APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1983, p. 919–934
0099-2240/83/030919-16$02.00/0

Vol. 45, No. 3

Natural Atmospheric Microbial Conditions in a Typical Suburban Area

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Received 27 July 1982/Accepted 22 November 1982

Ambient outdoor concentrations and size distributions of airborne microbial particles were measured approximately weekly for 2 years in a Washington, D.C., suburban area. The study objective was to characterize microbial air quality in the vicinity of a proposed sewage sludge composting facility. During the study, 379 samples were taken at 17 stations, using Andersen microbial samplers. Concentration ranges (in viable particles per cubic meter) were as follows: airborne mesophilic fungi, 0 to 7,220 with a geometric mean of 273; thermophilic fungi, 0 to 193 with a median of 2.1; *Aspergillus fumigatus*, 0 to 71 with a median of 1.0; aerobic bacteria, 4.2 to 1,640 with a geometric mean of 79; and fecal streptococci, 0 to 5.7 with a median of 0. No fecal coliforms were recovered. The potentially respirable fraction (<8 μm) averaged 34% for total bacteria, 56% for mesophilic fungi, 91% for thermophilic fungi, and 95% for *A. fumigatus*. The specific sampling location was not a major factor affecting microbial particle concentrations or size distributions. Conversely, the time of year was an important determinant of viable particle concentrations for all groups of microorganisms studied. The highest concentrations were observed in summer and fall, with significantly lower levels detected in winter. In general, the microbial data did not correlate with other variables, including weather conditions, measured in this study.

An investigation of background microbial air quality in the vicinity of a proposed sewage sludge composting site near Washington, D.C., was conducted from September 1978 to December 1980. The study was completed before any actual composting activity began. Thus, the results characterize natural atmospheric microbial populations in the study area for a 2-year period.

Composting is a microbiological process for treating sewage sludge. A properly designed and operated system will stabilize the organic materials and destroy the pathogens in sludge, rendering it acceptable for recycling or ultimate disposal. However, composting can result in the emission of microbial aerosols, some of which are potentially hazardous (5). *Aspergillus fumigatus*, an opportunistic fungal pathogen and allergen, has caused considerable concern because of the large numbers generated by certain composting methods (6, 20). An objective assessment of the impact of compost-associated microbial aerosols on public health requires a knowledge of preexisting microbial conditions. Quantitative monitoring before and after startup of a composting facility can demonstrate changes in local microbial air quality, and the data can be used to evaluate potential risks posed by the plant. A goal of the present study was to establish an adequate background data base for assessing the effect of a specific composting plant on airborne microorganism levels in the surrounding community.

Few long-term quantitative studies of ambient airborne microorganism populations have been published. Notable exceptions are the studies of airborne bacteria by Bovallius et al. (3) and Mancinelli and Shulls (17) and of airborne fungi and *Aspergillus* species by Hudson (11) and Solomon et al. (26). Most available data describe microbial aerosols in the vicinity of significant point sources, especially wastewater treatment plants (10, 22), with limited attention given to background conditions. Much of the published data also was collected over a relatively short time period. The lack of adequate data, plus the extreme variability in the data that have been published, made this investigation necessary. The present monitoring program was designed to determine the average and peak levels of viable airborne particles of specific microbial groups; the effects of sampling location, time of year, time of day, local activity, and weather on microbial conditions; the approximate viable-particle size distributions; and the interrelationships among the various microorganisms.

(This work was presented in part at the 1981

Annual Meeting of the American Society for Microbiology [B. L. Jones, J. T. Cookson, and D. R. Vogt, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, Q30, p. 205].)

MATERIALS AND METHODS

Overall field monitoring strategy. Ambient outdoor concentrations of viable airborne particles of bacteria, fecal coliforms, fecal streptococci, mesophilic fungi, thermophilic fungi, and *A. fumigatus* were measured approximately weekly over a 27-month period ending in December 1980. The sampling method also separated the airborne particles by aerodynamic size, classifying them by their potential for human respirability. Selected weather parameters were measured simultaneously with each microbial sample.

The monitoring program was conducted in the vicinity of a planned sewage sludge composting facility in Montgomery County, Md., approximately 8 km north of Washington, D.C. The study area had a mixed land use pattern and included residential, commercial, agricultural, and light industrial zones. No large point sources of microbial aerosols were identified in the area during the sampling period. A map of the study area and sampling network is given in Fig. 1.

Samples were collected regularly at 10 stations, designated by letters A through J, and less frequently at 7 supplementary locations, designated R_1 through R_7. The most important criteria for locating stations were topography, prevailing wind direction, and applicability of the resultant data to an assessment of effects on public health. The stations were not evenly distributed around the composting site but were skewed toward downwind residential and commercial areas. The resultant data, therefore, best describe suburban areas with moderate to heavy human activity.

Four stations were sampled on each sampling day, and sampling days were spaced throughout the year. Sample year 1979 extended from 27 September 1978 to 5 November 1979 and included 47 sampling days. Sample year 1980 consisted of 49 sampling days between 5 December 1979 and 15 December 1980. The primary station set (stations A, B, C, and D) was usually sampled twice a month, and the two secondary station sets (stations E, F, and G and stations H, I, and J) were sampled once a month. Each supplementary station was sampled three or four times a year. The station sampling order, although not formally randomized, was varied on each sampling day. Because of method limitations, samples were not taken at temperatures lower than 0°C or during heavy snow or rain storms.

Air sampling procedures. All microbial air samples were collected by using reusable two-stage Andersen microbial air samplers (Andersen Samplers, Inc., Atlanta, Ga.). These samplers are made of aluminum and have 200 holes in a radial pattern on each stage. The operating principle is similar to that described for other Andersen samplers (1, 18). Particles are collected by impaction onto an agar medium and are sized aerodynamically as a result of differential velocities of the airstream within the sampler. The 50% effective cutoff diameter is 8 μm.

Andersen samplers were used for several reasons.

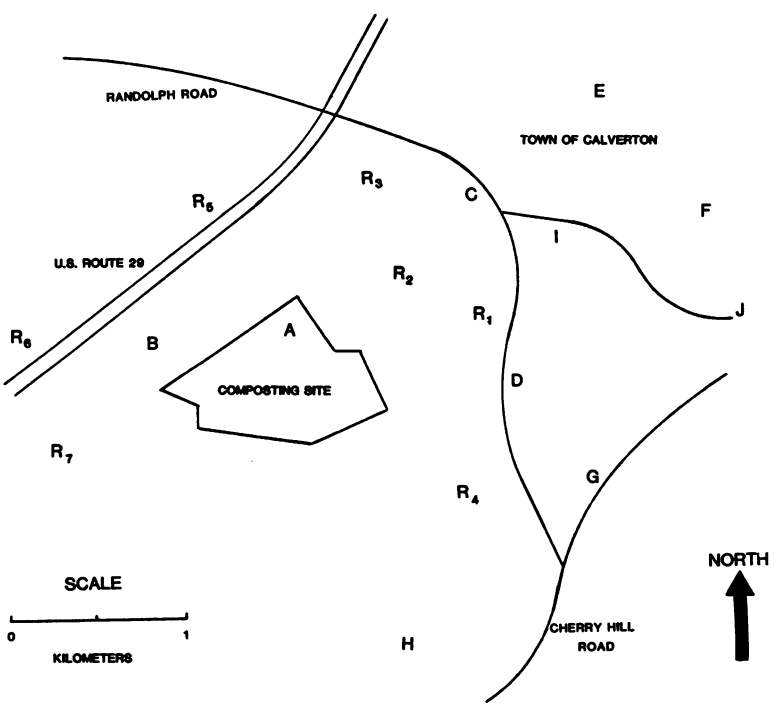

FIG. 1. Map of study area, with sampling stations designated by letters. The study area was approximately 9 km^2.

