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Size Distribution of Airborne Fungi in Vehicles Under Various Driving Conditions

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Size Distribution of Airborne Fungi in Vehicles Under Various Driving Conditions

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ABSTRACT. Circulation or air conditioning (AC) system was proven to improve the air quality inside the vehicles; however, the quantified study was limited. In this study, fungal concentration under various driving mode inside the vehicle was proposed. The driving conditions were classified into 4 states: (1) window closed without AC and circulation, (2) window open without AC and circulation, (3) window closed with only circulation on, and (4) window closed with only AC on. Results show that at state 4, the mean respirable fraction was 83.3%, with a number median diameter of the fungi being 1.73 μm . More attention should be paid for these smaller fungi easily penetrating into the alveoli and probably lead to allergic alveolitis. Turning on AC for reducing the normalized concentration for each size range of fungi was suggested; however, the respirable fraction increased. Those who are prone to allergies or asthma are suggested to switch between AC and the circulation mode while driving a long time.

KEYWORDS: fungi, number median diameter, size distribution, vehicles

Bioaerosols are airborne particles that are living or originate from living organisms. They are often associated with asthma,¹ rhinitis,² sick building syndrome,³ and hypersensitivity pneumonitis.⁴ Fungal contamination is also related to fungal allergies.⁵ An estimated 20 million Americans suffer from asthma, and allergic-asthma cases are around 50%.⁶ Additionally, the prevalence of allergenic rhinitis has been estimated as 15~20% in Finland.⁷ So does it in Taiwan, asthma prevalence has also increased over the last 2 to 3 decades, especially in children. Fungal exposure has been implicated as one of the causative or triggering factors

for asthma.¹ It has also long been recognized as an important source of allergens in patients with atopic disease.

An automobile is an enclosed microenvironment that researchers are interested in evaluating personal exposure. Numerous studies have been performed to assess exposure to air pollution in vehicles. These studies tend to focus on exposure to particulate matter (PM),^{8,9} carbonyl compounds,¹⁰ and volatile organic compounds (VOCs).^{8,11} Especially, traffic-related fine particles have been reported to be harmful.^{12,14} However, few studies have investigated the level of bioaerosols in vehicles. Most bioaerosol studies focused

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on seasonal and diurnal variability in airborne molds in indoor residential environments¹⁵ or measured concentrations in offices and hospitals,^{16–19} but seldom in vehicles.²⁰ Jo and Lee²¹ investigated airborne fungal and bacterial levels under automobile air conditioner or heater use. They indicated that bioaerosol concentrations increased between 5 and 15 minutes after the use of an automobile air conditioner. Hamada and Fujita²² also evaluated the effect of air conditioners on fungal contamination. Their results showed that fungal contamination increased only inside air conditioners that were used daily.

Some drivers enjoy driving with windows closed, and some prefer windows open. The states of the circulation system and the air conditioner also affect the air quality and comfort inside vehicles. Contaminants, including PM,²³ pollen,⁷ and VOCs,^{8,24} in cars have been carefully studied; however, the size distribution of fungi in cars, especially under various driving conditions, has not been studied. This study tried to investigate the distribution of airborne fungal concentrations and the size distribution inside vehicles under various driving conditions. This is the first study to present size distribution data of bioaerosol associated with driving conditions. The results of this study will provide information for further research on exposure assessment.

