

Research Article

Real-time Monitoring of Bioaerosol in a Residential Property in Central Tokyo

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ABSTRACT Real-time onsite monitoring of indoor airborne microbes in a residential property in central Tokyo was carried out in 2020 and 2021, following the onset of the COVID-19 pandemic. A microbial sensor utilizing fluorescence emitted by microorganisms was used to measure bioaerosol concentrations in the living room and children's bedroom as well as on the balcony. Indoor PM_{2.5} was also monitored simultaneously at certain time points using a PM_{2.5} sensor. The behavior of the residents was also recorded during some monitoring periods. The average number concentration of microbes as fungi in the living room was 15,100, 58,800, and 10,600 counts m⁻³ in spring, summer, and winter, respectively, increasing in summer when the outside temperature was high. Microbial number concentrations were closely related to human behavior, increasing rapidly during periods of physical activity, but decreasing again within 20–30 min of the activity ending. There was no clear correlation between indoor microbial number concentrations and PM_{2.5} concentrations, suggesting that indoor microorganisms are concentrated in coarse particles, such as dust, which are quickly removed via gravitational settling. The concentration of indoor airborne microorganisms decreased significantly after ventilation, and although an occasional increase was observed immediately after ventilation, concentrations decreased again rapidly within 10–20 min. These results suggest that even a short period of ventilation can significantly reduce the indoor bioaerosol.

KEY WORDS Bioaerosol, Indoor air pollution, Human behavior, Real-time monitoring, Ventilation

1. INTRODUCTION

Japanese spend more than 80% of their day indoors (Shiotsu *et al.*, 1998), and the time spent at home is thought to have increased because of the COVID-19 pandemic, with the implementation of lockdown measures and increase in telecommuting. Maintaining a healthy indoor air environment has therefore become even more important.

Indoor air contains a number of chemical components, such as CO₂, NO_x, and volatile organic compounds (VOCs), as well as bioaerosols, such as fungi and bacteria (e.g., Salthammer, 2020; Salonen *et al.*, 2019; Wolkoff, 2018; Wolkoff and Nielsen, 2017). Bioaerosols in indoor air are a major component of total indoor aerosols, in some case accounting for almost 50% (Jaenicke, 2005). A number of reports

on bioaerosols in indoor air have already been published. For example, Hospodsky *et al.* (2012) studied the particle size distribution of bioaerosols in a university classroom and found that bacteria were more concentrated in PM₁₀ than PM_{2.5}. Meanwhile, Mirhoseini *et al.* (2016) reported a positive correlation between microbial and PM₁ concentrations in indoor air in several different types of building, including offices, laboratories, and apartments. These findings suggest that microorganisms are distributed over a range of particle sizes.

Bioaerosols can enter the human body and cause infectious diseases, as well as increasing health risks by emitting microbial VOCs, a form of metabolite, into the room. Hospodsky *et al.* (2012) previously reported that airborne bacteria and fungi in indoor environments are linked to the development and exacerbation of chronic respiratory illnesses, such as asthma. Meanwhile, Yen *et al.* (2020) revealed a correlation between the amount of indoor fungi and endotoxin exposure and the prevalence of allergies and asthma in children. Understanding the status of indoor microbial contamination is therefore important in reducing the human health risks associated with exposure to microbes.

In most residential properties, a major source of microorganisms in indoor air is human activities (Mandal and Brandl, 2011; Stetzenbach, 1997). Talking, sneezing, coughing, walking, washing, and using the bathroom all generate bioaerosols (Mandal and Brandl, 2011), as do activities such as vacuuming and cleaning (Veillette *et al.*, 2013). Indoor microbial concentrations therefore tend to be higher when people are present (e.g., Hospodsky *et al.*, 2012), although food, pets, carpets, and indoor plants are also sources of bioaerosols (Mandal and Brandl, 2011; Kalogerakis *et al.*, 2005; Cox and Wathes, 1995).

Outdoor air is also an important source of indoor microorganisms. Zhou *et al.* (2020) studied the sources of indoor airborne microorganisms in eight commercial buildings in Hong Kong, and found that outdoor air and occupants' skin were the main contributors. Moreover, comparisons of indoor emissions (typically from occupancy and resuspension) and ventilation (outside air infiltration) as sources of airborne bacteria and fungi in a typical house in Oklahoma, USA, revealed that indoor emissions were the main source of particulate matter, bacteria, and fungi, accounting for about 94% (PM₁₀), 97%, and 91% of indoor air levels, respectively (Kwan *et al.*, 2020). Thus, the sources of indoor airborne microorganisms vary greatly depending on the location and the building

type, with the concentration and composition affected by factors including indoor population, behavior, and ventilation. The above findings were mainly obtained by collecting indoor bioaerosols and identifying the species and amount of microorganisms through cultivation or polymerase chain reaction (PCR) measurement, and the temporal resolution was not high. If the indoor bioaerosols can be continuously monitored with high temporal resolution, further knowledge about indoor bioaerosols may be obtained.

