# Examination of Fungi in Domestic Interiors by Using Factor Analysis: Correlations and Associations with Home Factors

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Factor analysis was utilized to investigate correlations among airborne microorganisms collected with Andersen samplers from homes in Topeka, Kans., during the winter of 1987 to 1988. The factors derived were used to relate microbial concentrations with categorical, questionnaire-derived descriptions of housing conditions. This approach successfully identified groups of common aboveground decay fungi including Cladosporium, Alternaria, Epicoccum, and Aureobasidium spp. The common soil fungi Aspergillus and Penicillium spp. were also separated as a group. These previously known ecological groupings were confirmed with air sampling data by a quantitative evaluation technique. The aboveground decay fungi sampled indoors in winter were present at relatively high concentrations in homes with gas stoves for cooking, suggesting a possible association between these fungi and increased humidity from the combustion process. Elevated concentrations of the soil fungi were significantly  $(P = 0.05)$  associated with the dirt floor, crawl-space type of basement. Elevated concentrations of water-requiring fungi, such as Fusarium spp., were shown to be associated with water collection in domestic interiors. Also, elevated mean concentrations for the group of fungi including Cladosporium, Epicoccum, Aureobasidium, and yeast spp. were found to be associated ( $P = 0.03$ ) with symptoms reported on a health questionnaire. This finding was consistent with our previous study of associations between respiratory health and airborne microorganisms by univariate logistic regression analysis.

Traditionally, data resulting from sampling for airborne microorganisms have been analyzed in a relatively qualitative fashion, usually because of the small number of samples available. Furthermore, the complexity of airborne microbial populations has discouraged a more quantitative approach. Qualitative presentation of data is valuable and has provided substantial information. However, many studies have been inconclusive, and broader implications have not been inferred, because of a lack of quantitative and statistical evaluation (17, 18, 20, 24). From a health perspective, understanding relationships among airborne microorganisms within a population plays a key role in objectively establishing the association between patterns of exposure and respiratory symptoms.

Factor analysis has been used to analyze data on various environmental pollutants (12, 21, 26). However, it has not been applied to the study of potential associations among airborne fungi that can be cultured. The studies reported here represent a preliminary application of these methods to aerosols of fungi in domestic interiors and evaluate the relationships between airborne-fungus populations and questionnaire-derived information on a variety of home factors and health symptoms.

## MATERIALS AND METHODS

A sample of <sup>150</sup> households in Topeka, Kans., was selected from a larger cohort of 350 homes participating in a detailed, year-long indoor-air-quality and respiratory-health study (25). The larger study included questionnaires that evaluated housing characteristics and respiratory symptoms in resident children. Originally, there were about 1,000 grade school children engaged in the respiratory health study 2 years prior to an outdoor- and indoor-air-quality investigation. Home characteristics questionnaires, designed to evaluate the residential environments, were administered by field technicians during air sampling visits (11). Included were questions about the type of cooking fuel and the presence of regular smokers in the family. On the basis of the responses, air-monitoring homes were categorized into four groups as follows: group A, no gas stove and no resident smoker; group B, gas stove and no resident smoker; group C, no gas stove but resident smoker; and group D, gas stove and resident smoker. Because homes with gas stoves and/or resident smokers had substantially greater variations in nonbiological-contaminant concentrations, an unbalanced design was defined with a housing-group sampling ratio of 1:2:2:4 (groups A, B, C, and D, respectively). In accordance with this ratio, 25 phone calls were made every day to invite participation in the air-monitoring study. A total of <sup>350</sup> homes in Topeka was available for the general air-monitoring project, and among these, 150 homes were used for biological sampling. A statistical comparison showed that these 150 homes are representative of the larger population on the basis of the housing factors used in this study.

For the data analyses presented here, self-reported respiratory-health questionnaires for the school-age children (5), distributed at the end of the sampling year, were used. Specific questions were as follows: (i) (hay fever) "Has the child had hay fever during this year?"; (ii) (wheezing condition and/or asthma) "Has the child had wheezing symptoms this year, or does the child have asthma confirmed by a physician?"; (iii) (lower respiratory illness [bronchitis and/or cough and/or chest illness]) "Has this child had bronchitis, persistent cough, or a chest illness that kept him or her at home for <sup>3</sup> days or more?".

