CRANFIELD UNIVERSITY

Louise J. Pankhurst

# The Effect of Green Waste Composting on the Concentration and Composition of Ambient Bioaerosols

School of Applied Sciences

Doctor of Philosophy

Supervisors: Dr. S. F. Tyrrel and Dr. G. H. Drew

January 2010

**CRANFIELD UNIVERSITY**

**SCHOOL OF APPLIED SCIENCES**

**DOCTOR OF PHILISOPHY**

**Academic Year 2009 - 2010**

**Louise J. Pankhurst**

# The Effect of Green Waste Composting on the Concentration and Composition of Ambient Bioaerosols

Supervisors: Dr. S. F. Tyrrel and Dr. G. H. Drew

**January 2010**

This thesis is submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

## Abstract

The emission and dispersal of bioaerosols from commercial composting facilities has become an issue of increasing concern over the past decade, as historical evidence links bioaerosol exposure to negative human health impacts. As a result, recommended concentrations and risk assessment limits were imposed in 2001. However, more recent research has suggested that these limits may be exceeded under certain circumstances. For example, underestimation of bioaerosol concentrations may occur through 'snapshot' sampling, and the use of methods that may reduce culturability of bioaerosols. This study aimed to address several gaps in knowledge, including quantification of bioaerosol concentrations downwind from sites, analysis of the effect that operational and environmental influences have on emission and downwind concentrations, and investigation of methods for the enumeration of non-culturable bioaerosols. The concentrations of bioaerosols upwind, on-site and downwind from two open-air green waste windrow composting facilities were enumerated in extensive detail, producing the first detailed and validated database of bioaerosol concentrations at green-waste composting facilities. The effects of composting processing activities, season, and meteorological conditions on concentrations were also investigated utilising this dataset. Results from these studies suggested that bioaerosols are able to disperse in elevated concentrations to distances beyond the 250 m risk assessment limit. Downwind peaks in concentration were directly linked to compost processing activities on-site, with the risk of sensitive receptor exposure to bioaerosols during non-operational hours minimal. Further, it was found that patterns in downwind concentrations of bioaerosols are likely to be governed by buoyancy effects, as a second peak in concentrations was found at 100-150m downwind. This finding was further supported through the use of a novel direct counting method. Finally, molecular methods allowed the composition of bioaerosols emitted from composting to be determined and showed that composting significantly alters the aerobiotic community at distances downwind. The methods investigated provide the potential for detailed, continuous measurements of bioaerosols, alongside identification of potentially pathogenic microorganisms, and could ultimately lead to source apportionment of bioaerosols.

## Acknowledgements

First and foremost, I would like to thank my supervisors Sean Tyrrel and Gill Drew for their endless knowledge, support and guidance. Their kindness and humour have made even the hardest times enjoyable.

I would like to thank Lewis Deacon for providing me with a source of inspiration and true friendship in both the field and laboratory. Other people essential to this project were Fred Coulon, Lolita Huet, Karine Desoir, Lee Larcombe and Pat Bellamy. Each of these people has provided support and assistance, without which I would have floundered.

Without my sponsors, SITA UK and SUEZ, with the Environmental Sustainability Knowledge Transfer Network, and the particular support of Philippa Yates, this project would never have been a success. I would also like to thank the staff in the Centre for Resource Management and Efficiency at Cranfield University, each of them has provided me with both personal and professional assistance during my time at Cranfield University. In particular, thanks to Phil Longhurst for setting a wonderful example through his endless generosity to myself, and everybody else within the department.

The team within the Centre for Research in Biomedicine and the Air Quality Management Resource Centre at the University of the West of England, Bristol; Simon Jackson, Enda Hayes, and Jian Liu provided crucial and exciting data for this project, as well as a ready source of inspiration. I would also like to thank the team at Essex University for welcoming me into their laboratory, and Manchester University for the WIBS P-TRAK collaboration.

I have enjoyed my time at Cranfield University with the help of my friends and relations. In particular João, Audrey, Ai and Niamh, for helping me through the final year. My family, including my parents, Steve, Hay, and Ben, gave me support and the belief that I would achieve my ambitions. And finally, Joe, for being eternally enthusiastic and reminding me of the bigger things in life.

## Table of Contents

## List of Figures

### SECTION I - The Emission and Dispersal of Bioaerosols

**Chapter 1**



**Chapter 2**



**Chapter 3**

**Chapter 4**



**Chapter 5**

**Chapter 6**



**SECTION II - The Composition of Bioaerosols**

**Chapter 8**

**Chapter 9**



**Chapter 10**

**Chapter 11**



**Chapter 12**



## List of Tables

**SECTION I - The Emission and Dispersal of Bioaerosols**

**Chapter 1**



**Chapter 2**



**Chapter 3**



**Chapter 5**

**Chapter 6**



**Chapter 8**



**Chapter 9**



**Chapter 10**

**Chapter 11**



## List of Equations



**APPENDIX I**

**APPENDIX II**

# SECTION I

# The Emission and Dispersal of Bioaerosols from Composting Facilities

# 1 The Bioaerosols Emitted from Composting Facilities: Introduction

## 1.1 Background

### 1.1.1 Introduction

Despite over a decade of research into bioaerosols emitted through green waste composting and their potential negative impacts on human health, understanding of their emission, dispersal, and composition remains limited. The requirement for composting to form a permanent part of the UK's strategy for recycling biodegradable waste has made knowledge of bioaerosol dispersal essential in order to inform the planning and regulation of composting facilities. Proportionate and appropriate measures concerning the emission of composting bioaerosols are required in order to protect human health, with current data available in the literature unable to allow development of these measures.

The dispersal distance of bioaerosols remains disputed, with a variety of contradictory data available (Fischer *et al*, 2008; Reinthaler *et al*, 1997). This may be due to the variety of sampling and enumeration methods available for bioaerosol analysis, making direct comparison between investigations difficult. In addition, many studies rely on a limited number of sampling occasions and locations. It has now been shown that bioaerosols are released episodically during compost processing activities (Albrecht *et al*, 2008; Taha *et al*, 2006). The reliance on short-term 'snapshot' sampling therefore increases the risk of missing peak emissions (Swan *et al*, 2003). Compounding this issue is the presentation of average bioaerosol concentrations; once again, this does not reflect the episodicity of emission, although peak, as well as mean exposure, is of concern with regards to human health (Becher and Lichtnecker, 2002; Bünger *et al*, 2000; Douwes *et al*, 2003; Hollingdale, 1974; Millner *et al*, 1994). There is a requirement for an extensive and validated database to be established regarding the concentrations of bioaerosols downwind from composting facilities, taking into account

both best and worst case scenarios. This will provide other researchers, site operators, and regulators with reliable data concerning the emission and dispersal of bioaerosols from composting facilities.

Another poorly understood area is the composition of bioaerosols, and impact of composting on the composition of ambient bioaerosols. Typically, sampling has focused on the enumeration of a limited number of culturable microorganisms of concern with regards to human health (Association for Organics Recycling, 2009). However, a large fraction of bioaerosols are not enumerated in this way, being non-culturable, non-viable, or unable to grow according to the conditions selected for culture (Head *et al*, 1998; Hugenholtz and Goebel, 2001; Swan *et al*, 2003; Ward *et al*, 1990). These fractions remain of concern with regards to human health (Swan *et al*, 2003). There are many available methods for the enumeration of these fractions of bioaerosols, including those based on direct counting and molecular analysis. However, few have been commonly or consistently used within bioaerosol analysis. These methods have the potential to increase knowledge of the composition of bioaerosols, including non-culturable fractions such as endotoxins, and the diversity of microbial communities emitted through composting. Use of these methods alongside culture-based methods would allow evaluation of their utility and applicability to bioaerosols studies, along with better knowledge of the impact that composting has on ambient bioaerosols and the bioaerosols sensitive receptors may be exposed to.

This project aims to address the issues outlined above, contributing to knowledge surrounding the concentration and composition of bioaerosols emitted through composting. Initially, a review of the information available regarding bioaerosols emitted through composting is presented. This review aims to critically analyse the available information regarding bioaerosols from composting facilities, evaluating their contribution to bioaerosol research. An analysis of the main factors affecting bioaerosol composition, emission and dispersal is provided, along with assessment of potential receptor exposure. Through this review, gaps in knowledge that are essential to the successful understanding of bioaerosols emitted through green waste composting are identified.

### 1.1.2 Legislative background to composting

Composting is the process by which biodegradable waste is decomposed under controlled conditions. The process is carried out aerobically, predominantly by thermophilic and thermotolerant microorganisms, converting biodegradable waste into a useful, stable, end-product (Duckworth, 2005; Office of the Deputy Prime Minister, 2004). The amount of waste being composted has increased in recent years, with this trend in growth predicted to continue due to changes in waste management policy. To date, most composting in the UK has been carried out in open air turned windrows (Slater *et al*, 2005), where biodegradable waste is heaped into piles (windrows) on composting pads in the open air. Windrows are regularly turned to aerate and homogenise them, with shredding carried out prior to, and screening carried out post composting. Activities such as turning, screening and movement of waste have been shown to cause aerosolisation of the thermophilic and thermotolerant microorganisms involved in the composting process (Taha, 2004; Taha and Pollard, 2005).

Most waste currently composted comes from Municipal Solid Waste (MSW); defined as either the waste fraction produced by households, or any waste collected by a local authority (Slater *et al*, 2005). In 2004 it was calculated that 29 million tonnes of MSW is produced every year in the UK, of which 66% was still sent to landfill (DEFRA, 2004). To improve sustainability and reduce greenhouse gas emissions (DEFRA, 2004; Duckworth, 2005) a series of targets for the diversion of MSW from landfill sites have been set through several European directives and UK legislations. The EU Landfill Directive (EC/31/99) of 1999 initiated the process of waste diversion from landfill through the establishment of the following targets:

- By 2010, the amount of Biodegradable Municipal Waste (BMW) sent to landfill must be reduced to 75% of the amount sent in 1995;
- By 2013, the amount of BMW sent to landfill must be reduced to 50% of the amount sent in 1995;
- By 2020, the amount of BMW sent to landfill must be reduced to 35% of the amount sent in 1995;

(Strategy Unit, 2002)

In the UK, the above targets were implemented through the 2000 Waste Strategy (Department of the Environment, Transport and the Regions, 2000; Pollard *et al*, 2004), the 2002 Landfill Regulations (DEFRA, 2004), and development in England has continued with the 2007 Waste Strategy (DEFRA, 2007). The Waste and Emissions Trading Act (WET act) and the Landfill Allowance Trading Scheme (LATS) implemented in 2004 and 2005 respectively (DEFRA, 2005) have introduced progressively tighter restrictions on the amount of biodegradable waste allowed to be sent to landfill (Slater *et al*, 2005). Below is an idealised hierarchy of how to deal with waste:

i. Reduction
ii. Re-use
iii. Recovery
iv. Disposal

(DEFRA, 2004)

Composting is seen as an essential part of this hierarchy, recovering the value of biodegradable waste. This has led to an increasing amount of waste being sent to composting facilities as Local Authorities strive to meet governmental and EU targets.

The number of composting facilities in the UK increased by 49% between the 2001/02 and 2003/04 Association for Organics Recycling surveys (Slater *et al*, 2005). The total quantity of waste composted increased over the same period by 20%, equating to an average increase in throughput of 10% per year (Slater *et al*, 2005). This increase in throughput continues, with a further 5% increase shown between 2005 and 2006/07 (Smith and Pocock, 2008). 58% of composting waste was handled at centralised[1], dedicated composting sites in 2006/07, with the remainder handled at on-farm sites[2] and landfill based sites (Smith and Pocock, 2008). Most of this waste (79%) is composted

[1] Sites run by dedicated compost producers or waste management companies (Slater *et al*, 2005)
[2] Sites run by individuals or organisations involved in farming or linked to agricultural activity (Slater *et al*, 2005)

using open-air turned windrow facilities (Smith and Pocock, 2008). In 2005 it was estimated that 1.8 million tonnes of waste had to be managed through facilities other than landfill in order to meet the Landfill Directive targets (Duckworth, 2005). With an estimated increased throughput of 600,000 tonnes anticipated to be found in 2008/09, the growth of the composting industry continues (Smith and Pocock, 2008).

The expansion in the number of facilities has increased the need for risk assessments and regulations surrounding composting, as links between bioaerosol exposure and compost worker health have been shown (Bünger *et al*, 2007; Ivens *et al*, 1997a; Ivens *et al*, 1997b; Wouters, 2003), coupled with evidence that bioaerosols can travel significant distances downwind (Albrecht *et al*, 2008; Crook *et al*, 2006; Fischer *et al*, 2008; Herr *et al*, 2003a; Recer *et al*, 2001). Regulations must be aimed at protecting sensitive receptors[3] (Association for Organics Recycling, 2009; Environment Agency, 2009a; The Composting Association, 1999). Current reference levels of 1000 CFU $m^{-3}$ are suggested for total bacteria and total fungi, and 300 CFU $m^{-3}$ for gram-negative bacteria, which must be achieved by the nearest sensitive receptor (Environment Agency, 2001a; Environment Agency, 2009b). Waste Management licenses stipulate that bioaerosol monitoring must be carried out regularly at composting facilities, and actions to reduce bioaerosol release taken if levels are excessive. Furthermore, composting facilities should not be situated within 250 m of sensitive receptors, unless it can be shown that bioaerosol release can be sufficiently reduced to minimise risk (Environment Agency, 2001a; Environment Agency, 2009a; Environment Agency, 2009b). These regulations were set by the Environment Agency in 2001 following the recommendations of a review of past data and studies of bioaerosol dispersal (Environment Agency, 2001a). The regulations are seen by many as being precautionary (Environment Agency, 2001a; Pollard *et al*, 2004); however, this precautionary approach is perhaps prudent given the uncertainties surrounding bioaerosol release and dispersal from composting facilities and the potential health impacts (Bond *et al*, 2005; Crook *et al*, 2006; Swan *et al*, 2003; Sykes *et al*, 2007).

[3] Any building, structure or installation where at least one person normally lives, works or attends school, other than a building, structure or installation within the same ownership or control of the operator/owner of the composting facility (Environment Agency, 2009a; The Composting Association, 1999)

## 1.2 Introduction to bioaerosols

Bioaerosols are airborne particles that are produced by living organisms (Curtis *et al*, 2006). This can include dust, fungi, bacteria, spores, pollen, dust mites, fragments of plants and animals and the constituent parts of cells (ADAS and SWICEB, 2005; Millner *et al*, 1994; Wouters, 2003). However, the largest proportion of bioaerosols consists of microorganisms and their cellular components (ADAS and SWICEB, 2005); including bacteria, fungal spores, actinomycetes, endotoxins, mycotoxins and glucans. Composting in open-air turned windrows leads to the proliferation of thermophilic and thermotolerant microorganisms, which contribute to bioaerosol loading in the atmosphere as composting activities are carried out. The different types of bioaerosols released from composting facilities are described below.

### 1.2.1 Bacteria

Bacteria are prokaryotic, single-celled organisms. They are ubiquitous, found in every habitat available, playing many essential roles within ecosystems (Prescott *et al*, 1990). The gram stain is a method used to distinguish between two of the main bacterial groups, by showing differences in cell wall structure. The two groups separated by the stain are termed gram-positive bacteria and gram-negative bacteria (Prescott *et al*, 1990). Gram-positive bacteria retain the gram-stain in their cell walls, which contain additional compounds, teichoic acids. These acids give the cell walls of gram-positive bacteria a negative charge, and are absent in the cell walls of gram-negative bacteria, which will not retain the gram-stain (Prescott *et al*, 1990).

Both gram-positive and gram-negative bacteria can be found as components of bioaerosols, as thermophilic and thermotolerant species proliferate during the composting process. For example, the gram-positive *Bacillus* spp. have been shown to increase during the thermic composting phase (Amir *et al*, 2008). A decline in bacteria is often seen towards the end of the composting process, as microorganisms better able to degrade recalcitrant substrates dominate (Amir *et al*, 2008), although it has been found that gram-negative bacteria can increase in number during the maturation phase

(Cahyani *et al*, 2002). It is actinomycetes, a type of gram-positive bacteria (Swan *et al*, 2003), and endotoxins, which can be found in the cell wall of gram-negative bacteria (Epstein, 1994; Liebers *et al*, 2006; Swan *et al*, 2003), that cause most concern for human health when considering bioaerosol release from composting facilities.

### 1.2.2 Actinomycetes

Actinomycetes are filamentous, spore producing, gram-positive bacteria (Swan *et al*, 2002; Swan *et al*, 2003). Although gram-positive bacteria are frequently found in aerosols of animal origin, filamentous bacteria such as actinomycetes are more common to soil and plant materials (Swan *et al*, 2002). Their thermophilic nature and ability to break down celluloses and lignin's means actinomycetes are essential to the composting process, proliferating during the thermic composting phase (Cahyani *et al*, 2002; Swan *et al*, 2003).

Actinomycetes have a mesophilic range of 20 to 50ºC (Taha *et al*, 2007) and a thermophilic range of 30 to 60ºC. 55ºC is their optimal temperature, at which numbers can exceed 10 million spores per gram of compost (Swan *et al*, 2002; Swan *et al*, 2003). Spores range in size from 1 to 3 μm and are readily aerosolised (Swan *et al*, 2002; Swan *et al*, 2003). The small size and abundance of actinomycete spores presents a hazard to human health, as they are able to penetrate deep into the human lung (Swan *et al*, 2003).

### 1.2.3 Endotoxins

Endotoxins are complex macromolecules that are found in the cell walls of gram-negative bacteria found living on the surfaces of animals and plants (Epstein, 1994; Swan *et al*, 2003). Endotoxins are composed of protein, lipid, and lipopolysaccharide (LPS) molecules found in the cell wall of gram-negative bacteria (Liebers *et al*, 2008). The endotoxins of gram-negative bacteria are typically composed of three regions: Lipid A, the hydrophobic and endotoxically active part of the molecule; the core section; and an *O*-polysaccharide, or *O*-chain. The chemical, and therefore structural, composition of these regions varies between species of gram-negative bacteria, leading

to variations in immuno-toxicity (Erridge *et al*, 2002). Although endotoxins are ubiquitous in the environment (Douwes *et al*, 1995), the volume released into the inhalable fraction of particulates through composting activities (Liebers *et al*, 2006; Schlosser *et al*, 2009; Swan *et al*, 2003) is a cause for concern regarding human health; as endotoxins are some of the main pulmonary immuno-toxicants, implicated in several respiratory conditions (Liebers *et al*, 2008; Swan *et al*, 2003).

### 1.2.4 Fungi

Fungi are eukaryotic organisms, most of which are characterised by filamentous growth as multicellular hyphae. Fungi reproduce by the means of spores (Prescott *et al*, 1990). Fresh, green waste is dominated by 'field' fungi such as *Cladosporium* spp., and *Alternaria* spp (Swan *et al*, 2003). Field fungi are most commonly found on living things, particularly plants. As the composting process progresses, these fungi are replaced by others, termed 'storage' fungi, as they proliferate in stored organic material (Swan *et al*, 2003). Fungi have been shown to increase towards the end of the composting process due to their ability to utilise recalcitrant substrates (Amir *et al*, 2008; Cahyani *et al*, 2002) Species commonly found in compost include *Penicillium* spp., *Eurotium* spp. and *Aspergillus* spp., all of which are storage fungi (Swan *et al*, 2003). In terms of bioaerosol release from composting facilities, *Aspergillus* species are frequently regarded as the most important, as they can be pathogenic in susceptible humans. The genus *Aspergillus* is ubiquitous in the environment and contains over 300 different fungal species (Epstein, 1994). The spores of the species *Aspergillus fumigatus* are commonly found in composting bioaerosols, as this species is thermotolerant and able to decompose celluloses and hemicelluloses (Epstein, 1994; Swan *et al*, 2002). The optimum temperature that *A. fumigatus* proliferates at is 37ºC, although thermophilic and thermotolerant fungi grow up to 65ºC (Swan *et al*, 2002). *A. fumigatus* is able to produce large numbers of inhalable spores, 2 - 3 μm in diameter, increasing the risk to human health as their small size can penetrate into bronchioles and alveoli of the lungs (Swan *et al*, 2003).

### 1.2.5 Mycotoxins

Mycotoxins are toxic secondary metabolites produced by certain fungal species as they proliferate within organic material (Swan *et al*, 2003). Both *Aspergillus* (including *A. fumigatus*) and *Penicillium* species produce mycotoxins. The role of mycotoxins within bioaerosols has rarely been studied (Swan *et al*, 2003). It has been suggested that fungi found as a product of composting do not produce highly toxic mycotoxins (Fischer *et al*, 1999), this may discourage research as health impacts are presumed to be less severe than impacts from exposure to other bioaerosols.

### 1.2.6 Glucans

Glucans are another component of fungal cells that can be aerosolised during the composting process. Within composting bioaerosols, (1-3)β-D-glucan is considered the most important. It is a polyglucose compound found in the cell walls of fungi, some bacteria, and plants (Swan *et al*, 2003). It has been shown to be a potent inflammatory agent (Swan *et al*, 2003). Glucans have also been infrequently studied as a component of composting bioaerosols. This may be for similar reasons as mycotoxins, as health impacts of glucan exposure are presumed to be less severe than exposure to other bioaerosols. However, it may also be a symptom of the focus of bioaerosol sampling methodologies on culturable bioaerosols, excluding non-culturable bioaerosol fractions such as endotoxins, mycotoxins and glucans. This is discussed in further detail within section 1.8 of this review.

## 1.3 Past research into bioaerosols emitted through composting

Bioaerosols emitted from composting facilities became popularised as a research area following Epstein's (1994) review paper, and a special research report that includes a comprehensive paper by Millner *et al*. (1994). While both publications focus on *A. fumigatus* and endotoxins, Epstein (1994) highlights health issues related to exposure from a residential and worker perspective. Conclusions suggested that the general

public would be protected from bioaerosol emissions through enclosure of activities, dampening compost, and the provision of buffer zones (Epstein, 1994). Millner *et al*. (1994) also assessed non-occupational exposure, and concluded that with the evidence available at the time, there was little risk posed to the general population by these bioaerosols from composting activities. There was, however, acknowledgement that immuno-suppressed and asthmatic fractions of the population have an increased risk of suffering from respiratory conditions as a result of bioaerosol exposure (Millner *et al*, 1994).

Current threshold values in the UK regarding bioaerosols and composting facilities were set following the recommendations of an Environment Agency report (Environment Agency, 2001a). Total bacteria and fungi, gram-negative bacteria, *Streptococcus*, and actinomycete emission and dispersal were measured at three composting sites and results compared to atmospheric dispersion modelling (Environment Agency, 2001a). The risk assessment limits were suggested following analysis of past data; recommended threshold levels of 1000 CFU $m^{-3}$ for total bacteria and fungi, and 300 CFU $m^{-3}$ gram-negative bacteria had been suggested in previous literature, and were adopted by the authors of the report (Environment Agency, 2001a). These values should be attained by the nearest sensitive receptor or by 250 m from site boundaries (Environment Agency, 2001a, b). Analysis of past data from Millner *et al*. (1994), and Gilbert (2002), supported these thresholds and the 250 m limit. However, within the Environment Agency report (2001) total bacteria and fungi were found above reference levels across all samples, and gram-negative bacteria threshold levels were exceeded on occasion at each site (Environment Agency, 2001a). Despite this the thresholds were supported, as concentrations did not exceed suggested sensitisation limits of $10^6$ to $10^8$ CFU $m^{-3}$ (Environment Agency, 2001a), and dispersal modelling also supported the thresholds. Uncertainties were highlighted regarding dose-response relationships, factors affecting particle deposition, and loss of viability. Despite these findings, health assessment of workers and past data analysis suggested little negative health impact on site workers, leading to a conclusion that there is little health risk posed to off-site sensitive receptors, and that the suggested thresholds are precautionary (Environment Agency, 2001a). However, since the implementation of these regulatory limits more

investigations into the background concentrations of bioaerosols have been carried out, particularly for *A. fumigatus* (O'Gorman and Fuller, 2008). This has led to a reconsideration of reference levels, with a suggestion that a 500 CFU $m^{-3}$ limit for *A. fumigatus* may be implemented (Dennis, 2009).

Although comprehensive, the Environment Agency study presented mean bioaerosol concentrations, gained through long-term sampling periods (Environment Agency, 2001a), neutralising peak emission data. Sampling was carried out using culture methods, which does not account for the non-viable fractions of bioaerosols, or non-culturable but viable cells (NCBV), which may recover viability under favourable conditions (Crook and Sherwood-Higham, 1997; Swan *et al*, 2003). Furthermore, modelling did not take into account different bioaerosols properties that affect deposition rate and viability (O'Gorman and Fuller, 2008). Dispersal was found to be highly variable, affected by type of waste, season, meteorological conditions, topography, and site operations. Taking into account these variables, the 250 m risk assessment limit, imposing a "presumption against permitting …of any new composting process…where the boundary of a facility is within 250 m of a workplace or the boundary of a dwelling,…" (Environment Agency, 2001b; Environment Agency, 2009b) was still recommended as a precautionary estimate (Environment Agency, 2001a).

After the Environment Agency (2001) report, little was published regarding bioaerosol emission and dispersal from composting facilities for several years, with data that was published often contradictory. For example, an ADAS and SWICEB (2005) report measuring total bacteria and fungi, actinomycetes, and *A. fumigatus* found 91% of data collected supported the Environment Agency's (2001) suggested limits, with concentrations below 1000 CFU $m^{-3}$ by 125 m from site boundaries, leading to a conclusion that bioaerosols return to upwind concentrations by 200 m from site boundaries. However, despite selection of sampling days where weather conditions were as similar as possible, less than half of samples taken showed the expected pattern in dispersal, consisting of low upwind concentrations, and higher on-site concentrations followed by a steady decline of bioaerosols to concentrations similar to those found

upwind (ADAS and SWICEB, 2005). Although bioaerosol modelling was carried out, confidence in the reliability of models was low due to the incomplete characterisation of how variables such as the presence and concentration of microorganisms in source material, impact of meteorological conditions on bioaerosol dispersal, and magnitude of activities on-site, affect emission and dispersal (ADAS and SWICEB, 2005). On the other hand, Recer *et al*. (2001), utilising a spore counting method, found 10 – 20% of *A. fumigatus* and actinomycete measurements 500 m downwind from a composting facility were two standard deviations or more above 'background' concentrations. Furthermore, a significant relationship was found between on-site emissions of bioaerosols and increases in concentration downwind (Recer *et al*, 2001). Despite historical support for the Environment Agency threshold levels, an increasing number of studies, such as Recer *et al*. (2001), report data suggesting bioaerosols may be transported to distances over 250 m from facilities in elevated concentrations (Albrecht *et al*, 2008; Fischer *et al*, 2008; Harrison, 2007; Sykes *et al*, 2007). The change in opinion regarding transport distances may be due to several factors, including sampling methods and experimental design.

The inherent problems in bioaerosol monitoring were highlighted through a comprehensive review of published data (Swan *et al*, 2003) . In particular, the exclusion of non-viable and NCBV fractions of bioaerosols through use of culture based methods; and the use of impaction samplers, which allow only short sampling times, were raised as issues. The importance of other bioaerosol sources was also shown, along with factors affecting emission and dispersal (Swan *et al*, 2003). It was suggested that short-term fluctuations in concentrations and differences in sampling strategy reduce the comparability of results, and only provide a 'snapshot' view of bioaerosol concentration. These factors combined to prevent the authors from answering questions surrounding the dispersal range and threshold values for nearby residential bioaerosol exposure (Swan *et al*, 2003), and are consistently cited as major issues within bioaerosol dispersal assessment (Albrecht *et al*, 2008; Fischer *et al*, 2008; Harrison, 2007; Sykes *et al*, 2007).

The issue of methods used in assessment of downwind concentrations was also raised by Douwes *et al*. (2003), particularly the lack of suitable quantitative methods. Within risk assessments, culture based methods can be useful as they show the presence of hazardous bioaerosols; however, it was suggested that they are of limited use within exposure assessments, where more sensitive measurements are required (Douwes *et al*, 2003). The report highlighted the need for validated non-culture quantitative methods, such as those based on fluorescence and direct counting (Chen and Li, 2005a; Chen and Li, 2005b; Prigione *et al*, 2004) to enable simpler risk assessments and allow the development of legal exposure limits (Douwes *et al*, 2003).

A recent Environment Agency report (in press) has also acknowledged the ongoing requirement for further data to support guidelines; this is despite the contribution of research since the 2001 report (Environment Agency, 2001a). Sampling results from Crook *et al.* (2006) suggested that there is a large decrease in bioaerosols from the source to 10 m downwind, followed by a similarly large decrease between 10 and 50 m. However, beyond 50 m patterns in deposition were unpredictable due to the influence of both climatic and physical variables (Crook *et al*, 2006). The suitability of the 250 m risk assessment limit was not discussed. Rather, the need for further data gathering to allow potential impacts on human health both on-site and at site boundaries to be quantified was highlighted (Crook *et al*, 2006; Environment Agency, in press).

The acknowledgement of failings within bioaerosol dispersal profiling and calls for improvement continues to be illustrated (Harrison, 2007; Sykes *et al*, 2007). Harrison (2007) described how several studies over the last decade show elevated bioaerosol concentrations 200 - 500 m downwind from composting activities. It was also suggested that all bioaerosol monitoring methods underestimate concentrations due to difficulties in retaining bioaerosol viability, the presence of the NCBV fraction, and the vast number of variables present within in sampling (Harrison, 2007). The acknowledgement of past issues within bioaerosol sampling has led to recent publication of studies presenting validated data regarding bioaerosol emission and dispersal (Albrecht *et al*, 2008; Fischer *et al*, 2008; Schlosser *et al*, 2009). These studies focus on repeat sampling, collecting a database of bioaerosol concentrations that can then be analysed

statistically to provide both mean and range of bioaerosol concentrations (Albrecht *et al*, 2008; Fischer *et al*, 2008; Schlosser *et al*, 2009). Research by Schlosser *et al.* (2009) concerned emission and on-site concentrations of total bacteria and fungi, gram-negative bacteria, actinomycetes and endotoxins, with an assessment of worker exposure according to composting strategy and activities carried out. The combined study by Albrecht *et al*. (2008) and Fischer *et al*. (2008) focused on dispersal of total bacteria and fungi, and actinomycetes, illustrating how periodically, bioaerosols could be found at concentrations greater than 1 order of magnitude higher than upwind concentrations 650 m from composting facilities. Although periodic increases in downwind concentrations were attributed to a combination of composting activity and meteorological conditions, these downwind concentrations were not directly traced to site as downwind concentrations were presented for distances over 200 m from site, omitting the distances from emission to 200 m. However, data suggested that typically, upwind concentrations of bioaerosols were achieved 300 – 500 m from site (Albrecht *et al*, 2008; Fischer *et al*, 2008).

The above literature goes some way towards quantifying downwind dispersal, and therefore potential sensitive receptor exposure to bioaerosols. However, in terms of specific exposure assessment, studies focus on waste management workers (Bünger *et al*., 2000; Douwes *et al*, 2000; Douwes *et al*, 2003; Heldal and Eduard, 2004; Ivens *et al*, 1997a, b; Lavoie and Dunkerley, 2002; Liebers *et al*, 2006; Neumann *et al*, 2002; Nielsen *et al*, 1997; Wouters *et al*, 2002; Wouters *et al*, 2003), omitting study of downwind receptors' potential exposure.

Herr *et al.* (2003a, b; 2004a, b) have examined residential exposure to composting bioaerosols, including total viable microorganisms, total fungi and bacteria, and actinomycetes. Through field measurement of bioaerosol concentrations and self-reporting of local residents' symptoms, evidence was presented suggesting that bioaerosol exposure can affect the health of local residents (Herr *et al*, 2003a, b, Herr *et al*, 2004a, b). Enumeration of bioaerosols suggested that background (upwind) levels were not reached until 550 m from the site. A distance dependent relationship between bioaerosol concentrations and irritative airway complaints amongst residents was also

found (Herr *et al*, 2004a), remaining evident up to 500 m from site (Herr *et al*, 2003a); although symptoms were not supported through clinical examination (Herr *et al*, 2003a, b, Herr *et al*, 2004a, b). Residents were found to have the highest exposure to bioaerosols 150 - 200 m from a large scale composting facility.

These more recent studies present peak concentrations of bioaerosols (Albrecht *et al*, 2008; Fischer *et al*, 2008; Herr *et al*, 2003a; Schlosser *et al*, 2009), unlike many previous studies which presented average concentrations. This evidence has contributed to the recent suggestions that bioaerosols may have the ability to travel in elevated concentrations to distances greater than 250 m from the site (Albrecht *et al*, 2008; Fischer *et al*, 2008; Herr *et al*, 2003; Schlosser *et al*, 2009). However, with little data specifically linking elevated downwind concentrations to on-site emissions, these data do not provide a complete profile of downwind bioaerosol concentrations, or provide complete analysis of the causes of elevated downwind concentrations.

In addition, the above review illustrates how sampling of bioaerosols emitted through green waste composting has focused on a minority of culturable microorganisms, namely total fungi and bacteria, *A. fumigatus*, actinomycetes and gram-negative bacteria. One exception to this is Schlosser *et al*. (2009), who analysed endotoxins alongside culturable bioaerosols. Furthermore, a recent study aiming to identify key microorganisms that may be utilised as indicators of composting processes, utilised non-culture based methods. This led to the qualification of bioaerosol communities emitted through composting (Le Goff *et al*, 2009), revealing the diversity of microorganisms emitted that is not accounted for through culture based enumeration of limited microorganisms. The lack of studies enumerating non-culturable fractions of bioaerosols and the focus on a small range of culturable microorganisms has limited knowledge of bioaerosol composition. The following section describes the health impacts of bioaerosols, illustrating how improved knowledge of composition would allow better understanding of potential health impacts from bioaerosol exposure.

## 1.4 Bioaerosols and their health impacts

### 1.4.1 Bioaerosols from composting facilities – summary

A variety of microorganisms, their metabolites and constituent parts can be aerosolised through the composting process. These bioaerosols all possess different physicochemical properties, affecting the way in which they interact, disperse in the atmosphere and the different health impacts that they can have. Table 1.1 presents a summary of bioaerosols that are released from composting facilities and are considered a health risk.

Table 1.1: Summary of key bioaerosols associated with green waste composting, including sources, known features of dispersal, potential health impacts, and legislation covering emission and dispersal.

| **Bioaerosol** | **Description** | **Species** | **Sources** | **Dispersal in the Atmosphere** | **Health Impacts** | **Legislation** |
|---|---|---|---|---|---|---|
| Bacteria | Both gram-positive and gram-negative bacteria can be found as components of bioaerosols (Swan *et al*, 2002). Can be found in the meso- (10 - 35ºC) to thermophilic (30 - 60ºC) range (ADAS and SWICEB, 2005; Swan *et al*, 2002). | Include most species of bacteria. Most thermophilic bacteria are gram-positive and *Bacillus* species (ADAS and SWICEB, 2005). Most common gram-positive types include corynebacteria and cocci (Dutkiewicz, 1997) | Ubiquitous in the environment. Larger quantities released through composting as gram-positive proliferate in stored plant material (ADAS and SWICEB, 2005; Dutkiewicz, 1997) Also found in other waste management activities (Bond *et al*, 2005; Swan *et al*, 2003). Food industry workers exposed to gram-negative and gram-positive bacteria (Dutkiewicz, 1997). Concentrations in air of region of $5.3\times10^3$ CFU $m^{-3}$ found at waste transfer sites (Lacey, 1997) | Tend to clump together forming larger aggregates (ADAS and SWICEB, 2005). Effect on dispersal relatively unknown (Herr *et al*, 2003a). Likely that bacterial aggregates fall out rapidly (ADAS and SWICEB, 2005; Environment Agency, 2001a) | Pose minimal health risk as aggregates cannot be inhaled deep into the lung (ADAS and SWICEB, 2005). Gram-negative bacteria can produce enterotoxins, implicated in gastrointestinal symptoms amongst sewage sludge workers (Dutkiewicz, 1997). Some constituent parts of gram-positive bacteria could be immuno-toxic (Dutkiewicz, 1997) | No legal threshold limit imposed. Suggested threshold levels of 1000 CFU $m^{-3}$ for total bacteria and 300 CFU $m^{-3}$ for gram-negative bacteria (Environment Agency, 2001a, b) |
| Actinomycetes | Filamentous, spore forming gram-positive bacteria (Swan *et al*, 2002). Present in compost in meso- and | Most common thermophilic species are *Saccharopolyspora (Faenia) rectivirgula*, *Saccharomonospora* spp, *Thermoactinomyces* | Commonly occur in stored plant material (Dutkiewicz, 1997). Many thermophilic species depend on heat generation during | Small, dry spores, less likely to aggregate (ADAS and SWICEB, 2005). Spores 1 - 5µm in diameter | Small spores can penetrate to the alveoli of the lungs (ADAS and SWICEB, 2005; Swan *et al*, 2002). Thermophilic | No legal threshold imposed. Affected by suggested threshold levels |

| | | | | | | |
|---|---|---|---|---|---|---|
| | thermophilic ranges (Swan *et al*, 2002; Swan *et al*, 2003; Taha *et al*, 2007). Often described as a cross between bacteria and fungi (ADAS and SWICEB, 2005). Thousands of spores 1-3 μm in diameter produced (Swan *et al*, 2002) | *thalpophilus*, *Thermoactinomyces vulgaris* and *Thermomonospora* spp. Most common mesophilic species include *Streptomyces* species (Lacey, 1997; Swan *et al*, 2003) | composting to flourish. Green wastes often dominated by *Streptomyces* species (Lacey, 1997) During compost shredding, *Saccharomonospora* spp., *Streptomyces. albus* and *Thermoactinomyces* frequently isolated (Lacey, 1997). Found at $5\times10^4$ CFU $m^{-3}$during shredding of green waste, and at $10^6$ CFU $m^{-3}$ during compost turning (Lacey, 1997). Isolated throughout other waste management activities (Bünger *et al*, 2000; Swan *et al*, 2002). Also found in agricultural activities (Dutkiewicz, 1997) | (Lacey and Crook, 1988). Emission rates from compost windrows estimated at $480 – 700 \times 10^6$ CFU/s (Taha and Pollard, 2004) | actinomycetes main cause of extrinsic allergic alveolitis (ADAS and SWICEB, 2005). Can also cause extrinsic allergic alveolitis. Exposure over 10's of millions of spores per $m^3$ of air can trigger immune responses (Swan *et al*, 2002). Repeated exposure leads to a cascade of responses, potentially leading to chronic bronchitis (Swan *et al*, 2003). Waste collectors and compost workers show sensitisation to actinomycetes (Bünger *et al*, 2000) | of 1000 CFU $m^{-3}$for total bacteria (Environment Agency 2001a, b) |
| Endotoxins | Complex phospholipid-polysaccharide macromolecules that are part of the cell wall of gram-negative bacteria (Epstein, 1994). | Produced by gram-negative bacteria (Epstein, 1994). Species including *Pantoea agglomerans*, *Pseudomonas* spp., *Klebsiella* spp., *Rahnella* spp. and *Alcaligenes faecalis* (Dutkiewicz, 1997) | Ubiquitous in the environment, have been isolated in household dust (Swan *et al*, 2003). Gram-negative bacteria grow on the surfaces of plants (Dutkiewicz, 1997), so found in large quantities in the arable agricultural and composting industries. Also found in textile | Released as discoid particles (microvesicles) 30 - 50 nm in diameter with a characteristic triple-tracked membrane (Dutkiewicz, 1997). | Main pulmonary immuno-toxicants (Dutkiewicz, 1997). Chronic exposure leads to symptoms such as inflammation, chronic bronchitis and COPD, leading to a decrease in lung function (Swan *et al*, 2003). Conversely, have also been linked to decreased | No legal threshold limit imposed. Affected by suggested threshold levels of 300 CFU $m^{-3}$ for Gram-negative bacteria (Environment Agency, 2001a, |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | industry, wood processing industry, metalworking industry, sewage works and paper mills (Dutkiewicz, 1997; Liebers *et al*, 2006). Found at levels below 0.5 µg $m^{-3}$ where compost processed (Clark *et al*, 1983). Occur in range of $10^{-1}$ to $10^{6}$ ng $m^{-3}$ in air of different occupational environments (Dutkiewicz, 1997). Found up 50 EU $m^{-3}$ in summer, usually below 10 EU $m^{-3}$ (Neumann *et al*, 2002) | | sensitisation to other allergens (Bush and Peden, 2006). Exposure at workplace linked to other symptoms including fever, cough, shortness of breath, wheezing, headache, nose and throat irritation and chest tightness (Liebers *et al*, 2006) | b). Committee on Occupational Health suggested threshold response ranges as 1000-2000 ng $m^{-3}$ for ODTS; 100 – 200 ng $m^{-3}$ for acute bronchoconstriction; 20 – 50 ng $m^{-3}$ for MMI (Millner *et al*, 1994) |
| Fungi and fungal spores | Field fungi dominate in fresh, green compost, replaced by storage fungi as the process advances. Thermophilic and thermotolerant species predominate. *A. fumigatus* has capacity to produce millions of spores, 2 - 3 µm in diameter throughout lifecycle (Swan *et al*, 2002; Swan *et al*, 2003). | Field fungi include *Cladosporium* spp., *Alternaria* spp. and *Verticillium* and are found on living things, particularly plants. Storage fungi include *Aspergillus*, *Eurotium*, and *Penicillium* species and decompose stored organic products (Dutkiewicz, 1997; Swan *et al*, 2002). *Aspergillus* genus contains over 300 species (Epstein, 1994), of which *A. fumigatus*, a thermotolerant fungi, is | Ubiquitous in the environment. Mid to late stage compost leads to proliferation of thermotolerant species (Swan *et al*, 2002). Also present in other waste management activities (Swan *et al*, 2002). *A. fumigatus* found to exceed exceeded $10^{6}$ CFU $m^{-3}$ at sites where compost processed (Clark *et al*, 1983). Field and storage fungi found in agricultural industry (Dutkiewicz, 1997). | Spores are small and dry, so unlikely to aggregate (ADAS and SWICEB, 2005). *A. fumigatus* spores 1 – 5 µm in diameter (Lacey and Crook, 1988), most fungi and fungal spores 5 - 19 µm in diameter (ADAS and SWICEB, 2005). Modelling of dispersal gives variable results – suggestions of 1 × | Size range means spores can penetrate to bronchi (ADAS and SWICEB, 2005). Pathogenic, so may be hazardous at low levels (Douwes *et al*, 2003). Can cause both allergenic and invasive disease. Several *Aspergillus* species implicated in extrinsic allergic alveolitis (Dutkiewicz, 1997). An opportunistic pathogen, most likely to infect the immuno-compromised | No legal threshold imposed. Suggested threshold levels of 1000 CFU $m^{-3}$ for total fungi (Environment Agency, 2001a, b). Suggestion of 500 CFU $m^{-3}$ limit for *A. fumigatus*, along with removal of 1000 CFU $m^{-3}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | considered most important amongst composting bioaerosols from fungal sources (Epstein, 1994); but also including *A. flavus, A. candidus, A. terreus, A. clavatus* and *A. niger* (Dutkiewicz, 1997) | Closed composting facilities during turning process showed > 5.0 x $10^5$ CFU $m^{-3}$fungi, proportion *A. fumigatus* of up to 64% (Reinthaler *et al*, 1997) | $10^6$ CFU $m^{-3}$*A. fumigatus* up to 400 m from compost facilities (Drew *et al*, 2005), also of reduction to background levels within 200 m of composting site (Gilbert *et al*, 2002; Herr *et al*, 2003a). *A. fumigatus* emission rates from compost windrows estimated at 240 – 890 × $10^6$ CFU/s (Taha and Pollard, 2004) | (Epstein, 1994). Can cause Invasive Aspergillosis (Swan *et al*, 2003). Symptoms of exposure include coughs, chest tightness and influenza type symptoms (Environment Agency, 2001). Immune responses triggered by high exposure, repeated exposure leads to cascade of responses resulting in chronic bronchitis (Swan *et al*, 2003). Also associated with gastrointestinal symptoms (Ivens *et al*, 1997a) | total fungi limit (Dennis, 2009). Classified as a level 2 hazard on the Health and Safety Executives approved list of biological agents (Advisory Committee on Dangerous Pathogens, 2004) |
| Mycotoxins | Low molecular weight toxic secondary metabolites produced by some species of filamentous fungi as they grow in organic materials (Swan *et al*, 2003) | Produced, amongst others, by *Penicillium* spp. and *Aspergillus* spp., including *A. fumigatus* (Swan *et al*, 2003) | Present in compost dusts and other waste management activities (Swan *et al*, 2002). Can be present in air in region of 0 - $10^2$ ng $m^3$ (Dutkiewicz 1997). No particularly toxic mycotoxins isolated from *A. fumigatus* cultures (Fischer *et al*, 1999). | Little known regarding dispersal in compost dusts (Swan *et al*, 2003) | Usual route of exposure through ingestion of contaminated food. Can cause neurotoxicity, carcinogenicity and teratogenicity in vertebrates (Swan *et al*, 2003). Can suppress activity of pulmonary defence system and alveolar macrophages, increasing risks of developing respiratory cancer (Dutkiewicz, | No legal threshold imposed. May be affected by current suggested threshold levels of 1000 CFU $m^{-3}$for total fungi (Environment Agency, 2001a, b) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | 1997). | |
| Glucans | Components of cell walls, found in fungi, some bacteria, and plants. Has been suggested as a marker of microbial exposure (Douwes *et al*, 2006; Swan *et al*, 2003) | Present in fungi, some bacteria and plants (Swan *et al*, 2003) | Usually associated with high fungal levels, including those from composting facilities (Swan *et al*, 2003). Has also been found in homes suffering from water damage (Douwes *et al*, 2006). (1-3)β-D-glucan can occur in air in region of $10^{-2}$ to $10^{2}$ ng $m^{-3}$ (Dutkiewicz, 1997) | Little known about glucans dispersal from composing facilities (Swan *et al*, 2003) | (1-3)β-D-glucan considered the most important in bioaerosols from a health perspective. It is a potent inflammatory agent, suspected to contribute to adverse lung function effects from bioaerosol exposure (Swan *et al*, 2003) | No legal threshold imposed. May be affected by current suggested threshold levels of 1000 CFU $m^{-3}$for total fungi and total bacteria (Environment Agency, 2001a, b) |

### 1.4.2 Allergic responses

Allergic responses[4], the hypersensitive reaction of the immune system to an allergen, are some of the most common complaints linked to bioaerosol exposure (Déportes *et al*, 1995). One of the most significant allergic risks posed is the exacerbation of existing conditions. In particular, high airborne fungal concentrations have been linked to increases in asthma mortality (Curtis *et al*, 2006). Further responses include Chronic Obstructive Pulmonary Disease (COPD) and allergic rhinitis (seasonal allergies) (Dutkiewicz, 1997). COPD is characterised by a general decline in lung function causing breathing to become forced and can develop from asthma (FARLEX, 2007).

Actinomycetes and fungi have been linked to these allergic responses (Epstein, 1994; Swan *et al*, 2002; Swan *et al*, 2003; Lacey and Crook, 1988). Thermophilic actinomycetes are acute allergens; while fungi and the constituent parts of fungi also commonly cause allergic responses (ADAS and SWICEB, 2005; Dutkiewicz, 1997). Fungi implicated include *Aspergillus* and *Penicillium* species (Dutkiewicz, 1997).

Concern has also been raised over possible sensitisation of people to bioaerosols, where immune markers linked to exposure to an antigen are found within a subject, despite no symptoms being evident. This may be caused by frequent exposure to low concentrations of bioaerosols. Clinical examinations of waste collectors and compost workers have revealed significant increases in blood antibodies against fungi and actinomycetes (Bünger *et al*, 2007; Hollingdale, 1974). One study showed that 19% of compost workers were sensitised to *A. fumigatus* (Becher and Lichtnecker, 2002); the same study found higher incidence of rhinitis symptoms (Becher and Lichtnecker, 2002) such as mild itching, watery eyes and nose, coughing, sneezing and wheezing (Millner *et al*, 1994) amongst these workers. Other studies verify this; with significantly more symptoms and diseases of the respiratory system found amongst waste collectors and compost workers when compared to control groups (Bünger *et al*, 2000; Hansen *et*

[4] Allergic response, or allergy, is defined as a 'hypersensitive state acquired through exposure to a particular allergen'. 'Hypersensitive state' describes an 'exaggerated immune reaction to what is perceived as a foreign substance' (FARLEX, 2007).

*al*, 1997; Wouters, 2003). Although, actual health complaints made by workers were not found to significantly deviate from control groups (Bünger *et al*, 2007).

The lack of reported respiratory conditions may be seen as indicative that observed symptoms do not significantly affect worker health. However, sensitisation is of concern as it may eventually lead to the development of a more serious condition, Extrinsic Allergic Alveolitis (EAA), also termed Hypersensitivity Pneumonitis. This is where repeated exposure to low levels of bioaerosols, sometimes over several years, sensitises the subject, causing a sudden and severe reaction to the allergen (FARLEX, 2007; Millner *et al*, 1994). The condition can also result from one high exposure event (FARLEX, 2007). Fungi identified as causes of EAA include several *Aspergillus* species, including *A. fumigatus, A. flavus, A. clavatus, A. terreus, A. versicolor*, and *A. umbrosus* (Dutkiewicz, 1997). Actinomycetes are also acute allergens, with thermophilic species cited as the main cause of EAA (ADAS and SWICEB, 2005; Dutkiewicz, 1997). It has also been suggested that endotoxin exposure can enhance immune response to other bioaerosols, activating and exacerbating responses to actinomycetes and fungi (Millner *et al*, 1994). Conversely, endotoxin exposure has also been linked to decreases in sensitivity to other allergens (Bush and Peden, 2006). Despite strong evidence linking these conditions to bioaerosol exposure, there is still little information regarding dose-response relationships (Douwes *et al*, 2003). This may be because bioaerosol exposure elicits different responses and degrees of response in each individual.

### 1.4.3 Inflammatory responses

Inflammation is the response of the immune system to harmful stimuli, in order to isolate and remove the stimuli. This may be caused through the hypersensitive reaction of the immune system to an allergen, or through exposure to a pathogen or irritant (FARLEX, 2007). Bioaerosol exposure can also lead to both acute and chronic inflammation of the lungs (Douwes *et al*, 2003), resulting in a range of symptoms and conditions. Symptoms include fever, cough, shortness of breath, wheezing, headache, chest tightness and acute airway flow restriction (Liebers *et al*, 2006). One of the more

common inflammatory conditions is Mucous Membrane Irritation (MMI) (Burge and Hodgson, 1988); MMI is a relatively mild and localised inflammatory reaction (Millner *et al*, 1994). Bioaerosols implicated in MMI include endotoxins, with suggestions that exposures of around 20 - 50 nanograms $m^{-3}$ can cause the condition (Millner *et al*, 1994). More chronic lung inflammation has been linked to (1-3)β-D-glucan exposure (Dutkiewicz, 1997); these conditions include Chronic Bronchitis, where inflammation reaches the bronchial mucous membrane (FARLEX, 2007). Particles capable of reaching and potentially causing inflammation in the bronchi include cell fragments (endotoxins and glucans), fungi and fungal spores, actinomycetes and bacteria (ADAS and SWICEB, 2005). It has been shown that waste collectors are at an increased risk of developing chronic bronchitis (Chun-Yuh *et al*, 2001; Hansen *et al*, 1997).

### 1.4.4 Infections

Infection is the growth of a pathogenic, foreign species, within a host species to the detrimental effect of the host (FARLEX, 2007). Infections, both tissue and systemic (Millner *et al*, 1994), are recognised as being one of the rarest responses to bioaerosol exposure (Swan *et al*, 2003) caused by exposure to pathogenic fungi and fungal spores. However, when respiratory infections do occur they can result in high mortality rates.

One possible infection is Aspergillosis; this is where *Aspergillus* species colonise lungs, creating balls of fungus termed Aspergillomas (Bond *et al*, 2005). *A. fumigatus* has been shown to cause over 90% of Aspergillosis cases (Denning, 2006; Lacey and Crook, 1988;). *A. fumigatus* has several properties that increase its pathogenicity; it is a thermotolerant and rapidly growing species, it has molecular properties allowing it to bind to airways and withstand host defences, and produces an inhibitor that may reduce immune response (Denning, 2006). Untreated Aspergillosis has a mortality rate of nearly 100% (Denning, 2006).

Although dangerous, these pathogens are highly opportunistic, meaning that they will usually only infect people who are immuno-compromised (Bond *et al*, 2005), or have existing lung damage. This includes people with diabetes, those who have recently

undergone surgery or treatment for cancer, people with immuno-suppressant diseases (Denning, 2006), and those who have suffered from diseases such as tuberculosis. It has been estimated that each person in the UK inhales hundreds of *A. fumigatus* spores each day with no adverse health effect (Swan *et al*, 2003).

### 1.4.5 Toxicity

Bioaerosols also have the potential to have toxic effects on the human body. Gram-positive bacteria pose minimal risk as they cannot be inhaled deep into the lungs (ADAS and SWICEB, 2005); however, some of their products and constituent parts such as enzymes and proteins could act as immuno-toxicants (Dutkiewicz, 1997). Immuno-toxic responses resulting from extreme exposure to fungi or endotoxins include Organic Dust Toxic Syndrome (ODTS, also known as Toxic Pneumonitis), which is one of the most common toxic effects and induces flu-like symptoms (Dutkiewicz, 1997; Lacey and Crook, 1988). A suggested threshold exposure capable of causing ODTS is 1000 - 2000 ng $m^{-3}$ of endotoxins (Millner *et al*, 1994). Fungal species cited as causes of ODTS include *Rhizopus microsporus* and another *Aspergillus* species, *A. candidus* (Dutkiewicz, 1997).

Fungi also produce mycotoxins, a secondary product of filamentous fungal metabolism (Swan *et al*, 2003). Although *A. fumigatus* has been shown to produce a range of mycotoxins, none have been found to be highly toxic (Fischer *et al*, 1999). However, it is possible that they can suppress the pulmonary defence system, making people more susceptible to other respiratory conditions (Dutkiewicz, 1997).

### 1.4.6 Other responses to bioaerosol exposure

There are a number of conditions also attributed to bioaerosol exposure, which do not fall into the classifications of allergy, infection or inflammation. The mycotoxins produced by fungi can suppress pulmonary defence systems and alveolar macrophages, making people more susceptible to other diseases (Dutkiewicz, 1997). In particular, this may increase the risk of developing respiratory cancer (Douwes *et al*, 2003; Dutkiewicz,

1997). Symptomatic conditions associated with bioaerosol exposure amongst waste collectors include unusual tiredness and headache (Heldal and Eduard, 2004). Thermophilic fungi have also been linked to mycotic abortion through infection (ADAS and SWICEB, 2005). Furthermore, there is evidence that increased exposure to fungi in homes can lead to an increase in neurotoxicity (Kilburn, 2003).

Gastrointestinal symptoms have frequently been attributed to bioaerosol exposure. As well as endotoxins, gram-negative bacteria can produce protein enterotoxins, which have been implicated in the development of gastrointestinal symptoms amongst sewage workers (Dutkiewicz, 1997; Marchand *et al*, 1995). Gastrointestinal symptoms amongst waste collectors also show higher prevalence than control groups (Poulsen *et al*, 1995), and this has been linked to increased exposure to fungi and gram-negative bacteria (Ivens *et al*, 1997a; Poulsen *et al*, 1995).

## 1.5 Exposure to bioaerosols

The average 70 kg male human inhales approximately 20 $m^{-3}$ of air daily (Curtis *et al*, 2006). Particulates, including bioaerosols, are inhaled with this air. Air pollution in general is estimated to cause 800,000 premature deaths worldwide every year (Swan *et al*, 2002). In 1999 approximately 1.8 million people were affected by occupational illnesses in the UK, of which 53,000 were respiratory and 14,000 of these cases were people employed in the agricultural sector (Swan *et al*, 2003).

Occupational illnesses amongst waste management workers linked to bioaerosol exposure include respiratory conditions caused through allergy, inflammation and infection; toxicity and gastrointestinal symptoms amongst other symptomatic afflictions. This has been shown in numerous studies. Within the available literature, there is a consensus that bioaerosol exposure is the cause of many occupational illnesses (Becher and Lichtnecker, 2002; Bünger *et al*, 2000; Bünger *et al*, 2007; Chun-Yuh *et al*, 2001; Curtis *et al*, 2006; Douwes *et al*, 2003; Dutkiewicz, 1997; Eduard *et al*, 2001; Hansen *et al*, 1997; Harrison, 2007; Ivens *et al*, 1997a; Ivens *et al*, 1997b; Liebers *et al*,

2008; Lundholm and Rylander, 1980; Wouters *et al*, 2002). However, the risk posed to the general public remains uncertain. In addition, although exposure can be defined as being subjected (exposed) to elevated concentrations of bioaerosols (FARLEX, 2007), there is no consistent method of determining exposure to bioaerosols. For example, exposure could be defined as the total quantity of bioaerosols that a sensitive receptor may be subject to over a defined period of time, typically the median values over sampling time and occasions; or the frequency of the receptor being subject to concentrations of bioaerosols above those found naturally, and the amount by which ambient concentrations were exceeded (Domingo and Nadal, 2009; Douwes *et al*, 2000).

Despite the quantity of available research, data regarding dose-response relationships are also limited for bioaerosols (Douwes *et al*, 2003). This makes the estimation of risk posed to off-site sensitive receptors difficult. Studies on the potential health effects posed to residents have been limited and results are often contradictory. Residents living between 150 and 200 m from a composting facility were shown to have increased prevalence of irritated airways. In this case thermophilic actinomycetes, bacteria and fungi were measured in the order of $10^5$ CFU $m^{-3}$ 200 m from the site (Herr *et al*, 2003a). The symptoms of irritated airways were found to be consistent with Mucous Membrane Irritation (MMI), although no increase in the prevalence of allergies and infection was shown (Herr *et al*, 2004a). However, the author recognises that other facilities only affect air quality up to 200 m from the site (Herr *et al*, 2003a). It has also been suggested that when atmospheric conditions are unstable, background levels of *A. fumigatus* are not reached until 500 m from sewage sludge composting facilities (Swan *et al*, 2003). In addition, recent studies have shown that elevated concentrations of bioaerosols could typically be found at 300 – 500 m from composting facilities, while periodically, elevated concentrations could be found over 650 m downwind (Albrecht *et al*, 2008; Fischer *et al*, 2008). Others have found elevated concentrations of bioaerosols in residential areas 500 m downwind from composting facilities, with 10% of *A. fumigatus* and 21% actinomycete measurements significantly higher than background concentrations (Recer *et al*, 2001). These results are significant in terms of the

Environment Agency's 250 m risk assessment limit, increasing doubts surrounding its validity.

Other studies, however, have suggested that exposure of residents to microorganisms from composting facilities is of little cause for concern; as annual median values of 170 - 330 CFU $m^{-3}$ for bacteria, 75 - 340 CFU $m^{-3}$ for fungi, and 15 - 52 CFU $m^{-3}$ for *A. fumigatus* were found in residential areas between 150 and 2000 m from large-scale composting facilities (Reinthaler *et al*, 1997). All of these values are well below the suggested thresholds (Environment Agency, 2001a; Environment Agency, 2009b; Environment Agency, 2001b). Peak quantities above the median may also have been attributable to alternative bioaerosol sources (Reinthaler *et al*, 1997), although no evidence is presented to support this. Further studies have found that 91% of bacteria and fungi samples were below the suggested thresholds by 125 m from composting facilities (ADAS and SWICEB, 2005); with 80 - 90% of bioaerosols deposited between 20 and 40 m from the site boundaries (Environment Agency., 2001).

The above data has been collected using a variety of sampling methods and strategies, contributing to the various uncertainties and differences in reported data. These discrepancies, along with numerous gaps in knowledge regarding bioaerosol dispersion and receptor exposure, have all served to undermine confidence in exposure assessment. This has led some to conclude that so far, there is insufficient data to prove or disprove any link between residential exposure to bioaerosols, and release from composting facilities (SLR Consulting Limited, 2003). However, determination of receptor exposure is essential to the process of defining dose-response relationships. Measurement of exposure will aid in the definition of 'dose', whether presented as median values over longer periods of time, such as 24 hours, or as the frequency of exposure to concentrations elevated over those found ambiently.

## 1.6 Bioaerosol Emission, Dispersal and Deposition

### 1.6.1 Emission from composting facilities

It has been shown that composting facilities release microorganisms and the constituent parts of microorganisms into the atmosphere. Measurements of the air 1 m downwind from windrows in open air composting facilities revealed *A. fumigatus* concentrations of 2 - 4 × $10^3$ CFU $m^{-3}$ (Kothary *et al*, 1984). Experiments using a portable wind tunnel to find emission rates of bioaerosols from static windrows found levels in the region of 13 - 22 × $10^3$ CFU $m^{-2}$/s for mesophilic actinomycetes and 8 - 11 × $10^3$ CFU $m^{-2}$/s for *A. fumigatus* (Taha *et al*, 2005b). Most bioaerosols are released through agitation activities such as compost shredding and windrow turning, where concentrations of approximately 10 - 37 × $10^6$ CFU $m^{-3}$ for *A. fumigatus* and 19 - 36 × $10^3$ CFU $m^{-3}$ for actinomycetes have been reported, levels 3 orders of magnitude higher than those from static windrows (Clark *et al*, 1983; Taha *et al*, 2006). It has been suggested that turning windrows generates more bioaerosols than compost shredding, with actinomycetes found below 5 × $10^4$ CFU $m^{-3}$ during the shredding of green waste, and up to $10^6$ CFU $m^{-3}$ while green waste windrows are turned (Lacey, 1997).

The condition and composition of compost is also likely to affect the bioaerosols emitted, although a lack of information regarding the release mechanisms of bioaerosols prevents detailed knowledge of these impacts. For example, although it is known that maintenance of compost at lower moisture contents increases emissions (Epstein, 1994), the effect of lower moisture on microbial composition of composting microorganisms, and hence bioaerosols emitted, remains unclear. This is despite evidence that maintenance of the composting processes with different physical characteristics results in the development of different microbial communities (Steger *et al*, 2005). In addition, it is acknowledged within available literature that different feedstocks are likely to result in different microbial populations throughout the composting process (Epstein, 1994). However, once again, there is little available data regarding the effect of feedstock on the composition of bioaerosols emitted.

The age of the compost can also affect the types of bioaerosols released. Bacteria are more common at the beginning of the process, actinomycetes and thermotolerant bacteria dominating in the thermic phase, followed by succession of fungi, actinomycetes, and mesophilic bacteria communities as the process continues and enters the maturation phase (Amir *et al*, 2008; Bond *et al*, 2005; Cahyani *et al*, 2002). The succession of microbial communities during composting is largely a result of selection through temperature and substrate availability. For example, early stage composting is characterised by the rapid growth of microorganisms able to successfully compete for readily available substrates, such as free sugars. These microbial communities are termed *r*-selected microorganisms within microbial ecology, and are characterised by rapid growth followed by population collapse to become dormant members of the microbial community as readily available substrates are exhausted (Begon *et al*, 1996). These *r*-selected populations are succeeded by communities of microorganisms termed *K*-selected, these are able to slowly grow in number through utilisation of recalcitrant substrates such as lignin, cellulose and hemicelluloses (Begon *et al*, 1996), becoming dominant community members in the later stages of composting. Although these changes in microbial community have been well shown, there remains a lack of knowledge regarding how these changes in microbial composition may affect bioaerosol composition. The focus of bioaerosol enumeration both on-site and downwind remains on a small selection of culturable bioaerosols. This has led to a lack of understanding of the consortium of bioaerosols that may be emitted through composting, with changes in bioaerosol composition likely to result from different feedstock, age, and condition of compost.

### 1.6.2 Factors affecting dispersal and deposition

Once released the dispersal and deposition of bioaerosols is determined according to parameters including the physical and chemical properties of the bioaerosol, topographical, and meteorological conditions (Colls, 2002). Dispersal in the atmosphere is determined by the movement of the bioaerosol plume as carried by the wind (advection), and turbulence, the random motion of air which causes the bioaerosol plume to spread perpendicular to the wind direction. Turbulence is affected by surface

roughness (the amount of vertical deviation from a 'smooth' surface), resulting from the movement of lower layers of air over the surface (Bell and Treshaw, 2002). Release of bioaerosols from composting facilities can be regarded as a series of sources and emissions, for example, as windrow turning moves from one end of the pile to the other (Swan *et al*, 2003). Activities such as turning and screening are typically elevated by 5 m (Swan *et al*, 2003), while windrows themselves can be found over 3 m high. This means that bioaerosols can be released as above ground sources, affecting plume dispersal (Swan *et al*, 2003). Ground point sources create the highest concentrations of a pollutant under stable meteorological conditions as dispersal is limited, while elevated point sources cause little local pollution in stable conditions, and are brought rapidly to ground in unstable meteorological conditions (Bell and Treshaw, 2002). Buoyancy effects (a rising air flow created by heating of local air by freshly exposed compost) may also increase the distance bioaerosols travel if released from an above ground source (Swan *et al*, 2003). The degree of pollutant dispersal over the first kilometre from release is largely determined by vertical temperature variations, and wind speed (Seinfeld and Pandis, 2006). Terrain also affects dispersal. In complex terrain both ground and elevated point sources cause most pollution under stable meteorological conditions (Bell and Treshaw, 2002). Also, higher surface roughness increases frictional drag, enhancing turbulent transfer of bioaerosols (Bell and Treshaw, 2002), and increasing deposition.

Particles, including bioaerosols, are transported to the ground through turbulent transfer, and deposited through sedimentation, Brownian diffusion, impaction, or interception (Colls, 2002; Bell and Treshaw, 2002; Seinfeld and Pandis, 2006). The size of particulates affects the way in which they are deposited and the rapidity of this deposition; bioaerosols can be found from 30 nm in diameter for some endotoxins, up to 19 μm for some fungi and fungal spores (ADAS and SWICEB, 2005; Dutkiewicz, 1997; Lacey and Crook, 1988). The individual properties of each bioaerosol, such as particle density, aerodynamic diameter, electrical charges and hydrophobicity will also affect the type and rate of deposition (Colls, 2002). Smaller particles have the ability to travel further in the atmosphere, however, they can also possess higher levels of thermal energy, which can cause particle aggregation and increase deposition. However, smaller

particles may also demonstrate more rapid deposition, as their passage through the air is impeded less by the presence of other airborne particles (Colls, 2002). For particles under 0.01 μm in diameter, Brownian diffusion is the dominant mechanism of deposition. This is the collision of particles with atmospheric molecules, resulting in the particle impacting on a surface. Particles over 1 μm in diameter are more likely to be deposited through sedimentation, where the particle falls onto surfaces through gravity (Colls, 2002). Bioaerosols ranging in size from 0.1 to 2.5 μm may accumulate in the atmosphere, as deposition mechanisms are least efficient in this range, with no deposition mechanism dominant (Colls, 2002; Seinfeld and Pandis, 2006). Bioaerosols could also become incorporated into cloud droplets, leading to wet deposition through precipitation (Bell and Treshaw, 2002; Seinfeld and Pandis, 2006; Smith, 1990). This process is partially determined by particle hydrophobicity and size, as the process is more effective for smaller particles (Smith, 1990).

The nature of bioaerosol emission, particularly episodicity of emissions due to composting activities (Clark *et al*, 1983; Lacey, 1997; Taha *et al* 2005b; Taha *et al*, 2006), is also likely to be reflected in downwind concentrations. This has been suggested through recent studies, which have shown periodic excursions of bioaerosols above background concentrations downwind from facilities, rather than constant high concentrations (Albrecht *et al*, 2008; Fischer *et al*, 2008; Recer *et al*, 2001). However, often these peaks are attributed to meteorological conditions rather than site activities, resulting in uncertainty regarding the causes of excursions above background concentrations downwind from site.

### 1.6.3 The prediction and measurement of bioaerosol dispersal

There are many differences and discrepancies in reported dispersal ranges for bioaerosols. The physical and chemical properties of bioaerosols determine deposition rate, however, most have not been fully characterised. Atmospheric modelling of bioaerosols must therefore make assumptions regarding bioaerosol properties. Bioaerosols can be released as clumps, aggregates or single cells (ADAS and SWICEB, 2005; Drew *et al*, 2006; Swan *et al*, 2003), the proportions of release in each form are

largely unknown, although recent currently unpublished work has contributed to knowledge in this area, suggesting that a large proportion are emitted as single cells or spores (Tamer Vestlund, 2009). Most models assume bioaerosols disperse as a gas, not accounting for the potential ability of bioaerosols to aggregate, which may result in behaviour as particles (Drew *et al*, 2006). It has been suggested that aggregations of *A. fumigatus* would increase their dispersal range, as their surface area increases (Drew *et al*, 2006). The aggregation potential is affected by spore size, hygroscopicity, aerodynamic diameter, humidity, moisture content, polarity, temperature, agitation, and light levels (Amanullah *et al*, 2001; Borrego *et al*, 2000). The incomplete characterisation of bioaerosol properties has therefore contributed to delays in the development of a successful bioaerosol dispersal model.

Characterisation of emission rates is another problem in bioaerosol dispersion modelling. Typically, hourly concentrations are used to model dispersal; this has the potential to miss episodic releases and smooth peaks or troughs in dispersal (Swan *et al*, 2003; Drew *et al*, 2005). In turn, this can lead to underestimation of the dispersal range of bioaerosols (Drew *et al*, 2005). Furthermore, most atmospheric dispersion models are designed to model dispersal over distances of 1 km or more (Drew *et al*, 2006; Drew *et al*, 2007a), but it is distances up to 1 km that are of interest in this context, particularly given the 250 m limit (Environment Agency, 2001a; Environment Agency, 2009b).

These assumptions and current gaps in knowledge have contributed to large variations in bioaerosol dispersal being found through both modelling and field measurements. Bioaerosols are expected to have reached current suggested threshold concentrations (1000 CFU $m^{-3}$ for total bacteria and total fungi, and 300 CFU $m^{-3}$ for gram-negative bacteria), or background concentrations (Environment Agency, 2001a; Environment Agency, 2009b) by 250 m from the site boundary. This limit was set by the Environment Agency using results from the SCREEN 3 dispersion model (SLR Consulting Limited, 2003; Swan *et al*, 2003). Gilbert *et al.* (2002) supported this, finding background concentrations ranging from 0 - 1 CFU $m^{-3}$ bacteria, to between $10^2$ and $10^3$ CFU $m^{-3}$ *A. fumigatus* (Gilbert *et al*, 2002) were achieved within 200 m of the site boundary (ADAS, 2005; Gilbert *et al*, 2002). Field studies also showed that 91% of

samples taken were below reference levels by 125 m from site boundaries (ADAS and SWICEB, 2005).

However, modelling using hourly meteorological data rather than Pasquill stability classes (as are typically used) has shown higher concentrations of bioaerosols at ground level over a smaller area (Drew *et al*, 2006; Drew *et al*, 2007a). Under very unstable atmospheric conditions *A. fumigatus* and actinomycetes were reduced to threshold levels by 50 and 100 m from the site respectively. In neutral weather conditions, threshold levels were achieved by 40 m for *A. fumigatus* and 100 m for actinomycetes. However, under very stable conditions threshold levels were not reached until 200 m for *A. fumigatus* and 300 m for actinomycetes (Drew *et al*, 2006). Levels of *A. fumigatus* reaching $1 \times 10^6$ CFU $m^{-3}$ 400 m from site boundaries under stable and convective weather conditions have been suggested (Drew *et al*, 2005). Field measurements have also demonstrated that SCREEN 3 predicts lower concentrations than are actually found on-site (Drew *et al*, 2007b). Other studies have suggested that background concentrations of actinomycetes and *A. fumigatus* are not reached until 300 m from composting facilities (Herr *et al*, 2003a, b), and that in some cases levels are 100 - 1000 times those reported as background concentrations between 150 and 320 m from bioaerosol sources (Herr *et al*, 2003a, b).

The variability between both bioaerosol and site properties also increases differences in downwind concentrations found at different locations. It has been suggested that some composting facilities influence ambient bioaerosol concentrations up to 550 m from the site (Herr *et al*, 2004a), while others were found to only influence concentrations up to 200 m from the site (Herr *et al*, 2003b). These differences may be caused by local weather and climate, topography, and the type and age of compost. ADAS and SWICEB (2005) found less than half of samples showed the expected dispersal patterns, more recently the Environment Agency (Crook *et al*, 2006) found dispersal over 50 m unpredictable. Above ground bioaerosol release along with variables including buoyancy effects may prevent a fraction of the bioaerosol plume from reaching ground level and being detected until some distance from site boundaries (Swan *et al*, 2003). As a result, sampling close to site boundaries may not detect the main bioaerosol plume,

only those that rapidly fall out of the atmosphere (Swan *et al*, 2003). The numerous variables affecting dispersal and discrepancies between measured and modelled bioaerosol concentrations have led some to conclude that in this context atmospheric dispersion models are imprecise (ADAS and SWICEB, 2005; Millner *et al*, 1994).

Many of these studies have also only used culture methods, not taking into account any non-viable or NCBV fractions. These non-viable and non-culturable bioaerosols can still pose a health risk (Crook and Sherwood-Higham, 1997; Swan *et al*, 2003), and their emission and dispersal remains to be quantified. As shown previously, as well as omitting non-culturable fractions the focus of bioaerosol enumeration on a minority of culturable bioaerosols has limited knowledge of the consortium of bioaerosols that sensitive receptors may be exposed to downwind from composting facilities.

A final issue is the quantification of 'background' bioaerosol concentrations. Customarily, samples taken upwind of site boundaries are taken as 'background' concentrations, with bioaerosol levels expected to reach this background by 250 m from composting site boundaries. However, there are numerous other sources of bioaerosols that can influence upwind concentrations and levels at receptor. It has been found that 17% of samples taken upwind of facilities showed bioaerosols, despite selection of sampling sites with no identifiable third-party source (ADAS and SWICEB, 2005). Furthermore, in some cases upwind concentrations were taken as little as 25 m from site boundaries (ADAS and SWICEB, 2005). It is possible that under light and variable wind conditions bioaerosols from the composting facility may disperse this short distance upwind; some reports have suggested that sampling 300 m upwind is more appropriate in order to reduce this risk (Albrecht *et al*, 2008; Fischer *et al*, 2008). In addition, as the composition of bioaerosols remains unknown, the effect of composting emissions on the composition of bioaerosols in ambient air, representing 'normal' sensitive receptor exposure, cannot be determined. Background levels could be more accurately described if alternative bioaerosol sources, as well as composting, are identified, qualified and quantified. Once this has been completed the impact of composting facilities on these background bioaerosols could be determined.

## 1.7 Bioaerosol sources

### 1.7.1 Background concentrations

Bioaerosols can be released from many different sources, both industrial and residential. These sources all contribute to the background concentrations of bioaerosols that receptors are exposed to on a daily basis. Background levels also vary with season, higher levels being reported in the summer and autumn months (Nielsen *et al*, 1997c). However, although many sources have been identified and shown to cause adverse health effects, residential exposure due to these sources is rarely investigated.

Little data is available regarding the background concentrations of bioaerosols within ambient environments, although a range of both rural and urban locations have been investigated. For example, mean *A. fumigatus* background concentrations, measured in an urban area, were suggested to be around 10 CFU $m^{-3}$, although periodically concentrations of 300 – 400 CFU $m^{-3}$ were found (O'Gorman and Fuller, 2008). Mesophilic bacteria have also been measured ambiently, with a background level of 0 – 1 CFU $m^{-3}$ suggested (Gilbert *et al*, 2002). In addition, background concentrations of endotoxins have been suggested to remain below 10 EU $m^{-3}$ (Liebers *et al*, 2008). The quantification of background concentrations of bioaerosols is complicated due to variations in land use, with bioaerosols emitted through many sources other than composting. For example, fungi depend upon the emission of fungal spores into the air for successful reproduction (Epstein, 1994; Swan *et al*, 2003), leading to potentially higher concentrations of fungal spore bioaerosols in areas with fungi present, such as woodland. The lack of data regarding background concentrations has been previously highlighted as a gap within knowledge regarding bioaerosols, limiting assessment of dispersal distances to site specific data regarding upwind concentrations (Albrecht *et al*, 2008).

### 1.7.2 The waste industry

The waste industry is a major source of environmental bioaerosols; with many routes for bioaerosol release other than composting. Waste collection is one of the more commonly investigated routes of bioaerosol exposure (Allmers *et al*, 2000; Becher and Lichtnecker, 2002; Bünger *et al*, 2000; Bünger *et al*, 2007; Hansen *et al*, 1997; Heldal and Eduard, 2004; Ivens *et al*, 1997a; Neumann *et al*, 2002; Poulsen *et al*, 1995; Wouters *et al*, 2002); with collection found to release $10^4$ CFU $m^{-3}$ total bacteria, and up to 50 EU $m^{-3}$ endotoxins in the summer (Neumann *et al*, 2002). Green waste collection has been found to release the highest quantities of bioaerosols, with workers exposed to 35 - 55 times the quantities of bioaerosols than those collecting other forms of waste. Concentrations up to $10^5$ CFU $m^{-3}$ *A. fumigatus* have been found from green waste collection (Dutkiewicz, 1997; Nielsen *et al*, 1997a). Sewage sludge is another source of bioaerosols within the waste industry. Sewage sludge workers can be exposed to endotoxins and gram-negative bacteria (Dutkiewicz, 1997); with up to 1 million colony forming units of *A. fumigatus* found in 1 g of sewage sludge (Kothary *et al*, 1984). Bioaerosols have even been shown to disperse into the atmosphere from anaerobically digested sewage sludge that has been spread onto land (Baertsch *et al*, 2007). Landfill sites are also sources of bioaerosols, particularly throughout the summer months (Bond *et al*, 2005; Rahkonen *et al*, 1987).

### 1.7.3 Agriculture

Another common source of bioaerosols is agricultural activities. Workers in this profession have a long recorded association with illnesses triggered by bioaerosol exposure (Eduard *et al*, 2001); such as Farmers Lung Disease (Extrinsic Allergic Alveolitis) and Mushroom Workers Lung. Crop farmers are commonly exposed to gram-negative bacteria, field and storage fungi, and thermophilic actinomycetes, from sources such as grain crops and silage (Dutkiewicz, 1997; Lacey, 1997). High endotoxin levels are particularly prevalent in the grain, seed and legume agricultural sector (Liebers *et al*, 2006; Spaan *et al*, 2005). Livestock farmers are also at risk of exposure, with animal keepers, breeders, and workers at slaughterhouses shown to be exposed to

high levels of gram-positive bacteria, and occasionally to endotoxins and fungi (Dutkiewicz, 1997; Lutgring *et al*, 1997; Seedorf and Hartung, 1999; Shale *et al*, 2006).

### 1.7.4 Other sources

Indoor bioaerosols are a common area of research, with illnesses such as sick building syndrome being attributed to bioaerosol exposure. Water damage in buildings has been cited as a cause of increased bioaerosol exposure in homes (Douwes *et al*, 2006; Trout *et al*, 2001). Storage of organic waste within houses can also increase bioaerosol exposure, with constituent parts of fungi (including *A. fumigatus*) and endotoxins found in quantities up to 7.6 times normal values on kitchen floors where organic waste was stored for over 1 week (Wouters *et al*, 2000).

Bioaerosols have also been linked to adverse health effects amongst workers in the wood and paper industries (Lacey, 1997), and some residents symptoms linked to odour from pulp mills (Deane *et al*, 1977). Wood processing workers are exposed to gram-negative bacteria and endotoxins in the early stages of processing, and filamentous fungi at later stages (Dutkiewicz, 1997; Liebers *et al*, 2006). The food industry, including food packing, can cause worker exposure to both gram-positive and gram-negative bacteria, along with endotoxins and occasionally fungi (Dutkiewicz, 1997; Lacey, 1997). Metalworkers can be exposed to gram-negative bacteria and endotoxins (Dutkiewicz, 1997); while biotechnology workers can also be exposed to endotoxins, along with the various other immuno-toxic products of fungi (Dutkiewicz, 1997; Lacey, 1997). Textile workers can also be exposed to bioaerosols, particularly endotoxins (Liebers *et al*, 2006); while workers in machining plants were shown to be exposed to endotoxins in quantities up to 790 EU $m^{-3}$, airborne fungi up to 470 CFU $m^{-3}$ and airborne bacteria up to 468,000 organisms $m^{-3}$ (Thorne *et al*, 1996).

It is possible that other sources of bioaerosols and human exposure remain undiscovered. For example, *A. fumigatus* can grow on a wide variety of media, including wood, green leaves, grass, paper, fabric, leather, rubber, plastics, aviation fuel and sludge (Epstein, 1994). As well as contributing to workplace exposure, growth on

all of these sources may contribute to daily residential exposure. Table 1.2 below provides a summary of bioaerosol concentrations found from a variety of sources, compared to those recovered at composting facilities.

Table 1.2: Summary of bioaerosol concentrations found to be emitted from, or present in, a range of sources.

| **Source** | **Bioaerosol (CFU $m^{-3}$)** | | | **Bioaerosol** | **Reference** |
|---|---|---|---|---|---|
| | **Bacteria** | ***A. fumigatus*** | **Fungi** | **Endotoxin** | |
| Suburban area | 42-$10^2$ | 0-$10^1$ | | | (Fuller, 2008; Kothary *et al*, 1984; Millner *et al*, 1994; O'Gorman and Fuller, 2008; Swan *et al*, 2003) |
| Indoors (UK homes) | | | 28 - > $10^3$ | | (Swan *et al*, 2003) |
| Grain stores | $10^5$ | | $10^4$ | $10^3$ ng $m^{-3}$ | (Swan *et al*, 2003) |
| Grain harvesting | $10^7$-$10^8$ | | $10^5$-$10^7$ | | (Swan *et al*, 2003) |
| Handling hay and grain | $10^8$ | | $10^8$ | | (Swan *et al*, 2003) |
| Animal feed mills | | | $10^3$ | $10^1$-$10^2$ ng $m^{-3}$ | (Swan *et al*, 2003) |
| Animal housing | $10^3$-$10^6$ | | $10^3$-$10^5$ | $10^1$-$10^4$ ng $m^{-3}$ | (Swan *et al*, 2003) |
| Handling mushroom compost | $10^7$ | | $10^5$ | 0-20 ng $m^{-3}$ | (Swan *et al*, 2003) |
| Picking mushrooms | $10^3$ | | $10^5$ | | (Swan *et al*, 2003) |
| Wood bark composting | $10^4$-$10^5$ | | $10^6$-$10^7$ | | (Swan *et al*, 2003) |
| Handling domestic waste | $10^3$-$10^4$ | | $10^4$-$10^5$ | | (Swan *et al*, 2003) |
| Domestic waste transfer station | $10^2$-$10^5$ | | $10^6$ | | (Lacey, 1997; Swan *et al*, 2003) |
| Domestic waste incineration | $10^7$ | | $10^7$ | | (Swan *et al*, 2003) |
| Domestic waste materials recycling | $10^5$ | | $10^5$ | $10^3$ ng $m^{-3}$ | (Swan *et al*, 2003) |
| Domestic waste landfill sites | $10^6$ | | $10^5$ | | (Swan *et al*, 2003) |
| Factories and mills | $10^1$-$10^6$ | | < $10^1$-$10^5$ | 0-$10^4$ ng $m^{-3}$ | (Swan *et al*, 2003; Thorne *et al*, 1996) |
| Compost waste collection | $10^3$ | $10^4$ | $10^4$-$10^5$ | | (Lavoie *et al*, 2006; Nielsen *et al*, 1997a) |
| Waste collection | $10^3$-$10^4$ | | $10^4$-$10^5$ | 10-50 EU $m^{-3}$ | (Neumann *et al*, 2002; Nielsen *et al*, 1997a; Nielsen *et al*, 1997b) |

## 1.8 The sampling and enumeration of bioaerosols

### 1.8.1 Sampling methods

There are many options available for sampling of bioaerosols; although the most commonly used are direct impaction, liquid impingement, and filtration (Dietrich, 2001). Direct impaction is where air is pumped across plates filled with a medium for microorganism growth (agar), allowing bioaerosols to impact upon the agar; filtration is where air is pumped through a filter, trapping bioaerosols on the filter surface; and liquid impingement is where the air is pumped into a liquid filled chamber, with bioaerosols impacting onto the liquid. The current standard protocol for bioaerosol sampling and enumeration, published by the Association for Organics Recycling (formerly the Composting Association), recommended only the use of direct impaction methods, where microorganisms are captured directly onto agar plates for the past decade (Association for Organics Recycling, 2009; The Composting Association, 1999), although recently, the standardised protocol has been updated to include filtration sampling (Association for Organics Recycling, 2009). The advised apparatus for direct impaction is the Andersen sampler, with one agar plate described as a 'stage', with each stage able to collect a different size fraction of airborne particles. This sampler can consist of either a single stage, which will only collect one particle size fraction, or multiple stages, which collect a range of size fractions (Andersen, 1966). The standardised protocol recommends at least two single-stage samplers should be used simultaneously (Association for Organics Recycling, 2009). It also recommends that vacuum pumps are used to draw air through the sampler at a constant rate of 28.3 L $min^{-1}$. Initial sampling times of between 20 and 30 minutes are recommended to prevent overloading of the plates and loss of microorganism viability (Association for Organics Recycling, 2009).

The accessibility of impaction samplers and advice from the recommended protocol (Association for Organics Recycling, 2009) has led to impaction being the most commonly used method for bioaerosol monitoring within the composting industry. Disadvantages of this method include expense, the labour intensive nature of the

sampling, bulky equipment and short sampling times (Environment Agency, in press). Sampling times are often further reduced to under 5 minutes, in order to prevent dehydration of the agar (Environment Agency, in press; Lin *et al*, 1999); it has been shown that sampling times over 3 minutes can significantly reduce the viability of captured bacteria and increase the variability of results (Godish and Godish, 2006) as more particles 'bounce' from the agar surface (Taha, 2005a). The short sampling times used as a result are less applicable to studies of bioaerosols at site boundaries and potential receptor exposure (Environment Agency, in press); where a profile of bioaerosol levels over a given time is of more use. Furthermore, options for microorganism enumeration with this method are restricted to culture based techniques.

Liquid impingement is the collection of bioaerosols directly into a liquid filled reservoir (Lin *et al*, 1999). As with impaction methods, bioaerosols are drawn into the sampler using a pump system at a set flow-rate. Sampling times can be longer through this method, although generally remain under one hour (Lin *et al*, 1999) to prevent evaporation of the liquid (Grinshpun *et al*, 1997). Low liquid levels also increase 'bounce' and cause re-aerosolisation of microorganisms, as they impact with the sampler sides rather than with liquid, reducing sampler efficiency (Grinshpun *et al*, 1997). However, viability of microorganisms can be higher with this method, as they are placed under less osmotic and physical stress throughout collection (Grinshpun *et al*, 1997; Lin *et al*, 1999). Both culture and non-culture methods can be used for microorganism enumeration with liquid impingement (Henningson and Ahlberg, 1994). However, the sampling equipment is delicate and expensive, making liquid impingement currently a less suitable method for regular field-based monitoring of bioaerosols (Environment Agency, in press).

Filtration methods also use pumps to draw air through the apparatus, capturing bioaerosols as air passes through a filter. There are many different types of filters available, of different pore sizes and materials (SKC Inc, 2007). Filtration also allows analysis through both culture and non-culture based methods (Henningson and Ahlberg, 1994). Filtration methods can be simpler to use than impaction and impingement, equipment cheaper, sampling can be carried out in a wider range of weather conditions

(Environment Agency, in press), and for longer periods of time. However, as with direct impaction viability of microorganisms can be reduced through dehydration and disruption of bioaerosol metabolism mechanisms (Chen and Li, 2005a; Chen and Li, 2005b). Filtration has also been shown to have lower particle collection efficiency than other methods (Henningson and Ahlberg, 1994; Martinez *et al*, 2004), possibly caused by re-aerosolisation of particles (Jankowska *et al*, 2000). Despite this, the ease of use and utility of this method has contributed towards its adoption as a standard method in Germany (Verein Deutscher Ingenieure, 2004a; Verein Deutscher Ingenieure, 2004b), and the recent adoption of filtration as an alternative method to direct impaction in the UK (Association for Organics Recycling, 2009). German guidelines suggest the use of gelatine filters, which can be used in a wider variety of temperature and humidity conditions than the more standard, polycarbonate, filter (Verein Deutscher Ingenieure, 2004a).

Currently, there are few other techniques for the sampling of bioaerosols, although novel methods continue to emerge. One such novel sampler continuously transports an air sample through a porous medium which is submerged in a liquid layer, splitting the sample into many small bubbles, which scavenge bioaerosols from the air sample (Agranovski *et al*, 2005; Agranovski *et al*, 2006; Agranovski, 2007). Although successful in experiments, and able to retain viability in most microorganisms, this method has not yet been adopted to the same extent as the other methods. Other novel samplers utilise centrifugal forces and rotating cups to separate particles (including bioaerosols) of certain inertia from an air stream (Henningson and Ahlberg, 1994; Schlosser *et al*, 2009), and electrical charges to induce electrostatic precipitation, causing bioaerosols to be deposited on a collection substrate (Mainelis *et al*, 1999).

Further development of existing sample methods has also continued, with the development of some samplers capable of size fractionation using porous foam (Kenny *et al*, 1999; Kenny *et al*, 1998), and the development of non-evaporating mediums for liquid impingement samplers (Lin *et al*, 1999). In addition, different types of filter have been developed, using novel media such as gelatine, which can increase the yield of

viable microorganisms as compared to polycarbonate filters (Verein Deutscher Ingenieure, 2004a).

### 1.8.2 Methods for bioaerosol enumeration

**Culture based methods**

The main methods of bioaerosol enumeration are those based on culture, direct counting and molecular methods. Through the advice given by the Association for Organics Recycling (Association for Organics Recycling, 2009) and the accessibility of culture-based methods (Environment Agency, in press), culturing is the most popular way of enumerating bioaerosols within the composting industry.

Culturing begins with the preparation of the agar plates before incubation. Samples collected through filtration are suspended, then diluted to several levels and spread onto plates containing an appropriate agar for the microorganism to be analysed (Association for Organics Recycling, 2009; Lin *et al*, 1999; Taha *et al*, 2007; The Composting Association, 1999; Verein Deutscher Ingenieure, 2004b). Liquid impingement samples can also be diluted and spread on appropriate plates. Samples collected through direct impaction can be incubated immediately. The prepared agar plates are incubated for periods appropriate for the microorganism to be analysed (Association for Organics Recycling, 2009; Lin *et al*, 1999; Taha *et al*, 2007; The Composting Association, 1999; Verein Deutscher Ingenieure, 2004b). Colonies formed after the chosen incubation periods are then counted and colony forming units[5] (CFU $m^{-3}$) calculated as advised by the Association for Organics Recycling (Association for Organics Recycling, 2009).

The culturing method has changed little over many years of microbiological analysis, with CFU calculating methods developed in the 1880's (Ritz, 2007). There are several problems with the culturing method and bioaerosol sampling. Firstly, bioaerosols contain fragments of plant, animal and microbial cells (ADAS and SWICEB, 2005; Wouters, 2003), which are not enumerated when culture methods are used. Culture is

[5] Colony Forming Units (CFU) defined as unit of 1 or more cells or spores which grow to form a single colony when inoculated onto a suitable growth medium (Swan *et al*, 2003)

also a highly selective method, with the only microorganisms enumerated being those capable of growing according to the substrate and temperature conditions selected (Giovannoni *et al*, 1990; Head *et al*, 1998; Ward *et al*, 1990). Microbial populations are also known to contain a fraction of microorganisms that are non-culturable but viable (NCBV). This fraction comprises microorganisms that have lost culturability due to environmental stress, such as nutrient deficit, that may recover culturability under favourable conditions; and also those that may be too sensitive to disruption to be successfully cultured in a laboratory environment (Giovannoni *et al*, 1990; Head *et al*, 1998; Ward *et al*, 1990). Although non-culturable, the NCBV fraction remains a health risk being toxic or allergenic (Crook and Sherwood-Higham, 1997; Swan *et al*, 2003). Through a combination of these factors, it has been found that only 20% of microorganisms may be enumerated through culture, even within microbial ecosystems that have been well studied over numerous years (Ward *et al*, 1990). As well as these issues, bioaerosol sampling may increase the proportion of bioaerosols that are not culturable. Bioaerosol collection methods, particularly filtration and impaction, may result in microorganisms being subject to stresses such as dehydration, and lead to a loss of microorganism viability and an underestimation of CFU. It has been suggested that fewer than 10% of aerosolised bacteria are capable of forming visible colonies (Heidelberg *et al*, 1997), while only 1% of soil bacteria can be represented through culture (Head *et al*, 1998). Hawksworth (2001) report similar findings for fungi, showing how species diversity is consistently underrepresented by spores captured. This has led to claims that culturing alone is no longer applicable within the field of microbiology (Ritz, 2007). However, culturing methods can provide information on the dynamics and structure of microbial communities (Nichols, 2007). Culturing and direct-counting methods in combination could provide the most complete information on microorganism samples and potential exposure risks (Nichols, 2007; Ritz, 2007)

Despite this debate, culturing remains a frequently used method in bioaerosol studies. This has encouraged the improvement and refinement of the methods used to culture samples. For example, both novel agar and novel enumeration methods for Anderson samplers have been developed. The novel ‘compost’ agar provides a compost based substrate, selecting for slower growing actinomycetes, and preventing more competitive

microorganisms masking actinomycetes (Taha *et al*, 2007). In this study, due to masking only 8% of nutrient agar plates could be enumerated, while 87% of compost agar plates could be enumerated (Taha, 2005a; Taha *et al*, 2007). Enumeration of samples collected from Anderson samplers can also be difficult due to plate overloading, leading to the development of a method to prevent masking of slower growing fungi species. This is done through random transfer of a subset of impaction points into sub-cultures, and has successfully allowed slower growing fungi to proliferate (Catranis *et al*, 2006). The continued development of culturing as well as its use to show community structure and dynamics means that this method is unlikely to become obsolete in the near future within bioaerosol studies.

**Non-culture based methods**

While it appears that culturing will remain the most commonly used method within the composting industry for the foreseeable future, researchers have explored many alternative methods for enumeration (Environment Agency, in press). Most of these methods are routinely used in other areas of environmental microbiology. In particular, nucleic acid based methods are now routinely used to identify specific microorganisms and community structure of microbial samples, these methods have the ability to rapidly and accurately identify captured bioaerosols (Agranovski *et al*, 2006; Orsini *et al*, 2002; Peccia and Hernandez, 2006; Pyankov *et al*, 2007).

Polymerase Chain Reaction (PCR) and reverse-transcriptase PCR (RT-PCR) are molecular methods that amplify, respectively, DNA and RNA. This process is carried out in order to synthesise and amplify concentrations of environmental nucleic acid, allowing analysis of the genotype of the microbial community. Through DNA amplification, both viable and non-viable or dead microorganisms are analysed, while through RNA amplification the active members of the microbial community are analysed as RNA is rapidly degraded within the environment (Miskin *et al*, 1999). The process begins with denaturation (splitting) of the nucleic acid through heating. Primers are added, which select the DNA or RNA required. These primers either target a section of DNA or RNA that represents a specific species or microbial family, such as actinomycetes, or a section of nucleic acid that is generic to a large group of

microorganisms, such as bacteria (Head *et al*, 1998; Hugenholtz and Goebel, 2001). The primers attach to each single strand of DNA or RNA, and polymerase creates copies of the single-strands of DNA, exponentially amplifying the selected DNA or RNA (Head *et al*, 1998; Hugenholtz and Goebel, 2001; Mukoda *et al*, 1994).

Community structure of the PCR product can be determined through a variety of methods that, once the laboratory equipment has been purchased, are fast and cost-effective. One such method is terminal restriction fragment length polymorphism (T-RFLP), shown to have a high efficiency and applicability to microbial community studies (Smith *et al*, 2005). Another is denaturing gradient gel electrophoresis analysis (DGGE). This method uses gel to separate microbial populations within a community and can identify populations that form only 1% of the community (Muyzer *et al*, 1993). This method also allows semi-quantitative analysis, as hybridization analysis can be used to identify particular DNA fragments (Muyzer *et al*, 1993). Often, several analyses can be run on the same PCR product, allowing a range of methods to be employed (Coulon *et al*, 2007). Cloning and sequencing of community profiles can also be carried out and results compared to those from other studies using computer software and databases. This allows identification of community components through matching of the found sequence to its closest relative available within a database of known genome sequences. Examples of such programs are Greengenes, BioEdit, BLAST, PSI-BLAST, CLUSTAL W and T-Align (Altschul *et al*, 1997; DeSantis *et al*, 2006; Smith *et al*, 2005; Thompson *et al*, 1994). The assignment of a closest relative to a DNA sequence effectively allows identification of the microorganism, providing variation between the sequences being matched is within a certain level of tolerance, typically a 97% match is required (Le Goff *et al*, 2009). In addition, the databases available, such as the Ribosomal Database Project (RDP) (Maidak *et al*, 1996; Maidak *et al*, 1999), and National Centre for Biotechnology Information (NCBI), allow identified microorganisms to be theoretically traced to source, through provision of information on environments where the microorganism has been previously identified from through sequencing (Le Goff *et al*, 2009). Quantitative PCR (Q-PCR) allows both qualification and quantification of microbial community genotype. It amplifies selected nucleic acid in the same way as PCR, with the addition of quantification after each amplification

round (Coulon *et al*, 2007; Environment Agency, in press). Following Q-PCR, the same qualitative methods can be used to discover the community profile and composition. Q-PCR allows more accurate quantification of microbial community constituents and abundance than PCR, as sequences with low abundance may be preferentially amplified through the PCR process. These issues are discussed in more depth within Chapter 10.

Despite the success of these methods within other areas of microbiology, these methods have not yet been widely adopted for bioaerosol sampling. There is some suggestion that this can be attributed to high-volume sampler efficiencies and size ranges not being fully characterised, lack of standardised preparation methods (Peccia and Hernandez, 2006), and the initial cost of equipment may be prohibitive. However, it has been suggested that these methods are applicable and useful tools within bioaerosol analysis, with the recent publication of a study analysing the genotypic community of bioaerosols emitted thorough composting (Le Goff *et al*, 2009). The study highlighted the importance of increasing the understanding of bioaerosol communities emitted through composting, with results suggesting preferential emission of spore forming microorganisms. This was suggested to be an important consideration within analysis of potential receptor exposure, as spores may show ability to disperse further than other bioaerosols (Le Goff *et al*, 2009).

Other methods that may have the potential to analyse bioaerosol composition include biochemical qualitative analyses, including endotoxin assay in order to quantify the emission and dispersal of these bioaerosols (Liebers *et al*, 2006; Liebers *et al*, 2008). Another method is phospholipid fatty acid analysis (PLFA), which identifies the lipid profile of a community, with lipid markers attributable to microbial groups. This allows analysis of phenotypic community structure, as well as the main taxonomic groups within the community (Macnaughton *et al*, 1999a; Macnaughton *et al*, 1999b). It has been suggested that PFLA in combination with PCR-based methods such as DGGE, provide the most complete profile for microbial communities, characterising both genotype and phenotype (Macnaughton *et al*, 1999b).

Other quantitative molecular methods available include DNA microarray (Franke-Whittle *et al*, 2005; Kim and Kim, 2007), fluorescent in situ hybridization (FISH) and Phylogenetic microarray. DNA microarray shows gene expression within a sample, while FISH and Phylogenetic microarray are quantitative methods which look at specific species within a sample, yielding information on community structure and dynamics (Coulon *et al*, 2007; Deloge-Abarkan *et al*, 2007).

Currently, the molecular methods described have not been routinely used for bioaerosol analysis, although they have been adopted for a limited number of investigations, and mostly shown to be successful (Agranovski *et al*, 2006; Deloge-Abarkan *et al*, 2007; Ishimatsu *et al*, 2007; Le Goff *et al*, 2009; Peccia and Hernandez, 2006; Pyankov *et al*, 2007). With further development and adaptation of methods from aquatic and soil microbiology, they could be powerful tools for the analysis of species, communities and activity within bioaerosol samples (Environment Agency, in press). This has already been shown through the development of *A. fumigatus* specific nucleic acid based methods (Brown *et al*, 2009; McDevitt *et al*, 2004).

Direct counting techniques are also being adopted from environmental microbiology for bioaerosol enumeration. Flow Cytometry (FCM) is a cell counting method adopted from aquatic microbiology, and combined with fluorescence (FL) to provide counts of microorganism viability and different communities. This combination (FCM/FL) has been shown to be successful in bioaerosol studies, yet remains little used or recommended (Chen and Li, 2005a; Chen and Li, 2005b). In order to attain the full potential of the method, bioaerosols from different sources need to be evaluated using FCM/FL, to create a database of bioaerosol communities from different sources (Chen and Li, 2005a; Chen and Li, 2005b). Once this has been done, FCM/FL may provide a powerful tool for bioaerosol analysis. Epifluorescence microscopy is another direct counting method (Prigione *et al*, 2004). The method was compared to FCM and attained closely comparable results for bioaerosol enumeration from each method, although FCM showed more precision and reliability (Prigione *et al*, 2004).

The above methods have been successful in enumerating bioaerosols, yet they are still not widely used. This may be a symptom of the volume of research regarding novel sampling and method development, rather than the dedicated development of a method as standard. In addition, these methods are more complex and expensive to implement, compared to the relatively simple and cheap culture based methods. As a result, while novel methods are regularly applied to bioaerosol studies, few are developed further, to a stage where they can be applied as a standard protocol to bioaerosol monitoring. For example, during 2007, the following methods have been explored for bioaerosol enumeration; Real-time/Q-PCR and PCR (Cayer *et al*, 2007; Chen and Li, 2005a; Chen and Li, 2005b; Ishimatsu *et al*, 2007; Pyankov *et al*, 2007), laser induced fluorescence (Cabredo *et al*, 2007), femtosecond filament-induced breakdown spectroscopy (Xu *et al*, 2007), mass spectroscopy (Chen and Li, 2007), FISH (Deloge-Abarkan *et al*, 2007), FCM/FL (Chen and Li, 2005b; Chen and Li, 2005a) and a cloning-sequencing method in combination with PCR (Cayer *et al*, 2007). The vast amount of research done on non-culture methods and sampling methods other than impaction is beginning to be recognised by policy makers (Environment Agency, in press). This may lead to a shift away from the original suggested protocols (Association for Organics Recycling, 2009), and to the development of a non-culture method for standard use in bioaerosol monitoring. In particular, molecular methods could be used, as they are applied with such success in other areas of microbiology. A combination of culture and non-culture based methods may provide more complete information on bioaerosol communities and their behaviour in the atmosphere when released from composting facilities (Environment Agency, in press; Nichols, 2007; Ritz, 2007).

## 1.9 Summary

Despite over a decade of research into composting as a source of bioaerosols, the distance to which bioaerosols are able to travel to remains disputed. In addition, the focus of enumeration through culture-based methods is likely to have led to underestimation of concentrations, as well as incomplete knowledge of the composition of bioaerosols able to disperse from composting facilities. Renewed calls for further

investigation into bioaerosol dispersal and fate in the environment highlight current doubts surrounding the guidelines currently in place (Crook *et al*, 2006; Harrison, 2007; Sykes *et al*, 2007). Detailed information, collected using the best available methods, is required. This will support or support a change to guidelines, as well as inform regulators of both the concentration and composition of bioaerosols that sensitive receptors may be exposed to. This is vital information for the implementation of proportionate and appropriate legislation to surround the expanding composting industry (Slater *et al*, 2005; Strategy Unit, 2002).

Developments within environmental microbiology have led to suggestions that the methods used to develop current guidelines are now out dated (Environment Agency, in press; Environment Agency, 2001a). Specifically, the accuracy of data gathered using direct impaction can be questioned, given evidence that it increases loss of viability (Godish and Godish, 2006), and has a lower collection efficiency than other methods (Godish and Godish, 2006; Taha, 2005a), and with this method only culture is used to enumerate bioaerosols. It is possible that through use of direct impaction (Association for Organics Recycling, 2009; The Composting Association, 1999) bioaerosol concentrations have been routinely underestimated. In addition, only a small fraction of the diversity of bioaerosols has been explored. The incorporation of filtration based sampling into standard protocols (Association for Organics Recycling, 2009) has provided the basis for further development of non-culture based methods for bioaerosol analysis, allowing enumeration of non-culturable and NCBV fractions, as well as qualification of bioaerosol communities. Prior to this, the development of novel methods with the ability to enumerate bioaerosols, rather than the dedicated development of one method, may also have hindered the adoption of a non-culture based method as standard. The variety of available methods has been shown, few of which have currently been developed beyond an experimental stage (Cabredo *et al*, 2007; Cayer *et al*, 2007; Chen and Li, 2007; Deloge-Abarkan *et al*, 2007; Ishimatsu *et al*, 2007; Pyankov *et al*, 2007; Xu *et al*, 2007)

The construction of dispersal profiles for bioaerosols is also hindered by the lack of suitable bioaerosol dispersion modelling programs, their development limited by the

scarcity of information surrounding individual bioaerosols properties and behaviour in the atmosphere. Although it has been acknowledged that bioaerosols are released episodically (Clark *et al*, 1983, Lacey, 1997; Taha *et al*, 2006) the effects of these episodic emissions on dispersal and receptor exposure is unknown; most researchers and modellers preferring to average bioaerosol concentrations over long periods of time. This may not be a suitable method for exposure assessment, as sudden peak exposures, as well as long term exposures, can have health impacts (Swan *et al*, 2003). The impact of climatic factors such as wind speed, relative humidity and buoyancy effects on individual bioaerosols transportation and deposition also remains uncertain. Until more information regarding the above factors is gathered, and models adapted to predict dispersal over short averaging times and short distances are available, atmospheric dispersion models cannot be used with confidence to accurately predict receptor exposure.

Intensive sampling, taking into account various meteorological, seasonal, and site conditions would allow more accurate bioaerosol assessment. Crucial to this, would be replication and validation of results, accounting for the variability inherent within the system rather than attempting to eliminate this variability through the production of average concentrations and exposure. Furthermore, although non-culture based methods are not currently available as standard methods, the non-viable fraction of bioaerosols should be accounted for where possible and knowledge regarding the composition of bioaerosols gained. The best available methods for bioaerosol sampling and enumeration should be used. These data combined would allow future development of proportionate and appropriate legislation and determination of sensitive receptor exposure. This data would also provide the basis for future determination of the 'dose' within dose-response relationships.

## 1.10 Aims and objectives

The aim of this project is to contribute towards knowledge surrounding the emission, dispersal and composition of bioaerosols from composting facilities. Following the

literature review, a series of research questions concerning the main gaps in knowledge identified have been created. Primarily, it has been found that knowledge of concentrations of bioaerosols downwind from composting facilities, and understanding of the composition of bioaerosols is incomplete. Following the creation of research questions, a series of hypotheses and objectives have been formulated, aimed at filling the identified gaps in literature. The results gained through achievement of these objectives will allow improved quantification of receptor exposure through analysis of the impact that green waste composting has on ambient bioaerosols. As well as contribution to the knowledge surrounding bioaerosol concentrations and composition, this will advise operators and regulators on best-practice methods for site operation and risk assessment.

### 1.10.1 Research questions and hypotheses

**Research question**: Is the 250 m limit for risk assessment imposed on compost facilities appropriate to the risk created by bioaerosol release?

**Hypothesis**: Bioaerosols decline rapidly from source, at times showing the ability to be found at elevated concentrations 250 m from site

The 250 m limit has been set by the Environment Agency, creating a requirement for risk assessments to be carried out if sensitive receptors can be found within 250 m of composting facilities. The Environment Agency currently recommends threshold limit values (TLV) of 1000 CFU $m^{-3}$ for both fungi and total bacteria and 300 CFU $m^{-3}$ for gram-negative bacteria (Environment Agency, 2009a, b). Dispersion models show that bioaerosol concentrations decrease rapidly with distance, however, studies have both modelled and measured peak concentrations over these limits at distances beyond 250 m from site (Albrecht *et al*, 2008; Fischer *et al*, 2008; Herr *et al*, 2003a; Recer *et al*, 2001). Based upon the evidence base, the 250 m limit requires verification through measurements of emission, concentrations at downwind locations, and through the determination of dispersal patterns using detailed field measurements.

**Research question**: Does episodic release lead to episodic downwind peaks in concentrations?

**Hypothesis**: Episodic releases will be reflected in the pattern of bioaerosol concentrations downwind

It has been shown that bioaerosols are released episodically from composting facilities. This is due to site activities, such as turning and shredding, causing aerosolisation of microorganisms and increasing their release by 2 - 3 orders of magnitude (Taha *et al*, 2006). However, the effects of these episodic releases on bioaerosol dispersal and receptor exposure have not been quantified and episodic releases not taken into account in atmospheric dispersion models (Drew *et al*, 2006). Qualification and quantification of the impact of episodic emission on downwind concentrations is vital to the development of dose-response relationships, as dose will be determined by receptor exposure to elevated bioaerosol concentrations. Given the large increase in concentrations on-site episodically, these events are likely to be reflected in concentrations at receptor. However, the extent of the effect of episodic release on downwind concentrations, duration of any downwind peak, and the period of time between the release and a downwind peak is currently unknown. Any episodic peaks in downwind concentrations are likely to be characterised by relatively small changes in concentration, remaining below threshold levels. This hypothesis is summarised in Figure 1.1 below.

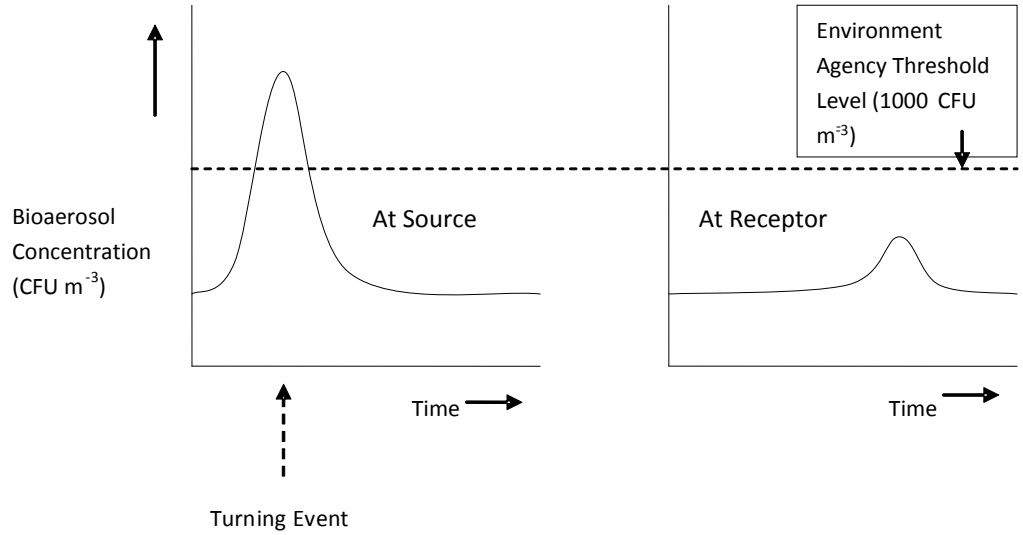



Figure 1.1: Pictorial representation of hypothesis for research question 'episodic emission leads to episodic dispersal'

**Research question**: Does composting significantly affect the composition of bioaerosols in ambient air?

**Hypothesis**: The microbial community of air influenced by composting will have a distinctive composition, significantly different from the microbial community composition of ambient air

Given the increase in bioaerosol concentrations found both on-site and downwind from composting facilities, it is likely that a change in community structure of bioaerosols will be seen. The magnitude of this change, however, is unknown. The change in community composition is likely to be visible downwind up to distances from site at which elevated bioaerosol concentrations may still be detected. Shifts in community are likely to be able to be traced to source, i.e. upwind or compost, through analysis of the composition of bioaerosol communities and the community fingerprint.

### 1.10.2 Objectives

Following the identification of research questions, a set of Objectives have been formulated to allow these questions to be answered.

i. Quantitative characterisation of bioaerosols emitted through composting activities and downwind concentrations up to and beyond 250 m from site at chosen case-study sites
    a. Assessment of the impact that episodic emission has on downwind concentrations
    b. Assessment of the relationships between bioaerosols and environmental conditions
ii. Characterisation of bioaerosol composition and communities including viable, non-viable and non-culturable components

The achievement of these Objectives is described through the following thesis, the structure of which is described in Figure 1.2, below.

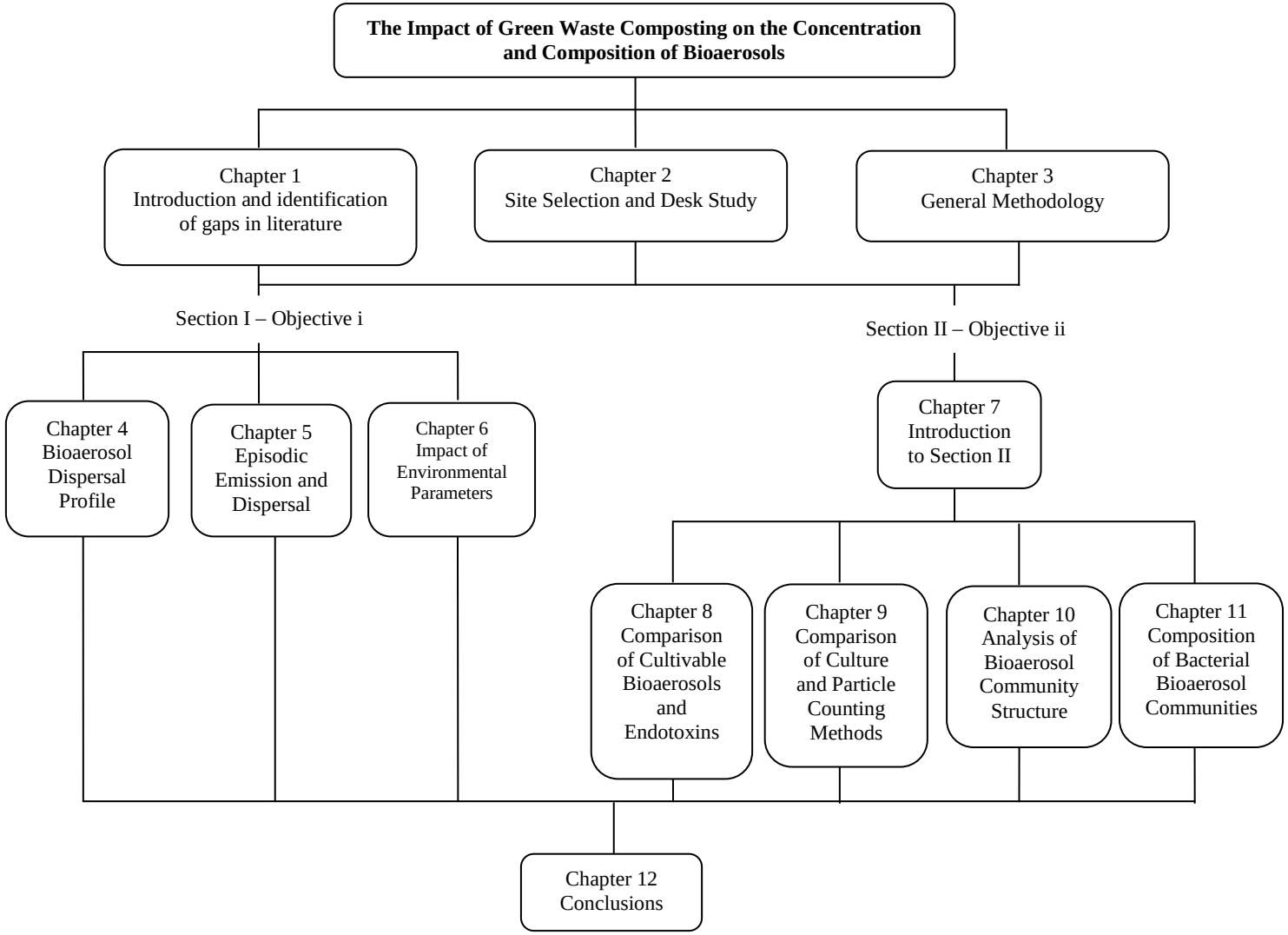



Figure 1.2: Flow diagram of thesis structure

# 2 Site Selection and Desk Study

## 2.1 Introduction

For the purposes of this study, an intensive sampling program at selected case-study sites was required. This would allow thorough investigation of bioaerosol release and dispersal, through repeated sampling, on a scale that has not been previously reported in the literature. This type of experimental plan would allow seasonal, diurnal, and meteorological variability to be accounted for, improving knowledge of the factors that influence bioaerosol release, dispersal patterns, and composition. A minimum of two sites were required. After answering the Objectives at the first site, it was necessary to test the results gained through experimentation at a second site. This ensured that results could be applied to other green waste composting facilities, and that they are not specific to one site. While it would be preferable to test results at more than two sites, time restrictions, and the amount of sampling that was required to satisfactorily meet the Objectives, resulted in this investigation being restricted to two sites.

