

**Drivers of Stormwater Runoff Characteristics from Non-Point Source
Urban Pollution**

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This dissertation is dedicated to the important women in my life, without whom I would not be the same person that I am today.

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ABSTRACT

As human populations continue to increase, so does the demand for drinking water and infrastructure. In urban areas, continued construction of impervious surfaces, such as rooftops and roadways, have the ability to generate considerable amounts of runoff; however associated runoff has been found to be heavily contaminated. The presence and resilience of fecal indicator bacteria in urban runoff could pose a threat to public health, as most non-point source runoff is not treated.

Through investigations of rooftop and roadway runoff, microbial communities and fecal indicator bacteria presence was consistent between storm events and over the course of storms, respectively. Runoff quality characteristics were found to be highly dependent on total precipitation, antecedent dry period, and runoff intensity. Further experiments using *Escherichia coli* (*E. coli*) isolated from rooftops revealed that the environmental *E. coli* was capable of attaching to and surviving on glass surfaces. The ability of the *E. coli* to survive was impacted by temperature, as a lower temperature slowed attachment but prolonged cell longevity.

Preliminary rooftop runoff experiments exhibited the consistency of runoff quality over the duration of a storm. With this discovery, rooftop runoff was collected and analyzed using random grab samples from housing developments in both the United States and China. Automatic flow-weighted samples were collected from the roadway and analyzed using 16S rRNA Sequencing technologies. Attachment and survival was analyzed using a modified acridine orange dye count method which paired epifluorescence microscopy with spatial analysis tools in ArcGIS to determine cell coverage on the glass surfaces.

Geographic location and storm characteristics seem to have a strong impact on non-bacterial quality from urban impervious surfaces. Roadway microbial populations were consistent between storm events, and *E. coli* concentrations were constant within a storm event in rooftop runoff. Environmentally isolated strains of *E. coli* have now been shown to be capable in surviving in environments which mimic roadway and rooftop conditions. This further supports the idea that *E. coli* is not a suitable choice for measuring fecal contamination, and that first flush diversion will not prevent bacterial contamination of cisterns; creating a need for new designs for runoff collection.

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INTRODUCTION

Motivation and Objectives

The human population is growing rapidly, and with such growth comes an increase for needs of clean drinking water and infrastructure. Non-point source pollution is under regulated, leading to impacts on human and environmental health. Roadways and rooftops are two causes of non-point source pollution, most of which drain directly into or towards nearby water sources with little to no treatment. An increase in similar impervious surfaces needed to keep up with population increase only raises the amount of pollution that can be generated and contaminate water sources.

One primary objective of this dissertation is determining the quality and microbiology associated with roadway and rooftop runoff, and the associated impact on a nearby water source. While it would not be advantageous to collect roadway runoff, rooftop runoff has reuse potential, and collection devices can be accommodated for small or large scale rooftops. Collection of rooftop runoff could also help to offset the increased flood potential caused by an upwelling in the development of new impervious surfaces, especially in urban areas. In developing countries, captured water could help provide a source of clean drinking water with the right treatment and catchment design. In order to design a better collection and storage system for rooftop runoff, more information is needed related to rooftop runoff characteristics.

A second objective which came from urban surface runoff quality studies is the potential for bacterial survival in roadways and on rooftops. The source of bacteria on urban surfaces is currently unknown, which limits the prevention of bacterial contamination in the affected stormwater runoff. Since roadway and rooftop surfaces are seemingly inhospitable to fecal indicator bacteria, which are not supposed to survive outside of a host, a reason for their presence in the associated runoff needs to be found.

Literature Review

Interstate Runoff Quality and Bacterial Contamination

With over 45,000 miles of interstate surfaces in the United States, roadways have the potential to affect a significant amount of stormwater runoff (USGS, 2006). Runoff from interstates, which are typically considered impervious surfaces, is rarely treated (CH2MHILL, 1998). As authorities are realizing that interstates may be contributing to watershed contamination, stricter requirements are being placed on departments of transportation across the country. Current best management practices (BMPs) are both inadequate and expensive, making treatment of interstate runoff unrealistic (Tennessee Water Resources Research Center, 2003). Efforts have begun to study interstate surface contribution to surrounding watershed quality, but interstate runoff without external influence is difficult to capture and study (Sansalone and Buchberger, 1997; Nason, Bloomquist, et al., 2012; Sansalone, Hird, et al., 2005; CH2MHILL, 1998; Van Metre and Mahler, 2003; Winston, Hunt, et al., 2012; Eck, Winston, et al., 2012;

Sansalone, Koran, et al., 1998). Due to inconsistent technique and pollutant monitoring, limited data is available from literature.

Common contaminants found in interstate runoff include nutrients (Miguntanna, Liu, et al., 2013; Hathaway, Tucker, et al., 2012; Helmreich, Hilliges, et al., 2010; Barrett, Irish Jr., et al., 1998; CH2MHILL, 1998; Gan, Zhuo, et al., 2008; Geldreich, Best, et al., 1968; Kayhanian, Fruchtman, et al., 2012; Opher and Friedler, 2010; Zuo, Fu, et al., 2011; McCarthy, Deletic, et al., 2008), heavy metals (Helmreich, Hilliges, et al., 2010; Zuo, Fu, et al., 2011; Legret and Pagotto, 1999; Barrett, Irish Jr., et al., 1998; CH2MHILL, 1998; Gan, Zhuo, et al., 2008; Kayhanian, Fruchtman, et al., 2012; Opher and Friedler, 2010; Lee, Kim, et al., 2011; Ellis, Revitt, et al., 1987), suspended solids (Helmreich, Hilliges, et al., 2010; Sansalone, Koran, et al., 1998; Legret and Pagotto, 1999; Barrett, Irish Jr., et al., 1998; Vaze and Chiew, 2004; Opher and Friedler, 2010; Lee, Kim, et al., 2011; Zuo, Fu, et al., 2011), and oil and grease (Helmreich, Hilliges, et al., 2010; Barrett, Irish Jr., et al., 1998; CH2MHILL, 1998; Opher and Friedler, 2010). Bacteria are rarely studied on roadway surfaces (Barrett, Irish Jr., et al., 1998; CH2MHILL, 1998; Opher and Friedler, 2010; Hathaway, Tucker, et al., 2012; Sidhu, Hodgers, et al., 2012). The antecedent dry period (ADP), rainfall intensity, and rainfall volume are the three most important precipitation-based contributors to runoff quality (Lee, Kim, et al., 2011; Helmreich, Hilliges, et al., 2010). Seasons, traffic volume, interstate surface material, atmospheric deposition, and maintenance practices are all additional possible factors affecting runoff quality. Interstate runoff is also considered to follow a first flush trend similar to that on rooftop surfaces (Sansalone and Buchberger, 1997; Dorchin and Shanas, 2013; Zhang, Chen, et al., 2010; Deletic and Maksimovic, 1998; Helmreich, Hilliges, et al., 2010; Barrett, Irish Jr., et al., 1998; Sansalone, Hird, et al., 2005; Opher and Friedler, 2010; Hathaway, Tucker, et al., 2012; Zuo, Fu, et al., 2011).

Sample Collection

Stormwater runoff from interstate surfaces is generally collected via mechanical or automatic samplers. Mechanical samplers are typically limited in volume and are used heavily for grab sampling and first flush samplers. Mechanical samplers exist in many styles, but common types are Vortex samplers, bottles with a ball stopper, or open bottles. Automatic samplers, such as ISCO samplers, are less personnel intensive, but cannot capture a first flush due to programming and priming requirements. ISCO samplers can excel at providing hydrograph data for entire storms, as well as sampling entire storms. Bottles can then be collected after a storm event, decreasing the amount of time spent in the field collecting samples (Barrett, Irish Jr., et al., 1998).

Once samples have been collected, they are oftentimes transferred into a separate container. Following sample collection, the EPA requires that samples be processed within a six hour limit to prevent sample degradation. This is particularly important for those that analyze bacterial contamination.

First Flush Effects

The first flush is a poorly defined part of stormwater runoff, but is commonly found and studied for impervious surface runoff. Typical definition of a first flush is a mass flushing of pollutants in the early stages of stormwater runoff. Just as in rooftop runoff, there is no set way to define a first flush. In roadway runoff, a first flush is often described with an 80/30 rule. 80% of the pollutant mass must be emitted in the first 30% of runoff (Bertrand-Krajewski, Chebbo, et al., 1998). There are many variations to this rule, but use is inconsistent (Hathaway, Tucker, et al., 2012). Using the 80/30 rule or a variation of it cannot be used as a design for first flush collection due to its relation to an amount of unknown runoff. First flush is commonly reported as a concentration rather than by mass. The first flush effects can also vary over season or within individual storm events. There is no consistent method that is used to measure the first flush between researchers.

A paper presented by Kayhanian et al. proposed the idea of a mass first flush (MFF) ratio (Kayhanian and Stenstrom, 2005). This ratio is able to quantitatively define the first flush in any initial section of a storm. Using the quotient of normalized mass fraction and runoff volume, any ratio greater than 1 would support a high MFF ratio. Following this method, chemical oxygen demand (COD), total kjeldahl nitrogen (TKN), dissolved organic carbon, copper, nickel, and zinc have all been found to be washed off in the beginning portion of a storm (Kang, Kayhanian, et al., 2008; Kayhanian and Stenstrom, 2005).

Physicochemical Constituents

Solids

Roadway runoff studies are not typically concerned with suspended solids as a whole, but with particle size (Sansalone, Koran, et al., 1998; Zuo, Fu, et al., 2011). Different particle sizes may wash off at different points during a storm and may also have attached metal, nutrient, or bacterial contamination (Opher and Friedler, 2010). Sediments are generally believed to rinse off as a part of a first flush. The removal of sediments in a BMP should reduce contamination to external water sources.

Heavy Metals

Metals have been studied extensively in roadway surface runoff due to the contribution from vehicular traffic and gasoline combustion. Due to the heavy nature of certain metals, they will not travel far from the roadway before being deposited. Until the 1970's, lead was used in gasoline and was found in high amounts in roadway runoff. Since lead has been removed from gas, lead concentrations have steadily decreased to values below $50 \mu\text{g L}^{-1}$ (Kayhanian, 2012).

Additional metals which are present in roadway runoff include zinc, copper, iron, cadmium, nickel, and chromium. Copper and zinc are metals which are frequently found in rooftop runoff. Similar to nutrients, metals can also come in either the dissolved or particulate form. Sansalone et al. found that aluminum, lead, and iron are mostly attached to particulates while zinc, cadmium, and copper were predominantly dissolved

(Sansalone, Koran, et al., 1998; Sansalone and Buchberger, 1997). They also found that first flush occurred for all metals bound to particulates, and even for most dissolved metals. Lead was not found to follow a first flush pattern. Since particulates follow a first flush pattern, contaminants attached to those particulates should also be a part of a first flush. Lead likely does not follow a first flush pattern due to past heavy use in gasoline.

Nutrients

Nutrients are not heavily studied for roadway surface runoff as a whole, but nitrogen and phosphorus tend to be of concern due to their ability to affect natural water sources, and oxygen demand, and can be in dissolved or particulate forms. TKN, nitrate, nitrite, ammonia, ortho-phosphate, and total phosphate are the commonly measured forms of nitrogen and phosphate (Miguntanna, Liu, et al., 2013; Vaze and Chiew, 2004; Davidson, Savage, et al., 2009). Hathaway et al. suggests that nitrogen and phosphorus do not typically follow a first flush pattern due to the dissolved nature of the nutrients (Hathaway, Tucker, et al., 2012). They did note that more did seem to flush off towards the beginning of the storm, but not in a concentration high enough to consider a first flush. Nitrogen showed more relation to a first flush than the phosphorus compounds.

Bacterial Contamination

Bacterial presence in roadway runoff is studied infrequently (CH2MHILL, 1998; O'Shea and Field, 1992; Barrett, Irish Jr., et al., 1998; Opher and Friedler, 2010; Hathaway, Tucker, et al., 2012). Fecal indicator bacteria (FIB) are the primary bacteria of concern; due to findings suggesting the ability of certain FIB to survive outside of host sources, scientists are debating the validity of FIB as a good indicator for fecal contamination. *Bacteroides* have emerged through polymerase chain reaction (PCR) techniques as a potential replacement for FIB. Microbial source tracking (MST) would also allow for the detection of host-specific bacteria or viruses without requiring cultivation. It is possible that in the future, MST will replace traditional FIB methodology as the primary detection methodology of bacterial contamination. Additional technologies which have seldom been used for roadway runoff is 16S rRNA sequencing. This allows for detection of a bacterial community.

Rooftop Runoff Water Quality

As the human population grows, so does the demand for fresh water. In areas lacking in adequate water treatment technologies, arid environments facing drought, and urban areas with high populations, meeting this demand can become problematic. Rainwater harvesting (RWH) is one possible solution to limited drinking water sources.

Many factors are known to affect rooftop runoff quality. Primary parameters are related to rooftop and environmental factors such as surface material and wind direction. Pollutants of concern for rooftop runoff are similar to those monitored for roadway surface and creek quality. No consistent methodology has been developed for rooftop quality monitoring. The majority of projects completed in relation to RWH focus on water quality in long-term storage, or cisterns. Cistern studies typically disregard influent quality, providing no baseline for the existing contaminants. A lack of data

related to influent water quality into rooftop-fed cisterns increases the importance of measuring contaminants coming directly from rooftop surfaces.

Current Collection and Sampling Methodologies

Even though RWH has been practiced since before the Common Era, many experts still disagree on what constitutes proper technique for collection and reuse. Areas of focus in literature for rooftop runoff focus on either cisterns or direct rooftop runoff. Typical direct rooftop and gutter runoff studies emphasize quality, but not necessarily for reuse purposes. Studies relating to direct runoff can increase understanding of public health impacts, runoff quality before entering a cistern for reuse, and future needs for stormwater runoff treatment. For this study, a focus will be made on rooftop runoff collection before it would enter a long term, reuse container such as a cistern. Three primary types of collection methodologies exist, including composite sampling, composite sampling following first flush diversion, and first flush sampling.

Composite Rooftop Runoff Collection

A more realistic model for modeling cistern input is the composite rooftop runoff analysis method. A composite method increases the number of sites which can be analyzed at one time. This advantage becomes more evident when pilot rooftops are the surfaces of interest. With only one set of incoming data from each site, more quality tests can be performed per sample. One major disadvantage of this methodology is a loss of potential kinetic wash off and rain intensity data. Another disadvantage is that the only comparisons that can be made are on roofing material, and runoff versus rain. Two separate articles containing studies conducted by Evans et al. (Evans, Coombes, et al., 2007; Craig A Evans, Coombes, et al., 2006) combine cistern quality and composite rooftop runoff. Other studies (Melidis, Akratos, et al., 2007; Winters and Graunke, 2014; Zhang, Wang, et al., 2014; Gromaire, Garnaud, et al., 2001; Farreny, Morales-Pinzón, et al., 2011; Lee, Yang, et al., 2010) also followed similar composite rooftop runoff collection but without attached cistern effluent quality.

First Flush Diversion

First Flush diversion is a widely used practice in rooftop rainwater collection systems. In some locations this is a legally required part of rainwater collection. While the first flush is a generally accepted theory regarding rooftop runoff quality, there is no set time or volume based frame for a first flush. First flush effects seem to vary by location, runoff intensity, and pollutant, making a strict frame difficult to define.

Two separate methods are available for achieving a first flush diversion. One prominent method is using an automatic sampler (Schriewer, Horn, et al., 2008; Förster, 1998; Förster, 1999; Förster, 1996; C. A. Evans, Coombes, et al., 2006). Automatic sampling offers the ability to study storms in portions without needing to be at the site for sampling. Another advantage of automatic sampling is the ability to form a composite sample and judge overall contamination. Many mechanical first flush diversion samplers follow a diversion-composite sample format. This methodology

provides an advantage over a study looking only at first flush analysis because it can measure contaminants throughout an entire storm.

Due to the lack of a set first flush period in stormwater runoff, the methodologies on how to divert a first flush vary. The most frequently utilized technique implements a volume based first flush measurement. An example of the variety of first flush analysis can be seen in the selected volumes for first flush analysis including 2 L (Mendez, Klenzendorf, et al., 2011), 10-20 L (Gikas and Tsihrintzis, 2012), and 3000 L (Kim, Lee, et al., 2005). This lack of consistency shows the need for a defined first flush frame.

First Flush Analysis

The first flush phenomenon is a highly debated occurrence in stormwater runoff. While it is widely agreed that the first flush exists in stormwater runoff situations, there is no agreement upon how long a first flush lasts, or which contaminants are affected. Inconsistencies between first flush duration makes consistent RWH technology design a challenging feat. While some areas require first flush diversion, other governing bodies do not require diversion, allowing whole storm collection for untreated reuse.

Very few studies related to rooftop runoff look at a first flush as its own entity. Studies that only use the first flush typically study a very low volume of water from a runoff surface. Volumes used for first flush analysis include the capture and analysis of 5 L (Uba and Aghogho, 2000; Yaziz, Gunting, et al., 1989), and 65 L (Van Metre and Mahler, 2003). The study done by Yaziz et al. is of particular interest because it collects five consecutive liters of water to explicitly determine a first flush by volume. The methodology proposed by Yaziz et al. was used as the primary method for first flush analysis for pilot rooftop runoff analysis and also as a guide for first flush collection for the data displayed in the previous works section of this proposal (Yaziz, Gunting, et al., 1989).

Pilot and Full Scale Roofing

Two separate methodologies exist for measuring rooftop contamination when considering selecting a rooftop type; choosing a roof attached to a building, or building pilot-scale roofs. It is possible to build pilot scale roofs, allowing for a control on specific independent variables which is not possible for full-scale roof projects.

One major advantage for projects utilizing pilot scale rooftops is the ability to control specific project variables. The projects most often take advantage of being able to control pilot roof location and material. The pilot roofs tend to be built immediately before the project begins, so studies can eliminate roof age as a potential source of contamination. While there are noticeable benefits that come with building pilot roofs, few researchers follow the pilot-scale roof runoff methodology. Chang et al. used their study on sixteen roofing structures to study specific roofing options such as orientation, slope, industrial treatments, materials, maintenance, and age (Chang, McBroom, et al., 2004). A study completed by Winters et al. focused on rooftop leaching, establishing that while new roofs seem to not leach polycyclic aromatic hydrocarbons (PAHs) or polybrominated diphenyl ethers (PBDEs), roof coatings can reduce further leaching from roofs using materials which contain copper and zinc. Copper and zinc are primary

pollutants commonly found in rooftop runoff (Winters and Graunke, 2014). Zhang et al. studied roof age, type, and roughness over varying seasons, with results suggesting that not only does the roof type matter, but that pollutant loading seems to be heavier in the spring and winter seasons (Zhang, Wang, et al., 2014).

A study completed by Mendez et al. utilized both pilot and full scale roofs (Mendez, Klenzendorf, et al., 2011). This study took seven storms, three for full scale, and four for pilot roofs, and used a first flush diversion methodology. Composite post-first flush samples were also collected. Findings suggest three main conclusions. The first conclusion is that roof type matters, and the second being that treatment is required before rooftop runoff can be reused. Treatment is especially important if the water will be used as a drinking water source. The final conclusion for the paper by Mendez et al. is that similar runoff can be seen between pilot scale roofs and aged full scale roofs. This implies that roof age may not play a strong role in rooftop runoff quality (Mendez, Klenzendorf, et al., 2011).

Existing rooftops are the primary focus of many rooftop runoff quality studies. Projects focused on aged rooftops tend to emphasize roof type and runoff quality. Additional experiments which found that flat roofs have a higher ability to store pollutants were completed by Bucheli et al., and Farreny et al. (Bucheli, Müller, et al., 1998; Farreny, Morales-Pinzón, et al., 2011). Roofing material is also known to be an important factor in pollutant presence in rooftop runoff (Gromaire, Garnaud, et al., 2001). A common conclusion for full scale rooftop papers is that treatment is needed before the runoff can be reused (Kim, Lee, et al., 2005). First flush and first flush diversion studies on full scale roofs were completed by Schriewer et al., Lee et al., Gikas et al., and Forster et al. (Schriewer, Horn, et al., 2008; Lee, Yang, et al., 2010; Gikas and Tsihrintzis, 2012; Förster, 1998; Förster, 1999; Förster, 1996). Dry deposition over dry periods was found to be a contributing factor in pollution, especially for bacteria by Evans et al., Yaziz et al. nearby sources onto rooftops. One unique study completed by Van Metre et al. hosed off the rooftop surfaces without relying on rainwater quality, removing independent variables related to environmental factors (Van Metre and Mahler, 2003). The majority of full scale rooftop runoff projects measure contaminants collected from both the rooftop and gutter system, but Uba et al. measured direct rooftop runoff without passage through a gutter system (Uba and Aghogho, 2000). Work by Uba et al. could lay groundwork for determining contaminants passed from gutter systems separate from rooftop runoff, as Melidis et al. believed that zinc was coming from drainage pipe erosion and roof age (Melidis, Akratos, et al., 2007).

Characteristics Affecting Runoff Quality

Disparities occur due to the inconsistent nature of rooftop runoff. Primary factors which seem to affect rooftop runoff quality are sampling techniques and rooftop and environmental factors. Additional parameters relate to transport kinetics of pollutants both depositing on and washing off of a rooftop. Various pollutants found on rooftops do not wash off at the same point in time during a storm.

Rooftop Characteristics

Rooftops can be separated by three major characteristics including type, age, and surface roughness. Roof age is a characteristic which has been shown to potentially affect rooftop leaching (Zhang, Wang, et al., 2014; Winters and Graunke, 2014). Chang et al. also investigated rooftop roughness, finding that cracks in roof surfaces encouraged the growth of organics on rooftop surfaces, which can wash off during storm events (Chang, McBroom, et al., 2004).

Rooftop material is the most commonly studied rooftop characteristic. Rooftop materials studied include shingle, sheet, tile, and green roofs. Intended rooftop slope can also affect selected roofing materials. Sloped roofs more regularly used shingles, tiles, and sheet materials. Ethylene propylene diene terpolymer (EPDM) (Winters and Graunke, 2014) which is a rubber roofing material, tar felt (Förster, 1999; Förster, 1998; Förster, 1996), fibrous cement (Förster, 1998; Förster, 1999; Förster, 1996), and bitumen (Mendez, Klenzendorf, et al., 2011) are frequently used for flat roofs. Flat roofs have been shown to be a pollutant sink, lowering the pollutant contribution from the rooftops into the surrounding watershed or groundwater system.

Common shingle materials include asphalt (Mendez, Klenzendorf, et al., 2011; Van Metre and Mahler, 2003; Zhang, Wang, et al., 2014), wood (Chang, McBroom, et al., 2004; Winters and Graunke, 2014), concrete (Zhang, Wang, et al., 2014; Gikas and Tsihrintzis, 2012; Förster, 1998; Förster, 1999; Förster, 1996; Egodawatta, Thomas, et al., 2009; Melidis, Akratos, et al., 2007), and composition (Chang, McBroom, et al., 2004). Asphalt shingle roof runoff has been found to have high amounts of zinc (Zhang, Wang, et al., 2014), lead (Van Metre and Mahler, 2003), and mercury (Van Metre and Mahler, 2003). Asphalt shingle roofs also show signs of first flush effects for pH, conductivity, and total and fecal coliforms (Mendez, Klenzendorf, et al., 2011). Wood roof shingles are less common, and need to be maintained to prevent excess decomposition. This decomposition leads to the release of extra ions, lowering pH, and increasing zinc and conductivity in the runoff (Chang, McBroom, et al., 2004). Winters et al. support the idea that extra ions and metals are released from wood roofing materials, as they found high concentrations of arsenic, copper, and zinc (Winters and Graunke, 2014). Concrete roofing materials may affect suspended solids, pH, and calcium, but are generally no worse than other standard roofing materials (Zhang, Wang, et al., 2014). Chang et al. found that new shingles, including composite shingles, are more likely to leach chemicals than aged shingles (Chang, McBroom, et al., 2004).

Tile roofs are constructed with ceramic (Zhang, Wang, et al., 2014), clay (Gikas and Tsihrintzis, 2012), slate (Gromaire, Garnaud, et al., 2001), or concrete (Egodawatta, Thomas, et al., 2009). Of all roof types tested, tile roofs seem to provide a cleaner runoff, similar to that of concrete shingle roofing. Zhang et al. found that clay tile had fewer ion contaminants than green and concrete roofs, and less total nitrogen and total organic carbon than asphalt shingle roofs. In winter however, ceramic tile runoff had the highest concentration of total organic carbon, total phosphorus, and zinc (Zhang, Wang, et al., 2014). Clay and concrete tiles were found to be susceptible to first flush effects for ions, but no trends were seen for bacterial contamination (Gikas and Tsihrintzis, 2012). Metals were found in higher quantities than in comparable literature

for lead, and in elevated levels for cadmium and zinc for slate, interlocking, and flat tile roofs (Gromaire, Garnaudo, et al., 2001). According to Egodawatta et al., particulate matter wash off does not seem to fluctuate between roofing materials (Egodawatta, Thomas, et al., 2009).

Sheet style roofs are commonly made from galvanized-metal (Van Metre and Mahler, 2003), specifically iron (Chang, McBroom, et al., 2004). Other metallic materials include copper (Winters and Graunke, 2014), zincalume (Winters and Graunke, 2014), galvalume (Mendez, Klenzendorf, et al., 2011), corrugated steel (Egodawatta, Thomas, et al., 2009), aluminum (Chang, McBroom, et al., 2004; Uba and Aghogho, 2000), and cast iron (Gromaire, Garnaudo, et al., 2001). One common trend between metal roofing materials is the increased occurrence of heavy metals found in runoff, especially for aluminum, galvanized metal, copper, zincalume, and zinc roofing materials (Chang, McBroom, et al., 2004; Van Metre and Mahler, 2003; Winters and Graunke, 2014; Uba and Aghogho, 2000; Gromaire, Garnaudo, et al., 2001). Sheet style roofing can also be made from non-metal materials such as polycarbonate plastic (Farreny, Morales-Pinzón, et al., 2011) or polyester (Bucheli, Müller, et al., 1998).

Green roofs are unfertilized grass or plant covered rooftops (Mendez, Klenzendorf, et al., 2011; Zhang, Wang, et al., 2014). Green roofs are a relatively uncommon roofing material, especially in the United States. Mendez et al. found that green roofs seemed to have higher concentrations of dissolved organic carbons (Mendez, Klenzendorf, et al., 2011). Zhang et al. determined that runoff quality from green roofs is so poor that they should not be used for rainwater harvesting (Zhang, Wang, et al., 2014). Pollutant parameters which were found to be unacceptable for green roofs studied by Zhang et al. include total nitrogen, total organic carbon, chemical oxygen demand, conductivity, copper, potassium, sodium, magnesium, chloride, sulfate, and silicate. The only advantage provided by Green roofs was a reduction in the amount of suspended solids and turbidity in the runoff (Mendez, Klenzendorf, et al., 2011).

Environmental Characteristics

Environmental characteristics which seem to affect rooftop runoff quality can be broken into two categories including storm characteristics, and geographical characteristics. Separation is necessary because the general location of sampled rooftops along with surrounding pollutant sources have been found to affect rooftop runoff quality.

Storm characteristics are variable, non-controllable influences on rooftop runoff quality. These characteristics include total precipitation (Farreny, Morales-Pinzón, et al., 2011; Zhang, Wang, et al., 2014), rainfall intensity (Schriewer, Horn, et al., 2008; Zhang, Wang, et al., 2014; Förster, 1998; Förster, 1999; Förster, 1996), and antecedent dry period (ADP) (C. A. Evans, Coombes, et al., 2006; Schriewer, Horn, et al., 2008; Zhang, Wang, et al., 2014; Förster, 1998; Förster, 1999; Förster, 1996). Of all storm characteristics, rainfall intensity seems to be the most influential. Positive correlations have been found repeatedly between rainfall intensity and pollutant wash off. The total amount of precipitation seems to have an inverse relationship with pollutant severity due to dilution (Farreny, Morales-Pinzón, et al., 2011). The ADP relates to atmospheric deposition. A longer dry period between rain events increases the quantity of pollutants

which are transported to roofs via atmospheric deposition. A dry deposition impact plateau is reached after about a week. Not all pollutants are related to atmospheric deposition, but ones that are include *Pseudomonas* spp. (C. A. Evans, Coombes, et al., 2006; Evans, Coombes, et al., 2007) and nitrate (Farreny, Morales-Pinzón, et al., 2011).

