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Assessment of home environments with a fungal index using hydrophilic and xerophilic fungi as biologic sensors

Abstract Previously, the author proposed a 'fungal index' that quantifies the capacity for fungal growth in a test environment where a device (fungal detector) encapsulating spores of a xerophilic sensor fungus *Eurotium herbariorum* was placed. It was also found that an extremely xerophilic fungus, *Aspergillus penicillioides*, was suitable as a sensor fungus at sites with lower relative humidity (RH). In this report, the hydrophilic fungus *Alternaria alternata* was added to sensor fungi for the determination of the index in extremely humid environments. Measurements of the index and observations of the formation of spores by the sensor fungi were made in stable climates in moisture chambers, under natural conditions in homes, and in bathrooms prepared in an artificial climate chamber. Higher index values and earlier sporulation were obtained at higher RH in stable climates. The hydrophilic *Alt. alternata* showed the greatest response at 100% and 97.3% RH, the moderately xerophilic *Eur. herbariorum*, at 94%, 84%, and 75% RH, and the extremely xerophilic *Asp. penicillioides*, at 71% RH. In homes, the hydrophilic fungus was most active in water-usage areas, and the xerophilic fungi were most active in non-water-usage areas. Sporulation was observed on sensor fungi in fungal detectors placed in rooms where the index exceeded 18 ru/week after one-month exposure. Sites where the index exceeded 18 ru/week were referred to as damp, where fungal contamination seems to be unavoidable. Evaluations of ventilation systems in bathrooms with extremely humid climates showed typical examples of a countermeasure to fungal contamination.

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Key words: Fungal index; Fungi; Microclimate; Contamination; Dampness; Home environment.

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Practical Implications

The purpose of this study is to establish a fungal index applicable in home environments with extremely high to relatively low relative humidity climates. The sensor fungus that showed the greatest response in a fungal detector (a device encapsulating spores of sensor fungi) served as not only a quantitative but also a qualitative indicator of the environment tested, indicating the type of fungi that would contaminate the site. A fungal index would be a good tool for detecting dampness that induces fungal contamination, which has adverse effects on human health. Evaluations of indoor climates would provide information useful to building owners, builders, designers, advisers, medical practitioners, and so on. Selection of the most suitable insulation systems in various buildings under different climates or evaluations of the drying process in water-damaged buildings could also be possible using fungal detectors and measurements of fungal indices.

Introduction

Fungi, heterotrophic organisms that grow on organic materials, can contaminate buildings and cultural assets, and affect the health of workers and occupants. Exposure to fungal contamination is associated with respiratory symptoms (e.g., coughing and wheezing), asthma, allergies, and immunologic reactions. Fungi produce a large quantity of airborne spores that can be inhaled. Repeated exposure to large amounts of spores increases the risk of developing allergic reactions (Wiszniewska et al., 2009). In the WHO guidelines for Indoor Air Quality (World Health Organization, 2009), it was reported that the health risk from biologic contaminants of indoor air could be addressed by considering dampness as a risk indicator. The health risk from dampness-associated microbial growth and contamination in homes is not a small problem.

Although fungal contamination is harmful, measures are not usually taken until the damage is visible or symptoms have developed. Also, one cannot accurately

predict which types of houses or which sites in buildings are susceptible to fungal contamination. Sunlight, floorplan, structure, and the local outdoor climate affect the microclimates of indoor environments. Lifestyle affects the amount of heat and moisture generated and distributed indoors. An uneven distribution of temperature can cause differences in RH and dew to condense at cold sites. In some cases, rain or water from pipes may be leaking into the building undetected.

For fungal contamination to occur, a microclimate that encourages fungal growth, that is a damp environment, must be maintained. If it can be determined in advance whether the environment at a particular site within a home is damp and supports fungal growth, countermeasures can be taken before contamination occurs. Therefore, a fungal index that quantifies the capacity for fungal growth in an environment was proposed (Abe, 1993).

The fungal index is a parameter for evaluating microclimates for potential fungal growth. For estimation of the index, a test-piece (fungal detector) encapsulating spores of fungi was exposed at the test site. The fungi were biologic sensors of the microclimate of the site. The growth of the fungus that showed the greatest response was measured, and the fungal index was estimated using the response and exposure period (Abe, 2010).

As biologic sensors, the moderately xerophilic *Eurotium herbariorum* (Abe, 1993; Abe et al., 1996) and extremely xerophilic *Aspergillus penicillioides* (Abe, 2010) were used. These fungi have shown good growth responses to microclimates in buildings, storage areas of museums, exhibition rooms, etc. However, a hydrophilic fungus should also be used to analyze indoor environments in homes, because fungal contamination can occur in highly humid locations such as bathrooms, dew-condensing sites such as north-facing walls in winter, air-conditioners in summer, and so on.

In this study, the hydrophilic fungus *Alternaria alternata* was added to the biologic sensors. Fungal indices were estimated using both hydrophilic and xerophilic fungi as the sensors in various stable climates controlled in moisture chambers, under natural conditions in homes, in water-usage and non-water-usage areas, and on the ceilings of bathrooms installed with ventilation systems. Fungal index values, above which an environment is conducive to fungal contamination in homes, are discussed. Potential applications of fungal detectors and fungal indices are also discussed.

Materials and methods

Sensor fungi

Three fungi differing in sensitivity to RH were used.

Eurotium herbariorum J-183, a moderately xerophilic fungus responsive to changes in the atmosphere over a wide range of RH values, has been used previously to assess indoor environments (Abe, 1993; Abe et al., 1996). The strain has been deposited in the National Institute of Technology and Evaluation Biological Research Center, with the access code NBRC 107902.

Aspergillus penicillioides K-712, a strongly xerophilic fungus, was selected to evaluate the storehouse of a museum (Abe, 2010). The strain was derived from a type culture of *Asp. penicillioides* NBRC 8155 and cultured continuously in our laboratory.

Alternaria alternata S-78, a hydrophilic fungus, was isolated outdoors in Aikougun, Kanagawa Prefecture, Japan. The strain was screened from ca. 20,000 colonies formed on plates of YS-10 agar (1% yeast extract, 10% sucrose, and 2% agar) incubated one week at 5°C so as to select a fungus that responds in places where dew condenses in winter. The strain had the following characteristics; (i) germinated within 24 h of exposure at 5°C and 100% RH, (ii) germinated within 1 week of exposure at 0°C and 100% RH, (iii) short hyphae in a fungal detector were clearly visible under a microscope, and (iv) the length of hyphae was more than 10,000 μm after 1-week incubation at 25°C and 100% RH. The strain has been deposited with the access code NBRC 107930.

Fungal detectors

For the preparation of conidia, which were encapsulated in fungal detectors, the hydrophilic strain and two xerophilic strains were cultured on agar plates of potato-dextrose medium (Nissui Co. Ltd., Tokyo, Japan) and YS-35 medium (Abe, 1993), respectively. The conidia that formed on the plates were collected and suspended in a medium as described previously (Abe, 1993) to give an approximate spore concentration of $10^3/\mu$l and used as spore suspensions.

The fungal detector (Figure 1) was prepared as follows: a double-sided adhesive sheet (13×27; thickness, 0.3 mm) with two holes, 7×7 and 7×12 mm, was stuck to a transparent plastic plate (13×40 mm). Then, a 3-μl spore suspension of each sensor fungus was spotted in the holes and air-dried. The inoculated spot was ca. 3 mm in diameter after drying. A gas-permeable sheet, 13×27 mm, was then attached to the other side of the adhesive sheet. Liquid water cannot enter into the holes covered with the gas-permeable sheet nor can the conidia of sensor fungi that have formed in the holes disperse into the surrounding air. The gas-permeable sheets were made from Minicopy film (Fujifilm Co. Ltd., Tokyo, Japan), a transparent negative film prepared by developing unexposed film. The sheets did not affect the responsiveness of the sensor fungi.

