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Effects of Lining Cooling Tower Interior Walls on Controlling the Growth of Legionella pneumophila

Kamal Rafiq Saeed Jaroor

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EFFECTS OF LINING COOLING TOWER INTERIOR WALLS ON CONTROLLING THE GROWTH OF \textit{LEGIONELLA PNEUMOPHILA}

By

Kamal Rafiq Saeed Jaroor

A thesis submitted to United Arab Emirates University in partial fulfillment of the requirements for the degree of M. Sc. in Environmental Science

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EFFECTS OF LINING COOLING TOWER INTERIOR WALLS ON CONTROLLING THE GROWTH OF LEGIONELLA PNEUMOPHILA

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ABSTRACT

The presence of *Legionella pneumophila* in cooling tower waters is a serious health hazard. If the contaminated mist from the cooling tower is inhaled, Legionnaires' disease could be contracted. Of the 2.4 million cases of pneumonia that occur each year in the United States, an estimated 8,000 to 18,000 are actually cases of Legionnaires' disease. Several factors have been suggested in the literature that enhance the high bacterial count in cooling towers including: temperature, pH, water stagnation, and presence of algae and protozoa.

On few occasions, water samples from the cooling tower at Dubai Hospital tested positive for *Legionella pneumophila* despite the routine application of chlorine. With additional doses of chlorine or bromine, *Legionella pneumophila* was not detected for a certain period of time after the shock treatment, but tested positive afterwards. The inner concrete surfaces of the cooling tower at Dubai Hospital were left without a smooth finish. Therefore, it is possible that the interior rough surfaces of the cooling tower enhance the development of *Legionella* due to water stagnation within the exposed large pores. Rough surfaces may also serve as a good environment for algal growth. No previous experimental work has been conducted to investigate the effect of lining cooling towers interior walls on the growth of *Legionella*. As such was the main objective of this study. Both the cooling tower at Dubai Hospital and a built prototype were used to determine *Legionella* count during the cold and the hot season of 2001. Results obtained in this study revealed that the growth of *Legionella* in the cooling tower and the prototype is linear under both lined and unlined conditions during the study duration. It was further
found that lining reduces the number of *Legionella* by about 37-68% but does not eliminate its presence in the cooling tower. In the prototype, the percent reduction in *Legionella* count due to lining appears to decrease with the time until it retains a constant value of approximately 30%. It was further found that, under similar conditions and sampling periods, the number of *Legionella* in the cooling tower were higher than those in the prototype despite the use of biocides in the cooling tower. This could be due to addition of make-up water to the cooling tower that may contain organic matter and some minerals which enhance the growth of *Legionella*. It was further demonstrated that there is an enhanced growth of *Legionella* during the hot season as compared to the cold season, but the increase was generally less than double with a 13.5°C change in temperature. A strong relationship ($R^2 = 0.86$) was found between *Legionella* count determined using swab sampling and that using water sampling.
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CHAPTER 1

INTRODUCTION

1.1 GENERAL

During the summer of 1976, the American Legion held a convention at the Bellevue-Stratford Hotel in Philadelphia (Stout and Yu, 1997; Adams, 1999; Sabria and Yu, 2002). Within days of the event, several attending veterans became deathly ill, 149 participants were stricken with a syndrome characterized by high unremitting fevers, rigors, and general prostration, frequently with rapidly progressive pulmonary infiltrates. The illness struck also 72 persons who did not attend the convention but were in or near the hotel over the same period. Thirty-four patients died of the syndrome, usually from rapidly progressive pneumonia or related complications. This mysterious illness was named "Legionnaires' Disease" by the press at that time (Freiji, 1996).

The offending agent, Legionella pneumophila, was identified by The Center for Disease Control in 1977 (Springston, 1999). As a result, a new bacterium was isolated from lung specimens. By comparing these bacteria with old bacterial samples, it became clear that the disease was not new but newly recognized. The first known hospital outbreak occurred in 1965 when eighty one patients at St. Elizabeth’s Hospital in Washington, D.C. developed pneumonia and 14 died. The cause of this pneumonia was not discovered at that time (Sabria and Yu, 2002). However, 12 years later, samples of frozen lung tissues from previous cases were tested and Legionella was found to be the cause (Freiji, 1996).

Legionnaires’ disease has flu like symptoms with muscle aches, headaches, loss of appetite, and dry cough. Slightly fewer than 50% of patients with Legionnaires’ disease suffer nausea, vomiting, and a watery diarrhea. Mental confusion is also not an uncommon symptom (Adams, 1999). Anyone may become infected but it is most
common among the elderly and those with impaired immune systems or underlying
diseases (e.g. cancer, kidney failure, diabetes, HIV infection, chronic lung disease or
heart failure); or medications (e.g. steroids, chemotherapy). Also, heavy smokers,
heavy drinkers, and people above 50 are at greater risk of acquiring the disease.
Furthermore, renal and heart transplant patients appear to have increased risk of
acquiring the disease (Freij i, 1996). However, there is no indication that Legionnai res’
disease can be transmitted from person to person (Abu Kwaik, 1998; Atlas, 1999).
Furthermore, the size of the infective dose of the organism required to produce
disease in human beings is still uncertain (O’Brien and Bhopal, 1993).

*Legionella* is found in virtually all natural aquatic environments, such as lakes, rivers,
and streams (Springston, 1999). It could be found in man-made habitats including
cooling towers, spas, and domestic water (hot and cold) distribution systems. Cultures
of soil have also indicated the presence of *Legionella* (Freiji and Barbaree, 1996).

### 1.2 Objectives

The aim of the present study was to investigate the impact of smoothening cooling
tower surface on the growth of *Legionella*. No previous experimental work has been
conducted to investigate the effect of lining cooling towers interior walls on
controlling the growth of *Legionella pneumophila*. It is hypothesized that rough
(unlined) surfaces facilitate formation of biofilm, which in return act as a harbor for
*Legionella* bacteria.

Had the objectives of this study been positive, cooling towers would be lined with a
smooth finish material to reduce the risk of Legionnaires’ disease and to reduce the
amount of chemical needed to control *Legionella* leading to substantial savings.
Furthermore, using fewer chemicals means less damage to the environment and
humans.

The specific objectives of this study were to:
1. Provide a baseline for existing bacteriological suitability of waters in cooling tower systems without lining.
2. Investigate the effect of lining of interior walls with a smooth finish material on the number of *Legionella pneumophila* on the wall surface and in the water.
3. Study the impact of seasonal variations on the colony forming units (CFU) of *Legionella* attached to the wall surface and in the cooling tower water.

### 1.3 SCOPE OF WORK

The present investigation was conducted using the cooling tower at Dubai Hospital for two seasons during the year 2001. Chemical analysis of the water was conducted along with the count of *Legionella pneumophila*. However, the presence of other bacteria and algae in the water and on the surface were not studied.

As the cooling tower could not be isolated into two sections, a prototype with two separate compartments (tanks) was constructed to simulate the cooling tower environment. One tank was constructed of concrete without lining and the other one was constructed of concrete but with ceramic-like-material lining. The idea was to investigate the impact of a rough surface on the growth of *Legionella pneumophila* in a well controlled environment. Colony forming units for *Legionella pneumophila* in the prototype were determined on both lined and unlined surfaces using swabs and water samples.

### 1.4 THESIS STRUCTURE

The thesis is organized into five chapters. This chapter describes the objectives and scope of work of this study. Chapter two reviews the literature pertaining to *Legionella* and the environmental and health aspects of the Legionnaires' Disease. Factors that affect *Legionella* growth were also explored in this chapter. Furthermore, Chapter 2 provides information on the amplifiers and disseminators of *Legionella* with emphasis on cooling towers. Chapter 3 provides description of the site where the experimental work was conducted. Site preparation including lining part of the
cooling tower and construction of the prototype are presented. Chapter 4 of this study presents the materials and methods used for chemical and bacteriological analysis of water and swab samples. The results of this study are presented in Chapter 5 along with a detailed discussion of the results. Finally, Chapter 6 concludes this work and sets some recommendations.
CHAPTER 2

LITERATURE REVIEW

2.1 LEGIONELLOSIS

Legionellosis refers to acute bacterial infections of humans caused by *Legionella pneumophila*. It has two distinct clinical forms: Legionnaires' disease and Pontiac fever (Adams, 1999). Legionnaires' disease is an acute respiratory infection caused by a distinctive gram-negative bacterium that was isolated from fatal cases of pneumonia among individuals attending an American Legion Convention in Philadelphia (Stout and Yu, 1997). On the other hand, Pontiac fever is a flu-like illness without pneumonia. In contrast to Legionnaires' disease, most of those who are exposed develop illness within 48 to 72 hours and most recover without antibiotic therapy. The first documented outbreak of Pontiac fever occurred in 1968 in Pontiac, Michigan in U.S.A. (Springston, 1999).

It is thought that Legionnaires' disease is rare, but the disease is quite common. Some cases go undetected because the required lab tests for confirmation are not ordered by the treating physician. Other cases are missed because the ordered lab tests are not sensitive enough to detect the disease (Freije, 1996). The Center for Disease Control estimates that 1,000 to 1,300 cases of Legionnaires' disease occur every year, but this is believed to be underestimated (Adams, 1999). However, the numbers of cases in the U.S.A. range from 8,000 to 18,000 per year (Kool et al., 1999).

2.1.1 The Organism

*Legionella* are aerobic, gram-negative bacteria (Springston, 1999) (see Fig. 2.1). In the laboratory, *Legionella* are fastidious in their growth requirements and will not grown on standard bacteriological media. A satisfactory medium consists of Buffered
Charcoal Yeast Extract (BYCE) agar at a pH of 6.9 and supplemented with L-cysteine, x-ketoglutanate (Bartie et al., 2003). By serological typing and other methods, at least 35 species and 54 serogroups of *Legionella* have been identified and some serogroups are further separated into subtypes (Fig. 2.2). More than 20 of these species have been linked to human disease. *Legionella pneumophila* is responsible for over 80% of Legionellosis and of its 16 serogroups, serogroup 1 is the most frequent to cause human infection (Springston, 1999; Rusin et al., 2003).

