

## ABSTRACT

HU, DI. Temporal and Spatial Variations of Bioaerosol Concentrations Emitted from an Egg Production Farm in Southeast U.S. (Under the directions of Dr. Lingjuan Wang-Li and Dr. Otto D. Simmons III).

As emission sources of bioaerosols, animal feeding operations (AFOs) cause high concentrations of bioaerosols in the areas surrounding the AFO facilities. People working on or living near AFOs are under the risk of exposures to relatively high concentrations of bioaerosols. In this study, concentrations of non-selective bacteria and fungi were measured in an egg production house and at four ambient stations surrounding the egg production farm in winter, spring and summer using Andersen six-stage samplers. Mean concentrations of in-house bacteria and fungi ranged from  $2.2 \times 10^5$  CFU/m<sup>3</sup> to  $1.1 \times 10^6$  CFU/m<sup>3</sup> and from  $1.5 \times 10^3$  CFU/m<sup>3</sup> to  $2.8 \times 10^3$  CFU/m<sup>3</sup>, respectively. Mean concentrations of ambient bacteria and fungi ranged from  $8.7 \times 10^2$  CFU/m<sup>3</sup> to  $1.3 \times 10^3$  CFU/m<sup>3</sup> and from  $2.8 \times 10^2$  CFU/m<sup>3</sup> to  $1.4 \times 10^3$  CFU/m<sup>3</sup>, respectively. It was observed that in-house bacteria concentrations were significantly different over the three seasons, but not in different time of day. In-house fungi concentrations were not significantly different over seasons or in different time of day. Ambient bacteria concentrations were not significantly different over different seasons, while ambient fungi concentrations were the highest in summer and the lowest in winter. Wind played an important role in affecting ambient bioaerosol concentrations. Among all influencing factors, air temperature (T), relative humidity (RH), and house ventilation rate had significant impacts on in-house bioaerosol concentrations. Downwind bacteria concentrations were mainly affected by RH, wind speed and direction, and emission rate,

while downwind fungi concentrations were affected by T, wind speed and direction, and emission rate. More than 50% of bioaerosols in the poultry house or at ambient were respirable. Therefore, exposures to bioaerosols emitted from the poultry house might bring higher risk of adverse health effects to people working on or living near the animal farm.

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by  
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# CHAPTER 1 INTRODUCTION

Bioaerosols are usually defined as microbial agents suspended in the air, which may consist of bacteria, fungi, viruses, endotoxins, pollen, etc. The sizes of bioaerosols range from sub-micrometers to over hundred-micrometers. Fungi are usually in the 10 – 50  $\mu\text{m}$  range, bacteria occupy the size range of 0.5 – 10  $\mu\text{m}$  and viruses represent the smaller size group of below 0.5  $\mu\text{m}$  (Hinds, 1998). Exposures to bioaerosols are associated with a wide range of adverse health effects, including but not limited to allergies, respiratory problems, infectious diseases, and hypersensitive reactions (Douwes, 2002).

While providing food for human-being, animal feeding operations (AFOs) also emit significant amounts of bioaerosols. Once emitted, bioaerosols can travel to short and long distances in the air. It is anticipated that concentrations of bioaerosols are higher than background level in the surrounding area of an AFO facility. Consequently, emissions of bioaerosols can bring health risks to people living in surrounding areas.

## **BIOAEROSOL SAMPLING TECHNIQUES**

Study of bioaerosols involves in taking bioaerosol samples and conducting biological analysis on the samples. In general, sampling bioaerosols is to separate the airborne viable particles from the air by various samplers utilizing different physical separation principles. Bioaerosol samplers can be divided into inertial devices and filters according to the separation mechanisms in use (Henningson and Ahlberg, 1994). Inertial devices include impactors, impingers, and centrifuges (Thompson et al., 1994; Baron and Wileke, 2001).

Decosemo and Griffiths (1992) classified sampling devices into three categories: those that collect samples onto solid surfaces, those that collect samples into liquids, and those that collect sample onto filters. Unlike sampling non-biological aerosols, there is lack of a standard or reference sampling device and method for bioaerosol sampling. Researchers have to make their own selections of suitable bioaerosol samplers according to their specific objectives of sampling and the types/levels of bioaerosols in the environment to be sampled (Decosemo and Griffiths, 1992; Nevalainen et al., 1992). Some commonly used samplers include the following:

### ***INERTIAL IMPACTORS***

This type of samplers collects bioaerosols onto solid media surfaces using inertial forces. Inside the sampler, the airstream is forced to change direction in the collection stage of the impactor, and particles with high inertia are impacted on a solid collecting surface (Henningson and Ahlberg, 1994; Thompson et al., 1994). Impactors with multiple collection stages, e.g. the Andersen six-stage sampler, provide information of the particle size distribution (PSD) of bioaerosols (Henningson and Ahlberg, 1994).

Several types of impactors have been used for bioaerosol sampling, including cascade impactors with one, two or six stages; slit samplers; and the ‘surface air system’ sampler (Thompson et al., 1993; Nevalainen et al., 1992). Among them, the Andersen six-stage sampler is the most commonly used for bioaerosol sampling in livestock buildings and often served as a reference sampler by some researchers in evaluating other sampling devices (Predicala et al., 2001; Cox and Wathes, 1995; Henningson and Ahlberg, 1994; Mehta et al.,

1996). The Andersen six-stage sampler allows simultaneous sizing and counting of viable microorganisms. It collects bioaerosols directly onto culture media (agar plate) for incubation and analysis without dilution or plating, which protects bioaerosols from inactivation during further treatment after sampling (Thorne et al., 1992). As stated by Andersen (1958), the wall losses in the Andersen samplers were extremely low.

One limitation with Andersen samplers is that they can only assess culturable bioaerosols because they collect bioaerosols directly onto nutrient agar (Griffiths and Decosemo, 1994). Even for culturable bioaerosol samples, the main problem associated with Andersen samplers that has been reported by several studies is the rapid overloading of the plates when bioaerosol concentration is relatively high in the environment to be sampled (Thorne et al., 1992). Other problems that have limited the use of Andersen samplers in remote locations include their dependence on external power supply (Mehta et al., 1996) and the inflexibility for simultaneously taking different species of biological samples for biological speciation analysis.

### ***LIQUID IMPINGERS***

This type of samplers works in the same way as impactors, except that the collection surface of an impinger is liquid nutrient broth and that of impactors is solid culture media (Henningson and Ahlberg, 1994). There are different types of impingers: all-glass impinger-30 (AGS-30), all-glass impinger-4 (AGS-40), SKC biosampler, and multistage all-glass liquid impingers (Thompson et al., 1993). The all-glass impinger method has been widely used since 1920s when it was firstly introduced (Thorne et al., 1992). This kind of sampler is

inexpensive and easy to use and it also allows the liquid samples to be diluted before conducting biological analysis. It is suitable when conducting simultaneous sampling at multiple locations or a great number of replicates are wanted. On the other hand, like the impactors, impingers also have a problem of damaging the viability of bioaerosols when hitting on the collection surfaces. The sampling duration cannot be too long in order to avoid liquid evaporation when using impinger (Henningson and Ahlberg, 1994).

### ***CENTRIFUGAL SAMPLERS***

This type of samplers also uses the inertial behavior of the particles. In centrifugal samplers, aerosol is forced into a centrifugal motion, and the particles with high inertia will deposit on the sampler wall (Henningson and Ahlberg, 1994). A centrifuge can be used to obtain the aerodynamic size distribution of irregular shaped particles, aggregate particles, and fiber as well as their concentrations (Baron and Willeke, 2001). On the other hand, centrifuge sampler is very expensive.

### ***FILTER SAMPLERS***

In filtration, inertial force also works to separate the particles from airstream, but besides that, other mechanisms, such as interception, diffusion, and electrostatic attraction contribute to the deposition of particles onto the filter (Nevalainen et al., 1992; Henningson and Ahlberg, 1994). Filtration samplers are relatively inexpensive and easy to use, and have been suggested for sampling some organisms within certain environmental limits for its simplicity and its suitability for a variety of environments (Thorne et al., 1992). The major problem associated with this method is the inactivation of microorganisms due to the dehydration

effects caused by the large volume of air passing over the filter and the damage or loss of bioaerosols when they are being removed from filters for lab analysis (Thorne et al., 1992; Cox and Wathes, 1995).

#### *COMPARISONS OF THE BIOAEROSOL SAMPLERS*

Although none of these samplers can be considered as a standard method, Brachman et al. (1964) have recommended the all-glass liquid impinge (AGI) and Andersen six-stage impactor to be considered as two reference methods. Several studies have been done to evaluate the performances of bioaerosol samplers or to compare the effectiveness of different bioaerosol sampling methods in experimental environments or in livestock facilities. Buttner and Stetzenbach (1993) have conducted a research to evaluate four sampling methods for sampling airborne fungal spores in an experimental indoor environment. The four samplers used to take air samples were Andersen six-stage, Surface Air System (SAS), Burkard, and depositional samplers. The authors reported that the Andersen and Burkard samplers showed the highest yields compared with the other two samplers, and the Andersen sampler turned out to have the highest levels of sensitivity and repeatability according to data from paired samplers (Buttner and Stetzenbach, 1993). Tseng et al. (2005) evaluated collection efficiencies of virus aerosols by Andersen one-stage impactor, AGI-30 impinger, gelatin filter, and nuclepore polycarbonate filter. The results revealed that the collection efficiencies of the Andersen impactor, impinger, and gelatin filter were 10 times higher than that of the nuclepore filter. It was suggested that airborne virus samples collected by an impinger should be processed as soon as possible to avoid loss of virus infectivity (Tseng et al., 2005). Jensen

et al. (1992) evaluated eight bioaerosol samplers under the controlled conditions in a horizontal bioaerosol chamber using AGI-30 as a reference. Bacterial aerosols were collected using Andersen one-stage, two-stage and six-stage impactors, a second AGI-30, Mattson-Gavin Slit-to-Agar (STA), membrane filter, SAS, and Biotest Reuter centrifugal sampler (RCS) individually. As reported by the authors, the Andersen six-stage impactor, the AGI-30, and the Andersen one-stage impactor gave comparable results, while the relative collection efficiencies of the membrane filter, SAS and RCS were extremely low (Jensen et al., 1992).

A research evaluating and comparing effectiveness of three portable samplers for sampling airborne fungi in a community building reported (Mehta et al., 1996) that the Burkard sampler retrieved equivalent volumes of fungi aerosols as the reference sampler (the Andersen 2-stage impactor) did. The other two evaluated samplers, the RCS Plus and the SAS Super 90 samplers, agreed with each other but showed significantly lower measurements than those obtained with the Andersen or Burkard samplers. Zimmerman et al. (1987) compared effectiveness of the Andersen two-stage impactor and the May three-stage glass impinger for sampling *Escherichia coli* generated by wastewater spray activities. The authors reported that the correlation between the two samplers were good after eliminated the data pairs containing potentially overloaded Andersen counts (Zimmerman et al., 1987).

Nieguitsila et al. (2011) evaluated a filtration sampler (airport MD8) and a centrifugal sampler (CIP 10-M) for sampling airborne culturable fungi in a poultry house and the two samplers showed comparable performance (Nieguitsila et al., 2011). Thorne et al. (1992) conducted a study in swine barns to compare the performances of the Andersen six-stage impactor, the all-glass impinger (AGI), and the Nuclepore filter. The authors reported that the

AGI and filter were suitable methods for sampling bacteria in this environment while the Andersen sampler was not because of overloading problem. All three evaluated methods correlated well with each other for fungi sampling (Thorne et al., 1992). Another study conducted in swine barns compared the Andersen six-stage impactor and a filtration sampler and suggested that the Andersen sampler showed significantly higher bioaerosol concentrations than the filtration sampler (Predicala et al., 2001).

The results of the above discussed studies show significant variations and even contradictory findings. This is mainly because that the strengths and weaknesses of the evaluated samplers, the environments to be sampled, sampling and analysis procedures, and the biological species of the investigations were different. No single sampling method is suitable for different case scenarios with different sampling objectives.

## **BIOLOGICAL ANALYSIS**

Once the bioaerosol samples are collected, it is important to choose a suitable biological analysis technique that will provide suitable information to meet project objectives. Various methods are available for biological analysis of the bioaerosol samples. The traditional methods include culture-based analyses and microscopic counting. More advanced methods include biochemical and molecular assays. While the traditional methods provide some fundamental evaluation of the bioaerosol concentrations, more advanced methods provide information about species that may present in a sampling environment as well as viability or infectivity of the sampled bioaerosols. Selection of a method for analysis should be based upon objectives of the study and availability of methods.

### ***TRADITIONAL METHODS: CULTURE-BASED***

Currently, cultivation on agar-based-media after sampling and counting of grown colonies is the most commonly used method for quantification of bioaerosols (Albrecht et al., 2007). The cultivation methods can only detect microbes that may survive from sampling and transportation, and reproduce after incubation (Albrecht et al., 2007). In some environments (e.g. Soil or freshwater) only 1-10% of the observable microorganisms are culturable when using standard cultivation techniques (Albrecht et al., 2007). So these methods are likely to underestimate the true concentration within a sample (Decosemo and Griffiths, 1992; Decosemo et al., 1992). The colony counting method is relatively cheap and easy but it can take 24 to 48 hours to obtain a result (Decosemo et al., 1992).

Several samplers (e.g. Andersen samplers, Casella Slit sampler, and SAS) directly collect bioaerosols onto an agar surface, and colonies in the agar plate are counted following incubation (Decosemo and Griffiths, 1992). There are non-selective agars and selective agars being utilized in research. The majority of culturable bioaerosols can survive and grown on non-selective agars, so it is suitable for measuring non-selective bioaerosol concentrations. In order to detect different species of bioaerosols, selective agar is employed, which only let a certain kind of bioaerosols to survive and grow on it (Cox and Wathes, 1995). A wide range of selective media are commercially available for different types of microorganisms. By changing the composition of the medium, or using specific nutrients, selected microorganisms can be cultivated or excluded. Bioaerosol samples for culturable microbes are incubated in an incubator at a specific temperatures depending upon the types of

microorganisms that are to be detected. In some instances, temperature alone can be selective for a specific group of microorganisms.

In the colony counting process of the culture-based method, for a plate with colonies being scattered over it, a numbers of areas or segments of the plate are counted and the total colony numbers are calculated based upon the counts in the selective segments. For overloaded plates collected by an Andersen sampler, the “positive hole” method or microscope were recommended to obtain accurate counts of the colonies conforming to the pattern of jets (Andersen, 1958). The positive hole method counts the jets that are occupied by multiple viable particles and convert the counts by using the “positive hole” conversion table to represent colony counts on the whole plate. This Method is usually not needed for the plates in the stages one and two of a multi-stage Andersen sampler because the deposition of particles does not follow the pattern of jets (Andersen, 1958; Macher, 1989).

#### ***ADVANCED METHODS: NON-CULTURE BASED***

The advanced method is usually referred to biochemical and molecular-biological assays. These non-culture methods can be more specific than conventional culture methods and could rapidly detect and identify bioaerosols (Cox and Wathes, 1995). Albrecht et al. (2007) compared culture and non-culture methods for detecting bioaerosols in a composting facility and reported that only 1.5-15.3% of the airborne bacteria detected by direct counting (non-culture) method formed countable colonies after incubation. Another study conducted in the swine confinement buildings reported that the bacteria concentration was 100 to 1000 times higher than the concentration of culturable bacteria (Nehme et al., 2008). While these two

studies have detected significantly more bacterial bioaerosols using non-culture method, they failed to distinguish living cells from dead ones. The non-culture methods require a relatively high level of training and expertise to provide accurate and meaningful results.

The selection of suitable biological analysis techniques is dictated by meeting the project objectives as well as availabilities of the samplers, analytical equipment and expertise.

## **BIOAEROSOLS INSIDE THE AFO FACILITIES**

As the rapid growth of livestock productions, concentrations of airborne microorganisms in animal houses have been reported to be notably higher than those found in other indoor environments (Banhazi et al., 2005; Heederik et al., 1991; Crook et al., 1991; Thorne et al., 2009; Predicala et al., 2002; Jo and Kang, 2005; Clark et al., 1983; Lee et al., 2006). High concentrations of bioaerosols in and emitted from livestock buildings affect animal and human health and welfare, and contribute to environmental air pollution. Several field studies have been conducted to assess airborne microorganisms in different types of animal houses.

Banhazi et al. (2005) measured and compared concentrations of bacteria in different types of swine buildings using the Andersen impactor. The median concentrations of bacteria were reported to range from 6.8 to  $9 \times 10^4$  CFU/m<sup>3</sup> in unbedded housing systems and to be  $2.17 \times 10^5$  CFU/m<sup>3</sup> in the deep-bedded shelters (Banhazi et al., 2005). Temperature and relative humidity (RH) were simultaneously measured during sampling. The authors reported that the type of building showed the most significant impact on bacteria concentration, and the deep-bedded shelters were most likely to have the highest concentration of airborne bacteria (Banhazi et al., 2005). In addition, this study revealed that temperature and RH had

insignificant impact on bacteria concentrations, which is contradictory to the literature. Meteorological conditions, such as temperature and RH, are supposed to be important influencing factors for bioaerosol concentrations.

Heederik et al. (1991) measured airborne bacteria concentrations in nursery buildings of swine farms using Andersen six-stage impactor with sampling duration of 1 min. The geometric mean concentration of bacteria was  $1.073 \times 10^5$  CFU/m<sup>3</sup> and that of gram-negative bacteria was  $7.7 \times 10^3$  CFU/m<sup>3</sup>. Their analysis showed a strong correlation between gram-negative bacteria levels and lung function symptoms (Heederik et al., 1991).

Impingement and filtration methods were applied in a study conducted in pig confinement houses to assess the concentration of airborne bacteria and fungi (Crook et al., 1991). Airborne microorganisms were simultaneously collected into the multistage liquid impingers (MSLI) and the aerosol monitoring cassettes for 40 min at each sampling site (Crook et al., 1991). Different species of bioaerosols were isolated and cultivated in laboratory after sampling. Bacteria were incubated at 25 °C and 37 °C, and airborne bacteria concentrations ranged from  $3 \times 10^5$  to  $8 \times 10^6$  CFU/m<sup>3</sup> and from  $2 \times 10^5$  to  $6 \times 10^6$  CFU/m<sup>3</sup>, respectively. Fungi were incubated at 25 °C and 40 °C, and the concentrations ranged from  $2 \times 10^3$  to  $10^5$  CFU/m<sup>3</sup> and from  $3 \times 10^2$  to  $10^4$  CFU/m<sup>3</sup>, respectively (Crook et al., 1991). According to the results, room temperatures were optimal for bacteria and fungi to grow. The MSLI also provided information of size distribution of bioaerosols. Most bacteria and fungi were deposited in the uppermost stage, which indicated that their aerodynamic diameters were greater than 6 µm and these large particles, if inhaled, will most likely deposit in the upper parts of the respiratory tract (Crook et al., 1991). The author also found that majority

of the detected airborne bacteria were gram positive, and this result agreed with that of other studies (Crook et al, 1991; Heederik et al., 1991).

Thorne et al. (2009) quantified and compared concentrations of airborne bacteria and fungi inside two types of AFOs. Bioaerosol samples were collected using AGI-30 liquid impingers for 15 min at a height of 2 m. The collected nutrient broth samples were diluted and incubated on three types of media at 25 °C before being enumerated. Bacteria were grown on tryptic soy agars (TSA) and R2A, and fungi were grown on malt extract agars (MEA). The geometric mean concentrations of TSA cultured bacteria ranged from  $1.48 \times 10^5$  to  $1.8 \times 10^7$  CFU/m<sup>3</sup> in hoop operations and from  $0.21 \times 10^4$  to  $8.24 \times 10^5$  CFU/m<sup>3</sup> in conventional confinement operations. The geometric mean concentrations of R2A cultured bacteria ranged from  $4.18 \times 10^4$  to  $1.59 \times 10^7$  CFU/m<sup>3</sup> in hoop operations and from  $0.21 \times 10^4$  to  $8.21 \times 10^5$  CFU/m<sup>3</sup> in conventional confinement operations. Fungi concentrations ranged from  $0.21 \times 10^4$  to  $4.28 \times 10^5$  CFU/m<sup>3</sup> in hoop operations and from  $0.21 \times 10^4$  to  $2.09 \times 10^5$  CFU/m<sup>3</sup> in conventional confinement operations (Thorne et al., 2009). The author concluded that the type of houses was the most significant factor affecting bioaerosol concentrations, which agreed with other research (Thorne et al., 2009; Banhazi et al., 2005, Jo and Kang, 2005). Meteorological factors, such as wind, temperature, and RH played important roles when interacting with seasonal factor (Thorne et al., 2009).

Predicala et al. (2002) assessed bioaerosol concentrations inside two swine finishing barns by sampling air using membrane filtration and the Andersen six-stage impactor. The researchers also measured and recorded the air temperature and RH inside the barns. The air temperatures ranged from 14.2 to 32 °C with means at 21.6 °C in naturally ventilated barn

and at 24.2 °C in mechanically ventilated barn. The RH ranged from 26% to 61% with a mean of 40% (Predicala et al., 2002). The mean concentrations of bioaerosols were  $6.6 \times 10^4$  CFU/m<sup>3</sup> measured by filtration and  $8.6 \times 10^4$  CFU/m<sup>3</sup> measured by the Andersen six-stage impactor (Predicala et al., 2002). The Andersen impactor collected significantly more viable bioaerosols than the filtration method did.

A study was conducted in Korea to assess concentrations of airborne bacteria and fungi in swine and poultry houses (Jo and Kang, 2005). The Andersen one-stage impactor was used for bioaerosol sampling and positive hole correction was applied to estimate the colony counts. In this study, sampling durations ranged from 0.5 to 2 min (Jo and Kang, 2005). The mean concentrations of bacteria were  $3.3 \times 10^4$  CFU/m<sup>3</sup>,  $1.34 \times 10^5$  CFU/m<sup>3</sup>, and  $2.80 \times 10^5$  CFU/m<sup>3</sup> for the summer swine, winter swine, and summer poultry, respectively. The mean concentrations of fungi were  $7.1 \times 10^3$  CFU/m<sup>3</sup>, 454 CFU/m<sup>3</sup>, and  $7.4 \times 10^3$  CFU/m<sup>3</sup> for the summer swine, winter swine, and summer poultry, respectively (Jo and Kang, 2005). In agreement with other research, the authors found the type of animal house to be a significant factor affecting bioaerosol concentrations (Jo and Kang, 2005; Thorne et al., 2009; Banhazi et al., 2005).

Clark et al. (1983) measured airborne bacteria and fungi concentrations in poultry and swine confinement buildings using the Andersen six-stage impactor. Sampling durations ranged from 0.5 to 4 min (Clark et al., 1983). The median concentrations of bacteria were  $3 \times 10^5$  CFU/m<sup>3</sup> in swine buildings and  $4.2 \times 10^5$  CFU/m<sup>3</sup> in poultry houses. The corresponding values of fungi were 300 CFU/m<sup>3</sup> in swine houses and 500 CFU/m<sup>3</sup> in poultry houses (Clark

et al., 1983). According to size distribution information, 26% of the gram-negative bacteria in swine houses and 40% of which in poultry houses were respirable (Clark et al., 2005).

Lee et al. (2006) assessed airborne microorganisms in three types of animal farms: swine, poultry, and dairy. A filtration sampler was used to conduct bioaerosol sampling. The collected samples were extracted for cultivation and microscopic counting after sampling. The mean bacteria concentrations ranged from  $3.0 \times 10^3$  CFU/m<sup>3</sup> to  $3.3 \times 10^8$  CFU/m<sup>3</sup>, and the mean fungi concentrations ranged from  $3.0 \times 10^2$  CFU/m<sup>3</sup> to  $7.4 \times 10^6$  CFU/m<sup>3</sup> (Lee et al., 2006). There were noticeable seasonal variations of bioaerosol concentrations on animal farms. The concentrations of bioaerosols measured on the swine and dairy farms were higher in summer than in winter (Lee et al., 2006).

## **BIOAEROSOLS IN THE VICINITIES OF THE AFO FACILITIES**

Like other aerosols, the concentrations of bioaerosol in the ambient air vary with the spatial and temporal effects (Hinds, 1998). Normally the further from emission source, the lower the concentration will be (Green et al., 2006). Another factor that affects bioaerosol concentrations is inactivation (Hinds, 1998). Bioaerosols lose their viability over time due to the condition they are exposed (Mohr, 1997). Meteorological factors (i.e. temperature, RH, and solar radiation) affect the fate and transport of bioaerosols in ambient air. The RH influences the viability of bioaerosols by working on membrane phospholipids and proteins of the microorganisms (Mohr, 1997). Bioaerosols tend to hardly maintain their original particle size and shape, and even lose their viability in low RH due to dehydration. Some gram-negative bacteria are most stable at an intermediate RH of 60%, and some species even

show a greater survival at high RH levels (Tang, 2006). However, there is a significant decrease in airborne bacteria inactivation rates at the RH level above 50% for some species (Peccia et al., 2001). Several studies reported increased death rates for some species of gram-negative bacteria at the RH levels above 50% (50%-90%) (Webb, 1959; Won and Ross, 1966). The viability of airborne microorganisms will decrease as the temperature increases. Microbes will also lose viability under extremely low temperatures (Mohr, 1997). Generally, bacteria survival rate decreased under temperatures above 24 °C (Tang, 2006). In contrast, airborne fungi seem to prefer higher temperatures. Studies confirmed that fungi concentrations were positively correlated with higher RHs and temperatures (Tang, 2006). Solar radiation is another important factor for the outdoor airborne microorganisms. Ulevičius et al.'s (2000) study showed a lethal effect of solar radiation on airborne fungi in ambient air.

Scarpino and Quinn (1998) conducted an experiment to determine the presence of microbes from swine facilities in downwind areas around them using the Andersen six-stage impactor. Bioaerosol concentration was found to be about 1430 CFU/m<sup>3</sup> downwind of the operations, and bioaerosol concentrations varied significantly at different ambient locations (Scarpino and Quinn, 1988).

Dungan et al. (2010) monitored concentrations of airborne bacteria and fungi in the vicinity of a concentrated dairy operation. All-glass liquid impingers were used for bacteria sampling and single-stage impactors were used for fungi sampling. Bacteria samples were collected at both upwind and downwind locations for 1 hour, then, the nutrient broth of impinge samples were diluted, spread onto plates and incubated before enumeration. The

average concentrations of bacteria were  $8.4 \times 10^3$  CFU/m<sup>3</sup> at the upwind location, and  $9.9 \times 10^5$  CFU/m<sup>3</sup> at the downwind location, respectively. Average fungi concentrations at the same upwind and downwind locations were 515 and 945 CFU/m<sup>3</sup>, respectively (Dungan et al., 2010). In this study, significant correlations between the ambient bioaerosol concentrations and the weather conditions were discovered. The ambient temperature ranged from 6.2 to 30.2 °C with an average of 14 °C and the RH ranged from 18% to 90% with an average of 48% (Dungan et al., 2010). This study showed decrease viability with decrease in RH and increase in temperature and solar radiation (Dungan et al., 2010).

Green et al. (2006) used the Andersen two-stage samplers to collect airborne bacteria samples inside, upwind and downwind of the swine houses. Plates of tryptic soy agar (TSA) were used to collect and cultivate bacteria samples. They reported that the minimum level of bacteria concentration was detected upwind of the operation, which averaged 82 CFU/m<sup>3</sup> (Green et al., 2006). Bacteria concentrations inside the animal houses averaged  $1.8 \times 10^4$  CFU/m<sup>3</sup>, which was comparable to another research conducted in the same season and type of farm (Green et al., 2006; Jo and Kang, 2005). This study found out that the respirable bacteria counted for half of the total concentration, which was not in agreement with the findings of Predicala et al. (2002) that only 32% of the bioaerosols were respirable (Green et al., 2006). This difference may be due to the use of different samplers and collection media.

## **PARTICLE SIZE DISTRIBUTIONS OF BIOAEROSOLS**

Although multiple stage impactors provide PSD information and have been widely used for bioaerosol sampling in animal facilities, few findings about size distributions of

bioaerosols were reported from the previous research. To provide information for studies of health effects, most researchers divided bioaerosols into two categories according to their sizes: respirable and nonrespirable (Green et al., 2006; Clark et al., 1983; Crook et al., 1991). If the Andersen six-stage sampler was applied, airborne particles deposited on the first two stages were considered as nonrespirable and particles went to the lower four stages were considered as respirable. In Green et al.'s (2006) study, 62% of the airborne bacteria upwind of the swine house and 47% of the bacteria inside the house were respirable. Clark et al. (1983) reported that the percentages of respirable bacteria were 26% in swine house and 40% in poultry house. If the multistage liquid impinger was used for sampling, airborne particles deposited in the uppermost stage were nonrespirable, and those went in the two lower stages were respirable. Crook et al. (1991) reported that most of the airborne bacteria and fungi collected in swine houses were nonrespirable. When study of fate and transport of bioaerosols, more detailed PSD information is required and is currently not available for most of AFO bioaerosols.

In summary, research has shown that elevated levels of bioaerosols were presented inside and in the vicinity of AFOs. Most of the AFO bioaerosol research utilized the Andersen impactors for sample collection and culture methods for biological analysis. TSA and R2A were two of the most commonly used collection media for non-selective bacteria, while MEA was generally used for non-selective fungi. Positive hole method was applied by some researchers for colony enumerating. Bioaerosol concentrations were found to be the highest inside the animal facilities when compared with ambient locations surrounding the facilities. Relatively higher concentrations were at downwind of the operations as compared to the

concentrations at upwind locations. Findings about bioaerosol size fractions were in disagreement among studies. Some found half bioaerosol to be respirable while others found only a small fraction of respirable bioaerosols. Knowledge gaps exist in how concentrations and size distributions of bioaerosols are affected by emission rate, meteorological factors, and other influencing factors.

## **COMMON FUNGI SPECIES**

*Aspergillus* spp. is a common and one of the oldest named fungi specie that can be found in almost all oxygen-rich environments (Machida and Gomi, 2010). *Aspergillus* spp. does not normally cause illness, but it can and does cause adverse health effects, for example, allergies, asthma, and infection on animals and human (Machida and Gomi, 2010). Sizes of *Aspergillus* spp. have been reported to range from 1.9  $\mu\text{m}$  to 2.2  $\mu\text{m}$  (Madelin and Johnson, 1992). Therefore, *Aspergillus* spp. should most probably deposit on stage 4 and stage 5 in the Andersen six-stage sampler.

*Cladosporium* spp. includes some of the most common indoor and outdoor fungi species. Similar to *Aspergillus* spp., *Cladosporioum* spp. is not a human pathogen except for individuals with weak immune systems (MBL, 2012). However, it is a common cause of respiratory problems, allergic reactions and can be an agent for hypersensitivity diseases (Lillard, 2004). *Cladosporium* spp. varies greatly in size and shape because they are frequently encountered as large branching chains, as well as individual spores. Size of *Cladosporium* spp. can range from near 1  $\mu\text{m}$  to many hundreds of micrometers in diameter

(Institute of Medicine, 2000). The large size range indicated that *Cladosporium* spores were possible to deposit on all stages of the Andersen six-stage sampler.

*Penicillium* spp. is commonly found in soil, food, grains, paint, carpet, wallpaper, and decaying vegetation. It can produce penicillin, an antibiotic which kills or stops the growth of certain species of bacteria and fungi. *Penicillium* exposures are associated with hypersensitivity pneumonitis, asthma, and allergic alveolitis in susceptible individuals (Toxic black mold information center, 2012). Size of *Penicillium* spp. ranges from 2.2  $\mu\text{m}$  to 3.9  $\mu\text{m}$  depending on humidity levels (Pasanen et al., 1991), therefore *Penicillium* spores are most likely to deposit on stage 3 and stage 4.

## **OBJECTIVES**

The goal of this research was to fill the knowledge gaps in quantifying bioaerosol concentrations and PSD of bioaerosols at the AFO emission sources and in the vicinity of the sources. The newly gained knowledge will provide fundamental information for studies of fate and transport of bioaerosols emitted from AFOs as well as for assessment of potential health effect of AFO bioaerosol emissions to the people living nearby the AFO facilities. The specific objectives of this study were to

1. Characterize concentrations and PSD of non-selective airborne bacteria and fungi at an emission source and in the vicinity of a commercial egg production farm
2. Investigate temporal and spatial variations of bioaerosol concentrations at source and ambient locations,
3. Identify factors causing the variations.

## CHAPTER 2 METHODOLOGY

This project was carried out through three steps, including field bioaerosol sampling, laboratory bioaerosol sample analysis, and data processing and analysis.

### RESEARCH SITE DESCRIPTION: THE LAYER FARM

Bioaerosol field sampling was conducted on a commercial egg production farm (also known as the layer farm) in North Carolina. The layout of the farm and the sampling locations are shown in Figure 1. This farm consisted of six mechanically ventilated high-rise layer houses (houses 1-4: tunnel-ventilated and houses 5-6: cross ventilated) and two naturally ventilated shallow-pit houses (houses 93 and 103).

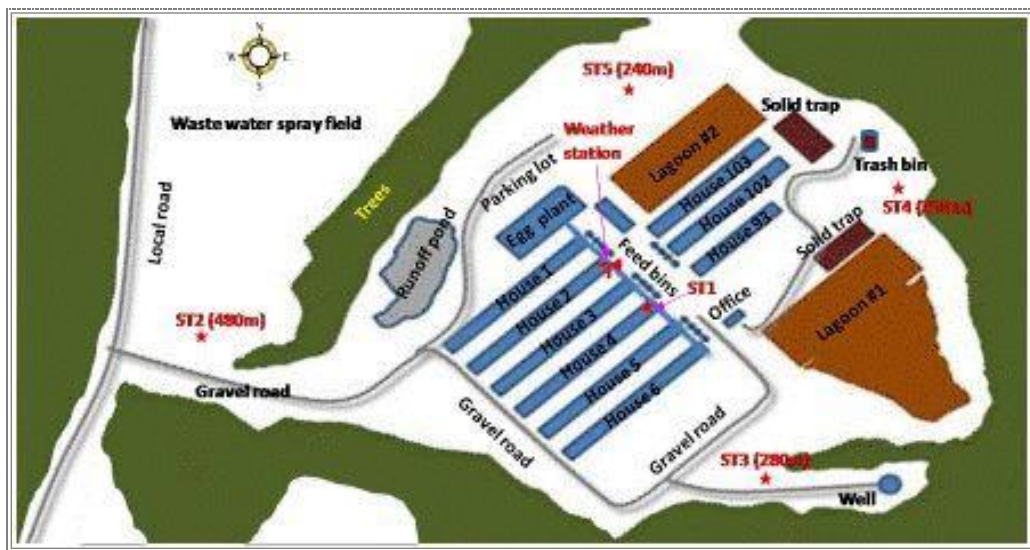


Figure 1. The layer farm layout and the bioaerosol sampling stations (Wang-Li et al., 2012a)

Five sampling stations established for various studies of air emissions on this farm were also used as sampling stations for this project. As illustrated in Figure 1, station 1 (ST1) was located at immediately upstream of the primary representative exhaust fan inside house 4, which was a tunnel-ventilated high-rise barn housing approximately 95,000 hens on the upper floor of the house. Manure fell down into the first floor and was stored there for up to one year (Wang-Li et al., 2012a). The other four sampling stations (ST2-ST5) were in the ambient locations surrounding the farm in four different directions. A 10 m weather tower was located between house 2 and house 3 at the east end and continuously measured ambient temperature, RH, wind speed and direction, and solar radiation.

## **EXPERIMENTAL DESIGN**

### ***FIELD SAMPLING FOR DETERMINATION OF TEMPORAL AND SPATIAL VARIATIONS AT AMBIENT***

#### **SAMPLING DESIGN**

For the purpose of studying bioaerosol dispersion and spatial variations, it is desired to collect samples simultaneously at all stations (upwind and downwind locations). Due to limited resources, only three six-stage viable samplers were available for simultaneous sampling at different locations. As a result, the five sampling stations were divided into two groups to simultaneously take samples of one group at a time. In this design, stations 1, 2, and 4 served as one group, whereas stations 1, 3, and 5 served as the other group. In each sampling test, bioaerosol samples were simultaneously taken at three locations (either ST1, 2, 4 or ST 1, 3, 5) with intention to capture bioaerosols at source (ST1), upwind and downwind locations.

A total of 14 sampling events (dates) were conducted from December 2010 (winter) to July 2011 (summer). Among the total 14 sampling event, 5 events were in winter and summer, and four events were in spring. Table 1 lists the detailed sampling times by season and the sampling timeline for taking bacteria and fungi samples on each sampling event (date). As shown in this table, the sampling plan produced 12 sampling tests for each sampling event (date) with 6 tests for bacteria and 6 tests for fungi from 10:00 a.m. to 2:25 p.m. on the sampling date.

