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An Alkaline Approach to Treating Cooling Towers for Control of *Legionella pneumophila*

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Earlier field and laboratory studies have shown that *Legionella* species survive and multiply in the pH range 5.5 to 9.2. Additionally, the technical feasibility of operating cooling towers at elevated alkalinities and pH has previously been documented by published guidelines. The guidelines indicate that these conditions facilitate corrosion control and favor chlorine persistence which enhances the effectiveness of continuous chlorination in biofouling control. This information suggests that control of *Legionella* species in cooling towers can be accomplished by operating the towers under alkaline conditions. To test this possibility, we collected water samples over a period of months from a hospital cooling tower. The samples were analyzed for a variety of chemical parameters. Subsamples were pasteurized and inoculated with non-agar-passaged *Legionella pneumophila* which had been maintained in tap water. Correlation of subsequent *Legionella* growth with corresponding pH and alkalinity values revealed statistically significant inverse associations. These data support the hypothesis that operating cooling towers outside of the optimal conditions for *Legionella* growth (e.g., at elevated alkalinities and a pH greater than 9) may be a useful approach to controlling growth in this habitat.

Cooling towers and evaporative condensers have been implicated in a number of outbreaks of Legionnaires disease (4, 8, 9, 17, 19). Surveys of various heat rejection systems, including well-maintained ones, have indicated that contamination with *Legionella* species and other bacteria is relatively common (1, 11, 14, 27, 28, 30, 40). A number of biocides have been shown to be effective against legionellae in the laboratory (20, 24, 28, 33, 39). Treatment with these biocides has been generally unsuccessful under field conditions since cooling towers typically contain organic matter and are exposed to light and aeration and since legionellae in this habitat may be protected from disinfectants by extracellular products of other microflora (5, 11, 16, 21, 27, 28). Chlorination appears to be the most reliable treatment to date (13, 14). However, *Legionella pneumophila* has been shown to be relatively resistant to disinfection with chlorine (25, 26), and heavy chlorination can cause increased cooling system corrosion. In light of these problems it has been suggested that better methods are needed to control *Legionella* contamination in cooling systems (4, 5, 8, 11, 31, 32).

Previous studies indicate that *Legionella* species are pH sensitive. Surveys of natural habitats have detected this organism in the pH range 5.5 to 8.1 (15). Similarly, laboratory studies have shown that *L. pneumophila* multiplies in tap water only in the pH range 5.5 to 9.2 (36).

The pH of cooling water is also an important consideration in the treatment programs of heat rejection systems. Corrosion, scaling, and biofouling are processes that can minimize heat transfer, reduce flow rates, and cause premature equipment failure in these systems. Recent guidelines for the chemical treatment of cooling towers have encouraged maintenance at higher pH levels (23). A higher-pH environment improves corrosion control. Additionally, owing to enhanced chlorine persistence, continuous chlorination at higher pH has been demonstrated to be more effective for general biofouling control than intermittent chlorination at low pH (7).

The documented pH limitations of *Legionella* species and the feasibility of maintaining higher pH levels in cooling systems suggest that operating under alkaline conditions can be a suitable method for controlling legionellae in cooling towers and evaporative condensers. To test this hypothesis, we conducted a study in which water samples were collected from the condenser and cooling tower basin of a hospital cooling system over 5 months. Chemical analyses were conducted on these samples. Subsamples were pasteurized and inoculated with naturally occurring *L. pneumophila*. Correlations were then calculated between *Legionella* growth and chemical composition. The purpose was to determine the extent to which pH, alkalinity, and other chemical parameters affect *Legionella* growth in this environment and to evaluate the potential utilization of these factors for bacterial control.

MATERIALS AND METHODS

Bacteria. A water sample was collected from another cooling tower located in the same hospital as the tower used in this investigation. This sample served as the initial source of the naturally occurring bacteria used in the growth studies. The sample contained 10^5 CFU of *L. pneumophila* per ml and a substantial number of unidentified non-*Legionellaceae* bacteria. Direct immunofluorescence testing indicated that the isolate of *L. pneumophila* belonged to serogroup 1.

