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# Fungal Bioaerosol Exposure and its Effects on the Health of Mushroom and Vegetable Farm Workers in Taiwan

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## ABSTRACT

Workers from specific occupational settings may be exposed to high fungal bioaerosol concentrations, causing detrimental health effects. Therefore, we conducted a study to evaluate the characteristics and health effects of fungal bioaerosols present on agricultural farms. By using IOM inhalable dust samplers, personal and area samples of airborne fungi were collected from five agricultural farms—two mushroom and three vegetable farms. A standardized questionnaire and spirometry were used to evaluate workers' health. The Kruskal–Wallis test was used to examine the distributions of fungal and environmental factors among the different farms, and regression analyses were performed to evaluate the effects of personal bioaerosol exposure on workers' health. In the personal samples, the geometric mean concentrations ranged from $4.3 \times 10^3$ to $3.0 \times 10^4$ CFU $m^{-3}$ for total culturable fungi and from $4.2 \times 10^3$ to $1.2 \times 10^5$ spores $m^{-3}$ for total fungal spores. The total fungal spore concentrations differed significantly among the personal samples ($p = 0.026$), but not among the area samples, from the five farms. The culturable fungal concentrations among the five farms did not differ significantly in the personal or area samples. Decreased lung functions of the workers were significantly associated with the concentrations of total fungi and several fungal taxa such as Ascospores, *Fusarium*, and *Periconia*. This study demonstrated that exposure to high fungal bioaerosol concentrations reduced the lung functions of the mushroom and vegetable farm workers. Superior ventilation and appropriate personal protection equipment are required to reduce occupational biohazards.

***Keywords:*** Bioaerosols; Personal exposure assessment; Occupational biohazard; Lung function.

## INTRODUCTION

Airborne particles that are living and those originating from living organisms are collectively called bioaerosols, including pollen, fungal spores, bacteria, viruses, animal dander, and mite-associated fragments (ACGIH, 1999; Krop *et al*., 2014; Chow *et al*., 2015). Fungi are almost omnipresent on the Earth. The essential environmental factors affecting fungal growth are water, temperature, light, and nutrients (ACGIH, 1999; Kavanagh, 2005). Fungal spores are often small (mostly 2–10 μm) and lightweight, and they can be easily suspended and carried through the air; because of their small size, spores can readily reach the lower respiratory airways (Burge and Rogers, 2000).

Agricultural products are crucial for our daily lives; however, agricultural workers are generally unaware of the biohazards in their work environments. Agricultural farms or greenhouses containing abundant organic substrates provide favourable environments for the growth of various fungi and bacteria (Tsapko *et al*., 2011). Agricultural activities, such as planting, threshing, harvesting, and watering, often cause crop and soil disturbances, resulting in high bioaerosol exposure (Lee *et al*., 2006). A study conducted in Taiwan

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indicated that workers were exposed to high concentrations of fungal contaminants on agricultural farms (Lee and Liao, 2014). Exposure to high bioaerosol concentrations has also been reported among Danish farmers working in greenhouses and on open fields (Hansen *et al*., 2012).

Occupational exposure to bioaerosols can cause various adverse health effects, such as infectious diseases, acute toxic effects, and allergies (Douwes *et al*., 2003; Bunger *et al*., 2007). Workers from specific occupational settings, such as agricultural farms and composting facilities, may be exposed to high fungal bioaerosol concentrations, resulting in increased respiratory symptoms and decreased lung functions (Bunger *et al*., 2007; Adhikari *et al*., 2011). Workers involved in the cultivation and marketing of mushrooms (such as shiitake) frequently exhibit allergic contact dermatitis, asthma, allergic rhinitis, conjunctivitis, and hypersensitivity pneumonitis (Aalto-Korte *et al*., 2005). In several case studies, hypersensitivity pneumonitis have been reported among mushroom farm workers resulting from occupational exposure to fungal spores (e.g., *Aspergillus glaucus* and *Penicillium citrinum*) (Yoshikawa *et al*., 2007; Ampere *et al*., 2012). A study conducted in the United States revealed that high fungal concentrations in greenhouses increased the prevalence of respiratory symptoms among workers compared with control subjects (Adhikari *et al*., 2011). In a cohort study, Riu *et al*. (2008) demonstrated that among greenhouse flower and ornamental plant workers, rhinitis was significantly associated with sensitization to workplace allergens; rhinitis was also significantly associated with the number of hours worked in the greenhouse per day.

Various studies have evaluated bioaerosol exposure on agricultural farms (Chang *et al*., 2002; Lee *et al*., 2006; Matkovic *et al*., 2009; Madsen, 2011; Hansen *et al*., 2012; Lee and Liao, 2014; Madsen *et al*., 2014; Viegas *et al*., 2014; Chow *et al*., 2015); of these, only a few have been conducted in Taiwan (Chang *et al*., 2002; Lee and Liao, 2014). In addition, studies focusing on the health risks of occupational fungal exposure on farms are scant (Yoshikawa *et al*., 2007; Adhikari *et al*., 2011; Ampere *et al*., 2012). Therefore, we conducted this study for evaluating fungal bioaerosol exposure and its effects on the health of farm workers in Taiwan.

## METHODS

### *Environmental Sample Collection*

We investigated mushroom and vegetable farms, which are two of the nine largest agricultural industries in Taiwan. Two mushroom farms situated in Taichung City and three vegetable farms located in Taoyuan County were selected. All farm workers willing to participate in this study were recruited.

Environmental sampling was performed from September 2014 to January 2015. A cross-sectional investigation over 1–3 days was conducted on each farm during the fall of 2014. Additional sampling was performed in winter at one of the vegetable farms to evaluate potential seasonal variations. Fungal bioaerosols were collected using an IOM inhalable dust sampler with polycarbonate filters (pore size 0.8 μm), connected to personal pumps (Universal Sampler & AirChek XR5000, SKC Inc., Pennsylvania, USA), at a flow rate of 2 L $min^{-1}$ (Gorner *et al*., 2010; Wang *et al*., 2015). For personal sampling, the sampler was attached to the workers' clothing within the breathing zone, and sampling was performed for the entire work shift (approximately 8 h). For area sampling, the samplers were placed at a height near the workers' breathing zone; the samples were collected from the major working areas such as the harvesting, cultivation, and packaging areas as well as from the outdoor environments as background samples. Area sampling was performed for a complete work shift, corresponding to personal sampling.

During area sampling, temperature, relative humidity, $CO_2$ concentrations, particulate matter concentrations, and wind speed were measured every 30 min by using direct reading instruments (YESAIR 7 Channel Indoor Air Quality Monitor, Critical Environment Technologies Canada Inc., Delta, British Columbia, Canada; Met One Instruments GT 521, Met One Instruments, Inc., Grants Pass, OR, USA; VelociCalc Plus Air Velocity Meters 8836A, TSI Inc., Shoreview, MN, USA). Other environmental parameters, such as building characteristics, ventilation types, vegetable types, and pesticide use were also recorded.

Before field sampling, the metallic parts of the IOM samplers were autoclaved, whereas the other parts were sterilized using ethylene oxide. The flows of the samplers were calibrated before and after each sampling by using a primary standard calibrator (Bio Defender 510-H, Bios International Co., Butler, NJ, USA). Fungal bioaerosol concentrations were calculated using the average flow rate. After sampling, the filters were placed in pyrogen-free tubes, immediately shipped to the laboratory, and stored at 4°C. Laboratory and area blanks were evaluated for quality control. All instruments had been calibrated by the vendors before sampling.

### *Sample Analyses*

Sample analyses were performed according to a previously validated method (Wang *et al*., 2015). Filter samples were extracted within 1 week after collection by using 5 mL of extraction buffer (0.01% Tween 80 in pyrogen free water). Extraction was performed by shaking the tube in a vortex mixer for 2 min (Touch Mixer, Model VM-2000, Digisystem, Taiwan), followed by ultrasonic agitation for 15 min (Powersonic 410, Hwashin Technology, Korea). After extraction, the eluted samples were allocated for subsequent analyses.

