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Evaluation of Two Water Reuse Applications: Cooling Tower Makeup Water and Residential HVAC Condensate Reuse

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Evaluation of Two Water Reuse Applications: Cooling Tower Makeup Water and Residential HVAC Condensate Reuse

by

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Abstract

Evaluation of Two Water Reuse Applications: Cooling Tower Makeup Water and Residential HVAC Condensate Reuse

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This study evaluated the potential impacts of two water reuse applications – urban cooling towers and residential homes. Large water demands make cooling towers an attractive target for water reuse applications. However, poorly operated cooling towers have historically been linked to diseases such as Legionnaire's Disease, and there is limited understanding on how transitioning from potable to alternative water sources will impact the microbial communities within cooling tower basins. Therefore, the microbial communities of three well-maintained and disinfected urban cooling towers were studied to assess the impact of source water on microbial populations, diversity, and the presence of potentially pathogenic organisms. Illumina sequencing results indicate that different makeup water sources do yield microbial basin communities that differ substantially in composition and diversity. Also, total bacterial loads in each basin decreased with increasing fraction of potable water used in the makeup water. *Legionella* spp. levels above 6 logGC/L were observed in a cooling tower basin that used reclaimed and potable water as makeup water sources. However, none of the basin or makeup water sources had

quantifiable levels of *L. pneumophila*, indicating the *Legionella* present in the cooling towers was mostly non-pneumophila *Legionella.*

Residential HVAC condensate was evaluated because it is a largely untapped water source that may be suitable for recovery and reuse. The two main challenges for HVAC condensate collection is estimating the condensate production volume and understanding the water chemistry of the condensate. Both production rate and water chemistry are crucial to understanding which reuse options are available for HVAC condensate. Thus, this study tested a method for estimating condensate production volumes and analyzed the water chemistry of condensate samples from three separate HVAC units at different residences. Measured condensate production volumes were within 12 to 25 percent of predicted values, and the water chemistry results identified the presence of both metals and organic species. Both studies indicate the importance of considering water quality for water reuse applications.

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Chapter 1: Introduction

1.1 Problem Statement

The combination of water stress and population growth in the Southwestern U.S. require an examination of alternative water sources to meet current and future demand. In 2010, the total water use in the United States was estimated to be 355 billion gallons per year, and water stressed states account for a disproportionately large fraction of total water use. For example, California and Texas, both water stressed states, account for 11 and 7 percent of the total U.S. water withdrawals, respectively (Maupin et al., 2014). The Southern and Western States, with warm and/or dry climates, accounted for the largest population growth regions in the United States (14.3 and 13.8%, respectively), and accounted for 84% of the total population growth in the U.S. from 2000-2010 (Maupin et al., 2014). Population growth coupled with the effects of climate change has forced municipalities to identify water reuse sources, or alternative water sources, to supplement traditional fresh water supplies especially in water scarce regions. Alternative water source options commonly include harvested rainwater, onsite stormwater, greywater, treated wastewater effluent (reclaimed water), and captured HVAC condensate. The applicability of these sources for particular end uses must be assessed based on the quantity and quality of the source water and the economic and technical feasibility of treatment to meet standards for the intended use. This work focused on two applications of water reuse: urban cooling tower makeup water and residential water reuse.

Urban cooling tower makeup water is being targeted because cooling towers require large volumes of water and therefore represent an attractive target for water reuse. Potable water is currently the dominant makeup water source for urban cooling towers, but use of HVAC condensate and reclaimed water has increased. Nevertheless, there are

no cooling tower water quality control regulations in a majority of the United States and using alternative water sources could create public heath concerns. Cooling towers have historically been associated with Legionnaires Disease (Ferré et al., 2008; Maisa et al., 2015; Nguyen et al., 2006; Shelton, Flanders, & Morris, 1994), and it is unknown how shifting from potable to alternative water sources will impact cooling tower microbial communities. This work aims to evaluate the microbial community impacts on cooling tower water to understand how the composition of the microbial community as a whole, (including potential pathogens) reacts to changing water sources.

Residential HVAC condensate was also evaluated because it is a largely untapped alternative water source that may be suitable for recovery and reuse. While greywater and harvested rainwater are commonly touted residential water reuse options, HVAC condensate potentially has higher water quality than either source. Furthermore, regions experiencing greater water stress tend to have lower levels of precipitation putting rainwater supply out of sync with demand. Condensate is a locally available water supply with potential to reduce municipal water demand in warm climates with moderate to high humidity levels. Furthermore, condensate production is constant, unlike harvested rainwater, and would require less storage space and biological growth inhibition.

1.2 OBJECTIVES

1.2.1 Cooling towers

The overall objective of the cooling tower study was to evaluate the effect that using alternative water sources in urban cooling towers has on the microbial community that develops in cooling tower water. To this end, the microbial community composition, water chemistry, and operations data were evaluated in three urban cooling towers. While each cooling tower was operated and maintained in a similar manner, each tower

received a different blend of potable water mixed with either tertiary-treated reclaimed wastewater effluent (reclaimed water) or water recovered from a variety of potential sources (e.g., HVAC system condensate, RO condensate, pumped groundwater from around building foundations, or swimming pool blowdown). The specific objectives for the study were as follows:

1. Use sequencing techniques to compare the microbial communities that develop in cooling tower basins supplied with different makeup water sources.
2. Quantify the level of *Legionella* spp., *L. pneumophila*, *L. pneumophila* sg. 1, and total bacteria in each cooling tower basin and the corresponding makeup water sources using qPCR.
3. Measure the major anions, cations, and nutrients present in each cooling tower and makeup water sample and use statistical tests to determine correlations between the total bacteria and *Legionella* spp. levels and water chemistry.

1.2.2 Residential HVAC condensate reuse

The overall objectives of the HVAC condensate study are to measure the volume of residential condensate produced and identify potential reuse options for condensate. The specific objectives were as follows:

1. Determine the accuracy of calculating theoretical condensate production using direct temperature and relative humidity measurements.
2. Evaluate the water quality of residential condensate with a focus on traditional water quality parameters and selected metal ions.
3. Identify potential reuse options based on the water chemistry analysis and production volumes.

Chapter 2: Literature Review

A literature review was conducted to examine the merits and concerns associated with water reuse in cooling towers as well as to assess the potential for reusing residential HVAC condensate.

2.1 Water Reuse in Cooling Towers

2.1.1 Cooling Tower Water Operation

Cooling towers are heat exchangers where heat is removed from water via evaporative cooling. In urban areas, cooling towers are typically part of indoor cooling systems (cooling systems) for large buildings (e.g., hospitals, hotels, etc.). They are large usually metal structures with a basin or pool of water at the bottom. Cooling towers release waste heat captured from cooling systems from indoor environments to the atmosphere. Figure 1 shows a cooling tower operation schematic with the typical hydraulic flows. To release captured waste heat, warmed liquid containing waste heat from the cooling system along with relatively cool basin water from the cooling tower are both pumped to a heat exchanger. In the heat exchanger, the cooling system liquid transfers some of its heat to the basin water. Thus, the basin water leaves the heat exchanger warmer and the cooling system liquid leaves the heat exchanger cooled and ready to return to the cooling system. The warmed basin water is then pumped to the top of the cooling tower where it is sprayed down, back to the basin. As the warmed basin water droplets fall, a small fraction of the water mass evaporates and cools the droplets. Evaporation of basin water leads to salt cycling in the basin. To avoid scaling in the heat exchanger, a certain volume of basin water is discharged to the sanitary sewer daily (blowdown water) and replaced with fresh makeup water.

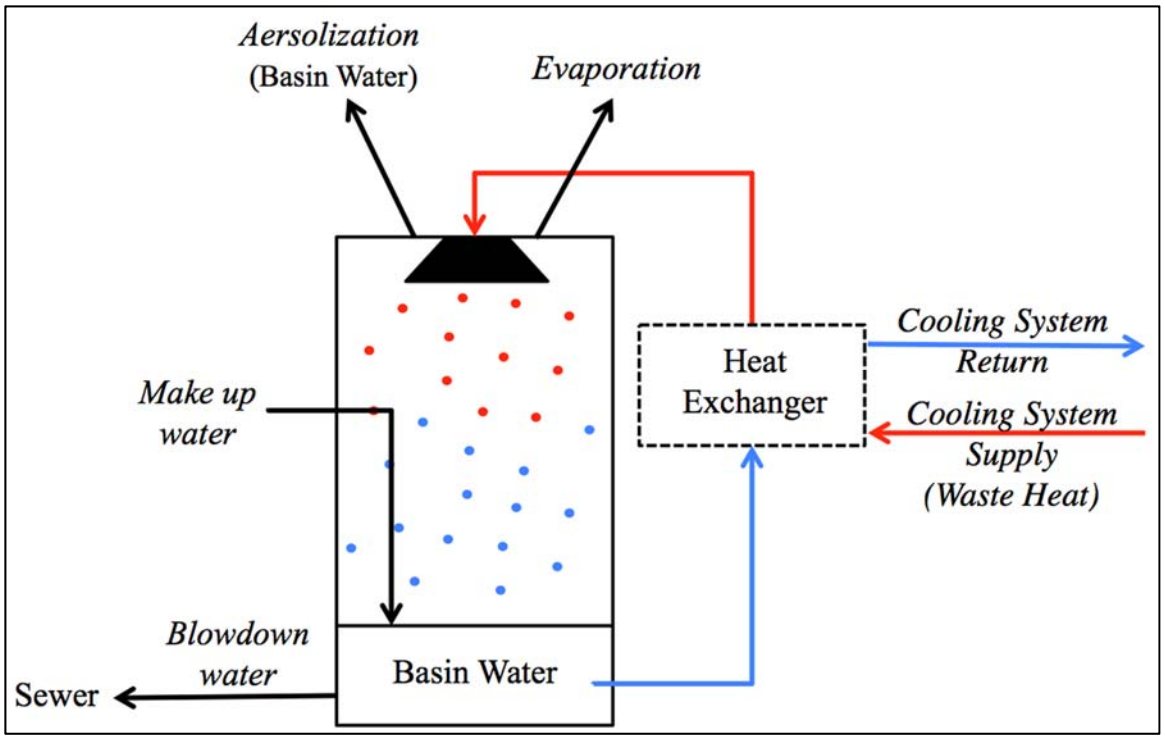

Figure 1: Typical Cooling Tower Operation Schematic. Basin water from the cooling tower and warmed liquid from the cooling system are pumped to a heat exchanger where the cooling system liquid transfers waste heat to the basin water. The basin water is then pumped to the top of the cooling tower and sprayed back down to the basin and is cooled by evaporative cooling. Water leaves the cooling tower via evaporation, aerosols, and blowdown water. Fresh make up water is added to maintain a relatively constant basin volume. Blue indicates relatively cool temperatures, and red indicates relatively warm temperatures.

To maximize airflow, and therefore evaporation, fans are usually installed at the top of cooling towers to pull air up through the cooling tower in the opposite direction of the falling basin water droplets. The walls of the cooling tower can also be open to increase airflow. The open top and sides allow small basin droplets to escape the cooling tower as aerosols. The aerosols retain the microbial community of the basin water.

2.1.2 Opportunities for Water Reuse in Cooling Towers

Cooling towers are an attractive target for reuse because of their large makeup water demands. The three cooling towers studied for this work used as much as 890, 1,200, and 1,600 m^3 of makeup water per day during the studied period. Where lower cost water supplies are available, utilities and managers can accrue significant cost savings by displacing part or all of the potable water used for makeup water with a lower cost water supply. Furthermore, municipalities with reclaimed water systems benefit from a constant consumer of the reclaimed water. For example, in Austin, Texas, cooling towers made up 38% of the total reclaimed water usage in 2013 (Austin Water, 2013).

2.1.3 Cooling Tower Water Quality and Human Health Concerns

Cooling towers can contain opportunistic pathogens including *Legionella* (Bentham, 2000; Ishimatsu, Miyamoto, Hori, Tanaka, & Yoshida, 2001; Shelton et al., 1994), *Pseudomonas* (Blasco, Esteve, & Alcaide, 2008), and non-tuberculosis mycobacteria (NTM) (Adrados et al., 2011a; Torvinen, Suomalainen, Paulin, & Kusnetsov, 2014). Cooling tower operation generates a fine mist which provides an inhalation exposure pathway for any pathogens present in the mist; infections have been attributed to exposure up to 7 kilometers away (Nguyen et al., 2006).

Legionella contaminated cooling towers have been responsible for several highly publicized Legionnaires' disease outbreaks around the world including in Philadelphia, PA (1976), Catalonia, Spain (2002), Pas-de-Calais, France (2003), Catalonia, Spain (2005), Warstein, Germany (2013), and New York City (2015), which infected 182, 113, 86, 55, 159, and 127 people, respectively (Ferré et al., 2008; Fraser et al., 1977; Maisa et al., 2015; Mazurkiewicz, 2015; Nguyen et al., 2006; Sabria et al., 2006). Legionnaires' disease is a serious bacterial pneumonia reported in 2-15% of all community-acquired pneumonia patients requiring hospitalization (Haubitz et al., 2014), with *Legionella*

pneumonophila serogroup (sg) 1 causing about 90% of cases (Touray, Newstein, Lui, Harris, & Knox, 2014).

Legionella contaminated cooling towers can also cause Pontiac Fever, a non-pneumonia, flu-like respiratory infection (Ambrose et al., 2014; Friedman, Spitalny, Barbaree, Faur, & McKinney, 1987). Because of the lack of specificity, Pontiac fever is often undiagnosed (Tossa, Deloge-Abarkan, Zmirou-Navier, Hartemann, & Mathieu, 2006).

Aside from *L. pneumophila*, at least 29 other *Legionella* species have been shown to be pathogenic (Cunha, Burillo, & Bouza, 2016). For example, *L. longbeachae* accounts for approximately 30% of Legionnaires' disease cases in Australia and New Zealand (Yu et al., 2002). More rare infections have been linked to *Legionella* including one case of a large frontal brain abscess caused by *L. micdadai* with no evident source (Charles et al., 2013). *L. micdadei* has also been reported to cause invasive lung infections and has been clinically mistaken for *Mycobacterium* (Waldron, Martin, & Ho, 2015).

The transmission of other opportunistic pathogens from cooling towers is largely unknown. There is currently little evidence in the literature to support NTM transmission via cooling tower aerosols. However, previous studies have shown 55-90% of samples from cooling towers contained NTM, and the occurrence of NTM disease outbreaks is likely underestimated since reporting NTM disease epidemiology is not required (Adrados et al., 2011a; Torvinen et al., 2014). NTM causes pulmonary disease (Field & Cowie, 2006), and epidemiological studies suggest the prevalence of NTM disease is growing in North America (Mirsaeidi et al., 2014).

2.1.4 Pathogens in Engineered Water Systems

Opportunistic pathogens including *Legionella*, *Pseudomonas*, and NTM are native to freshwater systems (Falkinham III, Hilborn, Arduino, Pruden, & Edwards, 2015), which provide the source water for cooling towers. *Legionella* has been identified in U.S. drinking water taps (Donohue et al., 2014), secondary treated wastewater samples (Palmer, Tsai, Paszko-Kolva, Mayer, & Sangermano, 1993), and secondary treated wastewater reused for irrigation (Alonso et al., 2006). NTM and *Pseudomonas* have also been found in potable water taps and potable distribution systems (Adrados et al., 2011b; Briancesco et al., 2014; Costa et al., 2015; Lecuona et al., 2016; Thomson et al., 2013; Wang, Edwards, Falkinham, & Pruden, 2012).

Opportunistic pathogens can survive for long periods in oligotrophic conditions at least in part because of biofilm association (Diederen, 2008; Wingender & Flemming, 2011). While able to survive in oligotrophic environments, higher concentrations of nutrients are required for replication. To this end, proliferation of opportunistic pathogens is a concern when using reclaimed effluent or recovered water because the elevated nutrient, dissolved organic carbon, and total bacteria concentrations relative to potable water have been shown to favor pathogen growth (Jjemba, Weinrich, Cheng, Giraldo, & LeChevallier, 2010; Solimini, Cottarelli, Marinelli, & De Giusti, 2014; Willey, Kieber, Eyman, & Avery Jr., 2000).

Biofilm association also provides opportunistic pathogens a demonstrated resistance to chlorine disinfectants (Cirillo et al., 1999; Cirillo, Falkow, Tompkins, & Bermudez, 1997; Cooper & Hanlon, 2010a; Donlan & Costerton, 2002; Murga et al., 2001; Steed & Falkinham, 2006). Cooling towers represent an ideal environment for the development of biofilms containing opportunistic pathogens due to the basin water aerosols coating the inside surfaces of the cooling tower. Furthermore, opportunistic

pathogens have been shown to have higher regrowth rates compared to typical indicator bacteria in reclaimed water distribution systems following disinfection (Jjemba et al., 2010) including *L. pneumophila* sg 1 (Buse, Schoen, & Ashbolt, 2012).

Several physiochemical parameters have been correlated with pathogen levels in water systems including several metals (Bargellini et al., 2011; Borella et al., 2004; Rakic, Peric, & Foglar, 2012; Stout et al., 1992; Zacheus & Martikainen, 1994), nitrate and sulfate (Zacheus & Martikainen, 1994), pH (Katz & Hammel, 1987; Ohno, Kato, Yamada, & Yamaguchi, 2003), and temperature (Kusnetsov, Ottoila, & Martikainen, 1996).

2.1.5 Current Cooling Tower Maintenance Regulations

Despite known risks, there are currently no federal regulations related to the maintenance and cleaning of urban cooling towers in the United States. In June 2015 ASHRAE published "ANSI/ASHRAE Standard 188-2015, Legionellosis: Risk Management for Building Water Systems" (ANSI/ASHRAE, 2015). The standard uses a risk-management approach to limit Legionnaires' disease infections associated with building water systems, including cooling towers. The standard provides guidance to owners, operators, and cooling tower designers but compliance is voluntary. The lone exception is The State of New York where the ANSI/ASHRAE standard was adopted by the state legislature following the 2015 Legionnaire's disease outbreak in New York City (Huchler, 2016).

Cooling towers are often not adequately maintained to inhibit biological growth. Inadequate or improper maintenance defined by a lack of regular inspections, faulty chemical pumps, and suboptimal or no disinfection have all been associated with cooling tower related disease outbreaks (Walser et al., 2014). ANSI/ASHRAE 2015 requires

microbiological activity control and a disinfection plan as part of each cooling tower's water management program. The water management program also must include when and where *Legionella* culture testing will be conducted if deemed necessary. However, both planktonic and biofilm associated Legionella have demonstrated chlorine resistance (Cooper & Hanlon, 2010b). Legionella can completely loose cultivability but remain viable following disinfection (Turetgen, 2008).

Guidelines containing action levels for the prevention of Legionnaire's disease are based on culture tests from the international standard ISO 11731. The method is complex and can require up to 14 days to obtain results. There is also a growing understanding of viable but non-culturable (VBNC) *Legionella* spp. that may be infectious to humans (Steinert, Emödy, Amann, & Hacker, 1997). There have been studies conducted to translate culture based action limits to qPCR based action limits (J. V. Lee et al., 2011), but there are still no recommendations containing qPCR based action limits. The lack of regulation of cooling tower waters is a concern for current operating systems, and the risk associated with using lower water quality sources in cooling towers is unknown.

2.2 RESIDENTIAL HVAC CONDENSATE POTENTIAL

2.2.1 Residential HVAC Condensate Production

Figure 2 shows a simple schematic of a residential HVAC system. Warm indoor supply air (supply flow) enters the HVAC unit where it contacts a cold cooling coil and chilled drier air is returned to the home (return flow). The moisture removed from the warm (condensate) condenses on the cooling coil and drips down into a collection pan and is usually drained away to either the lawn of the residence or is plumbed directly into the sewer system. Condensate forms as pure water on the cooling coil, but the low pH and alkalinity and mineral content make the condensate corrosive.