Table 1. Bioaerosol field sampling timetable

Winter (December 2010 – January 2011)										
Date	12/6/2010		12/8/2010		12/13/2010		12/15/2010		1/17/2011	
Station	ST1/2/4	ST1/3/5	ST1/2/4	ST1/3/5	ST1/2/4	ST1/3/5	ST1/2/4	ST1/3/5	ST1/2/4	ST1/3/5
10:00	TB*			TB	TF			TF	TB	
10:15	TF**			TF	TB			TB	TF	
10:30	TB			TB	TF			TF	TB	
10:45	TF			TF	TB			TB	TF	
11:00	TB			TB	TF			TF	TB	
11:15	TF			TF	TB			TB	TF	
13:00		TB	TB				TF	TF		TB
13:15		TF	TF				TB	TB		TF
13:30		TB	TB				TF	TF		TB
13:45		TF	TF				TB	TB		TF
14:00		TB	TB				TF	TF		TB
14:15		TF	TF				TB	TB		TF
Spring (March 2011 – May 2011)										
Date	3/7/2011		3/9/2011		4/21/2011		5/13/2011			
Station	ST1/2/4	ST1/3/5	ST1/2/4	ST1/3/5	ST1/2/4	ST1/3/5	ST1/2/4	ST1/3/5		
10:00	TB			TB	TF			TF		
10:15	TF			TF	TB			TB		
10:30	TB			TB	TF			TF		

Table 1 Continued

10:45	TF			TF	TB				TB	
11:00	TB			TB	TF				TF	
11:15	TF			TF	TB				TB	
13:00		TB	TB			TF	TF			
13:15		TF	TF			TB	TB			
13:30		TB	TB			TF	TF			
13:45		TF	TF			TB	TB			
14:00		TB	TB			TF	TF			
14:15		TF	TF			TB	TB			
Summer (May 2011 – July 2011)										
Date	5/31/2011		6/9/2011		6/24/2011		7/1/2011		7/7/2011	
Station	ST1/2/4	ST1/3/5	ST1/2/4	ST1/3/5	ST1/2/4	ST1/3/5	ST1/2/4	ST1/3/5	ST1/2/4	ST1/3/5
10:00	TB		TF			TB		TF	TB	
10:15	TF		TB			TF		TB	TF	
10:30	TB		TF			TB		TF	TB	
10:45	TF		TB			TF		TB	TF	
11:00	TB		TF			TB		TF	TB	
11:15	TF		TB			TF		TB	TF	
13:00		TB		TF	TB		TF			TB
13:15		TF		TB	TF		TB			TF
13:30		TB		TF	TB		TF			TB
13:45		TF		TB	TF		TB			TF

Table 1 Continued

14:00	TB	TF	TB	TF	TB
14:15	TF	TB	TF	TB	TF

TB\*= test of bacteria; TF\*\*=test of fungi

## THEORETICAL VALIDATION OF THE SAMPLING DESIGN

In this sampling plan (table 1), it was assumed that the time required for a particle to travel from the emission source to the ambient sampling locations was short enough that the ambient samplers may “simultaneously” capture the emissions from the houses. In order to verify this assumption when addressing source emission effects on the concentrations of the ambient stations, theoretical calculation of particle traveling time was conducted. Figure 2 shows the estimation of emission plume parameters. The physical stack height of the housing ventilation fans was around 1.5 m. Particle diameters were assumed to range from 0.5  $\mu\text{m}$  to 50  $\mu\text{m}$ , which is the size range of most airborne bacteria and fungi (Hinds, 1998). Particle density was assumed to be 1.25  $\text{g}/\text{cm}^3$  or 1250  $\text{kg}/\text{m}^3$ . The air was assumed to be at standard conditions with a density of 1.2  $\text{kg}/\text{m}^3$ , air viscosity of  $1.81 \times 10^{-5}$  Pa.s, and mean free path of 0.066  $\mu\text{m}$ .

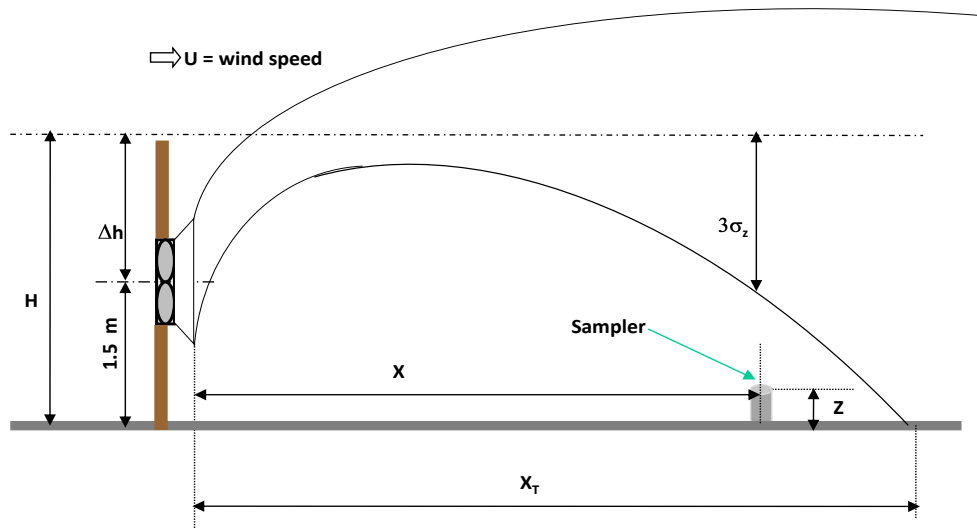


Figure 2. Emission plume parameter estimation

According to Hinds (1998), the particle relaxation time was calculated using

$$\tau = \frac{\rho_p \times d_p^2 \times C_c}{18\mu_a} \quad (1)$$

Where,  $\rho_p$  is particle density in  $\text{kg/m}^3$ ,  $d_p$  is particle diameter in  $\mu\text{m}$ ,  $C_c$  is the Cunningham correction factor, and  $C_c = 1 + \frac{2.5\lambda}{d_p}$  ( $\lambda$ = air mean free path =  $0.066\mu\text{m}$ ), and  $\mu_a$  is air viscosity in  $\text{Kg/m-s}$ . The time required for a particle to reach the terminal condition (wind speed) is:

$$t_\tau = 3 \times \tau \quad (2)$$

It was discovered that  $t \ll 1\text{s}$ , indicating that the particle velocity instantaneously reached wind speed. Thus, time for a particle traveling to a horizontal downwind location of the sampling station was:

$$t = \frac{x}{V_p} = \frac{x}{U} \quad (3)$$

Where  $V_p$  is the particle horizontal traveling velocity, and  $U$  is the wind speed.

Table 2 summarizes the particle traveling time from ST1 to individual ambient sampling locations (ST2-ST5) at three wind speeds. As it can be seen, the traveling time for a particle from ST1 to ST2 (the longest distance) was 2.67min at the wind speed in 2 m/s. These traveling times indicated that it was highly possible to simultaneously collect bioaerosols released from ST1 at the ambient stations when the ambient sampling time was set at 10 minute interval (greater than the particle traveling time).

Table 2. Particle traveling time calculated using equation 3

Station	Distance (m)	At 1m/s wind	At 2m/s wind	At 6m/s wind
ST2	480	8.00 min	2.67 min	1.33 min
ST3	280	4.67 min	1.56 min	0.78 min
ST4	290	4.83 min	1.61 min	0.81 min
ST5	240	4.00 min	1.33 min	0.67 min

In addition to check on the sampling time, further theoretical validation was conducted to verify if the sampling height was sufficient to reach and measure the emission dispersion plume at the ambient stations. It was assumed if a sampling height reached the plume bottom edge, or higher than the plume bottom edge, then, the sampling height was acceptable. When sampling was conducted, the Andersen samplers were placed on the top of the outdoor enclosure of a TEOM-PM monitor at each ambient station (Figure 3). Table 3 lists the sampling height at each station.

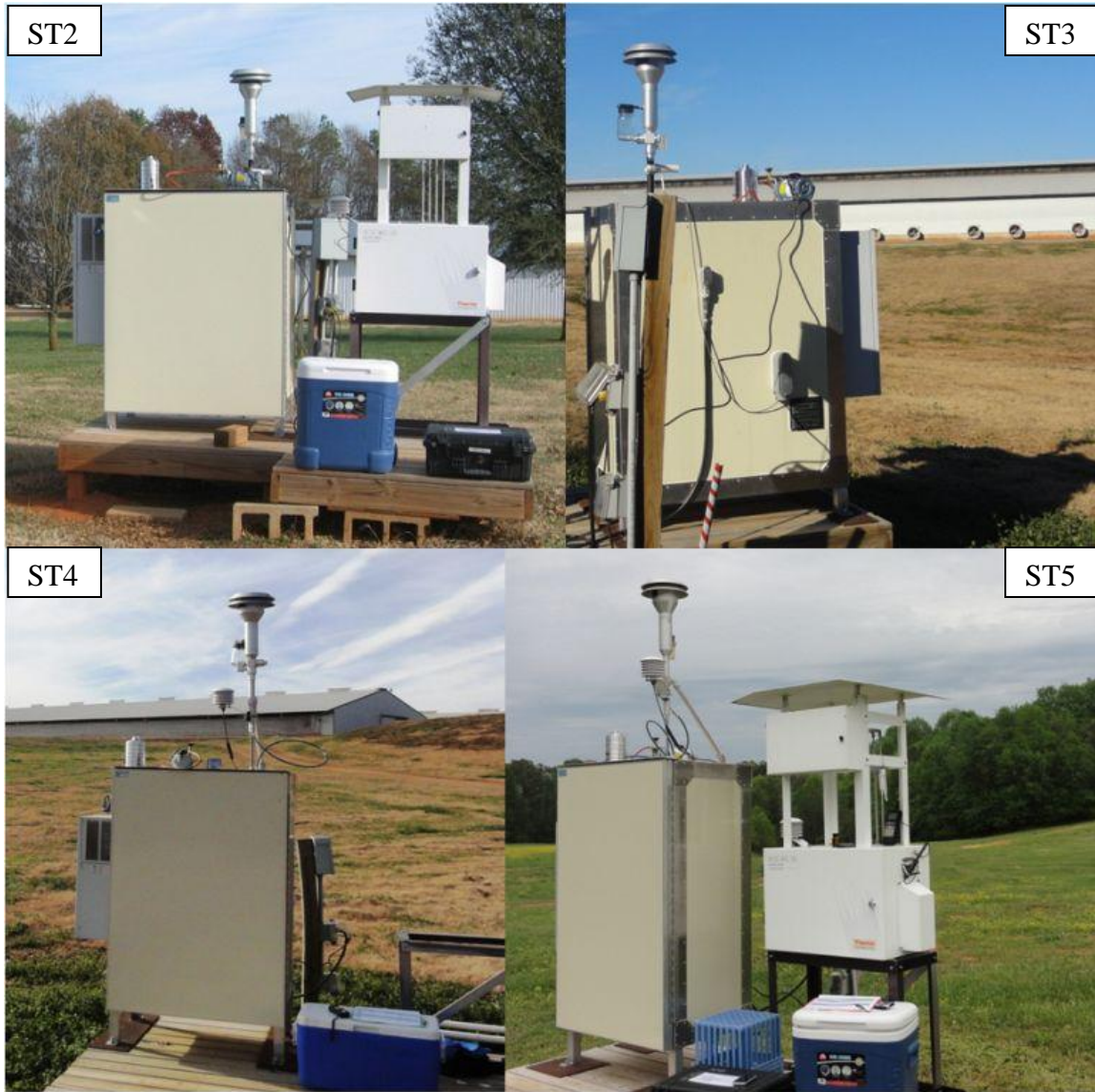


Figure 3. Placements of the Andersen six-stage viable samplers at ambient stations

Table 3. Sampling height at each sampling station

Station ID	ST1	ST2	ST3	ST4	ST5
Sampling height (cm)	60	175	168	194	194

In determining the minimum sampling height, the vertical plume spread parameter ( $\sigma_z$ ) was considered. According to Cooper and Alley (2002), the vertical plume spread parameter ( $\sigma_z$ ) may be calculated by

$$\sigma_z = c \times X^d + f \quad (4)$$

Where, X is the distance between ambient sampling station and the in-house station, c, d, f are stability class dependent parameters for plume spread calculation.

The result of calculated  $\sigma_z$  for each ambient station is listed in Table 4.

Table 4. Stability class dependent parameters

Stability*	Condition	C**	D	F
A	Very unstable	440.8	1.941	9.27
B	Moderately unstable	106.6	1.149	3.3
C	Slightly unstable	61.0	0.911	0
D	Neutral	33.2	0.725	-1.7
E	Slightly stable	22.8	0.678	-1.3
F	Stable	14.35	0.740	-0.35

\* According to the Pasquill-Gifford system, atmospheric stability has been divided into six classes, labeled A through F, with A being the most unstable (Cox and Wathes, 1995).

\*\* The letters c, d and f are constants in Gaussian dispersion model that are dependent on the stability class and on the distance X.

Table 5. The vertical plume spread parameter  $\sigma_z$  (m) at ambient sampling locations

Stability Class	ST2	ST3	ST4	ST5
A	115	47	49	37
B	49	28	29	24
C	31	19	20	17

Table 5 Continued

D	18	11	12	17
E	13	8	9	7
F	8	5	5	5

As reported by Jones, et al (2012), using Gaussian dispersion theory, the half of the plume width of dispersed emission could be represented by  $3\sigma_z$ . When the effective stack height (the physical stack height 1.5 m + plume-rise  $\Delta h$ , see Figure 2) is less than  $3\sigma_z$ , the ground level sampling is acceptable. According to Table 4, the smallest value of  $\sigma_z$  was 5 m, indicating that  $3\sigma_z$  was greater than the effective height even when the plume rise was up to 13.5 m. The plume rise varied over different seasons and under the different ventilation settings. As was observed on the field, the exhausted plume may reach about 14 m (Jones et al., 2012). This indicates that the sampling height at 1.5 to 2 m level (Table 3) is acceptable under most conditions.

#### FIELD SAMPLING FOR DETERMINATION OF TEMPORAL AND SPATIAL VARIATION AT SOURCE

To accurately estimation bioaerosol emission rates, five sampling events were conducted inside a high-rise house to assess the temporal and spatial variations of bioaerosol concentrations at sources in summer 2011. For this source concentration study, three sampling events were conducted in house 4 with 18 tests for bacteria and 15 tests for fungi on each sampling date to assess the temporal variation of bioaerosol concentrations inside the animal house. Besides, 3 tests for fungi were sampled outside the house immediately downstream of the primary representative exhaust fan while the fungi samples were also simultaneously taken inside the house at immediate upstream of the primary representative exhaust fan (Figure 4). These three tests were to assess the inside/outside variation of fungi concentration as impacted by ventilation air flow. For house spatial variation tests, two sampling events were conducted in house 3 with 10 tests for bacteria and 10 tests for fungi for each sampling event (date). In this group of sampling tests, samples were simultaneously taken at immediate upstream of the exhaust fans on the first and second floor (Figure 4).

Table 5 lists the detailed sampling times for taking bacteria and fungi samples on each sampling event for (date) for the investigation of source temporal and spatial variations.



[a] at upstream of the exhaust fans on the first 1<sup>st</sup> floor; [b] at downstream of exhaust fans; [c] at upstream of the exhaust fans on the 2<sup>nd</sup> floor; [d] changing the plates in the mobile lab for ST1 samples

Figure 4. Placements of the Andersen six-stage viable samplers in a high-rise layer house

Table 6. Bioaerosol sampling timetable for temporal and spatial variation investigation at source

Temporal and spatial (July 2011 – August 2011)										
Date	7/14/2011		7/21/2011		7/28/2011		8/15/2011		8/19/2011	
Station	ST1	Outside	ST1	Outside	ST1	Outside	1st floor	2nd floor	1st floor	2nd floor
10:00	TB		TB		TB		TB	TB	TB	TB
10:05	TF	TF	TF	TF	TF	TF	TF	TF	TF	TF
10:13	TB		TB		TB		TB	TB	TB	TB
10:18	TF	TF	TF	TF	TF	TF	TF	TF	TF	TF
10:26	TB		TB		TB		TB	TB	TB	TB
10:31	TF	TF	TF	TF	TF	TF	TF	TF	TF	TF
10:39	TB		TB		TB		TB	TB	TB	TB
10:44	TF		TF		TF		TF	TF	TF	TF
10:52	TB		TB		TB		TB	TB	TB	TB
10:57	TF		TF		TF		TF	TF	TF	TF
11:05	TB		TB		TB					
11:10	TF		TF		TF					
11:18	TB		TB		TB					
11:23	TF		TF		TF					
11:31	TB		TB		TB					
11:36	TF		TF		TF					
11:44	TB		TB		TB					
13:00	TB		TB		TB		TB	TB	TB	TB

Table 6 Continued

13:05	TF	TF	TF	TF	TF	TF	TF
13:13	TB	TB	TB	TB	TB	TB	TB
13:18	TF	TF	TF	TF	TF	TF	TF
13:26	TB	TB	TB	TB	TB	TB	TB
13:31	TF	TF	TF	TF	TF	TF	TF
13:39	TB	TB	TB	TB	TB	TB	TB
13:44	TF	TF	TF	TF	TF	TF	TF
13:52	TB	TB	TB	TB	TB	TB	TB
13:57	TF	TF	TF	TF	TF	TF	TF
14:05	TB	TB	TB				
14:10	TF	TF	TF				
14:18	TB	TB	TB				
14:23	TF	TF	TF				
14:31	TB	TB	TB				
14:36	TB	TB	TB				

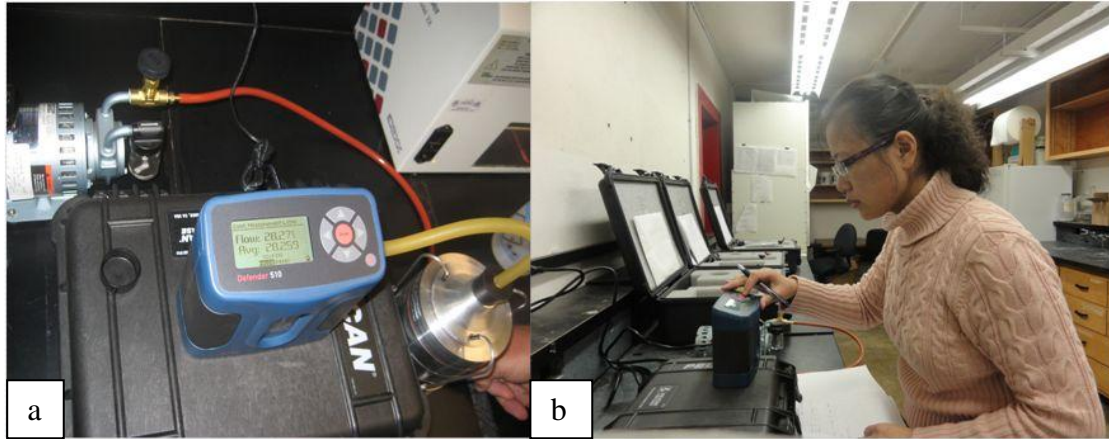
### *VIABLE SAMPLERS AND OPTIMAL SAMPLING DURATIONS*

Three identical Andersen six-stage samplers (Tisch 1 ACFM Six-stage Viable Particle Sampler, Tisch Environmental, INC. Village of Cleves, OH.) were used to collect samples of non-selective bacteria and fungi. The Andersen sampler consists of six perforated plates, each containing 400 holes. The size of the holes becomes smaller with each descending stage. Designed performance information about the samplers is provided in Table 7. The six-stage samplers provide information about bacteria and fungi concentration of each size range, and total concentrations of them. This information may link bioaerosol dispersion with particle size.

Table 7. Design characteristics of the three identical Andersen six-stage samplers (Tisch Environmental, Inc.)

Stage	Orifice Diameter (mm)	D <sub>50</sub> ( $\mu$ m)	Particle size range ( $\mu$ m)
1	1.18	5.8	7 & above
2	0.91	4.7	4.7-7.0
3	0.71	3.3	3.3-4.7
4	0.53	2.1	2.1-3.3
5	0.34	1.1	1.1-2.1
6	0.25	0.7	0.65-1.1

To ensure the performance of the viable samplers, the samplers' flow rates were calibrated to the design flow rate at 28.3 l/min before each sampling event, and were checked after sampling. Figure 5 shows an example of the flow rate calibration and checking in the lab before and after each sampling event. The reference flow meter for the calibrations was a digital flow meter, Bios drycal Defender 510-H (0.3-30 LPM) (Bios International Corporation, Bultter, NJ). In concentration calculation, the sampler's flow rate was the average value of the mean flows before and after sampling event for each of the samplers.



[a] flow calibrator; [b] flow calibration

Figure 5. Illustration of the bioaerosol sampler flow calibration and check

In the preliminary tests, sampling duration at all ambient locations was set for 10 min. per test for both bacteria and fungi samples and the durations for in-house sampling duration was set for 3 min. per test. The preliminary results indicated the acceptable sampling time for ambient stations, however, the sampling duration for in-house station for bacteria proved to be a challenge. Significant overloading problem was observed with 3 min. duration. Further try-and-error approach was taken to identify optimal sampling durations. The early sampling events were conducted in winter time (Table 1). Under winter condition, the house ventilation was minimal, thus the in-house concentration of bacteria was extremely high. In the first three sampling events, the in-house bacteria sampling time was adjusted from 3 minutes, to 1 minute, 30 seconds, 25 seconds, and 20 seconds (Figure 6). It was observed that all the plates were overloaded for those sampling durations. The overloading issue challenged the plate reading, and caused great uncertainty in colony forming unit (CFU) counting, and in the calculation of concentration. Therefore, starting at the fourth sampling event (12/15/2010), sampling duration of in-house bacteria was reduced to 15 seconds, which turned out to be an acceptable solution for the overloading problem. No overloading problem was observed for in-house fungi samples with duration of 3 min. Thus the sampling duration of in-house fungi was kept at 3 min. for all tests.

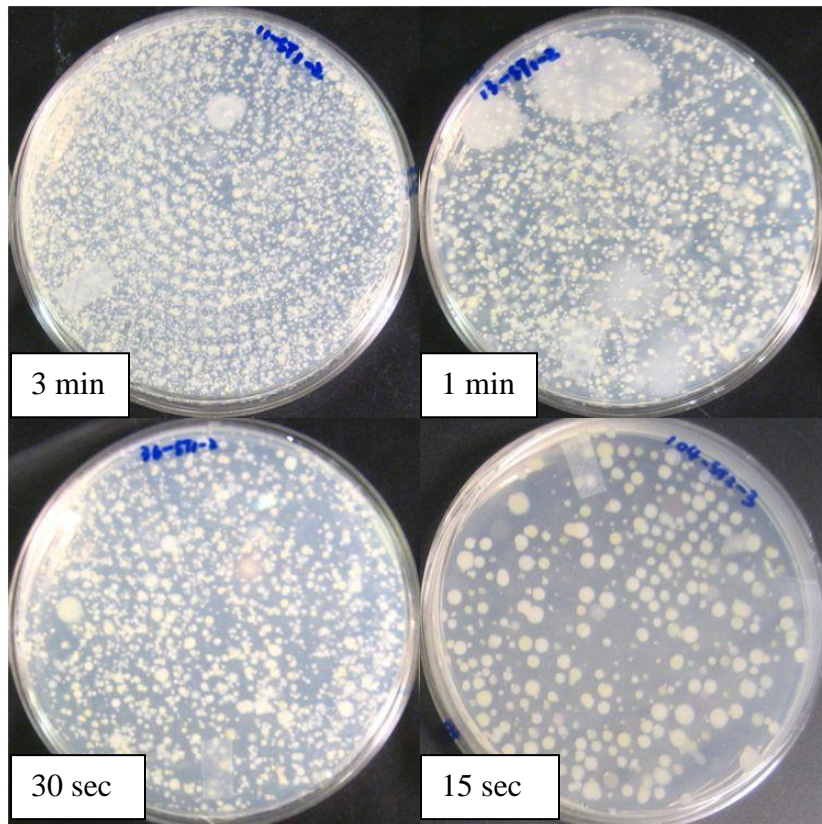


Figure 6. Agar plates under different sampling duration for in-house bacteria sampling

***COLLECTION MEDIA PREPARATION AND COLLECTED SAMPLE ENUMERATION***

The collection media for non-selective bacteria was R2A agar, which is a non-selective agar that has been found to allow the culturing of many species of bacteria. The collection media for non-selective fungi was Malt Extract Agar (MEA), which is commonly used for the isolation of fungi. To prepare the agar plate, 18.2 g R2A agar or 33.6 g MEA agar was added to each 1 L of DI water and mixed well. The liquid was autoclaved at 120 °C and 17 psi for one hour. After sterilization, the liquid were cooled in a water bath. When the liquid temperature dropped to 50 °C, 25 ml of liquid was dispensed into each 100 mm plate under a biosafety cabinet to avoid contamination on plates. Figure 7 shows the preparation of agar plates under the biosafety cabinet. To prevent bacteria growth on MEA agar, 1 ml 1000x streptomycin sulfate was added to each 1 L MEA agar after cooling and before dispensing.

The prepared agar plates were labeled and stored at 4 °C until use to avoid background contamination.



Figure 7. Agar plate preparation

As a quality control/quality assurance (QA/QC) procedure, a set of lab and field blanks for both collection media, R2A and MEA, were used on each sampling date. They were randomly chosen from the prepared plates before each sampling event. The lab blanks were stored in the refrigerator in the lab where the agar plates were prepared. The field blanks were transported to the research site with other sampling plates in coolers at each sampling event. The blanks were settled without covers being removed and were incubated with all other sampled plates after sampling.

After each sampling event, the bacteria and fungi samples collected at the research site (farm) were transported back on the same date to the lab in coolers with ice packs. The samples were then incubated immediately after arrived in the lab under consistent temperatures to allow the colonies to grow. Bacteria samples were incubated for 48 hours at 30°C, and fungi samples were incubated for 72 hours at room temperature, which was 25 °C (shown in Figure 8(a)). After incubation, colony counting was conducted under magnification and with the aid of a colony counter (Quebec Darkfield Manual Colony Counter, Reichert, INC.) Figure 8(b) and (c) show the colony counter and the use of the

colony counter for sample enumerating. Whenever possible, the numbers of colonies on the whole plate was counted. However, for crowded colonies (any overloaded plate), a special procedure was taken to obtain relatively accurate counts (Andersen, 1958). In this procedure, only 4 square areas with  $1\text{ cm}^2$  per square were randomly selected for counting. The numbers of colonies per  $\text{cm}^2$  were averaged and then multiplied by the areas of the plate to calculate the colony numbers for the whole plate. In this experiment, the 100 mm plate had an area of  $56\text{ cm}^2$ . Any colony count greater than 250 was considered to be estimated, zero count was reported as below detection.



[a] Plates in the incubator; [b] colony counter [c] counting the colony with the counter

Figure 8. Enumeration process for the collected bioaerosol samples

### ***FILED MEASUREMENTS OF THE ENVIRONMENTAL PARAMETERS***

In addition to bacteria and fungi concentration measurements, air temperature and RH were measured and recorded at each sampling station during each sampling test. A psychrometer was used in house to measure dry-bulb and wet-bulb temperatures. Two hand-held digital temperature/RH sensors (Digital Thermometer 11-661-21, Control Company, Friendswood, TX; Four in One Environmental Meter DLAf-8000, Taiwan) were used to measure the temperatures and RHs at the ambient locations. Started from summer 2011, two additional psychrometers were used to measure dry-bulb and wet-bulb temperatures at ambient stations.

A 10 m weather tower located between houses 2 and 3 (Figure 1) simultaneously took measurements of temperature, RH, wind speed and direction, and solar radiation at one minute interval. The wind sensor was Wind sentry model 03002VM anemometer (R.M. Young, Traverse, MI), the solar sensor was Pyranometer model LI-200SL (LI-COR, Lincoln, NE) and the Temp/RH sensor was capacitance type RH/T probe (Novus Automation, Porto Alegre, Brazil). All the sensors on this tower were 1.5m above the building ridges. The meteorological data were used to address meteorological effects on ambient bioaerosol temporal and spatial variations. Figure 9 shows the placement of the weather tower.

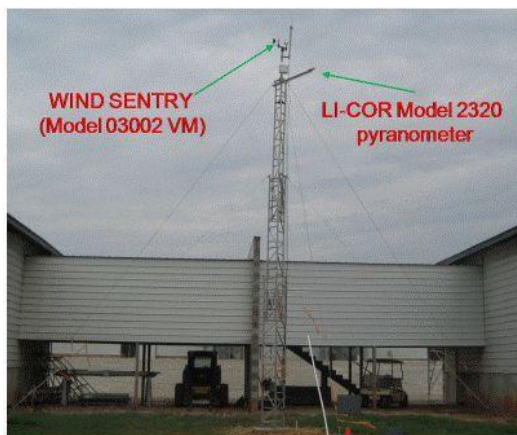
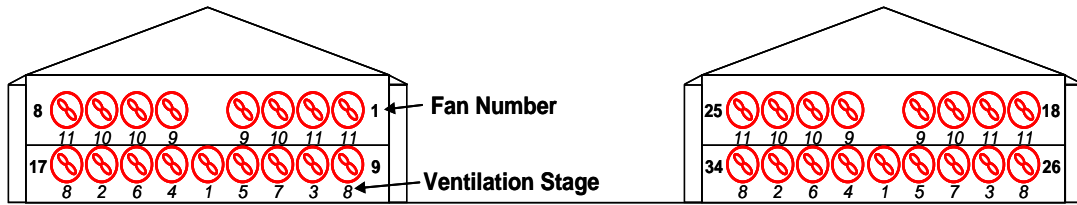


Figure 9. The 10m weather tower located between houses 2 and 3

## ***MONITORING HOUSE VENTILATION***

As shown in Figure 1, the in-house sampling station (ST1) was located in house 4. This production house was a tunnel ventilated high-rise layer barn. The ventilation settings for the tunnel ventilated high-rise layer houses are shown in Figure 10. To calculate bioaerosol emission rate, measurement of the house ventilation flow rate was required. To reduce monitoring cost, the operational status of some selective ventilation fans in the monitoring house were monitored using CR9380-NPN current switches (CR Magnetics Inc. St. Louis, MO) and recorded with HOBO data loggers in one minute interval. These selective fans were selected to represent each of the eleven ventilation stages (Table 7). It was observed that the ventilation fans in the same ventilation stage group had the same on/off status. Thus, monitoring one fan in each stage gave the information of the fan on/off status of all the ventilation fans in the house. The *in-situ* airflow measurements of all the ventilations fans in the house were conducted using a field-portable Fan Assessment Numeration System (FANS) (Gates et al., 2004). Detailed FANS measurement information and fan flow rate results are reported in Wang et al. (2012a). These *in-situ* measurement results of the fan flow rate were used for house air flow rate calculation for each of the sampling test.



Stage	Quantity	Fan ID
1	2	13, 30
2	2+2=4	13, 16, 30, 33
3	4+2=6	10, 13, 16, 27, 30, 33
4	6+2=8	10, 13, 14, 16, 27, 30, 31, 33
5	8+2=10	10, 12, 13, 14, 16, 27, 29, 30, 31, 33
6	10+2=12	10, 12, 13, 14, 15, 16, 27, 29, 30, 31, 32, 33
7	12+2=14	10, 11, 12, 13, 14, 15, 16, 27, 28, 29, 30, 31, 32, 33
8	14+4=18	9, 10, 11, 12, 13, 14, 15, 16, 17, 26, 27, 28, 29, 30, 31, 32, 33, 34
9	18+4=22	4, 5, 9, 10, 11, 12, 13, 14, 15, 16, 17, 21, 22, 26, 27, 28, 29, 30, 31, 32, 33, 34
10	22+6=28	3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17, 20, 21, 22, 23, 24, 26, 27, 28, 29, 30, 31, 32, 33, 34
11	28+6=34	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34

Figure 10. Ventilation settings for the tunnel-ventilated high-rise house (Wang-Li et al., 2012a)

Table 8. The monitored ventilation fans and their representation of the ventilation stages

Monitored fan ID	13	16	10	14	12	15	11	9	4	3	1
Representation of stages	1	2	3	4	5	6	7	8	9	10	11

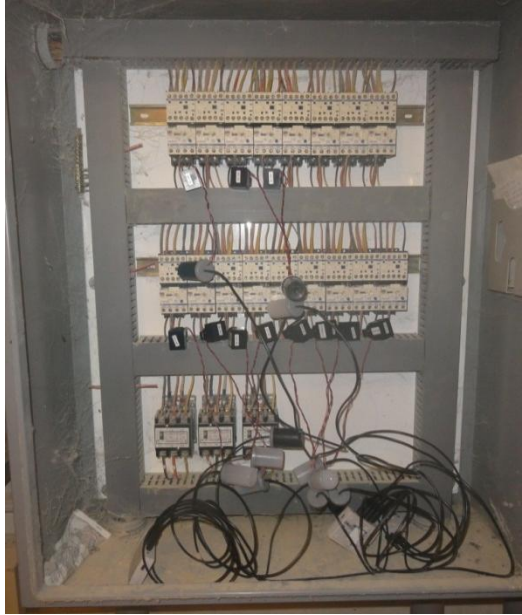


Figure 11. Current switches and data loggers installed in the farm ventilation control box for monitoring the on/off status of the selected fans

## **DATA PROCESSING AND ANALYSIS**

### ***BIOAEROSOL CONCENTRATION DETERMINATION***

After the plate reading, bioaerosol concentration (CFU/m<sup>3</sup>) was calculated using the following equation:

$$\text{Conc} = \frac{\text{CFU}}{T \times Q_s} \quad (5)$$

Where, CFU is the colony forming unit, which indicates the numbers of bacteria or fungi counts on the sampled plate; t is sampling duration; and Q<sub>s</sub> is sampler flowrate. As it is stated before, the sampler flow rate was calibrated before each sampling event, and was checked after the sampling event. The sampler flow rate (m<sup>3</sup>/min) was the average of pre-sampling flow rate and post-sampling flow rate.

### **BIOAEROSOL EMISSION RATE DETERMINATION**

Housing emission rate (CFU/min) was calculated using:

$$E = Q_{Ave} \times Conc_s \quad (6)$$

Where,  $Q_{ave}$  is the ventilation rate of the sampling house for each sampling test, and  $Conc_s$  is the measured bioaerosol concentrations at source. The ventilation rate was determined using the following equation:

$$Q_{Ave} = \frac{\sum(t_i \times Q_i)}{T} \quad (7)$$

Where,  $t_i$  is the operation time of each fan (monitored by the current switches);  $Q_i$  is the flow rate of each individual fan, which was measured by the FANS (Wang-Li et al., 2012a); and  $t$  is the sampling duration of each test.

### **AMBIENT WIND DATA PROCESSING**

For ambient stations, upwind and downwind locations were determined by the vectorial average of wind direction during each test (10 min). As shown in Figure 12, a line vertical to the line from ST1 (source) to an ambient station (e.g. ST2) to divide the directions of source and ambient station. If wind blew from source to one ambient station, then that ambient station was at downwind direction. Similarly, if wind blew from ambient station to source, then that ambient station was at upwind direction. Wind factor was used to describe the effects of wind speed and wind direction on downwind bioaerosol concentrations. It was calculated using:

$$W = U \times \cos\theta \quad (8)$$

Where,  $w$  is the wind factor;  $U$  is the vector average of wind speed during each test; and  $\theta$  is the angle between vector average wind direction and the line from source to an ambient station.



- The ratios of temperature and RH on the second floor to those on the first floor were two independent variables to predict the ratio of bacteria on the second floor to that on the first floor.
- For temporal and spatial variations at ambient locations
  - ANOVA test were applied to test the seasonal, time, and spatial variations of bioaerosol concentrations in the vicinity.
  - Temperature, RH, wind factor, solar radiation, and emission rate were the five predictors to predict downwind bioaerosol concentrations in the vicinity
  - Stepwise selections were applied to investigate the significant influencing factors affecting downwind bioaerosol concentrations.
- For bioaerosol size distributions at the source and ambient location
  - Mean concentrations of bacteria and fungi on each stage of the sampler were calculated by season at five sampling stations to test temporal effects on variations of bioaerosol size distributions.
  - Mean concentrations of bacteria and fungi on each stage of the sampler at different sampling locations (1<sup>st</sup> floor vs. 2<sup>nd</sup> floor, upstream vs. Downstream, and upwind vs. Downwind) to test spatial effects on variations of bioaerosol size distributions.
  - Percentages of bioaerosols in each size range were calculated by season at different locations to show portions of bioaerosols in each size ranges.

