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Characterization of indoor air quality and efficiency of air purifier in childcare centers, Korea

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ABSTRACT

Particulate matter (PM) samples were collected from inside ten childcare centers, and from their adjacent outdoor environments in Seoul, Korea during the summer, autumn and winter seasons. The concentrations and distribution of microbial size of the airborne bacteria and fungi in bio-aerosols were also investigated. The average indoor concentrations of fine particles less than 2.5 μm ($PM_{2.5}$) in the residential areas ranged from 37.1 μg/m^3 (±5.8 μg/m^3) to 45.2 μg/m^3 (±5.3 μg/m^3), while indoor $PM_{2.5}$ concentrations in centers with roadways nearby ranged from 48.9 μg/m^3 (±9.5 μg/m^3) to 52.9 μg/m^3 (±7.7 μg/m^3), and up to 51.1 μg/m^3 (±6.4 μg/m^3) in residential areas located near construction sites. The concentrations of particulate matter indoor in childcare centers were correlated with the corresponding outdoor locations, in residential areas (R^2 of 0.64 for PM_{10} and 0.66 for $PM_{2.5}$), near roadways (R^2 of 0.72 for PM_{10} and 0.76 for $PM_{2.5}$), and near construction areas (R^2 of 0.45 for PM_{10} and 0.62 for $PM_{2.5}$). The distribution of bio-aerosols showed that 69.4% to 78.1% of the airborne bacteria in the outdoor environments existed in stages 1–3 (over 3.3 μm), while from 59.2% to 78.6% existed in stages 2–4 (2.1–7.0 μm) inside the childcare centers. When the efficiency of air purifiers was compared with the location and characteristics of the indoor of child care centers, the removal efficiency of particulate matter with new data that may characterize indoor air quality.

1. Introduction

Indoor air quality has been attracting increasing amounts of public attention due to the fact that individuals spend over 80% of their time indoors [1–3]. Particulate matter (PM) concentrations indoors are about six times higher than outdoors [4,5]. Common indoor air contaminants include radon, tobacco smoke, mold, irritant and allergenic asthma triggers, combustion by-products and VOCs. Indoor contaminants may be of natural origin (e.g., radon, allergens, and molds), may derive from products used indoors (e.g., finishes, furnishings, and cleaning products) and may result from indoor processes and behaviors (e.g., smoking, use of unvented combustion sources; or cleaning, operation, and maintenance procedures) [6–10]. Building systems (e.g., heating, ventilating, and air conditioning) also have a direct influence on the type and amount of exposure building occupants may experience from environmental contaminants indoors [6,9]. Traffic is a significant PM source, causing higher PM levels in urban areas. Analysis of indoor particles less than 10 μm (PM_{10}) from motor vehicles showed that the coarse particles were mainly composed of particles from brake attrition and re-suspended road dust, whereas combustion processes were the main source of fine particles, in addition to the particles coming from outdoors by ventilation. Children living near areas with high traffic were shown to be more likely to have a greater number of asthma-related medical care visits per year, and a higher prevalence of respiratory symptoms, compared to those living near lower traffic conditions [4,11–13]. There have been many studies on the respiratory symptoms and diseases caused by long-term exposure to fine particles less than 2.5 μm ($PM_{2.5}$), and lung the function diseases and increase of hospitalization/death rates in the short-term [6,14–16].

Indoor $PM_{2.5}$ concentrations are generally affected by indoor emissions such as smoking, cooking (food handling), classroom cleaning, and re-suspension due to the number of people and indoor movements by people, outdoor–indoor transport processes

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such as leakage and ventilation, and movement mechanisms such as deposition during outdoor–indoor transport [6,7,17–20]. Previous research in this area has focused on the properties and behavior of $PM_{2.5}$ and PM_{10} [7,21–24]. These studies indicated significant penetration of particles of outdoor origin into indoor environments. In addition, the building shell was found to be ineffective at removing infiltrating particles. It is important to assess the penetration characteristics of particles into indoor environments.

Exposure to indoor PM is associated with a wide range of adverse effects, including respiratory, cardiovascular and allergy diseases [6,7,16,25,26]. The indoor pollutants standard proposed by the U.S. Environmental Protection Agency (EPA) has stimulated research on environmental diseases such as asthma, rhinitis and atopy, by exposure to humans, and subsequently, on the health issues of children [1,13,26–32]. More than 25% of children were estimated to suffer from allergic diseases in some countries of the European Union, and allergic diseases are also increasing rapidly throughout the world [26,33]. The health impact of air pollutants is much higher on small children than in adults in similar environments. Children are especially vulnerable to the effects of air pollution because their lungs and immune systems are still developing. In addition, they are more active in environments with high levels of pollutants, receiving higher doses than adults, because of differences in breathing rates and patterns.

Bio-aerosols may attach to other particles (rafting) and be transported together, and this highly polluted outdoor air (excluding auto vehicle emission) is the main source of particulate matter in the indoor environment. It follows that in air with high concentrations of particulates, the typical size distribution of bio-aerosols can be changed, resulting in altered size distributions of respirable bacteria and other bio-aerosols [14,18,34]. Bio-aerosols of 5–10 μm diameter deposited in the upper respiratory tract can cause rhinitis, and aerosols of less than 5 μm may induce allergies through the alveoli [6,35–39].

Many indoor bio-aerosols originate outdoors, but specific bio-aerosols may develop due to microbial growth in a building's heating, ventilation and air-conditioning (HVAC) system [39–41]. The NIOSH (National Institute for Occupation Safety and Health) reported that 5% of the biological pollutants influenced indoor air pollutants from 1971 to 1981, while from 35 to 50% of indoor pollutants were influenced by biological pollutants in the 1990s [14]. Most of the research on the distribution characteristics of the microorganisms in indoor air in Korea were conducted in hospitals and underground living spaces, and it is very necessary to obtain information about the indoor air quality including the distribution of microorganisms in school environments [35,42–45]. In addition, the impact could be more serious on children in daycare centers, since the children are smaller and have a higher metabolic rate than adults, which means that they breathe in more air per unit of body weight and are generally more susceptible to the effects of indoor air pollutants and airborne contaminants, which are likely to be more inhaled due to the outlying behaviors such as sucking, running, etc. [1,16,46]. The prevalence of children with asthma in Korea was only 3.4% in 1964, but it had more than doubled by the mid-1990s, with the highest airborne bacteria levels being shown in childcare centers in Korea, which caused the air quality in childcare centers to be announced as an important issue to be managed and controlled in the 2011 survey [26,46,47].

Therefore, our study aimed to (1) measure the indoor and outdoor particle and bio-aerosol concentrations in childcare centers, (2) to evaluate the correlation coefficient (R^2) of particulate matter and associated bio-aerosols (3) and to assess the effects of air purifier operation for indoor air quality in childcare centers.

2. Materials and methods

2.1. Sampling sites and descriptions

The study was conducted in ten childcare centers, including residential childcare centers which were near apartments and houses, childcare centers in commercial areas located near roadways adjacent to heavy traffic areas, and childcare centers existing near construction sites in Korea during the summer (6/29/2013–8/31/2013), fall (9/1/2013–11/30/2013) and winter (1/6/2014–2/21/2014) periods. Table 1 shows a general description of the sampling sites and environmental conditions of the ten daycare centers investigated in Korea. Sampling in the summer was conducted during the operation of mechanical ventilation air conditioning (MVAC) systems or window-type air conditioners. However, natural ventilation by opening and closing windows was always performed in the fall and summer, even though the child care centers were cooled by air conditioner all day. In addition, air purifiers were sometimes operated in the childcare centers.

2.2. Sampling and analysis of particles matter (PM_{10}, $PM_{2.5}$) and airborne bacteria and fungi

The samplers were worked for five consecutive days (24 h, 9:00 A.M.–9:00 A.M.) every week between July 29 and November 1. The indoor and outdoor particulate samples were simultaneously collected in two classrooms attended by children aged 5–7 in each childcare center. During the sampling period, the inlets of the indoor sampler were located at about 1.2–1.5 m above ground level at mid-points in the sampled classrooms. For the outdoor sampling, samplers were located on the rooftop of a 5 floor building, with the sampler inlets located 1.0–1.5 m above rooftop level. Indoor and outdoor particles levels were tested simultaneously at all observed sites to evaluate the relationship between indoor and outdoor particles.PM_{10} was assessed by the light scattering method (Light scattering method, Grimm 1.108, Germany) at an air flow rate of 1.2 L/min. The concentrations of $PM_{2.5}$ were analyzed using a portable sampler (SN3136, Air metrics, USA) per 1hr period. The $PM_{2.5}$ concentrations measured by the portable sampler were compared to the values measured using the gravimetrical method (DIN EN 12341). Data were analyzed by means of Spearman's test, using STATA 8.0 (Stata Corp., College Station, TX).

