7 AIRBORNE RELEASE OF *ASPERGILLUS FUMIGATUS* FROM HOME COMPOST BINS

7.1 INTRODUCTION

The potential risk to human health during home composting (HC) is associated with the release of pathogenic microoganisms and allergens (substance to which an individual is hypersensitive that causes an inappropriate immune response or allergy)(Lawrence 2000) thatare transported into the air by diffusion or dispersion mechanisms when the compost piles are disturbed. The range of airborne microflora is dependent on the initial contamination of the starting material, the microbial development between disposal and composting and, subsequently, during composting.

The organism of most concern is *Aspergillus fumigatus,* which is a fungus associated with crop plants and decaying vegetation although relatively common in homes, outdoor air, agriculture and public environments. The characteristics, which make it of primary concern with compost handlers is that, it is heavy sporulative, toxin-producing and is capable of surviving thermophilic temperatures (Epstein *et al*., 1994; Millner *et al*., 1994, Milner, 1995, Gilbert and Ward, 1998). These properties enable the fungus to cause a group of diseases collectively known as 'aspergilloses' (Alexopoulos, 1996), where inhalation of conidia may result in allergic reaction, growth of the fungus in air spaces within the body, or invasion of the fungus within the tissues.

Large centralised composting facilities may generate airborne emissions of the mould spores with potential health implications for neighbouring populations (Epstein *et al*., 1994; illner *et al*., 1994, Milner, 1995, Gilbert and Ward, 1998). However, exposure to *A. fumigatus* during home composting activity has not been previously assessed. Therefore, numbers of *A. fumigatus* were measured during compost handling and collection from HC bins by a standard protocol for airborne microorganisms (TCA, 1999b), to assess the potential exposure to the fungal spores released during compost agitation and the potential health implication associated with HC activity. Air was collected with a single stage impactor (to stimulate entry of particles into the respiratory tract) fitted with a nutritious laboratory media to enumerate the number of *A. fumigatus* between 1st -15th April 2002.

7.2 MATERIALS AND METHODS

7.2.1 Media preparation

Laboratory procedures, media preparation and sterilisation were carried out in accordance with the standard protocol described in BSI (1996). The composition of the malt extract agar medium used to culture *A. fumigatus* is shown in Table 7.1 (TCA, 1999b).

Table 7.1 Composition and directions for the preparation of malt extract agar (MEA) used to culture *Aspergillus fumigatus* (TCA, 1999)

Formulation	g l^{-1}
Malt extract (Oxoid Ltd., Basingstoke, Hampshire, UK)	20.0
Agar number 1 (Oxoid Ltd., Basingstoke, Hampshire, UK)	20.0
Penicillin G (Na^+ salt) (Sigma Aldrich, Gillingham, Dorset, UK)	20,000 units
Streptomycin sulphate (Sigma Aldrich, Gillingham, Dorset, UK)	40,000 units

Directions
Suspend the malt extract and agar in deionised water. Sterilize by autoclaving at 121 ^{o}C for 15 minutes and cool to 47 ^{o}C. Add the antibiotics and pour into petri dishes.

7.2.2 Sampling procedure

A portable single stage bioaerosol sampler with 100 apertures of 1 mm diameter (Burkard Manufacturing Co Ltd, Rickmansworth) was fitted with 90 mm MEA plates. In accordance with the manufacturer's instructions, the sampler was operated for a short period (30 s) to equilibrate. The apparatus was then mounted at the hinge of the bin lid to represent the typical distance of exposure to the mouth and respiratory tract and samples were taken over a 20 minutes period at a constant flow rate of 20 l min^{-1} Sampling was undertaken during the dismantling of the bins in April 2002 where there was maximum compost agitation. Sample collection was timed manually using a stopwatch and was set to provide a growth limit of approximately 30 *A. fumigatus* colonies. Replicate plates were used to monitor the maximum exposure to airborne particles during the dismantling of each HC bin in the study.

7.2.3 Confirmation and enumeration

Following exposure, plates were stored and transferred under chilled conditions to the laboratory. All plates were maintained at 4 ^{o}C until all samples had been collected in the bioaerosol investigation and subsequently the plates were inverted and incubated at 40 ºC for 48 h (M/R-253 Cooled Incubator, Sanyo Gallenkamp, PLC, Loughborough, Leicestershire, UK). The air sampling MEA plates were examined for typical colonies exhibiting *A. fumigatus* morphology (40 – 60 mm diameter, dense felt of dark green/grey) (Plate 7.1) and a selection of isolates (4) were subject to confirmation.