## CHAPTER 3 TEMPORAL AND SPATIAL VARIATIONS AT THE SOURCE

### TEMPORAL VARIATIONS

#### *AIRBORNE NON-SELECTIVE BACTERIA CONCENTRATIONS*

Based upon analyses of the bacteria samples taken at ST1 through all sampling events listed in Tables 1 and 5, non-selective bacteria concentrations in the poultry house have been found to range from  $2.8 \times 10^4$  to  $1.8 \times 10^6$  CFU/m<sup>3</sup>. It is suspected that the causes of such variation include temperature and RH changes, the consequent ventilation rate changes, the variations of gas pollutants concentrations (e.g. NH<sub>3</sub>, H<sub>2</sub>S, CO<sub>2</sub>, and volatile organic compounds) in the house, and the consequent variations of bacteria survival rate.

Table 9 lists the mean concentrations with standard deviations (SDs) of non-selective bacteria at ST1 in different seasons separated by the morning and afternoon sampling periods. An ANOVA test was conducted to compare the means. It was discovered that mean concentrations in morning and afternoon periods over different seasons were significantly different ( $p < 0.0001$ ). As shown in Table 9, there was no significant difference between mean concentrations of bacteria in winter and spring, while mean concentrations of summer bacteria were significantly lower than winter and spring. The main reason for the variation might be the extremely high ventilation rate in summer.

Table 9. Means with SDs of non-selective bacteria concentrations (CFU/m<sup>3</sup>) at ST1

Time of day	Winter		Spring		Summer	
	Mean <sup>*,**</sup>	SD	Mean	SD	Mean	SD
Morning	$8.0 \times 10^5$ <sup>[a]</sup>	$3.7 \times 10^5$	$1.1 \times 10^6$ <sup>[a]</sup>	$4.2 \times 10^5$	$2.6 \times 10^5$ <sup>[b]</sup>	$2.0 \times 10^5$
Afternoon	$9.7 \times 10^5$ <sup>[a]</sup>	$4.8 \times 10^5$	$1.0 \times 10^6$ <sup>[a]</sup>	$4.3 \times 10^5$	$1.9 \times 10^5$ <sup>[b]</sup>	$6.7 \times 10^5$

\* Means with different letters indicate significant difference at a 0.05 level.

\*\* Means were computed from 15, 15, 12, 12, 15, and 15 replicates for winter-morning, winter-afternoon, spring morning, spring afternoon, summer morning, and summer afternoon, respectively.

Figure 13 shows variations of mean bacteria concentrations in three seasons (winter, spring and summer) over different times of day. As it is observed, the mean bacteria concentrations in summer were constantly and significantly lower than those in other two seasons. Changes of mean concentrations over different times of day in summer were not as dramatic as in other two seasons. In summer time, the highest mean was observed at 10:00am, whereas in winter time the highest mean was at 1:00pm. For spring, the highest mean was observed before noon (around 11:00am) and the lowest means was at 1:00pm. It was hypothesized that the combination effects of the house temperature, RH and ventilation rates were the causes for the temporal variation patterns illustrated in Figure 13. Thus, temporal variations of these causing factors were further analyzed.

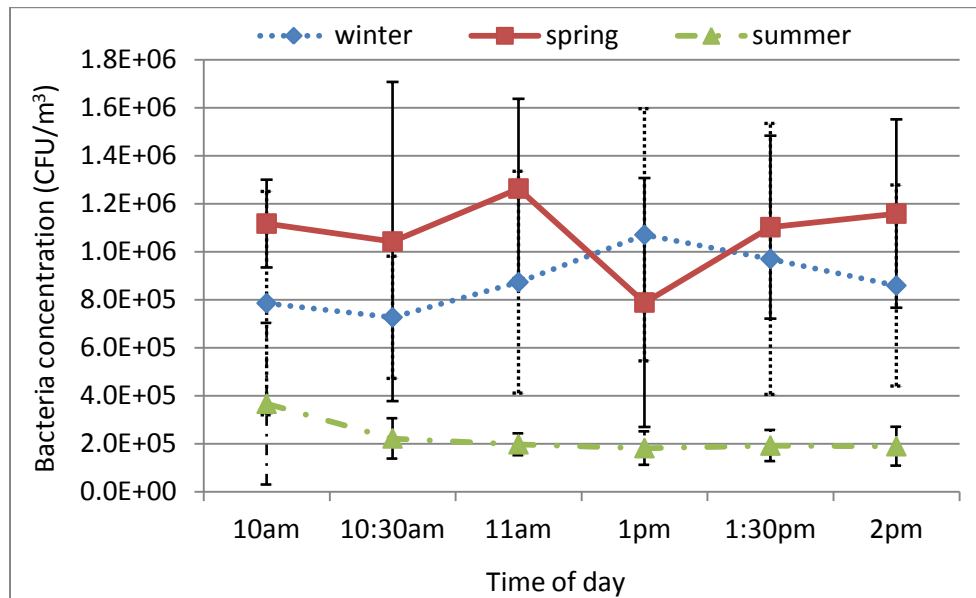


Figure 13. Mean bacteria concentrations  $\pm$  SD at source (ST1) in different seasons over different time of day

Figures 14-16 show the mean temperatures, RHs and ventilation rates (Q) at the times when the bacteria samples were taken in the house (ST1) in three seasons. It is shown in Figure 16 that the house ventilation rates in summer were significantly higher as compared to those in other seasons. High ventilation rate produced higher air exchange rate, consequently brought more fresh air into the house and removed more bacteria and other pollutants out of the house. It is also observed in Figures 14 and 15, the in-house mean temperatures were higher and the mean RHs were lower in summer time. High temperature and low RH levels were possible to cause bacteria dehydration and inactivation.

As shown in Figures 14 and 15, during summer, temperature increased with time while RH decreased with time in the same day. Summer bacteria concentrations were the highest in the morning because of the cooler and moister condition in the house. In winter, bacteria concentrations were at the lowest in morning because the temperature in the morning of winter was low that might cause bacteria to die. Winter bacteria concentrations increased with time in the morning, and reached to the peak at noon, when the temperature reached the highest in a day. After that, winter bacteria concentration decreased with time in the afternoon due to the decreasing temperature. In addition, the house ventilation rate in winter time was constantly low and had minimal impact on variation of the in-house bacteria concentration. In spring, morning had the highest bacteria concentration among three seasons, which might be because the air conditions (i.e., temperature and RH) were at the most optimal for bacteria to survive. Spring bacteria concentration dropped at noon, because of the increasing ventilation rate.

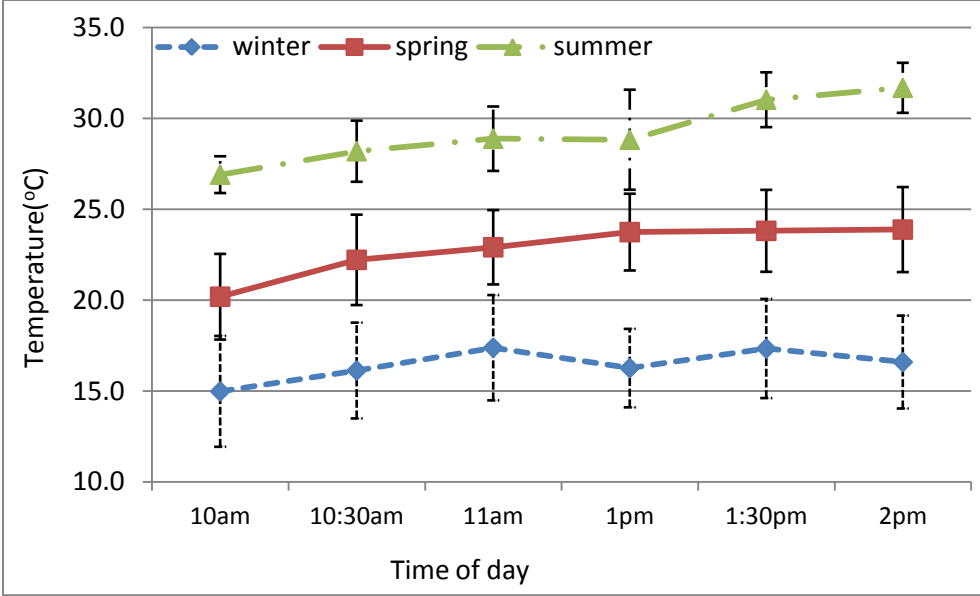


Figure 14. Mean temperatures ( $\pm$ SD) at bacteria sampling times

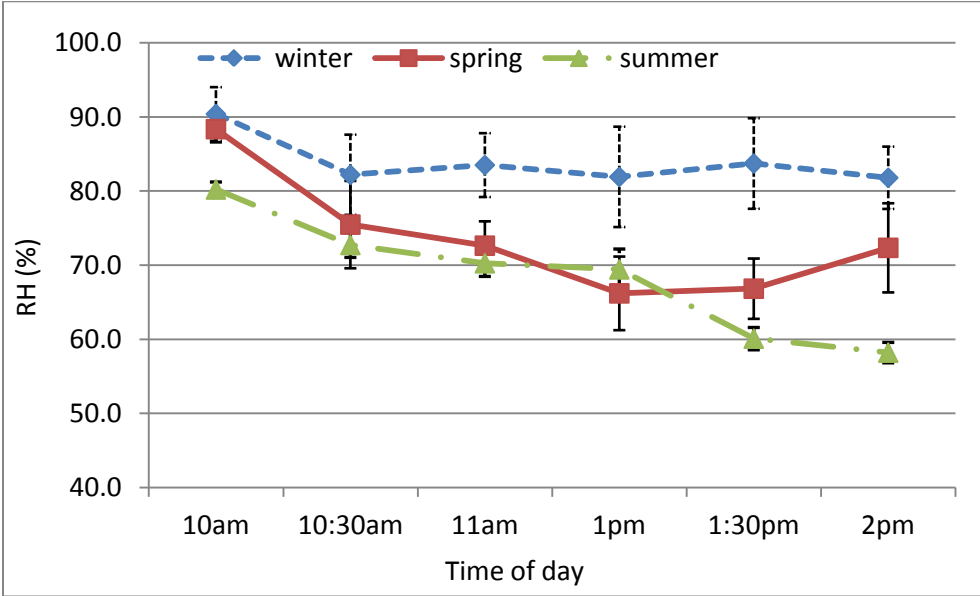


Figure 15. Mean RHs ( $\pm$ SD) at bacteria sampling times

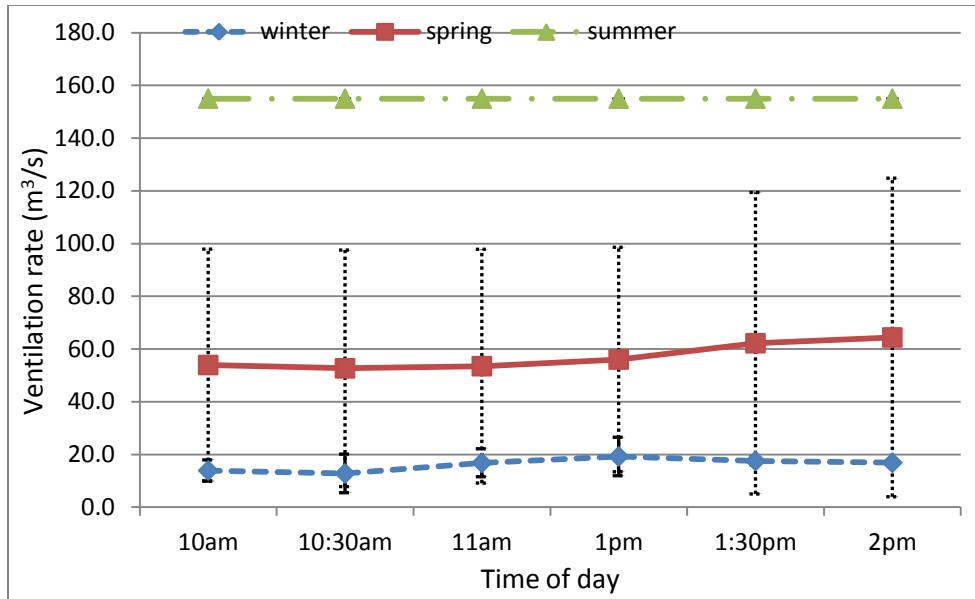


Figure 16. Mean ventilation rates ( $\pm$ SD) at bacteria sampling times

#### ***AIRBORNE NON-SELECTIVE FUNGI CONCENTRATIONS***

As done for the bacteria samples, analyses were also conducted on fungi samples taken at ST1 through all sampling events listed in Tables 1 and 5. Airborne fungi concentrations in the poultry house have been found to range from 220 to  $1.6 \times 10^4$  CFU/m<sup>3</sup>. Like bacteria concentrations, it is possible that the causes of such variations include temperature and RH changes, the consequent ventilation rate changes, the variations of gas pollutants concentrations in house, and the consequent variations of fungi survival rate.

Table 10 lists mean values with SDs of in-house fungi concentrations in different seasons and at different time of a day. An ANOVA test was conducted to compare the means. It was revealed that there were no significant differences in means over different seasons as well as between morning and afternoon periods ( $p = 0.1974$ ). As shown in Table 10, mean concentrations of fungi were relatively constant when compared to bacteria concentrations. It might be because that the temperature and RH changed among the three seasons were not great enough to cause a significant change in fungi survival rate. It also indicated that the

variations in ventilation rate did not have significant impact on fungi concentration in the house.

Table 10. Mean concentrations (CFU/m<sup>3</sup>) with SDs (CFU/m<sup>3</sup>) of fungi at source.

	Winter		Spring		Summer	
	Mean	SD	Mean	SD	Mean	SD
Morning	1.6×10 <sup>3[a]*</sup>	0.3×10 <sup>3</sup>	1.6×10 <sup>3[a]</sup>	0.9×10 <sup>3</sup>	2.5×10 <sup>3[a]</sup>	1.8×10 <sup>3</sup>
Afternoon	1.7×10 <sup>3[a]</sup>	0.8×10 <sup>3</sup>	1.5×10 <sup>3[a]</sup>	0.8×10 <sup>3</sup>	3.2×10 <sup>3[a]</sup>	4.5×10 <sup>3</sup>

\* Means with the same letters indicate no significant differences at a 0.05 level.

Figure 17 shows variations of mean fungi concentrations over different time of day separated by season. To better understand causes of the variation pattern shown in this figure, measurements of in-house temperature, RH and ventilation rates were also analyzed.

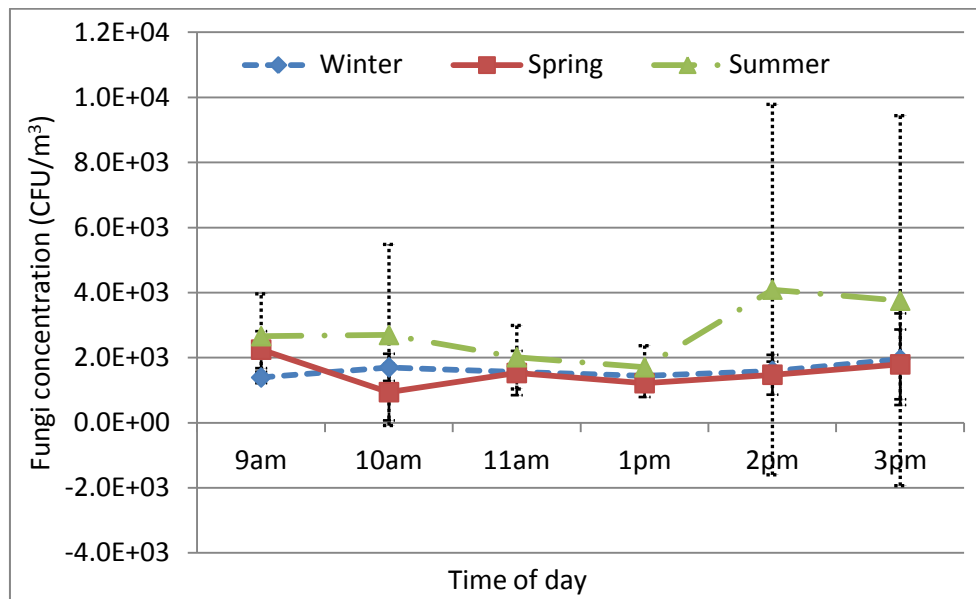


Figure 17. Mean fungi concentrations ± SD at source in different seasons over different time of day.

Figures 18 - 19 show the variations of the mean temperatures and RHs at the times when the fungi samples were taken in the house among three seasons. As shown in Figure 17, there were no significant differences between fungi concentrations in different seasons or at different time of day ( $p = 0.8143$ ). As shown in Figures 18 and 19, during summer, temperature increased with time while RH decreased with time in the same day. Besides that have been discussed above, possible reasons for the observed constant fungi concentrations at source also include that other factors (e.g. gaseous pollutants) might have greater effects on fungi concentration than temperature and RH.

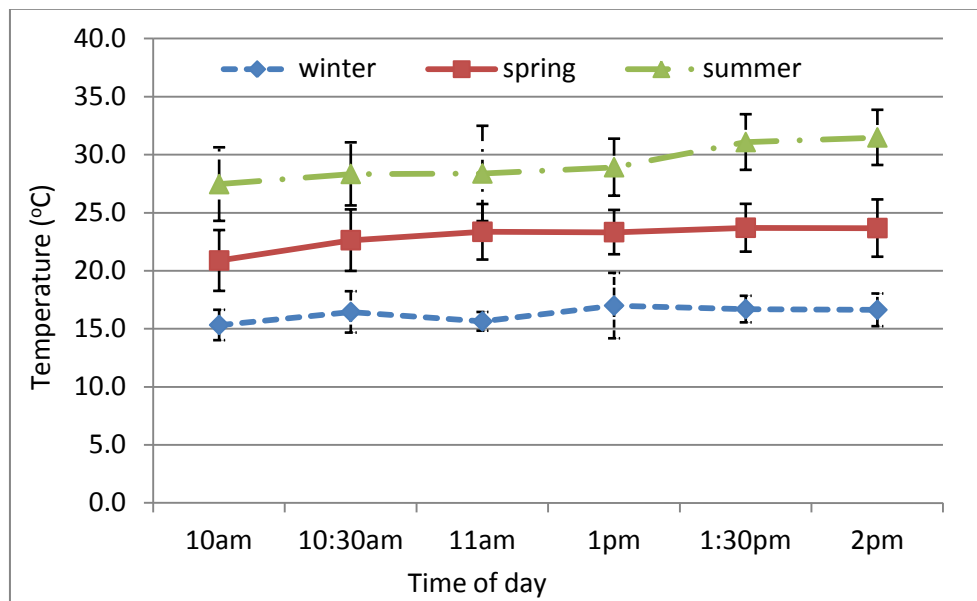


Figure 18. Mean temperature measurements ( $\pm$ SD) at fungi sampling times

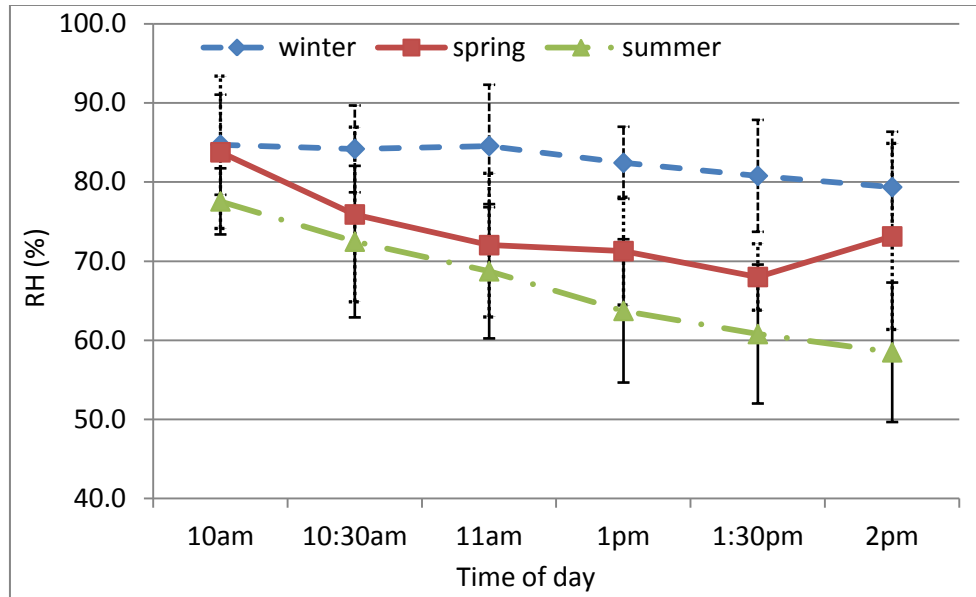


Figure 19. Mean RH measurements ( $\pm$ SD) at fungi sampling times

#### ***IMPACTS OF INFLUENCING FACTORS***

To better understand the observed seasonal and time of day variations in both bacteria and fungi concentrations at source, multiple linear regression models were applied to assess the relationship between in-house bacteria and fungi concentrations and affecting factors using SAS 9.2 software. In the regression models, concentrations of bacteria or fungi at source were dependent variables, and the influencing factors, temperature (T), RH, and ventilation rate (Q) were independent variables. To test the linear dependencies between the four variables, Pearson correlation coefficients were calculated for each two of them. It was shown that there were significant linear dependencies between bioaerosol concentrations and the three influencing factors, as well as between each two of the influencing factors ( $p < 0.0001$ ). It suggests that each independent variable had significant impact on bioaerosol concentrations at source. However, because the independent variables themselves were linearly correlated, they may not seem to be important when fitted in a model. The results of multiple linear regression models using T, RH, and Q as three predictors verified this hypothesis. The outputs of the models are summarized in Table 11.

Table 11. Multiple linear regression models for bioaerosol temporal variations.

Bioaerosol type		Estimate	Std. Error	<i>P</i> -value
Bacteria	Intercept	460000	280000	0.096
	T	21000	9400	0.029
	RH	3900	2300	0.084
	Q	-6900	870	<0.0001
Fungi	Intercept	5400	2100	0.010
	T	-52	68	0.44
	RH	-42	18	0.024
	Q	16	6.5	0.015

For modeling bacteria concentration as impacted by T, RH and Q, only T and Q played significant roles in affecting temporal variations of bacteria concentrations at a significant level of 0.05. The adjusted-R<sup>2</sup> of the model was 0.59.

For modeling fungi concentration as impacted by T, RH, and Q, only RH and Q played significant roles in affecting temporal variations of fungi concentrations at a significant level of 0.05. The adjusted-R<sup>2</sup> of the model was only 0.18, which means this model didn't explain the temporal variation of fungi concentration well. Furthermore, the estimated parameters for T and RH were negative while that for Q was positive. This observation didn't agree with common expectation. In general, the in-house concentration should negatively correlate with ventilation rate. The higher ventilation (Q) would cause the lower concentration.

To further modify the statistical models, bioaerosol concentrations were log transformed to be responses. Residual plots (see Figures A1-3 in appendix) between the responses and each predictor suggested that the relationship between bacteria concentration and T might be quadratic. Therefore, for predicting in-house bacteria concentration, T<sup>2</sup> were introduced as a new predictor. Among all possible regression models, a model using T, T<sup>2</sup> and Q as predictors was preferred by R-square and Mallows' CP selections (see Table A1 in appendix). For predicting in-house fungi concentration, residual plots (see Figures A3-6 in appendix)

suggested that the relationship between fungi concentration and T as well as RH were not linear.  $T^2$  and  $RH^2$  were introduced as new predictors, but the R-square and Mallows' CP selection showed that the model contained T, RH and Q as predictors were the most preferred (see Table A2 in appendix). Table 12 shows the results of selected models for predicting log transformed in-house bacteria and fungi concentrations.

Table 12. Selected multiple linear regression models for bacteria and fungi concentrations in log scales

Bioaerosol type		Estimate	Std. Error	P-value
Bacteria	Intercept	12	0.81	<0.0001
	T	0.21	0.069	0.0034
	$T^2$	-0.0042	0.0013	0.0020
	Q	-0.010	0.0016	<0.0001
Fungi	Intercept	8.1	0.59	<0.0001
	T	-0.018	0.020	0.3635
	RH	-0.0078	0.0052	0.1418
	Q	0.0058	0.0019	0.0021

As shown in this table, in bacteria model, all three predictors had significant impacts on bacteria concentration at a 0.05 level, and the adjusted R-square of this model was 0.59. However, in fungi model, neither T nor RH was important at a 0.05 level, and the adjusted R-square was 0.23. Although T and RH were not important in the linear regression models, it was aware that they had great impacts on fungi concentration indeed.

## SPATIAL VARIATIONS

### *1<sup>ST</sup> FLOOR & 2<sup>ND</sup> FLOOR VARIATION*

Based upon analyses of the bacteria and fungi samples simultaneously taken on two floors of the sampling house through all sampling events listed in Table 5, mean concentrations of bacteria and fungi on the two floors were computed. Figures 20 and 21

show the resultant mean concentrations of bacteria on the 1<sup>st</sup> and 2<sup>nd</sup> floors and the local air temperature and RH at the sampling times. It was observed that bacteria concentrations were significantly higher on the 2<sup>nd</sup> floor than on the 1<sup>st</sup> floor ( $p < 0.0001$ ).

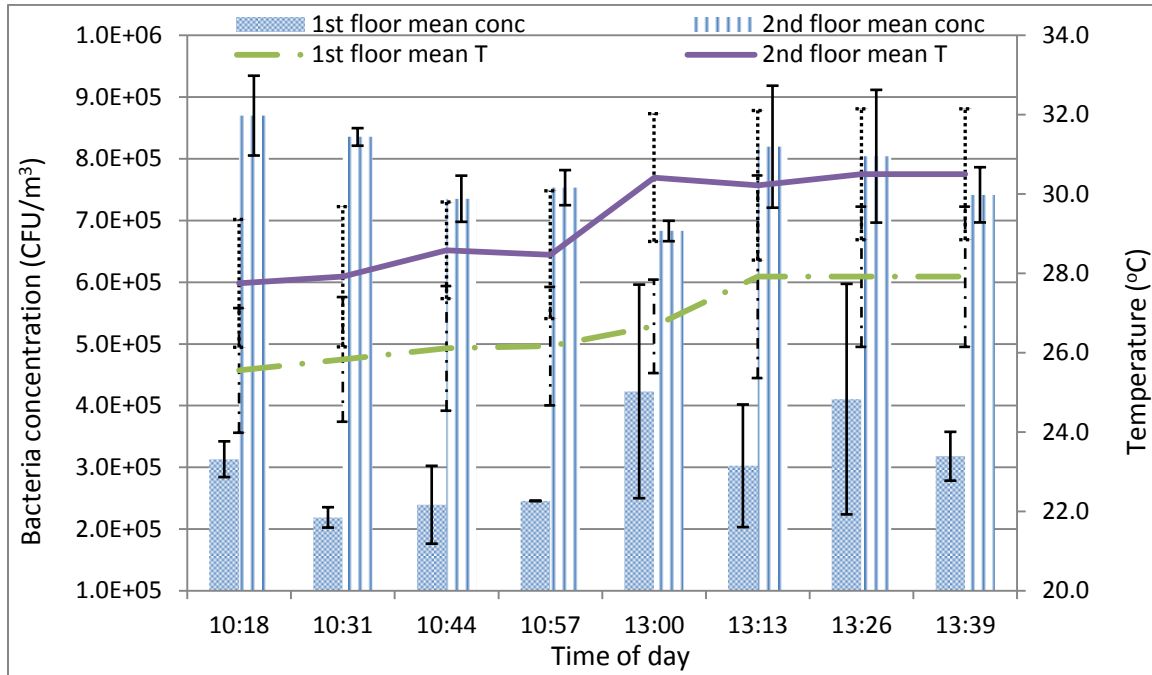


Figure 20. Comparisons of the mean temperatures and bacteria concentrations on the 1<sup>st</sup> and 2<sup>nd</sup> floors

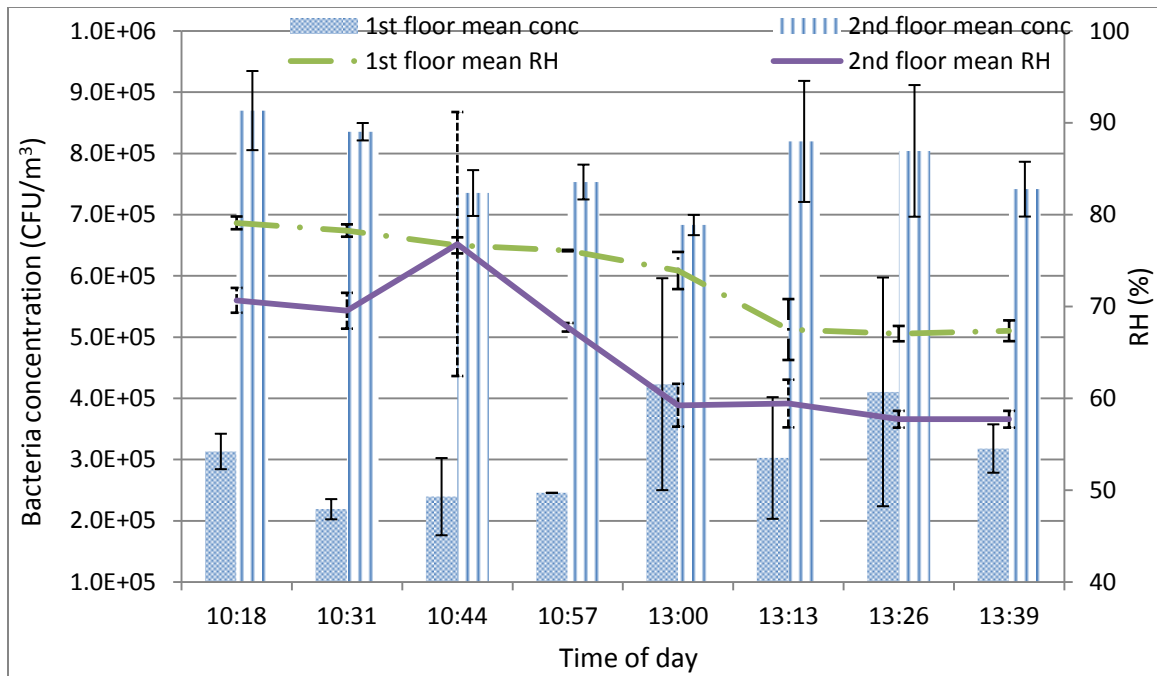


Figure 21. Comparisons of the mean RHs and bacteria concentrations on the 1<sup>st</sup> and 2<sup>nd</sup> floors

Figure 22 and 23 show the mean concentrations of fungi on the 1<sup>st</sup> and 2<sup>nd</sup> floors of the sampling house and the measured air temperature and RH during the sampling times. It was observed that there was no significant difference between fungi concentrations on the 1<sup>st</sup> and 2<sup>nd</sup> floors ( $p = 0.14$ ).

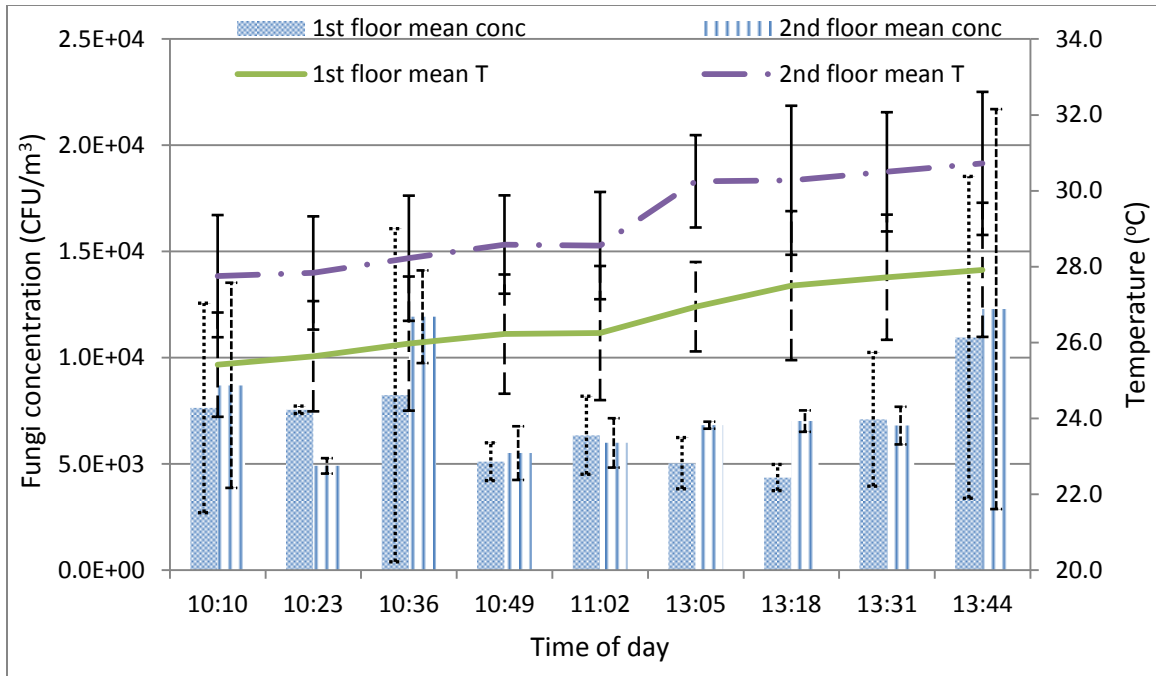


Figure 22. Comparisons of the mean temperatures and fungi concentrations on the 1<sup>st</sup> and 2<sup>nd</sup> floors

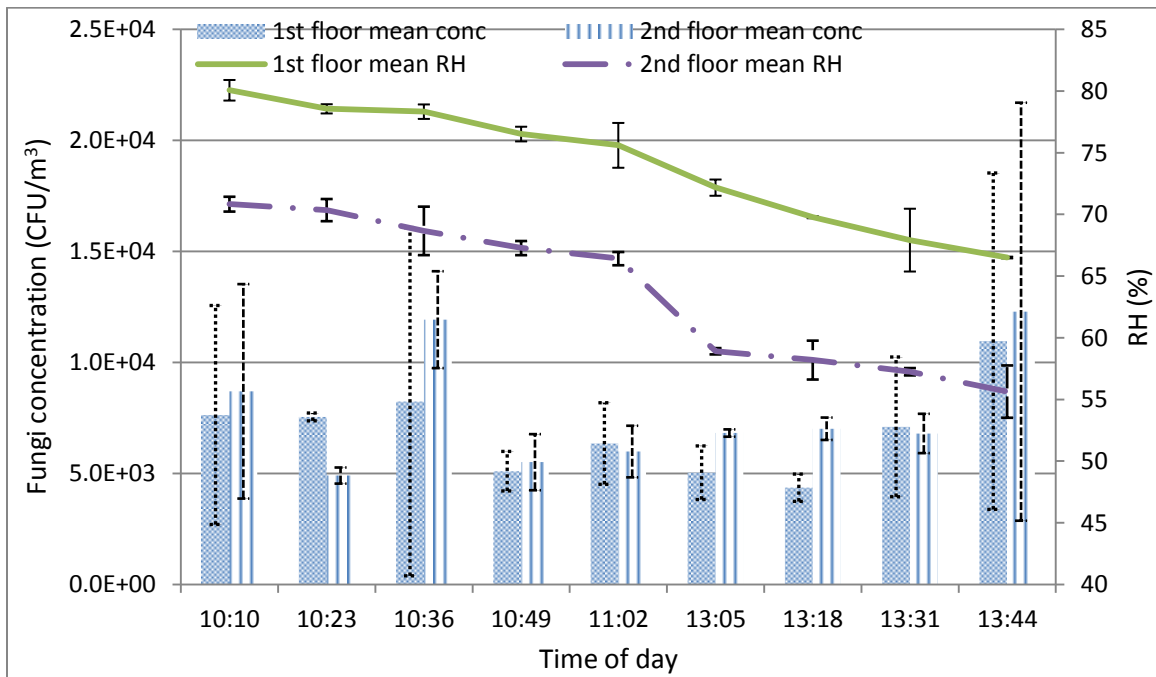


Figure 23. Comparisons of the mean rhs and fungi concentrations on the 1<sup>st</sup> and 2<sup>nd</sup> floors

Factors that might have impacts on in-house bioaerosol concentrations and survival rate include air conditions (e.g. temperature and RH levels), ventilation rate, and existence of gaseous pollutants. In this project, due to the limited budget and manpower, concentrations of gas pollutants were not able to be measured simultaneously at bioaerosol sampling. However, gas pollutants concentrations correlated with ventilation rate, the higher the ventilation rate, the lower the gas pollutant concentrations (Wang-Li et al., 2012b). As a farm practice, the ventilation rates were dictated by in-house temperature (Wang-Li et al. 2012a). Therefore, in-house air conditions (i.e. T and RH) were considered the main influencing factors for bioaerosol concentration. Simultaneously measured temperatures and RHs were then grouped into non-selective bacteria tests (TB) and non-selective fungi tests (TF) according to the corresponding bioaerosol sampling tests. Mean temperatures and RHs with SDs were calculated for both floors during morning and afternoon periods to investigate the variations of in-house air conditions at different locations and during different time periods. Table 13 shows the mean values with SDs of in-house temperature and RH grouped into morning and afternoon periods and separated by floors. For both bacteria and fungi tests, it is shown that mean temperatures were higher on the 2<sup>nd</sup> floor than on the 1<sup>st</sup> floor ( $p < 0.0001$ ), and were higher in afternoon than in morning. RHs were higher on the 1<sup>st</sup> floor than on the 2<sup>nd</sup> floor ( $p = 0.0001$ ), and were higher in morning than in afternoon. Temperatures were the highest on the 2<sup>nd</sup> floor during afternoon, and were the lowest on the 1<sup>st</sup> floor during morning. RHs were the highest on the 1<sup>st</sup> floor during morning, and were the lowest on the 2<sup>nd</sup> floor during afternoon.

