Routledge
Taylor & Francis Group

Archives of Environmental & Occupational Health

ISSN: 1933-8244 (Print) (Online) Journal homepage: http://www.tandfonline.com/loi/vaeh20

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To cite this article: Wan-Kuen Jo PhD & Ji-Hyun Lee PhD (2008) Airborne Fungal and Bacterial Levels Associated With the Use of Automobile Air Conditioners or Heaters, Room Air Conditioners, and Humidifiers, Archives of Environmental & Occupational Health, 63:3, 101-107, DOI: 10.3200/AEOH.63.3.101-107

To link to this article: http://dx.doi.org/10.3200/AEOH.63.3.101-107

Archives of Environmental & Occupational Health, Vol. 63, No. 3, 2008

Airborne Fungal and Bacterial Levels Associated With the Use of Automobile Air Conditioners or Heaters, Room Air Conditioners, and Humidifiers

Wan-Kuen Jo, PhD; Ji-Hyun Lee, PhD

ABSTRACT. The authors performed 3 experiments to measure temporal variation in airborne bacterial and fungal levels associated with the use of air conditioners (ACs), heaters, and humidifiers. The concentrations of bioaerosols that they measured inside vehicles and a seminar room prior to use of an AC were lower than or similar to those for outdoor air. In most cases, elevated concentrations occurred 5 to 15 minutes after the use of an automobile or household AC, and these concentrations decreased over time. For 3 of 5 cars, however, the bacterial concentrations did not vary significantly. For cars, the maximum bacterial concentration (2,550 CFU m^{-3}) was 46 times higher than the in-vehicle background concentration (55 CFU m^{-3}). Three fungi (*Cladosporium, Penicillium*, and *Aspergillus*) exhibited the highest concentrations for most sampling periods of the ACs and heater. The use of automobile heaters and household humidifiers could suppress in-vehicle and in-room microbial concentrations.

KEYWORDS: bioaerosol, fungus, temporal variation

Exposure to bioaerosols is possibly associated with adverse health effects.[1–3] Owing to the ubiquitous presence of bioaerosols in nature,[1,3] this exposure is almost inevitable within enclosed environments. Several studies have reported that exposure to high concentrations of airborne microbes is often associated with asthma and rhinitis, hypersensitivity pneumonitis, and sick-building syndrome.[4–7] Endotoxins and mycotoxins of bioaerosols also may play a major role in inflammatory reactions, deterioration of lung functions, and a number of other health effects, including infections.[8–10] However, researchers[11,12] in many scientific studies have not found conclusive evidence that airborne microbes are associated with adverse health effects.

Certain electronic appliances, such as air conditioners (ACs) and humidifiers, are used in various enclosed spaces to maintain a comfortable environment. However, the use of these appliances in such spaces is often associated with the proliferation of microorganisms.[13] ACs are often contaminated with microorganisms,[14–17] which they discharge with strong air currents, causing microbial proliferation into the air.[13,17] People will often smell an unpleasant odor or begin coughing when the AC is switched on, which is related to microbial growth inside the AC. Hodgson et al[18] observed a relation between bronchial disease and bacterial, actinomycetal, or fungal proliferation by the operation of ACs. Humidifiers that are intended to make residents feel more comfortable in winter also can influence bioaerosol levels in enclosed environments.[19]

An automobile cabin is another enclosed microenvironment associated with AC use. Many individuals spend time inside vehicles,[20,21] and taxicab, public bus, and truck drivers in particular spend much more time inside vehicles than do

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other vehicle users.[22] Similar to household ACs, when in-vehicle ACs are turned on, certain microbes can be emitted into the air and thus elevate in-vehicle microbial levels. Moreover, just as indoor ACs stir up bioaerosols in carpets and textiles,[23] automobile heaters and ACs can cause air turbulence, suspending bioaerosols from floor mats, textile seats, and occupants' clothes, thereby elevating airborne-microbe levels. There is scant information available regarding the effects of automobile ACs or heaters on bioaerosol levels inside vehicles.[13,24]

In the present study, we evaluated the relation between bioaerosol levels inside vehicles, a university seminar room, and an apartment, and the use of ACs, heaters, or humidifiers. We focused on viable bacteria and fungi, which exist in the airborne state as single cells or clumps.[1] In a series of longitudinal studies, we measured temporal variation in indoor bioaerosol concentrations with respect to the operating conditions of electronic appliances.

