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Exposure to culturable airborne bioaerosols during noodle manufacturing in central Taiwan

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ABSTRACT

Biological hazards associated with the manufacturing of noodles have not been well characterized in Taiwan. This is an issue that flour workers can be exposed to bioaerosols (airborne fungi and bacteria) resulting flour-induced occupational asthma or allergic diseases. This study is to survey the species and concentrations of bioaerosols at different sites within a noodle factory for one year, and to investigate the effects of environmental factors on concentrations of bioaerosols. Air samples were taken twice a day, one day each month using a MAS-100 bioaerosol sampler.

Nine species of culturable fungi were identified, with the main airborne fungi being *Cladosporium*, *Penicillium*, *Aspergillus* spp., non-sporing isolates and yeasts. *Cladosporium*, *Penicillium* and *Aspergillus* were the dominant fungal isolates in the indoor and outdoor air samples. *Micrococcus* spp. and *Staphylococcus xylosum* were the dominant bacterial isolates. Peak fungal and bacterial concentrations occurred at the crushing site, with mean values of 3082 and 12,616 CFU/m³. Meanwhile, the most prevalent fungi and bacteria at the crushing site were in ranges of 2.1–1.1 µm and 1.1–0.65 µm, respectively. Significant seasonal differences in total bacterial concentration were observed at all sampling sites (*P<0.05). Moreover, significant seasonal differences were observed for most of the fungal genera except *Fusarium*. Levels of *Aspergillus* and *Rhizopus* differed significantly during the two sampling times, as did levels of *Micrococcus* spp. and *Staphylococcus arlettae*.

Regarding the same operation procedures, relative humidity affected fungi levels more than temperature did. However, crushing generated the highest concentration of bioaerosols among all operation procedures. Furthermore, levels of bacteria at sites fitted with ventilation systems were lower than at sites without ventilation systems, especially at the crushing site. Therefore, we recommend these workers at the crushing site wear breathing protection and improve the local ventilation systems to minimize the biological hazards.

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1. Introduction

Noodle factories represent an occupational environment that contains flour, flavorings, and other ingredients. Workers in such environments may face exposure to bioaerosols, includ-

ing bacteria, viruses, fungi, algae, and dust. Nevertheless, the automation of modern noodle factories limits dust and bioaerosol diffusion in the workplace. Therefore, many people regard noodle factories as microbiologically safe working environments. Previous investigations have reported high

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levels of potentially hazardous bacteria and fungi in flour mills (Abdel Hameed, 2007). Organic dust in such mills contained high levels of microorganisms and microbial products, such as bacterial endotoxins, fungal glucans, and mycotoxins (Berghofer et al., 2003). For example, the flour aeroallergen (geometric mean $>500 \mu\text{g}/\text{m}^3$) was identified during certain flour mill operations (Nieuwenhuijsen et al., 1995a). Additionally, flour-induced occupational asthma (OA) has been reported in flour mill workers exposed to the flour dust, and is one of the main causes of chronic respiratory illnesses such as asthma (Nieuwenhuijsen et al., 1995b; Meo, 2004; Talini et al., 2002).

Due to the lack of internationally accepted occupational exposure limits (OELs) for microorganisms and threshold limit values (TLVs) for endotoxins, some recommendations have been published for controlling indoor microbiological contamination (ACGIH, 1986, 1989; AIHA, 1996). For instance, the guideline proposed by the American Conference of Governmental Industrial Hygienists (ACGIH) is between 100 and 1000 CFU/m³ for total fungi, while the National Institute of Occupational Safety and Health (NIOSH) set a limit of 1000 CFU/m³ for the total number of bioaerosol particles. Moreover, the ACGIH committee also indicated that total bacterial levels in excess of 500 CFU/m³ in workspaces of affected individuals (1986). The guideline set by the World Health Organization (WHO, 1990, 2002) for the bioaerosol counts of total bacteria is 500 CFU/m³. Fungal and bacterial concentrations greater than 10^4 CFU/m³ should be considered a health threat (Heida et al., 1995).

According to the study carried out by Eduard et al. (2001), irritation of the eyes and nose increased after exposure to fungal spores in the range of 2×10^4 – 5×10^5 CFU/m³. Many fungal species were regarded as producers of type I allergens (IgE binding allergens), and IgE sensitization to common fungal genera such as *Cladosporium*, *Alternaria*, *Penicillium*, and *Aspergillus* were found to be closely associated with allergic respiratory diseases, especially asthma (Douwes et al., 2003). Previous studies indicated that *Aspergillus* spp. and *Penicillium* spp. were classified as food-relevant dangerous, toxin-producing fungi (Weidenbörner et al., 2000). *Cladosporium*, *Aspergillus*, and *Penicillium* were considered to be allergenic fungi frequently found in flour mills (Abdel Hameed, 2007). Generally, the effects of fungal spores on human health depend on their concentration, composition (genera and species) and size. Larger fungal spores (diameter $>5 \mu\text{m}$) are deposited in the nasal or thoracic passages and may cause allergic rhinitis and asthma. Smaller fungal spores, such as those produced by *Aspergillus* and *Penicillium* (diameter $<5 \mu\text{m}$), penetrate the alveoli and cause allergic alveolitis (Lacey and Crook, 1988). Consequently, elevated concentrations of *Cladosporium* were usually associated with respiratory symptoms, and increased indoor levels of *Cladosporium* and *Penicillium* can cause allergic diseases (Li et al., 1995). In contrast, some bacterial genera such as *Bacillus subtilis*, *Bacillus licheniformis* and actinomycetes species such as *Streptomyces albus*, *Saccharomonospora viridis* and *Saccharopolyspora rectivirgula* have been regarded as allergens (Krysířska-Traczyk et al., 2005).

The biohazards associated with concentration, species, and size distribution of airborne culturable fungi and bacteria in Taiwan noodle factories have not been well characterized.

The determination of microbiological contamination provides useful indices for evaluating indoor air quality and may provide a reference value for assessing the acceptability of indoor air. Therefore, eleven sampling sites were chosen, and the concentrations and compositions of airborne fungal and bacterial spores were surveyed in order to assess the exposure to bioaerosols in a noodle-manufacturing environment in Taiwan over a 1-year period. The aim of this survey is not only to evaluate the levels of microbiological organisms, but also to investigate the effects of environmental factors, such as season, sampling period, and the presence/absence of ventilation systems, on airborne bioaerosol distribution.

