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Short communication

Particle size distribution of airborne *Aspergillus fumigatus* spores emitted from compost using membrane filtration

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ABSTRACT

Information on the particle size distribution of bioaerosols emitted from open air composting operations is valuable in evaluating potential health impacts and is a requirement for improved dispersion simulation modelling. The membrane filter method was used to study the particle size distribution of *Aspergillus fumigatus* spores in air 50 m downwind of a green waste compost screening operation at a commercial facility. The highest concentrations (approximately 8×10^4 CFU m^{-3}) of culturable spores were found on filters with pore diameters in the range 1–2 μm which suggests that the majority of spores are emitted as single cells. The findings were compared to published data collected using an Andersen sampler. Results were significantly correlated ($p < 0.01$) indicating that the two methods are directly comparable across all particles sizes for *Aspergillus* spores.

1. Introduction

The implementation of the Landfill Directive (EC/31/99) has stimulated the composting industry in Europe. For example, in the United Kingdom (UK), the amount of waste composted increased from 0.06 million tonnes in 1994 to an estimated 3.6 million tonnes in 2007 (Smith and Pocock, 2008). The majority of UK organic waste (79%) is composted using mechanically turned open-air windrows, a technology which has limited controls over emissions of particulates to the air (Smith and Pocock, 2008). The release into the air of microorganisms which originate in the feedstock or which participate in the composting process, particularly as a result of activities such as shredding, turning and screening has been well documented (Lacey, 1997; Reinthaler et al., 1997; Wheeler et al., 2001; Recer et al., 2001; Taha et al., 2006). Some of the bioaerosols emitted from compost have the potential to cause harm to humans if inhaled. The mould *Aspergillus fumigatus* is an example. *A. fumigatus* flourishes within stored organic material, and is released in elevated quantities during composting operations (in concentrations exceeding 10^6 colony forming units (CFU) m^{-3}) (Dutkiewicz, 1997; Reinthaler et al., 1997; Swan et al., 2002, 2003; Taha et al., 2006). Repeat exposure to *A. fumigatus* is associated with a range of respiratory conditions, including extrinsic allergic alveolitis and chronic bronchitis (Dutkiewicz, 1997; Swan et al., 2003). Although gross exposure to spores ($>10^6$ CFU m^{-3}) is usually required to trigger an immune response (Swan et al., 2003), *A. fumigatus* is an opportunistic pathogen. Low levels of exposure may pose an increased risk of invasive aspergillosis in the immunocompromised (Douwes et al., 2003; Swan et al., 2003). Immunological investigations have found increased levels of antibodies to *A. fumigatus* amongst compost workers, raising concerns about the long-term implications of occupational exposure (Bünger et al., 2000). The health impacts of particulate matter depend partly upon where they are deposited in the respiratory tract and this in turn is influenced by particle size (Park et al., 2009). The size distribution of bioaerosols emitted from composting facilities is also of interest to researchers because of the link to dispersion (Taha et al., 2007). Individual cells are estimated to settle at comparatively low rates (approximately 3 cm min^{-1}) (Swan et al., 2003) which are unlikely to affect airborne transport off site. Wheeler et al. (2001) postulated that bioaerosols released during composting may include a range of particle sizes, due to clumping, which may reduce distance of travel from source due to preferential deposition of larger particles. No measurements were made by Wheeler et al. (2001) of particle size

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to substantiate the hypothesis however. The current lack of information on the particle size distribution of bioaerosols emitted from composting limits our ability to model dispersion (Wheeler et al., 2001; Swan et al., 2003). Although culturable microorganisms represent only a small fraction of total airborne microorganisms, cultivation methods are still widely used and remain the principal available means of generating species-specific information (Parat et al., 1999). The Andersen 6-stage impactor is widely used to enumerate airborne culturable microorganisms and to separate them according to particle size (Andersen, 1958; Reinthaler et al., 1997; The Composting Association, 1999; Gorny et al., 1999; Tham and Zuraimi, 2005; Godish and Godish, 2007). Andersen samplers separate out particles according to their aerodynamic diameter and impact the different fractions on to agar for viable cell enumeration. Each stage of the sampler is representative of the human respiratory system and the physical barriers that prevent movement of particles into the lungs (Table 1): stage 1 [nose and mouth], stage 2 [pharynx], stage 3 [trachea and primary bronchi], stage 4 [secondary bronchi], stage 5 [terminal bronchi] and stage 6 [alveoli] (Andersen, 1958; Reinthaler et al., 1997). Gorny et al. (1999) used the 6-stage Andersen sampler in a study of size distribution of bacterial and fungal bioaerosols in indoor air. They concluded that the majority of Aspergillus spp. In indoor air were present as single spores. In a study of bioaerosols in ambient outdoor air, Jones and Cookson (1983) were able to demonstrate using a 2-stage Andersen sampler that 95% of airborne *A. fumigatus* were present in the potentially respirable fraction (<8 μm). As with all bioaerosol measurement devices, the Andersen sampler has some disadvantages. Specifically in the context of composting facilities, it has the drawback of becoming easily overloaded where bioaerosol concentrations are high (Reinthaler et al., 1997). There is growing interest in the use of the membrane-filtration sampling method as an alternative to the Andersen sampler for capturing bioaerosols in waste processing environments (Kildesø and Nielsen, 1997; Taha et al., 2006; Adhikhari et al., 2006). German standards have recently been developed for the sampling of moulds in air using gelatine and polycarbonate membrane filters (VDI 4252). These standards have been adopted as the basis for new International Organisation for Standardisation air quality standards currently under development (ISO 16000-17). In the context of the wider use of membrane filtration in bioaerosol studies at composting facilities and the importance of improving our knowledge of bioaerosol size characteristics in this environment this paper describes an attempt to quantify the particle size distribution of *A. fumigatus* emitted from a composting facility. This short communication concludes with a comparison of measured data with previously published data collected under similar conditions using an Anderson sampler.

