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|---|---|
| Title | Online Bioaerosol Sensing (OLBAS) |
| Authors | Sodeau, John;O'Connor, David;Feeney, Patrick;Quirke, Michael;Daly, Shane;Fennelly, Mehael;Buckley, Paul;Hellebust, Stig;McGillicuddy, Eoin;Wenger, John C. |
| Publication date | 2019-02-01 |
| Original Citation | Sodeau, J., O'Connor, D., Feeney, P., Quirke, M., Daly, S., Fennelly, M., Buckley, P., Hellebust, S., McGillicuddy, E. and Wenger, J. (2019) Online Bioaerosol Sensing (OLBAS). Available at: http://www.epa.ie/pubs/reports/research/air/Research_Report_269.pdf (Accessed: 22 March 2019) |
| Type of publication | Report |
| Link to publisher's version | http://www.epa.ie/pubs/reports/research/air/Research_Report_269.pdf |
| Rights | © 2019, Environmental Protection Agency. |
| Download date | 2026-11-17 11:50:39 |
| Item downloaded from | https://hdl.handle.net/10468/7661 |

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Report No. **269**

# Online Bioaerosol Sensing (OLBAS)

Authors: John Sodeau, David O'Connor, Patrick Feeney, Michael Quirke, Shane Daly, Mehael Fennelly, Paul Buckley, Stig Hellebust, Eoin McGillicuddy and John Wenger



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**EPA RESEARCH PROGRAMME 2014–2020**

# Online Bioaerosol Sensing (OLBAS)

# (2014-CCRP-MS.19)

# EPA Research Report

Prepared for the Environmental Protection Agency

by

University College Cork

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## ACKNOWLEDGEMENTS

This report is published as part of the EPA Research Programme 2014–2020. The EPA Research Programme is a Government of Ireland initiative funded by the Department of Communications, Climate Action and Environment. It is administered by the Environmental Protection Agency, which has the statutory function of co-ordinating and promoting environmental research. An IRC research studentship was awarded to Patrick Feeney in association with this project.

The authors would like to acknowledge the members of the project steering committee, namely John McEntagart (EPA), Caoimhin Nolan (EPA), David Dodd (Department of Communications, Climate Action and Environment), Professor Rob Kinnersley (English Environment Agency), Dr Warren Stanley (University of Hertfordshire), Dr Ray McGrath (Met Éireann) and Dr Saji Varghese (Met Éireann). Thanks also to Professor Martin Gallagher (University of Manchester) for the loan of the multi-parameter bioaerosol sensor, Dr Cathy O'Sullivan (University College Cork) for help with statistical analyses and José María Maya-Manzano (University of Extremadura, now at Dublin Institute of Technology) for primary biological atmospheric particle counting. Finally, a large debt of gratitude is owed to Professor Paul Kaye and his team at the University of Hertfordshire for designing and building the upgrade to our wideband integrated bioaerosol sensor (WIBS-4), which became WIBS-4$^{+}$.

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The EPA Research Programme addresses the need for research in Ireland to inform policymakers and other stakeholders on a range of questions in relation to environmental protection. These reports are intended as contributions to the necessary debate on the protection of the environment.

**EPA RESEARCH PROGRAMME 2014–2020**
Published by the Environmental Protection Agency, Ireland

ISBN: 978-1-84095-815-7 February 2019

Price: Free Online version

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# Executive Summary

This report presents a description of field and laboratory studies directed towards understanding the time behaviours of sources, removal pathways and number concentrations of airborne fungal spores and pollen. To achieve these aims novel real-time spectroscopic instrumentation based on fluorescence detection and optical scattering was developed, commissioned and deployed at two contrasting locations: (1) a dual windrow/in-vessel composting site and (2) an open field at the Met Éireann Valentia Observatory site. The real-time monitoring results were contrasted with those obtained by traditional Andersen sampling and coupled with impaction/optical microscopy techniques (SporeWatch).

Three short on-site campaigns were carried out at an Irish green-waste management facility and these were the first to provide real-time data on bioaerosol emissions as a set of site-characterising, continuous profiles. The wideband integrated bioaerosol spectrometer (WIBS) results showed that the fluorescence aerosol particle (FAP)/bioaerosol counts varied enormously depending on working activity, time of day/week and weather conditions. The Andersen counting method provided no insight into the activities because the measurements were performed off-site on just one occasion, in line with current licensing requirements.

A longer term campaign using the WIBS in tandem with a SporeWatch impactor was then launched. The results showed that the numbers of FAPs can be counted in the WIBS sizing regime 0.5–15 µm. However, only larger spores, such as *Ustilago maydis* (8–10 µm), can be related to optical microscopy identification. Key composting microbial releases, such as *Aspergillus fumigatus* and *Penicillium notatum* (2–5 µm), cannot be distinguished by either WIBS or optical microscopy. Nonetheless, all fine particulate matter ($PM_{2.5}$), particularly examples that are fluorescent in nature, can lead to adverse health effects and so simple counts of FAPs may prove to be useful for future regulations. The field campaigns led to two further avenues for real-time methodology to be deployed. The first was to develop a strategy to identify and distinguish between airborne primary biological atmospheric particles (PBAPs) using a novel device called the multi-parameter bioaerosol spectrometer (MBS). The second was to investigate levels of airborne PBAPs present in the staff cabin.

The MBS allows fluorescence spectral distributions of between 300 and 615 nm to be recorded. The data obtained are then treated statistically to provide further information on spore agglomerations and sizing. Three fungal spores – *A. fumigatus*, *Aspergillus niger* and *P. notatum* – were investigated in the commissioning laboratory study, both individually and as spore mixtures. It was shown that, using a combination of fluorescence distributions, propensity for aerosolisation and size measurements, the spores could be identified and distinguished. A subsequent field campaign performed at the composting site indicated that if a much larger library of MBS spore data were available this could be used to identify and distinguish between airborne PBAPs.

There were no prior measurements of bioaerosol levels in the offices or recreational areas where employees work, eat lunch or change clothes at composting sites. Employees entering their offices or staff cabins can act as vectors for carrying biological particles indoors on their clothes and bodies. Furthermore, when office and cabin doors are opened a draft is created that brings material indoors or disturbs settled dust/PBAPs. Hence, the WIBS was deployed in the staff cabin for a short period. The data show that FAP number concentrations averaged over the 4 days are associated with three major events occurring each day: at opening time, lunchtime and just before site closure.

One final campaign was performed in an open field site at the Met Éireann Valentia Observatory as a necessary precursor to the establishment of an Irish pollen and spore network. The study utilised a traditional impaction collector as well as an entirely novel upgrade to the WIBS device, which allowed FAPs of up to 40 µm to be analysed over an extended fluorescence wavelength range. The instrument, WIBS-$4^{+}$, was commissioned initially in the laboratory and the results provided clear evidence that PBAP exhibiting long wavelength fluorescence, such as

grass pollen, could be detected and distinguished from other airborne particulates. The results obtained in the field campaign also show that the WIBS-4$^{+}$ results tracked the diurnal variation of the pollen as counted by optical microscopy. A distinction between daytime and night-time pollen release patterns was shown using both techniques although the data acquisition time for the online WIBS-4$^{+}$ method was much quicker (seconds) than for the SporeWatch traditional methodology (hours/days) also employed here because the latter methodology requires both an impaction and collection step on-site before undergoing optical microscopy analysis in the laboratory afterwards.

# 1 Introduction

## 1.1 Background Information

The need to monitor the occurrence and transformation of aerosols in our atmosphere has increased dramatically over recent years (Sodeau and O'Connor, 2016). The necessity is based on the undesirable effects that they can have on our health and the role that they play in climate change. Of course, the atmospheric aerosol does not consist of abiotic chemical components alone. Field measurements have shown that primary biological atmospheric particles (PBAPs) are also present and comprise materials such as viruses, bacteria, fungal spores, pollen, sub-pollen and plant fragments. The diameters of these materials range between nanometres and hundreds of microns and display a wide variety of morphologies; they are often termed bioaerosols (Lacey and West, 2007).

Fungal spores generally range from 1 to 10 µm in size, although some are larger, and their shapes range from spherical to rod-like. It has been estimated that typical fungal spore mass concentrations of ≈1 µg $m^{-3}$ are present in continental boundary layer air. Their global emissions are ≈50 Tg $year^{-1}$ (Elbert *et al.*, 2007; Poehlker *et al.*, 2012). In contrast, the size of pollen generally ranges between 10 and 100 µm. Pollen also displays a wide range of shapes and structures. It is much more familiar to the public because it is often visible to the eye and its adverse health effects on those with hay fever are well known. However, anybody who has encountered "black mould" in damp bathrooms or "green mould" on bread will also be aware of fungal spores.

The air quality data determined for both ambient and occupational environments are affected significantly by the levels of particulate matter (PM) present. The health risks associated with small chemical PM ($PM_{10}$, $PM_{2.5}$ and $PM_1$), mainly generated by combustion processes and from non-exhaust vehicle emissions, are well known (Pöschl, 2005; Cohen *et al.*, 2005). Therefore, online urban monitoring of chemical particulates, especially beside roads, is now well developed in networks throughout Europe. Although the effects of bioaerosols on humans and plant life have been studied for many years (Pinnick *et al*., 1995; Després *et al*., 2012; Poehlker *et al*., 2012), the real-time monitoring of small-sized airborne material at known "hotspot" locations, such as composting sites, has lagged behind. This type of information is particularly necessary with regard to enforcing any legal framework that underpins local air quality and/or licensing activities. However, there are few studies published that quantify, in real time, the number and type of fungal spores, even though they are also often sized in the $PM_{2.5–10}$ range and released in places where people (particularly those who are immunocompromised) are present or where susceptible crops may be affected on a routine basis. Green-waste management sites are a good example of such outdoor spaces, often being located in rural areas close to crops, served by staff and visited by clients.

One of the most important aims of the waste industry is to maximise the benefits that can be recovered from global waste. However, this has now become an important industrial activity, which impacts on waste management targets. Composting is a traditional method of waste management based on the biological degradation and stabilisation of organic matter performed under aerobic conditions (Beffa *et al*., 1998; Hryhorczuk *et al*., 2001; Sanchez-Monedero *et al*., 2005; Avery *et al*., 2012; Wéry, 2014). Therefore, a substantial increase in activity has become apparent throughout the world in terms of the use of local composting facilities to manage and utilise green and food waste (Hryhorczuk *et al*., 2001; Recer *et al*., 2001; Wéry, 2014). This strategy then also leads to a reduction in the amount of waste sent to landfill sites.

Despite the obvious social benefits of such activities, it is also known that exposure to composting-released PBAPs (e.g. *Aspergillus fumigatus*) can be detrimental to human health (Horner *et al*., 1995; Simon-Nobbe *et al*., 2007). Concerns then arise because bioaerosols emitted from composting sites (including home composting) can remain airborne for some time and, like small chemical PMs, travel well off-site. The average size of these spores range between 2 and 10 µm ($PM_{2.5}$ and $PM_{10}$) (Simon-Nobbe *et al*., 2007) and so can penetrate deep into the inner lining of

the lungs into the alveoli. This behaviour can lead to numerous health problems linked with agricultural and composting work, including farmers' lung, aspergilliosis, pneumonitis and chronic obstructive pulmonary disease (COPD) (Millner *et al*., 1980; Simon-Nobbe *et al*., 2007; Chaudhary and Marr, 2011).

Furthermore, the presence of PBAPs has long been associated with asthma and other reactive airway diseases such as allergic bronchopulmonary mycoses, rhinitis, allergic sinusitis and hypersensitivity pneumonitis (Simon-Nobbe *et al*., 2007; Chaudhary and Marr, 2011). Deterioration in the pulmonary function of people with chronic asthma and cystic fibrosis are of particular concern to the medical community.

Many of the biochemical pathways involved in the degradation and transformation processes from green waste to fertiliser are now understood, and it has become clear that airborne bioaerosols are a natural, unavoidable outcome of the processing. Fungal spores are the most abundant of the PBAPs to be found in many local environments (Womiloju *et al*., 2003; Elbert *et al*., 2007; Després *et al*., 2012) and occur wherever decaying vegetation is present, as this is a food/energy source for the fungus. However, harmful fungal spores (containing significant concentrations of hazardous mycotoxins) can be released at composting sites, particularly during activities involving the vigorous movement of material such as shredding, compost pile-turning and screening of green-waste deliveries (Millner *et al*., 1980; Hryhorczuk *et al*., 2001; Sanchez-Monedero and Stentiford, 2003; Sanchez-Monedero *et al*., 2005; Taha *et al*., 2005, 2006; Gillum and Levetin, 2008; Pankhurst *et al*., 2011; Wéry, 2014).

There are licensing guidelines set by national and regional environmental agencies for commercial composting operations. These primarily focus on site workers and people inhabiting the surrounding areas. On Irish composting sites the monitoring of a limited number of harmful biological particles, such as *A. fumigatus*, is performed only once a year for a short time period, with the results returned some days later to the local management. Therefore, no indication of minute-to-minute or hour-to-hour changes in bioaerosol releases are made, even when the normal agitation processes associated with the composting process occur or when deliveries are made. The English Environment Agency guidance specifies that monitoring should be carried out at times of high site activity, e.g. turning, shredding and screening.

The current regulation for the licensing and operation of compost sites set by the English Environment Agency [also adopted by the Environmental Protection Agency (EPA) and local authorities] is for their management to demonstrate so-called "acceptable levels" of (1) mesophilic bacteria [$10^3$ CFU (colony-forming units) $m^{-3}$] and (2) the fungal spore *A. fumigatus* (500 CFU $m^{-3}$) (Pankhurst *et al*., 2011; Williams *et al*., 2013). These benchmarks have been set as a ×10 factor relative to ambient levels. (Few epidemiological studies have been performed to provide an appropriate limit based on actual health outcomes.) *A. fumigatus* spores are monitored because they are a known human pathogen (Horner *et al*., 1995; Fischer *et al*., 1998; Abba, 2004; Simon-Nobbe *et al*., 2007), with compost heaps being a major environmental source. Spore release occurs not only because of the internal heating process associated with composting but also because of the intrinsic thermo-tolerance of the spore (Millner *et al*., 1977; Vincken and Roels, 1984; van der Werf, 1996; Gillum and Levetin, 2008).

Actual number concentrations of bioaerosols present on-site or close by have not been extensively characterised at any location. Indeed, the occupational monitoring is carried out off-site only for short time periods (5–15 min), with the results returned in CFU $m^{-3}$ some days later to the local management (Williams *et al*., 2013; O'Connor *et al*., 2015). Therefore, no real-time indication of increases in ambient bioaerosol are ever made on-site even when the normal agitation processes associated with commercial composting activities occur or when deliveries are made.

Reports on emissions from waste management sites have indicated that levels in the $10^2$–$10^4$ CFU $m^{-3}$ range can be measured (Frederickson *et al*., 2013; Fletcher *et al*., 2014). Interestingly, there did not appear to be a relationship between the age of the media, air flow rate, residence or emission concentrations over a range of sites incorporating several different biofilter abatement systems, which often incorporate adsorption materials. Furthermore, in contrast to odour removal, the performance of the biofilters for bioaerosol reduction efficiency was found to be extremely variable and the same abatement

systems did not appear to be able to achieve significant, consistent removals of *A. fumigatus*, even during 1-day periods at a single site. In fact, some of the tested abatement systems produced an increase in *A. fumigatus*, with the presence of a scrubber appearing to have little effect on performance regardless of whether the subsequent biofilter was open or closed. It was concluded that an enclosed biofilter system results in a ≈30–45% drop in *A. fumigatus*, whereas an open biofilter leads to ≈40–80% reductions. The detailed understanding of localised effects on the air related to the different abatement systems used awaits experimental scrutiny by the waste management site operators and the licensing authorities.

In summary, the data outlined in the above reports and obtained from a variety of sites suggest that:

> The concentration of bioaerosols in the exhaust air from enclosed biowaste treatment facilities is extremely variable and appears to be dependent upon the site operating parameters at the time of sampling and, from the data obtained, it was not possible to determine if the different treatments systems or the type of waste being treated produces a typical bioaerosol emission profile. (Frederickson *et al*., 2013; Fletcher *et al*., 2014)

Such a statement indicates the complexities associated with measuring bioaerosol releases from composting sites. The problems of inconsistency may be related not only to the wide range of variables involved in biowaste treatments (temperature, humidity, pH, agitation, etc.) but also to the reliability and accuracy of the current measurement strategy employed, which involves Andersen samplers, as discussed in section 1.2, and general parameters such as TVCs (total viable counts).

Composting plant operations in the UK that are located < 250 m from any other property in the vicinity must complete a Specific Bioaerosol Risk Assessment for the Environment Agency. Furthermore, the increasing commercial growth of the industry is expected to lead to increased emissions of bioaerosols (Sanchez-Monedero *et al*., 2005). This aspect is currently causing concerns related to potential occupational health impacts on staff, clients and visitors at the facilities, as well as people living in the region, because PM the size of most fungal spores can be dispersed over hundreds of kilometres (van der Werf, 1996; Sanchez-Monedero *et al*., 2005; Wéry, 2014).

Local residents (and certain crops) in the site vicinity have the potential to be harmed because many bioaerosols are allergenic or plant pathogenic. The four genera most commonly associated with the development of allergies are *Alternaria*, *Cladosporium*, *Penicillium* and *Aspergillus* (Twaroch *et al*., 2015). The first two make large ambient contributions to the fungal spore load in continental climates (Corden *et al*., 2003; Grinn-Gofroń and Rapiejko, 2009; Grinn-Gofroń *et al*., 2015); however, in Mediterranean regions, all four are abundant (Rodolfi *et al.*, 2003; Pepeljnjak and Klarić, 2005; El-Akhdar and Ouda, 2009; Lanier *et al.*, 2010; Fernández-Rodríguez *et al.*, 2014; Pasquarella *et al.*, 2015; Pyrri and Kapsanaki-Gotsi, 2015). The actual range of fungal spore types (propagules) along with their number concentrations found at waste management sites or in farms, cities and towns have been little studied in Ireland to date. In other words, epidemiological studies relating human health to the small-sized bioaerosol releases specific to the composting process cannot be performed currently.

Although the human health aspect is the reason why licensing authorities in some countries have put in place regimes where regular monitoring of bioaerosols is performed, particularly in the early years of site operations, there are also potential adverse effects on crops and other plants. For example, *Ustilago maydis* is a smut fungus that is pathogenic to maize (Carlile *et al.*, 2001; Brefort *et al.*, 2009) and is associated with the type of green waste delivered by brewers and distillers to composting sites.

In contrast to fungal spores, the size of pollen is > $PM_{10}$. The main function of pollen is to transport DNA/genetic material in plants, but its dimensions and biochemical composition have led to many studies on its potential health effects (Mullins and Seaton, 1978; Garrett *et al.*, 1998; Kirkhorn and Garry, 2000). For example, components such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidases cause oxidant stress in the lung epithelium and drive allergic lung inflammation, whereas the presence of adenosine represents a potent immunoregulatory substance (Gilles *et al.*, 2011). Thus, allergens can become trapped in the nasopharynx and trachea, leading to adverse reactions such as pollinosis (hay

fever), diseases such as asthma and even mortality (Nunes and Ladeira, 2007; D'Amato *et al.*, 2001). In this regard, it has been reported that, for studies performed in the Netherlands, a strong association exists between the day-to-day variation in pollen concentrations and death owing to cardiovascular disease and COPD (Brunekreef *et al.*, 2000). In addition, there is evidence that, under wet conditions or during thunderstorms, pollen grains may be ruptured to make sub-pollen units and/or release a portion of their biochemical content, thereby inducing asthmatic reactions in patients with pollinosis (D'Amato *et al.*, 2012).

There are, of course, linkages between the above health issues and climate change, because increased air temperatures significantly influence pollen growth patterns whereas temperature as well as relative humidity and rainfall contribute to airborne number concentrations of fungal spores. Longer pollination seasons and increasingly widespread releases of fungal spores are then likely to increase the duration of allergic, respiratory and cardiac reactions in sensitised subjects.

In light of these direct impacts on the general public it is important that society develops "early warning" systems for bioaerosol detection at national and local levels. Recognising these pressures, the USA, France, Switzerland and the UK have been mounting research-led campaigns to achieve such an aim over recent years. For example, the UK has many established aerobiological monitoring stations, often organised by the British Aerobiology Federation. Therefore, many studies relevant to spore concentrations, their distributions and their correlation with meteorological parameters have been performed (Harvey, 1967; Corden and Millington, 1994; Hollins *et al*., 2004). Monitoring campaigns for ambient *Cladosporium* concentrations and also the fungal genus *Ganoderma* have been carried out throughout the country (Sreeramulu, 1963; Lewis *et al*., 2000; Hollins *et al*., 2004). Furthermore, related studies have highlighted the significance of *Alternaria*, in conjunction with other PBAPs, in the exacerbation of symptoms in those with asthma (Langenberg *et al*., 1977; Corden *et al*., 2003).

In contrast, Ireland's current capability to provide accurate, indigenous bioaerosol assessments is limited. Even the summer pollen count is currently based on monitoring data and models obtained and generated in the UK. This situation is quite different from the period between 1976 and the early 2000s, when a predictive model was developed for pollen measurements made in Dublin, Dartry and Baldonnel. The service provided members of the public who read the *Irish Times* with country-wide pollen levels over the hay fever season. This Irish-based network is no longer functioning, which means that Ireland is one of only five countries in Europe (including Bosnia and Herzegovina and Moldavia) that cannot be part of the European Aeroallergen Network (EAN) Pollen Database and therefore cannot access data used by scientists to create statistics and calculate climate change trends for the distribution of pollen on national and international scales.

However, a few research-based studies on pollen and spores have been performed in order to investigate atmospheric concentrations of selected PBAPs in Ireland. These include grass pollen, *Cladosporium*, basidiospores and other spores (with *Aspergillus*, *Penicillium* and *Botrytis* counted jointly) (McDonald and O'Driscoll, 1980). The studies centred on airborne species detected in Galway City during the late 1970s. During a similar period of time, collections of *Cladosporium* and basidiospores were made in Dublin, with the results analysed much later, in 1990 (Stephen *et al.*, 1990). A more recent study has focused on the use of culture techniques for sampling ambient fungal spore concentrations in Galway (O'Gorman and Fuller, 2008). Since then, few aerobiological surveys have been carried out in Ireland but the International Phenological Garden network does exist and comprises four sites (Valentia Observatory, JFK Arboretum, Johnstown Castle and the National Botanic Garden), with phenological data records extending back to the 1960s. At these locations, chosen observers record and monitor phenophases such as budburst, flowering and leaf-fall for a range of species, including downy birch (*Betula pubescens*). In fact, a recent publication using computer modelling to determine possible climate change effects on birch budburst has been built on such observational work recorded at Valentia Observatory between 1969 and 2012 (with only the 1994 record missing) (Caffarra *et al.*, 2014).

It is of little use to the public, particularly those with allergies and respiratory disease, to simply be made aware of historical records of PBAPs in their locality.

It is equally suspect not to provide staff and visitors to sites known to release bioaerosols with real-time measurements alongside real-time reporting that at the very least enumerate the number of potentially harmful particles in the air. The problem is that traditional (impaction-based) methods for identifying and counting fungal spores and pollen are time-consuming and labour intensive.

Real-time monitoring of airborne pollen and other PBAPs using a variety of spectroscopic and light scattering techniques represents an area of growing development and consequence (Pan *et al.*, 1999, 2011b; Agranovski *et al.*, 2003; Kanaani *et al.*, 2008; Gabey *et al.*, 2010; Huffman *et al.*, 2010; Mitsumoto *et al.*, 2010; Kiselev *et al.*, 2011; Sivaprakasam *et al.*, 2011; Healy *et al.*, 2012a). This approach, in tandem with traditional monitoring strategies, forms the basis of the Online Bioaerosol Sensing (OLBAS) project.

## 1.2 Impaction Methods for the Detection of Fungal Spores

In the past, detection techniques for fungal spore types were generally confined to methods such as the impaction of air samples onto adhesive sample substrates before analysis using optical microscopy, although scanning electron microscopy has also been employed (Vestlund *et al.*, 2014). This undertaking relies on the intrinsic skill of the identifier and is also very labour intensive because careful preparation of the substrate is required for accurate analysis. In fact, the approach represents one that is often employed for pollen counting and forecasting, but such a combination of techniques has also been used previously to show that very large number concentrations of spores can build up at agricultural sites. For example, using the above methodology it has been shown that air sampled from a cowshed can contain as many as 16,000,000 spores per $m^3$; the preponderance of these spores was deemed to be *Aspergillus–Penicillium* as a grouping because they are difficult to distinguish by optical microscopy as their sizes and morphology are very similar (Baruah, 1961).

Other, more modern methods for determining fungal spore concentrations and identifying species include (1) the Institute of Occupational Medicine (IOM) (inhalable dust) filtration sampler, which can be deployed close to the source of the bioaerosol emissions (Kenny *et al.*, 1999), and (2) the Andersen sampler, an impaction device that is prone to overloading and cannot be used reliably in highly contaminated environments (Solomon and Gilliam, 1970). Although both of these culture-based techniques can be more exact in their determination of differing species that cannot be detected using optical microscopy, they also require considerable time for the sampled fungal spore to grow on a suitable agar medium. Furthermore, the culture-dependent method suffers from the possibility of providing an underestimation of the total fungal content because a portion of the spores is generally unviable (Mandrioli *et al.*, 2003). Therefore, culture-independent studies are now performed, but only on occasion because of their expense, using quantitative polymerase chain reaction (q-PCR) sequencing (Chen and Li, 2007; Le Goff *et al.*, 2011; Pankhurst *et al.*, 2012; Galès *et al.*, 2015).

Notwithstanding its inherent limitations, Andersen sampling remains the current method of choice used for determining the concentrations of mesophilic bacteria and *A. fumigatus* at compost sites in Ireland. The samples are collected, after pumped-air impaction, onto agar gel plates, which are then returned to the laboratory for further cultivation and counting (Després *et al.*, 2012). However, the procedure allows for the measurement of spores and bacteria that are viable for specific media only. More importantly, only a small volume of air is collected, off-site, for a short 5- to 15-min period of time (Hryhorczuk *et al.*, 2001) because, as the micro-organisms are impacted directly onto the agar surface, there is a danger of the plate becoming overloaded in environments that give rise to high concentrations. Clearly, composting sites are a good example of this possibility and so sampling rarely occurs on-site; rather, sampling takes place 100–250 m upwind and downwind, off-site from the source (Eduarda and Heederik, 1998; Cartwright *et al.*, 2009; Williams *et al.*, 2013). Commercial costs for such a test can currently approach €10,000, with the measurements being performed only once a year.