They measure only viable particles, ones which can grow under the culture conditions used. They measure unmodified particles, as they naturally occur. They collect particles volumetrically, allowing a quantitative assessment of microbial air quality, and have been shown to be more efficient than other types of sampling devices (16). The samplers also differentiate particles roughly by size, allowing an estimate of their respirable potential. Andersen samplers are reliable and durable, and the sampling techniques relatively simple; these are important considerations for an extended field study. Their major drawback is a lack of sensitivity in measuring low particle concentrations. Andersen samplers have been recommended for viable particle sampling by the U.S./International Biological Program (7).

Plastic petri plates with 27 ml of agar medium were used in the Andersen samplers. The 50% effective cutoff diameter of 8 μm given by the manufacturer for the two-stage samplers is based on a 20-ml medium volume. A larger medium volume could possibly alter the distribution of particles between the two stages. However, the manufacturer of the sampler has conducted extensive calibration tests which indicate that the distribution of particles on the two stages is not significantly affected by medium volume as long as the distance from the sampler jets to the collection surface is between 1 and 10 times the jet diameter (M. Smith, Andersen Samplers, Inc., personal communication). This condition was met in the present study. Thus, microbial particles recovered on stage 1, herein designated the large-particle fraction, were predominantly greater than 8 μm, whereas those on stage 2, the small-particle fraction, were predominantly less than 8 μm. The small-particle fraction included the vast majority of respirable particles, i.e., those less than 5 μm in aerodynamic diameter, which are deposited in human tracheobronchial and alveolar regions (19).

All sampling equipment was portable and was transported between stations on sampling days. At each station the Andersen samplers were disinfected by wiping with 70% isopropyl alcohol swabs and then were loaded with petri plates containing the appropriate agar medium. The absence of spurious contamination was verified by loading and unloading control plates during the initial stages of the sampling program. After loading and reassembly, samplers were placed upright on the sampling platform approximately 1.5 m above ground level. Airflow rates were controlled at 0.028 m^3/min by the critical orifice incorporated into each Andersen sampler. Sample periods varied in length, depending on the anticipated microbial concentration. All samples were collected between 8 a.m. and 5 p.m., with 91% taken between 10 a.m. and 4 p.m. After sampling, the petri plates were brought back to the lab and incubated.

Concurrently with the microbial sampling at each station, measurements of air temperature, relative humidity, and wind speed and direction were taken with portable instruments. Dry and wet bulb temperatures were read directly from a psychrometer (Psychron model 566; Bendix Corp., Baltimore, Md.), and relative humidity was computed from these readings. Wind direction and velocity were estimated with a hand-held anemometer and wind vane (model 6052; Belfort Instrument Co., Baltimore, Md.). Unusual local activity and traffic conditions also were recorded during the sampling period.

Microbial assay procedures. Several groups of microorganisms were measured simultaneously at each sampling location. It should be emphasized that the methods used measured viable CFU, not total viable microorganisms. Aerobic bacteria were enumerated by collecting samples on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.), incubating at 35°C, and counting the colonies after 2 days. Cycloheximide (Calbiochem-Behring, La Jolla, Calif.) at a final concentration of 0.5 mg/ml, which has been previously shown not to affect bacterial counts (3, 4), was added to the media to inhibit growth of fungi. Littman's oxgall medium with antibiotics, developed by Littman (15) and used by Millner et al. (21) to isolate compost-associated fungi, was used to collect samples for the quantitation of mesophilic and thermophilic fungi, including *A. fumigatus*. Plates were incubated at temperatures varying from 22 to 25°C and were counted after 5 days for mesophilic fungi determinations. Thermophilic fungi were counted after incubation at 45°C for 3 days. *A. fumigatus* was identified morphologically on the thermophilic fungi plates, with final counts made at 3 days. Microscopic confirmation of *A. fumigatus* was based on descriptions and photographs in standard texts (8, 14). In all cases colonies were counted rather than estimated by the "positive hole" method described by Andersen (1).

Fecal coliforms and fecal streptococci were determined by replica plating from Trypticase soy agar onto selective media. This approach has been used for specific organism determinations in previous air monitoring studies (6, 12, 23). Indirect methods such as this are necessary because selective agars are not suitable for impaction samplers (13, 23) and are not recommended for use in Andersen samplers (Operating Manual for Andersen Samplers, 1976).

MacConkey agar (BBL) was used to differentiate fecal coliforms, and KF Streptococcus agar (BBL) was used to select for fecal streptococci. Both sets of plates were incubated at 35°C and examined after 24 and 48 h. Suspected positive colonies were inoculated into liquid media. Gas production in EC broth (BBL) fermentation tubes at 44.5°C was considered presumptive positive isolation of fecal coliforms. Growth in ethyl violet azide broth (BBL) culture tubes at 35°C was considered presumptive positive isolation of fecal streptococci. The replica plating procedures used in this study were verified qualitatively, but their exact sensitivity and specificity were not determined. Because of the indirect assay method, the qualitative nature of the procedures, and the low particle detection frequency, the fecal indicator data were analyzed and are presented separately from those of the other four microbial groups.

Data analysis. The sampling method measured CFU of a specific microbial group in a given volume of air. Resultant microbial particle counts were converted into concentrations (CFU per cubic meter of air) for comparison. Each CFU represents a viable airborne microorganism, a clump of viable microorganisms, or a particle or droplet bearing viable microorganisms.

The study results were analyzed by several approaches, and descriptive statistics were calculated for various data groupings. For many statistical comparisons it was desirable to convert microbial particle

concentrations to logarithms. Log transformation generally normalized the concentration data, facilitating the use of parametric statistical methods. The problem of transforming thermophilic fungal and *A. fumigatus* particle concentrations, many of which were zero, to logarithms was overcome by adding 1 to each value (2, 25). Details of specific statistical methods are given in the appropriate section below. Except where noted, the significance level for statistical testing was 5% ($P < 0.05$).

RESULTS

Frequency distributions of the microbial data. The frequency distributions of various subsets of the sampling data were analyzed in an attempt to identify underlying probability distributions. Particle concentration data for each organism were grouped into classes, and frequency histograms were constructed. Moment coefficients of skewness and kurtosis were calculated from the grouped data to assist in determining goodness-of-fit to the normal distribution (24, 25). The untransformed particle concentrations were not normally distributed for any of the microbial groups. However, logarithms of bacterial and mesophilic fungal particle concentrations closely followed a normal distribution. As an example, frequency distributions of both concentrations and log concentrations are given in Fig. 2 for 1979 bacterial data. The 1980 bacterial and the 1979 and 1980 mesophilic fungal particle concentrations were distributed similarly. Based on these findings concerning the sample frequency distributions, parametric statistical methods were used in the further analysis of log-transformed bacterial and mesophilic fungal particle concentrations.

In contrast, log transformation of thermophilic fungal and *A. fumigatus* particle concentrations resulted in somewhat skewed frequency distributions. However, the log-transformed concentrations approximated a normal distribution much more closely than did the untransformed data. Robust (not significantly affected by slight disparities from normality) parametric methods were used for statistical comparisons of the log-transformed data (24, 25).