2. Materials and methods

2.1. Description of the noodle factory

A three-story noodle factory located in Nantou, Taiwan was selected as the study location. This factory has been producing noodles for 20 years, and specialized in manufacturing dried instant noodles and ramen. The ventilation system employed positive pressure, forcing the external air into the internal environment by a mechanical ventilator. Fig. 1 illustrates the noodle production process in this factory. The first step is to mix salt, flavorings, and other ingredients with water, then add flour to produce dough for maturity. After maturation, the

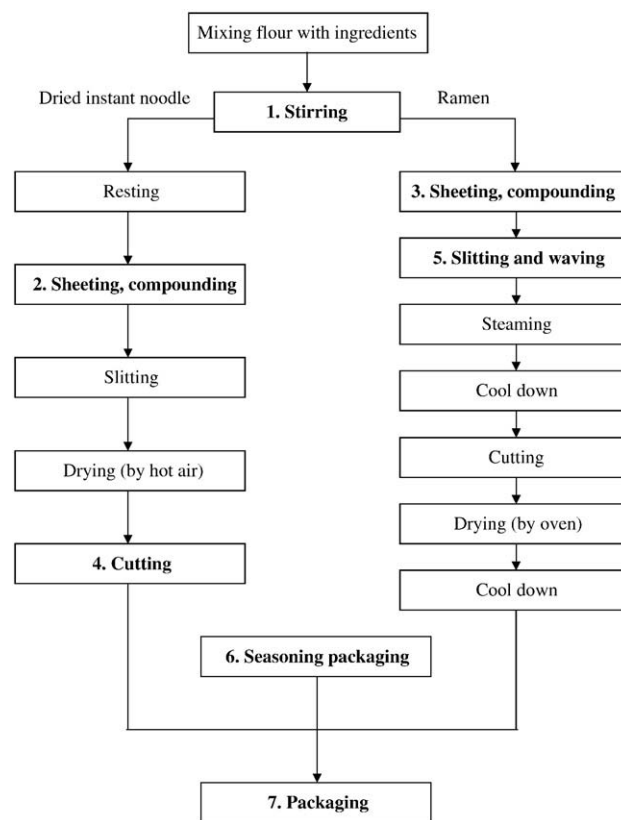


Fig. 1 – Flow chart of the noodle production process in the factory under study. (Air sampling for microbiological analyses are taken from area indicated in bold).

dough is passed through two rollers to produce two sheets, which later are combined. After adjusting its thickness, the sheet is cut and molded into blocks or other suitable shapes. The next step is drying and testing for moisture, shape, and other quality characteristics, followed by packing with various seasonings. Finally, unused noodles are collected and mechanically crushed at an assigned site for recycling.

2.2. Air sampling locations

Parallel outdoor and indoor air samples were taken simultaneously. Air samples were collected from the following sites: (1) stirring site, (2) dried instant noodle sheeting and compounding site, (3) ramen sheeting and compounding site, (4) dried instant noodles cutting site, (5) ramen slitting and waving site, (6) seasoning packaging site, (7) packaging site, (8) the unused noodle crushing site (the crushing site), (9) warehouse site, (10) staff offices, and (11) outdoors. This noodle factory was constructed and divided into clean areas, quasi-clean areas, and general areas (also called contaminated areas). The clean areas included the seasoning packaging site and packaging site, and quasi-clean areas included the mixing and stirring sites. Sites 8, 9, 11 were grouped to general areas. The requirement for cleanliness in the clean area was highest, followed by the quasi-clean areas, and finally, the general areas. The total workforce at all of these sites was 47 workers. Air samples collected from the staff office were taken as the control, whereas samples collected from sites 1 to 10 were indoor air samples. Air samples collected at sites 3, 5, 6, and 7 were defined as ‘with ventilation system’ samples, while air samples from sites 1, 2, 4, 9, and 10 were defined as ‘without ventilation system’ samples. The distance between workers and the air sample collection points was always less than 1 m.

2.3. Air sampling of bioaerosols

First, a pilot study of the sampling times for fungi and bacteria was conducted in the 11 sampling locations. Each air sample was collected over a three-minute period, except for the samples from the crushing site and the ramen sheeting and compounding site, which were collected over 0.5 min and 1 min periods, respectively, because they appeared to adequately represent the overall pattern of exposure concentrations. Each trial involved two time periods, namely morning (8:00–11:00) and afternoon (13:00–17:00). However, the samples from the crushing site were collected from 8 PM to 8 AM because that was the time for crushing. Triplicate samples were taken at each sampling site, providing a total of 1512 samples.

Outdoor and indoor air samples were collected at a height of 1.5 m above ground level to coincide with the worker breathing zone. An exception was made at the packaging site and staff office, where samples were collected at a height of 0.85 m, since the workers were seated. This survey was comprised of 12 sampling trials, one during each season. Sampling trials were carried out from December 2006 to November 2007. According to the weather data, including relative humidity and average temperature, the seasons in Taiwan can be classified as follows, spring (March–May),

summer (June–August), autumn (September–November) and winter (December–February). The meteorological factors, including temperature (°C) and relative humidity (RH) (%), were recorded using a Thermo Hygrometer (SHINYEI CTH-888, Osaka, Japan) during each sampling event.

Airborne bioaerosols were collected with a microbiological air sampler (MAS-100) consisting of a plate containing 400 holes with cut diameter of 0.74 μm (Merck, Darmstadt, Germany). The MAS-100 is based on the principles described by Andersen. It is comprised of a high-performance suction pump and continuously monitors the incoming air flow. Regulation of the aspirated air to a constant flow follows (Buttner et al., 2002). Air samples were collected at a flow rate of 100.0 l min⁻¹ and impacted on 90-mm Petri dishes containing malt extract agar (MEA) or trypticase soy agar (TSA), for sampling fungi and total viable bacteria, respectively.

To investigate the size distribution of the bioaerosols in the general areas, namely the warehouse, outdoors, and at the crushing sites, a six-stage Andersen impactor (Andersen Sampler Inc. Atlanta, CA) was employed for sampling at each of the three sites. The Andersen 6-stage sampler was a cascade impactor with 400-holes per stage, drawing in air at a flow rate of 28.3 l min⁻¹. Airborne particles were separated into six fractions on the basis of their 50% cut diameters in the six stages: $\geq 7.0 \mu\text{m}$ (stage 1), 7.0–4.7 μm (stage 2), 4.7–3.3 μm (stage 3), 3.3–2.1 μm (stage 4), 2.1–1.1 μm (stage 5), and 1.1–0.65 μm (stage 6).

2.4. Bioaerosol analysis

The fungal (MEA) and bacterial plates (TSA) were incubated at 25 °C for 3–5 days and 37 °C for 2 days, respectively. First, plates that were either contaminated or had produced considerable overgrowth were excluded. The resultant colonies were counted using visual inspection and converted to an effective count by the “positive-hole” correction table (Feller, 1950). Concentrations of bioaerosols were measured and expressed as colony forming units per cubic meter of air (CFU/m³). Fungal isolates were identified according to colony appearance, spore and morphological characteristics (De Hoog et al., 2000; Larone, 2002). Fungi that were subcultured into pure cultures that did not sporulate after 14 days were described as non-sporulating isolates (St. Germain and Summerbell, 1996; Barnett and Hunter, 1999). Bacterial species were identified using microscopic and biochemical methods, as recommended by Bergey’s Manual. The selected bacterial isolates were identified using microtests such as API (Analytical Profile Index) system 50 CHB, STAPH, 20E, 20NE, and CORYNE. The isolation frequency of fungi or bacteria was defined as the percentage of isolates of individual species in relation to the total number of isolates.