This situation means that detailed long-term profiles of green-waste site bioaerosol emissions cannot be constructed as a function of important variables such as site location, weather conditions or agitation activities such as turning and loading.

## 1.3 Impaction Methods for the Detection of Pollen

Although the existence of flowering pollen "dust" has been recognised since about 1540, it was only the development of optical microscopy some 200 years later that allowed us to establish their differing shapes, sizes and colours (Kennedy and Wakeham, 2015). In fact, it was not until the 1950s that JM Hirst invented an air sampler that allowed the detection and capture of "air flora", such as bacterial spores, fungal spores and pollen, to become routine. The device has subsequently become known as the Hirst trap. Further modifications were later made to the device (e.g. Hirst-type Burkhard volumetric trap or the "whirling arm") but its essential nature and use continue to this day (Lacey and West, 2007; Caruana, 2011).

The commercial instrument SporeWatch: Electronic Spore and Pollen Sampler (manufactured by Burkard Scientific, Uxbridge, UK) was used in the OLBAS project to collect spore and pollen samples, in line with many previously published reports. It was positioned 3 m from the ground. A narrow orifice, directed into the wind, sucks in fungal spores onto a silicone tape surface that is moved across the orifice at 2 mm hour$^{-1}$, with a suction rate of 10 L min$^{-1}$. The silicone tape is adhered onto a rotating drum with a 7-day sampling period. At the end of this time the drum is returned to the laboratory and the tape cut into seven equal segments, each representing a 24-hour period, i.e. a sample comprising 1 day of collection is deposited on a tape area of 48 × 14 mm (Hirst, 1952), which is then affixed to a microscope slide. The microscope slides were prepared, stored in a protective casing and sent to the University of Extremadura, Badajoz, Spain, where the counting was performed by members of the aerobiology research group. The optical microscope used for counting was a Nikon Eclypse model with a magnification of ×1000.

This off-line strategy is, of course, quite labour intensive and requires considerable training to prepare the samples and perform the optical analyses. The results give only a scaled-up estimate for the numbers of impacted PBAPs but accurate identification of particles > 2 µm in size is obtainable with experienced operators using high-magnification microscopes. The data is then made available to bodies such as Meteo Swiss where a meteorological wind dispersion model is applied for predictive purposes. The information may take days to disseminate to the public.

In Ireland, the daily pollen count published in newspapers or specialist websites comes from impaction collections made near Worcester (and elsewhere) in England. The UK Met Office then provides predictions such as "low", "moderate", "high" or "very high" as blanket descriptions for four large regions of the island. No information on allergenic fungal spore levels is available despite the fact that their sizes are found in the $PM_{2.5}$ range and can have adverse health effects on those at risk, such as asthmatics.

## 1.4 Spectroscopic, Real-time Methods for the Detection of PBAPs

Light-absorbing chromophores such as tryptophan, tyrosine, flavins and NADPH are present in PBAPs; these chromophores also fluoresce (Roshchina and Karnaukhov, 1999; Roshchina, 2003; Roshchina *et al.*, 2004; Roshchina, 2008; O'Connor *et al.*, 2011, 2014a). Hence, real-time bioaerosol detectors employing light-induced fluorescence (LIF) quantification techniques have been developed over the last 25 years. The techniques of data filtering are now much more advanced and so it is now relatively straightforward to distinguish between fluorescing chemical particles and droplets such as secondary organic aerosols (SOAs) and PBAPs. Nonetheless, it is generally accepted that the primary measurements of the LIF techniques give number counts of fluorescing aerosol particles (FAPs), which include PBAP contributions.

One of the first available reports on the real-time monitoring of bioaerosols was published in 1990. It was based on a rapidly scanning light detection and ranging (LIDAR) system called the laser cloud mapper (LCM); this was operated to determine its sensitivity for the remote characterisation of airborne biological organisms such as *Bacillus subtilis* var. *niger* sp. *globiggi* in spore form (Ho *et al.*, 1990). However, within 5 years the use of fluorescence plus optical scattering measurements had become established by military organisations such as the US Office for Naval Research, using prototype devices such as the single particle fluorescence analyser (SPFA) and the fluorescence aerodynamic particle sizer (FLAPS) (Ho *et al.*, 1999; Eversole *et al.*, 2001). However, it

was not for another 5 years that publications began to appear that were directed more towards the use of such technology for environmental analytical purposes (Gabey *et al.*, 2010; Huffman *et al.*, 2010; Pan *et al.*, 2011a; Healy *et al.*, 2014).

The wideband integrated bioaerosol sensor, universally called WIBS, was produced and designed originally by Professor Paul Kaye and co-workers at the University of Hertfordshire, UK (Kaye *et al.*, 2005). Newer versions are now commercially available from Droplet Measurement Technologies (DMT). WIBS is currently the most popular technique for monitoring PBAPs in real time. In summary, WIBS is an online fluorescence spectrometer with the ability to characterise the sizes and asymmetry/asphericity (shape) of individual, fluorescent and non-fluorescent particles. It does so by evaluating laser scatter parameters both forward and sideways along with the spectrally unresolved fluorescence intensity of single particles at a millisecond time resolution. The exact modus operandi of this instrument has been demonstrated in a number of publications, described below. Briefly, fluorescence intensities resulting from two xenon lamp excitations (280 nm and 370 nm) are evaluated using three detector channels, termed FL1_280, FL2_280 and FL3_370. These channels capture the total, spectrally unresolved fluorescence signals over two wavelength ranges, with FL1 = 310–400 nm and both FL2 and FL3 = 420–650 nm. Therefore, each particle is excited sequentially at 280 nm (FL1 and FL2) and then 370 nm (FL3). In total, WIBS datasets consist of particle numbers, size and "shape" parameters as well as three separate fluorescent intensity properties (if fluorescent).

The initial prototypes of the WIBS technology also incorporate a size selection utility acting via a dual gain function. This feature allows the operator to designate the size fraction of the ambient air sample on which to focus and perform data analysis. Hence, there are two sensitivity settings for WIBS-4: high gain (HG) and low gain (LG). For this model and older prototype versions, particles of sizes between 0.5 and 12 µm are monitored in HG whereas LG allows particles from 3 to 31 µm to be analysed. Therefore, the HG mode is suitable for fungal spore detection and the LG mode is suitable for some spores and some pollen. In contrast, the newer version (WIBS-4A) has a single gain, which evaluates particles between 0.5 and 15 µm, a range essentially suited for fungal spore detection.

Data are collected on a dedicated computer and stored for analysis using multi-variant statistical packages. Increasingly, these methods are based on the use of machine-learning algorithms and pattern recognition that require computer programmes such as MATLAB (MathWorks, Inc., USA). It is this step that currently represents the most challenging task for distinguishing between PBAPs, non-fluorescent particles and fluorescing chemical pollutants because very large amounts of data can be collected in a campaign (> 10,000,000 particles with five variable parameters for each).

In the OLBAS project, a WIBS-4 was used to monitor airborne particle releases from a green-waste management site to produce real-time bioaerosol profiles. A novel instrumental prototype called the multi-parameter bioaerosol spectrometer (MBS) was also deployed in one of the field campaigns because it provides higher resolution spectral distributions than the WIBS approach.

Pollen was monitored using a modified version of the WIBS-4 instrument, which was subsequently termed the WIBS-4$^{+}$. Essentially, the instrumental modifications allowed (1) detection of particles between 0.5 and 40 µm and (2) extension of the fluorescence detection wavelength range to 700 nm.

The OLBAS project links to the outcomes of previous research sponsored by the EPA and awarded as "Analyses of the Development and Occurrence of Biological and Chemical Aerosols (BioCheA)". The associated synthesis report (CCRP Report 18) was published in 2012 and is entitled "A New Approach to Bioaerosol Monitoring in Ireland" (http://www.epa.ie/pubs/reports/research/climate/ccrpreport18.html).

## 1.5 Objectives of OLBAS

- To provide and publish a comprehensive review of sources, monitoring methods, removal pathways and number concentrations of bioaerosols/PBAPs in ambient conditions and at waste management sites. This review was published as "Bioaerosol monitoring of the atmosphere for occupational and environmental purposes" in *Comprehensive Analytical Chemistry* (Sodeau and O'Connor, 2016).
- To provide a scientific foundation for assessing the potential application of the WIBS and related

real-time technology to the online detection of fungal spores such as *A. fumigatus* at a green-waste management site in Ireland.
- To compare and contrast the real-time monitoring results with those obtained by traditional Andersen sampling and coupled impaction/optical microscopy techniques.
- To deploy WIBS and a SporeWatch impaction device at the Met Éireann Valentia Observatory site at Cahersiveen and thereby to improve the limited database that exists for pollen and spore measurements in Ireland.
- To deploy novel instrumentation at the Met Éireann site to enhance the capabilities of WIBS to detect pollen in real time.
- To assess the benefits of the development of an indigenous pollen monitoring network for Ireland.

These objectives for OLBAS, as set in the original proposal, were achieved as described in Chapters 2 and 3.

# 2 Field Campaigns at a Green-waste Management Site in Ireland (2014–2016)

## 2.1 Site Description

The PBAP/bioaerosol monitoring campaigns were performed at a single commercial green-waste/ composting site in Ireland. The site has a 6000-tonne capacity per year for source-segregated green wastes such as grass, leaves and grain delivered from domestic and commercial sources located over different municipalities in the surrounding areas. Traditional, off-line impaction methods (Andersen sampling and Hirst trap) were employed alongside newer spectroscopic, online instrumentation to detect and count individual particles, as described in sections 1.2 and 1.4. Meteorological measurements such as temperature, wind speed, wind direction and relative humidity were obtained using a combination of a Davis Vantage-Pro2 permanent site station and a mobile Casella Nomad weather station.

At the site, the green waste was shredded mechanically and stored in both open windrows and composting vessels, where the compost was left to mature. A schematic of the arrangement is shown in Figure 2.1. The staff offices were built ≈20 m from the outdoor product compost area and ≈20 m from the delivery/loading/green-waste area. The bio-filter was positioned at the north end of the site.

Most of the monitoring studies were performed outdoors, with the equipment located close to windrows, as shown in Figure 2.1. However, as a control, the spectroscopic instrumentation was located, on occasions, in the staff quarters and the site office. The annual Andersen grab sampling for the site was carried out ≈100 m upwind (north-east) and then downwind (south-west). The point sources of the bioaerosols were not monitored.

The site was located in a remote, rural location ≈6 km due north of the Irish coastline and therefore only sea-salt particles were expected to make any large contribution to aerosol loading other than PBAPs. South-westerly winds from the Atlantic generally dominate weather patterns in Ireland although variations in speeds and directions often occur.

The typical working week for the employees at the facility is 08:30–17:00 from Monday to Thursday and 08:30–16:00 on Friday. The site is not open at the weekend. During the operating hours of the facility

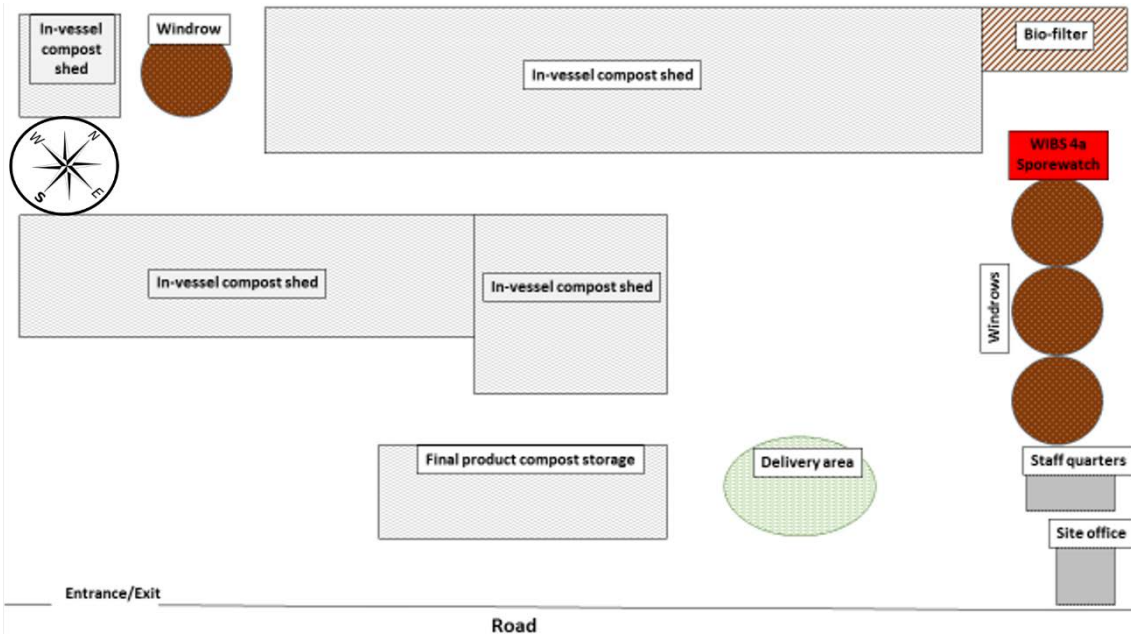



**Figure 2.1. A schematic of the green-waste site used for the three field campaigns.**

there was constant on-site activity, including deliveries of green waste and the moving of finished compost to the storage shed for eventual sale to the public. Front-end loaders were constantly in use for sorting deliveries, loading compost sales, moving the green waste to the shredder, pile-turning or loading of the screening machine to separate the oversized waste from the compost. Visitors also drove to the site for various reasons and so anthropogenic airborne particulate material from the site machinery and road transport may be present at the site, although the closest main road was some 2 km away.

## 2.2 Field Campaign 1: October 2014

Three periods of time were chosen to provide preliminary bioaerosol profiles of the site: (1) 30 September–1 October 2014 (a period logged by the site manager as representing a "heavy" workday with many deliveries of waste and compost loading taking place); (2) 10–13 October 2014, a weekend, when the site was closed; and (3) 15–17 October 2014 (a period logged by the site manager as representing a "light" workday, when few deliveries or little loading took place).

Commercial Andersen grab sampling was carried out between 13.00 and 14.00 on 16 October 2014, ≈100 m north-east and then ≈100 m south-west of the site, with the prevailing wind originating from the south, as shown in the wind rose plot in Figure 2.2. The CFU $m^{-3}$ levels of *A. fumigatus* and mesophilic bacteria were obtained using 15-min sampling of each type.

The WIBS-4 instrument and laptop were housed inside the office site hut, with an outdoor sampling inlet connected to the WIBS by a 2-m length of non-conductive tubing, as used in many previous studies of bioaerosol real-time sampling, to minimise potential

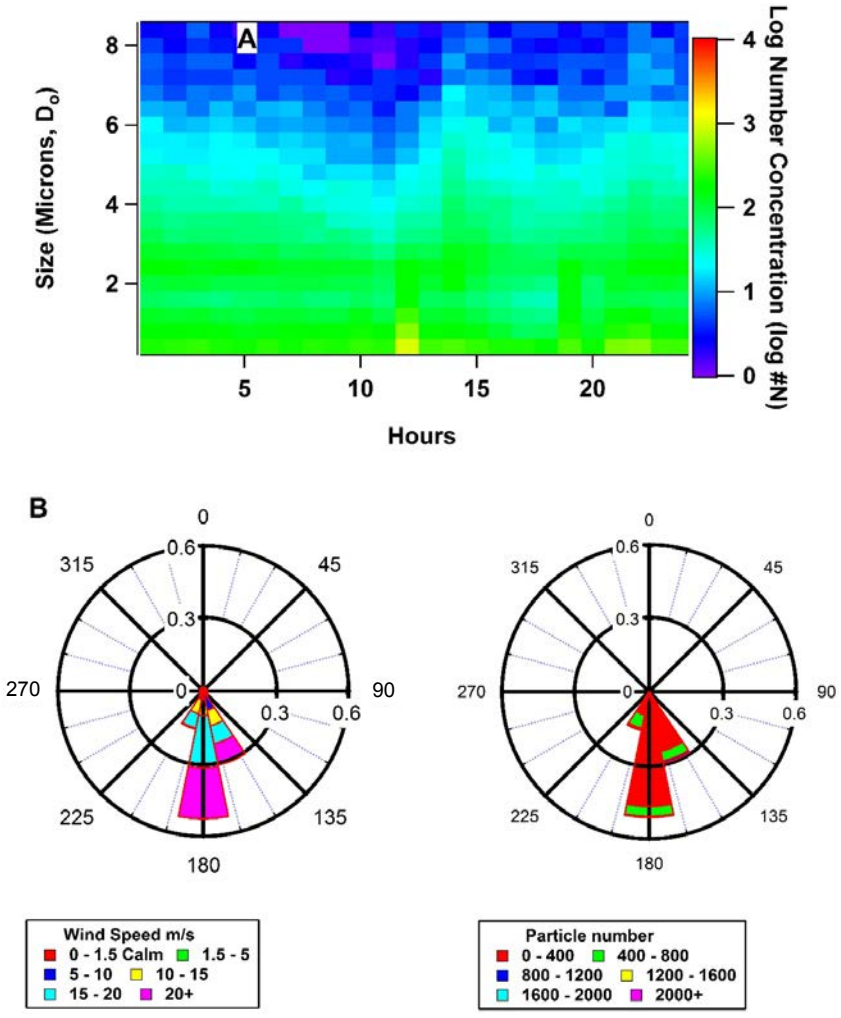



**Figure 2.2. Time-dependent particle size/number concentration plot (a) and corresponding wind rose graphic (b) for the "light" workload days.**

static effects. It extended vertically to a height of 1.5–2.0 m above ground level and can be considered to be a total suspended inlet. The length of tubing was kept to a minimum to reach the sampling point from the instrument location, as were bends. However, wall losses would always occur in sampling and very high winds could affect particle collection. A rain guard was employed to keep droplets and moisture away. If real-time techniques are eventually used at waste management sites to monitor bioaerosols such physical sampling parameters would need to be optimised over many on-site studies at various locations. The purpose of OLBAS, of course, was to provide a proof of principle for the future.

Both fluorescing and non-fluorescing particles were pumped individually into the spectrometer. Only those with sizes of 0.5–12 µm were analysed further as this range would include most fungal spores. FAPs were defined by the application of normal threshold techniques, as described in many publications (e.g. Sodeau and O'Connor, 2016). The results were used in combination with meteorological data to construct bioaerosol site profiles for each of periods studied: "heavy" workload, "light" workload and weekends, when the site was formally closed.

For each of the three campaign periods, particles not reaching both the required size and the fluorescence threshold criteria were filtered out from the analyses. The real-time, on-site data obtained by WIBS was used to construct the following types of fluorescent particle profile:

- size (µm) versus time (hour) fluorescent number concentration plots;
- asymetry facor (AF) value or "shape" versus time (hour) fluorescent number concentration plots;
- wind rose graphics for fluorescent number concentrations with wind speeds and directions;
- relative humidity effects on the fluorescent number count.

### *2.2.1 "Light" workload period*

Data were collected for a 3-day period between 11.00 on 15 October 2014 and 12.00 on 17 October 2014. The time-dependent particle size plot indicating the averaged fluorescent number concentrations (as a logarithmic function to enable all of the data to be presented on one scale) between 00.00 and 00.00 is shown in Figure 2.2a.

The colour-coded bands used for Figure 2.2a (and also Figures 2.3a and 2.4a) to express the logarithms of the fluorescent particle number concentration ranges versus size can also be expressed as actual number concentrations. The appropriate ranges are shown in Table 2.1.

The data show that at one single time point (about 11.00) a maximum number of fluorescent particles with sizes of < 1.5 µm (≈1000–1500 $m^{-3}$) was reached. At all other times the counts in this small size range, as well as between 2 and 3 µm, were about half that of the 11.00 level. These data indicate some type of "event" occurring late morning at the site. Very few, if any, particles were counted with sizes of > 6 µm.

The wind rose graphics with WIBS sited at the central point, the green-waste loading/unloading bay at 225° and the composting area at 315° are of considerable relevance for interpreting all of the particle data and, for the period from 15 to 17 October 2014, the data are summarised in Figure 2.2b.

Clearly, this "light" working day represented a period of time when there were relatively high-speed winds (> 20 m $s^{-1}$) from due south, i.e. directly from the Irish coast, which is about 6 km away.

The annual Andersen sampling exercise for the site was performed by a commercial company on 16 October between 13.00 and 14.00. This period represented a time when very few fluorescent particles were detected by WIBS. Hence, at the upwind/control

**Table 2.1. Number concentration limits for the monitored fluorescent particles**

| Number concentrations $m^{-3}$ | Colour bands in Figure 2.2 |
|---|---|
| 5000–10,000 | Red |
| 1500–5000 | Orange |
| 1000–1500 | Yellow |
| 80–1000 | Green |
| 0–80 | Blue |

location, 0 CFU $m^{-3}$ of *A. fumigatus* were detected alongside 14.2 CFU $m^{-3}$ of mesophilic bacteria, whereas the corresponding figures for the downwind site were 9.5 CFU $m^{-3}$ and 28.4 CFU $m^{-3}$, respectively. All of these values are well below the ambient levels expected for these species, even as "background". However, it could be deduced from Figure 2.2a that somewhat higher levels of *A. fumigatus* and/or mesophilic bacteria *might* have been expected to be sampled off-site if the timing had been about 2 hours earlier or, as shown in the following section, on another day when the workload was regarded as "heavy", as defined by the site manager.

### *2.2.2 "Heavy" workload period*

Data were collected for a 30-hour period, between 11.00 on 30 September 2014 and 15.00 on 1 October 2014. The time-dependent particle size plot indicating the fluorescent number concentrations (as a logarithmic function to enable all of the data to be presented one scale) between 00.00 and 00.00 is shown in Figure 2.3a.

The data show that during the working day (≈08.00–15.00) a maximum in fluorescent particle number concentrations (≈1500–5000 $m^{-3}$) is reached, with sizes of < 1.5 µm. Levels of ≈1000–1500 $m^{-3}$ were monitored for the rest of the day, except between ≈01.00 and 07.00 when the facility was

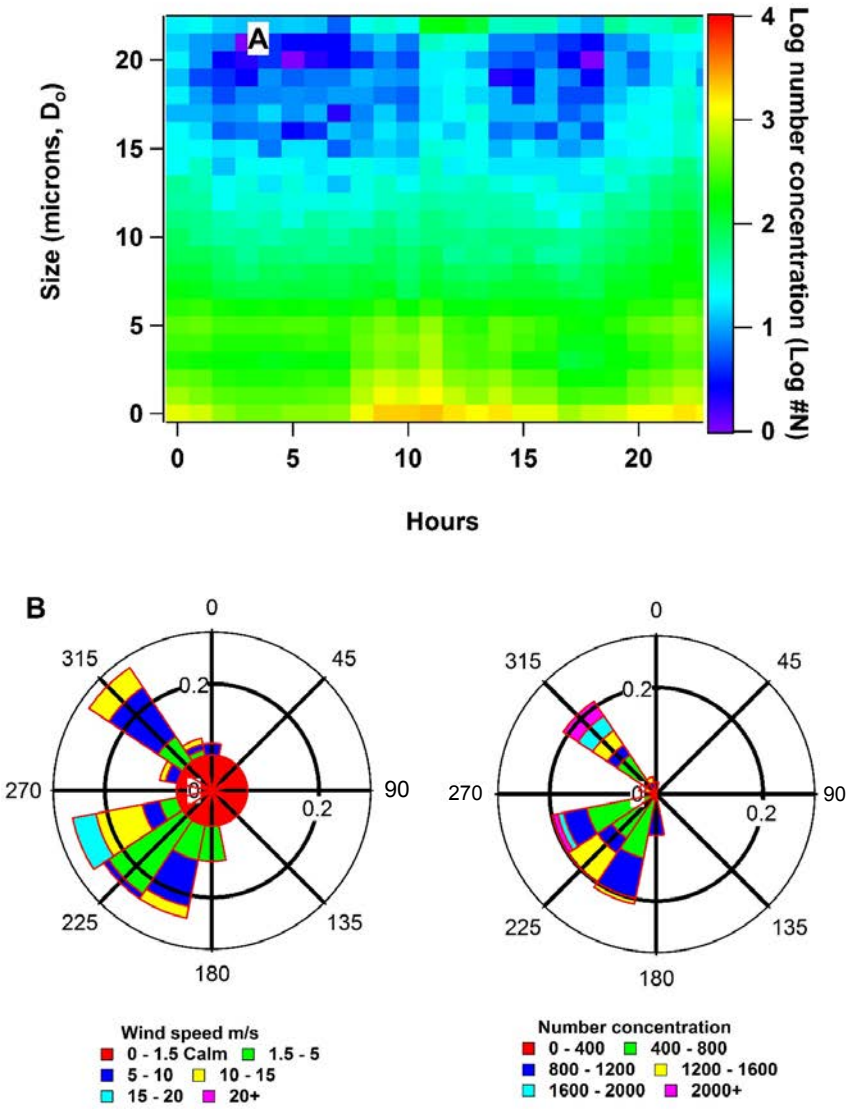



**Figure 2.3. Time-dependent particle size/number concentration plot (a) and corresponding wind rose graphic (b) for the "heavy" workload day.**

closed. Over this period, ≈80–1000 $m^{-3}$ fluorescent particles in this size range were monitored. There were few fluorescent particles of < 1.5 µm monitored between 03.00 and 07.00. Throughout the working day fluorescent particles in the 2.0 to 2.5 µm size range were also measured at ≈1000–1500 number concentrations. Few if any particles of > 6 µm in size were analysed. All of these data are summarised in Figure 2.3a.

The wind rose graphic (Figure 2.3b) for this time period shows that the fluorescent particle counts tracked the two main wind directions (south-west and north-west) throughout the day. It is of note that the waste loading and waste composting areas are also located in the same two directions relative to the WIBS location (225° and 315°, respectively). The wind speeds were very low/calm for about 20% of the day but mainly ranged between 1.5 and 10 m $s^{-1}$ for the rest of the time.