2. Methods

Membrane filtration was used to re-create the particle size distribution obtained using a 6-stage Andersen sampler. A range of pore-sizes was chosen that matched closely the range of the Andersen sampler (Table 1). Sampling was performed at a green waste windrow composting facility in central England, with a capacity of 25,000 tonnes per annum. The facility is approximately 500 m from the nearest residence, and approximately 600 m upwind of a village. The samples were collected 50 m downwind from two screening processes, close (10 m) to the compost windrows. The wind was predominantly from the S-W, the site is aligned along an S-S-S-W/N-N-E axis, therefore the prevailing wind roughly follows the long axis of the site. Medium flow, personal aerosol filter samplers (SKC Universal dust and vapour sampling pumps, model SKC PCXR8) operated at a flow rate of 2.2 ± 0.1 L min^{-1}. Pumps were attached to SKC IOM (Institute of Occupational Medicine) particulate sampling heads, loaded with IOM multi-dust plastic cassettes, with Tygon™ tubing and positioned equidistant along a sampling bar, at a height of 1.7 m (Taha et al., 2007). Each sample cassette contained one of the six sizes of polycarbonate filter used (Table 1). Sampling pumps were run for 30 min. Three sampling runs were completed whilst compost was being screened to permit assessment of reproducibility. Weather conditions were favourable, with partial cloud cover, a maximum temperature of 16 °C and wind speeds between 3 and 6 m s^{-1}. After sampling, filter cassettes were removed from the sampling heads and placed into a sterile container containing buffer solution (NaCl 1 g L^{-1} and 3 drops of Tween 80 L^{-1}, made up to 1 L with sterilised ddH_2O), agitated to ensure buffer covered the polycarbonate filter (to avoid cell desiccation) and placed at 4 °C for transport. Once in the laboratory (within 24 h), filters were removed from the sampling head under aseptic conditions and re-suspended in the vial by shaking for 2 min. The suspensions were diluted to a common logarithm order (10^{-1} and 10^{-2}) and aliquots of 100 μL of each dilution transferred onto the centre of Malt Extract Agar (MEA) 90 mm single-vented Petri dishes (with 0.1 g L^{-1} Chloramphemicol to suppress bacterial growth). The sample was spread evenly over the agar using a sterile spreader and once absorbed, the Petri dish was inverted and incubated at 37 ± 2 °C in the dark for 3–5 days. Colony forming units (CFUs) were enumerated visually and recorded as CFU m^{-3} of sampled air (Taha et al., 2005, 2006, 2007).

Table 1
Cut-off sampling characteristics of the membrane filter and the Andersen 6-stage samplers. Ranges of stages shown in square brackets.

Stage number	Polycarbonate filter size (μm)	Andersen pore size (μm)
1	8.0 [≥8.0]	7.0 [≥7.0]
2	5.0 [5.0–8.0]	4.7 [4.7–7.0]
3	3.0 [3.0–5.0]	3.3 [3.3–4.7]
4	2.0 [2.0–3.0]	2.1 [2.1–3.3]
5	1.0 [1.0–2.0]	1.1 [1.1–2.1]
6	0.65 [0.65–1.0]	0.65 [0.65–1.1]

3. Results and discussion

The filter sizes equivalent to the Andersen sampler stage sizes (Table 1) were plotted against *A. fumigatus* colony forming units

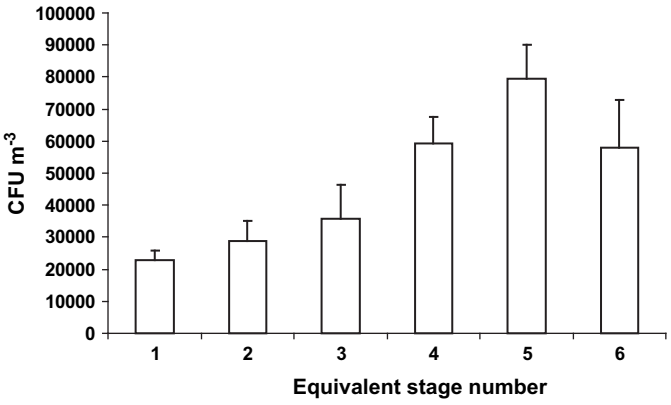

Fig. 1. Particle size distribution of *Aspergillus fumigatus* as captured using membrane filtration, represented as colony forming units m^{-3} of air, isolated at 50 m downwind of screening activities at an industrial scale green waste composting facility. Bars indicate means (n = 3); whiskers show standard error. Equivalent stage numbers indicate pore-sizes of filters used that are similar to the stage sizes in an Andersen sampler; stage 1 = 8.0 μm, 2 = 5.0 μm, 3 = 3.0 μm, 4 = 2.0 μm, 5 = 1.0 μm and 6 = 0.65 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)