2.5. Statistical analysis

Three-Way Analysis of Variance (ANOVA) was performed, including all interactions between independent variables (season, sampling period and the presence or absence of ventilation systems). Non-parametric Mann–Whitney tests and Kruskal–Wallis tests were applied to compare data sets. The level of significance was set at 0.05. Statistical analyses

were carried out using the SPSS statistical software package (Version 10.0 for Windows).

3. Results

3.1. Concentration and identification of airborne bacteria at different sampling sites

During this study, the mean outdoor temperature was 30.0–36.9 °C, and the mean indoor temperature within the noodle factory was 18.5–31.9 °C. At the sampling sites, the highest mean temperature (31.9 °C) occurred at the ramen slitting and waving site in autumn. The lowest mean temperature, 18.5 °C, was recorded at the seasoning packaging site in spring because this site used two air conditioning units. As for the RH, the indoor average RH was 43.1–75.7% and the outdoor average RH was 33.1–55.8%. The average RH was lower during winter than the other seasons. The lowest RH was 43.1% and occurred at the office site in winter, and the highest RH (75.7%) occurred at ramen slitting and waving site during summer. One of the main reasons for this phenomenon was significant vapor formation during noodle steam molding.

Staphylococcus xylosus, *Micrococcus* spp. and *Staphylococcus arlettae* were the dominant bacterial isolates found in the indoor environment, with relative frequencies of 22.3%, 19.1% and 9.9%, and mean values of 196, 109 and 75 CFU/m³, respectively (Table 1). The remaining bacterial isolates were *Staphylococcus cohnii*, *Staphylococcus kioosii*, *Staphylococcus intermedius*, *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus sciuri*, *Brevibacterium* spp. and *Bacillus firmus*, which varied in relative frequencies ranging from 8.0% to 0.8%. As for the bacterial isolates from the external environment, *S. xylosus*, *Micrococcus* spp. and *S. cohnii* were the predominant species, with relative frequencies of 18.0%, 12.1% and 9.9%, and with mean values of 62, 26 and 20 CFU/m³, respectively. Other bacterial isolates, in decreasing order of frequency from 8.9% to 0.7%, included *S. arlettae*, *S. aureus*, *B. cereus*, *S. sciuri*, *S. kioosii*, *Brevibacterium* spp. and *B. firmus*. Overall, *Micrococcus* spp. and *S. xylosus* were the dominant bacterial isolates obtained from indoor and

outdoor air samples in this study. However, few Gram-negative bacteria were identified in this survey. *Aeromonas hydrophila*, *Aeromonas sobria* and *Pseudomonas aeruginosa* were the dominant Gram-negative bacteria identified in indoor environments and had low relative frequencies.

The concentrations of most bacteria, apart from *S. cohnii*, *Brevibacterium* spp., *S. sciuri* and *B. firmus*, differed significantly among all the sampling sites (Table 2). The highest airborne bacterial concentration was measured at the crushing site. The bacterial concentrations before and during the crushing work periods, with mean values of 747 and 9024 CFU/m³, were statistically different according to the Mann–Whitney test. Furthermore, the peak bacterial concentration for all isolates except *Brevibacterium* spp. and *S. sciuri* was found at the crushing site. Significant seasonal differences in total bacterial concentration were observed among all the sampling sites (Table 5). For most sampling sites, the mean bacterial concentrations were higher in spring compared to those found during other seasons. At the site where the dried instant noodles were cut, the peak bacterial concentration occurred in summer, whereas the peak concentrations at the packaging and seasoning packaging sites occurred in winter.

3.2. Concentration and identification of airborne fungi at different sampling sites

A total of 49,629 fungi colonies were isolated, and the cultured fungi were classified as *Cladosporium*, *Penicillium*, *Aspergillus*, *Fusarium*, *Mucor*, *Acremonium*, *Alternaria*, *Rhizopus*, *Syncephalastrum*, yeast and non-sporulating species (Table 3). The mean concentration of total indoor fungi was 347 CFU/m³ (2144 to 42 CFU/m³). Meanwhile, the dominant indoor fungi identified were *Cladosporium* (41.2%), *Penicillium* (21.4%) and *Aspergillus* (17.5%), with mean concentrations of 148, 69 and 69 CFU/m³, respectively. The remaining fungi, which varied in relative frequency from 8.1% to 0.1%, were non-sporulating, yeast, *Mucor*, *Fusarium*, *Syncephalastrum*, *Rhizopus* and *Acremonium*. Analysis of outdoor air samples showed that *Cladosporium* (71.1%), *Penicillium* (8.0%) and *Aspergillus* (7.0%) were also the predominant fungal isolates, with mean concentrations of

Table 1 – The relative frequency (%), range, and mean concentrations of bacteria (CFU/m³) in indoor and outdoor air samples

Species	Relative frequency (%)		Mean ^a /range	
	Indoor (N=684)	Outdoor (N=72)	Indoor (N=684)	Outdoor (N=72)
Total bacteria	100.0	100.0	706/320.6–1091.4	354/0.0–739.4
<i>Staphylococcus xylosus</i>	22.3	18.0	196/153.7–238.3	62/0.0–128.5
<i>Micrococcus</i> spp.	19.1	12.1	109/59.1–158.9	26/7.1–33.1
<i>Bacillus cereus</i>	4.2	7.6	25/9.8–40.2	17/2.2–31.8
<i>Staphylococcus arlettae</i>	9.9	8.9	75/40.3–109.7	16/0.0–40.8
<i>Staphylococcus aureus</i>	2.7	8.1	61/17.0–105.0	11/0.0–28.6
<i>Staphylococcus cohnii</i>	8.0	9.9	95/58.3–131.7	20/0.0–51.2
<i>Staphylococcus kioosii</i>	7.7	4.3	74/32.7–115.3	15/0.0–40.0
<i>Staphylococcus intermedius</i>	4.5	0.0	56/28.0–84.0	0/0.0–0.0
<i>Staphylococcus sciuri</i>	1.8	4.7	12/0.0–25.6	10/4.9–15.1
<i>Brevibacterium</i> spp.	1.2	3.8	23/8.9–37.1	24/11.7–36.3
<i>Bacillus firmus</i>	0.8	0.7	21/4.7–47.3	6/0.0–18.2

^a Each value is the mean of samplings.