### 2.1.2 Epidemiology

The natural habitat of *Legionella* is fresh water of streams, lakes and thermal springs, moist soil and mud (Springston, 1999). They have been found worldwide in waters with temperatures varying from 5-62°C and pH of 5.4-8.2. Furthermore, it was found that *Legionella* can survive in marine environments (Heller, 1998). *Legionella* are inhibited by sodium chloride and are found in only small numbers, forming parts of the consortium of micro-organisms that makes up the biofilm (Kim et al., 2002).
2.2 EFFECTS OF LEGIONELLA ON HEALTH

Transmission of the organism to humans occurs by three routes. In the most common method, the individual inhales tiny droplets containing *Legionella* (Yu, 1993; Stout et al., 1997). However, according to Akbas and Yu (2001), aerosolization has been widely publicized but is relatively uncommon. This mechanism often involves devices that aerosolize contaminated water (Fig. 2.3). The second means for transmission is aspiration of water containing *Legionella* (Sabria and Yu, 2002). In aspiration, choking causes secretions in the mouth to go into the lungs rather than down the esophagus. Nasogastric tubes containing residual water contaminated with *Legionella* have been implicated in this process (Yu, 1993; Stout et al., 1997; Adams, 1999; Akbas and Yu, 2001). Bathing wounds in water containing *Legionella* comprises the third method (Adams, 1999). However, transmission of Legionnaires' disease from person to person has not been documented (Abukwaik, 1998; Atlas, 1999).

![Fig. 2.2 Legionella Species.](image-url)
Legionnaires' disease typically starts fairly abruptly, following an incubation period of 2-10 days, with high fevers, shivers, headache, and muscle pain. Pneumonia is demonstrated on X ray. Sometimes non-respiratory features, such as confusion and delirium or diarrhea are among the signs of Legionnaires' disease (Adams, 1999).

2.2.1 Clinical Manifestations

*Legionella pneumophila* infections occur in at least two distinct syndromes: Pontiac fever and Legionnaires' disease. Pontiac fever is an acute influenza-like illness characterized by fever, muscle pains, and headache. Cough, sore throat, diarrhea, confusion, and chest pain occur but are not usually prominent. Pneumonia does not occur, although one patient had a pleural friction rub during convalescence. The illness is debilitating for two to seven days, but all patients recover completely (Adams, 1997).

Legionnaires' disease varies in severity from a mild gripe to a severe multi-system disease affecting lungs, liver, kidney, gastrointestinal tract, and central nervous system. Chest radiographs can not be used to distinguish Legionnaires' disease from other pneumonias. As pneumonia progresses, death usually occurs. However, in few cases a syndrome resembling septic shock precedes death (Stout and Yu, 1997).

![Fig 2.3 Transmission of Legionella through aerosol.](image)
2.2.2 Laboratory Diagnosis

There is no distinctive clinical, biochemical or radioactive pattern that allows the early differentiation of Legionella infection from other causes of pneumonia. A range of microbiological procedures that can be used to diagnose Legionellosis in the laboratory, include:

- Culture on a permissive medium such as BCYE (buffered charcoal yeast extract) agar. It is considered to be the most sensitive method (Adams, 1999). Legionella does not grow on standard microbiologic medium in the lab (Stout and Yu, 1997; Sabria and Yu, 2002).
- Polymerase chain reaction. This method can be used to detect Legionella DNA in throat swab samples, serum, urine, and bronchoalvelar lavage fluid (Murdoch et al., 1999).
- Urinary antigen detection that can detect only Legionella pneumophila serogroup 1 (Lepine, 1998; Adams, 1999).
- Serological response.

2.2.3 Therapy

Antibiotics such as penicillin, cephalosporin, and aminoglycosides are not effective against Legionella infections (Adams, 1999). Azithromycin and clarithromycin have been used effectively in the treatment of Legionnaires’ disease (Stout et al., 1997; Stout and Yu, 1997; Adams, 1999). Delay in starting the appropriate treatment for Legionnaires’ disease significantly increases the chances of death (Stout and Yu, 1997).

2.3 FACTORS AFFECTING LEGIONELLA GROWTH

Several factors are favoring the growth of the Legionella bacteria including water temperatures, and presence of sediments, sludge, scale, and organic matter. Biofilm, sludge, and corrosion are protection for the Legionella against chemical and hot
treatments. Factors affecting the growth of *Legionella* in the environment are explored below.

**Temperature:**
Temperature is the most important factor that determines the survival of *Legionella* (Heller et al., 1998). The ideal temperature for the growth of the bacteria is between 35 to 46°C, indicating that *Legionella* is a mesophilic bacteria (Metcalf and Eddy, 1982). However, the bacteria can be present from 20 to 50°C (Springston, 1999). Figure 2.4 shows the optimum growth range. As noted by Cowles (2000), *Legionella* can not survive at temperatures above 60°C.

![Fig. 2.4 Optimum growth range for *Legionella* bacteria.](image)

**Scale:**
Scale is a source of nutrient for the *Legionella* bacteria (Cowles, 2000). In cooling towers, calcium carbonate is probably the most common form of scale. It is generally of a dense crystalline nature that adheres firmly to the underlying metal surface.
Biofilm:

Microorganisms seek solid surfaces to attach itself to. As the microorganisms grow and multiply, newly formed cells attach to each other and to the surface by creating slime. This deposition and growth result in a slime layer or biofilm which is widespread in nature and in man-made water systems. According to Atlas (1999), biofilm is identified as ecological niches in which *Legionella* survives, proliferates, and lies in wait for a susceptible host.

Bacteria and other organisms (host):

Bacteria and protozoa found in water systems can promote the replication of *Legionella* (Atlas, 1999; Kool et al., 1999). *Legionella* can infect and replicate within various protozoa found in soil and water (Springston, 1999). The virulence of *Legionella* may be increased by replicating in amoebae that can be isolated from the air (Stout et al., 1997). Droplets up to 100 µm in size can be suspended in the air and travel for long distances, so a few deeply inhaled *Legionella*-laden amoebae could cause infection, especially since *Legionella* successfully outgrow the host cell at body temperature (O'Brien et al., 1993). Atlas (1999) reported that high counts of *Legionella* were found when amoebae concentrations were also high.

Water stagnation:

Stagnant water allows bacteria to multiply rapidly as compared to non stagnant water. Freije and Barbaree (1996) observed that when stagnant water moves throughout the piping system, the bacteria would spread. Stagnation takes place in dead ends, showers, and faucets that are not used. Freije (2002) recommended that all unused faucets and showerheads be flushed regularly to avoid water stagnation that promotes multiplication of bacteria.

pH:

Research conducted by Freije (1996) has shown that the favorable range of pH for *Legionella* to grow is from 5.0 to 6.9. However, the favorable range of pH for *Legionella* to grow is from 2.0 to 9.5 (Springston, 1999).
Corrosion:
Corrosion is the destruction of material by interaction with the environment. Depending on the inherent corrosivity of the cooling water, corrosion control in open re-circulating cooling water systems may consist merely of controlling the pH, conductivity, and alkalinity levels (Freije, 1996). However, in systems with extensive piping networks, applications of corrosion inhibitors are often utilized to protect materials in the system. These inhibitors can include the following compounds: zinc salts, molybdate salts, triazoles, orthophosphates, polyphosphates, and polysilicates. Often, two or more of these inhibitors are used together. Oxidizing or nonoxidizing biocides also are required in cooling tower systems to inhibit biological activity. Rust is thought as a source of nutrient for the Legionella bacteria (Freije, 1996).

2.4 COOLING TOWERS

A cooling tower is generally used to cool liquid, mostly water that is being used for the cooling system of a machine or equipment. Cooling towers, as in the case of this study, are used to remove heat absorbed by the refrigerant gas during compression process of the water chiller at the condensing unit. The main purpose of a cooling tower is to provide a cost-effective means of a cooling system by re-circulating the same water, especially in large installations, where a large volume of cooling water is required (Freije, 1998).

The main components of a cooling tower system are sump tank, condenser pump unit complete with piping system, structure complete with forced draft fan, drift eliminators and fill material (Fig. 2.5). The condenser pump unit re-circulates the water through a piping system from the sump tank to the condensing unit of the water chiller, then to the ceramic cooling tower, equally distributed by low pressure nozzle and back to the sump tank. The re-circulated water absorbs the heat from the water chiller condensing unit and in turn, the forced draft fan and ceramic tiles remove the heat absorbed by the water.
Cooling towers have been linked to many outbreaks of Legionnaires' disease (Freije, 1998). Incoming makeup water may bring colloidal silts and organic materials, scale and corrosion buildup over time. The temperature and pH are within favorable range for bio-fouling. Also, cooling towers attract solid particles from large volumes of air drawn by the fans, especially if there is a building work or other dust generating activity taking place in the vicinity of the cooling tower. This leads to buildup of *Legionella* and the bio-film that protects it.

### 2.4.1 Types of Cooling Towers

Cooling towers are classified according to their types of ventilation used in cooling the re-circulated cooling water. Natural draft cooling towers are concrete counter flow hyperbolic structures and are used normally in large power generation industries. This type of cooling tower is generally used to minimize the operating cost. Mechanical draft cooling towers are concrete counter flow structure with one or multiple extract fans of multiple configurations and are used in medium size industries.

However, both types of cooling towers are using tile fill or PVC cellular fill material as a means of absorbing heat from the cooling water. Each cooling tower is custom designed to meet the needs of individual installations.

### 2.4.2 Design of Cooling Towers

During the normal operation of a cooling tower, aerosols are formed which will be carried into the environment through the tower exhaust. If *Legionella* is present in the tower water, breathing the formed aerosols can result in infection. Poorly maintained cooling towers have been implicated in outbreaks of Legionnaires' disease. According to the guidance issued by the Health and Safety Executive (1997), several precautions are recommended to control the risk associated with the spread of Legionnaires' disease from cooling towers. Among these recommendations are:

1. The cooling tower should be designed and constructed such that the release of aerosol is minimized (Freiji, 2002).
2. The materials used in its construction should not harbor or provide nutrients for bacteria and it should be readily be completely drained and cleaned.

