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Exposure Level and Distribution Characteristics of Airborne Bacteria and Fungi in Seoul Metropolitan Subway Stations

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Abstract: The exposure level and distribution characteristics of airborne bacteria and fungi were assessed in the workers' activity areas (station office, bedroom, ticket office and driver's seat) and passengers' activity areas (station precinct, inside the passenger carriage, and platform) of the Seoul metropolitan subway. Among investigated areas, the levels of airborne bacteria and fungi in the workers' bedroom and station precincts were relatively high. No significant difference was found in the concentration of airborne bacteria and fungi between the underground and above ground activity areas of the subway. The genera identified in all subway activity areas with a 5% or greater detection rate were *Staphylococcus*, *Micrococcus*, *Bacillus* and *Corynebacterium* for airborne bacteria and *Penicillium*, *Cladosporium*, *Chrysosporium*, *Aspergillus* for airborne fungi. *Staphylococcus* and *Micrococcus* comprised over 50% of the total airborne bacteria and *Penicillium* and *Cladosporium* comprised over 60% of the total airborne fungi, thus these four genera are the predominant genera in the subway station.

Key words: Seoul, Subway station, Airborne bacteria, Airborne fungi, Underground

Introduction

As Seoul, the capital of South Korea, is increasing in size, the subway is increasing in popularity and is gradually being used as the principal method for public transportation. Thus, the indoor environment of subway stations, which about 10 million Seoul citizens use every day, is a major public health concern. Because subway stations are mostly located in closed underground spaces, their indoor air quality is worse than that of other public buildings above ground[1)]. In addition, the potential exposure of subway riders' to various airborne contaminants from both indoor and outdoor sources could be extremely high when considering the amount of time spent underground during travel or work.

In other foreign cities that have a subway system, field assessments of the subway stations' pollution have been focused on assessing the concentrations and characteristics of gaseous and particulate aerial pollutants[1–11)]. There is, however, relatively little information related to biological contaminants' distribution characteristics in subway stations[12, 13)].

It is very essential to assess exposure level of airborne biological contaminants in subway stations as they are considered as environmental factors provoking or aggravating respiratory disorders such as pneumonia, asthma and rhinitis and contagious diseases[14, 15)].

The central hypothesis of this study is that underground Seoul subway stations are contaminated to some extent by airborne bacteria and fungi, based on the fact

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that so many people pass through Seoul subway stations. People and their activities are one of the principal causes of indoor bioaerosol formation[16, 17]. Thus, this field-based study, focusing on subway passengers' and workers' indoor activity areas, was performed to investigate the distribution characteristics of airborne bacteria and fungi using both quantitative and the qualitative assessments.

Materials and Methods

Selection of subjects

This research was performed from Nov. 2006 to Feb. 2007 at 22 randomly-selected stations of lines 1–4, operated by the Seoul Metropolitan Subway (12 stations were evaluated for subway workers' working conditions and 10 stations for passengers' conditions). Of those stations, 8 were above-ground stations and 14 were underground. For each station, measurements were taken at the subway workers' main activity areas —station offices, bedrooms, ticket offices, and driver's seats, and the passengers' main movement areas —station precincts, platforms, and passenger carriages. For the comparative assessment of indoor subway pollution levels, one additional outdoor spot was investigated on the same day of indoor pollution measurement. The measured point was 1.5 m above floor level, and each point was measured twice. Measurements were taken between 10 a.m. and 4 p.m. to avoid rush hours that could violate the spatial restrictions of the airborne bacteria-measuring instrument.