Geographical influences on rooftop runoff quality include non-storm related weather such as climate, season, air quality, humidity, wind speed, wind direction, and location based influences. Location based influences include the distance of sampling points from roads, and rooftop orientation. Wind speed and wind direction are the most important factors caused by geography on rooftop runoff quality. Wind velocity appears to show evidence of wet deposition from bacteria, specifically heterotrophic bacterium. This finding is important because the organisms are likely being lifted and transported from nearby sources in relation to wind direction (C. A. Evans, Coombes, et al., 2006; Evans, Coombes, et al., 2007). Humidity is likely inversely related to heterotrophic bacterium (C. A. Evans, Coombes, et al., 2006). Zhang et al. studied geographical characteristics with a focus on seasonal, atmospheric, and surrounding sources of pollution. They found that seasons do indeed affect runoff quality, with increased pollutants found in winter and spring (Zhang, Wang, et al., 2014). Chang et al. studied the effects of roof orientation, finding that only wooden roofs which orient in the direction of incoming storms tend to have increases in conductivity, manganese, magnesium, and zinc (Chang, McBroom, et al., 2004). One predominant geographical influence is the distance of the target roof from a nearby busy roadway. Van Metre et al. found that the closer a rooftop is to a major roadway the fewer pollutants were found (Van Metre and Mahler, 2003).

Contaminants of Concern and Risks to Public Health

Pollutants found in rooftop runoff and in cisterns vary based on different characteristics such as location, storm event characteristics, and rooftop characteristics. Commonly measured parameters measured include pH, conductivity, total suspended solids (TSS), turbidity, anions, cations, metals, and organic pollutants. While less frequently monitored, bacteria and pathogens can pose a more serious threat to public health. Since rooftop runoff is used in drinking water applications, comparison to EPA primary and secondary drinking water standards is common (Table 1).

pH and Conductivity

pH is a concern for rainwater due to acid rain, water chemistry, and bacterial growth and survival. Conductivity can provide insight into ionic activity from runoff samples. A review of rooftop runoff literature suggests that most pH values fall within drinking water standards (Table 2) (Gikas and Tsihrintzis, 2012; Melidis, Akrotos, et al., 2007; Farreny, Morales-Pinzón, et al., 2011; Zhang, Wang, et al., 2014; Kim, Lee, et al., 2005; Lee, Kim, et al., 2011; Yaziz, Gunting, et al., 1989; Uba and Aghogho, 2000; Mendez, Klenzendorf, et al., 2011). Only two groups reported pH values outside of the acceptable range, one with highly acidic conditions being reported in the rooftop runoff (Schriewer, Horn, et al., 2008; Chang, McBroom, et al., 2004). Conductivity seems to be generally inconsistent within rooftop runoff. A decrease in conductivity and a general

change of pH towards a more acidic condition was found by Yaziz et al. in their first flush study (Yaziz, Gunting, et al., 1989).

Table 1. EPA Primary and Secondary Drinking Water Standards.

(Environmental Protection Agency, 2009; Environmental Protection Agency, 2016b).

Contaminant	MCLG (mg/L)	MCL or TT (mg/L)	Secondary MCL
Heterotrophic bacteria	n/a	500 colonies per mL	-
Total coliforms	zero	5.00%	-
Turbidity	n/a	>0.3 NTU 95%	-
Copper	1.3	Action Level=1.3	1.0 mg/L
Lead	zero	Action Level=0.015	-
Mercury	0.002	0.002	-
Nitrate-N	10	10	-
Nitrite-N	1	1	-
Aluminum	-	-	0.05 to 0.2 mg/L
Chloride	-	-	250 mg/L
Iron	-	-	0.3 mg/L
Sulfate	-	-	250 mg/L
Zinc	-	-	5 mg/L
pH	-	-	6.5 - 8.5

Total Suspended Solids and Turbidity

Total suspended solids (TSS) and turbidity are frequently measured indicators of water quality. Total suspended solids and turbidity tend to be related, but the methodology for turbidity is generally cheaper and faster than that for TSS. Rooftop runoff solids content tends to be relatively low (Table 3), which may necessitate the use of turbidity to determine particulate content in rooftop runoff. Many studies use turbidity or TSS to measure rooftop runoff quality (Gromaire, Garnaudo, et al., 2001; Gikas and Tsihrantzis, 2012; Farreny, Morales-Pinzón, et al., 2011; Zhang, Wang, et al., 2014; Kim, Lee, et al., 2005; Uba and Aghogho, 2000; Mendez, Klenzendorf, et al., 2011; Van Metre and Mahler, 2003), and work completed by Yaziz et al. showed a flushing effect for both (Yaziz, Gunting, et al., 1989).

Bacteria and Pathogens

Bacterial and pathogenic content in rooftop runoff is not a new concept; however more studies have focused on cistern water quality rather than direct rooftop runoff due to the increased likelihood of bacterial ingestion. There is no evidence that a first flush is present for any type of bacterium or pathogen found in rooftop runoff (Yaziz, Gunting, et al., 1989). Bacteria seem to remain constant or even show up in random spiking increases throughout the duration of a storm.

Table 2. pH and Conductivity of Various Rooftop Runoff Projects.

Country	Roof Type	pH		Conductivity ($\mu\text{S/cm}$)		Reference
		Range	Mean	Range	Mean	
Germany	Zinc	5.8-8.4	6.7	10-242	50	(Schriewer et al., 2008)
Greece	Clay Tiles	-	6.49	-	394	(Gikas & Tsihrintzis, 2012)
	Concrete	-	6.55	-	256	
	Concrete	-	7.04	-	272	
	Clay Tiles	-	6.45	-	203	
	Clay Tiles	-	6.44	-	67	
Greece	Concrete	-	8.3	-	-	(Melidis et al., 2007)
	Mosaic	-	7.8	-	-	
	Mosaic	-	7.8	-	-	
	Clay Tiles	-	7.7	-	-	
	Concrete	-	7.9	-	-	
	Concrete	-	7.8	-	-	
	Iron-Sheet	-	7.8	-	-	
	Mosaic	-	7.9	-	-	
	Maxitherm	-	7.5	-	-	
	Mosaic	-	7.4	-	-	
Spain	Clay Tiles	6.75-8.3	7.6	20-200	75	(Farreny et al., 2011)
	Metal	6.7-8.4	7.6	10-80	60	
	Plastic	6.55-8.4	7.4	15-85	50	
	Flat Gravel	7.15-8.35	7.7	45-230	150	
China	Concrete	6.75-8.4	7.7	25-325	120	(Zhang et al., 2014)
	Asphalt	6.4-7.75	7	25-325	100	
	Ceramic Tile	6.5-8.75	7.1	25-150	50	
	Green	6.25-7.5	6.75	60-450	200	
Korea	Cement	-	6.9	-	28.5	(Kim et al., 2005)
	Green	-	7.8	-	167.5	
	Green	-	6.5	-	84.3	
Korea	PVC	6.7-7.8	7.3	50-340	170	(J. Y. Lee, Yang, Han, & Choi, 2010)
Malaysia	Galvanized Iron a	-	6.6	-	97	(Yaziz et al., 1989)
	Galvanized Iron b	-	6.6	-	85.5	
	Galvanized Iron c	-	6.6	-	72.1	
	Galvanized Iron d	-	6.5	-	59.3	
	Galvanized Iron e	-	6.4	-	50.7	
	Concrete Tile a	-	6.9	-	135.2	
	Concrete Tile b	-	6.9	-	121.1	
	Concrete Tile c	-	6.9	-	106.7	
	Concrete Tile d	-	6.9	-	94.7	
	Concrete Tile e	-	6.8	-	86.5	
Nigeria	Aluminum	-	7.08	-	-	(Uba & Aghogho, 2000)
	Zinc	-	7.3	-	-	
United States	Wood Shingle	3.33-6.89	5.07	7.0-232.0	38.78	(Chang et al., 2004)
	Composition Shingle	4.08-8.25	6.69	6.0-179.0	30.19	
	Aluminum	4.78-7.26	6.2	2.20-57.0	14.53	
	Galvanized Iron	3.62-7.41	6.59	4.0-172.0	20.34	
United States	Asphalt Fiberglass Shingle (Pilot)	6.7-6.9	-	18-57	-	(Mendez et al., 2011)
	Asphalt Fiberglass Shingle (Full)	5.8-6.5	-	20-102	-	
	Galvalume (Pilot)	6.0-6.8	-	9-56	-	
	Galvalume (Full)	5.4-6.3	-	18-60	-	

Table 3. Turbidity and Total Suspended Solids Reported for Rooftop Runoff.

Country	Roof Type	TSS (mg/L)		Turbidity (NTU)		Reference
		Range	Mean	Range	Mean	
France	zinc sheet, slate, tile	7-74	17	-	-	(Gromaire et al., 2001)
Greece	Clay Tiles	-	39.5	-	-	(Gikas & Tsihrintzis, 2012)
	Concrete	-	10.5	-	-	
	Concrete	-	9.5	-	-	
	Clay Tiles	-	12.9	-	-	
	Clay Tiles	-	10.2	-	-	
Spain	Clay Tiles	0-21	4	-	-	(Farreny et al., 2011)
	Metal	0-11	4	-	-	
	Plastic	0-18	5	-	-	
	Flat Gravel	0-20	5	-	-	
China	Concrete	0-375	90	-	-	(Zhang et al., 2014)
	Asphalt	0-150	50	-	-	
	Ceramic Tile	0-100	25	-	-	
	Green	0-50	20	-	-	
Korea	Cement	-	-	-	2.2	(Kim et al., 2005)
	Green	-	-	-	2.8	
	Green	-	-	-	2.8	
Malaysia	Galvanized Iron a	-	91	-	22	(Yaziz et al., 1989)
	Galvanized Iron b	-	81	-	18	
	Galvanized Iron c	-	73	-	15	
	Galvanized Iron d	-	63	-	12	
	Galvanized Iron e	-	52	-	10	
	Concrete Tile a	-	153	-	56	
	Concrete Tile b	-	141	-	47	
	Concrete Tile c	-	125	-	41	
	Concrete Tile d	-	106	-	33	
	Concrete Tile e	-	95	-	24	
Nigeria	Aluminum	-	0.28	-	-	(Uba & Aghogho, 2000)
	Zinc	-	0.43	-	-	
United States	Asphalt Fiberglass Shingle (Pilot)	12-54	-	8-24	-	(Mendez et al., 2011)
	Asphalt Fiberglass Shingle (Full)	20-150	-	6-23	-	
	Galvalume (Pilot)	20-87	-	7-30	-	
	Galvalume (Full)	10-50	-	5-35	-	
United States	Metal	-	122	-	-	(Van Metre & Mahler, 2003)
	Metal	-	278	-	-	
	Asphalt Shingle	-	297	-	-	
	Asphalt Shingle	-	128.33	-	-	

Table 4. Bacteria and Pathogens Commonly Studied in Rooftop Runoff Settings.

Country	Roof Type	Total Viable Bacteria (1/mL)		Heterotrophic Plate Count (CFU/mL)		Total Coliform (CFU/100 mL)		Fecal Coliform (CFU/100 mL)		<i>Pseudomonas</i> spp. (CFU/100 mL)		Reference
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	
Australia	Colourbond	-	-	-	1362	-	<4	-	<2	-	-	(C. A. Evans, Coombes, & Dunstan, 2006)
Korea	PVC	-	-	-	-	0-320	70	-	-	-	596	(J. Y. Lee, Yang, Han, & Choi, 2010)
Korea	Cement	-	351	-	-	-	-	-	-	-	-	(Kim et al., 2005)
	Green	-	341	-	-	-	-	-	-	-	-	
	Green	-	271	-	-	-	-	-	-	-	-	
Malaysia	Galvanized Iron a	-	32	-	-	-	46	-	4	-	-	(Yaziz et al., 1989)
	Galvanized Iron b	-	31	-	-	-	63	-	8	-	-	
	Galvanized Iron c	-	25	-	-	-	63	-	4	-	-	
	Galvanized Iron d	-	21	-	-	-	38	-	0	-	-	
	Galvanized Iron e	-	22	-	-	-	25	-	0	-	-	
	Concrete Tile a	-	51	-	-	-	75	-	13	-	-	
	Concrete Tile b	-	46	-	-	-	46	-	8	-	-	
	Concrete Tile c	-	48	-	-	-	48	-	8	-	-	
	Concrete Tile d	-	44	-	-	-	44	-	0	-	-	
	Concrete Tile e	-	41	-	-	-	41	-	0	-	-	
Nigeria	Aluminum	-	-	-	1.2x10 ³	-	39	-	0	-	880	(Uba & Aghogho, 2000)
	Zinc	-	-	-	5.9x10 ³	-	<3	-	0	-	0	
United States	Asphalt Fiberglass Shingle (Pilot)	-	-	-	-	117-1367	-	9-87	-	-	-	(Mendez et al., 2011)
	Asphalt Fiberglass Shingle (Full)	-	-	-	-	102-353	-	73-253	-	-	-	
	Galvalume (Pilot)	-	-	-	-	117-770	-	<1-8	-	-	-	
	Galvalume (Full)	-	-	-	-	64-173	-	37-127	-	-	-	

Even though coliform and bacterial counts tend to be relatively low, colony forming units present in wash off are still higher than allowed by drinking water quality laws (Table 4) (C. A. Evans, Coombes, et al., 2006; Lee, Kim, et al., 2011; Kim, Lee, et al., 2005; Yaziz, Gunting, et al., 1989; Uba and Aghogho, 2000; Mendez, Klenzendorf, et al., 2011). Uba et al. measured many different types of pathogens not shown in Table 4. This includes Fecal *Streptococcus*, *Vibrio* spp., *Salmonella* spp., *Shigella* spp., filamentous fungi, and yeast. Only fecal *Streptococcus* was not detected (Uba and Aghogho, 2000). *Escherichia coli* was only measured in one study, likely due to the low abundance of coliform bacteria found in rooftop runoff (Lee, Kim, et al., 2011). One frequently occurring and measured pathogen is *Pseudomonas* spp. (Uba and Aghogho, 2000; Lee, Kim, et al., 2011). *Pseudomonas* spp. is commonly found in RWH tanks, even after long term storage (Lye, 1987). Further studies into bacterial wash off are necessary for rooftop runoff.

Ions

Ionic contamination, especially in the form of hydrogen, nitrogen, or phosphorus, can have a profound effect on hydrological ecosystems. Increased ionic concentrations can upset bacterial balances and be hazardous to human health. Nitrogen in the form of nitrate, nitrite, and ammonium are important indicators of aquatic health. Excess nitrogen washing off a rooftop eventually reaches a formed watershed, with the potential to cause algal blooms. Nitrates and sulfates are also known byproducts of fossil fuel combustion, increasing chances for presence in atmospheric deposition. Seasonal effects are also prevalent when considering chloride and sodium, which tend to be found in increased quantities during winter months due to salting. It is likely that first flush tendencies exist for certain anions and cations, including sulfate, nitrate, nitrite, chloride, sodium, potassium, ammonium, and calcium (Gikas and Tsihrintzis, 2012).

Anions (Table 5) commonly found in rooftop runoff include chloride, sulfate, nitrate, and nitrite (C. A. Evans, Coombes, et al., 2006; Gikas and Tsihrintzis, 2012; Melidis, Akratos, et al., 2007; Farreny, Morales-Pinzón, et al., 2011; Zhang, Wang, et al., 2014; Lee, Kim, et al., 2011; Uba and Aghogho, 2000; Mendez, Klenzendorf, et al., 2011). Fluoride (Zhang, Wang, et al., 2014) and phosphate (Uba and Aghogho, 2000) occur at a relatively low concentration when detected and measured. Phosphorus is also more commonly measured as total phosphorus. Concentrations for all anions were lower than drinking water standards (Environmental Protection Agency, 2009). Separate studies have found that nitrates may be dependent on dry deposition (Mendez, Klenzendorf, et al., 2011; Förster, 1998). Support for this theory can be found in a study completed by Melidis et al. where areas with higher traffic volumes and population density showed greater concentrations of nitrate in rooftop runoff (Melidis, Akratos, et al., 2007).

Table 5. Commonly Reported Anions in Rooftop Runoff.

Country	Roof Type	Chloride (mg/L)		Sulfate (mg/L)		Nitrate (mg/L)		Nitrite (mg/L)		Reference
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	
Australia	Colourbond	-	15.75	-	7.59	-	2.24	-	-	(C. A. Evans, Coombes, & Dunstan, 2006)
Greece	Clay Tiles	-	-	-	-	-	0.46	-	0.14	(Gikas & Tsihrintzis, 2012)
	Concrete	-	-	-	-	-	0.68	-	0.15	
	Concrete	-	-	-	-	-	0.62	-	0.04	
	Clay Tiles	-	-	-	-	-	0.36	-	0.08	
	Clay Tiles	-	-	-	-	-	0.59	-	0.12	
Greece	Concrete	-	-	-	0.09	-	0.29	-	-	(Melidis et al., 2007)
	Mosaic	-	-	-	0.025	-	0.51	-	-	
	Mosaic	-	-	-	0.02	-	0.35	-	-	
	Clay Tiles	-	-	-	0.02	-	0.36	-	-	
	Concrete	-	-	-	0.045	-	0.61	-	-	
	Concrete	-	-	-	0.21	-	0.32	-	-	
	Iron-Sheet	-	-	-	0.05	-	0.46	-	-	
	Mosaic	-	-	-	0.02	-	0.19	-	-	
	Maxitherm	-	-	-	0.025	-	0.29	-	-	
	Mosaic	-	-	-	0.09	-	0.195	-	-	
Spain	Clay Tiles	-	-	0-5.8	2.5	0.1-1.75	1.5	-	-	(Farreny et al., 2011)
	Metal	-	-	0.5-6	2.7	0.75-1.8	1	-	-	
	Plastic	-	-	0.5-6	2.7	0-2.5	1	-	-	
	Flat Gravel	-	-	1-11.5	5.9	0-9	1.5	-	-	
China	Concrete	3-13	6	1-110	30	-	-	-	-	(Zhang et al., 2014)
	Asphalt	3-9	5	1-110	20	-	-	-	-	
	Ceramic Tile	3-8	4	1-40	15	-	-	-	-	
	Green	2-60	10	20-125	30	-	-	-	-	
Korea	PVC	5-18	7.5	2-7.2	4.1	2.9-9.8	6.8	-	-	(J. Y. Lee, Yang, Han, & Choi, 2010)
Korea	Cement	-	-	-	-	-	0.46	-	-	(Kim et al., 2005)
	Green	-	-	-	-	-	1.68	-	-	
	Green	-	-	-	-	-	1.44	-	-	
Nigeria	Aluminum	-	0.06	-	0.071	-	6.2	-	0.02	(Uba & Aghogho, 2000)
	Zinc	-	0.067	-	0.075	-	5.61	-	0.05	
United States	Asphalt Fiberglass Shingle (Pilot)	-	-	-	-	0.0-1.8	-	0.01-0.04	-	(Mendez et al., 2011)
	Asphalt Fiberglass Shingle (Full)	-	-	-	-	0.3-4.7	-	0.01-0.06	-	
	Galvalume (Pilot)	-	-	-	-	0-2	-	0.01-0.03	-	
	Galvalume (Full)	-	-	-	-	0.4-4.1	-	0.01-0.05	-	

Cations (Table 6) of primary interest include ammonium, calcium, potassium, and sodium (C. A. Evans, Coombes, et al., 2006; Gikas and Tsihrintzis, 2012; Melidis, Akratos, et al., 2007; Farreny, Morales-Pinzón, et al., 2011; Zhang, Wang, et al., 2014; Lee, Kim, et al., 2011; Uba and Aghogho, 2000). Ammonium concentrations varied, but were higher than expected for a study in Greece (Gikas and Tsihrintzis, 2012). Calcium was found in high concentrations in China (Zhang, Wang, et al., 2014).

Metals

Heavy metals are of great concern to public health. Metal leaching more commonly occurs on metal rooftop surfaces. For water reused for drinking water purposes, this could pose major health threats. Copper, iron, zinc, and lead are the four primary metals of concern in rooftop runoff, but aluminum, magnesium, and manganese have also been found (Table 7) (Gromaire, Garnaud, et al., 2001; Schriewer, Horn, et al., 2008; Melidis, Akratos, et al., 2007; Zhang, Wang, et al., 2014; Kim, Lee, et al., 2005; Lee, Kim, et al., 2011; Yaziz, Gunting, et al., 1989; Uba and Aghogho, 2000; Mendez, Klenzendorf, et al., 2011; Chang, McBroom, et al., 2004). Aluminum (Chang, McBroom, et al., 2004; Uba and Aghogho, 2000; Lee, Kim, et al., 2011), copper (Zhang, Wang, et al., 2014; Lee, Kim, et al., 2011), lead (Gromaire, Garnaud, et al., 2001; Schriewer, Horn, et al., 2008; Mendez, Klenzendorf, et al., 2011; Chang, McBroom, et al., 2004), zinc (Schriewer, Horn, et al., 2008; Zhang, Wang, et al., 2014; Lee, Kim, et al., 2011; Chang, McBroom, et al., 2004), and iron (Gromaire, Garnaud, et al., 2001; Lee, Kim, et al., 2011) were all reported to exceed primary and secondary drinking water standards. A commonly held belief about rooftop runoff is that both gutter and rooftop materials may leach metals, requiring further investigation into alternative roofing materials. Gromaire et al. believes that rooftops are the source of heavy metals in rooftop runoff (Gromaire, Garnaud, et al., 2001; Förster, 1999). The study conducted by Yaziz et al. shows that first flush effects are unlikely for both zinc and lead, as concentrations increased during collection (Yaziz, Gunting, et al., 1989). Another study conducted suggests that zinc does follow a first flush pattern (Schriewer, Horn, et al., 2008). Mendez et al. found that aluminum, arsenic, copper, iron, lead, and zinc all showed signs of first flush removal (Mendez, Klenzendorf, et al., 2011). Inconsistencies between data and methodologies show that the lack of understanding of first flush effects on rooftop runoff is a serious detriment to the future of RWH.

Total Organic Carbon, Total Nitrogen, and Total Phosphorus

Organic compounds have been found in multiple rooftop runoff sources. Primary organic concerns center around total organic carbon (TOC), total phosphorous (TP), and total nitrogen (TN) (Table 8) (Schriewer, Horn, et al., 2008; Gikas and Tsihrintzis, 2012; Farreny, Morales-Pinzón, et al., 2011; Zhang, Wang, et al., 2014; Kim, Lee, et al., 2005; Mendez, Klenzendorf, et al., 2011). Nitrogen, phosphorus, and carbon are directly responsible for microbial and algal growth.

Table 6. Cations Common to Rooftop Runoff.

Country	Roof Type	Ammonium (mg/L)		Calcium (mg/L)		Potassium (mg/L)		Sodium (mg/L)		Reference
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	
Australia	Colourbond	-	-	-	2.80	-	-	-	10.64	(C. A. Evans, Coombes, & Dunstan, 2006)
Greece	Clay Tiles	-	32.97	-	-	-	-	-	-	(Gikas & Tsihrintzis, 2012)
	Concrete	-	8.31	-	-	-	-	-	-	
	Concrete	-	2.37	-	-	-	-	-	-	
	Clay Tiles	-	10.84	-	-	-	-	-	-	
Greece	Clay Tiles	-	1.71	-	-	-	-	-	-	(Melidis et al., 2007)
	Concrete	-	0.39	-	-	-	-	-	-	
	Mosaic	-	0.15	-	-	-	-	-	-	
	Mosaic	-	0.7	-	-	-	-	-	-	
	Clay Tiles	-	0.3	-	-	-	-	-	-	
	Concrete	-	0.25	-	-	-	-	-	-	
	Concrete	-	0.18	-	-	-	-	-	-	
	Iron-Sheet	-	1.3	-	-	-	-	-	-	
	Mosaic	-	0.41	-	-	-	-	-	-	
	Maxitherm	-	0.53	-	-	-	-	-	-	
Spain	Mosaic	-	0.5	-	-	-	-	-	-	(Farreny et al., 2011)
	Clay Tiles	0.05-0.35	0.2	-	-	-	-	-	-	
	Metal	0.2-0.9	0.4	-	-	-	-	-	-	
	Plastic	0.03-1.1	0.45	-	-	-	-	-	-	
China	Flat Gravel	0.05-0.2	0.1	-	-	-	-	-	-	(Zhang et al., 2014)
	Concrete	-	-	5-57	25	2-9	3	0-3.5	1	
	Asphalt	-	-	5-45	11	0-3	1	0-2.5	0.5	
	Ceramic Tile	-	-	5-17	10	1-4	2	0.5-1.5	0.75	
Korea	Green	-	-	8-70	27	0-35	5	1.75-6.5	3	(J. Y. Lee, Yang, Han, & Choi, 2010)
	PVC	0.06-0.39	0.09	3.24-15.4	6.4	1.3-5.9	3.1	2.2-6.1	3.2	
Nigeria	Aluminum	-	-	-	0.71	-	0.074	-	0.05	(Uba & Aghogho, 2000)
	Zinc	-	-	-	0.22	-	0.038	-	0.043	

Table 7. Common Metals Found in Rooftop Runoff.

Country	Roof Type	Aluminum (mg/L)		Copper (mg/L)		Iron (mg/L)		Lead (mg/L)		Magnesium (mg/L)		Manganese (mg/L)		Zinc (mg/L)		Reference
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	
France	zinc sheet, slate, tile	-	-	0.014-0.240	0.043	-	-	0.076-2.458	0.392	-	-	-	-	0.582-12.357	2.998	(Gromaire et al., 2001)
Germany	Zinc	-	-	-	-	-	-	nd-0.031	-	-	-	-	-	0.3-30	6.8	(Schriewer et al., 2008)
Greece	Concrete	-	-	-	-	-	0.15	-	-	-	-	-	0.028	-	0.052	(Melidis et al., 2007)
	Mosaic	-	-	-	-	-	0.021	-	-	-	-	-	0.01	-	0.03	
	Mosaic	-	-	-	-	-	0.021	-	-	-	-	-	0.007	-	0.02	
	Clay Tiles	-	-	-	-	-	0.039	-	-	-	-	-	0.01	-	0.4	
	Concrete	-	-	-	-	-	0.019	-	-	-	-	-	0.01	-	0.54	
	Concrete	-	-	-	-	-	0.03	-	-	-	-	-	0.011	-	0.1	
	Iron-Sheet	-	-	-	-	-	0.195	-	-	-	-	-	0.068	-	0.08	
	Mosaic	-	-	-	-	-	0.09	-	-	-	-	-	0.037	-	0.07	
	Maxitherm	-	-	-	-	-	0.03	-	-	-	-	-	0.013	-	0.34	
	Mosaic	-	-	-	-	-	0.03	-	-	-	-	-	0.005	-	0.05	
	Concrete	-	-	0-10	5	-	-	-	-	0-3	1	-	-	0-30	10	(Zhang et al., 2014)
	Asphalt	-	-	0-15	5	-	-	-	-	0-1.75	0.5	-	-	0-150	50	
China	Ceramic Tile	-	-	0-5	2.5	-	-	-	-	0-2	0.6	-	-	0-75	25	
	Green	-	-	0-45	9	-	-	-	-	1-11	5	-	-	0-225	35	
	Cement	-	0.1	-	0.04	-	0.02	-	-	-	-	-	-	-	0.08	(Kim et al., 2005)
	Green	-	0.07	-	0.17	-	0.06	-	-	-	-	-	-	-	0.14	
Korea	Green	-	0.18	-	0.18	-	0.12	-	-	-	-	-	-	-	0.17	
Korea	PVC	100-400	225	70-120	85	-	-	10-40	27	0.5-2.7	1.2	70-170	115	120-280	160	(J. Y. Lee, Yang, Han, & Choi, 2010)
Malaysia	Galvanized Iron a	-	-	-	-	-	-	-	0.235	-	-	-	-	-	0.343	(Yaziz et al., 1989)
	Galvanized Iron b	-	-	-	-	-	-	-	0.254	-	-	-	-	-	0.489	
	Galvanized Iron c	-	-	-	-	-	-	-	0.194	-	-	-	-	-	0.497	
	Galvanized Iron d	-	-	-	-	-	-	-	0.166	-	-	-	-	-	0.494	
	Galvanized Iron e	-	-	-	-	-	-	-	0.145	-	-	-	-	-	0.294	
	Concrete Tile a	-	-	-	-	-	-	-	0.102	-	-	-	-	-	0.049	
	Concrete Tile b	-	-	-	-	-	-	-	0.213	-	-	-	-	-	0.048	
	Concrete Tile c	-	-	-	-	-	-	-	0.271	-	-	-	-	-	0.193	
	Concrete Tile d	-	-	-	-	-	-	-	0.232	-	-	-	-	-	0.082	
	Concrete Tile e	-	-	-	-	-	-	-	0.169	-	-	-	-	-	0.096	
Nigeria	Aluminum	-	0.95	-	-	-	0.16	-	nd	-	0.01	-	0.17	-	0	(Uba & Aghogho, 2000)
	Zinc	-	0.14	-	-	-	0.04	-	nd	-	0.043	-	0.38	-	0.88	
United States	Asphalt Fiberglass Shingle (Pilot)	-	-	-	-	-	-	0.0004-0.0012	-	-	-	-	-	0.008-0.085	-	(Mendez et al., 2011)
	Asphalt Fiberglass Shingle (Full)	-	-	-	-	-	-	0.0007-0.0086	-	-	-	-	-	0.001-0.015	-	
	Galvalume (Pilot)	-	-	-	-	-	-	0.0003-0.0023	-	-	-	-	-	0.077-0.362	-	
	Galvalume (Full)	-	-	-	-	-	-	0.0021-0.0058	-	-	-	-	-	0.018-0.023	-	
United States	Wood Shingle	0.008-2.343	0.382	0.001-5.41	0.029	-	-	0.025-0.7	0.045	0.082-6.680	0.982	0.001-0.404	0.044	0.0-109.7	16.317	(Chang et al., 2004)
	Composition Shingle	0.008-6.736	0.495	0.001-0.126	0.025	-	-	0.025-0.203	0.038	0.023-5.063	0.713	0.001-0.369	0.028	0.043-13.590	1.372	
	Aluminum	0.008-4.077	0.381	0.001-0.248	0.026	-	-	0.025-0.134	0.037	0.004-1.478	0.372	0.001-0.117	0.015	0.514-16.6	3.23	
	Galvanized Iron	0.008-6.884	0.435	0.001-0.224	0.028	-	-	0.025-0.255	0.049	0.001-3.659	0.362	0.001-0.252	0.017	0.124-212.33	11.788	

Table 8. Total Organic Carbon, Total Phosphorus, and Total Nitrogen in Rooftop Runoff.

