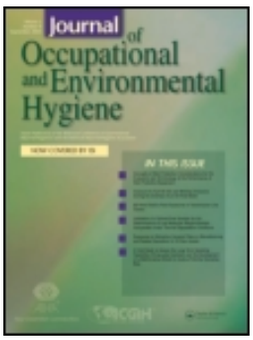

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Personal Exposure to Airborne Dust and Microorganisms in Agricultural Environments

Shu-An Lee, Atin Adhikari, Sergey A. Grinshpun, Roy McKay, Rakesh Shukla, and Tiina Reponen

Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio

Airborne dust and microorganisms are associated with respiratory diseases and increased mortality and morbidity. Farmers are at high risk of exposure to both of these hazards. Very limited information, however, is available on the combined exposures to both hazards on different types of farms. Moreover, most of the previous studies have measured the mass concentration of particles ignoring the particle size. In this study, farmers' exposure to airborne dust and microorganisms was studied using our newly developed personal sampling system. Particle number concentration and size distribution were measured with an optical particle counter. Simultaneously, particles were collected on a filter and analyzed for microorganisms. The field measurements were conducted in animal confinements (swine, poultry, and dairy) and during grain harvesting (corn and soybean). The results show the following average concentrations on the workers' breathing zone: 1.7×10^6 to 2.9×10^7 particles/m^3 for total dust, 0.9×10^3 to 3.9×10^4 spores/m^3 for total fungal spores, 0.3×10^3 to 3.6×10^4 CFU/m^3 for culturable fungal spores, 0.3×10^4 to 3.3×10^8 CFU/m^3 for culturable bacteria, and limit of detection (LOD) to 2.8×10^3 CFU/m^3 for culturable actinomycetes in animal confinements. The respective concentrations were 4.4×10^6 to 5.8×10^7 particles/m^3, 3.4×10^4 to 6.1×10^6 spores/m^3, 8.2×10^4 to 7.4×10^6 CFU/m^3, 0.4×10^5 to 1.4×10^6 CFU/m^3, and LOD to 2.6×10^4 CFU/m^3 during grain harvesting. The highest contribution of large particles (3–10 μm) in total particles was found during grain harvesting, whereas the size distribution was dominated by smaller particles (<3 μm) in animal confinements. High fraction (up to 37%) of particles between 2–10 μm was found to be fungal spores. The results indicate that an increase in the concentration of large dust particles (2–10 μm) during grain harvesting was partially attributed to the increase in the concentration of the fungal spores. Overall, the combined exposure to airborne dust and microorganisms was found to be more severe during harvesting than in animal confinements.

Keywords agricultural farms, bacteria, dust, fungi

Address correspondence to: Tiina Reponen, Department of Environmental Health, University of Cincinnati, P.O. Box 670056, Cincinati, OH 45267-0056; e-mail: reponeta@ucmail.uc.edu.

Several studies have shown that human exposures to airborne dust and microorganisms, such as bacteria and fungi, can cause respiratory diseases. Agricultural workers have been found to be at high risk of exposures to airborne particles of biological and nonbiological origin.[1–10]

Agricultural operations such as plowing, harrowing, cultivating, sowing, harvesting, threshing, animal feeding, and grain handling increase farmers' risk of exposures to airborne dust and microorganisms.[11] Airborne concentrations of bacteria and fungi in farm environments have previously been reported to be about 10^3–10^7 spores/m^3 in cow barns and grain storage facilities.[5,12–16] This is up to four to five orders of magnitude higher than the concentrations measured in nonagricultural indoor air environments (10^2–10^3 spores/m^3).[17] The mass concentrations for total dust in agricultural environments (0.7 to 95.4 mg/m^3)[8,11] have also been found to be much higher than those measured in nonagricultural indoor environments (0.02 to 0.44 mg/m^3)[18] and often exceed the exposure limit for total dust (15 mg/m^3, OSHA, 1994)[19] and organic dust (5 mg/m^3, Swedish National Board of Occupational Safety and Health, 1994).[20] Therefore, exposures to airborne dust and microorganisms on agricultural farms are common but poorly controlled.

Most of the previous studies estimated particulate exposure by measuring the total mass concentration; very few studies investigated the particulate exposure with respect to particle size on agricultural farms. The size of particles, however, affects their respiratory deposition, resulting in different types of health effects.

Another important aspect that helps to better understand workers' exposure patterns to airborne dust and microorganisms on agricultural farms is personal sampling. Airborne allergens obtained by stationary sampling have been shown to be significantly lower than those obtained by personal sampling.[21,22] Similar conclusion was also made by Rautiala et al.[23] who investigated the concentration of airborne culturable fungi during remediation of moldy buildings and by Toivola et al.[24] who collected particles and biaerosols in home

TABLE I. Environmental Characteristics of Agricultural Farms

Farm Types	Sampling Season	n[A]	Site Dimensions	T[B] (°C)	RH[C] (%)	Farming Activity
Swine	Summer	1	7 m × 10 m	30–31	45–∼54	Animal feeding, taking care of baby pigs, and mother pigs
	Winter	4		11–15	33–∼37	
Poultry	Summer	2	10 m × 50 m	32–∼34	44–∼52	Egg packing, routine cleaning, facility maintenance, and examination
Dairy	Summer	2	30 m × 100 m	22–∼26	44–∼52	Animal feeding, animal manure cleaning, stalls bedding, and milking
	Winter	3		11–∼15	38–∼58	
Corn1	Fall	2	200 m × 500 m	22–∼24	38–∼44	Corn harvesting—Grain Farm 1
Corn2	Fall	4	300 m × 500 m	4–∼6	37–∼53	Corn harvesting—Grain Farm 2
Soybean1	Fall	2	200 m × 500 m	18–∼20	70–∼75	Soybean harvesting—Grain Farm 3
Soybean2	Fall	1	30 m × 20 m	18–∼20	70–∼75	Soybean unloading and handling—Grain Farm 3

[A] n is number of human subjects tested.
[B] T is temperature measured during sampling.
[C] RH is relative humidity measured during sampling.

and work environments. In our previous study,[25] we found that individual stationary sampling underestimated personal exposures to airborne fungi in agricultural environments. We have recently developed a personal sampling system,[26] which was used in a concurrent study[27] to investigate the protection provided by respirators against dust and bioaerosols in agricultural farms.

This article reports the data on the personal exposure to dust and bioaerosols collected on farms using this new personal sampling system. The size distribution and number concentration of airborne dust and microorganisms were measured in the size range of 0.7 to 10 μm, which covers the size range of most bacteria and fungi. Furthermore, the contribution of microorganisms in airborne dust in the corresponding size range was investigated for different agricultural farms.

