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# Environmental Risk Assessment Of Community Acquired Legionellosis From Recycled Water Use In Los Angeles County California

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M.P.H. Thesis

ENVIRONMENTAL RISK ASSESSMENT OF COMMUNITY ACQUIRED  
LEGIONELLOSIS FROM RECYCLED WATER USE IN LOS ANGELES COUNTY  
CALIFORNIA

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Class of 2013

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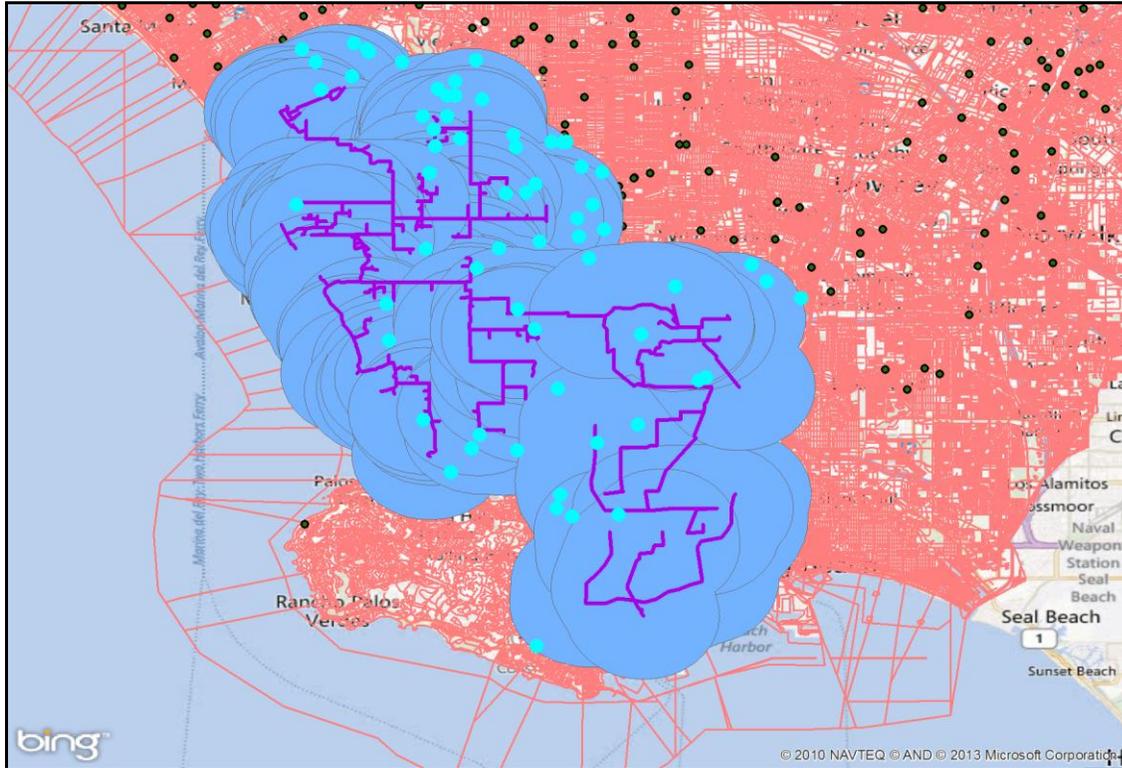
## Overview

The scope and magnitude of recycled water supplementation in Los Angeles County has increased over the last seven years to meet the increasing water demands of the County. Recycled water may facilitate exposure to *Legionella pneumophila*, the causal agent of Legionellosis by inhaled transmission. The Protection of Public Water Supply and Backflow Prevention Guidelines for Water Service Rule 16-D may lack the standard biological decontamination required to reduce the risk of Legionellosis to an acceptable level in wastewater treatment plants. Epidemiological evidence such as is presented in this paper must be the basis for stringent water cleanliness standards that more accurately promote public health. This study mapped the cases of Legionellosis in Los Angeles County from 2005-2012, in order to identify cases of the disease that were associated with exposure of recycled water pipelines. Recycled water may be aerosolized into droplets, which facilitate Legionellosis. A “buffer” was created to characterize the maximum distance in which recycled water may travel. By buffering the recycled water pipeline exposure as directed by the World Health Organization, we may more accurately identify Legionellosis cases that live within the infectious zone of the bacteria *L. pneumophila*. A relative risk was used to assess the magnitude of the risk posed by recycled water pipeline within Los Angeles County and an infectious zone map will illustrate the risk of *L. pneumophila* infection. The relative risk comparing exposed Legionellosis patients to unexposed Legionellosis patients was 1.408 (1.3088-1.6735). It is hoped that the results of this study will demonstrate how a water-standard that is deemed safe may not adequately protect against infection from *L. pneumophila*. The results of this analysis may form the foundation upon which future studies hoping to address similarly novel and important questions may be built.