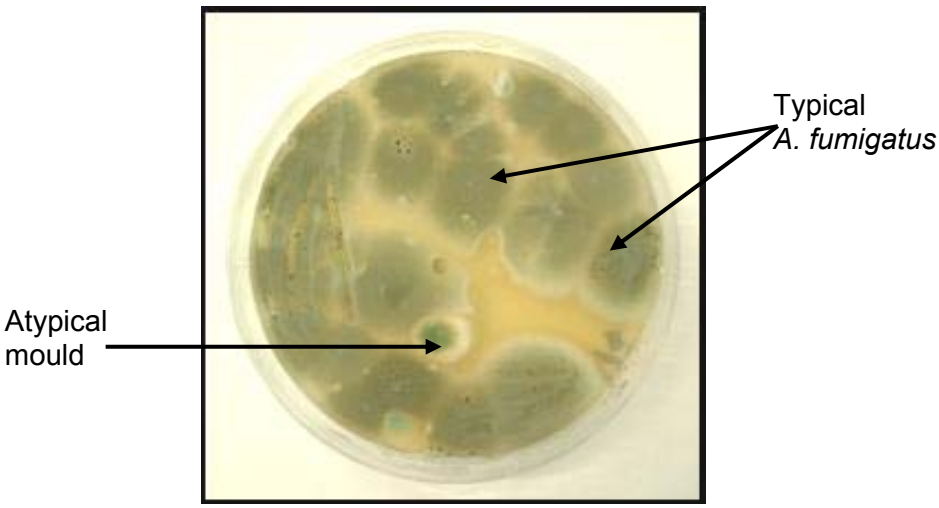

Plate 7.1 Typical and atypical cultures of *Aspergillus fumigatus*

For confirmation, spores from typical and atypical mould colonies were sub-cultured into 1 ml of sterile distilled water to form a turbid solution. A single point inoculum (10 ul) was performed on MEA and incubated at 40 ºC for 48 h (Plate 7.2). Speciation of the fungi was determined by an external mycologist (Williams & Neaves, Leatherhead, Surrey) using light microscopy.

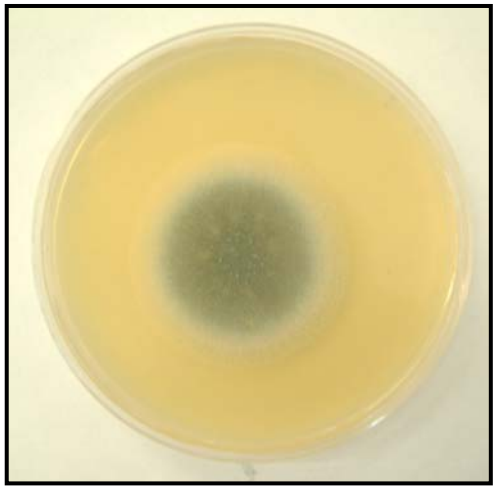

Plate 7.2 Single point inoculum culture of *Aspergillus fumigatus* used for confirmation

The numbers of *A. fumigatus* were calculated and reported as colony forming units per cubic metre of air (cfu m^{-3}). This represented the unit that results in the growth of a single colony on the MEA medium at the impaction site. Furthermore, it may represent a single microbial cell or spore, or cluster of cells which behave as a single aerodynamic particle.

The concentration was calculated as follows:

$$\textit{A. fumigatus} \text{ concentration (cfu } m^{-3}) = \frac{\text{Corrected Number of Colonies*}}{\text{Sample Duration x Flow Rate}}$$

* The corrected number of colonies was calculated from the measured Positive Hole Correction Table (Macher, 1989).

7.2.4 Quality assurance

Sampling was carried out in accordance with the principles of Good Laboratory Practice (DOH, 1990). Precautionary measures were taken to reduce the potential for microbial contamination by cleaning the sampling equipment between each sample collection cycle using a 70 % (v/v) aqueous solution of industrial methylated spirit. Equipment and surfaces in the laboratory were disinfected with 1 % solution of virkon during plating operation and preparation. Examination of plates and inoculation procedures were carried out in a class 2 safety cabinet to minimise aerial contamination.

Control plates of MEA were placed away from the compost bins and exposed for the same period as the respective samples to determine whether any indigenous *A. fumigatus* were present. No colony growths were observed on the control plates.

7.3 RESULTS AND DISCUSSION

7.3.1 Enumeration of *A. fumigatus*

All pure cultures were identified as *A. fumigatus* and an atypical culture was identified as *Aspergillus niger*. *A. niger* is a mesophilic mould capable of growth and survival within the temperature range: 7 – 47 ºC. It is associated with post-harvest decay of a wide variety of fresh fruit and stored foods, causing spoilage due to metabolite and toxin

release (Pitt & Hocking, 1995). Therefore, its presence within the composting environment was not unexpected.

