

Identification of Bioaerosols Released from an Egg Production Facility in the Southeast United States

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Abstract

This field study investigated biological characteristics of aerosols emitted from a commercial egg production farm (layer operation). Bioaerosol samples were taken on this farm at five sampling locations covering emission source (inside a layer barn) and four ambient surrounding stations at four wind directions. All-glass impingers (AGI) were used for the field sampling. AGI fluid samples were plated in duplicate on Trypticase Soy Agar for growth of bacteria and Sabouraud Dextrose Agar for growth of fungi. The most prominent bacterial colony types were identified using a combination of methods that include recording characteristics of colony morphology; performing a Gram staining method and metabolic analyses using the Biolog system. Results from thirty-five AGI samples taken at the five stations through seven sampling events over four seasons indicate that there were significantly lower total bacterial concentrations in the samples collected from ambient stations as compared with the samples collected in the layer house; the mean bacterial concentration at the in-house sampling station was $3.86 \times 10^5 \pm 1.74 \times 10^5$ cfu/m³, whereas the mean bacterial concentrations at four ambient stations in the vicinity of the farm ranged from 1.3×10^3 to 6.2×10^3 cfu/m³ with no significant differences in mean among ambient stations. There were also no significant differences in fungi concentrations among all sampling stations. Mean fungi concentrations at the in-house station was $3.0 \times 10^3 \pm 4.45 \times 10^3$ cfu/m³, whereas the mean concentrations at the ambient stations ranged from 7.4×10^3 to 1.7×10^4 cfu/m³. The most prominent bacterial species differed among all five stations. Three of the most prominent bacteria from samples taken at all five stations were gram positive. Fungal type also differed from station to station.

Introduction

IN THE UNITED STATES, animal feeding operations (AFOs), a vital link in America's food supply, may pose a major risk to the environment and to people working in or near the facilities (Donham, 1990, 1991; Whyte, 1993; Donham and Comro, 1999). In animal production facilities, air often contains high concentrations of particulate matter (PM; organic and/or inorganic particles) and airborne microorganisms and other biologically active substances that are also known as bioaerosols (Thedell *et al.*, 1980; Clark *et al.*, 1983; Heber *et al.*, 1988a, b; Donham, 1990; Maghirang *et al.*, 1997; Donham and Comro, 1999; Bilic *et al.*, 2000; Chang *et al.*, 2001; Predicala *et al.*, 2001; Allen *et al.*, 2003; Ko *et al.*, 2008, 2010). The main sources of bioaerosols are animal skin, feed, bedding materials, and fecal materials. These bioaerosols are subjected to various stresses (e.g., dehydration, toxic air pollutants, etc.) that affect viability and/or recoverability of the injured mi-

croorganisms. Therefore, housing type and growing conditions greatly influence the nature and magnitude of bioaerosol emissions from AFO buildings. Among cattle, swine, broiler, and layer operations, the airborne bacterial and fungal concentrations and emission rates were reported to be the highest in broiler houses followed by houses of layers, fattening pigs and sows (Seedorf *et al.*, 1998).

While it is well known that bioaerosols, once released through ventilation systems of AFO buildings, may transport off the farms and raise health concerns for the people living nearby, limited research has been done to assess the impact of those emissions on the levels of bioaerosols in local areas surrounding the production facilities. Scarpino and Quinn (1998) reported high levels of airborne bacteria and fungi at downwind locations of two swine production facilities and in a nearby residence. Green *et al.* (2006) also observed the elevated bioaerosol concentrations in the vicinity of a swine confinement operation and reported that bacterial concentrations at locations within 100 m downwind of the facility were recovered in levels that could cause a potential human health hazard. It was also further recommended that the optimal setback distance of a swine facility should be at least 200 m from a residence area. Further investigations of AFO

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bioaerosol emissions and their impacts on levels of bioaerosols in local communities for different animal species (e.g., poultry operations) are needed.

The United States is one of the largest poultry producers in the world, producing almost 9 billion heads per year for a total live weight of 22.7 million metric tons (USPEA, 2010; National Chicken Council, 2010). Most poultry are produced in the southeastern states (USDA NASS, 2011). In North Carolina, the poultry industry plays a significant role in the state's agricultural economy and ranks 4th nationally with more than 10% of the nation's poultry production (USDA NASS, 2011). This research focuses on characterizing the biological nature of aerosols emitted from a large commercial poultry facility (layer farm, also known as egg production) in the southeastern United States. The specific objectives of the study were to (1) quantify airborne bacterial and fungal concentrations in an egg production house and at the ambient locations surrounding the layer farm, and (2) identify the predominant airborne bacterial and fungal types or species in the house and in the vicinity of the layer farm.