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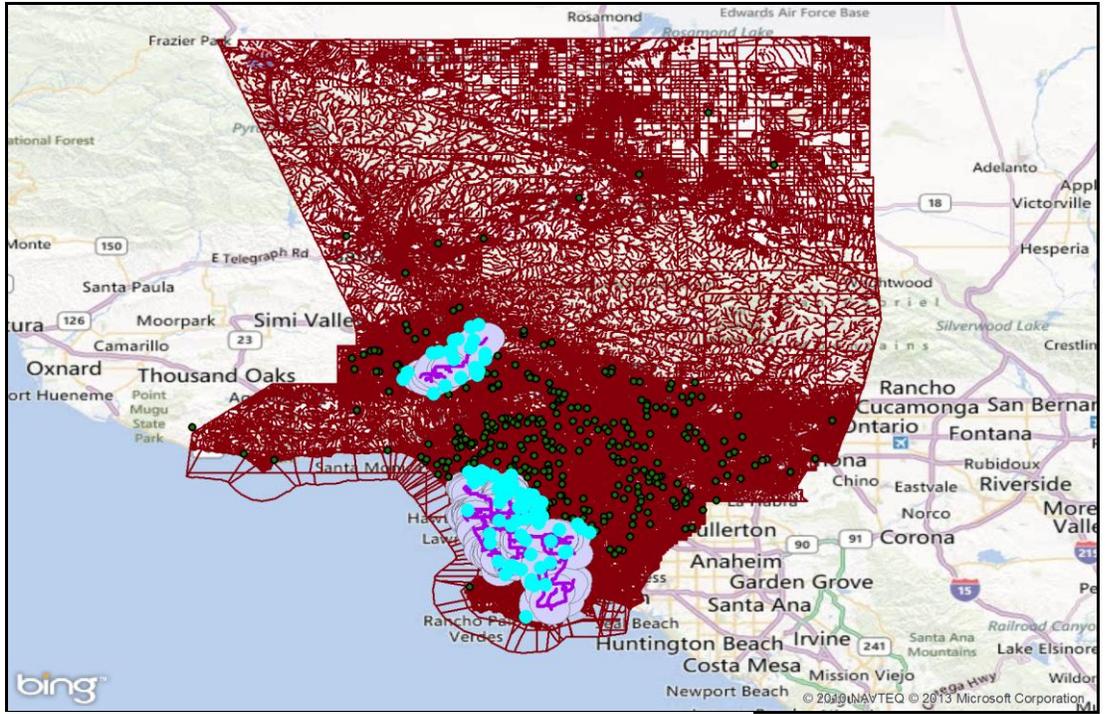
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**Map 1.** Edward C. Little Water Recycling Facility, 17140 Avalon Blvd, Carson, CA

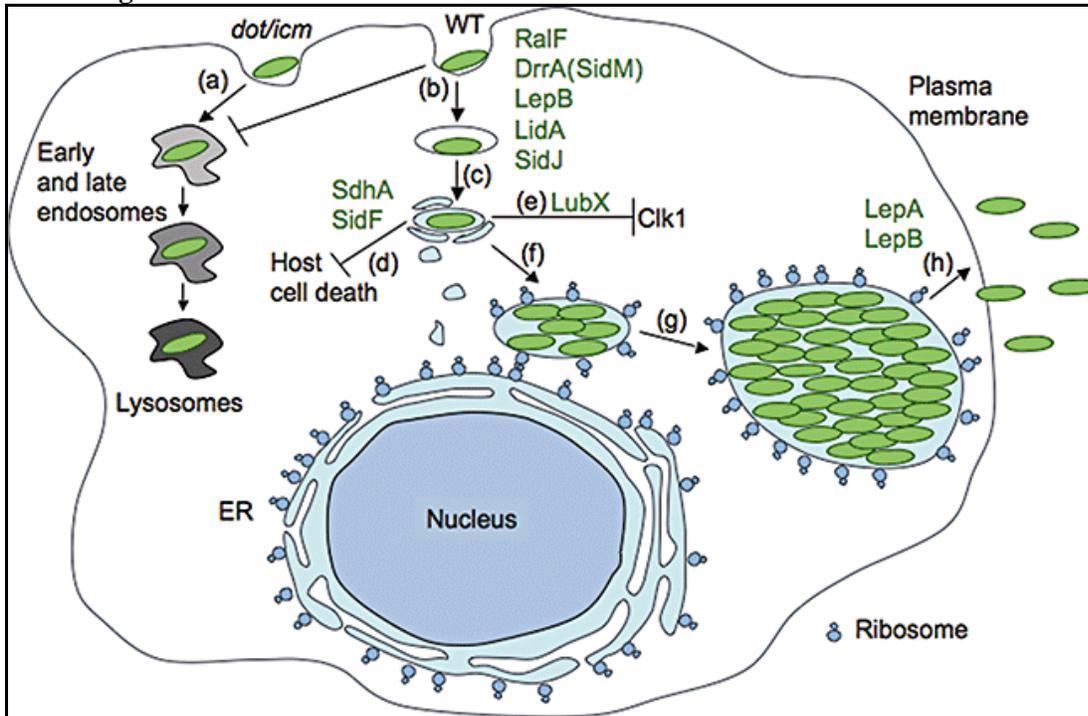
Legend	
	Exposed Cases
	Unexposed Cases
	Recycled Water Pipeline
	Street Lines
	3.2km buffer



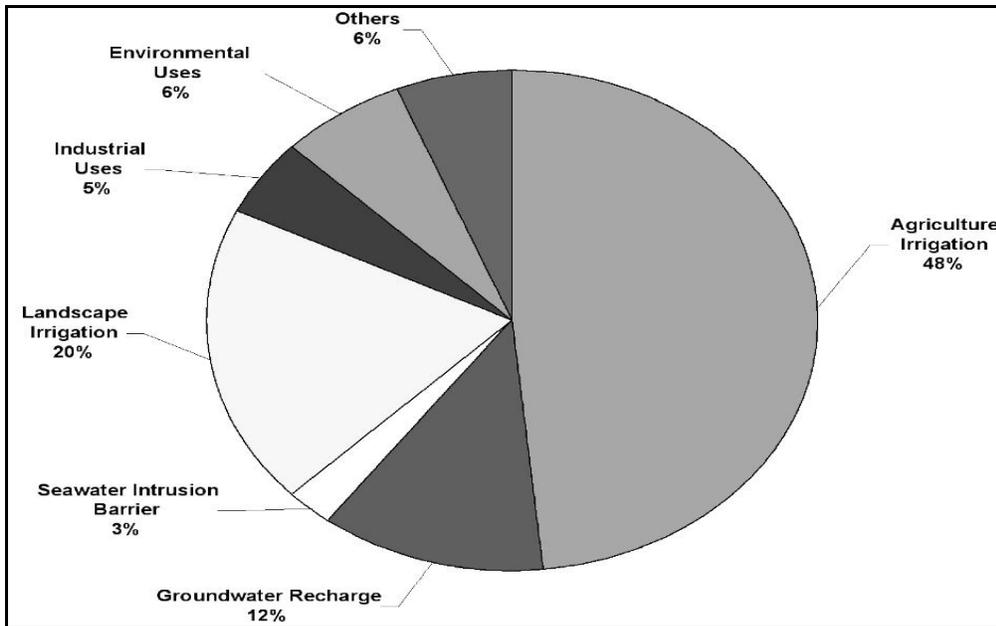
**Map2.** Los Angeles County Recycled water pipeline map 2012.

