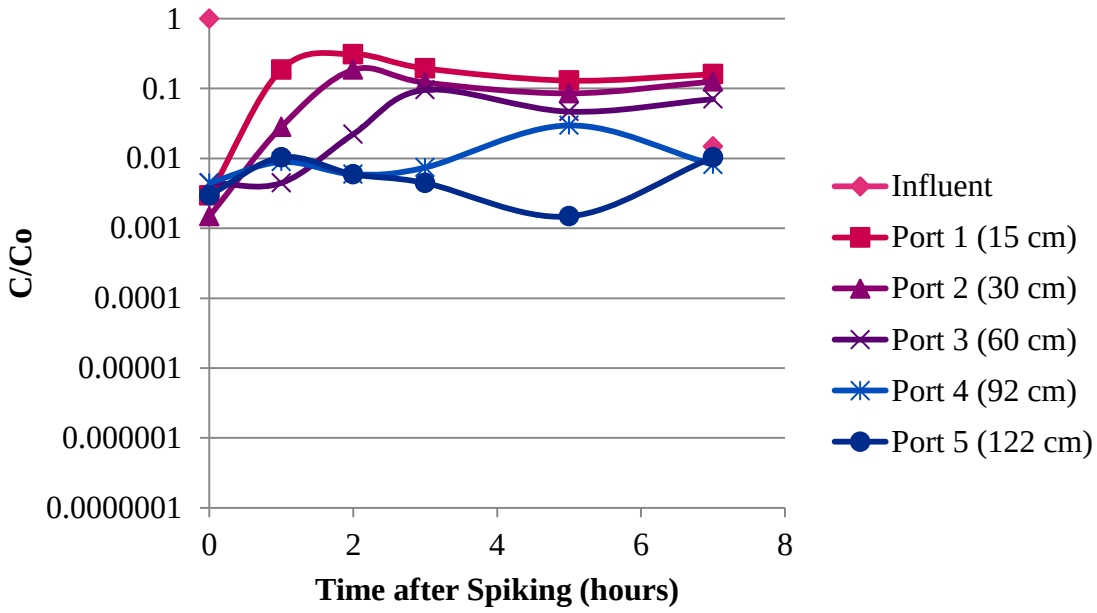

Figure 4.4 *E. coli* Transport through Recharge Media- Column A Test 3 (Time)

Test 3 showed the concentration at Port 1 and 2 peak between hours 1 and 2, and between hours 2 and 3, respectively. The concentration at Port 5 began to increase after hour 5.

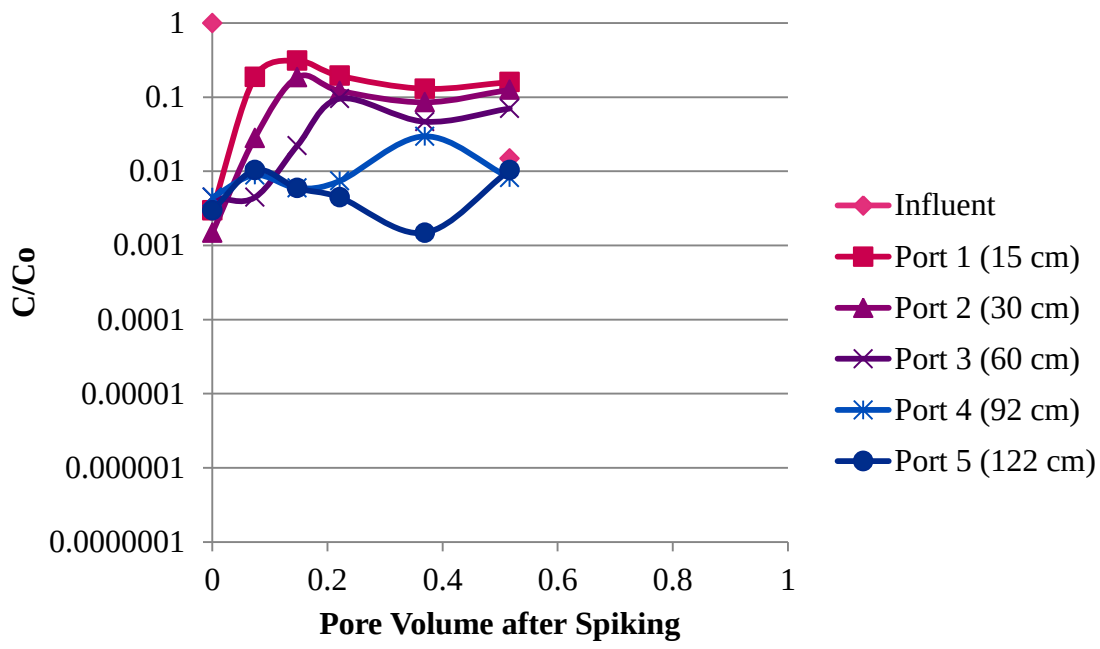

Figure 4.5 *E. coli* Transport through Recharge Media- Column A Test 3 (PV)

***E. coli* Transport Test 4.** One mL of the appropriate dilution was added to the head water of Column A. The column flow rate was set to 2.0 m/day. Samples were collected from Ports 1 through 5 at 1, 2, 3, 5 and 7 hours after the column was spiked. Samples were analyzed using culture based technique and data are presented in Figure 4.6 and 4.7.

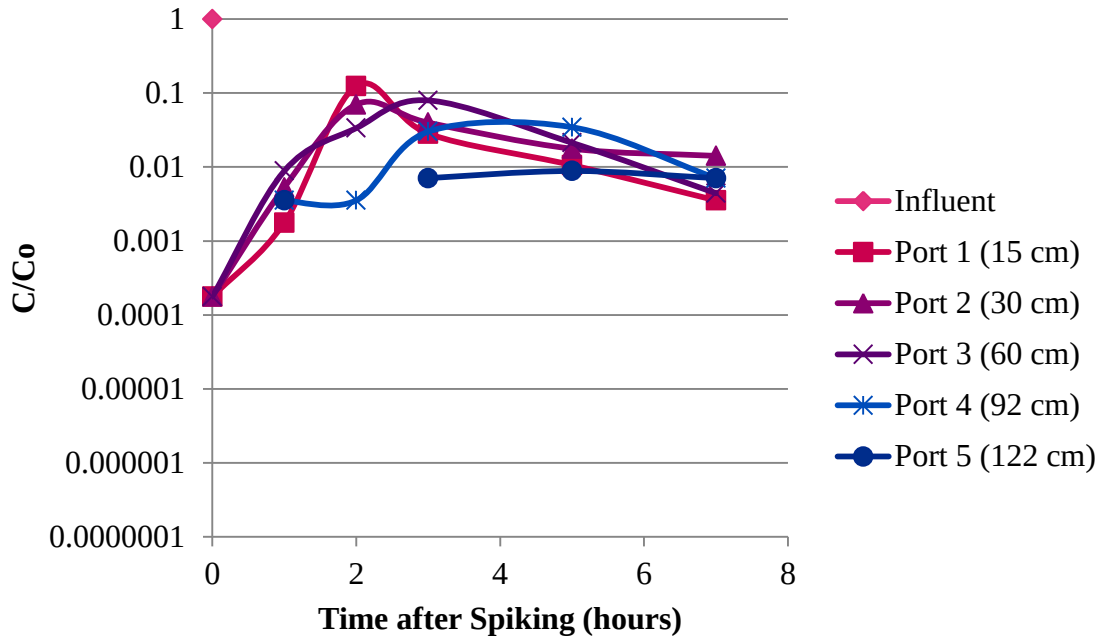

Figure 4.6 *E. coli* Transport through Recharge Media- Column A Test 4 (Time)

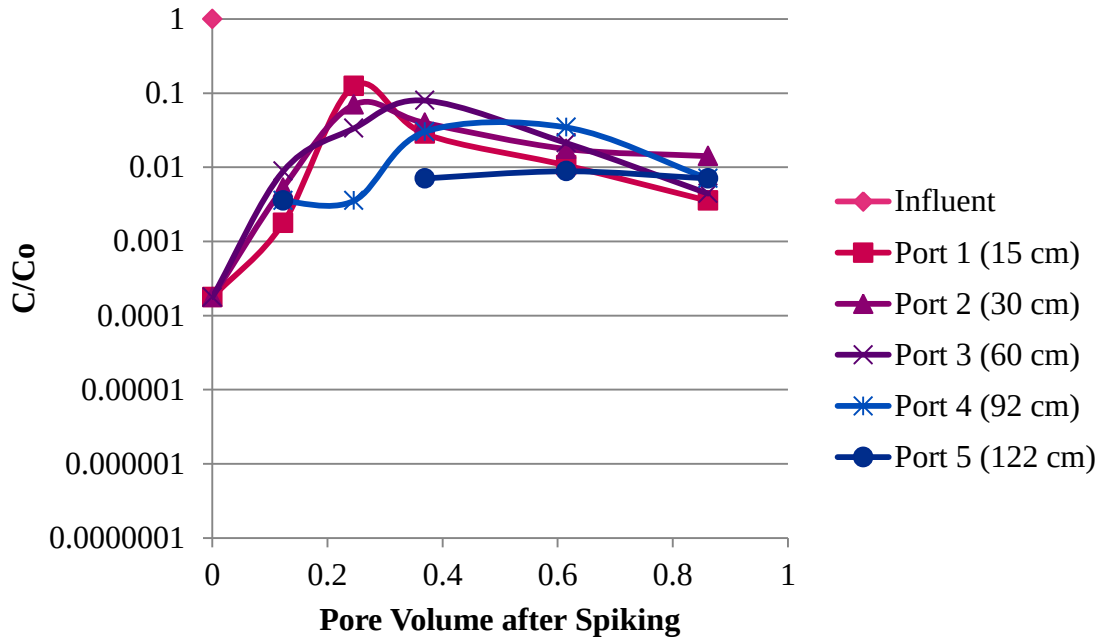

Figure 4.7 *E. coli* Transport through Recharge Media- Column A Test 4 (PV)

***E. coli* Transport Test 5.** Five mL of the *E. coli* culture was spiked into the head water of Column A. The column flow rate was set to 2.8 m/day. Samples were collected from Ports 1, 2 and 5 at 1, 2, 3, 6 and 30 hours after the culture was spiked. All samples were analyzed using culture based technique and data are presented in Figure 4.8 and 4.9.