Table 13. Means with SDs of temperature and RH on the 1<sup>st</sup> and 2<sup>nd</sup> floors

Bioaerosol type	In house air condition	First floor		Second floor					
		Morning		Afternoon		Morning		Afternoon	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Bacteria	Temperature (°C)	25.9 <sup>[c]*</sup>	1.1	27.5 <sup>[b][c]</sup>	1.5	28.1 <sup>[b]</sup>	1.2	30.3 <sup>[a]</sup>	1.3

Table 13 Continued

	RH (%)	77.5 <sup>[d]</sup>	1.3	69.1 <sup>[e]</sup>	3.2	71.0 <sup>[e]</sup>	5.8	58.2 <sup>[f]</sup>	1.3
	Temperature	25.9 <sup>[c]</sup>	1.2	27.5 <sup>[b]</sup>	1.2	28.2 <sup>[b]</sup>	1.2	30.3 <sup>[a]</sup>	1.3
Fungi	(°C)								
	RH (%)	77.8 <sup>[d]</sup>	1.8	68.9 <sup>[e]</sup>	2.4	68.7 <sup>[e]</sup>	2.0	57.7 <sup>[f]</sup>	1.5

\* Means with different letters indicate significant differences at a 0.05 level.

Factors causing spatial variation in bacteria concentrations between two floors include but not limited to (1) differences in ventilation rates on the two floors; (2) temperature difference; (3) difference in RH; (4) differences in sources generation of bacteria. As is shown in Figure 10, there was one less fan on the 2<sup>nd</sup> floor as compared on the 1<sup>st</sup> floor. Therefore, during summer, when all the fan stages were on, the ventilation rates were higher on the 1<sup>st</sup> floor than on the 2<sup>nd</sup> floor. Besides affecting bioaerosol concentrations, the difference in ventilation rates may also influence the local air temperature and RH. The generation sources of bacteria might be different on two floors. Bacteria detected on the 1<sup>st</sup> floor were most probably generated from animal waste while bacteria on the 2<sup>nd</sup> floor were most probably generated from animal feed and animal activities.

Two main reasons for the higher airborne bacteria concentration on the 2<sup>nd</sup> floor were the lower ventilation rate and lower RH there. Lower ventilation rate means there were less bioaerosols being exhausted to the ambient. An intermediate RH level (i.e., 60%) is the most optimal for many kinds of bacteria (Tang, 2006), and RH on the 2<sup>nd</sup> floor was closer to the intermediate level. As compared to RH, temperature might play a less significant role in affecting in-house bacteria concentration. This may be because that the temperatures on the 1<sup>st</sup> and 2<sup>nd</sup> floor were not high enough to cause bioaerosol inactivation.

Unlike bacteria, fungi concentrations did not show any significant variation on different floors. This may be because that the high RHs inside the house during sampling times were not in favor of the survival of many fungi species. Many fungi might have been dead at the

high RH levels; therefore fungi concentration did not show significant spatial variation as bacteria did.

A linear regression model was applied to assess the relationship between spatial variations of air conditions and spatial variations of airborne bacteria concentrations. The two independent variables representing air conditions were temperature ratio (T ratio) and RH ratio, and the dependent variable was concentration ratio (Conc ratio). All these ratios were defined by the following equations:

$$\text{T ratio} = \frac{t_2}{t_1} \quad (9)$$

$$\text{RH ratio} = \frac{\text{RH}_2}{\text{RH}_1} \quad (10)$$

$$\text{Conc ratio} = \frac{\text{Conc}_2}{\text{Conc}_1} \quad (11)$$

Where:  $t_1$ ,  $\text{RH}_1$ , and  $\text{Conc}_1$  are temperature, RH, and bacteria concentration on the 1<sup>st</sup> floor, respectively;  $t_2$ ,  $\text{RH}_2$ , and  $\text{Conc}_2$  are temperature, RH, and bacteria concentration on the 2<sup>nd</sup> floor, respectively.

The difference in ventilation rate between two floors was constant because all fan stages were on during summer sampling time; therefore it was not included as one independent variable. But the fact that ventilation rate had significant impact on bioaerosol concentrations was realized. SAS 9.2 software was used to carry out the statistical analysis. The results of regression model are summarized in Table 14.

As shown in the regression (Table 14), the two affecting factors (i.e. T ratio and RH ratio) both had significant levels below 0.05. This multiple linear regression model had an adjusted- $R^2$  of 0.53. The results suggest that when ventilation rate stayed unchanged, both

temperature and RH played significant roles in causing bacteria concentration differences between two floors.

Table 14. Multiple linear regression model for bacteria spatial variation

	Estimate	Std. Error	<i>P</i> -value
Intercept	22	8.0	0.014
T ratio	-22	6.8	0.0057
RH ratio	5.2	2.0	0.019

#### *UPSTREAM & DOWNSTREAM VARIATION*

Fungi samples taken at upstream and downstream locations of the ventilations fans (Figure 4 and table 5) were analyzed to investigate changes of the concentration as impacted by the ventilation fans. Figure 24 shows comparison of fungi concentrations at the upstream of the fans (inside the house) and that at the downstream of the fans (outside the house). It was noticed that there was significant difference between fungi concentration at upstream and downstream locations ( $p = 0.0007$ ). It is possible that high air flow and impaction on the fan blades/surfaces caused injury or damage of the airborne fungi, thus reduced viability of fungi spores. Due to the limited sample size, no reliable statistical conclusion can be drawn. Further investigation is needed to quantify the impacts of high speed air flow and impaction on bioaerosol survival and concentration so that such impact may be taken into consideration in quantifying bioaerosol emission rates.

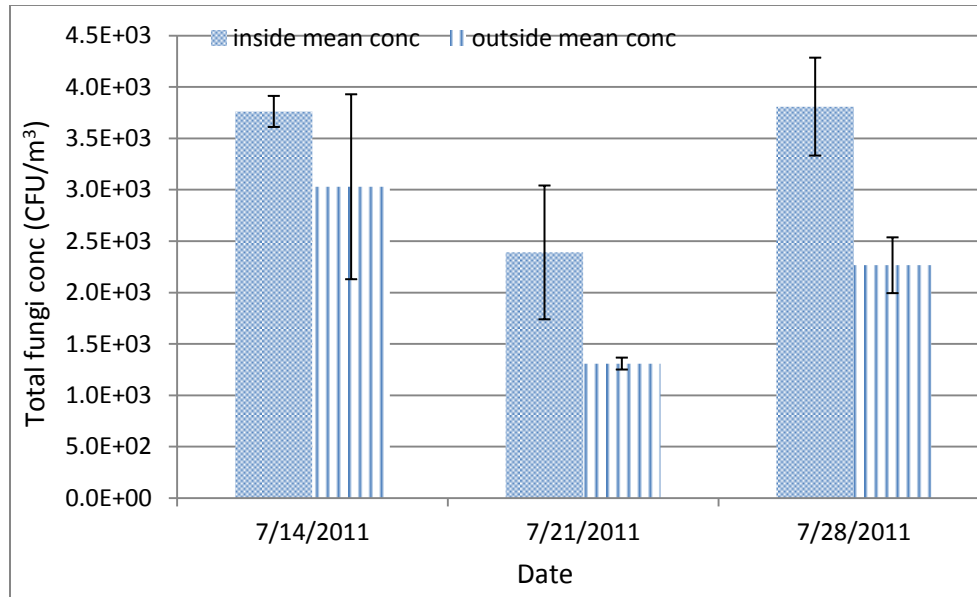


Figure 24. Comparison of mean fungi concentrations upstream and downstream of house ventilation fans

### PREDOMINANT FUNGI SPECIES

To identify spatial variations in fungi species, two sets of sample plates were analyzed for top three predominant fungi organism at the genus level at the Center for Microbial Community Systems & Health Research, RTI International (RTP, NC). These two sets of sample plates were simultaneously collected by two Andersen six-stage viable samplers on the two floors of the poultry house (Figure 4) in July 2011. The biological speciation analysis revealed that the top three predominant fungi species on the sampled plates were: *Aspergillus* spp., *Cladosporium* spp., and *Penicillium* spp. The percentages of the three predominant species on each sample plate are listed in Table 15. Figures 25 - 26 show the sample plates with the predominant species being indicated on each plate.

As shown in Table 15, percentages of the three predominant species were not significantly distinguished on different floors ( $p = 0.67, 0.57,$  and  $0.06$  for *Aspergillus* spp., *Cladosporium* spp., and *Penicillium* spp. respectively).

Table 15. Identifications and percentages of three predominant fungi species

	Sampler Stage	<i>Aspergillus</i> spp.		<i>Cladosporium</i> spp.		<i>Penicillium</i> spp.	
		Counts	Percentage	Counts	Percentage	Counts	Percentage
1 <sup>st</sup> Floor Samples	1			25	ND*	OG**	
	2	2	5.7%	19	54.3%	13	37.1%
	3	2	3.6%	31	55.4%	23	41.1%
	4	4	7.3%	41	74.5%	10	18.2%
	5	5	11.4%	10	28.6%	21	60.0%
	6	17	21.5%			62	78.5%
2 <sup>nd</sup> Floor samples	1	2	2.8%	53	74.6%	14	19.7%
	2	4	7.7%	33	63.5%	13	25.0%
	3	7	10.9%	46	71.9%	9	14.1%
	4	5	17.2%	17	58.6%	7	24.1%
	5			6	ND	OG	
	6					OG	

\* ND= not determined; \*\* OG = overgrown

As shown in Table 15, *Aspergillus* spp. were detected on the upper stages on both floors, and those *Aspergillus* spores might be attached to other particles with larger sizes and deposited on the first three stages. *Aspergillus* spp. includes several hundred mold species; therefore it can form colonies of different colors, such as dark green, black, white, and yellow. As shown in Figure 25 and 26, most *Aspergillus* spp. colonies produced in this project were dark gray and black. *Aspergillus* spp. concentrations were measured to vary from 0 to 85 CFU/m<sup>3</sup> in urban and rural environments (Guinea et al., 2006). In this project, *Aspergillus* spp. concentrations were 350 CFU/m<sup>3</sup> on the first floor, and were 210 CFU/m<sup>3</sup> on the second floor. First floor might have higher concentration of *Aspergillus* spp.

In this project, *Cladosporium* spp. was sampled in large numbers on the upper four stages, which might be because the fungi spores attached to greater aerosols and deposited on the

upper stages. *Cladosporium* spp. usually produces grey, dark green or black colonies. As shown in Figures 25 and 26, most *Cladosporium* spp. sampled in this project appeared black. Pini et al. (2004) measured concentrations of airborne fungi in a haematology ward over two years and reported that the average concentration of *Cladosporium* spp. was 24 CFU/m<sup>3</sup> in the rooms, 78 CFU/m<sup>3</sup> in the corridors and 318 CFU/m<sup>3</sup> outside. *Cladosporium* spp. concentration detected in this project was 1500 CFU/m<sup>3</sup> on the first floor and second floor.

As shown in Table 15, a great number of *Penicillium* spores sampled on the first floor had penetrated stage 3 and stage 4 and deposited on the lower stages, especially on stage 6. One possible reason was the contamination on plate before or after sampling. Besides the animal and animal waste, antibiotic in animal feed might be another; even greater source of *Penicillium* spp. *Penicillium* spp. produces green-grey or blue-grey colonies. Pini et al. (2004) reported that *Penicillium* spp. concentrations was 7 CFU/m<sup>3</sup> in the rooms, 19 CFU/m<sup>3</sup> in the corridors and 37 CFU/m<sup>3</sup> outside. *Penicillium* spp. concentration was 1800 CFU/m<sup>3</sup> on the first floor and 530 CFU/m<sup>3</sup> on the second floor. It seemed like that first floor contained more *Penicillium* spores, but it was not necessarily the fact. As shown in Table 15, *Penicillium* colonies were over grown on two of the six plates sampled on the second floor, which might cause to underestimate *Penicillium* spp. concentration on the second floor.

In general, the three predominant fungi species detected inside the animal house don't normally cause illness on health human or animals. But exposures to those fungi aerosols could result in adverse health effects on individuals with weaken immune systems. Due to lack of replicates, no reliable conclusion about predominant species can be drawn at this time. Also, because the bioaerosol samples collected in this project were only culturable microorganisms, the detected predominant species could not represent the true predominant species in the animal house.

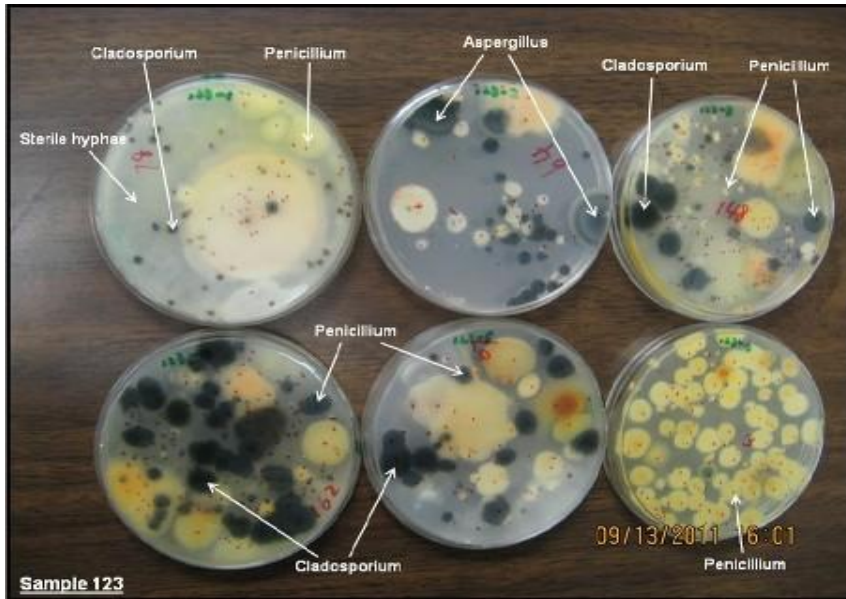


Figure 25. Predominant species on sample plates collected on the first floor

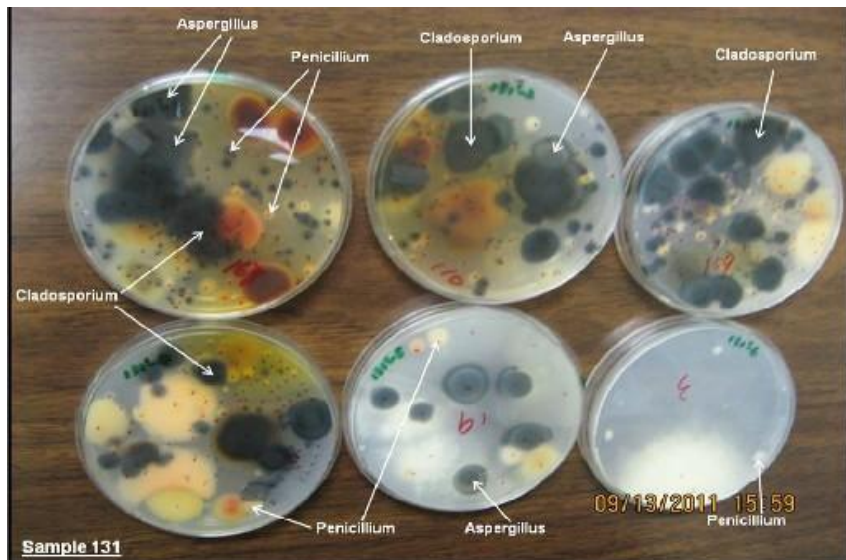


Figure 26. Predominant species on sample plates collected on the second floor

## EMISSION RATE VARIATION

The overall mean emission rates of bacteria and fungi from winter 2010 to summer 2011 in house 4 were  $7.0 \times 10^7$  CFU/s (SD= $8.2 \times 10^7$  CFU/s) and  $3.3 \times 10^5$  CFU/s (SD= $6.9 \times 10^5$  CFU/s), respectively.

The average emission rates of bacteria and fungi in three seasons are summarized in Table 16. For both bacteria and fungi, the emission rates were the highest in summer and the lowest in winter. Since the in-house bacteria concentration was at the lowest in summer (Figure 13), the high ventilation rates in summer contributed significantly to the high bacteria emission rates in summer. Similar observation was also reported for non-viable aerosols, i.e. Particulate matter (Li, et al. 2012).

Table 16. Emission rates (CFU/s) of bacteria and fungi in three seasons.

	Bacteria		Fungi	
	Mean	SD	Mean	SD
Winter	$1.6 \times 10^7$	$1.1 \times 10^7$	$2.7 \times 10^4$	$1.2 \times 10^4$
Spring	$7.2 \times 10^7$	$9.1 \times 10^7$	$8.9 \times 10^4$	$9.9 \times 10^4$
Summer	$1.2 \times 10^8$	$8.2 \times 10^7$	$8.3 \times 10^5$	$9.8 \times 10^5$

Figures 27 and 28 show the variations of bacteria and fungi mean emission rates in different time of the day separated by season. As shown in Figure 27, winter bacteria emission rates showed the same trend as winter concentration variation, and were the highest at noon. During the winter time, the house ventilation was at minimum through the day to keep in-house temperature. Consequently, ventilation impact on emission variation in winter was minimal. On the other hand, in spring time, bacteria concentrations were relatively high in morning but emission rates were contradictorily low. This is because great variation in ventilation rate through the day in response to big fluctuation of ambient temperature. In the morning, the ventilation rate was limited due to low temperature, whereas around the noon, significant increase in ventilation occurred in response to higher temperature. Consequently

higher emission rate was observed. In summer time, the house ventilation was set at full capacity through all the sampling events. The constant ventilation rate did not contribute to variation of emission rate over time of day. Consequently bacteria emission rate variations followed the same trend as concentration variations (Figures 13 and 27). Summer bacteria emission rates were the highest in early mornings and lower in the rest of the day.

As shown in Figure 28, fungi emission rates in winter and spring were relatively constant during the day. Summer fungi emission rates were higher in the afternoons, which might be due to the higher fungi concentrations in summer afternoons.

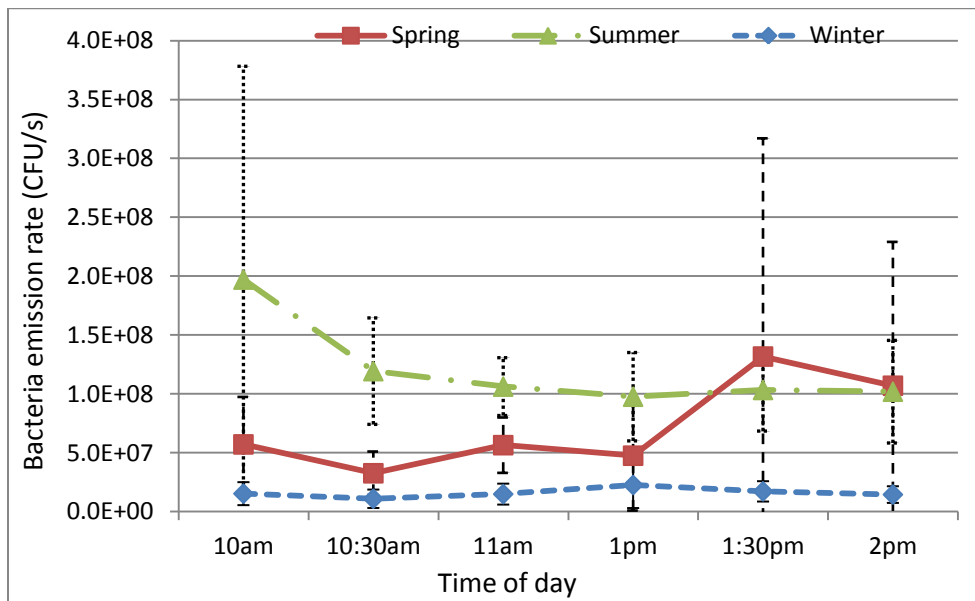


Figure 27. Mean emission rates of bacteria separated by season

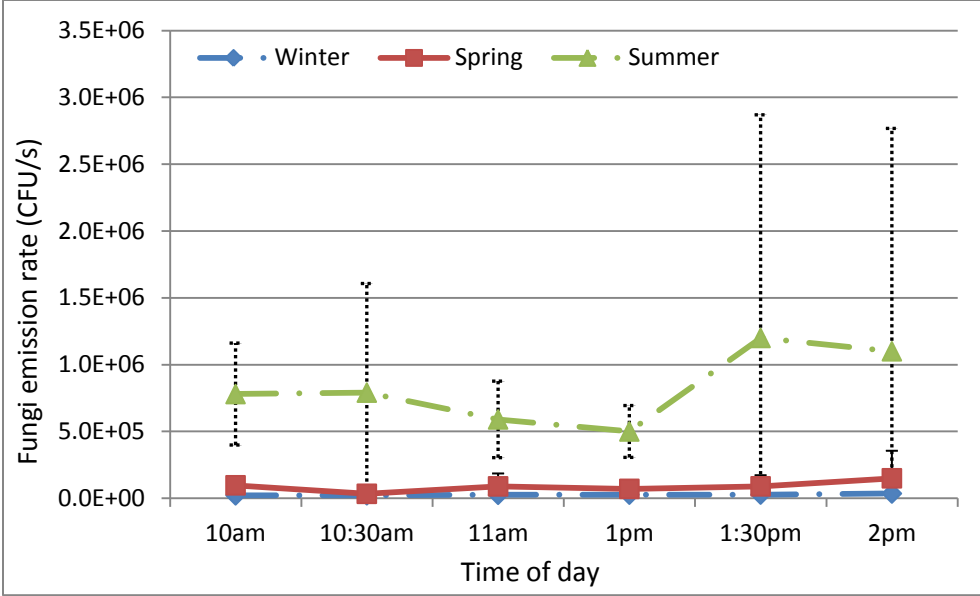


Figure 28. Mean emission rates of fungi emission separated by season

## CHAPTER 4 TEMPORAL AND SPATIAL VARIATIONS IN THE VICINITY

### TEMPORAL VARIATIONS

#### *SEASONAL EFFECTS*

The mean concentrations of bacteria and fungi among all ambient stations (ST2-ST5) were computed by season. The mean concentrations  $\pm$  SDs of bacteria were  $869 \pm 1003$  CFU/m<sup>3</sup>,  $1263 \pm 1955$  CFU/m<sup>3</sup>, and  $1193 \pm 1497$  CFU/m<sup>3</sup> for winter, spring and summer, respectively. An ANOVA test was run to test the differences between ambient bacteria concentrations in different seasons. It was observed that there was no significant difference between ambient bacteria concentrations among three seasons ( $p$ -value=0.33). The mean concentrations  $\pm$  SDs of fungi were  $280 \pm 373$  CFU/m<sup>3</sup>,  $1403 \pm 1461$  CFU/m<sup>3</sup>, and  $2558 \pm 2276$  CFU/m<sup>3</sup> for winter, spring and summer, respectively. Mean ambient fungi concentrations were the highest in summer and the lowest in winter ( $p < 0.0001$ ).

In order to investigate the impacts of atmospheric conditions on ambient bioaerosol concentrations, means of the potential affecting meteorological factors measured during field sampling were calculated by season. Temperature and RH were measured simultaneously at each sampling station, and the two measurements of each sampling test were averaged to represent ambient air temperature and RH at that sampling time. Solar radiations were measured by the weather tower. Table 17 lists the average values with SDs of meteorological factors that might have impact on bioaerosol survival, including temperature, RH, and solar radiation.

The observation of the lowest fungi concentration in winter may be due to the inactivation of fungi at low temperature and RH level. Fungi concentrations were higher in summer than in spring although solar radiations were significantly higher in summer than in spring ( $p < 0.0001$ ). This might be because that the higher temperature and lower RH in summer was in favor of fungi survival. Bacteria concentrations did not show a trend to vary

with season although atmospheric conditions changed significantly, which might indicate that the background bacteria level were high.

Table 17. Meteorological data in the vicinity in three seasons.

	Temperature (°C)		RH (%)		Solar Radiation (W/m <sup>2</sup> )	
	Mean*	SD	Mean	SD	Mean	SD
Winter	5	4	39	15	366	156
Spring	15	5	62	13	454	218
Summer	34	3	54	11	783	189

\* Means of 166 measurement points

#### ***SEASONAL EFFECTS BY STATION***

Bioaerosol concentrations at each ambient station were averaged by season to illustrate the seasonal effects at different stations. Figure 29 shows the mean values of ambient bacteria and fungi concentrations at four ambient stations (ST2-ST5) in three seasons (winter, spring and summer). As shown in this figure, seasonal variations of bacteria concentrations varied at ambient stations. As mentioned in Chapter 2, ST2 and ST4 served as one group for simultaneous upwind and downwind sampling, and ST3 and ST5 served as the other group during field sampling. At ST2 and ST5, bacteria concentrations were the highest in spring and the lowest in winter, while at ST3 and ST4, bacteria concentrations were the lowest in spring and higher in winter and summer. This observation suggested that wind played an important role in affecting ambient airborne bacteria concentration because there was significant difference ( $p < 0.0001$ ) between concentrations at downwind locations and upwind locations. Unlike bacteria, fungi concentrations showed coincident pattern of seasonal variations at all four ambient stations. Fungi concentrations were the lowest in winter and increased from winter to summer.

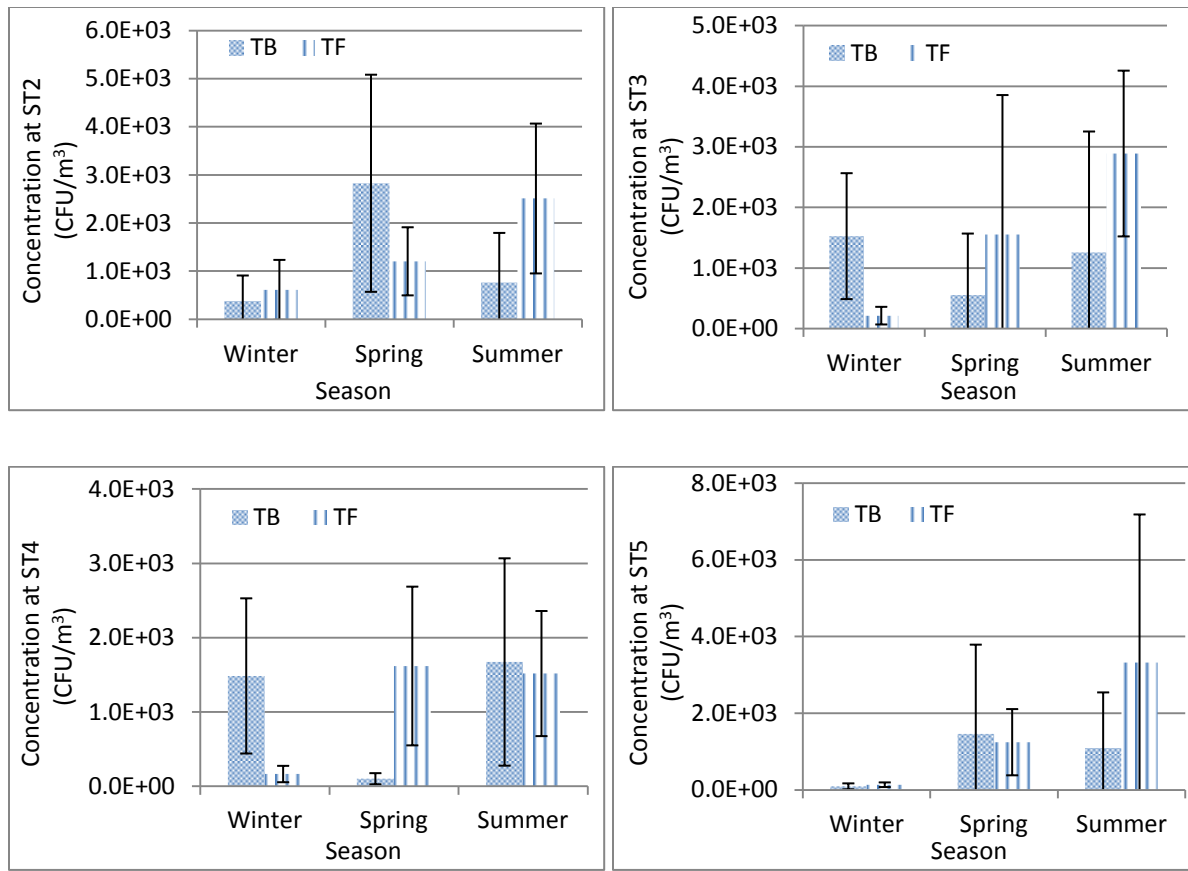


Figure 29. Mean concentrations  $\pm$ SDs of bacteria (TB) and fungi (TF) at ambient stations in three seasons

***TIME OF DAY EFFECT BY SEASON***

It was observed on the field that temperature and RH changed not only upon seasons, but also upon time of day in each sampling day. In order to estimate the time effect on ambient bioaerosol concentrations in different seasons, mean concentrations of bacteria and fungi at different time of day were computed by season.

Figures 30 and 31 show bacteria and fungi mean concentrations measured at ST2-ST5 in three seasons at different time of day. In winter, due to the low temperature and RH, concentrations of bacteria and fungi were low and only slightly changed during the day. In spring, there was no consistent pattern observed for both bacteria and fungi. Bacteria

concentrations were the lowest in afternoon, while fungi concentrations were the lowest in early morning and at noon. In summer, bacteria and fungi variations showed similar pattern.

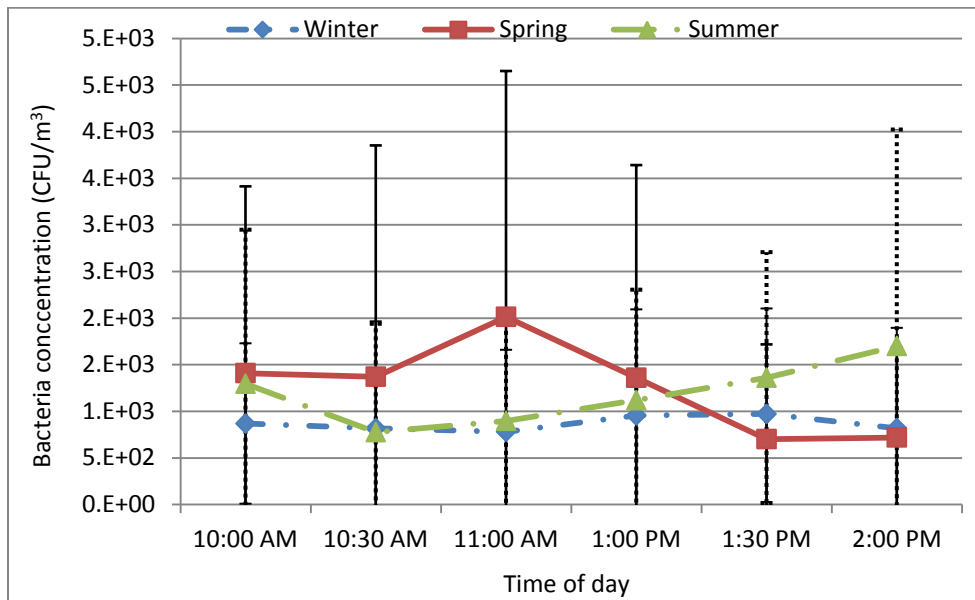


Figure 30. Mean concentrations  $\pm$  SDs of bacteria measured at four ambient stations at different time of day separated

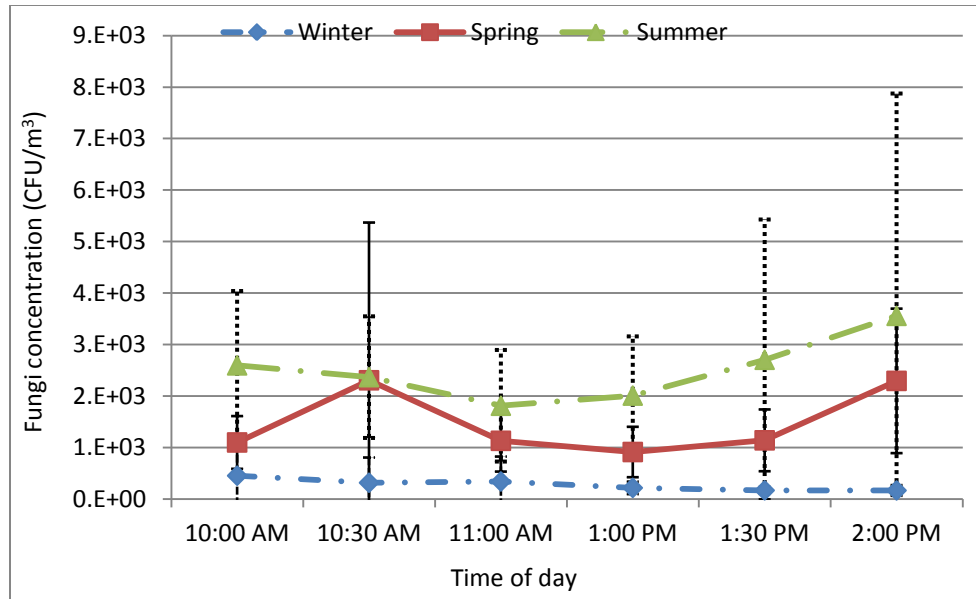


Figure 31. Mean concentrations  $\pm$  SDs of fungi measured at four ambient stations at different time of day separated by season

#### ***TIME OF DAY EFFECT BY STATION***

To investigate time effect on bacteria and fungi concentrations at different locations, mean concentrations of bacteria and fungi at different time of day were calculated by station. Figure 32 shows the time of day effects at different ambient stations.

As shown in Figure 32, time variations of bioaerosol concentrations varied greatly at different stations. At ST2 and ST3, bacteria concentrations were higher in the afternoons than mornings. At ST4 and ST5, bacteria concentrations were higher in the mornings than afternoons. At ST2 and ST4, fungi concentration decreased from morning to afternoon. At ST3, fungi concentrations were the highest at noon. At ST5, fungi concentrations were the lowest at noon. Bacteria and fungi concentrations had distinguished variations upon time at different stations, which was mainly because of the spatial effects.

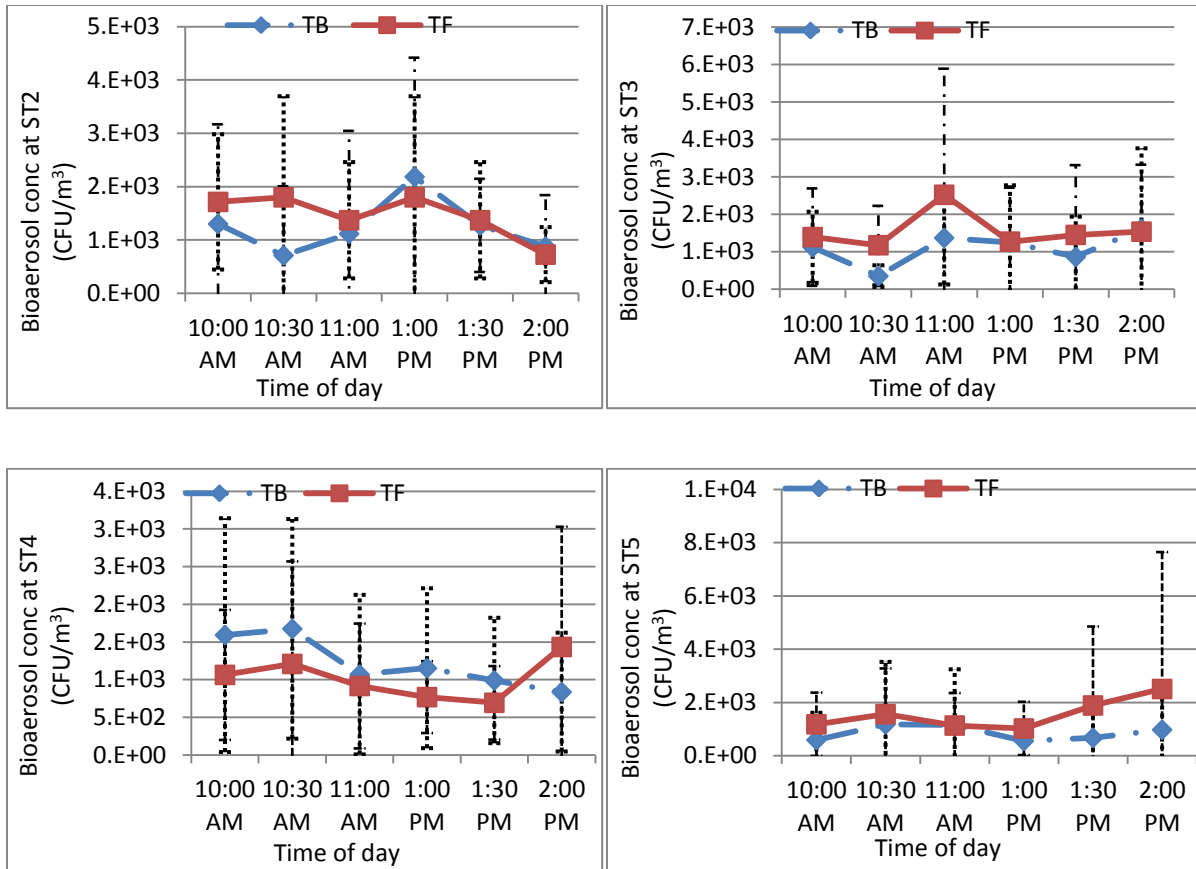


Figure 32. Mean concentrations  $\pm$  SDs of bacteria (TB) and fungi (TF) at different time of day, separated by sampling station

### SPATIAL VARIATIONS

To investigate spatial effect, data were grouped into the upwind and downwind classes according to upwind/downwind definitions in Chapter 2. During each test, if one ambient station was at upwind while the other station was at downwind, the two bacteria or fungi concentrations measured at this test were a valid group of measurements. There were totally 75 valid groups of bacteria measurements and 78 valid groups of fungi measurements. Statistical tests indicated that there were significant differences between downwind and upwind bioaerosol concentrations in one test ( $p < 0.0001$  for bacteria,  $p = 0.025$  for fungi).