METHODS

Sampling information

Four vehicles, including 2 cars and 2 vans, whose engine size, model year, and mileage ranged from 1800 to 2000 cc, 1996 to 2000, and 77,733 to 96,117 miles, respectively, were chosen. All vehicles used the 95 lead-free gasoline and the seats were artificial leather chairs. The in-vehicle airborne bioaerosol samples were taken while the car engines were idling at the campus of Chung Yuan Christian University during the sampling period (from 9:00 AM to 11:00 AM) between November and December. The averaged temperature was 19~32°C, the relative humidity was 48%~76%, and the fungal concentration was between approximately 212 and 482 colony-forming units (CFU)/m³ outside. The driving conditions were classified into 4 states: state 1, window closed without air conditioning (AC) and circulation; state 2, window open without AC and circulation; state 3, window closed with only circulation on; and state 4, window closed with only AC on. The sampling sequence was as follows: state 1 → state 3 → state 4 → state 2. Interior bioaerosol levels were equilibrated for 15 minutes before each sampling. A 6-stage microbial cascade impactor (TE-10-830; Tisch Environmental, Cleves, OH, USA) was placed on the armrest of the front seat to sample airborne fungi. A total of 56 samples (336 plates) were analyzed. The aerodynamic diameter ranges for stages 1 to 6 were >7, 4.7~7, 3.3~4.7, 2.1~3.3, 1.1~2.1, and 0.65~1.1 μm, respectively. The sampling flow rate was 28.3 L/min, the sampling time was 5 minutes, and the sampling height was around 70 cm to mimic the height

of a seated driver's nose. The field blanks were obtained and analyzed side by side with the actual samples. Plates were put in the sampler; however, the pump was not turned on during sampling. The results showed that there were no fungi grown for the field blanks.

Measurement and analysis

Malt extract agar (MEA) with chloramphenicol was used for capturing fungal samples. Field blanks were also collected during the sampling. After sampling, the Petri dishes were incubated for 120 hours at 25°C (Taiwan EPA method E401.11C) for fungi. The concentration of airborne fungi (CFU/m³) was calculated by dividing the total colony counts on the agar plate by the air volume. The fractions for each stage of bacteria and fungi were defined as follows:

$$F_i = C_i / C_f \times 100\% \quad (1)$$

where F_i is the fungal fraction (%), C_i is the stage i concentration for fungi, and C_f is total concentration of fungi.

$$R_f = (C_3 + C_4 + C_5 + C_6) / C_f \times 100\% \quad (2)$$

where R_f is the respirable fraction for fungi.¹⁸

Respirable fraction represents the fraction of bioaerosol easily penetrating into human trachea and bronchiole. Additionally, the environmental parameters (temperature and relative humidity) were recorded using an AirBoxx monitoring system (KD Engineering, Tuscon, AZ, USA) during sampling.

Data analysis

The Bonferroni-Dunn test using SPSS 13.0 (SPSS, Chicago, IL, USA) was conducted to determine the result of statistics. The Dunn test, a kind of multiple comparison, compares the difference in the sum of ranks between 2 columns with the expected average difference. The test was performed to examine the different concentrations in airborne fungi over various driving habits and environmental factors.

RESULTS AND COMMENT

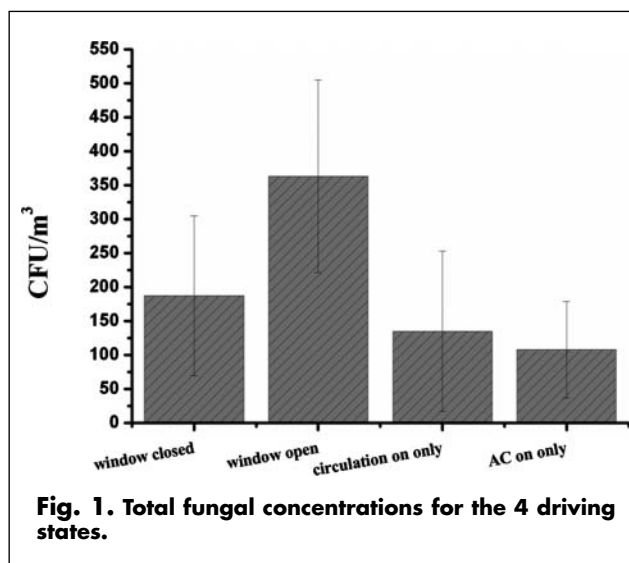
Limitation

The sampling vehicles including 2 cars and 2 vans with model years ranging from 1996 to 2000. During this time range, AC and car technology changed a lot. This is the limitation that the many intercorrelated factors could often not be included in the statistical analysis. Additionally, the small sample size is a limitation in this study. The result was probably due to the sample size not big enough to distinguish differences among various driving states.