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Variable		In $CFU/m^3$ for":											
	<b>ALT</b>	ASP	<b>AUR</b>	<b>BACT</b>	<b>CLAD</b>	<b>CUR</b>	<b>DRE</b>	EPI	<b>FUS</b>	<b>OTH</b>	PEN	PIT	<b>UNK</b>
<b>ASP</b>	0.057												
<b>AUR</b>	0.309	$-0.009$											
<b>BACT</b>	0.173	0.220	0.255										
<b>CLAD</b>	0.411	0.075	0.274	0.073									
<b>CUR</b>	0.100	0.107	0.166	0.181	0.181								
<b>DRE</b>	0.035	$-0.162$	0.007	$-0.131$	0.106	0.160							
EPI	0.288	$-0.027$	0.209	0.203	0.305	0.001	0.202						
<b>FUS</b>	0.151	0.151	0.061	$-0.029$	0.031	0.025	0.061	0.089					
<b>OTH</b>	0.125	0.228	0.108	$-0.097$	0.145	0.105	0.064	0.091	0.146				
<b>PEN</b>	$-0.084$	0.202	$-0.024$	$-0.248$	0.013	$-0.005$	$-0.074$	$-0.004$	$-0.049$	0.180			
<b>PIT</b>	0.224	$-0.008$	0.314	0.212	0.064	0.110	0.078	0.151	0.033	0.004	$-0.042$		
<b>UNK</b>	0.156	0.184	0.195	0.190	0.151	0.081	$-0.079$	0.262	0.098	0.265	$-0.074$	0.169	
<b>YEA</b>	0.283	0.213	0.210	0.246	0.239	0.052	$-0.108$	0.077	0.097	0.106	$-0.005$	$-0.045$	0.217

TABLE 1. Correlation matrix among microbial variables

<sup>a</sup> Boldface type indicates a significant ( $P < 0.05$ ) association between the paired variables.

Available also from the respiratory-health questionnaire, questions regarding the type of cooking fuel, the type of basement (crawl space or other types, such as occupied space), whether water collection was observed in interiors, and the presence of mold or mildew, among other questions, were used to study associations with the airborne-fungus concentrations measured. Discrete responses of "yes" or "no" to these questions on the respiratory-health questionnaire were recorded as <sup>1</sup> or 0 for further statistical analyses.

Indoor-air samples were collected at each home by using duplicate Andersen single-stage impactors operating at 28.4 liters/min for 30 <sup>s</sup> to <sup>1</sup> min (2, 15). The samplers were generally located 2 to 3 ft above the floor, indoors in the living or family room (e.g., on top of the coffee table). All samples were collected on malt extract agar (1). Only winter sampling data were used in this analysis, to maximize the probability that we were dealing with indoor-generated aerosols (24).

Samples were incubated at room temperature for 4 to 7 days under fluorescent or near-UV illumination, following preliminary studies to ensure that no taxa were selectively inhibited by these conditions (5a). All colonies were counted, colony counts were converted for possible multiple impactions by using Andersen's conversion table (2), and CFUs per cubic meter of air were calculated. Fungi were identified to genus level when possible. The classifications recorded included bacteria (BACT), Alternaria spp. (ALT), Aspergillus spp. (ASP), Aureobasidium spp. (AUR), Cladosporium spp. (CLAD), Curvularia spp. (CUR), Drechslera spp. (DRE), Epicoccum spp. (EPI), Fusarium spp. (FUS), Penicillium spp. (PEN), Pithomyces spp. (PIT), yeasts (YEA), unknown fungi (UNK), and other fungi (OTH), which included several less frequently encountered fungi.

Original fungal concentrations (in CFU per cubic meter) were transformed by natural logarithm to approximate normality in the analysis. Statistical analysis utilized the SAS statistical package (22); data entry and management used dBASE  $III^+$  (3). Factor analysis was used to group 14 different microbial genera into different factors on the basis of loading directions for each variable within each factor. Factor scores were calculated from the resulting factor structure. These factor scores were used in tests for association with variables of home characteristics and respiratory symptoms.