The particle size distribution data, as represented by the small-particle fraction, closely followed a normal distribution for bacteria and mesophilic fungi. The small-particle fractions for thermophilic fungi and *A. fumigatus* were not normal, their distributions being negatively skewed.

Concentrations of airborne microbial particles. The entire set of viable-particle concentration measurements for each microbial group was reduced to the descriptive statistics given in Table 1. The table includes the aggregate concentration range, arithmetic and geometric means, median, and appropriate 95% confidence interval for the four microbial groups during

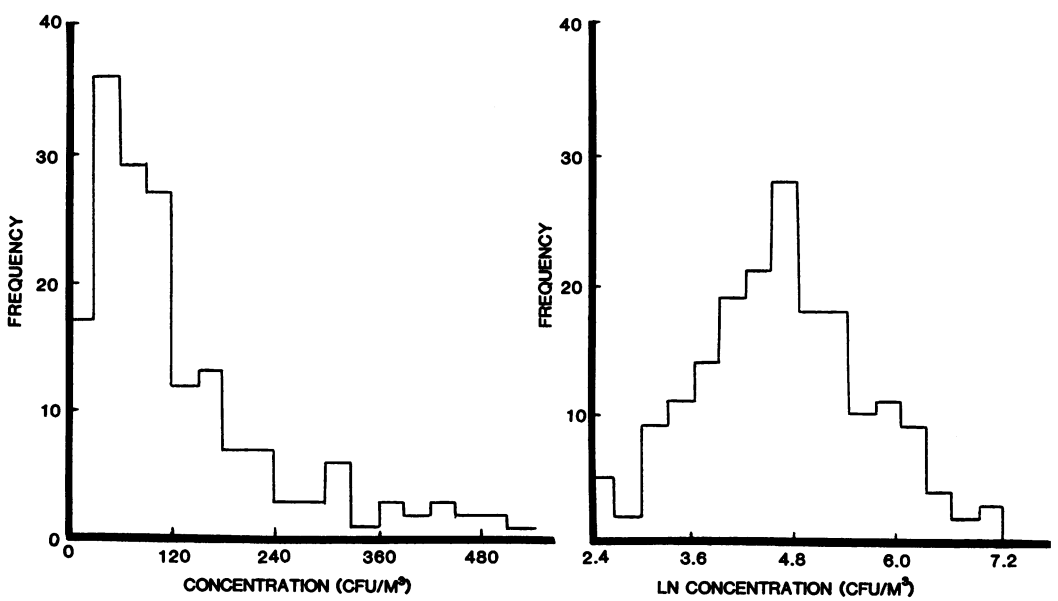

FIG. 2. Frequency distributions of untransformed and natural log-transformed bacterial particle concentrations for 1979. The parameters of the untransformed distribution were as follows: arithmetic mean, 169 CFU/m^3; skewness coefficient, >1.51; kurtosis coefficient, >4.70. The parameters of the natural log-transformed distribution were as follows: arithmetic mean, 4.65 CFU/m^3; skewness coefficient, 0.19; kurtosis coefficient, 2.65.

TABLE 1. Aggregate airborne microbial particle concentrations

Microbial group	Sample yr	No. of samples	Concn (CFU/m³)				
			Range	Arithmetic mean	Median	Geometric mean	95% Confidence interval
Aerobic bacteria	1979	184	11–1,280	169	99.5	104	90–121[a]
	1980	195	4.2–1,640	124	54	60	50–71[a]
Mesophilic fungi	1979	184	0–7,220	656	323.5	279	225–346[a]
	1980	191	7.0–6,550	736	325	267	212–337[a]
Thermophilic fungi	1979	180	0–193	4.5	1.6		1.2–2.4[b]
	1980	194	0–171	6.3	2.7		2.1–3.2[b]
A. fumigatus	1979	180	0–71	2.4	0.8		0.8–1.2[b]
	1980	194	0–58	3.5	1.2		0.9–1.8[b]

[a] Normally distributed interval for the geometric mean.
[b] Distribution-free interval for the median.

both sample years. Viable-particle concentrations were extremely variable throughout the study for all of the microbial groups. Mesophilic fungal particle concentrations were significantly higher ($P < 0.001$) than those of all other microbial groups measured. Concentrations of bacterial particles were significantly higher ($P < 0.001$) than thermophilic fungal or *A. fumigatus* particles, which were consistently detected at much lower concentrations.

Size distributions of airborne microbial particles. Table 2 gives the basic descriptive statistics derived from the aggregate particle size data, expressed as small-particle fractions. Subtracting the small-particle fraction from 100% yields the large-particle fraction. The concentration of either small or large particles can be calculated by multiplying the total concentration by the appropriate fraction.

The aggregate small-particle fraction range, arithmetic mean, median, and 95% confidence interval for each microbial group during both study years are given in Table 2. The small-particle fraction was significantly lower ($P < 0.001$) for bacteria than for the other three microbial groups and also was significantly lower ($P < 0.001$) for mesophilic fungi than for thermophilic fungi or *A. fumigatus*. Small-particle fractions were relatively consistent for each microbial group, with much less variability than the concentration data.

Effects of sampling location. The data were disaggregated and analyzed by sampling station to determine whether location affected measured particle concentrations. Tables 3 through 6 give the basic descriptive statistics, including appropriate confidence intervals, for the 10 sampling stations. A close examination of these tables suggests that there were few concentration differences attributable to the specific sampling location. This was confirmed by statistical testing, which revealed relatively few significant

TABLE 2. Aggregate airborne microbial particle size distributions

Microbial group	Sample yr	No. of samples	Small-particle fraction (% of total)			
			Range	Median	Arithmetic mean	95% Confidence interval[a]
Aerobic bacteria	1979	184	0–82	30	32.3	30.1–34.5
	1980	195	0–99	33	35.3	32.7–37.9
Mesophilic fungi	1979	183	0–100	60	58.3	55.3–61.3
	1980	191	0–100	56	54.4	51.7–57.1
Thermophilic fungi	1979	130	0–100	100	90.6	87.4–93.8
	1980	181	32–100	100	92.1	90.2–94.0
A. fumigatus	1979	114	0–100	100	92.7	88.8–96.6
	1980	169	0–100	100	95.9	94.1–97.7

[a] Normally distributed interval for the arithmetic mean.

TABLE 3. Concentrations of viable airborne particles of bacteria by sampling location

Sampling station	Concn (CFU/m³)							
	1979				1980			
	Arithmetic mean	Median	Geometric mean	95% Confidence interval[a]	Arithmetic mean	Median	Geometric mean	95% Confidence interval[a]
A	234	90	118	69–201	243	133	115	65–203
B	178	155	129	88–187	72	36	46	30–68
C	171	129	134	100–180	80	31	43	27–69
D	184	155	144	103–200	134	80	75	45–125
E	44	34.5	37	24–57	38	30.5	31	20–48
F	159	76.5	73	32–163	76	61.5	55	31–96
G	122	89.5	87	51–149	107	69	69	37–128
H	213	105	116	56–239	283	77.5	93	34–255
I	162	83	88	48–158	128	52.5	55	25–119
J	219	133	137	70–266	102	60.5	63	29–135
Other	102	93	84	54–129	95	39	49	29–83
Total	169	99.5	104	90–121	124	54	60	50–71

[a] Normally distributed interval for the geometric mean.

concentration differences in two-station comparisons, i.e., 13% (24 of 180) in 1979 and 14% (26 of 180) in 1980 (Table 7). The differences were fairly evenly distributed among the four microbial groups. Within each microbial group, however, the differences were mainly attributable to only one or two sampling stations. For example, station E had lower average concentrations of airborne bacterial particles than all other stations during both sample years, accounting for 12 of the 16 significant differences. Similarly, most (8 of 12) significant station-specific differences in mesophilic fungal particle concentrations were due to low levels at stations E and G, most (8 of 12) differences in thermophilic fungal concentrations were due to high levels at stations F and J, and most (8 of 10) differences in *A. fumigatus* concentrations were due to high levels at stations A and F.