Table 1 shows the environmental conditions such as temperature and relative humidity. The U.S. NIOSH recommend sampling for 10 min at a flow rate of 28.3 L/min in order to prevent the medium from drying out, with control of the suction time according to the airborne concentration if necessary [48]. A study by Yang, Park [49] indicated that increasing the time from 5 min to 10–15 min rather caused a 57–66% decrease in the measured concentrations of airborne bacteria. Therefore, the samples of airborne bacteria and fungi in this study were prepared following the Anderson principle (Solomon, 2003), and were conducted using single stage Anderson samplers with 400(N)-0.25 mm holes (MAS-100 Eco, MERCK, USA), equipped with a high performance suction pump that drew at a flow rate of 100 L/min for 2 min (Buck, A.P. Buck, Inc., USA). Each sample was nominally collected for 2 min every 20 min during the investigation periods (10:00–16:00), following [Nevalainen, Pastuszka [50]], on Tryptic Soy Agar (HANIL KOMED CO., LTD. KOREA) for airborne bacteria, and on Malt Extract Agar (DIFCO., USA) for fungi in Petri-dishes (100 × 15 mm) located on the sampler impactor. In cases of the low concentrations of airborne bacteria and to compare the results of concentrations every 1 min for 5 min intervals, a sampling time of 3 min was used, allowing evaluation of the lower concentrations of airborne bacteria by colony overgrowth.

Table 1
General description of sampling sites and environmental conditions at the ten childcare centers investigated in Korea.

Childcare center	Yrs[a]	Air purifier operation	Location	Site	Temp.[°C] mean ± S.D.	R.H.[%] mean ± S.D.	Number of children/ area (m^2)	Ventilation types
A	10+	Yes	Residential	A-1	24.8 ± 0.1	53.6 ± 0.4	18/33.6	Natural ventilation
				A-2	24.3 ± 0.2	56.2 ± 0.8	20/31.6	
B	5+	Yes	Residential	B-1	23.7 ± 0.2	54.6 ± 0.5	20/35.2	MVAC system
				B-2	24.8 ± 0.1	53.7 ± 0.3	19/32.3	
C	20+	Yes	Residential[b]	C-1	22.4 ± 0.3	58.6 ± 0.5	20/39.7	Natural ventilation
				C-2	23.7 ± 0.4	57.4 ± 0.3	20/41.3	
D	30+	Yes	Commercial	D-1	29.8 ± 0.2	44.0 ± 0.3	17/115.7	Window type air conditioner
			Near road way	D-2	27.2 ± 0.1	52.3 ± 0.7	20/100.6	
E	10+	No	Residential	E−1	25.3 ± 0.2	40.2 ± 0.7	17/32.4	Exhaust fan
				E−2	24.8 ± 0.2	41.3 ± 0.5	19/30.9	
F	15+	No	Residential	F-1	25.8 ± 0.1	52.2 ± 0.5	21/66.1	Natural ventilation
				F-2	24.3 ± 0.2	53.6 ± 0.4	20/38.3	
G	20+	Yes	Commercial[c]	G-1	23.0 ± 0.1	54.1 ± 0.4	13/19.8	Natural ventilation
			Near road way	G-2	24.1 ± 0.2	56.3 ± 0.3	18/23.9	
H	15+	No	Residential	H-1	29.5 ± 0.1	55.3 ± 0.7	20/57.8	Exhaust fan
				H-2	28.2 ± 0.2	56.3 ± 0.6	18/57.3	
I	20+	Yes	Residential	I-1	29.6 ± 0.1	51.8 ± 0.5	20/63.8	Natural ventilation
				I-2	28.2 ± 0.2	52.7 ± 0.6	17/45.9	
J	30+	Yes	Commercial[c]	J-1	27.9 ± 0.3	62.3 ± 0.2	20/40.0	Window type air conditioner
			Near road way	J-2	25.9 ± 0.2	57.3 ± 0.1	19/37.6	

[a] Construction years.
[b] Near construction.
[c] Near subway station.

The samplers were calibrated prior to and following the collection of each sample with a flow calibrator (DCL-H, Bios, Butler, NJ). The average of these two rates was then used as the sample flow rate for all the volume calculations. Tryptic Soy Agar (TSA) and Malt Extract Agar (MEA) plates were incubated at 30–35 °C for TSA plates and 25–30 °C for MEA plates during 1–2 days and 5–7 days, respectively. The counts for air sample plates were corrected for multiple impactions using the positive hole conversion method, and reported as colony-forming units per cubic meter of air (CFU/m^3) [51].

Biological activity of the bio-aerosol particles during and after collection is an important concern, which differs from physical aerosol particle sampling. Furthermore, sample handling and storage, as well as the analysis of the collected biological particles, are considerably different from the procedures used in general particle sampling [51]. Therefore, eight blank petri dishes were prepared and analyzed to compensate for the error which can occur during handling and transportation of the samples, and the samples results were corrected with the average of the blank concentrations. The results were reported as an average value of colony-forming units per cubic meter of air (CFU/m^3).

2.3. Distribution of microbial size for airborne bacteria and fungi

The distribution of microbial size for bio-aerosols that represented viable airborne bacteria and fungi were sampled employing a six stage viable particulate cascade impactor (TISCH six-stage viable sampler, USA) operated with a high-performance suction pump on the principle introduced by Anderson (Solomon, 2003). The range of the aerodynamic diameter consisted of stage 1 (≥7.0 μm), stage 2 (4.7 ~ 7.0 μm), stage 3 (3.3 ~ 4.7 μm), stage 4 (2.1 ~ 3.3 μm), stage 5 (1.1 ~ 2.1 μm) and stage 6 (0.65 ~ 1.1 μm). Airborne bacteria were measured by impacting onto a petri dish containing TSA for 10 min. The dishes were incubated for 48 h at 35 °C. To count the viable fungi, air samples were collected on MEA and incubated for 5 days at 30 °C.

2.4. Effects of air purifier operation

The effects of an air purifier (LA-R119SWF, Korea) were assessed by analyzing the concentrations of particulate matter (PM_{10}, $PM_{2.5}$) and bio-aerosols (airborne bacteria and fungi). First, the concentrations of particulate matter (PM_{10}, $PM_{2.5}$) and bio-aerosols (airborne bacteria and fungi) were measured without operation of the air purifier. Second, the air purifier was operated for five days. Finally, the concentrations of particulate matter and bio-aerosols were analyzed during twenty days, and were compared before and after air purifier operation.

3. Results and discussion

3.1. $PM_{2.5}$ and PM_{10}

3.1.1. Common evaluation

Fig. 1 shows the comparison of the average PM_{10} and $PM_{2.5}$ concentrations measured indoors and outdoors at the childcare centers. The average indoor concentrations of PM_{10} at the six sites (A, B, E, F, H, I) located in residential areas ranged from 66.7 μg/m^3 (±4.8 μg/m^3) to 81.3 μg/m^3 (±9.2 μg/m^3), which were slightly higher than those of 61.5 μg/m^3 (±5.3 μg/m^3) to 80.3 μg/m^3 (±6.3 μg/m^3) measured outdoors (Fig. 1a). The Pearson paired *t*-test between indoor and outdoor PM_{10} levels centers B and D revealed differences between those measured outdoors ($p < 0.01$). In addition, the average concentrations of PM_{10} inside centers D, G and J, which were located in urban districts and adjacent to roads with heavy traffic and ranged from 78.9 μg/m^3 (±4.3 μg/m^3) to 91.3 μg/m^3 (±11.2 μg/m^3), were lower than those at center C, which was located in a residential area near a construction site and ranged from 86.9 μg/m^3 (±4.8 μg/m^3) to 101.0 μg/m^3 (±5.6 μg/m^3) ($p < 0.01$).