For culturable fungi, 100-μL aliquots of the eluted samples with different dilutions (1:1, 1:10, and 1:100) were immediately cultivated on malt extract agar (MEA; Merck, Taiwan) supplemented with chloramphenicol (Sigma, USA) to inhibit bacterial growth. MEA plates were incubated at 25°C for 5 days before enumeration and identification. Appropriate dilutions of the eluted samples were selected for analyses. All fungal colonies were identified morphologically by using a stereo microscope and a light microscope at 800× magnification. Airborne culturable fungal concentrations were calculated as follows:

$$C_{culturable\ fungi} = CFU \times D \times (V_1/V_2)/Air\ volume\ sampled \quad (1)$$

where $C_{culturable\ fungi}$ denotes total culturable fungal concentration (in CFU $m^{-3}$), CFU denotes the number of colony forming units on the plate, D denotes the dilution fold of the eluted sample, $V_1$ denotes the amount of extraction buffer (5 mL), $V_2$ denotes the amount of eluted sample used for cultivation (0.1 mL), and Air volume sampled is given by flow rate × sampling time (min) × 0.001 ($m^3\ L^{-1}$).

For fungal spores, a 1-mL aliquot of the eluted samples was centrifuged at 8,000 rpm for 10 min, and 900 μL of the supernatant was discarded; the remaining 100-μL eluate was then vortexed for 2 min. Half of the eluate (50 μL) was spread on a glass slide, and allowed to dry completely on a slide warmer plate. The slide was stained using glycerin jelly, covered with a 25 × 25 mm cover glass, and sealed using transparent nail enamel. Fungal spores were counted and identified using a light microscope at 800× magnification (Orthoplan, Leitz, Wetzlar, Germany). We identified 24 fungal spore taxa according to the classification of the American Academy of Allergy, Asthma & Immunology (AAAAI) Aeroallergen Network (Muilenberg, 1999)—Ascospores, Basidiospores, *Alternaria*, *Arthrinium*, *Aspergillus*/*Penicillium*, *Botrytis*, *Cercospora*, *Cladosporium*, *Curvularia*, *Drechslera*/*Helminthosporium*, *Epicoccum*, *Fusarium*, *Nigrospora*, *Oidium*/*Erysiphe*, *Periconia*, *Peronospora*, *Pithomyces*, *Polythrincium*, Rusts, Smuts, *Stemphylium*, *Tetraploa*, *Torula*, and *Ulocladium*. Fungal spores not belonging to any of the aforementioned taxa were categorized as "other," and those that were unidentifiable because of coverage or damage were categorized as "unidentified." Fungal spore concentrations were calculated as follows:

$$C_{total\ fungal\ spores} = N \times (V_1/V_2) \times 2/Air\ volume\ sampled \quad (2)$$

where $C_{total\ fungal\ spores}$ denotes total fungal spore concentration (in spores $m^{-3}$), N denotes the spore count (spores), $V_1$ denotes the amount of extraction buffer (5 mL), $V_2$ denotes the eluted sample amount used for analysis (1 mL), 2 denotes 100 μL/50 μL, and Air volume sampled ($m^3$) is given by flow rate × sampling time (min) × 0.001 ($m^3\ L^{-1}$).

### *Health Evaluation*

Workers' health was evaluated using a structured questionnaire and lung function tests. We used the questionnaire to collect data on workers' demographics, job characteristics, perceived health, medical history, and personal protection equipment use. The questionnaire was validated by three experts, pretested by the workers, and approved by the Taipei Medical University-Joint Institutional Review Board. A questionnaire survey was conducted concurrently with environmental sampling.

Workers' pulmonary functions were measured before and after work on the study farms by using the Micro Lab ML3500 spirometer (Micro Medical Ltd., Kent, UK) (Reynolds *et al*., 2012). The measurements were performed by trained technicians, following the American Thoracic Society recommendation (Redlich *et al*., 2014). The lung function measurements included forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), $FEV_1$/FVC ratio (FEV1%), peak expiratory flow rate (PEF), forced expiratory flows at 25%, 50%, and 75% of expired volume ($FEF_{25}$, $FEF_{50}$, and $FEF_{75}$, respectively), and mid expiratory flow rate 25–75% ($FEF_{25–75\%}$). The study protocol was approved by the Taipei Medical University-Joint Institutional Review Board (TMU-JIRB No.: 201407037). Written informed consent was obtained from each participant.

### *Data Analysis*

We used the SAS statistical package (Version 9.3; SAS Institute, Cary, NC, USA) for statistical analyses. The Kruskal–Wallis test was used to compare the distributions of fungal concentrations and environmental factors among the agricultural farms. The Mann–Whitney *U* test was used to compare the fungal concentrations and environmental factors between the two farm types (mushroom vs. vegetable). The Wilcoxon signed-rank test was used to compare the distributions of fungal concentrations between area and personal samplings as well as between seasons. The Spearman correlation coefficient was used to examine the correlation among environmental factors. Mixed effects models and generalized estimating equations were used to evaluate the health effects of fungal bioaerosols. Interchangeable covariance models were used to adjust for the correlation among the participants working on the same agricultural farm. *P* values of $< 0.05$ were considered statistically significant.

In the regression analyses of health effects, we evaluated the effects of fungal concentrations, job duties, and personal protection equipment use. Other significant demographic variables such as sex, age, ethnicity, education, working years, working hours per day, and smoking status were also adjusted in the final models. Total fungal and individual fungal taxon concentrations were analyzed. The concentrations of fungal taxa, including *Aspergillus*/*Penicillium*, Basidiospores, and *Cladosporium*, total fungal spores, and total culturable fungi, were divided by 10,000 in regression analyses to avoid small beta coefficients. Job duties were categorized according to whether the workers engaged in activities involving high dust exposure, such as harvesting and packaging mushrooms and vegetables. Personal protection equipment use indicated whether the workers wore masks at work.

Several lung function measurements were used as dependent variables in regression analyses: (1) Original (value) and predicted (predicted %) values measured before and after work: original and predicted values of $FEV_1$, FVC, FEV1%, PEF, $FEF_{25}$, $FEF_{50}$, and $FEF_{75}$, and original values of $FEF_{25–75\%}$. (2) Decreased lung function measurements after work: if the original values ($FEV_1$, FVC, FEV1%, PEF, $FEF_{25}$, $FEF_{50}$, $FEF_{75}$, and $FEF_{25–75\%}$) after work were lower than those before work (i.e., after work measurement – before work measurement $< 0$), then the lung function was considered decreased. Otherwise, the variable was considered not decreased. (3) Abnormal lung function: predicted FVC before or after work $< 80\%$; predicted FEV1% before or after work $< 70\%$. In univariate analyses, the model was

adjusted for sex and age if the original values of the lung functions were analyzed, but not for predicted values and abnormal lung function.

## RESULTS

### *Collected Samples and Worker Characteristics*

In total, 79 fungal bioaerosol samples were collected, including 55 personal samples and 24 area samples. Furthermore, 59 questionnaires and 55 sets of lung function measurements were collected (Table 1). Six workers from the first vegetable farm participated in both fall and winter samplings. In total, 53 workers participated in this study.

Demographic characteristics of the participants are presented in Supplementary Table S1. Approximately 80% of the workers were women. The mean age of the workers was 45 years. Most participants (75.47%) were common workers, in charge of planting, watering, packaging, and other regular tasks, not requiring specific skills. On average, the participants had worked 8 h per day on their current farms for approximately 7 years. Most workers did not use masks at work (79.25%).