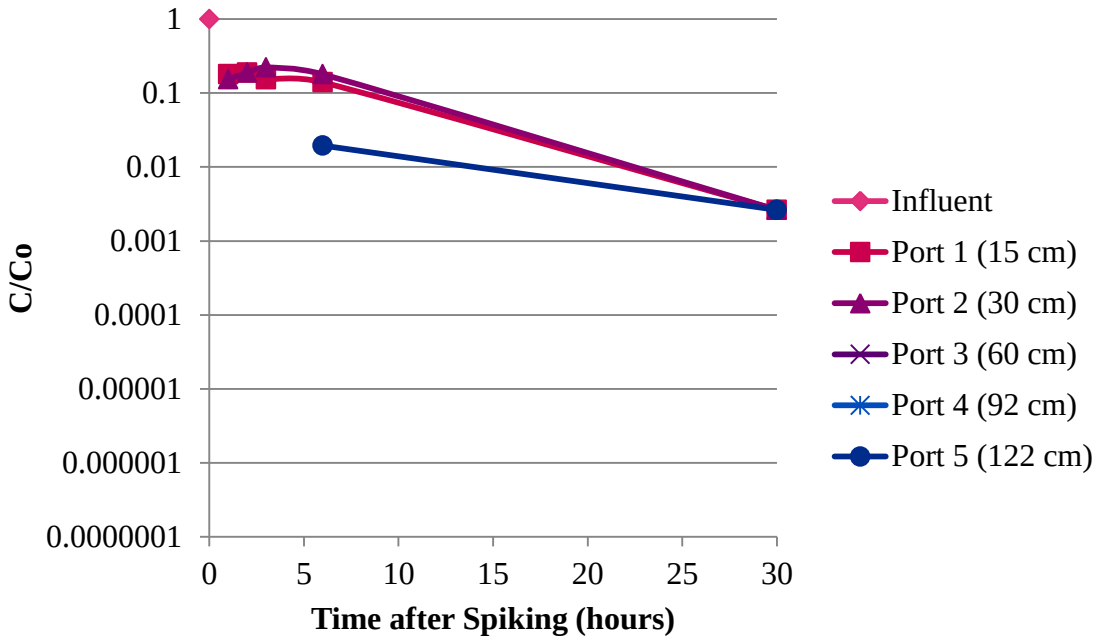

Figure 4.8 *E. coli* Transport through Recharge Media- Column A Test 5 (Time)

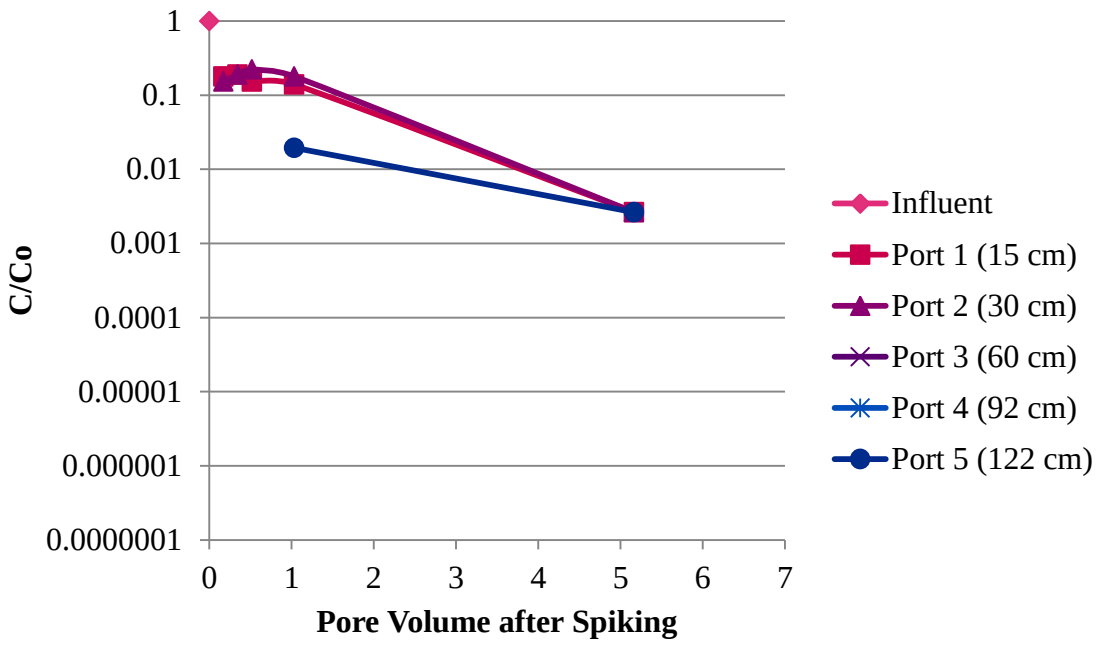

Figure 4.9 *E. coli* Transport through Recharge Media- Column A Test 5 (PV)

***E. coli* Transport Test 6.** Five mL of the *E. coli* culture was spiked into the head water of Column A. The column flow rate was set to 2.8 m/day. Samples were collected from Ports 1, 2 and 5 at 1, 2, 3, 6, and 24 hours after the. All samples were analyzed using culture based technique and data are presented in Figure 4.10 and 4.11.

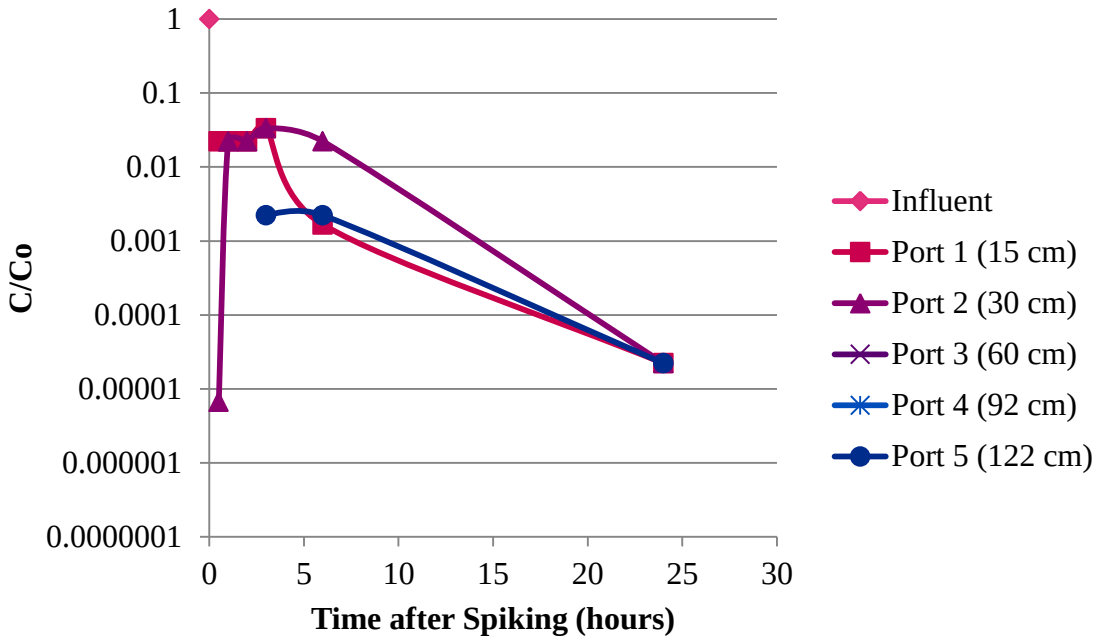

Figure 4.10 *E. coli* Transport through Recharge Media- Column A Test 6 (Time)

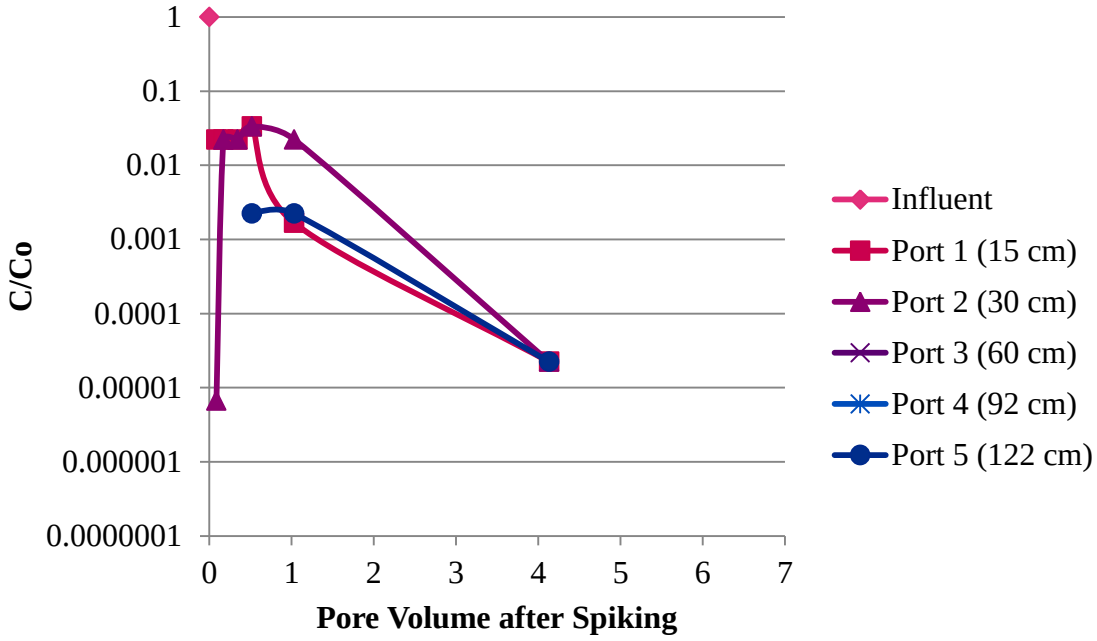

Figure 4.11 *E. coli* Transport through Recharge Media- Column A Test 6 (PV)

Single Spiked Dose *E. coli* Experiments in Column B

***E. coli* Transport Test 7.** One mL of the dilution was added to the head water of Column B. The column flow rate was set to 2.8 m/day. Samples were collected from Ports 1, 2 and 5 at 1, 2, 3, 6 and 30 hours after the culture was spiked. All samples were analyzed using culture based technique and data are presented in Figure 4.12 and 4.13.