Quantitative analysis

Air samples were collected for 10 min using a one-stage viable particulate cascade impactor (Model 10-800, Andersen Inc, USA) that was set for a flux of 28.3 l per minute[18]. The overall procedure of sampling and analysis for airborne bacteria and fungi was based on Kim and Kim's report[19]. Before collecting samples, the equipment's internal part was sterilized with 70% alcohol. To examine airborne bacteria, Trypticase Soy Agar (Lot 2087730, Becton Dickinson and Company, USA) was used as the growth medium with 500 mg cycloheximide added to suppress any fungal growth. To examine airborne fungi, 2% Malt Extract Agar (Lot 3111376, Becton-Dickinson, Sparks, MD, USA) was used as the growth medium with 100 mg chloramphenicol added to suppress any bacterial growth. After a bacterial or fungal sample was collected, the media was instantly moved to a microbe analysis room. Airborne bacteria were cultured for 1–2 d in an incubator at 35 ± 1°C, and airborne fungi were cultured for 3–5 d at room temperature (20–25°C). The concentration of airborne microbes (cfu m^{-3}) was calculated by dividing the value that was counted for the colony formed in the media after culturing by the air volume (m^3) (see equations 1 & 2).

CFU (Colony Forming Unit)/m^3 = Colony counted on agar plate / Air volume (m^3).....Eq. (1)

Air volume (m^3) = 28.3 l/min × sampling time (min)/10^3 .....Eq. (2)

Qualitative analysis

All cultured airborne bacteria were identified to the level of genera according to Bergey's manual[20]. After gram's staining of bacteria, additional identification was carried out by conducting biochemical test through the automated microbial identification system, VITEK (Model VITEK 32 system, bioMerieux Inc., France). Airborne fungal genera were identified according to the classification method of Ainsworth and Baron[21] by observing the form, shape and color of colony and spore through the optical.

Data analysis

The concentration difference of airborne bacteria and fungi for measurement points per area of subway workers and passengers was calculated with ANOVA using the SAS package (SAS/Stat 9.1, SAS Institute, Cary, USA). The concentration difference between an above ground station and an underground station was analyzed for statistical significance with the Student's *t*-test.

Results and Discussion

Concentration distribution of airborne bacteria and fungi in different areas of Seoul subway stations

As represented in Table 1, the mean concentrations of airborne bacteria in the station offices, bedrooms, ticket offices and driver's seats, which are the subway workers' main activity areas, were measured as 156 cfu m^{-3}, 246 cfu m^{-3}, 164 cfu m^{-3} and 118 cfu m^{-3}, respectively. Statistically, the bedroom had the highest concentration and the driver's seat had the lowest ($p<0.05$). The difference between the station office and the ticket office was not statistically significant ($p>0.05$). The mean outdoor concentration of airborne bacteria was 140 cfu m^{-3}. All three areas except for the driver's seat had higher airborne bacterial concentrations than measured outdoors.

For airborne fungi, the mean concentrations in the station offices, bedrooms, ticket offices and driver's seats were 89 cfu m^{-3}, 114 cfu m^{-3}, 82 cfu m^{-3} and 64 cfu m^{-3}, respectively. Statistically, the bedroom had the highest concentration of fungi and the driver's seat

Table 1. Concentrations of airborne bacteria and fungi in subway station areas

		Total airborne bacteria				Total airborne fungi			
		cfu m^{-3}							
		Mean	SD	Max.	Min.	Mean	SD	Max.	Min.
Workers' activity areas	Station office	156[a]	87	314	26	89[a]	53	207	22
	Bedroom	246[b]	168	686	28	114[b]	96	459	26
	Ticket office	164[a]	69	322	49	82[a]	27	128	35
	Driver's seat	118[c]	16	131	94	64[c]	4	67	58
Passengers' activity areas	Station precinct	224[a]	102	353	111	117[a]	36	157	71
	Inside train	165[b]	32	192	121	93[a]	19	110	71
	Platform	134[b]	36	176	91	105[a]	34	144	62
Outdoor		140	39	204	28	53	25	103	9

*a, b, c indicates that averaged values within the column by the same letter are not significantly different.

had the lowest ($p<0.05$). The difference between the station office and the ticket office was not statistically significant ($p>0.05$). The mean outdoor concentration of airborne fungi was 53 cfu m^{-3}. All four underground areas had higher airborne fungi concentrations than the outdoors.