Table 2 – Total culturable bacteria and some selected bacteria genera from eleven sampling sites^a over the four seasons

Species	Mean concentration of bacteria (CFU/m ³) ^b											P-value ^c
	1	2	3	4	5	6	7	8	9	10	11	
<i>Micrococcus</i> spp.	79	54	68	82	35	29	52	926	52	29	26	<0.0001*
<i>Staphylococcus xylosus</i>	76	50	93	84	33	22	51	2203	73	28	62	<0.0001*
<i>Bacillus cereus</i>	10	9	11	14	17	15	9	168	21	7	17	<0.0001*
<i>Staphylococcus arlettae</i>	62	31	42	35	21	21	38	1339	32	0	36	<0.0001*
<i>Staphylococcus aureus</i>	48	44	30	57	19	6	13	623	57	12	33	<0.0001*
<i>Staphylococcus cohnii</i>	62	80	38	72	67	6	26	1113	0	0	60	0.1191
<i>Staphylococcus kioosii</i>	48	40	59	84	29	14	31	818	19	11	34	<0.0001*
<i>Staphylococcus sciuri</i>	10	7	8	0	40	11	10	8	11	0	10	0.0281*
<i>Staphylococcus intermedius</i>	50	27	66	45	13	17	52	551	19	7	0	<0.0001*
<i>Brevibacterium</i> spp.	18	13	14	13	30	0	18	40	41	0	24	0.4750
<i>Bacillus firmus</i>	86	28	24	17	11	14	0	940	8	0	29	0.4010

^a Sites 1–11 are assigned as (1) stirring site, (2) dried instant noodle sheeting and compounding site, (3) ramen sheeting and compounding site, (4) dried instant noodles cutting site, (5) ramen slitting and waving site, (6) seasoning packaging site, (7) packaging site, (8) the unused noodle crushing site (the crushing site), (9) warehouse site, (10) staff offices, and (11) outdoors.

^b Each value is the mean of 72 samples except site 8 is the mean of 36 samples.

^c The differences among seasons are compared by nonparametric Kruskal–Wallis test (*P<0.05).

286, 40 and 34 CFU/m³, respectively. Other fungal isolates decreased in frequency from 4.9% to 0.1%, and were identified as non-sporulating species, *Mucor*, *Fusarium*, yeast, *Syncephalastrum*, *Rhizopus* and *Acremonium*. In conclusion, *Cladosporium*, *Penicillium* and *Aspergillus* were the dominant fungal isolates in both indoor and outdoor air samples.

All of the fungi genera identified, except *Rhizopus* and *Syncephalastrum*, reached peak concentrations at the crushing site (Table 4). The total fungal concentration reached its maximal value at the crushing site compared to other sampling sites. The difference in fungal concentration before and during crushing (with mean concentrations of 1164 and 2010 CFU/m³) is statistically significant based on the Mann–Whitney test (*P<0.05). Regardless of sampling site, significant differences were found between the total fungal concentrations (Table 5). Furthermore, fungi concentrations at each sampling site were higher during spring than the other three seasons, except for at the crushing site. The concentration of airborne fungi, in particular, exceeded 1000 CFU/m³ at the warehouse site in

spring and at the crushing site during spring, summer and autumn.

3.3. Effects of environmental factors on the distribution of airborne bioaerosols

To examine the relationship between environmental factors and culturable bacterial and fungal genera, ANOVAs were performed and the results are listed in Tables 6 and 7, respectively. As shown in Table 6, significant seasonal differences were observed for all the bacterial genera isolated. Only the concentrations of *Micrococcus* spp. and *S. arlettae* differed significantly between the two sampling periods. Clearly, concentrations of bacteria were lower at sites with a ventilation system than at those without a ventilation system. For instance, the levels of *Micrococcus* spp., *S. xylosus*, *S. aureus* and *Acinetobacter* differed significantly.

As shown in Table 7, significant seasonal differences were observed for most of the fungal genera (*P<0.05), with only

Table 3 – The relative frequency (%), range, and mean concentrations of fungi (CFU/m³) in indoor and outdoor air samples

Species	Relative frequency (%)		Mean ^a /range	
	Indoor (N=684)	Outdoor (N=72)	Indoor (N=684)	Outdoor (N=72)
Total fungi	100	100	347/0.0–916.9	410/0.0–827.2
<i>Cladosporium</i>	41.2	71.1	148/0.0–418.5	286/2.9–569.1
<i>Penicillium</i>	21.4	8.0	69/0.0–185.4	40/2.7–77.3
<i>Aspergillus</i>	17.5	7.0	69/0.0–282.9	34/0.0–72.5
Non-sporing	8.1	4.9	43/0.0–161.2	25/6.4–43.6
Yeast	4.7	1.1	15/3.0–27.0	19/0.0–38.1
<i>Mucor</i>	1.4	1.4	11/0.0–26.5	10/4.7–15.3
<i>Fusarium</i>	0.8	1.4	8/1.7–14.3	13/2.8–26.3
<i>Syncephalastrum</i>	0.1	0.4	5/1.8–8.2	10/5.8–14.2
<i>Rhizopus</i>	0.1	0.1	6/2.7–9.3	3/3.0–3.0
<i>Acremonium</i>	0.1	0.1	6/2.3–9.7	3/3.0–3.0

^a Each value is the mean of samplings.

Table 4 – Total culturable fungi and some selected fungi genera from eleven sampling sites^a over the four seasons

Species	Mean concentration of fungi (CFU/m ³) ^b											P-value ^c
	1	2	3	4	5	6	7	8	9	10	11	
<i>Cladosporium</i>	64	152	92	97	31	30	39	573	500	39	286	<0.0001*
<i>Penicillium</i>	73	76	81	87	51	13	27	315	48	12	40	<0.0001*
<i>Aspergillus</i>	85	43	32	46	28	9	26	495	49	18	34	<0.0001*
Non-sporing	18	27	18	25	30	10	15	303	35	16	25	<0.0001*
Yeasts	17	20	14	19	18	7	14	40	10	13	19	<0.0001*
<i>Mucor</i>	6	7	9	6	15	9	7	50	8	8	10	<0.0001*
<i>Fusarium</i>	5	6	6	6	13	3	3	27	11	8	13	<0.0001*
<i>Rhizopus</i>	6	3	7	6	0	0	3	0	9	0	3	0.0727
<i>Alternaria</i>	4	6	6	4	27	3	3	30	3	4	5	0.0008*
<i>Syncephalastrum</i>	6	7	3	5	9	4	3	0	5	0	10	<0.0001*
Unknown	12	15	14	15	28	7	9	110	20	9	16	<0.0001*

^a Sites 1–11 are assigned as the same as Table 2.^b Each value is the mean of 72 samples except site 8 is the mean of 36 samples.^c The differences among seasons are compared by nonparametric Kruskal–Wallis test (**P*<0.05).