### RESULTS

Factor analysis was conducted to select subsets of 14 microbiological variables. In general, factor analysis is a multivariate technique for explaining the relationships among difficult-to-interpret, correlated variables with a few conceptually meaningful, relatively independent factors (16). Specifically, it is assumed that apart from random fluctuation, each variable of an original set of  $p$  variables can be explained by a linear combination of a smaller set of  $k$ mutually uncorrelated factors with mean zero and unit variances. The correlation between a factor and a standardized original variable is called the factor loading for that variable. There are several methods for determining the number of factors and estimating factor loadings. In this paper, we have used the estimation method of principal component solution (PCS) (14). All resulting factor structures have been examined for consistency by comparing the estimated factor loadings with those generated by another method of estimation, maximum-likelihood solution (MLS).

The overall correlation coefficients were small (Table 1). Nonetheless, their statistical significances were confirmed by an overall test (Bartlett's  $\chi^2$  test,  $P \le 0.001$ ) (10) that rejected the hypothesis of no correlation among the microbiological variables. In addition, none of the pairwise correlations appeared to be driven by extreme values, as shown by an analysis of the pairwise correlations obtained by omitting one sample unit at a time (23). By using the correlation matrix, factor analysis initially included all 14 microbial variables. A scree plot of eigenvalues from PCS indicated the presence of a single dominant factor emerging from these data and suggested that six factors suffice to explain most of the variability in the data. Table 2 presents the analysis on the basis of a model with six factors. Of the total variance, 64% was explained by using six factors, in contrast to 56% explained by retaining only five factors.

Also, six factors explained a large proportion of the variance for individual biological variables (e.g., 78% for CUR and 52% for AUR), while five factors explained less than 50% of the variance for a number of variables, such as AUR, PIT, and UNK. Therefore, <sup>a</sup> six-factor structure was regarded as an optimal solution and used for further statistical analysis.

Both PCS and MLS consistently showed that CUR was the single dominant variable in the last factor. Because CUR

Solution	In $CFU/m^3$ for <sup>b</sup> :									
and variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6				
<b>PCS</b>										
<b>CLAD</b>	0.788	$-0.051$	$-0.005$	0.117	$-0.035$	0.170				
<b>ALT</b>	0.706	0.038	0.186	$-0.100$	0.113	0.015				
<b>YEA</b>	0.553	0.511	$-0.157$	$-0.078$	0.136	0.044				
EPI	0.525	$-0.301$	0.312	0.063	0.208	$-0.240$				
<b>AUR</b>	0.433	0.091	0.550	$-0.007$	$-0.069$	0.126				
<b>ASP</b>	$-0.012$	0.570	$-0.023$	0.316	0.362	0.267				
<b>BACT</b>	0.114	0.528	0.353	$-0.439$	$-0.028$	0.309				
<b>DRE</b>	0.125	$-0.711$	$-0.017$	$-0.063$	0.167	0.350				
<b>PIT</b>	$-0.006$	$-0.085$	0.833	$-0.042$	$-0.010$	0.085				
<b>PEN</b>	$-0.024$	0.068	$-0.036$	0.850	$-0.154$	$-0.008$				
<b>OTH</b>	0.126	0.016	0.081	0.532	0.506	0.128				
<b>FUS</b>	0.040	$-0.061$	$-0.079$	$-0.141$	0.777	0.009				
<b>UNK</b>	0.176	0.261	0.447	0.057	0.468	$-0.117$				
<b>CUR</b>	0.098	$-0.040$	0.114	0.030	0.017	0.869				
<b>MLS</b>										
<b>CLAD</b>	0.650	0.024	$-0.021$	$-0.022$	0.111	0.156				
<b>ALT</b>	0.599	$-0.072$	0.066	0.181	0.126	0.026				
EPI	0.432	0.014	$-0.161$	0.186	0.188	$-0.078$				
<b>AUR</b>	0.398	$-0.009$	0.099	0.380	0.061	0.116				
<b>YEA</b>	0.382	$-0.020$	0.413	$-0.078$	0.148	0.027				
<b>PEN</b>	$-0.019$	0.998	0.043	$-0.030$	0.014	$-0.003$				
<b>BACT</b>	0.123	$-0.258$	0.547	0.321	$-0.072$	0.213				
<b>ASP</b>	$-0.013$	0.177	0.447	$-0.032$	0.335	0.138				
<b>DRE</b>	0.115	$-0.052$	$-0.429$	0.042	0.043	0.185				
<b>PIT</b>	0.106	$-0.018$	$-0.048$	0.685	0.035	0.053				
<b>OTH</b>	0.095	0.174	$-0.029$	$-0.033$	0.579	0.089				
<b>UNK</b>	0.173	$-0.079$	0.190	0.215	0.445	0.003				
<b>FUS</b>	0.083	$-0.052$	$-0.002$	0.005	0.288	$-0.006$				
<b>CUR</b>	0.093	0.002	$-0.013$	0.091	0.067	0.729				