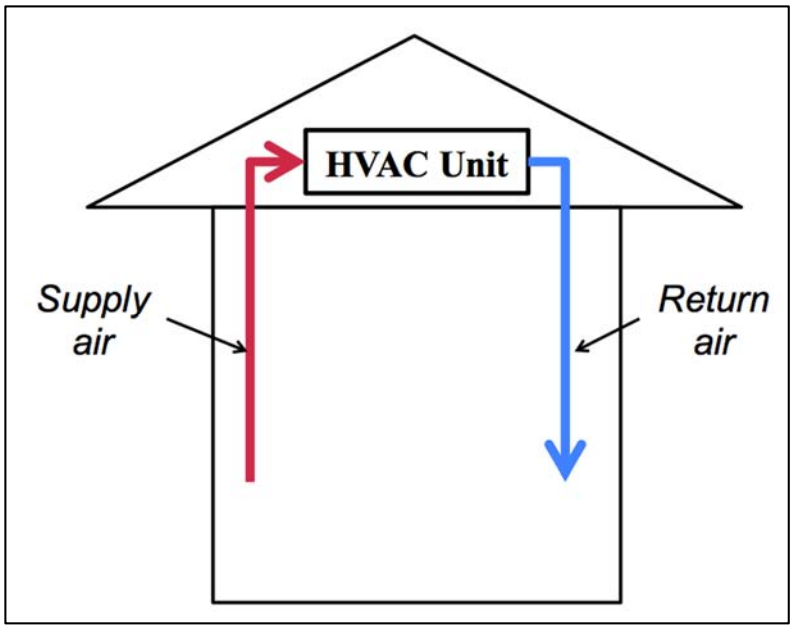

Figure 2: Schematic of residential HVAC system air flow. Red indicates relatively warm temperatures and blue indicates relatively cool temperatures.

2.2.2 Potential Water Recovery

HVAC condensate production is a function of HVAC system size, weather conditions, and building use (e.g., occupancy and human activities). Warm humid climates will produce the most condensate. In San Antonio, Texas, a mall captures 250 gallons per day from its air handlers, and a central library system captures 43,200 gallons per month. Bahrain Airport Services captures and reuses 2.3 million gallons per year of HVAC condensate (Guz, 2005). Collection has also been shown to be practical in northern U.S. cities in summer months when the moisture levels and temperature of the outdoor air are sufficiently high (Guz, 2005; T. M. Lawrence, Perry, & Dempsey, 2010b; T. Lawrence, Perry, & Dempsey, Jan 2010a).

Outdoor air ventilation is required to maintain acceptable indoor air quality. In commercial buildings, ASHRAE Standard 62.1-2004 recommends ventilation levels based on building and occupancy types. Additional outdoor air ventilation has been

shown to decrease rates of sick building syndrome (Fisk, Mirer, & Mendell, 2009). When HVAC systems are in use, outdoor air typically has a higher water vapor concentration and temperature compared to desired indoor conditions. Therefore, outdoor air is conditioned by reducing the temperature and moisture levels via the HVAC system. The moisture removed from the air forms condensate. Thus, higher rates of ventilation during warm, moist weather produce larger volumes of condensate.

The humidity ratio difference across an HVAC system determines the potential condensate production and has been described by Lawrence et al. 2010a. Painter 2009 used estimated humidity ratio differences across cooling coils to predict the volume of condensate produced. Daily outdoor temperature and relative humidity data were used for calculations along with selected indoor comfort conditions. The study estimated that a particular medical research laboratory in San Antonio, TX, could produce 1,887,031 gallons of condensate annually, enough to reduce the cooling tower makeup potable water demand by 16%. Lawrence et al. 2010b measured the outdoor and building supply temperature and humidity to calculate theoretical condensate production for a system conditioning 100% outdoor air. The actual volume collected exceeded the theoretical condensate production estimate by 28%.

Loveless, Farooq, & Ghaffour 2013 also used climate data and design indoor comfort conditions to calculate humidity ratios and then theoretical condensate production values for several regions around the world. Based on condensate potential and water scarcity issues, the Arabian Peninsula, West Africa, Southeast Asia, and Central and South America were identified as experiencing water scarcity issues while having high condensate production potential. Using climate data, buildings in Manila, Phillippines, Lagos, Nigeria, and Houston, Texas, were estimated to produce 330.3, 327.1, and 140.3 $m^3/(m^3/s)$ outdoor air/year of condensate, respectively.

In residential homes, HVAC systems typically do not condition 100% outdoor air. Therefore, using climate models for the HVAC system supply to estimate condensate production in residential homes would not be accurate. One approach to estimating residential condensate production is to use humidity ratios calculated from in-home temperature and relative humidity data. A review of published literature shows there are no studies that calculate theoretical residential condensate production using measured temperature and relative humidity data from both sides of the HVAC system cooling coil.

2.2.3 Condensate Water Chemistry and Reuse Potential

Condensate forms as pure water on cooling coils. The low levels of minerals and low pH make the condensate corrosive. Therefore, condensate likely contains some level of the metals it contacts in the HVAC and drainage systems. Condensate can also pick up biological contamination when it forms and as it drains.

A review of the current peer reviewed literature indicates that there is only limited information regarding the water chemistry of condensate waters and none of these studies provided sufficient data for heavy metals. Water quality parameters such as pH, hardness, and total dissolved solids can impact potential water reuse options. Even trace levels of certain heavy metals could change what reuse options are considered.

One study found the pH, conductivity, and turbidity of condensate samples from three central air conditioning systems ranged from 4.37-6.87, 18-27 μS/cm, and 0.041-0.15 NTU, respectively (Loveless et al., 2013). Low dissolved solids and turbidity are generally desired water quality characteristics, but low pH and mineral content increases the potential for dissolution of metal piping. Roll, Halden, & Pycke 2015 used condensate to measure indoor air contaminants. Antimicrobials, flame-retardants, solvents,

fragrances, and a pesticide were also identified. The results indicate and there is sufficient time for indoor air pollutants to partition into the condensate before it is drained.

Condensate can be reused for non-potable uses including landscape irrigation, cooling tower makeup water, water features (e.g., fountains or decorative ponds), and other non-potable building demand (e.g., toilet flushing) (Guz, 2005; Loveless et al., 2013). Loveless et al. 2013 suggests condensate could be reused as a potable water source following simple treatment based on the study's pH, conductivity, and turbidity results. However, conductivity and turbidity are indicators of overall water quality, but provide no information on heavy metal concentrations that could require significant treatment before the condensate is safe for potable reuse or other intended reuse alternatives. Table 1 below shows the inorganic primary drinking water maximum containment levels (MCLs) (US EPA, 2016b), secondary drinking water MCLs (US EPA, 2016a), and recommended concentrations for irrigation (US EPA, 2012) for relevant inorganics.

Table 1: Drinking water standards and irrigation recommendations for relevant inorganics and pH

Analyte	Primary Drinking Water Standard[1]	Secondary Drinking Water Standard[2]	Irrigation Limit Recommendations[3]
Aluminum	--	0.05-0.2	5.0
Arsenic	0.010	--	0.10
Barium	2	--	--
Boron	--	--	0.75
Cadmium	0.005	--	0.01
Chloride	--	250	--
Chromium	0.1	--	0.1
Copper	1.3	1.0	0.2
Fluoride	4.0	2.0	1.0
Iron	--	0.3	5.0
Lead	0.015	--	5.0
Manganese	--	0.05	0.2
Nickel	--	--	0.2
Nitrate	10.0	--	--
Nitrite	1.0	--	--
pH	--	6.5-8.5	--
Sulfate	--	250	--
Zinc	--	5.0	2.0

Units = mg/L

(1) Primary Drinking Water Maximum Contaminant Levels (US EPA, 2016)

(2) Secondary Drinking Water Standards (US EPA, 2016)

(3) Recommended water quality criteria for irrigation. (US EPA, 2012)

2.3 SUMMARY

Both alternative water use in cooling towers and residential HVAC condensate reuse present opportunities to reduce municipal potable water demand. However, additional water quality data is required for both water sources. The quality of alternative water sources for cooling tower makeup water influences nutrient availability and disinfectant levels required. The water quality of residential HVAC condensate limits the

potential reuse options for a particular source and the quantity of water available from HVAC systems dictates the feasibility of use.

Chapter 3: Materials and Methods

This chapter details the materials and methods used to investigate the microbial community impacts of using alternative water sources in urban cooling towers and the reuse applications of residential HVAC condensate.

3.1 Cooling Tower Study

3.1.1 Research Plan

Basin water, makeup water, and biofilm samples were collected from three urban cooling towers. Each cooling tower was sampled three times over a two-week period. The cooling towers follow a typical operation pattern. Cool water from the reservoir (basin water) is continuously recirculated up through a heat exchanger where it is warmed and then sprayed downward back to the basin. Evaporation cools the water as it returns to the basin. Basin water must be discharged (blowdown water) to avoid scaling issues, and influent fresh water must continuously be added (makeup water) to maintain a constant basin water volume.

Potable water has traditionally been the sole makeup water source of cooling towers. However, the studied cooling towers used a blend of potable, reclaimed, and recovered water. The three cooling towers were selected because they were operated similarly (i.e., same inspectors, routine sampling, annual cleaning), which reduces variations due to maintenance plans (or lack thereof). Sampling was conducted over a short interval to investigate short-term (i.e., not inter-seasonal) variability within each cooling tower.

Microbial analyses were conducted on each water and biofilm sample. The microbial community was characterized and the *Legionella* spp., *L. pneumophila*, *L. pneumophila* sg. 1, and total bacteria concentrations were quantified. Illumina sequencing

was used to characterize the cooling tower microbial communities. Quantitative real-time PCR (qPCR) was employed to quantify the levels of total bacteria (16S rRNA) (Harms et al., 2003), *Legionella* spp. (*ssrA* gene) (Thurman, Warner, Cowart, Benitez, & Winchell, 2011), *L. pneumophila* (*mip* gene) (Engleberg, Carter, Weber, Cianciotto, & Eisenstein, 1989), and *L. pneumophila* sg. 1 (*wzm* gene) (Merault et al., 2011). The Illumina sequencing and qPCR results were used to evaluate whether a cooling tower's makeup water source impacts the basin microbial community.

Major anions, cations, alkalinity, and total phosphorus were also measured for each water sample. The water chemistry results were evaluated together with the qPCR results to identify correlations between the water chemistry parameters and *Legionella* spp. and total bacteria levels. The *Legionella* qPCR and water chemistry results were also used to assess if higher levels of nutrients led to higher levels of *Legionella* spp, *L. pneumophila*, or *L. pneumophila* sg 1.

The total bacteria qPCR results were coupled with the Illumina sequencing results for select genera containing opportunistic pathogens, *Mycobacterium* and *Pseudomonas*, to estimate the quantity of each genera in the water samples.

3.1.2 Methodology

3.1.2.1 Cooling Tower Description

Three urban cooling towers (CT A, B, and C) in Austin, Texas, were sampled three times (Day 1, 2, and 3) over a two week period in September 2015. CTs A and C are located 1 mile from each other with CT B located approximately in the middle. Table 2 displays the makeup water sources for each cooling tower along with the physical characteristics of each basin. Each basin is dosed with 12% hypochlorite to maintain a total chlorine concentration of 0.6-1.5 ppm as Cl_2 and a maximum free chlorine residual

of 0.5 ppm as Cl_2. Basins B and C can also be dosed with chlorine dioxide if the maximum free chlorine residual is not adequately maintained.

Table 2: Cooling tower physical information

Cooling Tower	**Make-up Water Sources**	**Basin Dimensions (LxWxD) (m)**	**Volume (m^3)**
A	Potable, Recovered	302 x 207 x 8	416
B	Potable, Reclaimed	590 x 164 x 3-13[1]	643
C	Potable, Recovered	420 x 144 x 8	416
Cooling Tower	**Disinfectant**	**Cooling Capacity (Tonnage)**	**Pre-Treatment**
A	12% Sodium Hypochlorite	11	N/A
B	12% Sodium Hypochlorite, Chlorine Dioxide	13	Reclaimed water is sand filtered upstream of basin
C	12% Sodium Hypochlorite, Chlorine Dioxide	15	N/A

(1) Basin floor is sloped towards middle of basin

The pH values of CT basins A, B, and C were controlled with sulfuric acid and held between 8.4-8.7, 7.0-7.6, and 8.0-8.3, respectively. U.S. Water/ChemCal Microbiocide 1560 comprised of 1.1% 5-chloro-2-methyl-3(2H)-Isothiazolone, 0.39% 2-methyl-3(2H)-Isothiazolone, and <1.0% Cupric Acid was dosed to each CT basin weekly according to supplier recommendations. The water chemistry targets for each cooling tower are shown in Table 3. Each cooling tower is also taken off line and drained once a year and cleaned to remove any accumulated sediment and biofilms. As part of the cooling tower maintenance plan, samples of basin water from each cooling tower are routinely sent to an outside certified lab for culture-based *Legionella* testing.

Table 3: Cooling tower water chemistry targets

Cooling Tower	**pH**	**Conductivity** (umhos)	**Total Chlorine** (mg/L as Cl_2)	**Free Chlorine** (mg/L as Cl_2)	**Chlorine Dioxide** (mg/L as ClO_2)
A	8.4-8.7	2300-2600	0.6-1.5	0.5 max	N/A
B	7.0-7.6	4500-5100	0.6-2.0	0.5 max	1.5 max
C	8.0-8.3	2300-2600	0.6-2.0	0.5 max	1.5 max
Cooling Tower	**Phosphate** (mg/L as PO_4)	**Ortho Phosphate** (Filtered mg/L as PO_4)	**Iron** (mg/L as Fe)	**Copper** (mg/L as Cu)	**Target ORP**
A	10 max	N/A	1 max	0.5 max	350
B	15 min	15 min	1 max	0.5 max	275
C	10 max	10 max	1 max	0.5 max	320

The reclaimed water used in CT B comes from a municipal wastewater treatment plant with a typical treatment train. Raw wastewater is pretreated with grit chambers and primary clarifiers. Secondary treatment is achieved using the activated sludge process that includes aeration basins and secondary clarifiers. The treated water is then chlorinated and discharged to the reclaimed water system.

3.1.2.2 Sample Collection

Potable and recovered water are the makeup water sources for CTs A and C. Potable and reclaimed water are the makeup water sources for CT B (Table 2). At each cooling tower on each sampling day, two 1-L water samples were collected from the basin and from each makeup water source. The 1-L glass bottles used for collection were first acid washed and autoclaved. Basin water samples from Day 1, and all collected water samples from Days 2 and 3 were measured for pH, temperature, total dissolved solids (TDS), oxidation-reduction potential (ORP), and conductivity using a MyRon L

Ultrameter 2 (Myron L Company, Carlsbad, CA). Two biofilm samples were collected from each cooling tower during each sampling event using a sterile swab wetted with phosphate buffered saline (PBS) solution. Biofilm samples were collected from the visible biofilms occurring on the sideboards of the basin and along the walls of the CT enclosure.

All water and biofilm samples were kept in coolers on ice during sampling and were returned to the laboratory within 2 hours of collection and stored at 5°C until further processing. Up to 1-L of water from each water source was filtered through a 0.2 µm MicroFunnel Filter Unit (Pall Corporation, Port Washington, NY) within 4 hours of collection. All 0.2 µm filters and biofilm samples were stored at -20°C for later DNA extraction. The remaining 1-L sample from each water source was partitioned into an (1) unpreserved unfiltered aliquot, (1) unpreserved filtered (0.45 µm) aliquot, and (1) 2% nitric acid preserved filtered (0.45 µm) aliquot for later analytical analysis.

Basin water samples from all three cooling towers were submitted to Special Pathogens Laboratory (Pittsburgh, PA) for *Legionella* culture analysis (SPL Modified ISO Standards 11731-1:1998 and 11731-2:2004). Samples were collected and shipped according to laboratory instructions. Basin water samples from Days 1 and 3 were submitted to Eurofins/Eaton Analytical for assimilable organic carbon (AOC) analysis (Weinrich, Giraldo, & LeChevallier, 2009). Samples were collected and shipped according to laboratory instructions.

Bacterial DNA was extracted from all filters and biofilm swabs using a PowerWater DNA Isolation Kit (MoBio Labs, Carlsbad, CA). Cell lysis by multidirectional beating was conducted in the FastPrep-24 homogenizer (MP Biomedicals LLC, Solon OH), following manufacturer recommendations of 30 seconds at 5.0 m/s. DNA was eluted in 75 µL solution C6, quantified using PicoGreen dsDNA

assay (Invitrogen Life Technologies, Grand Island, NY), and stored at -20^{o}C until sequencing or qPCR analysis.

The total volume per day of each makeup water source added to each basin and the total blowdown water volume from each basin was recorded for each sampling day (from 0:00 to time of sampling) and each day prior to sampling (from 0:00 to 24:00) (Table 4). Approximate percent makeup of each basin was calculated by the ratio of the total volume of each makeup water source on the day of sampling. Residence times were approximated by dividing the basin water volume by the total makeup water added to each basin using the flow data from the days prior to sampling.

Table 4: Total volumes of makeup water sources and blowdown per day ($m^{3/}$day)

Basin	Sampling Day	Blowdown	Potable	Recovered	Reclaimed
A	1	886	596	-	-
A	2*	122	844	49	-
A	2	79	455	30	-
A	3*	89	638	42	-
A	3	-	350	26	-
B	1	-	435	-	708
B	2*	225	-	-	477
B	2	62	4	-	17
B	3*	169	-	-	845
B	3	-	-	-	1,221
C	1	-	971	371	-
C	2*	180	1,098	508	-
C	2	-	6,019	329	-
C	3*	188	1,267	318	-
C	3	-	556	190	-

* indicates total flows from the day prior to sampling
Units = m^3

Free chlorine residual was measured in all basins and the reclaimed water on Days 1 and 3 by the utility team that operates the CTs. Free chlorine was measured the day before Day 2 sampling, and this value was considered the approximate free chlorine for Day 2. Recovered water is the only makeup water source not disinfected prior to entering the CTs.

3.1.2.3 Analytical Methods

Aqueous chloride, nitrate, sulfate, fluoride, bromide concentrations were quantified from the 0.45 μm filtered aliquot using ion chromatography with conductivity detection (Dionex ICS-2100, 4x250 mm IonPac AS-19). A five point standard calibration curve was developed at the beginning of each run with IC stock solutions. A sample blank was measured at the beginning of run after the calibration curve was established.

Aqueous potassium, calcium, copper, iron, magnesium, manganese, and sodium were quantified from the 0.45 μm filtered nitric acid preserved aliquot using inductively coupled plasma-optical emission spectroscopy (ICP-OES). All samples were measured using a Varian 710-ES ICP-OES and Autosampler with 2% concentrated nitric acid mobile phase. A five-point standard calibration curve was developed at the beginning of each run with ICP stock solutions. A sample blank was measured at the beginning of each run after the calibration curve was established. Matrix interference was tested using a 1 mg/L strontium addition to each water source. All samples had >90% recovery except Basin B (80%) and Basin C (87%).

Alkalinity was measured by titration on unfiltered samples, following Standard Methods (Clesceri, Greenberg, & Eaton, 1998). Total phosphorus was measured from unfiltered samples using the Standard Methods 4500-P persulfate digestion method followed by the ascorbic acid method (APHA, AWWA, & WPCF, 1980). A standard curve was included with each run, and a sample blank was measured at the beginning of each run after the calibration curve was established. Method detection limits were measured following the US EPA minimum detection limit (MDL) procedure for all methods (U.S. EPA, 1997).

