Final Report:

Defra Project WR 1121 Bioaerosols and odour emissions from composting facilities

Authors:

Melanie Williams Baptiste Lamarre David Butterfield	NPL
Sean Tyrrel Phil Longhurst Gillian Drew Reyna Al-Ashaab Alan Nelson	Cranfield University
Toni Gladding Annika Simpson	Open University
Deborah Coughlin Anna Hansell	Imperial College London

20 August 2013

Executive Summary

Government policy requires that valuable resources should be recovered and recycled from biodegradable waste. A successful and growing organics recycling industry delivers this policy with composting being one of the principal technologies deployed to process suitable feedstock such as garden and food waste. Composting inevitably generates bioaerosols – particulate matter comprising cells or cellular components that are released into the air as a result of processing of composting feedstock and the preparation of final product. Exposure to bioaerosols has the potential to be harmful to human health. The Environment Agency adopts a precautionary approach to the regulation of composting facilities which was developed on the basis of research by Wheeler et al. (2001). As new evidence has become available no information has suggested a material change to this approach. The Environment Agency also requires site operators to monitor bioaerosols if they have sensitive receptors within 250m around their facilities using methods specified in a standard protocol which relies upon classical microbiology methods which are tried and tested but which are labour-intensive, slow and offer only a snapshot view of a highly dynamic system. A recent IOM review commissioned by Defra (Searl, 2009) on exposure-response relationships for bioaerosol emissions from waste treatment processes identified significant gaps in knowledge of exposure to bioaerosols and recommended that more research was needed into alternatives to viable microbial monitoring such as endotoxin and potential surrogates such as particulate matter. The IOM review also concluded that there is a lack of information to support the relaxation of current precautionary stand-off distances.

The overall aim of this project was to provide evidence on bioaerosol production, dispersion and potential exposures from composting facilities in support of future developments in policy and regulation of biowaste facilities. The objectives were: (i) to undertake a comprehensive set of standard and novel bioaerosol measurements at representative composting sites to assess comparability between different methods and also to measure spatial and temporal variations; and (ii) to determine the odour emissions and then compare these with bioaerosol emissions to see if odour is a marker of significant bioaerosol exposure. Standard (AfOR, 2009) and novel (CEN filter method, endotoxin, glucan, qPCR, real-time particulates) bioaerosols measurements were taken on a minimum of three to a maximum of six occasions over a twelve month period at four different composting facilities in England. The composting facilities were selected to represent sites of varying sizes (tonnages) and feedstocks and to allow a comparison of bioaerosol concentrations at standard open windrow sites as well as mixed open and in-vessel and a fully-contained site. Additional supporting information was collected including meteorological data at the time of sampling, observation of site operations and measurements of odour at one of the sites. Supporting bioaerosol and odour dispersion modelling was conducted at the site where the odour measurements were made.

The spatial trend of bioaerosol concentrations described by Wheeler et al., (1991) and upon which EA regulatory policy is based was broadly corroborated by this dataset. Excursions above the EA acceptable levels at or beyond 250m from source were rare. Bioaerosol concentrations at the enclosed site were generally lower than at the open windrow sites. There was no evidence of a seasonal pattern in bioaerosol concentrations at any of the sites

whereas between-sampling day variations were apparent. The cause(s) of these variations were not identified.

No consistent relationship was observed between the concentrations of bioaerosols measured by the two AfOR standard methods. The two methods displayed certain strengths and weakness in different situations. The IOM method (as defined in the AfOR protocol) proved to be well-suited to situations where high bioaerosol concentrations were encountered (close to source) but less appropriate at the lower concentrations typically found upwind of sites or at 250 m downwind from source. Conversely, the Andersen sampler is comparatively sensitive in the lower concentration range but is not the most appropriate device for situations where higher concentrations are expected due to its vulnerability to overloading. The higher volume filtration device tested in this project (referred to as the CEN method) produced data that did not consistently match either of the AfOR standard methods. This device demonstrated greater sensitivity than the IOM filter method (it has a lower limit of detection) but suffered drawbacks associated with its weight and a lack of ease of use in the field.

Endotoxin concentrations were normally below the level recommended by the Dutch Expert Committee on Occupational Safety (90 EU/m^3 as a marker of effects on lung function after 'chronic occupational exposure') but occasional exceedances of this standard were detected at the larger open windrow sites (up to 281 EU/m^3 in one instance). The majority of glucan measurements were below a widely referred to 10ng/m^3 threshold. However, significantly elevated concentrations of glucan (in eleven measurements of between 74 to 4093 ng/m^3) were detected at one of the larger open windrow sites.

The dynamic range of the qPCR method is wider (4-5-log) than either of the AfOR and the CEN methods. It is also quicker to carry out and has the potential for automation. The results from the qPCR method are mainly higher than standard AfOR methods, as the method does not distinguish viable and non-viable spores. The spatial distribution of *Aspergillus fumigatus* spores (by qPCR) along sampling transects, gives similar results compared to AfOR (and CEN) methods. Real time particle detection showed that both TSP and PM_{10} are correlated to *Aspergillus fumigatus* spore concentration. This gives rise to the possibility that particles could be considered as a "tier 1" screening measurement.

No consistent relationship was observed between odour and bioaerosol concentrations (although this was a limited dataset). The envelope of modelled (back-extrapolated) bioaerosol emission rates straddles several orders of magnitude. Distinguishing the influences of individual meteorological parameters on this variability was not possible. It was not possible to predict bioaerosol or odour emission rates with confidence. This continues to hamper confidence in modelling of odours and bioaerosols from open windrow facilities.

The findings of this research have implications for the current standard monitoring protocol which should be reviewed accordingly. The findings of this multi-site survey accord with existing regulatory policy. They are also supportive of the concept of using enclosed facilities to mitigate bioaerosol emissions. Notwithstanding this, continuing research is needed to enhance the database on emission from bioaerosol and odour abatement technologies (e.g. biofilters), to determine the cause(s) of occasional bioaerosol peaks, to improve exposure assessments through longer duration sampling and better modelling protocols, and to link enhanced exposure information to future health impact studies.

Table of contents

1. Background to the study

1.1 Introduction

Government policy requires the diversion of biodegradable waste from landfill with the objectives of reducing methane emissions; compliance with Landfill Directive targets; and recovering the maximum value from waste through increased recycling. As a low cost alternative to landfill, composting successfully processes several millions of tonnes of biodegradable waste per year. The composting industry has grown significantly in recent years with approximately 280 licensed sites in the UK currently operational (Gilbert et al., 2011) and the number expected to rise in the coming years.

As a natural microbiological process, composting generates large numbers of microorganisms including fungi and bacteria which are involved in the biodegradation of the organic feedstock. When composting materials are moved or processed these microbial cells and associated by-products may be emitted into the air as bioaerosols. It is inevitable that composting processes will lead to the release of bioaerosols into the air to some extent.

Bioaerosols emitted into the air have the potential to be transported off site and therefore people who are living or working nearby may be exposed. If humans are exposed to bioaerosols there is a risk of adverse health impacts. The magnitude of that risk and the relationship between exposure and health outcomes are not well understood. Human exposure to bioaerosols has the potential to rise over the next decade as a result of the increasing diversion of biodegradable municipal waste from landfill to composting sites. It should also be recognised that other production processes such as intensive livestock production and the recycling of organic fertilisers to land also have the potential to generate bioaerosol. Whilst this report focusses on composting, there is potential for new knowledge to be transferred to the regulation of emissions from related industries in the future.

The Environment Agency currently adopts an approach which is developed on the basis of research by Wheeler et al.(2001) which showed that concentrations of bioaerosols mostly decayed to background levels within 250 metres of open composting sites. The report presented measured and modelled data on the dispersion of bioaerosols from composting facilities and reviewed available literature. It recommended that:

 "Based on the available limit values and the modelling of the bioaerosol emissions, as well as the evidence from other studies, it is recommended that composting sites should not be sited closer than 250 metres from housing or other sensitive receptors such as schools and hospitals. Where composting technology or mitigation techniques are employed which can be demonstrated to reduce the emission below the reference levels discussed in this report, then this distance can be reviewed on a case-by-case basis. Factors that need to be considered in the assessment are the scale of operation, wastes treated and the containment" (Wheeler et al., 2001).

Since 2001 in England and Wales, the Environment Agency's policy has been one of a presumption against authorising new sites less than 250 m from dwellings or workplaces, unless a site specific bioaerosol risk assessment (SSBRA) shows that bioaerosol levels can be maintained at acceptable levels (as defined by the Environment Agency) at the dwelling or workplace (Environment Agency, 2007). Since 2010, the Environment Agency has required additional controls on sites processing large amounts of waste within 250 m of a sensitive receptor:

WR1121 Final Report (20.8.13)

"The interim position for such sites ...[i.e. if the quantity of waste handled exceeds 500 tonnes].....is that, subject to the SSBRA assessment, applicants will be issued permits....[only if]...the operations are carried out in a way and with the necessary measures (e.g. negative aeration, enclosure) to ensure that they are not likely to result in the uncontrolled release of high levels of bioaerosols" (Environment Agency, 2010).

Subsequent research from the Health and Safety Laboratory (HSL)(Stagg *et al.,* 2010) and Cranfield University (Pankhurst *et al.* 2011) has added to and broadly supported the main thrust of the 2001 conclusions from Wheeler et al., i.e. that bioaerosol concentrations from composting sources tend to fall towards background levels within 250 m of their source. It has been evident from this research that these concentrations may be quite variable and that periodically, elevated concentrations may be detected at some distance from the emission source. It should be noted however that background concentrations are subject to temporal variability and that measurements taken at distance from composting facilities are subject to interference from other emission sources.

Since the publication of the Environment Agency's latest position statement on composting and bioaerosols in November 2010, acceptable levels have been defined as:

"the concentrations of bioaerosols (as predicted or as derived from direct measurements) at the sensitive receptors which are attributable to the composting operations. The acceptable levels are 300, 1000 and 500 CFU/m^3 for Gram negative bacteria, total bacteria and Aspergillus fumigatus respectively, as measured by the standardised monitoring Protocol" (AfOR, 2009; Environment Agency, 2010).

These acceptable levels were originally proposed within the report by Wheeler *et al*. (2001) based on data available in the literature (the value of 1000 CFU/m^3 for fungi originally proposed was later made species specific (for *A. fumigatus*) and then subsequently reduced to 500 CFU/m^3 based on available information on background concentrations). Wheeler et al. (2001) originally proposed these levels as modelling benchmarks – effectively recognising the variability of background air quality – as opposed to safe levels from a public health perspective. It remains the case that the scientific evidence on health risks from bioaerosol exposures is unclear and exposure information is incomplete. A recent IOM review commissioned by Defra (Defra, 2009) concluded there were insufficient data to set exposure guidelines for most components of bioaerosols except endotoxin and identified significant gaps in knowledge of both exposures and health effects. In the absence of health-based guidelines the Environment Agency considers the existing acceptable levels to be appropriately precautionary.

Research carried out for Defra/EA in 2009, by the National Physical Laboratory focussed on new, more rapid measurement techniques for *A. fumigatus* (Brown et al, 2009). A method (qPCR) based on DNA detection was tested at an open-windrow site. The method detects both viable and non-viable spores rather than just the viable ones which form colonies, and thus gives a higher result than "traditional" methods, but one which is sensitive, reproducible and easier to carry out. This method has been further tested in this project. Concentrations of particulates (PM$_{10}$), measured at the same site, showed potential as an indicator for high bioaerosol concentrations and this is also investigated further. The method is suitable for automation into the future which means it has the potential to be less costly than the traditional microbiological method.

Whilst important progress has been made since the publication of the report by Wheeler *et al* in 2001 in the field of bioaerosols and composting, there remain a number of key questions that the research described in

2

this report sought to address and which have important implications for the evolving policy and regulatory situation. These include:

i.   Is the current standard monitoring Protocol fit for purpose or are modifications required to take into account new sampling and analytical technologies?

ii. Does the Environment Agency's current precautionary regulatory position need to be reviewed as a result of the emergence of new evidence?

iii. To what extent are bioaerosol concentrations surrounding composting sites affected by factors such as tonnage of waste on site or by the degree of containment?

iv. To what extent is dispersion of odours and bioaerosols correlated?

v.   How well do dispersion models simulate measurements of bioaerosol concentrations?

vi. Can particulate measurement be used routinely as an indicator for high bioaerosols concentrations?

vii. With what level of confidence can we describe the extent of exposure of communities neighbouring composting facilities to bioaerosols?

1.2 Objectives

Objective 1

To undertake a comprehensive set of standard and novel bioaerosol measurements at representative composting sites to assess comparability between different methods and also to measure spatial and temporal variations. Objective 1 is further refined into the following sub-objectives:

1.1 Undertake bioaerosol measurements at representative composting sites using standard AfOR techniques to measure spatial and temporal variations

1.2 Compare measurements using standard AfOR techniques with the CEN method (an alternative filter method)

1.3 Compare measurements using standard AfOR techniques with real-time particulate detection and a PCR-based detection method for *Aspergillus fumigatus*

1.4 Compare measurements using standard AfOR techniques with measurements of the priority bio-markers endotoxin and glucan

Objective 2

To determine the odour emissions and then compare these with bioaerosol emissions to see if odour is a marker of significant bioaerosol exposure. Objective 2 is further refined into the following sub-objectives:

2.1 Undertake olfactometric measurements of odour concentration at representative composting sites concurrent with bioaerosol measurements

2.2 Model the dispersion of odour and bioaerosols from site- sampled emissions to determine the extent to which odour is a marker of significant bioaerosol exposure

1.3 Approach

This report describes the findings of a study undertaken by a team from NPL, Open University, Cranfield University and Imperial College working in collaboration with project advisors from Defra and the Environment Agency. Standard and novel bioaerosols measurements were taken on a minimum of three and a maximum of six occasions over a twelve month period at four different composting facilities in

England. The composting facilities were selected to represent sites of varying sizes (tonnages) and to allow a comparison of bioaerosol concentrations at standard open windrow sites versus a fully contained site. Additional supporting information was collected including meteorological data at the time of sampling, observation of site operations and measurements of odour at one of the sites. Supporting bioaerosol and odour dispersion modelling was conducted at the site where the odour measurements were made.

2. Sites

2.1 Site selection

Four sites were included in this study. The principal factors governing their selection included:

- Sites must span the range of tonnages typical of UK composting.
- One site should be fully-enclosed and the remaining three should use standard open windrow techniques.
- The sites should accept feedstocks typical of UK composting i.e. garden waste with some food waste.
- The sites should have reasonable access for sampling on-site and on neighbouring land up to 1km away.
- The site operators must provide reasonable access for sampling and supporting information.

2.2 Site descriptions

The sites are referred to as sites A-D.

<u>Site A</u>

This is one of the largest operational compost sites in the UK, and has been operational since 2006. There are two processes on this site, a green waste windrow pad and a separate IVC facility and maturation pad. The green waste process accepts garden and similar biodegradable materials and the pad processes up to 30,000 per annum. It is estimated by the site managers that 7,000 tonnes of material are on site at each pad at any one time. The IVC/maturation pad accepts food and other biodegradable materials and processes up to 30,000 per annum. The IVC facility is enclosed with enforced aeration, which vents via a biofilter consisting of woodchip and compost material. Material from the IVC is then placed out onto an open windrow system for final maturation. The area has fields on two sides, adjoins a main road and is part of a larger commercial complex. The nearest sensitive receptor is a caravan site within 250m of the permitted area of the site. Both composting areas are adjacent to an adjoining aggregates yard.

<u>Site B</u>

This is a family-run business and has been producing fine quality compost for use in agriculture, horticulture, landscaping and gardening since 1997. Their soil improver products are produced in open windrows over a period of 8 weeks; the organic feedstocks processed include garden waste, fruit & vegetable waste, straw, stable waste, paper and card, or any other organic material not included in the Animal By-Product Regulations and permitted by BSI PAS100. The operational area is about 6 hectares which sits within a farm of about 22 hectares. The site managers estimate that the tonnage of material at any one time would be 6,000 tonnes at peak times in the summer falling to 4,000 tonnes at other times of year. The site is fully licensed by the Environment Agency and their products are BSI PAS100 certified. The site is well served with paved and unpaved roads which allow easy access in all directions for sampling. It is situated in a rural location, surrounded by relatively flat arable agricultural land with few buildings, hills or other large obstructions. The nearest sensitive receptors are located about 500 m (NE) and over a kilometre (NW) away.

WR1121 Final Report (20.8.13)

<u>Site C</u>

This site uses a 'table top' system of batch composting which lasts 8 or 16 weeks, with a maximum capacity of the site up to 75,000 tonnes per annum, consisting of a limit of 50,000 tonnes per annum of green waste and the remainder wood waste. At any instance in time the maximum amount of material being composted is 12,000 tonnes plus a maximum of 600 tonnes of raw material waiting to be shredded. This will mean a total of pre shredded and composting material on site at any one time of 12,600 tonnes. A maximum of 750 tonnes of processed wood and 750 tonnes of unprocessed wood waiting to be shredded is also permitted on site. The perimeter of this site is 220 meters away from the boundary of the nearest residential property. The presence of the blast walls and the soil bund between the composting site and the housing, plus planted trees is intended to screen bioaerosol spread.

<u>Site D</u>

All treatment and maturation takes place within a building which has negative pressure (hence air is drawn in on opening of doors). Materials accepted include food wastes. Currently the facility operates at around its operation capacity of 28,000 tonnes per annum. The site managers estimate approximately 3,000 tonnes of material are being processed at any time. There is a 20m stack and two biofilters on site, one containing a woodchip medium and the other a shell medium. The wood chip biofilter is open whilst the shell biofilter vents via the stack. The area around the site is surrounded by fields on three sides, with a long roadway entrance from a main road. Sensitive receptors are present at the end of the entrance road at a distance of around 150m.

3. Site sampling overview

3.1 Site sampling criteria

Sampling events were possible when:

- The site operators approved a sampling visit on a particular date.
- There was no rain or fog on the sampling day (sampling was avoided where possible when there had been heavy rain on the previous day). Wet or humid conditions prevent the proper operation of the sampling equipment and prevented access to some sampling locations.
- The wind was blowing in a direction that permitted downwind sampling.
- Temperatures were above 0 ^{o}C (it should be noted that the protocol specifies 3°C but this was expanded for the purposes of this research to accommodate more sampling days, with the proviso that temperatures below 3°C may adversely affect some of the sampling equipment).

3.2 Site sampling schedule

Site A

1. March 2012
2. June 2012
3. September 2012
4. November 2012

Site B

1. July 2011 (trial run)
2. October 2011
3. February 2012
4. June 2012
5. September 2012
6. December 2012

Site C

1. September 2011
2. January 2012
3. May 2012

Site D

1. September 2011
2. February 2012
3. May 2012
4. September 2012

3.3 Site sampling approach

Figure 1 represents an idealised sampling arrangement in which bioaerosol measurements are taken along a central transect running parallel to the direction of the wind. The intention is to be able to compare bioaerosol concentrations upwind of the site (no influence of the site), to observe the impact of the site (by sampling on site – subject to health and safety considerations), and then to establish at what distance bioaerosol concentrations return to background levels. In practice, the sampling pattern did not conform to the idealised arrangement due to changing wind direction during the sampling day and an inability to access certain sampling points (Figure 2).The precise location of each sampling point was recorded using a GPS.
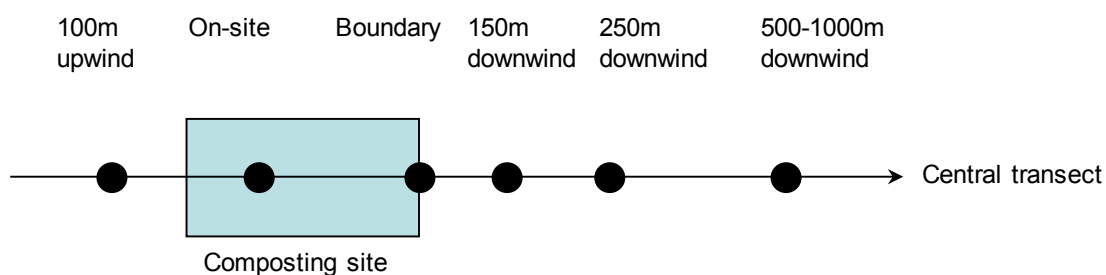

Figure 1 Idealised central traverse sampling arrangement for sequential sampling assuming no change in wind direction during sampling day
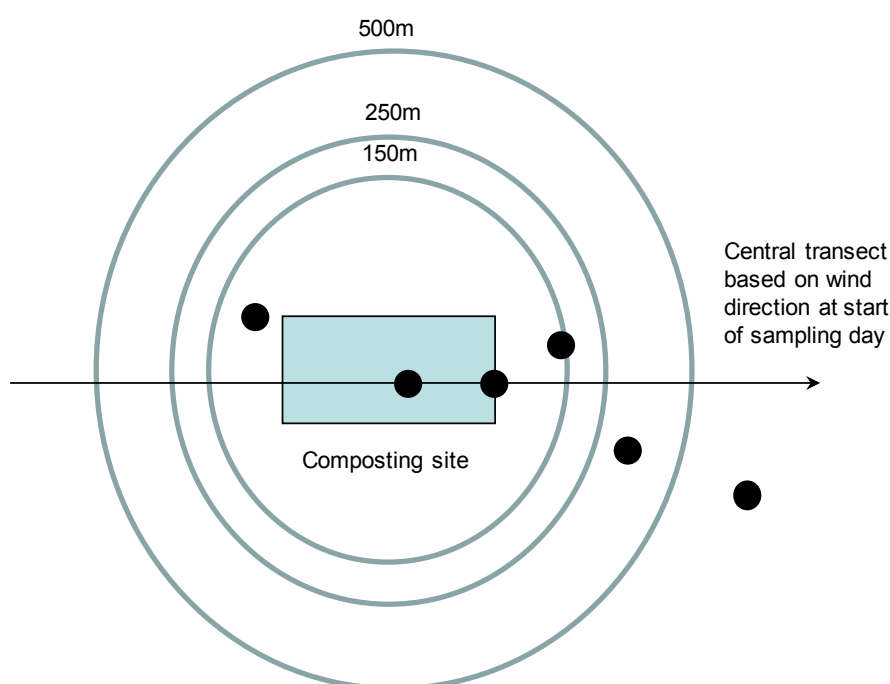

Figure 2 Illustration of an actual sampling arrangement for a sampling day where wind direction is variable during sampling day. Designated sampling distances are not always achievable due to site access constraints.

3.4 Bioaerosol sampling and analysis

Standard AfOR sampling methods

Wherever practically possible, samples for subsequent analysis of the viable microorganisms specified in the AfOR Protocol (*Aspergillus fumigatus* and total mesophilic bacteria) were taken at each sampling point using

8

both the Andersen and IOM filter sampling approaches for comparison. Andersen samplers were deployed in multiples of four and IOM filters in triplicate at each sample point (except that the Andersen was not deployed close to source as it is well established that this device is prone to overloading, e.g. Stagg *et al* 2010, Zhao *et al* 2011). Samples for the enumeration of Gram negative bacteria were also collected. Sampling times, agars and incubation time / temperature combinations were used as specified in the AfOR Protocol for total bacteria and A. fumigatus for both Andersen and IOM filter method samples. It should be noted that for viable Gram negative bacteria MacConkey Agar plates were used. These are not specified in the AfOR protocol, but are taken from EA risk assessment guidance (EA 2009) and consist of incubation at 37°C for 48 hours and a further check on growth after 3-4 days. Andersen samplers operate at 28.3 l/min for between 2 and 10 minutes, and hence the volume of air sampled was between 56 to 280 litres. Filters operate at 2 l/min for between 30 and 45 minutes, equating to 60-90 litres. The limits of detection (LOD) were calculated by the project team for both methods, at 4 cfu.m^{-3} and 185-278 cfu.m^{-3} for the Andersen and IOM methods respectively.

Alternative filter method - CEN/TS 16115-1:2011

A potential drawback of the IOM filter method presently used in the AfOR standard Protocol is its high lower limit of detection relative to the Andersen sampler. This makes it relatively insensitive to the concentrations of bioaerosols that might typically be expected upwind and, in some cases, further downwind of a composting facility. This is in part attributable to the relatively low flow rate used. A CEN Technical Specification CEN/TS 16115-1:2011based on an established German standard (VDI 4252) is available which uses a higher flow rate. A Sven Leckel sampler and bioaerosol sampling head were used with pre-loaded cassettes consisting of 80mm diameter gelatine filters, with a polycarbonate filter backing of 90 mm trimmed to the appropriate size (Sartorius Stedim Ltd 17528-80-ACD). This equipment was operated at 50 l/min for 20 minutes which was compliant with the CEN standard Technical Specification. This sampling method is designed for longer duration/higher flow rate sampling of fungi, and hence was used to take samples for subsequent analysis of *A. fumigatus*. Samples were normally taken at two or three of the sampling points during a sampling event (50m, 100m and 150m from source) depending upon the practicalities of the deployment on a given day (each sampler is 25kg and needs a 24v electrical supply which does limit deployment in the field). Samples taken using the CEN method were analysed using the AfOR standard agar and incubation time / temperature specifications to enable like-with-like comparison with samples taken using the Andersen and IOM filter methods. This method is able to sample for 8 hours continuously, but in this study times were limited to 20 minutes to enable comparisons to be made with other shorter running equipment such as the Andersen and filters. This means total air sampled was 1000 litres. The limit of detection for this sampler is quoted as 1 colony on a plate (CEN/TS 16115-1:2011), based on the sample time and extraction method used in this study, the LOD was calculated by the project team as 67 cfu/m^{3}.

Bertin Coriolis cyclone sampler

The sampler draws air at a flow rate of 630 litres per minute through a fine mesh to remove large airborne material, such as small leaves etc. and feed into a cyclone to form a vortex. The sampler pumps liquid into the cyclone before sampling and during sampling to keep the inner wall of the cyclone wet. The centrifugal forces within the vortex push any airborne particulate matter to the walls of the cyclone, where it is entrained in the liquid film and trapped. At the end of the sample, the sample pump stops and the liquid and trapped

particulate mass drain into a chilled container. This container is then removed from the Bertin and transported under refrigeration, back to NPL for analysis.

- Sample flow rate   630 L/min

- Sample time   10 minutes

- Total air volume sampled   6300 litres

Topas particulate matter monitor

The sampler uses a light scattering technique to determine the concentration of airborne particles and dust in the size range from ~0.4 µm to ~20 µm in optical (as opposed to aerodynamic) diameter. Above 20 µm, all particles are sized as 20µm. *Aspergillus* spores are generally in the size range 2 – 3 µm so are easily detected.

Air sample is continuously drawn into the instrument by a pump with a flow rate of 600cc per minute. The incoming air passes through a laser beam and photometer and then through a filter to remove the particles before reaching the pump. The light scattered by the individual particles of dust is converted into an electrical pulse, which is proportional the size of the particle. From using an assumed density for particulate mass, the mass concentration is calculated from the measured size. The standard assumed mass for particulate analysers is that of Arizona Road Dust, which has a density of 0.9 – 1.2 $g.m^{-3}$. This is similar to the expected density range for fungal spores (Eduard, 2009) 0.4 - 1.5 $g.cm^3$, therefore the mix of spores and other particulates does not significantly affect the concentration measured by the Topas analyser.

- Sample flow rate   0.6 L/min

- Sample time   30 to 45 minutes

- Total air volume sampled   18 - 27 litres

qPCR method for *A. fumigatus* spores detection and quantitation:

During air sampling, bioaerosols are impinged into a liquid phase that facilitates the biomolecular analysis of specific micro-organisms such as *Aspergillus fumigatus* (http://randd.defra.gov.uk/Document.aspx?Document=WR0605_8574_FRP.pdf).