### *Comparison of Fungal Concentrations and Environmental Factors on the Study Farms*

Table 2 displays a comparison of fungal concentrations and environmental factors on the study farms. The highest concentrations of total fungal spores (geometric means [GMs]: $1.2 \times 10^5$ and $4.0 \times 10^4$ spores $m^{-3}$ in the personal and area samples, respectively) were observed on the second mushroom farm, whereas the lowest were observed on the second vegetable farm (GM: $4.2 \times 10^3$ and $1.1 \times 10^3$ spores $m^{-3}$ in the personal and area samples, respectively). The personal samples ($p = 0.026$) of fungal spore concentrations differed significantly, but the area samples ($p = 0.063$) did not among the five farms. The highest culturable fungal concentration among the personal samples was observed on the first vegetable farm (GM: $3.0 \times 10^4$ CFU $m^{-3}$), whereas the lowest was observed on the second vegetable farm (GM: $4.3 \times 10^3$ CFU $m^{-3}$). Regarding the area samples, the highest culturable fungal concentration was observed on the second mushroom farm (GM: $5.8 \times 10^3$ CFU $m^{-3}$), whereas the lowest was observed on the second vegetable farm (GM: 763 CFU $m^{-3}$). However, the culturable fungal concentrations exhibited nonsignificant variation among the five farms. Detailed area sampling results are listed in Supplementary Table S2. On comparing concentrations between farm types, we observed that fungal concentrations did not differ significantly between the mushroom and vegetable farms (Table 2). Fungal concentrations in the personal samples were consistently higher than those in the area samples for both fungal spores and culturable fungi. However, no statistically significant differences were observed (for total fungal spores, $p = 0.093$; for culturable fungi, $p = 0.218$).

The highest mean relative humidity (74%) was observed on the first mushroom farm, and the highest mean $CO_2$ concentration (mean: 1,322 ppm) was observed on the second mushroom farm. The highest concentrations of fine

**Table 1.** Sample numbers and characteristics of the study farms.

| Farm type | Number of personal samples | Number of area samples | Number of questionnaires collected | Number of workers evaluated for lung functions | Area ($m^2$) | Sampling date | Farming activities |
|---|---|---|---|---|---|---|---|
| First Mushroom | 24 | 4 | 24 | 24 | 53,000–60,000 | 9/1–3/2014 | Compost preparation, container filling, inoculation, harvesting, packaging, and handling of used containers |
| Second Mushroom | 8 | 4 | 10 | 8 | 12,000–16,000 | 10/2–3/2014 | Compost preparation, container filling, inoculation, harvesting, packaging, and handling of used containers |
| First Vegetable (fall) | 7 | 4 | 9 | 7 | 20,000 | 10/13/2014 | Tilling soil, planting, watering, harvesting, and packaging |
| First Vegetable (winter) | 6 | 4 | 6 | 6 | 20,000 | 01/19/2015 | Tilling soil, planting, watering, harvesting, and packaging |
| Second Vegetable | 5 | 4 | 5 | 5 | 7,000–9,000 | 10/24/2014 | Tilling soil, planting, watering, harvesting, and packaging |
| Third Vegetable | 5 | 4 | 5 | 5 | 300,000 | 10/30/2014 | Tilling soil, planting, watering, harvesting, and packaging |
| Total | 55 | 24 | 59 | 55 | | | |

**Table 2.** Comparison of fungal bioaerosol concentrations and environmental factors in the personal and area samples from the five study farms [a].

| Farm type | Fungal spores (spores $m^{-3}$) | Culturable fungi (CFU $m^{-3}$) | Temperature (°C) | Relative humidity (%) | $CO_2$ (ppm) | Wind speed ($m\ s^{-1}$) | $PM_{2.5}$ ($\mu g\ m^{-3}$) | $PM_{10}$ ($\mu g\ m^{-3}$) | TSP ($\mu g\ m^{-3}$) |
|---|---|---|---|---|---|---|---|---|---|
| **First mushroom** | | | | | | | | | |
| Personal samples | $2.6 \times 10^4 \pm 7.43$ | $1.0 \times 10^4 \pm 7.50$ | | | | | | | |
| Area samples | $4.0 \times 10^3 \pm 5.76$ | $1.0 \times 10^3 \pm 7.90$ | 26 ± 3 | 74 ± 8 | 428 ± 114 | 0.12 ± 0.07 | 13 ± 3 | 63 ± 4 | 69 ± 4 |
| **Second mushroom** | | | | | | | | | |
| Personal samples | $1.2 \times 10^5 \pm 7.82$ | $1.2 \times 10^4 \pm 22.98$ | | | | | | | |
| Area samples | $4.0 \times 10^4 \pm 4.88$ | $5.8 \times 10^3 \pm 4.70$ | 26 ± 8 | 55 ± 5 | 1,322 ± 1,622 | 0.34 ± 0.30 | 23 ± 8 | 92 ± 1 | 112 ± 2 |
| **First vegetable (fall)** | | | | | | | | | |
| Personal samples | $6.5 \times 10^4 \pm 4.62$ | $3.0 \times 10^4 \pm 5.39$ | | | | | | | |
| Area samples | $4.5 \times 10^3 \pm 1.25$ | $1.4 \times 10^3 \pm 1.85$ | 27 ± 3 | 50 ± 8 | 367 ± 140 | 0.34 ± 0.14 | 10 ± 3 | 60 ± 4 | 82 ± 4 |
| **First vegetable (winter)** | | | | | | | | | |
| Personal samples | $8.4 \times 10^3 \pm 3.92$ | $2.8 \times 10^4 \pm 5.58$ | | | | | | | |
| Area samples | $3.1 \times 10^3 \pm 6.03$ | $1.2 \times 10^3 \pm 3.31$ | 16 ± 0 | 59 ± 3 | 353 ± 57 | 0.34 ± 0.14 | 13 ± 1 | 70 ± 2 | 101 ± 3 |
| **Second vegetable** | | | | | | | | | |
| Personal samples | $4.2 \times 10^3 \pm 2.25$ | $4.3 \times 10^3 \pm 2.05$ | | | | | | | |
| Area samples | $1.1 \times 10^3 \pm 1.96$ | $763 \pm 2.09$ | 29 ± 5 | 53 ± 10 | 727 ± 651 | 0.30 ± 0.33 | 0.31 ± 45 | 3 ± 25 | 5 ± 24 |
| **Third vegetable** | | | | | | | | | |
| Personal samples | $2.8 \times 10^4 \pm 2.03$ | $4.8 \times 10^3 \pm 2.67$ | | | | | | | |
| Area samples | $1.0 \times 10^4 \pm 9.81$ | $3.7 \times 10^3 \pm 9.59$ | 33 ± 2 | 46 ± 5 | 364 ± 18 | 1.19 ± 0.07 | 5 ± 1 | 24 ± 2 | 33 ± 2 |
| *p* value [b] | **0.026**/0.063 (personal/area) | 0.408/0.418 (personal/area) | 0.112 | **0.034** | 0.292 | 0.250 | **0.048** | 0.104 | 0.113 |
| *p* value [c] | 0.395/0.154 (personal/area) | 0.841/0.133 (personal/area) | 0.097 | **0.012** | 0.135 | 0.263 | 0.069 | 0.058 | 0.069 |

[a] Geometric means and geometric standard deviations are listed for the concentrations of fungal bioaerosols, $PM_{2.5}$, $PM_{10}$, and TSP; means and standard deviations are listed for other parameters.

[b] Comparison of the five farms using the Kruskal–Wallis (exact) test. Data of winter sampling were not included.

[c] Comparison between the types of farms (mushroom vs. vegetable farms) using the Mann–Whitney *U* test. Data of winter sampling were not included.

particulate matter ($PM_{2.5}$; GM: 23 μg $m^{-3}$), $PM_{10}$ (GM: 92 μg $m^{-3}$), and total suspended particulates (TSPs; GM: 112 μg $m^{-3}$) were observed on the second mushroom farm, whereas the lowest $PM_{2.5}$ (GM: 0.31 μg $m^{-3}$), $PM_{10}$ (GM: 3 μg $m^{-3}$), and TSP (GM: 5 μg $m^{-3}$) were observed on the second vegetable farm. Among all the environmental factors, only relative humidity ($p$ = 0.034) and $PM_{2.5}$ concentrations ($p$ = 0.048) differed significantly among the five farms. Relative humidity also differed significantly between the mushroom and vegetable farms ($p$ = 0.012; Table 2).