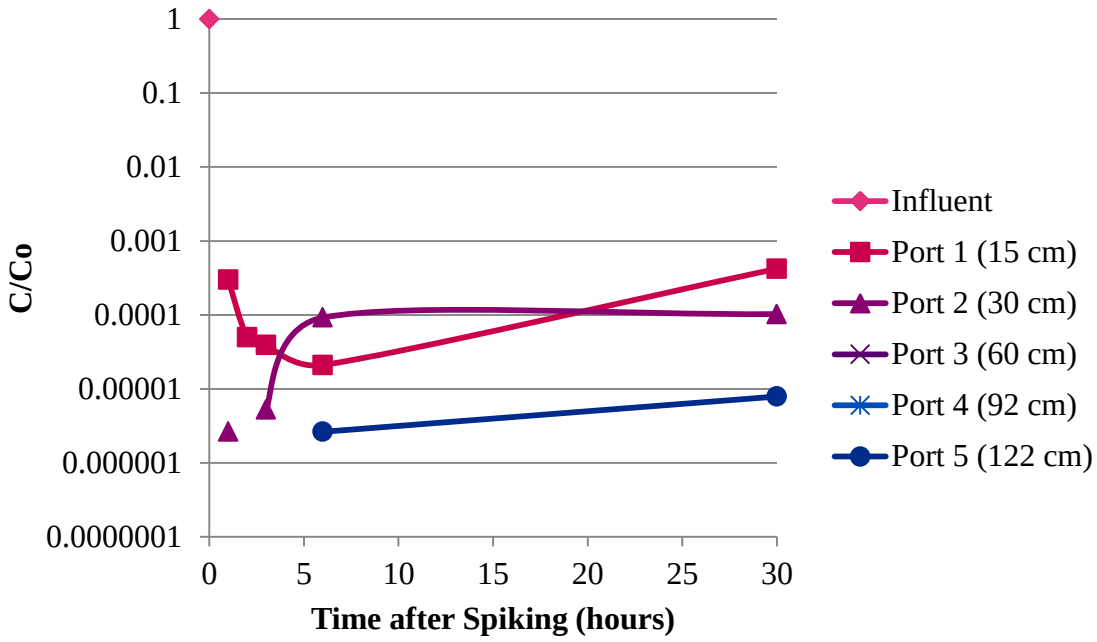

Figure 4.12 *E. coli* Transport through Recharge Media- Column B Test 7 (Time)

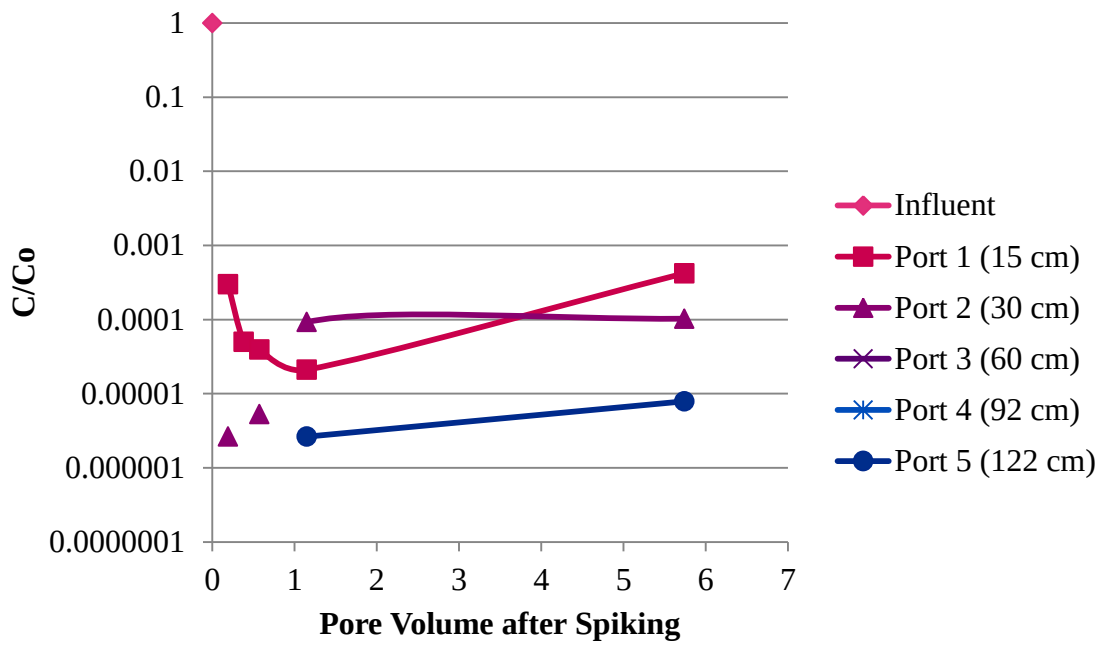

Figure 4.13 *E. coli* Transport through Recharge Media- Column B Test 7 (PV)

***E. coli* Transport Test 8.** Five mL of the culture was added to the head water of Column B. The column flow rate was set to 2.8 m/day. Samples were collected from Ports 1, 2 and 5 at 1, 2, 3, and 6 hours after the culture was spiked. All samples were analyzed using culture based technique and data are presented in Figure 4.14 and 4.15.

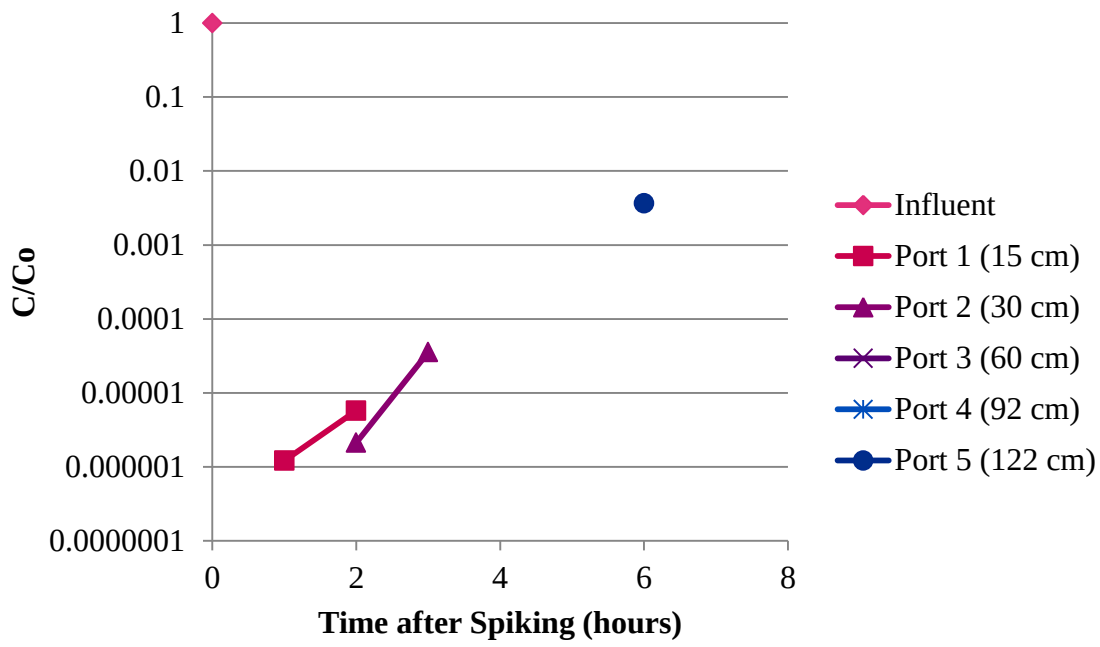

Figure 4.14 *E. coli* Transport through Recharge Media- Column B Test 8 (Time)

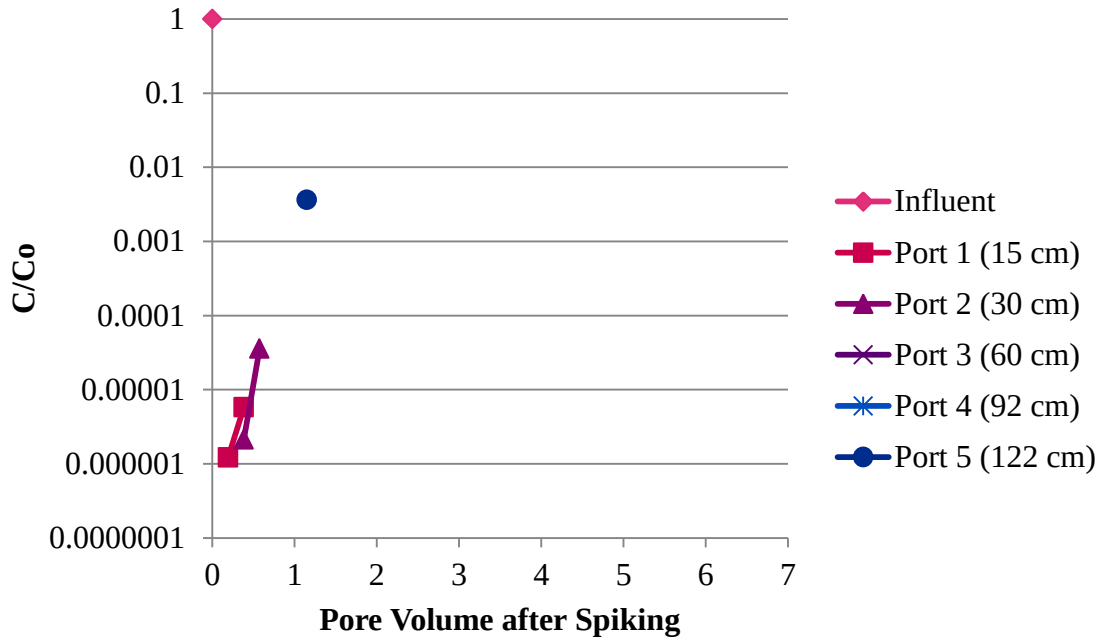

Figure 4.15 *E. coli* Transport through Recharge Media- Column B Test 8 (PV)

***E. coli* Transport Test 9.** Five mL of the culture was added to the head water of Column B. The column flow rate was set to 2.8 m/day. Samples were collected from Ports 1, 2 and 5 at 1, 2, 3, 6, and 24 hours after the culture was spiked. All samples were analyzed using culture based technique and data are presented in Figure 4.16 and 4.17.

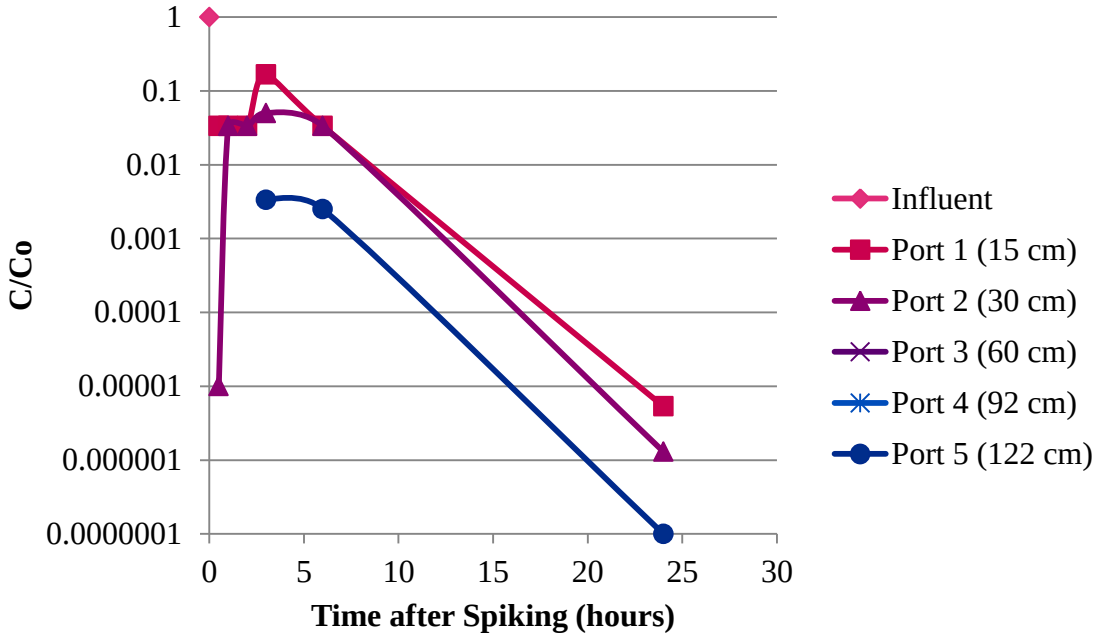

Figure 4.16 *E. coli* Transport through Recharge Media- Column B Test 9 (Time)