### *2.2.3 Weekend period (site closed)*

Data were collected for a 3-day period between 14.00 on Friday 10 October 2014 and 12.00 on Monday 13 October 2014. The time-dependent particle size plot indicating the averaged fluorescent number concentrations (as a logarithmic function to enable all of the data to be presented on one scale) for the 24-hour period between 00.00 and 00.00 is shown in Figure 2.4a.

The data show that, in contrast to the working weekdays, the non-operating site releases very high number concentrations of fluorescent particles

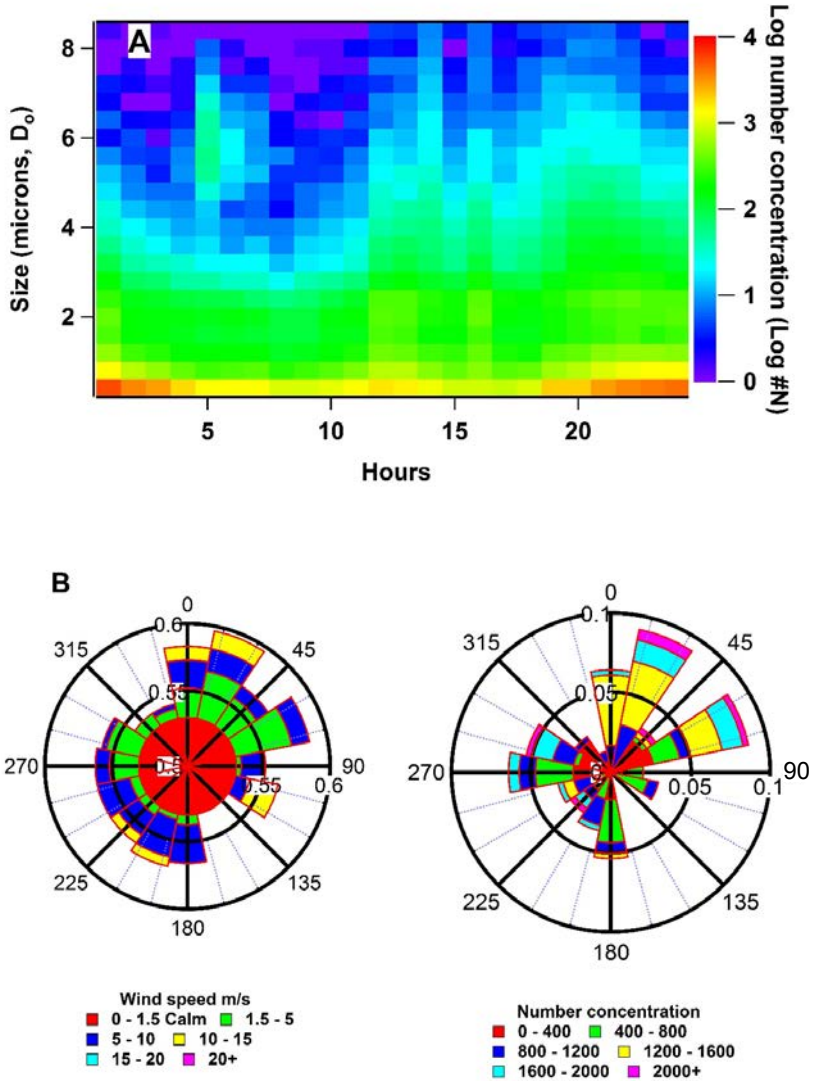



**Figure 2.4. Time-dependent particle size/number concentration plot (a) and corresponding wind rose graphic (b) for the weekend days.**

(≈5000–10,000 $m^{-3}$) of sizes of < 1 µm, which were detected at night-time between ≈19.00 and 05.00. During the daytime, particles of < 1.5 µm were collected, with number counts of ≈1000–1500 $m^{-3}$. Particles with number counts of ≈80–1000 $m^{-3}$ were counted in the 2- to 3-µm size range throughout the whole period. As observed during the weekdays, very few, if any, particles were counted with sizes of > 6 µm. However, the results indicate that periods when waste management sites are formally closed deserve monitoring studies of their own in the future.

The wind rose graphic for the weekend period is shown in Figure 2.4b. The wind conditions are quite different from the wind conditions during the 2 working day periods, with calm conditions (0–1.5 m $s^{-1}$) prevailing for > 50% of the time. When there was wind it came from all directions other than the north-north-west and the south-east, although it came predominantly from the prevailing south-west direction. As for the "heavy" working day data the fluorescent particles were collected with a similar dispersion profile to the wind directions.

Several points are worth raising about this novel WIBS sampling study at a green-waste management site. For example, although the site is unmanned at the weekends and would not be accepting waste or actively turning material, the composting process is still taking place and operating systems are likely to be set to some sort of automatic mode. Furthermore, different air-handling/extraction rates may be applied to the composting materials, which would influence temperature profiles of the waste and potential bioaerosol release rates.

In summary, waste sites are very complicated locations and it is important to observe and log exactly what is happening in real time to be able to fully interpret air/bioaerosol/odour emissions data. Our real-time instrumental measurements are a first, promising step on this pathway, but other defining aspects remain the responsibility of the operators and the licensing authorities.

### *2.2.4 Comparison between the data collected over the three time periods*

Number concentrations of non-fluorescent particles were also collected simultaneously with the fluorescence data. The number concentrations of fluorescent particles as a proportion of total particles counted amounted, on average, to ≈1% for the "light" workday period, as shown in Figure 2.5a and Table 2.2. It is likely that many of the non-fluorescent particles monitored in this period were sea salt from the coast because of the wind conditions described above from due south.

The proportion of fluorescent particles as a proportion of total particles counted for the "heavy" workday period showed a great variation over the day. More than 50% of the particles were determined as fluorescent at a time of considerable site activity (≈10.00), but between 08.00 and 16.00 (the full workday period) > 20% of the particles were determined as fluorescent. A much smaller percentage was shown to fluoresce in the analyses performed when the site was closed at night. Many non-fluorescent particles were also monitored during working hours and so over the whole day the average loading of fluorescent particles was ≈7%. The results are summarised in Figure 2.5b and Table 2.2.

The proportion of fluorescent particles detected also showed a great variation over the 2 weekend days. Some 60–70% of the particles were determined as fluorescent at about 08.00 on each of the two mornings but a substantial percentage (> 40%) was captured during the night/early morning period between 00.00 and 10.00. However, far fewer particles were collected over the weekend than on days when the site was operating, likely because of inactivity and the calm wind conditions prevailing for half of the period. Overall, it was calculated that about 18% of the collections were because of fluorescing PM at the weekend, as shown in Figure 2.5c and Table 2.2.

The above results show the key importance of wind speed, wind direction and working activity on the real-time capture and detection of non-fluorescent and fluorescent particles at the site. For the remote rural location studied here the fluorescent particles are most likely bioaerosols. Clearly, their measured number concentrations depend on a number of time-dependent factors and therefore single, annual Andersen sampling visits cannot be representative of any meaningful green-waste site profile for bioaerosol dispersion.

Further bioaerosol site profile information for the three campaign periods can be deduced by use

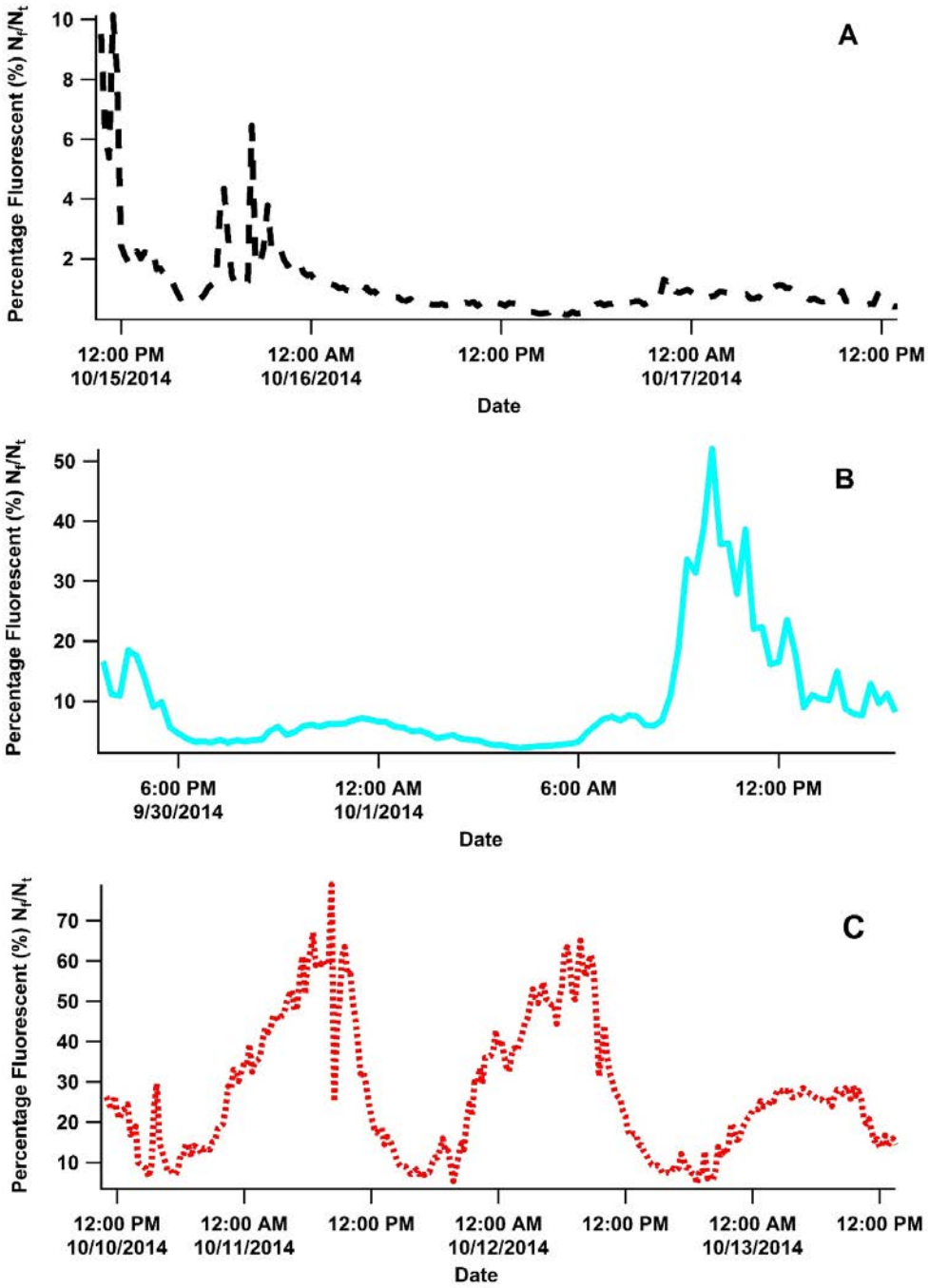



**Figure 2.5. The number concentrations of fluorescent particles as a proportion of total particles counted for (a) "light" workload days, (b) "heavy" workload days and (c) weekend days.**

of comparative descriptive statistics for all of the particles captured over the full campaign, as shown in Table 2.2. As the data indicate, there are clear physical distinctions between the non-fluorescent "dust" and the fluorescent particles analysed.

Table 2.2 shows that the total number concentrations of particles generated on weekdays (both fluorescent and non-fluorescent) were much larger than those generated at weekends. Furthermore, the bioaerosols (the fluorescent particles) collected in all three time periods fell into one main size regime: ≈0.5 µm to ≈3 µm; in contrast, the non-fluorescent "dust" was generally smaller. Interestingly, the weekend bioaerosol counts showed somewhat different size and "shape" distributions from the 2 working day periods. Hence, at the weekend the fluorescent particles were generally more elongated (average AF ≈33) but in the weekday periods they appeared to be more ellipsoidal/

**Table 2.2. Comparative descriptive statistics for the bioaerosols monitored over the three campaign periods**

| Parameter | Statistic | "Heavy" workload period | Weekend | "Light" workload period |
|---|---|---|---|---|
| *Non-fluorescent particles* | | | | |
| Size (µm) | Average | 1.2 | 0.8 | 1.1 |
| | 25th percentile | 0.5 | 0.4 | 0.5 |
| | 50th percentile | 0.9 | 0.5 | 0.9 |
| | 75th percentile | 1.6 | 1.0 | 1.5 |
| AF value | Average | 23.7 | 33.1 | 21.7 |
| | 25th percentile | 13.3 | 17.5 | 12.6 |
| | 50th percentile | 19.9 | 30.6 | 18.2 |
| | 75th percentile | 31.3 | 45.9 | 28.3 |
| Concentration | Particles/L | 3912.4 | 1460.8 | 11,412.2 |
| *Fluorescent particles* | | | | |
| Size (µm) | Average | 1.8 | 1.2 | 2.1 |
| | 25th percentile | 0.5 | 0.4 | 0.7 |
| | 50th percentile | 1.2 | 0.6 | 1.7 |
| | 75th percentile | 2.5 | 1.6 | 2.9 |
| AF value | Average | 26.7 | 32.9 | 24.2 |
| | 25th percentile | 14.9 | 18.5 | 13.5 |
| | 50th percentile | 23.0 | 30.7 | 20.6 |
| | 75th percentile | 35.6 | 44.6 | 31.4 |
| Concentration | Particles/L | 304.2 | 320.2 | 109.9 |

spherical (average AF ≈25). This observation may be related to a number of variables, for example the different bioaerosol sources present at the composting centre, including grain and grass, or alternatively direct biofilter emissions. Future studies should be directed towards a more complete understanding of this phenomenon at various locations on-site, including close to the biofilter. Finally, meteorological conditions are a likely influence on behaviour but a very long-term study would be required to establish this.

The weekend particles analysed were also somewhat smaller. Although the WIBS technique cannot currently provide information on the identity of PBAPs sampled in the field it is of note that the main types of spore/bacteria associated with green-waste composting sites, that is, actinomycetes/mesophilic bacteria and *A. fumigatus/Penicillium*, are of appropriate geometric diameters to account for the WIBS signals (Ashraf *et al.*, 2007). For example, the physical sizes of three actinomycetes spores measured by microscopy and image analysis appear to be somewhat ellipsoidal, with width/length dimensions of 0.68/0.84, 0.55/0.72 and 0.66/0.79 µm for *Staphylococcus albus*, *Micromonospora halophytica and Thymus vulgaris*, respectively (Reponen *et al.*, 1998, 2001). By contrast, *Penicillium* spp. are spherical or slightly ellipsoidal and are in the 2- to 4.5-µm range (Reponen *et al.*, 2001).

Previous studies using LIF/scatter detection have shown that the mean aerodynamic diameter for *A. fumigatus* and *Penicillium* fungal particles at age 1 and 2 weeks are 2.40 ± 0.12 and 3.55 ± 0.14 µm, respectively (Kanaani *et al.*, 2007, 2008). These measurements are in good agreement with previously obtained data for the spores (Latgé, 1999). The particle size distribution of *A. fumigatus* has also been assessed by filtration when directly emitted from compost; peak recovery was found between 2 and 3 µm (Deacon *et al.*, 2009). The results obtained in the current study are also in full agreement with a recent publication using q-PCR to determine the composition of bacterial cells, such as *Saccharopolyspora rectivirgula*, released from the composting process (Galès *et al.*, 2015).

It is of further interest that, in contrast to the working days, the highest proportion of bioaerosol was detected at the weekends, specifically at night-times. This loading is mainly because of the contribution from

particles in the < 1-µm size range and, as discussed above, the "shapes" are distinctly different from weekday counterparts.

In order to further probe these observations, measurements of the fluorescent particle profile as a function of relative humidity and wind speed over the weekend sampling period were made and details are shown in Figure 2.6. The plot shows that fewer fluorescent particles (in any/all of the three channels) were sampled in low relative humidity conditions and high wind speed conditions than when high relative humidity and low wind speeds prevailed.

These results are not surprising given that weather conditions such as humidity, rainfall and wind are known extrinsic factors controlling the release of spores in rural settings (Santarpia *et al.*, 2013; O'Connor *et al.*, 2014b). It is known, for example, that many ascomycetes are released in high relative humidity, a condition occurring at night-time. Minimal spore release occurs during daylight hours when there are naturally low relative humidity conditions and light–dark cycles can also affect release mechanisms. Basidiospores (size range 5–8 µm) also show a distinct diurnal variation, with pre-dawn or night-time peaks observed in response to high relative humidity; they are likely to be present in areas where mushrooms are found. However, very few, if any, fluorescent particles were detected in their size range.

It is of final note that dust particles have been shown to weakly fluoresce and could therefore produce some false-positive signals (Pöhlker *et al.*, 2012; Toprak and Schnaiter, 2013). Humic and fulvic acids, which are present in some soils and often termed HULIS (humic-like substances), are also known to exhibit excitation–emission matrixes that could affect fluorescent loads measured by the WIBS-4. It remains a little-studied area, but the size regimes involved are likely not relevant here (Pöhlker *et al.*, 2012).

The campaigns discussed above were the first of their type to provide real-time data on bioaerosol emissions as profiles from a composting/green-waste centre. The WIBS results show that the bioaerosol (FAPs) and non-fluorescent particle counts vary enormously depending on working activity, time of day and weather conditions, especially wind speeds (O'Connor *et al.*, 2015).

What could not be ascertained in the three campaigns were the identities of the full range of spores that were released at the site; hence, a further campaign using the WIBS in tandem with a SporeWatch impactor (followed by optical microscopy analysis) was mounted.

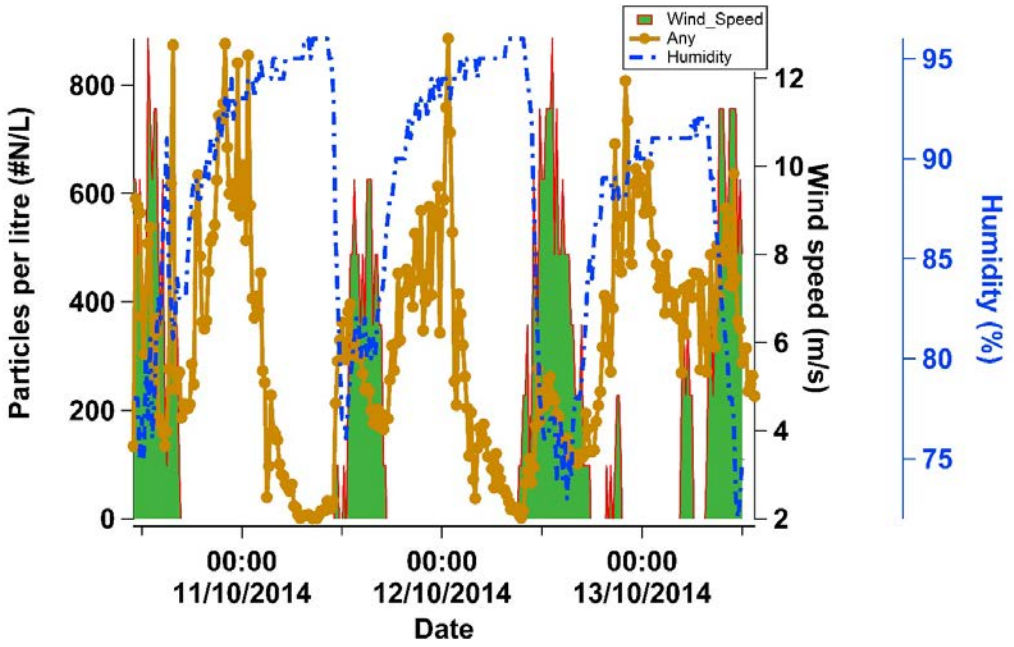



**Figure 2.6. Fluorescence counts over the weekend period as a function of wind speed and relative humidity.**

## 2.3 Field Campaign 2: February/ March 2016

### *2.3.1 Monitoring PBAPs with SporeWatch/ microscopy and WIBS*

This field campaign took place over a 1-week period from Monday 29 February to Monday 7 March 2016. The monitoring equipment was positioned on the site as indicated in Figure 2.1. The trailer housing the WIBS-4A was located in the northern corner of the compost site. To the east there was a ditch separating it from a farm, which was approximately 30 m away. A compost windrow was set to the south of the trailer and the screener was located to the west, with the latter used to separate fine and coarse compost; in operation it caused rigorous agitation of the compost. Clearly, such an activity would be expected to lead to large FAP concentrations in the surrounding air. The largest compost shed on-site was located north-west of the trailer and the biofilter was positioned due north. Wind rose data for the period are shown in Figure 2.12, with the results discussed in the accompanying text.

The following 22 types of fungal spores were measured on-site using the SporeWatch/optical microscope combination: *Aspergillus–Penicillium*, *U. maydis*, *Coprinus*, *Leptosphaeria*, *Cladosporium cladosporioides*, *Ustilago cynodontis*, *Diatrype*, *Arthrinium*, *Cladosporium herbarum*, *Venturia*, *Pleospora*, *Alternaria*, *Drechslera*, *Xanthoria*, *Massaria*, *Agrocybe*, *Oidium*, *Epicoccum*, *Ganoderma*, *Thelephora*, *Stemphylium solani* and *Curvularia*.

As is generally the case, *Aspergillus* and *Penicillium* were grouped together when counting. This is a common protocol as they are similar in size and indistinguishable under an optical microscope (Hryhorczuk *et al.*, 2001; Gillum and Levetin, 2008).

The six most dominant spores, with their contributing percentages in relation to total spore numbers, are displayed in Figure 2.7.

Clearly, the highest airborne concentrations were of *Aspergillus–Penicillium*, similar to that found previously in a study at a composting facility using Andersen sampling over a 10-day campaign (Hryhorczuk *et al.*, 2001). In fact, the spores here contributed 32.80% of the total spores measured over the full campaign, showing an average hourly concentration of 319 spores $m^{-3}$ and a total of 53,942 spores.

The spore with the second highest concentration measured on the compost site was *U. maydis*, which

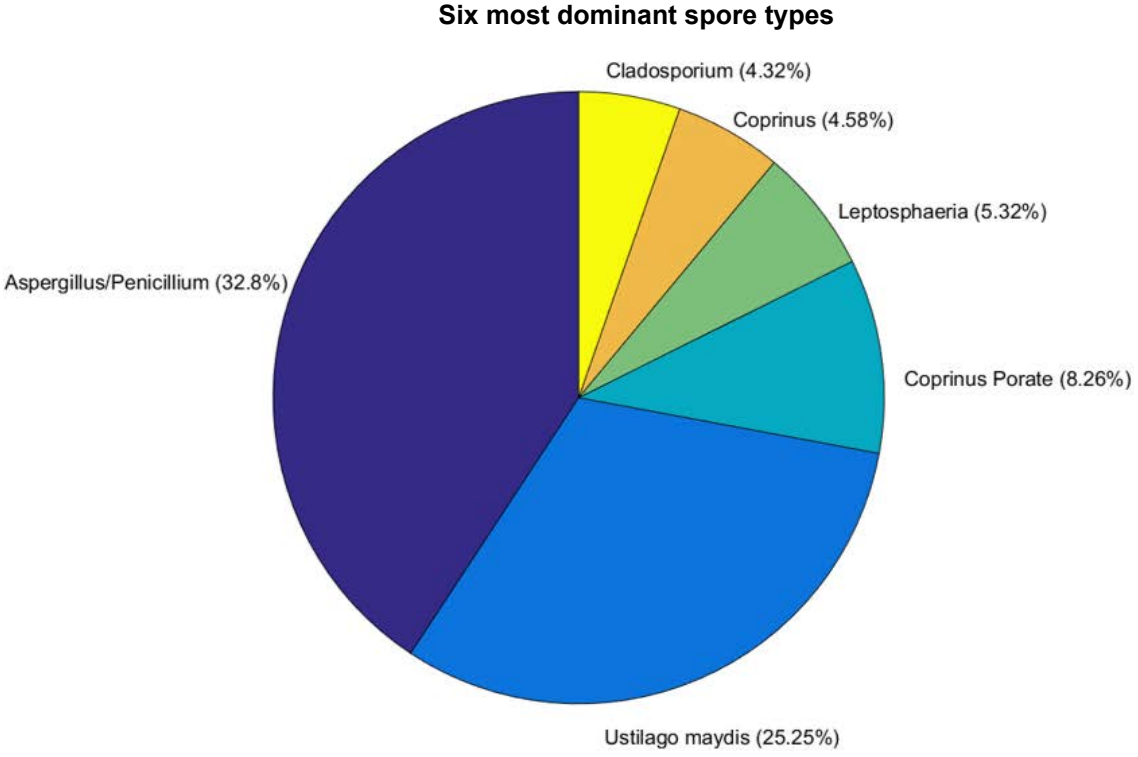



**Figure 2.7. Pie chart of the six most common spores released from the composting site.**

made up 25.25% of the total spores measured over the entire campaign. *U. maydis* is a basidiospore in the class Ustomycetes in the order Ustilaginales or smuts (Carlile *et al.*, 2001). The total number of spores measured over the campaign was 41,530, with an average hourly concentration of 246 spores $m^{-3}$. The standard deviation from the average was very high at 1672, which indicated that large variations occurred from the average hourly concentration. This observation is likely because the spores were measured mainly at certain major spore sampling events; concentrations were low at other times throughout the campaign. The behaviour is discussed below.

*Coprinus*, *Leptoshaeria* and *C. cladosporioides* occurred at much lower concentrations, with percentage of total spores of 8.26%, 4.58%, 5.32% and 4.32%, respectively.

A similar monitoring campaign using a SporeWatch has been performed previously. However, on that occasion the impactor was located 710 m downwind from the composting site rather than on-site, as in the current study. In agreement with the OLBAS study, *Aspergillus–Penicillium*, smut spores (in which *U. maydis* were grouped) and *C. cladosporioides* were all detected during the monitoring campaign, although *Coprinus* and *Leptosphaeria* were not recorded (Gillum and Levetin, 2008).

A graph of total spores counted versus time over the full 7-day measurement campaign is shown in Figure 2.8a. Fungal spore time-series profiles for the two most abundant spores found were also constructed to relate the SporeWatch measurements to site activity. Such graphs, spanning the entire campaign (13:30 on 29 February to 13:30 on 7 March), are shown in Figure 2.8b.