Particle size data also were analyzed for differences between individual stations (Table 8). Even fewer significant differences were detected among small-particle fractions at the various stations than among particle concentrations. In both 1979 and 1980 only 7% (13 of 180) of possible two-station comparisons were significantly different. Thus, in this study particle size distributions varied less with sampling location than did particle concentrations. Moreover, 15 of the 26 significant differences in the small-particle fractions were attributable to low values (i.e., relatively more large particles) at only two stations, D and G.

The locational effect also was assessed by

TABLE 4. Concentrations of viable airborne particles of mesophilic fungi by sampling location

Sampling station	Concn (CFU/m³)							
	1979				1980			
	Arithmetic mean	Median	Geometric mean	95% Confidence interval[a]	Arithmetic mean	Median	Geometric mean	95% Confidence interval[a]
A	294	245	190	119–302	514	396	221	142–343
B	774	429	358	191–667	859	325	229	103–511
C	790	657	434	247–762	625	304	273	147–506
D	965	355	496	284–867	595	339	261	139–489
E	229	148	88	25–298	471	437.5	271	108–676
F	357	364	186	73–471	686	306	303	111–821
G	127	158	118	61–230	382	251.5	218	98–481
H	1,110	545.5	504	191–1,330	1,120	243	389	123–1,230
I	343	286.5	202	77–525	948	296	258	72–911
J	719	227	282	103–772	967	239	310	96–991
Other	856	564	370	183–744	1,080	568.5	318	140–722
Total	656	323.5	279	225–346	736	325	267	212–337

[a] Normally distributed interval for the geometric mean.

TABLE 5. Concentrations of viable airborne particles of thermophilic fungi by sampling location

Sampling station	Concn (CFU/m³)							
	Arithmetic mean	Median	Geometric mean[a]	95% Confidence interval[b]	Arithmetic mean	Median	Geometric mean[a]	95% Confidence interval[b]
A	3.3	2.0	1.9	0.9–3.4	7.5	2.8	3.8	2.1–6.6
B	3.3	1.7	1.9	0.9–3.4	3.4	3.2	2.3	1.4–3.7
C	13	1.7	1.9	1.3–6.3	9.5	2.1	2.6	1.3–4.6
D	6.2	2.4	2.2	1.0–4.1	2.9	2.1	2.2	1.4–3.3
E	1.5	1.0	0.9	0.2–2.1	6.9	6.75	4.7	2.2–9.1
F	5.2	1.5	1.7	0.2–4.6	7.0	7.35	5.4	2.8–9.7
G	1.5	1.2	1.1	0.3–2.2	2.9	2.1	2.1	1.0–3.9
H	2.7	0.8	1.3	0.2–3.3	3.0	1.35	2.0	0.8–3.9
I	2.3	1.6	1.5	0.5–3.1	5.7	3.65	3.4	1.3–7.4
J	2.0	1.6	1.5	0.6–2.8	12	9.55	6.5	2.5–15
Other	3.7	2.4	2.3	1.2–3.9	7.3	2.65	3.1	1.5–5.6
Total	4.5	1.6	1.9	1.4–2.3	6.3	2.7	3.1	2.5–3.7

[a] Geometric mean calculated by the offset method.
[b] Normally distributed interval for the geometric mean.

comparing data from individual stations with aggregate data (minus the station being compared). This analysis determined whether the particle concentration or size distribution of a station was significantly different from annual aggregate mean values for the entire study area. Again, relatively few significant differences were seen (Table 9); only 10 of 80 concentration comparisons and 12 of 80 particle size comparisons showed significant differences. For the most part, the stations with values significantly different from aggregate means were the ones which differed from several individual stations. Low bacterial particle concentrations at station E and high small-particle fractions of *A. fumigatus* at station B were the only two results repeated in both sample years, indicating a consistent and possibly real difference at these stations.

Effects of station-specific characteristics. As discussed above, few station-specific differences in particle concentration and size distribution were detected in this study. Two station characteristics, local traffic density and nearby ground cover, were examined closely for associations with the microbial data. The stations were classified according to their specific characteristics (Table 10), and the station groups were examined for consistent relationships with particle concentrations and size distributions.

No strong associations were found between a station's traffic density and its particle concentration ranking. Stations with heavy traffic tend-

TABLE 6. Concentrations of viable airborne particles of *A. fumigatus* by sampling location

Sampling station	Concn (CFU/m³)							
	1979				1980			
	Arithmetic mean	Median	Geometric mean[a]	95% Confidence interval[b]	Arithmetic mean	Median	Geometric mean[a]	95% Confidence interval[b]
A	1.5	1.2	1.0	0.5–1.7	5.9	1.6	2.5	1.2–4.6
B	1.6	0.8	1.1	0.5–1.8	1.6	0.6	1.1	0.5–1.7
C	6.5	1.2	2.1	0.9–4.2	1.4	0.9	1.1	0.6–1.6
D	3.3	1.0	1.2	0.4–2.4	1.7	1.5	1.3	0.8–2.0
E	0.8	0	0.4	0–1.1	4.3	4.25	2.7	1.0–5.9
F	2.8	0.8	1.1	0.1–2.8	4.3	3.8	3.3	1.6–5.9
G	0.8	0.4	0.6	0.1–1.3	1.8	0.9	1.2	0.4–2.4
H	1.7	0	0.8	0–2.2	1.8	0.75	1.2	0.4–2.5
I	1.7	1.0	1.1	0.3–2.2	3.9	1.75	2.3	0.8–4.9
J	1.1	0.8	0.8	0.2–1.6	7.5	4.75	3.5	1.0–8.8
Other	2.3	0.9	1.4	0.6–2.4	5.5	0.9	1.8	0.7–3.5
Total	2.4	0.8	1.1	0.8–1.4	3.5	1.2	1.7	1.4–2.1

[a] Geometric mean calculated by the offset method.
[b] Normally distributed interval for the geometric mean.