The average indoor concentrations of $PM_{2.5}$ in centers located in residential areas ranged from 37.1 μg/m^3 (±5.8 μg/m^3) to 45.2 μg/m^3 (±5.3 μg/m^3), while those located near roadways ranged from 48.9 μg/m^3 (±9.5 μg/m^3) to 52.9 μg/m^3 (±7.7 μg/m^3), and that nearby a construction site ranged from 51.1 μg/m^3 (±6.4 μg/m^3) (Fig. 1b). For all observed sites, the indoor concentrations of PM_{10} ranged from 33.6 to 105.9 μg/m^3 with an average of 80.0 μg/m^3, while indoor $PM_{2.5}$ concentrations ranged from 20.3 to 63.2 μg/m^3 with an average of 45.9 μg/m^3. The corresponding outdoor levels varied between 55.9 and 121.3 μg/m^3 with an average of 81.4 μg/m^3 (±5.8 μg/m^3) for PM_{10}, and between 21.3 and 66.3 μg/m^3 with an

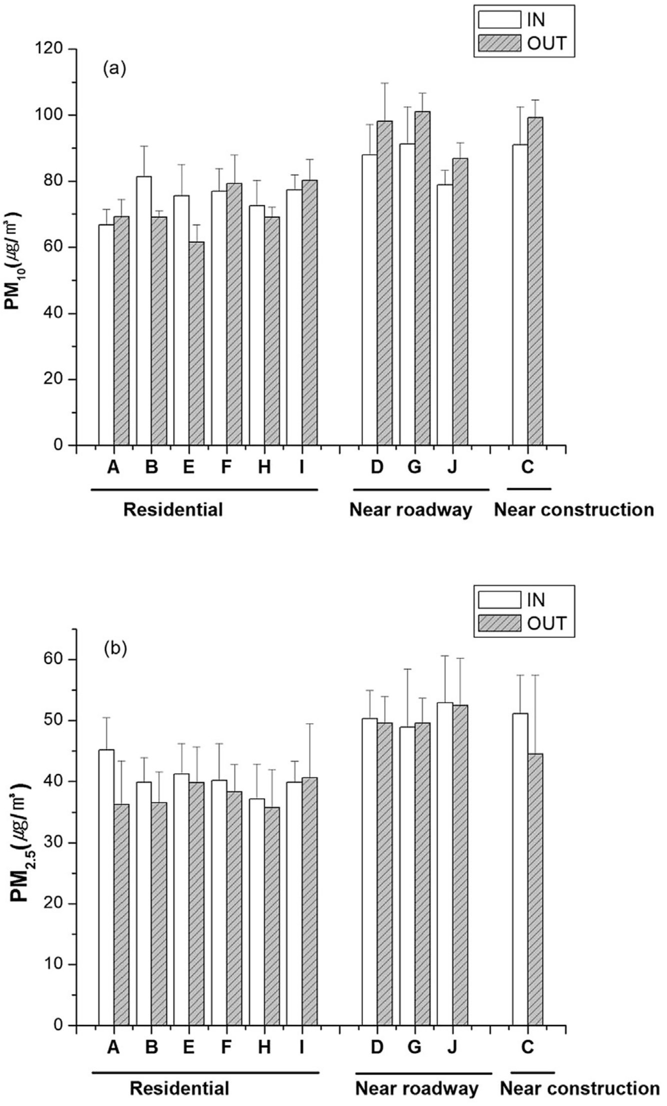

Fig. 1. Comparison of average indoor and outdoor (a) PM_{10} and (b) $PM_{2.5}$ concentrations at childcare centers.

average of 42.3 μg/m^3 (±6.7 μg/m^3) for $PM_{2.5}$. The indoor levels of PM_{10} did not exceed 150 μg/m^3, the outdoor PM_{10} standard recommended by the USEPA (1997), and those of $PM_{2.5}$ were not higher than the USEPA standard (65 μg/m^3), Korea (IAQ 24hr average of 50 μg/m^3, 2015). However, by the WHO regulation (24hr average of 25 μg/m^3), the concentrations of $PM_{2.5}$ exceeded the recommend values in the childcare centers. Table 2 summarized the statistics of indoor and outdoor concentrations of PM_{10} and $PM_{2.5}$, together with the indoor/outdoor (I/O) ratios and $PM_{2.5}$/PM_{10} ratios in the 20 sites of the ten childcare centers (2 classrooms/center).

The $PM_{2.5}$/PM_{10} ratios were found to range from 0.4 to 0.9, with an average of 0.7 for indoor air, and from 0.2 to 0.7, with an average of 0.5 for outdoor air. The average indoor ratio of $PM_{2.5}$/PM_{10} was higher than outdoors, indicating that fine particles comprised a large fraction of PM_{10} in the childcare centers (Fig. 2). Generally, $PM_{2.5}$/PM_{10} levels ranged from 0.6 to 0.9 in indoor air nearby traffic areas, and from 0.7 to 0.9 in the respective outdoor air. Furthermore, the PM_{10} levels were well correlated with the $PM_{2.5}$ loadings [32,52]. This indicated that the portions of $PM_{2.5}$ in PM_{10} were high. Therefore, high $PM_{2.5}$/PM_{10} ratios were found in the childcare centers due to the infiltration of $PM_{2.5}$ by natural ventilation. In addition, child activities causing re-suspension will induce higher indoor particle concentrations.

Table 2
Statistics of $PM_{2.5}$ and PM_{10} concentrations in classrooms and outdoors at ten childcare centers (μg/m^3).

Sites	Statistics	Indoors			Outdoors			I/O ratios	
		$PM_{2.5}$	PM_{10}	$PM_{2.5}$/ PM_{10}	$PM_{2.5}$	PM_{10}	$PM_{2.5}$/ PM_{10}	$PM_{2.5}$	PM_{10}
A-1	Min.	39.6	60.5	0.5	22.8	62.0	0.4	1.0	0.8
	Max.	55.7	72.8	0.8	51.2	77.3	0.7	1.6	1.0
	Mean.	45.8	68.1	0.7	37.2	70.4	0.5	1.3	1.0
A-2	Min.	38.2	61.3	0.5	21.2	66.3	0.5	1.1	0.7
	Max.	52.2	71.2	0.7	44.3	71.3	0.6	1.4	0.9
	Mean.	44.6	65.3	0.7	35.2	68.2	0.5	1.2	0.7
B-1	Min.	34.9	71.2	0.4	31.2	66.3	0.5	0.8	0.9
	Max.	47.6	99.1	0.6	50.3	72.6	0.7	1.4	1.4
	Mean.	41.6	81.7	0.5	35.5	69.3	0.7	1.1	1.2
B-2	Min.	32.2	76.3	0.5	35.2	65.2	0.4	0.7	0.6
	Max.	46.7	98.2	0.6	48.2	71.2	0.7	1.3	1.3
	Mean.	38.2	80.9	0.5	37.5	68.9	0.5	0.9	0.8
C-1	Min.	40.9	67.3	0.5	25.2	91.6	0.2	0.9	0.7
	Max.	62.9	115.7	0.7	66.3	108.8	0.6	1.7	1.0
	Mean.	47.9	109.4	0.6	45.4	100.0	0.6	1.2	0.9
C-2	Min.	41.3	66.9	0.6	28.3	89.6	0.4	0.8	0.8
	Max.	65.3	89.3	0.7	67.5	96.2	0.7	1.6	0.9
	Mean.	54.3	72.6	0.6	43.6	91.2	0.6	1.2	0.7
D-1	Min.	42.2	72.0	0.5	42.7	72.3	0.4	1.0	0.8
	Max.	58.3	105.9	0.6	57.3	121.4	0.6	1.1	1.0
	Mean.	47.4	94.6	0.6	54.9	95.0	0.5	1.0	0.9
D-2	Min.	46.3	75.6	0.4	45.3	71.2	0.5	0.9	0.7
	Max.	59.3	98.3	0.6	59.8	113.2	0.6	1.1	1.1
	Mean.	53.2	81.2	0.5	44.3	101.2	0.5	1.0	0.8
E–1	Min.	37.7	61.0	0.4	30.1	55.9	0.5	0.9	1.0
	Max.	49.2	91.3	0.7	51.3	69.2	0.8	1.2	1.4
	Mean.	38.6	76.6	0.5	38.3	51.8	0.6	1.0	1.2
E–2	Min.	36.6	68.2	0.5	32.9	69.3	0.6	0.8	0.7
	Max.	48.3	72.3	0.7	55.6	78.9	0.9	1.1	1.1
	Mean.	43.8	74.6	0.6	41.3	71.2	0.7	0.9	1.0
F-1	Min.	32.2	63.9	0.5	31.2	65.2	0.5	0.9	0.8
	Max.	54.3	95.1	0.7	51.2	101.3	0.6	1.3	1.2
	Mean.	35.1	85.9	0.6	27.3	82.4	0.5	1.1	1.0
F-2	Min.	33.6	66.2	0.5	33.6	72.3	0.5	0.8	0.7
	Max.	56.3	78.2	0.6	55.9	88.9	0.6	1.2	1.1
	Mean.	45.3	67.9	0.5	49.3	76.2	0.5	0.9	0.9
G-1	Min.	20.3	53.1	0.4	43.6	94.4	0.4	0.4	0.5
	Max.	62.6	100.0	0.7	57.9	113.2	0.7	1.1	1.1
	Mean.	48.9	91.3	0.5	48.0	110.8	0.5	1.0	0.9
G-2	Min.	21.3	65.2	0.5	45.6	89.6	0.6	0.9	0.6
	Max.	56.3	101.2	0.6	59.6	97.7	0.5	1.1	1.2
	Mean.	48.6	93.3	0.5	51.2	91.2	0.5	1.0	0.8
H-1	Min.	30.3	62.4	0.4	27.6	34.2	0.4	0.9	0.9
	Max.	49.2	88.8	0.7	51.2	76.0	0.6	1.5	1.2
	Mean.	32.9	67.0	0.5	32.9	44.0	0.5	1.1	1.1
H-2	Min.	32.3	77.6	0.6	32.2	101.3	0.5	0.7	0.9
	Max.	55.9	98.3	0.7	55.3	66.9	0.6	1.3	1.4
	Mean.	41.3	78.2	0.6	38.5	94.2	0.5	1.1	1.2
I-1	Min.	31.9	61.2	0.4	27.9	69.0	0.3	0.7	0.9
	Max.	46.3	87.9	0.6	59.7	97.6	0.7	1.4	1.0
	Mean.	42.9	75.5	0.5	38.3	78.9	0.5	1.0	1.0
I-2	Min.	33.9	75.3	0.5	32.3	66.3	0.6	0.9	0.7
	Max.	48.2	89.2	0.6	66.8	89.2	0.7	1.2	0.9
	Mean.	36.9	79.3	0.5	42.9	81.7	0.5	1.1	0.6
J-1	Min.	32.9	71.2	0.8	32.9	75.6	0.4	0.9	0.9
	Max.	69.3	88.2	1.0	62.3	92.3	0.7	1.1	1.0
	Mean.	52.9	78.9	0.9	47.8	84.5	0.6	1.0	1.0
J-2	Min.	33.9	75.3	0.8	39.6	78.9	0.5	0.8	0.9
	Max.	72.3	89.3	0.9	65.2	97.2	0.6	1.2	1.0
	Mean.	50.5	78.6	0.8	57.2	89.3	0.5	0.9	0.9