In order to focus on the project aims and objectives, case-study sites were required to minimise certain variables. As Chapter 1 has shown, there are many different microorganisms that can proliferate throughout the composting process. The presence and proliferation of these microorganisms will depend on the source of feedstock, the quantities and composition of feedstock, and the processing activities utilised on-site (Epstein, 1994; Swan *et al*, 2003). Therefore, to prevent major differences in microorganism community composition, it was necessary to select sites that received feedstock in both similar quantities and sources. It was also necessary for the sites to utilise the same methods to process the waste received. Furthermore, as this study examines the dispersal of several bioaerosols, it was beneficial to use case-study sites with topographical similarities, to ensure that any pattern in dispersal found was not site-specific.

The first site was recommended as suitable for sampling by the sponsors of this project, as there were no obvious secondary sources of bioaerosols and both the site and its

surrounding areas were easily accessible. As a result of this, the second site was selected as the closest match to the feedstock, processing, and topography of the initial sampling site.

This chapter describes the main features and processing activities of each site. The information compiled can then be used at a later stage to aid the analysis of bioaerosol release and dispersal patterns.

## 2.2 Lount Organic Waste Composting

Lount Organic Waste Composting (OWC) is located 3 km north-east of Ashby-de-la-Zouch in Leicestershire (Golder Associates, 1998) (Figures 2.1). The site is primarily a green waste composting facility, although a civic amenity site is also located on the premises.

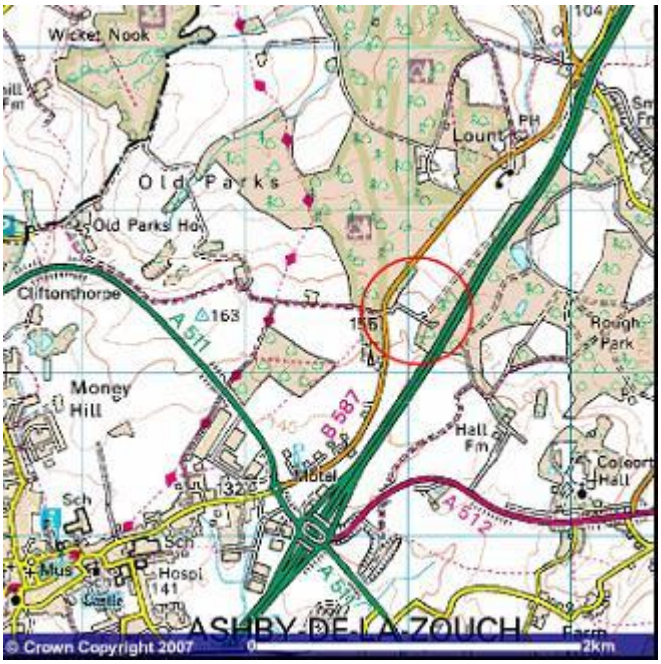



Figure 2.1: Map showing the location of Lount OWC (circled) (Ordnance Survey, 2008)

The composting facility is located on the site of a former landfill. The landfill site was placed on a void created through open cast clay and coal mining carried out in the 1960's and 1970's (Golder Associates, 1998). The original landfill site covers an area of approximately 19 ha, 900 m long and 230 m wide. The landfill site has now been capped with a low-permeability clay seal (engineered clay to specification of permeability less than $1 \times 10^{-9}$ m/s) and restoration soils (Golder Associates, 1998). The landfill cap has a minimum thickness of 1 m over most of the site, with a minimum of 1.1 m beneath the composting area. The site slopes from south-west to north-east with a shallow gradient, not exceeding 1:25 (Golder Associates, 1998). The western side of the site is bordered by the B587 road, which itself is bordered by regenerating woodland. The borders of the composting facility are surrounded with earthen bunding and a woodland buffer (Figures 2.2, 2.3) (Golder Associates, 1998). The nearest sensitive receptors are visitors to the civic amenity site, within site boundaries. The nearest residential area is Lount village, 1 km north east from the site (Figure 2.1).

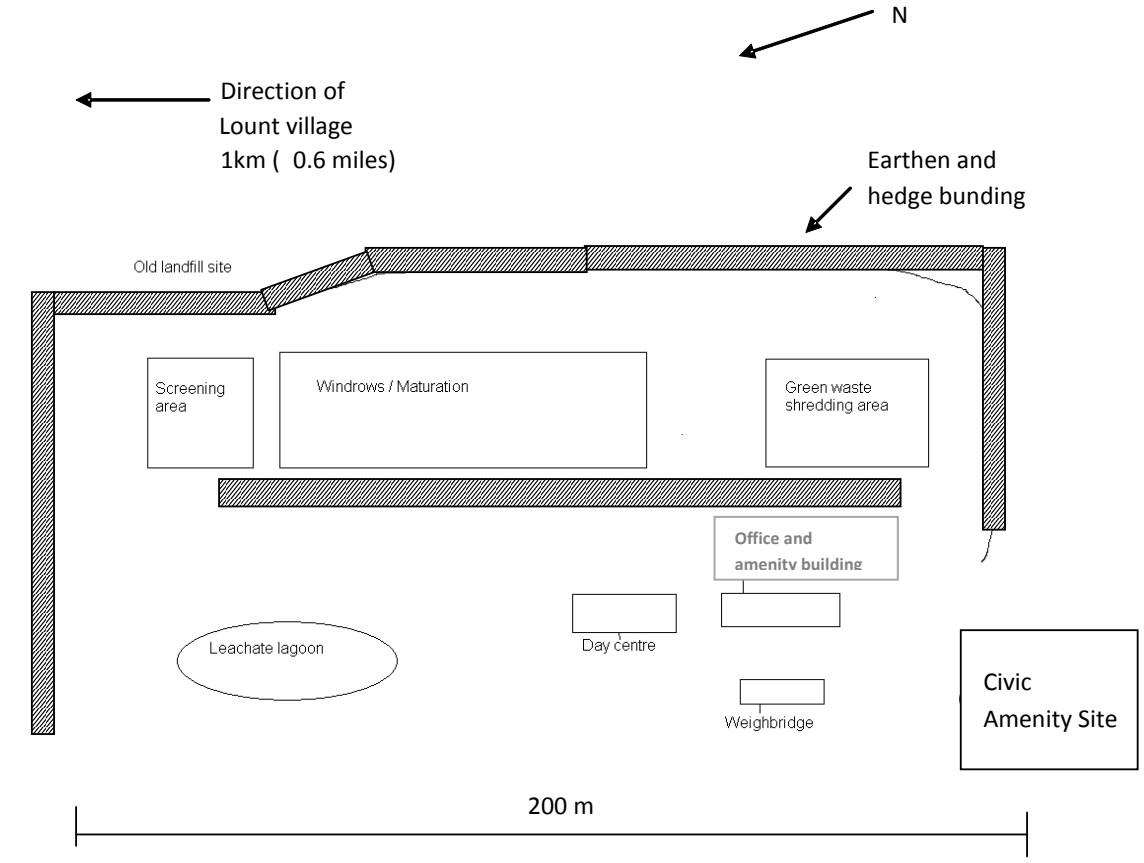



Figure 2.2: Schematic of Lount OWC, plan view (Environment Agency, 2001c)


Figure 2.3: View of the south-eastern site boundary, looking in a southerly direction. The meadow to the south and east is the closed landfill site.

The site is operated by SITA UK, a nationwide waste management company. The current waste management licence (issued in 1999) states that the site can receive up to 25,000 tonnes of green waste per annum (Environment Agency, 1999c). Approximately 60% of the waste comes from kerbside collections, with the remainder from civic amenity sites. The site may operate from 07:30 until 17:00 hours Monday to Friday, 07:30 until 14:00 Saturdays and 08:00 until 14:00 Sundays and bank holidays (Golder Associates, 1998). However, normally the site is only operated from Monday to Friday; Saturdays are worked during particularly busy periods.

Waste from both kerbside collection and civic amenity sites consists of green waste only, compost largely of plant matter such as hedge and grass clippings. Upon receipt, the waste is sorted and contaminants, such as plastics, are removed. Waste loads are received between 8 am and 1 pm and are tipped onto the reception pad, at the south end of the site (Figure 2.4). Waste is then shredded using a dedicated shredding machine to provide a more homogenous size and mix of waste material. Shredding is carried out for a minimum of 2 hours daily throughout the busier summer months. After shredding the waste is piled into windrows approximately 3 m high and 3 m wide (Golder Associates, 1998). The windrows are turned regularly, a minimum of once per week to homogenise and aerate the material, if necessary, fresh water is added to maintain moisture content between 30 and 60% (Golder Associates, 1998). This moisture content was verified through drying of compost samples from separate windows at 105°C $\pm$ 2°C for 24 hours. However, it was found that only one sample was within the stipulated range at 56%, with two of the samples found to have moisture content over 60% (Table 2.1). On average three windrows are turned each day using a mechanical front loader. Windrows are lifted and re-built further along the compost pad before reaching the end of the site and the maturation pad. The whole composting process takes between 6 and 9 weeks. The material, once composted and matured, is screened into 40 and 10 mm fractions (Figure 2.5) before being passed to the consumer (Golder Associates, 1998).

Table 2.1: Average moisture content (triplicate samples) found within three separate windrows of different ages at Lount OWC.

| Windrow | Moisture Content (%) |
|---|---|
| 1 | 62.4 |
| 2 | 55.9 |
| 3 | 65.0 |

Figure 2.4: Waste reception pad and sorting at Lount OWC

Figure 2.5: Compost screening and windrows at Lount OWC

Prevailing winds in the area come from the west-south-west. The site has a weather station that is in constant use, allowing operations to be adjusted if abnormal weather conditions are recorded that may result in receptor exposure to bioaerosols or odour (SITA SUEZ, 2006).

The waste management licence in place stipulates that bioaerosol monitoring is to be carried out twice yearly. This is carried out by D and F Associates, an environmental monitoring company who specialise in bioaerosols. D and F Associates follow the monitoring guidelines suggested by the Association for Organics Recycling (Association for Organics Recycling, 2009; D and F Associates, 2007; D and F Associates, 2006; Environment Agency, 2009b). A Merck MAS-100 Eco air sampler is used; this is equivalent to stage 5 of an Anderson impaction sampler, collecting particles over 1 μm in diameter. No data are available regarding the upper particle size limit of this sampler. Two samplers are used in tandem to sample, with a total of four plates for every sample being taken at each location. Samples are taken 100 m upwind of the site, 100 m downwind, on the composting pad, and at the nearest sensitive receptors, in this case at the civic amenity site adjacent to the composting pad. Sampling times are decided according to the estimated bioaerosol load, with times ranging from 3 minutes when concentrations are estimated to be high, to 8 minutes when estimated to be low.

Control plates that are not exposed are taken to the site and agar is checked against known microorganisms to provide quality control (D and F Associates, 2007; D and F Associates, 2006). Temperature, wind speed, and percentage relative humidity are also measured while sampling is carried out. Mesophilic and gram-negative bacteria and fungi, including *Aspergillus fumigatus* are measured. Nutrient agar, MacConkey agar and Malt Extract agar respectively are used to enumerate each microorganism. Sampling from May 2006 to June 2007 has suggested that at all sampling locations bioaerosol levels are acceptable, below the Environment Agency's current suggested threshold levels. Maximum values of 216 CFU $m^{-3}$ (found for bacteria) were found downwind from composting activities, 460 CFU $m^{-3}$ (found for bacteria) on-site, 72 CFU $m^{-3}$ (found for bacteria) upwind from site, and 600 CFU $m^{-3}$ (found for bacteria) at the nearest sensitive receptor (D and F Associates, 2007; D and F Associates, 2006).

The site is also operated to minimise the risks of receptor exposure to dust, odour and bioaerosols. If wind is found to be blowing towards the nearest sensitive receptors, (civic amenity site) activities such as turning and shredding are not carried out. Moisture levels are maintained at a level to minimise dust and bioaerosol release. If a windrow is found to be becoming anaerobic, oversize material is mixed in to minimise odour. Furthermore, if particularly contaminated or odorous material is delivered, the waste is not accepted. An odour neutraliser is also in place and operated when the prevailing wind moves towards receptors in the village of Lount, or material is found to be odorous (SITA SUEZ, 2006).

Complaints regarding the site are mainly placed with the Environment Agency, although they are sometimes given directly to the site. When a complaint is received, it is verified through analysis of the weather conditions as recorded by the weather station and by a visit to the location of the complaint by the site manager. If substantiated, action is taken to alter site activities to remove the reason for complaint. Since 2005 the amount of complaints regarding odour from the site have reduced. 22 were received in 2005, 4 in 2006 and 2 from January until June 2007 (Yates, 2007). Most complaints are received from residents living in the village of Lount, 1 km north east of the site (Figure 2.1).

## 2.3 Lower Trent Composting Plant (Flixborough)

The Lower Trent (Flixborough) Composting Plant is located to the north of Scunthorpe, on the eastern bank of the River Trent. It is unusually located, in that there are sensitive receptors located within 250 m of the site boundaries (Glover, 2008). The site was opened as a dedicated composting facility, with the waste management licence first issued in 2004 (Environment Agency 2005) Figures 2.6 and 2.7 below show the location of the plant, adjacent to the Flixborough industrial estate.

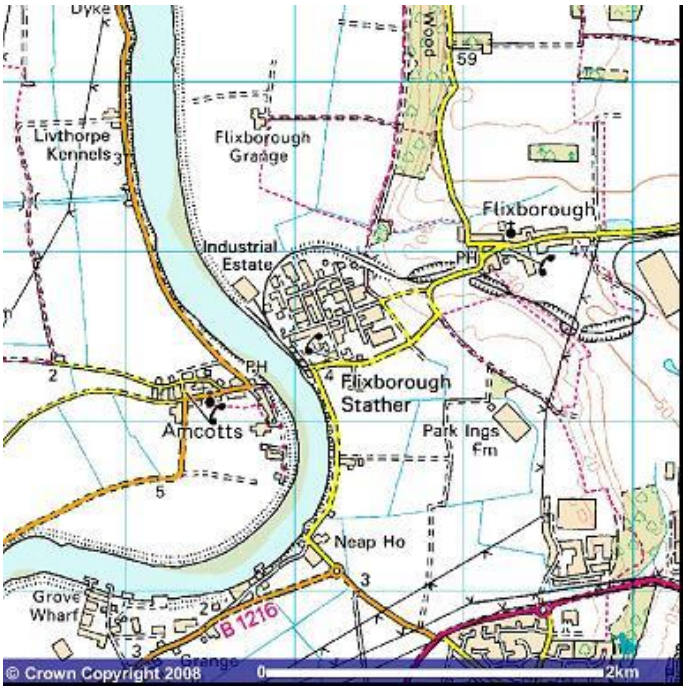



Figure 2.6: Flixborough Industrial Estate, Flixborough and Amcotts village (Ordnance Survey, 2008)

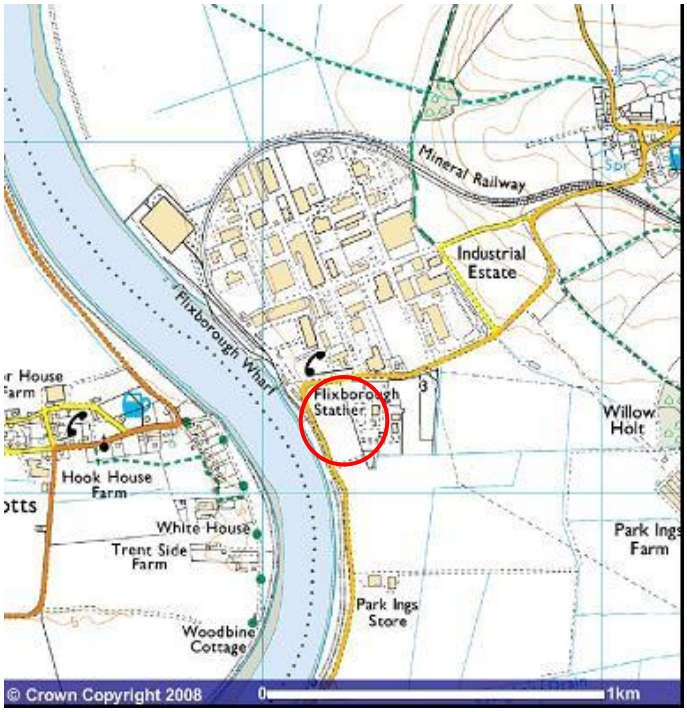



Figure 2.7: Flixborough Industrial Estate, showing composting facility (circled) (Ordnance Survey, 2008)

The site is situated on the southern edge of an industrial site. To the west the site is bordered by a road and the River Trent. This river enters the Humber estuary approximately 5 miles to the north, a dedicated Site of Special Scientific Interest (SSSI) (Glover, 2008; Ordnance Survey, 2008). Amcotts village is located on the opposite bank of the river, approximately 280 m from the site boundary. To the south, the site is bordered by arable fields, including a grain store approximately 250 - 300 m away (Glover, 2008). Hedging borders the western site boundary, with a mixture of hedging fencing, and concrete bunding on the southern and northern edges. Concrete bunding is present on the northern composting pad boundary. The eastern side of the site is bounded by fencing (Figure 2.8). To the east of the site is PET Polymers, a business currently out of operation, but with a limited workforce in place at the time of sampling (Glover, 2008). To the north of the site is Flixborough Industrial Estate, which contains a range of other industrial operations. Workers on this estate, including PET Polymers, have the potential to be affected by emissions from the composting facility, with many working within 250 m from the site boundaries. Flixborough village itself is located three quarters of mile to the north east of the site (Glover, 2008) (Figures 2.6 - 2.8).

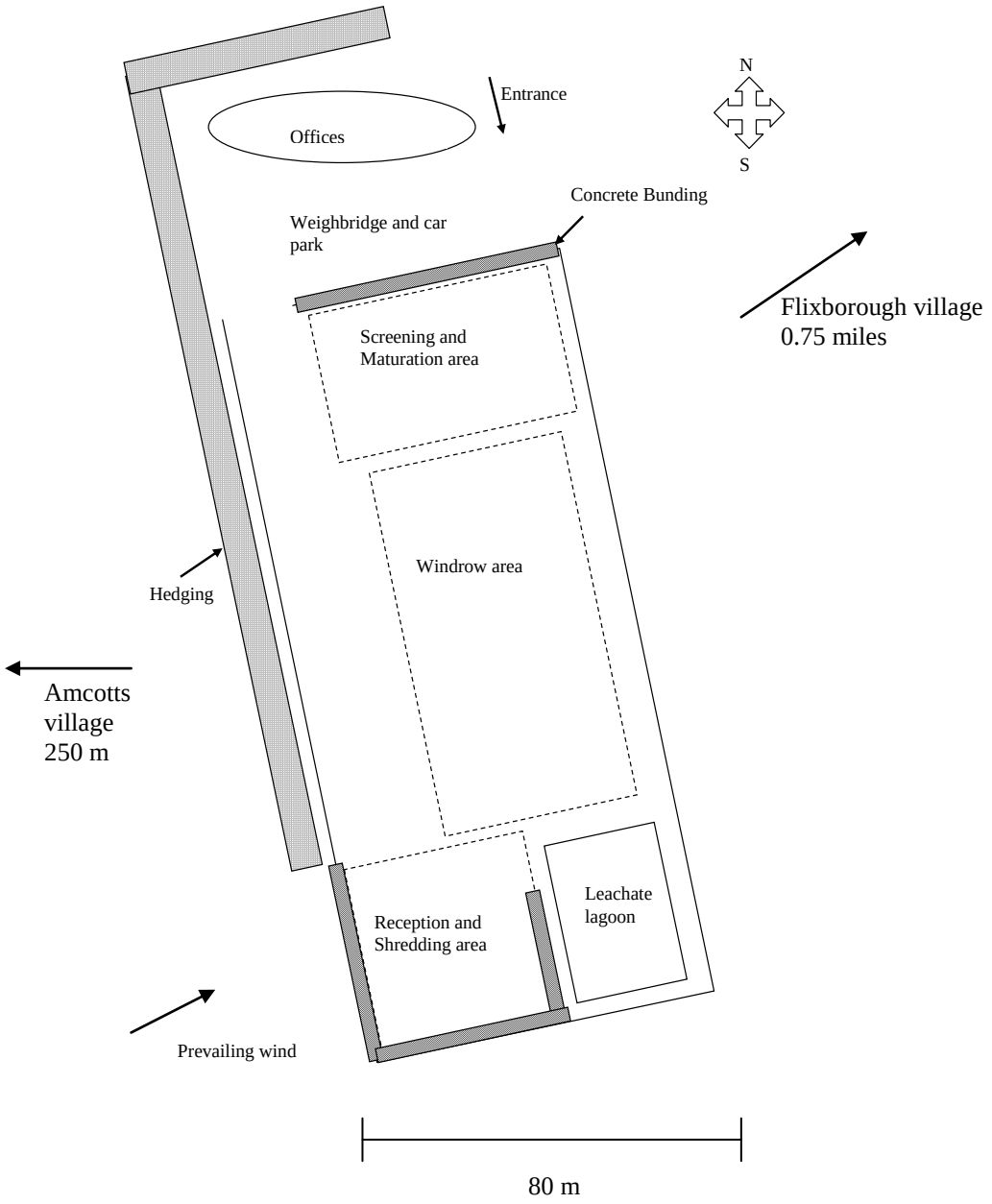



Figure 2.8: Schematic of Lower Trent Composting Plant (Flixborough)

The current waste management licence permits 25,000 tonnes of green waste to be processed each year, with no more than 12,000 tonnes allowed on-site at any one time (Environment Agency, 2005). This compost is sourced from kerbside collection (16,000 tonnes per year) and civic amenity sites (up to 7000 tonnes per year) (Glover, 2008). Kerbside collections, run by North Lincolnshire Council, include household green waste

which encompasses both kitchen and garden biodegradable waste. All composting is carried out on an impermeable concrete pad, incorporating drainage channels that direct leachate to a liquor lagoon (Figure 2.8). Compost is formed into windrows approximately 50 m long, 3 m wide and 3 m high, each containing approximately 250 tonnes of compost (Figure 2.9). They are typically turned using a dedicated windrow turning machine (Figure 2.10), although occasionally a front loader is used. The windrows are maintained at a minimum of 55ºC for at least 14 consecutive days with a minimum of 5 turnings, or 65ºC for at least 7 consecutive days with a minimum of 3 turnings. The entire composting process takes a minimum of 12 weeks. Moisture content is purportedly maintained between 40 and 60%. This moisture content was verified through drying of compost samples from separate windows at 105°C $\pm$ 2°C for 24 hours (Table 2.2), with a range of moisture contents found. Both moisture and temperature are monitored daily; if outside of the required boundaries remedial action is taken. Temperature can be altered through changing turning frequency, high moisture content is remediated though increasing turning frequency or adding drier feedstock. Low moisture content is remediated through addition of water (Environment Agency, 2005; SITA UK, 2007).

Table 2.2: Average moisture content (triplicate samples) found within four separate windrows of different ages at Flixborough.

| Windrow | Moisture Content (%) |
|---|---|
| 1 | 24.1 |
| 2 | 59.1 |
| 3 | 40.3 |
| 4 | 30.1 |

Figure 2.9: Compost windrows at Flixborough

Figure 2.10: Turning using a dedicated windrow turning machine at Flixborough

The proximity of the site to receptors and associated risk is acknowledged within the waste management licence (Environment Agency, 2005) (Figure 2.11 and 2.12). Several measures are therefore stipulated to reduce risk of public exposure to odour, dust and

bioaerosols. In particular, the 5 day weather forecast is used to identify suitable days for composting activities, such as turning, shredding and screening. If possible, these activities are only carried out when wind is not carrying any emissions towards sensitive receptors, or when wind speed is low (Finney, 2007). The dedicated turning machine also minimises emissions as compost is less dense and turning is quicker, only 1 - 2 hours up to 2 times per week, reducing the periods of higher emission rates; although, screening and shredding activities are carried out on most days (Finney, 2007; Glover, 2008). Olfactory monitoring is also carried out by the site manager or supervisor at least twice per day, at the site boundary downwind from activities. If necessary, activities are halted or the deodoriser is used (Finney, 2007; Environment Agency 2005).

Figure 2.11: Entrance to Flixborough composting plant, showing proximity to sensitive receptors

Figure 2.12: Compost shredding, with PET Polymers in background, approximately 60 m from site boundaries

Standard bioaerosol monitoring as stipulated by the Environment Agency is also carried out following the AFOR standard protocol (Association for Organics Recycling, 2009; Environment Agency, 2009b; The Composting Association, 1999). This included pre-operational levels being taken, along with sampling three, six and twelve months after operations began. Thereafter, monitoring is carried out every six months (Environment Agency, 2005; SITA UK, 2007). However, the last two monitoring occasions have shown bioaerosol levels above the suggested guidelines (Glover, 2008). Both odour and dust complaints have been received from the workers in closest proximity to the site (Glover, 2008).

## 2.4 Conclusions

This Chapter has described the location, features, and processing activities of each site. Both sites receive approximately 25,000 tonnes of green waste per year. The source of the waste is around 60% kerbside collection and 40% civic amenity at each site;

although Flixborough received more household vegetable derived waste than Lount. Processing activities are similar. At both sites composting is carried out on an impermeable pad. Waste is shredded upon reception using dedicated machinery. There are some differences in turning method; while Lount uses a front loader, Flixborough has the use of a dedicated turning machine. At both sites the final stage of the processing is screening, which is carried out using a dedicated machine. Topography at each site is similar, although Lount has more slope than Flixborough.

One of the main differences between the sites is the surrounding area. Lount is surrounded by a capped landfill site, while Flixborough is surrounded by arable land and an industrial estate. This may lead to more secondary sources of bioaerosols at Flixborough. It also has implications for potential receptors, as workers on the industrial estate are within 250 m of site boundaries. Furthermore, Flixborough has two villages within close proximity. In particular, Amcotts village is 250 m from site boundaries. This has led to extra measures being taken in order to reduce odour; in particular, the compost is maintained at low moisture content, around 40%. This reduces odour, but may lead to increases in the quantities of dust released from the site during processing activities (Epstein, 1994). The above information can be used to aid analysis of data, in order to explain any site specific differences in bioaerosol release and dispersal; as well as to explore the implications of findings for sensitive receptors. The following Chapter describes the general methodologies that were used at each site in order to characterise bioaerosol emissions and dispersal.

# 3 General Methodology

This Chapter describes the general methods that were common to all experiments throughout this project. Methods described include sample collection, processing, enumeration, and statistical analysis. The protocol detailed below uses the best available and practicable techniques to ensure that the highest possible quality of data was attained. A review of methods and their limitations is also provided.

## 3.1 Sampling Protocol

### 3.1.1 Pre-sampling and agar preparation

Prior to sampling, all necessary equipment was sterilised at 121°C for 15 minutes and dried. This includes filter cassettes (IOM multi-dust cassette assembly 225-71A, SKC Ltd, UK) loaded with 25 mm polycarbonate filters, with a pore size of 0.8 μm (225-1601, SKC Ltd, UK). Filters were loaded according to the manufacturer's instructions; this entails placing the filter so that bioaerosols are collected onto the shinier side. The shinier side is more appropriate for sample collection as is provides a suitable surface for microscope analysis (filters handled with forceps only) (SKC Ltd, 2009). Buffer solution (1 g NaCl and 3 drops Tween 80 per $L^{-1}$ sterilised deionised $H_2O$) was also prepared, and under aseptic conditions 10 mL was transferred into each sterilised Nalgene jar, and 9 mL into each sterilised universal. The principles of the Collection of Airborne Microorganisms on Nucleopore Filters (CAMNEA) method were used throughout the following methodologies (Palmgren *et al*, 1986; Taha *et al*, 2006; Taha *et al*, 2007).

***Aspergillus fumigatus***

*A. fumigatus* was cultured using Malt Extract Agar (MEA) (Oxoid, UK). The media was prepared by dissolving the powder in deionised $H_2O$ (50 g per 1000 mL). To prevent bacterial growth 0.1 g $L^{-1}$ chloramphenicol (Fisher, UK) was added to the media. This

media was autoclaved at 115ºC for 10 minutes. Once cooled below 50ºC the agar was poured into the required number of Petri dishes (triple-vented 90 mm diameter, Fisher, UK). This was done under aseptic conditions with approximately 20 mL agar poured into each Petri dish. Once set, the plates were loosely re-wrapped in their original packaging, inverted to prevent moisture build-up on the agar surface, and stored at 4ºC until required (Taha, 2005a).

**Actinomycetes**

Actinomycetes were cultured using a novel agar designed to allow slower-growing actinomycetes to dominate (Taha *et al*, 2007). This compost agar (CA) was prepared prior to sampling. 100 g $L^{-1}$ John Innes No.1 compost was suspended in deionised $H_2O$ and stirred for 5 minutes. The mixture was centrifuged at 700 relative centrifugal force (RCF) for 10 minutes (Falcon 6/300R, Sanyo, UK). The resulting compost slurry was filtered through 150 mm quantitative filter paper (QT 260, Fisherbrand, UK). 14 g $L^{-1}$ agar-agar (Oxoid, UK) was added to the mixture. The solution was autoclaved at 121ºC for 15 minutes. Once cooled below 50ºC, antifungal solution was added under aseptic conditions. This comprises 0.2 g of 95% cycloheximide (Fisher, UK) in 2 mL of 100% ethanol $L^{-1}$ (Fisher, UK). After gentle mixing, approximately 12 mL of agar was poured into each Petri dish. Once set, the plates were loosely re-wrapped in their original packaging, inverted, and stored at 4ºC until required (Taha, 2005a; Taha *et al*, 2007).

**Gram-negative bacteria**

MacConkey (MAC) agar (Oxoid, UK) was used to select for gram-negative bacteria within this experimental design. Initially, this was pre-prepared and poured as with the above methods. However, this allowed motile bacteria to proliferate, masking the growth of countable colonies. Therefore, a more suitable pour-plating method was adopted, inhibiting the spread of motile bacteria, allowing effective quantification of colonies (Seeley *et al*, 1991; Warcup, 1955). Agar was prepared after sampling has been completed, as samples are ready to be plated. 52 g per $L^{-1}$ MacConkey agar powder was mixed with deionised $H_2O$, autoclaved at 121ºC for 15 minutes and left to cool to below 50ºC. Meanwhile, under aseptic conditions 100 µL aliquot of the sample was transferred to a sterile Petri dish. The agar solution was poured into the plate (approximately 12 mL

per plate), and agitated in order to evenly disperse the inoculums. This involved sliding the plate gently in a vertical direction, then in a horizontal direction, and then in a circular motion, keeping the plate flat against the bench surface at all times (Figure 3.1). The plate is then left to set (Seeley *et al*, 1991; Warcup, 1955).

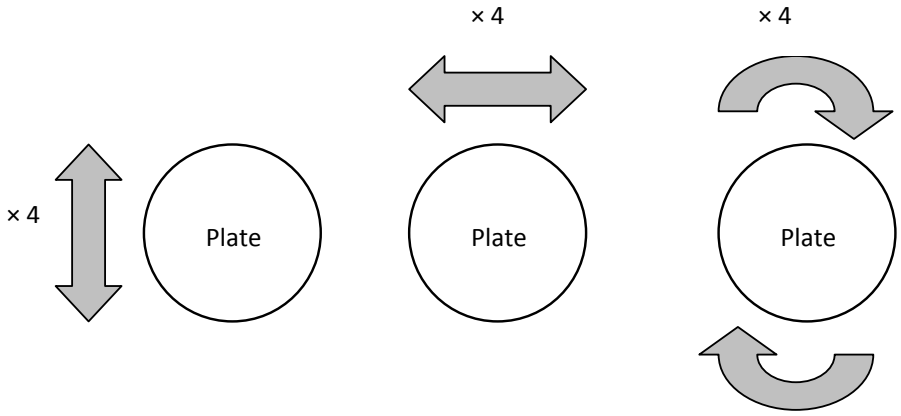



Figure 3.1: Schematic of pour-plating mixing method, plan view

### 3.1.2 Quality control

Standard techniques were employed in order to minimise microbiological contamination. This included sterilisation of field sampling kit (sampling heads, sampling cassettes, filters, Nalgene jars, caps and clips) and laboratory equipment (universal bottles, water for buffer solution, pipette tips). These items were all autoclaved at 121ºC for 15 minutes (TL AC-EV, PriorClave, UK) to ensure sterilisation, standard autoclaving procedures were followed. This included leaving jar and bottle lids loose, and wrapping small items in tin foil. This procedure allows steam penetration during autoclave procedure, as well as preserving sterility. Foil wrapped items were left wrapped until required, while lids were tightened immediately upon removal from the autoclave. Filter cassettes, filters and pipette tips were also dried at 40ºC for a minimum of 8 hours after autoclaving.

Any surface used during pre- or post-sampling laboratory work was sterilised using 70% ethanol solution. All sterile equipment was handled within 30 cm of a lit Bunsen burner, or within a class 2 laminar-flow cabinet (SC18-R, Labcaire Systems, UK) to maintain their condition. Furthermore, 70% ethanol solution was also used to sterilise hands prior to handling equipment. This was done both in the laboratory and field.

Once equipment had been used, any that came into contact with samples was again autoclaved at 121ºC for 15 minutes. All kit was washed in 10% Decon 90 solution, rinsed with deionised $H_2O$, and dried before being re-used. Blank samples were also periodically taken to ensure sample quality.

### 3.1.3 Sampling

Several experimental designs were employed in order to meet all aims and objectives. These are described in full within the experimental designs for each experiment (Chapters 4, 5, 6). The following protocol was used as standard throughout all experiments.

All sterilised equipment (pre-loaded filter cassettes, sampling heads, Nalgene jars containing buffer solution, caps and clips if endotoxin sampling is to be carried out) were loaded into a cool box, together with pre-frozen ice packs, to allow transportation to and from the sampling locations at approximately 4ºC.

A camcorder (SONY® Handycam DCR-SR35E) was set up at a central location, aimed to record site activity throughout the day. These recordings were later used to determine whether activities were present or absent during each sampling period, with those taken during periods throughout which no activities were taking place designated as ‘no activity’. A camera tripod (Adjustable tripod 570/322, Argos, UK) was raised and sampling platform constructed, schematic shown in Figure 3.2 and photographed in Figure 3.3 below, allowing SKC pumps to be mounted and sampling heads positioned at 1.7 m, corresponding to breathing height (Association for Organics Recycling, 2009; Environment Agency, 2001; Taha *et al*, 2006; The Composting Association, 1999;

Verein Deutscher Ingenieure, 2004). A Kestrel® weather station (Kestrel® 3000, Nielsen Kellerman, USA), measurement parameters illustrated in Table 3.1 below, was used to monitor weather conditions at the sampling location.

Table 3.1: Relevant parameters for Kestrel 3000 device (Nielsen-Kellerman, 2009).

| Feature | Units | Operational Parameters | Resolution | Accuracy (+/-) | Specification Range |
|---|---|---|---|---|---|
| Wind Speed | Metres per second (m/s) | 0.4 – 60.0 | 0.1 | Larger of least 3% of reading or least significant digit | 0.4 – 40.0 m/s |
| Temperature | Degrees Celsius (°C) | -45 - 125 | 0.1 | 1.0 °C | -29°C - 70°C |
| Relative Humidity | RH Percent (%) | 0 - 100 | 0.1 | 3% RH | 5% – 95% RH non-condensing |
| Dew Point | Degrees Celsius (°C) | 0 – 100% RH<br>-45 – 125°C | 0.1 | 2°C | 20 – 95% RH<br>-29.0 – 70.0°C |

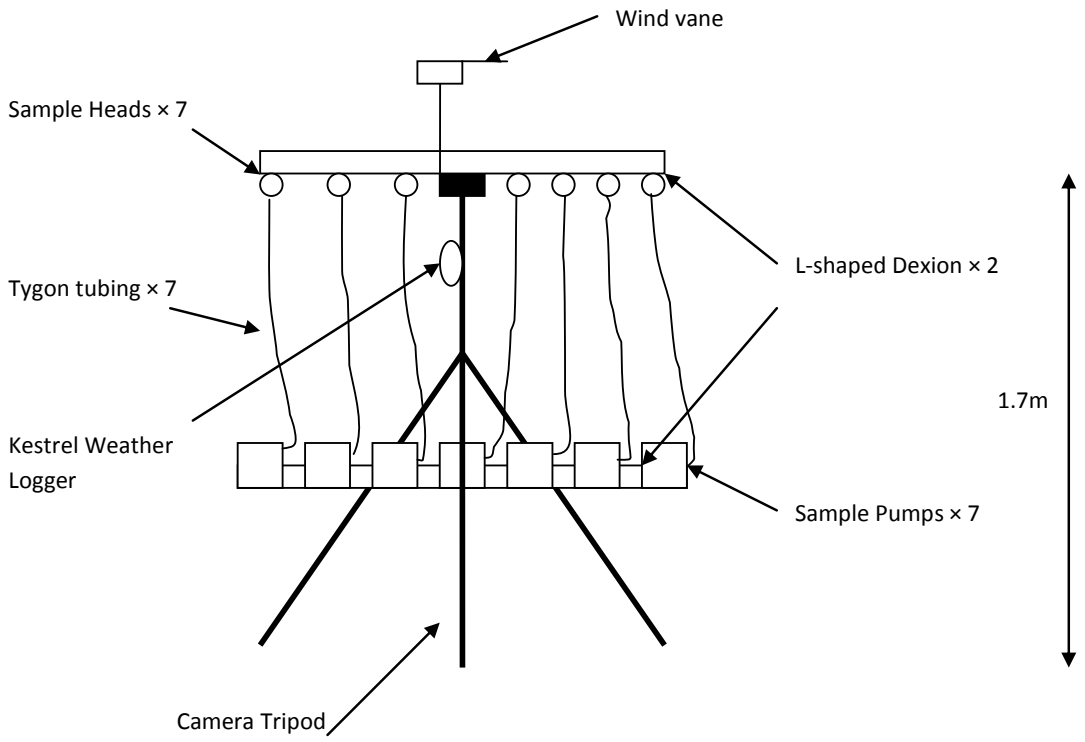



Figure 3.2: Schematic of sampling equipment set-up. SKC's were clipped onto L-shaped Dexion, which was fastened to the camera tripod. A second piece of Dexion was fastened to the top of the camera tripod, and raised to 1.7 m. The sampling heads were attached, evenly spaced, along this piece of Dexion. They were positioned to face the direction from which the sample is to be taken. A wind vane, and anemometer were also attached to the camera tripod.

Figure 3.3: Photograph of sampling set-up detailed in Figure 3.1; compost turning visible in background. Taken on the 17th October 2007 at Lount OWC.

Personal sample pumps (SKC PCXR8, SKC Ltd, UK), in combination with Tygon® tubing, were used (see Figures 3.3, 3.4 and 3.5). At the end of the tubing, the sterilised particulate sampling heads (225-70A SKC IOM Sampler, SKC Ltd, UK) were attached to the apparatus. As the sampling heads are directional, they were aligned to face towards the area being sampled from, as shown in Figures 3.3 and 3.5. Sampling pumps were pre-set to a sampling time and pump time of 30 minutes (Taha *et al*, 2005b; Taha and Pollard, 2004; Taha *et al*, 2006; Taha *et al*, 2007). Filter cassettes (IOM multi-dust plastic cassettes) were loaded into the sampling heads and the pumps started. Sampling heads and filter cassettes were only handled after hands have been washed with 70% ethanol, and care was taken not to touch the filter itself.


Figure 3.4: SKC PCXR8 personal sample pump

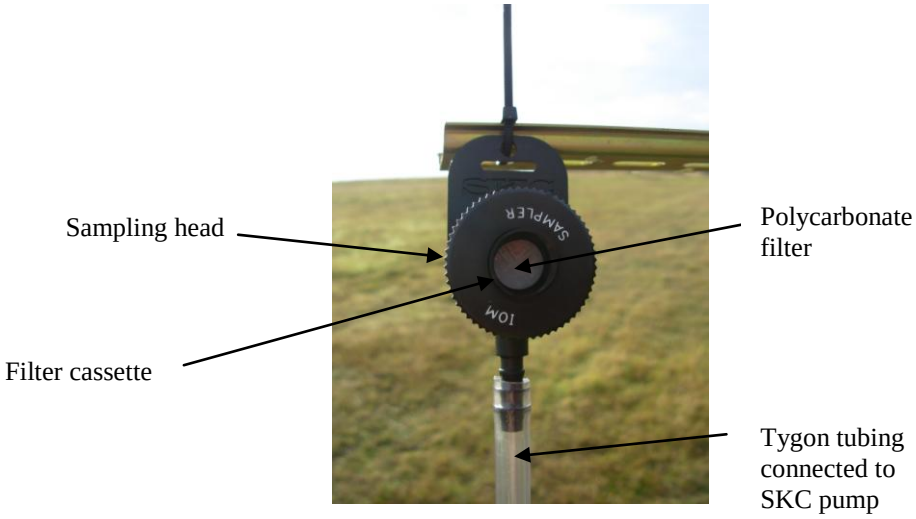



Figure 3.5: SKC IOM particulate sampling head, polycarbonate filter visible inside the sampling head

Sampling location was taken using a GeoExplorer 3 GPS system (GeoExplorer 3, Trimble, USA). A data table (Table 3.2) was completed, including continuous notes on activities throughout the sampling time.

Once the sampling was complete (pumps were set to auto-stop), hands were again cleaned with 70% ethanol. Filter cassettes were removed from the sampling heads and immediately placed into a labelled Nalgene jar containing aseptic buffer solution (Taha and Pollard, 2004; Taha *et al*, 2005b; Taha *et al*, 2006; Taha *et al*, 2007). The Nalgene

and filter cassette were then shaken to ensure the buffer covered the polycarbonate filter, to avoid desiccation of microbial cells. These were then sealed and placed back into the cool box. If the sample was to be used for endotoxin analysis, it was placed inside an aseptic cap and clip (supplied by manufacturer - IOM), and stored in an aseptic polyethylene bag. All samples were stored at 4°C for transportation to the laboratory. At the laboratory samples continued to be stored at 4°C until processing.

Table 3.2: Example of field sampling data recording sheet

| Sample I.D. | Location | Distance from Closest Windrow (m) | Time (hr:min) | Sample Time (min) | Height (m) | Wind Direction | Wind Speed (av. m/s) | Temp (ºC) | Humidity (%) | Dew Point (ºC) | Cloud Cover (%) | On-site Activities | Age of Compost (weeks) | Other |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *1. Upwind* | *E 438031.19 N 318470.14* | *100* | *9:58* | *30* | *1.7* | *S-S-W* | *5.7* | *4* | *89* | *0.1* | *50* | *None* | *0-12* | *Wind gusts up to 10 m/s* |

## 3.2 Sample processing

Samples for endotoxin analysis were packaged and sent to Bristol UWE for analysis, a brief description of the methods used is provided below. Samples for analysis through culture were processed according to the method described in section 3.2.2.

### 3.2.1 Endotoxin assay

The sampling parameters and extraction methods suggested by Park *et al*. (2001) were utilised for endotoxin assay. Extraction of endotoxins was carried out according to methods described by Spaan *et al*. (2007). An extraction solution of Limulus Amebocyte Lysate (LAL) grade water (Lonza Wokingham Limited, UK) and 0.05% Tween 20 was prepared. Filters (stored at -20 °C) were transferred into pyrogen-free six-well tissue culture plates, each well was loaded with 4 mL extraction solution. The extraction solution was transferred into pyrogen-free tubes and centrifuged at 1000 RCF for 15 minutes. Supernatant was collected, vortexed, and split into two pyrogene-free tubes. The samples could then be stored at -20 °C until analysis using the PyroGene rFC Endotoxin detection kit (Lonza Wokingham Limited, UK) (Park *et al*, 2001; Spaan *et al*, 2007).

### 3.2.2 Culture sample inoculation

Within 48 hours of sample collection, samples were plated in order to prevent loss of viability or microbial growth. Sample inoculation was carried out under aseptic conditions within a class 2 laminar flow cabinet. All equipment, laminar flow cabinet surfaces, and gloved hands placed inside the cabined were sterilised with 70% ethanol. The procedure outlined below was followed:

i. Using flame sterilised forceps (re-sterilised and cooled between each sample), filter was separated from the filter cassette
ii. Filter and cassette were placed back into the Nalgene jar and agitated by hand for ~2 minutes

iii. Samples were diluted along a common algorithm order. Each Nalgene jar (10°) was again agitated by hand (~10 s) before transfer of 1 mL inoculum by pipette to pre-prepared universal bottle containing 9 ml of buffer ($10^{-1}$). After manual agitation of the $10^{-1}$ solution (~10 s), 1 mL of inoculum was transferred into a second pre-prepared universal bottle containing 9 ml of buffer ($10^{-2}$). This $10^{-2}$ solution was also manually agitated (~10 s).

(Taha, 2005a; Taha *et al*, 2005b; Taha *et al*, 2006; Taha *et al*, 2007)

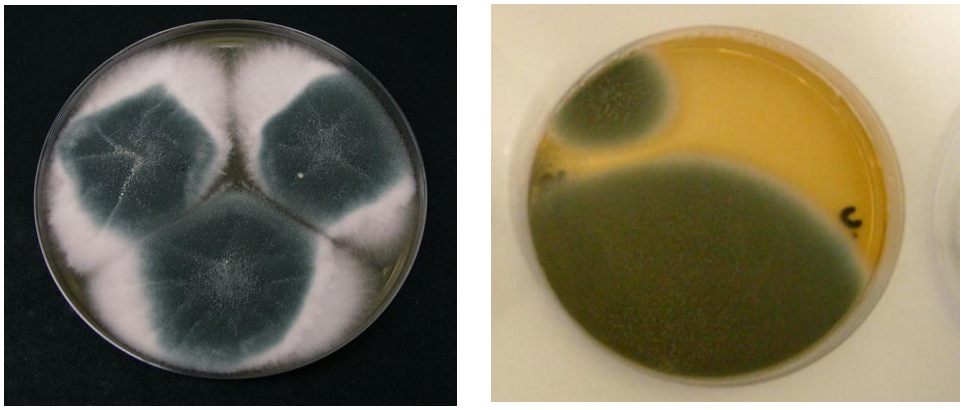
Once dilutions were prepared, the samples were inoculated onto the growth media. Petri dishes containing MEA and CA were ventilated in the laminar flow hood until excess moisture had evaporated and labelled with sample collection date, sample I.D. and dilution. Each sample was again manually agitated (~10 s) and aliquots of 100 µL were dispensed by pipette onto the centre of the agar (Taha *et al*, 2007; Taha, 2005a). The inoculum was spread evenly over the agar surface using aseptic microbiological spreaders (Spreader microbiological L-shaped high impact polystyrene, LPS-140-041X, Fisherbrand, UK), before the plate lid was closed. For MAC agar, the pour-plating method described in section 3.1.1 was followed.

### 3.2.3 Culture incubation

Once all samples were been plated, they were stacked, loosely re-wrapped in their original packaging and inverted for incubation (Searle Laboratory Thermal Equipment, UK, and Genlab 175L, M17SCF, UK) until visible colonies developed. MEA and MAC agar were incubated at 37 ± 2ºC in the dark (Searle) for 3 days, CA was incubated at 44 ± 2ºC (Genlab) in the dark for 7 days (Taha, 2005a; Taha *et al*, 2007).

### 3.2.4 Culture enumeration

Colonies were counted once visible. All observations regarding number, colour and shape of colonies were recorded, along with any details regarding contamination or other observations. Figures 3.6 through to 3.8 show and describe recognisable characteristics of each microorganism.



a) Midgley, 2006 b) Personal photograph

Figure 3.6: *Aspergillus fumigatus* on MEA incubated for 4 days at 37 ± 2 ºC (Midgley, 2006).

*Aspergillus fumigatus* colonies are velvety to floccose in texture, with blue-green to grey-green colouration, yellow, green, or red-brown shades shown on the reverse. Conidial heads are developed, columnar, up to 400 × 50 µm, although are often shorter or smaller. Conidiophores rise from submerged hyphae, or as short branches from aerial hyphae, and are often greenish (Epstein, 1994).

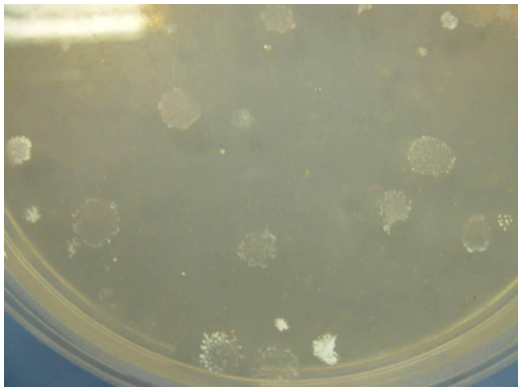


Figure 3.7: Actinomycetes on CA incubated at 44 ± 2 ºC for 7 days (personal photograph).

Actinomycetes have a branching growth, with short chains of spherical spores that are 0.7-1.3 µm in diameter (Taha *et al*, 2007).


Figure 3.8: Gram-negative bacteria, both lactose fermenting (pink) and non-lactose fermenting (colourless) colonies shown (Forbes *et al,* 2007). On MAC, incubated for 3 days at 37 ± 2 ºC (personal photograph).

The visually enumerated colony count was then used to calculate colony forming units per cubic metre of air (CFU $m^{-3}$).

Initially, one of the two calculations detailed below was used, depending upon the total number of colonies isolated on each plate (Equations 3.1, 3.2). Calculations were modified from British Standard 5763-0 (British Standards Institution, 1996) in order to prevent pseudo-replication caused through the replication of samples in the laboratory after they had already been repeated in the field. The formulae used are described below:

If any plate retained had more than 15 and less than 300 colonies:

$$N = \frac{\Sigma C}{V \times [n_1 + (0.1 \times n_2)] \times d}$$

[Equation 3.1: (British Standards Institution, 1996)]

Where

$N$ is the concentration of bioaerosols in the sample solution per millilitre

$\Sigma C$ is the sum of colonies counted on all plates retained from two successive dilutions, and where one plate contains between 15 and 300 colonies

$V$ is the volume of inoculums applied, in millilitres

$n_1$ is the number of plates retained at the first dilution

$n_2$ is the number of plates retained at the second dilution

d is the dilution factor corresponding to the first dilution retained (where $10^o = 1$, $10^{-1} = 0.1$ and $10^{-2} = 0.01$)

If none of the plates retained had more than 15 colonies, the estimated number of microorganisms ($N_E$) present was calculated:

$$N_E = \frac{\Sigma C}{V \times n \times d}$$ [Equation 3.2: (British Standards Institution, 1996)]

Where

$\Sigma C$ is the sum of colonies on all plates retained

$V$ is the volume of inoculums applied, in millilitres

$n$ is the number of dishes retained

$d$ is the dilution factor corresponding to the first dilution retained

The resulting figure ($N_E$) was then used in order to calculate CFU per sample. This was done through multiplication of the result by the volume of the sample; in this case, 10 mL. $N_E$ then represents total colonies in the sample (British Standards Institution, 1996).

Following this, CFU $m^{-3}$ was calculated using the following equation:

$$B_{con} = \frac{N_E}{(F_s \times t / 1000)}$$ [Equation 3.3]

Where

$B_{con}$ is the concentration of colony forming units per cubic metre of air (CFU $m^{-3}$)

$N_E$ is the total number of colony forming units in the sample solution

$F_s$ is the sampling flow rate (litres per minute)

$t$ is the sampling period (per minute)

## 3.3 Critical Evaluation of Method

### 3.3.1 Lower limits of detection

As with all methods, the one used as part of this project is subject to limitations. One of the main limitations is lower detection limits. Below are calculations for the detection limit of this method.

$$\text{LLOD} = \frac{\Sigma C \times N_2 \times S}{(F_s \times t / 1000)} \qquad \text{[Equation 3.4]}$$

Where

LLOD is the lower limit of detection

$\Sigma C$ is the sum of colonies on all plates retained (minimum 1 colony = 0.5)

$N_2$ is the number of colonies per millilitre of sample (accounting for volume of inoculum)

$S$ is the sample volume

$F_s$ is the sampling flow rate (litres per minute)

$t$ is the sampling period (minutes)

Worked example: $\frac{0.5 \times 10 \times 10}{0.066}$

Where

0.5 is average of one colony across two plates retained

10 accounts for the volume of inoculum (0.1 mL)

10 accounts for sample volume

0.066 is the amount of air sampled ($m^3$)

Despite the lower limit of detection using this method being 757 CFU $m^{-3}$, using the calculations necessary to gain CFU $m^{-3}$ (Equations 3.1 – 3.3) 1 colony equals 1515 CFU

$m^{-3}$. As a result, confidence in any concentrations below this level, as may happen when several samples are averaged, is low.

Current Environment Agency limits for total bacteria and total fungi are 1000 CFU $m^{-3}$, with limits for gram-negative bacteria at 300 CFU $m^{-3}$ (Environment Agency, 2009a; Environment Agency, 2009b). The lower limit of detection (LLOD) here is close to, or higher, that these limits. Therefore, using this method identification of the point at which these levels are achieved will not be possible. Furthermore, background concentrations of *A. fumigatus* have been suggested to be 1 – 2 CFU $m^{-3}$ (Kothary *et al*, 1984), and < 10 CFU $m^{-3}$ (O'Gorman and Fuller, 2008). For actinomycetes, estimates range from 10 CFU $m^{-3}$ (Millner *et al*, 1994) to $10^2$ CFU $m^{-3}$ (Herr *et al*, 2003a). For *A. fumigatus*, estimated background concentrations are well below lower limits of detection, while for actinomycetes, the estimate from Herr *et al.* (2003a) may be detectable, but not the estimate from Millner *et al.* (1994). These data mean that through the use of this method, background levels will most likely not be measured. While it would be possible to lower detection limits further through an increase in sampling time or reduction of sample dilution (from 10 mL to 5 mL), this may errors. For while increasing sampling time would reduce lower limit of detection, it would also increase the chance of bioaerosol loss of viability due to desiccation on the filter surface and osmotic stress (Chen and Li, 2005a).

In addition, bioaerosols are ubiquitous in the environment, with sources such as agriculture, and decomposing organic matter, and household waste contributing to bioaerosol concentrations in the ambient environment. Therefore, 'background' concentrations will vary from place to place, making accurate estimation of concentrations difficult. Taking the LLOD and the difficulty of quantifying background into account, in this case upwind levels will be quantified and achievement of these concentrations used to measure limits of downwind dispersal, rather than background.

### 3.3.2 Review of the method

Although lower limits of detection are relatively high for this method, there are some benefits to the use of the IOM sampling method. The reproducibility of the method means than many samples can be taken, allowing a repeated, validated data-set to be created. The relatively low costs of reproducing samples, ease of use, and robust nature of the equipment make filtration a more viable option for this type of sampling than impaction or impingement (Environment Agency, in press). Furthermore, filtration sampling offers flexibility, with a variety of methods available for bioaerosol enumeration. It is also able to capture high concentrations of bioaerosols, over longer sampling times than the most established method used to measure bioaerosols, namely impaction. This makes it more suitable for sampling close to source, sampling for exposure assessment, and for capturing episodic emissions of bioaerosols (Eduard and Heederik, 1998). Both laboratory and field tests against other samplers have suggested that the IOM sampler retains more precision at higher wind speeds than other samplers tested (Kenny *et al*, 1997). This suggests that the IOM sampler may be suited to environmental sampling. However, all samplers tested showed a poor performance in high wind speeds (Kenny *et al*, 1997). Given the attributes of filtration sampling, standard sampling protocols in Germany are based on filtration sampling, with UK guidelines recently updated to include the filtration method described here (Association for Organics Recycling, 2009; Environment Agency, 2009a; Verein Deutscher Ingenieure, 2004).

The cut-off point of IOM samplers is an aerodynamic diameter of 100 µm, although it has been found that IOM samplers have 75% collection efficiency with particles of this aerodynamic diameter. However, this is compared to an efficiency of 100% with smaller particles (Kenny *et al*, 1997). In addition, particles with smaller aerodynamic diameters are commonly of more concern with regards to human health, particularly those with an aerodynamic diameter < 5 µm that are able to penetrate alveolar spaces (Palmgren *et al*, 1986).

The face velocity of bioaerosol samplers contributes to yield, as higher face velocities may increase desiccation and dehydration of microorganisms, reducing viability. At a sampling rate of 2.2 L $min^{-1}$, taking into account effective filter diameter, face velocity is 20.7 cm $s^{-1}$. While there is little data considering the effect of face velocity on the retention of viability, the VDI guidelines recommend a method with similar face velocity, of 21.7 cm $s^{-1}$ (Verein Deutscher Ingenieure, 2004).

## 3.4 Data Analysis

Once a database of repeated bioaerosol samples had been established, it was necessary to develop a method for analysis. Initial analysis of the data-set revealed a skewed distribution. Figure 3.9 below shows an example of the fit of the data to this distribution, utilising *A. fumigatus* observations at Lount OWC. In this case, *A. fumigatus* observations correlated with expected observations (of a skewed distribution) with an $r^2$ of 0.99; indicating a very strong fit.

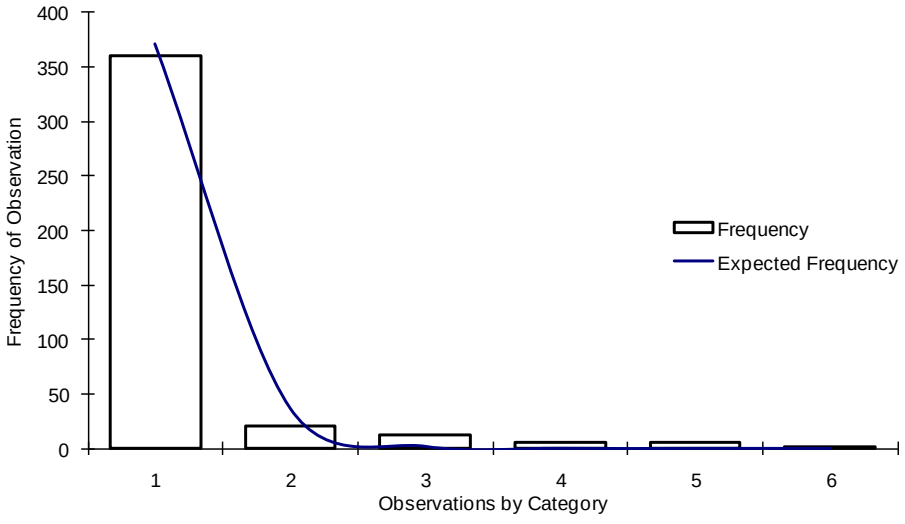



Figure 3.9: *A. fumigatus* frequency of observations by concentration category (e.g. category 1 = 0 to 12 × $10^3$ CFU $m^{-3}$. Range of each category = 24 × $10^3$ CFU $m^{-3}$). Bars represent frequency of observations within raw data; line represents expected frequency according to a Poisson distribution.

This distribution, often termed the 'Poisson' distribution, often describes environmental data, which is frequently comprised of a large number of '0' values, with a small number of very large values. This is precisely the case with bioaerosol data. However, the small number of large values, 'outliers', can make effective analysis challenging. Figure 3.10 below illustrates this, with a high amount of residual variance representing the inability the statistical program (STATISTICA 8; StatSoft 2008) to predict values utilising the raw data.

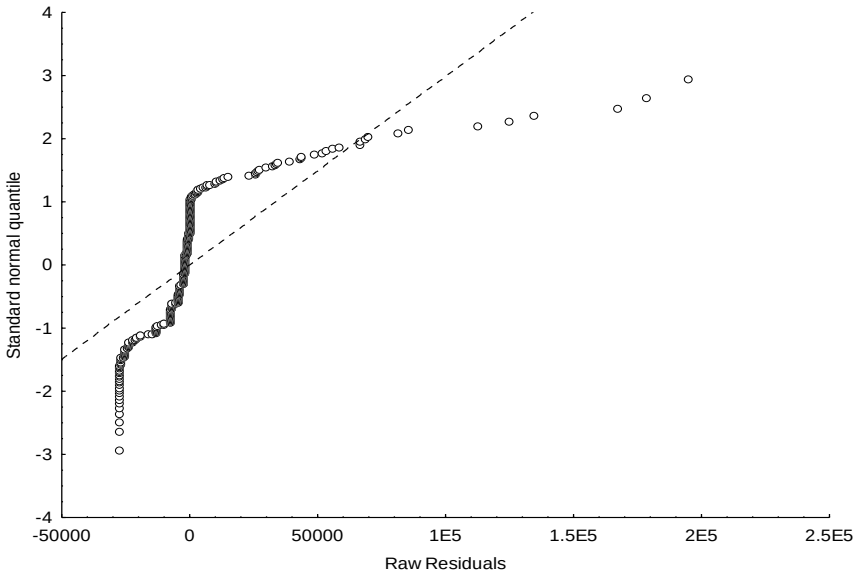



Figure 3.10: Q-Q plot of data for *A. fumigatus* at Lount OWC. Dotted line represents predicted value using a Normal model; circular markers represent raw data values.

In order to effectively analyse the data, it was therefore necessary to create a normal distribution through transforming the data. A natural log-transformation of the data was performed, with the resulting Quantile-Quantile (Q-Q) plot of the data shown in Figure 3.11. In order to perform a natural log transformations, any '0' values within the data-set must be adjusted. For the purposes of this data-set, within the raw culturable microorganism (*A. fumigatus*, actinomycetes, and gram-negative bacteria) results any '0' values were converted to 757 CFU $m^{-3}$, the lower limit of detection using the methods described within this Chapter. This value was chosen given the detection limit,

and taking into account reduced confidence in samples below 1515 CFU $m^{-3}$ (Section 3.3.1). Within the endotoxin raw data, and any '0' values were converted to 0.152 EU $m^{-3}$, the lower detection limit of Pyrogene endotoxin assay following standard procedures.

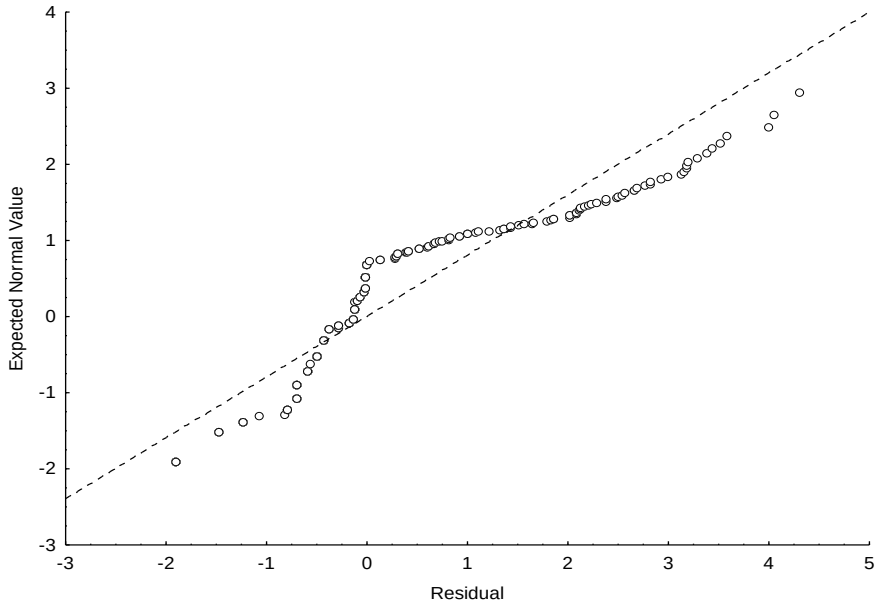



Figure 3.11: Q-Q plot of residuals for transformed *A. fumigatus* data at Lount OWC. Dotted line represents Normal value; circular markers represent transformed data values.

Figure 3.11 shows that the data can be assumed to follow a normal distribution for the transformed data, better than using raw data. However, the fit remained variable. This variation can be explained by Figure 3.12 below, illustrating the spread of residual data points. This spread is responsible for the variation seen in the Q-Q plot (Figure 3.11). The even nature of this spread also indicated that the log-transformation of data and subsequent analysis is likely to be the most effective method for bioaerosol data analysis in this case, despite the residual variation seen.

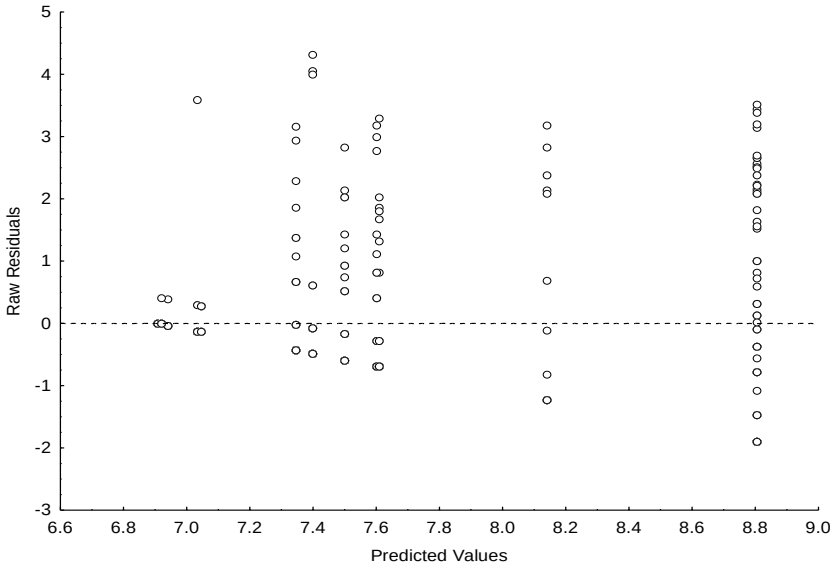



Figure 3.12: Spread of *A. fumigatus* residuals around predicted residuals. Dotted line represents Normal values, circular markers represent transformed data values.

The application of a log-transformation to data collected throughout this project has allowed analysis through a General Linear Model (GLM). The data were analysed in STATISTICA. This analysis allowed a variety of graphical and statistical outputs to be generated. In particular, a GLM analysis minimises the sum of the squared differences to estimate the parameters of the model (Pentecost, 1999). The natural logarithm transformation implies the comparisons will be of geometric, rather than arithmetic mean values for the fixed effects. Log transformation and hence geometric mean comparisons are often used for positively skewed data, and have been commonly used for bioaerosol analysis (Dytham, 1999; Schlosser *et al*, 2009). The natural logarithm transformation standardises and normalises the data (Figure 3.10). This allows smaller and more frequently found values to exert a larger influence on the data-set, therefore, accounting for the spread seen within this data-set and the skewed distribution through presentation of geometric mean. On the other hand, an arithmetic mean, or 'average', allows a minority of large values to influence the overall mean, not taking into account the frequency of lower values. The utilisation and presentation of geometric mean has enabled more accurate representation of mean bioaerosol concentrations. However, it must be acknowledged that this has also reduced comparability to previous bioaerosol investigations, where only arithmetic mean values are presented.

Once geometric mean values were calculated using the GLM, a back transformation was carried out (exponential of transformed data). This allowed presentation of the dispersal profile using CFU $m^{-3}$, rather than the logarithmic values. In order to show statistical similarity or difference between sampling locations a post-hoc analysis on the transformed data within the GLM was carried out. A Fisher Least Significant Difference (LSD) test provided a measure of statistical similarity to a 95% confidence level ($p = < 0.05$) between pairs of sampling locations. (Meier, 2006; Rowntree, 2004). An example of the output from this analysis is shown in Table 3.3 below.

Table 3.3: Pairwise testing of significance ($p = < 0.05$) between sampling locations for *A. fumigatus* at Lount OWC, calculated through Fisher LSD test. Treatment = sample location; Aspergillus log = geometric mean of *Aspergillus fumigatus* concentrations found at each sampling location; a, b, c, d, e = grouped statistical similarity generated through pairwise comparisons; **** = statistical similarity ($p = < 0.05$), i.e. all sampling locations in group 'a' with **** denotation show concentrations that are statistically similar.

| Treatment | Aspergillus log | a | b | c | d | e |
|---|---|---|---|---|---|---|
| Downwind 280m | 6.629363 | **** | **** | **** | | |
| Downwind 300m | 6.648638 | | **** | | | |
| Downwind 80m | 6.687189 | **** | **** | **** | | |
| Upwind | 6.778740 | | **** | **** | | |
| Downwind 180m | 6.860657 | **** | **** | **** | | |
| Downwind 250m | 7.148770 | **** | **** | **** | | |
| Downwind 100m | 7.226366 | **** | | **** | | |
| Downwind 150m | 7.350552 | **** | | | **** | |
| Downwind 50m | 7.432451 | **** | | | **** | |
| Site no activity | 7.443140 | **** | | | **** | |
| Site boundary | 7.987143 | | | | **** | |
| Site activity | 8.728112 | | | | | **** |

The groups of statistical similarity generated through the pairwise analysis were then assigned an annotation, and represented on the final output in order to provide a measure of statistical similarity or difference between sampling locations in place of a standard error whisker. This concept is illustrated in Figure 3.13 below, with Figure 3.14 showing an example from the data-set analysed as part of this study. In this example, the only groups of statistical similarity that site boundary and site activity

belong to are groups d and e, respectively. However, downwind 150 m, downwind 50 m, and site no activity samples also belong to group d, hence are statistically similar to site boundary. These results are shown in the final output for *A. fumigatus* at Lount OWC, shown below in Figure 3.14.

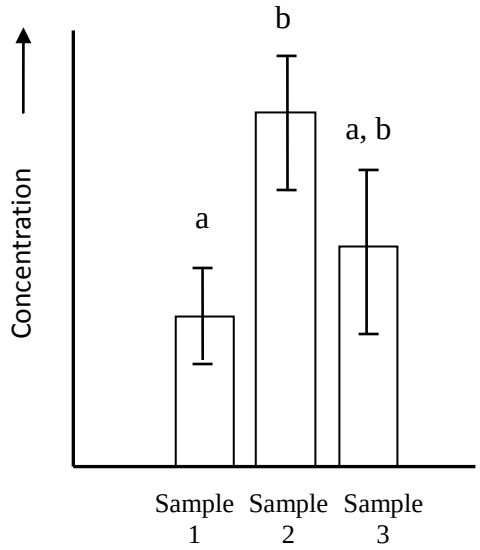



Figure 3.13: Theoretical representation of statistical difference, illustrated through conventional standard deviation whiskers, with the associated Fisher LSD generated annotations showing grouped significant difference ($p = < 0.05$).

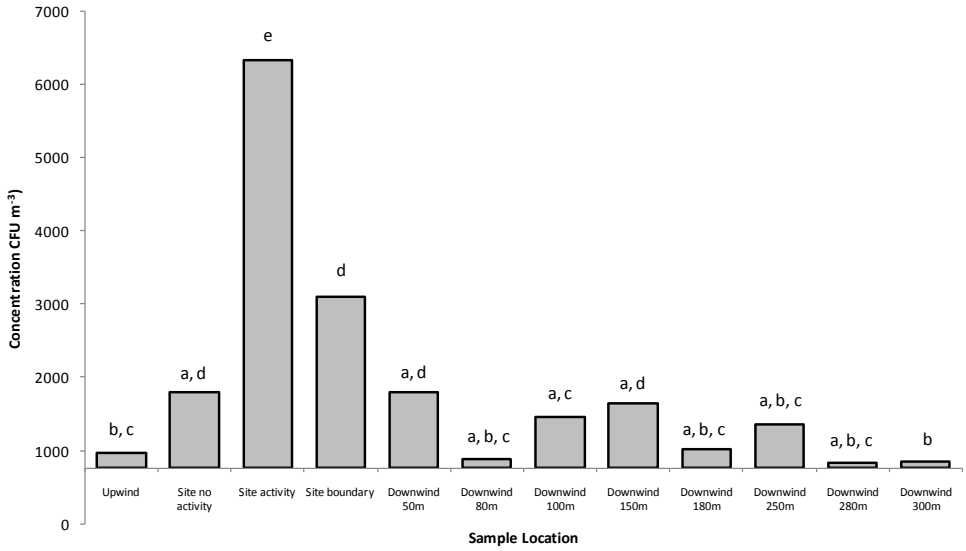



Figure 3.14: *A. fumigatus* at Lount OWC in CFU $m^{-3}$. Bars represent geometric mean, annotations represent groups of statistical similarity ($p = < 0.05$). X-axis crosses at lower limit of detection (757 CFU $m^{-3}$).

The application of a natural log-transformation also allowed the analysis of data including environmental and mechanical parameters, and assessment of the amount that these parameters contribute to total variation. Environmental parameters, as continuous measurements, were included as raw data. However, the data concerning mechanical activities at the time of sampling, gathered using the camcorder described in Section 3.1.3, was entered into the GLM as 'presence' or 'absence' data. Namely, if during a sampling period screening was taking place but turning wasn't, the sample was assigned a '1' for screening and '0' for turning. The Q-Q plot of data including environmental and mechanical parameters is shown in Figure 3.15. Once again, the fit of data to predicted values has been improved. This illustrates how some of the variation within the data-set can be accounted for by the inclusion of environmental and mechanical parameters.

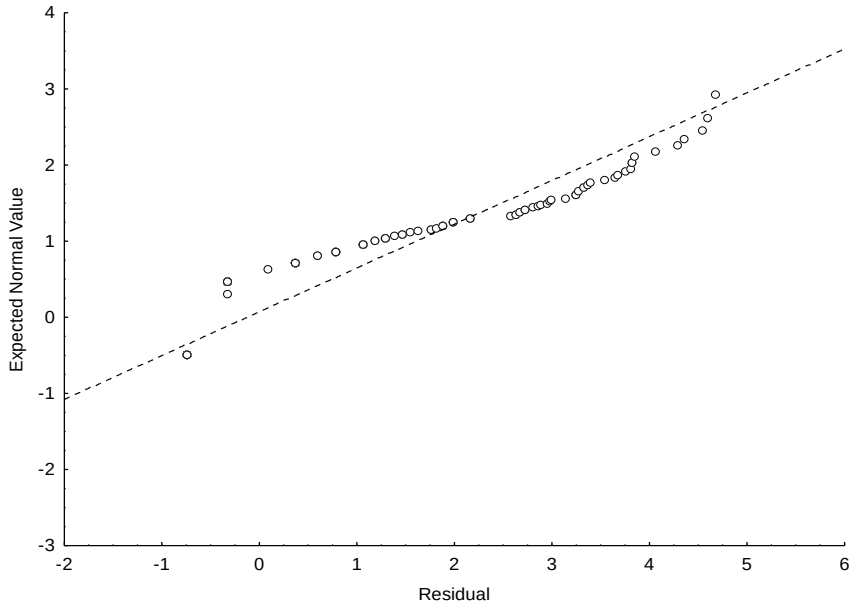



Figure 3.15: Q-Q plot of transformed *A. fumigatus* data at Lount OWC, including environmental parameters. Dotted line represents Normal value; circular markers represent transformed data values.

The general linear model is able to generate several outputs once environmental and mechanical factors have been incorporated into the design. For continuous parameters (environmental), geometric means for bioaerosols can be recalculated using means of these parameters. This allows re-presentation of bioaerosol dispersal under average environmental conditions. Furthermore, the influence that each parameter has on bioaerosol concentrations can be determined. This was done through the calculation of parameter coefficients, which give positive or negative values depending on whether the relationship with the bioaerosol is positive or negative. Furthermore, the significance of the relationship was shown, with significant relationships highlighted, and p-values of the relationship also provided. An example of this output is shown below (Table 3.4).

Table 3.4 Example of p-values and parameter coefficients for *A. fumigatus* at Lount OWC. Significant values highlighted.

| Parameter | P-value |
|---|---|
| Season | 0.007 |
| Sample Location | 0.000 |
| Wind direction (Degrees) | 0.807 |
| Wind speed (m/s) | 0.000 |
| Temperature (°C) | 0.390 |
| Relative Humidity (%) | 0.978 |
| Cloud cover (%) | 0.058 |

| Parameter | Coefficients |
|---|---|
| Autumn | 0.358 |
| Winter | 0.281 |
| Spring | 0.881 |
| Summer | - |
| Wind direction (Degrees) | 0.000 |
| Wind speed (m/s) | 0.209 |
| Temperature (°C) | 0.031 |
| Relative Humidity (%) | -0.000 |
| Cloud cover (%) | 0.007 |

However, analysis of the relationship between environmental parameters and bioaerosols was not straightforward. Results may be confounded by the cross-correlation of environmental parameters, which may enhance an impact on the bioaerosol being analysed, or cancel each other out, removing any impact. An example of this effect is shown below, with a scatter plot between temperature and relative humidity in Figure 3.16, and a table of correlations between all environmental parameters at Lount OWC in Table 3.5.

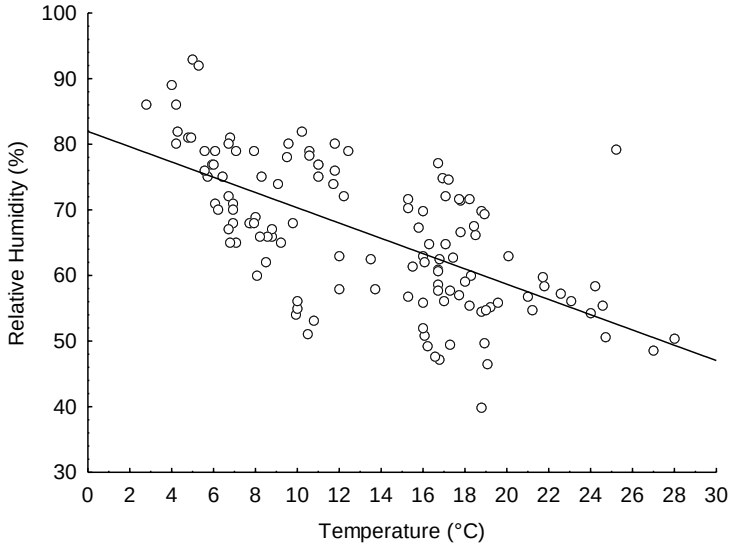



Figure 3.16: Scatterplot of temperature (°C) versus relative humidity (%) at Lount OWC showing strong negative correlation.

Table 3.5: Correlations between environmental parameters at Lount OWC. Significant correlations highlighted.

| | Wind direction (°) | Wind speed | Temperature (°C) | Humidity (%) | Cloud (%) |
|---|---|---|---|---|---|
| **Wind direction (°)** | 1.000 | | | | |
| **Wind speed** | -0.125 | 1.000 | | | |
| **Temperature (°C)** | -0.109 | -0.444 | 1.000 | | |
| **Humidity (%)** | 0.102 | 0.481 | -0.638 | 1.000 | |
| **Cloud (%)** | 0.194 | 0.075 | -0.061 | 0.300 | 1.000 |

These correlations mean it was necessary to separate correlating parameters, in order to determine the true impact of parameters on the bioaerosol in question. Figure 3.16 and Table 3.5 above illustrate how temperature and relative humidity were strongly correlated. Furthermore, humidity was significantly correlated with all other environmental parameters, and temperature was also significantly correlated with wind direction and wind speed. Therefore, analysis was carried out without each parameter in turn. An extract from this type of analysis is shown below, in Table 3.6. The analysis including all parameters showed wind speed and cloud cover as being significantly positively correlated with actinomycetes. However, removing temperature allowed the significance of correlations with wind direction and humidity to be revealed. Removing humidity in turn allowed the significance of relationships between temperature and actinomycetes to be revealed.

Table 3.6: The effect of environmental parameters on actinomycetes at Lount OWC, with all parameters, and without temperature and humidity in turn.

| ALL | | WITHOUT TEMPERATURE | | WITHOUT HUMIDITY | |
|---|---|---|---|---|---|
| **Parameter** | **Coefficient** | **Parameter** | **Coefficient** | **Parameter** | **Coefficient** |
| Wind Direction | -0.002 | Wind Direction | -0.003 | Wind Direction | -0.002 |
| Wind Speed (m/s) | 0.400 | Wind Speed (m/s) | 0.362 | Wind Speed (m/s) | 0.357 |
| Temperature (°C) | 0.079 | Humidity (%) | -0.027 | Temperature (°C) | 0.106 |
| Humidity (%) | -0.022 | Cloud cover (%) | 0.014 | Cloud cover (%) | 0.017 |
| Cloud cover (%) | 0.018 | | | | |

Although this type of analysis leads to differences in the strength of correlation between individual environmental parameters and the bioaerosol depending upon the analysis used, it is whether a parameter is significant, and whether this significance is positive or negative that is of greatest importance. In this case, as removal of temperature allowed most correlations to be revealed, these coefficients, along with the coefficient for temperature gained through the removal of humidity, could be used. However, it is clear that the decision regarding which coefficients to use is subjective. In order to create the most valid analysis, several methods were employed. Firstly, the general linear model was tested, meaning that the ability of the model to account for variability (Multiple $R^2$) was assessed. From this, coefficients from the model with the best ability to account for variability were favoured. In addition, it was necessary to use expert knowledge. This included review of the available literature; for example, several studies have shown a correlation between airborne bacteria, and temperature, and humidity (Korzeniewska *et al*, 2009; Seinfeld and Pandis, 2006; Tham and Zuraimi, 2005). It was therefore expected that these factors would correlate with actinomycetes, and analyses were carried out that would allow these correlations to be revealed.

## 3.5 Conclusions

The methods described above were used to enumerate bioaerosols through culture and were integral to all experimental designs and analyses used throughout this project. Initial implementation of the methods was through the experimental design aimed at characterising site emissions and bioaerosol dispersal.

# 4 Bioaerosol Dispersion Profile

## 4.1 Introduction

In order to fully quantify the dispersal of bioaerosols, it is necessary to understand patterns of emission and dispersal from release on-site, to distances greater than the 250 m risk assessment limit recommended by the Environment Agency (Environment Agency 2001a; Environment Agency 2009a). As introduced in Chapter 1, the dispersal range of bioaerosols remains uncertain. For example, Albrecht *et al.* (2008) suggest that sites influence bioaerosol concentrations 300 – 500 m from site, while Gilbert *et al.* (2002) found bioaerosols reached background concentrations 200 m from site. Many past studies present data based on 'snapshot' sampling strategies (Swan *et al*, 2003). Therefore, it is likely that both studies are correct, based upon the samples taken. However, the concentrations found may not represent concentrations that may be found on another day, with a limited number of samples unable to accurately represent the variability found in bioaerosol concentrations.

It is now well acknowledged that composting processes lead to episodic releases of bioaerosols, with emissions up to 3 orders of magnitude higher during agitation activities (Clark *et al*, 1983; Taha *et al*, 2006; Taha *et al*, 2007), with snapshot sampling potentially failing to account for this. The comparability of studies is also hampered by the variety of sampling strategies and methods used. Most studies utilise culture-based methods for bioaerosol enumeration, however, it is acknowledged that a large fraction of bioaerosols may not be culturable, although these bioaerosols remain a health risk (Crook and Sherwood-Higham, 1997; Swan *et al*, 2003). Until a recent update of guidelines (Association for Organics Recycling, 2009; Environment Agency, 2009b) the only standardised method in the UK for bioaerosol sampling used impaction methods for sample collection (The Composting Association, 1999) followed by enumeration through culture. This is despite that fact that many have suggested impaction leads to an increased loss of microorganism viability compared to other samplers, as well as easily becoming overloaded, leading to shorter sampling times (Environment Agency in press;

Godish and Godish, 2006). The use of this method may have led to underestimation of bioaerosol concentrations through both enhancing the 'snapshot' nature of the sampling strategy, and failing to enumerate non-culturable and non-viable fractions of bioaerosols, such as endotoxins (Chapter 1).

*Aspergillus fumigatus* is one of the bioaerosols released from composting facilities of most concern with regards to human health, being an opportunistic pathogen (Chapter 1). Hence, it is one of the most studied. Most measurements of *A. fumigatus* concentrations adjacent to processing activities reach $10^6$ CFU $m^{-3}$ (Clark *et al,* 1983; Taha 2004), with turning suggested to release the most *A. fumigatus* (Albrecht *et al,* 2008). A recent study determining emission of *A. fumigatus* suggested that emission rates reach between $6 \times 10^3$ and $10^4$ CFU $m^{-2}$/s from green waste (Fletcher *et al,* 2008). These estimates are significantly higher than measurements of background concentrations, where levels of 0 to 2 CFU $m^{-3}$, and less than 10 CFU $m^{-3}$ have been reported (Kothary *et al,* 1984; O'Gorman and Fuller, 2008). Concentrations of *A. fumigatus* downwind vary, with Kothary *et al.* (1984) finding concentrations 200 – 1000 CFU $m^{-3}$ by 50 m downwind from site. Another study found that 10% of measurements 540 m from site within a residential area were at least two standard deviations above background concentrations; leading to a conclusion that concentrations were significantly higher when the residential area was downwind from composting activities (Recer *et al,* 2001).

Actinomycetes are gram-positive spore forming bacteria associated with both green waste composting and negative health impacts (Swan *et al,* 2002; Swan *et al,* 2003). Concentrations adjacent to composting activities have been reported to reach $5 \times 10^4$ CFU $m^{-3}$ and $10^6$ CFU $m^{-3}$ (Lacey, 1997; Taha *et al,* 2004), with one study reporting higher concentrations are released during compost screening (Albrecht *et al,* 2008). However, less data are available regarding the dispersal of actinomycetes from composting facilities. One study found that 21% of samples taken 540 m from a composting site were two standard deviations or more above background concentrations (Recer *et al,* 2001). Herr *et al.* (2003a) found that actinomycetes had reduced to background concentrations by 300 m from site. Gram-negative bacteria are also released

through the composting process. Concentrations adjacent to activity at open-air windrow facilities have been found in the range of $0 – 2.8 \times 10^5$ CFU $m^{-3}$ (Environment Agency 2001a); while Clark *et al.* (1983) found concentrations were below those measured for *A. fumigatus*. However, both these bacteria and actinomycetes are rarely studied individually, with most published data focusing on meso- or thermophilic total bacteria, in accordance with guidelines (Association for Organics Recycling, 2009; Environment Agency, 2009b; The Composting Association 1999). Even when considered as total bacteria, less information is available regarding bacteria emission and dispersal than for *A. fumigatus.* It has been found that in locations 150 – 2000 m from composting facilities, annual median values of bacteria were 170 – 330 CFU $m^{-3}$ (Reinthaler *et al,* 1997). Gram-negative bacteria are considered a concern from a health perspective due to their ability to release endotoxins (Dutkiewicz, 1997). However, while endotoxin concentrations at composting facilities has been measured (Schlosser *et al*, 2009), downwind concentrations, to the authors' knowledge, have not.

The Environment Agency, based upon the available data in 2001 (Environment Agency, 2001a), set a risk assessment limit of 250 m for composting facilities. Either by the nearest sensitive receptor or by 250 m from site boundaries, total bacteria and fungi was expected to have reached 1000 CFU $m^{-3}$, while gram-negative bacteria should reach 300 CFU $m^{-3}$. In addition, it is now anticipated that *A. fumigatus* reference levels of 500 CFU $m^{-3}$, to be achieved by 250 m from site boundary, may be set (Dennis, 2009). From the above evaluation, it is clear that there is disagreement as to whether bioaerosols emitted through composting achieve these targets. The main reason for this is the lack of significant, comparable datasets based on repeated sampling occasions available in the literature to describe bioaerosol dispersal. Therefore, it is not currently possible to state with confidence whether, and how often, reference limits are exceeded due to a lack of validated data. While there has been examination of the dispersal profile of bioaerosols from 200 m to over 1000 m from site (Fischer *et al*, 2008), concentrations from source to downwind distances over 250 m from site have not been presented as a dispersal profile. In addition, Fischer *et al.* (2008) were not able to carry out statistical analysis of data due to a lack of comparability between samples. This lack of valid data from source to downwind has also prevented successful use of atmospheric modelling

programs, with no data available to verify results from dispersal models, including basic knowledge such as whether bioaerosols follow a Gaussian dispersal pattern, or a pattern more atypical. Other concerns surround the potential systematic underestimation of bioaerosol concentrations due to standard sampling methodologies (Environment Agency, in press; Godish and Godish, 2006; The Composting Association 1999) and the failure to account for non-culturable fractions of bioaerosols (ADAS and SWICEB, 2005; Crook and Sherwood-Higham 1997; Heidelberg *et al,* 1997; Ritz, K. 2007; Swan *et al,* 2003).

There is evidence that bioaerosols are able to travel beyond the 250 m risk assessment limit in concentrations above background levels (Albrecht *et al*, 2008; Fischer *et al*, 2008; Herr *et al* 2003a), and also that there may be a health risk associated with these bioaerosols (Herr *et al*, 2003a, b; Herr *et al*, 2004a, b). Herr *et al.* (2003a, b; 2004a, b) found an association with self-reported respiratory symptoms amongst populations living downwind from composting facilities. A distance dependent relationship between bioaerosol concentrations and composting site was demonstrated up to 550 m, with residents symptoms consistent with Mucosal Membrane Inflammation (MMI) (Herr *et al*, 2003a, b; Herr *et al*, 2004a, b). These findings further emphasise the importance of gathering a significant, validated database describing bioaerosol dispersal up to and beyond the 250 m risk assessment limit.

One of the main questions raised by the literature review carried out (Chapter 1) is:

Is the 250 m trigger distance for risk assessment imposed on compost facilities appropriate to the risk created by bioaerosol release?

The literature review presented within Chapter 1, along with the above review has shown that while data regarding both the emission and downwind concentrations of bioaerosols exists, often this data is based upon ‘snapshot’ samples. Furthermore, the focus of such studies typically concerns the differences between composting sites and processes, or samples taken at limited downwind locations of interest to the individual study (Albrecht *et al*, 2008; Fischer *et al*, 2008; Recer *et al*, 2001; Schlosser *et al*,

2009). To the authors' knowledge, no dataset profiling bioaerosol concentrations from emission, up to and beyond 250 m from site, exists; meaning that it is currently not possible to answer the above question with confidence. Therefore, one of the main aims of this Chapter is to provide the statistically valid bioaerosol dispersal profiles required in order to answer this question. To achieve this aim, the following Objective was set:

i. Quantitative characterisation of bioaerosols emitted through composting activities and downwind concentrations up to and beyond 250 m from site at chosen case-study sites

Taking into account the above review, as well as Chapter 1, it can be hypothesised that bioaerosols will be emitted in concentrations several orders of magnitude above upwind concentrations during site activities, and will show the ability at times to travel beyond 250 m from site in elevated concentrations.

## 4.2 Experimental Design

Methods used in order to sample on-site and process samples followed the general methodology described in Chapter 3. Sampling at Lount OWC took place from September 2007 until July 2008. Sampling at Flixborough took place from August 2008 until July 2009. A detailed list of sampling dates is provided in Appendix I.

### 4.2.1 Endotoxin Analysis

Alongside culturable microorganisms at Lount OWC, samples were also taken for endotoxin assay (Chapter 3). Access to endotoxin assay methods was achieved through a Natural Environmental Research Council (NERC grant NE/E008534/1) funded project entitled 'Environmental exposure to endotoxin emission from commercial composting activities'; carried out in conjunction with the University of the West of England (UWE), Bristol.

### 4.2.2 Lount OWC

In order to construct a dispersal profile for culturable bioaerosols and endotoxins, extensive site sampling was carried out. The method used to enumerate bioaerosols was based on replicated filtration sampling, followed by culture selecting for *A. fumigatus*, actinomycetes and gram-negative bacteria. Endotoxin samples were collected using the same apparatus, and sent to UWE, Bristol for assay. Sampling apparatus was highly portable, allowing flexibility and mobility within experimental designs. The method used was described in full within Chapter 3. Initially, a test sampling day was formulated, to inform the design of a detailed and achievable experiment. The test day included samples taken at locations upwind, downwind, adjacent to turning and screening, and between two static windrows. Following the test sampling day, three types of experiment were implemented:

1. Baseline – performed on three sampling occasions. This sampling strategy was aimed at providing information regarding site characteristics, for example, concentrations on-site, upwind, and at nearby downwind locations. Specifically:

    a. Upwind 100 m
    b. Adjacent to activity – maximum of three samples performed on each sampling occasion
    c. Between two windrows
    d. Downwind from site boundary 50 m , 100 m and 150 m

2. Transect – performed on three sampling occasions. The aim of transect sampling days was to expand upon the upwind and downwind samples taken as part of the baseline sampling days. A profile of the site to distances beyond the 250 m risk assessment limit would provide data regarding the potential of bioaerosols to disperse beyond this distance, to areas where receptors could potentially be exposed. Specifically:

a. Samples taken every 100 m from 400 m upwind of the centre of the site, to 400 m downwind of the centre of the site

3. Modified transect – performed on two sampling occasions. The modified transect was implemented after baseline and transect sampling days has been completed. The aim of this sampling regime was to build upon interesting results found on previous days, providing validated data where gaps in the sampling profile had been identified, confirming results where unusual findings were reported, and omitting sample locations where results were not contributing to the overall picture of bioaerosol dispersal, such as distances greater than 100 m upwind. Specifically:

    a. Upwind 100 m
    b. Every 100 m from 400 m downwind until 0 m (centre of the site)
    c. Adjacent to activity, maximum of three samples performed on each sampling occasion
    d. Between two windrows

Data gathered through these experimental designs were then aggregated into one large data-set for analysis and presentation of findings. The total numbers of samples taken for each bioaerosol at Lount OWC were: *A. fumigatus,* 411; actinomycetes, 411; gram-negative bacteria, 348; and, endotoxins, 177. Figure 4.1 below provides a generalised diagram of sampling strategies.

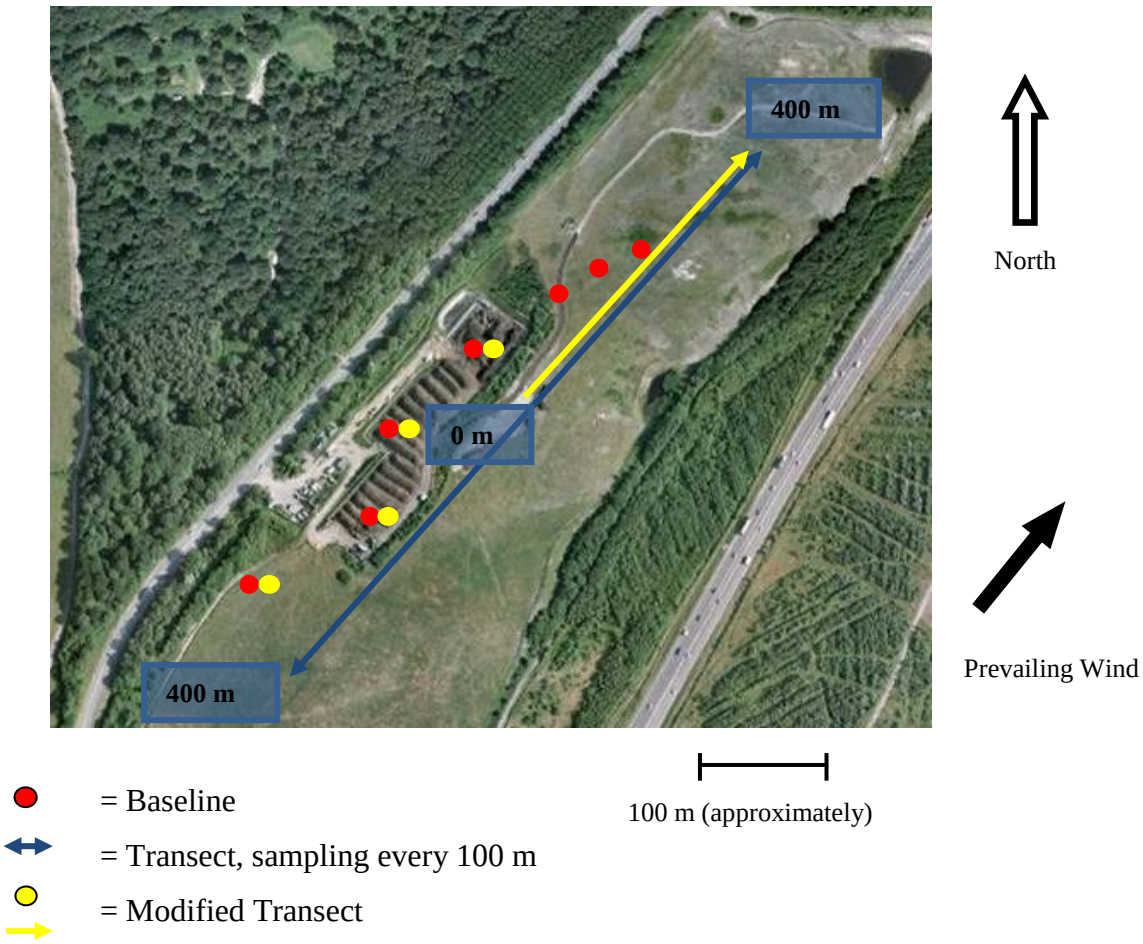



Figure 4.1: Aerial image of Lount OWC (Infoterra Ltd and Bluesky and Tele Atlas, 2009) with generalised sampling location for each experimental protocol detailed in Section 4.2.2. Upwind and downwind sampling directions determined through assessment of wind direction on each sampling day.

### 4.2.3 Flixborough

Site characterisation at Flixborough was achieved through the application of two types of experiment, designed based on the experience gained from Lount OWC sampling. Once again, the methods used were based on filtration followed by culture or endotoxin assay, and are described in full within Chapter 3. Two sampling strategies were implemented:

1. Baseline:
    a. Upwind 100 m
    b. Adjacent to activity × 3 (maximum)
    c. Between two windrows
    d. Downwind from source × 3 (maximum)

2. Downwind transect:
    a. Upwind 100 m
    b. Adjacent to activity
    c. Downwind from source, every 100 m until 600 m

Data gathered through these experimental designs were then aggregated into one large data-set for analysis and presentation of findings. The total numbers of samples taken for each bioaerosol at Flixborough were: *A. fumigatus*, 391; actinomycetes, 391; gram-negative bacteria, 391; and, endotoxins, 89. Figure 4.2 below provides a generalised diagram of sampling strategies.

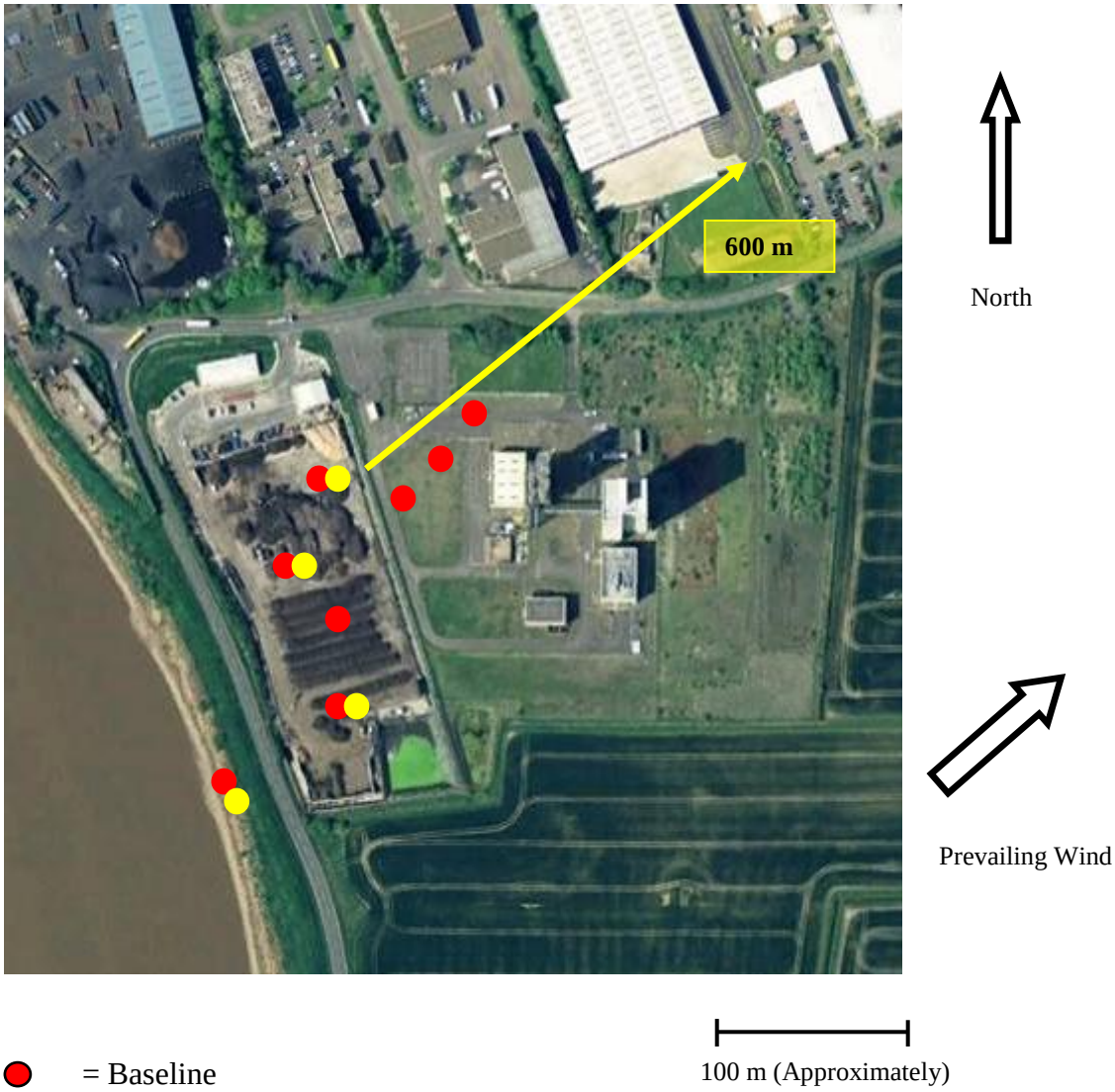



Figure 4.2: Aerial image of Flixborough (Infoterra Ltd and Bluesky and Tele Atlas, 2009) with generalised sampling location for each experimental protocol detailed in Section 4.2.3. Upwind and downwind sampling directions determined through assessment of wind direction on each sampling day.

## 4.3 Results

Dispersal profiles are presented for all bioaerosols measured within this section. Wherever 'significance' or 'statistical similarity/difference' is referred to, p-values are less than 0.05; indicating that the chance that the null hypothesis (i.e., that there is no relationship between the parameters being examined) is true is less than 5%, or less than one in twenty. For example, if a correlation between two samples has a p-value of < 0.05 this means there is less than a 5% chance that the two samples not correlated (Dytham, 1999). In-figure statistical similarity/difference data generated through the Fisher LSD test, where sampling locations are compared in pairs. Statistical similarity/difference (p = < 0.05) is presented for each individual sampling location compared to all other sampling locations. Details of the number of samples taken per location can be found in Appendix I.

### 4.3.1 *Aspergillus fumigatus*

Figures 4.3 and 4.4 illustrate the dispersal pattern of *A. fumigatus* at both Lount OWC and Flixborough. Both figures show geometric mean concentrations, with a scaled x-axis allowing analysis of dispersal patterns in direct comparison to geographic features (according to the prevailing wind direction). As anticipated, some of the highest concentrations were returned on-site, adjacent to composting activities. At this sampling location the geometric mean returned values of approximately $6.2 \times 10^3$ CFU $m^{-3}$ at Lount OWC, and $3.7 \times 10^3$ CFU $m^{-3}$ at Flixborough. However, at Flixborough the highest geometric mean concentrations were found at site boundary, where they reached $9.3 \times 10^3$ CFU $m^{-3}$.

Figure 4.5 (a, b) illustrates log-transformed (Ln) mean, inter-quartile range, outlying and extreme values for *A. fumigatus* at both sites. The data shows how median values at both sites are typically 6.63 natural log (Ln) units, therefore below the lower limit of detection (757 CFU $m^{-3}$). Highest mean and inter-quartile range values are returned from site activity and site boundary locations. For Lount OWC median values at site activity reached approximately $4.9 \times 10^3$ CFU $m^{-3}$ (8.5 Ln), and ranged from < 757

CFU $m^{-3}$ to $2.4 \times 10^5$ CFU $m^{-3}$ (6.63 to 12.4 Ln). At Flixborough highest median values were found at site boundary, however, the range at site activity was higher. At both sites minimum values measured at site activity were below detection limits (BDL), with values ranging up to $2.2 \times 10^5$ CFU $m^{-3}$ at Lount OWC and $2.9 \times 10^5$ CFU $m^{-3}$ at Flixborough (Appendix I, Tables 1.1, 1.2).

Figure 4.3 shows how Lount OWC concentrations at site activity are statistically different from any other sampling location, despite the range shown by Figure 4.5a. At Flixborough (Figure 4.4), concentrations at site activity and site boundary are statistically similar, but are different from all other sampling locations. During periods of no activity on site, concentrations at Lount remained significantly higher than upwind (Figure 4.3), while at Flixborough concentrations fell to under 1000 CFU $m^{-3}$, and were statistically similar to upwind. At Lount OWC mean concentrations at downwind 50 m and 150 m were above those found upwind. By 80 m downwind concentrations were statistically similar to those upwind; this was followed by an increase to levels statistically different from upwind at 150 m downwind. Following this secondary peak, concentrations once again fell to levels statistically similar to upwind. At Flixborough (Figure 4.4), no values from 100 m - 600 m downwind were significantly higher than upwind, despite mean concentrations being higher. At times maximum concentrations upwind reached $4.1 \times 10^4$ CFU $m^{-3}$ at Lount OWC, and $1.5 \times 10^3$ CFU $m^{-3}$ at Flixborough. Maximum concentrations at 50 m, 100 m, 200 m, and 600 m could be found 1 order of magnitude higher than those upwind at Flixborough, with downwind 100 m 1 order of magnitude higher at Lount OWC (Appendix I, Tables 1.1, 1.2). Figure 4.5 (a, b) shows how median and inter-quartile range values follow the patterns illustrated in Figures 4.1 and 4.2. However, at downwind locations outlying and extreme values that exceed inter-quartile range by up to $9.7 \times 10^4$ CFU $m^{-3}$ (4 Ln units) could be found. Concentrations of *A. fumigatus* were often not detectable, illustrated by median values in Figure 4.5, along with modal values and frequency of mode (Appendix I, Table 1.1, 1.2).

While concentrations downwind are not significantly different from those upwind at both sites (Figures 4.3, 4.4), and many samples taken were BDL, some samples taken at

distances over 250 m from site were above the Environment Agency recommended concentration for total fungi of 1000 CFU $m^{-3}$ (Environment Agency, 2001b; Environment Agency, 2009b). At Lount OWC approximately 20% of samples taken at distances at or over 250 m from site were above 1000 CFU $m^{-3}$, while at Flixborough approximately 5% of samples taken over 250 m from site were over this threshold. It is likely that a new recommended limit of 500 CFU $m^{-3}$ for *A. fumigatus* will be introduced in the near future (Dennis, 2009). However, as the LLOD of this method was 757 CFU $m^{-3}$, analysis of excursions above the new *A. fumigatus* reference levels was carried out using detection limits. At Lount OWC approximately 30% of samples and at Flixborough approximately 14% of samples taken at and beyond 250 m from site, were above detection limits.

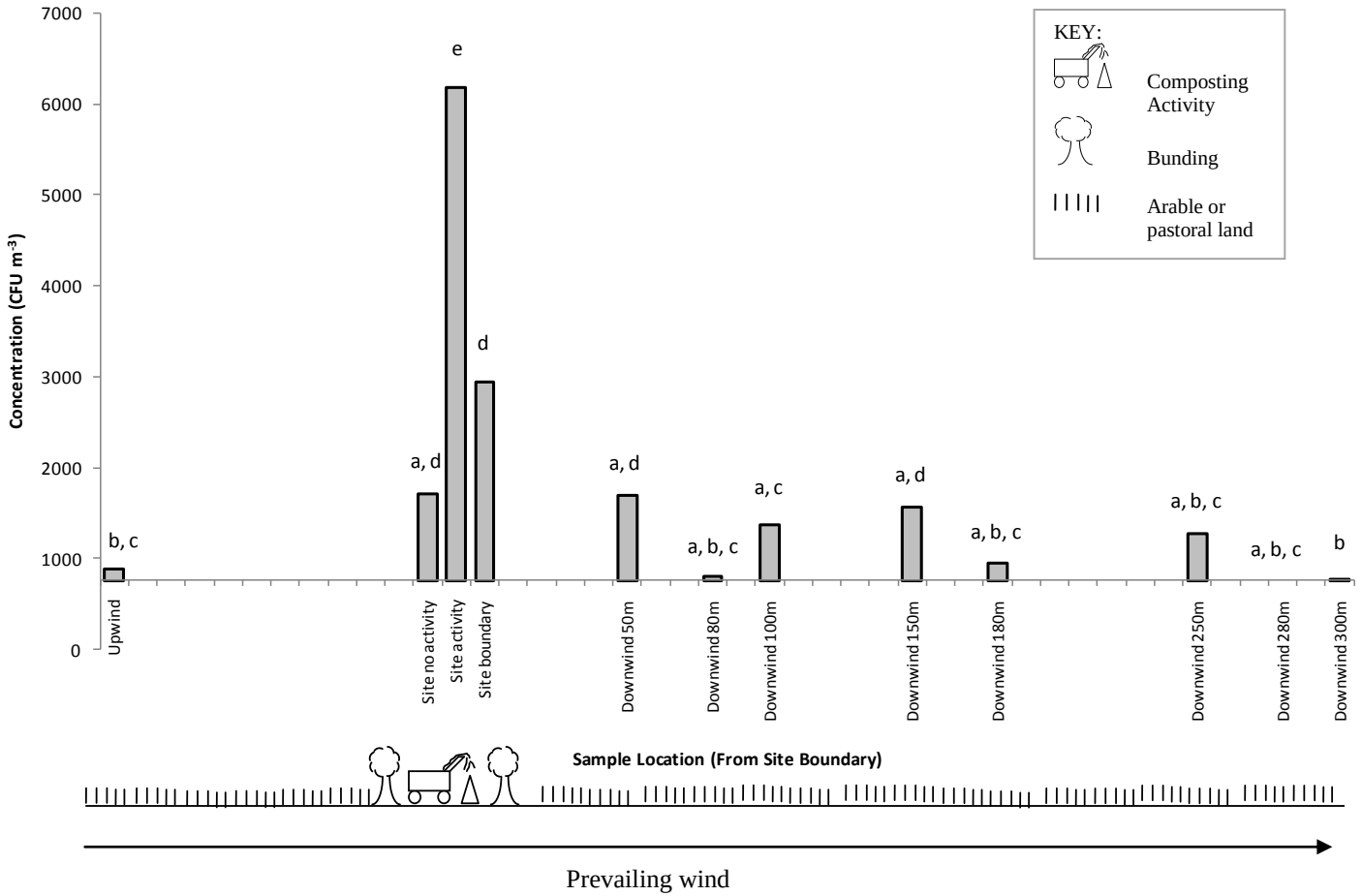



Figure 4.3: *A. fumigatus* dispersal at Lount OWC plotted on scaled x-axis. Bar represents geometric mean of data calculated through least square mean, labels represent statistical similarity ($p = < 0.05$) generated through pairwise comparisons. X-axis crosses at lower limit of detection (757 CFU $m^{-3}$).

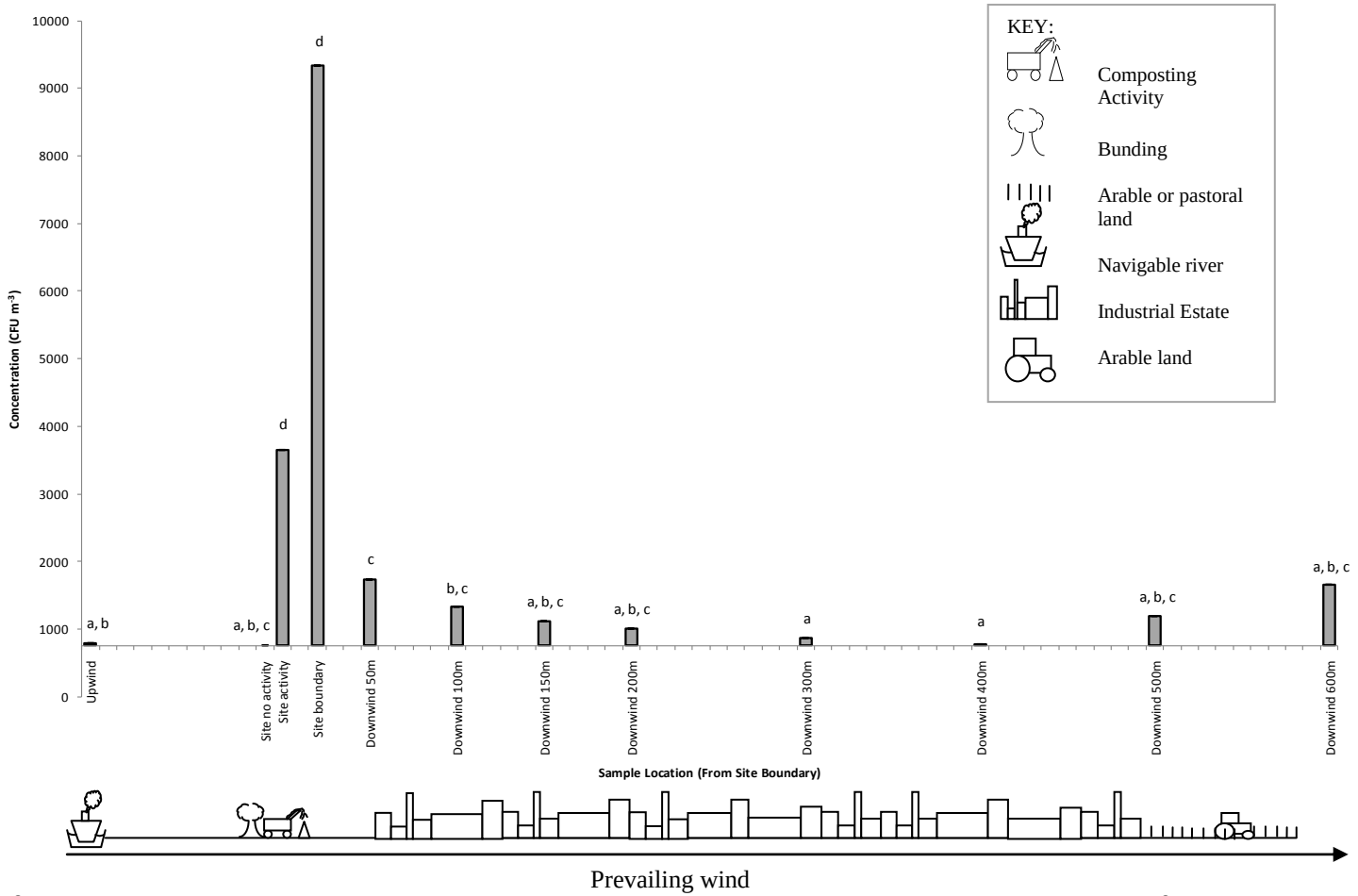
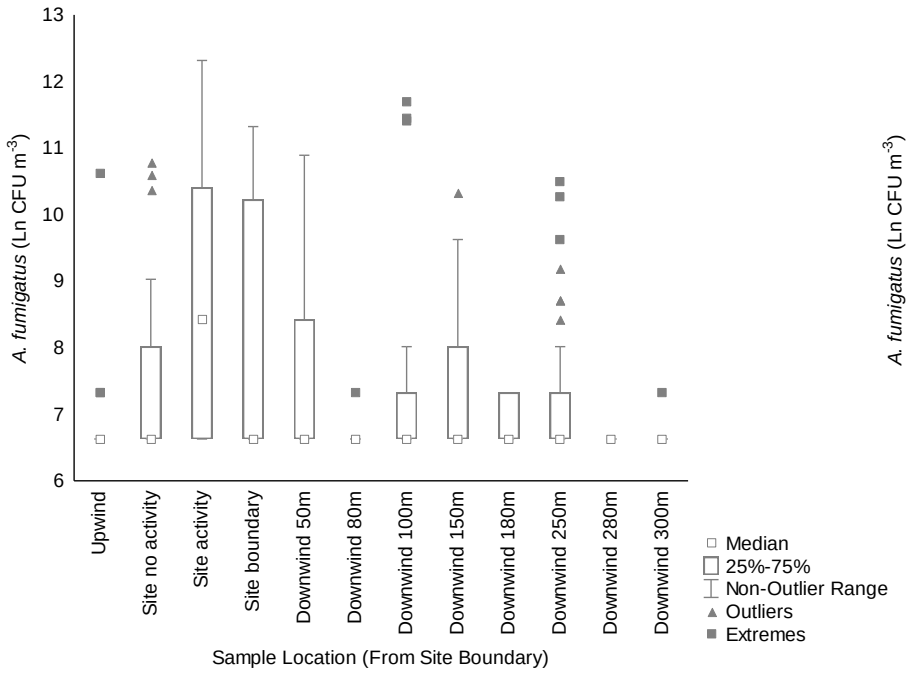
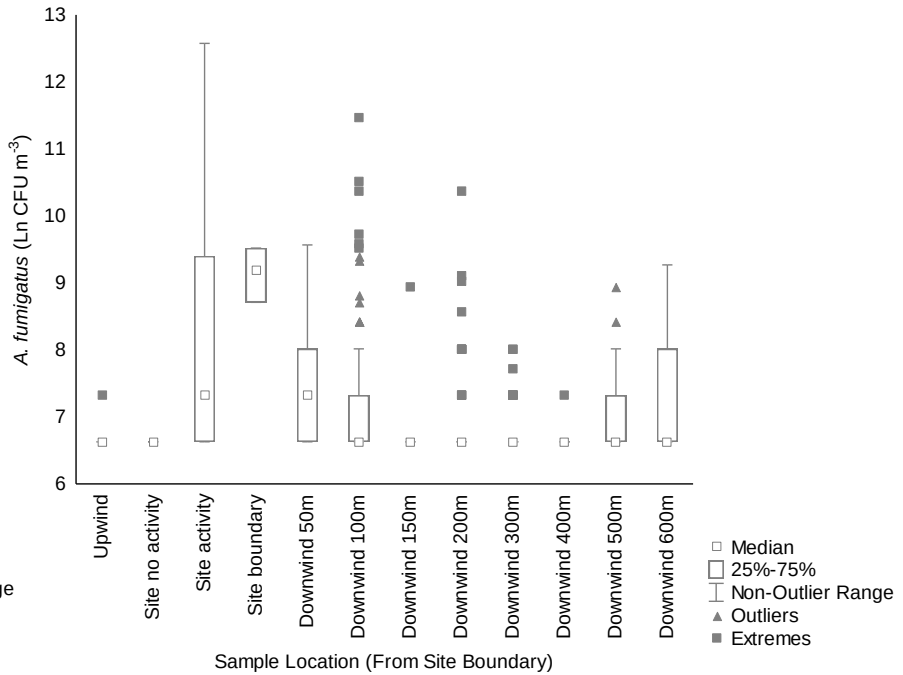



Figure 4.4 *A. fumigatus* dispersal at Flixborough plotted on scaled x-axis. Bar represents geometric mean of data calculated through least square mean, labels represent statistical similarity (p = < 0.05) generated through pairwise comparisons. X-axis crosses at lower limit of detection (757 CFU m-3).




