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A prevention strategy against fungal attack for the conservation of cultural assets using a fungal index

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ABSTRACT

The present study aimed to establish a prevention strategy to protect cultural assets from fungal attack. A fungal index that assesses conditions critical for fungal growth was determined using a fungal detector in the storerooms of historical buildings in Higashiomi area, Japan. The index measurements were repeated after 4 weeks' exposure of the detectors during the seasons when relative humidity outdoors and/or indoors was high. The index values obtained were from below the measurable lower limit to above the upper limit. The prevention strategy proposed was as follows. Each microclimate was categorized into three levels, A, B, or C, depending on the index values, <1.8, 1.8–18 or >18, respectively. If all microclimates in a room maintain level A continuously, the room is considered free of contamination. If some microclimates maintain level B, fungal contamination might occur. If microclimates maintain level C, fungal contamination is unavoidable, and countermeasures should be taken promptly. Finally, fungal indices are measured for evaluation of the countermeasures and for level-A confirmation. The systematic use of fungal indices will provide practically useful information for conservation and must be applicable to IPM (Integrated Pest Management) in museums and libraries.

1. Introduction

The conservation of cultural assets in the storerooms of historical buildings is not an easy task to accomplish on a limited budget and without specialized conservators, especially in local districts. Growth of fungi causes the deterioration of cultural assets. Mycelia penetrate the spaces between fibers of paper or cloth and weaken their structure. Namely, fungi use them as substrates and destroy them using enzymes (i.e. proteolytic, cellulolytic activities, etc.). Moreover, fungal growth discolors materials: Spores usually have their own colors, and mycelia produce pigments. Foxing (Meynell and Newsam, 1978; Arai, 2000), the formation of brown spots on surfaces colonized by xerophilic fungi, occurs even at the growth stage when spores or pigments are not visible to the naked eye. Fungal contamination of artifacts has detrimental effects on both the cultural and historical legacy.

A fungal index together with a fungal detector, which quantifies the potential for fungal growth in the microclimate at an examination point, has been established by one of the present authors (Abe, 1993a). For measuring the index, a fungal detector encapsulating the spores of sensor fungi is exposed at each survey point. The spores show a growth response in a given microclimate if the microclimate has enough potential for fungal growth. The index is assessed based on the growth response of the sensor fungi in a given exposure period.

Dependence of the index on microclimatic factors, temperature and relative humidity (RH) was mentioned previously (Abe, 1993a). The index increased approximately twofold with a 5 °C elevation of temperature in the range between 10 and 20 °C, and approximately twofold with 5% elevation of the RH in the range between 80 and 90%. The fungal index varied depending on the temperature and RH.

We know that spores of fungi are always floating in the air, infiltrating storerooms and attaching to items stored in the rooms. If there are microclimates in a storeroom that have enough potential for fungal growth, fungal spores will germinate, extend hyphae, produce new spores, and finally scatter the spores, resulting in inescapable fungal contamination. Under such conditions, not only contamination of items stored in the room, but also negative effects on health, such as fungi-induced allergy, might develop in people who enter the storeroom. It was also noted that children in all homes with fungal index >18 in their living rooms (10 out of 100 investigated homes) in summer were found to have allergies (Abe, 2012). To avoid fungal contamination of items and

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negative effects on health, we need to detect the microclimates that facilitate the growth of fungi and take suitable countermeasures before detrimental damage occurs. By using the fungal index, it is possible to detect such conditions.

Here it should be noted that, in this report, the aerial environment around a survey point during exposure of a fungal detector is referred to as a microclimate and that the group of microclimates in a given room is referred to as the indoor environment.

In this study, the fungal index was introduced for systematic monitoring of microclimates in selected rooms where artifacts were stored. The primary aim was to establish a prevention strategy against fungal attack for the conservation of cultural assets, providing a method to detect points with high potential for fungal growth using fungal detectors and to take countermeasures before fungal contamination occurs. Also, the application of the fungal index to IPM, namely Integrated Pest Management (Strang and Kigawa, 2009; Winsor et al., 2011) was mentioned.