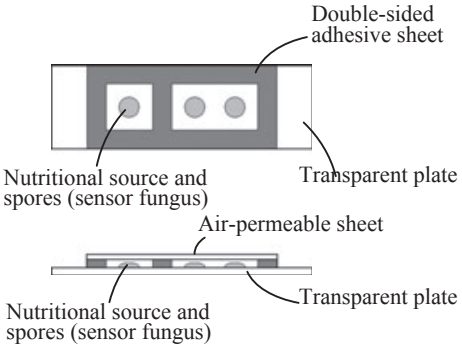

Fig. 1 Fungal detector. A fungal detector containing three sensor fungi was used to determine a fungal index in non-water-usage areas

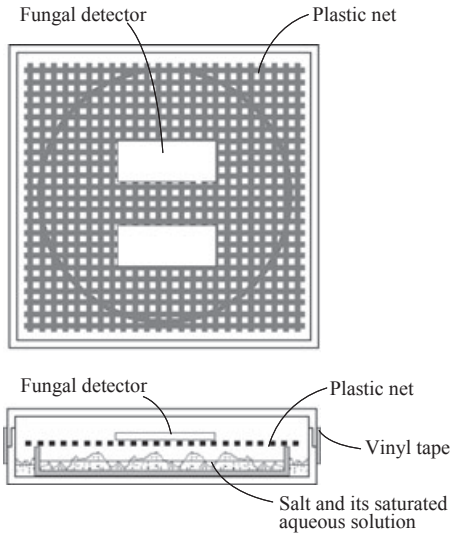

Fig. 2 A moisture chamber. Front and side views of the inner moisture chamber with two fungal detectors. A humidity-controlling agent, either an aqueous saturated salt solution with crystallized salt (K_2SO_4, KNO_3, KCl, NaCl, or $SrCl_2{\cdot}6H_2O$) or distilled water, was placed in the chamber

Determination of the fungal index

The fungal index was described previously in detail (Abe, 2010). The procedure used was as follows. (i) A fungal detector was exposed to a test environment; (ii) after the exposure, the detector was placed in a container with silica gel, and the development of hyphae was terminated by desiccation with silica gel; (iii) photographs of fungal growth in the detector were taken under a microscope; (iv) the length of hyphae was measured on the photographs; (v) the number of fungal response units (ru), was determined from the length of hyphae using a standard curve; and (vi) the fungal index was calculated using the greatest response among the sensor fungi in the detector.

For the estimation of the length of hyphae to determine response units, the five longest hyphae in the photograph were measured. The five were selected from among all hyphae in the photograph if hyphae were short. If the hyphae were long (elongating more than 100 μm outside from the edge of the inoculated spot), the five were selected from among hyphae that cross 1000 μm of the spot edge. Then, the longest and shortest values were omitted, and the lengths of the second, third, and fourth longest hyphae were averaged. The average value was defined as the length of hyphae estimated on the photograph (Abe, 2010). Figure 2 shows examples of short hyphae (A), long hyphae (B), and long hyphae, which extended more than the measurable limit (C).

The standard curve, which shows the relationship between hyphal length (μm) and response units (ru), was reported previously (Abe, 2010). The index was obtained by dividing the number of response units (ru) by the exposure period (in weeks).

The sensor fungus that showed the greatest response was shown after the index value. *Alt.a*, *Eur.h*, and *Asp.p* indicate *Alt. alternata*, *Eur. herbariorum*, and *Asp. penicillioides*, respectively.

In this report, individual fungal indices estimated using the response units of *Alt. alternata*, *Eur. herbariorum*, and *Asp. penicillioides* were also measured and represented by Alt.a index, Eur.h index, and Asp.p index, respectively.

Evaluation of stable climates

For the precise evaluation of stable climates, double moisture chambers, consisting of inner and outer compartments with controlled RH, were prepared. In both inner and outer chambers was placed the same humidity-controlling agent, either distilled water or an aqueous saturated salt solution with crystallized salt. The equilibrium RH of the aqueous saturated solution of K_2SO_4, KNO_3, KCl, NaCl, and $SrCl_2{\cdot}6H_2O$ was 97.3%, 93.6%, 84.3%, 75.3% (ASTM E 104-85), and 70.8% (Acheson, 1965), respectively, at 25°C. At 5°C, the equilibrium RH of K_2SO_4, KNO_3, KCl, and NaCl was 98.8%, 96.3%, 87.7%, and 75.7%, respectively (ASTM E 104-85). To prevent alterations in RH, the lids of all chambers were sealed with vinyl tape.

Two fungal detectors were placed in the upper part of the inner chamber, 10 cm square and 3 cm deep (Figure 3). The exposure lasted 2, 4, 7, 14, or 28 days depending on the climate to be tested. After the exposure, the fungal index of each climate was determined. The number of inoculated spots in each hole in a fungal detector was one or two, each spot with a separate sensor fungus. If the length of hyphae exceeded the measurable limit after exposure, the data were omitted. For measuring longer hyphae, a spot was placed closer to one side in the larger hole (7×12 mm).

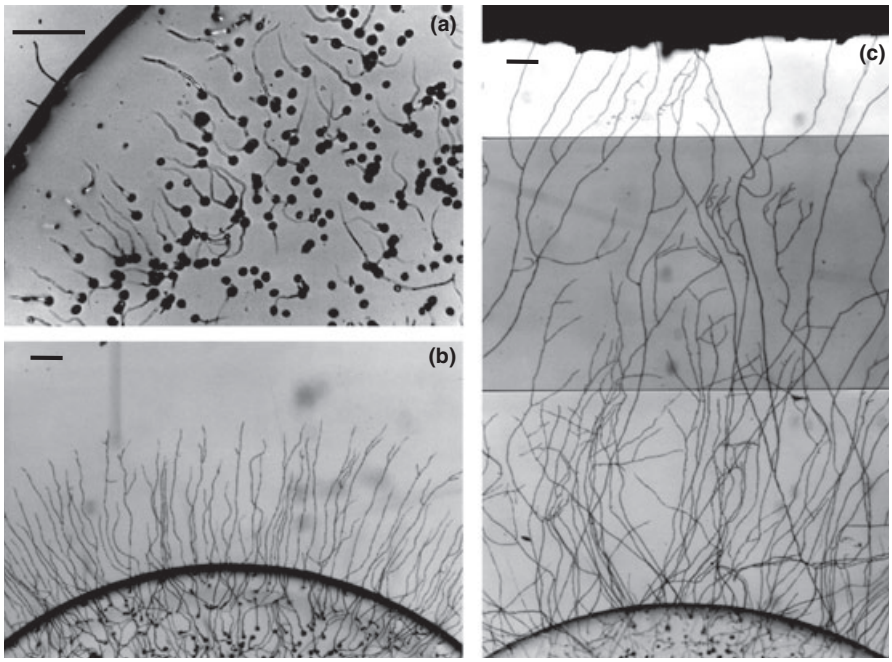

Fig. 3 Typical growth responses to the environment. (a) Shows short hyphae, (b) shows long hyphae that developed from the spot edge outward into the region without nutrients, and (c) shows extremely extended hyphae that exceeded the measurable limit, reaching to the edge of the double-sided adhesive sheet (scale = 100 μm)

Evaluation of daily climates in homes

Daily climates were evaluated in 15 homes. In seven homes, investigations were performed at one site in water-usage areas. In eight homes, investigations were performed at two sites in non-water-usage areas.

In water-usage areas, fungal detectors with two holes inoculated with *Alt. alternata* and *Eur. herbariorum* were used to determine the fungal index. The investigations were performed in June. The detectors were fixed in place on the wall ca. 60 cm above the floor by attaching the plastic at both ends with vinyl tape and were exposed 7 days.