The overall mean concentrations of bacteria and fungi at downwind locations were  $1856 \pm 1688$  CFU/m<sup>3</sup> and  $1155 \pm 1179$  CFU/m<sup>3</sup>, respectively. The overall mean concentrations of bacteria and fungi at upwind locations were  $291 \pm 606$  CFU/m<sup>3</sup> and  $1690 \pm 2321$  CFU/m<sup>3</sup>, respectively. Mean concentration of bacteria was significantly higher at downwind than at upwind ( $p < 0.0001$ ). In the contrast, mean concentration of fungi at downwind was not significantly different from which at upwind ( $p = 0.1206$ ).

This observation indicated that wind factor (i.e., wind direction and wind speed effects) played important role in affecting bioaerosol concentrations in the vicinity of the farm.

#### *UPWIND/DOWNWIND EFFECT BY SEASON*

The overall mean concentrations of bacteria and fungi at downwind location were computed by season. Figure 33 shows concentrations of bioaerosols at downwind location in three seasons.

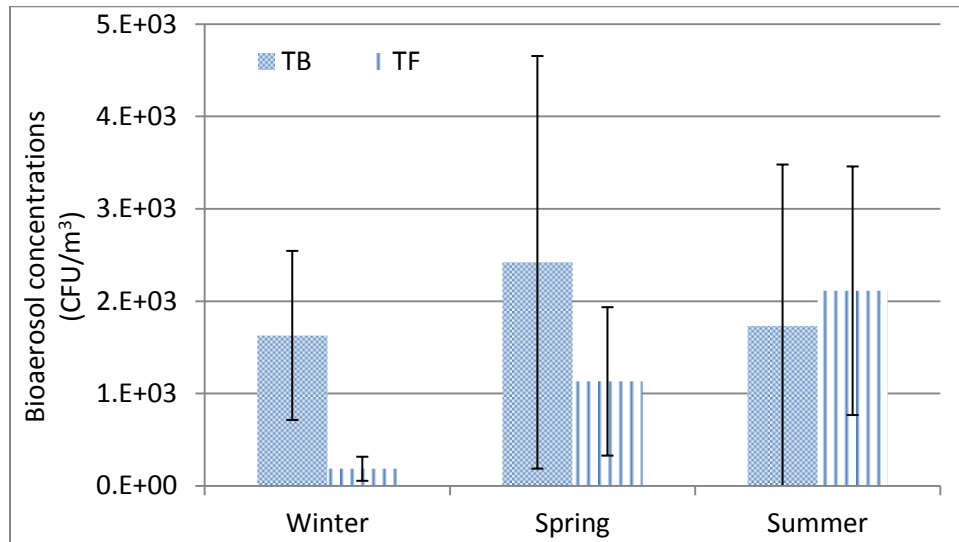


Figure 33. Mean concentrations  $\pm$  SDs of bacteria (TB) and fungi (TF) at downwind location, separated by season

Downwind concentrations of bacteria were not significantly different in three seasons ( $p = 0.51$ ). Fungi concentration at downwind were the highest in summer and the lowest in

winter ( $p < 0.0001$ ). The variations of downwind bioaerosol concentrations followed the same patterns as the overall mean bioaerosol concentrations in the vicinity of the farm did.

#### *UPWIND/DOWNWIND EFFECT BY STATION*

The average downwind and upwind concentrations of bacteria and fungi at each ambient station were calculated to illustrate the spatial variation of bioaerosol concentrations at different locations. Table 18 lists the averages and standard deviations of bioaerosol concentrations in the ambient under downwind and upwind conditions.

Table 18. Average concentrations of bioaerosols (CFU/m<sup>3</sup>) at four ambient stations under downwind and upwind conditions.

	Bacteria				Fungi			
	Downwind		Upwind		Downwind		Upwind	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
ST2	2223	2045	457	850	1585	1278	1393	1390
ST3	1737	1547	284	685	870	1421	2727	2022
ST4	1911	1175	266	370	776	591	1368	997
ST5	1611	2042	133	224	1636	969	1685	3574

As shown in Table 18, mean concentrations of bacteria at each station were higher at downwind than at upwind (ST2:  $p = 0.0005$ , ST3:  $p = 0.0004$ , ST4:  $p = 0.0002$ , ST5:  $p = 0.0016$ ), which was in agreement with the variations of overall mean ambient bacteria concentration. The mean concentrations of downwind bacteria at four ambient stations were not significantly different ( $p = 0.70$ ). Similarly, there was no significant difference between the mean concentrations of upwind bacteria at four ambient stations ( $p = 0.36$ ). In contrast, there were no significant differences between upwind and downwind fungi concentration at each station but ST3 (ST2:  $p = 0.64$ , ST3:  $p = 0.0019$ , ST4:  $p = 0.15$ , ST5:  $p = 0.92$ ). This observation suggests that wind direction and speed seemed to have low impact on fungi concentration variations. The mean concentrations of downwind fungi at ST5 and ST2 were higher than which at ST3 and ST4 ( $p = 0.03$ ). Whereas the mean concentrations of upwind

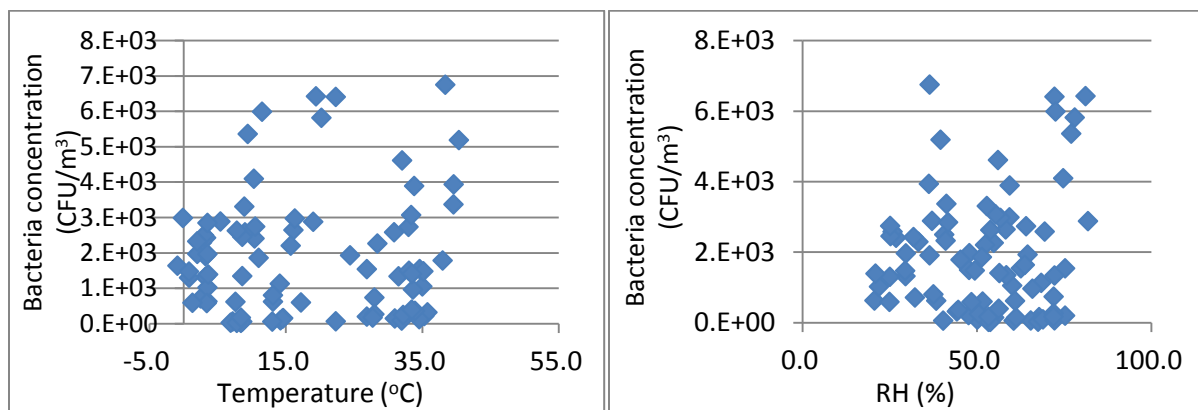
fungi at four ambient stations were not significantly different ( $p = 0.28$ ). The variations might be mainly because of the location of each station. As shown in Figure 1, ST2 was the furthest away from the animal houses and close to a local road and a waste water spray field. There was also a line of trees between ST2 and the houses that might block transport of bioaerosol from animal houses to ST2. However, ST2 had the higher mean value of fungi downwind concentration. It was probably that the spray field, instead of the farm, was the largest source of fungi for ST2. ST3 was very close to the cross-ventilated house (House 6), and the fans of house 6 could blow bioaerosols directly to ST3. ST4 was close to a large waste treatment lagoon. ST5 was further away from the animal houses than ST3 and ST4. In addition, there was an egg packing plant located between animal houses and ST5. However, bacteria and upwind fungi concentrations at ST5 were not lower than other ambient stations, and downwind fungi concentrations were even higher than ST3 and ST4. There might be other source of airborne fungi that had impact on bioaerosol concentrations at ST5.

## INFLUENCING FACTORS

In comparison to bioaerosol concentrations at source (ST1), there were more factors affecting bioaerosol concentrations at ambient stations in the vicinity of the farm.

### *DOWNWIND BACTERIA CONCENTRATIONS AND INFLUENCING FACTORS*

Figures 34 and 35 illustrate the impacts of meteorological factors and emission rate on ambient bacteria and fungi concentrations at downwind locations.



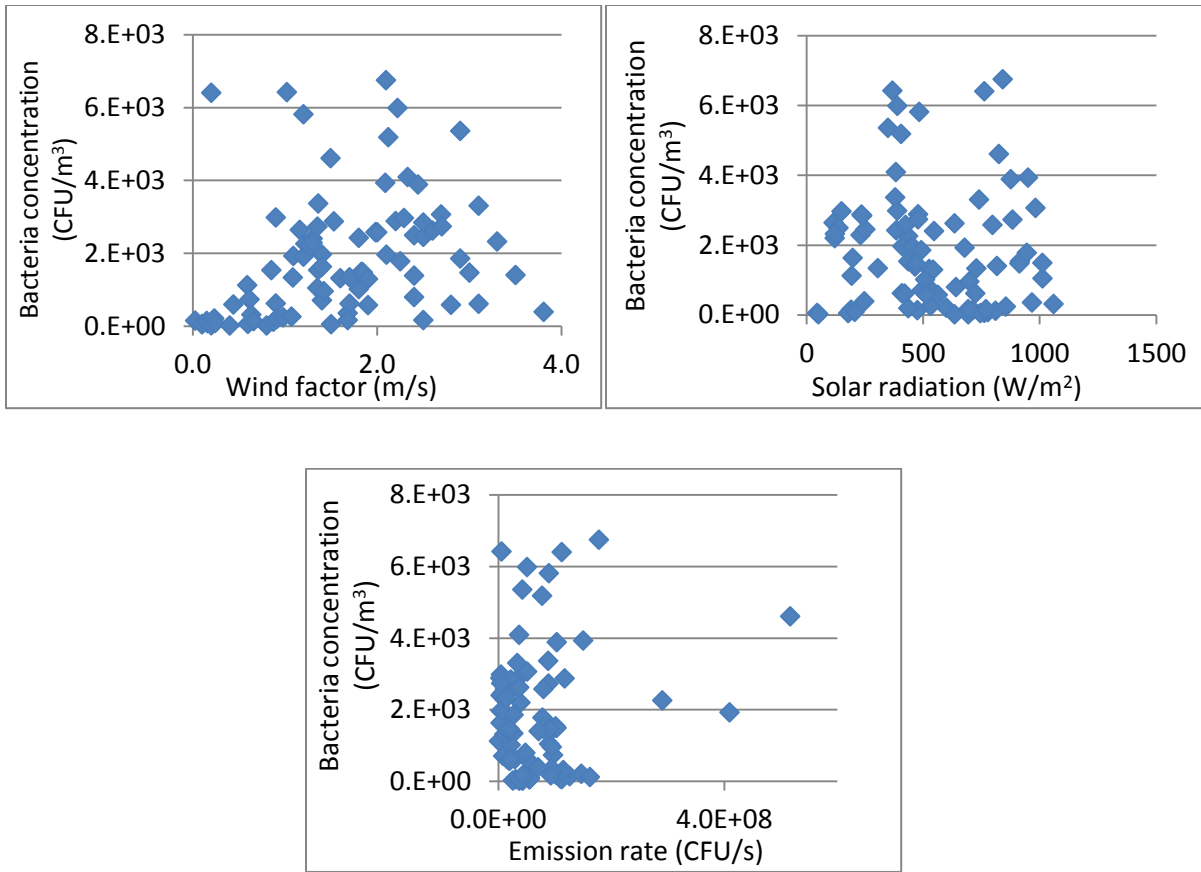


Figure 34. Responses of downwind bacteria concentrations to various influencing factors

As shown in Figure 34, no obvious linear relationships between downwind bacteria concentrations and air temperature, RH, or solar radiation were observed from the plots. High concentrations of bacteria were more likely to be detected at temperatures from 20 °C to 40 °C; and medium to high RH levels (40% to 80%) were more in favor of high bacteria concentrations. Higher concentrations of bacteria were detected with moderate to strong solar radiation (>300 W/m<sup>2</sup>). Bacteria concentrations were observed to be positively related to wind factor and emission rate.

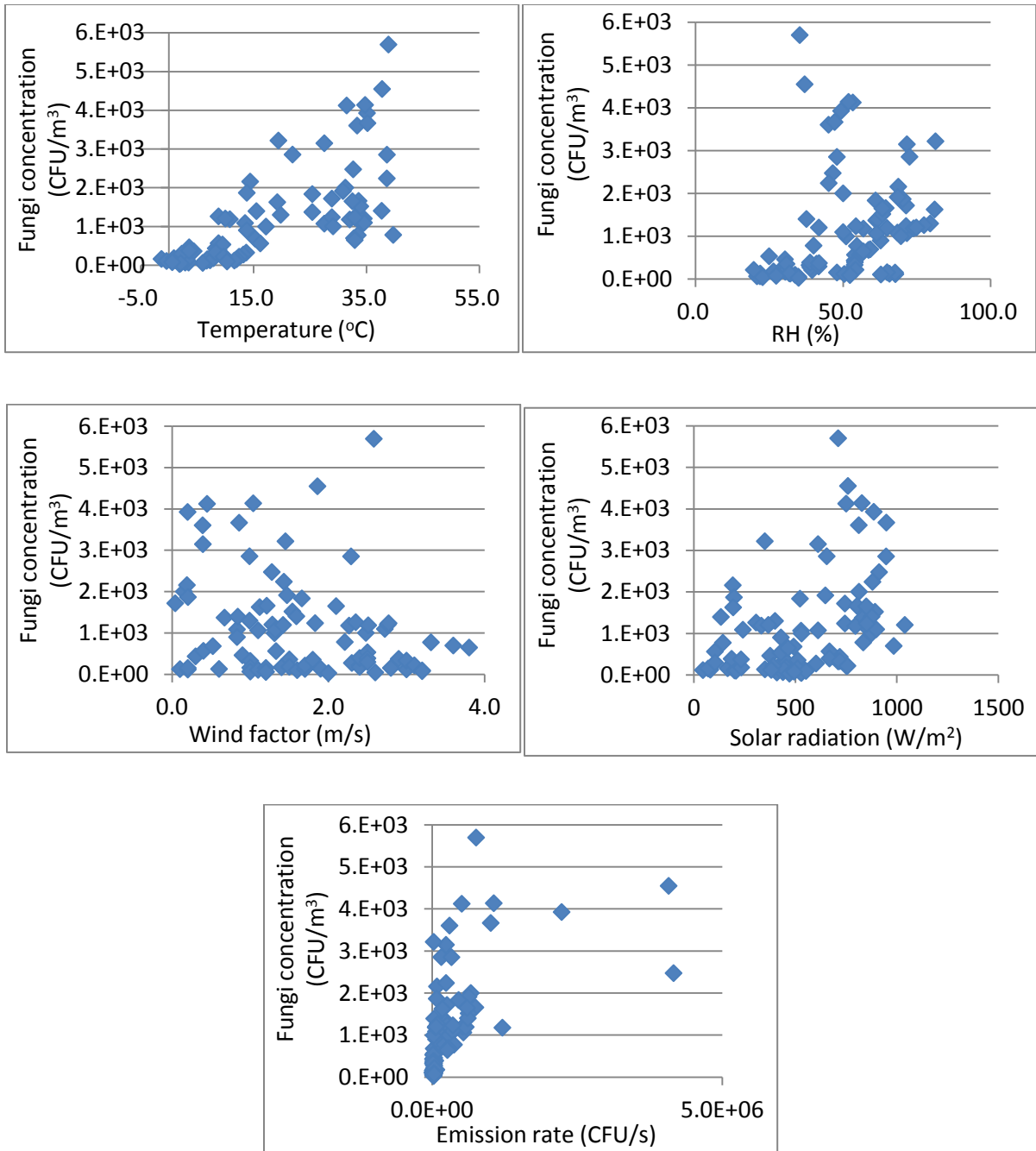


Figure 35. Responses of downwind fungi concentrations to various influencing factors

As shown in Figure 35, downwind fungi concentrations were positively related to T, RH, solar radiation, and emission rate, and were negatively related to wind factor.

Both bacteria and fungi concentrations were found to be higher at stronger solar radiation, although solar radiation was reported to cause bioaerosol damage (Ulevičius et al., 2000). This might be because that strong solar radiation with low wind speed (<5 m/s) resulted in unstable to very unstable atmosphere, and the consequent unstable atmosphere caused high bioaerosol concentrations at local downwind locations (<2 km away from the sources).

To better understand the impacts of influencing factors on downwind bioaerosol concentrations, multiple linear regression models were applied to assess the relationships between ambient bioaerosol concentrations and affecting factors using SAS 9.2 software. In the regression models, temperature (T), RH, wind factor, solar radiation, and emission rate were independent variables, and the concentrations of bacteria and fungi measured at downwind stations were dependent variables. To test the linear dependencies between the six variables, Pearson correlation coefficients (see Tables A3-4 in appendix) were calculated for each two of them.

It was shown that downwind bacteria concentration was only linearly dependent on wind factor ( $p=0.0046$ ) at a significant level of 0.05. However, it didn't mean that other influencing factors (i.e. T, RH, solar radiation, and emission rate) were not important in impacting downwind bacteria concentration, because there were strong linear dependencies between them. T had significant linear dependencies with RH ( $p=0.0022$ ), solar radiation ( $p<0.0001$ ), and emission rate ( $p<0.0001$ ); RH had significant linear dependencies with T, wind factor ( $p=0.0022$ ), and emission rate ( $p=0.0169$ ); wind factor had significant linear dependencies with RH; solar radiation had significant linear dependencies with T and emission rate ( $p=0.0002$ ); and emission rate had significant linear dependencies with T, RH, and solar radiation.

Pearson correlation coefficients suggested that downwind fungi concentration was significantly linearly dependent on all five influencing factors at a 0.05 level. Similarly, there were strong linear dependencies between the influencing factors. T had significant linear dependencies with RH ( $p=0.0062$ ), solar radiation ( $p<0.0001$ ), and emission rate ( $p<0.0001$ );

RH had significant linear dependencies with T and wind factor ( $p=0.0488$ ); wind factor had significant linear dependencies with RH; solar radiation had significant linear dependencies with T and emission rate ( $p<0.0001$ ); and emission rate had significant linear dependencies with T and solar radiation.

The linear correlation between variables might cause some predictors to be not important when fitted in a linear regression model. The results of multiple linear regression models using T, RH, wind factor, solar radiation and emission rate as five predictors verified this hypothesis. The outputs of the models are summarized in Table 19.

Table 19. Multiple linear regression models for downwind bioaerosol concentration predictions.

		Estimate	Std. Error	P-value
Bacteria	Intercept	-27	880	0.98
	T	12	20	0.56
	RH	15	12	0.22
	Wind factor	770	210	0.0003
	Solar radiation	-1.2	0.89	0.20
	Emission rate	0.0000040	0.0000027	0.15
Fungi	Intercept	470	440	0.28
	T	57	11	<0.0001
	RH	2.8	6.3	0.66
	Wind factor	-260	95	0.0066
	Solar radiation	-0.40	0.49	0.43
	Emission rate	0.00049	0.00015	0.0021

As shown in Table 19, not all predictors were important in the models. To better study the impacts of influencing factors on downwind bioaerosol concentrations, log transformed bioaerosol concentrations were used as new responses. As suggested by plots (see Figure A7

in appendix), emission rate was also log transformed. Quadratic forms of wind factor ( $\text{wind}^2$ ) and emission rate ( $\text{emission}^2$ ) were introduced as two new predictors for predicting bacteria concentration downwind. Among all subset models, the model included T, wind factor,  $\text{wind}^2$ , solar radiation, emission rate, and  $\text{emission}^2$  was preferred by R-square and Mallow's CP selections (see table A5 in appendix). For predicting downwind fungi concentration, residual plots suggested that the relationships between fungi downwind concentration and emission or wind factor were not quadratic. Among all subset models, the model included every influencing factor but solar radiation was preferred by R-square and Mallow's CP selections (see Table A6 in appendix). Table 20 lists the results of the selective regression models.

Table 20. Selective models for downwind bioaerosol concentration prediction

		Estimate	Std. Error	P-value
Bacteria	Intercept	56	19	0.0040
	T	0.034	0.016	0.037
	Wind factor	3.0	0.46	<0.0001
	Wind <sup>2</sup>	-0.62	0.13	<0.0001
	Solar radiation	-0.0016	0.00058	0.0064
	Emission rate	-6.0	2.3	0.0099
	Emission <sup>2</sup>	0.17	0.067	0.0131
Fungi	Intercept	2.0	1.1	0.060
	T	0.045	0.013	0.0006
	RH	0.021	0.0047	<0.0001
	Wind factor	-0.19	0.078	0.0187
	Emission rate	0.24	0.11	0.0231

As shown in this table, wind factor had different effects on bacteria and fungi concentrations. On the one hand, high speed wind might bring more bioaerosol to a sampling station more quickly; on the other hand, strong wind might also cause damage to bioaerosol. Therefore, increasing wind factor resulted in increasing in bacteria concentrations and

decreasing in fungi concentrations. For the influencing factors that were not included in the regression models, it does not mean that they were not important. The results of the models only indicated that when other factors were controlled, RH was no longer an important predictor of bacteria concentration downwind while solar radiation was no longer an important predictor of fungi concentration downwind. Due to the differences in size and specie characteristics, bacteria and fungi responded differently to the influencing factors. Temperature, wind parameters, and emission rate were important predictors for both bacteria and fungi variations.

## **CHAPTER 5 PARTICLE SIZE DISTRIBUTIONS OF BIOAEROSOLS**

### **BIOAEROSOL SIZE DISTRIBUTIONS AT SOURCE**

In order to investigate information of particle size distributions (PSDs) of bioaerosols in the poultry house, mean concentrations of bacteria and fungi on each stage of the Andersen six-stage viable sampler were calculated by season and by different time of day. Tables 21-22 list mean concentrations with SDs of in-house bacteria and fungi on each stage in three seasons over different time of day.

As shown in Table 21, stage 6 (0.65-1.1  $\mu\text{m}$ ) had the lowest bacteria concentrations at all time, which indicated that only a small portion of bacteria in the animal house were in this size range. Stage 4 (2.1-3.3  $\mu\text{m}$ ) had the highest level of bacteria concentrations at all times, and stage 3 (3.3-4.7  $\mu\text{m}$ ) had the highest level of bacteria concentrations in winter and spring but a lower level in summer. Stages 1 (>7  $\mu\text{m}$ ) and 2 (4.7-7  $\mu\text{m}$ ) had intermediate levels of bacteria concentration in winter and spring but lower levels in summer. Stage 5 (1.1-2.1  $\mu\text{m}$ ) had low levels of bacteria concentrations in winter and spring mornings but intermediate levels in spring afternoon and summer. The mean concentrations on different stages indicate that most in-house bacteria were in the size range from 2.1 to 4.7  $\mu\text{m}$  (deposited on stages 3 and 4). The observed variations of in-house bacteria size distributions suggest that seasonal or temporal effects had impacts on bacteria size distributions. The results show that under higher temperature and lower RH levels, there were more bacteria in small size ranges. This variation might be due to dehydration of bacteria caused by air conditions.

As shown in Table 22, similar to bacteria, the lowest fungi concentrations were discovered on stage 6 at most of time. Stages 1, 3 and 4 had the high fungi concentrations in all seasons over different time periods of day, which indicated there were a great portion of fungi were in the intermediate to large size range. It was observed that sometimes, there was no significant difference among fungi concentrations on the six stages (e.g. Spring morning).

No obvious seasonal or temporal variation of fungi size distribution was observed in the animal house.

Based upon mean concentrations on each stage, the percentages of bioaerosol concentrations in each size range in three seasons at different time of day were calculated to illustrate the portion of bioaerosols in each size range. Figures 36-37 show percentages of in-house bacteria and fungi concentrations in each size range in three seasons over different time of day.

As shown in Figure 36, in winter and spring, bacteria in 3.3-4.7  $\mu\text{m}$  accounted for the greatest portion (~25%) of bacteria at source. But in summer, bacteria in 2.1 to 3.3  $\mu\text{m}$  accounted for the greatest portion (~28%) to the total bacteria collected in 6 stages at source. Bacteria that deposited on the four lower stages (stage 3-6) were considered to be respirable while those on the first two stages were nonrespirable. Most bacteria detected in the animal house were respirable. The portion of respirable bacteria ranged from 60% (in winter) to 80% (in summer).

As shown in Figure 37, fungi greater than 7  $\mu\text{m}$  accounted for 20% to 25% of total measured fungi at source in winter and spring, but accounted less than 20% in summer. Fungi with intermediate sizes in 2.1 to 4.7  $\mu\text{m}$  accounted for more than 40% of fungi at source. The portion of respirable fungi ranged from 60% (in winter and spring) to 70% (in summer). This observation indicated that exposure to bioaerosols in the animal house might bring risks to human beings and animals because most of the bioaerosols can be inhaled and cause adverse health effects.

Table 21. Overall mean concentrations  $\pm$ SD (CFU/m<sup>3</sup>) of bacteria at source on each stage of the sampler.

		Particle size range ( $\mu$ m)						
		>7	4.7-7	3.3-4.7	2.1-3.3	1.1-2.1	0.65-1.1	Total
Winter morning	Mean	$1.4 \times 10^{5[a][b]*}$	$1.7 \times 10^{5[a]}$	$2.0 \times 10^{5[a]}$	$1.6 \times 10^{5[a][b]}$	$8.7 \times 10^{4[c]}$	$4.0 \times 10^{4[c]}$	$7.9 \times 10^5$
	SD	$6.2 \times 10^4$	$9.0 \times 10^4$	$8.1 \times 10^4$	$8.5 \times 10^4$	$5.9 \times 10^4$	$2.9 \times 10^4$	
Winter afternoon	Mean	$2.0 \times 10^{5[a][b]}$	$1.8 \times 10^{5[a][b]}$	$2.2 \times 10^{5[a]}$	$2.0 \times 10^{5[a][b]}$	$1.2 \times 10^{5[b][c]}$	$5.4 \times 10^{4[c]}$	$9.7 \times 10^5$
	SD	$9.4 \times 10^4$	$8.5 \times 10^4$	$1.1 \times 10^5$	$1.0 \times 10^5$	$8.1 \times 10^4$	$4.2 \times 10^4$	
Spring morning	Mean	$2.1 \times 10^{5[a][b]}$	$1.9 \times 10^{5[a][b]}$	$2.8 \times 10^{5[a]}$	$2.6 \times 10^{5[a][b]}$	$1.7 \times 10^{5[b]}$	$3.4 \times 10^{4[c]}$	$1.1 \times 10^6$
	SD	$8.5 \times 10^4$	$8.4 \times 10^4$	$1.1 \times 10^5$	$1.0 \times 10^5$	$7.6 \times 10^4$	$1.5 \times 10^4$	
Spring afternoon	Mean	$1.6 \times 10^{5[a]}$	$1.6 \times 10^{5[a]}$	$2.6 \times 10^{5[a]}$	$2.5 \times 10^{5[a]}$	$1.5 \times 10^{5[a]}$	$3.8 \times 10^{4[b]}$	$1.0 \times 10^6$
	SD	$8.5 \times 10^4$	$9.4 \times 10^4$	$1.3 \times 10^5$	$1.1 \times 10^5$	$6.3 \times 10^4$	$2.2 \times 10^4$	
Summer morning	Mean	$2.9 \times 10^{4[c]}$	$2.9 \times 10^{4[c]}$	$4.6 \times 10^{4[b]}$	$6.9 \times 10^{4[a]}$	$5.8 \times 10^{4[a][b]}$	$1.5 \times 10^{4[d]}$	$2.5 \times 10^5$
	SD	$2.1 \times 10^4$	$1.7 \times 10^4$	$2.8 \times 10^4$	$3.5 \times 10^4$	$1.9 \times 10^4$	$8.7 \times 10^3$	
Summer afternoon	Mean	$2.8 \times 10^{4[c]}$	$2.9 \times 10^{4[c]}$	$4.8 \times 10^{4[b]}$	$6.8 \times 10^{4[a]}$	$5.6 \times 10^{4[b]}$	$1.5 \times 10^{4[d]}$	$2.4 \times 10^5$
	SD	$2.5 \times 10^4$	$1.7 \times 10^4$	$2.0 \times 10^4$	$2.2 \times 10^4$	$1.5 \times 10^4$	$7.8 \times 10^3$	

\* In each row, means with different letters indicate significant difference at a 0.05 level

Table 22. Overall mean concentrations  $\pm$ SD (CFU/m<sup>3</sup>) of fungi at source on each stage of the sampler.

		Particle size range ( $\mu$ m)						
		>7	4.7-7	3.3-4.7	2.1-3.3	1.1-2.1	0.65-1.1	Total
Winter	Mean	$4.0 \times 10^{2[a]*}$	$2.3 \times 10^{2[a]}$	$3.7 \times 10^{2[a]}$	$3.8 \times 10^{2[a]}$	$8.0 \times 10^{[b]}$	$1.5 \times 10^{[b]}$	$1.5 \times 10^3$
morning	SD	$1.5 \times 10^2$	$1.1 \times 10^2$	9.8 $\times 10$	$1.3 \times 10^2$	5.1 $\times 10$	9	
Winter	Mean	$3.5 \times 10^{2[a][b]}$	$2.6 \times 10^{[a][b]}$	$4.0 \times 10^{2[a]}$	$3.9 \times 10^{2[a]}$	$1.4 \times 10^{2[b]}$	$1.3 \times 10^{2[b]}$	$1.7 \times 10^3$
afternoon	SD	$1.1 \times 10^2$	$1.3 \times 10^2$	$1.3 \times 10^2$	$2.0 \times 10^2$	$2.2 \times 10^2$	$3.9 \times 10^2$	
Spring	Mean	$3.6 \times 10^{2[a]}$	$3.0 \times 10^{2[a]}$	$3.3 \times 10^{2[a]}$	$3.6 \times 10^{2[a]}$	$2.0 \times 10^{2[a][b]}$	$2.3 \times 10^{[a]}$	$1.6 \times 10^3$
morning	SD	$1.9 \times 10^2$	$2.0 \times 10^2$	$2.2 \times 10^2$	$2.6 \times 10^2$	$2.1 \times 10^2$	3.3 $\times 10$	
Spring	Mean	$3.5 \times 10^{2[a][b]}$	$2.3 \times 10^{2[a][b][c]}$	$3.0 \times 10^{2[a][b]}$	$4.2 \times 10^{2[a]}$	$1.9 \times 10^{2[b][c]}$	$1.3 \times 10^{[c]}$	$1.5 \times 10^3$
afternoon	SD	$1.8 \times 10^2$	$1.4 \times 10^2$	$2.0 \times 10^2$	$2.9 \times 10^2$	$1.8 \times 10^2$	3	
Summer	Mean	$7.5 \times 10^{2[a][b][c]}$	$5.6 \times 10^{2[b][c]}$	$8.8 \times 10^{2[a][b]}$	$1.1 \times 10^{3[a]}$	$5.1 \times 10^{2[b][c]}$	$4.2 \times 10^{2[c]}$	$4.3 \times 10^3$
morning	SD	$1.0 \times 10^3$	$4.4 \times 10^2$	$6.5 \times 10^2$	$7.2 \times 10^2$	$3.5 \times 10^2$	$1.1 \times 10^3$	
Summer	Mean	$6.3 \times 10^{2[a][b][c]}$	$5.5 \times 10^{2[b][c]}$	$1.0 \times 10^{3[a][b]}$	$1.3 \times 10^{3[a]}$	$6.1 \times 10^{2[b][c]}$	$2.3 \times 10^{2[c]}$	$4.3 \times 10^3$
afternoon	SD	$4.9 \times 10^2$	$4.5 \times 10^2$	$9.3 \times 10^2$	$1.0 \times 10^3$	$7.6 \times 10^2$	$7.4 \times 10^2$	

\* In each row, means with different letters indicate significant difference at a 0.05 level

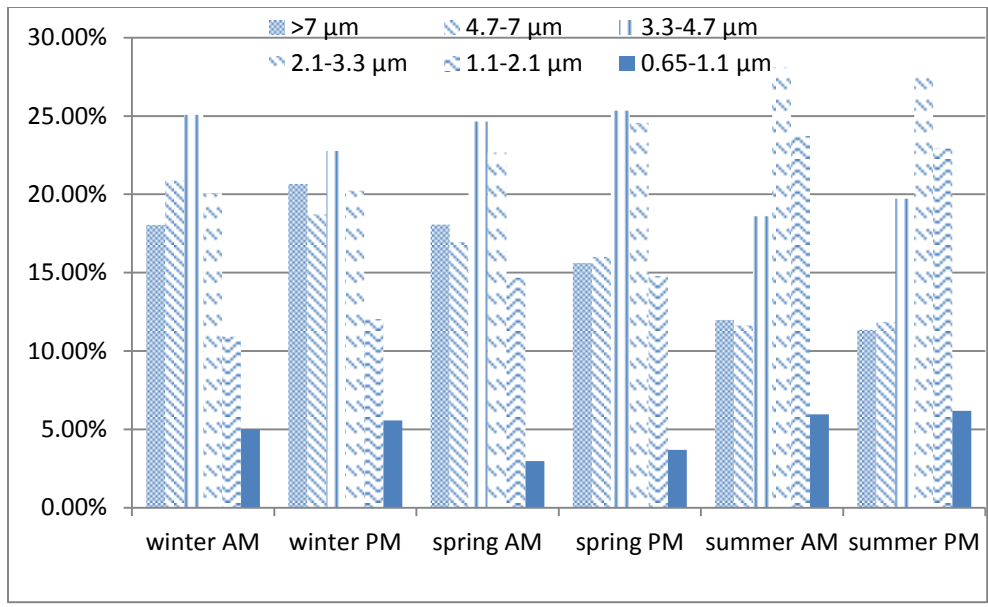


Figure 36. Percentages of in-house bacteria concentrations on each stage in different seasons and time of day.

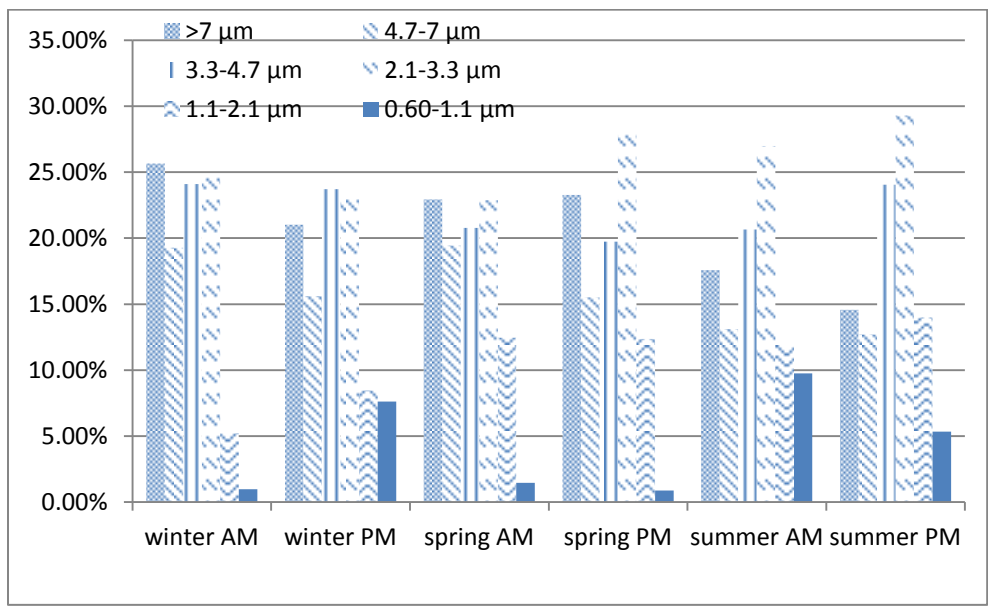


Figure 37. Percentages of in-house fungi concentrations on each stage in different seasons and time of day.