3.1.2.4 Illumina Sequencing

Bacterial DNA was analyzed at the Genomic Sequencing and Analysis Facility (GSAF) at the University of Texas at Austin (Austin, TX, USA) for Illumina® paired-end (2×250) sequencing on the MiSeq platform. For bacteria, first-round PCR was used to amplify the V4/V5 regions of the 16S rRNA gene using the primers 515F (5'-GTGYCAGCMGCCGCGGTA-3') (Baker et al., 2003) and 909R (5'-CCCCGYCAATTCMTTTRAGT-3') (Wang & Qian 2009). Primers included appropriate

Illumina adapters with reverse primers also having an error correcting 12-bp barcode unique to each sample to permit multiplexing of samples. PCR amplification was performed using Qiagen Taq polymerase (Qiagen Corporation, Valencia, CA). After the PCR amplification, samples were prepared for their Illumina® sequencing run. This first round of PCR amplification was run in triplicate for each sample, pooled, and then cleaned using AMPure beads (New England Biolabs, Ipswich, MA). Second-round PCR amplification was performed with different primers that added sample-specific barcodes. Both rounds of PCR amplification (a total of 30 cycles) used Taq polymerase NEB Q5 (New England Biolabs, Ipswich, MA). The final PCR products for each sample after both rounds of amplification were then size-purified by removing amplicons less than 300 bp in length using AMPure beads (New England Biolabs, Ipswich, MA) and quantified using PicoGreen (Life Technologies, Carlsbad, CA). Samples were then normalized by amplicon mass and pooled for the Illumina® run. In addition, a random subset of samples was assessed on an Agilent BioAnalyzer (Agilent Technologies, Santa Clara, CA) to ensure correct amplicon size. Negative PCR controls were included to test for contamination.

3.1.2.5 Illumina Sequencing Data Processing and Statistical Analysis

Bacterial DNA sequences were processed and analyzed in QIIME v.1.8 (Caporaso et al., others, 2010). Sequences were demultiplexed and forward and reverse reads were merged using FLASH v.1.2.11 (Magoč & Salzberg 2011) with maximum overlap of 250bp. Sequences were quality-filtered (-q 19), and chimeras were removed via QIIME and USEARCH (Edgar 2010). High-quality sequences were clustered into operational taxonomic units (OTUs) at 97% similarity using QIIME's USEARCH-based open-reference OTU clustering workflow (pick_open_reference_otus.py). Global singleton

OTUs were removed and taxonomy was assigned using the Ribosomal Database Project classifier (Wang et al. 2007) with the reference database Greengenes13_8 16S rRNA (McDonald et al. 2012). In order to quantify the possible contamination due to background DNA in reagents or introduced during sample processing, negative controls (extraction kit controls) were analyzed and sequenced. Negative controls showed a small number of total reads (<5% of those obtained in the samples, then not passing the rarefaction threshold), indicating the likelihood of contamination was negligible.

Pair-wise dissimilarities between communities (e.g. principle coordinate analysis (PCoA)) were calculated using Bray Curtis dissimilarities and samples were rarefied to the minimum number of sequences present in any individual sample (16,414 counts).

3.1.2.6 qPCR Assay Conditions

Three separate real time PCR assays were conducted on an Applied Biosystems Viia™ 7 Real-Time PCR System (Applied Biosystems, Foster City, CA): a duplex assay for *Legionella* spp. and *L. pneumophila*, an assay for *L. pneumophila* sg 1, and a total bacteria assay. The swabs were only tested using the duplex and simplex *Legionella* assays and were not tested for total bacteria. The primers and probes used to target the *ssrA*, *mip*, and *wzm* genes were previously described (Benitez & Winchell, 2013). Probe dye and reporter modifications were adopted for PCR system compatibility. Total bacterial (16S) levels were measured using a modified method from Harms et al. 2003, which amplifies a conserved region of the 16S gene. All primers and probes are shown in Table 5.

Table 5: *Legionella* and total bacteria qPCR primers and probes

Name	Sequence (5'-3')	Target	Product (bp)
LsppF	GGCGACCTGGCTTC	*ssrA*	101
LsppR	GGTCATCGTTTGCATTTATATTTA		
LsppP	FAM-ACGTGGGTTGCAA-MGBNFQ[1]		
LpnF	TTGTCTTATAGCATTGGTGCCG	*mip*	115
LpnR	CCAATTGAGCGCCACTCATAG		
LpnP	TAM-CGGAAGCAATGGCTAAAGGCATGCA-BHQ2[2]		
Lsg1F	TGCCTCTGGCTTTGCAGTTA	*wzm*	70
Lsg1R	CACACAGGCACAGCAGAAACA		
Lsg1P	VIC-TTTATTACTCCACTCCAGCGAT-MGBNFQ		
1055F	ATGGCTGTCGTCAGCT	16S	340
1055R	ACGGGCGGTGTGTAC		
1055P	HEX-CAACGAGCGCAACCC-TAMRA		

The duplex assay reaction volume was 30 µL consisting of 600 nM of primers for ssrA, 400 nmol/l of primers for mip, 150 nmol/l of each probe, 15 uL of Taq Man Environmental Mastermix 2.0 (Life Technologies, Carlsbad, CA, USA), and 10 µL of extracted DNA. The *L. pneumophila* sg1 assay total reaction volume was 15 µL consisting of 0.40 µM of primers, 0.15 µM of probe, 7.5 µL of TaqMan Environmental Mastermix 2.0, and 5 µL of extracted DNA. The thermal reaction conditions for both *Legionella* assays were 95°C for 10 minutes followed by 45 cycles of 95°C for 15 seconds and 60°C for 1 minute (Collins, Jorgensen, Willis, & Walker, 2015).

The total bacteria assay reaction volume was 20 µL consisting of 0.25 µM of primers and probe, 10 µL of Taq Man Environmental Mastermix 2.0, and 2 µL of extracted DNA. The thermal reaction conditions were 50°C for 2 minutes followed by

95°C for 10 minutes and then 40 cycles of 95°C for 30 seconds, 50°C for 1 minute, and 72°C for 45 seconds.

Serial dilutions of ten replicates were used to determine the limit of quantification (LOQ) for each qPCR assay. The LOQ was defined as the lowest concentration yielding a positive result with 100% confidence and a coefficient of variation below 5% (Collins et al., 2015). The cycle threshold standard deviation for each sample triplicate also had to be <0.50 to be considered above the LOQ. The LOD was defined as any sample having two positive results out of the three sample replicates. Biofilm samples were evaluated using the LOD criterion only.

The LOQ for *Legionella* spp. and *L. pneumophila*, and *L. pneumophila* sg 1 was 50, 27, and 27 gene copies (GC)/reaction, respectively. The LOQ expressed as GC/L of the original water samples vary based on the volume of water filtered during sample processing (Table 6).

Table 6: LOQs for *Legionella* assays converted to logGC/L

Basin	Source Water	Sample Date	Volume Filtered (mL)	*Legionella* spp.	*L. pneumophila*	*L. pneumophila* sg 1
A	Basin	1	244	4.19	3.92	3.92
A	Basin	2	270	4.14	3.87	3.87
A	Basin	3	235	4.2	3.93	3.93
A	Potable	1	1000	3.57	3.3	3.3
A	Potable	2	1000	3.57	3.3	3.3
A	Potable	3	1000	3.57	3.3	3.3
A	Recovered	2	1100	3.53	3.26	3.26
A	Recovered	3	1000	3.57	3.3	3.3
B	Basin	1	170	4.34	4.07	4.07
B	Basin	2	91	4.61	4.34	4.34
B	Basin	3	82	4.66	4.39	4.39
B	Potable	1	1000	3.57	3.3	3.3
B	Potable	2	1000	3.57	3.3	3.3
B	Potable	3	1000	3.57	3.3	3.3
B	Reclaimed	1	476	3.9	3.63	3.63
B	Reclaimed	2	349	4.03	3.76	3.76
B	Reclaimed	3	357	4.02	3.75	3.75
C	Basin	1	420	3.95	3.68	3.68
C	Basin	2	173	4.34	4.07	4.07
C	Basin	3	208	4.26	3.99	3.99
C	Potable	1	1000	3.57	3.3	3.3
C	Potable	2	1000	3.57	3.3	3.3
C	Potable	3	1000	3.57	3.3	3.3
C	Recovered	1	510	3.87	3.6	3.6
C	Recovered	2	522	3.86	3.59	3.59
C	Recovered	3	405	3.97	3.7	3.7

3.1.2.7 Standard DNA

L. pneumophila sg1 DNA was used to generate the standard curves for the *L. pneumophila* sg1 assay, and *L. Pneumophila* sg 2 DNA was used for the duplex assay. Both DNA aliquots were obtained at a concentration of 10 ng/µL from the Pneumonia

Response and Surveillance Laboratory, Centers for Disease Control and Prevention (Atlanta, Georgia). *E. coli* O157 purchased from Sigma Aldrich (St. Louis, MO) was used as the control DNA for the total bacteria assay. Five point standard curves were generated and each standard concentration was run in triplicate for each assay. Each standard curve had a $R^2 > 0.99$. Figure 3 shows typical standard curves for the *ssrA*, *mip*, *wzm*, and 16S rRNA total bacteria qPCR assays. The *ssrA* and *mip* assays detected the control *L. pneumophila* sg. 1 and 2 100% of the time. The *wzm* assay detected the control *L. pneumophila* sg. 1 strain but did not detect the control *L. pneumophila sg. 2* strain, indicating 100% specificity. Matrix interference was tested by spiking each water sampled with 4.5 logGC/reaction in triplicate. Minimal inhibition was measured (>95% recovery) in all water and biofilm sources.

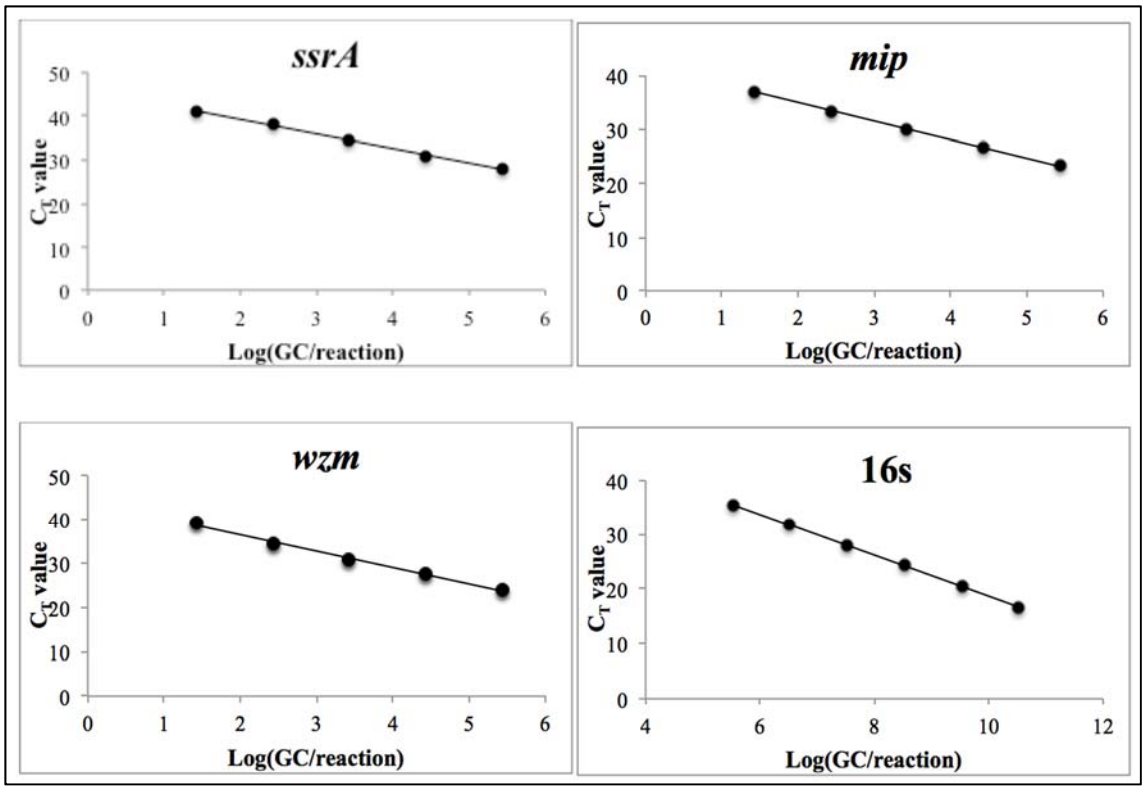

Figure 3: Typical qPCR standard curves for *Legionella* spp (ssrA), *L. pneumophila* (mip), *L. pneumophila* sg 1 (wzm), and total bacteria (16S). Standard curves were established before each qPCR run and required an $R^2 > 0.99$. All concentration units are gene copies (GC) per qPCR reaction.

3.2 Residential HVAC Condensate Study

3.2.1 Research Plan

Three private residences with central air conditioning systems were studied. At each residence, temperature and relative humidity of the supply and return air streams were monitored to calculate theoretical condensate production using the difference of humidity ratios across the HVAC system. While temperature and relative humidity data were being logged, the HVAC system's actual condensate was collected to compare the calculated theoretical condensate volume to the actual collected volume. Condensate

samples collected from each home were also measured for organic content, metals, anions, total phosphorus, and alkalinity to assess reuse options.

3.2.2 Methodology

3.2.2.1 Residence Information

Three private residences were used for this study: Residence A, Residence B, and Residence C. Residences A and B were single-family homes and Residence C was an apartment unit. Residence A has a total of three HVAC units, but only one was studied. Residence B also has three HVAC units and two were studied. The two systems use the same drainage system so the collected and predicted condensate volumes represent the sum of the two systems. Residence C has only one designated HVAC system. Table 7 below contains a summary of the HVAC units sampled.

Table 7: HVAC Unit residence information

Unit	Dwelling Type	System Flow Rate (m^3/hr)	Cooling Coil Material
A	Single Family Home	2529	Copper tubing with aluminum fins with galvanized steel collection pan. PVC drain pipe.
B	Single Family Home	3182	Copper tubing with PVC drain pipe
C	Apartment Unit	510	Aluminum tubing with steel collection pan.

3.2.2.2 Temperature and Humidity Data Collection

Onset (Bourne, MA) HOBO temperature and relative humidity data U12-013 loggers were attached using zip ties to the return and one supply grate for each HVAC unit assessed. The loggers had an accuracy of ±0.35°C for temperature and ±2.5% for relative humidity. Each logger recorded a temperature and relative humidity reading

every minute. Following the monitoring period, the data from each logger was uploaded using Onset's HOBOware software.

3.2.2.3 Condensate Volume Measurements

The actual volume of condensate produced from each HVAC unit studied was collected for each residence over the same time period that temperature and humidity data were being logged. A volumetrically marked 18-L drum was either connected to the HVAC drain pipe directly or clear vinyl tubing was connected to the drain pipe and routed to the drum. Approximately once per day the volume in the drum was recorded and the condensate was discarded. The time between condensate volume recordings is denoted as "t" in Section 3.2.2.4 below.

3.2.2.4 Humidity Ratio Calculation

The humidity ratio difference across each HVAC unit was calculated to predict the theoretical condensate production rate. To calculate the humidity ratios, the temperature and humidity data from the supply vent were first transformed as described below. An example temperature cycle transformation is shown in Figure 4 for reference.

HVAC units run in pulses of a few to several minutes. Supply temperature data should have a relatively high baseline temperature, indicating the HVAC unit is not operating, and pulses of cooler air when the HVAC system is operating. However, because of the plastic casing of the data loggers and other factors, the raw data contain gradual, parabolic-like temperature declines and rises that reflect when the HVAC unit is running instead of strict pulses. The parabolic cycles of the raw data were transformed to binomial pulse cycles. Figure 4 shows an example temperature operation cycle transformation with the parabolic raw data and the transformed data. Similarly, the humidity data should have a relatively low baseline and pulses of higher humidity when

the HVAC system is operating, but the raw data contain gradual rises and declines. The supply raw humidity data were also transformed to strict pulses.

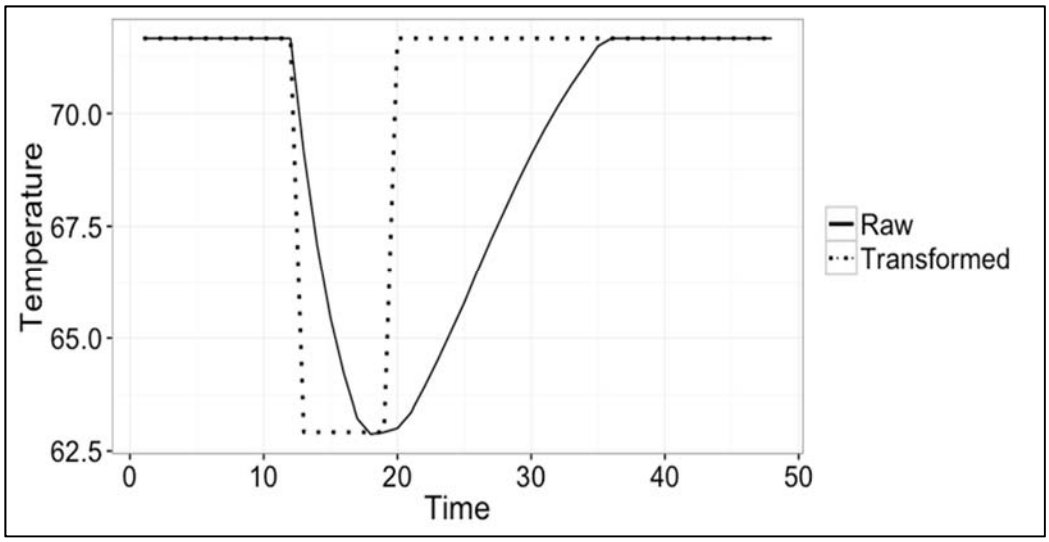

Figure 4: Example supply air temperature operation cycle transformation. HVAC units operate on cycles and the supply air temperature should contain lower temperature pulses, indicating HVAC unit operation. However, the temperature loggers captured a gradual decrease and then increase in temperature (solid "Raw" curve above). Therefore, each supply air temperature operation cycle was transformed to strict pulses (dashed "Transformed" curve above).

Condensate is only produced while the HVAC unit is actually operating. Therefore, only the temperature and humidity data capturing the HVAC unit operating were used for calculating humidity ratios. For the supply temperature, each minute reading that had a lower temperature than the previous minute indicated that the HVAC system was operating. Each operating minute's temperature was converted to the lowest temperature point of that operation cycle. The HVAC unit was assumed not to be operating for each minute that had a higher temperature reading than the previous minute (Figure 4).

Once the raw supply data was transformed, the humidity ratio for both the supply and return air can be calculated for each minute of HVAC unit operation. The steps taken to calculate the supply and return humidity ratios are below (ASHRAE, 1987):

1. Convert all temperatures to Kelvin (T)
2. Use the following empirical formula to calculate the saturated pressure of water vapor (P_{WS}) for the temperature at each operating time point

   $\ln(P_{WS}) = C_8/T + C_9 + C_{10}T + C_{11}T^2 + C_{12}*T^3 + C_{13}*\ln(T)$

   Where: C_8=-5800.2206, C_9=1.3914993, C_{10}=-0.04860239, C_{11}=0.41764768e-4, C_{12}=-0.14452093e-7, C_{13}=6.5459673
3. Multiply the P_{WS} by either the transformed (supply) or raw (return) relative humidity fraction to find the partial pressure of water vapor for the given relative humidity (P_W) in pascals
4. Calculate the humidity ratio (W) by $W = 0.62198(P_W) / (101325 - P_W)$ where 101325 is the pressure in pascals at sea level and 0.62198 is the approximate ratio of the molecular weight of water vapor to the molecular weigh of dry air. W is in units of mass of water vapor per mass of dry air.

