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Fungal Monitoring Network and Algorithm

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Rialtas na hÉireann
Government of Ireland

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2. Office of Environmental Enforcement
3. Office of Evidence and Assessment
4. Office of Radiation Protection and Environmental Monitoring
5. Office of Communications and Corporate Services

The EPA is assisted by advisory committees who meet regularly to discuss issues of concern and provide advice to the Board.

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Identifying pressures

Bioaerosols are biological particles in the air such as pollen, fungal spores, bacteria and viruses. While most are harmless, some can trigger adverse effects such as hay fever or respiratory diseases. For example, many people with existing respiratory ailments, such as asthma, have their symptoms exacerbated by breathing in fungal spores. The agricultural sector is also affected: crop yields can be substantially decreased by diseases caused by airborne plant pathogens. As no monitoring system is in place, no forecast based on local data is available in Ireland. The forecast provided by the University of Worcester (UK) is not based on Irish data. A reliable bioaerosol monitoring and forecasting system would be a valuable tool for the Irish health and agricultural sectors.

Thus, the impacts of bioaerosols on the health of the Irish population and agriculture and the linkages to climate remain underexplored. The health and well-being implications place substantial pressures on people suffering from allergic rhinitis and asthma. This, in turn, places undue pressure on national healthcare services.

This project seeks to address this problem by undertaking the required monitoring and by developing a forecast model.

Informing policy

The direct impact of bioaerosols and fungal spores in particular can be seen throughout the year, leading to reduced quality of life and loss of productivity. Hence, this impact can exert significant and far-reaching societal and economic pressures, placing financial burdens on employees and employers alike.

While most who suffer from allergies find their symptoms more irritating than debilitating, this cannot be said for people who have asthma. As Ireland has the fourth highest incidence of asthma in the world, this is a significant sub-section of the population. Many find that their conditions are exacerbated by fungal spores and chemical particulates, placing significant strains on the public health infrastructure. Furthermore, the agricultural sector is considerably affected by fungal diseases such as potato blight, with substantial losses incurred every year.

In the light of these impacts on the general public, our society must develop "early warning" systems for bioaerosol detection at both national and local levels. FONTANA set out to develop such systems to mitigate the people of those negatively affected by fungal spores.

Developing solutions

FONTANA produced the first Irish bioaerosol network, sampling and determining the concentrations of ambient fungal species in both rural (Carlow/Sligo) and urban (Dublin/Cork) settings. This was done using both traditional impaction methodologies and real-time light-scattering and light-induced fluorescence (novel) approaches. The traditional methods highlighted the difference between the sites used, with grass pollen more prevalent at the rural site.

These data were collated with fungal data collected in the 1980s to create the first fungal calendar for Ireland, displaying the start, end and peak release periods for each fungal spore type, and highlighting the most allergenic species present in the Irish environment.

The data from the novel approaches showed decent correlation with those from the traditional methodologies, had a greater time resolution and were outputted in a far timelier manner. These data, in tandem with other air quality data, could be useful for air quality modelling and risk assessment. However, the novel approaches were unable to differentiate between fungal species. Therefore, they are more useful as bulk bioaerosol monitors than species-specific detectors.

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Fungal Monitoring Network and Algorithm

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Prepared for the Environmental Protection Agency

by

Dublin City University

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DISCLAIMER

This report is based on research carried out/data from July 2017 to August 2021. More recent data may have become available since the research was completed.

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.

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Executive Summary

Primary biological aerosol particles consist of a number of particle types, from bacteria to fungal spores and even pollen. The detrimental impacts of such particles on both human and plant health have grown in importance as our understanding of their concentration and composition has developed. Fungal spores in particular have a significant effect on the public and are present throughout the majority of the year; however, the community at large knows little of the species and concentrations that they have to deal with in their daily lives. The incidence of fungal allergy is less than that of pollen and has been found to range from 6% to 24% in the general population (Salvaggio and Aukrust, 1981; Simon-Nobbe *et al*., 2008; Tariq *et al*., 1996).

While many suffering from allergy (allergenic rhinitis) see it as an inconvenience and a quality-of-life issue, the same cannot be said for those with underlying respiratory diseases. In fact, it has been suggested that between 45% and 80% of asthma sufferers have an allergy (Corey *et al*., 1997; Hsieh and Shen, 1988; Lopez and Salvaggio, 1985). Ireland has the fourth highest rate of asthma in the world, and therefore fungal spores present a serious risk to the Irish public, as they can trigger and exacerbate asthma attacks.

However, there is as yet no established aeroallergen network in Ireland to provide detailed and accurate forecasts for those at risk from such particles. Thus, mitigation of exposure to fungal spores is virtually non-existent.

This report describes the establishment of Ireland's first aeroallergen monitoring network to determine concentrations and species of airborne fungal spores. Following this, the process of creating fungal forecasts from the data collected is described and the forecasts presented. A number of recommendations for a potential Irish fungal monitoring network are also made. Thus the name "FONTANA", or "Fungal mONitoring neTwork ANd Algorithm", was chosen to represent the project.

Extended ambient sampling in both urban (Dublin) and rural (Carlow) settings was undertaken using traditional microscopy methodologies (Hirst-type sampler). These contemporary data were combined with previously unanalysed historical data (from the 1970s). This allowed the construction of the first Irish fungal calendar that highlights the times of year at which the greatest concentrations of significant fungal species are present in the atmosphere. Sites in Cork and Sligo were established and run in tandem with the Dublin and Carlow sites in summer 2021. Differences in the overall concentrations and the proportions of the most common fungal types were seen between the sites, with rural sites (Carlow and Sligo) displaying more ascospores than basidiospores and the reverse being the case in the urban environments (Cork and Dublin).

In addition to using traditional fungal spore analysis methodologies, real-time instrumentation that uses light scattering and fluorescence was also used at the Dublin site. This was done to evaluate its ability compared with traditional methods and to gauge the possibility for its use in an automated network. A wideband integrated bioaerosol sensor – new electronics option (WIBS-NEO) and a FLIR instantaneous biological analyser and collector (IBAC-2) device were compared with the traditional impaction methodologies. Correlations between the WIBS data and fungal spore concentrations were observed, with Pearson correlation coefficients of approximately 0.5 noted between the fluorescent BC cluster (fluorescent particles that are detected only in the FL2 and FL3 channels, with excitation at 280 and 370 nm, respectively, and detection at between 420 and 650 nm) and total fungal spores, and the analysis being done in a fraction of the time taken by traditional methods. The Pearson correlation coefficient was higher for the BC cluster than for basidiospores ($r = 0.67$). However, the instruments were generally unable to differentiate between fungal spores at the species level, acting as more of a broad bioaerosol detector. The WIBS-NEO data were also compared with total pollen and Urticaceae pollen concentrations, returning correlations of $r = 0.7$–0.8. The IBAC-2 fared less well, with little correlation between its values and those obtained from the Hirst device. It did, however, display reasonable

relationships with other ambient pollution data and those obtained from the WIBS-NEO.

Using the ambient fungal data collected in conjunction with concurrently collected meteorological parameters and phenological observations, several predictive fungal models were created and tested. Both numerical and classification models were used and, in the case of the numerical forecasts, we found that a number of the classification models yielded accuracies above 60%. It should be noted, however, that additional data are needed to refine the modelling.

Finally, we draw conclusions and make recommendations related to the future of the work initiated here.

1 Introduction

1.1 Background

The air contains a significant number of particles that can be described as biological in nature. These are known as primary biological aerosol particles (PBAPs) and range in size from only a few nanometres (the size of individual virus or bacterial cells) all the way up to hundreds of micrometres, or the size of some small insects or fragments of insects (Després *et al*., 2012; Kasprzyk, 2008). One of the most prevalent PBAPs, found in all parts of the world, in all climates and at all times of year, are fungal spores. The fungus kingdom consists of entirely eukaryotic, heterotrophic organisms, existing as both single-celled and multi-celled species. Fungi can form groups of hyphae, and a grouping of hyphae is termed a mycelium. Ecologically, fungi can be parasitic, mutualistic or commensalistic, and there are as many as 5 million possible species of fungi in existence (McLaughlin and Spatafora, 2014; Simon-Nobbe *et al*., 2008). Fungi have various cell structures, including single-celled fungi (yeasts), cross-walled fungi, single-nucleated fungi and multinucleated fungi (coenocytes). Fungal spores are non-chlorophyllous and non-mobile and have chitin in their cell walls (Simon-Nobbe *et al*., 2008).

Fungal spores are of interest because of both their abundance and their impact on all aspects of the biosphere. Fungal spores can be found in the air, soil and water. They can proliferate from fungi growing on decaying or dead flora and fauna and in nutrient-rich soils. Some fungi grow on living plants and animals (Köhler *et al*., 2017). Areas with abundant life, such as farms, forests and urban green spaces, and dark, damp areas all provide perfect conditions for fungal development and proliferation.

Fungal spores have many colours and pigments, shapes and sizes, allowing their optical identification and differentiation (Dijksterhuis, 2019; Dilcher and Sheffy, 1971). Fungal spores spread and are released both passively by the fungus (e.g. wind or rain dispersal) and actively by discharge mechanisms such as sporangium explosions or bursts. Spores dispersed in the air tend to remain suspended there for a long time and can travel on air currents across and between countries and even over large bodies of water before being deposited. An example of this is the almost 1000-km distance that large *Cladosporium* clouds have travelled over the North Sea between England and Denmark (Carlile *et al*., 2001). Rain falling on fungal spores deposited on the ground often results in the aerosolisation of certain types of fungal spores (e.g. ascospores); hence, rain events can lead to further spore dispersal, in a similar way to strong wind events. It should also be noted that spores often spread not as dry clouds but as part of larger aerosols, which can allow their dispersal over hundreds or thousands of kilometres. Spores spread and settle in areas to allow further growth of the fungi, continuing the cycle of fungal growth and spore dispersal (Carlile *et al*., 2001; Grinn-Gofroń *et al*., 2018; Ingold, 1971, 1999; Kasprzyk, 2008; Pringle *et al*., 2005; Smith, 1984, 1986).

These characteristics have allowed fungi to play an important part in human history, particularly in relation to their impact on agricultural crops as humanity developed farming practices and monocultures. Such impacts include that of *Phytophthora infestans*, the cause of late blight in potatoes, which resulted in famine in Ireland. Severe fungal infections can also devastate vineyards, caused when the aphid-like pest *Daktulosphaira vitifoliae* (phylloxera) feeds on roots and leaves, leaving behind open wounds. Such fungi have had effects both economically and societally, changing the trajectory of entire cultures and civilisations through famine (Banerjee *et al*., 2007; Ristaino, 2002). Many of the most common fungal species in Ireland, such as *Alternaria*, *Aspergillus* and *Cladosporium*, have been found to contaminate food in storage (Vagelas *et al*., 2011). Fungal spores can also affect fauna directly, including humans. Asthma attacks and seasonal respiratory allergy syndromes can be caused by airborne fungal spores, and the term “sick-building syndrome” describes respiratory illnesses relating to fungal spores, among other PBAPs, in residences or workplaces (D’Amato *et al*., 1997; Gostic *et al*., 2020; Rapiejko *et al*., 2004). The significant, severe impacts that fungi have on human life, both directly and indirectly, highlight the need for further research and study in and around this area of aerobiology.

1.2 Fungal Spore Monitoring in Ireland

Annual and seasonal monitoring of fungal spore varieties and concentrations has occurred globally for decades and is ongoing. Examples include studies across Europe from the Iberian Peninsula, Poland, Slovakia and other regions, all showing that peak fungal spore concentrations are found from the start of summer until the end of autumn, when all spore concentrations begin to decline (Bednarz and Pawłowska, 2016; Reyes *et al*., 2016; Ščevková and Kováč, 2019). These studies all highlight the fact that fungal spore concentrations do not begin to significantly decline until mid- to late autumn in continental Europe. Ireland has a climate that is more maritime than the inland nations of Slovakia and Poland, and it is further north than the Iberian Peninsula and has much cooler summers. As the majority of studies have been carried out on the continent, it was thought that these factors might influence the fungal spore season in Ireland, and gave reason to monitor fungal spores here.

Little in the way of published fungal spore data exist for the Irish environment, and what data are available are relatively old. One Irish study was carried out during the summers of 1977 and 1978 in Galway, on the west coast. The study looked at the concentrations and seasonality of different types of fungal spores and pollen. Varying meteorological conditions were thought to be the major factor contributing to changes in adverse allergenic reactions (McDonald and O'Driscoll, 1980).

Peak spore concentrations in the above study differed slightly from those in Europe, with the 1977 spore peak around July and consistently high values right throughout the May to September period. The 1978 period saw spore numbers starting to increase in June, reach their peak in July and decrease before reaching a seasonal low in September. Fungi such as *Cladosporium* spp*.,* the most common fungal spore type in the Irish atmosphere, had its highest values in August and September, just as sampling was halted. Concentration of all fungal spores appeared to be rising towards a high peak right as the season's sampling was stopped. As sampling in Ireland has never previously been carried out after September, and there is strong evidence that some fungal spore concentrations increase in mid-autumn, this increased the importance of the FONTANA project as a way of filling this knowledge and information gap and charting fungal spore concentrations in the autumn in Ireland for the first time.

More studies have been undertaken in the UK, which has a similar climate to Ireland. A 5-year study carried out in Worcester, England, from 2006 to 2010 covered each calendar year (Sadyś *et al*., 2016a) and provides a more complete dataset of fungal spore distributions. In the Worcester study, 9 of the 20 spore types recorded reached their annual peak month in or after September. These are all spore types commonly found in the Irish atmosphere. Large concentrations of spores were found throughout the winter, and any decrease in fungal spore concentrations was not enough to warrant halting spore counting in winter (Sadyś *et al*., 2016a).

The fungal spore studies both in and around Ireland, and further across Europe, all show that it is necessary to continue year-round sampling and that many spore types, and possibly a majority, reach their peak fructification periods in the August–October period. The FONTANA project aims to address this knowledge gap by forming a comprehensive monitoring network and, furthermore, by developing predictive bioaerosol models.

1.2.1 Traditional fungal monitoring

Because of the many decades of experience of its widespread use and refinement, the traditional air sampler, known as the Hirst sampler, is still used in the vast majority of fungal spore sampling studies published to this day (over 70 years of use) (Hirst, 1952). The Hirst 7-day sampling device comprises an electrically powered motor that sucks air into the device's sampling head at a rate of 10 L/min. Thus, particles contained in ambient air impact an adhesive surface stored inside the device. The adhesive surface is then removed and brought to a laboratory for full manual analysis using optical microscopy.

As a motor powers the device, a constant, reliable electricity supply is required. This is the major weakness of this device, as it means that sampling fungal spores in remote locations is not possible without altering the functionality of the device. Previous studies using this device have specifically reported on fungal spore concentrations over time,

their impacts as potential allergenic particles and their possible negative impacts on crop production (Grinn-Gofroń *et al*., 2018; Martínez-Bracero *et al*., 2019; O'Connor *et al*., 2014a). Results from these studies, particularly that of O'Connor *et al*. (2014a), highlight the ability of the device to accurately monitor fungal spore concentrations up to hourly and to use these data in tandem with other publicly available datasets, such as meteorological data, to identify diurnal and seasonal trends and construct fungal spore predictive models, without the need for any additional equipment, instrumentation or other specialist materials.

1.2.2 Real-time monitoring

Traditional methods of fungal spore monitoring were developed decades ago. Various technologies and measurement methods have been developed since with the aim of complementing traditional sampling methods or directly replacing them. These real-time methods can offer a suitable alternative to the traditional Hirst-type sampling, which tends to be very time-consuming, requires a lot of laboratory training and cannot yield results until over a week after sampling has taken place.

The first real-time methods focused mainly on the development of early warning systems for identification of bio-toxins that could harm the military or citizens. One of the samplers used in this project, the instantaneous biological analyser and collector (IBAC), was developed for this purpose (Huffman *et al*., 2020; Pazienza, 2013; Santarpia *et al*., 2013). For this reason, the majority of real-time monitoring methods currently rely on rapid identification of the physical characteristics of particles, such as their size, shape and fluorescence, and a range of techniques also investigate the potential use of chemical properties to differentiate between bioaerosols (Fennelly *et al*., 2018; Huffman *et al*., 2020).

The instrument that has shown the most potential in previous studies, and is the main focus of the FONTANA project, is the wideband integrated bioaerosol sensor (WIBS; several generations have been used). The WIBS is a three-channel single aerosol particle fluorescence monitor. It detects fluorescent biological aerosol particles (FBAPs) in real time using light-induced fluorescence (LIF). It was invented at the University of Hertfordshire by Professor Paul Kaye for the purpose of detecting airborne particles in a defensive setting (Fennelly *et al*., 2018). It is based on UV-LIF technology and uses xenon flashlamp sources, in place of the more expensive solid-state UV lasers more often used, to allow the production of a low-cost, more accessible real-time bioaerosol monitoring device (University of Hertfordshire, 2022). The individual shapes and sizes of both fluorescent and non-fluorescent particles are obtained by measuring the forward and side optical scatter and the spectrally unresolved fluorescence intensity of individual particles to a time resolution of 1 ms (Fennelly *et al*., 2018).

The WIBS-NEO (new electronics option) operates broadly as follows: ambient air is drawn into the device, where a 635-nm laser irradiates the particles. Light from each particle is converted to an electrical signal for the purpose of particle sizing and triggering of the Xe1 280-nm flash lamp, followed by the Xe2 370-nm xenon flash lamp. Detector wavelength bands of 310–400 nm and 420–650 nm are then used to detect the fluorescence emissions that result from the interaction of the particles with the two flash lamps. Fluorescence signals are divided into three detector channels, namely FL1 (excitation at 280 nm and detection at between 310 and 400 nm), FL2 and FL3 (excitation at 280 and 370 nm, respectively, and detection at between 420 and 650 nm) (Després *et al*., 2012; Fennelly *et al*., 2018; Huffman *et al*., 2020; O'Connor *et al*., 2014b). A breakdown of the fluorescence characteristics can be seen in Table 1.1.

Field studies have used the WIBS to sample large quantities of air, using the unique fluorescence and physical characteristics of fungal spores to distinguish them from other aerosols and bioaerosols and then quantify their concentrations in the atmosphere (Perring *et al*., 2015; Spracklen and Heald, 2014). Indoor and laboratory studies have also been carried out, testing the capabilities and limits of the device in a controlled setting, as well as testing the impacts and volumes of outdoor particles, such as fungal spores, upon indoor environments (Healy *et al*., 2012a,b; O'Connor *et al*., 2013; Xie *et al*., 2017). This technology has also been used in a similar manner to discriminate between different types of pollen (grass and tree pollen), with researchers concluding that the technology and methods used could be applied to PBAPs such as fungal spores (O'Connor *et al*., 2014c).

Table 1.1. WIBS-NEO particle fluorescence classification

Name	Fluorescence must be above background for:
All	All particles
Excited	Particles excited by the flash lamp
Fluorescent	Fluorescent particles detected in any channel
FL1	Fluorescent particles detected in channel FL1 (excitation at 280 nm, emission at 310–400 nm)
FL2	Fluorescent particles detected in channel FL2 (excitation at 280 nm, emission at 420–650 nm)
FL3	Fluorescent particles detected in channel FL3 (excitation at 370 nm, emission at 420–650 nm)
A	Fluorescent particles detected in channel FL1 only
B	Fluorescent particles detected in channel FL2 only
C	Fluorescent particles detected in channel FL3 only
AB	Fluorescent particles detected in channels FL1 and FL2 only
AC	Fluorescent particles detected in channels FL1 and FL3 only
BC	Fluorescent particles detected in channels FL2 and FL3 only
ABC	Fluorescent particles detected in channels FL1, FL2 and FL3

Source: Droplet Measurement Technologies (2017).

The other real-time instrument tested as part of the project was the IBAC-2. ICx Biodefense initially developed this LIF continuous air monitor for detecting potential threats related to biological aerosols. It was commercialised by FLIR Systems (FLIR, 2021). It differentiates between biological and non-biological particles via elastic scattering (photomultiplier tubes) and particle fluorescence, using a 405-nm laser as an excitation and scattering source. A 405-nm laser excites the particles, and size and particle concentrations are discerned from the light scatter. A fluorescence range of 450–600 nm is used to determine whether a particle is potentially fluorescent/biological. The device also groups particles into small (0.7–1.5 µm) and large (1.5–10 µm) particle sizes. Thus, there are four different groups based on both size and fluorescence (Anchlia, 2015; DeFreez, 2009; Jonsson and Kullander, 2014; Pazienza, 2013; Santarpia *et al*., 2013).

The IBAC-2 has some distinct advantages over other biological particle sensors and detectors, including its ability to work efficiently over prolonged periods. As the IBAC-2 is designed with threat detection and warning in mind, its filter sampler commences sample collection only upon detection of a possible biological threat. This allows analysis of the most important desired air samples without requiring continuous sampler maintenance and replacement of collectors (FLIR, 2021). Recent developments and advances were researched and compiled in a publication by Martínez-Bracero *et al.* (2022a).

1.3 Fungal Spore Modelling and Forecasting Methods

Many different modelling techniques have been applied to predict and forecast ambient bioaerosol concentrations (Maya-Manzano *et al*., 2021; Vélez-Pereira *et al*., 2021). The majority of literature studies have tended to focus on the predictions of ambient pollen concentrations rather than that of ambient fungal spore concentrations, although fungal spores present a greater risk to human and plant health and are more prevalent in the atmosphere (Damialis and Gioulekas, 2006). Having said that, fungal spore modelling studies are becoming more topical and receiving increased interest as a result of the health concerns associated with fungal spores, including their ability to readily transmit plant pathogens. In recent times a variety of methods have been used to model fungal spore concentrations, including observation-based, process-based and source-orientated models.

1.3.1 Observation-based models

Observational models are used to predict the behaviour of a dependent variable using related independent variables. The independent variables (model inputs) and model outputs are usually site specific, meaning that these models are often location limited (not easily adapted to other sites). The release, dispersion and transport of fungal spores are largely dependent on a range of environmental

factors, including meteorological, geographical and phenological parameters. As a result, fungal spore prediction models typically include such meteorological and phenological parameters (independent variables). However, the significance of different parameters tends to vary depending on the fungal species and its release mechanism (Sesartic and Dallafior, 2011). The impact of various meteorological conditions on different fungal spore types and their correlation has been well documented in the literature (Damialis and Gioulekas, 2006; Filali Ben Sidel *et al*., 2015; Grinn-Gofroń and Bosiacka, 2015; Grinn-Gofroń and Mika, 2008; Ianovici, 2016; Jones and Harrison, 2004; Li and Kendrick, 1995; Lyon *et al*., 1984a; Sadyś *et al*., 2018). By investigating this relationship, rough estimates of what conditions result in the release of specific spores can be developed. As a result, several genera of fungi, such as *Cladosporium* and *Alternaria*, have been categorised as "dry spore types", highlighting their significant association with dry weather (Grinn-Gofroń and Mika, 2008; Ianovici, 2016; Sadyś *et al*., 2015a; Stępalska and Wołek, 2012). Conversely, other fungi such as *Leptosphaeria* and ascospores have displayed similar correlations with high relative humidity and rainfall (Li and Kendrick, 1995; Lyon *et al*., 1984a), leading to their categorisation as "wet spore types".

Regression analysis is a popular approach for aerobiological forecasting. Linear regression is the simplest approach. Using a straight line, this method establishes the relationship between one dependent and one independent variable. For complex systems such as the release and dispersion of fungal spores, more than one independent variable is often needed to fully explain the behaviour of the dependent variable. The use of more than one independent variable to predict dependent variable concentrations is known as multiple regression analysis. A range of regression techniques have been used to predict fungal spore concentrations to date. These include simple linear regression models (Mediavilla Molina *et al*., 1998; Rodríguez-Rajo *et al*., 2005), multiple regression models (Aira *et al*., 2008; Burch and Levetin, 2002; Fernández-González *et al*., 2013; Grinn-Gofroń and Mika, 2008; Hollins *et al*., 2004; Rodríguez *et al*., 2020; Stępalska and Wołek, 2005), and other multiple regression techniques including step-wise multiple regression (Burch and Levetin, 2002; Filali Ben Sidel *et al*., 2015; Recio *et al*., 2012), backwards elimination regression (Lyon *et al*., 1984a) and logistic regression (De Linares *et al*., 2010; Vélez-Pereira *et al*., 2019).