3. It should be positioned as far away as possible from air conditioning and ventilation inlets.

4. All cooling towers, whether new or existing, should have effective efficiency drift eliminators (Freiji, 2002). Drift eliminators are normally positioned at the top of the tower where air is discharged in order to intercept water droplets.

5. The area above the cooling tower pond should be as well enclosed as possible to reduce the effects of the wind that can cause drift to escape through the sides, especially if it is poorly enclosed. This is particularly significant when the tower runs with its fan off. It may also be necessary to screen the tower or its pond to prevent the entry of birds, vermin or other debris or contaminants.

6. The water distribution system within the cooling tower should be designed to create as little spray as possible.

7. It should be constructed of materials which can be readily disinfected and which do not support microbial growth.

8. It should be maintained in a clean and sound condition.

9. The water quality of cooling towers should be well controlled.

2.4.3 Control of 
Legionella in Cooling Towers

To reduce or eliminate the risk of Legionellosis, it is necessary to minimize the concentrations of 
Legionella in the affected system. Cleaning of the system is very important in the overall control of 
Legionella. Without cleaning, a buildup of algae, fungi, protozoa, dirt and biofilm can occur in the system. Such buildup can place a greater demand on a biocide and may prevent it from making contact with microorganisms. Studies have indicated that bacteria sequestered in amoebae are afforded a 30- to 120-fold increase in protection from water treatment regimens (Springston, 1999). At a minimum, cooling tower systems should be bled (i.e., blowdown) and flushed twice a year. All surfaces should be thoroughly cleaned and allowed to air dry.
Because even well-maintained towers can still be colonized by *Legionella*, it is important to eliminate any possible transmission of drift from the tower to people. The first step is the use of high-efficiency drift eliminators. However, such
eliminators may actually aid in *Legionella* growth by increasing the system temperature. Another important step is to locate, if possible, the cooling towers as far away as possible from operable windows, outdoor air intakes, and outdoor areas frequented by people. At a minimum, outdoor air intakes should be located at least 7.5 m from cooling towers (Springston, 1999). In addition, prevailing wind directions must be taken into account with regard to air intakes and cooling towers.

The main purpose of adding chemicals to a cooling tower system is to maintain the system in a cost effective way and to control the growth of bacteria that are hazardous to health. However, extreme care should be taken in selecting the types of the chemicals, and ensure, whenever possible, that these chemicals are authorized by competent regulating society and are environmentally acceptable. Several chemicals are commonly used in the cooling tower system as described below.

**Scale and corrosion inhibitors:**

Scale and corrosion inhibitor chemicals are used to prevent anodic corrosion and to inhibit crystalline scale formation on the ferrous and non-ferrous surfaces of the cooling water system. These chemicals also help in improving the heat transfer efficiency and protect the system from the costly effects of corrosion.

**Biocide augment and mineral dispersant:**

Biocide augment and mineral dispersant chemicals are used to disperse sludge, silt, oil and clay in the cooling water system. It will increase the effectiveness of microbiocide by dispersing and penetrating bio-matters, slime and other foulants.

**Microbiocide:**

Microbiocide chemicals are used to control the growth of algae, bacteria, and fungi in re-circulating cooling water system and control of slime forming bacteria in heat exchangers. For cooling towers that have been implicated in outbreaks of Legionnaires' disease, or where high levels have been found, emergency treatment is necessary. As quoted by Springston (1999), one such procedure is to use a slug chlorination of the system with 50 ppm of free residual chlorine, along with a
A 10 ppm of free residual chlorine should than be maintained for 24 hours. The pH must be within a range of 7.5 to 8.0 for the chlorine to be effective. The entire system is drained and the chlorination process repeated. Bromine, which is less affected by pH levels, can be used in place of chlorine.

Biological treatment of cooling towers usually consists of the addition of algacides and bactericides, such as gluteraldehyde. These chemicals help to control multiplication of bacteria, protozoa and algae. This, in turn, reduces the amount of microbiota available to Legionella as a nutrient source. However, the control of other microorganisms does not necessarily indicate the control of Legionella, so any testing performed to determine the effectiveness of treatment must include sampling for viable Legionella bacteria. Biocides should be administered via a continuous feed rather than slug dosing, to maintain consistent concentrations in the system.

2.5 OTHER AMPLIFIERS AND DISSEMINATORS

Aside from cooling towers, several cases of Legionnaires' disease have been linked to domestic water system, spas and whirlpools, humidifiers, decorative fountains, faucets and shower heads (Atlas, 1999 and Mietzner et al., 2002), and fire fighting systems (Freije, 1998; Springston, 1999). These amplifiers and disseminators are briefly discussed in the following subsections.

2.5.1 Domestic Water System

Legionella is a common inhabitant of human-made water distribution system (Mietzner et al., 2002; Sabria and Yu, 2002). The calorifier is a major source of proliferation if it, or parts of it, contains water at 20-45°C (Freije, 1998). Water in storage calorifiers often stratifies so that although temperatures in the bulk of the calorifier may be high, there will be a cooler zone below the heating coils where Legionella can multiply due to presence of scale and biofilm. The scale and biofilm are not only food for the bacteria but also they provide protection from high temperature and any chemical disinfectants (Springston, 1999). This is rarely a
problem if the calorifier is in constant use and the off-take is steady. However, if use is intermittent, or there are sudden increases in demand so that the cooler water is not heated for a sufficient time, or if standby calorifiers are brought into use before the water is adequately heated throughout, *Legionella* laden water may be drawn into the distribution system. *Legionella* is therefore most likely to multiply in calorifiers where water temperatures are insufficiently high and in pipe work leading to taps and showers. Proliferation may occur if water temperatures are below 50°C (Freije, 1996).

In many investigations, Legionnaires' disease has been linked to hospital water systems (Goetz et al., 1998; Yu, 1998). According to Freiji (2001), the hot water system at a hospital in Pamplona, Spain was blamed for 18 cases of Legionnaires' disease that resulted in three deaths. As reported by Darelid et al. (2002), an outbreak of Legionnaires' disease happened in 1991 in a district general hospital in Sweden, which was traced to the hospital water system. The hot water temperature was raised from 45 to 60°C. The high temperature was maintained and *Legionella* became undetected in the water samples.

Cold water services present little risk in normal circumstances. However, there may be some danger from large cold water systems if use is intermittent, if water temperature exceeds 20°C, for example. This could occur due to high ambient temperatures and inadequate insulation, or if water is taken from sources such as boreholes. While the risk is generally small, it may be significant in premises where occupants are particularly susceptible. Furthermore, *Legionella* can be found in stagnated water present in dead legs or unused sections of piping (Springston, 1999).

### 2.5.2 Spas and Whirlpools

A spa is a bath or small pool in which bathers sit while warm water is constantly recirculated, often through high velocity jets or with the injection of air to agitate the water. The water is not changed after each user; instead it is filtered and chemically treated. The water temperature typically exceeds 30°C and the deliberate agitation can create a spray or aerosol above the surface of the water. Spas have been linked with
various infections including Legionnaires' disease (Freije, 1998; Benkel et al., 2000; Akbas and Yu, 2001). Careful maintenance, frequent cleaning of equipment such as filters, and regular water treatment is essential if the danger of infection is to be avoided.

A whirlpool bath is a bath fitted with high velocity water jets and/or air injection, but it does not incorporate water re-circulation as the water is discharged after each use. They do not, therefore, present the same dangers as spas but the precautions which apply to hot water services are appropriate (Cowles, 2000; Akbas and Yu, 2001).

2.5.3 Humidifiers

Atomizing humidifiers and spray-type air washers may incorporate, or take water from reservoirs or tanks which can store water at temperatures in excess of 20°C. Unless they are regularly cleaned and maintained they can become very heavily contaminated, especially in industrial environments (Cowles, 2000). The water is sprayed in the air conditioning system or, in some industrial applications, directly in the workplace.

The equipment and the water supplied to humidifiers that create a spray should be kept clean. Regular cleaning and disinfecting and not allowing water to stand for more than a day or so in equipment or tanks, especially in warm conditions is a must. Using water direct from the mains water supply rather than re-circulated or stored water will also reduce microbiological contamination, but equipment will need approval under water bylaws.

2.5.4 Decorative Fountains

Fountains can be ideal spreaders of Legionella if the water temperature reaches to 25°C or higher. According to Freije (2001), several cases of Legionnaires' disease were linked to decorative fountains.
2.5.5 Faucets and Shower Heads

When outlets are not in regular use and the water stays stagnant, the *Legionella* bacteria multiply. Therefore, weekly flushing can significantly reduce the number of *Legionella* discharged from the outlet. When this procedure is initiated it has to be sustained and logged as lapses can result in a critical increase in the *Legionella* at the outlet. Risk assessment may indicate the need for more frequent flushing - e.g. where there is a susceptible population present.

It is well documented that within 3 - 4 days *Legionella* has been shown to multiply in showers and taps to potentially hazardous levels; more frequent flushing should be considered (Straus et al., 1996). In one of the studies it was shown that the number of *Legionella* is directly related to the number of sediments in the water outlets (Heller et al., 1998).

Outlets usage cannot normally be monitored in private environments therefore the outlet will normally have to be flushed irrespective of their use to satisfy health and safety monitoring requirements. Designated shower flushers are potentially at increased risk.