Level of airborne fungi for the 4 driving states

Figure 1 and Table 1 show a comparison of the total airborne fungal concentrations for 56 samples for the 4 driving states. The mean concentration of fungi was 363 CFU/m³ for state 2 (window open without AC and circulation). Lee et al²⁵ found that the total fungal spore concentration outdoors was usually greater than that indoors. Rizzo et al²⁶ indicated the good relationship of fungal concentrations between indoors and outdoors. Dassonville et al²⁷ also found that outdoor levels and season largely contributed to the variability of indoor airborne fungal concentrations. Thus, the higher mean concentration for state 2 in this study was possibly associated with the concentrations outside the vehicle, which were between 212 and 482 CFU/m³. This was consistent with the results in winter in Lee and Jo's²⁰ study, which indicated in-vehicle sources were not strong enough to outweigh the outdoor concentrations that penetrated inside the vehicles. However, there was no statistical significant difference for fungal concentration in-vehicles and outdoors. This was because the sampling in-vehicles and outdoors were not taken simultaneously and the sampling size was not big enough.

Except state 2, it was interesting to find the levels of fungal concentration were in the order state 1 > state 3 > state 4. According to statistical analysis, windows open or closed showed statistically significant difference on fungal concentration ($p < .05$). Turning on circulation or AC would help to reduce the fungal concentration in cars in this study. The filters in the AC system removed a certain amount of airborne fungi comparing with the situation in state 3; however, the



difference between state 3 and state 4 was not so obvious, probably due to the small sampling size. The fungal concentration in state 4 was 108 CFU/m³, which was the lowest under the 4 various driving states in this study. The result was lower than that in Hamada and Fujita's²² study, but similar to that in Jo and Lee's²¹ study. An average of 200 CFU/m³ airborne fungal concentration in cars with AC was found in Hamada and Fujita's²² study. The temporal variation in bacterial and fungal levels with the use of ACs was investigated in Jo and Lee's²¹ study. The highest concentration was obtained at 5 minutes after turning on the AC and the levels

Table 1.—Driver's Exposure to Airborne Fungi Under Various Driving Conditions

Overall	State 1		State 2		State 3		State 4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fungi (CFU/m ³) ^a	187*	118	363*	142	135	118	108	71
F1 (%) ^b	8.15	3.13	8.72	3.50	16.6	23.6	7.57	11.7
F2 (%)	5.73	4.30	15.1	2.87	16.5	19.0	9.12	7.03
F3 (%)	13.6	8.59	20.5	9.36	13.0	12.4	15.2	10.2
F4 (%)	30.3	7.14	34.8	5.40	22.7	19.2	22.3	12.0
F5 (%)	39.0	15.1	19.9	14.3	27.3	20.3	38.0	15.4
F6 (%)	3.32	4.49	1.01	0.86	3.85	5.53	7.78	7.65
R _f (%)	86.1	6.02	68.7	31.6	66.8	33.5	83.3	16.5
CO ₂ (ppm)	3,952*	1,908	402*	83	2899	2452	1732	1198
CO (ppm)	8.10	4.82	6.90	5.76	9.20	6.39	8.70	5.54
HCHO (ppm)	<LOD		<LOD		<LOD		<LOD	
Temperature ^c (°C)	25.7	3.25	21.0	4.14	27.6	3.73	24.8	4.65
Relative humidity (%)	71.0*	11.2	62.0	13.9	65.0	11.7	53.0*	5.03

^an = 56 (14 for each state).

^bF1 (>7 μm), F2 (4.7~7 μm), F3 (3.3~4.7 μm), F4 (2.1~3.3 μm), F5 (1.1~2.1 μm), and F6 (0.65~1.1 μm).