Such models have been used to predict a range of different outputs, including daily (Mediavilla Molina *et al*., 1998; Rodríguez *et al*., 2020; Vélez-Pereira *et al*., 2019), weekly (Filali Ben Sidel *et al*., 2015) and annual (De Linares *et al*., 2010) fungal spore concentrations. These models have mainly focused on predicting concentrations of known allergenic and pathogenic fungal spores, including the genera *Alternaria* (Aira *et al*., 2008; Burch and Levetin, 2002; De Linares *et al*., 2010; Filali Ben Sidel *et al*., 2015; Recio *et al*., 2012; Rodríguez-Rajo *et al*., 2005; Stępalska and Wołek, 2005), *Cladosporium* (Aira *et al*., 2008; Burch and Levetin, 2002; Hollins *et al*., 2004; Lyon *et al*., 1984a; Mediavilla Molina *et al*., 1998; Recio *et al*., 2012; Rodríguez-Rajo *et al*., 2005; Vélez-Pereira *et al*., 2019), *Epicoccum* (Burch and Levetin, 2002; Ščevková *et al*., 2019; Stępalska and Wołek, 2005), *Ganoderma*, *Leptosphaeria*, *Didymella* (Stępalska and Wołek, 2005) and *Botrytis* (Rodríguez *et al*., 2020; Stępalska and Wołek, 2005).

These models can be developed with computational ease, making them a popular choice. However, they often fail to fully mirror the seasonal trends in aerobiological data, which can affect model accuracy (Astray *et al*., 2010). One alternative approach that can identify such seasonal and underlying trends, without being confined by assumptions of normality or linearity, is time-series analysis. Time-series models attempt to predict future values by reviewing past values and extracting general and seasonal trends (Maya-Manzano *et al*., 2021). ARIMA (autoregressive integrated moving average) is the time-series approach that has been most widely used to predict fungal spore concentrations. This method has been used to predict ambient concentrations of several fungal spore types, including *Alternaria* (Damialis and Gioulekas, 2006; Escuredo *et al*., 2011), *Cladosporium* (Damialis and Gioulekas, 2006; Stephen *et al*., 1990), *Botrytis* (Rajo and Jato, 2009; Rodríguez-Rajo *et al*., 2010) and other phytopathogenic taxa such as *Erysiphe* and *Plasmopara* (Fernández-González *et al*., 2016).

The traditional observational methods cannot always accurately model the complex nature of biological systems, such as the interactions between fungal spores and environmental parameters. More recently,

there has been a push towards developing more complex machine learning techniques in an effort to rectify these limitations. The most popular forecasting method that has been applied to fungal spore data is artificial neural networks (ANNs). This method is designed to mimic biological processing systems and has been shown to work well for aerobiological data. ANNs have been used to model an array of hourly (Grinn-Gofroń and Strzelczak, 2009) and daily fungal spore concentrations, including *Alternaria* (Astray *et al*., 2010; Bruno *et al*., 2007; Grinn-Gofroń and Strzelczak, 2008a, 2009; Tomassetti *et al*., 2013, 2009), *Cladosporium* (Grinn-Gofrón and Strzelczak, 2008b, 2009, 2013; Grinn-Gofrón *et al*., 2011; O'Connor *et al*., 2014a), *Ganoderma* (Jedryczka *et al*., 2015; Kasprzyk *et al*., 2011; Kumar *et al*., 2013; O'Connor *et al*., 2014a; Sadyś *et al*., 2016b), *Pleospora* (Bruno *et al*., 2007; Tomassetti *et al*., 2009, 2013) and others (Sadyś *et al*., 2018; Verma and Pathak, 2009).

In addition to ANNs, advanced methods using decision trees have also been routinely applied to fungal spore forecasting. Multiple regression trees have been used in several fungal spore modelling studies, many of which also employed ANNs. This method is based on clustering data repeatedly and representing data graphically as decision trees (De'ath, 2002). Multiple regression trees have been applied to forecasting *Alternaria*, *Cladosporium* (Grinn-Gofroń and Strzelczak, 2009; O'Connor *et al*., 2014a), *Didymella* (O'Connor *et al*., 2014a) and *Ganoderma* (Kasprzyk *et al*., 2011; Sadyś *et al*., 2016b) spore concentrations. Other decision tree methods, namely random forest, have been applied to other aerobiological data (Maya-Manzano *et al*., 2021). The random forest method has not been thoroughly applied to fungal spore data in the literature, although it has been used to forecast *Alternaria* and *Cladosporium* spore concentrations (Grinn-Gofroń *et al*., 2019). More recently, random forest models have been used to forecast *Ganoderma* spore concentrations by using typical observational data along with back trajectory analysis and land cover data (Grinn-Gofroń *et al*., 2021).

Although these methods are an improvement on more simplistic techniques, recent studies have shown that these advanced techniques might be less accurate in predicting specific exposure risks for those with allergies (Jedryczka *et al*., 2015). However, this could also show a need for more street-level sampling, which could improve these predictions.

1.3.2 Process- and source-based modelling

Phenological observations can be used to determine the main phases in plant development and have been regularly used to determine flowering periods for predicting pollen concentrations (Grundström *et al*., 2019; Tormo *et al*., 2011). In the case of fungal spore modelling, phenological studies can be used to determine the key phenophases of the plants that act as hosts for fungal plant pathogens. These observations can be coupled with meteorological data to forecast the presence of fungal pathogens on plants (Fernández-González *et al*., 2013). This type of method is popular for predicting periods of crop disease risk to ensure that accurate fungicide treatments can be applied. This method has been well documented for known vineyard pathogens such as *Uncinula necator* (Fernández-González *et al*., 2013; Martínez-Bracero *et al*., 2019) and *Erysiphe necator* (syn. *U. necator*) (González-Fernández *et al*., 2019), *Botrytis cinerea* (Fernández-González *et al*., 2012; Martínez-Bracero *et al*., 2019; Rodríguez-Rajo *et al*., 2010) and *Plasmopara viticola* (Martínez-Bracero *et al*., 2019).

The prime source of fungal spore emissions is infected plants and decaying organic matter, and so an examination of land use can also aid in predicting airborne spore concentrations (Ansari *et al*., 2015; Apangu *et al*., 2020; Crandall and Gilbert, 2017; Kallawicha *et al*., 2015; Qi *et al*., 2020). Ambient fungal spore composition is ultimately dependent on location and, in some cases, vegetation type (Redondo *et al*., 2020). Analysis of land cover can aid in determining major sources of potentially pathogenic and allergenic fungal spores (Apangu *et al*., 2020) and aid in predicting ambient concentrations.

Source-orientated models can predict the distribution of fungal spores while also requiring less extensive monitoring datasets (Ansari *et al*., 2015). These models use modified chemistry transport models, which were developed to model bioaerosol dispersal. This method was initially applied to ambient pollen data but has, more recently, been applied to fungal spore data. Several transport models have been applied to fungal spore modelling, including the

COSMO-ART and WRF-Chem models (Ansari *et al*., 2015; Hummel *et al*., 2015). Other transport models, such as the Zefir source–receptor model, have also recently been applied to investigate the origin of ambient fungal spores (Sarda-Estève *et al*., 2019). Such models have been applied in predicting concentrations of *Alternaria* (Apangu *et al*., 2020; Sadyś *et al*., 2015b; Sarda-Estève *et al*., 2019; Skjøth *et al*., 2012), *Ganoderma* (Sarda-Estève *et al*., 2019; Skjøth and Kennedy, 2014) and *Cladosporium* (Sarda-Estève *et al*., 2019) spores.

For areas that are subject to long-distance transport of fungal spores, the inclusion of transport data and modelling can provide better forecasting potential than relying on local meteorological data alone (Grinn-Gofroń *et al*., 2021).

2 Traditional Fungal Spore Monitoring

2.1 Historical Monitoring Data

The ambient concentrations of fungal spores in the grounds of Trinity College Dublin were monitored over the springs and summers of 1978–1980, from as early as the beginning of March to as late as the end of September, depending on the year. The traditional volumetric–microscopic instrument, the 7-day Hirst volumetric spore sampler (Hirst, 1952), was used for fungal spore collection during the entirety of the historical monitoring campaign. The purpose of the monitoring was to identify the prevalent ambient fungal spore types present in the region at the time.

2.2 Overview of Prevalent Fungal Types and Trends

Over the course of the 1978–1980 seasons, 10 different fungal spore types were consistently sampled and categorised. In total, 23 unique fungal spore types were categorised during the three seasons of the sampling campaign. The most prevalent spore types identified in each of the three years can be seen in Figure 2.1.

The predominant fungal spore types identified during the study period were basidiospores (40%), *Cladosporium* spp. (32%), ascospores (24%) and rusts (3%), which together represented 99% of fungal spores identified during the study period. Other fungal spore types that were present in all three years of the study included *Tilletiopsis*, *Erysiphe*, downy mildew, *Epicoccum*, *Alternaria* and *Botrytis*.

Figure 2.2 shows a set of box and whisker plots for each month of each year of the campaign period, allowing direct comparison between the data for each year, as well as an overview of the seasonal trends

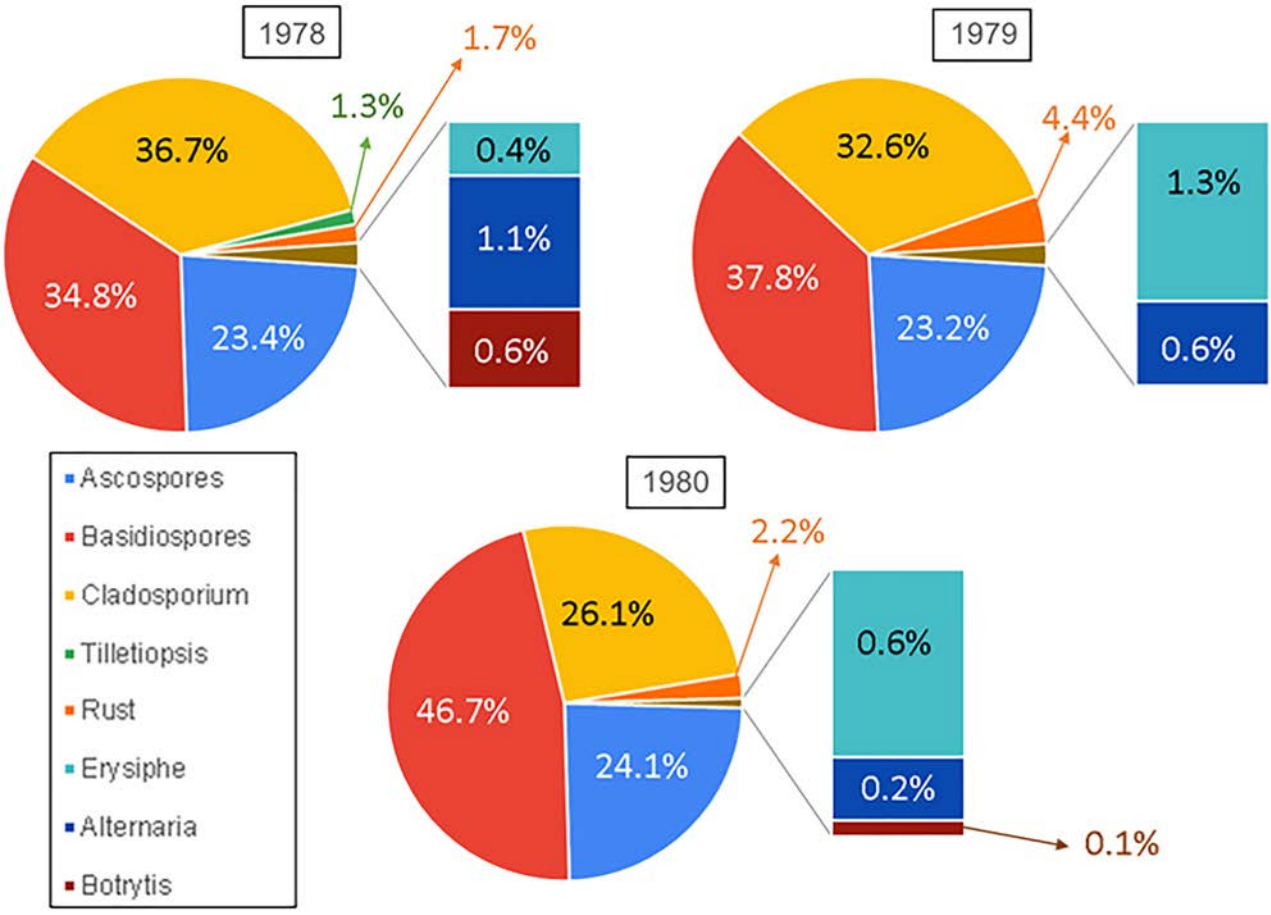

Figure 2.1. Pie charts showing fungal spore composition for the years 1978–1980.

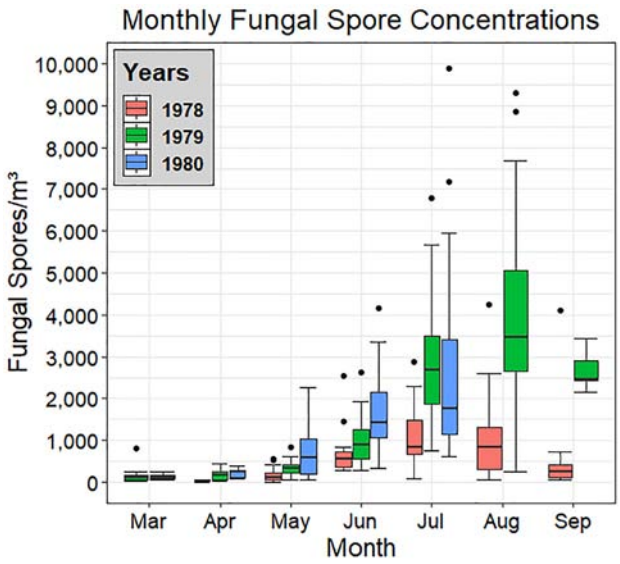

Figure 2.2. Monthly fungal spore concentrations for each year of the 1978–1980 study period.

in total fungal spore concentrations. Fungal spore concentrations for all years were low in March and April, before beginning to rise in May, with 1980 data best illustrating this, as the median concentration more than doubled between April and May. All years saw significantly higher values in June, with concentrations rising into the summer months. Concentrations in 1979 continued to rise, before peaking in August, while concentrations in 1978 were similar in both July and August. September saw the first sign of a marked decrease in concentrations for all months studied, signifying the end of the peak fungal fructification period.

When the three years are combined into a "seasonal distribution", basidiospores reached their peak on 30 July, on average. On that date, an average of 6650 spores/m^3 was determined. On three occasions average *Cladosporium* spore concentrations peaked above that value, with the highest being an average of 8931 fungal spores/m^3, reached on 29 July. Ascospores appeared to have a less defined "peak" than basidiospores, with average fungal spore numbers in the run-up to the peak fructification period similar to those in the rest of the season. Some days in the middle of the expected "peak period" for ascospores had very low spore counts, such as 16 July, when the average for the study period was only 269 spores/m^3.

Figure 2.3 is a seasonal distribution chart of fungal spore concentrations. It was created to determine the peak fructification periods of fungi whose spores were identified during this study period. The longest peak fructification periods were for *Scopulariopsis* and yeasts, each totalling more than 4 months, extending from April to the start of September for *Scopulariopsis* and from the start of May to mid-September for yeasts. The shortest peak fructification period belonged to *Venturia*, which peaked for less than 4 weeks, during parts of May and June. *Scopulariopsis* reached its peak earliest, and it and *Ganoderma* were the only

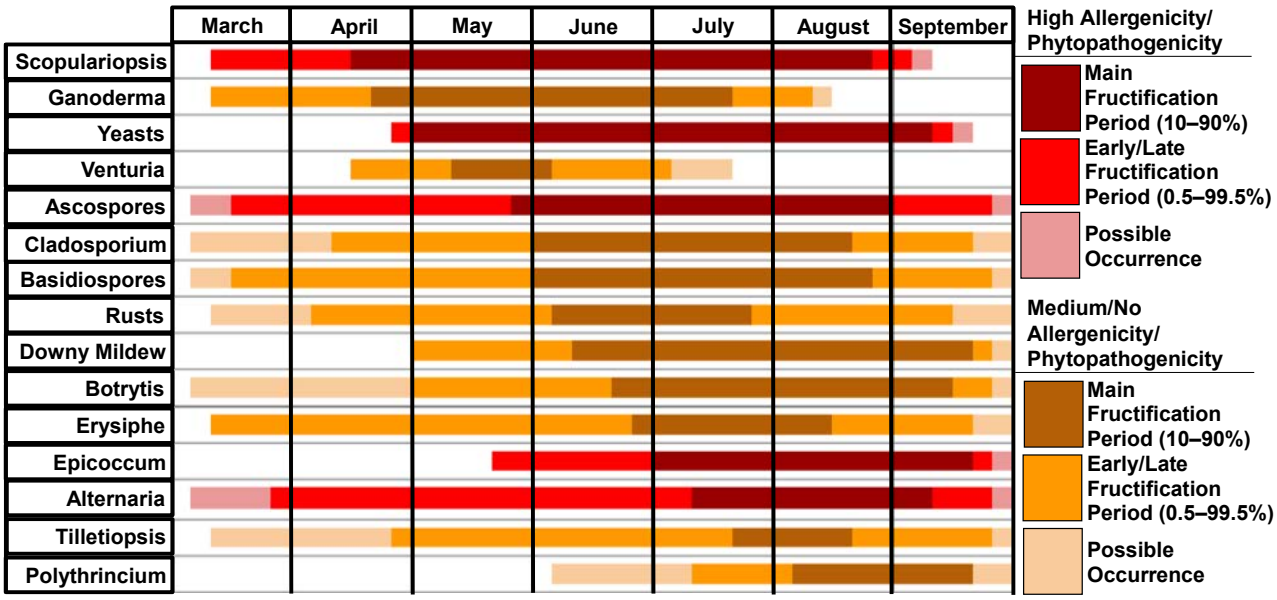

Figure 2.3. Seasonal and peak fructification periods of allergenic fungi detected in the 1978–1980 study.

genera to have released 10% of their annual totals before May. *Polythrincium*, on the other hand, did not reach peak fructification until August, suggesting that it is very likely that the lack of sampling after September may have had an effect on the total spore concentrations recorded.

Basidiospores had the highest seasonal spore integral (SSIn), followed by *Cladosporium* and ascospores (Martínez-Bracero *et al*., 2022b). As there is no description of how basidiospores were identified during this campaign, the explanation for such high concentrations of this spore type may lie in the similar results obtained by McDonald and O'Driscoll (1980), whose definition of basidiospores was very broad, encompassing all spores of a darker pigmentation or without identifying features such as being contained within an ascus. While *Cladosporium* had the second highest seasonal spore integral concentrations, it was the spore type with the highest daily peak concentrations.

During the 1970s and 1980s, the majority of studies using the Hirst volumetric sampler focused their attention on pollen grains, which are larger and more easily identified (Gregory, 1978; Mandrioli *et al*., 1982; Mullins *et al*., 1977; Viander and Koivikko, 1978). While many studies on fungal spores were carried out at this time, the focus of a large portion of these was on instrumental evaluation. The usefulness and practicability of the Hirst device, as well as its possible applications, were often investigated with regard to fungal spore counting and analysis; it was also compared with other instrumentation (Burge *et al*., 1977; Käpylä and Penttinen, 1981; Lyon *et al*., 1984b; Perrin, 1977). Investigations into the seasonality of individual spore types, or comparisons between regions in certain countries, were carried out in some instances, such as a study investigating the amount of rainfall and humidity required to activate ascospore discharge (Johnson, 1979).

One long-term study involved the running of Hirst volumetric samplers at four locations across Finland in the mid-1970s (1974–1976) (Rantio-Lehtimäki, 1977). Two of the Finnish sites are at latitudes much higher than Ireland and are classified as a "boreal" climate (Beck *et al*., 2018). The two southern sampling sites are coastal and are classified as a "humid continental" climate (Beck *et al*., 2018). With monthly mean temperatures ranging from a low of –3.8°C to a high of 18.1°C across the year (Finnish Meteorological Institute, 2022), these two sites can be compared with the 1979–1980 data obtained in Dublin.

This Finnish study found an initial peak in spore concentrations in mid-July at the two southern stations, similar to that identified in the Dublin dataset. Interestingly, a second, larger, fungal spore peak concentration in late August was also identified in Finland. This again shows that halting fungal spore counting in Dublin in the late summer of each year of the study was possibly premature, particularly because the majority of fungal species' spore concentrations had not yet dropped significantly below their "peak" values when counting was halted.

The most common spore type recorded in the south of Finland was *Cladosporium*, which accounted for 53% of all spores identified. *Cladosporium* dropped as a proportion of total spores as latitude increased, falling to 16% and then to 4% with increasing latitude and distance from continental Europe. Further in-depth analysis of the historical monitored data has been completed and published (Martínez-Bracero *et al*., 2022b).

2.3 Contemporary Monitoring Sites

Contemporary traditional fungal sampling was carried out across Ireland using data collected from four sites: Carlow, Dublin, Sligo and Cork (Figure 2.4). Given the restrictions imposed during the COVID-19 pandemic, concurrent sampling at all sites was not possible, with persistent lock-downs stifling this objective. Long-term studies were carried out at two of the sites, one rural (Carlow) and one urban (Dublin). The two sites were chosen to reflect the maximum possible difference in population density and anthropological setting without any major change in climactic factors or weather conditions.

For the long-term datasets, the Dublin sampling site ran from 2017 to 2019, with instrumentation placed on the rooftop of the five-storey Technical University Dublin Kevin Street building (53° 20′12.4″N, 6° 16′ 4.25″W) (approx. 20 m high). The Carlow sampler (52° 43′23.6″N, 6° 39′36.0″W) ran during 2020 and 2021. This site is a rural, agricultural inland area, and the spore trap is located in a field on farmland. Both sites have lower rainfall than the national average (1200 mm annually), at 758 mm and 868 mm,

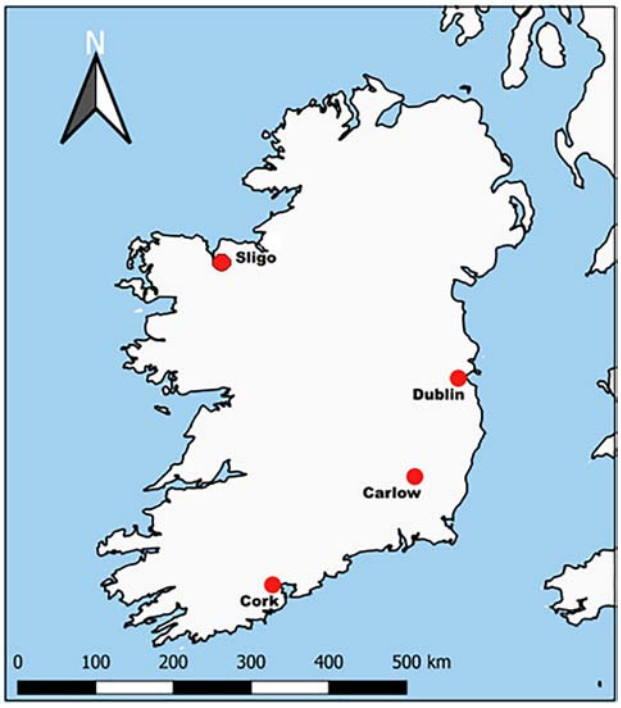

Figure 2.4. Contemporary fungal spore sampling sites.

respectively, and average annual temperatures of 9.9°C (Met Éireann, 2022). The Cork and Sligo sites ran intermittently over the course of the project. All four sites were in operation for the peak fungal season in 2021. The results will be discussed in detail below.

2.3.1 Overview of prevalent fungal types and trends (Dublin and Carlow)

For Dublin, 26 different fungal spore types were identified over the full campaign. The predominant fungal spore types identified were *Cladosporium* (50.8%), ascospores (35.6%) and basidiospores (7.2%). These three spore types combined represented 93.6% of all fungal spore types classified during the sampling period (Figure 2.5).