Country	Roof Type	TOC (mg/L)		TP (mg/L)		TN (mg/L)		Reference
		Range	Mean	Range	Mean	Range	Mean	
Germany	Zinc	1-51	4.3	-	-	-	-	(Schriewer et al., 2008)
Greece	Clay Tiles	-	-	-	7.16	-	-	(Gikas & Tsihrintzis, 2012)
	Concrete	-	-	-	2.23	-	-	
	Concrete	-	-	-	1.33	-	-	
	Clay Tiles	-	-	-	2.98	-	-	
	Clay Tiles	-	-	-	1.21	-	-	
Spain	Clay Tiles	2-17	13	-	-	-	-	(Farreny et al., 2011)
	Metal	2-5	4	-	-	-	-	
	Plastic	1-9	5	-	-	-	-	
	Flat Gravel	8-55	28	-	-	-	-	
China	Concrete	0-25	10	0-0.5	0.1	0-15	4	(Zhang et al., 2014)
	Asphalt	0-75	30	0-0.3	0.1	0-20	5	
	Ceramic Tile	0-30	10	0-0.3	0.1	0-13	3	
	Green	25-175	25	0-0.15	0.1	5-35	17	
Korea	Cement	-	-	-	0.21	-	-	(Kim et al., 2005)
	Green	-	-	-	1.18	-	0.8	
	Green	-	-	-	1	-	1.6	
United State	Asphalt Fiberglass Shingle (Pilot)	-	-	-	-	-	3	(Mendez et al., 2011)
	Asphalt Fiberglass Shingle (Full)	-	-	-	-	-	-	
	Galvalume (Pilot)	-	-	-	-	-	-	
	Galvalume (Full)	-	-	-	-	-	-	

Zhang et al. found that green roof runoff outputs a large range of total organic carbon (Zhang, Wang, et al., 2014). Total phosphorus concentrations were especially high for clay tiles in Greece, but were low in China (Gikas and Tsihrintzis, 2012; Zhang, Wang, et al., 2014).

Microbial Surface Attachment

Initial biofilm development begins with microbial surface attachment, which can cause problems in environments which require sterility. Microbial surface attachment can be detrimental in medical, industrial, water transport, and food preparation settings. Biofilms have been studied heavily in these settings due to impacts on public health. Studies focus on previously developed films and how to remove them in addition to how they develop. Varying materials are known to affect surface attachment differently. Common work surface materials include stainless steel, aluminum, glass, and plastics. A question of critical importance is how biofilms form, beginning with surface attachment. Biofilms have already been found developed on glass, stainless steel, aluminum, and plastics, but few studies have dared to go past common materials in sterile or water transport settings. Unfortunately the lack of information on biofilms does not stop at how microbes attach to a surface, but extends into non-work materials that dominate our society. Examples of other surfaces which need to be studied include urban materials such as concrete, asphalt, brick, tile, and shingles. These surfaces are man-made materials which can be found all around us on a daily basis: roads, sidewalks, and rooftops.

Attachment Surfaces

Microbial surface attachment and biofilm formation is a known health hazard in many settings, especially when related to food preparation surfaces (Joseph, Otta, et al., 2001; Avery and Buncic, 2003; Dourou, Beauchamp, et al., 2011; Rivas, Fegan, et al., 2007; Ortega, Hagiwara, et al., 2010; Chia, Goulter, et al., 2009; Rivas, Dykes, et al., 2007), medical settings (Otto, 2008; Palmer, Flint, et al., 2007), and clean water pipe flow (Beale, Barratt, et al., 2013; Percival, Knapp, et al., 1998). Bacteria are also known to attach and develop films in natural environments including in natural water bodies (Lyautey, Lacoste, et al., 2005; Leff, Dana, et al., 1995), materials with a potential for high surface area such as sand (Baker and Duke, 2006; Elliot, Stauber, et al., 2006), glass (Mafu, Roy, et al., 1990; Chia, Goulter, et al., 2009), and metals (Tsuji and Yokoigawa, 2012; Rivas, Dykes, et al., 2007; Rivas, Fegan, et al., 2007; Goulter-Thorsen, Taran, et al., 2011; Li, Hauer, et al., 2007; Mafu, Roy, et al., 1990).

The majority of studies on biological adhesion center on medical, food, and environmental settings. Medical and food prep settings are studied more predominantly due to immediate risks to public health. Since stainless steel is common in both medical and food settings, many studies either include or focus on adhesion to stainless steel (Dourou, Beauchamp, et al., 2011; Goulter-Thorsen, Taran, et al., 2011; Mafu, Roy, et al., 1990; Nguyen, Turner, et al., 2010; Ortega, Hagiwara, et al., 2010; Rivas, Fegan, et al., 2007; Tsuji and Yokoigawa, 2012; Wong, Chung, et al., 2002; Eginton, Gibson, et al., 1995). Glass (Tsuji and Yokoigawa, 2012; Goulter-Thorsen, Taran, et al., 2011; Chia, Goulter, et al., 2009; Wong, Chung, et al., 2002; Mafu, Roy, et al., 1990), polypropylene (Tsuji and Yokoigawa, 2012; Mafu, Roy, et al., 1990), polyethylene (Tsuji and Yokoigawa, 2012; Eginton, Gibson, et al., 1995), polyurethane (Chia, Goulter, et al., 2009), polyvinylchloride (PVC) (Tsuji and Yokoigawa, 2012; Coquet, Cosette, et al., 2002), polystyrene (Moreira, Simões, et al., 2014; Tsuji and Yokoigawa, 2012; Eginton, Gibson, et al., 1995), aluminum foil (Tsuji and Yokoigawa, 2012), titanium (Tsuji and Yokoigawa, 2012), polytetrafluoroethylene (Goulter, Gentle, et al., 2010; Eginton, Gibson, et al., 1995; Chia, Goulter, et al., 2009), formica (Eginton, Gibson, et al., 1995), and nitrile butyl-rubber (Chia, Goulter, et al., 2009; Mafu, Roy, et al., 1990) were studied in food and medical settings. Coquet et al. studied attachment in fish farm materials. Wood, fiberglass, PVC, and most importantly concrete, were studied (Coquet, Cosette, et al., 2002). Coquet et al. found that *Yersinia ruckeri* was able to attach to concrete in fish farms.

Bacteria Commonly Utilized in Biofilm Studies

In order to determine public health threats, most bacteria used in adhesion studies focus on pathogenic or potentially pathogenic microorganisms. Many papers focused on multiple different strains of *E. coli* (Dourou, Beauchamp, et al., 2011; Goulter, Gentle, et al., 2010; Goulter-Thorsen, Taran, et al., 2011; Moreira, Simões, et al., 2014; Ortega, Hagiwara, et al., 2010; Rivas, Fegan, et al., 2007; Tsuji and Yokoigawa, 2012; Eginton, Gibson, et al., 1995). This is likely due to the frequency with which *E. coli* is used as a detection method for human contamination in food, medical, and environmental settings. Additional bacterium which were studied include *Campylobacter jejuni*

(Nguyen, Turner, et al., 2010), *Salmonella typhimurium* (Chia, Goulter, et al., 2009), *Salmonella infantis* (Chia, Goulter, et al., 2009), *Salmonella virchow* (Chia, Goulter, et al., 2009), *Salmonella sofia* (Chia, Goulter, et al., 2009), *Pseudomonas aeruginosa* (Eginton, Gibson, et al., 1995), *Staphylococcus epidermis* (Eginton, Gibson, et al., 1995), *Streptococcus thermophiles* (Boulangé-Petermann, Rault, et al., 1997), *Yersinia ruckeri* (Coquet, Cosette, et al., 2002), *Vibrio parahaemolyticus* (Wong, Chung, et al., 2002), and *Listeria monocytogenes* (Mafu, Roy, et al., 1990).

Bacterial Preparation for Attachment

No consistent method is provided in the literature for bacterial preparation for attachment, even for studies using the same type of bacteria. Variations are seen in incubation time, media or broth, and solutions. Cultures were typically grown on a solid media plate and then transferred to a glycerol stock, stored in a -80°C freezer, for future use. Following growth or removal from a glycerol stock, cultures were either grown in a liquid broth (Rivas, Fegan, et al., 2007; Tsuji and Yokoigawa, 2012; Eginton, Gibson, et al., 1995; Wong, Chung, et al., 2002; Coquet, Cosette, et al., 2002; Mafu, Roy, et al., 1990), or were centrifuged in order to work with a pellet (Rivas, Fegan, et al., 2007; Ortega, Hagiwara, et al., 2010; Moreira, Simões, et al., 2014; Dourou, Beauchamp, et al., 2011; Goulter-Thorsen, Taran, et al., 2011; Goulter, Gentle, et al., 2010; Nguyen, Turner, et al., 2010; Chia, Goulter, et al., 2009; Boulangé-Petermann, Rault, et al., 1997). Pellets were washed twice and re-suspended before use for attachment. Cell density was measured following suspension using ocular density (Nguyen, Turner, et al., 2010; Goulter, Gentle, et al., 2010; Tsuji and Yokoigawa, 2012).

Techniques for Purposeful Bacterial Attachment

Two common methodologies have arisen for purposeful bacterial attachment. The less common method is using a flow chamber with recirculation (Moreira, Simões, et al., 2014). A more popular method is submergence. Small pieces of the surface material of interest are placed face down or completely submerged in the bacterial solution (Dourou, Beauchamp, et al., 2011; Goulter, Gentle, et al., 2010; Goulter-Thorsen, Taran, et al., 2011; Nguyen, Turner, et al., 2010; Ortega, Hagiwara, et al., 2010; Rivas, Fegan, et al., 2007; Tsuji and Yokoigawa, 2012; Eginton, Gibson, et al., 1995; Coquet, Cosette, et al., 2002; Boulangé-Petermann, Rault, et al., 1997; Wong, Chung, et al., 2002). Solution contents were dependent upon bacteria of choice and previously used solutions, but phosphate buffer solution (PBS) was used frequently (Chia, Goulter, et al., 2009; Nguyen, Turner, et al., 2010; Goulter-Thorsen, Taran, et al., 2011; Goulter, Gentle, et al., 2010; Rivas, Fegan, et al., 2007). Thin material coupons were placed face down in the bacterial solution for twenty minutes with gentle swirling every five minutes at room temperature (Rivas, Fegan, et al., 2007; Tsuji and Yokoigawa, 2012; Goulter-Thorsen, Taran, et al., 2011; Goulter, Gentle, et al., 2010; Chia, Goulter, et al., 2009). Studies following total submergence often incubated at times much greater than twenty minutes (Dourou, Beauchamp, et al., 2011; Nguyen, Turner, et al., 2010; Wong, Chung, et al., 2002; Boulangé-Petermann, Rault, et al., 1997; Coquet, Cosette, et al., 2002;

Eginton, Gibson, et al., 1995). Following incubation, coupons were rinsed with a bacteria free solution of either phosphate buffer solution (PBS) or water.

Determination of Attachment and Biofilm Development

Successful attachment was typically measured using an epifluorescence microscope (Rivas, Fegan, et al., 2007; Goulter-Thorsen, Taran, et al., 2011; Goulter, Gentle, et al., 2010; Nguyen, Turner, et al., 2010; Chia, Goulter, et al., 2009; Wong, Chung, et al., 2002). Prior to using the microscope, coupons were stained with either acridine orange (Chia, Goulter, et al., 2009; Goulter-Thorsen, Taran, et al., 2011; Goulter, Gentle, et al., 2010; Rivas, Fegan, et al., 2007) or a Live/Dead BacLight viability kit (Wong, Chung, et al., 2002; Nguyen, Turner, et al., 2010). An alternative method to staining is using nutrient agar plates. This method involves multiple agar plate transfers of the coupon, face down on the agar plate (Tsuji and Yokoigawa, 2012). Colonies on the final plate which appeared between the coupon and the agar were counted. Moreira et al. used a constantly recorded microscope to monitor growth on coupons in the flow chamber (Moreira, Simões, et al., 2014).

Purposeful Removal of Attached Bacteria

Multiple methods were presented for removing bacterial cells from surface coupons. Following rinsing, coupons withstood sonication (Boulangé-Petermann, Rault, et al., 1997; Coquet, Cosette, et al., 2002; Wong, Chung, et al., 2002), plate transfers (Eginton, Gibson, et al., 1995; Goulter-Thorsen, Taran, et al., 2011; Nguyen, Turner, et al., 2010), vortexing (Dourou, Beauchamp, et al., 2011), or swabbing (Ortega, Hagiwara, et al., 2010). Liquid samples were diluted and plated following rinsing and non-plating bacterial removal techniques.

Conclusions from Effective Attachment and Detachment Projects

A wide variety of factors have been tested when relating to parameters which affects attachment and detachment from common surfaces. Common conclusions between scientists have been drawn around temperature, bacterial growth stage, and incubation time. Multiple studies reported that the temperature of the incubation did affect the attachment process. Colder temperatures near 4°C inhibited attachment, while warmer temperatures, 15-55°C dependent on bacterium, led to increased surface attachment. This increased attachment also prevented detachment as well (Nguyen, Turner, et al., 2010; Tsuji and Yokoigawa, 2012; Dourou, Beauchamp, et al., 2011). Dourou et al. found that after three days of incubation, temperature began to affect attachment (Dourou, Beauchamp, et al., 2011). In situ attachment studies are typically done with bacteria in either the exponential growth or the stationary phase (Wong, Chung, et al., 2002). Literature studying the effects of time on attachment found that attachment counts stopped increasing after 2-3 hours (Wong, Chung, et al., 2002; Nguyen, Turner, et al., 2010). Depending on bacterial strain and surface type, full biofilms were able to form in six hours (Wong, Chung, et al., 2002; Eginton, Gibson, et al., 1995). Wong et al. found that cell attachment actually began to decline after 8 hours of incubation (Wong, Chung, et al., 2002).

Inconsistent results were found for hydrophobicity and surface roughness. Studies on surface roughness showed that roughness does not affect the ability of microorganisms to attach to surfaces, but does affect detachment. Smooth surfaces, such as glass, had increased detachment when compared with a rougher surface like stainless steel (Boulangé-Petermann, Rault, et al., 1997; Goulter-Thorsen, Taran, et al., 2011; Ortega, Hagiwara, et al., 2010; Rivas, Fegan, et al., 2007). Rougher stainless steel coupon surfaces also showed less detachment than smoother stainless steel coupons. Coquet et al. found that for *Yersinia ruckeri*, surface roughness played a significant role in the ability of bacteria to adhere to the fish farm materials (Coquet, Cosette, et al., 2002). Studies have not been able to pinpoint how hydrophobicity affects the ability of bacteria to attach to various surfaces. Rivas et al. and Chia et al. both found no influence on attachment from hydrophobicity or bacterial source (Rivas, Fegan, et al., 2007; Chia, Goulter, et al., 2009). Moreira et al. found that more hydrophobic materials had higher adhesion than hydrophilic materials (Moreira, Simões, et al., 2014), while Goulter et al. stated that *E. coli* was more likely to bind to hydrophilic surfaces (Goulter, Gentle, et al., 2010).

CHAPTER I
ROADWAY STORMWATER RUNOFF IMPACT ON RECEIVING
STREAM MICROBIAL CONTAMINATION

A version of this chapter was originally submitted for publication by Kristen N. Wyckoff, Si Chen, Andrew J. Steinman, and Qiang He:

Citation

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Kristen is the first author on this paper, with writing, editing, and revisions completed by both Kristen and Qiang He. Si Chen aided in 16S rRNA sequencing techniques, while Andrew Steinman assisted with sample collection.

Abstract

Stormwater runoff from roadways has increasingly become a regulatory concern for water pollution control. Recent work has suggested roadway runoff as a potential source of microbial pollutants. It is important to determine the impact of roadway runoff on the microbiological quality of receiving streams. In this study, the water quality of roadway stormwater runoff and the receiving stream was monitored during storm events. Enumeration of total coliform as a measure of fecal microbial pollution found consistently lower total coliform counts in roadway runoff than those in the stream water, suggesting that roadway runoff was not a major contributor of microbial pollutants to the receiving stream. Further characterization of the microbial community in the stormwater samples by high-throughput sequencing revealed that the microbial composition of stormwater runoff from the roadways and the receiving stream differed significantly, demonstrating that stormwater runoff did not appear to have a major influence on the stream in terms of microbiological quality and sources other than the roadway runoff were likely the primary contributors of microbial loading to the stream, which needs to be validated in other watersheds. Understanding gained in this study could support the development of more effective strategies for stormwater management in sensitive watersheds.

Introduction

Non-point source pollution caused by stormwater runoff is of major concern for improving water quality in impaired watersheds. Non-point source pollution from stormwater runoff is primarily generated from impervious surfaces such as roadway surfaces, parking lots, and roof tops. Pollutants from these sources can come in many forms, including microbial contamination, nutrients, metals, solids, and chemicals contaminants (Sansalone, Koran, et al., 1998; Sansalone and Buchberger, 1997; Sansalone, Hird, et al., 2005; Winston, Hunt, et al., 2012; Flint and Davis, 2007; Geldreich, Best, et al., 1968; He, Valeo, et al., 2010).

Recent research has focused on microbial contamination in streams due to the detection of excessive levels of fecal indicator organisms (McLellan, Fisher, et al., 2015). Stormwater management installations for transportation infrastructure are frequently designed to direct water towards streams, rivers, and lakes without preliminary treatment, raising concerns with microbial contamination from roadway

stormwater runoff. Monitoring programs for roadway stormwater runoff quality have reported the presence of fecal indicator organisms, suggesting that fecal contamination in water bodies could potentially result from the discharge of stormwater runoff from roadways (Clark, Burian, et al., 2006; Falbo, Schneider, et al., 2013). It is important however, to determine whether roadways are a primary contributor of microbial pollutants to receiving streams. Identification of the contribution of roadway stormwater runoff to microbial contamination in receiving streams will assist in the design of effective water quality protection strategies.

Monitoring of microbial contamination of water has mostly relied on the enumeration of fecal indicator bacteria (FIB) (Barrett, Irish Jr., et al., 1998; Hathaway, Tucker, et al., 2012; Kayhanian, Fruchtmann, et al., 2012), which however represent only selected members of the microbial community in fecal materials. Prior studies have reported the persistence and propagation of certain members of FIB in non-fecal environments, including soils and sediments (Lechevallier, Cawthorn, et al., 1988; Anderson, Whitlock, et al., 2005; Van Donsel, Geldreich, et al., 1967; Nshimyimana, Ekklesia, et al., 2014; Parhad and Rao, 1974; Lyautey, Wilkes, et al., 2010; Sawyer, English, et al., 2008). These findings have raised uncertainties in the validity of conventional methods that rely on the enumeration of FIB to evaluate the occurrence of fecal contamination. Therefore conventional methods targeting only selected members of the fecal microbial community, i.e. FIB, might not accurately represent the presence of fecal contamination in the environment of interest. Recent development in high-throughput sequencing has made it possible to target all microbial populations of the microbial community, not only FIB, allowing assessment based on the entire microbial community in the environment with reduced uncertainty (Hazen, Rocha, et al., 2013).

In this study, the microbial community composition of stormwater runoff from the roadways and the receiving stream was characterized by high-throughput sequencing. Subsequently microbial community composition was compared to determine the impact of stormwater runoff on the microbiological quality of the receiving stream. Our results show that the microbial community of the stormwater runoff from roadways differed significantly from those of the receiving stream; this indicates that stormwater runoff had minimal influence on the stream in terms of microbiological quality, and sources other than the roadway runoff might be primary contributors of microbial loading to the stream. Results from this study provide much needed insight into the microbial impact of roadways on the environment which could be used to develop more effective strategies for stormwater management in sensitive watersheds.

Methodology

Study Site Characteristics

The sampling sites were located in an urbanized watershed in north Knoxville, Tennessee (Fig. 1). The three sampling locations selected include the upstream site at the beginning of a local stream referred to as Stream 1 (C1), a roadway runoff discharge point thought to be a source of contamination to the receiving stream referred to as Runoff (R), and a down-stream location after the Runoff (R) site referred to as

Stream 2 (C2). The water collected at R was at a topographical high point, i.e. an overpass bridge, so that no stormwater other than the runoff from the roadway was collected. These sampling sites were selected due to the ability to collect stream water which had been both contaminated and uncontaminated by roadway runoff. The roadway sampling location was chosen so that the physical area providing runoff could be defined. Selecting a topographical high point on a bridge also prevented contamination from other factors affecting roadways such as soil, grass, or runoff ponding. The upstream location, C1, was at the head of the stream, and had no contributions from the roadway runoff. The stormwater ran off from the north-bound side of the roadway only, flowed through a pipe, and exited on the south-bound side of the road where the runoff was collected. The downstream location, C2, had impacts from multiple roads, parking lots, restaurants, stores, and a park in addition to the roadway stormwater runoff. As a result, the sampling design enabled the collection of stormwater runoff from the road only (R), stream water with no roadway runoff (C1), and stream water with roadway runoff (C2), which allowed for the determination of the impact that roadway runoff had on the microbiological quality of the stream. As a result, water samples at C1 and C2 were collected directly from different points in the same stream, while water samples at R were collected from a roadway section (Table 9). The sub-watersheds draining to R, C1, and C2 differed in land use patterns (Table 9).

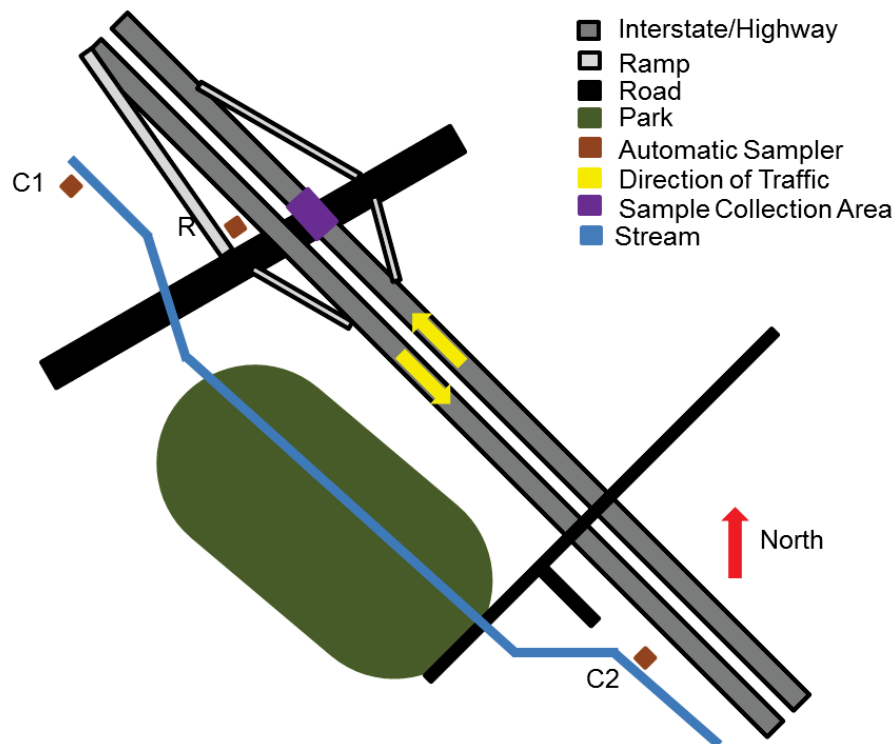


Figure 1. Schematic of sampling locations at the study site. R, runoff; C1, upstream; C2, downstream.

Table 9. Characteristics of Sample Sites.

Site	Impervious Surface (%)	Drainage Area (km ²)	Land Use of Drainage Area		
			Commercial (%)	Residential (%)	Other (%)
R	100	0.3	-	-	100
C1	19.5	231	15.1	80.5	4.4
C2	29.3	510	27.4	70.1	2.5

Storm Characteristics

Two storms with contrasting characteristics were selected for analysis in this study (Table 10). The antecedent dry period (ADP) for storm 1 was nearly five times greater than that for storm 2. A longer ADP might increase the accumulation of pollutants on the roadways via dry deposition and vehicular traffic. Similar buildup of pollutants elsewhere in the watershed would increase as well due to dry deposition and other mechanisms such as wildlife defecation and lawn fertilization. Two summer storms were selected as a baseline, to do a preliminary investigation of roadway runoff microbiology and its relation to stream microbiology.

The total rainfall and storm intensity were also much greater during storm 1 than those during storm 2. Greater rainfall and rainfall intensity would be expected to facilitate the mobilization of particulate pollutants accumulated on the surfaces of roadway, vegetation, and soil, resulting in higher levels of these pollutants in stormwater runoff. In contrast, if the target pollutant was not present in significant quantities in parts of the watershed, increased rainfall or rainfall intensity would have minimal impact on the level of this particular pollutant in stormwater runoff from those portions of the watershed.

Table 10. Monitored Storm Event Characteristics.

	Antecedent Dry Period (Days)	Storm Duration (hr)	Average Rainfall Intensity (cm hr ⁻¹)	Total Rainfall (cm)
Storm 1	10	3.13	0.30	0.99
Storm 2	2.86	3.00	0.20	0.61

Water Sampling and Analysis

Stormwater samples were collected using three automatic samplers (ISCO 6712, Lincoln, NE, USA) during two separate storm events in late summer following previously described procedures (Barrett, Irish Jr., et al., 1998). Runoff was collected by using a v-notch weir to calculate flow; flow based samples were collected over the course of the

storm. This method accounted for any lag times that may have been experienced between the roadway and the receiving stream. Water quality analysis used flow-weighted composite samples collected during each storm at the three sampling sites. For high-throughput sequencing, aliquots of 50-mL from the composite samples were centrifuged and the pellets were stored at -80°C until deoxyribonucleic acid (DNA) extraction.

Water quality analysis included measurements of pH, nitrate, suspended solids, and total coliform, as a measure of fecal microbial contamination following standard methods. Total coliform was enumerated using Standard Method 9222 (APHA; AWWA; WEF, 2005) with Difco™ m-Endo Broth MF™ medium (Sparks, MD, USA). An Ion Chromatograph (IC) system (Dionex ICS-2100 & ICS-1100, Sunnyvale, CA, USA) was used to measure nitrate concentrations. Total suspended solids (TSS) were measured using Standard Methods 2540 (APHA; AWWA; WEF, 2005). Total rainfall, rainfall intensity, antecedent dry period were determined from precipitation data recorded by the weather station installed at the study site.

