

Field Survey on Generation Patterns of Airborne Fungi in a Livestock Manure Composting Plant in South Korea

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Article

Field Survey on Generation Patterns of Airborne Fungi in a Livestock Manure Composting Plant in South Korea

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1. Introduction

There is a current trend of expanding the supply of renewable energy to minimize the environmental pollution load of organic waste and efficiently utilize energy resources worldwide. For example, in the case of livestock manure, the direction of the treatment method is to recycle resources through composting for solids and converting biogas to energy through anaerobic digestion for liquids. However, suppose composting and energy facilities for organic waste resources, which are expected to increase in demand in the future, are expanded. In that case, as more facilities begin to operate, it is expected that the generation of large amounts of various air pollutants will increase.

Among the air pollutants generated from resource-processing workplaces that treat organic waste resources, such as livestock manure as primary materials, the emission rate of bioaerosols, such as airborne bacteria and fungi, is reported to be significantly higher than

that of other workplaces [1]. Airborne microorganisms, which are biologically harmful, are known to cause infectious or allergic respiratory system diseases, such as asthma, rhinitis, bronchitis, etc. [2,3]. In particular, it has been reported by many researchers that workers in composting facilities that directly deal with organic waste resources are exposed to high concentrations of bioaerosol, so the damage to their health is relatively higher than that of nearby residents [4–8].

In terms of resource recycling, because organic compost was recognized years ago as a renewable energy source in the EU and was widely used, special guidelines for the protection of workers' health in the operation of composting facilities have been established and revised since 2000 [9]. In addition, the monitoring system for the operation of composting facilities emphasizes occupational health aspects centered on pathogenic bioaerosols such as *Aspergillus fumigatus* [10].

However, most of these previous studies were conducted abroad. In the case of Korea, there are no investigations related to workers' exposure to bioaerosol generated from organic waste recycling facilities. Therefore, this study aims to provide fundamental data for the prevention of the health of workers employed at this workplace by evaluating the distribution characteristics of exposure concentrations of airborne fungi among bioaerosol factors for workplaces that compost livestock manure among organic waste resources.

2. Materials and Methods

2.1. Subject

Three types of livestock manure composting plants in Jeju city, Korea, were selected based on the fermentation mode of the compost pile, be it screw type, rotary type or natural dry type. Table 1 summarizes the main characteristics of the livestock manure composting plant investigated in this study.

Table 1. Profile of livestock manure composting plant investigated in this study.

Site	Reactor Type	Turning Mode	Treatment Capacity	Location
1	Cross	Screw	10 (7.5) * ton/day	Jeju
2	Cross	Rotary	5 (1) ton/day	
3	Pile	Natural dry	3 (1.5) ton/day	

* (): Practical treatment capacity.

2.2. Measurement

During the period from Sep. 2019 to Aug. 2020, three composting plants were visited once a month, and 12 visits were made to each composting plant for a year. Air sampling for measuring airborne fungi was conducted 1 m from the middle location of the livestock manure composting plant between 13:00 and 17:00. For each measurement, a sample was collected by repeating it three times, and the average was taken as a representative value.

The six-stage viable particulate cascade impactor (Model 10-800, Andersen Inc., OH, USA) with a flow amount of 28.3ℓ/min was used for sampling airborne fungi, and the aerodynamic diameter ranges for each stage are as follows: stage 1 (>7.0 μm), stage 2 (4.7–7.0 μm), stage 3 (3.3–4.7 μm), stage 4 (2.1–3.3 μm), stage 5 (1.1–2.1 μm), and stage 6 (0.65–1.1 μm). Air sampling was conducted for 8 to 12 min according to the environmental situation of the measurement locations. Before sampling, the inside of the sampler was disinfected with 70% alcohol and then inserted with an agar plate according to the collection protocol. Malt extract agar (MEA; Cat No. 218610, Becton Dickinson and Company, USA) was used for fungi and chloramphenicol (2%) was added to inhibit bacterial growth. The culture media for which sample collection was finished were immediately taken to the microbe laboratory and cultured in the incubator for 3–5 days at room temperature. The counts for the air sample plates were corrected for multiple impactions using the positive hole conversion method. The concentration of airborne fungi, i.e., cfu/m³, was calculated by dividing by air volume (m³) the value obtained from counting the colonies formed on the culture medium after the process of culturing (Equation (1)). Regarding representing

the levels of airborne fungi, total concentration and respirable concentration means the colony number obtained from all the stages (stages 1 through 6) and from stage three to stage six, respectively.

$$C_f = F \times A_v \quad (1)$$

C_f : CFU (Colony Forming Unit)/m³

F: Number of colonies counted on agar plates

A_v : Air volume (m³)

The airborne fungal genera were identified by observing the colony's form, shape and color and spores with a scanning electron microscope. This method for identifying airborne fungi is based on the classification technique suggested by Ainsworth and Baron (1961). They used this to identify the major morphological characteristics of the fungus and to distinguish the fungal species after performing molecular characterization using the amplification of ITS sequences.