2. Methods

2.1. Investigation rooms and the survey points

The storerooms of several historical shrines and temples in the district of Higashiomi, and Storeroom I and II of the Archaeological Center of Higasiomi City, Japan, were selected as the rooms for this investigation. Buddha statues, silk books, traditional paper books etc. were placed in the storerooms of the temples, and Noh costumes, hanging scrolls, mikoshi (portable shrines) and items for local festivals were placed in the storerooms of the shrines, some of which have been designated as national treasures or important cultural assets of Japan. In storeroom I of the Archaeological Center, unearthed articles, clay vessels, iron swords, etc. are stored, and storeroom II contains documents such as photos, books, and maps. Higashiomi is an area known for having historical temples and shrines. The area is located on the east side of Lake Biwa, which is the largest and most well-known lake in Japan. On the east side of Higashiomi are the Suzuka Mountains. Therefore, the area is full of greenery, and moisture could easily infiltrate the buildings from the surroundings.

The number of survey points in each room was three to ten. The survey points included (1) the lower parts of the room corners, which are the moistest in a room in general, (2) the middle of the room, which is generally relatively dry, and (3) other points where important items were placed in the room.

2.2. Survey period and seasons

Focusing on fungal growth, the investigations were conducted from June to October 2011 and 2012. The periods included the rainy season, summer, and autumn. The temperature and humidity in Japan are relatively high during these seasons, and therefore fungi are liable to grow at a higher rate. Fungal index measurements were repeated four times during the following periods: June 22 to July 20, July 20 to August 17, August 17 to September 14, and September 14 to October 12.

2.3. Fungal index

The fungal index was determined biologically using the fungal detector illustrated in Fig. 1, which was mentioned in the previous paper (Abe, 2012). The detector comprises a device encapsulating spores of sensor fungi. In the survey more than 10 years ago, spore-containing spots were sandwiched between a gas-permeable cover film and a support film in a fungal detector (Abe et al., 1996). The design of the detector was changed to completely sealed type,

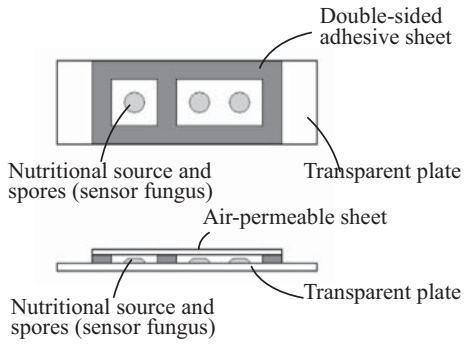

Fig. 1. A fungal detector. The upper and lower figures show the frontal and cross-sectional view of the detector, respectively, encapsulating three sensor fungi. (from Abe, 2012).

because there were incidents in which hyphae were eaten by mites, such as *Tyrophagus* spp., invading the detectors of the previous sandwiched type. After changing the design of the detector, that is, the cover and support films were sealed using a frame of a double-sided adhesive sheet, mites could no longer eat hyphae. The change of the design did not affect the fungi in the detector.

The measuring procedure of the index was described in detail previously (Abe, 2010). The procedure employed in this investigation was as follows: (1) A fungal detector was exposed for 4 weeks at each survey point; (2) after exposure, the detector was placed in a container with silica gel and the development of hyphae was terminated by desiccation; (3) the length of hyphae in each sensor fungus was measured under a microscope; (4) the number of response units, ru (Abe, 2012), was determined from the length of hyphae in each sensor fungus; and (5) the fungal index was calculated using the greatest growth response among the sensor fungi in the detector. The value of the index was defined as the growth response (ru) per exposure period (week).