Materials and Methods

Layer farm and the bioaerosol sampling locations

The bioaerosol sampling was conducted on a commercial layer farm in North Carolina. The layout of the farm and the sampling locations are shown in Fig. 1. This farm consists of four tunnel-ventilated high-rise barns, two cross-ventilated high-rise barns and three naturally ventilated shallow-pit barns. Among these nine egg production barns, two tunnel-ventilated barns (3 and 4) were monitored for the baseline emissions of PM, ammonia (NH_3), hydrogen sulfide (H_2S), carbon dioxide (CO_2), and volatile organic compounds under the National Air Emission Monitoring Study (NAEMS; Wang-Li *et al.*, 2012a), which was overseen by the U.S. Environmental Protection Agency under the AFO Air Compliance

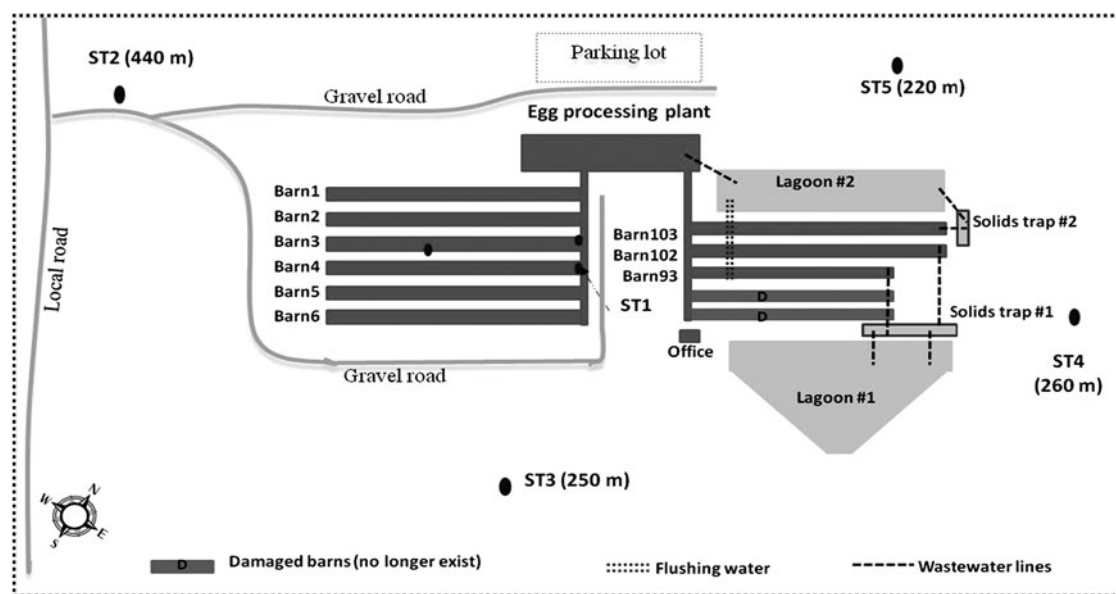
Agreement (EPA, 2005). The baseline emission data of these pollutants are reported elsewhere (Wang-Li *et al.*, 2012a, 2012b; Li *et al.*, 2012a, 2012b; Wang *et al.*, 2012a, 2012b).

Each tunnel-ventilated high-rise barn had dimensions of 175 m long and 18 m wide, housing approximate 95,000 hens in six rows of 4-tier A-frame/curtain backed cages on the upper floor. Manure fell onto the curtain backed cages and then down into the first floor (pit), where it was stored for up to 1 year. The tunnel barn had 34, 122-cm (48-in) diameter, 480 VAC, 3-phase, belt-driven single speed ventilation fans (Chore-time, Milford, IN) located on the east and west end walls, with a spacing of 0.2 m (9 inches). Each end wall had 9 fans on the first floor and 8 fans on the second floor (Fig. 2). Each tunnel barn was ventilated in 11 stages. Two stage-1 fans were located in the middle of each end wall on the first floor. These two stage-1 fans were also known as the primary representative exhaust fans (PREFs). Each of the next six stages (i.e., stages 2–7) added 2 fans, while stages 8–9 added 4 fans each, and stages 10–11 added the final 6 fans.

Bioaerosol sampling locations (stations) are illustrated in Fig. 1. The sampling station 1 (ST1) was located inside barn 4 immediately upstream of the PREF. The sampling stations 2–5 (ST2–5 in Fig. 1) were placed around the farm in four different directions. Each of the sampling stations (ST1–5) was equipped with colocated tapered element oscillating microbalance-PM and a Partisol 2300 $\text{PM}_{2.5}$ speciation sampler (Fig. 3) for measurement of concentrations of PM_{10} , $\text{PM}_{2.5}$, and $\text{PM}_{2.5}$ chemical speciation. A 10 m weather tower was located between barns 2 and 3 at the east end, and sensors for solar radiation, wind speed and direction, ambient temperature and relative humidity (RH) were mounted on the tower for continuous measurements.

Bioaerosol field sample collection

As shown in Figs. 1 and 3, bioaerosol samples were collected at the emission source (in barn 4) and at prescribed



ST1 = station 1 (inside the barn 4); ST2 = station 2; ST3 = station 3; ST4 = station 4; ST5 = station 5

FIG. 1. Layer farm (not to scale) and the locations of the bioaerosol sampling stations.