2.5.6 Firefighting Systems

Firefighting systems involving sprinklers or hose reels may be permanently charged with water, often forming long 'dead-legs'. In the event of the system being employed, the risk from *Legionella* bacteria would be insignificant in comparison to the risk from the fire. However, there will be a possible risk during the testing and maintenance of these systems. The control measures as applied to cold water systems should be adopted so far as is practicable for firefighting systems. Testing procedures adopted should minimize the generation of aerosols, and the tests should be carried out at a time when the minimum number of people is likely to be affected.
2.6 RISK ASSESSMENT LEVELS

Today, there are no well-established standard limits to categorize the severity of contamination of water with *Legionella*. However, the fact that *Legionella* is present, either in heat rejection systems or water services, will not in itself cause disease. High numbers of *Legionella* have been noted in cooling towers and other sources with no associated disease (Springston, 1999). As noted by Springston (1999), most outbreaks from cooling towers and evaporative condensers have been associated with high numbers of *Legionella* of about 1,000 colony forming units per ml (CFU/ml) or more, in the implicated water source. In a potable water system associated with an outbreak, the reported numbers of *Legionella* averaged 160 CFU/ml (range less than 1 to 1500 CFU/ml), while as few as 10 CFU/ml in a fogger reservoir may have caused disease. As referenced by the Occupational Safety and Health Association (OSHA) and based on the various available data regarding outbreaks, a quantitative *Legionella* risk assessment criteria was developed as demonstrated in Table 2.1.

Table 2.1 Suggested *Legionella* risk assessment levels (from Springston, 1999).

<table>
<thead>
<tr>
<th>Legionella per ml</th>
<th>Cooling towers</th>
<th>Hot water systems</th>
<th>Humidifiers/Fogger</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>low</td>
<td>low</td>
<td>low</td>
</tr>
<tr>
<td>1-9</td>
<td>low</td>
<td>Low but increasing</td>
<td>Moderate</td>
</tr>
<tr>
<td>10-99</td>
<td>Low but increasing</td>
<td>Moderate</td>
<td>High</td>
</tr>
<tr>
<td>100-999</td>
<td>Moderate</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>&gt;1000</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
</tbody>
</table>
CHAPTER 3

SITE DESCRIPTION AND PREPARATION

3.1 SITE DESCRIPTION

The cooling tower in Dubai Hospital (Fig. 3.1) -model ct 797- was manufactured, built and installed by Ceramics Cooling Towers Company in October 1978. The cooling tower structure is constructed with concrete material and has ceramic tile fill. There are four cells installed to dissipate absorbed heat to the atmosphere, and the tower is sited in a separate structure from the central air-conditioning plant at the back of the main hospital building (Fig. 3.2).
The cooling tower is the forced induced draft type. Each cooling tower cell is designed to collect and dissipate the heat rejected from the centrifugal water chiller and absorbed by the cooling tower at a rate of 9.5 m$^3$ per minute. Typically, the water temperature is reduced by such action from 39 to 33°C.

![Diagram of cooling tower](image)

**Fig. 3.2** Cross section of cooling tower in Dubai Hospital.

The cooling water system circulates to the piping system and equipments and flows down from the nozzle spray system at a controlled pressure of 4 psig (Fig. 3.3), through the ceramics tile fill, and down to the common sump tank structure. The nozzles ensure that the entering condenser water is evenly distributed over the top of the ceramics tile fill surface area, to maximize the effective area of the ceramics tile fill in contact with the condenser water, thereby increasing heat transfer efficiency.

![Nozzle in cooling tower](image)

**Fig. 3.3** One of the nozzles in a cooling tower.
The ceramic tile fills on the cooling towers are of high-grade materials with long and proven reliability and meets the stringent specifications set by well-known society of materials testing. This hard-burned clay tile fill remains resistant to water treatment chemicals because of its water absorption rate of less than 4%, and if maintained properly, will have a life expectancy of about 50 years (Fig. 3.4).

![Fig. 3.4 Ceramic fill, corresponds to (K) in Fig 2.5.](image)

The four cells of the cooling tower are equipped with suitable drift eliminators to ensure extremely low water droplets carry over or mist that is carried out by the air which is forced through the ceramics tile fill by the forced draught fan installed on the uppermost portion of the cooling tower structure. The drift eliminators (Fig. 3.5) are made of chemical resistant reinforced molded fiberglass, and are firmly supported by stainless steel rods attached to the concrete structure.

![Fig. 3.5 Drift eliminators, corresponds to (C) in Fig. 2.5.](image)
The forced induced draught fan is made from fiberglass reinforced epoxy resin with a very high strength to weight ratio. It has very good chemical and corrosion resistant. The fan is driven by a 50 horsepower electric motor. Its blades are individually balanced to minimize vibration on the cooling tower structure. Temperature sensors fitted on the suction pipeline of the centrifugal condenser water pump automatically set the forced induced draft fan to operation.

The sump tank capacity of the cooling tower is 152 m$^3$, and within the structure of the sump, there are five individual pockets, which collect the ingress of waterborne sand and other foreign materials. The arrangement is such that the individual pockets are provided with individual drain valves to be able to flush out the accumulated ingress of waterborne materials.

The circulation of cooling water through the cooling tower system is maintained by four centrifugal condenser pumps installed adjacent to the cooling tower structure. The condenser pumps are arranged to operate on a duty basis with particular matching chiller and cooling tower unit. Each condenser pump is capable of re-circulating the cooling water of the cooling tower system at the rate of 8 m$^3$ per minute at 24.4m discharge head.

The main source of the heat absorbed by the cooling tower water-cooling system is from the four self-contained type centrifugal water-cooled chillers installed in the separate chiller plant room on the ground floor at the back of the main hospital building. Each centrifugal water chiller is capable of cooling down 6.4 m$^3$ per minute of chilled water, when supplied with 8 m$^3$ per minute of condenser water at 33°C (approximately 800 tons of refrigeration).

The four water cooled centrifugal chillers supply the required chilled water for the operation of air handling units, and fan coil units, which provide the necessary air conditioning system in the hospital. The heat absorption and heat dissipation between the centrifugal water chillers and the cooling towers is a continuous process and
3.2 SITE PREPARATION

3.2.1 Lining Material

The commercial name for the selected lining material is Nitocote EP405. This material comes in two separate tins: resin base tin and hardener tin. In this way the material in the two tins will not be active and it will be active only when the hardener is mixed with the base. This material was selected because it provides non-toxic coating which is chemical and corrosion resistant, its cost is low and it has long life, easy to apply and to clean, and has smooth gloss finish. Although the roughness of lined and unlined surfaces was not characterized, it was visually observed that lining provided smoother surfaces.

The application method is as follows:

- The surface was cleaned and dried.
- The contents of the resin base tin were thoroughly stirred to disperse any possible settlements.
- The entire contents of the hardener were poured into the base container.
- The two materials were mixed thoroughly using a slow speed drill fitted with a mixing paddle until both uniform color and consistency were obtained.
- The mix was then applied on the surface. The first coat was left to dry and then the second layer was applied to obtain a smooth finish.

3.2.2 Cooling Tower

The following preparations were carried out:

- The cooling tower was drained completely. This was done in conjunction with routine cleaning that is done twice a year.
- An area of four square meters was selected and divided into two equal sections.
- Both areas were cleaned and disinfected as well as the rest of the cooling tower.
- The surface of the first area was covered with the ceramic like material that has a smooth finish (Fig. 3.6).

![Fig. 3.6 Lining of one area in the cooling tower at Dubai Hospital.](image)

- The two adjacent areas were separated by a partition (Fig. 3.7). This way it was ensured that the two areas were totally isolated to prevent cross contamination.

![Fig. 3.7 Partition between the lined and the unlined areas.](image)
- The lined area was fitted with a second partition to prevent water current coming from cell 1 to cause any disturbance to the lined area.
- The surface of the second area was not lined as it was considered to be the control.
- The cooling tower was filled with water again to resume operation. Water treatment chemicals were added (Table 3.1).

Table 3.1 Chemicals used in the cooling tower.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Function of chemical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymate 5830</td>
<td>Inhibits scale and corrosion</td>
</tr>
<tr>
<td>Spertrus NX 1164</td>
<td>Aids the control of bacterial, fungal, and algal slime</td>
</tr>
<tr>
<td>Spertrus NX 1422</td>
<td>Control of microbial growth</td>
</tr>
<tr>
<td>Flomate 781</td>
<td>Antifoulant that inhibits the deposition of fouling material on heat exchange surfaces.</td>
</tr>
</tbody>
</table>

3.2.3 Prototype

As the four cells of the cooling tower could not be isolated from each other (to line one cooling tower at least with ceramic like material), a prototype with two separate compartments (tanks) was constructed to simulate the cooling tower environment (Fig. 3.8). One tank was constructed of concrete without lining (Fig. 3.9) and the other one was constructed of concrete but with ceramic like material lining (Fig. 3.10). The idea was to investigate the impact of rough surface on the growth of *Legionella* bacteria. In this way, it was possible to have a controlled environment that could help in the main study.

The prototype was built to represent one cell in the cooling tower but on a smaller scale. The dimensions of the prototype were: Length = 150 cm, width = 50 cm, and depth = 30 cm. The water was filled in each tank to a depth of 20 cm, resulting in a water volume of 0.180 m³ whereas the volume of water in one cell within the cooling
tower was 35.9 m$^3$. This makes the ratio between the two volumes 1:198. To better simulate flow conditions within the cooling tower, the flow rates should be at the same ratio so that similar hydraulic residence times would be obtained in the two systems. It should be indicated that the estimated hydraulic residence time of the cooling tower was based on the flow rate and the volume of the system rather than by determining the actual residence time using a tracer.

Fig. 3.8 Drawing of prototype.
The flow rate passing through one cell within the cooling tower was 8 m³/min, which means that the flow rate of the prototype pump should be approximately 0.038 m³/min. However, the design flow rate of the pump (Davey, model XF92) that was used was 0.028 m³/min.

Fig. 3.9 Unlined tank.

Fig. 3.10 Lined tank.
To make sure that the two tanks were subjected to the same conditions with the exception of lining, the following precautions were taken:

- The two tanks were identical in the dimensions and were kept under the same conditions. Both tanks were housed in a small secured shack (Fig. 3.11).
- The two tanks were kept close to each other to be under the same conditions, but separated so no cross contamination could take place.
- The water for both tanks came from the same source (potable water), and the water temperature was the same.
- The tanks were secured enough to prevent animal tampering.
- The tanks were protected against rainwater.
- The make up water was added equally to both tanks.
- No chemicals were added to any of the two tanks.
- The experiments using the two tanks were conducted at the same time.