MATERIALS AND METHODS

Personal Sampling System

Field samples were collected using a personal sampling system previously described in detail by Lee et al.[26,28] In short, particle samples were collected using an ambient sampling line in the sampling system. Airborne dust and microorganisms were simultaneously sampled through the sampling probe at a flow rate of 10 L/min and drawn through Tygon tubing to a metal sampling chamber. A portion of each aerosol flow (2.8 L/min) was sampled from the chamber into an optical particle counter (OPC, model HHPC-6; ARTI Inc., Grants Pass, Ore.) for dust measurement. The rest of the aerosol flow (7.2 L/min) passed through a filter sampler that collected the airborne microorganisms. The concentrations of particles in five size classes ranging from 0.7 μm to 10 μm were recorded and instantly displayed on the OPC screen as 1-min averages. The filter samples were analyzed for microorganisms by microscopic counting and/or cultivation as specified further below.

Description of Agricultural Farms

Six farms were included in this study: three types of animal confinements (swine, poultry, and dairy), and three grain farms. The field tests were conducted during the following farming activities: animal feeding on a swine and a dairy farm, routine examination of facilities on a poultry farm, grain harvesting in two corn fields and in a soybean field, and soybean unloading near a silo. All these farming activities were done between 30 to 60 min. The sampling covered the time the specific work task lasted and thus represents the presumed worst-case exposures. The characteristics of these agricultural environments and farming activities are summarized in Table I. Volunteer farmers were recruited by contacting the Ohio Agricultural Research and Development Center extension agents and local farmers.

The swine farm was situated at the agricultural experimental station of the Ohio State University (Ohio Agricultural Research and Development Center, Western Branch, South Charleston, Ohio). To investigate seasonal variation in concentrations of airborne dust and microorganisms, the experiments were conducted on this farm during summer and winter. The activities performed during testing included pig feeding, working with baby pigs in the nursery, and taking care of mother pigs in the farrowing room. The experiments were conducted in a fattening room during pig feeding. There were 10 pens in this room (7 m × 10 m), each having 8 to 10 pigs. Two sides of the room were open to provide sufficient ventilation during summer. In winter, these two sides were partially closed with a curtain to preserve heat. A water sprayer

was installed to reduce the temperature in summer. During pig feeding, feed was delivered to a trough from an outside silo, where this feed was stored. A farmer had to examine feed in the trough and turn on the automated delivery system three to four times a day to maintain sufficient amount in the trough. The task was performed for 30 min each time. The temperature was 30°C to 31°C, and the relative humidity was 45% to 54% in summer; the respective values were 11°C to 15°C and 33% to 37% in winter.

The poultry farm was situated in an agricultural field near Ottawa, Ohio. The activities performed on this farm included egg packing, routine cleaning, maintenance, and examination of the facility. The experiments were performed during the routine examination of the facility (10 m × 50 m) including removal of unhealthy chickens and assuring the operation of the conveyor belt, which transported the eggs. All egg production lines were automated. There were about 70,000 chickens and around 100,000 eggs produced each day. After eggs were laid, they slipped onto a conveyer and were transported to a packing machine. Eggs were packed into boxes and transported to storage or shipping dock. The farm was dusty and the floor was covered with feathers, grains, and poultry manure. Small windows were open on the top of the side walls to allow the flow of fresh air into the poultry facility. Experiments were conducted only in summer because the poultry farmers did not allow the investigators to enter the facility in winter due to bird flu epidemic. The temperature inside the facility was 32°C to 34°C and the relative humidity was 44% to 52%.

The dairy farm was located in an agricultural field also near Ottawa, Ohio. The farming activities performed during testing were removing cow manure, bedding stalls with grinding soil, feeding cows with hay, and milking cows. The farm housed approximately 600 cows in a barn (30 m × 100 m). Four sides were partially open for ventilating airflow. The floor was wet and covered with soil and hay. Farmers usually cleared away the animal fecal material from inside the pen when cows were in the milking room. Trucks were used to distribute hay twice a day for feeding. Experiments on this farm were conducted in summer and winter. The temperature on the dairy farm was 22°C to 26°C, with a relative humidity ranging from 44% to 52% in summer; respective values were 11°C to 15°C and 38% to 58% in winter.

The corn harvesting sites (Farms 1 and 2) were situated in open cornfields in Hamilton, Ohio (Farm 1: 200 m × 500 m) and in Clarksville, Ohio (Farm 2: 300 m × 500 m). The temperature in the cornfield was 22°C to 24°C and the relative humidity was 38% to 44% on Site 1; respective values were 4°C to 6°C and 37% to 53% on Site 2. The field tests were conducted while a farmer was driving a combine and doing corn harvesting in fall. Study subjects wearing a respirator stood on the combine outside the cab in order to assess the worst-case exposure during corn harvesting.

Field experiments for the farming activities of soybean harvesting and unloading were performed on the same agricultural farms in Clarksville, Ohio (Farm 3). The samples were collected during the period when a farmer drove a combine to harvest soybeans in the soybean field (200 m × 500 m) and unloaded soybeans from a semi truck to an underground soybean storage room (30 m × 20 m). The experiments were conducted while (a) study subjects were standing on the combine outside the cab during soybean harvesting, and (b) a subject was walking around to observe and examine soybean unloading from the truck. The temperature inside the facility was 18°C to 20°C and the relative humidity was 70% to 75%.

Field Sample Treatment

Airborne dust was sampled and recorded by an OPC operated at a flow rate of 2.8 L/min. The recorded data were later downloaded from the instrumental data logger into a laboratory computer for data analysis. One-minute average particle concentrations were averaged size selectively over the entire sampling time (30–60 min). Airborne microorganisms were collected on a filter (white polycarbonate, with a pore size of 3.0 μm and a diameter of 25 mm; Osmonics Inc., Westborough, Mass.). One composite sample was obtained for the entire 30–60 min sampling time. Prior to field test, the filter was placed into a sterilized and cleaned filter cassette and sealed with sterile aluminum foil at both ends during transportation. The filter sample was collected at a flow rate of 7.2 L/min. The total flow rate of 10 L/min for the sampling line was calibrated prior to sample collection using a dry calibrator (Bios Drycal DC-Lite Calibrator; Zefon International Inc., Petersburg, Fla.).