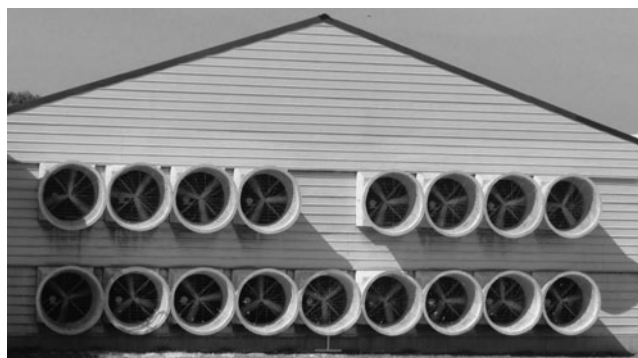


FIG. 2. External view of a tunnel-ventilated high-rise layer barn.

distances and directions from the emission source near the farm property lines using all-glass impingers (AGI). One channel of the PM_{2.5} speciation sampler at each station was used to provide air flow and the flow rate control. The air sampling flow rate was set at 12.4–12.5 L/min for 30 min at all five stations with the same starting time. Consequently, the AGI samples were simultaneously taken at five stations for each sampling event. Seven sampling events were conducted, on May 14, July 14, August 5, October 6, November 17, December 9, and December 15, 2009, with attempt to cover different weather conditions.

In addition to sampling flow rate and time control and calculating valid sampled air volume, this sampler is also capable of measuring and computing average air temperature, barometric pressure, and RH.

All of the AGI impingers were prepared at the RTI International microbiological laboratory. The nutrient broth of impinger fluid consisted of (4.8 g, BD BBL™), NaCl (3.0 g), Antifoam Y-30 Emulsion (1.5 mL, Sigma) in 600 mL water and it was sterilized by autoclaving. The targets for the reported sampling events include (1) total bacterial counts, (2) identification and counts for the most prominent bacterial colonies observed by types (gram-positive, gram-negative, *Bacillus* spp., *Actinomyces*), (3) numbers of fungal types (for samples in May and July), and (4) total fungal counts (for samples in August through December).

Bioaerosol sample analysis

The biological analysis was conducted at the RTI International microbiological lab following standard method for the examination of water and waste water (Clesceri *et al.*, 1989) and Manual of Clinical Microbiology (Lennette *et al.*, 1974). After each sampling event, all the impinger samplers were transported to the RTI International microbiological lab with 24 h in coolers with ice pack (4°C). Upon receipt in this lab, the AGI fluid from each sample was transferred to a sterile tube and its volume determined. The fluid samples, and/or dilutions in F-tab containing 0.1% Tween 80, were plated in duplicate on Trypticase Soy Agar (TSA) for growth of bacteria and on Sabouraud Dextrose Agar (SDA) for growth of fungi. The TSA plates were incubated at 37°C and the SDA plates were incubated at 23°C. TSA at 37°C was selected because the primary focus was the isolation and identification of potential pathogens, not environmental bacteria; whereas SDA was selected because it gives discrete colonies compared with Malt Extract Agar. There was an average time of 2 weeks between

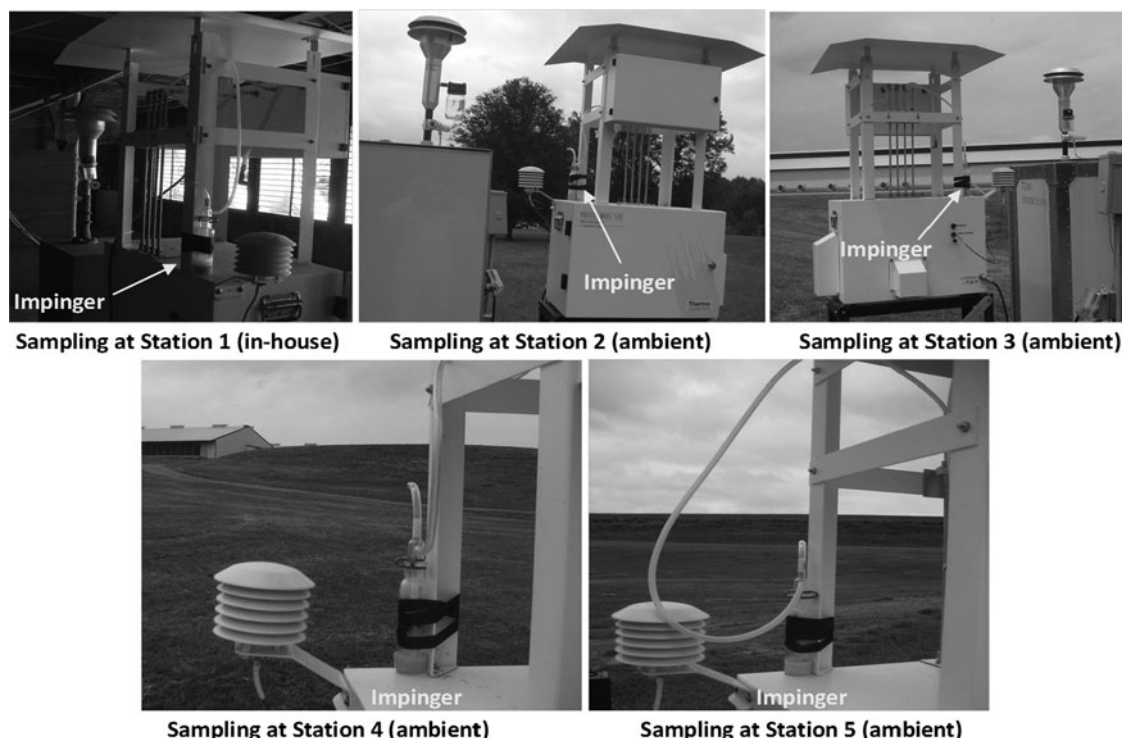


FIG. 3. The all-glass impinger bioaerosol sampler attached to the flow control system of a Partisol 2300 PM_{2.5} speciation sampler at the five sampling stations.