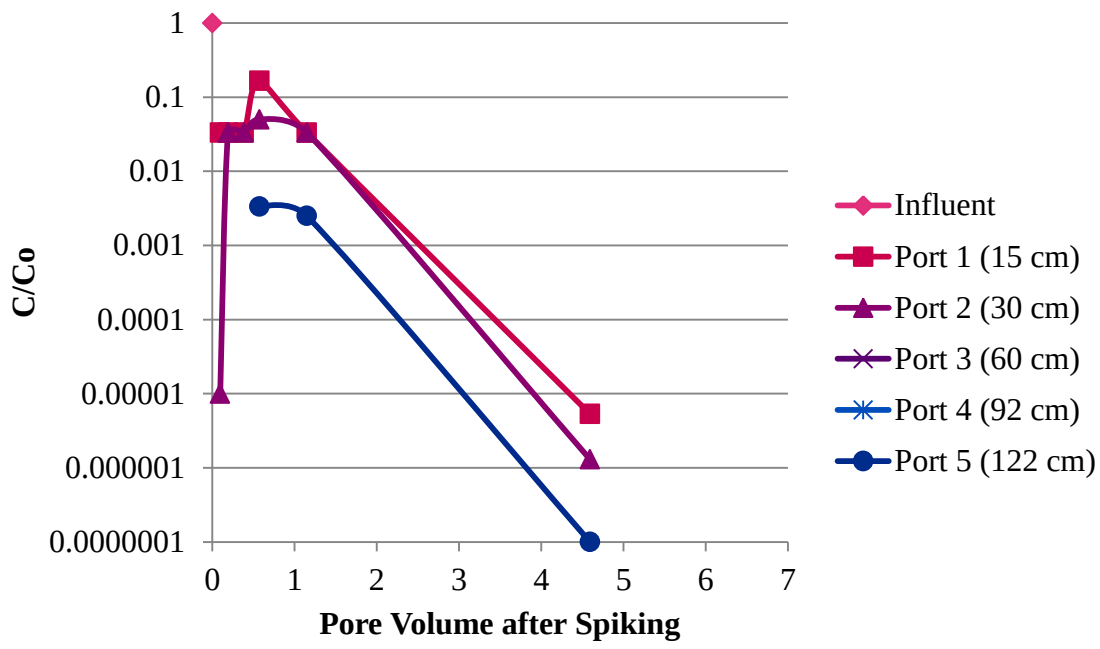

Figure 4.17 *E. coli* Transport through Recharge Media- Column B Test 9 (PV)

Single Spiked Dose *Legionella* Experiments

Legionella transported through Column A was evaluated for a flow rate ranging from 1.0 m/day to 2.75 m/day. The spiked head water concentration for the *Legionella* transported through Column A ranged from5×10^4 CFU/mL to 2.7×10^5CFU/mL. *Legionella* transported through Column B was evaluated for a flow rate ranging from 1.2 m/day to 1.75 m/day. The spiked head water concentration for the *Legionella* transported through Column B ranged from1.8×10^5CFU/mL to2.7×10^5CFU/mL. A description of each of the *Legionella* Transport Tests, including the test initiated, column flow rate, and the head water spiked concentration is listed (Table 4.6).

Table 4.6 Single Spiked Dose *Legionella* Experimental Plan

Test	Column	Date Initiated	Flow rate (m/day)	Spiked Concentration in the Head Water (*Legionella* CFU/mL)
10	A	03-19-14	1.5	5×10^4
11	A	04-09-14	1.5	n/a
12	A	05-01-14	1.0	2.7×10^5
13	A	07-10-14	2.75	2.7×10^5
14	B	04-09-14	1.5	n/a
15	B	05-01-14	1.2	1.8×10^5
16	B	07-10-14	1.75	2.7×10^5

n/a: not determined

Initial breakthrough sampling times and pore volume fractions are summarized for the Port 1 and Port 5 column sampling ports, located at 15 cm and 122 cm deep in the recharge basin media. *Legionella* was transported to Port 1 at the initial sampling time for each test in Column A. Initial sampling occurred between 0.06 and 0.17 pore volumes. This was also the case for *E. coli.* However, in Column B, *Legionella* was not observed to breakthrough Port 1 until 3 hours after spiking the column, between 0.25 and 0.36 pore volumes.

Legionella was shown to initially breakthrough Port 5 (122 cm) at sampling times between 24 and 48 hours for Column A and between 24 and 30 hours for Column B. These sampling times occurred at 4.06 and 4.43 pore volumes for Column A and between 2.46 and 2.87 pore volumes for Column B.

Table 4.7 Single Spiked Dose *Legionella* Breakthrough Summary

Test	Column	Flow rate (m/day)	Port 1 (15 cm) (hours)	Port 1 (15 cm) (PV)	Port 5 (122 cm) (hours)	Port 5 (122 cm) (PV)
10	A	1.5	1*	0.09	48	4.43
11	A	1.5	n/a	n/a	n/a	n/a
12	A	1.0	1*	0.06	n/a	n/a
13	A	2.75	1*	0.17	24	4.06
14	B	1.5	n/a	n/a	n/a	n/a
15	B	1.2	3	0.25	30	2.46
16	B	1.75	3	0.36	24	2.87

PV: pore volume fraction
n/a: not available
*The first sample collected

Single Spiked Dose *Legionella* Experiments in Column A

***Legionella* Transport Test 10.** Figure 4.18 shows the sampled concentrations, recorded as C/Co, as a function of the time after the column head water was spiked. Five mL of the culture was added to the head water of Column A. The column flow rate was set to 1.5 m/day. Samples were collected from Ports 1 through 5 at 1, 2, 3, 4, 5, 24, and 48 hours after the culture was spiked. All samples were analyzed using culture based technique and data are presented in Figures 4.18 and 4.19.

As seen in the graph, the concentration in Port 1 declines from the first sample through the 48 hour sample. This observation is probably the result of rapid breakthrough. Suction caused by opening the other sampling ports may have caused this breakthrough trend.

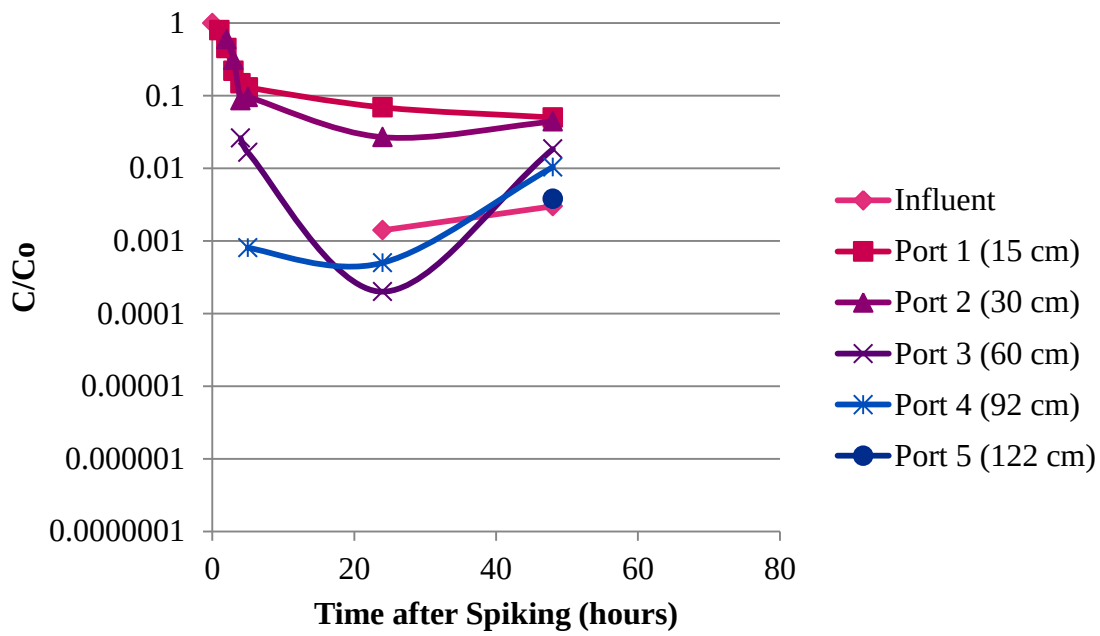

Figure 4.18 *Legionella* Transport through Media- Column A Test 10 (Time)

Figure 4.19 displays the same concentration (C/Co) trends; however, this graph is shown as a function of pore volumes after the column was spiked. After 3 pore volumes, the concentration in throughout the column was highest in the top ports and lower in the bottom ports.

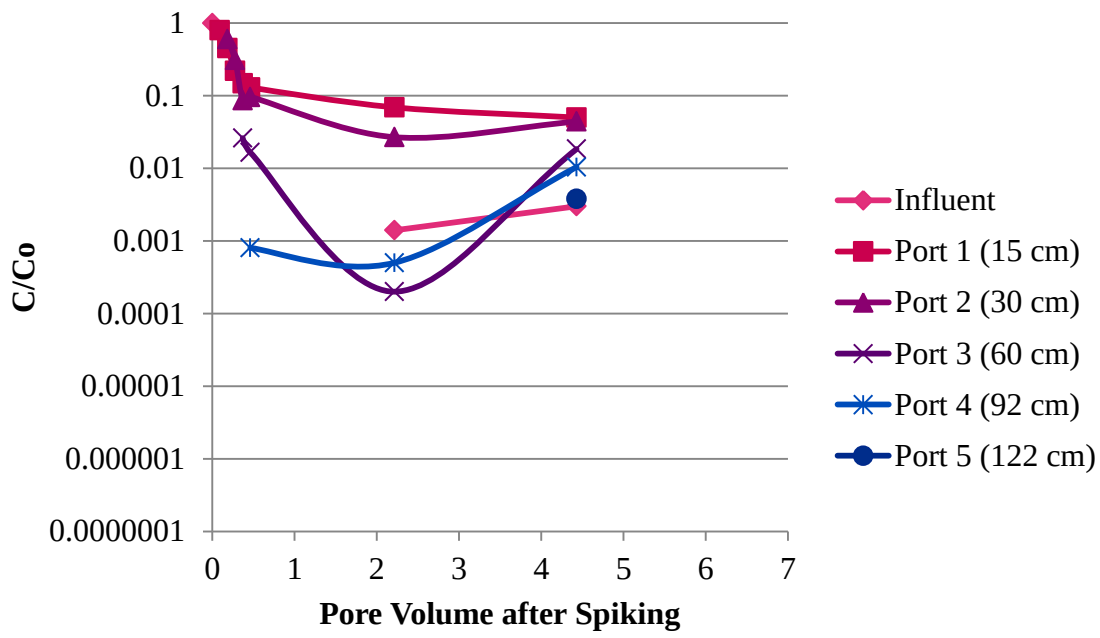

Figure 4.19 *Legionella* Transport through Recharge Media- Column A Test 10 (PV)

Figures 4.20 through 4.25 show the observed concentration of *Legionella* throughout the column. In order as presented, Influent, Port 1, 2, 3, 4, and 5.

