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Original Research

Sedimentation with the Omeliansky Formula as an Accepted Technique for Quantifying Airborne Fungi

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Abstract

The aim of the present study was to determine the reliability of sedimentation using the "Omeliansky formula" as an accepted method for quantifying airborne fungi, by comparing concentrations collected using sedimentation, impingement, and filtration methods. Significant correlation coefficients ($P \leq 0.01$) and non-significant differences ($P \geq 0.01$) were found between fungal concentrations using the three sampling methods. Sedimentation had a stronger correlation with filtration in comparison with the impingement method. Sedimentation may be accepted as a method for quantifying airborne fungi.

Keywords: air, fungi, sampling, sedimentation, filtration, impingement

Introduction

The sampling method has proven to be critical for recovery of air microorganisms. The passive and active methods are the main types for collecting air microorganisms. The advantages and disadvantages of different collection techniques, filtration, impingement, impaction, and sedimentation have previously been reviewed [1]. Filtration is a widely used method because of its ease of use, low exposure, and high capture rate in heavily contaminated environments [2]. However, it is not suitable for evaluating vegetative cells, desiccation of the microorganisms, and inefficient extraction of nucleic acids from filter surfaces [3].

Liquid impinge sampler (AGI-30), low and high rates, is a widely used sampler [4]. It is low cost, efficient for collecting microorganisms, and cell aggregates are broken apart [5], but its fluid evaporates quickly, and it is not efficient for the collection of hydrophobic particles [6].

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A sedimentation (passive sampling) method is used for collecting airborne microorganisms on Petri dishes containing suitable media, on which particles are deposited by gravity. Sedimentation is the simplest method, and commonly used due to its practical usage and low cost [7]. But it gives a rough approximation of the levels, and its reliability is affected by the size of the particle and motion of the surrounding air [8]. The aim of the present paper was to confirm the reliability of the sedimentation method with the "Omeliansky Formula" as an accepted technique for quantifying airborne fungi.

Materials and Methods

Indoor and outdoor air samples were collected at soybean and cotton industry workplaces. The samples were taken at a height of 1.5 m, the breathing zone, and at the middle of the workplaces. And outdoor comparison samples were taken ~10 m from the indoor workplaces.

Table 1. The concentrations and ratios of airborne fungi collected using impingement (IM), filtration (FM), and sedimentation (SM) at different industry workplaces.

Sampling technique	CFU·m^{-3} × 10^3					
	Soybean mill			Cotton mill		
	Wet line	Store	Outdoor	Carding	Spinning	Outdoor
Impingement	0.51-6.3	0.52-24	0.28-1.6	0.51-24	0.07-6.8	0.13-4.7
	(2.75±1.9)	(3.93±6.45)	(0.9±0.72)	(5.75±8.48)	(1.97±2.76)	(1.33±1.28)
	[2.1]	[2.25]	[0.56]	[2.00]	[0.63]	[1.25]
Filtration	0.52-7.3	0.16-12	0.1-1.8	0.1-13	0.06-7.5	0.08-2.9
	(2.46±1.88)	(3.02±3.08)	(0.66±0.46)	(2.6±3.66)	(1.92±2.69)	(1.27±1.46)
	[2.35]	[2.2]	[0.52]	[1.15]	[0.59]	[0.81]
Sedimentation	1.11-4.58	0.6-16.6	0.33-18	0.27-7.08	0.05-13.8	0.116-19.4
	(2.76±1.08)	(4.35±4.4)	(2.36±4.96)	(3.3±3.16)	(3.07±4.17)	(2.97±5.54)
	[2.85]	[2.9]	[0.94]	[2.48]	[1.58]	[1.09]
S/I	1.3	1.28	1.6	1.24	2.5	0.87
S/F	1.2	1.3	1.8	2.15	2.6	1.34

Range, (mean ± SD), [median], S – sedimentation, I – impingement, F – filtration

The samples were taken twice per month with two sequential samples collected during each sampling event.