Legend	
	Exposed Cases
	Unexposed Cases
	Recycled Water Pipeline
	Street Lines
	3.2km buffer

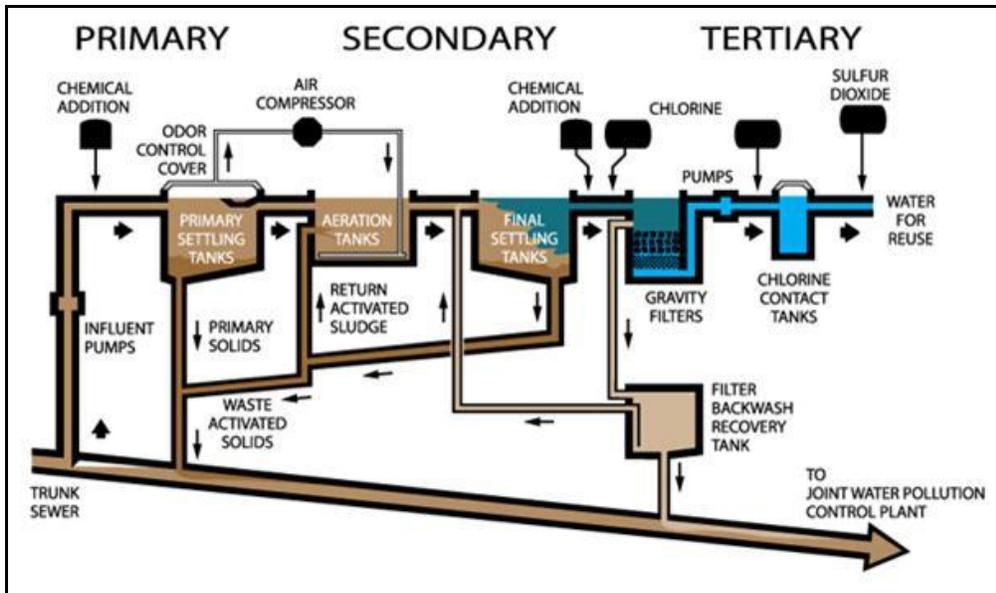
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**Figure 1.** *Legionella pneumophila* uptake and Legionella Containing Vacuole (LCV) replication. (Shin, 2008).



**Figure 2. Recycle water use distribution.** The figure below shows the distribution of recycled water usage in Los Angeles in 2006-7. Water reuse summary for fiscal year 2006-2007. Retrieved November 20, 2008, from Sanitation Districts of Los Angeles County Web site:



**Figure 3.** Graphical Flow Schematic of a Water Reclamation Plant. (Sanitation District of Los Angeles, 2012).

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Purveyor	Potable Water (\$/AF)	Recycled Water (\$/AF)	Discount (%)
Long Beach Water Department	755.33	377.67 - 528.82	30 - 50
City of Cerritos	614.20	326.70	47
City of Lakewood	740.52	444.31	40
Central Basin MWD	525.00	255.00 - 322.00	38 - 51
Pomona Water Department	604.19	422.93	30
Walnut Valley Water District	762.30	647.96	15
Rowland Water District	635.98 - 853.78	544.50	14 - 36
San Gabriel Water Company	642.77	201.51 - 546.94	15 - 69
Valencia Water Company	424.27	356.32	12

**Table 1**

Acre-feet per year (AFY) 2006-2007. In Los Angeles, recycled water costs are significantly lower than those of potable water. Table 1 shows a rate comparison in a number of WRPs (water reclamation plants) in Los Angeles County. Water reuse summary for fiscal year 2006-2007. Retrieved November 20, 2008, from Sanitation Districts of Los Angeles County Web site: <http://www.lacsd.org>

## Appendix

**Gray-water** – Untreated domestic wastewater from bathtubs, showers, bathroom wash basins, clothes washing machines, and laundry tubs, but excluding toilets, kitchen sinks, dishwashers, photo development sinks and laundry water from soiled diapers. This is not the same as treated recycled water (Los Angeles County Recycled Water Advisory Committee, 2005).

**Irrigation Period** – The time, from start of water flow to end, which a specific area receives recycled water by direct irrigation application, no matter how often the specific area is irrigated - that is length of the duty cycle (Los Angeles County Recycled Water Advisory Committee, 2005).

**Irrigation Use** – An approved use of recycled water for landscape irrigation as defined for recycled water under Title 22, Chapter 3 of the California Code of Regulations (Los Angeles County Recycled Water Advisory Committee, 2005).

**Non-Potable Water** - water which is unfit for human or animal consumption due to contaminants that exceed the current permissible Maximum Contaminant Level (MCL) in drinking water (Guidelines for pipeline construction and instillation for the safe use of recycled/reclaimed wastewater, 2009) (Los Angeles County Recycled Water Advisory Committee, 2005).

**Potable Water** – That water that is pure and wholesome, does not endanger the lives or health of human beings, and conforms to the latest edition of the California Safe Drinking Water Act, or other applicable standards (Los Angeles County Recycled Water Advisory Committee, 2005).

**Recycled/Reclaimed Water** – Non-potable water that is treated to the California Code of Regulations, Title 22, Chapter 3 and used for approved purposes other than drinking water (Los Angeles County Recycled Water Advisory Committee, 2005).

**Recycled Water Treatment Plant** - Although secondary-treated effluent may also be reused, its applications are limited and subject to much greater restrictions, and it will not be addressed in this Manual.) The facilities that produce recycled water are known as Water Recycling (or Reclamation) Plants that are owned and operated by “Recycled Water Producers.” The recycled water produced by these plants is delivered to users through distribution systems owned and operated by “Recycled Water Agencies.” Recycled Water Producers and Agencies can be one and the same entity (Los Angeles County Recycled Water Advisory Committee, 2005).

**Windblown Spray** – Dispersed, airborne particles of recycled water that can be transmitted through the air to locations other than those approved for the direct use of recycled water (Los Angeles County Recycled Water Advisory Committee, 2005).