Nucleic acids are extracted from samples by mechanical cell wall disruption using bead beating. Specific fungal mitochondrial DNA target is amplified by qPCR during thermo-cycling using a Smartcycler (Cepheid). During the polymerase chain reaction, the 3'-5' exonuclease activity of DNA polymerase degrades a specific probe that leads to the emission of fluorescence. The fluorescence intensity values are measured during each cycle. The Ct (Cycle threshold) value is defined as the number of cycles required for the fluorescent signal to cross the fluorescence threshold value corresponding to 15 times the background fluorescence. Ct levels are inversely proportional to the amount of target nucleic acid in the sample. So, the total amount of spore DNA in an air sample could be measured accurately by referring to a standard curve processed in the same way using a known concentration of spores. The concentration of spores is then correlated to the volume of liquid collected and the total volume of air sampled to express results in spores per cubic metre. The qPCR method is quite sensitive and it can be subject to the effects of interfering molecules and/or chemicals that may be present within air samples. To identify the presence of qPCR inhibitors, an internal control was run for each sample. If the difference between the expected Ct value and the Ct value obtained

WR1121 Final Report (20.8.13)

experimentally for the internal control assay was higher than 1 cycle, this may indicate the presence of qPCR inhibitors/competitors. In most cases, the inhibition was minimised to negligible levels by diluting the sample. The diluted sample was then re-analysed and the spore concentration was re-calculated taking into consideration the dilution factor.

- Limit of detection:

The genomic target of the qPCR is a multi-copies gene present in each *Aspergillus fumigatus* spore. The genomic target of the qPCR is a multi-copy mitochondrial tRNA gene (Genebank accession number: L37095). The number of mitochondrial genes that are amplified is estimated to 9–10 per single copy gene. (Costa and al. 2001; Bretagne and al. 1995, 1998)

We measured the lower LOD as equal to 0.8 spore equivalent per qPCR reaction, which is slightly better than that published in Brown and al. (2009). We have optimised the extraction procedure since the earlier report was published, resulting in less damage to the DNA, which leads to higher Ct values and so a better LOD.

The volume of air sample used per reaction is equal to 5 microliters that makes 160 spores per millilitre. The average volume of liquid recovered from the Coriolis sampler is 16 millilitres. A sampling time of 10 minutes represents a volume of air of $6.3m^3$. So, the LOD per cubic metre is calculated as followed:

(Volume of liquid / Volume of air sampled)* LOD per millilitre

So, 16/6.3*160= 406 spores per cubic metre
  - < 1 spore per qPCR reaction
  - < 500 spores per cubic metre

- Dynamic range:
  - Four to five orders of magnitude

Endotoxin and β glucan

Endotoxin and glucan were sampled as per the IOM AfOR (2009) methodology at 50m, 100m and 150m. For analysis, a kinetic chromogenic LAL assay (ACC, Associates of Cape Cod, Inc.) was used for quantification of endotoxin at 37°C, with kinetic readings recorded automatically every 30 seconds for a period of 90 minutes (British Standards Institute, 2003). Five concentrations of Control Standard Endotoxin (CSE) were prepared and utilised, 50EUml^{-1} at serial dilution to 0.005EUml^{-1}. CSE was reconstituted with pyrogen-free reagent water (ACC), and the LAL (Pyrotell-T) with Glucashield buffer (to prevent interference from Glucans).

For analysis of (1-3)-β-D-glucan, a kinetic chromogenic Glucatell kit (ACC) was used for quantification of glucans at 37°C, with kinetic readings recorded automatically every 30 seconds for a period of 90 minutes (British Standards Institute, 2003). Six concentrations of Glucan standard were prepared and utilised,

100pgml-1 at serial dilution to 3.125pgml-1. The standard was reconstituted with pyrogen-free water (ACC), and the Glucatell lysate with pyrosol buffer and pyrogen-free water.

Samples were tested in triplicate. The data was converted to EUm^{-3} and ngm^{-3} for endotoxin and glucan data, respectively. The limit of detection of this method is 0.1 EUm^{-3} for endotoxin and 0.1ngm^{-3} for glucan.

3.5 Meteorological measurements

Meteorological measurements were made on the day of each sampling event using a combination of a Skye Instruments MiniMet wind speed and direction system along and a Davis Instruments Vantage Pro Plus weather station for other meteorological parameters. The specifications of these measurements are given below:

<u>Wind speed and direction</u>

Skye Instruments MiniMet system:

Wind speed	0.3 – 75 m/s	Accuracy	0.1m/sor 2%, whichever is largest
Wind direction	Resolution:	5°	
Averaging period	1 minute		

<u>Other Meteorological Parameters</u>

Ambient temperature	0.1 ^oC
Ambient Pressure	1 mBar
Dew point	0.1 ^oC
UV Radiation	UV index
Solar Radiation	$W.m^2$
Rain	mm

<u>Additional meteorological measurements made at each sampling location</u>

Paramount to selecting the sampling point is the wind direction. The Kestrel 4000 Pocket Weather Tracker allowed for instant accurate readings of environmental conditions (wind speed and direction, humidity, temperature) throughout the sampling period. It complemented the portable vane normally used and offers a digital representation to the weather confirming wind direction. The Kestrel (and thus the IOM filters) was directed against the most prominent wind, therefore recording maximum wind velocity. A compass was also used to ascertain and record wind direction (NSEW); furthermore, downwind sampling points were also selected by observation of steam emitted from windrows, cloud movement, and if available, the site's weather vane, typically placed at or near the site's office.

3.6 Odour sampling

Site B was selected as the site at which odour measurements would be made for comparison with bioaerosols measurements. The site was sampled for odours on two occasions, in June and September 2012. Emissions were sampled directly from windrows using a Lindvall hood to enable the measurement of surface flux (Gostelow et al., 2003). A series of pumped samples were taken from masts located at locations co-incident with the bioaerosol sampling programme at heights of 1.5 m and 4.5 m. All odour samples were

collected in Tedlar™ bags which have a low absorbency for odour. These samples were analysed using dynamic dilution olfactometry in a BSI-accredited controlled laboratory environment working to the CEN Standard EN13725:2003.

3.7 Supporting information on site operations

Information on site operations was collected at each site visit and at each sampling point. Where visible, the machinery operating on site at the time of sampling was recorded along with observations of vehicle movements in and out of the site, weather, apparent odour or other potential sources of bioaerosols in the immediate vicinity of the sampling point. Additional information on site operations was acquired from the site operators.

The location of the principal bioaerosol sources (e.g. windrow turning) on site on any given sampling day was recorded. This information was used in conjunction with the GPS data to calculate an actual distance from source to a given sampling location. It should be noted that whilst this approach is relatively straightforward for small sites where normally only one processing machine is operating at one time, these distances are less reliable at the larger sites where it is highly likely that multiple sources are emitting bioaerosols simultaneously.

3.8 Modelling

Short term modelling runs were undertaken with the objective of determining the range of possible emission rates from an area source under the conditions found during monitoring at Site B. The range of concentrations measured simultaneously with different monitoring techniques provided a unique opportunity to determine the potential range of emission rates and the impact different monitoring techniques have on this approach. In addition, long term simulations were run with the objective of determining the average and worst case scenario conditions at each site, based on three years of meteorological conditions.

Modelling and analysis was undertaken using ArcGIS and ADMS (version 4 for long term runs and version 5 for short term runs) for all sites in the following sequence:

i.   GIS base maps were prepared for all sites to allow for data modelling across each sampling period to be incorporated. These include the data collected at each sampling visit e.g. extent of composting active area and location of sampling points using GPS co-ordinates.

ii. A set of model inputs were defined and used to prepare a standard modelling method for each site.

iii. The GIS database was designed to incorporate measured and modelled odour and bioaerosol concentrations, as well as site activity data and meteorological variables (measured on site). In order to facilitate analysis of the data and modelling results, and for incorporation into the GIS database, all results collected to date have been collated into a single Excel spread sheet for each composting facility.

Modelling Approach

The model inputs for each site are described in Table 1. The sites were modelled initially using the Pasquill stability classes, which facilitated model set up and testing of the model inputs. Subsequently, the four sites were modelled using the meteorological data collected on-site during sampling. A new bioaerosol pollutant was defined using the ADMS default values for a gas pollutant, as there is insufficient information available to fully define an appropriate pollutant. The initial approach used an emission rate of 1 CFU/m^2/s for an area

13

source, defined as the area where all active composting activities occur for each of the sites. The results from these short term (ST) runs were used to back calculate emission rates for each of the sites. Calm conditions auxiliary files were used in short term runs when wind speed was less than 0.75 m/s at the sampling point.

Preliminary results analysed indicated that the model significantly overestimated measured concentrations (data not shown). A disparity between modelled and measured concentrations has been evident in previous modelling studies, with the model tending to overestimate concentrations (Drew et al., 2007; Taha et al., 2007). The decision was then taken to refine the source geometry, to include a low emission rate for static composting windrows (Taha et al., 2006), a minimum of two point sources representing screening and shredding activities, and a line source representing turning activities. The emission rates for these sources used in the long term (LT) runs are shown in Table 1.

Table 1. Input parameters defined for ADMS modelling

Input parameter	Value used / assumption	Source of value / assumption
Source definition	**Composting area**: where composting activity occurs **Turning**: line source **Screening and shredding**: point sources	GPS co-ordinates of composting active area. Cranfield PhD (Philippa Douglas, in progress)
Emissions data	ST runs: Model with emission rate of 1 CFU/m^2/s and back calculate LT runs (refined source geometry): Composting area: 10^3CFU/m^2/s Mature Compost: 10^2CFU/m^2/s Screening: 10^6CFU/s Shredding: 10^3CFU/s Turning: 10^5CFU/m/s Site D doors: 10^5CFU/m/s Site D biofilter: 10^3CFU/m^2/s	Email discussion with CERC Cranfield PhD (Philippa Douglas, in progress), existing databases and reported literature Site D doors – same as activity Site D biofilter – same as composting area
Emission velocity	1-2 m/s	Cranfield PhD (Philippa Douglas, in progress)
Emission temperature	Ambient plus 3°C for short term runs Ambient for long term runs	Cranfield PhD work(Philippa Douglas, in progress)
Inclusion of wet or dry deposition	No	Cranfield PhD work(Philippa Douglas, in progress)
Model as gas or particle	Model as a gas, without deposition or plume depletion	Insufficient evidence on particle properties to model as a particle

Meteorological data	Pasquill stability classes NPL monitored met data for period sampled Met Office observed data for 3 years	The Pasquill stability class runs allowed us to set up the models and test our input. The NPL met monitored data provides short term model output, covering only the periods when sampling was carried out. The Met Office data will provide long term averages and allow the calculation of exceedances of EA acceptable concentrations.

The odour modelling was undertaken in the same manner as the bioaerosol modelling for Site B. However, the short term run, based on an emission rate of 1 $ou_E/m^2/s$ produced very low concentrations, which results in very high emission rates on back calculation. There were also only two points where the model produces an output concentration (mostly 0), so we were not able to produce a range of emission rates. These back calculated emission rates are unrealistic in comparison to the sampled concentrations and were therefore not used for the long term run. We therefore used an average of the sampled emission rates for the composting area (66.5 $ou_E/m^2/s$) and undertook a back extrapolation modelling process to determine emission rates for the activities using the odour concentration sampled at source, and the Pasquill stability class D as the average UK stability class to ensure an output concentration was calculated. The three activities were defined as a group of sources and were allocated the same emission rate to each in absence of any data to support more detailed separation. The tested emission rates were $1 \times 10^3 ou_E/s$, $1 \times 10^5 ou_E/s$ and $1 \times 10^6 ou_E/s$. An emission rate of $3 \times 10^5 ou_E/m^2/s$ was decided upon, as the output concentration at point SP2 most closely matched the sampled concentration (modelled concentration of 3601 ou_E/m^3 with sampled concentration of 3774.5 ou_E/m^3). Emission rate units for turning as a line source were $ou_E/m/s$.

Back Calculations

Accurately modelling dispersion of emissions from any source depends on the use of accurate data for both source emissions as well as weather conditions, within the calculating period. It is not always straightforward to record this data, even for static processes. However, determining source data measurements that accurately reflect the dynamic nature of composting facilities is particularly difficult. The continual movement of the source during activities as well as the potential hazards from moving machinery make sampling at source impractical. The result is that source measurements often do not accurately reflect emissions at the time of measurement, or the likely range that may be experienced. We are therefore often reliant on measuring emissions downwind to reflect the source and plume characteristics.

Where samples can be taken downwind from the source and weather conditions recorded, dispersion models can be used to 'back calculate' the source emissions likely to have occurred at the time of sampling.

A pre-defined emission value is required as the starting point for the dispersion model run where source emissions are not well characterised.

Back calculated emission rates were estimated for site B, using all sampling methods for all distances downwind, and for all three micro-organisms. ADMS 5 was run using the related weather conditions data provided by NPL with an input emission rate (Er) of 1 $CFU/m^2/s$ for an area source, with no deposition.

Emission rates were then determined using an iterative process of testing emission rates until the appropriate downwind concentration was achieved. For example, if the emission rate of 1 CFU/m^2/s is used and gives an output of 10 CFU/m^3, then in order to achieve an output concentration of 1000 CFU/m^3, an emission rate of 100 CFU/m^2/s is required. Back calculated emission rates were then plotted against distance of the data point being used from the source.

WR1121 Final Report (20.8.13)

4. Results & Discussion

4.1 Comparison of the AfOR methods

The AfOR Protocol permits bioaerosols to be sampled using the Andersen and IOM sampling methods. This investigation provides the first opportunity to compare a substantive dataset of bioaerosol measures derived from samples taken simultaneously.

The Andersen and IOM sampling methods do not produce comparable data across the range of sampling locations selected for this research. In part, this is attributable to the known, inherently-different designs and operational features of these samplers. The Andersen is prone to overloading at high bioaerosol concentrations (over 200 colonies per plate, particularly for fungi). As such samples were not taken close to source, whereas IOM is capable of sampling in this environment. The IOM has a higher lower limit of detection compared to the Andersen due to the relatively low volumes of air it samples. As such, the Andersen can detect bioaerosols at relatively low concentrations typical of "background" air (in single figures cfu/m^3) whereas the IOM cannot. These characteristics mean that there are a number of situations of high and low ambient bioaerosol concentrations in which reliable measures cannot be made for both samplers and therefore comparisons are impossible. Figures 3 and 4 present the combined dataset for all sites for total bacteria with paired IOM and Andersen data as an illustration of the performance of the two measurement approaches. The measurements are weakly correlated. There are occasions when the samples are in close accordance, when the Andersen detects more bacteria than the IOM, and vice versa. Figure 4 confirms considerable data scatter and deviation from the 1:1 line.
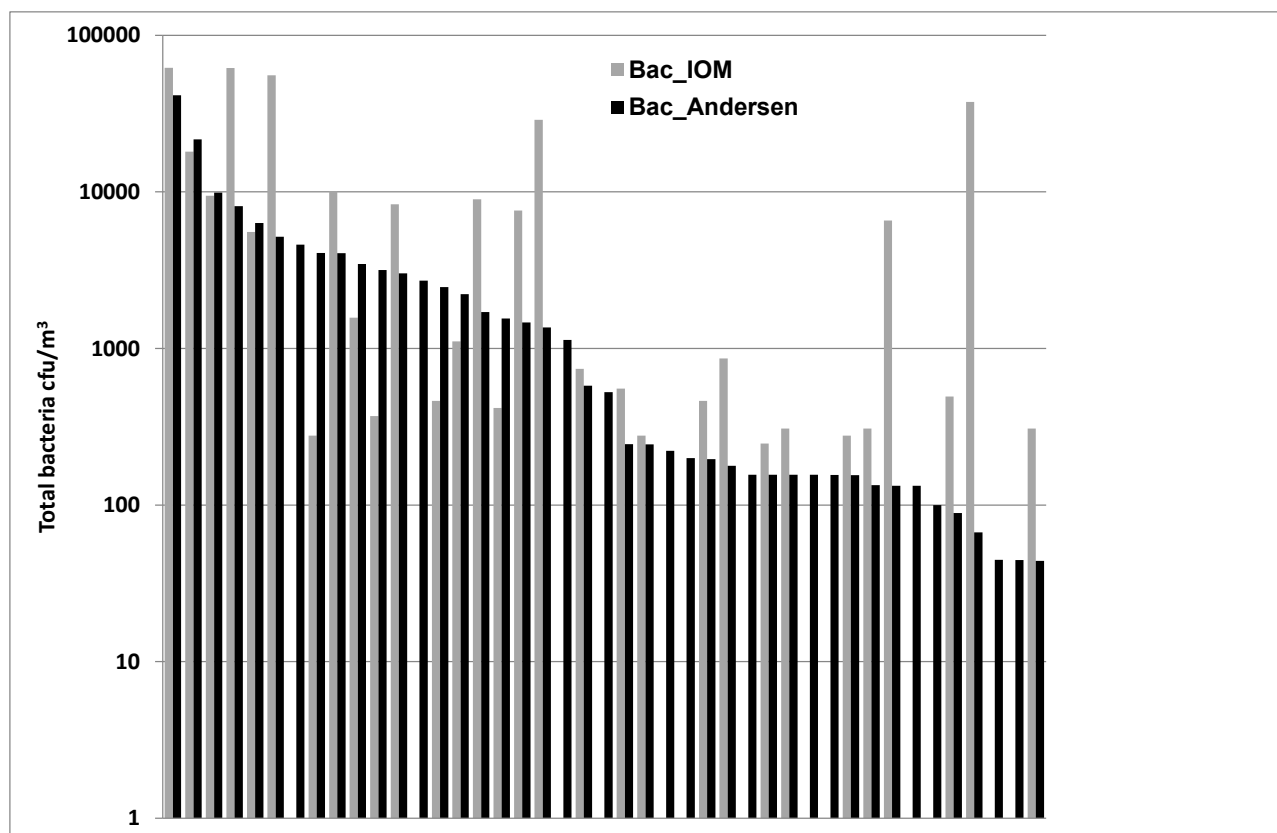

Figure 3 Comparison of total bacteria concentrations from paired IOM and Andersen samples from all four sites

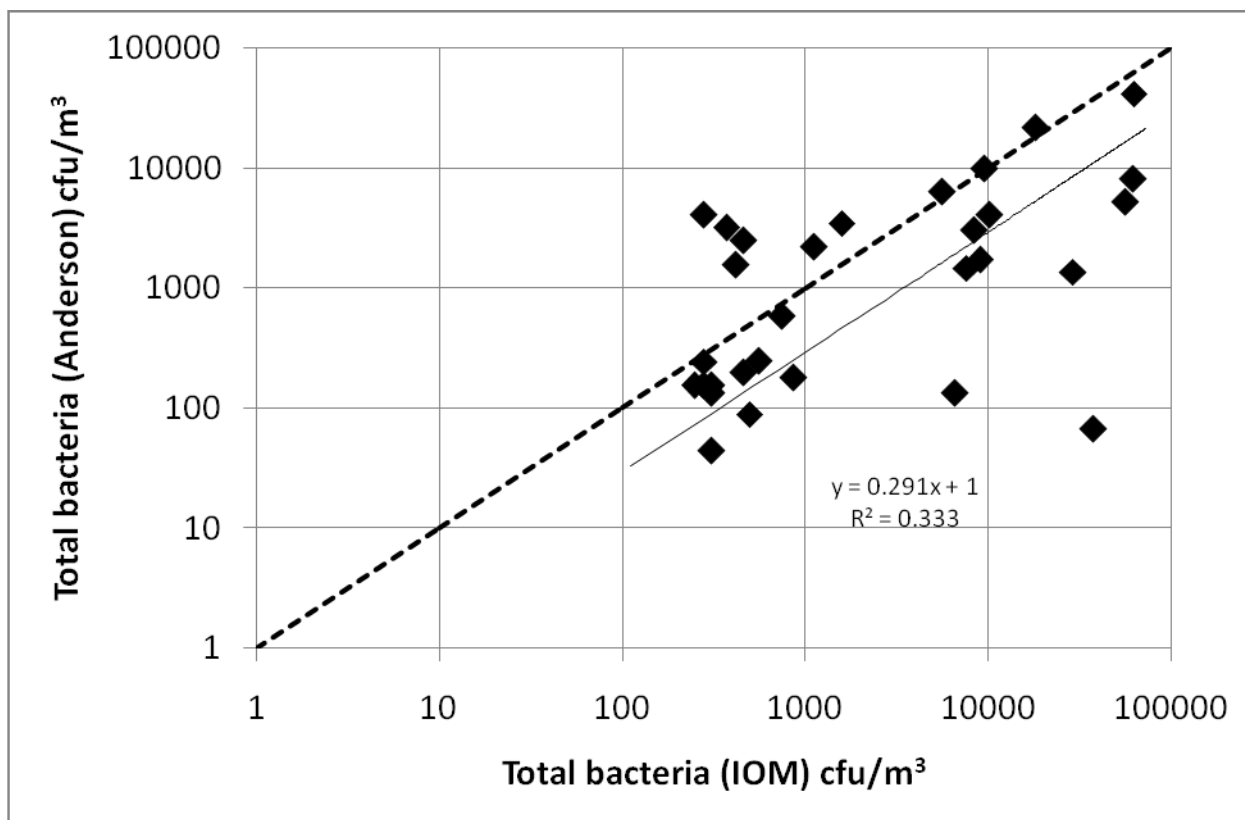WR1121 Final Report (20.8.13)

Figure 4 Scatterplot of total bacteria concentrations from IOM and Andersen samples (log-transformed for clarity) from all four sites. The dashed line is the theoretical 1:1 relationship and the continuous line is the linear regression.

4.2 Spatial and temporal variation using standard AfOR techniques

The spatial distribution of the standard AfOR determinants (*A. fumigatus* and total bacteria) is presented in Figures 5 and 6. A general trend of low (<1000 CFU/m^3) bioaerosol concentrations upwind of the site, significantly elevated concentrations immediately downwind of an emissions source (peak total bacteria >10^6 CFU/m^3 for IOM) and then declining concentrations with distance away from the site was observed. As previously described, the IOM sampler is able to detect higher concentrations (maximum total bacteria >10^6 CFU/m^3, maximum *A. fumigatus*>5 x 10^5 CFU/m^3) close to source than the Andersen. *A. fumigatus* was below the limit of detection in all upwind IOM samples. Conversely, the Andersen is much more sensitive at the lower concentrations found upwind and at sample points more distant from the site – especially for *A. fumigatus*. Whilst concentrations generally decline at distance from site, there is evidence that samples do not consistently return to background concentrations. This is particularly evident for total bacteria where concentrations >1000 CFU/m^{3}are more common beyond 250 m from source than in upwind samples.
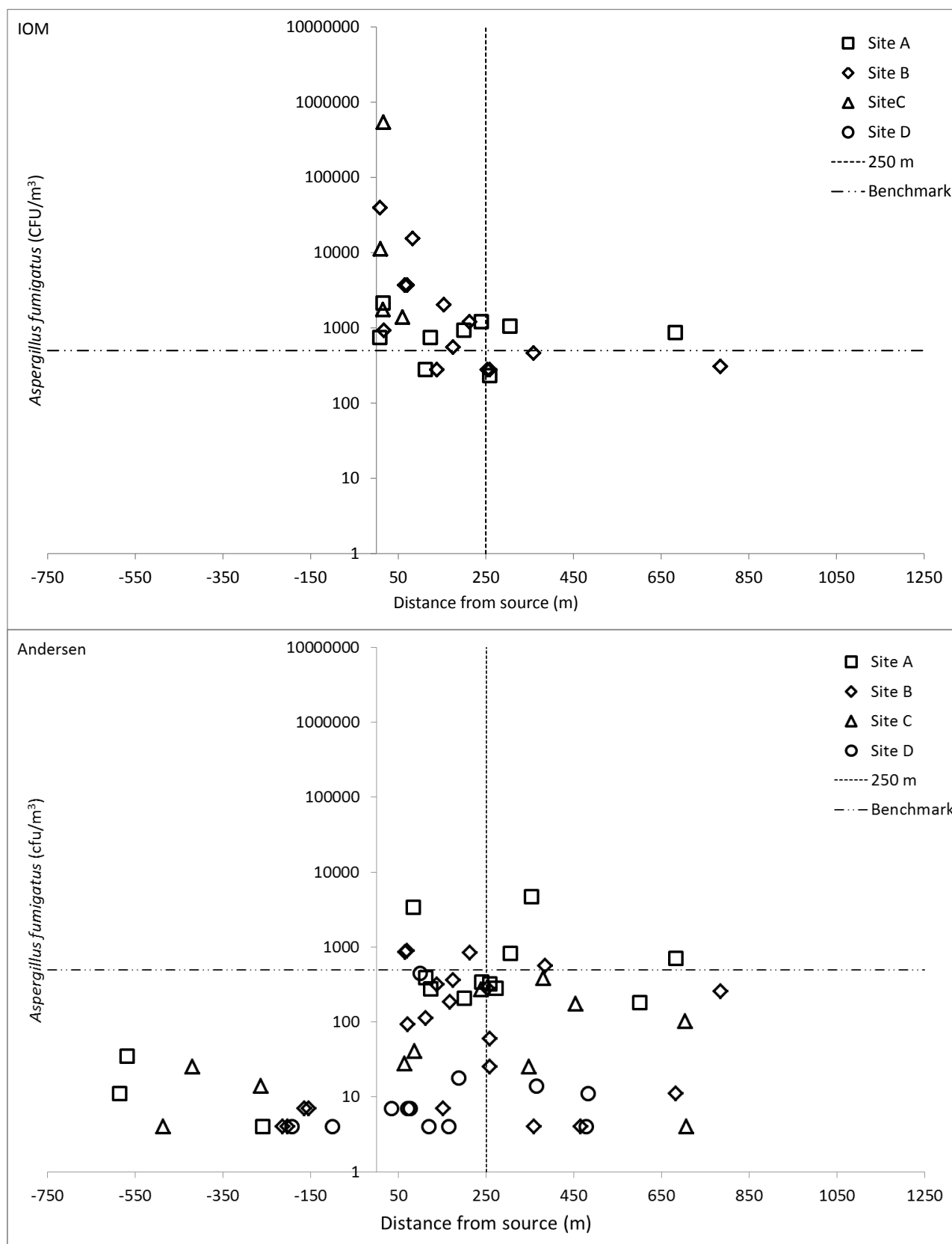
Figure 5 Spatial distribution of *A. fumigatus* along the sampling transects as measured by the IOM (upper panel) and Andersen samplers (lower panel). Data from all four sites presented. The Environment Agency acceptable concentration of 500 CFU/m³ is presented as a comparative benchmark rather than as a measure of compliance.
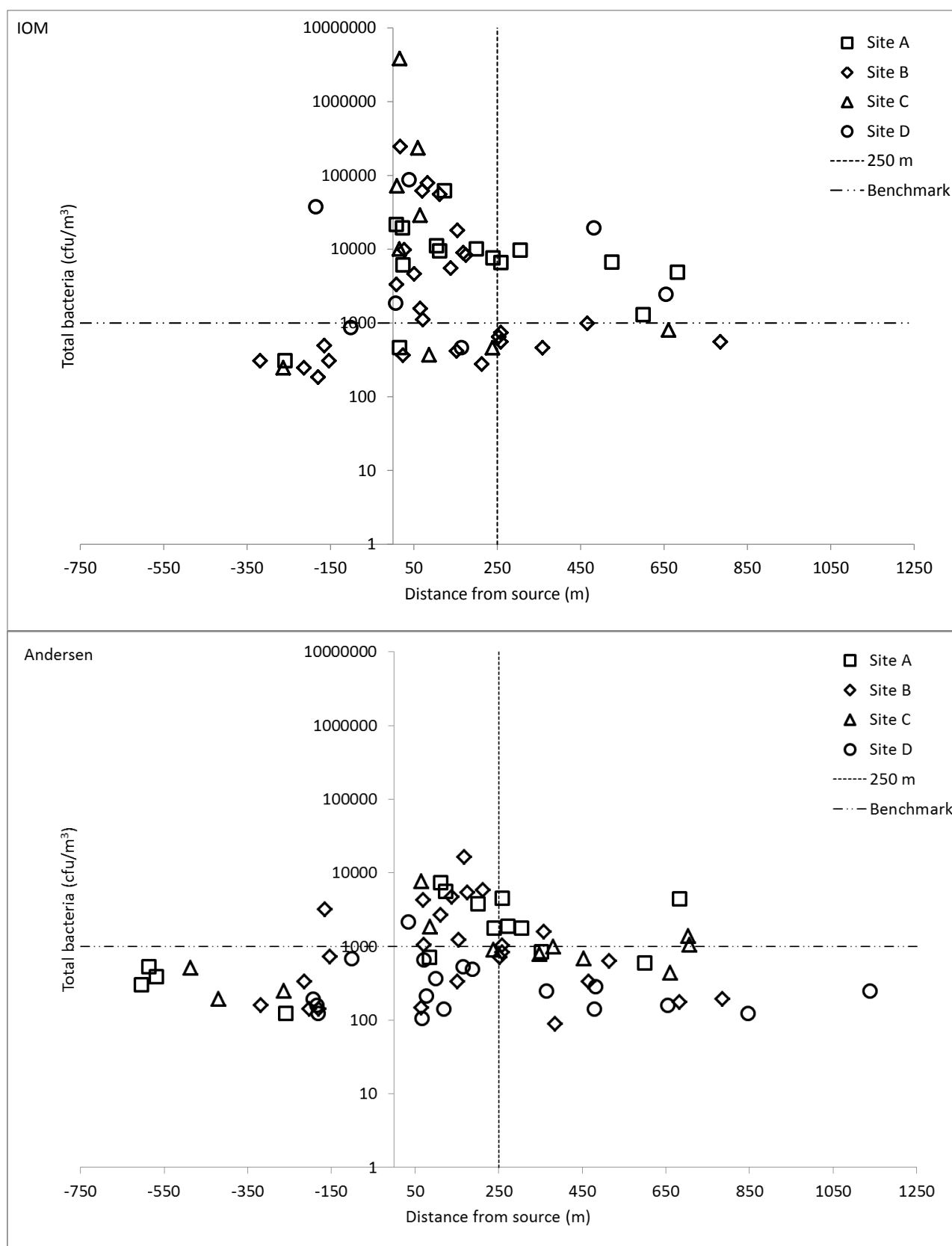
Figure 6 Spatial distribution of total bacteria along the sampling transects as measured by the IOM (upper panel) and Andersen samplers (lower panel). Data from all four sites presented. The Environment Agency acceptable concentration of 1000 CFU/m³ is presented as a comparative benchmark rather than as a measure of compliance.