Fig. 3.11 Prototype housing.
CHAPTER 4

MATERIALS AND METHODS

4.1 NATURE OF SAMPLES AND SAMPLING FREQUENCY

Both swabs and aqueous samples were collected from the cooling tower and the prototype at different periods of time. For each time, three swab samples (from untreated and treated areas) and two aqueous samples were collected and analyzed for the cooling tower. However, one aqueous and two swab samples were collected and analyzed for each tank in the prototype. Each swab sample was taken from a different location within the tested area. The number of samples and the duration of the study were limited by the cost and workload of the laboratory. The samples were taken both in winter and in summer of 2001. Five intervals were considered for each season (Table 4.1)

Table 4.1 Summary of biological samples collected from the cooling tower and prototype.

<table>
<thead>
<tr>
<th>Nature of sample</th>
<th>Number of samples and Frequency</th>
<th>Location</th>
<th>Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cooling Tower</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water sample</td>
<td>Two every two weeks</td>
<td>Cooling tower</td>
<td>5</td>
</tr>
<tr>
<td>Swab sample</td>
<td>Three every two weeks</td>
<td>Unlined area</td>
<td>5</td>
</tr>
<tr>
<td>Swab sample</td>
<td>Three every two weeks</td>
<td>Lined area</td>
<td>5</td>
</tr>
<tr>
<td><strong>Prototype</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water sample</td>
<td>One every two weeks</td>
<td>Unlined tank</td>
<td>5</td>
</tr>
<tr>
<td>Swab sample</td>
<td>Two every two weeks</td>
<td>Unlined tank</td>
<td>5</td>
</tr>
<tr>
<td>Swab sample</td>
<td>Two every two weeks</td>
<td>Lined tank</td>
<td>5</td>
</tr>
<tr>
<td>Water sample</td>
<td>One every two weeks</td>
<td>Lined tank</td>
<td>5</td>
</tr>
</tbody>
</table>
4.2 SAMPLE COLLECTION AND HOLDING TIME

Water samples for *Legionella* testing were collected in sterilized glass bottles while water samples for chemical analysis were collected in an un-sterilized glass bottles for the cooling tower but in sterilized bottles for the prototype. Swab samples for *Legionella* testing were collected using sterilized swabs and were placed in sterilized container for preservation and transportation.

**A. Water sampling procedure from cooling tower:**

The drain valve of the cooling tower was opened for 5 minutes to flush the stagnated water in the drain line. A 0.5 liter sterilized bottle was opened and completely filled with water sample. Then, the bottle was immediately closed with the cap and properly marked. Two water samples were collected for each sampling time. The bottles were collected and sent to the laboratory within 30 minutes after collection for microbiological analysis of *Legionella*.

Furthermore, one more water sample was collected in un-sterilized bottle for chemical analysis at site. One water sample was collected for chemical analysis for each sampling time.

**B. Water sampling procedure from prototype:**

A 0.5 liter sterile bottle was handled with sterile plastic gloves so it would not be contaminated from the outside. The bottle was opened and lowered into the unlined tank until it became full. Then, it was immediately closed with the cap. The same was done for another bottle. Both bottles were dried and disinfected with Hycolin from the outside to remove any contaminants. Finally, both bottles were properly marked and one was sent to the laboratory within 30 minutes after collection for microbiological analysis of *Legionella* while the other one was kept on site for chemical analysis. The same procedure was done for the lined tank.
C. Swab sampling procedure from cooling tower:

A four sided metal box (Fig. 4.1) and a lifting frame were fabricated in Dubai Hospital workshop to be used in the swab collection procedure. The length of the box was 2.44 m, the width was 2.44 m, and the height was 1.22 m. The box was put in position in the cooling tower with the help of a crane (Fig. 4.2 and 4.3). The lifting frame was lifted and positioned in the cooling tower using the crane as well (Fig. 4.4).

Fig. 4.1 Fabrication of the metal box.

Fig. 4.2 Lifting of the metal box with crane.
The idea was to lower the box, using the lifting frame, to circumference the selected areas for the study in the cooling tower and to drain the water which was housed by the box to be able to collect the swab samples. The lifting frame was used to lower and lift the box. The box was lowered in the water and lifted over the water using a hoist fixed on the lifting frame, which was kept in the cooling tower (Fig 4.5). The box was lowered slowly in the water so the bottom of the cooling tower was not disturbed. The water, which was housed by the box, was emptied using a submersible
pump to a depth of 5 cm due to technical limitation of the pump. The hanging ladder, which was fixed in a way that it would not touch the floor and it would not disturb the surface in any way (Fig. 4.3), was used to collect the swab sample. The swab seal cover was opened carefully. The swab was rubbed against a small area (0.5 cm²) evenly for two full rotations. This was done to assure that enough sample was collected. The wooden handle of the swab was cut one centimeter above the swab itself using sterile scissors. Each swab was kept in a sterile container with 2 ml of sterile distilled water. The same procedure was followed in collecting the swab samples from the unlined area and lined area. Three swabs from each area were taken for each sampling time. The containers were properly marked and were transported to the laboratory within 30 minutes after collection. In the laboratory, the sample volume was increased to 30 ml by adding sterile distilled water. The swabs were plated and the same techniques for water samples were followed.
D. Swab sampling procedure from prototype:

As the depth of the water in the two tanks was about 30 cm, the swabs were collected without draining the water. The swab seal cover was opened carefully. The swab was rubbed against a small area (0.5 cm²) evenly for two full rotations. This was done to assure that enough sample was collected. The wooden handle of the swab was cut 1 cm above the swab itself using sterile scissors. Each swab was kept in a sterile container with 2 ml of sterile distilled water. The containers were properly marked. This was done for the unlined tank and for the lined one as well. Three swabs from each tank were taken for each sampling time. Then, the samples were transported to the laboratory within 30 minutes after collection.

4. 3 MICROBIOLOGICAL ANALYSIS FOR LEGIONELLA

Experimental work was conducted at Dubai Hospital using its cooling tower while the microbiological testing for the presence of *Legionella* was conducted at the laboratories located at AL Maktoum Hospital, Dubai.

At the laboratory, the sample bottle was shaken to re-suspend any deposits that may have settled and 400 ml of the water sample from the bottle was poured into a sterile centrifuge bottle. The sample was centrifuged at 3000 rpm for 10 minutes. The supernatant was poured out gently and was discarded and the deposit was re-suspended in 30 ml sterile distilled water, which was poured into sterile container. This constitutes the prepared sample. Then, the prepared sample was divided into three portions of 10 ml each in three labeled sterile containers. This was done such that each would receive a different type of treatment including acid treatment, heat treatment, and no treatment (Bartie et al., 2003; Mietzner et al., 2002)

First, a petri dish of BCYE agar with PAV (Buffered charcoal yeast. Extract agar with Polymyxin B, Cycloheximide and Vancomycin medium) was inoculated with 0.1 ml of re-suspended deposit from container #1. The inoculum was redistributed with a spreader. This portion of the prepared sample was called the untreated portion.
Second, the 10 ml which was kept in container #2 was poured into a sterile centrifuge bottle and centrifuged at 3000 rpm for 10 minutes. Nine ml of the supernatant were drawn off with sterile pipette. The deposit was suspended by vigorous shaking. Nine ml of acid solution (KCl with pH=2.1) were added to the deposit and mixed gently to prevent over growth of competing flora (Mietzner et al., 2002). This was referred to as acid treatment. The mix was allowed to stand for 5 minutes. Another petri dish that has BCYE with PAV was inoculated with 0.1 ml of the mix. This was referred to as the second dish. A third dish that has BYCE with L-cysteine was inoculated with 0.1 ml of the mix. Third, one ml was taken from container #3 (prepared sample) into a sterile container and placed in a water bath at 56°C for 15 minutes. This was referred to as heat treatment. According to Mietzner et al. (2002), this procedure was useful if heavy growth of Psedomonas aeruginosa is present. A fourth dish of BYCE with PAV was inoculated with 0.1 ml of the heat treated sample. The plates were kept in the CO₂ incubator at 36°C for 10 days.

The plates were checked for growth after 3, 5, 7, and 10 days. The number of colonies in the plate were counted and multiplied by 10 to get the number of colony forming units per ml. Mietzner et al. (2002) reported that the colonies of Legionella are generally blue/grey in appearance, the colonies usually have an entire edge, a more dense lighter colored central region and a ground glass appearance (Figs. 4.7 a and c).

The purpose of using acid treatment and heat treatment was to kill other organisms in the sample. This would allow Legionella to grow freely without much competition. However, acid and heat treatments sometimes kill the Legionella bacteria as well. That is why one sample was left untreated. Representative pictures of the results of the different treatment methods are shown in Figs. 4.6 and 4.7.

