

## Efficacy of oligodynamic metals in the control of bacteria growth in humidifier water tanks and mist droplets

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### ABSTRACT

Antimicrobial capsules were evaluated for their effectiveness to control bacterial contamination of cool mist humidifiers. These capsules contain a mixture of silver and copper promoted alumina beads designed to release low concentrations of these oligodynamic metals into the reservoir water for bacteria control. The reservoir water and mist droplets from the humidifier units were tested for the presence of bacteria over a three-week period. A control unit (without capsule) showed significant bacterial contamination by day three, which increased throughout the three-week test period, in both the reservoir and mist droplets, whereas the antimicrobial capsules reduced contamination during the first week, and minimized the presence of bacteria, in both the reservoir water and mist droplets, to less than 2% of the control unit throughout the three-week test period. It was also observed that, after each inactive weekend, the initial discharge of bacteria via the mist droplets in the control unit was significantly higher than during daily use. However, initial bacterial discharge from the test unit following weekend inactivity never exceeded 0.5% of the control unit. In conclusion, these capsules containing oligodynamic metals are effective in controlling bacteria growth in humidifier water tanks and mist droplets.

**Key words** | cool mist humidifiers, silver and copper promoted alumina, oligodynamic metals, bacteria control, mist droplets

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### INTRODUCTION

Oligodynamic metals, such as silver and copper, have long been utilized as disinfectants for non-spore-forming bacteria and viruses (Thurman & Gerba 1988). Silver can serve as a disinfectant at concentrations about 1,000 times lower than the toxic level to mammalian life (Warrington 1996). The sulfhydryl-binding propensity of silver ion disrupts cell membranes, disable proteins and inhibit enzyme activities (Thurman & Gerba 1988; Semikina & Skulacher 1990). Copper, another oligodynamic metal, can be combined with silver resulting in a synergistic disinfection effect on bacterial cells. The positively charged copper ion distorts the cell wall by bonding to negatively charged groups and allowing the silver ion into the cell (Hambidge 2001). Silver ions bind to DNA, RNA, enzymes and cellular proteins causing cell damage and death (Hambidge 2001). Silver and

copper ions are easily immobilized on a solid substrate. Our previous studies have shown that the disinfection property of silver and copper promoted aluminas increased when the alumina support is highly hydroxylated. These hydroxyl groups are thought to improve the disinfectant action of silver or copper in two distinct ways: (1) the metal concentration on the supporting surface is much higher than the allowable level in free solution, and (2) the surface increases the frequency and length of the metal/bacteria interactions. Thus, the metal ion immobilization and modification of the supporting surface act in concert to increase the activity of a given metal concentration, without having to introduce the free metal into the solution. One of the drawbacks of this method is the possibility of biofilm formation on the supporting surface that may significantly

reduce the antibacterial activity of the supporting surface (Camper *et al.* 1986; Costerton *et al.* 1987; Brown & Gilbert 1993).

Humidifiers are subject to contamination by a broad spectrum of microorganisms (Burge *et al.* 1980; Harpin & Rutter 1985; Parat *et al.* 1996). In addition, some of the bacteria form biofilms and are therefore more difficult to control. Therefore, a product to control bacterial growth must be effective against a broad spectrum of microorganisms and must be able to function under a variety of water conditions. Recently, a number of humidifiers have been introduced into the marketplace that contain biocidal or biostatic chemical additives in the plastic components of the humidifier. The manufacturers claim that the chemical additives control bacteria that may grow on the plastic surfaces of the humidifier. However, since these additives are found only in the plastic components of the humidifier they offer little or no protection to the growth of bacteria or fungi in the water itself, as our results demonstrate (unpublished results).

The antimicrobial capsule (7 cm long and 2 cm in diameter) from K2 Concepts, Inc. (Atlanta, GA) contains 15 g of a proprietary mixture of silver and copper promoted alumina beads. We therefore evaluated this antimicrobial capsule for its effect on controlling bacterial contamination in a cool mist humidifier. Water samples from the unit's reservoir, as well as the mist droplets, were tested for the presence of bacteria over a three-week test period. The experiment was carried out following normal operating instructions using tap water. In this study, we compared the bacterial growth in a commercially available humidifier both with and without the use of the antimicrobial capsule.