In terms of site comparison, the most noteworthy feature arising from a visual assessment of these data is

the comparatively low bioaerosol concentrations measured at Site D (the enclosed site). For example at Site

WR1121 Final Report (20.8.13)

D, no measurements of *A. fumigatus*>500 CFU/m^3 were detected by either IOM or Andersen at any sampling location, even close to source (the only site to achieve this). The IOM data for Sites B and C indicates occasional very high concentrations of bioaerosols close to source (for example four samples exceeding 10^4*A. fumigatus*>1000 CFU/m^3 detected by the IOM). There is some evidence that Site A, (the site with the largest tonnage on site)has a tendency for occasional higher bioaerosol concentrations (above benchmark values) at distance (>250 m) from source – consistent across IOM and Andersen samplers.

The spatial distribution of Gram negative bacterial concentrations for all four sites measured by the IOM and Andersen methods and delineated by site are presented in Figure 7. The overall spatial pattern of Gram negative bacterial concentrations is similar to that of the total bacteria and *Aspergillus fumigatus*. With IOM data in particular we can observe a general trend of low (BLOD) concentrations upwind of the site, significantly elevated concentrations immediately downwind of the emissions source (peak concentration >30,000 CFU/m^3) and then a decline in concentrations (often to BLOD) with distance away from the site. We can see that the IOM sampler detected high concentrations close to source and the Andersen was able to detect much lower concentrations at all sampling locations. For example Andersen upwind concentrations were generally between 10-100 CFU/m^3 (i.e. below the limit of detection of the IOM sampling configuration). Andersen data in particular suggest that several samples at Site A had Gram negative bacterial concentrations > 1000 CFU/m^3 up to 250 m from source and above background up to 650 m from source on two occasions (no other sources of bioaerosols were seen in the area). As with total bacteria and *A. fumigatus*, there were occasional higher concentrations (above benchmark values) at distance (>250 m) from source at Site A (the higher tonnage site).

The higher sensitivity of the Andersen sampler provides an opportunity to assess the variability of upwind concentrations of cultivable bioaerosols. The ranges for *A. fumigatus*, total bacteria and Gram negative bacteria are typically <10-<100 CFU/m^3, 100-1000 CFU/m^3 and 10-100 CFU/m^3 respectively.
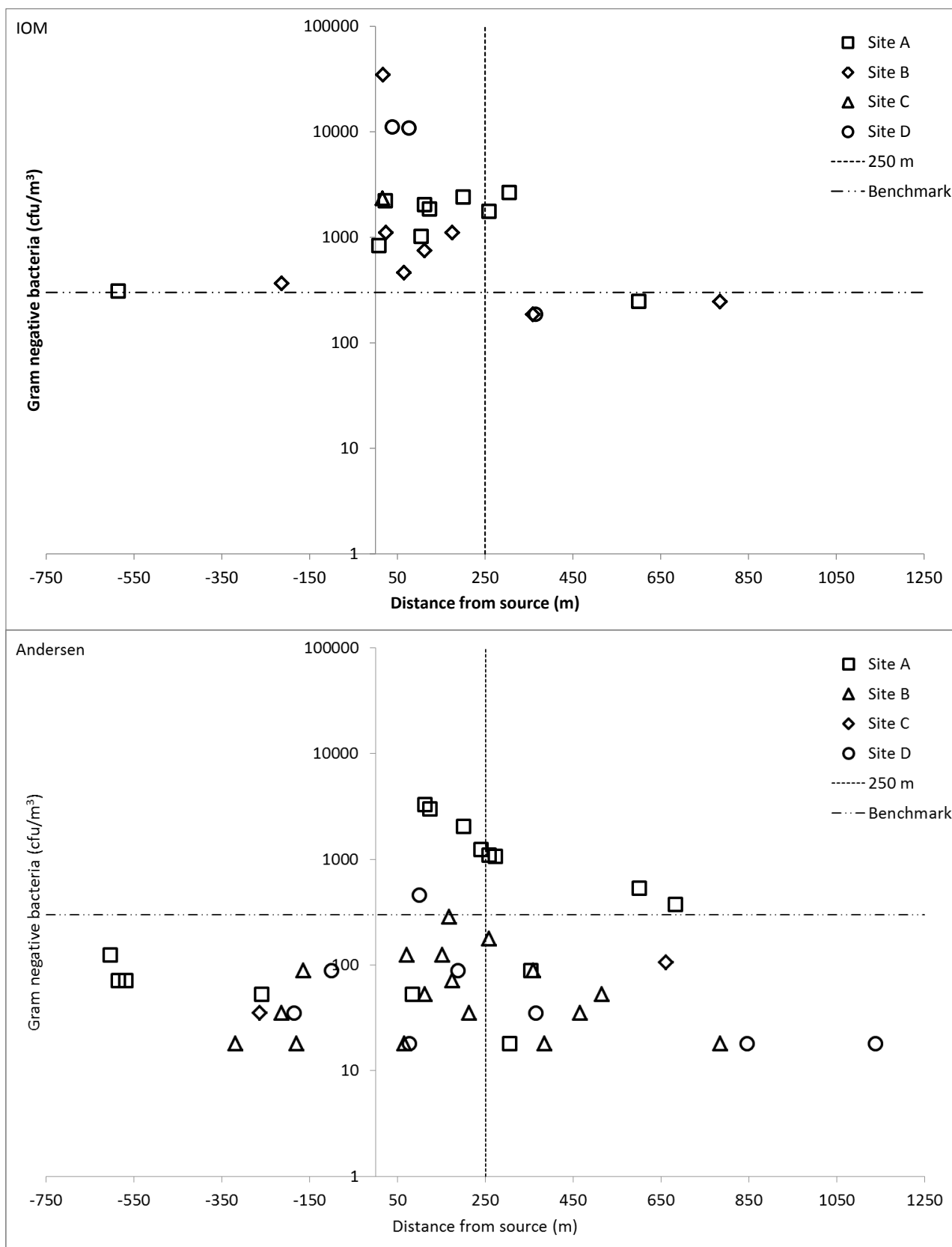
Figure 7 Spatial distribution of Gram negative bacteria along the sampling transects as measured by the IOM (upper panel) and Andersen (lower panel) samplers. The Environment Agency acceptable concentration of 300 CFU/m^3 is presented as a comparative benchmark rather than as a measure of compliance.

A quantitative assessment of these data was made by comparing the measured values to the Environment Agency's acceptable levels. It is important to emphasise that this analysis is not a measure of compliance with such levels. In practice, exceedance would be assessed on a sample by sample basis by comparison to the background on that sampling day. In this analysis we have used the acceptable levels as benchmarks to enable an assessment of the variability of concentrations at each site. Tables 2, 3 and 4 present the percentage exceedance of Environment Agency acceptable levels (presented as a comparative benchmark - not a measure of compliance)for total bacteria, *Aspergillus fumigatus* and Gram negative bacteria. The results are classified as upwind samples, samples taken between source and 249 m downwind, samples taken at or beyond 250 m from source, and for the whole dataset. Exceedance of benchmark levels:

- Occurred occasionally upwind of a composting facility
- Occurred occasionally at ≥250 m from source
- Occurred more frequently close to source
- Occurred more frequently at Site A than any other site
- Occurred less frequently and showed no spatial pattern at Site D
- Occurred more frequently for total bacteria than *Aspergillus fumigatus* and Gram negative bacteria
- Was generally consistent between sampling devices

This analysis has highlighted Site A (the highest tonnage site) for having a higher proportion of samples with elevated concentrations at distance from site. For example, the majority of samples taken ≥250 m from source were >1000 CFU/m^{3}total bacteria, irrespective of sampling device.

The dataset of bioaerosol concentrations collected from the four sites under investigation using the standard AfOR protocol sampling and analysis methodologies was reviewed with a view to identifying if there were any detectable temporal variations. In Figures 8 and 9, the data were categorised as either "summer" (April-September) or "winter" (October-March). There is no indication of any seasonal effect for either measured parameter or either sampler. There is evidence of between-sampling day variability in this dataset however. If we take Site B as an example (Figure 10), it can be seen that *A. fumigatus* concentrations vary between sampling dates. For example the *A. fumigatus* concentrations on December 2012 were elevated for both IOM and Andersen data (peak concentrations approx. 10^4 and 10^3 CFU/m^3 respectively) by comparison with September 2012 (peak concentrations approx. 10^3 and 10^2 CFU/m^3 respectively).[1]This data spread tends to be greatest close to source compared to upwind or more distant downwind locations for the IOM data. The Andersen data shows such a spread at all downwind sampling locations, probably due to this sampler's greater sensitivity at lower concentrations. This demonstrates the usefulness of each sampler in different situations – the IOM providing a picture of variable concentrations close to source and the Andersen picking up this variability further downwind. These data are suggestive of time-varying emissions. Furthermore they suggest that these variations may persist but at reduced magnitude further downwind (as detected by the Andersen). This has implications for representative monitoring at composting facilities as occasional snapshot sampling may not represent anything other than the conditions at a particular point in time.

---

[1]It is not possible to attribute definitively these peaks to specific meteorological or operational conditions although it was noted that there was high activity / green waste shredding in close proximity to the sampler on this occasion (Table 5).

WR1121 Final Report (20.8.13)

Table 2 Percentage of samples exceeding Environment Agency acceptable level for total bacteria of 1000 CFU/m^3 (presented as a comparative benchmark - not a measure of compliance). Number of samples in brackets. Light grey shading >50% of samples exceed benchmark. Dark grey shading >75% of samples exceed benchmark.

	Site A		Site B		Site C		Site D	
	IOM	Andersen	IOM	Andersen	IOM	Andersen	IOM	Andersen
Upwind	0 (4)	0 (4)	0 (6)	17 (6)	0 (3)	0 (3)	25 (4)	0 (4)
0-249 m	80 (10)	80 (5)	81 (16)	80 (10)	71 (7)	67 (3)	18 (11)	13 (8)
≥250 m from source	71 (7)	67 (6)	0 (9)	22 (9)	0 (6)	33 (6)	33 (6)	0 (6)
All samples	62 (21)	53 (15)	42 (31)	44 (25)	31 (16)	33 (12)	24 (6)	6 (18)

Table 3 Percentage of samples exceeding Environment Agency acceptable level for *Aspergillus fumigatus* of 500 CFU/m^3 presented as a comparative benchmark - not a measure of compliance). Number of samples in brackets. Light grey shading >50% of samples exceed benchmark. Dark grey shading >75% of samples exceed benchmark.

	Site A		Site B		Site C		Site D	
	IOM	Andersen	IOM	Andersen	IOM	Andersen	IOM	Andersen
Upwind	0 (4)	0 (4)	0 (6)	0 (6)	0 (3)	0 (3)	0 (4)	0 (4)
0-249 m	55 (11)	20 (5)	50 (16)	33 (9)	57 (7)	0 (3)	0 (11)	0 (8)
≥250 m from source	29 (7)	50 (6)	0 (9)	11 (9)	0 (6)	0 (6)	0 (6)	0 (6)
All samples	33 (21)	27 (15)	26 (31)	17 (24)	25 (16)	0 (12)	0 (21)	0 (18)

Table 4 Percentage of samples exceeding Environment Agency acceptable level for Gram negative bacteria of 300 CFU/m^3 presented as a comparative benchmark - not a measure of compliance). Number of samples in brackets. Light grey shading >50% of samples exceed benchmark. Dark grey shading >75% of samples exceed benchmark.

	Site A		Site B		Site C		Site D	
	IOM	Andersen	IOM	Andersen	IOM	Andersen	IOM	Andersen
Upwind	25 (4)	0 (4)	25 (4)	0 (4)	0 (1)	0 (1)	0 (2)	0 (2)
0-249 m	60 (10)	80 (5)	45 (11)	0 (7)	100 (1)	0 (0)	40 (11)	50 (2)
≥250 m from source	29 (7)	67 (6)	0 (6)	11 (6)	0 (1)	0 (1)	0 (3)	0 (4)
All samples	43 (21)	53 (15)	29 (21)	0 (17)	25 (4)	0 (2)	20 (10)	13 (8)

The operational and meteorological conditions coincident with peak or other notably high bioaerosol concentrations are summarised in Table 5. This information was synthesised from photographs, videos and documented observations taken by the Cranfield University team on the corresponding sampling day. The causal factor(s) responsible for unusually high bioaerosol emissions or poor dispersion leading to elevated bioaerosol concentrations on particular sampling days are often debated but little evidence exists to confirm the comparative importance of the plausible operational and meteorological candidates. The generation of such evidence is hampered by the challenges in isolating the effects of factors that are entirely (e.g. weather) or largely (e.g. site operations) beyond the control of investigators. These challenges are evident in this dataset. Typically, the peak concentration of bioaerosol at each site was recorded by the IOM sampler close to source as previously described in this report. These peak concentrations close to source tended to occur in calm conditions which would not favour their rapid dispersion. No association between peak concentrations and cloud cover, humidity and temperature was observed. Observations made at the time of sampling suggests an association between peak concentrations and a generally higher than normal level of site activity (vehicle movements and feedstock / product manipulations which would be expected to increase emissions. The highest bioaerosol concentrations recorded at any site (Site C, May 2012, *A. fumigatus,* 541,667 CFU/m^3 and total bacteria, 3,855,556 CFU/m^3) were associated with high operational activity, low wind speed (max 1.3 m/sec), low relative humidity (55%). On this date the IOM filter heads contained significant visible deposits of dust which had the appearance of compost. The concentrations at greater distance from source were not unusually high on this date suggesting that these high emissions settled rapidly in the still air. This indicates that high concentrations of bioaerosol close to source do not necessarily translate into high downwind concentrations. As previously stated, higher concentrations (exceeding benchmark values) occurred at sample points >250 m from source more frequently at Site A than at any other site. The March 2012 data were an example of this phenomenon. This sampling date was notable for significant visible dusty emissions and gusting winds. A combination of high emission from this large, busy site and local factors leading to poor dispersion may be responsible but such a conclusion is speculative.
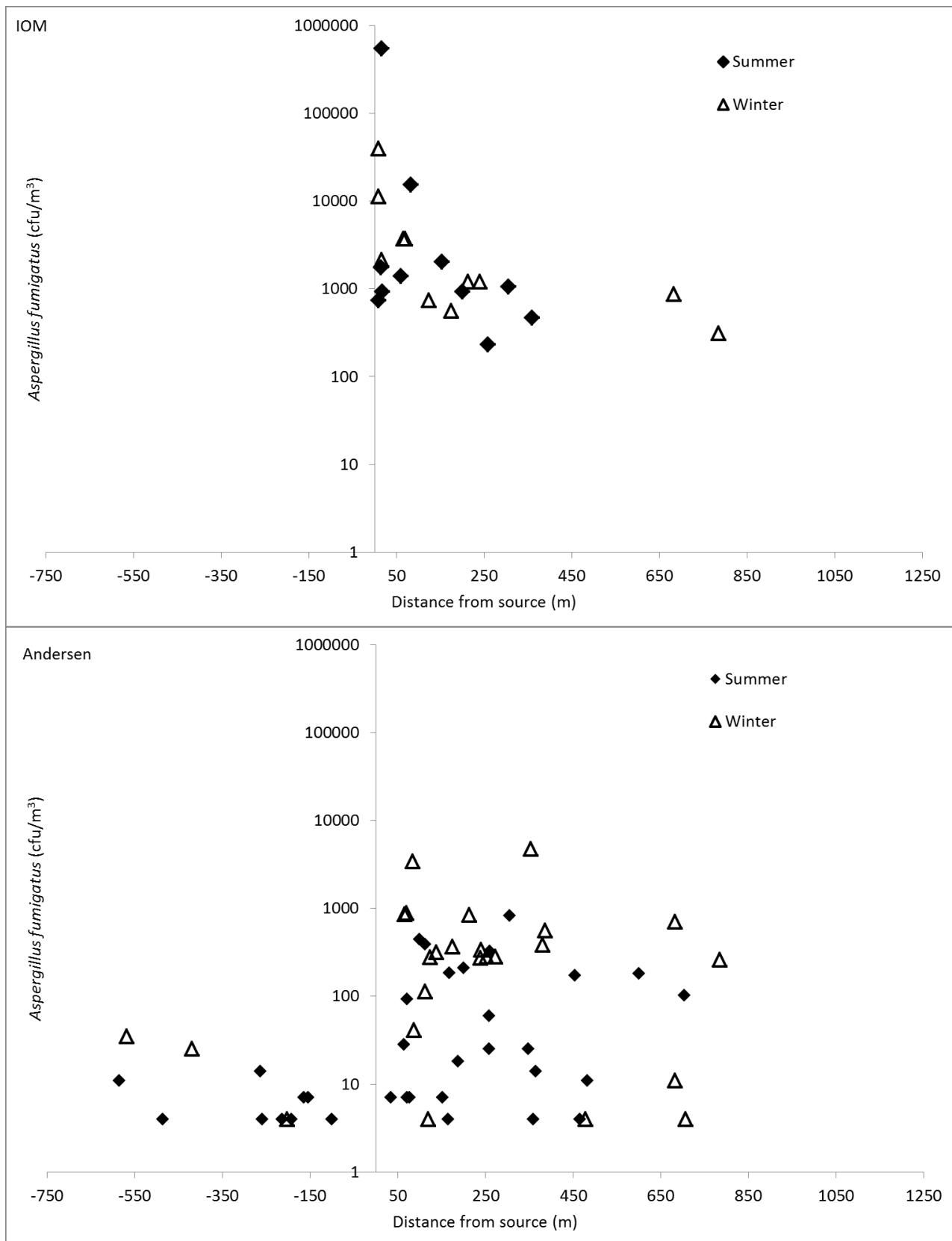
Figure 8 Spatial distribution of *A. fumigatus* along the sampling transects as measured by the IOM and Andersen samplers with data categorised as Summer (April-September) or Winter (October-March). IOM data below limit of detection not presented for clarity.
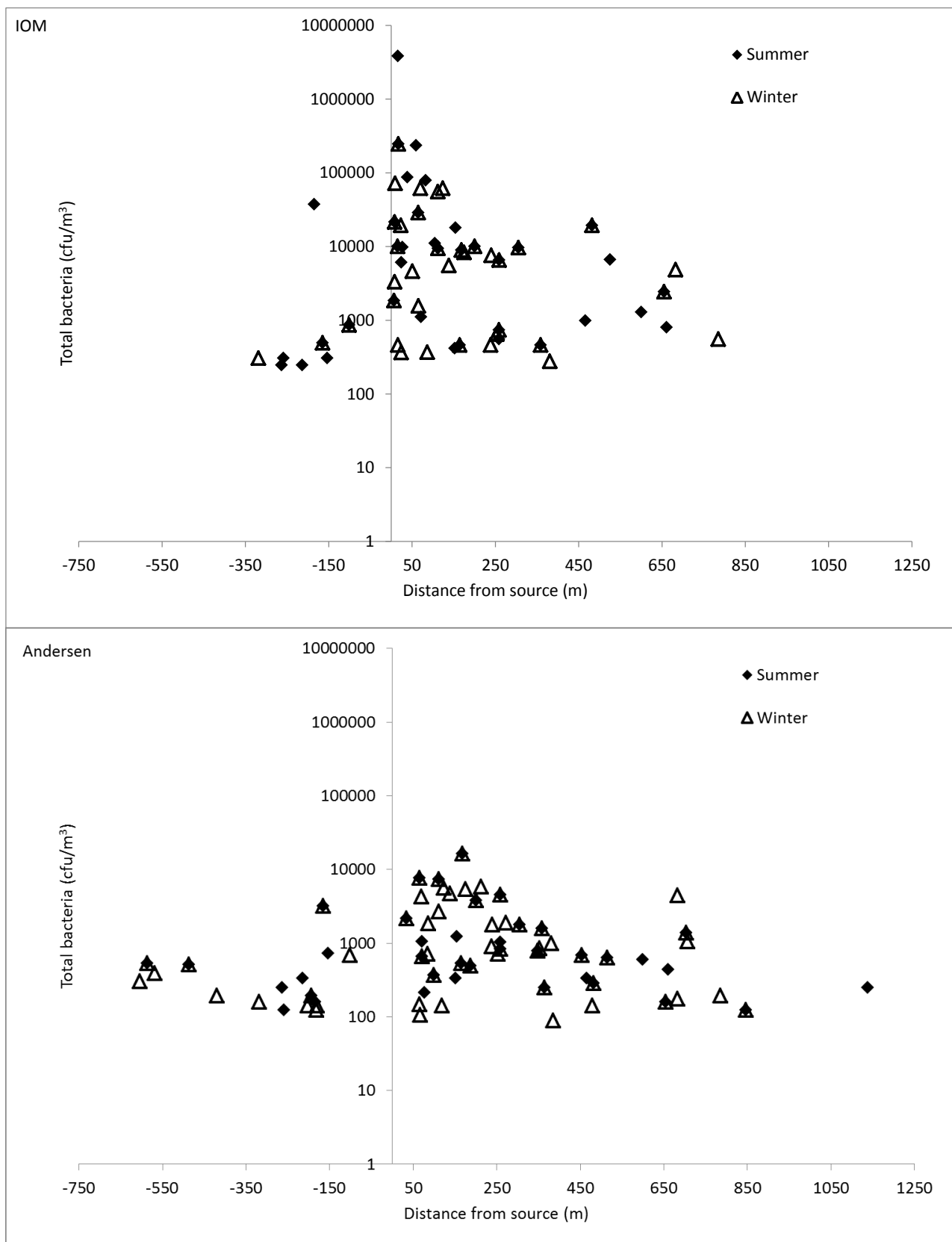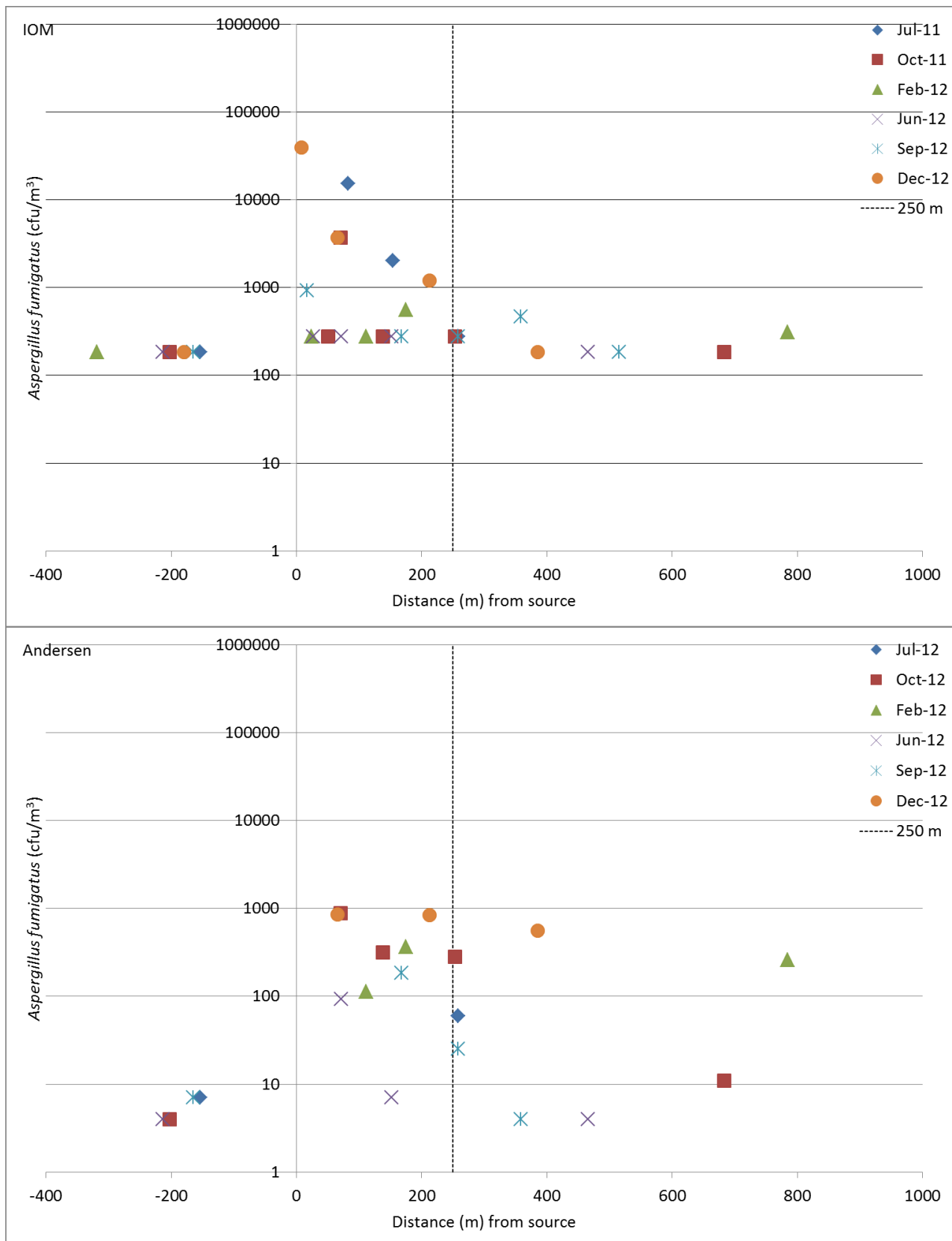
Figure 9 Spatial distribution of total bacteria along the sampling transects as measured by the IOM and Andersen samplers with data categorised as Summer (April-September) or Winter (October-March). IOM data below limit of detection not presented for clarity.