^cAverage temperature.

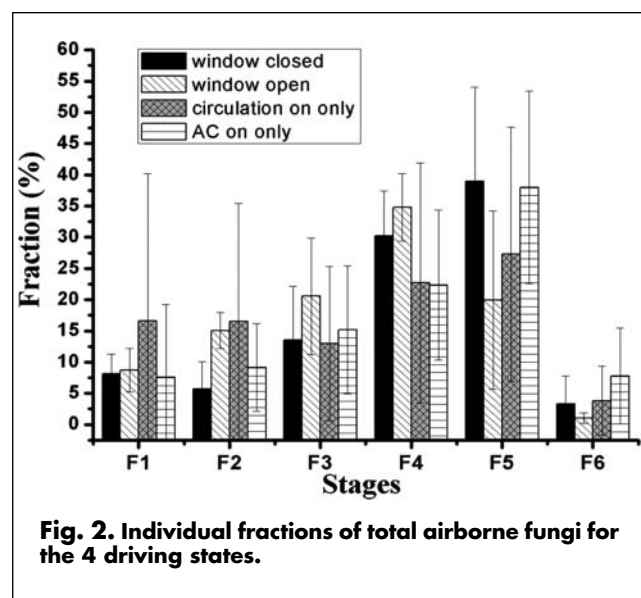
*Statistical significance is found ($p < .05$).

LOD = limit of detection.

decreased with duration of use. The sampling in this study was taken with the interior bioaerosol levels equilibrated for 15 minutes. The fungal concentrations in these vehicles might reach equilibrium with the background concentrations after 15 minutes.

The temperatures for states 1 to 4 were 26°C, 21°C, 28°C, and 25°C, respectively. The humidities for states 1 to 4 were 71%, 62%, 65%, and 53%, respectively. Windows open or closed showed statistically significant difference in fungal concentration ($p < .05$) and CO₂ concentration ($p < .05$). Turning on or off AC also showed statistically significant difference on humidity ($p < .05$) and fungal concentration ($p < .05$). The preliminary test showed that *Cladosporium* was the prevalent fungal genus in this study. Jo and Lee²¹ have also identified the *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* was the 4 most prevalent fungi in cars. The same result was found in Lee and Jo's¹⁹ study, which investigated the indoors and outdoors bioaerosols at high-rise apartment buildings. Katial et al²⁸ further indicated that the *Cladosporium* spp. concentration was positively correlated with temperature and relative humidity. In this study, a higher relative humidity led to a higher total fungal concentration was found, except for state 2 (window open). Additionally, Pastuszka et al²⁹ indicated spores of *Alternaria*, *Aspergillus*, *Penicillium*, and *Cladosporium* spp. are responsible for causing an array of respiratory conditions, from allergic rhinitis to asthma. Rivas and Thomas³⁰ also illustrated the airborne spores of *Cladosporium* species are significant allergens, and in large amounts they can severely affect asthmatics and people with respiratory diseases. Accordingly, to those who are sensitive to allergies should be aware of the effect of *Cladosporium* spp. while driving.

Figure 2 shows the fractions of airborne fungi for the 4 driving states, where F1~F6 are the 6 stages, respectively. The highest fractions were obtained for F4 (2.1~3.3 μm)