**Water-Stressed Area** -  $<1,700\text{m}^3$  of renewable water per capita. (Postel, 2000)

## **Environmental risk assessment for community acquired legionellosis from recycled water use in Los Angeles County.**

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### **Abstract**

Legionellosis is caused by *Legionella pneumophila*, resulting in 8,000-18,000 yearly hospitalizations and 4,000 deaths in the United States (Bouwknegt, 2012) (Toshiaki, 2004) (World Health Organization, 2012). *L. pneumophila* may persist in the built environment due to unique intracellular characteristics. Although the mode of transmission of *L. pneumophila* is inhalation of aerosolized contaminated water, this bacterium is not transmitted human to human (World Health Organization, 2012). *L. pneumophila* infection may be facilitated by the increased use of recycled water in Los Angeles County. In a water stressed area such as Los Angeles County, a limited potable water supply may not sustain the growing metropolitan population. Recycled and reclaimed water may help lessen the demand by supplying non-drinking water to industries such as agriculture, water table restoration, and recreation use. To be more cost effective, recycled water has been mandated to receive less treatment than the treatment used for county potable water. Recycled water treatment has less stringent backflow and purification procedures compared to county potable water treatment. The Environmental Health division prioritizes monitoring and treatment of potable water. Monitoring and treating recycled water will be limited. Mapping the cases of non-hospital acquired Legionellosis cases exposed to recycled water pipeline compared to potable water pipeline will show the difference in risk associated with Legionellosis. There was a statistically significant difference in the number of cases within the recycled water pipe-lined area including the infectious zone, compared to the unexposed recycled water pipeline area. The Chi-squared P-value was 0.0013 comparing the exposed Legionellosis cases to unexposed. Individuals exposed to *L. pneumophila* from recycled water pipeline had a 1.4806 (1.3088, 1.6735) times greater risk of developing Legionellosis compared to individuals who were not exposed to *L. pneumophila* from recycled water pipeline. The current standard of recycled water cleanliness may not promote the public health; a more stringent standard involving more concentrated disinfection and filtration steps is needed. Public Water Supply Rule 16-D and Section 2.12 of the Rules Governing Water and Electric Services in the City of Los Angeles must include inhaled exposure of *L. pneumophila*. The first steps to reduce Legionellosis may include standardize of Filtration, Ozonation, Disinfection, and/or Fluorination for the Los Angeles-Glendale Water Plant, the Donald C. Tillman Water Reclamation, the Hyperion Waste-Water, and the Terminal Island Water Reclamation Plant to the degree that is used for potable water treatment.

## Introduction

Los Angeles County California is home to 9,372,269 residents as of 2010 (US Census, 2010) (Cities within the County of Los Angeles County, 2010), whose water usage demands have placed potable water resources under stress (Water Replenishment District of Southern California, 2012). Polluted freshwater (such as municipal wastewater) may be discharged to the sea, but this will almost certainly adversely affect the integrity of coastal marine systems, or have long-term unhealthy environmental consequences (Hamilton, 2007). Reclaiming/ recycling wastewater is recognized as one means of alleviating such pressures (Hamilton, 2007). As the population increases, greater demands are placed on water supplies. To meet the demand for new water, water recycling is one nontraditional way to help stretch the available supplies and result in improved reliability of supply for everyone in Southern California, as well as Phoenix Arizona (Ruffer and Bair, 1981), the Santa Fe New Mexico (Postel, 2000), the Colorado and Columbia water basin (Kundzewicz , 2008), Hebei Province, China, (Zhu, 2006), and Mexico (Molden, 2007).

In Los Angeles County, recycled water may alleviate the environmental stress brought on by the growing demand for water. Recycled water use is categorized into four groups in Los Angeles County (Los Angeles County Recycled Water Advisory Committee, 2005); Urban Landscape, Agriculture, Impoundments, and Industrial. Industrial makes up the majority of recycled water, yet none of these water types are fit for human consumption (Los Angeles County Recycled Water Advisory Committee, 2005). The Los Angeles County Plumbing Code, Section 218.0 defines potable water as: "Water which is satisfactory for drinking, culinary, and domestic purposes and meets the requirements of the Health Authority having jurisdiction (Los Angeles Cross Connection and Water Pollution Control, 2009)." Potable water undergoes a stern five step treatment including aeration, flocculation, sedimentation, filtration, and a disinfectant step (Water Education Foundation, 2006). To this extent, recycled water may not share, backflow, or interconnect with the public water supply (Los Angeles Cross Connection and Water Pollution Control, 2009). This requirement is intended to spatially separate potable and recycled water. This requirement includes individual residences using recycled water for landscape irrigation as part of an approved area of use (Rule Governing water and electric service in the city of Los Angeles) (Los Angeles Department of water and power, Water Service rule 16-d, Aug 2006).

## Background

*L. pneumophila* is naturally found to live in temperate to warm bodies of waters such as rivers and lakes and may persist in temperatures between 20-50 degrees Celsius (World Health Organization, 2012). Anthropogenic water systems like air conditioning pipes and showers that are within the habitable rate of *L. pneumophila* have tested positive for this bacterium (Boyle, 1991). Boyle et al suggest that *L. pneumophila* may live in recycled water pipes, because this water is likely not sterile. *L. pneumophila* within the pipes and processing components of water reclamation systems poses a potential health hazard (Boyle, 1991). Benin et al clearly explain how the bacteria *L. pneumophila* may enter a human host through inhalation of Legionella-containing water droplets. Benin et al suggest that Legionellosis is primarily a hospital borne disease (nosocomial), however; the environmental risk of non hospital (community) acquired Legionellosis is unknown.

## **Pathology**

*L. pneumophila* infections may be acquired from sources that create aqueous aerosols; such as rivers, lakes, fountains, saunas, cooling towers, rainwater tanks, showerheads, bubblers, and whirlpools (Bencini, 2005) (Vieritz, 2010). Inhalation of these aerosols contaminating *L. pneumophila* are subsequently deposited in the alveolar region of the lungs can lead to phagocytosis of *L. pneumophila* by local macrophages (Bouwknegt, 2012). For *L. pneumophila* a dose-response relationship was estimated, which indicated that low inhaled doses of the bacterium are likely to cause infection in humans (i.e., 1-12 inhaled viable LCV cells gave a 50% probability of infection (Bouwknegt, 2012). Aerosol formation is deemed necessary to cause *L. pneumophila* disease, but aspiration following ingestion of contaminated water, ice, and food has also been implicated as the route of infection in some cases (Marrie, 1991) (Blatt, 1993).