METHODS

Survey Protocol

We performed 3 experiments to measure the airborne bacterial and fungal levels associated with the use of an AC, heater, and humidifier. For the first experiment, we examined 5 passenger cars (4 Hyundai vehicles and 1 Kia) over summer and winter (see Table 1). The 5 cars had engine sizes ranging from 1,500 to 2,000 cc, model years 1994 to 2000, and mileage 45,000 to 90,625. While the passenger cars idled at an outdoor stadium of Kyungpook National University, we measured the time-series, in-vehicle airborne bioaerosol concentrations prior to and after the use of an AC during the summer and a heater during the winter. We did not control for other conditions that may have differentiated the ACs or heaters prior to each sampling day. We positioned the vehicles so as to minimize the amount of exhaust inside the test vehicle. Prior to idling, we opened the car's windows for a minimum of 1 hour to equilibrate the interior bioaerosol levels to ambient levels and to allow the engines to cool to the ambient temperature. We then closed the windows and collected a 2-minute, in-vehicle air sample prior to starting the engine. We collected these samples at 5, 15, 25, and 35 minutes after turning on the automobile AC or heater. The temperature level was set at an optimum comfort level, which we deemed to be between 19°C and 23°C. Relative humidity (RH) ranged from 39% to 74%. We also collected a 2-minute outdoor air sample prior to and after the in-vehicle experiment (20 m upwind of the automobile).

We conducted the second experiment in a university seminar room (90.2 m^3) during the summer. We measured the time-series, in-room airborne bioaerosol concentrations prior to and after running a household AC for 4 hours. This experiment was repeated 4 times (once a day). In the room was a standing-type household AC positioned in the corner. Except for the AC, ten 2.5-m tables and 20 chairs were the only furniture in the room. The floor was made of marble; there was no carpet. At the beginning of all experiments, we opened the room windows and doors for a minimum of 1 hour to equilibrate the interior bioaerosol levels to ambient levels. We then closed the windows and doors and collected a 2-minute air sample of the room prior to switching on the AC. We collected these samples at 5, 15, 30, 60, 120, 180, and 240 minutes after we turned on the AC. We set the temperature to an optimum comfort level of 20°C; during the 4 hours, the room's actual temperature ranged from 19°C to 23°C. RH ranged from 33% to 82%. We collected subsequent 2-minute samples at 5, 15, 30, 60, 120, 180, and 240 minutes after switching off the AC. We collected a 2-minute outdoor sample prior to and after this seminar room experiment.

We conducted the last experiment in an apartment room during winter by measuring time-series, in-room bioaerosol concentrations prior to and after the use of a humidifier for 3, 12, and 72 hours. The humidifier was ultrasonic, with a storage capacity of 7 L and a humidifying rate of 0.4 L/min. When necessary, we refilled it with water every 12 hours. We conducted the experiment in an empty 46.8-m^3 room of an apartment, with a portable humidifier in the middle. The floor was covered with linoleum. At the beginning of the experiment, we left open the windows and door for a minimum of 1 hour to equilibrate the interior bioaerosol levels to ambient levels. We then closed the windows and door and collected a 2-minute air sample prior to and at specified time periods after using the humidifier. We measured RH concurrently using a humidity meter (Thermo Recorder TR-72S, T & D Co., Tokyo, Japan). We opened the door only during bioaerosol

Table 1.—Information on Passenger Cars in This Survey

Car	Manufacturer	Engine size (L)	Model/year	Mileage (m)	Fuel type
1	Hyundai	1.5	Accent 1995	128,312	Gasoline
2	Hyundai	1.5	Accent 1994	145,473	Gasoline
3	Hyundai	1.5	Elantra 1996	138,351	Gasoline
4	Hyundai	2.0	Sonata 1994	140,237	Gasoline
5	Kia	1.8	Carens 2000	72,556	LPG

Note. We collected the outdoor air sample prior to and after each time-series indoor experiment; $n = 5$ for each data set of indoor samples. LPG = liquefied petroleum gas.

sampling and water refills. We collected a 2-minute outdoor air sample prior to and after this experiment.