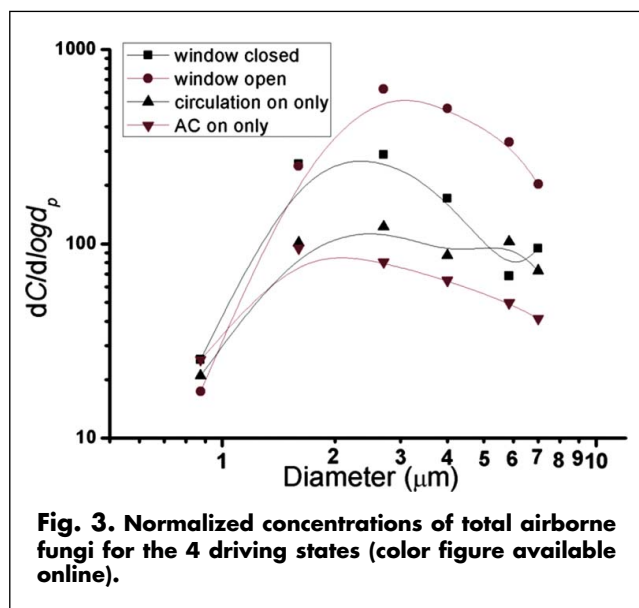
and F5 (1.1~2.1 μm) for all driving states. The fractions for states 1, 3, and 4 were highest in F5 and lowest in F6. However, the fraction for state 2 was highest in F4 and lowest in F6. For F1 and F2, the fractions were highest for state 3. For F3 and F4, the fractions were highest for state 2. For F5, the fraction was highest for state 1. For F6, the fraction was highest for state 4. The sum of the fourth and fifth fractions were 69.2%, 54.7%, 50.0%, and 60.3% for states 1 to 4, respectively. There were no significant differences ($p > .05$) neither between window open or not nor between AC on or off. Furthermore, the mean respirable fractions for states 1 to 4 were 86.1%, 68.7%, 66.8%, and 83.3%, respectively. The standard deviation between states 1 and 4 is lower than that between states 2 and 3. It probably implied that the states 1 and 4 provided more stable environment rather than states 2 and 3. The higher R_f in states 1 and 4 probably made inhalable fungi circulated inside vehicles, but there was no statistical significance found for respirable fractions among states owing to the small sample size.

The diameter of most fungal spores falls in the range of 1~30 μm ,³¹ allowing the spores to penetrate into the airways of the human respiratory tract. Infections can be caused by pathogens and the subsequent dissemination. However, respirable fraction (R_f) receives more attention because these smaller bioaerosol fungi easily penetrate into the alveoli and can lead to allergic alveolitis and other illnesses.³² R_f was in the range of 66.8% to 86.1% in this study, which is higher than the values obtained in the study of Kim and Kim.¹⁸ R_f values in this study were 86.1% and 83.3% for states 1 and 4, respectively, which are much higher than those found in Kim and Kim's study. These values are comparable to those found in Pastuszka et al's study,²⁹ which compared healthy and moldy homes in Upper Silesia, Poland. The results showed that the R_f values were 70% and 80% in the summer and winter, respectively. Those prone to allergies or asthma who spend lots of time driving with the window closed with or without AC should be aware of the potential adverse health effects of the high respirable fraction of fungi in vehicles.

Size distribution of airborne fungi for the 4 driving states

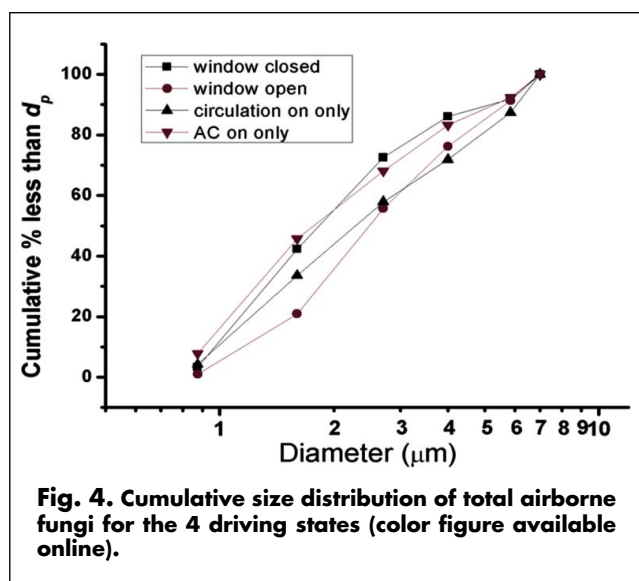
The concentrations of airborne fungi collected on the 6-stage median of the Andersen sampler were recorded. The normalized concentrations of airborne fungi ($dC/d\log dp$ versus dp) obtained from the test cars are shown in Figure 3. All of the fungal concentrations have a major peak in the size range of 2~3 μm for all driving states. The size distributions for the 4 driving states are all monomodal. The patterns of the normalized concentration distribution for state 1 and state 2 are similar.