Fusarium showing no significant seasonal differences. In addition, only the levels of *Aspergillus* and *Rhizopus* showed significant differences during the two sampling periods. Regarding the ventilation system factor, the concentrations of fungi at sites with ventilation systems were lower than at sites without ventilation systems. The difference in the levels of most of the identified fungi between sites with and without ventilation systems reached a level of significance. Nevertheless, the ventilation system did not exhibit significant influence on the levels of *Mucor*, *Alternaria*, *Acremonium* and yeast (Table 7).

3.4. Particle size distribution of airborne bacteria and fungi

Among the different sampling sites, the peak airborne bio-aerosol concentration occurred at the crushing site, followed by the warehouse and outdoor sites. These three areas were

grouped into general areas. Since the levels of airborne bio-aerosols at the crushing site exceeded the guideline of 1000 CFU/m³ proposed by NIOSH, a further examination of the particle size distribution of bioaerosols was performed. As illustrated in Fig. 2, most of bacteria at the crushing site were in the size range of 1.1–0.65 µm (21.2%), while the least prevalent size range was 7.0–4.7 µm (11.3%). When the season was considered, it was found that the largest proportion of bacteria at the crushing site were in 2.1–1.1 µm size range during winter, spring and summer, and in 3.3–2.1 µm during autumn, with mean values of 1746, 1903, 1861, and 1472 CFU/m³, respectively. Fig. 2 showed that the highest and lowest proportions of bacteria by size category for the warehouse site fell in the ranges of ≥7.0 µm (22.7%) and 2.1–1.1 µm (11.7%), respectively. Regarding the seasons, the dominant size distributions for total bacteria at the warehouse site occurred in the ranges of ≥7.0 µm, 7–4.7 µm, 4.7–3.3 µm and 1.1–0.65 µm, in winter,

Table 5 – Seasonal concentrations of airborne bacteria and fungi at different sites

Sampling sites	Mean concentration of bacteria ^a (CFU/m ³)				P-value ^b	Mean concentration of fungi ^a (CFU/m ³)				P-value ^b
	Winter (n=189)	Spring (n=189)	Summer (n=189)	Autumn (n=189)		Winter (n=189)	Spring (n=189)	Summer (n=189)	Autumn (n=189)	
Stirring	323	578	323	291	0.0235*	183	447	177	283	0.0007*
Dried instant noodles sheeting and compounding	128	413	293	227	<0.0001*	160	612	194	287	<0.0001*
Ramen noodles sheeting and compounding	210	332	340	432	<0.0001*	175	456	101	262	<0.0001*
Dried instant noodles cutting site	139	421	491	436	0.0001*	163	513	134	306	<0.0001*
Ramen noodles slitting and waving	132	201	190	131	0.0378*	110	302	194	179	0.0002*
Seasoning packaging	129	36	66	85	<0.0001*	46	86	57	23	<0.0001*
Packaging	255	139	186	186	0.0189*	62	168	84	102	0.0010*
The unused noodles crushing	7424	12616	8204	7851	0.0112*	907	2144	3082	1907	<0.0001*
Warehouse	219	581	292	203	<0.0001*	732	1592	209	446	<0.0001*
Staff office	90	93	62	104	<0.0001*	92	101	42	86	<0.0001*
Outdoors	351	675	271	119	<0.0001*	395	882	166	199	<0.0001*

^a Each value is the mean of all samplings.^b The differences are compared by nonparametric Kruskal–Wallis test (**P*<0.05).

Table 6 – Factors affecting airborne bacteria concentrations^a (CFU/m³): result of three-way ANOVA

Species	Season					Sampling period			Ventilation system		
	Winter (n=162)	Spring (n=162)	Summer (n=162)	Autumn (n=162)	P ^b	Morning (n=324)	Afternoon (n=324)	P ^b	Yes (n=288)	No (n=360)	P ^b
<i>Micrococcus</i> spp.	70 (14.3–125.7)	49 (6.3–91.7)	65 (2.6–127.4)	43 (16.2–69.8)	<0.0001*	61 (4.9–117.1)	51 (8.7–93.3)	0.0479*	48 (6.4–89.6)	62 (7.3–117.7)	0.0006*
<i>Staphylococcus xylosus</i>	52 (19.2–84.8)	54 (6.4–101.6)	62 (13.8–110.2)	62 (24.8–99.2)	<0.0001*	59 (15.5–102.5)	57 (15.9–98.1)	0.7460	51 (7.4–94.6)	65 (25.0–105.0)	0.0111*
<i>Bacillus cereus</i>	15 (0.0–36.8)	8 (2.2–13.8)	12 (0.0–26.7)	15 (0.2–29.8)	<0.0001*	14 (0.0–30.3)	12 (0.0–25.9)	0.3814	13 (0.0–29.5)	13 (0.0–27.2)	0.2996
<i>Staphylococcus arlettae</i>	42 (11.8–72.2)	32 (0.0–85.5)	0 (0.0–0.0)	0 (0.0–0.0)	<0.0001*	49 (0.0–102.7)	26 (0.0–62.5)	0.0192*	33 (0.8–65.2)	41 (0.0–92.0)	0.4772
<i>Staphylococcus aureus</i>	13 (3.8–22.2)	49 (1.8–96.2)	22 (0.0–60.3)	0 (0.0–0.0)	<0.0001*	46 (0.0–110.5)	27 (0.0–54.1)	0.0532	19 (1.7–33.3)	50 (0.0–102.7)	0.0001*
<i>Staphylococcus cohnii</i>	0 (0.0–0.0)	52 (18.8–85.2)	0 (0.0–0.0)	0 (0.0–0.0)	<0.0001*	49 (19.9–78.1)	56 (19.6–92.4)	0.6499	37 (7.5–66.5)	72 (45.1–98.9)	0.4919
<i>Staphylococcus kioosii</i>	0 (0.0–0.0)	0 (0.0–0.0)	41 (5.5–76.5)	37 (0.0–86.3)	<0.0001*	40 (0.0–82.7)	39 (0.0–80.7)	0.7071	35 (0.0–72.0)	43 (0.0–88.5)	0.1124
<i>Staphylococcus sciuri</i>	12 (0.0–25.6)	0 (0.0–0.0)	0 (0.0–0.0)	0 (0.0–0.0)	<0.0001*	11 (4.1–17.9)	14 (0.0–30.3)	0.1619	14 (0.0–30.0)	9 (2.3–15.7)	0.0712
<i>Staphylococcus intermedius</i>	0 (0.0–0.0)	0 (0.0–0.0)	0 (0.0–0.0)	41 (13.0–69.0)	<0.0001*	37 (11.0–63.0)	45 (15.5–74.5)	0.5034	48 (15.6–80.4)	36 (12.5–59.5)	0.5574
<i>Brevibacterium</i> spp.	0 (0.0–0.0)	0 (0.0–0.0)	21 (6.9–35.1)	0 (0.0–0.0)	<0.0001*	18 (9.3–27.7)	25 (7.1–42.9)	0.6379	21 (12.1–29.9)	21 (5.4–37.6)	0.0814
<i>Bacillus firmus</i>	29 (0.8–57.2)	0 (0.0–0.0)	0 (0.0–0.0)	0 (0.0–0.0)	<0.0001*	29 (5.4–52.6)	29 (0.0–60.9)	0.9345	16 (7.5–24.5)	38 (4.5–71.5)	0.0575
<i>Acinetobacter</i>	0 (0.0–0.0)	0 (0.0–0.0)	32 (6.2–57.8)	0 (0.0–0.0)	<0.0001*	30 (4.2–55.8)	32 (6.2–57.8)	0.5844	22 (5.7–38.3)	38 (9.8–67.2)	0.0485*

^a Each value is the mean of all samplings except for the samples from the crushing site and outdoor.