Fig. 2 shows examples of the responses of a sensor fungus *Eurotium herbariorum* J-183; A is "below the measurable lower limit" with no germination, B is when hyphal length is ca. 500 μm, and C is "above the measurable upper limit" with hyphal length >2600 μm.

The responses shown in Fig. 2-A, -B, and -C, which are visible as hyphal lengths, correspond to the growth responses of <7, 24, and >72 ru, respectively, which are expressed as response units. When the exposure period of fungal detectors was 4 weeks, the values of the fungal index (response units divided by exposure weeks, 4) were calculated to be <1.8, 6.0, and >18.0, respectively. If the exposure periods were different, the values of the fungal index would differ. For example, if the exposure period was 8 weeks, the values of the index would be <0.9, 3.0, and >9.0, respectively.

Three fungi differing in sensitivity to RH were chosen as the sensors for measuring the index, which were described in the previous paper (Abe, 2012). The sensor fungi were moderately xerophilic *Eurotium herbariorum* J-183, strongly xerophilic *Aspergillus penicillioides* K-712, and hydrophilic *Alternaria alternata* S-78. The fungus *E. herbariorum* J-183 was the standard sensor fungus screened from candidates isolated from more than 10,000 colonies formed on agar plates for isolation of xerophilic fungi floating in the air. The fungal strain showed the greatest growth response in various test climates (Abe, 1993b), but its sensitivity was low at higher RH of >96% and lower RH of <72%. To compensate for the low sensitivity at higher and lower RH, hydrophilic *A. alternata* S-78 and strongly xerophilic *A. penicillioides* K-712 were added, respectively, to the fungal detector.

The strains *E. herbariorum* J-183 and *A. alternata* S-78 were deposited in the National Institute of Technology and Evaluation

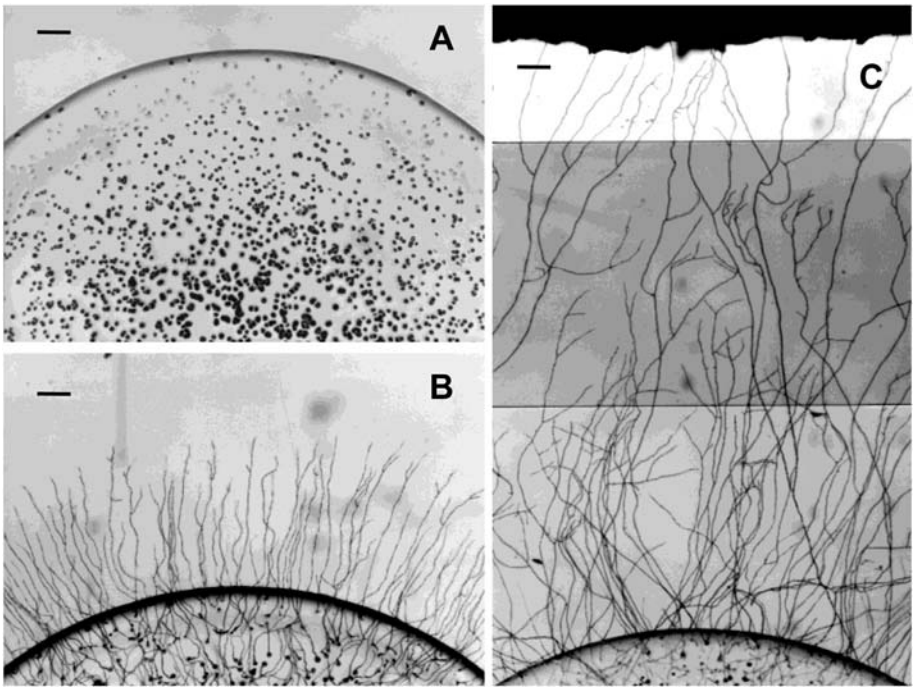

Fig. 2. Typical growth responses to the environment. A shows no spore germination, hyphal length below the measurable lower limit, which corresponds to <7 ru. B shows hyphae that developed from the spot edge outward into the region without nutrients, which corresponds to 24 ru. C shows extremely extended hyphae that exceed the measurable limit, reaching the edge of the double-sided adhesive sheet of the detector, which corresponds to >72 ru. Fungal indices estimated from A, B, and C after an exposure period of 4 weeks were <1.8, 6.0, and >18, respectively. Bar: 100 μm (Figs. 2B and C from Abe, 2012).