Immediately after sampling, the filters were charge neutralized and transferred from filter cassettes to sterile 50-mL tubes in a clean area. The tubes with filters were stored in an icebox during transportation to the laboratory. For each field test, one field blank and one transportation blank were obtained. The field blank sampling was performed with a filter sampler connected to the sampling system without any airflow passing through it, whereas the transportation blank was a sample taken to the field without exposing it to the sampling environment.

Microbial Analysis

Microorganisms were extracted from the filters with 20 mL buffer solution, which was made by mixing 1 L of deionized distilled water, 1 gram of peptone, and 50 μL of Tween 80. Before using, the freshly prepared autoclaved buffer was filtered through a 0.2-μm polycarbonate filter. The extraction was performed by 2-min vortex and 15-min ultrasonic vibration as described in our earlier study.[29] This extract was used for cultivation and microscopic counting. Cultivation was done immediately after extraction. The rest of the suspension was treated by adding 1% (v/v) of formaldehyde and kept at 4°C for subsequent microscopic analysis for up to 3 days.

Series of dilutions (10^0, 10^1, and 10^2) were prepared for microbial cultivation. Each dilution was cultivated in triplicate to assure the accuracy of colony enumeration. The average of colonies on three agar plates was used to calculate the

concentration of culturable microorganisms. To recover bacteria and actinomycetes, tryptic soy agar (40 g/L) supplemented with cycloheximide to inhibit the growth of fungi (0.5 g/L; Sigma-Aldrich Co., St. Louis, Mo.) was used. Fungi were recovered on malt extract agar supplemented with streptomycin sulfate to prevent the growth of bacteria (40 mg/L; Sigma-Aldrich Co.). Both types of agar plates were incubated at 28°C ± 2°C. The total number of bacterial colonies was counted after 3 days of incubation. Actinomycete colonies were counted after 2 weeks of incubation. For fungi, the incubation time was 1 week. The colonies were counted with a colony counter (Scienceware Electronic Handheld Colony Counter, Fisher Scientific, Fairlawn, N.J.), and the airborne concentration of culturable microorganisms (expressed as colony forming units per cubic meter of air, CFU/m^3) was calculated as described by Wang et al.[29]

For microscopic enumeration of fungal spores, an aliquot of 15 mL of each extract was filtered through a 13-mm mixed cellulose ester membrane filter in a sterile filter funnel inside a biological safety cabinet (Sterilchem-Gard Class II, Type B2; Baker Co., Sanford, Maine). The filter was removed after filtration and placed on a microscopic slide inside the biological safety cabinet until it was completely dry. The filter disc was made transparent for microscope analysis by acetone vapor generated by a modified acetone-vaporizing unit (Quickfix model; EMS, Charleston, S.C.).[30] The sample was mounted by using glycerin jelly (gelatin: 20 g, phenol crystals: 2.4 g, glycerol: 60 mL, sterile deionized water: 70 mL) with a 25 × 25 mm cover glass and was sealed by transparent nail enamel. The fungal spores were counted and identified to the genus or group level using a high-resolution light microscope (Labophot 2; Nikon Corp., Tokyo, Japan) in 40 microscopic fields at 400× magnification and the counts were converted into airborne concentration (spores/m^3).[30]

Calculation of Aerodynamic Size for Airborne Dust and Fungal Spores

The penetration efficiency through the sampling line depends on the aerodynamic size. In order to obtain accurate concentrations of airborne dusts and microorganisms in the field, the size information of dust particles and individual microorganism is needed. For airborne dust, the aerodynamic size, d_a, was calculated from the optical size measured by the OPC, assuming that the particles were spherical with a density equal to 1 g/cm^3. As the OPC cannot distinguish biological particles from dust particles, we measured the optical size (length and width) of spores under the microscope. At least 20 spores of each group/genus were measured from samples collected through the ambient sampling line. The spore d_a was calculated following procedures described earlier by this research group.[31] Because the shape of fungal spores was slightly or considerably ellipsoidal, the volume equivalent diameter (d_{volume}) was calculated as follows:

$$d_{volume} = \sqrt[3]{W^2 L} \quad \textbf{(1)}$$

where W is the width and L is the length of the spore. The representative d_{volume} for each type of fungal spores was the average of 20 measurements of d_{volume}. The aerodynamic size was calculated by the following equation:

$$d_a = \sqrt{\frac{\rho_p}{\rho_0 \chi}} d_{volume} \quad \textbf{(2)}$$

where ρ_p is the density of the particle and χ is the dynamic shape factor. As the density of the microorganisms was not known, we assumed that $\rho_p = 1$ g/cm^3 for all species. The information on the shape factor of spores is available in the aerosol literature.[32–34] The mean d_a, which represents the particle size for both airborne dust and microorganisms, was the arithmetic average of upper and lower size of each aerodynamic size range.

Penetration Factor for Particle Losses in the Sampling Lines

In our previous study,[26] the particle losses occurring during the aerosol transmission through the personal sampling system were investigated by accounting for gravitational, inertial, and turbulent deposition. In order to determine the actual aerosol concentration in the agricultural environments and to facilitate the comparison of the results with other studies, a penetration factor accounting for particle losses in the ambient sampling line were included. The calculations of the penetration factors for particles in the size range of 0.7 μm to 10 μm were performed separately for the following sections of the ambient sampling line: a straight section of Tygon tubing, one bend of the Tygon tubing, the sampling probe, and the metal sampling chamber as described in Lee et al.[26] At the constant sampling flow rate of 10 L/min, the following dimensions were used to calculate particle losses in Tygon tubing: tubing diameter of ½ inch (1.27 cm); tubing length of 40 inches (101.6 cm) and one bend (angle = 180° and radius = 12.7 cm). The diameter of 0.25 cm and the length of 1.11 cm were used for evaluating particle losses in the sampling probe. The particle losses occurring during the aerosol transport through the metal sampling chamber were also determined for calculating the penetration factor, as described in detail in Lee et al.[26]

The accuracy of the correction was determined by comparing the corrected fungal spore concentrations to those obtained simultaneously with the Button Aerosol Samplers (SKC, Inc., Eighty Four, Pa.), attached to the human subject wearing the respirator and the new personal sampling system on a swine farm. The methodology was described in detail by Adhikari et al.[25]

RESULTS AND DISCUSSION

The measured optical and the estimated aerodynamic particle sizes and the penetration factor for airborne dust and microorganisms are presented in Table II. As seen from this table, *Aspergillus/Penicillum* had the smallest aerodynamic size of 2.6 μm to 4.8 μm in the field samples, whereas the

TABLE II. Particle Size Parameters and the Correction Factor to Account for Losses of Airborne Dust and Fungal Spores in the Sampling Line