DNA Extraction and High-Throughput Amplicon Sequencing

Prior to sequencing of the microbial community in each water sample, DNA extraction, polymerase chain reaction (PCR) amplification, and purification were completed as previously described (S. Chen, Cheng, et al., 2016). A FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH, USA) was used for DNA extraction, and a DNA Clean & Concentrator™-5 kit (Zymo Research, Irvine, CA, USA) was used to for DNA purification. PCR amplification targeting the V4 region of the 16S rRNA gene was performed using Golay primers 515-F and 806-R (Caporaso, Lauber, et al., 2012). Thermal cycling began with 3 minutes at 94°C, followed by 25 cycles in three stages, including 94°C for 45 seconds, 50°C for 60 seconds, and 72°C for 90 seconds. The final 10 minutes of thermal cycling were completed at 72°C.

Purification of DNA amplicons from PCR was conducted by gel electrophoresis followed by clean-up with the Wizard® SV Gel and PCR Clean-Up system (Promega, Madison, WI, USA). An Invitrogen Quant-It Pico Green DNA quantification kit was used to determine the concentrations of the DNA amplicons (Life Technologies, Carlsbad, CA, USA). Samples were then pooled at equimolar ratios and paired-end sequencing of the 16S rRNA gene amplicon library was completed using the Illumina MiSeq platform (San Diego, CA, USA) as previously described (Wu, Yang, et al., 2016).

Sequence Analysis

Analysis of sequence reads from the Illumina MiSeq platform was completed using the Mothur platform v.1.35 following the Mothur MiSeq SOP (Si Chen, Cheng, et al., 2016; Schloss, 2009). Sequence quality processing required barcode and primer trimming, denoising, and chimera check. RDP Classifier was used to assign all validated sequences of each sample to taxonomic ranks with a confidence threshold of 80% (Wang, M., et al., 2007). Operational Taxonomic Units (OTUs), a statistical representation of microbial taxonomic ranks, were defined using a 97% similarity cutoff for 16S rRNA genes. Comparisons of microbial composition between samples were

conducted with population overlap proportions and principle coordinate analysis (PCoA) following procedures specified by the Mothur platform (Zhang and He, 2013; S. Chen, Cheng, et al., 2016; Kozich, Westcott, et al., 2013; Wu, Yang, et al., 2016). The microbial population overlap proportion of each pairwise comparison was computed as the Nonparametric Maximum Likelihood Estimator based on microbial community composition represented by OTUs derived from high-throughput sequencing of 16S rRNA gene amplicon libraries (Yue and Clayton, 2005). Sequence alignment and construction of phylogenetic trees were completed with BioEdit (Hall, 1999) and MEGA6 (Tamura, Stecher, et al., 2013), respectively. All sequences can be accessed at the NCBI Sequence Read Archive with accession number SRP091994.

Statistical Analysis

Statistical data analysis was completed using SAS Enterprise Guide software version 6.1 of the SAS System. All statistical analyses were completed using $\alpha=0.05$. Tests run included one way ANOVA, Tukey's Honest Significant Difference (HSD) testing, and Student's *t*-test.

Results and Discussion

Quality of Stormwater Runoff and Stream Water

Corresponding to the contrast in storm characteristics, the stormwater quality exhibited considerable differences between storm 1 and storm 2 (Table 11). Stormwater pollutants, including total coliform, TSS, and nitrate, were overall higher during storm 1 than storm 2 in both stream water and roadway runoff, confirming that storms with longer ADP, higher rainfall total, and greater rainfall intensity would result in greater pollutant loadings to the receiving stream.

Roadway runoff did not appear, however, to be a major contributor to the pollutant loading in the watershed of interest in this study. As a measure of fecal microbial pollutants, total coliform counts during the more intense storm 1 were high in the stream water, reaching 26×10^4 and 606×10^4 CFU/100 mL at the upstream and downstream sites respectively (Table 3). In comparison, the total coliform count in the roadway runoff was only 8×10^4 CFU/100 mL, which was two orders of magnitude lower than that in the downstream water. The much higher total coliform level in the downstream indicates that roadway runoff was unlikely to be a major contributor of fecal microbial pollutants to the receiving stream. Sources downstream of the roadway runoff collection point R were instead more likely to be responsible for the majority of the fecal microbial pollutant loading. It should be noted that, during the less intense storm 2, the total coliform counts did not vary considerably between upstream and downstream, ranging between 4×10^4 and 8×10^4 CFU/100 mL (Table 3), which were much lower than those during the stronger storm 1. This observation could be attributed to the lack of mobilization of fecal microbial contaminants by the weaker storm 2. Interestingly, the difference in total coliform counts in roadway runoff was minimal between storm 1 and storm 2 (Table 3), indicating that storm characteristics had no impact on the export of fecal microbial pollutants from roadways. One likely explanation was the absence of

fecal microbial buildup on the roadway; consistent with the indication that roadway runoff was not a major contributor of fecal microbial pollutants to the receiving creek.

Table 11. Quality and characteristic parameters from stormwater runoff.

		C1	C2	R
pH	Storm 1	7.7	7.9	7.8
	Storm 2	7.6	7.9	8.9
Total Coliform ($\times 10^4$ CFU/100 mL)	Storm 1	26	606	8
	Storm 2	8	4	6
TSS (mg/L)	Storm 1	124	95	57
	Storm 2	91	24	19
Nitrate- NO_3^- (mg/L)	Storm 1	0.52	0.62	0.60
	Storm 2	0.12	0.09	0.14
Average Flow Rate (L s^{-1})	Storm 1	25.2	164.4	2.9
	Storm 2	84.6	255.5	14.9

TSS, another measure of stormwater pollutants, exhibited similar patterns as total coliform, as TSS levels in the roadway runoff were consistently lower than those in the stream water samples (Table 11). These observations suggest that roadways were not likely the primary sources of TSS in the streams during these storm events. On the other hand, nitrate, as a measure of nutrient pollution in stormwater, had concentrations in roadway runoff similar to those in the stream water, which was different from the patterns of total coliform and TSS (Table 11). Thus the possibility of roadway runoff as a major source of nitrogen input to the receiving streams could not be excluded.

Microbial Composition of Stormwater Runoff and Stream Water

To further study the impact of roadway stormwater runoff on the microbiological quality of receiving streams, the microbial composition of roadway runoff and stream water during both storm events was characterized by high-throughput sequencing of the 16S rRNA gene amplicon libraries. A total of 15,845 OTUs were identified between all water samples, with *Proteobacteria* representing the most predominant phylum in the watershed, accounting for 52.8% of the sequencing reads (Fig. 2). *Actinobacteria* was the second most abundant population, representing 15.1% of the microbial community, which was followed by *Bacteroidetes* as the only other phylum with more than 5% representation.

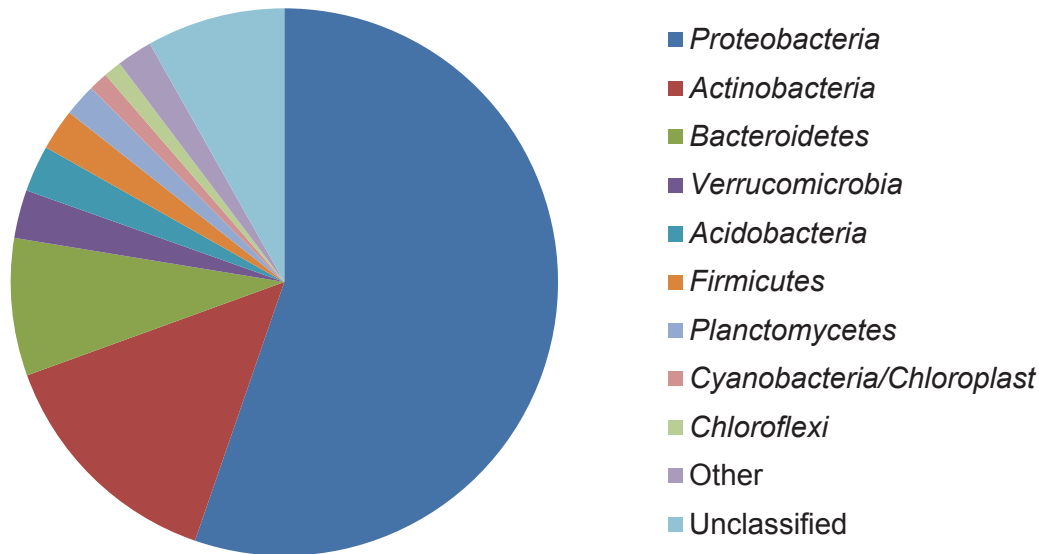


Figure 2. Relative abundance of identified microbial populations (phylum) of all combined samples.

Since coliform bacterial populations belong to the phylum *Proteobacteria*, a closer examination of the stormwater microbial communities was conducted at the OTU level to determine the presence of coliform bacterial populations at a finer taxonomic resolution (Fig. 3). Consistent with the phylum-level analysis showing the dominance of *Proteobacteria* (Fig. 2), 17 of the top 20 most abundant OTUs belonged to the phylum of *Proteobacteria* (Fig. 3). Among the most abundant OTUs, the bacterial family of *Comamonadaceae* was most frequently represented, accounting for 8.7% of the total stormwater microbial community. Bacterial populations in the family of *Comamonadaceae* are not classified as coliform bacteria, as they are predominantly found in aquatic and soil habitats (Nemergut, Anderson, et al., 2007). In fact, 19 of the top 20 OTUs belonged to non-coliform bacterial populations with the exception of OTU10, which was associated with the family of *Enterobacteriaceae* (Fig. 3). The identification of *Enterobacteriaceae* populations in stormwater with high-throughput sequencing was consistent with the detection of total coliform using cultivation-based standard methods (Table 3), given that coliform bacteria are part of *Enterobacteriaceae*.

Comparison of Microbial Community Composition

When roadway runoff is a primary source of the microbial input to the receiving stream, it could be expected that the microbial composition of the receiving stream would resemble that of the roadway runoff. Therefore the impact of roadway runoff on the receiving stream could be deduced from the comparison of the microbial community composition between the roadway runoff and receiving stream. Principle Coordinates Analysis (PCoA) was performed to visualize the relationships between stormwater samples based on microbial community composition derived from high-throughput

results (Fig. 4). The roadway runoff samples from storm 1 and 2 formed a distinctive cluster away from all stream water samples, indicating that the roadway runoff samples differed significantly in microbial composition from the stream water samples. These results provided another line of evidence to support that roadway runoff was not likely to be a major source of microbial input to the receiving streams.

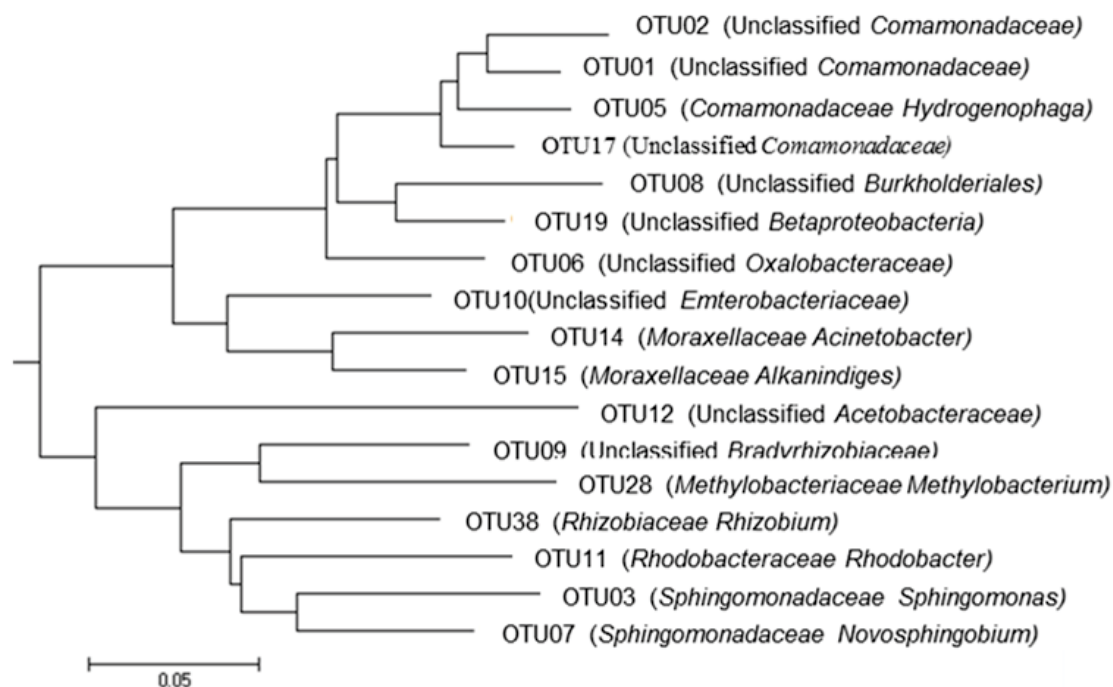


Figure 3. Phylogenetic tree showing the taxonomic classification of representative partial 16S ribosomal RNA gene sequences from *Proteobacteria* in the 20 operational taxonomic units (OTUs) with the highest relative abundance in stormwater. Taxonomic classification of the OTUs at the family and genus level is indicated next to the OTU designation in parentheses.

It is interesting to note that the upstream and downstream water samples from storm 1 formed a cluster, while the upstream and downstream water samples from storm 2 formed another cluster (Fig. 4), indicating that the upstream and downstream samples shared similar microbial composition during the same storm event; however the microbial composition shifted significantly from storm 1 to storm 2 at the same upstream or downstream sites. Evidence suggests that the microbial composition in the stream water was influenced primarily by storm characteristics instead of roadway runoff, possibly through mechanisms such as enhanced transport of sediment and microorganisms from sources other than roadways in the watershed by strong storms. Total suspended solids decreased for storm 2 in comparison with storm 1, similar to fecal indicator bacteria. Principle coordinates analysis uses a matrix of similarities and dissimilarities to graphically represent the distances between data points that accurately represent original dissimilarities.

The microbial composition of the stormwater samples could be further compared using microbial population overlap proportions between stormwater samples (Fig. 5). The population overlap proportion between roadway stormwater runoff samples from storm 1 and storm 2 was close to 1.0, indicative of high levels of similarity, which is consistent with results from PCoA analysis (Fig. 4). In contrast, all other pairwise population overlap proportions between roadway runoff samples and stream water samples were below 0.5, demonstrating the lack of similarity in microbial composition between roadway runoff and stream water. Thus, these results further support the conclusion that the receiving stream was not influenced by stormwater runoff from roadways with regard to microbial pollutants.

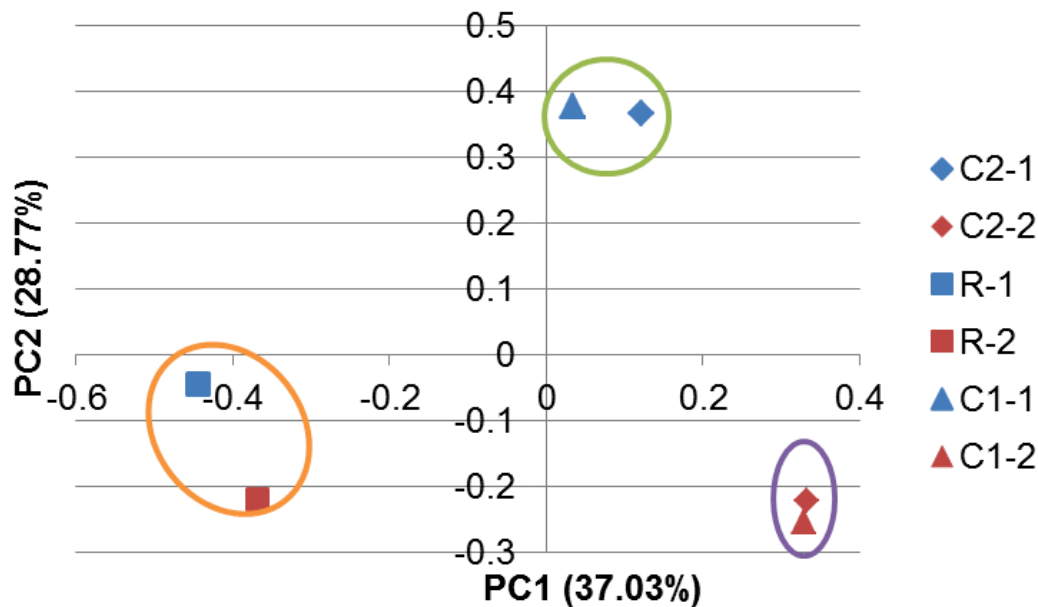


Figure 4. Principle coordinate analysis of microbial composition of stormwater samples in relation to storm characteristics: C1, upstream (triangle); C2, downstream (diamond); R, roadway stormwater runoff (square); 1, Storm 1 (blue); and 2, Storm 2 (red).

It should be noted that due to the limited scope of stormwater sampling, observations made in this study need to be validated in watersheds with distinct characteristics, such as land use pattern, climate, and topography. Since storm characteristics had major impact on stormwater quality (Table 11), further efforts should also be made to study the impact of storm characteristics.

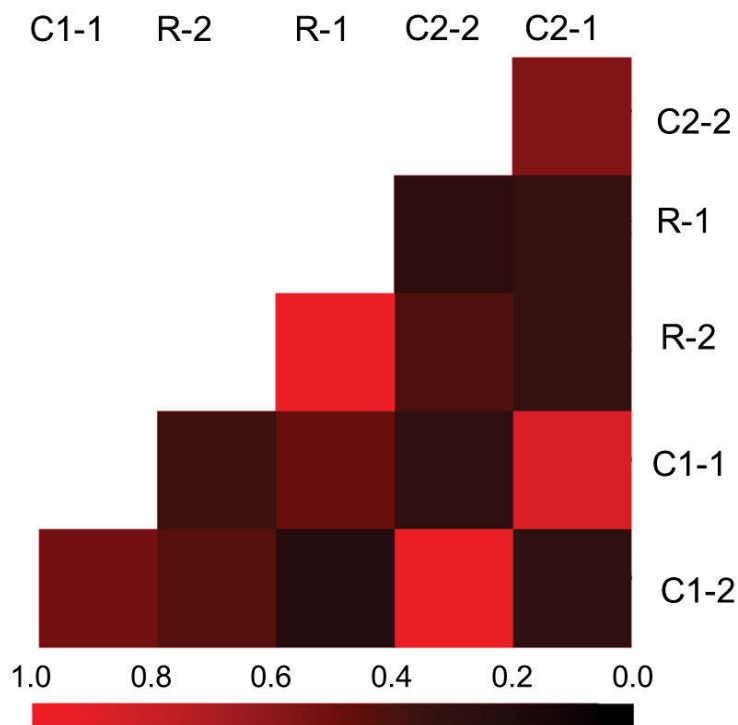


Figure 5. Microbial population heat map overlapping proportions between water samples in the receiving stream and roadway stormwater runoff during two storm events. The overlap proportion is scaled between 0.0 and 1.0, with 0.0 being no overlap and 1.0 being complete overlap. Water sample designation: C1, upstream; C2, downstream; R, roadway stormwater runoff; 1, Storm 1; and 2, Storm 2.

Conclusion

Nonpoint-source pollution has been a major concern for the protection of stream water quality. It is important to determine the impact of roadway runoff as non-point source pollution on the microbiological quality of receiving streams.

Two storm events with contrasting characteristics were monitored for stormwater runoff quality parameters. It was found that total coliforms, as a measure of fecal microbial pollutant loading, were consistently lower in roadway runoff than those in the stream water, suggesting that roadway runoff was not a major contributor of microbial pollutants to the receiving stream.

High-throughput sequencing of the microbial community in stormwater runoff and stream water further revealed the lack of similarity in microbial composition between roadway runoff and stream water, providing another line of evidence that the influx of roadway runoff did not have a significant impact on the microbial composition of the receiving stream.

This work represented the first effort to determine the impact of roadway runoff on the microbiological quality of receiving streams with microbial community analysis.

Further efforts extending to watersheds with distinct characteristics are needed to validate the conclusions of this study.

Although microbial pollutants in stormwater runoff from roadways were not found to be a major contributor to receiving streams, stormwater runoff from roadways could serve as a likely source of nitrate (Table 11), which might affect microbial communities in the receiving stream as a nutrient, as well as a terminal electron acceptor. It is thus likely that other pollutants not monitored in this study, including heavy metals and organics, could be discharged into receiving streams and influence the indigenous microbial communities as stressors or substrates. Further studies are needed to evaluate the source, loading, and impact of these pollutants in stormwater runoff from the roadways.

It should also be noted that, due to the limited scope of stormwater runoff and stream water sampling, observations made in this study need to be validated in watersheds with distinct characteristics, such as land use pattern, climate, and topography. Since storm characteristics had major impact on the quality of stormwater runoff and stream water (Table 11), further efforts should also be made to study the significance of storm characteristics.

CHAPTER II
ROOFTOP RUNOFF QUALITY IN SINGLE FAMILY HOMES OVER THE
COURSE OF ENTIRE STORMS

Abstract

Rooftop runoff collection is common practice in many countries, serving as a source of drinking water in areas facing water shortages or lacking access to potable water. Current design of rooftop runoff collection devices are inconsistent, heavily relying on the presence of first flush diversion to increase the quality of the water stored within the capturing cistern. Additional inconsistencies were identified within available literature for what volume constituted a first flush for rooftop runoff. This project attempted to identify runoff quality trends for pollutants commonly found in rooftop runoff. Additional attempts were made to determine bacterial wash off throughout a storm event. For bacteria, total suspended solids, metals, and ions, no first flush effects were detected. While some flushing effects were seen throughout the storms, most pollutant concentrations were irregular, increasing and decreasing inconsistently throughout the storms. Runoff quality was found to vary by storm and rooftop. The most contaminated rooftop, roof 1, showed no statistical differences for quality over the course of a storm, while the cleaner rooftop, roof 3, showed significant differences between samples for ammonia, dissolved carbon, dissolved organic carbon, TSS, VSS, sulfate, and fluoride. Rooftop qualities appear to have an impact on runoff quality, but the driving factors are not currently obvious. Nitrate concentrations showed a flushing effect for rooftop 2. This research suggests that first flush elimination may not be effective at providing clean water for storage and reuse. Given variations in runoff quality by storm event, one single design for rooftop runoff capture is unlikely to work for every rooftop.

Introduction

The storage of rainwater is commonly referred to as rain water harvesting (RWH). RWH is common in the United States, Australia, Sudan, Afghanistan, New Guinea, Korea, Bangladesh, and Micronesia. In the Virgin Islands, cistern collection of rooftop rainwater is required by law (Lye, 1992). What stands out between reported techniques is the lack of consistency. Collection techniques for rooftop runoff vary on one significant point, first flush diversion. First flush diversion is commonly practiced in order to remove contaminants found in the beginning portion of the runoff. This practice is usually completed on a by-volume basis; however the range of volumes utilized in rooftop quality studies ranges anywhere from 2 to 3000 liters of runoff (Kim, Lee, et al., 2005; Mendez, Klenzendorf, et al., 2011; Gikas and Tsihrintzis, 2012). First flush for rooftops does is not calculated on a roofing area basis.

Rainstorms producing rooftop runoff provides an opportunity for reuse, but thus far general rooftop runoff quality is severely understudied. Many studies on rooftop runoff quality tend to focus more on cistern quality or contamination from metals and nutrients; few studies emphasize rooftop runoff quality and microbiological contamination directly from gutter outfalls. Studies that do consider the presence of microorganisms in rooftop runoff rarely consider the same forms of bacteria (C. A. Evans, Coombes, et al., 2006; Lee, Yang, et al., 2010; Kim, Lee, et al., 2005; Yaziz, Gunting, et al., 1989; Uba and Aghogho, 2000; Mendez, Klenzendorf, et al., 2011).

Typical rooftop runoff is heavily contaminated with metals, solids, nutrients, and viruses and bacteria (Chang, McBroom, et al., 2004; Lee, Yang, et al., 2010; Lye, 2009;

Craig A Evans, Coombes, et al., 2006; Evans, Coombes, et al., 2007; Kim, Lee, et al., 2005). Since cisterns, rooftops, and gutters are not regularly maintained, pollutants build up not only inside cisterns, but additionally on the roof and in the gutters. In addition to the general lack of attention to the collection system, both new and aging rooftop materials are known to leach out metals and other toxics (Mendez, Klenzendorf, et al., 2011; Townsend, Powell, et al., 2007; Jiannan Chen, Kyle J. Groves, Liangquan Wang, Tuncer B. Edil, 2013; Winters and Graunke, 2014). While literature strongly supports the idea that rooftop runoff as a whole is not currently possible to use as a potable water source, no recent efforts have been made to help mitigate this problem. In order to be able to reuse rooftop runoff, it is imperative to know and understand when pollutants in rooftop runoff are being flushed during a storm event.

The primary objective of this study was to capture rooftop runoff for the entire duration of a storm. Complete storm capture by grab sampling has not been done, as most sampling is mechanical or automatic and the sample aliquots are combined as they would be in a cistern. This allowed for the quality to be compared over the duration of the storm, between storms, and between different rooftops.

Methodology

Sites

Preliminary rooftop runoff studies were completed on three rooftops in different areas of Knoxville, Tennessee. Sample collection sites were located in north Knoxville (site 1), near campus (site 2), and west Knoxville (site 3). All three rooftops were constructed using asphalt shingles, but varied in age and tree cover. Roof 1 was the oldest roof with the most tree cover. Roof 2 had minimal tree cover and was less aged than Roof 1. Roof 3 was the youngest roof and had no tree cover. Exact roof age was unavailable. The goal of the rooftop runoff pilot study was to determine changes in water quality over the course of a storm for various contaminants. Three storms were collected for two of the roofs while only two storms were collected for the third roof. Since the rooftop characteristics were dissimilar, a more broad view of runoff pollutant loading could be investigated.

Sampling Technique

Sampling technique was personnel-heavy, requiring one person per rooftop. Sampling was completed using a grab sampling methodology. Water was collected directly from a gutter outfall into a sterile nalgene container. As soon as water began running from the gutter, the first sample was taken. Every fifteen minutes another 1L grab sample was taken until five samples had been taken. Samples were taken at 0, 15, 30, 45, and 60 minutes. Sample bottles which were not filled in fifteen minutes were sealed; with the next sample being taken immediately. Water which exited the gutter between grab samples was not collected. This 60 minute time frame covered the total runoff duration for all three storms. Samples were analyzed within the recommended 6 hour time frame. Samples were kept cool between collection and analysis.

Sample Analysis

All samples were monitored for bacterial, chemical, and physical contamination upon collection. Pollutants measured in rooftop runoff include bacteria (total heterotrophic bacteria, and *Escherichia coli*), suspended solids (total, and volatile), pH, dissolved carbon (total, organic, inorganic), dissolved nitrogen (which represents nitrate, nitrite, ammonia, and organic nitrogen), dissolved metals (sodium, potassium, magnesium, calcium, aluminum, copper, iron, manganese, zinc, cadmium, cobalt, lead, and sulfur), and dissolved ions (fluoride, sodium, potassium, magnesium, calcium, nitrate, nitrite, ammonia, chloride, and sulfate).

Methodologies utilized for sample analysis all followed Standard Methods or the Environmental Protection Agency Preferred Methods (APHA; AWWA; WEF, 2005; Environmental Protection Agency, 2012). *Escherichia coli* (*E. coli*) was the primary bacteria of interest, and was monitored using m-ColiBlue24[®] (HACH, Loveland, Colorado, USA) and sterile, gridded 0.45 µm Millipore filter papers (Millipore, Molsheim, France) and were incubated for 24 hours at 35°C. Total suspended and volatile solids were measured using aluminum trays and Whatman[®] glass microfiber filter papers (GE Healthcare Bio-Sciences, Piscataway, New Jersey, USA). Nutrients, including NO₃⁻, NO₂⁻, PO₄³⁻, SO₄²⁻, and Cl⁻ were measured using a Dionex[™] ion chromatography unit AS-AP, ICS-1100, ICS-2100 (ThermoScientific[™]) (APHA; AWWA; WEF, 2005). Heavy metals were analyzed with iCAP 7000 Series ICP Spectrometer (Thermo Scientific, Waltham, MA, USA), total dissolved carbon (DC) was measured using a TOC-L Total Organic Carbon Analyzer and ASI-L Auto Sampler (Shimadzu, Kyoto, Kyoto Prefecture, Japan) and heterotrophic bacteria were measured using the heterotrophic plate count (HPC) method (APHA; AWWA; WEF, 2005).