A. fumigatus were detected during the physical disturbance of composting residues in all HC bins and the confirmed numbers enumerated during collected is shown in Table 7.2. Each cfu is the result of the growth of one colony on the selective medium at the impaction site on the medium and may represent a single microbial cell or spore, a cluster of cells, which behave as a single aerodynamic particle. Colony forming units were calculated based on advice by the HSE (pers com. HSE) and research guidance given by Macher (1989), which provided a 'positive-hole' correction table to calculate the number of colonies that can be adjusted for the probability that more than one viable particle was collected through a sampling hole and merged with other microorganisms at the impaction site to produce a single colony for a 100-hole impactor.

Table 7.2 Plate counts of *Aspergillus fumigatus* concentrations of airborne fungal particles

	Direct colony count on agar plates	Corrected colony number (Macher, 1989)	Colony forming units per volume of air (cfu m^{-3})
Min	10.0	11.0	36.7
Max	30.0	37.0	123.3
Average	20.9	23.7	78.7
Median	21.0	24.0	80.0

The mean airborne concentration of *A. fumigatus* was 79 cfu m^{-3} and the maximum number enumerated was 123 cfu m^{-3}, which are within the recommended tolerable concentration of 10^3 – 10^6 cfu m^{-3} that are reported to cause sensitisation, which is an acquired sensitivity or allergy (Millner *et al*. 1994). These values for airborne exposure are based on published scientific data derived from a variety of studies. However, they maybe conservative given that health effects were not reported downwind of composting sites despite evidence of bioaerosol emissions exceeding the limits (Bagni *et al*., 1977; Bovallius *et al*. 1978; Millner *et al*. 1994,). Nevertheless, there is relatively little data on the effects of exposure times for *A. fumigatus* and the effects on human health, and there are uncertainties about the appropriateness of recommended tolerable limits with respect to different time periods of exposure. Further research is therefore necessary to examine the exposure time/environmental standards assigned to protect human health.

Due to the ubiquitous nature of airborne fungal spores within the environment, small quantities of fungus are unlikely to produce any harmful effects although health issues may arise with extensive exposure to fungal particles that overwhelm the immune defence mechanisms (TCA, 1998b; Wheeler *et al*, 2001). However, the response and sensitisation to *A. fumigatus* is highly variable and even exposure to background doses maybe harmful to certain individuals. Nevertheless, the presence of spores or even growth of particular fungi is not always indicative of toxin production. For example, only small amounts of toxin may be produced, maybe unstable or they may only be toxic if produced in large amounts (Samson and Hoekstra, 1996). In addition, other factors such as physical, meteorological and operational characteristics have not been quantitatively assessed in relation to *A. fumigatus* (TCA, 1998b and Wheeler *et al*, 2001).

7.3.2 Effects of compost bin management on airborne emissions of *A. fumigatus*

Analysis of variance (ANOVA) was performed to statistically assess the effects of the four main home compost bin management treatments: (garden size, mixing, earthworm

inoculum and accelerator addition) on the airborne release of *A. fumigatus* during the physical handling of the composting waste residue (Table 7.3).

Table 7.3 Mean values and F probabilities for the effects of management treatments on *A. fumigatus*

	Statistic	Garden size		Accelerator		Inoculum		Mixing	
Colony forming units (cfu m^{-3})		**Large**	**Small**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**
	Mean values	75.4	82.0	77.5	79.9	73.3	84.1	78.4	79.0
	F probabilities	0.199		0.637		0.038		0.918	

A statistically significant difference (F_{PR} <0.05) in airborne emissions was detected with the earthworm inoculum and the spore concentration was measured with the addition of earthworms compared to the unamended condition (Table 7.3). There was no statistically significant effect (F_{PR} >0.05) of garden size, accelerator addition or mixing. Mean values confirmed that the significant correlation was attributable to positive inoculum addition (Table 7.3).

Waste treatment in small-scale units is highly biodynamic and organic matter is present at different stages of decomposition, which also depends on the activities of invertebrate animals, particularly earthworms. Therefore, earthworm inoculum addition may potentially influence the biological activity in the composters, including the activity of microorganisms, and therefore the emission of *A. fumigatus* spores. However, the mean values in Table 7.3 should be viewed with caution due to the correction factor of colony number inclusion (Table 7.2).

7.3.3 *A. fumigatus* in relation to waste inputs

The relationship between *A. fumigatus* and annual food, paper and garden waste is shown in Figures 7.1 – 7.3. Results suggest that *A. fumigatus* exposure was independent of waste type and inputs and no significant correlations were detected.