In the time-series analysis shown in Figure 2.6, both 2017 and 2019 had relatively similar fungal spore concentrations throughout the year. In 2017, the peak period began earlier than in 2019, with concentrations starting to rise at the end of June. In 2019, a similar increase in concentrations was not seen until the middle of July. The 2017 peak period also rose to higher overall concentrations than in 2019. In 2018,

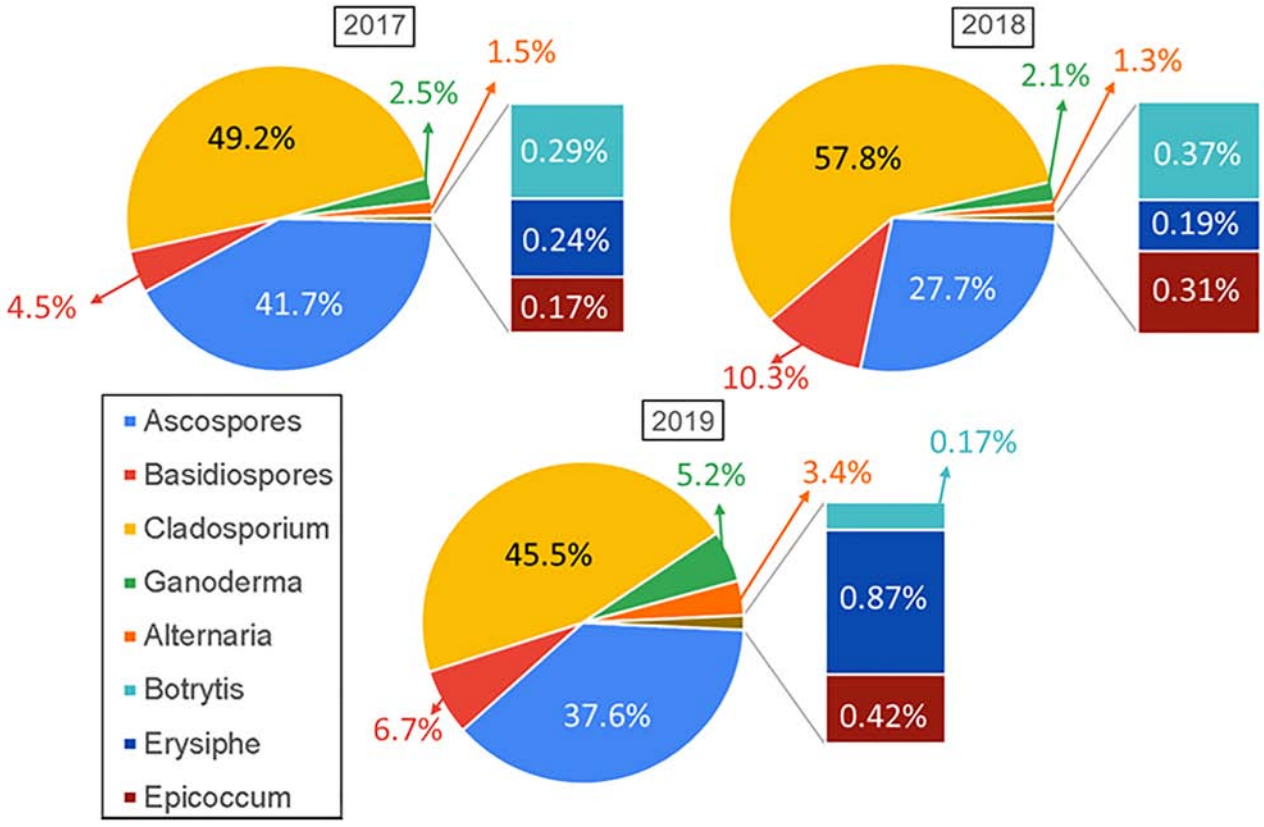

Figure 2.5. Pie charts showing the distribution of major fungal spore types in Dublin in the years 2017–2019.

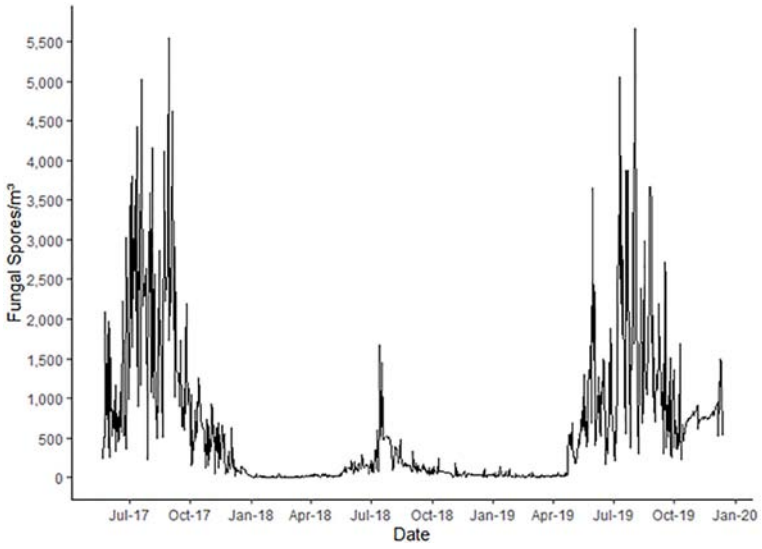

Figure 2.6. Time-series chart showing contemporary fungal spore counts in Dublin from July 2017 to January 2020.

fungal spore concentrations were extremely low in comparison with the preceding and following year.

This was not entirely unexpected, as Ireland experienced multiple long periods of both heatwaves and droughts throughout what would have been the peak fungal fructification period in 2018 (Falzoi *et al*., 2019). The drought affected all aspects of the Irish ecosystem, and it was a Europe-wide event that had impacts across the continent (Caloiero *et al*., 2018; O'Dwyer *et al*., 2020). In previous studies carried out during periods of drought or prolonged heatwaves, researchers also found large decreases in expected spore concentrations. In one study, which charted fungal spore concentrations in Niğde, Turkey, in 2014, researchers saw a 50% decrease in fungal spore concentrations during drought associated with low rainfall (Arslan, 2017; Çeter *et al*., 2020). The drought in Ireland during the summer of 2018, depending on how drought is defined, lasted as long as 53 days (Moore, 2020).

Figure 2.7 shows a set of box plots representing the average start and end dates of the fructification phenophase of each major fungal spore type, as well as aggregate figures for "total spores". From the plots it can been seen that *Alternaria* had the shortest fructification season, beginning after all the other spore types shown and ending earliest. Conversely, ascospores had the longest season, having the earliest start and the latest end to the season. The width of each box plot also gives us some indication of the variation in the start and end dates for the release of each spore type, across all years studied. Given that "other spores" is an aggregation of many spore types, it had the least well-defined season, with both

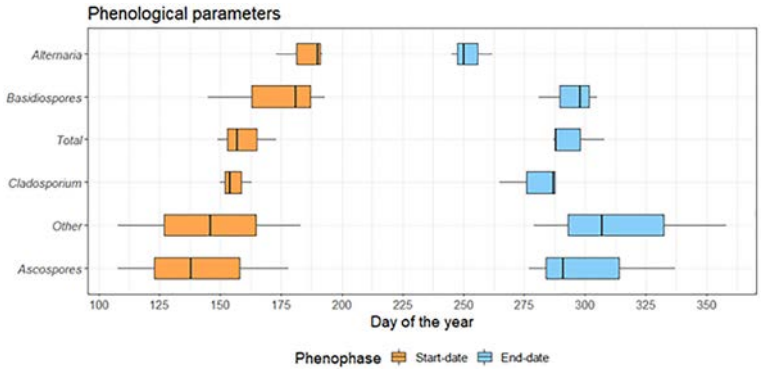

Figure 2.7. Box plots showing start and end dates of major spore types detected in Dublin during the campaign.

box plots having an interquartile range that extends over 30-day periods. *Cladosporium* and *Alternaria* had the most well-defined seasons, with interquartile ranges for their start and end dates narrowed down to a week (from year to year). The fructification period for "total spores" was driven by *Cladosporium* spore concentrations because of the large quantities of this type present. However, because ascospores, basidiospores and "other spores" had end dates that were later in the season, the overall fungal spore average, or "total spores", had the same median end date as *Cladosporium*, with the box plots of "total spores" and *Cladosporium* not aligned with each other.

For Carlow, 20 different fungal spore types were identified over the campaign period (2020–2021). The predominant fungal spore type identified was again *Cladosporium* (similar to that seen in Dublin). This fungal spore type made up 74% of the total fungal spores identified in 2020 and 87% of total fungal spores in 2021 (Figure 2.8).

Other spore types that were identified (>1%) were ascospores (9% in 2020, 5% in 2021), basidiospores (2% in 2020, 3% in 2021) and *Alternaria* (3% in 2020, 2% in 2021). A variety of other fungal spore types accounted for the remainder of the spores identified (12% in 2020, 3% in 2021).

One reason for the increase in concentrations of *Cladosporium* spores relative to other fungal spore types between the two years of the study in Carlow was that while, in 2020, sampling took place throughout the main fructification period (March to August inclusive), in 2021 sampling data are available only from the start of June to the end of August. This is the time of year when *Cladosporium* concentrations are expected to be at their highest and thus can explain the apparent increase, year on year.

As this system of determining the start and end dates is the standard and most widely used across the aerobiology field, direct comparisons could be made with similar studies. A study in France found that the length of the *Cladosporium* season decreased as sampling moved further from the equator (Sindt *et al.*, 2016). The season lasted 220 days in southern France and 160 days in central France. Our Dublin study, at a higher latitude again, had a season length of between 130 and 135 days for each of the years studied, thus in keeping with this trend.

The shortest season length for any spore type in our study was for *Alternaria*, lasting on average only 8 weeks from 9 July to 7 September. Again, this is shorter than the seasons found in other continental studies, by the degree expected based on latitude. A study in Kraków, Poland, found the same peak

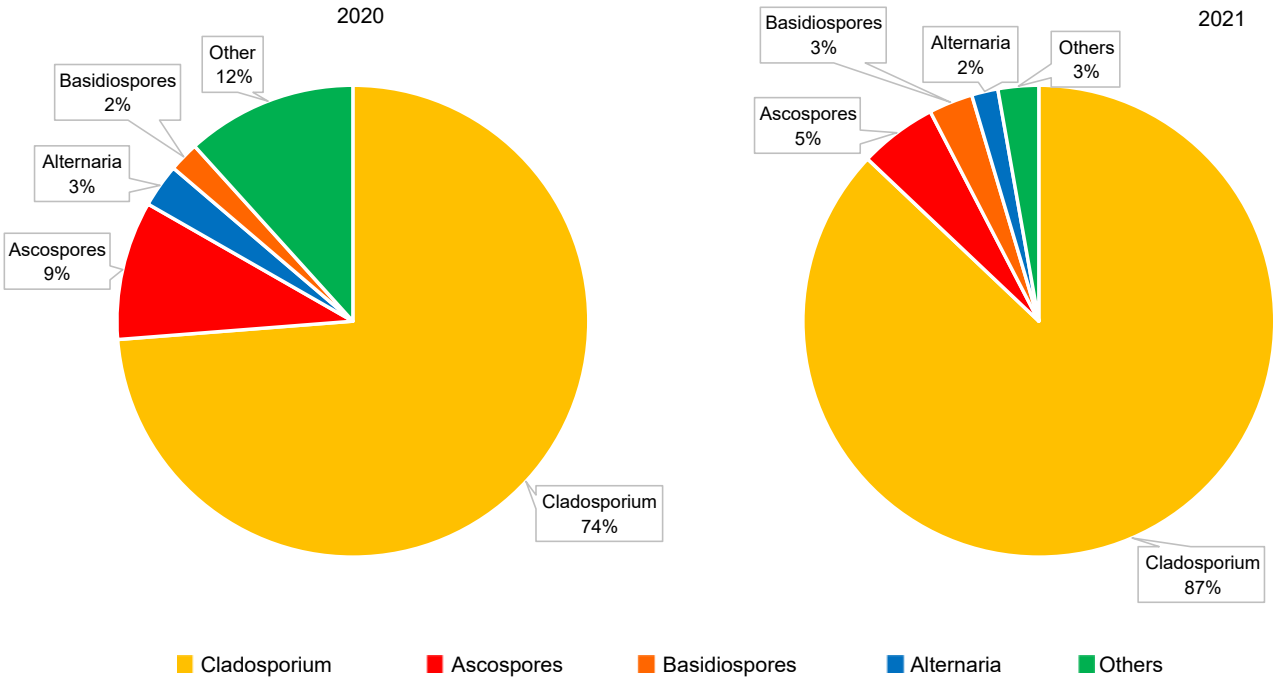

Figure 2.8. Pie charts showing the distribution of major fungal spore types in Carlow in the years 2020 and 2021.

period for *Alternaria* as in Ireland, but with the season starting 3 weeks earlier, and ending 1 week later (Stępalska and Wołek, 2005). Other spore types in the Kraków study also reflect this trend, showing that, although Ireland is on the fringe of Europe, extending into the Atlantic Ocean, its fungal spore seasonal patterns are largely related to and representative of the European continental seasonal patterns.

While the data outlined here show the current state of the fungal spore season in Ireland, they should be regarded as a snapshot in time, rather than a constant. Studies in the UK have observed that the length, start dates and end dates of various fungal spore seasons are shifting over time and require constant monitoring (Andrew *et al*., 2018; Corden and Millington, 2001). A 25-year study of the start date for *Alternaria* spore release in Derby, UK, observed that it shifted by an entire month between 1972 and 1997, from the end of June to the start of the month (Corden and Millington, 2001). Such shifts are occurring for all fungal spore types as a result of climate change. Keeping track of the shifts is essential to ensure that models designed to forecast and predict these seasons accurately reflect spore release patterns over time.

2.4 Multi-site Traditional Monitoring Campaign in Summer 2021

The sampling locations used in the project can be seen in the CORINE land cover maps (Figure 2.9). These sites were operated concurrently from the beginning of June until approximately mid-August 2021. As can be seen in the figure, there is a significant difference been the rural sites (Carlow and Sligo) and urban sites (Cork and Dublin). The urban sites are surrounded by land associated with urban life, industry and

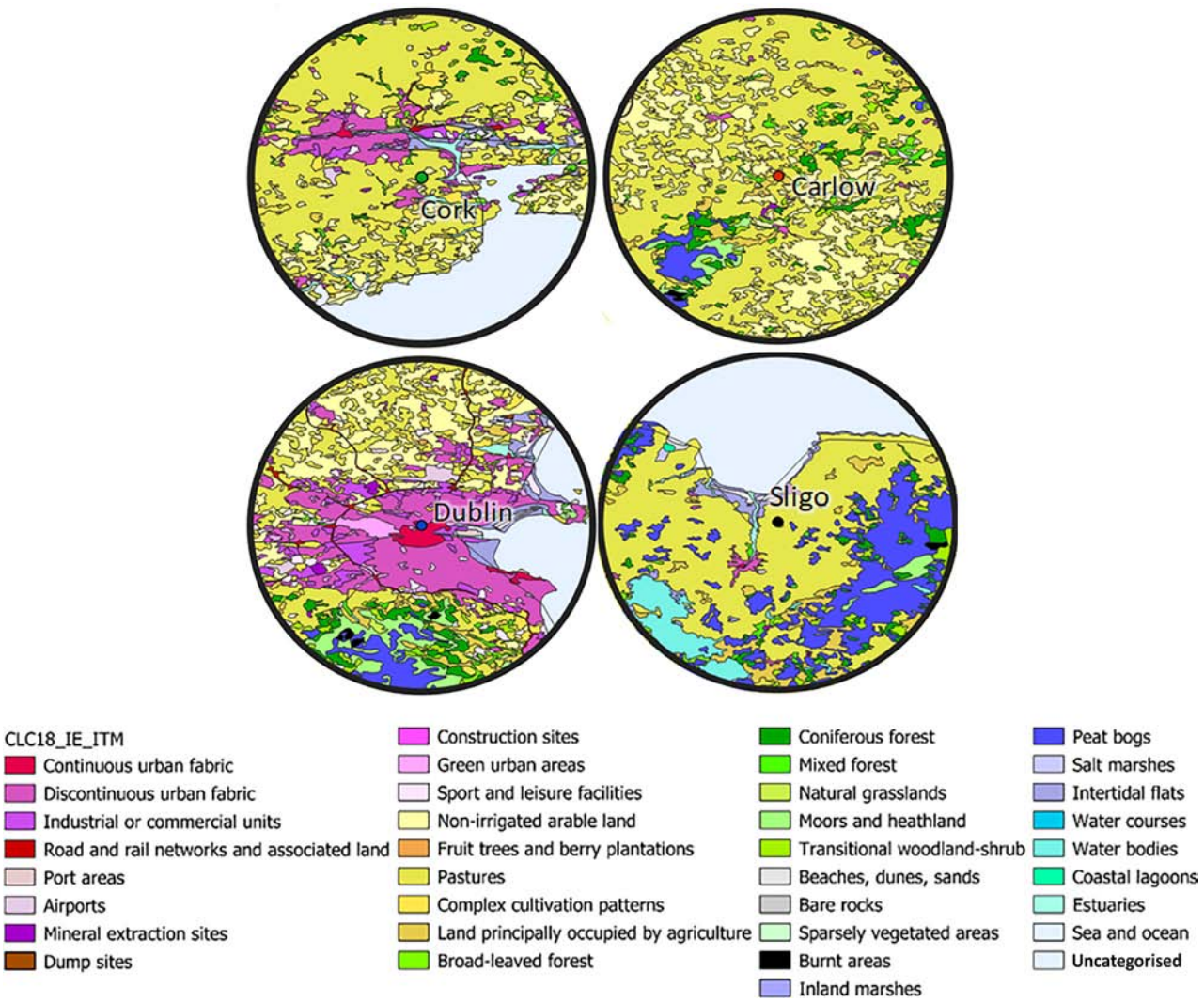

Figure 2.9. CORINE land cover maps of the four fungal spore sampling sites.

commercial activities (purple), while the rural sites have a preponderance of pastures, agricultural land, unirrigated arable land and peat bogs in their vicinities.

While the main fructification seasons and times of highest fungal spore concentration in both Dublin and Carlow are similar, several differences were noted between the urban and rural sites. During all years, the ratio of *Cladosporium* to other fungal spores was the most obvious difference, as alluded to in the previous sections. In Dublin, approximately 50% of all fungal spores monitored were identified as *Cladosporium*, compared with around 75% of all fungal spores in Carlow (Figures 2.5 and 2.8).

This was not an unexpected discovery, as the Carlow sampling site is surrounded by grassland, which is a host for *Cladosporium*, while the Dublin sampling site is in the centre of a metropolitan area, surrounded by buildings rather than grassland. Additionally, the Carlow sampling device was raised just above the ground on a wooden platform to allow its sampling inlet to be positioned above long grass, whereas the Dublin sampler was positioned on top of a five-storey building, increasing the difference in distance *Cladosporium* spores had to travel before being collected by the samplers.

Figure 2.10 shows a spider plot of the relative ratios of ascospores, basidiospores and *Alternaria* at all four locations – Carlow, Cork, Dublin and Sligo – during the peak fructification period of June–August 2021. The counties are represented in different colours, and the distance from the centre of the circle indicates the proportion of the spore type found at a given location. The most notable aspect of the spider plot is the urban–rural divide in relation to relative ascospore and basidiospore concentrations. At the two urban sampling sites in Dublin and Cork cities, the relative concentrations of basidiospores were clearly higher than those of ascospores. At the rural sampling sites of Sligo and Carlow, this observation is reversed, and the relative concentrations of ascospores were clearly higher than those of basidiospores.

Figure 2.11 shows a comparison of spore concentrations and proportional densities between the four sampling locations over the course of the peak fructification period of 2021. From the data we can see that the peak fructification period in Ireland was in the second week of July, with relatively high concentrations of spores seen at all four sampling locations. The season is bookended by higher relative concentrations in Carlow at the start of the fructification period and in Dublin at the end of the period. Carlow and Cork show some evidence of a bimodal peak in July, with one peak in the first week of the month separated from a second peak (approx. 21 July) by a dip in spore concentrations. Indeed, in the month of July the patterns of fungal spore concentrations in Cork and Carlow mirror each other quite well.

This bimodal pattern, however, is not reflected in the Dublin dataset, and is less evident in the Sligo time series, which appears as more of a continuum after the early peak in July. While little if any fungal spores were recorded in Dublin at the beginning of the

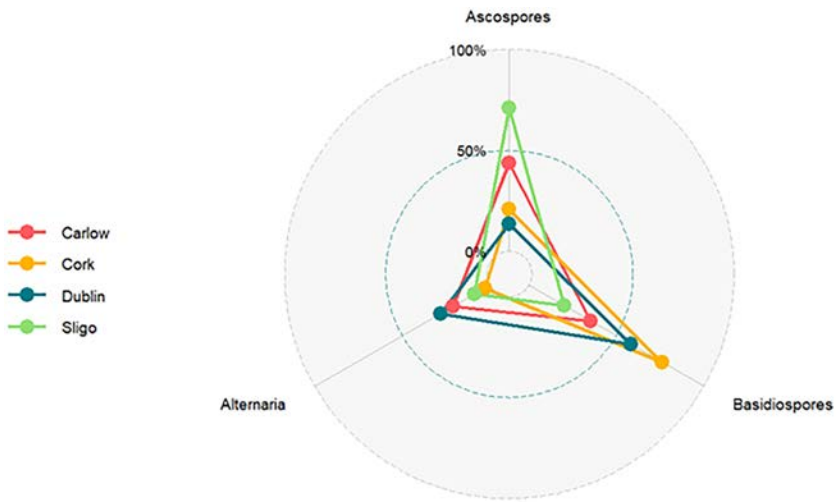

Figure 2.10. Spider plot showing the compositional distribution of the major fungal spore types (minus *Cladosporium*) at all four locations where sampling took place in summer 2021.

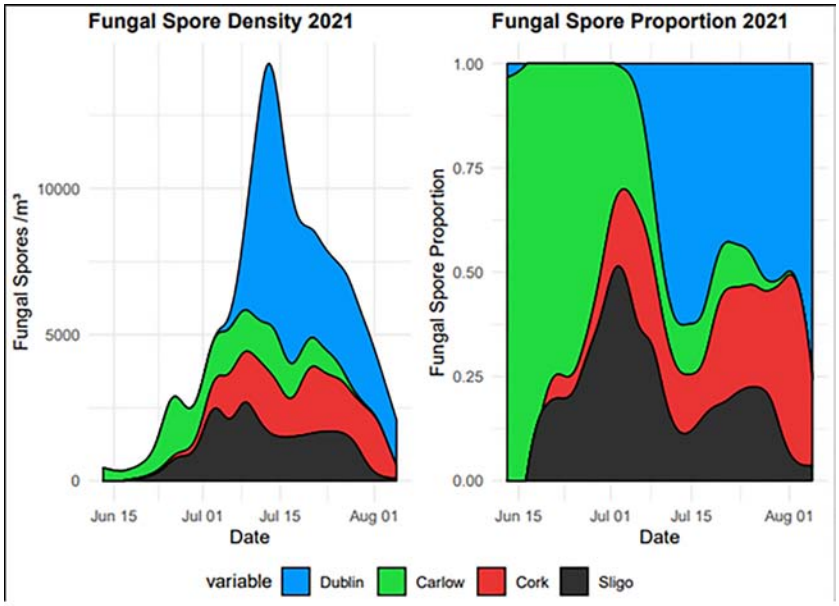

Figure 2.11. Fungal spore concentrations and proportional densities in Dublin, Carlow, Cork and Sligo in summer 2021.

sampling period, a sharp peak in concentration is clear in mid-July. The fact that this peak was not mimicked at any of the other sites highlights the influence of localised climate or weather and indeed sources on spore concentrations.

These initial data highlight the need to monitor fungal spore concentrations across the island and develop a full monitoring network. The major peak in Dublin was not reflected at the other sampling locations, and the release patterns of fungal spores were related but different at every location monitored.