These naturally occurring *L. pneumophila* and associated bacteria were maintained in the laboratory as a water stock culture by periodic transfer into filter-sterilized tap water. Details on the maintenance of the water stock culture have been presented previously (34). Use of non-agar-passaged legionellae from a water stock culture is advantageous in light of the demonstrated changes in growth, virulence, and

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TABLE 1. Chemical characteristics of cooling tower samples

Parameter	Basin			Condenser			Tap water (control) (avg)[a]
	Range[a]	Avg	SD	Range[a]	Avg	SD	
pH	6.83–8.63	7.83	0.53	6.98–8.46	7.82	0.44	7.88
Hardness	192–1,284	776	301	528–1,276	853	167	102
Ca	46–442	244	106	154–434	273	62	30
Mg	9.70–55.4	40.2	12.5	34.0–53.5	42.5	5.64	6.8
Alkalinity	36–208	131	56	84–210	143	39.2	30
Cl	80–340	196	68	160–310	219	43	20
Total organic carbon	3.76–18.1	11.0	3.84	7.61–18.5	11.9	3.58	1.66
Total dissolved solids	221–1,840	1,002	430	682–1,670	1,118	223	154
SO_4	363–1,088	747	201	450–1,075	819	141	105

[a] All results expressed as milligrams per liter except pH.

resistance to disinfection exhibited by bacteria maintained on artificial medium (6, 12, 25, 30).

L. pneumophila was enumerated by plating dilutions of water cultures on differential glycine-vancomycin-polymyxin B (DGVP) agar (37). Non-*Legionellaceae* bacteria, when counted, were enumerated by plating dilutions of the culture on UNBCYE agar (38). Before counting, the plates were incubated for 6 days at 35°C in sealed plastic bags to prevent dehydration.

Chemical analyses. Thirteen sets of water samples were collected at approximately 2-week intervals from the hospital cooling tower basin and condenser during July through November 1984. A chemical analysis was conducted on each sample. Chloride, sulfate, alkalinity, pH, dissolved solids, hardness, calcium, and magnesium were measured by mercuric nitrate, turbidimetric, sulfuric acid titrimetric, electrometric, conductivity, and EDTA methods as described by the American Public Health Association (2). Organic carbon content was determined with a Dohrman Envirotech (Santa Clara, Calif.) DC54 total organic carbon analyzer. The concentrations of nine metals (see Table 2) were measured by flame or graphite furnace techniques with an atomic absorption spectrophotometer (model 503; The Perkin-Elmer Corp., Norwalk, Conn.).

Multiplication studies. Each of the basin and condenser samples collected from the cooling system was evaluated for its ability to support *Legionella* growth. After collection, the samples were stored in the dark at 4°C. At the beginning of the experiment, 100-ml unfiltered portions of each of the samples were pasteurized in a water bath by incubation for 30 min at 60°C. While this treatment eliminates legionellae and associated bacteria, it does not remove the dead organisms and may not eliminate certain microorganisms such as amoebic cysts. Membrane filtration was not employed since a portion of the metals in the samples was in suspended rather than dissolved form and filtration would have altered the chemical composition of the samples. After pasteurization, each 100-ml sample was inoculated with 1.0 ml of the water stock culture containing *L. pneumophila* in the late exponential phase. The initial *Legionella* concentration in the sample was approximately 10^3 CFU/ml. The inoculated samples were then incubated in the dark in air at 35°C. Each sample was cultured weekly on DGVP agar for a 5-week period to monitor the growth of *L. pneumophila*.

RESULTS

Chemical survey. The results of wet chemical and organic carbon analysis of the 13 basin and condenser samples are summarized in Table 1. The concentrations of nine metals in these samples are described in Table 2. The chemical characteristics of a tap water sample typical of that used for the cooling system makeup water have been included for purposes of comparison. As the data indicate, chemical conditions varied widely for almost all parameters over the 5-month period (e.g., alkalinity varied from 36 to 210 mg/liter; Mo concentrations ranged from 0.53 to 45.5 mg/liter). Additionally, with the exception of pH, Mn, and Cd, the values of all chemical factors in the samples differed substantially from those in tap water.

Multiplication experiment. To investigate the effects of various chemical conditions on *Legionella* growth, we inoculated the basin and condenser samples with water stock culture. Over a 5-week period the observed growth varied substantially from sample to sample. Multiplication ranged from a low of 0.19 log CFU/ml to a high of 2.59 log CFU/ml. Computer-assisted linear correlation analyses were performed to examine the association between *L. pneumophila* growth and chemical characteristics. Simple correlation co-