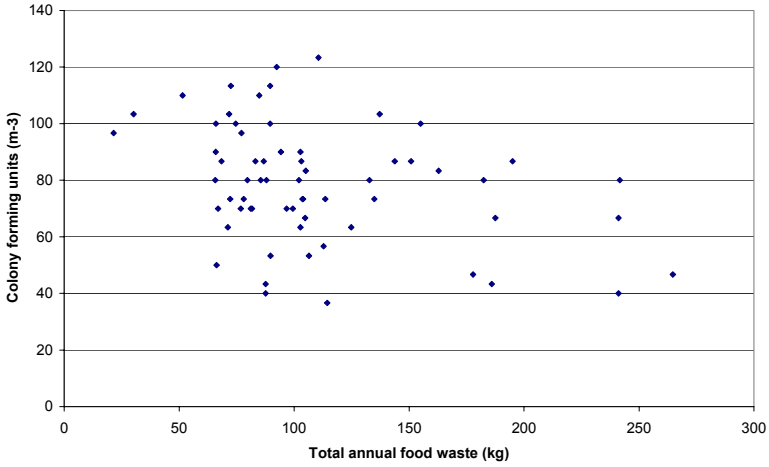

Figure 7.1 *Aspergillus fumigatus* in relation to total annual food waste, April 2002

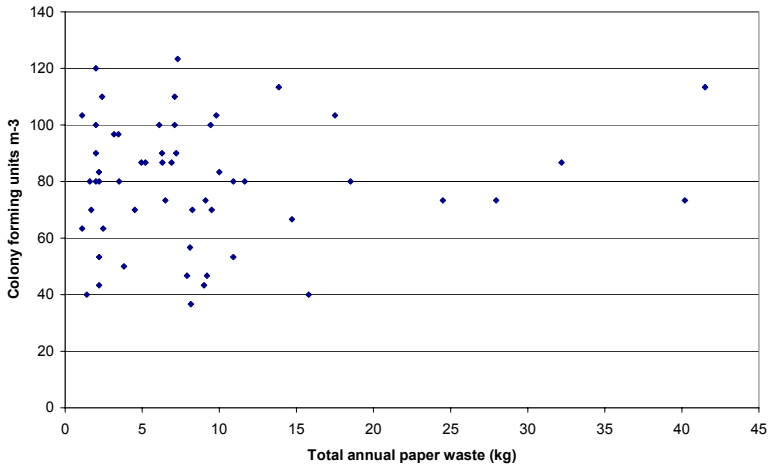

Figure 7.2 ***Aspergillus fumigatus*** **in relation to total annual paper waste, April 2002**

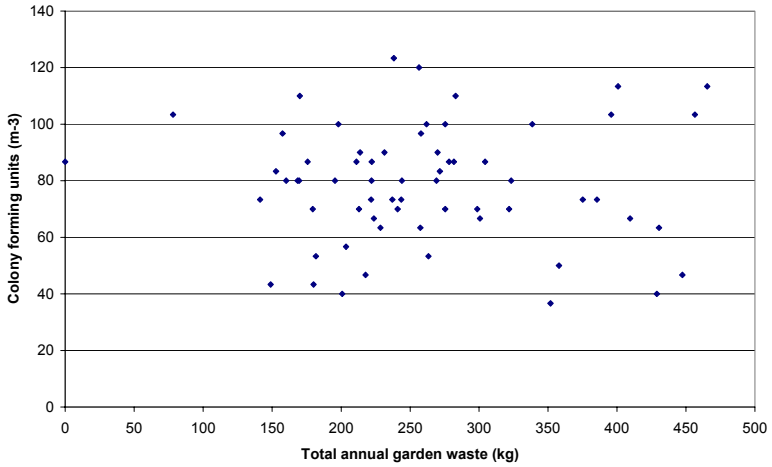

Figure 7.3 ***Aspergillus fumigatus*** **in relation to total annual garden waste, April 2002**

7.3.4 Guidance to home compost producers

Emission of *A. fumigatus* is associated with home composting activities and therefore, guidance to individuals involved in these activities is required. Gardeners handling (agitating) any organic substrate may generate bioaerosols causing exposure to microbial particles. The amount generated depends on the moisture status of the compost and other factors such as physical properties and the extent of the disturbed area (Miller *at al*., 1994). The results presented here are the first data produced on the potential bioaerosol emissions of *A. fumigatus* from HC bins and numbers were significantly below published health thresholds, which implied there is little risk to health from HC activity.

7.4 SUMMARY

A. fumigatus is a ubiquitous fungus in the environment and it is an opportunist allergen that can potential cause ill effects in individuals exposed to airborne spores. Airborne concentrations of *Aspergillus* spp. were detected during the physical disturbance of composting residues in all bins monitored in the study. However, these were well below the recommended tolerable concentration that may cause sensitisation (Millner *et al*., 1994). Although statistical analysis of fungus production and compost management factors revealed a significant correlation with earthworm inoculum addition, other

management factors such as garden size were not significant. However, it was not possible to offer an explanation for this occurrence. In general, the data suggests that airborne fungal microorganisms arising from HC activities do not pose a potential health hazard.