Numerous studies have analysed the performance of multiple Hirst-type devices at multiple locations in the same state or country over the same period, such as one peak season or one year (Hollins *et al*., 2004; Lacey, 1962; Oliveira *et al*., 2010; Palmas and Cosentino, 1990; Patel *et al*., 2018; Rodríguez-Fernández *et al*., 2022). Studies comparing urban and rural settings in Portugal (Oliveira *et al*., 2010) and Sardinia (Palmas and Cosentino, 1990) both found that the rural locations tended to have higher overall spore concentrations than urban locations. The one exception was that of basidiospores, which were found in significantly higher concentrations in the urban area in Portugal (Porto) than in the rural area (Amares). This trend in increased basidiospore concentrations in urban settings is directly reflected in our study (Figure 2.10).

Although trends show that the majority of spore types are found at higher concentrations in rural environments, major differences can be seen between different urban environments, due to local influences. One study investigating the difference in fungal spore concentrations across five samplers all within the same urban centre (Las Vegas, USA) found that, even within the same urban centre, there was a large variation between samplers (Patel *et al*., 2018). The authors advocated increasing the number of bioaerosol samplers within “microenvironments” so that local influences, such as the growth of younger plants in “newer developments” within the city, could be accounted for.

A study in Saudi Arabia (Hasnain *et al*., 2005) comparing basidiospore concentrations in three different coastal cities determined that one (Jizan) had significantly higher concentrations than the other two locations (Jeddah and Dammam). This was not expected because Jizan and Jeddah are close, both situated on the coast of the Red Sea, whereas Dammam lies on the coast of the Persian Gulf. The researchers determined that the proximity of Jizan to the Yemeni border was influencing the basidiospore concentrations. This could be of relevance to the Irish context and opens up the idea of cross-border monitoring with Northern Ireland. Considering the impact of anthropogenic aerosols as well as bioaerosols is important, as aerosols and emissions do not respect political boundaries, only natural ones.

3 WIBS Real-time Monitoring Campaign

3.1 Campaign Overview

The data obtained from the manual counting of fungal spores using the traditional microscopy method were compared with the fluorescence channel outputs observed using a WIBS-NEO over the same date range (7 August to 15 September 2019). The sampling took place at the Technical University Dublin Kevin Street site, where both the Hirst volumetric trap and WIBS-NEO were located over the course of the campaign.

3.2 Fungal Monitoring Data

During the monitoring period, the single most commonly identified spore type was *Cladosporium*, which accounted for 66.3% of all fungal spores counted during the study period.

Ascospores constituted 13.9%, basidiospores 7.8%, *Alternaria* 4.7% and "other spores" 7.3% of all fungal spores identified. One large peak in total spore counts was observed over a 3-day period, with relative "troughs" of low concentrations seen 5 days before and 5 days after the peak. *Cladosporium* drove the concentration of total spores for the majority of the period, as it acounted for the vast majority of total fungal spores, as shown in Figure 3.1. One major exception to this was during the first week of the study, where a small rise in total fungal spore concentrations was seen during a time when *Cladosporium* concentrations were low. During this period, high increases in ascospore concentrations, as well as increases in the total amount of other spores, were observed, leading to this peak.

3.3 WIBS Monitoring Data

In parallel with the traditional sampling, the WIBS-NEO was used to sample particles and determine their fluorescence characteristics. During the 40-day campaign, a total of 56,818,969 particles were detected and categorised by the WIBS-NEO

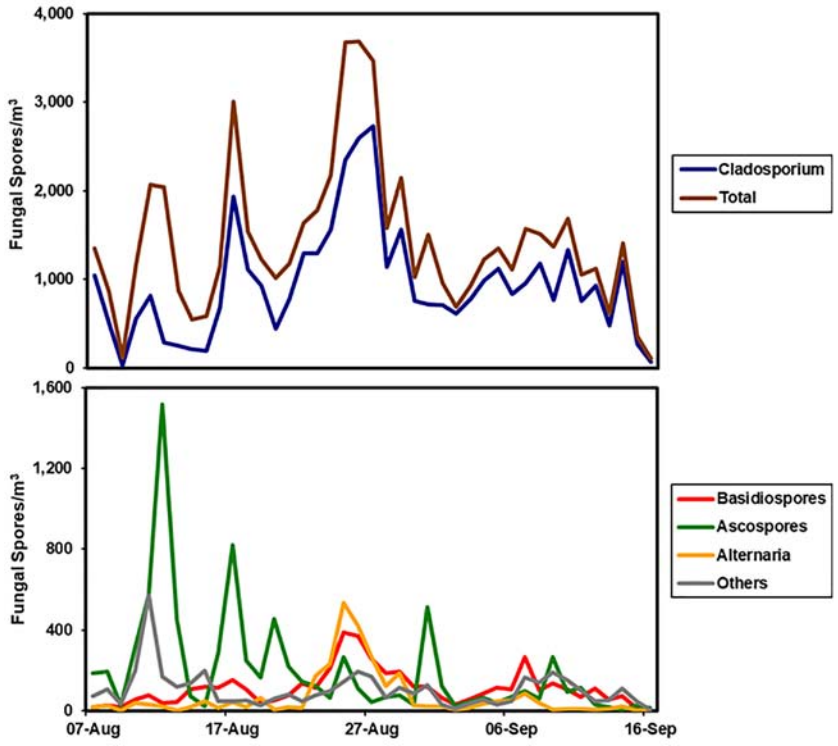

Figure 3.1. Daily concentrations of major fungal spore types and "other" fungal spores identified during the study period.

(Perring *et al*., 2015). Before the analysis could be conducted, the baseline fluorescence intensity of each channel was determined as follows.

The force trigger mode in the WIBS was initiated. This effectively causes the xenon flash lamps in the device to fire on empty space (within the optical chamber). This background measurement was continued for 5 minutes until sufficient data had been collected and hence a baseline threshold for each fluorescence channel calculated. These baselines were determined as the average value of the measured force trigger intensities (in each channel FL1, FL2 and FL3) plus three times the standard deviation of the values obtained. This is known as the 3-sigma (3σ) value and described as such throughout this document. Similarly, a value of 6-sigma would represent the average value of the force trigger fluorescence intensity plus six times the standard deviation.

Figure 3.2 depicts the daily concentrations and overall breakdown of all fluorescence categories (as described in Table 1.1) over the study period for 3-sigma, 6-sigma and 9-sigma filters. It also shows a set of pie charts showing the overall proportion each of the fluorescence categories contributes to the fluorescent particle distribution. One of the initial observations is that the AC category (pink) contains so few particles that it is not visible on any of the charts in this figure. In raw numbers, a total of 19,716 AC particles were detected throughout the study period at the 3-sigma level, but this is negligible in comparison with the other bands. For example, at the same sigma level, approximately 2.7 million B particles were detected over the same period. Another observation that can be made from Figure 3.2 is that, while at the 3-sigma level the C particles (orange) constitute a relatively low proportion of the total particles identified, once the fluorescence baseline is raised to the 6-sigma level, C particle concentrations drop dramatically in comparison with all other particles (from 9% to 1%).

The ratios of all other particles and bands appear to remain consistent. Looking at the bar charts at the various sigma levels, what stands out most clearly are the four “peak” days that appear at the 3-sigma level. When applying the 6-sigma filter level, the two peaks at the earlier part of the study period begin to show less prominently, while the two at the end of the study period are more conspicuous in relation to the other days around them. This effect is seen again at the 9-sigma level, where there now appear to be two peak days in the second week of September and a high concentration period appears in the third week of August. These high and low particle concentration days will be compared with fungal spore concentrations over the same period later in this chapter.

3.4 Comparison of Fungal Data from the Hirst Device and WIBS

Analyses of various fungal spore groups were compared with detected FBAPs. Increasing the fluorescence threshold from 3-sigma to 6-sigma, and from 6-sigma to 9-sigma, was required to reduce the impact of interfering particles (anthropogenic fluorescent particles) and to extract representative fungal fractions. BC-type particles were determined to be the most representative FBAP fraction for total fungal spore concentrations. Although several studies have highlighted the importance of the B and C fluorescence channels (FL2 and FL3) for certain fungal spore fractions (Healy *et al*., 2014), the majority of studies have linked the FL1 fluorescence channel to fungal spores or the A channel (Healy *et al*., 2012a; Hernandez *et al*., 2016; O’Connor *et al*., 2015a; Savage *et al*., 2017).

In our own study, very little or no correlation with fungal spores was observed for the majority of FL1-type particles. There are several potential reasons for this. Firstly, a range of different WIBS models (WIBS-3, WIBS-4, etc.) have been used in previous studies, and this study is employing yet another variation of the WIBS technology – the WIBS-NEO. Any possible differences in fluorescence sensitivity between previous WIBS models and the WIBS-NEO, even very minute ones, such as a slight increase in FL2 or FL3 channel sensitivity (compared with other channels), could result in a shift towards particles with B- and C-type fluorescence (Ila Gosselin *et al*., 2016).

Secondly, FBAPs detected by the WIBS in more complex urban environments show that anthropogenic or combustion particles favour the FL1 channel over the FL2 or FL3 channels (Savage *et al*., 2017; Yu *et al*., 2016). The study by Yu *et al*. (2016) using the WIBS-4 showed that anthropogenic particles such as black carbon almost exclusively possessed FL1-type fluorescence, inhibiting the differentiation of PBAPs

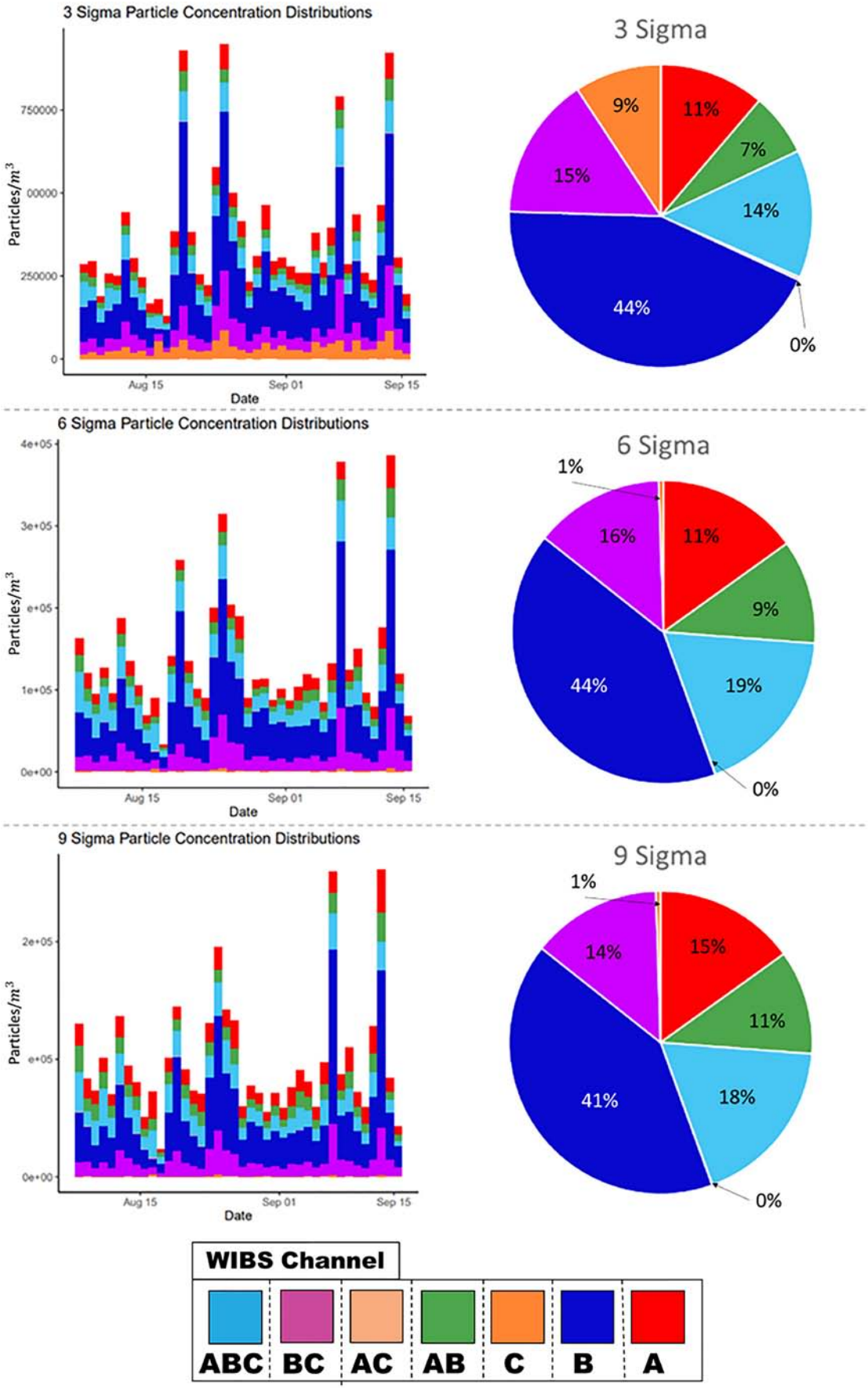

Figure 3.2. Concentrations of the WIBS fluorescent particle types at 3-, 6-, and 9-sigma levels during the study period.

from such anthropogenic pollution. A recent study by Forde *et al*. (2019) compared the WIBS-NEO with its predecessor the WIBS-4. A notable difference in fluorescence intensity was observed when examining various PBAP samples. This deviation was driven by higher fluorescence intensities detected in FL1. It is therefore possible that, because of the high fluorescence sensitivity of the FL1 channel and the urban environment of the sampling site, any potential detection of PBAPs in the FL1 channel could be masked by the fluorescence of anthropogenic aerosols.

BC particles of between 2 and 30 µm at the 9-sigma level showed a relatively good correlation with total fungal spore concentrations, yielding a Pearson correlation coefficient of 0.54 (Figure 3.3). Although the WIBS-NEO performed well in detecting peak fungal spore concentrations observed on 25 August and 14 September, there are notable periods in which BC particles failed to account for high concentrations of fungal spores. Periods where there are large peaks in total fungal spore concentrations that are not reflected in the WIBS-NEO data correspond to periods in which ascospore concentrations are high. This illustrates that some spore types are not being detected in the BC fraction.

As a result, a second subset of data was created using a new variable, “dry spores”, that did not include ascospores. This increased the correlation between total spores and BC particles from $r = 0.54$ to $r = 0.60$. A notable correlation was also observed between C-type particles and “dry spores” ($r = 0.50$). Further analysis was attempted to correlate prevalent fungal spore types with FBAP fractions. Both basidiospores and *Alternaria* spores exhibited notable correlations with BC-type particles when compared exclusively. Basidiospores correlated well with BC particles in the size range of 2–30 µm ($r = 0.66$), as illustrated in Figure 3.4.

Although a good correlation between the two devices was observed for basidiospores, there are periods when the WIBS data do not correlate with peaks in spore concentrations. Similarly, there are periods when peaks in BC particles do not correspond to increases in spore concentrations. As it is known that BC particles also correlate well with other PBAP types, albeit at lower fluorescence thresholds, these peaks

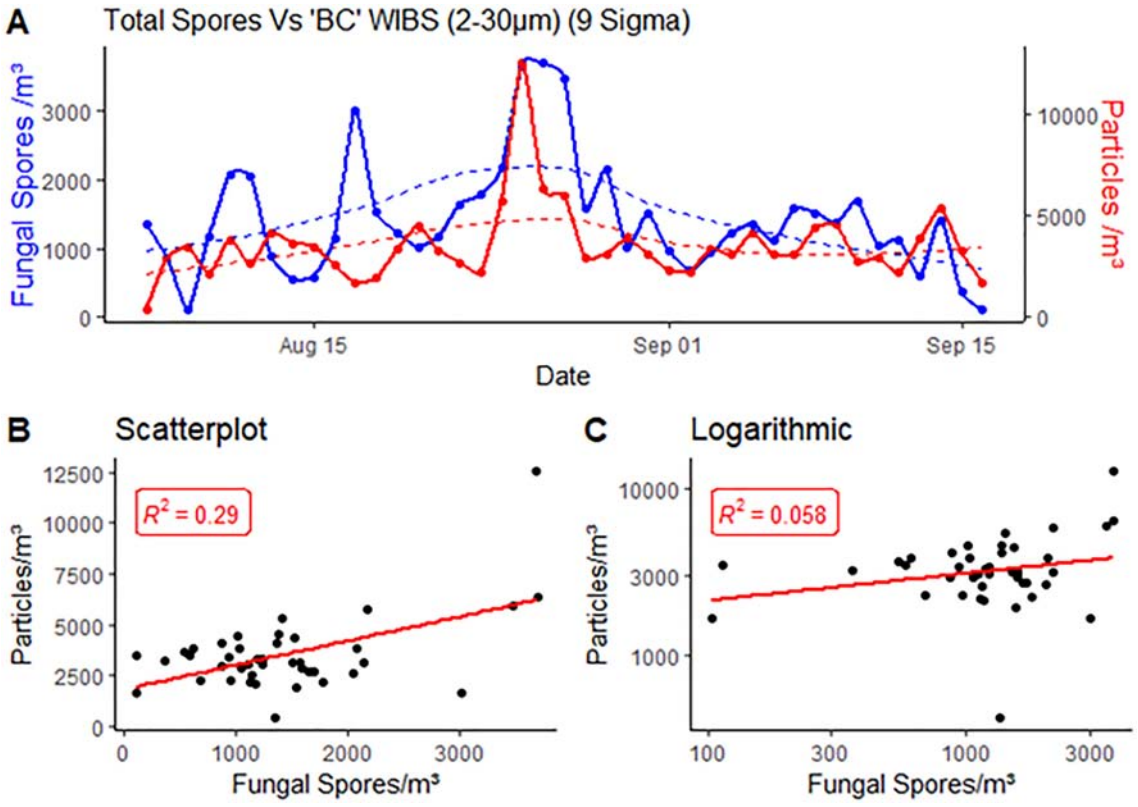

Figure 3.3. (A) Time series of Hirst device fungal spore and WIBS BC particle concentrations (daily); (B) linear scatterplot of daily values ($R^2 = 0.29$); and (C) logarithmic scatterplot of daily values ($R^2 = 0.058$).

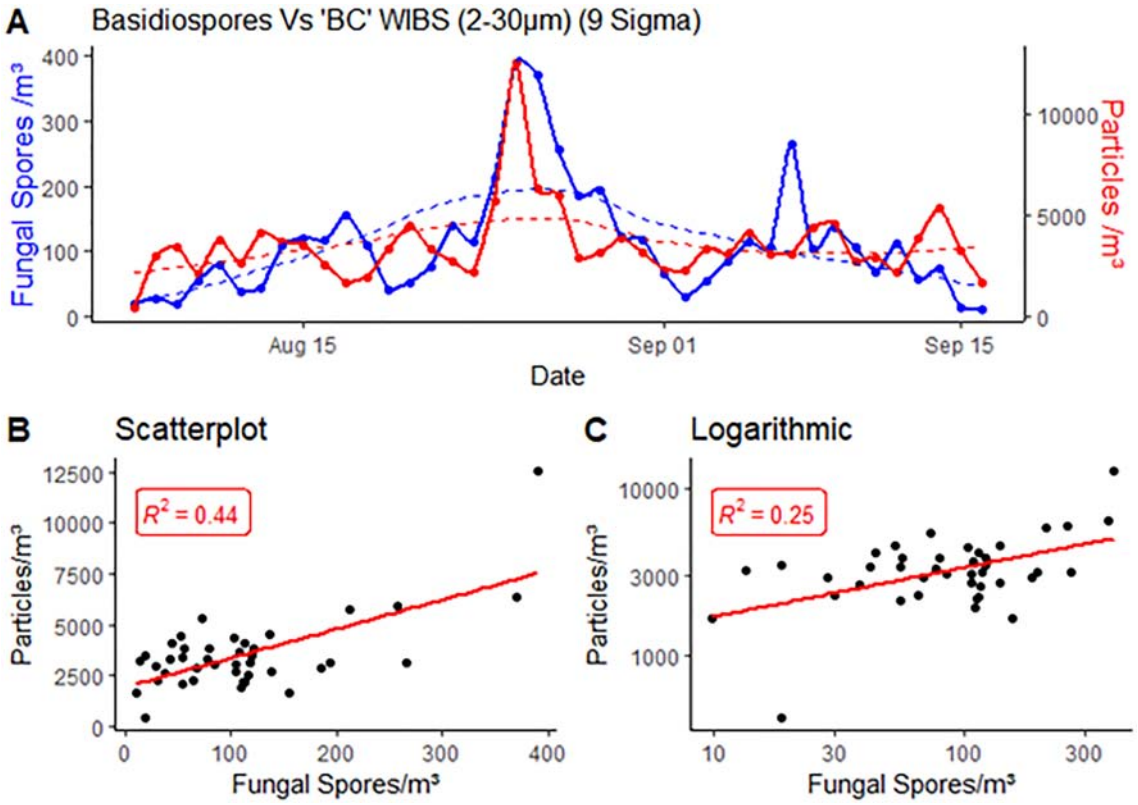

Figure 3.4. (A) Time series of Hirst device basidiospore and WIBS BC particle concentrations (daily); (B) linear scatterplot of daily values (R^2 = 0.44); and (C) logarithmic scatterplot of daily values (R^2 = 0.25).

that do not equate to basidiospore concentrations could be indicative of other PBAPs or potential interferences that are not removed using fluorescent filtering.

With regard to other fungal spore types, *Alternaria* correlated well with BC particles greater than 8 µm at the 6-sigma level (r = 0.6). A poor correlation was observed for *Cladosporium* spores, although they were the most prevalent spore type sampled. Several reasons for this have been discussed previously in the literature: *Cladosporium* spores have been found in previous studies to be commonly miscounted by multiple versions of the WIBS (Fernández-Rodríguez *et al*., 2018; Healy *et al*., 2014; O'Connor *et al*., 2015b). In particular, the WIBS has shown an inability to fully characterise sharp increases and decreases in *Cladosporium* concentrations. Several reasons for this have been put forward, including the physical characteristics of *Cladosporium* spores and their tendency to be released in clusters, which can lead to increased cell wall loss, as well as the photophysical properties of the spore, which could inhibit the absorption of light by bio-fluorophores within the cell (O'Connor *et al*., 2015a).

This spore type has typically also shown an association with FL1 (A) fluorescence. The likely contamination of FL1-type particles with anthropogenic interferences led to the WIBS being unable to differentiate *Cladosporium*-like particles from other FL1 particles (O'Connor *et al*., 2015a). In similar campaigns conducted in less diverse ambient environments, *Cladosporium* has shown considerable affinity for FL1 or A-type particles. Similarly, in a study by Healy *et al*. (2014), basidiospores showed considerably higher percentage contributions to total spore concentrations than *Cladosporium*. In that study, fungal spores showed stronger correlations with the FL2 and FL3 channels, corroborating the link observed between basidiospores and BC particles during the current study. This could explain why a significant correlation was observed for certain spore types and not for others.

3.4.1 Influence of weather on fluorescent particles and fungal spores

The impact of meteorological parameters on fungal spore and fluorescent particles was also investigated.

Figure 3.5 shows the daily trends in a number of weather parameters (pressure, grass minimum temperature, minimum temperature and wind speed) and the correlations with fluorescent aerosol particle (FAPs; i.e. any particle deemed fluorescent) concentrations over the campaign period.