(a) Lount OWC




(b) Flixborough

Figure 4.5: Box and Whisker plots for *A. fumigatus* presented in natural log values at Lount OWC and Flixborough. Lower limit of detection is 6.63 natural log units.

### 4.3.2 Actinomycetes

Actinomycete dispersal patterns show some similarity to *A. fumigatus*, with composting activities resulting in significant emissions of bioaerosols. One significant difference, however, is the frequency of detectable concentrations, which is higher for actinomycetes (Figure 4.8; Appendix I, Tables 2.1, 2.2). Highest geometric mean concentrations reach $5.5 \times 10^4$ CFU $m^{-3}$ at Lount OWC site activity (Figure 4.6), and $3.2 \times 10^6$ CFU $m^{-3}$ at Flixborough site boundary (Figure 4.7). These patterns are also reflected through median and range values (Figure 4.8). Concentrations adjacent to composting activities at both sites are significantly different from any other sampling location, with site boundary concentrations at Flixborough also statistically different from all other sampling locations, and significantly higher than those at site activity (Figures 4.6, 4.7). At Flixborough, while both highest geometric and arithmetic mean values can be found at site boundary, highest maximum concentrations and inter-quartile range was found adjacent to activities, reaching $1.1 \times 10^7$ CFU $m^{-3}$ (Figures 4.7, 4.8; Appendix I, Tables 2.1, 2.2).

Downwind from Lount OWC, concentrations at 50 m, 80 m and 100 m are similar to those found upwind, with a secondary peak at 150 m showing a geometric mean statistically higher than upwind. Beyond this, concentrations reduce to levels statistically similar to upwind, and at 300 m downwind mean concentrations are significantly lower than those found upwind (Figure 4.6). The large inter-quartile and non-outlier ranges shown in Figure 4.8 suggests that variation in actinomycete concentrations remains even across the dispersal profile at Lount OWC, although median values support the dispersal pattern shown in Figure 4.6. This is further supported by upwind concentrations, with an arithmetic mean of $2.5 \times 10^4$ CFU $m^{-3}$, the same order of magnitude or higher than arithmetic means at all other sampling locations. Dispersal patterns at Flixborough are more distinct. Concentrations are similar to those found upwind at 50 m, then significantly higher at 100 m, 150 m and 200 m downwind (Figure 4.5). By 300 m downwind concentrations have reduced to levels similar to those found upwind. At both sites geometric and arithmetic mean actinomycete concentrations (Figures 4.6, 4.7; Appendix I, Tables 2.1, 2.2) were above

1000 CFU $m^{-3}$ at all sampling locations. Approximately 52% of samples taken at or above 250 m from site at Lount OWC were above 1000 CFU $m^{-3}$, with 37% of samples at Flixborough found above this threshold.

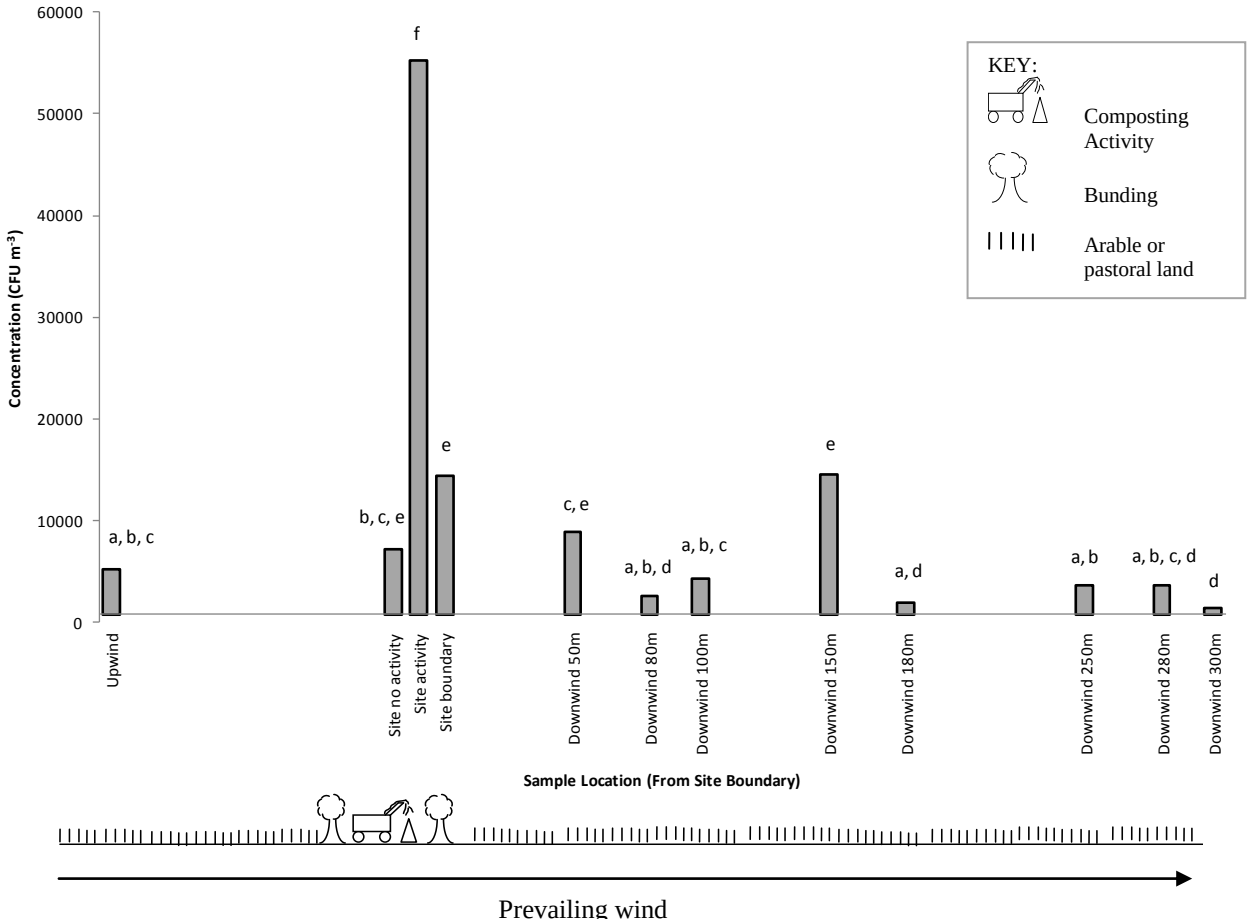



Figure 4.6 Actinomycete dispersal at Lount OWC plotted on scaled x-axis. Bar represents geometric mean of data calculated through least square mean, labels represent statistical similarity ($p = < 0.05$) generated through pairwise comparisons. X-axis crosses at lower limit of detection (757 CFU $m^{-3}$).

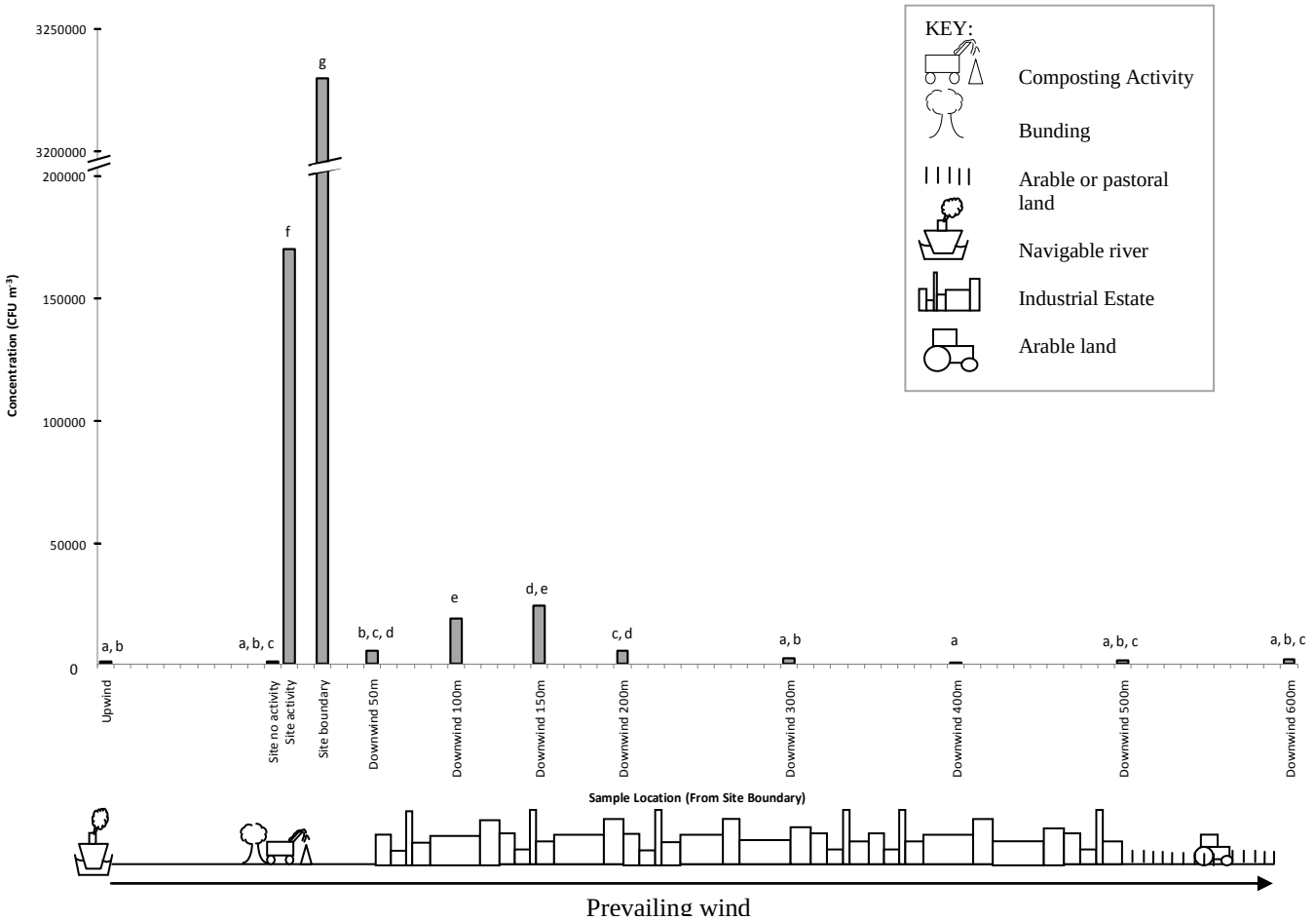
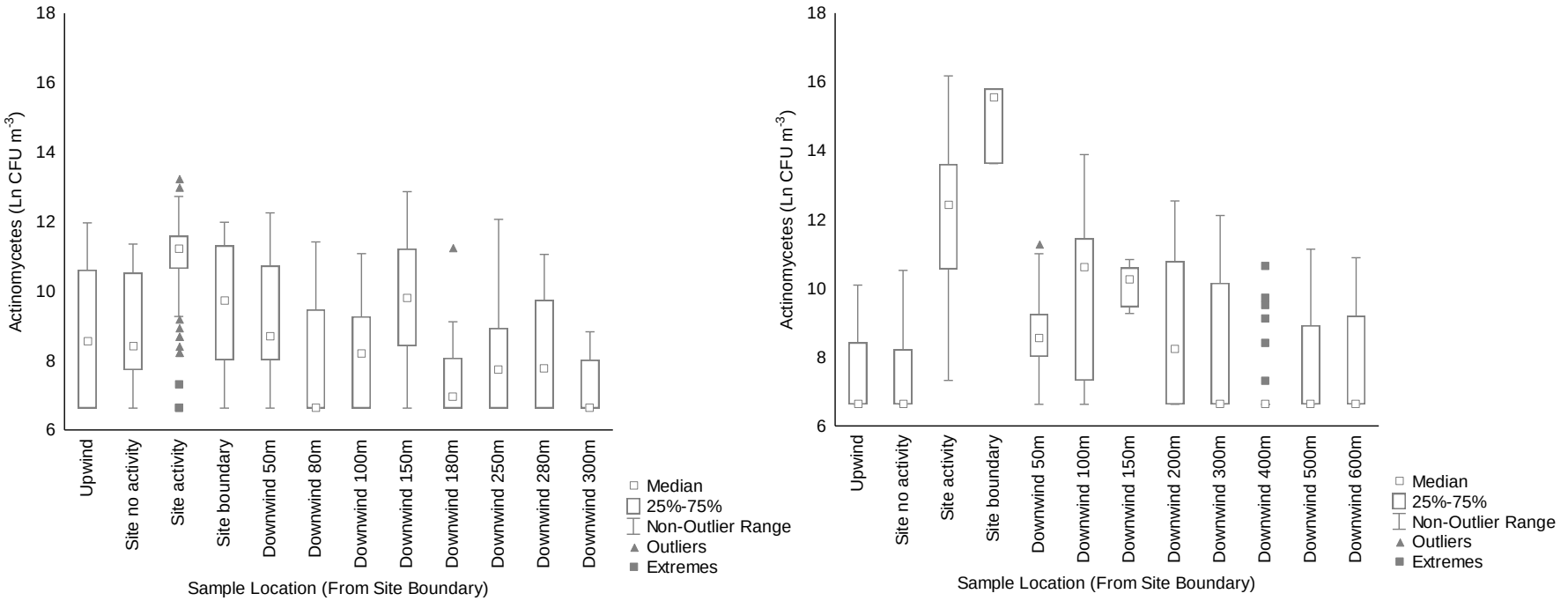



Figure 4.7: Actinomycete dispersal at Flixborough plotted on scaled x-axis. Bar represents geometric mean of data calculated through least square mean, labels represent statistical similarity ($p = < 0.05$) generated through pairwise comparisons. X-axis crosses at lower limit of detection (757 CFU $m^{-3}$).




(a) Lount OWC (b) Flixborough

Figure 4.8: Box and Whisker plots for actinomycetes presented in natural log values at Lount OWC and Flixborough. Lower limit of detection is 6.63 natural log units.

### 4.3.3 Gram-negative Bacteria

A similar pattern of dispersal emerged for gram-negative bacteria. At Lount OWC (Figure 4.9) highest geometric means can be found at site activity, reaching $1.6 \times 10^4$ CFU $m^{-3}$. This is reflected in Figure 4.11a, where median values are highest at site activity, with non-outlier range at this location reaching over $1.2 \times 10^6$ CFU $m^{-3}$ (14 Ln). Figure 4.9 illustrates how concentrations at site activity are similar to those at site boundary, but are statistically different from all other sampling locations. At Flixborough (Figure 4.10) the highest geometric mean values of $7.8 \times 10^5$ CFU $m^{-3}$ were found at site boundary. However, analysis of median and non-outlier range (Figure 4.11b) shows how although median concentrations are lower at site activity, non-outlier range reaches over $8.9 \times 10^6$ CFU $m^{-3}$ (16 Ln) units at this location, with no higher than $1.2 \times 10^6$ CFU $m^{-3}$ (14 Ln) being found at site boundary. Statistical similarity reflects this (Figure 4.10), with concentrations at site boundary statistically similar to those found on-site during periods of activity, and at 150 m downwind. Once again, the range of values gained is high, with the largest range shown at Flixborough site activity (Figure 4.11b), where a minimum value close to the lower confidence limit was found while maximum values were measured at $1.1 \times 10^7$ CFU $m^{-3}$ (Appendix I, Tables 3.1, 3.2).

A similar pattern of dispersal to actinomycetes has also been recorded, with a secondary peak found at 100 - 150 m downwind through analysis of both geometric and arithmetic mean values. Once again, concentrations on-site, at site boundary, and at 50 m downwind are significantly higher than those upwind. At Lount OWC (Figure 4.9) geometric mean reached $8 \times 10^3$ CFU $m^{-3}$ at the secondary peak, while arithmetic mean reached $4.2 \times 10^4$ CFU $m^{-3}$; 1 order of magnitude higher than those found at 80 m and 100 m downwind (Appendix I, Table 3.1). At Lount OWC range at most locations was also high (Figure 4.11a), up to approximately $1.8 \times 10^6$ CFU $m^{-3}$ (6 Ln) units, with median values reflecting the patterns shown in Figure 4.9. At Flixborough (Figures 4.10, 4.11a), beyond the secondary peak concentrations do not return to levels similar to upwind until 400 m downwind. Furthermore, at 600 m downwind geometric mean is $2.4 \times 10^3$ CFU $m^{-3}$, statistically similar to upwind, site no activity, downwind 50 m, 200 m,

300 m, 400 m, and 500 m. Arithmetic mean values suggest that upwind concentrations are not achieved at any other sampling location, with downwind locations 1 – 2 orders of magnitude higher than upwind. The high range of gram-negative bacteria concentrations at all sampling locations may contribute to these results (Figure 4.11b; Appendix I, Table 3.2).

In terms of the 300 CFU $m^{-3}$ Environment Agency limit (Environment Agency, 2001b), as detection limit for the sampling method used here is 757 CFU $m^{-3}$ analysis of excursions above thresholds is carried out using samples that were above detection limits, and therefore above 300 CFU $m^{-3}$. At Lount OWC 37% of samples taken at or over 250 m from site were found above detection limits, while at Flixborough 44% of samples were above detection limits at distances over 250 m from site.

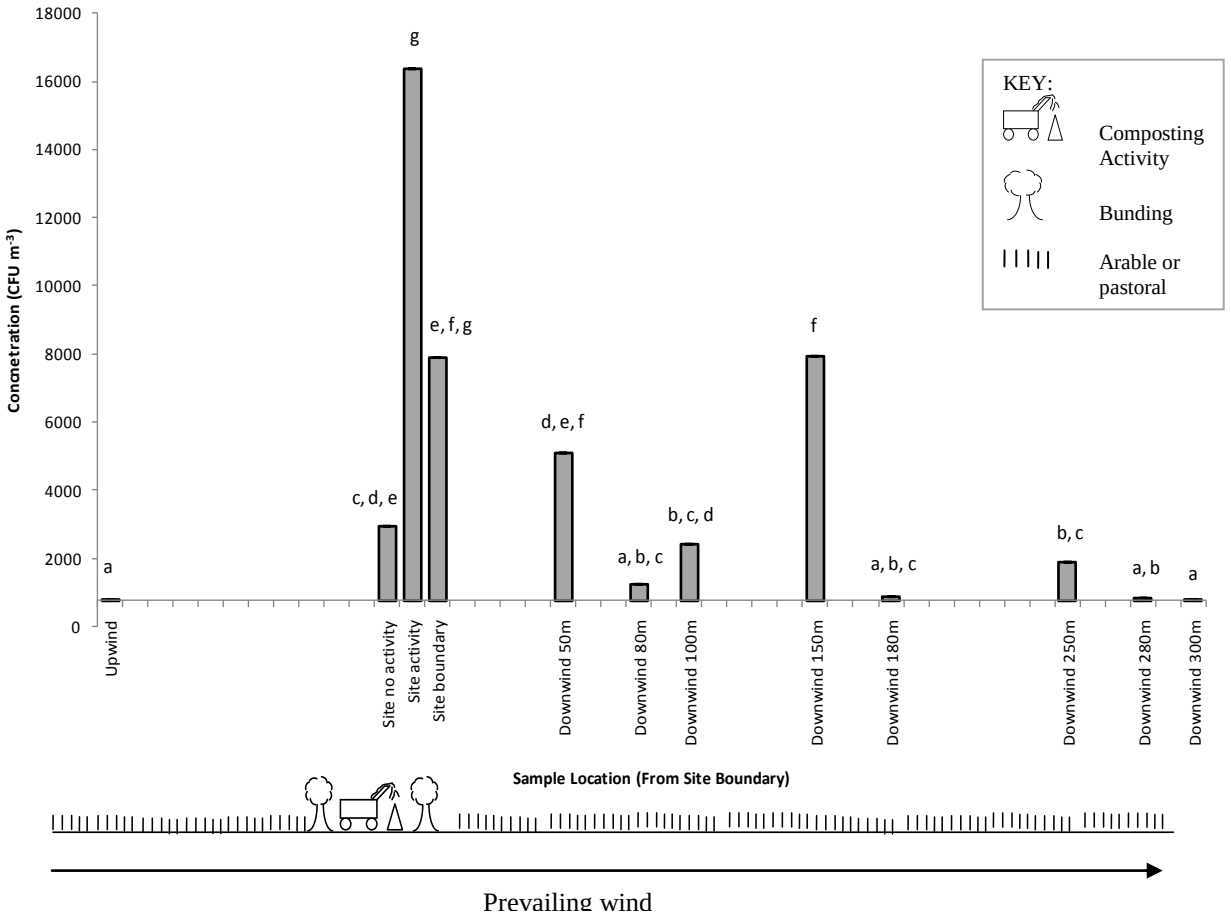



Figure 4.9 Gram-negative bacteria dispersal at Lount plotted on scaled x-axis. Bar represents geometric mean of data calculated through least square mean, labels represent statistical similarity ($p = < 0.05$) generated through pairwise comparisons. X-axis crosses at lower limit of detection (757 CFU $m^{-3}$).

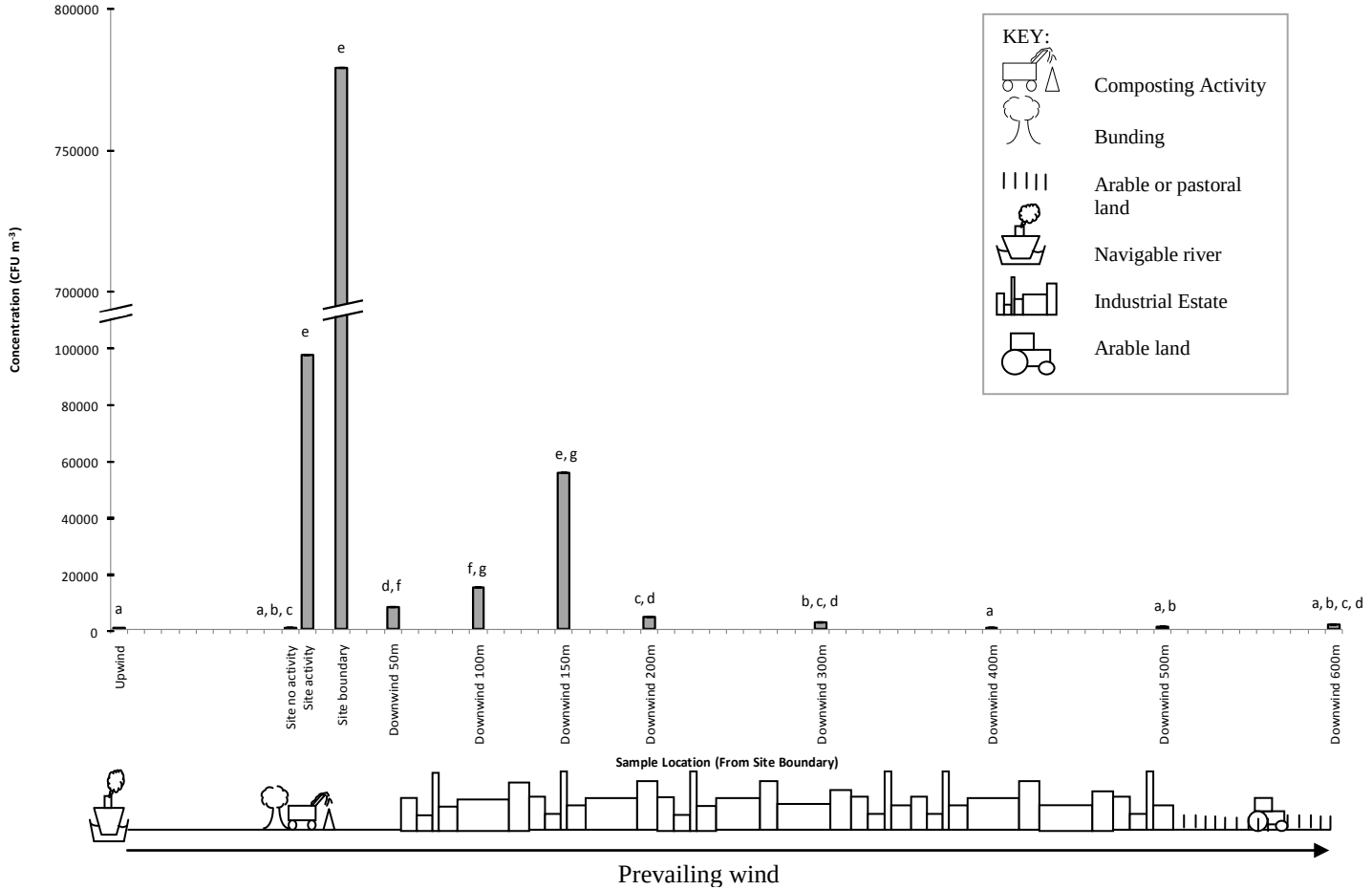
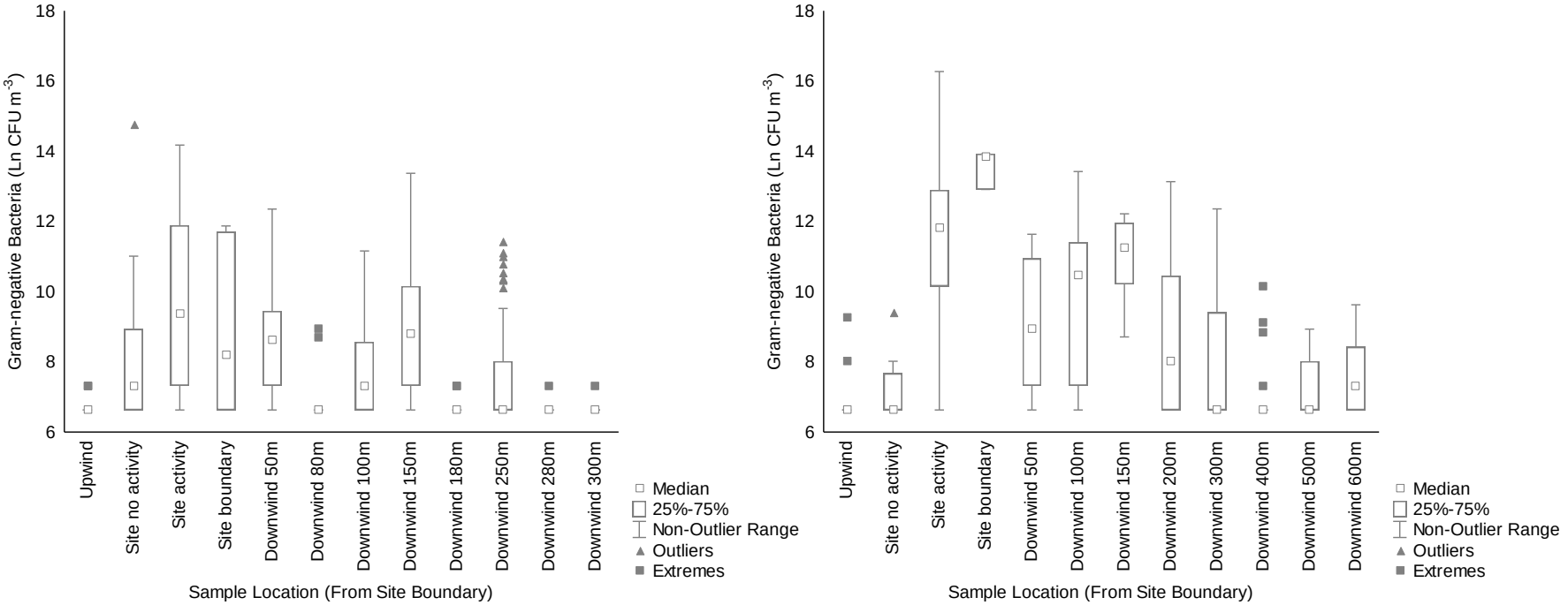



Figure 4.10: Gram-negative bacteria dispersal at Flixborough plotted on scaled x-axis. Bar represents geometric mean of data calculated through least square mean, labels represent statistical similarity ($p = < 0.05$) generated through pairwise comparisons. X-axis crosses at lower limit of detection (757 CFU $m^{-3}$).




(a) Lount OWC (b) Flixborough

Figure 4.11: Box and Whisker plots for gram-negative bacteria presented in natural log values at Lount OWC and Flixborough. Lower limit of detection is 6.63 natural log units.

### 4.3.4 Endotoxins

The trend of high on-site releases is also found for endotoxins. At Lount OWC the highest geometric mean concentrations (Figure 4.12) can be found at site activity, followed by site no activity. At these locations geometric mean concentrations reach 2.3 and 1.4 EU $m^{-3}$. At Flixborough (Figure 4.13) the highest concentrations can be found at site boundary, with a geometric mean of approximately $1.7 \times 10^2$ EU $m^{-3}$ and arithmetic mean of $1.3 \times 10^3$ EU $m^{-3}$ (Appendix I, Table 4.1). These values are reflected in the medians shown in Figure 4.14 (a, b), although median values at Flixborough are similar at site activity and site boundary. Inter-quartile and non-outlier range values at Lount OWC are similar at site activity and site boundary locations, reaching approximately 20 EU $m^{-3}$ (4 Ln).

Dispersal patterns at each site show some differences. At Lount OWC (Figure 4.12), geometric means suggest that concentrations decline between site and 80 m downwind, followed by a second peak at 100 m; in a similar fashion to dispersal patterns shown by actinomycetes and gram-negative bacteria. Concentrations at site activity are statistically different from those at site boundary, 50 m and 80 m downwind; and are statistically similar to concentrations at 100 m and 150 m downwind. At Lount OWC endotoxin concentrations show a third peak at 280 m downwind, where concentrations reach 1.3 EU $m^{-3}$ and are statistically similar to those found adjacent to composting activities. Figure 4.14a illustrates this pattern further, showing how median values reflect geometric means. Arithmetic mean concentrations, however, show some differences, with downwind 180 m returning concentrations 2 order of magnitude higher than any other sampling location. This pattern of dispersal is less evident at Flixborough, with median and inter-quartile ranges in Figure 4.14b showing less variation in concentrations from 200 – 600 m downwind. Concentrations adjacent to site activity and at site boundary are statistically similar to each other. However, by 100 m downwind concentrations are all statistically similar to upwind (Figure 4.13).

As there is no set threshold value for occupational or residential endotoxin concentrations, it is not possible to analyse whether the values presented here are

significant in terms of a threshold level. It is possible, however, to compare downwind concentrations to upwind in order to assess the impact of endotoxin release from composting on the ambient environment. At Lount OWC endotoxins were below detection limits upwind (Figure 4.12; Appendix I, Table 4.1), meaning that at no point downwind were upwind concentrations achieved. At Flixborough, arithmetic mean concentrations can be found 1 order of magnitude above upwind concentrations at downwind 50 m, 100 m, 200 m, 300 m, 400 m, 500 m and 600 m, although geometric mean concentrations show concentrations within the same order of magnitude as upwind at downwind 150 m, then from 300 – 600 m downwind (Appendix I, Table 4.2).

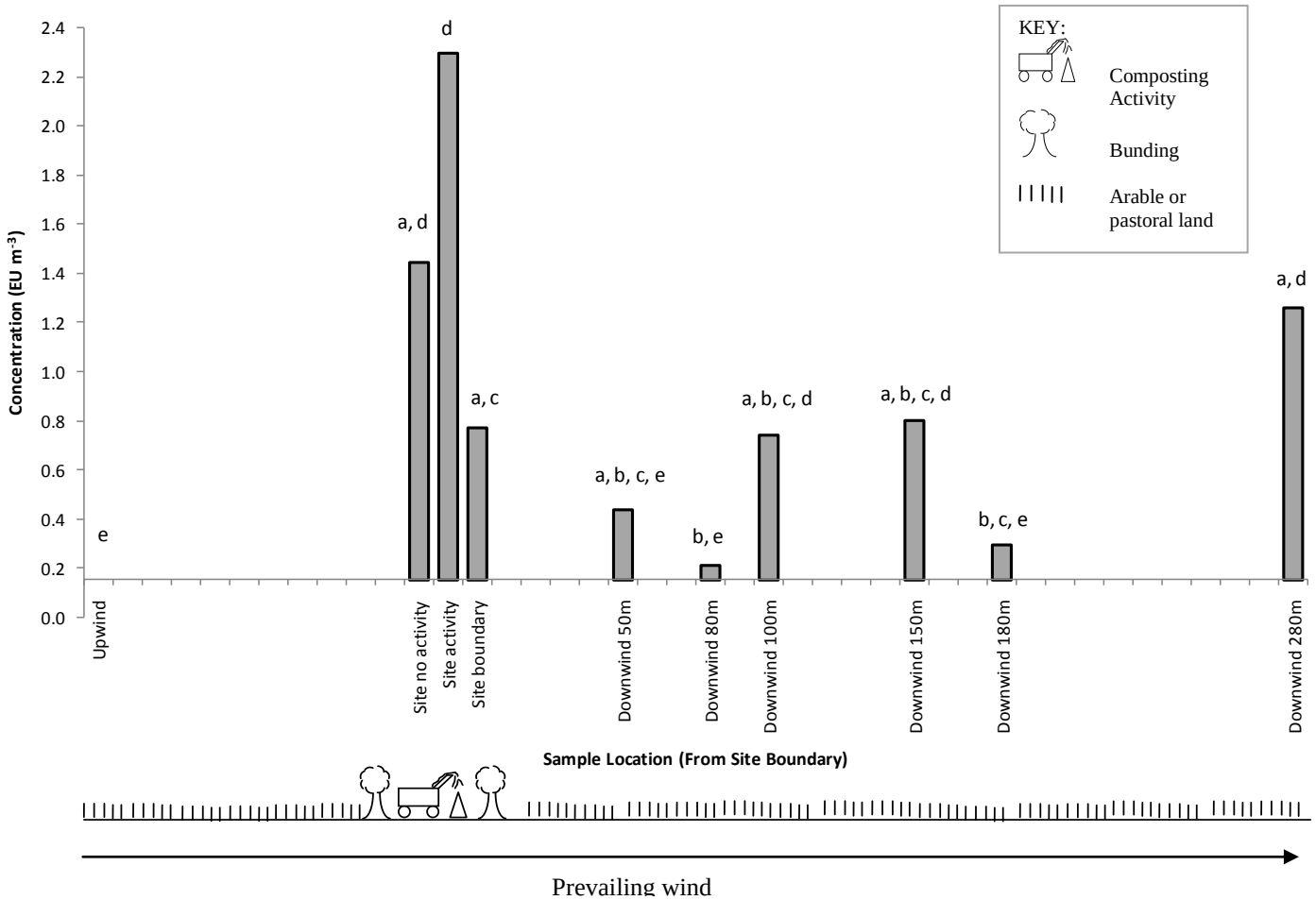



Figure 4.12 Endotoxin dispersal at Lount OWC plotted on scaled x-axis. Bar represents geometric mean of data calculated through least square mean, labels represent statistical similarity ($p = < 0.05$) generated through pairwise comparisons. X-axis crosses at lower limit of detection (0.152 EU $m^{-3}$).

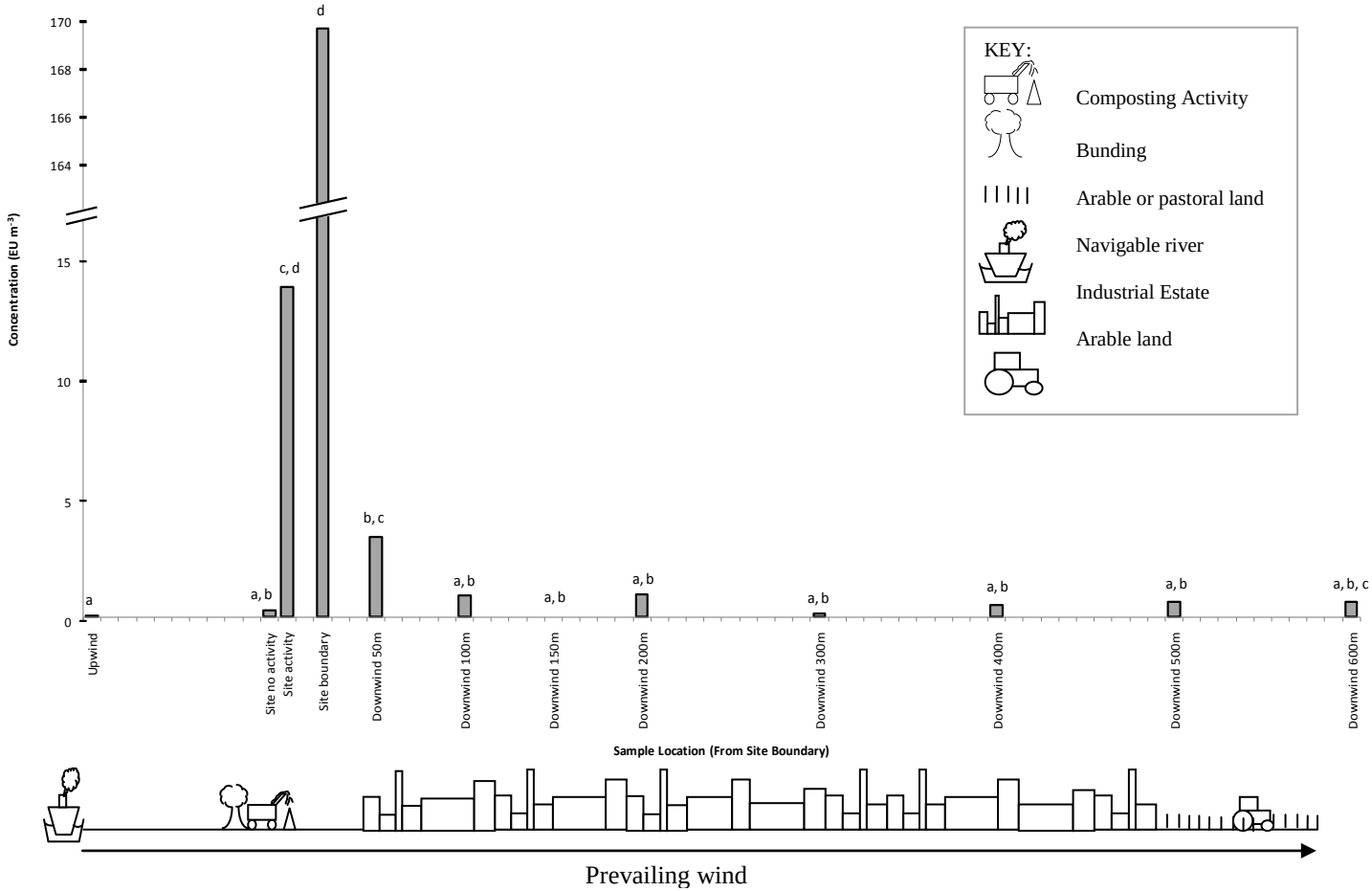
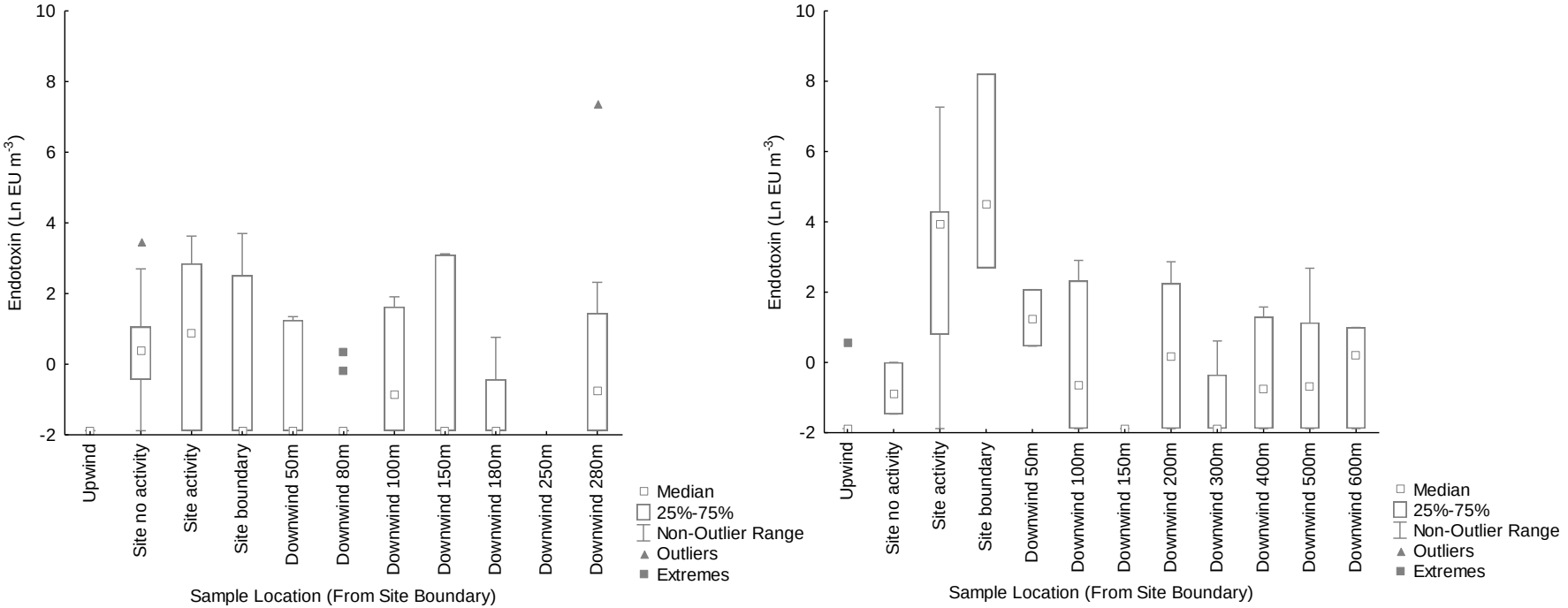



Figure 4.13: Endotoxin dispersal at Flixborough plotted on scaled x-axis. Bar represents geometric mean of data calculated through least square mean, labels represent statistical similarity ($p = < 0.05$) gerenated through pairwise comparisons. X-axis crosses at lower limit of detection (0.152 EU $m^{-3}$).




(a) Lount OWC (b) Flixborough

Figure 4.14: Box and Whisker plots for endotoxins presented in natural log values at Lount OWC and Flixborough. Lower limit of detection is -1.88 log units.

## 4.4 Discussion

The introduction to this chapter described how several Objectives aimed at addressing gaps in literature surrounding bioaerosol emission and dispersal were to be met through the construction of dispersal profiles for each bioaerosol. In particular, the Objective to be met was:

i. Quantitative characterisation of bioaerosols emitted through composting activities and downwind concentrations up to and beyond 250 m from site at chosen case-study sites

Through striving to achieve this Objective, a large dataset describing the release and dispersal of bioaerosols, has been created. While many findings are in agreement with those found by other researchers, some other, novel findings are also reported. This section analyses and evaluates the results described in section 4.3 above.

Concentrations of all bioaerosols were found to be highest on-site, or at site boundary. This result was anticipated, as many past studies have identified that composting activities result in higher releases of bioaerosols (Clark *et al*, 1983; Taha *et al*, 2006; Taha *et al*, 2007). While concentrations at Lount were highest at site activity, site boundary returned the highest values at Flixborough. Figures depicting dispersal at Flixborough (4.6, 4.7, 4.10, 4.13) illustrate how samples taken on-site and at site boundary sampling locations were relatively close together in terms of location. Indeed, these locations may be adjacent to each other as activity was often carried out close to site boundary (Chapter 2; Figure 2.8). Therefore, it is likely that site boundary locations are strongly affected by activities on-site. The Flixborough site boundary is largely un-bounded by tree or earthen bunding, unlike Lount OWC (Chapter 2), which may allow increased concentrations to be found at Flixborough site boundary. The presence of a tree-line at the site boundary of Lount OWC may reduce concentrations of bioaerosols, either due to increased deposition through turbulence (Seinfeld and Pandis, 2006), or through the forced passage of air above the barrier, moving bioaerosols above the sampling point. In addition, Tables 1.2, 2.2, 3.2, and 4.2 (Appendix I) show how site

boundary statistics are based on only 3 replicates, whereas site activity is based on 82 replicates; resulting in a more accurate measure of variance and mean values at site activity. For culturable bioaerosols, maximum concentrations on-site were all within previously published ranges. *A. fumigatus* maximum concentrations found at either site were $10^4$ CFU $m^{-3}$ (Clark *et al*, 1983; Taha *et al*, 2006; Schlosser *et al*, 2009; Sykes *et al*, 2007); actinomycetes up to $10^4$ CFU $m^{-3}$ at Lount OWC, and $10^6$ CFU $m^{-3}$ at Flixborough (Environment Agency, 2001a; Lacey 1997; Schlosser *et al*, 2009; Sykes *et al*, 2007; Taha *et al*, 2004; Taha *et al*, 2006;); gram-negative bacteria were found up to $10^5$ and $10^6$ CFU $m^{-3}$ at Lount OWC and Flixborough respectively. While these gram-negative bacteria concentrations are in agreement with some studies (Environment Agency, 2001a), Schlosser *et al.* (2009) reported mean gram-negative bacteria up to $10^8$ CFU $m^{-3}$, a value 2 orders of magnitude higher than maximum found here. This may be a reflection of sampling method, with Schlosser *et al.* (2009) employing a method based on liquid impingement, which may improve yield (Chapter 1). In addition, sampling sites used by Schlosser *et al*. (2009) were not directly comparable, with emissions at in-vessel composting facilities enumerated alongside open-air facilities.

It has been suggested that background concentrations of endotoxins are below 10 EU $m^{-3}$ (Liebers *et al*, 2008), if this is the case all geometric means values at Lount OWC, and all except site activity and site boundary locations at Flixborough are below background concentrations. However, here, the low upwind concentrations (< 1 EU $m^{-3}$) meant that a significant impact on concentrations was found on-site and at some locations downwind, even when concentrations remained below 10 EU $m^{-3}$ (Figure 4.12). Arithmetic mean values found by Tolvanen *et al.* (2005), in a drum composting plant were $2.3 \times 10^3$ EU $m^{-3}$; values ranging from 8 to $30 \times 10^2$ EU $m^{-3}$ have also been reported (Liebers *et al*, 2008; Bünger *et al*, 2007). Both of these reports concur with concentrations found, which reached $3.6 \times 10^3$ EU $m^{-3}$ on-site (Appendix I, Table 4.2). Others, however, have reported higher concentrations. Wouters *et al.* (2006) found compost workers were exposed to geometric means reaching $10^2$ EU $m^{-3}$, with a maximum exposure of $3.7 \times 10^4$ EU $m^{-3}$ reported. Schlosser *et al.* (2009) found maximum concentrations on-site reached $1.5 \times 10^6$ EU $m^{-3}$, with median values of $1.6 \times 10^4$ EU $m^{-3}$. However, these values are also in excess of the others reported (Bünger *et*

*al*, 2007; Liebers *et al*, 2008; Tolvanen *et al*, 2005). Once again, these differences may be due to sampling methods and differences in composting facilities studied. As well as the use of in-vessel composting facilities reducing comparability, the sampling methods used by Schlosser *et al.* (2009) reportedly capture 10 – 100 times more endotoxins than filtration based methods.

As anticipated, compelling evidence that bioaerosol emissions are significantly affected by on-site activities was found. For example, *A. fumigatus* geometric mean concentrations on-site were 70% lower during periods of no activity than concentrations during activity at Lount OWC, and 80% lower at Flixborough; although Schlosser *et al.* (2009) found concentrations were 1 – 2.5 orders of magnitude lower on-site away from processing activities, and Taha *et al.* (2006) found increases of 2 – 3 orders of magnitude adjacent to activity. For actinomycetes geometric mean concentrations on site during periods of no activity were 1 order of magnitude lower than during activity at Lount OWC (Figure 4.6) and 2 orders of magnitude lower at Flixborough (Figure 4.7). This is in agreement with the 2 – 3 orders of magnitude increase previously reported (Taha *et al*, 2006). For gram-negative bacteria concentrations on-site also reduced by 1 order of magnitude during periods of no activity (Figures 4.9 and 4.10). This finding is comparable with Schlosser *et al.* (2009) who found a decline of 1 – 2.5 orders of magnitude when sampling away from activity. Endotoxin data is also in agreement with the same study (Schlosser *et al*, 2009), with activities increasing on-site concentrations by 2 orders of magnitude at Flixborough; and although within the same order of magnitude, activity increased site concentrations by 40% at Lount OWC (Figures 4.12, 4.13).

For all bioaerosols, concentrations were found at higher levels at Flixborough, with this difference most marked for actinomycetes, gram-negative bacteria and endotoxins; where peak concentrations were 1 – 2 orders of magnitude higher at Flixborough than Lount OWC. There may be several reasons for this. The higher emissions at this site may be attributable to differences in feedstock, as Flixborough receives household-derived waste, including vegetable matter, which Lount OWC does not (Chapter 2). It has been shown that the composting process, and microbial community, is affected by

feedstock composition (Adams and Frostick, 2009). Therefore, the bioaerosol composition is also likely to be affected. In addition, as shown in Chapter 3, the composting process at Flixborough is maintained at lower moisture content than at Lount OWC. This may allow increased emissions of bioaerosols, as shown previously within the literature (Epstein, 1994). Intuitively, it could be anticipated that drier compost would increase liberalisation of dust and other particles, including bioaerosols (Jones and Harrison, 2004); drier compost may also favour the proliferation and emission of spore forming microorganisms, as spores are more able to survive environmentally challenging conditions (Le Goff *et al*, 2009). This may lead to increases in emissions of bioaerosols such as fungi and actinomycetes (Le Goff *et al*, 2009); although this does not describe the increase in gram-negative bacteria at Flixborough. Despite these differences in concentration of emissions at each site, dispersal patterns remained similar. This suggests that the patterns may be extrapolated to other green waste composting facilities.

Most dispersal profiles show a decrease of over 80% between peak concentrations and downwind 50 – 80 m. This initial reduction in bioaerosols has been previously reported, for example, it has been suggested that 90% of samples taken show concentrations under 1000 CFU $m^{-3}$ by 125 m from site (ADAS and SWICEB, 2005; Kothary *et al*, 1984), however, the authors did not continue sampling downwind. The decrease found here was followed by a secondary peak, seen at 100 – 150 m downwind. For actinomycetes and gram-negative bacteria, this secondary peak returned concentrations to levels significantly higher than those found upwind, and for gram-negative bacteria statistically similar to concentrations found on-site (Figures 4.6, 4.7, 4.9, 4.10). While this pattern has not been fully explored within the available literature, there is a suggestion that bioaerosols may be subject to a buoyancy effect as they are released above ground, in a parcel of air that is warmer than the surrounding air (Seinfeld and Pandis, 2006; Swan *et al*, 2003). This dispersal pattern may be a result of this, as bioaerosols lose their buoyancy and return to ground some distance from composting facilities. Figure 4.15 below shows a conceptual diagram of the buoyancy effect, along with a Gaussian plume.

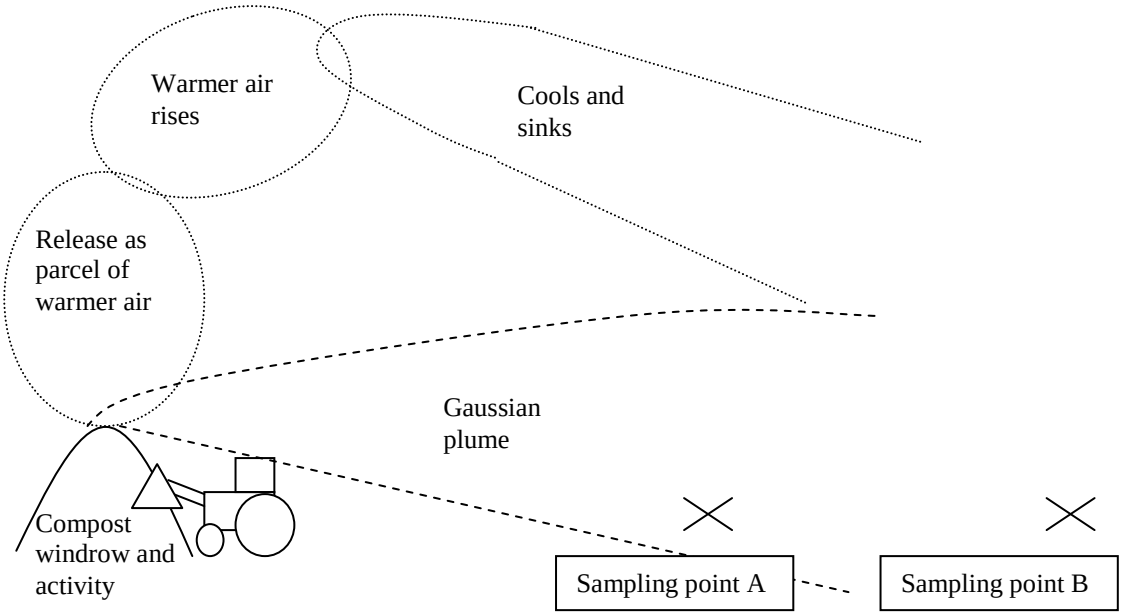



Figure 4.15: Conceptual model of buoyancy effect (···) versus Gaussian plume model (- -). Sampling point A would detect dispersal from the Gaussian plume, whereas release of bioaerosols within a package of warmer air would cause them to rise above sampling point A. Sampling point B would detect both plume models.

Some differences were shown in the dispersal of endotoxins from Flixborough (Figure 4.13), although it can be assumed that dispersal was governed by the same forces, with a trough shown at 150 m downwind rather than 50 – 80 m, and a small secondary peak at 200 m downwind rather than 100 – 150 m. Furthermore, *A. fumigatus* concentrations at Flixborough did not follow the same pattern, with the secondary peak not evident (Figure 4.4). This highlights how the individual properties of bioaerosols and site topography, such as the presence of bunding at Lount OWC (Chapter 2) may affect dispersal. However, as the remainder of dispersal profiles showed the same pattern, it can be assumed that this dispersal pattern represents the majority of bioaerosols and is governed by buoyancy, regardless of the presence of bunding. This finding has not been previously reported within the literature, and once again may be extrapolated to other green waste composting facilities.

From the secondary peak, bioaerosol concentration typically showed a gradual reduction. At Lount OWC, all culturable microorganisms were found at concentrations consistently statistically similar to upwind by 180 m downwind. There were some differences in the achievement of this at Flixborough; where *A. fumigatus* achieved upwind levels by 100 m downwind, while actinomycetes and gram-negative bacteria achieved upwind levels by 300 – 400 m downwind (Figures 4.4, 4.7, 4.10). The finding for actinomycetes is in agreement with Herr *et al.* (2003a), who found a reduction to background concentrations by 300 m from site. However, data regarding actinomycetes and gram-negative bacteria dispersal is scarce. In order to compare their dispersal to previously reported literature, they must often be considered part of 'total bacteria' measurements. Gilbert *et al.* (2002) found total bacteria had reduced to background concentrations by 200 m from site, while Reinthaler *et al.* (1997) found annual median values of 170 – 330 CFU $m^{-3}$ total bacteria in residential areas 150 – 2000 m from site. The dispersal distances found at Flixborough are in disagreement with Gilbert *et al.* (2002); although median values downwind were typically BDL, suggesting concentrations more in agreement with Reinthaler *et al.* (1997). The increased dispersal distances in elevated concentrations for actinomycetes and gram-negative bacteria may be related to the higher emissions seen at Flixborough as compared to Lount OWC, as discussed above.

*A. fumigatus* concentrations at Flixborough, as previously described, did not show the secondary peak. No reason for this difference could be found, given that all other bioaerosols show the same dispersal pattern independently of site. Dispersal here was characterised by a gradual decrease from site, followed by a small increase in geometric mean 500 m and 600 m downwind. Although concentrations at these sampling locations are statistically similar to those found upwind (Figure 4.4), geometric mean is 1 order of magnitude higher. These results must be interpreted with caution due to differences in site topography and surrounding land-use. Figure 4.4 shows how at these sampling locations land-use changes from a built environment, to agriculture. It is known that concentrations of *A. fumigatus* may increase in an agricultural environment, which may have resulted in an increase in concentrations at these locations (Dutkiewicz, 1997). However, the ability of bioaerosols to disperse to similar distances at levels above those

found upwind has also been previously reported; Albrecht *et al.* (2008) suggested that thermotolerant fungi could be found up at concentrations 1 – 2 orders of magnitude higher than background 600 – 1000 m from site, while Herr *et al.* (2003a) found concentrations 1 – 2 orders of magnitude higher than background 150 – 320 m from site.

For endotoxins, at Lount OWC a tertiary peak, not seen for culturable microorganisms, can be found at 280 m downwind (Figure 4.12). This peak returns geometric mean concentrations to levels similar to those found on-site. However, analysis of median, range and outlying values (Figure 4.14a) shows how this peak can largely be attributed to one outlying value, with no alternative source present (Chapter 2). The reasons for this outlying value are unknown; it may be due to filter contamination, or a one-off emission event from an alternative source. Although statistically similar to concentrations found upwind from 100 m downwind, geometric means at Flixborough remain above those found upwind for the remainder of the dispersal profile (Figure 4.13). No comparison can be made to previous studies, as to the author's knowledge; there are none available regarding endotoxin transport from composting facilities.

The increase of bioaerosol emission from site during composting activities has been shown above, and is likely to result in high levels of variability in downwind concentrations. While the discussion has focused on dispersal in terms of mean values, it is vital that range of values is also considered, as this provides data regarding best- and worst-case scenarios of potential sensitive receptor exposure, up to and beyond 250 m from site. For *A. fumigatus*, Figure 4.5 illustrates outlying and extreme values at both sites. At both sites the inter-quartile range for both site activity locations is $10^3$ CFU $m^{-3}$. At Lount OWC, maximum downwind concentrations reached levels 3 orders of magnitude higher than upwind geometric mean at 100 m, and 1 – 2 orders of magnitude higher at all other sampling locations (except downwind 280 m). At Flixborough, maximum concentrations of $3.1 \times 10^4$ CFU $m^{-3}$ were found at 200 m downwind. At all sampling locations apart from site boundary at Flixborough, minimum values were below detection limits; in addition, lower quartile concentrations were typically BDL, showing the frequency with which no *A. fumigatus* counts were returned. This range is

in agreement with Recer *et al.* (2001), who found even within 30 m of composting sites, concentrations were highly variable and often close to detection limit. Gram-negative bacteria and endotoxins showed similar ranges, with maximum values for gram-negative bacteria found from $10^3$ to $10^4$ CFU $m^{-3}$, 150 – 600 m from site, while maximum upwind concentrations are typically 1 order of magnitude lower. Endotoxins at Flixborough showed maximum concentrations 1 order of magnitude higher than those found upwind at 100 m, 200 m, and 500 m downwind (Appendix I, Tables 1.1 – 4.2).

Some differences were seen for variability within actinomycetes concentrations. Range values at downwind locations at both sites are high (Figure 4.11). As with other culturable microorganisms, there are also several outlying or extreme values at downwind locations seen at each site. However, while geometric mean values (Figures 4.6, 4.7) show how composting results in significantly higher releases of actinomycetes, Figure 4.8a and Table 4.3 show how at Lount OWC, concentrations are more even across the dispersal profile, with maximum concentrations upwind found in the same order of magnitude as those found 50 m, 150 m, and 250 m downwind. This finding is supported by Flixborough, where concentrations were found at a maximum of $5.4 \times 10^4$ CFU $m^{-3}$ 600 m from site; in the same order of magnitude as maximum found at upwind locations. This finding suggests that actinomycetes can be found at high concentrations within the ambient environment. This has been previously suggested, (Korzeniewska *et al*, 2009) but largely unacknowledged within the literature. Although minimum values returned reached detection limit, comparison of mode (BDL in most cases) and frequency of mode values between *A. fumigatus* and actinomycetes reveals how concentrations BDL were detected more frequently for *A. fumigatus* than for actinomycetes (Appendix I, Tables 1.1 - 2.2). This is particularly evident for on-site samples, and reinforces the suggestion that actinomycetes can be found in high concentrations ambiently within the environment, whereas the presence of *A. fumigatus* is strongly linked to composting. The comparable concentrations upwind and downwind from site are in disagreement with Recer *et al.* (2001), who found 21% of samples taken 500 m from site showed actinomycete concentrations two standard deviations or more above background; and Herr *et al.* (2003) who reported concentrations 2 – 3 orders of magnitude higher than background 150 – 320 m from site.

These data illustrate how bioaerosol concentrations downwind are highly variable, with this variability contributing to the amount of times threshold and background concentrations are exceeded at downwind locations. Based upon Wheeler *et al.*, authors of the Environment Agency report (2001), EA guidance values state that total bacteria and fungi should be found at concentrations below 1000 CFU $m^{-3}$ by 250 m from site (Environment Agency, 2001a). In addition, gram-negative bacteria should achieve 300 CFU $m^{-3}$ by 250 m downwind (Environment Agency, 2001b; Environment Agency, 2009a). The variability of bioaerosol concentrations found downwind means that *A. fumigatus*, actinomycetes, and gram-negative bacteria can all be found above these concentrations on occasion at distances over 250 m downwind. For *A. fumigatus*, 20% of samples taken at or over 250 m from site were over 1000 CFU $m^{-3}$ at Lount OWC, and 5% at Flixborough. This finding is supported by some studies within the available literature, with one reporting 10% of samples taken 540 m from site were two standard deviations or more higher than concentrations found in control areas (Recer *et al,* 2001). Furthermore, it is suggested that a limit of 500 CFU $m^{-3}$ may be set for *A. fumigatus* within the near future (Dennis, 2009). It was found that at or beyond 250 m from site boundaries 30% of samples at Lount OWC and 14% of samples at Flixborough was above detection limits, and therefore above the 500 CFU $m^{-3}$ proposed limit. The results of this study suggest that 500 CFU $m^{-3}$ is unlikely to be consistently achieved 250 m from the site boundary at either site.

For actinomycetes, 52% of samples at Lount OWC, and 37% of samples at Flixborough, taken at or over 250 m from site were over 1000 CFU $m^{-3}$, although the high ambient concentrations of actinomycetes are likely to have contributed to this. In terms of the 1000 CFU $m^{-3}$ total bacteria risk assessment limit, concentrations above this value could be found at all sampling locations, including upwind, at both sites. This suggests that 1000 CFU $m^{-3}$ total bacteria recommended limit is not an appropriate benchmark for actinomycetes. Excursions above the 300 CFU $m^{-3}$ threshold for gram-negative bacteria cannot be accurately assessed here, due to the limitations of the method used here leading to a lower detection limit of 757 CFU $m^{-3}$ (Chapter 3). However, it was found that 37% of samples at Lount OWC and 43% of samples at

Flixborough (taken at or over 250 m from site) were above this detection limit, and therefore above 300 CFU $m^{-3}$. No threshold values exist for endotoxins to enable a similar comparison. It was suggested that worker exposure should not exceed 200 EU $m^{-3}$ over an 8-hour period (Schlosser *et al*, 2009). Taking this limit, concentrations were not exceeded at any downwind location (Appendix I, Tables 4.1, 4.2), particularly given that samples were not taken over 8-hour periods. The only location where 200 EU $m^{-3}$ was exceeded was downwind 300 m at Lount OWC, where concentrations were attributed to an anomalous result, as described above.

The variability in concentrations downwind was anticipated, and may be due to site activities. This issue was explored in Chapter 1 (section 1.10), leading to the conceptual diagram describing how episodic on-site emissions may lead to peak concentrations being found downwind from site. This diagram is reproduced in Figure 4.16 below.

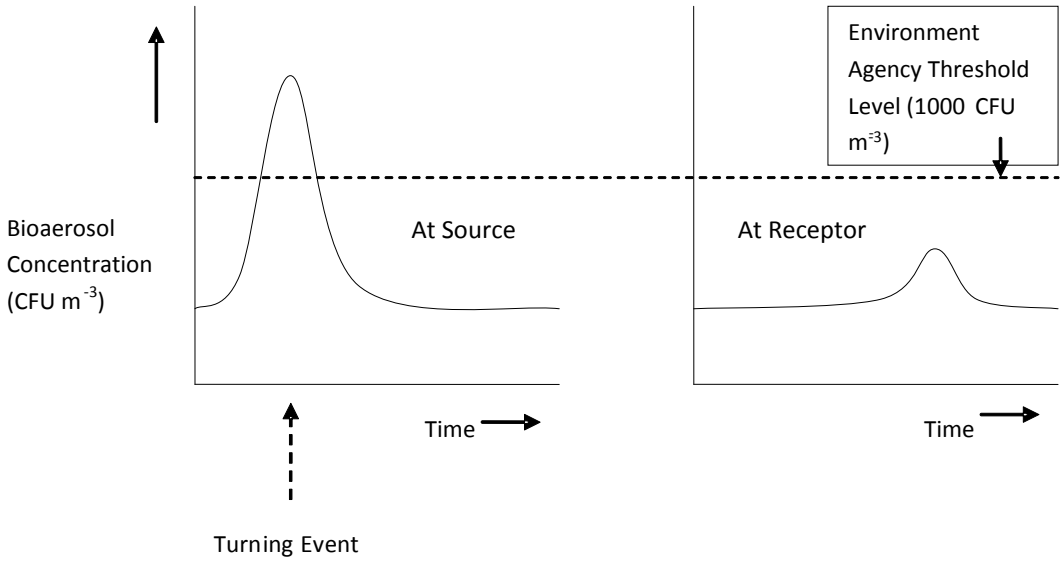



Figure 4.16: Pictorial representation of hypothesis for research question 'episodic emission leads to episodic dispersal'

The findings from this study show how culturable bioaerosols may be found in concentrations exceeding the 1000 CFU $m^{-3}$ recommended threshold downwind from composting facilities. Therefore, this conceptual model has been revised, with Figure 4.17 below presenting this revised version.

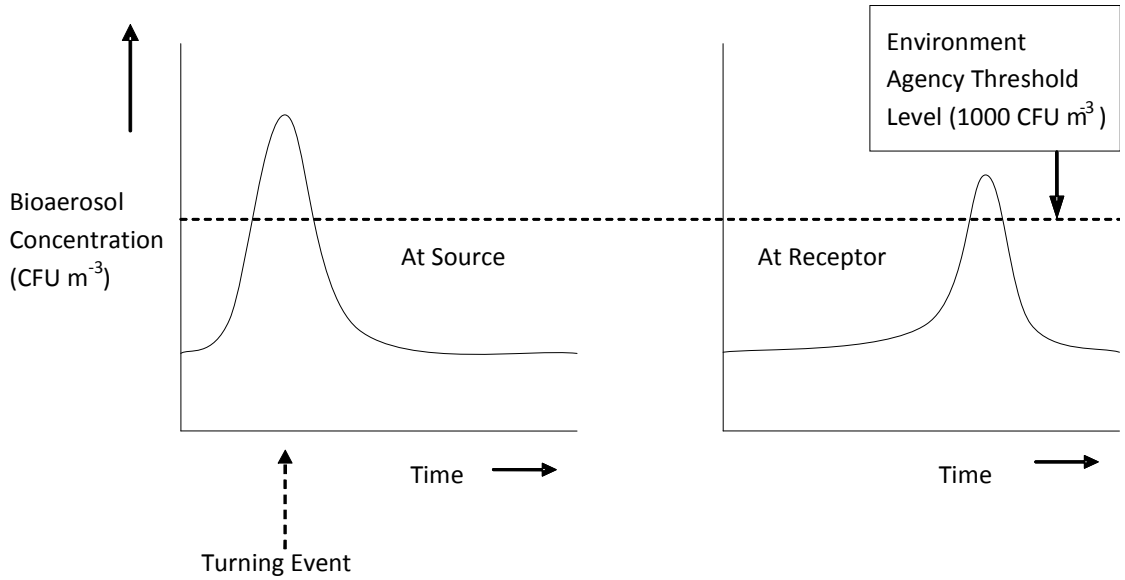



Figure 4.17: Revised version of the pictorial representation of the hypothesis for the research question 'episodic emission leads to episodic dispersal', originally represented in Figures 1.1 and 4.16

The hypothesis described by Figure 4.17 (episodic emission leads to episodic dispersal) suggests that site activities may result in downwind excursions above threshold values, contributing towards the variability in concentrations seen within this study. While site activities have been linked to changes in concentrations on-site within this Chapter, the impacts of site activities on downwind concentrations and their variability is further explored in Chapter 5.

Although excursions above threshold values, or LLOD for *A. fumigatus* and gram-negative bacteria, have been found, the lack of ability to detect concentrations below 757 CFU $m^{-3}$ has reduced ability within this study to quantify 'background' concentrations. Therefore, where dispersal limits of bioaerosols are analysed, upwind levels are used as a measure to calculate the significance of downwind concentrations. These upwind levels, and LLOD, may not represent 'background' concentrations; for example, previous literature has suggested background level of 0 – 1 CFU $m^{-3}$ for mesophilic bacteria (Gilbert *et al,* 2002). Of particular concern, is the frequency of *A. fumigatus* samples that were BDL. It is possible that composting activities affect downwind concentrations of *A. fumigatus* to a greater extent than has been presented

here, with sampling methods used for this project unable to detect the concentrations present. It has been suggested that *A. fumigatus* background concentrations are below 10 CFU $m^{-3}$, well below detection limits of this method (O'Gorman and Fuller, 2008). Resulting from this, actual background concentrations and the return of bioaerosols to this background has not been quantified.

However, despite the lack of sensitivity to low concentrations, it has been shown that bioaerosols have the ability to disperse in concentrations above those found upwind up to and beyond 250 m from site. Despite differences in site topography, surrounding land-use, feedstock, and concentrations of bioaerosols released, a dispersal pattern has been found that applied to all bioaerosols measured. The variability of bioaerosol concentrations has been shown, and partially traced to site activities. All of these factors have led to the achievement of Objective i, 'Quantitative characterisation of bioaerosols emitted through composting activities and downwind concentrations up to and beyond 250 m from site at chosen case-study sites'; leading to support of the hypothesis that bioaerosols are emitted in elevated concentrations through composting, and show the ability to travel beyond 250 m from site. One of the most important findings of this work has been the frequency with which concentrations may be found above threshold values at and beyond 250 m from site, suggesting that such excursions are not exceptional occurrences, but part of the normal downwind concentration range at composting facilities. The following Chapter attempts to further explore the causes of these excursions above threshold values, following the hypothesis described in Figure 4.17, and the theory that on-site activities may be responsible for downwind peaks in concentration.

## 4.5 Conclusions

Dispersal profiles of *A. fumigatus*, actinomycetes, gram-negative bacteria and endotoxins from green-waste composting facilities have been constructed. To the authors' knowledge, this is the first time that these bioaerosols have been analysed in such detail, providing statistically valid data to quantify dispersal patterns. In particular, no such data for endotoxins has previously existed. The main conclusions from this study are described below.

- Site activities lead to emission of bioaerosols, including endotoxins, in elevated concentrations
- Endotoxins are able to be transported in elevated concentrations from composting facilities
- Dispersal patterns are likely to be strongly affected by buoyancy, leading to a secondary peak in concentrations 100 – 150 m from site boundary
- More than 50% of samples can be found above threshold values (or detection limits for *A. fumigatus* and gram-negative bacteria) at and beyond 250 m from site boundary
- *A. fumigatus* is unlikely to consistently reach 500 CFU $m^{-3}$ by 250 m from site boundary
- The usefulness of actinomycetes as a microbial group indicative of composting is questionable, due to high ambient concentrations

A significant finding is that dispersal does not follow a simple Gaussian model, with impacts from buoyancy, site design, topography, and feedstock, affecting dispersal patterns and range. While the dispersal pattern related to buoyancy shown here has been suggested previously (Environment Agency, 2001a), it remains widely un-publicised or acknowledged within the literature. This has led to claims that as concentrations reduce significantly by 50 – 80 m downwind, background would be achieved by 250 m downwind (ADAS and SWICEB, 2005). However, the dispersal profiles presented here suggest that buoyancy has a significant impact on dispersal patterns, as well as transport distances. Although, it must be acknowledged that dispersal distances in elevated

concentrations are based on upwind, rather than background concentrations due to the limitations induced by the sampling detection limit. Despite this limitation, all bioaerosols were found 1 – 2 orders of magnitude higher than upwind concentrations more than 250 m from site, with 5 – 52% of samples taken over 1000 CFU $m^{-3}$ at distances beyond 250 m from site. These findings may have implications for future sampling and legislation of bioaerosols emitted through composting. Current recommended guidelines (Association for Organics Recycling, 2009; Environment Agency, 2009a) do not take into account either the potential for buoyancy to affect downwind concentrations, or the ability of bioaerosols to be transported to distances beyond 250 m from site.

The increase seen during site activity along with evidence within the literature (Chapter 1) suggests that the dominant factor determining bioaerosol release and downwind concentrations is compost processing activities. Understanding of the impact of these activities, as well as their influence on downwind concentrations is the focus of the following Chapter.

# 5 Episodic Emissions and Dispersal

## 5.1 Introduction

It has now been widely acknowledged that bioaerosols can be released episodically as composting activities are carried out (Chapter 1) (Lacey, 1997; Schlosser *et al*, 2009; Taha *et al*, 2006). However, while it has been established that a temporal effect between releases on-site and downwind concentrations exists (Domingo and Nadal, 2009), the nature of this relationship (the effect of on-site episodic releases on downwind concentrations), has not previously been quantified. Knowledge of this relationship is essential in order to determine both 'worst-case' dispersal distances and potential receptor exposure to bioaerosols downwind from composting facilities.

In terms of site emission and downwind concentrations, it is now established that composting activities result in an increase of bioaerosol concentrations on-site by 2 – 3 orders of magnitude (Clark *et al,* 1983; Kothary *et al*, 1984; Sykes *et al,* 2007; Taha *et al,* 2004; Taha *et al,* 2006). This episodicity was also shown by Recer *et al.* (2001) who found that concentrations as little as 30 m from composting sites could be highly variable, with this variability attributed to episodic composting activities. This has also been shown through modelling of bioaerosol dispersal, which has suggested that even close to source bioaerosol concentrations can be highly variable; although this variability was attributed to environmental rather than operational influences (Lighthart and Mohr, 1987). Further, some authors have suggested that certain bioaerosols may be associated with particular composting activities (Albrecht *et al*, 2008; Fischer *et al*, 2008). It has been found that turning leads to higher emissions of actinomycetes than shredding, with levels reaching $10^6$ CFU $m^{-3}$ (Lacey, 1997). Schlosser *et al.* (2009) found that lowest concentrations of actinomycetes and *A. fumigatus* were associated with shredding activity, while screening was associated with higher levels of gram-negative bacteria, actinomycetes, and endotoxins.

Other studies have investigated bioaerosol concentrations downwind from facilities, and found variations in concentration that could be related to specific factors such as environmental and operational conditions. For example, Albrecht *et al.* (2008) and Fischer *et al.* (2008) found that concentrations 600 – 1400 m from facilities could be found 1 – 2 orders of magnitude higher than background concentrations, with dispersal distances dependent on meteorological factors and turnover of the specific facility (Albrecht *et al*, 2008; Fischer *et al*, 2008). It has also been suggested by Herr *et al.* (2003) that bioaerosols can be found 2 – 3 orders of magnitude higher than background concentrations 150 – 320 m from composting site boundaries; although in this case variability in concentrations was not attributed to meteorological or operational conditions. Another study found that 10% of *A. fumigatus* concentrations and 21% of actinomycete concentrations were two standard deviations or more above background concentrations up to 500 m from a composting facility (Recer *et al*, 2001). These studies suggest that elevated concentrations of bioaerosols may periodically be found at distance from composting facilities. However, no 'cause and effect' study has yet directly related downwind changes in concentration to site activities. Furthermore, concentrations emitted from site have also been found to achieve background concentrations by 200 m (Gilbert *et al*, 2002). Reinthaler *et al.* (1997) report a median range of 15 – 52 CFU $m^{-3}$ for *A. fumigatus* and 170 – 330 CFU $m^{-3}$ for bacteria concentrations in residential areas 150 m and 2000 m from composting facilities, well below recommended threshold limits (Environment Agency, 2009b). However, this study also reported occasional higher counts, up to 350 CFU $m^{-3}$ for *A. fumigatus*, with these concentrations attributed to environmental influences and site activities (Reinthaler *et al*, 1997). In the investigations reported within this thesis, analysis of downwind concentrations of bioaerosols from composting facilities (Chapter 4) has revealed variability in concentrations similar to that found within the literature. Across all culturable bioaerosols measured, 5 – 52% of samples taken were over 1000 CFU $m^{-3}$ at distances beyond 250 m from site. All bioaerosols could, at times, be found 1 to 2 orders of magnitude higher than upwind concentrations at distances at or beyond 250 m from site boundary.

Through assessment of past literature and analysis of the impact that on-site activity has upon concentrations, it is clear that episodic emission on-site is likely to affect downwind concentrations of bioaerosols. While it is also possible that environmental factors affect downwind concentrations, the strength of evidence for on-site episodic emissions and past acknowledgement of downwind transient peaks in concentrations suggests these features may be linked. The lack of studies aiming to directly analyse peak downwind concentration in relation to operational activities has contributed to the absence of certainty regarding the variability found in bioaerosol concentrations; for example, whether downwind peaks in concentration can be attributed to site operational activities, meteorological conditions, or a combination of both.

With the theory that episodic emission of bioaerosols leads to variation in downwind concentrations, it could be said that episodic emission leads to 'episodic dispersal' of bioaerosols, and hence episodic sensitive receptor exposure at distances over 250 m from site. This hypothesis is illustrated in Figure 5.1 below. This Figure was previously shown in Chapter 1 and revised according to findings within Chapter 4, as concentrations higher than the 1000 CFU $m^{-3}$ limit were found. However, peak downwind concentrations have not yet been directly related to episodic emission on-site.

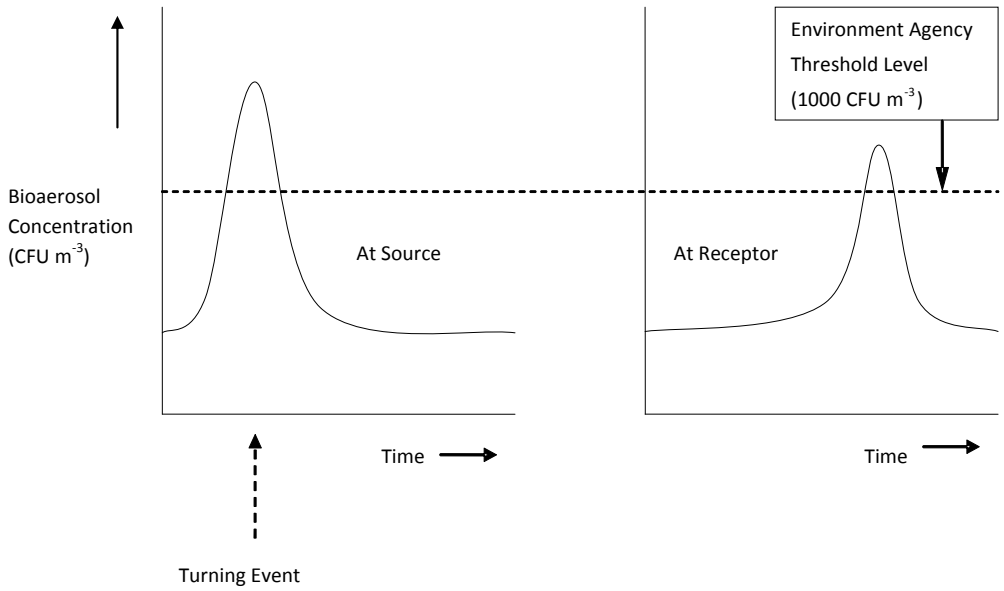



Figure 5.1: Revised version of the pictorial representation of the hypothesis for the research question 'episodic emission leads to episodic dispersal', originally represented in Figures 1.1 and 4.16

Past studies have highlighted the importance of quantifying peak bioaerosol concentrations both on-site and downwind, given the potential health impacts of both low-level long term exposure and single exposure events to high concentrations (Becher and Lichtnecker, 2002; Bünger *et al*, 2000; Douwes *et al*, 2003; Hollingdale, 1974; Millner *et al*, 1994). Exposure to high concentrations of fungal spores ($10^8$) following a period of sensitisation through exposure to lower concentrations ($10^5$ – $10^6$ spore $m^{-3}$) has been suggested to cause Hypersensitivity Pneumonitis (Bünger *et al*, 2000; Hollingdale, 1974). This sensitisation has also been linked to an increase in the prevalence of rhinitis, along with other upper airway afflictions (Becher and Lichtnecker, 2002; Bünger *et al*, 2000; Millner *et al*, 1994). Other studies have reported that toxic-irritative reactions occur after single exposure events to fungal spores around $10^6$ – $10^9$ spores $m^{-3}$ (Herr *et al*, 2003a). Quantification of on-site and downwind concentrations of bioaerosols and potential health impacts has been carried out within a minority of studies. Although not supported by clinical evidence, Herr *et al*. (2003b) found that residents 150 m from site self-reported increased levels of breathlessness and respiratory health complaints; with measured bioaerosol concentrations around $10^6$ CFU

$m^{-3}$ at this location (Herr *et al*, 2003a, b). Furthermore, complaints of airway irritation were anticipated after frequent exposures to concentrations of bioaerosols around $10^4$ – $10^5$ CFU $m^{-3}$ (Herr *et al*, 2003a, b). However, no further studies have attempted to quantify exposure to bioaerosols from composting facilities and relate the results to health impacts.

The above brief review has highlighted two areas within the literature where knowledge is incomplete. These are the direct relation of episodic site emissions to downwind variations in concentration, and the attribution of these variations in downwind concentration to potential receptor exposure patterns. Understanding of these relationships is vital to the understanding of bioaerosol 'dose', necessary in order to assist the future development of dose-response relationships. However, past sampling studies have focused on direct impaction and culture-based analysis (Association for Organics Recycling, 2009; Environment Agency, 2009a; The Composting Association, 1999). As shown in Chapters 1 and 4, this may lead to underestimation of concentrations enhanced through short sampling times, or 'snapshot' sampling protocols, where peak concentrations may be missed. For the purposes of exposure assessment, longer-term and repeat sampling is required (Drew *et al*, 2005; Recer *et al*, 2001). This enables any short-term exposure events, such as the episodic emission and dispersal of bioaerosols, to be accounted for.

These gaps in knowledge will be met thorough the achievement of the following thesis Objective:

i. Quantitative characterisation of bioaerosols emitted through composting activities and downwind concentrations up to and beyond 250 m from site at chosen case-study sites
    a. Assessment of the impact that episodic emission has on downwind concentrations

Through the literature review carried out above and within Chapter 1, it can be hypothesised that episodic emission will have a significant impact on downwind

dispersal of bioaerosols, and will be shown to be responsible for downwind peaks and excursions above upwind concentrations.

Chapter 4 presented results from a statistically valid data-set profiling downwind concentration of *A. fumigatus*, actinomycetes, gram-negative bacteria, and endotoxins. In order to achieve Objective i (a), this data-set was re-analysed here with a focus on the impact that composting activities have on downwind concentrations. In addition, data specifically for the purposes of episodic emission and dispersal analysis were collected at each site.

## 5.2 Experimental Design

Methods used in order to sample on-site and process samples followed the general methodology described in Chapter 3. Sampling at Lount OWC took place from September 2007 until May 2008. Sampling at Flixborough took place from August 2008 until July 2009. A full list of sampling dates can be found in Appendix I.

### 5.2.1 Lount OWC

For the purposes of this analysis, where samples had been previously measured from site boundary (such as within Chapter 4), distances were re-calculated with nearest activity as the ‘0 m’ point. Alternatively, if no activities were present at the time of sampling the nearest compost windrow was taken as the ‘0 m’ point. The re-calculation of downwind distances is illustrated in Figure 5.2 below.

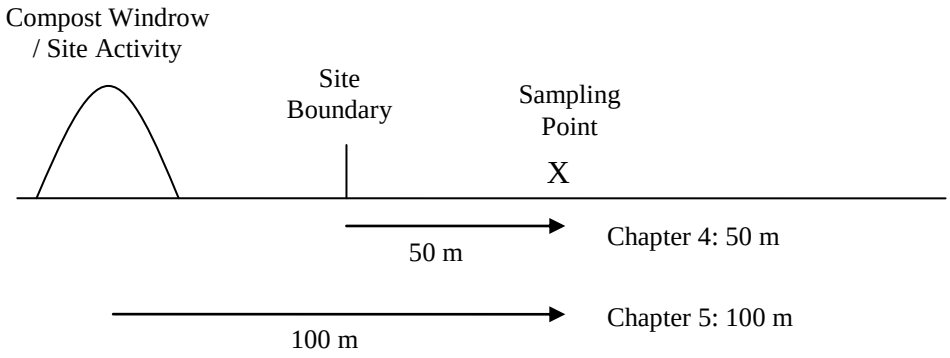



Figure 5.2: Illustration of re-calculation of downwind distances for the purposes of analysis of the impacts that site activities have on downwind bioaerosol concentrations.

Downwind samples were also reclassified as having been taken during periods of activity or no activity. 'Activity' was defined as any period during which composting activities, such as shredding, turning, screening, or moving, was taking place. 'No activity' was defined as any time period during which no composting activities were taking place. Times of activity or no activity were determined through either direct observation, or video recording (Chapter 3). In addition, samples specifically for the purpose of quantification of episodic emission and dispersal were taken. These involved simultaneous sampling at two points in order to determine real-time impacts of emission on downwind concentrations. These sampling events were planned in order to capture the beginning or cessation of composting activity, to allow determination of any effect on downwind concentrations. Sampling points included:

1. On-site and 100 m downwind
2. 100 m downwind and 200 m downwind
3. 200 m downwind and 300 m downwind
4. 250 m downwind and 350 m downwind
5. 300 m downwind and 355 m downwind (downwind distance limited by hedging and access boundary)

However, as up to 100 m separated the sampling locations, immediate removal of the filter post-sampling (Chapter 3) was logistically impossible to carry out for both

sampling points. As one of the points would then inevitably have the filters removed after several minutes, the risk of additional bioaerosols impacting on the filter outside of the sampling period would increase, reducing accuracy of the sample (Chapters 1, 3). In order to eliminate this risk, the two sampling locations were operated with a 15 minute time-lag.

### 5.2.2 Flixborough

At Flixborough all downwind sampling distances were measured from emission source (adjacent to site activity), rather than site boundary. This enabled samples to be classified as being taken during periods of activity or no activity without a re-calculation of distance from source, as occurred with Lount OWC samples. Times of activity or no activity were determined through either direct observation, or video recording (Chapter 3), as described in section 5.2.1. In addition, samples were taken specifically for the purpose of characterising episodic emission and dispersal, and timed in order to capture beginning or cessation in activity. Sampling points included:

1. On-site and 100 m downwind
2. 100 m downwind and 200 m downwind
3. 200 m downwind and 300 m downwind
4. 300 m downwind and 400 m downwind
5. 400 m downwind and 500 m downwind
6. 500 m downwind and 600 m downwind

As at Lount OWC each sampling occasion consisted of the two sampling points being simultaneously maintained with a 15 minute time-lag.

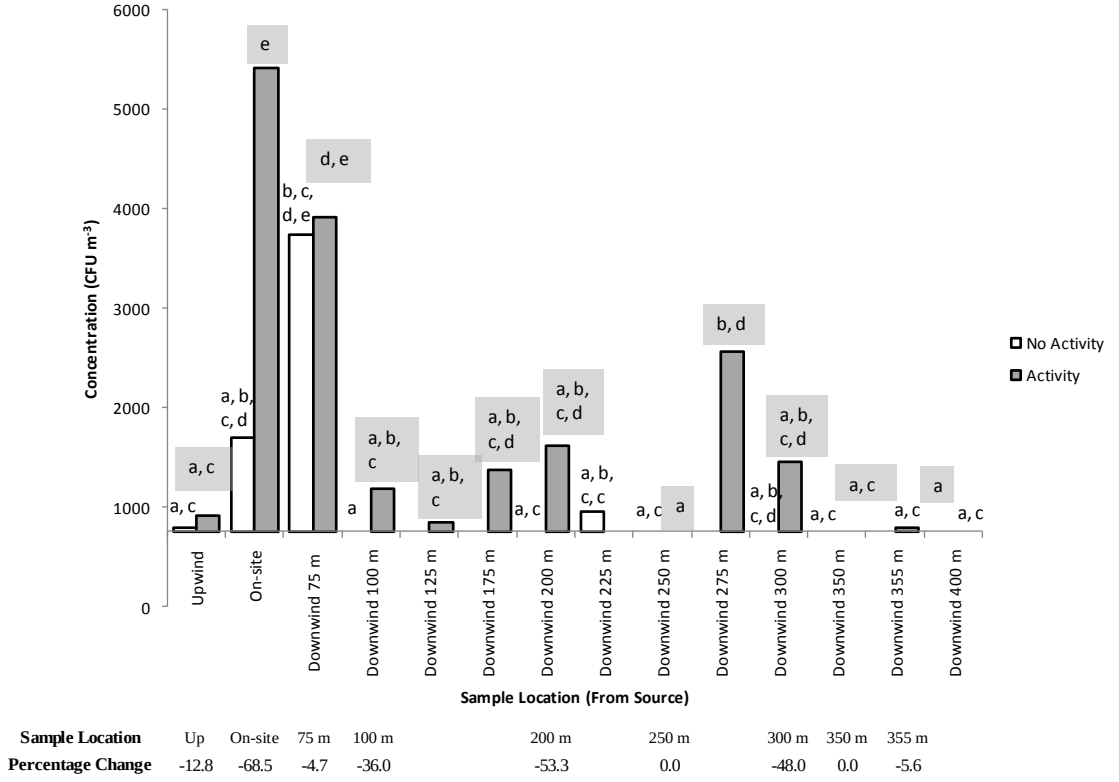
## 5.3 Episodic Emission and Dispersal Results

Results for the analysis of episodic emission and dispersal are presented for all bioaerosols measured within this section. All references to downwind distances are taken from source (adjacent to composting activity or windrow). Samples were qualified as being taken during periods of activity or no activity according to both site observations and recording taken by camcorder (Chapter 3). Wherever 'significance' or 'statistical similarity/difference' is referred to, p-values are less than 0.05. In-figure statistical similarity/difference data generated through the Fisher LSD test, where sampling locations are compared in pairs. Statistical similarity/difference ($p = < 0.05$) is presented for each individual sampling location compared to all other sampling locations. For *A. fumigatus*, actinomycetes, and gram-negative bacteria, mean values are presented, with data for individual episodic sampling days presented within Appendix I. For endotoxins, only mean data is presented as endotoxin samples specifically for the purpose of episodic analysis were not collected. In order to easily compare the difference between concentrations during periods of activity and no activity at each sampling location, the percentage difference between each was calculated. Concentrations found during periods of activity were taken as 100%, with concentrations during periods of no-activity calculated as a percentage of this and the difference between these two figures presented as percentage change.

### 5.3.1 *Aspergillus fumigatus*

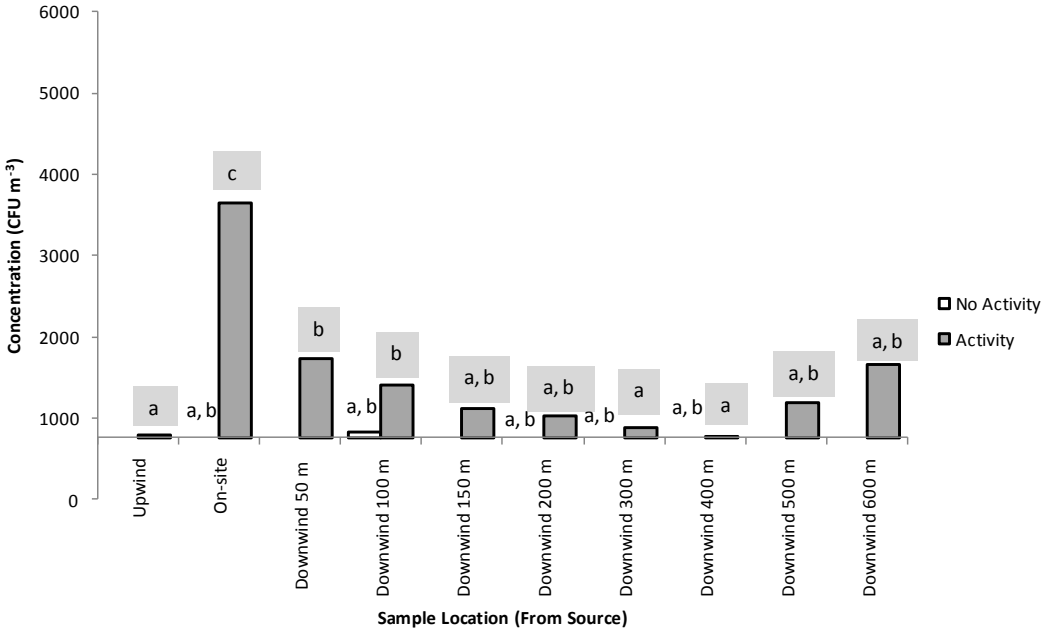
Figures 5.3 and 5.4 show mean data across all samples taken during periods of activity and no activity. At both sites all samples taken during periods of activity were higher than those taken during periods of no activity, with this difference most pronounced at on-site sampling locations. At Lount OWC, concentrations on-site increased by 68% during periods of activity, while at Flixborough concentrations increased 79%. Concentrations on-site at Lount OWC reached $5.4 \times 10^3$ CFU $m^{-3}$, a level statistically different from all other sampling locations apart from 75 m downwind. At Flixborough, however, on-site samples during activity were significantly higher than any other

samples taken, reaching $3.6 \times 10^3$ CFU $m^{-3}$. Percentage differences between periods of activity and no activity at comparable sampling locations at each site (Figures 5.3, 5.4) varied between 0 and 79%. The largest increases between periods of no activity and activity occurred on-site at both Lount OWC and Flixborough. At Lount OWC a small increase of approximately 5% is seen at 75 m downwind, with increases up to 50% at downwind 100 m, 200 m, and 300 m. At Flixborough, percentage increase is highest on-site, with a steady decrease seen to 400 m downwind. Arithmetic mean shows how at both sites samples taken during periods of no activity were largely below detection limits, with only 100 m downwind at Flixborough showing measurable *A. fumigatus* concentrations, while at Lount OWC levels were measurable upwind, on-site, 75 m and 225 m downwind (Appendix I, Tables 5.1, 5.2). However, at both sites concentrations taken during periods of activity were consistently higher, and showed the dispersal patterns previously described in Chapter 4. These patterns were also supported by arithmetic mean values (Appendix I, Tables 5.1, 5.2).




| Sample Location | Up | On-site | 75 m | 100 m | 200 m | 250 m | 300 m | 350 m | 355 m |
|---|---|---|---|---|---|---|---|---|---|
| Percentage Change | -12.8 | -68.5 | -4.7 | -36.0 | -53.3 | 0.0 | -48.0 | 0.0 | -5.6 |

Figure 5.3: *A. fumigatus* concentrations at Lount OWC. Bars represent geometric mean, annotations represent statistical similarity ($p = < 0.05$) generated through pairwise comparison, shaded labels assigned to 'activity' samples, blank to 'no activity'. Table incorporated into the Figure represents percentage change between activity and no activity, concentrations during activity representing 100%, where data is available. X-axis crosses at lower limit of detection (757 CFU $m^{-3}$). Where no bar or measure of statistical similarity is presented, no samples were gained (e.g. 270 m downwind no activity).




| Sample Location | On-site | 100 m | 200 m | 300 m | 400 m |
|---|---|---|---|---|---|
| Percentage Change | -79.2 | -41.0 | -26.2 | -15.1 | -2.8 |

Figure 5.4: *A. fumigatus* concentrations at Flixborough. Bars represent geometric mean, annotations represent statistical similarity ($p = < 0.05$) generated through pairwise comparison, shaded annotations assigned to 'activity' samples, blank to 'no activity'. Table incorporated into the Figure represents percentage change between activity and no activity, concentrations during activity representing 100%, where data is available. X-axis crosses at lower limit of detection (757 CFU $m^{-3}$). Where no bar or measure of statistical similarity is presented, no samples were gained (e.g. Upwind no activity).

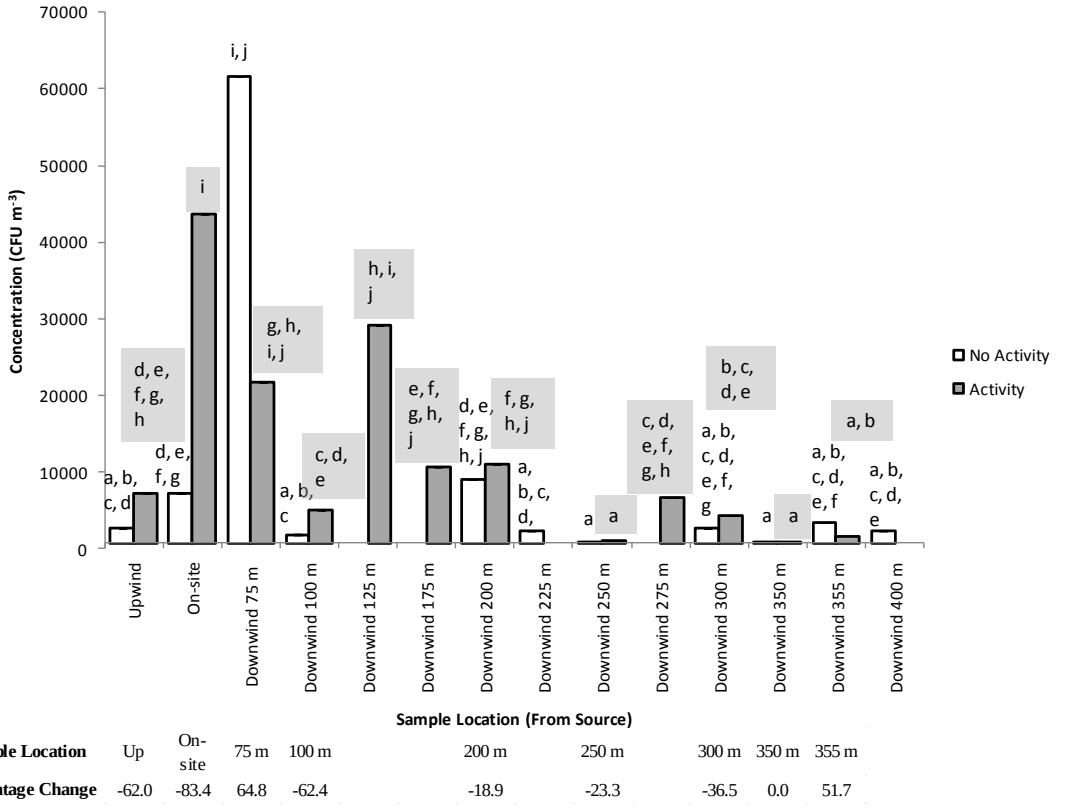
The data collected through individual episodic sampling days, where bioaerosol concentrations were simultaneously collected at two sampling locations, are presented in Appendix I (Figures 1.1 – 1.6). For *A. fumigatus* a general pattern reflecting a decrease in concentrations during periods of no activity is shown. This pattern is more discernable at Lount OWC (Appendix I, Figure 1.1), and is reflected at both sampling locations measured on individual sampling days. This pattern, however, cannot be seen at Flixborough (Appendix I, Figure 1.2), where concentrations are lower than at Lount OWC and no clear trend is seen.

### 5.3.2 Actinomycetes

Highest concentrations of actinomycetes at Lount OWC (Figure 5.5) were returned at 75 m downwind during no activity, with levels 65% higher than those found during activity. However, these concentrations are statistically similar to those taken on-site, 125 m, 175 m and 200 m downwind, indicating that they are variable. Concentrations on-site during periods of activity were 83% higher than those taken on-site during periods of no activity, reaching $4.3 \times 10^4$ CFU $m^{-3}$. At Flixborough (Figure 5.6) concentrations on-site during periods of activity were significantly higher than any other sampling location, with levels reaching $1.6 \times 10^5$ CFU $m^{-3}$, 98% higher than samples taken on-site during periods of no activity. At both sites, all samples taken during periods of no activity were similar to those found upwind. Dispersal patterns during periods of activity were similar at both sites, and were described in Chapter 4. One difference can be seen at Lount OWC, where variability in concentrations resulted in most downwind sampling locations being statistically similar to both each other and upwind concentrations. Furthermore, an increase is visible at 275 m downwind that is not shown in Chapter 4; despite this, concentrations at 225 – 400 m downwind are statistically similar to those found upwind. At Flixborough, dispersal patterns were previously described in Chapter 4, with upwind concentrations consistently achieved by 300 m downwind.

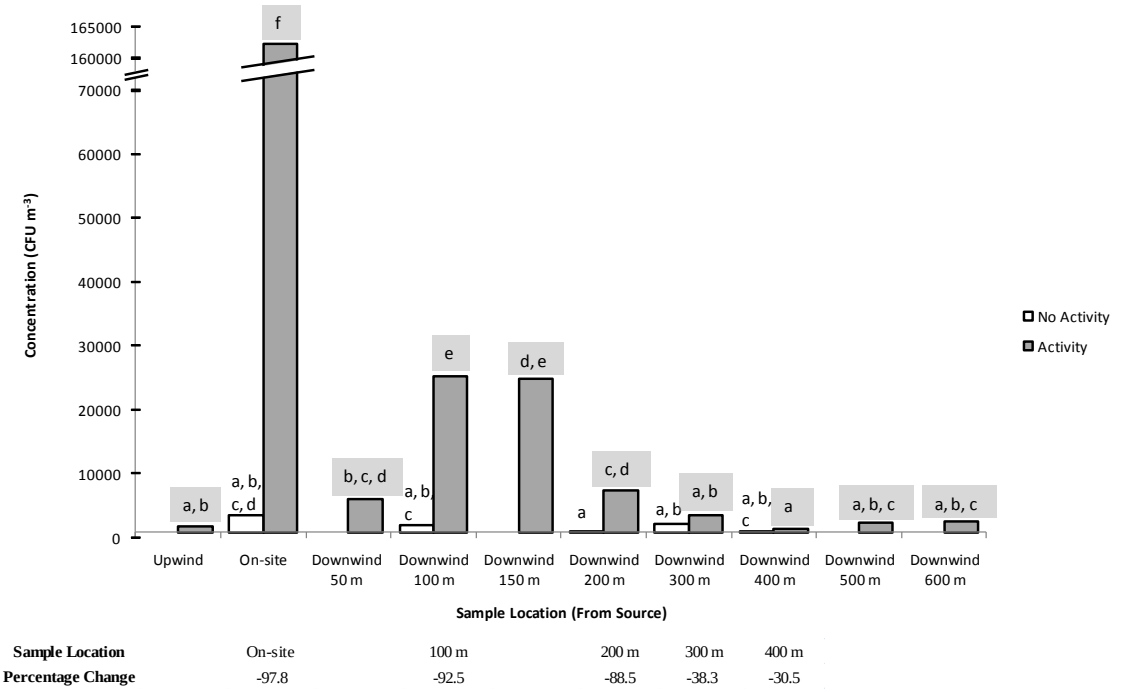
There is considerable variability in percentage change between periods of no activity and activity at Lount OWC (Figure 5.5). At upwind locations, concentrations increased by 62% during activity. At some downwind locations concentrations were higher during periods of no activity, such as at downwind 75 m, 350 m, and 355 m. At Flixborough, however, differences in concentration between periods of no activity and activity at on-site and downwind 100 m are both over 90%. From these sampling locations differences between periods of no activity and activity gradually decrease to downwind 400 m, where a difference of 31% was found. The variability shown in concentrations at Lount OWC is reflected in arithmetic mean, where concentrations during periods of no activity are within the same order of magnitude as those taken during activity at on-site to downwind 250 m. Arithmetic mean concentrations at Flixborough are up to 2 orders of

magnitude higher during periods of activity, and maximum concentrations 3 orders of magnitude higher (Appendix I, Tables 6.1, 6.2).




| Sample Location | Up | On-site | 75 m | 100 m | 200 m | 250 m | 300 m | 350 m | 355 m |
|---|---|---|---|---|---|---|---|---|---|
| Percentage Change | -62.0 | -83.4 | 64.8 | -62.4 | -18.9 | -23.3 | -36.5 | 0.0 | 51.7 |

Figure 5.5: Actinomycete concentrations at Lount OWC. Bars represent geometric mean, annotations represent statistical similarity ($p = < 0.05$) generated through pairwise comparison, shaded annotations assigned to 'activity' samples, blank to 'no activity'. Table incorporated into the Figure represents percentage change between activity and no activity, concentrations during activity representing 100%, where data is available. X-axis crosses at lower limit of detection (757 CFU $m^{-3}$). Where no bar or measure of statistical similarity is presented, no samples were gained (e.g. 275 m downwind no activity).




| Sample Location | On-site | 100 m | 200 m | 300 m | 400 m |
|---|---|---|---|---|---|
| Percentage Change | -97.8 | -92.5 | -88.5 | -38.3 | -30.5 |

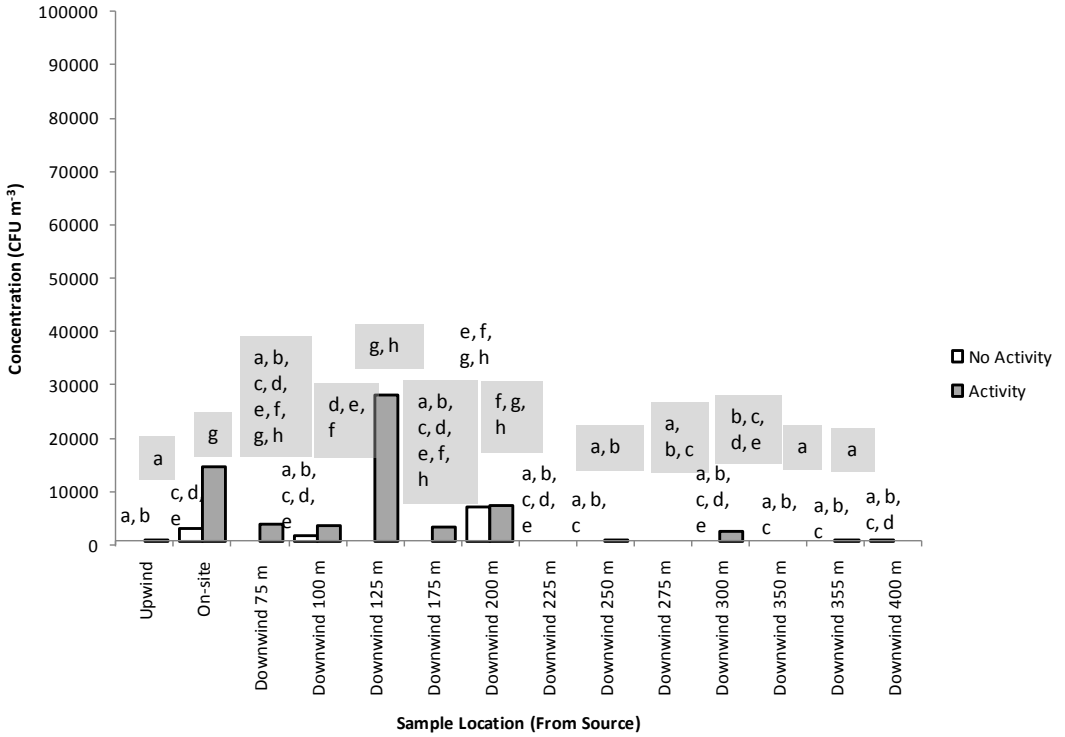
Figure 5.6: Actinomycete concentrations at Flixborough. Bars represent geometric mean, annotations represent groups similarity ($p = < 0.05$) generated through pairwise comparison, shaded annotations assigned to 'activity' samples, blank to 'no activity'. Table incorporated into the Figure represents percentage change between activity and no activity, concentrations during activity representing 100%, where data is available. X-axis crosses at lower limit of detection (757 CFU $m^{-3}$). Where no bar or measure of statistical similarity is presented, no samples were gained (e.g. Upwind no activity).

Data gathered during individual episodic sampling occasions (where bioaerosol concentrations were measured at two sampling locations simultaneously) is presented in Appendix I (Figures 1.1 – 1.6). For actinomycetes (Appendix I, Figures 1.3 and 1.4) patterns found on individual days are similar, with decreases in concentrations visible upon cessation of activity at downwind 100 m and 200 m, then 250 m and 350 m at Lount OWC; and at on-site and 100 m downwind at Flixborough. Start of activities appeared to result in an increase in actinomycete concentrations at downwind 100 m and 200 m, then downwind 200 m and 300 m, and downwind 300 m and 400 m at Flixborough.

### 5.3.3 Gram-negative Bacteria

As with actinomycetes, at Lount OWC gram-negative bacteria concentrations are variable and show much statistical similarity across the dispersal profile (Figure 5.7). Peak concentrations are seen during activity at 125 m downwind, with concentrations statistically similar to those found on-site during activity, downwind 75 m, 175 m, and 200 m. At Flixborough (Figure 5.8) peak concentrations are on-site during activity. At Lount OWC concentrations on-site increase by 80% to $1.5 \times 10^4$ CFU $m^{-3}$ during activity, while at Flixborough they increase by 89% to $9.2 \times 10^4$ CFU $m^{-3}$. At both sites, concentrations found during periods of no activity are largely similar to those found upwind. Concentrations during periods of activity follow the dispersal pattern described in Chapter 4. Percentage differences between periods of activity and no activity show how the largest increases are found on-site, although all other sampling locations apart from 350 m downwind at Lount OWC and 400 m downwind at Flixborough also show an increase in concentrations during activity. Unlike *A. fumigatus* and actinomycetes, large increases in concentration can be found downwind, with a 70% increase at 300 m downwind at Lount OWC, and an 87% increase at 200 m downwind at Flixborough.

These patterns are once again reflected by arithmetic mean values, with concentrations during periods of no activity BDL, or up to 2 orders of magnitude lower than those found during periods of activity (Appendix I, Tables 7.1, 7.2).




| Sample Location | Up | On-site | 100 m | 200 m | 250 m | 300 m | 350 m | 355 m |
|---|---|---|---|---|---|---|---|---|
| Percentage Change | -10.9 | -79.8 | -52.9 | -6.2 | -15.9 | -70.0 | 0.0 | -5.6 |

Figure 5.7: Gram-negative bacteria concentrations at Lount OWC. Bars represent geometric mean, annotations represent statistical similarity ($p = < 0.05$) generated through pairwise comparison, shaded annotations assigned to 'activity' samples, blank to 'no activity'. Table incorporated into the Figure represents percentage change between activity and no activity, concentrations during activity representing 100%, where data is available. X-axis crosses at lower limit of detection (757 CFU $m^{-3}$). Where no bar or measure of statistical similarity is presented, no samples were gained (e.g. 275 m downwind no activity).

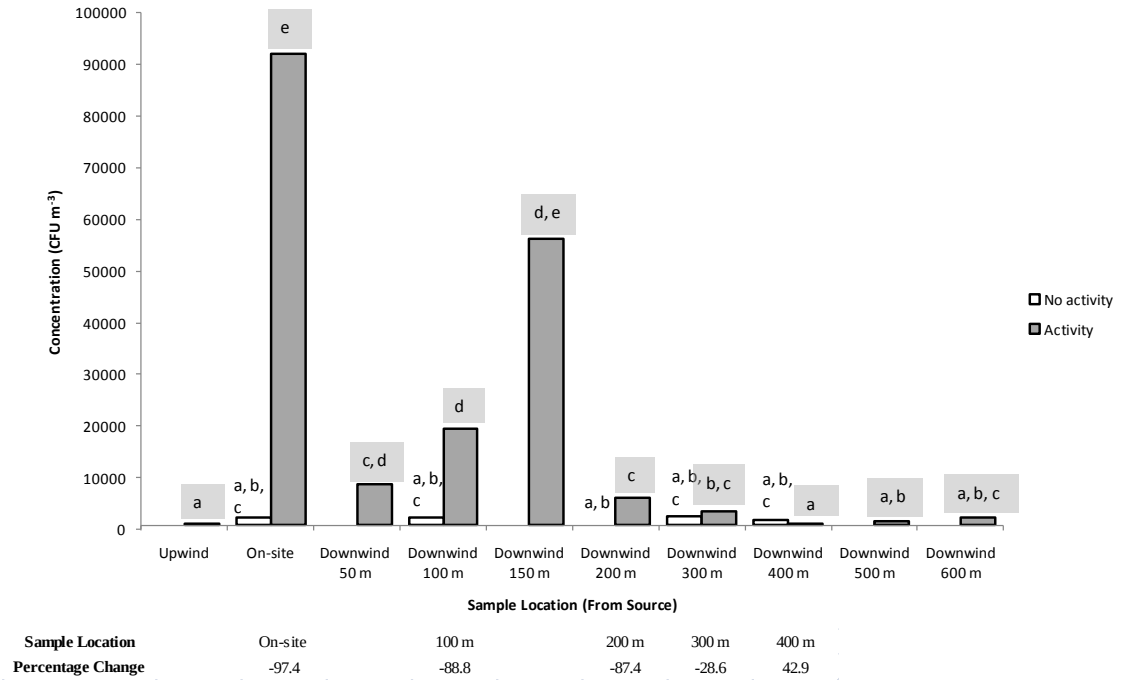



Figure 5.8: Gram-negative bacteria concentrations at Flixborough. Bars represent geometric mean, annotations represent statistical similarity ($p = < 0.05$) generated through pairwise comparison, shaded annotations assigned to 'activity' samples, blank to 'no activity'. Table incorporated into the Figure represents percentage change between activity and no activity, concentrations during activity representing 100%, where data is available. X-axis crosses at lower limit of detection (757 CFU $m^{-3}$). Where no bar or measure of statistical similarity is presented, no samples were gained (e.g. Upwind no activity).

As with *A. fumigatus* and actinomycetes, results from individual episodic sampling days are presented in Appendix I (Figures 1.1 – 1.6). Simultaneous sampling at two locations was undertaken in order to illustrate real-time differences in bioaerosol concentrations at two sampling locations, as the level of composting activity altered. The data show how response of gram-negative bacteria concentrations to the start or end of activity is slow (Appendix I, Figures 1.5 and 1.6). At both sites, there is little difference in concentrations taken during periods of no activity, although cessation of activity appears to result in a decrease in concentrations at some sampling locations at Lount

OWC, such as downwind 100 m and 200 m. Patterns are once again reflected in samples taken at the further upwind and downwind samples on individual occasions.

### 5.3.4 Endotoxins

A slightly different pattern of emission is visible for endotoxins at Lount OWC (Figure 5.9), with concentrations on-site similar whether taken during periods of activity or no activity. However, downwind samples taken during periods of no activity are statistically similar to upwind, while most downwind samples taken during periods of activity are similar to those found on-site. At Flixborough (Figure 5.10), emission and dispersal patterns are more similar to culturable microorganisms, with an increase of 97% (reaching 18 EU $m^{-3}$) between samples taken on-site during periods of activity compared to no activity. Patterns of dispersal are unclear at Lount OWC, with geometric mean concentrations during activity remaining largely similar across the dispersal profile. Samples taken during periods of activity reach levels similar to upwind at downwind 200 m and 300 m only. At Flixborough, patterns of concentrations during periods of activity are similar to those described in Chapter 4; by 150 m downwind concentrations are statistically similar to those found upwind. Percentage increases show how at Lount OWC only an 11% increase is seen on-site during activity, as opposed to 80% seen at downwind 75 m. At Flixborough, increases of over 90% were seen at on-site and 100 m downwind locations.

As found in Chapter 4, at Lount OWC concentrations are relatively low at all sampling locations. During periods of activity, arithmetic mean concentrations at 275 m downwind show the highest concentrations, being 2 orders of magnitude higher than those found at on-site locations. Although an increase is seen at this location in geometric mean values, the peak is less significant (Figure 5.9; Appendix I, Table 8.1). This dispersal pattern was previously discussed in Chapter 4. At Flixborough comparison between samples taken during periods of no activity with periods of activity is difficult due to a lack of endotoxin samples taken during no activity. Comparing the available no activity samples shows how arithmetic mean on-site concentrations are 3 orders of magnitude higher during periods of activity (Appendix I, Table 8.2). Individual sampling days for analysis of the episodic emission of bioaerosols were not

carried out; as a result data for simultaneous sampling is not presented as for the other bioaerosols.