Particle Type	Measured Size Parameter[A]	Measured Size Range (μm)[A]	Aerodynamic Size Range (μm)[B]	Mean Aerodynamic Size (μm)[C]	Particle Loss Correction Factor[D]
Dust	OPC-measured diameter (d_{opt})	0.7–1	0.7–1	0.9	0.986
		1–2	1–2	1.5	0.966
		2–3	2–3	2.5	0.908
		3–5	3–5	4.0	0.778
		5–10	5–10	7.5	0.607
Aspergillius/Penicillium	Optical width Optical length	2.6–4.8[E]	2.6–4.8	3.7	0.862
Ascospores	Optical width	2.6–5.9	3.7–7.5	5.6	0.840
	Optical length	6.6–16.7			
Basidiospores	Optical width	4.8–7.3	5.2–8.3	6.8	0.712
	Optical length	5.2–12.4			
Cladosporium	Optical width	4.3–9.2	5.2–10.9	8.1	0.663
	Optical length	7.8–16.6			
Smut spores	Optical width Optical length	8.3–11.0[E]	8.3–11.0	9.7	0.559
Alternaria	Optical width	7.9–12.6	11.2–17.8	14.5	0.517
	Optical length	30.2–57.0			
Epicoccum	Optical width Optical length	15.7— 22.0[E]	15.7–22.0	18.9	0.253

[A] For dust, the measured size is referred to the aerosol particle size measured by the OPC. For fungal spores, the measured size is referred to the optical length and width measured under the microscope.
[B] Aerodynamic size for dust particles was calculated from the optical particle size measured by the OPC assuming that the particles are spherical with a density = 1g/cm^3. Aerodynamic size of fungal spores was calculated based on their optical dimensions (length and width) measured by the microscope assuming the spore density = 1 g/cm^3.
[C] Mean aerodynamic size is an arithmetic average of the upper size and lower size, determined for each aerodynamic size range.
[D] Correction factor is applied to the calculated value of the particle penetration efficiency to account for the particle losses through the sampling system.[35] This factor was used to correct the concentrations of particles sampled into the sampling lines.
[E] If the fungal spore is spherical, the optical size is referred to as a diameter (width = length), and for the particle density of 1 g/cm^3 is equal to the aerodynamic particle size.

Epicoccum had the largest aerodynamic size of 15.7 μm to 22.0 μm. The most prevalent airborne fungal spores observed in agricultural environments are listed in the ascending order according to their aerodynamic size: *Aspergillus/Penicillum* < Ascospores < Basidiospores < *Cladosporium* < Smut spores < *Alternaria* < *Epicoccum*. The penetration factors accounting for losses in the ambient sampling line ranged from 0.607 to 0.986 for dust particles with the mean aerodynamic size ranging from 0.9 μm to 7.5 μm, and from 0.253 to 0.862 for fungal spores with the mean aerodynamic size of 3.7 μm to 18.9 μm (Table II). These factors were used to correct the concentrations of particles and predominant fungal spores on agricultural farms. For bacteria, the penetration factor was 0.986 assuming that the mean aerodynamic size of all bacteria was approximately 0.9 μm. Thus, the bacterial losses are negligible and no correction was needed. The validation experiment performed on a swine farm showed that the concentration of fungal spores measured in the ambient sampling line and corrected for predominant fungi was close to the ones measured simultaneously with two Button Samplers. The concentration of total fungal spores obtained with the ambient sampling line and corrected for losses was 3.3×10^4 spores/m^3; both Button Samplers measured concentration of 3.4×10^4.

The total number concentration of particles (non-normalized) ranging from 0.7 μm to 10 μm observed in animal confinements was between 1.7×10^6 and 2.9×10^7 particles/m^3. Figure 1 presents the distribution of particle concentrations as a function of particle size on different agricultural farms. The number concentration of airborne particles in each size range (ΔC_N) was normalized by dividing it by a logarithmic interval of the corresponding particle size range. As shown in Figure 1A, the concentration of particles was higher on the poultry farm than on other types of animal confinements. This can be attributed to the low ventilation rate and continuous particle aerosolization from moving poultry.