The majority of fluorescent WIBS particle classes exhibited significant negative correlations with minimum temperature, grass minimum temperature and wind speed. Daily trends in and regression analysis of such parameters versus FAP concentrations are shown in Figure 3.5. This negative association with both temperature parameters during the late summer and early autumn months is indicative of ambient bacterial activity. Although removing WIBS particles below 2 µm diameter is likely to remove a large fraction of FAPs associated with bacteria, ambient bacterial concentrations have been shown to peak at size ranges up to 4–5 µm (Brągoszewska and Pastuszka, 2018; Gong *et al*., 2020). The main reason for this decline in FAPs could also be related to the significant correlation seen between grass minimum

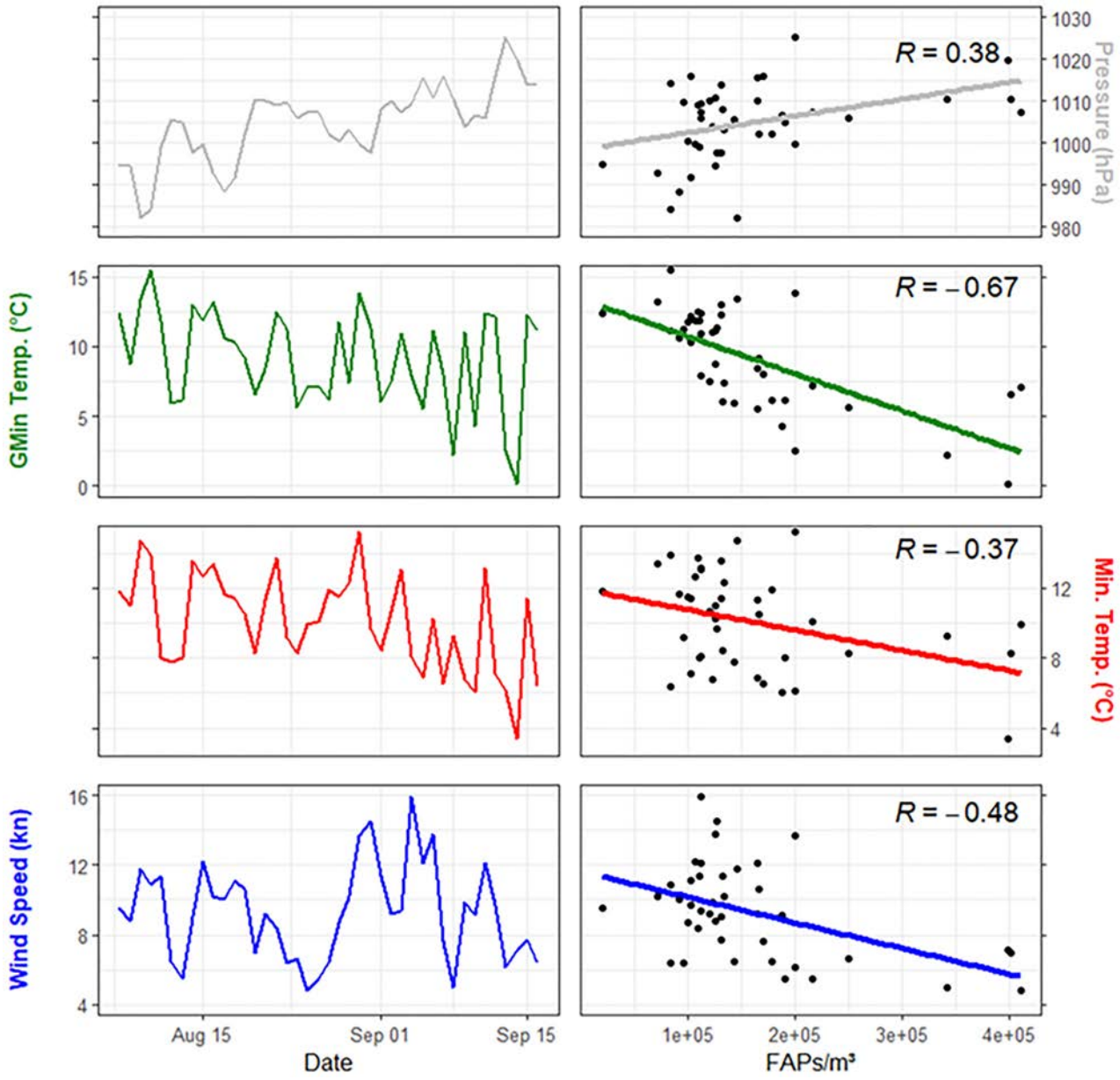

Figure 3.5. Daily trends in pressure (grey), grass minimum temperature (green), minimum temperature (red) and wind speed (blue) during the campaign and correlations between FAP concentrations and weather observations.

temperature, rain and wind speed, which all contribute to the transport and deposition of certain bioaerosols (Brągoszewska and Pastuszka, 2018; Davies and Smith, 1974; Hart *et al*., 1994; Oliveira *et al*., 2009). A notable positive correlation was also observed for FAPs detected by the WIBS with pressure. Such correlations with pressure have been well documented in past literature for many pollen and fungal spore types (Kruczek *et al*., 2017; O'Connor *et al*., 2014a), potentially indicating a degree of contribution of bioaerosols to the FAP fraction.

An initial investigation into the correlation between fungal spore concentrations and different meteorological parameters was also undertaken. This yielded unexpected results for ascospores. Traditionally, ascospores are considered "wet" spores (released in association with rain). However, the correlation coefficient between ascospore concentration and rainfall over the WIBS campaign period was found to be 0.01.

To further investigate this apparent anomaly, plots comparing ascospore concentration and rainfall were constructed. Figure 3.6A shows a time-series plot in which it becomes apparent that, while the two factors may not be correlated according to the correlation coefficient currently applied, as shown in the linear regression line and scatterplot in Figure 3.6B, there is an apparent, strong relationship between rainfall and ascospore concentrations. Visually, it appears that there is a delay, or lag, between increases in rainfall and increases in ascospore concentrations. To test this hypothesis, a cross-correlation test was performed. Cross-correlation measures the similarity between two datasets, as a function of the displacement of one relative to the other, such as a time lag. An example of its use would be investigating the correlation

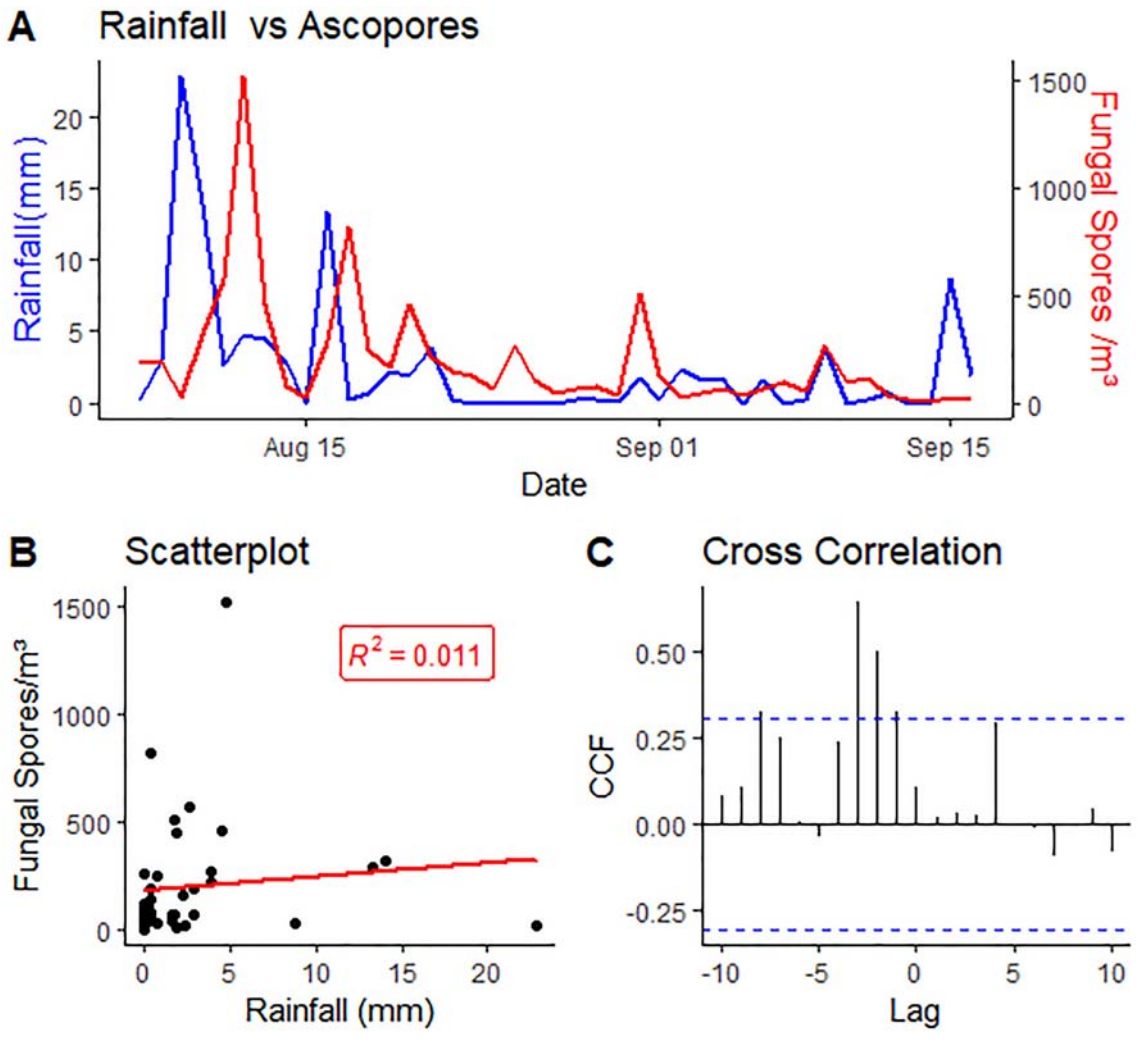

Figure 3.6. (A) Time series of Hirst device ascospore concentration and rainfall (daily); (B) linear scatterplot (R^2 = 0.011); and (C) cross-correlation lag regression plot.

between river pollution and crashes in aquatic fauna populations, as these events, if related, would not occur simultaneously (Croke *et al*., 2015).

The cross-correlation between ascospores and rainfall is shown in Figure 3.6C. It can be seen that, while the cross-correlation coefficient at a lag of 0 days was around 0.1, as the lag is adjusted, the cross-correlation coefficient increases for each day the lag in rainfall is increased, up until 3 days. At this point, the correlation between the two parameters begins to decrease again. The peak cross-correlation coefficient appeared to be at a lag of 3 days and the value was approximately 0.64. This is hard to tell from the graph provided. To further investigate the exact values, a grid of scatterplots, each lagging by 1 day more than the previous scatterplot, was constructed (Figure 3.7).

It becomes clear from the scatterplot grid that there is significant cross-correlation when a 2-day lag (0.51) and 3-day lag (0.64) are applied to the rainfall data. The apparent increase in correlation at an 8-day lag is a result of ascospore concentrations from the first major concentration peak aligning with rainfall values from the second major concentration peak.

This pattern indicates that a relationship between ascospores and rainfall does exist, even if not evident on statistical analysis, and offers scope for the development of new and more comprehensive statistical tests in the future relating to lagged data and

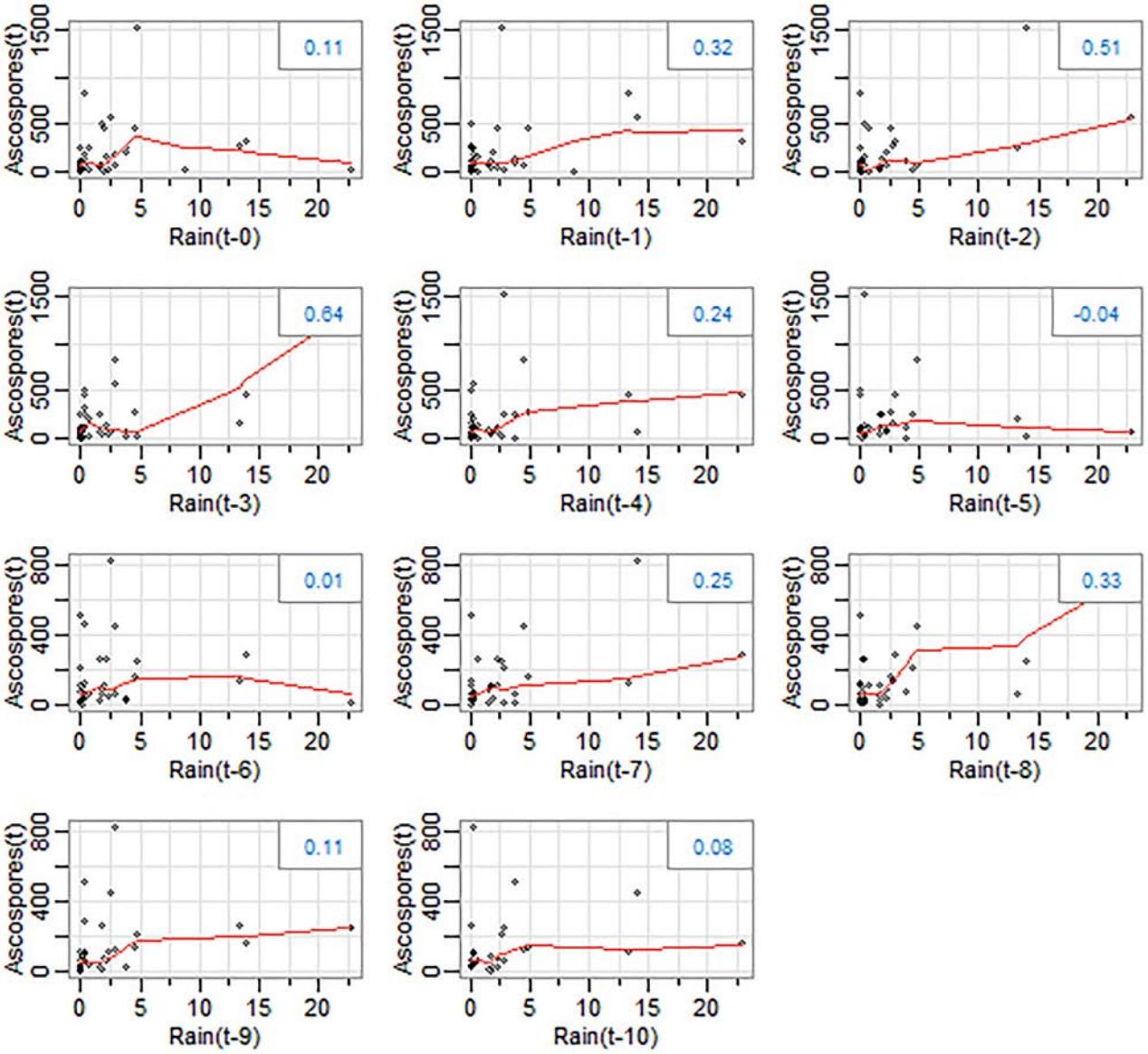

Figure 3.7. Grid of scatterplots showing an increasing lag in rainfall.

variable lags and using them directly for fungal spore concentration forecasting and modelling.

This analysis was then applied to the WIBS-NEO data, to identify whether different meteorological parameters had an impact on the levels of correlation between fungal spore concentrations and WIBS-NEO particle outputs. After a comprehensive analysis of various parameters and concentration thresholds, one strong pattern identified was the relationship between ascospores and AC-type particles. At a 9-sigma filtration, and particle size resolution of between 2 and 30 µm, this relationship had been previously noted, with a relatively high Pearson correlation coefficient ($r = 0.68$). But when meteorological parameters were taken into account, much higher levels of correlation were identified (Figure 3.8).

In Figure 3.8 the result of plotting ascospores against AC-type WIBS particles can be seen, when "days of rainfall" (> 1 mm in a day) were removed from the dataset. By removing these days, the Pearson correlation coefficient rose to $r = 0.93$. The very strong positive correlation, present only when rainfall days are removed, shows both the relationship between ascospores and rainfall and the possible impact that rainfall may have upon the sensors within the WIBS-NEO.

Diurnal comparisons

When we look at diurnal data (average daily cycle of particles) for the WIBS-NEO and fungal spores at the site in Dublin, a clear pattern is visible in WIBS-NEO data. When all fluorescent WIBS particle types are included (3-sigma level of filtration), the majority of WIBS categories follow the same trend (Figure 3.9). This does not follow the expected diurnal trends seen in previous studies. Although other studies have seen

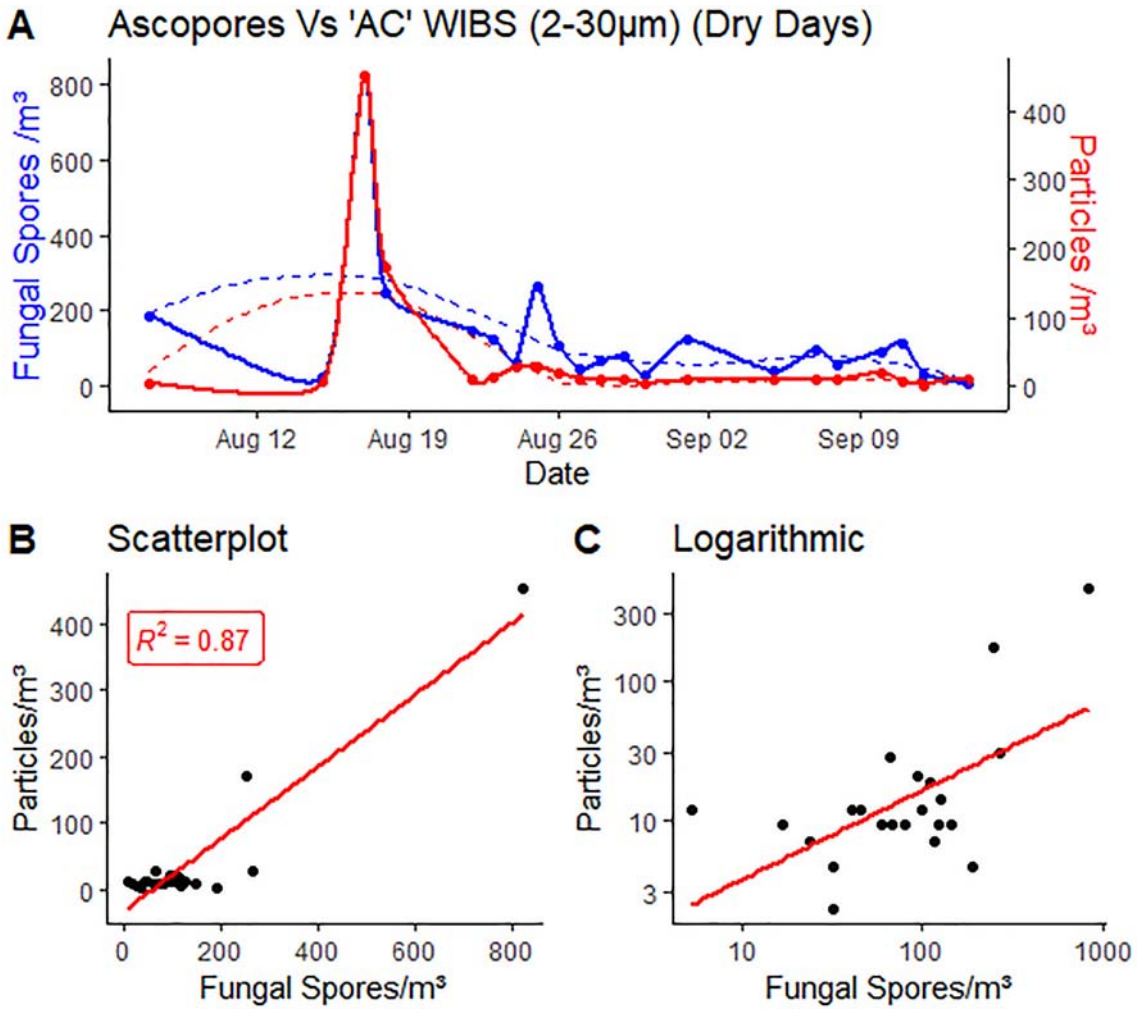

Figure 3.8. (A) Time series of Hirst ascospores and WIBS AC particle concentrations (days with 1 mm of rainfall or less); (B) linear scatterplot of daily values ($R^2 = 0.87$); (C) logarithmic scatterplot of daily values.

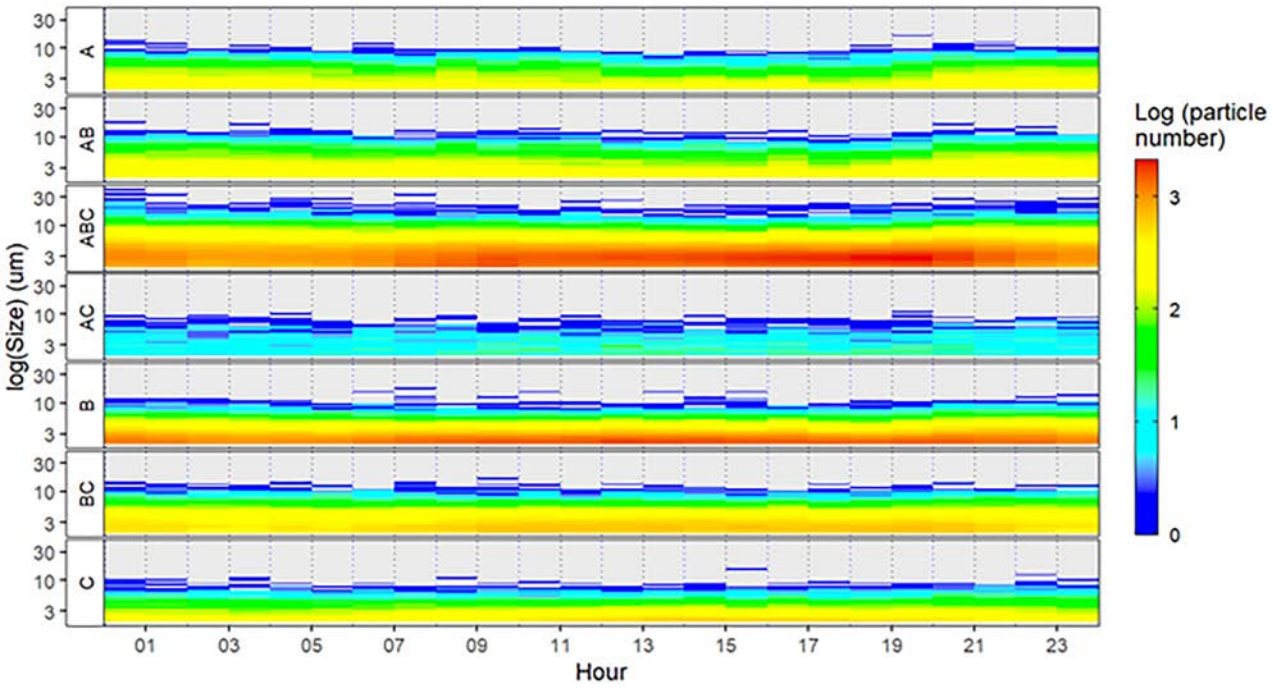

Figure 3.9. Log of diurnal WIBS particle distributions.

increases in fluorescent particle concentrations during the night, reaching a peak in the early morning before dawn, between 2 a.m. and 6 a.m., the data from the Dublin site show exactly the opposite trend. As the sun sets, the concentrations begin to decrease, reaching a minimum concentration between 2 a.m. and 6 a.m. While other studies have seen the concentration decrease as the sun comes out, with minimum values reached in the mid-afternoon, the Dublin data appear to show an increase in concentration as the sun rises, reaching a peak at 9 a.m., and then dropping during the afternoon, with the decrease continuing until 7 p.m.

The potential impact of anthropogenic pollution was considered, and a second dataset was created in which only particles with a diameter of 2 µm or above were included, as this would remove the majority of the anthropogenic pollution and 2 µm is the minimum size for visible optical analysis of fungal spores in the laboratory. In this dataset, we see diurnal patterns that are far more reflective of expected WIBS particle outputs and can be seen in numerous studies (Abdel Hameed *et al*., 2009; Calderon *et al*., 1995; Garland *et al*., 2009; Huffman *et al*., 2012; Khan *et al*., 2016; Saari *et al*., 2015).

To examine the possible diurnal correlations between individual WIBS bands and fungal spore types, and to account for possible discrepancies in sampling volume between methods, normalised WIBS particle concentration curves were created (Figure 3.10). Each graph shows all seven categories, and one separate band is highlighted in colour in each chart, so that the relative positions of each band can be easily distinguished. Two clear “pairs” of WIBS bands become visible when the data are viewed in this way. First are the B and BC bands, which show the highest seasonal correlations with basidiospores and other “dry spores”. These spore types both fluoresce in the FL2 channel. Both follow the same pattern of rising to a sharp peak in the early morning, and slowly decreasing in concentration over the course of the day, before reaching their lowest concentration in the middle of the night, before dawn.