TABLE 2. Orthogonal factor patterns determined by principal component factor analysis with all microbial variables<sup>a</sup>

<sup>a</sup> Cumulative proportion of variance explained from the PCS as follows: factor 1. 0.1904; factor 2, 0.3017; factor 3, 0.4064; factor 4, 0.4868; factor 5, 0.5629; factor 6, 0.637.

<sup>b</sup> Boldface type indicates variables that were considered significant and included as components of the factors identified.

occurred in only 5.7% of the samples and because the last factor determined by the analysis is generally unstable and statistically the least interpretable, CUR was eliminated from the data base. On reanalysis without CUR, the composition of the first five factors remained consistent with that of the factors determined by using all fourteen variables. Therefore, the rationale to drop CUR from the analysis appeared to be sufficient.

A subsequent concern was whether to keep BACT in the analysis. BACT was consistently associated with the presence of some fungal species, as shown by data for factor 2 and factor 4 (Table 2). Malt extract agar, however, is not an optimal medium for culturing environmental bacteria, and growth is specific to that medium and not representative of the population. A new factor analysis according to the procedures described above was conducted with the subset of data resulting from the elimination of both BACT and CUR. The dominant variables contained in the factors did not vary with this subset by either the PCS or the MLS method. The resulting factor loadings of individual variables are shown in Table 3. The five factors determined by PCS from this modified data base explained about 61% of the total variance of the data. For individual variables, the least variance explained corresponded to UNK (49%) and the largest variance explained corresponded to PEN (75%).

To confirm the proposed factor structure, a randomnumber selection was utilized to divide the original data base into two subsets ( $n_1 = 75$  and  $n_2 = 74$ ), both of which were

analyzed by the same procedure (14), which included (i) eliminating BACT and CUR or (ii) dropping CUR only. Factor scores resulting from these two subsets were plotted against each other to check the consistency of the resulting structures. This graphical analysis supported the factor structure determined as described above. For all these analyses, unrotated and rotated methods, as well as PCS and MLS, were conducted, and results from these procedures were consistent. In addition, analysis results suggested consistently that orthogonal and oblique rotations did not differ significantly in either the rotated patterns or the loadings of each variable. Therefore, values of factor loading presented in Tables 2 and <sup>3</sup> are from the orthogonal rotation (varimax method) only.

Statistical evaluation of the consistency between PCS and MLS was conducted to reaffirm the factor structure (14). The resulting factor scores for each factor from PCS and MLS were plotted against each other, and approximately 45° lines were observed (slope = 1 at  $P < 0.05$ ), indicating a close agreement between the two different methods. Also, there was reasonable consistency between matching factors with respect to their constituent fungal variables. Therefore, factor scores from PCS were used for further analysis, since PCS maximized the proportion of the variance explained for the collected data.

The two-sided Wilcoxon rank sum test (27) was employed to assess the associations between each home condition recorded in the home characteristics questionnaire data





<sup>a</sup> Cumulative proportion of variance explained from the PCS as follows: factor 1, 0.2072; factor 2, 0.3369, factor 3, 0.4341; factor 4, 0.5230; factor 5, 0.6112. **b Boldface type indicates all variables that were considered significant and included as components of the factors identified by PCS.** 

base, and the corresponding score for each factor was determined from the final structure by the factor analysis. Results are shown in Table 4. Factor <sup>1</sup> was significantly associated ( $P < 0.05$ ) with the use of gas stove cooking; homes with gas cooking stoves presented <sup>a</sup> comparatively high mean factor score. Factor <sup>3</sup> was associated with the type of basement (crawl space versus others); homes with crawl space showed a comparatively high mean factor score  $(P \le 0.05)$ . Factor 2 was negatively associated with reported water damage at the significance level of  $P = 0.02$ .