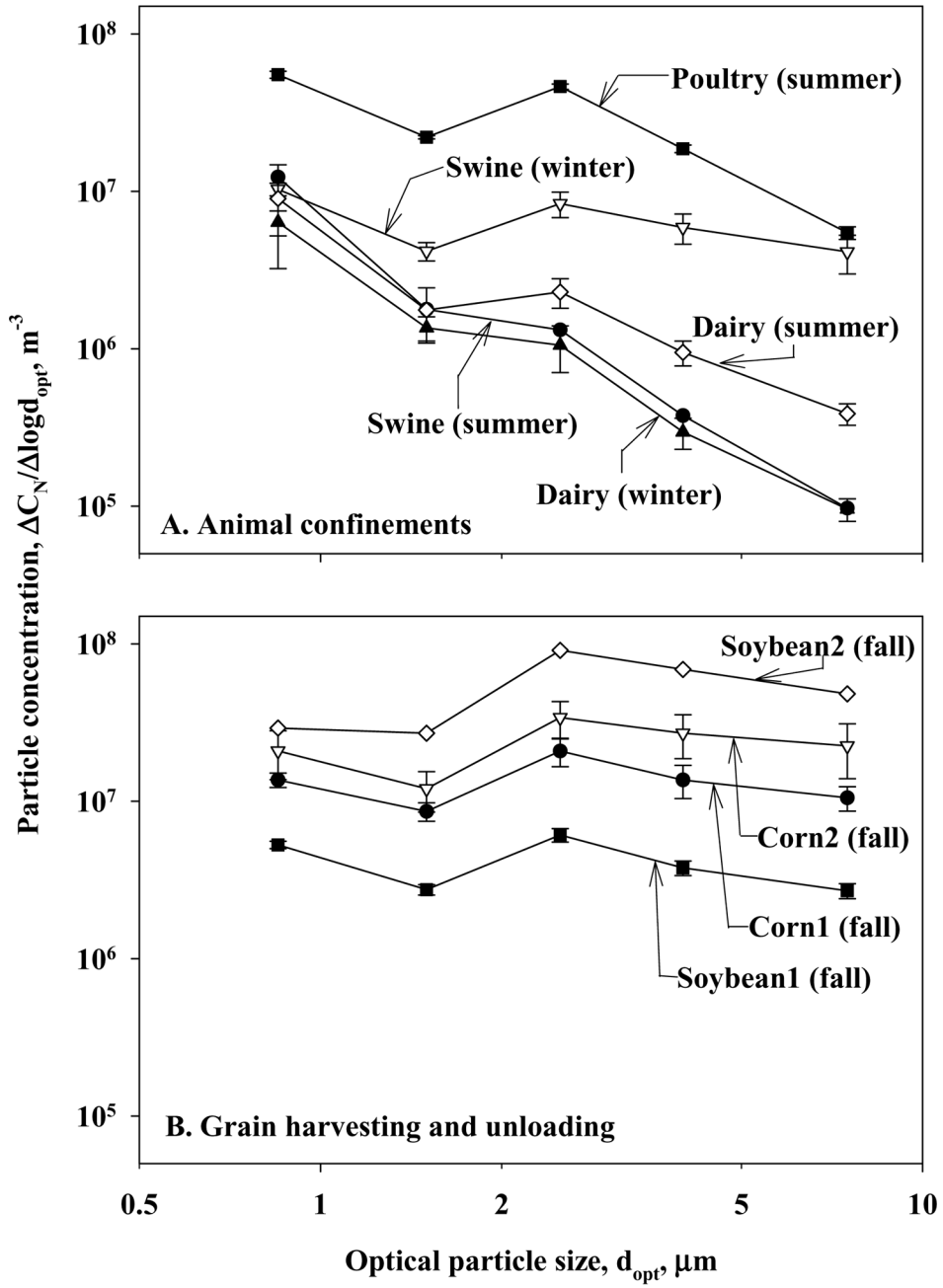

FIGURE 1. Normalized total particle concentrations as a function of particle size measured in animal confinements during different work procedures (A), and outdoors during grain harvesting (B). The concentrations have been corrected for particle losses with sampling lines using the correction factors presented in Table II.

In all animal confinements, the number concentrations of small particles (0.7 μm to 3 μm) were higher than those of large particles (3 μm to 10 μm). On the swine farm, the concentrations of particles were higher in winter than in summer. This might be due to additional space enclosure that was introduced in winter to protect hogs from the cold weather. Thus, the aerosols generated during pig feeding activities were not diluted by outdoor air, resulting in higher aerosol concentrations in winter months as compared with the summer months when the swine confinement was more open.

On the dairy farm, no additional enclosing was used in the barn during the year. Therefore, the seasonal difference in the particle concentrations measured on the dairy farm was not as distinct as in the swine confinement. The particle concentration decreased with increasing particle size on the dairy farm during summer and winter and on the swine farm during summer.

This occurred because there was no continuous aerosolization source for large particles in the dairy and swine confinements compared with the poultry confinement. Feed was delivered directly to the trough in the swine confinement, and hay was distributed near the cow feeding area by a truck in the dairy confinement. There were no visible particles observed in these two confinements. In contrast, in the poultry confinement, the high poultry activity aerosolized large particles. For instance, we observed feathers and grain dust suspended in the air and sometimes poultry manure splashed on clothes and face.

Our results support those of Bakutis et al.,[35] who found significantly higher dust concentration in pig and poultry houses than in cowsheds. In addition, they found higher dust concentration in insulated cowsheds than in uninsulated ones. With respect to mass concentration, Molocznik found previously that respirable dust represented up to 10% from the total dust in both cattle and swine farms.[19] Lee et al.[36] showed that respirable dust was 3% of inhalable dust during fruit harvesting.

During grain harvesting, the non-normalized total number concentrations of particles in the size range of 0.7–10 μm were between 4.4×10^6 and 5.8×10^7 particles/m^3 (Figure 1B). The concentration levels were rather consistent across the five particle size ranges as compared with the concentrations in the confinements. As seen in Figure 1B, the contribution of large particles (> 2 μm) generated during harvesting and unloading was greater than that measured in animal confinements, whereas the contributions of small particles were about the same in both types of agricultural environments. Interestingly, the size range of these "large" particles (2–10 μm) represents most species of airborne fungal spores.

Table III presents the culturable airborne microbial concentrations for bacteria, actinomycetes and fungi, total airborne fungal spore concentration, fungal culturability, and contribution of fungal spores to the total particle concentration in the size range of 2–10 μm. This information is presented based on the test performed in different agricultural environments in different seasons. The mean microbial concentrations ranged from 3.0×10^3 to 3.3×10^8 CFU/m^3 for culturable bacteria, from <LOD to 2.6×10^4 CFU/m^3 for culturable actinomycetes, from 3.0×10^2 to 7.4×10^6 CFU/m^3 for culturable fungal spores, and from 9.0×10^2 to 6.1×10^6 spores/m^3 for total fungal spores. It was noticeable that the concentration of microorganisms varied more than the concentration of particles in different agricultural farms. The highest bacterial concentration (3.3×10^8 CFU/m^3) was observed in the swine confinement during summer. Highest concentrations of actinomycetes were found during corn harvesting and on the poultry farm. The concentrations of fungal spores were found to be higher during grain harvesting in the fields than during animal feeding in animal confinements.

The results also show the seasonal variation of microbial concentrations. The concentration of bacteria and fungal spores measured on the swine and dairy farms in summer was higher than in winter. Since most fungi cannot grow and sporulate properly at the low temperature during winter, the lower concentration of fungal spores in winter is expected. Snow cover in winter also reduces the background concentration of fungal spores in the outdoor air.

There are no guidelines or threshold limit values for microbial concentrations in occupational or nonoccupational environments. Therefore, the results have to be assessed in comparison with other studies. Table IV shows the concentrations of airborne dust and microorganisms previously presented in the literature. The comparison of concentrations obtained in this study with those reported in the literature has to be done with caution because of different sampling methods. Therefore, Table IV includes only those studies that used filter sampling similarly to our study. Concentration of culturable bacteria obtained in our study was about the same level as that reported in previous studies for swine confinements,[37–39] corn harvesting,[38,40,41] and soybean harvesting and unloading.[8] It appears, however, that our results obtained in poultry and dairy confinements were 10^2 to 10^4 times lower than those measured in previous studies.[37,38,42] For actinomycetes, our data show about tenfold higher than those measured earlier for harvesting.[41] When comparing our results obtained for fungal spores with the earlier findings, the concentrations of culturable fungi were about the same range as those reported previously for animal confinements[13,15,37,39,41,42] and harvesting.[8,41] With regard to total fungi, it appears that the concentrations of total fungi obtained in our study were approximately 10 to 1000 times lower than those presented previously for animal confinements[13,15,37,38,43] and about the same as those reported previously for grain harvesting.[38] As seen in Table IV, previous studies also show that the dust concentrations obtained in poultry confinements[37,38] and grain harvesting[38,40] were higher than those obtained in swine[37,38] and dairy confinements.[15,38,42]

