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Outbreak of Bioaerosols with Continuous Use of Humidifier in Apartment Room

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The effect of continuous humidifier use on the bioaerosol concentration in an indoor environment was investigated. An ultrasonic humidifier was operated for 10 hr per day for 15 days in an apartment room. During this time period, viable bioaerosol samples were taken using a single-stage Andersen sampler containing culture media plates for bacteria and fungi. The culture plates were then incubated at room temperature for 2~7 days depending on the media. The counts for the air sample plates were corrected for multiple impactions using the positive hole conversion method and are reported as the colony forming units per cubic meter of air (CFU/m^3). While the bacterial concentration measured using the tryptic soy agar (TSA) did not show any significant change during the first 3 days, the concentration increased from the 6^{th} day (6979 CFU/m^3) and reached a maximum on the 9^{th} day (46431 CFU/m^3). The concentration then decreased to 2470 CFU/m^3 on the 12^{th} day, at which point the fungal concentration increased rapidly to 14424~16038 CFU/m^3. Also, while the fungal concentration showed a significant change until the 9^{th} day of humidifier use, fungal growth was observed on the wallpaper and increased rapidly from the 12^{th} day. However, the bacterial concentration increased rapidly after the fungi were removed by remediation. The major fungal species identified in the samples were Penicillium representing 34%, Aspergillus representing 31%, Cladosporium representing 24%, and Alternaria representing 1%. The results also indicated that a relative humidity over 80% was easily achieved with continuous humidifier use. Yet, maintaining a high humidity in a room can cause a rapid outbreak of microbial growth.

***Key words*:** Bioaerosol, Humidifier, Apartment room, Mold, Fungus, Bacteria

INTRODUCTION

There is a recent growing concern over exposure to microbial aerosols (bioaerosols), due to related adverse health effects. Exposure to relatively large concentrations of airborne microbes is often associated with asthma and rhinitis (Denning *et al*., 2006), hypersensitivity pneumonitis (Ando *et al*., 1991; Fung and Hughson, 2003), sick-building syndrome (Cooley *et al*., 1998; Straus *et al*., 2003), and a number of other health effects, including infections (Fischer and Dott, 2003). The nutrient availability, indoor temperature, and moisture are the key elements required to support microorganisms in an indoor environment. The water activity (A_w) describes the moisture holding capacity of building materials, and indicates how much water is available for microbial growth. The relative humidity (RH) of room air in relation to the substrate A_w/ERH (equilibrium RH) also plays important role in the water content of materials in a room (ACGIH, 1999). Koreans residing in apartments usually prefer a lower relative humidity during winter time. As the Korean winter is very cold and dry, the relatively high temperature maintained in apartments lowers the relative humidity in the rooms. Therefore, many Korean apartment residents frequently use humidifiers during winter time to control the humidity. Furthermore, most Korean apartments are wallpapered and include wooden furniture, beds, and blankets, all of which support additional microbial growth in the case of sufficient moisture.

Accordingly, this report investigated the indoor bioaerosol concentration in an apartment in the case of extreme use of a humidifier during winter. An apartment was rented for 10 days and a humidifier operated for certain time periods to create a sufficient relative humidity. The bioaerosols, including bacteria and fungi, were then sampled and the relative humidity recorded during the investigation period.

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MATERIALS AND METHODS

Site and humidifier operation. The current study sampled the airborne viable bacteria and fungi in an apartment room during winter in the case of continuous operation of a humidifier. The apartment building was constructed using concrete and iron rods, and no mold was present when the experiment was initiated. The humidifier was ultrasonic and manufactured by the R company (Korea) with a storage capacity of 3.4 *l* and humidifying rate of 0.35 *l*/hr. Tap water was used to fill the humidifier, which was then operated for 10 hr/day using a timer. The experiment was conducted in an empty 46.8/m^3 room with the portable humidifier in the middle. The floor was covered with linoleum. At the beginning of the experiment, the windows and door were left open for a minimum of 1 hour to equilibrate the interior bioaerosol level to an ambient level. The windows and door were then closed and a 2-minute air sample collected prior to and at specified time periods while using the humidifier. The RH was also measured concurrently using a humidity meter (Thermo Recorder TR-72S, T & D Co., Tokyo, Japan). The door was only opened for bioaerosol sampling and refilling the water. A 2-minute outdoor air sample was also collected prior to and after the experiment.

Sampling and analytical methods. The viable bioaerosol sampling was conducted using a single-stage Andersen sampler with 400 0.25-mm holes, drawing air at a flow rate of 28.3 *l*/min (NIOSH 0800, 2004). The indoor samples were collected at the center of the room at a height of 1.0~1.5 m during the afternoon (usually between 3~8 pm). The samplers were calibrated prior to and following the collection of each sample using a flow calibrator (DCL-H; Bios, Butler, NJ). The average of the two rates was then used as the sample flow rate for all the volume calculations. No samples departed more than 10% from the initial flow rate during the study. During sampling, the temperature and relative humidity were both recorded. Each bioaerosol sample was nominally collected for 2 min, following Lee and Jo (2006), on nutrient media (specific to either fungi or bacteria) in petri dishes located on the impactor. A malt extract agar (MEA) and dichloran glycerol 18 agar (DG-18) were both used for fungi, where chloramphenicol was added to inhibit any bacterial growth. Meanwhile, a trypticase soy agar (TSA) was used for bacteria, where cycloheximide was added to inhibit any fungal growth. The MEA, DG-18, and TSA plates were incubated at room temperature for 3~5 days, 5~7 days, and 2~3 days, respectively. The counts for the air sample plates were corrected for multiple impactions using the positive hole conversion method (Andersen, 1958), and are reported as colony forming units per cubic meter of air (CFU/m^3).