Sampling and Analysis

We conducted viable bioaerosol sampling using single-stage Anderson samplers with 400 0.25-mm holes (Graseby-Anderson, Atlanta, GA) that drew air at a rate of 28.3/min (corresponding to a velocity of 24 m/s). We calibrated the samplers prior to and after collecting each sample with a flow calibrator (DCL-H, Bios, Butler, NJ). We used the average of these 2 rates as the sample flow rate for all volume calculations. No sample deviated more than 10% from the initial flow rate during the study. During sampling, we recorded temperature and RH.

We collected each bioaerosol sample for 2 minutes, in the method of Nevalainen et al,[25] on nutrient media (specific to either fungi or bacteria) in Petri dishes located on the impactor. We applied both malt extract agar (MEA 2%) and dichloran glycerol 18 agar (DG-18) for fungi, adding chloramphenicol to inhibit bacterial growth. We used trypcase soy agar (TSA) for bacteria, adding cycloheximide to inhibit fungal growth. We incubated the MEA, DG-18, and TSA plates at room temperature for 3 to 5 days, 5 to 7 days, and 2 to 3 days, respectively. Using the positive hole conversion method, we corrected counts from air sample plates for multiple impactions and reported the amounts as the number of colony-forming units per cubic meter of air (CFU m^{-3}). For the bioaerosol samples collected during the AC and heater experiments, we also identified the fungal genera on the basis of their morphological characteristics, using standard taxonomic keys.

RESULTS

Bioaerosol Concentrations Prior to and After Use of Automobile AC or Heater

Figures 1–4 display the time series microbial concentrations measured in 5 passenger cars prior to and after the use of an AC and a heater. We observed neither unusual moisture sources nor obvious fungal sources in the cars. We present data on the total fungal concentrations obtained using DG-18 but not MEA plates for the matched figures because the time-series concentration trends were similar for the 2 plates. The concentrations of total bacteria, total fungi, and fungal species measured inside the vehicles prior to turning the AC on were lower than or similar to those for outdoor air (see Figures 1 and 2 and Table 2). For Car 1 and Car 2, the total bacterial concentrations were elevated 5 minutes after turning the AC on, and they declined gradually with the duration of the AC use, whereas for the other 3 cars, the bacterial concentrations did not significantly vary prior to or after switching on the AC, or with the duration of operation. We were unable to collect the information necessary to better understand the time-series concentration trend for those 3

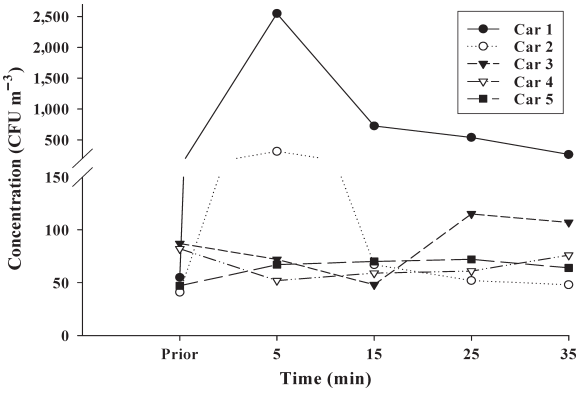

Fig. 1. Airborne total bacterial concentrations measured in 5 passenger cars prior to and after switching on the air conditioner. The average outside levels of total bacteria were 235, 247, 65, 285, and 58 CFU m^{-3} for Car 1, 2, 3, 4, and 5, respectively.

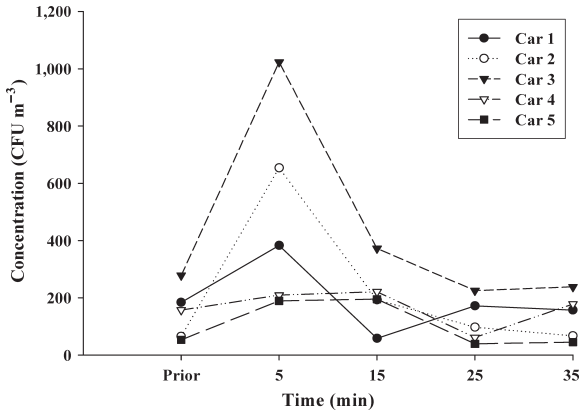

Fig. 2. Airborne total fungal on dichloran glycerol 18 agar (DG-18) concentrations measured in 5 passenger cars prior to and after switching on the air conditioner. The average outside levels of total fungi were 198, 77, 265, 161, and 48 CFU m^{-3} for Car 1, 2, 3, 4, and 5, respectively.