In non-water-usage areas, fungal detectors inoculated with the three sensor fungi (Figure 1) were used. *Alt. alternata* was placed in the 7×7 mm hole, and *Eur. herbariorum* and *Asp. penicillioides* in the 7×12 mm hole. The detectors were fixed on a north wall 10 cm above the floor in two rooms of each home. The exposure period was one month. The investigations were performed monthly from July to November 2006.

Evaluation of bathrooms controlled by ventilation systems

The ventilation systems in bathrooms prepared in an artificial climate chamber were evaluated using fungal detectors. The climate in the artificial chamber was controlled at 20°C and 50% RH. The ventilating equipment was DS-10BP, DS-10BS3, or DS15BW (Hitachi Appliances Inc, Tochigi, Japan). Ten fungal detectors with two holes (7×7 mm each) inoculated with *Alt. alternata* and *Eur. herbariorum* were placed on the ceiling of the bathroom with the door closed: two detectors in the center, four at corners, and four in central areas of the ceiling. To replicate conditions when taking a bath, the bathtub was filled with hot water (40°C), the bathroom was showed with hot water for 6 min, the stopper in the bathtub was pulled out, and hot water was spread on the walls in the bathroom for 1 min. This process was repeated three times, at 20-min intervals, i.e., a total of 1 h, everyday. DS-10BP, which has an exhaust function only, and DS-10BS3, with dual functions (both removing and supplying air), were operated 6 h after the bath hour everyday. DS-15BS3, also with dual functions, was operated continuously, 24 h everyday. The exposure period for fungal detectors was 4 days.

Results

Fungal indices in stable climates

Table 1 shows the fungal indices in various stable climates at 25°C. Different periods of exposure were used. Hyphal length, sporulation, response units (R-units in the table), and the fungal index estimated using the response units in each sensor fungus are also listed in the table for different exposure periods. The hyphal length marked with A or B is the length of hyphae between the spore and hyphal tip, and the length of hyphae between the edge of the inoculated spot and hyphal tip, respectively.

Table 1 Fungal indices in stable climates at 25°C

Experimental conditions			Sensor fungi in fungal detectors														
			Alternaria alternata S-78					*Eurotium herbariorum* J-183					*Aspergillus penicillioides* K-712				
Relative humidity (%)[a]	Period (day)	Average fungal index (ru/week)[b]	Hyphal length (μm)[c]	S[d]	R-unit (ru)[e]	Alt.a index (ru/week)[f]	*N*[g]	Hyphal length (μm)[c]	S[d]	R-unit (ru)[e]	Eur.h index (ru/week)[f]	*N*[g]	Hyphal length (μm)[c]	S[d]	R-unit (ru)[e]	Asp.p index (ru/week)[f]	*N*[g]
100	2	306.1 (*Alt.a*)	3336 (527) B	+	87.5 (11.7)	306.1 (41.0)	8	312 (36) B	–	19.8 (0.8)	69.3 (2.9)	4	0 A	–	<7.0	<24.5	4
	4	318.6 (*Alt.a*)	7593 (1814) B	+	182.1 (40.3)	318.6 (70.6)	4	297 (41) B	–	19.5 (0.9)	34.1 (1.7)	4	0 A	–	<7.0	<12.3	4
	7	>190 (*Alt.a*)	>8000 B	+	>190	>190	4	322 (53) B	–	20.1 (1.2)	20.1 (1.2)	4	0 A	–	<7.0	<7.0	4
97.3 (0.5)	2	228.1 (*Alt.a*)	2332 (274) B	+	65.2 (6.1)	228.1 (22.3)	4	926 (229) B	–	36.1 (5.2)	126.3 (18.2)	4	157 (18) B	–	16.3 (0.5)	56.9 (1.6)	4
	4	233.3 (*Alt.a*)	5374 (570) B	+	132.8 (12.7)	233.3 (22.2)	4	3092 (655) B	+	82.1 (14.5)	143.6 (25.4)	4	220 (36) B	–	17.7 (0.8)	31.0 (1.4)	4
	7	>190 (*Alt.a*)	>8000 B	+	>190	>190	4	6036 (925) B	+	147.5 (20.6)	147.5 (20.6)	4	248 (25) B	–	18.4 (0.4)	18.4 (0.5)	4
93.6 (0.6)	2	167.6 (*Eur.h*)	413 (12) B	–	22.1 (2.0)	77.4 (7.0)	14	1555 (153) B	–	47.9 (3.4)	167.6 (11.9)	18	376 (53) B	–	21.3 (1.2)	74.6 (4.2)	4
	4	178.8 (*Eur.h*)	1357 (211) B	–	43.5 (4.7)	76.1 (8.3)	6	3882 (594) B	+	99.6 (13.2)	174.3 (23.1)	6	1479 (93) B	–	46.2 (2.1)	80.9 (3.6)	4
	7	168.5 (*Eur.h*)	2686 (108) B	+	73.0 (2.4)	73.0 (2.4)	6	6984 (735) B	+	168.5 (16.3)	168.5 (16.3)	6	3165 (577) B	+	83.7 (12.8)	83.7 (12.8)	4
84.3 (0.3)	2	69.9 (*Eur.h*)	0 A	–	<7.0	<24.5	4	318 (37) B	–	20.0 (0.8)	69.9 (2.9)	16	92 (3) A	–	11.5 (0.2)	40.0 (0.6)	4
	4	74.0 (*Eur.h*)	0 A	–	<7.0	<12.3	4	1305 (194) B	–	42.3 (4.3)	74.0 (7.5)	12	351 (75) B	–	20.7 (1.7)	36.2 (3.0)	4
	7	74.3 (*Eur.h*)	0 A	–	<7.0	<7.0	4	2744 (309) B	+	74.3 (6.8)	74.3 (6.8)	10	1192 (185) B	–	39.7 (4.1)	39.7 (4.1)	6
75.3 (0.1)	7	13.5 (*Eur.h*)	0 A	–	<7.0	<7.0	4	141.0 (67.0) A	–	13.5 (1.7)	13.5 (1.7)	56	61.9 A (24.2)	–	10.0 (1.1)	10.0 (1.1)	22
	14	13.5 (*Eur.h*)	0 A	–	<7.0	<3.5	4	626 (91) B	–	27.0 (2.1)	13.5 (1.0)	16	362 (68) B	–	21.0 (1.5)	10.5 (0.8)	16
	28	12.9 (*Eur.h*)	0 A	–	<7.0	<1.8	4	1724 (525) B	–	61.7 (11.7)	12.9 (3.0)	18	1181 (294) B	–	39.5 (6.6)	9.9 (1.6)	10
70.8	7	<7.0	0 A	–	<7.0	<7.0	4	0 A	–	<7.0	<7.0	10	0 A	–	<7.0	<7.0	6
	14	3.9 (*Asp.p*)	0 A	–	<7.0	<3.5	4	0 A	–	<7.0	<3.5	14	17 (14) A	–	7.8 (0.7)	3.9 (0.4)	14
	28	3.9 (*Asp.p*)	0 A	–	<7.0	<1.8	4	78 (59) A	–	10.9 (2.3)	2.7 (0.6)	28	132 (48) B	–	15.7 (1.2)	3.9 (0.3)	32

[a]The values in parentheses are s.d. reported elsewhere (ASTM E 104-85).

[b]Fungal index under the experimental conditions. The sensor fungus used to determine the index, which showed the greatest response among the three sensor fungi, is shown in parentheses. *Alt.a*, *Eur.h,* and *Asp.p* indicate *Alternaria alternata* S-78, *Eurotium herbariorum* J-183, and *Aspergillus penicillioides* K-712, respectively.

[c]The values in parentheses are s.d. 'A' indicates the length of hyphae between the spore and hyphal tip. The hyphae were short and less developed. 'B' indicates the length of hyphae between the edge of the inoculated spot and the hyphal tip. The hyphae developed from the spot into the region without nutrients by more than 100 μm.