Biological Research Center, with the access codes NBRC 107902 and NBRC 107930, respectively. The strain *A. penicillioides* K-712 was derived from a type culture of *A. penicillioides* NBRC 8155 and cultured continuously in our laboratory.

3. Results

3.1. Current status assessment

Table 1 shows typical results of the first investigation in 2011. The fungal index values differed from point to point, and from season to season at the same point. There was marked variation in the indoor environments of the rooms investigated, from all of the fungal index values in all seasons in the room being below the measurable lower limit to all being above the upper limit.

In the storeroom of Hachiman shrine, all index values at all points in all seasons surveyed were below the measurable lower limit (<1.8); no spores of sensor fungi germinated in any detectors. We concluded that this storeroom was free of fungal contamination. However, in the front room, fungal indices were relatively high.

Also, in Storeroom I of the Archaeological Center of Higashomi City, all index values were <1.8 at all points in all seasons surveyed. The fungal index measurements indicated that fungi could not grow in this storeroom.

In contrast, all index values were extremely high in the storeroom of Enmeiji temple. The indices exceeded the measurable upper limit at all points throughout the seasons surveyed. The hyphae of the xerophilic *A. penicillioides* K-712 and (or) *E. herbariorum* J-183 reached the surrounding frames, and new spores were formed, although hydrophilic *A. alternata* S-78 showed no response. The storeroom apparently maintained an indoor environment that facilitates the growth of xerophilic fungi. In the front room, fungal indices also exceeded the upper limit. In this room, the hydrophilic *A. alternata* S-78 responded in addition to the xerophilic sensors, indicating that not only xerophilic but also hydrophilic fungi would grow. Fungal contamination that would eventually scatter spores occurred in these rooms.

At Oshitate shrine, the fungal indices were distributed in rather a low range. The highest index of 4.9 was found in the north corner in August to September. Also, relatively higher values were found in the same season at other points. Some sites might allow fungal growth and consequent contamination of this storeroom.

In Storeroom II of the Archaeological Center of Higashomi City, fungal indices were relatively high in 2011 (Table 2). Values >15 were obtained in the north corner during June to July and July to August. The highest fungal index was observed at every survey point in the storeroom in July to August.

3.2. Countermeasures

In Storeroom II of the Archaeological Center of Higashomi City, dehumidification was adopted in 2012 as a countermeasure. After dehumidifiers were installed, fungal index values fell to <1.8 (Table 2).

In the storeroom of Hachiman shrine, in which all index values were below the measurable lower limit (Table 1), a dehumidifier was already installed and operated continuously during the seasons surveyed. Also, in Storeroom I of the Archaeological Center where all index values were below the measurable lower limit (Table 1), the room had air-conditioning set at 23 °C and 50% RH. Humidity control by dehumidification or air-conditioning was effective as a countermeasure for the whole room.

In 2012, the fungal indices in the storeroom of Enmeiji temple were extremely high, just as in the first investigation in 2011. However, most of the cultural assets had been moved to the air-conditioned room before the investigation in 2012. The movement of cultural assets from the non-air-conditioned to the air-conditioned room was a practical solution to keep them free from fungal attack.

Table 1
Fungal indices at the first investigation in storerooms in 2011.