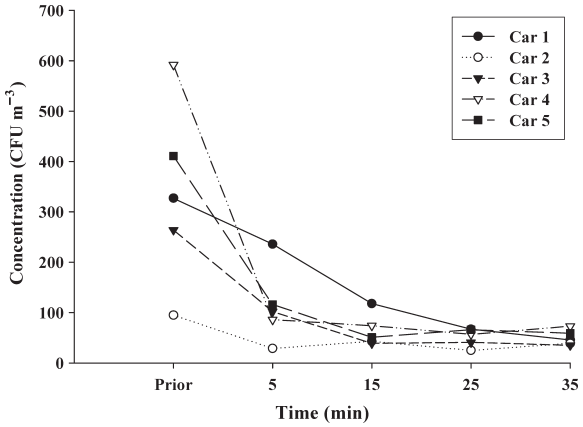

Fig. 3. Airborne total bacterial concentrations measured in 5 passenger cars prior to and after switching on the heater. The average outside levels of total bacteria were 336, 107, 279, 635, and 438 CFU m^{-3} for Car 1, 2, 3, 4, and 5, respectively.

cars. For Car 1, the maximum bacterial concentration (2,550 CFU m^{-3}) was 46 times higher than was the in-vehicle background concentration (55 CFU m^{-3}). A substantial amount of dust was ejected from Car 1's AC vent; the sampling day was the first day of summer use for that AC, wheras other cars' ACs had been used at least once prior to sampling. As such, Car 1's AC likely had a long-term accumulation of bacteria with dust, suggesting that the history of an AC is an important factor associated with exposure to bioaerosols during AC operation.

The trend in bacterial concentrations obtained from Cars 1 and 2 was similar to that from the office heating ventilation and air conditioning system reported by Law et al.[17] For all test cars, elevated concentrations of total fungi occurred either at 5 or 15 minutes after the automobile AC was turned on, and the levels decreased with duration of use. A possible explanation is that the maximum number of bacteria and fungi accumulated in AC filters was ejected through the AC vent within the first 5 minutes of use and that the lower concentrations we recorded at 15, 25, and 35 minutes were due to the lower number of bacteria and fungi present in the AC filter. Findings from recent longitudinal AC studies[13,17] support this hypothesis. As such, opening automobile windows during the early phase of AC operation can minimize exposure to bioaerosols. Another possible explanation for the early, higher concentrations is that the filters in the AC systems removed a certain amount of airborne microorganisms from the outdoor air or the recirculated indoor air, thereby lowering the in-vehicle airborne microbe levels. This hypothesis is supported by findings from Kumar et al,[24] who reported significantly lower mold concentrations inside passenger cars with a filter compared with cars without filters. Air turbulence caused by the vehicle's AC might resuspend microorganisms from vehicle floor mats or fabric seats, thereby elevating in-vehicle airborne microbial concentrations. However, our results suggest that the resuspension of microorganisms because of car ACs was not significant enough to elevate microbial concentrations.

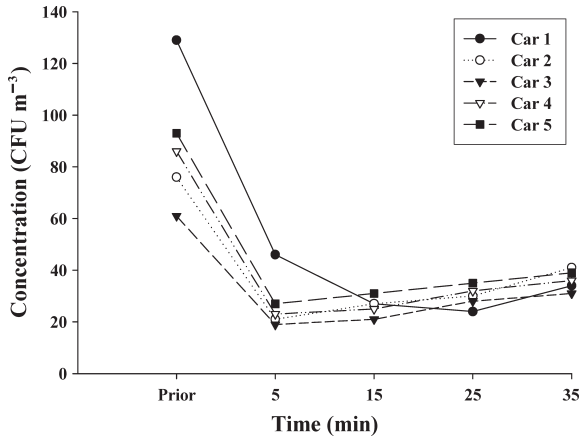

Fig. 4. Airborne total fungal on dichloran glycerol 18 agar (DG-18) concentrations measured in 5 passenger cars prior to and after switching on the heater. The average outside levels of total fungi were 137, 85, 77, 97, and 104 CFU m^{-3} for Car 1, 2, 3, 4, and 5, respectively.