[d]Sporulation at the sensor fungus. + and – indicate with and without spore formation, respectively.

[e]Response units at that hyphal length. The values in parentheses are s.d.

[f]Fungal index determined with each sensor fungus using each response unit. The values in parentheses are s.d.

[g]Number of inoculated spots of the sensor fungus used under the experimental conditions.

The number of devices, fungal detectors encapsulating spores, used for the calculation ranged from 2 to 56. The index measurements at 25°C and 75.3% RH with *Eur. herbariorum*, which were repeated 56 times, were used as a positive control in many experiments.

Higher indices were obtained at higher levels of RH although the sensor fungus that showed the greatest response differed depending on the climate tested. The measurements in each climate were almost constant; the values did not vary depending on the period of exposure.

At 100% RH, *Alt. alternata* showed the greatest response. The Alt.a index reached ca. 310 ru/week. The hyphal length of *Alt. alternata* exceeded the measurable limit at 7 days in the climate. Sporulation of the fungus was observed in 2, 4, and 7 days. *Eur. herbariorum* did not respond well, that is, the spores germinated, but the hyphal growth stopped. *Asp. penicillioides* showed no response.

At 97.3% RH, again *Alt. alternata* showed the greatest response. The Alt.a index reached ca. 230 ru/week. Sporulation of the fungus was observed in 2, 4, and 7 days. Hyphal length exceeded the measurable limit at 7 days. *Eur. herbariorum* responded well, though less well than *Alt. alternata*. Sporulation of the fungus was observed in 4 and 7 days. *Asp. penicillioides* did not respond well; it germinated but its hyphal elongation ceased.

At 93.6% RH, *Eur. herbariorum* showed the greatest response. The Eur.h index reached ca. 170 ru/week. Sporulation of the fungus was observed in 4 and 7 days. *Alt. alternata* and *Asp. penicillioides* showed weaker responses than *Eur. herbariorum*. These fungi sporulated in 7 days.

At 84.3% RH, *Eur. herbariorum* again showed the greatest response. The Eur.h index reached ca. 70 ru/week. Sporulation of the fungus was observed in 7 days. *Asp. penicillioides* showed less of a response. *Alt. alternata* showed no response below 84.3% RH.

At 75.3% RH, once more *Eur. herbariorum* showed the greatest response. The Eur.h index reached ca. 13 ru/week. *Asp. penicillioides* showed less of a response.

At 70.8% RH, *Asp. penicillioides* showed the greatest response. Asp.p index reached 3.9 ru/week. No germination was observed at 7 days in this climate. *Eur. herbariorum* showed less of a response.

Table 2 shows the fungal index in various test climates at 5°C. Although higher values were obtained at higher RH, the values were lower than at 25°C. Again, values did not vary depending on the period of exposure.

At 5°C, *Alt. alternata* showed the greatest response at 100% RH and 98.5% RH. The Alt.a index reached ca. 40 and 32 ru/week, respectively. Sporulation of the sensor fungus was observed within 7 days in both climates. At 96.3% RH, the responses of *Eur. herbariorum* and *Alt. alternata* were similar, and the Alt.a and Eur.h indices reached ca. 15 ru/week. Sporulation was observed at 28 days for both fungi. At 87.7% RH, *Eur. herbariorum* showed the greatest response and the Eur.h index reaching ca. 8 ru/week. *Alt. alternata* showed no response. At 75.7% RH, no sensor fungi exhibited a response.

Climate in water-usage areas in homes

Table 3 shows the fungal indices in water-usage areas in homes. The highest index obtained was 82.8 (*Alt.a*) ru/week on a wall of the bathroom in home D. Sporulation of *Alt. alternata* was observed in the detector. The next highest value was 43.4 (*Alt.a*) ru/week (home E, also on a wall in the bathroom). The response of *Alt. alternata* was greater than that of *Eur. herbariorum* in all bathrooms. At these sites, *Eur. herbariorum* showed less of a response or no response. Only on the wall above the faucet in the washroom, located in front of the bathroom of home F, did *Eur. herbariorum* show a greater response than *Alt. alternata*. On the kitchen walls (homes C and G), no germination was observed, and the indices were below the measurable limit. Fungal contamination was visible in all bathrooms. On the wall above the faucet in the washroom, fungal contamination was visible. No fungal contamination was visible on the kitchen walls examined.

Climate in non-water-usage areas in homes

Tables 4 and 5 shows the fungal indices in non-water-usage areas, on north walls of 16 rooms of eight homes, surveyed monthly from July to November. Table 4 shows the experimental conditions where fungal indices were measurable. Table 5 shows the conditions where fungal indices were below the measurable limit, and no germination occurred among any of the sensor fungi. Table 4 was arranged from the experimental conditions with higher to lower fungal index levels.

The xerophilic *Eur. herbariorum* and *Asp. penicillioides* showed good responses, but the hydrophilic *Alt. alternata* showed less of a response. *Eur. herbariorum* showed the greatest response at the sites with a high fungal index. *Eur. herbariorum* formed the longest hyphae among sensor fungi at all locations where the index exceeded 6.1 ru/week, but did not form as many spores as *Asp. penicillioides*. Sporulation by *Eur. herbariorum* was observed in some areas where the index exceeded the measurable limit.

Asp. penicillioides showed the most frequent response. The fungus showed the greatest response at the sites with a relatively low fungal index. Sporulation of the fungus was observed at all sites with fungal indices exceeding 16.7 (*Eur.h*) ru/week, where the

Table 2 Fungal indices in stable climates at 5°C

Experimental conditions			Sensor fungi in fungal detectors													
			Alternaria alternata S-78					*Eurotium herbariorum* J-183					*Aspergillus penicillioides* K-712				
Relative humidity (%)[a]	Period (day)	Average fungal index (ru/week)[b]	Hyphal length (μm)[c]	S[d]	R-unit (ru)[e]	Alt.a index (ru/week)[f]	*N*[g]	Hyphal length (μm)[c]	S[d]	R-unit (ru)[e]	Eur.h index (ru/week)[f]	*N*[g]	Hyphal length (μm)[c]	S[d]	R-unit (ru)[e]	Asp.p index (ru/week)[f]	*N*[g]
100	2	42.6 (*Alt.a*)	115 (50) A	–	12.2 (1.9)	42.6 (6.6)	10	0 A	–	<7.0	<24.5	10	0 A	–	<7.0	<24.5	10
	4	32.9 (*Alt.a*)	267 (61) B	–	18.9 (1.5)	32.9 (2.5)	8	0 A	–	<7.0	<12.3	8	0 A	–	<7.0	<12.3	8
	7	41.1 (*Alt.a*)	1121 (217) B	+	41.1 (2.6)	41.1 (2.6)	8	0 A	–	<7.0	<7.0	8	0 A	–	<7.0	<7.0	8
98.5 (0.9)	2	32.3 (*Alt.a*)	45 (9) A	–	9.3 (0.5)	32.3 (1.6)	2	0 A	–	<7.0	<24.5	2	0 A	–	<7.0	<24.5	2
	4	30.3 (*Alt.a*)	203 (58) B	–	17.4 (1.3)	30.3 (2.3)	2	8 (5) A	–	7.3 (0.3)	12.8 (0.5)	2	0 A	–	<7.0	<12.3	2
	7	31.7 (*Alt.a*)	833 (9) B	+	31.7 (0.2)	31.7 (0.2)	2	129 (23) A	–	12.9 (0.8)	12.9 (0.5)	2	0 A	–	<7.0	<7.0	2
96.3 (2.1)	7	17.0 (*Alt.a*)	188 (84) B	–	17.0 (2.0)	17.0 (2.0)	4	164 (52) B	–	16.4 (1.3)	16.4 (1.3)	4	0 A	–	<7.0	<7.0	4
	14	15.3 (*Alt.a*)	786 (369) B	–	30.6 (8.3)	15.3 (4.2)	4	572 (130) B	–	25.3 (3.0)	13.3 (1.6)	4	23 (11) A	–	8.0 (0.6)	4.0 (0.3)	4
	28	14.0 (*Eur.h*)	1828 (340) B	+	54.0 (7.6)	13.5 (1.9)	4	1916 (460) B	+	55.9 (10.2)	14.0 (2.6)	4	153 (19) B	–	16.2 (0.5)	4.1 (0.1)	4
87.7 (0.5)	7	7.4 (*Eur.h*)	0 A	–	<7.0	<7.0	4	10 (5) A	–	7.4 (0.3)	7.4 (0.3)	4	0 A	–	<7.0	<7.0	4
	14	8.4 (*Eur.h*)	0 A	–	<7.0	<3.5	2	174 (4) B	–	16.7 (0.1)	8.4 (0.1)	2	5 (3) A	–	7.1 (0.1)	3.6 (0.1)	2
	28	7.7 (*Eur.h*)	0 A	–	<7.0	<1.8	2	786 (51) B	–	30.7 (1.2)	7.7 (0.3)	2	160 (52) A	–	13.7 (1.4)	3.4 (0.3)	2
75.7 (0.3)	7	<7.0	0 A	–	<7.0	<7.0	2	0 A	–	<7.0	<7.0	2	0 A	–	<7.0	<7.0	2
	14	<3.5	0 A	–	<7.0	<3.5	2	0 A	–	<7.0	<3.5	2	0 A	–	<7.0	<3.5	2
	28	<1.8	0 A	–	<7.0	<1.8	2	0 A	–	<7.0	<1.8	2	0 A	–	<7.0	<1.8	2