To verify the statistical correlation with airborne fungi, the environmental factors in the livestock manure composting plant were measured simultaneously at the site temperature, relative humidity, particulate matter (TSP, PM₁₀, PM_{2.5} and PM₁) and odor. They were monitored directly using the Digital Thermohygrometer (608-H1, Testco, Germany) for temperature and humidity, Portable Dust Monitor (Dustmate, TurnKey Instruments Ltd., NJ, USA) for particulate matters, and Hand-Held Odor (OMX-SR, Shinyei, Japan) for odor.

2.3. Data Analysis

The SAS package (SAS/Stat 9.1, SAS Institute Inc., Cary, NC, USA) was used for the analysis of the measured field data. First, the Shapiro–Wilk test found that the measured data had a normal distribution, and the measured values were presented as the arithmetic mean and standard deviation. ANOVA and Duncan's multiple comparison analysis methods were applied to compare the concentration difference of the internal airborne fungi according to the livestock manure composting plant type and the emission amount of airborne fungi generated at each turning time of the compost pile. The correlation between airborne fungi and environmental factors in the livestock manure composting plant was verified for statistical significance by applying Pearson's correlation test method.

3. Results and Discussion

3.1. Monthly Concentration Distribution of Airborne Fungi According to the Type of Livestock Manure Composting Plant

Figure 1 shows the monthly concentrations of airborne fungi from livestock manure composting plants operated in the three types of compost pile turning modes. In the case of the screw type, the mean concentration of airborne fungi was 1143 (± 106)CFU m⁻³, and its maximum and minimum levels were 5424 (± 573)CFU m⁻³ in June and 112 (± 10)CFU m⁻³ in February, respectively. In the case of the rotary type, the mean concentration of airborne fungi was 552 (± 146)CFU m⁻³, and its maximum and minimum levels were 2367 (± 702)CFU m⁻³ in June and 32 (± 15)CFU m⁻³ in February, respectively. In the case of the dry type, the mean concentration of airborne fungi was 434 (± 73)CFU m⁻³, and its maximum and minimum levels were 1466 (± 175)CFU m⁻³ in June and 58 (± 7)CFU m⁻³ in February, respectively. Based on the results obtained from this study, the mean concentration of airborne fungi was highest in the livestock manure composting plant operated with the screw type, followed by the rotary type and dry type ($p < 0.05$). Regardless of the compost pile turning mode, the monthly level of airborne fungi in livestock manure composting plants was the highest in June and the lowest in February, respectively. Regarding mean values, the livestock manure composting plants operating with the screw type exceeded the domestic indoor standard guideline (500CFU m⁻³) of airborne fungi.

Based on the results obtained from this study, the monthly concentration distribution of airborne fungi in the livestock manure composting plant did not show a consistent trend, regardless of the fermentation mode. Generally, it was found that the concentration of

airborne fungi increased during the warm season when the temperature was high, but it is estimated that the variation in environmental conditions (temperature, humidity, airflow, etc.) in the livestock manure composting plant at the time of measurement had a more significant effect on the level of airborne fungi. This finding can be explained by another finding, that the concentration of airborne fungi measured between May and August when the outdoor temperature is mild increased relative to the concentrations monitored in other months. Compared to previous results [11–15], a significant difference in the level of airborne fungi was found among different researchers, and there is also a substantial difference in those results compared with the results of this study. This is presumed to be due to the difference in composting raw materials with different properties. Additionally, these differences could be attributed to the composition of the culture medium, as well as the area-to-volume ratio, conditions of the growth physiology of the fungi and the activation of differential sporulation processes.