Microbial sensors able to easily and automatically measure microbial concentrations in the air without pretreatment were recently developed (Weber *et al.*, 2019; Tomi-naga *et al.*, 2017). By utilizing fluorescence emitted by the microorganisms themselves, these sensors have potential in real-time onsite measurements of microorganism concentrations in the air at a temporal resolution of approximately 10 min. In this technique, microorganisms are detected by monitoring intracellular fluorescent molecules such as riboflavin, NADH, and tryptophan (Tomi-naga *et al.*, 2017). Although these sensors cannot identify the species or whether they are alive, and have the possibility of detecting fluorescence of abiotic substances (e.g., polycyclic aromatic hydrocarbons: PAHs, soot; Huffman *et al.*, 2010), the approach is considered valuable from the viewpoint of screening for microorganisms in the air. In this study, we therefore preliminarily monitored indoor bioaerosols continuously using a recently developed microbial sensor with high temporal resolution, and evaluated the effects of indoor population, behavior, and ventilation on indoor bioaerosol concentrations.

2. EXPERIMENTAL METHODS

Indoor air monitoring was carried out in the living room and children's bedroom in a single-family dwelling with four family members in central Tokyo. The outline of the rooms is shown in Fig. 1. The living room was located on the second floor of the three-story building, with the south side facing a balcony. Two ventilation ports (naturally aspirated) installed on the walls of the balcony side were open throughout. The children's bedroom (bedroom number 1 in Fig. 1) was located to the east of the living room, with a window on the east side of the room. A ventilation port (naturally aspirated) installed on the east wall was kept open.

Indoor air monitoring was carried out in spring (17–26 April) and summer (17–19 August) of 2020 and in win-

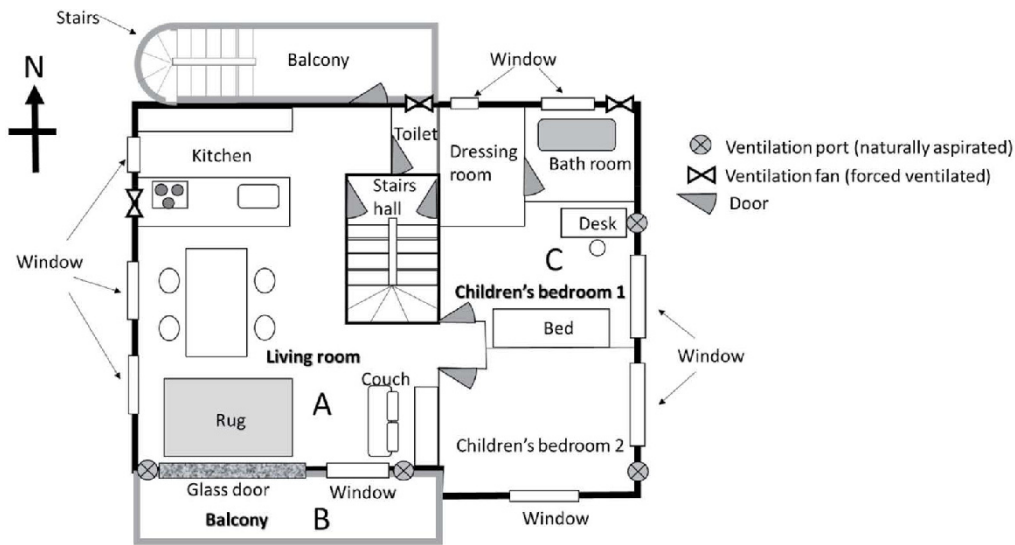


Fig. 1. Outline of the house and rooms used for monitoring. A, B and C represent the monitoring points.

ter (1–4 February) of 2021. These periods were after the COVID-19 had spread in Japan, and thus the number of people staying at home was deemed higher than normal owing to lockdown measures and government recommendations to work from home. A microbe fluorescence biosensor (BM-300C, Sharp) was installed on the south side of the living room (point A in Fig. 1) or in the children's bedroom (point C in Fig. 1) at a height of 100 cm from the floor. The sensor selectively draws particles below 10 μm diameter by means of a cyclone. The sensor relies on the microbes emitting a characteristic fluorescence when illuminated by radiation at a certain frequency. However, this method is unable to identify the species or distinguish between fungi and bacteria. In addition, the method is not applicable to viruses, and it is not possible to ascertain whether the microbes are alive. The measurement value therefore represents the concentration of all microbes (fungi and bacteria). This instrument measures the fluorescent intensity, which is converted into fungal and bacterial equivalent number concentrations. In this study, we use the concentrations as fungi. The concentration as bacteria is calculated 2.75 times the concentration as fungi in this system. The sensor rapidly and automatically measures the amount of airborne microbes, including bacterial microbes and mold spores, in a fixed volume of air over a specified period of time. Hence, it is well suited to analyses of the temporal variation in airborne microbial concentrations in indoor air. The amount of microbial biomass in the air was monitored at