^b The differences are compared by three-way ANOVA (*P<0.05).

Table 7 – Factors affecting airborne fungi concentrations^a (CFU/m³): result of three-way ANOVA

Species	Season					Sampling period			Ventilation system		
	Winter (n=162)	Spring (n=162)	Summer (n=162)	Autumn (n=162)	P ^b	Morning (n=324)	Afternoon (n=324)	P ^b	Yes (n=288)	No (n=360)	P ^b
<i>Cladosporium</i>	116 (0.0–295.4)	232 (0.0–365.9)	25 (0.0–61.4)	69 (0.0–174.7)	<0.0001*	129 (0.0–362.3)	109 (0.0–347.6)	0.3813	46 (0.0–120.7)	187 (0.0–492.6)	<0.0001*
<i>Penicillium</i>	29 (6.1–51.9)	63 (0.8–125.2)	26 (7.1–44.9)	102 (0.0–271.7)	<0.0001*	49 (0.0–122.8)	62 (0.0–176.0)	0.1248	45 (0.0–133.4)	63 (0.0–164.2)	0.0080*
<i>Aspergillus</i>	26 (0.0–53.5)	70 (0.0–205.9)	43 (3.9–82.1)	23 (3.8–42.2)	<0.0001*	46 (0.0–141.4)	34 (0.0–7.3)	0.0432*	25 (0.0–52.7)	52 (0.0–143.8)	<0.0001*
Non-sporing	12 (6.4–17.6)	18 (6.0–30.0)	19 (3.2–34.8)	32 (8.2–55.8)	<0.0001*	23 (2.5–43.5)	21 (4.4–37.6)	0.3136	17 (3.4–30.6)	25 (4.4–45.6)	<0.0001*
Yeast	12 (4.2–19.8)	14 (4.3–23.7)	19 (2.0–36.0)	14 (6.3–21.7)	0.0069*	14 (5.7–22.3)	16 (2.1–29.9)	0.0518	13 (4.4–21.6)	17 (2.8–31.2)	0.1680
<i>Mucor</i>	7 (2.9–11.1)	7 (2.6–11.4)	10 (3.0–17.0)	7 (3.3–10.7)	<0.0001*	8 (2.5–13.5)	7 (1.8–12.2)	0.3869	9 (2.1–15.9)	7 (2.9–11.1)	0.5892
<i>Fusarium</i>	8 (3.2–12.6)	9 (1.2–16.8)	7 (3.6–10.4)	7 (2.0–12.0)	0.2379	8 (1.6–14.4)	7 (2.6–11.4)	0.9809	6 (1.8–10.2)	8 (2.3–13.7)	<0.0001*
<i>Rhizopus</i>	3 (3.0–3.0)	7 (4.4–9.6)	6 (2.5–9.5)	0 (0.0–0.0)	0.0256*	7 (3.8–11.2)	5 (2.3–7.7)	0.0446*	6 (2.9–9.1)	6 (2.7–9.3)	0.0292*
<i>Alternaria</i>	0 (0.0–0.0)	0 (0.0–0.0)	7 (0.0–14.9)	5 (2.8–7.2)	<0.0001*	6 (0.2–11.8)	7 (0.0–14.7)	0.6617	9 (0.0–19.2)	4 (2.5–5.5)	0.2498
<i>Syncephalastrum</i>	6 (3.0–9.0)	5 (1.7–8.3)	0 (0.0–0.0)	0 (0.0–0.0)	<0.0001*	4 (2.1–5.9)	7 (3.3–10.7)	0.0927	5 (2.1–7.9)	6 (1.4–8.6)	0.0112*
<i>Acremonium</i>	6 (2.6–9.7)	0 (0.0–0.0)	0 (0.0–0.0)	0 (0.0–0.0)	<0.0001*	7 (2.9–11.1)	6 (2.8–9.2)	0.7265	8 (4.7–11.3)	6 (2.8–9.2)	0.2163

^a Each value is the mean of all samplings except for the samples from the crushing site and outdoor.

^b The differences are compared by three-way ANOVA (*P<0.05).

spring, summer and autumn, with mean values of 707, 748, 766 and 730 CFU/m³, respectively. The most prevalent and least prevalent size ranges for bacteria occurring in the external environment were ≥ 7.0 μm (27.1%) and 1.1–0.65 μm (9.8%), respectively. The dominant size ranges for total outdoor bacteria were in the ranges of ≥ 7.0 μm , 2.1–1.1 μm , 7–4.7 μm and ≥ 7.0 μm , in winter (919 CFU/m³), spring (3121 CFU/m³), summer (736 CFU/m³) and autumn (636 CFU/m³), respectively.

As illustrated in Fig. 3, the highest and lowest proportions of fungi at the crushing site fell within 2.1–1.1 μm (42.4%) and 7.0–4.7 μm (6%) size ranges, respectively. Regardless of the season, most fungi at the crushing site were in the size range of 2.1–1.1 μm , with values of 1078, 3003, 3204 and 1095 CFU/m³, in winter, spring, summer and autumn, respectively. Additionally, the highest and lowest proportions of total fungi at the warehouse site occurred in the size ranges of 2.1–1.1 μm (30.7%) and 1.1–0.65 μm (7.7%), respectively. During winter, spring and summer, the highest proportions of fungi at the warehouse site were all in the size range of 2.1–1.1 μm , with mean concentrations of 574, 7615 and 942 CFU/m³, respectively. During autumn at this site, the highest proportion of fungi was found to be in the size range of 3.3–2.1 μm , with a mean concentration of 1148 CFU/m³.