### *Distributions and Characteristics of Fungal Taxa on the Study Farms*

Distributions and characteristics of the most prevalent fungal taxa in the personal samples from the study farms are shown in Table 3. For fungal spores, the most prevalent fungal taxa on the mushroom farms were *Aspergillus*/*Penicillium* (GMs of the first and second farms: $9.2 \times 10^3$ and $9.6 \times 10^4$ spores $m^{-3}$, respectively), Basidiospores ($8.0 \times 10^3$ and $5.4 \times 10^3$ spores $m^{-3}$, respectively), and *Cladosporium* ($1.9 \times 10^3$ and $4.2 \times 10^3$ spores $m^{-3}$, respectively). The most prevalent fungal taxa on the vegetable farms were *Aspergillus/Penicillium* (GMs of the first, second, and third farms: $4.1 \times 10^4$, $2.8 \times 10^3$, and $1.5 \times 10^4$ spores $m^{-3}$, respectively) and *Cladosporium* ($5.5 \times 10^3$, 571, and $9.4 \times 10^3$ spores $m^{-3}$, respectively). For culturable fungi, the most prevalent fungal taxa were *Aspergillus*, *Cladosporium*, *Penicillium*, and yeast on both the mushroom and vegetable farms.

### *Seasonal Variations in Fungal Bioaerosol Concentrations and Environmental Factors on the First Vegetable Farm*

Seasonal variations in fungal concentrations in the personal and area samples are presented in Fig. 1. In personal sampling, the median total fungal spore concentration ($9.7 \times 10^4$ spores $m^{-3}$) was higher during fall than during winter, whereas the median culturable fungal concentrations were almost equal during the two seasons ($3.1 \times 10^4$ CFU $m^{-3}$). A significant seasonal difference was observed for fungal spore concentrations ($p$ = 0.031), but not for culturable fungal concentrations. In area sampling, higher median concentrations of fungal spores ($4.8 \times 10^3$ spores $m^{-3}$) and culturable fungi ($1.1 \times 10^3$ CFU $m^{-3}$) were observed during fall. However, seasonal variation was nonsignificant for both fungal concentrations.

Higher relative humidity and particulate ($PM_{2.5}$, $PM_{10}$, and TSP) concentrations were observed during winter than during fall. Temperature and $CO_2$ concentrations were higher during fall (Table 2). Temperature and relative humidity varied significantly between seasons (both $p$ = 0.03).

### *Regression Analyses*

The prevalence rates of work-related symptoms were low among the study farms. One participant reported work-related eye tiredness. Work-related respiratory symptoms such as dry throat, runny nose, sputum, and wheezing were only reported by one participant in each symptom category. Because of the low frequencies of reported symptoms, regression analyses were only performed on lung function measurements and not on work-related symptoms. Distributions of the worker lung function measurements on the five study farms are presented in Supplementary Table S3. The mean values of several lung function measurements (i.e., PEF, FEV1%, $FEF_{25}$, and $FEF_{75}$) decreased after work on most of the study farms.

Table 4 presents the results of the univariate and

**Table 3.** Distributions and characteristics of prevalent fungal taxa on the five study farms in the personal samples [a].

| Fungus | First mushroom ($n$ = 24) | Second mushroom ($n$ = 8) | First vegetable (Fall, $n$ = 7) | Second vegetable ($n$ = 5) | Third vegetable ($n$ = 5) |
|---|---|---|---|---|---|
| **Fungal spore taxa (spores $m^{-3}$)** | | | | | |
| *Alternaria* | ND [b] | 136 ± 2.57 | 57 ± 3.94 | ND | 163 ± 1.83 |
| Ascospores | 466 ± 2.82 | 990 ± 2.44 | 827 ± 2.79 | 112 ± 1.87 | 286 ± 2.54 |
| *Aspergillus/Penicillium* | $9.2 \times 10^3$ ± 6.02 | $9.6 \times 10^4$ ± 10.22 | $4.1 \times 10^4$ ± 5.64 | $2.8 \times 10^3$ ± 1.12 | $1.5 \times 10^4$ ± 2.46 |
| *Arthrinium* | 150 ± 2.14 | 249 ± 2.58 | 467 ± 2.70 | 64 ± 1.91 | 160 ± 3.28 |
| Basidiospores | $8.0 \times 10^3$ ± 13.55 | $5.4 \times 10^3$ ± 27.48 | 884 ± 2.67 | 63 ± 1.94 | 358 ± 1.40 |
| *Cladosporium* | $1.9 \times 10^3$ ± 4.20 | $4.2 \times 10^3$ ± 2.27 | $5.5 \times 10^3$ ± 2.53 | 571 ± 3.44 | $9.4 \times 10^3$ ± 2.11 |
| *Curvularia* | 61 ± 1.7 | 243 ± 2.35 | 113 ± 2.70 | 73 ± 1.13 | 52 ± 1.03 |
| *Fusarium* | 84 ± 3.32 | 207 ± 1.82 | 113 ± 2.12 | 40 ± 1.01 | 200 ± 1.49 |
| *Nigrospora* | 71 ± 1.7 | 87 ± 2.10 | 121 ± 2.23 | ND | 73 ± 1.53 |
| Smuts | 109 ± 2.86 | 94 ± 1.49 | 795 ± 5.54 | 68 ± 1.85 | 91 ± 1.90 |
| **Culturable fungal taxa (CFU $m^{-3}$)** | | | | | |
| *Aspergillus* | 529 ± 5.40 | 79 ± 1.65 | $3.0 \times 10^3$ ± 6.51 | 126.80 ± 1.37 | 171 ± 7.37 |
| *Cladosporium* | 332 ± 2.93 | 210 ± 3.30 | $1.4 \times 10^3$ ± 30.07 | 716.29 ± 5.15 | 814 ± 10.13 |
| *Fusarium* | ND | ND | 651 ± 14.34 | 31 ± 3.64 | 123 ± 2.56 |
| *Penicillium* | 790 ± 37.32 | $6.4 \times 10^3$ ± 17.22 | $1.0 \times 10^4$ ± 8.59 | 678 ± 2.63 | 735 ± 3.99 |
| *Rhizopus* | 228 ± 4.58 | 129 ± 1.30 | 612 ± 22.2 | ND | ND |
| Yeast | $4.3 \times 10^4$ ± 20.28 | $2.4 \times 10^3$ ± 15.28 | $3.0 \times 10^3$ ± 3.62 | $1.6 \times 10^3$ ± 1.67 | 437 ± 1.97 |

[a] Data presented are geometric means and geometric standard deviations.
[b] ND denotes not detected.