TABLE 2. Metals in cooling tower samples

Metal	Concn (mg/liter)						
	Basin			Condenser			Tap water (control) (avg)
	Range	Avg	SD	Range	Avg	SD	
Fe	0.109–0.924	0.372	0.262	0.365–2.158	1.324	0.625	0.038
Mn	0.018–0.097	0.047	0.023	0.036–0.103	0.066	0.023	<0.010
Al	1.18–8.81	5.85	2.24	3.26–8.78	6.77	2.08	0.820
Na	14.60–32.44	29.93	5.71	31.54–32.40	32.15	0.24	8.60
K	2.50–15.46	10.79	3.98	8.60–15.38	12.35	1.60	1.50
Zn	0.472–2.368	1.156	0.746	0.680–2.829	1.343	0.744	<0.010
Cd	0.001–0.010	0.002	0.004	0.001–0.010	0.002	0.004	<0.001
Cu	0.145–2.670	0.713	0.866	0.261–2.807	1.031	0.982	<0.010
Mo	0.53–41.33	18.05	12.71	7.00–45.50	21.70	12.23	0.130

TABLE 3. Correlation coefficients for chemical parameters correlated with multiplication of *L. pneumophila* in inoculated cooling tower samples

Location of sample	Simple correlation coefficients			Multiple correlation coefficients		
	Independent parameter	*r*	Significance of *r*	Independent parameter	*R*	Significance of *R*
Basin (n = 13)	—[a]	—	—	—	—	—
Condenser (n = 13)	Alkalinity	−0.806	0.001	Alkalinity, pH	0.691	0.005
	pH	−0.725	0.01			
Basin and condenser combined (n = 26)	Alkalinity	−0.546	0.005	Alkalinity, pH, Mn	0.493	0.005
	pH	−0.542	0.005			
	Mn	+0.533	0.01			

[a] —, None.

efficients were calculated for the association between each chemical parameter measured in the samples and *L. pneumophila* multiplication expressed as log CFU per milliliter. All parameters which correlated with multiplication at the $P \geq 0.01$ level of significance were combined, and a multiple correlation coefficient was calculated for *L. pneumophila* growth. Table 3 lists the significant correlation coefficients. The multiple correlations suggest that the chemical environment, in general, significantly affected *Legionella* growth ($P = 0.005$). The simple correlation coefficients indicate a significant inverse relationship between *Legionella* multiplication and alkalinity and pH ($P = 0.01$ to 0.001). Mn is positively correlated with growth to a lesser extent ($P = 0.01$). The observed inverse relationships between alkalinity and pH with growth are graphically depicted in Fig. 1 and 2, respectively. *L. pneumophila* multiplication was least (≤0.65 log CFU/ml) when pH exceeded 8.2 and alkalinity exceeded 200 mg/liter. Growth was greatest (>2.0 log CFU/ml) when pH levels were less than 7.3 and alkalinity values were lower than 100 mg/liter.

DISCUSSION

Cooling towers provide an ideal ecological niche for the amplification and dissemination of *Legionella* species and a variety of other bacteria, algae, and protozoa (13, 18). Not all cooling towers or evaporative condensers have a regular water treatment program. Of those that do, the primary concern is usually with corrosion and scale. Only the larger systems incorporate programs for the control of microorganisms (29). The cooling tower utilized in this study is located in a hospital that has experienced sporadic cases of nosocomial legionellosis during the past several years. This tower and another in the same building have been positive for *Legionella* species at various times in the past and continued to be positive during the period of study at a level of 10^2 CFU/ml. This particular cooling system is constructed of a variety of materials including galvanized steel and copper. At the time of the study, treatment consisted of weekly doses of a molybdate compound for corrosion inhibition and polyacrylate and a dispersant for scale and fouling control. Chlorine, in the form of liquid sodium hypochlorite, was added intermittently for disinfection. The ineffectiveness of the disinfection program led to consideration of an alternate approach.

The levels of certain chemical factors in the cooling system samples were elevated relative to those of the tap water control (Tables 1 and 2). This resulted from a combination of concentration, treatment, and corrosion effects. Dissolved solids, hardness, sulfate, alkalinity, Cl, Ca, Mg,

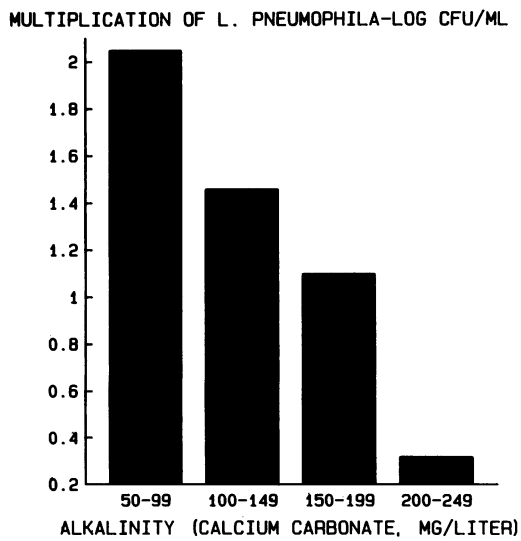

FIG. 1. *L. pneumophila* multiplication and alkalinity in cooling tower condenser samples.