The second “pair” of WIBS bands are the A and AB bands (and, to a lesser extent, AC). These bands all share the FL1 fluorescence channel and show the highest seasonal correlations with the “wet” ascospores. These particles show a very clear bimodal pattern. A first peak in the early morning, similar to that seen in the B and BC bands, is followed by a drop in concentration throughout the day. The difference is that in the evening, at around 6 p.m., concentrations begin to rise once again and reach a second equal peak around 7 p.m. This bimodal distribution was noted for comparison with diurnal fungal spore concentrations, which were also determined over the same study period.

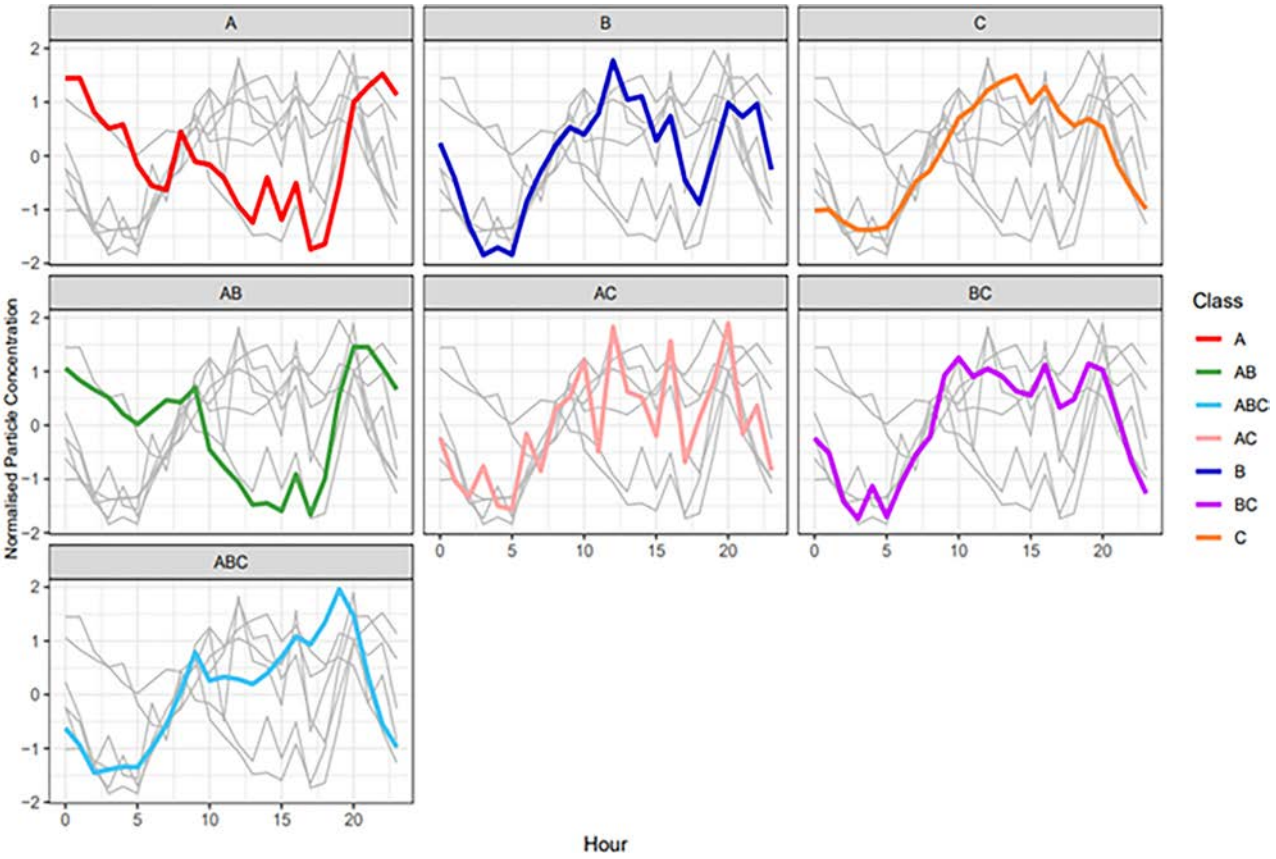

Figure 3.10. WIBS-NEO diurnal charts for each band (particles > 2 µm, 3-sigma).

Additionally, a short, sharp, small peak in fluorescent particles is seen in all seven bands at 3 p.m. This is the only diurnal trend that is identifiable in every single band (though the pattern is very weak in the C band). It is possible that an anthropogenic source at the same time every day could be responsible for this reading.

As fungal spores are counted using a Hirst-type device, they can be analysed at a time resolution as short as 1 hour. This allows the creation of diurnal plots, the outputs of which are shown in Figure 3.11. The normalised diurnal spore concentration curves for *Alternaria*, ascospores, basidiospores and *Cladosporium* show very different diurnal trends. Basidiospore diurnal trends did not follow the expected early peak, and concentration declined throughout the day, as was seen with B and BC particles. Ascospores, however, did appear to have a bimodal distribution, similar to that of the A and AB bands.

In fact, the presence of two daily peaks in the data, seen in the A and AB bands, particularly reflects the shape of the diurnal ascospore concentration curve. Both the WIBS-NEO bands (> 2 µm) and the ascospore diurnal charts see similar drops in average concentrations around 8–9 p.m. This analysis took place during August and September, meaning that the decreases in particle and fungal spore concentrations are aligned with the setting of the sun in the evening. At the start of August the sun sets from 9:20 p.m., and by the middle of September the sun sets at 7:40 p.m., in each case aligning with predicted decreases in particle concentrations. This could be one explanation for the decrease in concentrations around sunset, with "wet spores" able to proliferate at the start and end of the day, when there is sunlight along with damp and dew, but not in the heat of the day, when the atmosphere is too dry and devoid of moisture (Almaguer *et al*., 2014).

To further investigate the apparent relationship between ascospores and the A and AB particles, a normalised distribution curve (Figure 3.12) was constructed. This shows that the evening peaks of spores and particles coincide precisely, with ascospore concentrations rising much higher than the WIBS particles during the morning peak, as well as "leading" the WIBS particles by about 3 hours. Many factors

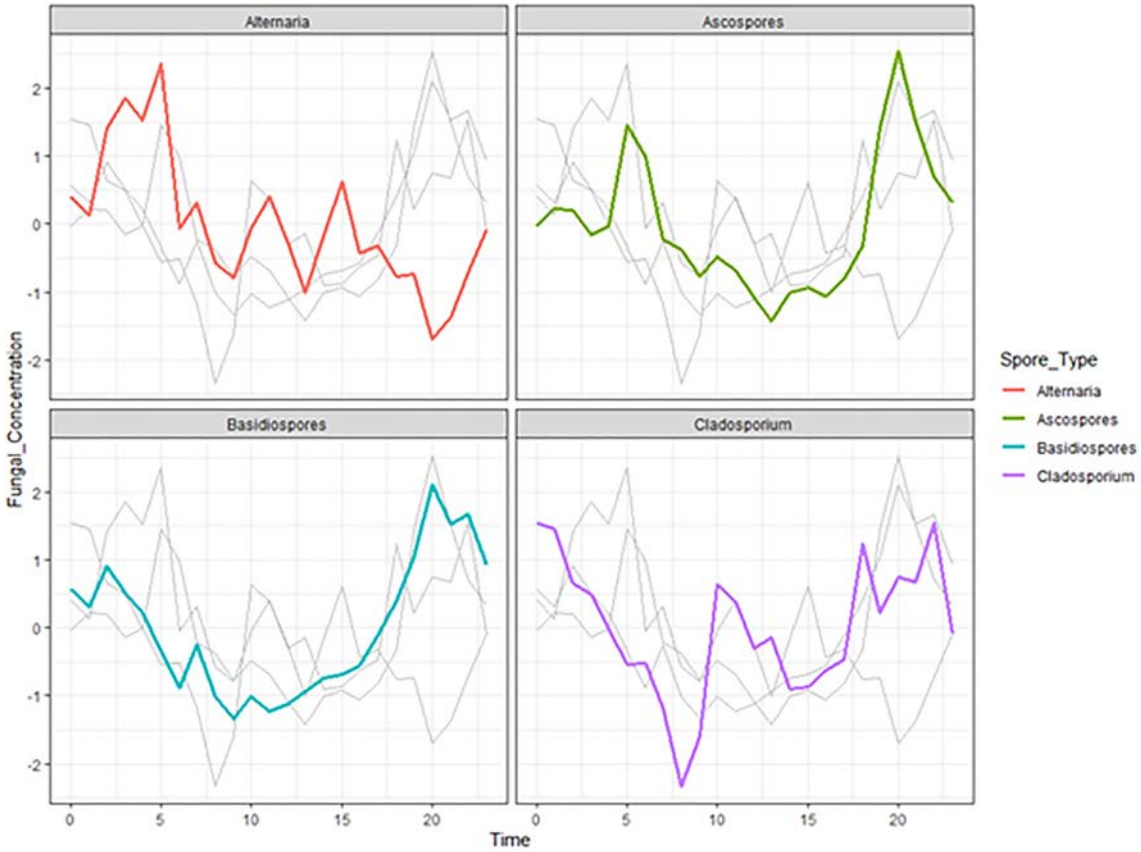

Figure 3.11. Diurnal charts showing concentrations of major fungal spore types.

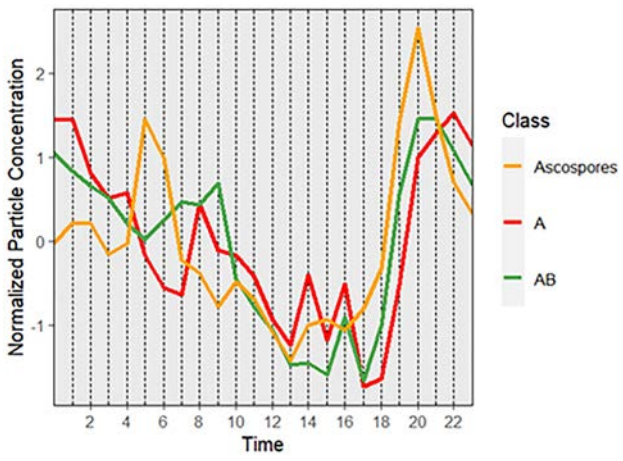

Figure 3.12. Normalised diurnal concentrations of particles in A and AB WIBS bands and ascospores.

could account for the apparent 3-hour delay in the morning, not least the devices' very different sampling methods and the fact that, as the Hirst device has an inlet width of 2 mm, and rotates at a rate of 2 mm per hour, accuracy is diminished at this smaller time resolution (Apangu *et al*., 2018; Orlandi *et al*., 2014).

3.5 Application for Monitoring Other Bioaerosols

The WIBS has also shown promising performance for the detection and monitoring of different pollen taxa (Healy *et al*., 2012a; O'Connor *et al*., 2014b). During the Dublin monitoring campaign, pollen grains were also sampled and recorded using the same optical Hirst–microscopic analysis method used for the traditional monitoring of fungal spores. Comparisons of major pollen types with WIBS particles were also made in efforts to evaluate the WIBS's suitability for monitoring pollen, as well as fungal spores. Good correlations were observed for both total ($R^2 = 0.6$) and Urticaceae ($R^2 = 0.64$) pollen with BC particles greater than 10 µm in size at 6-sigma, as illustrated in Figure 3.13.

Thus, there is potential for such an instrument to be used for monitoring both fungal spores and pollen. Indeed, given the ability of the WIBS to sample all aerosol particles, it could have a use as an air quality monitoring instrument rather than as a specific bioaerosol monitoring device.

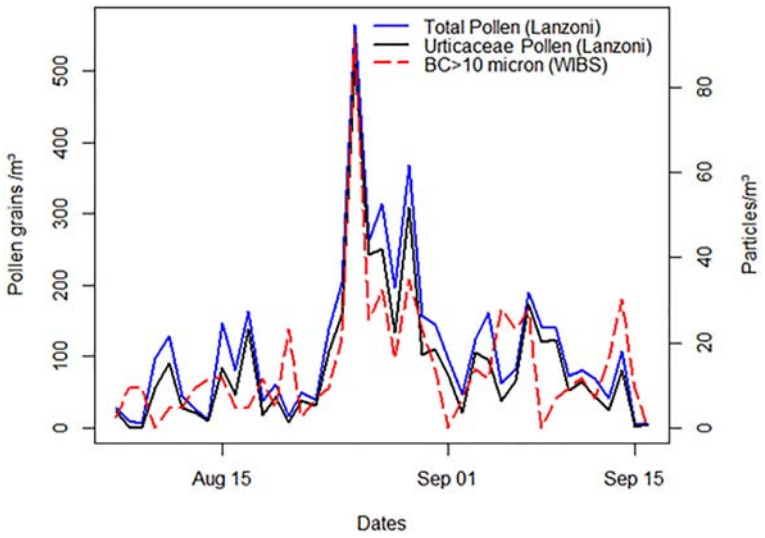

Figure 3.13. Time series for daily total and Urticaceae pollen concentrations (Hirst, or Lanzoni, device) and daily BC particle concentrations >10 µm at 6-sigma (WIBS) recorded in the 2019 Dublin campaign.

4 IBAC-2 Real-time Monitoring Campaign

4.1 Campaign Overview

Another device we tested for its suitability for detecting fungal spores as part of the project is the IBAC-2 (a successor to FIDO B2 and the original IBAC). The IBAC is a UV-LIF continuous air monitor initially developed by ICx Biodefense for detecting potential threats related to biological aerosols and acting as an early warning system. It is commercialised by FLIR Systems (FLIR, 2021). Similarly to the WIBS, it can differentiate between biological and non-biological particles via elastic scattering (photomultiplier tubes) and particle fluorescence using a 405-nm laser as an excitation source. Particles are drawn into the device at a rate of 3.8 L/min. They pass through an optical illumination region, where the 405-nm laser excites the particles. The size and concentration of particles are measured from the light scatter. If the integrated fluorescence emitted by a particle falls between 450 and 600 nm, and exceeds a pre-set threshold, it is determined to be fluorescent/biological. Individual particle analysis is possible at a rate of up to 500,000 particles/L, allowing 1,500,000 particles/min to be individually interrogated, as the maximum count rate is 25,000 particles/s.

The device also categorises particles into two sizes: “small” (0.7–1.5 µm in diameter) and “large” (1.5–10 µm in diameter). Thus, there are four different groups defined by the instrument: “small biological” (fluorescent particles between 0.7 and 1.5 µm), “large biological” (fluorescent particles between 1.5 and 10 µm), “all small” (all particles between 0.7 and 1.5 µm) and “all large” (all particles between 1.5 and 10 µm). The device can potentially detect spores and bacteria, among other bioaerosols. Primary customers of the first IBAC included homeland security and defence agencies (Anchlia, 2015; DeFreez, 2009; Jonsson and Kullander, 2014; Pazienza, 2013; Santarpia *et al*., 2013).

The advantages of the IBAC over other biological particle sensors and detectors include its ability to work more efficiently and to run continuously for long periods. A disadvantage of the IBAC is possible confusion arising when detecting particles from anthropogenic sources, as the device occasionally struggles to correctly determine whether or not some particles from these sources are biological in origin (FLIR, 2021).

The IBAC-2 operated almost continuously (7 August to 15 September 2019), with the exception of 28 August.

4.2 IBAC-2 Monitoring Campaign

Fungal spore concentrations were compared with biological particles detected by the IBAC-2 over the campaign period; however, the correlation between the two methods was poor, as shown in Table 4.1. No significant correlations were found between the IBAC-2 particles and any of the fungal spore types. The highest correlation, of 0.2 between *Alternaria* and small biological particles (0.7–1.5 µm), is not of use, given that small particles are categorised as below 1.5 µm, whereas *Alternaria* is the largest of the studied spore types, with sizes of 2 µm and above (generally far larger). All other Pearson correlation values were

Table 4.1. Pearson correlation coefficients for concentrations of IBAC-2 particles and fungal spores

	Particle type			
Spore type	Small	Large	Small bio	Large bio
Cladosporium	0.01	–0.15	0.06	–0.07
Alternaria	0.09	–0.08	0.2	0.1
Ascospores	–0.08	–0.04	–0.1	0
Basidiospores	0.02	–0.18	0.11	–0.04
Other spores	–0.24	–0.2	–0.18	–0.12
Total spores	–0.03	–0.18	0.03	–0.06

below 0.2 (a correlation of 0.11 between basidiospores and small biological particles being the next highest), showing that, at least in this study, the instrument was not a strong indicator of fungal spore fructification levels.

Figures 4.1 and 4.2 are box plots of daily IBAC-2 particle concentrations over the course of the monitoring campaign. Each box plot is made up of 24 points, each representing the average value for each hour of the day, in order to reduce the massive number of outliers occurring when plotted at the maximum temporal resolution.

The peak point in IBAC-2 particle concentrations was on 9 August. This occurred during a period of extremely heavy rainfall; when looking at data from other sources, such as the WIBS, and fungal spore concentrations at the time, we can conclude that, while the rainfall supressed fungal spore growth, the IBAC-2 recorded the rainfall as an increase in "particulate matter". During the main peak in fungal spore concentrations in the last week of August, the IBAC-2 did not noticeably track or predict it, resulting in the low correlation coefficients seen in Table 4.1.

4.3 Comparison Between Real-time Devices

The WIBS-NEO and IBAC-2 were both run over the same period in summer 2019, allowing a direct comparison between the two sensors. While both devices use similar technology, there is a significant difference in the quantity of information they output. This is due to the WIBS monitoring a wide range of particle sizes (0.5–30 µm) and outputting them as single particle data. The IBAC-2 has two defined particle size bands: 0.7–1.5 µm and 1.5–10 µm. Thus, the IBAC data are far more agglomerated/aggregated. Equally, individual particle fluorescence intensities are determined by the WIBS (with two excitation wavelengths and emission bands) while the IBAC-2 uses only one excitation source.

Table 4.2 shows the correlation coefficients for comparison of the two instruments. While the WIBS

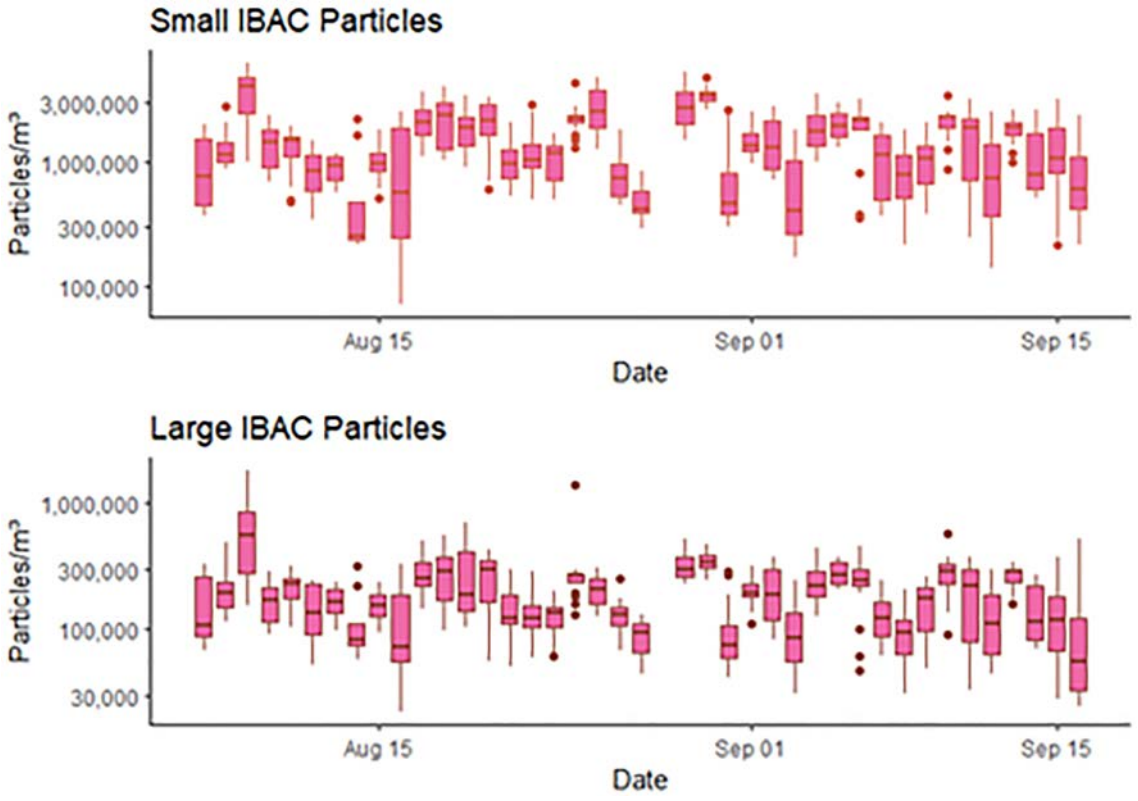

Figure 4.1. Box plots showing daily concentrations of all small (0.7–1.5 µm) and large (1.5–10 µm) particles detected by the IBAC-2.

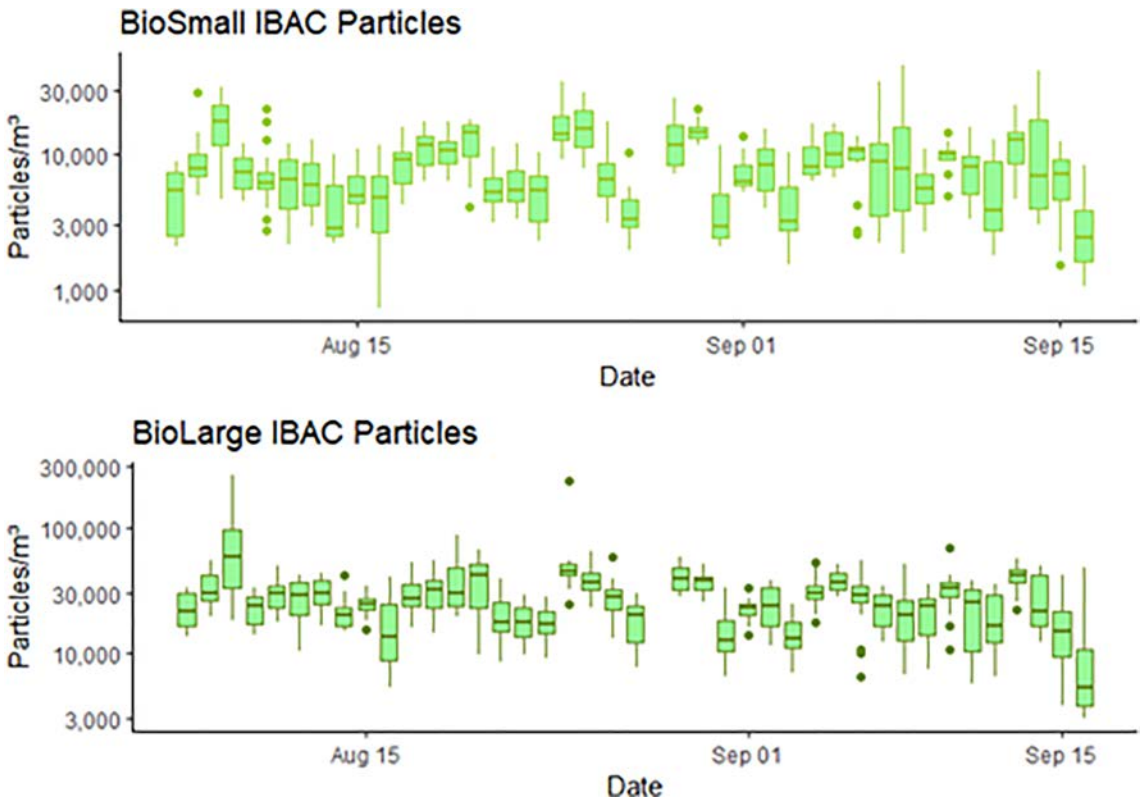

Figure 4.2. Box plots showing daily concentrations of small biological (0.7–1.5 µm) and large biological (1.5–10 µm) particles detected by the IBAC-2.