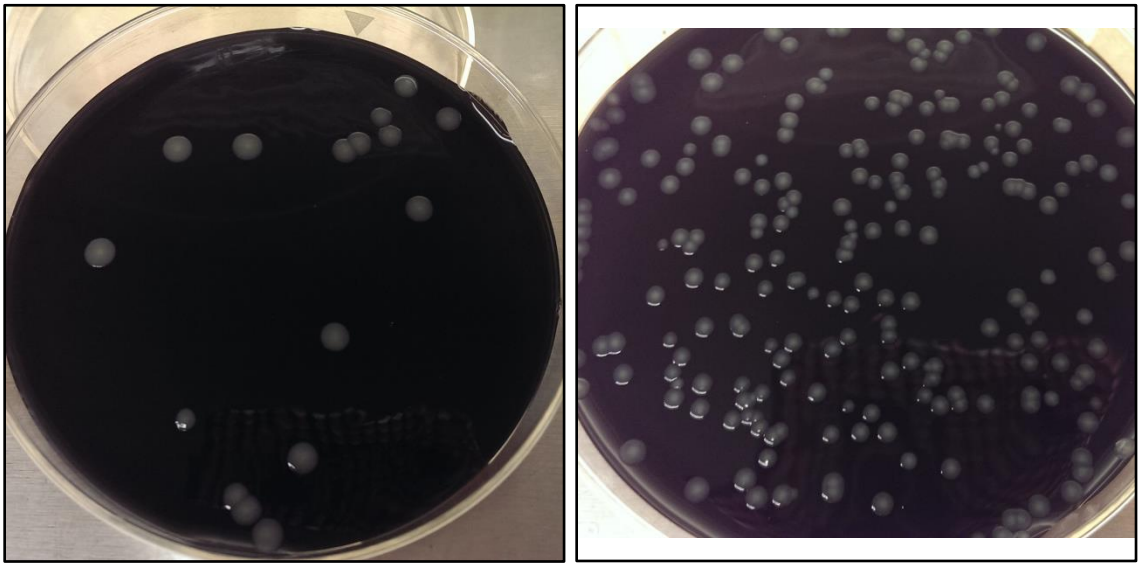

Figure 4.20 *Legionella*- Column A Test 10 (48 Hours after Spiking) Influent

Figure 4.21 *Legionella*- Column A Test 10 (48 Hours after Spiking) Port 1 (15 cm)

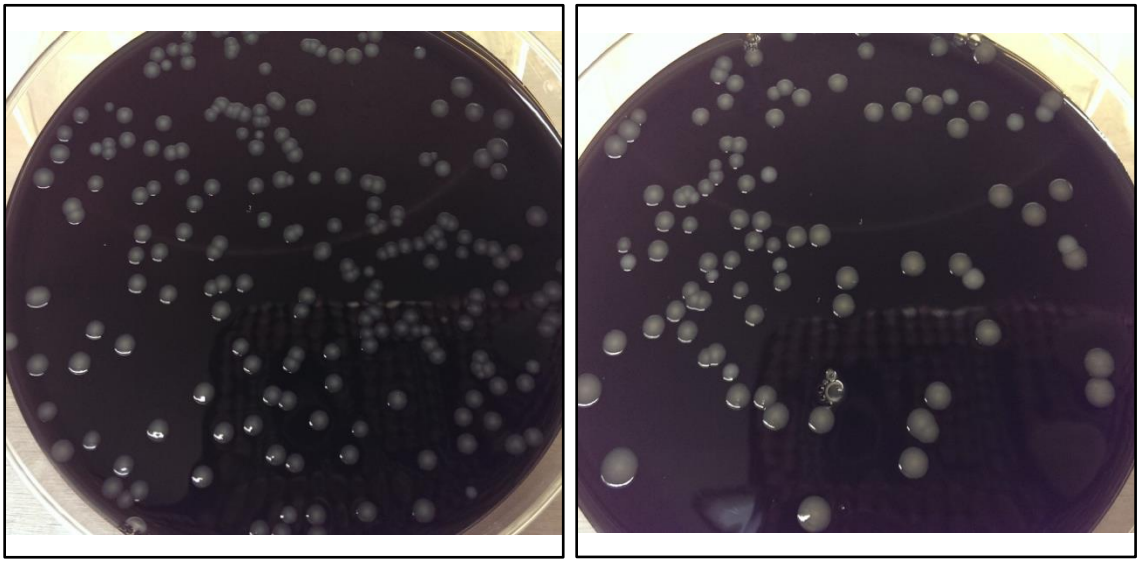

Figure 4.22 *Legionella*- Column A Test 10 (48 Hours after Spiking) Port 2 (30 cm)

Figure 4.23 *Legionella*- Column A Test 10 (48 Hours after Spiking) Port 3 (60 cm)

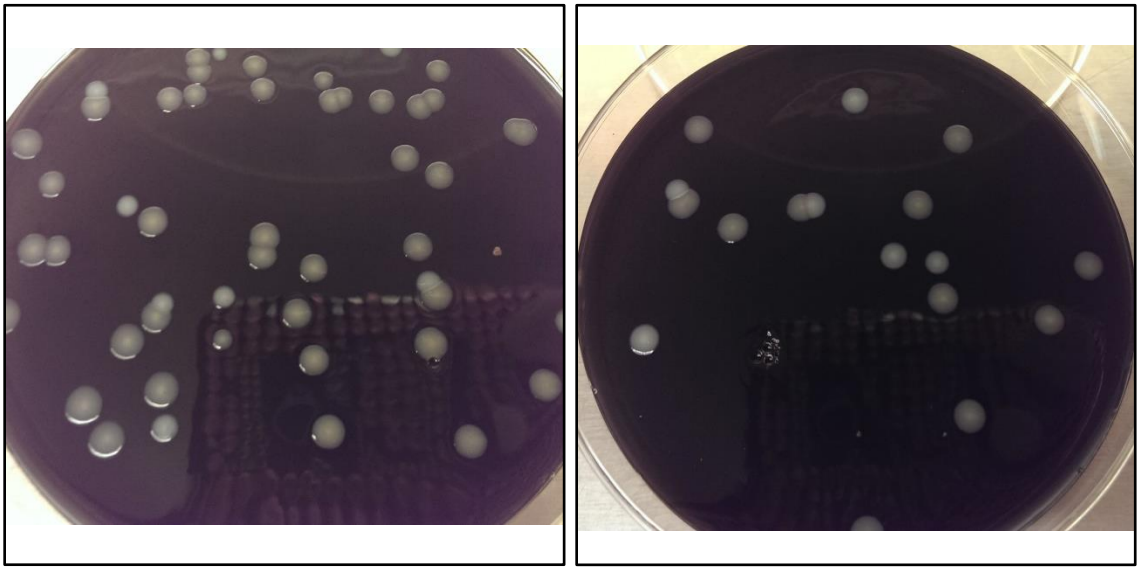

Figure 4.24 *Legionella*- Column A Test 10 (48 Hours after Spiking) Port 4 (92 cm)

Figure 4.25 *Legionella*- Column A Test 10 (48 Hours after Spiking) Port 5 (122 cm)

***Legionella* Transport Test 11.** Five mL of the culture was added to the head water of Column A. The column flow rate was set to 1.5 m/day. Samples were collected from Ports 1 through 5 at 1, 2, 3, 4, 5, 24, and 48 hours after the culture was spiked. All samples were analyzed using culture based technique.

This experiment did not proceed as planned. There were numerous errors that may have inhibited the *Legionella* colonies from growing. Several issues occurred with the media production. The media may have been poured too hot or too cold. The antibiotics may have been added at too high of a concentration. The L-cysteine may have been added before the media had cooled off correctly. The original *Legionella* culture may have been too old or unhealthy in general. The broth media may have been made incorrectly. In the end, the plates in this experiment did not culture any *Legionella*.

***Legionella* Transport Test 12.** Five mL of the culture was added to the head water of Column A. The column flow rate was set to 1.0 m/day. Samples were collected from Ports 1 through 5 at 1, 2, 3, 6, 9, 30, 56, and 77 hours after the culture was spiked. All samples were analyzed using culture based technique and data are presented in Figures 4.26 and 4.27.

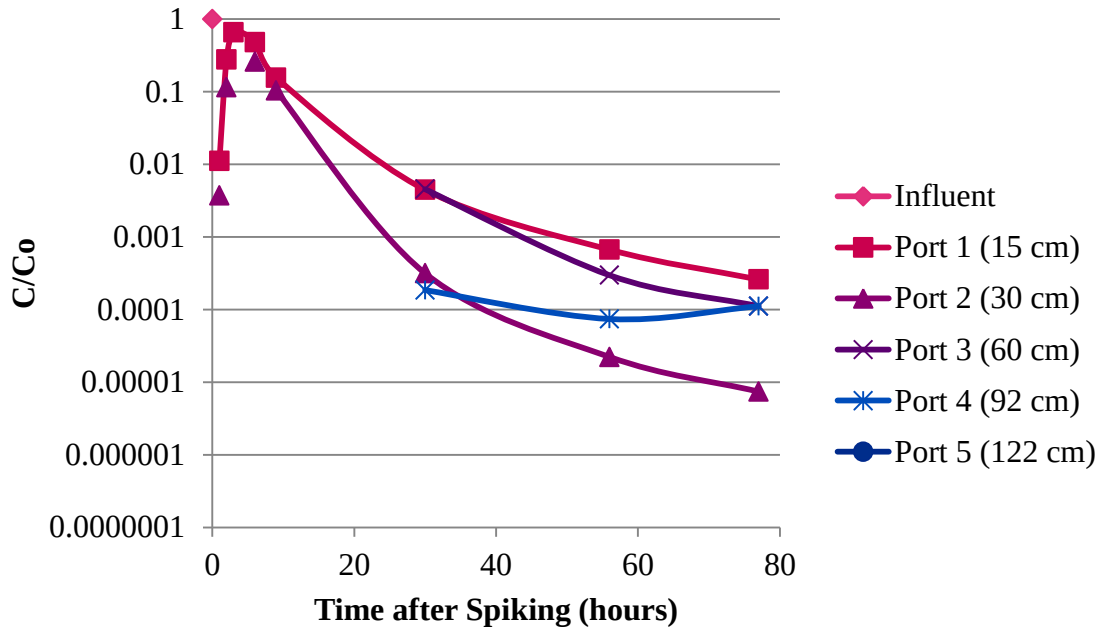

Figure 4.26 *Legionella* Transport through Media- Column A Test 12 (Time)