As shown in Table 5, factor scores were also tested for association with selected respiratory symptoms reported in the self-administered health questionnaires. Both wheezing condition and/or asthma and hay fever were significantly associated with factor 1. No other significant associations were seen.

### DISCUSSION

As shown in Table 3, the dominant variables identified in factor <sup>1</sup> are CLAD, ALT, AUR, EPI, and YEA. Except for YEA, these fungi grow mainly on aboveground dead organic material with temperature optima between 18 and 25°C and are generally considered outdoor fungi (6). They are all adaptable with respect to nutritional requirements and contain melanin in their cell walls, rendering them resistant to UV damage, and many require UV for sporulation (13). The colony incubation and identification procedures used in this study included UV illumination. However, laboratory studies had been conducted previously to ensure that such UV exposure would not result in a selection bias against nonmelanin-containing fungi.

TABLE 4. Wilcoxon rank sum test among factors and housing variables

Housing variable <sup>a</sup>	$P$ value for:							
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5			
Gas stove	0.046	0.803	0.651	0.394	0.881			
Visible mildew	0.519	0.744	0.411	0.925	0.815			
Water collection	0.629	0.965	0.298	0.441	0.090			
Water damage	0.246	0.015	0.443	0.269	0.804			
Basement <sup><i>b</i></sup>	0.854	0.241	0.050	0.251	0.998			
Kerosene heater <sup>c</sup>	0.350	0.633	0.111	0.899	0.524			

<sup>a</sup> All are categorical variables (either yes or no).<br><sup>b</sup> All participants reported having some form of basement. Basements were grouped into either crawl-space type (no) or occupied (yes) basements.

<sup>c</sup>Only <sup>5</sup> homes had kerosene space heaters in this data base of 148. Therefore, the information presented is regarded as less reliable. All other variables had sufficient participants in either category.

Respiratory-healthy variable	P value for:							
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5			
$LRI^a$	0.976	0.722	0.594	0.970	0.450			
Wheezing and/or asthma	0.008	0.982	0.575	0.906	0.313			
Hay fever	0.003	0.608	0.617	0.303	0.242			

TABLE 5. Wilcoxon rank sum test among factors and respiratory-health variables

<sup>a</sup> LRI, lower respiratory illness (a composite variable combining the occurrence of cough and/or bronchitis and/or chest illness).

The major variables (taxa) in factor <sup>2</sup> included PIT, AUR, UNK, EPI, and ALT. PIT has characteristics similar to those of AUR, EPI, and ALT; these characteristics are discussed above. It is of note that PIT occurred at a lower frequency (10.7%) than the other fungi in this group, which all appeared in more than 40% of the sampled homes. However, the correlation between PIT and AUR was among the highest (Table 1). UNK often includes nonsporulating colonies of basidiomycetes (i.e., fungi with clamp connections), Alternaria and Pithomyces spp., and other dark-spore outdoor fungi. Basidiomycetes are almost exclusively outdoor fungi. In general, then, factor 2 contains outdoor fungi, with CLAD (often the dominant fungus indoors and outdoors) missing.

Factor <sup>3</sup> includes PEN, ASP, and OTH (less frequently encountered fungi). Penicillium and Aspergillus spp. are both well-known soil fungi and are commonly considered indoor fungi in aerobiology (19, 24). They are taxonomically related, and neither has cell wall melanin. Both genera contain species that can grow at elevated temperatures (i.e., greater than 32°C). Both genera also include some species that can live in osmotically stressful environments, and some can utilize human skin scales by excreting keratinases. These characteristics are shared by several taxa that are included in OTH (Wallemia, Paecilomyces, Trichoderma, and Scopulariopsis spp.).

DRE and EPI were dominant in factor 4. They are outdoor fungi with characteristics similar to those of the organisms in factor 1. On the other hand, ASP and YEA, which showed negative correlations in factor 4, are the indoor fungi of factor 3. It should be noted that occurrence frequency for DRE was extremely low (only 3.4%).