Statistical Analysis

Statistical analysis was completed using NCSS 10 (NCSS 10 Statistical Software (2015). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/ncss). Correlation provided the framework for statistical analysis, but the Wilcoxon-Mann-Whitney and Kruskal-Wallis tests were used to confirm the similarities and differences between samples, sampling locations, and storm events. Principle Components Analysis (PCA), Factor Analysis, and K-means Cluster Analysis were also used for analysis. PCA uses an orthogonal transformation to create a values of variables from a set of observations; in this case rooftop runoff quality measurements.

Results and Discussion

With three independent rooftops and three storms (Table 12), a fully captured storm allows for analysis of the change in pollutant concentrations over the course of an entire storm event, differences in rooftop quality, and storm-by-storm variances in runoff quality. In general, the weather characteristics are similar, but storm 3 had a greater amount of precipitation during the sampling period. Storm 2 did not last the full hour for capture. No data normalization was done based on the volume of collected runoff.

Table 12. Storm and Weather Characteristics.

Storm Event	Temp °C	Humidity %	Wind speed km h ⁻¹	Precipitation mm	Antecedent Dry Period days
1	19.4	67	14.48	0.25	3.83
2	18.9	68	17.70	0.76	3.25
3	19.4	77	8.05	4.06	2.42

Through K-means clustering and Kruskal-Wallis analysis, roof 3 was confirmed to produce cleaner runoff than the other two rooftops. Clustering analysis for storm 2 and 3, which were the only two storms sampled for roof 3, show separate clusters for roof 3 versus the clustering of roofs 1 and 2 together (Figure 6). Major quality differences shared for both storms 2 and 3 which were statistically significant were for HPC, pH, nitrate, nitrite, and dissolved inorganic and organic carbon, with either one or both of the other two rooftops having significantly higher concentrations or measurements than those for roof 3. When considering roof 3 as a standalone rooftop, clustering revealed two clusters, one with samples 2-5 from each storm, and one with the first samples from the two storms (Figure 7). Pollutants which appear to be more contaminated in the first sample include ammonia, dissolved carbon, and dissolved organic carbon. TSS, VSS, sulfate, and fluoride, were statistically lower in samples 3, 4, and 5 than in sample 1. Magnesium ions were a special case, as the first sample was only statistically different from the second and fifth samples, but not the third or fourth. Storm 1 analysis separated samples 1-3 for roof 2 with sample 1 from roof 1 (Figure 6). This cluster had lower bacteria, but higher concentrations of TSS, nitrite, chloride, and fluoride. However statistically, nitrite was the only of these contaminants which was statistically different between the rooftops. Nitrite and potassium were higher for roof 2, but nitrate was higher for roof 1. For roof 2, storm 2 clustered separately from storm 1 and samples 3-5 of storm 3. The second cluster had less heterotrophic bacteria, but a higher pH, and higher concentrations of sulfate, fluoride, potassium, magnesium, and calcium. The only statistical differences for roof 2 between samples were for nitrate and magnesium. For nitrate, sample 1 was higher than samples 3-5; sample 2 was also higher than sample 5. Magnesium was only different for samples 1 and 4. Roof 1 was unique in the fact that there were no statistical differences between samples. Clustering analysis did however separate sample 1 from storm 2 and samples 1 and 2 from storm three into their own small cluster (Figure 7). The smaller cluster had a lower pH and fluoride concentration, but notably higher concentrations for nitrate, chloride, sulfate, and magnesium. All clustering was impacted by a mix of the following constituents: pH, nitrate, nitrite, chloride, sulfate, magnesium, calcium, potassium, fluoride, dissolved organic carbon, dissolved inorganic carbon, dissolved nitrogen, total suspended solids, volatile suspended solids, copper, silicate, *E. coli*, and heterotrophic plate count.

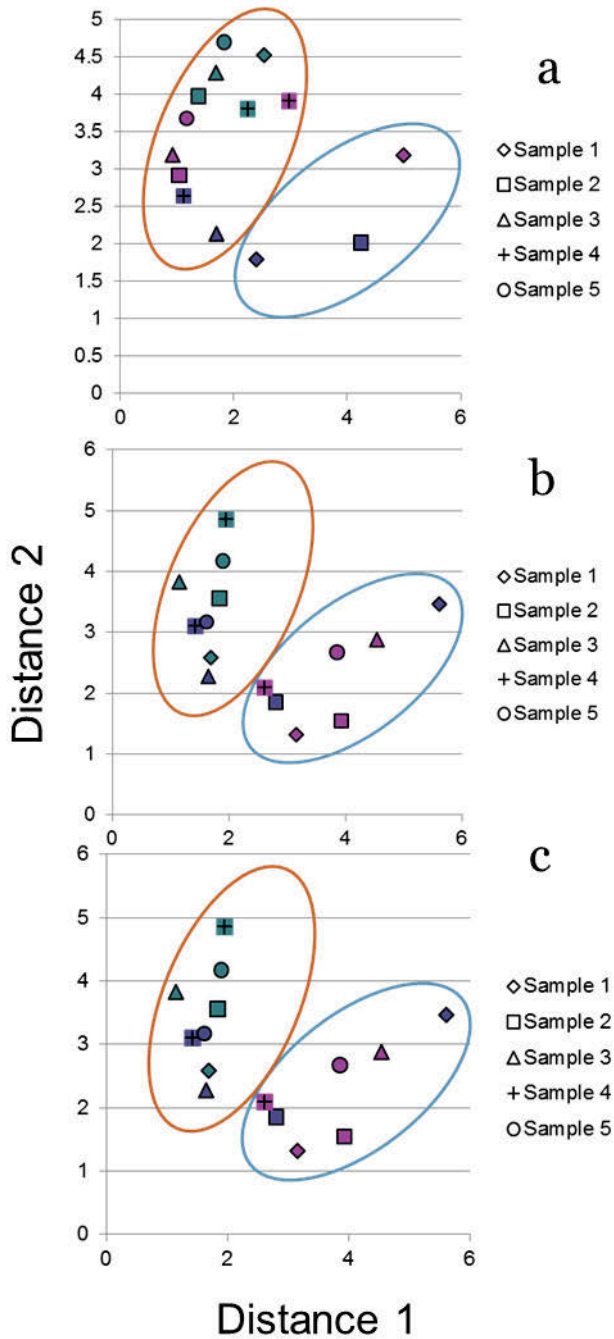


Figure 6. k-means Clustering separated by sample. Cluster 1 is outlined in orange, while cluster 2 is outlined in blue. Storm 1 (turquoise), storm 2 (indigo), and storm 3 (purple) are shown in different colors to further assist in understanding clustering breakdown. A) Roof 1 clustering, b) Roof 2 clustering, c) Roof 3 clustering.

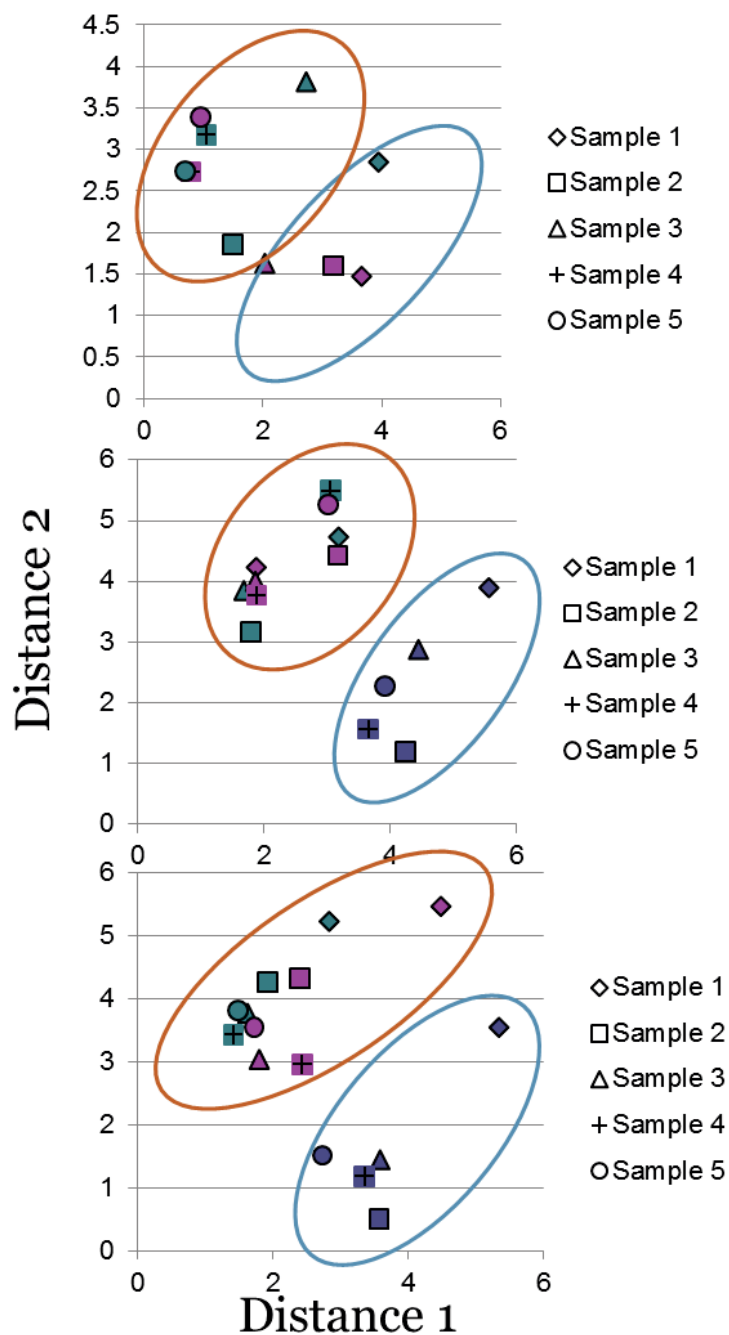


Figure 7. k-means Clustering separated by sample. Cluster 1 is outlined in orange, while cluster 2 is outlined in blue. Roof 1 (turquoise), Roof 2 (indigo), and Roof 3 (purple) are shown in different colors to further assist in understanding clustering breakdown. A) storm 1 clustering, b) storm 2 clustering, c) storm 3 clustering.

Common metal contaminants were relatively similar or lower in concentration than what was found in literature. Common contaminants of concern in rooftop runoff include aluminum, copper, iron, lead, and zinc (Figure 8). Aluminum, lead, and zinc appear to be lower in concentration in comparison with values reported in literature for rooftop runoff from across the world. Iron appears to be equivalent, but copper appears to be higher than values from literature.

Although bacteria are commonly found in cisterns, bacterial contamination is not frequently monitored in relation to rooftop runoff quality. Measurements taken across the duration of the storm events show that bacterial contamination are relatively consistent (Figure 9). The highest measurements were typically found within the middle three samples in the storm event for independent rooftops. It is possible that there is a lag during the storm, which contributes to increased bacterial loading in the middle of a storm, but transport appears to be uniform. This finding suggests that any rooftop runoff that would be reused would need some kind of treatment.

Conclusions

Multiple conclusions can be drawn from this small pilot study. The most important conclusion is that for most of the monitored parameters, contamination is consistent over the course of a storm. Parameters that did show variations over the course of a storm varied between rooftops. More importantly, rooftops with overall less contamination were the ones that showed an improvement as storms progressed. The significance of this is that if a rooftop is contaminated and causing polluted rooftop runoff, then this water will remain contaminated throughout the course of a storm, not wash off all at once.

Bacterial loading is infrequently studied in rooftop runoff, but *E. coli* and heterotrophic bacterial contamination were found throughout the storm; rooftop runoff would therefore need treatment prior to reuse, even for agricultural uses. The need for pre-treatment of collected rooftop runoff would be necessary to treat multiple contaminants, not just bacteria.

It is important to note that rooftops in this study were often significantly different from one another. The most contaminated rooftop was older and had more tree cover than the two other rooftops. Runoff quality could also be related to population density or traffic, as roof 3, the least contaminated roof, was in a less populated and traveled area than the other two rooftops. Any of these factors have the ability to contribute to rooftop runoff contamination, especially since atmospheric deposition is considered to be a major contributor to contaminant loading. For proper treatment, one design would not fit all rooftops. Current designs for water catchment from rooftops do not adequately account for runoff contamination, and typically remove the first portion of runoff, or first flush, which may not necessarily contain the highest concentration of pollutants as previously believed.

One benefit of discovering the consistency of quality in rooftop runoff during a storm is for sample collection. Quality for one rooftop over the course of a storm is consistent, but grab samples can be analyzed for most parameters without concern of inconsistent values as the storm progresses.

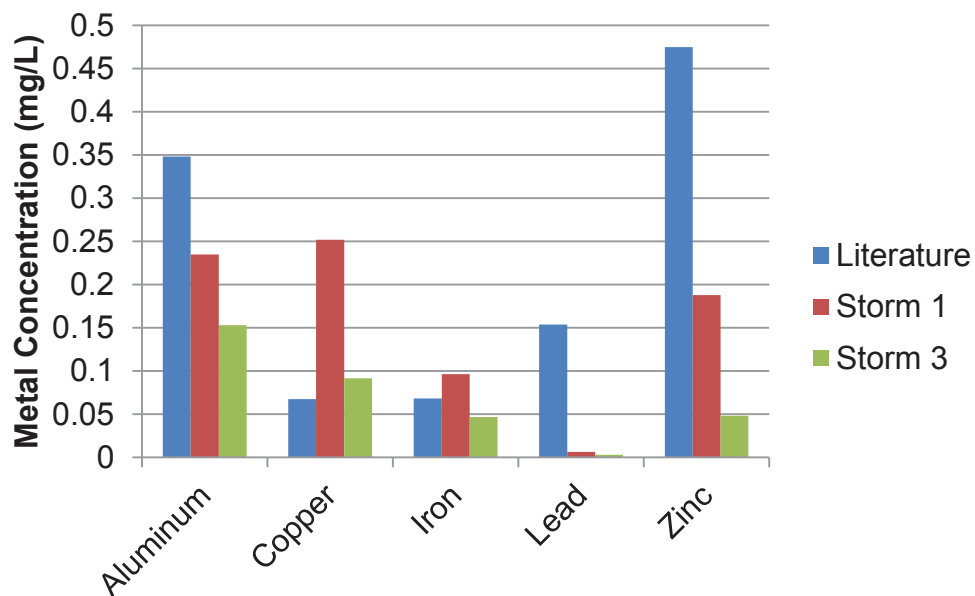


Figure 8. Comparison of common metals found in rooftop runoff in literature with concentrations found in storms 1 and 3.

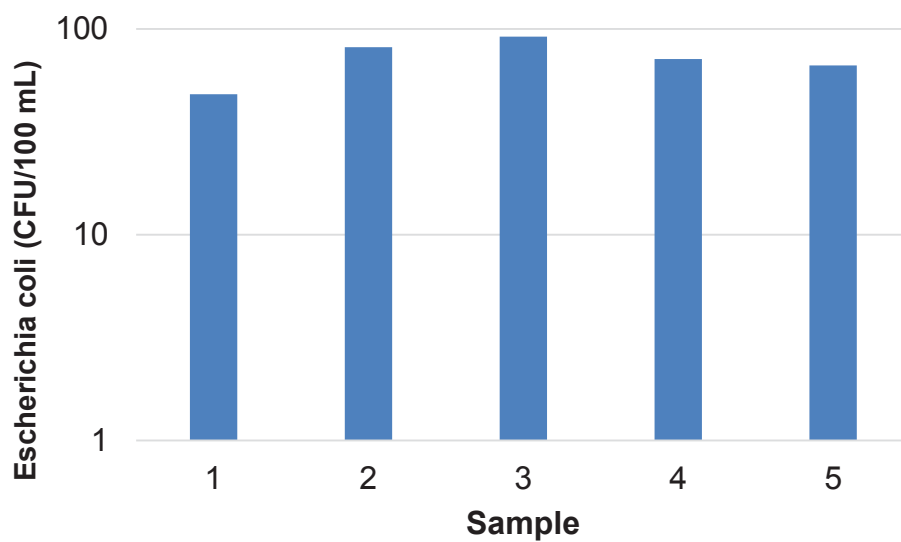


Figure 9. Figure 4. Average *E. coli* concentration over the course of the collected storms.

Advances in rooftop runoff collection could be particularly useful in developing countries and areas which experience significant drought events. The potential source of potable water will only be useful if technologies can be designed and implemented in rooftop runoff collection devices.

Though access to the rooftops used in this study is no longer available, more studies of this nature would help solidify the change in quality over time. Current literature tends to group what is considered as the first flush with the remaining quality of the storm. The change in quality can therefore be masked by the total collected runoff volume. Even though runoff quality seems to vary by rooftop, what does not vary is the generally consistent values across the entirety of a storm event on one rooftop.

CHAPTER III
ROOFTOP RUNOFF QUALITY AND MICROBIOLOGY FOR
APARTMENT COMPLEXES IN KNOXVILLE, TN, USA

Abstract

While rooftop runoff water collection and harvesting is becoming increasingly popular around the world and in the United States, the lack of quality data directly from gutter outfalls is worrisome. Designing cisterns without influent quality data does not allow for proper storage design or treatment prior to reuse. Since most water collection occurs at houses, five buildings from each of three large apartment complexes were selected for this study. Grab samples were collected from one gutter downspout per building near the mid-point of the storm. Runoff was monitored for metals, organics, ions, and *Escherichia coli*. Comparisons made between storm events and apartment complexes revealed that the antecedent dry period and precipitation seem to be primary driving factors in rooftop runoff quality. Roofing material derivatives in the form of aluminum, silicate, and calcium were prevalent. Aluminum and silicate were related to each other, suggesting roof erosion, but calcium was found to be strongly correlated with the antecedent dry period. Bacterial counts were inconsistent between storm events and apartment complexes, which suggests that the first flush treatment method may not be appropriate for bacterial contamination prevention in future rainwater harvesting designs. As a whole, the rooftop runoff system is complex, and the network between environmental conditions, weather, and quality cannot be easily separated.

Introduction

As rainwater harvesting continues to become a popular practice in the United States, it is important to understand the influent water quality from rooftops and gutter outfalls into small-scale cisterns. Houses would be the most likely buildings to make use of rainwater harvesting technologies. Capturing rooftop runoff could also help with flood mitigation and provide solutions for long-term storage in drought conditions. The problem associated with current literature however, is the lack of influent rooftop runoff quality before entry into a cistern.

Rooftop runoff is known to contain a variety of contaminants. Metals (Gromaire, Garnaud, et al., 2001; Schriewer, Horn, et al., 2008; Melidis, Akratos, et al., 2007; Zhang, Wang, et al., 2014; Kim, Lee, et al., 2005; Lee, Yang, et al., 2010; Yaziz, Gunting, et al., 1989; Uba and Aghogho, 2000; Mendez, Klenzendorf, et al., 2011; Chang, McBroom, et al., 2004), ions (Craig A Evans, Coombes, et al., 2006; Gikas and Tsihrintzis, 2012; Melidis, Akratos, et al., 2007; Farreny, Morales-Pinzón, et al., 2011; Zhang, Wang, et al., 2014; Lee, Yang, et al., 2010; Uba and Aghogho, 2000; Mendez, Klenzendorf, et al., 2011), and organics (Schriewer, Horn, et al., 2008; Gikas and Tsihrintzis, 2012; Farreny, Morales-Pinzón, et al., 2011; Zhang, Wang, et al., 2014; Kim, Lee, et al., 2005; Mendez, Klenzendorf, et al., 2011) are the most commonly reported water quality parameters reported for rooftop runoff. Bacteria is less commonly measured, but needs to be studied more regularly (Craig A Evans, Coombes, et al., 2006; Lee, Yang, et al., 2010; Kim, Lee, et al., 2005; Yaziz, Gunting, et al., 1989; Uba and Aghogho, 2000), as bacterial presence in rooftop runoff has the potential to pose a risk to public health should the stored water provide a sustainable environment for incoming bacteria. As people attempt to design effective rainwater harvesting systems

(Thomas, Kirisits, et al., 2014), more information needs to be collected on the influent rooftop runoff water.

This study was designed to monitor rooftop runoff quality in relation to weather and other environmental characteristics at apartment complexes. Three individual apartment complexes were selected in North, South, and Central Knoxville, Tennessee, with five separate buildings sampled from each complex. Quality data was compared between complexes and sampled storms for more information on predictors for runoff quality. Runoff was analyzed for metals, organics, selected ions, and *Escherichia coli* (*E. coli*).

Methodology

Site descriptions

Three apartment complexes within Knoxville, Tennessee city limits were selected for rooftop runoff collection. One complex each was selected from north (36° 1' 13.740" N, 83° 56' 27.279" W), central (35° 57' 42.308" N, 83° 55' 47.254" W), and south (35° 57' 18.045" N, 83° 54' 1.659" W) Knoxville. Five individual buildings were selected from each apartment complex to represent the overall runoff quality. Complexes and buildings were selected based upon access to open gutter systems. Roof age and material was consistent within the complexes. Roofing materials for each complex were all aged asphalt shingles. Exact roof age was unavailable.

Sample collection

Three late-winter storm events were collected, generating a total of 45 individual samples. Storms were frontal storms which affected all three sites at the same time (Figure 10). One liter volumes were collected as individual grab samples in sterile Nalgene bottles. Runoff was collected directly from the output of the gutter downpipe. Individual bottles were stored on icepacks until sampling was complete and they were delivered to the lab for analysis. Runoff from all three of the apartment complexes were collected using the same three storm events. Storms were collected in late winter-early spring seasonal conditions.

Sample analysis

Chemical, physical, and bacterial analyses conducted to characterize and assess contamination were completed in accordance with Standard Methods (APHA; AWWA; WEF, 2005). *Escherichia coli* (*E. coli*) was measured using m-ColiBlue24® (Hach) paired with the membrane filtration method. Dissolved metal and nutrient ions were assessed using inductively coupled plasma (ICP iCAP 7000 Series Spectrometer, Thermo Scientific). Dissolved carbon and nitrogen were analyzed with (TOC-L, ASI-L, Total Organic Carbon Analyzer, Shimadzu). pH and Turbidity were also recorded for base water quality background. Weather data was collected using local weather stations near the apartment complexes. Atmospheric quality data was recorded from the Tennessee Department of Environment and Conservation (TDEC) database using the Air Quality Index (AQI) for ozone and particulate matter (PM_{2.5}) presence.

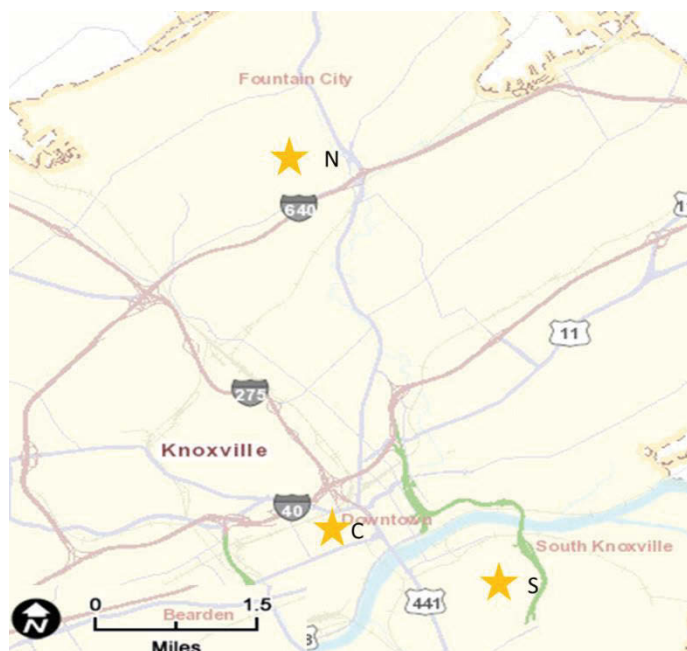


Figure 10. Knoxville, Tennessee map depicting apartment complex locations.

Statistical analysis

NCSS 10 was the primary package used for statistical analysis on this data set (NCSS 10 Statistical Software (2015)). Data screening revealed no outliers, but did show a lack of normality, requiring data to be processed utilizing non-parametric analysis. Principle Components Analysis (PCA) and Factor Analysis (FA) results were used to perform k-means clustering analysis. The Kruskal-Wallis test for one-way ANOVA was supported using Wilcoxon Rank Sum Test for paired samples. Paired t-testing was also completed to verify sample-by-sample mean comparisons. All statistical analyses were completed with $\alpha=0.05$.

Results and Discussion

Runoff pollutant concentrations were found to vary by site and environmental factors, making rooftop runoff quality a multifaceted system. Interpretation of rooftop runoff quality can be accomplished more easily by breaking down the system into factors related to sample collection location and environmental parameters.

Preliminary statistical analysis showed no outliers. PCA and FA revealed a total of four important factors, including pH, magnesium, calcium, aluminum, silicate, precipitation, and ADP for the first factor, zinc and sodium for the second, temperature and humidity for the third, and manganese, pressure, and a repeated calcium and zinc for the fourth. These factors control the clustering output (Figure 11). Cluster 1 is comprised of only storm 2 samples, which had an ADP around 5 days. Cluster 2 encompassed storm 1 and storm 3 samples. Both storms 1 and 3 have an ADP of around 2.4 days (Table 13). Since the variation in precipitation is so large between

storms 1 and 3, it is unlikely that it is a driving factor in clustering (Table 14). ADP appears to be a driving force for clustering, and should be considered when analyzing rooftop runoff quality.

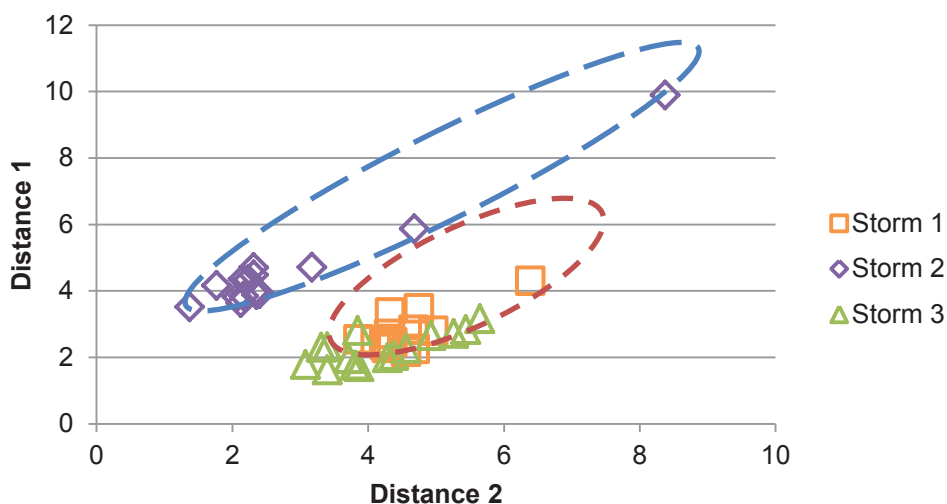


Figure 11. K-means cluster analysis. Clusters seem to be dependent upon the antecedent dry period. Cluster 1 is outlined in blue, while cluster 2 is outlined in red. Storm 1 is depicted with orange squares; storm 2 is depicted with purple diamonds; storm 3 is depicted with green triangles.

Rooftop runoff quality is rarely checked for bacterial contamination. In order to address this problem, *E. coli* was measured for each sample. Bacterial data was highly inconsistent between sites and storms (Figure 12). What is important to note about the *E. coli* counts is that they can be found throughout the storm and therefore do not completely wash off in the beginning of the storm. This has the potential to impact current collection technologies, as it is all geared towards first flush avoidance; this would not be a successful method for collecting less contaminated water if bacterial contamination is coming prevalent throughout a storm event in irregular patterns. The amount of *E. coli* found in the runoff was more equivalent to what others have found for total viable bacteria or total coliform bacteria (Yaziz, Gunting, et al., 1989; Kim, Lee, et al., 2005). The same idea can be supported for other quality measurements from this study such as potassium, dissolved carbon, and dissolved nitrogen. While first flush comparisons cannot be made, the presence of these parameters mid-storm suggests that they do not wash off as thoroughly as expected in the initial runoff volume. There was no relationship between copper, a known biocide, and *E. coli* concentrations in the runoff.

Pollutants in the apartment complex runoff were found to be lower in concentration in comparison with reported quantities in literature. Metal concentrations reported from the apartment complexes are lower in comparison with reported quantities in literature (Melidis, Akratos, et al., 2007; Zhang, Wang, et al., 2014; Kim, Lee, et al., 2005; Lee, Yang, et al., 2010; Yaziz, Gunting, et al., 1989; Mendez, Klenzendorf, et al., 2011). Zinc is the only metal which comes close to being comparable to previous findings. Organic carbon and nitrogen concentrations were within similar ranges to literature, but were on the low side of reported values (Schriewer, Horn, et al., 2008; Farreny, Morales-Pinzón, et al., 2011; Zhang, Wang, et al., 2014; Kim, Lee, et al., 2005; Mendez, Klenzendorf, et al., 2011).