If there was a growth, a subculture was done on blood agar and on MWY (modified Wadowsky Yee) agar (Bartie et al., 2003). After 3 days of incubation, if there was growth on the blood agar then there was no Legionella present in the sample as Legionella does not grow on blood agar (Mietzner et al., 2002). However, if there was a growth on the MWY agar and there was no growth on the blood agar, the sample
has *Legionella*. To determine if the *Legionella* is *pneumophila*, a direct fluorescent antibody (DFA) test was done (Bartie et al., 2003). If the DFA was positive, then the sample contains *Legionella pneumophila*. If the test was negative, the following procedure is used in the DFA using the kit from Mardx Diagnostic:

Suspensions of cultures of known or suspected *Legionella* bacteria in 1% natural formalin that gave a light turbidity were done in the biological safety cabinet. Smears were prepared on the slide and were air dried and heat fixed. Sufficient polyvalent test and negative control conjugate were added to one of each of the six serogroup wells. Also sufficient polyvalent test and negative control conjugate to the two wells of each test slide were added. The slides were placed in a moisture chamber and were stained for 20-30 minutes at room temperature. The excess conjugate was tapped off the slides were rinsed with a gentle stream of PBS (phosphate buffer saline with pH of 7.5) assuring that the test conjugate did not run over negative control conjugate reacted wells. The slides were immersed in PBS for 10 min and were rinsed briefly in

![Fig. 4.6 Untreated sample with fungus and Legionella bacteria.](image.png)
distilled water. The slides were removed from the PBS and were placed, antigen side up, on a dry paper towel. The 18 well blotter was carefully placed over the control slide so that the blotter was carefully indexed so as not to come in contact with the reaction wells. The blotter was held from the edge with one hand to keep the slide in place and sufficient gentle pressure with the microscope slide roller was applied to

Fig. 4.7 Petri dishes showing the same sample subject to (a) acid and (c) heat treatments with sub cultures of MWY plates shown in (b) and (d).
remove the moisture surrounding antigen wells. Care was taken not to allow the antigen wells to dry. Four to five drops of *Legionella* mounting medium were added to the slides which were covered with a cover slip avoiding air bubbles. The slides were read with a fluorescence microscope. The microscope was switched on, for at least five minutes before the slides were read. The slides were read within one hour to ensure proper readings. The slides were examined using a 40X objective initially. The observations were confirmed with 100X oil immersions objective if necessary. Brilliant yellow-green cell wall indicates a positive *Legionella* pneumophila while the absence of yellow green specific fluorescence indicates a negative sample. The control slides were read before the test slides to confirm the integrity of the test.

4.4 CHEMICAL ANALYSIS

Hardness, alkalinity, conductivity, chloride level, and pH for the water samples in the cooling tower and the prototype were determined using the well established techniques by the American Public Health Association (APHA). Furthermore, the water temperatures for the cooling tower and the prototype were determined.
CHAPTER 5

RESULTS AND DISCUSSION

5.1 TEMPERATURE VARIATIONS

Water temperatures in the cooling tower and the prototype were recorded each time a sample was taken. The results are presented in Fig. 5.1. The figure shows average variations in temperature between the two seasons was about 14°C. The figure also shows trends of increase of temperature within any season for both cooling tower and prototype although this increase was slight as indicated by the small standard deviation of any group of data which did not exceed 2.6°C (see Appendix B).

Fig. 5.1 Temperature variations in the cold and hot seasons for cooling tower and prototype.
Small variations between average temperature of cooling tower and prototype within the same season are possibly due to:

1. The cooling towers functions as a means for reducing the temperature of the water that comes back from the condenser side of the chiller. Such function does not exist in the prototype as there was just circulation of water.

2. The prototype was housed in a small shack that did not provide an open ventilation system.

Figure 5.1 also depicts that there appears to be a trend of increase in temperature over the study duration. This would be expected since the hot and the cold experiments were initiated at the beginning of the each season where an increase in ambient air temperature (see Appendix B) was observed. To further investigate whether such variations in temperature within each study duration was statistically significant, a test for significance of relationship between time and temperature was conducted.

Specifically, a test of the hypothesis that the slope of the relationship is different from zero (Anderson, 1987) was conducted. If the slope equals zero, then changing values of one variable are not related to changes in the other. Table 5.1 summarizes the results of the statistical analysis of the data. In the table the value of B is the slope of the best fit line of the data within a certain season for the prototype or the cooling tower. The values of t were obtained from the following equation

\[ t = \frac{|B|}{s_{y,x} \sqrt{\frac{1}{n} \left( \sum X^2 - \frac{(\sum X)^2}{n} \right)}} \]

where \( s_{y,x} \) is the standard deviation of temperature on time, X are the values of time and n is the number of data points. These values were compared to the critical value of \( t (t_{\text{critical}}) \) listed in student's t tables (double-sided).
Table 5.1 Significance of relationship between time and temperature.

<table>
<thead>
<tr>
<th>Location</th>
<th>Season</th>
<th>Slope (B)</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prototype</td>
<td>Hot</td>
<td>0.414</td>
<td>4.11</td>
</tr>
<tr>
<td>Prototype</td>
<td>Cold</td>
<td>0.643</td>
<td>5.04</td>
</tr>
<tr>
<td>Cooling tower</td>
<td>Hot</td>
<td>0.343</td>
<td>5.48</td>
</tr>
<tr>
<td>Cooling tower</td>
<td>Cold</td>
<td>0.357</td>
<td>3.73</td>
</tr>
</tbody>
</table>

$t_{critical} = 2.78$.

In all cases the $t$ values are greater than $t_{critical}$, indicating that the slope is different from zero. This means that there is a change of temperature with time within the same season. It should be noted that there was a daily fluctuation in temperature with each season. These fluctuations are much higher than fluctuations that were observed at the time when samples were taken. However, daily temperature fluctuations were not recorded.

5.2 CHEMICAL ANALYSIS

Variations in alkalinity, total dissolved solids (TDS) and chloride levels during the study period for water samples collected from the prototype and cooling tower are presented in Fig 5.2. Data are also listed in Appendix B. The figure shows that variations in these parameters for the prototype water over the study period do not fluctuate as is the case with results obtained from the cooling tower. The figure also illustrates that the values of the parameters are higher for the cooling tower water than corresponding values determined for the prototype water. Higher values are due to the addition of chemicals that would contribute to the three tested parameters. These chemicals are typically added to the cooling tower every two to three days. Fluctuation in the values is attributed to the difficulty of maintaining a constant level of chemicals that are added in the cooling tower.
Fig. 5.2 Alkalinity, TDS and chloride in the cooling tower and the prototype.
To investigate whether variations in the chemical parameters (i.e., alkalinity, TDS and chloride) within each season are statistically significant, a test for significance of relationship between time and the chemical parameter was conducted as explained above for the case of temperature. The results are summarized in Table 5.2. As shown in all cases the $t$ value is smaller than $t_{critical}$, indicating that the slope is not different from zero. This means that there is no significant change of alkalinity, TDS, or Cl levels with time within the same season.

<table>
<thead>
<tr>
<th>Season</th>
<th>Parameter</th>
<th>$t$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot</td>
<td>Alkalinity</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>TDS</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Chloride</td>
<td>0.03</td>
</tr>
<tr>
<td>Cold</td>
<td>Alkalinity</td>
<td>2.35</td>
</tr>
<tr>
<td></td>
<td>TDS</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>Chloride</td>
<td>0.28</td>
</tr>
</tbody>
</table>

$t_{critical} = 2.78$

5.3 Baseline of Legionella in Cooling Tower.

Results of swab samples taken from the unlined area of the cooling tower during the two seasons are presented in Fig. 5.3. The figure shows that Legionella count increases with time. The rate of growth in the hot season, however, appears to be higher than that in the cold season. The data for the hot season are more scattered than those collected in the cold season. The data were regressed using a linear fit and the regression equation along with the coefficient of determination ($R^2$) are shown on the figure. The close value of $R^2$ to 1.0 indicates a good fit of the data to the model used. The regression equations indicate that the growth of Legionella is almost double in the hot season as compared to the growth in the cold season.
Results of analysis of water samples from the cooling tower are shown in Fig. 5.4. Again, the results illustrate an increase in *Legionella* count within each season. The numbers of *Legionella* found are higher in the hot season as compared to those in the cold season for a given time after initiation of the study. Contrary to the results of the swabs, the number of *Legionella* in the water does not appear to start from zero at initial times. This is due to the fact that the floor of the cooling tower was properly cleaned and disinfected before the beginning of the experiment. However, for water samples, it appears from Fig. 5.4 that at $t = 0$ the CFU/ml should not be zero; even though the source water was free from *Legionella*. This is possibly due to presence of *Legionella* on the interior and exterior walls of the piping system within the cooling tower. It could also be due to the presence of *Legionella* in some areas in the cooling tower that were not accessed for disinfection. It should be realized that while the floor of the cooling tower was cleaned and disinfected properly, the piping system was not thoroughly cleaned due to technical limitations. Among such limitations is the inability to scrub the inside of all piping system to remove the biofilm. Formation of
biofilm has been noticed when the elbow joining the piping system with the chiller was disconnected for routine maintenance.

It should be indicated that once the cooling tower was filled with water and became ready for operation, two water samples were collected to obtain the first results at $t = 0$. The results were considered zero as *Legionella* was not detected in the samples. This could be true because the fresh water filling the cooling tower, which was tested negative for *Legionella*, did not have enough time to circulate and become contaminated to a level that can be detected in the laboratory. Such observation confirms that once the cooling tower was infected with *Legionella*, it becomes very hard or impossible to eradicate the *Legionella*.

The data presented in Fig. 5.4 were regressed with a linear fit with a non zero intercept. This resulted in a better fit to data than when a line forced through the origin was selected. It should be emphasized that the selection of linear model to fit the data in this case was possibly not the best choice.

![Fig. 5.4 Baseline of *Legionella* count in the water of the cooling tower.](image-url)
5.4 Effects of Lining Cooling Tower

Figure 5.5 compares between Legionella count in the swabs for lined and unlined conditions of the cooling tower during the cold (Fig. 5.5a) and hot (Fig. 5.5b) seasons.

![Graph showing Legionella count in swabs for lined and unlined conditions during cold and hot seasons.](image)

Fig. 5.5 Cooling tower swab for lined and unlined conditions during (a) cold and (b) hot seasons.
Obviously, lining reduces the number of *Legionella* detected in the swabs but does not eliminate the presence of this bacteria in the cooling tower.

Qualitative assessment of the effects of lining on the growth of *Legionella* was made through comparison of the slopes of the best fit lines of the data presented in Fig. 5.5. A linear fit with zero intercept was selected for data fitting. The appropriateness of using linear model with zero intercept over a model with a non-zero intercept was statistically verified using the method outlined by Anderson (1987). The slopes of the regression equations along with the values for the coefficient of determination ($R^2$) are shown in Table 5.3. The difference between the slopes of the lines for the lined and unlined case was obvious. To confirm the significance of this apparent difference, the confidence limits of the slopes were determined as shown in Table 5.3. The confidence interval for the slopes of the lined and unlined regression equations are not overlapping, indicating that there was a significant difference between the two conditions. Thus, lining reduces the number of *Legionella* in cooling towers.