Indoor-outdoor paired-sample *t*-tests of PM_{10} and $PM_{2.5}$ were also conducted to determine whether the indoor or outdoor mean concentrations were significantly higher. PM_{10} levels were correlated with $PM_{2.5}$ loadings with correlation coefficients (R^2) of 0.20–0.28 for indoor and 0.47–0.94 for outdoor levels in childcare

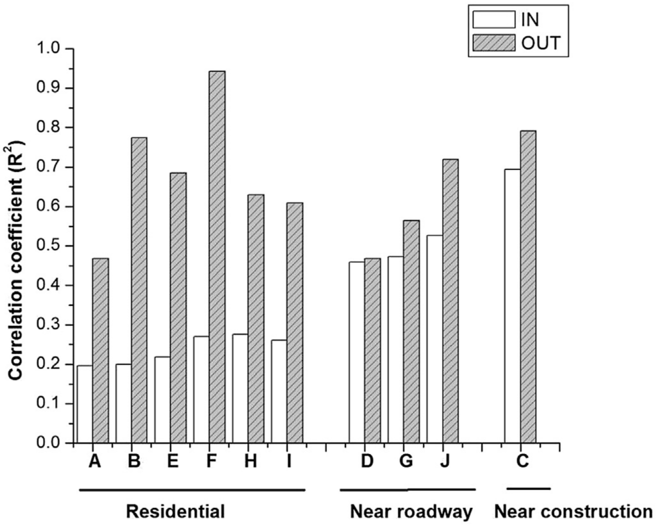

Fig. 2. Relationship between indoor and outdoor PM$_{10}$ and PM$_{2.5}$ concentrations according to the location of childcare centers.

centers located in residential areas. The PM$_{10}$ levels were correlated with PM$_{2.5}$ loadings with correlation coefficients (R^2) of 0.46–0.53 for indoor and 0.47–0.72 for outdoor levels in the centers located near roadways (Fig. 2).

Therefore, the PM$_{2.5}$/PM$_{10}$ ratios in centers located near roadways showed higher values more often than those located near residential areas, and the PM$_{10}$ levels were well-correlated with PM$_{2.5}$. In addition, indoor sources such as cooking, cleaning, child activities, wind-induced natural ventilation, and re-suspended particles depended strongly on the distribution of the ambient particles in the daycare centers.

The indoor/outdoor (I/O) ratio is an indicator of whether the indoor levels are influenced by significant indoor sources of particulates, or if indoor levels are the result of outdoor particle concentrations. I/O ratios of the observed sites varied from 0.7 to 1.4, with an average of 1.1 for PM$_{10}$, and from 1.2 to 1.9, with an average of 1.4 for PM$_{2.5}$. The average I/O ratio of PM$_{2.5}$ was higher than that of PM$_{10}$, indicating higher penetration of PM$_{2.5}$ than PM$_{10}$ from outdoors, and stronger indoor sources of PM$_{2.5}$ than PM$_{10}$, considering the effects of human activities and ventilation types on the indoor particulate levels.

Indoor particles mainly affect the indoor air quality, including those generated by processes such as cleaning, cooking (food handling), organic and silicate particles due to human activity, and re-suspension due to child activities, even though pollutants are also generated by the operation of ventilation systems and the penetration of outdoor air pollutants [13,28,53]. Outdoor particulate concentrations may not be reliable indicators of indoor and personal particulate exposures, due to the poor associations between outdoor and indoor particulate levels or personal levels, which may result from the presence of indoor particulate sources. On an equal weight base, the PM$_{10}$ of indoor air has been shown to be toxicologically more active than outdoor PM$_{10}$, with the main difference being a higher concentration of organic and silicate particles in the indoor air.

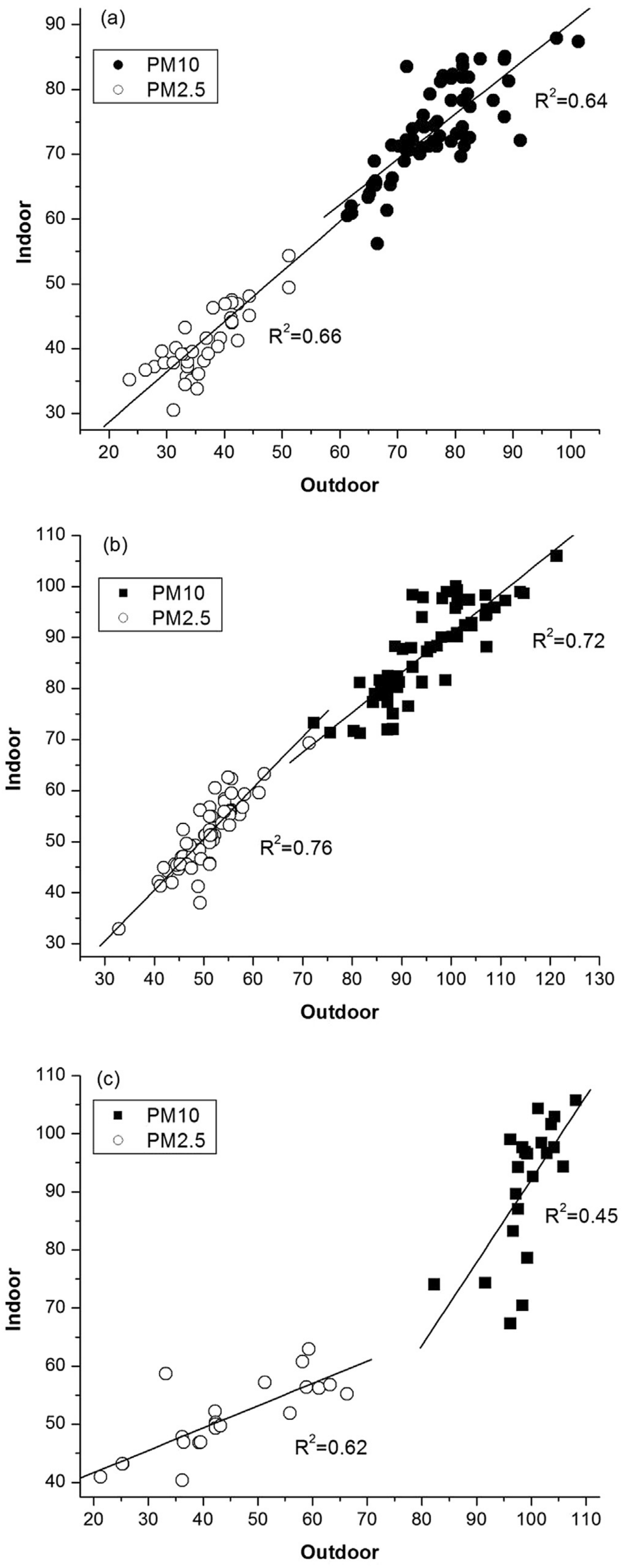

Fig. 3. Relationship between indoor and outdoor PM$_{10}$ and PM$_{2.5}$ concentrations based on location: (a) residential; (b) near roadway; (c) near construction.

3.1.2. Impact of different outdoor environments

The indoor concentrations of PM$_{2.5}$ and PM$_{10}$ in the childcare centers were related to the outdoor environmental conditions. The relationship between the outdoor and indoor particulates was investigated using linear regression analysis. Fig. 3 shows the correlation coefficient (R^2) for the indoor and outdoor measurements, according to the classification of the outdoor environments such as residential, near roadways, and near construction sites.