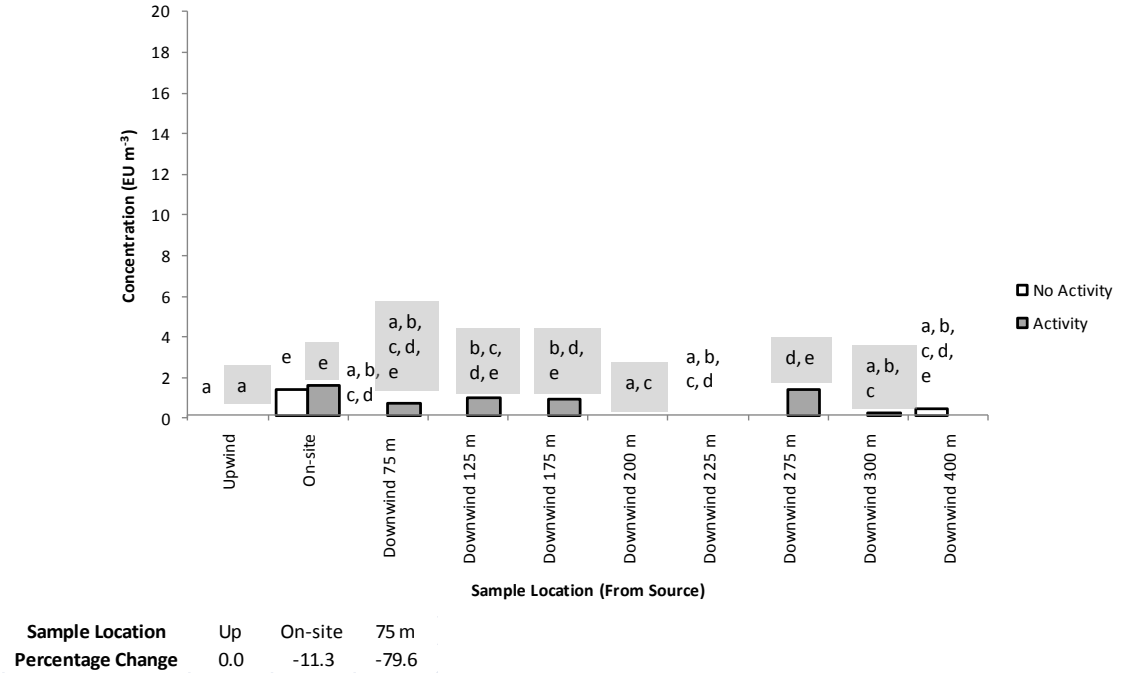
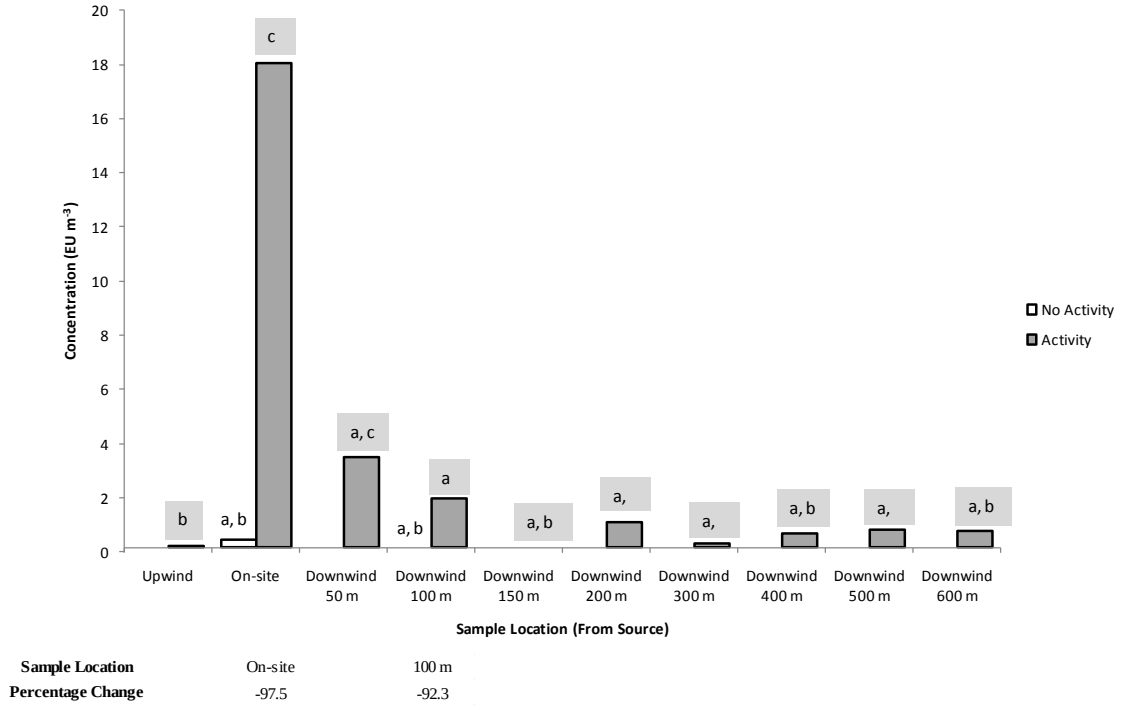



Figure 5.9: Endotoxin concentrations at Lount OWC. Bars represent geometric mean, annotations represent statistical similarity ($p = < 0.05$) generated through pairwise comparison, shaded annotations assigned to 'activity' samples, blank to 'no activity'. Table incorporated into the Figure represents percentage change between activity and no activity, concentrations during activity representing 100%, where data is available. X-axis crosses at lower limit of detection (0.152 EU $m^{-3}$). Where no bar or measure of statistical similarity is presented, no samples were gained (e.g. 275 m downwind no activity).




| Sample Location | On-site | 100 m |
|---|---|---|
| Percentage Change | -97.5 | -92.3 |

Figure 5.10: Endotoxin concentrations at Flixborough. Bars represent geometric mean, annotations represent statistical similarity ($p = < 0.05$) generated through pairwise comparison, shaded annotations assigned to 'activity' samples, blank to 'no activity'. Table incorporated into the Figure represents percentage change between activity and no activity, concentrations during activity representing 100%, where data is available. X-axis crosses at lower limit of detection (0.152 EU $m^{-3}$). Where no bar or measure of statistical similarity is presented, no samples were gained (e.g. Upwind no activity).

## 5.4 Operational Parameter Analysis Results

Using the data shown above, it was possible to include operational parameters (site activities) into the analysis, generating results assessing the impact of these parameters on bioaerosol concentrations. This analysis was carried out in the STATISTICA general linear model (GLM) (Chapter 3). Data were subjected to a natural log (Ln) transformation, enabling inclusion into the GLM alongside presence and absence data for operational activities. For example, if screening was occurring at any time during a

sample, it was classified as ‘presence’, or ‘1’, while samples taken in periods during which no screening occurred were classified as ‘absence’, or ‘0’. If during the course of one sampling period screening and shredding were present, but turning and moving were not, the sample would be assigned the code ‘1,1,0,0’. Calculation of geometric mean and the significance of differences between concentrations found during presence and absence of each operational process could then be calculated, and concentrations back transformed (exponential; Chapter 3) to give colony forming units (CFU). The results from the analysis are presented in this section. Wherever ‘significance’ or ‘statistical similarity/difference’ is referred to, p-values are less than 0.05. In-figure statistical similarity/difference data generated through the Fisher LSD test, where sampling locations are compared in pairs. Statistical similarity/difference ($p = < 0.05$) is presented for each individual sampling location compared to all other sampling locations.

### 5.4.1 *Aspergillus fumigatus*

Figure 5.11 and Table 5.1 show the impact of mechanical influences on *A. fumigatus* concentrations. At Lount OWC, screening, turning and moving all increase concentrations, although the difference in geometric mean is not significant for moving. Screening and turning result in the largest increases in concentrations, from less than $1.5 \times 10^3$ CFU $m^{-3}$ to over $3.0 \times 10^3$ CFU $m^{-3}$. Shredding, however, has a negative impact on *A. fumigatus* geometric mean, with the coefficient for this parameter showing no significant effect. At Flixborough, coefficients show how absence of shredding and front loader turning has a significant negative impact on concentrations. This is reflected in Figure 5.11, with the other mechanical parameters showing a reduction in concentrations, although coefficients reveal none of these parameters significantly affect *A. fumigatus*. Peak concentrations at Flixborough are lower than those found at Lount OWC, with a maximum of $1.8 \times 10^3$ CFU $m^{-3}$ during periods of front loader turning.

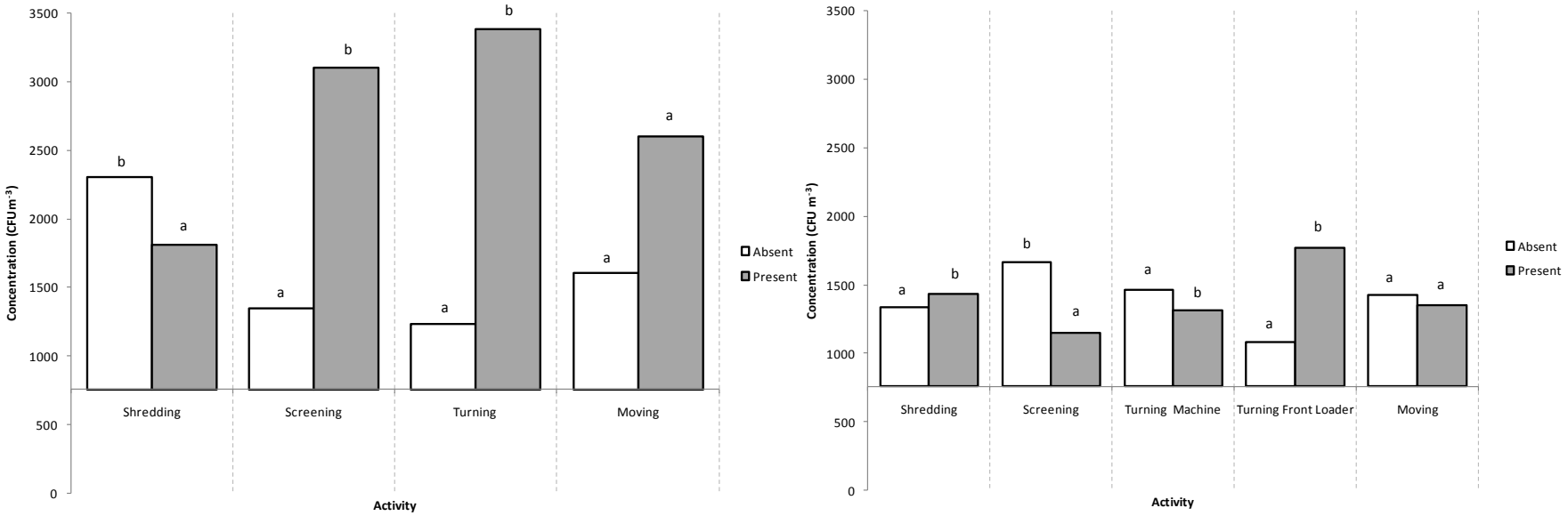
Table 5.1: Coefficients for the relationship between *A. fumigatus* and mechanical parameters at both sites. Significant coefficients are highlighted

a) Lount OWC

| Parameter | Coefficients |
|---|---|
| Shredding absent | 0.244 |
| Screening absent | -0.833 |
| Turning absent | -1.010 |
| Moving absent | -0.484 |

b) Flixborough

| Parameter | Coefficients |
|---|---|
| Shredding absent | -0.067 |
| Screening absent | 0.372 |
| Turning Machine absent | 0.110 |
| Turning Front Loader absent | -0.489 |
| Moving absent | 0.054 |




(a) Lount OWC (b) Flixborough

Figure 5.11: *A. fumigatus* concentrations during periods of presence and absence of each operational parameter at Lount OWC and Flixborough. Bars represent geometric mean, recalculated using means of environmental parameters. Annotations represent statistical similarity ($p = < 0.05$) generated through pairwise comparison. X-axis crosses at lower limit of detection, 757 CFU $m^{-3}$.

### 5.4.2 Actinomycetes

The impact of mechanical influences is shown is Figures 5.12 and Table 5.2. Patterns in the response of actinomycete concentrations to the presence or absence of activities were largely different at each site. Similarities were found for shredding, where concentrations were lower during periods of activity, although coefficients show no significant effect. At both sites presence of screening led to significantly higher quantities of actinomycetes. However, turning was also associated with higher concentrations at Lount OWC; with both types of turning and moving associated with lower concentrations at Flixborough. Figure 5.12 also shows how concentrations at Lount OWC during screening and turning activities are 1 order of magnitude higher than at Flixborough.

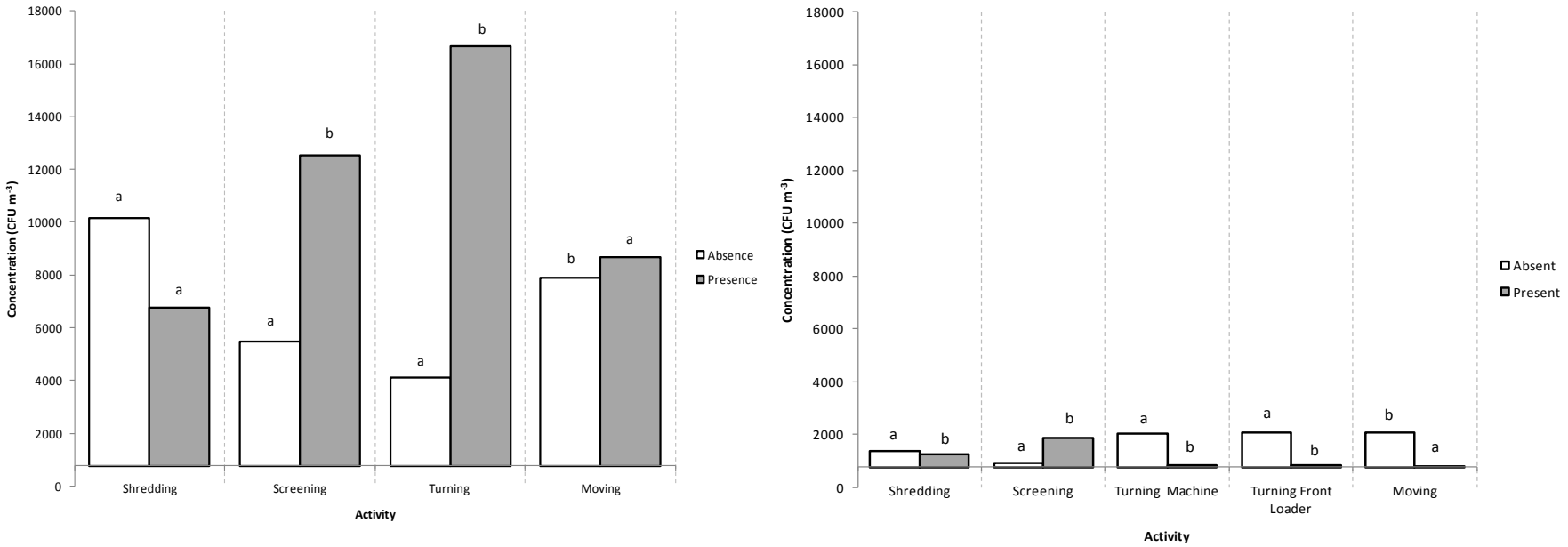
Table 5.2: Coefficients for the relationship between actinomycetes and mechanical parameters at both sites. Significant coefficients are highlighted

a) Lount OWC

| Parameter | Coefficients |
|---|---|
| Shredding absent | 0.407 |
| Screening absent | -0.826 |
| Turning absent | -1.397 |
| Moving absent | -0.094 |

b) Flixborough

| Parameter | Coefficients |
|---|---|
| Shredding absent | 0.106 |
| Screening absent | -0.724 |
| Turning Machine absent | 0.906 |
| Turning Front Loader absent | 0.933 |
| Moving absent | 0.951 |




(a) Lount OWC (b) Flixborough

Figure 5.12: Actinomycete concentrations during periods of presence and absence of each operational parameter at Lount OWC and Flixborough. Bars represent geometric mean, recalculated using means of environmental parameters. Annotations represent statistical similarity ($p = < 0.05$) generated through pairwise comparison. X-axis crosses at lower limit of detection, 757 CFU $m^{-3}$.

### 5.4.3 Gram-negative bacteria

Figures 5.13 and Table 5.3 illustrate the impact of site activities on gram-negative bacteria concentrations. Highest concentrations were found at Lount OWC, although data was largely within the same order of magnitude at both sites. Both sites showed the same relationship for screening, with significantly higher mean concentrations during screening and negative coefficients during the absence of screening showing the significance of this relationship. However, while at Lount OWC turning also resulted in significant increases in concentrations, at Flixborough shredding, both types of turning, and moving exerted a negative impact on gram-negative bacteria, although coefficients shows how the negative relationship with turning machine is not significant, despite the difference in mean values.

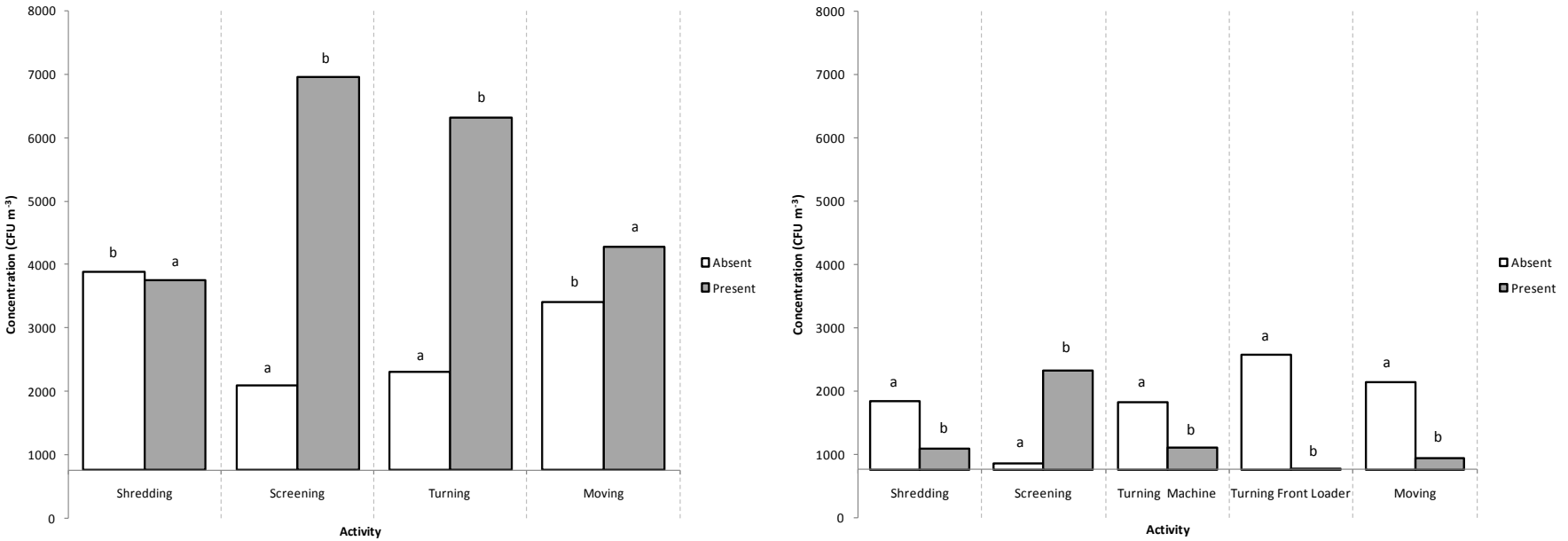
Table 5.3: Coefficients for the relationship between gram-negative bacteria and mechanical parameters at both sites. Significant coefficients are highlighted

a) Lount OWC

| Parameter | Coefficients |
|---|---|
| Shredding absent | 0.032 |
| Screening absent | -1.199 |
| Turning absent | -1.008 |
| Moving absent | -0.228 |

b) Flixborough

| Parameter | Coefficients |
|---|---|
| Shredding absent | 0.521 |
| Screening absent | -1.000 |
| Turning Machine absent | 0.502 |
| Turning Front Loader absent | 1.197 |
| Moving absent | 0.825 |




(a) Lount OWC (b) Flixborough

Figure 5.13: Gram-negative bacteria concentrations during periods of presence and absence of each operational parameter at Lount OWC and Flixborough. Bars represent geometric mean, recalculated using means of environmental parameters. Annotations represent statistical similarity ($p = < 0.05$) generated through pairwise comparison. X-axis crosses at lower limit of detection, 757 CFU $m^{-3}$.

### 5.4.4 Endotoxins

The influence of mechanical parameters on endotoxin concentrations is shown in Figure 5.14 and Table 5.4. At Lount OWC, shredding, screening and turning all led to increased levels of endotoxins, with significant differences between concentrations found during absence and presence of these activities. However, coefficients for these relationships were not significant. At Flixborough only turning showed the same relationship as Lount OWC, with coefficients showing a significant relationship between the absence of turning and endotoxin concentrations; this was also shown for the absence of moving. However, at Flixborough, coefficients also showed a significant positive relationship between the absence of screening and endotoxin concentrations. Highest concentrations at Flixborough were found during front loader turning, reaching $1.4 \times 10^4$ EU $m^{-3}$, although turning machine activity also caused an increase in endotoxin concentrations to levels significantly higher than those found during absence of this activity. Concentrations at Flixborough are higher than those at Lount, with most samples showing a difference of 3 – 4 orders of magnitude.

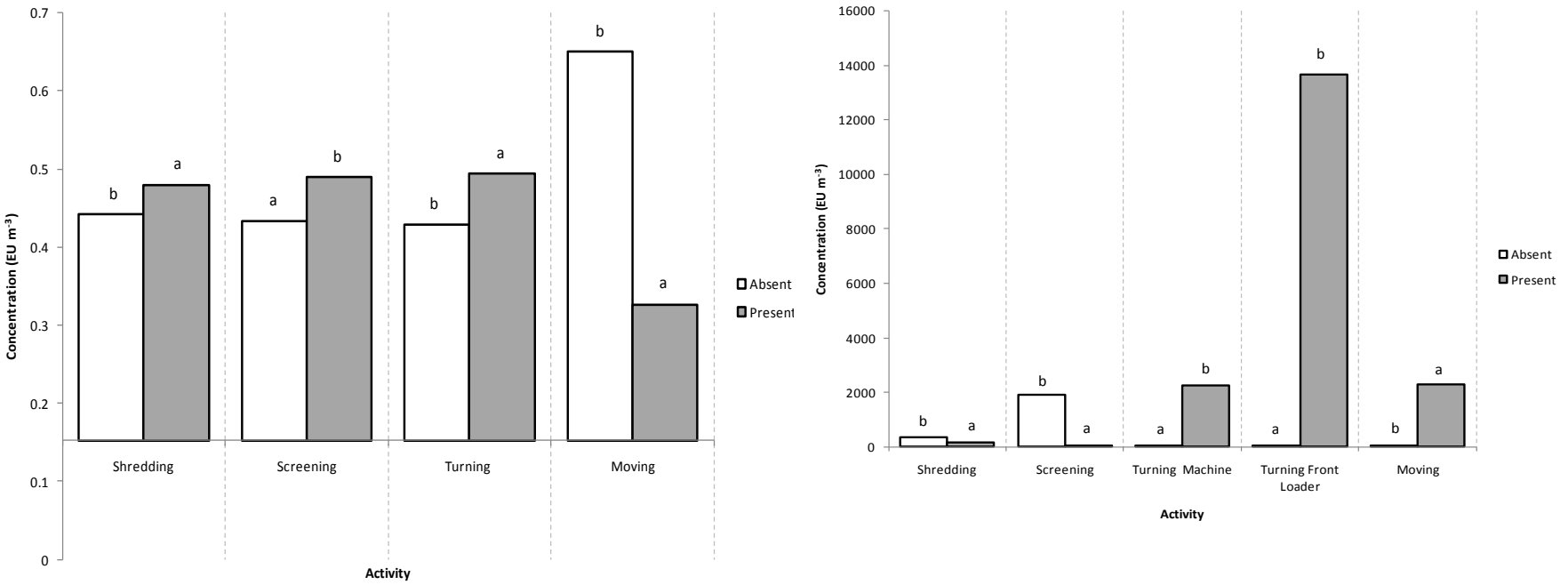
Table 5.4: Coefficients for the relationship between endotoxins and mechanical parameters at both sites. Significant coefficients are highlighted

a) Lount OWC

| Parameter | Coefficients |
|---|---|
| Shredding absent | -0.080 |
| Screening absent | -0.121 |
| Turning absent | -0.142 |
| Moving absent | 0.691 |

b) Flixborough

| Parameter | Coefficients |
|---|---|
| Shredding absent | 0.763 |
| Screening absent | 4.152 |
| Turning Machine absent | -4.473 |
| Turning Front Loader absent | -8.087 |
| Moving absent | -4.502 |




(a) Lount OWC (b) Flixborough

Figure 5.14: Endotoxin concentrations during periods of presence and absence of each operational parameter at Lount OWC and Flixborough. Bars represent geometric mean, recalculated using means of environmental parameters. Annotations represent statistical similarity ($p = < 0.05$) generated through pairwise comparison. X-axis crosses at lower limit of detection, 0.152 EU $m^{-3}$.

## 5.5 Discussion

Chapter 4 presented the dispersal profile of each bioaerosol, focusing on the patterns of dispersal found, and reasons for these patterns. The results presented within this chapter are based upon a re-analysis of the data, along with additional dedicated sampling, in order to further explore the relationship between emissions on-site, and concentrations downwind. This data could then be used to assess potential receptor exposure in best and worst case scenarios. This aim would be met through the achievement of the following objective:

i. Quantitative characterisation of bioaerosols emitted through composting activities and downwind concentrations up to and beyond 250 m from site at chosen case-study sites
    a. Assessment of the impact that episodic emission has on downwind concentrations

In order to meet this Objective data were collected aimed at quantifying both peak and trough concentrations on-site and downwind up to and beyond 250 m from site. Simultaneous, detailed observations of site activity throughout sampling occasions (Chapter 3) also allowed analysis of the impact that individual operational activities have on bioaerosol concentrations.

### 5.5.1 Limitations

The data presented here was subject to many limitations, within both sampling strategy and execution. One of the major logistical limitations was the difficulty of capturing 'no activity' samples. This was particularly difficult at Flixborough. Figures 5.15 and 5.16 below represent estimated typical daily operational activities at Lount OWC and Flixborough, based upon observations taken on each sampling day. The Figures illustrate how at Lount OWC, it was possible to collect samples during periods of no activity when sampling coincided with the workers' lunch break. However, at Flixborough sampling during periods of activity was more difficult to achieve as the

workers' employed a staggered lunch break system, preventing complete cessation of activity throughout the working day. Furthermore, at both sites no access was possible outside of working hours. This prevented sampling, for example, at the end of the working day in order to capture the reduction of concentrations to upwind levels. The result from this is either absence of activity or no activity samples at some sampling locations, or a lack of repeated samples at some locations. For example, peak concentrations were found at 75 m downwind during periods of no activity for actinomycetes at Lount OWC (Figure 5.5). This may be a result of the lack of samples taken, as only 3 were gained (Appendix I, Table 6.1), therefore the data may reflect a single 'peak' emission event (due to operational or meteorological factors), and lower confidence is held in the data shown. In effect, where few samples were gained the mean values represent a 'snapshot' of bioaerosol concentrations, the pitfalls of which were discussed in Chapters 1 and 4, as well as section 5.1 above.


Figure 5.15: Estimated typical daily operational activity at Lount OWC. Assumptions based upon site observations. Solid shaded bars represent periods of activity; empty bars represent periods of no activity; diagonally shaded bars represent the workers' lunch break


Figure 5.16: Estimated typical daily operational activity at Flixborough. Assumptions based upon site observations. Solid shaded bars represent periods of activity; empty bars represent periods of no activity

Figures 5.15 and 5.16 also illustrate another of the major limitations within this study, the difficulty of capturing samples while only one activity, or none at all, were being carried out. This limitation has mainly affected results presented in section 5.4, where some bioaerosols (Figures 5.11 – 5.14) showed higher concentrations during the absence of activity. This was particularly evident at Flixborough. Figures 5.15 and 5.16 show how opportunities to capture occasions where only one activity was being carried out were rare, leading to difficulties in the interpretation of concentrations during the absence or presence of individual activities. For example, Figure 5.13 suggests that during the absence of shredding, moving and turning gram-negative bacteria concentrations increased. However, Figure 5.16 shows how these activities were rarely carried out without other activities, such as screening (a near constant activity at Flixborough) also being present on-site. Therefore, within this example the increase during the absence of these activities may be due to an increase in concentrations caused by screening. Likewise, several activities being carried out concurrently on-site, may result in concentrations reflecting an accumulation of bioaerosols from these activities, while the analysis of data in this way suggests that concentrations during one activity result solely from that activity.

### 5.5.2 Episodic Emission and Dispersal

Despite the limitations of this study outlined above, the impact of on-site activities on bioaerosol concentrations downwind has been shown. For all bioaerosols measured, concentrations during periods of no-activity were largely statistically similar to upwind, or below detection limits (BDL) (Figures 5.3 – 5.10; Appendix I, Tables 5.1 – 8.2). This result suggests that during non-operational periods (outside of the working day) bioaerosol dispersal from site is minimal; meaning that during times of no-activity the risk of sensitive receptor exposure to elevated concentrations of bioaerosols is negligible. While dispersal patterns during periods of activity were largely similar to those found in Chapter 4, some differences in downwind concentrations were seen. For example, the peak concentrations were seen at 75 m downwind during periods of no activity for actinomycetes; and at 125 m downwind during activity for gram-negative bacteria (Figures 5.5, 5.7). However, in both cases this peak may be due to the sampling

limitations described above, with a lack of comparable samples reducing confidence in results (Appendix I, Tables 6.1, 7.1). Another minor difference was seen in that peak values at Flixborough were found on-site rather than at site boundary, which was found to be highest in Chapter 4 (Figures 5.4, 5.6, 5.8, and 5.10). This difference could be expected, as site boundary measurements were often carried out close to operational activities at Flixborough (Chapter 2), leading to re-classification of site boundary samples as adjacent to activity for the purposes of episodic analysis (Section 5.2).

Increases in the concentrations of all bioaerosols were found on-site during periods of activity, as compared to on-site during periods of no-activity and upwind concentrations. In most cases, however, these increases were less significant than those previously reported in the literature. This was the case for *A. fumigatus*, where concentrations increased by up to 1 order of magnitude (Figures 5.3, 5.4), gram-negative bacteria, where an increase of 1 – 2 orders of magnitude was observed (Figures 5.7, 5.8), and endotoxins, which showed an increase of up to 1 order of magnitude (Figures 5.9, 5.10). This is opposed to the 2 – 3 orders of magnitude difference between background and on-site, and 1 – 2.5 orders of magnitude difference in concentrations between compost processing areas reported for all bioaerosols by Schlosser *et al.* (2009), as well as the 2 – 3 order of magnitude increase reported by Taha *et al.* (2006). However, increases in concentrations in response to activity on-site were more comparable for actinomycetes, with increases of 1 - 2 orders of magnitude (Figures 5.5, 5.6) (Schlosser *et al*, 2009). As discussed in Chapter 4, the lower peak concentrations seen as compared to Schlosser *et al.* (2009) within this study may be due to differences in sampling method and site processing methods, as these were not directly comparable, leading to potential differences in sampling efficiency, yield, and concentrations returned (Chapters 1, 4). However, this cannot be said for be comparison with Taha *et al*. (2006), where sampling methods were similar.

Almost all samples taken during periods of no activity and across all culturable bioaerosols measured, showed concentrations statistically similar to those found upwind (Figures 5.3 – 5.10). For actinomycetes, however, arithmetic mean values during periods of no activity are often within the same order of magnitude as those found

during activity (Appendix I, Tables 6.1, 6.2). This may be due to the ubiquitous nature of actinomycetes, previously discussed in Chapter 4. Analysis of upwind concentrations at Lount OWC shows how samples taken during periods of activity achieved upwind activity concentrations consistently by 75 m downwind and upwind no activity concentrations consistently by 250 m downwind (Figure 5.5), reinforcing the theory that high concentrations may be found ambiently. For endotoxins, comparison between concentrations during periods of activity and no activity was hindered by the difficulty of obtaining samples during periods of no activity. This limitation was discussed in section 5.5.1 above, and was enhanced for endotoxins as fewer samples were collected for endotoxin assay than for culture-based analysis due to the collaborative nature of this work (Section 5.5.1; Chapters 2, 4; Appendix I, Tables 8.1, 8.2). However, samples that were gathered downwind during periods of no activity showed concentrations statistically similar to upwind, while those found during activity achieved this by 150 – 200 m downwind (Figures 5.9, 5.10). Dispersal distances and patterns for all bioaerosols during periods of activity are similar to those discussed in Chapter 4, and illustrate how the pattern described is due to bioaerosol emissions from site activity.

Some increases in upwind concentrations were seen during periods of activity, with the largest increase being 62% for actinomycetes at Lount OWC. This suggests that bioaerosols may also be able to disperse some way upwind during periods of activity. This has been found previously in the literature, with suggestions that sampling upwind should be done at distances over 300 m from site in order to eliminate the possibility of upwind dispersal through turbulence (Albrecht *et al*, 2008). However, despite the increases in upwind concentrations, geometric mean remains statistically similar to concentrations found during periods of no activity, illustrating how any upwind dispersal was relatively insignificant.

The presentation of individual sampling days dedicated to capturing episodic emissions was intended to illustrate the changes in bioaerosol concentrations at two locations in response to changes in site activity. However, the Figures presented (Appendix I, Figures 1.1 – 1.6) suggest a more complex relationship. Generally, patterns in concentration throughout each sampling day were the same at the two separate sampling

locations. This supports the suggestion that patterns in emission would be reflected in downwind concentrations, however, this was not clearly shown in all Figures. For example, at both sites actinomycetes responses were inconsistent, although decreases in concentrations upon cessation of activity and increases upon commencement were seen. This lack of consistency was seen within data for all bioaerosols, with changes in concentrations not always associated with changes in site activity. This result may reflect the influence of other factors, such as meteorological conditions, which potentially alter the response of downwind concentrations to changes in site activity on each sampling day (Albrecht *et al*, 2008; Fischer *et al*, 2008). The data for these Figures was based on individual sampling days dedicated to measuring episodicity. Therefore, the data once again highlights the inability of 'snapshot' samples, i.e. based on one sampling occasion, to adequately describe this relationship.

### 5.5.3 Relationships between Bioaerosols and Operational Parameters

Individual mechanical parameters have been shown to affect concentrations in different ways for each bioaerosol, and differently at each site. For most bioaerosols, however, shredding was found to cause no significant increase in concentrations (Tables 5.1 – 5.4, Figures 5.11 – 5.14). This could be anticipated, as upon receipt fresh green waste would not be expected to contain a high abundance of microorganisms associated with composting, and has been found previously for some bioaerosols (Hermann and Shann, 1997; Schlosser *et al*, 2009). It could be anticipated that mid stage composting processes (turning, screening and moving) would generate high numbers of composting bioaerosols, as the high temperatures and degradation process has encouraged the proliferation of these microorganisms (Hermann and Shann, 1997; Lacey, 1997). However, the response shown to mid stage processing activities varies between sites and bioaerosols. Turning at Lount OWC is carried out with a front-loader, so can be directly compared with front-loader turning at Flixborough (Chapter 2). Significant positive relationships between front-loader turning and culturable microorganisms, but not endotoxins, were found at Lount OWC; while at Flixborough only *A. fumigatus* and endotoxin showed significant positive relationships (Tables 5.1 – 5.4, Figures 5.11 – 5.14). It was anticipated that machine turning would also result in the generation of high

concentrations of bioaerosols, as this process is highly energetic and turning has previously been associated with increased in concentrations (Albrecht *et al,* 2008; Schlosser *et al*, 2009). This was found not to be the case for culturable microorganisms, with only an increase in endotoxin concentrations associated with machine turning (Tables 5.1 – 5.4, Figures 5.11 – 5.14). It is possible that the high energy of this process resulted in increased disruption of gram-negative bacteria and increased endotoxin emission (Dutkiewicz, 1997). However, the lack of a positive relationship with culturable microorganisms cannot be readily explained. Screening showed a significant positive relationship with actinomycetes and gram-negative bacteria at both sites; and with *A. fumigatus* at Lount OWC (Tables 5.1 – 5.3, Figures 5.11 – 5.13); once again, these relationships have been previously reported (Albrecht *et al*, 2008; Fischer *et al*, 2008; Schlosser *et al*, 2009). However, it has also been suggested that endotoxins are positively associated with screening (Schlosser *et al*, 2009), which was not found here (Table 5.4, Figure 5.14).

The variations seen between bioaerosols, sites, and individual activities may partially be due to differences in feedstock composition and condition. For example, it is known that at Flixborough the feedstock contains vegetable derived matter and is maintained at lower moisture content than Lount OWC (Chapter 2). These differences may affect bioaerosol concentrations in two ways, through differences in the microbial composition of the green waste, and through differences in emission (Jones and Harrison, 2004; Swan *et al*, 2003). Due to the lower moisture content at Flixborough, it would be anticipated that higher concentrations of bioaerosols would be found as compost processes carried out at higher moisture contents have been shown to reduce bioaerosol emissions, as dust liberation and spore release is decreased (Jones and Harrison, 2004; Epstein, 1994). However, concentrations of all culturable bioaerosols associated with activity were found to be higher at Lount OWC. Lower concentrations may have been found at Flixborough due to an artefact of the analysis. Either higher ambient levels of bioaerosol at Flixborough effectively reduced the magnitude of the effect that activities appear have on concentrations; or, as average meteorological parameters were assumed for this analysis, emission at Flixborough is strongly related to meteorological conditions not described here (Albrecht *et al,* 2008; Fischer *et al,* 2008). Once again the

lack of refinement and control within this study prevented the accurate enumeration of bioaerosols emitted through different composting processes.

The inconsistency of trends shown reduces confidence in their interpretation, suggesting that variables not accounted for within this analysis are affecting relationships between bioaerosol concentrations and individual site activities. The limitation described in section 5.5.1 may also have affected the results presented here, as two or more compost processing activities may run simultaneously, and where a sample is, for example, described as 'absence of turning', the concentrations recovered may reflect the presence of other activities (Figures 5.15, 5.16). As samples were taken within an operational composting facility with no control over processing activities, post-hoc analysis attempting to analyse the effect of individual activities was applied. The inability of this analysis to show reliable patterns in emission of bioaerosols from individual activities highlights the difficulty of this type of analysis. There remains a requirement for more dedicated and controlled studies into the factors affecting the emission of different bioaerosols from different activities, such as compost age, condition, and feedstock composition.

### 5.5.4 Potential Receptor Exposure

In terms of potential receptor exposure, this data demonstrates that excursions above upwind concentrations and Environment Agency recommended limits (discussed in full in Chapters 1 and 4) (Environment Agency, 2001a; Environment Agency, 2009a, b) are due to site activities, as samples taken downwind during periods of no activity were rarely statistically different from upwind levels. Therefore, it can be said that sensitive receptors present at the 250 m limit or beyond may be periodically exposed to concentrations of bioaerosols significantly higher than those found upwind, with these exposures dependent on site activity. For example, at 275 m downwind from source, *A. fumigatus* at Lount OWC can be found at $2.6 \times 10^3$ CFU $m^{-3}$ (Figure 5.3), while 300 m downwind from source at Flixborough, gram-negative bacteria can be found at $3.4 \times 10^3$ CFU $m^{-3}$ (Figure 5.8). While within the same order of magnitude as Environment Agency guideline concentrations (Environment Agency, 2009a, b), as no dose-response

data is available it cannot be said how much of an additional risk these concentrations may constitute. Furthermore, as these figures represent geometric mean, maximum concentrations may be found at higher concentrations. These data are shown in Appendix I (Tables 5.1 – 8.2). For the two sampling locations described above, maximum concentrations reach $1.2 \times 10^5$ CFU $m^{-3}$ and $2.4 \times 10^5$ CFU $m^{-3}$ respectively; levels 2 orders of magnitude higher than recommended Environment Agency concentrations (Environment Agency, 2001a; Environment Agency, 2009a, b). These data further illustrate how during periods of activity, bioaerosols may disperse to distances greater than 250 m from emission source. As emission source is often adjacent to site boundary (Chapter 2), this also implies that dispersal to distances greater than 250 m from site boundary is possible. This implication was shown to be valid through analysis of data in Chapter 4, where downwind distances were calculated from site boundary, with elevated concentrations found to distances similar to those reported here.

These data suggest that during non-operational periods (outside of the working day) the risk of bioaerosol dispersal in elevated concentrations beyond 250 m from site boundaries is minimal. Other than 75 m downwind for actinomycetes at Lount OWC, all downwind concentrations during periods of no activity were BDL, or statistically similar to those found upwind. Consequently, it can be said that static windrows pose little risk to sensitive receptors in terms of bioaerosol emission and downwind concentrations. This may have implications for the analysis of risk at composting facilities, as sensitive receptor exposure to bioaerosols during non-operational hours is potentially of little concern. Flixborough is an example of a site where these findings could affect assessment of risk, as workforces surround the site and may be exposed to elevated concentrations of bioaerosols during operational hours (discussed in more detail below). However, potential residential exposure could be low, as residents are likely to be frequently absent during operational hours. Likewise, when residents are present, such as evenings and weekends, the risk of exposure to elevated bioaerosol concentrations is minimal. Although, it must be acknowledged that more vulnerable members of the community, such as the elderly and very young, may be present during operational hours.

The data presented here has gone some way towards quantifying potential receptor exposure, illustrating how exposure is likely to be low during periods of no activity, with exposure to elevated concentrations of bioaerosols likely during periods of activity. However, downwind peaks remain transitory, illustrated through the variability of concentrations within the data presented, along with results shown in Chapter 4. At Lount OWC transient downwind peaks are unlikely to result in actual receptor exposure, as there are no sensitive receptors within 500 m of the site (Chapter 2) and even during periods of activity all bioaerosols achieved concentrations statistically similar to those found upwind by 400 m downwind (Figures 5.3 – 5.10). At Flixborough, however, there are many opportunities for receptors to be exposed to elevated bioaerosol concentrations, with workplaces surrounding the site from approximately 50 – 500 m to the north and east, while a residential area may be found 250 m to the west (Chapter 2). Although most workplaces are enclosed providing some form of protection through provision of a barrier between workers and bioaerosols, the presence of a café approximately 125 m from the north-eastern site boundary and a mobile catering vehicle approximately 220 m from site means that unprotected workers are often present in the surrounding streets and car parks. As a result, the likelihood of actual sensitive receptor exposure to elevated bioaerosol concentrations during operational hours is high. Figure 5.17 below illustrates the location of these potential points of exposure in relation to site boundary.

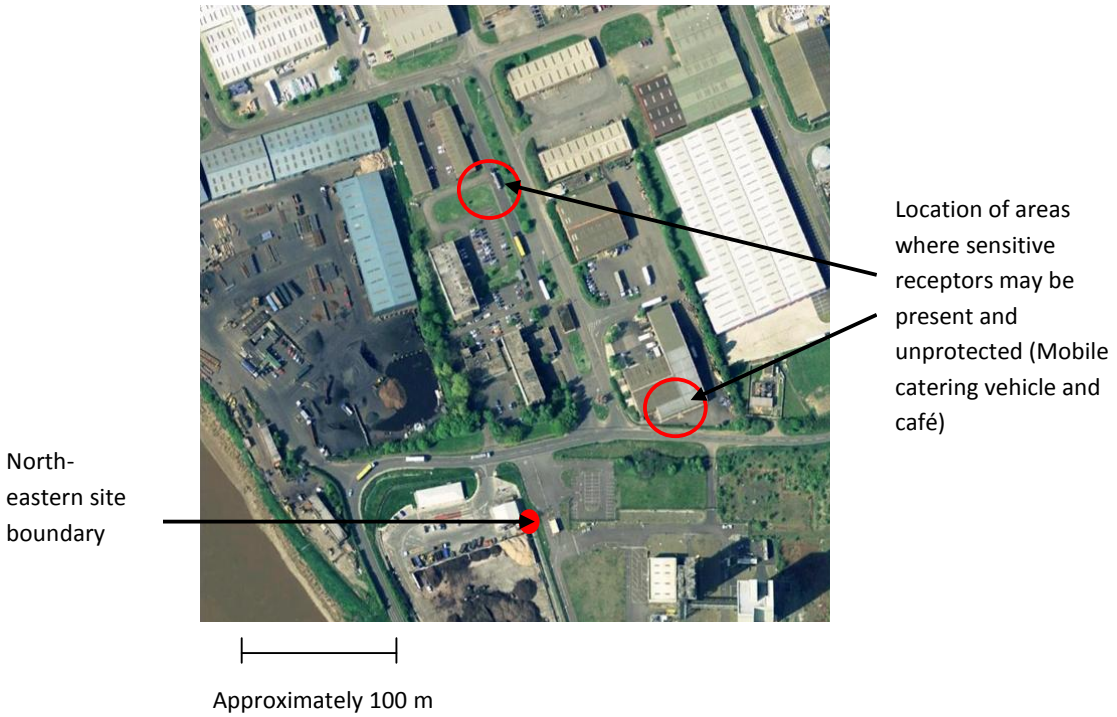



Figure 5.17: Aerial photograph of Flixborough, annotated to illustrate location of areas where unprotected sensitive receptors may be present and the site boundary (Infoterra Ltd and Bluesky and Tele Atlas, 2009).

This study aimed to contribute further to knowledge surrounding potential receptor exposure through quantification of the response of downwind bioaerosol concentrations to changes in operational activity on-site. In addition, an analysis aiming to determine the contribution of each type of activity carried out on-site to bioaerosol concentrations was carried out (Section 5.5.3). The results from these two analyses were not easily interpretable. This was due to the limitations described in section 5.5.1, including the difficulty of capturing samples during periods of no activity, and the difficulty of separating the impact of each operational activity as most were carried out alongside at least one other activity. Figure 5.18 below reproduces the pictorial re-presentation of the hypothesis that 'episodic emission leads to episodic dispersal', also shown in section 5.1. The Figure illustrates how it was anticipated that a single emission event on-site would also be able to be detected downwind, at locations where sensitive receptors may be exposed.

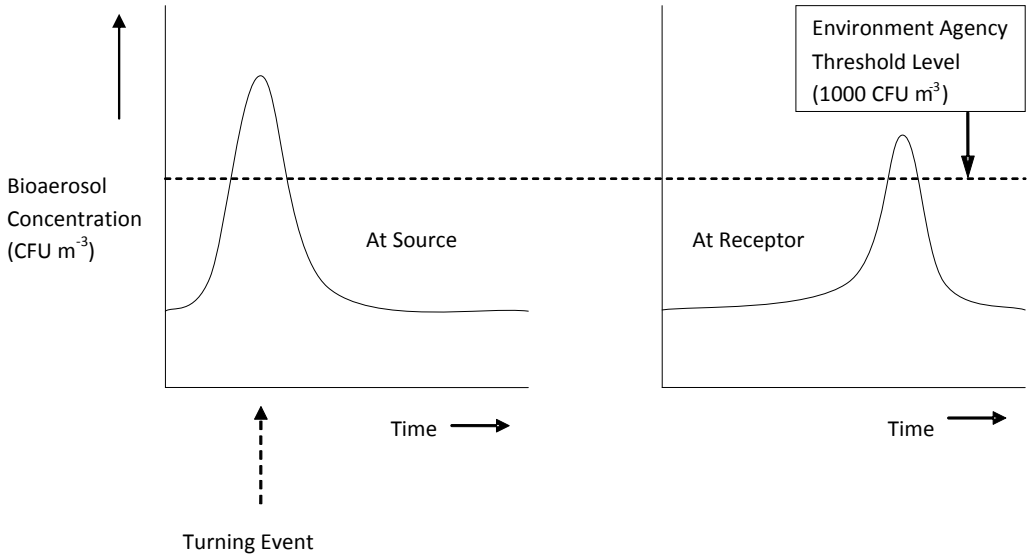



Figure 5.18: Revised pictorial representation of 'episodic emission leads to episodic dispersal' hypothesis.

However, through analysis of the data presented as part of this Chapter, it has become apparent that fluctuations in bioaerosol concentrations are not easily predictable. This is largely due to the fact that activities on-site often run concurrently, and that other factors, such as compost age, feedstock, and meteorological conditions may also affect downwind concentrations of bioaerosols and were not accounted for as part of this study (Albrecht *et al*, 2008; Fischer *et al*, 2008). This information can contribute to a new conceptual model for the relationship between site emissions, episodicity, and downwind concentrations, illustrated through Figure 5.19. The key differences are that activities run concurrently at the sites studied, leading to a 'blurring' of episodic releases on-site, and preventing the separation of individual episodic emissions. The result of this is shown as an increase in downwind concentrations during periods of activity, with periodic increases to concentrations above those recommended by the Environment Agency (Environment Agency, 2001a; Environment Agency, 2009a, b). If this is the case, the strong relationship between downwind elevated concentrations and site activities also suggests that at busy centralised sites, such as the case-study sites used here, worst-case receptor exposure may be present throughout the whole working day. However, downwind concentrations are not clearly traceable back to episodic

source on a day-to-day basis utilising the methods employed within this study, due to the issues of 'snapshot' sampling (Section 5.5.1, Chapters 1, 4).

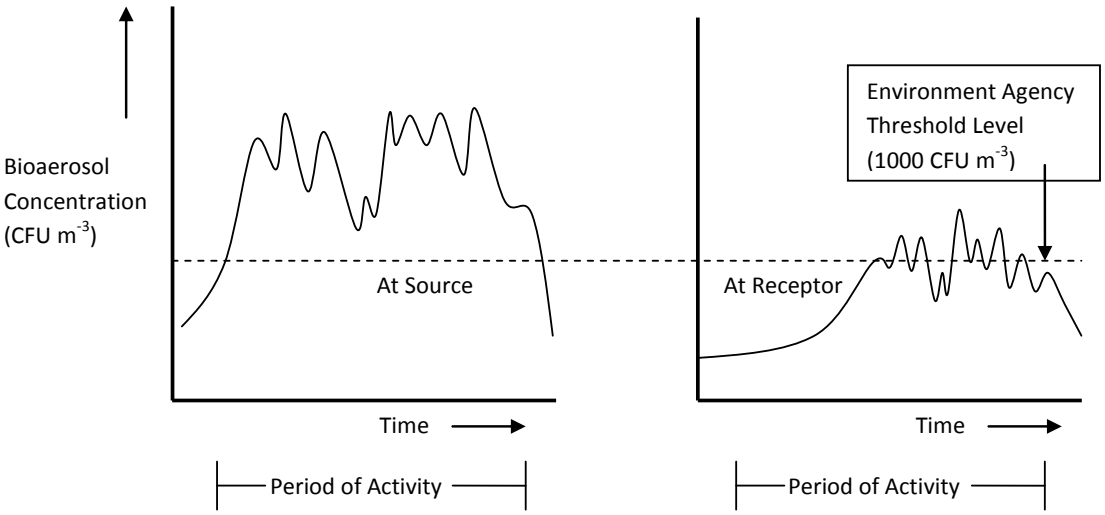



Figure 5.19: Pictorial representation of modified 'episodic emission leads to episodic dispersal' hypothesis.

Combining Figure 5.19 with the suggestion that concentrations on-site and downwind from site may be elevated throughout the working day, with relatively insignificant emissions from static windrows, can be expressed through the conceptual model below. Figure 5.20 illustrates how over a 24 hour period, bioaerosol concentrations may alter according to site activities.

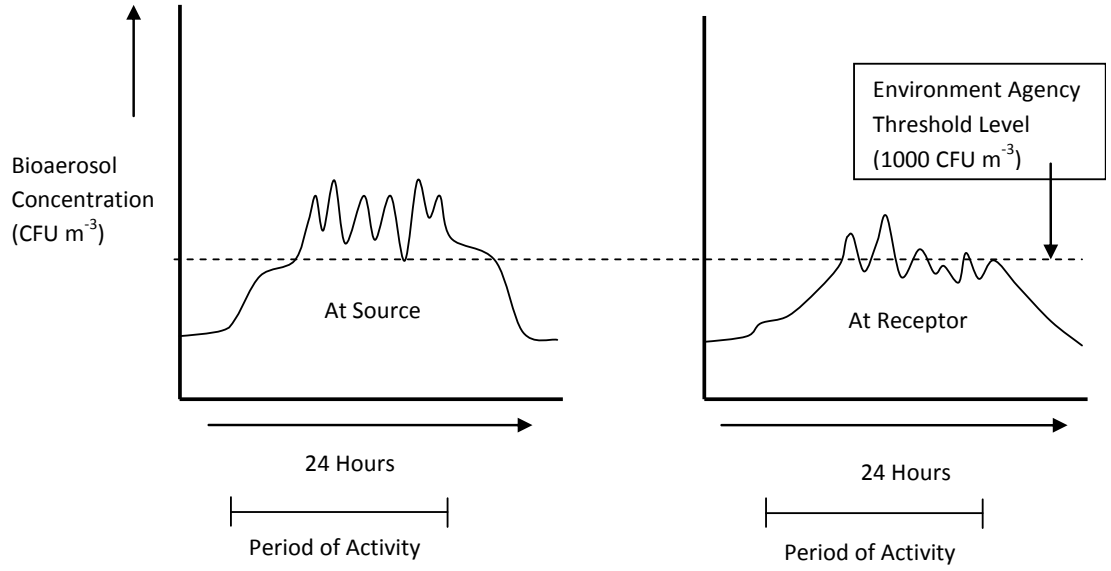



Figure 5.20: Pictorial representation of conceptual model illustrating hypothetical bioaerosol concentrations over a period of 24 hours.

However, although this study has contributed to understanding of the reasons for elevated concentrations being found downwind from composting facilities, a considerable amount of variability remains unaccounted for. This was particularly illustrated by the inability to account for variations seen in bioaerosol concentrations between different activities, and the presence or absence of these activities (section 5.5.3). As discussed in Chapters 1 and 4, accounting for variability in bioaerosol concentrations is of interest as it may allow determination of both best- and worst-case scenarios for concentrations downwind, and the causes of these scenarios. It has been suggested previously within the literature that environmental parameters, such as season and meteorological conditions will also affect the concentrations of bioaerosols found downwind from composting facilities (Albrecht *et al*, 2008; Fischer *et al*, 2008; Recer *et al*, 2001). In a continued attempt to account for this variability, the relationships between bioaerosols and environmental parameters are explored in Chapter 6.

## 5.6 Conclusions

The main conclusions gained through this study are summarised below.

- Composting activities are responsible for downwind patterns in concentrations seen, including buoyancy, and elevated downwind concentrations
- During periods where no activities are being carried out on-site, concentrations are typically below detection limits (757 CFU $m^{-3}$) at downwind locations
- Mid- and late-stage, and more vigorous composting activities result in the largest increase in bioaerosol emissions, including endotoxins
- Periodic sensitive receptor exposure to elevated concentrations of bioaerosols at distances beyond 250 m from source may be a direct result from composting activities
- Static windrows are a relatively insignificant source of bioaerosols, with the risk for sensitive receptor exposure to elevated concentrations of bioaerosols minimal during non-operational hours

The results presented have shown the impact that activities have on bioaerosol emissions and downwind dispersal, with downwind dispersal patterns and ranges shown to be attributable to site activity. One of the major limitations to this study was the inability to separate the impact of individual activities on bioaerosol concentrations. This prevented higher resolution analysis of the impacts which individual site activity and combinations of activity have on downwind concentrations and potential exposure. The lack of repeated samples was also an issue, caused by an inability to gain samples during periods of no activity. However, this finding has also highlighted how intense activity at centralised composting sites may lead to frequent excursions of threshold values at distances over 250 m from site throughout a working day; although outside of operational hours potential sensitive receptor exposure is likely to be minimal. This study was also limited by the same factors discussed in Chapter 4, namely, the relatively high detection limit of this method. Relationships between individual activities and bioaerosols also showed some variability. This is likely to be due to individual site differences, which may affect emissions from different operational activities. However,

results were broadly as expected with increased emissions of bioaerosols in mid- to late-stage composting processes and associated with vigorous activities such as turning and screening.

For future studies the outcomes of this project can be used to make several recommendations. More detailed and dedicated studies aimed at quantifying the individual impacts of each operational activity on bioaerosol concentrations are required. Controlled study of the effect that each activity has on bioaerosols would remove the need for post-hoc attempts to separate these effects, increasing accuracy. As shown through this study, the post-hoc analysis is unable to adequately describe the effects of different operational activities on different bioaerosols. The gathering of further data quantifying bioaerosol concentrations during non-operational hours would allow more detailed evaluation of risk to sensitive receptors during best-case exposure scenarios. In addition, while it has been shown that sensitive receptors may be affected by episodic dispersal, therefore studies utilising more sensitive methods at receptor would allow further quantification of exposure.

The hypothesis that episodic emission will be shown to be responsible for downwind transient peaks in bioaerosol concentrations has been supported, although this relationship was not as definitive as was anticipated. The study was limited in many ways, with further, dedicated investigation required to analyse the effect of different combinations of activities on downwind concentrations. Despite these limitations, the impact of activities on downwind concentrations is clear, although variability remains high. The relationship of bioaerosols with operational activities appears to be affected by other variables, such as meteorological factors, feedstock, and moisture content of compost. The following Chapter attempts to further this work through analysis of the effect that environmental parameters have on bioaerosol concentrations, and how these factors also contribute to the variability seen in concentrations.

# 6 The Impact of Environmental Parameters on Bioaerosol Concentrations

## 6.1 Introduction

It has been suggested through many studies that the emission and dispersal of bioaerosols from composting facilities is affected by environmental conditions. Changes in conditions such as temperature and wind speed may result in differences in emission, dispersal pattern and range, as well as viability (Albrecht *et al*, 2008; Fischer *et al*, 2008; Griffiths *et al*, 2001; Grinn-Gofroń and Strzelczak, 2008; Harrison *et al*, 2005; Jones and Harrison, 2004; Marthi *et al*, 1990; Reinthaler *et al*, 1997; Schlosser *et al*, 2009; Tham and Zuraimi, 2005; Vega-Maray *et al*, 2003; Walter *et al*, 1990). Bioaerosol composition and concentration is also shown to alter according to season (Bauer *et al*, 2008; Dutkiewicz, 1997; Jones and Cookson, 1983; Nielsen *et al*, 1997; Recer *et al*, 2001; Schlosser *et al*, 2009; Vega-Maray *et al*, 2003). However, the nature of these relationships and how they affect bioaerosols emitted through the composting process remains uncertain.

Chapters 4 and 5 have both shown a high level of variability within bioaerosol concentrations. Although Chapter 5 has gone some way towards describing this variability, there remain discrepancies within the results. In particular, bioaerosols did not always respond to individual site activities as was anticipated (section 5.5.3). Results from Chapter 5 also suggested that activities throughout the course of a day result in an increase in bioaerosol concentrations, with levels periodically increasing above recommended limits downwind from site (section 5.5.4; Figure 5.26). This was also shown by Chapter 4, where bioaerosol concentrations could be found within a wide inter-quartile range, with outlying and extreme concentrations also found downwind from site (section 4.4). Although elevated concentrations were related to site activities, reasons for these outlying and extreme values, as well as excursions above threshold values, remain unclear. Taking into account the suggestion that environmental conditions may affect the type and concentration of bioaerosols emitted, and their

dispersal and deposition, some of this variability may be accounted for through analysis of the impact that environmental parameters have on bioaerosol concentrations. Understanding of the causes of worst-case bioaerosol dispersal and peak downwind concentrations is necessary in order to allow site management and operational activities to be managed accordingly; with the aim of minimising the risk of sensitive receptor exposure to elevated concentrations of bioaerosols.

The current uncertainty surrounding the impact of environmental conditions may partially be due to the experimental design typically applied to investigate composting bioaerosols. As shown in Chapters 1, 4, and 5, sampling of bioaerosol concentrations at composting facilities is often carried out on a 'snapshot' basis, reducing the ability to capture transient peaks in concentrations that may be attributable to both operational and meteorological conditions (Recer *et al*, 2001). In addition, these relationships are known to be complex, with each bioaerosol likely to respond in different ways to the same environmental influences. This is due to the individual physicochemical properties that each bioaerosol possesses, affecting its interactions with the surrounding environment. Properties implied in these interactions include hydrophobicity, aerodynamic diameter, oxygen sensitivity, and electrical charge. These physicochemical properties lead to a different interaction with parameters such as relative humidity, temperature, pressure, air currents, UV radiation and other airborne particles. Knowledge of these properties for composting bioaerosols, and how these bioaerosols interact with the environment, is severely limited. As a result, investigations attempting to understand these interactions often reduce complexity through quantification of either indoor bioaerosols or focusing on specific species (Cole and Cook, 1998; Colls, 2002; Mainelis *et al*, 2002; Marthi *et al*, 1990; Tham and Zuraimi, 2005; Walter *et al*, 1990). There are some studies that have attempted to quantify the impact of environmental parameters on both fungal spores and airborne bacteria in outdoor environments (Grinn-Gofroń and Strzelczak, 2008; Harrison *et al*, 2005; Ho *et al*, 2005), with the finding that ambient bioaerosol concentrations are affected by factors including wind direction, air temperature and relative humidity.

While these studies have been carried out using meteorological data collected from automated weather stations, most studies that have qualified or quantified the behaviour of bioaerosols released from composting facilities analyse the response to environmental parameters in a post-hoc fashion. This means that spot meteorological and concentration data has been collected simultaneously, then analysed (Schlosser *et al,* 2009). The accuracy of the analysis is therefore reduced through the inevitable introduction of other variables, such as composting green waste condition, composition, and site operational activities. However, it has been suggested that due to the small spatial scale of bioaerosol sampling (typically less than 1 kilometre from site), sampling strategies based on site specific sampling meteorological measurements are more applicable (Albrecht *et al*, 2008; Fischer *et al*, 2008).

Despite limitations within sampling strategies, it is accepted that environmental conditions significantly affect bioaerosols emitted through composting, with relationships encompassing the emission, dispersal and survival of bioaerosols. For emission, the depth of penetration of wind into compost surface has been shown to alter *Aspergillus fumigatus* liberation from green waste by up to 1 order of magnitude (Fletcher *et al,* 2008). The release of microorganism spores and cells is also affected by other environmental parameters, with tension and bonding forces affected by interactions between the surrounding air and surface itself. Temperature, moisture, and radiation of both the surrounding air and surface have also been shown to result in changes in tension and bonding forces, leading to the release of particles (Gorny, 2004; Jones and Harrison, 2004). This has been demonstrated within the literature, with drier compost associated with increases in dust and bioaerosol emission (Epstein, 1994). In terms of dispersal, it has been shown that under certain meteorological conditions, such as air drainage flow conditions, bioaerosols can be transported up to 1000 m from site (Albrecht *et al,* 2008; Fischer *et al*, 2008); while Reinthaler *et al.* (1997) found that peak fungal counts could be related to wind speed. It has also been suggested that environmental parameters such as relative humidity and temperature are likely to affect culturability of *A. fumigatus* (Ho *et al,* 2005); while solar radiation, desiccation through interaction with other airborne particles, and extremes of temperature may cause rapid loss of bacterial bioaerosol viability (Harrison *et al*, 2005; Korzeniewska *et al,* 2009;

Lighthart and Mohr, 1987; Marthi *et al*, 1990; Tham and Zuraimi, 2005). Likewise, some environmental conditions have been found to preserve viability, such as relative humidity from 70 – 80%, and temperatures under 12°C (Marthi *et al*, 1990; Walter *et al*, 1990). However, little is known regarding the relative importance of the effects that individual environmental conditions have on the emission, dispersal, or viability of bioaerosols. In addition, the evidence is often contradictory. While some authors have suggested that high relative humidity increases desiccation of bioaerosols and therefore reduces concentrations (Lighthart and Mohr, 1987; Tham and Zuraimi, 2005), others suggest that high relative humidity is necessary both for the aerosolisation and preservation of bioaerosols (Griffiths *et al*, 2001; Marthi *et al*, 1990; Seinfeld and Pandis, 2006).

Bioaerosol concentrations also vary seasonally, with studies reporting higher concentrations throughout the summer and autumn months (Nielsen *et al*, 1997a; Recer *et al*, 2001; Schlosser *et al*, 2009). It has been suggested that different bioaerosols respond differently to changes in season. For example, all bioaerosols have been shown to decline in winter, with declines for actinomycetes shown to be less significant (Recer *et al*, 2001). Once again, the reasons for seasonal differences are not clear. Site influences, including operational activities, may result in seasonal trends (Recer *et al*, 2001). For example, microorganism composition in green waste is known to alter according to the stage of composting (Amir *et al*, 2008; Cahyani *et al*, 2002; Hermann and Shann, 1997; Steger *et al*, 2005), and this may lead to differences in the emission of different bioaerosols as composting progresses. Changes in throughput of green waste over the course of a year (Chapter 2) are therefore also likely to result in changes in composting microbial populations; for example, through prolonging of the mesophilic stage during times of low site throughput the microorganisms associated with this composting phase will proliferate.

The data available within the literature illustrates how the interaction of bioaerosols with environmental parameters is complex. Figure 6.1 below presents a conceptual model of these influences, in order to summarise the information above.

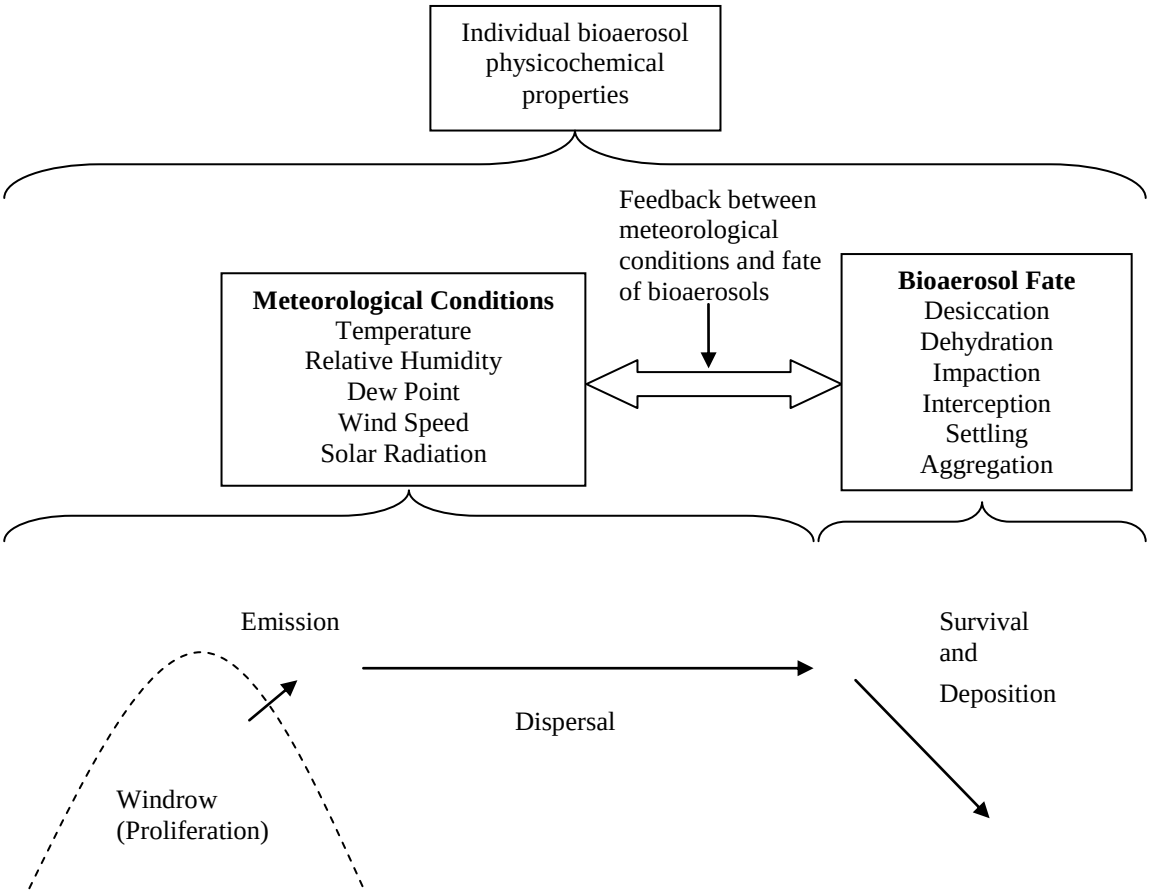



Figure 6.1: Simple conceptual model of bioaerosol emission, dispersal and deposition from composting facilities, and the environmental conditions that may affect these processes.

As shown through the brief review above and within Chapter 1, there is compelling evidence that bioaerosol concentrations will be affected by environmental conditions. However, the precise nature of these relationships remains uncertain. In particular, it is acknowledged that each bioaerosol will possess a different range of physicochemical properties. Despite this, studies comparing the different responses of, for example, fungi and bacteria or different species of bacteria, to environmental conditions are uncommon, with most investigations focusing on specific microbial species. In addition, the lack of validated and statistically significant data concerning composting bioaerosols (Chapter 1) has limited ability to determine the nature of the individual bioaerosols response. It may therefore be possible to increase understanding of bioaerosol concentrations downwind from site and the reasons for excursions above

threshold and upwind values (Chapters 4 and 5) through analysis of the impact of environmental parameters.

The collection of data regarding meteorological parameters within this study was outlined in Chapter 3, with local meteorological conditions only accounted for. In addition, measurement of some parameters, such as cloud cover, was subjective, although this subjectivity was reduced as the same person estimated cloud cover on each sampling day. Preferably, weather stations provided by the Meteorological Office (Met office) should be used to provide long-term and reliable data (Grinn-Gofroń and Strzelczak, 2008; Harrison *et al*, 2005; Ho *et al*, 2005). Two Meteorological stations can be found approximately 9 miles either west or north-east from Lount OWC, while the closest two to Flixborough can be found 2 miles north-east or and 32 miles to the south (Met Office, 2009). However, use of these monitoring stations may not allow full understanding of bioaerosol emission and dispersal from composting facilities due to the influence of site specific local meteorology. As shown in Chapters 4 and 5, bioaerosols were measured up to 600 m from site, making understanding of local microclimate potentially more important than the wider environmental conditions. In addition, site specific features such as the location of Flixborough adjacent to an estuarine river, will reduce comparability of the microclimate to regional environmental conditions (Chapter 2). It has been suggested that measurement of specific microclimate and meteorological conditions should be considered of high importance within bioaerosol sampling strategies (Albrecht *et al*, 2008; Fischer *et al*, 2008). Therefore, only spot measurements of environmental conditions with a handheld device (Chapter 3) at the time of sampling were obtained.

As shown above, the interaction of environmental parameters with bioaerosols is highly complex. Therefore complete analysis of these interactions was impossible within the time-frame available to this project. While this study was not aimed at providing in-depth analyses of the impact that environmental parameters have on bioaerosol concentrations, it was possible to contribute to this area of knowledge through incorporation of meteorological conditions into the analysis. Although meteorological data was limited through methodology, no other studies, to the author's knowledge,

have been able to apply site-specific meteorological data to the analysis of a large and validated dataset, as created through this project. This will allow the impact of environmental parameters on bioaerosol concentrations to be interpreted with more confidence.

This phase of the project was aimed at meeting thesis Objective i (b):

i. Quantitative characterisation of bioaerosols emitted through composting activities and downwind concentrations up to and beyond 250 m from site at chosen case-study sites
    b. Assessment of the relationships between bioaerosols and environmental conditions

Through Chapter 5, it has been shown that site activities result in elevated bioaerosol concentrations downwind from site. However, the review above illustrates how environmental conditions may also affect downwind concentrations. Therefore, the research question to be answered through this study is; do environmental influences significantly affect bioaerosols independently from and alongside operational parameters? It can be hypothesised that environmental parameters, particularly temperature, relative humidity, and wind speed, will have a significant impact on bioaerosol concentrations (Fletcher *et al*, 2008; Grinn-Gofroń and Strzelczak, 2008; Ho *et al*, 2005; Schlosser *et al*, 2009); although site activities will remain the most important factor determining bioaerosol concentrations (Chapter 5). Other parameters, such as wind direction, may have a more site-specific influence depending upon the potential for alternative bioaerosol sources (Harrison *et al*, 2005).

## 6.2 Experimental Design

Methods used in order to sample on-site and process samples followed the general methodology described in Chapter 3. Sampling at Lount OWC took place from September 2007 until May 2008. Sampling at Flixborough took place from August 2008 until July 2009. A full list of sampling dates can be found in Appendix I.

### 6.2.1 Meteorological Conditions

Several meteorological parameters were measured at each site. These were wind speed, temperature, relative humidity and cloud cover. In addition, dew point was measured at Flixborough. A summary of weather conditions, and how interactions between weather conditions affect statistical analysis, is presented here.

Median wind speeds and range were highest at Lount OWC during spring and lowest during summer. At Flixborough, highest median values were found during autumn. These data are similar to climatic data from the UK Meteorological Office, which suggests that lowest wind speeds can be found in summer, with an increase in wind speed in autumn (Met Office, 2009). As could be anticipated, at both sites the highest median air temperatures were found during summer and lowest during winter, this is also reflected in data gathered by the nearest weather stations to each site, from the period of January 2007 – November 2009 (Met Office, 2009). Dew point temperature, measured at Flixborough only, was also found to follow air temperature patterns. At Lount OWC lowest relative humidity was found during summer, with relative humidity at Flixborough lowest in spring and summer. Lowest cloud cover estimations were found during winter at Lount OWC, and spring at Flixborough. Samples were collected over the course of a year at both sites, although more samples were taken during summer at Lount OWC and spring at Flixborough.

In order to analyse the relationship between environmental conditions and bioaerosol concentrations, it was necessary to test the correlations between continuous environmental parameters measured. Correlating environmental parameters can affect

the analysis of bioaerosols within the STATISTICA General Linear Model (GLM) (Chapter 3); positive correlations between parameters can result in an enhanced effect being shown for the parameter and the bioaerosol being analysed; while negative correlations can 'cancel' each other, leading to no significant relationship being shown between the parameter and the bioaerosol. For example, as temperature increases relative humidity decreases. It is desirable to know the effects of these parameters on individual bioaerosols. However, analysing their effect on a bioaerosol at the same time will show no significant impact on the bioaerosol as the parameters 'cancel' out each other's effect. If analysed separately, it may be found that the parameters still have no significant effect, or it may be found that one or both of the parameters individually have a significant impact on the bioaerosols' concentrations. Therefore separate analyses should be carried out in order to fully understand the interaction of the bioaerosol with meteorological parameters (Chapter 3).

Tables 6.1 and 6.2 show parameter coefficients for environmental variables at both sites, with significant correlations highlighted. At Lount OWC significant correlations exist between all environmental parameters apart from between temperature and cloud, and wind speed and cloud. At Flixborough Table 6.2 shows how, once again, most parameters are associated with each other; only wind speed and cloud, and wind direction and dew point show no significant correlation. Taking into account these relationships, for both sites separate analysis excluding each environmental parameter in turn must be carried out in order to determine whether correlation between different environmental parameters is significantly affecting the results generated as part of this analysis. Without accounting for these correlations, false positive or negative relationships between environmental parameters and bioaerosols may be generated.

Table 6.1: Correlation coefficients for continuous environmental parameters at Lount OWC. Significant negative correlations highlighted.

| | Wind direction (°) | Wind speed | Temperature (°C) | Humidity (%) | Cloud (%) |
|---|---|---|---|---|---|
| **Wind direction (°)** | 1.000 | | | | |
| **Wind speed** | -0.125 | 1.000 | | | |
| **Temperature (°C)** | -0.109 | -0.444 | 1.000 | | |
| **Humidity (%)** | 0.102 | 0.481 | -0.638 | 1.000 | |
| **Cloud (%)** | 0.194 | 0.075 | -0.061 | 0.300 | 1.000 |

Table 6.2: Correlation coefficients for continuous environmental parameters at Flixborough. Significant negative correlations highlighted.

| | Wind direction (°) | Wind speed | Temperature (°C) | Humidity (%) | Cloud (%) | Dew Point (°C) |
|---|---|---|---|---|---|---|
| **Wind direction (°)** | 1.000 | | | | | |
| **Wind speed** | 0.102 | 1.000 | | | | |
| **Temperature (°C)** | 0.160 | 0.253 | 1.000 | | | |
| **Humidity (%)** | -0.420 | -0.119 | -0.575 | 1.000 | | |
| **Cloud (%)** | -0.413 | 0.018 | -0.100 | 0.441 | 1.000 | |
| **Dew Point (°C)** | -0.019 | 0.242 | 0.894 | -0.158 | 0.115 | 1.000 |

## 6.3 Results

As each sample was taken (Chapters 4, 5), meteorological conditions were simultaneously monitored and recorded using a handheld Kestrel device (Chapter 3). This enabled the impact of meteorological parameters on concentrations to be assessed. The database created through Chapter 4 and 5 was used for this analysis. Within the STATISTICA general linear model (GLM) the meteorological conditions measured alongside bioaerosol concentrations were added to the database, alongside bioaerosol concentrations and the 'presence' and 'absence' data used to analyse operational

parameters (Chapter 5). This allowed determination of the relationships between bioaerosol concentrations and environmental conditions, both with and without the influence of operational parameters (Chapter 3). Analysing the effect of environmental parameters both with and without the influence of site activities allows the significance of relationships found to be determined alongside operational influences. The results from these analyses are presented in this section. Wherever 'significance' or 'statistical similarity/difference' is referred to, p-values are less than 0.05. In-figure statistical similarity/difference data generated through the Fisher LSD test, where sampling locations are compared in pairs. Statistical similarity/difference ($p = < 0.05$) is presented for each individual sampling location compared to all other sampling locations. Details of the number of samples taken per location can be found in Appendix I.

### 6.3.1 *Aspergillus fumigatus*

The environmental and seasonal parameters outlined above were input into the STATISTICA GLM, allowing the impact of these parameters on *A. fumigatus* concentrations calculated with and without site operational parameters to be analysed. Removal of correlating environmental parameters exerted no significant impact upon the analysis output. Therefore the results presented here include all variables.

Tables 6.3 and 6.4 below illustrate the impact of environmental parameters on *A. fumigatus* concentrations both without operational parameters (Table 6.3), and with (Table 6.4). For both data-sets season and sample location significantly affect concentrations, indicated by p-values. Correlations with meteorological parameters alter depending on whether analysis was carried out with or without operational parameters. Without operational parameters (Table 6.3), only wind speed shows a positive correlation with concentrations. With operational parameters also included (Chapter 5), wind direction, wind speed and temperature show significant positive correlations at Lount OWC, while dew point is significantly positively correlated at Flixborough (Table 6.4). However, the amount of variability within samples that is accounted for ($R^2$) increases for both sites with the inclusion of operational parameters. As this data is likely to represent the most accurate measurement of *A. fumigatus* concentrations,

operational parameters were included in the analysis of differences in concentrations across seasons, shown in Figure 6.2. The parameter coefficients show how for Lount OWC, sampling during spring correlates with higher concentrations, while at Flixborough, samples taken in winter and spring were negatively correlated (Tables 6.3 and 6.4). This pattern is also shown by Figure 6.2, which illustrates average concentrations across the seasons for all sampling locations. At Lount OWC *A. fumigatus* concentrations are significantly higher during spring and lower during summer. The pattern differs at Flixborough, where significantly higher concentrations were found during autumn and summer.

Table 6.3: Coefficients and significance of parameters included in analysis for *A. fumigatus* at Lount OWC and Flixborough, parameters significantly affecting concentrations highlighted.

(a) Lount OWC $R^2$ 33%

| | P-value | Coefficient |
|---|---|---|
| Sample Location | 0.000 | |
| Season | 0.007 | |
| Autumn | | 0.367 |
| Winter | | 0.298 |
| Spring | | 0.910 |
| Summer | | 0.000 |
| Wind direction (Degrees) | 0.774 | 0.000 |
| Wind speed (m/s) | 0.000 | 0.213 |
| Temperature (°C) | 0.376 | 0.033 |
| Relative Humidity (%) | 0.957 | 0.000 |
| Cloud cover (%) | 0.060 | 0.007 |

(b) Flixborough $R^2$ 44%

| | P-value | Coefficient |
|---|---|---|
| Sample Location | 0.000 | |
| Season | 0.000 | |
| Autumn | | -0.031 |
| Winter | | -1.349 |
| Spring | | -1.217 |
| Summer | | 0.000 |
| Wind direction (Degrees) | 0.449 | 0.001 |
| Wind speed (m/s) | 0.039 | 0.176 |
| Temperature (°C) | 0.744 | -0.037 |
| Relative Humidity (%) | 0.720 | -0.009 |
| Cloud cover (%) | 0.129 | 0.003 |
| Dew Point (ºC) | 0.608 | 0.062 |

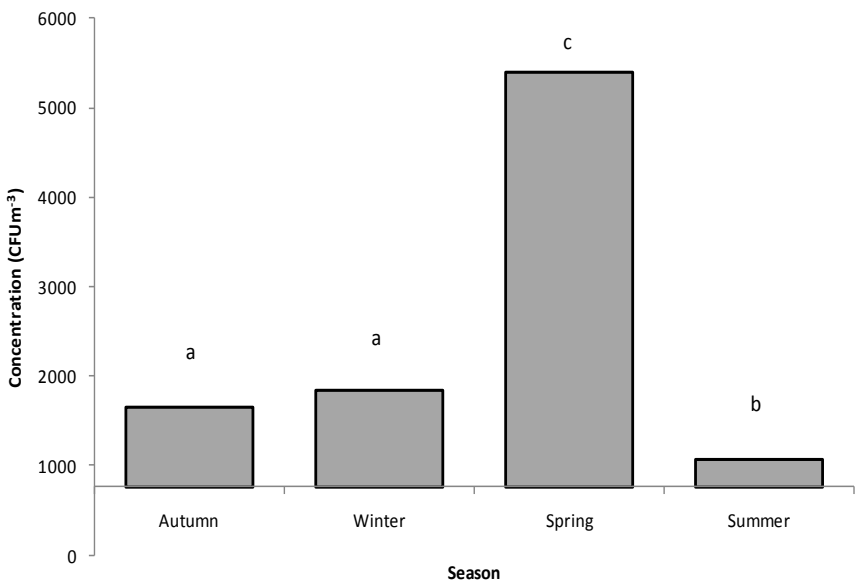
Table 6.4: Coefficients and significance of parameters included in analysis for *A. fumigatus* at Lount OWC and Flixborough, operational parameters accounted for. Parameters significantly affecting concentrations highlighted.

(a) Lount OWC $R^2$ 44%

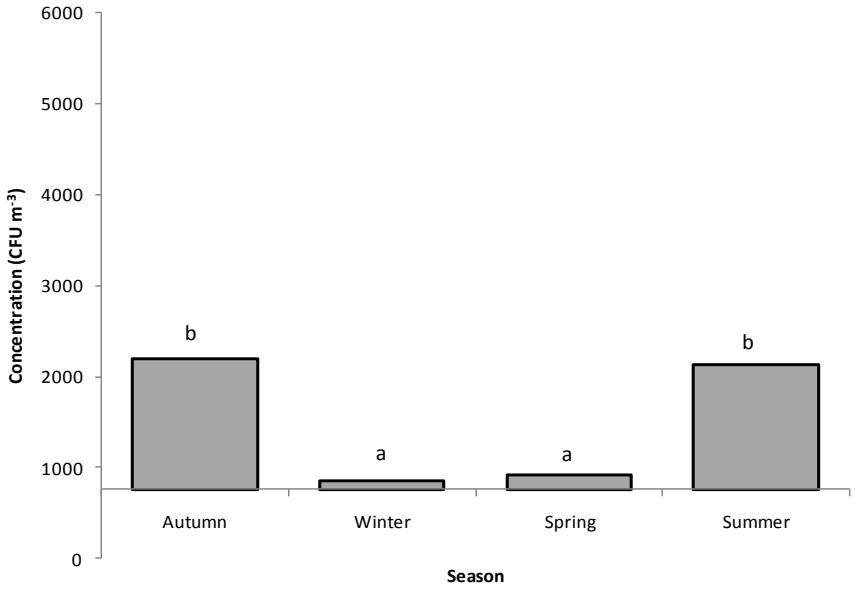
| Parameter | P-value | Coefficients |
|---|---|---|
| Sample Location | 0.000 | |
| Season | 0.000 | |
| Autumn | | 0.447 |
| Winter | | 0.555 |
| Spring | | 1.627 |
| Summer | | - |
| Wind direction (Degrees) | 0.000 | 0.004 |
| Wind speed (m/s) | 0.000 | 0.314 |
| Temperature (°C) | 0.001 | 0.133 |
| Relative Humidity (%) | 0.937 | 0.001 |
| Cloud cover (%) | 0.147 | 0.006 |

(b) Flixborough $R^2$ 46%

| Parameter | P-value | Coefficients |
|---|---|---|
| Sample Location | 0.000 | |
| Season | 0.001 | |
| Autumn | | 0.029 |
| Winter | | -0.910 |
| Spring | | -0.847 |
| Summer | | - |
| Wind direction (Degrees) | 0.845 | 0.000 |
| Wind speed (m/s) | 0.087 | 0.166 |
| Temperature (°C) | 0.905 | -0.013 |
| Relative Humidity (%) | 0.918 | -0.003 |
| Cloud cover (%) | 0.901 | 0.000 |
| Dew Point (°C) | 0.047 | 0.039 |




(a) Lount OWC




(b) Flixborough

Figure 6.2: Average *A. fumigatus* concentrations across each season and all sampling locations, including environmental and operational parameters at Lount OWC and Flixborough. Bars represent geometric mean, annotations represent statistical similarity ($p = < 0.05$) generated through pairwise analysis. X-axis crosses at lower limit of detection, 757 CFU $m^{-3}$.

### 6.3.2 Actinomycetes

For the purposes of this analysis meteorological parameters that correlated were analysed independently in order to show their individual influences on actinomycete concentrations (Section 6.2.1). The parameter coefficients and significance of the impact for each environmental parameter on actinomycete concentrations for analysis both with and without operational parameters is shown in Table 6.5 and 6.6. At Lount OWC all parameters were found to significantly affect actinomycete concentrations in both analyses, while at Flixborough, all parameters except for wind speed and cloud cover were found to significantly affect actinomycete concentrations. At both sites temperature showed a positive correlation with actinomycete concentrations, while relative humidity showed a negative correlation. At Lount OWC wind speed and cloud cover also showed positive correlations with concentrations. Through analysis without operational parameters (Table 6.5) wind direction also positively correlated with actinomycetes, while once mechanical parameters had been included, a negative correlation was seen (Table 6.6). However, the analysis with operational parameters included accounted for 57% of the variability seen ($R^2$) within actinomycete concentrations, as compared to 41% accounted for by analysis without operational parameters, lending more confidence to results shown in Table 6.4. At Flixborough, within both analyses (Tables 6.5, 6.6) wind direction showed a significant negative correlation with actinomycetes, while dew point temperature showed a significant positive correlation.

The impact of season on actinomycete concentrations is shown in both Tables 6.5 and 6.6, and Figure 6.3. Once again, while for both analyses autumn, winter and spring samples showed significant positive correlations with actinomycetes at Flixborough; there were some differences seen between analyses at Lount OWC. Without operational parameters included only winter shows a significant positive correlation (Table 6.5), while with operational parameters accounted for (Table 6.6), autumn, winter and spring showed significant positive correlations. As shown above, $R^2$ values indicate that analysis with operational parameters included is able to account for more variability at Lount OWC, this is also the case for Flixborough (Tables 6.5, 6.6); therefore analysis

with operational parameters is used to plot actinomycete concentrations across seasons (Figure 6.3). Both sites show how geometric mean concentrations are highest in winter, and lowest in the summer. However, geometric mean during winter is only significantly different from the other seasons at Lount OWC.

Table 6.5: Coefficients and significance of parameters included in analysis for actinomycetes at Lount OWC and Flixborough, parameters significantly affecting concentrations highlighted.

(a) Lount OWC $R^2$ 41%

| | P-value | Coefficient |
|---|---|---|
| Sample Location | 0.022 | |
| Season | 0.000 | |
| Autumn | | 0.374 |
| Winter | | 1.563 |
| Spring | | 0.340 |
| Summer | | 0.000 |
| Wind direction (Degrees) | 0.000 | -0.003 |
| Wind speed (m/s) | 0.005 | 0.368 |
| Temperature (°C) | 0.024 | 0.105 |
| Relative Humidity (%) | 0.012 | -0.028 |
| Cloud cover (%) | 0.001 | 0.014 |

(b) Flixborough $R^2$ 73%

| | P-value | Coefficient |
|---|---|---|
| Sample Location | 0.000 | |
| Season | 0.000 | |
| Autumn | | 2.573 |
| Winter | | 4.994 |
| Spring | | 2.298 |
| Summer | | 0.000 |
| Wind direction (Degrees) | 0.000 | -0.007 |
| Wind speed (m/s) | 0.681 | -0.052 |
| Temperature (°C) | 0.000 | 0.287 |
| Relative Humidity (%) | 0.000 | -0.066 |
| Cloud cover (%) | 0.226 | -0.003 |
| Dew Point (ºC) | 0.000 | 0.436 |

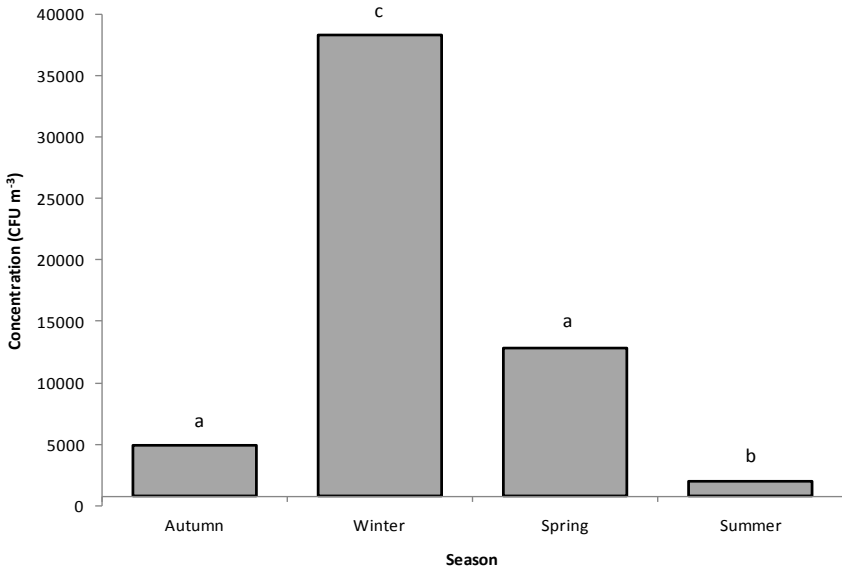
Table 6.6: Coefficients and significance of parameters included in analysis for actinomycetes at Lount OWC and Flixborough, operational parameters accounted for. Parameters significantly affecting concentrations highlighted.

(a) Lount OWC $R^2$ 57%

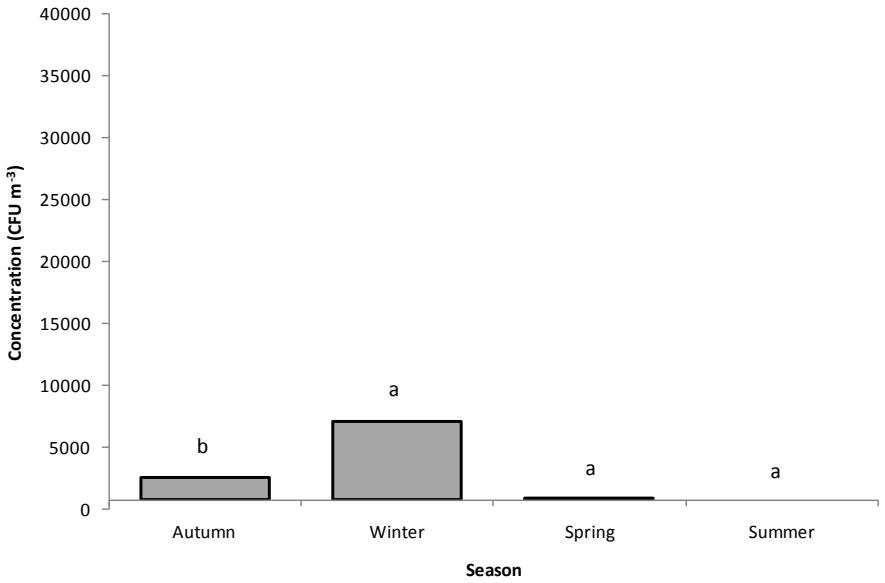
| Parameter | P-value | Coefficients |
|---|---|---|
| Sample Location | 0.000 | |
| Season | 0.000 | |
| Autumn | | 0.927 |
| Winter | | 2.980 |
| Spring | | 1.886 |
| Summer | | - |
| Wind direction (Degrees) | 0.025 | 0.003 |
| Wind speed (m/s) | 0.000 | 0.453 |
| Temperature (°C) | 0.000 | 0.327 |
| Relative Humidity (%) | 0.006 | -0.030 |
| Cloud cover (%) | 0.006 | 0.013 |

(b) Flixborough 75%

| Parameter | P-value | Coefficients |
|---|---|---|
| Sample Location | 0.000 | |
| Season | 0.000 | |
| Autumn | | 2.762 |
| Winter | | 3.770 |
| Spring | | 1.749 |
| Summer | | - |
| Wind direction (Degrees) | 0.002 | -0.004 |
| Wind speed (m/s) | 0.768 | -0.041 |
| Temperature (°C) | 0.000 | 0.205 |
| Relative Humidity (%) | 0.000 | -0.046 |
| Cloud cover (%) | 0.457 | -0.002 |
| Dew Point (°C) | 0.048 | 0.338 |




(a) Lount OWC




(b) Flixborough

Figure 6.3: Average actinomycete concentrations across each season and all sampling locations, including environmental and operational parameters at Lount OWC and Flixborough. Bars represent geometric mean, annotations represent statistical similarity ($p = < 0.05$) generated through pairwise analysis. X-axis crosses at lower limit of detection, 757 CFU $m^{-3}$.

### 6.3.3 Gram-negative bacteria

Once again, analysis of meteorological parameters affecting gram-negative concentrations was carried out with care, taking into account correlations described in Section 6.2.1. Tables 6.7 and 6.8 show how there are significant relationships between season and sampling location at both sites, shown in both analyses. Wind direction significantly negatively correlates with gram-negative bacteria at both sites without operational parameters accounted for (Table 6.7). However, once these operational parameters are included in the analysis, wind direction shows no significant relationship with concentrations at Lount OWC (Table 6.8). Wind speed positively correlates with gram-negative bacteria at Lount OWC (Table 6.7, 6.8), although this is not shown at Flixborough. At both sites temperature shows a positive and relative humidity a negative correlation with concentrations. The inclusion of operational parameters also resulted in some differences in the relationship of gram-negative bacteria and cloud cover. At Lount OWC both analyses show a positive correlation for cloud cover, while at Flixborough the positive relationship only become significant once operational parameters are included in the analysis (Table 6.7, 6.8). Dew point temperature, however, is significantly positively correlated with concentrations within both analyses.

As with both *A. fumigatus* and actinomycetes, more variability in concentrations is accounted for when operational parameters are included in the analysis ($R^2$; Tables 6.7, 6.8). As a result this data is used to express the differences in concentration across seasons seen in Figure 6.4. Coefficients in Tables 6.7 and 6.8 show how for both analyses concentrations at Lount OWC were significantly positively correlated with spring, while at Flixborough without operational parameters (Table 6.7) positive correlations were found for winter and spring, while with operational parameters (Table 6.8) positive correlations were found for autumn and winter. Figure 6.4 illustrates how these correlations result in significantly higher geometric mean during spring at Lount OWC, while at Flixborough highest geometric mean can be found in winter, although this concentration is not significantly different from those found in spring.

Table 6.7: Coefficients and significance of parameters included in analysis for gram-negative bacteria at Lount OWC and Flixborough, parameters significantly affecting concentrations highlighted.

(a) Lount OWC $R^2$ 47%

| | P-value | Coefficient |
|---|---|---|
| Sample Location | 0.000 | |
| Season | 0.000 | |
| Autumn | | - |
| Winter | | -0.100 |
| Spring | | 1.280 |
| Summer | | 0.000 |
| Wind direction (Degrees) | 0.033 | -0.004 |
| Wind speed (m/s) | 0.002 | 0.304 |
| Temperature (°C) | 0.002 | 0.146 |
| Relative Humidity (%) | 0.170 | -0.015 |
| Cloud cover (%) | 0.000 | 0.024 |

(b) Flixborough $R^2$ 68%

| | P-value | Coefficient |
|---|---|---|
| Sample Location | 0.000 | |
| Season | 0.000 | |
| Autumn | | 0.676 |
| Winter | | 3.332 |
| Spring | | 0.982 |
| Summer | | 0.000 |
| Wind direction (Degrees) | 0.000 | -0.007 |
| Wind speed (m/s) | 0.120 | 0.209 |
| Temperature (°C) | 0.000 | 0.207 |
| Relative Humidity (%) | 0.000 | -0.046 |
| Cloud cover (%) | 0.764 | 0.001 |
| Dew Point (ºC) | 0.000 | 0.418 |

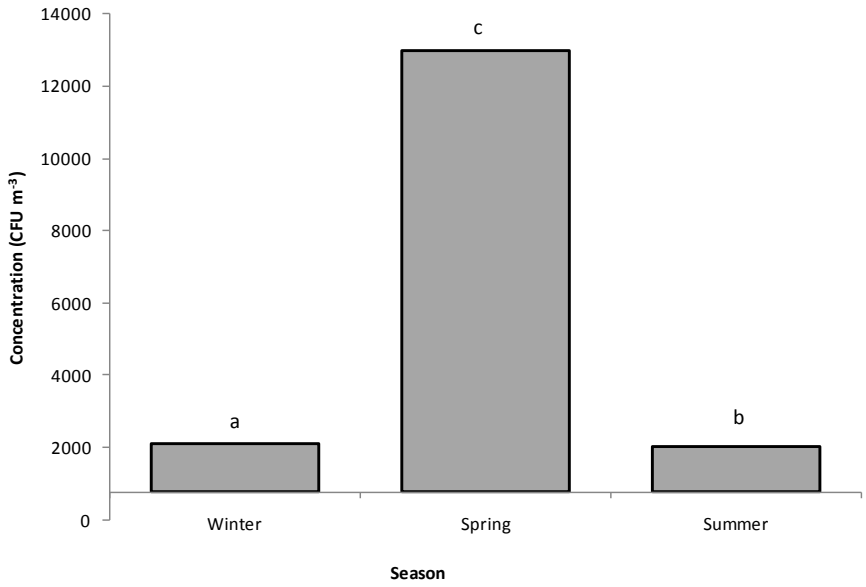
Table 6.8: Coefficients and significance of parameters included in analysis for gram-negative bacteria at Lount OWC and Flixborough, operational parameters accounted for. Parameters significantly affecting concentrations highlighted.

(a) Lount OWC $R^2$ 54%

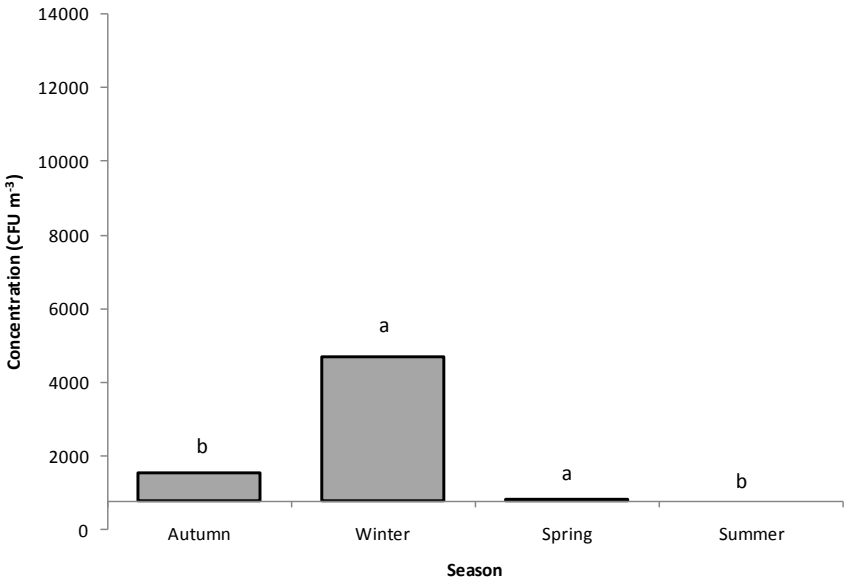
| Parameter | P-value | Coefficients |
|---|---|---|
| Sample Location | 0.000 | |
| Season | 0.000 | |
| Autumn | | - |
| Winter | | 0.037 |
| Spring | | 1.854 |
| Summer | | - |
| Wind direction (Degrees) | 0.284 | 0.002 |
| Wind speed (m/s) | 0.030 | 0.219 |
| Temperature (°C) | 0.001 | 0.210 |
| Relative Humidity (%) | 0.732 | 0.004 |
| Cloud cover (%) | 0.000 | 0.025 |

(b) Flixborough $R^2$ 72%

| Parameter | P-value | Coefficients |
|---|---|---|
| Sample Location | 0.000 | |
| Season | 0.000 | |
| Autumn | | 0.760 |
| Winter | | 1.885 |
| Spring | | 0.080 |
| Summer | | 0.000 |
| Wind direction (Degrees) | 0.008 | -0.004 |
| Wind speed (m/s) | 0.079 | 0.250 |
| Temperature (°C) | 0.002 | 0.145 |
| Relative Humidity (%) | 0.002 | -0.032 |
| Cloud cover (%) | 0.014 | 0.006 |
| Dew Point (°C) | 0.000 | 0.397 |




(a) Lount OWC




(b) Flixborough

Figure 6.4: Average gram-negative bacteria concentrations across each season at all sampling locations, including environmental and operational parameters at Lount OWC and Flixborough. Bars represent geometric mean, annotations represent statistical similarity ($p = < 0.05$) generated through pairwise analysis. X-axis crosses at lower limit of detection, 757 CFU $m^{-3}$.

### 6.3.4 Endotoxins

Analysis of endotoxin concentrations at both sites was carried out including all environmental parameters, as removal of correlated parameters produced no significant difference in significance of relationships and correlation coefficients for endotoxins. The results of analysis both without and with operational parameters are shown in Tables 6.9 and 6.10. There are more differences between each site and analysis seen in environmental parameter relationships for endotoxins than for culturable microorganisms. At Lount OWC analysis without operational parameters (Table 6.9) shows a positive correlation between endotoxins and wind speed and cloud cover; at Flixborough wind direction shows a positive relationship with endotoxins. When operational parameters are included, however (Table 6.10), no significant relationships are seen at Lount OWC for meteorological parameters. At Flixborough wind direction remains positively correlated with endotoxins, with a significant positive correlation between wind speed and relative humidity, and a significant negative correlation for concentrations and temperature, being found.

Correlations between endotoxin concentrations and season show more similarities across the analyses with and without mechanical parameters. At Lount OWC, autumn and winter are negatively correlated with endotoxins (Tables 6.9, 6.10). At Flixborough, no significant relationship is found between seasons and concentrations when analysed without operational parameters (Table 6.9), while with these parameters included (Table 6.10) a significant negative correlation is seen between samples taken in autumn and endotoxin concentrations. As with culturable microorganisms, more variability within endotoxin concentrations is accounted for by the analysis including operational parameters ($R^2$; Tables 6.9, 6.10). Therefore, this data is used to represent the effect of season on concentrations, shown in Figure 6.5. At Lount OWC concentrations are highest in spring, and significantly different from any other season. At Flixborough, however, although geometric mean is highest in summer, concentrations are statistically similar to those found in autumn. In addition, concentrations at Flixborough are shown to peak at levels 6 orders of magnitude higher than peak concentrations at Lount OWC.

Table 6.9: Coefficients and significance of parameters included in analysis for endotoxins at Lount OWC and Flixborough, parameters significantly affecting concentrations highlighted.

(a) Lount OWC $R^2$ 48%

| | P-value | Coefficient |
|---|---|---|
| Sample Location | 0.000 | |
| Season | 0.000 | |
| Autumn | | -1.769 |
| Winter | | -1.249 |
| Spring | | - |
| Summer | | - |
| Wind direction (Degrees) | 0.940 | 0.000 |
| Wind speed (m/s) | 0.018 | 0.233 |
| Temperature (°C) | 0.778 | -0.022 |
| Relative Humidity (%) | 0.455 | -0.014 |
| Cloud cover (%) | 0.005 | 0.022 |

(b) Flixborough $R^2$ 54%

| | P-value | Coefficient |
|---|---|---|
| Sample Location | 0.000 | |
| Season | 0.476 | |
| Autumn | | -1.499 |
| Winter | | -6.560 |
| Spring | | - |
| Summer | | - |
| Wind direction (Degrees) | 0.000 | 0.022 |
| Wind speed (m/s) | 0.457 | -0.341 |
| Temperature (°C) | 0.441 | 1.299 |
| Relative Humidity (%) | 0.257 | 0.356 |
| Cloud cover (%) | 0.592 | 0.007 |
| Dew Point (ºC) | 0.286 | -1.723 |

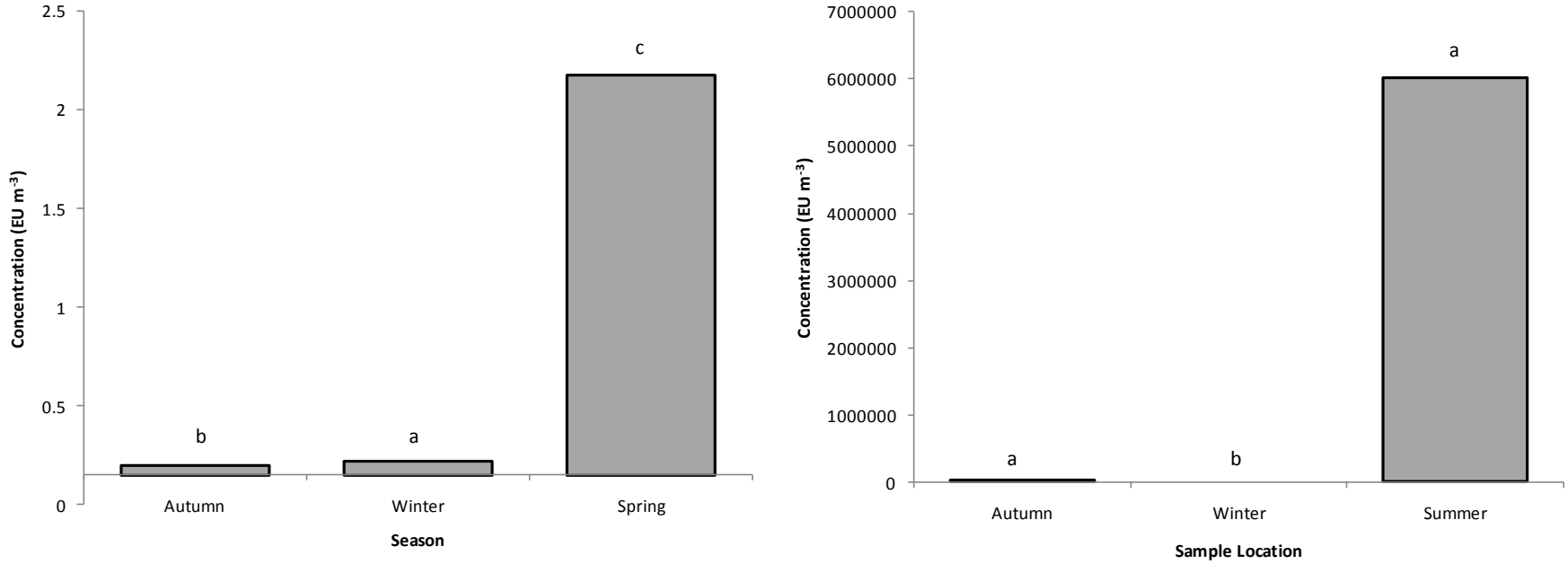
Table 6.10: Coefficients and significance of parameters included in analysis for endotoxins at Lount OWC and Flixborough, operational parameters accounted for. Parameters significantly affecting concentrations highlighted.

(a) Lount OWC $R^2$ 52%

| Parameter | P-value | Coefficients |
|---|---|---|
| Sample Location | 0.000 | |
| Season | 0.000 | |
| Autumn | | -2.377 |
| Winter | | -2.286 |
| Spring | | - |
| Summer | | - |
| Wind direction (Degrees) | 0.455 | -0.002 |
| Wind speed (m/s) | 0.533 | -0.072 |
| Temperature (°C) | 0.802 | 0.021 |
| Relative Humidity (%) | 0.097 | 0.037 |
| Cloud cover (%) | 0.174 | 0.011 |

(b) Flixborough $R^2$ 65%

| Parameter | P-value | Coefficients |
|---|---|---|
| Sample Location | 0.001 | |
| Season | 0.026 | |
| Autumn | | -10.228 |
| Winter | | -20.159 |
| Spring | | - |
| Summer | | - |
| Wind direction (Degrees) | 0.010 | 0.009 |
| Wind speed (m/s) | 0.036 | 0.716 |
| Temperature (°C) | 0.008 | -2.009 |
| Relative Humidity (%) | 0.027 | 0.284 |
| Cloud cover (%) | 0.123 | 0.024 |
| Dew Point (°C) | 0.393 | 1.786 |




(a) Lount OWC (b) Flixborough

Figure 6.5: Average endotoxin concentrations across each season and all sampling locations, including environmental and operational parameters at Lount OWC and Flixborough. Bars represent geometric mean, annotations represent statistical similarity ($p = < 0.05$) generated through pairwise analysis. X-axis crosses at lower limit of detection, 0.152 CFU $m^{-3}$.

## 6.4 Discussion

### 6.4.1 Meteorological Parameters

As shown in Section 6.1, there are many factors that prevent the results from this study being used to create a model of bioaerosol response to environmental conditions. These include the post-hoc nature of analysis, the use of a hand-held device to collect meteorological data, and the subjectivity of some measurements, such as cloud cover. Despite this, the advantage of this study is that site specific measurements of the microclimate were directly linked to samples taken on-site. In addition, the data were extensive and validated, with past studies unable to assess environmental parameters within a data-base of this size and significance. While most studies of bioaerosols emitted through composting focus on only one or two factors affecting concentrations, such as those affecting emission and worker exposure (Schlosser *et al*, 2009), or the effect of meteorological conditions on downwind dispersal (Albrecht *et al*, 2008; Fischer *et al*, 2008), the creation of the dataset used within this study has allowed determination of the significance of a range of influences. These are summarised in Figure 6.6 below.

| Parameter | *A. fumigatus* | | Actinomycetes | | Gram-negatives | | Endotoxins | |
|---|---|---|---|---|---|---|---|---|
| | Lount | Flix. | Lount | Flix. | Lount | Flix. | Lount | Flix. |
| Wind Direction (º) | + | | + | - | | - | | + |
| Wind Speed (m/s) | + | | + | | + | | | + |
| Temperature (°C) | + | | + | + | + | + | | - |
| Humidity (%) | | | - | - | | - | | + |
| Cloud Cover (%) | | | + | | + | + | | |
| Dew Point (°C) | N/A | + | N/A | + | N/A | + | N/A | |

Figure 6.6: Summary of the significant impacts ($p = < 0.05$) that environmental parameters were found to have on bioaerosol concentrations, including operational parameters. Lount = Lount OWC; Flix. = Flixborough; N/A = Parameter not measured; + = Positive correlation; - = Negative correlation; Blank boxes = No significant relationship found.

Wind speed was found to significantly affect bioaerosols at both sites (Figure 6.6), although different individual bioaerosols were affected at each site. This result could be anticipated for *A. fumigatus* (Tables 6.3, 6.4), having been reported by several studies (Fletcher *et al*, 2008; Korzeniewska *et al*, 2009; O'Gorman and Fuller, 2008). It is possible that this relationship with wind speed is related to physiology. More *A. fumigatus* spores may be released from both static windrows while wind speed is higher; due to the release of spores from aerial hyphae when disturbed, although largely, the increased physical suspension of particles during times of higher wind speed is likely to be a major cause of increased concentrations. This disruption is enhanced during composting activities, with a combination of disruption through composting activity and higher wind speed likely to increase emissions further (Epstein, 1994; Jones and Harrison, 2004; Swan *et al*, 2003). Actinomycetes, gram-negative bacteria, and endotoxins were also found to positively correlate with wind speed at Lount OWC, although the relationship with endotoxins is more tenuous as it was no longer significant when analysed with operational parameters (Figure 6.6; Tables 6.5 - 6.10). Although relationships between these bioaerosols and wind speed have not been previously

reported in the literature, wind speed has been reported to affect airborne bacteria concentrations (Korzeniewska *et al*, 2009). The same mechanisms resulting in this relationship for *A. fumigatus* may be responsible; namely, increased disturbance and liberation of dust, spores and cells with increased wind speed. Less significance was seen for wind speed at Flixborough, with only *A. fumigatus* without mechanical parameters (Table 6.4), and endotoxins with mechanical parameters (Figure 6.6; Table 6.10) showing a significant positive correlation. This may be due to numerous features, such as site design, activities, or higher emissions at Flixborough (Chapters 4, 5), resulting in wind speed having a less significant impact on concentrations. Although discussed above in terms of emission, the positive correlations found for wind speed are likely to result from two aspects, increase in bioaerosol emissions, and the increased dispersal of elevated concentrations of bioaerosols. However, it must also be acknowledged that increased wind speed would increase the rate of bioaerosol dilution in the atmosphere, potentially reducing concentrations. Therefore wind speed is unlikely to have a simple cause and effect relationship with bioaerosol concentrations. There is evidence supporting both of these relationships within the available literature (Albrecht *et al*, 2008; Fischer *et al*, 2008; Fletcher *et al*, 2008).The site specificity of the results supports the supposition that a simple correlation between wind speed and bioaerosol concentrations cannot be found. In addition, other factors, such as operational activity or overall site concentrations, may affect the impact of wind speed and increase the site specificity of the relationship.

Wind direction also showed a significant impact on bioaerosol concentrations at both sites. It is likely that wind direction affects the amount of ambient bioaerosols that may be found through the influence of external bioaerosol sources. At Lount OWC the effect of wind direction on concentrations is variable between bioaerosols, and between analyses with and without mechanical parameters, reducing confidence in any interpretation. At Flixborough relationships with wind direction were more consistent (Tables 6.5 - 6.10). A significant negative relationship was found for actinomycetes and gram-negative bacteria (Figure 6.6), meaning that as wind direction moved towards a more westerly and northerly source, concentrations decreased. This effect may be a result of topography and land use, with westerly or northerly wind directions resulting

in air flowing over a river rather than agricultural land including grain farms and a grain store prior to sampling (Chapter 2). As agricultural land is an acknowledged source of bioaerosols (Dutkiewicz, 1997), and the river is unlikely to contribute to the concentrations of bioaerosols analysed throughout this study as they are mainly associated with organic matter, these results are not unexpected. For endotoxins, however, concentrations increased with a more westerly or northerly wind (Figure 6.6). This would not be anticipated for the same reasons as discussed for actinomycetes and gram-negative bacteria; namely, it could be expected that wind passing over the grain fields and store (southerly) prior to sampling would lead to increased concentrations (Dutkiewicz, 1997). Without more in-depth monitoring of the impact of wind direction at each site and modelling of the effect of land-use and topography on concentrations, these findings cannot be verified.

While it has been suggested that *A. fumigatus* concentrations are associated with temperature and relative humidity (Ho *et al*, 2005; O'Gorman and Fuller, 2008), results here did not fully support this, with temperature only significantly correlating with *A. fumigatus* at Lount OWC with the inclusion of operational parameters (Figure 6.6; Table 6.4). Temperature was significantly positively correlated with actinomycetes and gram-negative bacteria at both sites, while relative humidity had a significant negative effect on actinomycetes at both sites, and gram-negative bacteria at Flixborough (Figure 6.6; Tables 6.5 - 6.8). There is evidence both supporting and contradicting the relationships found here within the literature. For example, Tham and Zuraimi (2005) found temperatures over 20°C resulted in loss of bacteria viability due to thermal stress. This result could be anticipated, as it is common practice within microbiology to preserve viability through storage at colder temperatures (Chapter 3) (Seeley *et al*, 1991). For this reason, it can be assumed that the increase in actinomycetes and gram-negative bacteria seen at warmer temperatures is not a result of improved preservation of viability. Another reason for this correlation may be increased proliferation of the microorganisms in compost; Harrison *et al*. (2005) found a correlation between temperature and airborne bacteria related to the increase of growth rates of bacteria in warmer conditions. However, as composting is a self-heating process (Swan *et al*, 2003)

it is unlikely that air temperature would significantly affect the composting temperatures reached, and alter proliferation.