FUS dominates the last factor, with some contribution from UNK, OTH, and ASP. Fusarium spp. produce wet spores, in contrast to the hydrophobic spores produced by the common dark-spore fungi of factor <sup>1</sup> and factor <sup>2</sup> and to the Penicillium and Aspergillus spp. of factor 3. Fusarium species are often found in standing water, such as in the water of humidifiers and other fluid reservoirs (7). UNK includes fungi related to *Fusarium* spp. that produce slimy spores and are difficult or impossible to identify from culture, such as species of the genus Acremonium. Several fungi in OTH also require excess water for growth (Sporobolomyces spp., in the order Mucorales). The genus Sporobolomyces is a basidiomycetous yeast that forcibly discharges spores into the air. It has been associated with farm-related hypersensitivity pneumonitis (9). The order Mucorales (included in the class Zygomycetes) includes the black bread molds and fungi that cause soft rot of fruits such as strawberries. These fungi utilize relatively simple nutrient sources and probably require more water than the nonzygomycetous fungi. These discussions of biological characteristics have been based on generic identifications. It is possible that, for example, the species of *Aspergillus* that fit in factor 3 are different from those in factor 5.

The mean factor <sup>1</sup> score was higher in homes with gas ranges than in homes with electric ranges ( $P \le 0.05$ ). Factor <sup>1</sup> contains mostly fungi that are usually considered to come from outdoor sources. It is likely that homes that are supplied with natural gas may have both gas stoves and other gas appliances such as furnaces and water heaters. However, the respiratory-health questionnaire from which the data presented here were taken did not address these other home factors. Use of natural gas may be associated with increased penetration of outdoor air due (at least in part) to venting requirements for gas appliances.

Gas combustion also produces water vapor that could increase relative humidity. Associations between indoor fungal growth and relative humidity have been demonstrated in previous research (8). In addition, gas combustion produces potentially toxic products. For example,  $NO<sub>2</sub>$  is produced, and its sorption on surfaces leads to nitrate and nitrite formation and the release of nitrous acid (4). Fungi in factor <sup>1</sup> may be more resistant to combustion products than fungi included in other factors. The effects of chemical pollutants on survival of fungal spores have not been studied.

The mean factor score for factor <sup>3</sup> was higher for homes with crawl spaces than for homes with occupied basements. Factor <sup>3</sup> includes the common soil fungi (Aspergillus and Penicillium spp.). A crawl space usually has <sup>a</sup> dirt floor and is often damp and poorly ventilated. The presence of soil and, possibly, high humidity would predispose these environments to contamination with soil fungi.

The mean factor score for fungi requiring a greater quantity of water for growth (i.e., factor 5) was significantly high  $(P \le 0.09)$  in homes reporting water collection problems. Although they are not conclusive, these results suggest that water reservoirs may be an important questionnaire variable and that further studies specifically directed at wet-spore fungi should be of value.

The Wilcoxon rank-sum test suggested a significant difference in factor 2 ( $P \le 0.02$ ) for mean factor scores between those homes reporting water damage (group A) and those not reporting such damage (group B). However, the mean value of the factor score for group A is lower than it is for group B. Water damage is known to present a risk for fungal contamination (24). The reasons for these apparently inverse correlations are unknown.

Factor scores were also used to study the associations between airborne-fungus concentrations and respiratory symptoms. For homes in which resident children reported hay fever, a comparatively high mean score for factor <sup>1</sup> was observed ( $P \le 0.003$ ) (Table 5). This implies that higherthan-average concentrations of factor <sup>1</sup> fungi (CLAD, EPI, YEA, and AUR) were found in the homes of these children.

These studies provide quantitative evidence connecting biological characteristics and potential interactions among common airborne fungi. Positive associations were found between mean factor scores and both housing characteristics

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and health variables. Although it is extremely time-consuming, species identification for environmental fungi is necessary to accurately assess both the relationships described above and health-related characteristics such as possible cross-reactivity between allergens. It is apparent that the discussion of biological characteristics for each factor was compromised because of the lack of speciation of the fungi. Nearly all genera contain more than one species that have characteristics that are distinct from the others. In addition, identification of other microbial components such as viruses, bacteria, and/or microbial effluents would strengthen this type of analysis. Research aimed at obtaining more-detailed information, together with simplification of sampling and analysis procedures, is crucial. Only when large speciesspecific data bases have been accumulated can we conclusively document the correlations among airborne microorganism populations and accurately examine health risks derived from respiratory exposure.

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