Table 2 presents the concentrations of the 4 most prevalent fungi (*Alternaria, Aspergillus, Cladosporium*, and *Penicillium*) measured in 5 passenger cars prior to and after using the AC. Other fungi we found included *Arthrinium, Aureobasidium, Botrytis, Chatomium, Epicoccum, Exosporiella, Fusarium, Mucor, Nigrospora, Paecilomyces, Pestalotia, Phoma, Rhizopus, Stachybotrys*, and *Streptomyces*. However, we do not report these species because their concentrations were

Table 2.—Mean Concentrations in the Air (CFU m^{-3}) of 4 Fungal Species Found Inside and Outside Passenger Cars Prior to and After Switching on the Air Conditioner (AC) During the Summer and Heater During the Winter

				Inside after (min)				
Fungus	Type	Measuring agent	Inside prior	5	15	25	35	Outside
Alternaria	AC	MEA	ND	ND	ND	ND	ND	33
		DG-18	39	19	27	27	19	42
	Heater	MEA	ND	ND	ND	ND	ND	ND
		DG-18	ND	ND	ND	ND	ND	ND
Aspergillus	AC	MEA	41	138	23	67	41	37
		DG-18	53	176	85	57	49	43
	Heater	MEA	19	ND	ND	ND	ND	29
		DG-18	25	ND	ND	ND	ND	38
Cladosporium	AC	MEA	42	132	58	38	44	201
		DG-18	67	153	66	49	57	235
	Heater	MEA	23	ND	ND	ND	19	41
		DG-18	38	19	19	ND	19	38
Penicillium	AC	MEA	48	134	35	88	43	41
		DG-18	43	135	69	98	40	57
	Heater	MEA	19	ND	ND	ND	19	40
		DG-18	19	22	15	25	9	29

Note. We collected the outdoor air sample prior to and after each time-series indoor experiment; $n = 5$ for each data set of indoor samples. MEA = malt extract agar; DG-18 = dichloran glycerol 18 agar; ND = not detected.

considerably lower than those of the 4 major fungi. In most cases, the concentrations of 3 fungi (*Aspergillus, Cladosporium*, and *Penicillium*) were similar for the matched time periods and were much higher than those of *Alternaria*. This result is supported by previous study findings, which report these 3 species' predominance in AC filters.[13,19] Similar to the total amount of bacteria and fungi, we recorded the maximum concentrations of the 3 prevalent fungi 5 minutes after the automobile AC was turned on, and these decreased with duration of use.

We also measured the time-series bacterial and fungal concentrations in 5 passenger cars prior to and after the use of a heater during winter, to examine the effect of automobile heaters on in-vehicle microbial levels. Similar to the AC study, the concentrations of total bacteria, total fungi, and fungal species measured inside vehicles prior to the use of the heater were lower than or similar to those levels for outdoor air (see Figures 3 and 4, and Table 2). However, in contrast to the AC study, the total bacterial and fungal concentrations for all cars decreased gradually with the duration of heater operation to much lower levels than the background, in-vehicle levels (see Figures 3 and 4). For certain fungi, the in-vehicle concentrations declined to undetectable levels (see Table 2). The heat from the automobile's heating system would disinfect the recirculating microbes when the heater was in operation, thereby decreasing the number of viable microbes inside the vehicle. Moreover, the use of a heater could reduce RH and microbial growth. Further studies are needed to confirm these assertions. Similar to the automobile AC experiment, the results from the heater experiment suggested that the heater fan's resuspension of microorganisms was not significant enough to elevate in-vehicle microbial concentrations. Further studies are needed to confirm these assertions.