TABLE 7. Significant ($P < 0.05$) station-specific differences in particle concentrations

Microbial group	1979		1980	
	Station difference	P^a	Station difference	P^a
Aerobic bacteria	D > E	<0.001	A > E	<0.001
	C > E	<0.001	A > B	<0.01
	B > E	<0.001	A > C	<0.01
	A > E	<0.001	D > E	<0.01
	J > E	<0.01	D > B	0.04
	H > E	<0.01	D > C	0.04
	G > E	0.01	G > E	0.03
	I > E	0.02	H > E	0.04
Mesophilic fungi	B > G	0.01		
	B > A	0.01		
	B > E	0.04		
	C > G	<0.01		
	C > A	<0.01		
	C > E	0.02		
	D > G	<0.01		
	D > A	<0.01		
	D > E	<0.01		
	H > I	<0.01		
	H > G	0.01		
	H > E	0.02		
Thermophilic fungi	C > E	0.03	J > H	0.02
	C > G	0.04	J > D	0.03
			J > B	0.04
			J > G	0.04
			F > D	0.02
			F > G	0.02
			F > H	0.03
			F > B	0.03
			E > G	0.04
			A > D	0.04
A. fumigatus	C > E	0.01	F > C	<0.01
	C > G	0.02	F > B	<0.01
			F > D	0.02
			F > H	0.04
			F > G	0.05
			A > C	0.03
			A > B	0.03
			A > D	0.05

[a] Approximate P value resulting from two-tailed t-test of geometric means.

ed to be higher in bacterial particle concentration ranking, especially during 1979. There was no discernible association within the other three microbial groups. Also, traffic density class was not related to the station concentration differences given in Table 7. Although local traffic may affect microbial particle concentrations, it was clearly not the overriding determinant in this study. In contrast, there was a strong association between traffic density and particle size distribution for bacteria and mesophilic fungi. A chi-square analysis demonstrated that stations with high traffic density ranked significantly ($P < 0.01$) lower in small-particle fraction than other stations (i.e., high traffic density was correlated with relatively more large particles). In addition, 18 of the 21 significant differences given in Table 8 were due to lower small-particle fractions at high-traffic stations relative to low-traffic stations.

The relationship between particle concentration and the nearby ground cover (hard surface, gravel, or vegetation) of a station was evaluated by chi-square analysis. No consistent associations were found within any of the microbial groups.

One variable which was thought to possibly affect the microbial data and which was observed during sampling was the presence of visible dust. Dusty samples were compared with

TABLE 8. Significant ($P < 0.05$) station-specific differences in small-particle fractions

Microbial group	1979		1980	
	Station difference	P^a	Station difference	P^a
Aerobic bacteria	B > G	<0.01	C > D	<0.01
	F > G	<0.01	E > D	<0.01
	H > G	<0.01	J > D	0.02
	I > G	0.02	F > D	0.05
			H > D	0.05
Mesophilic fungi	I > D	<0.01	F > C	0.02
	I > C	<0.01	F > D	0.02
	I > B	0.03	F > B	0.05
	I > G	0.04		
	A > D	<0.01		
	A > C	0.03		
	E > D	0.01		
	E > C	0.05		
	H > D	0.04		
Thermophilic fungi			J > A	<0.01
			J > F	0.04
A. fumigatus			G > A	0.02
			H > A	0.02
			B > A	0.04

[a] Approximate P value resulting from two-tailed t-test of arithmetic means.

nondusty samples taken the same day, using a paired t-test, and the results for all four microbial groups during both sample years were analyzed. Only bacterial particle concentrations in 1979 were significantly higher when visible dust was present ($P = 0.02$).

Year-to-year differences. The data reported here represent 2 successive years of sampling. The 2 years of data were analyzed separately to make it possible to compare annual particle concentrations and size distributions. The distribution of sampling dates by month is given for both years in Table 11, and differences must be considered when interpreting data from the 2 sample years.

Aggregate and station-specific microbial particle concentrations for the 2 sample years are given in Table 1 and Tables 3 through 6. Comparisons between 1979 and 1980 geometric means were made by using t-tests. Except for mesophilic fungi, the aggregate particle concentrations differed significantly in the 2 years. Bacterial particle concentrations at stations B, C, and D were significantly higher in 1979 than in 1980. Mesophilic fungal and *A. fumigatus* particle concentrations at stations E, F, and J

TABLE 9. Significant differences ($P < 0.05$) between individual station and aggregate mean values[a]

Microbial group	Stations significantly different from aggregate mean values			
	Particle concn		Small-particle fraction	
	1979	1980	1979	1980
Aerobic bacteria	E < Agg (<0.001)	E < Agg (<0.01) A > Agg (0.02)	G < Agg (<0.01)	D < Agg (<0.01)
Mesophilic fungi	G < Agg (<0.01) E < Agg (0.04) D > Agg (0.05)		D < Agg (0.02) I > Agg (0.02)	F > Agg (0.02)
Thermophilic fungi	E < Agg (0.04)	C < Agg (0.02)	E > Agg (<0.001)	J > Agg (<0.001)
A. fumigatus		B < Agg (0.05) F > Agg (0.05)	F > Agg (<0.001) B > Agg (<0.01)	G > Agg (<0.001) H > Agg (<0.001) B > Agg (0.02)

[a] Agg, Appropriate aggregate mean derived from all data except the station being compared. Values within parentheses represent approximate P values resulting from two-tailed t-tests of appropriate means.

TABLE 10. Sampling-station characteristics

Station	Traffic density[a]	Local ground cover		Description
		Primary	Secondary	
A	Low	Gravel	Vegetation	Vacant gravel lot
B	Moderate	Hard surface	Vegetation	Center of grassy traffic circle
C	High	Vegetation	Hard surface	Roadside—beside cultivated fields
D	High	Vegetation	Hard surface	Roadside—beside cultivated fields
E	Moderate	Hard surface	Vegetation	Paved parking lot
F	Low	Vegetation	Gravel	Edge of large grassy playground
G	High	Hard surface	Gravel	Roadside—busy intersection
H	Low	Vegetation	Gravel	Open grassy spot in densely wooded area
I	Moderate	Vegetation	Hard surface	Roadside—quiet residential area
J	High	Hard surface	Vegetation	Roadside—busy intersection

[a] Estimated average density. Low, <12 vehicles per h; moderate, 12 to 120 vehicles per h; high, >120 vehicles per h.

were significantly higher in 1980 than in 1979. These results demonstrate that detectable annual variations in concentrations of airborne microbial particles can occur at specific locations and over an entire sampling area.

Aggregate small-particle fractions (Table 2) did not differ significantly in the 2 years for any microbial group. There were only two significant station-specific differences. The small-particle fraction for bacteria at station G was lower in 1979, and the small-particle fraction for mesophilic fungi at station I was higher in 1979. As with locational differences, annual differences were more pronounced among particle concentrations than among size distributions.

Monthly and seasonal variations. It became obvious during the initial data analysis that the concentration of viable airborne particles detected varied greatly with the time of year for all four microbial groups. The concentration data were analyzed by month for each microbial group. Monthly values from the 2 sample years were combined for this analysis to enlarge the sample size and average out year-to-year variations. The monthly data are given in Tables 12 through 15 and indicate substantial variation for each of the microbial groups. Concentrations of viable airborne particles were lowest in winter months for all four groups. Particle concentrations of bacteria, thermophilic fungi, and *A. fumigatus* were highest in late summer or fall, whereas mesophilic fungi peaked earlier, with the highest levels observed in late spring and summer. The variation in the relative monthly viable-particle concentrations of bacteria, mesophilic fungi, and thermophilic fungi is shown in Fig. 3.

The small-particle fractions also were analyzed for monthly variation. Statistically insignificant monthly fluctuations were detected for bacteria, thermophilic fungi, and *A. fumigatus*, with no seasonal or other trends discernible. However, the mesophilic fungal particle size distribution varied significantly by month. This variation appeared to be seasonal, with relatively more small mesophilic fungal particles recovered in summer and fall. The monthly variation in small-particle fraction for bacteria, mesophilic fungi, and thermophilic fungi is shown in Fig. 4.