Identification of fungi. To identify the genera of the sampled fungi, the fungi that grew to a certain size were stored in a slant agar and fixed on a microscope slide. The genera of the fungi were then identified using an atlas (Koneman *et al*., 1978).

RESULTS

Humidity change with use of humidifier. The RH started at 45% before using the humidifier, and then reached 68% after 3 hr of use, 84% after 24 hr, and 93% after 3 days. After adjusting the humidifier use to 10 hr/day, the RH remained at 80% until the 9th day, and then increased to 97% on the 12th day. The room temperature during the humidifier use ranged from 22~25°C.

Bacterial concentration changes during humidifier use. While the bacterial concentration measured by the TSA did not show any significant change during the first 3 days, the concentration increased from the 6th day (6979 CFU/m^3) and reached its maximum on the 9th day (46431 CFU/m^3). The concentration then decreased to 2470 CFU/m^3 on the 12th day at which point the fungal concentration increased rapidly (Fig. 1). However, the bacterial concentration increased rapidly after the fungi were removed by remediation.

Fungal concentration changes during humidifier use. While the fungal concentration changed significantly until the 9th day of humidifier use (Fig. 2B), fungal growth was observed on the wallpaper and increased rapidly from the 12th day (14424~16038 CFU/m^3) (Fig. 2). After the contaminated wall paper was removed, the fungal concentration decreased rapidly (Fig. 2C).

Identification of fungi. The major fungal species identified in the samples were Penicillium representing 34%,

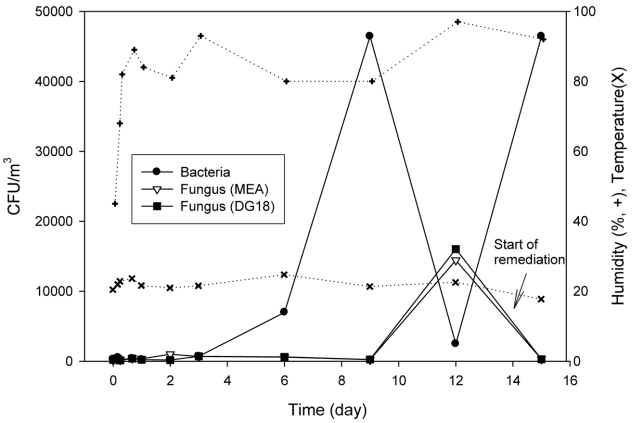
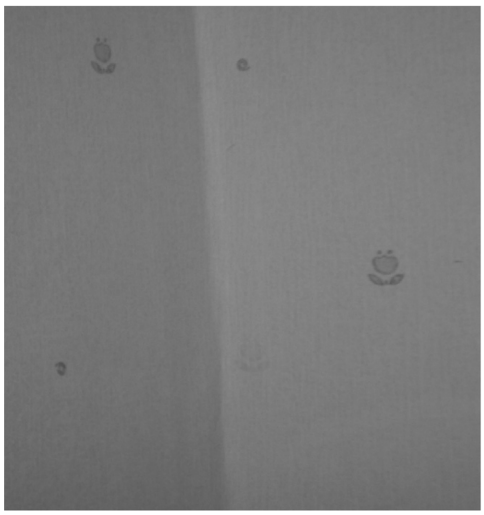


Fig. 1. Bacterial and fungal concentrations with continuous humidifier use for 15 days. The bacterial and fungal concentrations (CFU/m^3) were measured during 15 day of humidifier operation. The remediation started from the 14th day. +, humidity; ×, temperature; ●, bacteria; ■, fungus (DG18 media); ▽, fungus (MEA media).

A. Before humidifier use



B. After 9 days of humidifier use

C. After 12 days of humidifier use

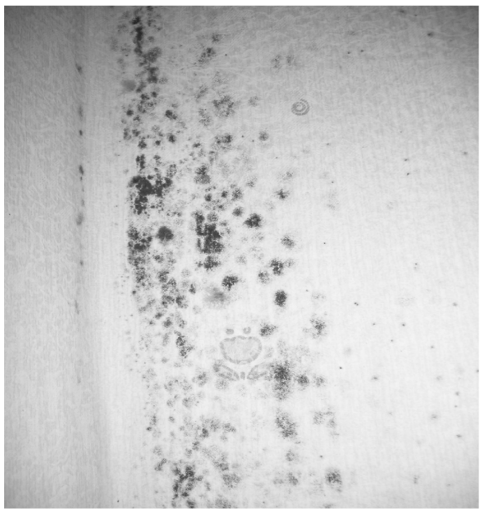


Fig. 2. Fungal growth on apartment wallpaper with continuous humidifier use. The condition of wall paper was photographed during 15 day of humidifier operation.