A proper linear relationship with the correlation coefficients (R^2) of 0.64 for PM_{10} and 0.66 for $PM_{2.5}$ in the residential childcare centers (Fig. 3a), 0.72 for PM_{10} and 0.76 for $PM_{2.5}$ (Fig. 3b) in those near roadways, and 0.45 for PM_{10} and 0.62 for $PM_{2.5}$ in that near a construction area (Fig. 3c) could be found, implying that the outdoor levels strongly influenced the indoor levels. Regarding the outdoor factors, first, three of the childcare centers (D, G, J) were situated in close proximity to a heavy-traffic road where a lot of vehicles often cause traffic jams. The effects of traffic conditions and motor vehicle exhaust outdoors on the indoor levels of $PM_{2.5}$ and PM_{10} in the childcare centers were very significant. Second, construction activities were conducted nearby daycare C during sampling. The construction activities significantly increased the ambient suspended particulate matter, which inevitably influenced the indoor air levels of $PM_{2.5}$ and PM_{10}. Finally, the effect of child activities in the classrooms on the indoor air particulate levels in the daycare centers was very important.

3.1.3. *Effects of ventilation patterns*

While window-type air conditioners were used in the childcare centers during the summer (6/29/2013–8/31/2013), natural ventilation by opening and closing windows was mainly used in all seasons. In addition, only one of the childcare centers operated MVAC (mechanical ventilation air conditioning) systems, while some classrooms had window type air conditioners and exhaust fans installed for ventilation. Therefore, the concentrations of indoor particulates were related with the ventilation patterns in the childcare centers. Fig. 4 shows the effects of the different ventilation types on the I/O ratios of PM_{10} and $PM_{2.5}$. Ventilation using exhaust fans and window-type air conditioners showed high I/O ratios of 1.37 and 1.40 for PM_{10} and 1.30 and 1.85 for $PM_{2.5}$, respectively, while natural ventilation and the MVAC (mechanical ventilation air conditioning) system showed lower I/O ratios of 0.7 and 0.9 for PM_{10} and 1.2 and 1.1 for $PM_{2.5}$, respectively. This indicated that the filtration processes of natural ventilation and the MVAC system were relatively effective to remove the particles. MVAC systems are usually equipped with air filters, and were found to be the most effective to remove coarse particles [52]. Wang, Bi [52] showed I/O ratios much lower than 1, produced by use of the MVAC system and natural ventilation. In this study, we could not prove the effects of the MVAC system due to the high $PM_{2.5}$ concentrations from indoor sources in the childcare centers. In addition, the I/O ratios in childcare centers using natural ventilation were also higher than 1. This indicated that the filtration system was not effective to remove the particles from childcare centers. Overall, the window-type air conditioner was the most inefficient at removing particles, even leading to accumulation of the particles. The indoor environmental quality at childcare centers depended on the operation of ventilation, infrequent and inadequate cleaning of indoor surfaces, and a large number of students per classroom area and volume, with constant re-suspension of the particles from the surfaces along with suspension of soil material due to the activity of the children. Therefore, we evaluated the effect of another method, using an air purifier, on the indoor particles.

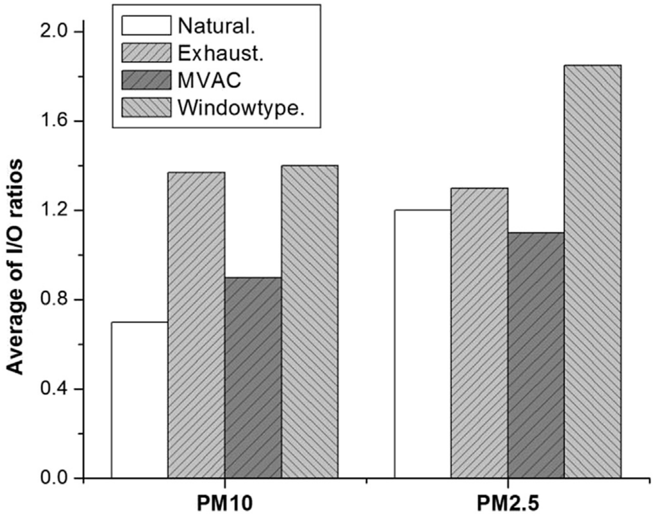

Fig. 4. Comparison of average I/O ratios of particulate materials according to different ventilation types. (Natural: natural ventilation; Exhaust: exhaust fan; MVAC: mechanical ventilation air conditioning,; Window type: window-type air conditioner).

3.2. *Total airborne bacteria and fungi*

3.2.1. *Evaluation of concentrations*

The concentrations of total airborne bacteria and fungi indoors and outdoors at the childcare centers are shown in Fig. 5. The

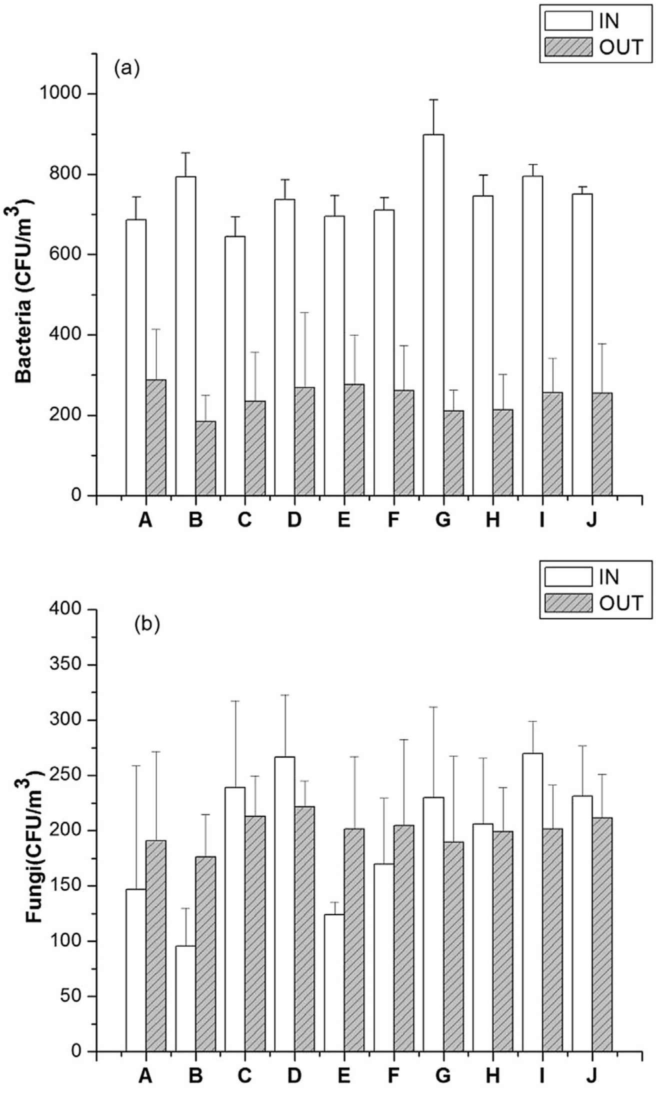

Fig. 5. Concentrations of total airborne (a) bacteria and (b) fungi located indoors and outdoors at childcare centers.

average concentrations of airborne bacteria indoors ranged from 645.3 CFU/m^3 (±49.0 CFU/m^3) to 898.5 CFU/m^3 (±86.5 CFU/m^3), which were higher the respective outdoor values, which ranged from 184.9 CFU/m^3 (±65.0 CFU/m^3) to 288.3 CFU/m^3 (±125.5 CFU/m^3). The concentrations of airborne bacteria in childcare center G, which had a high child to area ratio, exceeded the Indoor Air Quality (IAQ) Standard of Korea (limit of 800 CFU/m^3). The concentrations of airborne bacteria in centers B, H and I also exceeded the IAQ standard of Korea when considering the maximum values. The average indoor concentrations of airborne fungi ranged from 95.6 CFU/m^3 (±34.2 CFU/m^3) to 269.6 CFU/m^3 (±29.6 CFU/m^3), which was higher than the outdoor range of 176.0 CFU/m^3 (±38.5 CFU/m^3) to 221.6 CFU/m^3 (±23.5 CFU/m^3).

The concentrations of airborne fungi in the childcare centers did not exceed the WHO standard (limit of 500 CFU/m^3) (Fig. 5). However, fungal spores can utilize living plants to grow well when provided with adequate moisture at over 25 °C, which occurs from September to October more than in summer. Therefore, center D showed higher concentrations (266.7 ± 56.3 CFU/m^3) than the other centers. The year of construction of the building, according to the degree of aging, also reflects the fungi concentrations. Specific bio-aerosol sources may develop due to microbial growth in the building's heating, ventilation and air-conditioning systems, or in the building's structure itself [18]. These results are similar to another report on the health assessment and efficient management of the airborne bacteria in childcare centers [54].