Once the humidity ratio (W) is determined for each minute the HVAC unit is operating for both the supply (W_{Supply}) and return (W_{Return}) flows, the humidity ratio difference across the HVAC system and the theoretical condensate volume can be calculated using the following steps:

1. Calculate the humidity ratio difference for each minute of HVAC unit operation by $\Delta W = W_{Supply} - W_{Return}$
2. Average the ΔW values within each time interval that correspond to condensate measurement times (ΔW_{Ave})

3. Calculate theoretical condensate volume (V) for each condensate measurement time interval by $V = \Delta W_{Ave}$ Q t

W

here: Q = return air flow rate for HVAC unit and t = interval between condensate measurement times

The theoretical volumes (V) for each time interval were then compared to the actual condensate volume measurements, and percent differences between the two values were calculated.

3.2.2.5 Sample Collection for Analytical Testing

Approximately 500 mL of condensate was collected from each residence directly from the HVAC condensate drain line into an acid washed and autoclaved glass container. The HVAC units were purposefully operated to collect samples and sample collection occurred within 2-10 minutes. The container was then put on ice and brought back to the laboratory within 1 hour of collection. At the lab, one aliquot was left unfiltered, one aliquot was filtered (0.45 μm), and a final aliquot was filtered (0.45 μm) and preserved with concentrated nitric acid to a final concentration of 2% (v/v).

3.2.2.6 Analytical Methods

Aqueous chloride, nitrate, sulfate, fluoride, bromide concentrations were quantified from the 0.45 μm filtered aliquot using ion chromatography with conductivity detection (Dionex ICS-2100, 4x250 mm IonPac AS-19). A five point standard calibration curve was developed at the beginning of each run with IC stock solutions. A sample blank was measured at the beginning of each run after the calibration curve was established.

To substantiate the presence of both acetate and formate, condensate samples were spiked with acetate and formate standard solutions at a concentration of 5 mg/L and measured via IC analysis as described above. An example chromatogram from Unit B of the spiked and raw sample is below in Figure 5. The IC method was altered to separate the acetate and formate peaks for concentration analysis. Samples were also separately spiked with acetate and formate to determine which peak was acetate and which was formate.

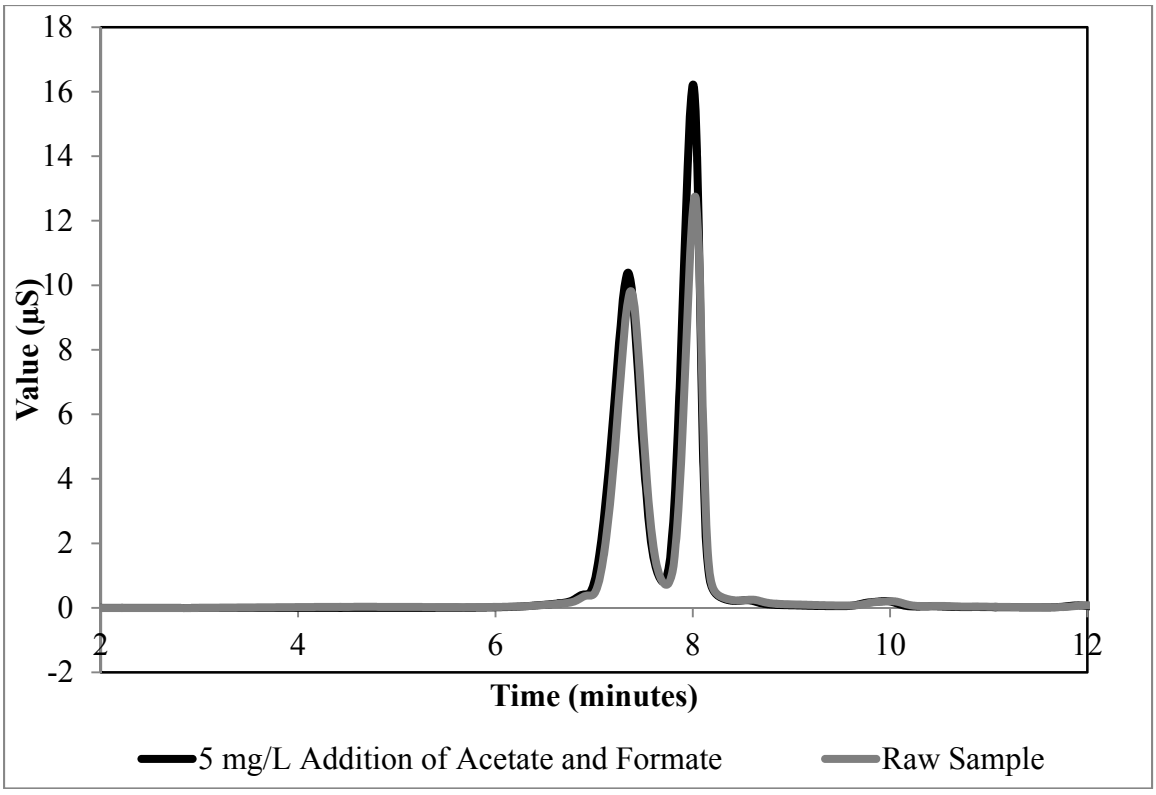

Figure 5: Chromatorgraph of formate and acetate addition to Unit B condensate. The raw condensate sample peaks and the spiked condensate peaks overlap without peak spreading. The IC method was altered to further separate the peaks for quantification.

Dissolved organic carbon was measured on Unit B and C condensate samples using a Total Organic Carbon Analyzer TOC-LCPH/CPN (Shimadzu, Japan). Raw samples were filtered through a 0.45 μm filter before analysis. A standard curve was established and a sample blank was run before running the samples. Both units were spiked with 5 mg/L of acetate to test recovery.

Nitrite was confirmed for the condensate using Unit C using ultraviolet-visible spectroscopy (UV-Vis). Samples were measured on an Agilent 8453 UV-Vis System (Aglient Technologies, California). Standards were developed using sodium nitrite. A sample blank was measured after the standard curve was developed and before samples. Raw Unit C condensate and Unit C condensate spiked with 10 mg/L as nitrite were quantified. All standards, blanks, and samples were measured at 210 nm, the maximum absorbance for nitrite (Sung, 2011). 101% of the nitrite in the spiked nitrite sample was recovered.

Aqueous potassium, calcium, copper, iron, magnesium, manganese, and sodium were quantified from the 0.45 μm filtered nitric acid preserved aliquot using inductively coupled plasma-optical emission spectroscopy (ICP-OES). All samples were measured using a Varian 710-ES ICP-OES and Autosampler with 2% concentrated nitric acid mobile phase. A five-point standard calibration curve was developed at the beginning of each run with ICP stock solutions. A sample blank was measured at the beginning of each run after the calibration curve was established. Matrix interference was tested using a 1 mg/L strontium addition to each water source. All samples had >90% recovery except Basin B (80%) and Basin C (87%).

Alkalinity was measured by titration on unfiltered samples, following Standard Methods (Clesceri et al., 1998). Total phosphorus was measured from unfiltered samples using the Standard Methods 4500-P persulfate digestion method followed by the ascorbic

acid method (APHA et al., 1980). A standard curve was included with each run, and a sample blank was measured at the beginning of each run after the calibration curve was established. Method detection limits were measured following the US EPA minimum detection limit (MDL) procedure for all methods (U.S. EPA, 1997).

3.2.2.7 Solubility Plots

Copper and aluminum solubility plots were generated using Visual MINTEQ v3.1, a chemical equilibrium model and the MINTEQ database (Gustafsson, 2014).

Chapter 4: Cooling Tower Experimental Results and Discussion

4.1 Cooling Tower Experiment

The three cooling towers studied used a blend of potable and alternative water in their makeup water supplies. Cooling towers A and C used mostly potable water supplemented with recovered water which consisted of an unknown mixture of HVAC condensate, recreational pool blowdown, and groundwater pumped from building foundations. Cooling tower B used mostly reclaimed water supplemented with potable water. Cooling tower B was expected to have the highest levels of nutrients, TDS and conductivity because the reclaimed water was expected to contain higher levels of most, if not all, of the measured dissolved species relative to potable water.

4.1.1 Comparison of Cooling Tower Operating Data

Table 8 shows the pH, temperature, TDS, ORP, conductivity, free chlorine, and AOC of each water source. Basin B has approximately double the TDS and conductivity levels compared to Basins A and C, which follows from the reclaimed water having 2-5 times more TDS and conductivity relative to the other make-up water sources. Positive ORP values indicate aerobic conditions for all water samples, which was expected since the recirculating spray aerates the basins and the makeup water sources should contain dissolved oxygen. The basins have higher TDS and conductivity compared to the makeup water sources due to salt cycling in the basins. The reclaimed water had the highest TDS and conductivity followed by the recovered water at Basin A, potable water, and then recovered water at Basin C. These results indicate the water chemistry of the reclaimed water was different at Basin A compared to the reclaimed water at Basin C. The potable water pH values are consistent with drinking water operations at a water treatment plant

that utilizes lime softening and recarbonation to produce a relatively low alkalinity and moderate hardness.

The variability of the potable water samples can be use to evaluate the variability of sampling since there were six potable water samples measured for TDS, ORP, and conductivity, and the potable water was assumed to be relatively constant in terms of water chemistry. The average (standard deviation) of TDS, ORP, and conductivity for the potable water samples was 217 mg/L (2.4 mg/L), 264.8 mV (57.4 mV), and 328.8 $\mu S/cm^2$ (2.4 $\mu s/cm^2$), respectively. The TDS and conductivity both have standard deviations of about 1% of the average value, indicating that the level of dissolved constituents was relatively constant on each sampling day.

The reclaimed water entering Basin B had minimal to no free chlorine (Table 8). Basin C had the highest single value for residual chlorine (0.70 mg/L as Cl_2), but the ranges were similar for each basin. A free residual chlorine level <0.50 mg/L has been positively associated with *Legionella* colonization, and all basin samples were below this level except for the maximum value from Basin C (Mouchtouri, Goutziana, Kremastinou, & Hadjichristodoulou, 2010). The AOC results were also similar for each basin on sampling days 1 and 3. The temperatures of the basins were relatively constant over the three sampling days, which is not surprising given that the sampling plan was conducted over a two week period to capture short term variability instead of seasonal variability.

Table 8: Water quality data for the three cooling tower basins and the corresponding makeup water sources

Cooling Tower	Water Source	pH	Temperature (°C)	TDS (mg/L)	ORP (mV)	Conductivity (µs/cm^2)	Free Chlorine (mg/L as Cl_2)	Assimilable Organic Carbon (mg/L)
A	Basin	8.27-8.47	22.7-26.2	1506-1953	290-377	2040-2543	0.09-0.10	0.47-0.57
B	Basin	7.14-7.29	23.5-26.7	3840-4021	248-319	4870-5063	0.05-0.21	0.46-0.68
C	Basin	8.37-8.57	25.3-27.8	1682-1772	322-447	2310-2471	0.17-0.70	0.43-0.66
A	Potable	9.18-9.38	28.7-29.3	216-218	235-290	329-331	NA	NA
B	Potable	8.93-9.20	27.2-27.8	214-216	243-371	325-328	NA	NA
C	Potable	9.19-9.28	26.6-27.6	217-221	222-228	328-332	NA	NA
B	Reclaimed	6.19-6.20	29.4-29.7	781-805	229-348	1132-1145	0-0.06	NA
A	Recovered	7.27-7.42	28.2-28.3	403-418	287-292	588-623	NA	NA
C	Recovered	7.40-7.68	27.2-27.3	113-198	253-273	174-301	NA	NA

Table 9 shows each basin's hydraulic residence time and the approximate percentage of each makeup water source in each basin. For example, on Day 3, Basin A was made up of approximately 93% potable water and 7% recovered water. Basins A and C were mostly comprised of potable water whereas Basin B was mostly reclaimed water Basin B also had the longest hydraulic residence time (1.1 days), followed by Basin A (0.5 days) and then Basin C (0.1 days).

Table 9: Makeup water percent contribution and hydraulic residence time

Cooling Tower	Sampling Date	Potable Percent	Recovered Percent	Reclaimed Percent	Residence Time (days)
A	1	100	0	0	0.5
A	2	94	6	0	
A	3	93	7	0	
B	1	38	0	62	1.1
B	2	19	0	81	
B	3	0	0	100	
C	1	72	28	0	0.1
C	2	95	5	0	
C	3	75	25	0	

4.1.2 Water Chemistry Analysis

The complete water chemistry analysis for each sample is included in the Appendix. Cooling tower operation (i.e., evaporation) leads to higher levels of anions and cations in the basins relative to the makeup water sources. The reclaimed water samples contained higher levels of chloride, fluoride, nitrate, sulfate, potassium, calcium, magnesium, sodium, and total phosphorus compared to all other makeup water sources, which follows from the reclaimed water having higher TDS and conductivity relative to the other makeup sources (Table 8).

4.1.3 Basin and Makeup Water Microbial Communities Assessment

Figure 6 shows the relative abundance of the top twenty OTUs identified to the genus level in all water samples. All samples were grouped by water source and are displayed by basin. The potable water samples are dominated by *Rheinheimera*, *PSB-M-3*, *Pseudomonas*, and *Hydrogenophaga*. However, none of those genera were present at high relative abundance in the basin samples even in Basins A and C that were supplied greater than 70% potable water (Table 9). The reclaimed water was dominated by *Cupriavidus*, *Sphingomonas*, *Sphingopyxis*, and *Novophingobium*. Basin B was made up of at least 60% reclaimed water (Table 9) and contained *Cupriavidus* and *Sphingomonas* at large relative abundances as well. The recovered water sources contained the greatest number of unique genera at significant relative abundances compared to any other water source including the basins. However, the only genus present in the both the recovered water and Basin A and C samples at a significant relative abundance was *Sphingomonas*. These results indicate that the microbial community of the reclaimed water is potentially the more conserved than the other three makeup water sources.

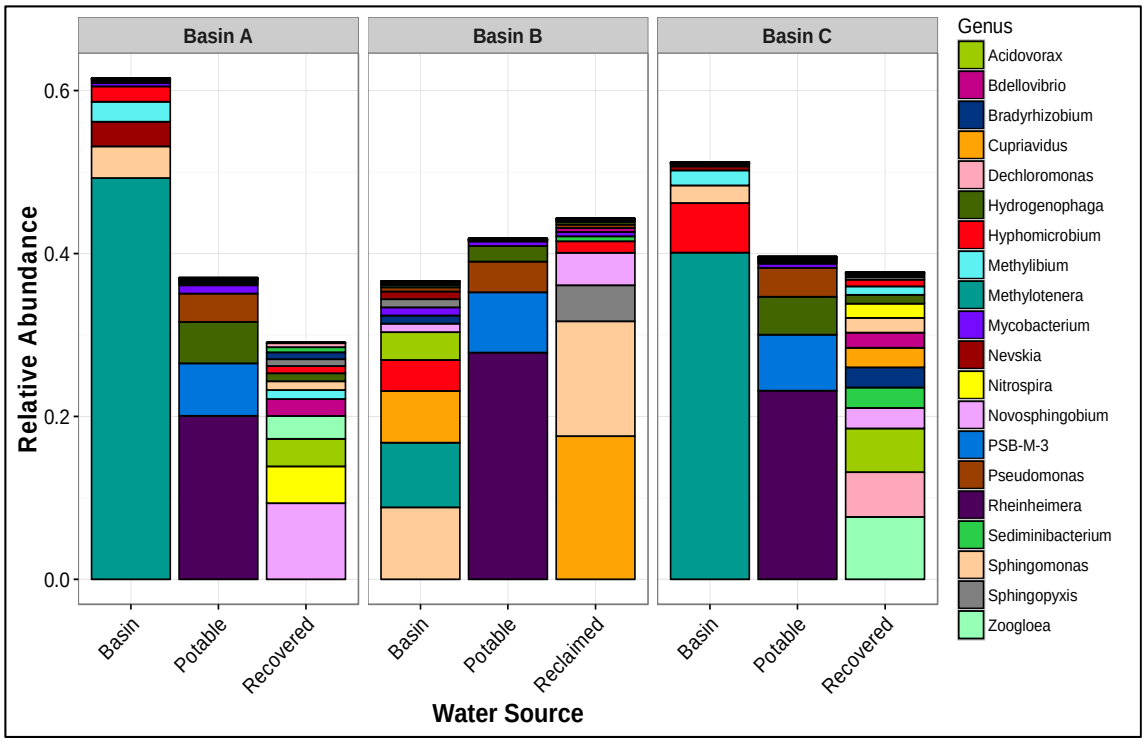

Figure 6: Relative abundance of top twenty OTUs identified to the genus level in each water source. Samples are grouped by water source. Basin samples are shown in panels with their corresponding makeup water sources. Note the similarities of Basins A and C compared to Basin B.

Basins A and C, with the same makeup water sources, are dominated by *Methylotenera* and also contain large relative abundances of *Hyphomicrobium*, *Methylibium*, and *Sphingomonas*. *Methylotenera, Hyphomicrobium,* and *Methylibium* are all methylotrophs that make up a significant fraction of the microbial communities in Basins A and C, but are not present in significant quantities in the potable or recovered water sources. For example, *Methylotenera* made up 50% and 40%, of the average relative abundance of Basins A and C, respectively. Conversely, while all makeup water samples contained *Methylotenera,* it was never at a relative abundance greater than 0.2% in the makeup waters. Two obvious explanations for the discrepancy between basin water

and makeup water samples are: (1) basin water is scrubbing the bacteria from the air as it is recirculated (Figure 1) or (2) the genera are being enriched for within the basins.

It is unknown what contribution the atmosphere has on the microbiome that develops within the basin water samples. However, at least in the case of the three methylotrophic genera listed above, it is likely that they are being enriched within the basins since *Methylotenera* and *Hyphomicrobium* can both be found in engineered water systems (Gliesche, Fesefeldt, & Hirsch, 2005; Inkinen et al., 2016) and all three were present in all makeup water samples.

Basin B contains large relative abundances of the same genera that dominate Basins A and C, *Methylotenera, Hyphomicrobium*, and *Sphingomonas* but contains a much smaller relative abundance of *Methylotenera*, a higher fraction of *Sphingomonas* and contains several other genera with significant relative abundances including *Cupriavidus* and *Acidovorax*. *Sphingamonas* is an animal pathogen that can readily degrade copper pipes and has the capacity to degrade refractory pollutants. It has been isolated from hospital water supplies, stocked distilled water, and patients with different types of infections (White, Sutton, & Ringelberg, 1996). *Cupriavidus* has been found in potable water systems and can degrade haloacetic acid (HAA) (Berthiaume et al., 2014). HAAs are a common undesirable byproduct of chlorine disinfection, which are likely elevated in the reclaimed water relative the other makeup water sources. Several *Acidovorax* strains reduce nitrate and oxidize iron (Chakraborty & Picardal, 2013; Chakraborty, Roden, Schieber, & Picardal, 2011), and the nitrate and iron levels are higher in Basin B compares to Basins A and C (Appendix). The different genera present and the increased number of unique genera in Basin B compared to Basins A and C indicates that Basin B had a different and potentially more diverse microbiome than Basins A and C.

The water chemistry parameters including elevated nitrate, iron, and HAAs could be selecting for the different microbiomes between basins. Thus, the AOC levels in the basins were compared since AOC is considered an important biological growth predictor. Basins A, B, and C had 0.47, 0.46, and 0.66 mg/L of AOC on Sampling Day 1 and 0.57, 0.68, and 0.43 mg/L of AOC on Sampling Day 3, respectively (Table 8). Therefore, it does not appear that the AOC concentrations in the basins helped drive the community differences between the basins. However, the higher levels of most water chemistry parameters in Basin B, including phosphorous, (Appendix) likely contributed to the microbial community differences, but additional studies would be required to determine what specific parameters were responsible for the differences observed.