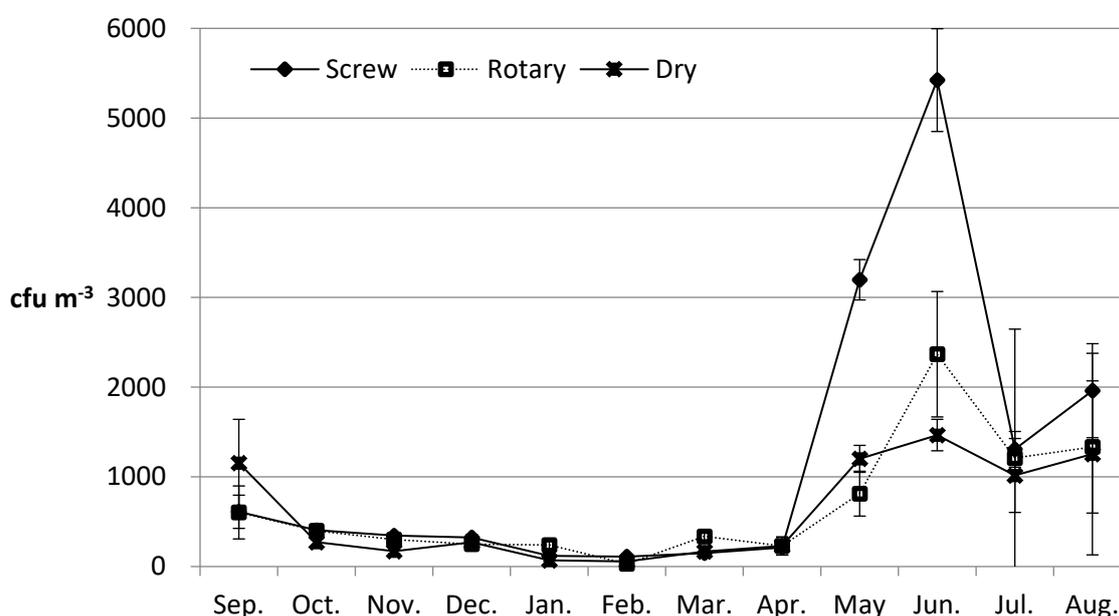


Figure 1. Monthly mean concentration trend of airborne fungi in livestock manure composting plants.

The composting plant based on livestock manure was the investigation subject in this study, whereas the plants that general compost waste, such as municipal solid waste and food waste, in the case of previous studies. Additionally, the fact that micrometeorological factors such as temperature, humidity, wind speed, and season that affect the generation of airborne fungi during the composting process were different from each other at the time of measurement may be one of the reasons [16–18].

3.2. Comparison of Airborne Fungi Emitted from Livestock Manure Composting Plant According to Agitation Time of Compost Pile

Figure 2 shows the concentration pattern of airborne fungi according to the agitation time (before, during, and after turning) of the livestock manure compost pile. The turning period was one hour, and air samples were taken for one hour after turning. Mean values of airborne fungi were 230 (± 100)CFU m⁻³ before turning, 247 (± 50)CFU m⁻³ on turning, and 256 (± 62)CFU m⁻³ after turning, respectively. As a result of the measurement, it was analyzed that the difference in the concentration of airborne fungi according to agitation time of compost pile was not statistically significant ($p > 0.05$) based on ANOVA. This finding differed from the result of the previous report [11] that a large amount of microorganisms distributed in the compost pile are generally released into the air through turning. The reason why the results obtained from this study differ from those of previous

research is presumed to be due to the time of year in which it was carried out, the ecosystem conditions used, or the ambient atmospheric conditions.

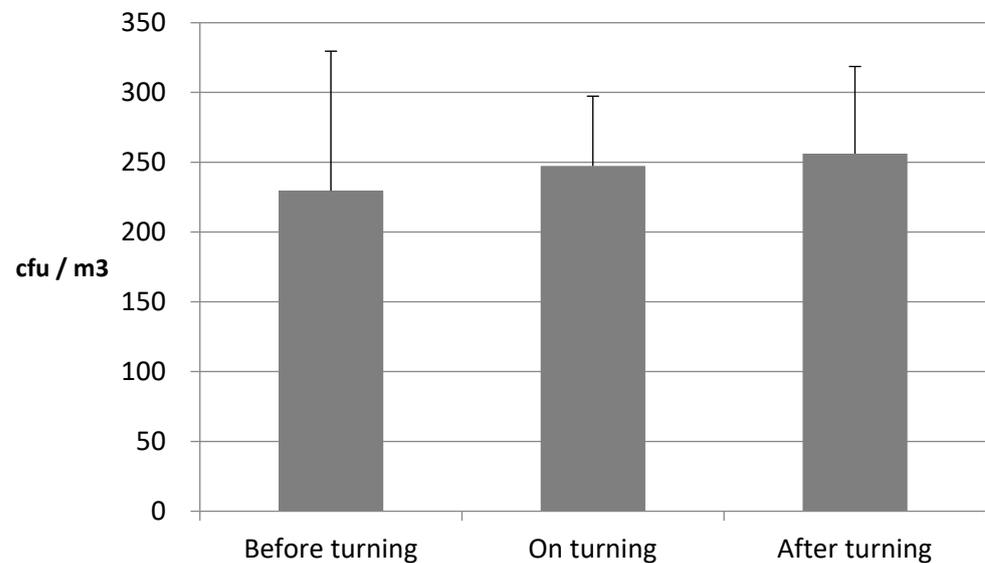


Figure 2. Mean level of airborne fungi in livestock manure composting plants according to agitation time of compost pile.