when samples were plated and when final counts were made. Plates were checked daily for growth of colonies and moved to 4°C when colonies were of appropriate size for identification and counting. Impinger fluid not used for immediate plating was stored in the refrigerator. In cases where fewer than 20 bacterial colonies were present on plates of undiluted samples, the remaining impinger fluid stored in the refrigerator was filtered through a sterile Nalgene 0.2 μM filter unit to lower the detection limit. The filters were then removed from the filter units and placed on the agar surface of TSA plates to allow colony formation.

The most prominent bacterial colony types were identified using a combination of methods. First, characteristics of colony morphology were recorded. Second, a Gram staining method was performed and cell morphology of resulting slide mounts was noted. Finally, metabolic analyses were performed using the Biolog system (Biolog MicroLog Microbial Identification System DP database version 6.12).

Upon lab analysis of the colony counts of individual AGI samples, the total bioaerosol (bacteria or fungi) concentration of each AGI sample was calculated using the following equation:

$$Con. = \frac{N}{Q \times t} \quad (1)$$

where *Con.* is the total bacterial or fungal concentration (colony forming units [cfu]/ m^3), *N* is the total bacterial or fungal counts in an AGI sample (cfu), *Q* is the sampling air flow rate (m^3/min), and *t* is the sampling time (min).

Results and Discussion

Total bacteria concentrations

Measurements of total bacterial concentrations, air temperatures and RHs for the seven sampling events at ST1 are shown in Fig. 4. It was observed that the total bacteria concentrations at the source (ST1) varied from 2.5×10^4 to 5.3×10^5 cfu/ m^3 ; the lowest concentration occurred on December 9, 2009 and the highest concentration occurred on August 9, 2009. The reason for the lowest concentration on December 9, 2009 was unknown, but there was a tendency of higher bacterial concentrations in warmer months and under higher RH conditions at this station (ST1).

The mean concentration (\pm SD) at ST1 (in-house) was $3.86 \times 10^5 \pm 1.74 \times 10^5$ cfu/ m^3 . In an environmental study of poultry confined buildings, Jones *et al.* (1984) reported average concentrations of 10^5 cfu/ m^3 and 10^4 cfu/ m^3 for bacteria and fungi, respectively. In another study of airborne microorganism emissions from livestock buildings, Seedorf *et al.* (1998) reported a mean bacteria concentration of 10^6 cfu/ m^3 in poultry houses. This study observed the in-house mean bacterial concentration close to the reported value by Jones *et al.* (1984), but it was one 10-fold less than the reported value by Seedorf *et al.* (1998). Different housing type, growing conditions, waste management practices, and animal types contributed to the differences in in-house air quality including bacterial concentrations.

Measurements of total bacterial concentrations, air temperatures, and RHs for the seven sampling events at ST2–5 are shown in Fig. 5. The total bacterial concentrations at four

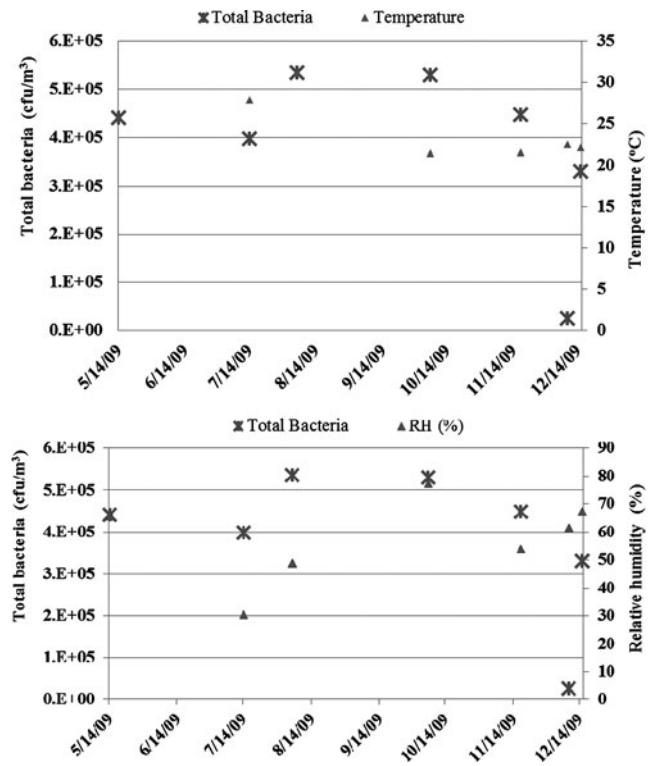


FIG. 4. Measurements of total bacteria concentrations, air temperature, and relative humidity (RH) at sampling station 1 (ST1) for all seven sampling events.