Based on the percentage of respirable bioaerosols in each test, mean percentages with SDs of respirable bioaerosols at source were calculated by different seasons and time of day. Figure 38 shows the portions of respirable bacteria and fungi at source. It was shown that respirable percentages of bacteria at source were not significantly different in different time periods of day ( $p=0.5063$ ). But they were significantly higher in summer, and were not significantly different in spring and winter at a 0.05 level. Fungi respirable percentages showed the similar trend. The percentages of respirable fungi at source were not significantly different over different time of day ( $p=0.5301$ ), but were significantly higher in summer, and were not significantly different in spring and winter at a 0.05 level. The overall mean respirable percentage of bacteria was significantly higher than fungi ( $p<0.0001$ ).

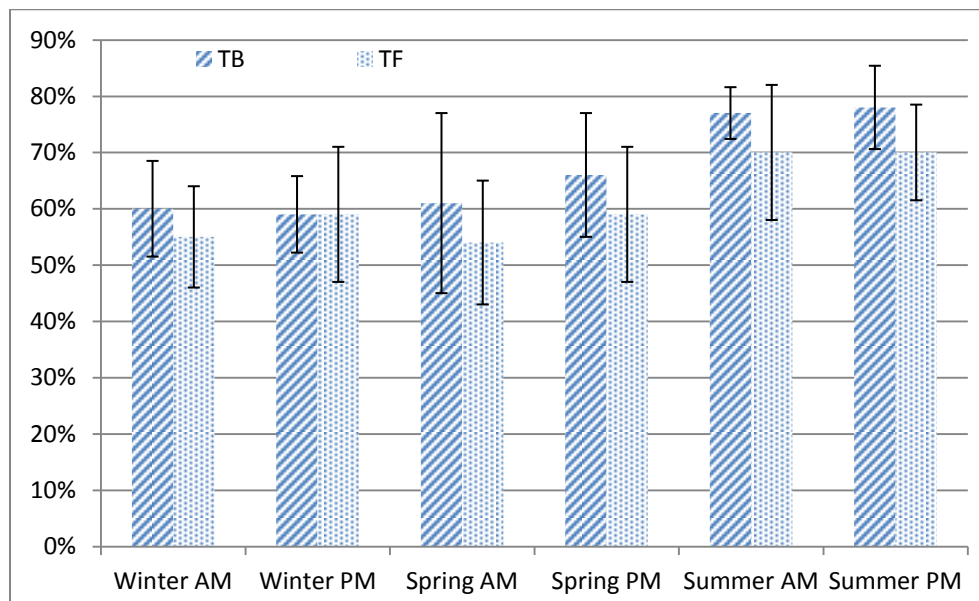


Figure 38. Respirable percentages of bacteria and fungi at source in different seasons and time of day

As described in Chapter 2, 19 tests have been carried out simultaneously on the first and second floors in the animal house in summer 2011 for bacteria and fungi sampling. In order to investigate the spatial variations of bioaerosol size distributions at different location in the animal house, mean concentrations with SDs of bacteria and fungi on each stage on different

floors were calculated. Tables 23-24 list mean concentrations with SDs of bacteria and fungi on each stage on the first and second floors, respectively.

As shown in Table 23, stage 6 had the lowest bacteria concentrations on both floors. On the first floor, stage 4-5 had the highest concentrations of bacteria, which indicated that most bacteria on the first floor ranged from 1.1 to 3.3  $\mu\text{m}$ . Bacteria concentrations on other four stages were not significantly different. On the second floor, stage 3 had the highest bacteria concentration. Stage 1-2 had intermediate levels of bacteria concentration. Stage 5-6 had the lowest bacteria concentrations. It was observed that bacteria collected on the second floor were in larger sizes.

As shown in Table 24, on the first floor, the highest fungi concentrations were detected on stage 4. There was no significant difference among fungi concentrations on the other 5 stages. On the second floor, the first four stages had higher fungi concentrations than the lower two stages. However, there was no significant difference among fungi concentrations on stages 1-4, or between fungi concentrations on stages 5-6.

Table 23. Overall mean concentrations  $\pm$ SD (CFU/m<sup>3</sup>) of bacteria on 1<sup>st</sup> and 2<sup>nd</sup> floors on each stage of the sampler.

		Particle size range ( $\mu$ m)						
		>7	4.7-7	3.3-4.7	2.1-3.3	1.1-2.1	0.65-1.1	Total
1 <sup>st</sup> floor	Mean	3.8 $\times$ 10 <sup>4</sup> [c][d]*	3.4 $\times$ 10 <sup>4</sup> [c][d]	5.3 $\times$ 10 <sup>4</sup> [b][c]	7.8 $\times$ 10 <sup>4</sup> [a]	6.8 $\times$ 10 <sup>4</sup> [a][b]	2.0 $\times$ 10 <sup>4</sup> [d]	2.9 $\times$ 10 <sup>5</sup>
	SD	3.6 $\times$ 10 <sup>4</sup>	2.4 $\times$ 10 <sup>4</sup>	3.0 $\times$ 10 <sup>4</sup>	2.7 $\times$ 10 <sup>4</sup>	1.4 $\times$ 10 <sup>4</sup>	8.6 $\times$ 10 <sup>3</sup>	
2 <sup>nd</sup> floor	Mean	1.3 $\times$ 10 <sup>5</sup> [c]	1.4 $\times$ 10 <sup>5</sup> [b][c]	1.8 $\times$ 10 <sup>5</sup> [a]	1.6 $\times$ 10 <sup>5</sup> [b]	1.0 $\times$ 10 <sup>5</sup> [d]	5.5 $\times$ 10 <sup>4</sup> [e]	7.7 $\times$ 10 <sup>5</sup>
	SD	3.3 $\times$ 10 <sup>4</sup>	2.7 $\times$ 10 <sup>4</sup>	3.2 $\times$ 10 <sup>4</sup>	2.0 $\times$ 10 <sup>4</sup>	1.0 $\times$ 10 <sup>4</sup>	1.3 $\times$ 10 <sup>4</sup>	

\* In each row, means with different letters indicate significant difference at a 0.05 level

Table 24. Overall mean concentrations  $\pm$ SD (CFU/m<sup>3</sup>) of fungi on 1<sup>st</sup> and 2<sup>nd</sup> floors on each stage of the sampler.

		Particle size range ( $\mu$ m)						
		>7	4.7-7	3.3-4.7	2.1-3.3	1.1-2.1	0.65-1.1	Total
1 <sup>st</sup> floor	Mean	8.5 $\times$ 10 <sup>2</sup> [b]*	9.2 $\times$ 10 <sup>2</sup> [a][b]	1.5 $\times$ 10 <sup>3</sup> [a][b]	1.8 $\times$ 10 <sup>3</sup> [a]	1.0 $\times$ 10 <sup>3</sup> [a][b]	8.3 $\times$ 10 <sup>2</sup> [b]	6.9 $\times$ 10 <sup>3</sup>
	SD	3.8 $\times$ 10 <sup>2</sup>	6.2 $\times$ 10 <sup>2</sup>	1.3 $\times$ 10 <sup>3</sup>	1.1 $\times$ 10 <sup>3</sup>	4.8 $\times$ 10 <sup>2</sup>	1.1 $\times$ 10 <sup>3</sup>	
2 <sup>nd</sup> floor	Mean	1.5 $\times$ 10 <sup>3</sup> [a]	1.7 $\times$ 10 <sup>3</sup> [a]	2.1 $\times$ 10 <sup>3</sup> [a]	1.6 $\times$ 10 <sup>3</sup> [a]	5.3 $\times$ 10 <sup>2</sup> [b]	2.2 $\times$ 10 <sup>2</sup> [b]	7.7 $\times$ 10 <sup>3</sup>
	SD	1.2 $\times$ 10 <sup>3</sup>	1.2 $\times$ 10 <sup>3</sup>	1.0 $\times$ 10 <sup>3</sup>	6.8 $\times$ 10 <sup>2</sup>	2.3 $\times$ 10 <sup>2</sup>	3.0 $\times$ 10 <sup>2</sup>	

\* In each row, means with different letters indicate significant difference at a 0.05 level

The percentages of bioaerosol concentrations on each stage on the first and second floors were calculated to illustrate the portion of bioaerosols in each size range. Figure 39 shows percentages of bacteria and fungi concentrations on each stage on different floors.

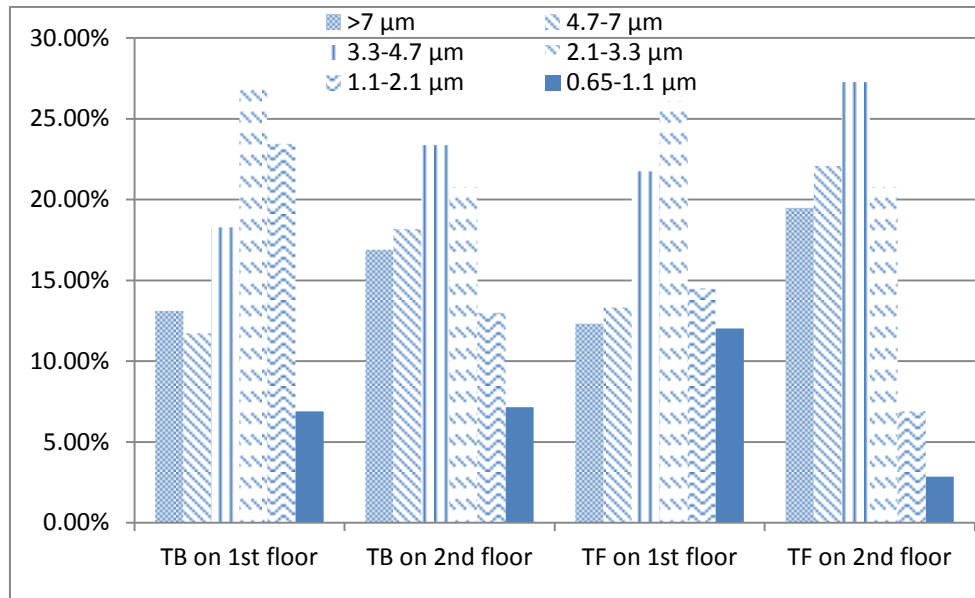


Figure 39. Percentages of bacteria and fungi concentrations on each stage on different floors.

As shown in Figure 39, most of the bioaerosols on first floor ranged from 2.1 to 3.3 μm while most of the bioaerosols on second floor were in the range 3.3-4.7 μm. Based on the percentage of respirable bioaerosols in each test, mean percentages with SDs of respirable bioaerosols on two floors were calculated. Figure 40 shows the portions of respirable bacteria and fungi on the 1<sup>st</sup> floor and 2<sup>nd</sup> floor. It was shown that respirable percentages of bacteria and fungi were significantly higher on the 1<sup>st</sup> floor than on the 2<sup>nd</sup> floor ( $p < 0.0001$ ). Although bacteria concentration was higher on the 2<sup>nd</sup> floor, since the portions of respirable bioaerosol were lower, the risks associated with bioaerosols on two floors were hard to be compared with the known information.

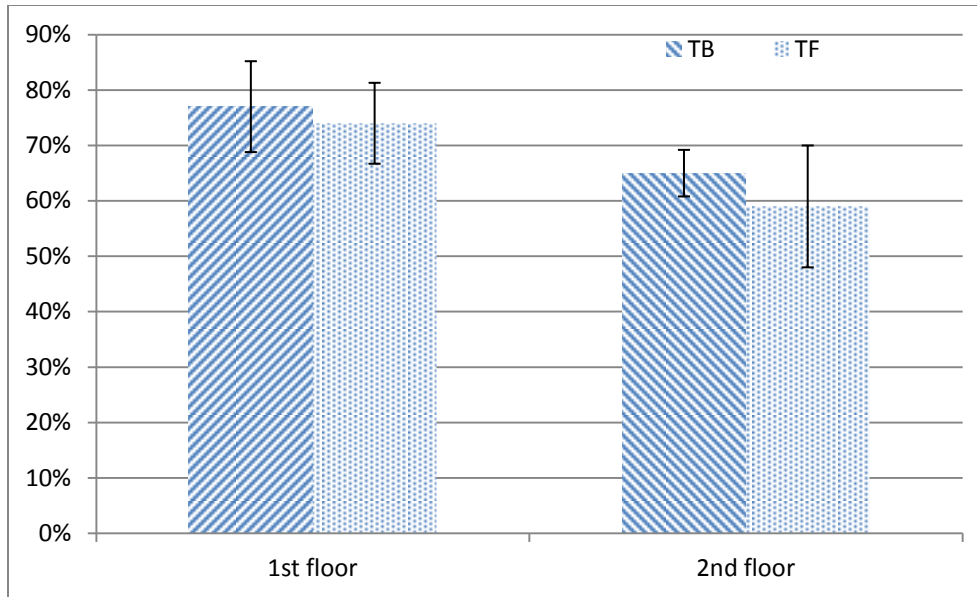


Figure 40. Respirable percentages of bacteria and fungi on different floors

Nine tests of fungi were conducted simultaneously at upstream and downstream of the exhaust fans in summer 2011. Table 25 lists the mean concentrations with SDs of fungi concentrations on each stage at upstream and downstream locations. As shown in this table, fungi PSDs at downstream and upstream of exhaust fans were similar to each other. Fungi concentrations at both locations were at the highest on stage 4. The only difference was that stage 3 had a larger portion of fungi concentrations at upstream than at downstream. Percentage of fungi concentrations on each stage at upstream and downstream locations were calculated and are shown in Figure 41. As shown in this figure, the portions of respirable fungi at upstream and downstream were close. During summer, more than 70% of the fungi emitted from the animal house were respirable.

Table 25. Mean concentrations  $\pm$ SD (CFU/m<sup>3</sup>) of fungi on each stage of the sampler at upstream and downstream of the fans.

		Particle size range ( $\mu$ m)						Total
		>7	4.7-7	3.3-4.7	2.1-3.3	1.1-2.1	0.65-1.1	
Downstream	Mean	3.2 $\times$ 10 <sup>2</sup> [b][c]*	2.1 $\times$ 10 <sup>2</sup> [b][c]	3.7 $\times$ 10 <sup>2</sup> [b]	7.9 $\times$ 10 <sup>2</sup> [a]	4.3 $\times$ 10 <sup>2</sup> [b]	8.7 $\times$ 10 <sup>2</sup> [c]	2.2 $\times$ 10 <sup>3</sup>
	SD	1.3 $\times$ 10 <sup>2</sup>	9.0 $\times$ 10	9.6 $\times$ 10	3.1 $\times$ 10 <sup>2</sup>	2.4 $\times$ 10 <sup>2</sup>	1.3 $\times$ 10 <sup>2</sup>	
Upstream	Mean	4.5 $\times$ 10 <sup>2</sup> [b][c]	4.1 $\times$ 10 <sup>2</sup> [b][c]	8.2 $\times$ 10 <sup>2</sup> [a]	1.1 $\times$ 10 <sup>3</sup> [a]	4.5 $\times$ 10 <sup>2</sup> [b]	1.3 $\times$ 10 <sup>2</sup> [c]	3.4 $\times$ 10 <sup>3</sup>
	SD	1.4 $\times$ 10 <sup>2</sup>	9.1 $\times$ 10	1.4 $\times$ 10 <sup>2</sup>	4.3 $\times$ 10 <sup>2</sup>	2.2 $\times$ 10 <sup>2</sup>	1.9 $\times$ 10 <sup>2</sup>	

\* In each row, means with different letters indicate significant difference at a 0.05 level

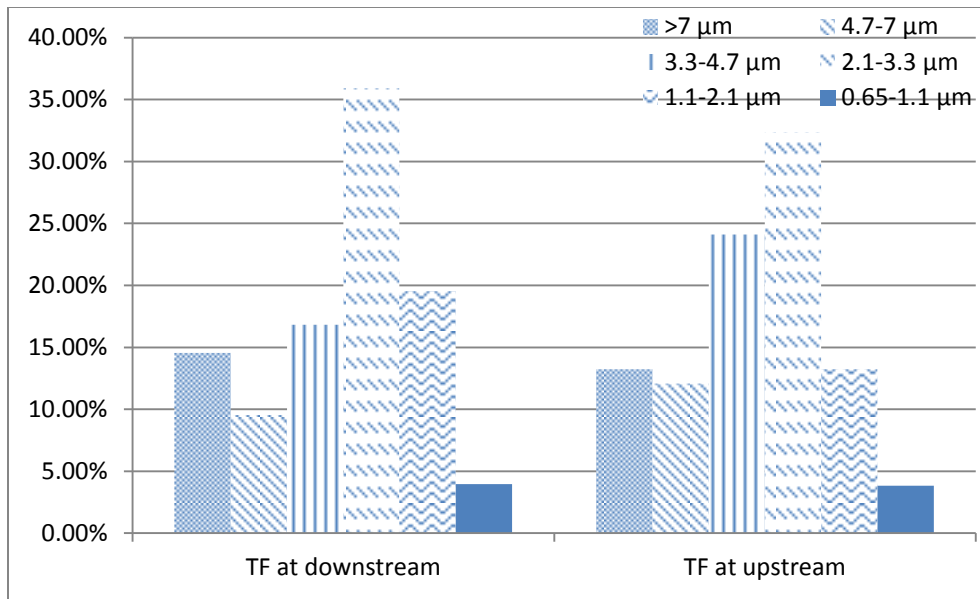


Figure 41. Percentages of fungi concentrations on each stage of the sampler at downstream and upstream of the exhaust fans.

#### BIOAEROSOL SIZE DISTRIBUTION AT AMBIENT LOCATIONS

To investigate seasonal effects on ambient bioaerosol PSDs, mean concentrations with SDs of ambient bacteria and fungi on each stage at each ambient station were calculated by season. Table 26 lists the mean concentrations with SDs of bacteria at ST2-5 in three seasons. As shown in this table, bacteria concentrations on each stage were significantly different in winter at ST3-5. But there were not significant differences among bacteria concentrations on each stage in spring and summer at ST2-5. This observation indicated that seasonal effects had impacts on bacteria size distributions in the ambient locations. Table 27 lists the mean concentrations with SDs of fungi at ST2-5 in three seasons. As shown in Table 27, there were significant differences among fungi concentrations on each stage in three seasons at ST2-5. In winter, stage 5 had the highest fungi concentrations; while in spring and summer, stage 4 had the highest fungi concentrations. The observed variations of fungi size distributions indicated that in warmer weathers, larger particles accounted for more of ambient fungi.

Table 26. Mean concentrations  $\pm$ SD (CFU/m<sup>3</sup>) of bacteria at ST2-5 in three seasons on each stage of the sampler.

		Particle size range ( $\mu$ m)								
			>7	4.7-7	3.3-4.7	2.1-3.3	1.1-2.1	0.65-1.1	Total	
Winter	ST2	Mean	4.3 $\times$ 10 <sup>[a]*</sup>	3.8 $\times$ 10 <sup>[a]</sup>	6.4 $\times$ 10 <sup>[a]</sup>	1.1 $\times$ 10 <sup>2[a]</sup>	1.1 $\times$ 10 <sup>2[a]</sup>	1.5 $\times$ 10 <sup>[a]</sup>	3.8 $\times$ 10 <sup>2</sup>	
		SD	5.0 $\times$ 10	3.4 $\times$ 10	6.5 $\times$ 10	2.0 $\times$ 10 <sup>2</sup>	2.1 $\times$ 10 <sup>2</sup>	2.0 $\times$ 10		
	ST3	Mean	1.5 $\times$ 10 <sup>2[b]</sup>	1.2 $\times$ 10 <sup>2[b]</sup>	2.3 $\times$ 10 <sup>2[b]</sup>	4.7 $\times$ 10 <sup>2[a]</sup>	4.9 $\times$ 10 <sup>2[a]</sup>	6.4 $\times$ 10 <sup>[b]</sup>	1.5 $\times$ 10 <sup>3</sup>	
		SD	1.0 $\times$ 10 <sup>2</sup>	9.6 $\times$ 10	1.7 $\times$ 10 <sup>2</sup>	3.2 $\times$ 10 <sup>2</sup>	3.4 $\times$ 10 <sup>2</sup>	5.8 $\times$ 10		
	ST4	Mean	1.3 $\times$ 10 <sup>2[b][c]</sup>	8.6 $\times$ 10 <sup>[c]</sup>	1.9 $\times$ 10 <sup>2[b][c]</sup>	4.0 $\times$ 10 <sup>2[a][b]</sup>	4.9 $\times$ 10 <sup>2[a]</sup>	1.8 $\times$ 10 <sup>2[b][c]</sup>	1.5 $\times$ 10 <sup>3</sup>	
		SD	1.2 $\times$ 10 <sup>2</sup>	6.4 $\times$ 10	1.5 $\times$ 10 <sup>2</sup>	3.3 $\times$ 10 <sup>2</sup>	4.0 $\times$ 10 <sup>2</sup>	4.3 $\times$ 10 <sup>2</sup>		
	ST5	Mean	2.7 $\times$ 10 <sup>[a]</sup>	1.4 $\times$ 10 <sup>[a][b]</sup>	1.7 $\times$ 10 <sup>[a][b]</sup>	1.3 $\times$ 10 <sup>[a][b]</sup>	1.3 $\times$ 10 <sup>[a][b]</sup>	8 <sup>[b]</sup>	9.2 $\times$ 10	
		SD	3.4 $\times$ 10	1 $\times$ 10	1.9 $\times$ 10	1.1 $\times$ 10	1.2 $\times$ 10	7		
	Spring	ST2	Mean	6.2 $\times$ 10 <sup>2[a]</sup>	4.3 $\times$ 10 <sup>2[a]</sup>	5.5 $\times$ 10 <sup>2[a]</sup>	6.3 $\times$ 10 <sup>2[a]</sup>	5.2 $\times$ 10 <sup>2[a]</sup>	7.7 $\times$ 10 <sup>[a]</sup>	2.8 $\times$ 10 <sup>3</sup>
			SD	7.3 $\times$ 10 <sup>2</sup>	4.3 $\times$ 10 <sup>2</sup>	4.9 $\times$ 10 <sup>2</sup>	4.5 $\times$ 10 <sup>2</sup>	3.6 $\times$ 10 <sup>2</sup>	6.8 $\times$ 10	
		ST3	Mean	5.7 $\times$ 10 <sup>[a]</sup>	4.8 $\times$ 10 <sup>[a]</sup>	8.1 $\times$ 10 <sup>[a]</sup>	1.7 $\times$ 10 <sup>2[a]</sup>	1.8 $\times$ 10 <sup>2[a]</sup>	2.9 $\times$ 10 <sup>[a]</sup>	5.7 $\times$ 10 <sup>2</sup>
			SD	7.2 $\times$ 10	1.0 $\times$ 10 <sup>2</sup>	1.5 $\times$ 10 <sup>2</sup>	3.4 $\times$ 10 <sup>2</sup>	3.5 $\times$ 10 <sup>2</sup>	3.2 $\times$ 10	
ST4		Mean	3.2 $\times$ 10 <sup>[a]</sup>	1.6 $\times$ 10 <sup>[a]</sup>	1.9 $\times$ 10 <sup>[a]</sup>	1.4 $\times$ 10 <sup>[a]</sup>	6 <sup>[a]</sup>	1.4 $\times$ 10 <sup>[a]</sup>	1.0 $\times$ 10 <sup>2</sup>	
		SD	3.6 $\times$ 10	1.4 $\times$ 10	2.0 $\times$ 10	1.2 $\times$ 10	3	1.6 $\times$ 10		
ST5		Mean	1.4 $\times$ 10 <sup>2[a]</sup>	1.7 $\times$ 10 <sup>2[a]</sup>	2.4 $\times$ 10 <sup>2[a]</sup>	4.4 $\times$ 10 <sup>2[a]</sup>	4.1 $\times$ 10 <sup>2[a]</sup>	6.6 $\times$ 10 <sup>[a]</sup>	1.5 $\times$ 10 <sup>3</sup>	
		SD	1.9 $\times$ 10 <sup>2</sup>	2.4 $\times$ 10 <sup>2</sup>	3.7 $\times$ 10 <sup>2</sup>	7.7 $\times$ 10 <sup>2</sup>	6.6 $\times$ 10 <sup>2</sup>	1.1 $\times$ 10 <sup>2</sup>		

Table 26 Continued

Summer	ST2	Mean	$1.5 \times 10^{2[a]}$	$8.5 \times 10^{[a]}$	$1.6 \times 10^{2[a]}$	$1.8 \times 10^{2[a]}$	$1.5 \times 10^{2[a]}$	$3.0 \times 10^{[a]}$	$7.6 \times 10^2$
		SD	$1.9 \times 10^2$	$8.8 \times 10$	$2.6 \times 10^2$	$3.1 \times 10^2$	$2.2 \times 10^2$	$3.4 \times 10$	
	ST3	Mean	$1.4 \times 10^{2[a]}$	$1.4 \times 10^{2[a]}$	$2.1 \times 10^{2[a]}$	$3.5 \times 10^{2[a]}$	$3.5 \times 10^{2[a]}$	$7.5 \times 10^{[a]}$	$1.3 \times 10^3$
		SD	$2.1 \times 10^2$	$2.6 \times 10^2$	$3.8 \times 10^2$	$6.0 \times 10^2$	$5.3 \times 10^2$	$8.5 \times 10$	
	ST4	Mean	$1.4 \times 10^{2[a]}$	$2.3 \times 10^{2[a]}$	$3.5 \times 10^{2[a]}$	$4.1 \times 10^{2[a]}$	$3.4 \times 10^{2[a]}$	$2.0 \times 10^{2[a]}$	$1.7 \times 10^3$
		SD	$1.0 \times 10^2$	$2.0 \times 10^2$	$2.9 \times 10^2$	$4.0 \times 10^2$	$3.6 \times 10^2$	$2.9 \times 10^2$	
	ST5	Mean	$1.4 \times 10^{2[a]}$	$1.8 \times 10^{2[a]}$	$2.5 \times 10^{2[a]}$	$2.3 \times 10^{2[a]}$	$2.5 \times 10^{2[a]}$	$4.1 \times 10^{[a]}$	$1.1 \times 10^3$
		SD	$1.7 \times 10^2$	$2.8 \times 10^2$	$3.8 \times 10^2$	$3.4 \times 10^2$	$3.2 \times 10^2$	$2.5 \times 10$	

\* In each row, means with different letters indicate significant difference at a 0.05 level

Table 27. Mean concentrations  $\pm$ SD (CFU/m<sup>3</sup>) of fungi at ST2-5 in three seasons on each stage of the sampler.

			Particle size range ( $\mu$ m)						
			>7	4.7-7	3.3-4.7	2.1-3.3	1.1-2.1	0.65-1.1	Total
Winter	ST2	Mean	$5.5 \times 10^{[b]*}$	$1.6 \times 10^{[b]}$	$3.1 \times 10^{[b]}$	$1.9 \times 10^{2[a][b]}$	$3.2 \times 10^{2[a]}$	$4.3^{[b]}$	$6.2 \times 10^2$
		SD	$1.3 \times 10^2$	$1.1 \times 10$	$2.4 \times 10$	$2.4 \times 10^2$	$3.4 \times 10^2$	$1.9$	
	ST3	Mean	$4.1 \times 10^{[b]}$	$1.8 \times 10^{[b]}$	$1.2 \times 10^{[b]}$	$4.3 \times 10^{[b]}$	$9.3 \times 10^{[a]}$	$7.3^{[b]}$	$2.1 \times 10^2$
		SD	$7.2 \times 10^2$	$2.9 \times 10$	$7.3$	$2.5 \times 10$	$8.0 \times 10$	$8.2$	
	ST4	Mean	$2.4 \times 10^{[b][c]}$	$1.3 \times 10^{[b][c]}$	$1.4 \times 10^{[b][c]}$	$4.0 \times 10^{[a][b]}$	$6.5 \times 10^{[a]}$	$8^{[c]}$	$1.6 \times 10^2$

Table 27 Continued

	SD	3.3×10	1.4×10	1.3×10	2.8×10	4.6×10	9.5		
	ST5	Mean	1.4×10 <sup>[b]</sup>	1.0×10 <sup>[b]</sup>	1.6×10 <sup>[b]</sup>	3.7×10 <sup>[a]</sup>	5.1×10 <sup>[a]</sup>	4.4 <sup>[b]</sup>	1.3×10 <sup>2</sup>
		SD	1.3×10	8.9	1.0×10	2.0×10	3.4×10	1.4	
Spring	ST2	Mean	1.2×10 <sup>2[b][c]</sup>	1.2×10 <sup>2[b][c]</sup>	2.4×10 <sup>2[b][c]</sup>	4.6×10 <sup>2[a]</sup>	2.4×10 <sup>2[b]</sup>	2.1×10 <sup>[c]</sup>	1.2×10 <sup>3</sup>
		SD	1.5×10 <sup>2</sup>	1.6×10 <sup>2</sup>	2.3×10 <sup>2</sup>	2.7×10 <sup>2</sup>	8.7×10	4.4×10	
	ST3	Mean	1.7×10 <sup>2[a]</sup>	2.3×10 <sup>2[a]</sup>	3.2×10 <sup>2[a]</sup>	4.1×10 <sup>2[a]</sup>	4.2×10 <sup>2[a]</sup>	6.4 <sup>[a]</sup>	1.6×10 <sup>3</sup>
		SD	3.6×10 <sup>2</sup>	6.4×10 <sup>2</sup>	6.5×10 <sup>2</sup>	4.5×10 <sup>2</sup>	3.7×10 <sup>2</sup>	2.7	
	ST4	Mean	2.0×10 <sup>2[b][c]</sup>	1.7×10 <sup>2[b][c]</sup>	3.2×10 <sup>2[a][b][c]</sup>	5.5×10 <sup>2[a]</sup>	3.6×10 <sup>2[a][b]</sup>	6.7 <sup>[c]</sup>	1.6×10 <sup>3</sup>
		SD	2.7×10 <sup>2</sup>	2.0×10 <sup>2</sup>	3.7×10 <sup>2</sup>	2.2×10 <sup>2</sup>	2.8×10 <sup>2</sup>	5.4	
	ST5	Mean	1.0×10 <sup>2[b][c]</sup>	1.1×10 <sup>2[b][c]</sup>	1.8×10 <sup>2[b][c]</sup>	5.1×10 <sup>2[a]</sup>	3.2×10 <sup>2[a][b]</sup>	8.1 <sup>[c]</sup>	1.2×10 <sup>3</sup>
		SD	1.8×10 <sup>2</sup>	1.8×10 <sup>2</sup>	1.7×10 <sup>2</sup>	3.6×10 <sup>2</sup>	2.3×10 <sup>2</sup>	1.1×10	
Summer	ST2	Mean	4.4×10 <sup>2[a][b]</sup>	4.4×10 <sup>2[a][b]</sup>	6.0×10 <sup>2[a]</sup>	5.7×10 <sup>2[a]</sup>	3.0×10 <sup>2[a][b]</sup>	2.0×10 <sup>2[b]</sup>	2.6×10 <sup>3</sup>
		SD	3.6×10 <sup>2</sup>	2.8×10 <sup>2</sup>	3.9×10 <sup>2</sup>	2.9×10 <sup>2</sup>	2.4×10 <sup>2</sup>	4.1×10 <sup>2</sup>	
	ST3	Mean	5.0×10 <sup>2[a][b]</sup>	4.4×10 <sup>2[b]</sup>	6.9×10 <sup>2[a][b]</sup>	7.8×10 <sup>2[a]</sup>	4.7×10 <sup>2[a][b]</sup>	1.8×10 <sup>[c]</sup>	2.9×10 <sup>3</sup>
		SD	3.0×10 <sup>2</sup>	3.0×10 <sup>2</sup>	3.9×10 <sup>2</sup>	3.2×10 <sup>2</sup>	2.7×10 <sup>2</sup>	1.6×10	
	ST4	Mean	2.1×10 <sup>2[b]</sup>	2.1×10 <sup>2[b]</sup>	3.4×10 <sup>2[a][b]</sup>	5.1×10 <sup>2[a]</sup>	2.4×10 <sup>2[b]</sup>	1.2×10 <sup>[c]</sup>	1.5×10 <sup>3</sup>
		SD	1.4×10 <sup>2</sup>	1.6×10 <sup>2</sup>	1.6×10 <sup>2</sup>	2.7×10 <sup>2</sup>	1.7×10 <sup>2</sup>	1.6×10	
	ST5	Mean	5.6×10 <sup>2[a][b]</sup>	7.2×10 <sup>2[a][b]</sup>	9.4×10 <sup>2[a]</sup>	6.3×10 <sup>2[a][b]</sup>	4.0×10 <sup>2[a][b]</sup>	5.9×10 <sup>[b]</sup>	3.3×10 <sup>3</sup>
		SD	6.4×10 <sup>2</sup>	1.0×10 <sup>3</sup>	1.5×10 <sup>3</sup>	4.8×10 <sup>2</sup>	5.4×10 <sup>2</sup>	1.1×10 <sup>2</sup>	

\* In each row, means with different letters indicate significant difference at a 0.05 level

Percentages of bacteria and fungi concentrations on each stage in different seasons at ST2-5 were calculated and are shown in Figures 42-47.

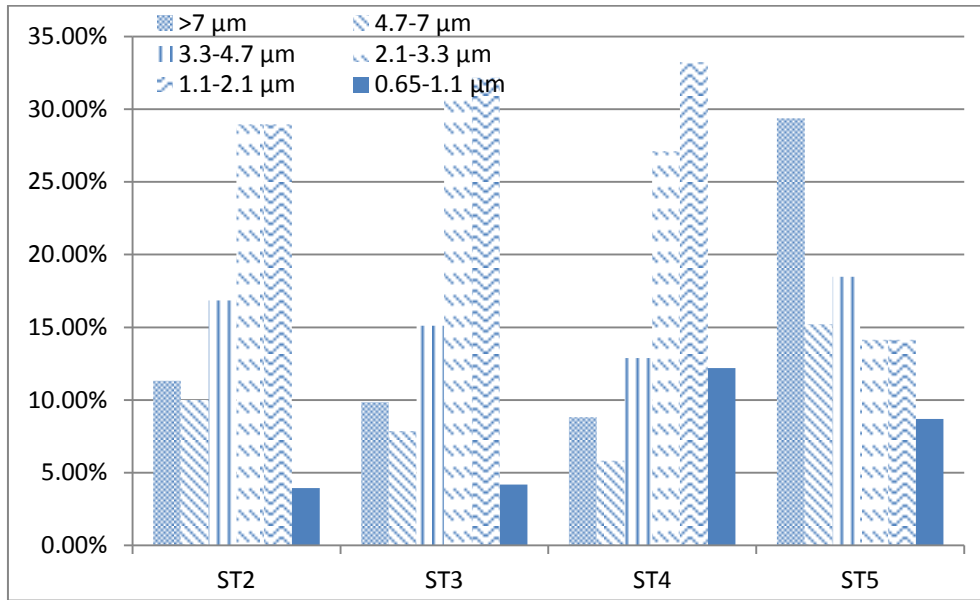


Figure 42. Percentages of bacteria concentrations on each stage of the sampler at ST2-5 in winter.

As shown in Figure 42, in winter, bacteria concentrations were the highest on stages 4-5 at ST2-4. ST5 had the highest bacteria concentration on stage 1 in winter. In winter, 80% of bacteria collected at ST 2-4 were respirable, but only 55% at ST5 were respirable.

As shown in Figure 43, in spring, stages 4-5 had the highest bacteria concentration at ST3 and ST5. Bacteria were evenly distributed on the first five stages at ST2 and were the highest on stage 1 at ST4. Portions of respirable bacteria ranged from 50% to 80% at different ambient stations in spring.