A Shannon diversity index plot (Figure 7) was created to investigate the diversity observed in each of the cooling tower basins as well as in the makeup water sources. Figure 7 corroborates the trends evident in Figure 6. Specifically, the microbial community in the recovered water was the most diverse makeup water source and Basin B was more diverse than Basins A and C. The recovered water was expected to be the most diverse makeup water source since it is the only source that was not disinfected. According to Figure 6, Basin B was more diverse than its two makeup water sources (potable and reclaimed), while Basins A and C were less diverse than their two makeup water sources (potable and recovered). The water chemistry differences of the three basins could account for this difference as described above, including the elevated levels of most water chemistry parameters, including nutrients, in Basins B compared to Basins A and C.

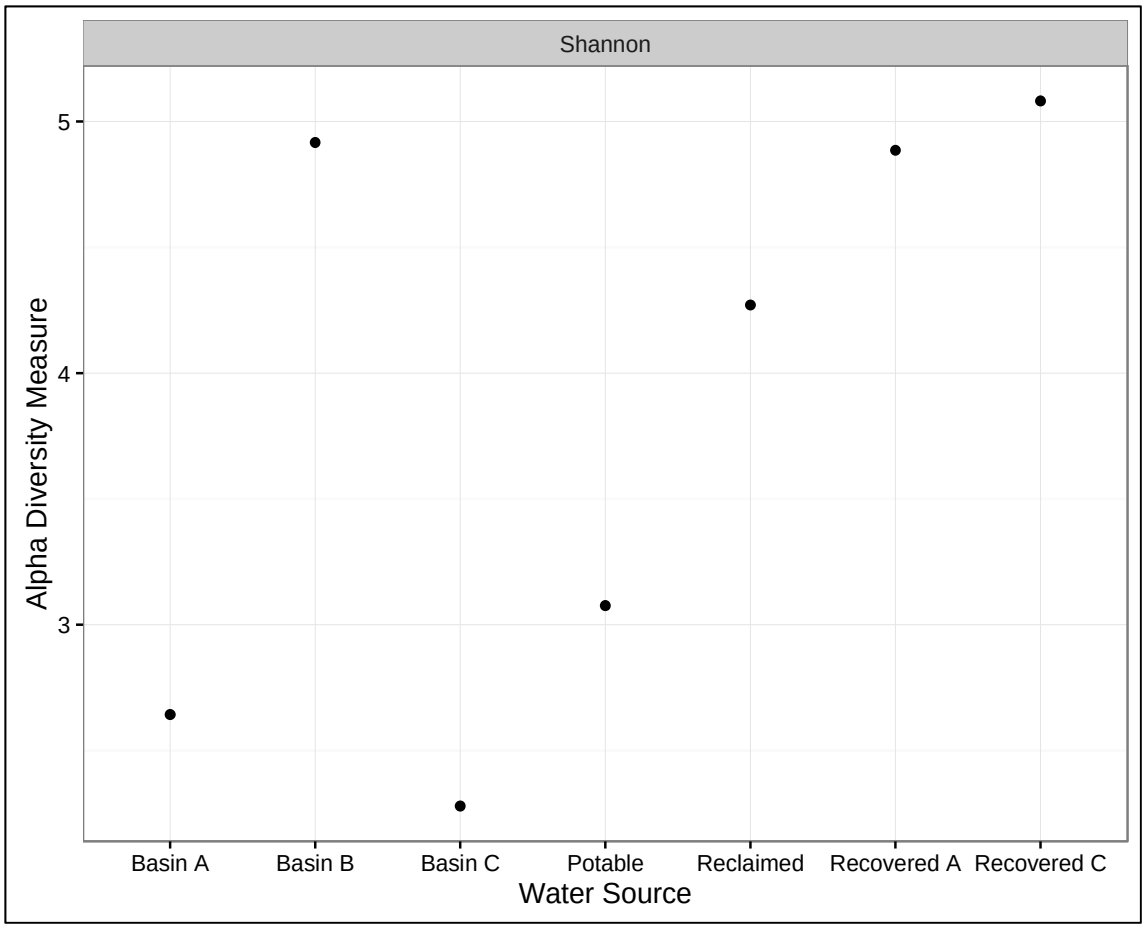

Figure 7: Shannon alpha diversity index of water samples. Individual samples are grouped by water source. The recovered water sources are the most diverse, which was expected since they are the only water source not disinfected. Basin B is the most diverse basin water. The higher levels of nutrients in Basin B relative to Basins A and C could explain the higher level diversity.

Figure 8 is a Bray Curtis dissimilarity PCoA plot and provides an additional method for highlighting microbial community diversity differences across the water samples. Figure 8 allows for microbial community comparisons with the full sequence dataset as compared to Figure 6 which only examined the top 20 genera. Figure 8 supports the previous results indicating that the microbial communities present in Basins A and C are more similar to each other than that which develops in Basin B. Similarly,

the community in Basin B is distinct from both Basins A and C. Also, Basin B and the reclaimed water samples are clustered near each other whereas Basins A and C's samples are not located near the potable or the recovered samples, corroborating that the reclaimed water microbial community was the most conserved microbial community of the makeup water sources.

The recovered water supply to the cooling towers have several potential sources (ie, HVAC condensate, pool water blowdown, etc.), but Figure 8 indicates that all recovered water samples were very similar with respect to microbial community composition. This suggests that the recovered water was from the same unknown water source on each sampling day for both Basins A and C.

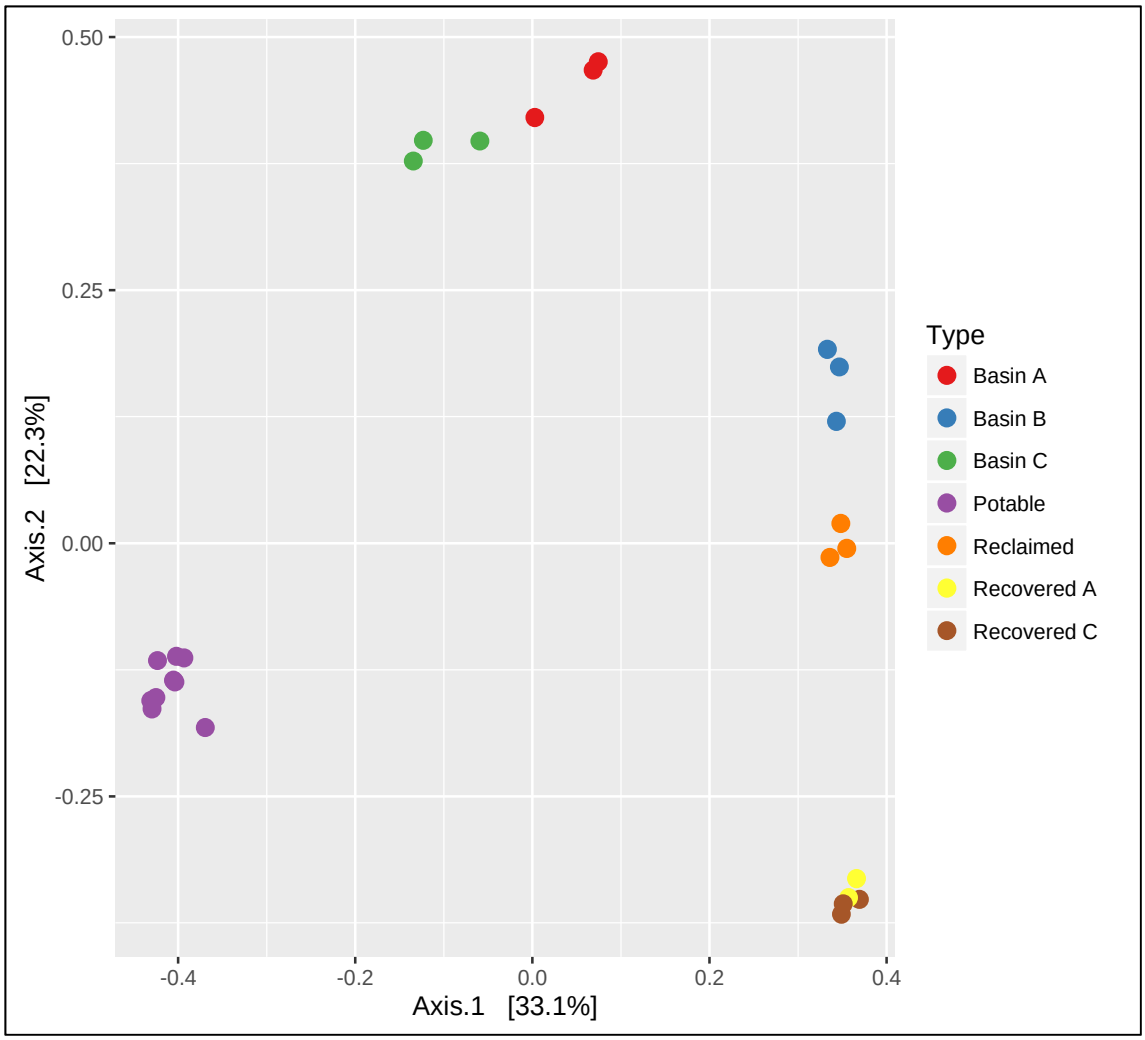

Figure 8: Bray Curtis dissimilarity PCoA analysis of the bacterial community detected in each cooling tower basin and makeup water source. All samples were rarified to the minimum read depth of all water samples (16414). Each point represents a single sample. Note that Basin B samples are clustered closer to reclaimed water samples compared to Basins A and C. All of the recovered water samples at both Basins A and C appear to be from the same source.

4.1.4 qPCR Results

The plot of total bacteria by water source (Figure 9) shows that basin, recovered, and reclaimed water samples have about 2-3 orders of magnitude higher levels of total bacteria compared to the potable water samples. The bars indicate the mean and the

whiskers on Figure 9 indicate the minimum and maximum value for each water source. A guideline for maximum biological growth in cooling towers is 10^4 CFU/mL as measured by the standard heterotrophic plate count procedure (Ludensky, 2005). Assuming that HPCs capture as low as 1% of the present bacteria (Lewis, 2013), Basins A, B, and C contain approximately 2.7, 3.4, and 2.7 logGC/mL, respectively, which is within range of this guideline.

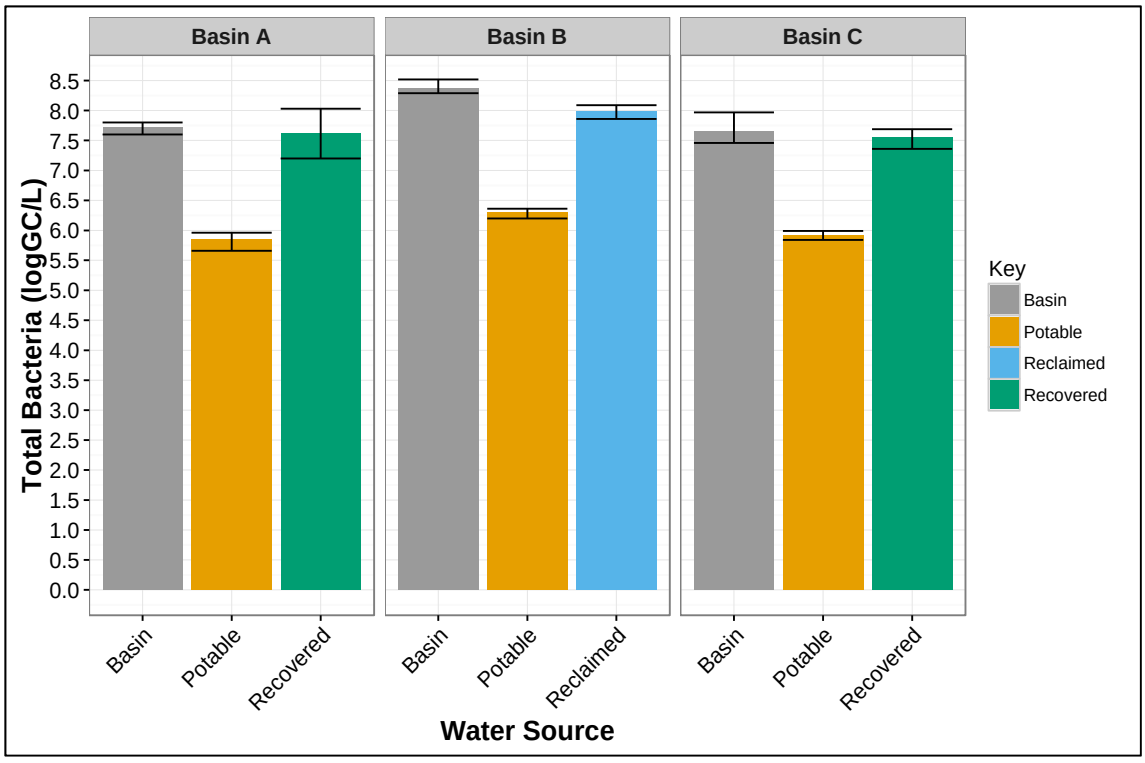

Figure 9: Total bacteria levels by water source. Total bacteria levels were measured using qPCR. The bars show the average total bacteria for each source and the bars indicate the min and max value for each water source. Note that Basin B has the highest level of total bacteria, but all basins are within one order of magnitude. Basin samples are shown in panels with their corresponding makeup water sources. (n=3 for each water source, except Basin A Recovered where n=2). All concentration units are in gene copies (GC) per liter.

All potable water samples contained lower levels of bacteria compared to the other makeup water sources (Figure 9). In Figure 10, the total bacteria levels in the basins were plotted as a function of the corresponding percent of potable makeup water from Table 9 to investigate if lower levels of bacteria in a makeup water source resulted in lower levels of bacteria in the basin. As evident in Figure 10, total bacteria concentrations were negatively correlated to the fraction of potable water contributing to each basin (R^2 = 0.83). However, it is unclear if the correlation is due to the bacterial input or the higher nutrient levels.

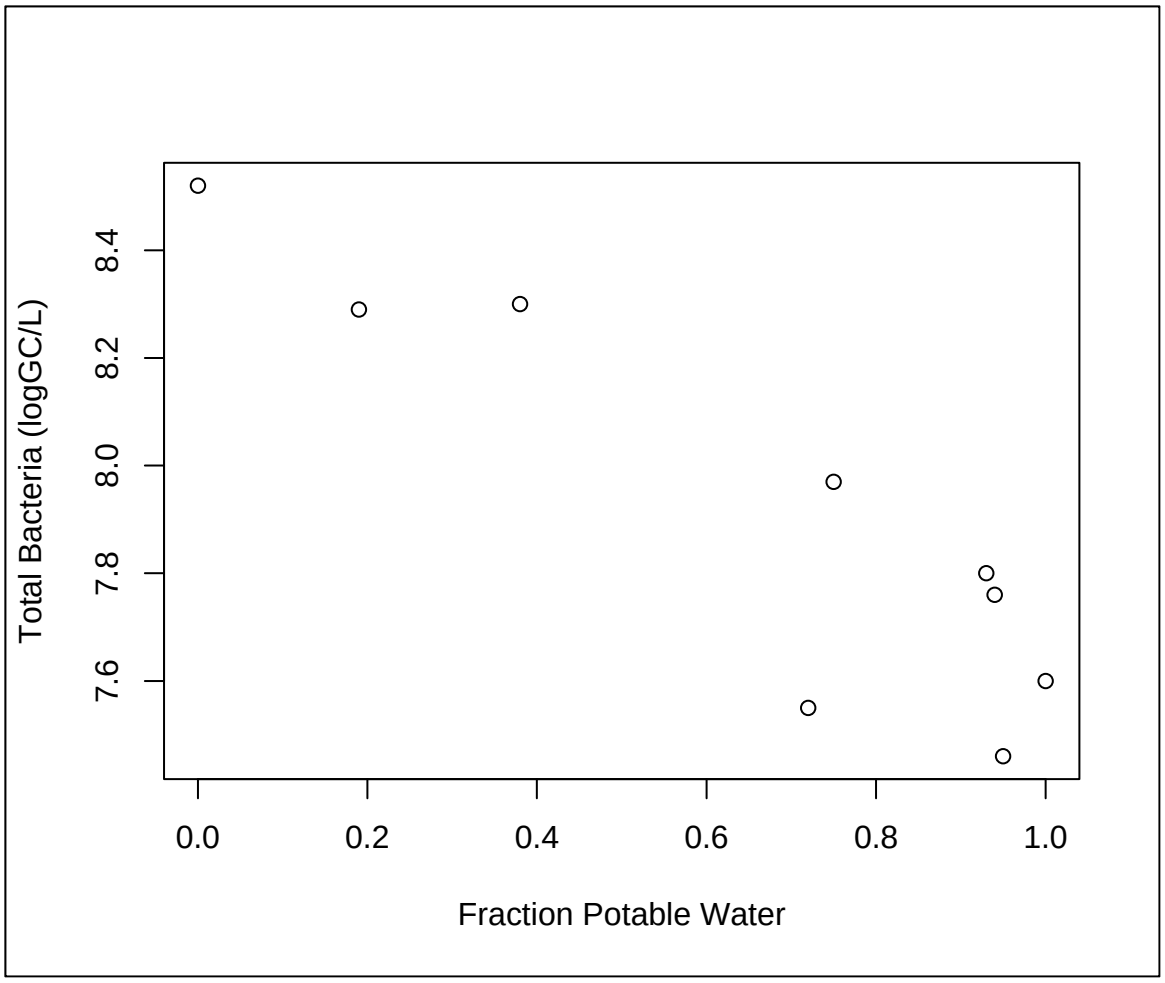

Figure 10: Total bacteria versus fraction potable water for basin water samples. Each point represents one basin sample. Note the strong negative correlation ($R^2 = 0.83$) between the fraction of potable water in the basin and the total bacteria.

The *Legionella* spp. and *L. pneumophila* qPCR results for each water sample are summarized in Table 10. *L. pneumophila* sg. 1 was not detected at levels above the LOD in any sample. Basins A, B, and C contained 5.26 to 6.21, 6.18 to 6.4, and <LOD to 4.81 log GC/L of *Legionella* spp., respectively (Figure 11). The bars indicate the mean and the whiskers on Figure 11 indicate the minimum and maximum value for each water source.

Recovered water had the highest average *Legionella* spp. concentrations of all makeup water sources. All *Legionella* culture analyses were negative.