Figure 10 Spatial distribution of *A. fumigatus* along the sampling transects at Site Bas measured by the IOM and Andersen samplers with data categorised by sampling date

Table 5 Operational and meteorological conditions coincident with peak or other notably high bioaerosol concentrations

Site	Date	Justification for selection	Distance from specified source	Specified source	Intensity of traffic activity	Additional comments on site activity	Cloud cover (oktas)	Max wind strength (m/sec)	Beaufort wind scale NUMBER	Beaufort wind scale DESCRIPTION	Temp	Humidity
Site A	30-Nov-12	Maximum *A. fumigatus* concentrations for IOM (2,130 CFU/m^3)(Also highest recorded value for Andersen (4,739 CFU/m^3) on this date).	16	Screening	High	High vehicle activity in front of sampling	8	0.6	1	Light air	1	84
Site A	20-Mar-12	Maximum total bacteria concentrations for IOM (61,582 CFU/m^3) (Also elevated total bacteria concentrations for IOM and Andersen (~4 x 10^3 CFU/m3) at 682 m from source on this date).	123	Screening	Normal	Significant dowsing as it was first visit. Although normal for this site higher vehicle activity compare to other sites. Several activities occurring at same time. JCB occasionally moving final or near to final products (as it was still steaming), approx. 95m – 120m away in windrows upwind, causing considerable particulate and dust clouds.	2	5.1	3	Gentle breeze	12	66
Site B	03-Sep-12	Maximum total bacteria concentrations for IOM (247,222 CFU/m^3).	17	Screening	High	Site busier than usual.	4	1.2	1	Light air	17	78
Site B	11-Dec-12	Maximum *A. fumigatus* concentrations for IOM (39,630 CFU/m^3).	8	Shredding	High	Moving materials from shredder in front sampling. Shredding fresh green waste trees conifers.	3	0.5	1	Light air	1	89
Site B	03-Sep-12	Maximum *A. fumigatus* concentrations for Andersen (16,272 CFU/m^3).	168	Screening	Normal	Site busier than usual. Strong odour smells at P3 also on recently mown grass.	1	0.8	1	Light air	20	68
Site C	22-May-12	Maximum *A. fumigatus* (541,667 CFU/m^3) and total bacteria concentrations for IOM (3,855,556 CFU/m^3).	16	Screening	Normal	Shredding, turning and screening activities. Considerable dust / particulates. For health and safety reasons sampler had to leave sampling location whilst sample being taken. All filters had unusually large amounts of visible dust.	0	1.3	1	Light air	24	55
Site D	17-May-12	Maximum total bacteria concentrations for IOM (87,500 CFU/m^3)(N.b. elevated total bacterial counts recorded upwind and very low *A. fumigatus* on this date).	38	In-vessel	Enclosed site	Foul odour was noted. No sprinklers working on the biofilter during sampling .	7	0.4	1	Light air	13	62
Site D	22-Sep-11	Maximum total bacteria concentrations for Andersen (2,173 CFU/m^3)(Noteworthy as this was a rare occasion in which the Andersen was used close to source due to history of low counts at this site).	34	In-vessel	Enclosed site	Location between bio filter and stack on concrete. A sheltered position between bio filter and stack. Noted bio filter being dowsed during sampling.	2	1.7	2	Light breeze	16	67
Site D	22-Sep-11	Second highest total bacteria concentrations for IOM (19,444 CFU/m^3)(N.b. anomalous reading in context of other measurments on that day and recorded at distance from source).	482	In-vessel	Enclosed site	Recently combined crops and ploughing in nearby fields.	6	3.7	3	Gentle breeze	17	52

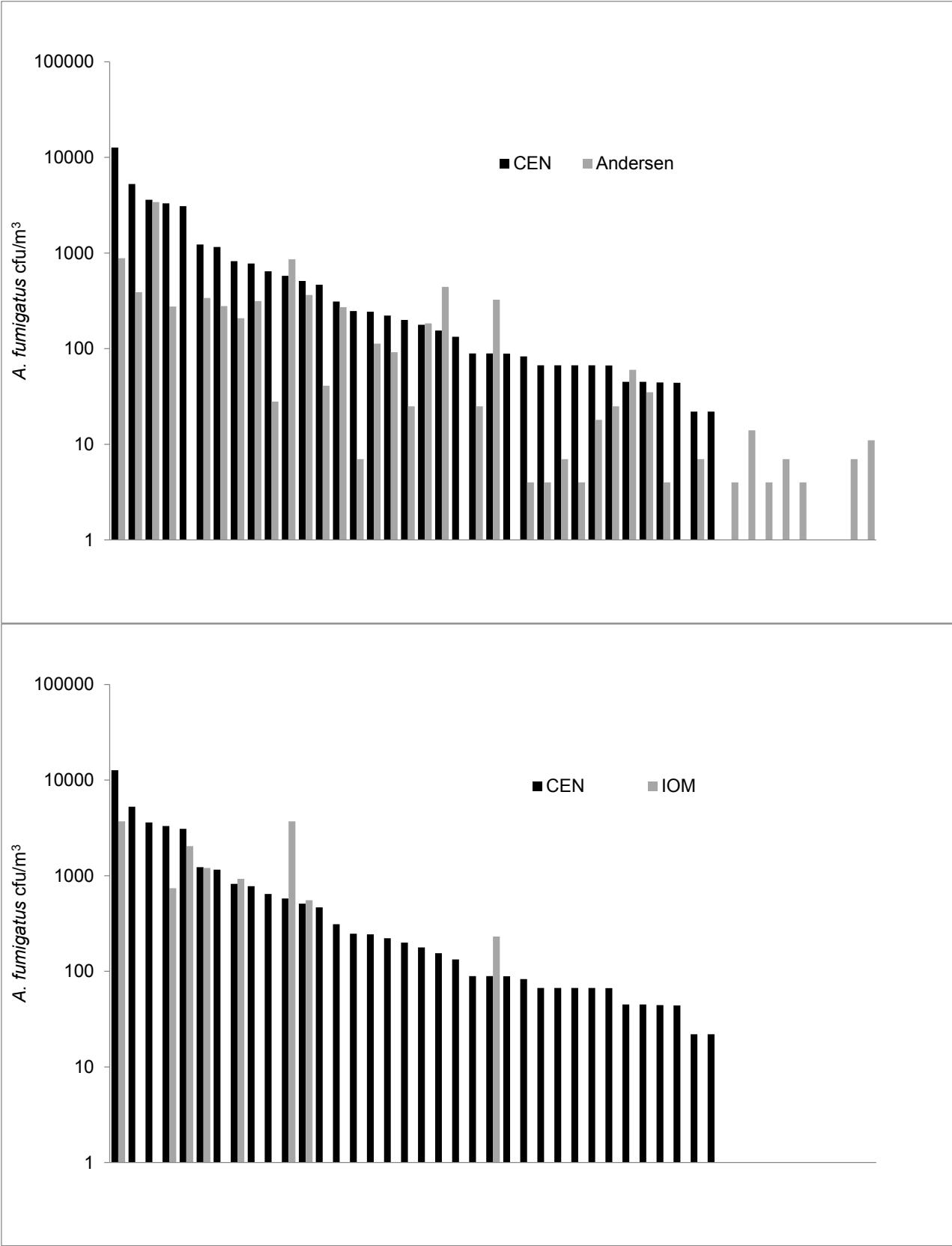## 4.3 Comparison of standard AfOR techniques with CEN

Figure 11 Comparison of *A. fumigatus* concentrations from paired samples from the CEN and Andersen samplers (upper panel) and CEN and IOM samplers (lower panel) from all four sites. IOM data below limit of detection removed for clarity.
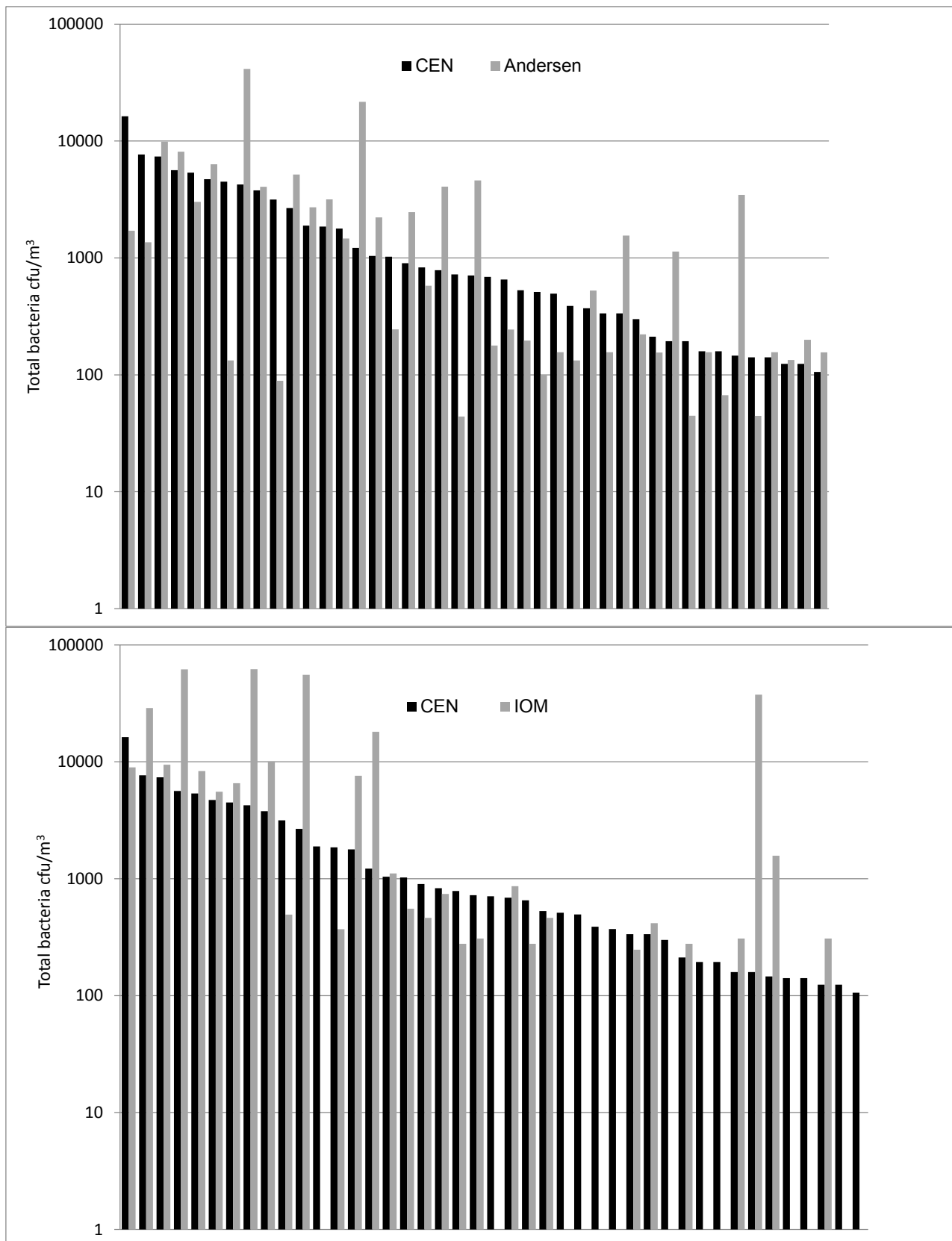
WR1121 Final Report (20.8.13)

Figure 12 Comparison of total bacteria concentrations from paired samples from the CEN and Andersen samplers (upper panel) and CEN and IOM samplers (lower panel) from all four sites. IOM data below limit of detection removed for clarity.

In total there were 45 data points over the four sites where the CEN, IOM and Andersen samplers were operated in parallel. 27 of the 45 samples (60%) can be considered of the same order (ignoring the 1-10 scale) between the IOM and CEN, with the IOM being generally higher 73% of the time. In comparison 31 were of the same order between the CEN and Andersen (68%) although the CEN is generally higher than the Andersen 68% of the time. (This assumes 'low' Andersen counts (below 20 colonies) are the same order as when the CEN recorded a zero). The majority of disagreements between the CEN and IOM are where the IOM records at the limit of detection (185 cfu/m^3) but the CEN records a zero, which it does 9 of 45 points (20%).

The CEN records a magnitude higher than either of the other samplers on four separate occasions (8%) but is higher than both samplers on 9 occasions (20%) 8 of which were at 50m. Hence it may capture more colonies in more highly contaminated environments, but this would need further investigation. However it is generally in more agreement with the Andersen 17 of 45 times (37%) and the IOM 12 of 45 (26%) (the remainder being classified as agreement (28%) or disagreement (7%) with both of the other samplers).

A further illustration of the lack of consistency between the different sampling devices, the total bacteria results from the CEN sampler are presented in a scatterplot in comparison to the IOM and Andersen samplers (Figure 13). Given the logarithmic scaling of this presentation, whilst there is a general positive correlation (as would be expected), it is clear that there are occasions where there are large discrepancies (on occasions several orders of magnitude) between measurements made by the different sampling devices.

It should be noted that an advantage of the CEN method is that it allows for significantly longer sampling duration than those feasible within the sampling constraints of this project. Sampling for one hour or longer samples may have resulted in less variable results.

In summary, the CEN tends to record concentrations below the IOM and above the Andersen with a few notable exceptions, and agrees more with the Andersen at the lower detection levels (often recording a zero where less than 20 colonies are seen on the Andersen but 185 cfu/m^3 for the IOM) which is a limitation of the IOM rather than the CEN.

The CEN method may not lend itself to deployment on all sites. Access is the major concern as a 4x4 vehicle and two people are needed to get it to some sampling locations. This would double the costs of a monitoring exercise as both the Andersen and IOM only need one person on site and are powered by batteries (the CEN requires an appropriate generator). Similarly it is not very practical to lift it over fences or carry it any distance, particularly on uneven ground, due to its weight. Another problem encountered with the CEN device was that the gelatine filters proved to be extremely sensitive to moisture, and were found to crack and flake when the humidity was high.
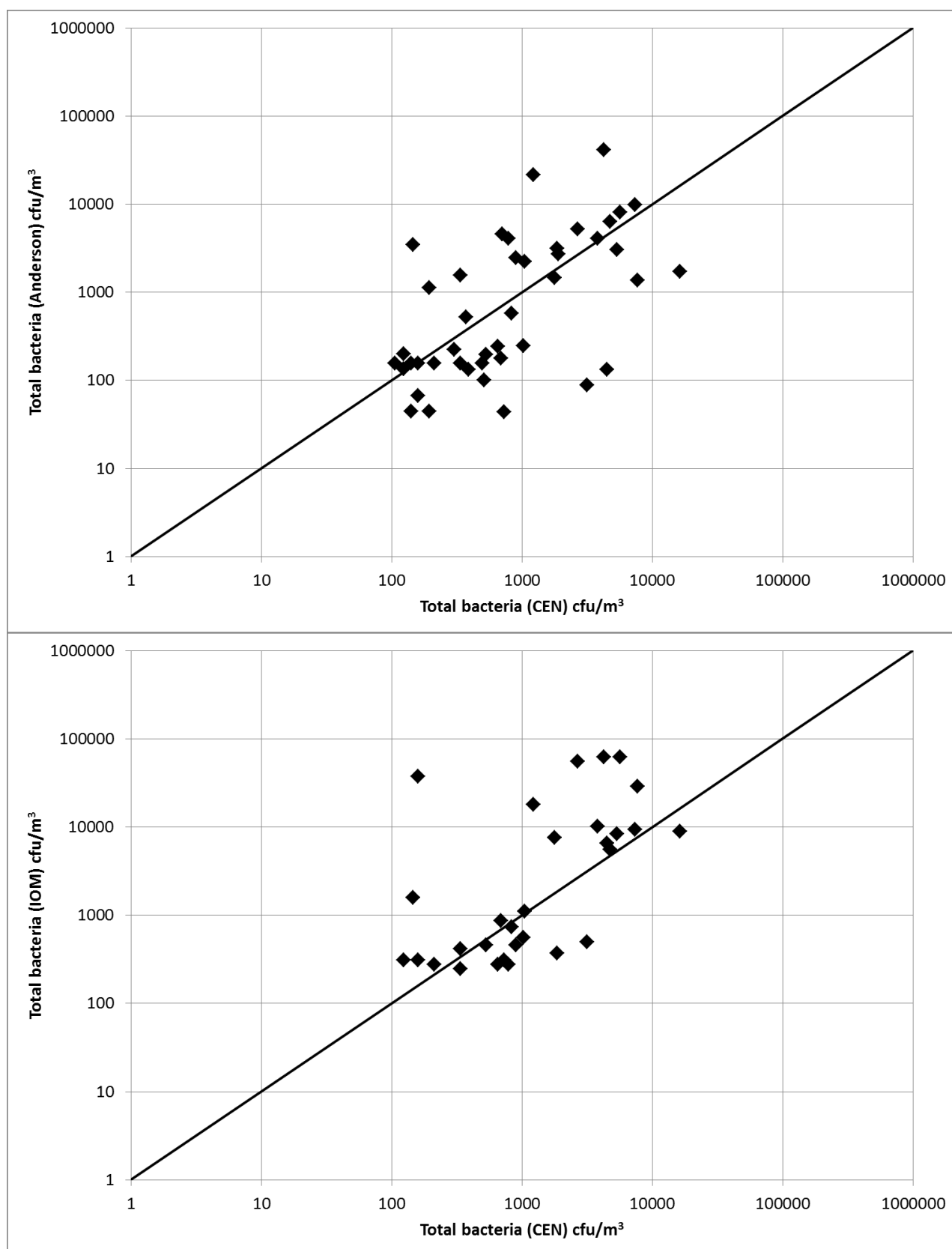
Figure 13 Scatterplots of log-transformed total bacteria concentrations from CEN and Andersen samples (upper panel) and CEN and IOM samplers (lower panel) from all four sites. IOM data below limit of detection not presented for clarity).

An analysis of replicate variability for the AfOR and CEN methods is presented in Table 6. As a comparative indicator of variability, a percentage co-efficient of variation (CoV%) has been calculated for each device at each site and for each bioaerosol determinand. The values presented in the table is grand mean of CoV%

WR1121 Final Report (20.8.13)

taking into account the individual CoV% for all of the sampling points and as such provides an comparative overview of the performance of the sampling devices. The Andersen sampler consistently demonstrates a lower CoV% (i.e. has lower between replicate variability) compared to the filtration sampling devices (it should be noted that the sampling duration for the CEN was at its lower limit of its operational capability – longer sampling times are needed to properly evaluate this device). Examples of the comparatively low variability of the Andersen compared to the more variable IOM are presented for illustrative purposes in Figure 14.

Table 6 Mean values of the co-efficient of variation % for IOM, Andersen and CEN samplers by site and by bioaerosol

Site	Bioaerosol	Sampler	Mean co-efficient of variation %
A	Total bacteria	IOM	83
B	Total bacteria	IOM	79
C	Total bacteria	IOM	77
D	Total bacteria	IOM	135
A	Total bacteria	Andersen	27
B	Total bacteria	Andersen	26
C	Total bacteria	Andersen	32
D	Total bacteria	Andersen	33
A	Total bacteria	CEN	79
B	Total bacteria	CEN	89
C	Total bacteria	CEN	85
D	Total bacteria	CEN	84
A	*A. fumigatus*	IOM	62
B	*A. fumigatus*	IOM	72
C	*A. fumigatus*	IOM	77
D	*A. fumigatus*	IOM	#
A	*A. fumigatus*	Andersen	23
B	*A. fumigatus*	Andersen	39
C	*A. fumigatus*	Andersen	33
D	*A. fumigatus*	Andersen	43
A	*A. fumigatus*	CEN	41
B	*A. fumigatus*	CEN	71
C	*A. fumigatus*	CEN	70
D	*A. fumigatus*	CEN	13
A	Gram negative bacteria	IOM	90
B	Gram negative bacteria	IOM	76
C	Gram negative bacteria	IOM	153
D*	Gram negative bacteria	IOM	82
A	Gram negative bacteria	Andersen	36
B	Gram negative bacteria	Andersen	65
C	Gram negative bacteria	Andersen	47
D*	Gram negative bacteria	Andersen	60

Typically below limit of detection
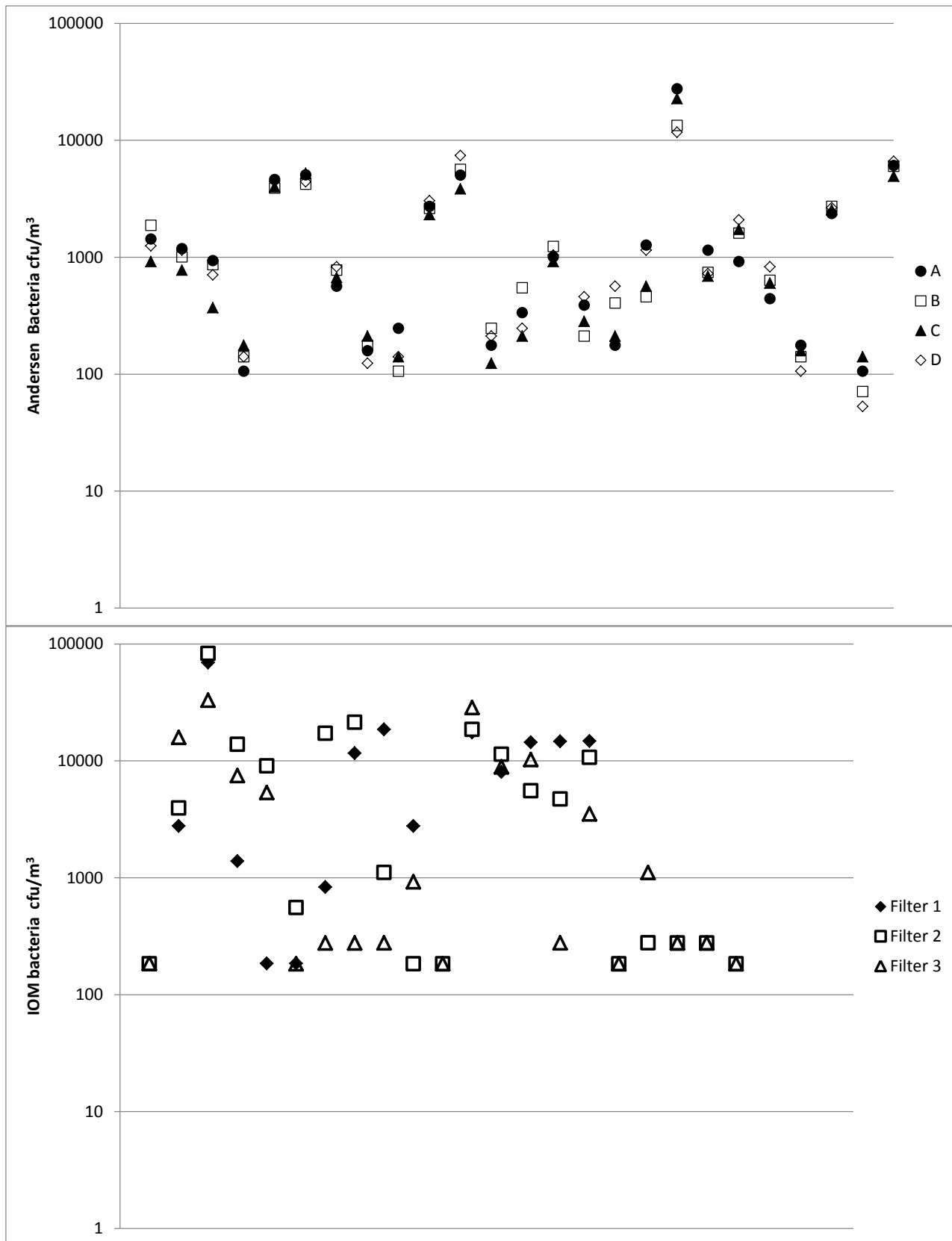* Gram negative bacteria not assessed with CEN

Figure 14 Illustrative graphs of between-replicate data spread for bacteria sampled with the Andersen (site B) and the IOM (site A)

4.4 Comparison of standard AfOR techniques with real-time particulate detection and a PCR-based detection method for *Aspergillus fumigatus*

Previous sections describe comparison between AfOR and CEN methods for *Aspergillus fumigatus* spore emission (in cfu/m^3) from composting facilities. In this section, we aim to compare standard AfOR techniques with a PCR-based detection method and real-time particulate detection for *Aspergillus fumigatus*. It is important to note that a direct comparison is difficult as the measurands are different depending on the method used:

    i.   AfOR and CEN methods measure colony-forming units (cfu) per cubic metre i.e. viable spores only.

    ii.   qPCR based detection method measures a number of genomic copies that is correlated to the number of spores (both viable and non-viable) equivalent per cubic metre.

    iii.   The real-time particle detection method measures particle mass per cubic metre.

So, due to the different nature of the measurands, we decided to directly compare results in a similar way to section 4.1 of this report.