The differences in airborne bacterial and fungi concentrations in the subway workers' activity areas compared to outdoors could be caused by the number of workers, whether windows were open or closed, and efficiency differences in the forced ventilation systems. This field research suggests that indoor spaces with a large number of workers and poor natural ventilation with either closed windows or a low-efficiency ventilation system have higher concentrations of airborne bacteria and fungi. This is based on the fact that one of the main sources of aerosols, especially airborne bacteria, is the person who resides in the indoor space[16, 17]. Given that all windows are closed during this winter field survey, the indoor space has poor natural ventilation due to closed windows or a low-efficiency ventilation system, and therefore shows a high concentration of airborne fungi[22, 23].

Bedroom measurements were taken during the day. For the most part, there were no workers present during the measurements, except for sleeping shift workers. The bedrooms were not clean, and had untidy bedding. In most of the bedrooms, ceiling ventilating openings were closed, or ventilating systems were not in operation at all, which may be why bedrooms had the highest concentrations of airborne bacteria and fungi. To assess the pollution level of airborne bacteria and fungi in bedrooms of subway workers clearly, a case-by-case study should be conducted to compare airborne microbe pollution levels according to whether a window is open or closed.

Because there has not been any domestic or foreign research that has documented the concentration level of airborne bacteria and fungi in relation to the activity areas of subway workers prior to this study, it is not possible to assess the exposure level of subway workers to airborne bacteria objectively. However, based on the fact that the concentration of airborne bacteria was below 800 cfu m^{-3} which is the domestic actual indoor regulation standard in multi-purpose facilities, and that generally, the concentration of airborne fungi in an indoor environment is $10–10^4$ cfu m^{-3} [24–27]. The pollution levels of airborne bacteria and fungi in subway worker areas is not considered threatening. However, except for the airborne bacteria measured at the driver's seat, all concentrations of airborne bacteria and fungi were higher than outdoors. Because the indoor/outdoor concentration ratio of airborne bacteria and fungi pollution is above one, the pollution must come from indoor contaminants[28, 29], indoor air quality should be continuously controlled. The subway drivers' seats were expected to have a higher concentration of airborne bacteria and fungi compared to the station offices, ticket offices and bedrooms, since they are located at a lower level the other areas. However, our field research indicated a lower level of contaminants in the drivers' seats. Most pollutants generated in the subway operation section are not organic substances that can be used as food sources by airborne bacteria and fungi, but are inorganic particulate substances which contain a metal component[30, 31]. The bioaerosol of airborne bacteria and fungi distributed in the underground space of the subway operation is less than 1% of the total aerosol[32].

The mean concentrations of airborne bacteria and fungi in the station precincts, passenger carriages and platforms, which are the main activity areas of subway passengers, were 224 cfu m^{-3} and 117 cfu m^{-3}, 165 cfu m^{-3} and 93 cfu m^{-3}, and 134 cfu m^{-3} and 105 cfu m^{-3}, respectively. The station precincts had

the highest airborne bacterial concentrations ($p<0.05$). There were no concentration differences between the passenger carriage and the platform ($p>0.05$). While station precincts had a concentration higher than 140 cfu m^{-3} (the mean outdoor concentration), the passenger carriage and the platform had concentrations lower than the outdoor concentration. For airborne fungi, the station precincts had the highest concentrations and the passenger carriage had the lowest concentrations, but this difference was not statistically significant ($p>0.05$). The mean outdoor concentration of airborne fungi was 53 cfu m^{-3}, which was lower than the three passenger areas.

Due to the structural limitations of the measuring instrument that was used in this research, it was not possible to collect air samples during rush hour. When considering that bacteria and fungi are dispersed into the air from subway passengers' clothes and hairs[10], further research during the rush hour may clarify the subway passengers' actual exposure level to airborne bacteria and fungi. Since indoor airborne bacteria and fungi flow indoors from the outside via air flow from the ventilation[33], and this research was conducted in the winter when the outdoor concentration is at its lowest of the four seasons[34], this research may underestimate the exposure level of subway passengers. Thus, additional research is needed to better clarify the concentrations of airborne bacteria and fungi in the subway during the different seasons.