Table 10: *Legionella* spp. and *L. pneumophila* qPCR results

Cooling Tower	**Water Source**	**Sampling Day**	***Legionella* spp (logGU/L)**	***L. pneumophila* (logGU/L)[1]**
A	Basin	1	5.26	<LOD
A	Basin	2	5.34	<LOD
A	Basin	3	6.21	<LOD
A	Potable	1	<LOD	<LOD
A	Potable	2	<LOD	<LOD
A	Potable	3	<LOD	<LOD
A	Recovered	2	5.53	+
A	Recovered	3	6.14	+
B	Basin	1	6.4	+
B	Basin	2	6.26	+
B	Basin	3	6.18	+
B	Potable	1	<LOD	<LOD
B	Potable	2	<LOD	<LOD
B	Potable	3	+	<LOD
B	Reclaimed	1	5.69	<LOD
B	Reclaimed	2	4.6	<LOD
B	Reclaimed	3	4.9	<LOD
C	Basin	1	4.81	<LOD
C	Basin	2	+	<LOD
C	Basin	3	4.21	<LOD
C	Potable	1	<LOD	<LOD
C	Potable	2	<LOD	<LOD
C	Potable	3	+	<LOD
C	Recovered	1	6.04	+
C	Recovered	2	5.62	<LOD
C	Recovered	3	5.75	+

(1) + Indicates sample was below the LOQ and above the LOD

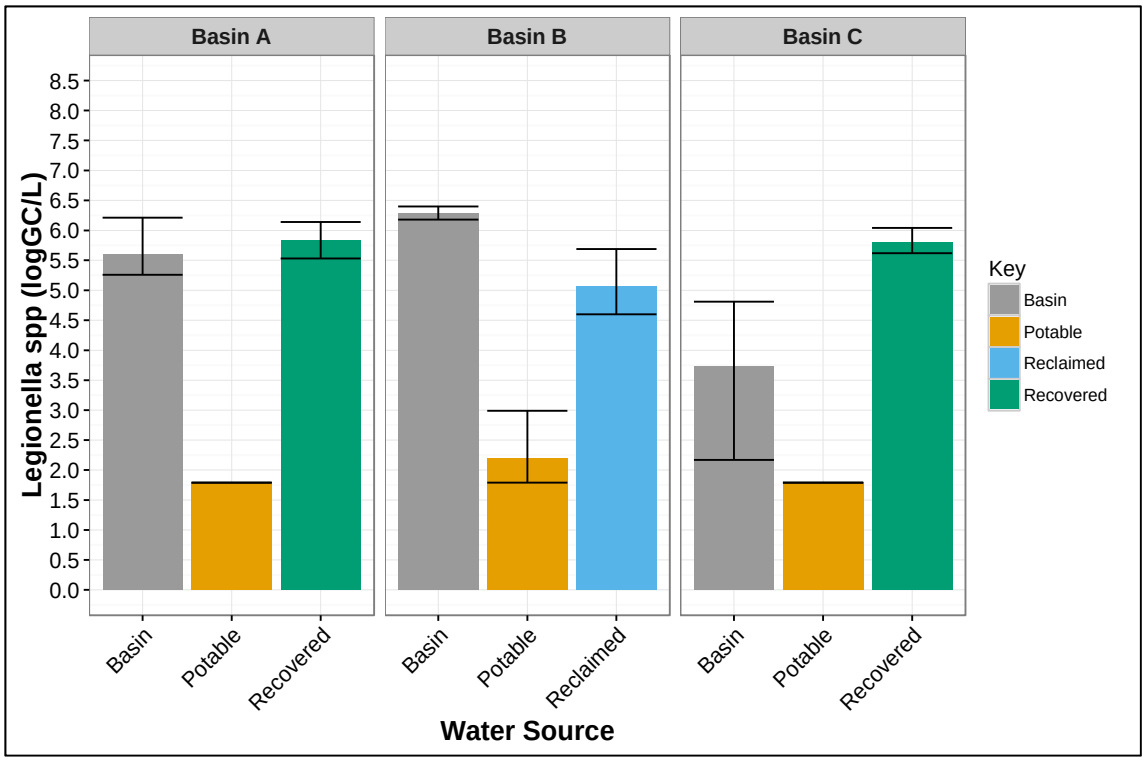

Figure 11: *Legionella* spp. levels measured in each water source and basin. Legionella spp. levels were measured using qPCR. The bars show the average Legionella spp. for each source and the bars indicate the min and max value for each water source. Samples below the LOQ are represented has half the LOQ. Basin samples are shown in panels with their corresponding makeup water sources. (n=3 for each water source, except Basin A Recovered where n=2).

No samples contained *L. pneumophila* above the LOQ. Table 10 shows that only recovered water and Basin B samples contained *L. pneumophila* above the LOD. The LOQ for the recovered water and Basin B samples ranged from 3.26-3.70 logGC/L and 4.07-4.39 logGC/L (Table 6). Lee et al. 2011 proposed alert and action *L. pneumophila* levels of 3.70 and 4.70 logGC/L as measured by qPCR. Therefore, it is likely the recovered water samples are below the proposed alert level, but the Basin B samples could have *L. pneumophila* concentrations above the proposed alert level.

Current guidelines for *Legionella* testing are based on culturing tests, but culturing tests are difficult to compare to qPCR results. Lee et al. 2011 correlated the amount of *Legionella* DNA gene units per liter (GU/L) as measured by qPCR with the concentration of *Legionella* (CFU/L) as measured by traditional culturing methods in matched samples. They found weak correlations for *Legionella* spp, but strong correlations for *L. pneumophila*. Reasons cited for the weak correlations included *Legionella* spp. plating isolation methods were originally designed for *L. pneumophila* and that some non-pneumophila *Legionella* species grow either poorly or not at all on the *Legionella* growth medium BCYE.

Weak correlations between qPCR and culturing methods make it difficult to compare the *Legionella* spp. levels to established culturing limits. For example, alert and action levels for *Legionella* spp. of >5 log GC/L and >6 log GC/L, respectively, based on qPCR and culturing correlations have been proposed (J. V. Lee et al., 2011). Given these alert and action levels, all of Basin A and B samples exceeded either the alert or action levels for *Legionella* spp. However, it is possible that our study's samples contain a higher fraction of unculturable *Legionella* relative to the Lee et al. 2011 study, and therefore would correspond with higher alert and action levels of *Legionella* spp if correlated to culture results.

Wéry et al. 2008 measured *Legionella* spp. and *L. pneumophila* in a single cooling tower for 10 months. The *Legionella* spp. concentration ranged from approximately 6.5 logGU/L to below the detection threshold of 3.3 logGU/L, which aligns with the *Legionella* spp. basin concentrations of this study. The *L. pneumophila* concentrations were below the detection threshold (2.8 logGC/L) for 8 out of 10 months. For the other two months *L. pneumophila* proliferated at concentrations of approximately 5 logGC/L. The study also tracked the *Legionella* spp. population diversity, and the beginning of the

L. pneumophila proliferation period was concurrent with a major decline in *Legionella* spp. diversity. This finding could support the idea that the *Legionella* spp. communities of the cooling towers in this study are likely diverse given the low levels of *L. pneumophila*.

A similar *Legionella* pattern was found in the biofilm swabs (Table 11). All but one biofilm sample contained *Legionella* spp., but only three samples were positive for *L. pneumophila*. No biofilm samples tested positive for *L. pneumophila* sg 1. The *Legionella* communities in the biofilms are likely also diverse for the same reasons the *Legionella* communities in the water samples are potentially diverse.

Table 11: Biofilm *Legionella* qPCR results

Basin	**Sampling Date**	***Legionella* spp.**	***L. pneumophila***
A	1	+	-
A	1	-	-
B	1	+	-
B	1	+	-
C	1	+	+
C	1	+	-
A	2	+	-
A	2	+	-
B	2	+	+
B	2	+	+
C	2	+	-
C	2	+	-
A	3	+	-
A	3	+	-
B	3	+	-
B	3	+	-
C	3	+	-
C	3	+	-

4.1.5 Water Chemistry and qPCR Correlations

Previous studies have found correlations between *Legionella* concentrations and specific water quality parameters. Manganese, heterotrophic plate counts, and hardness have been shown to have a positive association (Bargellini et al., 2011; Borella et al., 2004) with *Legionella* concentrations. Copper has been negatively correlated with *Legionella* (Bargellini et al., 2011), and chlorine has been shown to be both positively and negatively correlated with Legionella (Bargellini et al., 2011; Flannery et al., 2006; Zacheus & Martikainen, 1994). The total bacteria and *Legionella* spp. levels in the current study were correlated with the water chemistry and operational parameters using Spearman rank correlation tests to evaluate if similar correlations occurred in the basin and makeup water samples. Makeup water and basin water samples were tested separately, and the results are shown in Tables 12 and 13. pH, nitrate, calcium, iron, and manganese were significantly ($p<0.05$) correlated with the total bacteria levels in the makeup water samples. Chloride, fluoride, iron, magnesium, manganese, residence time, percent potable water, and percent reclaimed water were found to be significantly ($p<0.05$) correlated to the total bacteria in the basin water samples.

Table 12: Spearman correlations between water chemistry parameters and total bacteria levels

Parameter	Makeup Water Samples		Basin Water Samples	
	rho	p	rho	p
Total P	0.086	0.743	0.500	0.178
pH	**-0.816**	**6.41E-05**	-0.650	0.067
Alkalinity	-0.040	0.879	-0.583	0.108
Chloride	0.128	0.626	**0.817**	**1.08E-02**
Fluroide	-0.010	0.968	**0.778**	**1.35E-02**
Nitrate	**0.888**	**1.98E-06**	0.550	0.130
Sulfate	0.465	0.060	0.483	0.194
Potassium	0.366	0.148	0.667	0.059
Calcium	**0.670**	**3.30E-03**	0.533	0.148
Copper	0.445	0.074	0.444	0.232
Iron	**0.587**	**1.32E-02**	**0.667**	**4.98E-02**
Magnesium	0.266	0.302	**0.717**	**3.69E-02**
Manganese	**0.607**	**9.80E-03**	**0.762**	**1.69E-02**
Total Bacteria	N/A	N/A	N/A	N/A
Residence Time	N/A	N/A	**0.791**	**1.12E-02**
Percent Potable	N/A	N/A	**-0.783**	**1.72E-02**
Percent Recovered	N/A	N/A	-0.479	0.192
Percent Reclaimed	N/A	N/A	**0.822**	**6.57E-03**

Table 13: Spearman correlations between water chemistry parameters and *Legionella* spp. levels

Parameter	Makeup Water Samples		Basin Water Samples	
	rho	**p**	**rho**	**p**
Total P	-0.181	0.486	0.583	0.108
pH	**-0.743**	**6.33E-04**	**-0.883**	**3.10E-03**
Alkalinity	0.304	0.236	**-0.733**	**3.11E-02**
Chloride	0.027	0.949	0.002	0.900
Fluroide	-0.300	0.243	0.494	0.177
Nitrate	**0.736**	**7.57E-04**	0.400	0.291
Sulfate	0.380	0.132	0.550	0.133
Potassium	0.176	0.499	0.500	0.178
Calcium	**0.761**	**3.91E-04**	0.350	0.359
Copper	**0.757**	**4.36E-04**	0.469	0.203
Iron	0.251	0.331	**0.861**	**2.90E-03**
Magnesium	-0.118	0.653	0.583	0.108
Manganese	0.280	0.276	**0.753**	**1.93E-02**
Total Bacteria	**0.834**	**3.13E-05**	**0.717**	**3.69E-02**
Residence Time	N/A	N/A	**0.896**	**1.08E-03**
Percent Potable	N/A	N/A	-0.550	0.133
Percent Recovered	N/A	N/A	-0.531	0.141
Percent Reclaimed	N/A	N/A	0.653	0.056

Nitrate, pH, calcium, copper, and total bacteria were significantly ($p<0.05$) correlated with the *Legionella* spp. levels in the makeup water samples. Alkalinity, pH, iron, manganese, total bacteria, and residence time were significantly ($p<0.05$) correlated with the *Legionella* spp. levels in the basin water samples. These results are consistent with the previous studies above that manganese and total bacteria were correlated with *Legionella* spp. levels. However, co-linearity exists between water chemistry parameters

in both the makeup and basin water samples, and the low number of samples limits additional conclusions.

4.1.6 Estimated *Pseudomonas* and *Mycobacteria* Levels

The total bacteria qPCR results were coupled with the relative abundance data for the *Pseudomonas* and *Mycobacteria* genera to provide an estimated level of each genus in each sample (Figures 12 and 13). Samples were averaged by water source. The bars indicate the mean and the whiskers on Figures 12 and 13 indicate the minimum and maximum value for each water source. A previous study found $1.6x10^3$ - $3.9x10^3$ CFU/mL of *Pseudomonas* in cooling towers from fluorescent counts (Blasco et al., 2008), which is the approximate range measured in Basin B. A minimum dose of 10^7 cells/mL was found to cause illness in mice (George et al., 1989), which is higher than any basin level detected in this study. A study of 53 cooling towers found a *Mycobacteria* concentration range of $4.6x10^3$ to $1.79x10^6$ cells/L using qPCR (Adrados et al., 2011a). Basins A and C are within that same range with Basin B potentially containing slightly higher concentrations.

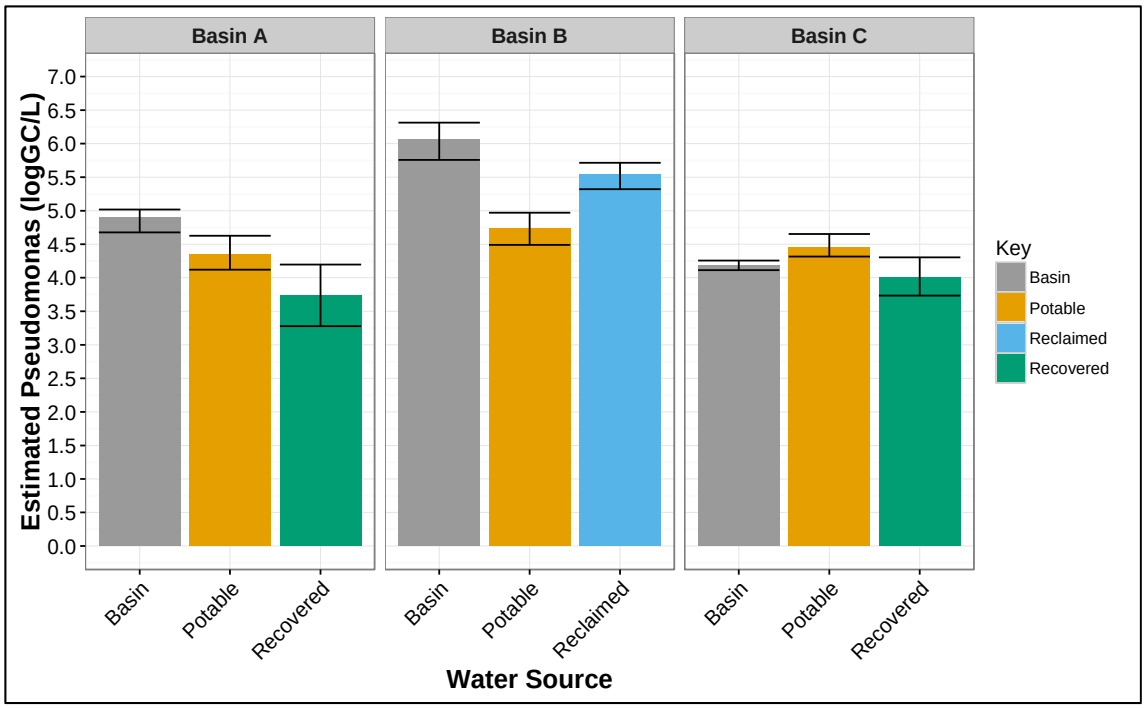

Figure 12: Pseudomonas levels in water samples. Pseudomonas levels were calculated by multiplying the total bacteria concentration measured using qPCR by the relative abundance of Pseudomonas detected in each water sample. Basin samples are shown in panels with their corresponding makeup water sources. The bars show the average total bacteria for each source and the bars indicate the min and max value for each water source (n=3 for each water source, except Basin A Recovered where n=2).

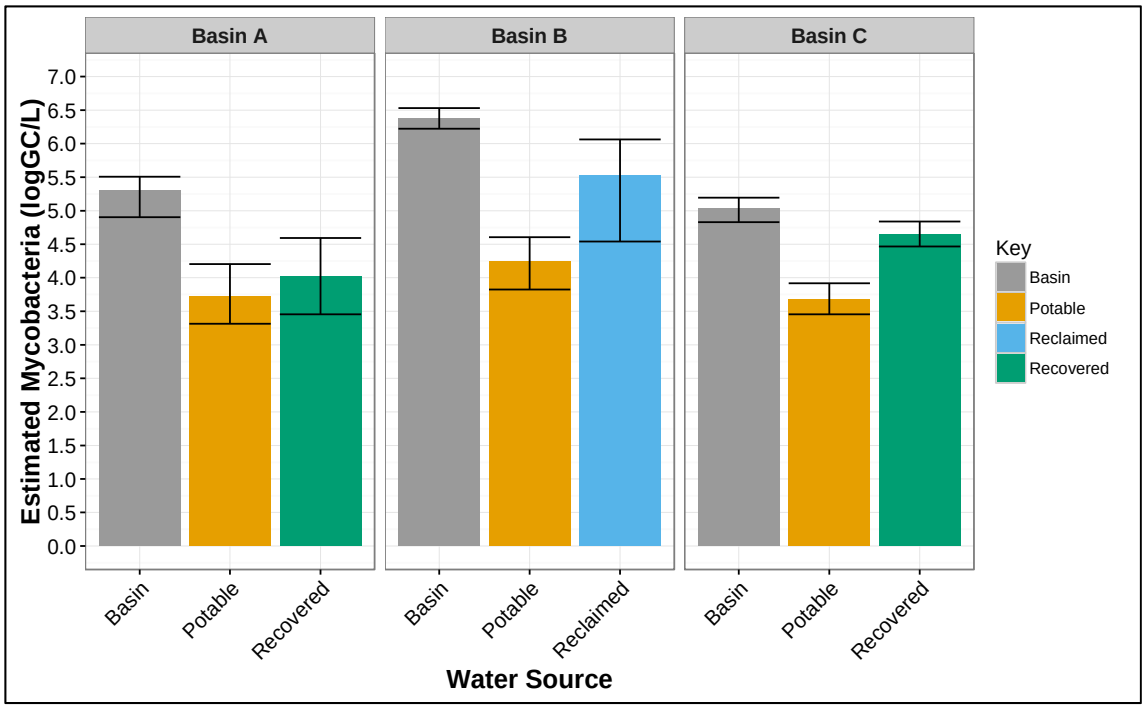

Figure 13: Mycobacteria levels in water samples. Mycobacteria levels were calculated by multiplying the total bacteria concentration measured using qPCR by the relative abundance of Mycobacteria for each water sample. Basin samples are shown in panels with their corresponding makeup water sources. The bars show the average total bacteria for each source and the bars indicate the min and max value for each water source. (n=3 for each water source, except Basin A Recovered where n=2).

Chapter 5: HVAC Condensate Collection Study Results and Discussion

5.1 HVAC Condensate Collection Study

Three HVAC units were studied to investigate the accuracy of using humidity ratios, with in-home temperature and relative humidity data, to predict the volume of HVAC condensate produced. The water quality was expected to be high given that HVAC condensate is a pure water source when it forms on the coil. However, due to the low pH, alkalinity, and mineral content, the condensate was assumed to be corrosive and could contain heavy metals that it contacted on the cooling coil, drip pan, and/or the drain pipe. Both the volumes produced and the water chemistry of the condensate will drive which reuse options are available for the condensate.

5.2 Condensate Collection Volumes

Table 14 below compares the predicted volume of HVAC condensate produced for each HVAC unit to the measured volumes. The average HVAC condensate that would be produced in a 24-hour period from units A, B, and C was 8.6, 39.3, and 3.9 liters, respectively. The mean difference between the predicted and collected HVAC condensate volumes was 18%. Most predicted values overestimated the volume of condensate that would be produced. The Onset data loggers have an error range of 2.5% for relative humidity, which contributes some to the uncertainty of the predicted volume. Formed condensate also could attach to the cooling coil, collection plate, or drainpipe and not enter the collection vessel. Any leaks in the duct bringing supply air to the HVAC unit would also lead to less air passing over the cooling coil than predicted. The remaining unaccounted for condensate could be in the air returning to the residence if equilibrium conditions were not achieved (i.e., air supersaturated with water vapor).

However, at Residence B, six measurements underestimated the condensate produced. This could be due to uneven air flow where the data loggers were installed.