Time-of day and day-of-week effects. The data were evaluated to determine whether viable-

TABLE 11. Monthly sampling-date distribution

Mo	No. of samples		
	1979	1980	Total
Jan.	19	12	31
Feb.	0	8	8
Mar.	19	24	43
Apr.	0	12	12
May	16	20	36
June	16	16	32
July	16	8	24
Aug.	18	12	30
Sep.	21	28	49
Oct.	27	16	43
Nov.	20	16	36
Dec.	12	23	35
Total	184	195	379

TABLE 12. Monthly concentrations of airborne bacterial particles

Mo	Concn (CFU/m³)			Mo with significantly lower concn ($P < 0.05$)
	Median	Geometric mean	95% Confidence interval	
Sep.	171	178	138–230	All others
Nov.	110.5	118	87–160	Jan., Feb., Mar., Apr., May, Aug.
Dec.	121	110	78–155	Jan., Feb., Mar., Apr.
Oct.	98	106	76–148	Jan., Feb., Mar., Apr.
July	79	85	53–134	Jan., Feb., Mar., Apr.
June	68.5	72	48–109	Feb., Mar., Apr.
May	74	70	47–103	Feb., Mar., Apr.
Aug.	77.5	68	46–99	Feb., Mar., Apr.
Jan.	45	47	33–65	Feb.
Mar.	41	36	25–51	None
Apr.	25	30	16–54	None
Feb.	22	24	16–36	None

particle concentrations were related to station sampling order, time of day of sampling, or day of week of sampling. These analyses were done primarily to validate and generalize the data obtained. Chi-square contingency testing revealed no association between sampling order and specific sampling stations. Thus, sampling order was not a confounding variable masking locational effects.

The data were evaluated to determine whether time of day of sample collection was related to the measured particle concentration. Concentrations at stations A, B, C, and D during a full year were compared by using two-factor analysis of variance, with sampling date as the row variable and sampling order as the column variable. Sampling order was used as a surrogate for time of day. This approach assumes, on the basis of the data presented above, that particle concentrations were not affected by location. No relationship between particle concentration and sampling order was shown for the four microbial groups. The analysis confirmed, however, the strong relationship between concentration and sampling date.

Because sampling order was only a rough approximation of time of day, a second approach was used to evaluate the association between time of day and concentration. All samples for a single month were analyzed, thereby removing time of year as a confounding variable. The samples, regardless of location, were grouped into four time periods, and mean particle concentrations were compared by using *t*-tests. This approach also assumes that sampling location did not affect concentration. In general, there was no association between time of day and particle concentration for any microbial group for the months analyzed.

The relationship between day of week of sample collection and measured particle concentration also was examined. Daily concentration averages were classified as either above or below the appropriate monthly average and tested for association with day of week by a chi-square analysis. There was no evidence for association

TABLE 13. Monthly concentrations of airborne mesophilic fungal particles

Mo	Concn (CFU/m³)			Mo with significantly lower concn ($P < 0.05$)
	Median	Geometric mean	95% Confidence interval	
Sep.	1,210	1,290	1,000–1,650	All others
July	781	778	565–1,080	Jan., Feb., Mar., Apr., Oct., Nov., Dec.
June	687	680	507–912	Jan., Feb., Mar., Apr., Oct., Nov., Dec.
May	479.5	576	424–784	Jan., Feb., Mar., Apr., Nov., Dec.
Aug.	576.5	558	430–725	Jan., Feb., Mar., Apr., Nov., Dec.
Oct.	527	404	279–582	Jan., Feb., Mar., Apr., Dec.
Nov.	302	245	159–376	Jan., Feb., Mar.
Dec.	150	155	112–213	Jan., Feb., Mar.
Apr.	142.5	126	61–258	Jan., Feb., Mar.
Mar.	51	53	41–67	Jan., Feb.
Jan.	29	33	21–50	Feb.
Feb.	17	16	10–24	None

TABLE 14. Monthly concentrations of airborne thermophilic fungal particles

Mo	Concn (CFU/m³)			Mo with significantly lower concn ($P < 0.05$)
	Median	Geometric mean	95% Confidence interval	
Nov.	6.4	6.3	4.1–9.4	Jan., Feb., Mar., Apr., May, June, July, Oct.
Dec.	4.7	4.6	3.3–6.3	Jan., Feb., Mar., Apr., May, June, July, Oct.
Sep.	3.1	3.9	2.7–5.4	Jan., Mar., Apr., May, June, July
Aug.	2.4	3.2	1.7–5.6	Jan., Mar., Apr.
Oct.	2.9	2.7	1.8–3.8	Jan., Mar., Apr.
July	1.6	2.0	1.1–3.4	Mar., Apr.
May	1.8	1.7	1.2–2.4	Mar., Apr.
June	1.2	1.7	1.0–2.8	Mar., Apr.
Feb.	2.25	1.7	0.5–4.0	Apr.
Jan.	1.2	1.3	0.8–2.1	Apr.
Mar.	0.8	0.9	0.6–1.2	Apr.
Apr.	0	0.3	0.1–0.5	None

between day of week and particle concentration for any of the microbial groups.

Effect of measured weather parameters. Correlation analyses between sampling-day temperature, wind velocity, and rainfall and the microbial data were done. The high degree of seasonality in the concentration data suggested a possible correlation with temperature. However, the only microbial group with a significant correlation was mesophilic fungi. Monthly mean log concentrations of mesophilic fungal particles correlated relatively well with monthly mean sampling-day temperatures ($y = 0.121x + 3.267$, where y = monthly mean log concentration and x = monthly mean sampling-day temperature in degrees Celsius; $r^2 = 0.760$). A linear regression analysis between 1980 daily mean log concentrations and temperatures yielded a similar equation ($y = 0.121x + 3.388$; $r^2 = 0.508$). No relationship was found between temperature and particle size data.

No direct correlation with wind velocity existed for individual sample concentrations or daily or monthly means. However, an association was found for mesophilic fungi by taking the monthly variation into account, suggesting that the effect of wind velocity was possibly being masked by seasonal effects. A chi-square contingency test in which daily particle concentration means were classified by wind velocity and by whether they were higher or lower than the appropriate monthly mean demonstrated a significant positive association for mesophilic fungi ($P = 0.035$), but not for the other microbial groups. The result indicates that high wind velocities may be associated with higher-than-average concentrations of airborne mesophilic fungal particles. In addition, on all five sampling days with an average wind velocity of greater than 3.1 m/s, higher-than-average particle concentrations were measured for all four microbial groups. This suggests a possible relationship between wind velocity and particle concentration for all four microbial groups, an association limited to higher velocities than generally measured in this study (75% of the sampling days had average

TABLE 15. Monthly concentrations of airborne *A. fumigatus* particles

Mo	Concn (CFU/m³)			Mo with significantly lower concn ($P < 0.05$)
	Median	Geometric mean	95% Confidence interval	
Nov.	3.05	3.5	2.1–5.6	Jan., Feb., Mar., Apr., May, June, July, Oct.
Dec.	1.8	2.3	1.5–3.5	Jan., Feb., Mar., Apr., May, July
Sep.	1.6	2.1	1.5–2.9	Jan., Feb., Mar., Apr., May, July
Aug.	0.8	1.7	0.9–3.1	Jan., Mar., Apr.
Oct.	1.6	1.6	1.0–2.2	Jan., Mar., Apr.
June	0.8	1.3	0.7–2.2	Mar., Apr.
May	0.9	1.1	0.7–1.7	Mar., Apr.
July	1.0	1.1	0.5–1.8	Mar., Apr.
Feb.	0.9	0.8	0.1–1.8	None
Jan.	0.6	0.7	0.4–1.1	Apr.
Mar.	0.3	0.4	0.3–0.6	None
Apr.	0	0.2	0–0.5	None