## **Dose- Response**

The infectious dose for humans has been suggested be as low as 1 to 12 inhaled and viable *L. pneumophila* organisms from the LCV-amoeba complex (Bouwknegt, 2012). *L. pneumophila* infections have frequently been traced to contaminated aerosols generated at distances of up to 3.2 kilometers (Addiss, 1989) (World Health Organization, 2012). The suggested infectious zone of 3.2 kilometers as suggested by the World Health Organization is the reason for the 3.2 kilometer radius buffer zone presented in the recycled water pipeline maps. The World Health Organization uses the 3.2 kilometer buffer for cooling tower the release aerosolized droplets of water that contain the bacterium. This idea was adapted to recycled water pipeline exposure, as there is a gap in the literature regarding an infectious zone for inhaled aerosolized *L. pneumophila*. What both Bouwknegt et al and Addiss et al suggest is that due to the extremely high infectiousness of *L. pneumophila*, any exposure may result in the disease, where patients must be tested as soon as can be.

## **Mode of Infection**

*L. pneumophila* is transmitted to an unsuspecting person by inhalation of water droplets that contain the bacterium already present within the protozoa. The Legionella-containing-vacuole (LCV) will be inhaled by a host, where the bacterium complex will then travel by means of the respiratory tract, until it reaches alveolar macrophage (Urwyler, 2009) (Hubber, 2010). *L. pneumophila* may hijack the normal host cell machinery to create a niche where the bacteria can replicate (Urwyler, 2009). The pathogenesis of *L. pneumophila* is derived from its growth within lung macrophages (Hubber, 2010) (Urwyler, 2009). One of the *L. pneumophila*'s type IV secretion systems, the Dot/Icm secretion system, is of critical importance for its ability to replicate and to cause disease. The Dot/Icm substrates modulate multiple host cell processes and in particular, redirect trafficking of the *L. pneumophila* phagosome and mediate its conversion into an ER-derived organelle competent for intracellular bacterial replication (Urwyler, 2009). *L. pneumophila* also manipulates host cell death and survival pathways in a way that allows continued intracellular replication (Hubber, 2010). The LCV becomes surrounded by the host rough endoplasmic reticulum in order to evade lysosomes, but may then release pore-forming toxins in order to destroy the host alveolar macrophages (Molmeret, 2002).

## **Funds saved by recycling water**

Recycled water treatment may be cost effective. Depending on the infrastructural cost such as pipeline construction and upkeep, Biagtan concluded that there are three provable

scenarios which may result in county profits. The quantifiable summary statistic for each scenario is follows: Scenario 1 is \$794/acre-ft, Scenario 2 is \$1,093/acre-ft, and Scenario 3 is \$1,162/acre-ft. Qualitative factors was also recognized. For example, Scenario 1's future demands, which extend beyond the time set time horizon of planning, are highly uncertain and environmental impacts may be more significant. Scenario 2 has relatively lower capital and unit cost compared with Scenario 3 (Biagtan, 2008). Because these models extrapolate savings into 2030, it is difficult to have precise saving values, as cost of pipeline upkeep, water treatment, and water technology changes (Sanitation Districts of Los Angeles County, 2008) (Biagtan, 2008).

## **Reasons to Improve Recycled Water Treatment**

*L. pneumophila* may infect an individual if the LCV is inhaled. This is because recycled water treatment is not as effective as potable water in Los Angeles County in removing this bacterium. Although there is no current literature investigating Legionella cases in Los Angeles County from recycled water exposure, it is biologically plausible that a water treatment system that does not meet potable water standards may in fact disperse infected water droplets. These water droplets may infect unsuspected populations within Los Angeles County. Recycled water pipelines affect a minimum of 1,531,048 people (not including travelers, or unregistered people from the US 2010 census) (US Census Bureau, 2010). The aerosolized water droplets containing the LCV may continuously expose these people to Legionellosis. The urgency to satisfy water demands have led to an increase in recycled water popularity.

Los Angeles County is far from self-sufficient in its fresh water supplies. The growth in the Los Angeles County population is dependent on limited natural water sources, which is quickly being depleted (Fuller, 2010) (Los Angeles Cross Connection and Water Pollution Control, 2009). Sierra Nevada mountain range holds snowpack that when melted, annually accounts for one third of California's drinking water and irrigation supply. As temperatures increase, this snowpack is predicted to melt faster and therefore not provide the steady stream of water in the summer and fall that cities and farmers rely (Fuller, 2010). In 2007 the snowpack had shrunk 10% below its wintertime average, which have strained Los Angeles County's fixed water supply (Fuller, 2010). Recycling waste water may have become an imperative means to alleviate the water demands of this fast growing metropolis of almost 10 million.

As the Southern California population increases, more demands are placed on water supplies (Water Replenishment District of Southern California, 2012). Traditional sources for the water replenishing district of southern California (Water Replenishment District of Southern California) service area have been local groundwater and imported surface water through the Metropolitan Water District of Southern California from the Colorado River and State Water Project. To meet the demand for new water, water recycling is one nontraditional way to help stretch the available supplies and result in improved reliability of supply for everyone in Southern California in the long run (Water Replenishment District of Southern California, 2012). Recycled water standards regarding sanitation may not reduce the risk of Legionellosis to the level of potable water risk of Legionellosis (Water Education Foundation, 2006) (Water Replenishment District of Southern California, 2012).

## **Recycled Water Regulation**

Management for recycled water distribution is standardized to regulate the use. Periodic inspections, cleaning, emergency procedures and modifications, and discharge are the primary methods to control recycled water distribution (Water District Recycled Water Program, 2012).

