

Note

An experimental verification of fungal overgrowth in temporary houses at the site of the Great East Japan Earthquake

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Abstract: Fungal contamination in the indoor air of prefabricated temporary houses at the site of the Great East Japan Earthquake revealed extremely high levels compared to those found in conventional residences. We experimentally investigated fungal growth levels on different interior materials to support fungal overgrowth in prefabricated temporary houses. Three species each of allergenic fungi and invasive fungi observed in temporary housing were selected for inoculation tests with various interior materials. The experiments with fungal inoculation were conducted in conformance with standards for industrial products described in the Japanese “JIS Z 2911:2018 Methods of test for fungus resistance” with small modifications. After incubation, visual and stereomicroscopic assessments were performed to determine fungal growth levels. The viability of the fungi varied according to the interior material type. Our findings demonstrate the importance of antifungal measures in indoor environments and the need for additional research on the growth levels of fungal species on various interior materials.

Keywords : the Great East Japan Earthquake / fungal overgrowth / temporary house / indoor environment / gypsum board.

Fungal contamination in a residential environment can cause health issues such as fungal allergies in humans. Previous researchers performed field surveys in the indoor air of prefabricated temporary houses at the site of the Great East Japan Earthquake from 2012 to 2013 and revealed severe fungal contamination compared to those found in conventional residences (Shinohara et al. 2014; Shinohara et al. 2018; Oshikata et al. 2020; Watanabe et al. 2021). They found an unusual fungal overgrowth in the interior materials, such as floorboards or ceiling boards, used in these buildings (Watanabe et al. 2021). They suggested that the fungal

overgrowth was probably due to high humidity and dew condensation (Shinohara et al. 2014; Shinohara et al. 2018; Watanabe et al. 2021). Indeed, several investigations into the health status of residents in the temporary houses in Miyagi prefecture found that many of them had developed asthma related to exposure to fungal and mite allergens (Oshikata et al. 2020). Moreover, it was reported that a patient with allergic bronchopulmonary mycosis (ABPM) was induced by *Eurotium herbariorum*, which had unusually grown in his house (Oshikata et al. 2017). The large exposure to fungal cells potentially causes residents to develop diseases, including allergies. The residents of prefabricated temporary houses could face a serious health risk due to fungal overgrowth. Therefore, it is essential to confirm the

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TABLE 1. Fungal growth levels on the interior materials used in temporary houses

Species	Fungal growth level ^a			
	Ceiling panel			Carpet tile
	Gypsum board 1	Gypsum board 2	Plywood board	
<i>Aspergillus fumigatus</i>	1 ^b (1) ^c	2 (2)	1 (1)	0 (1)
<i>Aspergillus restrictus</i>	0 (0)	0 (0)	1 (1)	0 (0)
<i>Aspergillus versicolor</i>	0 (0)	2 (2)	1 (1)	0 (1)
<i>Cladosporium cladosporioides</i>	1 (1)	2 (2)	1 (1)	0 (0)
<i>Eurotium herbariorum</i>	0 (1)	0 (1)	1 (1)	0 (0)
<i>Trichosporon asahii</i>	0 (0)	0 (0)	1 (1)	0 (0)

^aThree fungal growth levels have been defined: 0, no mycelial growth is visible on the inoculated sample; 1, mycelial growth is visible on the inoculated sample in less than one-third of the entire area; and 2, mycelial growth is visible on the inoculated sample in more than one-third of the entire area, regardless of spore maturity.

^bResult confirmed by visual evaluation.

^cResult confirmed by stereomicroscopic evaluation.

viability and growth level of fungi on the interior materials of these temporary houses in a high-humidity environment for preventive health management. In this study, we experimentally investigated the growth levels of several target fungal species on interior materials in temporary houses.

We collected commercially available interior materials for our inoculation experiments to verify the growth levels of each fungal species on them. The most commonly used ceiling panel in prefabricated temporary houses in Miyagi prefecture was the plywood board type, though the gypsum type was used in temporary houses in some areas. In this study, we used three types of ceiling panels: gypsum board 1, gypsum board 2, plywood board, and one type of polypropylene carpet tile. Each material was cut into 30-mm squares and then sterilized with ethylene oxide gas.