Table 4.2. Pearson correlation coefficients for concentrations of IBAC-2 and WIBS-NEO particles

	IBAC particle type			
WIBS particle type	**All small**	**All large**	**Small bio**	**Large bio**
All small	0.6*	0.49*	0.6*	0.44*
All large	0.67*	0.63*	0.7*	0.6*
Small fluorescent	0.21	0.07	0.46*	0.26
Large fluorescent	0.21	0.11	0.51*	0.32

***Correlation is significant at the $p \leq 0.05$ level.**

is capable of monitoring a larger range of particle sizes than the IBAC-2, its data were size filtered for this comparison, so the size ranges and groups detected by the IBAC-2 remain true for the WIBS in this analysis. There is a relatively good level of correlation between the two machines, particularly when comparing the "small biological" particle output of the IBAC-2 with the different outputs of the WIBS.

Correlation coefficients of between approximately 0.5 and 0.7 can be seen for small biological IBAC groups compared with those of the WIBS. It should be noted that fluorescent particles in the WIBS groups reflect any particle that fluoresces.

A direct comparison of a combination of the two size bands for total particles, fluorescent particles and non-fluorescent particles was also undertaken, as shown in Figure 4.3. Overall, the IBAC-2 sampled more particles than the WIBS. The IBAC-2 recorded 1.5 times more particles than the WIBS, which may be due to differences in the flow rate of the WIBS-NEO (0.3 L/min) compared with that of the IBAC-2 (3.8 L/min). Equally, while each particle analysed in the WIBS-NEO is interrogated by both the sizing laser and two independent xenon flash lamps, the IBAC-2 deduces all sizing and fluorescence information from just the one 405-nm laser. Thus, the potential for missing particles in the IBAC-2 is far lower than

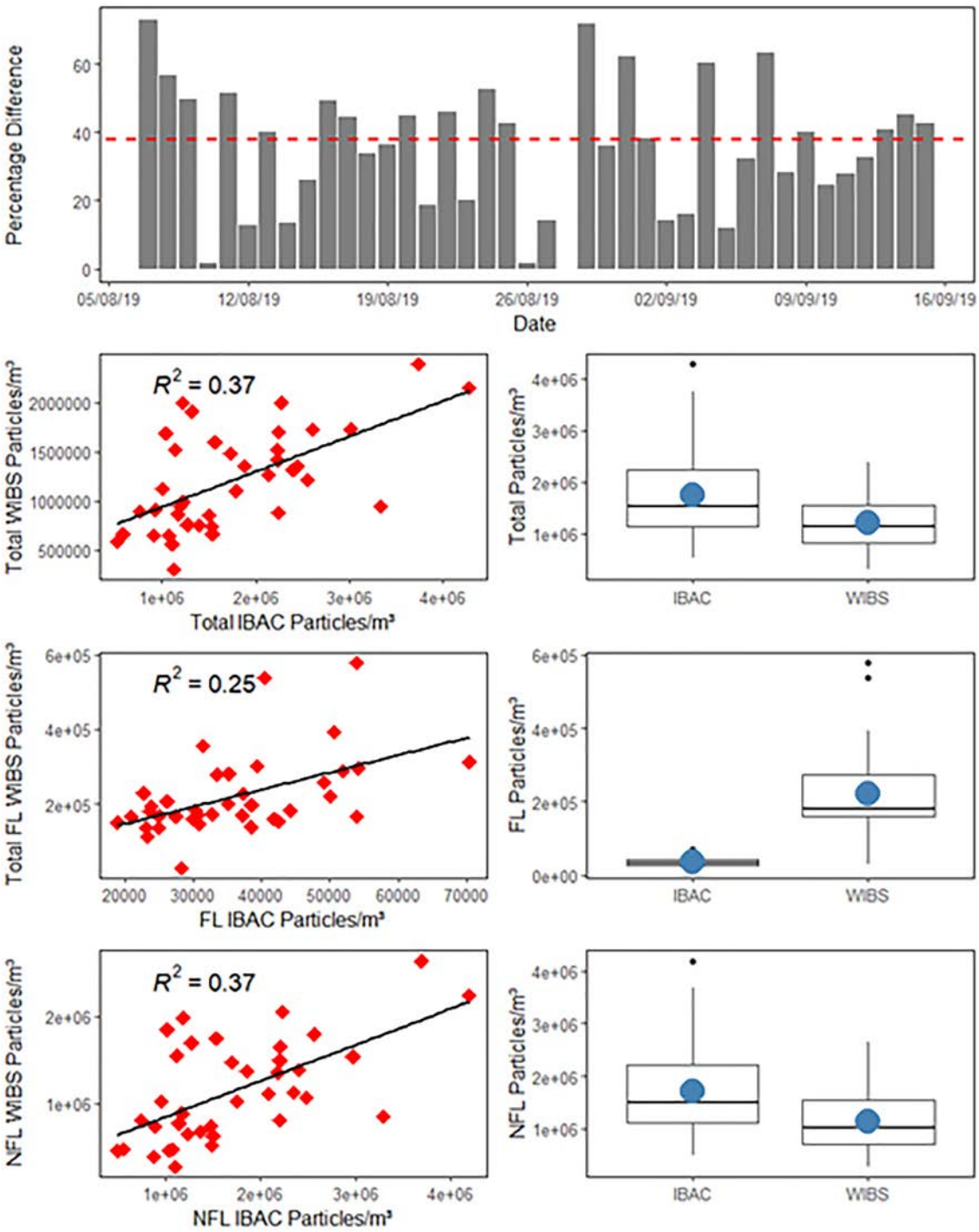

Figure 4.3. Comparison between the WIBS-NEO and the IBAC-2 of total, fluorescent and non-fluorescent particle concentrations in the size range 0.7–10 µm.

in the WIBS, given that the sequence of analysis involves fewer variables, less instrumentation, etc. Similarly, taking into account the fact that the IBAC was created for biological weapon detection, its efficiency at counting smaller particles may be greater than that of the WIBS. While the counting efficiency of the WIBS has been estimated (Healy *et al*., 2012b; Lieberherr *et al*., 2021), this has not been carried out for the IBAC-2. Thus, this is only a potential cause of the discrepancies observed between the two devices. Interestingly, the WIBS-NEO recorded a greater concentration of particles in the larger size

range (1.5–10 µm), which is potentially explained by the WIBS being designed to detect much larger particles, making it more efficient at sampling particles at the 10 µm size limit of the IBAC-2. Similarly, more fluorescent particles were detected by the WIBS, with the IBAC-2 recording more non-fluorescent particles. This is due to the WIBS possessing more excitation and emission channels than the IBAC-2 and therefore being capable of detecting fluorescent particles that go undetected by the IBAC-2. The greatest agreement between the two instruments was seen for non-fluorescent particles in the range 1.5–10 µm ($r = 0.67$) and fluorescent particles in the range 0.7–1.5 µm ($r = 0.60$). In efforts to improve the correlation between the two instruments, individual fluorescence channels of the WIBS were compared directly with the IBAC-2. The highest correlation was observed for the FL1 channel ($r = 0.4$), specifically for fluorescent particles between 0.7 and 1.5 µm ($r = 0.6$). Although the FL3 channel is more similar to the IBAC-2 in terms of excitation and emission wavelengths, the increased sensitivity of the FL1 channel in the WIBS-NEO appears to be more representative of the ambient FAPs detected by both instruments.

4.4 Comparison of IBAC and WIBS in Detecting Other Pollutants

In an attempt to identify other potential uses for the IBAC-2, the coefficients of determination of common anthropogenic pollutants in the area against the device outputs over the monitoring campaign period were calculated. There was little or no relationship for most pollutants (nitrogen oxides $R^2 = 0.009$, fine particulate matter $R^2 = 0.19$), whereas there was a stronger relationship between dust and particles detected by the IBAC-2 (Figure 4.4). This indicates that the lower concentrations of fungal spores detected by the IBAC-2 could be due to the IBAC also detecting particles from anthropogenic sources and that potentially the IBAC-2 could be used for measuring these pollutants in the future.

It should be noted that the pollution data were taken from a nearby EPA monitoring station and thus will be more influenced by local sources than the IBAC-2 data. Analysing the IBAC-2 outputs and the EPA particulate matter measurements from the same location could lead to a greater understanding of how the IBAC-2 performs and how its fluorescence

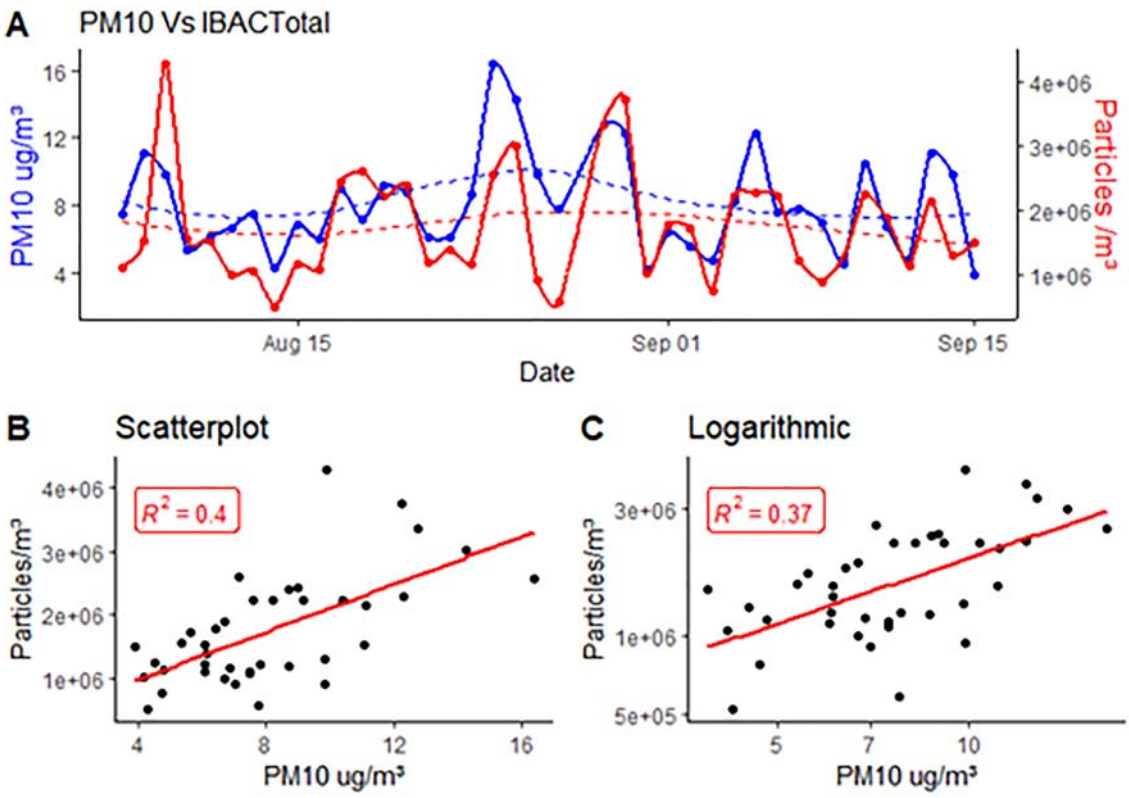

Figure 4.4. Comparison of concentrations of IBAC-2 particles and PM_{10} (dust) emissions during the monitoring campaign.

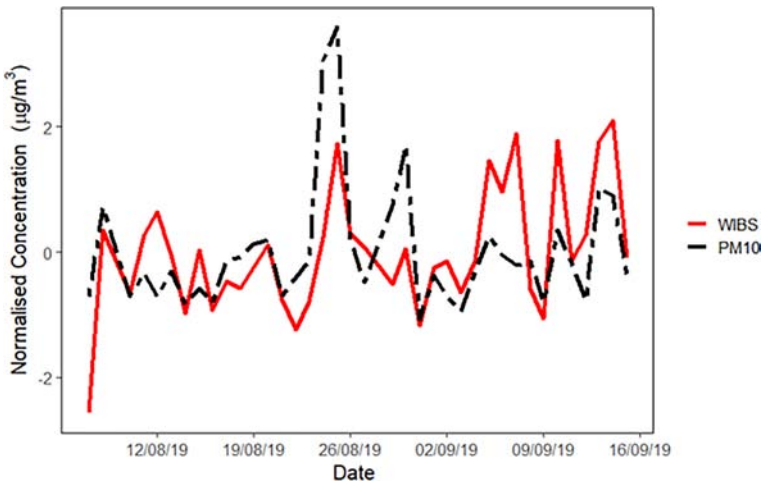

Figure 4.5. Normalised comparison of concentrations of WIBS particles and PM_{10} (dust) emissions during the monitoring campaign.

measurements could be used to determine air quality and the Air Quality Index.

Similar trends were found when comparing concentrations of WIBS particles with PM_{10} (dust) concentrations (Figure 4.5). Again, it would be of interest to evaluate the WIBS's potential as an instrument for measuring air quality, particularly as several anthropogenic aerosol particles fluoresce (polycyclic aromatic hydrocarbons and secondary organic aerosols) and could potentially be distinguished by the WIBS.

5 Fungal Spore Forecasting and Modelling

Fungal spores are considered some of the most important aeroallergens, triggering allergenic symptoms and further exacerbating existing respiratory conditions. Predicting periods when ambient concentrations of allergenic fungal spores will be high would provide an important resource for allergy sufferers and those suffering from respiratory conditions such as asthma. As previously discussed, fungal spore calendars can be used as a rough graphical approximation of general/average seasonal trends in fungal fructification and depict periods when exposure is likely to be high. However, the resolution of such methods is relatively low and they do not provide specific data for upcoming days and weeks. Thus, more sophisticated mathematical methods need to be applied to fungal spore monitoring data to predict future concentrations more accurately. However, these algorithmic approaches do require sufficient quantities of fungal and meteorological data to construct and train the models appropriately. To date several regression and classification models have been developed for specific fungal spores. The Dublin monitoring site has the most sampling data available, being the site first established within the network. As a result, this dataset has a greater potential for developing more accurate models than those from the other sampling locations.

Typically, prediction models are developed for specific fungal spores of concern. In this case, allergenic fungal spores represent the most concerning fungal spore types, owing to the prevalence of respiratory conditions in Ireland (Asthma Society of Ireland, 2022). *Alternaria* and *Cladosporium* spores are two of the most prevalent and allergenic fungal spores (Kasprzyk *et al*., 2021), and both have been readily observed at all FONTANA monitoring sites. The remainder of this chapter will describe several predictive model types that have been developed for *Alternaria* and *Cladosporium* ambient spore concentrations. These modelling techniques can be applied to other allergenic and/or pathogenic fungal spores in future as sampling efforts continue and expand. The input variables varied depending on fungal spore type and model algorithm. This is not unexpected, as fungal spore release mechanisms are largely dependent on a range of environmental factors. The importance of predictor variables has been shown to vary between different fungal species.

5.1 Classification Models

Both classification and regression-type models were developed for *Alternaria* and *Cladosporium* spore forecasting. *Alternaria* and *Cladosporium* spore concentration data from the period 2017–2019 sampled from the Technical University Dublin Kevin Street site were used to develop several classification models. Meteorological data were obtained from the Met Éireann website (Met Éireann, 2022) and from the Dublin Airport, Oak Park (Carlow), Cork Airport (Cork) and Bemullet (Sligo) weather stations. All meteorological input variables are defined as daily mean values unless specified. Classification models predict a characteristic or label a class, e.g. “high”, “medium”, “low”. Classification models differ from regression-type models, in which a numerical output is predicted. Compared with other aerobiological networks that have been undertaking sampling for decades, the Irish dataset available is relatively limited. As a result, the quality of model training data is low in comparison with other, more extensive, European networks. Since classification models do not predict a precise numerical result, but instead predict a broader class, they tend to be more accurate for the size of the Irish dataset.

Typically, models are trained with approximately 80% of the monitoring data and then validated using the remaining data, which is known as supervised learning. However, in this case, the models were developed and trained using all the Dublin monitoring data. The models were then tested using the Carlow, Cork and Sligo site data, during which the predicted levels were compared with the actual observed levels. Model input variables initially included both past daily *Alternaria* or *Cladosporium* concentrations (dependent variables) and various meteorological and phenological parameters. These parameters are summarised in Table 5.1. Each model algorithm assesses the input–output relationship using the Dublin training data. The underlying principle by which

Table 5.1. Classification model input variables

Variable class	Input variable
Fungal spore inputs	*Alternaria*/*Cladosporium* concentration level class ("high", "medium" or "low" for previous days)
Phenological inputs	Growing degree-days
	(base temperature = 2–10°C)
Meteorological inputs	Wind direction (degrees)
	Wind speed (knots)
	Highest gust (knots)
	Highest 10-min mean wind speed (knots)
	Rainfall (mm)
	Maximum temperature (°C)
	Minimum temperature (°C)
	Mean temperature (°C)
	Sunshine duration (h)
	Grass minimum temperature (°C)
	Mean CBL (atmospheric) pressure (hPa)
	Global radiation (J/cm^2)
	Mean soil temperature (°C)
	Evapotranspiration (mm) (derived estimates and not directly observed)
	Potential evapotranspiration (mm) (derived estimates and not directly observed)
	Soil moisture deficit, well drained (mm) (derived estimates and not directly observed)
	Soil moisture deficit, moderately drained (mm) (derived estimates and not directly observed)
	Soil moisture deficit, poorly drained (mm) (derived estimates and not directly observed)

CBL, convective boundary layer.

this is conducted varies slightly depending on the algorithm used. After adequate training, the model should then be capable of estimating a predicted concentration using inputted independent variables. Each model was optimised by selecting significant model inputs from the initial parameters given in Table 5.1. *Cladosporium* and *Alternaria* levels were predicted using the validation datasets (July and August 2021 – Carlow, Cork, Sligo) and the trained model.

Two types of classification models were developed, random forest and support vector machine models. These models assess the input–output relationship slightly differently using the training data. The models developed were then tested using external validation data (July and August 2021 – Carlow, Cork, Sligo), during which predicted values were compared with the observed concentrations to evaluate the efficacy of the models (Figures 5.1 and 5.2).

Two different classification levels were applied to the *Alternaria* spore concentration data. Overall, the support vector machine models showed greater accuracy when applying the developed *Alternaria* models to the data collected form the Cork and Sligo sampling sites, exceeding 60% and 75% accuracy, respectively. The random forest models, on the other hand, performed best for the data from the Carlow sampling site, with model accuracy exceeding 60% when high levels are defined as *Alternaria* spore concentrations exceeding 10 spores/m^3 and low levels are defined as concentrations of less than 10 spores/m^3. Only one set of level classifications were applied to the *Cladosporium* spore concentration data, where fungal spore levels exceeding 500 spores/m^3 were deemed high and anything less was considered low. The support vector machine model performed best for both the Carlow and Sligo validation data, yielding model accuracies of greater than 75% and 40%, respectively, whereas the random forest model performed best for Cork validation data, with model accuracy exceeding 60%.

5.2 Regression Models

In addition to standard classification models, several regression-type models were also developed for both *Alternaria* and *Cladosporium* spore concentrations.

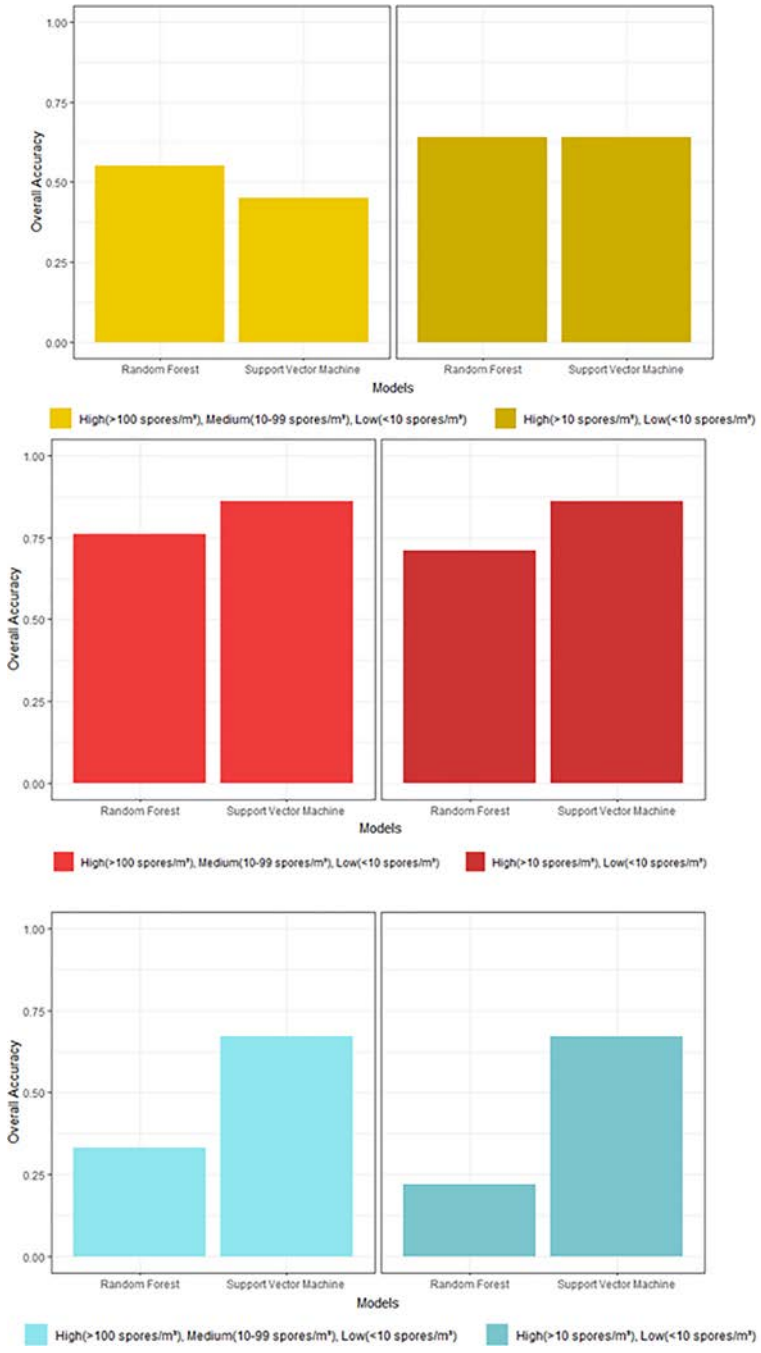

Figure 5.1. Comparison of the accuracy of the results from the *Alternaria* classification model using data from three sites.

A least trimmed squares robust regression model (robust model), generalised linear ensemble model (ensemble model) and generalised additive model (GAM) were developed. Input variables, namely the fungal spore inputs, varied slightly from those used in the classification models. Changes in input variables are summarised in Table 5.2. Each model was then optimised to include only significant variables. This optimisation varied depending on fungal type and model algorithm, as summarised in Table 5.3.