Examined room	Survey point	Season	Fungal index	Growth of each sensor fungus[a]		
				A. p	*E. h*	*A. a*
Hachiman shrine	Storeroom					
	North corner	Jun.–Jul.	<1.8	−	−	−
		Jul.–Aug.	<1.8	−	−	−
		Aug.–Sep.	<1.8	−	−	−
		Sep.–Oct.	<1.8	−	−	−
	Center	Jun.–Jul.	<1.8	−	−	−
		Jul.–Aug.	<1.8	−	−	−
		Aug.–Sep.	<1.8	−	−	−
		Sep.–Oct.	<1.8	−	−	−
	Front room	Jun.–Jul.	12.3	++++	++++	−
		Jul.–Aug.	7.0	+++	+++	−
		Aug.–Sep.	14.1	+++	++++	−
		Sep.–Oct.	6.0	++	++	−
Archaeological center	Storeroom I					
	North corner	Jun.–Jul.	<1.8	−	−	−
		Jul.–Aug.	<1.8	−	−	−
		Aug.–Sep.	<1.8	−	−	−
		Sep.–Oct.	<1.8	−	−	−
	South corner	Jun.–Jul.	<1.8	−	−	−
		Jul.–Aug.	<1.8	−	−	−
		Aug.–Sep.	<1.8	−	−	−
		Sep.–Oct.	<1.8	−	−	−
	Center	Jun.–Jul.	<1.8	−	−	−
		Jul.–Aug.	<1.8	−	−	−
		Aug.–Sep.	<1.8	−	−	−
		Sep.–Oct.	<1.8	−	−	−
Enmeiji temple	Storeroom					
	North corner	Jun.–Jul.	>18.0	+++++	+++++	−
		Jul.–Aug.	>18.0	+++++	+++++	−
		Aug.–Sep.	>18.0	+++++	+++++	−
		Sep.–Oct.	>18.0	+++++	+++++	−
	Center	Jun.–Jul.	>18.0	+++++	+++++	−
		Jul.–Aug.	>18.0	+++++	+++++	−
		Aug.–Sep.	>18.0	+++++	+++++	−
		Sep.–Oct.	>18.0	+++++	+++++	−
	Front room	Jun.–Jul.	>18.0	+++++	+++++	++
		Jul.–Aug.	>18.0	+++++	+++++	++
		Aug.–Sep.	>18.0	+++++	+++++	++
		Sep.–Oct.	>18.0	+++++	+++++	++
Oshitate shrine	Storeroom					
	North corner	Jun.–Jul.	2.6	+	−	−
		Jul.–Aug.	4.0	+	+	−
		Aug.–Sep.	4.9	++	++	−
		Sep.–Oct.	2.0	−	+	−
	South corner	Jun.–Jul.	<1.8	−	−	−
		Jul.–Aug.	2.3	+	−	−
		Aug.–Sep.	4.6	+	++	−
		Sep.–Oct.	<1.8	−	−	−
	Center	Jun.–Jul.	<1.8	−	−	−
		Jul.–Aug.	<1.8	−	−	−
		Aug.–Sep.	2.9	+	+	−
		Sep.–Oct.	<1.8	−	−	−

[a] *A. p*, *E. h*, and *A. a* indicate *Aspergillus penicillioides* K-712, *Eurotium herbariorum* J-183, and *Alternaria alternata* S-78, respectively. +++++, ++++, +++, ++, and + indicate that the hyphal length of each sensor fungus from each spot edge was >2000 μm, 2000 to 1000 μm, 1000 to 500 μm, 500 to 200 μm, and <200 μm, respectively. − indicates no spore germination.

4. Discussion

4.1. Prevention strategy against fungal attack for conservation

Fig. 3 shows the proposed prevention strategy against fungal attack for the conservation of cultural assets using a fungal index. In the first investigation for current status assessment, fungal indices are measured to evaluate microclimates in the storeroom concerned. The measurements are repeated with 4-week exposure during the seasons when RH outdoors and/or indoors is high. The survey points include the moistest area, a relatively dry area, and other areas where important items are placed in the room. From the results of the measurement, each microclimate is categorized into level A, B or C, depending on the fungal index value, <1.8, 1.8–18, or >18, respectively. Level A is ideal, as no spores germinate during exposure for 4 weeks (cf. Fig. 2A). Level B requires careful attention, as spores germinate and extend hyphae within 4 weeks (cf. Fig. 2B). Level C is the worst since spores germinate, extend long hyphae, and produce new spores within 4 weeks (cf. Fig. 2C).