The response of green waste and composting microorganisms to warmer temperatures may suggest another potential cause of this relationship; the drying of windrows leading to higher emissions of dust and associated bacteria. Jones and Harrison (2004) show how bonding of the particle to the surface it is on depends upon temperature and humidity of the surrounding air, with changes in these parameters potentially causing release. Dry surfaces are known to facilitate particle release, therefore drier compost may result in increasing bioaerosol emissions (Jones and Harrison, 2004). This drying of compost is also related to relative humidity, with a lower relative humidity (found at higher temperatures – Tables 6.1, 6.2) further drying compost windrows. However, it has also been suggested that higher humidity increases airborne desiccation of bacteria, due to the increased impaction of water vapour with airborne microorganisms (Lighthart and Mohr, 1987; Tham and Zuraimi, 2005), potentially reducing culturable bacteria. This issue is complicated by other reports showing that a higher relative humidity increases aerosolisation of particles and preserves viability through the increased size of droplets containing microorganisms (Griffiths *et al*, 2001; Marthi *et al*, 1990; Seinfeld and Pandis, 2006). The negative correlation between actinomycetes and humidity here would seem to support Tham and Zuraimi's (2005) and Jones and Harrison's (2004) work. Furthermore, the suggestion that dry compost increases liberation of bioaerosols supports the increased concentrations of bioaerosols seen at Flixborough, discussed in Chapters 4 and 5, as compost at Flixborough is maintained at 40% moisture, rather than the 60% maintained at Lount OWC (Chapter 2). It has also been previously reported that lower composting moisture increases emissions (Epstein, 1994). In addition, the positive relationship with temperature and negative with relative humidity has been previously reported for other, ambient, bioaerosols (Grinn-Gofroń and Strzelczak, 2008).

Relationships between temperature, relative humidity and endotoxins were variable. Without mechanical parameters no relationship was found between temperature and humidity and concentrations (Table 6.9). However, with the inclusion of mechanical

parameters at Flixborough a significant positive relationship was seen for relative humidity, and a significant negative relationship with temperature (Figure 6.6; Table 6.10). Endotoxins may be released in higher concentrations as gram-negative bacteria are disturbed (Dutkiewicz, 1997). This may provide some explanation for the positive relationship with relative humidity, with increased relative humidity suggested to increase the desiccation of airborne bacteria as collisions with airborne particles, namely water vapour, increase (Lighthart and Mohr, 1987; Tham and Zuraimi, 2005). However, as shown above, it cannot be determined whether correlations of bioaerosols with meteorological parameters result from the response of compost and emissions to the environmental conditions or from the response of airborne bioaerosols to these parameters. In addition, the relationship was only found for one of the analyses for endotoxins (Table 6.10), reducing confidence in findings; whereas relationships with actinomycetes and gram-negative bacteria were consistently found.

Cloud cover was found to significantly positively correlate with actinomycetes and gram-negative bacteria both with and without mechanical parameters at Lount OWC; and with gram-negative bacteria at Flixborough once mechanical parameters were included (Figure 6.6; Tables 6.5 - 6.8). It could be proposed that increased cloud cover affects the viability of these microorganisms, as increasing cloud would reduce solar radiation and potentially allow airborne bacteria to remain viable for longer (Korzeniewska *et al*, 2009; Lighthart and Mohr, 1987). However, at Lount OWC cloud cover was also found to be significantly positively correlated with endotoxins (Table 6.10), with increased solar radiation providing no explanation for an increase in endotoxin concentrations. In addition, as cloud cover was a subjective estimate, less confidence can be had in this finding. There is little evidence within the literature considering the impact of solar radiation on bioaerosol concentrations, particularly endotoxins, therefore, no satisfactory explanation for this relationship can be found.

Dew point temperature was also measured at Flixborough, with positive correlations found for all culturable bioaerosols (Figure 6.6; Tables 6.4 - 6.8). This could be anticipated, given the close relationship between dew point temperature and air temperature, shown in Table 6.2, and the positive relationship found between these

microorganisms and temperature. Furthermore, it has been previously reported in the literature than dew point temperature shows a significant positive correlation with spore and pollen concentrations (Grinn-Gofroń and Strzelczak, 2008; Vega-Maray *et al*, 2003). Although results here show the same relationship can be found for composting bioaerosols, the reasons for this relationship again remain unclear.

### 6.4.2 Season

The effect of meteorological parameters, along with alterations in the duration of the composting process and feedstock throughout the year could be expected to result in bioaerosols being released in different quantities depending on the time of year. For this reason, average bioaerosol concentrations across winter, spring, summer and autumn were analysed. *A. fumigatus* concentrations across seasons were found to differ at each site. At Lount OWC, concentrations in spring were significantly higher (Table 6.3, 6.4, Figure 6.2); while at Flixborough samples taken in autumn and summer had similar concentrations and were higher than winter and spring (Figure 6.2). Despite these differences, none of these results are unexpected. It could be anticipated that warmer months would lead to increased emission of bioaerosols, as reception and processing of waste on-site increases during these months, and as discussed above, warmer temperatures may facilitate release of bioaerosols. The increased concentration of *A. fumigatus* in summer and autumn shown at Flixborough is in agreement with previous studies (Dutkiewicz, 1997; Grisoli *et al*, 2009; Nielsen, 1997a; Recer *et al*, 2001; Schlosser *et al*, 2009). For actinomycetes, winter was found to have the highest geometric mean concentrations (Figure 6.3). This is contrary to some published studies (Recer *et al*, 2001; Schlosser *et al*, 2009); although others have suggested that certain species of actinomycetes, such as *Streptomyces* spp. may dominate green wastes during winter months due to lower temperatures (Lacey, 1997). This may lead to increased emissions of actinomycetes as composting activities are carried out.

Differences in correlations for seasons between sites and analyses with and without mechanical parameters (Tables 6.7, 6.8) led to gram-negative bacteria concentrations being significantly higher during spring at Lount OWC (Figure 6.4), while at

Flixborough concentrations during winter showed highest geometric mean but were statistically similar to those found in spring (Figure 6.4). This data differs from other published data for both gram-negative and total mesophilic bacteria, where concentrations were higher in summer than winter (Grisoli *et al*, 2009; Schlosser *et al*, 2009). The results found here may be a feature of processing activities and feedstock altering microorganism composition of compost seasonally. For example, the maturation phase of composting allows mesophilic bacteria to predominate (Amir *et al*, 2008; Cahyani *et al*, 2002; Hermann and Shann, 1997; Schlosser *et al*, 2009; Steger *et al*, 2005). Decreased throughput during winter may therefore allow gram-negative bacteria to proliferate in maturing compost. During spring, throughput begins to increase, potentially releasing higher concentrations of gram-negative bacteria as more composting activities are carried out.

Samples taken in autumn and winter had negative correlations with endotoxin concentrations. However, these correlations were only significant at Lount OWC, and for autumn when analysed with mechanical parameters (Tables 6.9, 6.10). This led to higher concentrations being found in spring at Lount OWC, while at Flixborough highest geometric mean concentrations were found in summer (Figure 6.5). Decreases in endotoxin concentration during winter have been previously reported (Schlosser *et al*, 2009), and again, may be associated with decreased throughput and composting processing activities during these months. Figure 6.5 also shows how concentrations for endotoxins at Flixborough were found at levels up to 6 orders of magnitude higher than at Lount OWC. This may be due to site-specific influences, such as feedstock, which contains household vegetable waste at Flixborough which is not found at Lount OWC (Chapter 2). In addition, Chapter 5 shows how mechanical turning activities led to high emissions of endotoxins, an activity that is only carried out at Flixborough. This may also enhance endotoxin concentrations at this site. However, as little is known with regards to mechanisms of endotoxin release, this finding cannot be supported.

### 6.4.3 Summary of the impact of environmental parameters

The variability seen between individual bioaerosols and the impact that described environmental parameters have on them, along with variation across seasons, illustrates the complex nature of bioaerosol emission and dispersal. Although some variation in concentrations has been accounted for within this study, much remains uncertain. For example, one of the chief factors behind increased bioaerosol release may be drier compost. However, while warmer temperatures facilitate drying, not all bioaerosols showed higher concentrations during warmer months, as would be anticipated. Including environmental parameters and operational parameters allowed between 44% and 75% of variability to be accounted for, showing how understanding of the relationships between bioaerosol emission, dispersal, and environmental and operational parameters remains incomplete. However, within this study accounting for both operational and environmental parameters allowed the most variation within the data-set to be accounted for. The fact that environmental parameters remained significant after the inclusion of operational parameters also showed how although site activities are the main causes of bioaerosol emission (Chapter 5), environmental parameters also exert a significant influence on concentrations.

There remains little evidence within the literature to fully describe the reasons behind relationships found. Although the data presented here is based on a validated and extensive data-set, there remain many questions surrounding the response of bioaerosols to environmental parameters. One of the most pressing is whether the environmental parameters measured here affect bioaerosol concentrations once released, such as increased relative humidity increasing airborne desiccation of bacteria (Lighthart and Mohr, 1987; Tham and Zuraimi, 2005); or whether these parameters more affect the emission of bioaerosols through interaction with compost. Through this study, it is impossible to separate these factors. Other issues inherent to the experimental design used were highlighted previously and include ‘snapshot’ meteorological data and post-hoc analysis. In addition, it has been found that weather conditions several days prior to the sampling event should be taken into account in order to evaluate the impact of environmental parameters on pollen and fungal spore concentrations (Grinn-Gofroń and

Strzelczak, 2008; Vega-Maray *et al*, 2003); which was not possible here with the use of a handheld device. Dedicated experiments for the purpose of determining the effect of environmental parameters on compost, emissions, and dispersal are required to analyse whether environmental parameters affect bioaerosols more within compost or once aerosolised, in order to complete understanding.

Despite these limitations, this study has contributed towards the future understanding of the environmental conditions that affect bioaerosol concentrations. Combined with data presented in Chapter 5, it could be suggested that 'worst case' bioaerosol emissions and dispersal would occur during periods of site activity, when meteorological conditions include higher temperatures, higher dew point temperature, lower relative humidity and higher wind speed. While others have suggested these relationships, (Fletcher *et al*, 2008; Grinn-Gofroń and Strzelczak, 2008; Ho *et al*, 2005; Schlosser *et al*, 2009), prior to this study it has not been shown through analysis of an extensive and validated dataset that environmental parameters affect bioaerosol concentrations independently from, and alongside, operational parameters. This data will inform both future studies into the emission and dispersal of bioaerosols from composting, and site operation. Currently site operations are typically only modified if wind direction and strength on-site suggests that sensitive receptors may be exposed to bioaerosols emitted through composting activity (Chapter 2). However, the data here suggests that conditions such as temperature and relative humidity may also be relevant measures of the potential risk posed to sensitive receptors. In addition, the accumulation of data within Chapter 4, 5 and the current Chapter, suggests that maintaining compost at lower moisture content may significantly increase bioaerosol emissions.

## 6.5 Conclusions

The main findings of this study are described below.

- Typically, air and dew point temperature and wind speed are positively associated with bioaerosol concentrations, while relative humidity is negatively associated with bioaerosol concentrations

- The prediction of the response of bioaerosols to environmental parameters is hindered by the impact of site specific features and physicochemical properties of individual bioaerosols that have not been characterised
- Level of throughput appears to dominate seasonal changes in bioaerosol concentrations rather than meteorological conditions

The data presented within this study will inform the design and implementation of future studies exploring the emission and dispersal of bioaerosols. Understanding of the interaction of bioaerosols with environmental influences will allow informed site operation. For example, when environmental conditions are known to be conducive to emission, composting activities may be avoided. This study was carried out in a similar fashion to most comparable studies, utilising a post-hoc analysis of the data in relation to environmental parameters after sampling for the purpose of emission and dispersal quantification. Despite the fact that data here is based on an extensive and validated data-set, interpretation remained difficult. An experimental design aimed at separating the effect of meteorological parameters on compost and aerosolised microorganisms and endotoxins is required, as well as study of the effect that feedstock and throughput has on seasonal emissions. This could be achieved, for example, through experimental control of the composting process, with pre-determined feedstock compositions and moisture contents. The feasibility of this type of study has already been shown through analysis of composting microbial communities at different oxygen levels (Steger *et al*, 2007). Controlled aerosolisation events of the different compost types along with control of environmental parameters such as relative humidity and air temperature would then allow determination of the relative significance of these parameters.

Despite the limitations of this study, the data presented and discussed shows the concentrations of bioaerosols in response to environmental parameters, taking into account the influence of operational parameters (Chapter 5). This study has also highlighted the importance of gaining measures of the effect of environmental parameters on a range of bioaerosols, as they cannot be expected to respond in the same way.

The combination of data presented in Chapters 4, 5, and 6 has provided information regarding the emission and dispersal of bioaerosols up to and beyond 250 m from site, taking into account both the effect of operational and environmental parameters; concluding Section I of this thesis. However, while endotoxins have been enumerated, the use of culture based methods has prevented full understanding of the consortia of bioaerosols released from composting facilities, and how they affect ambient aerobiota. In addition, the detection limits of this method (757 CFU $m^{-3}$) mean that low levels of bioaerosols cannot be enumerated, further hindering the analysis of the effect that composting has on ambient aerobiota. Section II of this these explores the community structure and composition of bioaerosols released from composting facilities through both culture and non-culture based methods. This aims to further understanding of the composition of composting bioaerosols, and their impact on the ambient aerobiota and sensitive receptor exposure.

# SECTION II

# Characterisation of Bioaerosol Components and Communities

# 7 Characterisation of Bioaerosol Components and Communities: An Introduction

The bioaerosols emitted from composting facilities include both culturable microorganisms (fungi and bacteria), and the fractions or by-products of microorganisms, such as endotoxins, mycotoxins and glucans (Swan *et al*, 2003). The measurement of bioaerosols at composting facilities has typically been centred on enumeration of culturable bioaerosols, following guidelines issued by the Association for Organics Recycling (AFOR) in 1999 (updated in 2009) and recommendations from the Environment Agency (Environment Agency, 2009b; The Composting Association, 1999). Culture based methods have also been recommended on a European-wide scale (Verein Deutscher Ingenieure 2004a, b). Sampling methods in the UK have been largely based on direct impaction, with guidelines encouraging use of the Andersen sampler (Environment Agency, 2009b; The Composting Association, 1999), while German guidelines are based upon filtration with gelatine filters (Verein Deutscher Ingenieure 2004a, b). Recent revision to AFOR guidelines, however, describes filtration sampling as a suitable alternative to direct impaction (Environment Agency, 2009a). This alteration in recommendations will provide the means for development of non-culture based methods, as one of the major benefits of filtration based sampling is that samples can be analysed through both culture and non-culture based methods, while direct impaction only allows enumeration through culture (Eduard and Heederik, 1998; Rinsoz *et al*, 2008).

The utilisation of filtration sampling has been illustrated through the use of this method throughout this project (Chapter 3). The same method has been used for culture of *Aspergillus fumigatus*, actinomycetes and gram-negative bacteria, as well as for endotoxin assay. The enumeration of endotoxin emission and dispersal from composting facilities has represented a significant advance in the understanding of non-culturable bioaerosols (Chapters 4, 5, 6). However, there is compelling evidence within the literature that analysis of microorganisms may be incomplete if only culture-based methods are applied. The reasons for this are numerous. Microorganisms may enter into

a ‘non-culturable but viable’ (NCBV) state, where they cannot be enumerated through culture, yet remain viable and hazardous to human health (Crook and Sherwood-Higham, 1997; Swan *et al*, 2003). In addition, certain microorganisms may be particularly sensitive to substrate or culture conditions, making enumeration through culture challenging (Hugenholtz and Goebel, 2001). The culture-based method is also highly selective, only allowing enumeration of microorganisms that are able to form visible colonies on the substrate and under the conditions selected. This requires a prior knowledge of the conditions suitable for cultivation of the desired microorganisms (Head *et al*, 1998; Hugenholtz and Goebel, 2001; Ward *et al*, 1990). Comparison between direct counting and culture of marine microorganisms found an underestimation of several orders of magnitude through culture (Giovannoni *et al*, 1990). It has been estimated, and supported through experimental work, that only 20% of species within a microbial community may be enumerated through culture, leaving up to 80% unqualified and unquantified (Ward *et al*, 1990). The advance of non-culture based methods over the last 20 years, primarily applied in soil and aquatic microbiology, has allowed extensive qualification and quantification of environmental microbial communities; revealing a far greater diversity of microorganisms than was anticipated after decades of application of culture-based methods (Aguilera *et al*, 2006; Giovannoni *et al*, 1990; Head *et al*, 1998; Hugenholtz and Goebel, 2001).

The issues inherent to culture-based methods may be enhanced through the sampling of airborne microorganisms. In particular, loss of microorganism viability once aerosolised may increase, as cells and spores are subject to desiccation, dehydration, changes in temperature, pressure, and relative humidity, and solar radiation (Chen and Li, 2005b; Crook and Sherwood-Higham, 1997; Tham and Zuraimi, 2005; Walter *et al*, 1990). Furthermore, the sampling process itself may lead to desiccation and dehydration of microorganisms (Chen and Li, 2005b; Verein Deutscher Ingenieure, 2004). Through studies comparing culture counts to direct counting it has been suggested than only 0.3 to 55% of airborne fungi, and fewer than 10% of aerosolised gram-negative bacteria can be enumerated using culture based methods due to a combination of these factors (Cheng *et al*, 2008a; Heidelberg *et al*, 1997; Lappalainen *et al*, 1996; Lee *et al*, 2006). These data are supported by more in-depth study of soil bacterial communities, where it

is suggested that only 1% of the community can be represented through use of culture-based methods (Cheng *et al*, 2008a; Head *et al*, 1998; Heidelberg *et al*, 1997; Ritz, 2007). While non-culture based methods have provided fresh insight into soil and aquatic microbial communities, these methods have rarely been applied to airborne microorganisms in the environment. This is despite the above evidence, which shows that bioaerosol diversity and concentrations have almost certainly been routinely underestimated.

Bioaerosol sampling has focused on culturable microorganisms known to have detrimental impacts on human health; including *A. fumigatus*, actinomycetes, and gram-negative bacteria as the producers of endotoxins, although guidelines only suggest measurement of total bacteria and fungi, and gram-negative bacteria (Association for Organics Recycling, 2009; Environment Agency, 2009; The Composting Association, 1999). This focus has prevented knowledge of communities of bioaerosols. While there have been studies on the microbial composition of compost (Cahyani *et al*, 2002; Peters *et al*, 2000; Steger *et al*, 2005; Steger *et al*, 2007; Swan *et al*, 2003), the proportion of these communities that can be aerosolised and disperse into the ambient environment remains largely unknown. This has omitted a large proportion of total airborne microbial community, although they may also pose a health risk (Crook and Sherwood-Higham, 1997; Dutkiewicz, 1997; Liebers *et al*, 2008). Understanding of the composition of bioaerosols may be important in the future quantification of human health risk. For example, while it has been shown that endotoxins can be released and are able to disperse from composting facilities (Chapters 4, 5) (Schlosser *et al*, 2009), the composition of aerosolised gram-negative bacteria able to release these endotoxins remains unknown. Endotoxin, or lipopolysaccharide (LPS), possesses different characteristics depending on the source bacteria, leading to differences in immuno-toxic potency (Erridge *et al*, 2002). Therefore, knowledge of the community structure of bioaerosols, particularly bacteria, as well as measurement of endotoxin emission and dispersal, may enhance ability to determine health risks.

The identification of bioaerosol community structure may also allow the development of a methodology for the source apportionment of bioaerosols. Bacteria and fungi are

released into the air from many different sources, such as waste management activities, agriculture and household waste bins (Dutkiewicz, 1997; Wouters *et al*, 2000). Given the difference in sources, it is likely that different communities of bioaerosols are released. Through analysis of the bioaerosol communities from composting, in ambient air, and downwind, it may be possible to identify key microorganisms that are indicative of the composting process and a community structure representative of composting. This principle has been shown to be effective in the determination of composting phase through identification of key microorganisms (Peters *et al*, 2000; Steger *et al*, 2007). A recently published investigation has also utilised analysis of bioaerosol microbial communities emitted through composting in an attempt to identify key bioaerosols associated with composting (Le Goff *et al*, 2009). As well as identification of airborne microorganisms and a community 'fingerprint' indicative of the composting process, it may be possible to apply this to other sources of bioaerosols, leading to the ability to attribute airborne microorganisms to source through community profiling.

A range of methods have been developed that allow enumeration of NCBV and non-culturable fractions, and can be used both independently from and alongside culture based methods in order to provide the best possible quantification and qualification of microorganism communities (Chen and Li, 2005b; Nichols, 2007). However, few of these methods have been used with regularity in the field of bioaerosol research (Environment Agency, in press), and few of them are developed specifically for bioaerosol enumeration. The experimental work for this project has used filtration with enumeration through culture as the basic method (Chapter 3). However, in light of the possibility for underestimation of bioaerosol concentrations, along with the potential that non-culture based methods hold for improving the understanding of bioaerosols, opportunities for sampling with novel and non-culture based methods were investigated.

Novel methods that may be utilised for bioaerosol enumeration can be broadly split into two categories, those based on direct counting and those based on molecular analysis. While traditional direct counting is performed using microscopy-based methods, these can be laborious and time-consuming (Crook and Sherwood-Higham, 1997); factors that are prohibitive to the extensive repeat sampling strategies necessary in order to

characterise bioaerosol emission and dispersal. However, direct counting methods have been developed that exploit the fluorescence of biological particles in order to separate them from non-biological particles, and enable rapid counting. Methods based on this technique and suggested as of use in bioaerosol sampling include Flow Cytometry with Fluorescence (FCM/FL) (Chen and Li, 2005a, b), Fourier Transform Infrared Spectroscopy (FTIR) and Laser Induced Fluorescence (LIF) (Cabredo *et al*, 2007; Wlodarski *et al*, 2009). In addition, it is also possible to directly count fine particle fractions (< 1µm) through condensation particle counters (CPC), which utilise condensation to enable fine particles to increase in size until they are detectable by a laser beam (Zhu *et al*, 2006); although this method is unable to separate biological from non-biological particles. Therefore, these methods may be able to provide counts of both airborne biological particles, including microorganisms, and endotoxins, as these have been suggested to comprise part of the fine fraction of particles (Dutkiewicz, 1997). A more comprehensive review of novel fluorescence based direct counters can be found in Chapter 9. Few of these methods have been tested in order to enumerate bioaerosols emitted from composting facilities, with many remaining in developmental stages (Wlodarski *et al*, 2009). In addition, while these methods allow enumeration of biological particles including NCBV and non-culturable fractions, they cannot determine the type of particle counted, such as whether the particle is a fungal spore or bacterial cell. While microscopy may provide insight into the type of bioaerosol, fine taxonomic identification is difficult as morphology of similar microorganisms may not be distinct (Ward *et al*, 1990). These methods are therefore of use in the determination of total bioaerosols and physical properties of these bioaerosols, such as particle size, but not in the analysis of bioaerosol composition.

The development of methods based on molecular analysis of microbial communities is well established within soil and aquatic microbiology, allowing increased understanding of microbial community composition. This may allow a relatively simple transfer of these methods to the area of airborne microbial enumeration. Some of these methods are based upon biochemical analysis, such as Phospholipid Fatty Acid (PLFA), ergosterol or lipopolysaccharide (LPS) analysis (Cheng *et al*, 2008a, b; Zelles, 1999), while others focus on nucleic acid analysis, such as DNA microarray and Polymerase Chain

Reaction (PCR) based methods. PLFA, for example, analyses the fatty acids present within environmental samples, with signature fatty acids able to be assigned to microbial groups allowing determination of phenotypic microbial community structure (Macnaughton *et al*, 1999). PCR allows the amplification of nucleic acid, with the PCR product able to be subjected to a range of different methods in order to determine genotypic microbial community structure. These analysis methods largely work through separation of microbial groups of species based upon the physical properties of the nucleic acid itself, such as length of the molecule. Examples of such methods include Denaturing Gradient Gel Electrophoresis (DGGE), Temperature Gradient Gel Electrophoresis (TGGE), and Terminal-Restriction Fragment Length Polymorphism (T-RFLP) (Head *et al*, 1998; Muyzer *et al*, 1993; Smith *et al*, 2005). Further, analysis of the microbial community composition can be carried out through cloning and sequencing methods (Hugenholtz and Goebel, 2001; Head *et al*, 1998; Macnaughton and Stephen, 2001; Margulies *et al*, 2005; Parameswaran *et al*, 2007).

These methods provide the potential for qualification and quantification of microbial communities at numerous levels. Both active and inactive or dead microorganisms can be targeted. For example, PLFA analysis and PCR amplification of ribosomal nucleic acid (RNA) followed by analysis provides information on active members of the microbial community, as both PLFA and RNA are rapidly degraded upon cell death, with RNA concentration also positively correlated with cell activity (Miskin *et al*, 1999). Alternatively, deoxyribonucleic acid (DNA) amplification through PCR allows analysis of community including dead and inactive microorganisms, as DNA is persistent and stable within the environment (Miskin *et al*, 1999). Both PLFA and PCR based methods can be used to compare community structure between different samples, showing major changes in community in response to environmental conditions (Malik *et al*, 2008). There are also numerous options for taxonomic classification of microbial communities. PLFA markers can be assigned to microbial groups, such as fungi, or gram-negative bacteria. However, this method does not allow further taxonomic refinement (Malik *et al*, 2008). Through DNA or RNA based analysis, either specific microorganisms can be selectively amplified through PCR, allowing their identification, or whole communities can be amplified and subsequently sequenced. This will allow

identification of community components. However, while PLFA allows simultaneous analysis of both fungal and bacterial communities, with PCR based methods these microorganism communities must be analysed separately. Biomass can also be analysed through PLFA and quantitative PCR (Felske *et al*, 1998; Malik *et al*, 2008), while sequencing of PCR or quantitative PCR product, allows the dominance of each microorganism identified to be determined (Felske *et al*, 1998). A more comprehensive review of these methods and their application can be found in Chapters 10 and 11.

Although these methods provide detailed knowledge surrounding microbial community structure, there are no standardised methods available that could be applied to bioaerosol analysis. While it has been suggested that both biochemical and nucleic acid based methods are applicable and useful in the analysis of airborne microbiota (Baertsch *et al*, 2007; Brooks *et al*, 2007; Harrison *et al*, 2005; Macnaughton *et al*, 1999; McDevitt *et al*, 2004; Mukoda *et al*, 1994; Orsini *et al*, 2002; Peccia and Hernandez, 2006; Rinsoz *et al*, 2008), these methods have rarely been applied to bioaerosol analysis, particularly those emitted through composting. This may be due to a lack of standardised methods and the past focus of composting bioaerosol studies on a limited range of culturable, pathogenic, microorganisms. Both direct counting and molecular based analyses hold great potential for increasing the breadth and depth of knowledge surrounding the emission and dispersal of bioaerosols from composting facilities. This is despite the infrequent use of these methods in this area of research, and the lack of standardised methods. In order to better understand the composition and community structure of bioaerosols emitted from composting facilities, the second main Objective of this thesis, "Characterisation of bioaerosol composition and communities including viable and non-viable components" was formulated.

Of particular interest within this part of the thesis was the enumeration of non-culturable fractions of bioaerosols. This has been achieved to a great extent within Chapters 4 – 6, with the detailed enumeration of endotoxin emission and dispersal. However, direct counting methods based on fluorescence may be able to provide further insight into the emission and dispersal of bioaerosols, including non-culturable and NCBV fractions. Comparison of direct counting and filtration based methods would potentially also

allow the efficiency of each method to be determined. In order to enhance understanding, particularly regarding the potential health impact, of endotoxins at composting facilities, it is also desirable to identify key gram-negative bacteria that may contribute to endotoxins found at composting facilities. In addition, the use of selective culture-based methods has prevented the qualification of airborne microbial communities. Through the use of molecular methods it may be possible to identify a composting bioaerosol community fingerprint, and compare this to ambient air and the green waste itself. This will reveal information on the similarity of these communities, as well as determining the influence of composting on ambient airborne microorganisms. Qualification of the microbial composition of these communities will allow identification of key microorganisms that significantly contribute to any alterations in the community structures seen within compost and bioaerosols. This type of analysis may also enable identification of signature bioaerosols or bioaerosol community structures from specific sources, allowing the bioaerosols sampled to be traced back to source, namely, 'source apportionment'. The application of some of the methods reviewed above would allow all of these aims to be met. However, as many of these methods have not yet been applied to environmental bioaerosol sampling, their application would be as 'proof of concept' studies, designed to illustrate the potential of these methods and the ability of sampling by filtration to be used in combination with these methods.

The achievement of Objective ii, and the above aims, is described in Chapters 8 - 11. In turn, these Chapters perform the following tasks:

1. Comparison of the relationships between culturable bacteria, fungi and endotoxins enumerated as part of Section I of this thesis and identification of key gram-negative bacteria;
2. Direct comparison of enumeration through culture to novel direct counting methods;
3. Analysis of the community structure of bioaerosols and comparison to upwind and compost samples, and;
4. Identification of the species comprising bacterial bioaerosol communities, and comparison to upwind and compost sample species composition.

# 8 The Composition of Bioaerosols Released from Composting Facilities: Cultivable Fraction and Endotoxins

## 8.1 Introduction

Throughout Section 1 different components of the total bioaerosol load released from composting facilities were measured. The components measured include members of the culturable and non-culturable fractions that are of most concern with regards to human health, and are indicative of the composting process. These are *Aspergillus fumigatus*, actinomycetes, gram-negative bacteria, and endotoxins (Dutkiewicz, 1997; Environment Agency, 2001a; Millner *et al*, 1994; Swan *et al*, 2003). The creation of the dataset profiling downwind bioaerosol concentrations presented in Chapter 4, and analysis of the impact of operational parameters in Chapter 5, has provided an opportunity to compare the proportions of these bioaerosols emitted and capable of downwind dispersal.

The dispersal profiles presented in Chapter 4, along with the analysis of the impact that different composting activities and environmental conditions have on bioaerosol concentrations in Chapters 5 and 6, has highlighted some of the factors affecting emission and downwind concentrations. The results have also illustrated how the response of each bioaerosol to environmental and operational influences varies. For example, each bioaerosol was shown to respond differently to individual composting activities (Chapter 5). Within the literature, there is little discussion and comparison of the emission and dispersal patterns of individual bioaerosols. Compost of different ages is known to possess different microbial community structure (Cahyani *et al*, 2002; Herrmann and Shann, 1997; Steger *et al*, 2005; Steger *et al*, 2007), suggesting that activities carried out at different compost stages may emit bioaerosols in different proportions. This has been previously suggested, with turning and screening shown to emit different ratios of actinomycetes and fungi (Albrecht *et al*, 2008; Fischer *et al*, 2008). Chapters 4 and 5 illustrated how dispersal distances of bioaerosols are different depending upon site. It was suggested that dispersal range may be affected by the

concentrations of bioaerosols emitted. This was particularly the case for actinomycete and gram-negative bacteria concentrations at Flixborough, which showed higher on-site concentrations than at Lount OWC, and the capability to disperse in elevated concentrations further downwind. It may be possible to provide additional information regarding the potential of each bioaerosol to disperse in elevated concentrations through analysis of ratios of bioaerosols within dispersal profiles. These differences in dispersal may also be attributable to the individual physicochemical properties of bioaerosols. For example, the smaller size of endotoxins (30 – 50 nm) may aid their deposition, resulting in the shorter dispersal distances seen in Chapter 4 (Dutkiewicz, 1997; Harrison *et al*, 2005; Korzeniewska *et al*, 2009; Lighthart and Mohr, 1987; Marthi *et al*, 1990; Seinfeld and Pandis, 2006; Tham and Zuraimi, 2005). Data regarding the proportions of bioaerosols released may highlight differences in behaviour related to individual bioaerosol properties as well as concentration of emissions.

Through analysis of the ratios of culturable bioaerosols and endotoxins found within Chapters 4 and 5, it may be possible to contribute to knowledge surrounding the relationships between these bioaerosols, and the overall composition of bioaerosols. For example, Chapter 4 showed how composting significantly increases the concentrations of bioaerosols in ambient air. Through analysis of the ratios of these bioaerosols, it may be possible to provide a measure of the impact that composting has on the composition of bioaerosols, and at what point downwind from site ratios return to those found in upwind. In addition, it has been suggested that analysis of the ratio of actinomycetes to fungi may allow determination of the source of bioaerosols, namely, whether from turning or screening (Albrecht *et al*, 2008; Fischer *et al*, 2008). This suggestion may be tested through analysis of the ratios of bioaerosols emitted through each operational activity (Chapter 5), and if supported, provide evidence for the individual impact that each operational activity has on bioaerosol emissions.

The relationship between gram-negative bacteria and endotoxins is also of interest, as while the presence of endotoxins is due to gram-negative bacteria; little data exists comparing their distributions and concentrations within the environment. Endotoxins are composed of proteins, lipids, and lipopolysaccharides (LPS) molecules found in the

cell membrane of gram-negative bacteria (Liebers *et al*, 2008). The molecules referred to with the term 'endotoxins' are LPS molecules, leading to the more accurate description of endotoxins as LPS within most research studies (Erridge *et al*, 2001; Liebers *et al*, 2008). The endotoxin of gram-negative bacteria is typically composed of three regions: Lipid A, the hydrophobic and endotoxically active part of the molecule; the core section; and an *O*-polysaccharide, or *O*-chain. The structure of these regions varies between species of gram-negative bacteria, resulting in different levels of immuno-toxicity (Erridge *et al*, 2002). While it has been shown that endotoxins are released in elevated concentrations from composting facilities (Chapters 4, 5, and 6) (Schlosser *et al*, 2009), these endotoxins have not yet been directly related to gram-negative bacteria species. Knowledge of this may be important in order to assess the potential for endotoxins to cause negative health impacts in people exposed to these elevated concentrations. Preliminary results gained through collaboration with the University of the West of England (UWE), Bristol, suggest that endotoxins collected from on-site have a higher immuno-stimulatory potential than laboratory strain endotoxins (*Escherichia coli*) (Liu *et al*, submitted 2009). Intuitively it may be assumed that gram-negative bacteria and endotoxins follow similar patterns in emission and dispersal. This was shown by Dutkiewicz *et al*. (1992), who found a correlation between gram-negative bacteria and endotoxins within stored timber. The dynamics of the relationship between gram-negative bacteria and endotoxin emission and dispersal throughout the composting process and downwind concentrations remain unknown. The ability to attribute endotoxins emitted from composting facilities to the gram-negative species of bacteria responsible, along with knowledge of the relationships between these bioaerosols' dispersal patterns, would allow more in-depth analysis of the potential health impacts of endotoxin exposure (Domingo and Nadal, 2009; Liebers *et al*, 2006; Liebers *et al*, 2008).

The initial aim of this Chapter is therefore to analyse the proportions of culturable bioaerosols released through different composting activities, and downwind from site. As shown in Section I, each bioaerosol responded differently to operational and environmental influences, and showed differences in ability to disperse downwind. This analysis will allow further knowledge to be gained concerning the proportions of

different culturable bioaerosols and endotoxins on-site and downwind from site. A second aim of this chapter is to identify culturable aerosolised gram-negative bacteria in order to find the species that may be responsible for endotoxin emissions at composting facilities. Achievement of this aim will provide additional information on the type of gram-negative bacteria that may be responsible for endotoxin emissions at composting facilities, increasing knowledge of potential health impacts through bioaerosol exposure.

This phase of the experiments contributes to thesis Objective ii: Characterisation of bioaerosols released from composting facilities, including viable and non-viable components. In order to achieve a contribution to this Objective, a Chapter Objective has been designed:

'Analysis of relationships between culturable bioaerosols and endotoxins, and identification of endotoxin sources'

Through the above review, as well as Chapter 1, it can be hypothesised that the ratios of culturable bioaerosols and endotoxins will vary according to composting activity. As suggested by Albrecht *et al*. (2008) and Fischer *et al*. (2008), ratios of actinomycetes to fungi emitted from turning and screening may be different. Furthermore, the data shown in Chapters 4, 5, and 6 illustrates how all bioaerosols measured are able to disperse in elevated concentrations to different distances. This suggests that analysis will highlight changes in ratios from site to downwind locations as the proportions of bioaerosols change. While studies have provided insight into species composition of compost and bioaerosols (Cahyani *et al*, 2002; Herrmann and Shann, 1997; Le Goff *et al*, 2009; Steger *et al*, 2005; Steger *et al*, 2007), the data for gram-negative bacteria has not yet been directly related to endotoxin emissions.

## 8.2 Experimental Design

The ratios of *A. fumigatus*, actinomycetes, gram-negative bacteria, and endotoxin concentrations were calculated for on-site and downwind sampling locations (measured from site boundary) and in relation to different sampling activities. The data used for this analysis was generated through Section 1, with colony forming unit counts per cubic metre of air used (CFU $m^{-3}$). As described in Chapter 5, operational parameters were altered to 'presence' (1) and 'absence' (0) data for inclusion into the STATISTICA General Linear Model (GLM), allowing average concentrations for the presence and absence of each operational activity to be calculated. Although ratios between all bioaerosols were measured, it was found that the patterns and ratios shown between fungi and gram-negative bacteria and actinomycetes were similar. Therefore, the decision was taken to aggregate actinomycete and gram-negative bacteria as 'total bacteria' in order to prevent repetition within presentation of results and discussion. Non-metric multidimensional scaling (MDS) was also used in order to describe the difference between the individual bioaerosols enumerated across all samples taken. This method is a spatial analysis, illustrating the closeness of relationships between the bioaerosols measured (McCune et al., 2002). Results through this analysis are presented in terms of 'similarity' percentage. For example, if bioaerosol concentrations within Sample A have more similarity to those found in Sample B than Sample C, Sample A will be placed closer to Sample B than Sample C on the MDS plot. The configuration of similarity presented within the MDS plot is the one with least stress; this is the configuration with the least distortion between the similarity of samples and the MDS generated plot (Clarke and Warwick, 2001). In addition, gram-negative bacteria colonies isolated and enumerated from on-site samples at Flixborough (10th July 2009) through typical culture analysis were identified through the API miniaturised biochemical test kits to identify enterobacteriaceae and non-enterobacteriaceae (bioMérieux API 20E, 20100; bioMérieux API 20 NE, 20050; available at http://www.biomerieux-diagnostics.com/servlet/srt/bio/clinical-diagnostics/home).

Procedures for gram-negative bacteria identification included: purification of isolates based on colony morphology to gain a single-strain (Seeley *et al*, 1991); suspension of

colonies in a saline solution (0.85% NaCl); and inoculation of test strip according to the manufacturer's instructions. Reading of the test strip is carried out 24 – 48 hours after inoculation. An 18 – 21 part code is generated through a sequence of either positive or negative reactions after incubation. The code is submitted to the apiweb$^{TM}$ database (40011-6) for comparison to known taxa on the API database. The proximity of the submitted code to that of known taxa is analysed, generating an identification and likelihood of correct identification.

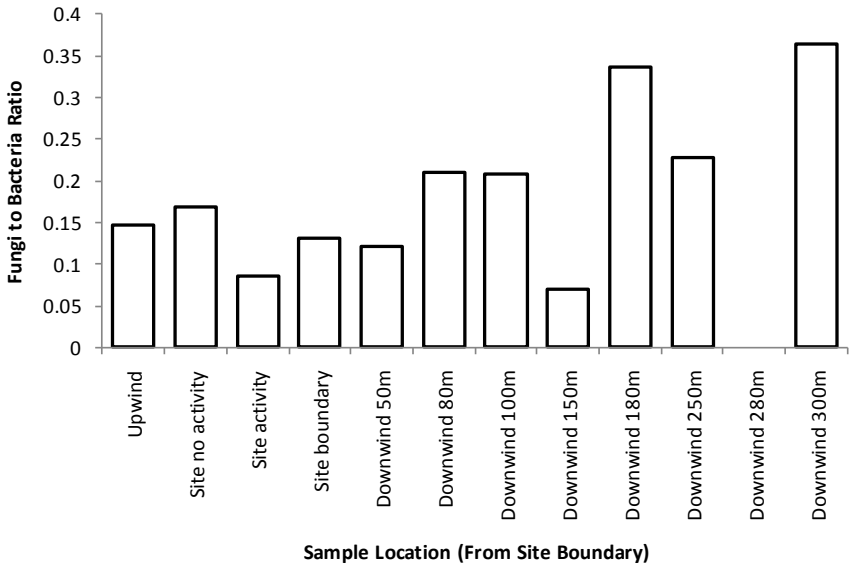
## 8.3 Results

Figures 8.1 – 8.4 below illustrate the ratios of fungi (*A. fumigatus*) (Figures 8.1, 8.3) and endotoxins to bacteria (actinomycetes and gram-negative bacteria) (Figures 8.2 and 8.4) for downwind dispersal profiles, and different composting activities. The raw data for these Figures was presented in Chapters 4 and 5, with CFU $m^{-3}$ data used for this analysis. Figure 8.1 illustrates the ratio of fungi (*A. fumigatus*) to bacteria (actinomycetes and gram-negative bacteria) for dispersal profiles presented in Chapter 4. The Figures show some site-specific features. At Lount OWC concentrations of fungi are more comparable to bacteria, with higher concentrations of bacteria found at Flixborough. The increase in the proportions of actinomycetes and gram-negative bacteria to *A. fumigatus* at Flixborough is shown by the lower ratio at this site (Figure 8.1b); however, at both sites the ratio increases further from site. Figure 8.2 shows the ratio of endotoxins (measured in EU $m^{-3}$) to gram-negative bacteria for dispersal profiles presented in Chapter 4. While ratios remain similar across most sampling locations, some increases are seen downwind. Primarily, this is noticeable at 280 m downwind at Lount OWC, where an increase in ratio is seen as a result of the outlying endotoxin value described and discussed in full as part of Chapter 4. At Flixborough an increase in the ratio of endotoxins to gram-negative bacteria is also seen from 400 – 600 m downwind.

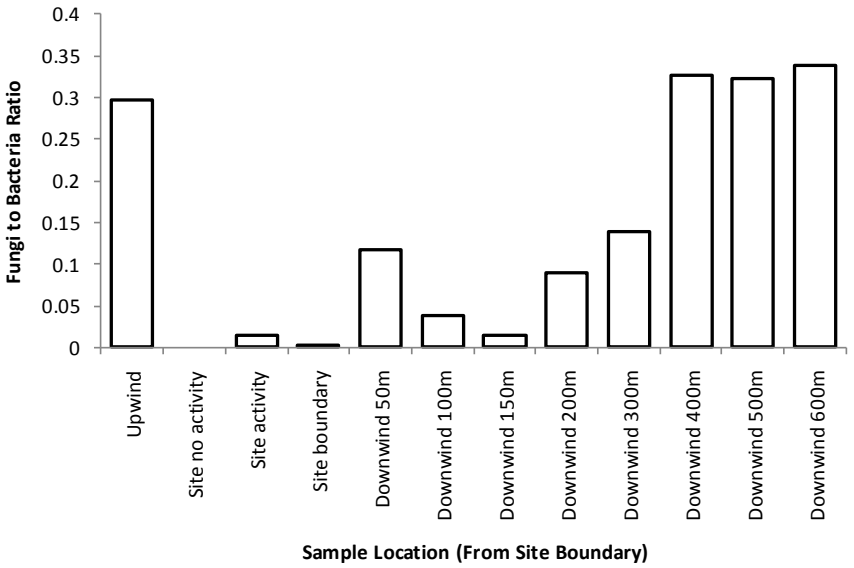
The ratios of fungi to bacteria during different composting activities also differed at each site (Figure 8.3). At Lount OWC proportions are similar across activities,

independent of the presence or absence of individual activities. However, at Flixborough increases in the ratio are seen during shredding, both types of turning and moving, and during the absence of screening; showing an increase in the amount of fungi compared to bacteria. The ratio of endotoxins to gram-negative bacteria (Figure 8.4) increases during absence of most activities, suggesting a greater increase in gram-negative bacteria than endotoxins during activities. However, this result must be interpreted with caution due to limitations presented in Chapter 5; namely, that 'absence' of one activity does not remove the influence of other activities which may be occurring on-site. At Flixborough the ratio increased during both types of turning and moving. In addition, the ratio is up to 6 orders of magnitude higher at Flixborough, showing a proportionally higher concentration of endotoxins than Lount OWC.

Figures 8.5 and 8.6 show differences between concentrations of cultured bacteria and endotoxins at all sampling locations. Data were log-transformed (Ln) and plotted using Bray-Curtis similarity and non-metric MDS (Clarke and Warwick, 2001). At both sites there is up to 60% similarity between the concentrations of cultured microorganisms, while endotoxins only show up to 20% similarity to other bioaerosols. Table 8.1 outlines gram-negative bacteria species identified through API tests, presenting the species alongside a summary of their key features. A diverse range of gram-negative bacteria were identified, covering 11 genera. Most species found are common to environmental niches, although others common to water (*Vibrio fluvialis*) and parts of normal animal flora (*Pasteurella* spp.) were also found.

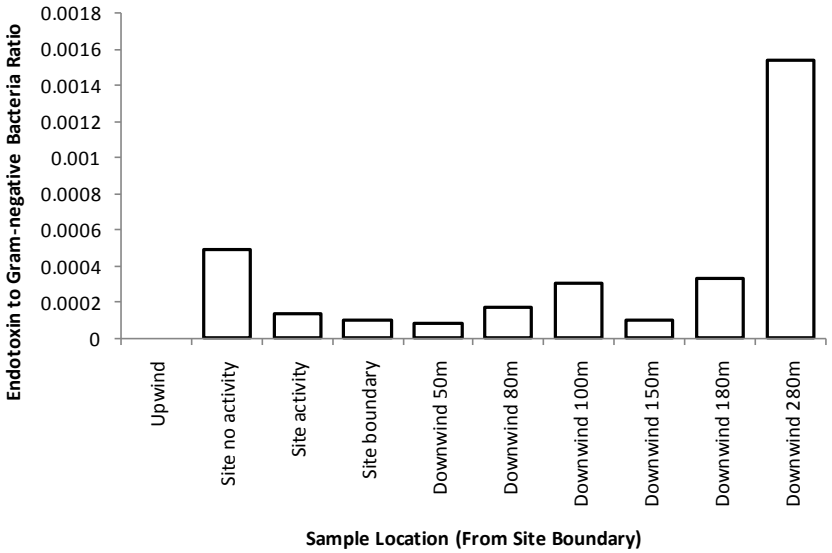



(a) Lount OWC

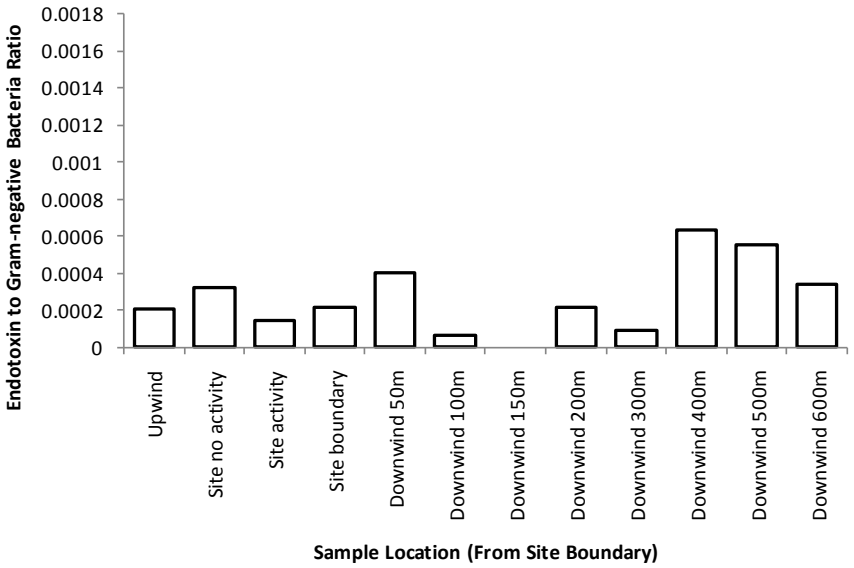



(b) Flixborough

Figure 8.1: Ratios of fungi (*A. fumigatus*) to total bacteria (actinomycetes and gram-negative bacteria), measured in CFU $m^{-3}$, at Lount OWC and Flixborough

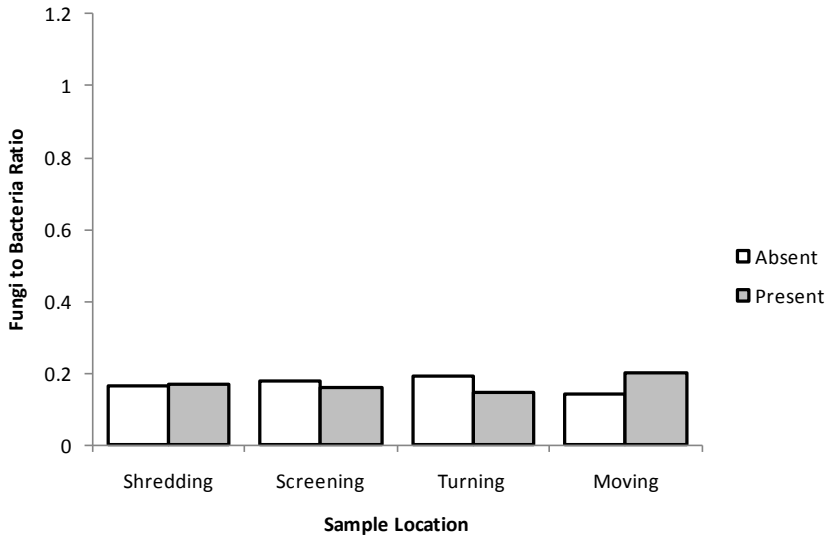



(a) Lount OWC

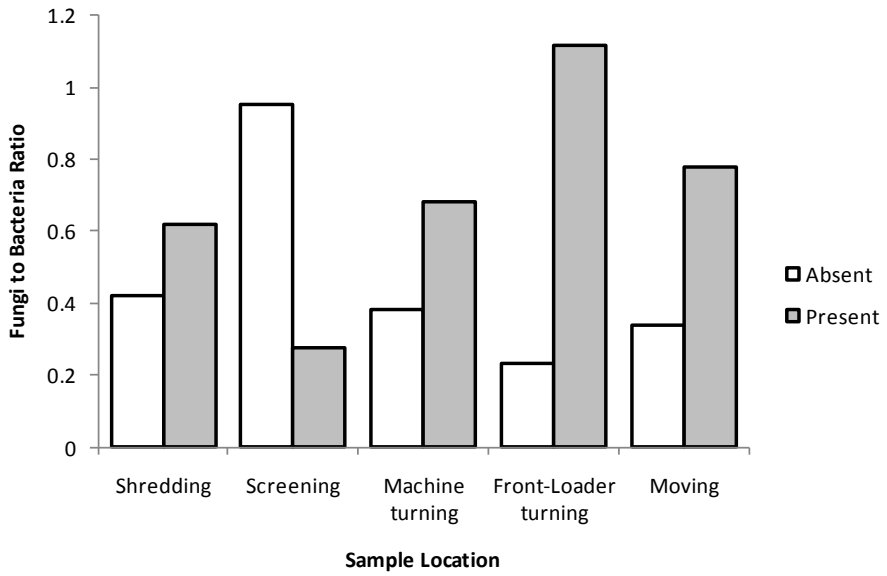



(b) Flixborough

Figure 8.2: Ratios of endotoxins to gram-negative bacteria, measured in EU $m^{-3}$ and CFU $m^{-3}$ respectively, at Lount OWC and Flixborough

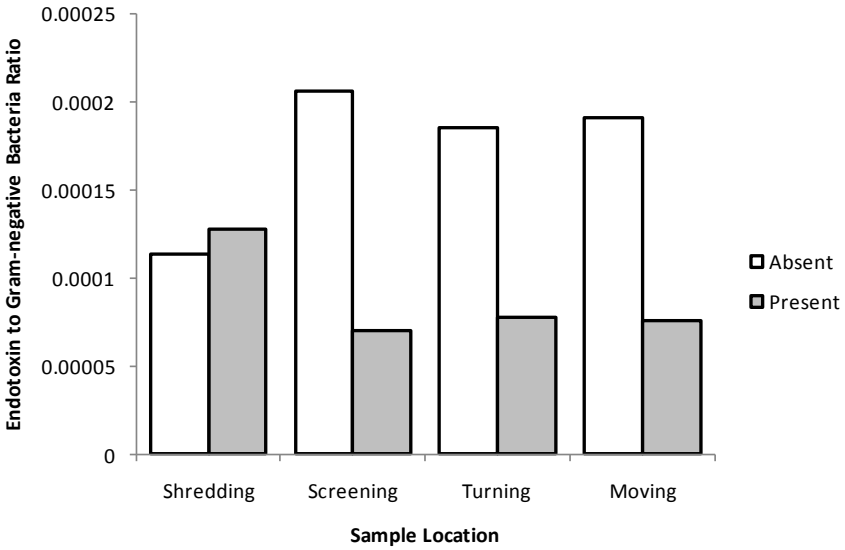



(a) Lount OWC

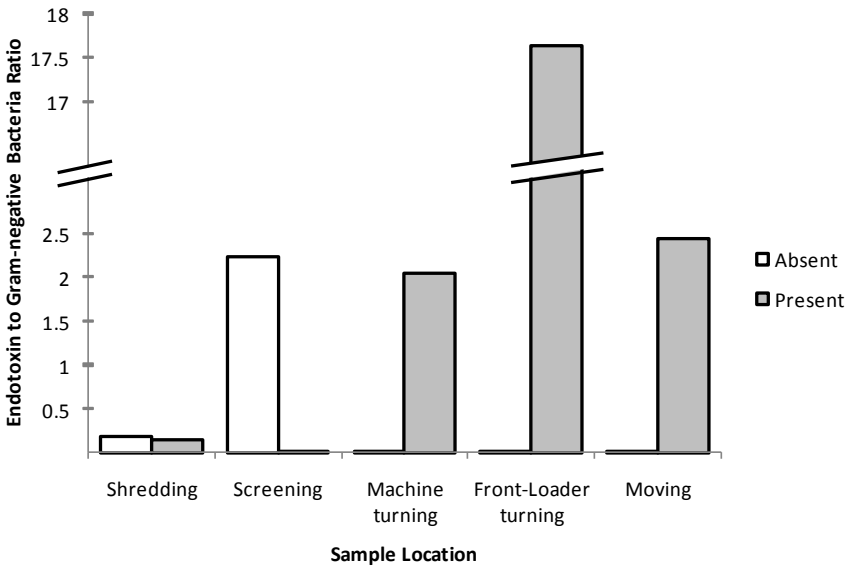



(b) Flixborough

Figure 8.3: Ratios of fungi (*A. fumigatus*) to total bacteria (actinomycetes and gram-negative bacteria), measured in CFU $m^{-3}$, at Lount OWC and Flixborough according to composting activity




(a) Lount OWC




(b) Flixborough

Figure 8.4: Ratios of endotoxins to gram-negative bacteria, measured in EU $m^{-3}$ and CFU $m^{-3}$ respectively, at Lount OWC and Flixborough according to composting activity

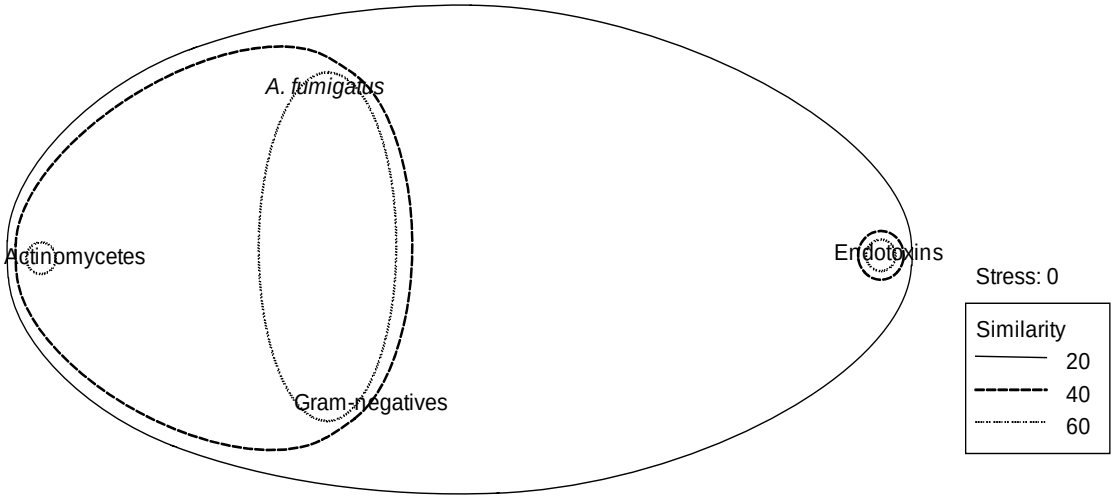



Figure 8.5: Non-metric MDS plot of *A. fumigatus*, actinomycete, gram-negative bacteria, and endotoxin concentrations at Lount OWC. Calculated using Bray-Curtis similarity, stress = 0.

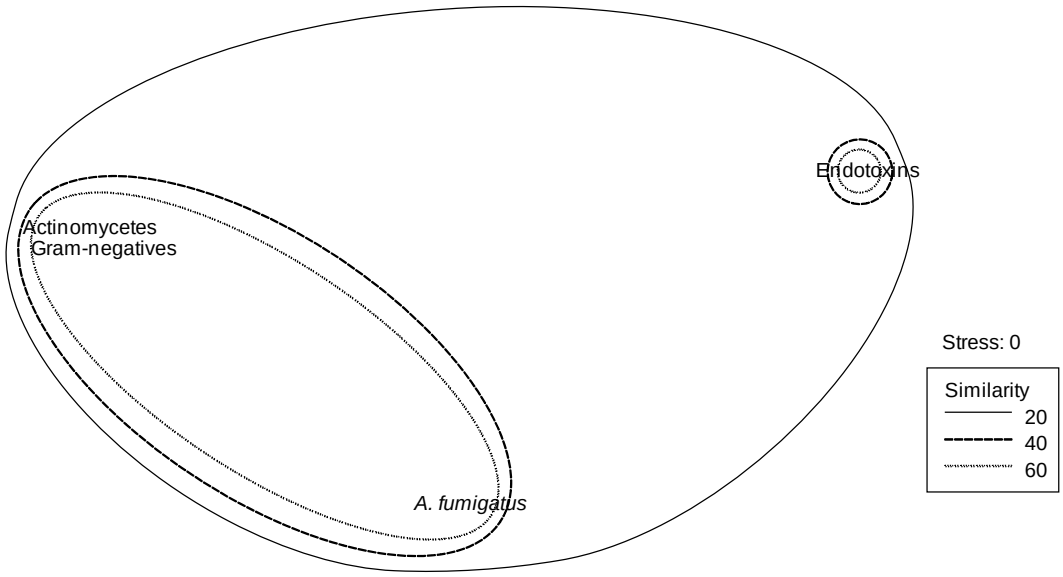



Figure 8.6: Non-metric MDS plot of *A. fumigatus*, actinomycete, gram-negative bacteria, and endotoxin concentrations at Flixborough. Calculated using Bray-Curtis similarity, stress = 0.

Table 8.1: Identification of culturable gram-negative bacteria including enterobacteriaceae (E) and non-enterobacteriaceae (NE); with summary of key features and virulence factors

| Identification | Percentage Identification | E or NE | Key Features | Sources |
|---|---|---|---|---|
| *Acinetobacter lwoffi* | 99.1 | NE | *Acinetobacter* spp. bacilli widely distributed in nature, not part of normal human flora, and can be opportunistic pathogens. *A. lwoffi* common coloniser of food and can infect immuno-compromised people. Genus LPS Lipid A form associated with highest level of immune system activation seen for LPS. | (Forbes *et al*, 2007; Leone *et al*, 2007; Van Looveren *et al*, 2004) |
| *Acinetobacter radioresistens* | 96.7 | NE | *Acinetobacter* spp. bacilli widely distributed in nature, not part of normal human flora, and can be opportunistic pathogens. Genus LPS Lipid A form associated with highest level of immune system activation seen for LPS. | (Forbes *et al*, 2007; Leone *et al*, 2007; Van Looveren *et al*, 2004) |
| *Brevundimonas vesicularis* | 99.5 | NE | Formerly part of *Pseudomonas* group. Bacilli, common in the environment and not part of normal human flora. | (Forbes *et al*, 2007) |
| *Burkholderia cepacia* | 93.3 | NE | Formerly part of *Pseudomonas* group. Mesophilic bacilli, commonly found in soil, water, and living on plant matter. Opportunistic pathogen, particularly for those suffering from cystic fibrosis and the immunocompromised. *Burkholderia cepacia* complex (BCC) consists of at least 10 species, all have an unusual Lipid A structure within LPS. BCC LPS indices increased IL-8 production from epithelial cells. Found to have cytokine producing capability comparable or greater than other pathogens. | (De Soyza *et al*, 2008; Forbes *et al*, 2007; Mahenthiralingam *et al*, 2005; Vinion-Dubieland Goldberg, 2003) |
| *Klebsiella pneumoniae* ssp. *rhinoscleromatis* | 99.9 | E | Bacilli; and opportunistic pathogen. Commonly found in soil and water. Clinically most important member of *Klebsiella* genus, particularly capable of causing pneumonia. Lipid A portion has been shown to induce inflammatory mediators (IL-8). | (Forbes *et al*, 2007; Regueiro *et al*, 2006; Williams and Tomas, 1990) |
| *Mannheimia haemolytica / Pasteurella trehalosia* | 93.4 | NE | Known cause of pneumonia in livestock, particularly cattle. LPS shown to induce IL-8 production in bovine alveolar macrophages and implicated in inflammation during bovine pneumonia. | (Lafleur *et al*, 2001) |

| | | | | |
|---|---|---|---|---|
| *Ochrobactrum anthropi* | 94 | E | Found in environmental niches, particularly those with high moisture content. May be transient coloniser of human intestinal tract. | (Forbes *et al*, 2007) |
| *Pasteurella* spp. | 52.5 | N<br>E | Bacilli, part of general animal flora.<br>Opportunistic pathogen. | (Forbes *et al*, 2007) |
| *Pseudomonas luteola* | 99.6 | N<br>E | Found in variety of environmental niches, not part of normal human flora. Opportunistic pathogen. | (Forbes *et al*, 2007) |
| *Sphingomonas paucimobilis* | 99.2 | N<br>E | Common to environmental niches, not part of normal human flora. Contains glycosphingolipids (GSL) rather than LPS. Some GLS shown to be capable of inducing IL-6 and IL-1 production, shows less potency than LPS. | (Forbes *et al*, 2007; Krziwon *et al*, 1995) |
| *Stenotrophomonas maltophilia* | 99.7 | N<br>E | Formerly part of *Pseudomonas* group. Widely distributed in environment, particularly soil and plants. Has role in rhizosphere processes. Opportunistic pathogen, shown to stimulate immune system, but only weakly invade system, suggesting may contribute to airway inflammation. | (Forbes *et al*, 2007; Ryan *et al*, 2009) |
| *Vibrio fluvialis* | 89 | E | Commonly found in marine or brackish aquatic environments, also sometimes in freshwater. Gastroenteritis and primary septicemia can result from ingestion of contaminated seafood or water. Skin infection from exposure to contaminated water. | (Forbes *et al*, 2007; Tantillo *et al*, 2004) |

## 8.4 Discussion

The first aim of this phase of the study was to utilise the database formed through Section I of the thesis to analyse the difference in composition of cultured bioaerosols and endotoxins, and how emissions through composing affect the ratios of bioaerosols in ambient air. A second aim was to identify the key culturable species of gram-negative bacteria isolated. Figures 8.1 and 8.2 illustrate changes in the ratio of fungi (*A. fumigatus*) to total culturable bacteria (actinomycetes and gram-negative bacteria), and endotoxins to gram-negative bacteria throughout the dispersal profiles constructed in Chapter 4. The changes in ratio seen reflect patterns in dispersal presented in Chapter 4. In addition, at both sites an increase in the ratio of fungi to bacteria towards the end of the dispersal profile is seen (Figures 8.1). These increases coincide with the reduction of

bioaerosol concentrations to levels statistically similar to those found upwind or below detection limits (Chapter 4). This was shown to occur at 300 – 400 m for bacteria, and 100 m downwind for *A. fumigatus* at Flixborough, and 180 m at Lount OWC. The increase in ratio may therefore be indicative of a return of ratios to levels found ambiently in the environment, after alteration by emissions from composting. Further data at Flixborough support this (Figure 8.1), with the ratio at 400 m downwind returning to levels similar to those found upwind. Ratios were more even across the dispersal profile at Lount OWC, indicating that higher concentrations of fungi in proportion to bacteria were recovered from this site. The higher concentrations of actinomycetes and gram-negative bacteria at Flixborough were discussed in Chapters 4, 5, and 6, and may be due to differences in feedstock, or increased emission of particles due to the maintenance of compost at lower moisture content at Flixborough (Chapter 2) (Jones and Harrison, 2004). Changes in the ratio of endotoxins to gram-negative bacteria (Figure 8.2) also reflected dispersal patterns and reduction to upwind concentrations seen in Chapter 4, including the outlying endotoxin value found at 280 m downwind at Lount OWC.

The ratios of fungi to bacteria and endotoxins to gram-negative bacteria were also examined according to site activity (Figures 8.3, 8.4). The data for these Figures were calculated using average meteorological conditions (Chapters 3, 5). This resulted in differences being found between ratios measured without meteorological parameters (Figures 8.1, 8.2), from those calculated taking into account average meteorological parameters (Figure 8.3, 8.4). This was most evident at Flixborough, where higher ratios are evidenced within Figure 8.3, indicating a higher proportion of fungi as compared to total bacteria emitted assuming average meteorological conditions. Despite these differences, Figure 8.3 shows how at Lount OWC ratios of fungi to bacteria were similar across all sampling locations, indicating little change in the relative concentrations of bacteria or fungi emitted by individual activities. However, at Flixborough shredding, both types of turning, and moving led to increases in the ratio, indicating a proportionally higher increase in fungi from these activities. This may be indicative of the ease with which fungal spores may be emitted in comparison to bacterial cells, as *A. fumigatus* reproduction relies upon the emission of spores from

aerial hyphae (Epstein *et al*, 2001; Swan *et al*, 2003). The higher ratio during absence of screening suggests a greater increase in bacteria during screening than fungi, potentially indicative of a decrease in the prevalence of fungi during later stages of composting. However, this is contradictory to other studies, which have suggested that concentrations of fungi increase during the mesophilic composting phase (Cahyani *et al*, 2002; Herrmann and Shann, 1997). As little is known with regards to the aerosolisation of different bioaerosols throughout the composting process, this finding cannot be analysed further. It was suggested by Albrecht *et al.* (2008) and Fischer *et al.* (2008) that the ratio of actinomycetes to fungi may be of use to analyse the source of bioaerosols. The data here support this theory at Flixborough, with higher proportions of fungi found for turning, and higher levels of bacteria for screening. However, Lount OWC did not show the same relationship, suggesting that differences in site feedstock and composting processes (Chapter 2) reduce the usefulness of comparing ratios.

Comparison of the ratio of endotoxins to gram-negative bacteria also shows site-specific features (Figure 8.4). At Lount OWC the decreased ratio during activities suggests that proportionally higher levels of gram-negative bacteria are released during activities than endotoxins. At Flixborough, ratios exceed those found at Lount OWC by several orders of magnitude, reflecting the higher endotoxin concentrations found at this site (Chapters 4, 5). It can be suggested that the higher ratio at Flixborough may be due to feedstock composition, with Flixborough receiving household vegetable derived waste (Chapter 2). This suggestion is supported by the higher ratio seen during shredding at Flixborough, suggesting more endotoxin is present in fresh green waste than at Lount OWC. At Flixborough ratios during activities increase up to 2 orders of magnitude, while at Lount OWC they remain within the same order of magnitude across all activities. The increases in the ratio illustrate the higher relative increase of endotoxins than gram-negative bacteria at Flixborough. This may be due to differences in the condition of the compost, with lower moisture content and vigour of the activity combining to increase emissions (Chapter 5). It was suggested in Chapter 5 that the high energy of machine turning may cause more disruption of gram-negative cells and therefore increased emission of endotoxins (Dutkiewicz, 1997); while lower moisture content may enhance emissions of particulates, including endotoxins (Jones and

Harrison, 2004). The data here supports and enhances the findings and discussion presented in Chapter 5, showing how machine turning activity results in a higher increase in emissions of endotoxins compared to gram-negative bacteria. These results suggest that the emission of endotoxins is related to both composting processes at each site, and the condition of the feedstock.

Through MDS it was shown how at both sites endotoxins show the least similarity to the other bioaerosols emission and dispersal patterns. The dispersed positioning of all bioaerosols in Figure 8.5 indicated that even when sharing 60% similarity in concentration patterns, bioaerosols retain individual features of emission and dispersal; although at Flixborough, the close proximity of actinomycetes and gram-negative bacteria indicates more similar patterns. None of the bioaerosols measured showed more than 60% similarity at either site, negating the assumption that all bioaerosols will be emitted and disperse in the same way. This is particularly illustrated by the relationships between gram-negative bacteria and endotoxins. Despite the fact that gram-negative bacteria are responsible for the presence of endotoxins at composting facilities, Figure 8.4 showed how they are emitted in different proportions according to activity, while Figures 8.5 and 8.6 showed how endotoxin dispersal patterns are the most dissimilar from any other bioaerosol measured. This may be due to the different physicochemical properties of gram-negative bacteria and endotoxins. In particular, while most gram-negative bacteria can be found around 1 – 3 μm in size (Tham and Zuraimi, 2005), it has been suggested that endotoxins can be found from 30 – 50 nm in size (Dutkiewicz, 1997). This difference is likely to result in different behaviour once released through composting activities, however, there is not enough knowledge regarding their physicochemical properties to predict dispersal patterns and ranges based on particle size.

In addition, the relationship between gram-negative bacteria and endotoxins must be interpreted with caution. Little is known with regards to the release mechanisms of endotoxins. For example, while gram-negative bacteria are likely to produce endotoxins when alive, it is unknown whether upon cell death, disruption, or desiccation, endotoxins are emitted in higher concentrations. Further, it remains unknown whether

endotoxins will remain persistent within compost upon cell death, potentially being emitted as bioaerosols, or be broken down. The results here compare culturable aerosolised gram-negative bacteria to endotoxins. The enumeration of culturable gram-negative bacteria alone has left the non-culturable and dead bacteria unaccounted for, although these may also be a source of bioaerosols. It is also possible that contribution to endotoxin concentrations may have been made by gram-negative bacteria that have not been aerosolised, and therefore also not enumerated. In order to fully understand the relationships between gram-negative bacteria and endotoxins, it may be necessary to enumerate the consortia of gram-negative bacteria within green waste, as well as the non-viable and non-culturable fractions.

Gram-negative bacteria cultured from site were also identified in order to discover some of the species responsible for endotoxin emission at composting facilities. This information was intended to complement studies investigating the immuno-stimulatory properties of endotoxins (Liu *et al*, submitted 2009), and provide further information on the potential health impacts of bioaerosol exposure. The species identified and a summary of their main features, particularly known LPS properties, are shown in Table 8.1. Of particular interest is *Acinetobacter* spp., whose LPS has been shown to contain the most potent known immuno-stimulatory Lipid A structure. Also of interest is *Burkholderia cepacia*. The *Burkholderia cepacia* complex (BCC) comprises at least 10 species of bacteria. They have been shown to stimulate the immune system with potency similar to that found for *Escherichia coli*. BCC is an emerging opportunistic pathogen, meaning that it is increasingly being implicated in hospital acquired infections. BCC has been shown to particularly affect those with cystic fibrosis. In cystic fibrosis patients BCC can cause 'cepacia syndrome', characterised by uncontrollable deterioration and death (Mahenthiralingam *et al,* 2005). The endotoxic potential of BCC is one of the factors that increases risk to immuno-compromised patients, with many studies now available investigating the properties of BCC and its LPS (De Soyza *et al*, 2008; Mahenthiralingam *et al,* 2005; Vinion-Dubiel and Goldberg, 2003). *Klebsiella* spp. were also found, and have been shown to induce an immune response (Regueiro *et al,* 2006). In addition, *Mannheimia haemolytica*, a well known cause of pneumonia in livestock, particularly cattle, was identified (Lafleur *et al*, 2001).

The identification of these gram-negative species has highlighted the importance of understanding sources of endotoxins at composting facilities. All species found are *Proteobacteria* (*Alpha, Beta* and *Gamma*), in agreement with gram-negative bacteria dominance within the composting bioaerosols recently identified by Le Goff *et al* (2009). The species shown in Table 8.1 were enumerated through culture; comprising the key airborne gram-negative species that can be cultured from green-waste composting facilities. Due to the selectivity of culture, the presence of the NCBV (Chapter 7), and the limited scale of this study (based on one sampling occasion) this identification is unlikely to represent the full diversity of gram-negative bacteria, and therefore sources of endotoxin. Chapter 4 illustrated how emissions of endotoxins from composting facilities reach concentrations well above proposed threshold values. For example, on-site at Flixborough geometric mean concentrations were found 1 order of magnitude higher than the 50 EU $m^{-3}$ workplace exposure threshold that has been previously suggested (Chapter 4) (Schlosser *et al*, 2009). The combination of these high concentrations with a consortium of gram-negative bacteria possessing LPS with high endotoxic properties, suggests a potential human health risk from exposure to composting endotoxins. This risk may be posed to compost site workers, as well as off-site sensitive receptors; Chapter 4 demonstrated the ability of endotoxins to disperse off-site in elevated concentrations. However, as little dose-response data is available the health response to exposure cannot be determined.

## 8.5 Conclusions

The results from this study have resulted in a number of conclusions, outlined below.

- Analysis of the ratios of bioaerosols is of use in the determination of the effect that composting bioaerosols have on the ambient composition of bioaerosols
- Site specific differences in feedstock composition and conditions reduces the usefulness of determining source of bioaerosols through ratio analysis

- Differences in emission and dispersal patterns of bioaerosols result in no more than 60% similarity in patterns between any bioaerosol measured
- Gram-negative bacteria emitted through the composting process contain a range of endotoxins of concern with regards to human exposure and health impacts
- The dispersal range and patterns of endotoxins is largely unrepresented by culturable gram-negative bacteria dispersal range and patterns

The importance of understanding the emission and dispersal patterns of bioaerosols, particularly gram-negative bacteria and endotoxins was highlighted through this study. Composting may result in the emission of gram-negative bacteria possessing endotoxin of significance in terms of human health. However, the lack of a relationship between endotoxins and culturable gram-negative bacteria suggests other, un-enumerated gram-negative bacteria also act as endotoxin sources. The importance of basing dispersal studies on site data was illustrated, as under- or over-estimation of sensitive receptor exposure may result from the assumption that bioaerosols have similar patterns of emission and dispersal. The hypothesis that bioaerosol composition will alter according to distance downwind and composting activities has been supported; although these alterations were not as consistent as was anticipated. In addition, as could have been anticipated following the recent publication from Le Goff, *et al.* (2009), key gram-negative species found belonged to the bacterial phylum, *Proteobacteria*.

Although this study has gone some way towards characterising culturable bioaerosols and their relationship with endotoxins, the use of culture based methods means that NCBV fractions have not been accounted for. While there is a suggestion that differences in dispersal patterns may result from the differing sizes of bioaerosols, there remains little information on the individual physicochemical properties of bioaerosols, including aerodynamic diameter. Although recent studies have suggested that the majority of bioaerosols are emitted as single cells (Tamer Vestlund, 2009), few published studies have examined the size distribution of bioaerosols emitted through composting. This is despite the fact that size may affect behaviour within the atmosphere (ADAS and SWICEB, 2005; Borrego *et al*, 2000; Jones and Harrison, 2004). These differences cannot be enumerated with the methods used as standard

throughout this project (Chapter 3). In order to further understand non-culturable fractions and determine particle sizes of bioaerosols, direct counting methods can be used. The following Chapter continues the characterisation of bioaerosols emitted by composting through use of a novel, real-time direct counting method alongside the filtration based methods used as standard throughout this project.

# 9 Direct comparison between filtration sampling and a novel particle counting method

## 9.1 Introduction

Although sampling through filtration followed by culture is a well established method (Eduard and Heederik 1998; Palmgren *et al,* 1986; Taha *et al,* 2005a; Verein Deutscher Ingenieure, 2004a, b), there is acknowledgement within the available literature that non-culture based methods are required in order to provide a complete picture of bioaerosol composition and concentration (Chapter 1). Within known microbial communities a proportion of microorganisms remain uncultured. This may be due to the microorganisms being sensitive to disruption, or unable to grow within the substrate and temperature conditions selected for culture analysis. In addition, a fraction of microorganisms may be non-culturable but viable (NCBV) (Giovannoni *et al*, 1990; Head *et al*, 1998; Ward *et al*, 1990). Bioaerosol sampling has also been shown to reduce culturability further, through sampling stresses resulting in microorganisms becoming NCBV, or causing loss of viability (Cheng *et al,* 2008a; Crook and Sherwood-Higham 1997). Through studies comparing culturable fungi with direct spore counts it has been suggested that only 0.3 to 55% of airborne fungi are enumerated through culture (Cheng *et al,* 2008a; Lappalainen *et al,* 1996; Lee *et al,* 2006). Furthermore, Heidelberg *et al.*, (1997) demonstrated through controlled environmental chamber testing and enumeration through epifluorescence microscopy that under 10% of aerosolised gram-negative bacteria were culturable (Heidelberg *et al,* 1997). Within soil microbiology, where non-culture based methods are arguably further advanced, it has been suggested that only 1% of bacterial cells are represented using culture based methods (Ritz, 2007; Head *et al*, 1998). In addition, bioaerosols are also composed of the products and constituent parts of microorganisms, such as endotoxins and glucans (Liebers *et al,* 2006; Swan *et al,* 2003); which cannot be enumerated using culture-based methods. However, non-culturable, NCBV, and non-viable fractions may remain a health risk for sensitive receptors (Crook and Sherwood-Higham, 1997).

Within soil and aquatic microbiology, there has been a range of both direct counting and molecular methods developed to be used both independently from, and alongside, culture based methods in order to provide the best possible quantification and qualification of microorganism communities. However, few of these methods have been used with regularity in the field of bioaerosol research (Environment Agency, in press), and few of them are developed specifically for bioaerosol enumeration. The experimental work for this project has used filtration with enumeration through culture as the basic method (Chapter 3). However, in light of the possibility for underestimation of bioaerosol concentrations using these methods, opportunities for sampling with novel and non-culture based methods were investigated.

Many direct counting methods for microorganism enumeration exploit their natural fluorescence in order to separate biological from non-biological particles. Methods used to enumerate bioaerosols include Flow Cytometry combined with fluorescence (FCM/FL) and Epifluorescence microscopy (Chen and Li, 2005a, b; Prigione *et al*, 2004). However, an alternative utilisation of the fluorescence of biological particles has been implemented in the design of a novel biological particle counter developed specifically to detect bioaerosols. The prototype wide issue bioaerosol sensor (WIBS) uses the fluorescence of tryptophan and nicotinamide adenine dinucleotide (NADH) to enumerate the total number of biological particles within the particle size fraction of 0.8 – 20 μm in real-time (Kaye *et al*, unpublished material). A second real-time airborne particle counter, P-TRAK, uses condensation to enable ultrafine particles to reach detectable sizes (Kaur *et al*, 2006); providing a count of particles 0.02 – 1 μm in size. Together these instruments are being utilised to provide novel data on airborne biological and ultrafine particles.

The results from a collaborative project, allowing simultaneous enumeration of bioaerosols through filtration followed by culture, endotoxin assay, WIBS, and P-TRAK is presented within this Chapter. The combination of cutting-edge bioaerosol enumeration with more traditional culture methods provided the opportunity to directly compare these methods, and their effectiveness for enumerating bioaerosols. The provision of data regarding the non-culturable fractions of bioaerosols meets one of the

central aims of this project, contributing to knowledge surrounding the composition of bioaerosols emitted through composting. In addition, direct comparison with filtration and culture-based methods will allow evaluation of the non-viable and NCBV fractions that may be omitted through culture-based analysis (Cheng *et al,* 2008a; Heidelberg *et al,* 1997).

The comparison between these methods made a significant contribution towards the achievement of thesis Objective ii; 'Characterisation of bioaerosol composition and communities including viable, non-viable and non-culturable components'

With the Chapter Objective:

'Direct comparison between biological particles enumerated through novel direct counting methods and culture-based methods'

Given that it has been frequently acknowledged that culture methods may underestimate bioaerosols (Cheng *et al,* 2008a; Heidelberg *et al,* 1997), it can be hypothesised that WIBS3 would return concentrations higher than those found using culture based methods. This was anticipated as the size range encompassed by WIBS3 (1 – 20 μm) would include both bacteria and fungi, with the ability of WIBS3 to enumerate biological particles also providing counts of non-viable and NCBV bioaerosols, as well as those that can be enumerated through culture. The size of endotoxins released from composting in the atmosphere is unknown, although according to Dutkiewicz (1997), it is more likely that P-TRAK would detect these with its particle size range of 0.02 – 1 μm. A second hypothesis can therefore be formed, that patterns of endotoxin concentrations will show similarities with measurements from P-TRAK, while *A. fumigatus*, actinomycetes and gram-negative bacteria patterns in concentration will show similarities with WIBS3 measurements.

## 9.2 WIBS3

WIBS3 is the third generation in a series of multi-parameter prototype sensors that can count particles, assess their size and shape, and indicate the presence of biological fluorophores (Kaye *et al,* unpublished material). Although past emphasis for such particle counters has been for biohazard detection purposes, WIBS3 is being tested in a variety of settings as interest in bioaerosols within the field of atmospheric and climate research increases (Kaye *et al,* unpublished material).

Causing biological particles to fluoresce is a well established method to separate them from non-biological particles. The WIBS sensors use miniature xenon flashtubes to allow selection of optimal excitation wavelengths for biological fluorophores, while also providing sufficient energy to cause fluorescence (Kaye *et al,* unpublished material). This has allowed the technology to be incorporated into portable sensors, where traditionally lasers have been required to provide intense excitation illumination. WIBS3 draws in ambient air through a laminar flow system, this places suspended particles into single file. Air is sampled at a flow rate of 2.3 L $min^{-1}$ with around 200 mL $min^{-1}$ of the sample filtered and used to sheath the sample flow (Kaye *et al,* unpublished material). The single file of particles traverses a focused beam from a 635 nm continuous-wave diode laser (Figure 9.1). The intersection of the particulates with the laser beam defines the scattering volume. As each particle enters the scattering volume it produces a scattered light signal, which is captured by a quadrant photomultiplier detector (Figure 9.2). Each of the four quadrant detector elements receives a light distribution, which can be used to calculate an asymmetry factor for each particle. The sum of the four quadrant detector elements output is total scatter, which is used to estimate particle size. Particles that are found to be over ~1 µm in size trigger the firing of the two xenon UV flashlamps (Figure 9.1), which excite particle fluorescence (Kaye *et al,* unpublished material).

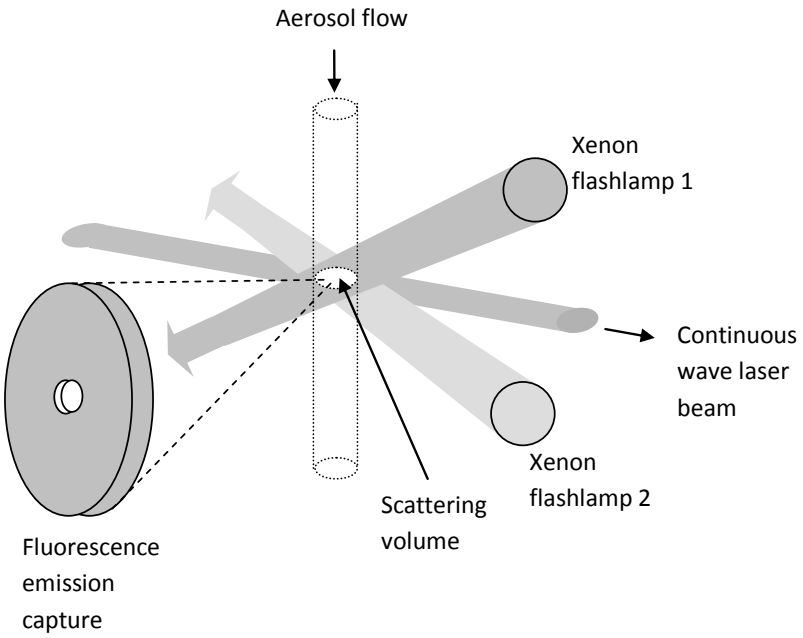



Figure 9.1: Schematic of the WIBS3 operating system, reproduced from Kaye, *et al*. Actual size of sensor head is approximately 26 × 22 × 28 cm (Kaye *et al*, unpublished material).

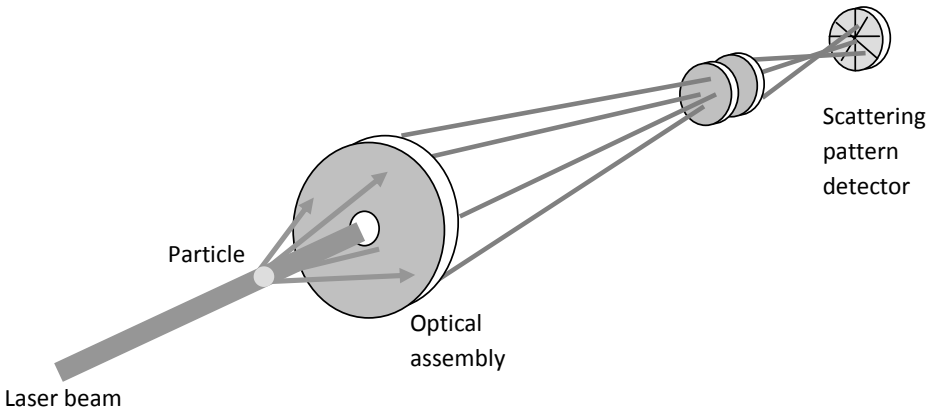



Figure 9.2: Schematic of the particle shape detecting elements of WIBS3, reproduced from Kaye, *et al*. (Kaye *et al*, unpublished material).

The flashlamps are optically filtered, allowing the desired wavelengths to be achieved. These are peak wavelengths of 280 nm, the fluorescence excitation of the amino acid, tryptophan; and 370 nm, the fluorescence excitation of nicotinamide adenine dinucleotide

(NADH). Tryptophan is produced by living organisms, including both microorganisms and plants; while NADH is a coenzyme used in various cellular processes (Green *et al,* 1990). The measurement of the fluorescence of tryptophan and NADH together gives a measure of the amount of biological particles and their viability contained in the sample (Kaye *et al,* unpublished material). The fluorescence detection channels are also optically filtered. The first detector (FL1) measures fluorescence across a band from approximately ~310 – 400 nm. This contains the maximum emission spectrum of tryptophan. The second (FL2) measures from approximately ~420 – 600 nm, encompassing the NADH emission band (Kaye *et al,* unpublished material).

The magnitude of the scattered light signal produced by each particle as it enters the scattering volume is typically larger than the particle fluorescence, this allows side scatter, and the fluorescence pulse of FL2 to be recorded using the same photomultiplier tube. An approximate measure of particle size can also be obtained using this scatter (Kaye *et al,* unpublished material).

The result is that each particle passing through WIBS3 has several parameters measured, these include: particle side scatter, fluorescence from ~310 – 400 nm with 280 nm excitation, fluorescence from ~420 – 600 nm with 280 nm and 370 nm excitation, particle time of flight through laser beam, four quadrants of forward scatter from shape detector photomultiplier tube, calculated asymmetry factor, and size, calculated from scatter and a calibration table (Kaye *et al,* unpublished material).

## 9.3 P-TRAK

P-TRAK is an Ultrafine Particle Counter manufactured by TSI Inc. (TSI Incorporated, 2006). This experiment used model 8525. P-TRAK utilises the condensation of fine particles when mixed with alcohol to count particles 0.02 – 1 μm in size (Kaur *et al,* 2006).

P-TRAK is a battery operated, portable instrument. Air is drawn through the instrument via a pump. The particles drawn in pass through a saturator tube, which mixes the particles with alcohol vapours (Zhu *et al,* 2006) produced by isopropyl alcohol that has been pre-loaded into the machine (TSI Incorporated, 2006). Once passed through the saturator tube, the alcohol and particle mixture passes into the condenser tube. This cools the air stream causing alcohol to condense onto the particles, increasing their size until they are detectable droplets (Zhu *et al,* 2006). These droplets then pass through a focused laser beam; each one causes light to scatter as it passes through the beam. These flashes of scattered light are detected via a photodetector. The total count of flashes is assumed to represent particle number concentration (Zhu *et al,* 2006).

P-TRAK can record ultrafine particle concentrations every minute, allowing rapid detection of peaks in concentration, rather than giving time-averaged concentrations (Kaur *et al,* 2006). It can detect ultrafine particles up to concentrations of 500,000 particles per cubic centimetre (pt/cc), although it has been suggested that above 100,000 pt/cc there can be some under-estimation of concentrations, due to coincidence errors (Kaur *et al,* 2006).

P-TRAK can be used alongside WIBS3, allowing real-time measurements of particles 0.02 – 20 μm in diameter to be taken. For the purposes of this experiment, both instruments were used alongside filtration, followed by culture; and endotoxin assay carried out by UWE, Bristol (Chapter 3).

## 9.4 Experimental Design

The aim of this experiment was to test filtration followed by analysis through culture, with real-time particle counts generated by both WIBS3 and P-TRAK. Experimental work for this study was carried out at Flixborough, on the 26$^{th}$ November 2008. Samples were taken

at the following locations, with each sampling device used alongside each other and operated simultaneously:

- 100 m Upwind
- Adjacent to turning activities
- Adjacent to shredding activities (also downwind from turning)
- 50 m Downwind from activities
- 100 m Downwind from activities

These sampling locations are illustrated in Figure 9.3 below.

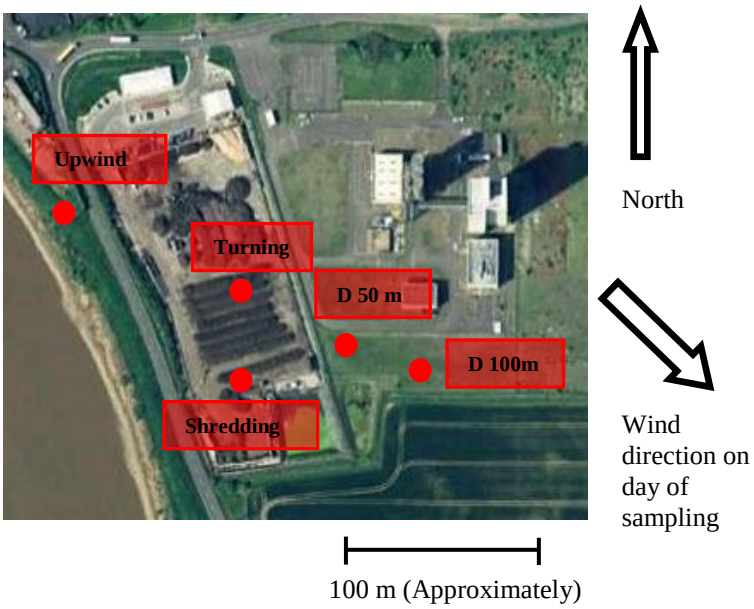



Figure 9.3: Aerial view of Flixborough, superimposed with sampling locations (Infoterra Ltd and Blueskyand Tele Atlas, 2009). Each sampling location occupied by co-located filtration, WIBS3 and P-TRAK samplers.

Sampling time for all instruments was 30 minutes, with samples taken at a height of 1.8 m. Triplicate sampler were obtained for culture and endotoxin analysis. For WIBS3 and P-TRAK measurements were taken every minute and averaged over the 30 minute time period.

Following the sampling day, WIBS3 and P-TRAK were transported back to Manchester University for data extraction and analysis. Filters used for endotoxin collection were sent to UWE Bristol for assay, while filters used for microbiological collection were returned to Cranfield University for enumeration of *A. fumigatus*, actinomycetes and gram-negative bacteria through culture (Chapter 3). Results from each sampling location were averaged, and presented in the following results section.

## 9.5 Results

### 9.5.1 Culturable Microorganisms and Endotoxins

As has been seen on previous sampling occasions, the highest concentrations of culturable bioaerosols were measured during composting activities. Mean *A. fumigatus* concentrations at shredding reached over $1.6 \times 10^4$ CFU $m^{-3}$ (Figure 9.4). Unlike other sampling occasions, turning was not the greatest source of *A. fumigatus*, with concentrations at this location over 1 order of magnitude less than those found at screening. Actinomycetes differed from *A. fumigatus* in that the highest concentrations were gained adjacent to turning rather than shredding (Figure 9.4). As has been found previously, actinomycetes were detectable at all sample locations (Chapters 4, 5, 6). Gram-negative bacteria showed a very similar release and dispersal pattern to actinomycetes (Figure 9.4). Concentrations at turning were the highest, at $4.4 \times 10^4$ CFU $m^{-3}$ (Figure 9.4). The pattern of endotoxin distribution was more similar to *A. fumigatus* (Figure 9.5). The highest concentrations were recorded adjacent to shredding activities, returning a mean value of 203 EU $m^{-3}$. Endotoxins were detectable at

all sampling positions (Figure 9.5). For all bioaerosols, dispersal patterns were similar to those found in Section I, and fully described as part of Chapter 4, with an initial drop in bioaerosol concentrations from source, followed by a second peak in concentrations at 100 m downwind (Figure 9.4).

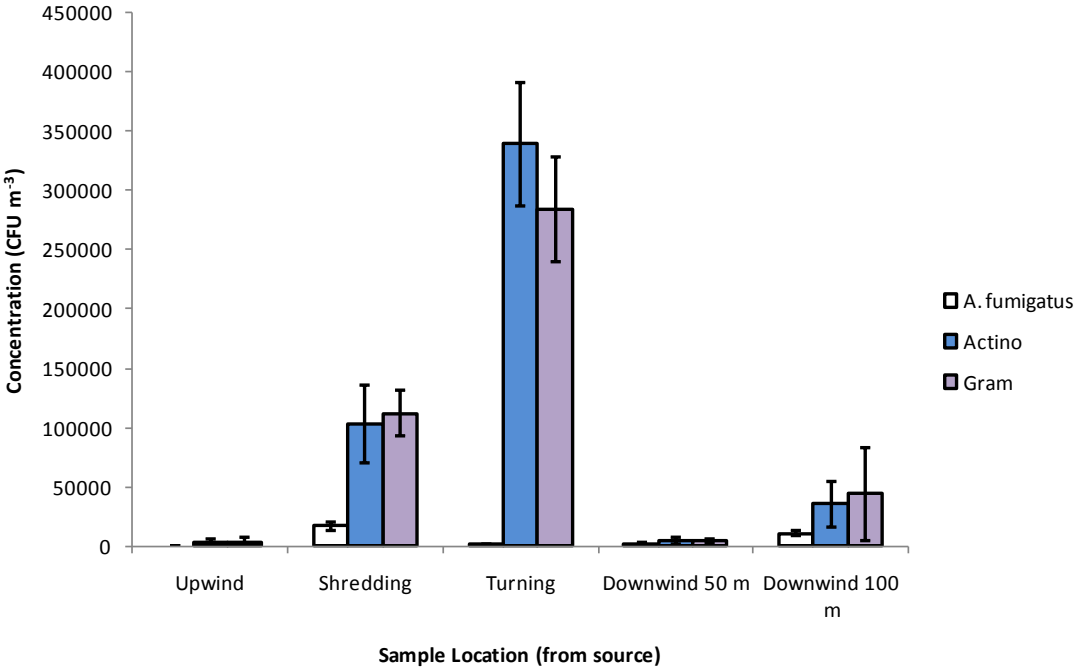



Figure 9.4: Mean microbial concentrations presented in colony forming units per cubic metre (CFU $m^{-3}$) represented by bar, standard deviation represented by whisker. 'A. fumigatus = *Aspergillus fumigatus*; 'Actino' = actinomycetes; 'Gram' = Gram-negative bacteria.

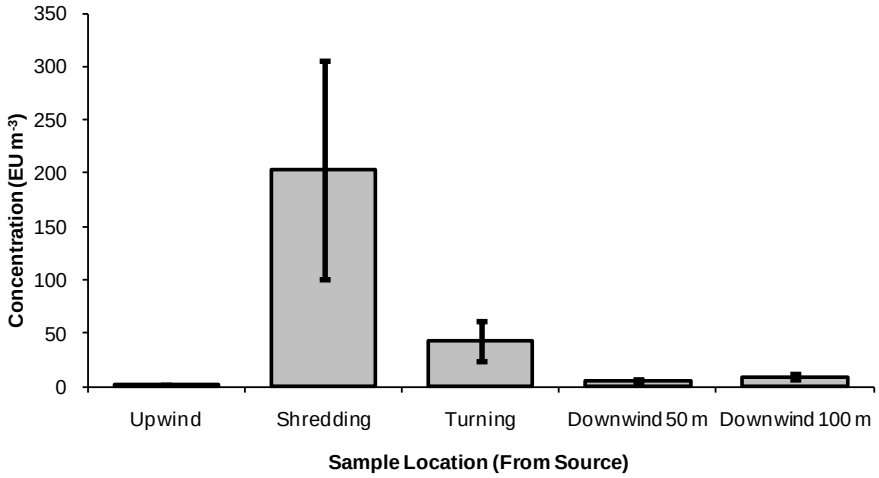



Figure 9.5: Mean endotoxin concentrations presented in endotoxin units per cubic metre (EU $m^{-3}$) represented by bar, standard deviation represented by whisker.

### 9.5.2 WIBS3 and P-TRAK

A key to results generated by WIBS3 is given in Table 9.1 below:

Table 9.1: Description of the identification given to results generated by WIBS3.

| ID | Description |
|---|---|
| Total | Total number of particles passing through the instrument |
| Bio 1 | Number of particles that excite and fluoresce like tryptophan |
| Bio 2 | Number of particles that excite like tryptophan but fluoresce like NADH |
| Bio 3 | Number of particles that excite and fluoresce like NADH |
| 1 and 3 | Total number of particles that appear to contain both tryptophan and NADH |
| 1 or 3 | Total number of particles that contain either tryptophan or NADH |

P-TRAK returned total counts of particles 0.01 – 1 μm in size passing through the instrument. All particle counts produced by WIBS3 and P-TRAK were averaged and re-

calculated to produce a mean number of particles per cubic metre of air over the sampling period (30 minutes).

The total number of particles passing through WIBS3 reached $2.9 \times 10^6$ (mean value) at shredding (Figure 9.6). Particles were detectable at all sampling locations, with a gradual decline recorded between the peak at shredding, and 100 m downwind. At this final sampling location, concentrations remained an order of magnitude above those taken upwind. Although standard deviation is high, this is anticipated as the samples presented are means of samples taken every minute, for 30 minutes, and concentrations can vary greatly minute to minute (Figures 9.6 and 9.7).

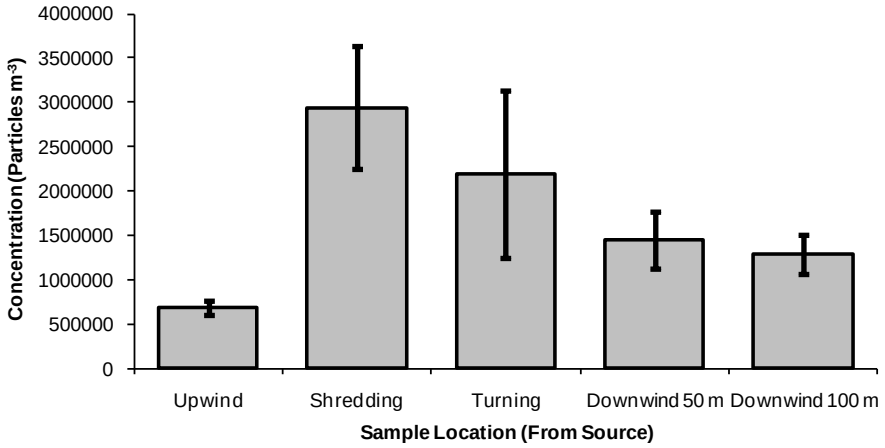



Figure 9.6: Mean total particle 1 – 20 μm in diameter concentrations per cubic metre ($m^{-3}$) represented by bar, standard deviation represented by whisker.

The separation of biological (containing tryptophan and/or NADH) from non-biological particles results in a different dispersal pattern being produced. This was reflected in all biological particle parameters measured (Bio 1, 2, 3, 1 and 3, and 1 or 3) (Figure 9.7). Again, standard deviations remain high due to minute-by-minute fluctuations in particle counts.

For particles exciting and fluorescing like Tryptophan (Bio 1), highest mean concentrations were recorded adjacent to turning activities (Figure 9.7). Shredding returned the next highest mean values. Concentrations declined between turning and 50 m downwind by an order of magnitude, before increasing again at 100 m downwind. Patterns returned by Bio 2 particles (excite like tryptophan but fluoresce like NADH) were similar to those returned by Bio 1, being highest at shredding and turning activities. Again, between turning and 50 m downwind a decrease in concentrations of approximately 1 order of magnitude was observed. At 100 m downwind concentrations increased to $1.1 \times 10^5$ particles $m^{-3}$, similar to those found at shredding ($1.0 \times 10^5$ particles $m^{-3}$). A strikingly similar pattern of dispersal was also observed for Bio 3 (particles that excite and fluoresce like NADH). The two highest means were returned by samples taken at turning, and 100 m downwind; with quantities reaching over $2.0 \times 10^5$ particles $m^{-3}$ in both cases (Figure 9.7).

Mean concentrations of Bio 1 or 3 (particles that both excite and fluoresce like tryptophan or NADH) also showed similar dispersal pattern (Figure 9.7). Turning emissions reached $3.9 \times 10^5$ particles $m^{-3}$, while the reduction of concentrations between turning and 50 m downwind was less pronounced in this case, with concentrations remaining within the same order of magnitude. A peak remained at 100 m downwind, although this is less significant than for other data-sets. Bio 1 and 3 particles (those that appear to contain both tryptophan and NADH) also show this dispersal pattern. Concentrations reach $1.1 \times 10^5$ particles $m^{-3}$, with the remaining locations recording concentrations one order of magnitude lower than this. Concentrations at 50 m downwind return to similar quantities to those found upwind ($2.0 \times 10^5$ and $2.8 \times 10^5$ respectively), with a further peak also seen at 100 m downwind, returning concentrations to $8.7 \times 10^5$ particles $m^{-3}$ (Figure 9.7).

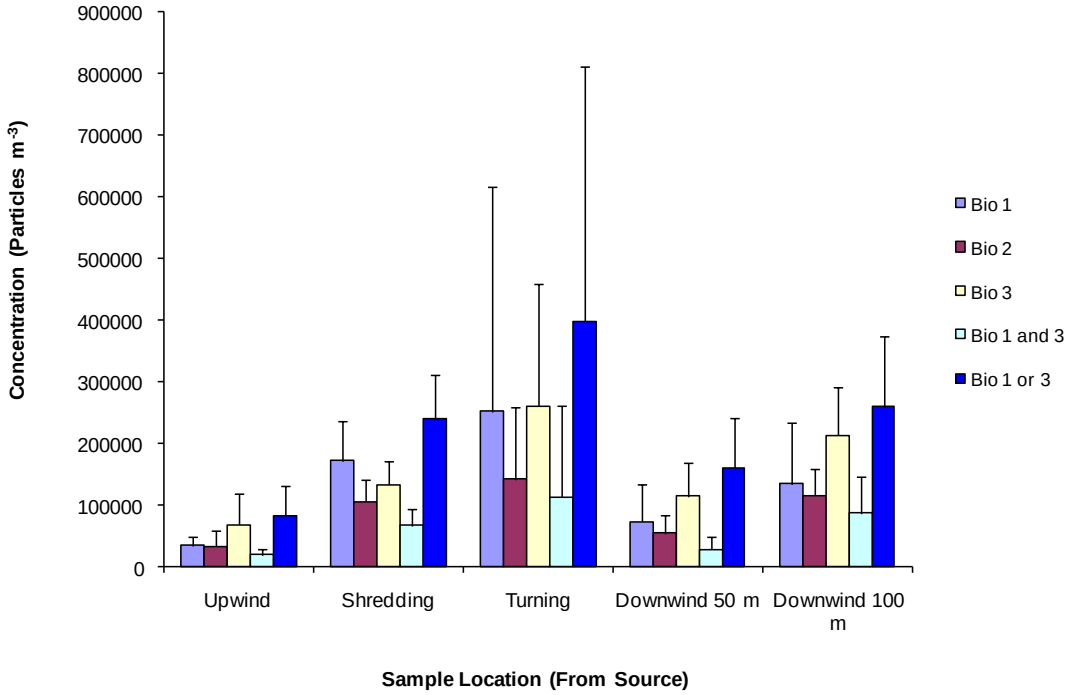



Figure 9.7: Mean Bio 1, Bio 2, Bio 3, Bio 1 and 3, and Bio 1 or 3 concentrations per cubic metre (m-3) represented by bar, standard deviation represented by whisker.

Ultrafine particle counts returned by P-TRAK show a slightly different distribution pattern (Figure 9.8), with concentrations also reaching level 6 orders of magnitude higher than those found with WIBS3. Highest concentrations were returned by shredding, with a mean value of $1.6 \times 10^{11}$ particles $m^{-3}$. Concentrations upwind were significantly below this, reaching a mean of $9.4 \times 10^{9}$ particles $m^{-3}$. From shredding, ultrafine particles reduced until 100 m downwind, where, as with previous datasets, a second peak was observed (Figure 9.8).

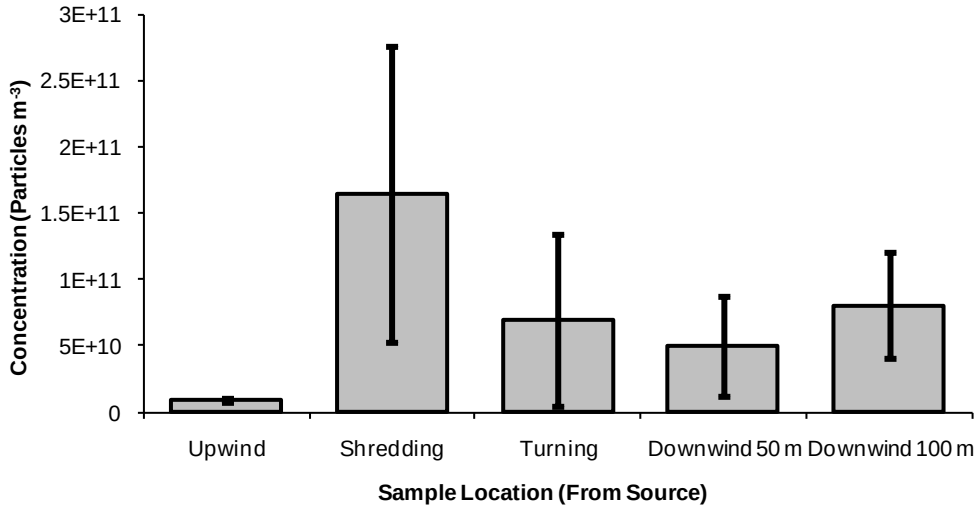



Figure 9.8: Mean total particle 0.02 – 1 μm in diameter (P-TRAK) concentrations per cubic metre ($m^{-3}$) represented by bar, standard deviation represented by whisker.

### 9.5.3 Analysis of relationships

Following the presentation of the above graphs (Figures 9.4 – 9.8) analysis of the relationships between data was carried out through calculation of p-values using product-moment and partial correlation analysis in STATISTICA (Chapter 3). The data are presented in Appendix II (Table 1.2) In order to explore this data further, Figure 9.9 represents total cultured bioaerosols, along with total viable particles counted by WIBS3 (Bio 1 or 3) as 100%; with the proportion of this total represented by each parameter also shown. Table 9.2 shows that data used to generate these Figures in particles $m^{-3}$ for WIBS3 measurements, and CFU $m^{-3}$ for cultured bioaerosols. Presenting the data in this way illustrates how, at activities, bioaerosols enumerated through culture represent over 60% of the total count of viable and culturable cells. However, at up- and downwind sampling locations the percentage of the total represented by culture methods falls to below 25%. Figure 9.10 presents the total particles counted by WIBS3, along with bioaerosols enumerated through culture as 100%, with the composition of this data presented according to parameter measured (WIBS3 total, Bio 1 or 3, gram-negative bacteria, actinomycetes,

and *A. fumigatus).* This Figure illustrates how culturable and biological particles comprise a relatively small fraction of total particles measured, with these fractions remaining below 40% of total particles.

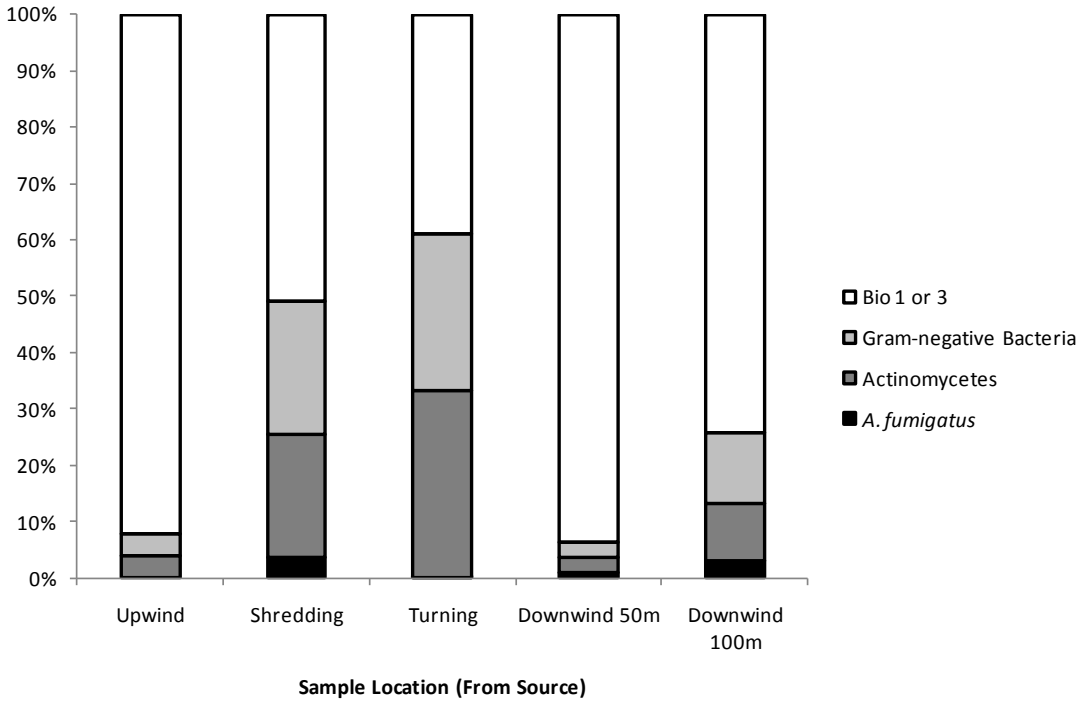



Figure 9.9: Mean *A. fumigatus*, actinomycetes, gram-negative bacteria, and Bio 1 or 3 counts as a percentage of the total enumerated through all methods.

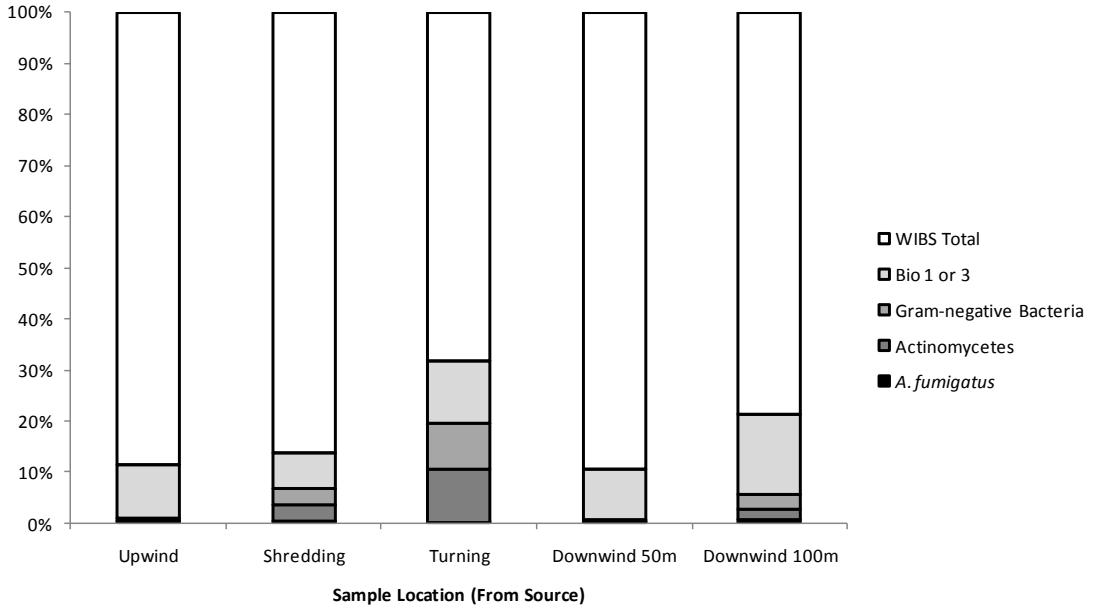



Figure 9.10: WIBS3 total and mean *A. fumigatus*, actinomycetes, gram-negative bacteria, and Bio 1 or 3 counts as a percentage of the total enumerated through all methods.

Table 9.2: Values for WIBS total, Bio 1 or 3, gram-negative bacteria, actinomycetes, and *A. fumigatus* at each sampling location

| | Particles $m^{-3}$ | | CFU $m^{-3}$ | | |
|---|---|---|---|---|---|
| | WIBS Total | Bio 1 or 3 | Gram-negative Bacteria | Actinomycetes | *A. fumigatus* |
| Upwind | 688000 | 83000 | 3535 | 3535 | BDL |
| Shredding | 2944000 | 240000 | 112029 | 102847 | 16850 |
| Turning | 2187000 | 399000 | 284068 | 339302 | 1010 |
| Downwind 50m | 1447000 | 161000 | 5051 | 4545 | 1515 |
| Downwind 100m | 1288000 | 260000 | 43893 | 35744 | 10859 |

# 9.5 Discussion

### 9.5.1 Comparison between methods

Initial viewing of results (Figures 9.4 – 9.8) reveals how there are two main patterns of bioaerosol release and dispersal, these are represented within both cultured

microorganisms, endotoxin analysis, WIBS3, and P-TRAK counts. The two main patterns are as follows:

1. Highest concentrations resulting from shredding activities, concentrations decrease by approximately one order of magnitude between shredding and 50 m downwind. A second, smaller peak in concentrations seen at 100 m downwind.

Samples taken that display this pattern include *A. fumigatus*, endotoxin, and particles sized 0.02 – 1 μm.

2. Highest concentrations resulting from turning activities. Concentrations fall by approximately one order of magnitude between turning and 50 m downwind. A second, smaller peak in concentrations seen at 100 m downwind.

Samples taken that display this second pattern of distribution include actinomycetes, gram-negative bacteria, Bio 1, Bio 2, Bio 3, Bio 1 or 3, and Bio 1 and 3.

Both dispersal patterns shown are reflected by the data-set presented as part of Section I of the thesis, with highest emissions adjacent to activity, followed by a steep decline and subsequent secondary peak (Chapter 4). Confirmation of the dispersal pattern found previously through an independent, non-culture based method, further validates the results and discussion presented in detail in Chapter 4; that buoyancy effects significantly impact upon the dispersal patterns of bioaerosols.

*A. fumigatus* concentrations, upon initial viewing, appear to have a similar dispersal pattern to particle counts from P-TRAK (0.02 – 1 μm in diameter). Analysis of the relationships (Appendix II, Table 1.1) confirmed this, returning a correlation coefficient ($r^2$) of 0.811 with a significant p-value of 0.037. This suggests the likelihood that the relationship is generated through chance is <5%. This result was not anticipated. The physical size of *A. fumigatus* spores is reported to be 1 – 5 μm (Deacon *et al*, 2009; Lacey and Crook, 1988;

Swan *et al*, 2003), while P-TRAK is designed to count particles ranging from 0.02 – 1 μm in size (Section 9.3). It may be possible that P-TRAK is detecting those cells that are released into the 1 μm size fraction; however, this cannot be verified. A more likely reason for this relationship is that *A. fumigatus* and the ultrafine particle fraction are both being emitted in higher concentrations through shredding. The suggestion that elevated concentrations of *A. fumigatus* are associated with shredding does not correspond to data presented within Section I of this thesis, where shredding was not associated with higher *A. fumigatus* concentrations (Chapter 5). However, as the data for this Chapter was collected on one sampling day only, it is possible that the waste shredded on this day contained *A. fumigatus* in concentrations elevated above those typically found, reflecting the variability shown in Section I.

Actinomycete dispersal patterns had many similarities with Bio 1, Bio 2, Bio 3, and Bio 1 or 3 samples taken by WIBS3 (Figures 9.4, 9.7). It could be anticipated that WIBS3 would detect actinomycetes, as they have been reported within the 1 – 3 μm (Swan *et al*, 2002; Swan *et al*, 2003) and 1 – 5 μm (Lacey and Crook, 1988) size ranges. Unpublished results investigating the size range of bioaerosols from composting facilities suggest that actinomycetes could be found in equal quantities from 0.65 to 8 μm in size, possibly due to the release of spores as chains (Tamer Vestlund, 2009). Whichever estimate of the size range of actinomycetes is more accurate; they are all within the capabilities of WIBS3 to detect (1 – 20 μm).