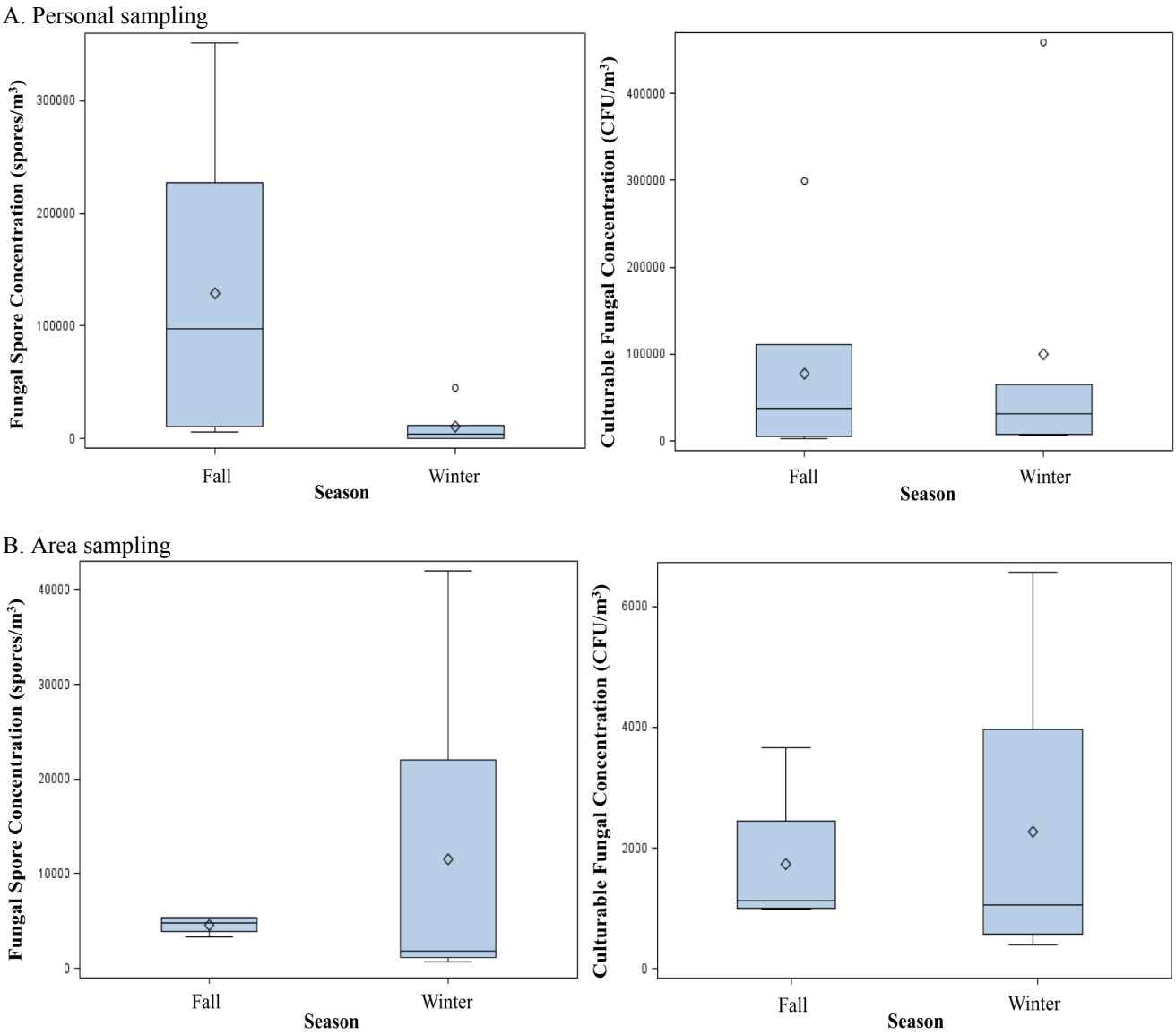


**Fig. 1.** Seasonal variations in the concentrations of the total fungal spores and total culturable fungi in personal and area samplings. The box plots show medians and means; 10th, 25th, 75th, and 90th percentiles; and outliers.

multivariate analyses of lung function measurements. Among the continuous variables, *Nigrospora* concentration was negatively associated with original PEF after work ($p = 0.0406$). Furthermore, the total culturable fungal concentrations was negatively correlated with predictive FEV1% before work ($p = 0.0138$). The Basidiospore concentration was positively associated with abnormal FVC before work ($p = 0.0198$), and the Basidiospore ($p = 0.0397$) and *Nigrospora* ($p = 0.0084$) concentrations were positively associated with abnormal FVC after work. The total fungal spore concentrations were associated with decreased $FEV_1$, FVC, $FEF_{25}$, and $FEF_{50}$, and the total culturable fungal concentrations were associated with decreased $FEF_{50}$. The concentrations of various fungal taxa were also associated with decreased lung functions of the workers on the study farms. We also evaluated the effects of job duties, such as harvesting and packaging, and personal protection equipment use on lung functions. Harvesting and packaging were negatively associated with predictive $FEF_{75}$ before work ($p = 0.0479$ and 0.0451, respectively). However, wearing a mask showed insignificant protective effect on workers' lung functions.

We also performed multivariate regression analyses of lung functions (Table 4). Because only one variable each was associated with original and predictive lung functions and abnormal lung functions (Table 4), no multivariate model could be presented. In the multivariate regression models of decreased lung functions after work, the concentrations of Ascospores, *Fusarium*, and *Periconia* were positively associated with decreased $FEV_1$, and the concentrations of total culturable fungi and Ascospores were positively associated with decreased $FEF_{50}$. Several fungal taxa demonstrated significant relationships with decreased lung functions in univariate analyses (Table 4); however, the significance of these relationships diminished in multivariate models because of collinearity.

**Table 4.** Univariate and multivariate regression models of lung function measurements.

| | Parameter | β coefficient | SE | *p* value |
|---|---|---|---|---|
| **Univariate regression models** | | | | |
| **Continuous lung function measurements** [a] | | | | |
| PEF_after work | *Nigrospora* | −0.00598 | 0.002835 | 0.0406 |
| Predictive FEV1%_ before work | Total culturable fungi | −0.2495 | 0.09709 | 0.0138 |
| $FEF_{75}$_before work | Harvesting | −28.3088 | 13.9375 | 0.0479 |
| $FEF_{75}$_before work | Packaging | −28.9624 | 14.0713 | 0.0451 |
| **Abnormal lung function measurements** [b] | | | | |
| FVC_before work | Basidiospores | 0.0131 | 0.0056 | 0.0198 |
| FVC_after work | Basidiospores | 0.0084 | 0.0041 | 0.0397 |
| | *Nigrospora* | 0.0038 | 0.0014 | 0.0085 |
| **Decreased lung functions after work** [c] | | | | |
| $FEV_1$ | Total fungal spores | 0.0103 | 0.0024 | < 0.0001 |
| | Ascospores | 0.0009 | 0.0003 | 0.0007 |
| | *Arthrinium* | 0.0014 | 0.0007 | 0.0353 |
| | *Cladosporium* | 0.0782 | 0.0393 | 0.0466 |
| | *Curvularia* | 0.0142 | 0.0061 | 0.0196 |
| | *Fusarium* | 0.0091 | 0.0027 | 0.0009 |
| | *Nigrospora* | 0.0077 | 0.0037 | 0.0388 |
| | *Periconia* | 0.0032 | 0.0011 | 0.0029 |
| FVC | Total fungal spores | 0.0086 | 0.0022 | < 0.0001 |
| | Ascospore*s* | 0.0006 | 0.0003 | 0.0415 |
| | *Aspergillus/Penicillium* | 0.0122 | 0.0022 | < 0.0001 |
| | *Fusarium* | 0.0072 | 0.0035 | 0.0407 |
| PEF | Basidiospores | 0.0217 | 0.0078 | 0.0057 |
| $FEF_{25}$ | Total fungal spores | 0.0065 | 0.0030 | 0.0300 |
| | Ascospores | 0.0007 | 0.0003 | 0.0155 |
| | *Aspergillus/Penicillium* | 0.0096 | 0.0043 | 0.0261 |
| $FEF_{50}$ | Total fungal spores | 0.0120 | 0.0040 | 0.0028 |
| | Total culturable fungi | 0.0300 | 0.0058 | < 0.0001 |
| | Ascospores | 0.0007 | 0.0001 | < 0.0001 |
| | *Aspergillus/Penicillium* | 0.0401 | 0.0081 | < 0.0001 |
| $FEF_{75}$ | *Arthrinium* | 0.0015 | 0.0006 | 0.0167 |
| | *Nigrospora* | 0.0038 | 0.0010 | 0.0001 |
| | Smuts | 0.0002 | 0.0001 | 0.0146 |
| **Multivariate regression models of decreased lung functions after work** [c] | | | | |
| $FEV_1$ | Ascospores | 0.0006 | 0.0002 | 0.0052 |
| | *Fusarium* | 0.0052 | 0.0023 | 0.0225 |
| | *Periconia* | 0.0020 | 0.0010 | 0.0328 |
| $FEF_{50}$ | Total culturable fungi | 0.0205 | 0.0055 | 0.0002 |
| | Ascospores | 0.0005 | 0.0002 | 0.0128 |

[a] The dependent variable was a continuous variable of original or predictive lung function measurements. The model of original values was adjusted for sex and age, but the model of predictive values was not.
[b] Abnormal lung function included predictive FVC of < 80% before or after work and FEV1% of < 70% before or after work.
[c] The dependent variable was a categorical variable. If the original value of the lung function after work was lower than that before work, then it was defined as decreased (1). Otherwise, the variable was defined as not decreased (0). The multivariate models were adjusted for significant demographic variables.