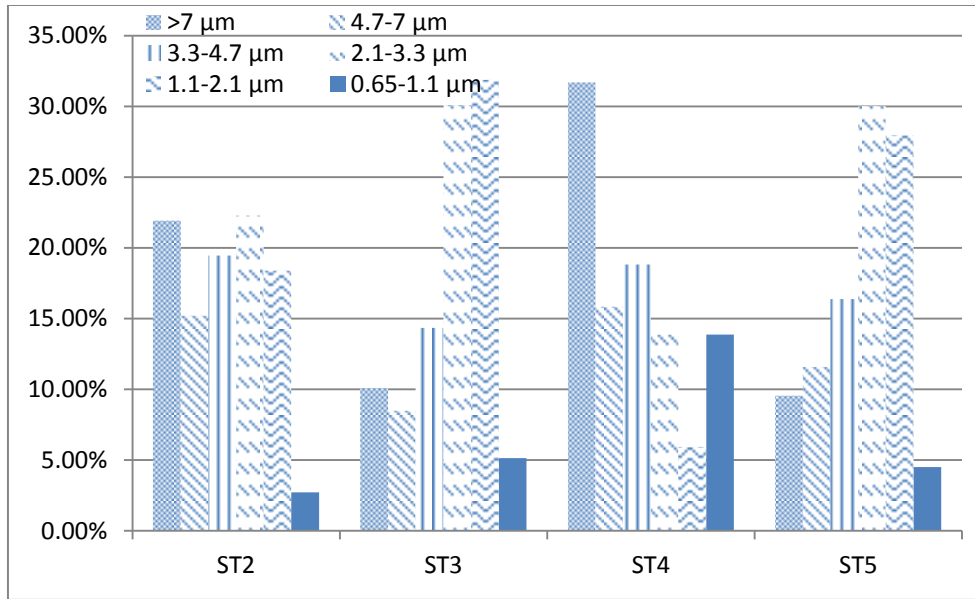


Figure 43. Percentages of bacteria concentrations on each stage of the sampler at ST2-5 in spring.

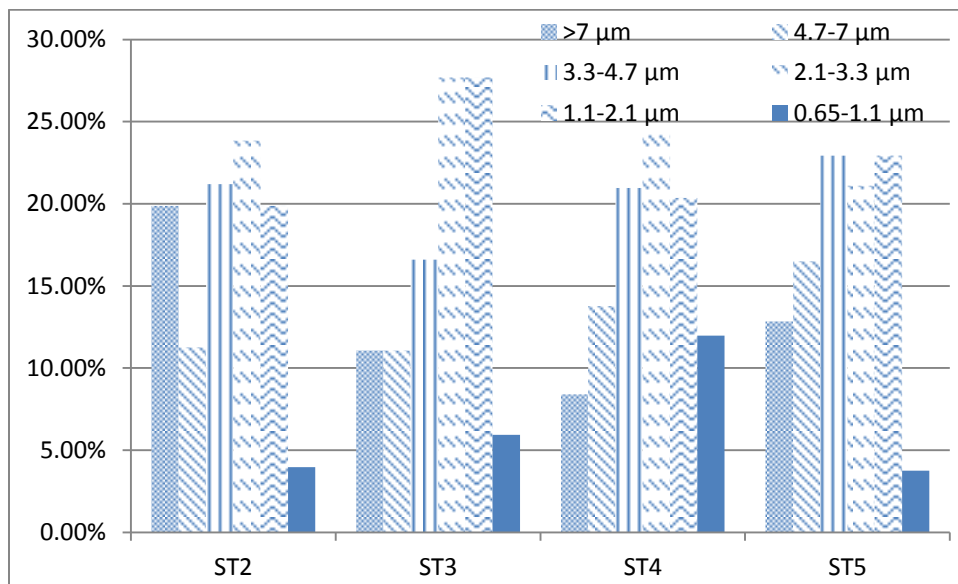


Figure 44. Percentages of bacteria concentrations on each stage at ST2-5 in summer.

As shown in Figure 44, in summer, stages 3-5 had the highest bacteria concentrations at ST2, ST4 and ST5. Stages 4-5 had the highest bacteria concentrations at ST3. 68% to 78% of

bacteria at ambient stations were respirable in summer. It was observed that sometimes, stage 1 had the highest bacteria concentrations at some stations. This might be because that these stations were at upwind for most time of a season, and the bacteria collected at this station were mainly from background or other sources.

As shown in Figure 45, in winter most of fungi deposited on stage 5 at all stations. 70% to 90% of fungi at ambient were respirable in winter.

As shown in Figure 46, stage 4 had the highest fungi concentrations at all stations in spring. Stage 3 also had high fungi concentration at ST3. 74% to 83% of fungi at ambient were respirable in spring.

As shown in Figure 47, stages 3 and 4 had high level of fungi concentrations at all stations in summer. Stage 2 also had high fungi concentration at ST5. 60% to 70% of fungi at ambient were respirable in summer.

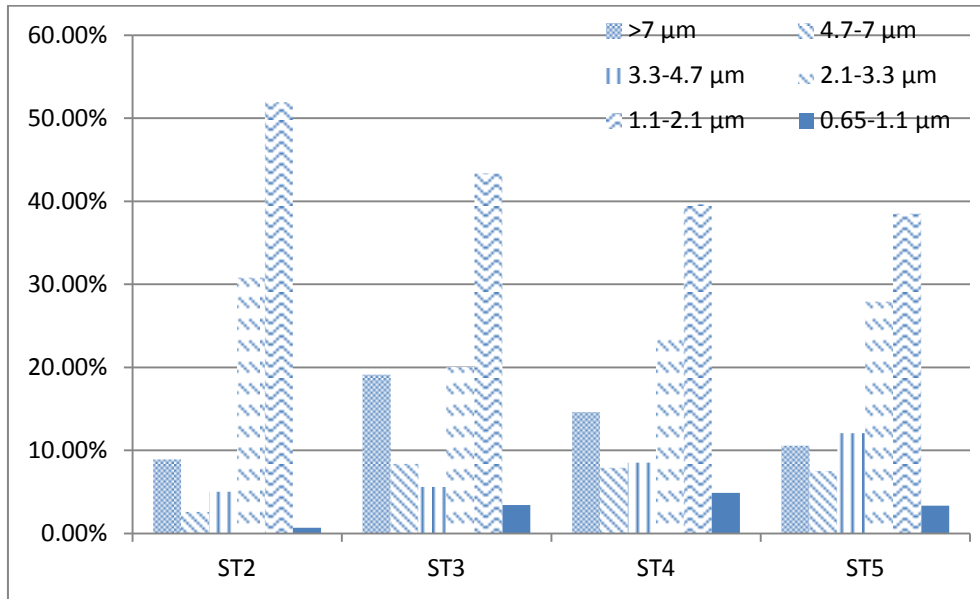


Figure 45. Percentages of fungi concentrations on each stage of the sampler at ST2-5 in winter.

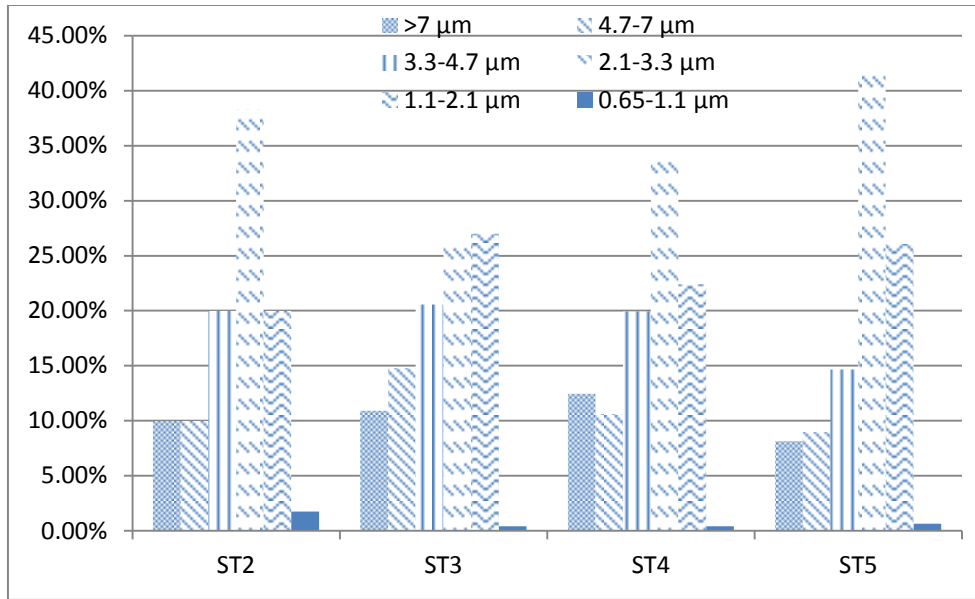


Figure 46. Percentages of fungi concentrations on each stage of the sampler at ST2-5 in spring.

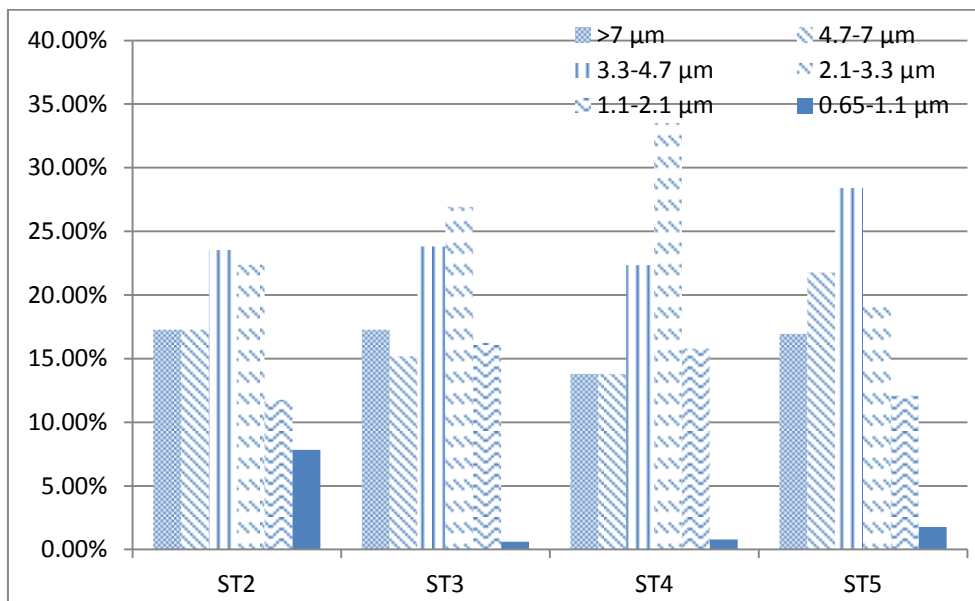


Figure 47. Percentages of fungi concentrations on each stage of the sampler at ST2-5 in summer.

It was observed that as weather got warmer, the portions of larger fungi particles increased at ambient. Therefore, portions of respirable fungi decreased from winter to summer.

Based on the percentage of respirable bioaerosols in each test, mean percentages with SD of respirable bioaerosols at each ambient station were calculated by different seasons and time of day. Figures 48-51 show the portions of respirable bacteria and fungi at ambient stations.

As shown in Figure 48, at ST2, bacteria respirable percentages were not significantly different over different time of day ( $p=0.4494$ ), nor different over three seasons ( $p=0.9606$ ). Contradictorily, fungi respirable percentages were greater in the morning than afternoon ( $p=0.0067$ ). Fungi respirable percentages were the smallest in summer, and were not significantly different from winter to spring at a 0.05 level at ST2.

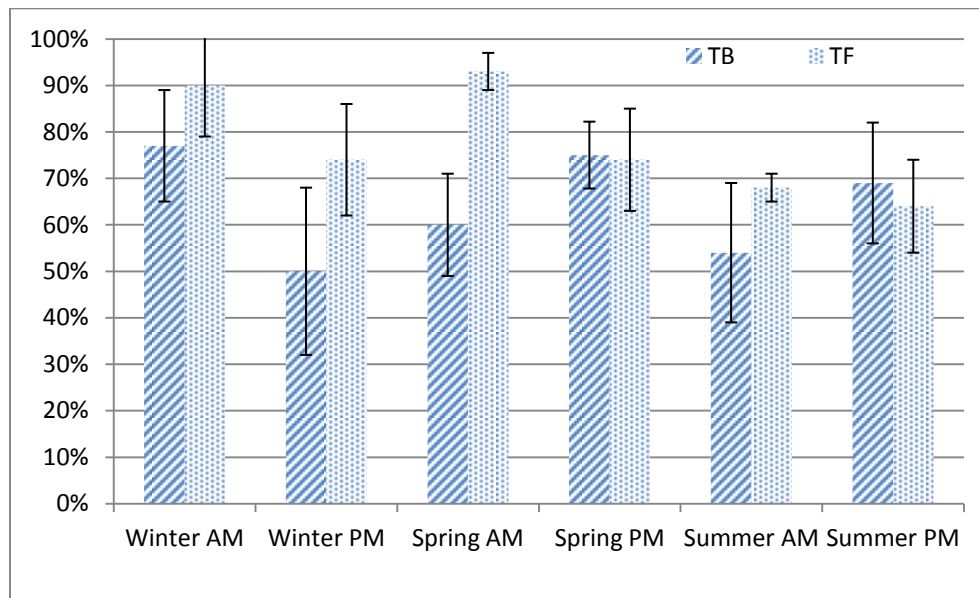


Figure 48. Respirable percentages of bacteria and fungi at ST2 in different seasons and time of day

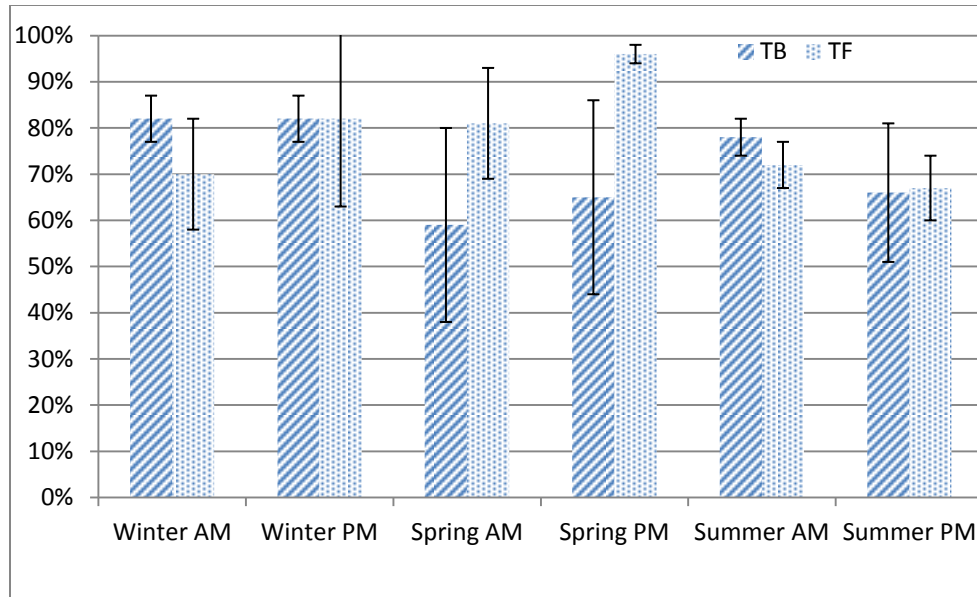


Figure 49. Respirable percentages of bacteria and fungi at ST3 in different seasons and time of day

As shown in Figure 49, at ST3, bacteria respirable percentages were not significantly different over different time of day ( $p=0.8978$ ). Bacteria respirable percentages were the greatest in winter and the lowest in spring ( $p=0.0014$ ). Fungi respirable percentages were not significantly different over different time of day either ( $p=0.2529$ ), and were the greatest in spring and the smallest in summer ( $p=0.0019$ ).

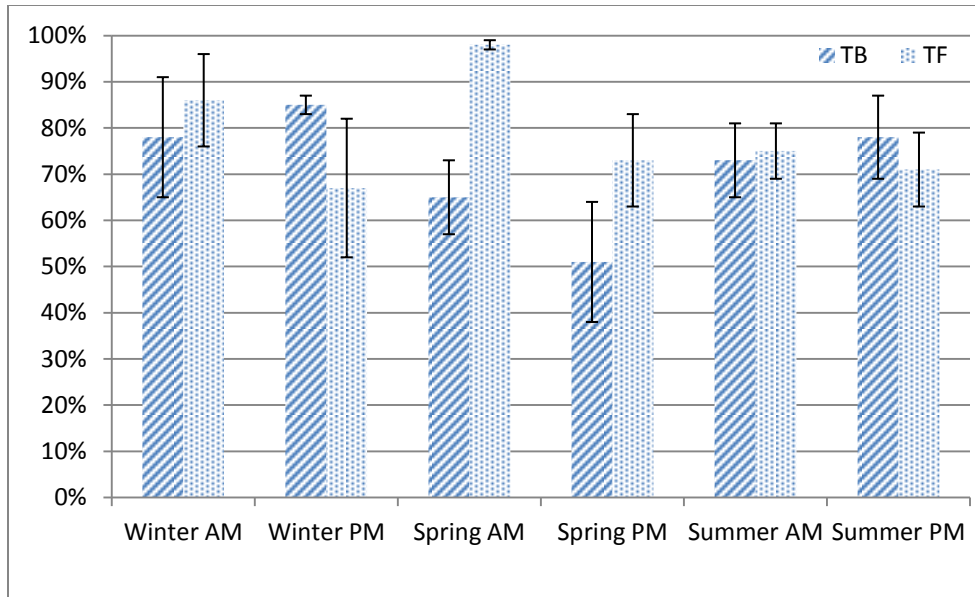


Figure 50. Respirable percentages of bacteria and fungi at ST4 in different seasons and time of day

As shown in Figure 50, at ST4, bacteria respirable percentages were not significantly different over different time of day ( $p=0.54$ ), and were the smallest in spring ( $p<0.0001$ ). There was no significant difference between bacteria respirable percentages in winter and summer at a level of 0.05. Fungi respirable percentages were higher in the morning than in the afternoon at ST4 ( $p=0.001$ ). There was no significant difference among fungi respirable percentages in winter, spring and summer ( $p=0.3067$ ).

As shown in Figure 51, at ST5, bacteria respirable percentages were not significantly different over different time of day ( $p=0.2496$ ), or over different seasons ( $p=0.0897$ ). Fungi respirable percentages were not significantly different over different time of day, either ( $p=0.5897$ ). Fungi respirable percentages were the smallest in summer, and were not significantly different in winter and spring at a 0.05 level.

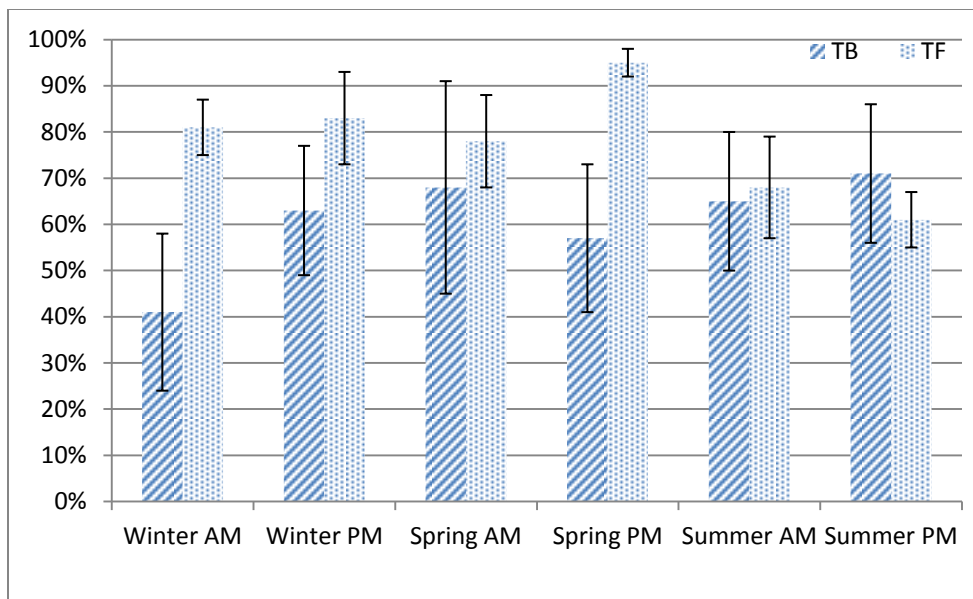


Figure 51. Respirable percentages of bacteria and fungi at ST5 in different seasons and time of day

The overall mean bacteria respirable percentages were the greatest at ST4 and ST3, and were the smallest at ST5 at a 0.05 level. The overall mean fungi respirable percentages were not significantly different over the four ambient stations ( $p=0.989$ ).

To investigate upwind/downwind effects for ambient bioaerosol size distribution, mean concentrations with SDs of ambient bacteria and fungi on each stage were calculated by upwind and downwind locations. Tables 28-29 list concentrations with SDs of bacteria and fungi on each stage at upwind and downwind locations, respectively.

As shown in Table 28, there was no significant difference among bacteria concentrations on each stage at upwind. It indicated that sizes of background bacteria distributed evenly from 0.65  $\mu\text{m}$  to  $>7 \mu\text{m}$ . Stage 4-5 had the highest bacteria concentrations at downwind, which might be because that more small particles transported to ambient stations with wind than larger particles.

As shown in Table 29, at upwind, fungi concentrations on the first five stages (Stage 1-5) were not significantly different. At downwind, stages 3-4 had the highest fungi concentrations. This variation was similar to bacteria except that the majority of fungi were in a larger size range.

Percentages of bacteria and fungi concentrations on each stage at upwind and downwind were calculated and are shown in Figures 52. As shown in this figure, downwind locations had a larger portion of smaller bacteria particles. Fungi didn't show a spatial variation in size distributions.

Based on the percentage of respirable bioaerosols in each test, mean percentages with SDs of respirable bioaerosols upwind and downwind were calculated. Figure 53 shows the portions of respirable bacteria and fungi at downwind and upwind locations. As shown in this figure, bacteria respirable percentages were significantly greater upwind than downwind ( $p < 0.0001$ ) while fungi respirable percentages were not significantly different upwind and downwind ( $p = 0.7444$ ). The variations on respirable portions of bacteria and fungi might be because that they acted differently to influencing factors.

Table 28. Over all mean concentrations  $\pm$ SD (CFU/m<sup>3</sup>) of bacteria at upwind and downwind on each stage of the sampler.

		Particle size range ( $\mu$ m)						
		>7	4.7-7	3.3-4.7	2.1-3.3	1.1-2.1	0.65-1.1	Total
Upwind	Mean	6.3 $\times$ 10 <sup>[a]*</sup>	3.7 $\times$ 10 <sup>[a]</sup>	5.6 $\times$ 10 <sup>[a]</sup>	5.3 $\times$ 10 <sup>[a]</sup>	5.3 $\times$ 10 <sup>[a]</sup>	3.2 $\times$ 10 <sup>[a]</sup>	
	SD	1.1 $\times$ 10 <sup>2</sup>	6.0 $\times$ 10	1.4 $\times$ 10 <sup>2</sup>	1.6 $\times$ 10 <sup>2</sup>	1.4 $\times$ 10 <sup>2</sup>	9.1 $\times$ 10	
Downwind	Mean	2.2 $\times$ 10 <sup>2[b][c]</sup>	2.1 $\times$ 10 <sup>2[b][c]</sup>	3.3 $\times$ 10 <sup>2[b]</sup>	5.0 $\times$ 10 <sup>2[a]</sup>	4.9 $\times$ 10 <sup>2[a]</sup>	1.0 $\times$ 10 <sup>2[c]</sup>	
	SD	3.4 $\times$ 10 <sup>2</sup>	3.7 $\times$ 10 <sup>2</sup>	3.4 $\times$ 10 <sup>2</sup>	4.7 $\times$ 10 <sup>2</sup>	4.3 $\times$ 10 <sup>2</sup>	3.1 $\times$ 10 <sup>2</sup>	

\* In each row, means with different letters indicate significant difference at a 0.05 level

Table 29. Overall mean concentrations  $\pm$ SD (CFU/m<sup>3</sup>) of fungi at upwind and downwind on each stage of the sampler.

		Particle size range ( $\mu$ m)						
		>7	4.7-7	3.3-4.7	2.1-3.3	1.1-2.1	0.65-1.1	Total
Upwind	Mean	2.6 $\times$ 10 <sup>2[a]*</sup>	2.8 $\times$ 10 <sup>2[a]</sup>	4.0 $\times$ 10 <sup>2[a]</sup>	4.3 $\times$ 10 <sup>2[a]</sup>	3.2 $\times$ 10 <sup>2[a]</sup>	2.0 $\times$ 10 <sup>[b]</sup>	
	SD	4.2 $\times$ 10 <sup>2</sup>	5.8 $\times$ 10 <sup>2</sup>	7.8 $\times$ 10 <sup>2</sup>	4.0 $\times$ 10 <sup>2</sup>	3.2 $\times$ 10 <sup>2</sup>	6.0 $\times$ 10	
Downwind	Mean	1.6 $\times$ 10 <sup>2[b]</sup>	1.5 $\times$ 10 <sup>2[b][c]</sup>	2.3 $\times$ 10 <sup>2[a][b]</sup>	3.4 $\times$ 10 <sup>2[a]</sup>	2.3 $\times$ 10 <sup>2[b]</sup>	4.2 $\times$ 10 <sup>[c]</sup>	
	SD	2.1 $\times$ 10 <sup>2</sup>	2.1 $\times$ 10 <sup>2</sup>	3.0 $\times$ 10 <sup>2</sup>	3.5 $\times$ 10 <sup>2</sup>	2.7 $\times$ 10 <sup>2</sup>	1.8 $\times$ 10 <sup>2</sup>	

\* In each row, means with different letters indicate significant difference at a 0.05 level

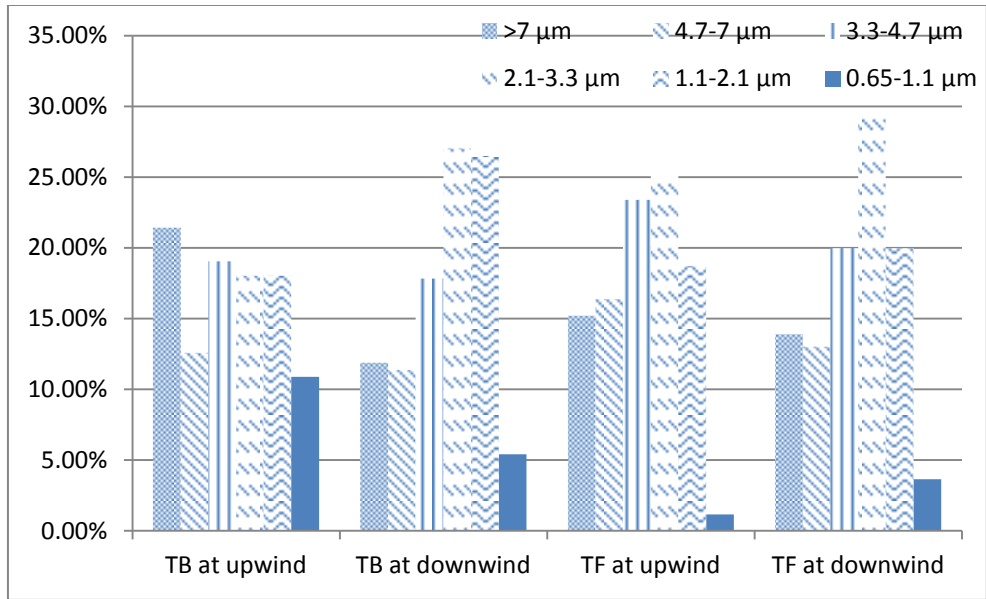


Figure 52. Percentages of bacteria and fungi concentrations on each stage of the sampler at upwind and downwind

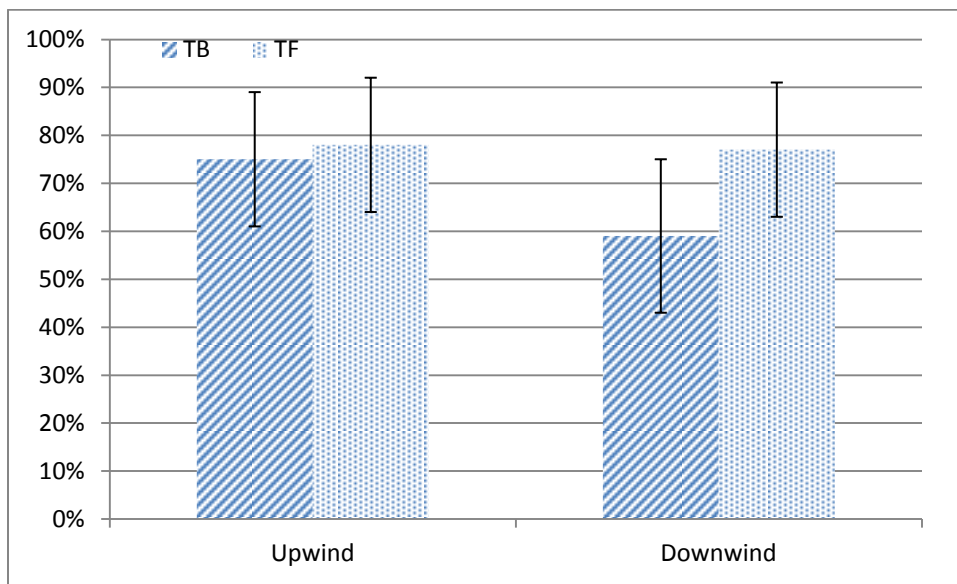


Figure 53. Respirable percentages of bacteria and fungi upwind and downwind

## CHAPTER 6 CONCLUSIONS AND FUTURE WORK

### CONCLUSIONS

In this project, concentrations of bacteria and fungi in an animal house and at ambient locations on an egg production farm were measured in three seasons. To investigate temporal effects on variations of bioaerosol, temperature (T), RH, and ventilation rate (Q) were measured simultaneously at source; in addition, meteorological data including T, RH, wind direction and speed, and solar radiation were measured simultaneously at ambient stations.

It was discovered that in-house bacteria concentrations were significantly different over three seasons, but the in-house fungi concentrations were relatively constant. In-house bacteria concentrations were shown to correlate with T and RH and negatively correlate with Q; in-house fungi concentrations negatively correlated with T and RH but correlated with Q. Bacteria concentrations on the second floor of the poultry house were significantly higher than the first floor. No significant difference between fungi concentrations on different floors was observed. Concentrations of fungi at upstream of the exhaust fans were higher than that at downstream, which indicated that there were injury or damage of the airborne fungi caused by high air flow and impaction on the fan blades/surfaces. The three predominant fungi species detected on both floors in the poultry house were *Aspergillus* spp., *Cladosporium* spp., and *Penicillium* spp. Both bacteria and fungi had the highest emission rates in summer, which indicated that ventilation rate had dominant effects on variations of bioaerosol emissions from the poultry house.

Ambient bacteria concentrations did not show a trend to vary with season although atmospheric conditions changed significantly. The lowest ambient fungi concentration was observed in winter time. Ambient fungi concentrations were higher in summer than in spring although solar radiations were significantly higher in summer. There were significant differences between downwind and upwind bacteria and fungi concentrations. Downwind bacteria and fungi concentrations responded differently to the influencing factors. Wind

speed and direction, and emission rate were two important predictors for both bacteria and fungi variations. Bacteria concentrations were more affected by RH while fungi concentrations were more affected by T.

Bacteria particle size distributions at source were different among seasons. Under higher temperature and lower RH conditions, more bacteria fell into small size ranges. No obvious seasonal or temporal variation of fungi size distribution was observed in the animal house. The portion of respirable bacteria ranged from 60% to 80%, and that of respirable fungi ranged from 60% to 70% in the poultry house in three seasons. Bioaerosol size distributions at ambient were different among stations in winter but were not in spring and summer. There were significant differences among fungi size distributions among ambient stations in three seasons. The percentages of respirable bacteria at ambient ranged from 50% to 80%, and that of fungi ranged from 60% to 90% in different seasons. These results indicated that exposures to bioaerosols emitted from the poultry house may bring higher risk of adverse health effects to people working on or living near the animal farm.

## **FUTURE WORK**

Due to limited resources, this project only collected non-selective culturable bacteria and fungi samples for three seasons. To better investigate the variations of bioaerosols on an animal farm, there are many further studies that can be conducted:

1. Study different bioaerosol species: detecting different species of bioaerosols on animal farm and in the vicinity using selective agars; employing non-culture based methods to detect non-culturable bioaerosol species, which can provide a more comprehensive understanding of AFO bioaerosols.
2. Study mechanisms of meteorological impacts on bioaerosol viabilities: different species of bioaerosols acted differently to environmental stresses. It is important to investigate how the bioaerosol viabilities are impacted by influencing factors (i.e. T, RH, solar radiation).

3. Improve statistical models: since the mechanisms of meteorological impacts on bioaerosol viabilities are unclear, it is difficult to determine the relationship between bioaerosol concentrations with influencing factors. Also, some important factors (e.g. gas pollutants) were not measured and included in the models in this project.