    (a) <u>Comparison of standard AfOR techniques with a PCR-based detection method for *Aspergillus fumigatus*</u>

The qPCR method has the wider dynamic range (4-5-Log) comparing to AfOR methods. The scatter plot (Fig. 15) reflects the lower limit of detection (LOD) for AfOR methods, 4 cfu.m^{-3} and 185-278 cfu.m^{-3} for the Anderson and IOM methods respectively. So, it is justified to eliminate points at 185 and 278 cfu.m-^{3} for the IOM method and at 4 cfu.m^{-3} for the Andersen method.
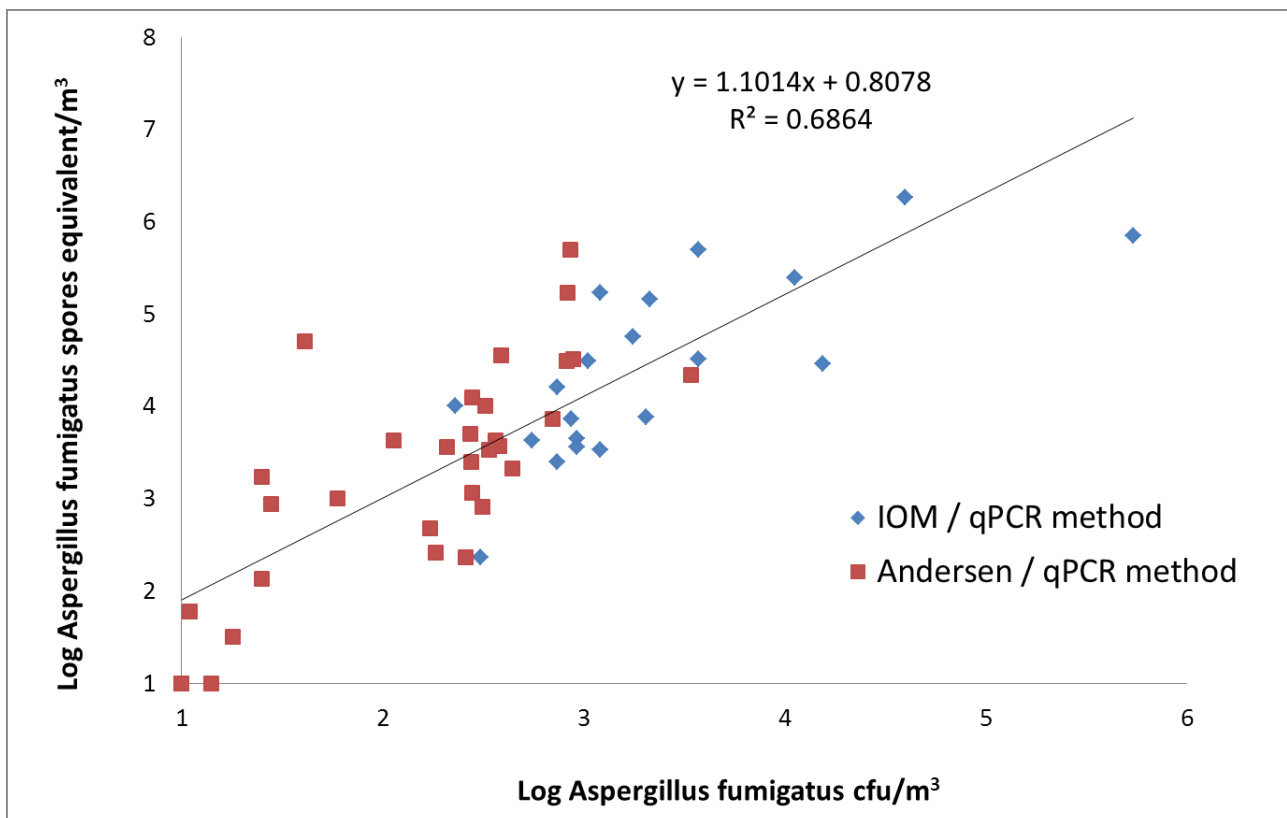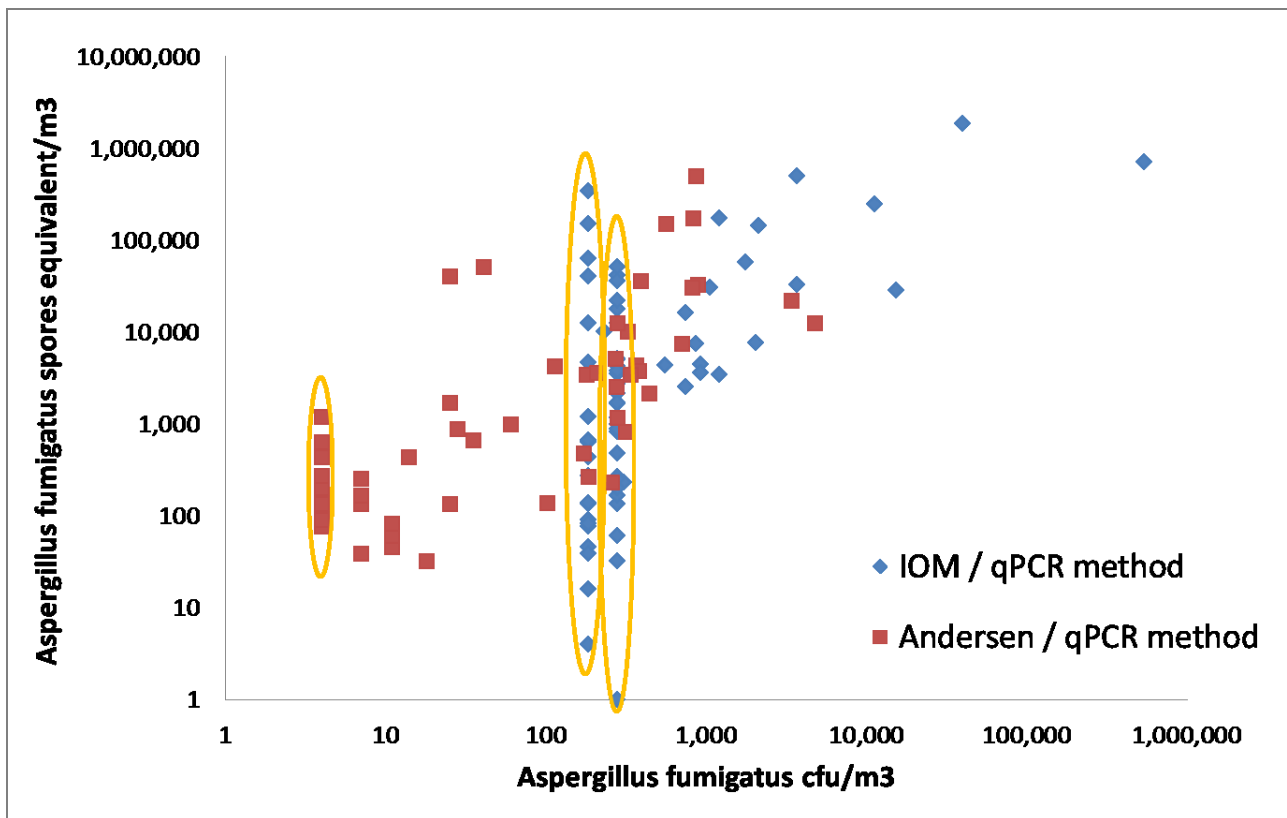
Figure 15.1: Comparison of standard AfOR methods and qPCR based method. On the top figure, points showing the limit of detection of IOM and Andersen methods are surrounded in orange. On the bottom figure, points showing LOD for both AfOR methods have been removed to clarify the plot. A trend line is added to correlate methods.
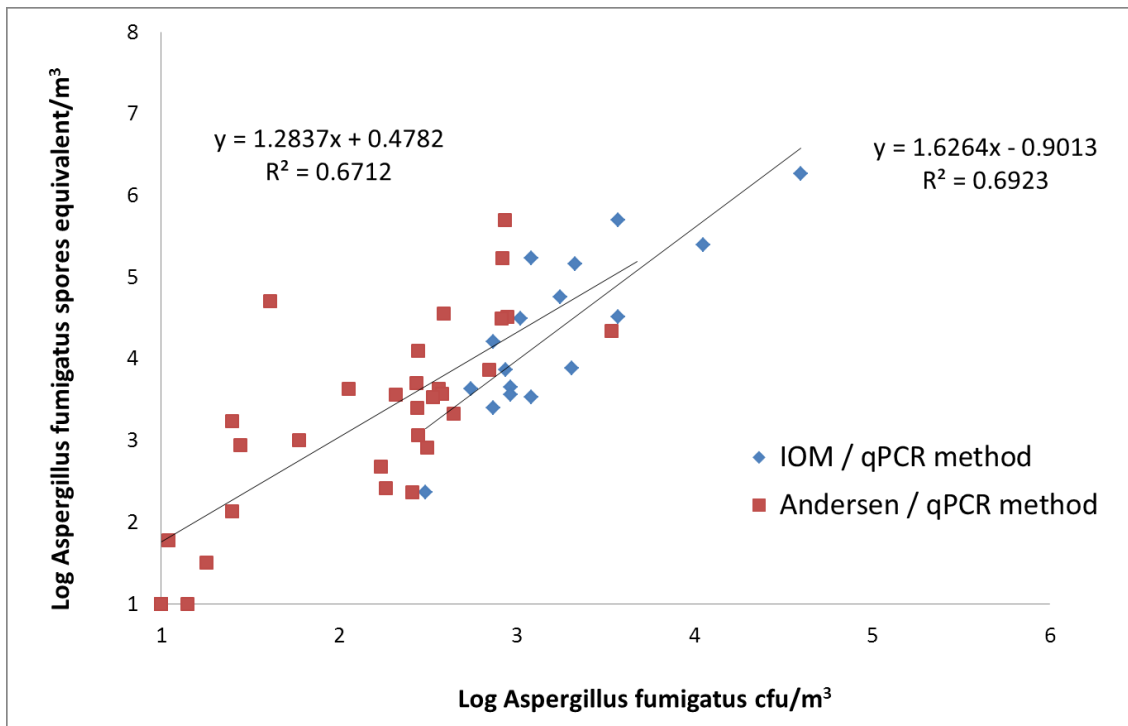
Figure 15.2: Comparison of standard AfOR methods and qPCR based method. Trend lines have been added for each AfOR method. Comparison of IOM method to qPCR method shows a slightly better relationship with an r^2 value of 0.69.

Interestingly, results for the CEN method cover the medium range of the dynamic range from 100 to 10,000 cfu/ m^3 (Fig.16, below).
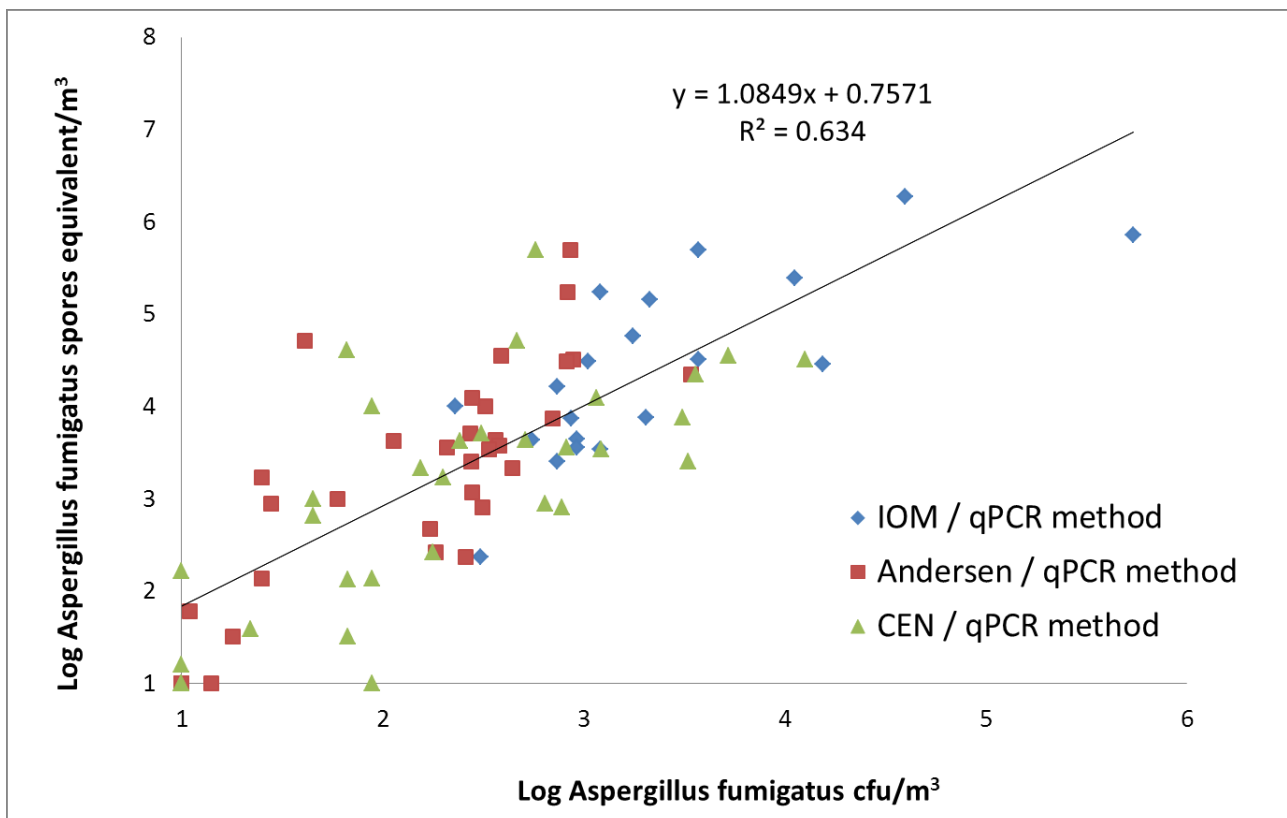

Figure 16: Comparison of standard AfOR, CEN methods and qPCR based method. A trend line is added to correlate methods.

WR1121 Final Report (20.8.13)

Scatter plots (Fig 15 and 16) don't show an obvious relationship between AfOR methods and the qPCR method, probably due to different ratios between viable and non-viable spores at each site. However a trend could be identified more clearly when plotting qPCR results and IOM results for individual sites. (Fig. 17).
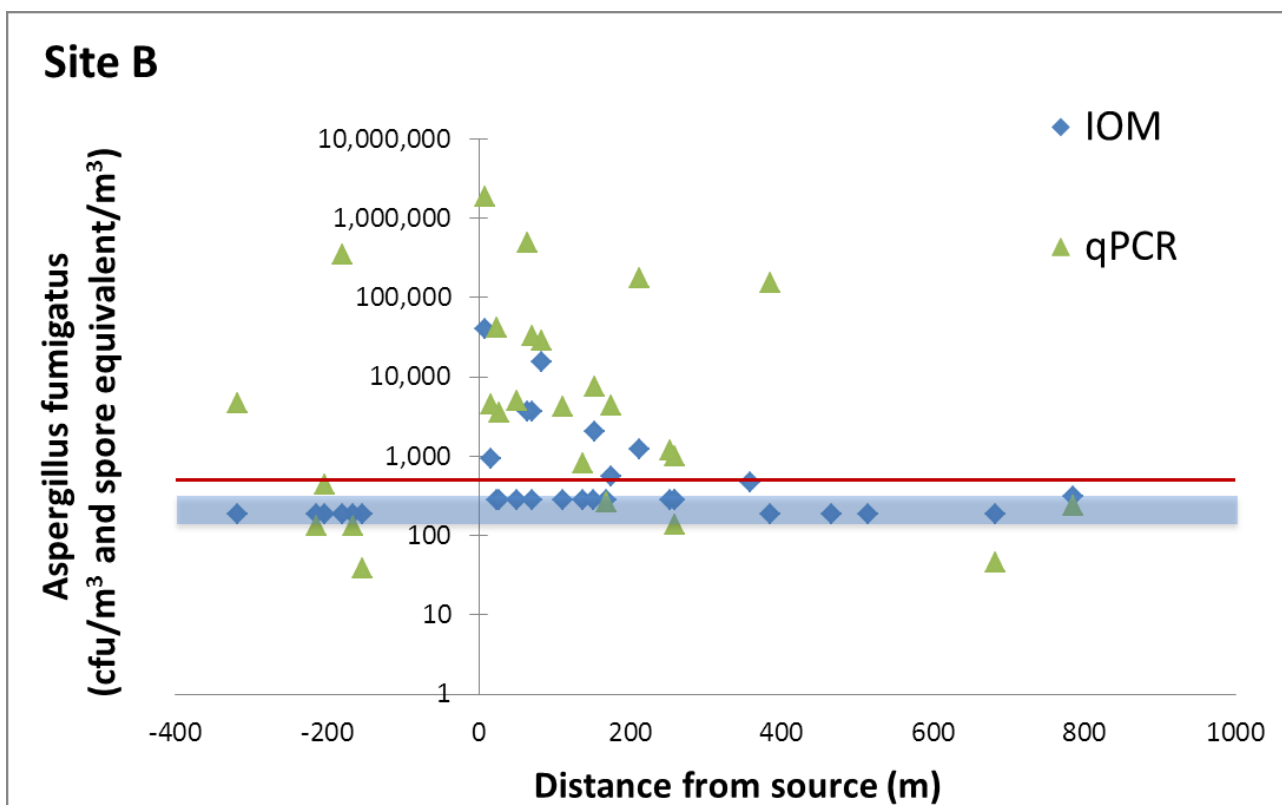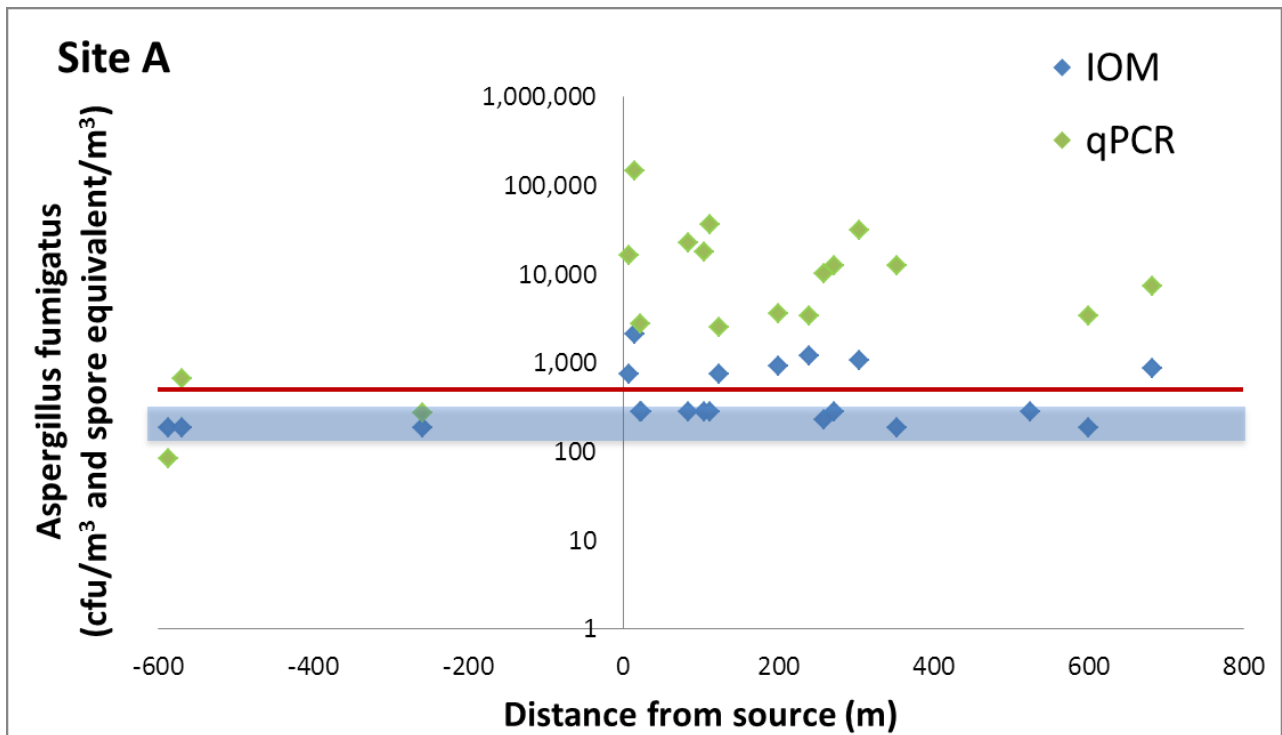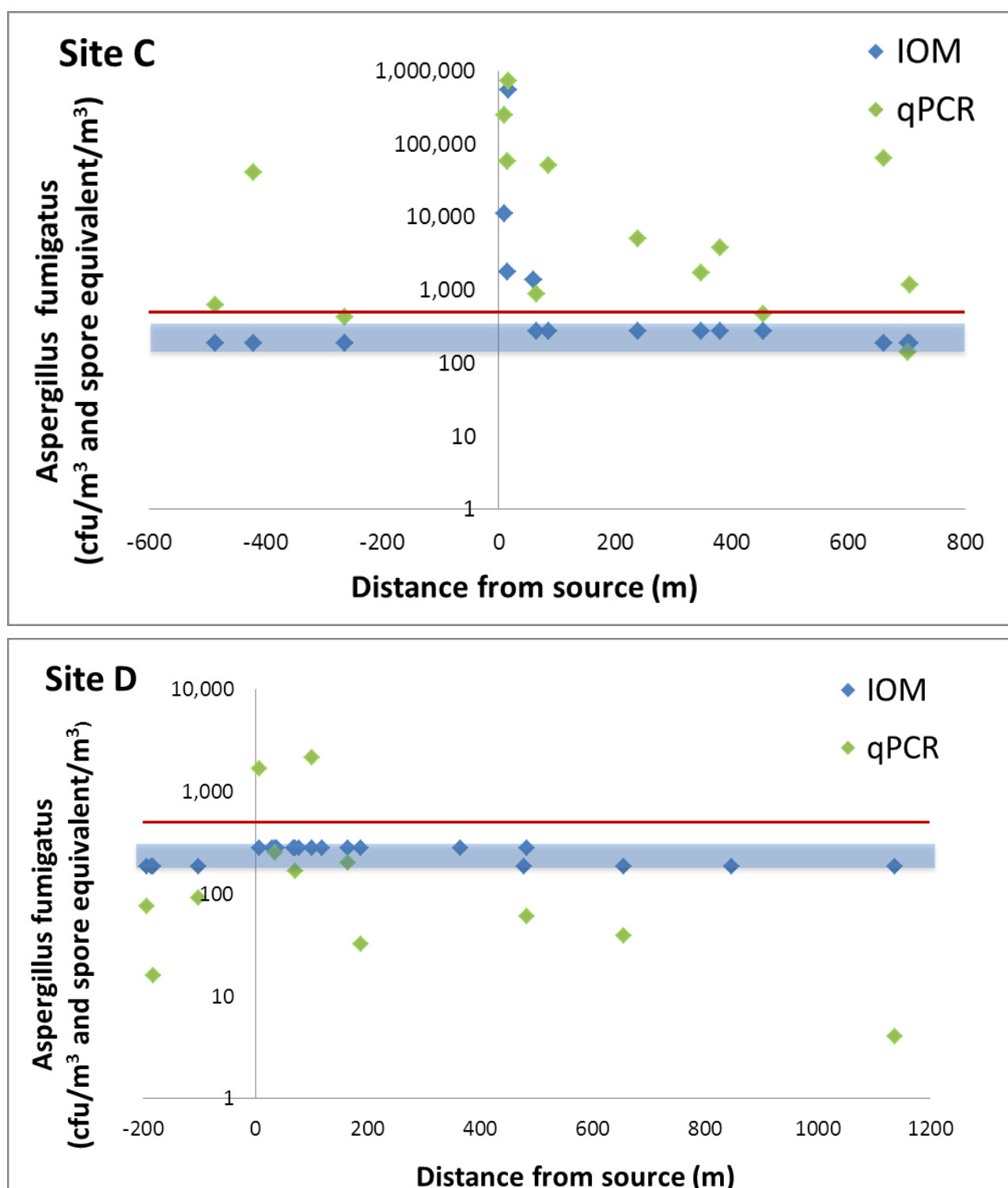
Figure 17: Comparison *Aspergillus fumigatus* bioaerosols emission for all sites (IOM method and qPCR). Blue rectangles show limits of detection of IOM method. The Environment Agency acceptable concentration of 500 CFU/m^3 is presented as a comparative benchmark (red line)

Generally, the qPCR method gives higher spore values per cubic metre compared with the IOM method, as the method measures the total amount (viable and non-viable) of *Aspergillus fumigatus spores* present in the sample. This information may be useful for health studies. However, lower spore values are also measured by qPCR, below the limit of detection of IOM method.

For all sites, the qPCR method shows a similar spore concentration decay as a function of the distance from the source as AfOR methods. In a few cases (listed below), the spores decay trend is still observable and similar to the IOM method but needs to be assessed in regard to higher background concentrations of spores measured by qPCR. This phenomenon has been observed for Site B (autumn and winter samples) and for Site C during winter under meteorological conditions when there is a high haze due to low wind speeds and poor dispersion.

The qPCR method shows some potential benefits over culture based methods, as the dynamic range of this method is wider (4-5-log) than either of the AfOR and the CEN methods. It is also quicker to carry out and has the potential for automation that could be a cost effective methods to monitor spores emissions from composting facilities.

Spatial distribution of *Aspergillus fumigatus* spores along sampling transects, gives similar results compared to AfOR (and CEN) methods.

(b) Comparison of standard AfOR techniques with real time particle detection method for *Aspergillus fumigatus*

The real time particle detector, measured total particle mass (TSP), and the mass of particles with an aerodynamic diameter of 10 μm or less (PM_{10}), during the sampling periods. In order to facilitate a comparative analysis, we have calculated the arithmetic mean of the particle mass measured over the sampling time. The scatter plots in Fig. 18 compare the AfOR methods results and the real time particle detection method for TSP and PM_{10}.

Figure within plot:
$y = 0.217x + 1.0585$
$R^2 = 0.2905$

Axis labels:
- Y-axis: Log PM10 ($\mu g/m^3$)
- X-axis: Log *Aspergillus fumigatus* cfu/m^3
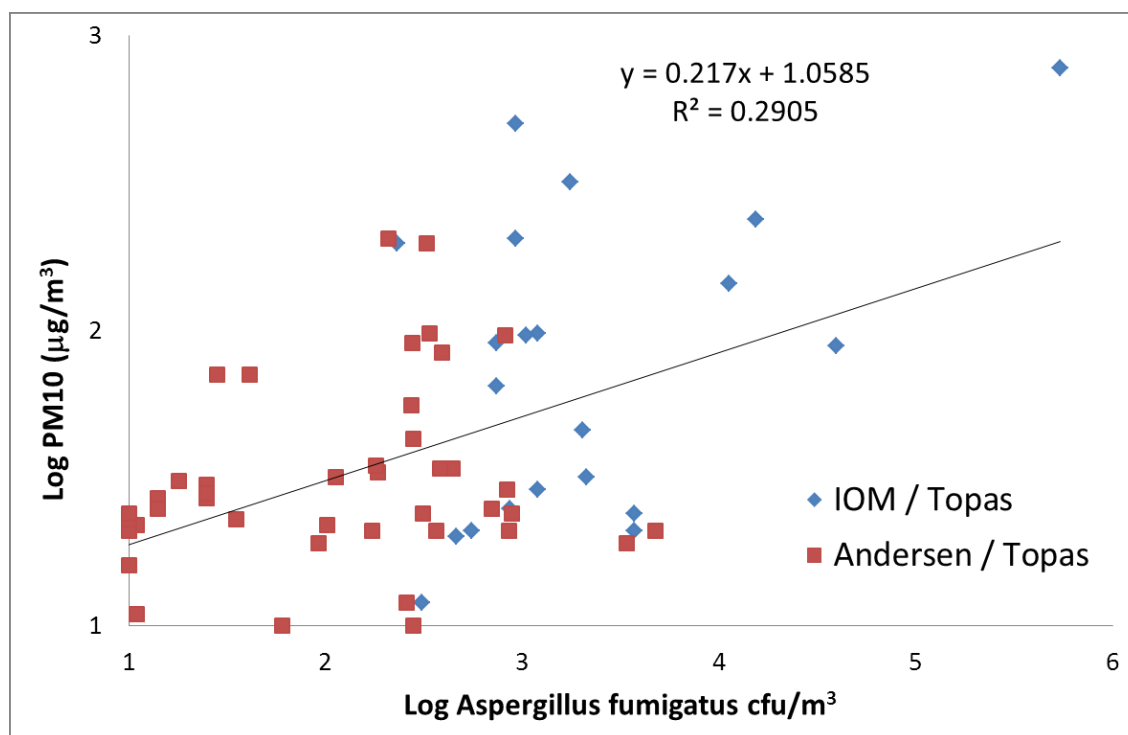
Legend:
- IOM / Topas
- Andersen / Topas

Figure 18.1 Comparison of AfOR methods and real time particle detection for all sites. The top panel shows the total suspended particle and the bottom panel shows the PM_{10} fraction. For clarity, LOD have been removed for both AfOR methods.