## MATERIALS AND METHODS

The HumidiSafe<sup>®</sup> antimicrobial capsule, a product of K2 Concepts, Inc. (Atlanta, GA), was evaluated for its effectiveness to control bacterial contamination of cool mist humidifiers. The HumidiSafe capsule is a small porous capsule that contains a proprietary mixture of silver and copper promoted alumina beads designed to release low concentrations of these oligodynamic metals into the reservoir water for bacteria control.

The free chlorine levels in the Atlanta municipal tap water were measured using a Hach Cl test kit for water analysis (Hach Company, Loveland, CO). This test kit uses the DPD method for free chlorine. Samples were read using a Hach DR/2010 Portable Datalogging Spectrophotometer.

At the end of each week day, a 10 ml sample of reservoir water was collected, 1 drop of 12 N HNO<sub>3</sub> was added as a preservative and the samples were analyzed for Ag and Cu using a PerkinElmer Elan 6100 Inductively Coupled Plasma–Mass spectrometer (PerkinElmer, Boston, MA).

## Test background

Two Duracraft humidifiers model DF-1/DF-12 (3.8 liters, Whisper Mist Cool Moisture Humidifier) (Duracraft<sup>®</sup> Corp., Southborough, MA) were purchased for this study (see Figure 1). The Duracraft humidifier Model DF-1/DF-12 is equipped with a polarized AC line plug that connects to a 120 V power source and produces a cool mist, using a spinning disk, when in operation. The humidifier units were rinsed 5 times thoroughly with ordinary tap water to remove any plasticizers or chemical residues that may have been present in the manufactured unit. The antimicrobial capsule was placed into one of the units and used as the test unit. The other humidifier was used as the control with no capsule added. Both humidifiers were filled with Atlanta municipal chlorinated tap water. The units were turned on at the beginning of each day and operated for 4–6 h. At the end of each 4–6 h operational period, water



Figure 1 | Humidifier test units.

and mist samples were collected from each humidifier and the bacteria concentration measured. Water was removed from the reservoir and assayed on R2A agar plates incubated at 20°C for 5 d. When plating our samples we did not use a thionneutralizer as we felt it was not justified for this particular application. In particular, published work shows that using a thionneutralizer has no benefits when the exposure time of the silver to the bacteria exceeds 10–20 min (Tilton & Rosenberg 1978; Landeen *et al.* 1989). To collect mist droplet samples, a R2A agar plate was placed directly in front and 2" away from the "misting port" of the humidifier for 2 s and then incubated at 20°C for 5 d. This sampling technique provides primarily a qualitative measure of the humidifier mist bacterial discharge (Burge *et al.* 1980). The humidifiers were then allowed to sit overnight. The next day, both humidifier reservoirs were topped-off with tap water and operated for another 4–6 h. The samples of reservoir water and mist droplets were collected and cultured as described before. Reasoner's 2A (R2A) agar was specifically used to test for viable and injured organisms that may continue to grow (Reasoner & Geldreich 1985). This cycle was repeated for a period of three weeks. The units were not operated and no samples were collected on the weekends.