The culturability of fungal spores ranged from 29% to >100%. The culturablity of greater than 100% for fungal spores was found in this study and this observation may be attributed in part to the growth of fungal mycelium fragments on agar plates. Fungal mycelium was not counted as fungal spores under microscope. Both fungal spores and mycelium, however, can form colonies on the agar medium. This can cause the culturability of fungal spores to be greater than 100% in some cases. Moreover, the spores immediately released from the substrates in agricultural environments may have culturability close to 100%, which may be detected as >100% due to the variability in the counting results. The culturabilty of greater than 100% was also reported in Adhikari's study[44] and was attributed to the difference in sampling methods used for culturable and total count. We found that the culturability of fungal spores was higher during harvesting than during animal feeding. Furthermore, it was higher on the dairy farm than on the swine farm. The culturability of fungal spores measured on the dairy farm decreased in winter, whereas it remained about the same in the swine farm in both the summer and the winter. This variation in the culturability of airborne spores is likely caused by the variation in the spore culturability in the fungal

TABLE III. Culturable and Total Airborne Microbial Concentrations, Culturability of Fungal Spores, and Relative Contribution of Fungi to the Concentration of Total Aerosol Particles (2–10 μm) in Different Farm Environments

	Swine		Poultry	Dairy		Corn Harvesting		Soybean	
Measured Parameter	**Summer (n = 1)**	**Winter (n = 4)**	**Summer (n = 2)**	**Summer (n = 2)**	**Winter (n = 3)**	**Corn1 Fall (n = 2)**	**Corn2 Fall (n = 4)**	**Soybean1 Fall (n = 2)**	**Soybean2 Fall (n = 1)**
Concentration of culturable bacteria (CFU/m^3)	3.3×10^8	$(1.0 \pm 0.5) \times 10^4$	$(3.4 \pm 1.4) \times 10^5$	$(1.3 \pm 0.3) \times 10^4$	$(0.3 \pm 0.4) \times 10^4$	$(3.5 \pm 0.6) \times 10^5$	$(1.4 \pm 1.1) \times 10^6$	$(4.0 \pm 0.4) \times 10^4$	7.5×10^4
Concentration of culturable actinomycetes (CFU/m^3)	0.3×10^3	<LOD	$(2.8 \pm 2.7) \times 10^3$	$(0.2 \pm 0.2) \times 10^3$	$(0.2 \pm 0.2) \times 10^3$	$(2.6 \pm 0.1) \times 10^4$	$(1.7 \pm 1.4) \times 10^4$	<LOD	<LOD
Concentration of culturable fungal spores (CFU/m^3)	4.1×10^3	$(2.0 \pm 2.7) \times 10^3$	$(2.8 \pm 2.1) \times 10^4$	$(3.9 \pm 0.6) \times 10^4$	$(0.3 \pm 0.3) \times 10^3$	$(1.6 \pm 0.01) \times 10^6$	$(7.4 \pm 2.3) \times 10^6$	$(7.5 \pm 2.8) \times 10^5$	8.2×10^4
Concentration of total fungal spores (spores/m^3)[A]	14.2×10^3	$(5.8 \pm 4.3) \times 10^3$	$(1.8 \pm 0.1) \times 10^4$	$(3.6 \pm 0.6) \times 10^4$	$(0.9 \pm 0.2) \times 10^3$	$(1.2 \pm 0.02) \times 10^6$	$(6.1 \pm 2.1) \times 10^6$	$(8.3 \pm 0.01) \times 10^5$	3.4×10^4
Culturability of fungal spores (%)[B]	29	29 ± 22	162 ± 130	110 ± 22	43 ± 48	141 ± 3	124 ± 17	91 ± 34	238
Fraction of fungal spores (%)[C]	4.12	0.14 ± 0.09	0.13 ± 0.01	4.88 ± 0.16	0.31 ± 0.07	12.00 ± 2.21	36.98 ± 18.54	30.61 ± 3.34	0.07

Notes: The concentrations have been corrected for particle losses using the correction factors presented in Table II. Each cell (except the first and the last columns) presents mean ± standard deviation. LOD is limit of detection (ranged from 71 to 308 CFU/m^3); n is number of observations.

[A]Concentration of total fungal spores, determined by microscopic analysis, represents the sum of the concentrations of spores from all fungal genera. Genera/group species comprising the total concentration of fungal spores are presented in Table V.

[B]Culturability of fungal spores: [concentration of culturable fungal spores]/[concentration of total fungal spores].

[C]Fraction of fungal spores: [concentration of total fungal spores]/[concentration of all particles in the size range of 2 μm to 10 μm].

TABLE IV. Airborne Dust and Microbial Concentrations Measured in Agricultural Environments in Previous Studies

Measured Parameter	Swine	Poultry	Dairy	Grain	Soybean
Concentration of culturable bacteria (CFU/m^3)	LOD–1.6×10^8, $5.8\times10^{6\,A(36)}$ $(1.5\times10^7)^{B,C(37)}$ 4.1×10^4–3.3×10^6 $(4.7\times10^5)^{(38)}$	5.7×10^5–1.6×10^9, $(7.9\times10^7)^{A(36)}$ $(4.8\times10^7)^{B,C(37)}$	$(2.8\times10^6)^{B,C(37)}$ 8.9×10^3–5.2×10^6 $(3.4\times10^5)^{B(41)}$	$(1.1\times10^7)^{B,C(37)}$ $(7.6\times10^4)^{(39)}$ 5.8×10^4–$1.0\times10^{9(40)}$	LOD–3.1×10^5 $(2.2\times10^4)^{B(8)}$
Concentration of culturable actinomycetes (CFU/m^3)				LOD–$2.3\times10^{3(40)}$	
Concentration of culturable fungal spores (CFU/m^3)	LOD–4.3×10^6 $(3.8\times10^5)^{A(36)}$ 1×10^3–6.5×10^3 $(3.4\times10^3)^{(38)}$	1.4×10^4–1.1×10^8 $(4.4\times10^5)^{A(36)}$	10^1–$10^{7(13)}$ 1.7×10^3–1.6×106 $(1.9\times10^4)^{B(41)}$ 5.5×10^3–3.7×10^6 $(7.6\times10^4)^{B(42)}$	$(2.1\times10^4)^{(39)}$ 1.8×10^3–$1.3\times10^{7(40)}$	LOD–$9.7\times10^6(5\times10^5)^{(8)}$
Concentration of total fungal spores($spores/m^3$)	LOD–1.4×10^8 $(8.7\times10^6)^{A(36)}$ $(2.0\times10^5)^{B(37)}$	LOD–1.1×10^9 $(2.0\times107)^{A(36)}$ $(3.0\times105)^{B(37)}$	$(4.0\times10^4)^{B(37)}$ 8.7×10^5–9.8×10^6 $(1.7\times10^6)^{B(42)}$	$(2.0\times10^6)^{B(37)}$	
Total dust (mg/m^3)	1.1–13.8 $(4.0)^{A(36)}$ $(3.1)^{B(37)}$	0.4–21.8 $(7.0)^{A(36)}$ $(5.0)^{B(37)}$	$(1.2)^{B(37)}$ 0.01–6.5 $(0.74)^{B(41)}$ 0.5–3.4 $(1.4)^{B(42)}$	$(5.1)^{B(37)}$ 2.8–15.2 $(7.6)^{(39)}$	2.7–62.8 $(11.9)^{B(8)}$