There is no obvious correlation between real-time particle monitoring (PM_{10} fraction) and AfOR methods.
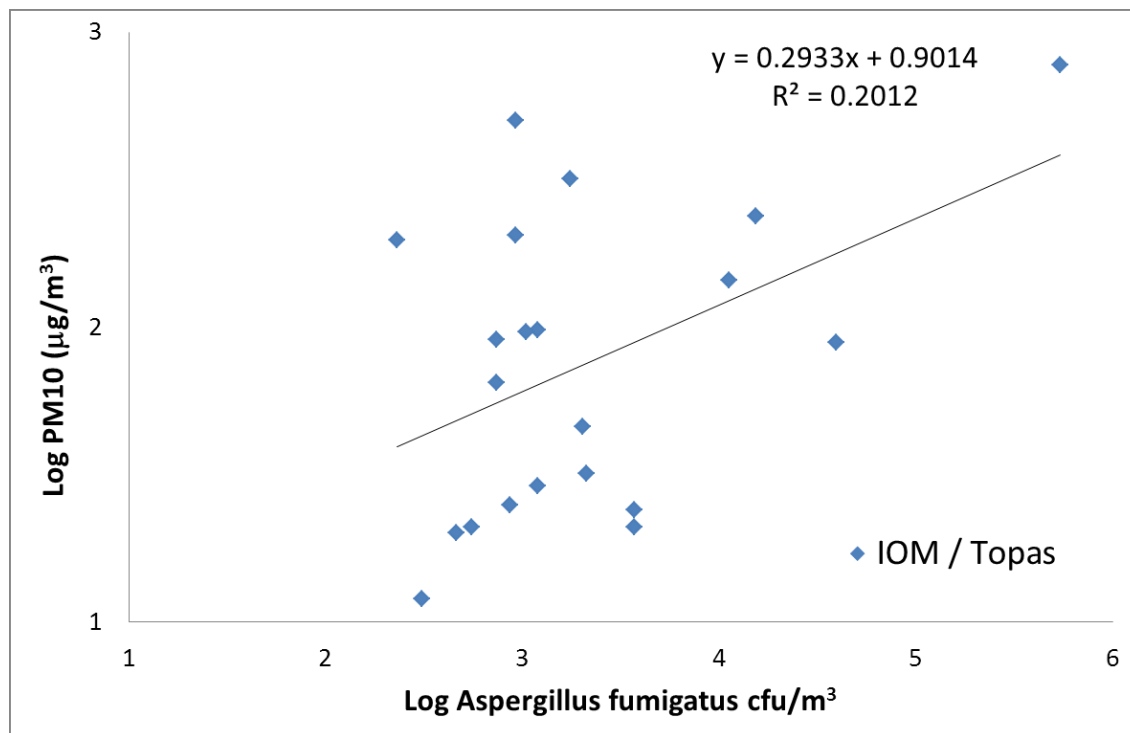

Figure 18.2 Comparison of IOM method and real time particle detection for PM_{10} fraction
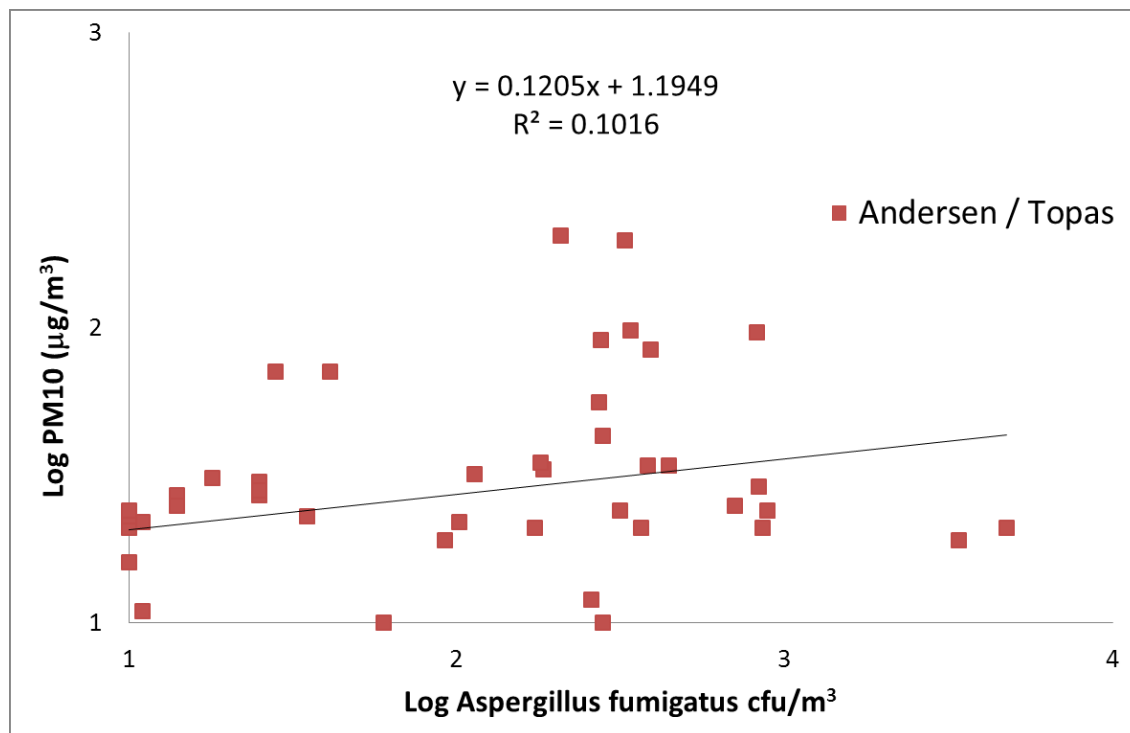

Figure 18.3 Comparison of Andersen method and real time particle detection for PM_{10} fraction

No obvious correlation between individual IOM or Andersen methods and PM_{10} fractions could be observed in Fig.18.2 and 18.3. Additional measurements points would be required to establish a better relationship between methods.

However, when data is plotted on an individual site basis, the spatial distribution of spores measured with the IOM method and real time particle detection have a similar trend for all sites (Fig. 19).
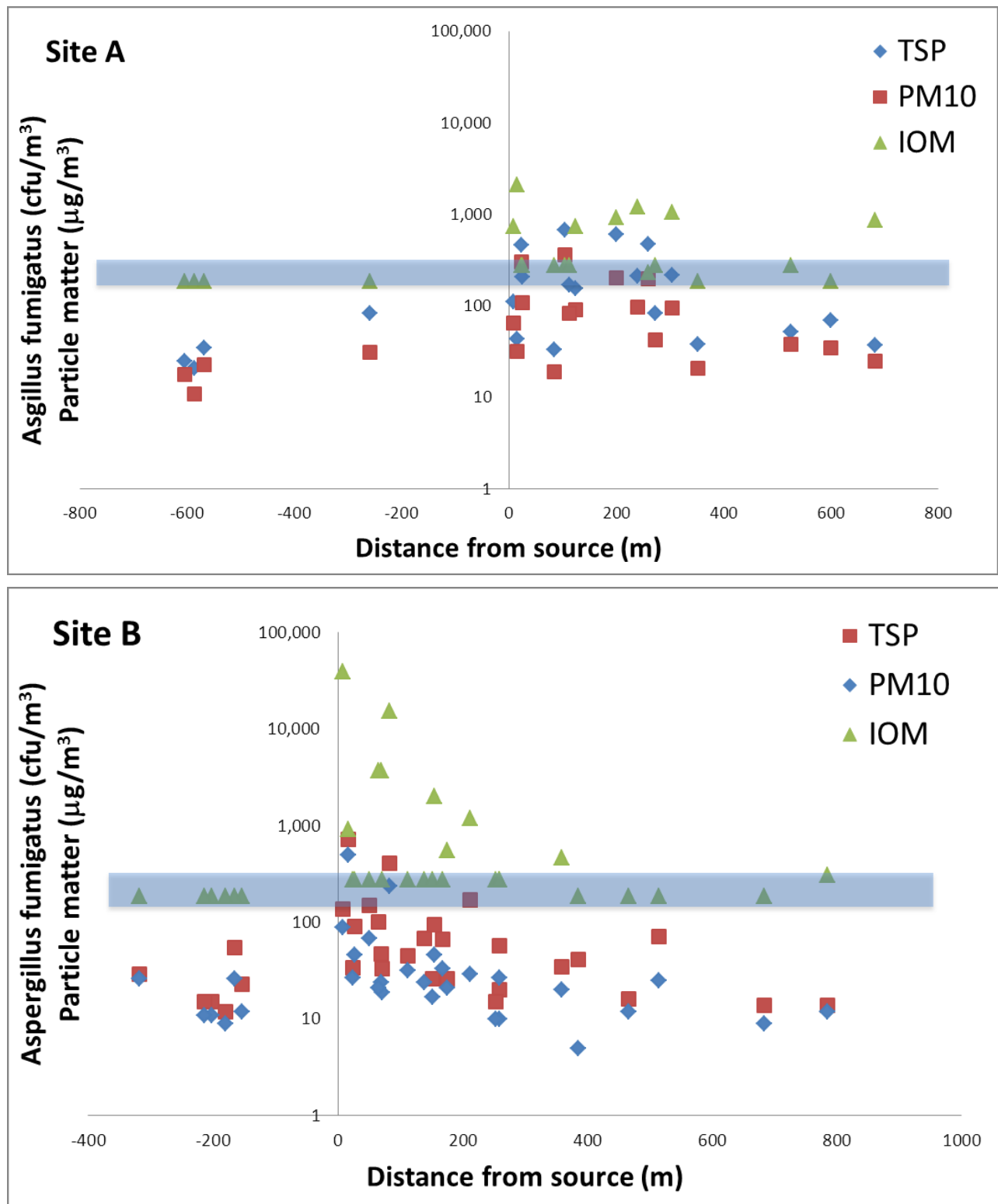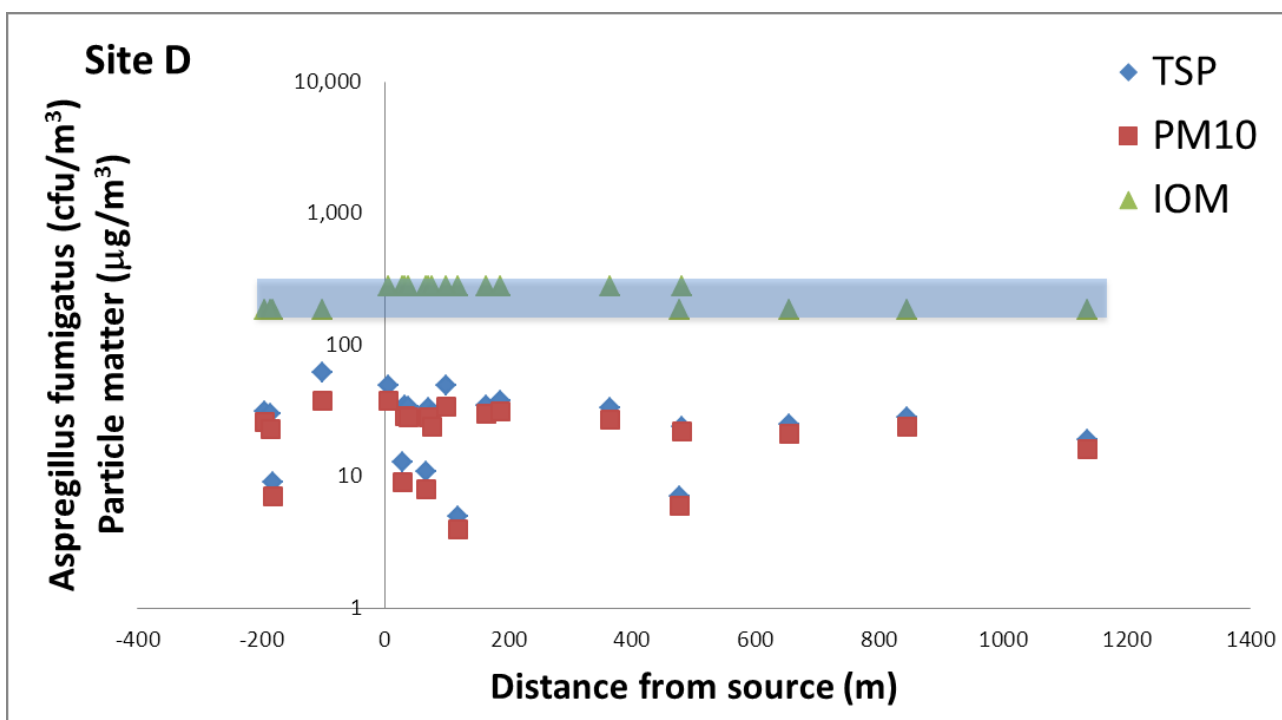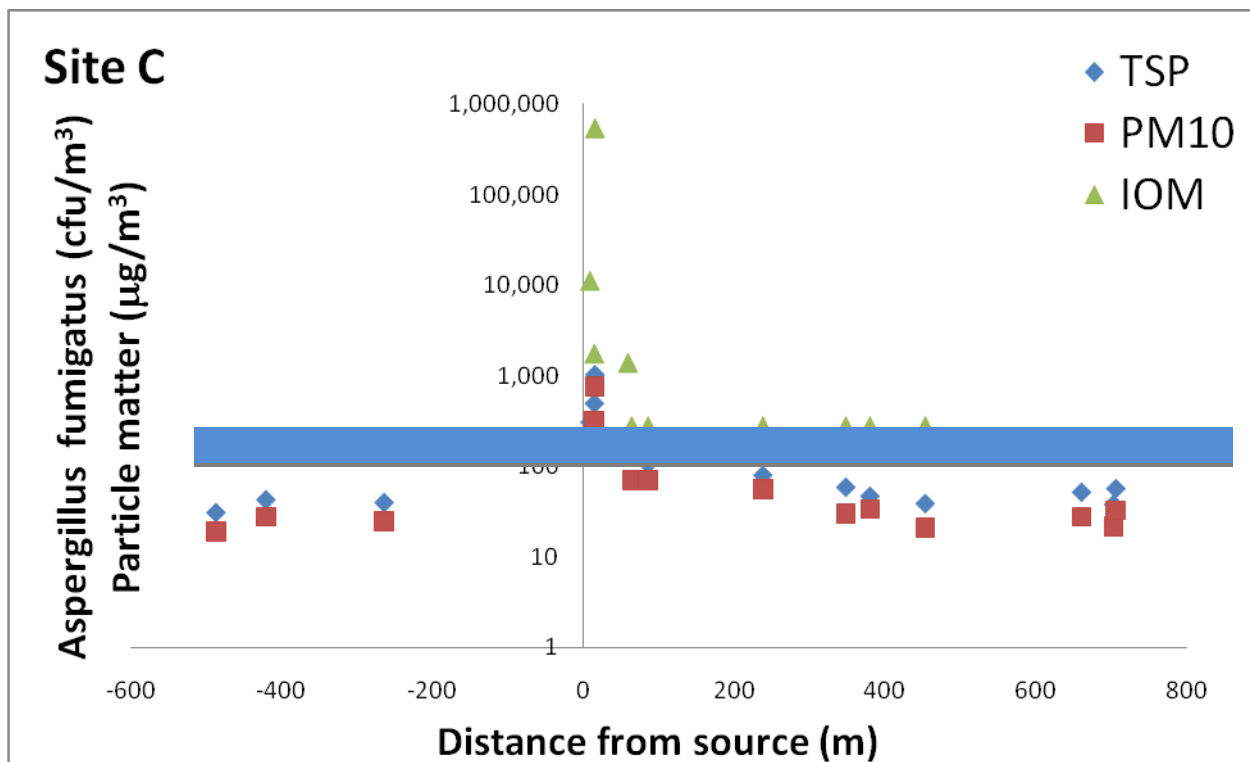
Figure 19: Comparison of IOM method and real-time particle detection (blue for TSP and red for PM10) method for all sites. Blue rectangles show limits of detection of IOM method.

The difference in signal magnitude is consistent between the IOM method and real time particle detection for all sites. As expected, TSP values are always higher than the PM_{10} fractions, but both of them reflect the presence of *Aspergillus fumigatus* spores.

Real time particle detection shows that both TSP and PM_{10} are correlated to *Aspergillus fumigatus* spore concentration. The best correlation is observed between the IOM method and the PM_{10} fraction. An attempt has been made to correlate cfu values and particle mass (TSP and PM_{10}) in order to define a threshold value

WR1121 Final Report (20.8.13)

for the real time detection method that match with the acceptable level of *A. fumigatus* spores (500 cfu/m^3). This assessment is based on the actual data set and takes into consideration the fact that emissions of bioaerosols could fluctuate during the sampling time. However, values of 100 μg/m^3 of TSP and 50 μg/m^3 of PM$_{10}$ represent a good indicator and threshold value for real time *Aspergillus fumigatus* monitoring. Note that PM$_{10}$ levels in excess of 50 μg/m^{3,} might also indicate the possibility of exceedances of dust limit values.
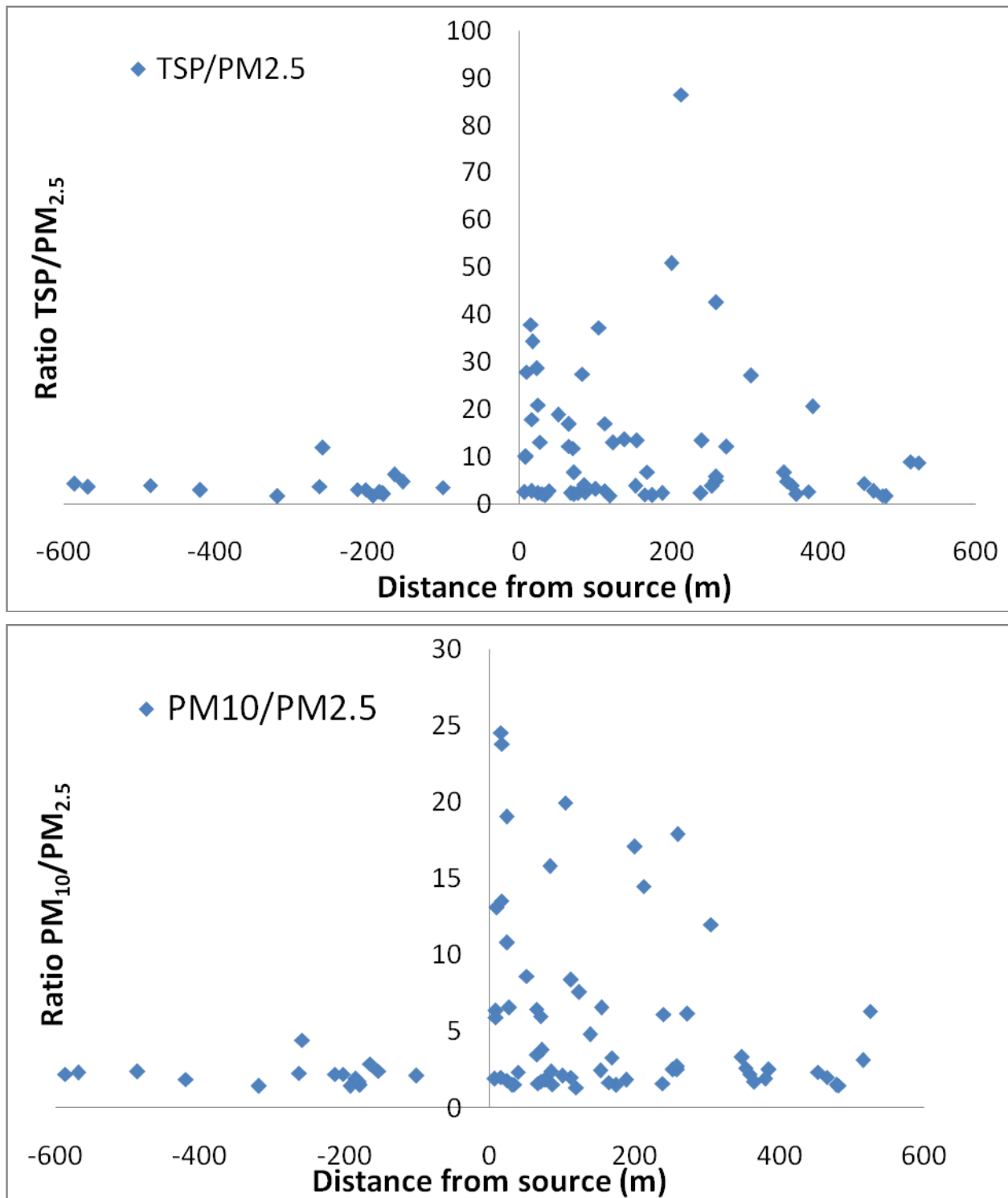

Figure 20: Comparison of TSP/PM2.5 ratio (top panel) and PM10/PM2.5 ratio (bottom panel) for all sites in function of distance from source.

WR1121 Final Report (20.8.13)

The potential of real time particle detection is emphasised when plotting the ratio of TSP/PM$_{2.5}$ and PM$_{10}$/PM$_{2.5}$ fractions. Figure 20 plots the distribution of ratios as a function of distance from source for all sites. As an example, TSP/PM$_{2.5}$ ratio superior to 9 matches at 89% with IOM results above the acceptable *Aspergillus fumigatus* threshold value (500 cfu/m^3). Similarly, PM$_{10}$/PM$_{2.5}$ ratio superior to 3 matches at 79% with IOM results above the acceptable *Aspergillus fumigatus* threshold value (500 cfu/m^3). Those ratio values are given for information; deeper correlation analyses would be required to evaluate appropriate, TSP/PM$_{2.5}$ and PM$_{10}$/PM$_{2.5}$ ratios.

4.5 Comparison of standard AfOR techniques with priority bio-markers endotoxin and glucan

Components such as endotoxin and glucan are present in both viable and non-viable bioaerosols and require separate measurement and enumeration.
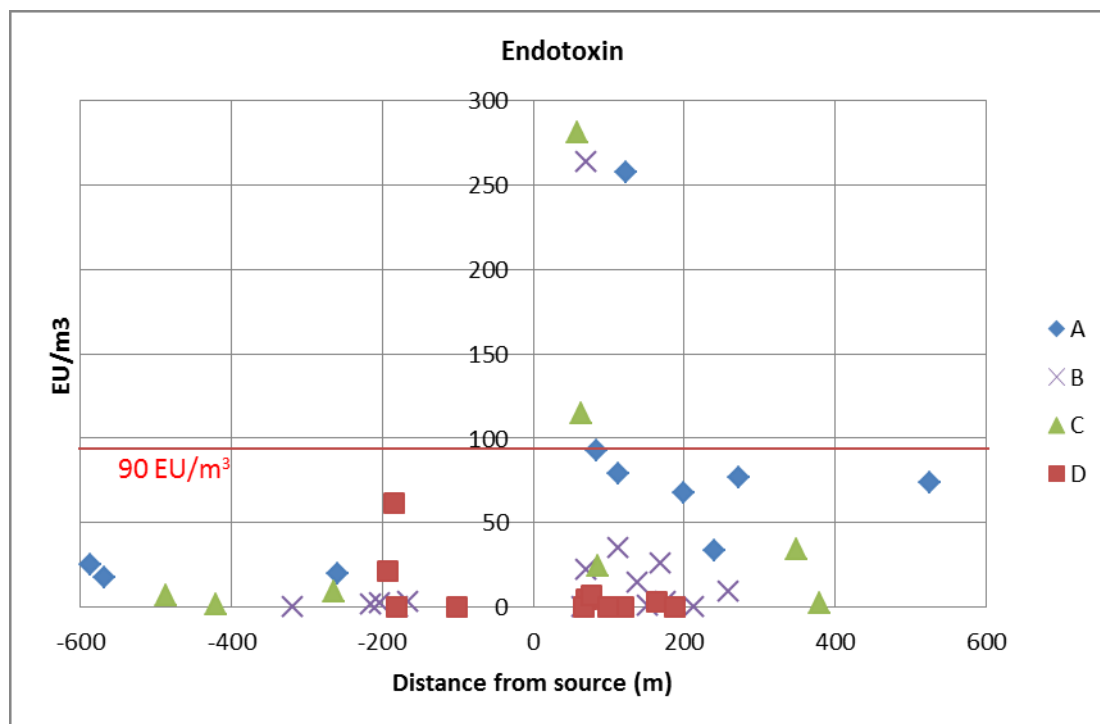

Figure 21 Spatial distribution of endotoxin along the sampling transects at all sites with data categorised by site

The Dutch Expert Committee on Occupational Safety (DECOS) recently signed an agreement with other Nordic countries to push through a new occupational endotoxin standard (European Agency for Safety and Health at Work (EU-OSHA), 2011). The new current standard is 90EU/m^3 (NordicExpertGroup, 2011). This was based on the conclusion that no adverse health effects are expected after chronic occupational exposure at 90 EU/m^3.

The data (Figure 21) shows five points over this guideline, which were all recorded within 59 to 123m downwind of the open sites (Site C and Site A, the larger sites had two each). All three open sites showed at least one measurement in excess of the guideline within this distance, and distance from site appears to be the main factor rather than any particular activity.

Upwind concentrations of endotoxin tended to be very low, ranging from undetectable to generally less than 20 EU/m^3. There is one notable exception with a measurement of 62 EU/m^3 upwind at Site D during Spring 2012. As there is farming activity surrounding the site this may be attributable to this.

There does not appear to be a strong correlation with viable Gram negative bacteria measured as per the AfOR protocol. In particular Site D showed concentrations of 1 x 10^4 cfu/m^3 of Gram negative bacteria on IOMs running simultaneously with the IOMs sampling for endotoxin, but the measurement of endotoxin at this point was below 7 EU/m^3. Conversely the elevated background concentration of 62 EU/m^3 mentioned above showed viable Gram negative concentrations of only 185 cfu/m^3 on the IOM and 34 cfu/m^3 on the Andersen. This could point to an inverse relationship between endotoxin and viable Gram negatives (where endotoxin is released as cells die). However, instances across the other sites where 70-80 EU/m^3 correspond with either 200 or 3000 cfu/m^3 on the AfOR equipment suggest a correlation inverse or otherwise is very unlikely.
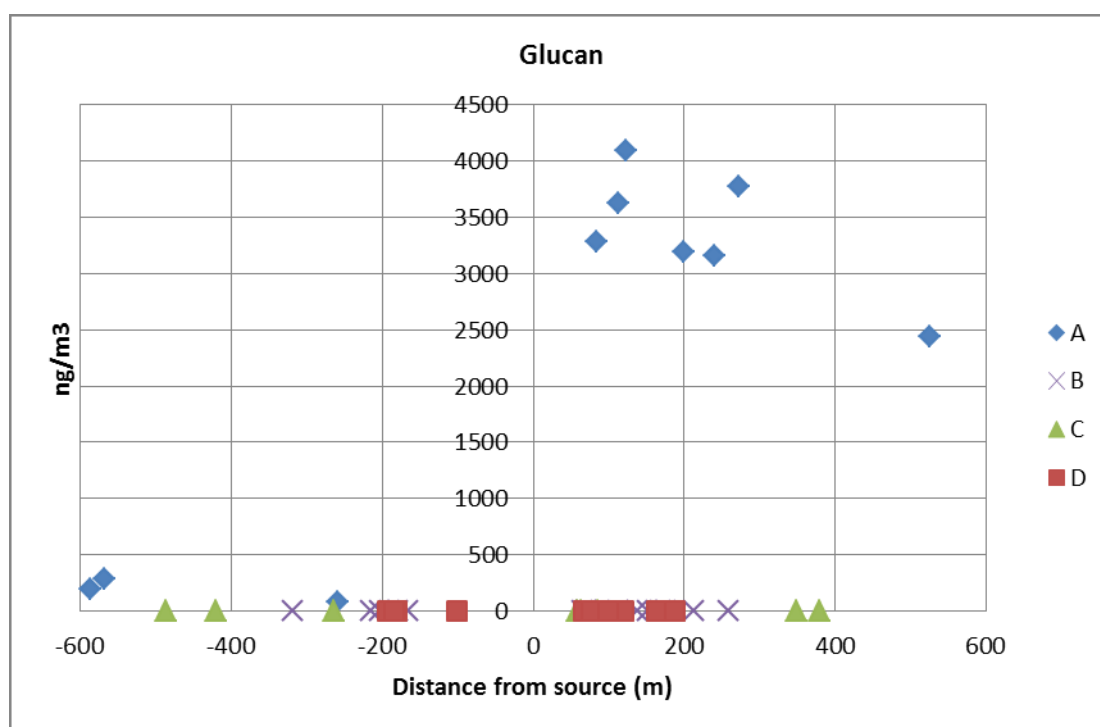

Figure 22 Spatial distribution of glucan along the sampling transects at all sites with data categorised by site

Fewer exposure standards have been proposed for (1-3)-β-D-glucan, and papers generally tend to quote 10ng/m^3 based on work in Sweden by Rylander (1997).