10-min intervals. During summer and winter monitoring, the $\text{PM}_{2.5}$ concentration was also measured simultaneously at 1-min intervals using a $\text{PM}_{2.5}$ sensor (P-sensor, Industrial Hygiene Device Calibration, Inc.) placed close to the microbe sensor. Temperature and relative humidity (RH) in the living room were also recorded by a household thermo-hygrometer at 1-hour intervals during the observation term of residents' behavior in spring. In summer and winter, temperature and RH in the living room were monitored at 5-min intervals using a temperature and humidity sensor (2JCIE-BU01, OMRON).

During some observations, the behavior of the residents in each room, the usage of air conditioning equipment, and opening and closing of windows and external glass doors were also recorded. In addition, microbial number concentrations on the balcony (point B in Fig. 1) were also measured in each season at intervals of 10 min. Microbial number concentrations after opening and closing the windows and cleaning the children's bedroom were also measured at 10-min intervals.

3. RESULTS AND DISCUSSION

3.1 Temperature and RH in the Living Room and Number Concentrations of Indoor Airborne Microbes

The average temperature in the living room in spring, summer and winter were $20(\pm 1)^{\circ}\text{C}$, $25.4(\pm 0.8)^{\circ}\text{C}$,

Table 1. Concentrations of airborne microbes as fungi in the living room in each season (counts m⁻³).

	Spring				Summer				Winter			
	Total	I ¹⁾	II ¹⁾	III ¹⁾	Total	I ¹⁾	II ¹⁾	III ¹⁾	Total	I ¹⁾	II ¹⁾	III ¹⁾
Average	15,100	26,600	11,900	5,330	90,300	160,900	54,700	17,900	10,600	30,600	2,030	994
Max	176,000	176,000	94,200	107,000	390,000	390,000	119,000	24,300	319,000	319,000	77,100	25,300
Min	ND ¹⁾	ND ¹⁾	ND ¹⁾	ND ¹⁾	13,400	48,300	22,000	13,400	ND ¹⁾	ND ¹⁾	ND ¹⁾	ND ¹⁾
SD ³⁾	17,700	22,700	12,900	9,820	78,400	69,400	28,600	2,790	35,200	57,300	8,540	3,880

¹⁾Behavior categories: I, someone in the room and involved in a physical activity; II, someone in the room but not involved in physical activity; and III, no one in the room

²⁾Not detected

³⁾Standard deviation

and 20.5 (± 1.2)°C, respectively. The average RH in the living room in spring, summer and winter were 50 (± 3)%, 65 (± 3)%, and 53 (± 4)%, respectively. There was little variation in the room temperature and RH within the same season. Both temperature and RH were higher in summer, while they were about the same level in spring and winter.

Concentrations of bioaerosol (as fungi, same as below) in the living room in each season are shown in Table 1. The data were obtained during a period of continuous measurement for 12 h or more using the fixed microbial sensor. Data obtained during periods of time when the sensor was moved were excluded. During the data collection period, the air conditioner in the living room was always operated except late at night in summer and winter, while it was not operated in spring. The glass doors, windows and doors in the living room were always closed except for a very short time when people were entering or leaving, according to the behavioral records of the residents. The average concentrations of bioaerosol in the living room were 15,100, 58,800, and 10,600 counts m⁻³ in the spring, summer, and winter, respectively, and tended to be higher in the summer when inside and outside temperatures were also high. In fact, the temperature in the living room during the summer season was about 5°C higher than in the spring and winter seasons. This was consistent with microbial activity, which tended to increase in warmer weather. However, there was no significant difference in maximum concentrations between summer and winter. Microorganisms in indoor air are also generated by textiles such as rugs, duvets, and clothes (Mandal and Brandl, 2011), all of which are habitats for microorganisms; thus, even in winter, the concentration of indoor microorganisms can temporarily increase with increased movement of residents and air currents.

Table 1 also shows movement of the residents within the living room and the effect of different behaviors on indoor air concentrations of bioaerosol. Behavior categories I, II, and III represent the following: someone in the room and involved in physical activity (e.g., cooking, cleaning, walking, or folding laundry), someone in the room but not involved in a physical activity (e.g., reading or resting on the couch), and no one in the room, respectively. Airborne microbial number concentrations in the living room were highest during category I in all seasons, followed by categories II and III. These findings suggest that indoor microbes increase following periods of physical activity. In addition, the microbial number concentration increased notably while tidying away futons and folding laundry, suggesting that microorganisms on bedding and clothing have a significant impact on indoor microbial number concentrations. These results are consistent with previous studies showing that indoor bioaerosols are generated from factors including human activities, food preparation, the presence of pets, and carpets (Hospodsky *et al.*, 2012; Mandal and Brandl, 2011).