3.3. Size Distribution Characteristics of Airborne Fungi According to the Type of Livestock Manure Composting Plant

As shown in Figure 3, the size distribution characteristics of airborne fungi in livestock manure composting plants were as follows: 27% (Screw), 33% (Rotary) and 40% (Dry) in stage 1 (>7.0 μm); 7% (Screw), 20% (Rotary) and 5% (Dry) in stage 2 (4.7–7.0 μm); 7% (Screw), 7% (Rotary) and 2% (Dry) in stage 3 (3.3–4.7 μm); 20% (Screw), 13% (Rotary) and 10% (Dry) in stage 4 (2.1–3.3 μm); 13% (Screw), 7% (Rotary) and 3% (Dry) in stage 5 (1.1–2.1 μm); 26% (Screw), 20% (Rotary) and 40% (Dry) in stage 6 (0.65–1.1 μm).

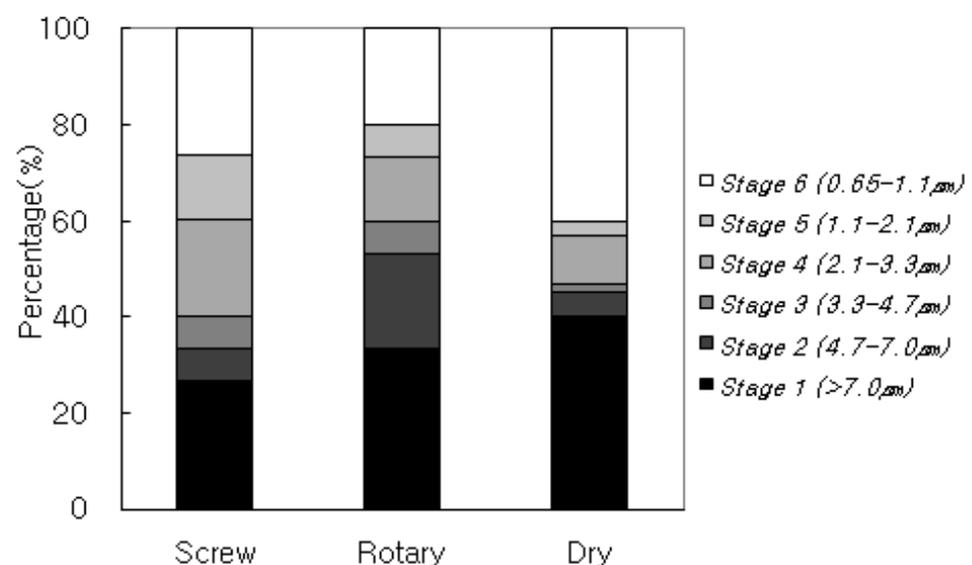


Figure 3. Size distribution characteristics of airborne fungi in livestock manure composting plants.

Stage 1 (>7.0 μm) showed the highest frequency rate among particle size ranges regardless of the livestock manure composting plant type. In the six-stage viable particulate cascade impactor, the proportion of airborne fungi of 0.65 μm or more and 4.7 μm or less

(stage 3–6) that is within the respiratory particle size range to the total concentration were 66% for the screw mode, 47% for the rotary mode, and 55% for dry mode, respectively.

Kim and Kim [19] and Kim et al. [20], who studied multi-use facilities, reported that the concentration ratio of airborne fungi was the highest in stage 5 (1.1–2.1 μm) and the ratio of respiratory concentration to the total concentration was 55–70%. Kim et al. [21], who studied the particle size distribution characteristics of airborne fungi in the working environment of a feed manufacturing factory, reported that the highest ratio was in stage 1 (>7.0 μm) and the lowest was in stage 3 (3.3–4.7 μm). The ratio of the concentration corresponding to the respiratory particle size to the total concentration was about 30%. The distribution characteristics by particle size were generally similar as compared with the result measured in this study.