It is immediately apparent (Figure 22) that the majority of measurements at three of the four sites are well below this guideline. Sites B, C and D all recorded concentrations of under 5 ng/m^3 at all points. Site A showed significant concentrations of glucan, with elevated concentrations up to 525m downwind of the site. However, it is also notable that all four upwind measurements of glucan at Site A also exceed this guideline significantly, with concentrations recorded ranging from 75 to 286 ng/m^3. This could point to a local source (although no off-site activity was seen that could account for this) or there may be a re-aerosolisation of previously deposited particles. However, the concentration range is much greater downwind of the site which indicates that it is a major contributory factor to these measurements.

In comparison with AfOR methods, glucan is associated with fungal biomass rather than *A. fumigatus* specifically so it is difficult to draw a direct comparison between the two measurements. Figure 6 indicates that the IOM does not necessarily show higher concentrations of *A. fumigatus* specifically at Site A compared to the other open sites, although the Andersen does indicate this.

Glucan results could indicate a diverse fungal load generated by Site A, but what is unclear is what other local sources may be contributing, or whether the site itself may have raised general concentrations in the area by deposition over a period of time.

4.6 Comparison of bioaerosol and odour measurements

Micro-organism counts (total bacteria, *Aspergillus fumigatus* and Gram negatives) and odour concentrations were sampled simultaneously in June and September at Site B. Bioaerosols were captured using the IOM filter method, the Andersen method, the CEN method and the Coriolis method for spores. The results of the odour emissions sampled directly from windrows using the Lindvall hood are shown in Tables 7 and 8 for June and September 2012. As would be expected, the odour concentration of the samples collected at source was higher than those detected at downwind sampling locations (which have been subject to dispersive processes). The principal purpose of collecting odour samples by this means was to generate a measured value of the odour emission rate from different materials on site as in input to the odour modelling. An average of these emission rates was used in the modelling to represent static sources and a back extrapolation method was used to determine emission rates for agitation activities, as explained in section 3.8.

Table 7. Results from the Lindvall hood measurements made in June 2012

Sampling time (Hrs.)	Sample source and position	Sample Odour concentration geometric mean (ou_E/m^3)	Odour emission rate ($ou_E/m^2 s$)	Material temperature (°C)	Air speed under hood, (m/s)
11:47	Fresh shredded	9,335	74	42.9	0.28
12:22	Mature 1	891	9	53.3	0.37
12:55	Mature 2	11,050	115	70.4	0.37
14:16	Fine screen	5,700	57	41.6	0.36
15:28	Course screen 1	8,130	74	35.7	0.33
13:30	Course screen 2	7,630	70	35.7	0.33

Table 8. Results from the Lindvall hood measurements made in September 2012

Sampling time (Hrs.)	Sample source and position	Sample Odour concentration geometric mean (ou_E/m^3)	Odour emission rate ($ou_E/m^2 s$)	Material temperature (°C)	Air speed under hood, (m/s)
13:11	1 week old	2,845	6	42.6	0.07
13:31	2 weeks old	1,825	8	78.1	0.16
13:59	15 weeks old	4,447	34	72.1	0.27

Only the IOM filter method provided sufficient data to permit a comparison with the odours data obtained at breathing (1.5 m) and 4.5 m heights on both sampling dates as shown in Figures 23 and 24. A general trend

of a reduction in bioaerosol concentration with distance away from source was observed in both June and September samplings. Although odour trend in June at 1.5 m matches this trend of bioaerosols, the September results do not. Odour concentrations at 4.5 m on both sampling occasions were approaching the lower limit of detection and show no clear trend. These data suggest no consistent correlation between bioaerosols and odour. Caution should be applied in drawing conclusions from a relatively limited dataset however and further repeats of the simultaneous odour and bioaerosol sampling are needed to establish if there is a consistent relationship. The present information does not support using odour as a proxy indicator for exposure to bioaerosols.
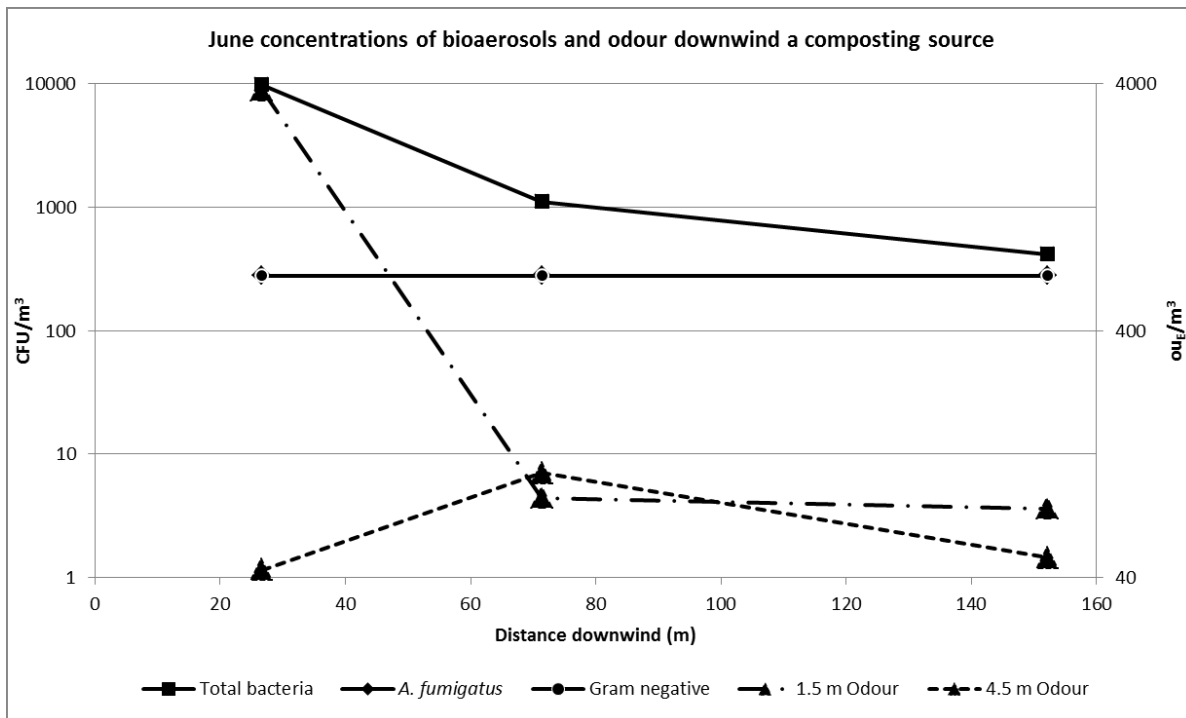

Figure 23. Downwind concentrations of bioaerosols and odours at Site B site in June 2012 (n.b. only total bacteria were detectable in the June sampling. The Lower Limit of Detection for *Aspergillus fumigatus* and Gram negative bacteria is plotted for reference)
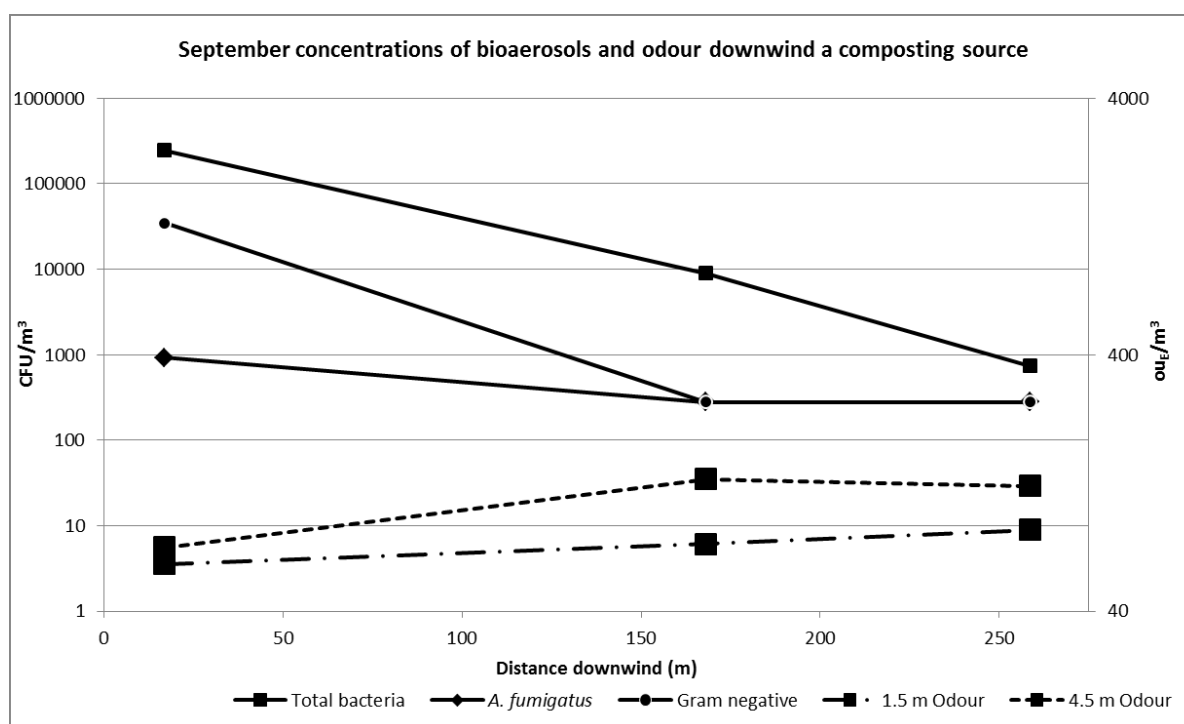
Figure 24. Downwind concentrations of bioaerosols and odours at Site B site in September 2012.

4.7 Comparison of modelled and measured bioaerosol and odour concentrations

Model results are available for all sites, however only the results for Site B are presented in this report. This is the site with the most complete bioaerosol data set and the only site at which odour sampling was undertaken.

Short term modelling

Figures 25, 26 and 27 show the envelope of emission rates calculated through the back calculation process. The distributions are widely scattered. A similar study by Shi and Hodson (2012) used data collected up to 250m downwind of various sites and based on a point source modelling approach. Whilst the emission rates from this project encompass the range reported by Shi and Hodson, their results showed a significantly narrower range of emission rates (spanning up to four orders of magnitude compared with up to thirteen orders of magnitude from our dataset). The large spread is in part explained by the range of monitored data upon which the back-calculated emission rates are derived. For example, the _Aspergillus fumigatus_ dataset generated by Coriolis/qPCR spans seven orders of magnitude. However, the variability in measured values does not account for the extent of the range of back-calculated emission rates. A further contribution to this range may be associated with the modelling approach taken in this project (e.g. area source as opposed to point source in the Shi and Hodson study) and/or the uncertainties inherent with the dispersion model itself. This exercise corroborates Shi and Hodson's finding that back-calculated emission rates derived from downwind monitoring of active open composting are highly variable. Whilst representative emission rate values may be taken from this distribution to conduct long term modelling exercises, appropriate caveats are necessary to highlight the extent of uncertainty that exists over such values. Additional research being undertaken in PhD research due to be completed in 2013 (Philippa Douglas, Cranfield University) is expected to improve our current understanding of the open composting source term.

Long term modelling

Figure 28 shows the modelled concentration of bioaerosols for Site B, with the *Aspergillus fumigatus* concentrations measured using the IOM and Andersen samplers, presented for illustrative purposes. The ADMS model generates a concentration at each point on a user-defined grid, for each hour of meteorological data provided. However, to reduce the amount of data produced, the model only saves summary statistics at each of the grid nodes. These include defaults such as the average, but the user can define additional output, such as percentiles or the number of hours a certain concentration is exceeded. The approach taken here was to request the 50th percentile value, which represents the median of the dataset, as well as the 99th percentile, which reflects a near worst case scenario. The 50th percentile model results (data not shown) show concentrations of less than 100 CFU/m^{3}across the entire mapped area, which does not correspond with the monitoring results. The 99th percentile output visually resembles the pattern of measured data more closely than the 50th percentile model results (concentrations up to 10,000 CFU/m^3 close to source, but by 250m concentrations have mostly decreased to below 1,000 CFU/m^3)[2]. The relationship between the 99[th]percentile modelled data and the measured data were compared graphically. Figures 29 and 30 show scatter plots of the measured and modelled data for *Aspergillus fumigatus* and total bacteria and show no clear relationship between the modelled and measured data. The measured data cover a much wider range than the modelled data. The model simulation does not generate concentrations as high as those in the upper range of measured data. This may indicate that the single emission rate value used for the modelling was not high enough to generate modelled outputs that matched those higher measured values. A stochastic approach to inputting emission rates to encompass the back-calculated emission rate range may be a possible further step.

Odour modelling

The odour modelling approach has produced high concentrations, particularly at the 99th percentile (Figure 31). The model results are consistently greater than the measured results. Typically, a site would be expected to not exceed a threshold value of 5-10 ouE/m^3 for 2 percent of the time. However, the modelled concentrations clearly exceed this, although the data shown is the 99th percentile and thus represents the near worst case scenario. This is likely to be related to the methods used to calculate the emission rate. Data collected on site represented only static sources, so back calculation was used to estimate emission rates for the agitation sources. There is currently a lack of information available on the emission rate of odour from composting agitation activities, due to the health and safety implications associated with sampling at source. A method of sampling for bioaerosol emissions close to source has been developed at Cranfield University (Philippa Douglas PhD research) and could be adapted to sample for odours. In addition, the model setup for the odour was possibly not ideal as the model inputs were determined by the setup for bioaerosols. This was undertaken in order to attempt to compare modelled emissions of odour and bioaerosols. However, the data collected on site for odours was limited due to the expense associated with odour sampling and due to health and safety issues. There does not appear to be any similar trends in the model results for odour and bioaerosols, however both sets of model outputs highlight the difficulties of appropriately defining the source for this complex scenario.

---

[2]It should be noted that the measured concentrations represent single snapshots in time, whereas the modelled data shows the near worst case scenario.
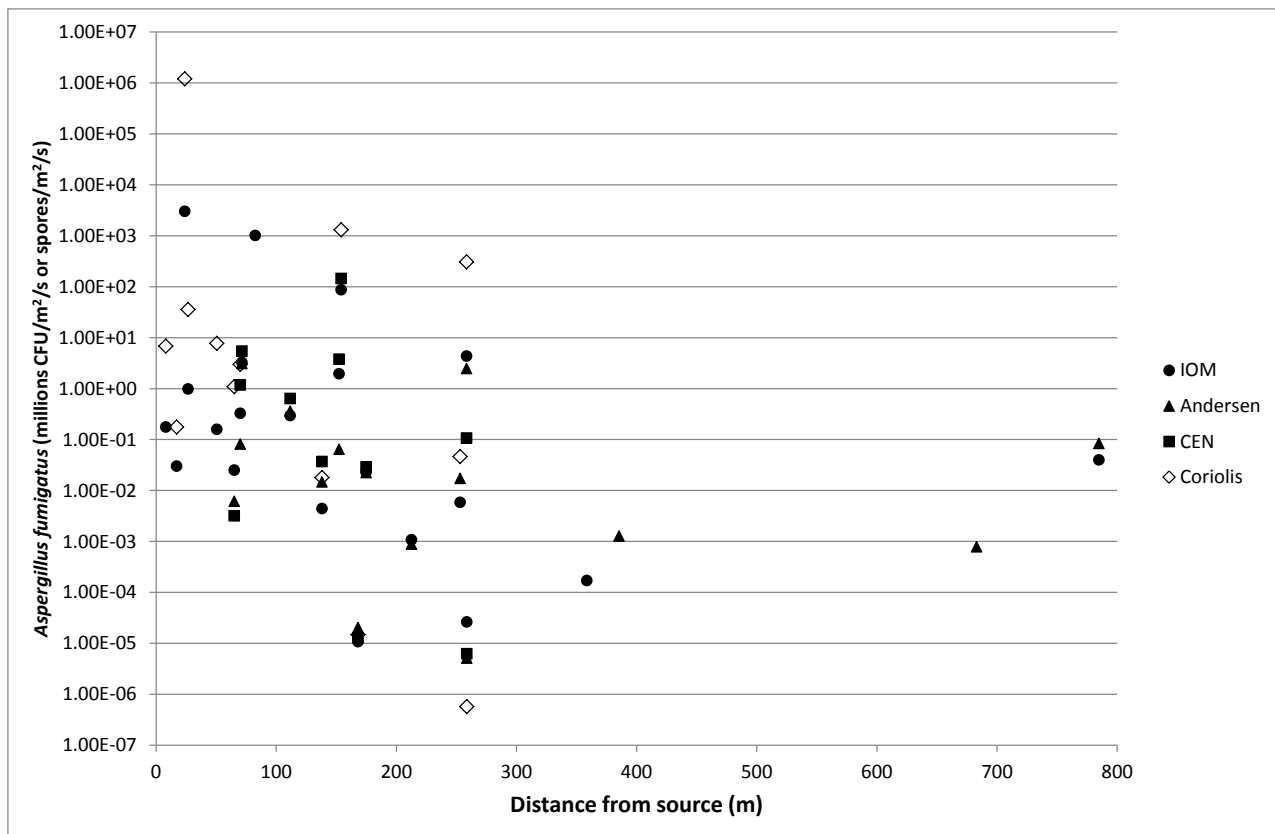
Figure 25 Back-calculated emission rates for Site B, *Aspergillus fumigatus*(corrected for background)
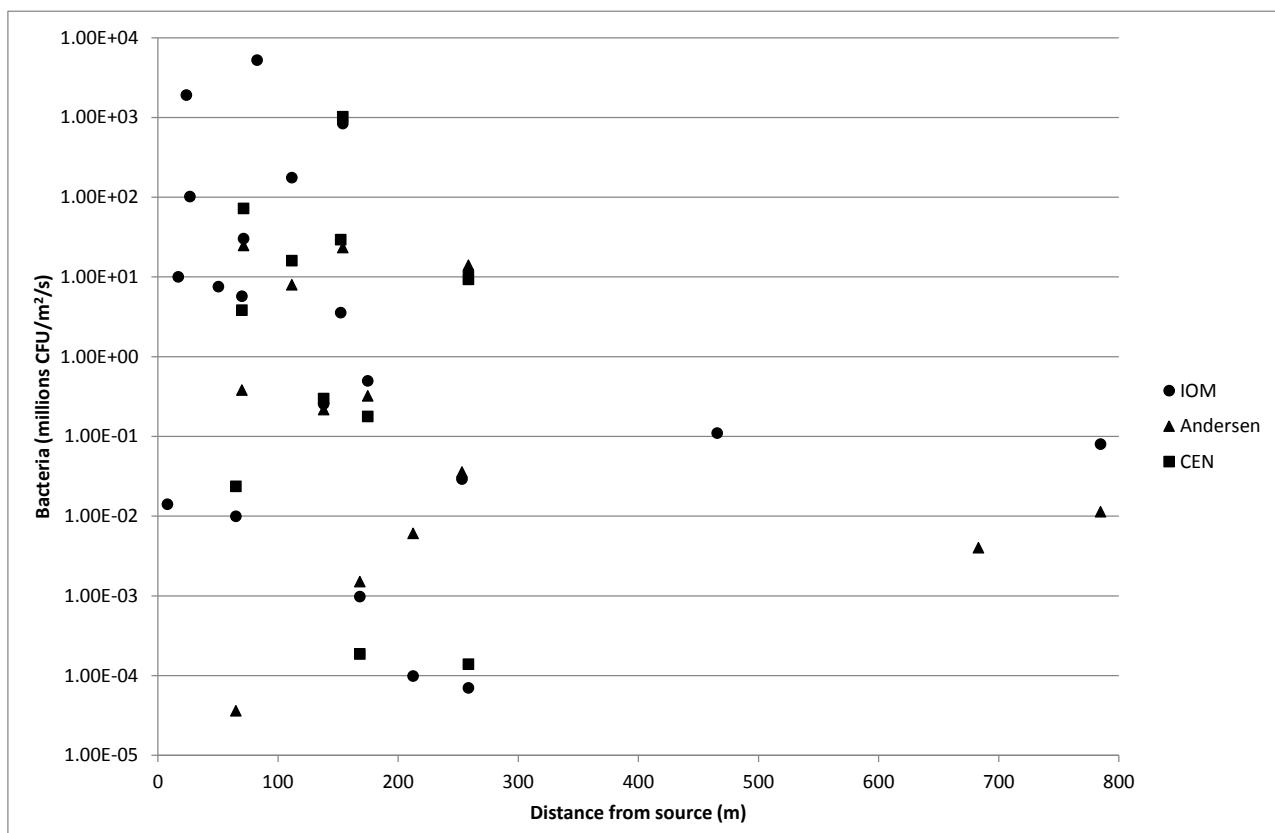

Figure 26 Back-calculated emission rates for Site B, total bacteria (corrected for background)
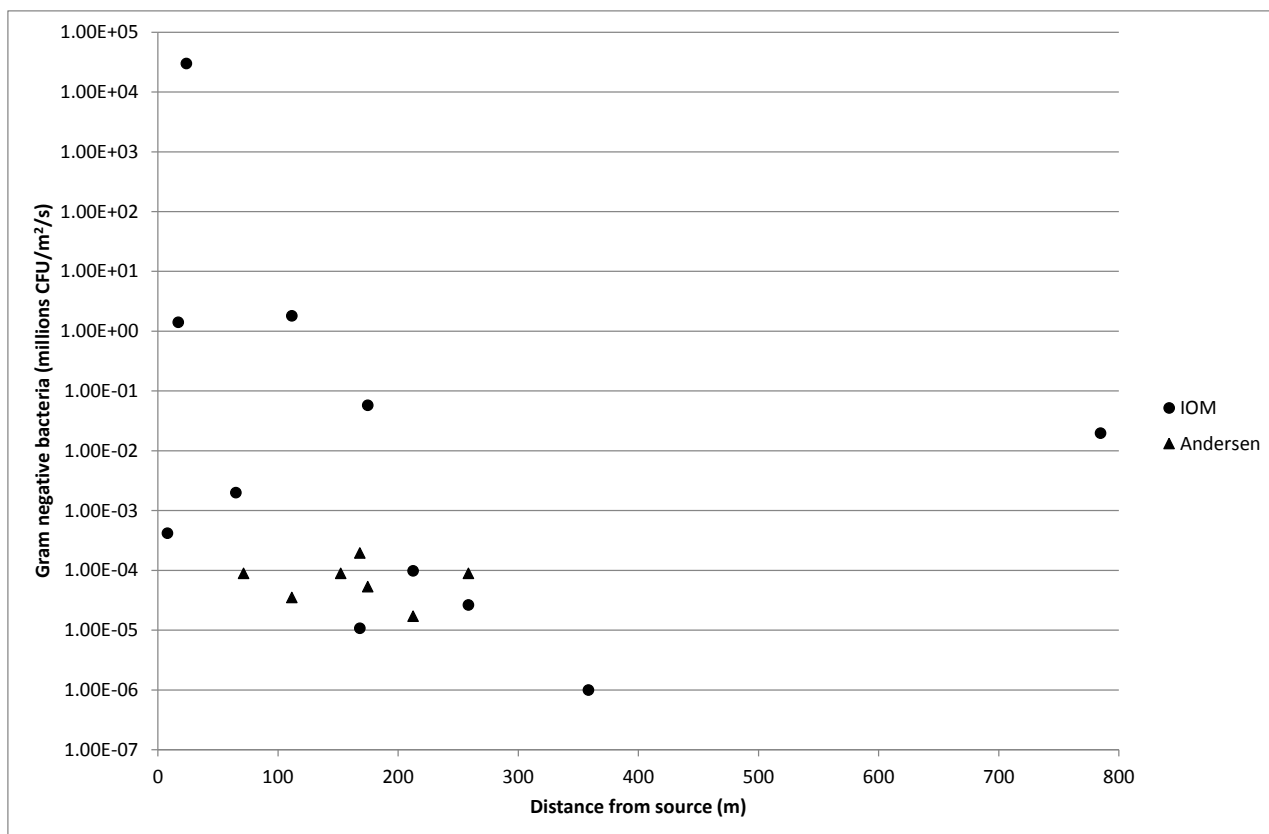
Figure 27 Back-calculated emission rates for Site B, Gram negative bacteria (corrected for background)
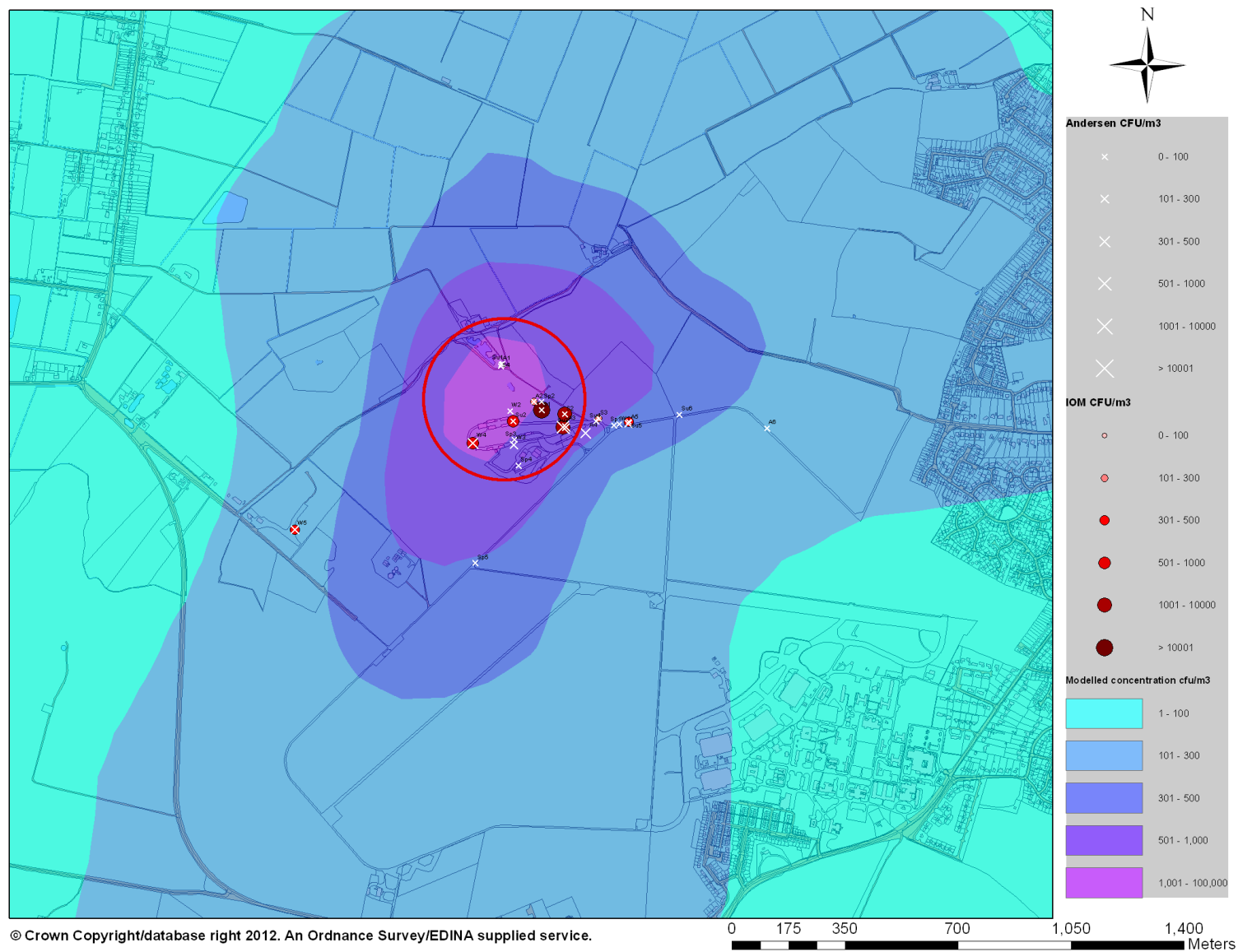
Figure 28. Map showing the 99th percentile of model output (contours) in comparison to IOM sampled *Aspergillus fumigatus*

© Crown Copyright/database right 2012. An Ordnance Survey/EDINA supplied service.