3.2 Effect of Population on Concentrations of Airborne Microbial Biomass

Fig. 2 shows the relationship between the number of people in the living room and the concentration of bioaerosol across four days in spring 2020. A positive correlation was observed between the two factors ($r^2 = 0.30$), with an increase in airborne microbes with an increasing number of people in the room. These findings confirm the aforementioned results whereby physical movement caused an increase in microbial number concentrations (Table 1). In other words, indoor microbial number concentrations can be roughly expressed as a function of the indoor population. Because of the COVID-19 pandemic at the time of this experiment, more time was being spent

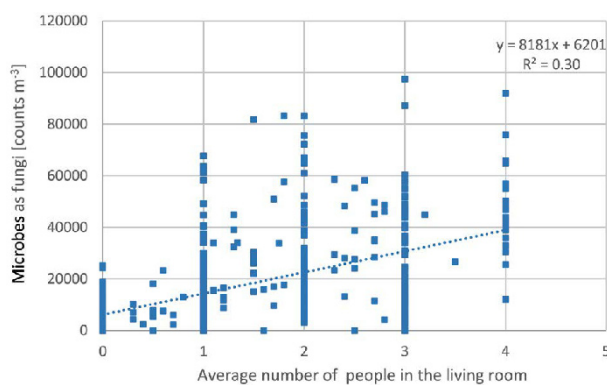


Fig. 2. Relationship between the average number of people in the living room and the number concentration of airborne microbes.

indoors than average, and therefore, the concentrations of airborne microorganisms observed were thought to have been higher than those before the pandemic.

3.3 Effect of Human Behaviors on Number Concentrations of Airborne Microbes in the Living Room

Fig. 3 shows the relationship between airborne microbial number concentrations over time and behavior across four days in spring 2020. The microbial number concentration was low from midnight to dawn when the room was empty, and was intermittently high at all other times, in line with periods of activity. Behaviors corresponding to high microbial number concentrations included physical activities such as eating, taking in and folding laundry and futons, exercising on the rug, and cleaning (Category I, see Table 1 and section 3.1). Although the microbial number concentration in the indoor air increased sharply with each of these behaviors, values tended to decrease sharply about 10 to 20 min after each activity ended. The indoor concentration of microbes therefore fluctuated rapidly within a short period of time depending on the behavior.

Even with similar behavior, some variation in microbial number concentrations was observed. For example, the maximum microbial number concentration at breakfast was about 60,000 counts m^{-3} on April 22, 23, and 25, while the maximum was about 40,000 counts m^{-3} on April 24. The maximum microbial number concentration during lunch was about 60,000 counts m^{-3} on April 23, while the maximum concentration was about 40,000 counts m^{-3} on April 22, 24, and 25. Furthermore, microbial number concentrations was about 80,000–90,000

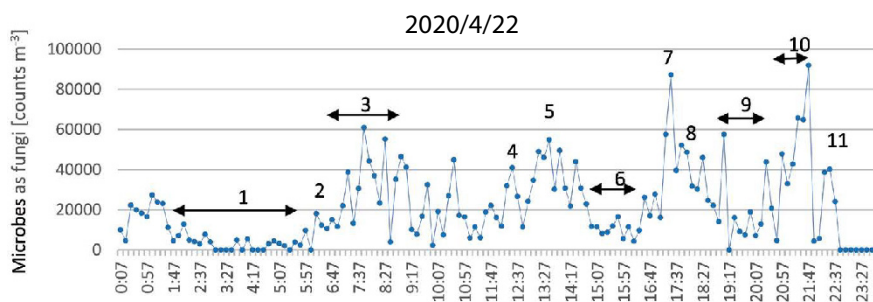
counts m^{-3} during dinner on April 22 and 23, 2020, but was about 20,000 counts m^{-3} during dinner on April 24. In contrast, all of the breakfasts on these four days were Japanese meals (rice, miso soup, and unheated side dishes such as natto and seaweed) with no grilled cooking. Lunch consisted mainly of noodles such as ramen and soba noodles, and no grilled cooking was also done. Dinner, on the other hand, consisted of grilled meat on April 22 and 23, and no grilled food on April 24 and 25.

Here, grilled cooking of meat can generate PAHs (e.g. Saito *et al.*, 2014; Tanaka *et al.*, 2012), which can affect the measurements of the microbial sensor because PAHs emit fluorescence (Huffman *et al.*, 2010). As already mentioned, Fig. 3 shows that the microbial number concentration tended to increase during meals regardless of the cooking method. On the other hand, the microbial number concentration was slightly higher during the grilled cooking of meat (dinner on April 22 and 23) when PAHs tends to be generated. This suggested that PAHs emitted by cooking may affect the measured values of the microbial sensor, but the effect was not considered to be significant. In other words, the signal values of the microbial sensor in this study were considered to be mostly derived from the fluorescence of the microorganisms.