When compared with the MEA plates, the DG-18 plates produced better counts for the target fungi in both the AC and heater experiments. This finding is supported by Ren et al,[2] who reported a significantly higher CFU m^{-3} on the DG-18 plates than on the MEA plates for *Alternaria, Aspergillus*, and *Cladosporium* but not *Penicillium*. However, although Ren et al[2] also reported significantly higher total culturable fungi concentrations on the MEA plates than DG-18 plates via 1034 and 846 CFU m^{-3} ($p < .0005$), respectively, they suggested that the sole use of DG-18 to collect fungal samples was adequate for residential levels of those fungi and that using one medium for sampling is more economical and practical.

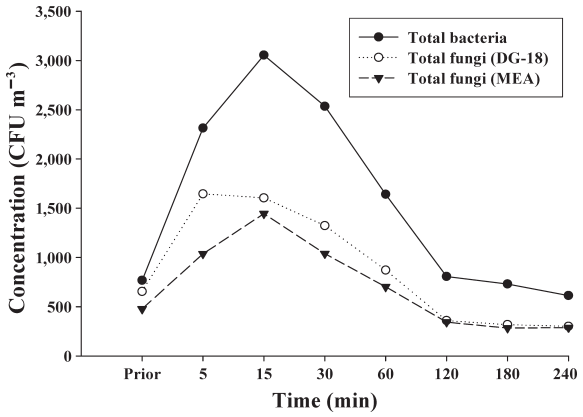

Fig. 5. Airborne total bacterial and total fungal concentrations measured in a seminar room prior to and after switching on the air conditioner. The room was surveyed 4 times, and average values are presented. The outside levels of total bacteria, total fungi on malt extract agar (MEA), and total fungi on DG-18 were 837, 762, and 646 CFU m^{-3}, respectively.

Bioaerosol Concentrations Prior to and After Use of Seminar Room AC

We measured the airborne concentrations of total bacteria, total fungi, and 4 fungal species in a seminar room prior to and after the use of an AC. We observed neither unusual moisture sources nor obvious fungal sources in the room. Similar to the automobile AC experiment, the concentrations of the total bacteria, total fungi, and fungal species measured prior to turning on the room AC were lower than or similar to those for outdoor air (see Figure 5 and Table 3). The maximum concentrations of total bacteria, total fungi,

Table 3.—Mean Concentrations in the Air (CFU m^{-3}) of 4 Fungal Species Identified Inside and Outside a Seminar Room Prior to and After Switching on the Air Conditioner During the Summer

			Inside after (min)							
Fungus	Measuring agent	Inside prior	5	15	30	60	120	180	240	Outside
Alternaria	MEA	31	184	203	137	67	31	45	36	39
	DG-18	78	190	248	151	71	40	62	53	85
Aspergillus	MEA	114	210	181	133	76	49	31	27	132
	DG-18	117	258	221	153	58	40	53	36	123
Cladosporium	MEA	155	318	376	315	270	103	78	87	198
	DG-18	208	353	397	274	264	155	105	101	184
Penicillium	MEA	136	325	301	235	128	32	45	62	140
	DG-18	172	401	322	261	146	53	75	53	158

Note. We collected the outdoor air sample prior to and after each time-series indoor experiment; $n = 4$ for each data set of indoor samples. MEA = malt extract agar; DG-18 = dichloran glycerol 18 agar.

Table 4.—Concentrations in the Air (CFU m^{-3}) of Total Bacteria and Total Fungi Identified Inside an Apartment Prior to and After Switching on the Humidifier During the Winter

			After (h)					
Measurement	Measuring agent	Prior	0.5	1.0	1.5	2.0	2.5	3.0
Total bacteria	TSA	325	275	290	295	284	204	247
Total fungi	MEA	325	253	216	270	143	198	233
	DG-18	382	253	325	325	307	325	288
Relative humidity (%)	—	40	43	46	48	50	53	57

Note. TSA = Trypcase soy agar; MEA = malt extract agar; DG-18 = dichloran glycerol 18 agar.

Table 5.—Concentrations (CFU m^{-3}) of Total Bacteria and Total Fungi Identified Inside Air of an Apartment Room Prior to and After Switching on a Humidifier During the Winter

			After (h)						
Measurement	Measuring agent	Prior	0.5	1.0	2.0	3.0	6.0	9.0	12.0
Total bacteria	TSA	457	205	249	207	336	395	287	305
Total fungi	MEA	175	183	96	60	37	64	41	54
	DG-18	191	164	134	78	73	82	47	54
Relative humidity (%)	—	46	51	55	59	62	69	74	75

Note. TSA = Trypcase soy agar; MEA = malt extract agar; DG-18 = dichloran glycerol 18 agar.