Periodic Site Inspections of the User's recycled water irrigation system are mandated in the Water Code (Section 13523.1(b)(5)). Such inspections include, at a minimum, the visual inspection of all back-flow prevention devices, pump rooms, exposed piping, valves, pressure reducing stations, points of connection, sprinklers, controllers, lakes, storage facilities, signs, labeling, tags, etc. Back-flow involves the reverse of the flow of the treated water, which has the capability to contaminate potable water (Water District Recycled Water Program, 2012).

### **Emergency Procedures/ Modifications**

In case of a major earthquake, the Site Supervisor should immediately inspect the potable and recycled water systems for damage. If either system appears damaged, both water systems (recycled and potable water) should be shut off at their points of connection (Water District Recycled Water Program, 2012). Emergency modifications or repairs can be made by the user to the recycled water system without the prior approval of the Recycled Water Agency to prevent contamination, damage or a public health hazard (Water District Recycled Water Program, 2012). As soon as possible the User must notify the Recycled Water Agency of the emergency modifications and file a written report. Standardizing recycled water use is extensive and may inhibit many public health concerns; however the risk of Legionellosis may persist. Sprinkler heads, and spray control may limit the extent of the risk of Legionellosis (Water District Recycled Water Program, 2012).

Recycled Water treatment process in Los Angeles County includes a three step treatment process, which is done at recycled or reclaimed water treatment plants such as the Los Angeles-Glendale Water Plant (4600 Colorado Blvd) or the Donald C. Tillman Water Reclamation Plant (6100 Woodley Ave, Van Nuys), the Hyperion Waste-Water plant (12000 Vista Del Mar) or the Terminal Island Water Reclamation Plant (445 Ferry Street, Los Angeles). The first step in the treatment of waste water is the settlement of greasy, waxy, and heavy material to in order to remove these impurities (Sanitation District of Los Angeles, 2012). The second step involves introducing aerobic microbes to the water, in order to digest organic material. After digestion, these compounds settled out of the water (Sanitation District of Los Angeles, 2012). The final step use coal and sand to filter out the remaining material (Los Angeles Cross Connection and Water Pollution Control, 2009).

#### Primary Treatment

Incoming water that will be treated for recycling water usage may contain many harmful contaminants (Sanitation District of Los Angeles, 2012). The heavier solid materials will settle to the bottom of large concrete tanks, and the lighter materials, like plastic and grease, which float to the top, are called primary sludge (Sanitation District of Los Angeles, 2012). The primary sludge is removed and returned to the sewers for further treatment. The remaining wastewater containing dissolved and suspended materials (mostly organic) moves to the second phase of treatment in aeration tanks and secondary settling basins (Los Angeles Cross Connection and Water Pollution Control, 2009).

#### Secondary Treatment

In the secondary treatment, aeration tanks at the treatment plants allow air bubbles through the water to supply oxygen (Sanitation District of Los Angeles, 2012). The same microorganisms in the wastewater grow as they feed on the organic materials in these tanks. In the secondary treatment settling tanks, the microorganisms clump together and settle to the bottom, where they are removed and some are recycled back into the treatment process (Sanitation District of Los Angeles, 2012). (Los Angeles Cross Connection and Water Pollution Control, 2009).

### Tertiary Treatment

Finally, the clean water soaks into the earth beneath the river and joins the underground water supply (Los Angeles Cross Connection and Water Pollution Control, 2009). This clean water is then collected by the treatment plants by filters, which remove any remaining suspended materials from the water (Sanitation District of Los Angeles, 2012). Typically, the filters contain layers of anthracite coal, sand, and gravel (Sanitation District of Los Angeles, 2012). The recycled water is then disinfected. It is now legally free of harmful bacteria and viruses and safe for human contact (not consumption), recharging groundwater, irrigational use, or other recycled water approved uses.

Recycled water may provide a new and innovative means to satisfy the urgent demand for non consumption water in Los Angeles County, but the more relax standard of water treatment may be a serious health concern. Some human pathogens, such as *L. pneumophila*, do not have to be consumed in order to cause harm. World Health Organization suggest that *L. pneumophila* may infect a host exposed to water way systems such as air conditioning systems, hotels, or other facilities (Benin, 2002) (World Health Organization, 2012). The bacteria live in water and colonize hot-water systems at temperatures of 20–50 °C (optimal 35–46 °C) (WHO, 2012). More stringent regulation of both sprinkler heads and spray control, as well as multiple biological disinfection steps may have a greater impact in the reducing the risk of Legionellosis.

### **Symptoms**

Legionellosis may be hard to diagnose as it may follow other pneumonia symptoms. The Center for Disease Control's Legionellosis symptoms include: a high fever, chills, and a cough. Some people may also suffer from muscle aches and headaches. Chest X-rays are needed to find the pneumonia caused by the bacteria, and other tests can be done on sputum (phlegm), as well as blood or urine to find evidence of the bacteria in the body. Legionellosis may be fatal in 5% to 30% of cases if not treated (Center for Disease Center, 2011). Most cases can be treated successfully with antibiotics and healthy people usually recover from infection (Center for Disease Center, 2011). Azithromycin and levofloxacin have become the standard Legionellosis treatments (Garrido, 2005). All the cases of Los Angeles County used in this analysis tested positive for urinary antigen test (UApos) and/or direct fluorescent-antibody (DFA)-positive, culture-negative specimens. DFApos is test performed on sputum/respiratory specimens from patients. UApos is urine antigen positive, the test most frequently performed when Legionellosis is suspected.

### **Objective**

To evaluate the environmental health risk posed by recycled water treatment and usage in Los Angeles County compared to potable water usage. Legionellosis is caused by the *microorganism L. pneumophila*, and is the causal agent that will be investigated (Figure 1). In this way, recycled water exposure was compared to non-recycled water exposure within Los Angeles County as it pertained to Legionellosis cases (Map1 and 2). This evaluation will assess if there is a statistically significant public health risk of Legionellosis posed by recycled water use in Los Angeles County Department of Public Health from 2005 to 2012. The risk of

Legionellosis from recycled water was found by conducting a relative risk of the all Legionellosis cases in Los Angeles County.