Moreover, the high concentrations of airborne microbes observed while handling laundry and bedding was likely due to the dispersal of microorganisms on each item, in addition to those originating from the residents themselves.

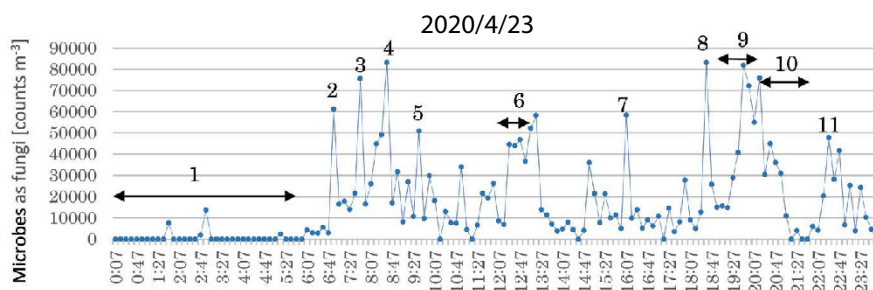
3.4 Relationship between Airborne Microbes and $PM_{2.5}$

Fig. 4 shows the results of simultaneous measurement of airborne microbes and $PM_{2.5}$ concentrations in the living room during the summer. In general, the temporal changes in microbial number concentrations and $PM_{2.5}$ concentrations tended to differ (Fig. 4A, C, and D); however, similar trends were observed during some time periods (Fig. 4B). The relationship between the microbial number concentration and $PM_{2.5}$ concentration (10-min average) during the time periods corresponding to (A) through (D) in Fig. 4 is shown in Fig. 5. The correlation coefficient (r^2) between the microbial number concentration and $PM_{2.5}$ concentration during the time period shown in Fig. 4B was 0.77, while the r^2 values during the other time periods ranged from 0.088 to 0.27. These results suggest that while airborne microorganisms tend not to be concentrated in small particles of less than



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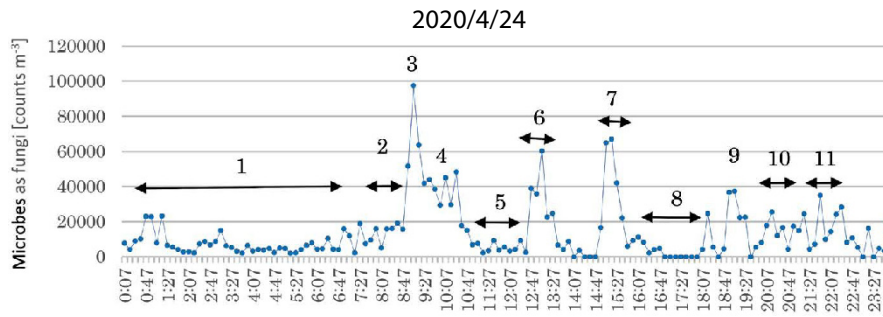
ID	Behavior
1	Room empty
2	Breakfast preparation
3	Intermittent breakfast
4	Lunch
5	Putting away laundry
6	Room empty
7	Snack time
8	Folding laundry on the rug
9	Dinner preparation
10	Broiling meat on the table
11	Exercise on the rug



4/23

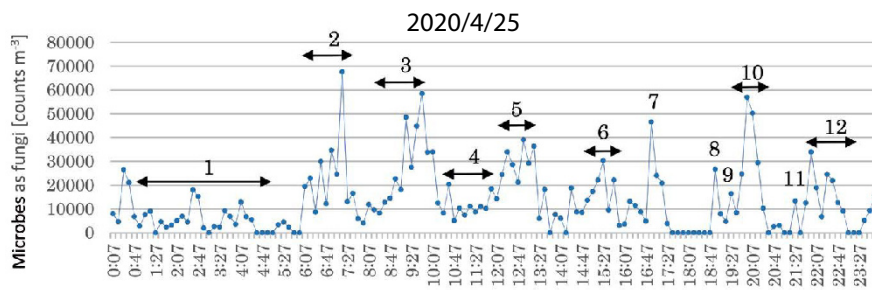
ID	Behavior
1	Room empty
2	Breakfast
3	Cleaning
4	Exercise on the rug
5	Breakfast
6	Lunch preparation and lunch
7	Putting away laundry
8	Frequent movement within the living room
9	Dinner preparation
10	Dinner
11	Cleaning up of dinner

Fig. 3. Temporal changes in airborne microbes in the living room across four days in spring 2020. A key to each behavior type is shown below each figure.