In general, the concentrations of airborne bacteria and fungi were highest in the workers' bedrooms, which were in poor sanitary conditions with poor ventilation, and in the station precincts through which many people passed.

Indoor level of airborne bacteria and fungi in above-ground and underground subway stations

In Fig. 1, the concentrations of airborne bacteria and fungi in the station precincts, a platform, a station office, and a ticket office in the above ground station are compared with those of underground stations. Our results show that the concentration of both airborne bacteria and fungi was higher in the station precincts and the ticket office below-ground, and was higher in the platform and the station office in the above-ground station. However, the differences between the above-ground and the underground stations were not statistically significant ($p>0.05$). Therefore, the location of a subway station, whether above- or below-ground, did not affect the concentration of airborne bacteria and fungi.

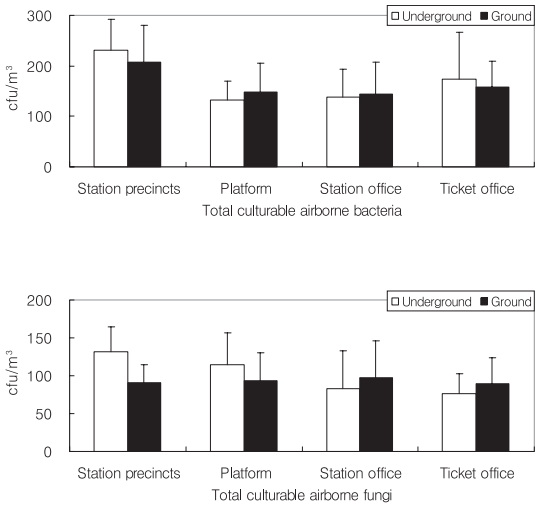

Fig. 1. Comparison of concentrations of airborne bacteria and fungi between underground and above-ground subway station areas.

Identification of airborne bacteria and fungi from the subway stations

Tables 2 and 3 show genera and detection rates of airborne bacteria and fungi identified in the main activity areas of subway workers and passengers in underground stations. In all areas researched, *Staphylococcus, Micrococcus, Bacillus, Corynebacterium* comprised over 5% of the total airborne bacteria detected. Of these bacteria, the airborne bacteria *Staphylococcus* and *Micrococcus* accounted for more than 50%. Generally, *Staphylococcus* was detected the most overall, but *Micrococcus* was predominantly detected in the bedrooms and platforms. This distribution of airborne bacteria is very similar to foreign research reports that identified airborne bacteria in other indoor areas[23, 35–37]. Subway areas were divided into a station office, a ticket office, the station precincts, a driver's seat, a passenger carriage, a platform and a bedroom. In the station office, the ticket office and the station precincts, the only other genus of airborne bacteria identified was *Enterobacteriaceae* with a detection rate below 1%, whereas in the driver's seat, the ticket office and the station precincts, *Escherichia (E-Coli), Pseudomonas*, and *Aeromonas* were identified. In the bedroom, *Nocardia* and *Pseudomonas* were identified with a detection rate below 1%. The lack of detection of *Nocardia* in all other areas is characteristic of this genus.