Figure 2.8a clearly shows that, as expected for the location, spores were present throughout the campaign. Most strikingly, there was a major spore release (20,000 spores $m^{-3}$) measured on Thursday 3 March between 16:00 and 17:00, which dominated the time series for the campaign. Notable peaks also occurred on 1 March at 14:00 and between 09:00 and 10:00 on that day. A further peak was observed on 4 March at 09:00. All of these times corresponded to the working hours of the compost site.

Figure 2.8b details the time series *for Aspergillus–Penicillium* and shows that spore releases throughout the campaign occurred irregularly; the reason for this is probably because of spores being present in large chains/clumps contained in bubbles on the adhesive substrate. However, differing site activities also likely contribute to the irregularities observed.

In contrast, *U. maydis* spores were released on only a few occasions, as shown in Figure 2.8b. They accounted for the large spore event measured on Thursday 3 March (16:00–17:00). This conclusion is clear when comparing its specific time series with that for total spores (Figure 2.8a). *U. maydis* is a species of fungus that appears on maize, which was one of the green-waste types delivered to the compost site. On examination of the delivery logbook it was found that there were regular deliveries from a local distillery. Waste products from the distillation process include wheat, barley and maize and therefore represent the most likely source of the *U. maydis* smut fungus determined on-site. The largest spore releases of *U. maydis* spores were measured on Thursday 3 March between 16:00 and 17:00 and Friday 4 March at 09:00. The smallest spore release measurements were made on Tuesday 1 March from 09:00 to 14:00.

The FAP data obtained from the WIBS measurement campaign were used to construct profiles that were complementary to the spore count numbers. The data included measurements of (1) time series; (2) size distributions; (3) accumulated concentration diurnal plots; and (4) correlations with meteorology data.

A total of 3,482,073 particles (fluorescent and non-fluorescent) were measured over the campaign from 29 February (13:30) to 6 March (23:59). FAPs were counted by applying the measured forced trigger threshold values (as defined by the generally accepted 3σ of the variation plus the average forced trigger in each channel). In total, 516,172 FAPs were measured. This value represents 14.8% of the overall particles measured at the site. Two separate FAP site profiles were constructed for the measurement campaign. One was for the weekday period (Monday–Friday), over which time the compost site was operational during the working hours of 08:30–17:00, and the other was for the weekend period (Saturday and Sunday), when the compost site was formally closed and locked up.

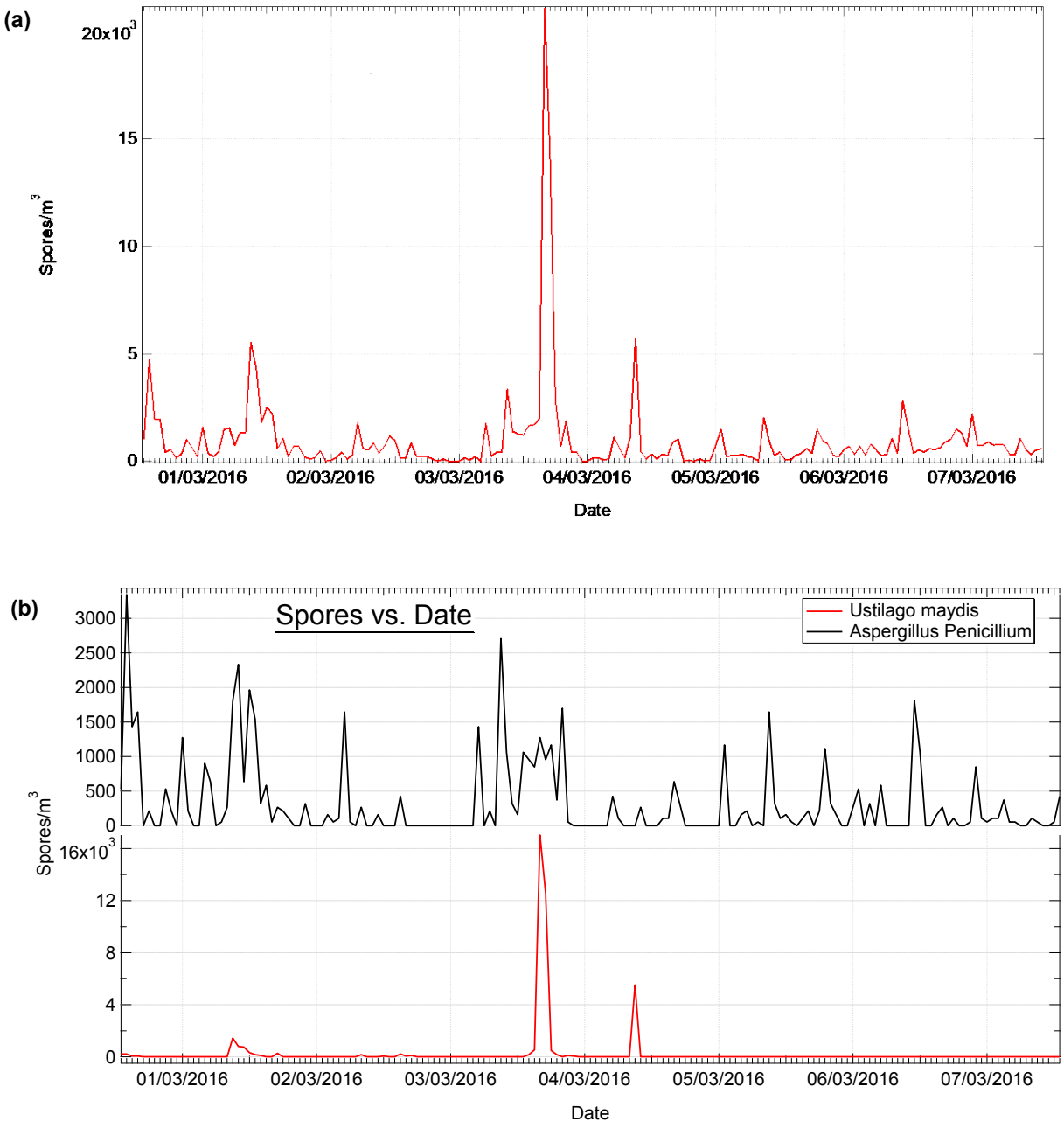



**Figure 2.8. (a) Time series of total spore counts vs. time over the 7-day measurement campaign. (b) Time series of spore counts vs. time for *Aspergillus–Penicillium* (black line) and *U. maydis* (red line) over the 7-day measurement campaign.**

The weekday FAP site profile was obtained over the period from 29 February 2016 (13:30) to 4 March 2016 (23:59). The weekend measurement period, from Saturday (00:00) to Sunday (23:59), was analysed separately as the measurements made could not be affected by normal working operations at the compost site.

Time-series graphs for each period were constructed and are shown in Figures 2.9a and b. The graphs also provided information on size and fluorescence intensities measured in the FL1 channel. Each time series was constructed with a 1-hour resolution.

Figure 2.9a shows that a major fluorescent particle event occurred on Thursday 3 March at 15:00. This feature coincides with the *U. maydis* event recorded by the SporeWatch, shown in Figure 2.8b. The evolution of the event is clearly observable using the WIBS because of its superior time resolution. The

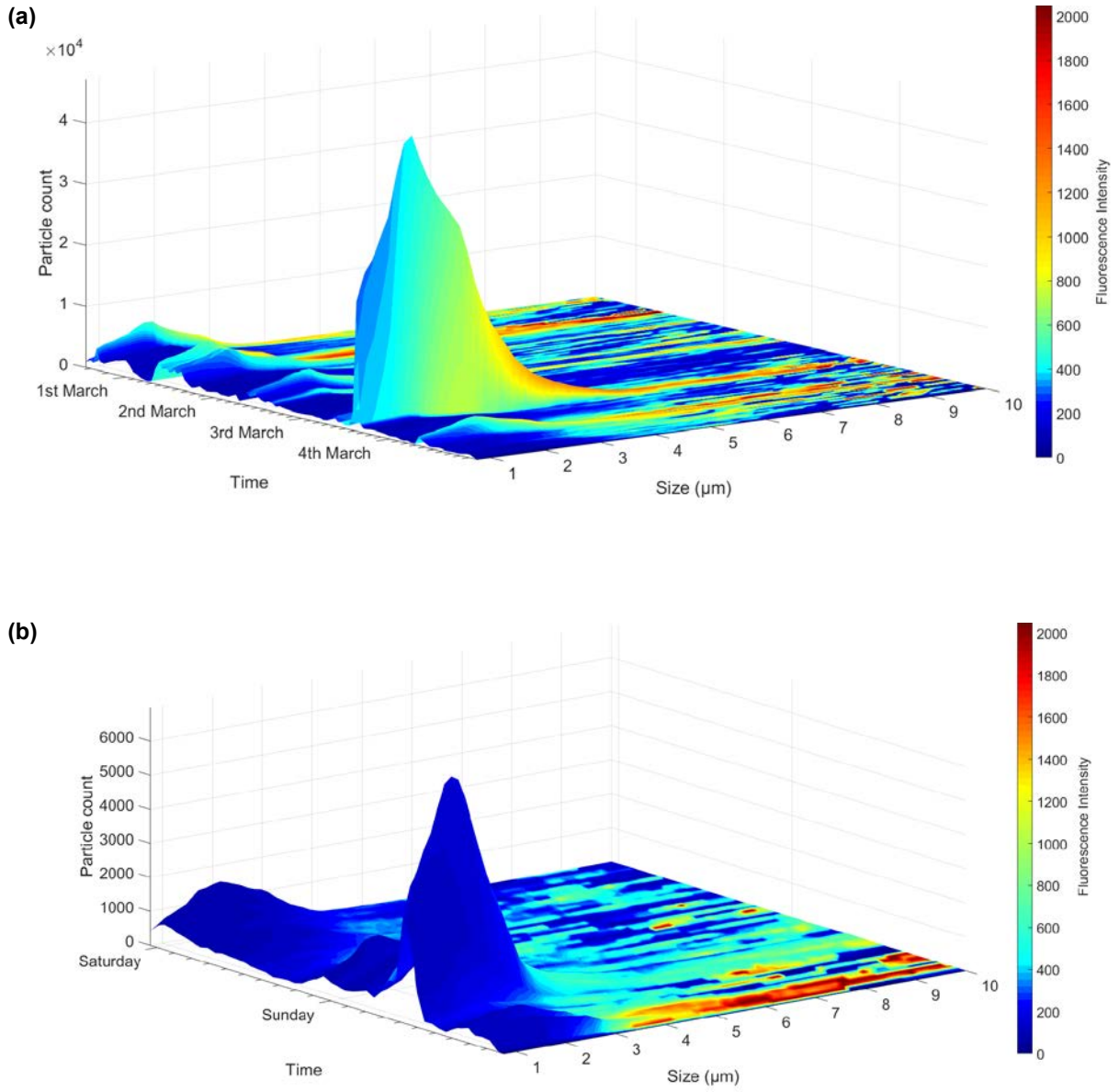



**Figure 2.9. (a) A time-series profile of size and fluorescence intensity for the weekdays. (b) A time-series profile of size and fluorescence intensity for the weekend.**

fluorescence intensity of the particles was high and ranged from ≈400 to 800 for particles of size < 4 μm. The event itself dominated the time series for the full weekday period. The strongest fluorescent intensities of > 1400 were all associated with larger particles (> 4 μm) and were measured in much lower number concentrations than the smaller particles.

Figure 2.9b illustrates that there was a large number concentration of FAPs measured on Sunday from 07:00 to 11:00. The majority of the spores released were < 3 μm in size and had a low fluorescence intensity. The higher fluorescence intensity was associated with somewhat larger particles; moderate intensities are shown in turquoise. Particles with very intense fluorescence (indicated on the graph in deep-red/crimson) occurred late on Sunday night. These were even larger particles ranging in size from 4 to 10 μm and were present in very low concentrations.

To investigate the size of the particles measured over the weekday and weekend periods, accumulated and averaged hourly concentration diurnal image plots of size (μm) versus time (hour), with the FAP counts in a colour key, were constructed for both the weekday and weekend periods, as seen in Figures 2.10a and b.

Figure 2.10a highlights the fact that a high density of particles was measured during the weekday operational hours of the site (08:30–17:00), as can be seen by the light-blue/green region. This result was consistent with the findings made in the first OLBAS campaigns, as shown in Figure 2.3 (O'Connor *et al.*, 2015).

There was also an extremely high concentration of particles on 3 March at 16:00, which dominated the image plot with its deep-red/crimson profile. This feature can be attributed to the large FAP event measured on Thursday 3 March from 15:00 to 16:00. The size of the particles measured during the workday period (08:30–17:00) was mostly in the 1- to

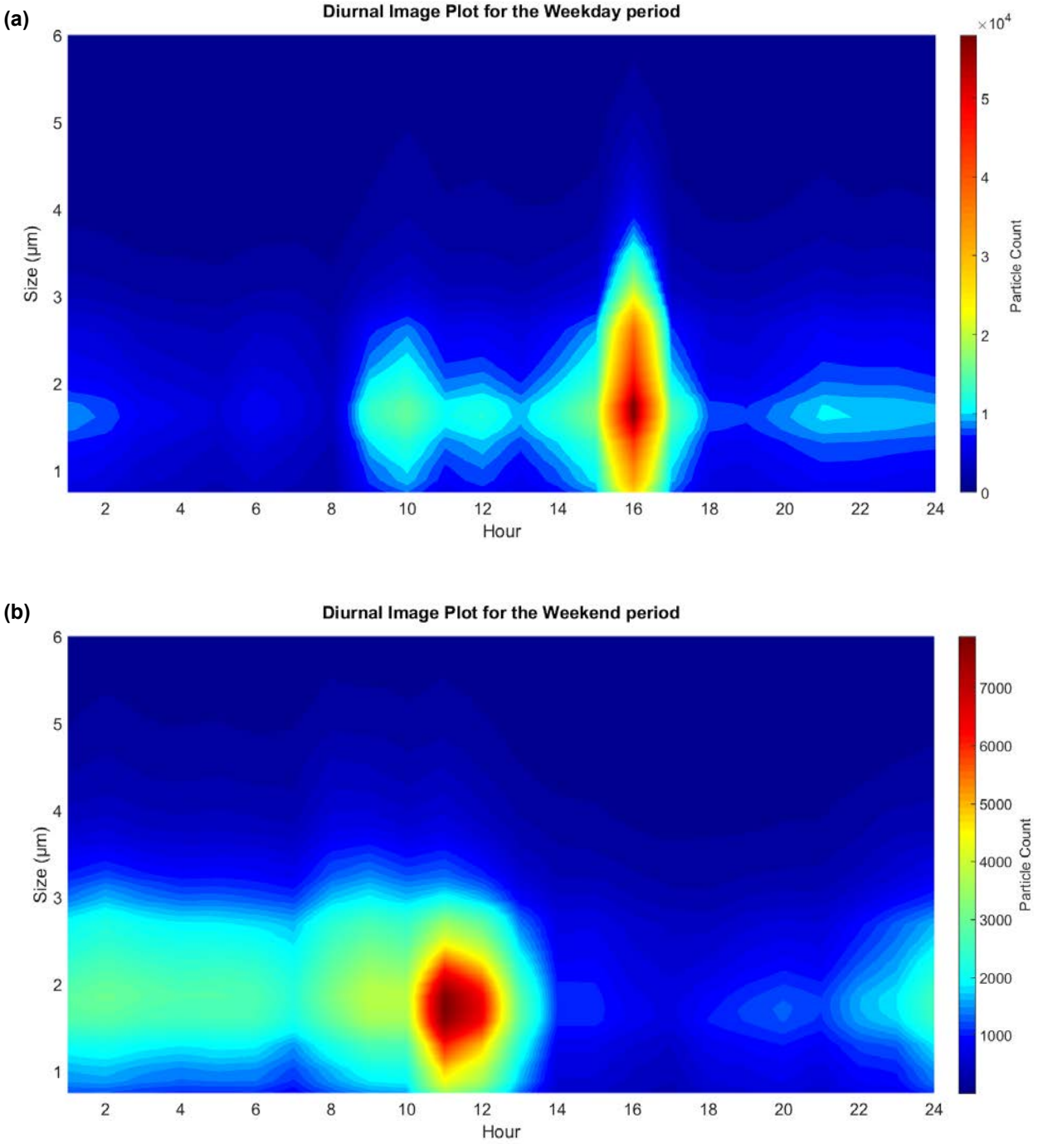



**Figure 2.10. (a) Diurnal image plot over the weekday period with size on the *y*-axis and particle count as the colour index. (b) Diurnal image plot over the weekend period with size on the *y*-axis and particle count as the colour index.**

3-µm range, which is a dominant size range when monitoring FAPs (Toprak and Schnaiter 2013; Gabey *et al.*, 2013; O'Connor *et al.*, 2015), with highest levels at ≈1.7 µm. An increased number of these particles was measured from 21:00 to 24:00, most prominently at 21:00. The image plot clearly demonstrates that activities at the site had a major impact on FAP concentrations when it was operational (08:30–17:00), especially in the *A. fumigatus* and mesophilic bacteria size ranges. These species have been well studied in many previous off-line campaigns and their presence forms the basis of site licensing activities in the UK and Ireland (van der Werf, 1996; Recer *et al.*, 2001; Sanchez-Monedero and Stentiford, 2003; Taha *et al.*, 2005).

Figure 2.10b illustrates that most of the higher accumulated concentrations at the weekend occurred from 00:00 to 13:00, as can be seen by the light-blue/green colour. It is important to note that the colour scales for Figures 2.10a and b represent different concentrations, i.e. Figure 2.10a represents ≈10× higher concentrations than Figure 2.10b.

At the weekend, the FAP concentrations were low during the hours between 14:00 and 22:00 and then increased from 23:00 onwards. Therefore, the higher concentrations of FAPs occurred during the night and morning and low measurements were recorded in the afternoon and evening. Most of the particles measured were in the 1- to 3-µm size range. A very large number concentration of FAPs was measured at 11:00 in the size range between 1.4 and 2.1 µm, as indicated by the deep-red/crimson colour. There was a stark difference between both FAP profiles as the weekday period is dominated by the activities of the compost site, leading to higher concentrations of FAPs, whereas the weekend appears to revert to a more rural profile in which fungal spores are mainly released at night (Toprak and Schnaiter, 2013; Crawford *et al.*, 2014).

Owing to the high time resolution of the WIBS it was possible to construct a profile of FAP trends according to meteorological conditions. The mobile weather station recorded parameters such as rainfall, temperature and humidity at 5-min intervals, which were then averaged to 1-hour resolution periods. The values were then plotted against the 1-hour particle bin data obtained with the WIBS-4A. The values for wind speed and wind direction were taken from the site weather station at 15-min intervals, which were also averaged into 1-hour intervals. Subsequently, diurnal weather/particle concentration profiles for the measurement campaign were constructed for the campaign period and are presented in Figure 2.11.

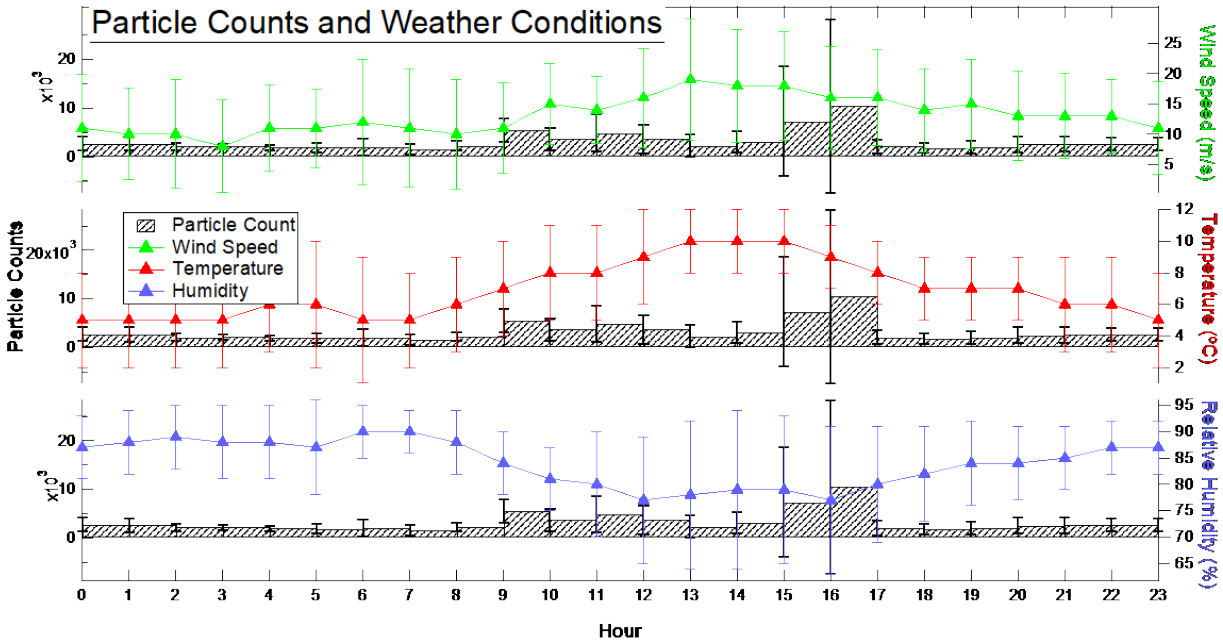



**Figure 2.11. Average diurnal profiles of FAP number concentrations plotted against the weather parameters of humidity (blue line), temperature (red line) and wind speed (green line) measured over the measurement campaign.**

Figure 2.11 shows that no striking relationship was recorded during this campaign. Instead, occupational activities (such as agitation), which are evident between 09:00 and 17:00, appear to overwhelm any effect that meteorological conditions may have had on FAP concentrations.

Figure 2.12a provides a profile of wind speed and direction for the duration of the campaign. These parameters are important as they determine the directions in which the bioaerosols are dispersed (Recer *et al.*, 2001) because many PBAPs have low settling velocities, e.g. *A. fumigatus* (0.03 cm $s^{-1}$) (Millner *et al.*, 1980). It has been previously found that wind speed caused major variations in bioaerosol/FAP detection at this particular compost site (O'Connor *et al.*, 2015). The prevalent winds in the March study were from the west-north-west, north-west and north-north-west with a large range of wind speeds. These conditions can be compared with those in the October campaign, in which the winds monitored during the working week were from the south and south-west, again with a large range of wind speeds.

Figure 2.12b illustrates that the highest concentrations of FAPs originated from the west-north-west and north-west. North-west was one of the three prevailing wind directions. The screening machine was west of the trailer and therefore many of the subsequent FAP emissions would have been carried by the north-west wind into the path of the WIBS-4A.

The other two prevailing wind directions originated from the north-west and north-north-west directions. Surprisingly, the concentrations associated with these two directions were relatively low, as can be seen from the colour key (low concentrations indicated in red). This observation is best explained by the location of the compost shed north-west of the WIBS because it would obstruct winds from blowing FAPs in the direction of the trailer. In summary, wind speeds and directions do play a part in moving FAPs around the site (Recer *et al.*, 2001; O'Connor *et al.*, 2015) but are less relevant when site activities happen.

The most important question remains: do the WIBS measurements track with the traditional impactor/ optical microscopy approach?

Figure 2.13 shows time-series graphs constructed to summarise the daily number concentrations recorded using the two approaches. Each hourly concentration was summed over the individual 24-hour periods.

Figure 2.13 shows consistent trends for the two contrasting methods of PBAP detection. Both FAP and spore measurements fluctuated over most of the 7-day period. Of particular note is that there was a large consistent difference in the FAP concentrations compared with the measured spore concentrations. In fact, there were ≈200× the number of FAPs measured by the WIBS approach than spores counted by optical microscopy, as shown by comparison between the primary and the secondary axes.