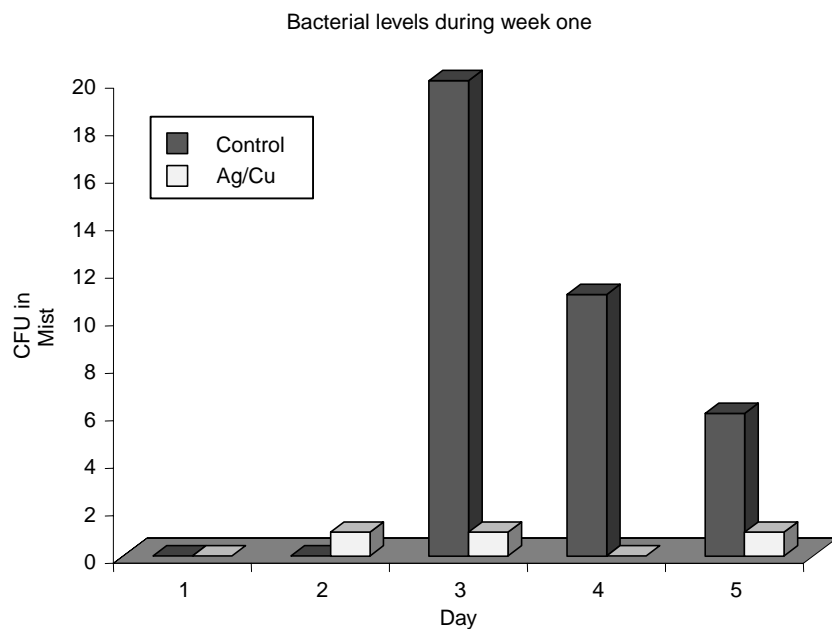
## RESULTS AND DISCUSSION

The antimicrobial capsule was evaluated for its effectiveness to control bacterial contamination in a cool mist humidifier for three weeks. Bacterial growth in the mist droplets rose dramatically in the control unit as compared to the test unit. There were 20 colony forming units (CFU) detected in the mist droplets of the control unit on day three while only 1 CFU was observed in the mist droplets of the treated unit (Figure 2). R2A agar (Reasoner & Geldreich 1985) was used to test for viable and injured organisms in our samples. We have noticed that R2A plates give us higher colony forming unit counts than do MacConkey plates because of their ability to allow injured organisms to recover and grow (unpublished data). As mentioned earlier we did not utilize a thionneutralizer in plating our samples because the thionneutralizer has no benefits when the exposure time of the silver to the bacteria exceeds 10–20 min (Tilton &

Rosenberg 1978; Landeen *et al.* 1989). Since a humidifier tank is a static environment, the exposure time of the silver to the bacteria is of the order of days and therefore this exposure time would negate any benefit of applying a thionneutralizer. In addition, the Ag<sup>+</sup> concentration in the humidifier tank water in our experiments is of the order of 100 µg per liter, which is well above what Tilton & Rosenberg (1978) indicate is sufficient to create 100% kill in less than 20 min.

Throughout the test period the level of bacterial contamination in the control unit continued to rise in both the mist (Figure 3) and the reservoir (Figure 4). The number of CFU in the mist samples of the control unit reached 6,000, while only 3 CFU were detected in mist samples of the treated unit at the end of the third week (Figure 3). These results demonstrate that the antimicrobial capsule was effective in reducing the presence of bacteria in both the reservoir water and mist droplets in the test humidifier throughout the three-week test period. By week three the bacterial levels in the mist samples of the test unit were less than 0.05% of those of the control unit. Similar results were observed in the reservoir water samples (Figure 4). By week three bacterial levels in the water in the control unit reservoir reached 1,000,000 CFU/ml, while the bacterial levels in the reservoir water of the test unit remained as low as 2% of the control unit.