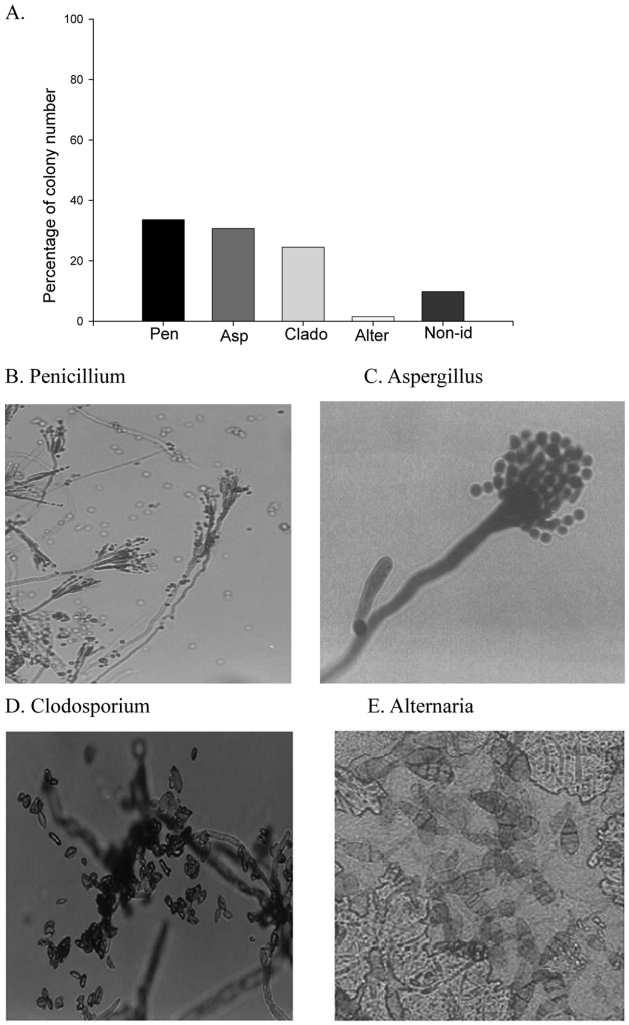


Fig. 3. Fungal species found after humidifier use. The major fungal species identified in the samples were plotted. Major species identified were photographed. Pen, Penicillium; Asp, Aspergillus; Clado, Clodosporium; Alter, Alternaria.

Aspergillus representing 31%, Cladosporium representing 24%, and Alternaria representing 1% (Fig. 3). The remaining fungi representing 10% were not identified.

DISCUSSION

Continuous operation of a humidifier during winter can create a favorable environment for fungal growth by increasing the water activity of the building materials. The present results indicated that a relative humidity over 80% was easily achieved with continuous operation of a humidifier. Maintaining a high humidity in a room can quickly cause an outbreak of microbial growth. A recent investigation conducted by the Korean National Institute of Environmental Research announced that 70% of residential homes in Korea exceeded the indoor air quality limit for bacteria

2.2 times and the WHO recommended limit for fungi 1.5 times. Therefore, the report recommended frequent cleaning of air conditioners, humidifiers, and bath rooms and lowering the humidity below 60% to avoid airborne microbial contamination (Gonggam, 2012). The present results indicated that bacterial outgrowth preceded fungal outgrowth. Plus, while the fungal outgrowth decreased the airborne bacterial concentration, the airborne bacteria increased after the fungal growth was removed. The reason for this phenomenon is competition for food and habitat between the bacteria and the fungi (Madigan *et al*., 2011; ACGIH, 1999). Although MEA is widely used as a medium for sampling airborne fungi, DG 18 can be a supplement for rapidly growing fungi, such as Rhizopus, Mucor, Monilia, and Trichoderma (ACGIH, 1999), which is why this study used both media. The indicator species according to the water activity (A_w) were as follows: $A_w > 0.9$~0.95 for Aspergillus fumigatus, Trichoderma, Exophiala, Stachybotrys, Phialophora, Fusarium, Ulocladium, and Rhodotorula, A_w 0.85~9.0 for Aspergillus versicolor, and $A_w \leq 0.85$ for Aspergillus versicolor, Eurotorium, Wallemia, and Penicillium (Samson *et al*., 1994). The indicator species, such as Penicillium and Aspergillus, found in this study also indicated that continuous use of a humidifier caused a high humidity inducing a high water activity. The naked eye observation of fungal growth on the wallpaper in the apartment room also indicated airborne fungal spread. The Korean Indoor Air Quality Control in Public Use Facilities, etc. Act (MOE, 2011) prescribes a total airborne bacterial concentration below 800 CFU/m^3 for hospitals, nurseries, public nurseries and hospitals for the elderly, and post partum care centers. However, the Act does not yet recommend an airborne fungal concentration.

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