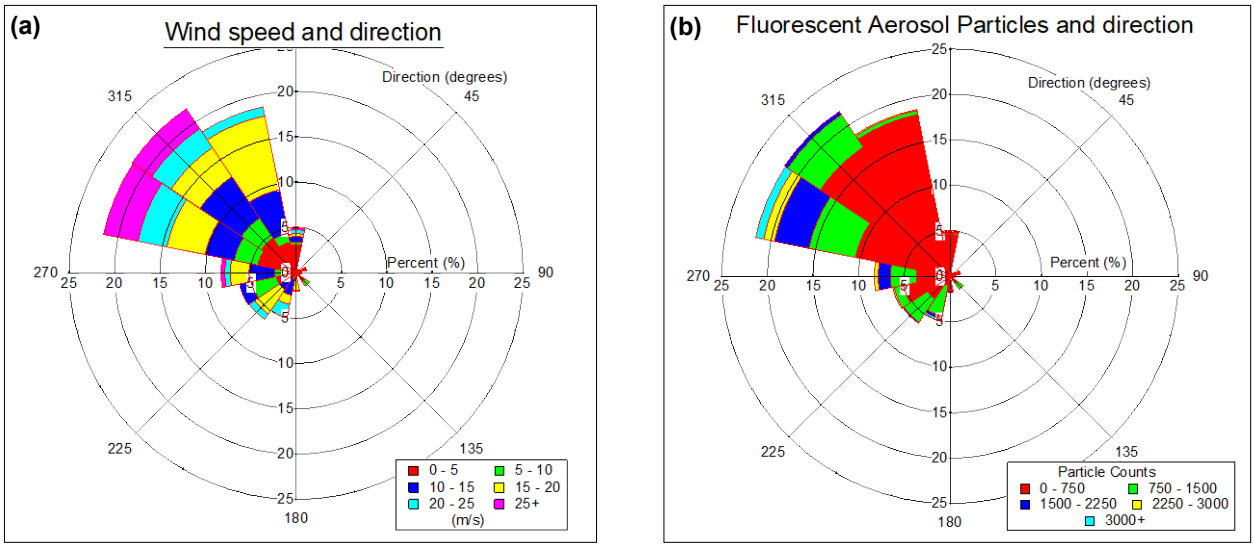



**Figure 2.12. (a) Wind rose diagram of wind speed and direction and (b) spore concentrations in terms of wind direction.**

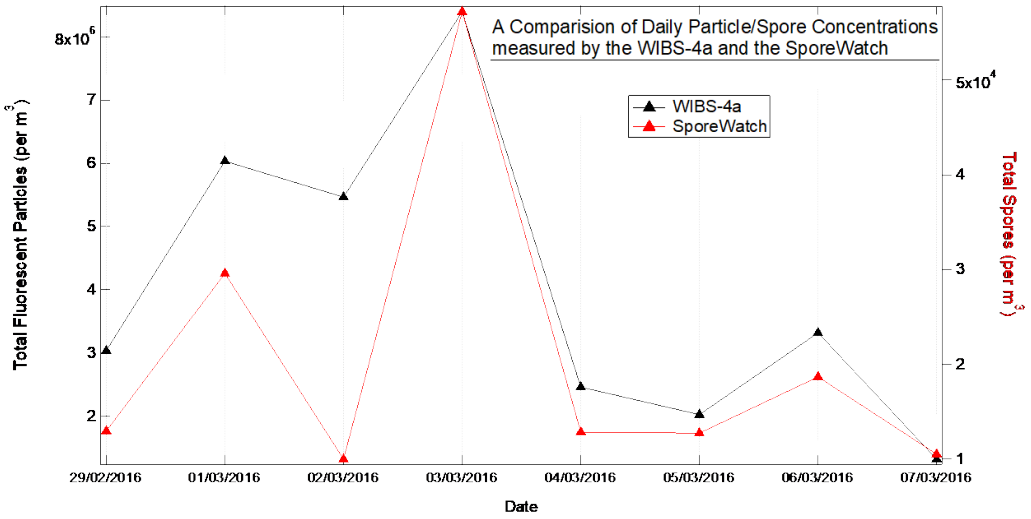



**Figure 2.13. A comparison between the daily particle/spore concentrations measured by the WIBS-4A and those measured by the SporeWatch.**

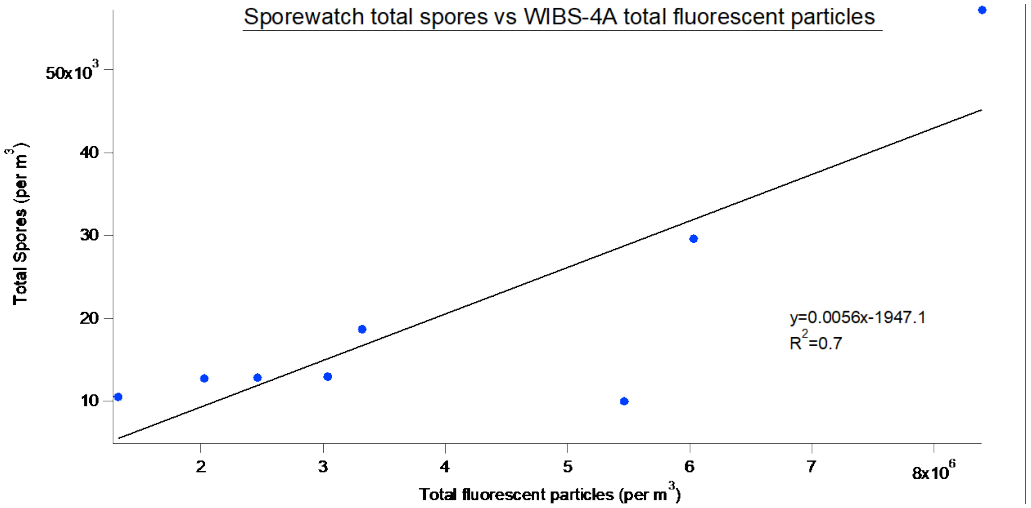



**Figure 2.14. Linear regression for SporeWatch and WIBS for the daily concentrations measured by each method during the campaign.**

In part, this important observation can be explained by consideration of the differing particle size regimes that the two techniques monitor. Thus, the WIBS can measure sizes down to ≈0.7 µm and therefore monitor bacteria (Hernandez *et al.*, 2016) present on the compost site. In contrast, owing to magnification limitations associated with many optical microscopes, the impactor method has a low efficiency for determining microorganisms of < 2 µm in size. This drawback makes it a poor method for measuring bacteria (Eduarda and Heederik 1998; Cartwright *et al.*, 2009).

As mentioned above, there are numerous potential fluorescent interferents that might be expected at a rural green-waste site, for example HULIS originating from soils or SOAs from vehicle activity. In principle, false positives can be monitored by WIBS (Poehlker *et al.*, 2012; Gabey *et al.*, 2013). However, it has previously been noted that the fluorescence quantum yields of such interferents are very low (Poehlker *et al.*, 2012) in comparison with the PBAP biofluorophores (Poehlker *et al.*, 2012; Healy *et al.*, 2012b; Gabey *et al.*, 2013).

To provide a more detailed statistical treatment of the counting by the two contrasting techniques, linear regression was applied to the data, as shown in Figure 2.14. Hence, a coefficient of determination, correlation coefficient and variance were calculated.

The resulting coefficient of determination ($R^2$ value) was 0.7. An important source of this variance was associated with the very low measurements made using the SporeWatch on Wednesday 2 March. This observation may be a result of the high wind speeds recorded on that day, with an average speed of 24 m s$^{-1}$, thereby interfering with the impaction efficiency of the SporeWatch. The correlation coefficient ($r$) was 0.84.

As discussed, an important spore counted on the compost site during this campaign was *U. maydis*, a smut fungus that is pathogenic to maize (Carlile *et al.*, 2001; Brefort *et al.*, 2009). It is therefore associated with the type of green waste delivered by brewers and distillers. High concentrations of it were recorded on 1, 3 and 4 March, as shown in Figure 2.15. Generally, its size ranges from ≈8 to 10 µm and the spore is spherical. In contrast to *A. fumigatus*, it is a type of PBAP that does not agglomerate into physical clusters. Hence, a size range filter of 8–12 µm was applied to the WIBS-4A fluorescence measurements.

There is a poor correlation between the WIBS and impaction results obtained on 1 March when *U. maydis* was recorded to be present over several hours. However, there was much on-site activity over that day as it was the first day of the working week. This interference possibility is in agreement with the fact that many fluorescent particles were recorded by WIBS-4A on that day.

In contrast, two excellent correlations between *U. maydis* optical microscopy counts and FAP counts were observed over much shorter time scales on 3 and 4 March at 16.00 and 09.00, respectively. The 4 March measurement corresponded with a green-waste delivery made by a local distillery. The source of the spore is therefore likely to be associated with wheat, barley and maize. This finding is important because it shows that the WIBS approach can provide an early warning signal for *U. maydis*, which is an important plant pathogen that can lead to severe crop losses. Monitoring of other occupational environments such as farms, breweries and distilleries may therefore prove instructive.

The advantage of co-locating the SporeWatch with the WIBS-4A was that the traditional impaction approach determined *U. maydis* to be present and the number counts tracked FAP counts over the appropriate size range. The former measurements took days to be analysed, whereas the WIBS-4A results were immediately available and with a much superior time resolution.

Figure 2.16a shows the real-time emissions of FAPs over a short (18 min) period on 3 March from 15:26 to 15:44. Figure 2.16b presents a shorter period (4 min) on 4 March from 09:06 to 09:10. This treatment again highlights the excellent time-resolution possibilities of WIBS (seconds) for real-time measurement and real-time reporting compared with impaction/microscopy, which has a 1-hour resolution and requires several days of analysis.

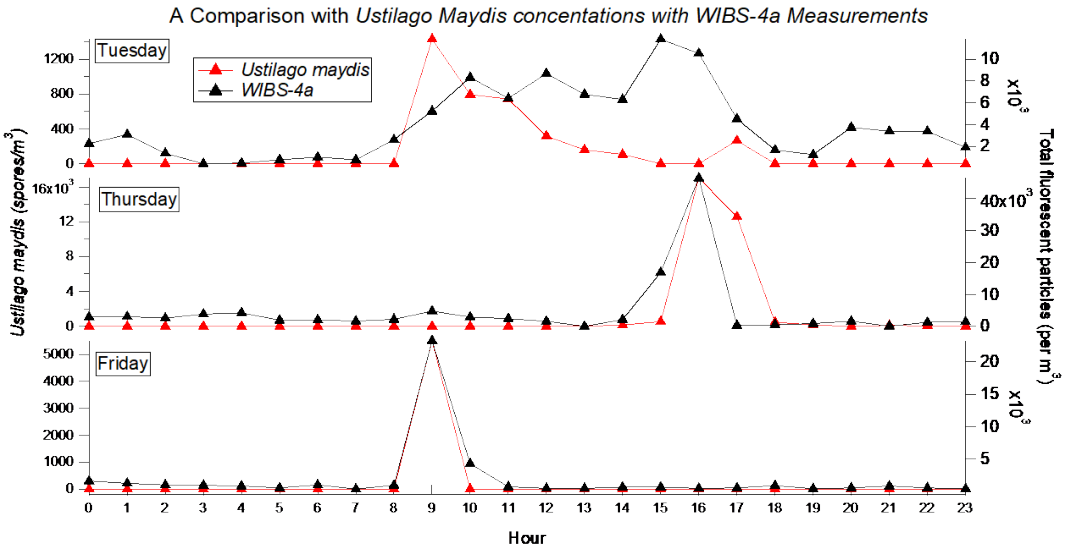



**Figure 2.15. Hourly concentration diurnal graphs for 1, 3 and 4 March.**

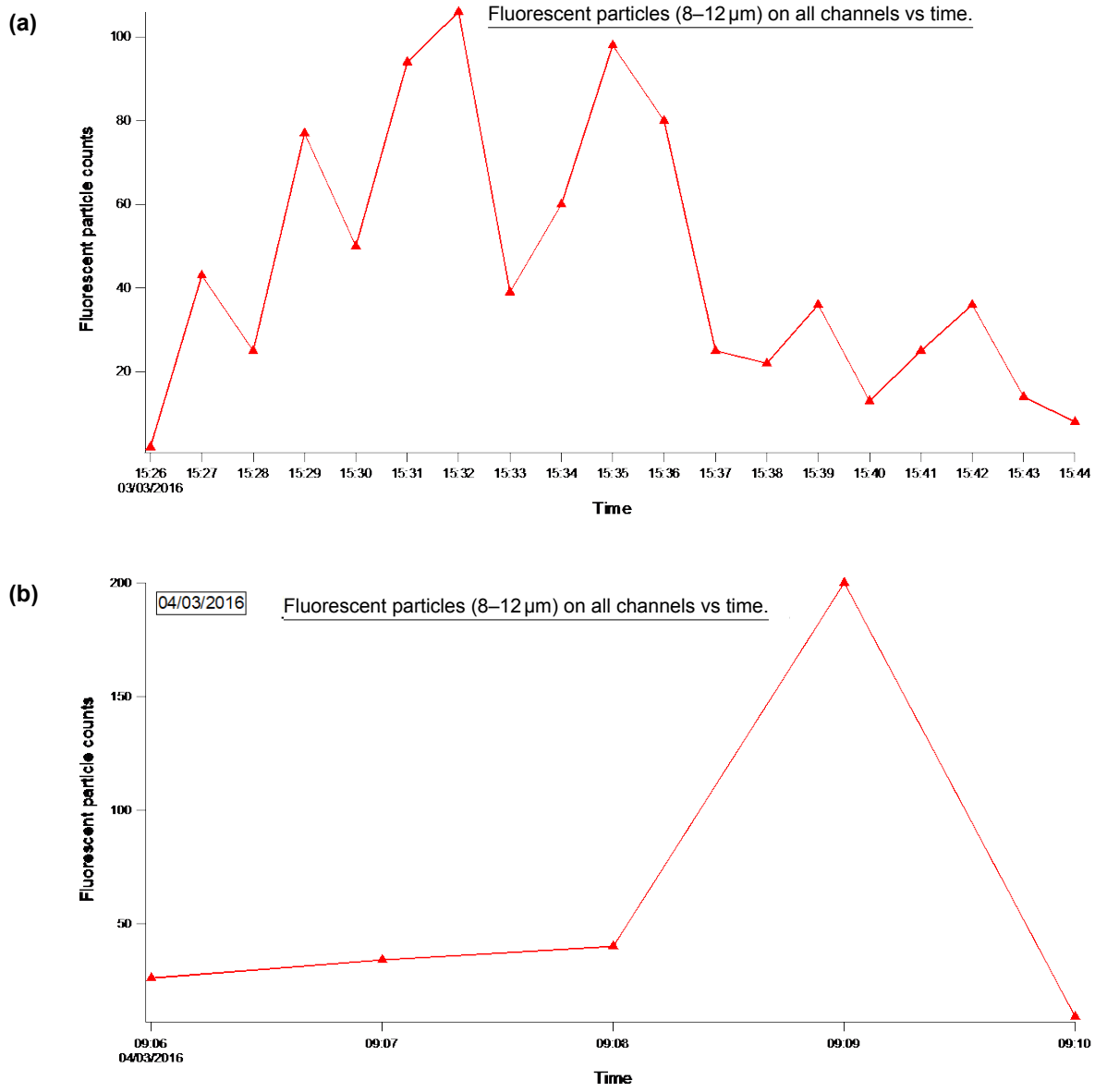



**Figure 2.16. (a) Time series of fluorescent particles vs time. The particles plotted are in the 8–12 µm size range over an 18-min period on 3 March. (b) Time series of fluorescent particles vs time. The particles plotted are in the 8–12 µm size range over a 4-min period on 4 March.**

The fluorescence trends of the *U. maydis* spores were investigated across the FL1, FL2 and FL3 channels. Their intensities are shown in Figure 2.17a and b for the time periods over which their emissions were highest, i.e. 3 and 4 March. The FL1 channel counts were dominant, with much higher intensities than for the FL2 and FL3 channels, as is clear from the different scalings required to illustrate the measurements. In fact, the FL2 and FL3 intensities tracked closely with each other. These fluorescence intensity trends, coupled with the sizing data, could be used to selectively distinguish *U. maydis* spores as a routine approach when they are suspected to be present. It has been found in previous fluorescence studies that most fungal and bacterial spores are dominant in the FL1/A channel (Hernadaz *et al*., 2016). Thus, it is unsurprising to see such a result, and the fluorescence signal is most likely caused by amino acids and proteins.

### *2.3.2 Comparison between the SporeWatch/ microscopy and WIBS methodologies*

The SporeWatch/optical microscopy approach to fungal spore and pollen counting and identification is discussed in section 1.3.

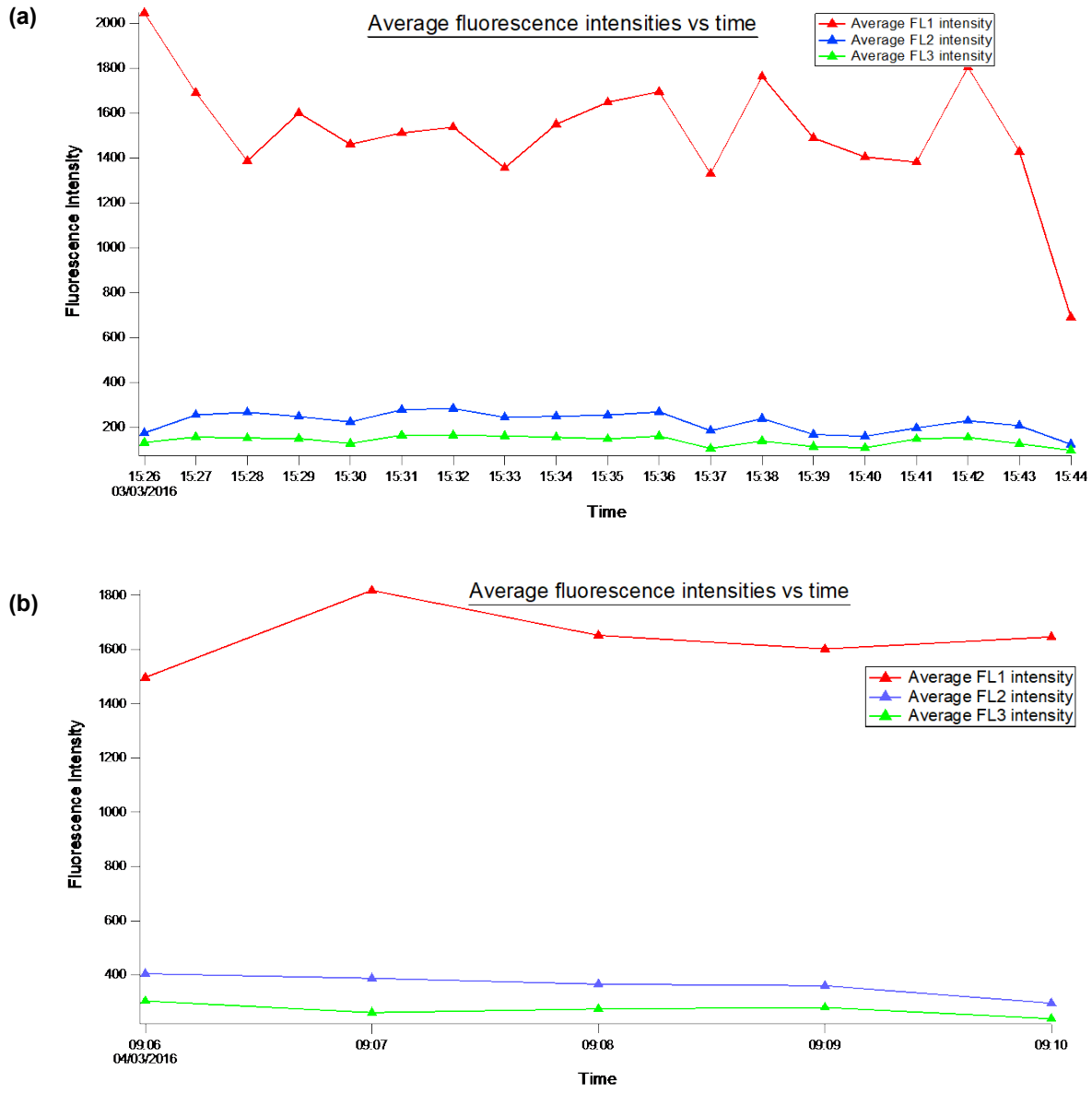



**Figure 2.17. (a) Fluorescence intensities vs time for the 18-min period on 3 March. (b) Fluorescence intensities vs time for the 4-min period on 4 March.**

There were somewhat higher number concentrations of spores recorded by impaction/optical microscopy on weekdays than at weekends at the green-waste site, with an average hourly concentration of 361 spores $m^{-3}$ on weekdays and 252 spores $m^{-3}$ at weekends. Looking at the averages, however, neglects the fact that sampled concentration patterns of the fungal spores were different for the two periods. During the weekdays the highest concentrations of spores occurred during the working hours of the compost site (08:30–17:00) and can be attributed to the disturbance of spores by necessary activities such as deliveries or turning, shredding and screening of compost (Millner *et al.*, 1980; van der Werf, 1996; Recer *et al.*, 2001; Gillum and Levetin, 2008; O'Gorman, 2011). At the weekend, fewer numbers were recorded.

From the results, the WIBS-4A instrumentation has been shown to be capable of providing a far superior time resolution for detecting on-site FAPs (and therefore potential PBAPs) than the traditionally employed off-site technique, Andersen sampling, and also the SporeWatch/microscopy approach. The fluorescence spectroscopy measurements were able to provide continual data with a 1-second time resolution, in contrast to the two other techniques that provide only "snapshots" of bioaerosol releases. The WIBS, real-time, on-site approach should be of

particular interest and assistance when investigating specific events that may occur from time to time when producing compost not only from commercial green-waste sites but also from domestic composting. The PBAP events recorded here would simply not be picked up as a function of time by use of Andersen sampling. An example here is the measurement of high levels of FAPs recorded on 3 March at 15:26–15:44, as shown in Figure 2.16a.

Although short-term deployments of real-time instrumentation may be most useful to licensing authorities and site operators, the OLBAS study shows that it would be possible to leave a WIBS-4 operating full-time at any commercially operated compost site or occupational environment for an indefinite period of time using remote off-site computer control, which would enable more complete descriptive temporal profiles of spore release to be provided even when the facility is closed. Furthermore, the technique readily provides a more accurate profile of all bioaerosols (as well as non-fluorescent particles) as a function of normal working activities.

A total FAP loading to determine whether or not a composting license should be issued might prove useful, especially if it is activity dependent. Although not strictly equivalent to the number of *A. fumigatus* CFU counts that currently underpin regulations in the UK and Ireland, it could be argued that all $PM_{2.5}$, particularly examples that are fluorescent in nature, are likely to lead to adverse health effects. Perhaps the most powerful tool to advise authorities at this stage would be to co-deploy the traditional and modern instrumentation. Although, currently, the cost of deploying real-time monitoring devices is high, it may prove to be an acceptable cost if future legislation requires site operators to provide relevant information about PM levels to their staff, visitors and local residents. Such developments will need to be driven by the licensing authorities themselves.

The results specific to *U. maydis* indicate that it is possible to propose a future measurement campaign near an agricultural environment in which maize is grown. There is a strong potential to distinguish *U. maydis* in real time by applying size filters and investigating WIBS fluorescence channel ratios. As the spore is pathogenic to this crop it would be of importance to maize producers to determine spore concentrations in the ambient air around the crop. The measurements could lead to an indication of crop contamination by this fungus (Feeney, 2016; Feeney *et al.*, 2018).

Finally, it is worth noting that the OLBAS study suggests that the WIBS methodology could be used as a research tool to inform and improve standard site sampling regime designs. Of course, a limitation of the WIBS as a regulatory instrument is that it measures all organisms alive, dead and culturable; in contrast, current regulatory instruments are based on counting viable organisms only.

The field campaigns described in sections 2.2 and 2.3 led to two further avenues for the real-time methodology to be deployed in the OLBAS study of green-waste management sites: (1) to develop a strategy to identify and distinguish between airborne PBAPs outdoors using MBS and (2) to investigate on-site, indoor levels of airborne PBAPs present in the staff cabin.

## 2.4 Deployment of the Multi-parameter Bioaerosol Spectrometer

Wideband integrated bioaerosol sensor technology provides three channels of fluorescence intensity data (FL1, FL2 and FL3) covering just two different wavelength ranges between 310 and 400 nm and 420 and 650 nm after excitation by two xenon flashlamps rapidly firing at 280 nm and 370 nm. Although the technique provides good high time-resolution FAP/PBAP count data, it is much more difficult to identify airborne fluorescent species in a multi-component mixture because of its low spectral resolution.

The MBS was therefore developed by Professor Paul Kaye and his team at the University of Hertfordshire. It provides a more sophisticated LIF approach to identifying FAPs/PBAPs than WIBS by means of an eight-channel detector; hence, spectral distributions for individual particles are measurable. Large amounts of data are generated in this process and therefore statistical clustering analysis or machine-learning algorithms are required to provide meaningful identifications.

Only one paper has been published on the use of the MBS in the laboratory, to distinguish between PBAPs such as puffball spores and aspen pollen (Ruske *et al.*, 2017). No previous studies have been reported

that have used the MBS deployed in the field and therefore the OLBAS campaign, which was carried out at a green-waste management site, is unique. Initially, control experiments using pure samples of fungal spores expected to be present were tested in the laboratory. Specific attention was paid to *Aspergillus* spp. (2–3.5 µm) and *Penicillium* (3–5 µm), which are not separable using traditional impaction/optical microscopy methods of identification because of their similar small sizes and morphologies.

### *2.4.1 Laboratory studies*

A full description of the operation of the MBS used in this study and associated data analysis used is available (Quirke, 2016). However, in summary, the optical chamber comprises two lasers. The first, a high-power pulsed laser with an output at 637 nm, is coupled with a second low-power continuous wave laser operating at 635 nm. Initial detection is made by the low-power laser, which sizes individual particles. Firing of the high-powered (637 nm) pulsed laser follows as the particle makes its way through the sensing volume of the optical chamber and gives an indication of particle shape. Subsequent electronic excitation of the particle is provided by a xenon flash source similar to the one found in the conventional WIBS instrument; its output is tuned at 280 nm. However, in contrast to the three channels of the WIBS, the MBS photomultiplier tube detection wavelengths are split into eight channels ranging from 300 to 655 nm in ≈40-nm segments.

The spectral distributions between 300 and 615 nm of three types of spores, *A. fumigatus*, *Aspergillus niger* and *Penicillium notatum*, were obtained in the laboratory study. Initially, they were investigated individually and then as spore mixtures. The individual spores aerosolised with differing efficiencies. For example, *A. fumigatus* gave rise to about twice as many particles as *A. niger* and about three times as many particles as *P. notatum*. Therefore, in studies of mixtures, *A. fumigatus* would be expected to dominate population numbers but not necessarily the fluorescence intensities.

The sampling set-up was based on one previously employed for WIBS studies (Healy *et al.*, 2012a). A purified air feed was passed through a flowmeter into a 10-L Nalgene chamber. The flowmeter played an important role in the process as the amount of sample (spores + air) introduced into the chamber had to be close to the rate at which it was removed by the MBS (1.12 L $min^{-1}$). The flow was kept constant throughout the experiments, although some fungal spores were harder to nebulise than others. High-efficiency particulate air (HEPA) filters were used to ensure that any fungal spores that were not sampled were filtered out and therefore posed no threat to the air quality inside the laboratory. A standard operating procedure was carried out for the experiment detailing the amount of sample to be used, duration of agitation and initial mixing period. A detailed process for cleaning the chamber out between spore samples was also followed.

The raw fluorescence data obtained for the individual particles in the laboratory were transferred into an IBM SPSS statistics (version 20) package for analysis. Outliers (extreme observations) were removed from the datasets using both univariate and multivariate techniques by application of MATLAB. Initial outlier removal was necessary to allow the most accurate determination of ‘k’, which is the number of clusters to be applied for K-means clustering. Then, all data could be sorted into appropriate functions of fluorescence channel, size, shape and time. The output was a series of histograms (and boxplots) that allowed the fungal spores to be distinguished from each other. The results were compared with the K-mean results generated from the SPSS statistical program.

All three fungal spores, introduced separately into the sampling chamber, gave rise to three statistical groupings termed as clusters. One cluster in each case was clearly dominant and comprised 60–75% of the fluorescence particles. Figures 2.18–2.20 show the spectral distributions obtained for the dominant cluster of each fungal spore. The minor clusters gave identical spectral patterns to the dominant cluster in all three cases. Each channel, from (XE)1 to (XE)7, shown in these figures corresponds to 35- to 45-nm “bands” between 300 nm and 615 nm.