Two of the analyses (Bio 1 and Bio 1 or 3, Table 9.2) returned correlation coefficients with actinomycetes ($r^2$) of 0.824 and 0.788 respectively. P-values were 0.033 and 0.044 respectively; both confirming that data is significantly related to the 95% level. Bio 2 and Bio 3 however, showed weaker correlations with actinomycetes, as p-values of 0.12 and 0.13 respectively were found (Appendix II, Table 1.1). Bio 1 provides counts of particles over 1 μm in size that excite and fluoresce like tryptophan. The relationship of these particles with bacteria, including actinomycetes, could be anticipated, as tryptophan will be present in bacterial cells. This also explains the relationship with Bio 1 or 3, as the

relationship with Bio 1 would cause a similar correlation to be demonstrated. Although confidence levels are lower, some correlation is seen between actinomycetes and Bio 2 and 3 (Appendix II, Table 1.1); suggesting some relationship between the presence of NADH and actinomycete concentrations, or particles that excite like tryptophan but fluoresce like NADH.

The presence of NADH could be seen as indicative of cell activity, as it is crucial to aerobic respiration. Likewise, tryptophan is indicative of viable cells. Bio 1, 2 and 3 therefore give an indication of cell viability and activity, while Bio 1 or 3 could be seen as a total count of viable cells. It could be hypothesised that actinomycete CFU's should be comparable to particle counts for these parameters. This is the case, as actinomycetes concentrations can be found in the same order of magnitude as Bio 1, 2, 3 and Bio 1 or 3, with both found up to $10^4$ CFU $m^{-3}$/particles $m^{-3}$ (Figures 9.4, 9.7). There is, however, more variation within actinomycete concentrations, particularly at the upwind and 50 m downwind sampling locations. At these locations actinomycetes concentrations show the most pronounced decline when measured through culture, where they fall to an order of magnitude lower than those recorded by WIBS3 (Figures 9.4, 9.7).

Gram-negative bacteria demonstrate an almost identical relationship with WIBS3 data as actinomycetes. The strongest correlations are found with Bio 1 (p-value 0.018) and Bio 1 or 3 (p-value 0.034), both giving a significance level of 95%. Gram-negative bacteria are also positively correlated with Bio 2 and Bio 3, although confidence in these relationships are lower at 90% (p-value 0.091) and 85% (p-value 0.123) respectively (Appendix II, Table 1.1). The cause for these relationships is likely to be the same as for actinomycetes, the presence of tryptophan and NADH in bacterial cells. As with actinomycetes, concentrations of culturable gram-negative bacteria were counted in the same order of magnitude ($10^4$) as was measured by WIBS3; with gram-negative CFU's showing more variation, falling to an order of magnitude lower than WIBS where concentrations were lower (upwind and 50 m downwind) (Figures 9.4, 9.7). The pattern of decline to 50 m followed by secondary peak at 100 m is also less pronounced for results shown by WIBS3 than for gram-negative bacteria.

In order to further explore the relationship between viable particles measured by WIBS3 (represented by Bio 1 or 3, particles that excite and fluoresce like Tryptophan or NADH), and cultured bioaerosols, further analysis was carried out. Figure 9.9 represents total viable particles and culturable bioaerosols as 100%. The composition of the total is then shown, with Bio 1 or 3, *A. fumigatus*, actinomycetes, and gram-negative bacteria represented as a percentage of the total (Figure 9.9). This data is supported by Table 9.3, showing the data represented by percentages in Figure 9.9. At sampling locations adjacent to activities (shredding and turning), cultured bioaerosols represented approximately 50% and 63% of the total culturable and viable count. This shows that at point of emission, enumeration through culture and counts by WIBS3 are comparable, and suggests that culture accurately represents bioaerosol concentrations emitted through composting activities. This has been suggested previously, with microorganisms emitted through composting suggested to have high culturability (Adams and Frostick, 2009). However, at downwind locations the percentage of cultured bioaerosols falls to under 10% at 50 m, and remains under 30% at 100 m (Figure 9.9). The fact that WIBS3 is able to detect additional particles at these locations suggests that bioaerosols may become non-culturable but viable. While there have been suggestions within the literature that a large proportion of bioaerosols released through composting may be NCBV (Crook and Sherwood-Higham, 1997; Heidelberg *et al*, 1997; Swan *et al*, 2003), this has not been quantified previously; therefore there is no way of verifying the data presented above. Figure 9.10 shows how no more than 40% of total particles and culturable bioaerosols enumerated by WIBS3 are part of the biological and culturable fractions. Once again, this data is supported by Table 9.2, showing the data represented by percentages in Figure 9.10. This Figure suggests that of particles emitted through composting, between 10 and 35% are microbial in origin, with the bulk of emissions likely to comprise dust, and other inorganic particulates such as water vapour.

The relationship between endotoxins and P-TRAK particle counts has also been shown. A positive correlation with significance of over 95% was found, with a p-value of 0.031 (Appendix II, Table 1.1). P-TRAK is able to measure particles ranging from 0.02 – 1 μm

(TSI Incorporated, 2006). The strong correlation between endotoxin concentrations and those measured by P-TRAK may provide some insight into the physical properties of endotoxin as released from composting facilities. Dutkiewicz (1997) suggested that endotoxins can be released as microvesicles 0.003 – 0.005 μm (30 – 50 nm) in size, placing then into a particle size range too small to be detected by P-TRAK. However, little is known regarding the release and behaviour of endotoxins from composting facilities. For example, it is possible that they are not released as microvesicles, that they may aggregate in the atmosphere, and that they may be released attached to other atmospheric particles.

In addition, P-TRAK is designed to detect vehicle exhaust fumes, as these are released into the ultrafine particle range (Kaur *et al*, 2005; Kaur *et al*, 2006) and P-TRAK does not distinguish between biological and non-biological particles. The variations seen between sampling locations for P-TRAK may partially be due to fluctuations in exposure to vehicle exhaust (Kaur *et al*, 2005), as shredding resulted in the highest concentrations being found and involves the use of two pieces of heavy machinery. Another contributor to the ultrafine particle load is likely to be ultrafine inorganic materials such as dust (Kaur *et al*, 2005). These other sources of ultrafine particles are reflected in overall concentrations, where P-TRAK recorded up to a mean of $1.6 \times 10^{11}$ particles $m^3$, with endotoxins only measuring 203 EU $m^{-3}$ for the same sample. This correlation suggests that endotoxins may be emitted into the sub-micron particle size range, although further study is required in order to qualify and enumerate other sources of ultrafine particles at composting facilities. Both P-TRAK and endotoxins also show a correlation with total counts by WIBS3 (Appendix II, Table 1.1), with p-values of 0.037 and 0.047 respectively; suggesting that ultrafine and total counts may reflect the emission and dispersal of inorganic dust and exhaust rather than bioaerosols.

### 9.5.2 Review of methods

Comparison between these sampling methods and the results presented has allowed several important findings. One of the most interesting is the validation of dispersal patterns, as

influenced by buoyancy, by an independent, novel method. This provides additional confidence in the dispersal patterns found throughout Section I of this thesis. In addition, it has been found that actinomycetes and gram-negative bacteria can be found within the same order of magnitude as biological particle counts from WIBS3 at point of emission, supporting evidence that microorganisms retain high levels of culturability upon emission from composting facilities (Adams and Frostick, 2009). However, the proportion of biological particles accounted for through culture enumeration declines with distance from site, and is also low upwind (Figure 9.9). This suggests that a proportion of bioaerosols may lose their culturability. This may be due to osmotic stress or desiccation through collision with other airborne particles (Chapter 1 and 6) (Harrison *et al*, 2005; Korzeniewska *et al*, 2009; Lighthart and Mohr, 1987; Marthi *et al*, 1990; Tham and Zuraimi, 2005). However, due to a lack of information regarding the culturability of bioaerosols emitted through composting, these data cannot be verified. The implications for future sampling of bioaerosols may be several-fold. This study has suggested that culture-based methods accurately describe bioaerosols adjacent to source, but may not allow full enumeration of bioaerosols in ambient air, or downwind from source. This may lead to underestimation of concentrations at these locations. WIBS3 is able to provide real-time counts of viable bioaerosols. This provides several advantages: the ability to eliminate the risk of missing peak emissions of bioaerosols, and, the ability to enumerate bioaerosols that are non-culturable; as discussed in Chapters 1, 4, 5, and 6, it is possible to miss peak emissions of bioaerosols using culture-based methods due to sampling times being limited in order to retain culturability. P-TRAK also proved an applicable method for the analysis of bioaerosol emission through composting. However, in this case it is not possible to discriminate between bioaerosols, and non-biological ultrafine particles. Although ultrafine particles may be of concern with regards to human health, the utilisation of P-TRAK as a tool for the analysis of bioaerosols may be confounded by the quantity of ultrafine particles emitted that may be non-biological. This was illustrated by the high concentrations returned by P-TRAK within this study.

The real-time direct counting methods used here may provide a direction for the future development of bioaerosol samplers, primarily through their ability to collect real-time data, and enumerate non-culturable bioaerosols. However, due to the cost of these instruments, they are unlikely to be used with regularity within the near future. In addition, WIBS3 only separates particles on the basis of the presence of NADH and tryptophan. This does not allow the separation of, for example, the *A. fumigatus*, actinomycetes, and gram-negative bacteria, able to be enumerated separately through culture based analysis. The following Chapter continues the exploration into the composition of bioaerosols, through the analysis of bioaerosol community composition through molecular analysis. Molecular methods also allow analysis of non-culturable fractions, as well as providing options for the further taxonomic refinement of samples, and may provide another option for the future development of bioaerosol sampling methodologies.

## 9.6 Conclusions

The main limitation of this study was that data were based on only one sampling occasion due to time restrictions. Consequently, all data must be interpreted with care and requires further investigation and replication in order to verify findings. Despite this, several conclusions can be drawn from this investigation.

- The dispersal pattern presented in Chapter 4 is also visible through analysis with a novel direct counting method, verifying the potential impact of buoyancy on bioaerosol dispersal
- Culturable bioaerosols can be found in the same order of magnitude as biological particles enumerated through direct counting, suggesting that upon emission bioaerosols are highly culturable
- Filtration followed by culture allows an accurate measurement of bioaerosol emissions at source

- Aerosolised endotoxins may be found in the 0.02 – 1 µm particle size range

Most of the hypotheses tested within this Chapter were supported. WIBS3 returned higher concentrations than those found using culture based methods, although culturable bioaerosols were more comparable to counts from WIBS3 than was anticipated. However, *A. fumigatus* did not correlate with WIBS3 patterns in concentration, as was expected, although endotoxins correlated with P-TRAK, and culturable actinomycetes and gram-negative bacteria correlated with WIBS3 patterns in concentration.

While concentrations returned through culture were found in the same order of magnitude as those returned by WIBS3, at downwind locations the proportion of cultured bioaerosols decreased, although they remained higher than those found upwind. This may be due to previously culturable bioaerosols becoming NCBV. However, this cannot be verified without further investigation. The data suggests that WIBS3 may provide a suitable method for the real-time monitoring of bioaerosols, being able to enumerate bioaerosols that are non-culturable.

WIBS3 and P-TRAK provided real-time counts of both biological and ultrafine fractions of bioaerosols. Combined with culture-based analysis this has provided valuable information regarding the characterisation of bioaerosols emitted through composting. Further investigation and verification of results is required to build upon the results presented here. Despite this, the investigation has contributed towards knowledge surrounding the composition of composting bioaerosols, and the development of non-culture based methods for bioaerosol enumeration. However, this method has not allowed detailed analysis of biological fractions of bioaerosols, and their composition. The following Chapter uses non-culture based methods to further understand the composition of microorganisms emitted from composting facilities.

# 10 Characterisation of Bioaerosol Communities

## 10.1 Introduction

The analysis of bioaerosols from composting facilities has largely focused on the qualification and quantification of emission and dispersal patterns. This has been achieved through culture-based methods (Chapters 4, 5, 6), and a novel direct counting method (Chapter 9). In addition, endotoxin assay was utilised alongside culture analysis, allowing analysis of part of the non-culturable fraction of bioaerosols. However, the focus on culture-based enumeration of selected microorganisms has provided little information on the microbial community that can be found as part of composting bioaerosols. With the advance of non-culture based microbial community qualification and quantification it has become clear that culture-based methods are largely unable to represent true microbial diversity (Giovannoni *et al*, 1990; Peters *et al*, 2000; Ward *et al*, 1990). Culture-based methods are inherently highly selective, with only microorganisms suited to the substrate and conditions chosen for culture able to be enumerated (Giovannoni *et al*, 1990; Head *et al*, 1998; Ward *et al*, 1990). In addition, as identified in Chapters 1 and 7, sole enumeration through culture-based methods can lead to underestimation of microorganism concentrations (Head *et al*, 1998; Hugenholtz and Goebel, 2001). This is of particular concern for bioaerosols, where underestimation may occur due to the inability of culture to account for non-culturable but viable (NCBV) fractions. This fraction may include microorganisms that are already NCBV, as well as those that are rendered NCBV due to environmental conditions once emitted or the sampling process itself (Chen and Li, 2005b; Crook and Sherwood-Higham, 1997; Tham and Zuraimi, 2005; Marthi *et al*, 1990). Other microorganisms may also be emitted in a non-viable state, or once again, lose viability during emission and sampling due to desiccation and dehydration.

Comparison of the culturable and non-culturable fractions of bioaerosols has suggested that between 0.3 and 55% of airborne fungi can be enumerated, while fewer than 10% of gram-negative bacteria have been shown to be culturable (Cheng *et al*, 2008; Heidelberg *et al*, 1997; Lappalainen *et al*, 1996; Lee *et al*, 2006). Within available literature the focus of studies concerning composting bioaerosols has been on those of concern regarding human health; enumerated through culture-based methods (Chapters 1, 7). It has been found that even within well-studied microbial ecosystems, large numbers of community members have never been isolated through culture-based analysis (Giovannoni *et al*, 1990; Ward *et al*, 1990). The authors of this study speculate that this would be true of nearly all microbial ecosystems where analysis has focused on culture, with levels of identified microorganisms estimated at only 20% within these ecosystems (Ward *et al*, 1990). Although methods for non-culture based community analysis have existed for several decades, this speculation would include airborne microbial communities. It is therefore almost certain that both diversity and biomass of bioaerosols has been routinely underestimated.

Utilisation of molecular methods for the characterisation of bioaerosol communities will allow the identification of composting bioaerosol community structure. Comparison of this structure to upwind bioaerosols and samples taken directly from green waste will allow analysis of several factors. Namely, the contribution of upwind and green waste microorganisms to on-site and downwind bioaerosol community structure; and the significance of the effect that composting has on bioaerosol community structure. The identification of bioaerosol community structure will also increase understanding of the communities of microorganisms that sensitive receptors may be exposed to, rather than focusing on those with health impacts that have already been demonstrated. Further, identification of a typical composting microbial community structure, along with microbial community structures from alternative bioaerosol sources, may allow attribution of samples taken at receptor to a source; or, ‘source apportionment’ of bioaerosols found at sensitive receptor. For example, bioaerosols are emitted from many different sources, such as agricultural activities and some industrial activities (Swan *et al*, 2003). It is unlikely, given the range of sources, that the same bioaerosol communities are emitted from each source.

Currently, analysis of composting bioaerosols focuses on microorganisms that may be commonly found within the environment (*A. fumigatus*, actinomycetes, and gram-negative bacteria) (Swan *et al*, 2003). This has often led to difficulties in determining the source of measured bioaerosols (ADAS and SWICEB, 2005; Reinthaler *et al*, 1997), and was shown through Chapters 4, 5, and 6 where bioaerosols were also found upwind. However, it is likely that other members of the microbial community, not enumerated through culture, are more unique to particular sources, or a particular community structure of microorganisms may be unique to a source. The identification of signature composting bioaerosol microorganisms was also recently attempted by Le Goff *et al*. (2009). Molecular methods may allow the identification of microbial community 'fingerprints' from individual sources. Future development of this type of work would allow the sampling of bioaerosols at receptor, and subsequent identification of the source of these bioaerosols through analysis of microbial community fingerprint and the identification of signature microorganisms (Le Goff *et al*, 2009), namely, 'source apportionment'. This capability would also allow the contribution of composting to sensitive receptor exposure to be contextualised thorough the tracing of bioaerosols at receptor to source.

As shown in Chapters 1 and 7, there are several options for the biochemical and molecular analysis of microorganism community structure. However, two methods in particular are readily available and have the ability to be utilised for bioaerosol analysis. These methods are phospholipid fatty acid analysis (PLFA), which allows phenotypic analysis, and nucleic acid based techniques, which allows genotypic analysis. Both methods have been used in both the analysis of green waste and airborne microorganisms (Adams and Frostick, 2009; Cahyani *et al*, 2002; Hermann and Shann, 1997; Le Goff *et al*, 2009; Macnaughton *et al*, 1999), and have been suggested to be highly complementary (Macnaughton and Stephen, 2001).

### 10.1.1 PLFA Analysis

PLFA analysis is a biochemical approach widely used in soil, sediment and water microbial community analysis to determine viable microbial biomass concentrations and provide a whole community fingerprint (Macnaughton *et al*, 1999). PLFA's are essential components of cell membranes of living organisms. Figure 10.1 below illustrates the context of phospholipids within the cell membrane.

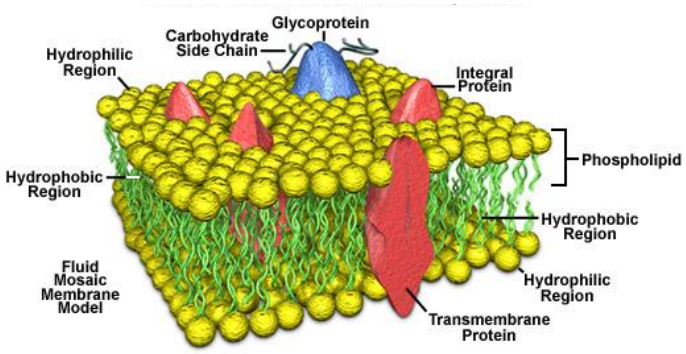



Figure 10.1: Graphic illustrating the context of phospholipids within the cell membrane (Davidson, 2004)

PLFA have a high level of structural diversity, with certain structures specific to certain groups of organisms (Amir *et al*, 2008; Frostegård *et al*, 1993a; Stoeck *et al*, 2002; Zelles, 1999). They are easily extractable, making them ideal for analysis of microbial communities (Macnaughton *et al*, 1999). In addition, there is some evidence that some of these fatty acids may be unique to specific taxonomic groups. PLFA analysis is typically used to indicate broad changes in the phenotypic structure of microbial communities, such as a shift from a community dominated by fungi to a community dominated by gram-negative bacteria (Amir *et al*, 2008; Frostegård *et al*, 1993a; Macnaughton *et al*, 1999).

PLFA analysis can be used to quantify the biomass, phenotypic composition, and effect of changing conditions on communities of microorganisms (Frostegård *et al*, 1991; Frostegård

*et al*, 1993a, b; Macnaughton *et al*, 1999; Macnaughton and Stephen, 2001; Stoeck *et al*, 2002). In particular, PLFA's are synthesised during microbial growth, then rapidly degraded following death. This attribute means changes in phenotypic community structure can be rapidly assessed (Macnaughton *et al*, 1999; Macnaughton and Stephen, 2001; Zelles, 1999). PLFA analysis has been used with success within the area of compost microbiological analysis (Amir *et al*, 2008; Cahyani *et al*, 2002; Hermann and Shann, 1997), and suggested to be a useful method in the determination of compost maturity. Particular microbiological communities develop and succeed each other during composting due to selection by changing environmental conditions, such as temperature, and substrate availability, with these changes detectable through PLFA analysis (Hermann and Shann, 1997). However, PLFA analysis must also be interpreted with caution at times. Some PLFA are common to many organisms, meaning these must often be excluded from analysis in order to accurately describe community similarities and differences (Zelles, 1999). For example, many fatty acids indicative of fungi are common to eukaryotic cells, including human skin (Macnaughton *et al*, 1999). Furthermore, interpretation of individual PLFA markers is often carried out based upon data gained from pure cultures; which are subject to the same limitations of any culture-based sample (Chapters 1, 7). These data are also often contradictory. Therefore, analysis of PLFA markers in environmental samples and assignment of these markers to a specific microorganism or microbial groups may lead to misinterpretation and underestimation of diversity or biomass. For this reason it has been suggested that analysis should be based upon functional groups of PLFA marker and microbial community rather than specific markers (Zelles, 1999).

PLFA nomenclature demonstrates the key differences in fatty acid structure that allow determination of the microbial group. For example; A:BωC where 'A' represents the number of carbon atoms present in the fatty acid; 'B' represents the number of double bonds from the aliphatic (ω) end of the molecule; and 'C' represents the closest unsaturated carbon atom from the aliphatic end of the molecule. In addition, a 'c' or 't' suffix indicates *cis* and *trans* geometric isomers, respectively; while the prefixes 'i' and 'a' indicate iso or anteiso methyl branches; 'cy' indicates cyclopropyl rings; and mid-chain branching is

represented by the carbon atom at which the methyl branching occurs, and ‘me’. Finally, hydroxyl substitution is represented by the carbon atom at which the substitution occurs, followed by hydoxyl group (-OH). Where unsaturated fatty acids have unknown double bond locations, the fatty acid classification is followed by a capital letter. For example; 16:1ω5 shows a fatty acid with 16 carbon atoms, 1 double bond from the aliphatic end (monoenoic), with carbon atom 5 (from the aliphatic end) the first unsaturated one (Cahyani *et al*, 2002; Hermann and Shann, 1997; Macalady *et al*, 2000; Macnaughton *et al*, 1999). Table 10.1 below describes the PLFA that are commonly attributed to microbial groups within available literature.

Table 10.1: PLFA associated with microorganism groups throughout the literature

| PLFA | Group | Reference |
|---|---|---|
| i15:0, a15:0, 15:0, i16:0, a17:0, i15:0 3OH, 15:1 iG, 16:1ω9, a16:0, i16:1 G, i16:1 H, 17:0, i17:0, 15:0 2OH, 15:0 3OH, 17:0 2OH, i17:1ω8 | Bacteria | (Frostegård *et al*, 1991; Zhang *et al*, 2009) |
| 18:1ω7 c | Anaerobia | (Zhang *et al*, 2009) |
| 16:1ω7 c, 16:1ω7 t, 18:1ω7, 18:1ω7 t, cy17:0, cy19:0 | Gram-negative bacteria | (Bartlett *et al*, 2007; Frostegård *et al*, 1991; Macnaughton and Stephen, 2001) |
| Cyclopropyl | Gram-negative Bacteria, anaerobic gram-positive bacteria | (Amir *et al*, 2008; Zelles, 1999) |
| 2OH | *Pseudomonas*, Gram-negative bacteria | (Amir *et al*, 2008) |
| *Iso-anteiso* and branched chain. 18:1 ω9 t, 18:1ω9 c, 19:0 c. i15:0, i16:0, 10Me16:0, 18:1ω9 also common | Gram-positive bacteria | (Amir *et al*, 2008; Frostegård *et al*, 1991; Zelles, 1999;) |
| Branched chain in which position of methyl branching is other than *iso-anteiso* (10Me, 18:0, 17:0) | Actinomycetes | (Amir *et al*, 2008; Macnaughton and Stephen, 2001; Stoeck *et al*, 2002; Zelles, 1999; Zhang *et al*, 2009) |
| Branched chain (*i*, *a*, and branched) 16:1ω5 | *Flavobacterium-Bacteroides* phylum | (Frostegård *et al*, 199; Stoeck *et al*, 2002; Zelles, 1999) |
| 3OH | *Thiobacillus* | (Amir *et al*, 2008) |
| 16:1ω5 | Arbuscular mycorrhizal fungi | (Frostegård *et al*, 1991; Gavito and Olsson, 2008) |
| 18:2ω6, 18:3ω3, ergosterol, 18:1ω9 c, 18:3ω6 c | Fungi | (Macnaughton and Stephen, 2001; Stoeck *et al*, 2002; Zelles, 1999; Zhang *et al*, 2009) |
| 16:0 | Type I methanotrophs | (Bartlett *et al*, 2007) |
| 16:1ω5 c | Methanotrophs | (Zhang *et al*, 2009) |
| 18:1ω8 c | Type II methane oxidisers | (Macnaughton and Stephen, 2001) |
| 16:1ω8 c | Type I methane oxidisers | |
| 16:0 10Me | Sulphate reducing | (Zhang *et al*, 2009) |
| High amounts of branched saturated and monounsaturated (i17:1ω7 c) | *Desulfovibrio* | (Macalady *et al*, 2000; Macnaughton and Stephen, 2001) |
| High amounts methyl branched and cyclopropyl (10Me16:0, cy17:0, cy19:0) | *Desulfobacter* | |
| High amounts unbranched (17:1, 15:1, 15:0) | *Desulfobulbus* | |
| i15:0, a15:0, i17:0, a17:0 | *Bacillus* or *Arthrobacter* | (Macnaughton and Stephen, 2001) |
| Plasmalogens, dipicolinic acid | *Clostridia* | |
| 20:1ω6, 20:3ω6, 20:4ω6 | Protozoa | |
| 16:1ω13 t, 18:3ω3 | Algae | |
| 18:2ω6, 18:3ω3, 20:5ω3, 26:0 | Higher plants | |

As well as identification of microbial groups, PLFA analysis can provide information regarding the physiological status of the community. *Trans*-mono-unsaturated PLFA increase the ordering of bacterial cell membranes, decreasing their fluidity (Macnaughton and Stephen, 2001). It has been shown that gram-negative bacteria synthesise *trans* fatty acids during times of environmental stress, such as desiccation and exposure to pollutants (Macnaughton *et al*, 1999; Macnaughton and Stephen, 2001; Stoeck *et al*, 2002).

A number of studies describing the composting process using PLFA analysis are available (Amir *et al*, 2008; Cahyani *et al*, 2002; Hermann and Shann, 1997; Steger *et al*, 2005). However, there appears to be limited published evidence investigating the qualification of airborne microbial communities using PLFA analysis. Indoor airborne communities have been qualified using PLFA analysis (Macnaughton *et al*, 1999). PLFA found within industrial buildings were compared to those found outdoors. This study utilised a high-efficiency filtration sampler, sampling a total volume of 500 – 1500 $m^{-3}$ of air. Indoor and outdoor samples were dominated by biomass of eukaryotic origin, although bacterial PLFA were also found, particularly close to a suspected source of bacteria. Microbial phenotypic community structure was found to be different indoors and outdoors, with indoor air strongly influenced by the presence of gram-negative bacteria. In addition, 16:1ω7 and 18:1ω7 *trans* and *cis* showed a ratio of 0.1 or less within air samples, indicative of a non-stressed microbial community. It was found that 1 to 3 orders of magnitude more biomass could be detected through PLFA than culture-based methods (Macnaughton *et al*, 1999). However, although this study proved that airborne microorganisms could be successfully sampled and analysed for PLFA, the sampling apparatus used was relatively bulky at 1.6 m in diameter, and could only be used to sample in rooms with an indoor volume over 500 $m^{-3}$, in order to prevent double sampling of air (Macnaughton *et al*, 1999). The method, therefore, would benefit from refinement to enable more flexible sampling procedures.

Studies assessing phenotypic microbial community structural dynamics during the composting processes have shown that the phenotypic microbial diversity decreases as the process progresses, due to selection through temperature and substrate availability

(Hermann and Shann, 1997). For example, within microbial decomposers species able to utilise readily available substrates, such as amino-acids and sugars, are termed '*r*-selected species', and show explosive growth during the initial phases of composting before decreasing in number and becoming dormant members of the community when these substrates are exhausted. Slower growing members of the microbial community that are able to utilise recalcitrant substrates such as lignin and cellulose are termed '*K*-selected species' and are able to slowly grow to become dominant community members in the later stages of composting (Begon *et al*, 1996). The initial thermophilic phase of composting is characterised by intense activity and *r*-related microbial growth. During this phase thermophilic microorganisms, including gram-positive bacteria and actinomycetes, become dominant (Hermann and Shann, 1997). PLFA biomarkers associated with the presence of actinomycetes have been shown to increase from 0.53% of the total PLFA composition to 2.95% during the first week of composting, while fungal markers decreased from 36% to 4.8% (Cahyani *et al*, 2002). An increase in 15 and 17 carbon PLFA chains has been suggested to be indicative of an increase in thermophilic *Bacillus* sp (Amir *et al*, 2008; Steger *et al*, 2005). During the latter stages of composting, the amount of simple carbohydrate based substrates declines as they are utilised by microbial populations, leading to a reduction in bacterial PLFA and *r*-selected species. At this stage *K*-selected species increase in number, with actinomycete markers persisting and fungal markers increasing. PLFA indicative of gram-negative bacteria have also been found to increase during the mesophilic and maturation phases (Amir *et al*, 2008; Cahyani *et al*, 2002; Hermann and Shann, 1997; Steger *et al*, 2005).

### 10.1.2 Nucleic Acid Based Analysis

The use of nucleic acid based analysis to investigate microbial community composition within environmental samples has increased in popularity and publication frequency. The technique has facilitated identification of previously unforeseen levels of microbial diversity (Head *et al*, 1998; Hugenholtz and Goebel, 2001). There are two major routes for nucleic acid based analysis, the profiling of total communities through polymerase chain

reaction (PCR) amplification of rDNA (in order to represent rRNA, which is one of the most highly conserved sequences) (Hurst *et al*, 1997) or the analysis of metabolically active community members through reverse-transcriptase PCR (RT-PCR), which targets RNA for amplification (Felske *et al*, 1998; Miskin *et al*, 1999). PCR amplification of total bacterial communities through 16S rRNA (rDNA) amplification is possible as 16S rRNA copies remain consistent within cells, and DNA is relatively stable within the environment (Miskin *et al*, 1999). Therefore, this type of amplification includes inactive and non-viable cells. RT-PCR, on the other hand, allows amplification of only metabolically active community members as RNA is rapidly degraded in the environment, due to the abundance of RNases, while DNA is more persistent (Miskin *et al*, 1999). In addition, metabolically active microorganisms contain more ribosomes than those that are inactive; with the quantity of ribosomes and rRNA within a cell approximately proportional to the activity and growth of the cell. It has been suggested that upon cell death RNA only persists within the environment for a few minutes (Miskin *et al*, 1999). Cloning of community 16S rRNA has become a popular tool within microbial ecology (Alfreider *et al*, 2002; Ward *et al*, 1990). Rapid screening of clones, through methods such as restriction fragment length polymorphism (RFLP) allows identification of related sequences (Head *et al*, 1998). Further, sequencing of each clone can identify the composition of the microbial community (Alfreider *et al*, 2002; Head *et al*, 1998; Hugenholtz and Goebel, 2001). Although it is also possible to amplify fungal communities, typically through 18S rRNA amplification, it is arguably more difficult to perform due to the ubiquitous presence of eukaryotic cells containing 18S rRNA, and the associated risk of contamination (Adams and Frostick, 2009).

The process of PCR involves initial lysis of cells to extract the DNA, after which the DNA is purified. PCR can then be used to amplify the DNA region of choice. PCR consists of three stages; first is denaturation of the DNA, splitting double DNA strands into single strands known as ‘templates’. Following this is annealing, where selected primers attach to the single DNA strands. The primers chosen determine which genes are amplified. Primers that select an area of DNA or ribosomal RNA (rRNA) ubiquitous to microorganisms (e.g.

16S or 18S) can lead to whole community analysis. Alternatively, primers that select for genes expressed in microorganisms of interest can be selected, leading to amplification of the desired components of the microbial community only (Head *et al*, 1998; Hugenholtz and Goebel, 2001). After annealing is extension, where the selected region of DNA is amplified (Mukoda *et al*, 1994). This method enables concentrations of DNA or rRNA to reach levels that are detectable and measurable through subsequent analysis methods. Figure 10.2 below illustrates the whole process of PCR diagrammatically.

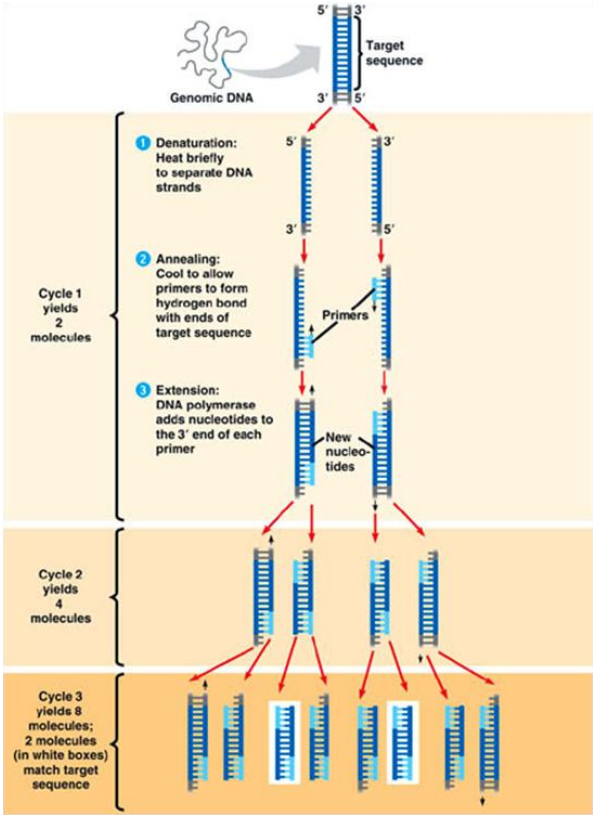



Figure 10.2: Diagrammatic representation of PCR process, illustrating selection of target DNA fragment, use of primers to target and amplify selected DNA fragment, and amplification of DNA (University of Miami Department of Biology).

The initial amplification of PCR is termed direct PCR. If insufficient DNA is present within the sample, through low community numbers or inefficient lysis of cells, nested PCR can

be carried out. Nested PCR uses primers that are internal to the region of the gene that was initially selected and amplified through direct PCR (Head *et al*, 1998). This increases the specificity of the PCR process, reducing the possibility of amplification of the wrong fraction of DNA. While nested PCR is able to increase yield by 1 – 2 orders of magnitude (Peccia and Hernandez, 2006) the level of bias inherent to PCR amplification (discussed below) is also increased, as the amplification process is repeated on the initial product. In addition, microbial diversity of the sample is further reduced through the exclusion of the part of the 16S rRNA gene that lies outside of the areas selected through nested PCR primers (Peccia and Hernandez, 2006). The principal of nested PCR is illustrated in Figure 10.3 below.

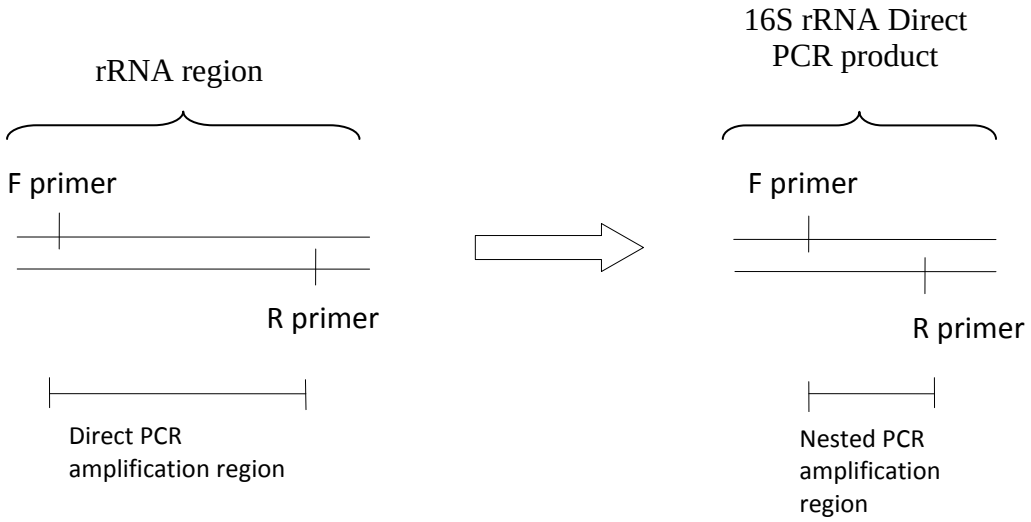



Figure 10.3: Diagrammatic representation of direct and nested PCR. ‘F’ indicates forward primer position; ‘R’ indicates reverse primer position. After direct PCR a region of the gene internal to the original selected region is selected and amplified. This is to increase the specificity of the PCR amplification.

After amplification of the RNA or rRNA (rDNA) of interest, a number of different analysis methods can be used in order to determine community structure of the sample. One of these methods is denaturing gradient gel electrophoresis (DGGE), which creates a bacterial community fingerprint through profiling of the variable V3 region of the 16S rRNA gene

(Muyzer *et al*, 1993). Samples are injected into a polyacrylamide gel, and electrophoresed in a linearly increasing gradient. DNA migrates through the gel, until it becomes denatured, which dramatically reduces mobility through the gel. The size of the DNA fragment and its structure also affects mobility (Heuer *et al*, 2001). The resulting fingerprint shows a band for each population, represented by DNA, within a community. Figure 10.4 below shows an example schematic of the resulting community fingerprint. Community members identified in each sample are converted to 'presence' or 'absence' data, based upon the presence or absence of DNA bands across several samples, and the resulting data can be used to compare between the community structures of samples. DGGE is particularly of use in studying the changes in microbial community structure, as many samples can be analysed in parallel and the method is relatively rapid (Head *et al*, 1998). In addition, the resulting bands can be excised, re-amplified and sequenced in order to identify community members (Macnaughton and Stephen, 2001).

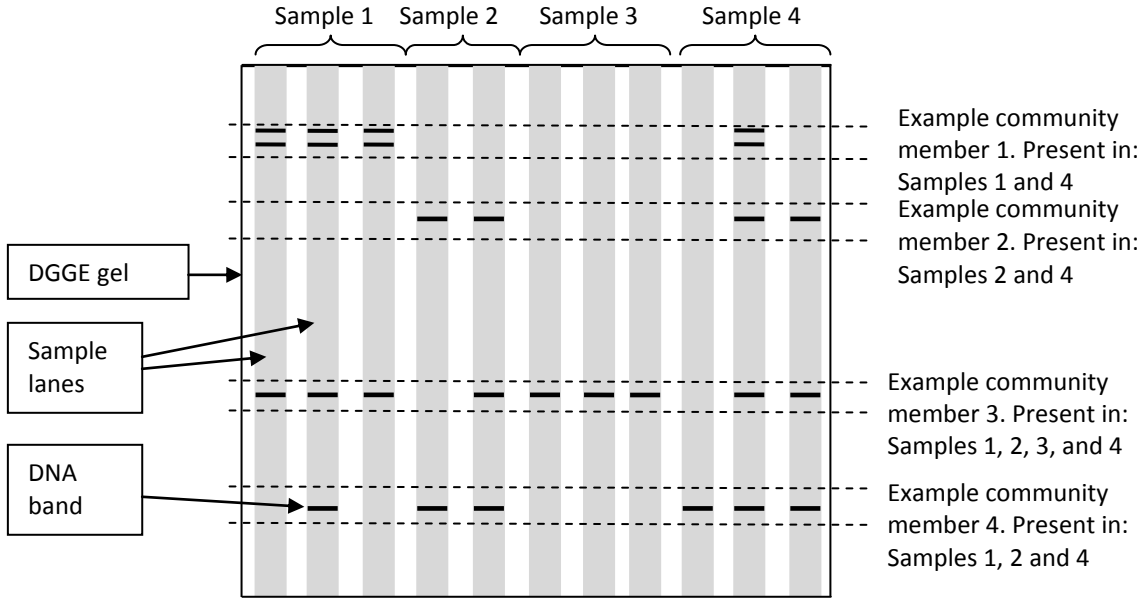



Figure 10.4: Example schematic of community fingerprint, annotated to illustrate microbial community structure determination, generated through PCR followed by DGGE analysis. Example bands representing microbial community members that are present in one sample but not another are highlighted

PCR amplification of DNA followed by DGGE offers many benefits over culture-based sampling. Key advantages include the potential to characterise inactive, non-culturable and non-viable microorganisms, although it is estimated that a microbial population must represent 1% of the community to be determined through DGGE analysis. In addition, DGGE provides the option to identify community members through DNA sequencing (Head *et al*, 1998; Heuer *et al*, 2001; Muyzer *et al*, 1993). However, as with most methods there remain sources of error. These include: poor extraction of nucleic acid from the sample; co-extraction of compounds inhibitory to the PCR process, such as humic acids (particularly prevalent in environmental samples); biased PCR amplification, where not all nucleic acid is amplified equally and primers exclude microorganisms not containing the selected gene; and the formation of chimeric DNA, where similar strands of DNA from different microorganisms are merged. These sources of error can lead to the dominant sequences post-amplification not representing the organisms that are numerically dominant in the environment. In addition, PCR is a sensitive method, so any contamination can be amplified, thus distorting final results (Head *et al*, 1998; Hugenholtz and Goebel, 2001; Felske *et al*, 1998; Suzuki and Giovannoni, 1996). This can be a particular problem when analysing eukaryota, as contamination from human cells can occur, although fungi specific primers are available. For this reason, many studies prefer to focus on bacterial DNA, in particular 16S rRNA, which is ubiquitous throughout prokaryota and highly conserved (Hurst *et al*, 1997; Ishimatsu *et al*, 2007). Minimisation of these potential sources of bias and error can be carried out through careful experimental and laboratory protocol design, with many reviews and studies available advising on the most suitable procedures (Aguilera *et al*, 2006; De Lipthay *et al*, 2004; Felske *et al*, 1998; Hugenholtz and Goebel, 2001; Marchesi, 2001; Tsai and Rochelle, 2001).

PCR and DGGE have been widely used and established as standard methods within soil and aquatic microbiology (Heuer *et al*, 2001; Hugenholtz and Goebel, 2001), and it has been suggested and shown that PCR based methods would also be highly applicable and informative for airborne microbiological community analysis (Mukoda *et al*, 1994; Peccia

and Hernandez, 2006). However, they remain little used in airborne microbial characterisation studies (Peccia and Hernandez, 2006). Often, the focus for PCR based methods within bioaerosol science is indoor microorganisms, particularly pathogens or viruses (Ishimatsu, *et al*, 1997; Pyankov *et al*, 2007). In other studies of aerobiota through PCR methods, rather than whole sample amplification and subsequent community analysis, specific microorganisms have been targeted and amplified (Baertsch *et al*, 2007; Cayer *et al*, 2007; McDevitt *et al*, 2004). The testing of nucleic acid based analysis in bioaerosol science has illustrated the ability of the method to combat some of the limitations induced by culture based analysis (Chapter 7). For example, Peccia and Hernandez (2006) were able to detect aerosolised *Legionella pneumophilia* in 9 out of 12 samples from hospital settings using PCR based methods (nested PCR), and only 3 of these samples using culture-based methods. The applicability of PCR based methods for airborne microbial community analysis in the outdoor environment and activities related to waste processing has been shown. Some authors have used PCR amplification to track the effect of land-applied biosolids on aerobiota (Baertsch *et al*, 2007; Brooks *et al*, 2007). Results demonstrated that the biodiversity of aerobiota increased during land application of biosolids, with a measurable shift in bacterial community structure. However, no one genus was solely recovered from biosolids and aerosols (Brooks *et al*, 2007). Other studies have successfully used these methods to perform community fingerprinting of ambient bioaerosols and emissions from specific sources, although the relative scarcity of these studies and variety of methods used prevents overarching trends from being identified (Harrison *et al*, 2005; Negrin *et al*, 2007; Orsini *et al*, 2002; Rinsoz *et al*, 2008).

PCR based methods have also been utilised for the characterisation of microbial communities directly from compost (Adams and Frostick, 2009; Franke-Whittle *et al*, 2005; Peters *et al*, 2000; Steger *et al*, 2005). These studies have highlighted the ability of these methods to characterise community diversity and dynamics, with a suggestion that community diversity increases throughout the composting process, as species succeed each other due to changes in physical and nutritional conditions, yet do not disappear as a new microbial group becomes dominant (Hermann and Shann, 1997). This was not shown

through culture-based analysis, where samples from end-stage composting showed 290 individual isolates, which comprised only 6 different bacterial species (Peters *et al*, 2000). In addition, nucleic acid based analysis has shown composting to be characterised by rapidly changing microbial communities in response to alterations in the physicochemical conditions of the green waste; with specific marker microorganisms, such as members of *r*-selected to *K*-selected species, able to be traced to particular composting stages (Adams and Frostick, 2009; Alfreider *et al*, 2002; Begon *et al*, 1996; Peters *et al*, 2000; Steger *et al*, 2005). DGGE has also been commonly used to qualify changes in microbial community during composting. Although it has been found through DGGE analysis that previously reported microbial succession profiles were not conformed to within their study of open-air windrow composting, illustrating discrepancies between results depending upon method used and site specific conditions, such as feedstock and composting processes (Adams and Frostick, 2009). In addition, the focus of most PCR based studies of compost on 16S rRNA amplification is highlighted, with an assumption that bacteria perform the major role in composting presumed to be one of the reasons for this (Adams and Frostick, 2009). Despite the progress made in terms of refining methods for microorganism community and bioaerosol analysis, there is little data available regarding environmental aerobiota community structure, and the effect of sources of bioaerosols on this structure. One recent study has illustrated the utilisation of PCR based methods for enumeration of composting bioaerosols, although in this case the identification of key microbial species rather than analysis of community and changes in community structure (Le Goff *et al*, 2009). While the community dynamics of microorganism communities in compost have been determined, it therefore remains unknown which aspects of the community are emitted into the air through composting activity, and the impact these emissions have on ambient microbial communities.

### 10.1.3 Implementation of Methods

PLFA and DGGE were identified throughout the course of this project as potential methods to contribute towards the achievement of thesis Objective ii; ‘Characterisation of

bioaerosols released from composting facilities, including bioaerosol community structure, and non-viable components'.

The information within the literature suggested that PLFA could be successfully used to analyse microbial communities directly from composted waste. However, as only a single published example of analysis of airborne microorganisms exists, and utilised a different method to the one available within this project (Chapter 3) (Macnaughton *et al*, 1999), it was uncertain whether PLFA could be successfully analysed from airborne samples. It is possible that the methodology used within this study (Chapter 3) may not capture sufficient biomass to determine PLFA from airborne samples. However, Chapters 4, 5, and 6 show how concentrations of bioaerosols found on-site are high, suggesting that composting sites would be suitable for a 'proof of concept' study, exploring the use of PLFA to analyse airborne microbial communities using SKC samplers and polycarbonate filters.

Given the fact that PCR amplification and DGGE has been successfully carried out for a wide variety of environmental samples, including airborne microbial communities, it was anticipated that these methods could be used to analyse the change in community composition of bacterial bioaerosols (16S rRNA) due to composting activities. It has been suggested that filtration is currently the most suitable method for airborne microorganism collection for PCR amplification (Harrison *et al*, 2005; McDevitt *et al*, 2004); suggesting that the same methods as used for culture based analysis (Chapter 3), could be used for PCR and DGGE.

The aims of this Chapter were therefore to implement a 'proof of concept' experimental protocol and procedures for identifying and characterising bioaerosol community and structure using PLFA and DGGE analysis, and to compare bioaerosol community composition within green waste, along with bioaerosols on-site, downwind from site, and at 'background' sites using PLFA and DGGE analysis. These aims were designed in order to contribute to thesis Objective ii of this project (above), to be achieved through the Chapter Objective:

'Analysis of the microbial community structure of green waste, ambient and composting bioaerosols through molecular methods'

Taking into account the available literature regarding bioaerosols and microbial communities (Chapters 1 and 7), it could be hypothesised that airborne microbial communities would be significantly different upwind, on-site, and downwind from site.

## 10.2 Experimental Design and Methodology

Sampling occasions dedicated to this phase of the project and details of the sampling regime are shown in Table 10.2 below. All samples were taken at Flixborough.

Table 10.2: Details of occasions where samples were taken for the purposes of molecular analysis, including date, location, replicates taken, and the duration of sampling

| Date | Sample Location | Sample details | Replicates | Duration (minutes) |
|---|---|---|---|---|
| 22nd April 2009 | Downwind from site activity | DGGE | 3 | 60 |
| | 100 m downwind | DGGE | 3 | 60 |
| | Green waste grab samples (100g) | DGGE | 2 | N/A |
| 6th May 2009 | Upwind | DGGE/PLFA | 6 | 60 |
| | Downwind from site activity | DGGE/PLFA | 6 | 60 |
| | 100 m downwind | DGGE/PLFA | 6 | 60 |
| 21st May 2009 | Upwind | DGGE/PLFA | 6 | 120 |
| | Downwind from site activity | DGGE/PLFA | 6 | 120 |
| | Downwind from site activity | DGGE | 3 | 34* |
| | 100 m downwind | DGGE | 3 | 120 |
| | Green waste grab samples (100 g) | DGGE/PLFA | 8 | N/A |

* Sampling time reduced due to adverse weather conditions

Sampling duration was initially doubled from 30 minutes, used for analysis through culture, to 60 minutes, with a subsequent increase to 120 minutes for upwind samples due to low biomass found on upwind sample for 60 minutes. Culture analysis was routinely performed with a sampling duration of 30 minutes as this provided adequate numbers of bioaerosols for enumeration, while an extension of sampling time was undesirable as this would increase the risk of loss of microorganism culturability (Chen and Li, 2005a; Godish and Godish, 2006). It was not necessary to preserve culturability for PLFA or DGGE analysis, allowing extension of sampling times. On the 21$^{st}$ May failure of sampling pumps due to adverse weather conditions resulted in three 34 minute samples being acquired. These samples were retained to be used in the refinement of analysis methods. Samples collected for the purposes of PLFA and DGGE analysis were placed within sterilised caps and clips for transportation back to the laboratory, as described for endotoxin analysis (Chapter 3).

### 10.2.1 PLFA Methodology

PLFA analysis was carried out following a modified version of the method described by Frostegård *et al.* (1991; 1993a). The samples (5 g of green waste and filters) were initially freeze-dried (Alpha 1-2 LD freezedrier; Christ freeze driers, Osterode am Harz, Germany) and placed into sterile glass media bottles (28 ml BTS-160-016G). PLFA were extracted through addition of 15 mL Bligh Dyer solution, which comprised of 200 mL citrate buffer (0.15M citric acid dehydrate with 0.15M trisodium citrate), 250 mL chloroform, and 500 mL methanol at a ratio of 0.8:1:2; plus 50 mg $L^{-1}$ butylated hydroxyl toluene. At this stage PTFE tape was added as a barrier to prevent plasticide contamination from the lid (PTFE tape SEL-530-010P). After 30 minutes sonication and subsequent centrifugation at 700 relative centrifugal forces (RCF) for 10 minutes (Falcon 6/300; Sanyo, UK), the upper layer was removed and placed into a clean glass media bottle. This solution was separated into two phases through the addition of 4 mL chloroform and 4 mL citrate buffer. After a separation time of 24 hours, the upper phase was removed and discarded. The lower layer was dried under a constant stream of nitrogen ($N_2$) at < 37ºC.

Fractionation of the lipid extract follows in order to separate neutral, glycol- and polar lipids. Chloroform (1 mL) was added to each sample in order to re-suspend the lipid extract. The extract was then loaded onto SPE cartridges (3 ml / 500 mg silica Sep-pak Vac$^{TM}$; Waters Chromatography WAT 020810;). Selective elution of neutral, glycol- and polar lipids was carried out through consecutive addition of chloroform (5 mL), acetone (12 mL), and methanol (8 mL). The final elution (polar lipids) was collected in a clean glass media bottle, and dried under a constant stream of $N_2$ at 37°C.

The polar lipids, or phospholipids, were converted to fatty acid methyl esters (FAME) by mild acid methanolysis (Dowling *et al,* 1986*).* Toluene:methanol (1 mL) (1:1) and 1 mL 0.2M methanoloic potassium hydroxide was added to each sample, following which, samples were incubated for 30 minutes at 37°C. The reaction was stopped through the addition of 0.25 mL 1M acetic acid. The final extraction of FAMEs was carried out through addition of 5 mL hexane:chloroform mixture (4:1) and 3 mL deionised water. The samples were sonicated for 30 minutes at 30°C and subsequently centrifuged (700 RCF) to separate the sample into two phases. The upper phase was discarded, while the lower phase was cleaned through addition of 3 mL 0.3M sodium hydroxide solution. This top layer was filtered through sodium sulphate (Whatman N$^{o}$ 4 filter paper) into a clean glass media bottle, and dried under a constant stream of $N_2$ at room temperature $\pm$ 2°C.

The extracts were re-suspended in 200 µL hexane and analysed using gas chromatography (GC). The GC (Agilent Technologies 6890N; Software Agilent G2070 ChemStation) was fitted with a HP-5 (Agilent Technologies) fused silica capillary column (30 m length, 0.32 mm ID, 0.25 µm film). The carrier gas used was helium, introduced at 1 mL.minute$^{-1}$. The program for separating FAMEs was: 50°C for 1 minute (splitless hold time); increased at 25°C per minute to 160°C; increased at 2°C per minute to 240°C; increased at 25°C per minute to 310°C; inject samples (1 µL) using an autosampler, injector temperature 310°C. FAMEs were detected using a flame ionisation detector (FID) at 320°C. Standards of known PLFA (SUPELCO) were used to identify common PLFA's. Results are expressed

on a Mol% basis (each peak expressed as a percentage of the total area of the identified peaks on the chromatogram).

Each sample was analysed in triplicate, apart from upwind bioaerosols where the third filter showed unacceptable contamination (21$^{st}$ May). Peak identification was performed by comparing the relative retention times of each peak with those of the standard materials. The GC retention time of the resulting FAME depends upon the length of the fatty acid chain and the presence and position of double bonds. These retention times cover a certain 'peak area', depending upon how much of the FA identified by the retention time is present. The peak area was measured by triangulation and the percentage of the fatty acids and unsaponifiables were calculated as (area of each peak/area of all peaks) x 100, thus normalising the data across different samples. This allows the composition of a community according to the FA to be identified and peak area to be determined. An example of the GC output, allowing visualisation of the peak area and retention time, is shown below in Figure 10.5. Similarity between PLFA profiles was calculated using binary data for presence of particular PLFA (Jaccard coefficient) and a MDS (multidimensional scaling) plot produced from the resulting proximity matrix (Software: Primer 6 version β, 2001).

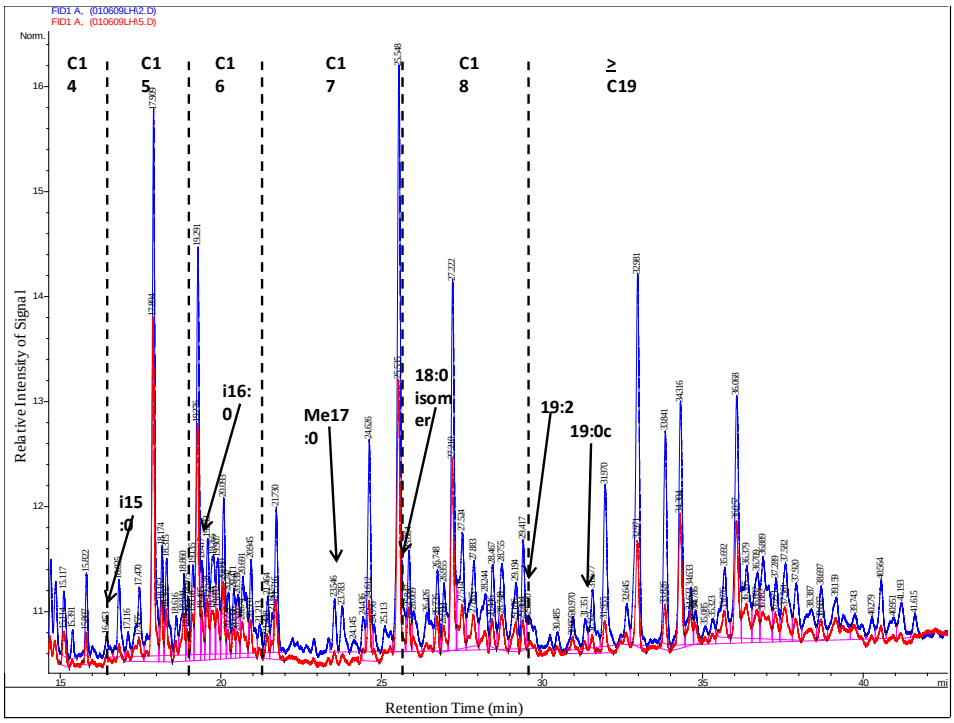



Figure 10.5: Example of GC chromatograph output for one upwind (red) and one downwind (blue) sample. Example signature fatty acids, corresponding peaks carbon chain lengths indicated.

### 10.2.2 PCR and DGGE analysis

PCR amplification consists of several steps. Initially, DNA must be extracted from the sample, purification procedures must then be used in order to ensure the process is not inhibited and contaminants are removed, following this, amplification can be carried out. The initial step, extraction, can be one of the most challenging, as the nucleic acid should be relatively intact and co-extraction of inhibitory compounds such as humic acid should be minimised. Given the wide range of microorganisms, sample substrates used, and potential procedures for extraction, there is no generalised protocol for DNA extraction from environmental samples, although a number of nucleic acid extraction kits are widely available (Tsai and Rochelle, 2001).

**DNA extraction and amplification**

Extractions and amplifications were carried out on both dry filters for bioaerosols, and compost. A preliminary step applied to all filters involved the halving, and the further sub-division of one half of the filter into small fragments, before transfer of the filter to a bead beating tube. For the green waste, two procedures were carried out: (i) 0.2 g (fresh weight) weighed and transferred to bead beating tubes, and; (ii) 0.4 g green waste suspended in 3 mL CTAB/Phosphate buffer (120 mM, pH8), vortexed and centrifuged at 3000 RCF for 10 minutes, 500 µL supernatant transferred to bead beating tube. Cell lysis (i and ii) was initiated through the addition of 500 µL CTAB/Phosphate buffer, and 500 µL chloroform: Isoamyl alcohol (25:24:1) was added to remove proteins and humic acid. Bead beating was then carried out for 30 seconds at 2000 rpm (Mikro-dismembrator U); this step was carried out twice. The tubes were centrifuged for 5 minutes at 16,100 RCF, before the top aqueous layer was removed and transferred to clean tubes. If additional cleaning and purification of the sample was required (i.e. for green waste samples), 500 µL phenol-chloroform: isoamyl alcohol (24:1) was added and the tubes centrifuged for 5 minutes at 16,100 RCF.

The top aqueous layer was removed and transferred to a polyvinylpolypyrrolidone (PVPP) tube, where 500 µL chloroform: isoamyl alcohol (24:1) was added. After incubation at 4°C for 60 minutes, tubes were centrifuged for 5 minutes at 16,000 RCF. The supernatant was transferred to a new tube, and 1 mL 30% PEC 6000/1, 6M NaCl added to the aqueous layer, before leaving samples to incubate at room temperature overnight. Tubes were then centrifuged for 5 minutes at 16,100 RCF to obtain DNA pellets. The pellets were washed with 200 µL ethanol (70%, ice-cold) and centrifuged for 20 minutes at 16,100 RCF. The supernatant was removed, and samples left to air-dry (~20 minutes). Once dry, the pellets were re-suspended in 50 µL pure water.

A direct PCR with pA and pH′ primers (Table 10.3; (Edwards *et al*, 1989)) was performed as follows: 2 µL of the DNA template (sample); 5 µL buffer (×10 + 5mM $MgCl_2$); 5 µL 2mM dNTP; 2 µL each pA and pH′ primers 10 µM; and 0.5 µL Taq polymerase was added to 30.5 µL DEPC water. PCR (Gen Amp PCR System 9700) was performed according to

the following temperature programme: 94°C for 5 minutes; 30 cycles at 94°C for 30 seconds; 55°C for 30 seconds; 72°C for 1 minute; and a final extension at 72°C for 10 minutes.

Nested PCR was carried out using Muyzer primers (Table 10.3; (Muyzer *et al*, 1993)). PCR conditions were as follows: 1 µL direct PCR product; 5 µL buffer (×10 + 5mM $MgCl_2$); 5 µL 1mM dNTP; 2 µL of 10 µM each primer 341F-GC clamp and 534R; and 0.5 µL Taq polymerase. The PCR program (Gen Amp PCR System 9700) used began with a hot start at 95°C for 2 minutes; 25 cycles of 94°C for 30 seconds; 57°C for 30 seconds; 72°C for 30 seconds; and a final extension at 72°C for 10 minutes.

After each method had been applied, yield of nucleic acid was analysed by electrophoresis in 1% agarose gel. Electrophoresis conditions: 100 volts, 142 mA for 25-30 minutes after which the gel was stained for 20 minutes in ethidium bromide; and washed in water for 10 minutes. In addition, spectrophotometry ratio of $A_{260}$:$A_{280}$ was used to check for inhibitory compounds (Tsai and Rochelle, 2001).

Table 10.3: Details and references of primers used

| Primer | Sequence 5’ - 3’ | Target Gene | Reference |
|---|---|---|---|
| 63F | CAGGCC TAA CAC ATG CAA GTC | 16S rRNA (bacteria) | Marchesi *et al,* 1998) |
| 1492R | TACGG(C/T)TACCTTGTTACGACTT | 16S rRNA (bacteria) | (Heuer *et al*, 1997) |
| pA | AGAGTTTGATCCTGGCTCAG | 16S rRNA (bacteria) | (Edwards *et al*, 1989) |
| pH’ | AAGGAGGTGATCCAGCCGCA | 16S rRNA (bacteria) | (Edwards *et al*, 1989) |
| 341G GC clamp | CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G CC TAC GGG AGG CAG CAG | 16S rRNA (bacteria) | (Muyzer *et al,* 1993) |
| 534R | ATT ACC GCG GCT GCT GG | 16S rRNA (bacteria) | (Muyzer *et al,* 1993) |

**DGGE Protocol**

Denaturing gradient gel electrophoresis was carried out using the Bio-Rad D Code system. Similarly sized PCR products were separated on gel consisting of 8% (w/v) polyacrylamide and a denaturing gradient from 40% to 60% (formamide and urea), in 1 x TAE (40 mM Tris-acetate; 1 mM di-sodium-EDTA; pH 8.0), at a constant setting of 60 V and 60°C for 16 hours. After electrophoresis, the gel was stained with silver nitrate. After fixation by gentle shaking in 200 mL of an absolute ethanol solution (100 mL/L) and acetic acid (5 ml/L) for 30 minutes at room temperature, staining was performed with gentle shaking in 200 mL of a 1 g/L silver nitrate solution for 20 minutes at room temperature. The gel was developed in a solution containing, per litre, 15 g of NaOH and 8 mL of formaldehyde, then incubated in the same solution, with gentle rotary shaking, until DNA bands were visible (approximately 20 minutes). To allow further development, the gel was placed back into the solution of absolute ethanol and acetic acid, with gentle rotary shaking for 10 minutes. Following this the gel was washed by rocking in distilled water.

All samples were analysed in triplicate, apart from upwind where only duplicate samples were obtained. Similarity between DGGE profiles was calculated using binary data for presence of particular bands (Jaccard coefficient) and a MDS (multidimensional scaling) plot produced from the resulting proximity matrix (Software: Primer 6 version β, 2001).

### 10.2.3 Safety and Quality Control

Normal laboratory COSHH and risk assessment procedures were carried out and adhered to (Chapter 3); all solvents were handled within fume hood and disposed of in an appropriate manner (according to ISO14001). Electrophoresis included both positive (*Methonococcus aureus*) and negative control samples. 20” Hg vacuum on the SPE manifold was not exceeded when performing solid phase extraction for PLFA extraction.

## 10.3 Results

The data gained through PLFA and DGGE analysis are presented below. Through PLFA analysis, both bacterial and fungal phenotypic communities are analysed, with qualitative assignment of PLFA markers to taxonomic groups and abundance possible. However, for DGGE only bacterial genotypic communities are analysed, with a focus on change in community structure as qualification of taxonomic groups and abundance is not possible through this analysis.

### 10.3.1 PLFA Analysis

The samples used for analysis were 3 downwind 60 minute, 2 upwind 120 minute, 3 fresh green waste, 3 medium green waste, and 3 old green waste. Due to time constraints the other samples were not analysed. The data were aligned to standard FAs (standardised peaks for Cranfield University GC - SUPELCO), analysed for presence or absence of PLFA, and normalised to provide a measure of PLFA abundance. Similarity between samples was calculated using a binary matrix (Jaccard coefficient) and a non-metric multidimensional scaling (MDS) plot was produced from the resulting proximity matrix (Clarke and Warwick, 2001; McCune et al., 2002). MDS presents the similarity or dissimilarity of results (based on presence or absence of community component, in this case PLFA markers) spatially. Where 'similarity' is referred to, it has been calculated through MDS to the best configuration possible, i.e. the one with least stress. For example, if Sample A has a more similar community structure to Sample B than Sample C, it will be placed closer to Sample B than Sample C on the resulting MDS plot (Clarke and Warwick, 2001). Stress for the analysis here was low, <0.01; giving a good representation of orientation and low possibility of misinterpretation (Clarke and Warwick, 2001). There were two clear groups of 20% similarity; one contained upwind samples, while the other contained green waste and downwind samples. There was 40% similarity between green waste and downwind samples, and 60% similarity between all green waste samples.

The data presented in Figure 10.6 clearly shows some significant differences between green waste, upwind and downwind communities, with green waste and downwind samples sharing 40% similarity and upwind showing no similarity to any other samples. Analysis of PLFA groups and abundance expanded upon the MDS results. In total, forty-five peaks were found and aligned to standard FAs, of these 34 were identifiable; these are shown and assigned to microorganism groups in Table 10.4 below. Samples taken downwind from site showed 13 additional known PLFA's compared to upwind, namely i15.0, a15:0, 15:0, 16:1 isomer, i16:0, 16:1ω7 t, 16:1ω5, Me17:0 isomer, 18:0 isomer, 18:2ω6 c, 18:1ω9 c, 18:0 and 19:2. Of these PLFA's, 12 were also found in green waste samples with only 16:1ω7 c an exception, identified as gram-negative bacteria. Of these 12, 3 were unidentified, 2 identified as belonging to the *Bacillus* or *Arthrobacter* groups, 2 to gram-negative bacteria, 1 as aerobic bacteria, 1 as gram-positive bacteria, 1 was identified as belonging to both arbuscular mycorrhizal fungi and the *Flavobacterium-Bacteroides* group, 1 to both fungi and gram-positive bacteria, and 1 one to fungi.

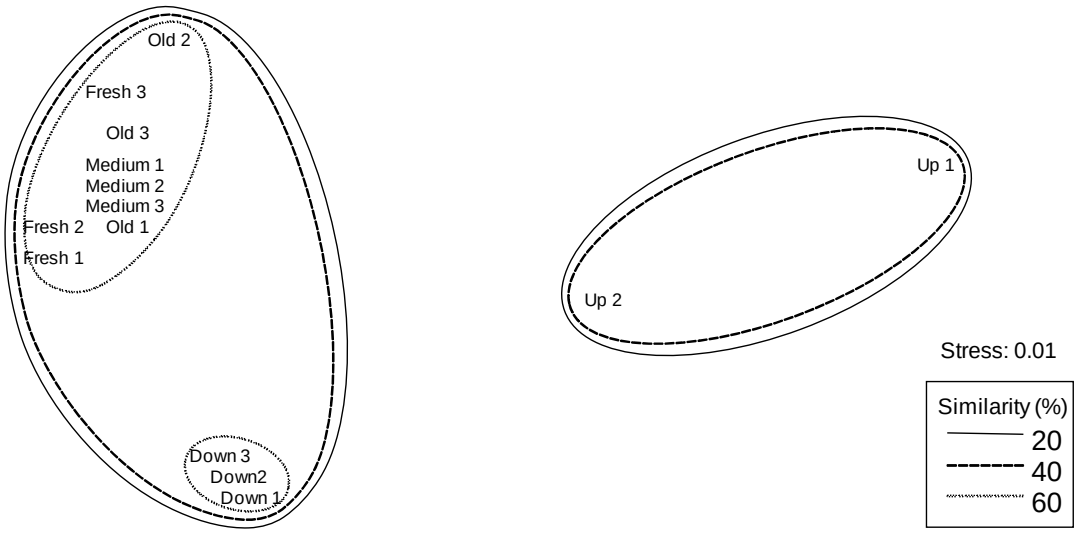



Figure 10.6: MDS plot of PLFA results, similarity of individual samples according to the presence or absence of DNA bands represented by percentage. Samples taken on 6th May: Down 1-3 (downwind from site activities). Samples taken on 21st May: Upwind 1, 2; Fresh green waste 1-3; Medium green waste 1-3; Old green waste 1-3. Stress 0.01.

Table 10.4: PLFA's identified at each sampling location with taxonomy as identified in sources shown in Table 10.1.

| Upwind | Downwind | Fresh Green Waste | Medium Green Waste | Old Green Waste | Taxonomy | | |
|---|---|---|---|---|---|---|---|
| 14:0 | 14:0 | 14:0 | 14:0 | 14:0 | | | |
| | i15:0 | i15:0 | | | Bacteria | Gram-positive | *Bacillus* or *Arthrobacter* |
| | a15:0 | a15:0 | a15:0 | a15:0 | Bacteria | Gram-positive | *Bacillus* or *Arthrobacter* |
| | | 15:1 | 15:1 | 15:1 | Bacteria | Gram-negative | *Desulfobulbus* |
| | 15:0 | 15:0 | 15:0 | 15:0 | Bacteria | Gram-negative | *Desulfobulbus* |
| 2OH14:0 | 2OH14:0 | 2OH14:0 | 2OH14:0 | 2OH14:0 | Bacteria | *Pseudomonas* /Gram-negative bacteria | |
| | 16:1 isomer | 16:1 isomer | 16:1 isomer | 16:1 isomer | | | |
| | i16:0 | i16:0 | i16:0 | i16:0 | Bacteria | Gram-positive | |
| a16:0 | a16:0 | a16:0 | a16:0 | a16:0 | Bacteria | | |
| 16:1*w*7 c | 16:1*w*7 c | 16:1*w*7 c | 16:1*w*7 c | 16:1*w*7 c | Bacteria | Gram-negative bacteria | |
| | 16:1*w*7 t | | | | Bacteria | Gram-negative bacteria | |
| | 16:1*w*5 | 16:1*w*5 | 16:1*w*5 | 16:1*w*5 | Fungi / Bacteria | AMF/*Flavobacterium-Bacteroides* | |
| 16:0 | 16:0 | 16:0 | 16:0 | 16:0 | Bacteria | Type I Methanotrophs | |
| | Me17:0 isomer | Me17:0 isomer | Me17:0 isomer | Me17:0 isomer | Bacteria | Gram-positive | Actinomycetes |
| | | i17:0 | i17:0 | i17:0 | Bacteria | Gram-positive | *Bacillus* or *Arthrobacter* |
| | | a17:0 | a17:0 | a17:0 | Bacteria | Gram-positive | *Bacillus* or *Arthrobacter* |
| | | 17:0 isomer? | | | Bacteria | | |
| | | 17:0 c | 17:0 c | 17:0 c | | | |
| | | 17:1 isomer | 17:1 isomer | 17:1 isomer | Bacteria | | |
| | | | | 17:0 | Bacteria | | |
| | | 17:0 isomer | 17:0 isomer | 17:0 isomer | Bacteria | | |
| | 18:0 isomer | 18:0 isomer | 18:0 isomer | 18:0 isomer | | | |
| | 18:2*w*6 c | 18:2*w*6 c | 18:2*w*6 c | 18:2*w*6 c | Fungi | | |
| | 18:1*w*9 c | 18:1*w*9 c | 18:1*w*9 c | 18:1*w*9 c | Fungi / Bacteria | Fungi/Gram-positive bacteria | |
| 18:1*w*9 t | 18:1*w*9 t | 18:1*w*9 t | 18:1*w*9 t | 18:1*w*9 t | Bacteria | Gram-positive bacteria | |
| 18:1*w*7 t | 18:1*w*7 t | 18:1*w*7 t | 18:1*w*7 t | 18:1*w*7 t | Bacteria | Gran-negative bacteria | |
| 18:1 isomer | 18:1 isomer | 18:1 isomer | 18:1 isomer | 18:1 isomer | | | |
| | 18:0 | 18:0 | 18:0 | 18:0 | | | |
| | 19:2 | 19:2 | 19:2 | 19:2 | | | |
| | | 19:0 c | 19:0 c | 19:0 c | Bacteria | Gram-positive bacteria | |

It has been suggested that some PLFA's, for example, 16:0, should be excluded from analysis of community structure as they are ubiquitously found. However, on this occasion no PLFA was found to be dominant amongst the data-set, allowing inclusion of all PLFA's found into a Principal Components Analysis (PCA) in STATISTICA. Data were normalised and PLFA peak normalised prior to PCA analysis (Figure 10.7). Principal component one (PC1) was found to account for the majority of variation between communities, at 82.66%. One-way ANOVA and Fisher's Least Significant Difference (LSD) was applied to PC1 in order to quantify the significance of differences between samples. The results from this analysis showed that all compost samples were statistically similar (p-value = > 0.05), indicating only minor changes in the phenotypic microbial community structure of fresh, medium, and old green waste samples. Upwind and downwind samples, however, were both statistically different from compost (p = <

0.05). In addition, upwind and downwind samples were significantly different from each other (p = < 0.05).

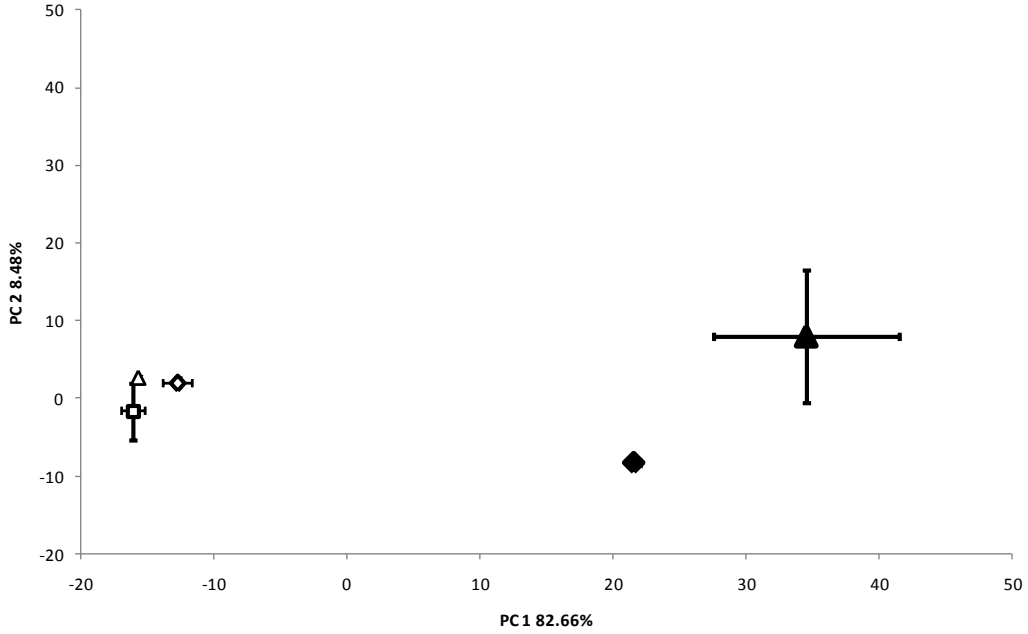



Figure 10.7: Principal components analysis of PLFA peak areas for fresh green waste (□); medium green waste (△); old green waste (◇); upwind (▲); and downwind (◆). Whiskers represent standard error.

Detailed analysis of the PLFA data (Figure 10.8) shows a clear group of PLFA's, 18:1ω9 t, 2-OH14:0, and an unknown, which appear to be contributing to the significant difference observed between up- and downwind bioaerosol samples, and green waste samples; while green waste samples appear to be significantly affected by 17:1 isomer, 18:0, 16:1ω5, and two unknown PLFA's.

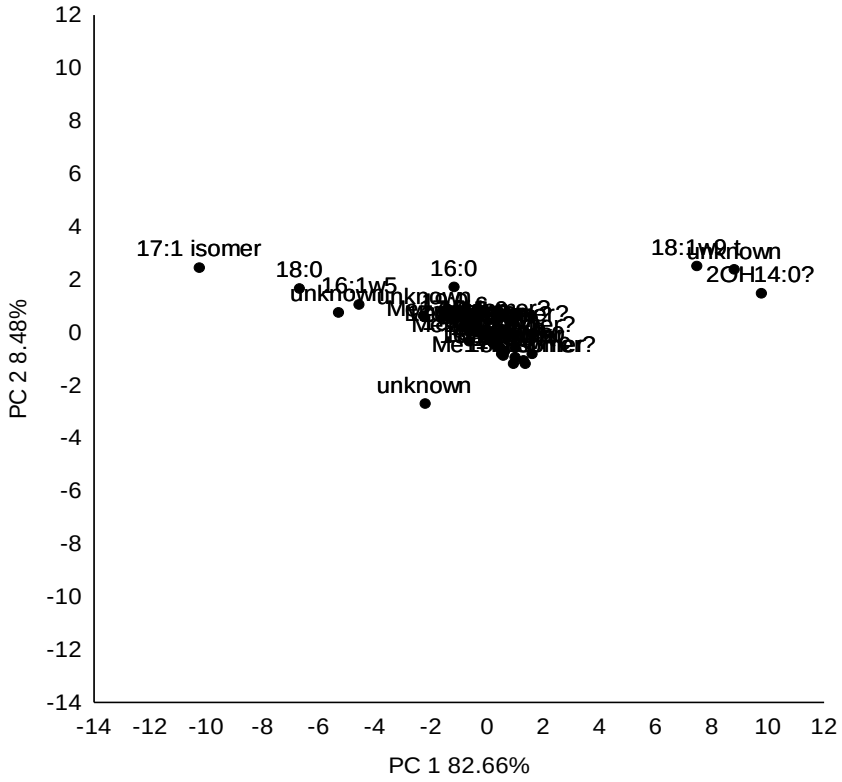



Figure 10.8: Projection of the PLFA variables ( ●) contributing to Figure 10.7.

Figures 10.9 and 10.10 present PCA plots and projections of PLFA variables for bioaerosols and green waste samples individually. Figure 10.9 illustrates how the downwind samples are largely influenced by a cluster of PLFA's, while upwind mean and standard error is a result of a disperse range of PLFA's rather than change in a specific microbial component. A similar situation is seen in Figure 10.10, where medium and old compost samples are close on the PCA, most likely largely represented by the cluster of PLFA's on the projection graph.

Table 10.5 shows percentage composition of PLFA's at each sampling location. At upwind, 2OH14:0, an unknown, and 18:1ω9 t dominated, with over 20% of the composition attributable to each. These PLFA's are suggested to signify gram-negative and gram-positive bacteria. This pattern is reflected in both Figures 10.9 and 10.10. The remainder of the PLFA's for upwind hold similar levels of abundance, all under 10%. Only 10 PLFA's were found in upwind samples, while downwind samples showed 30; although the same three PLFA's showed highest percentage abundance at both sampling locations. Similar diversities of PLFA were found in green waste samples, with fresh,

medium, and old green waste showing between 39 and 42 PLFA's. For fresh compost, 17:1 isomer was the most abundant, and is assumed to belong to bacteria. The PLFA's 16:0, 18:0, and an unknown all showed abundances over 10%. In samples from medium green waste, 17:1 isomer also showed over 20% abundance, with 16:1ω5 (assigned to both fungi and gram-positive bacteria), 18:0, and an unknown PLFA also showing over 10%. Abundances were similar in old green waste, with only minor changes in percentage (Table 10.5).

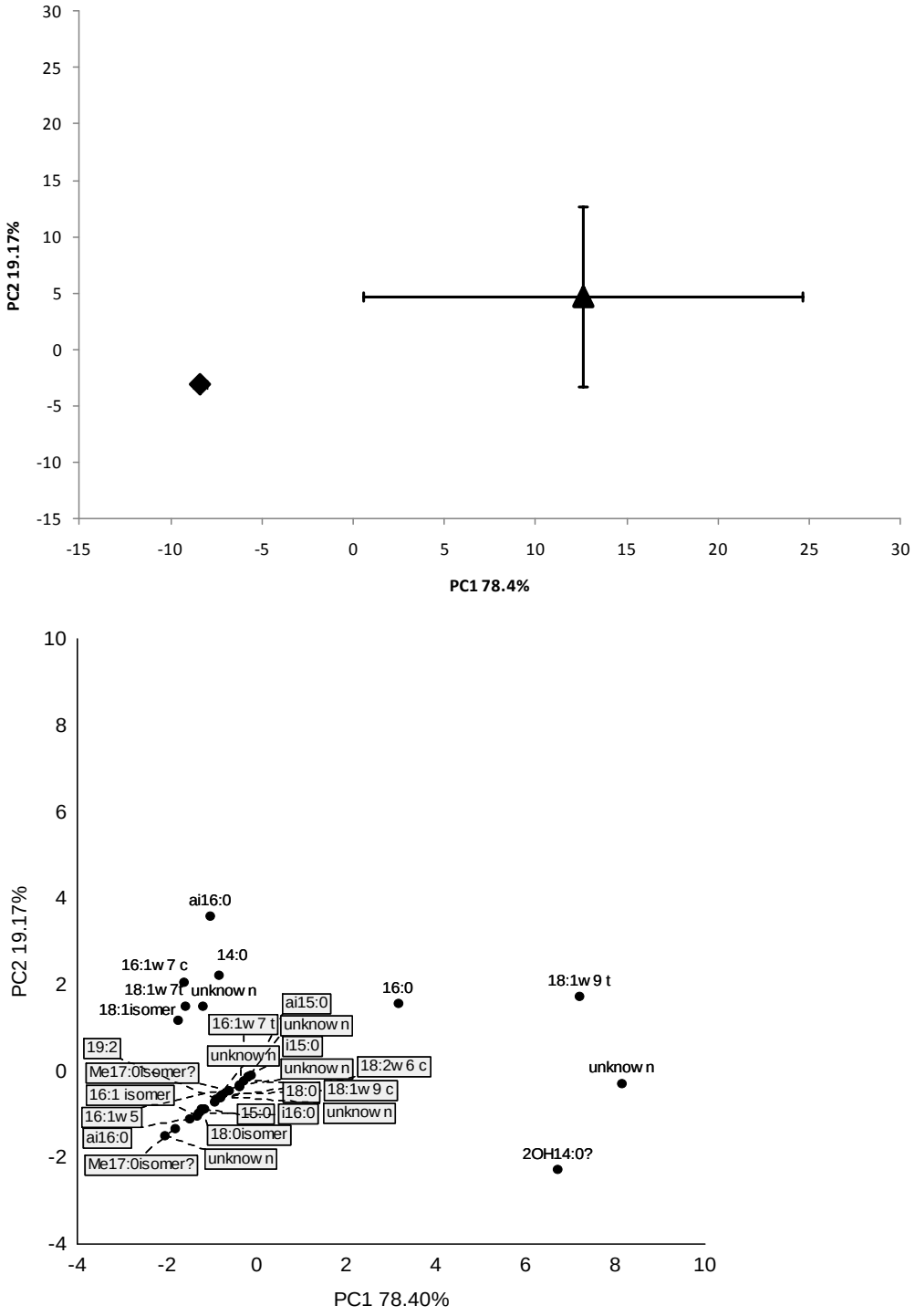



Figure 10.9: PCA and projection of PLFA variables for upwind (▲); and downwind (◆). The PCA illustrates the difference between the distribution of PLFA variables, represented by the projection plot, within each sample (upwind and downwind). The PCA whiskers represent standard error. Boxed PLFA variables on the projection represent those within the cluster.

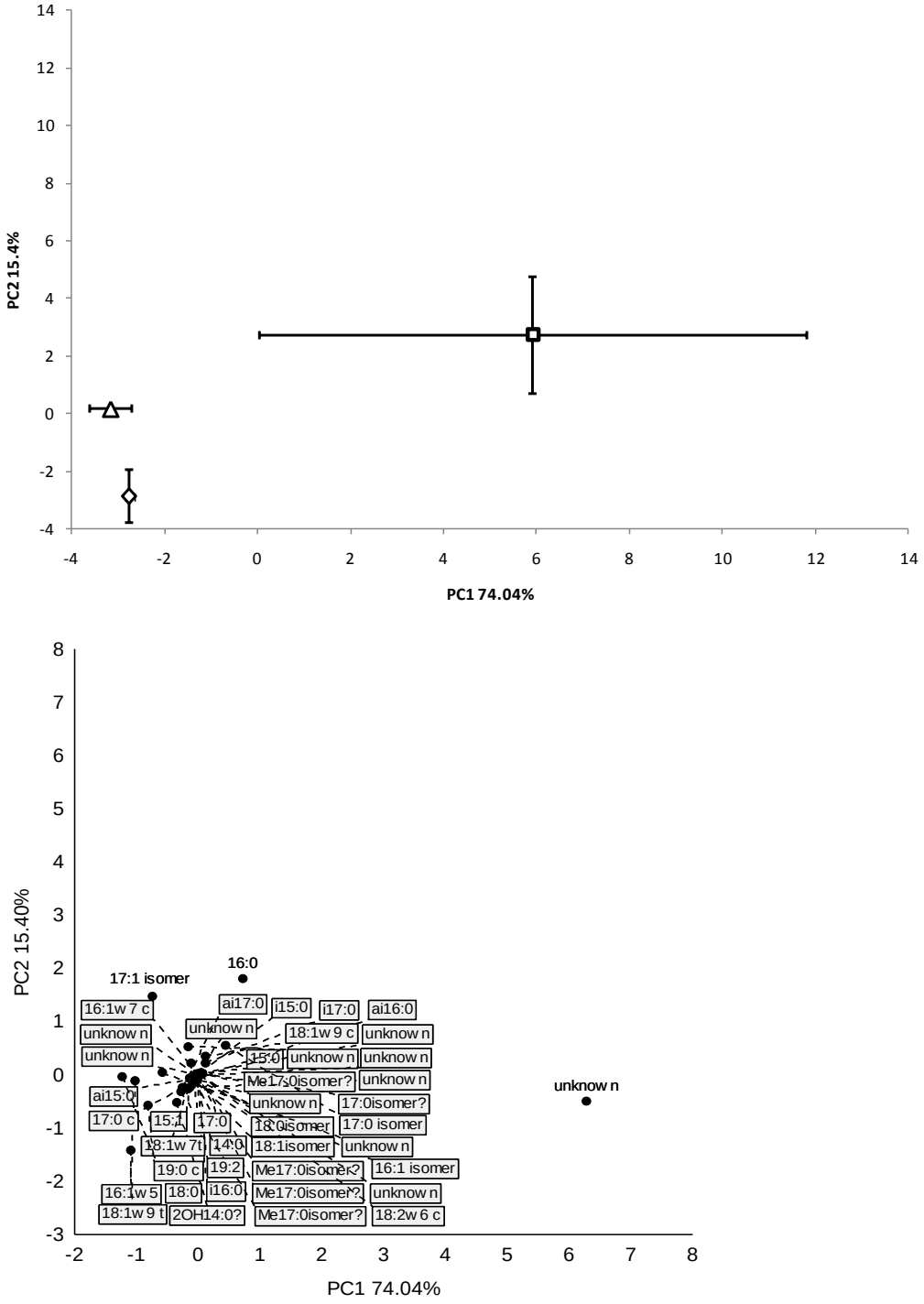



Figure 10.10: PCA and projection of PLFA variables for fresh green waste (□); medium green waste (△); and old green waste (◇). The PCA illustrates the difference between the distribution of PLFA variables, represented by the projection plot, within each sample (fresh, medium and old green waste). The PCA whiskers represent standard error. Boxed PLFA variables on the projection represent those within the cluster.

Table 10.5: Percentage composition of PLFA in each sample; calculated from sum of peak area across replicates and presented as percentage composition.

| ID | Upwind | Fresh Green Waste | Medium Green Waste | Old Green Waste | Downwind |
|---|---|---|---|---|---|
| 14:0 | 4.14 | 0.17 | 0.17 | 0.33 | 1.97 |
| i15:0 | | 0.06 | | | 0.52 |
| a15:0 | | 0.25 | 0.39 | 0.46 | 0.35 |
| 15:1 | | 2.05 | 1.77 | 1.66 | |
| 15:0 | | 0.19 | 0.15 | 0.26 | 2.69 |
| 2OH14:0? | 21.26 | 0.07 | 0.68 | 1.47 | 16.16 |
| 16:1 isomer | | 0.29 | 0.44 | 0.56 | 3.05 |
| unknown | | 0.03 | 0.11 | 0.08 | 1.00 |
| unknown | 21.79 | 0.57 | 0.63 | 1.09 | 12.04 |
| i16:0 | | 0.14 | 0.22 | 0.38 | 2.96 |
| a16:0 | | 0.54 | 0.47 | 0.71 | 3.38 |
| a16:0 | 6.37 | | | | 2.61 |
| 16:1*w*7 c | 4.75 | 0.87 | 0.82 | 0.91 | 3.85 |
| 16:1*w*7 t | | | | | 1.04 |
| 16:1*w*5 | | 9.37 | 10.17 | 10.47 | 1.05 |
| 16:0 | 8.65 | 11.25 | 7.51 | 6.39 | 2.58 |
| unknown | | 0.16 | 0.15 | 0.16 | 2.23 |
| Me17:0isomer? | | 0.21 | 0.18 | 0.27 | 1.37 |
| Me17:0isomer? | | 0.04 | 0.03 | 0.06 | 4.08 |
| Me17:0isomer? | | 0.92 | 1.46 | 1.87 | |
| unknown | | 0.40 | 0.43 | 0.48 | |
| Me17:0isomer? | | 0.05 | | | |
| i17:0 | | 0.97 | 0.60 | 0.38 | |
| a17:0 | | 0.44 | 1.10 | 1.21 | |
| 17:0isomer? | | 0.17 | | | |
| 17:0 c | | 0.64 | 1.03 | 1.39 | |
| 17:1 isomer | | 21.81 | 22.12 | 19.21 | |
| 17:0 | | | | 0.45 | |
| 17:0 isomer | | 2.58 | 0.47 | 0.91 | |
| unknown | | 8.33 | 4.29 | 4.39 | 4.52 |
| unknown | | 0.96 | 0.37 | 0.84 | |
| 18:0isomer | | 0.14 | 0.16 | 0.58 | 2.67 |
| unknown | | 0.42 | 0.29 | 0.52 | 0.56 |
| 18:2*w*6 c | | 0.16 | 0.11 | 0.18 | 1.85 |
| 18:1*w*9 c | | 2.43 | 2.33 | 1.93 | 1.74 |
| 18:1*w*9 t | 22.27 | 0.76 | 2.41 | 4.16 | 11.02 |
| 18:1*w*7t | 3.88 | 0.79 | 1.01 | 1.47 | 3.60 |
| 18:1isomer | 3.46 | 0.17 | 0.24 | 0.34 | 3.84 |
| 18:0 | | 12.76 | 15.43 | 13.66 | 0.87 |
| unknown | | 12.55 | 11.25 | 11.58 | 1.77 |
| 19:2 | | 0.23 | 0.27 | 0.45 | 1.93 |
| unknown | | 4.00 | 5.66 | 4.24 | |
| unknown | | 0.07 | 0.28 | 0.20 | |
| 19:0 c | | 0.97 | 3.66 | 2.98 | |
| unknown | 3.42 | 1.06 | 1.12 | 1.33 | 2.66 |
| **Number PLFA** | 10 | 42 | 39 | 40 | 30 |

The ratios of fungal to bacterial PLFA were also analysed. For the purposes of this analysis unidentified and PLFA's where taxonomy remains unclear were removed, as they would not contribute to the analysis. Results are presented in Figure 10.11 below. No PLFA's indicating fungal genera were found in upwind samples. In all compost samples, the fungi to bacteria ratios are similar, being below 0.005, with an increase to over 0.03 in downwind samples.

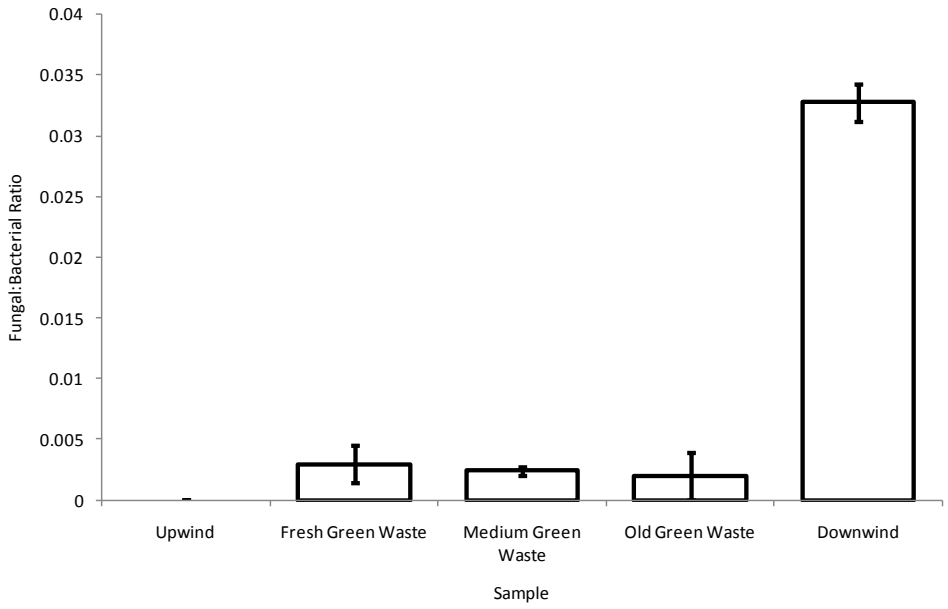



Figure 10.11: Ratios of fungi to bacteria for known PLFA's for upwind, downwind, and green waste samples. Bar represents mean ratio ($n$ = 3 for downwind and compost and $n$ = 2 for upwind samples). No representation of upwind ratio as no fungal PLFA detected. Whisker represents standard error.

Shannon's Index of species diversity and evenness was also applied to the data. Shannon's index is commonly used to describe biodiversity, and can be applied to describe the diversity and evenness of PLFA within each sample. The index provides an enumeration of the number (or diversity) of unique PLFA found within each sample, comparing the diversity within each sample, and the evenness of the distribution of these PLFA according to the abundance of each PLFA (Mouillot and Leprêtre, 1999). Equations 10.1 and 10.2 describe this process.

$$H' = \Sigma_{i=1}^{S} \rho_i \ln \rho_i \quad \text{[Equation 10.1]}$$

Where:

*H'* = Shannon Index

*S* = Number of PLFA's encountered

$p_i$ = The relative abundance of PLFA's *i* (abundance of individual PLFA / total number PLFA)

Σ = Sum of PLFA's found

ln = Natural log

$$E = \frac{H'}{(\ln H')} \quad \text{[Equation 10.2]}$$

Where:

E = PLFA envenness

*H'* = Shannon index

ln = Natural log

As shown in Table 10.6, microbial community diversity is lowest at the upwind location and similar Shannon indexes were obtained for the remaining samples. Evenness is high across all samples, with medium green waste showing the highest PLFA evenness.

Table 10.6: Shannon and evenness index for upwind, downwind, and green waste PLFA profiles.

| | Shannon | Evenness |
|---|---|---|
| **Upwind** | 2.24 | 0.97 |
| **Downwind** | 3.36 | 0.99 |
| **Fresh Green Waste** | 3.71 | 0.99 |
| **Medium Green Waste** | 3.65 | 1.00 |
| **Old Green Waste** | 3.64 | 0.99 |

### 10.3.2 DGGE analysis

Community fingerprint generated through DGGE (Figure 10.12) was transformed to a binary matrix (1 = presence of DNA band; 0 = absence of DNA band) and analysed according to Jaccard coefficients using Primer 6, version β (2001) software. The results from a non-metric MDS scaling plot are shown in Figure 10.13. The MDS plot was calculated with a stress of 0.07, indicating the plot has good orientation and structure, although interpretation must still be carried out with care (Clarke and Warwick, 2001). The data clearly showed two groups, each containing samples of > 20% similarity, with one upwind sample un-related to these groups. Upwind 1a, however, shows 20% similarity with green waste 2, and site samples 3 and 4. All of these samples were taken on the same sampling day (21$^{st}$ May 2009). The other group of 20% similarity contains green waste 1, site samples 1 and 2, and downwind samples 1 and 2. Green waste 1, site 1, and downwind 1 were all taken on the 22$^{nd}$ April, while site 2 and downwind 2 were taken on the 6$^{th}$ May. While compost 1 only shares 20% similarity with any other sample, site samples 1 and 2 and downwind samples 1 and 2 share up to 60% similarity. Further analysis, as has been carried out for PLFA analysis, has not been carried out for DGGE data, as abundance and identification of markers for specific communities or groups is not possible with this analysis.

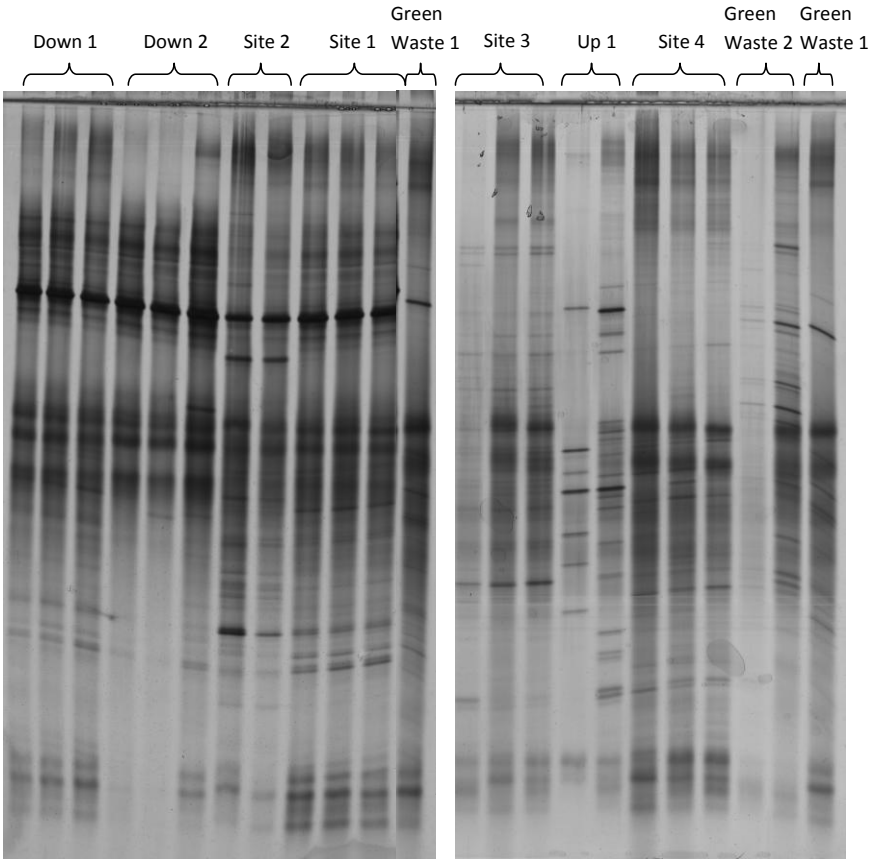



Figure 10.12: Image of DGGE gel stained by nitrate silver, all samples shown, comparing bioaerosol and green waste samples by PCR-DGGE analysis of 16S rDNA fragment 341-534. Bands analysed according to Jaccard coefficient.

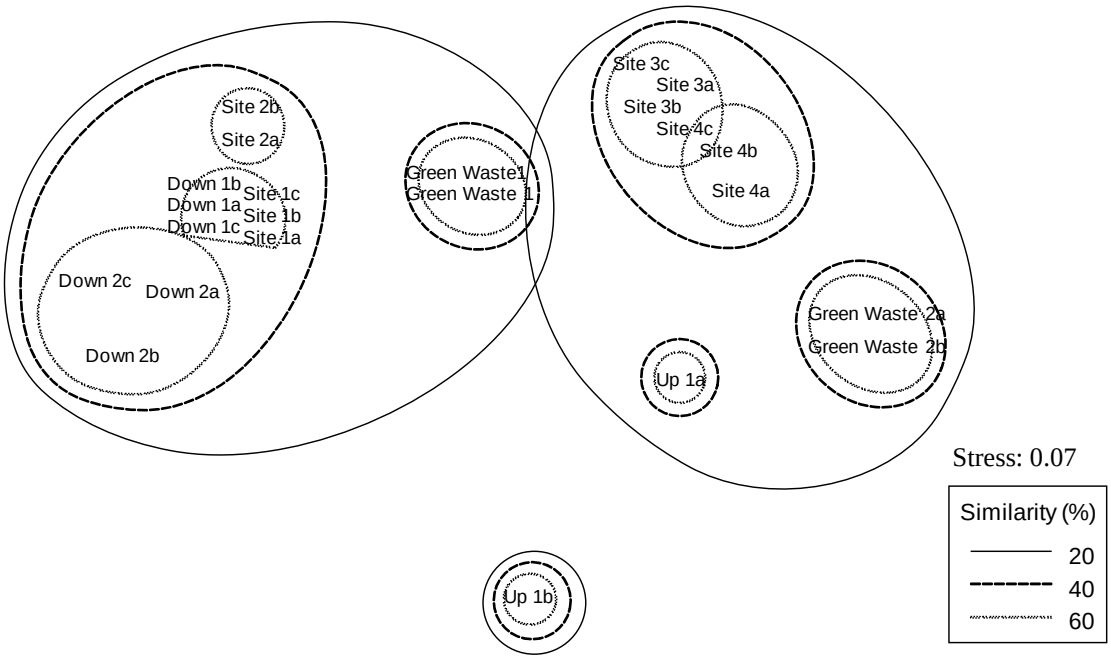



Figure 10.13: MDS plot of DGGE results, similarity of individual samples according to the presence or absence of DNA bands represented by percentage. Samples taken on 22nd April: Green waste 1 (sample re-analysed as part of DGGE control); Site 1a-c; Down 1a-c. 6th May: Site 2a-b; Down 2a-c. Samples taken on 21st May: Upwind 1a-b; Green waste 2a-b; Site 3a-c; Site 4a-c. Stress 0.07.

## 10.4 Discussion

### 10.4.1 Bioaerosol Microbial Communities

Both PLFA and DGGE analyses clearly show significant differences between composting, upwind, and downwind microbial communities. The MDS plots for each method are comparable, although must be interpreted with care. Although PLFA analysis represents the whole microbial community, DGGE only includes bacterial commmunities (Section 10.2.2), reducing the ability to directly compare between results. In addition, in order to increase PCR yield for DGGE analysis, nested PCR was carried out. The principle of nested PCR is that new primers are used in order to target part of the 16S rRNA product from the direct PCR (Figure 10.3), this may increase the

level of bias found due to PCR amplification, and reduce microbial diversity (Peccia and Hernandez, 2006). However, in this case nested PCR was necessary to gain detectable concentrations of DNA for DGGE analysis. Despite the increased potential error in DGGE analysis, and the lack of fungal community characterisation, significant and comparable results were found.

Figure 10.6 shows relative similarities between PLFA profiles for each sample, while Figure 10.13 shows the same data for DGGE, determined through analysis of DNA band presence or absence within each sample. While PLFA can be described as showing the active members of the microbial community, DGGE also shows non-viable microorganisms. In this case, the additional qualification of non-viable microorganisms through DGGE did not lead to a significant difference in community structure. This may be due to the high levels of viability that are suggested to be found within composting environments (Adams and Frostick, 2009). Furthermore, the additional qualification of fungal community through PLFA did not lead to significant differences in results gained through MDS. Both figures highlight that within site, downwind, and compost samples the microbial communities detected are typically tightly clustered. Upwind samples show more variation, indicating more disperse biota across samples, with fewer PLFA showing higher abundance. For PLFA, the upwind profile is > 60% similar to any other sample, while for DGGE Upwind 1a shows 20% similarity to green waste sample 2 and site samples 3 and 4. The samples within this 20% similarity cluster were all taken on the same day, explaining their similarity. However, this also highlights how samples taken from similar locations, but on different days, contain different microbial communities, illustrated by the lack of similarity between site samples 1 and 2, and 3 and 4 (Figure 10.13). This difference may be due to day-to-day variations, such as meteorological conditions, feedstock composition, and type of processing activity on-site. This finding again suggests that ‘snapshot’ samples, defined as those taken on only one sampling occasion and discussed in Section I of the thesis, reduce confidence in data. Both MDS plots, however, show how composting alters the community composition of bioaerosols, with > 20% similarity between green waste, on-site and downwind samples shown through both analyses (Figures 10.6, 10.13). The results from DGGE analysis effectively show changes in bacterial bioaerosol

community composition due to a particular source, in this case composting. This is in agreement with other studies based on PCR, showing the same effect for land-application of biowaste (Brooks *et al*, 2007), and for composting bioaerosols where aerosolised microbial community members were traced to compost samples from which the same species had been isolated (Le Goff *et al*, 2009). The analysis of PLFA composition can provide further information regarding phenotypic community composition, as both qualitative identification and abundance of microbial groups contributing to community similarity or diversity can be analysed.

The inital analysis of identifiable PLFA (Table 10.4) showed how samples downwind from site contained 13 more PLFA than upwind samples, with 12 of these PLFA also found in compost samples. Of the additional PLFA that have been identified, two have been attributed to gram-positive bacteria (Amir *et al*, 2008; Frostegård *et al*, 1991; Zelles, 1999), possibly belonging to the *Bacillus-Arthrobacter* group (Macnaughton and Stephen, 2001); two have been associated with gram-negative bacteria (Bartlett *et al*, 2007; Frostegård *et al*, 1991; Macnaughton and Stephen, 2001); one with fungi (Macnaughton and Stephen, 2001; Stoeck *et al*, 2002; Zelles, 1999; Zhang *et al*, 2009), and one with actinomycetes (Amir *et al*, 2008; Macnaughton and Stephen, 2001; Stoeck *et al*, 2002; Zelles, 1999; Zhang *et al*, 2009). However, the caution with which these assignments must be interpreted with is highlighted through the fact that two PLFA found amongst this group have been associated with both bacterial and fungal groups (Amir *et al*, 2008; Frostegård *et al*, 1991; Gavito and Olsson, 2008; Macnaughton and Stephen, 1002; Stoeck *et al*, 2002; Zelles, 1999; Zhang *et al*, 2009). The assignment of PLFA markers to these microbial groups, particularly the absence of biomarkers purely attributed to fungi in upwind samples, suggests that composting had a significant impact upon the microbial species composition of the aerobiota. The increased difference between upwind communities and green waste and bioaerosol samples shown through PLFA analysis (Figure 10.6) may also be due to the presence of fungi, which were not included in DGGE analysis.

In order to further explore this relationship, PCA was carried out. The results from this analysis were clearly defined and show a high level of similarity to the MDS plot

(Figures 10.6, 10.7). Green waste samples were shown to be statistically similar in PLFA composition, while upwind and downwind samples were both significantly different from all green waste samples, as well as each other. Error was higher for upwind samples; this is likely due to fewer replications being obtained at this location as a result of contamination and reflects the MDS results (Section 10.3.1). The PCA suggests that the emission of composting microorganisms significantly affected PLFA composition downwind. Projection of the variable input for the PLFA (Figure 10.8) showed that upwind and downwind samples were largely affected by PLFA identified as a gram-positive marker (18:1ω9 t) and a gram-negative marker (2-OH); as well as an unknown PLFA (Amir *et al*, 2008; Frostegård *et al*, 1991; Zelles, 1999). Green waste community dispersal was found to be affected by a 17:1 isomer, only found in green waste samples; 18:0, found in green waste and downwind samples; 16:1ω5, also found in green waste and downwind samples, and attributed to both fungal and bacterial groups (Frostegård *et al*, 1991); and two unidentified PLFA also affected compost community composition.

As shown by Figure 10.8 a large cluster of PLFA prevented some markers, such as those responsible for differences between upwind and downwind samples, from being discerned. A break-down of the data into bioaerosol and compost samples followed by separate PCA's was therefore carried out. Figure 10.9 shows the results of this for bioaerosol samples. A cluster shown on the projection of PLFA contributing to PCA results appears to be largely responsible for the downwind community profile, while upwind PLFA are more disperse, contributing to the large error seen. Fifteen individual identified PLFA can be found within the cluster, with 5 unidentified. Of the identified PLFA, only two are found in both upwind and downwind samples, while all can be found in green waste. This again highlights the influence of composting microorganisms on species composition of aerobiota downwind from site.

The contribution of different PLFA to differences in PCA for green waste samples is more difficult to interpret (Figure 10.10). While there is some suggestion that 17:1 isomer, 16:0, and an unknown PLFA may be responsible for the variation seen in fresh green waste samples, 16:0 was found across all samples, and 17:1 isomer was found in

all green waste samples. Table 10.5 illustrates how the variability of fresh green waste samples is related to abundance of PLFA markers found for fresh green waste. This could be anticipated, as diversity has previously been found to be highest in fresh green waste (Hermann and Shann, 1997). Within both upwind and downwind samples, the same three PLFA dominated in terms of percentage peak area, with two of these PLFA identified as belonging to *Pseudomonas* or gram-negative bacteria, and gram-positive bacteria (Amir *et al*, 2008; Frostegård *et al*, 1991; Zelles, 1999). However, the number of PLFA found was three times higher in downwind samples, with the additional PLFA directly attributable to green waste. Comparison of the ratio of *trans* to *cis* gram-negative PLFA markers within different samples could not be carried out, as trans markers for gram-negative PLFA were only found in downwind samples (16:1ω7 t) (Macnaughton *et al*, 1999). However, in itself, this may indicate that gram-negative bacteria are more stressed in airborne samples, given that 16: 1ω7 c could be found in all samples.

Similar numbers of PLFA were identified in all green waste samples, although fresh green waste showed a slightly higher number of PLFA. One PLFA, 17:0 isomer, dominated in all green waste samples, although differences in the dominant markers within fresh, medium and old green waste were seen. This suggests an alteration in composting microorganisms over time, as could be anticipated from the earlier literature review (Section 10.1). It has been suggested that 16:0 and 18:0 are widely represented within environmental samples (Hermann and Shann, 1997), explaining their dominance across compost samples. Another PLFA, 16:1ω5, has been associated with both fungal and bacterial groups (Frostegård *et al*, 1991); here, it could be assumed that the marker represents a thermophilic microorganism, that has proliferated during the early stages of composting (Hermann and Shann, 1997) as its dominance declines once again as green waste ages and returns to a mesophilic state. The proportional diversity of other species in old green waste increases; with less dominance shown for 17:0 isomer in particular. This may be related to an increase in mesophilic communities the cooling phase of green waste (Hermann and Shann, 1997). Other PLFA's attributed to microbial groups have shown changes in community structure as green waste ages. For example, four markers of actinomycetes (Methyl branched) were found, with three of these increasing

in dominance from fresh to old green waste; this is in agreement with Cahyani *et al.* (2002). Some markers indicative of *Bacillus* spp. increased (Amir *et al*, 2008; Steger *et al*, 2005), such as a17:0 which increased from 0.44% in fresh green waste to 1.21% in old green waste. However, other markers for this group decreased (Tables 10.1, 10.5). No difference in the ratio of bacterial to fungal PLFA markers was seen (Figure 10.11) in green waste samples, in disagreement with other studies reported within the literature; however increases in markers for gram-negative bacteria (18:1ω7 t) were seen, in agreement with published literature (Amir *et al*, 2008; Cahyani *et al*, 2002; Hermann and Shann, 1997; Steger *et al*, 2005).

Figure 10.11 illustrates the difference in fungal:bacterial ratios between green waste and bioaerosol samples. While ratios are similar within green waste samples, downwind samples show a far higher ratio of bacteria to fungi. This is a result of an increase in the proportion of fungi found as compared to bacteria, and may reflect the ease with which fungal spores are released through composting (Fletcher *et al*, 2008) as compared to bacterial cells (Chapter 1). This is also supported by Le Goff *et al*. (2009), who showed how sporulating bacteria dominated within bacterial bioaerosols emitted through composting, with a suggestion that these spores were emitted with greater ease than bacterial cells (Le Goff *et al*, 2009). It can be largely assumed that fungal markers found are a product of composting activities, as none were found in upwind samples. These data differ from the fungal:bacterial ratios presented from culture analysis in Chapter 8 (Figure 8.1), where ratios were higher at upwind and downwind locations. This may reflect the inability of PLFA to detect all fungal markers. In addition, the Shannon index of diversity and evenness does not show any large differences between samples taken, as fungi markers comprised a small fraction of the total PLFA (<1%) within each green waste and downwind bioaerosol, reducing impact on the Shannon index (Table 10.6). The remaining bacterial PLFA did not vary greatly in composition or abundance, shown by the high evenness. However, as could be anticipated, upwind samples showed less species diversity and evenness than the other samples taken. This information was also found through analysis of Table 10.5. Downwind and compost samples diversity indexes were similar, again reinforcing the suggestion that species composition of bioaerosols was significantly affected by composting emissions.

### 10.4.2 Evaluation of Methods

This phase of the study study was intended as a 'proof of concept' project, illustrating the potential use of PLFA analysis and PCR followed by DGGE for bioaerosol communities. While there are other studies utilising PCR based methods for bioaerosol analysis, these methods have not been used for study of composting bioaerosols. In addition, only one study using PLFA analysis for bioaerosol analysis is available, and utilised an impractical sampling method and practice. Specifically, the apparatus was large and would not provide a practical field based method (Macnaughton *et al*, 1999). This study, however, has demonstrated how PLFA analysis and PCR followed by DGGE can be successfully performed using small, portable equipment that is typically used for culture-based analysis (Chapter 3). While 60 minute sampling times were sufficient in areas of high bioaerosol concentration, 120 minutes was required at the upwind sampling location, where concentrations were lower. Despite this, the fact that bioaerosol PLFA and suitable concentrations of cells for PCR were recovered at upwind locations illustrates how culture based methods and their related selectivity are unable to represent the aerobiotic community; as concentrations were often below detection limits when sampling through culture (Chapters 4 and 5). Furthermore, the pump speed and volume of air sampled as part of this methodology was the same as that used to preserve culturability of microroganisms (Chapter 3); for future studies in this area, it may be possible to increase pump speed, therefore reducing sampling time for non-culture based analysis and the chance of contamination.

Although this study has demonstrated the major differences in microbial community structure upwind, downwind, and within green waste; interpretation of several aspects of the dataset must be carried out with caution. As described in sections 10.1.1 and 10.1.2, there are methodological limitations affecting both PLFA and DGGE. For PLFA, interpretation of markers for microbial groups must be carried out with caution, as this information is largely based on markers determined through culture analysis, and therefore subject to the same limitations as culture; in addition, contradictory data exists within the literature atributing PLFA to different taxonomic groups (Chapter 7) (Zelles, 1999). For DGGE, limitations exist in the PCR amplification of samples. As described

in section 10.1.2, potential for error can be found in the extraction of nucleic acid, the exclusion of sections of 16S rRNA through primer selection, enhanced through the use of nested PCR, the formation of chimeric DNA, and the bias of amplification leading to preferential amplification of sequences that do not form dominant components of the community (Felske *et al*, 1998; Head *et al*, 1998; Hugenholtz and Goebel, 2001; Suzuki and Giovannoni, 1996). However, the majority of these aspects affect analysis at the sequencing stage, where DNA is profiled and assigned to taxonomic groups. This analysis was not performed as part of this study, where focus is instead on quantifying differences between microbial communities. A limitation that may have affected the analysis here, however, is the positioning of two DNA bands in the same location within the DGGE gel (Figure 10.2). This may lead to underestimation of true diversity (Heuer *et al*, 1997).

Despite all these factors, the main limitation to this study was time. As a result, sampling occasions and the amount of samples processed were limited. Along with the 'proof of concept' nature of this study, the limited sampling occasions means results must be interpreted with caution. Until verification of findings through more extensive study, the data must therefore be viewed as preliminary. The lack of certainity in some results gained was shown. For example, comparison between composting communities in fresh, medium and old green waste showed some agreement with previous studies (Cahyani *et al*, 2002; Hermann and Shann, 1997), while other aspects disagreed. In particular, no net shift in the predominance of species was seen in compost samples as the material aged (Amir *et al*, 2008; Steger *et al*, 2005). However, this study was not intended to provide a measure of composting community dynamics, as many other robust studies exist on this topic (Hermann and Shann, 1997). In addition, the lack of identification of actinomycete PLFA markers in upwind samples is contradictory to the high levels of actinomycetes found ubiquitously through culture-based analysis (Chapter 4). This indicates that PLFA analysis was unable to account for or identify all actinomycete markers, as many PLFA remain unidentified or of disputed origin. The abundance of actinomycetes within the environment has been shown through Section I of this thesis, as well as several, independent studies (Korzeniewska *et al*, 2009; Tamer Vestlund, 2009) The lack of repeated samples has also restricted the amount of

statistical analyses that could be applied, while the methods that have been used, such as the Shannon index, would likely be significantly improved through analysis of further samples.