## DISCUSSION

This study demonstrated that the workers employed at both the mushroom and vegetable farms were exposed to high fungal bioaerosol concentrations, with a 10-fold higher concentration on the mushroom farms than on the vegetable farms. The total fungal spore and culturable concentrations were 5–373 and 8–1,816 folds higher in personal samples (based on individual data) than those in outdoor area samples, respectively. The mean fungal concentrations observed in the present study were slightly lower than those observed in another Taiwanese study investigating a mushroom farm (median concentrations for culturable fungi: $1.4 \times 10^4$ to $1.5 \times 10^7$ CFU $m^{-3}$; median concentrations for fungal spores: $1.7 \times 10^6$ to $1.3 \times 10^8$ spores $m^{-3}$) (Lee and Liao, 2014). The mean airborne fungal concentrations in the personal and

area samples of this study were similar to those in Danish tomato and cucumber greenhouses (median concentrations of stationary samples vs. personal samples: $3.4 \times 10^3$ to $4.1 \times 10^5$ CFU $m^{-3}$ vs. $6.1 \times 10^3$ to $1.0 \times 10^7$ CFU $m^{-3}$) (Hansen *et al*., 2012). Currently, no limit values for airborne fungal exposure on agricultural farms or similar occupational settings have been recommended. However, the airborne fungal concentrations observed in this study exceeded the indoor air quality standard in Taiwan: maximum concentration < 1,000 CFU $m^{-3}$ (Taiwan EPA, 2016).

In this study, we performed both personal and area samplings on the study farms. Personal sampling accurately measures workers' exposure to inhalable airborne fungi. Monitoring major work sites can aid in substantially evaluating site- and task-specific microbial exposure of workers. However, we found that the fungal concentrations in the personal samples were consistently higher than those in the area samples, although the difference was not significant, probably because of a small sample size. Similar results were observed in a Danish study conducted in vegetable greenhouses (Madsen *et al*., 2014). Because workers often disturbed bioaerosol sources during activities, such as mixing material, picking vegetables, digging soil, and packaging, thus increasing the personal exposure concentrations. Therefore, exposure may be underestimated if area sampling results are used to estimate personal exposure. We also observed that the fungal concentrations varied among different sampling sites (Supplementary Table S2). On the mushroom farms, higher fungal concentrations were observed in the harvesting and packaging areas than in the compost preparation and container filling areas and outdoors. However, on the vegetable farms, higher fungal concentrations were frequently observed in uncultivated fields. Among all the area samples, the concentrations of total fungi and various fungal taxa were the lowest outdoors. The ideal indoor fungal concentrations should be lower than or comparable to those outdoors because the indoor fungal spore concentrations are strongly dependent on the outdoor concentrations. If the fungal concentration indoors is higher than that outdoors, then indoor contamination sources are likely present (Burge *et al*., 1982). Here, mushrooms, plants, and soil were the essential fungal sources on the study farms.

In this study, the fungal concentrations and categories may have been affected by many environmental factors including season, temperature, relative humidity, plant type, ventilation type, and farm activities. Temperature (26°C–33°C) and relative humidity (46%–74%) were favorable for fungal growth in this study. The most prevalent fungal spore taxa were *Aspergillus*/*Penicillium*, Basidiospores, and *Cladosporium* on the mushroom farms and *Aspergillus/Penicillium* and *Cladosporium* on the vegetable farms. Furthermore, the most prevalent culturable fungi were consistent with the most prevalent fungal spore taxa. Similar findings were observed in a previous study investigating three agricultural confinements: hog, dairy, and grain farms (Adhikari *et al*., 2004); the most prevalent fungi were *Cladosporium*, *Aspergillus*/*Penicillium,* Basidiospores, and Ascospores. In the present study, a major difference in the fungal taxa between the two farm types was the much higher Basidiospore concentrations observed on the mushroom farms than on the vegetable farms; we observed high white-hyaline Basidiospore concentrations on the mushroom farms produced by mushrooms. High concentrations of *Cladosporium* were observed on our study vegetable farms, probably because soil and plants are essential sources of *Cladosporium*. Because most sampling sites on the vegetable farms were semiopen, the observed fungal categories were common outdoor fungi such as *Aspergillus*/*Penicillium, Cladosporium*, Ascospores, and Basidiospores.

To examine the potential seasonal variations in fungal concentrations, we performed two samplings on one vegetable farm. The median fungal concentrations were higher during fall than during winter, but a significant difference in fungal spore concentrations was observed only among the personal samples. In addition, among the environmental factors, temperature, relative humidity, $CO_2$ concentrations, and particulate matter concentrations showed significant seasonal variations. Differences in temperature and relative humidity in the two seasons probably contributed to significant temporal variations in fungal spore concentrations (Kang *et al*., 2015; Saari *et al*., 2015). Our observations support the findings of previous studies: total fungal spore concentrations were higher during fall than during winter in a Norwegian grain and compound feed industry (Halstensen *et al*., 2013), and in Ohio, USA, the total fungal spore concentrations were higher during summer than during winter in swine and dairy farm environments (Lee *et al*., 2006).

Various studies have demonstrated that exposure to high microbial contaminant concentrations results in respiratory disorders and decreased lung functions on various farms, including swine, grain, and poultry farms (Post *et al*., 1998; Kirychuk *et al*., 2003; Chattopadhyay *et al*., 2007; Chenard *et al*., 2007; Rimac *et al*., 2010; Szczyrek *et al*., 2011). However, scientific information on health risks to vegetable farm workers and the effect of their exposure to specific microbial agents, particularly fungi that are present on agricultural farms is scant. To our knowledge, this was the first study to examine the effects of fungal exposure on workers' lung functions on vegetable farms in Taiwan. In this study, > 40% of the workers' pulmonary function measurements declined after work. The FEV1% values reduced on average from 2% to 10%, the $FEV_1$ and FVC values decreased on average 0.3–1 L, and the PEF, $FEF_{25}$, $FEF_{50}$ values reduced 0.1–1.1 L $s^{-1}$. These reductions might be associated with asthma, hypersensitivity reaction of the airways, and chronic obstructed pulmonary disease (COPD) due to the increased levels of fungal contamination during harvesting, packaging, and tilling soil on the study farms (Broaddus *et al*., 2016).