The experiments involving fungal inoculation were conducted in conformance with standards for industrial products described in the Japanese “JIS Z 2911:2018 Methods of test for fungus resistance” with small modifications (Japanese Industrial Standards Committee 2018). In this study, we used the following three allergenic species (Larone 2011; Oshikata et al. 2017) as inocula to materials: *Aspergillus fumigatus* (NIHS 5504), *Trichosporon asahii* (NBRC 103889), and *E. herbariorum* (NIHS 5223), and three additional species frequently detected in Japanese homes, including prefabricated temporary houses, (Ara et al. 2004; Shinohara et al. 2014; Hashimoto et al. 2018; Shinohara et al. 2018; Oshikata et al. 2020; Watanabe et al. 2021) as follows: *Aspergillus restrictus* (NIHS 5531), *Aspergillus versicolor* (NIHS 5500), and *Cladosporium cladosporioides* (NIHS 5656). Each fungal species was cultured on either potato dextrose agar (PDA agar; Eiken Chemical Co. Ltd., Tokyo, Japan) or dichloran 18% glycerol (DG18) agar (Oxoid Limited, Waltham, MA, USA) at 26°C for 7 days. For the inoculation experiments, an inoculum of spore suspension prepared in humectant-added sterilized water

(0.005% dioctyl sulfosuccinate sodium salt) with spore concentration adjusted to 10⁶/ml was prepared for each species. Then, 0.5 ml of the inoculum was sprayed onto a 900-mm² piece of the interior material, which was placed in a sterilized Petri dish and stored together with a water tank for humidification in a sealed container with incubation at 26 ± 2°C and >95% relative humidity for 28 days. After incubation, visual and stereomicroscopic (20X magnification) evaluations were performed to determine fungal growth levels based on the following scoring system: 0, no mycelial growth is visible on the inoculated sample; 1, mycelial growth is visible on the inoculated sample in less than one-third of the entire area; and 2, mycelial growth is visible on the inoculated sample in more than one-third of the entire area, regardless of spore maturity. These experiments were conducted in triplicate to confirm the repeatability of the results. The results of this triplicate were referred to and evaluated comprehensively.

The results of the evaluation of fungal species' viability on various interior materials showed in Table 1. We confirmed the growth of *A. fumigatus* and *C. cladosporioides* on the gypsum board 1 and that of *A. fumigatus*, *A. versicolor*, and *C. cladosporioides* on the gypsum board 2, showing that the growth levels between two gypsum board types were different. Both of gypsum boards for ceiling panels tested in this study are decorative boards laminated with synthetic resin or paper on the surface. Although the materials of both products used in this study are not known in detail, laminates typically vary among manufacturers or products, indicating that moisture levels and surface textures may have been different between them. It was thought that these may also have influenced the differences in fungal growth between the two gypsum board products used in this study.

A previous study reported that the fungal growth on a gypsum board significantly promoted the decrease in the tensile strength and weight loss of the board (Kazemian et

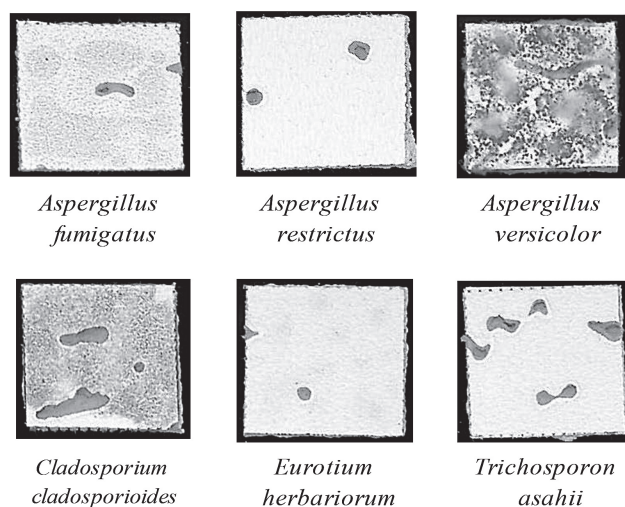


FIG. 1. Growth of tested fungal species on gypsum board 2 used for ceiling panels after 28-day incubation.

al. 2019). They also mentioned that exposure to moisture and dust accelerated the biodeterioration of a gypsum board. In a previous study, we measured surface temperature on a ceiling panel with fungal overgrowth and observed the attic space behind the ceiling panel in some prefabricated temporary houses at the site of the Great East Japan Earthquake (Watanabe et al. 2021). We actually confirmed a decline in the surface temperature on a part of a ceiling panel and a lack of thermal insulation construction, coinciding with the area of the ceiling panel with fungal overgrowth. One house had thermal insulation construction with uplift of heat shield materials, and another house had construction with discontinuity of airtight sheet. It was a possibility that the fungal overgrowth on the panels in the fabricated houses at that site was caused by dew condensation or an increase in water content with cooling below the dew point. The results of this study support the idea that gypsum boards are vulnerable to the development of some kinds of fungi in highly humid conditions. However, the most commonly used ceiling panels made of gypsum are not expected to be used in highly humid conditions. When using gypsum boards indoors in a prefabricated home in a cold climate, it is recommended that the thermal insulation performance be carefully checked.