The purpose of the first investigation is to confirm the present protection of stored items from fungal attack in the storeroom, and to determine whether countermeasures are necessary. If a given storeroom has microclimates categorized into level A at all points throughout the periods surveyed, as in the storeroom of Hachiman shrine or Storeroom I in the Archaeological Center (Table 1), the

Table 2
Fungal indices in Storeroom II at the first investigation in 2011 and after taking preventive measures in 2012 (dehumidifiers were installed in the storeroom).

Year	Survey point	Season	Fungal index	Growth of each sensor fungus[a]		
				A. p	*E. h*	*A. a*
2011	North corner	Jun.–Jul.	15.7	+++++	++++	−
		Jul.–Aug.	16.2	++++	+++++	−
		Aug.–Sep.	7.0	++	+++	−
		Sep.–Oct.	<1.8	−	−	−
	Center	Jun.–Jul.	13.2	++++	++++	−
		Jul.–Aug.	13.3	++++	++++	−
		Aug.–Sep.	5.0	++	++	−
		Sep.–Oct.	<1.8	−	−	−
2012	North corner	Jul.–Aug.	<1.8	−	−	−
	Center	Jul.–Aug.	<1.8	−	−	−
	Under a mobile rack	Jul.–Aug.	<1.8	−	−	−
	Container of photos	Jul.–Aug.	<1.8	−	−	−
	Container of maps	Jul.–Aug.	<1.8	−	−	−
	South corner	Jul.–Aug.	<1.8	−	−	−
	East corner	Jul.–Aug.	<1.8	−	−	−
	West corner	Jul.–Aug.	<1.8	−	−	−

[a] *A. p*, *E. h*, and *A. a* indicate *Aspergillus penicillioides* K-712, *Eurotium herbariorum* J-183, and *Alternaria alternata* S-78, respectively. +++++, ++++, +++, ++, and + indicate that the hyphal length of each sensor fungus from each spot edge was >2000 μm, 2000 to 1000 μm, 1000 to 500 μm, 500 to 200 μm, and <200 μm, respectively. − indicates no spore germination.

storeroom is considered free of contamination, and additional countermeasures are not necessary. If a given storeroom has microclimates categorized into level B at some points in some periods surveyed, as in the storeroom of Oshitate shrine (Table 1) or Storeroom II of the Archaeological Center (Table 2), suitable countermeasures are necessary because foxing might occur on the items stored in the room. If a given storeroom has microclimates categorized into level C at many points in many seasons surveyed, as in the storeroom of Enmeiji temple (Table 1), countermeasures should be taken for the whole room as soon as possible, because fungal contamination is difficult to avoid and, in addition, negative effects on health might occur in people who enter the room on a daily basis.

At the final investigation for confirmation, fungal indices are measured for evaluation of the countermeasures employed and for level-A confirmation. If index values are still measurable, additional countermeasures and subsequent re-investigation for confirmation are necessary.