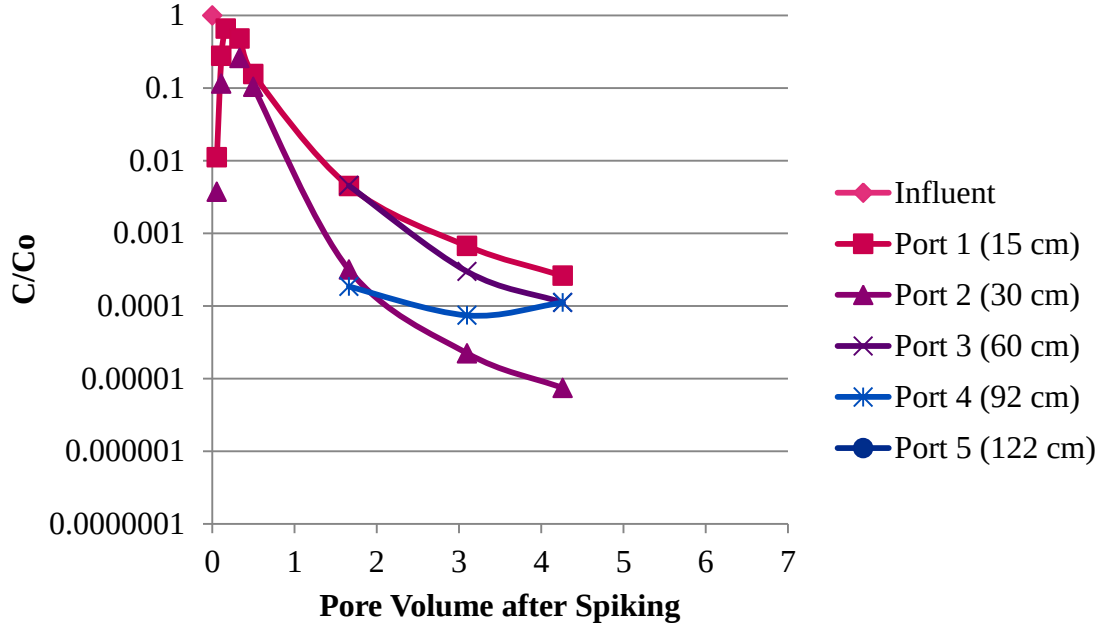

Figure 4.27 *Legionella* Transport through Recharge Media- Column A Test 12 (PV)

***Legionella* Transport Test 13.** Five mL of the culture was added to the head water of Column A. The column flow rate was set to 2.75 m/day. Samples were collected from Ports 1, 2, 3 and 5 at 1, 2, 3, 6, 24, 75, 96, and 125 hours after the culture was spiked. All samples were analyzed using culture based technique and data are presented in Figures 4.28 and 4.29.

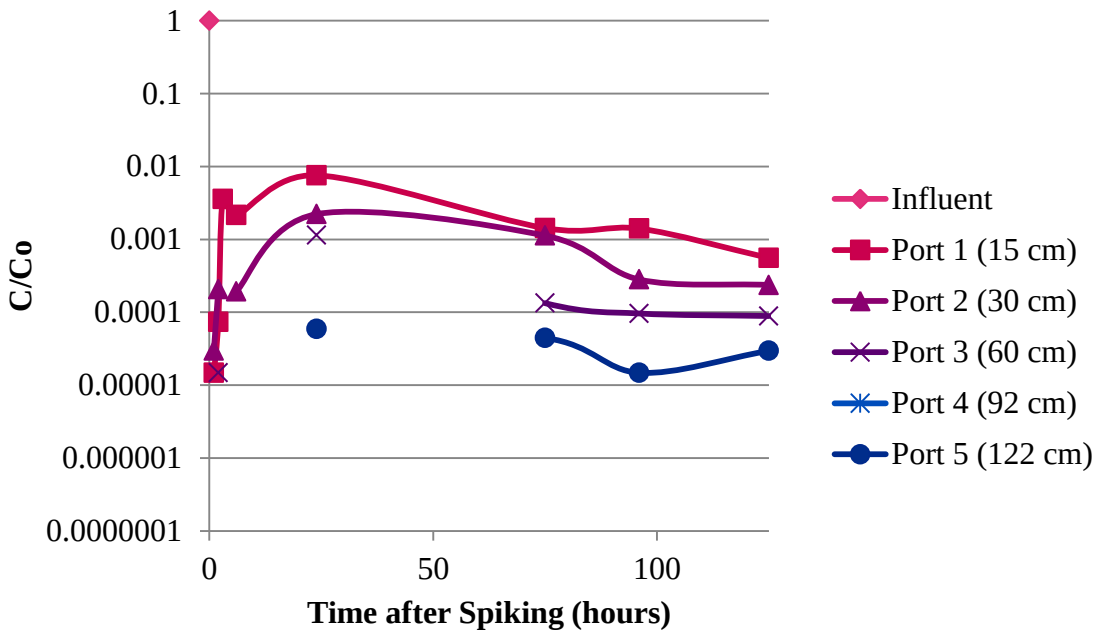

Figure 4.28 *Legionella* Transport through Media- Column A Test 13 (Time)

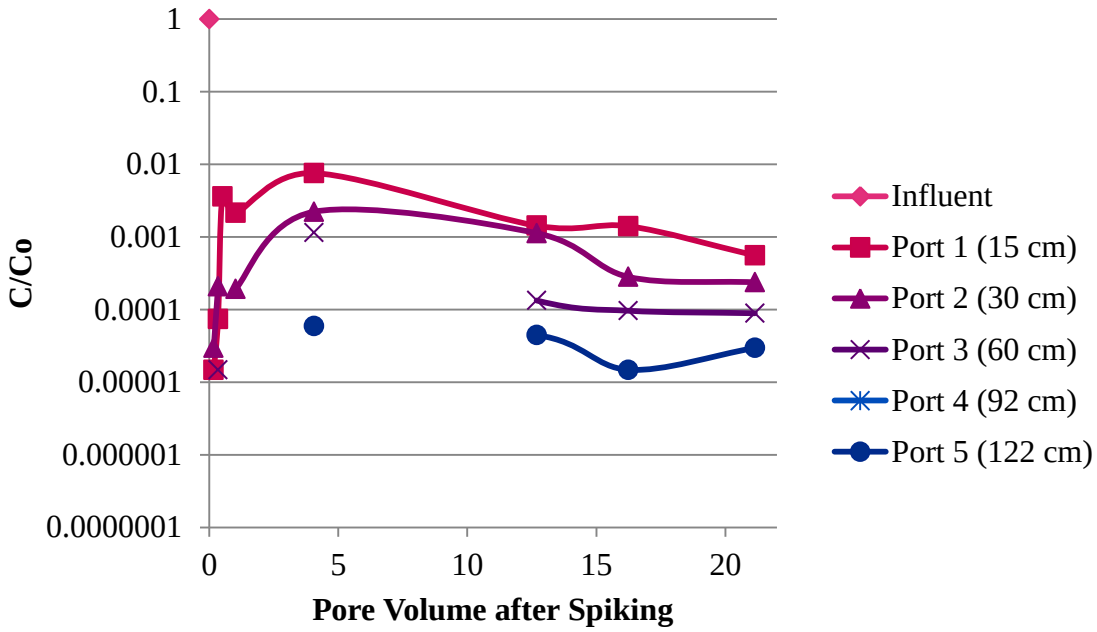

Figure 4.29 *Legionella* Transport through Recharge Media- Column A Test 13 (PV)

Single Spiked Dose *Legionella* Experiments in Column B

***Legionella* Transport Test 14.** Five mL of the culture was added to the head water of Column B. The column flow rate was set to 1.5 m/day. Samples were collected from Ports 1 through 5 at 1, 2, 3, 4, 5, 24, and 48 hours after the culture was spiked. All samples were analyzed using culture based technique.

This experiment did not proceed as planned. There were numerous errors that may have inhibited the *Legionella* colonies from growing. Several issues occurred with the media production. The media may have been poured too hot or too cold. The antibiotics may have been added at too high of a concentration. The L-cysteine may have been added before the media had cooled off correctly. The original *Legionella* culture

may have been too old or unhealthy in general. The broth media may have been made incorrectly. In the end, the plates in this experiment did not culture any *Legionella*.

***Legionella* Transport Test 15.** Five mL of the culture was added to the head water of Column B. The column flow rate was set to 1.2 m/day. Samples were collected from Ports 1 through 5 at 1, 2, 3, 6, 9, 30, 56, and 77 hours after the culture was spiked. All samples were analyzed using culture based technique and data are presented in Figure 4.30 and 4.31.

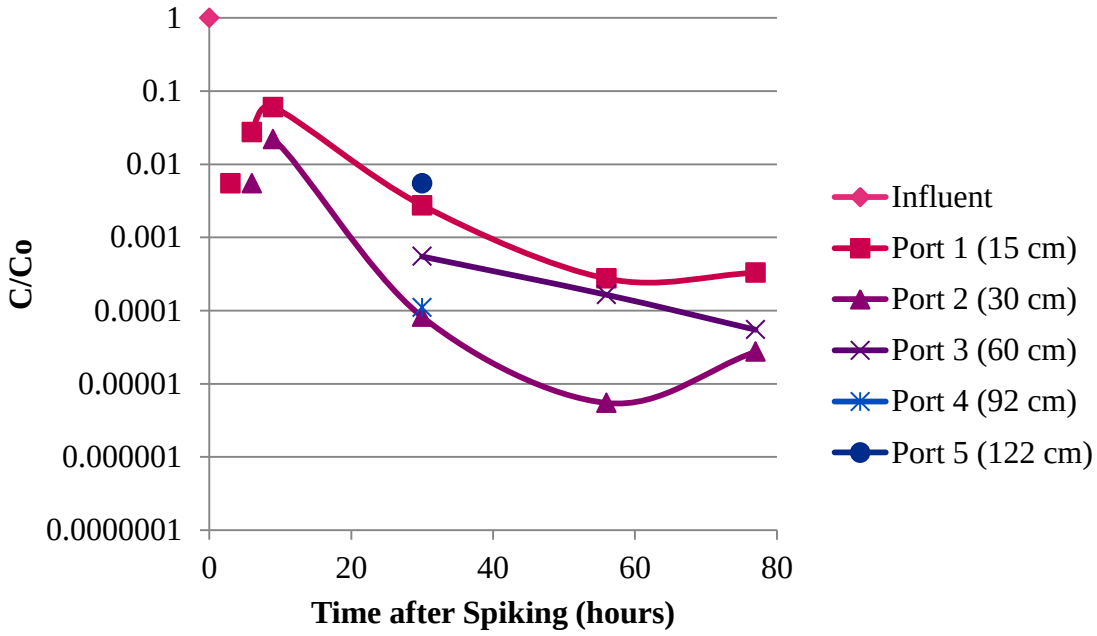

Figure 4.30 *Legionella* Transport through Media- Column B Test 15 (Time)