In this study, we examined the effects of fungal bioaerosol exposure on the farm workers' pulmonary functions both before and after work, as well as on cross shift declines. Due to the normal diurnal variation in human lung function, usually higher values in the afternoon than in the early morning (Borsboom *et al.*, 1999; Medarov *et al.*, 2008;

Movaseghi *et al*., 2016), we evaluated the associations between fungal bioaerosol exposure and lung function both before and after work as long–term occupational exposure effects. We also examined the cross shift declines to evaluate the acute effect of fungal bioaerosol exposure. In univariate analyses, we observed that the total fungal spore concentrations were associated with decreased $FEV_1$, FVC, $FEF_{25}$, and $FEF_{50}$ after work; total culturable fungal concentrations were associated with predictive FEV1% before work and decreased $FEF_{50}$ after work. The concentrations of various fungal taxa were also associated with decreased lung functions of the workers from the agriculture farms. In multivariate analyses, we observed that the concentrations of Ascospores, *Fusarium*, and *Periconia* were associated with decreased $FEV_1$. The concentrations of total culturable fungi and Ascospores were correlated with decreased $FEF_{50}$ (Table 4). The results of a study conducted in New Taipei City, Taiwan, revealed that ambient *Cladosporium* concentrations were strongly associated with lung function changes among schoolchildren (Chen *et al*., 2014). Thus, the allergic and respiratory symptoms have probably been caused by the allergic and inflammatory components of fungal contaminants at the study sites.

Regarding abnormal lung functions, the concentrations of Basidiospores and *Nigrospora* were significantly associated with abnormal FVC. Thus, the high Basidiospore concentrations are of great concern on mushroom farms. Harvesting and packaging mushrooms and vegetables were also associated with decreased $FEF_{75}$ (Table 4). These associations may have occurred because of the frequent movement of mushrooms and vegetables during packaging as well as plant and soil disturbance during harvesting. Various particulates are easily suspended in the air; this may have resulted in higher fungal concentrations in the packaging and harvesting areas on the study farms. A Danish study indicated high fungal concentrations during the packaging of *Campanula* and *Rhipsalideae* in flower greenhouses (Thilsing *et al*., 2015). Another study indicated that among mushroom farm workers, chronic cough was associated with spore inhalation at mushroom farms; the spore concentrations were higher in harvesting and packaging rooms than in offices (Tanaka *et al*., 2002). Therefore, farmers performing job duties involving high fungal exposure should wear suitable protective gear, such as masks, gloves, and working coats, to prevent exposure, as demonstrated effective in a previous study (Odo *et al*., 2015). Washing hands thoroughly after completing specific duties is necessary for preventing extended contact with farm dust.

This study had some limitations. First, our sample size was small; we only evaluated the effects on the health of 53 farm workers from two mushroom and three vegetable farms, thus limiting the generalizability of our results. However, the adverse effects of fungal exposure on farmers' lung functions are evident. In addition, susceptible workers might have left the industry due to the healthy worker effect and resulted in an underestimation of health impacts (Bunger *et al*., 2007; Chenard *et al*., 2007; Rimac *et al*., 2010). Second, we used a self-reported questionnaire to evaluate the working conditions and perceived health among the workers. Recall bias and social pressure may have resulted in symptom misclassification or underreporting; because very low frequencies of work-related symptoms were reported, we could not analyze the effect of fungal exposure on health symptoms, and only lung function measurements were analyzed. Finally, our study was mostly cross-sectional. Seasonal variations were investigated only on one farm. Therefore, future studies should longitudinally investigate bioaerosol exposure and health risks in a large cohort.

## CONCLUSIONS

Workers' exposure to fungal contaminants at the study agricultural farms was high, with higher concentrations observed on the mushroom farms than on the vegetable farms. Decreased lung functions of the workers were associated with total fungi and several fungal taxa. Job duties such as harvesting and packaging were also associated with decreased lung functions. Our results demonstrated that exposure to high fungal concentrations reduced the lung functions of the agricultural workers. Therefore, increasing ventilation and using suitable personal protection equipment are required for reducing occupational biohazards. In addition, health promotion among agricultural workers is warranted to aid them in recognizing potential health hazards at work. Future studies should further evaluate the health effects among farm workers longitudinally by using a large cohort.

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## SUPPLEMENTARY MATERIAL

Supplementary data associated with this article can be found in the online version at http://www.aaqr.org.

## REFERENCES

Aalto-Korte, K., Susitaival, P., Kaminska, R. and Makinen-Kiljunen, S. (2005). Occupational protein contact dermatitis from shiitake mushroom and demonstration of shiitake-specific Immunoglobulin E. *Contact Dermatitis* 53: 211–213.

ACGIH (1999). *Bioaerosols: Assesment and Control*, American Conference of Governmental Industrial Hygienists, Cincinnati.

Adhikari, A., Reponen, T., Lee, S.A. and Grinshpun, S. A. (2004). Assessment of human exposure to airborne fungi in agricultural confinements: personal inhalable sampling versus stationary sampling. *Ann. Agric. Environ. Med.*

11: 269–277.

Adhikari, A., Gupta, J., Wilkins, J.R. 3rd., Olds, R.L., Indugula, R., Cho, K.J., Li, C. and Yermakov, M. (2011). Airborne microorganisms, endotoxin, and (1-->3)-beta-D-glucan exposure in greenhouses and assessment of respiratory symptoms among workers. *Ann. Occup. Hyg.* 55: 272–285.

Ampere, A., Delhaes, L., Soots, J., Bart, F. and Wallaert, B. (2012). Hypersensitivity pneumonitis induced by Shiitake mushroom spores. *Med. Mycol.* 50: 654–657.

Borsboom, G.J., van Pelt, W., van Houwelingen, H.C., van Vianen, B.G., Schouten, J.P. and Quanjer, P.H. (1999). Diurnal variation in lung function in subgroups from two Dutch populations: Consequences for longitudinal analysis. *Am. J. Respir. Crit. Care Med.* 159: 1163–1171.

Broaddus, C.V., Mason, J.R., Ernst, D.J., King, E.T., Lazarus, C.S., Murray, F.J., Nadel, A.J. and Slutsky, S.A. (2016). *Murray and Nadel's Textbook of Respiratory Medicine 6th Edition*, Saunders, Elsevier Inc. Philadelphia.

Bunger, J., Schappler-Scheele, B., Hilgers, R. and Hallier, E. (2007). A 5-year follow-up study on respiratory disorders and lung function in workers exposed to organic dust from composting plants. *Int. Arch. Occup. Environ. Health* 80: 306–312.

Burge, H.A., Solomon, W.R. and Muilenberg, M.L. (1982). Evaluation of indoor plantings as allergen exposure sources. *J. Allergy Clin. Immunol.* 70: 101–108.

Burge, H.A. and Rogers, C.A. (2000). Outdoor allergens. *Environ. Health Perspect.* 108: 653–659.

Chang, C.W., Ho, C.K., Chen, Z.C., Hwang, Y.H., Chang, C.Y., Liu, S.T., Chen, M.J. and Chen, M.Y. (2002). Fungi genus and concentration in the air of onion fields and their opportunistic action related to mycotic keratitis. *Arch. Environ. Health* 57: 349–354.

Chattopadhyay, B.P., Das, S., Adhikari, A. and Alam, J. (2007). Exposure to varying concentration of fungal spores in grain storage godowns and its effect on the respiratory function status among the workers. *Ind. Health* 45: 449–461.

Chen, B.Y., Chao, H.J., Wu, C.F., Kim, H., Honda, Y. and Guo, Y.L. (2014). High ambient *Cladosporium* spores were associated with reduced lung function in schoolchildren in a longitudinal study. *Sci. Total Environ.* 481: 370–376.

Chenard, L., Senthilselvan, A., Grover, V.K., Kirychuk, S.P., Lawson, J.A., Hurst, T.S. and Dosman, J.A. (2007). Lung function and farm size predict healthy worker effect in swine farmers. *Chest* 131: 245–254.

Chow, J.C., Yang, X., Wang, X., Kohl, S.D., Hurbain, P.R., Chen, L.W.A. and Watson, J.G. (2015). Characterization of ambient $PM_{10}$ bioaerosols in a California agricultural town. *Aerosol Air Qual. Res.* 15: 1433–1447.

Douwes, J., Thorne, P., Pearce, N. and Heederik, D. (2003). Bioaerosol health effects and exposure assessment: progress and prospects. *Ann. Occup. Hyg.* 47: 187–200.