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# APPENDIX

## SAS CODES

### *SOURCE BIOAEROSOL VARIATION*

```
proc print data=tempbac;
run;
proc reg data=tempbac;
model conc=t rh q;
plot residual.*predicted. residual.*t residual.*rh residual.*q;
run;
data tempbac; set tempbac;
logconc=log(conc) t2=t*t;
run;
proc reg data=tempbac;
model logconc=t--q t2/selection=rsquare adjrsq cp;
run;
```

```
proc print data=tempfungi;
run;
proc reg data=tempfungi;
model conc=t rh q;
plot residual.*predicted. residual.*t residual.*rh residual.*q;
run;
data tempfungi; set tempfungi;
logconc=log(conc) t2=t*t rh2=rh*rh;
run;
proc reg data=tempfungi;
model logconc= t--q t2 rh2/selection=rsquare adjrsq cp;
run;
```

### *AMBIENT BIOAEROSOL VARIATIONS*

```
/*proc corr data=downwindbac;
title'correlation coefficient of downwind bac';
var conc t rh wind solar emission;
run;
/*
data downwindbac; set downwindbac;
conc=log(conc);
emission=log(emission);
emission2=emission*emission;
wind2=wind*wind;
proc reg data=downwindbac;
var emission;
model conc=t wind wind2;
plot (residual.)*emission;
run;
endsas;
symbol value=dot i=rq;
proc gplot;
plot conc* emission;
```

```

run;
*/
proc reg data=downwindbac;
title 'all subsets regression';
model conc=t--emission wind2 emission2/selection=rsquare sse adjrsq cp;
run;
proc reg data=downwindbac;
title 'full regression model';
*model conc=t rh wind wind2 solar emission emission2;
model conc=t wind wind2 solar emission emission2;
*plot residual.*predicted. residual.*t residual.*rh residual.*wind
residual.*solar residual.*emission;
run;

/*proc corr data=downwindfungi;
var conc t rh wind solar emission;
run;
*/
data downwindfungi; set downwindfungi;
conc=log(conc);
emission=log(emission);
emission2=emission*emission;
wind2=wind*wind;
proc reg data=downwindfungi;
title 'fungi all subsets regression';
model conc=t--emission emission2 wind2/selection=rsquare sse adjrsq cp;
run;
proc reg data=downwindfungi;
title 'fungi full regression model';
*model conc=t rh wind solar emission;
model conc=t rh wind emission ;
*plot residual.*predicted. residual.*t residual.*rh residual.*wind
residual.*solar residual.*emission;
run;

```

### ***BIOAEROSOL SIZE DISTRIBUTION AT SOURCE***

```

/*proc glm data=respirablest1;
class season time agar;
model respirable=season*time*agar;
means season*time*agar;
run;*/
/*proc glm data=respirablest1;
title'compare psd of source bac in different seasons';
where agar='TB';
class season;
model respirable=season;
means season/tukey lines;
run;*/
/*
proc glm data=respirablest1;
title'compare psd of source bac in different seasons';
where agar='TB';

```

```

class time;
model respirable=time;
means time/tukey lines;
run;
*/
/*proc glm data=respirablest1;
title'compare psd of source fungi in different seasons';
where agar='TF';
class season;
model respirable=season;
means season/tukey lines;
run;
proc glm data=respirablest1;
title'compare psd of source fungi in time of day';
where agar='TF';
class time;
model respirable=time;
means time/tukey lines;
run;*/

proc glm data=respirablest1;
title'compare psd of bac and fungi at source';
class agar;
model respirable=agar;
means agar/tukey lines;
run;

```

### ***BIOAEROSOL SIZE DISTRIBUTION AT AMBIENT LOCATIONS***

```

/*proc print data=psdambient;
run;
proc glm data=psdambient;
title 'compare psd at each station';
class season time location station agar;
model respirable=season*time*station*agar;
means season*time*station*agar;
run;
*/
proc glm data=psdambient;
title'compare psd of bac at ST2';
where agar='TB'and station='ST2';
class season;
model respirable=season;
means season/tukey lines;
run;
proc glm data=psdambient;
title'compare psd of bac at ST2';
where agar='TB'and station='ST2';
class time;
model respirable=time;
means time/tukey lines;
run;
proc glm data=psdambient;

```

```

title 'compare psd of fungi at ST2';
where agar= 'TF' and station='ST2';
class season;
model respirable=season;
means season/tukey lines;
run;
proc glm data=psdambient;
title 'compare psd of fungi at ST2';
where agar= 'TF' and station='ST2';
class time;
model respirable=time;
means time/tukey lines;
run;

```

### SAS OUTPUT FIGURES

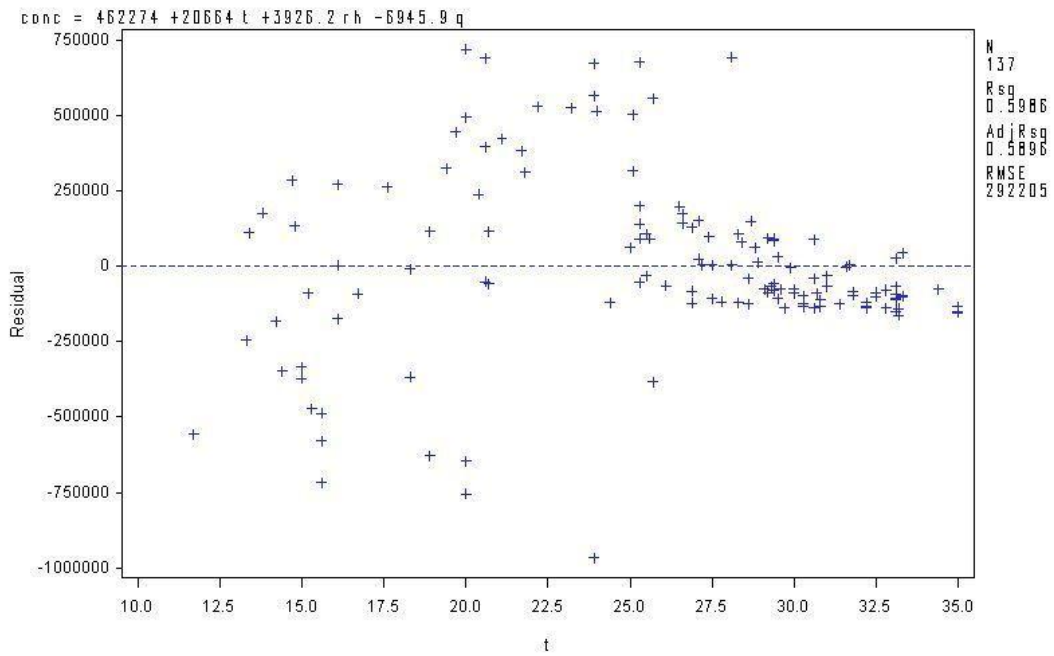


Figure A1. Residuals vs. T plots of bacteria concentrations at source

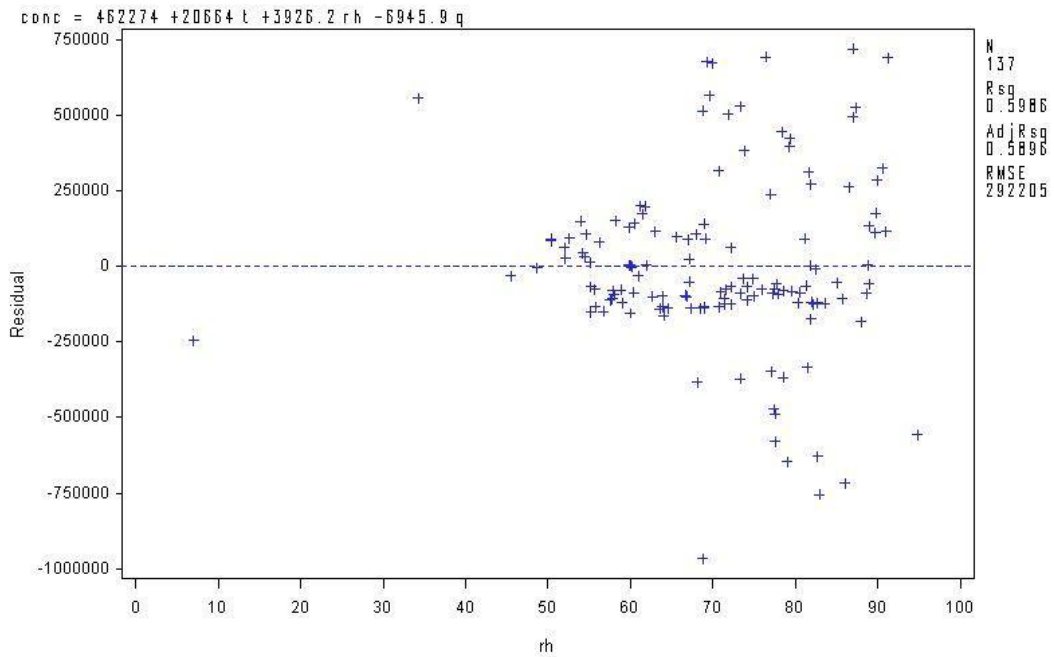


Figure A2. Residuals vs. RH plots of bacteria concentrations at source

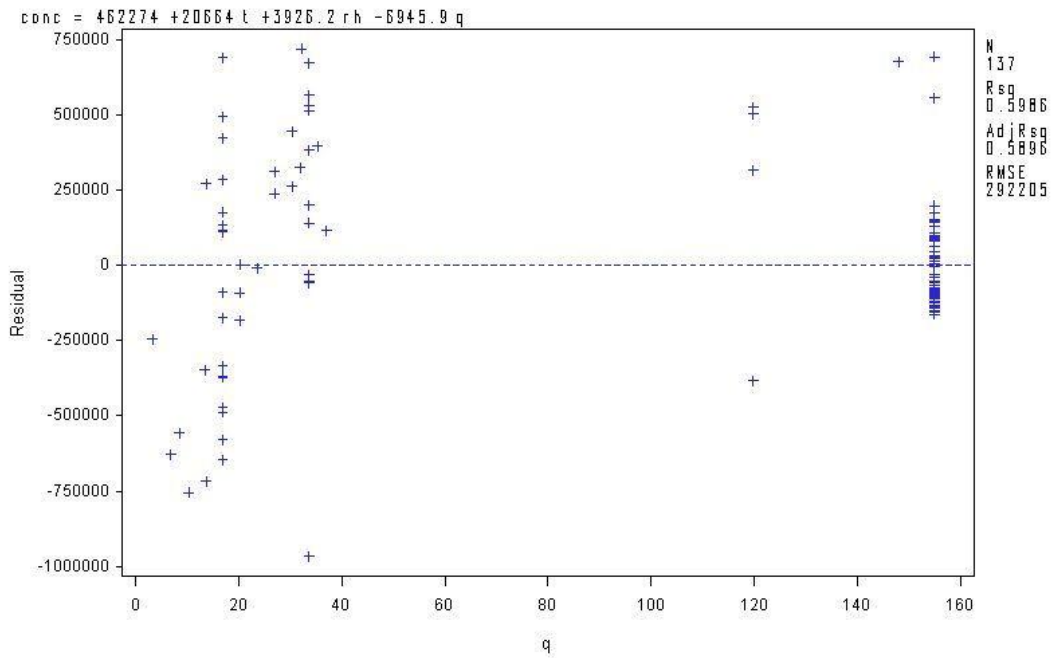


Figure A3. Residuals vs. Q plots of bacteria concentrations at source

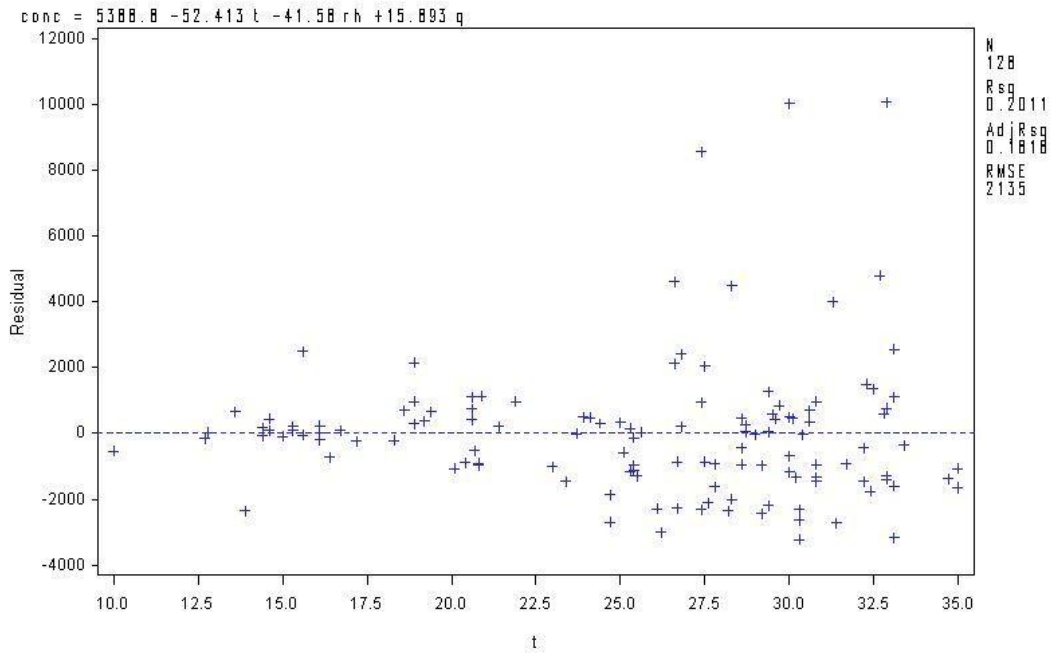


Figure A4. Residuals vs. T plots of fungi concentrations at source

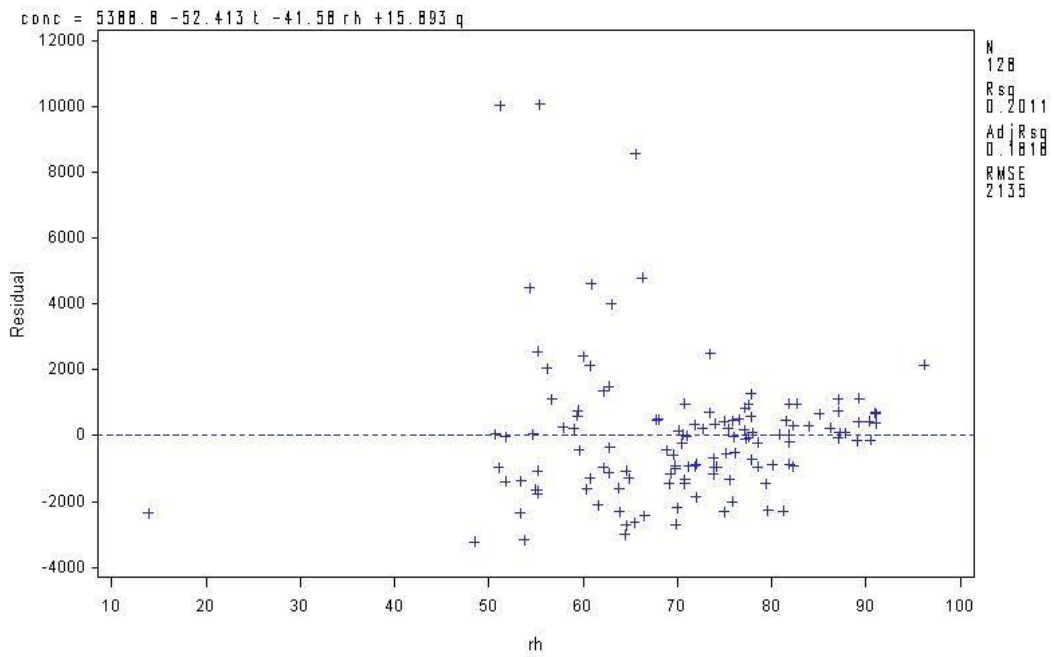


Figure A5. Residuals vs. RH plots of fungi concentrations at source

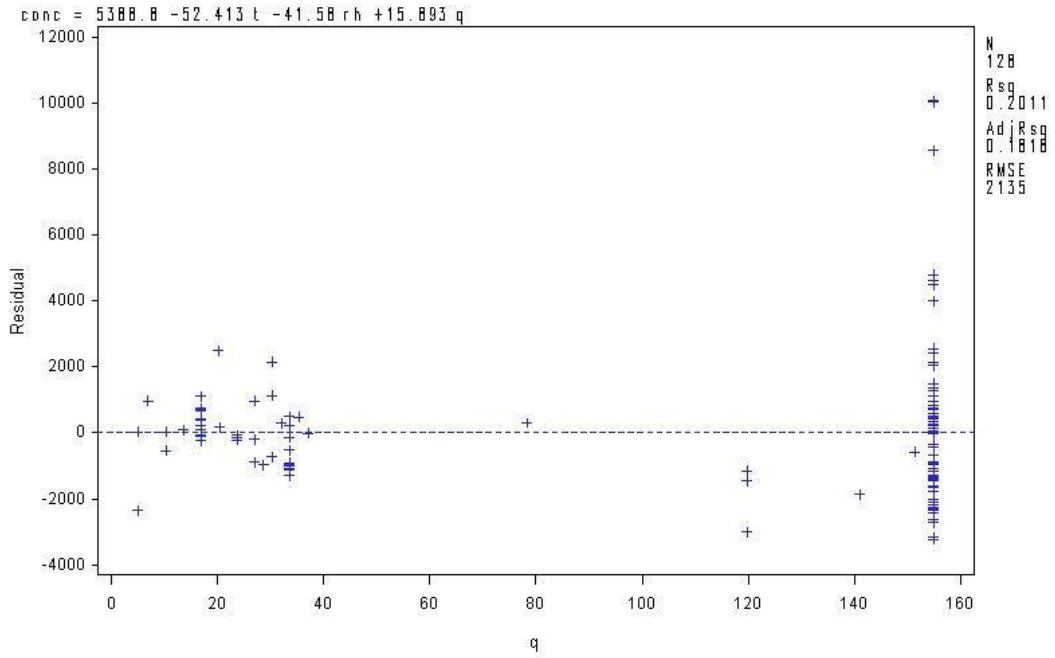


Figure A6. Residuals vs. Q plots of fungi concentrations at source

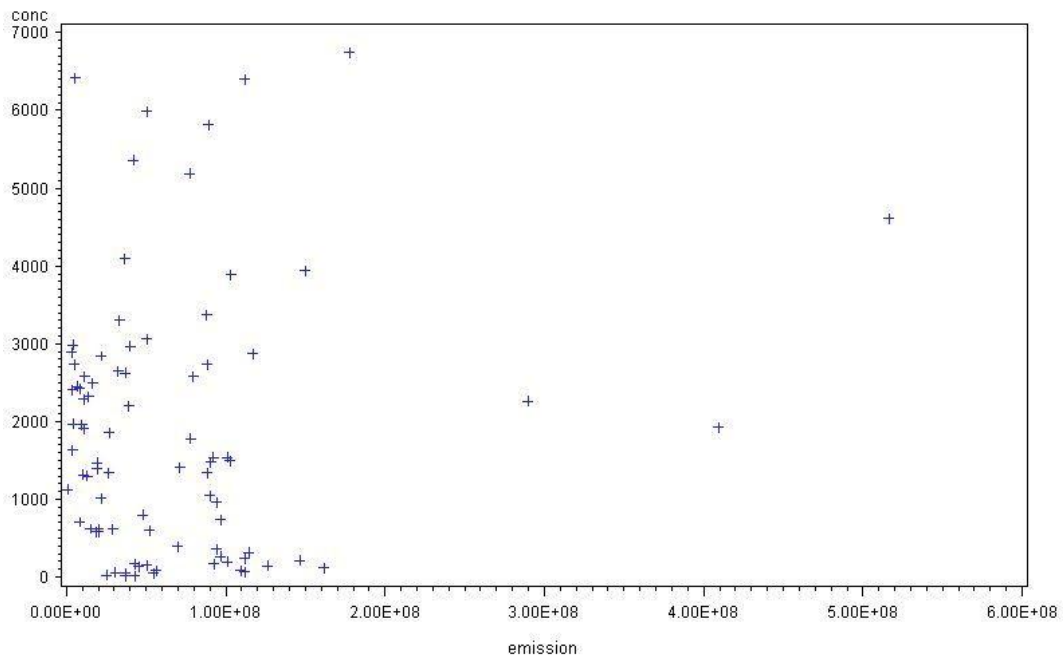


Figure A7. Bacteria concentration vs. emission plots at ambient

## SAS OUTPUT TABLES

Table A1. R-square and CP selections of source bacteria model

Number in Model	R-Square	Adjusted R-Square	C(p)	Variables in Model
1	<b>0.5569</b>	0.5536	9.0235	q
1	<b>0.4631</b>	0.4591	39.0810	t2
1	<b>0.4505</b>	0.4464	43.1118	t
1	<b>0.1236</b>	0.1171	147.8998	rh
2	<b>0.5605</b>	0.5540	9.8583	q t2
2	<b>0.5573</b>	0.5507	10.8952	rh q
2	<b>0.5572</b>	0.5506	10.9055	t q
2	<b>0.4646</b>	0.4566	40.5955	t t2
2	<b>0.4632</b>	0.4552	41.0339	rh t2
2	<b>0.4505</b>	0.4423	45.1100	t rh
3	<b>0.5881</b>	0.5788	3.0313	t q t2
3	<b>0.5605</b>	0.5506	11.8583	rh q t2
3	<b>0.5575</b>	0.5475	12.8341	t rh q
3	<b>0.4648</b>	0.4528	42.5233	t rh t2
4	<b>0.5881</b>	0.5757	5.0000	t rh q t2

Table A2. R-square and CP selections of source fungi model

Number in Model	R-Square	Adjusted R-Square	C(p)	Variables in Model
1	<b>0.2117</b>	0.2054	1.7965	q

Number in Model	R-Square	Adjusted R-Square	C(p)	Variables in Model
1	<b>0.1561</b>	0.1494	10.6630	t2
1	<b>0.1492</b>	0.1424	11.7736	t
1	<b>0.1069</b>	0.0999	18.5096	rh2
1	<b>0.0972</b>	0.0900	20.0686	rh
2	<b>0.2223</b>	0.2098	2.1072	rh q
2	<b>0.2201</b>	0.2076	2.4601	q rh2
2	<b>0.2138</b>	0.2012	3.4537	t q
2	<b>0.2120</b>	0.1994	3.7476	q t2
2	<b>0.1700</b>	0.1567	10.4524	rh t2
2	<b>0.1690</b>	0.1557	10.6063	t2 rh2
2	<b>0.1660</b>	0.1526	11.0881	t rh
2	<b>0.1648</b>	0.1515	11.2711	t rh2
2	<b>0.1584</b>	0.1449	12.3018	t t2
2	<b>0.1105</b>	0.0962	19.9497	rh rh2
3	<b>0.2274</b>	0.2088	3.2802	t rh q
3	<b>0.2253</b>	0.2066	3.6157	t q rh2
3	<b>0.2251</b>	0.2064	3.6544	t q t2
3	<b>0.2250</b>	0.2062	3.6739	rh q rh2
3	<b>0.2245</b>	0.2058	3.7466	rh q t2
3	<b>0.2223</b>	0.2034	4.1089	q t2 rh2
3	<b>0.1710</b>	0.1510	12.2817	t rh t2

Number in Model	R-Square	Adjusted R-Square	C(p)	Variables in Model
3	<b>0.1704</b>	0.1503	12.3858	t t2 rh2
3	<b>0.1701</b>	0.1500	12.4357	rh t2 rh2
3	<b>0.1661</b>	0.1459	13.0666	t rh rh2
4	<b>0.2345</b>	0.2096	4.1565	t rh q t2
4	<b>0.2331</b>	0.2081	4.3856	t q t2 rh2
4	<b>0.2290</b>	0.2039	5.0372	t rh q rh2
4	<b>0.2266</b>	0.2014	5.4230	rh q t2 rh2
4	<b>0.1711</b>	0.1441	14.2783	t rh t2 rh2
5	<b>0.2355</b>	0.2041	6.0000	t rh q t2 rh2

Table A3 Pearson correlation coefficients between variables in ambient bacteria model

Pearson Correlation Coefficients, N = 86 Prob >  r  under H0: Rho=0						
	conc	t	rh	wind	solar	emission
conc	1.00000	0.08655	0.09939	0.30285	-0.03492	0.12974
		0.4281	0.3626	0.0046	0.7495	0.2338
t	0.08655	1.00000	0.32622	-0.13298	0.61274	0.58821
	0.4281		0.0022	0.2223	<.0001	<.0001
rh	0.09939	0.32622	1.00000	-0.32602	-0.03950	0.25698
	0.3626	0.0022		0.0022	0.7180	0.0169

Pearson Correlation Coefficients, N = 86 Prob >  r  under H0: Rho=0						
	conc	t	rh	wind	solar	emission
wind	0.30285 0.0046	-0.13298 0.2223	-0.32602 0.0022	1.00000	0.04515 0.6798	-0.19206 0.0765
solar	-0.03492 0.7495	0.61274 <.0001	-0.03950 0.7180	0.04515 0.6798	1.00000	0.39559 0.0002
emission	0.12974 0.2338	0.58821 <.0001	0.25698 0.0169	-0.19206 0.0765	0.39559 0.0002	1.00000

Table A4. Pearson correlation coefficients between variables in ambient fungi model

Pearson Correlation Coefficients, N = 86 Prob >  r  under H0: Rho=0						
	conc	t	rh	wind	solar	emission
conc	1.00000	0.70000 <.0001	0.26000 0.0156	-0.24038 0.0258	0.42562 <.0001	0.56234 <.0001
t	0.70000 <.0001	1.00000	0.29295 0.0062	-0.00809 0.9411	0.68485 <.0001	0.51839 <.0001
rh	0.26000 0.0156	0.29295 0.0062	1.00000	-0.21317 0.0488	-0.07116 0.5150	-0.01309 0.9048

<b>Pearson Correlation Coefficients, N = 86</b> <b>Prob &gt;  r  under H0: Rho=0</b>						
	<b>conc</b>	<b>t</b>	<b>rh</b>	<b>wind</b>	<b>solar</b>	<b>emission</b>
<b>wind</b>	-0.24038	-0.00809	-0.21317	1.00000	0.06984	-0.06457
	0.0258	0.9411	0.0488		0.5229	0.5547
<b>solar</b>	0.42562	0.68485	-0.07116	0.06984	1.00000	0.41601
	<.0001	<.0001	0.5150	0.5229		<.0001
<b>emission</b>	0.56234	0.51839	-0.01309	-0.06457	0.41601	1.00000
	<.0001	<.0001	0.9048	0.5547	<.0001	

Table A5. R-square and CP selections of ambient bacteria model

<b>Number in Model</b>	<b>R-Square</b>	<b>Adjusted R-Square</b>	<b>C(p)</b>	<b>SSE</b>	<b>Variables in Model</b>
1	<b>0.2547</b>	0.2459	40.7661	133.15446	wind
1	<b>0.1368</b>	0.1265	60.1939	154.22616	wind2
1	<b>0.0237</b>	0.0120	78.8332	174.44268	emission
1	<b>0.0209</b>	0.0093	79.2856	174.93337	emission2
1	<b>0.0126</b>	0.0009	80.6530	176.41649	rh
1	<b>0.0007</b>	-.0112	82.6098	178.53886	t
1	<b>0.0005</b>	-.0114	82.6537	178.58651	solar
2	<b>0.3988</b>	0.3843	19.0421	107.42293	wind wind2
2	<b>0.2638</b>	0.2460	41.2771	131.53939	t wind

<b>Number in Model</b>	<b>R-Square</b>	<b>Adjusted R-Square</b>	<b>C(p)</b>	<b>SSE</b>	<b>Variables in Model</b>
2	0.2593	0.2415	42.0149	132.33963	wind emission
2	0.2578	0.2399	42.2632	132.60895	rh wind
2	0.2575	0.2397	42.3045	132.65373	wind emission2
2	0.2567	0.2388	42.4402	132.80099	wind solar
2	0.1471	0.1266	60.4959	152.38450	emission wind2
2	0.1445	0.1239	60.9228	152.84750	wind2 emission2
2	0.1426	0.1220	61.2371	153.18840	t wind2
2	0.1371	0.1163	62.1434	154.17142	rh wind2
2	0.1370	0.1162	62.1611	154.19063	solar wind2
2	0.0714	0.0490	72.9728	165.91716	t emission
2	0.0670	0.0445	73.6946	166.70003	t emission2
2	0.0611	0.0385	74.6616	167.74893	emission emission2
2	0.0278	0.0043	80.1594	173.71193	solar emission
2	0.0269	0.0035	80.2967	173.86083	rh emission
2	0.0245	0.0010	80.6875	174.28474	rh emission2
2	0.0245	0.0010	80.6985	174.29661	solar emission2
2	0.0172	-.0065	81.9037	175.60380	t rh
2	0.0133	-.0105	82.5407	176.29475	rh solar
2	0.0031	-.0209	84.2237	178.12008	t solar
3	0.4157	0.3944	18.2469	104.39117	rh wind wind2

<b>Number in Model</b>	<b>R-Square</b>	<b>Adjusted R-Square</b>	<b>C(p)</b>	<b>SSE</b>	<b>Variables in Model</b>
3	0.4154	0.3940	18.2969	104.44546	wind solar wind2
3	0.4069	0.3852	19.7082	105.97611	t wind wind2
3	0.4004	0.3785	20.7745	107.13270	wind emission wind2
3	0.3996	0.3776	20.9085	107.27803	wind wind2 emission2
3	0.3581	0.3346	27.7390	114.68647	wind emission emission2
3	0.3078	0.2825	36.0251	123.67376	t wind emission
3	0.3012	0.2756	37.1193	124.86062	t wind emission2
3	0.2815	0.2552	40.3596	128.37506	t wind solar
3	0.2665	0.2397	42.8275	131.05176	rh wind emission
3	0.2646	0.2377	43.1493	131.40079	t rh wind
3	0.2639	0.2370	43.2612	131.52217	rh wind emission2
3	0.2597	0.2326	43.9580	132.27793	rh wind solar
3	0.2594	0.2323	43.9942	132.31725	wind solar emission
3	0.2579	0.2308	44.2407	132.58454	wind solar emission2
3	0.2360	0.2081	47.8525	136.50200	emission wind2 emission2
3	0.2003	0.1710	53.7425	142.89042	t emission wind2
3	0.1933	0.1638	54.8882	144.13305	t wind2 emission2
3	0.1489	0.1178	62.1944	152.05751	solar emission wind2
3	0.1486	0.1175	62.2500	152.11781	t solar wind2
3	0.1476	0.1164	62.4214	152.30373	rh emission wind2

<b>Number in Model</b>	<b>R-Square</b>	<b>Adjusted R-Square</b>	<b>C(p)</b>	<b>SSE</b>	<b>Variables in Model</b>
3	0.1458	0.1145	62.7145	152.62160	solar wind2 emission2
3	0.1448	0.1135	62.8797	152.80082	rh wind2 emission2
3	0.1445	0.1132	62.9351	152.86088	t rh wind2
3	0.1373	0.1058	64.1067	154.13161	rh solar wind2
3	0.0850	0.0515	72.7312	163.48596	t emission emission2
3	0.0762	0.0424	74.1854	165.06322	t rh emission
3	0.0722	0.0383	74.8343	165.76700	t solar emission
3	0.0718	0.0378	74.9044	165.84299	t rh emission2
3	0.0681	0.0341	75.5050	166.49439	rh emission emission2
3	0.0679	0.0338	75.5452	166.53806	t solar emission2
3	0.0625	0.0282	76.4357	167.50391	solar emission emission2
3	0.0294	-0.0062	81.8948	173.42494	rh solar emission
3	0.0264	-0.0092	82.3745	173.94523	rh solar emission2
3	0.0261	-0.0095	82.4336	174.00934	t rh solar
4	0.4669	0.4405	11.8234	95.25495	wind emission wind2 emission2
4	0.4653	0.4389	12.0750	95.52783	t wind solar wind2
4	0.4338	0.4058	17.2695	101.16185	t wind emission wind2
4	0.4329	0.4049	17.4143	101.31888	rh wind solar wind2
4	0.4296	0.4014	17.9682	101.91974	t wind wind2 emission2
4	0.4241	0.3957	18.8618	102.88892	rh wind emission wind2

<b>Number in Model</b>	<b>R-Square</b>	<b>Adjusted R-Square</b>	<b>C(p)</b>	<b>SSE</b>	<b>Variables in Model</b>
4	<b>0.4221</b>	0.3936	19.1986	103.25416	rh wind wind2 emission2
4	<b>0.4185</b>	0.3897	19.7989	103.90526	t rh wind wind2
4	<b>0.4180</b>	0.3893	19.8654	103.97738	wind solar wind2 emission2
4	<b>0.4167</b>	0.3879	20.0823	104.21266	wind solar emission wind2
4	<b>0.3684</b>	0.3372	28.0451	112.84929	t wind emission emission2
4	<b>0.3630</b>	0.3316	28.9276	113.80644	wind solar emission emission2
4	<b>0.3620</b>	0.3305	29.1043	113.99816	rh wind emission emission2
4	<b>0.3208</b>	0.2873	35.8837	121.35113	t wind solar emission
4	<b>0.3145</b>	0.2806	36.9257	122.48133	t wind solar emission2
4	<b>0.3131</b>	0.2792	37.1551	122.73018	t rh wind emission
4	<b>0.3060</b>	0.2718	38.3155	123.98873	t rh wind emission2
4	<b>0.2816</b>	0.2461	42.3395	128.35326	t rh wind solar
4	<b>0.2666</b>	0.2304	44.8055	131.02793	rh wind solar emission
4	<b>0.2639</b>	0.2275	45.2606	131.52157	rh wind solar emission2
4	<b>0.2507</b>	0.2137	47.4396	133.88492	t emission wind2 emission2
4	<b>0.2360</b>	0.1983	49.8466	136.49556	solar emission wind2 emission2
4	<b>0.2360</b>	0.1983	49.8514	136.50083	rh emission wind2

<b>Number in Model</b>	<b>R-Square</b>	<b>Adjusted R-Square</b>	<b>C(p)</b>	<b>SSE</b>	<b>Variables in Model</b>
					emission2
<b>4</b>	<b>0.2037</b>	0.1644	55.1769	142.27699	t solar emission wind2
<b>4</b>	<b>0.2004</b>	0.1609	55.7257	142.87220	t rh emission wind2
<b>4</b>	<b>0.1969</b>	0.1572	56.2975	143.49233	t solar wind2 emission2
<b>4</b>	<b>0.1934</b>	0.1535	56.8787	144.12274	t rh wind2 emission2
<b>4</b>	<b>0.1536</b>	0.1118	63.4313	151.22978	t rh solar wind2
<b>4</b>	<b>0.1502</b>	0.1082	63.9922	151.83819	rh solar emission wind2
<b>4</b>	<b>0.1466</b>	0.1044	64.5869	152.48323	rh solar wind2 emission2
<b>4</b>	<b>0.0921</b>	0.0472	73.5667	162.22285	t rh emission emission2
<b>4</b>	<b>0.0857</b>	0.0405	74.6212	163.36665	t solar emission emission2
<b>4</b>	<b>0.0794</b>	0.0339	75.6591	164.49230	t rh solar emission
<b>4</b>	<b>0.0751</b>	0.0295	76.3534	165.24541	t rh solar emission2
<b>4</b>	<b>0.0683</b>	0.0223	77.4852	166.47299	rh solar emission emission2
<b>5</b>	<b>0.4948</b>	0.4632	9.2283	90.27099	wind solar emission wind2 emission2
<b>5</b>	<b>0.4832</b>	0.4509	11.1291	92.33264	rh wind emission wind2 emission2
<b>5</b>	<b>0.4831</b>	0.4508	11.1413	92.34588	t wind solar emission wind2
<b>5</b>	<b>0.4798</b>	0.4473	11.6940	92.94533	t wind solar wind2 emission2

<b>Number in Model</b>	<b>R-Square</b>	<b>Adjusted R-Square</b>	<b>C(p)</b>	<b>SSE</b>	<b>Variables in Model</b>
5	0.4746	0.4417	12.5554	93.87960	t wind emission wind2 emission2
5	0.4678	0.4345	13.6776	95.09680	t rh wind solar wind2
5	0.4536	0.4194	16.0101	97.62663	t rh wind emission wind2
5	0.4489	0.4144	16.7865	98.46877	t rh wind wind2 emission2
5	0.4333	0.3979	19.3513	101.25059	rh wind solar emission wind2
5	0.4329	0.3975	19.4117	101.31607	rh wind solar wind2 emission2
5	0.3823	0.3437	27.7471	110.35682	t wind solar emission emission2
5	0.3719	0.3326	29.4686	112.22395	t rh wind emission emission2
5	0.3650	0.3253	30.6078	113.45957	rh wind solar emission emission2
5	0.3223	0.2799	37.6381	121.08479	t rh wind solar emission
5	0.3157	0.2729	38.7227	122.26118	t rh wind solar emission2
5	0.2540	0.2073	48.8940	133.29315	t solar emission wind2 emission2
5	0.2507	0.2038	49.4359	133.88091	t rh emission wind2 emission2
5	0.2361	0.1883	51.8430	136.49175	rh solar emission wind2 emission2
5	0.2038	0.1540	57.1592	142.25780	t rh solar emission wind2

<b>Number in Model</b>	<b>R-Square</b>	<b>Adjusted R-Square</b>	<b>C(p)</b>	<b>SSE</b>	<b>Variables in Model</b>
5	0.1971	0.1469	58.2673	143.45967	t rh solar wind2 emission2
5	0.0955	0.0390	75.0002	161.60842	t rh solar emission emission2
6	0.5221	0.4858	6.7256	85.38725	t wind solar emission wind2 emission2
6	0.5029	0.4651	9.8939	88.82362	rh wind solar emission wind2 emission2
6	0.4901	0.4514	11.9949	91.10241	t rh wind emission wind2 emission2
6	0.4898	0.4511	12.0423	91.15385	t rh wind solar emission wind2
6	0.4861	0.4470	12.6599	91.82372	t rh wind solar wind2 emission2
6	0.3829	0.3360	29.6612	110.26365	t rh wind solar emission emission2
6	0.2546	0.1980	50.7844	133.17428	t rh solar emission wind2 emission2
7	0.5265	0.4840	8.0000	84.60028	t rh wind solar emission wind2 emission2

Table A6. R-square and CP selections of source fungi model

<b>Number in Model</b>	<b>R-Square</b>	<b>Adjusted R-Square</b>	<b>C(p)</b>	<b>SSE</b>	<b>Variables in Model</b>
1	0.6348	0.6304	34.5191	50.68387	t

<b>Number in Model</b>	<b>R-Square</b>	<b>Adjusted R-Square</b>	<b>C(p)</b>	<b>SSE</b>	<b>Variables in Model</b>
1	<b>0.5982</b>	0.5934	46.1981	55.76402	emission
1	<b>0.2469</b>	0.2379	158.2914	104.52276	rh
1	<b>0.2000</b>	0.1904	173.2500	111.02949	solar
1	<b>0.0431</b>	0.0317	223.3088	132.80423	wind
2	<b>0.7107</b>	0.7037	12.2981	40.14814	t rh
2	<b>0.6953</b>	0.6879	17.2208	42.28945	rh emission
2	<b>0.6752</b>	0.6674	23.6156	45.07107	t wind
2	<b>0.6531</b>	0.6447	30.6934	48.14980	t solar
2	<b>0.6524</b>	0.6440	30.8969	48.23831	t emission
2	<b>0.6303</b>	0.6214	37.9659	51.31319	wind emission
2	<b>0.6012</b>	0.5916	47.2469	55.35029	solar emission
2	<b>0.4809</b>	0.4684	85.6248	72.04403	rh solar
2	<b>0.2577</b>	0.2398	156.8394	103.02118	rh wind
2	<b>0.2573</b>	0.2394	156.9711	103.07849	wind solar
3	<b>0.7314</b>	0.7216	7.6918	37.27449	t rh wind
3	<b>0.7302</b>	0.7203	8.0925	37.44879	t rh emission
3	<b>0.7116</b>	0.7011	14.0105	40.02305	t rh solar
3	<b>0.7087</b>	0.6981	14.9276	40.42196	rh wind emission
3	<b>0.6972</b>	0.6861	18.6143	42.02559	rh solar emission
3	<b>0.6895</b>	0.6782	21.0598	43.08937	t wind emission

<b>Number in Model</b>	<b>R-Square</b>	<b>Adjusted R-Square</b>	<b>C(p)</b>	<b>SSE</b>	<b>Variables in Model</b>
3	<b>0.6884</b>	0.6771	21.4005	43.23758	t wind solar
3	<b>0.6729</b>	0.6609	26.3758	45.40173	t solar emission
3	<b>0.6314</b>	0.6179	39.6128	51.15961	wind solar emission
3	<b>0.4981</b>	0.4798	82.1192	69.64920	rh wind solar
4	<b>0.7481</b>	0.7356	4.3732	34.96099	t rh wind emission
4	<b>0.7322</b>	0.7189	9.4551	37.17155	t rh wind solar
4	<b>0.7316</b>	0.7183	9.6481	37.25550	t rh solar emission
4	<b>0.7113</b>	0.6970	16.1103	40.06644	rh wind solar emission
4	<b>0.7046</b>	0.6900	18.2381	40.99202	t wind solar emission
5	<b>0.7493</b>	0.7336	6.0000	34.79867	t rh wind solar emission