The driver behind instigating a study investigating the use of these methods for bioaerosol analysis was 'source apportionment', the theory that through study of the microbial community structure, bioaerosols may be traced to source. Both methods used here have shown the potential to allow source apportionment. However, a lack of time and resources has prevented this being trialed, for example, through the analysis of alternative sources of bioaerosols. The additional taxonomic refinement of PLFA within has shown the best potential, within this study, for use in future source apportionment. The contributions of composting microorganisms to the PLFA profile downwind from site created a significantly different PLFA 'fingerprint' from that found upwind. In order to confirm that composting may have a specific PLFA fingerprint, further samples of composting bioaerosols are initially required, as there may be differences in microroganism communities emitted through composting on a day-to-day basis (shown through DGGE results). PLFA analysis of alternative sources of bioaerosols would also be required, with the aim of confirming a composting PLFA fingerprint. However, PLFA analysis is limited in two major ways, the inability to identify all PLFA markers, shown through the lack of PLFA markers for actinomycetes upwind, and the lack of further taxonomic refinement. All markers identified are, at best, assigned to groups of microorganisms. This may result in similar PLFA profiles being gained for two samples, when further taxonomic refinement may illustrate signficant differences in community structure. For example, it is likely that a large proportion of composting PLFA originate from thermophilic and thermotolerant microorganisms, with analysis unable to determine this.

DGGE analysis has also shown the potential for use in the development of a composting DNA 'fingerprint' for bioaerosols. This may be done through comparison of the gel (Figure 10.12), used to generate the MDS results shown in Figure 10.13, to the fingerprint from other samples. This was not possible during the course of this study due to a lack of time and resources. The issue of a lack of taxonomic refinement is

enhanced for DGGE, as no taxonomic information is gained through this analysis. Once again, in order to confirm a composting DNA fingerprint, further samples of both compost bioaerosols and those from alternative sources are required, with analysis of fungal as well as bacterial DNA. One advantage of DNA analysis, however, is the potential for further refinement of the composting microbial community fingerprint, as each band on a DGGE gel may represent a bacterial species, oppose to the relatively broad taxonomic groups assigned to PLFA markers. In addition, DNA analysis provides the option for further study and detailed taxonomic refinement through sequencing.

To summarise, the data presented could arguably have been obtained through culture-based analysis. However, the potential for the use of these methods for the future analysis of bioaerosols is great. Both methods would allow rapid analysis of bioaerosols, including non-culturable fractions, with DNA analysis also allowing enumeration of non-viable fractions. This study has demonstated that they may be used with success alongside culture-based analysis, using the same equipment. Although limitaitions remain within both methods, alongside culture-based analysis they may provide more complete understanding of the composition of bioaerosols. Development of these methods may also allow source apportionment of bioaerosols within future studies. The theory of source apportionment through analysis of the composition of bioaerosol communities is explored further in the following Chapter.

## 10.5 Conclusions

The aim of this study was to show the differences and similarities in community profiles upwind, downwind, and within compost through PLFA analysis. This aim has been achieved with the hypothesis; ‘Airborne microbial communities will be significantly different upwind, on-site, and downwind from site’, supported.

Green waste, upwind, and downwind samples were significantly different ($p = < 0.05$), showing no more than 40% similarity in microorganism community structure between green waste, upwind and downwind. PLFA markers in air samples downwind from site

were also found within green waste samples, but not found upwind, suggesting that the changes in bioaerosol community seen downwind could be attributed to composting. MDS plots for both PLFA and DGGE analysis show increased similarity between green waste, on-site, and downwind samples as compared to upwind, indicating the contribution of composting microorganisms to the microbial community.

These data have allowed several conclusions to be drawn, outlined below.

- Upwind, green waste, and downwind bioaerosol community compositions are significantly different from each other
- Sampling on different days leads to different community structures of bioaerosols
- Green waste significantly contributes to bioaerosol community structure downwind from composting activities

The results presented here have shown the influence that composting has on aerobiota through two independent methods, PLFA analysis and DGGE analysis. It is clear that specific conditions such as feedstock composition, presence and type of composting activity, and meteorological conditions will affect community composition on a diurnal basis. Yet due to incomplete knowledge surrounding the types of microorgansims released through composting and the mechanisms of these releases, reasons for differences between sampling days cannot be discerned. Another area that requires verification is the ratio of *trans* to *cis* PLFA markers. There is some suggestion that airborne communities may be more stressed than those found in green waste, due to the presence of *trans* gram-negative markers downwind, with this information requiring verification through additional experimental work.

These data have illustrated the potential for further understanding of the impact that composting emissions have on ambient bioaerosols to be gained through implementation of molecular methods. However, the limitations of this study leave many options for further investigation. In particular, the differences found between different sampling days suggests further work investigating the day-to-day variations in

community structure of bioaerosols and the factors affecting community structure would be appropriate. Further refinement of sampling methods may also increase reproducibility of results, allowing rapid, repeated sampling of bioaerosols for the purposes of molecular analysis.

Further study of composting microbial fingerprints would also allow investigation of the differences in microbial populations; characterising aerobiota from alternative sources, and exploring the contribution of composting bioaerosols to aerobiota further downwind and potential receptor exposure through source apportionment The analysis of further samples would also allow microbial biomass through PLFA analysis to be quantified; not carried out as part of this study due to time and sampling limitations. Further taxonomic refinement of PLFA data is not possible (Adams and Frostick, 2009). However, continued exploration into the bacterial community composition of the PCR product gained as part of this study can be performed through DNA sequencing. Chapter 11 carries out this task, presenting the results for pyrosequencing of the PCR product, and identification of the specific microorganisms that have resulted in the differences in community structure seen here.

# 11 The Diversity and Structure of Bioaerosol Communities at Composting Facilities

## 11.1 Introduction

The microbial diversity of bioaerosols studied through phospholipid fatty acid analysis (PLFA) and denaturing gradient gel electrophoresis (DGGE) was carried out in Chapter 10. This study revealed significant differences between green waste microbial community structure, and upwind, on-site and downwind bioaerosol community structure. Through analysis of PLFA markers, it was possible to identify the microbial groups responsible for some of the changes in community structure observed. In particular, the PLFA markers i15.0, a15:0, 15:0, 16:1 isomer, i16:0, 16:1ω5, Me17:0 isomer, 18:0 isomer, 18:2ω6 c, 18:1ω9 c, 18:0 and 19:2 originated from bioaerosol compost samples and were also found in downwind samples but not in upwind ones. These markers were attributed to all of the major microbial groups associated with composting, including gram-negative and gram-positive bacteria, actinomycetes and fungi (Chapter 10). However, the lack of refinement of this taxonomic identification means that the specific microbial families or species responsible for changes in microbial community remains poorly described. The importance of identification of microorganisms responsible for bioaerosols at composting facilities was highlighted in Chapter 8, where identification of key gram-negative bacteria revealed several known opportunistic pathogens present on-site. Further identification of communities may reveal the species responsible for the alterations in microbial community found in Chapter 10, as well as provide information on the consortium of microorganisms that sensitive receptors may be exposed to through composting, and key aerosolised microorganisms indicative of composting (Le Goff *et al*, 2009).

As shown in Chapter 10, the amplification of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) through polymerase chain reaction (PCR) allows the nucleic acid of microbial community members to reach concentrations that can be analysed (Mukoda *et al*, 1994). In this case, only bacterial communities were amplified. This was

largely due to the increased difficulty in amplification of fungal DNA, as the risk contamination is suggested to be higher (Felske *et al*, 1998; Head *et al*, 1998; Hugenholtz and Goebel, 2001; Suzuki and Giovannoni, 1996). In addition, the lack of time and resources within this study also limited ability to analyse fungal communities. After amplification, there are a number of options available for microbial community analysis. Differences between microbial communities within different samples may be analysed through methods such as DGGE, described in detail in Chapter 10. Following analysis of the community structure, the identification of microbial community can be performed through DNA cloning and sequencing (Alfreider *et al*, 2002; Ward *et al*, 1990). It is possible to excise individual DNA bands gained through DGGE, re-amplify the band through PCR, and sequence the product. This type of procedure allows selection of key community members from the DGGE gel; namely, bands that are common to all communities can be excised, as well as bands that are unique to certain samples, identifying the community members that result in similarities and differences between samples. Alternatively, the entire PCR product may be sequenced, allowing identification of the whole community amplified.

Sequencing may be carried out through the Sanger method, or through 454 pyrosequencing (pyrosequencing) (Wicker *et al*, 2006). Pyrosequencing, a method developed over the last decade for commercial usage (Margulies *et al*, 2005), allows rapid sequencing of multiple samples (Parameswaran *et al*, 2007; Wicker *et al*, 2006). The method is based on the synthesis of a complementary strand of DNA from single-strands of DNA, with the nucleotides adenine, thymine, cytosine and guanine (A, T, C, G) added sequentially. As a nucleotide complements a corresponding unpaired base in the single strand of DNA, organic phosphate is released, leading to a cascade enzymatic response and the emission of a light signal (Metzker, 2005). Through the sequential addition of each nucleotide and analysis of light signals, the sequence of nucleotides within the DNA can therefore be determined. The ability to sequence multiple samples simultaneously, saving both cost and time, has made pyrosequencing an essential tool for the sequencing of genomes (Margulies *et al*, 2005; Parameswaran *et al*, 2007; Wicker *et al*, 2006). However, as a method that has only been available for the last 5 years (Margulies *et al*, 2005), it remains novel within the analysis of environmental

microorganism community structure. Although pyrosequencing offers a unique opportunity to rapidly and accurately explore microbial diversity, the analysis of this data provides a new challenge for microbial ecologists. For example, many environmental DNA sequences found have not yet been attributed to specific microorganisms. Best practice methods for the treatment and analysis of data are also yet to emerge (Head *et al,* 1998).

Following sequencing, the library created is typically submitted to publically available programs, such as Greengenes (http://greengenes.lbl.gov) (DeSantis *et al*, 2006a, b), which provide services such as alignment of sequences and classification through comparison of sequences to genome databases such as the Ribosomal Database Project (RDP) (http://rdp.cme.msu.edu/) (Maidak *et al*, 1996; Maidak *et al*, 1999), and the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/). This allows assignment of the sequences to the closest taxonomic match available. The development of non-culture based methods and rapid sequencing has allowed identification of unforeseen microbial diversity, making them indispensible tools in microbial ecology (Head *et al*, 1998; Ward *et al*, 1990).

Sequencing has been employed in many studies of microbial community composition, including those of green waste and airborne microorganisms. However, few studies considering the impact of composting microorganisms on airborne communities have been carried out. A recently published investigation characterised the microbial diversity of bioaerosol communities emitted from five different composting facilities in order to identify whether a common microbial signature of key organisms could be defined (Le Goff *et al*, 2009). The diversity of samples was high, with 685 sequences obtained through analysis of the 16S clone libraries, distributed across 220 phylotypes. The dominant bacterial phylotypes were *Actinobacteria* and *Firmicutes,* which accounted for 49 and 37% of the phylotypes respectively. The remainder of the phylotypes were *Proteobacteria* (*Alpha*, *Beta* and *Gamma*), *Bacteroidetes, Chloroflexi, Gemmatimonadetes, Planctomycetes, Thermotogae* and TM7 (Le Goff *et al*, 2009). The authors suggest that the dominance of *Actinobacteria* and *Firmicutes* is due to their thermophilic and spore forming nature, (Le Goff *et al*, 2009). Further comparison to

existing sequences available in the RDP indicated that soil and compost were the dominant sources of the species identified (Le Goff *et al*, 2009). This study compared only single samples of bioaerosol from five different sites, deliberately selected for their different feedstock types and operating systems. Although this has allowed comparison between composting bioaerosols from different sources, the impact that composting has on ambient bioaerosols has not been determined. In addition, microbial community composition of the feedstock itself was not analysed, preventing direct analysis of which microbial components may be aerosolised.

Other studies have considered the composition of microorganisms within compost itself. Clone library construction, alongside DNA single strand-conformation polymorphism (SSCP) was used by Alfreider *et al.* (2002) to identify bacterial community dynamics (16S) throughout the composting process. Bacterial clones within fresh green waste were assigned to *Gamma-proteobacteria*, the *Bacillus-Clostridium* group, and to *Bacteroidetes*, previously known as the *Cytophaga-Flavobacteria-Bacteroides* (CFB) group. These data were anticipated as the taxa found are common in compost (Alfreider *et al*, 2002). During the thermophilic composting phase, most clones were assigned to low-G+C content gram-positive bacteria, which includes the *Bacillus-Clostridium* group. A significant change in bacterial community was observed as the process entered the maturation phase. Clones were assigned to the *Bacteroidetes* group, and the *Thermus-Deinococcus* group. This indicated a community shift resulting from high temperatures and a reduction in the amount of readily available substrates. Microorganisms belonging to these groups, known to thrive in the thermic phase of composting, and possessing the ability to degrade more recalcitrant substrates such as cellulose and lignin were found (Alfreider *et al*, 2002). Other studies have focused on specific composting stages or microorganisms. Steger *et al.* (2007) studied *Actinobacteria* populations during composting. The communities found appeared to be largely governed by changes in temperature. A shift in the *Actinobacteria* group community structure was observed, from *Corynebacterium, Rhodococcus* and *Streptomyces* in the fresh green waste to thermotolerant species such as *Saccharomonaspora viridis*, *Thermobifida fusca* and *Thermobispora bispora* as composting reached the maturation phase. *Streptosporangium* and other thermophilic

microorganisms were also detected during the hottest composting phases. At the later stages of composting, *Microbacterium* and *Arthrobacter* were identified (Steger *et al*, 2007). These results were interpreted as evidence of the importance of *Actinobacteria* in composting, due to their ability to proliferate during the hottest phases of composting, as well as degrade more recalcitrant substrates (Steger *et al*, 2007).

Studies concerning the composition of ambient bacterial bioaerosols are also scarce. Brooks *et al*. (2007) identified background aerosols as part of their study analysing the impact of land applied biosolids on bioaerosol communities. The majority of the unique clones identified were *Proteobacteria* (*Alpha*, *Beta* and *Gamma*) with a large dominance of *Gamma-proteobacteria*, *Actinobacteria* and *Firmicutes* (Brooks *et al*, 2007). Baertsch *et al*. (2007) also analysed the impact of land application of biosolids through PCR and sequencing. In this case, background bacterial aerosols were identified as 48% *Proteobacteria*, 36% gram-positive bacteria, 14% *Actinobacteria*, and 2% *Bacteroidetes* (Baertsch *et al*, 2007).

Sequencing will allow identification of the bacteria that comprise the community, and contributed to differences in the bacterial community structure described as part of Chapter 10. This will significantly contribute towards the achievement of thesis Objective ii:

'Characterisation of bioaerosols released from composting facilities, including bioaerosol community structure, and non-viable components'

The data developed throughout this part of the thesis is aimed at achieving the Chapter Objective:

'Taxonomic identification of the composition of bioaerosol bacterial communities and comparison to green waste bacterial community'

These data will provide novel knowledge on the bacterial diversity, structure and dynamics in bioaerosols and composting microorganisms, and identify potential

indicators of composting microorganisms emitted into upwind air samples during compost activities. In addition, the study will act as a 'proof of concept' that advanced nucleic acid based analysis can be successfully applied to bioaerosol investigations utilising the same methods as used for culture-based analysis (Chapter 3).

## 11.2 Experimental Design and Methodology

Five samples, representative of the samples used for DGGE analysis (Chapter 10), were selected for sequencing. These were:

| | |
|---|---|
| 22nd April 2009 - | 100 m downwind |
| | Green waste |
| 6th May 2009 - | Downwind from site activity (Site 1) |
| 21st May 2009 - | Downwind from site activity (Site 2) |
| | Upwind |

A full description of these samples can be found in Chapter 10. Sampling times ranged from 60 – 120 minutes. All samples were collected onto polycarbonate filters according to the methods outlined in Chapters 3 and 10.

The selected samples had nucleic acid extracted and 16S rRNA amplified through PCR as part of Chapter 10. Those selected for sequencing were re-amplified using the Muyzer primers shown in Table 11.1. PCR conditions were as follows: 2 µL of the DNA template (sample); 5 µL buffer (×10 + 5 mM $MgCl_2$); 5 µL 1mM dNTP; 5 µL $MgCl_2$; 2 µL each of 454 (Parameswaran *et al*, 2007) and 534R (Muyzer *et al*, 1993) primers 10 µM; and 0.5 µL Taq polymerase was added to 28.5 µL DEPC water. PCR (Gen Amp PCR System 9700) was performed according to the following temperature programme: 95°C for 2 minutes; 25 cycles at 94°C for 30 seconds, 57°C for 30 seconds, 72°C for 30 seconds; and a final extension at 72°C for 10 minutes.

Table 11.1: Details and references of primers used

| Sample | Primer | Sequence 5′ - 3′ | Target Gene | Reference |
|---|---|---|---|---|
| Upwind | 454 Forward | GCC TCC CTC GCG CCA TCA GCA GGT GGC ATC CTA CGG GAG GCA GCA G 454-BAC-357f-BC9 | 16S rRNA (bacteria) | (Parameswaran *et al*, 2007) |
| Site 1 | 454 Forward | GCC TCC CTC GCG CCA TCA GCA TTG AAG CTC CTA CGG GAG GCA GCA G 454-BAC-357f-BC10 | 16S rRNA (bacteria) | (Parameswaran *et al*, 2007) |
| Downwind | 454 Forward | GCC TCC CTC GCG CCA TCA GCT AAG TTC AGC CTA CGG GAG GCA GCA G 454-BAC-357f-BC11 | 16S rRNA (bacteria) | (Parameswaran *et al*, 2007) |
| Green Waste | 454 Forward | GCC TCC CTC GCG CCA TCA GCT AAG AAC GTC CTA CGG GAG GCA GCA G 454-BAC-357f-BC12 | 16S rRNA (bacteria) | (Parameswaran *et al*, 2007) |
| Site 2 | 454 Forward | GCC TCC CTC GCG CCA TCA GCT GGA GGA CTC CTA CGG GAG GCA GCA G 454-BAC-357f-BC13 | 16S rRNA (bacteria) | (Parameswaran *et al*, 2007) |
| All | 534R | ATT ACC GCG GCT GCT GG | 16S rRNA (bacteria) | (Muyzer *et al*, 1993) |

After amplification, yield was tested by electrophoresis in 1% agarose gel. Electrophoresis conditions: 100 volts, 142 mA for 25 - 30 minutes after which the gel was stained for 20 minutes in ethidium bromide; and washed in water for 10 minutes. After electrophoresis, DNA bands were excised and processed according to the QIAquick Gel Extraction Kit. 100 – 200 µL diffusion buffer (0.5M ammonium acetate; 10mM magnesium acetate; 1 mM ethylenediaminetetraacetic acid (EDTA); pH 8.0; 0.1% sodium laurel sulphate (SDS)) was added per 100 mg of gel. Samples were incubated at 50°C for 30 minutes, and then centrifuged at 10,000 RCF for 1 minute. The supernatant was removed and passed through the supplied column. Buffer QG (QIAquick Gel Extraction Kit) was added in a 3:1 ratio to the supernatant. The sample was placed in a spin column and centrifuged for 30 – 60 seconds at 10,000 RCF. With flow-through discarded, 0.75 mL Buffer PE (dissolved in ethanol) (QIAquick Gel Extraction Kit) was added and the sample centrifuged for 1 minute at maximum speed. DNA was eluted through addition of 50 µL Buffer EB (10 mM Tris·Cl, pH 8.5), or water, to the spin column. Finally, the samples were centrifuged for 1 minute at 10,000 RCF.

The NanoDrop ND1000 spectrophotometer was used to calculate DNA concentrations. Results were as follows:

Upwind - 12.81 ng/µL
Green waste - 64.66 ng/µL
Site 1 - 17.03 ng/µL
Site 2 - 51.88 ng/µL
Downwind - 33.66 ng/µL

These samples were combined with others from the same laboratory (not shown) in equimolar amounts. DNA concentration was measured at 47.3 ng/µL (NanoDrop ND1000). The Qiagen PCR purification kit (cat. n$^{o}$. 28106) was used to concentrate the sample to 50 µL. Buffer PB was added to the sample in the volume 5 parts buffer to 1 part sample. The sample was transferred to a QIAquick spin column placed in a collection tube, and centrifuged for 30 – 60 seconds. After discarding the flow-through, 0.75 mL Buffer PE was added and the sample centrifuged for 30 – 60 seconds. The flow-through was again discarded, before centrifuging the sample for 1 minute. With the column placed into a clean microcentrifuge tube, DNA was eluted through addition of 50 µL Buffer EB (10 mM Tris·Cl, pH 8.5), water can also be used. Centrifuge for 1 minute. The sample was then sent to the Advanced Genomic Facility of Liverpool for sequencing (Margulies *et al*, 2005) (http://www.liv.ac.uk/cgr//454sequencing.html).

In Microsoft Excel, the barcode relating to each sample (Table 11.1, highlighted section of primer sequences) was removed and sequences were organised into samples within individual files. Files were imported into BioEdit (version 7.0.5) and aligned using the Greengenes NAST alignment tool (http://greengenes.lbl.gov) (DeSantis *et al*, 2006a). As sequences were < 400 base pairs (bp), performing a chimera check was not possible. Aligned sequences were classified using the Greengenes classification tool. This tool compares aligned sequences to the prokMSA, finding near-neighbours using Simrank. Divergence from near-neighbours was calculated using the DNAML option of DNADIST (PHYLIP package) (DeSantis *et al*, 2006a, b). One hundred nucleotide bases were compared in order to classify a sequence, as sequence length ranges from > 150 to 300 bp; 75% similarity between sequences was required in order to be considered a match. Classification was determined using the NCBI database. Classified sequences

were filtered, with a 90% cut-off for phylum and 97% for genus and species identification.

Alignment of sequences was repeated using CLUSTAL W (Version 2.0.12; http://www.clustal.org/); pairwise alignments were set to FAST/APROXIMATE using the parameters: gap penalty, 3; K-tupule, 1; Number of top diagonals, 5; window size, 5. Multiple alignment parameters were: gap opening penalty, 10; gap extension penalty, 0.2; delay divergent sequences, 30%; DNA transitions weight, 0.5; DNA weight matrix, IUB; and use negative matrix, off. This alignment allowed the inclusion of all sequences (maximum of 500 bp aligned through Greengenes) (Thompson *et al*, 1994). The UNIFRAC webserver (http://bmf2.colorado.edu/unifrac/index.psp) was used to analyse differences between aligned sequences within each sample. UNIFRAC calculates the distance (difference) between microbial communities based upon the sequence lineages contained within them (Lozupone *et al*, 2006; Lozuponeand Knight, 2005). Principal components analysis was carried out using the aligned sequences within UNIFRAC. The Bonferroni correction was also applied to analyse the significant differences. Figure 11.1 below provides a summary of the process from sampling to phylogenetic analysis.

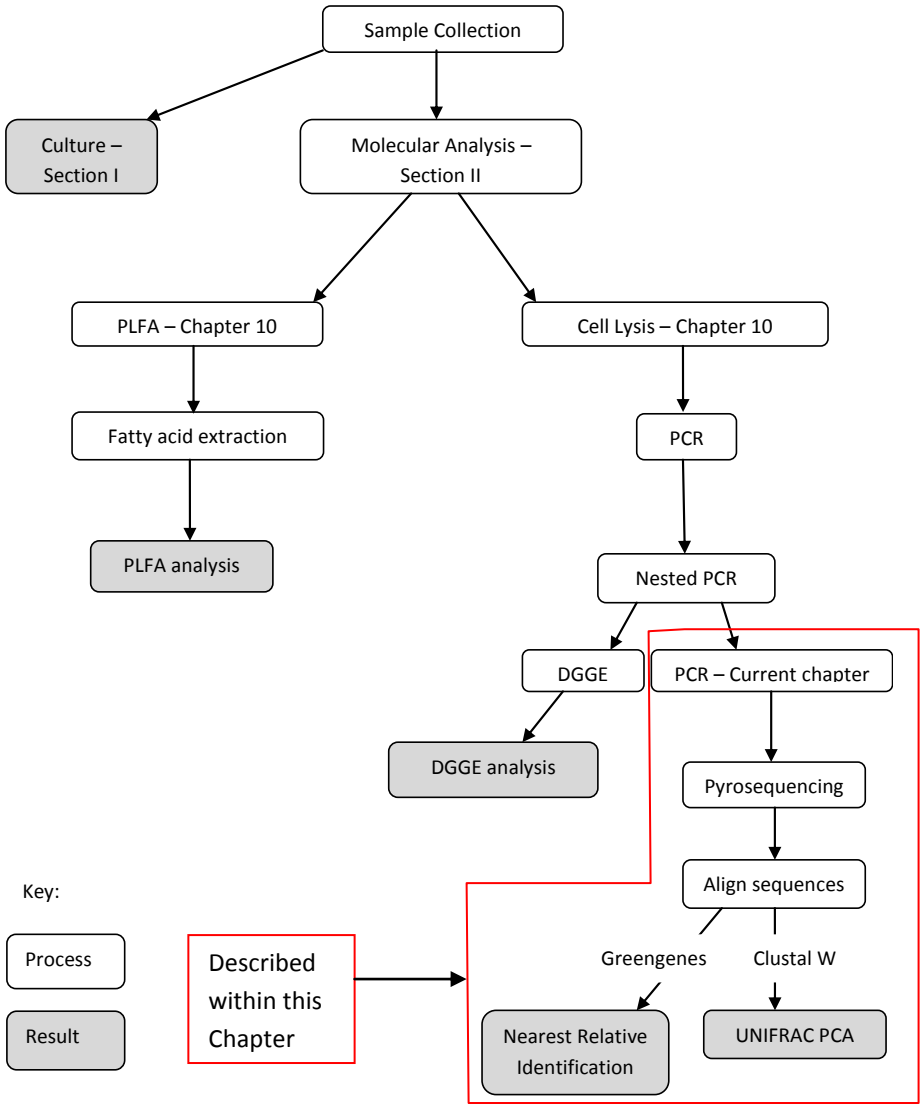



Figure 11.1: Flow diagram of analysis carried out with samples from collection to identification. Highlighted area is described within this Chapter.

## 11.3 Results

Three main groups of bacteria were observed across all samples, *Firmicutes*, *Proteobacteria* and *Actinobacteria*. However the distribution of these groups showed significant differences across the samples. Pyrosequencing revealed an abundance of sequences, covering 7 bacterial groups. Figure 11.2 below illustrates the division of these groups within samples analysed, with a 90% similarity threshold. Upwind

bacterial bioaerosols showed an even distribution between *Proteobacteria* (*Alpha, Beta* and *Gamma*), *Actinobacteria*, and *Firmicutes*, each accounting for over 30% of the sequences identified. Green waste was dominated by two groups, *Proteobacteria* (*Alpha, Beta* and *Gamma*) and *Firmicutes* accounting for 44 and 39% of the sequences respectively. The remaining 16% was assigned to *Actinobacteria, Bacteroidetes* and *Deinococcus-Thermus.* Differences were observed between samples taken on-site (Site 1 and Site 2). Bacterial diversity of site 1 was dominated by *Proteobacteria* (49%) followed by *Actinobacteria* (29%) and *Firmicutes* (20%). In contrast Site 2 was largely dominated by *Firmicutes* (76%) and the 24% remaining was comprised of *Actinobacteria*, *Proteobacteria*, *Bacteroidetes* and *Deinococcus-Thermus*. *Proteobacteria* phylotypes dominated downwind sample, accounting for 74% of sequences, followed by the *Firmicutes* (22%) and the remainder assigned to *Acidobacteria, Actinobacteria, Bacteroidetes* and *Deinococcus-Thermus*.

Table 11.2 (a, b) shows the dominant bacteria identified to genus level within each sample with a 97% similarity threshold. Upwind gram-positive bacteria were dominated by *Streptomyces* within *Actinobacteria, Geobacillus* and unclassified members of *Bacillales*, within *Firmicutes*. Gram-negative bacteria were dominated by *Hyphomicrobium*, a member of the order *Rhizobiales*. Percentage distribution of gram-positive bacteria was more even in green waste samples, although *Geobacillus* showed a similar abundance to that shown in upwind. Gram-negative bacteria were also more evenly distributed in green waste, although some dominance was shown by *Sinorhizobium*, along with *Acinetobacter* and *Pseudomonas*. Samples taken on-site showed significant differences. In site 1, gram-positive bacteria were dominated by *Arthrobacter* and unidentified Bacillus, whilst *Streptomyces* and *Geobacillus* were the two dominant bacteria in site 2. For gram-negative bacteria, site 1 sequences were largely dominated by *Pseudomonas* sp., while no overall dominance was seen at site 2. Downwind sequences were also dominated by *Pseudomonas* in gram-negative bacteria, while gram-positive bacteria were dominated by an unknown member of the *Bacillales* order (accession number AF071858).

In total, 97 unique sequences were identified with a 97% similarity threshold. These 97 unique sequences were obtained from a database of over 35,000 returned through the pyrosequencing analysis. The number of sequences returned illustrates the diversity and abundance of microorganisms within these environments. Across the samples taken, upwind contained 18 unique sequences, green waste, site 1 and site 2 samples contained similar numbers of sequences, 53, 54 and 43 respectively. Downwind bioaerosols contained 27 unique sequences. Of the 97 sequences, 5 were found across all samples. Sequences found on-site contained a mixture of those found in upwind (8) and green waste (18) samples. Of sequences found in green waste, 12 were also found in both on-site and downwind samples, although 17 further sequences were only found in green waste. Genus that were found across green waste, on-site and downwind samples are highlighted within Table 11.2 (a, b).

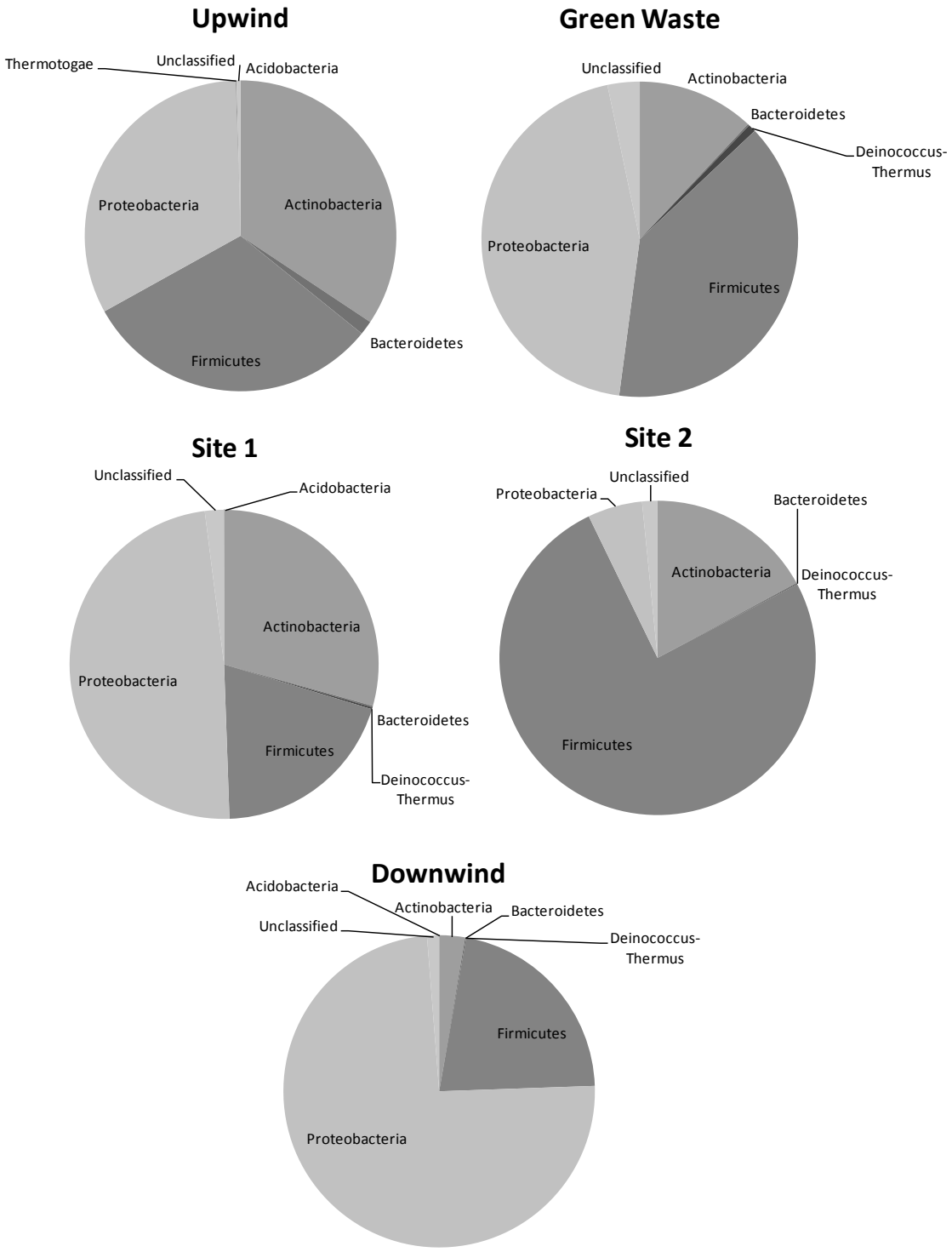
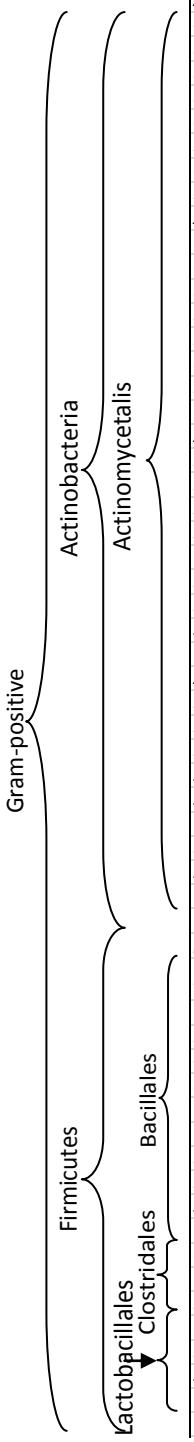



Figure 11.2: Major bacterial groups identified in samples (upwind, green waste, site 1, site 2 and downwind), 90% similarity threshold.

Table 11.2a: Genus and species identified in each sample with percentage composition of total number of unique sequences (100% across Tables 11.2 a, b), and similarity threshold. Light shaded = found across green waste and site 1 and/or 2; dark shaded = found across green waste, site 1 and/or site 2, and downwind. * = Well-described pathogen; ** = Opportunistic pathogen; *** = Potential pathogen; ¥ = Well-described plant pathogen (De León *et al*, 2009; Forbes *et al*, 2007).

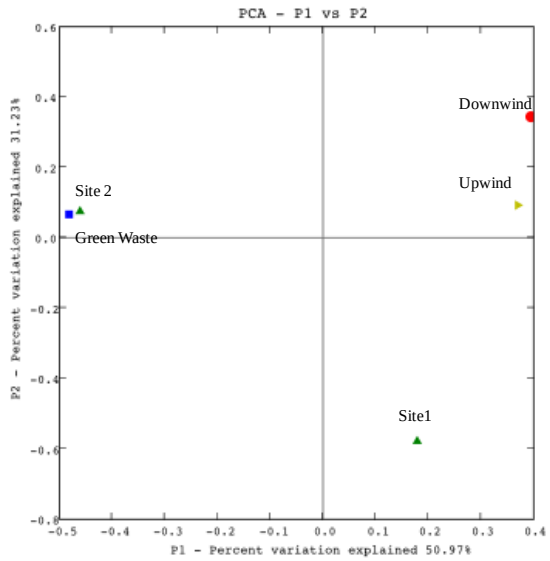



| NEAREST RELATIVE (accession number) | | | | COMMUNITY COMPOSITION (% Unique Sequences) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Genus | Species | Similarity (%) | | Upwind | Green Waste | Site 1 | Site 2 | Downwind |
| ***Actinobacteria* 778 sequences, 38 genuses** | | | **Total (%)** | 30.80 | 11.35 | 32.01 | 16.08 | 2.26 |
| *Gordonia* (AM411960) ** | | 98 | | 0.00 | 0.09 | 0.00 | 1.01 | 0.00 |
| *Mycobacterium* (EF564379)* | | 100 | | 1.52 | 0.00 | 0.12 | 0.00 | 0.00 |
| *Nocardia* (AB084445)** | | 98 | | 0.00 | 0.00 | 0.25 | 0.00 | 0.00 |
| *Rhodococcus* (AJ786666) ** | | 100 | | 0.00 | 0.35 | 1.24 | 0.07 | 0.00 |
| *Geodermatophilus* (L40621)*** | | 97 | | 0.00 | 0.00 | 0.12 | 0.00 | 0.00 |
| *Kineococcus* (EU543662.1) | | 98 | | 0.00 | 0.00 | 0.00 | 0.07 | 0.00 |
| *Cellulomonas* (FJ200382.1) | | 98 | | 4.18 | 0.00 | 0.12 | 0.27 | 0.00 |
| *Brachybacterium* (AJ415379.1) | | 99 | | 0.00 | 0.18 | 0.50 | 0.47 | 0.13 |
| *Janibacter* (AY522568.1) ** | | 100 | | 0.00 | 1.41 | 0.12 | 0.00 | 0.00 |
| *Tetrasphaera* (AF409018.1) | | 98 | | 0.00 | 0.00 | 0.12 | 0.00 | 0.00 |
| *Clavibacter* (D45051.1) ¥ | | 99 | | 0.00 | 0.18 | 0.25 | 0.00 | 0.00 |
| *Leucobacter* (AJ746337.1) | | 99 | | 0.00 | 0.00 | 0.37 | 0.00 | 0.00 |
| *Microbacterium* (AY785738.1) | | 100 | | 4.18 | 1.50 | 2.23 | 0.27 | 0.03 |
| *Microcella* (AJ717387.1) | | 98 | | 0.00 | 0.00 | 0.25 | 0.00 | 0.00 |
| *Okibacterium* (AB042097.1) | | 98 | | 0.00 | 0.00 | 0.00 | 0.07 | 0.03 |
| *Plantibacter* (DQ339591) | | 99 | | 0.00 | 0.00 | 0.12 | 0.00 | 0.00 |
| Unclassified *Microbacteriaceae* (DQ177485) | | 98 | | 0.00 | 0.00 | 0.00 | 0.07 | 0.00 |
| *Arthrobacter* (AY651317) | | 100 | | 1.90 | 5.45 | 22.33 | 0.41 | 0.03 |
| *Kocuria* (Y16264) *** | | 100 | | 1.52 | 0.00 | 0.50 | 0.00 | 0.00 |
| *Liuella* (DQ372937) | | 99 | | 0.00 | 0.00 | 0.12 | 0.00 | 0.00 |
| *Micrococcus* (EU660215) ** | | 99 | | 0.00 | 0.00 | 0.12 | 0.00 | 0.00 |
| *Rothia* (AJ131121) *** | | 99 | | 0.76 | 0.00 | 0.12 | 0.00 | 0.00 |
| *Cellulosimicrobium* (AB116667) ** | | 100 | | 0.00 | 0.00 | 0.12 | 0.00 | 0.00 |
| *Isoptericola* (EU249579) | | 97 | | 0.00 | 0.00 | 0.00 | 0.07 | 0.00 |
| *Promicromonospora* (EU274374) | | 100 | | 0.00 | 0.53 | 0.00 | 0.00 | 0.00 |
| *Sanguibacter* (DQ339590) | | 100 | | 1.14 | 0.00 | 0.25 | 0.00 | 0.00 |
| Unclassified *Micrococcineae* (AY289118) | | 98 | | 0.00 | 0.09 | 0.00 | 0.00 | 0.00 |
| *Actinoplanes* (AB048219) | | 98 | | 0.00 | 0.09 | 0.00 | 0.00 | 0.00 |
| *Nocardioides* (DQ673618) ** | | 98 | | 0.00 | 0.00 | 0.37 | 0.41 | 0.03 |
| *Pimelobacter* (X53213) | | 98 | | 0.00 | 0.00 | 0.12 | 0.00 | 0.00 |
| *Propionibacterium* (AJ704571) *** | | 99 | | 0.76 | 0.00 | 0.00 | 0.07 | 0.00 |
| *Streptacidiphilus* (AB180766) | | 99 | | 0.00 | 0.00 | 0.12 | 0.00 | 0.00 |
| *Streptomyces* (X95968) *** | | 100 | | 14.45 | 1.23 | 1.74 | 12.16 | 1.98 |
| Unclassified *Streptosporangiceae* (AY464542) | | 98 | | 0.00 | 0.00 | 0.00 | 0.07 | 0.03 |
| *Nonomuraea* (AJ582011) | | 100 | | 0.00 | 0.26 | 0.12 | 0.41 | 0.00 |
| *Streptosporangium* (X89945) | | 98 | | 0.00 | 0.00 | 0.12 | 0.00 | 0.00 |
| Unclassified *Actinomycetalis* (EU368819) | | 98 | | 0.00 | 0.00 | 0.00 | 0.07 | 0.00 |
| Unclassified *Actinobacteria* (EF612290) | | 99 | | 0.38 | 0.00 | 0.00 | 0.14 | 0.00 |
| ***Firmicutes* 2401 sequences, 19 genuses** | | | **Total (%)** | 55.51 | 35.44 | 21.34 | 77.16 | 16.92 |
| *Bacillus* | *Bacillus cereus group* (EU240386)** | 97 | | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 |
| Unclassified *Bacillus* (AF071858) | | 100 | | 26.24 | 14.69 | 4.84 | 36.96 | 10.47 |
| *Exiguobacterium* (AM072763) | | 98 | | 0.00 | 0.00 | 0.12 | 0.00 | 0.00 |
| *Geobacillus* (AY608961) | | 100 | | 17.11 | 17.33 | 3.35 | 36.35 | 5.57 |
| *Oceanobacillus* (AB275883) | | 97 | | 0.00 | 0.00 | 0.00 | 0.07 | 0.00 |
| Unclassified *Bacillaceae* (AY960768) | | 98 | | 0.00 | 0.18 | 0.00 | 0.00 | 0.00 |
| *Ureibacillus* (AB176540) | | 100 | | 0.00 | 0.35 | 0.25 | 2.36 | 0.38 |
| *Brevibacillus* (AY372923)** | | 98 | | 0.00 | 0.18 | 0.12 | 0.00 | 0.00 |
| *Paenibacillus* (DQ522106)** | | 98 | | 0.00 | 0.79 | 0.00 | 0.68 | 0.25 |
| *Sporosarcina* (AJ514408)*** | | 100 | | 0.00 | 0.53 | 0.12 | 0.00 | 0.03 |
| Unclassified *Staphylococcus* (AY918868) | | 100 | | 11.79 | 0.00 | 12.41 | 0.61 | 0.13 |
| *Clostridium* (Y18184)* | | 98 | | 0.00 | 0.00 | 0.00 | 0.07 | 0.00 |
| *Tepidimicrobium* (AB332033) | | 98 | | 0.00 | 0.18 | 0.00 | 0.00 | 0.00 |
| *Eubacterium* (AF044945)** | | 97 | | 0.00 | 0.44 | 0.00 | 0.07 | 0.03 |
| *Carnobacterium* (M58816) | | 100 | | 0.38 | 0.62 | 0.00 | 0.00 | 0.00 |
| *Enterococcus* (AJ276462)* | | 97 | | 0.00 | 0.09 | 0.00 | 0.00 | 0.00 |
| *Streptococcus* (X78825)* | | 98 | | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 |
| Unclassified *Weissella* (AM157446) | | 98 | | 0.00 | 0.00 | 0.12 | 0.00 | 0.00 |
| Unclassified *Firmicutes* (AB116133) | | 98 | | 0.00 | 0.09 | 0.00 | 0.00 | 0.00 |

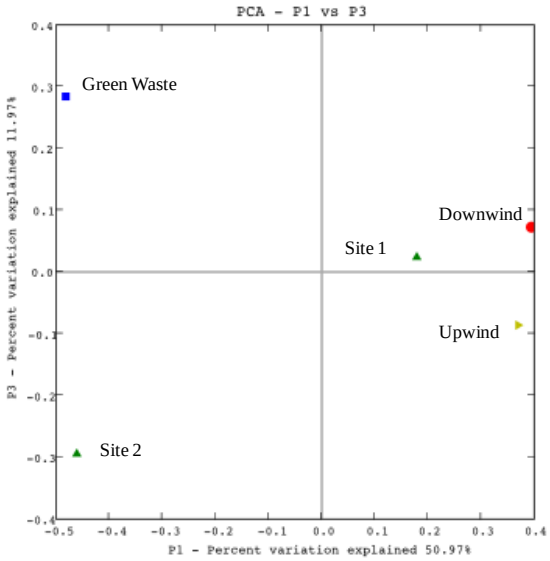
Table 11.2b: Genus and species identified in each sample with percentage composition of total number of unique sequences (100% across Tables 11.2 a, b), and similarity threshold. Light shaded = found across green waste and site 1 and/or 2; dark shaded = found across green waste, site 1 and/or site 2, and downwind. * = Well-described pathogen; ** = Opportunistic pathogen; *** = Potential pathogen; ¥ = Well-described plant pathogen (De León *et al*, 2009; Forbes *et al*, 2007).


| NEAREST RELATIVE | | | | COMMUNITY COMPOSITION (% Unique Sequences) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Genus | Species | | | Upwind | Green | Site 1 | Site 2 | Downwind |
| ***Deinococcus-Thermus* 16 sequences, 1 genus** | | | **Total (%)** | 0.00 | 1.06 | 0.37 | 0.07 | 0.00 |
| *Thermus* (AE017221) | | 100 | | 0.00 | 1.06 | 0.37 | 0.07 | 0.00 |
| ***Alpha-proteobacteria* 329 sequences,26 genuses** | | | **Total (%)** | 13.31 | 13.90 | 5.83 | 5.00 | 0.47 |
| *Brevundimonas* (AF296678) | | 100 | | 0.00 | 0.09 | 0.00 | 0.07 | 0.00 |
| *Bartonella* (AF143446)* | | 98 | | 0.00 | 0.18 | 0.00 | 0.00 | 0.00 |
| *Afipia* (U87770)*** | | 100 | | 0.76 | 0.00 | 0.00 | 0.00 | 0.03 |
| *Nitrobacter* (L35502) | | 97 | | 0.38 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Brucella* (AY513518)* | | 99 | | 0.00 | 0.44 | 0.25 | 0.00 | 0.00 |
| *Ochrobactrum* (AY040351)** | | 98 | | 0.00 | 0.44 | 0.00 | 0.00 | 0.00 |
| Unclassified *Brucellaceae* (AY994315) | | 98 | | 0.00 | 0.09 | 0.00 | 0.00 | 0.00 |
| *Devosia* (AJ548825) | | 99 | | 0.00 | 0.09 | 0.25 | 0.61 | 0.03 |
| *Hyphomicrobium* (AF279789) | | 100 | | 12.17 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Pseudodevosia* (EF012357) | | 100 | | 0.00 | 0.00 | 0.37 | 0.88 | 0.00 |
| *Aminobacter* (AJ011759) | | 98 | | 0.00 | 0.09 | 0.00 | 0.00 | 0.00 |
| *Mesorhizobium* (EU999235) | | 100 | | 0.00 | 0.09 | 0.50 | 0.00 | 0.00 |
| *Nitratireductor* (AB257592) | | 97 | | 0.00 | 0.00 | 0.12 | 0.00 | 0.00 |
| *Phyllobacterium* (AY512821) | | 99 | | 0.00 | 0.26 | 0.12 | 0.00 | 0.03 |
| *Pseudaminobacter* (AJ294416) | | 99 | | 0.00 | 0.09 | 0.00 | 0.20 | 0.00 |
| *Agrobacterium* (D13943)**¥ | | 100 | | 0.00 | 2.99 | 0.37 | 1.49 | 0.00 |
| *Rhizobium/Agrobacterium group* | *Agrobacterium* (EU295450) **¥ | 98 | | 0.00 | 0.00 | 0.00 | 0.07 | 0.00 |
| | *Rhizobium* (AM085997)* | 99 | | 0.00 | 0.35 | 0.12 | 0.00 | 0.00 |
| *Rhizobium* (Y17047)** | | 100 | | 0.00 | 0.09 | 0.87 | 0.41 | 0.16 |
| *Sinorhizobium* (AY505134) | | 100 | | 0.00 | 8.09 | 0.74 | 0.14 | 0.00 |
| *Ketogulonicigenium* (AF136846) | | 100 | | 0.00 | 0.18 | 0.00 | 0.20 | 0.00 |
| *Paracoccus* (D32243) | | 100 | | 0.00 | 0.00 | 0.74 | 0.00 | 0.13 |
| Unclassified *Rhodobacteraceae* (AM403163) | | 98 | | 0.00 | 0.09 | 0.12 | 0.00 | 0.00 |
| *Sphingomonas* (AY026948)* | | 100 | | 0.00 | 0.18 | 0.74 | 0.41 | 0.06 |
| *Sphingopyxis* (D13723) | | 100 | | 0.00 | 0.09 | 0.37 | 0.47 | 0.03 |
| Unclassified *Alpha-proteobacteria* (DQ985036) | | 100 | | 0.00 | 0.00 | 0.12 | 0.07 | 0.00 |
| ***Beta-proteobacteria* 2 sequences, 2 genuses** | | | **Total (%)** | 0.38 | 0.09 | 0.00 | 0.00 | 0.00 |
| *Nitrosomonas* (AB079053) | | 98 | | 0.38 | 0.00 | 0.00 | 0.00 | 0.00 |
| Unclassified *Beta-proteobacteria* (AY049943) | | 98 | | 0.00 | 0.09 | 0.00 | 0.00 | 0.00 |
| ***Gamma-proteobacteria* 3267 sequences, 9 genuses** | | | **Total (%)** | 0.00 | 35.53 | 39.83 | 0.41 | 79.77 |
| *Citrobacter* (AF025372)** | | 100 | | 0.00 | 0.18 | 0.00 | 0.00 | 0.00 |
| *Enterobacter* | *Enterobacter cloacae complex* (AY787819)* | 97 | | 0.00 | 0.35 | 0.00 | 0.00 | 0.00 |
| *Enterobacter* (AB114268)* | | 100 | | 0.00 | 2.46 | 0.12 | 0.00 | 0.00 |
| *Klebsiella* (AY291290)** | | 100 | | 0.00 | 0.35 | 0.00 | 0.00 | 0.00 |
| *Pantoea* (EF602554)** | | 98 | | 0.00 | 0.09 | 0.00 | 0.00 | 0.00 |
| Unclassified *Enterobacteriaceae* (AY374105) | | 100 | | 0.00 | 0.09 | 0.00 | 0.00 | 0.00 |
| *Acinetobacter* (AM423152)* | | 100 | | 0.00 | 17.41 | 0.37 | 0.20 | 0.06 |
| *Psychrobacter (* AM410897)*** | | 98 | | 0.00 | 0.00 | 0.00 | 0.14 | 0.00 |
| *Pseudomonas* (AM411994)* | | 100 | | 0.00 | 14.60 | 39.33 | 0.07 | 79.71 |
| **Unclassified *Proteobacteria* 2 sequences, 1 phylotype** | | | **Total (%)** | 0.00 | 0.00 | 0.00 | 0.07 | 0.03 |
| Unclassified *Proteobacteria* (EU250939) | | 99 | | 0.00 | 0.00 | 0.00 | 0.07 | 0.03 |
| **Unclassified Bacterium 70 sequences, 1 phylotype** | | | **Total (%)** | 0.00 | 2.64 | 0.62 | 1.22 | 0.53 |
| Unclassified Bacterium (AB208731) | | 100 | | 0.00 | 2.64 | 0.62 | 1.22 | 0.53 |
| **TOTAL SEQUENCES** | | | | 18 | 53 | 54 | 43 | 27 |

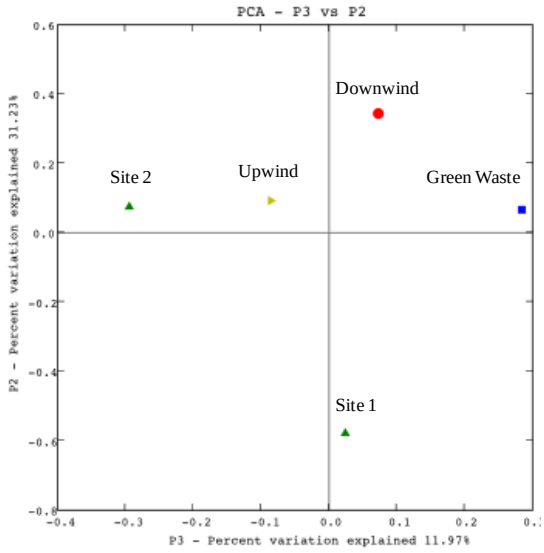
Figure 11.3 below shows results from the UNIFRAC principal components analysis. Three principal components were found and compared. Principal component (PC) 1 versus PC 2 accounts for over 80% of the variation seen within the dataset. Site 2 and green waste samples are shown to be similar, with site 1 isolated on PC 2, but close to upwind and downwind samples on PC 2. Upwind and downwind samples show some similarity on both PC analyses. PC1 versus PC3 accounts for approximately 63% of the variation found. Once again, site 1, downwind and upwind samples are shown to be spatially close, showing similarities were found between sequences at these locations. Although close on PC 1, PC 3 shows little relationship between site 2 and green waste. PC 3 versus PC 2 accounts for approximately 43% of the variation found. In this graph, site 2, upwind and green waste are similar on PC 2, but show little relationship on PC 3, while site 1 and downwind are similar on PC 3, but show little relationship on PC 2. Bonferroni's correction results suggested no significant difference between each sample (p-values > 0.05), suggesting the differences seen in the PCA are due to variances in abundance within bacterial groups, rather that diversity.




(a)




(b)




(c)

Figure 11.3: Principal Component's (PC) analysis comparing sequences obtained from upwind, green waste, site 1, site 2 and downwind. Graph (a) = PC 1 vs. PC 2; Graph (b) = PC 1 vs. PC 3; Graph (c) = PC 3 vs. PC 2.

## 11.4 Discussion

Analysis of the structure of bacterial groups within green waste and bioaerosol communities showed differences depending on sampling days (Figure 11.2). For example, upwind and site 2 samples were taken on the same day, with the change in dominance of communities to 76% *Firmicutes* highlighting clearly the impact of green waste microorganisms, as *Firmicutes* were found at higher abundance within green waste than upwind air. The thermophilic bacteria belonging to *Geobacillu*s was identified as largely responsible for this increase. The differences in structure of site 1 and site 2 bioaerosol communities suggests, as previously shown in Chapter 10, that specific site parameters, such as activity and meteorological conditions, may affect the communities of bioaerosols emitted on a day-to-day basis. Across all samples, the contribution of compost bioaerosols to ambient bioaerosols resulted in a decrease of *Actinobacteria* with a concurrent increase of *Proteobacteria* and *Firmicutes* phylotypes. *Firmicutes* increase was also observed by Le Goff *et al.* (2009), and it was suggested to be due to the sporulating nature of these bacteria. However, here *Proteobacteria* was the other most dominant bacterial phylotype, rather than *Actinobacteria*.

Identification of bacteria responsible for the changes seen in community composition and structure is shown in Table 11.2 (a, b). The diversity of species found increased from 18 unique sequences in upwind samples, to 54 and 43 unique sequences in site 1 and 2 samples. Of these additional unique sequences, 18 could also be found in green waste, suggesting not all bioaerosols originated from the composting facility. Identification of these bacteria showed 5 belonged to the *Actinobacteria* phylotype, one to *Firmicutes*, one to *Deinococcus-Thermus*, 10 to *Alpha-proteobacteria*, and one to *Gamma-proteobacteria.* This suggests that *Alpha-proteobacteria* contributed the most to community change through contribution of green waste microorganisms to bioaerosols. However, each of the 18 genuses identified contributed less than 1% to the overall site 1 and site 2 bacterial bioaerosol communities, suggesting an increase in diversity rather than dominance through the addition of these unique sequences. In addition, not all sequences identified in green waste were also found in on-site samples, with 17 unique sequences found in green waste, showing that a sizable proportion,

approximately one third, of composting microorganisms were not detectable in bioaerosols.

Thirteen sequences belonging to *Actinobacteria* (*Brachybacterium*), *Firmicutes* (*Ureibacillus, Paenbacillus, Sporosarcina, Eubacterium*), *Alpha-proteobacteria* (*Devosia, Phyllobacterium, Rhizobium, Sphingomonas, Sphingopyxis*), and *Gamma-proteobacteria* (*Acinetobacter, Pseudomonas*) were present in green waste, on-site and downwind samples. This finding, suggests that they could constitute good indicators of compost aerosols emission. The remaining sequence was an unclassified bacterium (accession number AB208731). These results are significant, suggesting that both *Firmicutes* and *Alpha-proteobacteria* are capable of emission from green waste and downwind dispersal. In particular, of the 5 *Firmicutes* genus's found in green waste and on-site, 4 were also found in downwind bioaerosols (*Ureibacillus, Paenbacillus, Sporosarcina, and Eubacterium*). This supports findings from Le Goff *et al*. (2009) who suggest that *Firmicutes* would be capable of dispersal downwind from composting facilities. Although, downwind bioaerosol was dominated by the genus *Pseudomonas*, suggesting that this genus may be capable of downwind dispersal in elevated abundances. The genus described above may all have the potential for use as indicators of the composting process. However, as highlighted recently by Le Goff *et al.* (2009), in order to become a successful indicator species the natural occurrence of the genus or species within the environment and as a bioaerosol must be well qualified and quantified. Given the current paucity of data surrounding the bacterial communities of bioaerosols, an indicator cannot therefore be currently recommended (Le Goff *et al*, 2009). As well as contribution from green waste composting microorganisms, on-site and downwind samples were shown to contain sequences also identified in upwind samples. For example, 8 sequences found on-site were also found upwind and 5 sequences were found across all samples taken.

The data found suggests that both *Firmicutes* and *Proteobacteria* are preferentially emitted from composting facilities, with both phylotypes able to be transported downwind. There may be two reasons for the increase in emission of these phylotypes. As suggested by Le Goff *et al.* (2009), *Firmicutes* spores may be emitted more readily

than other bacterial cells. However, this does not provide an explanation of the preferential emission of *Proteobacteria*. It may also be due to dominance in green waste, with both of these phylotypes present in equal abundance within green waste (Figure 11.2). However, *Actinobacteria* were also found in the same abundance in green waste. It could be anticipated that *Actinobacteria* would also be found in high abundances in on-site and downwind samples, due to their spore-forming nature and evidence from Le Goff *et al*. (2009). In this case, however, *Actinobacteria* were found in highest abundance in upwind samples. Although this result would not be anticipated following Le Goff *et al.* (2009), culture-based analysis data presented within Section I of this thesis supports the results found here as actinomycetes were recovered in high concentrations at all sampling locations, including upwind (Chapters 4, 5, 6). Regarding the preferential emission of *Firmicutes* and *Proteobacteria*¸ and the downwind dispersal of *Pseudomonas*, a combination of dominance within green waste and ease of aerosolisation may result in the dominance observed on-site and downwind. However, further investigation on the aerosolisation of microorganisms from composting processes is needed in order to fully support this hypothesis.

Many of the sequences found have also been identified in previous studies on compost. *Lactobacillus*, *Enterobacteria* and *Pseudomonas* have been identified in fresh green waste (Alfreider *et al*, 2002), while sequences assigned to the *Deinococcus-Thermus* group have been found in the maturation phase of composting (Alfreider *et al*, 2002). *Rhodococcus*, a member of the *Actinobacteria* has been found in fresh green waste (Steger *et al*, 2007), although *Streptomyces*, also found in fresh green waste (Steger *et al*, 2007), was found to be a dominant community member in upwind bioaerosols here. *Streptosporangium*, identified at the hottest composting phases by Steger *et al.* (2007) was also found here in on-site bioaerosols.

A number of the genus's identified in this study were also identified through API testing, described in Chapter 8. These include *Acinetobacter, Brevundimonas, Klebsiella, Pseudomonas* and *Sphingomonas*. None of these genus's were found in upwind samples through pyrosequencing, being identified from green waste and on-site samples. Both results demonstrate the ability of API test to identify successfully

bioaerosols emitted through composting. However, API was not able to identify the diversity shown through pyrosequencing. This is likely due to the limitations of culture-based sampling, which has been shown to allow only a small fraction of microbial diversity to be enumerated (Giovannoni *et al*, 1990; Head *et al*, 1998; Ward *et al*, 1990).

Of the 97 identified unique sequences, 39 of these have been found to be pathogenic. Ten of the genus's, along with the species *Enterobacter cloacae* complex, were identified as well known human pathogens. Of particular note is the genus *Mycobacterium*, within which species responsible for tuberculosis may be found (Forbes *et al*, 2007). *Clostridium* is another well known pathogenic genus, containing the species *C. botulinum*, responsible for botulism (Forbes *et al*, 2007). A further 18 sequences were identified as potential opportunistic pathogens, with 8 sequences also identified as possible human pathogens, with pathogenicity poorly defined (Forbes *et al*, 2007). A number of the genus identified as pathogenic through API were also identified here, with their culturability indicative of potential pathogenicity (*Acinetobacter, Klebsiella, Pseudomonas* and *Sphingomonas).* Also of note was the identification of 2 plant pathogens. In particular, the genus *Clavibacter* contains species that are quarantined through European-wide legislation, as major pathogens of tomato crops, with infection resulting in major economic losses (De León *et al*, 2009). Of these identified potential pathogens the genus's *Nocardioides, Paenbacillus*, *Sporosarcina, Eubacterium, Rhizobium, Sphingomonas, Acinetobacter* and *Pseudomonas* were identified in downwind samples, but not in upwind samples. In addition, all genuses apart from *Nocardioides* were able to be traced directly to green waste. These findings are significant, not only suggesting that potentially pathogenic species may be emitted through composting, but that they may also be able to disperse from site. However, it must also be acknowledged that 6 of these potential pathogens were also found in upwind bioaerosols. In addition, as dose-response relationships for bioaerosols are not established, whether any health risk is posed remains unknown.

Within all PCA figures (Figure 11.3), little similarity is shown between communities, suggesting there are differences in abundance of bacterial groups within each sample. However, PC1 versus PC2, accounting for the most variability within data (80%),

showed site 2 and green waste samples grouped together, indicating a similar community structure and abundance. This observation is unclear, as these samples were taken on different days, with most samples taken on different days then showing differences in community composition and structure. In order to refine and enhance these results, it would be necessary to take more samples for pyrosequencing. Here, although a large number of sequences were obtained, the number of samples analysed was too small to allow significance of the differences between community structures to be determined. This was illustrated by Bonferroni's correction, which resulted in no significant difference being found between each sample.

One of the main limitations of the methods used here was the amount of sequences recovered. Most available programs for sequence processing and analysis are only able to analyse up to 500 sequences per run (e.g. Greengenes, UNIFRAC), as a result analysis is time-consuming and sub-optimal. For example, the amount of sequences recovered through this study prevented further analysis of the results, such as through a phylogenetic tree. Another limitation is the potential bias, and other sources of error within PCR amplification, leading to inaccurate assessment of abundance (Felske *et al*, 1998; Head *et al*, 1998; Hugenholtz and Goebel, 2001; Suzuki and Giovannoni, 1996). These PCR limitations were discussed in full in Chapter 10. For this reason, analysis of abundances identified through pyrosequencing is restricted within this study. Despite these limitations, this study has illustrated how pyrosequencing may be used with success to analyse the composition of bacterial bioaerosol communities.

## 11.5 Conclusions

The results from this study can lead to several conclusions.

- Composting microorganisms make a significant contribution to the composition of bioaerosols
- Bacterial diversity was largely dominated by *Firmicutes, Proteobacteria* and *Actinobacteria*

- Dominant bacteria were represented by the genus *Streptomyces, Geobacillus, Acinetobacter* and *Pseudomonas*
- Potential indicators of aerosol emitted were represented by 13 phylotypes
- The influence of composting microorganisms declines further downwind
- *Firmicutes* and *Pseudomonas (Proteobacteria)* show the best ability to disperse downwind
- 39 potential pathogens were identified, with 7 of these found downwind from site and traced directly to the composting bacterial community

The limitations of this study, particularly the sample size (rather than the number of sequences obtained), have prevented the significance of differences between community compositions being determined. The study has, however, provided evidence that composting leads to a significant alteration in the composition of bioaerosols that may be traced downwind. In addition, it has been shown that potentially pathogenic microorganisms, with many unable to be identified through culture-based analysis, are able to be emitted through composting and disperse off-site. For future studies, several areas of research merit further investigation. These include the preferential emission of *Proteobacteria* and *Firmicutes*, the influence of the feedstock, composting conditions, and composting processing activities on their dominance and aerosolisation; and the ability of individual bioaerosols, particularly potential pathogens, to disperse downwind. This study highlighted the importance of understanding the diversity of bioaerosols in order to improve knowledge of sensitive receptor exposure. Comparison to API identified gram-negative bacteria demonstrated the inability of culture-based methods to account for this diversity. In addition, the preferential dispersal of *Firmicutes* and *Pseudomonas* suggests sensitive receptors may be exposed to particular fractions of bioaerosols that may be traceable to source. These methods may therefore be potentially of use in the future development of source apportionment, reinforcing the hypothesis that bioaerosol community analysis at receptor may be traced to source through identification of community components.

# 12 Summary and Conclusions

## 12.1 Project Background and Drivers

The rapid growth of the composing industry is anticipated to continue as more sustainable options for the disposal of biodegradable waste are sought. This prediction has increased the urgency with which understanding of the ability of composting to emit bioaerosols and potentially result in receptor exposure is required. Despite several decades of research into the emission and dispersal of bioaerosols from commercial composting, a reliable data-set quantifying the concentrations of bioaerosols on-site and downwind had not been established. This is due to many factors. One major influence is the reliance on sampling through direct impaction and culture-based analysis of bioaerosols. The inability to sample for long periods of time, particularly close to source, using this method has created a 'snapshot' view of bioaerosol concentrations. The labour intensive nature of this method is also prohibitive to sampling strategies incorporating repeat sampling in order to create statistically valid data-sets. As a result, bioaerosol sampling has often been 'snapshot' in two ways, both through sampling times on-site, and amount of sampling occasions. As shown by more recent work, this sampling approach is insufficient, as bioaerosol emissions are highly variable, leading to a large range in concentrations that can be found on-site. 'Snapshot' sampling can therefore lead to both under- and over-estimation of concentrations. In addition, while sampling has focused on direct impaction due to recommendations from the Composting Association, now the Association for Organics Recycling (AFOR) (The Composting Association, 1999), many alternative methods are also available. The variety of methods used within bioaerosol sampling strategies has reduced comparability between studies, also preventing the compilation of a validated data-set. Despite these limitations, it was determined that enough evidence existed to impose a 250 m risk assessment trigger limit in 2001 by the Environment Agency (Environment Agency, 2009b; Environment Agency, 2001a; Environment Agency, 2001b).

The position of the Environment Agency is that there will be a "presumption against permitting...of any new composting process...where the boundary of the facility is within 250 m of a workplace or boundary of a dwelling...". However, if through "independent scientific evidence" bioaerosols can be shown to reach "appropriate levels" at the dwelling or workplace, an exemption to the proposed facility or modification to an existing facility, may be permitted. In this case, acceptable levels are suggested as 1000 CFU $m^{-3}$ for both total bacteria and total fungi, and 300 CFU $m^{-3}$ for gram-negative bacteria (Environment Agency, 2001a). Although there is a body of scientific evidence to support these limits, as shown above, many of these studies may have underestimated concentrations due to sampling and methodological limitations. In addition, several recent studies have suggested that these limits are inappropriate, with concentrations found above the acceptable levels beyond 250 m from site (Fischer *et al*, 2008; Herr *et al*, 2003a; Recer *et al*, 2001). The inconsistency between measurements of bioaerosol concentrations downwind from composting facilities has led several authors to suggest that more evidence is required in order to support, or support a change, to the 250 m risk assessment limit (Harrison, 2007; Swan *et al*, 2003; Sykes *et al*, 2007). This uncertainty and the continued development of research into bioaerosols has led to recent changes in the regulatory guidance surrounding composting facilities, including the introduction of filtration based sampling as a standard method (Association for Organics Recycling, 2009; Environment Agency, 2009a), and the proposed introduction of a 500 CFU $m^{-3}$ *A. fumigatus* reference level (Dennis, 2009).

From the human health perspective, knowledge of the concentrations of bioaerosols that people may be exposed to is vital. While dose-response levels for bioaerosols do not exist, the first step in developing such data is through establishment of the concentrations of bioaerosols downwind from composting facilities; particularly at locations where people may be exposed. For example, at the nearest sensitive receptor, or beyond 250 m from composting facilities. Furthermore, knowledge of the composition of these bioaerosols will be vital in the determination of potential receptor dose. While previously it had been shown that endotoxins can be emitted in elevated concentrations through composting, the ability of these endotoxins to disperse downwind and contribute to receptor exposure has thus far been unknown. This may be

due to the focus of sampling methods on culture-based techniques. It is well understood that different gram-negative bacteria species contain endotoxins with variations in structure. These variations lead to individual types of endotoxin having different levels of toxicity. Knowledge surrounding the composition of gram-negative bacteria present throughout composting and within bioaerosols is limited. As a result, the potential health impact of composting endotoxins remains poorly understood. The focus on culture-based analysis of bioaerosols with known health impacts (*Aspergillus fumigatus*, actinomycetes and gram-negative bacteria) has also limited understanding of the communities of bioaerosols that can be emitted through composting. Although culture-based analysis can identify bacteria and fungi, through culture identification understanding of true abundance and diversity of these microorganisms is limited. It is well understood within microbial ecology that culture-based analysis may only be able to account for 1% of bacteria, and 0.3 – 55% of fungi (Cheng 2008a, b; Heidelberg *et al*, 1997; Lappalainen *et al*, 1996; Lee *et al*, 2006). Understanding of bioaerosol composition and concentrations, including both non-culturable and non-viable fractions would allow improved knowledge of sensitive receptor exposure and key composting bioaerosols that may contribute to sensitive receptor exposure, leading to an improved understanding of exposure, 'dose' and health impacts, and the future development of dose-response relationships.

In order to fully understand bioaerosols, including both the non-culturable and non-viable fractions, it may therefore be necessary to employ non-culture based methods, such as those based on molecular analysis and direct counting. The use of molecular analysis would contribute to understanding of receptor exposure, as the bioaerosol community can be characterised. Information of this type would allow the development of source apportionment capabilities, as well as identifying any bioaerosols that may pose a health risk. This characterisation would also represent a significant advance in understanding of bioaerosols, particularly the contribution of composting, and other sources of bioaerosols, to ambient bioaerosols. While there is evidence within the literature that these molecular and direct counting methods may be used with success to qualify and quantify bioaerosols, they remain little used.

In view of the above outline, this project aimed to fill two major gaps in the available literature; focusing on the quantification of bioaerosols on-site and at distance downwind and the characterisation of bioaerosol composition and communities. The two aims of this project were therefore to:

1. Provide a reliable, validated data-set describing the emission and dispersal of bioaerosols from composting facilities
2. Provide 'proof of concept' data describing the composition and community structure of bioaerosols utilising non-culture based methods

In order to achieve these aims the following thesis Objectives were implemented:

i. Quantitative characterisation of bioaerosols emitted through composting activities and downwind concentrations up to and beyond 250 m from site at chosen case-study sites
    a. Assessment of the impact that episodic emission has on downwind concentrations
    b. Assessment of the relationships between bioaerosols and environmental conditions
ii. Characterisation of bioaerosol composition and communities including viable, non-viable and non-culturable components

The achievement of these Objectives has provided knowledge of bioaerosols previously not present within the literature. However, through their achievement the limitations of this study, as well numerous areas for future research, have been revealed.

## 12.2 Conclusions

### 12.2.1 Thesis section I

Thesis Objective i was achieved through Chapters 4 – 6. Chapter 4 described the emission and dispersal patterns, and downwind concentrations, of *A. fumigatus*, actinomycetes, gram-negative bacteria, and endotoxins. The first achievement of this section, and one of the most informative, was the measurement of endotoxin emission and dispersal. Prior to this study, the ability of endotoxins to disperse from composting facilities had not been investigated. The focus of this project on only two sites, studied in great detail, allowed the creation of a statistically valid data-set describing the emission and downwind concentrations of these bioaerosols. While other studies that investigate both the emission and dispersal of bioaerosols exist, these have focused either on site concentrations or concentrations at point of potential exposure, such as within residential areas (Albrecht *et al*, 2008; Fischer *et al*, 2008; Herr *et al*, 2003a; Recer *et al*, 2001; Schlosser *et al*, 2009).

**The Dispersal of Bioaerosols from Composting Facilities**

The focus of this study on emissions and near-site to at distance concentrations has revealed a dispersal pattern for bioaerosols that has not been previously reported in the literature. Namely, evidence suggests that bioaerosols decrease rapidly from site, with an apparent secondary peak 100 – 200 m from site. Although it cannot be confirmed due to a lack of air temperature measurements of the compost emission, it is likely that this pattern is due to buoyancy. This dispersal pattern, alongside dispersal pattern that would be anticipated given a Gaussian plume, is shown in Figure 12.1 below.

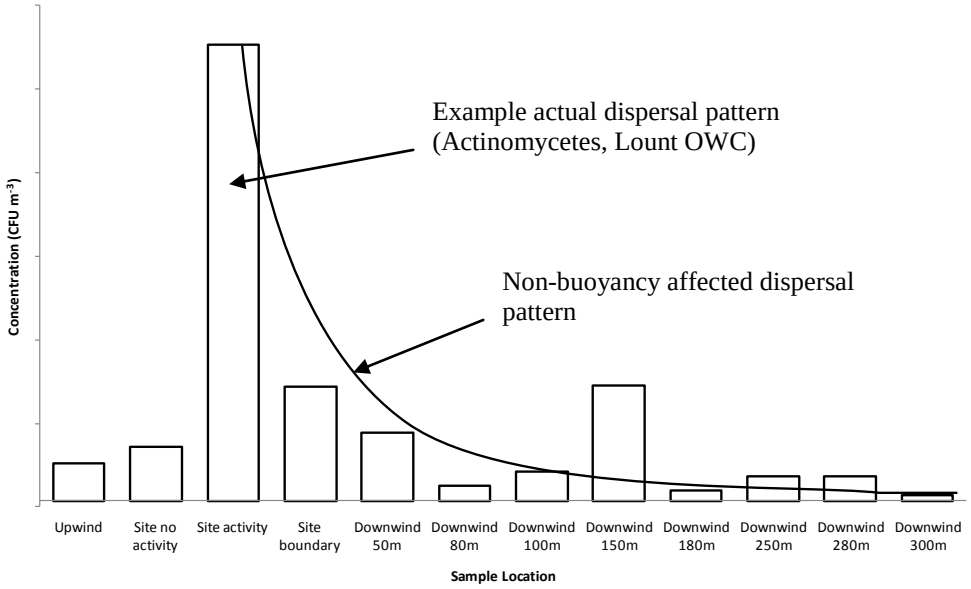



Figure 12.1: Example of actual patterns in downwind concentrations of bioaerosols, versus a pattern that would result from Gaussian dispersal.

It was shown through investigation of downwind concentrations that between 5 and 52% of culturable bioaerosol samples taken at or beyond 250 m from site were greater than 1000 CFU $m^{-3}$. This finding provides a suggestion that the new 500 CFU $m^{-3}$ reference limit for *A. fumigatus* (Dennis, 2009) may be difficult to achieve consistently, as *A. fumigatus* was found to periodically (5 – 20% of samples) exceed 1000 CFU $m^{-3}$ at 250 m from site. The frequent excursion of gram-negative bacteria above 1000 CFU $m^{-3}$ 250 m downwind from site also suggests that the 300 CFU $m^{-3}$ limit is frequently exceeded. However, due to the LLOD of the method used here (757 CFU $m^{-3}$) the number of excursions above the gram-negative bacteria and proposed *A. fumigatus* limit could not be calculated; although up to 20% of *A. fumigatus* samples were above 1000 CFU $m^{-3}$ at and beyond 250 m from site. This suggests that excursions above suggested limits for these microorganisms may be more frequent than was suggested in Chapter 4.

**The Effect of Operational and Environmental Influences on Bioaerosol Concentrations**

The degree to which operational and environmental influences affect bioaerosol concentrations on-site and downwind was considered in Chapters 5 and 6. These studies aimed to provide an explanation for the variability seen in bioaerosol concentrations

within Chapter 4. The ability of bioaerosols to disperse in concentrations elevated above both upwind and recommended concentrations was linked to site activity, described as part of Chapter 5. Within this Chapter it was shown that during periods of 'no activity' concentrations were rarely statistically higher than those found upwind, or were below detection limits. Elevated downwind concentrations could therefore be attributed to composting site activity, with the influence of composting on ambient bioaerosols during times of no activity shown to be minimal. In addition, this provided evidence that sensitive receptors may be exposed to elevated concentrations of bioaerosols, although the lack of dose-response relationships means that no health impact can be associated with the concentrations found as part of this study. Efforts to determine the individual impacts of activities on bioaerosols within Chapter 5 were largely unsuccessful, with no consistent trends found.

This was also one of the major findings of Chapter 6, where the effect of environmental conditions on bioaerosol concentrations was investigated. Both of these Chapters are based on a post-hoc analysis; namely, one that has been carried out after collection of data through comparison of two data-sets, for example, bioaerosol concentrations and meteorological conditions. This has limited the ability of this study to evaluate the impact of both environmental and mechanical parameters, largely through inability to separate parameters. For example, where screening may be absent, other activities may be present. This will create a result suggesting that absence of screening creates higher concentrations of the bioaerosol in question, although the increase in concentrations will be due to the presence of other activities. Chapter 6 did show how some consistent correlations may be found between environmental conditions and bioaerosols. These include air temperature and dew point temperature, which showed positive correlations with most bioaerosols, and relative humidity, which showed negative correlations with most bioaerosols. However, the reasons for these relationships remain unknown. Environmental parameters are likely to affect the emission, dispersal, and survival of bioaerosols, with the individual physicochemical properties of each bioaerosol also affecting response. Evidence for this uncertainty was shown through comparison between endotoxins and bacteria, where each bioaerosol showed an opposite response to environmental conditions. Knowledge surrounding the properties of each bioaerosol and

response to environmental conditions is not developed sufficiently to explain the relationships found here, for example, evidence both supporting and contradicting the results found as part of this study are present within the literature (Lighthart and Mohr, 1987; Marthi *et al*, 1990; Seinfeld and Pandis, 2006; Tham and Zuraimi, 2005). However, the data here show how bioaerosol concentrations are significantly affected by environmental conditions within a 'real-world' scenario. Another conclusion drawn from Section I of the thesis is in regards to the suitability of actinomycetes as a measure of composting influence on ambient bioaerosols. Actinomycetes were found in concentrations above 1000 CFU $m^{-3}$ regularly at all sampling locations. This suggests that they may be present at high concentrations ambiently within the environment, reducing their usefulness as an indicator species.

### 12.2.2 Thesis section II

Section II of the thesis aimed to achieve thesis Objective ii, focusing on the characterisation of bioaerosol components and communities. While several conclusions have been drawn from this work, one of the major findings of this section of the thesis is that non-culture based methods can be used with success alongside culture-based methods. Indeed, the same sampling equipment may be used for both culture and a variety of non-culture based analyses.

**Comparison between Different Bioaerosols Dispersal Patterns, and Verification of Patterns through Direct Counting**

While Section I of the thesis quantified the emission and dispersal of endotoxins, the data suggested that endotoxins may behave differently to other bioaerosols. This was confirmed through Chapter 8, which showed that endotoxin concentration patterns were the most different from any other bioaerosol. However, none of the bioaerosols measured were more than 60% similar, illustrating how a 'one-size fits all' prediction of bioaerosol concentrations may not be possible. The differences in behaviour may be due to particle size, a suggestion supported by Chapter 9, where a novel direct counting method was used alongside the filtration followed by culture and endotoxin assay methods used throughout this project. In particular, this Chapter showed how

endotoxins were statistically associated with the P-TRAK Condensation Particle Counter, which measures particles < 1 µm in size. The other main finding from Chapter 9 was that the dispersal pattern shown through analysis of Section I, was also shown by WIBS3, independently confirming the presence of a secondary peak at 100 – 150 m downwind. Culturable bioaerosols were also found in comparable concentrations to biological particle counts by WIBS. This suggests, supported through available literature, that bioaerosols emitted through composting may be largely culturable upon release. This study provided, for the first time, a direct comparison between a culture-based method and a novel direct counting method for bioaerosol enumeration. In addition, the application of the WIBS3 real-time particle counter to composting bioaerosol enumeration has not been carried out previously. This study has therefore shown the applicability of WIBS3 to composting bioaerosol studies, with this method potentially able to provide reliable, continuous data concerning emissions of bioaerosols. This would eliminate any remaining issues with 'snapshot' sampling, and allow direct links to be made between, for example, specific composting analysis and bioaerosol concentrations.

**Identification of Culturable Aerosolised Gram-negative Bacteria**

Given the differences in gram-negative bacteria and endotoxin concentrations seen throughout Chapters 8 and 9, as well as the dispersal data gathered in Section I, it was desirable to identify the key aerosolised gram-negative bacteria that may contribute to the endotoxins found. This identification, not seen before within the literature, showed how several species of gram-negative bacteria possessing endotoxin with a known high endotoxic potential, were present. This suggests that health effects may result from exposure to endotoxin derived from composting microorganisms. However, the lack of dose-response relationships prevents further conclusions regarding potential health impacts.

**Analysis of the Microbial Community Composition of Bioaerosols and Green Waste**

While Chapters 8 and 9 broadly compared the composition of bioaerosols in terms of culturable and non-culturable fractions, the composition of the microorganism

communities emitted through composting were investigated in Chapters 10 and 11. Chapter 10 utilised molecular methods to examine the impact of composting on bioaerosol community structure; these were PLFA and DGGE. Both methods showed how the passage of air across a composting facility leads to a significant change in the community composition of bioaerosols. In particular, PLFA markers found in 'compost-influenced air' and not upwind were also found in the green waste itself. The lack of taxonomic refinement of these methods was addressed through DNA sequencing with results presented in Chapter 11, although only bacterial communities were characterised as part of this study. Bacterial communities, identified to genus and species levels, within green waste and aerosolised communities upwind, on-site and downwind were compared. Bioaerosol bacterial communities on-site were found to be comprised of both microorganisms from green-waste and upwind, with a decrease in the influence of green waste derived microorganisms visible downwind from site. The main groups affecting bioaerosol composition and traced to green waste were *Proteobacteria* and *Firmicutes,* with *Firmicutes* and *Pseudomonas* (*Proteobacteria*) showing particular ability to disperse downwind and therefore the potential to be used as indicators of the influence of composting bioaerosols. In addition, numerous genera were identified as potential pathogens, to both humans and plants. Seven of these genera were found within green waste and downwind samples, illustrating how composting may lead to the emission and dispersal of potentially pathogenic microorganisms. The data shown reveals the true diversity of bioaerosols, that was not represented through culture-based methods, and illustrated how sensitive receptors may be exposed to microorganisms directly linked to green waste.

### 12.2.3 Scientific Achievements

**Composting activities lead to emission and dispersal of bioaerosols, including endotoxins, in elevated concentrations.**

Although the episodic emission of bioaerosols has been previously shown in the literature, here, endotoxins were also linked to emissions resulting from site activity. One of the major achievements of this study has been the provision of a detailed, validated data-set quantifying *A. fumigatus*, actinomycete, gram-negative bacteria, and

endotoxin concentrations on-site and downwind from composting facilities, up to and beyond 250 m from site boundaries. A dataset of this type and incorporating endotoxins has not been seen previously within the available literature, and has allowed confirmation of previous findings as well as novel findings. One novel finding was the relative insignificance of static windrows as a source of bioaerosols. Concentrations were typically below detection limits (757 CFU $m^{-3}$) at downwind locations during periods of no activity. Consequently, composting activities were directly linked to peak concentrations downwind from site, whereas previously, environmental conditions and the influence of other sources have been cited as the major cause of peaks in downwind concentrations. It was also found that site activities resulted in up to 52% of bioaerosol samples taken at and beyond 250 m from site being above recommended limits (or detection limits for *A. fumigatus* and gram-negative bacteria). Recently published studies (Albrecht *et al,* 2008; Fischer *et al,* 2008) have obtained similar findings; although this study is the first to directly link these elevated concentrations to composting activity through detailed analysis of concentrations from site to 600 m downwind. Another significant novel finding was the evidence suggesting that dispersal patterns of all bioaerosols are strongly affected by buoyancy, leading to a secondary peak in concentrations 100 – 150 m from site boundary. While it was suggested that buoyancy may affect bioaerosol emissions previously (Swan *et al,* (2003), this had not been shown experimentally and was therefore an unexpected and significant finding.

These patterns of emission and dispersal were found consistently between bioaerosols and sites. This suggests that these patterns and dispersal distances may be extrapolated to other green waste composting facilities. Although, slight changes in dispersal distances could be anticipated at different sites, due to differences in emission concentrations and site topography.

**The influence of operational and environmental parameters on bioaerosol concentrations is significant.**

It was found that mid-stage activities lead to the greatest increases in bioaerosol emissions, with the maintenance of compost at lower moisture content also potentially increasing emissions. Environmental conditions were also significantly related to

bioaerosol concentrations, with air temperature, dew point temperature and wind speed positively associated, and relative humidity negatively associated with bioaerosol concentrations. While these results have been suggested previously within the literature, here the provision of a large and validated dataset has confirmed these relationships. However, the lack of characterisation of the response of individual bioaerosols to operational and environmental influences was highlighted, with reasons for many of these relationships either unavailable or contradictory. Therefore, one of the more significant revelations of these findings is that understanding of the interactions of the numerous parameters with bioaerosols at composting facilities appears impossible to achieve even with the benefit of a large and validated data-set. As a result of this study, it can be suggested that more dedicated investigations into the effect of operational and environmental conditions on bioaerosol concentrations are required.

**Novel direct counting and molecular methods can be used with success alongside culture-based methods**

One of the major findings of Section II of the thesis was the ability to use the same methods for both culture-based and molecular analysis of bioaerosols. The use of the recently standardised (Association for Organics Recycling, 2009) filtration method for both culture and non-culture based analysis has not been shown previously within the literature. The direct comparison of culture-based methods with a novel direct counting method also resulted in a significant finding, with the dispersal patterns found through culture based analysis and presumed to be a result of buoyancy, also found by a real-time direct counting method. This has provided verification that bioaerosols show a steep decline in concentrations from source, followed by a secondary peak in concentrations 100 – 150 m from source. In addition, the use of WIBS3 alongside culture based methods suggested that although highly culturable at source, bioaerosols may lose culturability downwind from site. This has provided an additional reason for furthering investigation into non-culture based methods for regular assessment of bioaerosol concentrations.

**The microbial community composition of ambient bioaerosols is significantly affected by composting**

Through the successful use of non-culture based methods, it has also been shown that composting causes a significant change in the community composition of bioaerosols. One aspect of novelty within this study was the use of PLFA and DGGE to analyse the differences between upwind, on-site, and downwind bioaerosols. No other information of this type is available regarding composting bioaerosols within the literature. Although, with the recent publication by Le Goff *et al.* (2009), there is a suggestion that DNA based methods are increasing in popularity within the study of airborne microorganisms in the environment. The results from the application of non-culture based methods illustrated the significant impact that composting has on the aerobiota. Also for the first time, endotoxin analysis was carried out alongside identification of the culturable airborne species of gram-negative bacteria. The results from this study suggested that gram-negative bacteria with known high endotoxic potential are emitted through green waste composting activities and remain culturable, and therefore potentially pathogenic. Comparison between the emission and dispersal patterns found highlighted another significant finding; that the patterns found in gram-negative bacteria and endotoxin concentrations are largely unrelated. This has illustrated how further information is required with regards to the release mechanisms of endotoxins from gram-negative bacteria. Further data regarding the emission and dispersal of potentially pathogenic bacteria through composting was shown through pyrosequencing, with a suggestion that potential pathogens are able to disperse off-site. The data also supported recent suggestions by Le Goff *et al.* (2009) that *Firmicutes* form a large fraction of bioaerosols, and are able to disperse downwind from site.

It is well acknowledged within the literature that culture is unable to account for both the full diversity and concentration of microorganisms within the environment. This was supported by evidence in Chapter 9 showing how although potentially highly culturable on-site, the culturability of bioaerosols may reduce downwind from site. Currently, the study of airborne microorganisms is stunted in that soil and aquatic microbiological studies regularly employ molecular methods, while the study of airborne microorganisms emitted through composting remains focused on culture. This study has demonstrated that these methods are applicable to bioaerosol investigations. For the first time, bioaerosols emitted through composting have been compared to the

compost microbial community itself, and upwind bioaerosol communities. This has, with great detail, demonstrated the composition of bacterial bioaerosols, including potential pathogens that sensitive receptors may be exposed to. While providing significant results, the full potential of these methods remains unexplored. Furthermore, as well as qualification of microbial communities, there is the potential for real-time monitoring of bioaerosols through WIBS3, which would eliminate any remaining issues with 'snapshot' sampling. Further utilisation of molecular methods may allow future source apportionment, allowing the contribution of composting to sensitive receptor exposure to be contextualised. They would also allow identification of unique composting community fingerprints and indicator species, which may also be utilised in future culture-based methods, allowing bioaerosols to be traced more effectively to composting.

### 12.2.4 Implications of findings

One of the main implications of these findings is that *A. fumigatus* is unlikely to consistently fall to 500 CFU $m^{-3}$ by 250 m from site boundary, and periodic sensitive receptor exposure to elevated concentrations of all bioaerosols at distances beyond 250 m from site may result directly from composting activities. This may have ramifications for both risk assessment of composting facilities, as well as the future planning permission of composting facilities; under current trigger distances for risk assessment (250 m), consistent achievement of both former and proposed recommended limits is doubtful. In addition, the effect of buoyancy may lead to modification of sampling regimes, in order to account for both the initial large drop in bioaerosol concentrations, followed by a secondary peak. The information found here may aid in the management of composting facilities. The relationship of increased levels of bioaerosols with high temperatures, and low relative humidity may mean that intense composting activities should be avoided on days with these meteorological conditions. In addition, the maintenance of compost at higher moisture levels is likely to reduce bioaerosol emissions and dispersal in elevated concentrations. From a regulatory perspective, the findings here suggest that bioaerosols may be found above recommended threshold levels at distances up to and beyond 250 m from site boundaries on a regular basis. The

verification of all findings at two composting facilities suggests that these results may be extrapolated to other green waste composting facilities.

The use of direct counting and molecular methods for bioaerosol analysis has demonstrated their utility. The methods chosen for investigation within this study have shown to have the potential to provide real-time counts and particle size fractionation of bioaerosols; analysis of the potentially pathogenic fractions of bioaerosols; and analysis of the community structure of bioaerosols along with the contribution of composting microorganisms to ambient bioaerosols. As culture-based analysis is well acknowledged to result in underestimation of microbiological diversity and concentrations, it is suggested that non-culture based analysis may provide the refined and detailed information required in order to advance bioaerosol exposure assessment. This was demonstrated at Flixborough, where it has been shown that sensitive receptors may be exposed to elevated concentrations of bioaerosols due to their proximity to the site. Analysis of the bacterial community composition through pyrosequencing then revealed that potentially pathogenic bacteria, not identified through culture-based analysis, may comprise a fraction of these bioaerosols. This demonstrates how the focus of sampling on culture-based analysis has prevented full understanding of the aerobiota, and omitted fractions of bioaerosols (NCBV, non-viable, non-culturable) from analysis. This may have led to underestimation of concentrations, and omission of potentially pathogenic microorganisms. Regular use and the construction of a database of airborne microbiological communities as emitted from a variety of sources may allow determination of receptor exposure through source apportionment. For the composting industry and regulators, this ability would demonstrate the proportion of bioaerosol exposure that may be attributed to composting.

## 12.3 Limitations

One of the main limitations that must be acknowledged and is relevant to the main findings of this project is the relatively high detection limit of the methods used here. This limitation means that background bioaerosol concentrations have not been

quantified. Therefore, the distance at which bioaerosols achieve background has not been described through this study. Despite this, a proportion of the bioaerosols measured were found at concentrations significantly higher than those found upwind beyond 250 m from site. This suggests that more sensitive sampling methods may detect bioaerosols in elevated concentrations to distances beyond those found here. Another major limitation of this study became apparent within the analysis of the effect of operational and environmental conditions on bioaerosol concentrations. Methodologically, the presence or absence of activity on-site was at times difficult to determine due to practical reasons, i.e. managing both sampling points at downwind locations (out of sight) of site meant some changes in activity were inevitably unobserved. In addition, meteorological data were collected using a hand-held device and based on observations during sampling only. In order to increase accuracy of meteorological parameter measurement a permanent weather station should be employed. As discussed above, the post-hoc nature of this analysis also prevented influences on bioaerosol emission and dispersal to be separated and therefore accurately quantified. This is likely to be one of the influences preventing conclusions following this analysis to be formed, due to inconsistencies and a lack of clarity within the results. Another factor preventing accurate conclusions to be drawn was the lack of data, and contradictory data, available within the literature. For example, as the individual physicochemical properties of bioaerosols remain un-described, consistent theories to explain relationships between environmental parameters and bioaerosols were absent. This was highlighted for gram-negative bacteria and endotoxins, where patterns in concentrations and responses to external influences were largely dissimilar, with little information within the literature to explain the differences found. In addition, although efforts were made to account for both best- and worst-case bioaerosol concentrations on-site and downwind, the method used here still employed 30 minute sampling periods, and was therefore to some degree snapshot.

Section II of the thesis was largely limited by a lack of time and resources. The data presented as part of this section was ‘proof of concept’, meaning that while the investigations allowed some significant and novel data to be presented, the methods must be further developed, optimised, and repeated through additional studies in order

to increase confidence in the findings. In addition, only bacterial communities were classified taxonomically due to the difficulties of amplifying fungi, as well as a lack of time and resources. In order to fully understand the contribution of composting microorganisms to bioaerosols these fungal communities should also be characterised.

## 12.4 Future Work

This study, while significantly contributing to knowledge surrounding the emission, dispersal, and composition of bioaerosols, has raised many more questions and revealed additional gaps in knowledge that may be addressed within future work. Some of these gaps may be addressed through the following research directions:

1. Quantification of background bioaerosol concentrations and study of the dispersal of bioaerosols in light of this information. This will allow a more accurate dispersal distance of bioaerosols to be quantified
2. Dedicated study of the impact that compost composition and condition, in combination with individual site activities, have on bioaerosol emission and dispersal. This would allow better understanding of the reasons for worst-case emissions and bioaerosol dispersal, and therefore provide the ability to prevent these worst-case situations through control of the causes.
3. Detailed controlled study of the impact of environmental conditions on bioaerosol emissions and concentrations. This would also allow better control and prevention of worst-case emission and dispersal scenarios.
4. Investigation of the release mechanisms of endotoxins from gram-negative bacteria. A study investigating the relationships of these bioaerosols may provide strategies for the reduction of endotoxin emission through composting; for example, through the minimisation of conditions under which the production and emission of endotoxins is encouraged.
5. Exploration of the health impacts of bioaerosol exposure, leading to the future development of dose-response relationships

6. Continued development and optimisation of molecular methods, leading towards characterisation of bioaerosol communities from a variety of sources and future source apportionment through community analysis, as well as identification of bioaerosols indicative of composting, and potentially pathogenic microorganisms
7. Continued development of real-time biological particle counters in order to allow continuous monitoring of bioaerosol concentrations.

# References

Adams, J. D. W. and Frostick, L. E. (2009), Analysis of bacterial activity, biomass and diversity during windrow composting, *Waste Management*, vol. 29, no. 2, pp. 598-605.

ADAS and SWICEB (2005), *Bioaerosol Monitoring and Dispersion from Composting Sites*, 1, ADAS, UK.

Advice Committee on Dangerous Pathogens (2004), *The Approved List of Biological Agents*. Health and Safety Commission, UK.

Agranovski, I. E. (2007), Personal sampler for viable airborne microorganisms: main development stages, *Clean*, vol. 35, no. 1, pp. 111-117.

Agranovski, I. E., Safatov, A. S., Borodulin, A. I., Pyankov, O. V., Petrishchenko, V. A., Sergeev, A. N., Sergeev, A. A., Agranovski, V. and Grinshpun, S. A. (2005), New personal sampler for viable airborne viruses: feasibility study. *Journal of Aerosol Science*, vol. 36, no. 5-6, pp. 609-617.

Agranovski, I. E., Safatov, A. S., Sergeev, A. A., Pyankov, O. V., Petrishchenko, V. A., Mikheev, M. V. and Sergeev, A. N. (2006), Rapid detection of airborne viruses by personal aerosol sampler combined with the PCR device. *Atmospheric Environment*, vol. 40, no. 21, pp. 3924-3929.

Aguilera, A., Gómez, F., Lospitao, E. and Amils, R. (2006), A molecular approach to the characterization of the eukaryotic communities of an extreme acidic environment: Methods for DNA extraction and denaturing gradient gel electrophoresis analysis, *Systematic and Applied Microbiology*, vol. 29, no. 7, pp. 593-605.

Albrecht, A., Fischer, G., Brunnemann-Stubbe, G., Jäckel, U. and Kämpfer, P. (2008), Recommendations for study design and sampling strategies for airborne microorganisms, MVOC and odours in the surrounding of composting facilities, *International Journal of Hygiene and Environmental Health*, vol. 211, no. 1-2, pp. 121-131.

Alfreider, A., Peters, S., Tebbe, C. C., Rangger, A. and Insam, H. (2002), Microbial community dynamics during composting of organic matter as determined by 16S ribosomal DNA analysis, *Compost Science and Utilization*, vol. 10, no. 4, pp. 303-312.

Allmers, H., Huber, H. and Baur, X. (2000), Two year follow-up of a garbage collector with allergic bronchopulmonary aspergillosis (ABPA), *American Journal of Industrial Medicine*, vol. 37, no. 4, pp. 438-442.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. (1997), Gapped BLAST and PSI-BLAST: A new generation of protein database search programs, *Nucleic Acids Research,* vol. 25, no. 17, pp. 3389-3402.

Amanullah, A., Leonildi, E., Nienow, A. W. and Thomas, C. R. (2001), Dynamics of mycelial aggregation in cultures of aspergillus oryzae, *Bioprocess and Biosystems Engineering,* vol. 24, no. 2, pp. 101-107.

Amir, S., Merlina, G., Pinelli, E., Winterton, P., Revel, J. and Hafidi, M. (2008), Microbial community dynamics during composting of sewage sludge and straw studied through phospholipid and neutral lipid analysis, *Journal of hazardous materials,* vol. 159, no. 2-3, pp. 593-601.

Andersen, A. A. (1966), A sampler for respiratory health hazard assessment. *American Industrial Hygiene Association Journal,* vol. 27, no. 2, pp. 160-165.

Association for Organics Recycling (2009), *A Standardised Protocol for the Monitoring of Bioaerosols at Open Composting Facilities,* Association for Organics Recycling, UK.

Baertsch, C., Paez-Rubio, T., Viau, E. and Peccia, J. (2007), Source tracking aerosols released from land-applied class B biosolids during high-wind events, *Applied and Environmental Microbiology,* vol. 73, no. 14, pp. 4522-4531.

Bartlett, M. D., James, I. T., Harris, J. A. and Ritz, K. (2007), Interactions between microbial community structure and the soil environment found on golf courses, *Soil Biology and Biochemistry,* vol. 39, no. 7, pp. 1533-1541.

Bauer, H., Schueller, E., Weinke, G., Berger, A., Hitzenberger, R., Marr, I. L. and Puxbaum, H. (2008), Significant contributions of fungal spores to the organic carbon and to the aerosol mass balance of the urban atmospheric aerosol, *Atmospheric Environment,* vol. 42, no. 22, pp. 5542-5549.

Becher, S. and Lichtnecker, H. (2002), Immunological aspects and affections of rubbish collectors caused by bioaerosols, *Journal of Occupational Health,* vol. 44, no. 3, pp. 125-130.

Begon, M., Harper, J. L. and Townsend, C. R. (1996), *Ecology,* Blackwell Science Limited, London, UK.

Bell, J. N. B. and Treshaw, M. (2002), *Air Pollution and Plant Life,* 2nd ed, Wiley, UK.

Bond, A., Fawell, J., Harrison, R., Kay, D., Kemm, J., Kibble, A., Matthews, I., Mullins, J., Morgan, H., Palmer, S., Parry, J., Stoner, J., Thomas, H. and Walker, D. (2005), *Health Impact Assessment of Waste Management: Methodological Aspects and Information Sources,* Environment Agency, UK.

Borrego, S., Niub, E., Ancheta, O. and Espinosa, M. E. (2000), Study of the microbial aggregation in Mycobacterium using image analysis and electron microscopy, *Tissue and Cell*, vol. 32, no. 6, pp. 494-500.

British Standards Institution (1996), *Methods for Microbiological Examination of Food and Animal Feeding Stuff - Part 0: General Laboratory Practices*, British Standards Institution, UK.

Brown, A., Knight, A., Kumarswami, N., Lamarre, B., Lipscombe, B., Robinson, R. and Williams, M. (2009), *Rapid and Responsive Monitoring Network for Bioaerosol Emissions: Final Report*, National Physical Laboratory, UK.

Brooks, J. P., Gerba, C. P. and Pepper, I. L. (2007), Diversity of aerosolised bacteria during land application of biosolids, Journal of Applied Microbiology, vol. 103, pp. 1779-1790.

Bünger, J., Antlauf-Lammers, M., Schulz, T. G., Westphal, G. A., Müller, M. M., Ruhnau, P. and Hallier, E. (2000), Health complaints and immunological markers of exposure to bioaerosols amongst biowaste collectors and compost workers. *Occupational and Environmental Medicine*, vol. 57, pp. 458-464.

Bünger, J., Schappler-Scheele, B., Hilgers, R. and Hallier, E. (2007), A 5-year follow-up study on respiratory disorders and lung function in workers exposed to organic dust from composting plants, *International Archives of Occupational and Environmental Health*, vol. 80, no. 4, pp. 306-312.

Burge, H. A. and Hodgson, M. (1988), Health risks of indoor pollutants, *ASHRAE Journal*, vol. 30, no. 7, pp. 34-38.

Bush, R. K. and Peden, D. B. (2006), Advances in environmental and occupational disorders, *Journal of Allergy and Clinical Immunology*, vol. 117, no. 6, pp. 1367-1373.

Cabredo, S., Parra, A. and Anzano, J. (2007), Bacteria spectra obtained by laser induced fluorescence, *Journal of Fluorescence*, vol. 17, no. 2, pp. 171-180.

Cahyani, V. R., Watanabe, A., Matsuya, K., Asakawa, S. and Kimura, M. (2002), Succession of microbiota estimated by phospholipid fatty acid analysis and changes in organic constituents during the composting process of rice straw, *Soil Science and Plant Nutrition*, vol. 48, no. 5, pp. 735-743.

Catranis, C. M., Anagnost, S. E., Zhang, L., Zhou, S., Fernando, A., Morey, S. and Wang, C. J. K. (2006), A new sub-sampling method for analysis of air samples collected with the Andersen single-stage sampler. *Aerobiologia*, vol. 22, pp. 177-184.

Cayer, M., Veillette, M., Pageau, P., Hamelin, R., Bergeron, M., Mériaux, A., Cormier, Y. and Duchaine, C. (2007), Identification of mycobacteria in peat moss processing plants: Application of molecular biology approaches, *Canadian Journal of Microbiology*, vol. 53, no. 1, pp. 92-99.

Chen, P. -S. and Li, C. -S. (2005a), Bioaerosol characterization by flow cytometry with fluorochrome, *Journal of Environmental Monitoring,* vol. 7, pp. 950-959.

Chen, P. -S. and Li, C. -S. (2005b), Sampling performance for bioaerosols with flow cytometry with fluorochrome, *Aerosol Science and Technology,* vol. 39, pp. 231-237.

Chen, P. -S. and Li, C. -S. (2007), Real-time monitoring for bioaerosols - Flow cytometry, *Analyst,* vol. 132, no. 1, pp. 14-16.

Cheng, J. Y. W., Lau, A. P. S. and Fang, M. (2008a), Assessment of atmospheric fungal prevalence through field ergosterol measurement I – Determination of the specific ergosterol content in common ambient fungal spores and yeast cells, *Atmospheric Environment,* vol. 42, pp. 2256-5533.

Cheng, J. Y. W., Lau, A. P. S. and Fang, M. (2008b), Assessment of atmospheric fungal prevalence through field ergosterol measurement II: Establishing the conversion factor, *Atmospheric Environment,* vol. 42, pp. 5534-5541.

Chun-Yuh, Yang, Wen-Tzong Chang, Hung-Yi Chuang, Shang-Shyue Tsai, Trong-Neng Wu and Fung-Chang Sung. (2001), Adverse health effects among household waste collectors in Taiwan. *Environmental Research Section A,* vol. 85, pp. 195-199.

Clark, C. S., Rylander, R. and Larsson, L. (1983), Levels of gram-negative bacteria, *Aspergillus fumigatus,* dust, and endotoxin at compost plants. *Applied and Environmental Microbiology,* vol. 45, no. 5, pp. 1501-1505.

Clarke, K. R. and Warwick, R. M. (2001), *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation*, 2nd ed., Primer-E Ltd., Plymouth Marine Laboratory, UK.

Cole, E. C. and Cook, C. E. (1998) Characterisation of infectious aerosols in health care facilities: An aid to effective engineering controls and preventative strategies. *American Journal of Infection Control,* vol. 24, no. 4, pp 453-464.

Colls, J. (2002), *Air Pollution,* 2nd ed, Span Press, London, UK.

Coulon, F., McKew, B. A., Osborn, A. M., McGenity, T. J. and Timmis, K. N. (2007), Effects of temperature and biostimulation on oil-degrading microbial communities in temperate estuarine waters, *Environmental Microbiology,* vol. 9, no. 1, pp. 177-186.

Crook, B. and Sherwood-Higham, J. L. (1997), Sampling and assay of bioaerosols in the work environment. *Journal of Aerosol Science,* vol. 28, no. 3, pp. 417-426.

Crook, B., Stagg, S. and Uwagboe, U. C. (2006), *Bioaerosols in Waste Composting: Deriving source terms and characterising profiles*, Environment Agency, UK.

Curtis, L., Rea, W., Smith-Willis, P., Fenyves, E. and Pan, Y. (2006), Adverse health effects of outdoor air pollutants, *Environment International,* vol. 32, no. 6, pp. 815-830.

D and F Associates (2006) *Bio-aerosol Monitoring at Lount Composting Facility, Leicestershire*, 1st June 2006. D and F Associates, 2006.

D and F Associates (2007) *Bio-aerosol Monitoring at Lount Composting Facility, Leicestershire*, 17th May 2007. D and F Associates, 2007.

Davidson, M. W. (2004), *Molecular Expressions*, available at: http://micro.magnet.fsu.edu/cells/plasmamembrane/images/plasmamembranefigure1.jpg (accessed 24th December 2009).

De León, L., Rodríguez, A., Llop, P., López, M. M. and Siverio, F. (2009), Comparative study of genetic diversity of Clavibacter michiganensis subsp. Michiganensis isolates from the Canary Islands by RAPD-PCR, BOX-PCR and AFLP, *Plant Pathology*, vol. 58, no. 5, pp. 862-871.

De Lipthay, J. R., Enzinger, C., Johnsen, K., Aamand, J. and Sørensen, S. J. (2004), Impact of DNA extraction method on bacterial community composition measured by denaturing gradient gel electrophoresis, *Soil Biology and Biochemistry*, vol. 36, no. 10, pp. 1607-1614.

Deacon, L.J., Pankhurst, L.J., Drew, G.H., Hayes, E.T., Jackson, S., Longhurst, P. J., Longhurst, J.W.S., Liu, J., Pollard, S.J.T. and Tyrrel, S.F. (2009) Particle size distribution of airborne *Aspergillus fumigatus* spores emitted from compost using membrane-filtration, *Atmospheric Environment*, DOI: 10.1016/j.atmosenv.2009.07.042.

Deane, M., Sanders, G. and Jonsson, E. (1977), Trends and community annoyance reactions to odors from pulp mills Eureka, California 1969-1971. *Environmental Research*, vol. 14, pp. 232-244.

DEFRA (2004), *Options for the Diversion of Biodegradable Municipal Waste from Landfill*, DEFRA, UK.

DEFRA (2005), *Landfill Allowance Trading Scheme*, available at: http://www.defra.gov.uk/environment/waste/localauth/lats/index.htm (accessed 18th July 2007).

DEFRA (2007), *Waste Strategy for England 2007*, DEFRA, UK.

Deloge-Abarkan, M., Ha, T. -., Robine, E., Zmirou-Navier, D. and Mathieu, L. (2007), Detection of airborne Legionella while showering using liquid impingement and fluorescent in situ hybridization (FISH), *Journal of Environmental Monitoring*, vol. 9, no. 1, pp. 91-97.

Denning, D. W. (2006), *Aspergillosis*, Schering-Plough Corporation.

Dennis, V. (2009) *E-mail regarding Aspergillus fumigatus recommended limits.* Sent to Pankhurst, L. J. December 2009.

Department of the Environment, Transport and the Regions (2000), *Waste Strategy 2000 for England and Wales*, Department of the Environment, Transport and the Regions, UK.

Déportes, I., Benoit-Guyed, J. and Zmiroub, D. (1995), Hazard to man and the environment posed by the use of urban waste compost: A review, *Science of the Total Environment*, vol. 172, pp. 197-222.

DeSantis, T. Z., Hugenholtz, P., Keller, K., Brodie, E. L., Larsen, N., Piceno, Y. M., Phan, R. and Andersen, G. L. (2006a), NAST: A multiple sequence alignment server for comparative analysis of 16S rRNA genes, *Nucleic acids research*, vol. 34.

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., Huber, T., Dalevi, D., Hu, P. and Andersen, G. L. (2006b), Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB, *Applied and Environmental Microbiology*, vol. 72, no. 7, pp. 5069-5072.

De Soyza, A., Silipo, A., Lanzetta, R., Govan, R. J. and Molinaro, A. (2008), Chemical and biological features of *Burkholderia cepacia* complex lipopolysaccharides, *Innate Immunity*, vol. 14, no. 3, pp. 127-144.

Dietrich, D. F. (2001), Measuring Bioaerosols. *Occupational Health and Safety*, vol. 70, no. 5, pp. 62-69.

Domingo, J. L. and Nadal, M. (2009), Domestic waste composting facilities: A review of human health risks, *Environment International*, vol. 35, pp. 382-389.

Douwes, J., Versloot, P., Hollander, A., Heederik, D. and Doekes, G. (1995), Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. *Applied and Environmental Microbiology*, vol. 61, no. 5, pp. 1763-1769.

Douwes, J., Siebers, R., Wouters, I. M., Doekes, G., Fitzharris, P. and Crane, J. (2006), Endotoxin (1→3)-β-D-glucans and fungal extra-cellular polysaccharides in New Zealand homes: A pilot study. *Annals of Agricultural and Environmental Medicine*, vol. 13, pp. 361-365.

Douwes, J., Thorne, P., Pearce, N. and Heederik, D. (2003), Bioaerosol health effects and exposure assessment: progress and prospects, *Annals of Occupational Hygiene*, vol. 47, no. 3, pp. 187-200.

Douwes, J., Wouters, I., Dubbeld, H., van Zwieten, L., Steerenberg, P., Doekes, G. and Heederik, D. (2000), Upper airway inflammation assessed by nasal lavage in compost workers: a relation with bio-aerosol exposure. *American Journal of Industrial Medicine*, vol. 37, pp. 459-468.

Dowling, N. J. E., Widdel, F. and White, D. C. (1986), Phospholipid ester-linked fatty acid biomarkers of acetate-oxidizing sulphate-reducers and other sulphide-forming bacteria, *Journal of General Microbiology*, vol. 132, no. 7, pp. 1815-1825.

Drew, G. H., Smith, R., Gerard, V., Burge, C., Lowe, M., Kinnersley, R., Sneath, R. and Longhurst, P. J. (2007a), Appropriateness of selecting different averaging times for modelling chronic and acute exposure to environmental odours, *Atmospheric Environment*, vol. 41, no. 13, pp. 2870-2880.

Drew, G. H., Smith, R. S., Pollard, S. J. T., Longhurst, P. J. and Kinnersley, R. (2005), Amenity impacts of episodic emissions from composting facilities, *10th European Biosolids and Biowaste Conference, Workshop and Exhibition*, session 25, paper 84, November 2005, Wakefield, Aqua Environmental Technology Transfer, UK.

Drew, G. H., Tamer, A., Taha, M. P. M., Smith, R., Longhurst, P. J., Kinnersley, R. and Pollard, S. J. T. (2006), Dispersion of bioaerosols from composting facilities, *Waste 2006 Conference*, 19-21st September, Stratford, UK.

Drew, G. H., Tamer Vestlund, A., Tyrrel, S. F., Longhurst, P. J. and Pollard, S. J. T. (2007b), *Bioaerosol and Odour Monitoring at Three Compost Sites*, UKPIR12, SEPA/SNIFFER, http://www.sniffer.org.uk.

Duckworth, J. (2005), *Composting Industry Code of Practice*, The Composting Association, Northamptonshire, UK.

Dutkiewicz, J. (1997), Bacteria and fungi in organic dust as potential health hazard, *Annals of Agriculture and Environmental Medicine*, vol. 4, no. 1, pp. 11-16.

Dytham, C. (1999), *Choosing and Using Statistics. A Biologist's Guide*, Blackwell Science Limited, Oxford, UK.

Eduard, W. and Heederik, D. (1998), Methods for quantitative assessment of airborne levels of noninfectious microorganisms in highly contaminated work environment, *American Industrial Hygiene Association Journal*, vol. 59, no. 2, pp. 113-127.

Eduard, W., Douwes, J., Mehl, R., Heederik, D. and Melbostad, E. (2001), Short term exposure to airborne microbial agents during farm work: exposure-response relations with eye and respiratory symptoms. *Occupational and Environmental Medicine*, vol. 58, pp. 113-118.

Edwards, U., Rogall, T., Blocker, H., Emde, M. and Bottger, E. C. (1989), Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA, *Nucleic acids research*, vol. 17, no. 19, pp. 7843-7853.

Environment Agency (1999), E*nvironmental Protection Act 1990 Waste Management License*, Environment Agency, UK.

Environment Agency (2001a), *Health Effects of Composting. A study of three compost sites and review of past data*, Environment Agency, UK.

Environment Agency (2001b), *Technical Guidance on Composting Operations*, Environment Agency, UK.

Environment Agency (2001c), *Monitoring the Environmental Impact of Waste Composting Plants*, R&D Technical Summary P1-216, Environment Agency, UK.

Environment Agency (2005), *Environmental Protection Act 1990 Waste Management License*. Environment Agency, UK.

Environment Agency (2009a), *Guidance on the evaluation of bioaerosol risk assessments for composting facilities*, Environment Agency, UK.

Environment Agency (2009b), *Position Statement. Sustainable management of biowastes. Composting - maximising the benefits and minimising the environmental impacts*, Environment Agency, UK.

Environment Agency (in press), *Updated Review of Methods for Monitoring Compost Bioaerosols*. Environment Agency, UK.

Epstein, E. (1994), Composting and bioaerosols. *BioCycle*, vol. 35, no. 1, pp. 51-59.

Epstein, E., Wu, N., Youngberg, C. and Croteau, G. (2001), Controlling dust and bioaerosols at a biosolids composting facility, *BioCycle*, vol. 42, no. 4, pp. 50-55.

Erridge, C., Bennett-Guerrero, E. and Poxton, I. R. (2002), Structure and function of lipopolysaccharides, *Microbes and Infection*, vol. 4, no. 8, pp. 837-851.

FARLEX (2007), *The Free Dictionary*, available at: http://medical-dictionary.thefreedictionary.com/ (accessed 21st August 2008).

Felske, A., Akkermans, A. D. L. and De Vos, W. M. (1998), Quantification of 16S rRNAs in complex bacterial communities by multiple competitive reverse transcription-PCR in temperature gradient gel electrophoresis fingerprints, *Applied and Environmental Microbiology*, vol. 64, no. 11, pp. 4581-4587.

Finney, A. and Bacon, J. (2007) *Risk Assessment for Bioaerosols for Lower Trent OWC*, SITA Organics, UK.

Fischer, G., Albrecht, A., Jäckel, U. and Kämpfer, P. (2008), Analysis of airborne microorganisms, MVOC and odour in the surrounding of composting facilities and implications for future investigations, *International Journal of Hygiene and Environmental Health*, vol. 211, no. 1-2, pp. 132-142.

Fischer, G., Müller, T., Ostrowski, R. and Dott, W. (1999), Mycotoxins of *Aspergillus fumigatus* in Pure Culture and in Native Bioaerosols from Compost Facilities. *Chemosphere*, vol. 38, no. 8, pp. 1745-1755.

Fischer, G., Müller, T., Schwalbe, R., Ostrowski, R. and Dott, W. (2000), Exposure to airborne fungi, MVOC and mycotoxins in biowaste-handling facilities. *International Journal of Hygiene and Environmental Health*, vol. 203, pp. 97-104.