AGI-30, containing 20 ml of sterilized phosphate buffer, was used to collect fungi at a recommended flow rate of 12 l/min for 15 minutes. Aliquots (0.1 ml) of the original sample and its serial dilutions were spread-plated, in duplicate, onto the surface of malt extract agar (MEA), (BD, Sparks, Maryland, USA) supplemented with 50 ppm chloramphenicol (Oxoid, England).

Membrane filter (0.25 µm pore size, and 25 mm diameter) was used to collect fungi. One hour samples were obtained using an open face holder, and a vacuum pump calibrated to draw 8 l/min. The filters were washed with 20 ml phosphate buffer supplemented with 0.1 ml Tween 80 and shaken vigorously for 30-60 min. Aliquots (0.1 ml) of the original sample and its dilutions were spread-plated, in duplicate, onto the surface of MEA supplemented with chloramphenicol.

Samples also were collected using passive sedimentation on Petri dishes (90 mm diameter), in duplicate containing the previously mentioned medium for 10 min.

The inoculated plates were incubated at 28ºC for 5-7 days. The resultant colonies were counted and the concentration was expressed as colony forming units per cubic meter of air (CFU m^{-3}).

When the sedimentation technique was used, the concentration was calculated according to Omeliansky [9] using the following formula:

$$N=5a \times 10^4 (bt)^{-1}$$

...where: N = CFU·m^{-3}, a = number of colonies per Petri dish, b = dish square centimeter, t = exposure time (min).

Spearman's rank correlation coefficient and student's significant-t-test ($P \leq 0.01$) were used to examine the significance of correlation and difference between fungal concentrations collected using the different sampling methods.

Results

Fungal concentrations ranged between 10^1 and 10^4 CFU/m^3 indoors and 10^1 and 10^3 CFU/m^3 outdoors using different sampling methods (Table 1). Sedimentation method gave the higher concentrations. The medians were $2.85\times10^3/2.9\times10^3$ CFU·m^{-3} at wet line/store units (in the soybean mill), and $2.3\times10^3/3.054\times10^3$ CFU·m^{-3} at carding/spinning units (in the cotton mill) using the sedimentation method (Table 1).

Sedimentation gave ~1-2 times higher ratios than both impingement and filtration, except impingement gave a higher ratio outside the cotton mill (Table 1).

Spearman's rank correlation coefficient showed significant relationships ($P \leq 0.001$) between sedimentation with both the impingement and filtration methods (Table 2). The relationships ranged between 0.73-0.99, with the stronger relationships found between sedimentation and filtration. Non-significant differences ($P \geq 0.01$) were found between concentrations obtained using the three sampling methods.

Discussion

Airborne fungal concentrations exceeded the guideline limit value, 500 CFU·m^{-3} recommended by the World Health Organization [10], and the industry-workplaces may not be safe for the workers. Sedimentation with

Table 2. Spearman's rank correlation coefficients between sampling methods in different industry workplaces.

Technique	Soybean mill									Cotton mill								
	Wet line			Store			Outdoor			Carding			Spinning			Outdoor		
	I	F	S	I	F	S	I	F	S	I	F	S	I	F	S	I	F	S
Soybean																		
I	1	o.96	0.98	1	0.92	0.95	1	0.96	0.87									
F		1	0.99		1	0.97		1	0.87									
S			1			1			1									
Cotton mill																		
I										1	0.73*	0.77*	1	0.98	0.94	1	0.98	0.85
F											1	0.93		1	0.96		1	0.88
S												1			1			1

I – impingement, F – filtration, S – sedimentation, P < 0.001, *P < 0.01

"Omeliansky Formula" gave the greatest concentrations. Kruczalak et al. [11] found that the number of microorganisms measured by impaction method were 14 times lower than the case of sedimentation. Sedimentation gave higher concentrations than the impaction method [12], but sedimentation and impaction methods gave similar airborne fungal concentrations [13].

In the present study, sedimentation showed stronger correlations with filtration than impingement, as the liquid impinger sampler is not efficient for the collection of hydrophobic particles (e.g. some fungal spores) [14].

Non-significant differences were found between the sampling methods as indication of the reliability of using sedimentation with the Omeliansky Formula to quantify fungi. It is concluded that sedimentation using "Omeliansky Formula" is a good method for sampling airborne fungi, but further investigation is needed under different sampling conditions.

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