[a]The values in parentheses are s.d. reported elsewhere (ASTM E 104-85).

[b]Fungal index under the experimental conditions. The sensor fungus used to determine the index, which showed the greatest response among the three sensor fungi, is shown in parentheses. *Alt.a* and *Eur.h* indicate *Alternaria alternata* S-78 and *Eurotium herbariorum* J-183, respectively.

[c]The values in parentheses are s.d. 'A' indicates the length of hyphae between the spore and hyphal tip. The hyphae were short and less developed. 'B' indicates the length of hyphae between the edge of the inoculated spot and the hyphal tip. The hyphae developed from the spot into the region without nutrients by more than 100 μm.

[d]Sporulation at the sensor fungus. + and – indicate with and without spore formation, respectively.

[e]Response units at that hyphal length. The values in parentheses are s.d.

[f]Fungal index determined with each sensor fungus using each response unit. The values in parentheses are s.d.

[g]Number of inoculated spots of the sensor fungus used under the experimental conditions.

Table 3 Fungal indices in water-usage areas

Experimental conditions			Sensor fungi in each fungal detector							
			Alternaria alternata S-78				*Eurotium herbariorum* J-183			
Home	Survey site	Fungal index in each fungal detector (ru/week)[a]	Hyphal length (μm)[b]	Sp[c]	R-unit (ru)[d]	Alt.a index (ru/week)[e]	Hyphal length (μm)[b]	Sp[c]	R-unit (ru)[d]	Eur.h index (ru/week)[e]
A	Wall, bath room	11.3 (*Alt.a*)	90 A	–	11.3	11.3	0 A	–	<7.0	<7.0
B	Wall, bath room	31.4 (*Alt.a*)	820 B	–	31.4	31.4	133 A	–	13.1	13.1
C	Wall, kitchen	<7.0	0 A	–	<7.0	<7.0	0 A	–	<7.0	<7.0
D	Wall, bath room	82.8 (*Alt.a*)	3127 B	+	82.8	82.8	247 B	–	18.3	18.3
E	Wall, bath room	43.4 (*Alt.a*)	1353 B	–	43.4	43.4	0 A	–	<7.0	<7.0
F	Wall above the faucet, wash room	37.1 (*Eur.h*)	190 B	–	17.0	17.0	1070 B	–	37.1	37.1
G	Wall, kitchen	<7.0	0 A	–	<7.0	<7.0	0 A	–	<7.0	<7.0

[a]Fungal index under the experimental conditions during a 7-day exposure. The fungus used to determine the index, which showed the greater response of the two sensor fungi, is shown in parentheses. *Alt.a* and *Eur.h* indicate *Alternaria alternata* S-78 and *Eurotium herbariorum* J-183, respectively.
[b]'A' indicates the length of hyphae between the spore and hyphal tip. The hyphae were short and less developed. 'B' indicates the length of hyphae between the edge of the inoculated spot and the hyphal tip. The hyphae developed from the spot into the region without nutrients by more than 100 μm.
[c]Sporulation at the sensor fungus. + and – indicate with and without spore formation, respectively.
[d]Response units at that hyphal length.
[e]Fungal index determined with each sensor fungus.

hyphal length of *Eur. herbariorum* was more than 2627 μm (corresponding to 71.7 ru) after one-month exposure, ca. 30 days. Sporulation occurred much more frequently in *Asp. Penicillioides* than *Eur. herbariorum*.

Alt. alternata showed little or no response, except at a few locations where the Asp.p and Eur.h indices exceeded the measurable limit. One major exception was in a bedroom of home L in November, where sporulation was observed for all sensor fungi.

The fungal index varied among homes and rooms; for example, in home H, extremely high values exceeding the measurable limit, 18 (*E* & *A*) ru/week (hyphal length of both *Eur. herbariorum* and *Asp. penicillioides* exceeded the measurable limit), were recorded in July in the two rooms, whereas in home J, relatively low values were recorded and the index readings were 10.2 (*Eur.h*) ru/week in the bedroom and 2.7 (*Asp.p*) ru/week in the living room in July. Also, the seasonal variation differed among rooms. In many rooms, the highest indices were recorded in July. In July, after the detectors had been placed in the middle of the rainy season, the index exceeded the measurable limit in eight of 16 rooms, and fungal responses were detected in every room. The values in August to November were lower than those in July in many rooms, although high indices remained at some sites. In November, the indices were below the measurable limit in 13 of 16 rooms, but in the bedroom of home L, the highest values were obtained in November.

Climate in bathrooms with ventilation systems

Table 6 shows fungal indices on the ceiling in bathrooms installed with different ventilation systems. Readings for each detector and average values and s.d. for 10 readings were obtained.

Without ventilation, the average fungal index was 62.1 ± 7.2 (*Alt.a*) ru/week, and the highest and lowest readings were 75.6 and 52.9 (*Alt.a*) ru/week, respectively. In the bathrooms with DS-BP or DS-10BS3 operated 6 h everyday, the indices were 21.5 ± 6.8 (*Alt.a*) ru/week or < 14.5 (*Alt.a*) ru/week, respectively. In the bathroom with DS-15BW operated continuously, the index was < 12.3 ru/week, below the detectable limit. All of Eur.h indices were lower than Alt.a indices. *Eur. herbariorum* did not respond under ventilation.

Discussion

The fungal index was first proposed as a parameter of microclimates in 1993. In the same stable climate, ideally, the same readings should be obtained for different periods of exposure of fungal detectors, and higher values should be obtained at higher levels of RH. As shown in Tables 1 and 2, the measurements for the three exposure periods were almost constant; the same fungal index values were obtained irrespective of the length of the exposure. Also, higher values were obtained at higher RH levels at both 25 and 5°C. These findings support that the fungal index is suitable for representing humid climates that allow fungal growth.