3.4. Association between Airborne Fungi and Environmental Factors in Livestock Manure Composting Plant

Table 2 represents the statistical relationship between airborne fungi and the environmental factors in livestock manure composting plants. There was no significant correlation relationship among them except for temperature/airborne fungi ($r = 0.708$), temperature/ $\text{PM}_{2.5}$ ($r = -0.776$), R.H./ PM_{10} ($r = 0.752$), and $\text{PM}_{2.5}/\text{PM}_1$ ($r = 0.722$). Based on the statistical analysis, temperature was found to be the environmental factor that significantly affects the generation of airborne fungi, which thus determined the amount of evaporation and therefore water activity. This parameter limits or triggers sporulation processes in fungi.

Table 2. Correlation relationship between airborne fungi and indoor environmental factors.

	Airborne Fungi	Temp.	RH	TSP	PM_{10}	$\text{PM}_{2.5}$	PM_1	Odor
Airborne fungi		0.708 **	−0.085	0.011	−0.071	−0.298	−0.130	0.035
Temp.			−0.367	0.078	−0.266	−0.776 **	−0.419	0.150
RH				0.470 *	0.615 **	0.383	0.127	0.043
TSP					0.752 **	−0.303	−0.302	−0.017
PM_{10}						0.150	−0.178	0.009
$\text{PM}_{20.5}$							0.722 **	0.069
PM_1								0.144
Odor								

* $p < 0.05$, ** $p < 0.01$.

This study has a limitation in analyzing only the correlation of airborne fungi with atmospheric environmental factors such as fine dust and odor. Therefore, in future research, it is necessary to understand the relationship between various factors, such as information about the storage conditions of manure for composting, the physical and chemical composition of the manure used for composting and the technical characteristics of the composting facilities that affect the emission of airborne fungi derived from the compost pile.

It was found that there were no environmental factors showing a significant correlation with the airborne fungi in the livestock manure composting plant. However, the confidence in statistical analysis is relatively low because researchers have different opinions on how temperature and relative influence the generation of airborne fungi [22–25]. In addition, this statistical analysis was performed with a very small number of samples. Thus, further studies should investigate a clear scientific conclusion on the relationship between airborne fungi and environmental factors.

3.5. Qualitative Analysis of Airborne Fungi According to the Type of Livestock Manure Composting Plant

Table 3 presents the identification results of airborne fungi from livestock manure composting plants according to fermentation mode. The predominant species of airborne fungi identified in over 10% detection rates were *Aspergillus* spp.(29.3%), *Penicillium* spp.(20.2%) and *Cladosporium* spp.(18.5%) for screw type, *Aspergillus* spp.(26.2%), *Cladosporium* spp.(24.2%)

and *Penicillium* spp.(19.8%) for rotary type and *Aspergillus* spp.(35.3%), *Cladosporium* spp.(19.2%) and *Penicillium* spp.(17.6%) for dry type, respectively. As a result, the species profile of airborne fungi in livestock manure composting plants was generally similar regardless of compost pile turning mode. This finding would be attributed to using the same resource, namely livestock manure, as composting material. *Cladosporium* spp. and *Penicillium* spp. were the predominant species of airborne fungi found in other indoor facilities [19,20,26–29], which is generally identical to the airborne fungi profile in the livestock manure composting plant investigated through this study.

Table 3. Identification of airborne fungi in livestock manure composting plant.

	Rotary Type	Screw Type	Dry Type
<i>Aspergillus</i> spp.	26.2	29.3	35.3
<i>Chrysosporium</i> spp.	3.8	8.2	2.4
<i>Cladosporium</i> spp.	24.2	18.5	19.2
<i>Fusarium</i> spp.	3.6	4.1	5.6
<i>Mucor</i> spp.	7.2	6.4	5.8
<i>Penicillium</i> spp.	19.8	20.2	17.6
<i>Ulocladium</i> spp.	2.1	1.0	3.7
Yeasts	6.2	4.3	1.2
Unknown	6.9	8.0	9.2
Total	100.0	100.0	100.0

(Unit: %).

4. Conclusions

The livestock manure composting plant of the screw type showed the highest concentration of airborne fungi, followed by the rotary and dry types. The monthly level of airborne fungi was highest in June and lowest in February, regardless of fermentation mode. The ratio of respirable size to total airborne fungi was approximately 40–60%. The predominant genera of airborne fungi identified were *Aspergillus* spp., *Cladosporium* spp. and *Penicillium* spp.

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Conflicts of Interest: The authors declare no conflict of interest.

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