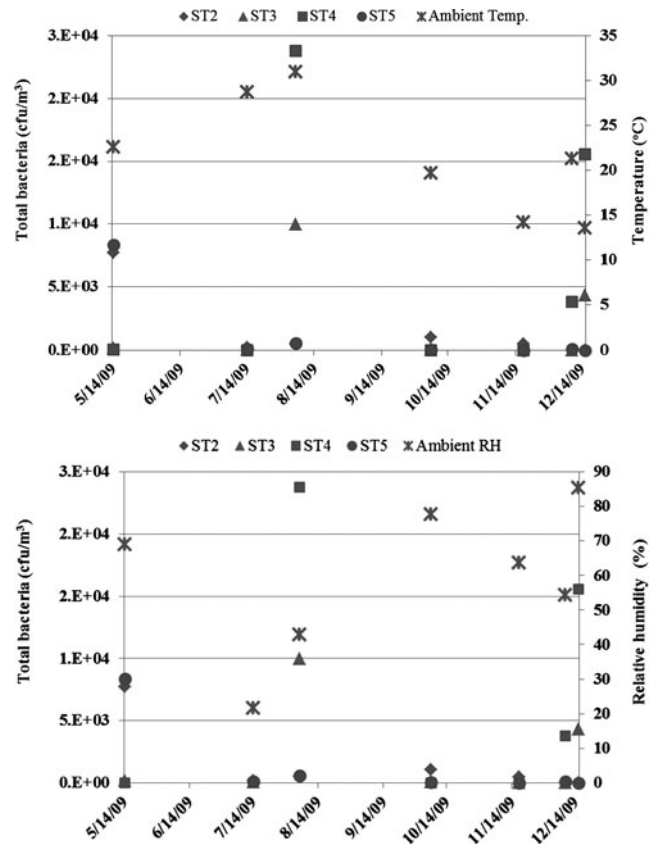


FIG. 5. Measurements of total bacteria concentrations, air temperature, and RH at ST2–5 for all seven sampling events.

ambient stations varied from nondetectable to 2.4×10^4 cfu/m³ with the highest concentration occurred at ST4 on August 5, 2009. Similar to the in-house bacterial concentration, there was a tendency that warmer months had higher bacterial concentrations at the ambient stations.

The mean concentrations of all sampling events at four ambient locations were in the range of 1.3×10^3 (ST5) to 6.2×10^3 cfu/m³ (ST4). Scarpino and Quinn (1998) reported ambient bacteria concentrations of 3.88×10^2 and 1.3×10^3 cfu/m³ in autumn at two sampling locations a few hundred yards away from a swine facility. When examining the data for October and November, the mean ambient bacterial concentrations were in the range of the levels reported by Scarpino and Quinn (1998). Analysis of variance (ANOVA) test revealed no significant difference in mean among ambient stations (ST2–5; $p = 0.2829$) although ST4 seems to have higher mean concentrations. Figure 6 shows the comparison of mean bacterial concentrations, temperature, and RH of all ambient stations combined over different sampling months. As it is illustrated, high concentrations occurred at high air temperature. The sampling event on August 5, 2009 showed the highest mean ambient bacterial concentrations as compared with other months. This is because the highest temperature on this date was in favor of bacterial survival. Moreover, during summer time, the production house ventilation rates were at maximum causing high air emission rate, consequently high bioaerosol concentration in local ambient area.

To compare in-house and ambient bacterial levels, the mean concentrations of all the sampling events at individual sampling stations are shown in Fig. 7. As can be seen, there

was a significant reduction of mean concentrations in ambient stations as compared with the in-house station. The differences in mean between in-house (ST1) and ambient stations (ST2–5) were significant ($p < 0.0001$) over the seven sampling events.

To examine wind effect on the transport of the bacteria emitted from the farm, wind-rose maps were developed for all the sampling events and three of them are illustrated in Fig. 8 along with total bacterial concentrations at each ambient station. Although there was no clear and consistent tendency showing higher downwind concentrations and lower upwind concentrations, it is observed that when the north wind occurred, ST3 and ST4 tended to have higher concentrations than other stations, or higher concentrations at these two stations as compared with other wind directions. When the east or southeast wind occurred, ST2 tended to have higher concentration. South or southwest wind caused higher concentrations at ST5 when compared with other wind directions. There is an indication that wind direction had an impact on the ambient airborne bacterial concentrations at various locations. High upwind concentration at ST2 indicates that there may be other background sources affecting bacterial concentration at this location.