Table 6, fungal index readings on the ceilings in bathrooms installed with different ventilation systems, shows typical examples evaluating countermeasures to fungal contamination. The data were obtained during the development of new products by a consumer-electronics maker. Other Japanese companies have used fungal detectors and the indices in the

Table 4 Fungal indices on north walls examined monthly, where fungal indices were measurable

Experimental conditions					Sensor fungi in each fungal detector								
					Aspergillus penicillioides K-712			*Eurotium herbariorum* J-183			*Alternaria alternata* S-78		
Home	Room	Season	Exposure period (day)	Fungal index in each fungal detector (ru/week)[a]	Hyphal length (μm)[b]	Sp[c]	Asp.p index (ru/week)[d]	Hyphal length (μm)[b]	Sp[c]	Eur.h index (ru/week)[d]	Hyphal length (μm)[b]	Sp[c]	Alt.a index (ru/week)[d]
L	Bedroom	November	25	>22 (*Eur.h*)	1863 B	+	15.3	>2920 B	+	>22	1487 B	+	13.0
H	Living room	July	28	>19 (*E* & *A*)	>2880 B	+	>20	>2900 B	+	>19	180 B	–	4.4
H	Bedroom	July	28	>19 (*E* & *A*)	>2860 B	+	>19	>2900 B	+	>19	107 B	–	3.9
H	Living room	August	30	>18 (*E* & *A*)	>2820 B	+	>18	>2800 B	+	>18	0 A	–	<1.6
H	Bedroom	August	30	>18 (*E* & *A*)	>2850 B	+	>18	>2800 B	–	>18	0 A	–	<1.6
M	Bedroom-1	July	30	>18 (*E* & *A*)	>2820 B	+	>18	>2850 B	+	>18	0 A	–	<1.6
K	Bedroom	July	30	>18 (*E* & *A*)	>2880 B	+	>18	>2850 B	–	>18	0 A	–	<1.6
I	Living room	July	31	>18 (*Eur.h*)	1630 B	+	11.2	>2960 B	–	>18	0 A	–	<1.6
K	Living room	July	30	>18 (*Eur.h*)	1826 B	+	12.6	>2820 B	–	>18	0 A	–	<1.6
M	Bedroom-2	July	30	>18 (*Eur.h*)	1717 B	+	12.0	>2820 B	–	>18	0 A	–	<1.6
O	Bedroom	July	30	>18 (*Eur.h*)	2100 B	+	14.0	>2900 B	–	>18	0 A	–	<1.6
O	Bedroom	September	29	>19 (*Eur.h*)	1500 B	+	11.2	>2920 B	–	>19	0 A	–	<1.7
L	Bedroom	October	31	>18 (*Eur.h*)	1623 B	+	11.2	>2930 B	–	>18	0 A	–	<1.6
H	Living room	September	30	16.7 (*Eur.h*)	1703 B	+	11.9	2627 B	–	16.7	0 A	–	<1.6
L	Living room	July	30	15.5 (*Eur.h*)	1100 B	–	8.8	2400 B	–	15.5	0 A	–	<1.6
I	Bedroom	July	31	14.1 (*Eur.h*)	983 B	–	7.9	2217	–	14.1	0 A	–	<1.6
O	Living room	July	30	13.7 (*Eur.h*)	877 B	–	7.6	2050 B	–	13.7	0 A	–	<1.6
H	Bedroom	September	30	13.2 (*Eur.h*)	1150 B	+	9.1	1953 B	–	13.2	0 A	–	<1.6
L	Bedroom	July	30	12.6 (*Eur.h*)	983 B	–	8.2	1833 B	–	12.6	0 A	–	<1.6
M	Bedroom-1	September	30	11.7 (*Eur.h*)	1247 B	–	9.6	1660 B	–	11.7	0 A	–	<1.6
J	Bedroom	July	28	10.2 (*Eur.h*)	620 B	–	6.7	1227 B	–	10.2	0 A	–	<1.8
M	Bedroom-2	September	30	10.2 (*Eur.h*)	777 B	–	7.0	1377 B	–	10.2	0 A	–	<1.6
L	Bedroom	September	29	9.8 (*Eur.h*)	413 B	–	5.3	1227 B	–	9.8	0 A	–	<1.7
N	Bedroom	November	30	9.4 (*Eur.h*)	457 B	–	5.4	1220 B	–	9.4	0 A	–	<1.6
M	Bedroom-1	August	31	9.3 (*Eur.h*)	597 B	–	5.9	1253 B	–	9.3	0 A	–	<1.6
H	Living room	October	31	6.1 (*Eur.h*)	323 B	–	4.5	617 B	–	6.1	0 A	–	<1.6
N	Bedroom	July	31	6.1 (*Eur.h*)	610 B	–	6.0	630 B	–	6.1	0 A	–	<1.6
O	Bedroom	August	30	5.0 (*Asp.p*)	380 B	–	5.0	257 B	–	4.3	0 A	–	<1.6
H	Living room	November	29	4.6 (*Eur.h*)	75 A	–	2.6	277 B	–	4.6	0 A	–	<1.7
I	Living room	September	30	4.4 (*Eur.h*)	240 B	–	4.2	273 B	–	4.4	0 A	–	<1.6
I	Living room	August	30	4.3 (*Eur.h*)	247 B	–	4.3	250 B	–	4.3	0 A	–	<1.6
H	Bedroom	October	31	3.7 (*Eur.h*)	147 B	–	3.6	170 B	–	3.4	0 A	–	<1.6
M	Bedroom-2	August	31	3.5 (*Asp.p*)	130 B	–	3.5	177 A	–	3.3	0 A	–	<1.6
J	Bedroom	September	30	3.2 (*Eur.h*)	105 A	–	2.8	147 A	–	3.2	0 A	–	<1.6
O	Living room	August	30	3.0 (*Asp.p*)	132 A	–	3.0	0 A	–	<1.6	0 A	–	<1.6
J	Living room	July	28	2.7 (*Asp.p*)	73 A	–	2.7	42 A	–	2.3	0 A	–	<1.8
L	Bedroom	August	31	2.6 (*Asp.p*)	93 A	–	2.6	75 A	–	2.4	0 A	–	<1.6
N	Living room	July	31	2.6 (*Asp.p*)	98 A	–	2.6	0 A	–	<1.6	0 A	–	<1.6
O	Bedroom	October	33	2.4 (*Eur.h*)	52 A	–	2.0	93 A	–	2.4	0 A	–	<1.5
I	Bedroom	September	30	2.3 (*Asp.p*)	57 A	–	2.3	48 A	–	2.2	0 A	–	<1.6
I	Bedroom	August	30	1.8 (*Asp.p*)	20 A	–	1.8	10 A	–	1.7	0 A	–	<1.6
J	Bedroom	October	31	1.8 (*Asp.p*)	18 A	–	1.8	7 A	–	1.6	0 A	–	<1.6
L	Living room	October	31	1.6 (*Asp.p*)	10 A	–	1.6	0 A	–	<1.6	0 A	–	<1.6

[a]Fungal index under the experimental conditions. The sensor fungus used to determine the index, which showed the greatest response, is shown in parentheses. *Eur.h* and *Asp.p* indicate *Eurotium herbariorum* J-183 and *Aspergillus penicillioides* K-712, respectively. '*E* & *A*' indicates that responses of both *Eurotium herbariorum* J-183 and *Aspergillus penicillioides* K-712 exceeded the measurable limit.

[b]'A' indicates the length of hyphae between the spore and hyphal tip. The hyphae were short and less developed. 'B' indicates the length of hyphae between the edge of the inoculated spot and the hyphal tip. The hyphae developed from the spot into the region without nutrients by more than 100 μm.

[c]Sporulation at the sensor fungus. + and – indicate with and without spore formation, respectively.