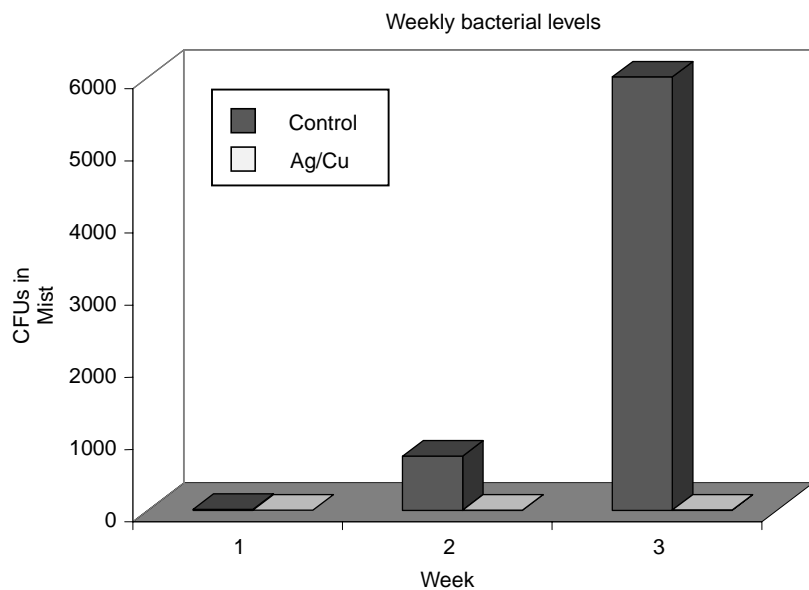
Shown in Figure 5 are the daily bacterial numbers in the mist of the control and test units throughout the three-week study. We observed a common trend following the weekend inactivity; the bacterial levels in the control reservoir had risen significantly. This "weekend" effect was much less pronounced for the treated unit. Following the weekend the control mist sample contained as many as 10,000 CFU, while the mist samples of the test unit never exceeded 3 CFU (Figure 5). The weekend down time is thought to mimic real-life situations where units are not in constant operation. During the "weekend down times" it has been observed that bacteria concentrations increase significantly and thus when the humidifier is first used following this down time the initial discharge of bacteria via the mist can be significantly higher than during daily use. This weekend growth cycle has been found to cause acute illness in people subjected to the mist upon exposure on Mondays (Parrott & Blyth 1980; Kateman *et al.* 1990; Fridkin *et al.* 1996).



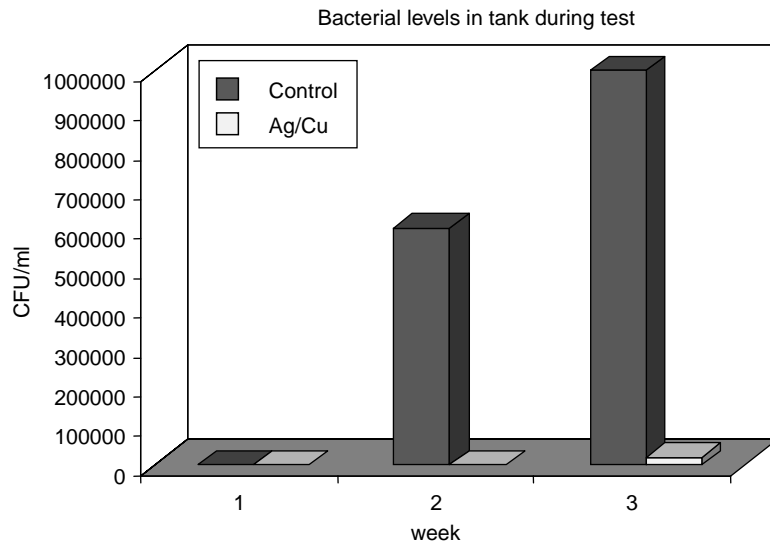
**Figure 2** | Bacteria in the mist samples during the first week.

However, the growth cycle should not be confused with an illness termed “Monday morning fever” (Parrott & Blyth 1980; Kateman *et al.* 1990; Fridkin *et al.* 1996). Monday morning fever results from some individuals developing a tolerance to some of the microorganisms found in the

humidifier reservoir water during exposure. However, this resistance may be lost after a day or two of absence from exposure. The combined effect is that on Mondays workers have lost any tolerance to the contaminating microorganisms and at the same time are being exposed to higher levels