Table 14: Predicted and observed condensate production

Unit	Measurement Period (hrs)	System Operating Time[1] (hrs)	Predicted Volume (L)	Collected Volume (L)	Percent Difference[2]
A	19.2	4.2	16.0	13.2	19%
A	22.7	0.9	2.9	2.2	26%
A	26.9	2.8	12.3	9.0	31%
A	24.0	1.5	7.1	5.5	26%
A	27.6	3.7	15.7	12.4	23%
B	11.3	5.8	13.0	12.1	7%
B	13.3	6.8	10.9	14.0	-25%
B	9.2	7.8	16.6	15.2	9%
B	8.8	9.8	16.3	18.0	-10%
B	7.8	5.2	10.8	12.0	-10%
B	7.2	7.1	14.9	14.6	2%
B	5.1	6.7	11.3	13.2	-16%
B	8.5	6.7	13.0	16.5	-24%
B	11	7.9	17.9	17.0	5%
B	2.8	1.0	1.9	2.6	-32%
C	34.4	5.4	6.2	5.3	16%
C	50.3	13.6	12.0	10.0	18%
C	50.1	13.6	10.9	8.5	24%
C	34.7	7.8	6.1	5.0	20%
C	32.3	6.5	5.3	4.5	16%

(1) Total time HVAC unit was operating during measurement period

(2) Percent difference = (Predicted-Collected)/[Average(Predicted, Collected)]

5.3 HVAC CONDENSATE CHEMISTRY

The pH, alkalinity, anion, and organic acid concentrations for the HVAC condensate samples are shown in Table 15. Given the low pH of the water, the alkalinity

was higher than expected and suggests the presence of organic acids with pKa's near the CO_2 equivalence point for which the alkalinity titrations were performed. Therefore, the alkalinity measurements are probably unreliable because of the low alkalinity and because the alkalinities were dominated by weak organic acids.

Table 15: Alkalinity, pH, and anion concentrations of condensate samples

Analyte	Unit A	Unit B	Unit C
Acetate	72.9	54.1	39.7
Alkalinity (meq/L)	0.5	0.2	0.5
Bromide	<0.0026	<0.0026	<0.0026
Chloride	0.265	0.1803	<0.0018
Fluoride	<0.0010	<0.0010	<0.0010
Formate	26.9	20.1	10.5
Nitrate	0.0757	0.0843	0.26
Nitrite	0.0283	0.0739	7.11
pH (pH units)	4.8	4.5	5.7
Sulfate	0.1535	<0.0120	<0.0120

(1) Units = mg/L (unless otherwise noted)
(2) All samples filtered (0.45 μm filter) before analysis

The presence and concentrations of the acetate and formate were unexpected and further investigated by DOC analysis to substantiate. DOC was measured in the condensate from Units B and C and compared to the calculated organic carbon concentrations assuming the only organic carbon contributers were acetate and formate to both corroborate the acetate and formate concentrations in Table 15 and to further investigate if additional organics were present (Table 16). The measured DOC levels were larger than the DOC concentration calculated from assuming formate and acetate were the only contributors to DOC. Thus, there were more organics present in both

samples than just acetate and formate, and the acetate and formate levels were not inconsistent with the measured DOC results. Unit C's measured DOC level was much higher (100.60 mg/L) than the calculated value (18.62 mg/L). This indicates again that there were additional organics present the sample. However, the condensate samples used for the DOC measurements were collected at a different time than the condensate samples used to quantify the acetate and formate via IC analysis, which could be contributing to the variability between the measured and calculated DOC levels.

Table 16: Calculated and measured dissolved organic carbon values

Parameter	Unit A	Unit B	Unit C
Acetate DOC Contribution (mg/L)	29.16	21.64	15.88
Formate DOC Contribution (mg/L DOC)	7.02	5.24	2.74
Total Calculated DOC (mg/L DOC)	36.18	26.88	18.62
Measured DOC (mg/L DOC)	NA	31.83	100.60
Difference[1]	NA	-17%	-138%

(1) Difference = (Calculated - Measured) / [Average(Calculated, Average)]

Further investigating the acetate and formate concentrations, Table 17 shows the required corresponding vapor concentrations of acetic and formic acid in the return air that must have been present if the total acetate and formate concentrations from Table 15 originated from the indoor air assuming equilibrium was achieved. Units A and B have the highest required volatile acetate concentrations (0.12 ppm). This level is below the low range of the odor threshold for acetic acid (U.S. Department of Health and Human Services & U.S. Department of Labor, 1978a) and the OSHA Permissible Exposure Limit (PEL) of 10 ppm (Occupational Safety and Health Administration, 2016). Unit B had the

highest level of required volatile formic acid (0.0073 ppm), which is well below the odor threshold of 21 ppm (U.S. Department of Health and Human Services & U.S. Department of Labor, 1978b) and the OSHA PEL (Occupational Safety and Health Administration, 2016). Since the vapor concentrations of both acetate and formate were calculated to be below the corresponding odor thresholds, and none of the residences smelled of acetic or formic acid, the aqueous concentrations in Table 15 are not unreasonable. These results are consistent with those from Table 16.

Table 17: Required Vapor Concentrations of Acetic and Formic Acid

Parameter	Acetic Acid	Formic Acid
Equilibrium Constant[1] ($logK_a$)	-4.757	-3.744
Henry's Constant[2,3] (L_L/L_G)	8.17E-06	4.60E-06
Vapor Concentrations (ppm)		
Odor Threshold[4,5]	0.2-24	21
OSHA PEL[6]	10	5
Unit A	0.12	0.0053
Unit B	0.12	0.073
Unit C	0.014	0.0003

(1)Visual Minteq database (Gustafsson, 2014)
(2) Acetic acid's Henry's Constant source (Benjamin & Lawler, 2013)
(3) Formic acid's Henry's Constant source (Johnson, Betterton, & Craig, 1996)
(4) Acetic acid odor threshold source (U.S. Department of Health and Human Services & U.S. Department of Labor, 1978a).
(5) Formic acid odor theshold source (U.S. Department of Health and Human Services & U.S. Department of Labor, 1978b)
(6) OSHA Permissible Exposure Limit for General Industry (Occupational Safety and Health Administration, 2016)

The presence of acetate and formate was initially unexpected, but are volatile components of many cleaning compounds and are present in other consumer products. Furthermore, acetate and formate are known to cause “ant-nest” or formicary corrosion of copper cooling coils, which can lead to premature coil failure (Bastidas, Cayuela, & Bastidas Rull, 2006), indicating that acetate and formate are present in other residences as well.

The nitrite concentration from Unit C was not consistent with Units A or B. Unit C condensate was measured using UV-Vis at 210 nm to substantiate the nitrite concentrations. The nitrite concentration was measured to be 10.3 mg/L in Unit C

condensate. This is higher than the nitrite concentration measured using IC (Table 15), but the condensates used for measurement were collected on different days.

The cation analysis showed aluminum, boron, copper, iron, manganese, silica, tin, and zinc were above the method detection limit for all samples (Table 18). Most of the cations were measured with both ICP and ICP-MS analyses and the results were consistent. Unit C usually had the lowest concentration of any given cation indicating it was either less corrosive, the coil and/or metal collection system was more corrosion resistant, or the condensate was in contact with the coil and/or metal collection system for less time. Unit C condensate could have been less corrosive since it had the highest pH of all condensates (Table 15).

Table 18: HVAC Condensate ICP and ICP-MS analysis results

Cation	Unit A	Unit B	Unit C
Aluminum	7,817[a]	2,040[a]	1,851
Arsenic	<0.029	<0.029	<0.029
Boron	45	614	66
Barium	<0.087	<0.087	<0.087
Calcium	60	<50.241	<50.241
Cadmium	<0.008	<0.008	<0.008
Chromium	<0.057	<0.057	<0.057
Copper	1,552[a]	993[a]	97
Iron	67	74	10
Potassium	<4.711	<4.711	<4.711
Magnesium	<4.601	<4.601	<4.601
Manganese	10	1	3
Sodium	<49.656	<49.656	<49.656
Nickel	<.030	<.030	<.030
Phosphorus	7	<2.657	<2.657
Lead	2	1	<0.008
Silica	274	274	403
Tin	2	2	2
Strontium	<0.627	<0.627	<0.627
Zinc	596	205	49

(1) Units = μg/L
(2) All samples filtered (0.45 μm filter) before analysis
(a) denotes values measured with ICP analysis. All other measurements from ICP-MS analysis.

5.3.1 Evaluation of Water Quality of HVAC Condensate with respect to reuse options

Table 19 displays current U.S. primary drinking water standards, secondary drinking water standards, irrigation limit recommendations, and the HVAC condensate sample range for several water chemistry parameters. Unit A exceeded the primary drinking water standard for copper, and Unit B approached the limit. Unit C's cooling coil was made of 100% aluminum and the copper concentration in Unit C condensate was

an order of magnitude lower than the copper primary and secondary drinking water standard. It was also lower than the irrigation limit recommendation for copper. These results suggest if all cooling coils were manufactured with no copper, the resulting condensate would be lower than applicable limits. Unit C exceeded the primary drinking water standard for nitrite, which would be difficult to remove below the standard in a cost effective way for this application. The nitrite levels in Units A and B were nearly two orders of magnitude less than the nitrite primary drinking water standard.

In addition to copper, aluminum and pH also exceeded the secondary drinking water standard and/or the irrigation limit recommendations in at least one condensate sample. All samples exceeded the secondary drinking water standard for aluminum, and Unit A exceeded the irrigation limit recommendation. All three of the cooling coils were made of aluminum, which is in contact with the condensate as it forms. As long as coils are made from aluminum, some amount of aqueous aluminum in the condensate is expected. The condensate is virtually void of minerals when it forms and therefore has low pH values. The condensate pH will have to be increased for many reuse applications.

The levels of DOC are significant, especially for Unit C's condensate. DOC will lead to biological growth and is a precursor to disinfection byproducts. Therefore, condensate samples should not be used for potable water reuse unless first treated for DOC and analyzed for levels of disinfection byproducts following disinfection. For non-potable reuse, the level of organics could cause biological growth in storage tanks and conveyance piping.

Table 19: Condensate water chemistry compared to drinking water and irrigation limits

Analyte	HVAC Sample Range	Primary Drinking Water Standard[1]	Secondary Drinking Water Standard[2]	Irrigation Limit Recommendations[3]
Aluminum	1.85-7.82	--	0.05-0.2	5.0
Arsenic	<0.000029	0.010	--	0.10
Barium	<0.000087	2	--	--
Boron	0.05-0.61	--	--	0.75
Cadmium	<0.000008	0.005	--	0.01
Chloride	<0.0018-0.265	--	250	--
Chromium	<0.000057	0.1	--	0.1
Copper	0.10-1.55	1.3	1.0	0.2
Fluoride	<0.0010	4.0	2.0	1.0
Iron	0.01-0.074	--	0.3	5.0
Lead	<0.000008-0.001	0.015	--	5.0
Manganese	0.001-0.01	--	0.05	0.2
Nickel	<0.000030	--	--	0.2
Nitrate	0.076-0.26	10.0	--	--
Nitrite	0.0283-7.11	1.0	--	--
pH	4.5-5.7	--	6.5-8.5	--
Sulfate	<0.0120-0.1535	--	250	--
Zinc	0.05-0.60	--	5.0	2.0

Units = mg/L

HVAC Sample range exceeds one or more limit

(1) Primary Drinking Water Maximum Contaminant Levels (epa.gov)

(2) Secondary Drinking Water Standards (epa.gov)

(3) Recommended water quality criteria for irrigation. 2012 Guildelines for Water Reuse (epa.gov)

5.4 COMPARISON OF HVAC CONDENSATE TO HARVESTED RAINWATER

Rainwater collection is commonly promoted as a water reuse source to supplement surface and groundwater. Table 20 below shows a comparison of the water quality of the HVAC condensate samples and rainwater samples collected in various

studies. The HVAC sample range exceeds the rainwater samples for three parameters: aluminum, nitrite, and TOC. Units A and B were in the same range as rainwater for nitrite and Unit C's nitrite level is significantly higher. Moreover, all condensate samples had the same order of magnitude of aluminum as rainwater samples collected off metal roofs. For all other parameters, the rainwater samples contained higher concentrations than the HVAC condensate samples. The organic carbon in the condensate samples was also higher than rainwater, especially for Unit C. Higher levels of organic carbon lead to biological growth and are the precursors for disinfection byproducts.

Table 20: HVAC Condensate water quality compared to rainwater quality

Parameter	HVAC Sample Range	Pure Rainwater [1]	Harvested Rainwater [2]	Metal Roof[3]	Shingle Roof [4]	Metal Roof[5]	Shingle Roof[6]	Tank Collection[8]
Aluminum	1.85-7.82	0.05-0.24	0.100-0.400	NA	NA	2	3.3	0.060 (0.560)
Arsenic	<0.000029	0-0.001	0-0.006	NA	NA	0.001	0.004	0.00025 (0.0037)
Barium	<0.000087	NA	NA	NA	NA	NA	NA	0.012 (0.120)
Cadmium	<0.000008	ND	0-0.004	NA	NA	NA	NA	NA
Calcium	<0.050-0.06	0.17-3.82	3.24-15.4	NA	NA	NA	NA	2.4 (21.0)
Chloride	<0.018-0.27	1.1-10	5.0-18	NA	NA	NA	NA	3.9 (76.1)
Chromium	<0.000057	0-0.005	0-0.010	NA	NA	NA	NA	0.00053 (0.0043)
Copper	0.10-1.55	0.020-0.080	0.070-0.120	NA	NA	ND	0.59	0.021 (1.6)
Iron	0.01-0.07	NA	NA	NA	NA	1.8	2	0.068 (4.4)
Lead	<8e-6-0.002	0.010-0.040	0.010-0.040	0.003-0.0058	0.004-.0086	0.006	0.005	0.0054 (0.084)
Magnesium	<0.0046	0.04-0.62	0.5-2.7	NA	NA	NA	NA	0.5 (11.4)
Manganese	0.001-0.01	0.020-0.080	0.70-0.170	NA	NA	NA	NA	0.0087 (0.140)
Nickel	<0.000030	NA	NA	NA	NA	NA	NA	0.0013 (0.016)
Nitrate	0.08-0.26	0.6-4.2	2.9-9.8	0-4.1	0.0-4.7	NA	NA	1.6 (14.2)
Nitrite	0.03-7.11	NA	NA	0.01-0.05	0.01-0.06	NA	NA	0.1 (0.6)

Table 20 (continued)

Phosphate	<0.002657-0.01	ND	0-0.04	NA	NA	NA	NA	0.1-1.1
Potassium	<0.004711	0.16-6.5	1.3-5.9	NA	NA	NA	NA	0.9 (11.2)
Sodium	<0.050	0.24-4	2.2-6.1	NA	NA	NA	NA	2.8 (38.4)
Sulfate	<0.0120-0.15	1.0-6.2	2.0-7.2	NA	NA	NA	NA	1.6 (31.5)
TOC	31.83-101.6	NA	2-50	NA	NA	NA	NA	1.35 (16.4)
Zinc	0.05-0.60	0.040-0.090	0.120-0.280	0.018-0.362	0.001-0.085	0.89	0.15	0.770 (26.0)

"ND"=non-detect

"NA" = not reported

Unit=mg/L

HVAC Sample Range exceeds rainwater samples

(1) Collected in a sterilized beaker. (J. Y. Lee, Yang, Han, & Choi, 2010)

(2) Harvested from a PVC storage tank via galvanized catchment and aluminum gutters/downpipes. (J. Y. Lee et al., 2010)

(3) Collected from 55% aluminum-zinc alloy coated steel after first flush. (Mendez et al., 2011).

(4) Collected from asphalt fiberglass shingles after first flush. (Mendez et al., 2011).

(5) Collected from 55% aluminum-zinc alloy coated steel first flush. Maximum values. (Mendez et al., 2011).

(6) Collected from asphalt fiberglass shingles first flush. Maximum values. (Mendez et al., 2011)

(7) Collected in concrete or PVC tanks of different sizes. (Kus, Kandasamy, Vigneswaran, & Shon, 2010)

(8) Collected from 32 different tanks material unknown (365 total samples). Mean(max). (Huston, Chan, Chapman, Gardner, & Shaw, 2012)

The production of condensate was compared with rainwater harvesting potential volumes in Table 21. Table 21 estimates the maximum size residence each HVAC unit could serve if each unit was the only unit at the residence. The rainwater collection volume was calculated assuming that the hypothetical residences are single story with the same roof area as the estimated residence area assuming 0.62 gallons per square foot of collection surface per inch of rainfall can be collected at 85% efficiency (Texas Water Development Board, 2005). For an Austin, TX, summer under average and drought conditions, significantly more rainwater is available for harvesting compared to the condensate volumes any of the three HVAC systems would have produced. However, there are several disadvantages to rainwater systems. Rain in Austin, Texas, does not fall at a constant rate over the summer period. A large percentage of the rainfall occurs on just a few days. Therefore, the rainwater harvesting cistern would need to be sized large enough to capture and store a large fraction of the Estimated Summer Rain Volume (Table 21) at any given time, which is infeasible for many private residences. For example, assuming 25% of all the rain in an average summer over a 24-hour period and no reuse occurred during that time, each hypothetical residence would need 7.8, 9.3, and 2.2 m^3 storage tanks, respectively. There are private residences with rainwater harvesting tanks this large in Texas (Texas Water Development Board, 2005), but they are unlikely to be used in urban areas. Also, since rain occurs only a few days a month, the harvested rainwater must be stored until reused. Storage provides biological growth potential that must be managed.

Table 21: HVAC Condensate and potential rainwater collection comparison

Unit	System Flow Rate (m^3/hr)	Estimated unit tonnage[1]	Estimated residence area[2] (ft^2)	Estimated Average Summer Rain Volume[3] (L)	Estimated Drought Summer Rain Volume[4] (L)	Estimated Summer 2016 HVAC Condensate Collection[5] (L)
A	2529	3.72	2100	19392	6186	774
B	3182	4.68	2500	23086	7364	3537
C	510	0.75	600	5541	1767	351

(1) Estimated using 400 x tonnage = air flow rate (cfm)

(2) Estimated using supplier design charts

(3) Assumes 0.62 gallons per square foot of collection surface per inch of rainfall can be collected at 85% efficiency (Texas Water Development Board, 2005) rain falling on estimated residence area will be captured. Average rain levels for Austin, TX, for June, July, and August, from 1856-2013 is 3.06, 2.16, 2.21 inches, respectively (NOAA, 2013) were used for calculation.

(4) Same assumptions as "Estimated Average Summer Rain Collection Volume". Precipitation levels from May, June, and July 2009 in Austin, TX (NOAA, 2013)

(5) HVAC Condensate collection estimate used the average 24-hour condensate production rate for each unit multiplied by 60 days

5.5 HVAC CONDENSATE REUSE POTENTIAL

HVAC Condensate reuse options include cooling tower makeup water, landscape irrigation, and water feature supply (e.g., decorative ponds, fountains, etc.) (Guz, 2005; Painter, 2009). The low levels of salt in the condensate also indicates that potable water reuse could be an option with minimal treatment. For all reuse options, the main concerns would be the concentrations of copper and aluminum along with the organic content and low alkalinity and pH.

Alkalinity and pH levels can be increased with passive treatment (i.e., limestone bed contactors) or more active technology (i.e., hydrated lime and carbon dioxide addition). Passive treatment is more realistic for reuse systems to keep operating costs low. Significant copper and aluminum removal has also been shown using uncoated

limestone (Sdiri & Higashi, 2013; Somasani, 2012). Thus, it is possible that copper and aluminum could also be reduced with limestone bed contactors while the pH and alkalinity was being increased. Batch and pilot testing would be required to determine required residence times and confirm performance.

However, aluminum and copper precipitation could occur if the pH of the condensate was increased without also actively reducing the aluminum and copper levels. Figure 14 shows a solubility diagram of aluminum. The thermodynamic data used to create Figures 14 and 15 (below) came from the Minteq Visual database (Gustafsson, 2014). Amorphous aluminum solid was assumed present at all conditions shown for calculations. Values of (pH, log Al concentration) that fall above the black line indicate conditions where condensate will be supersaturated with respect to amorphous aluminum hydroxide and precipitation will occur at equilibrium. As seen in Figure 14, if the pH of Unit A or B condensate was raised to the low end of the range required by secondary drinking water standards (pH=6.5~7.5), aluminum hydroxide would precipitate. The condensate from Unit C would not form aluminum hydroxide precipitate at any pH as its aluminum concentration is below the minimum point of the total dissolved aluminum curve.