Figure 4 shows the cumulative size distribution of total airborne fungi for the 4 driving states. The results indicate that 72.6%, 55.7%, 57.9%, and 68.1% of airborne fungi were



smaller than the range of $2.1 \sim 3.3 \mu\text{m}$ for states 1 to 4, respectively. The number median diameter (NMD) is the diameter that divides the total number into 2 halves.³³ The geometric standard deviation (σ_g) indicates the variability of fungal concentration. The NMD values of the total fungal concentration were 1.73, 2.82, 2.32, and $1.73 \mu\text{m}$ for states 1 to 4, respectively. The σ_g values of the total fungal concentration were 2.08, 2.33, 2.15, and $2.15 \mu\text{m}$ for states 1 to 4, respectively. The NMD for state 2 was the highest, and the NMD for state 4 was the lowest. The σ_g value for state 2 was the highest, and the σ_g value for state 1 was the lowest.

In the present study, turning on AC reduced the total fungal concentration (Figure 1) and lowered the normalized concentration for each size range (Figure 4). However, using AC gradually increased the ratio of the respirable fraction



(Figure 3) and lowered the NMD. To the best of our knowledge, there are only a few studies on the measurement of size distribution of airborne fungal concentrations in cars. Turning on AC or circulation certainly reduced the fungal concentrations in cars; however, it increased the prevalence of smaller fungi ($<2.1 \mu\text{m}$), as shown on Figure 2. Hamada and Fujita²² found that fungal contamination in house dust decreased with AC use; however, it increased in the AC filter. Jo and Lee²¹ found that the bioaerosol concentration decreased to the background concentration after using AC for 15 minutes. Vonberg et al.³⁴ also indicated after starting the AC system, the total number of microorganism, mold spores, and particles were all reduced by 81.7%, 83.3%, and 87.8%, respectively. According to these results and intercorrelating the variation of size distributions among the various driving states in this study, those who are sensitive to allergies or asthma are suggested to switch between AC and the circulation mode while driving. Airborne fungi are often associated with asthma, rhinitis, sick building syndrome, and hypersensitivity pneumonitis; however, the relationship between bioaerosol aerodynamic diameter and asthma prevalence warrants further investigation in the future.

Conclusion

1. Windows open or not influences the fungal concentrations based on statistical analysis. The lowest mean concentration ($108 \text{ CFU}/\text{m}^3$) for fungi in vehicle was found with windows closed with only AC on. The highest one ($363 \text{ CFU}/\text{m}^3$) was found with windows open without AC and circulation.

2. Fungal exposure has been implicated as one of the triggering factors for asthma. The mean respirable fractions for states 1~4 were 86.1%, 68.7%, 66.8%, and 83.3%, respectively, as well as the number median diameter values of the total fungal concentration were 1.73, 2.82, 2.32, and $1.73 \mu\text{m}$ for states 1 to 4, respectively. Although not as high statistically as the residential levels reported in other studies,^{19,35-37} more attention should be paid for the major peak fungal concentration among size range of $2 \sim 3 \mu\text{m}$, which could easily penetrate into the alveoli and probably lead to allergic alveolitis and other illnesses.

3. According to the variations of fungal concentrations at different modes, the drivers who are sensitive to allergies or asthma are suggested to switch between AC and the circulation mode while driving, especially during the first 15 minutes after starting the engine. More research and further investigation are warranted due to the limited sample sizes.

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