The clearest difference in the distributions is between the *Aspergillus* species, as shown in Figures 2.18 and 2.19. Hence, in the spectral region between 390 nm and 485 nm and designated XE1_3 (390–435 nm) and XE1_4 (440–485 nm), there are contrasting fluorescence intensities for *A. niger* versus *A. fumigatus*. On the other hand, the *P. notatum* and *A. fumigatus* distributions strongly resemble each

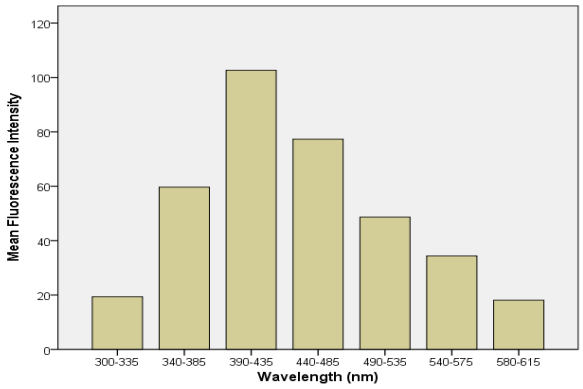



**Figure 2.18. Spectral fluorescence distribution of the dominant cluster for the spore *A. fumigatus.***

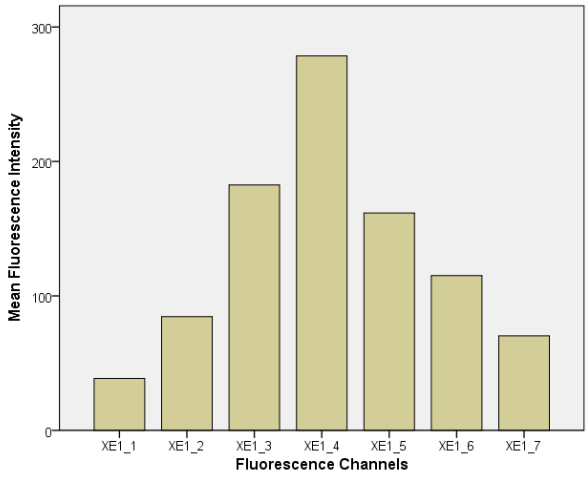



**Figure 2.19. Spectral fluorescence distribution of the dominant cluster for the spore *A. niger.***

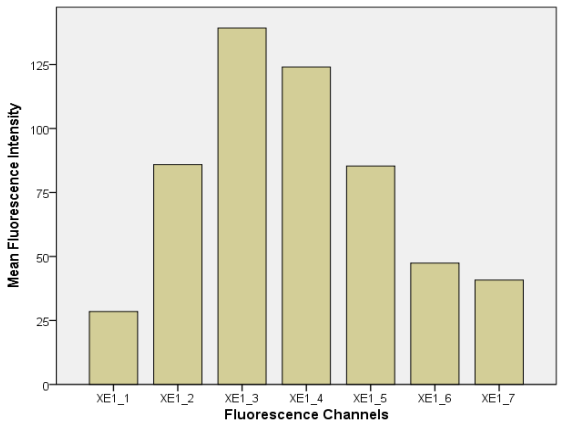



**Figure 2.20. Spectral fluorescence distribution of the dominant cluster for the spore *P. notatum.***

other, although are not identical. The dominance of the longer wavelength range XE1_4 in the spectral distribution of *A. niger* could be linked to its dark colour resulting from the presence of eumelanin,

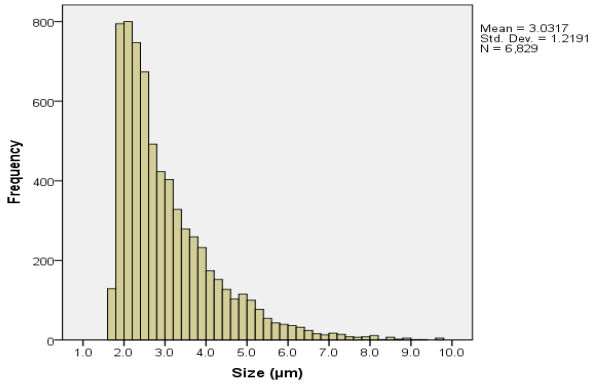



**Figure 2.21. Size histogram for the dominant *A. fumigatus* cluster.**

which photo-protects certain spores (although not *A. fumigatus* or *P. notatum).*

In contrast to the fluorescence intensity measurements for *A. fumigatus*, its three statistical groupings show quite different physical size distributions. The dominant statistical cluster, accounting for 72% of the total fluorescent particles, exhibits a size distribution that includes a large proportion in the 2- to 3-µm range, with a maximum population at ≈2.2 µm and a mean size for the full distribution of ≈3 µm. The two other clusters, which accounted for 22% and 6% of the total fluorescent population, gave much larger mean size values, 4.7 and 7.5 µm, respectively, and are likely to be caused by two main agglomerations, possibly duos and trios, because all three statistical clusters gave identical fluorescence distributions. The results for the dominant statistical cluster are shown in Figure 2.21.

It is of note that the size of individual *A. fumigatus* spores, as measured by optical microscopy, is between 2.0 and 3.5 µm (Latgé, 1999).

It is known that *A. fumigatus* mainly, although not entirely, disperses as single spores and the results found here are in full agreement with that idea (Afanou *et al.*, 2015). The fact that about one-quarter of the total spores appeared to clump together as agglomerated physical clusters may be a result of the introduction system for the spores used in the laboratory study. Of course, it might also reflect the natural dispersion process.

*Aspergillus niger* exhibited similar sizing behaviour to *A. fumigatus*, with sizes for the dominant (61%) cluster recorded to be mainly in the range from 2 to 3.5 µm and a maximum population at ≈2.2 µm. However, in

contrast to *A. fumigatus*, the range 3.5–6.5 µm also contained a substantial contribution, which leads to a skew in the mean size to 3.7 µm.

The two minor clusters for *A. niger* represented 26% and 13% of the fluorescent population and were associated with mean sizes of 5.9 µm and 8.5 µm, respectively.

Similar behaviour was observed for *P. notatum*, with the three clusters representing 67%, 25% and 8% of the fluorescent population and exhibiting similar, but not the same, sizing behaviour to that of *A. fumigatus.* For the dominant cluster the sizes were mainly in the range from 2 to 4.5 µm, with a maximum population at ≈2.5 µm and mean size of 3.3 µm, as shown in Figure 2.22.

These values are all slightly larger than that found for the dominant cluster of *A. fumigatus*, as expected from optical microscopy studies. In addition, in contrast to *A. fumigatus*, but similar to *A. niger*, the size range 3.5–6.5 µm contained a substantial contribution. The two minor clusters were associated with mean sizes of 5.6 µm and 8.4 µm.

In summary, the dominant clusters for *A. fumigatus*, *A. niger* and *P. notatum* gave maximum size populations of 2.2 µm, 2.2 µm and 2.5 µm, respectively. The mean sizes of their minor clusters were 4.7 µm and 7.5 µm for *A. fumigatus*, 5.9 µm and 8.5 µm for *A. niger* and 5.6 µm and 8.4 µm for *P. notatum*.

Therefore, a propensity to physically agglomerate was found for all three fungal spores studied using the MBS. Nonetheless, the dominant statistical grouping for each could be identified using airborne individual spores. These results indicate that unlike optical microscopy the possibility exists that airborne mixtures of *A. fumigatus* and *P. notatum*, along with their agglomerates, could be distinguished by size using an MBS. *A. niger* is readily distinguished from the other two by its fluorescence spectral distribution.

Laboratory experiments were then performed using the MBS to determine its capabilities for distinguishing between mixed samples of the fungal spores detailed above. The experiment was set up in exactly the same way as for individual testing. Different combinations of *A. fumigatus*, *A. niger* and *P. notatum* were introduced into the mixing chamber and sampled. Analysis of the results using statistical treatments was again performed in order to separate their characteristic fluorescence and sizing distributions from each other.

### *Aspergillus fumigatus and Aspergillus niger*

This mixture resulted in three clusters. One was dominant representing ≈70% of the total, with a mean size of 3.3 µm. The most minor of the contributions (mean size 8 µm) represented 10% of the population. The third cluster accounted for 20% of the total (mean size 5.5 µm).

Figure 2.23 shows the spectral distributions observed for the three clusters. The most populated cluster is highlighted in blue but it displays the weakest fluorescence intensity values, with XE1_4

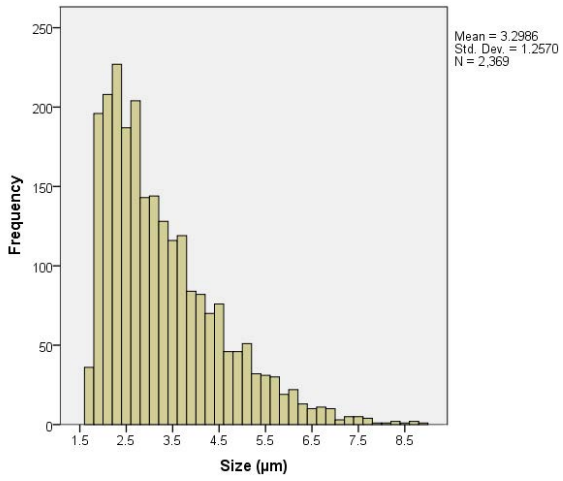



**Figure 2.22. Size histogram for the dominant *P. notatum* cluster.**

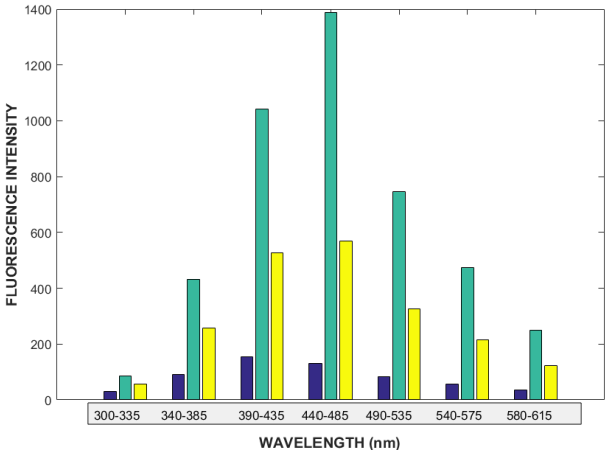



**Figure 2.23. Spectral distributions of *A. fumigatus* and *A. niger* mixtures (green, least populated cluster; yellow, second most populated cluster; blue, dominant cluster).**

(440–485 nm) being the dominant channel, as associated with *A. fumigatus*.

The least populated cluster (10%) is represented in green in Figure 2.23. It produced the most intense mean fluorescence values of all the spectral distributions. It closely resembled the MBS fluorescence pattern found for *A. niger* spores, with XE1_4 dominant. The intensities are seen to be almost double those measured for the second most populated cluster (20%, highlighted in yellow) and a magnitude stronger than the dominant cluster (70%, highlighted in blue).

Both the green and yellow spectral distributions showing high fluorescence intensities were clearly associated with *A. niger* because of the dominant XE1_4 channel. However, this channel is much more dominant in the green distribution than in the yellow distribution. Their mean sizes are different too, but both are much larger than the statistical cluster attributable to *A. fumigatus* (highlighted blue). From the individual spore results there is an expectation that agglomeration would occur. It would appear that the green distribution comprises trio agglomerates mainly containing *A. niger*, whereas the yellow distribution represents duo mixtures of the two *Aspergillus* types. The fact that the two clusters exhibit greater fluorescence intensities than the individual spores is explained by the fact that larger PBAPs contain more biofluorophores. The large population of *A. fumigatus* is related to it being much more efficiently aerosolised (×2) than *A. niger*.

*Aspergillus fumigatus and Penicillium notatum*

This mixture led to a best K-means fit of four clusters, which are highlighted purple, blue, green and yellow in Figure 2.24.

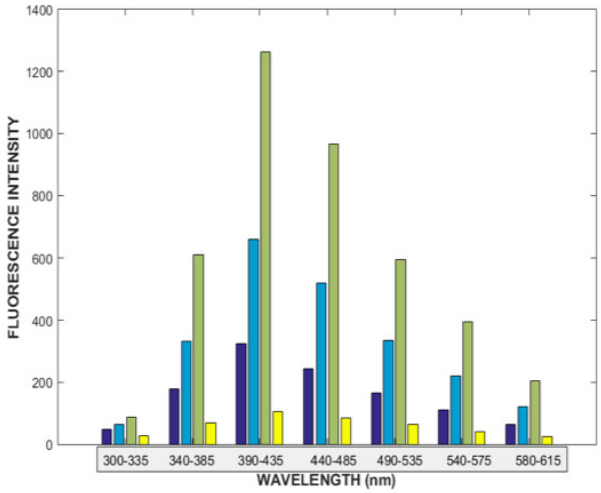



**Figure 2.24. Spectral distributions of *A. fumigatus* and *P. notatum* mixtures.**

A total of ≈3800 particles were deemed fluorescent and the K-means statistical treatment gave rise to the four clusters, with population and sizing data summarised in Table 2.3.

The spectral distributions of all four clusters closely resembled each other as expected from the results found for the individual spore measurements. However, it is possible to assign cluster 2 (yellow) to *A. fumigatus* and cluster 4 (purple) to *P. notatum* for the following reasons: (1) cluster 2 has over double the population of cluster 4, in agreement with the relative efficiency of aerosolisation of the two spores; (2) cluster 2 has a population that is smaller than that of cluster 4; (3) the fluorescence intensity of cluster 2 is smaller than that of cluster 4, in line with its smaller size and therefore fewer biofluorophores.

Cluster 1 likely represents duo agglomerates of the two spores, whereas cluster 3 shows a larger size range in line with trio agglomerates.

**Table 2.3. Clusters, populations and size distributions for airborne mixtures of *A. fumigatus* and *P. notatum***

| Cluster | Population | Percentage contribution | Most populated size bin (µm) | Size range (µm) |
|---|---|---|---|---|
| 1 (Blue) | 430 | 11 | ≈6 | 5–7 |
| 2 (Yellow) | 2237 | 59 | 2.2 | 2–3.5 |
| 3 (Green) | 202 | 5 | ≈8 | 7–9 |
| 4 (Purple) | 938 | 25 | 3.0 | 2–6 |

### 2.4.2 Outdoor monitoring campaign

A monitoring campaign for the detection of airborne fungal spores was performed at a commercial green-waste/composting site from 26 February to 7 March 2016, as described in section 2.3. The MBS was located in a housing next to the SporeWatch impactor for the 4-day period between 3 and 7 March. The area chosen for deployment was close to the biofilter at the north end of the facility, as shown in Figure 2.1. The main aim of this study was to relate the laboratory characterisation experiments carried out on *A. fumigatus*, *A. niger* and *P. notatum* to an occupational environment where these spores are known to be released into the air.

Fluorescence intensity spectral distributions were recorded and the statistical treatment described in section 2.4.1 was then applied to the resulting ambient air dataset.

The SporeWatch sampling drum and tape were sent off-site after the 4 days and the impacted particles were counted and identified by optical microscopy. The three spores, *A. fumigatus*, *A. niger* and *P. notatum*, were categorised, as is generally the case, in a grouping termed *Aspergillus–Penicillium* because they cannot be distinguished from each other by use of this traditional method of collection and analysis. Time periods when *Aspergillus–Penicillium* spores were counted with high number concentrations were then compared specifically with the corresponding MBS fluorescence data.

Figure 2.25 shows seven clear events over the campaign when the *Aspergillus–Penicillium* grouping was detected at "high" concentrations. This condition was defined as a contribution of at least 60% of the total number concentrations of spores.

The five major peaks were all analysed by K-means cluster analysis with the following criteria set: (1) > 60% *Aspergillus–Penicillium* and (2) > 1000 spores $m^{-3}$. Two of the peaks were selected for detailed statistical study: (1) 5 March 2016 between 01.00 and 02.00 and (2) 6 March 2016 between 22.00 and 23.00. These are illustrated in Figure 2.25 as the first and last peaks, representing the start and end of the campaign. The largest peak probably comprised many contributing types of bioaerosol because its statistical analysis proved inconclusive in terms of clustering. The two chosen peaks, 5 March 2016 between 01.00 and 02.00 and 6 March 2016 between 22.00 and 23.00, gave rise to concentrations from the SporeWatch/optical microscopy analysis of 1485 and 1326 total spores $m^{-3}$, respectively. The events at these times showed good correlations between the spores of interest and the total MBS counts, as shown in Table 2.4.

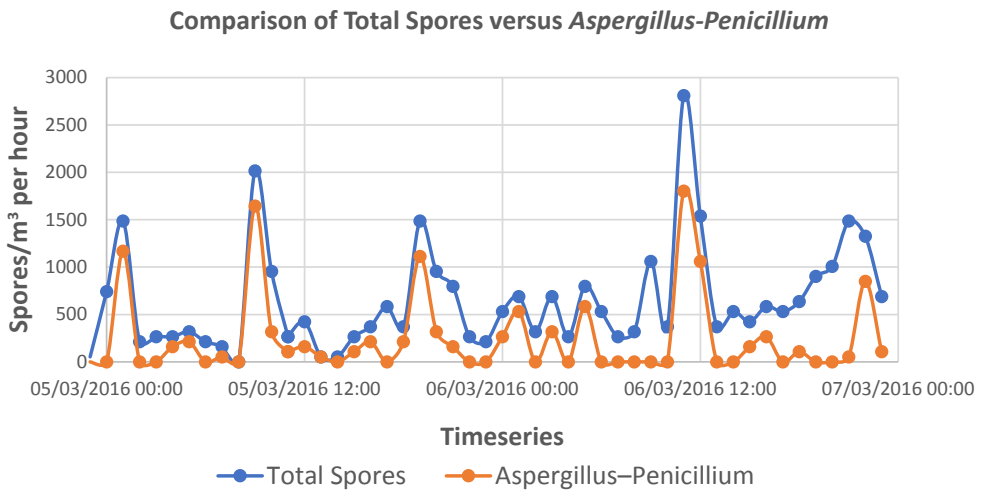



**Figure 2.25. Scatter chart comparing total spores with *Aspergillus–Penicillium* spores from 5 to 7 March.**

**Table 2.4. The two *Aspergillus–Penicillium* release events used for MBS correlation**

| Time | Total spores $m^{-3}$ | *Aspergillus–Penicillium* spores $m^{-3}$ |
|---|---|---|
| 5 March 2016, 01:00–02:00 | 1485.12 | 1113.84 (75%) |
| 6 March 2016, 22:00–23:00 | 1326 | 848 (64%) |

The MBS samples at a much lower rate than the SporeWatch (≈1.12 L min$^{-1}$ vs 10 L min$^{-1}$) and MBS data are collected in a time resolution of seconds rather than hourly, as for SporeWatch impaction. The MBS recorded over 500,000 particles for the 4-day period with the counts per second rate maintained at ≈3 s$^{-1}$. Individual counts recorded on the MBS do not necessarily represent FAPs. Rather, to determine which particles can be designated as FAPs and therefore possibly fungal spores requires sizing criteria as well as threshold fluorescence filtering. In this study, all particles of < 2 µm were discarded from the subsequent data analysis as they cannot represent members of the *Aspergillus–Penicillium* grouping. A number of treatment approaches were then attempted on the resulting ensemble for the statistical analysis. These included approaches based on forced trigger values, fluorescence averages or individual channel filtering, but they all removed almost all particles from the datasets. It was found eventually that the process of determining *total* fluorescence thresholds for the particles proved the best way to give good statistical fits, while not eliminating most data points.

*5 March 01:00–02:00 time period*

Ten percent of the total number of particles detected were deemed to be fluorescent, corresponding to ≈400 counts. Two clusters were determined from the K-means clustering approach. The dominant grouping accounted for 95% of the population and exhibited a much higher fluorescence intensity than the minor cluster.

Figure 2.26 gives the fluorescent spectral distribution produced by the dominant statistical cluster. Although it does not exactly reproduce any of the distributions obtained for the "pure" spores, a clear dominance of the XE1_4 channel is apparent. This behaviour was linked to the presence of *A. niger* in the laboratory studies. It is possible that this spectral distribution results from the 25% of spores not attributed to *Aspergillus–Penicillium*, for example *C. cladosporioides* spores, as shown in Figure 2.27, which were not investigated in the laboratory study. This idea of a different type of spore contribution is supported by the size histogram generated for the dominant cluster, as shown in Figure 2.27, which is different from any of those found for the individual spores studied.

Histogram peaks were observed at ≈2.2 µm and ≈2.9 µm, with the full distribution ranging between 2 and 3.5 µm. This profile indicates that no agglomeration is occurring.

*6 March 22:00–23:00*

Only 466 particles were deemed to fluoresce with sizes of > 2 µm in this hour-long period using the same threshold criteria employed in the first period. Three clusters resulted from K-means treatment with two of them each contributing about 50% to the population.

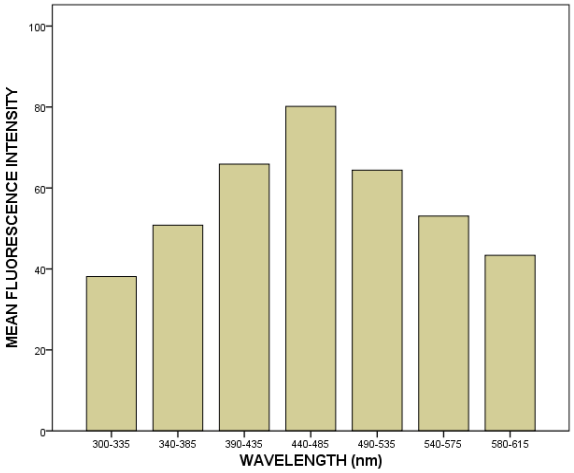



**Figure 2.26. Dominant cluster from the K-means analysis of 5 March.**

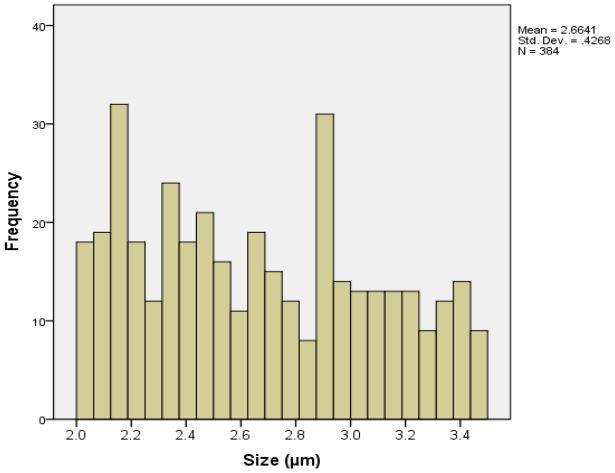



**Figure 2.27. Size histogram for the dominant cluster of 5 March.**

Figure 2.28 shows the spectral distribution of the first of these two clusters, with the XE1_3 channel being the most highly populated. Its adjacent channels (XE1_2 and XE1_4) displayed ratios between them that were similar to those observed for the dominant clusters of *A. fumigatus* and *P. notatum* in the laboratory studies, as shown in Figures 2.18 and 2.20. The mean fluorescence intensity reached a limit of about 300.

This cluster spectral distribution does not resemble *A. niger.*

The size profile for this cluster is shown in Figure 2.29. It clearly indicates a major particle population of size between 2 and 4 µm with very low counts above this limit (up to about 10 µm). Furthermore, the most populated size bin is 2.2 µm.

The second cluster, also accounting for about 50% of the total fluorescing particles, gave rise to the spectral distribution shown in Figure 2.30.

The dominant channel is again XE1_3 (390–435 nm). However, in contrast to the first cluster, XE1_2 (340–385 nm) rather than XE1_4 (440–485 nm) is the second most intense channel, representing a shift in the fluorescence spectral distribution to shorter wavelengths. Furthermore, much larger mean fluorescence intensities (> 1000) were measured than in the first cluster, with the measured intensity values being similar to those found for the agglomerated *Aspergillus* or *Penicillium* spores observed in the laboratory study.

Figure 2.31 shows the size profile of the particles associated with the second cluster. It indicates a wide

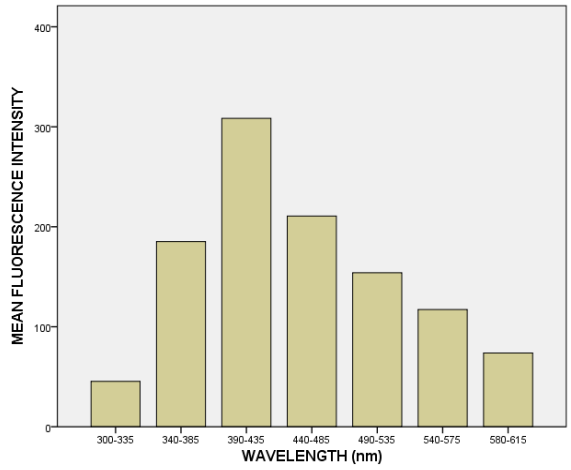



**Figure 2.28. Spectral distribution of the first cluster from the K-means analysis of 6 March.**

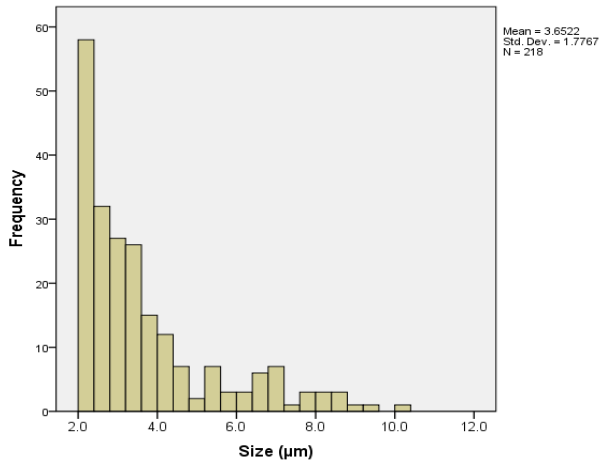



**Figure 2.29. Size histogram for the first cluster of 6 March.**

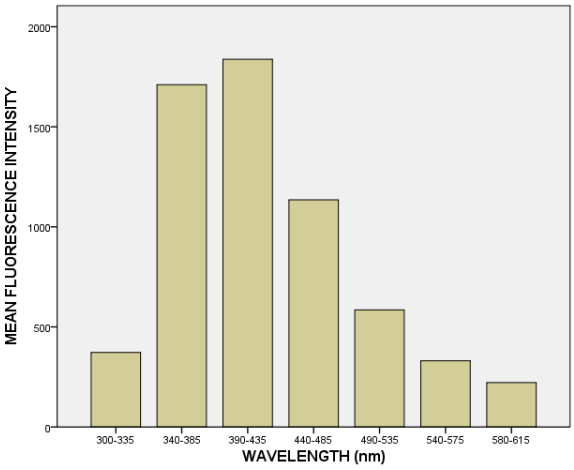



**Figure 2.30. Spectral distribution of the second cluster from the K-means analysis of 6 March.**

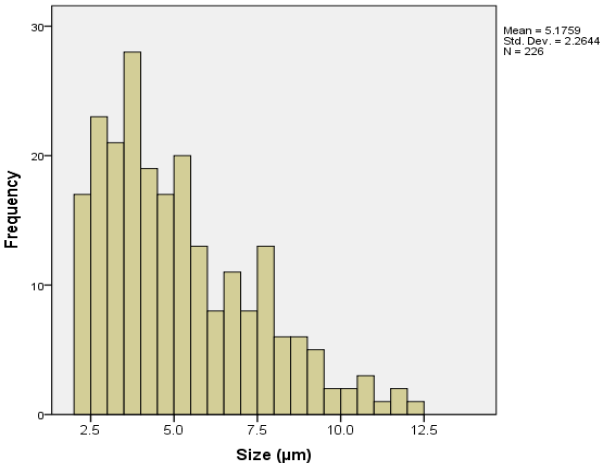



**Figure 2.31. Size histogram for the second cluster of 6 March.**

range of particles with sizes between 2 and 12.5 µm. Major features at ≈3 µm, ≈5 µm and ≈7.5 µm were observable, which is in line with the agglomeration results for *A. fumigatus*, *A. niger* and *P. notatum* found in the laboratory study.