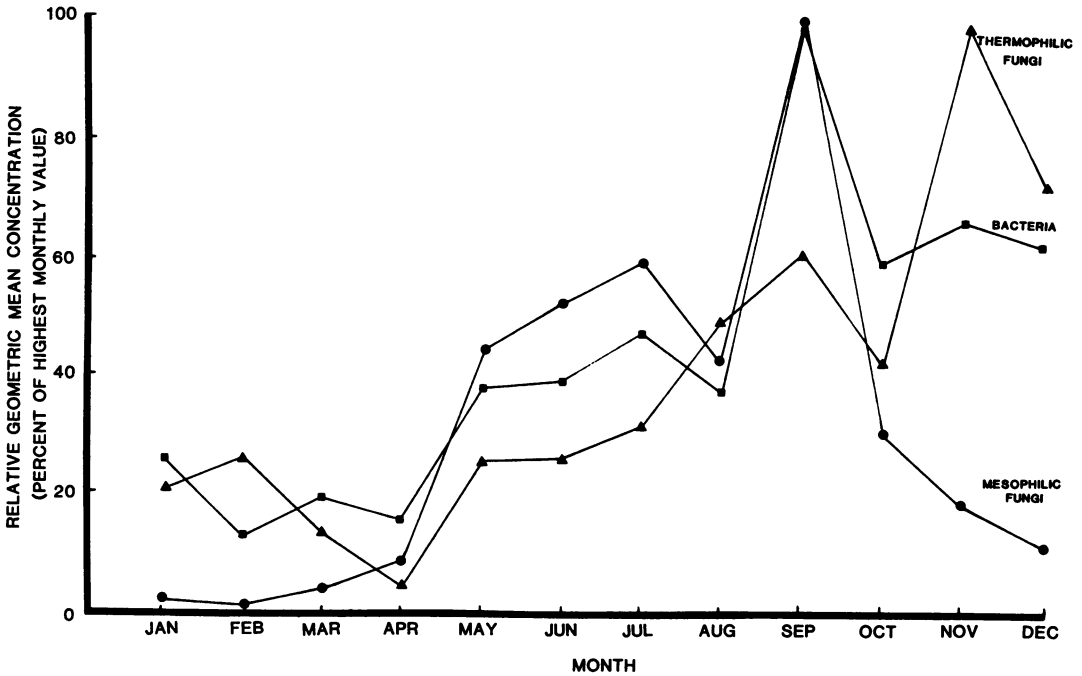

FIG. 3. Relative monthly geometric mean concentrations of viable airborne microbial particles.

wind velocities of less than 2.2 m/s).

The microbial data were examined for associations between precipitation and measured particle concentration. Daily mean concentrations were classified by occurrence of precipitation and by whether they were higher or lower than the monthly mean, and chi-square tests of association were done. No relationship between precipitation, on either the sampling day or the day before, and concentration relative to the mean was seen for any of the microbial groups.

No relationship was found between relative humidity and either microbial concentration or particle size distribution.

Interrelationships among the microorganisms. The particle concentration data for the various microbial groups were evaluated for consistent interrelationships. The particle concentration ratio between mesophilic fungi and bacteria varied widely, and no precise correlation existed. However, mesophilic fungal particle concentrations were generally higher than bacterial concentrations, especially in nonwinter months (88% of the time). During winter months, mesophilic fungal concentrations were higher 54% of the time.

The relationship between *A. fumigatus* and total thermophilic fungi was also closely examined. Because *A. fumigatus* particle concentrations were a subset of total thermophilic fungal concentrations, the ratio of *A. fumigatus* to total thermophilic fungi was always less than or equal to 1. Overall, *A. fumigatus* particles comprised 54% of the total thermophilic particles, but the percentage varied widely among individual samples. No consistent correlation between these two microbial groups was detected.

Recoveries of fecal indicator bacteria. Samples for fecal streptococcus and fecal coliform determinations were collected throughout the study. During the 2 years, approximately 225 m^3 of air was assayed for fecal indicators. No confirmed fecal coliforms were recovered. Assuming that fecal coliform particles in air follow a Poisson distribution and that the detection efficiency was 100% allows the calculation of 0.02 CFU/m^3 as the upper 95% confidence limit of concentration. Because there were no known sources of fecal coliform aerosols in the study area and because of the very rapid die-off of fecal coliforms in air (9, 27), this result is not surprising.

Confirmed fecal streptococci were recovered sporadically in low numbers. During sample year 1979, fecal streptococci were detected in 20 of the 184 samples (10.9%), with a total of 33 confirmed colonies recovered. Based on the assumptions described above, the 95% confidence interval for the estimated concentration is 0.20 to 0.43 CFU/m^3. During sample year 1980, 23 of 195 samples (11.8%) were positive for fecal streptococci, with a total of 47 confirmed colonies recovered (95% confidence interval, 0.30 to 0.55 CFU/m^3). No fecal streptococci were recovered in the winter in either sample year.

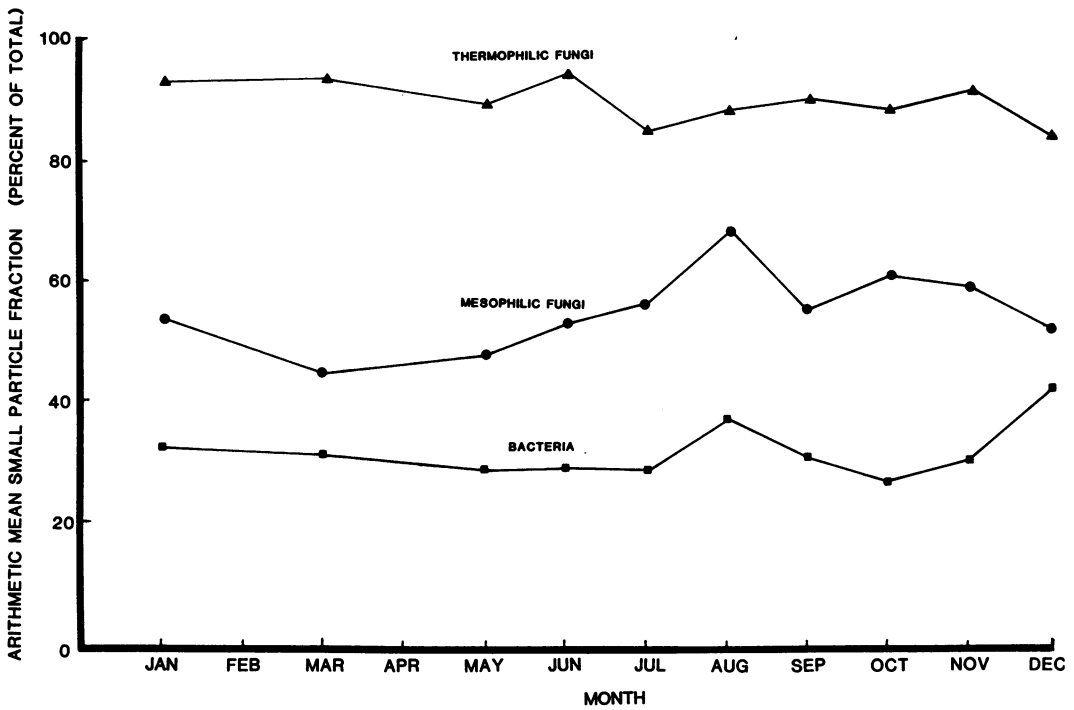

FIG. 4. Monthly arithmetic mean small-particle fractions. February and April were omitted due to insufficient samples.