## **Rationale**

*L. pneumophila* is a robust microorganism that may live in recycled water and can cause Legionellosis. *L. pneumophila* accounts for 29% of all drinking water outbreaks since being reported in the United States (Craun, 2010) and is credited with over 90% of Legionellosis cases (Bouwknegt, 2012). *L. pneumophila* live within free living protozoa within the genera *Acanthamoeba* and *Naegleria* in the environment (Figure 1) (Shin, 2008) (Helen, 2012). Los Angeles County treats potable water and recycled water differently, which will result in different levels of *L. pneumophila* (Sanitation District of Los Angeles, 2012) (Los Angeles Cross Connection and Water Pollution Control, 2009). Both potable and recycled water quality are regulated by the Los Angeles County Cross Connections (Los Angeles Cross Connection and Water Pollution Control, 2009). LACC – Title 28, Plumbing, Sections 609.0 and 720.0 states that due to the difference in cleanliness, both waters must always be separate and recycled water must not be consumed for health reasons (Los Angeles Cross Connection and Water Pollution Control, 2009). Although recycled water may not be consumed, consumption of recycled water is not the primary mode of transmission of *L. pneumophila*. Even if the public follow the law (Title 28), they will continue to be exposed to *L. pneumophila* by aerosolization of the droplets of recycled water.

## **Materials and Methods**

The recycled water maps of Los Angeles County were provided by the Los Angeles County Cross Connections Water Division and West Basin Water District. All epidemiological demographic and Legionellosis case data were provided by the Los Angeles County Epidemiology Division.

The GIS Los Angeles County base line map was made possible and provided by the Los Angeles County GIS data portal. ArcGIS10.1 soft ware and use was provided by the Yale Geographic Information System. The Los Angeles County GIS data portal base line map of Los Angeles County potable water pipeline and street was used as the Basemap. The GIS portable document formatted- maps (provided by the West Basin Water District and Los Angeles County Cross Connections) were geocoded to reference ArcGIS10.1 (Map 1 & 2), superimposed onto the Basemap, and pinned together using intersection matches. In this way, the recycled water pipeline maps matched the Basemap. Using the trace mapping function of ArcGIS10.1, every recycled water pipeline (in purple) was traced on the Basemap. The final product was a map of Los Angeles County with the recycled water pipelines visible and colored in purple. Next a 3.2 kilometer buffer was greater on the recycled water pipelines on the Basemap according to the Addiss (1989) suggestions of the bacterium's infectious zone. Now the Basemap contained the recycled water pipeline trace as well as the 3.2 kilometer buffer surrounding the entire pipeline. Next, all cases that were positively diagnosed with community acquired Legionellosis were placed as dots on the Basemap. The Basemap containing all the 379 cases were now visually placed either within the exposed (pipeline and buffer) or unexposed area (Map 1 & 2). Exposed cases and unexposed cases along with their respective populations were now calculated. Every city that was either fully emerged or partially emerged in the exposed area was associated with the exposed cases, and the same was true for the unexposed cases. The cities' populations were

found using 2010 US census data provided by the US Census Bureau. ArcGIS10.1 identified 85 cases residents within the exposed area and 294 cases outside of the exposed area.

Epidemiological data used to assess the environmental risk of Legionellosis was retrospectively collected over a seven year period, from 2005 to 2012. A chi-squared test of significance was used to determine if there was a statistically significant difference in the period prevalence of Legionellosis, and a relative risk was used to assess the magnitude of the risk posed by recycled water pipeline within Los Angeles County. The 95% confidence interval was generated by finding the square root of the standard error of the variance of the natural log of the relative risk. Although the Los Angeles County Epidemiology Division provided the case data, the Acute Communicable Disease Control, Department of Public Health County of Los Angeles determined if the Legionellosis cases were derived from the bacterium in a hospital setting (nosocomial) or community environment (non-nosocomial).

## Results

The epidemiological data collected retrospectively collected over 2005 to 2012. A chi-squared test of significance was used to determine if there was a statistically significant difference in the period prevalence of Legionellosis within Los Angeles County from 2005-2012. A two by two table of cases and populations was constructed to perform the chi-squared test

$$\chi^2 = \sum \frac{(\text{Observed frequency} - \text{Expected frequency})^2}{\text{Expected frequency}}$$

(figure 3 poster).

The observed frequency was 85 (exposed cases) and the expected frequency was 57.41 (unexposed cases). The expected frequency was found by finding the unexposed prevalence ratio. This was done by taking the unexposed cases (294) and dividing them by their associated population (7,840,927) (Cities within the County of Los Angeles County, 2010). The newly found rate of Legionellosis in the unexposed area was then multiplied by the exposed population (1,530,963) to find the expected frequency (Cities within the County of Los Angeles County, 2010). The chi-squared test which compared the exposed cases and associated population within Los Angeles County, and the unexposed cases and the rest of Los Angeles County found a significant difference. With a chi-squared P-value of 0.001337, the observed incidence 85 cases from the exposed areas are not due to randomness.

A relative risk ratio was then done to identify the difference in risk of Legionellosis between the exposed and unexposed cases of Los Angeles County. The exposed cases were divided by their associated population, while the unexposed cases were divided by their associated population in order to find exposed and unexposed incidence rates (exposed =  $5.55176 \times 10^{-5}$ , unexposed =  $3.7491 \times 10^{-5}$ ). A relative risk was found, in order to indicate how larger the prevalence of Legionellosis may be compared to the unexposed population. The relative risk was found to be 1.4806. The confidence interval was found by taking the natural log (ln) of the relative risk. Next, the variance of the Ln of relative risk, Var (Ln(RR)). The standard error was found from the variance (SE Var(Ln(RR)) =  $((1 - \text{Risk of exposed}) / (\text{Risk of exposed}) (\text{population of exposed cities}) + ((1 - \text{Risk of unexposed}) / (\text{Risk of unexposed}) (\text{population of unexposed cities}))$ . For a 95% Confidence interval of the Ln Relative risk was added and subtracted from the (SE Var(Ln(RR)) =  $\pm 1.96(.1229)$  Next, both the Ln of the lower confidence limit and the Ln of the upper confidence limit are raised by e ( $e^{Lcl}$  and  $e^{Ucl}$ ). Finally the 95%

confidence limit was found to be 1.3088-1.6735, allowing the relative risk of 1.4806 to be statistically significant.