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ID	Behavior
1	Room empty
2	Breakfast
3	Folding laundry on the rug
4	Breakfast
5	Room empty
6	Lunch preparation and lunch
7	Putting away and folding laundry and futons
8	Room empty
9	Dinner preparation
10	Dinner
11	Cleaning up of dinner



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ID	Behavior
1	Room empty
2	Exercise on the rug
3	Intermittent breakfast
4	Resting on the sofa
5	Lunch preparation and lunch
6	Resting on the rug
7	Snack time
8	Folding laundry on the rug
9	Dinner preparation
10	Dinner
11	Cleaning up of dinner
12	Watching a movie on the rug

Fig. 3. Continued.

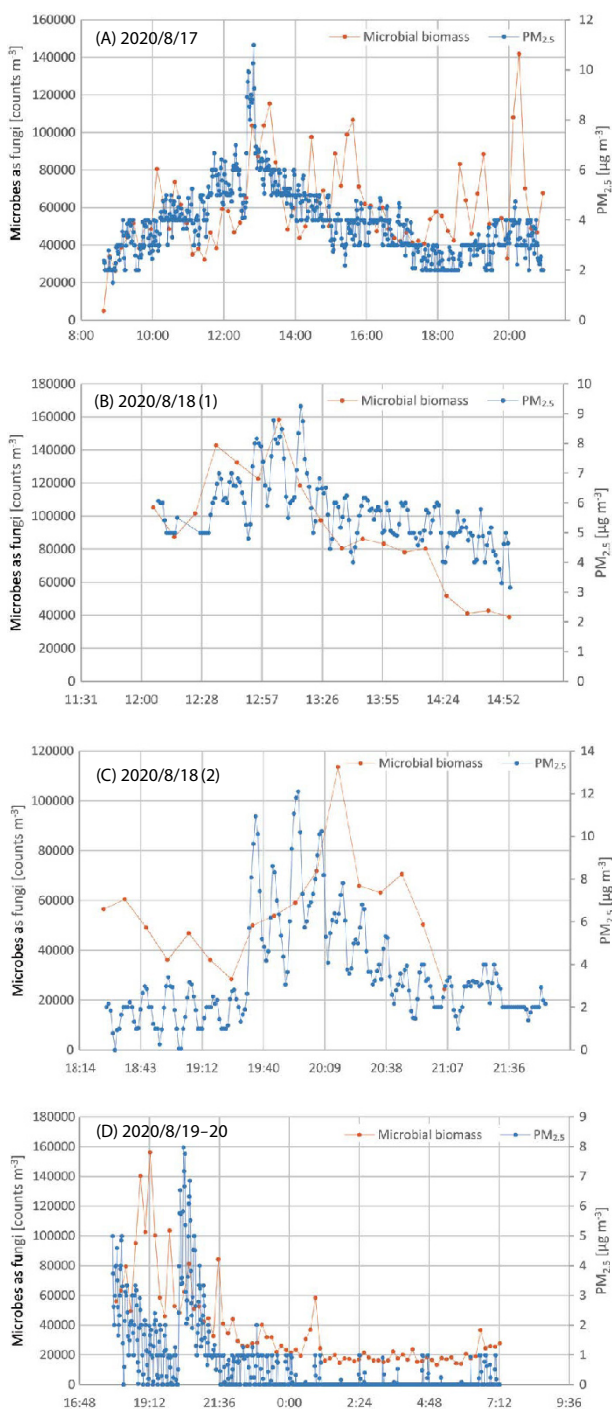


Fig. 4. Temporal variation in airborne microbes and $PM_{2.5}$ concentrations in the living room.

$2.5 \mu m$ in size, it is possible. In line with this, Hospodsky *et al.* (2012) measured the concentration of microorganisms in $PM_{2.5}$ and PM_{10} in a university classroom and found high concentrations in the latter. As shown in Fig.