Gorner, P., Simon, X., Wrobel, R., Kauffer, E. and Witschger, O. (2010). Laboratory study of selected personal inhalable aerosol samplers. *Ann. Occup. Hyg.* 54: 165–187.

Halstensen, A.S., Heldal, K.K., Wouters, I.M., Skogstad, M., Ellingsen, D.G. and Eduard, W. (2013). Exposure to grain dust and microbial components in the Norwegian grain and compound feed industry. *Ann. Occup. Hyg.* 57: 1105–1114.

Hansen, V.M., Meyling, N.V., Winding, A., Eilenberg, J. and Madsen, A.M. (2012). Factors affecting vegetable growers' exposure to fungal bioaerosols and airborne dust. *Ann. Occup. Hyg.* 56: 170–181.

Kang, S.M., Heo, K.J. and Lee, B.U. (2015). Why does rain increase the concentrations of environmental boaerosols during monsoon? *Aerosol Air Qual. Res.* 15: 2320–2324.

Kavanagh, K. (2005). *Fungi: Biology and Applications*. John Wiley & Sons Ltd, England.

Kirychuk, S.P., Senthilselvan, A., Dosman, J.A., Juorio, V., Feddes, J.J., Willson, P., Classen, H., Reynolds, S.J., Guenter, W. and Hurst, T.S. (2003). Respiratory symptoms and lung function in poultry confinement workers in Western Canada. *Can. Respir. J.* 10: 375–380.

Krop, E.J.M., Jacobs, J.H., Sander, I., Raulf-Heimsoth, M. and Heederik, D.J.J. (2014). Allergens and beta-Glucans in Dutch homes and schools: Characterizing airborne levels. *PLoS One* 9: e88871.

Lee, S.A., Adhikari, A., Grinshpun, S.A., McKay, R., Shukla, R. and Reponen, T. (2006). Personal exposure to airborne dust and microorganisms in agricultural environments. *J. Occup. Environ. Hyg.* 3: 118–130.

Lee, S.A. and Liao, C.H. (2014). Size-selective assessment of agricultural workers' personal exposure to airborne fungi and fungal fragments. *Sci. Total Environ.* 466–467: 725–732.

Madsen, A.M. (2011). Occupational exposure to microorganisms used as biocontrol agents in plant production. *Front. Biosci. (Schol Ed)* 3: 606–620.

Madsen, A.M., Tendal, K. and Frederiksen, M.W. (2014). Attempts to reduce exposure to fungi, beta-glucan, bacteria, endotoxin and dust in vegetable greenhouses and a packaging unit. *Sci. Total Environ.* 468–469: 1112–1121.

Matkovic, K., Vucemilo, M. and Vinkovic, B. (2009). Airborne fungi in dwellings for dairy cows and laying hens. *Arh. Hig. Rada. Toksikol.* 60: 395–399.

Medarov, B.I., Pavlov, V.A. and Rossoff, L. (2008). Diurnal variations in human pulmonary function. *Int. J. Clin. Exp. Med.* 1: 267–273.

Movaseghi, F., Kazemi, N. and Moein, E. (2016). Time of day effect on oxygen uptake changes and lung function of active female. *Turkish J. Sport Exercise* 18: 85–89.

Muilenberg, M. (1999). A Practical Guide to Aeroallergen Identification. Proceedings of the American College of Allergy and Immunology Annual Meeting. Chicago, USA.

Odo, N.U., Raynor, P.C., Beaudoin, A., Somrongthong, R., Scheftel, J.M., Donahue, J.G. and Bender, J.B. (2015). Personal protective equipment use and handwashing among animal farmers: A multi-site assessment. *J. Occup. Environ. Hyg.* 12: 363–368.

Post, W., Heederik, D. and Houba, R. (1998). Decline in lung function related to exposure and selection processes

among workers in the grain processing and animal feed industry. *Occup. Environ. Med.* 55: 349–355.

Redlich, C.A., Tarlo, S.M., Hankinson, J.L., Townsend, M.C., Eschenbacher, W.L., Von Essen, S.G., Sigsgaard, T., Weissman, D.N. and American Thoracic Society Committee on Spirometry in the Occupational Setting. (2014). Official American Thoracic Society technical standards: Spirometry in the occupational setting. *Am. J. Respir. Crit. Care Med.* 189: 983–993.

Reynolds, S.J., Clark, M.L., Koehncke, N., von Essen, S., Prinz, L., Keefe, T.J., Mehaffy, J., Bradford, M., Cranmer, B., Davidson, M.E., Yang, I.V. and Burch, J.B. (2012). Pulmonary function reductions among potentially susceptible subgroups of agricultural workers in Colorado and Nebraska. *J. Occup. Environ. Med.* 54: 632–641.

Rimac, D., Macan, J., Varnai, V.M., Vucemilo, M., Matkovic, K., Prester, L., Orct, T., Trosic, I. and Pavicic, I. (2010). Exposure to poultry dust and health effects in poultry workers: Impact of mould and mite allergens. *Int. Arch. Occup. Environ. Health* 83: 9–19.

Riu, E., Monso, E., Marin, A., Magarolas, R., Radon, K., Morera, J., Andreo, F. and Nowak, D. (2008). Occupational risk factors for rhinitis in greenhouse flower and ornamental plant growers. *Am. J. Rhinol.* 22: 361–364.

Saari, S., Niemi, J., Rönkkö, T., Kuuluvainen, H., Järvinen, A., Pirjola, L., Aurela, M., Hillamo, R. and Keskinen, J. (2015). Seasonal and diurnal variations of fluorescent bioaerosol concentration and size distribution in the urban environment. *Aerosol Air Qual. Res.* 15: 572–581.

Szczyrek, M., Krawczyk, P., Milanowski, J., Jastrzebska, I., Zwolak, A. and Daniluk, J. (2011). Chronic obstructive pulmonary disease in farmers and agricultural workers - an overview. *Ann. Agric. Environ. Med.* 18: 310–313.

Taiwan EPA (2016). Indoor Air Quality Standard. Taiwan: Environmental Protection Administration (EPA): Air Quality Management, http://ivy5.epa.gov.tw/docfile/200030.pdf, Last Accessed: 16 September 2016.

Tanaka, H., Saikai, T., Sugawara, H., Takeya, I., Tsunematsu, K., Matsuura, A. and Abe, S. (2002). Workplace-related chronic cough on a mushroom farm. *Chest* 122: 1080–1085.

Thilsing, T., Madsen, A.M., Basinas, I., Schlunssen, V., Tendal, K. and Baelum, J. (2015). Dust, endotoxin, fungi, and bacteria exposure as determined by work task, season, and type of plant in a flower greenhouse. *Ann. Occup. Hyg.* 59: 142–157.

Tsapko, V.G., Chudnovets, A.J., Sterenbogen, M.J., Papach, V.V., Dutkiewicz, J., Skorska, C., Krysińska-Traczyk, E. and Golec, M. (2011). Exposure to bioaerosols in the selected agricultural facilities of the Ukraine and Poland - A review. *Ann. Agric. Environ. Med.* 18: 19–27.

Viegas, C., Malta-Vacas, J., Sabino, R., Viegas, S. and Verissimo, C. (2014). Accessing indoor fungal contamination using conventional and molecular methods in Portuguese poultries. *Environ. Monit. Assess.* 186: 1951–1959.

Wang, C.H., Chen, B.T., Han, B.C., Liu, A.C., Hung, P.C., Chen, C.Y. and Chao, H.J. (2015). Field evaluation of personal sampling methods for multiple bioaerosols. *PLoS One* 10: e0120308.

Yoshikawa, S., Tsushima, K., Yasuo, M., Fujimoto, K., Kubo, K., Kumagai, T. and Yamazaki, Y. (2007). Hypersensitivity pneumonitis caused by *Penicillium citrinum*, not Enoki spores. *Am. J. Ind. Med.* 50: 1010–1017.

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