Although indoor airborne bacteria standards (800 CFU/m^3) are regulated by the Korean Ministry of Environment, it is impossible to present the exposure limits to pathogens [55], as the response to pathogens varies, and information on the dose–response relationship and epidemiological data are also insufficient. In addition, the guideline for fungal concentrations of indoor air was presented by the WHO (less than 500 CFU/m^3), OSHA (<1000 CFU/m^3), Singapore (<500 CFU/m^3), Brazil (<1000 CFU/m^3) and other relevant agencies. However, quantitative risk criteria for fungal species have not yet been established [56,57].

The I/O ratios of the observed sites varied from 2.3 to 4.2 for airborne bacteria, and from 0.5 to 1.3 for airborne fungi, indicating stronger indoor sources of airborne bacteria than fungi, considering activities of the children and skin particles (Fig. 6). The average I/O ratios of airborne bacteria were higher than those of airborne fungi, showing the significantly high value I/O ratios of airborne bacteria while the I/O ratios of airborne fungi were similar to other studies [57].

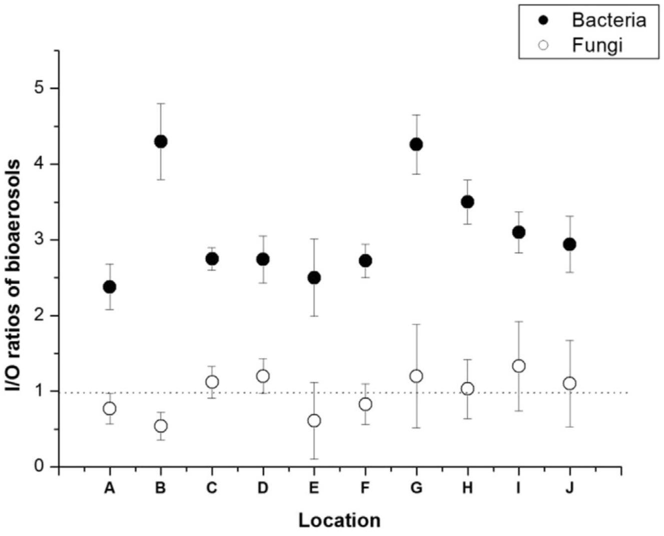

Fig. 6. I/O ratios of airborne bacteria and fungi in childcare centers.

Analysis of the 16s rRNA gene of the indoor samples indicated that most genus were in the sequence database, but step species of the gene showed over 90% homology. The results of Unifrac-based principal coordinate analysis (PCoA) of the bacteria originating from indoor air showed a noticeable difference from the outdoor bacteria. Bio-aerosols including airborne bacteria are ubiquitous both in indoor and outdoor air. Among all particles larger than 0.2 μm in the outdoor air, approximately 5–50% appeared to be of biological origin [51]. Outdoor bacteria are generally found in soil, water, plants, and animals. In air, bacteria may occur as vegetative cells or endospores. They may be carried by other particles, such as water droplet residues, plant materials, or the skin fragments of animals [35,51]. Human activities, such as the activity of children, classroom cleaning, and cooking could be a major source of the bacteria at childcare centers. The extent of bacteria-attached particles is governed by particle physic-chemical characteristics and environmental conditions such as temperature and humidity. Therefore, the indoor total bacteria count (TBC), as a concentration of airborne bacteria, depends on the concentration of indoor particles and the dynamics of the indoor environments, related to humidity and temperature. *Actinomycetes* are widely detected, as a main group of soil bacteria that can grow in either a yeast form or by producing mycelium. According to modern taxonomy based on genetic sequencing, they can cause hypersensitivity pneumonitis, and belong to the larger class of *Actinobacteria* such as *Actinomycetes*, *Micrococcus* and *Staphylococcus*, which inhabit the skin and dandruff [51].

Taxonomy based on genetic sequencing of the samples from the childcare centers revealed that 2–6% of the genes analyzed were *Staphylococcus*, which exist on human skin (data not shown). In addition, the genes of samples from center C, located adjacent to a construction site, indicated the presence of *Micrococcus* due to soil particles. Therefore, airborne bacteria could be carried with the infiltrating outdoor particles, and may occur alone or attached to other particles. Bacteria tend to grow in colonies in their natural habitats, such as water, and as biofilms on different surfaces in 60% humidity and 4–38 °C with nutrients such as organic materials. Therefore, whenever they become aerosolized, bacterial particles often occur as aggregates or microcolonies attached to other materials.

Most fungal aerosols can cause allergic reactions and diseases, such as asthma, allergic rhinitis, or in some cases, hypersensitivity pneumonitis. Studies of these allergens usually focus on fungal spores, although metabolites and fungal fragments can also become airborne. Although a few genera, such as *Cladosporium, Alternaria*, basidiospores, and ascospores dominate the outdoor aerosols in most of the world, there is some geographical variation. In areas of seasonal variation, the levels of fungal spores are highest in farming and food handling occupations, as well as in some industries [58].

Therefore, detailed research on the species of fungi which impact children indoors is needed for the characterization of bio-aerosols related to childcare centers, especially those which can handle food, and have certain environmental conditions.

3.2.2. *Distribution of the aerodynamic diameter for airborne bacteria and fungi*

Suspended bio-aerosols or spores were collected by a six-stage impactor (TISCH six-stage sampler) and the distribution of aerodynamic diameter of the airborne bacteria and fungi was investigated. The distribution of diameter of the suspended particles of this device is correlated with the respiratory tract, in which the lower level of the alveolar stage corresponds to the lower respiratory tract, and the upper stage is equivalent to the upper respiratory tract. Therefore, the results from this device can be useful to analyze risk assessment [57].

Fig. 7 shows the distribution of the aerodynamic diameter of the airborne bacteria in the childcare centers. Of the airborne bacteria, 59.2–78.6% was in stages 2–4 (2.1–7.0 μm), indicating a higher correlation with PM_{10}, especially PM_{10}-$_{2.5}$ (3.3–7.0 μm for coarse part), than with $PM_{2.5}$ from the outdoor to indoor distribution, with a more similar distribution with PM_{10} than $PM_{2.5}$. This result is similar to the observation that the correlation coefficient (R^2) was higher for PM_{10} than $PM_{2.5}$ (Fig. 8).

Larger particles can be suspended for a longer time than particles with a small diameter, which may be present for a short time in the atmosphere. In addition to the difficulty of considering the spatial mobility, small particles cannot be detected. Unlike outdoor environments, stage 4 (2.1–3.3 μm) particles can be exist on the floors in the childcare centers by re-suspension.

The distribution of aerodynamic diameter of the fungi was similar to that of outdoor environment, showing that 67.0%–84.4% existed in stages 3–5 stage (1.1–4.7 μm) in the outdoor environments, and at 72.4%–85.5% in the indoor environments.

Generally, the distribution of aerodynamic diameter of airborne bacteria indoors exist in stages 1–3 (over 4.7 μm); however, suspended particles of stage 4 (2.1–3.3 μm) were circulating in the indoor air, indicating re-suspension of the fine particles in indoor environments, which is often compared to outdoor environments to see if indoor air quality control is deemed necessary [57].

3.3. *Association between particulate matter and bacteria/fungi concentrations*

Fig. 8 shows the correlation coefficient (R^2) between airborne bacteria and particulate matter in the childcare centers. The correlation coefficient varied from 0.29 to 0.70 for PM_{10} and from 0.01 to 0.43 for $PM_{2.5}$. The airborne bacteria in centers B, E, F and I were more highly affected by PM_{10} than at the other childcare centers. However, such relatively strong correlations were found in the residential childcare centers. The relative higher fidelity of correlation to PM during September and October may be due to the natural ventilation system, due to other outdoor pollution sources. PM_{10} concentrations occasionally showed high peaks, mainly due