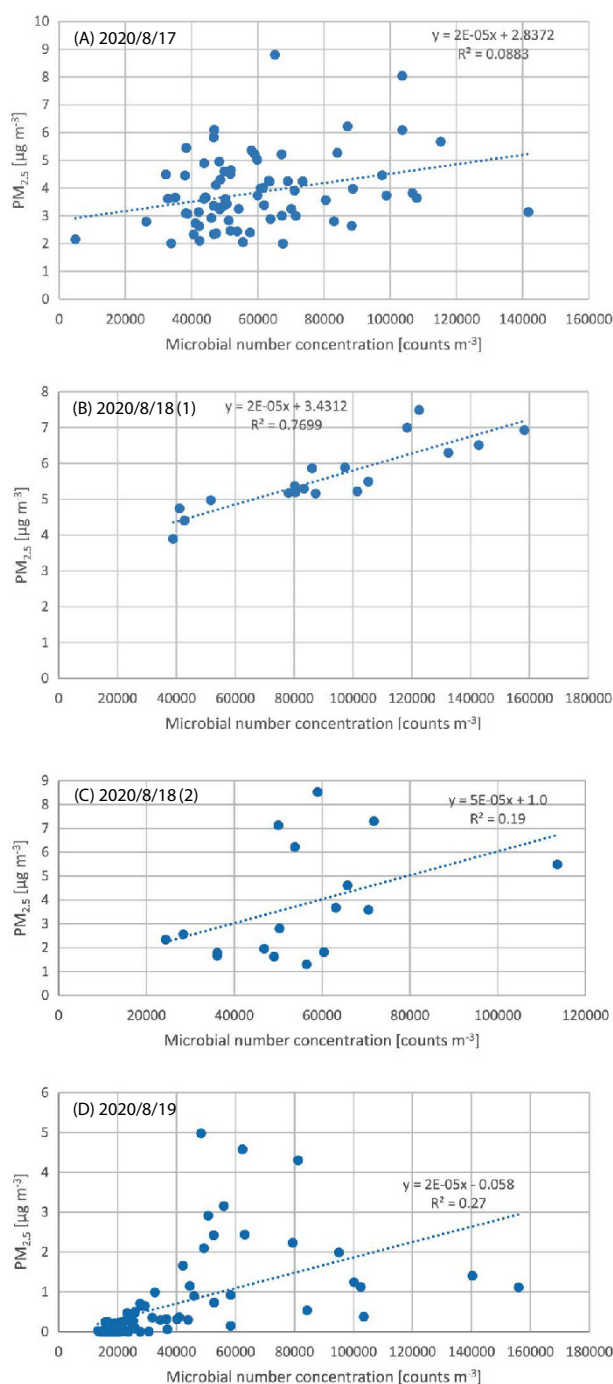


Fig. 5. Relationship between microbial number concentration and $PM_{2.5}$ in the living room. (A) to (D) correspond to (A) to (D) in Fig. 4, respectively.

3, the airborne microbial number concentration temporarily increased with physical activity of the residents, although levels tended to decrease rapidly within a short period of time of 10–20 min after the activity ended. This

is consistent with the idea that microorganisms are concentrated in coarse particles, since they are removed rapidly from the air via gravitational settling. On the other hand, Mirhoseini *et al.* (2016) reported a modest positive correlation between PM₁ concentrations and microbial concentrations in schools, offices, and residential properties. The reason for these differences is unknown, but in case most of the coarse particles in the indoor air are removed, the correlation between the concentration of PM_{2.5} and the concentration of microbial may be high. In fact, in Fig. 4B, where a high correlation between the microbial number concentration and the PM_{2.5} concentration was observed, the coarse particles may have been removed from the indoor air via gravitational settling because no one was in the living room after 12:30. As a result, most of the microbes may have been concentrated into PM_{2.5}. In addition, they may be due to differences in the indoor particle concentration and size distribution. Frequent cleaning and the use of air purifiers are thought to remove most coarse particles, such as dust, from indoor air, while less frequent cleaning and the absence of air purifiers are thought to increase the presence of coarse particles. Thus, these differences may also affect the particle size distribution of microorganisms.

3.5 Effect of Ventilation on Microbial Number Concentrations in Indoor Air

Airborne microbial number concentrations in the living room (point A in Fig. 1) and on the balcony (point B in Fig. 1) in each season are shown in Table 2. Since only one microbe sensor was available, concentrations were measured alternately between the two locations at 10-min intervals. Microbial number concentrations in both the living room and balcony were highest in the summer and lowest in the winter. In spring and winter, the microbial number concentration was lower on the balcony than in the living room, while in summer, concentrations were slightly higher on the balcony than the living room. This result may reflect the fact that in spring and winter, the indoor temperature was higher than the outdoor temper-

ature, thereby increasing the number of microorganisms. In contrast, in summer, the living room was air-conditioned and the temperature was lower than the outdoor temperature, thereby inhibiting microbial growth. Here, it is considered that the microbial sensor used in this study also can detect fluorescence emitted by pollen. Since the amount of pollen derived from flowers such as Japanese cedar and Japanese cypress is high in the spring in Japan, the signal value of the sensor may be high due to fluorescence by pollen. In general, pollen concentration is much higher outdoor than indoor (Hugg and Rantio-Lehtimäki, 2007; Ohashi *et al.*, 2005; Kiyosawa and Yoshizawa, 2001), so if the microbial sensor detected fluorescence from pollen, the signal would be higher outdoor than indoor. However, as shown in Table 2, the microbial number concentration indoors was higher than that outdoors during spring, indicating that the effect of pollen on the measured values of the microbial sensor is considered to be small. For seasons other than summer, the concentration of indoor microorganisms is thought to be reduced as a result of ventilation, since the concentration of microorganisms outside is lower than that inside.