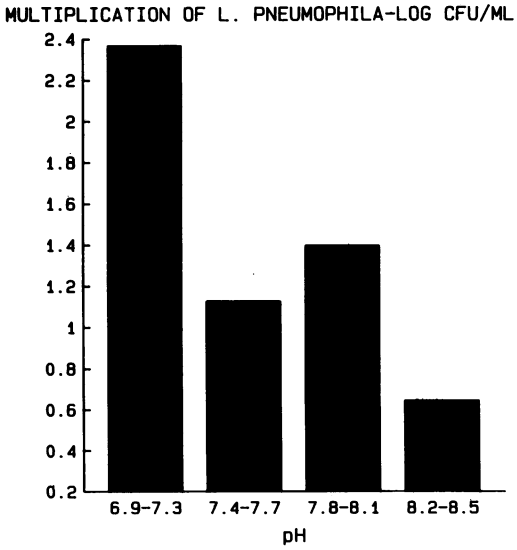

FIG. 2. *L. pneumophila* multiplication and pH in cooling tower condenser samples.

Na, K, and Al levels increased a number of times owing to cycles of concentration within the system. The increases in Mo and total organic carbon are treatment effects resulting from the use of molybdate and polyacrylate scale and corrosion inhibitors. The increased concentrations of Fe, Cu, and Zn are likely associated with corrosion and leaching of metallic components of the system. *Legionella* species and other organisms inhabiting the cooling system are exposed to this chemical environment. The question posed by the study is the extent to which they are affected and the potential use of certain of the factors in controlling these microorganisms.

Attempts have been made previously to elucidate environmental and operating factors that influence *Legionella* growth in cooling towers (21, 39). In the current investigation we found *L. pneumophila* multiplication to be inversely correlated with pH and alkalinity and, to a lesser extent, positively correlated with Mn concentrations (Table 3; Fig. 1 and 2). This observed inverse correlation with pH is consistent with the field and laboratory observations of Fliermans et al. (15) and Wadowsky et al. (36). The existence of a pH tolerance range for *Legionella* species reflects the fact that its natural habitat is the outdoor aquatic environment. The observed tolerance range for legionellae is pH 5.5 to 9.2, while the usual pH for open lakes varies between 6.0 and 9.0 (22). The inverse association with alkalinity appears to be equally strong. This effect could be due to changes in the availability of required or toxic metals and compounds as alkalinity changes (3, 41). Additionally, since absorption of microbial cells to solid surfaces in aquatic environments affects microbial distribution and activity and since factors such as electrolyte concentration influence absorption (18) the alkalinity effect may be related to the absorption process. The tendency for Mn to be positively associated with *L. pneumophila* multiplication is similar to our earlier findings on the effects of metals in hot water tanks and municipal water systems on *Legionella* growth (34, 35). The intensity of the relationship in this cooling system is less than that observed in hospital hot water tanks. This difference may reflect the relatively limited variation in metals concentration among samples in this study. Although added to the cooling system in substantial concentrations as a treatment agent (Table 1), the metal molybdenum did not appear to affect *Legionella* growth.

While there are no clear-cut guidelines concerning the extent to which cooling towers should be monitored or treated for control of *Legionella* species, general approaches have been recommended (5, 14). The results of the current investigation suggest that elevated pH and alkalinity would bring environmental conditions out of the tolerance range of *Legionella* species and could be useful in controlling multiplication in cooling systems. Since current recommendations encourage the use of higher pH for the control of corrosion and fouling, the use of such an approach would be additionally beneficial. On a practical basis, supplemental corrosion inhibitors (e.g., zinc, chromate, orthophosphates, polyphosphates, or combinations of these) may be needed to supplement this approach to control corrosion (23). Scaling, which can result from higher pHs and alkalinities, can be inhibited by the addition of organophosphorus compounds (10, 23). Additionally, in some cases, commercially available biodispersants, along with low-level continuous chlorination, may help to ensure that legionellae associated with a biofilm are exposed to disinfectant and high pH-alkalinity conditions.

The optimal pH and alkalinity levels for control of *Legionella* contamination must be determined in an operational cooling tower. Our laboratory is presently pursuing these studies.

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