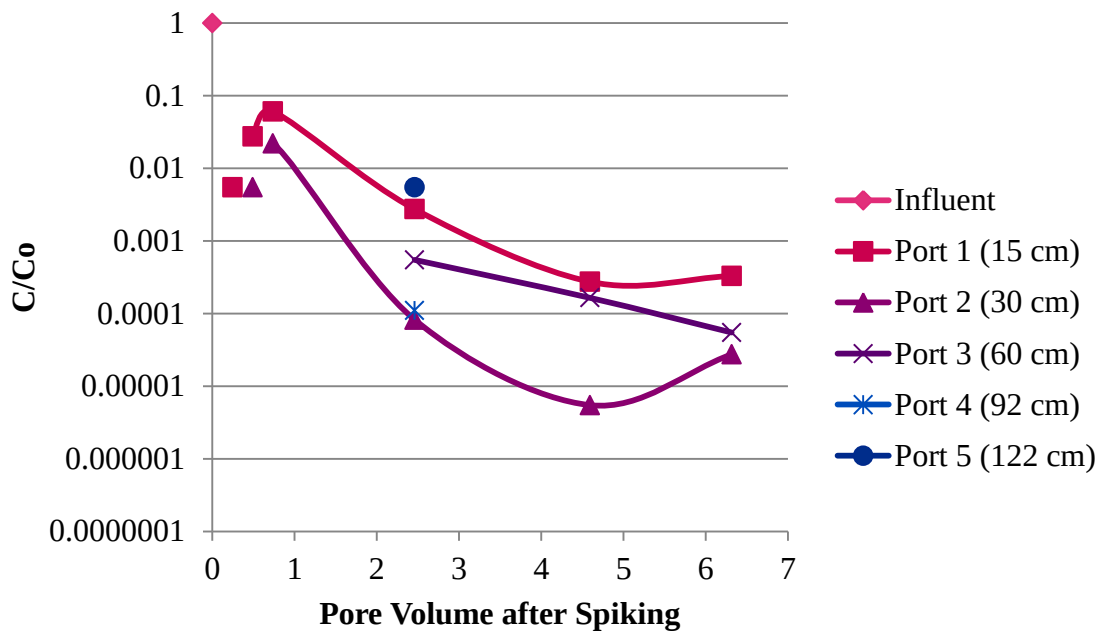

Figure 4.31 *Legionella* Transport through Recharge Media- Column B Test 15 (PV)

***Legionella* Transport Test 16.** Five mL of the culture was added to the head water of Column B, flow rate was set to 1.75 m/day. Samples were collected from Ports 1, 2, 3 and 5 at 1, 2, 3, 6, 24, 75, 96, and 125 hours after the culture was spiked. All samples were analyzed using culture based technique and data are presented in Figures 4.32 and 4.33.

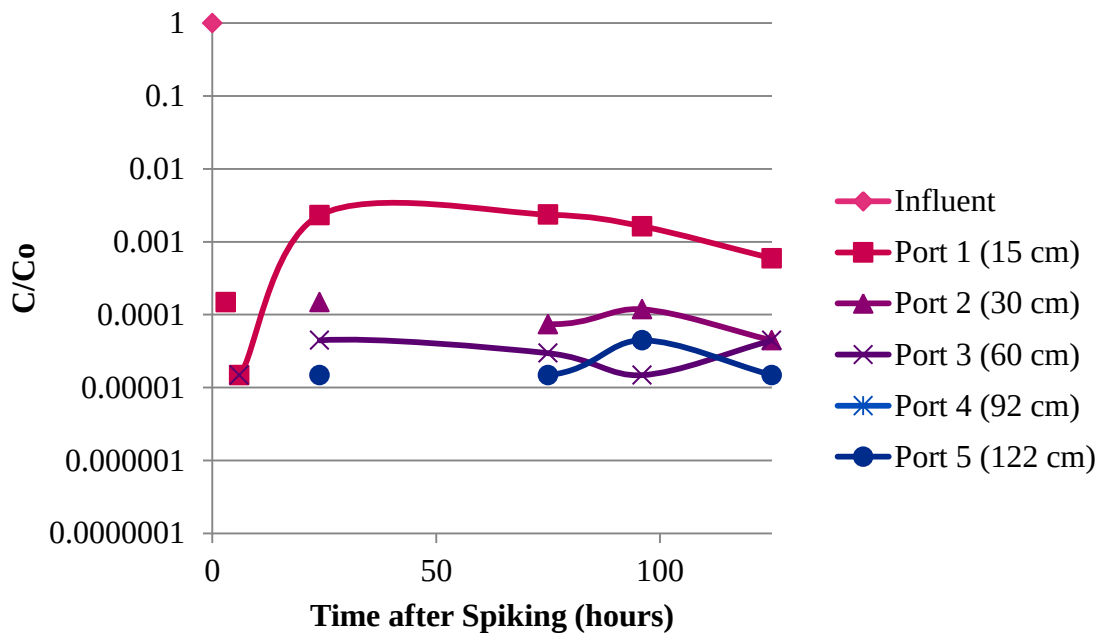

Figure 4.32 *Legionella* Transport through Media- Column B Test 16 (Time)

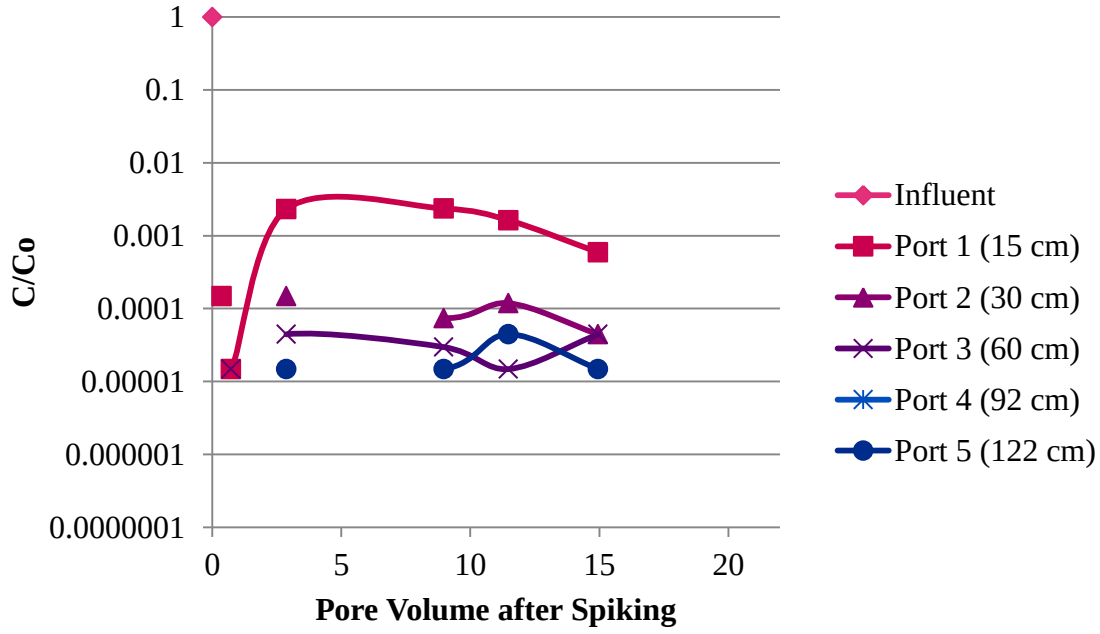

Figure 4.33 *Legionella* Transport through Recharge Media- Column B Test 16 (PV)

Discussion of Single Spiked Dose Experiments

Flow rate Variations. Column flow rates were controlled using a brass ball valve add additional info for the valve such as size etc. purchased at Homedepot. The experimental flow rates were inconsistent due to limitation in controlling the flows by the valves. After an extended period of time, Column B flow rate was at 2.8 m/day. When the flow rate was adjusted, the valve would eventually clogged and the flow rate droped below the desired 1 m/day mark. To minimize the variation in the flow rate in both columns, the flow was adjusted to 2.8 m/day.

Column Sampling Port Clogging. Eventually the top three sampling ports of both columns clogged. The columns were backwashed from Port 3 upward and the media was removed with minimal disruption the media beneath Port 3. The column was packed again using a wet packing technique as previously described

Soil Porosity. The column porosities (ranging from 0.30-0.35) were estimated based on the volume of water required to saturate the media, and based on breakthrough time for fluorescein tracer and bacterial cells in a 3 m packed column. The latter was estimated by dividing this breakthrough time by the porous media volume and volumetric flow rate.

Microbial Transport Comparison. Under similar transport conditions (distance, media and porosity) *Legionella* transport lagged a day behind *E. coli* as determined by culture based technique. *Legionella* are found normally attached to environmental surfaces and also associated with other bacteria via extracellular anionic matrix (Declerck 2010; Donlan 2002; Flemming and Walker 2002; Marrão et al., 1993; Rogers et al., 1994). *Legionella* cells (approximately 2 µm in length and 0.3-0.9 µm in width) are

larger than *E. coli* cells. The larger cell size and strong attachment of *Legionella* with surfaces and co-existing bacteria may be a reason for the observed differences in the breakthrough of *Legionella* (24 hour) versus *E. coli* (6 hour).

***Legionella* Survival in Columns.** It is important to point out that using molecular techniques (PCR) *Legionella* was detected a month after the original spike. This is an additional evidence of the survival and attachment of *Legionella* on surfaces, thus affecting their fate and transport in environment. This observation is further concerted by the *Legionella* exopolymer production observed in SEM electonmicrogrphs (data not shown), which may explain the travel time discrepancy between the *Legionella* and *E. coli* transport mechanisms.

***E. coli* Transport through Column A Compared with Column B.** *E. coli* cells were transported through both columns under a similar conditions. Test 6 and 9 are comparable because they were both performed on the same day and at the same flow rate. Since the column had formerly clogged, both columns had the media replaced through the first 3 sampling ports. Therefore, the acclimation period was the same for both columns. Test 6 and 9 were performed at a flow rate of 2.8 m/day. In Column A and B Port 5, breakthrough occurred at 0.52 pore volume (3 hours), and at 0.57 pore volumes (3 hours), respectively. Since this was the first time Port 5 had been sampled for either column, breakthrough may have occurred at an earlier time. However, these breakthrough in Test 6 and 9 were consistent with the other *E. coli* transport experiments. Therefore, it is unlikely that breakthrough occurred much sooner than recorded.

***Legionella* Transport through Column A Compared with Column B.** Unlike *E. coli* the soil grain size distribution differences between Column A and B may have

affected the *Legionella* transport mechanism. Tests 13 and 16 show this comparison. The flow rate in Column A and B was 2.75 and 1.75 m/day, respectively. However, due to the faulty ball valve the flow rate in Column B increased to nearly 2.8 m/day after the initial flow rate of 1.75 m/day was recorded. Test 13 and 16 show similar trends regarding the persistence of *Legionella* in the water samples long after 3 pore volumes passed through the columns. However, *Legionella* in Column A was transported differently than in Column B. This observation is demonstrated through the pore volume breakthrough at Port 5. Breakthrough occurred in Column A and B Port 5 after 4.06 pore volumes (24 hours) and 2.87 pore volumes (24 hours), respectively.

Recharge Field Site Study

Secondary and Tertiary Treated Wastewater. PCR assay confirmed the presence of *Legionella* in the secondary and tertiary treated waste water effluent in an East Valley wastewater reclamation facility. The tertiary treated wastewater from this facility is currently used for recharge applications. The secondary treated waste water samples were collected prior to GAC filtration, whereas the tertiary treated wastewater samples were collected after the GAC filtration. Both secondary and tertiary treated wastewater samples were analyzed using culture based technique, DNA extraction, and PCR assay.

- Secondary treated wastewater sample was culture positive
- Tertiary treated wastewater sample was culture negative
- PCR results for both samples were positive for *Legionella*

Recharge Basin. Groundwater samples were collected from an East Valley recharge basin. The samples were collected from monitoring wells 2 and 3. Groundwater

samples were collected from two monitoring wells at the recharge site. Groundwater quality data was collected at three times for each monitoring well. Initial groundwater quality was analyzed after flushing the monitoring well piping and the pump lines. The groundwater quality was again assessed after pumping 1,000 and 2,000 gallons from the monitoring well. (Tables 4.8 and 4.9).