Fletcher, L. A., Stentiford, E. I. and Kemp, A. A. (2008), Determining whether estimated spore release rates for Aspergillus fumigatus are compatible with their measured growth rates in composting systems, *13th - 15th October, Wageningen,* The Netherlands, pp. 1.

Forbes, B., Sahm, D. F. and Weissfeld, A. S. (2007), *Bailey and Scott's Diagnostic Microbiology,* 12th ed., Mosby Elsevier, USA.

Franke-Whittle, I. H., Klammer, S. H. and Insam, H. (2005), Design and application of an oligonucleotide microarray for the investigation of compost microbial communities, *Journal of Microbiological Methods,* vol. 62, pp. 37-56.

Frostegård, Å., Bååth, E. and Tunlid, A. (1993b), Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis, *Soil Biology and Biochemistry,* vol. 25, no. 6, pp. 723-730.

Frostegård, Å., Tunlid, A. and Baath, E. (1991), Microbial biomass measured as total lipid phosphate in soils of different organic content, *Journal of microbiological methods,* vol. 14, no. 3, pp. 151-163.

Frostegård, Å., Tunlid, A. and Baath, E. (1993a), Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals, *Applied and Environmental Microbiology,* vol. 59, no. 11, pp. 3605-3617.

Gavito, M. E. and Olsson, P. A. (2008), Foraging strategies of the external mycelium of the arbuscular mycorrhizal fungi Glomus intraradices and Scutellospora calospora, *Applied Soil Ecology,* vol. 39, no. 3, pp. 282-290.

Gilbert, E. J., Kelsey, A., Karnon, J. D., Swan, J. R. M. and Crook, B. (2002), Preliminary results of monitoring the release of bioaerosols from composting facilities in the UK: interpretation, modelling and appraisal of mitigation measures, *International Symposium Composting and Compost Utilization,* Colombus, Ohio, USA.

Gorny, R. L. (2004), Filamentous microorganisms and their fragments in indoor air - A review, *Annals of Agricultural and Environmental Medicine,* vol. 11, no. 2, pp. 185-197.

Giovannoni, S. J., Britschgi, T. B., Moyer, C. L. and Field, K. G. (1990), Genetic diversity in Sargasso Sea bacterioplankton, *Nature,* vol. 345, no. 6270, pp. 60-63.

Glover, G. (2008) *Discussion on Site Operational Activities* with Pankhurst, L.J. 20th February 2008.

Godish, D. R. and Godish, T. J. (2006), Relationship between sampling duration and concentration of culturable airborne mould and bacteria on selected culture media. *Journal of Applied Microbiology,* vol. 102, pp. 1479-1484.

Golder Associates (1998), *Green Waste Composting Facility at Lount Landfill, Leicestershire, Working Plan*. Golder Associates Ltd. UK.

Green, N.P.O.; Stout, G.W. and Taylor, D.J. (1990), *Biological Science 1 and 2*. Cambridge University Press, UK.

Griffiths, W. D., Stewart, I. W., Clark, J. M. and Holwill, I. L. (2001), Procedures for the characterisation of bioaerosol particles. Part II: Effects of environment on culturability. *Aerobiologia*, vol. 17, pp. 109-119.

Grinn-Gofroń, A. and Strzelczak, A. (2008), Artificial neural network models of relationships between Alternaria spores and meteorological factors in Szczecin (Poland), *International Journal of Biometeorology*, vol. 52, no. 8, pp. 859-868.

Grinshpun, S. A., Willeke, K., Ulevicius, V., Juozaitis, A., Terzieva, S., Donnelly, J., Stelma, G. N. and Brenner, K. P. (1997), Effect of impaction, bounce and reaerosolization on the collection efficiency of impingers, *Aerosol Science and Technology*, vol. 26, no. 4, pp. 326-342.

Grisoli, P., Rodolfi, M., Villani, S., Grignani, E., Cottica, D., Berri, A., Picco, A. M. and Dacarro, C. (2009), Assessment of airborne microorganism contamination in an industrial area characterised by an open composting facility and a wastewater treatment plant, *Environmental Research*, vol. 109, pp. 135-142.

Hansen, J., Ivens, U. I., Breum, N. O., Nielsen, M., Würtz, H., Poulsen, O. M. and Ebbehøj, N. (1997), Symptoms among Danish waste collectors. *Annals of Agricultural and Environmental Medicine*, vol. 4, pp. 69-74.

Harrison, E. Z. (2007), Health impacts of composting air emissions, *BioCycle*, vol. 48, no. 11, pp. 44-50.

Harrison, R. M., Jones, A. M., Biggins, P. D. E., Pomeroy, N., Cos, C. S., Kidd, S. P., Hobman, J. L., Brown, N. L. and Beswick, A. (2005), Climate factors influencing bacterial count in background air samples, *International Journal of Biometeorology*, vol. 49, pp. 167-178.

Head, I. M., Saunders, J. R. and Pickup, R. W. (1998), Microbial evolution, diversity, and ecology: A decade of ribosomal RNA analysis of uncultivated microorganisms, *Microbial Ecology*, vol. 35, no. 1, pp. 1-21.

Heidelberg, J. F., Shahamat, M., Levin, M., Rahmin, I., Stelma, G., Grim, C. and Colwell, R. R. (1997), Effect of aerosolization on culturability and viability of gram-negative bacteria. *Applied and Environmental Microbiology*, vol. 63, no. 9, pp. 3585-3588.

Heldal, K. K. and Eduard, W. (2004), Associations between acute symptoms and bioaerosol exposure during the collection of household waste. *American Journal of Industrial Medicine*, vol. 46, pp. 253-260.

Henningson, E. W. and Ahlberg, M. S. (1994), Evaluation of microbiological aerosol samplers: A review. *Journal of Aerosol Science,* vol. 25, no. 8, pp. 1459-1492.

Herr, C. E. W., Nieden, A. z., Bödeker, R. H., Gieler, U. and Eikmann, T. F. (2003b), Ranking and frequency of somatic symptoms in residents near composting sites with odor annoyance, *International Journal of Hygiene and Environmental Health,* vol. 206, no. 1, pp. 61-64.

Herr, C. E. W., zur Nieden, A., Jankofsky, M., Stilianakis, N. I. and et al. (2003a), Effects of bioaerosol polluted air on airways of residents: A cross sectional study. *Occupational and Environmental Medicine,* vol. 60, no. 5, pp. 336.

Herr, C. E. W., zur Nieden, A., Seitz, H., Harpel, S., Stinner, D., Stilianakis, N. I. and Eikmann, T. F. (2004b), Bioaerosols in outdoor air - Statement of environmental medical assessment criteria on the basis of an epidemiological cross sectional study, *Gefahrstoffe Reinhaltung der Luft,* vol. 64, no. 4, pp. 143-152.

Herr, C. E. W., zur Nieden, A., Stilianakis, N. I. and Eikmann, T. F. (2004a), Health effects associated with exposure to residential organic dust, *American Journal of Industrial Medicine,* vol. 46, pp. 381-385.

Herrmann, R. F. and Shann, J. F. (1997), Microbial community changes during the composting of municipal solid waste, Microbial ecology, vol. 33, no. 1, pp. 78-85.

Heuer, H., Wieland, G., Schönfeld, J., Schönwälder, A., Gomes, N. C. M. and Smalla, K. (2001), Bacterial community profiling using DGGE or TGGE analysis, in Rochelle, P. A. (ed.) *Environmental Microbiology: Protocols and Applications,* Horizon Scientific Press, Wymondham, UK, pp. 178-190.

Heuer, H., Krsek, M., Baker, P., Smalla, K. and Wellington, E. M. H. (1997), Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients, *Applied and Environmental Microbiology,* vol. 63, no. 8, pp. 3233-3241.

Ho, H. -M., Rao, C. Y., Hsu, H., Chiu, Y., Liu, C. and Chao, H. J. (2005), Characteristics and determinants of ambient fungal spores in Hualien, Taiwan, *Atmospheric Environment,* vol. 39, no. 32, pp. 5839-5850.

Hollingdale, M. R. (1974), Antibody responses in patients with farmer's lung disease to antigens from *Micropolyspora faeni*, *Journal of Hygiene,* vol. 72, no. 1, pp. 79-89.

Hugenholtz, A. and Goebel, B. M. (2001), The polymerase chain reaction as a tool to investigate microbial diversity in environmental samples, in Rochelle, P. A. (ed.) *Environmental Molecular Microbiology: Protocols and Applications,* Horizon Scientific Press, Wymondham, UK, pp. 31-41.

Hurst, C. J., Knudsen, G. R., McInerney, M. J., Stetzenbach, L. D. and Walter, M. V. (1997), *Manual of Environmental Microbiology,* American Society for Microbiology, Washington D.C. USA.

Infoterra Ltd and Bluesky and Tele Atlas (2009), *Google Earth*, Google.

Ishimatsu, S., Abe, H., Fukuda, K., Ishidao, T., Taniguchi, H. and Hori, H. (2007), Bioaerosol concentrations and the identification of aerosolized bacteria by 16S rDNA analysis in work environments, *Journal of Occupational Health,* vol. 49, no. 2, pp. 39-44.

Ivens, U. I., Ebbehøj, N., Poulsen, O. M. and Skov, T. (1997a), Gastrointestinal symptoms among waste recycling workers. *Annals of Agricultural and Environmental Medicine,* vol. 4, pp. 153-157.

Ivens, U. I., Hansen, J., Breum, N. O., Ebbehøj, N., Nielsen, M., Poulsen, O. M., Würtz, H. and Skov, T. (1997b), Diarrhoea among waste collectors associated with bioaerosol exposure. *Annals of Agricultural and Environmental Medicine,* vol. 4, pp. 63-68.

Jankowska, E., Reponen, T., Willeke, K., Grinshpun, S. A. and Choi, K. (2000), Collection of fungal spores on air filters and spore reentrainment from filters into air. *Journal of Aerosol Science,* vol. 31, no. 8, pp. 969-978.

Jones, B. L. and Cookson, J. T. (1983), Natural atmospheric microbial conditions in a typical suburban area, *Applied and Environmental Microbiology,* vol. 45, no. 3, pp. 919-934.

Jones, A. M. and Harrison, R. M. (2004), The effects of meteorological factors on atmospheric bioaerosol concentrations - A review, *Science of the Total Environment,* vol. 326, no. 1-3, pp. 151-180.

Kaur, S., Clark, R. D. R., Walsh, P. T, Arnold, S. J., Colvile, R. N., and Nieuwenhuijsen, M. J. (2006), Exposure visualisation of ultrafine particle counts in a transport microenvironment, *Atmospheric Environment,* vol. 40, pp. 386-398

Kaur, S., Nieuwenhuijsen, M. J. and Colvile, R. N. (2005), Pedestrian exposure to air pollution along a major road in Central London, UK, *Atmospheric Environment*, pp. 7307-7320.

Kaye, P.H.; Stanley, W.R.; Foot, V.E.; Barrington, S.J.; Gallagher, M. and Gabey, A. (unpublished material). *Low-cost real-time multi-parameter bio-aerosol sensors.*

Kenny, L. C., Aitken, R., Chalmers, C., Fabries, J. F., Gonzalez-Fernandez, E., Kromhout, H., Liden, G., Mark, D., Riediger, G. and Prodi, V. (1997), A collaborative European study of personal inhalable aerosol sampler performance, *Annals of Occupational Hygiene,* vol. 41, no. 2, pp. 135-153.

Kenny, L. C., Bowry, A., Crook, B. and Stancliffe, J. D. (1999), Field testing of a personal size-selective bioaerosol sampler. *Annals of Occupational Hygiene*, vol. 43, no. 6, pp. 393-404.

Kenny, L. C., Stancliffe, J. D., Crook, B., Stagg, S., Griffiths, W. D., Stewart, I. W. and Futter, S. J. (1998), The adaptation of existing personal inhalable aerosol samplers for bioaerosol sampling, *American Industrial Hygiene Association Journal*, vol. 59, no. 12, pp. 831-841.

Kilburn, K. H. (2003), Summary of the 5th International Conference on Bioaerosols, Fungi, Bacteria, Mycotoxins, and Human Health, *Archives of Environmental Health*, vol. 58, no. 8, pp. 538-542.

Kim, K. Y. and Kim, C. N. (2007), Airborne microbiological characteristics in public buildings of Korea, *Building and Environment*, vol. 42, no. 5, pp. 2188-2196.

Korzeniewska, E., Filipkowska, Z., Gotkowska-Płachta, A., Janczukowicz, W., Dixon, B. and Czułowska, M. (2009), Determination of emitted airborne microorganisms from a BIO-PAK wastewater treatment plant, *Water research*, vol. 43, no. 11, pp. 2841-2851.

Kothary, M. H., Chase Jr, T. and Macmillan, J. D. (1984), Levels of *Aspergillus fumigatus* in air and in compost at a sewage sludge composting site, *Environmental Pollution (Series A)*, vol. 34, pp. 1-14.

Krziwon, C., Zahringer, U., Kawahara, K., Weidemann, B., Kusumoto, S., Rietschel, E. T., Flad, H. and Ulmer, A. J. (1995), Glycosphingolipids from Sphingomonas paucimobilis induce monokine production in human mononuclear cells, *Infection and immunity*, vol. 63, no. 8, pp. 2899-2905.

Lacey, J. (1997), Actinomycetes in composts. *Annals of Agricultural and Environmental Medicine*, vol. 4, pp. 113-121.

Lacey, J. and Crook, B. (1988), Fungal and actinomycete spores as pollutants of the workplace and occupational allergens, *Annals of Occupational Hygiene*, vol. 32, no. 4, pp. 515-533.

Lafleur, R. L., Malazdrewich, C., Jeyaseelan, S., Bleifield, E., Abrahamsen, M. S. and Maheswaran, S. K. (2001), Lipopolysaccharide enhances cytolysis and inflammatory cytokine induction in bovine alveolar macrophages exposed to *Pasteurella (Mannheimia) haemolytica* leukotoxin, *Microbial Pathogenesis*, vol. 30, no. 6, pp. 347-357.

Lappalainen, S., Nikulin, M., Berg, S., Parikka, P., Hintikka, E. -L., and Pasanen, A. -L. (1996), *Fusarium* toxins and fungi associated with handling of grain on eight Finnish farms, *Atmospheric Environment*, vol. 30, no. 17, pp. 3059 – 3065.

Lavoie, J., Dunkerley, C. J., Kosatsky, T. and Dufresne, A. (2006), Exposure to aerosolized bacteria and fungi among collectors of commercial, mixed residential,

recyclable and compostable waste, *Science of the Total Environment,* vol. 370, no. 1, pp. 23-28.

Le Goff, O., Bru-Adan, V., Bacheley, H., Godon, J. and Wery, N. (2009), The microbial signature of aerosols produced during the thermophilic phase of composting, *Journal of Applied Microbiology,* vol. 108, pp. 325-340.

Lee, T., Grinshpun, S., Martuzevivius, D., Adhikari, A., Crawford, C. M. and Reponen, T. (2006), Culturability and concentration of indoor and outdoor airborne fungi in six single-family homes, *Atmospheric Environment* vol. 40, pp. 2902 – 2910

Leone, S., Sturiale, L., Pessione, E., Mazzoli, R., Giunta, C., Lanzetta, R., Garozzo, D., Molinaro, A. and Parrilli, M. (2007), Detailed characterization of the lipid A fraction from the nonpathogen *Acinetobacter radioresistens* strain S13, *Journal of Lipid Research,* vol. 48, no. 5, pp. 1045-1051.

Liebers, V., Brüning, T. and Raulf-Heimsoth, M. (2006), Occupational endotoxin exposure and possible health effects on humans. *American Journal of Industrial Medicine,* vol. 49, pp. 474-491.

Liebers, V., Raulf-Heimsoth, M. and Brüning, T. (2008), Health effects due to endotoxin inhalation (review), *Archives of Toxicology,* pp. 1-8.

Lighthart, B. and Mohr, A. J. (1987), Estimating downwind concentrations of viable airborne microorganisms in dynamic atmosphere conditions. Applied and Environmental Microbiology, vol. 53, no. 7, pp. 1580-1583.

Lin, X., A. Reponen, T., Willeke, K., Grinshpun, S. A., Foarde, K. K. and Ensor, D. S. (1999), Long-term sampling of airborne bacteria and fungi into a non-evaporating liquid, *Atmospheric Environment,* vol. 33, no. 26, pp. 4291-4298.

Liu, J., Pankhurst, L., Deacon, L. J., Wondwossen, A., Hayes, E. T., Drew, G. H., Longhurst, P. J., Pollard, S., Longhurst, J., Tyrrel, S. F. and Jackson, S. K. (2009) Evaluation of inflammatory effects of airborne endotoxin emitted from composting sources, *Environmental Health Perspectives,* submitted 27th October, 2009.

Lozupone, C., Hamady, M. and Knight, R. (2006), UniFrac - An online tool for comparing microbial community diversity in a phylogenetic context, *BMC Bioinformatics,* vol. 7.

Lozupone, C. and Knight, R. (2005), UniFrac: A new phylogenetic method for comparing microbial communities, *Applied and Environmental Microbiology,* vol. 71, no. 12, pp. 8228-8235.

Lundholm, M. and Rylander, R. (1980), Occupational symptoms among compost workers, *Journal of Occupational Medicine,* vol. 22, no. 4, pp. 256-257.

Lutgring, K. R., Linton, R. H., Zimmerman, N. J., Peugh, M. and Heber, A. J. (1997), Distribution and quantification of bioaerosols in poultry-slaughtering plants, *Journal of Food Protection,* vol. 60, no. 7, pp. 804-810.

Macalady, J. L., Mack, E. E., Nelson, D. C. and Scow, K. M. (2000), Sediment microbial community structure and mercury methylation in mercury-polluted Clear Lake, California, *Applied and Environmental Microbiology,* vol. 66, no. 4, pp. 1479-1488.

Macnaughton, S. J. and Stephen, J. R. (2001), A Combined Phospholipid and 16S-rDNA PCR-DGGE Analysis to Study Bioremediative Microbial Communities In Situ, in Rochelle, P. A. (ed.) *Environmental Molecular Microbiology: Protocols and Applications*, Horizon Scientific Press, Wymondham, UK, pp. 141-160.

Macnaughton, S. J., Cormier, M. R., Jenkins, T. L., Davis, G. A. and White, D. C. (1999a), Quantitative sampling of indoor air biomass by signature lipid biomarker analysis, *Journal of Industrial Microbiology and Biotechnology,* vol. 22, no. 2, pp. 80-87.

Macnaughton, S. J., Stephen, J. R., Venosa, A. D., Davis, G. A., Chang, Y. -. and White, D. C. (1999b), Microbial population changes during bioremediation of an experimental oil spill, *Applied and Environmental Microbiology,* vol. 65, no. 8, pp. 3566-3574.

Mahenthiralingam, E., Urban, T. A. and Goldberg, J. B. (2005), The multifarious, multireplicon *Burkholderia cepacia* complex, *Nature Reviews Microbiology,* vol. 3, no. 2, pp. 144-156.

Maidak, B. L., Cole, J. R., Parker Jr., C. T., Garrity, G. M., Larsen, N., Li, B., Lilburn, T. G., McCaughey, M. J., Olsen, G. J., Overbeek, R., Pramanik, S., Schmidt, T. M., Tiedje, J. M. and Woese, C. R. (1999), A new version of the RDP (Ribosomal Database Project), *Nucleic acids research,* vol. 27, no. 1, pp. 171-173.

Maidak, B. L., Olsen, G. J., Larsen, N., Overbeek, R., McCaughey, M. J. and Woese, C. R. (1996), The Ribosomal Database Project (RDP), *Nucleic acids research,* vol. 24, no. 1, pp. 82-85.

Mainelis, G., Górny, R. L., Reponen, T., Trunov, M., Grinshpun, S. A., Baron, P., Yadav, J. and Willeke, K. (2002), Effect of electrical charges and fields on injury and viability of airborne bacteria, *Biotechnology and Bioengineering,* vol. 79, no. 2, pp. 229-241.

Mainelis, G., Grinshpun, S. A., Willeke, K., Reponen, T., Ulevicius, V. and Hintz, P. J. (1999), Collection of airborne microorganisms by electrostatic precipitation, *Aerosol Science and Technology,* vol. 30, no. 2, pp. 127-144.

Malik, S., Beer, M., Megharaj, M. and Naidu, R. (2008), The use of molecular techniques to characterize the microbial communities in contaminated soil and water, *Environment International*, vol. 34, no. 2, pp. 265-276.

Marchand, G., Lavoie, J. and Lazure, L. (1995), Evaluation of bioaerosols in a municipal solid waste recycling and composting plant, *Journal of the Air and Waste Management Association*, vol. 45, no. 10, pp. 778-781.

Marchesi, J. R., Sato, T., Weightman, A. J., Martin, T. A., Fry, J. C., Hiom, S. J., and Wade, W. G. (1998) Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Applied and Environmental Microbiology*, vol. 64, no. 2, pp. 795-799.

Marchesi, J. R. (2001), Primer design for PCR amplification of environmental DNA targets, in Rochelle, P. A. (ed.) *Environmental Molecular Microbiology: Protocols and Applications*, Horizon Scientific Press, Wymondham, UK, pp. 43-54.

Margulies, M., Egholm, M., Altman, W. E., Attiya, S., Bader, J. S., Bemben, L. A., Berka, J., Braverman, M. S., Chen, Y. -., Chen, Z., Dewell, S. B., Du, L., Fierro, J. M., Gomes, X. V., Godwin, B. C., He, W., Helgesen, S., Ho, C. H., Irzyk, G. P., Jando, S. C., Alenquer, M. L. I., Jarvie, T. P., Jirage, K. B., Kim, J. -., Knight, J. R., Lanza, J. R., Leamon, J. H., Lefkowitz, S. M., Lei, M., Li, J., Lohman, K. L., Lu, H., Makhijani, V. B., McDade, K. E., McKenna, M. P., Myers, E. W., Nickerson, E., Nobile, J. R., Plant, R., Puc, B. P., Ronan, M. T., Roth, G. T., Sarkis, G. J., Simons, J. F., Simpson, J. W., Srinivasan, M., Tartaro, K. R., Tomasz, A., Vogt, K. A., Volkmer, G. A., Wang, S. H., Wang, Y., Weiner, M. P., Yu, P., Begley, R. F. and Rothberg, J. M. (2005), Genome sequencing in microfabricated high-density picolitre reactors, *Nature*, vol. 437, no. 7057, pp. 376-380.

Marthi, B., Fieland, V. P., Walter, M. and Seidler, R. J. (1990), Survival of bacteria during aerosolization, *Applied and Environmental Microbiology*, vol. 56, no. 11, pp. 3463-3467.

Martinez, K. F., Rao, C. Y. and Burton, N. C. (2004), Exposure assessment and analysis for biological agents. *Grana*, vol. 43, pp. 193-208.

Meier, U. (2006) A note on the power of Fisher's least significant difference procedure, *Pharmaceutical Statistics*, vol. 5, pp. 253 - 263

Metzker, M. L. (2005), Emerging technologies in DNA sequencing, *Genome research*, vol. 15, no. 12, pp. 1767-1776.

McCune, B., Grace, J. B. and Urban, D. L. (2002), Nonmetric Multidimensional Scaling, in *Analysis of Ecological Communities*, MjM Software Design, Oregon, USA., pp. 125-142.

McDevitt, J. J., Lees, P. S. J., Merz, W. G. and Schwab, K. J. (2004), Development of a method to detect and quantify *Aspergillus fumigatus* conidia by quantitative PCR for environmental air samples, *Mycopathologia,* vol. 158, no. 3, pp. 325-335.

Met Office (2009), *Synoptic and Climate Stations*, available at: http://www.metoffice.gov.uk/climate/uk/networks/ (accessed 20th November 2009).

Midgley, D. (2006), *Aspergillus fumigatus*, available at: http://upload.wikimedia.org/wikipedia/commons/0/08/Aspergillus_fumigatus.jpg (accessed 18th December 2007).

Millner, P. D., Olenchock, S. A., Epstein, E., Rylander, R., M.D., Haines, J., Walker, J., Ooi, B. L., Horne, E. and Maritato, M. (1994), Bioaerosols associated with composting facilities, *Compost Science and Utilization,* vol. 2, no. 4, pp. 6-57.

Miskin, I. P., Farrimond, P. and Head, I. M. (1999), Identification of novel bacterial lineages as active members of microbial populations in a freshwater sediment using a rapid RNA extraction procedure and RT-PCR, *Microbiology,* vol. 145, no. 8, pp. 1977-1987.

Mouillo, D., and Leprêtre, A. (1999), A comparison of species diversity estimators, *Researches on Population Ecology,* vol. 41, no. 2, pp. 203 - 205.

Mukoda, T. J., Todd, L. A. and Sobsey, M. D. (1994), PCR and gene probes for detecting bioaerosols, *Journal of Aerosol Science,* vol. 25, no. 8, pp. 1523-1532.

Muyzer, G., DeWaal, E. C. and Uitterlinden, A. G. (1993), Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA, *Applied and Environmental Microbiology*, vol. 59, no. 3, pp. 695-700.

Negrin, M. M., Del Panno, M. T. and Ronco, A. E. (2007), Study of bioaerosols and site influence in the La Plata area (Argentina) using conventional and DNA (fingerprint) based methods, *Aerobiologia,* vol. 23, no. 4, pp. 249-258.

Neumann, H., Balfanz, J., Becker, G., Lohmeyer, M., Mathys, W. and Raulf-Heimsoth, M. (2002), Bioaerosol exposure during refuse collection: Results of field studies in the real-life situation. *Science of the Total Environment,* vol. 293, pp. 219-231.

Nichols, D. (2007), Cultivation gives context to the microbial ecologist. *Federation of European Microbiological Societies Microbial Ecology,* vol. 60, pp. 351-357.

Nielsen-Kellerman (2009), *Kestrel 3000 Pocket Wind Meter Specifications*, available at: http://www.nkhome.com/ww/3000-fs.html (accessed 20th November 2009).

Nielsen, E. M., Breum, N. O., Nielsen, B. H., Würtz, H., Poulsen, O. M. and Midtgaard, U. (1997a), Bioaerosol exposure in waste collection: A comparative study on the

significance of collection equipment, type of waste, and seasonal variation. *Annals of Occupational Hygiene*, vol. 41, no. 3, pp. 325-344.

Nielsen, B. H., Würtz, H., Breum, N. O. and Poulsen, O. M. (1997b), Microorganisms and endotoxin in experimentally generated bioaerosols from composting household waste, *Annals of Agriculture and Environmental Medicine*, vol. 4, no. 1, pp. 159-168.

Nielsen, M., Würtz, H., Nielsen, B. H., Breum, N. O. and Poulsen, O. M. (1997c), Relationship between different bioaerosol parameters sampled from the breathing zone of waste collectors - Identification of the most important parameters, *Annals of Agriculture and Environmental Medicine*, vol. 4, no. 1, pp. 81-85.

Office of the Deputy Prime Minister (2004), *Planning for Waste Management Facilities. A research study*, Office of the Deputy Prime Minister, London, UK.

O'Gorman, C. M. and Fuller, H. T. (2008), Prevalence of culturable airborne spores of selected allergenic and pathogenic fungi in outdoor air, *Atmospheric Environment*, vol. 42, no. 18, pp. 4355-4368.

Ordnance Survey (2008), Get-a-map, http://www.ordnancesurvey.co.uk/oswebsite/getamap/. (Accessed 28th March 2008).

Orsini, M., Laurenti, P., Boninti, F., Arzani, D., Ianni, A. and Romano-Spica, V. (2002), A molecular typing approach for evaluating bioaerosol exposure in wastewater treatment plant workers. *Water Research*, vol. 36, pp. 1375-1378.

Parameswaran, P., Jalili, R., Tao, L., Shokralla, S., Gharizadeh, B., Ronaghi, M. and Fire, A. Z. (2007), A pyrosequencing-tailored nucleotide barcode design unveils opportunities for large-scale sample multiplexing, *Nucleic Acids Research*, vol. 35, no. 19.

Palmgren, U., Strom, G., Blomquist, G. and Malmberg, P. (1986), Collection of airborne micro-organisms on Nucleopore filters, estimation and analysis - CAMNEA method, *Journal of Applied Bacteriology*, vol. 61, pp. 401-406.

Park, J. -H., Gold, D. R., Spiegelman, D. L., Burge, H. A. and Milton, D. K. (2001), House dust endotoxin and wheeze in the first year of life, *American Journal of Respiratory and Critical Care Medicine*, vol. 163, no. 2, pp. 322-328.

Peccia, J. and Hernandez, M. (2006), Incorporating polymerase chain reaction-based identification, population characterisation, and quantification of microorganisms into aerosol science: A review, *Atmospheric Environment*, vol. 40, no. 21, pp. 3941-3961.

Pentecost, A. (1999), *Analysing Environmental Data*, Pearson Education Limited, Essex, UK.

Peters, S., Koschinsky, S., Schwieger, F. and Tebbe, C. C. (2000), Succession of microbial communities during hot composting as detected by PCR-single-strand-

conformation polymorphism-based genetic profiles of small- subunit rRNA genes, *Applied and Environmental Microbiology*, vol. 66, no. 3, pp. 930-936.

Pollard, S., Tyrrel, S. and Longhurst, P. (2004), *An Environmental Risk Mangement Framework for Composting Facilities in England and Wales*, Environment Agency, UK.

Poulsen, O. M., Breum, N. O., Ebbehøj, N., Hansen, A. M., Ivens, U. I., van Lelieveld, D., Malmros, P., Matthiasen, L., Nielsen, B. H., Nielsen, E. M., Schibye, B., Skov, T., Stenbaek, E. I. and Wilkins, C. K. (1995), Collection of domestic waste. Review of occupational health problems and their possible causes. *Science of the Total Environment*, vol. 170, pp. 1-19.

Prescott, L. M., Harley, J. P. and Klein, D. A. (1990), *Microbiology*, 2nd ed, Wm. C. Brown Publishers, USA.

Prigione, V., Lingua, G. and Filipello Marchisio, V. (2004), Development and use of flow cytometry for detection of airborne fungi, *Applied and Environmental Microbiology*, vol. 70, no. 3, pp. 1360-1365.

Pyankov, O. V., Agranovski, I. E., Pyankova, O., Mokhonova, E., Mokhonov, V., Safatov, A. S. and Khromykh, A. A. (2007), Using a bioaerosol personal sampler in combination with real-time PCR analysis for rapid detection of airborne viruses. *Environmental Microbiology*, vol. 9, no. 4, pp. 992-1000.

Rahkonen, P., Ettala, M. and Loikkanen, I. (1987), Working conditions and hygiene at sanitary landfills in Finland, *Annals of Occupational Hygiene*, vol. 31, no. 4 A, pp. 505-513.

Recer, G. M., Browne, M. L., Horn, E. G., Hill, K. M. and Boehler, W. F. (2001), Ambient air levels of *Aspergillus fumigatus* and thermophilic actinomycetes in a residential neighborhood near a yard-waste composting facility, *Aerobiologia*, vol. 17, no. 2, pp. 99-108.

Regueiro, V., Campos, M. A., Pons, J., Albertí, S. and Bengoechea, J. A. (2006), The uptake of a Klebsiella pneumoniae capsule polysaccharide mutant triggers an inflammatory response by human airway epithelial cells, *Microbiology*, vol. 152, no. 2, pp. 555-566.

Reinthaler, F. F., Marth, E., Eibel, U., Enayat, U., Feenstra, O., Friedl, H., Kock, M., Pichler-Semmelrock, F. P., Pridnig, G. and Schlacher, R. (1997), The assessment of airborne microorganisms in large-scale composting facilities and their immediate surroundings, *Aerobiologia*, vol. 13, no. 3, pp. 167-175.

Rinsoz, T., Duquenne, P., Greff-Mirguet, G. and Oppliger, A. (2008), Application of real-time PCR for total airborne bacterial assessment: Comparison with epifluorescence microscopy and culture-dependent methods, *Atmospheric Environment*, vol. 42, no. 28, pp. 6767-6774.

Ritz, K. (2007), The plate debate: Cultivable communities have no utility in contemporary environmental microbial ecology, *Federation of European Microbiological Societies Microbial Ecology*, vol. 60, pp. 358-362.

Rowntree, D. (2004), *Statistics Without Tears. A Primer for Non-Mathematicians.* Pearson Education Incorporated, USA.

Ryan, R. P., Monchy, S., Cardinale, M., Taghavi, S., Crossman, L., Avison, M. B., Berg, G., van der Lelie, D. and Dow, J. M. (2009), The versatility and adaptation of bacteria from the genus *Stenotrophomonas*, *Nature Reviews Microbiology*, vol. 7, no. 7, pp. 514-525.

Schlosser, O., Huyard, A., Cartnick, K., Yanez, A., Catalan, V. and Do Quang, Z. (2009), Bioaerosols in composting facilities: Occupational health risk assessment, *Water Environment Research*, vol. 81, no. 9, pp. 866-877.

Seedorf, J. and Hartung, J. (1999), Reduction efficiencies of a biofilter and a bioscrubber for bioaerosols from two different piggeries, *Berliner und Munchener Tierarztliche Wochenschrift*, vol. 112, no. 12, pp. 444-447.

Seeley, H. W. J., VanDemark, P. J. and Lee, J. J. (1991), *Microbes in Action*, 4th ed, W.H. Freeman and Company, New York, USA.

Seinfeld, J. H. and Pandis, S. N. (2006), *Atmospheric Chemistry and Physics*, 2nd ed, John Wiley and Sons Incorporated, New Jersey, USA.

Shale, K., Lues, J. F. R., Venter, P. and Buys, E. M. (2006), The distribution of staphylococci in bioaerosols from red-meat abattoirs. *Journal of Environmental Health*, vol. 69, no. 4, pp. 25-33.

SITA UK (2007), *Lower Trent Composting Plant Working Plan*, SITA Organics, UK.

SITA SUEZ (2006), *Amenity and Report Management Reporting Plan (AMRP) for Operation of an In-Vessel Composter*, SITA Organics, UK.

SKC inc (2007), *Air Sampling Filters and Accessories*, available at: http://www.skcinc.com/filters.asp (accessed 8th August 2008).

Slater, R., Davies, P. and Gilbert, E. J. (2005), *The State of Composting in the UK 2003/04*, The Composting Association, UK.

SLR Consulting Limited (2003), *Welbeck Composting Facility. Site specific risk assessment of potential bioaerosol releases.* 4C-197-174, SLR Consulting Limited, UK.

Smith, E. H. (1990), *Air Pollution and Forests*, 2nd ed, Springer-Verlag, USA.

Smith, R. and Pocock, R. (2008), *The State of Composting and Biological Waste Treatment in the UK 2006/07*, Association for Organics Recycling.

Smith, C. J., Danilowicz, B. S., Clear, A. K., Costello, F. J., Wilson, B. and Meijer, W. G. (2005), T-Align, a web-based tool for comparison of multiple terminal restriction fragment length polymorphism profiles, *Federation for European Microbiologist Societies Microbiology Ecology,* vol. 54, no. 3, pp. 375-380.

Spaan, S., Heederik, D. J. J., Thorne, P. S. and Wouters, I. M. (2007), Optimization of airborne endotoxin exposure assessment: Effects of filter type, transport conditions, extraction solutions, and storage of samples and extracts, *Applied and Environmental Microbiology,* vol. 73, no. 19, pp. 6134-6143.

Spaan, S., Wouters, I. M., Oosting, I., Doekes, G. and Heederik, D. (2005), Exposure to inhalable dust and endotoxins in agricultural industries. *Journal of Environmental Monitoring,* vol. 8, pp. 63-72.

Steger, K., Eklind, Y., Olsson, J. and Sundh, I. (2005), Microbial community growth and utilization of carbon constituents during thermophilic composting at different oxygen levels, *Microbial Ecology,* vol. 50, no. 2, pp. 163-171.

Steger, K., Sjögren, Å. M., Jarvis, Å., Jansson, J. K. and Sundh, I. (2007), Development of compost maturity and Actinobacteria populations during full-scale composting of organic household waste, *Journal of Applied Microbiology,* vol. 103, no. 2, pp. 487-498.

Stoeck, T., Kröncke, I., Duineveld, G. C. A. and Palojärvi, A. (2002), Phospholipid fatty acid profiles at depositional and non-depositional sites in the North Sea, *Marine Ecology Progress Series,* vol. 241, pp. 57-70.

Strategy Unit (2002), *Waste not, Want not - A strategy for tackling the waste problem in England*, Strategy Unit, UK.

Swan, J. R. M., Crook, B. and Gilbert, E. J. (2002), Microbial emissions from composting sites, environmental and health impact of solid waste management activities. R.S.C. *Issues in Environmental Science and Technology,* vol. 18.

Swan, J. R. M., Kelsey, A., Crook, B. and Gilbert, E. J. (2003), *Occupational and Environmental Exposure to Bioaerosols from Composts and Potential Health Effects – A critical review of published data*, Health and Safety Executive, UK.

Suzuki, M. T. and Giovannoni, S. J. (1996), Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR, *Applied and Environmental Microbiology*, vol. 62, no. 2, pp. 625-630.

Sykes, P., Jones, K. and Wildsmith, J. D. (2007), Managing the potential public health risks from bioaerosol liberation at commercial composting sites in the UK: An analysis of the evidence base, *Resources, Conservation and Recycling,* vol. 52, no. 2, pp. 410-424.

Taha, M. P. M. (2005a), *Bioaerosol Releases from Composting Facilities* (unpublished PhD thesis), Cranfield University, UK.

Taha, M. P. M., Drew, G. H., Longhurst, P. J., Smith, R. and Pollard, S. J. T. (2006), Bioaerosol releases from compost facilities: Evaluating passive and active source terms at a green waste facility for improved risk assessments, *Atmospheric Environment,* vol. 40, pp. 1159-1169.

Taha, M. P. M., Drew, G. H., Tamer-Vestlund, A., Aldred, D., Longhurst, P. J. and Pollard, S. J. T. (2007), Enumerating actinomycetes in compost bioaerosols at source – Use of soil compost agar to address plate 'masking'. *Atmospheric Environment,* vol. 41, pp. 4759-4765.

Taha, M. P. M. and Pollard, S. J. T. (2004), *Emission and dispersal of bioaerosol during the agitation of green waste compost piles,* 28-30, Proc. Integrated Waste Management and Pollution Control: Policy Practice, Research and Solutions, Stratford-upon-Avon.

Taha, M. P. M., Pollard, S. J. T., Sarkar, U. and Longhurst, P. (2005b), Estimating fugitive bioaerosol releases from static compost windrows: Feasibility of a portable wind tunnel approach. *Waste Management,* vol. 25, pp. 445-450.

Tamer Vestlund, A. (2009), *Characterisation and Dispersal of Bioaerosols Emitted from Composting Facilities*. Personal communication to Pankhurst, L.J., unpublished PhD thesis.

Tantillo, G. M., Fontanarosa, M., Di Pinto, A. and Musti, M. (2004), Updated perspectives on emerging vibrios associated with human infections, *Letters in Applied Microbiology,* vol. 39, no. 2, pp. 117-126.

Tham, K. W. and Zuraimi, M. S. (2005), Size relationship between airborne viable bacteria and particles in a controlled indoor environment study, *Indoor Air Supplement,* vol. 15, no. 9, pp. 48-57.

The Composting Association (1999), *Standardised Protocol for the Sampling and Enumeration of Airborne Micro-organisms at Composting Facilities*, The Composting Association, UK.

Thompson, J. D., Higgins, D. G. and Gibson, T. J. (1994), CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice, *Nucleic Acids Research,* vol. 22, no. 22, pp. 4673-4680.

Thorne, P. S., DeKoster, J. A. and Subramanian, P. (1996), Environmental assessment of aerosols, bioaerosols, and airborne endotoxins in a machining plant, *American Industrial Hygiene Association Journal,* vol. 57, no. 12, pp. 1163-1167.

Tolvanen, O., Nykänen, J., Nivukoski, U., Himanen, M., Veijanen, A. and Hänninen, K. (2005), Occupational hygiene in a Finnish drum composting plant. *Waste Management*, vol. 25, pp. 427-433.

Trout, D., Bernstein, J., Martinez, K., Biagini, R. and Wallingford, K. (2001), Bioaerosol lung damage in a worker with repeated exposure to fungi in a water-damaged building. *Environmental Health Perspectives,* vol. 109, no. 6, pp. 641-644.

Tsai, Y. -L. and Rochelle, P. (2001), Extraction of nucleic acids from environmental samples, in Rochelle, P. A. (ed.) *Environmental Molecular Microbiology: Protocols and Applications,* Horizon Scientific Press, Wymondham, UK, pp. 15-30.

TSI Incorporated (2006). *Model 9525 P-Trak Ultrafine Particle Counter. Operation and Service Manual.* Revision J. TSI Incorporated, USA.

University of Miami Department of Biology , *PCR*, available at: http://www.bio.miami.edu/~cmallery/150/gene/Taq.htm (accessed 7th November 2009).

Van Looveren, M., Goossens, H., Baquero, F., Bruce, J., Cookson, B., Cornaglia, G., Dijkshoorn, L., Gould, I., Kahlmeter, G., Krcmery, V., Monnet, D., MacKenzie, F., Mollison, J., Struelens, M., Towner, K., van den Broek, P. J., van der Meer, J., Vila, J., Voss, A. and Wagner, D. (2004), Antimicrobial resistance of Acinetobacter spp. in Europe, *Clinical Microbiology and Infection*, vol. 10, no. 8, pp. 684-704.

Vega-Maray, A. M., Valencia-Barrera, R. M., Fernández-González, D. and Fraile, R. (2003), Urticaceae pollen concentration in the atmosphere of North Western Spain, *Annals of Agriculture and Environmental Medicine*, vol. 10, no. 2, pp. 249-255.

Verein Deutscher Ingenieure (2004a), *Measurement of airborne microorganisms and viruses in ambient air. Active sampling of bioaerosols,* VDI 4252 Part 1, Verein Deutscher Ingenieure, Düsseldorf.

Verein Deutscher Ingenieure (2004b), *Measurement of airborne microorganisms and viruses in ambient air. Culture based method for the determination of the concentrations of moulds in air*, VDI 4253 Part 2, Verein Deutscher Ingenieure, Düsseldorf.

Vinion-Dubiel, A. D. and Goldberg, J. B. (2003), Lipopolysaccharide of *Burkholderia cepacia* complex, *Journal of endotoxin research*, vol. 9, no. 4, pp. 201-213.

Walter, M. V., Marthi, B., Fieland, V. P. and Ganio, L. M. (1990), Effect of aerosolization on subsequent bacterial survival, *Applied and Environmental Microbiology*, vol. 56, no. 11, pp. 3468-3472.

Warcup, J. H. (1955), Isolation of fungi from hyphæ present in soil, *Nature*, vol. 175, no. 4465, pp. 953-954.

Ward, D. M., Weller, R. and Bateson, M. M. (1990), 16S rRNA sequences reveal numerous uncultures microorganisms in a natural community, *Nature,* vol. 345, no. 6270, pp. 63-65.

Wicker, T., Schlagenhauf, E., Graner, A., Close, T. J., Keller, B. and Stein, N. (2006), 454 sequencing put to the test using the complex genome of barley, *BMC Genomics*, vol. 7.

Wikipedia (2007) *Wikipedia* http://www.wikipedia.org/. Accessed 6th September 2007

Williams, P. and Tomas, J. M. (1990), The pathogenicity of *Klebsiella pneumoniae*, *Reviews in Medical Microbiology*, vol. 1, no. 4, pp. 196-204.

Wlodarski, M., Kopczynski, K., Kaliszewski, M., Kwasny, M., Mularczyk-Oliwa, M. and Kastek, M. (2009), Application of advanced optical methods for classification of air contaminants, in Brebbia, C. A. and Popov, V. (eds.), *Air Pollution XVII*, Vol. 123, 20th - 22nd July 2009, Talinn, Estonia, WIT Press, UK, pp. 237.

Wouters, I. M. (2003), *Man and His Waste. Bioaerosol exposure and respiratory health effects in waste management.* (unpublished PhD thesis), Utrecht Universtiy, The Netherlands.

Wouters, I. M., Douwes, J., Doekes, G., Thorne, P. S., Brunekreef, B. and Heederik, D. J. J. (2000), Increased levels of markers of microbial exposure in homes with indoor storage of organic household waste, *Applied and Environmental Microbiology*, vol. 66, pp. 627-631.

Wouters, I. M., Hilhorst, S. K. M., Kleppe, P., Doekes, G., Douwes, J., Peretz, C. and Heederik, D. (2002), Upper airway inflammation and respiratory symptoms in domestic waste collectors. *Occupational and Environmental Medicine,* vol. 59, pp. 106-112.

Xu, H. L., Méjean, G., Liu, W., Kamali, Y., Daigle, J. -., Azarm, A., Simard, P. T., Mathieu, P., Roy, G., Simard, J. -. and Chin, S. L. (2007), Remote detection of similar biological materials using femtosecond filament-induced breakdown spectroscopy, *Applied Physics B: Lasers and Optics*, vol. 87, no. 1, pp. 151-156.

Yates, P (2007) *Conversation regarding composting facilities*. Personal Communication to Pankhurst, L.J. 3rd July 2007.

Zelles, L. (1999), Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: A review, *Biology and Fertility of Soils*, vol. 29, no. 2, pp. 111-129.

Zhang, Q. -F., Liu, B., Lin, Y. -Z., Shi, H., Yang, S. -S. and Zhou, X. -Z. (2009), The diversity of phospholipid fatty acid (PLFA) biomarker for the microbial community in soil, *Shengtai Xuebao/ Acta Ecologica Sinica*, vol. 29, no. 8, pp. 4127-4137.

Zhu, Y., Yu, N., Kuhn, T. and Hinds, W. C. (2006), Field comparison of P-Trak and Condensation Particle Counters, *Aerosol Science and Technology*, vol. 40, pp. 422-430.

# Glossary

**Actinomycetes –** Filamentous, spore producing, gram-positive bacteria (Swan *et al*, 2003)

**Activity –** Used to describe composting activities, i.e. the shredding, turning, moving and screening of compost

**Aerosols –** Solid particles or liquid droplets that are suspended in the air (Wikipedia, 1997)

**Aggregation –** The collection of particles into an unorganised whole, also, the state of being so collected (Wikipedia, 1997)

**Allergy –** Where a person's immune system becomes hypersensitive to a particular allergen. The allergen itself may be completely harmless, for example, pollen (FARLEX, 2007)

**Arithmetic mean –** Statistical term, meaning the average of the data (Wikipedia, 1997)

**Bacteria –** Prokaryotic, single-celled organisms. They are ubiquitous, found in every habitat available, playing many essential roles within ecosystems (Wikipedia, 1997)

**Below Detection Limits –** Where no results were gained from a measurement as concentrations were below the detection limit of the chosen method

**Bioaerosol –** Airborne liquid or solid particulate matter of microbial, plant or animal origin (Wouters *et al,* 2003)

**Biodegradable –** Material that can be degraded through the action of living organisms (Wikipedia, 1997)

**Brownian Diffusion –** The collision of airborne particles with atmospheric molecules, resulting in the particle impacting on a surface (Colls, 2002)

**Coefficient –** The statistical relationship between observed data (Wikipedia, 1997)

**Colony Forming Units (CFU $m^{-3}$) –** Defined as unit of 1 or more cells or spores which grow to form a single colony when inoculated onto a suitable growth medium (Swan *et al,* 2003)

**Compost –** Solid particulate material that is the result of composting, that has been sanitised and stabilised and that confers beneficial effects when added to soil, used as a component of a growing medium, or is used in another way in conjunction with plants (Duckworth, 2005)

**Composting –** Process of controlled biological decomposition under managed conditions that are predominantly aerobic and that allow the development of thermophilic temperatures as a result of biologically produces heat (Duckworth, 2005)

**Culture –** Agar plates are inoculated with microorganisms and incubated. Colonies formed after the incubation period are then counted and colony forming units (CFU $m^{-3}$) calculated (The Composting Association, 1999)

**Culturable –** A microorganism that may be successfully enumerated through culture

**Direct Impaction –** Microorganisms are captured directly onto agar plates as air is drawn through a sampler by vacuum pump (The Composting Association, 1999)

**Dose-response –** The term used to describe a certain dose (a specified quantity) of an agent that a person is subject to, results in a defined medical response (FARLEX, 2007).

**Emission –** In this case defined as bioaerosol concentrations adjacent to the source, therefore the amount of bioaerosols that has been emitted from the source

**Endotoxins –** Complex macromolecules that can be found in the cell walls of gram-negative bacteria found living on the surfaces of animals and plants (Epstein, 1994; Swan *et al*, 2003).

**Episodic –** Occurring sporadically or periodically (Wikipedia, 1997)

**Exponential –** Statistical term referring to the exponential function ($e^x$), or the inverse of a natural logarithm (Wikipedia, 1997)

**Exposure –** Defined as the condition of being exposed, in this case refers to the condition of being exposed to bioaerosols (FARLEX, 2007)

**Extreme –** Statistical term referring to a value that occurs at the limits of acceptability within the dataset (Wikipedia, 1997)

**Filtration –** Filtration uses vacuum pumps to draw air through sampling apparatus, capturing bioaerosols as air passes through a filter (SKC Inc, 2007)

**Fungi –** Ekaryotic organisms, most of which are characterised by filamentous growth as multicellular hyphae. Some species grow as single-cells. Fungi reproduce by the means of spores (Wikipedia, 1997)

**Genotype –** The genetic constitution of an organism (Wikipedia, 1997)

**Genus –** A term essential in biological nomenclature, dividing organisms according to their similarity and family. For example, *Canis Lupus*, where the genus '*Canis'* refers to the generic dog, and the species *'Lupus'* refers to the wolf (Wikipedia, 1997)

**Geometric mean –** Statistical term referring to the multiplication of the numbers within a set, followed by division of the result by the *n*th root. This provides a mean of the data, taking into account the spread of data (Wikipedia, 1997)

**Glucans –** Components of fungal cells; (1-3)β-D-glucan is a polyglucose compound found in the cell walls of fungi, some bacteria, and plants and is considered the most important glucan within composting bioaerosols (Swan *et al,* 2003)

**Gram-negative Bacteria –** Gram-negative bacteria do not contain teichoic acids within their cell wall, so respond negatively to the Gram stain (Prescott *et al* 1990)

**Gram-positive Bacteria –** Gram-positive bacteria contain teichoic acids within their cell wall. These acids give the cell walls of Gram-positive bacteria a negative charge and cause these cells to give a positive reaction to the Gram stain (Prescott *et al,* 1990)

**Health –** The World Health Organisation (WHO) define health as a "state of complete physical, social, and mental wellbeing and not merely the absence of disease or infirmity", including the ability to lead an "socially and economically productive life" (Wikipedia, 1997)

**Hydrophobicity –** Degree to which a particle is repelled by water (Wikipedia, 1997)

**Impaction -** Where airborne particles following streamlines of air around objects are deposited on the object if particle inertia is too high (Colls, 2002)

**Infection –** The colonisation of a host organism by a foreign species (pathogen) (Wikipedia, 1997)

**Inflammation –** The response of vascular tissues to pathogens or irritants, aimed at removing the damaging substance or stimulating a healing process (Wikipedia, 1997)

**Interception –** Where airborne particles are intercepted by an object and impact upon it (Colls, 2002)

**Inter-quartile range –** A statistical term referring to the values of data between the upper and lower 25% of the data range found (Wikipedia, 1997)

**Least square mean –** A method for gaining the best fit of data to a distribution; an instance of the model for which the sum of squared residuals has its least value, where a residual is the difference between the observed value and the value provided by the model (Pentecost, 1999; Wikipedia, 1997)

**Liquid Impingement –** The collection of bioaerosols directly into a liquid filled reservoir as air is drawn through the apparatus via a vacuum pump (Lin *et al,* 1999)

**Log10 –** The common logarithm, or logarithm with a base 10 (Wikipedia, 1997)

**Lower Limit of Detection (LLOD) –** The lowest value able to be enumerated through the chosen methods (also limit of detection; LOD)

**Median –** The value of the data point that divides the total frequency into two halves (Pentecost, 1999)

**Microorganism –** Organism in the microscopic or sub-microscopic size range (Wikipedia, 1997)

**Mode –** The value of the data point possessed by the greatest number of the population, or other data points (Pentecost, 1999)

**Mycotoxins –** Toxic secondary metabolites produced by certain fungal species as they proliferate within organic material (Swan *et al,* 2003)

**Natural Log –** The natural logarithm, or logarithm to the base *e* (Euler's number). The natural logarithm of a number *x* is the power to which *e* would have to be raised to equal *x* (Wikipedia, 1997)

**No Activity –** Term used to describe periods of time on-site when no compost processing activities were being carried out

**Non-Culturable but Viable (also known as viable but non-culturable) –** Fraction of microorganisms that are initially non-culturable, may recover culturability under favourable conditions (Crook and Sherwood-Higham, 1997; Swan *et al,* 2003)

**Outlier –** Statistical term to describe a number that is numerically distant from the rest of the dataset (Wikipedia, 1997)

**P-value –** Measure of the statistical similarity between samples. Typically taken at the 95% level, i.e. when the p-level is < 0.05 there is a 5% chance that the null hypothesis is correct (Dytham, 1999)

**Pathogen –** An infectious agent (FARLEX, 2007)

**Phenotype –** Any observable trait of an organism, such as morphology, substrate usage or development (Wikipedia, 1997)

**Phylogenetic –** Evolutionary relatedness between groups of microorganisms (Wikipedia, 1997)

**Physicochemical –** The description of chemical systems in terms of physical concepts, for example, describing mass as well as chemical properties alone (Wikipedia, 1997)

**Post-hoc –** Term used to describe examining the data after the experiment has been concluded, or after the event (Wikipedia, 1997)

**Qualitative –** A type of study relating, or comparing, qualities (Wikipedia, 1997)

**Quantitative -** A type of study determining or measuring quantities. For example; may be used to determine the quantities of the chemicals within a mixture, where such chemicals may already have been identified through qualitative study (Wikipedia, 1997)

**Range –** Statistical term for the interval between the minimum and maximum values within a dataset (Wikipedia, 1997)

**Risk –** Risk is defined as a "concept that denotes a potential negative impact to an asset or some characteristic of value that may arise from some present process or future event" (Wikipedia, 1997)

**Screening –** The process by which compost is separated into different size fractions. Can be carried out both at the start and end of the composting process (Taha *et al*, 2006)

**Sedimentation –** Where particles fall onto surfaces through gravity (Colls, 2002)

**Sensitive Receptor –** Any building, other structure or installation, in which at least one person normally lives or works, other than a building, structure or installation within the

same ownership or control as the operator/owner of the composting facility (The Composting Association, 1999)

**Standard deviation –** The square root of variance within a sample set, used as a measure of variability (Wikipedia, 1997)

**Standard error –** Statistical term to describe the standard deviation of a sample group. Namely, the standard error of the mean values, each with their own standard deviation, from a sample set (Wikipedia, 1997)

**Taxa –** A group of one or more organisms that are deemed to be a unit taxonomically, according to their genotype and phylogenetic relationships (Wikipedia, 1997)

**Thermophilic –** Heat-loving; in the case of bacteria, grow best at high temperatures (50-60ºC) (Wikipedia, 1997)

**Thermotolerant –** Organisms that can tolerate/survive higher temperatures; used to describe microorganisms (Wikipedia, 1997)

**Toxicity –** Where exposure to a certain substance has a deleterious effect on organisms (Wikipedia, 1997)

**Turbulent Transfer –** Turbulence is caused by the movement of the lower layers of air over a rough surface; turbulent transfer is the transfer of particles to the ground with varying rapidity according the level of turbulence caused by the surface roughness (Bell and Treshaw, 2002)

**Turning –** The process by which compost windrows are mechanically turned in order to homogenise and aerate them (Taha *et al*, 2006)

**Viable –** A microorganism that is alive and may be able to grow and reproduce

**Windrow composting –** Where compost is heaped into long piles (windrows) to encourage the composting process (Taha *et al,* 2006)

# Appendix I – Section I of Thesis

## Sampling Dates and Experimental Design

Lount OWC

- Preliminary test of methods – 27th September 2007
- Baseline – 17th October 2007, 16th January 2008, 5th March 2008
- Transect – 14th November 2007, 13th February 2008
- Modified Transect – 1st May 2008, 21st May 2008
- Episodic – 30th January 2008, 9th April 2008, 5th June 2008, 19th June 2008, 10th July 2008, 16th July 2008, 30th July 2008

Flixborough

- Baseline – 20th August 2008, 5th November 2008, 26th November 2008, 4th March 2009, 18th March 2009
- Transect – 10th September 2008, 10th December 2008, 11th February 2009, 10th July 2009
- Episodic – 8th January 2009, 4th March 2009, 18th March 2009, 8th April 2009, 22nd April 2009, 6th May 2009, 21st May 2009

## Basic statistical data supporting Chapter 4

Table 1.1: Basic statistical data for *A. fumigatus* (CFU $m^{-3}$) at Lount OWC

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Upwind | 36 | 1221 | BDL | BDL | 33 | BDL | 40909 | BDL | BDL | 1135 |
| Site no activity | 33 | 4802 | BDL | BDL | 19 | BDL | 48209 | BDL | 3030 | 2029 |
| Site activity | 84 | 27977 | 4545 | BDL | 23 | BDL | 223140 | BDL | 33058 | 5382 |
| Site boundary | 18 | 13640 | BDL | BDL | 10 | BDL | 82645 | BDL | 27548 | 5678 |
| Downwind 50m | 27 | 4487 | BDL | BDL | 17 | BDL | 53719 | BDL | 4545 | 2095 |
| Downwind 80m | 12 | 126 | BDL | BDL | 11 | BDL | 1515 | BDL | BDL | 126 |
| Downwind 100m | 42 | 7789 | BDL | BDL | 26 | BDL | 121212 | BDL | 1515 | 4102 |
| Downwind 150m | 45 | 2710 | BDL | BDL | 24 | BDL | 30303 | BDL | 3030 | 837 |
| Downwind 180m | 12 | 505 | BDL | BDL | 8 | BDL | 1515 | BDL | 1515 | 215 |
| Downwind 250m | 54 | 2345 | BDL | BDL | 38 | BDL | 36364 | BDL | 1515 | 902 |
| Downwind 280m | 12 | BDL | BDL | BDL | 12 | BDL | BDL | BDL | BDL | BDL |
| Downwind 300m | 36 | 42 | BDL | BDL | 35 | BDL | 1515 | BDL | BDL | 42 |

Table 1.2: Basic statistical data for *A. fumigatus* (CFU $m^{-3}$) at Flixborough

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Upwind | 18 | 84 | BDL | BDL | 17 | BDL | 1515 | BDL | BDL | 84 |
| Site no activity | 8 | BDL | BDL | BDL | 8 | BDL | BDL | BDL | BDL | BDL |
| Site activity | 82 | 21866 | 1515 | BDL | 36 | BDL | 289256 | BDL | 12121 | 5487 |
| Site boundary | 3 | 9848 | 9848 | Multiple | 1 | 6061 | 13636 | 6061 | 13636 | 2187 |
| Downwind 50m | 15 | 2597 | 1515 | BDL | 7 | BDL | 14286 | BDL | 3030 | 1047 |
| Downwind 100m | 79 | 3494 | BDL | BDL | 59 | BDL | 95041 | BDL | 1515 | 1372 |
| Downwind 150m | 6 | 1263 | BDL | BDL | 5 | BDL | 7576 | BDL | BDL | 1263 |
| Downwind 200m | 75 | 1030 | BDL | BDL | 62 | BDL | 31818 | BDL | BDL | 460 |
| Downwind 300m | 57 | 279 | BDL | BDL | 49 | BDL | 3030 | BDL | BDL | 98 |
| Downwind 400m | 27 | 56 | BDL | BDL | 26 | BDL | 1515 | BDL | BDL | 56 |
| Downwind 500m | 12 | 1263 | BDL | BDL | 9 | BDL | 7576 | BDL | 1515 | 718 |
| Downwind 600m | 9 | 2525 | BDL | BDL | 5 | BDL | 10606 | BDL | 3030 | 1312 |

Table 2.1: Basic statistical data for actinomycetes (CFU $m^{-3}$) at Lount OWC

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Upwind | 36 | 24962 | 5303 | BDL | 15 | BDL | 157025 | BDL | 40634 | 6666 |
| Site no activity | 33 | 20507 | 4545 | BDL | 6 | BDL | 85399 | 2273 | 37190 | 4341 |
| Site activity | 84 | 96324 | 73691 | Multiple | 3 | BDL | 571625 | 42011 | 107438 | 10574 |
| Site boundary | 18 | 43105 | 17045 | BDL | 3 | BDL | 159780 | 3030 | 81267 | 11266 |
| Downwind 50m | 27 | 28084 | 6061 | Multiple | 3 | BDL | 209366 | 3030 | 45455 | 9161 |
| Downwind 80m | 12 | 16334 | BDL | BDL | 7 | BDL | 90909 | BDL | 23933 | 8795 |
| Downwind 100m | 42 | 13897 | 3788 | BDL | 14 | BDL | 64738 | BDL | 10606 | 3308 |
| Downwind 150m | 45 | 53237 | 18182 | BDL | 7 | BDL | 385675 | 4545 | 74380 | 12412 |
| Downwind 180m | 12 | 8247 | 758 | BDL | 6 | BDL | 78512 | BDL | 4167 | 6446 |
| Downwind 250m | 54 | 18840 | 2273 | BDL | 19 | BDL | 173554 | BDL | 7576 | 5149 |
| Downwind 280m | 12 | 12661 | 2652 | BDL | 5 | BDL | 63361 | BDL | 20386 | 5907 |
| Downwind 300m | 36 | 1389 | BDL | BDL | 21 | BDL | 6818 | BDL | 3030 | 316 |

Table 2.2: Basic statistical data for actinomycetes (CFU $m^{-3}$) at Flixborough

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Upwind | 18 | 3114 | BDL | BDL | 10 | BDL | 24242 | BDL | 4545 | 1405 |
| Site no activity | 8 | 5596 | BDL | BDL | 5 | BDL | 37190 | BDL | 3788 | 4556 |
| Site activity | 82 | 855489 | 251377 | 3788 | 3 | 1515 | 10606061 | 38567 | 812672 | 188184 |
| Site boundary | 3 | 4568411 | 5606061 | Multiple | 1 | 826446 | 7272727 | 826446 | 7272727 | 1931853 |
| Downwind 50m | 15 | 14107 | 5195 | Multiple | 2 | BDL | 81267 | 3030 | 10390 | 6094 |
| Downwind 100m | 79 | 93631 | 41322 | BDL | 18 | BDL | 1074380 | 1515 | 93664 | 19040 |
| Downwind 150m | 6 | 28708 | 28926 | Multiple | 1 | 10606 | 50964 | 12879 | 39945 | 6364 |
| Downwind 200m | 75 | 34275 | 3788 | BDL | 28 | BDL | 279614 | BDL | 48209 | 6493 |
| Downwind 300m | 57 | 17532 | BDL | BDL | 33 | BDL | 183196 | BDL | 25758 | 4598 |
| Downwind 400m | 27 | 3265 | BDL | BDL | 21 | BDL | 42700 | BDL | BDL | 1732 |
| Downwind 500m | 12 | 12179 | BDL | BDL | 8 | BDL | 68871 | BDL | 19353 | 6649 |
| Downwind 600m | 9 | 11961 | BDL | BDL | 6 | BDL | 53719 | BDL | 9848 | 7108 |

Table 3.1: Basic statistical data for gram-negative bacteria (CFU $m^{-3}$) at Lount OWC

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Upwind | 21 | 144 | BDL | BDL | 19 | BDL | 1515 | BDL | BDL | 99 |
| Site no activity | 30 | 94591 | 1515 | BDL | 13 | BDL | 2603306 | BDL | 7576 | 86557 |
| Site activity | 66 | 142850 | 11742 | BDL | 10 | BDL | 1432507 | 1515 | 145455 | 33184 |
| Site boundary | 12 | 49093 | 5303 | BDL | 5 | BDL | 143251 | BDL | 121212 | 17980 |
| Downwind 50m | 24 | 20047 | 5682 | 1515 | 5 | BDL | 231405 | 1515 | 12879 | 10038 |
| Downwind 80m | 9 | 1515 | BDL | BDL | 7 | BDL | 7576 | BDL | BDL | 1010 |
| Downwind 100m | 36 | 7185 | 1515 | BDL | 16 | BDL | 70248 | BDL | 5303 | 2425 |
| Downwind 150m | 42 | 42190 | 6818 | Multiple | 6 | BDL | 641873 | 1515 | 25758 | 16755 |
| Downwind 180m | 9 | 337 | BDL | BDL | 7 | BDL | 1515 | BDL | BDL | 223 |
| Downwind 250m | 54 | 8544 | BDL | BDL | 34 | BDL | 92287 | BDL | 3030 | 2658 |
| Downwind 280m | 9 | 168 | BDL | BDL | 8 | BDL | 1515 | BDL | BDL | 168 |
| Downwind 300m | 36 | 42 | BDL | BDL | 35 | BDL | 1515 | BDL | BDL | 42 |

Table 3.2: Basic statistical data for gram-negative bacteria (CFU $m^{-3}$) at Flixborough

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Upwind | 18 | 758 | BDL | BDL | 16 | BDL | 10606 | BDL | BDL | 603 |
| Site no activity | 8 | 2083 | BDL | BDL | 5 | BDL | 12121 | BDL | 2273 | 1486 |
| Site activity | 82 | 760829 | 137741 | BDL | 5 | BDL | 11666667 | 25758 | 398072 | 189844 |
| Site boundary | 3 | 855831 | 1046832 | Multiple | 1 | 404959 | 1115702 | 404959 | 1115702 | 226311 |
| Downwind 50m | 15 | 28998 | 7576 | Multiple | 2 | BDL | 112948 | 1515 | 56474 | 9938 |
| Downwind 100m | 79 | 64682 | 35813 | BDL | 17 | BDL | 674931 | 1515 | 89394 | 11218 |
| Downwind 150m | 6 | 94858 | 88843 | Multiple | 1 | 6061 | 202479 | 27273 | 155647 | 32439 |
| Downwind 200m | 75 | 32836 | 3030 | BDL | 30 | BDL | 505510 | BDL | 34435 | 9089 |
| Downwind 300m | 57 | 24153 | BDL | BDL | 32 | BDL | 232782 | BDL | 12121 | 7135 |
| Downwind 400m | 27 | 1615 | BDL | BDL | 23 | BDL | 26171 | BDL | BDL | 1031 |
| Downwind 500m | 12 | 1768 | BDL | BDL | 7 | BDL | 7576 | BDL | 3030 | 765 |
| Downwind 600m | 9 | 4040 | 1515 | BDL | 3 | BDL | 15152 | BDL | 4545 | 1786 |

Table 4.1: Basic statistical data for endotoxins (EU $m^{-3}$) Lount OWC

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Upwind | 36 | BDL | BDL | BDL | 36 | BDL | BDL | BDL | BDL | BDL |
| Site no activity | 18 | 4.4 | 1.5 | BDL | 4 | BDL | 32.0 | 0.6 | 2.9 | 1.9 |
| Site activity | 39 | 9.8 | 2.4 | BDL | 12 | BDL | 37.6 | BDL | 17.2 | 2.0 |
| Site boundary | 18 | 7.6 | BDL | BDL | 12 | BDL | 40.5 | BDL | 12.3 | 3.1 |
| Downwind 50m | 9 | 1.2 | BDL | BDL | 6 | BDL | 3.8 | BDL | 3.5 | 0.6 |
| Downwind 80m | 12 | 0.2 | BDL | BDL | 10 | BDL | 1.4 | BDL | BDL | 0.1 |
| Downwind 100m | 12 | 2.2 | 0.6 | BDL | 6 | BDL | 6.7 | BDL | 5.2 | 0.8 |
| Downwind 150m | 9 | 7.4 | BDL | BDL | 6 | BDL | 22.7 | BDL | 22.1 | 3.7 |
| Downwind 180m | 12 | 0.4 | BDL | BDL | 8 | BDL | 2.1 | BDL | 0.6 | 0.2 |
| Downwind 280m | 12 | 136.3 | 0.7 | BDL | 6 | BDL | 1612.1 | BDL | 4.2 | 134.2 |

Table 4.2: Basic statistical data for endotoxins (EU $m^{-3}$) at Flixborough

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Upwind | 9 | 0.2 | BDL | BDL | 8 | BDL | 1.7 | BDL | BDL | 0.2 |
| Site no activity | 3 | 0.5 | 0.4 | Multiple | 1 | 0.2 | 1.0 | 0.2 | 1.0 | 0.2 |
| Site activity | 26 | 111.4 | 51.5 | BDL | 6 | BDL | 1430.3 | 2.2 | 73.3 | 55.1 |
| Site boundary | 3 | 1259.3 | 90.5 | Multiple | 1 | 14.7 | 3672.7 | 14.7 | 3672.7 | 1206.9 |
| Downwind 50m | 3 | 4.3 | 3.4 | Multiple | 1 | 1.6 | 7.9 | 1.6 | 7.9 | 1.9 |
| Downwind 100m | 12 | 4.7 | 0.9 | BDL | 6 | BDL | 18.2 | BDL | 10.2 | 1.9 |
| Downwind 150m | 3 | BDL | BDL | BDL | 3 | BDL | BDL | BDL | BDL | BDL |
| Downwind 200m | 9 | 4.5 | 1.2 | BDL | 4 | BDL | 17.5 | BDL | 9.5 | 2.1 |
| Downwind 300m | 6 | 0.4 | BDL | BDL | 4 | BDL | 1.8 | BDL | 0.7 | 0.3 |
| Downwind 400m | 6 | 1.7 | 0.7 | BDL | 3 | BDL | 4.8 | BDL | 3.6 | 0.9 |
| Downwind 500m | 6 | 3.2 | 0.9 | BDL | 3 | BDL | 14.6 | BDL | 3.1 | 2.3 |
| Downwind 600m | 3 | 1.3 | 1.2 | Multiple | 1 | BDL | 2.7 | BDL | 2.7 | 0.8 |

# Basic statistical data supporting Chapter 5

Table 5.1: Basic statistical data for *A. fumigatus* at Lount OWC

(a) During periods of no activity

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Upwind | 12 | 126.26 | 0.00 | 0.00 | 11.00 | 0.00 | 1515.15 | 0.00 | 0.00 | 126.26 |
| On-site | 33 | 4802.15 | 0.00 | 0.00 | 19.00 | 0.00 | 48209.37 | 0.00 | 3030.30 | 2028.82 |
| Downwind 75m | 3 | 4545.45 | 4545.45 | Multiple | 1.00 | 1515.15 | 7575.76 | 1515.15 | 7575.76 | 1749.55 |
| Downwind 100m | 12 | 0.00 | 0.00 | 0.00 | 12.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 200m | 6 | 0.00 | 0.00 | 0.00 | 6.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 225m | 3 | 505.05 | 0.00 | 0.00 | 2.00 | 0.00 | 1515.15 | 0.00 | 1515.15 | 505.05 |
| Downwind 250m | 6 | 0.00 | 0.00 | 0.00 | 6.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 300m | 3 | 0.00 | 0.00 | 0.00 | 3.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 350m | 6 | 0.00 | 0.00 | 0.00 | 6.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 355m | 6 | 0.00 | 0.00 | 0.00 | 6.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 400m | 6 | 0.00 | 0.00 | 0.00 | 6.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

(b) During periods of activity

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Upwind | 24 | 1767.68 | 0.00 | 0.00 | 22.00 | 0.00 | 40909.09 | 0.00 | 0.00 | 1702.97 |
| On-site | 102 | 25446.98 | 4166.67 | 0.00 | 33.00 | 0.00 | 223140.50 | 0.00 | 30303.03 | 4567.02 |
| Downwind 75m | 6 | 13119.83 | 6060.61 | 0.00 | 3.00 | 0.00 | 53719.01 | 0.00 | 12878.79 | 8496.55 |
| Downwind 100m | 36 | 1346.80 | 0.00 | 0.00 | 21.00 | 0.00 | 15151.52 | 0.00 | 1515.15 | 504.25 |
| Downwind 125m | 6 | 252.53 | 0.00 | 0.00 | 5.00 | 0.00 | 1515.15 | 0.00 | 0.00 | 252.53 |
| Downwind 175m | 12 | 3156.55 | 757.50 | 0.00 | 6.00 | 0.00 | 30303.03 | 0.00 | 1515.15 | 2477.45 |
| Downwind 200m | 36 | 2462.12 | 757.58 | 0.00 | 18.00 | 0.00 | 15151.52 | 0.00 | 3030.30 | 676.50 |
| Downwind 250m | 12 | 0.00 | 0.00 | 0.00 | 12.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 275m | 12 | 25367.31 | 0.00 | 0.00 | 9.00 | 0.00 | 121212.12 | 0.00 | 44765.84 | 13416.19 |
| Downwind 300m | 45 | 2881.85 | 0.00 | 0.00 | 27.00 | 0.00 | 36363.64 | 0.00 | 1515.15 | 1068.08 |
| Downwind 350m | 12 | 0.00 | 0.00 | 0.00 | 12.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 355m | 12 | 126.26 | 0.00 | 0.00 | 11.00 | 0.00 | 1515.15 | 0.00 | 0.00 | 126.26 |

Table 5.2: Basic statistical data for *A. fumigatus* at Flixborough

(a) During periods of no activity

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| On-site | 6 | 0.00 | 0.00 | 0.00 | 6.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 100m | 8 | 189.39 | 0.00 | 0.00 | 7.00 | 0.00 | 1515.15 | 0.00 | 0.00 | 189.39 |
| Downwind 200m | 6 | 0.00 | 0.00 | 0.00 | 6.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 300m | 12 | 0.00 | 0.00 | 0.00 | 12.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 400m | 3 | 0.00 | 0.00 | 0.00 | 3.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

(b) During periods of activity

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Upwind | 18 | 84.18 | 0.00 | 0.00 | 17.00 | 0.00 | 1515.15 | 0.00 | 0.00 | 84.18 |
| On-site | 87 | 20949.30 | 1515.15 | 0.00 | 38.00 | 0.00 | 289256.20 | 0.00 | 12121.21 | 5187.41 |
| Downwind 50m | 15 | 2597.40 | 1515.15 | 0.00 | 7.00 | 0.00 | 14285.71 | 0.00 | 3030.30 | 1047.47 |
| Downwind 100m | 71 | 3866.45 | 0.00 | 0.00 | 52.00 | 0.00 | 95041.32 | 0.00 | 1515.15 | 1521.09 |
| Downwind 150m | 6 | 1262.63 | 0.00 | 0.00 | 5.00 | 0.00 | 7575.76 | 0.00 | 0.00 | 1262.63 |
| Downwind 200m | 69 | 1119.89 | 0.00 | 0.00 | 56.00 | 0.00 | 31818.18 | 0.00 | 0.00 | 498.94 |
| Downwind 300m | 45 | 353.54 | 0.00 | 0.00 | 37.00 | 0.00 | 3030.30 | 0.00 | 0.00 | 121.82 |
| Downwind 400m | 24 | 63.13 | 0.00 | 0.00 | 23.00 | 0.00 | 1515.15 | 0.00 | 0.00 | 63.13 |
| Downwind 500m | 12 | 1262.63 | 0.00 | 0.00 | 9.00 | 0.00 | 7575.76 | 0.00 | 1515.15 | 718.30 |
| Downwind 600m | 9 | 2525.25 | 0.00 | 0.00 | 5.00 | 0.00 | 10606.06 | 0.00 | 3030.30 | 1312.16 |

Table 6.1: Basic statistical data for actinomycetes at Lount OWC

(a) During periods of no activity

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Upwind | 12 | 20202.02 | 757.58 | 0.00 | 6.00 | 0.00 | 157024.79 | 0.00 | 6060.61 | 13699.19 |
| On-site | 33 | 20506.72 | 4545.45 | 0.00 | 6.00 | 0.00 | 85399.45 | 2272.73 | 37190.08 | 4341.07 |
| Downwind 75m | 3 | 62901.74 | 70247.93 | Multiple | 1.00 | 45454.55 | 73002.75 | 45454.55 | 73002.75 | 8759.77 |
| Downwind 100m | 12 | 10112.49 | 0.00 | 0.00 | 7.00 | 0.00 | 104683.20 | 0.00 | 3787.88 | 8627.12 |
| Downwind 200m | 6 | 13578.97 | 6818.18 | 4545.46 | 2.00 | 3787.88 | 41322.31 | 4545.45 | 18181.82 | 5972.43 |
| Downwind 225m | 3 | 2777.78 | 3030.30 | Multiple | 1.00 | 0.00 | 5303.03 | 0.00 | 5303.03 | 1536.05 |
| Downwind 250m | 6 | 252.53 | 0.00 | 0.00 | 5.00 | 0.00 | 1515.15 | 0.00 | 0.00 | 252.53 |
| Downwind 300m | 3 | 3787.88 | 3787.88 | Multiple | 1.00 | 0.00 | 7575.76 | 0.00 | 7575.76 | 2186.93 |
| Downwind 350m | 6 | 252.53 | 0.00 | 0.00 | 5.00 | 0.00 | 1515.15 | 0.00 | 0.00 | 252.53 |
| Downwind 355m | 6 | 3914.14 | 4166.67 | 4545.46 | 2.00 | 1515.15 | 6818.18 | 2272.73 | 4545.45 | 768.03 |
| Downwind 400m | 6 | 3282.83 | 2651.52 | 0.00 | 2.00 | 0.00 | 9090.91 | 0.00 | 5303.03 | 1446.24 |

(b) During periods of activity

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Upwind | 24 | 27341.56 | 8333.33 | 0.00 | 9.00 | 0.00 | 121212.00 | 0.00 | 47520.66 | 7471.00 |
| On-site | 102 | 86932.13 | 68181.76 | 0.00 | 5.00 | 0.00 | 571625.34 | 27548.21 | 106060.61 | 9138.55 |
| Downwind 75m | 6 | 35950.41 | 27720.39 | Multiple | 1.00 | 5303.03 | 90909.09 | 7575.76 | 56473.83 | 13972.12 |
| Downwind 100m | 36 | 16720.23 | 4545.45 | 0.00 | 7.00 | 0.00 | 209366.39 | 1893.94 | 8712.12 | 6409.58 |
| Downwind 125m | 6 | 39095.50 | 42699.72 | 64738.29 | 2.00 | 9090.91 | 64738.29 | 10606.06 | 64738.29 | 10953.57 |
| Downwind 175m | 12 | 32988.98 | 12121.21 | Multiple | 2.00 | 0.00 | 90909.09 | 1515.15 | 76446.28 | 11065.25 |
| Downwind 200m | 36 | 55930.52 | 17424.24 | 0.00 | 8.00 | 0.00 | 385674.93 | 1136.36 | 68181.82 | 15296.76 |
| Downwind 250m | 12 | 883.84 | 0.00 | 0.00 | 8.00 | 0.00 | 4545.45 | 0.00 | 1515.15 | 435.73 |
| Downwind 275m | 12 | 24586.73 | 16597.80 | 0.00 | 4.00 | 0.00 | 64738.00 | 0.00 | 47520.54 | 7820.28 |
| Downwind 300m | 45 | 22271.20 | 2272.73 | 0.00 | 15.00 | 0.00 | 173553.72 | 0.00 | 9848.48 | 6058.55 |
| Downwind 350m | 12 | 252.53 | 0.00 | 0.00 | 11.00 | 0.00 | 3030.30 | 0.00 | 0.00 | 252.53 |
| Downwind 355m | 12 | 1830.81 | 2272.73 | 0.00 | 5.00 | 0.00 | 4545.45 | 0.00 | 3030.30 | 506.13 |

Table 6.2: Basic statistical data for actinomycetes at Flixborough

(a) During periods of no activity

| | Observations | Arithmetic | Median | Mode | Frequency | Minimum | Maximum | Lower | Upper | Standard |
|---|---|---|---|---|---|---|---|---|---|---|
| On-site | 6 | 12901.74 | 1515.15 | 0.00 | 3.00 | 0.00 | 37190.08 | 0.00 | 37190.08 | 7695.58 |
| Downwind 100m | 8 | 6680.44 | 0.00 | 0.00 | 5.00 | 0.00 | 41322.31 | 0.00 | 6060.61 | 5115.26 |
| Downwind 200m | 6 | 252.53 | 0.00 | 0.00 | 5.00 | 0.00 | 1515.15 | 0.00 | 0.00 | 252.53 |
| Downwind 300m | 12 | 11363.64 | 0.00 | 0.00 | 9.00 | 0.00 | 56473.83 | 0.00 | 16528.93 | 6108.71 |
| Downwind 400m | 3 | 505.05 | 0.00 | 0.00 | 2.00 | 0.00 | 1515.15 | 0.00 | 1515.15 | 505.05 |

(b) During periods of activity

| | Observations | Arithmetic | Median | Mode | Frequency | Minimum | Maximum | Lower | Upper | Standard |
|---|---|---|---|---|---|---|---|---|---|---|
| Upwind | 18 | 3114.48 | 0.00 | 0.00 | 10.00 | 0.00 | 24242.42 | 0.00 | 4545.45 | 1404.52 |
| On-site | 87 | 963478.83 | 254820.94 | 3787.88 | 3.00 | 0.00 | 10606060.61 | 35812.67 | 936639.12 | 200063.14 |
| Downwind 50m | 15 | 14107.31 | 5194.81 | Multiple | 2.00 | 0.00 | 81267.22 | 3030.30 | 10389.61 | 6094.26 |
| Downwind 100m | 71 | 103428.01 | 48209.37 | 0.00 | 13.00 | 0.00 | 1074380.17 | 6060.61 | 118457.30 | 20872.01 |
| Downwind 150m | 6 | 28707.53 | 28925.62 | Multiple | 1.00 | 10606.06 | 50964.19 | 12878.79 | 39944.90 | 6364.28 |
| Downwind 200m | 69 | 37233.00 | 5303.03 | 0.00 | 23.00 | 0.00 | 279614.33 | 0.00 | 57851.24 | 6947.40 |
| Downwind 300m | 45 | 19176.61 | 0.00 | 0.00 | 24.00 | 0.00 | 183195.59 | 0.00 | 25757.58 | 5594.45 |
| Downwind 400m | 24 | 3609.96 | 0.00 | 0.00 | 19.00 | 0.00 | 42699.72 | 0.00 | 0.00 | 1940.01 |
| Downwind 500m | 12 | 12178.60 | 0.00 | 0.00 | 8.00 | 0.00 | 68870.52 | 0.00 | 19352.62 | 6649.32 |
| Downwind 600m | 9 | 11960.51 | 0.00 | 0.00 | 6.00 | 0.00 | 53719.01 | 0.00 | 9848.48 | 7108.32 |

Table 7.1: Basic statistical data for gram-negative bacteria at Lount OWC

(a) During periods of no activity

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Upwind | 9 | 0.00 | 0.00 | 0.00 | 9.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| On-site | 30 | 94591.37 | 1515.15 | 0.00 | 13.00 | 0.00 | 2603305.79 | 0.00 | 7575.76 | 86557.01 |
| Downwind 75m | 0 | | | Multiple | 1.00 | | | | | |
| Downwind 100m | 12 | 2777.78 | 1515.15 | 0.00 | 5.00 | 0.00 | 15151.52 | 0.00 | 3030.30 | 1303.29 |
| Downwind 200m | 6 | 17676.77 | 4545.45 | 3787.88 | 2.00 | 1515.15 | 78030.30 | 3787.88 | 13636.36 | 12191.50 |
| Downwind 225m | 3 | 0.00 | 0.00 | 0.00 | 3.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 250m | 6 | 0.00 | 0.00 | 0.00 | 6.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 300m | 3 | 0.00 | 0.00 | 0.00 | 3.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 350m | 6 | 0.00 | 0.00 | 0.00 | 6.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 355m | 6 | 0.00 | 0.00 | 0.00 | 6.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 400m | 6 | 252.53 | 0.00 | 0.00 | 5.00 | 0.00 | 1515.15 | 0.00 | 0.00 | 252.53 |

(b) During periods of activity

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Upwind | 12 | 252.53 | 0.00 | 0.00 | 10.00 | 0.00 | 1515.15 | 0.00 | 0.00 | 170.25 |
| On-site | 78 | 128425.87 | 11363.64 | 0.00 | 15.00 | 0.00 | 1432506.89 | 1515.15 | 133608.82 | 28434.59 |
| Downwind 75m | 6 | 19272.27 | 2651.52 | 0.00 | 3.00 | 0.00 | 93663.91 | 0.00 | 16666.67 | 15111.04 |
| Downwind 100m | 36 | 13997.93 | 3030.30 | 0.00 | 9.00 | 0.00 | 231404.96 | 757.58 | 7954.55 | 6630.59 |
| Downwind 125m | 3 | 28971.53 | 24793.39 | Multiple | 1.00 | 22727.27 | 39393.94 | 22727.27 | 39393.94 | 5245.22 |
| Downwind 175m | 6 | 9377.87 | 2651.52 | 0.00 | 3.00 | 0.00 | 35812.67 | 0.00 | 15151.52 | 5809.68 |
| Downwind 200m | 33 | 48731.11 | 7575.76 | 0.00 | 7.00 | 0.00 | 641873.28 | 1515.15 | 25757.58 | 21139.55 |
| Downwind 250m | 12 | 378.79 | 0.00 | 0.00 | 10.00 | 0.00 | 3030.30 | 0.00 | 0.00 | 271.87 |
| Downwind 275m | 6 | 0.00 | 0.00 | 0.00 | 6.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 300m | 45 | 10555.55 | 1515.00 | 0.00 | 22.00 | 0.00 | 92286.50 | 0.00 | 6060.61 | 3117.20 |
| Downwind 350m | 12 | 0.00 | 0.00 | 0.00 | 12.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 355m | 12 | 126.26 | 0.00 | 0.00 | 11.00 | 0.00 | 1515.15 | 0.00 | 0.00 | 126.26 |

Table 7.2: Basic statistical data for gram-negative bacteria at Flixborough

(a) During periods of no activity

| | Observations | Arithmetic | Median | Mode | Frequency | Minimum | Maximum | Lower | Upper | Standard |
|---|---|---|---|---|---|---|---|---|---|---|
| On-site | 6 | 3661.62 | 2272.73 | 0.00 | 2.00 | 0.00 | 12121.21 | 0.00 | 5303.03 | 1880.42 |
| Downwind 100m | 8 | 6697.66 | 757.58 | 0.00 | 4.00 | 0.00 | 39944.90 | 0.00 | 6060.61 | 4874.95 |
| Downwind 200m | 6 | 0.00 | 0.00 | 0.00 | 6.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 300m | 12 | 13842.98 | 0.00 | 0.00 | 7.00 | 0.00 | 96418.73 | 0.00 | 7575.76 | 8689.79 |
| Downwind 400m | 3 | 3030.30 | 0.00 | 0.00 | 2.00 | 0.00 | 9090.91 | 0.00 | 9090.91 | 3030.30 |

(b) During periods of activity

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Upwind | 18 | 757.58 | 0.00 | 0.00 | 16.00 | 0.00 | 10606.06 | 0.00 | 0.00 | 603.21 |
| On-site | 87 | 746554.10 | 140495.87 | 0.00 | 8.00 | 0.00 | 11666666.67 | 21212.12 | 407713.50 | 179422.69 |
| Downwind 50m | 15 | 28997.77 | 7575.76 | Multiple | 2.00 | 0.00 | 112947.66 | 1515.15 | 56473.83 | 9938.46 |
| Downwind 100m | 71 | 71216.00 | 44077.13 | 0.00 | 13.00 | 0.00 | 674931.13 | 3030.30 | 95041.32 | 12237.19 |
| Downwind 150m | 6 | 94857.67 | 88842.98 | Multiple | 1.00 | 6060.61 | 202479.34 | 27272.73 | 155647.38 | 32438.62 |
| Downwind 200m | 69 | 35690.90 | 4545.45 | 0.00 | 24.00 | 0.00 | 505509.64 | 0.00 | 35812.67 | 9809.13 |
| Downwind 300m | 45 | 26902.36 | 0.00 | 0.00 | 25.00 | 0.00 | 232782.37 | 0.00 | 18181.82 | 8730.92 |
| Downwind 400m | 24 | 1437.67 | 0.00 | 0.00 | 21.00 | 0.00 | 26170.80 | 0.00 | 0.00 | 1113.23 |
| Downwind 500m | 12 | 1767.68 | 0.00 | 0.00 | 7.00 | 0.00 | 7575.76 | 0.00 | 3030.30 | 765.19 |
| Downwind 600m | 9 | 4040.40 | 1515.15 | 0.00 | 3.00 | 0.00 | 15151.52 | 0.00 | 4545.45 | 1785.62 |

Table 8.1: Basic statistical data for endotoxins at Lount OWC

(a) During periods of no activity

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Upwind | 12 | 0.00 | 0.00 | 0.00 | 12.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| On-site | 18 | 4.39 | 1.48 | 0.00 | 4.00 | 0.00 | 32.00 | 0.65 | 2.87 | 1.87 |
| Downwind 75m | 3 | 0.00 | 0.00 | 0.00 | 3.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 100m | 0 | | | Multiple | 1.00 | | | | | |
| Downwind 200m | 0 | | | Multiple | 1.00 | | | | | |
| Downwind 225m | 3 | 0.00 | 0.00 | 0.00 | 3.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 250m | 0 | | | Multiple | 1.00 | | | | | |
| Downwind 300m | 0 | | | Multiple | 1.00 | | | | | |
| Downwind 350m | 0 | | | Multiple | 1.00 | | | | | |
| Downwind 355m | 0 | | | Multiple | 1.00 | | | | | |
| Downwind 400m | 6 | 1.16 | 0.00 | 0.00 | 4.00 | 0.00 | 3.93 | 0.00 | 3.06 | 0.75 |

(b) During periods of activity

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Upwind | 24 | 0.00 | 0.00 | 0.00 | 24.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| On-site | 57 | 9.09 | 1.93 | 0.00 | 24.00 | 0.00 | 40.48 | 0.00 | 16.55 | 1.65 |
| Downwind 75m | 6 | 1.83 | 1.74 | 0.00 | 3.00 | 0.00 | 3.84 | 0.00 | 3.67 | 0.82 |
| Downwind 100m | 0 | | | Multiple | 1.00 | | | | | |
| Downwind 125m | 6 | 3.30 | 3.27 | 0.00 | 3.00 | 0.00 | 6.73 | 0.00 | 6.55 | 1.48 |
| Downwind 175m | 12 | 5.94 | 0.34 | 0.00 | 6.00 | 0.00 | 22.73 | 0.00 | 12.10 | 2.85 |
| Downwind 200m | 6 | 0.00 | 0.00 | 0.00 | 6.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 250m | 0 | | | Multiple | 1.00 | | | | | |
| Downwind 275m | 12 | 136.25 | 1.32 | 0.00 | 5.00 | 0.00 | 1612.10 | 0.00 | 4.24 | 134.17 |
| Downwind 300m | 12 | 0.24 | 0.00 | 0.00 | 9.00 | 0.00 | 1.41 | 0.00 | 0.31 | 0.13 |
| Downwind 350m | 0 | | | Multiple | 1.00 | | | | | |
| Downwind 355m | 0 | | | Multiple | 1.00 | | | | | |

Table 8.2: Basic statistical data for endotoxins at Flixborough

(a) During periods of no activity

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| On-site | 3 | 0.55 | 0.41 | Multiple | 1.00 | 0.23 | 1.00 | 0.23 | 1.00 | 0.23 |
| Downwind 100m | 3 | 0.00 | 0.00 | 0.00 | 3.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

(b) During periods of activity

| | Observations (Number) | Arithmetic Mean | Median | Mode | Frequency of Mode | Minimum | Maximum | Lower Quartile | Upper Quartile | Standard Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Upwind | 9 | 0.19 | 0.00 | 0.00 | 8.00 | 0.00 | 1.74 | 0.00 | 0.00 | 0.19 |
| On-site | 29 | 230.18 | 55.00 | 0.00 | 6.00 | 0.00 | 3672.70 | 7.88 | 78.03 | 132.49 |
| Downwind 50m | 3 | 4.31 | 3.41 | Multiple | 1.00 | 1.59 | 7.94 | 1.59 | 7.94 | 1.89 |
| Downwind 100m | 9 | 6.29 | 2.91 | 0.00 | 3.00 | 0.00 | 18.24 | 0.00 | 11.39 | 2.28 |
| Downwind 150m | 3 | 0.00 | 0.00 | 0.00 | 3.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Downwind 200m | 9 | 4.52 | 1.20 | 0.00 | 4.00 | 0.00 | 17.51 | 0.00 | 9.45 | 2.11 |
| Downwind 300m | 6 | 0.42 | 0.00 | 0.00 | 4.00 | 0.00 | 1.84 | 0.00 | 0.70 | 0.31 |
| Downwind 400m | 6 | 1.66 | 0.74 | 0.00 | 3.00 | 0.00 | 4.84 | 0.00 | 3.64 | 0.86 |
| Downwind 500m | 6 | 3.24 | 0.85 | 0.00 | 3.00 | 0.00 | 14.61 | 0.00 | 3.10 | 2.33 |
| Downwind 600m | 3 | 1.31 | 1.21 | Multiple | 1.00 | 0.00 | 2.71 | 0.00 | 2.71 | 0.78 |

## Graphs for Episodic Sampling Occasions

Figures 1.1 – 1.6 show individual samples taken on occasions dedicated to episodic sampling, with concentrations at two sampling locations measured simultaneously. Times during which no composting activities were being carried out are indicated within the Figures. The aim of this representation of the data was to illustrate the real-time difference in bioaerosol concentrations as the level of site activity changes at two sampling locations.

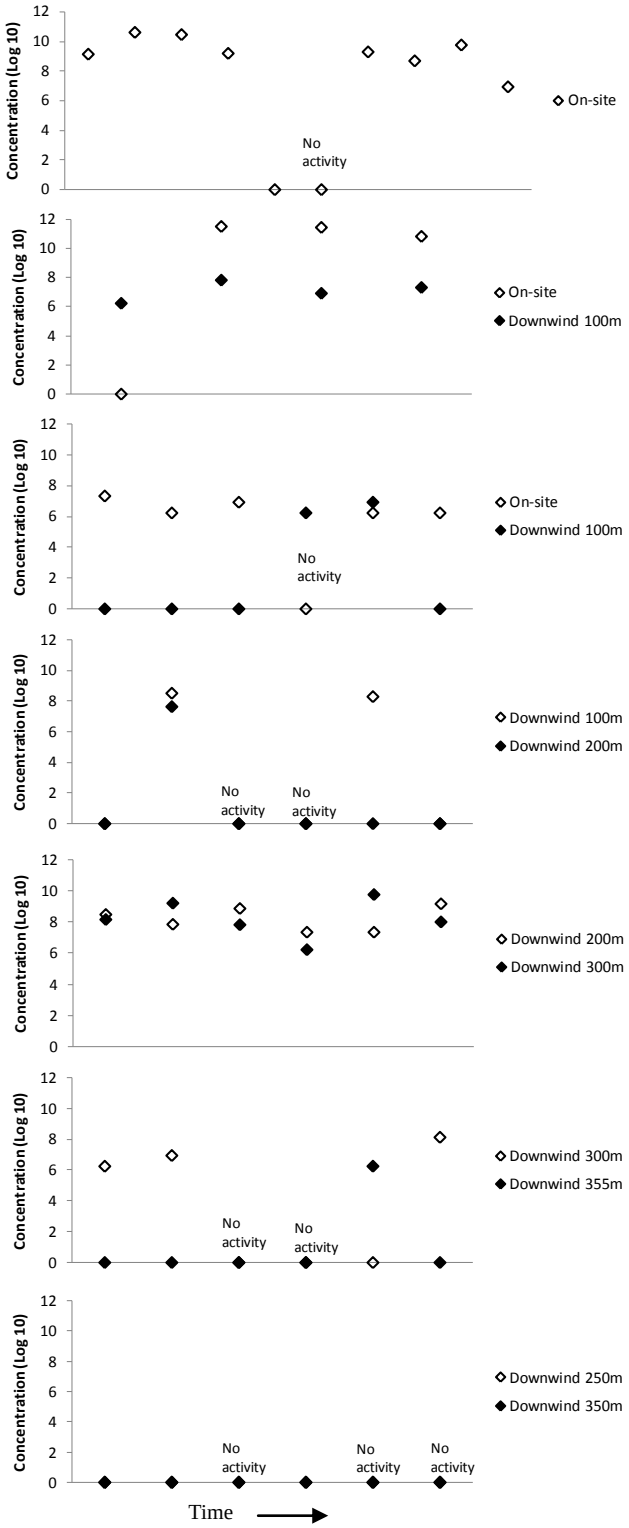



Figure 1.1: *A. fumigatus* concentrations measured simultaneously at two sampling locations at Lount OWC represented as a non-equidistant chronological sequence of sample repeats. Data presented as log 10 concentrations. 'No activity' annotations mark samples during which no operational activities occurred.

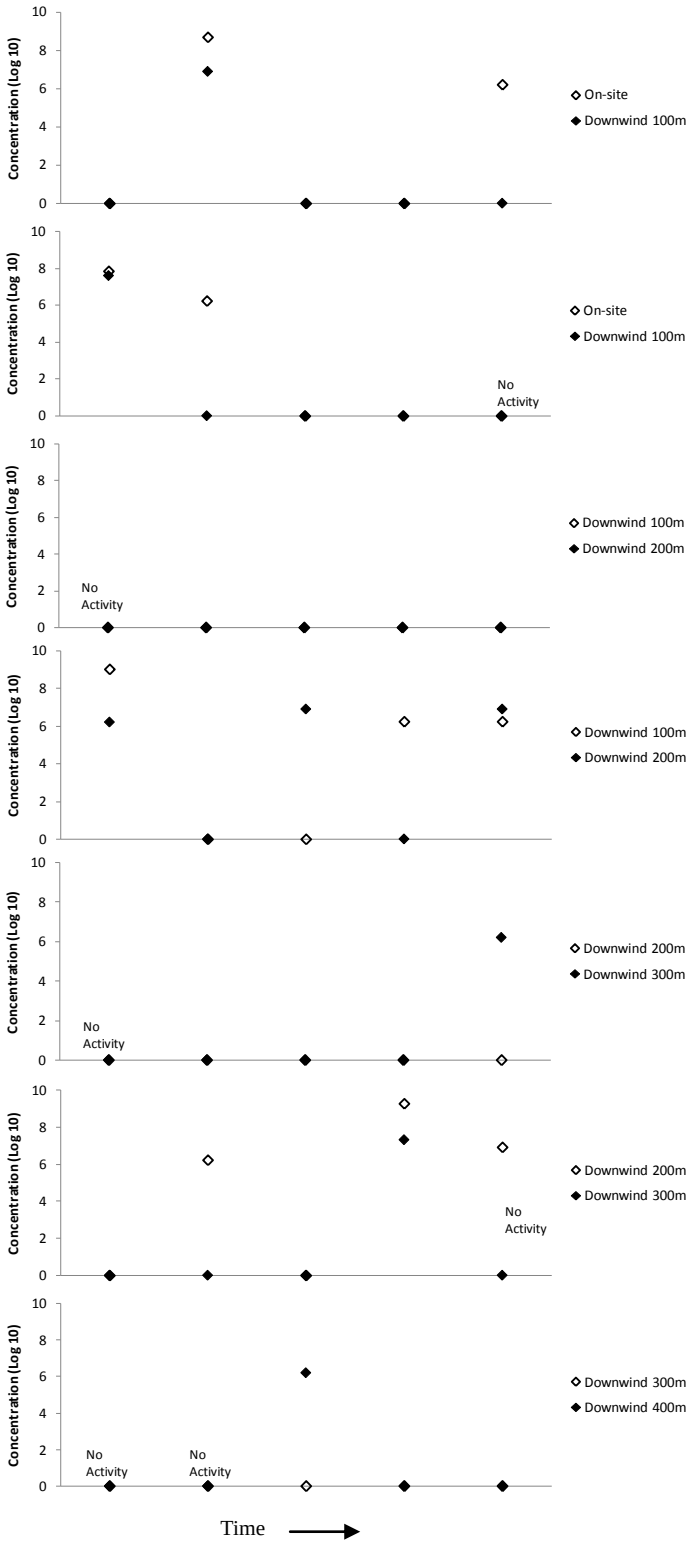



Figure 1.2: *A. fumigatus* concentrations measured simultaneously at two sampling locations at Flixborough represented as a non-equidistant chronological sequence of sample repeats. Data presented as log 10 concentrations. 'No activity' annotations mark samples during which no operational activities occurred.

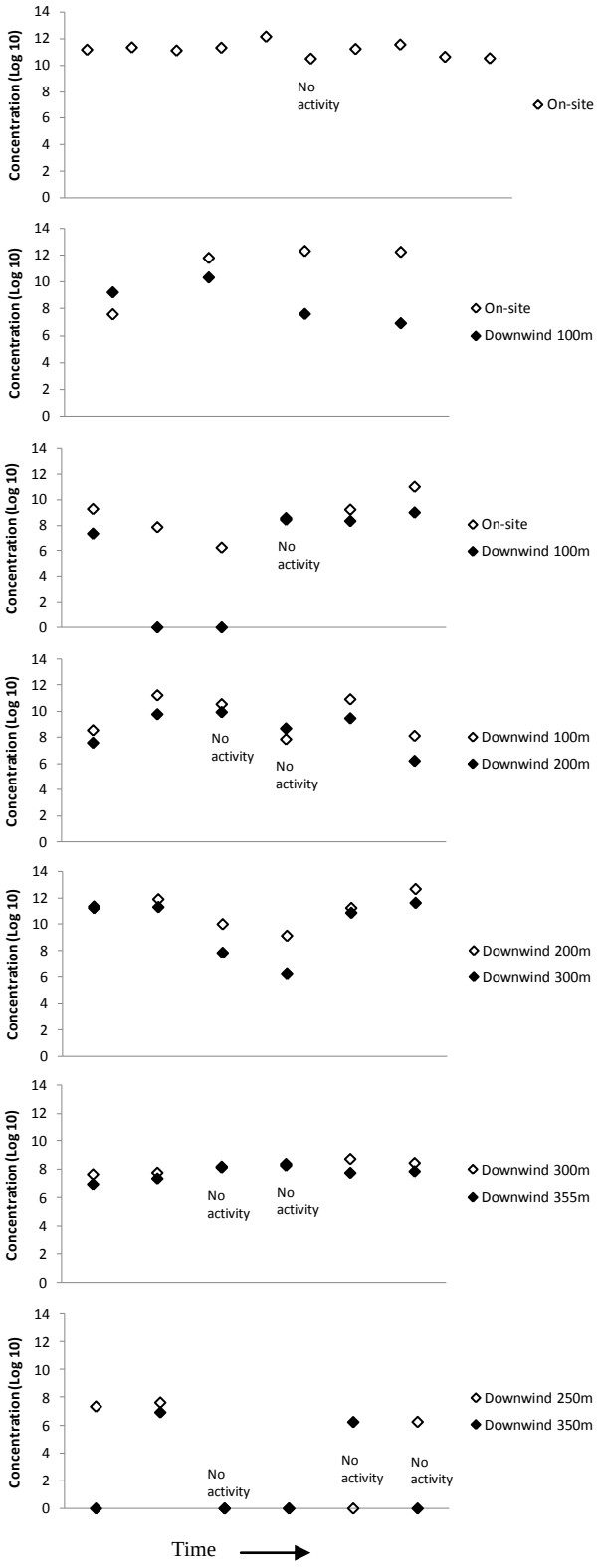



Figure 1.3: Actinomycetes concentrations measured simultaneously at two sampling locations at Lount OWC represented as a non-equidistant chronological sequence of sample repeats. Data presented as log 10 concentrations. 'No activity' annotations mark samples during which no operational activities occurred.

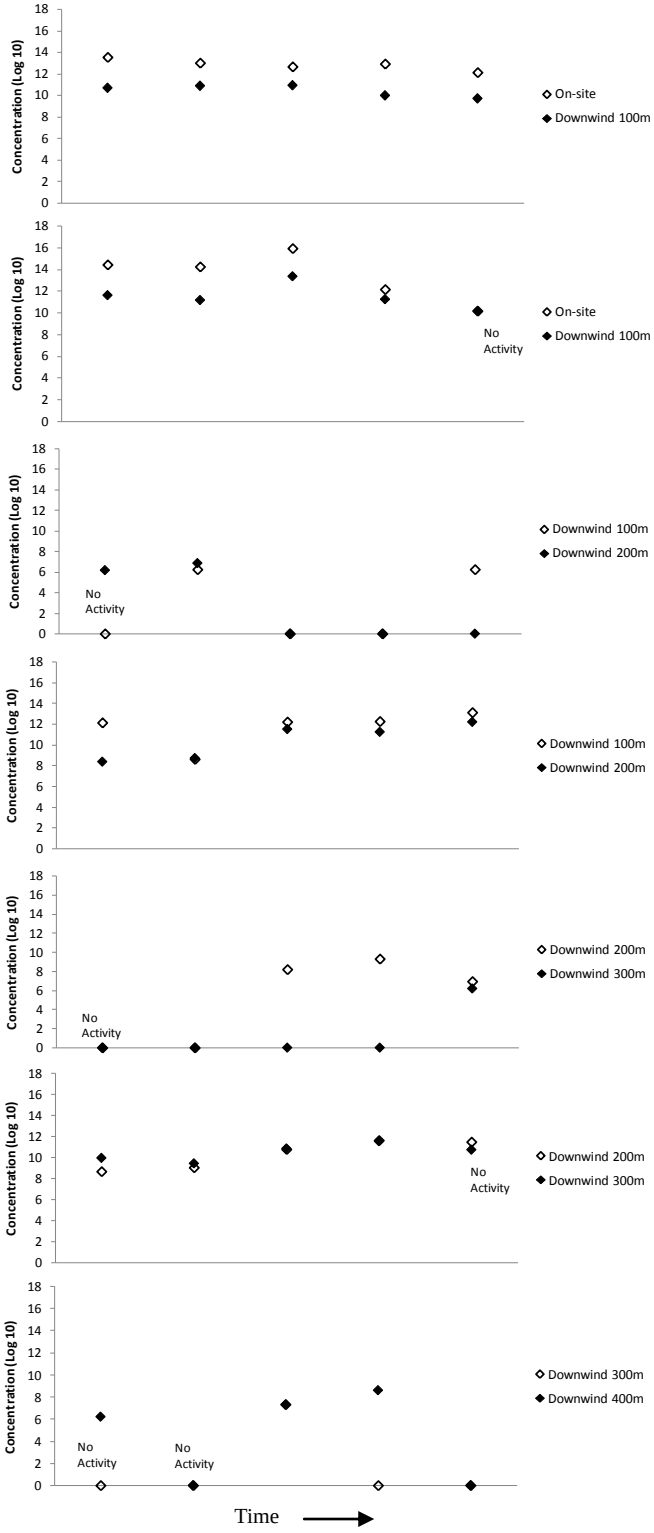



Figure 1.4: Actinomycete concentrations measured simultaneously at two sampling locations at Flixborough represented as a non-equidistant chronological sequence of sample repeats. Data presented as log 10 concentrations. 'No activity' annotations mark samples during which no operational activities occurred.

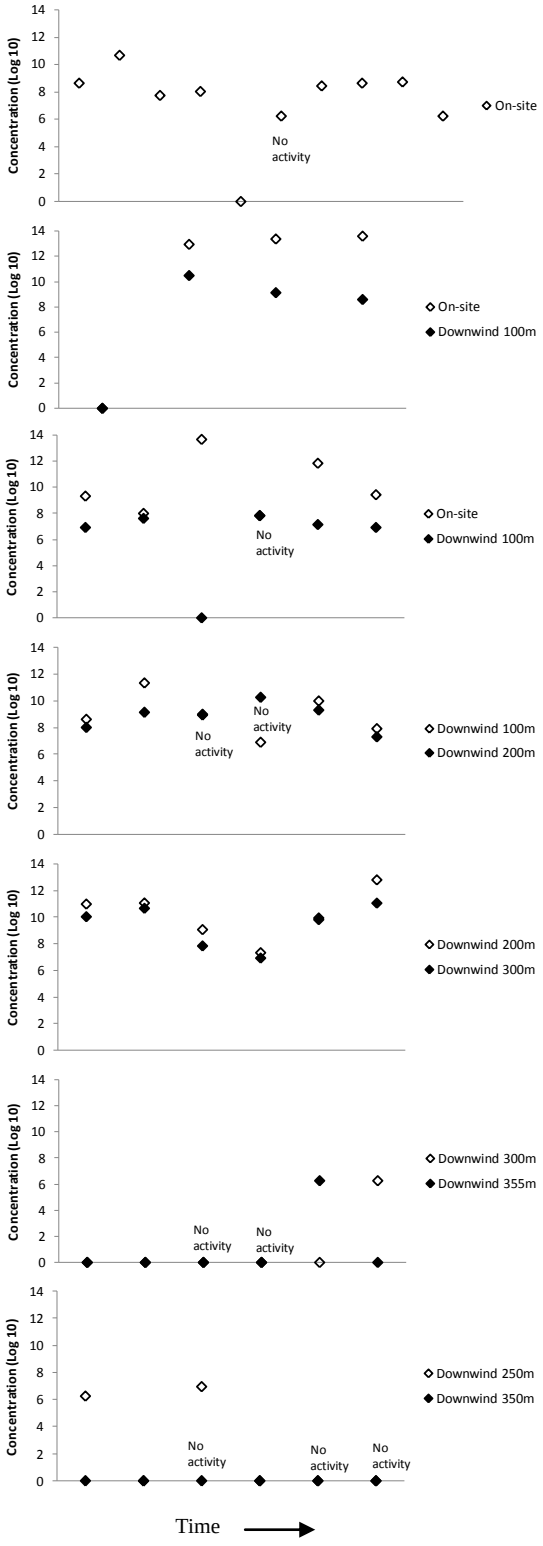



Figure 1.5: Gram-negative bacteria concentrations measured simultaneously at two sampling locations at Lount OWC represented as a non-equidistant chronological sequence of sample repeats. Data presented as log 10 concentrations. 'No activity' annotations mark samples during which no operational activities occurred.

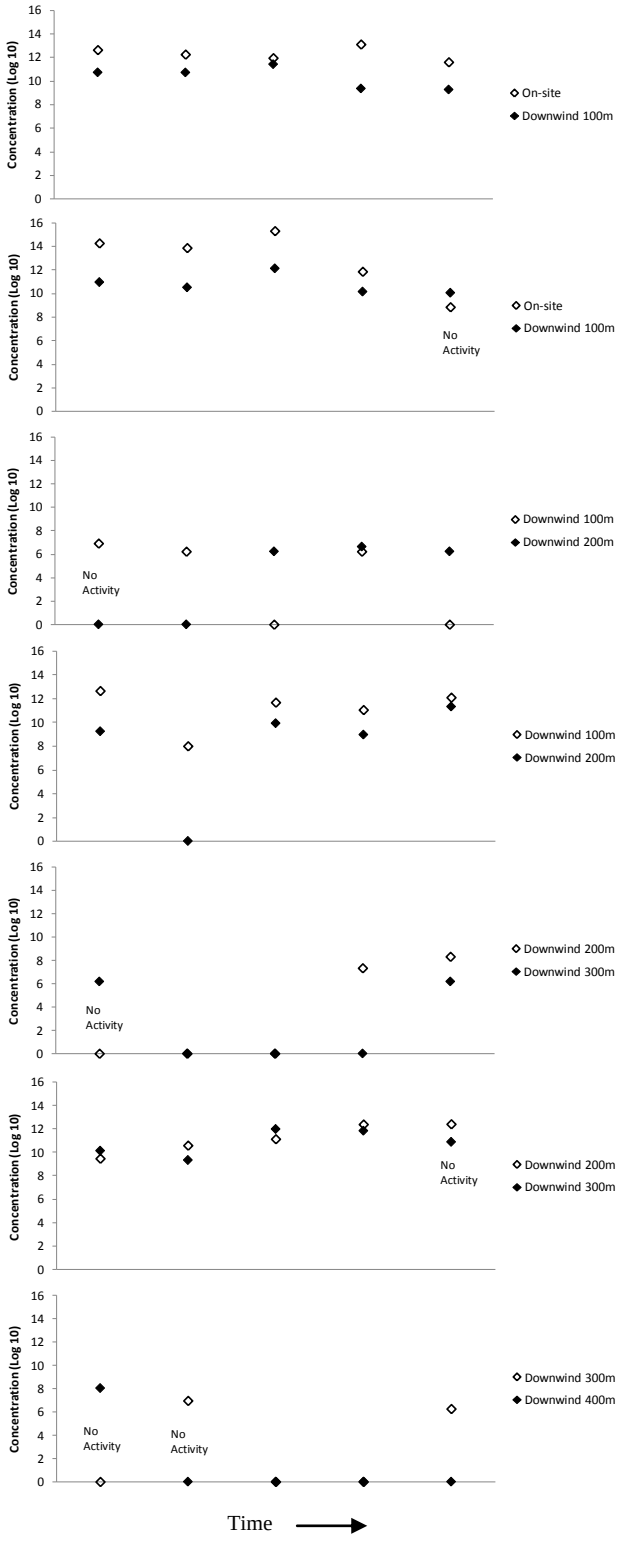



Figure 1.6: Gram-negative bacteria concentrations measured simultaneously at two sampling locations at Flixboroughrepresented as a non-equidistant chronological sequence of sample repeats and presented as log 10 concentrations. 'No activity' annotations mark samples during which no operational activities occurred.

# Appendix II – Section II of Thesis

Table 1.1 Correlation coefficients and p-values found when comparing results generated through culture, endotoxin assay, WIBS3 and P-TRAK. Significant results (those with p-value below 0.05), are highlighted. 'Total', 'Bio 1', 'Bio 2', 'Bio 3', '1 and 3', and '1 or 3' represent particle counts generated by WIBS3. 'P-TRAK' represents particle counts generated by P-TRAK. '*A. fumigatus'*, 'actinomycetes' and 'gram-negative' represent bioaerosols enumerated through culture. 'Endotoxin' represents endotoxins measured through assay. 'Total microb' represents total culturable bioaerosols (*A. fumigatus*, actinomycetes and gram-negative bacteria.

| | Total | Bio 1 | Bio 2 | Bio 3 | 1 and 3 | 1 or 3 | P-TRAK | A. fumigatus | Actinomycetes | Gram-negative | Endotoxin | Total microb |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Total** | 1 | | | | | | | | | | | |
| | p= --- | | | | | | | | | | | |
| **Bio 1** | 0.7459 | 1 | | | | | | | | | | |
| | p=.148 | p= --- | | | | | | | | | | |
| **Bio 2** | 0.6402 | 0.9518 | 1 | | | | | | | | | |
| | p=.245 | p=.013 | p= --- | | | | | | | | | |
| **Bio 3** | 0.3626 | 0.8598 | 0.932 | 1 | | | | | | | | |
| | p=.549 | p=.062 | p=.021 | p= --- | | | | | | | | |
| **1 and 3** | 0.5235 | 0.9338 | 0.9858 | 0.963 | 1 | | | | | | | |
| | p=.365 | p=.020 | p=.002 | p=.008 | p= --- | | | | | | | |
| **1 or 3** | 0.6029 | 0.9724 | 0.9656 | 0.9509 | 0.9677 | 1 | | | | | | |
| | p=.282 | p=.005 | p=.008 | p=.013 | p=.007 | p= --- | | | | | | |
| **P-TRAK** | 0.9011 | 0.561 | 0.5695 | 0.2558 | 0.4339 | 0.4275 | 1 | | | | | |
| | p=.037 | p=.325 | p=.316 | p=.678 | p=.465 | p=.473 | p= --- | | | | | |
| **A. fumigatus** | 0.6256 | 0.2773 | 0.3937 | 0.097 | 0.2715 | 0.1709 | 0.9004 | 1 | | | | |
| | p=.259 | p=.651 | p=.512 | p=.877 | p=.659 | p=.783 | p=.037 | p= --- | | | | |
| **Actinomycetes** | 0.545 | 0.908 | 0.7794 | 0.7683 | 0.8124 | 0.8877 | 0.2279 | -0.1111 | 1 | | | |
| | p=.342 | p=.033 | p=.120 | p=.129 | p=.095 | p=.044 | p=.712 | p=.859 | p= --- | | | |
| **Gram-negative** | 0.6112 | 0.9385 | 0.8179 | 0.7757 | 0.8389 | 0.9055 | 0.3173 | -0.0153 | 0.9953 | 1 | | |
| | p=.273 | p=.018 | p=.091 | p=.123 | p=.076 | p=.034 | p=.603 | p=.981 | p=.000 | p= --- | | |
| **Endotoxin** | 0.8833 | 0.4356 | 0.3468 | -0.0122 | 0.2199 | 0.2346 | 0.9117 | 0.778 | 0.2169 | 0.2987 | 1 | |
| | p=.047 | p=.463 | p=.567 | p=.985 | p=.722 | p=.704 | p=.031 | p=.121 | p=.726 | p=.625 | p= --- | |
| **Total microb** | 0.5947 | 0.9323 | 0.8104 | 0.7765 | 0.8345 | 0.9031 | 0.2952 | -0.0389 | 0.9974 | 0.9997 | 0.2772 | 1 |
| | p=.290 | p=.021 | p=.096 | p=.122 | p=.079 | p=.036 | p=.630 | p=.950 | p=.000 | p=.000 | p=.652 | p= --- |