Bacteria speciation

Speciation of the bacterial samples was conducted by identifying most predominant types. Table 1 lists the results of the bacterial speciation analysis. As shown in this table, the predominant bacterial populations varied among stations and across seasons. Greater diversity was observed in the larger bacterial populations (cfu) when compared with the smaller populations (cfu). Gram-positive bacteria, not including *Bacillus* spp., were most prominent in samples from all five stations at all times. In analysis, it was also observed that *Micrococci* were the predominant gram-positive bacterial species across all four seasons (not shown in Table 1). These organisms are generally considered harmless, and they may occur in a wide range of environments including water, dust, and soil (Moon *et al.*, 2009; Womack *et al.*, 2010). *Pseudomonas* was the predominant gram-negative bacteria found in ST1 samples between October and December (not shown in Table 1). Actinomycetes were observed in July samples at ST2, ST3, and ST5. These organisms are ubiquitous in nature and

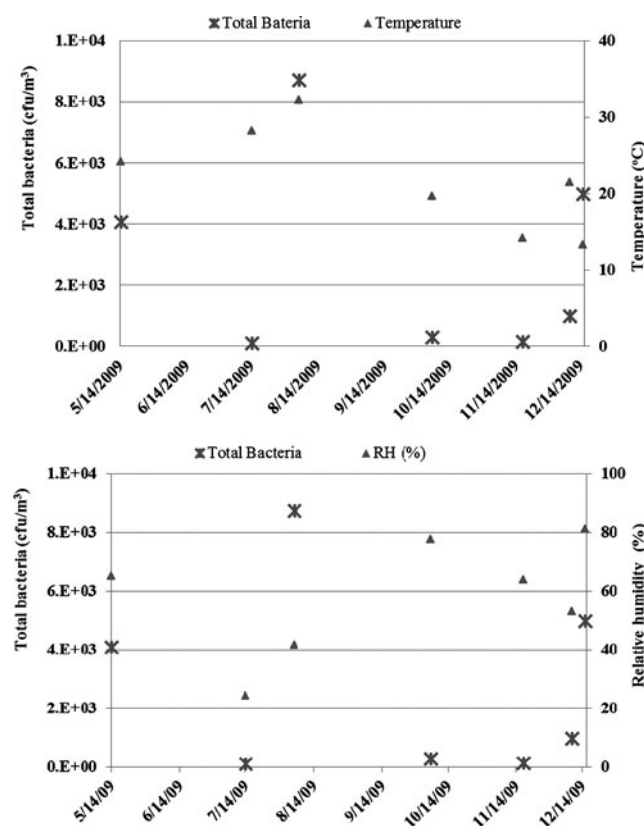


FIG. 6. Comparison of mean bacterial concentrations among different sampling events at ambient stations (ST2–5).

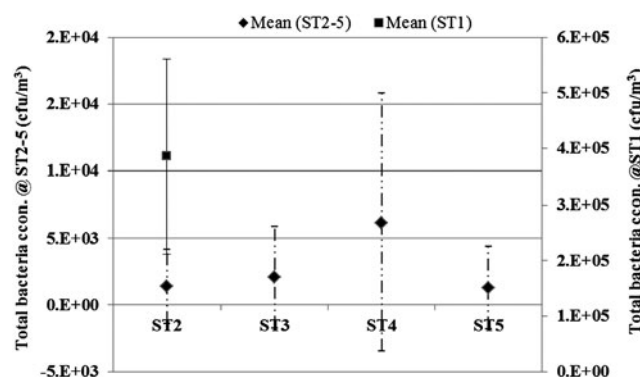


FIG. 7. Mean concentrations (\pm SD) of the total bacteria at ST1 (secondary y -axis on right) and ST2–5 (primary y -axis on left).