**Figure 3** | Weekly bacterial levels in the humidifier mist samples.

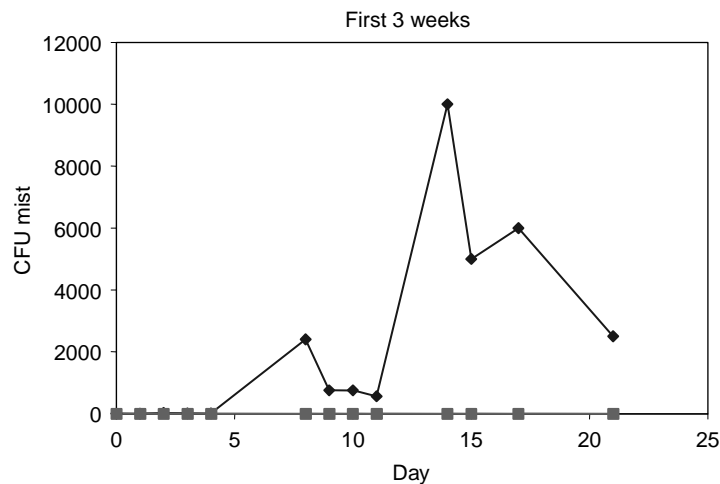


**Figure 4** | Weekly bacterial levels in the humidifier reservoirs.

of them in the air (Parrott & Blyth 1980). This data indicates that even when a humidifier is left inactive with the water remaining in the reservoir over the weekend the antimicrobial capsule still remained efficacious in preventing bacterial growth.

The ability of the antimicrobial capsule to prevent bacterial growth over the weekend can be understood by examining the data in Figure 6. Analysis of the Cu and Ag metal levels in the reservoir water demonstrates that small amounts of Cu (low ppm) and Ag (~100 ppb) are released

from the media and have a bacterial static or bacterial cydial effect. As described earlier, oligodynamic metals such as silver and copper can serve as disinfectants at concentrations about 1,000 times lower than levels at which it is toxic to mammalian life (Warrington 1996). Copper levels remained at approximately 1,000 ppb during the test period and Ag levels at approximately 100 ppb. These levels are sufficiently below the US Environmental Protection Agency's drinking water standards (US EPA 2004).



**Figure 5** | Bacterial levels in the humidifier mist samples throughout the entire test period.

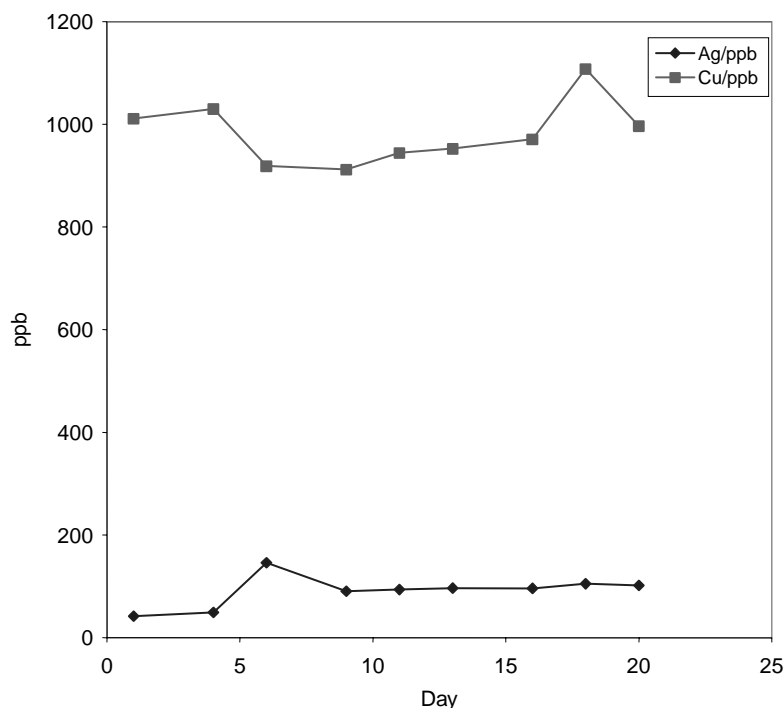


Figure 6 | Metal leaching data throughout the test period.