For airborne fungi, *Penicillium, Cladosporium, Chrysosporium* and *Aspergillus* showed detection rates over 5% in all research areas, and among these, the sum of detection rates of *Penicillium* and *Cladosporium* was over 60%. Even though *Penicillium* was detected

Table 2. Detection rate of airborne bacteria identified in underground areas of subway stations

	Workers' area				Passengers' area		
Genus	Station office	Bedroom	Ticket office	Driver's seat	Station precinct	Inside train	Platform
Staphylococcus spp.	42.8	16.2	50.5	35.2	55.8	33.8	30.5
Micrococcus spp.	26.3	62.4	24.6	30.2	16.4	28.5	32.9
Bacillus spp.	10.2	8.3	12.8	18.0	8.8	13.8	17.6
Corynebacterium spp.	9.5	6.3	6.2	5.2	7.3	9.3	4.3
Enterococcus spp.	5.3	-	1.8	2.8	3.2	7.4	3.5
Streptococcus spp.	0.8	2.8	0.5	0.8	1.2	1.5	2.3
Enterobacteriaceae spp.	0.3	-	0.9	-	0.8	-	-
Nocardia spp.	-	0.6	-	-	-	-	-
Escherichia (E-Coli) spp.	-	-	-	-	-	0.1	0.8
Pseudomonas spp.	-	0.2	-	-	-	-	0.4
Aeromonas spp.	-	-	-	0.4	-	0.3	-
Other species	4.8	3.2	2.7	7.4	6.5	5.3	7.7
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Table 3. Detection rate of airborne fungi identified in underground areas of subway stations

	Workers' area				Passengers' area		
Genus	Station office	Bedroom	Ticket office	Driver's seat	Station precinct	Inside train	Platform
Penicillium spp.	44.8	16.2	49.4	35.2	48.2	33.8	29.6
Cladosporium spp.	31.2	55.3	29.2	32.8	25.4	30.5	34.8
Chrysosporium spp.	6.8	11.1	4.7	7.0	6.3	5.9	6.6
Aspergillus spp.	10.6	8.2	12.5	15.4	8.2	13.6	9.8
Ulocladium spp.	-	4.3	-	4.5	-	6.8	3.8
Mucor spp.	-	-	-	-	-	-	7.2
Fusarium spp.	-	1.4	-	-	-	-	-
Yeasts	6.6	3.5	4.2	5.1	11.9	9.4	8.2
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

the most, *Cladosporium* was detected at the highest rate in the bedroom and the platform. Airborne fungi show a similar distribution tendency in other indoor areas to airborne bacteria[24, 38–40]. *Mucor* was detected on the platform and *Fusarium* was detected in the bedroom, though at very low frequencies, as would be expected.

The reason that different kinds of airborne bacteria and fungi were identified in different subway areas is most likely due to different internal sources. That is, most of the station office, the ticket office and the station precincts are located on the 1st floor below ground, while the driver's seat, the passenger carriage and the platform are mostly located on the 2nd floor below ground, though they are exposed above the ground from time to time. On a platform, for example, a puddle on the bottom of the railway that does not drain can be considered a source of new kinds of bacteria that can become airborne. Also, a bedroom with blankets and pillows that are not cleaned properly can be another source of airborne bacteria.

To clarify the health risk of airborne bacteria and fungi, an investigation into the concentration of airborne bacteria and fungi based on particle size, and the expansion of identification to species level are needed, while simultaneously collecting an outdoor sample to allow identification of internal subway sources. An analysis during rush hour should also be conducted to identify and quantify airborne bacteria and fungi when large numbers of people are present.

Another point to be considered when interpreting the results is a selection of agar media. The media of trypticase soy agar and malt extract agar used in this study for culturing airborne bacteria and fungi have a potential to underestimate their real concentrations in subway station because it is possible that all the airborne bacteria and fungi distributed in subway station are not detected in the media. For example, it should be difficult for several species of xerophilic fungi such as *A. penicilloides*, *A. restriscus*, *W. sebi*, and *Eurotium* spp. to be cultured in malt extract agar. This fact is a limitation of this study considered as interpreting results obtained from this field survey.

Conclusion

This study presents the distribution characteristics of airborne bacteria and fungi in the activity areas of workers and passengers corresponding to the stations of the Seoul subway lines 1–4. The survey results obtained from this study provide fundamental data which can be used by public health and industrial hygiene managers to develop preventative measures related to indoor air quality of subway stations.

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