Table 6.—Concentrations in the Air (CFU m^{-3}) of Total Bacteria and Total Fungi Identified Inside an Apartment Prior to and After Switching on a Humidifier During the Winter

			After (h)					
Measurement	Measuring agent	Prior	3	8	16	24	48	72
Total bacteria	TSA	283	218	126	367	218	180	703
Total fungi	MEA	263	220	190	267	218	99	683
	DG-18	290	266	126	235	248	180	703
Relative humidity (%)	—	45	68	82	89	84	81	93

Note. TSA = Trypcase soy agar; MEA = malt extract agar; DG-18 = dichloran glycerol 18 agar.

and 4 fungi also occurred either 5 or 15 minutes after turning on the room AC, and the levels decreased gradually until 120 minutes, at which time the levels evened. This result suggests that the ventilation fan could disseminate viable organisms into the air for a limited time period. *Cladosporium* exhibited the highest concentrations for most sampling periods, followed by *Penicillium, Aspergillus,* and *Alternaria,* in descending order. Most concentrations of fungi were higher on the DG-18 plates than on the MEA plates.

Bioaerosol Concentrations Prior to and After Use of Household Humidifier

We measured time-series bioaerosol concentrations in an apartment prior to and following the use of a humidifier for 3 time periods (3, 12, and 72 hours), to examine the longitudinal variations of bacterial and fungal concentrations. For the 2 time-period experiments (3 and 12 hours), although the total bacterial and fungal concentrations that we measured after the use of the humidifier exhibited no trends, they decreased with the duration of the humidifier in operation (Tables 4 and 5), as compared with the background room levels. A possible explanation for this is that airborne microorganisms collide with fine water droplets that are sprayed into the air through a humidifier, and both microorganisms and water droplets settle down together. Moreover, humidifiers can eliminate some indoor bioaerosols through their filters.[26] Although the water in humidifiers is a potential source of microbiological proliferation,[16,19] we found no significant water contamination. This assertion is supported by the fact that we emptied and then filled the humidifier with fresh, chlorine-disinfected tap water prior to every experiment. However, we observed a significant elevation in both the total bacterial and total fungal concentrations 72 hours after the use of the humidifier. RH also increased gradually up to 93% (see Table 6). The elevation in

bioaerosol concentrations is most likely due to the resuspension of microorganisms that accumulated on wallpaper under humid conditions. The amount of microorganisms on the wallpaper would increase as time passes, then more resuspension of microorganisms occurs, thereby elevating airborne microbial concentrations. Although the use of a humidifier for 72 consecutive hours does not represent normal conditions, our results may reflect bioaerosol proliferation in a humid, enclosed environment. Similar to the AC and heater experiments, the concentrations of total fungi were higher on the DG-18 plates than on the MEA plates for most experiments.

COMMENT

We examined the relationship between bioaerosol levels inside vehicles in a university seminar room and in an apartment, and use of an AC, heater, or humidifier, respectively. We measured the temporal variation of indoor bioaerosol concentrations through a series of longitudinal studies with respect to the operating conditions of the electronic appliances. The concentrations of total bacteria, total fungi, and fungal species measured inside the vehicles and in a seminar room prior to the use of an AC were lower than or similar to those levels for outdoor air. In most cases, elevated bioaerosol concentrations occurred between 5 and 15 minutes after the automobile or household room AC was turned on, and the levels decreased with an increased duration of use, suggesting that opening windows during the early phase of an AC operation is necessary to minimize exposure to bioaerosols. The present results also suggested that resuspension of microorganisms caused by an automobile AC or heater fan were not significant enough to elevate in-vehicle microbial concentrations. In addition, the use of an automobile heater and a household humidifier could suppress in-vehicle and in-room microbial concentrations under conditions similar to those in our study.

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This study was supported by Research Grant KNU-2005-3-3 from Kyungpook National University. The authors thank Mr Seo Y. for his sample collecting and analyses.

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