[d]Fungal index determined with each sensor fungus.

development of ventilation systems for bathrooms. Similar results were obtained in their experiments. The results obtained using 10 fungal detectors placed on the ceiling of a bathroom with no ventilation would help us to understand a fungal index value obtained using one detector at one test site. In the bathroom, the average fungal index was 62.1 (*Alt.a*) ru/week, and the standard deviation was 7.2 ru/week, that is, index readings did not fluctuate much, ca. 12%. With incomplete drying with DS-10BP (6 h per day), the standard deviation was higher, ca. 32%, suggesting uneven drying on the ceiling.

The sensor fungus that showed the greatest response expressed not only quantitative but also qualitative

Table 5 Fungal indices on north walls examined monthly, where fungal indices were below the measurable limit

Experimental conditions					Sensor fungi in each fungal detector								
					Aspergillus penicillioides K-712			*Eurotium herbariorum* J-183			*Alternaria alternata* S-78		
Home	Room	Season	Exposure period (day)	Fungal index in each fungal detector (ru/week)[a]	Hyphal length (μm)[b]	Sp[c]	Asp.p index (ru/week)[d]	Hyphal length (μm)[b]	Sp[c]	Eur.h index (ru/week)[d]	Hyphal length (μm)[b]	Sp[c]	Alt.a index (ru/week)[d]
J	Living room	August	31	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
J	Bedroom	August	31	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
K	Living room	August	30	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
K	Bedroom	August	30	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
L	Living room	August	31	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
N	Living room	August	31	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
N	Bedroom	August	31	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
J	Living room	September	30	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
K	Living room	September	29	<1.6	0 A	–	<1.7	0 A	–	<1.7	0 A	–	<1.7
K	Bedroom	September	29	<1.7	0 A	–	<1.7	0 A	–	<1.7	0 A	–	<1.7
L	Living room	September	29	<1.7	0 A	–	<1.7	0 A	–	<1.7	0 A	–	<1.7
N	Living room	September	30	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
N	Bedroom	September	30	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
O	Living room	September	29	<1.7	0 A	–	<1.7	0 A	–	<1.7	0 A	–	<1.7
I	Living room	October	30	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
I	Bedroom	October	30	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
J	Living room	October	31	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
K	Living room	October	31	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
K	Bedroom	October	31	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
M	Bedroom-1	October	31	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
M	Bedroom-2	October	31	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
N	Living room	October	31	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
N	Bedroom	October	31	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
O	Living room	October	33	<1.5	0 A	–	<1.5	0 A	–	<1.5	0 A	–	<1.5
H	Bedroom	November	29	<1.7	0 A	–	<1.7	0 A	–	<1.7	0 A	–	<1.7
I	Living room	November	30	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
I	Bedroom	November	30	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
J	Living room	November	30	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
J	Bedroom	November	30	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
K	Living room	November	30	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
K	Bedroom	November	30	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
L	Living room	November	25	<2.0	0 A	–	<2.0	0 A	–	<2.0	0 A	–	<2.0
M	Bedroom-1	November	30	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
M	Bedroom-2	November	30	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
N	Living room	November	30	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
O	Living room	November	31	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6
O	Bedroom	November	31	<1.6	0 A	–	<1.6	0 A	–	<1.6	0 A	–	<1.6

[a]Fungal index under the experimental conditions.
[b]'A' indicates the length of hyphae between the spore and hyphal tip.
[c]Sporulation at the sensor fungus. + and – indicate with and without spore formation, respectively.
[d]Fungal index determined with each sensor fungus.

differences in the test environment. The highest responding fungus must be an indicator of the type of fungi contaminating that environment. The hydrophilic fungus responded better in wet areas (Tables 3 and 6), while the xerophilic fungi did so in dry areas (Tables 4 and 5). The former and latter areas clearly maintained environments suitable for the growth of hydrophilic and xerophilic fungi, respectively, suggesting that wet and dry areas in homes are contaminated with hydrophilic and xerophilic fungi, respectively.

The hydrophilic sensor *Alt. alternata* was essential for the evaluation of water-usage areas, because the xerophilic fungi did not respond well to extremely humid environments (Tables 3 and 6). Fungal detectors encapsulating *Alt. alternata* have been used in the development of air-conditioners to avoid fungal contamination inside the equipment (Sato and Abe, 2004). The inside of air-conditioners in summer is extremely humid and easily contaminated by fungi. These environments could be evaluated with *Alt. alternata*.

Although data from eight homes from July to November were not sufficient to discuss seasonal change in homes, it was clear that many rooms showed high fungal index values in July and low values in November (Table 4). However, in one bedroom in Home L, located on the north side of an apartment

Table 6 Fungal indices on the ceiling in a bathroom

Experimental conditions		Fungal index (ru/week)[a]		Sensor fungi in each fungal detector					
				Alternaria alternata S-78			*Eurotium herbariorum* J-183		
Model number of ventilating equipments	Ventilation period per day	Average	Each value	Hyphal length (μm)[b]	R-unit (ru)	Alt.a index (ru/week)[c]	Hyphal length (μm)[b]	R-unit (ru)	Eur.h index (ru/week)[c]
No ventilation	0 h	62.1 (7.2) (*Alt.a*)	53.6 (*Alt.a*)	783 B	30.6	53.6	60 A	10.0	17.5
			59.5 (*Alt.a*)	933 B	34.0	59.5	45 A	9.2	16.0
			55.1 (*Alt.a*)	823 B	31.5	55.1	53 A	9.7	17.0
			65.6 (*Alt.a*)	1090 B	37.5	65.6	28 A	8.4	14.7
			63.5 (*Alt.a*)	1037 B	36.3	63.5	22 A	8.0	14.0
			66.3 (*Alt.a*)	1107 B	37.9	66.3	50 A	9.5	16.5
			75.6 (*Alt.a*)	1343 B	43.2	75.6	70 A	10.4	18.2
			61.3 (*Alt.a*)	977 B	35.0	61.3	50 A	9.5	16.6
			67.6 (*Alt.a*)	1137 B	38.6	67.6	53 A	9.7	17.0
			52.9 (*Alt.a*)	767 B	30.2	52.9	26 A	8.3	14.5
DS-10BP	6 h	21.5 (6.8) (*Alt.a*)	25.4 (*Alt.a*)	178 A	14.5	25.4	0 A	<7.0	<12.3
			23.3 (*Alt.a*)	140 A	13.3	23.3	0 A	<7.0	<12.3
			26.4 (*Alt.a*)	202 A	15.1	26.4	0 A	<7.0	<12.3
			14.5 (*Alt.a*)	27 A	8.3	14.5	0 A	<7.0	<12.3
			16.8 (*Alt.a*)	52 A	9.6	16.8	0 A	<7.0	<12.3
			28.7 (*Alt.a*)	252 A	16.4	28.7	0 A	<7.0	<12.3
			17.2 (*Alt.a*)	57 A	9.8	17.2	0 A	<7.0	<12.3
			16.6 (*Alt.a*)	50 A	9.5	16.6	0 A	<7.0	<12.3
			33.4 (*Alt.a*)	372 A	19.1	33.4	0 A	<7.0	<12.3
			13.1 (*Alt.a*)	12 A	7.5	13.1	0 A	<7.0	<12.3
DS-10BS3	6 h	<14.5 (*Alt.a*)	<12.3	0 A	<7.0	<12.3	0 A	<7.0	<12.3
			<12.3	0 A	<7.0	<12.3	0 A	<7.0	<12.3
			13.3 (*Alt.a*)	13 A	7.6	13.3	0 A	<7.0	<12.3
			14.0 (*Alt.a*)	22 A	8.0	14.0	0 A	<7.0	<12.3
			<12.3	0 A	<7.0	<12.3	0 A	<7.0	<12.3
			<12.3	0 A	<7.0	<12.3	0 A	<7.0	<12.3
			13.3 (*Alt.a*)	15 A	7.6	13.3	0 A	<7.0	<12.3
			17.3 (*Alt.a*)	58 A	9.9	17.3	0 A	<7.0	<12.3
			<12.3	0 A	<7.0	<12.3	0 A	<7.0	<12.3
			<12.3	0 A	<7.0	<12.3	0 A	<7.0	<12.3
DS15BW	24 h	<12.3	<12.3	0 A	<7.0	<12.3	0 A	<7.0	<12.3
			<12.3	0 A	<7.0	<12.3	0 A	<7.0	<12.3
			<12.3	0 A	<7.0	<12.3	0 A	<7.0	<12.3
			<12.3	0 A	<7.0	<12.3	0 A	<7.0	<12.3
			<12.3	0 A	<7.0	<12.3	0 A	<7.0	<12.3
			<12.3	0 A	<7.0	<12.3	0 A	<7.0	<12.3
			<12.3	0 A	<7.0	<12.3	0 A	<7.0	<12.3
			<12.3	0 A	<7.0	<12.3	0 A	<7.0	<12.3
			<12.3	0 A	<7.0	<12.3	0 A	<7.0	<12.3
			<12.3	0 A	<7.0	<12.3	0 A	<7.0	<12.3