DISCUSSION

This investigation fulfilled its primary objective by providing background microbial aerosol data for a planned sewage sludge composting facility. The results also have more general applicability as quantitative long-term data describing natural airborne microbial conditions in an unperturbed suburban area. The present study, in addition to yielding information on viable microbial particle concentrations, allowed an evaluation of the effect of several major variables on microbial conditions.

The measured concentrations of airborne microbial particles were highly variable, as reported elsewhere (3, 28), with the standard deviation often exceeding the mean. Because of the extreme variability in microbial aerosol data, a large number of measurements is required to confidently estimate sample parameters and to make sensitive statistical comparisons. With very few exceptions, the smallest subsets compared in the present study contained at least 12 samples each, allowing detection (at a 95% confidence level) of a difference in means greater than 85% of the standard deviation.

The summary statistics given in Tables 1 and 2 were derived from the complete data set and represent the maximum meaningful reduction of the microbial data. As aggregate statistics, they must be interpreted with caution. They are most useful as summary descriptions of the present study, a relatively large set of samples collected at various locations over 1-year periods. However, the data must be disaggregated and analyzed with respect to sampling variables, such as location, time, and local activity, before being compared with other sample sets. It would not be valid to simply compare these aggregate results with other microbial aerosol data collected by different methods under different sampling conditions.

A meaningful comparison of the present data with those of other published studies is extremely difficult because of differences in sampling locations, sampling methods, and microbial assay procedures. The large number of sampling variables that can affect the results makes even internal comparisons difficult. Nonetheless, the concentrations of viable airborne particles reported here are within the ranges observed in similar studies of airborne bacteria (3, 17) and *A. fumigatus* (11, 26). Of more general interest and applicability, however, are the findings pertaining to the effects of sampling variables on particle concentrations and size distributions.

Airborne microbial particles were quantitated, using the same protocol, regularly at 10 stations within the sampling area, with no two stations more than 3.5 km apart. Very little locational effect was detected for any of the microbial

groups. Of 360 two-station comparisons of particle concentration, 50 (14%) station pairs were different at a 95% confidence level. Only four differences—stations A, D, G, and H having higher airborne bacterial particle concentrations than station E—were significant in both sample years. A total of 12 of the 50 significant differences were specifically attributable to low bacterial particle concentrations at station E. Thus, the vast majority of comparisons between stations revealed no concentration differences at the different locations.

The particle size data varied even less with location than did the concentration data, with only 7% (26 of 360) of the station-specific differences being significant at a 95% confidence level. As shown in Table 8, 19 of the 21 differences for bacteria and mesophilic fungi were attributable to low small-particle fractions at only three stations, C, D, and G. There were no station pairs significantly different in both sample years, although the small-particle fraction for *A. fumigatus* at station B was significantly higher than the aggregate value in both years.

The conclusion drawn from the station-specific analysis is that, with the exception of bacterial particle concentration at station E and possibly *A. fumigatus* particle size distribution at station B, sampling location was not a consistently strong factor determining either particle concentration or size distribution. It is probable that there are actual locational differences in microbial particle concentrations and size distributions within the sample area, but the results of this study indicate that these differences are small relative to sampling variability. Therefore, very large sample sizes would generally be necessary to detect locational differences. As a practical matter, locational differences of the magnitude included here (less than 3.5 km) are probably negligible in most field monitoring studies, provided that sampling stations are not immediately adjacent to microbial aerosol generators. This should be verified at representative locations, however, in any field monitoring program.

Station characteristics, specifically local ground cover and nearby traffic density, did not correlate well with microbial particle concentrations. However, the small-particle fractions of bacteria and mesophilic fungi were negatively associated with traffic density, indicating that stations with higher nearby traffic density had higher percentages of large viable particles. Because of differential settling velocities of different-sized particles, aerosols of local origin generally have a higher percentage of large particles than do aerosols of distant origin (3). Thus, the present data suggest that microbial particles recovered at high-traffic stations were of local origin to a greater degree than particles recovered at the other stations. This observation is not surprising, since traffic could be expected to stir up and disperse particles.

Except for the bacterial data from 1979, visible dust observed during the sampling period was not related to concentrations of viable airborne particles. This was somewhat surprising in light of the large numbers of microorganisms in soil and reported correlations between suspended particulates and bacteria (17). The lack of association observed here may be due to the qualitative nature of the observation of visible dust or to the effect of confounding variables. Quantitative particulate measurements are required to better evaluate this possible association.

An evaluation of the 2 years of data separately allowed not only yearly comparisons of particle concentrations and size distributions, but also an assessment of the consistency of trends and correlations observed in the data. An important result of the present study is that aggregate concentrations of airborne bacterial thermophilic fungal and *A. fumigatus* particles were significantly different in successive years. For each of these microbial groups, 3 of 10 individual stations also had significant annual differences. These results were obtained by the same investigators using the same methods, with no important changes within the sampling area over the 2 years. Although the particle concentration differences may in part be due to different monthly sampling distributions in the 2 years, the fact that the same year did not have the higher concentration for each microbial group suggests that the observed differences are not entirely attributable to this. The yearly differences aptly demonstrate that one must use extreme caution in interpreting microbial aerosol data from different time periods, even when collected by identical sampling protocols. Statistically significant differences may reflect natural fluctuations in concentrations of airborne microbial particles. This suggests that the magnitude as well as the statistical significance level of any concentration difference should be considered when assessing the effects of a microbial aerosol source on local air quality by comparing "before" and "after" data.

The small-particle fraction showed much less year-to-year variation than did the concentration, with only two individual stations having significant differences. In the case of a microbial source that generates aerosols with a different size distribution than natural background, "before" and "after" particle size distributions may be a more sensitive and useful measure of local air quality effects than microbial particle concentration data.

Substantial monthly variation and definite seasonal trends in concentrations of viable airborne particles were evident. The annual periodicity for bacteria paralleled that observed by Bovallius et al. (3). The monthly and seasonal variability was large in magnitude, had a high statistical confidence level, occurred in both sample years, and was detected in all four microbial groups. Therefore, particle concentration data generated from substantially different monthly sampling distributions must be compared with caution unless the differences are accounted for in the data analysis. The extreme time-of-year variation in concentrations of airborne microbial particles implies that the common practice of assessing microbial air quality effects of a generating source on the basis of data collected a month or two before source start-up is inappropriate. As observed for locational and annual differences, particle size distributions varied much less with month than did particle concentrations.

The general lack of strong correlations between the microbial data and the meteorological variables was not entirely surprising. Other investigators have also reported few consistent associations (17, 28). This does not rule out an association, because in a field study such as this, subtle or complex interactions within the data are difficult to identify due to the number of uncontrollable and potentially confounding variables.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of all field and laboratory personnel who contributed to this study, especially Frank Wirsing. We are indebted to Patricia Millner and Regina Smith for critically reviewing the draft manuscript. We also acknowledge the contributions of Donald Vogt and Karl Deugwillo of the Washington Suburban Sanitary Commission.

This work was financially supported by the Washington Suburban Sanitary Commission.

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