The extent of reduction in *Legionella* for the two conditions as determined from the values of the two slopes ranges between 68% in the cold season and 37% in the hot season. This indicates that the effectiveness of lining on reducing *Legionella* was more pronounced in the cold season as compared to the hot season.

**Table 5.3** Confidence limits (CL) for the slope of the regression lines of *Legionella* count for lined and unlined conditions of the cooling tower.

<table>
<thead>
<tr>
<th>Season</th>
<th>Condition</th>
<th>Linear regression</th>
<th>95% CL for the slope</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Slope (B)</td>
<td>$R^2$</td>
</tr>
<tr>
<td>Cold</td>
<td>Lined</td>
<td>5.23</td>
<td>0.9915</td>
</tr>
<tr>
<td></td>
<td>Unlined</td>
<td>16.23</td>
<td>0.9972</td>
</tr>
<tr>
<td>Hot</td>
<td>Lined</td>
<td>18.58</td>
<td>0.9544</td>
</tr>
<tr>
<td></td>
<td>Unlined</td>
<td>29.44</td>
<td>0.9724</td>
</tr>
</tbody>
</table>
The ratio of *Legionella* count found in the swabs for the lined and unlined areas of the cooling tower is presented in Table 5.4. The table shows that during the cold season this ratio was almost constant, averaging 0.32. However, the ratio fluctuates during the hot season and averages 0.61. Again, since these two ratios were less than 1.0, it demonstrates that lining reduces *Legionella* growth.

<table>
<thead>
<tr>
<th>Time, weeks</th>
<th>Cold Season</th>
<th>Hot Season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lined</td>
<td>Unlined</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>36.7</td>
</tr>
<tr>
<td>4</td>
<td>23.3</td>
<td>60</td>
</tr>
<tr>
<td>6</td>
<td>28.3</td>
<td>96.7</td>
</tr>
<tr>
<td>8</td>
<td>41.7</td>
<td>128.3</td>
</tr>
<tr>
<td>10</td>
<td>53.3</td>
<td>165</td>
</tr>
</tbody>
</table>

**Table 5.4 Ratio of CFU/ml found in the swabs for the lined and unlined areas of the cooling tower**

5.5 *Legionella* in Prototype

The results of swab and water tests for the lined and unlined prototype tanks are presented in Figs. 5.6 and 5.7, respectively. In any season and for either swab or water testing, the number of *Legionella* count for the lined tank was lower than the corresponding one obtained in the unlined tank. Different than the behavior observed in the cooling tower, *Legionella* in the prototype required an acclimation period to a new system. This is why no or few *Legionella* count, in both swabs and water, were detected in the second week of the experiment. After the acclimation period, it appears that the growth of *Legionella* in the lined and unlined tanks follows a linear trend.
Fig. 5.6 *Legionella* count in swabs for lined and unlined Prototype tanks during (a) cold and (b) hot seasons.
Fig. 5.7 *Legionella* count in water for lined and unlined prototype tanks during (a) cold and (b) hot seasons.

Comparison between the number of *Legionella* in the cooling tower and those in the prototype for the same conditions reveals that the numbers in the cooling tower were higher than those in the prototype despite the fact that biocides were used in the
cooling tower and were not used in the prototype. This could be attributed to the following reasons: (1) the cooling tower system has already some *Legionella* attached to the interior walls of the pipes and the ceramic fills that were not cleaned while the prototype was properly disinfected, (2) due to excessive evaporation in the cooling tower, more water was added to replace such losses. Added tap water may contain substrate (organic matter) that acts as a source of food for *Legionella*, thus enhancing its growth. While the prototype did not receive much water as it was housed in a shack that reduced the evaporation rate and there were no fans as is the case with the cooling tower, and (3) chemicals added for treatment in the cooling tower have some minerals (phosphorus) that can promote the growth of the *Legionella*.

Percentage reduction in *Legionella* count using a lined tank as opposed to the use of an unlined tank is plotted in Fig. 5.8. There appears to be a decrease in the percent reduction with time and then reduction retains a constant value of approximately 30% starting week 8 and afterward. The solid line in Fig. 5.8 has been drawn manually to show such behavior.

![Graph showing percentage reduction of Legionella in the lined prototype tank as compared to the unlined tank.](image)

**Fig. 5.8** Percentage reduction of *Legionella* in the lined prototype tank as compared to the unlined tank.
5.6 Effects of Temperature on Legionella

To investigate the impact of temperature on the growth of Legionella in cooling towers, it was necessary that any potential impact of other parameters be eliminated or at least accounted for. Thus, it was necessary to prove that changes in chemical parameters are not statistically significant over the study duration. The average values of temperatures for the cooling tower and prototype within each season were analyzed to determine if they were really different from each other. For this, the F-test was used first to determine whether the unknown variances were equal or not. Then, the t-test was used to determine if the two average values are statistically different from each other. This was repeated for alkalinity, TDS, and chloride level in each system over the two seasons. The results are summarized in Table 5.5, which shows that temperature varies between the two seasons ($t > t_{critical}$) while the chloride level does not significantly vary ($t < t_{critical}$). As to alkalinity and TDS, it shows that there was no variation in the average value between the two seasons or the variation was slight ($t$ close to $t_{critical}$). Zanetti et al. (2000) investigated how the occurrence of Legionella count could be related to the chemical characteristics of water. These authors reported that there was no association between Legionella count and pH over the range of 7.4-8.1, or residual chlorine (0-0.2 mg/l), or hardness (0.7-2.6 mmol/l).

**Table 5.5** Significance of changes in temperature, alkalinity, TDS and chloride level between hot and cold seasons.

<table>
<thead>
<tr>
<th>Location</th>
<th>$t$ value</th>
<th>Temperature</th>
<th>Alkalinity</th>
<th>TDS</th>
<th>Chloride Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooling Tower</td>
<td>16.6</td>
<td>4.6</td>
<td>1.9</td>
<td>1.29</td>
<td></td>
</tr>
<tr>
<td>Prototype</td>
<td>11.3</td>
<td>1.8</td>
<td>6.4</td>
<td>0.59</td>
<td></td>
</tr>
</tbody>
</table>

$t_{critical} = 2.23$

All the data for the ratio of Legionella count found in hot and cold seasons were plotted in Fig. 5.9. Excluded from this figure are data points where the denominator was zero as well as data points collected at initial times. All the data were greater than
1.0, indicating that *Legionella* is more metabolically active during the hot season. It should be recalled that the difference in temperature between the hot (average 36°C) and cold (22.5°C) seasons is only 13.5°C.

Figure 5.9 further shows that for all cases, with the exception of swabs in the lined area, the ratio did not exceed 2.1. This indicates that growth in the hot season could only double the number of *Legionella* as compared to that in the cold season for a temperature difference of 13.5°C between the two seasons. For the case of swabs collected for the lined portion of the cooling tower, the ratio reached 4.5. The higher values obtained in this case could be due to abrasion that was caused by scratching the lining area while it was being cleaned after the cold season and before the start of the hot season.

The observed increase in *Legionella* count with the increase in temperature is well established. A relationship that is usually used to predict the rate of growth at a certain temperature from available data at another temperature is given by:

\[
\frac{r_{T_2}}{r_{T_1}} = \Theta^{(T_2-T_1)}
\]

where, \( r_{T_2} \) and \( r_{T_1} \) are the reaction rates at \( T_2 \) and \( T_1 \), respectively, and \( \Theta \) is the temperature activity coefficient. For a linear growth of *Legionella* in cooling towers, the ratio of the rate of growth at two different temperatures would be constant. For most of the data points in Fig. 5.9, the ratio between the *Legionella* counts in the hot season as compared to the cold season is 1.0–2.12. Given that the average temperature difference of the water between the two seasons is 13.5°C, the values of \( \Theta \) would range between 1.0-1.06 for most of the data points. This range of \( \Theta \) is comparable to that reported by Metcalf and Eddy (1982) for activated sludge system.

The increase in *Legionella* count observed in the hot season in the cooling tower and the prototype is in conflict with the results reported by Zanetti et al. (2002). These authors investigated the relationship between *Legionella* count and temperature (8-31°C) in dental units and found an inverse relationship between the two variables.
Heller et al. (1998) investigated the effects of different temperatures (4, 10, 20, 30 and 37°C) and various concentrations of sodium chloride solutions (0.1-3%) on the survival of *Legionella pneumophila*. They found that the combinations of high temperatures, i.e. 30 and 37°C, with NaCl concentrations over 1.5%, reduced cell numbers significantly.

![Graph showing the ratio of Legionella count found in hot-to-cold seasons.](image)

**Fig. 5.9** Ratio of *Legionella* count found in hot-to-cold seasons.

### 5.7 *Legionella* Count in Swab Versus Water Samples

Typically, swab samples and water samples are collected from faucets and shower heads to determine the *Legionella* count while in cooling towers only water samples are used to determine the *Legionella* count since it is difficult to obtain a swab from the floor of the cooling tower. Since, in this study, both water and swab samples were collected, it would be beneficial to see if a relationship between the two methods
Legionella count found in swab samples for both the cooling tower and the prototype were plotted against the Legionella counts obtained from the water samples as shown in Fig. 5.10. The figure contains 28 points out of an originally 36 data points obtained for all the cases studied with the exception of the swab data for the lined area of the cooling tower since the water samples in this case do not represent the behavior of a fully lined system. Eight results have been excluded from the graph as they were zero.

When the Legionella counts in the swabs versus corresponding ones in the water for the cooling tower were regressed, a coefficient of determination of 0.76 (n=10) was obtained (Table 5.6). As for the case of the prototype data an R² value of 0.82 (n=18) was obtained (Table 5.6). Since the slope of each regression line lies within the confidence interval of the slope of the other system, it is statistically justified to combine data from two systems and treat the pooled data as one set. By so doing, a strong linear relationship between Legionella count in swab and water samples exists (R²= 0.86) is observed. This suggests that either swab or water sampling can be used to determine the CFU for the Legionella. Given the difficulty encountered in collecting swab samples in the cooling tower, in particular, collection of water samples from the analysis of Legionella would then be sufficient.

