Indoor temperature, humidity, and microorganisms in traditional and modern houses in Japan

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Abstract. Microorganisms in our living environment may affect human health. Microbial suppression by air cleaners and disinfecting agents may provide protection from pathogenic materials. However, excessive microbial suppression can negatively affect human health; thus, an appropriate level of microbiome control is beneficial. It is not well understood how physical environmental conditions, such as temperature and relative humidity, and human lifestyles and behaviors affect indoor microorganisms. To understand the relationship between physical environmental conditions and microbial communities in the human living environment, we measured temperature and relative humidity and collected microbial samples in modern and traditional Japanese houses. In this study, bacteria and fungi were the target microorganisms. In both houses, the DNA concentration of microorganisms on floor surfaces was high when the average relative humidity of the room was high. The same tendency was observed for the beam and pillar surfaces in the traditional house. Although more careful consideration is needed for some indoor surfaces, such as storage ceilings and air conditioner outlets, seasonal changes in relative humidity and DNA concentrations of microorganisms on indoor surfaces exhibit some correlation.

1 Introduction

In modern life, we spend approximately 87% of our time indoors [1], and the impact of the living environment on human health is considered significant. The microbial community in each living environment is one of the elements that affects human health; this also called the microbiome. Previous studies have indicated that excessive microbial suppression may result in an increased number of pathogenic microorganisms and adverse health effects [2,3,4,5].

Several factors have been proposed to affect microbial communities in indoor environments, including temperature, relative humidity, air exchange rate, and occupant density [6,7]. However, it remains unclear how housing itself (thermal insulation performance, airtightness, building materials); the physical environment, such as temperature and relative humidity, which are affected by living conditions (ventilation, air conditioning); and the outdoor environment (meteorological conditions) affect microbial communities and abundances in the living environment. Moreover, microbial sampling methods for indoor environments have not yet been fully established.

Therefore, this study aimed to understand how temperature, relative humidity, and living conditions affect microbial communities and abundances in living environments. Additionally, we attempted to find a better method for examining microbial communities in indoor environments through field surveys of buildings that are currently in use. As the first step of this research, we focused on the relationship between the DNA concentration of microorganisms and the temperature and humidity of indoor surfaces in modern and traditional Japanese houses. We examined the differences in DNA concentrations seasonally and by house.

The final goal of this study is to propose housing and methods that inhibit microorganisms that may adversely affect the health of occupants.

2 Materials and methods

2.1 Research sites

In this study, we examined one modern Japanese house and one traditional Japanese house.
The modern house is a reinforced concrete house located in Okinawa City, Okinawa. Okinawa is a hot and humid region, where visible mold is often detected in buildings. This house is located in a residential area; reinforced concrete buildings are common in Okinawa. In this house, occupants use air conditioners in the living room and bedroom, during both summer and winter. Hereafter, we will call this “house A.”

The traditional house is a thatched-roof house built of natural materials, such as mud walls, and is located in Miyama, Nantan City, Kyoto. Hereafter, we will call this “house B.” Miyama is cold during winter and is surrounded by mountains and forests; some thatched-roof houses remain in this area. Low airtightness is one of the characteristics of house B, which is a common feature of traditional houses. During the summer, occupants open windows and room partitions to ensure ventilation and do not use air conditioners. In winter, they use a wood-burning stove installed in a Japanese-style room next to the dining room and earthen floor space.

2.2 Temperature and humidity measurements

The temperature and humidity were measured at several points in the houses. Fig. 1(a) and 1(b) show the measurement points in houses A and B, respectively. The indoor air temperature and humidity were measured using temperature/humidity sensors (HOBO UX100–011A, Onset Computer Corporation, Bourne, MA, USA), and the indoor surface temperatures were measured using thermocouples (HOBO UX100–014M or HOBO UX120-006M, Onset Computer Corporation, Bourne, MA, USA). Weather stations (HOBO Weather Station Kits, Onset Computer Corporation, Bourne, MA, USA) were used to measure outdoor air temperature and humidity. The measurement interval for all locations was 30 min. Temperature and humidity were measured for one month prior to microbial sampling. Table 1 shows the microbial sampling dates and corresponding temperature and humidity measurement periods.
2.3 Microbial sampling and DNA extraction

Microbial samples were collected twice in each house, once each during summer and winter (Table 1). Swabs (FLOQswab 5U005Sdual, COPAN, CA, USA) were used to collect surface microbial samples. When collecting the samples, we used only one of the two swab heads. The size of the sampling area was 100, 200, or 300 cm² depending on the surface condition.