Microbial contamination of water can make it unfit for use in cool mist humidifiers. Pathogenic bacteria, viruses, protozoa, parasites and heterotrophic bacteria may all pass out of the reservoir in the mist droplets of the humidifiers. Several studies have shown that portable room humidifiers are susceptible to bacteria growth within the water reservoir of the units (Kane *et al.* 1993; Patterson *et al.* 1998). Kane *et al.* showed that *Klebsiella oxytoca* growth in an ultrasonic cool air home humidifier could result in hypersensitivity pneumonitis (Kane *et al.* 1993; Patterson *et al.* 1998). The discharge of these bacteria into the air through the “misting” of reservoir water is a significant health concern for children, the elderly and immune-compromised individuals (Rhame *et al.* 1986). Contaminated humidifiers induce illnesses ranging from pneumonia-like diseases to those with flu-like symptoms (Zuravleff *et al.* 1983; Baur *et al.* 1988; Patterson *et al.* 1998).

Cool mist humidifiers and evaporative humidifiers are typically preferred over “steam” type humidifiers. This is primarily due to a concern that the user may come into contact with the hot steam of the steam type humidifier and receive severe burns. However, the cool mist humidifiers have been shown to be the most sensitive to bacterial

contamination. Ultrasonic humidifiers used in hospitals commonly have up to  $10^5$  CFU/ml bacteria in their reservoirs (Oie *et al.* 1992). Studies have shown that neither changing the reservoir water daily (Harpin & Rutter 1985) nor the addition of household bleach (Burge *et al.* 1980) eliminated bacterial contamination in the humidifiers tested. In addition, free residual chlorine in tap water in the reservoirs disappears within 30 min of filling the reservoir (Oie *et al.* 1988, 1992). Therefore, it is essential to develop a system that reliably and safely controls the growth of bacteria in the water reservoir and thus prevents bacterial discharge into the air. The growth of microorganisms in water reservoirs is not limited to portable room humidifiers and has been reported in a broad range of humidifiers in factories, air-conditioning systems and other units containing a water reservoir used for humidification (Burke *et al.* 1977; Parrott & Blyth 1980; Baur *et al.* 1988; Parat *et al.* 1996).

The results of this study indicate that the antimicrobial capsule containing silver and copper promoted alumina evaluated here was effective in controlling bacterial contamination during the three weeks of testing in both the



reservoir water and mist droplets. The level of bacterial growth continued to rise in both the reservoir water and mist droplets of the control unit throughout the entire test period. By week three bacterial levels in the control reservoir water reached 1,000,000 CFU/ml, and as high as 10 000 CFU in the mist droplets. The levels of bacterial growth in the reservoir water in the test unit never exceeded 3 CFU in mist droplets (2% of those in the control unit). During the entire test period only small amounts of Cu (~ 1 ppm) and Ag (~ 100 ppb) were released into the water reservoir water.

## CONCLUSIONS

In conclusion, the results of this study demonstrate that capsules containing silver and copper promoted alumina are effective in controlling bacteria growth in humidifier water tanks and mist droplets. The data demonstrates that cool mist humidifiers operated under normal conditions are prone to rapid bacterial growth in the reservoir water and subsequent discharge in the mist droplets. The data also show that the addition of antimicrobial capsules eliminates contamination during the first week of operation. In addition, during the entire three-week test period the capsules effectively minimized the presence of bacteria in both the reservoir water and mist droplets to below 2% of the bacteria counts found in the unit operated without the antimicrobial capsule (control unit). On Mondays, after each inactive weekend, the initial discharge of bacteria via the mist droplets in the unit without the antimicrobial capsule was significantly higher than during daily use. In comparison, the initial bacterial discharge from the test unit operated with the antimicrobial capsule following weekend inactivity never exceeded 0.5% of the control unit. In conclusion, oligodynamic metals are effective in preventing bacterial growth in humidifier tanks and mist droplets and may be useful for a wide variety of water and health applications.

## ACKNOWLEDGEMENTS

This material is based upon work supported in part by the STC Program of the National Science Foundation under Agreement Number CTS-0120978, NSF Grant no HRD9909014 and NIH RCMI Grant no G12RR03062.

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