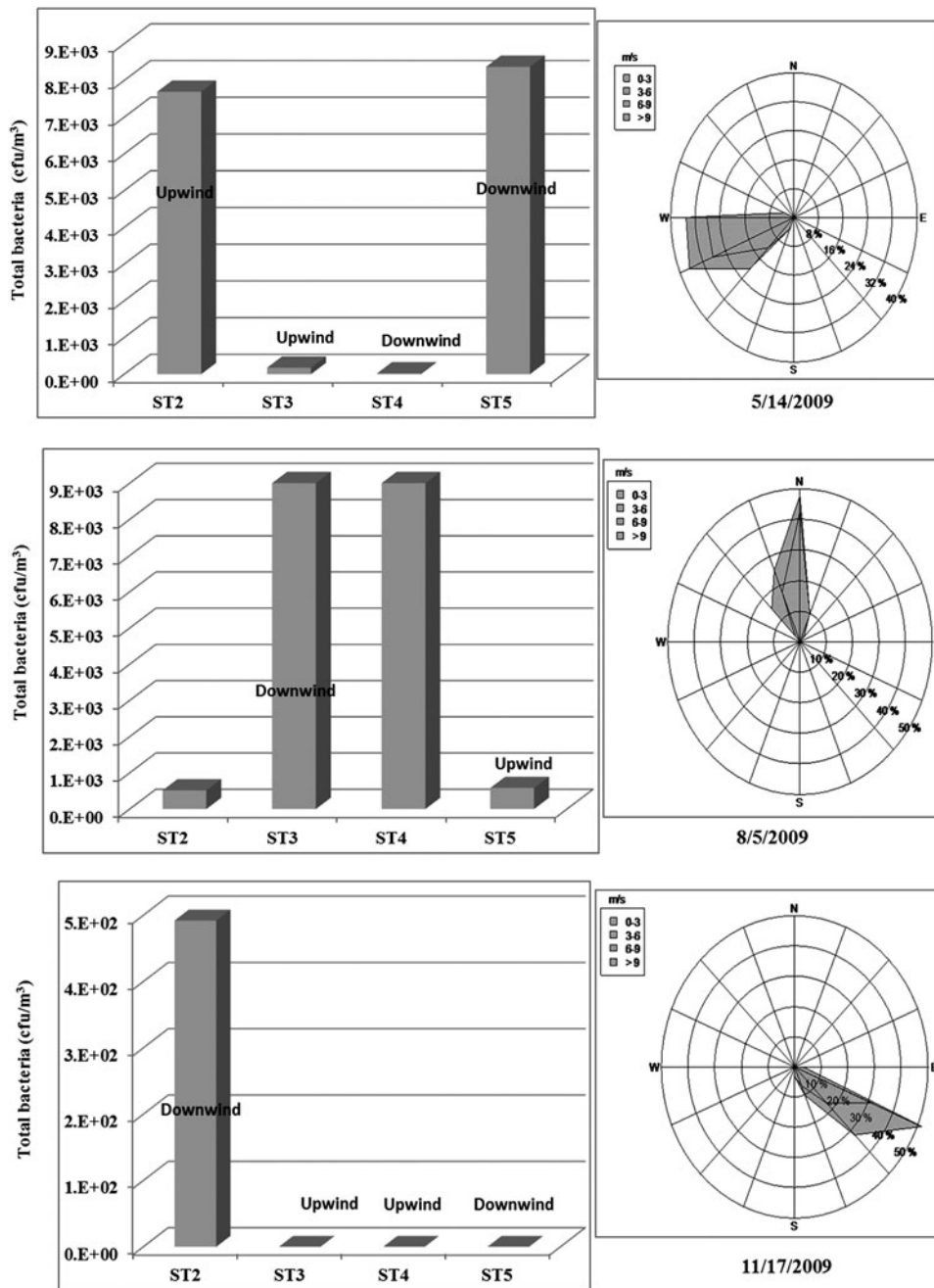


FIG. 8. Total bacteria concentrations at ST2–5 and the wind roses at three sampling events.

belong to a diverse group of gram positive, filamentous bacteria (Waksman, 1950; Mancinelli and Shulls, 1978). Many species of *actinomycetes* occur in soil and are considered harmless (Jeffrey, 2008). *Bacillus* spp. briefly appeared in August samples at ST2.

Fungi concentration and speciation

AGI samples were only analyzed for fungal colony formation counts for August sampling event and after. Table 2 lists the fungal concentrations at five sampling stations for five sampling events. Although there was some variation in the fungal concentrations among the stations, the ANOVA test suggests that the differences between stations were not

significant ($p=0.905$) over the five sampling events. On the other hand, differences in fungal concentrations between months with all the stations combined were significant ($p=0.0001$). The mean fungi concentration at ST1 was 3.0×10^3 cfu/m³ and the mean concentrations at the ambient stations ranged from 7.4×10^3 (ST3) to 1.7×10^4 cfu/m³ (ST4). As observed in bacterial concentration measurements, the lowest fungal concentration at ST1 occurred on December 9, 2009. Significantly higher concentrations were observed for the event on December 15, 2009 at all ambient stations. This was not observed in bacterial measurements. In cold time, the animal house ventilation was maintained minimal to minimize the heat losses. Low ventilation rate led to low air emission rates from the animal houses, and consequently had

TABLE 1. PROMINENT BACTERIAL TYPES AT FIVE SAMPLING STATIONS

Types	ST1	ST2	ST3	ST4	ST5
5/14/09					
Gram positive	31%	65%	100%	100%	30%
Gram negative	ND	ND	0	0	ND
<i>Actinomyces</i>	ND	ND	0	0	ND
<i>Bacillus</i>	ND	ND	0	0	ND
Unknown	ND	ND	0	0	ND
7/14/09					
Gram positive	30%	67%	0	0 ^a	33%
Gram negative	ND	0	0	0	0
<i>Actinomyces</i>	ND	33%	100%	0	66%
<i>Bacillus</i>	ND	0	0	0	0
Unknown	ND	0	0	0	0
8/5/09					
Gram positive	98%	31%	100%	97%	37%
Gram negative	2%	15%	0	3%	0
<i>Actinomyces</i>	0	0	0	0	0
<i>Bacillus</i>	0	31%	0	0	0
Unknown	0	23%	0	0	63%
10/6/09					
Gram positive	81%	50%	100%	0 ^a	100%
Gram negative	8%	4%	0	0	0
<i>Actinomyces</i>	2%	0	0	0	0
<i>Bacillus</i>	0	0	0	0	0
Unknown	9%	46%	0	0	0
11/17/09					
Gram positive	95%	85%	0 ^a	0 ^a	0 ^a
Gram negative	5%	15%	0	0	0
<i>Actinomyces</i>	0	0	0	0	0
<i>Bacillus</i>	0	0	0	0	0
Unknown	0	0	0	0	0
12/9/09					
Gram positive	76%	0 ^a	0 ^a	85%	100%
Gram negative	24%	0	0	15%	0
<i>Actinomyces</i>	0	0	0	0	0
<i>Bacillus</i>	0	0	0	0	0
Unknown	0	0	0	0	0
12/15/09					
Gram positive	87%	0 ^a	75%	85%	0 ^a
Gram negative	13%	0	25%	15%	0
<i>Actinomyces</i>	0	0	0	0	0
<i>Bacillus</i>	0	0	0	0	0
Unknown	0	0	0	0	0

Analysis and reporting format changed beginning with August sampling event.