All samples were obtained using aseptic techniques and appropriate negative controls. Each swab was directly placed in the head tube of a DNeasy PowerBiofilm Kit (QIAGEN, Germantown, MD, USA) within a laminar flow cabinet, and DNA was extracted according to the manufacturer’s protocol, with some modifications, as follows [8]. Instead of using glass beads in the PowerBiofilm bead tubes, 400 µL of sterilized 0.5 mm zirconia beads (TORAY, Tokyo, Japan) and two 5 mm zirconia beads (TORAY) were used for homogenization. The samples were beaten using a multi-bead shoker® (Yasui Kikai Corporation, Osaka, Japan) at 2700 rpm for 10 min. DNA was eluted in 100 µL of elution buffer. The concentration and purity of the DNA were measured using a DS-11FX + Spectro/Fluorometer (DeNovix, Wilmington, NC, USA).

3 Results and discussion

3.1 DNA concentration on indoor surfaces of the modern house

To determine how microbial abundances on indoor surfaces vary seasonally and by house, we collected microbial samples during summer and winter for houses A and B (Fig. 1, ●). Fig. 2 shows the DNA concentration per 100 cm² on indoor surfaces in house A and Table 2 lists the DNA concentrations on floor surfaces and relative humidity.

Fig. 2. DNA concentration on indoor surfaces in house B.

Microbial samples from the living room and bedroom walls were collected only in June 2022.

Table 2. DNA concentration and RH of floor in house A.

<table>
<thead>
<tr>
<th>Surface</th>
<th>DNA Concentration [ng/100 cm²]</th>
<th>Average RH [%]</th>
<th>Highest RH [%]</th>
<th>Lowest RH [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bedroom (June)</td>
<td>2.93</td>
<td>85.5</td>
<td>97.6</td>
<td>52.9</td>
</tr>
<tr>
<td>Bedroom (March)</td>
<td>0.53</td>
<td>80.9</td>
<td>96.9</td>
<td>50.7</td>
</tr>
<tr>
<td>Living room (June)</td>
<td>1.43</td>
<td>80.6</td>
<td>93.1</td>
<td>54.9</td>
</tr>
<tr>
<td>Living room (March)</td>
<td>0.31</td>
<td>74.4</td>
<td>93.9</td>
<td>50.6</td>
</tr>
</tbody>
</table>

Supplementary information for Table 2: RH in this table is the relative humidity of the air in each room, and the average, highest, and lowest values were calculated using the monthly data before each sampling.

In house A, the DNA concentrations of microorganisms on the floor surfaces were higher in June than in March in both the living room and bedroom (Fig. 3). Comparing relative humidity, the average and lowest relative humidity values were higher in June than those in March in both rooms, when DNA concentrations were also high (Table 2).

For samples collected in June 2022, the DNA concentration on the floor was higher than that on the wall in each room. This could be because microorganisms tend to accumulate more easily on the floor than on the wall by attaching themselves to dirt and dust.

3.2 DNA concentration on indoor surfaces of the traditional house

Subsequently, we examined the DNA concentrations of microorganisms on the indoor surfaces of house B. Fig. 3 shows the DNA concentration per 100cm² on indoor surfaces in house B and Table 3 lists the DNA concentrations on indoor surfaces and the relative humidity.

Table 3. DNA concentration and RH of floor in house B.

<table>
<thead>
<tr>
<th>Surface</th>
<th>DNA Concentration [ng/100 cm²]</th>
<th>Average RH [%]</th>
<th>Highest RH [%]</th>
<th>Lowest RH [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bedroom (June)</td>
<td>3.16</td>
<td>81.0</td>
<td>94.4</td>
<td>51.3</td>
</tr>
<tr>
<td>Bedroom (March)</td>
<td>1.24</td>
<td>78.9</td>
<td>92.7</td>
<td>50.0</td>
</tr>
</tbody>
</table>

Supplementary information for Table 3: RH in this table is the relative humidity of the air in each room, and the average, highest, and lowest values were calculated using the monthly data before each sampling.

In house B, the DNA concentrations of microorganisms on the floor surfaces were higher in March than in June in both the living room and bedroom (Fig. 4). Comparing relative humidity, the average and lowest relative humidity values were higher in March than those in June in both rooms, when DNA concentrations were also high (Table 3).