Even though the highest proportion of fungi fell in 2.1–1.1 μm size range for outdoor air samples, the proportion of fungi in the size range of 3.3–2.1 μm (52.8%) was almost equal to that in the size range of 2.1–1.1 μm (52.5%). The lowest proportion of total culturable fungi obtained from outdoor air samples fell within the size range of 1.1–0.65 μm (6.9%). Clearly, the size distributions of the total culturable fungi obtained from the warehouse site and the outdoor environment were identical. Due to the short distance between the outdoor and warehouse sampling sites, airborne fungi from the external environment sometimes influenced the concentration of airborne organisms in the internal environment. Meanwhile, the highest proportion of total outdoor fungi occurred in the size range of 2.1–1.1 μm , and appeared in spring (4841 CFU/m³) and summer (1113 CFU/m³). However, the highest proportions of total outdoor fungi fell within the

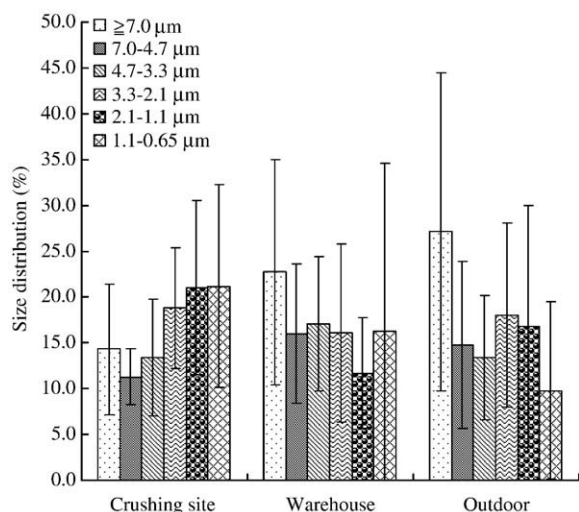


Fig. 2– The size distribution (%) of total bacterial at the crushing site, warehouse, and outdoors.

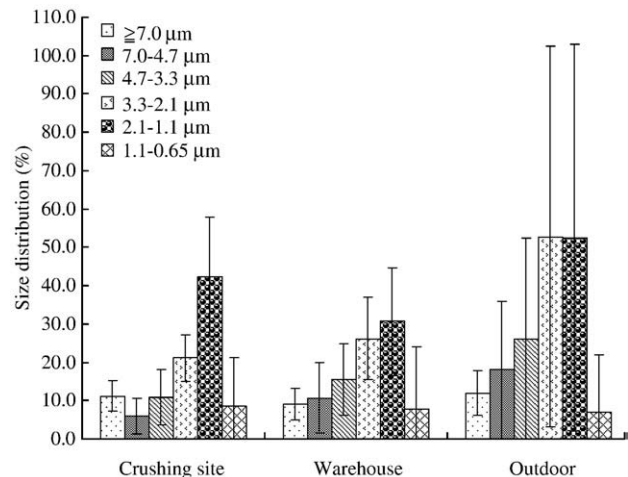


Fig. 3– The size distribution (%) of total fungi at the crushing site, warehouse, and outdoors.

size range of 3.3–2.1 μm , and occurred in winter (1608 CFU/m³) and autumn (1213 CFU/m³).

4. Discussion

According to the current measurements, fungal concentrations measured for most of the internal environment fell between 100 CFU/m³ and 1000 CFU/m³. The levels of fungi in clean areas such as the seasoning packaging site and packaging site were the lowest, followed by those in quasi-clean areas including the mixing and stirring sites. As for general areas, fungal concentrations at the warehouse site in spring and at the crushing site during spring, summer and autumn exceeded the guideline of 1000 CFU/m³ recommended by ACGIH (1989). In this study, the frequency of common allergy-inducing molds was ordered as follows: *Cladosporium*, *Penicillium* and *Aspergillus*, in both indoor and outdoor environments. Since airborne fungal spores could penetrate doors and windows, it was proposed that fungal flora indoors represented the outdoor atmosphere (Chew et al., 2003). These findings were also supported by Abdel Hameed's (2007) study, where *Aspergillus* and *Penicillium* were the major fungal types found in the interior of a flour mill, while *Cladosporium* and *Aspergillus* were the main fungi found outside the mill.

In addition, the Commission of the European Community (CEC) has established that fungal concentrations below 500 CFU/m³ be classified as low, between 500 and 999 CFU/m³ be intermediate, and above 1000 CFU/m³ be high (CEC, 1993). In our study, the level of *Cladosporium* at the crushing site exceeded intermediate values, and the *Aspergillus* concentration reached intermediate values, as did the *Cladosporium* concentration at the warehouse site. The high concentration of *Cladosporium* occurring at the crushing site can be expected to adversely affect employee health.

In this study, *Micrococcus* spp. and *S. xyloso* were the dominant bacterial isolates occurring within the indoor environment. This result is similar to the findings of a study by Abdel Hameed, (2007), in which *Micrococcus* spp. and

Staphylococci were the major bacteria types in the flour mill. In this survey, the total concentrations of culturable bacteria at the warehouse site and the stirring site during spring, and at the crushing site during all four seasons exceeded the guideline of 500 CFU/m³ proposed by ACGIH (1986). Accordingly, in this factory, there should be increased concern about the biohazards for workers at the warehouse site during spring and at the crushing site during all four seasons.

Many factors influence concentrations of culturable bioaerosols, such as relative humidity, temperature, different work procedures, sunlight, sampling time, seasons and the ventilation system. Meanwhile, relative humidity and temperature were regarded as the major factors since they have an important effect on the release and dispersal of fungal spores, and high average temperature and higher relative humidity favor microbiological growth (Jones and Harrison, 2004; Jo and Seo, 2005). In this investigation, high levels of total fungi occurred in spring, likely due to the average indoor temperature of 18.5–31.9 °C and the average indoor RH of 43.1–75.7%. This result was supported by Lin and Li (2000), who found that microbiological growth was favored by moderate temperatures of 25–30 °C and moderate relative humidity of 60–70%. With regard to the relationship between seasonal factors and total airborne fungal concentrations, a clear seasonal variation pattern for airborne fungi was observed at each sampling site. Except for the crushing site, high levels of total culturable fungi were observed during spring, regardless of the sampling site. As for mesophilic bacteria, because they were active at temperatures approaching ambient temperature ($T \leq 35$ °C) and were not affected by seasonal changes, high levels of these bacteria were observed in spring for most of the sites. This phenomenon can be attributed to the rainy season that occurs in the spring time in Taiwan, which often leads to high relative humidity in the air. At higher relative humidity, microbial growth was initiated more rapidly, even at lower temperatures. Meanwhile, common airborne molds including *Cladosporium* and *Aspergillus* were more prevalent inside the noodle factory during spring. This result indicated that workers in the studied factory potentially suffered from allergy symptoms during spring.