The statistical cluster indicates that physical agglomerations were present, although other spores or large bacteria not tested for in the OLBAS MBS laboratory experiments are also likely there. These studies are unique; in particular, the laboratory experiments provide a basis for developing a full database of MBS spectral profiles for fungal spores that may be encountered in the field. It could therefore act, in due course, as a method for identifying them in real time. Such a development would, of course, be of great importance to licensing authorities.

## 2.5 Distinguishing Fungal Spores Using the MBS

The study described in section 2.4 represents the first trial employing an MBS to detect airborne biological particles relevant to a green-waste management facility.

The laboratory results show great promise for the MBS approach to distinguish between spores that cannot be separated using optical microscopy. Particular emphasis was placed on *A. fumigatus* and *P. notatum* in this regard. The statistical clustering analysis approach was also able to isolate the fluorescence spectral distribution of *A. niger* from that of the fungal spores tested. Finally, differences between individual spores and their duo or trio agglomerates proved possible.

Instructive results were also obtained when the MBS was co-located with the particle impaction-based detection technique termed the SporeWatch. Good correlation was found between fungal spores counted and identified by the SporeWatch and particle counts measured by the MBS.

In fact, 22 different spore types were counted over the full campaign. However, not all of their MBS characteristics could be tested in the laboratory because of time and financial constraints. Instead, the project provided a strong indication that spores with similar morphology could be separated using fluorescence detection, sizing and statistical clustering analysis in the laboratory at the very least.

The range of skills required to acquire and interpret MBS results obtained in outdoor field campaigns is extensive. Furthermore, the instrumentation is not commercially available as yet. Nonetheless, the OLBAS studies do provide a proof of principle that the technique can be used in the laboratory to characterise the fluorescence spectral distributions of fungal spores.

There is no previous field study employing real-time LIF techniques that has proved able to count and potentially distinguish between important compost-related spores such as *A. fumigatus*, *A. niger* and *P. notatum*. However, the routine use of MBS to enable sites to become fully compliant with the requirements of licensing authorities remains far off, or at least until further development of the technique, in terms of both sampling and statistical analysis, is made possible. However, it is clearly a topic that deserves urgent further attention.

## 2.6 Real-time Monitoring of FAPs in the Staff Cabin

This 4-day indoor study was undertaken on the green-waste site between ≈13:00 on 19 October 2015 and 13:00 on 22 October 2015 at the staff cabin located in the south-east area of the site, as shown in Figure 2.1. The WIBS-4 was employed to continuously monitor particles, most importantly FAPs, as a function of time of day and staff cabin activities. Non-conductive tubing (2 m long) was used to connect to the inlet of the WIBS sampling system. The WIBS-4 was set ≈50 cm outside the open window to represent cabin air without overloading the spectroscopic detection system, which was the case when sampling inside the cabin. However, some contribution from outdoor air must be expected.

Bioaerosol monitoring at composting sites is not undertaken generally at their point sources and there are no prior measurements of their levels in the offices or recreational areas where employees work, take breaks, eat lunch or change clothes. Given the health effects of many PBAPs, described in section 1.1, it is surprising that such monitoring for occupational purposes is not performed continuously, or at least regularly, at these sites.

Employees entering their offices or staff cabins can act as vectors for carrying biological particles (including

skin-shale shedding) indoors on their clothes and bodies after their on-site working activities. Second, when the employees open office and cabin doors a draft is created that also brings material indoors or disturbs settled dust/PBAPs, which can be released through open windows. Therefore, it was expected that both of these effects would create conditions in which the WIBS would register enhanced levels of FAPs if it was located indoors. As mentioned above, however, the actual sampling was carried out 50 cm outside the window to prevent overloading, and some contributions from outdoor air are to be expected. However, these are likely to be small as the large increases in signal occur only when there are cabin activities.

In total, 4,276,591 particles were measured over the 4 days, with 537,235 (12.6%) being deemed fluorescent using normal threshold criteria for WIBS.

The size distribution of the fluorescent particles is presented in the histogram shown in Figure 2.32. A bimodal distribution with maxima at < 1 µm and at 2.2 µm was observed, which is fully consistent with bioaerosol measurements made in many previous LIF campaigns undertaken throughout the world. In the context of a composting site a peak at 2.2 µm would be expected as it corresponds well to the dimensions of *Aspergillus* spp.

A time series for fluorescent particles registering in the FL1 channel is shown in Figure 2.33. It presents the FAP behaviour recorded over the full campaign. It clearly highlights peak measurements being made on (1) 19 October at 15:00; (2) 20 October at 08:00–09.00 and 13:00–14.00; (3) 21 October at 08:00–09:00 and 13:00–14:00; and (4) 22 October at 08:00–09:00. On the first day measurements began during the afternoon when the WIBS-4 was being set up in the cabin by the

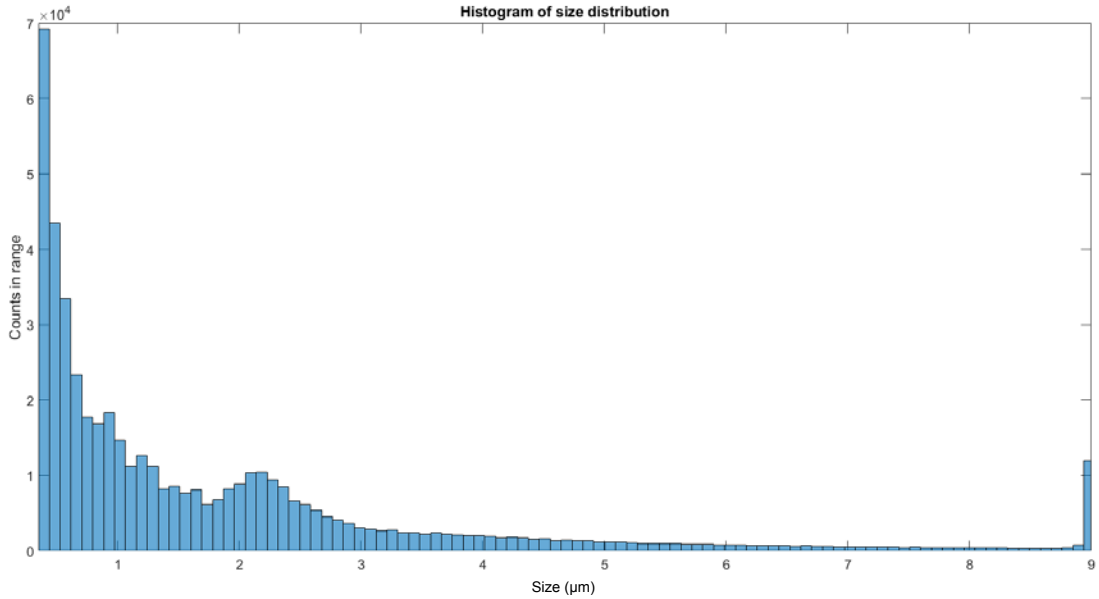



**Figure 2.32. Histogram of fluorescent particle size measured over the campaign.**

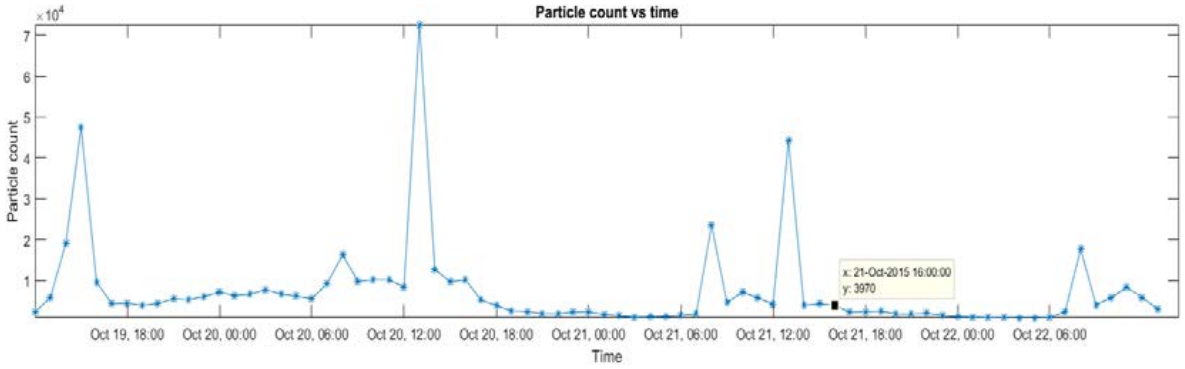



**Figure 2.33. Plot of FAP counts measured in the FL1 channel as a function of time of day.**

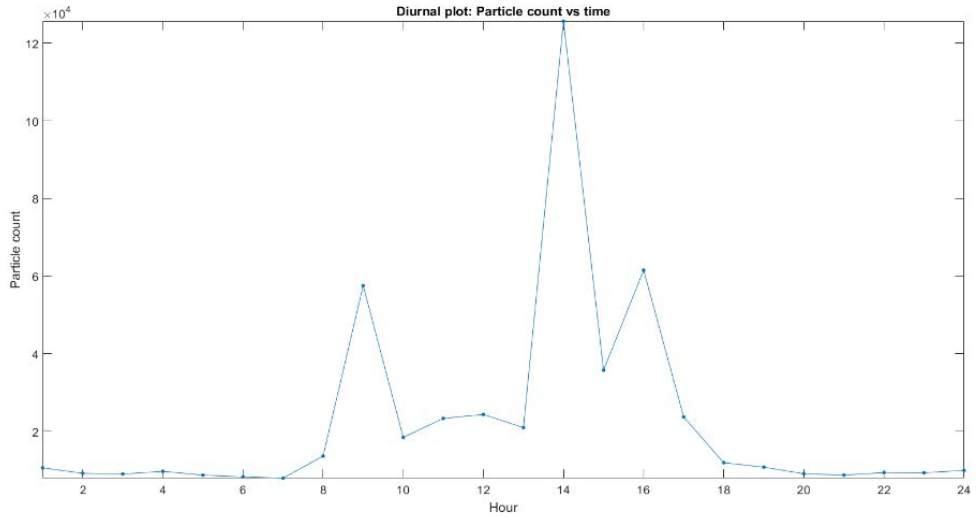



**Figure 2.34. Diurnal plot of averaged FAP counts in the FL1 channel as a function of time of day.**

team. The equipment was removed at lunchtime on the last day. The facility opens at 08:30 each day and the staff then put on their working clothes. The period from 13.00 to 14.00 is when the staff take lunch in the cabin.

Figure 2.34 shows the FAP data averaged over the 4 days and confirms that three major events occur each day in the cabin: at opening time, at lunchtime and at closing time. Clearly, the period from 13.00 to 14.00 reflects the greatest level of bioaerosol release and is the time when several of the staff are present together, having worked on-site during the morning. Figure 2.34 also shows that an equivalent peak in the timespan from 08:00 to 09:00 is present, coinciding with opening time (note that in this diurnal graph, particles are grouped from a full hour into the later time, e.g. all particles detected from 08.00 to 09.00 are assigned to 09.00).

It should be emphasised that only the FL1 channel gave rise to fluorescence signals, whereas clothes fibres tend to have different activity, mainly in the FL2 and FL3 channels. Skin-shale signals have not been studied previously. Hence, future studies in waste management site indoor locations should include parallel spore plate developments.

These measurements indicate potentially serious health consequences for those working on green-waste composting facilities, as has been found for those working at municipal solid waste management sites including active landfills and refuse bin collection points. Indeed, it has been suggested for such locations that exposure assessment with regard to total dust and bioaerosols should be focused on waste loaders and the truck cabins (Madsen *et al.*, 2016). In the current OLBAS study the results show that staff would be exposed to relatively concentrated levels of bioaerosols when they enter their communal cabin areas to eat, drink and socialise.

It would appear, at the very least, that good occupational practice should include enforced removal of site wear outside rigorously cleaned staff cabins, possibly at a dedicated facility, with showers taken before entry. Furthermore, one of the new generation of $PM_{2.5}$ indoor sensors could be easily (and cheaply) located in every office and communal facility on-site. All of these procedures would be fully in line with a “guilty until proven innocent” safety regime.

# 3 Field Campaign at the Met Éireann Valentia Observatory (2016)

## 3.1 Site Description

The location for this campaign was the Met Éireann Valentia Observatory site in Cahersiveen, County Kerry (51°56′23″N, 10°14′40″E; 25 m above mean sea level). The meteorological measurements were provided by Met Éireann staff using a station located 24 m above ground.

The novel technology WIBS-4$^+$ and the SporeWatch were located at the Brewer House, situated south-east of the main observatory buildings and east of the phenological garden. WIBS sampling was performed using ≈2-m non-conductive tubing and ≈1-m stainless steel tubing through an inlet located 0.5–1 m above the (flat) roof of the building. Other sampling configurations could be investigated in future studies to build on the OLBAS proof-of-principle campaign (e.g. arrangements to maximise the number of FAP counts in the > 10-µm range). The SporeWatch sampler was deployed just outside the Brewer House and so the two instruments can be considered to be sampling essentially the same air masses, albeit using very different methodologies.

Off-line identification and manual counting of spores and pollen were subsequently performed at the University of Extramadura, Spain, using a Zeiss KF2 optical microscope with ×400 magnification.

However, before this campaign was mounted the capabilities of the WIBS-4$^+$ for detecting pollen were assessed in a laboratory study because it represents a substantial modification to the WIBS-4 technology used in the green-waste management site campaigns described in Chapter 2. Essentially, the instrumental changes allowed (1) detection of particles in the 0.5- to 40- µm range and (2) fluorescence detection up to 750 nm. No changes to the inlet sampling were made.

## 3.2 Laboratory Testing of the WIBS-4$^+$

In 2014, a laboratory study was performed that provided the first fluorescence spectra and lifetime results for the intrinsic fluorescence of individual pollen grains (O'Connor *et al.*, 2014a). The most relevant result for the OLBAS campaign was that the biomolecule, chlorophyll-a, was shown, by measurement of its unique fluorescence peak at 670 nm, to be present in flower pollen, such as grass pollen. The finding was in contrast to the emission results from pollen produced by catkins, such as tree pollen, where no chlorophyll-a was determined to be present. In fact, this work built on an earlier study that provided fluorescence spectra for bulk samples of certain pollen and fungal spores. Here, it was shown that, as well as grass pollen, spores such as *C. cladosporioides* fluoresced at wavelengths > 600 nm (O'Connor *et al.*, 2011).

The sizes of pollen grains are much larger than the sizes of fungal spores, as described in section 1.1. Therefore, a strategy for uniquely identifying airborne grass pollen in real time and distinguishing it from both fungal spores and tree pollen became clear: develop WIBS instrumentation that could sample PBAPs with sizes between 0.5 and at least 40 µm and also detect PBAP fluorescence up to 750 nm.

Such an instrument was developed as an upgrade to the WIBS-4 and was termed the WIBS-4$^+$. The main change to the WIBS-4 was the addition of a third fluorescence detector, specifically to detect chlorophyll-a fluorescence across the wavelength range of approximately 600–750 nm. As a result, the system displayed two extra channels of fluorescence data (in addition to FL1, FL2 and FL3), termed FL4 and FL5. No changes to the sampling procedure were made to account for larger particles.

The FL4 channel provides the fluorescence intensities recorded in the 600- to 750-nm range when the 280-nm xenon flashlamp fires and FL5 data follow excitation by the 370-nm flashlamp.

Before a field campaign was mounted, laboratory testing of four contrasting types of particle was performed to assess the capabilities of the new technology. The sampling set-up was the same as that used in the MBS studies described in section 2.4.1.

Hence, the spectral distributions between 300 and 750 nm of salt (non-fluorescent), tryptophan (non-fluorescent at > 400 nm), a fungal spore, *C. cladosporioides*, and a grass pollen, *Poa pratensis*, were obtained, as shown in Figure 3.1.

The results are in full agreement with the known fluorescence behaviours of all four particle types, that is, common salt is non-fluorescent and tryptophan does not emit at wavelengths of > 600 nm whereas both *C. cladosporioides* and *P. pratensis* do emit at wavelengths of > 600 nm.

Hence, it was decided to deploy the WIBS-4$^+$ in the field during a period when grass and nettle pollination would become important and when hay fever sufferers would begin to experience symptoms.

## 3.3 Field Campaign: June/July 2016

The measurement campaign was carried out for a duration of 22 days between 20 June and 12 July 2016. Three separate films from the sampling drum of the SporeWatch were collected, representing 21 days of pollen measurements. The counting was performed by Jose María Maya-Manzano from the University of Extremadura, Spain, using a Zeiss KF2 microscope with ×400 magnification.

More than 20 species were identified from SporeWatch impaction, although some unidentified pollen was also measured (labelled as other). The pollen included (in order of decreasing concentration) Poaceae, Urticaceae spp., other, *Plantago* spp., *Juncus*, *Rumex* spp., Cupressaceae, *Quercus*, *Betula*, *Typha*, Amaranthaceae, Apiaceae, Pinaceae, *Juglans*, *Castanea sativa*, Cyperaceae, *Fraxinus–Phillyrea*, *Urtica membranacea*, *Helianthus*, *Senecio* and *Artemisia.*

Poaceae and Urticaceae spp. showed the highest number concentrations and were apparent on a daily basis. These two types are particularly important for health reasons because they include many hay fever-inducing species such as *P. pratensis* and the highly allergenic *Parietaria* (in the Urticaceae family).

The descriptive statistics for the total grains as well as the five most prevalent types of pollen collected over the campaign are summarised in Table 3.1.

In fact, these results represent the longest dataset collection for pollen obtained for anywhere in Ireland since early 2000. It is of note that all of the SporeWatch films had to be sent to Spain for analysis as no commercial service is currently available in Ireland.

A time series for the total pollen counts is shown in Figure 3.2 and indicates that pollen, as is normally the case, was rarely detected between 01:00 and 05:00, in line with previous aerobiological concentration studies.

The averaged diurnal behaviour of the total pollen grains is shown in Figure 3.3 and indicates peak number concentrations at ≈08:00, ≈14:30, ≈18:00 and ≈21:30. The individual pollen counts reveal that

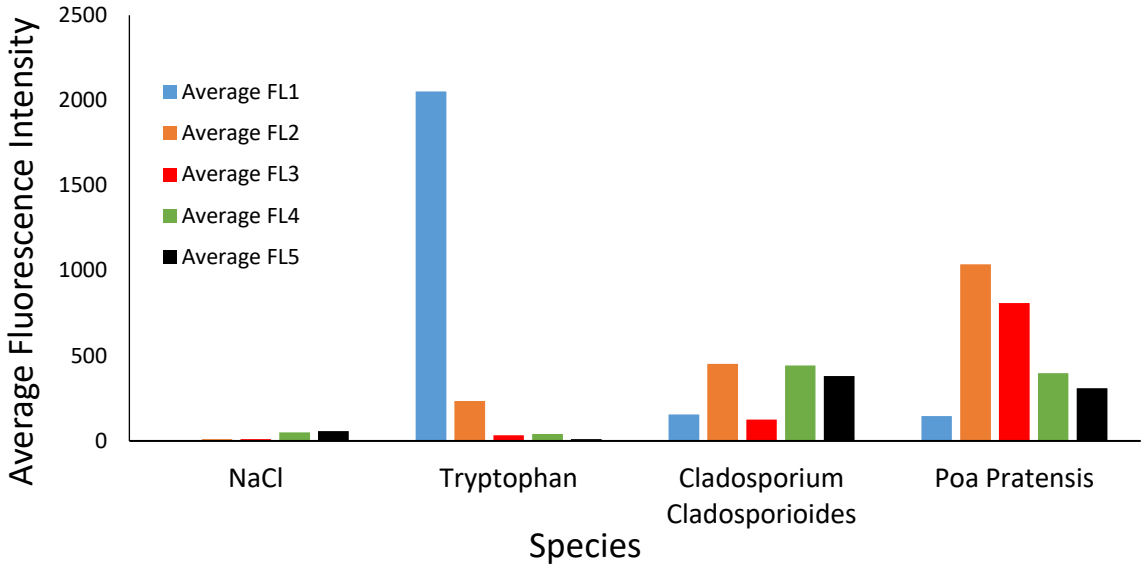



**Figure 3.1. Histogram of WIBS-4$^+$ fluorescence intensity for four particle types.**

**Table 3.1. Descriptive statistics for the hourly concentrations of named pollen species**

| Statistic | Total grains | Poaceae | Urticaceae spp. | Other (not identified) | *Plantago* spp. | *Juncus* |
|---|---|---|---|---|---|---|
| Sum | 25,620 | 19,215 | 4408 | 768 | 553 | 353 |
| Mean | 48 | 36 | 8 | 1 | 1 | 1 |
| Median | 15 | 0 | 0 | 0 | 0 | 0 |
| Mode | 0 | 0 | 0 | 0 | 0 | 0 |
| Standard deviation | 78 | 70 | 23 | 7 | 5 | 5 |
| Minimum | 0 | 0 | 0 | 0 | 0 | 0 |
| Maximum | 492 | 492 | 230 | 77 | 61 | 61 |

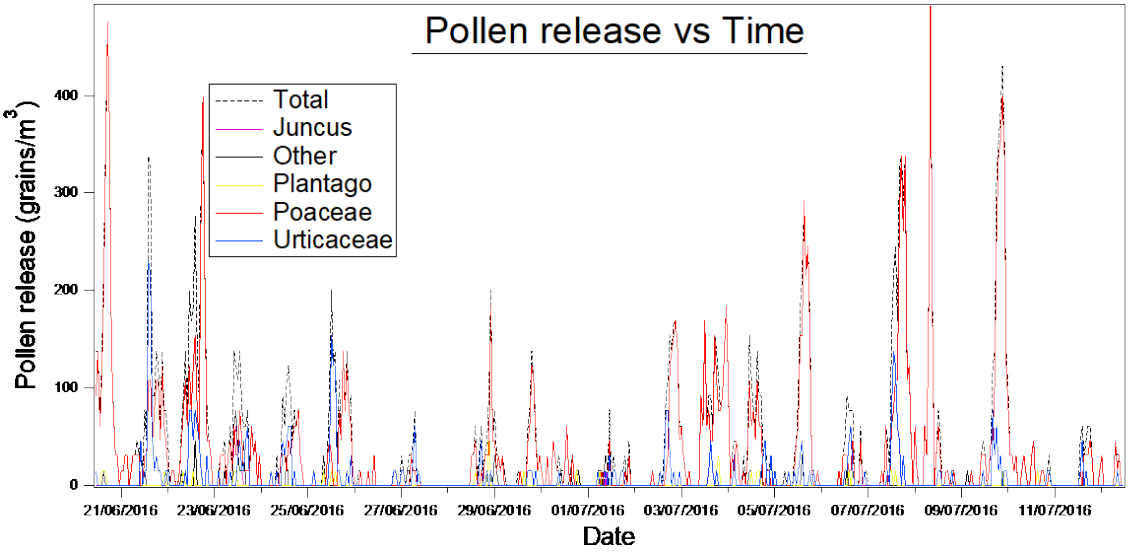



**Figure 3.2. Time series for total pollen grains detected at their greatest concentration during the campaign.**

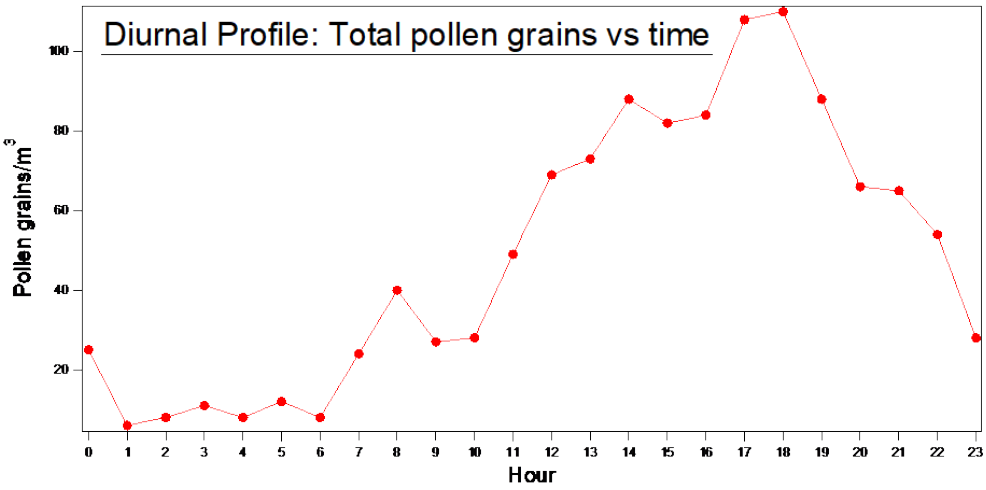



**Figure 3.3. Diurnal profile of total pollen grains as a function of time of day.**

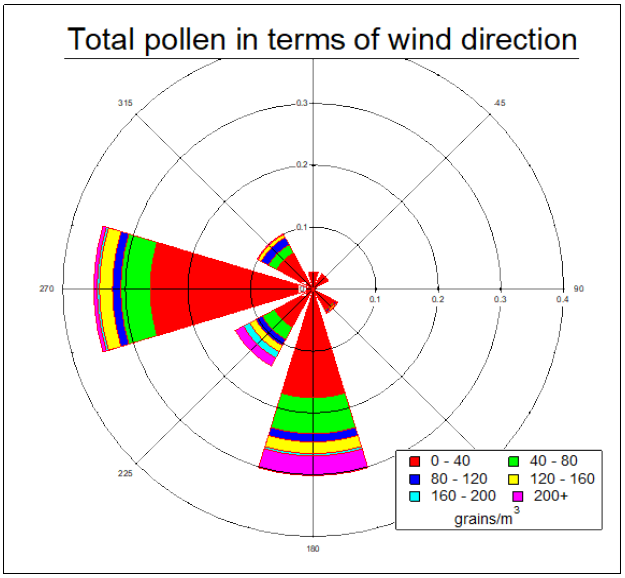



**Figure 3.4. Total pollen number concentrations as a function of wind direction over the full campaign.**

the earliest peak is caused by Poaceae, as are the third and final peaks, whereas Urticaceae (nettle) is responsible for the early afternoon maximum.