The Center for Disease Control's Mortality and Morbidity Weekly Report (MMWR) provided incidence rate data per 100,000 people. These statistics included national and pacific coast state incidence of Legionellosis were specific enough to confirm community acquired Legionellosis compared to hospital acquired Legionellosis.

## Discussion

There was a statistically significant difference in the number of cases within the recycled water pipe-lined area including the infectious zone, compared to the unexposed recycled water pipeline area. The Chi-squared P-value was 0.0013 comparing the exposed Legionellosis cases compared to the unexposed, suggesting that there is a statistically significant difference in what we should expect to see regarding Legionellosis cases. Individuals exposed to *L. pneumophila* from recycled water pipeline had a 1.4806 (1.3088, 1.6735) times greater risk of developing Legionellosis compared to individuals who were not exposed to *L. pneumophila* from recycled water pipeline.

Los Angeles County recognizes the risk of drinking recycled water, and therefore has taken steps to spread awareness of the presence of recycled water use. Signs, brochures, and pipeline color warn people to not consume this water (DPHEH, 2012). This may not reduce the risk of Legionella, as the mode of transmission is inhalation. Recycled water is essential to help alleviate the water stress of Los Angeles County. Although the current standard of recycled water cleanliness may not promote the public health, a more stringent standard involving more concentrated disinfection and filtration steps are needed. Public Water Supply Rule 16-D and Section 2.12 of the Rules Governing Water and Electric Services in the City of Los Angeles must include inhaled exposure of *L. pneumophila*. The first steps to reduce Legionellosis may include standardize of Filtration, Ozonation, Disinfection, and/or Fluorination for the Los Angeles-Glendale Water Plant, the Donald C. Tillman Water Reclamation, the Hyperion Waste-Water, and the Terminal Island Water Reclamation Plant to the degree that is used for potable water treatment.

A secondary recommendation involves a more standard means of recycled water treatment for the LCV associated amoebae. Treatment is recommended to be effective enough to eliminate the significant risk posed by *Acanthamoeba* and *Naegleria*. Although there are resources and monitoring of recycled water treatment, survey of recycled water in use is minimal. Recycled water monitoring in-use pipelines must not only be more done on a more regular basis, this basis must be standardized. The standard of cleanliness for potable water as defined by the (Los Angeles Cross Connection and Water Pollution Control, 2009) would serve a paragon template for future recycled water cleanliness.

Recycled water use compared to drinking water use may save the county 30-50% per year depending on the 3 scenarios depicted by Biagtan. In order to allocate more funding and environmental health specialists, it is in the best interest of the Environmental Health Department to find cost effective solution. Templates for cost effective strategies for recycled water cost can be found with both the Long Beach Water Department (Saving 30-50%) and City of Cerritos (Saving 47%). Cost effectiveness as well as the need to alleviate water stress caused by the increase in population may be the reason recycled water is used in agriculture/irrigation, groundwater- recharge, landscaping, and environmental use.

Another reason for the imperative changes needed to improve recycled water treatment is due to the risk infection to visiting populations. Recycle water pipelining is primarily found along popular locations such as South Bay beaches and northern Hollywood. It is biologically plausible to suspect that non-native Los Angeles County individuals that are exposed to LCV associated recycled water may be at a higher risk of Legionellosis. Foreign and domestic travel (particularly tourism) into these areas may significantly increase the risk of Legionellosis.

## **Limitations**

There are a number of limits and assumptions that were recognized in this paper. It was not possible to control for travel, travel restriction, residential changes, or variations both within Los Angeles County and Los Angeles County cities. Travelling within the exposure area of recycled water pipeline area would not have accurately been depicted from home address data. Therefore, travel into the exposed areas (such as vacationing, work, ect) was not controlled in this paper. Another limitation was the population variation within Los Angeles County. During the seven year period, (when this data was collected) there was no control for the residential movement of people.

Another limit that was not controlled was the individual dose of recycled water exposure measured in every individual of Los Angeles County. It may not be feasible to perform a urine antibody presence test for the 9,372,269 residence of Los Angeles County, nor is there an established biomarker to measure. A method to have controlled for recycled water usage may have been to provide recycled water use questionnaires. Selection random samples within both the exposed and unexposed populations (in order to more accurately reflect the population) may have shown differences in recycled water uses, and therefore shown difference in the relative risk of Legionellosis. It is important to remember that the exposures lay within tourist areas, and prime beach front property. Many of the cases may have been exposed to recycled water, however due to their address; these cases were not associated with the exposure. This limitation if corrected would only strengthen the already statistically significant association between recycled water exposure and Legionellosis.

Homeless cases of Legionellosis were not used in this analysis, because of the lack of residential address information. Due to this reason, the ArcGIS10.1 software would not have known where to place cases of Legionellosis without a permanent residence. Homeless individuals with Legionellosis were not identified either because they lacked a permanent address or expressed their homeless status. The relative risk found may not be the true Risk of Legionellosis from recycled water pipeline exposure. ArcGIS10.1 matched each address in question within Los Angeles County by percentage of likelihood. On a scale of 1-100, the likelihood factor determined how accurate the inputted address in ArcGIS10.1 was to the actual address in reality. Only addresses meeting a standard of sixty-five percent or higher were used in ArcGIS10.1.

Prevalence of Legionella over a seven year period is not a readily available statistic of disease surveillance within the United States. Not only was it not feasible to compare Los Angeles County recycled water exposed Legionellosis cases with national standards, a more important limit was the exposure of the national standards. This analysis did not compare to the national standards for two reasons. First, the extent of national recycled pipeline exposure with the same treatment standards and means such as in Los Angeles County was unknown. Second,

variations in population susceptibility, temperature, water droplet size and travel were not known.

Another limit to this paper was the need for standardization. Due to residential population variation over time, and variations in recycled water use points (end points), it was almost impossible to gauge magnitude of exposure. The magnitude of exposure refers to the proximity and frequency of recycled water use points. A recycled water use point in a section of the pipeline that releases the recycled water for the intended use.

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