The significant growth on gypsum board 2 of *A. versicolor*, another dominant species frequently detected with *Eurotium* in the indoor air of temporary houses in a previous report (Watanabe et al. 2021), was confirmed with extensive spore production and a growth rate higher than that of other species (Table 1, Fig. 1). Furthermore, gypsum board 2 also showed the growth of the representative allergenic fungal species, *A. fumigatus*. These results suggest that some gypsum board products, when exposed to high humidity conditions for extended periods, become a breeding ground for environmentally and medically important fungi, such as *A.*

fumigatus or *A. versicolor*, possibly due to external factors such as condensation. The indoor proliferation of these species would pose a significant health risk to the residents; therefore, fungus-eliminating measures such as the prevention of dew condensation should be implemented. Concerningly, *A. fumigatus* showed the highest growth on all tested materials among the fungal species tested in this study. Further research is required to understand the biological parameters that affect the growth of *A. fumigatus* on interior materials and to control contamination by this hazardous fungus in our living environments.

The growth at level 1 on plywood boards were confirmed with all five fungal species tested in this study (Table 1). Wooden boards were used as a positive control in many previous studies of fungal growth on building materials (Mensah-Attipoe et al. 2015; Claeson et al. 2019). Therefore, the fungal growth levels on wooden boards are considered relatively high. Our results were consistent with previous studies. On the polypropylene carpet tiles, which are used in almost all temporary housing specifications, only weak growth at level 1 were confirmed only with *A. fumigatus* and *A. versicolor* by stereomicroscopy, suggesting that polypropylene carpet is comparably resistant to fungal growth indoor environments.

We confirmed the growth of *E. herbariorum* on two gypsum board types by stereomicroscopic observation only. Previous research reported that substantial *Eurotium* contamination in the indoor air of a prefabricated temporary housing of an ABPM patient as the causative fungi (Oshikata et al. 2017). Our result suggests that there is a low possibility of ceiling panels or carpet tiles being involved in *Eurotium* contamination in the patient's house. We also confirmed no growth of *T. asahii*, a representative species responsible for summer-type hypersensitivity pneumonitis, on the tested materials except plywood board, suggesting that these materials have a low or no chance of becoming a breeding ground for *T. asahii*.

These results indicate that the level of fungal growth differ among species depending on a kind of interior materials, and the growth may be affected by the chemical (e.g., carbon source), physicochemical (e.g., pH, surface topography, and moisture absorption or desorption), and other properties of materials. Our findings imply that the importance of using antifungal measures in temporary houses, such as prevention of dew condensation, high humidity, and others. Our previous study reported that the moldy parts of the ceiling panel were frequently observed visually in a type of temporary housing-complexes (Watanabe et al. 2021). In those temporary housing-complexes, gypsum boards rather than plywood boards were used as ceiling panels. We should pay close attention to interior material specifications and avoid using gypsum ceiling panels with temporary housing constructed in the case of a disaster, especially at condensation-prone areas.

Our results provided insights into one of the causes of

fungal overgrowth in temporary houses. The growth level of each fungal species depends on various factors regarding interior materials. Further investigations, including of a field survey of fungal contamination in contemporary houses and sufficient data on the growth level of each fungal species on interior materials, are needed to validate our findings.

Author contributions

M. W. and R. K. designed the study. M. W., R. K., Na. K., Y. K., and K. T. developed the technique and carried out the experiments. M. W., R. K., K. H., and Na. K. analyzed the data. M. W., R. K., Na. K. and Y. K. prepared the manuscript. M. W., R. K., K. H., No. K., H. Y., and K. T. provided reagents and technical support. No. K., H. Y. and Y. H.-K. supervised the project. M. W., Y. K., Y. H.-K. provided funding. All authors approved the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Ethical approval

This study did not contain any human and animal subject.

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