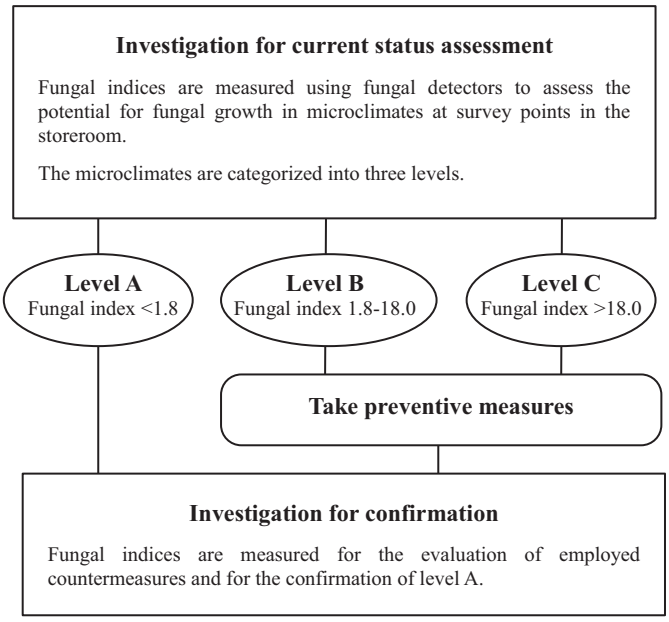

Fig. 3. Prevention strategy against fungal attack for the conservation of cultural assets.

4.2. Countermeasures applicable

The countermeasures could be taken either for the whole room or at selected sites where cultural assets have been stored. Humidity control by dehumidification or air-conditioning targets the whole room, which was evaluated in this investigation.

Storage of cultural assets in airtight containers to avoid the infiltration of moisture is a typical countermeasure that targets a partial space. Bags made of moisture-impermeable film are useful and helpful as containers. Although sealing in a moisture-impermeable bag was not attempted in the storerooms reported here, a preliminary exposure test of sealed bags in a humid climate of 25 °C and 93.6% RH showed that the usage of aluminum-laminated film as bag material was very effective (Abe and Murata, 2013). Usage of conservation bags made of aluminum-laminated film could be recommended for storerooms that have microclimates categorized into level B, such as at Oshitate shrine.

Usage of airtight containers for conservation is common in storerooms. Sealing with oxygen absorbers or humidity control agents in airtight containers and showcases is used by numerous museums. The internal microclimate should be evaluated using the fungal index. Measurement of the index will provide practically useful information to avoid fungal contamination even in such containers and showcases.

4.3. The fungal index as a meaningful indicator

The fungal index is the best tool available for monitoring microclimates where fungal contamination can occur. As we know, higher RH facilitates fungal growth, but the average RH of a room is not a direct quantitative indicator of growth. Moreover, 60%RH, which is thought to be safe from fungal contamination, does not ensure safety. For example, foxing occurred on a painting propped against a wall in the northeast area in a storeroom where dehumidifiers were in operation and the RH recorded at the center of the storeroom was around 60%. The measured fungal indices around the contaminated part of the painting were in the order of 7 during the rainy season and summer (Abe, 2010). A hygrometer placed to monitor room climate did not reflect the microclimate around the contaminated parts. Only on-site fungal index measurements could reveal the microclimate where fungi were growing.

Recently, the concept of IPM has been introduced by leading museums, libraries and related institutions (Strang and Kigawa, 2009; Winsor et al., 2011). IPM was originally developed in the field of agriculture, where various organic materials were vulnerable to destruction by pests such as insects, rodents, or mold (fungi). In the past, various chemicals have been applied in museums to eradicate pests, but chemicals used as pesticides have been found to damage collections and cause health problems for the museum's staff and visitors. The strategy of IPM was therefore employed to secure safety by reducing the usage of pesticides. The strategy includes multiple phases, such as monitoring, identification, inspection, cleaning, treatment action, evaluation, education, etc.

The prevention strategy against fungal attack for conservation reported here is applicable to the IPM system. It will work in monitoring the protection from fungal attack, assist in taking preventive actions and evaluating the actions as countermeasures against fungal attack. The fungal index acts as a direct indicator of the conditions of protection at a specific site in museums, libraries, and related institutions. The fungal index is therefore a useful concept and tool for supporting the IPM system by protecting property and staff from fungal attack. Fungal detectors are easy to handle and the fungal index could be a basic element in the IPM system.

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