Because most of this study was conducted indoors, the operation procedure taking place in a given area is another factor affecting the levels of culturable bioaerosol. As a result, the crushing procedure generated higher levels of bioaerosol than other operational procedures in the investigated factory. Although the highest mean temperatures (31.9 °C) and the highest RH (75.7%) occurred at the slitting and waving sites of ramen, the peak concentrations of culturable fungi and bacteria among the 11 sampling sites were found at the crushing site, which exhibited mean levels of 3082 CFU/m³ in summer and 12,616 CFU/m³ in spring. The peak fungi concentration in this study was lower than in other studies, with a maximum level of 1.7×10^4 CFU/m³ during grain crushing (Lugauskas et al., 2004). This lower peak fungi concentration could be attributed to different geographic locations, samplers, media, and sampling approaches (Adhikari et al., 2004). When considering the same working operation procedures, the sheeting sites for both dried instant noodles and ramen in spring had mean concentrations of fungi of 612 and 456 CFU/m³, however, the sites had mean temperatures of 22.2 °C and 25.2 °C, and a mean RH of 72.1% and

64.9%, respectively. This result indicated that levels of fungi were more closely associated with relative humidity than temperature for the same working operation procedures.

The indoor environment in the investigated factory was illuminated by numerous windows. Sunlight penetrated the windows and thus, sunlight radiation was one of the factors influencing the concentrations of culturable bacteria (Shaffer and Lighthart, 1997). In this study, high average temperatures and intense radiation were measured at the first sampling time in the morning (around 10 AM) and culturable bacteria were damaged by UV radiation, the bacterial counts should have been low. However, higher bacterial concentrations occurring in the morning was consistent with other reports (Lighthart and Shaffer, 1995; Zhu et al., 2003; Fang et al., 2007). This result can be attributed to the first sampling time roughly coincided with the busiest time in the morning for the noodle factory workers, resulting in high bacterial counts. In this study, all the bacterial isolates except *Micrococcus* spp. and *S. arlettae* exhibited significant differences in concentration between the two sampling times. In addition, very few Gram-negative bacteria were found in this investigated noodle factory. Although Gram-negative bacteria were naturally present in great abundance on flours, endotoxins may still exist. One of the main reasons for this phenomenon was that almost all the bacterial cells were killed during the production process.

With respect to the diurnal periodicity, the incidence of certain fungi was higher at specific times of the day. In our study, levels of *Aspergillus* and *Rhizopus* demonstrated a significant diurnal variation, while the levels of *Cladosporium*, *Penicillium*, and *Alternaria* did not. It was reported that sunlight was required to initiate ascospore discharge in many bitunicate fungi, and diurnal differences were to be expected (Li and Kendrick, 1994). For example, conidial fungi, such as *Cladosporium* and *Alternaria*, preferred to release spores around midday when the sunlight was at its most intense (Salvaggio and Aukrust, 1981). Similarly, the concentrations of *Cladosporium* in the morning were higher than those in the afternoon in this study. This finding was consistent with other reports of the maximum hourly *Cladosporium* concentration occurring in the morning (Nayak et al., 1998; Hasnain et al., 2004). Nevertheless, this result differed from that of Lin and Li (2000), who reported that *Cladosporium* and *Alternaria* showed the diurnal patterns. This discordance may be a result of the use of different culture media, sampling methods, and sampling environments.

Ventilation system also influenced the species and concentrations of bioaerosols. Based on this investigation, a high level of *S. cohnii* was found at the crushing site. This result demonstrated that the crushing procedure was performed in an inadequately ventilated room, since the dominance of *S. cohnii* has been shown to be an indication of inadequate ventilation (Morey et al., 1986). On the other hand, the operation of ventilation systems may also reduce relative humidity, and the mean absolute water content tends to be lower in their presence. The warm, dry weather conditions promoted the passive dispersal of dry-air-spores such as *Cladosporium*. Hence, the dry-air-spores were mostly blown out naturally by the air, and their concentrations were found to be higher without, rather than with a ventilation system.

In this study, levels of *Cladosporium* at sites with ventilation systems were lower than at sites without ventilation systems.

Aerodynamic diameter was critical in assessing respiratory exposure to flour, especially for the crushing site, warehouse and outdoor. This study observed varying proportions of different-sized bioaerosols at the crushing site, with the highest proportion of total culturable bacteria in the range of 1.1–0.65 μm , while most bacteria fell within the size range of $\geq 7 \mu\text{m}$ for both the warehouse and outdoor sampling areas. At the crushing site, the larger bacteria appeared to settle on the ground, while the smaller bacteria remained suspended in the air. Therefore, smaller bacteria such as *Micrococcus* spp. and *S. xylosum*, which were the dominant bacterial species in the air at the crushing site, could potentially penetrate the alveoli and cause disease. Bacteria of a similar size range were identified at the warehouse and outdoor sampling sites. Since the air in the warehouse was vented from the outdoors, some of the air in the warehouse may have originated from outside. On the other hand, the highest proportion of fungi at the crushing site, warehouse, and outdoors were observed to be in the same size range of 2.1–1.1 μm . Based on this result, it could be inferred that the predominant fungi found at the crushing site, such as *Aspergillus* and *Penicillium*, were in the small size range of 2.1–1.1 μm . Hence, the dominant fungi and bacteria occurring at the crushing site were small enough to produce bioaerosols and were known to be allergens and pathogens that could trigger allergic alveolitis.

5. Conclusions

Based on a 1-year survey, the total number of bioaerosol particles measured for most indoor environments was below the limit of 1000 CFU/m³ proposed by NIOSH. Notably, levels of fungi during spring at the crushing site and the warehouse exceeded 1000 CFU/m³. Meanwhile, the bioaerosol counts of total bacteria at the warehouse and the crushing, and stirring sites were found to exceed 500 CFU/m³, the guideline set by the WHO. Consequently, these workers may be at risk for a number of allergic and non-allergic microorganisms. It was thus recommended workers at these sites wear breathing protection.

Given the same work operation procedures, relative humidity affected fungi levels more than temperature. In the investigated factory, the crushing procedure was associated with the highest concentration of airborne bioaerosols. Crushing was performed in a workplace environment with inadequate ventilation and common allergic fungi such as *Cladosporium*, *Penicillium*, and *Aspergillus* dominated the identified fungal species. Staff working at the crushing site was at risk of exposure to bioaerosols generated from the aerosolization of microorganisms, since most fungi and bacteria at the crushing site were in the small size ranges of 2.1–1.1 μm and 1.1–0.65 μm , respectively. This result had important implications for evaluating the potential risk of exposed workers at the crushing site. Hence, increased ventilation was recommended to minimize the biological hazard at the crushing site.

These study results were limited to the single factory described here and may not be representative of what is typical for a noodle factory. Many parameters, such as geographic

location, culture media, and the microbiological sampler, appear to influence the concentrations and relative frequencies of airborne bacteria and fungi. Considerable further study regarding exposure to the identified bioaerosols and their relationship to diseases as well as disease control is necessary. Additionally, the possible role of bacterial endotoxins as an occupational hazard in the noodle industry should be addressed in future studies.

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