Table 5.6 Linear relationship (zero-intercept) between Legionella counts determined by the swab and the water sampling method.

<table>
<thead>
<tr>
<th>System</th>
<th>Slope</th>
<th>R²</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooling tower</td>
<td>0.79±0.24</td>
<td>0.76</td>
<td>10</td>
</tr>
<tr>
<td>Prototype</td>
<td>0.98±0.25</td>
<td>0.82</td>
<td>18</td>
</tr>
<tr>
<td>For all data points</td>
<td>0.805±0.20</td>
<td>0.86</td>
<td>28</td>
</tr>
</tbody>
</table>

Mietzner et al. (2002) reported that swab samples yielded greater number of Legionella than water samples collected from the water fixtures such as shower heads and faucets. Furthermore, they indicated that surface sampling is not recommended for cooling towers given the high levels of competing bacteria found in these systems.
Fig. 5.10 Relationship between *Legionella* counts in swabs and water samples.
CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

Based on the results obtained in this study, it was concluded that:

1. The growth of *Legionella* in the cooling tower was linear under both lined and unlined conditions.
2. Prototype results were consistent with those of the cooling tower with the exception of the first 4 weeks where bacterial growth in a new system requires an acclimation period.
3. Lining reduces the number of *Legionella* but does not eliminate its presence in the cooling tower.
4. The extent of reduction in *Legionella* under lined and unlined conditions ranges between 68% in the cold season and 37% in the hot season in the cooling tower. In the prototype, there appears to be a decrease in the percent reduction with time and then reduction retains a constant value of approximately 30% starting 8 weeks after the initiation of the experiment.
5. For similar conditions and sampling periods, the number of *Legionella* in the cooling tower was higher than the number in the prototype despite the use of biocides in the cooling tower. This was probably due to addition of makeup water that may contain organic matter and due to the presence of some minerals in the chemicals that are routinely used in the cooling tower treatment.
6. The ratio of *Legionella* count in hot-to-cold season was greater than 1.0. This indicates enhanced growth during the hot season. The ratio, however, did not exceed 2.1 for most of the cases examined.
7. A strong linear relationship ($R^2 = 0.86$) exists between Legionella count determined using swab sampling and that using water sampling.

RECOMMENDATIONS

1. Lining of concrete cooling towers is recommended as it reduces the growth of Legionella.
2. Since Legionella testing is costly and time consuming, it will be sufficient to analyze either water or swab samples; as it was shown that a strong relationship exists between the results of the two different types of samples.
3. Disinfection of Dubai Hospital cooling tower should be performed more than twice a year to reduce the number of Legionella in the system. However, this may not be practical during the hot season as the disinfection process requires a complete shut down of the tower for 4-6 hours.
4. Cleaning of the cooling tower should cover the inner of the piping system which may be accomplished mechanically by using cleaning rods.
REFERENCES


Table A.1 *Legionella* count in water and swab samples from the cooling tower.

<table>
<thead>
<tr>
<th>DESCRIPTION</th>
<th>UNLINED AREA</th>
<th>LINED AREA</th>
<th>WATER SAMPLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collection Date</td>
<td>Swab 1</td>
<td>Swab 2</td>
<td>Swab 3</td>
</tr>
<tr>
<td>8.1.2001</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>22.1.2001</td>
<td>30</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>5.2.2001</td>
<td>50</td>
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<td>60</td>
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<td>19.2.2001</td>
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<td>90</td>
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<td>5.3.2001</td>
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<td>19.3.2001</td>
<td>200</td>
<td>165</td>
<td>130</td>
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<td>0</td>
<td>0</td>
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<td>30</td>
<td>50</td>
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<td>4.6.2001</td>
<td>120</td>
<td>70</td>
<td>90</td>
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<td>18.6.200</td>
<td>235</td>
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<td>200</td>
</tr>
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<td>2.7.2001</td>
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<td>225</td>
</tr>
<tr>
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<td>320</td>
<td>275</td>
<td>285</td>
</tr>
</tbody>
</table>

Table A.2 *Legionella* count in water and swab samples from the prototype.

<table>
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<tr>
<th>DESCRIPTION</th>
<th>UNLINED TANK</th>
<th>LINED TANK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collection Date</td>
<td>Swab 1</td>
<td>Swab 2</td>
</tr>
<tr>
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<td>0</td>
</tr>
<tr>
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</tr>
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<td>30</td>
</tr>
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<tr>
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</tr>
<tr>
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<td>0</td>
</tr>
<tr>
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<td>0</td>
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<td>35</td>
</tr>
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</tr>
<tr>
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<td>75</td>
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</table>
### APPENDIX B

Table B.1 Chemical characteristics and temperature of water samples from the cooling tower.

<table>
<thead>
<tr>
<th>Date of collection</th>
<th>Temperature °C</th>
<th>pH</th>
<th>Alkalinity mg/L as CaCO₃</th>
<th>TDS mg/L</th>
<th>Chloride mg/L</th>
<th>Conductivity μS/cm</th>
</tr>
</thead>
<tbody>
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<td>2615</td>
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<td>3000</td>
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<td>2315</td>
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<td>380</td>
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<td>900</td>
<td>3360</td>
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</table>

Table B.2 Chemical characteristics and temperature of water samples from the prototype.

<table>
<thead>
<tr>
<th>Date of collection</th>
<th>Temperature °C</th>
<th>pH</th>
<th>Alkalinity mg/L as CaCO₃</th>
<th>TDS mg/L</th>
<th>Chloride mg/L</th>
<th>Conductivity μS/cm</th>
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</thead>
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<td>50</td>
<td>269</td>
<td>110</td>
<td>385</td>
</tr>
</tbody>
</table>
إن وجود ميكروب اللجنائيلا نيموفيلا في مياه أبراج التبريد يعتبر مشكلة صحية خطيرة. حيث أنه في حال تم استشاط الرودات الملوث فقد يتسبب ذلك بالإصابة بمرض (Legionnaires’ Disease) الذي له نفس أعراض مرض الأنتهار الرئوي. وتشير التقديرات أن من بين 2.4 مليون إصابة بالأنتهار الرئوي سنويًا في الولايات المتحدة هناك 8000 إلى 18000 إصابة فعلية بهذا المرض. وتوجد عدة عوامل محتملة تؤدي إلى زيادة في نمو هذه البكتيريا في أبراج التبريد ومنها: درجة الحرارة والإس الهيدروجيني وركود المياه ووجود الطحالب ووجود الألواح (البروتوزوا) التي يمكن أن تكون عائل للكثيرا اللجنائيلا نيموفيلا. وفي عدة مرات أظهرت عينات الماء المأخوذة من برج التبريد في مستشفى دبي وجود بكتيريا اللجنائيلا نيموفيلا على الرغم من استعمال الكلور بشكل دائم في برج التبريد. وتجدر الإشارة إلى أن الأسطح الحساسية الداخلية لبرج التبريد في مستشفى دبي غير منظمة. وعلى هذا فأنه من المحتمل أن يقوم السطح الداخلي بزيادة عملية نمو هذه البكتيريا بسبب ركود المياه. بالإضافة إلى هذا فإن الأسطح الخشنة ربما تعمل كتلة مناسبة للماء الطحالب. وهذا الاحتمال لا يزال بحاجة إلى إثبات علمي حيث أنه لم يتم دراسة سابقة في هذا الموضوع.

إن الهدف من هذه الرسالة هو دراسة تأثير تبطن أو عدم تبطن الجدران الداخلية لأبراج التبريد في مستشفى دبي في التحكم في نمو ميكروب اللجنائيلا نيموفيلا. وتمت هذه الدراسة في فصول منفصلتين خلال سنة 2001. وحيث أنه لم يكن بالإمكان عزل برج التبريد إلى مناطق تم تفضيلها مبلغاً على نمو بحثاً أولي (Prototype) من خزائن منفصلين من الخرسانية وتبطنين الخزان الأول أما الثاني فترك دون تبطن.

لقد كشفت النتائج التي تم الحصول عليها أن نمو بكتيريا اللجنائيلا نيموفيلا في برج التبريد والنموذج الأولي بشكل علامة خط مستقيم (linear) في حالة التبطن وفي حالة عدم التبطن. وتم أيضاً الكشف عن أن التبطن يقلل من نمو هذه البكتيريا بنسبة تتراوح ما بين 37-86% ولكننا لا منهجه نهائياً في برج التبريد. أما درجة التخفيض من نمو البكتيريا في النموذج الأولي المطلوب فقد تبين أنها تقل مع مرور الزمن حتى وصلت إلى نسبة 30% تقريباً. وتوصلت هذه الدراسة أيضاً أنه تحت نفس الظروف في نفس الوقت من الدراسة فإن عدد بكتيريا اللجنائيلا في برج التبريد كان أعلى من عدد البكتيريا الموجودة في النموذج الأولي على الرغم من استعمال (Biocides) في برج التبريد. وربما تكون المياه التي تصل إلى برج التبريد بما تحتويه من مواد عضوية ومعادن تعزز من نمو هذه البكتيريا. وتم إثبات أن هناك زيادة في نمو هذه البكتيريا في الفصل الحار إذا ما قورنت بالفصل البارد. ولكن هذه الزيادة هي أقل من الضعف في معظم الحالات التي تم دراستها. وقد أظهرت هذه الدراسة وجود علاقة قوية بين النتائج التي تم الوصول إليها عن طريق أخذ عينات من المياه (swabs) أو المسحات (water samples)
تأثير تبطين الجدران الداخلية لأبراج التبريد في المحكم في تمو ميكروب الليجاناتلا نيفوفيلالا

رسالة مقدمة من الطالب
كمال رفيق سعيد جمرور

رسالة مقدمة إلى
جامعة الإمارات العربية المتحدة
إسكلالا منطلبات الحصول على درجة الماجستير في علوم البيئة

2003