[a]Fungal index under the experimental conditions during a 4-day exposure. The values in parentheses are s.d. The fungus used to determine the index, which showed the greater response of the two sensor fungi, is shown in parentheses. *Alt.a* and *Eur.h* indicate *Alternaria alternata* S-78 and *Eurotium herbariorum* J-183, respectively.

[b]'A' indicates the length of hyphae between the spore and hyphal tip. The hyphae were short and less developed. 'B' indicates the length of hyphae between the edge of the inoculated spot and the hyphal tip. The hyphae developed from the spot into the region without nutrients by more than 100 μm.

[c]Fungal index determined with each sensor fungus.

block, the highest fungal index reading was obtained in November, indicating that lowered temperatures outdoors affected the microclimate at the test site.

A fungal index below 1.6, with no germination (corresponding to <7.0 ru) after exposure for 30 days, suggested no fungal contamination during that particular season, but not necessarily other seasons (Table 5). Microenvironments differ from house-to-house, site-to-site, and season-to-season. To analyze microenvironments in homes, much more testing will be needed. Examinations of fungal indices throughout the year, or at least in summer and winter, might be necessary for a precise evaluation. Rooms where fungal indices rise in winter cannot be detected in a survey lasting from July to November, as dew condensation or high RH on north walls facing outdoors occurs in cold weather, mainly from January to February in Japan.

Cleaning affected the actual visible growth of mold on surfaces in homes. One bathroom with a fungal index of 11.3 (*Alt.a*) ru/week (Table 3) showed fungal

contamination, but was not cleaned until the growth become visible. In another bathroom with a fungal index of 21.5 (*Aspergillus niger*) ru/week (*Asp. niger* NBRC 6342 was used instead of *Alt. alternata* in this examination), no fungal contamination was observed in the bathroom, although the room was cleaned after every bath. Cleaning might be a determining factor at sites with higher fungal indices.

In fungal detectors prepared without nutrients, *Alt. alternata* showed no growth at 25°C and 93.6% RH (the fungus in fungal detectors prepared with nutrients showed growth, Table 1). If detectors with no nutrients were placed in a plate of agar medium without touching the medium (not placed directly on the agar, that is, the agar plate was used as a moisture chamber in which the agar medium is a humidity-control agent), the spores germinated and grew, although the hyphae were shorter than those in fungal detectors prepared with nutrients and placed under the same conditions. This suggests that fungi can grow using volatile organic compounds in the air. Thus, cleaning at sites with high fungal indices would be essential even if few nutrients are present on the surface.

In the drying of water-damaged buildings, fungal detectors would again be helpful. One side of a fungal detector is air-permeable (an air-permeable sheet) and the other is not (a plastic plate). When the air-permeable side was placed on construction material, responses of the sensor fungi depended on the moisture content of the material. In a preliminary examination, a dried wood block was placed in a moisture chamber with 93.6% RH at 25°C. Fungal indices on the surface (the air-permeable side faced forward the block) were below the detectable limit in the first survey, increased gradually, and were the same as the chamber after 49 days. The drying of moist wood in a dry chamber might also be evaluated using fungal detectors.

The fungal index shows potential microbial contamination. There must be a fungal index level conducive to contamination in homes and reflecting damp conditions. In fungal detectors placed in rooms with a fungal index above 18 (*Eur.h*) ru/week, spore formation was observed for all *Asp. penicillioides* (Table 4). The observation of new spores indicates that the life cycle of the sensor fungus at these sites progressed to completion (spore germinated, extended hyphae, and new spores on the hyphae). Xerophilic fungi already present or settled around the tested sites should also complete their life cycle, because there are many organic compounds, which are nutrients of fungi, and because in our environment, many kinds of fungal spores including xerophilic fungi are always floating in the air and settling everywhere. If a microclimate suitable for the growth of fungi is reestablished every month or every year, the new spores will germinate, complete their life cycle, and scatter new spores repeatedly, thus perpetuating the contamination.

Fungal indices in living rooms and children's bedrooms were estimated in July (the rainy season in Japan), at 100 homes (62 having children with allergies and 38 controls), (Hamada et al., 2011). As a part of the research, the presence or absence of visible fungal contamination in living rooms and bedrooms was surveyed through the use of a questionnaire. In the rooms with fungal indices above 18, 5.0–18, and 2.5–5.0 (*Eur.h*) ru/week, fungal contamination was visible in 60% (12 of 20 rooms), 48% (31 of 64 rooms), and 18% (six of 33 rooms), and so seemed to occur in rooms with higher fungal indices. In addition, all the homes with readings above 18 (*Eur.h*) ru/week in the living room had children with allergies (unpublished data, Hamada et al.). These results also suggest that sites at which the index exceeded 18 ru/week could be referred to as damp, where fungal contamination would occur and child allergies seem to be unavoidable.

The effectiveness of methods to avoid fungal contamination could be evaluated using fungal detectors and fungal indices. Evaluations of indoor climate with fungal index readings like in Table 6 would provide information useful to occupants, building owners, builders, designers, building advisers, medical practitioners, and so on. The most suitable insulation systems in buildings in various climates, or air-conditioning systems, including cooling, dehumidifying, heating, and ventilation, could potentially be selected using fungal detectors and measurements of fungal indices.

Dampness in buildings is associated with symptoms of allergies and inflammation of the airway, wheezing, rhinitis (Jaakkola et al., 2010), and asthma and a risk factor for the occurrence of Sick Building Syndrome (Sahlberg et al., 2010; Saijo et al., 2009). Remediation aimed at sources of moisture has reduced asthma-related morbidity (Kercsmar et al., 2006). The fungal index can be used as an indicator of dampness and provides a good way to evaluate remediation. In addition, the sensor organism in the detector could potentially be replaced with the house-dust mite (Abe and Kusuki, 2004), pathogenic fungi like *Aspergillus fumigatus*, or wood-decaying fungi for the assessment of health risk and dampness in homes.

Conclusions

- A fungal index that assesses climates in homes quantitatively was established using three sensor fungi differing in sensitivity to RH, the hydrophilic *Alt. alternata*, moderately xerophilic *Eur. herbariorum*, and extremely xerophilic *Asp. penicillioides*.
- The sensor fungus that showed the greatest response expressed not only quantitative but also qualitative differences in the environment tested. The highest responding fungus suggests the type of fungi contaminating the test environment.

- The effectiveness of methods to avoid fungal contamination could be evaluated by using fungal detectors and measurement of fungal indices.
- Fungal detectors and fungal indices would be a good tool for detecting damp conditions that cause fungal contamination with a high risk to human health.
- Conditions with fungal indices exceeding 18 (*Eur.h*) ru/week were considered damp and conducive to contamination with xerophilic fungi, where life cycles of the fungi would proceed repeatedly.

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