Notes: Only studies that used filter sampling are included. The table lists the concentration range separately for different studies. The mean value is given in parentheses, if available. LOD is limit of detection.

[A] Median.

[B] Geometric mean.

[C] Bacteria measured by non-culture-based method.

TABLE V. Concentrations of Most Common Fungal Genera or Groups (spores/m^3) by Microscopic Analysis

	Swine		Poultry	Dairy		Corn Harvesting		Soybean	
Fungal Genera or Group	**Summer (n = 1)**	**Winter (n = 4)**	**Summer (n = 2)**	**Summer (n = 2)**	**Winter (n = 3)**	**Corn1 Fall (n = 2)**	**Corn2 Fall (n = 4)**	**Soybean1 Fall (n = 2)**	**Soybean 2 Fall (n = 1)**
Aspergillus/ Penicillium	6.1×10^3	$(1.7 \pm 0.4) \times 10^3$	$(1.8 \pm 0.6) \times 10^3$	$(4.7 \pm 0.5) \times 10^3$	$(0.7 \pm 0.2) \times 10^3$	$(1.6 \pm 0.9) \times 10^3$	$(3.5 \pm 3.4) \times 10^3$	$(2.7 \pm 0.9) \times 10^3$	6.1×10^3
Ascospores	2.2×10^3	$(1.9 \pm 0.9) \times 10^2$	$(5.0 \pm 1.9) \times 10^2$	$(9.9 \pm 4.8) \times 10^2$	$(0.5 \pm 0) \times 10^2$	$(7.4 \pm 10.4) \times 10^2$	<LOD	$(8.6 \pm 12.1) \times 10^2$	4.5×10^2
Basidiospores	5.3×10^2	$(0.3 \pm 0.5) \times 10^2$	$(1.1 \pm 0) \times 10^2$	$(1.1 \pm 0.5) \times 10^3$	<LOD	$(2.5 \pm 3.5) \times 10^2$	$(5.1 \pm 7.1) \times 10^3$	$(2.9 \pm 1.9) \times 10^3$	1.3×10^2
Cladosporium	4.5×10^3	$(1.4 \pm 1.0) \times 10^3$	$(1.2 \pm 0.02) \times 10^4$	$(2.7 \pm 0.5) \times 10^4$	<LOD	$(9.0 \pm 0.1) \times 10^5$	$(5.8 \pm 2.0) \times 10^6$	$(8.0 \pm 0.1) \times 10^5$	6.4×10^3
Smut spores	0.5×10^3	$(1.4 \pm 2.8) \times 10^3$	$(2.5 \pm 0.4) \times 10^3$	$(0.6 \pm 0.4) \times 10^3$	<LOD	$(2.1 \pm 0.2) \times 10^5$	$(2.2 \pm 1.0) \times 10^5$	$(6.8 \pm 4.5) \times 10^3$	1.1×10^4
Alternaria	<LOD	$(0.2 \pm 0.4) \times 10^2$	<LOD	$(2.4 \pm 3.0) \times 10^2$	<LOD	$(1.5 \pm 0.5) \times 10^4$	$(1.1 \pm 1.2) \times 10^4$	$(9.8 \pm 9.0) \times 10^3$	3.7×10^3
Epicoccum	<LOD	$(0.7 \pm 1.1) \times 10^3$	<LOD	<LOD	$(0.1 \pm 0.1) \times 10^3$	$(3.0 \pm 1.0) \times 10^4$	$(4.1 \pm 2.1) \times 10^4$	$(6.9 \pm 3.7) \times 10^3$	6.0×10^3
Other fungi	3.1×10^2	$(1.5 \pm 1.0) \times 10^2$	$(5.3 \pm 3.2) \times 10^2$	$(5.8 \pm 3.8) \times 10^2$	$(0.8 \pm 0.5) \times 10^2$	$(7.6 \pm 0.9) \times 10^2$	$(9.1 \pm 6.6) \times 10^2$	$(1.8 \pm 2.5) \times 10^2$	8.5×10^2

Notes: The concentrations have been corrected for particle losses using the correction factors presented in Table II. Each cell (except the first and the last columns) presents mean ± standard deviation. LOD is limit of detection (ranged from 38 to 95 spores/m^3); n is number of observations.

source, which in turn may be affected by environmental factors such as variation in relative humidity.

The fraction of fungal spores in the concentration of total particles in the size range of 2–10 μm ranged from 0.07% to 37%. This fraction was higher during grain harvesting (12–37%) than during animal feeding (0.13–5%). As discussed above, the concentration of large dust particles (2–10 μm) was also higher during grain harvesting than during animal feeding. These findings suggest that the increase in the particle concentration in the size range of 2 μm to 10 μm during harvesting is partially attributed to an increase in the concentration of the fungal spores. The contribution of biological particles in the total dust exposure has not been previously addressed for agricultural environments, but there are some data available for outdoor air.

Jaenicke[44] estimated that approximately 25% of the count of total atmospheric particles is contributed by all kinds of bioaerosols, including pollen, fungal spores, bacteria, and cellular material. Based on the concentration of phospholipids, Womiloju[45] estimated that cell materials of fungi and pollen could contribute 4–11% of the total PM2.5 mass in outdoor air. Thus, the fraction of biological particles to nonbiological particles measured in our study for fungal spores alone is about the same as previously reported for all biological particles in outdoor air.