Table 13. Cluster means.

Variables	Unit	Cluster 1	Cluster2
pH		6.99	6.78
Calcium	$\mu\text{g L}^{-1}$	2665	1104
Sodium	$\mu\text{g L}^{-1}$	508	138
Magnesium	$\mu\text{g L}^{-1}$	227	72
Manganese	$\mu\text{g L}^{-1}$	5.25	3.66
Silicate	$\mu\text{g L}^{-1}$	121	260
Aluminum	$\mu\text{g L}^{-1}$	7.74	29.63
Zinc	$\mu\text{g L}^{-1}$	96.95	18.66
Temperature	$^{\circ}\text{C}$	15.6	12.9
Humidity	%	75.3	85.5
Pressure	mm	751	754
Precipitation	mm	12.2	28.7
ADP	days	5.5	2.4
Count		15	30

Location-related variations in rooftop runoff quality

Using paired t-tests and non-parametric ANOVA tests, some differences between the runoff were statistically significant. The most notable parameters with statistical differences include zinc, calcium, copper, sodium, and inorganic carbon. Zinc was much higher at the northern apartment complexes, followed by central and then southern complexes with the lowest. Southern complexes had the most calcium, but the least copper. Northern rooftops had the least amount of dissolved inorganic carbon. Statistical differences were also detected between sites for pH, turbidity, and manganese (Table 15). There are no obvious reasons for the variations found between the apartment complexes, especially since storm and environmental conditions were similar between the collection sites for each respective storm event. These variations

may be caused by roof age and materials (Zhang, Wang, et al., 2014; Uba and Aghogho, 2000).

Table 14. Environmental characteristics for each storm event.

Environmental Characteristics	Unit	Storm 1	Storm 2	Storm 3
Precipitation	mm	17.5	12.3	39.8
ADP	days	2.5	5.5	2.3
Temperature	°C	8.9	15.6	17.2
Humidity	%	91	75	79
Wind Speed	km h ⁻¹	0.48	5.95	2.74
Pressure	mm	751	751	756

Impact of storm and weather characteristics on rooftop runoff quality

In order to better understand the potential impacts of storm and weather characteristics, it is necessary to examine the differences between storm events (Table 13). Storm 2 had the highest ADP and wind speed, but the lowest amount of precipitation. Storm 3 had the highest amount of precipitation at the warmest temperature. Storm 1 was the coldest storm collected, having the lowest wind speed but the highest humidity.

One common consistency between storm events was the relationship between aluminum and silicate (Figure 13). Aluminum and silicate are derivatives of kaolin, which is commonly used in the construction of asphalt shingles. Storms 1 and 3 both showed higher amounts of dissolved aluminum and silicate in the runoff. Storms 1 and 3 had similar ADP, but shared few other environmental characteristics. The antecedent dry period is strongly negatively correlated (-0.96) with aluminum, but not with silicate. Silicate was more directly related to humidity (-0.91) and wind speed (-0.81). Increased amounts of calcium were also found in storms with greater antecedent dry periods. Calcium is also a known derivative from roof material erosion. Knoxville is also home to mining locations which could introduce another dry-deposition source for aluminum, silicate, magnesium, and calcium in the runoff. Roof age may play a role, but evidence has been suggesting that there is statistically no difference between new and aged rooftop runoff for similar roofing materials (Mendez, Klenzendorf, et al., 2011; Schriewer, Horn, et al., 2008).

Other than impacts on aluminum, silicate, and calcium concentrations, atmospheric conditions monitored for this study to not appear to have a strong impact on rooftop runoff quality. Though factor analysis found ADP, precipitation, temperature, humidity, and pressure to be important in relation to runoff quality, additional impacts are not obvious. Separation into individual apartment complexes or storm events makes patterns a little more clear.

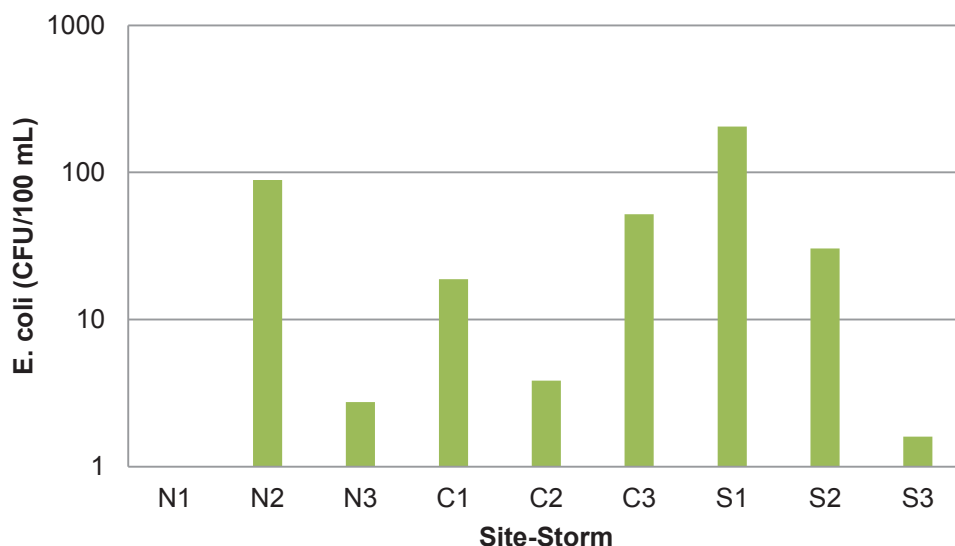


Figure 12. Average *Escherichia coli* counts for each apartment complex for each storm. Values are inconsistent, but generally present in values that make reuse or exposure a potential threat to public health.

Conclusion

Rooftop runoff quality is a highly complex system related to weather, environment, and sample collection location. Many factors play a role in predicting quality, and from this study it was determined that ADP and precipitation were important environmental and weather characteristics. Roofing material also seems to play a role in contaminant release, as primary contaminants found in the runoff, aluminum, silicate, and calcium, are likely derivatives of asphalt shingle breakdown and erosion. Clusters for rooftop runoff quality were separated by storm event, with the primary difference being the ADP. There is a chance that sand from mining and nails from rooftop construction could be an additional source of aluminum, silicate, and zinc in the runoff.

The concentrations of most metal and nutrient contaminants were relatively low, especially in relation to concentrations reported in relevant literature. Bacterial contamination, in the form of *E. coli*, was found to be higher than what was reported by other studies, as counts were more equivalent to total heterotrophic bacteria counts. Metals were mostly found to be lower in concentration than what was reported in literature with the exception of zinc which fell within reported ranges. Organic carbon and nitrogen, while low, were also similar to reported values.

Even within the same city, the location did matter for certain measured parameters. The three separate apartment complexes all had the same roofing material installed, but age was unknown. Since storm events were similar within themselves, there is no obvious reasoning for differences between the locations other than possible differences in geography. Roof age is unlikely to be a contributor to these differences.

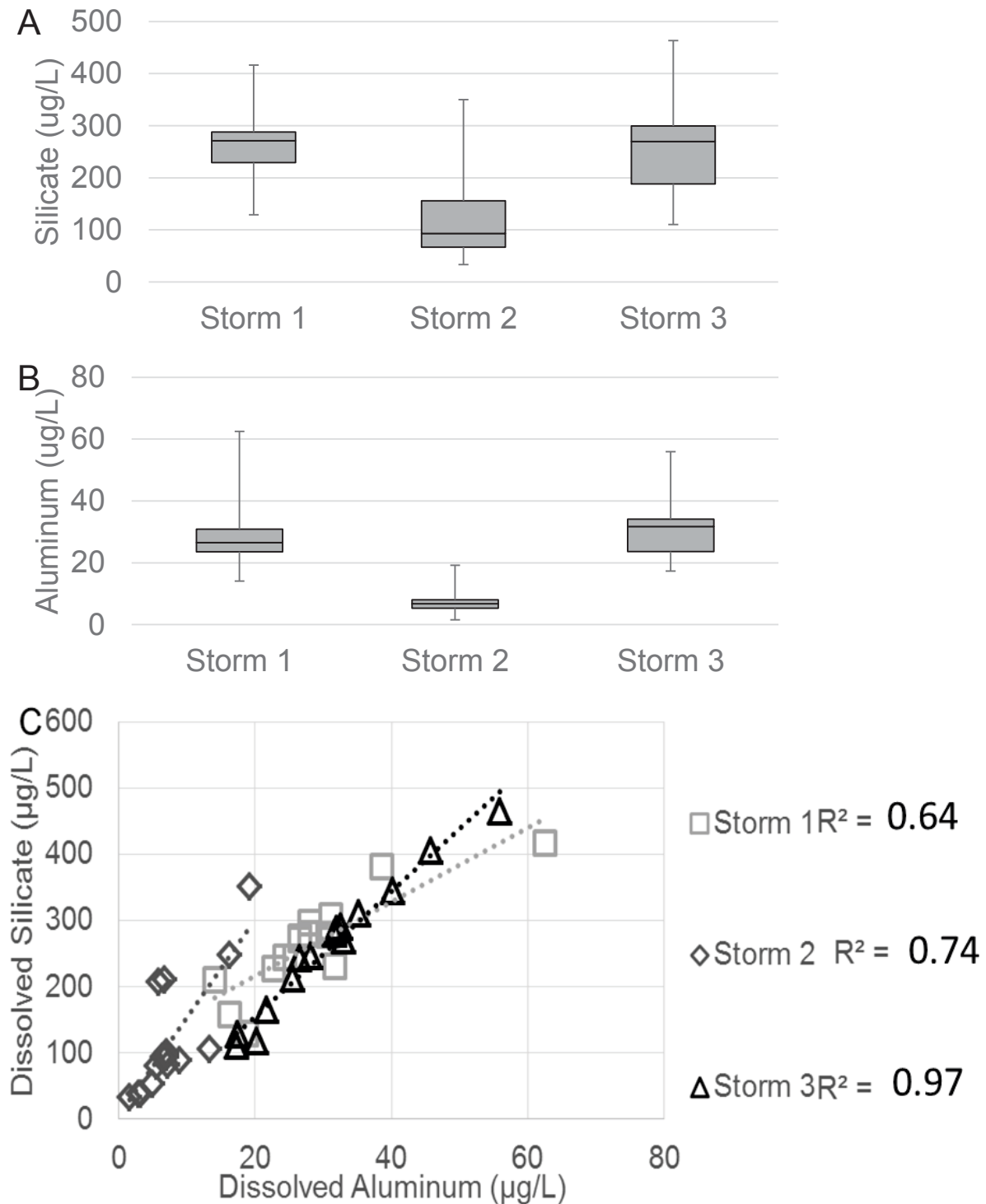


Figure 13. Relationship between aluminum and silicate, separated by storm event. A) silicate separated by storm event. B) aluminum separated by storm event. C) Relationship between aluminum and silicate in runoff, separated by storm event

Table 15. Rooftop runoff quality means results separated by storm event and apartment complexes.

Parameter	Unit	Storm Event			Apartment Complex			Collective	
		Storm 1	Storm 2	Storm 3	Central	North	South	Min	Max
pH		6.90	6.99	6.65	6.85	6.70	6.99	6.42	7.35
Turbidity	NTU	0.96	1.56	1.35	0.96	1.58	1.33	0.51	6.78
<i>E. coli</i>	CFU/100 mL	75	41	19	25	31	79	0	1024
Sodium	µg/L	118.08	508.36	158.09	102.76	502.61	179.17	0	2205.96
Potassium	µg/L	2603.70	296.29	269.86	470.30	417.84	2281.70	89.39	27855.75
Magnesium	µg/L	72.07	227.40	71.07	95.09	181.53	93.92	41.64	883.70
Manganese	µg/L	4.30	5.25	3.02	3.57	6.67	2.33	0.40	24.50
Calcium	µg/L	1306.13	2664.85	902.49	1275.03	1289.61	2308.82	279.52	4664.57
Sulfur	µg/L	207.57	621.43	217.05	216.40	492.85	336.81	141.59	2080.63
Silicate	µg/L	263.14	121.44	257.80	198.02	245.56	198.80	33.56	463.64
Aluminum	µg/L	28.36	7.74	30.89	19.27	25.38	22.35	1.52	62.52
Copper	µg/L	10.40	11.76	8.99	13.29	12.50	5.36	2.62	31.83
Iron	µg/L	2.68	1.43	3.04	1.92	3.49	1.74	0.15	9.70
Lead	µg/L	1.39	1.01	0.72	1.12	0.93	1.07	0	4.10
Zinc	µg/L	23.99	96.95	13.33	11.59	116.78	5.90	2.87	502.55
Organic Carbon	mg/L	2.38	3.03	1.43	1.69	3.56	1.60	0.95	13.58
Inorganic Carbon	mg/L	0.97	1.13	0.66	0.92	0.65	1.20	0.29	2.12
Carbon	mg/L	3.35	4.16	2.10	2.61	4.21	2.79	1.26	14.14
Nitrogen	mg/L	0.32	0.49	0.20	0.25	0.49	0.27	0.12	2.39

CHAPTER IV
ROOFTOP RUNOFF QUALITY AND MICROBIOLOGY FOR
RESIDENTIAL GROUP HOUSING ON THE TSINGHUA UNIVERSITY
CAMPUS IN BEIJING, CHINA

Abstract

As the world continues to develop, and populations increase, the demand for potable water will rise. China is a developing country which is currently unable to provide clean drinking water to all of its citizens. The rate of industrialization and growth in the country are also creating flooding problems, which are especially present in larger cities. One potential way to mitigate water availability and flooding is to capture stormwater runoff. Rooftop runoff could be available for capture and use wherever there is a rooftop and gutter system. One problem associated with developing countries however, is atmospheric pollution. This study was designed to answer two questions; the first is whether or not atmospheric quality in the form of particulate matter 2.5 contaminates the rooftop runoff quality, while the second relates to rooftop runoff quality between different rooftops and storm events. Nine residential buildings were sampled over three storms on the Tsinghua University campus in Beijing, China in the summer of 2017. Quality and microbiology analysis revealed that the majority of differences in runoff characteristics were caused by variations in storm events. Precipitation and runoff intensity during collection were the most influential parameters controlling runoff quality. Few differences were detected between buildings. Atmospheric quality may have affected the turbidity of the rooftop runoff, but did not appear to impact any other parameters. Since storm event characteristics appear to alter runoff quality, it will be difficult to design a device which could both capture and treat runoff for reuse.

Introduction

It is well known that citizens of China often lack access to clean drinking water (Yu, 2011). In more populated areas, increased amounts of impervious surfaces also increase flooding risks (Brody, Zahran, et al., 2008). Rooftop runoff collection and reuse could help mitigate both problems, providing a source of drinking water and reducing flood potential by collecting water that would normally run out onto the street or into a sewer when available (Zhang, Hu, et al., 2012).

Rooftop runoff has been studied only a few times in China (Zhang, Chen, et al., 2010; Zhang, Wang, et al., 2014), and no published works represent rooftops in Beijing. Runoff quality reported in China is notoriously poor, raising questions about the impact of atmospheric quality on rooftop runoff quality. Beijing has reported some of the worst atmospheric quality in the world (Chen, Yan, et al., 2015); however summer quality is not nearly as poor as in winter. Beijing was chosen for this study due to its poor history of air quality, flooding, and high population which could benefit from rooftop runoff collection and use. Particulate matter ($PM_{2.5}$) is a common contaminant in air, especially in China where there are few regulations and a growing industry. $PM_{2.5}$ have a diameter of less than 2.5 micrometers, and can be found in the form of combustion particles, organics, or metals. $PM_{2.5}$ is small and light, staying in the air for extended periods of time. They are also capable of entering through the nose or mouth and penetrate deeply into the lungs (Environmental Protection Agency, 2016a). Most atmospheric pollution in Beijing comes from vehicles, coal, dust storms, and construction dust. The major

pollutants emitted around the area are sulfate, nitrate, organic carbon, elemental carbon, potassium, silicon, calcium, iron, aluminum, calcium, and zinc (Chak, 2002).

In order to determine if rooftop runoff capture would be plausible for drinking water purposes, runoff quality from residential buildings needed to be investigated. Residential buildings were chosen since this is the primary location where most people would require access to clean drinking water. Nine residential buildings were chosen on the Tsinghua University campus, three each from staff, master's student, and international student housing. Each set of buildings had similar characteristics in relation to roofing material, age, upkeep, and tree cover.

Methodology

This project was completed with partial funding from Professor Yunfeng Yang at The School of Environment at Tsinghua University, the National Science Foundation, and the Chinese Academy of Sciences as a part of the East Asia & Pacific Summer Institutes Fellowship under award number 1713940. All sample collection and processing was completed in Beijing, China in summer 2017.

Sample Collection Locations

Collection locations were selected on the Tsinghua University campus. All sampled buildings were residential buildings, separated into staff, master's student, and international student housing (Figure 14). Buildings were selected in triplicates. Rooftops on the Tsinghua University campus were flat, using scupper designs for removing water from the rooftop (Figure 15). This design is different from traditional roofing designs used for residential buildings in the United States.



Figure 14. Tsinghua University campus maps. Stars show sample collection locations. Buildings to the far left are staff housing. Buildings in the middle are designated for master's students. Far right buildings are reserved for international students.

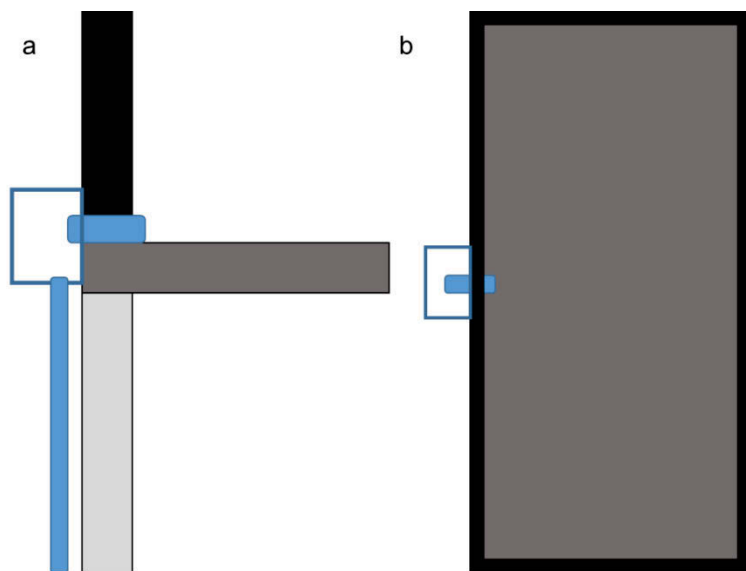


Figure 15. Scupper schematics. (a) Is the elevation view, showing how water leaves the roof and enters the gutter downspout. (b) Depicts the plan view of the roof, and how water enters the scupper.

Sample Collection and Processing

Three storms were collected over the course of the summer. Storms varied in precipitation and antecedent dry period (Table 16). A total of ten samples were collected per storm event, nine runoff samples and one pure rain sample. Samples were collected using the grab sampling methodology. The design of the rooftops was flat and concrete. All samples were taken while the storm was still in process; however the second and third storms were collected closer to the end of the storm event and took longer to collect due to low flow. In order to test runoff quality, pH, water temperature, turbidity, and *E. coli* were tested (APHA; AWWA; WEF, 2005). Samples were preserved through filtration to measure dissolved carbon and nitrogen, and additionally acidified with hydrochloric acid to measure metals. Aluminum, copper, iron, lead, zinc, and silicate were selected for metals analysis because they are commonly found in rooftop runoff. Filtered samples were preserved at 4°C until processing was possible. Only the first collected storm had enough collected volume to process all parameters, the other two storms were only tested for pH, turbidity, and *Escherichia coli*.

Metals were measured using an XSeries 2 ICP-MS. Organic carbon was analyzed using a TOC-V CPH Total Organic Carbon Analyzer from Shimadzu. Turbidity was measured using a HACH 2100N turbidity meter. pH and water temperature were measured using a Denver Instrument UB-7. Pre-mixed EC MUG media was purchased in China. Agar was added to follow a spread plate method. Fluorescing colonies under ultraviolet light were counted as positive. Large volumes were tested for *E. coli* by filtering with Whatman AH-934 filters.

Table 16. Storm characteristics for sampled events at Tsinghua University.

Date	Temp	Humidity	Wind	Precipitation	ADP	PM2.5
	°C	%	Km hr ⁻¹	mm	hr	
7/6/2017	24.4	88	9.66	22.1	38	133
7/18/2017	28.9	84	8.05	0.254	74	150
7/26/2017	22.2	82	6.44	1.016	119	55

Statistical Analysis

NCSS 10 was used for statistical analysis (NCSS 10 Statistical Software (2015). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/ncss). Data for this study were non-normal. The Kruskal-Wallis one-way ANOVA and Dunn's Test were used to determine differences in quality between storms and building sets. Principle components analysis and factor analysis were used to complete k-means clustering. All analyses were completed using $\alpha=0.05$.

Results and Discussion

Based upon statistical analyses, storm event characteristics strongly impact rooftop runoff quality for residential buildings at Tsinghua University. This is evident through both clustering analysis and Kruskal-Wallis One-way ANOVA testing with Dunn's Test.

ANOVA testing determined statistical differences existed across storms for pH, turbidity, water temperature, and dissolved organic carbon, nitrogen, aluminum, iron, copper, zinc, lead, and silicate. The first storm had significantly lower readings or concentrations for pH, dissolved organic carbon, aluminum, zinc, lead, and silicate as compared with the second and third storms. Dissolved iron and copper were both the highest in concentration for storm two, which had the lowest amount of precipitation, and was collected at the very end of the storm with a low intensity. Turbidity, runoff temperature, and dissolved nitrogen were all significantly lowest for storm three. There is no obvious answer for as to why storm three was the lowest for these three parameters; however the particulate matter was lowest for storm three which could have influenced the turbidity. The atmospheric temperature and humidity were also lowest for the third storm.

Robust principle components analysis showed a total of five possible factors. The first factor included dissolved components, including organic carbon, aluminum, iron, copper, and silicate. The second factor was influenced by the third storm, including turbidity, runoff water temperature, and dissolved nitrogen. The final three factors contained individual parameters, dissolved inorganic carbon, *E. coli*, and dissolved zinc respectively. Factor analysis reduced the number of factors to three. While no changes were made to the first or third factors as identified by PCA, factor two no longer contained turbidity. K-means cluster analysis separated the three storms and building

sets into two clusters (Figure 16). Sample quality for cluster 2 was notably worse than what was found for samples in cluster 1 (Table 17). The two clusters seemed to separate based upon precipitation and collection intensity. The lowest intensities during collection for storm two were the six samples which formed the second cluster, coming from the masters and international student housing. The remaining three samples from storm 2, staff housing, clustered with the first and third storm event. Intensity out of the gutter outfall was faster, improving quality for the staff housing in comparison with the masters and international student housing. Therefore it is logical that precipitation would be negatively correlated to dissolved organic carbon and lead.

Table 17. Variables selected for cluster analysis for Tsinghua University storm events.

Variables	Units	Cluster 1	Cluster 2
Temp	°C	24.0	26.8
Dissolved Organic Carbon	mg/L	14.68	36.90
Dissolved Inorganic Carbon	mg/L	2.20	6.36
Dissolved Nitrogen	mg/L	9.98	20.60
Dissolved Aluminum	mg/L	0.10	0.15
Dissolved Iron	mg/L	0.07	0.12
Dissolved Copper	mg/L	0.005	0.013
Dissolved Silicate	mg/L	1.11	2.99
Count		21	6

When considering variations between residential building sets, it is also important to consider differences caused by tree cover. The international student housing had large trees near and hovering above the rooftops. This led to increased amounts of inorganic carbon and silicate. The international student housing also had pvc gutter downspouts versus the metal ones used for staff and masters student housing, which could be related to the significantly smaller concentrations of zinc found from this rooftop (Chang, McBroom, et al., 2004). While these factors may not be the definitive cause of the statistical differences, they are strong potential influencers for future design work. Dissolved nitrogen was found to be higher for masters and international student housing as compared to staff housing. This could be due to lower flow rates during collection. Staff housing was always collected first, and therefore had higher flow rates for collection, especially when collection occurred near the end of storm events.

E. coli was found to be consistent in samples, and was even regularly found in rain samples (Figure 17). It did not correlate with any other contaminants or weather or environmental characteristics. There were no statistical differences between storms or buildings for *E. coli* concentrations. Though concentrations were inconsistent in rain samples, the presence of bacteria in the rain is concerning. Samples were taken under free sky over the duration of the storm and sampling.

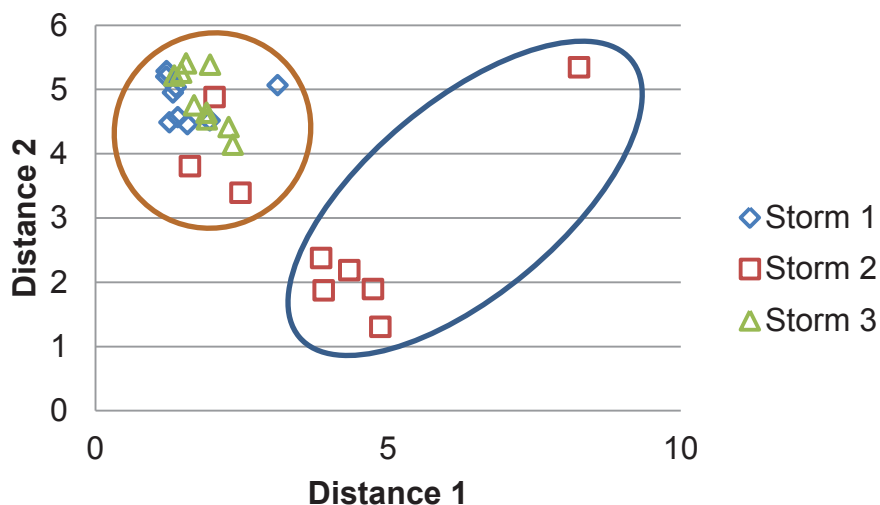


Figure 16. k-means cluster analysis for rooftop runoff at Tsinghua University. Storm 1 is depicted with blue diamonds, Storm 2 with red squares, and storm 3 with green triangles. Cluster 1 is outlined with an orange border, while cluster 2 is outlined with a blue border.

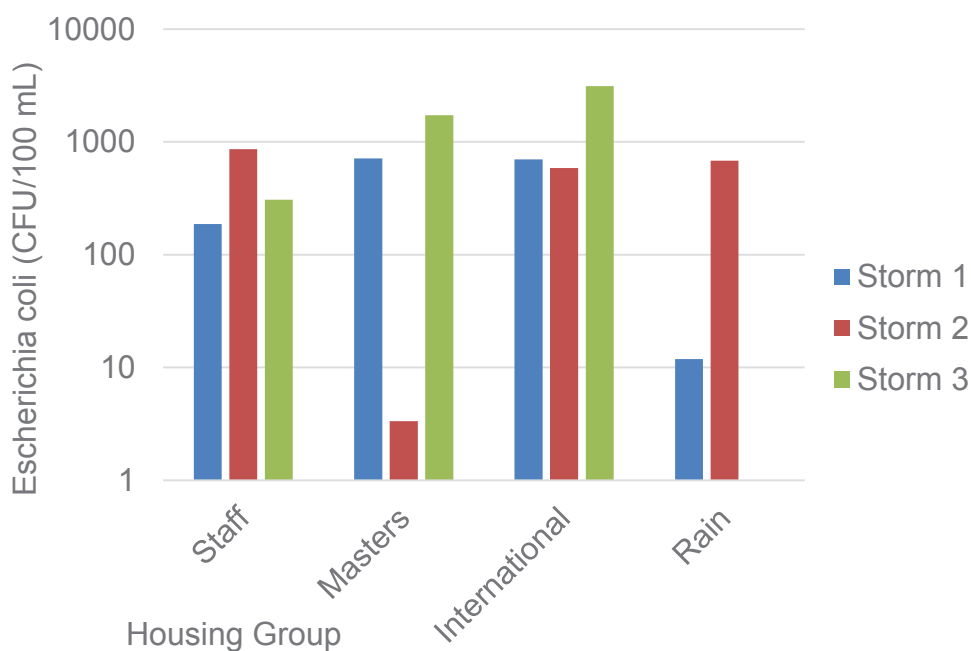


Figure 17. *Escherichia coli* comparisons between housing groups and rain samples at Tsinghua University.