^aBacteria counts were not detectable.

ST1–5, sampling stations; ND, not done or <1%.

low impact on ambient bioaerosol concentrations. Therefore, the observation of high fungal concentration at ambient locations suggests that there might be other fungi sources affecting ambient fungal concentrations in the vicinity of this farm.

The fungal speciation was conducted through identification of most predominant fungi types. As shown in Table 3, for the sampling events in May through August, *Fusarium* and *Acremonium*-like organisms were most prominent at all stations. However, these gave way to a variety of yeasts and sterile hyphae for sampling events in October through December. It is suspected that there may be some seasonal effects on fungal types and concentrations at ambient locations; more investigation needs to be conducted to help better understand this assumption.

Summary and Conclusions

Field study of bacterial and fungal concentrations in an egg production facility and its surrounding area was conducted. Analysis of thirty-five AGI samples taken at the five stations through seven sampling events over four seasons indicates that there were significant reductions in total bacterial concentrations in the samples collected from the ambient stations as compared with the samples collected in a layer house. The mean bacterial- and fungal concentrations at the in-house sampling station were $3.86 \times 10^5 \pm 1.74 \times 10^5$ cfu/m³ and $3.0 \times 10^3 \pm 4.45 \times 10^3$ cfu/m³, respectively. The mean bacterial and fungal concentrations at the four ambient stations in the vicinity of the farm ranged from 1.3×10^3 to 6.2×10^3 cfu/m³ and 7.4×10^3 to 1.7×10^4 cfu/m³, respectively. No significant differences in means among ambient stations were observed. The most prominent bacterial species were gram positive from all five stations at all times. Further, *Micrococci* were the predominant gram positive bacterial species across four seasons. Fungi types differed among all five stations.

While this study provides much needed information about airborne bacterial and fungal concentrations and their predominant species in a commercial AFO farm and its vicinity, we realized that seven sampling events with thirty-five AGI samples were not sufficient to address diurnal, seasonal, and spatial variations as impacted by changes of animal housing emission rates and meteorological conditions. Further investigation is recommended to verify the findings of this study with greater samples. It is also recommended that in speciation of bacteria species, focus should be given on microbes that may cause poultry diseases, and/or public health concerns.

Acknowledgments

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Disclosure Statement

No competing financial interests exist.

TABLE 2. FUNGAL CONCENTRATIONS (CFU/M³) AT FIVE SAMPLING LOCATIONS

Date	ST1	ST2	ST3	ST4	ST5
8/5/09	1388	831	1240	1348	1316
10/6/09	10,831	554	831	904	591
11/17/09	2258	313	425	441	490
12/09/09	123	504	1839	131	426
12/15/09	433	62,972	32,746	82,915	52,632

Analysis and reporting format changed beginning with August sampling event. No fungal colony counts were taken for samples on 5/14/09 and 7/14/09.

TABLE 3. PREDOMINANT FUNGI TYPES AT FIVE SAMPLING LOCATIONS

Date	ST1	ST2	ST3	ST4	ST5
5/14/09	1 type: <i>Cladosporium</i>	2 types: <i>Cladosporium</i> sterile hyphae	1 type: <i>Cladosporium</i>	None	2 types: <i>Penicillium</i> sterile hyphae
7/14/09	3 types: <i>Fusarium</i> <i>Acremonium</i> -like <i>Cladosporium</i>	2 types: <i>Fusarium</i> <i>Acremonium</i> -like	2 types: <i>Fusarium</i> <i>Acremonium</i> -like	3 types: <i>Acremonium</i> -like sterile hyphae unknown white colony	3 types: <i>Fusarium</i> <i>Acremonium</i> -like sterile hyphae
8/5/09	primarily <i>Cladosporium</i>	primarily <i>Acremonium</i> -like	primarily <i>Acremonium</i> -like	primarily <i>Fusarium</i>	primarily <i>Fusarium</i>
10/6/09	primarily white yeast	primarily <i>Fusarium</i> -like & pink yeast	primarily <i>Cladosporium</i> spp.	primarily <i>Geotrichum</i>	primarily <i>Scopulariopsis</i> -like
11/17/09	primarily white yeast	primarily sterile white hyphae	primarily sterile hyphae	primarily cream yeast	None
12/9/09	primarily white yeast	primarily sterile white hyphae	primarily sterile white hyphae	None	primarily sterile white hyphae
12/15/09	primarily <i>Paecilomyces</i> -like	primarily cream yeast	None	primarily cream yeast	primarily cream yeast

Analysis and reporting format changed beginning with August sampling event.

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