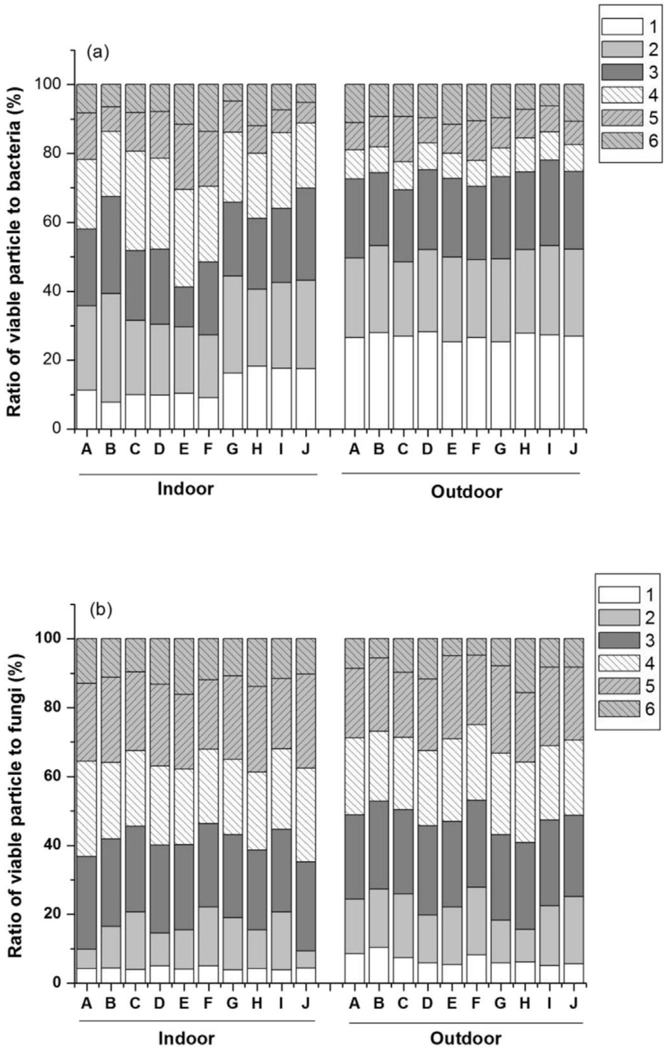

Fig. 7. Ratio of viable particles to (a) bacteria and (b) fungi, indoor and outdoor at childcare centers. (1: stage 1, over 7 μm; 2: stage 2, 4.7–7.0 μm; 3: stage 3, 3.3–4.7 μm; 4: stage 4, 2.1–3.3 μm; 5: stage 5, 1.1–2.1 μm; 6: stage 6, 0.65–1.1 μm).

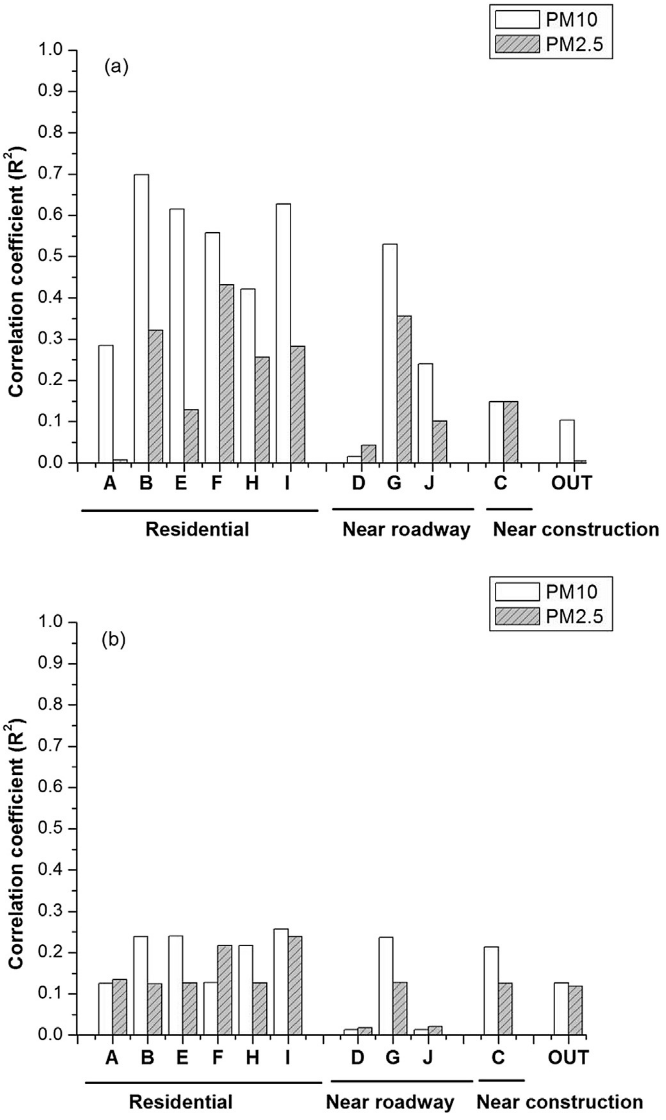

Fig. 8. Relationship between particles and bio-aerosols inside childcare centers. (a) Total airborne bacteria. (b) Total airborne fungi.

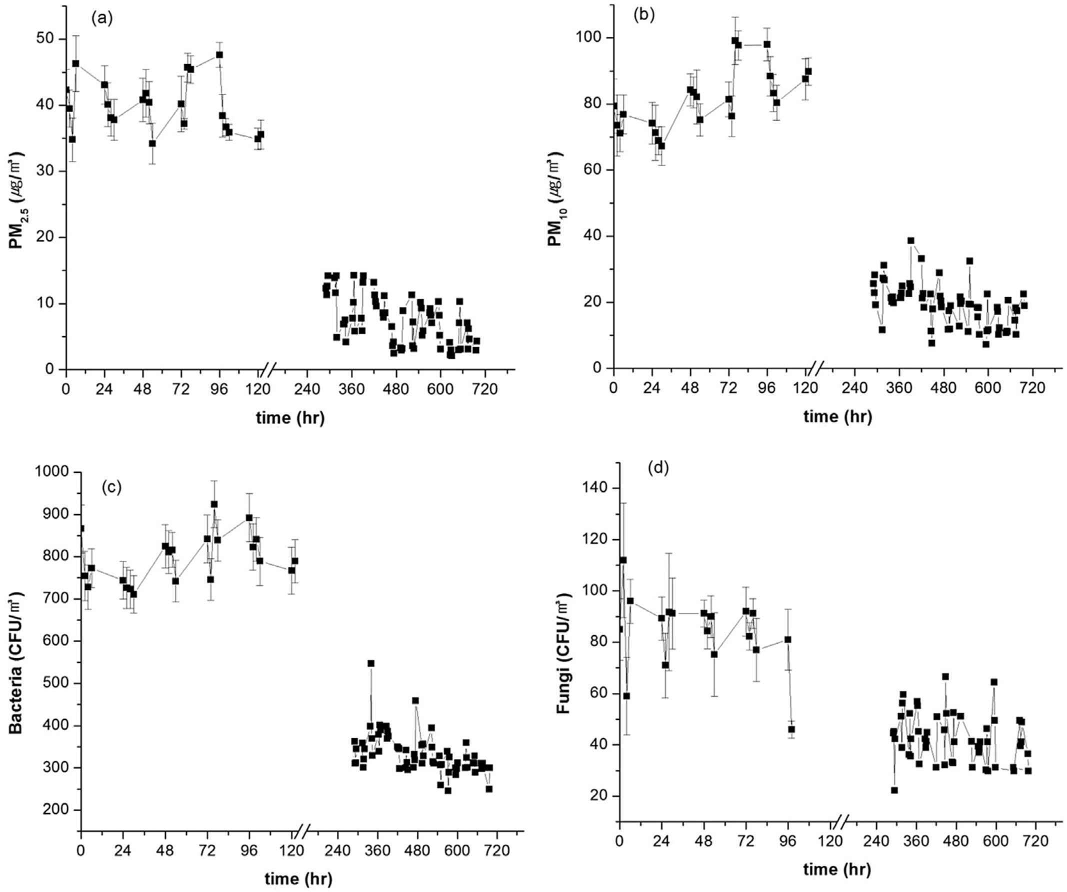

Fig. 9. The effect of air purifier operation on concentration during one month in residential childcare centers.

to outdoor sources, and airborne bacteria attached to the PM_{10} indoors originated from bacteria of outdoor particles/other sources, whereas the indoor $PM_{2.5}$ level was mainly affected by indoor sources, with some association with outdoor particles. The enrichment of organic particles in the indoor air may be explained by the presence of human skin particles, as they showed the same morphology and elemental composition as skin scrapings collected directly from healthy individuals [4]. Other authors also found organic carbon to be the major source of indoor PM_{10} [1]. Indeed, human keratin from skin was reported to contribute dominantly to indoor PM_{10} in schools [1], originating from the human personal cloud [1].

Many indoor bio-aerosol particles originate outdoors. The surfaces of living and dead plants are probable sources of airborne bacteria and fungal spores, whereas the presence of humans was reported to be the most important source of airborne bacteria [59]. In the activities of children, such as talking, sneezing, coughing, walking, washing, human skin and toilet flushing, can generate airborne biological particulate matter. In addition, handling of food, toy textiles, carpets, wood materials and furniture stuffing can occasionally release spores of *Alternaria, Aspergillus, Botrytis, Cladosporium, Penicillium,* or *Scopulariopsis* into the air [60,61].

Therefore, the mechanical movement of plants or soil, for example, through construction, can generate bio-aerosols together with the nonbiological dust. In nonindustrial indoor environments, the most important source of airborne bacteria is usually the presence of humans [18]. Air with a high concentration of human bacteria is not necessarily a health hazard, but may indicate the presence of humans and their physical activities.