Conclusion

Rooftop runoff quality from rooftops on Tsinghua University campus in Beijing, China was consistent between groups of rooftops, but varied heavily between storm events. Precipitation and intensity were the most important factors controlling quality; however precipitation did not impact bacterial concentrations. Clustering analysis revealed that intensity during collection and total precipitation are likely impacting runoff quality. The six samples that comprise cluster 2 were the last six samples taken at the end of the storm with the smallest amount of precipitation.

pH, dissolved organic carbon, and dissolved lead were all negatively correlated with precipitation. Water temperature, turbidity, and dissolved nitrogen were notable because of the third storm event, which had the longest antecedent dry period, but lowest atmospheric temperature, humidity, and particulate matter concentration. Storm characteristic influence is further supported by PCA and factor analysis.

Even though roofing materials were the same, gutter downspout materials were not. International student housing had pvc downspouts, while the staff and masters student housing had metal-based downspouts. Zinc concentrations were considerably smaller for the international student housing as compared to the other two building sets.

Since sampling only occurred three times, more samples and further analysis at locations off the Tsinghua University campus would be beneficial to further understand runoff quality in China. It would also be advantageous to take samples in the winter, as this is when air quality is the worst. This would help to identify any possible impacts that the atmospheric quality could have on stormwater runoff quality. What did come of this study however, is the importance of precipitation and intensity on rooftop runoff quality.

CHAPTER V
ATTACHMENT AND SURVIVAL OF ROOFTOP RUNOFF ISOLATED
***ESCHERICHIA COLI* IN VARIED TEMPERATURES**

Abstract

Rooftops are a well-known source of non-point source pollution. Rooftop runoff collection is common in developing countries, but the presence of bacteria in the runoff is a concern. The source of bacteria in rooftop runoff is unknown. In order to identify potential sources for bacteria in rooftop runoff, rooftops themselves must be investigated. In order to determine whether or not rooftops could harbor bacteria, three environmental strains of *Escherichia coli* were isolated from rooftop stormwater runoff and put in contact with glass microscope slides. Slides were allowed to incubate at either 20°C, 35°C, or 45°C for up to nine days. Environmental strains were then compared with one laboratory strain of *E. coli* K12. Analysis was completed using acridine orange dye paired with fluorescence microscopy. Images were then analyzed using pixel reclassification using ArcMAP 10.5. Imaging was able to show the growth and division of cells during incubation, and the potential development of biofilms. Temperature did appear to play a role in attachment speed for the *E. coli*. Two of the environmental strains reached the death stage after about five to seven days; the third had a long lag period during the beginning, delaying the death stage past the nine day experiment window. The implications of this study are that bacteria is very likely surviving and potentially propagating on rooftop surfaces, which could become a source of bacteria in rooftop runoff. A one-day window exists between introduction and attachment where easy removal could be possible.

Introduction

Literature has recently started to discuss the possibility that *E. coli* may be capable of surviving in the environment, which would cause current testing methods to monitor human fecal contamination to be inaccurate. Fecal indicator bacteria in urban stormwater runoff have been of great concern in recent times.

Non-point source pollution is a problem for stormwater quality, with little ability to regulate and monitor on a large scale. Two major generators of non-point source urban runoff are roadway and rooftop surfaces. A lingering problem for both rooftop and roadway runoff is the source of bacterial contamination. Bacterial contamination is common in non-point source runoff, but a clear source or reasoning for bacteria to be washed off has not yet been found.

Determining whether or not bacteria, specifically fecal indicator bacteria, are capable of surviving in the environment is an important question. A non-environmental strain of K-12 was used as a standard to test against capabilities of *E. coli* found in rooftop runoff. One K-12 strain of *E. coli* was chosen as the control strain due to its safe nature and individuality in comparison with other strains of *E. coli*. Environmental conditions on roadways and rooftops can be harsh and should kill microbes, yet they are still appearing in our runoff and waterways.

In order to tackle the problem of bacterial source in rooftop runoff, it is important to determine whether or not bacteria are capable of surviving on rooftops in seemingly inhospitable surfaces. Due to the nature of roofing materials, they cannot be tested directly. Glass surfaces modified specifically for acridine orange dye count were used for attachment surfaces. Since temperature is a major factor in bacterial survival

capabilities, temperature impact on three environmental strains and one lab strain of *E. coli* were tested on how well they attach and survive over the course of 9 days. Slides were kept in sterile containers away from light for the duration of experiment. Cell concentrations on the surfaces were large, so pixel analysis was used in lieu of direct counting for enumeration.

Methodology

The methodology for attachment to glass slides can be split into five parts: strain isolation, growth, attachment, variations to survival environments, and microscopy.

Bacterial Strain Isolation and Preparation

Three *Escherichia coli* strains were isolated from rooftop runoff samples from previous rooftop runoff experiments discussed in Chapter III. Two isolates were collected from the same rooftop, but at varying time points during different storms. One isolate was collected at the beginning of the storm (K1), while one was collected at the end of the storm (K5). The third isolate was cultured from runoff from an apartment complex in West Knoxville (S1). Similar to K1, S1 was collected from the initial rooftop runoff generated during a storm. Runoff samples were handled individually and were not mixed.

Stormwater samples were processed using the membrane filter method and mColiBlue24 as the growth medium, with incubation at 35°C for 24 hours. Samples were stored at 4°C until utilized for attachment studies. Using sterilized scissors, individual colonies were cut from the membrane filter and transferred to one well in a 96-well plate which contained 0.2 µL mColiBlue24. Following standard incubation procedures at 35°C for 24 hours, media in positive wells was transferred to a sterile test tube containing Tryptic Soy Broth (TSB). Test tubes were allowed to incubate for 24 hours, and ocular density (OD) was measured using a spectrophotometer to show positive growth. Strong colonies from the K roof in North Knoxville were selected, and the strongest growth from the S rooftop was selected for sample variety.

One lab strain of *E. coli* was grown from a stock solution in the Fozo lab at The University of Tennessee. It is a sequenced lab strain of K12 with the identification of MG1655. K12 samples were transferred from original plating material, LB Agar, to TSA. Colonies were selected and transferred to TSB following growth on TS agar plates (Rivas, Fegan, et al., 2007; Tsuji and Yokoigawa, 2012; Eginton, Gibson, et al., 1995; Wong, Chung, et al., 2002; Coquet, Cosette, et al., 2002; Mafu, Roy, et al., 1990). Colonies transferred to TSB in test tubes were grown to exponential growth as shown by growth curves (Figure 18). 2 mL of the well-mixed growth solution were transferred into a sterile 2 mL Eppendorf tube for centrifugation. Centrifugation was done three times. The first run was at 13,000 G for 3 minutes to separate the cells from the media. The remaining media was removed, and 1 mL 1X Phosphate Buffer Solution (PBS) was added to the Eppendorf tube to wash cells. The cells were washed twice with centrifuge runs set to spin at 10,000 G for 10 minutes. Following each run, the supernatant was removed, and 1 mL PBS was added to gently mix the cells in the pellet back into solution (Chia, Goulter, et al., 2009; Nguyen, Turner, et al., 2010; Goulter-Thorsen,

Taran, et al., 2011; Goulter, Gentle, et al., 2010; Rivas, Fegan, et al., 2007). After the second run, the supernatant was once again removed and the pellet was re-suspended in 1 mL PBS once again. The closed Eppendorf tube was then briefly vortexed at a setting of 2500 rpm to ensure that the cells were well mixed in the suspension (Nguyen, Turner, et al., 2010; Goulter, Gentle, et al., 2010; Tsuji and Yokoigawa, 2012). The cell suspension could then be stored at 4°C until use.

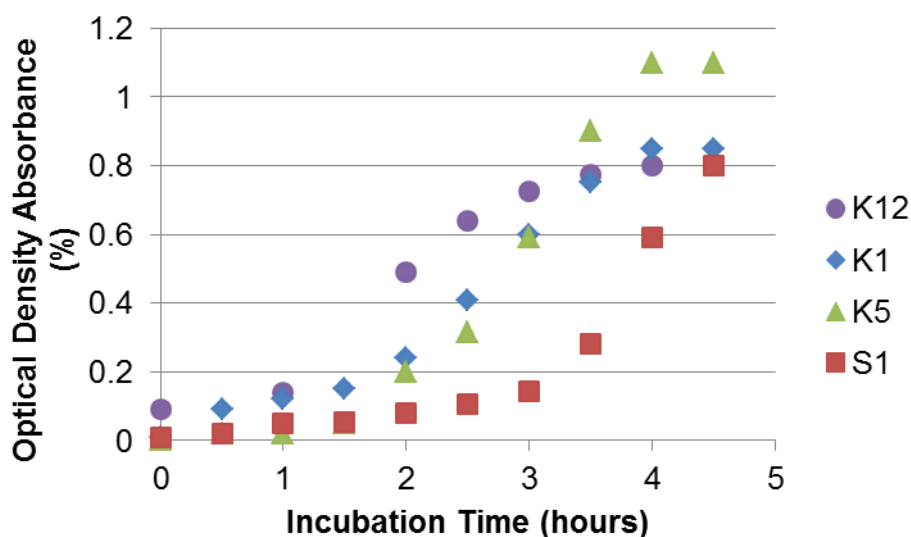


Figure 18. Optical density growth patterns for selected strains of *Escherichia coli*.

Once the cells were cleaned and isolated, they were preserved. 0.5 mL of the suspension was transferred into a clean 2 mL Eppendorf tube with sterile, 80% glycerol. The tube was then vortexed slowly and stored at -80°C as a stock solution. To revive cells and create live cell for attachment studies, the tube was removed from the freezer and a sterile instrument was used to scrape off the top layer of the stock. The top layer was then transferred to TSB or TSA for growth. This methodology was performed separately for each of the four strains used in this study.

Surface Attachment and Survivability Conditions

Cell suspensions were used to treat specialized glass slides used for Acridine Orange Dye Count (TEKDON Incorporated, Design 72-021-120) (Chia, Goulter, et al., 2009; Goulter-Thorsen, Taran, et al., 2011; Goulter, Gentle, et al., 2010; Rivas, Fegan, et al., 2007). These slides had 8 identical circles surrounded by a white frosted coating. The coating prevented cross contamination between samples. Slots 1-2 were reserved for the reference strain, K12. Slots 3-4 were for K1, 5-6 were for K5, and 7-8 were for S1. The dual slots served as replicates from different stocks of the same strain. 10 µL of each well-mixed cell suspension was transferred to the specialized glass slide through pipetting. A total of fifteen slides were prepared, with 5 each for 20°C, 35°C, and 45°C

temperatures. The five slides were used for Day 1, 3, 5, 7, and 9 data points. One additional slide was prepared for a Day 0 baseline. Due to the destructive nature of acridine orange dye, the same slide could not be used for more than one time point. Slides were then transferred to a sterile pipette tip box, face up, and covered with foil.

Microscopy and Attachment Analysis

After the slides reached their proper residence times, the surfaces had to be prepped for microscopy. Steps for acridine orange dyeing prep are as follows:

1. Rinse the slide with 20 mL 1X PBS
2. Heat fix the remaining cells to the slide with a Bunsen burner
3. Apply approximately 10 μ L acridine orange dye to each individual cell on the glass slide
4. Set the slide on the counter under foil for 2 minutes. The foil should not be in contact with the slide surface. Acridine orange dye is light reactive, and must be kept in the dark
5. Rinse at an angle with 20 mL PBS
6. Paper towel dry areas not of interest
7. Heat fix under foil on a hot plate until dry
8. Rinse the glass slide by dipping and removing from Milli-Q ultrapure water, and then again by dipping and gently swishing back and forth twice
9. Paper towel dry areas not of interest
10. Store the slides in a cold, dark place until analysis

Steps varied for the day 0 slide, as the initial rinsing step was skipped. Rinsing in this situation would have likely removed all bacteria from the surface. Instead, the procedure began at step 2.

Rinsing the slides was necessary to remove any dead or unattached bacteria from the slides. Any bacteria still attached to the slide following rinsing are assumed to be alive. This is because dead bacteria would not be able to remain attached to the surface.

Once microscopy was performed, slide oil and a glass cover slide were attached to the surface of the prepared slide. Slides were analyzed using a fluorescence microscope. Individual cells were photographed approximately 10 times each, creating around 20 images for each strain at each time point and temperature.

Due to the bacterial presence in the cells on the slide, counting the cells was not possible. Instead of following traditional cell counting techniques, images were analyzed using ArcMAP 10.5. Images were imported into the program and analyzed using the reclassification techniques, which can be found in the ArcToolbox under spatial analyst tools. Each image was originally set to 6 natural breaks, unless the image had extra background noise. The attribute table showed the total number of pixels in each break. The attribute table data was transferred to an excel file, and the breaks which best depicted the cells in the image were summed and divided by the total number of pixels. The total percentage of cell coverage was then averaged over the twenty images captured for each time point, temperature, and strain, and then compared. Reclassified

images were in vibrant colors changed for each break, allowing for easier cell depictions (Figure 19).

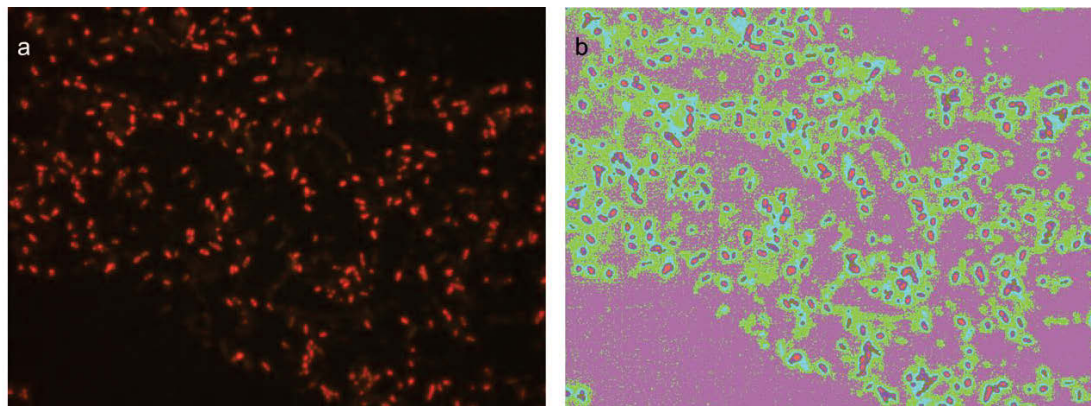


Figure 19. Comparison of original microscopy image with reclassified image.

Statistical Analysis

Statistical analysis was completed using NCSS10. The Kruskal-Wallis Test for One-Way ANOVA for nonparametric data was used to determine if any differences existed in percent coverage over the nine days and three temperatures. The Kruskal-Wallis Multiple Comparison Z-Value Test, also known as Dunn's Test, shows the difference between test medians. Any z-value greater than 1.96 is considered statistically significantly different. All analysis was completed using $\alpha=0.05$.

Results and Discussion

Visual and statistical examination of pixel analysis results showed that the *E. coli* isolated from the environment were capable of attaching and surviving, and that temperature does impact attachment and survival capabilities. At first glance, there even appears to be bacterial growth on the surfaces prior to eventual decay (Figure 20). There is also evidence of biofilm formation for the individual strains (Figure 21). Even more surprising is how well the stock K12 survives and attaches to the glass in comparison with the three environmental strains. Statistically, K12 and the K1 strains are similar, while the K5 and S1 strains behave more similarly to each other. As a whole, individual bacterium strains behave differently at each temperature (Figure 22). Over the various time points of the study, the day 1 samples are statistically lower than most other days; the only exceptions are day 9 for K12, Day 0 for K1, and Days 3 and 5 for S1 (Figure 21). This shows that there is an attachment and possibly adjustment period for the bacteria on the new surfaces. The S1 strain performed differently from the other two environmental strains, as it appeared to have a longer adjustment or attachment period than either of the K1 or K5 strains. While the control K12 strain did not appear to grow during the attachment period, all three environmental strains did grow. The K1 and K5 strains grew enough to be statistically larger than their initial

coverage percentage. K5 was statistically larger than its day 0 value by day 3, while K1 achieved this by day 5.

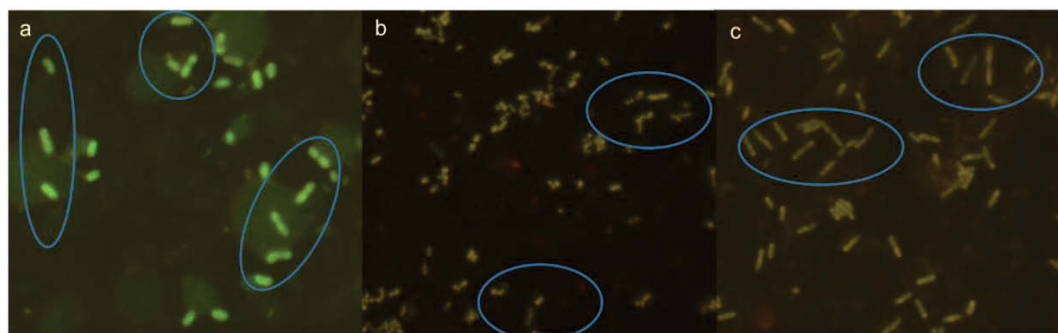


Figure 20. Evidence of bacterial growth on the slides for the environmental strains: (a) K1, (b) K5, and (c) S1.

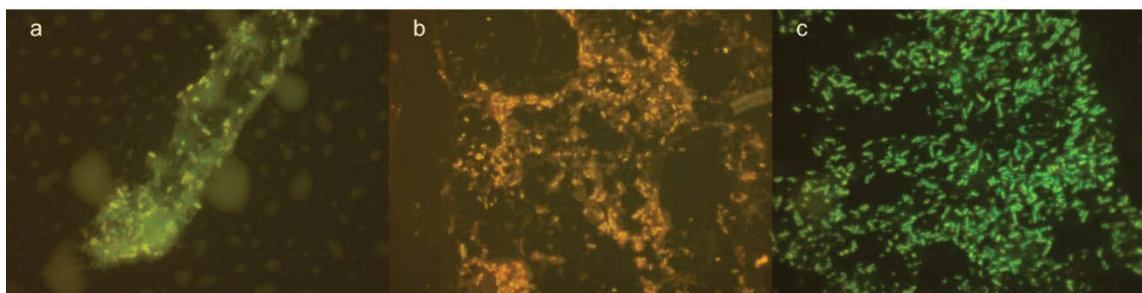


Figure 21. Clustered cells with signs of a film-like substance keeping them in place: (a) K1, (b) K5, and (c) S1.

S1 was never statistically larger than its day 0 value, but was able to maintain and surpass its day 0 values by day 7. It is likely that with additional time points past day 9, that the S1 strain would follow similar but delayed patterns to the K1 and K5 strains. Temperature affected each strain differently. For the K12, K1, and K5 strains, the 45°C temperature encouraged growth and attachment the most. For K12, the 20°C and 35°C temperatures were statistically similar, while each temperature was significantly different for K1. K5 was statistically higher than 20°C for both 35°C and 45°C. Temperature did not appear to have a significant impact on the S1 strain, as all temperatures produced between 13-14% coverage on average. The K12 strain performed the best at 20°C, followed by the K1 and S1 strains, and then the K5 strain which showed the lowest coverage statistically than the other three strains. The K1 strain outperformed all other strains at both 35°C and 45°C. K12 and K5 had statistically more coverage than S1 at 45°C, but not at 35°C. As a whole, K1 and K12 had the most coverage, statistically greater than either K5 or S1. Variations in environmental temperatures will impact surface temperatures for attachment. The strength of the K1 strain is concerning, as *Escherichia coli* is not supposed to be able to survive outside of a gut.

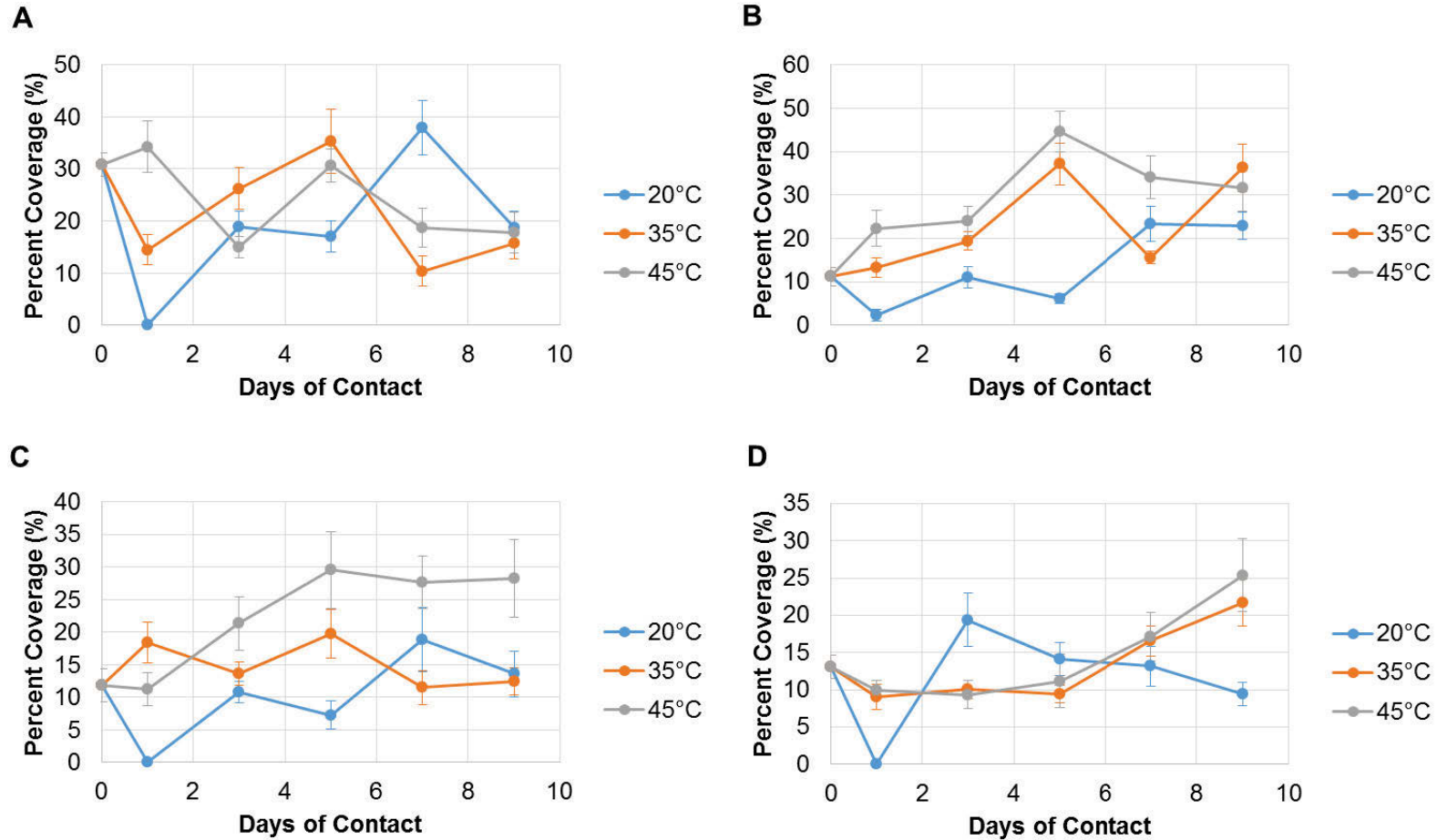


Figure 22. Comparison of strain growth capabilities over the 9 day sample period at 20°C, 35°C, and 45°C. (A) Is the control K12 strain. (B) Is the K1 strain, isolated from the beginning of a storm event. (C) Is the K5 strain, isolated from the end of a storm from the same roof as the K1 strain. (D) Is the S1 strain, which was isolated from the beginning of a storm on a separate roof from K1 or K5.

Temperature impacted growth differently during the attachment period. For both 35°C and 45°C, attachment was the highest at day 5. 20°C showed the most attachment at day 7, but day 7 was statistically similar to days 3 and 9 as well. For 35°C, attachment was similar for days 5 and 9. For 45°C, days 7 and 9 are statistically similar to each other, but lower than day 5. Days 1 and 3 are statistically lower than the day 7 and 9 attachment results.

Conclusion

Results from this study are incredibly revealing. First and foremost, the pure, environmentally-isolated *E. coli* cultures are capable of surviving and potentially even grow while attaching to a surface. Even the lab strain was able to survive over time, but did not show obvious signs of growth in comparison with the initial surface coverage. All of the strains behaved individually to variations in temperature over the experimental period, with the S1 strain being the most abnormal from the group.

One revealing factor is the temperature impact on bacterial attachment. While the lower temperature delayed attachment processes, the bacteria seemed to remain viable and maintain attachment over a longer period of time. Bacteria peaked at day 5 for the two higher temperatures, but showed notable signs of death and detachment following day 5 more so for the 45°C set. The 35°C temperature seemed relatively stable once reaching maximum attachment at day 5. This is important because the relative temperature is impacting how well and how soon the bacteria are attaching to the surfaces.

Roadway temperatures are known to reach 45°C or higher, and rooftops are no different. One advantage of rooftops however is increased surface area from asphalt shingle overlap, decreased temperature in overlap areas, and potential for shade in areas with substantial amount of greenery and wildlife.

Bacteria is known to be transferred through wet deposition, but could also be contributed by wildlife. Given the setup of this experiment, it is highly likely that bacteria would be able to survive on the rooftops for up to a week or longer if they can attach. The bacteria would have access to nutrients and increased surface area unlike in this experiment which used no light or nutrients on smooth glass surfaces. Adding nutrients and light, *E. coli* would be more than capable of attaching and surviving on rooftops or in roadways.

Implications of bacterial survival in built environments, especially fecal indicator bacteria, are numerous. Given that this study supports the idea that *E. coli* is capable of surviving outside of the human gut, *E. coli* becomes an unreliable indicator of human fecal contamination. The survival, attachment, and probable growth of bacteria shows that runoff collected on such surfaces of will likely be contaminated. There seems to be a small window where bacteria removal would be possible, preventing attachment and buildup on built surfaces. Even though the bacteria would eventually die off on their own, the introduction of new bacteria and nutrients may allow for conditions which support continuous growth. Wash-off events have the potential to transfer live bacteria to a nearby water source.

CONCLUSION

Summary and Conclusions

For this dissertation, water quality analysis, 16S rRNA sequencing, and fluorescence microscopy served as the foundation for urban stormwater runoff analysis from roadways and rooftops. Water quality analysis of roadways, streams, and rooftops located around the world was necessary to provide a basic mode of comparison to other studies, drinking water standards, and to each other. 16S rRNA sequencing revealed a more in-depth look at the microbiology of roadway runoff, allowing for an investigation into the impact that roadway runoff has on surrounding streams. Fluorescence microscopy is commonly used in addition to standard plate count methods for determining bacterial attachment to a surface. What was not standard was the use of spatial analysis tools to enumerate the percent of a surface which was covered by cells. This methodology reduced the chance of human error related to cell counting, and further permitted the analysis of cell attachment on glass slides.

Two common threads between both roadway and rooftop runoff were discovered. The first is that storm characteristics are important in determining potential runoff quality. The most important storm characteristics relating to runoff quality are precipitation, antecedent dry period, and intensity. The second is that bacterial populations seem to be persistent and capable of surviving between storm events. This was evident with the constant bacterial populations found on the roadway using 16S rRNA sequencing, and through attachment and survival experiments. Temperatures in the attachment studies were selected to reflect roadway and rooftop temperatures, so survival in conditions with little to no sunlight and possibly no nutrients is plausible.

One unique aspect of this dissertation was the ability to review rooftop runoff quality from two different countries. China and the United States could both benefit from rooftop runoff collection, though not necessarily for the same reasons. Rooftop runoff in both countries did not seem to be impacted by atmospheric quality. One major difference between storms in each country is the rainwater quality in China. Preliminary rain collection in the US was always negligible, but that was not the case for rainwater in China. Rain collected in China had notable amounts of *E. coli*. Relationships between storm characteristics were also more obvious from the runoff collected in China. One benefit of collection on the two different roof types was the ability to support a hypothesis generated from the apartment complex runoff in the United States was the relationship between aluminum and silicate as a degradation byproduct of asphalt shingles. An obvious relationship was detected between aluminum and silicate for rooftops covered with asphalt shingles, but not for concrete-based rooftops in China. Rooftops in China did release more metals in the runoff quality as compared with those in the United States, which were reported to be much lower than values found in other countries. The values reported in China were similar to metals reported by other studies from around the world.