WR1121 Final Report (20.8.13)

Figure 29.Scatter plot showing the 99th percentile of model output in comparison to IOM and Andersen sampled *Aspergillus fumigatus*

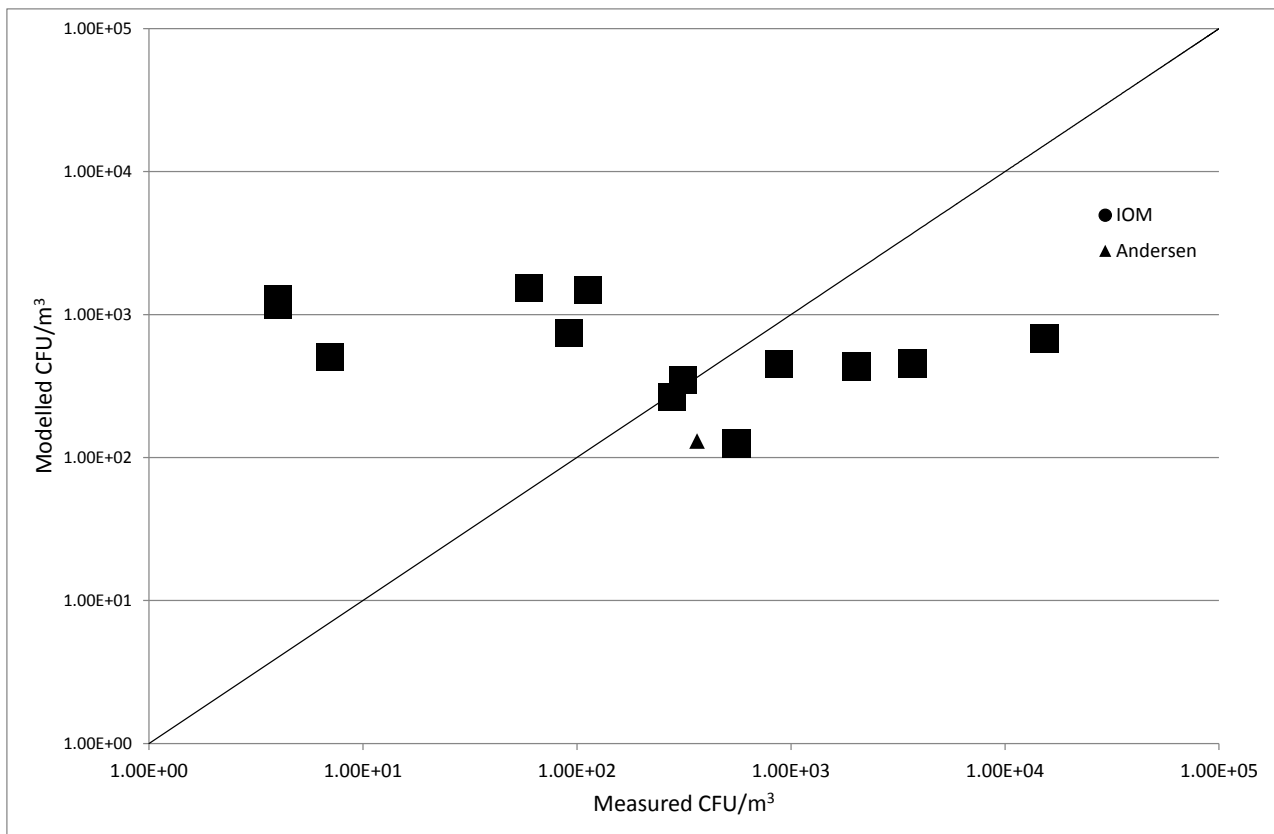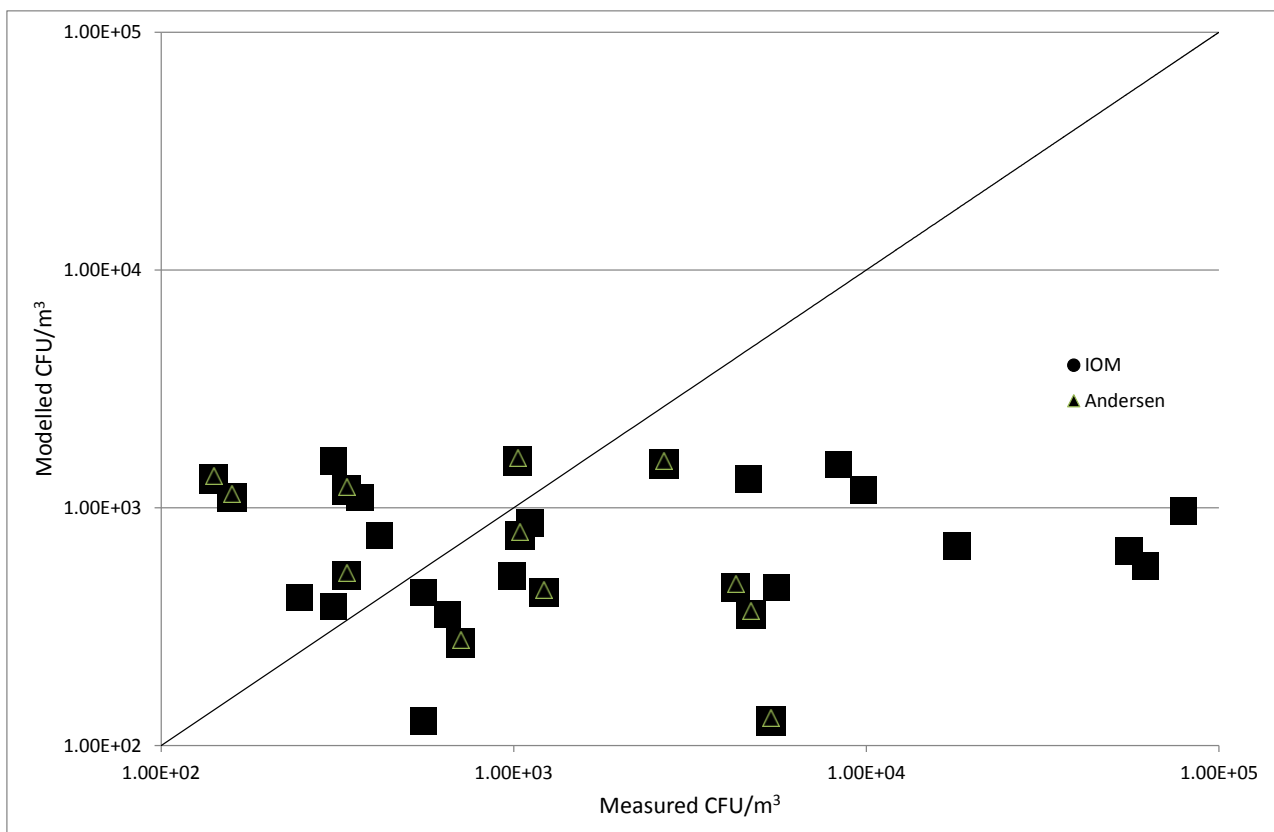**Figure 30.Scatter plot showing the 99th percentile of model output in comparison to IOM and Andersen sampled Total Bacteria**
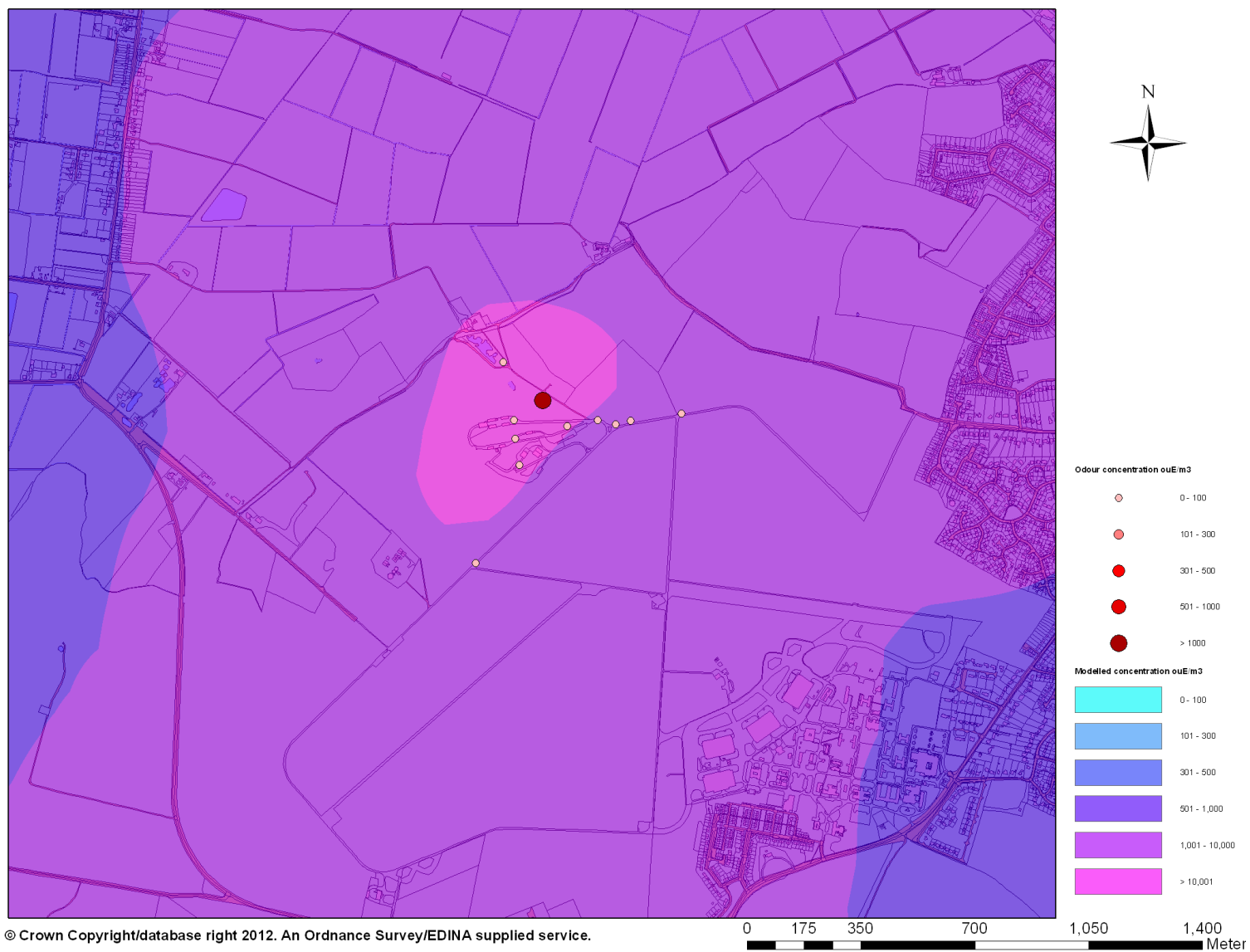
Figure 31. Map showing the 99th percentile of model output (contours) in comparison to sampled odour concentration

WR1121 Final Report (20.8.13)

<u>Modelling Conclusions</u>

The modelling results reported here are based on the IOM and odour sampling. All sites were modelled; however the site with the most comprehensive dataset was Site B. Emissions from Site B were also the only location where odour samples were taken. Therefore to use the most comprehensive data set and for the sake of brevity, the results presented are for Site B only. However, the discussion points raised are applicable to all sites modelled.

It is clear from site observations that process operations, e.g. screening, shredding and turning cause significant increases in emissions. However, without isolating single processes to measure individual process specific emissions it is not possible to take account of these variations in the modelling. This would require a dedicated investigation using sampling techniques under controlled conditions that may not accurately represent true operational activities. Whilst beyond the scope of this study the results could then be applied as generic values for each process.

Modelling has been completed within the constraints of our current knowledge and abilities. There are a number of improvements that can be identified, particularly around definition of the source term and collection of information regarding pollutant and source properties. Wherever possible, insights accruing from on-going EA-funded PhD research has informed the source term characterisation and modelling approach. However as this work has not been reported yet, the full recommendations were not available to fully deploy in this project. This will require follow-on work to make full value of the advances made in using ADMS that will come from that research coupled to the improved datasets that are now available for bioaerosols. Our continuing lack of confidence in modelling results here is not limited to bioaerosols, as the complexity of the composting facility as an emission source will cause difficulties in sampling any type of emission, including odours. Despite the limitations in accurately assessing emissions from a site, there is significant value in understanding the emission ranges from operational processes. Indeed, using the range of emissions as an input for future modelling studies will assist in determining the risk of exposure to bioaerosols downwind where no better data is available.

4.8 Implications of the results of this study for modelling community exposures as part of a small area health study

The outputs from this study have the potential to inform the design of bioaerosol exposure assessment for use in future small area health studies. The aim of such studies would be to examine health outcomes in relation to composting site proximity and estimated bioaerosol exposure using national routine data such as hospital admissions, birth registrations and, potentially, general practice data. The outputs from the current Defra study would be a key resource in developing an exposure assessment protocol to provide better estimates of community exposures than 'distance from site' at composting sites.

5. Conclusions

i. Whilst the bioaerosol concentrations generated by the Andersen, IOM and 'CEN' (Leckel sampler and head) samplers were positively correlated, significant disparities between the concentrations from the different samplers on individual sampling days were found.

ii. The between-replicate variability of the Andersen, IOM and CEN samplers was not comparable. The Andersen sampler consistently returned a lower level of variability around the mean compared to both of the filtration devices.

iii. The Andersen was able to enumerate viable bioaerosols within a lower concentration range (<1000 CFU / m^3 – i.e. at or close to background) than the IOM filter method. The Andersen is therefore well-suited to the type of sampling task specified in the AfOR protocol. It is not appropriate for sampling close to source or other situations in which higher concentrations might be expected.

iv. The IOM sampling device proved to be appropriate for situations in which high bioaerosol concentrations may be expected (>10,000 CFU / m^3) i.e. close to source. It is less appropriate for the type of sampling tasks specified in the AfOR protocol because its lower limit of detection is often higher than the prevailing concentration in ambient air. This device remains a valuable tool for bioaerosol measurements on-site and for personal monitoring due to its portability and the flexibility of analysis from filter suspensions.

v. The higher sampling rate of the Leckel sampler and head ('CEN' method) results in a lower limit of detection and as such greater sensitivity at lower concentrations compared to the IOM method. The CEN method proved to be cumbersome however and more difficult to place in terms of access in the field (due to its weight and need for an external power source). The between-replicate variability of this device was of the same order as that of the IOM method but this could have been affected by the short sampling times relative to its intended use as a longer duration sampler. Given its CEN TS status, this sampler deserves further evaluation – especially in the context of the need for longer duration sampling to complement the prevailing snapshot approach and in support of future exposure assessment.

vi. The spatial trend of bioaerosol concentrations described by Wheeler et al., (1991) is broadly corroborated by this dataset. This dataset provides additional information on concentrations expected beyond 250 m from source.

vii. Bioaerosol concentrations at the entirely enclosed site were generally lower than at the unenclosed and partially enclosed sites.

viii. There was no evidence of seasonal differences in bioaerosol concentrations at any of the sites.

ix. There was evidence of between-sampling day variations (not consistent according to season) in bioaerosol concentrations close to source and at >250 m from source. The precise cause(s) of these variations were not discernible due to a lack of control over the likely governing variables.

x. The dynamic range of the qPCR method is wider (4-5-log) than either of the AfOR and the CEN methods. It is also quicker to carry out and has the potential for automation. Into the future this has the potential to make qPCR a potential alternative to the traditional culture techniques.

WR1121 Final Report (20.8.13)

xi.   The results from the qPCR method are mainly higher than standard AfOR methods, as the method does not distinguish viable and non-viable spores. The best correlation is obtained when comparing qPCR and the IOM method. This information may be useful for health studies.

xii.   Spatial distribution of *Aspergillus fumigatus* spores (by qPCR) along sampling transects, gives similar results compared with AfOR (and CEN) methods.

xiii.   Real time particle detection shows that both TSP and PM_{10} are correlated to *Aspergillus fumigatus* spore concentration.

xiv.   Endotoxin concentrations are usually below the level recommended by the Dutch Expert Committee on Occupational Safety but occasional exceedances of this standard were detected at the larger open windrow sites, although always within 200m from source.

xv.   The majority of glucan measurements were below the $10ng/m^3$ threshold suggested by previous researchers. However significantly elevated concentrations were detected at the largest site, (comprising open and enclosed operations) even at distances greater than 250m. Further research would be needed to ascertain whether this is repeated at other large open sites or whether this is a local issue.

xvi.   No consistent relationship was observed between odour and bioaerosol concentrations (although this was a limited dataset).

xvii.   The envelope of modelled (back-extrapolated) bioaerosol emission rates straddles many orders of magnitude. Distinguishing the influences of individual meteorological parameters on this variability is not possible.

xviii.   We are not in a position to be able to predict emission rates with confidence. This reality continues to hamper confidence in modelling of odours and bioaerosols from open windrow facilities in particular and requires further work.

6. Considerations for policy-makers and regulators

6.1 Key questions

We posed a number of key questions in the introduction to this report. In the following section we reflect upon those questions in the light of the findings of this report

i.   *Is the current standard monitoring Protocol fit for purpose or are modifications required to take into account new sampling and analytical technologies?*

The monitoring methods tested all have particular strengths and weaknesses in the context of practical composting facility monitoring but are not considered to be comparable. A review of the standard monitoring Protocol is required to reconsider the use of IOM and Andersen methods as alternative methods and also to consider the options for and mechanisms of the future introduction of new samplers and measures of bioaerosol.

ii.   *Does the Environment Agency's current precautionary regulatory position need to be reviewed as a result of the emergence of new evidence?*

It would be prudent to review the regulatory position given the availability of new evidence. The current position statement requires a higher level of regulatory scrutiny where there are sensitive receptors within 250 m of composting operations. The findings from this project confirm that bioaerosol concentrations decline rapidly between the point of emission and 250 m from source. Concentrations exceeding normal upwind values may be detected ≥250 m downwind of source (the frequency of occurrence of such elevated concentration varies by site, determinand and sampling device). Evidence from this study suggests that full enclosure of composting operations can be an effective control measure. Further study of enclosed facilities and exhaust air control technologies should be pursued to support these initial findings.

iii.   *To what extent are bioaerosol concentrations surrounding composting sites affected by factors such as tonnage of waste on site or by the degree of containment?*

There is evidence that the site (A) which handles the greatest tonnage of waste in this study had the greatest impact on bioaerosol concentrations at ≥250 m downwind of source. This tentatively supports the concept of applying greater regulatory scrutiny to large sites. However this conclusion should be applied with caution and due consideration of the possible effect of other factors. There may be other variables in addition to tonnage which contribute to higher bioaerosol concentrations at Site A. As discussed in (ii), there is evidence that complete containment reduces downwind bioaerosol concentrations.

iv.   *To what extent is dispersion of odours and bioaerosols correlated?*

People living close to composting sites are often concerned that exposure to odour (which is detectable by individuals) may be associated with exposure to bioaerosols. No consistent relationship was observed between odour and bioaerosol concentrations. On the basis of this (small) dataset, there is no evidence to indicate these air quality measures are correlated.

v.   *How well do dispersion models simulate measurements of bioaerosol concentrations?*

Dispersion models are used successfully to simulate air pollutant transport in other contexts. There is no fundamental reason why air dispersion models should not be able to model bioaerosols. In the work presented in this report, the model simulations do not match the observed data well. We suggest that this is a reflection of continuing challenges with selecting appropriate input parameters for the model in the context of open windrow composting, rather than being a weakness of the model *per se.* The completion of an Environment Agency funded PhD later in 2013 (Philippa Douglas, Cranfield University) is expected to provide new insights and advances in this regard.

vi.   *Can particulate measurement be used routinely as an indicator for high bioaerosols concentrations?*

Particulate concentration was found to be correlated with *Aspergillus fumigatus* spore concentration, so it is possible that this method could be routinely used as an indicator for high bioaerosol concentration. Particulate concentrations could be established, which if exceeded, would indicate the need for targeted bioaerosol monitoring.

vii.   *With what level of confidence can we describe the extent of exposure of communities neighbouring composting facilities to bioaerosols?*

Anybody who is within 250 m of an active open composting operation is likely to be routinely exposed to bioaerosol concentrations which significantly exceed those found beyond 250m or upwind of a facility. The degree of exposure is likely to worsen with proximity to an emission source. Anybody who is greater than 250 m from an active, open emissions source will normally be exposed to concentrations of a similar order of magnitude to those found upwind of a facility. Occasionally, higher exposures will be experienced at distances greater than 250 m from an active, open emissions source. With short-term ("snapshot") monitoring it is impossible to characterise these exposures more quantitatively. Longer duration monitoring should be carried out in the future. While there may be occasional higher concentrations experienced outside a 250m buffer, results from this study suggest these will rarely be above the EA acceptable levels.

6.2 Recommendations

i.   The research-base upon which the Environment Agency's current regulatory position is founded is corroborated by these research findings. The current precautionary approach should be sustained until such a time as evidence for health-based threshold values is available.

ii.   There is preliminary evidence to suggest that enclosed facilities reduce the concentrations of bioaerosols in ambient air. Further monitoring of such facilities and their bioaerosol and odour abatement control technologies is warranted to improve the depth of the evidence base and to enhance confidence in these controls.

iii.   Equivalence of AfOR and qPCR methods with the CEN method should be investigated formally using a protocol established for this purpose.

iv.   Bioaerosol measurements (and modelled emission rates derived monitored data) suggest a wide range in emissions from open windrow processes. The particular processes or conditions responsible for elevated emissions remain unknown. High frequency emissions / close to source sampling coupled to site operational activity and met data logging at the same time step is recommended to establish if peak emissions sources / high risk dispersal conditions can be identified and ultimately controlled in the open windrow composting context.

v.   More extensive simultaneous odour and bioaerosol measurement is required to definitively assess their dispersion behaviour and relationship.

vi.   The GIS-based approach to visualising measurement data and simulated concentrations offers a user-friendly approach to assessing and communicating the risk of exposure of communities neighbouring composting facilities to bioaerosols (or odours) and its utility should be reviewed.

vii.   Modelling of open windrow composting continues to be hampered principally by our lack of confidence in defining sources and ascribing appropriate emission rates. Advances in knowledge arising from PhD work which is close to completion will demand a further review of this conclusion.

viii.   Good information on exposure is a pre-requisite for any future health study. However, the evidence base for community level exposure remains limited and further monitoring studies are needed to corroborate findings of this study. In particular there is a need for longer term fixed station monitoring to examine temporal variability in relation to meteorological variables (e.g. while raining) and for further evaluation of the use of particulate monitoring as a proxy indicator of bioaerosol exposure as well as its use as a screening tool to indicate need for specific bioaerosol measurement.

ix.   Personal sampling of exposure within communities has not been attempted and equipment used to conduct short-term personal monitoring within occupational settings is not practical in community settings. Funding of studies investigating possible biomarkers of bioaerosol exposures would be valuable, not only for potential use in evaluating exposure to bio-composting but also in medical research evaluating contribution of bioaerosol exposure to clinically apparent disease.

7. References

Association for Organics Recycling (2009) A standardised protocol for the monitoring of bioaerosols at open composting facilities. Published by the Environment Agency/AfOR. Ed. T Gladding.

Andrew Brown, Alex Knight, Neelam Kumarswami, Baptiste Lamarre, Bob Lipscombe, Rod Robinson, Melanie Williams (2009) Rapid and Responsive Monitoring Network for Bioaerosol Emissions: Final Report. NPL REPORT AS 33. Available at:
http://randd.defra.gov.uk/Document.aspx?Document=WR0605_8574_FRP.pdf

Bretagne, S., Costa, J.-M., Marmorat-Khuong, A., Poron, F.,Cordonnier, C., Vidaud, M., Fleury-Feith, J. Detection of Aspergillus species DNA in bronchoalveolar lavage samples by competitive PCR. J. Clin. Microbiol. 1995, 33, 1164–1168.

Bretagne, S., Costa, J.-M., Bart-Delabesse, E., Dhe´din, N., Rieux, C., Cordonnier, C. Comparison of serum galactomannan antigen detection and competitive polymerase chain reaction in diagnosing invasive aspergillosis. Clin. Infect. Dis. 1998, 26, 1407–1412.

Costa C, Vidaud D, Olivi M, Bart-Delabesse E, Vidaud M, Bretagne S. Development of two real-time quantitative TaqMan PCR assays to detect circulating Aspergillus fumigatus DNA in serum.

Microbiol Methods. 2001 Apr;44(3):263-9.PMID:11240049

Drew, G.H., Tamer Vestlund, A., Jordinson, G., Smith, R., Tyrrel, S., Longhurst, P.J. and Pollard, S.J.T. (2007). Developing a Best Practice Method for Modelling Dispersion of Bioaerosols from Composting Facilities. Eleventh International Waste Management and Landfill Symposium, Sardinia, 1-5 October 2007.

Eduard, W (2009) Fungal spores: A critical review of the toxicological and epidemiological evidence as a basis for occupational exposure limit setting, Informa Healthcare, Sept 2009, 799-864, DOI: 10.3109/10408440903307333.

Gilbert, J., Chen, J., Pocock, R. and French-Brooks, J. (2011) A study of the UK organics recycling industry in 2009. WRAP Project RES147.

Gostelow P, Longhurst P J, Parsons S A and Stuetz R M (2003) Sampling for Measurement of Odours, IWA Scientific and Technical Report No.17, IWA Publishing, London, ISBN 1-843390-33-7

Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals and the Dutch Expert Committee on Occupational Safety (2011) Endotoxins.http://gupea.ub.gu.se/bitstream/2077/26596/2/gupea_2077_26596_2.pdf

Pankhurst, L. J, Deacon, L. J., Liu, J., Drew, G. H., Hayes, E. T., Jackson, S., Longhurst, P. J., Longhurst, J. W. S., Pollard, S. J. T and Tyrrel, S. F. (2011) Spatial Variations in Airborne Microorganism and Endotoxin Concentrations at Green Waste Composting Facilities. International Journal of Hygiene and Environmental Health, 214, 376-383.

Rylander R. 1997. Airborne (1-3)-B-D-glucan and airway effects in a day care center before and after renovation. . Arch Environ Health 52: 281-285.

Searl, A. (2008) Exposure-response relationships for bioaerosol emissions from waste treatment processes. Defra Project WR 0606. Institute for Occupational Medicine for, UK.

WR1121 Final Report (20.8.13)

Shi, J.P. and Hodson, H. (2012) Bioaerosol monitoring data review project – open windrow source term analysis and its implication in the risk impact assessment. Unpublished internal report. AQMAU, Environment Agency.

Stagg, S., Bowry, A, Kelsey, A. & Crook, B. (2010) Bioaerosol emissions from waste composting and the potential for workers exposure.RR786 Research Report. Health and Safety Executive.

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, 40 (6) 1159-1169.

Taha, M.P.M., Drew, G.H., Tamer, A., Hewings, G., Jordinson, G., Longhurst, P.J. and Pollard, S.J.T., (2007). Improving bioaerosol exposure assessments of composting facilities – comparative modelling of emissions from different compost ages and processing activities. Atmospheric Environment, 41 (21) 4504-4519.

VDI 4252 Part 2:2004-06, Measurement of airborne microorganisms and viruses in ambient air —Active sampling of bioaerosols —Separation of airborne moulds on gelatine/polycarbonate filters. Berlin: Beuth-Verlag.

Wheeler, P. A., Stewart, I., Dumitrean, P., and Donovan, B. (2001).Health Effects of Composting. A study of three compost sites and review of past data. R&D Technical Report P1-315/TR. Environment Agency, UK.

Zhao Y, Aarnink AJA, Jong MCMd, Ogink NWM, Koerkamp PWGG. (2011). Effectiveness of Multi-Stage Scrubbers in Reducing Emissions of Air Pollutants from Pig Houses. *Transactions of the ASABE* 54(1): 285-293.