The effect of opening and closing the window and external glass doors on the indoor concentration of airborne microbial biomass in the children's bedroom and living room is shown in Fig. 6. In the children's bedroom, airborne microbial number concentrations tended to decrease after opening the window, especially in winter (Fig. 6A and B). These results suggest that ventilating the room by opening the window reduced the concentration of indoor airborne microorganisms. Meanwhile, in the living room, airborne microbial number concentrations increased for about 30 min after opening the external glass doors, before decreasing (Fig. 6C). The immediate increase in microbial number concentrations after opening the doors was thought to be due to the fact that microorganisms on the floor, for example on the rug, were disturbed by the influx of outside air. The living room data shown in Fig. 6C were all obtained in the morning, prior to which the room was empty for 8 h. Most of the micro-

Table 2. Concentration of airborne microbes in the living room and on the balcony (counts m⁻³).

	Spring		Summer		Winter	
	Living room	Balcony	Living room	Balcony	Living room	Balcony
Average	45,900	16,100	85,000	106,000	11,100	1,270
SD ¹⁾	19,700	6,620	33,300	17,100	31,800	2,700

¹⁾Standard deviation

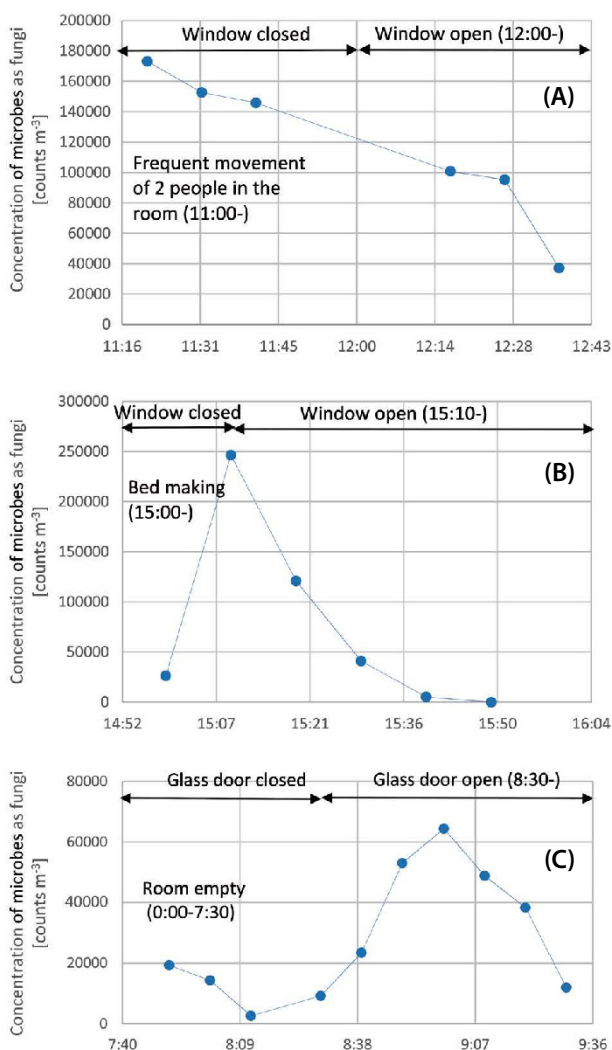


Fig. 6. Effect of ventilation on the concentration of airborne microbes in the child's room (A and B) and living room (C).

organisms suspended in the air within the living room will therefore have been removed via gravitational settling. Thus, as shown above, when the windows and external glass doors are opened in a room that has been unoccupied for a long time, the microbial number concentration may temporarily increase. In contrast, in the children's bedroom, the microbial number concentration did not increase after opening the window, possibly because the microorganisms on the floor and other surfaces had already been disturbed as a result of movement within the room, for example while making the beds. Overall, these results suggest that microbial number concentrations in indoor air can be reduced by opening windows or external doors for about 30 min.

4. CONCLUSIONS

In order to clarify the factors affecting microbial concentrations in indoor air, we studied the microbial number concentrations and PM_{2.5} concentrations in indoor air using a microbial sensor and PM_{2.5} sensor. The behavior of occupants in the room was also recorded and the relationship with indoor microbial number concentrations was analyzed. Indoor microbial number concentrations tended to increase in summer and decrease in winter, in line with the fact that microbial activity was higher in the summer when temperatures were higher. Microbial number concentrations also tended to increase with physical activity of the occupants, and with an increase in the number of occupants in the room, although concentrations decreased again rapidly within 20–30 min of the activity ending. There was no clear correlation between microbial number concentrations and PM_{2.5} concentrations in the indoor air except for some time period, suggesting that indoor microorganisms tend to be concentrated in coarse particles, which are quickly removed from the air by gravitational settling, even when disturbed by human activities. The concentration of microorganisms in the room also decreased rapidly after 10–20 min of ventilation, suggesting that microorganisms can be removed by only a short period of ventilation.

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