A summary of additional conclusions from work in this dissertation are:

1. Total coliform bacteria which are used as a measure of fecal microbial pollutant loading were decreased in roadway runoff as compared to

- concentrations in the stream water. This finding suggests that the receiving stream was not notably impacted by microbial pollutants generated by the roadway runoff.
2. High-throughput sequencing further revealed the variation in microbial composition between roadway runoff and stream water. This provided another line of evidence to support the hypothesis that the microbial composition of the receiving stream is not impacted significantly by the influx of roadway runoff.
 3. Bacterial contamination was found to be consistent over the course of a storm for rooftop runoff, especially in the form of *E. coli* and heterotrophic bacteria. This finding elicits a need for rooftop runoff to be treated prior to reuse.
 4. The need for pre-treatment of collected rooftop runoff would be necessary to treat multiple contaminants, but one design would not fit all rooftops.
 5. Current designs for water catchment from rooftops do not adequately account for runoff contamination. First flush removal may not necessarily be effective, as it likely does not contain the highest concentration of pollutants as previously believed.
 6. Grab samples can be analyzed for most parameters without concern of inconsistent values as the storm progresses.
 7. Sample location matters for runoff quality, as it can change across a city or even between countries.
 8. Gutter downspout materials show potential to impact rooftop runoff quality. Rooftops in China which used PVC piping for their downspouts released less zinc than other rooftops which used metal downspouts.
 9. Environmentally-isolated *E. coli* cultures are capable of survival and growth without access to light or growth while attaching to a surface, however all of the strains behaved differently to variations in temperature.
 10. The bacteria appeared to develop biofilms while attached to the surface. Attachment occurred at different times for different strains and temperatures.
 11. Temperature had a major impact on bacterial survival, with bacterial death stages starting after 5 days at the 45°C, but remaining in the stagnant phase at 35°C for multiple days prior to entering the death stage.
 12. During the first day of the attachment studies, bacteria struggle to stay attached to the surface during washing. There seems to be a small window where bacteria removal would be possible before strong attachment could happen to a roofing material.

Limitations and Recommendations

The biggest limitation for this dissertation is the weather. All chapters, minus Chapter V were all subject to weather patterns and collectable storm events. Events that were collected with grab samples had to occur during the daytime, and had to generate enough runoff for collection. The total number of collectable storms, and therefore collected storms, was determined by the weather. In order to better solidify conclusions from this dissertation, more samples could be taken. While it is unlikely that replicates

will be taken from the locations in China any time soon, there are plans to replicate studies in Chapter I. Chapter III could also be replicated or continued in the future. To get a better idea of the ability of bacteria to survive on roofing materials, additional conditions should be introduced to the glass slides, and eventual testing on real materials would be advantageous. Glass was selected as the attachment surface for many reasons, but the primary reason was the limitation caused by fluorescence microscopy. Dyes could not be applied successfully to actual roadway or rooftop materials, and materials were too thick to fit under the microscope. This portion of the dissertation could be further improved by expanding to roadway materials and bacteria. Another possible pathway for this dissertation is to sequence rooftop runoff samples for comparison with rooftop bacterial quality and roadway sequencing data.

REFERENCES

- Anderson, K. L., Whitlock, J. E., and Harwood, V. J. (2005) Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments. *Applied and environmental microbiology*, **71**(6), 3041–3048.
- APHA; AWWA; WEF (2005) *Standard methods for the examination of water & wastewater* (A. D. Eaton, L. S. Clesceri, E. W. Rice, and A. E. Greenberg, eds.), Baltimore, MD, American Public Health Association; American Water Works Association; Water Environment Federation.
- Avery, S. M. and Buncic, S. (2003) *Escherichia coli* O157 diversity with respect to survival during drying on concrete. *J Food Prot*, **66**(5), 780–786.
- Baker, D. L. and Duke, W. F. (2006) *Intermittent slow sand filters for household use - a field study in Haiti* (R. Gimbel, N. Graham, and M. R. Collins, eds.), London, IWA Publishing.
- Barrett, M. E., Irish Jr., L. B., Malina Jr., J. F., and Charbeneau, R. J. (1998) Characterization of highway runoff in Austin, Texas, area. *Journal of Environmental Engineering*, (February), 131–137.
- Beale, D. J., Barratt, R., Marlow, D. R., Dunn, M. S., Palombo, E. a, Morrison, P. D., and Key, C. (2013) Application of metabolomics to understanding biofilms in water distribution systems: a pilot study. *Biofouling*, **29**(3), 283–294.
- Bertrand-Krajewski, J.-L., Chebbo, G., and Saget, A. (1998) Distribution of pollutant mass vs volume in stormwater discharges and the first flush phenomenon. *Water research*, **32**(8), 2341–2356.
- Boulangé-Petermann, L., Rault, J., and Bellon-Fontaine, M. N. (1997) Adhesion of *Streptococcus thermophilus* to stainless steel with different surface topography and roughness. *Biofouling*, **11**(February 2016), 201–216.
- Brody, S. D., Zahran, S., Highfield, W. E., Grover, H., and Vedlitz, A. (2008) Identifying the impact of the built environment on flood damage in Texas. *Disasters*, **32**(1), 1–18.
- Bucheli, T. D., Müller, S. R., Heberle, S., and Schwarzenbach, R. P. (1998) Occurrence and behavior of pesticides in rainwater, roof runoff, and artificial stormwater infiltration. *Environmental Science and Technology*, **32**, 3457–3464.
- Caporaso, J. G., Lauber, C. L., Walters, W. a, Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J. a, Smith, G., and Knight, R. (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal*, **6**(8), 1621–1624.
- CH2MHILL (1998) *Highway stormwater runoff study*, Grand Rapids, MI.
- Chak, K. (2002) Characterization of PM_{2.5} chemical composition in the ambient air of Beijing, China. *Environmental Science and Engineering*, **47**(2), 634–635.
- Chang, M., McBroom, M. W., and Scott Beasley, R. (2004) Roofing as a source of nonpoint water pollution. *Journal of Environmental Management*, **73**(4), 307–315.
- Chen, S., Cheng, H., Liu, J., Hazen, T. C., Huang, V., and He, Q. (2016) Unexpected competitiveness of *Methanosaeta* populations at elevated acetate concentrations in methanogenic treatment of animal wastewater. *Applied Microbiology and Biotechnology*, **101**(4), 1729–1738.
- Chen, S., Cheng, H., Wyckoff, K. N., and He, Q. (2016) Linkages of *Firmicutes* and

- Bacteroidetes* populations to methanogenic process performance. Journal of Industrial Microbiology and Biotechnology, **43**(6), 771–781.
- Chen, W., Yan, L., and Zhao, H. (2015) Seasonal variations of atmospheric pollution and air quality in Beijing. Atmosphere, **6**(11), 1753–1770.
- Chia, T. W. R., Goulter, R. M., McMeekin, T., Dykes, G. a., and Fegan, N. (2009) Attachment of different *Salmonella serovars* to materials commonly used in a poultry processing plant. Food Microbiology, **26**(8), 853–859.
- Clark, S. E., Burian, S., and Pitt, R. (2006) Urban wet-weather flows. Water Environment Research, **78**(10), 1133–1192.
- Coquet, L., Cosette, P., Junter, G. A., Beucher, E., Saiter, J. M., and Jouenne, T. (2002) Adhesion of *Yersinia ruckeri* to fish farm materials: influence of cell and material surface properties. Colloids and Surfaces B: Biointerfaces, **26**(4), 373–378.
- Davidson, E. a., Savage, K. E., Bettez, N. D., Marino, R., and Howarth, R. W. (2009) Nitrogen in runoff from residential roads in a coastal area. Water, Air, & Soil Pollution, **210**(1–4), 3–13.
- Deletic, B. A. B. and Maksimovic, T. (1998) Evaluation of water quality factors in storm runoff. Journal of Environmental Engineering, **124**(September), 869–879.
- Van Donsel, D. J., Geldreich, E. E., and Clarke, N. A. (1967) Seasonal variations in survival of indicator bacteria in soil and their contribution to storm-water pollution. Applied microbiology, **15**(6), 1362–70.
- Dorchin, A. and Shanas, U. (2013) *Daphnia magna* indicate severe toxicity of highway runoff. Journal of environmental quality, **42**(5), 1395–401.
- Dourou, D., Beauchamp, C. S., Yoon, Y., Geornaras, I., Belk, K. E., Smith, G. C., Nychas, G.-J. E., and Sofos, J. N. (2011) Attachment and Biofilm Formation by *Escherichia coli* O157:H7 at Different Temperatures, on Various Food-Contact Surfaces Encountered in Beef Processing. International journal of food microbiology, **149**(3), 262–8. [online]
<http://www.ncbi.nlm.nih.gov/pubmed/21802758>.
- Eck, B. J., Winston, R. J., Hunt, W. F., and Barrett, M. E. (2012) Water quality of drainage from permeable friction course. Journal of Environmental Engineering-Asce, **138**(2), 174–181.
- Eginton, P. J., Gibson, H., Holah, J., Handley, P. S., and Gilbert, P. (1995) Quantification of the ease of removal of bacteria from surfaces. Journal of Industrial Microbiology, **15**(4), 305–310.
- Egodawatta, P., Thomas, E., and Goonetilleke, A. (2009) Understanding the physical processes of pollutant build-up and wash-off on roof surfaces. Science of the Total Environment, **407**(6), 1834–1841.
- Elliot, M. A., Stauber, C. E., Koksai, F., Liang, K. R., D.K., H., DiGiano, F. A., and Sobsey, M. D. (2006) “The operation, flow conditions and microbial reductions of an intermittently operated, household-scale slow sand filter” in Recent Progress in Slow Sand and Alternative Biofiltration Processes. IWA Publishing, 268–277.
- Ellis, J. B., Revitt, D. M., Harrop, D. O., and Beckwith, P. R. (1987) The contribution of highway surfaces to urban stormwater sediments and metal loadings. The Science of the Total Environment, **59**, 339–349.

- Environmental Protection Agency (2009) National primary drinking water regulations. , 1–6. [online] <https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations> (Accessed November 1, 2017).
- Environmental Protection Agency (2016a) Particulate matter (PM) pollution. [online] <https://www.epa.gov/pm-pollution/particulate-matter-pm-basics> (Accessed November 9, 2017).
- Environmental Protection Agency (2012) Preferred/Recommended Models. [online] http://www.epa.gov/scram001/dispersion_prefrec.htm (Accessed February 12, 2012).
- Environmental Protection Agency (2016b) Secondary drinking water standards: guidance for nuisance chemicals. [online] <https://www.epa.gov/dwstandardsregulations/secondary-drinking-water-standards-guidance-nuisance-chemicals> (Accessed June 27, 2016).
- Evans, C. A., Coombes, P. J., and Dunstan, R. H. (2006) Wind, rain and bacteria: the effect of weather on the microbial composition of roof-harvested rainwater. *Water Research*, **40**(1), 37–44.
- Evans, C. A., Coombes, P. J., Dunstan, R. H., and Harrison, T. (2007) Identifying the major influences on the microbial composition of roof harvested rainwater and the implications for water quality. *Water Science and Technology*, **55**(4), 245–253.
- Evans, C. A., Coombes, P. J., Dunstan, R. H., Harrison, T., Martin, A., and Harris, J. N. (2006) Coliforms, biofilms, microbial diversity and the quality of roof-harvested rainwater. *Water Research*, **40**(1), 34–44.
- Falbo, K., Schneider, R. L., and Buckley, D. H. (2013) Roadside ditches as conduits of fecal indicator organisms and sediment implications for water quality management. *Journal of Environmental Management*, **128**(10), 1050–1059.
- Farreny, R., Morales-Pinzón, T., Guisasola, A., Tayà, C., Rieradevall, J., and Gabarrell, X. (2011) Roof selection for rainwater harvesting: quantity and quality assessments in Spain. *Water Research*, **45**, 3245–3254.
- Flint, K. R. and Davis, A. P. (2007) Pollutant mass flushing characterization of highway stormwater runoff from an ultra-urban area. *Journal of Environmental Engineering*, **133**(6), 616–626.
- Förster, J. (1996) Patterns of roof runoff contamination and their potential implications on practice and regulation of treatment and local infiltration. *Water Science and Technology*, **33**(6), 39–48.
- Förster, J. (1998) The influence of location and season on the concentrations of macroions and organic trace pollutants in roof runoff. *Water Science and Technology*, **38**(10 pt 8), 83–90.
- Förster, J. (1999) Variability of roof runoff quality. *Water Science and Technology*, **39**(5), 137–144.
- Gan, H., Zhuo, M., Li, D., and Zhou, Y. (2008) Quality characterization and impact assessment of highway runoff in urban and rural area of Guangzhou, China. *Environmental monitoring and assessment*, **140**(1–3), 147–59.
- Geldreich, E. E., Best, L. C., Kenner, B. A., and Donsel, D. J. Van (1968) The bacteriological aspects of stormwater pollution. *Water Pollution Control Federation*,

- 40**(11), 1861–1872.
- Gikas, G. D. and Tsihrintzis, V. A. (2012) Assessment of water quality of first-flush roof runoff and harvested rainwater. *Journal of Hydrology*, **466–467**, 115–126.
- Goulter-Thorsen, R. M., Taran, E., Gentle, I. R., Gobius, K. S., and Dykes, G. a (2011) Surface roughness of stainless steel influences attachment and detachment of *Escherichia coli* O157. *Journal of food protection*, **74**(8), 1359–63.
- Goulter, R. M., Gentle, I. R., and Dykes, G. a (2010) Characterisation of curli production, cell surface hydrophobicity, autoaggregation and attachment behaviour of *Escherichia coli* O157. *Current microbiology*, **61**(3), 157–62.
- Gromaire, M., Garnaud, S., Saard, M., and Chebbo, G. (2001) Contribution of different sources to the pollution of wet weather flow in combined sewers. *Water Res*, **35**(2), 521–533.
- Hall, T. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95–98.
- Hathaway, J. M., Tucker, R. S., Spooner, J. M., and Hunt, W. F. (2012) A traditional analysis of the first flush effect for nutrients in stormwater runoff from two small urban catchments. *Water, Air, & Soil Pollution*, **223**(9), 5903–5915.
- Hazen, T. C., Rocha, A. M., and Techtmann, S. M. (2013) Advances in monitoring environmental microbes. *Current Opinion in Biotechnology*, **24**, 526–533.
- He, J., Valeo, C., Chu, A., and Neumann, N. F. (2010) Characterizing physicochemical quality of storm-water runoff from an urban area in Calgary, Alberta. *Journal of Environmental Engineering*, (November), 1206–1217.
- Helmreich, B., Hilliges, R., Schriewer, A., and Horn, H. (2010) Runoff pollutants of a highly trafficked urban road-correlation analysis and seasonal influences. *Chemosphere*, **80**(9), 991–7.
- Jiannan Chen , Kyle J . Groves, Liangquan Wang, Tuncer B . Edil, A. S. (2013) *Leaching characteristics of recycled asphalt shingles mixtures*, Madison, WI.
- Joseph, B., Otta, S. K., Karunasagar, I., and Karunasagar, I. (2001) Biofilm formation by *Salmonella* spp. on food contact surfaces and their sensitivity to sanitizers. *International Journal of Food Microbiology*, **64**(3), 367–372.
- Kang, J. H., Kayhanian, M., and Stenstrom, M. K. (2008) Predicting the existence of stormwater first flush from the time of concentration. *Water Research*, **42**(1–2), 220–228.
- Kayhanian, M. (2012) Trend and concentrations of legacy lead (Pb) in highway runoff. *Environmental Pollution*, **160**(1), 169–177.
- Kayhanian, M., Fruchtmann, B. D., Gulliver, J. S., Montanaro, C., Ranieri, E., and Wuertz, S. (2012) Review of highway runoff characteristics: comparative analysis and universal implications. *Water research*, **46**(20), 6609–24.
- Kayhanian, M. and Stenstrom, M. (2005) Mass loading of first flush pollutants with treatment strategy simulations. *Transportation Research Record*, **1904**(1), 133–134.
- Kim, R. H., Lee, S., Kim, Y. M., Lee, J. H., Kim, S. K., and Kim, S. G. (2005) Pollutants in rainwater runoff in Korea: their impacts on rainwater utilization. *Environmental technology*, **26**(March), 411–420.

- Kozich, J., Westcott, S., Baxter, N., Highlander, S., and Schloss, P. (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied and Environmental Microbiology*, **79**(17), 5112–20.
- Lechevallier, M. W., Cawthorn, C. D., Lee, R. G., Cawthon, C. D., and Lee, R. G. (1988) Factors promoting survival of bacteria in chlorinated water supplies. *Applied and Environmental Microbiology*, **54**(3), 649–654.
- Lee, J. Y., Kim, H., Kim, Y., and Han, M. Y. (2011) Characteristics of the event mean concentration (EMC) from rainfall runoff on an urban highway. *Environmental pollution*, **159**(4), 884–8.
- Lee, J. Y., Yang, J. S., Han, M., and Choi, J. (2010) Comparison of the microbiological and chemical characterization of harvested rainwater and reservoir water as alternative water resources. *Science of the Total Environment*, **408**(4), 896–905.
- Leff, L. G., Dana, J. R., McArthur, J. V, and Shimkets, L. J. (1995) Comparison of methods of DNA extraction from stream sediments. *Applied and environmental microbiology*, **61**(3), 1141–3.
- Legret, M. and Pagotto, C. (1999) Evaluation of pollutant loadings in the runoff waters from a major rural highway. *The Science of the total environment*, **235**(1–3), 143–50.
- Li, X. Z., Hauer, B., and Rosche, B. (2007) Single-species microbial biofilm screening for industrial applications. *Applied microbiology and biotechnology*, **76**(6), 1255–62.
- Lyautey, E., Lacoste, B., Ten-Hage, L., Rols, J.-L., and Garabetian, F. (2005) Analysis of bacterial diversity in river biofilms using 16S rDNA PCR-DGGE: methodological settings and fingerprints interpretation. *Water research*, **39**(2–3), 380–8.
- Lyautey, E., Wilkes, G., Miller, J. J., Van Bochove, E., Schreier, H., Koning, W., Edge, T. a., Lapen, D. R., and Topp, E. (2010) Variation of an indicator of *Escherichia coli* persistence from surface waters of mixed-use watersheds, and relationship with environmental factors. *International Journal of Limnology*, **47**(1), 11–19.
- Lye, D. J. (1987) Bacterial levels in cistern water systems of northern Kentucky. *Water Resources Bulletin*, **23**(6), 1063–1068.
- Lye, D. J. (1992) Microbiology of rainwater cistern systems: a review. *Journal of Environmental Sciences and Health*, **A27**(8), 2123–2166.
- Lye, D. J. (2009) Rooftop runoff as a source of contamination: a review. *Science of the Total Environment*, **407**(21), 5429–5434.
- Mafu, a. a., Roy, D., Goulet, J., and Magny, P. (1990) Attachment of *Listeria monocytogenes* to stainless steel, glass, polypropylene, and rubber surfaces after short contact times. *Journal of Food Protection*, **53**(9), 742–746.
- McCarthy, D. T., Deletic, A., Mitchell, V. G., Fletcher, T. D., and Diaper, C. (2008) Uncertainties in stormwater *E. coli* levels. *Water research*, **42**(6–7), 1812–24.
- McLellan, S. L., Fisher, J. C., and Newton, R. J. (2015) The microbiome of urban waters. *International Microbiology*, **18**(3), 141–149.
- Melidis, P., Akrotos, C. S., Tsihrintzis, V. A., and Trikilidou, E. (2007) Characterization of rain and roof drainage water quality in Xanthi, Greece. *Environmental Monitoring and Assessment*, **127**(1–3), 15–27.

- Mendez, C. B., Klenzendorf, J. B., Afshar, B. R., Simmons, M. T., Barrett, M. E., Kinney, K. A., and Kirisits, M. J. (2011) The effect of roofing material on the quality of harvested rainwater. *Water Research*, **45**, 2049–2059.
- Van Metre, P. C. and Mahler, B. J. (2003) The contribution of particles washed from rooftops to contaminant loading to urban streams. *Chemosphere*, **52**, 1727–1741.
- Miguntanna, N. P., Liu, A., Egodawatta, P., and Goonetilleke, A. (2013) Characterising nutrients wash-off for effective urban stormwater treatment design. *Journal of environmental management*, **120**, 61–7.
- Moreira, J. M. R., Simões, M., Melo, L. F., and Mergulhão, F. J. (2014) *Escherichia coli* adhesion to surfaces—a thermodynamic assessment. *Colloid and Polymer Science*, **293**(1), 177–185.
- Nason, J. A., Bloomquist, D. J., and Sprick, M. S. (2012) Factors influencing dissolved copper concentrations in Oregon highway storm water runoff. *Journal of Environmental Engineering*, (July), 734–742.
- NCSS 10 Statistical Software (2015). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/ncss
- Nemergut, D. R., Anderson, S. P., Cleveland, C. C., Martin, A. P., Miller, A. E., Seimon, A., and Schmidt, S. K. (2007) Microbial community succession in an unvegetated, recently deglaciated soil. *Microbial Ecology*, **53**(1), 110–122.
- Nguyen, V. T., Turner, M. S., and Dykes, G. A. (2010) Effect of temperature and contact time on *Campylobacter jejuni* attachment to, and probability of detachment from, stainless steel. *Journal of Food Protection*, **73**(5), 832–838.
- Nshimiyimana, J. P., Ekklesia, E., Shanahan, P., Chua, L. H. C., and Thompson, J. R. (2014) Distribution and abundance of human specific *Bacteroides* and relation to traditional Indicators in an urban tropical catchment. *Journal of applied microbiology*, **116**(5), 1369–1383.
- O’Shea, M. L. and Field, R. (1992) Detection and disinfection of pathogens in storm-generated flows. *Canadian Journal of Microbiology*, **38**(4), 267–76.
- Opher, T. and Friedler, E. (2010) Factors affecting highway runoff quality. *Urban Water Journal*, **7**(3), 155–172.
- Ortega, M. P., Hagiwara, T., Watanabe, H., and Sakiyama, T. (2010) Adhesion behavior and removability of *Escherichia coli* on stainless steel surface. *Food Control*, **21**(4), 573–578.
- Otto, M. (2008) Staphylococcal biofilms. *Current Topics in Microbiology and Immunology*, **322**, 207–228.
- Palmer, J., Flint, S., and Brooks, J. (2007) Bacterial cell attachment, the beginning of a biofilm. *Journal of Industrial Microbiology and Biotechnology*, **34**(9), 577–588.
- Parhad, N. M. and Rao, N. U. (1974) Effect of pH on survival of *Escherichia coli*. *Water Pollution Control Federation*, **46**(5), 980–986.
- Percival, S. L., Knapp, J. S., Edyvean, R., and Wales, D. S. (1998) Biofilm development on stainless steel in mains water. *Water Research*, **32**(1), 243–253.
- Rivas, L., Dykes, G. a., and Fegan, N. (2007) A comparative study of biofilm formation by Shiga toxigenic *Escherichia coli* using epifluorescence microscopy on stainless steel and a microtitre plate method. *Journal of Microbiological Methods*, **69**(1), 44–

51.

- Rivas, L., Fegan, N., and Dykes, G. a. (2007) Attachment of Shiga toxigenic *Escherichia coli* to stainless steel. *International Journal of Food Microbiology*, **115**, 89–94.
- Sansalone, J. J. and Buchberger, S. G. (1997) Partitioning and first flush of metals in urban roadway storm water. *Journal of Environmental Engineering*, **123**(2), 134–143.
- Sansalone, J. J., Hird, J. P., Cartledge, F. K., and Tittlebaum, M. E. (2005) Event-based stormwater quality and quantity loadings from elevated urban infrastructure affected by transportation. *Water Environment Research*, **77**(4), 348–365.
- Sansalone, J. J., Koran, J. M., Smithson, Z. J. A., and Buchberger, S. G. (1998) Physical characteristics of urban roadway solids. *Journal of Environmental Engineering*, (MAY), 427–440.
- Sawyer, C. B., English, W. R., Hayes, J. C., Jolley, L. W., Post, C. J., and Bridges, W. C. (2008) “Preliminary analysis of indicator bacteria within retention pond best management practices” in 2008 South Carolina Water Resources Conferences. Charleston.
- Schloss, P. (2009) Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, **75**(23), 7537–41.
- Schriewer, A., Horn, H., and Helmreich, B. (2008) Time focused measurements of roof runoff quality. *Corrosion Science*, **50**, 384–391.
- Sidhu, J. P. S., Hodggers, L., Ahmed, W., Chong, M. N., and Toze, S. (2012) Prevalence of human pathogens and indicators in stormwater runoff in Brisbane, Australia. *Water research*, **46**, 6652–60.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, **30**, 2725–2729.
- Tennessee Water Resources Research Center (2003) *Guide to the Selection & Design of Stormwater Best Management Practices (BMPs)*,
- Thomas, R. B., Kirisits, M. J., Lye, D. J., and Kinney, K. A. (2014) Rainwater harvesting in the United States: A survey of common system practices. *Journal of Cleaner Production*, **75**, 166–173.
- Townsend, T., Powell, J., and Xu, C. (2007) *Environmental issues associated with asphalt shingle recycling*, Gainesville, FL.
- Tsuji, M. and Yokoigawa, K. (2012) Attachment of *Escherichia coli* O157:H7 to abiotic surfaces of cooking utensils. *Journal of Food Science*, **77**(4), 194–199.
- Uba, B. N. and Aghogho, O. (2000) Rainwater quality from different roof catchments in the Port Harcourt district, Rivers State, Nigeria. *Journal of Water Supply: Research and Technology-AQUA*, **49**(5), 281–288.
- USGS (2006) *Materials in Use in U.S. Interstate Highways*, Denver, CO.
- Vaze, J. and Chiew, F. H. S. (2004) Nutrient loads associated with different sediment sizes in urban stormwater and surface pollutants. *Journal of Environmental Engineering*, **130**(4), 391–396.

- Wang, Q., M., G. G., Tiedje, J. M., and Cole, J. R. (2007) Naïve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology.*, **73**(16), 5261–7.
- Winston, R. J., Hunt, W. F., Kennedy, S. G., Wright, J. D., and Lauffer, M. S. (2012) Field evaluation of storm-water control measures for highway runoff treatment. *Journal of Environmental Engineering*, **138**(1), 101–111.
- Winters, N. L. and Graunke, K. (2014) *Roofing materials assessment investigation of toxic chemicals in roof runoff*, Olympia, WA.
- Wong, H., Chung, Y., and Yu, J.-A. (2002) Attachment and inactivation of *Vibrio parahaemolyticus* on stainless steel and glass surface. *Food Microbiology*, **19**(August 2001), 341–350.
- Wu, L., Yang, Y., Chen, S., Zhao, M., Zhu, Z., Yang, S., Qu, Y., Ma, Q., He, Z., Zhou, J., and He, Q. (2016) Long-term successional dynamics of microbial association networks in anaerobic digestion processes. *Water Research*, **104**, 1–10.
- Yaziz, M. I., Gunting, H., Sapari, N., and Ghazali, A. W. (1989) Variations in rainwater quality from roof catchments. *Water Research*, **23**(6), 761–765.
- Yu, C. (2011) China's water crisis needs more than words. *Nature*, **470**, 307.
- Yue, J. C. and Clayton, M. K. (2005) A similarity measure based on species proportions. *Communication in Statistics- Theory and Methods*, **34**(11), 2123–2131.
- Zhang, M., Chen, H., Wang, J., and Pan, G. (2010) Rainwater utilization and storm pollution control based on urban runoff characterization. *Journal of environmental sciences*, **22**(1), 40–46.
- Zhang, Q., Wang, X., Hou, P., Wan, W., Li, R., Ren, Y., and Ouyang, Z. (2014) Quality and seasonal variation of rainwater harvested from concrete, asphalt, ceramic tile and green roofs in Chongqing, China. *Journal of Environmental Management*, **132**, 178–187.
- Zhang, X., Hu, M., Chen, G., and Xu, Y. (2012) Urban rainwater utilization and its role in mitigating urban waterlogging problems-a case study in Nanjing, China. *Water Resources Management*, **26**(13), 3757–3766.
- Zhang, Y. and He, Q. (2013) Characterization of bacterial diversity in drinking water by pyrosequencing. *Water Science & Technology: Water Supply*, **13**(2), 358–367.
- Zuo, X., Fu, D., Li, H., and Singh, R. P. (2011) Distribution characteristics of pollutants and their mutual influence in highway runoff. *CLEAN - Soil, Air, Water*, **39**(10), 956–963.

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