3.4. Effects of air purifier operation

The use of an air purifier may be considered in cases where natural ventilation is used but concerns exist of indoor pollution from external sources, or if the ventilation system is not effective at removing particles. This is the best way to improve indoor air quality, through the reduction or removal of pollutants or indoor sources of particles.

Most of the childcare centers in Korea have the risk of indoor pollution due to the flux of the external sources, due to the use of natural ventilation or inadequate mechanical ventilation systems. Using air purifiers seems to be the most effective way to maintain a comfortable indoor environment, and reduces pollutants particles in the air to remove contaminants which could cause allergic diseases [31,62,63].

Because care is required for the safety of children who have high mobility/activity, the installation location of the air purifier was considered. We reviewed the liquidity of pollutants [64], and measured the efficiency of air purifiers in childcare centers located near residential areas (Fig. 9). Particles (PM_{10}, $PM_{2.5}$), airborne bacteria and fungi were measured four times every 2 h for five days without operation of an air purifier system, after which the air purifier system was operated during the week. All pollutants showed a statistically significant decrease in three weeks ($p < 0.01$).

The concentration of particulate matter went from 39. 9 μg/m^3(±4.0 μg/m^3) to 5.6 μg/m^3(±2.7 μg/m^3) for $PM_{2.5}$ and from 81.3 μg/m^3(±9.2 μg/m^3) to 15.0 μg/m^3(±2.5 μg/m^3) for PM_{10}, which showed an 86% efficiency of $PM_{2.5}$ and 69% efficiency of PM_{10} removal over three weeks. The bio-aerosol concentrations went from 794.1 CFU/m^3 (±58.6 CFU/m^3) to 304.4 CFU/m^3 (±25.3 CFU/m^3) and from 94.4 CFU/m^3 (±27.9 CFU/m^3) to 42.5 CFU/m^3 (±12.5 CFU/m^3) for airborne bacteria and fungi, respectively. The removal efficiency of bio-aerosols by the air purifier was 62% for airborne bacteria and 55% for fungi, respectively.

Fig. 10 shows the effects of air purification on the characteristics, according to location (residential, commercial), presence of people (moderate traffic, heavy traffic), and the year of construction (above or below 15 years) in classrooms where activities contributing to re-suspension were performed. When the efficiency of the air purifier was compared with the location and characteristics inside the childcare centers, the removal efficiency of particulate matters and bio-aerosols were 58–85% for $PM_{2.5}$, 49–86% for PM_{10}, 41–68% for airborne bacteria, and 40–58% for fungi.

The different sources of bio-aerosols and the environments require investigation, with specific investigation of the fungal hazard to the human body when considering the removal efficiency of fungi at lower levels by an air purifier [18].

The NIOSH and the ACGIH (American Conference of Governmental Industrial Hygienists) set the acceptable levels as 1000 CFU/m^3 for airborne bacteria and 500 CFU/m^3 for the culturable count of total bacteria [60,65–67]. However, the total airborne bacteria concentration was limited by the Korea Ministry of Environment to 800 CFU/m3 for Indoor Air Quality Control in Public Use Facilities, including childcare centers, while the concentration of airborne fungi has not yet been recommended.

There is insufficient information to assess the concentrations of airborne fungi, since the types and concentrations of indoor bio-aerosols are dependent on outdoor concentrations, which vary according to different environments [40]. The identification of causes of high concentrations of airborne fungi is required, even though there are regulations about the acceptable levels of airborne fungi.

It was determined that the performance and efficiency of the air purifiers depended on the structure and the filter. In addition, natural ventilation was most highly used in the childcare centers in Korea, but external pollutants can penetrate into the building. Therefore, use of an air purifier is one of the most effective and continuous ventilation systems. This system can be useful to improve indoor air quality through exchange filters and internal controls in the air purifier systems, which can prevent the loss of cost and adjust the temperature and humidity in childcare centers.

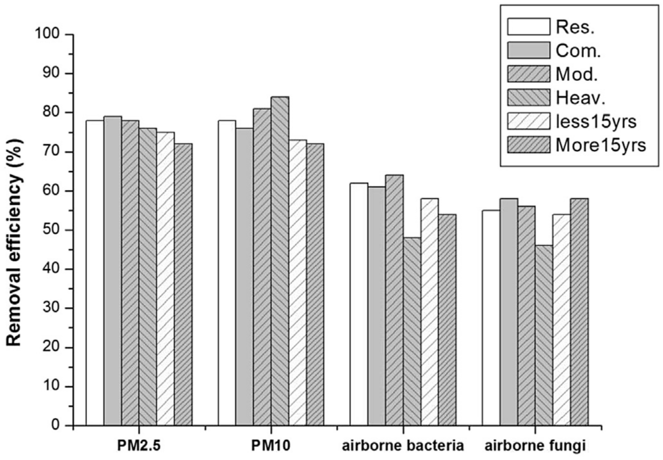

Fig. 10. Effects of air purifiers on the characteristics of childcare centers. (Res: residential; Com: commercial; Mod: moderate traffic; Heav: heavy traffic; less15yrs: building was constructed less than 15 years ago; More15yrs: building was constructed over 15 years ago).

A long term investigation over all four seasons with different outdoor environments, such as during the yellow dust season and smog events near the childcare centers, is needed to evaluate the effect of seasonal weather patterns and different events on indoor particles and bio-aerosol levels.

4. Conclusions

The characteristic of indoor air quality (PM_{10}/$PM_{2.5}$ and airborne bacteria/fungi) and the efficiency of air purifiers in childcare centers of Korea were investigated. The indoor levels of $PM_{2.5}$ and PM_{10} in childcare centers were influenced by the effects of traffic conditions and motor vehicle exhaust outdoors, with high concentrations of particulate matter observed in the three centers (D, G, J) which were located in close proximity to roads with heavy-traffic, where a lot of vehicles often cause traffic jams. The average indoor ratio of $PM_{2.5}$/PM_{10} was higher than outdoors, indicating that fine particles comprised a large fraction of the PM_{10} inside the childcare centers.

Window-type air conditioners seemed to be the most inefficient filtration system for removing particles, and even led to particulate accumulation. The indoor environmental quality at the childcare centers depended on the operation of ventilation, infrequent and inadequate cleaning of indoor surfaces, and a large number of students per classroom area and volume, with constant re-suspension of particles from the room surface, along with suspension of soil material due to the activity of the children.

The mean concentrations of airborne bacteria in childcare centers except G, which had a high child to area ratio, did not exceed the Indoor Air Quality (IAQ) Standard of Korea (limit of 800 CFU/m^3); however, the maximum values of airborne bacteria observed in childcare centers B, H and I also exceed the IAQ standard of Korea. A six-stage impactor (TISCH six-stage sampler), which can be compared to the respiratory system, was used to investigate the aerodynamic diameter for the risk assessment analysis of the bio-aerosols. The distribution of aerodynamic diameter showed that 69.4–78.1% of the airborne bacteria existed in stages 1–3 (over 3.3 μm) in the outdoor environments, while 59.2–78.6% existed in stages 2–4 (2.1–7.0 μm) inside the childcare centers. The distribution of indoor aerodynamic diameter for fungi was similar to that of outdoors, with 67.0%–84.4% of the microbial particles outdoors existing in stages 3–5 (1.1–4.7 μm) and 72.4% to 85.5% existing at that stage inside the childcare centers.

The removal efficiency of particulate matter and bio-aerosols revealed 75–78% for $PM_{2.5}$, 72–84% for PM_{10}, 48–64% for airborne bacteria, and 48–64% for fungi. The use of air purifiers may improve the indoor air quality through the exchange filters and internal controls in the system, which can prevent loss of cost and adjust the temperature and humidity in the childcare centers. A long term investigation over all four seasons with different outdoor environments, such as during yellow dust season and smog events near the childcare centers, is needed to evaluate the effects of seasonal weather patterns and different events on indoor particle and bio-aerosol levels. Most childcare centers in Korea installed window-type air conditioners and used natural ventilation, opening windows, which resulted in an equilibrium between the indoor and outdoor concentrations which also seemed effective to reduce the concentrations of particulate matter; however, the penetration of high levels of outdoor particulate matter in the external environments is sometimes possible, and there are other potential internal sources such as induced organic particles from skin of children or from cooking activities in the centers. Therefore, the use of air purifier should be considered to improve the air quality in childcare centers.

Above all, it is important to manage the concentrations of particulate matter which worsen the indoor air quality due to pollutants, including the penetrated particles from outdoors and the organic particles generated inside childcare centers, which mostly rely on natural ventilation. The use of air purifiers demonstrated efficient removal of not only particulate matter but also bioaerosols. Therefore, the use of air purifiers should be considered to improve indoor air quality, and should actively be reviewed.

Acknowledgment

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