It should, however, be kept in mind that Jaenicke and Womiloju et al. used different methods than those utilized in this study for the sampling and analysis of nonbiological and biological particles. The latter methods have also been employed in another study recently conducted in residential homes in the Cincinnati, Ohio, area, which revealed that the fraction of fungal spores from the total particle number in the size range of 2–10 μm varied from 0.08% to 6.5% (unpublished data). These values are lower than those that we found during harvesting and about the same as those obtained during animal feeding. This confirms that farmers' exposure to fungal spores during harvesting is more severe than in animal confinements. In addition, the fraction of fungal spores to total particles was found to be lower in winter than in summer on both the swine and dairy farms.

The concentrations of most common fungal genera or groups in different farm environments are presented in Table V. The mean microbial concentrations ranged from 7×10^2 to 6.1×10^3 spores/m^3 for *Aspergillus/Penicillium*, from

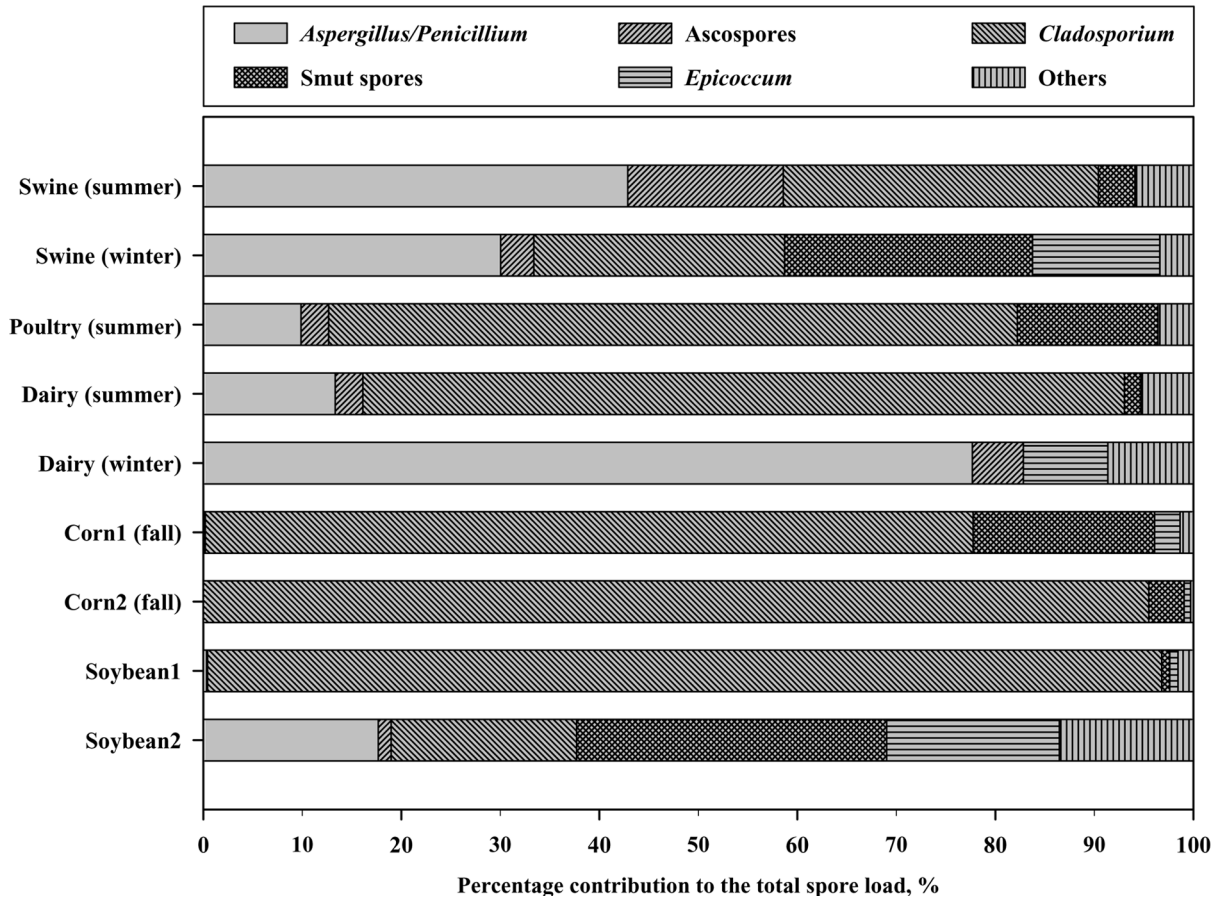

FIGURE 2. Contribution of different fungal genera or groups to the total spore load in different farm environments

< LOD to 2.2×10^3 spores/m^3 for Ascospores, from <LOD to 5.1×10^3 spores/m^3 for Basidiospores, from <LOD to 5.8×10^6spores/m^3 for *Cladosporium*, from <LOD to 2.2×10^5spores/m^3 for Smut spores, from <LOD to 1.5×10^4 spores/m^3 for *Alternaria*, from <LOD to 4.1×10^4 spores/m^3 for *Epicoccum*, and from 80 to 9.1×10^2 spores/m^3 for other fungi.

Figure 2 shows the contribution of different fungal genera or groups to total spore load in different farm environments. *Aspergillus/Penicillium*, *Cladosporium*, and Smut spores were the most prevalent fungi in all agricultural farms and composed about 68% to 99% of the total concentration of fungal spores.

Many of the airborne fungal spores commonly found in this study are potential human health hazards. *Cladosporium, Aspergillus/Penicillium*, and *Alternaria* are strongly associated with allergic respiratory disease, especially asthma.[46] Smuts and *Epicoccum* are reported as important aeroallergens.[46–48] Thus, with respect to farmers' exposure to these fungal spores, the exposure level to fungal spores on agricultural farms should be carefully addressed.

The limitation of this study is its small sample size. The study, however, presents unique data on personal exposures to dust and microorganisms in various types of agricultural environments. The dust particles were measured in the size range of 0.7–10 μm to match with the size of bacteria and fungal spores. Even larger dust particles (up to inhalable range) can cause adverse health effects.

CONCLUSIONS

The personal sampling system, which was used to evaluate protection provided by respirators against airborne dust and microorganisms in our concurrent study, was used to investigate the personal exposure of dust and bioaerosols on farms. Each type of agricultural environment was found to have specific characteristics for the exposure. Harvesting was dominated with exposure to large dust particles with high fraction of fungal spores, whereas the particle size distributions in animal confinements were dominated by small particles. Swine confinement had the highest exposures to bacteria. The results can be used in targeting intervention to address the specific exposures.

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