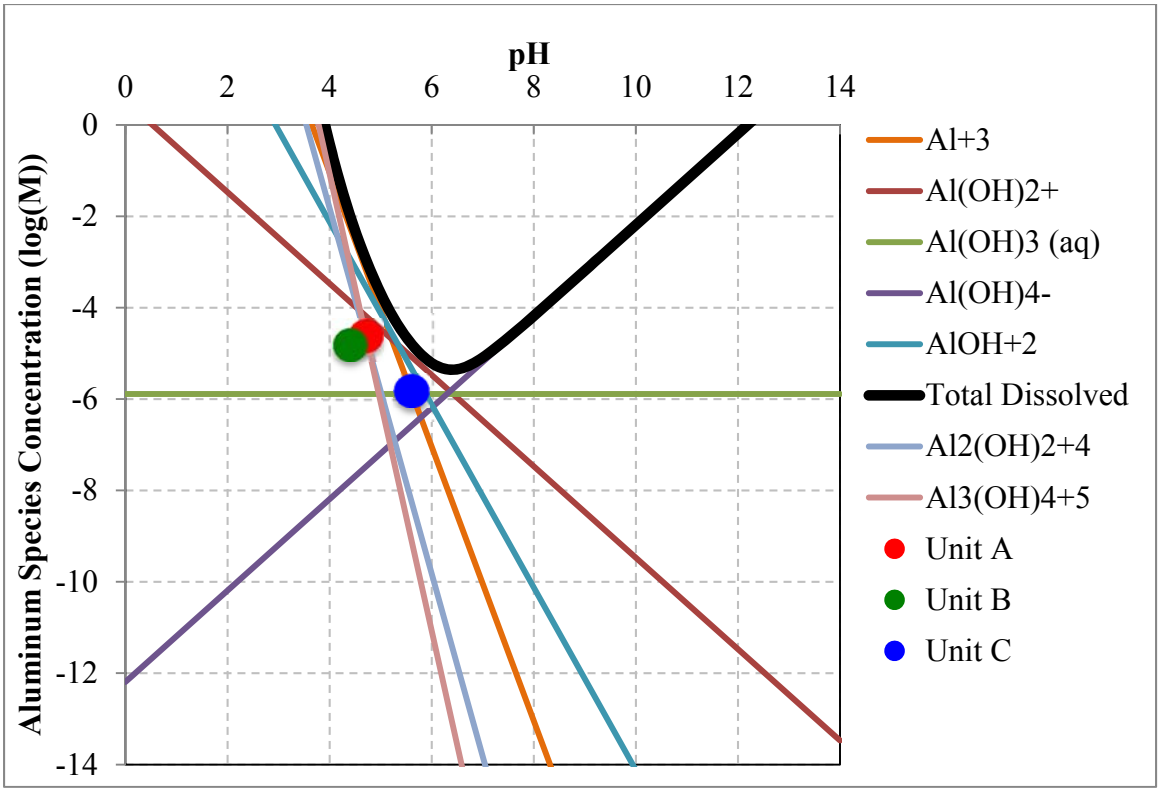

Figure 14: Aluminum solubility plot

Similarly, the saturation curve for copper hydroxide is shown in Figure 15. Copper hydroxide ($Cu(OH)_2$) solid was assumed present at all conditions shown for calculations. If the pH of the condensate for Units A and B was raised above 7.0 and above 8.0 for Unit C, copper precipitation will occur at equilibrium. Therefore, copper and aluminum removal would be recommended below the minimum point on the saturation curve for the pH range of 6.5-8.5 (1.5×10^{-7} M copper and 4.4×10^{-6} M aluminum).

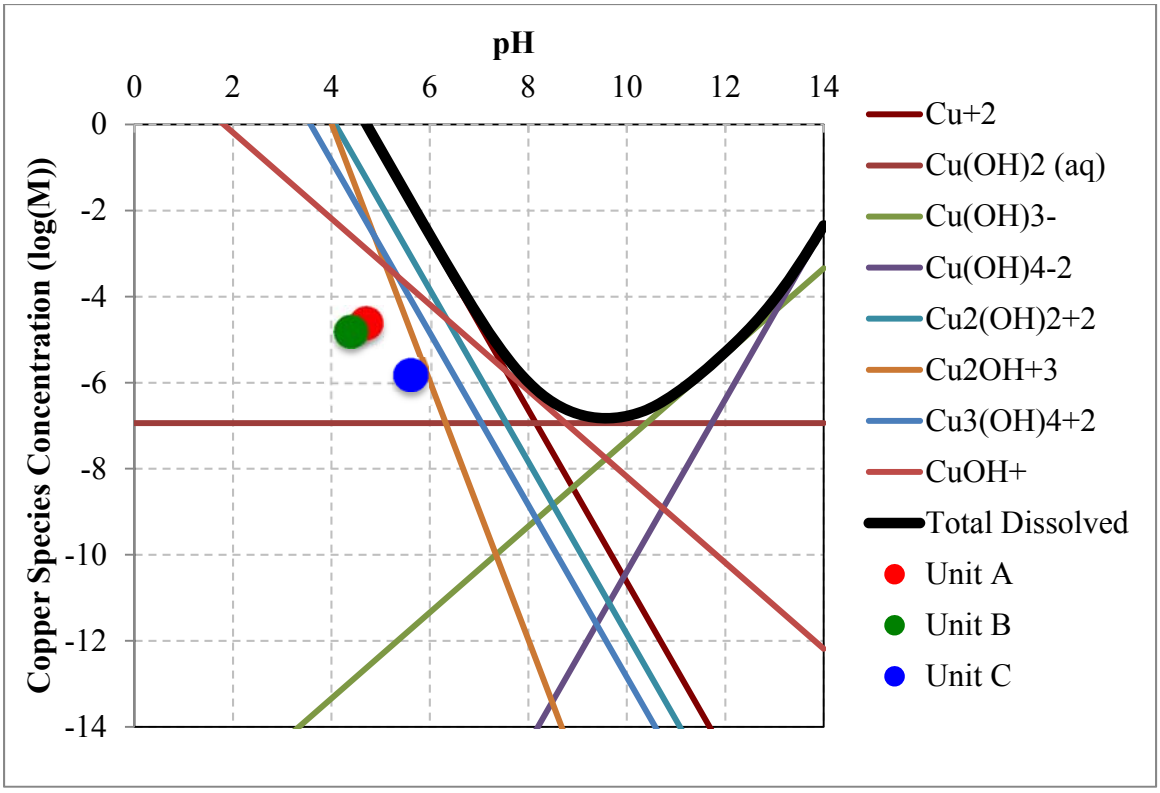

Figure 15: Copper solubility plot

The levels of acetate and formate, both excellent biological electron donors, could create biological control issues. Condensate conveyance systems could become clogged with biological growth. For non-potable reuse, the conveyance systems would have to be cleaned out at regular time intervals or disinfected to avoid blockages. This could also yield TOC values within acceptable ranges for a wider range of reuse options.

Given the volumes of production over the summer months, 8.6, 39.3, and 3.9 L/day for Units A, B, and C, respectively, irrigation or in-home non-potable reuse (i.e., toilet flushing) are currently the best options for condensate reuse. If condensate were used for toilet flushing, no pretreatment would be required. Standard toilets use 6 liters (1.6 gallons) per flush (EPA, 2016). Therefore, Unit B could significantly offset the water demand of toilets, and residences with multiple HVAC units could offset an even larger

portion of toilet water demand. Conveyance piping and a pressure tank would be required for the reuse system. However, Units A and C are likely too small to use for toilet flushing and are more suitable for irrigation purposes.

Chapter 6: Conclusions

6.1 Cooling Tower Experiments

The cooling tower study results indicate that the source water used to supply makeup water to cooling towers influences the microbial communities that develop in the cooling tower basins. However, the microbial communities present in the makeup water sources are not necessarily conserved within the basins. The microbial communities of Basins A and C, with the same makeup water sources (recovered plus potable water), appear distinct from Basin B (reclaimed and potable water) as well as from the microbial communities of potable and recovered water sources (Figures 6 and 8). Basin B contained a more diverse community (Figure 7), likely because its primary makeup water source (reclaimed water) was more diverse and it contained elevated levels of nutrients relative to Basins A and C. While the composition of the microbial community varied between the basins, the total bacteria levels in the three basins only varied by approximately one order of magnitude.

The sequencing results indicate that Basins A and C contained a large fraction of methylotrophic genera including *Methylotenera*, *Hyphomicrobium,* and *Methylibium*. It is unknown why more methylotropic genera are present in Basins A and C versus B, but it could be related to the types of organics present in each basin. Basin B contained higher relative abundances of *Sphingomonas*, *Cupriavidus*, and *Acidovorax*, which is potentially an artifact of higher levels of nutrients and more complex organics present in the reclaimed wastewater used to supply the makeup water to the basin.

The cooling towers evaluated in this study have pH control, continuous chlorine feeds, biocide controls, and are drained and cleaned out annually. Following this regimen, the qPCR results suggest using alternative water sources with higher levels of *Legionella* spp. compared to potable water does not result in basin waters with unacceptable levels

of *L. pneumophila*. All basins contain mostly non-pneumophila species of *Legionella*. However, there are at least 58 identified species of *Legionella* and at least 30 of them have been associated with pathogenicity (Cunha et al., 2016). Therefore, further research into which *Legionella* species are present is required to further elucidate any potential risks.

The results of this study align with previous studies that have concluded that proper maintenance of cooling towers can control *L. pneumophila* levels. Therefore, additional states within the United States should consider adopting cooling tower maintenance regulations like ANSI/ASHRAE Standard 188-2015. While the non-pneumophila *Legionella* risk levels are not as well understood, this study indicates that well maintained cooling towers can control *L. pneumophila* and *L. pneumophila* sg 1 levels. Given that *L. pneumophila* sg 1 causes an estimated 90% of Legionnaires disease cases, controlling it should significantly reduce the number of Legionnaires disease cases.

No information was found on what levels of NTM in cooling towers presented a public health risk. However, the NTM levels found in the cooling towers align with the levels measured by Adrados et al. 2011. That study concluded that if the level of *Legionella* that has been reported to cause infection (5 logCFU/L) were considered similar for NTM, then the NTM levels present in the cooling towers are high enough to warrant further investigation. Additional studies are required to develop alert and action levels for NTM in cooling towers.

This study focused on the short term variability of the microbial communities in cooling tower basins using alternative water sources for makeup water. Future studies should examine long term trends of microbial communities of cooling tower basins using alternative water sources to understand how the community evolves over time and as a function of season.

6.2 HVAC CONDENSATE STUDY

Humidity ratios calculated with in-home temperature and humidity data predicted condensate production rates within 25% of the actual measured production rates. On average, Units A, B, and C produced 8.6, 39.3, and 3.9 L/day of condensate during the study period. The volumes produced by Units A and C are likely too low to be reused for toilet flushing or landscape irrigation. However, useful volumes could be produced if multiple residences routed their condensate together. Unit C was a single apartment unit within an apartment complex of approximately 50 units. If the condensate from all 50 units was combined, approximately 195 L/day of condensate would be collected which would offset a significant amount of the potable water used for landscape irrigation.

The water quality analysis showed aluminum, copper, pH, and nitrite levels that were above either a primary or secondary drinking water standard or an irrigation recommendation. All of the units had either aluminum components in their cooling coil and/or aluminum drip pans. The two units with copper containing cooling coils contained copper levels that were also above the secondarydrinking water regulation or irrigation recommendation. The condensate from Unit C, which had no copper in its cooling coil, did not exceed any of the copper limits. If landscape irrigation were selected for collected condensate, treatment such as a limestone bed contactor would likely be required to reduce the aluminum and copper concentrations.

A surprising level of organics were found in the condensate, especially from Unit C. The level of organics could result in a nuisance issue for non-potable reuse. The organics will result in biological growth in piping, storage tanks, and potentially end use equipment, including toilets. Disinfection of the condensate is possible, but disinfection byproduct formation potential will need to be determined. Potable resuse is not suggested

without further study because of the disinfection byproducts that could form if chemically disinfected and the potential for microbial contamination.

6.3 RECOMMENDATIONS

Water quality should always be considered when evaluating a potential reuse application. In urban cooling towers, different makeup water sources can lead to different microbial communities and diversity levels. For HVAC condensate reuse projects, the levels of aluminum, copper, and organic carbon need to be addressed before a reuse option is selected for any given site.

Appendix: Water Chemistry of Cooling Tower Samples

Cooling Tower	Water Source	Sampling Date	Total P (mg/L P)	pH	Total Alkalinity (meq/L)
A	Basin	1	2.8	8.84	4.85
A	Potable	1	0.27	9.71	1.07
B	Basin	1	17.95	7.16	0.85
B	Potable	1	0.11	9.71	0.99
B	Reclaimed	1	4.54	6.91	0.71
C	Basin	1	2.27	8.9	4.87
C	Potable	1	0.25	9.65	1.02
C	Recovered	1	0.24	7.56	1.36
A	Basin	2	1.84	8.75	4.95
A	Potable	2	0.18	9.68	0.98
A	Recovered	2	0.05	7.58	3.42
B	Basin	2	13.39	7.39	1.21
B	Potable	2	0.13	9.63	0.92
B	Reclaimed	2	2.85	6.72	0.79
C	Basin	2	1.65	8.83	4.69
C	Potable	2	0.17	9.28	0.97
C	Recovered	2	0.06	7.63	1.3
A	Basin	3	1.51	8.69	4.63
A	Potable	3	0.15	9.67	0.94
A	Recovered	3	0.08	7.36	3.25
B	Basin	3	12.18	7.42	1.19
B	Potable	3	0.06	9.68	0.95
B	Reclaimed	3	2.8	6.78	0.63
C	Basin	3	1.54	8.95	5.91
C	Potable	3	0.12	9.74	0.95
C	Recovered	3	0.02	7.3	1.01
Cooling Tower	**Water Source**	**Sampling Date**	**Nitrate (mg/L as N)**	**Chloride (mg/L)**	**Fluoride (mg/L)**
A	Basin	1	0.72	458.67	2.36
A	Potable	1	0.09	46.62	0.24
B	Basin	1	58.75	787.44	4.65
B	Potable	1	0.08	46.55	0.3
B	Reclaimed	1	15.99	119.49	1.02

C	Basin	1	2.04	402.18	2.3
C	Potable	1	0.1	47.2	0.28
C	Recovered	1	0.51	54.78	0.29
A	Basin	2	1.13	483.71	2.5
A	Potable	2	0.01	47.17	0.35
A	Recovered	2	1.78	48.62	0.22
B	Basin	2	87.37	742.76	5.31
B	Potable	2	0.02	46.43	0.38
B	Reclaimed	2	15.75	129.73	1.04
C	Basin	2	1.34	441.35	2.39
C	Potable	2	0.02	47.93	0.36
C	Recovered	2	0.36	32.62	0.19
A	Basin	3	1.17	459.75	2.34
A	Potable	3	0.01	46.9	0.41
A	Recovered	3	1.76	45.52	0.21
B	Basin	3	78.58	717.21	4.65
B	Potable	3	0.02	46.76	0.4
B	Reclaimed	3	16.43	129.12	1.07
C	Basin	3	1.33	443.89	2.53
C	Potable	3	0.01	47.3	0.4
C	Recovered	3	0.32	18.41	0.09
Cooling Tower	**Water Source**	**Sampling Date**	**Bromide (mg/L)**	**Sulfate (mg/L SO_4^{-2})**	**Manganese (mg/L)**
A	Basin	1	NA	395.35	<0.0012
A	Potable	1	NA	30.88	<0.0012
B	Basin	1	NA	1186.83	0.09
B	Potable	1	NA	31.19	<0.0012
B	Reclaimed	1	NA	189.64	0.03
C	Basin	1	NA	464.55	<0.0012
C	Potable	1	NA	31.57	<0.0012
C	Recovered	1	NA	52.66	<0.0012
A	Basin	2	12.61	499.79	<0.0012
A	Potable	2	0.07	37.4	<0.0012
A	Recovered	2	0.25	64.73	<0.0012
B	Basin	2	8.96	1283.07	0.12
B	Potable	2	0.04	37.94	<0.0012
B	Reclaimed	2	0.15	211.74	0.01
C	Basin	2	10.03	511.83	<0.0012

C	Potable	2	0.07	38.52	<0.0012
C	Recovered	2	0.06	32.93	<0.0012
A	Basin	3	14.66	452.02	<0.0012
A	Potable	3	0.05	38.97	<0.0012
A	Recovered	3	0.2	61	<0.0012
B	Basin	3	10.92	1146.17	0.08
B	Potable	3	0.07	38.26	<0.0012
B	Reclaimed	3	0.21	216.14	0.02
C	Basin	3	7.8	422.73	<0.0012
C	Potable	3	0.07	38.99	<0.0012
C	Recovered	3	0	18.61	<0.0012
Cooling Tower	**Water Source**	**Sampling Date**	**Potassium (mg/L)**	**Calcium (mg/L)**	**Sodium (mg/L)**
A	Basin	1	35.45	126.51	296.85
A	Potable	1	3.89	15.11	25.25
B	Basin	1	69.01	260.5	661.52
B	Potable	1	3.99	11.59	26.22
B	Reclaimed	1	12.99	56.47	121.91
C	Basin	1	31.84	167.96	250.56
C	Potable	1	3.86	15.36	26.25
C	Recovered	1	6.13	31.1	30.04
A	Basin	2	41.79	132.54	294.48
A	Potable	2	5.01	15.38	25.72
A	Recovered	2	5.24	87.9	31.22
B	Basin	2	92.58	257.47	694.74
B	Potable	2	4.96	10.96	25.42
B	Reclaimed	2	15.66	50.89	118.82
C	Basin	2	40.17	142.51	265.47
C	Potable	2	4.96	14.86	25.52
C	Recovered	2	3.03	27.51	18.68
A	Basin	3	34.66	122.86	286.08
A	Potable	3	3.86	14.06	25.09
A	Recovered	3	4.71	81.08	31.87
B	Basin	3	74.94	258.02	657.44
B	Potable	3	3.83	11.67	24.77
B	Reclaimed	3	13.78	54.32	116.23
C	Basin	3	36.43	137.69	269.33
C	Potable	3	3.73	13.56	24.33
C	Recovered	3	1.79	19.96	11.28

Cooling Tower	Water Source	Sampling Date	Copper (mg/L)	Iron (mg/L)	Magnesium (mg/L)
A	Basin	1	0.13	0.05	119.95
A	Potable	1	<0.0032	<0.0019	14.98
B	Basin	1	0.21	0.17	197.42
B	Potable	1	<0.0032	<0.0019	16.83
B	Reclaimed	1	<0.0032	0.02	34.37
C	Basin	1	0.08	0.02	107.07
C	Potable	1	<0.0032	<0.0019	15.48
C	Recovered	1	0.01	<0.0019	16.94
A	Basin	2	0.25	0.03	109.13
A	Potable	2	<0.0032	<0.0019	13.61
A	Recovered	2	0.88	<0.0019	6.62
B	Basin	2	0.17	0.15	172.49
B	Potable	2	<0.0032	<0.0019	15.52
B	Reclaimed	2	<0.0032	0.03	33.52
C	Basin	2	0.08	0.03	104.61
C	Potable	2	<0.0032	<0.0019	13.79
C	Recovered	2	0.01	<0.0019	7
A	Basin	3	0.33	0.05	100.21
A	Potable	3	<0.0032	<0.0019	13.94
A	Recovered	3	0.52	<0.0019	6.08
B	Basin	3	0.16	0.13	175.58
B	Potable	3	<0.0032	<0.0019	15.14
B	Reclaimed	3	<0.0032	0.03	34.48
C	Basin	3	0.22	0.02	108.37
C	Potable	3	<0.0032	<0.0019	13.7
C	Recovered	3	0.02	<0.0019	3.19

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