Although pollen types were readily measured using impaction/optical microscopy, *Juncus* and many cultivated Poaceae pollen types exhibit sizes above the upper limit capability (40 µm) of the WIBS-4$^+$. Hence, they would present as saturated readings in the largest size bin of the WIBS-4$^+$. However, Urticaceae spp. would appear to be the most favourable grains for WIBS-4$^+$ detection as they are relatively small, normally 12–15 µm (although much larger than fungal spores), and the second most abundant type of pollen monitored at the site.

Meteorological data allowed the construction of wind roses to apportion total pollen number concentrations in terms of wind direction, as shown in Figure 3.4.

Wind speeds rarely exceeded 10 m s$^{-1}$ over the entire campaign and the average speed was 5 m s$^{-1}$. It was noted that number concentrations of pollen generally increased when wind speeds and temperatures were higher. The pollen impaction data followed the wind direction exactly.

## 3.4 Field Testing of the WIBS-4$^+$

This field campaign was the first to be performed anywhere using the novel WIBS-4$^+$ technology. Three main objectives were set for this proof-of-principle campaign: (1) to determine whether fluorescence signals in all of the channels were measurable but paying particular attention to the chlorophyll-a channels FL4 and FL5, that is, from 600 to 750 nm; (2) to determine whether or not such signals corresponded to fluorescent particles of size > 10 µm; and (3) to determine when (or whether) the WIBS-4$^+$ data corresponded with the concurrent impaction/optical microscopy measurements (SporeWatch) of pollen made at the Valentia Observatory.

### *3.4.1 Fluorescent particle detection using the WIBS-4$^+$*

The total number of particles measured over the campaign was 2,193,334, with about 10% of the particles being defined as FAPs from normal threshold filtering.

The majority of the FAPs were < 10 µm in size, with only ≈1000 of them being measured to be > 10 µm. Only three particles with sizes in the 30- to 40-µm range were recorded, coinciding with a grass-cutting event directly outside the Brewer House. It is likely that this small number reflects the 2-m length of the collection tubing and subsequent pumping of relatively large particles into the WIBS-4$^+$. It is clear that future campaigns should use very short lengths of non-conductive tubing that feed vertically into the detection system. Many *Poa* spp. are > 25 µm in size but Urticaceae pollen are much smaller, between 12 and 15 µm. In fact, about 95% of the FAPs of > 10 µm in size were found to be in the 10- to 20-µm range. The approach taken in the OLBAS study is entirely novel but acts as a proof of principle. Future studies should, of course, establish the limitations that apply to different sampling train set-ups and establish a standard procedure for how WIBS (or MBS) sampling should be performed. It is important that this be clarified for different scenarios as well, e.g. during high humidity conditions and if it is raining.

The results indicate that it would be essential for future studies to establish the limitations associated with various real-time sampling set-ups. Standard procedures can then be developed, but such steps were beyond the scope of the OLBAS project.

The mean diurnal fluorescence values recorded in the FL4 and FL5 channels throughout the campaign are shown in Figures 3.5a and b, respectively.

The results clearly show that data are recorded in the FL4 and FL5 channels, which track the diurnal variation of the pollen as counted by optical microscopy and shown in Figure 3.3. There is also a clear distinction between daytime and night-time using both techniques. It should be remembered that the data acquisition time for the WIBS-4$^+$ is very much quicker than that for the SporeWatch methodology and may show more detailed time behaviour than is possible with impaction. The total FAP count as a time series over the whole campaign is shown in Figure 3.6.

There were two main peak events found using the WIBS-4$^+$. The first was on 23 June at 15:00 and the second was on 27 June at 11:00. The first coincides with grass cutting outside the Brewer House and also led to the largest pollen sizes (> 30 µm). This event is also clearly observable in Figure 3.2, which provides the pollen count obtained by the traditional SporeWatch/optical microscopy methodology. Interestingly, most of the pollen types, especially *Poa* and Urticaceae spp., contribute to this release. The second also coincides with an Urticaceae spp. release. However, it should be noted that at other times the species were measured by optical microscopy, although no corresponding fluorescence signals were recorded by the WIBS-4$^+$.

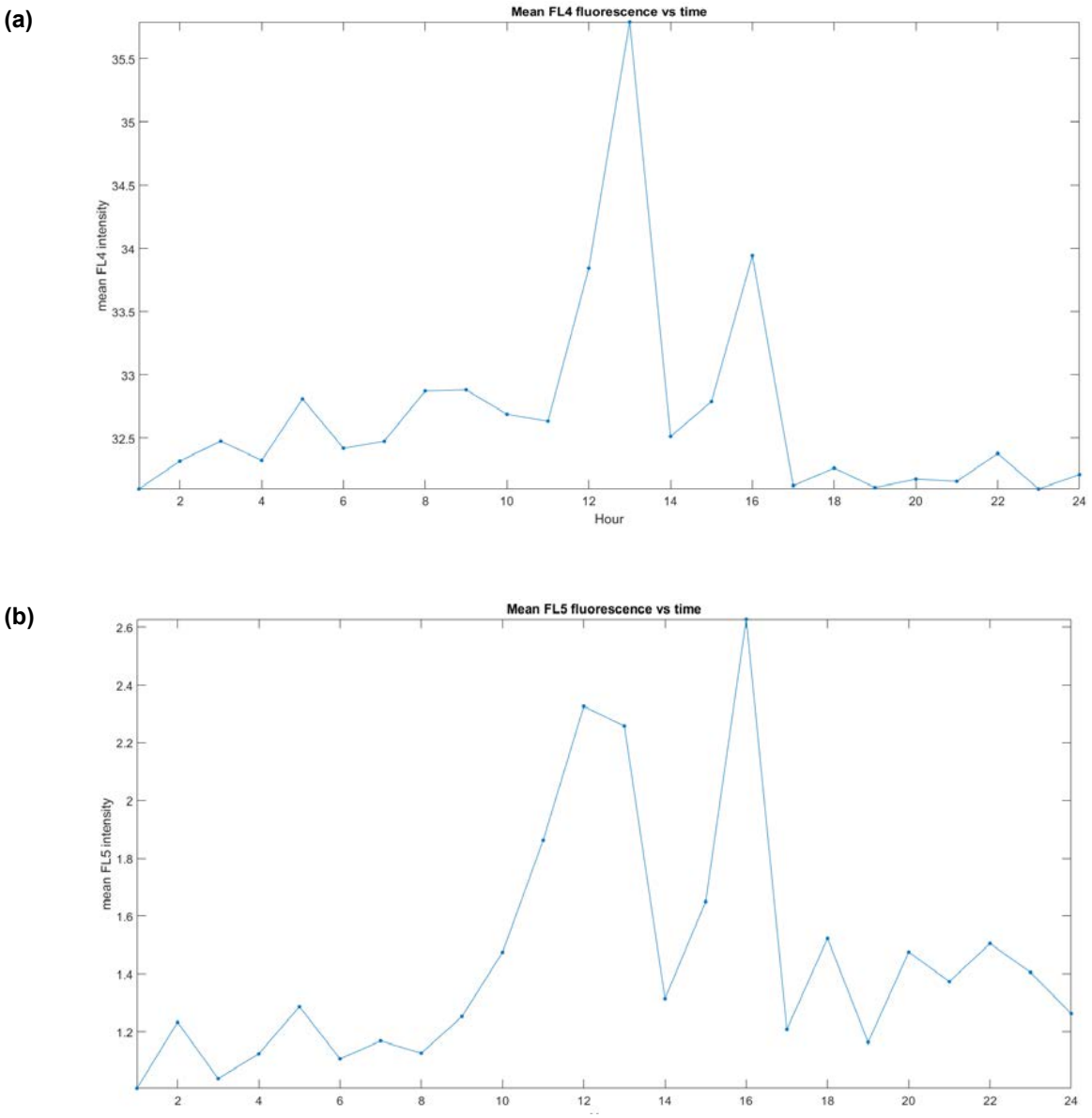



**Figure 3.5. Diurnal time series over the whole campaign for (a) the FL4 channel and (b) the FL5 channel.**

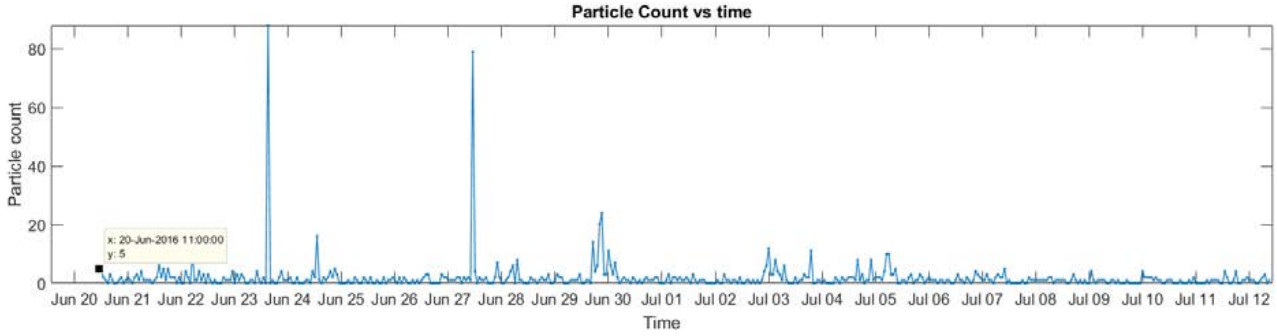



**Figure 3.6. Time series for FAP counts over the whole campaign.**

In conclusion, the main objectives of this proof-of-principle campaign were achieved. Crucially, it was demonstrated that the WIBS-4$^{+}$ could be successfully deployed in the field to provide real-time signals for pollen that tracked the traditional impaction/optical microscopy approach. Fluorescence signal detection in the 600- to 750-nm range was apparent, giving some confidence that flowering pollinators such as grass could be distinguished in future campaigns.

# 4 Afterword

In the 1870s John Tyndall discovered, using light scattering, that organic germ spores (microbes) existed in even the most carefully cleaned and filtered air. He then showed that this “floating matter of the air” was universal and stated that “it is as certain that urban air contains suspended germs as that chimneys produce smoke” (Tyndall, 1881). His optical work put the research field of aerobiology on a firm scientific basis and even led him to the recognition that *Penicillium* colonies inhibited bacterial growth many years before its use as an antibiotic. The studies also helped confirm his belief that certain particles of < 1 µm in size and always present in the air were a cause of epidemic diseases and putrefaction. Today, Tyndall’s “floating matter” is generically termed PBAPs or bioaerosols. Although fungal spores, some bacteria and pollen have been monitored for many years by methodologies based on impaction/visualisation, it can take considerable time (up to many days) for the public to be informed of the results. In other words, the measurements provide “historical” data that give context but are of little use to those at immediate risk from PBAP exposure.

Hence, in recent years bioaerosols have become a focus for real-time, online spectroscopic monitoring because it would be of clear benefit to our quality of life if some form of “early-warning” system, in terms of both pollen/bacterial/fungal spore number counts and identity, could be deployed both indoors and outdoors in a range of occupational and ambient environments.

The OLBAS study represents a proof-of-principle set of campaigns to help realise this ambition for the future; several dual approach studies were carried out at (1) a composting, green-waste management site and (2) a greenfield location in County Kerry.

The project involved both novel instrumental development and the application of established “big data” statistical techniques to the results and so intensive laboratory testing of these approaches was required to be performed before field deployment.

From the OLBAS study, it is clear that useful real-time measurements of bioaerosol counts can be made, with the possibility of real-time reporting to the public using dedicated data packages in the near future. However, identification of the bioaerosols in real time is an ambition that needs to be further developed, although the MBS laboratory experiments and campaign do point to a potentially successful outcome.

A number of important discoveries were made as outlined in the body of this report, regarding not only the occupational safety of staff working at waste management sites but also the idea of developing Ireland’s first national Irish Pollen and Spore Network (IPSN) using both real-time and traditional impaction/ microscopy approaches. The IPSN would both provide real-time reporting of PBAP levels to the public, especially those at risk such as the old, the young, asthmatics and those suffering from cardiac conditions, and allow Irish membership of the European Allergy Network for the first time.

In the future, locations associated with severe occupational risk such as hay barns and food waste and other agricultural facilities could, and perhaps should, be monitored. Furthermore, it might be possible to detect indoor/outdoor sources of *Legionella* bacteria in real time, as well as *Cryptosporidium* oocysts at water treatment plants, because the WIBS technique can be applied to aqueous droplets. However, all bioaerosols would be deemed spherical and of similar sizes. Again, such important developments would require more funding to be made available before field deployments could be made.

Indoors, real-time monitoring of hospital respiratory wards, operating theatres and cystic fibrosis units could be equally successful. Finally, real-time bioaerosol measurements made in museums and flooded housing stock that is being reoccupied would be of great utility to curators and insurance companies.

The final words of this afterword come from John Tyndall: “Believing as I do, in the continuity of nature, I cannot cease abruptly where our microscopes cease to be of use” (Heimann, 1972).

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# Abbreviations

| | |
|---|---|
| **AF** | Asymetry factor |
| **CFU** | Colony-forming units |
| **COPD** | Chronic obstructive pulmonary disease |
| **EPA** | Environmental Protection Agency |
| **FAP** | Fluorescencing aerosol particle |
| **HG** | High gain |
| **HULIS** | Humic-like substances |
| **ISPN** | Irish Pollen and Spore Network |
| **LG** | Low gain |
| **LIF** | Light-induced fluorescence |
| **MBS** | Multi-parameter bioaerosol spectrometer |
| **NADPH** | Nicotinamide adenine dinucleotide phosphate |
| **OLBAS** | Online Bioaerosol Sensing |
| **PBAP** | Primary biological atmospheric particle |
| **PM** | Particulate matter |
| **q-PCR** | Quantitative polymerase chain reaction |
| **SOA** | Secondary organic aerosol |
| **WIBS** | Wideband integrated bioaerosol sensor |

**AN GHNÍOMHAIREACHT UM CHAOMHNÚ COMHSHAOIL**
Tá an Ghníomhaireacht um Chaomhnú Comhshaoil (GCC) freagrach as an gcomhshaol a chaomhnú agus a fheabhsú mar shócmhainn luachmhar do mhuintir na hÉireann. Táimid tiomanta do dhaoine agus don chomhshaol a chosaint ó éifeachtaí díobhálacha na radaíochta agus an truaillithe.

## Is féidir obair na Gníomhaireachta a roinnt ina trí phríomhréimse:

**Rialú:** *Déanaimid córais éifeachtacha rialaithe agus comhlíonta comhshaoil a chur i bhfeidhm chun torthaí maithe comhshaoil a sholáthar agus chun díriú orthu siúd nach gcloíonn leis na córais sin.*

**Eolas:** *Soláthraímid sonraí, faisnéis agus measúnú comhshaoil atá ar ardchaighdeán, spriocdhírithe agus tráthúil chun bonn eolais a chur faoin gcinnteoireacht ar gach leibhéal.*

**Tacaíocht:** *Bímid ag saothrú i gcomhar le grúpaí eile chun tacú le comhshaol atá glan, táirgiúil agus cosanta go maith, agus le hiompar a chuirfidh le comhshaol inbhuanaithe.*

## Ár bhFreagrachtaí

### Ceadúnú
Déanaimid na gníomhaíochtaí seo a leanas a rialú ionas nach ndéanann siad dochar do shláinte an phobail ná don chomhshaol:
- saoráidí dramhaíola *(m.sh. láithreáin líonta talún, loisceoirí, stáisiúin aistrithe dramhaíola)*;
- gníomhaíochtaí tionsclaíocha ar scála mór *(m.sh. déantúsaíocht cógaisíochta, déantúsaíocht stroighne, stáisiúin chumhachta)*;
- an diantalmhaíocht *(m.sh. muca, éanlaith)*;
- úsáid shrianta agus scaoileadh rialaithe Orgánach Géinmhodhnaithe *(OGM)*;
- foinsí radaíochta ianúcháin *(m.sh. trealamh x-gha agus radaiteiripe, foinsí tionsclaíocha)*;
- áiseanna móra stórála peitril;
- scardadh dramhuisce;
- gníomhaíochtaí dumpála ar farraige.

### Forfheidhmiú Náisiúnta i leith Cúrsaí Comhshaoil
- Clár náisiúnta iniúchtaí agus cigireachtaí a dhéanamh gach bliain ar shaoráidí a bhfuil ceadúnas ón nGníomhaireacht acu.
- Maoirseacht a dhéanamh ar fhreagrachtaí cosanta comhshaoil na n-údarás áitiúil.
- Caighdeán an uisce óil, arna sholáthar ag soláthraithe uisce phoiblí, a mhaoirsiú.
- Obair le húdaráis áitiúla agus le gníomhaireachtaí eile chun dul i ngleic le coireanna comhshaoil trí chomhordú a dhéanamh ar líonra forfheidhmiúcháin náisiúnta, trí dhíriú ar chiontóirí, agus trí mhaoirsiú a dhéanamh ar leasúchán.
- Cur i bhfeidhm rialachán ar nós na Rialachán um Dhramhthrealamh Leictreach agus Leictreonach (DTLL), um Shrian ar Shubstaintí Guaiseacha agus na Rialachán um rialú ar shubstaintí a ídíonn an ciseal ózóin.
- An dlí a chur orthu siúd a bhriseann dlí an chomhshaoil agus a dhéanann dochar don chomhshaol.

### Bainistíocht Uisce
- Monatóireacht agus tuairisciú a dhéanamh ar cháilíocht aibhneacha, lochanna, uiscí idirchriosacha agus cósta na hÉireann, agus screamhuiscí; leibhéil uisce agus sruthanna aibhneacha a thomhas.
- Comhordú náisiúnta agus maoirsiú a dhéanamh ar an gCreat-Treoir Uisce.
- Monatóireacht agus tuairisciú a dhéanamh ar Cháilíocht an Uisce Snámha.

### Monatóireacht, Anailís agus Tuairisciú ar an gComhshaol
- Monatóireacht a dhéanamh ar cháilíocht an aeir agus Treoir an AE maidir le hAer Glan don Eoraip (CAFÉ) a chur chun feidhme.
- Tuairisciú neamhspleách le cabhrú le cinnteoireacht an rialtais náisiúnta agus na n-údarás áitiúil *(m.sh. tuairisciú tréimhsiúil ar staid Chomhshaol na hÉireann agus Tuarascálacha ar Tháscairí)*.

### Rialú Astaíochtaí na nGás Ceaptha Teasa in Éirinn
- Fardail agus réamh-mheastacháin na hÉireann maidir le gáis cheaptha teasa a ullmhú.
- An Treoir maidir le Trádáil Astaíochtaí a chur chun feidhme i gcomhair breis agus 100 de na táirgeoirí dé-ocsaíde carbóin is mó in Éirinn.

### Taighde agus Forbairt Comhshaoil
- Taighde comhshaoil a chistiú chun brúnna a shainaithint, bonn eolais a chur faoi bheartais, agus réitigh a sholáthar i réimsí na haeráide, an uisce agus na hinbhuanaitheachta.

### Measúnacht Straitéiseach Timpeallachta
- Measúnacht a dhéanamh ar thionchar pleananna agus clár beartaithe ar an gcomhshaol in Éirinn *(m.sh. mórphleananna forbartha)*.

### Cosaint Raideolaíoch
- Monatóireacht a dhéanamh ar leibhéil radaíochta, measúnacht a dhéanamh ar nochtadh mhuintir na hÉireann don radaíocht ianúcháin.
- Cabhrú le pleananna náisiúnta a fhorbairt le haghaidh éigeandálaí ag eascairt as taismí núicléacha.
- Monatóireacht a dhéanamh ar fhorbairtí thar lear a bhaineann le saoráidí núicléacha agus leis an tsábháilteacht raideolaíochta.
- Sainseirbhísí cosanta ar an radaíocht a sholáthar, nó maoirsiú a dhéanamh ar sholáthar na seirbhísí sin.

### Treoir, Faisnéis Inrochtana agus Oideachas
- Comhairle agus treoir a chur ar fáil d'earnáil na tionsclaíochta agus don phobal maidir le hábhair a bhaineann le caomhnú an chomhshaoil agus leis an gcosaint raideolaíoch.
- Faisnéis thráthúil ar an gcomhshaol ar a bhfuil fáil éasca a chur ar fáil chun rannpháirtíocht an phobail a spreagadh sa chinnteoireacht i ndáil leis an gcomhshaol *(m.sh. Timpeall an Tí, léarscáileanna radóin)*.
- Comhairle a chur ar fáil don Rialtas maidir le hábhair a bhaineann leis an tsábháilteacht raideolaíoch agus le cúrsaí práinnfhreagartha.
- Plean Náisiúnta Bainistíochta Dramhaíola Guaisí a fhorbairt chun dramhaíl ghuaiseach a chosc agus a bhainistiú.

### Múscailt Feasachta agus Athrú Iompraíochta
- Feasacht chomhshaoil níos fearr a ghiniúint agus dul i bhfeidhm ar athrú iompraíochta dearfach trí thacú le gnóthais, le pobail agus le teaghlaigh a bheith níos éifeachtúla ar acmhainní.
- Tástáil le haghaidh radóin a chur chun cinn i dtithe agus in ionaid oibre, agus gníomhartha leasúcháin a spreagadh nuair is gá.

### Bainistíocht agus struchtúr na Gníomhaireachta um Chaomhnú Comhshaoil
Tá an ghníomhaíocht á bainistiú ag Bord lánaimseartha, ar a bhfuil Ard-Stiúrthóir agus cúigear Stiúrthóirí. Déantar an obair ar fud cúig cinn d'Oifigí:
- An Oifig um Inmharthanacht Comhshaoil
- An Oifig Forfheidhmithe i leith cúrsaí Comhshaoil
- An Oifig um Fianaise is Measúnú
- Oifig um Chosaint Radaíochta agus Monatóireachta Comhshaoil
- An Oifig Cumarsáide agus Seirbhísí Corparáideacha

Tá Coiste Comhairleach ag an nGníomhaireacht le cabhrú léi. Tá dáréag comhaltaí air agus tagann siad le chéile go rialta le plé a dhéanamh ar ábhair imní agus le comhairle a chur ar an mBord.

# EPA Research Report 269

## Online Bioaerosol Sensing (OLBAS)

Authors: John Sodeau, David O'Connor, Patrick Feeney, Michael Quirke, Shane Daly, Mehael Fennelly, Paul Buckley, Stig Hellebust, Eoin McGillicuddy and John Wenger

Field and laboratory studies to monitor the real-time evolution of airborne fungal spores and pollen were performed. Novel instrumentation, based on fluorescence detection and optical scattering, was first commissioned and then deployed at both a commercial windrow/in-vessel green-waste composting site and the Met Éireann Valentia Observatory.

The research provided insight into the potential impacts of bioaerosols on the occupational health and safety of staff working at waste management sites and provided a proof-of-principle for the development of a National Pollen and Spore Network (IPSN) in Ireland using both real-time and traditional impaction/microscopy approaches.

## Pressures

The need to measure the occurrence and real-time development of bioaerosols related to natural emissions and agricultural and waste-management activities has increased dramatically over recent years. This necessity is based on the undesirable effects that they are known to have on human health and the role that they play in global warming. For example, Aspergillus fumigatus, which is released from both composting and harvesting activities, is the most important airborne fungal spore pathogen to cause life-threatening infections in immunocompromised patients. High levels of pollen can also be particularly serious to those in the population defined with "at risk" respiratory issues such as asthma.

## Developing Solutions

Three short, on-site campaigns were carried out at a green-waste management facility in Ireland and were the first to provide real-time data on bioaerosol emissions as a set of site-characterising, continuous profiles. The results showed that the fluorescence aerosol particle (FAP)/bioaerosol counts varied enormously depending on working activity, time of day/week and weather conditions. The Andersen counting method provided limited insight into the activities, because the measurements were performed off-site on just one occasion per year, in line with current licensing requirements. Averaged FAP monitoring data collected in the staff cabin showed that three major bioaerosol events occur each day, at opening time, lunchtime and closing time. Airborne grass pollen was identified for the first time in real time by measuring its chlorophyll signal.

## Future Policy

Indoor and outdoor locations likely to be associated with higher bioaerosol occupational risk such as green-waste composting sites, farms with hay barns and food waste or associated agricultural facilities could be continuously monitored as a matter of course.

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