For samples collected in March 2022, the DNA concentration on the floor was higher than that on the wall in each room. This could be because microorganisms tend to accumulate more easily on the floor than on the wall by attaching themselves to dirt and dust.

Table 4. DNA concentration and RH of floor in house B.

<table>
<thead>
<tr>
<th>Surface</th>
<th>DNA Concentration [ng/100 cm²]</th>
<th>Average RH [%]</th>
<th>Highest RH [%]</th>
<th>Lowest RH [%]</th>
</tr>
</thead>
<tbody>
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<td>50.0</td>
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</tbody>
</table>

Supplementary information for Table 4: RH in this table is the relative humidity of the air in each room, and the average, highest, and lowest values were calculated using the monthly data before each sampling.
Similar to house A, the dining room floor surface DNA concentration was higher in August than that in February, when the average and lowest relative humidity were also high. The same relationship between DNA concentration and relative humidity was found for the dining room beam and Japanese-style room pillar.

In contrast, for the storage room ceiling, the DNA concentration was higher in February than in August, although the relative humidity was lower in February than in August. This may be because microorganisms are less likely to adhere to ceilings than to floors, beams, or columns. Moreover, in this house, occupants used a wood-burning stove during winter; hence, the indoor air underwent convection, especially during winter. Therefore, investigations of both the DNA concentrations of microorganisms and the microbial community structures are necessary to understand whether the microbial communities on the floor and ceiling are related.

### 3.3 DNA concentration on indoor surfaces and the relative humidity

Fig. 4 shows the relationship between the DNA concentrations of microorganisms and the relative humidity of the indoor surfaces of houses A and B. No clear correlation was found between the two factors. However, for most samples, the higher the average relative humidity, the higher the DNA concentration, considering the seasonal changes in each sampling spot (arrow mark in Fig. 4). This correlation between DNA concentration and average relative humidity was found for the floor surfaces in both houses, whereas the DNA concentration of the ceiling in house B was not correlated with the relative humidity of the room, as mentioned above.

### 3.4 DNA concentration of air conditioner filters and outlets in the modern house

We also collected microbial samples from the air conditioner (AC) filters and outlets, under the assumptions that the AC filter represented the microbial community in the room air and that the AC outlet represented the microbial community in the air supplied by the AC. Occupants of house B do not use an AC; thus, AC sampling was conducted only in house A. There are two AC units in house A, one each in the living room and bedroom.

For the AC filter in both rooms, DNA concentrations were higher in June than in March (Fig. 5). In contrast, the DNA concentration at the AC outlet was higher in March than in June in both rooms (Fig. 6). Thus, the DNA concentrations of the AC filters and outlets were not correlated with each other in these
samples. We collected microbial samples from the AC outlets in the same place during both samplings for each AC. Hence, microorganisms on the AC outlet surfaces could have been reduced by the sampling in March and might not have recovered sufficiently by the time of sampling in June. To clarify the influences of sampling in the future, we will wash the filters and wipe the outlets with alcohol after each sampling. Another possible reason is that the AC units were used for cooling and heating. This should be examined in future studies by sampling from various houses.

![Graph](Fig. 5. DNA concentrations on AC filters.)

![Graph](Fig. 6. DNA concentrations on AC outlets.)

4 Conclusions

The purpose of this study was to understand the relationships between temperature and relative humidity and the microbial abundance in a living environment. In this study, we examined microbial abundance via the DNA concentrations on indoor surfaces in a modern Japanese house and a traditional Japanese house.

For the floor surfaces in both houses, the higher the average and lowest relative humidity, the higher the DNA concentration of microorganisms. In the traditional house, the same tendency was observed for the beam and column surfaces. The high relative humidity maintained may promote the attachment and growth of microorganisms.

The DNA concentrations of microorganisms at each sampling spot on the indoor surfaces was high when the average relative humidity of the month prior to sampling was high, with some exceptions. However, the AC requires further careful consideration, and we need to accumulate more samples.

These results show that relative humidity can affect the seasonal change of the DNA concentration of microorganisms on indoor surfaces. Although whether higher or lower the DNA concentration on indoor surfaces is better for human health is still unclear, it might be important to control indoor relative humidity which is affected by living conditions to control the indoor microbiome.

In future studies, we will not only focus on the DNA concentrations but also examine the microbial community structures to understand more clearly how indoor microbial communities differ from house to house and season to season.

Acknowledgments

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References