Table 4.8 Monitoring Well 2 Groundwater Quality

Parameter	Initial Sample	Sample after 1,000 gallons	Sample after 2,000 gallons
Depth to groundwater (ft)	42.3	45.3	45.6
pH	7.43	7.38	7.73
Conductivity (µS/cm)	2170	2160	2130
Temperature (°C)	20.3	20.4	21.3

Table 4.9 Monitoring Well 3 Groundwater Quality

Parameter	Initial Sample	Sample after 1,000 gallons	Sample after 2,000 gallons
Depth to groundwater (ft)	40.3	44.5	44.9
pH	7.02	7.15	7.11
Conductivity (µS/cm)	2270	2290	2310
Temperature (°C)	23.1	23.5	23.4

Groundwater samples collected from monitoring wells 2 and 3 were analyzed using culture based technique and DNA extraction. Minimally, 2 L were collected from each monitoring well.

- *Legionella* in the samples did not culture on BCYE media
- *Legionella* DNA detected in one sample

CHAPTER 5

CONCLUSIONS

Impact of the Project

This study documents the transport of bacterial indicator and a pathogen through soil columns (recharge basin) to subsurface. Although soil is commonly known as a medium for removal of waterborne contaminants, our study highlights the limitation of this phenomenon for removing microbial indicator and pathogenic bacteria. *Legionella* was transported through all 122 cm of the Columns A and B in under 24 hours. Samples collected from Port 5 (122 cm) resulted in *Legionella* transport ranging from 1.9×10^2 to 1×10^3 cfu/mL. In addition, *E. coli* column study resulted in a similar transport through the recharge media. *E. coli* was transported, through all 122 cm of the Columns A and B, in under 3 hours. Graphical data presented in this document for the pathogenic bacteria was confirmed by PCR results.

Although soil is commonly known as a medium for purification, the results show a discrepancy in this conclusion. Transport of *E. coli* and *Legionella* cells in the recharge basin media columns suggests the possibility of groundwater recharge basin to be susceptible to pathogen such as *Legionella* and vulnerabilities of recharge systems to microbial contamination. This proof of concept study demonstrates that bacterial removal by passage through soil may not be completely efficient and adequately reliable for preventing our groundwater aquifers from pollution.

Column Transport Study

E.coli breakthrough in the column was at a faster rate compared to *Legionella.* When comparing Column A to Column B, *E. coli* transport was similar and was not

impacted by the particle grain size distribution differences. *E. coli* breakthrough in Columns A and B at Ports 1 and 5 occurred with similar transport trend and with a similar range of pore volume suggesting similar transport phenomena despite different size distributions in the columns (Table 4.3). However, *Legionella* transport was affected by the differences in the particle grain size distributions of Columns A and B, indicated by a breakthrough differences at Port 1 and Port 5. The transport of *Legionella* within Column A occurred at a different rate from Port 1 to Port 5, whereas the breakthrough in Column B was occurred at a linear trend through the entire column. Such differences in transport at different depth within the same column (A) is not clear, however, this may have been due to some artifacts introduced during the maintenance of the column, the flow rate or the grain size particles, however such difference in transport rate cannot be explained by the data obtained.

Given the novel results of this proof of concept study, can be concluded that the transport of *Legionella* into groundwater aquifers through engineering recharge basin conditions, in Central Arizona. As environmental engineers, it is critical that we ensure the quality of groundwater for generations to come. The occurrence and potential for the long-term survival of *Legionella*, in groundwater aquifers is an issue that must be address in order to mitigate future risks to water resources in Central Arizona.

Community Benefit

This was the first study looking at the fate and transport of *Legionella* under control and field conditions in Central Arizona. The increasing use of groundwater recharge as a water management practice implies the need for more comprehensive data on the occurrence and distribution of *Legionella* in source and treated waters.

Additional Considerations

After further tests and analysis for the removal mechanism and additional field study we will have a better understanding of the threat of *Legionella* that may poses on groundwater systems. Additional data will characterize the potential of *Legionella* spp. incidence and persistence in Central Arizona aquifers under recharge conditions. This can be accomplished by routine sample collection and analyses from recharge basin and groundwater wells. Such data is needed for the spatial and temporal variability pertinent to microbial transport under recharge conditions.

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APPENDIX A

ABBREVIATIONS

ASU	Arizona State University
ATCC®	American Type Culture Collection
°C	degrees Celsius
CDC	Centers for Disease Control and Prevention
CFT	classic filtration theory
CFU	colony forming unit
cm	centimeter
cm^3	cubic centimeter
DI	deionized
DNA	deoxyribonucleic acid
DO	dissolved oxygen or die-off
EPA	U.S. Environmental Protection Agency
°F	degrees Fahrenheit
FC	fecal coliform
ft	feet
FVDW	van der Waals forces
g	gram
gal	gallon

g/cm^3	grams per cubic centimeter
gpd/ft	gallons per day per foot
GWR	Groundwater Rule
GWUDI	groundwater under the direct influence
IESWTR	Interim Enhanced Surface Water Treatment Rule
L	liter
LT1 ESWTR	Long-term I Enhanced Surface Water Treatment Rule
LT2 ESWTR	Long-term II Enhanced Surface Water Treatment Rule
M	mole
m	meter
m/day	meters per day
MGD	million gallons per day
mg/L	milligrams per liter
mL	milliliter
mL/min	milliliters per minute
mM	millimole
µL	microliter
µm	micrometer

N	newton or nitrogen
NaCl	sodium chloride
$NaHCO_3$	sodium bicarbonate
nm	nanometer
NTU	nephelometric turbidity unit
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PFU	plaque-forming unit
PV	pore volume
PVC	polyvinyl chloride
QA/QC	quality assurance/quality control
RE	recovery efficiency
rpm	revolutions per minute
SWTR	Surface Water Treatment Rule
TC	total coliforms
TDS	total dissolved solids
TSA	tryptic soy agar
TSB	tryptic soy broth

TSS	total suspended solids
USEPA	United States Environmental Protection Agency
USDA	United States Department of Agriculture

APPENDIX B

BUFFERED-CHARCOAL YEAST EXTRACT MEDIA

Table B.1 BD Sparks, MD: BBL™ BCYE Agar Base

Approximate Formula per Liter

Yeast Extract .....10.0 g

Ferric Pyrophosphate .....0.25 g

ACES Buffer .....10.0 g

Charcoal, Activated .....2.0 g

α-Ketoglutarate .....1.0 g

Agar .....15.0 g

Directions for Preparation from Dehydrated Product

1. To 500 mL of purified water, add 2.4 g KOH pellets and mix to dissolve.

2. Add 38.3 g of the powder and 500 mL of purified water. Mix thoroughly.

3. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.

4. Autoclave at 121ºC for 15 minutes.

5. Cool to 45 - 50°C and add 4 mL of a 10% filter-sterilized solution of L-cysteine HCl.

6. Mix thoroughly. Check pH; if not 6.85 ± 0.1, adjust using 1 N HCl or KOH.

7. Dispense into Petri dishes. Agitate while dispensing to keep charcoal in suspension.

8. Test samples of the finished product for performance using stable, typical control cultures.

Table B.2 Neogen Co., Lansing, MI: BCYE Agar Base (7728)

Formula per Liter

Yeast Extract .....10 g

ACES Buffer.....10 g

Charcoal, Activated .....1.5 g

α-Ketoglutarate.....1 g

Ferric Pyrophosphate .....0.25 g

Agar.....15 g

Supplements / 10 mL

L-Cysteine (4%), sterile

Final pH

6.9 ± 0.2 at 25°C

Directions

1. Suspend 38 g of the medium in 900 mL of purified water.

2. Adjust pH to 6.9 with 1N KOH.

3. Add water to bring volume to 1000 mL.

4. Heat to boiling with stirring to dissolve.

5. Autoclave at 121°C for 15 minutes. Cool to 45 - 50°C.

6. Aseptically add 10 mL of a sterile solution of L-Cysteine (4%).

7. Mix and add inhibitor solutions if required.

8. Dispense with agitation.

APPENDIX C

RECHARGE BASIN PHOTOGRAPHS

City of Chandler, Arizona

The following images were taken during environmental sampling conducted at the City of Chandler recharge basins of Veteran's Oasis Park.

Figure C.1 Photographs from City of Chandler Recharge Basins at Veterans Oasis Park

Figure C.2 Photograph from Mesa Recharge Basin Number 2 during Flooding Event

Source: Adapted from *Soil-Aquifer Treat for Sustainable Water Reuse* by Fox et al.

Figure C.3 Photograph from Tucson Sweetwater Recharge Site

Source: Adapted from *Soil-Aquifer Treat for Sustainable Water Reuse* by Fox et al.

APPENDIX D

LABORATORY SIEVING PHOTOGRAPHS

Figure D.1 Sieves Used for Soil Classification (left)

Figure D.2 Scale Used for Analysis (right)

APPENDIX E

LABORATORY COLUMN PHOTOGRAPHS

Figure E.1 Lauren McBurnett (right) and Sanya Mehta (left)

Figure E.2 Sampling from Port #4 of Column B

Figure E.3 Biological Activity at the Head of Column A

APPENDIX F

PCR RESULTS FOR RECLAIMED WATER AND COLUMN STUDY SAMPLES

Gel Electrosis Results from Ports #1 and #5 of Columns A and B and the Secondary and Tertiary Treated Wastewater of the City of Chandler.

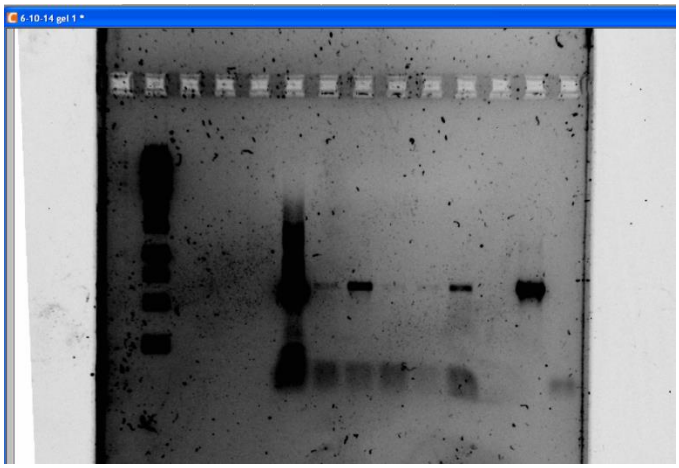

Figure F.1 PCR Results from 06/10/2014

Table F.1 PCR Gel Well Assignments

1	2	3	4	5	6	7	8	9	10	11	12	13	14
0*	Ladder	0*	0*	0*	2^{nd} WW	3^{rd} WW	A 6”	A 48”	B 48”	B 6”	-	+	0*

0* = Blank Well

WW = Treated wastewater

6” = water sampled from 6 inch deep column sampling port

48” = water sampled from 48 inch deep column sampling port

- = negative control

+ = positive control