Once again, the developed models were initially trained using Dublin data and then tested using external validation data from Carlow, Cork and Sligo (July and August 2021). The predicted model values were compared with the actual ambient concentrations recorded using traditional volumetric methods. In order

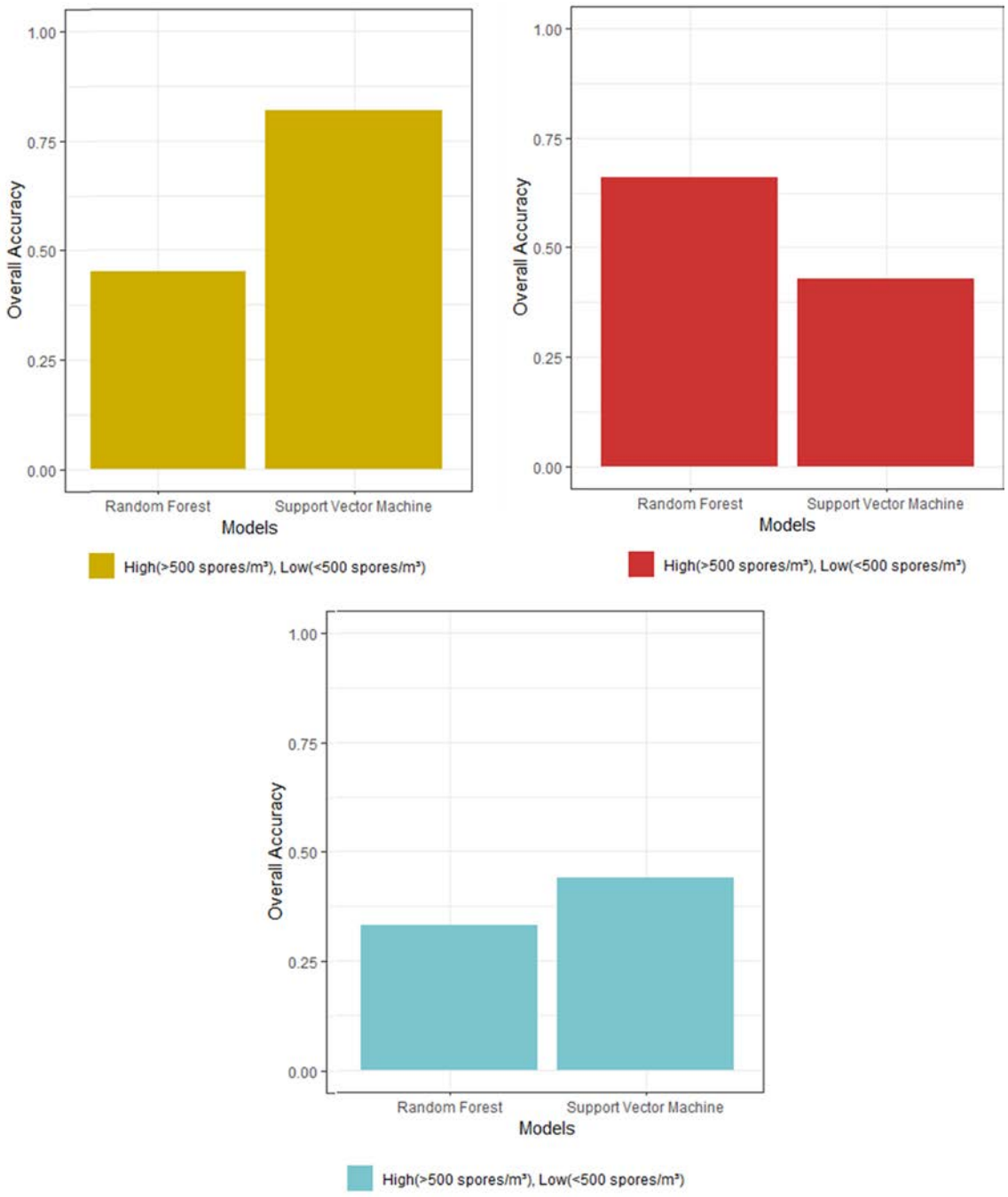

Figure 5.2. Comparison of the accuracy of the results from the *Cladosporium* classification model using data from three sites.

to evaluate the model performances for each site, an R^2 value was calculated between the predicted and expected concentrations for each model, as shown in Figure 5.3.

Comparing these predicted values with measured expected concentrations and calculating the R^2 value allowed the variance between these two measurements to be evaluated. The robust model performed best for predicting *Alternaria* spore concentrations in Carlow, whereas the ensemble model performed best for predicting *Alternaria* spore concentrations in Cork and Sligo. Overall, the models were less accurate in predicting *Cladosporium* concentrations, especially for Carlow. On the other hand, the robust model and GAM performed best for predicting *Cladosporium* spore concentrations in Cork and Sligo, respectively.

Table 5.2. Regression model input variables

Variable class	Input variable
Fungal spore inputs	*Alternaria*/*Cladosporium* daily concentrations (spores/m^3) Set limits/levels for daily *Alternaria* concentrations (spores/m^3), for example: • Days exceeding 10 spores/m^3 • Days exceeding 100 spores/m^3 Set limits/levels for daily *Cladosporium* concentrations (spores/m^3), for example: • Days exceeding 100 spores/m^3 • Days exceeding 500 spores/m^3 • Days exceeding 1000 spores/m^3
Phenological inputs	Unchanged
Meteorological inputs	Unchanged

Table 5.3. Optimised regression model input variables

Model type	Meteorological/phenological inputs used
Robust model	*Alternaria* (AIR): • Minimum temperature • Highest gust *Cladosporium* (CIR): • Potential evapotranspiration • Soil moisture deficit, poorly drained
Ensemble model	*Alternaria* (AEn): • Mean soil temperature • Soil moisture deficit, well drained • Maximum temperature *Cladosporium* (Clen): • Soil moisture deficit, well drained • Soil moisture deficit, poorly drained • Soil moisture deficit, moderately drained • Maximum temperature • Year • Wind speed • Highest 10-min mean wind speed • Wind direction • Highest gust
GAM	*Alternaria* (GAmA): • Time • Maximum temperature *Cladosporium* (GAmCl): • Time • Maximum temperature

It is important to note that, compared with other European networks that have been monitoring ambient fungal spore concentrations for decades, the Irish network is very much in its early stages. Many forecasting studies in the literature have vast quantities of monitoring data for model training, validation and optimisation. At the current time, when the models are applied to external validation data, they perform reasonably well, considering the relatively small quantity of training data available. Any notable deviations observed between predicted and expected values can be accounted for by deviations introduced by applying the models to different sampling locations, which are likely to experience different changes in environmental factors. In addition, at this early stage in model development it is expected that periods of extreme deviations in spore concentrations will not be accurately predicted by the models developed using only the available training data. We currently advise using classification models for *Alternaria* and *Cladosporium* forecasting rather than regression models. This is because of the improved accuracy achieved when broader level classes are predicted rather than specific numerical values. As monitoring efforts continue to grow and expand, incorporating additional data will greatly improve the models' performance and accuracy in the future. Increased monitoring will also provide an opportunity to develop location-specific models for the other sampling locations and spore types.

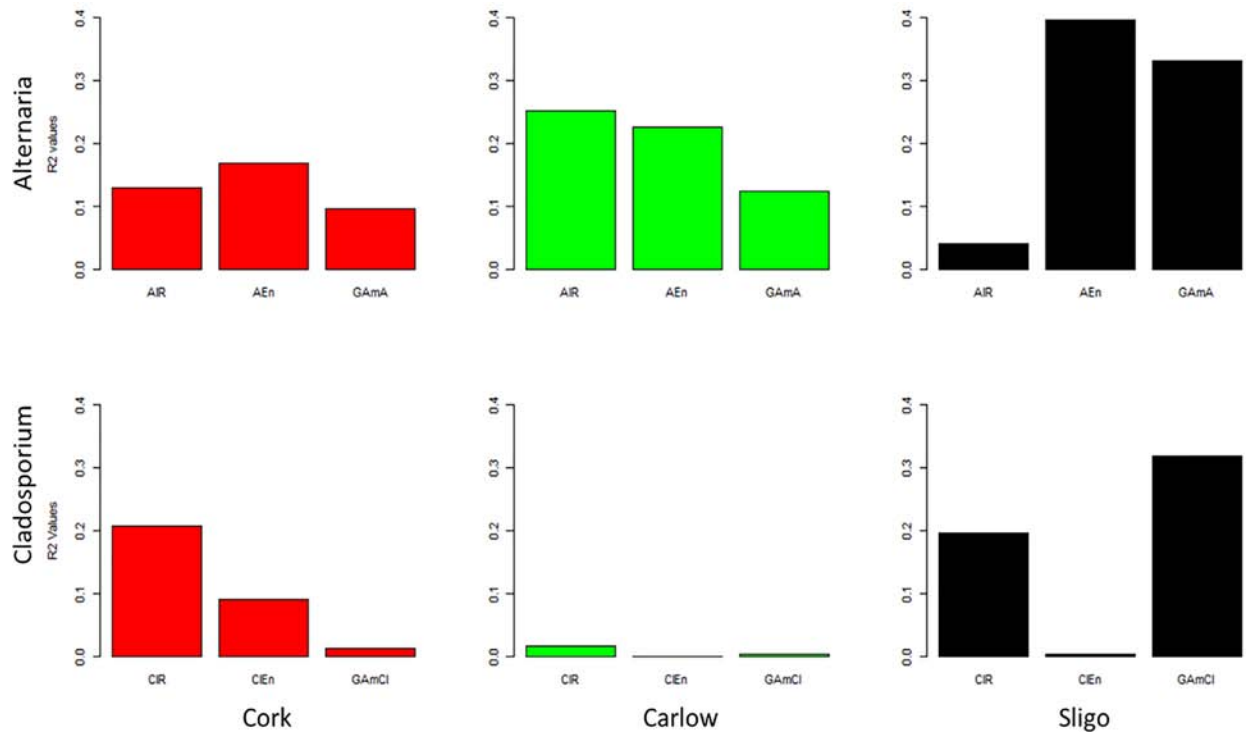

Figure 5.3. R^2 determination for each regression model.

6 Recommendations

Given the extensive work undertaken by the FONTANA project researchers in constructing a network and prototype forecast system, it is imperative that this work should act as the building blocks for a sustainable future rather than as a singular output in itself. Thus, we outline a number of recommendations in this chapter.

6.1 Establish a Fungal Spore Monitoring Network

We recommend that a network of fungal spore monitoring be established. Given the complexity and impact of these particles, it is imperative for human and plant health that we understand the factors influencing the release and distribution of fungal spores, species composition, and temporal and spatial trends. Equally, additional stakeholders should be integrated into such an endeavour (Met Éireann, Teagasc, etc.). Given the knowledge gained by members of the project team, their continued involvement in setting up this network is strongly suggested.

6.2 Integrate the Monitoring Network with Other Irish Networks

The Irish fungal spore monitoring network should be integrated with existing or planned ambient monitoring networks in Ireland. The fungal network and these other networks should have the following characteristics:

- A good geographical spread of stations should be ensured to allow the determination of the transport and dispersion of fungal spores over the island of Ireland. This will become more important should dispersion modelling be used in the future.
- The majority of stations should be placed in the most populated conurbations in the country (Dublin, Cork, Limerick, etc.). This would allow the greatest number of people to benefit from site-specific data on fungal spores, greatly aiding allergy sufferers and allowing them to mitigate their exposure during the fungal spore season. The cost–benefits of such an approach are discussed in Chapter 7.
- Some of the stations should be based in rural areas to allow additional work to determine the influence of agriculture on the production of fungal spores and the effect of fungi on crop yields. These stations should be located in regions where crop production is prominent.
- The spread of the stations should also be such that it permits an understanding of the climatic influences on fungal spore release (i.e. the stations should be positioned the length and breadth of the country). This final point is of importance, as in the coming years a shift in the timing, intensity and length of the fungal spore season is expected because of climate change. Hence, an understanding of the climatic impacts over the length and breadth of Ireland will be important.

6.3 Expand Work on Real-time Detection of Fungal Spores

Additional work on the real-time detection of fungal spores is required. While the instruments used in this project have been shown to correlate well with total fungal spore measurements in other investigations (Daly *et al.*, 2019; Fernández-Rodríguez *et al.*, 2018; Käpylä and Penttinen, 1981; O'Connor *et al.*, 2013, 2014b), the relationships found here seem to be significantly impacted by anthropogenic aerosols. Thus, additional work is needed to fully ascertain the instruments' abilities. Furthermore, newer devices have been developed in the intervening period between the beginning of this project and its completion. Thus, this newer generation of instruments (which massively increases the number of single particle data acquired) could be capable of differentiating fungal spores down to the species level and even detect fluorescent anthropogenic pollution particles, such as polyaromatic hydrocarbons or secondary organic aerosols. Indeed, instruments such as the SwisensPoleno Jupiter and Plair Rapid-E+ have already been able to identify pollen to the species level. Given the enhancements in fluorescence spectral ranges and fluorescence lifetime measurements these instruments can undertake in

real time, their use in an automated fungal network may be a reality and this should be evaluated.

6.4 Gather More Data to Develop the Models

We recommend collecting additional fungal data to allow the modelling aspect of this work to be fully developed. As data are collected, this will allow the refinement of the models over time. A network of operational samplers will allow the modelling work to be continued in the future. This is strongly recommended, as Ireland has a dearth of information on fungal species and their prevalence in the ambient environment.

6.5 Develop a "Traffic Light" System for Public Outreach

Both numerical and classification models were developed in this work; however, we recommend that the output to the public be in the form of a "traffic light" colour-coded system (red, orange, yellow) to indicate the potential for fungal spores to cause allergenic reactions. Red indicates high levels of a particular fungal spore type, orange medium levels and yellow low levels. Similar colour-coded systems have been widely implemented (e.g. European meteoalarm: https://meteoalarm.org/en/).

6.6 Make Forecasts Widely Available

A forecast should allow predictions to be made 2–3 days into the future, in line with what is currently produced. The forecast should be housed and displayed in the most public of forums so that it is disseminated to those who need it most. Hence, the forecast could be posted on social media (similarly to the Asthma Society of Ireland's pollen forecast) with a dedicated page and displayed on a web page similar to that currently used by Met Éireann.

Other European countries have started using specifically designed phone apps for the dissemination of pollen data. Such an information pathway could also be considered.

Good dissemination methods will be essential for those who have health concerns related to exposure to bioaerosols. This is important for both residents and tourists. Thus, being able to provide relevant information to visitors to the country would be an extra boon, ensuring that they enjoy their time in Ireland and perhaps influencing return visits.

7 Conclusions

In conclusion, the FONTANA project has produced the first fungal spore monitoring network in Ireland, in doing so sampling and determining the concentrations of ambient fungal species in both rural (Carlow and Sligo) and urban (Dublin and Cork) settings. Both traditional impaction methodologies and novel real-time light scattering/light-induced fluorescence approaches were used. The traditional methods highlighted the difference between the sites, with the ratio of *Cladosporium* to other fungal spores counted being notably different between Dublin and Carlow. Similarly, we also found that the ascospore to basidiospore ratio was different in rural and urban sites.

These data, along with previously collected fungal data from the period 1978–1980, were collated to create the first fungal calendar for Ireland, displaying the start and end of the season and the peak release periods for each fungal spore type and highlighting the most allergenic species present in the Irish environment.

The output from the WIBS showed reasonable correlation with that of the impaction methodologies ($r = 0.5–0.67$) and the WIBS has the added benefit of outputting data with a greater time resolution and in a far more timely manner than the traditional methodologies. These data, in tandem with other air quality data, could be very useful for air quality modelling and risk assessment, with possible risks including acute sinusitis or bronchial symptoms in susceptible people, or wider negative health impacts associated with chronic, long-term air pollution exposure. The WIBS, however, was unable to differentiate between fungal species. Thus, it is more useful as bulk fungal monitor than a species-specific detector. The IBAC-2 did not fare as well as the WIBS, and the data had little to no correlation with fungal spore concentrations. They did, however, correlate well with the non-fluorescent counts from the WIBS and with other ambient pollution particles (PM_{10}, or dust).

The fungal data collected, along with meteorological parameters, were used to develop a fungal forecast model for the main allergenic species (*Alternaria* and *Cladosporium*) in the Irish environment. Several modelling methodologies, including regression analysis and support vector machine learning, were used. Classification models offered better results than attempting to specify a numerical fungal spore concentration.

The project team makes a number of recommendations based on the work presented here. These include:

1. Create a fungal spore monitoring network that gives good spatial and population coverage throughout Ireland.
2. Consider using new iterations of real-time instruments in the network. A pilot study looking at such instrumentation in the Irish context would be useful in determining their potential for deployment in the recommended network. Such new-generation instrumentation has the ability to differentiate between pollen species and deliver the requisite data in real time (Tummon *et al*., 2021). Thus, it should be investigated whether it could also differentiate fungal spore types and potentially other ambient bioaerosols.
3. Collect additional data to further develop the modelling aspects of this project, as Ireland has very few historical data.
4. Involve the project team in the development of the network, given the knowledge and expertise the team members have developed throughout this project.
5. Develop a traffic light colour-coded system to display the fungal forecasts to the public.

The impact of this work will be far reaching, given the influence that PBAPs can have on the health and well-being of members of the public. Even at very low concentrations, fungal spores can have impacts on well-being such as congestion and eye and skin irritation. This work will be of particular help to those who suffer from asthma. Allergy-associated costs to countries in Europe are estimated to total as much as €150 billion a year (Clot *et al*., 2020; Zuberbier *et al*., 2014). The direct and indirect costs for an individual are estimated to be around €2400 per

year (Zuberbier *et al*., 2014). The impact in Ireland is likely to be even greater given its high prevalence of asthma – the fourth highest in the world. It is estimated that more than 1 in every 6 people in Ireland experience asthma at some point in their lives (Asthma Society of Ireland, 2022). The health-related cost of asthma to the state is estimated to be €472 million per annum. If we add the cost of health concerns related to air pollution to the health-related cost of asthma, the total cost to the state rises to approximately €2 billion per year. Thus, potential solutions that could ease the burden on the health system would effectively pay for themselves in the long run, while also improving the quality of life of asthma sufferers.

Adults with asthma in Ireland miss on average 12 days' work a year because of their condition. As many as 80% of people with asthma have been diagnosed with a fungal allergy (Salvaggio and Aukrust, 1981). The impact of seasonal allergies on a person's ability to work and their productivity and quality of life has also been discussed extensively in the literature (Blaiss, 2010; Kessler *et al*., 2001; Vandenplas *et al*., 2008, 2018). Bioaerosols and anthropogenic pollution are known to trigger and heighten "asthma attacks" and increase wheezing and other breathing difficulties. Indeed, in Ireland, one person dies every 6 days as a result of asthma, and every 4 minutes a person visits an emergency room because of complications associated with asthma. The findings of this research will therefore increase the mitigation of such events by allowing sensitised individuals to control their interactions with bioaerosols and pollution, helping them to manage their condition better, to improve their well-being and to lead lives that are far more productive economically.

Finally, the work could also be easily adapted to ascertain and mitigate the effects of bioaerosols on agriculture and forestry. Agriculture is one of Ireland's largest economic sectors. A significant benefit to the agricultural sector, and to the country as a whole, would come from an enhanced understanding of the bioaerosol factors affecting agricultural outputs. Historically, bioaerosols such as *P. infestans* (potato blight) have had a devastating effect on Ireland, with the resulting food insecurity costing millions their lives and precipitating mass emigration. *P. infestans* is still responsible for significant loss of crops each year – €1 billion worth of losses in the EU alone. It is estimated that €5 million is spent annually in Ireland on fungicides to mitigate the impacts of the disease, which amounts to between 15 and 20 fungicide applications per season (Teagasc, 2022). The development and use of accurate forecasting methods for fungal spore concentrations could help limit the use of fungicides in agricultural production by predicting periods of high risk. By providing information so that fewer applications are needed to reduce the risk of complete crop destruction, unnecessary pollution of the atmosphere, crops, soil, etc. is avoided (Frenguelli, 1998). Such work has been carried out for major agricultural sectors elsewhere in Europe, including extensive work on Mediterranean vineyards (Fernández-González *et al*., 2013; Martínez-Bracero *et al*., 2019; Rodríguez-Rajo *et al*., 2010). This work could also be used to determine the sources of and hotspots for crop and other plant pathogens. For example, invasive species such as the fungus that causes ash dieback have cost the state €2.6–5.8 million (as of 2018) and caused the death of hundreds of thousands of ash trees (Vasaitis and Enderle, 2017; Viney, 2020). Again, real-time measurements could target the areas in most need within Ireland and/or internationally.

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Abbreviations

ANN	Artificial neural network
FAP	Fluorescent aerosol particle
FBAP	Fluorescent biological aerosol particle
IBAC	Instantaneous biological analyser and collector
LIF	Light-induced fluorescence
PBAP	Primary biological aerosol particle
WIBS	Wideband integrated bioaerosol sensor
WIBS-NEO	Wideband integrated bioaerosol sensor – new electronics option

An Ghníomhaireacht Um Chaomhnú Comhshaoil

Tá an GCC freagrach as an gcomhshaol a chosaint agus a fheabhsú, mar shócmhainn luachmhar do mhuintir na hÉireann. Táimid tiomanta do dhaoine agus don chomhshaol a chosaint ar thionchar díobhálach na radaíochta agus an truaillithe.

Is féidir obair na Gníomhaireachta a roinnt ina trí phríomhréimse:

Rialáil: Rialáil agus córais chomhlíonta comhshaoil éifeachtacha a chur i bhfeidhm, chun dea-thorthaí comhshaoil a bhaint amach agus díriú orthu siúd nach mbíonn ag cloí leo.

Eolas: Sonraí, eolas agus measúnú ardchaighdeáin, spriocdhírithe agus tráthúil a chur ar fáil i leith an chomhshaoil chun bonn eolais a chur faoin gcinnteoireacht.

Abhcóideacht: Ag obair le daoine eile ar son timpeallachta glaine, táirgiúla agus dea-chosanta agus ar son cleachtas inbhuanaithe i dtaobh an chomhshaoil.

I measc ár gcuid freagrachtaí tá:

Ceadúnú

- Gníomhaíochtaí tionscail, dramhaíola agus stórála peitril ar scála mór;
- Sceitheadh fuíolluisce uirbigh;
- Úsáid shrianta agus scaoileadh rialaithe Orgánach Géinmhodhnaithe;
- Foinsí radaíochta ianúcháin;
- Astaíochtaí gás ceaptha teasa ó thionscal agus ón eitlíocht trí Scéim an AE um Thrádáil Astaíochtaí.

Forfheidhmiú Náisiúnta i leith Cúrsaí Comhshaoil

- Iniúchadh agus cigireacht ar shaoráidí a bhfuil ceadúnas acu ón GCC;
- Cur i bhfeidhm an dea-chleachtais a stiúradh i ngníomhaíochtaí agus i saoráidí rialáilte;
- Maoirseacht a dhéanamh ar fhreagrachtaí an údaráis áitiúil as cosaint an chomhshaoil;
- Caighdeán an uisce óil phoiblí a rialáil agus údaruithe um sceitheadh fuíolluisce uirbigh a fhorfheidhmiú
- Caighdeán an uisce óil phoiblí agus phríobháidigh a mheasúnú agus tuairisciú air;
- Comhordú a dhéanamh ar líonra d'eagraíochtaí seirbhíse poiblí chun tacú le gníomhú i gcoinne coireachta comhshaoil;
- An dlí a chur orthu siúd a bhriseann dlí an chomhshaoil agus a dhéanann dochar don chomhshaol.

Bainistíocht Dramhaíola agus Ceimiceáin sa Chomhshaol

- Rialacháin dramhaíola a chur i bhfeidhm agus a fhorfheidhmiú lena n-áirítear saincheisteanna forfheidhmithe náisiúnta;
- Staitisticí dramhaíola náisiúnta a ullmhú agus a fhoilsiú chomh maith leis an bPlean Náisiúnta um Bainistíocht Dramhaíola Guaisí;
- An Clár Náisiúnta um Chosc Dramhaíola a fhorbairt agus a chur i bhfeidhm;
- Reachtaíocht ar rialú ceimiceán sa timpeallacht a chur i bhfeidhm agus tuairisciú ar an reachtaíocht sin.

Bainistíocht Uisce

- Plé le struchtúir náisiúnta agus réigiúnacha rialachais agus oibriúcháin chun an Chreat-treoir Uisce a chur i bhfeidhm;
- Monatóireacht, measúnú agus tuairisciú a dhéanamh ar chaighdeán aibhneacha, lochanna, uiscí idirchreasa agus cósta, uiscí snámha agus screamhuisce chomh maith le tomhas ar leibhéil uisce agus sreabhadh abhann.

Eolaíocht Aeráide & Athrú Aeráide

- Fardail agus réamh-mheastacháin a fhoilsiú um astaíochtaí gás ceaptha teasa na hÉireann;
- Rúnaíocht a chur ar fáil don Chomhairle Chomhairleach ar Athrú Aeráide agus tacaíocht a thabhairt don Idirphlé Náisiúnta ar Ghníomhú ar son na hAeráide;
- Tacú le gníomhaíochtaí forbartha Náisiúnta, AE agus NA um Eolaíocht agus Beartas Aeráide.

Monatóireacht & Measúnú ar an gComhshaol

- Córais náisiúnta um monatóireacht an chomhshaoil a cheapadh agus a chur i bhfeidhm: teicneolaíocht, bainistíocht sonraí, anailís agus réamhaisnéisiú;
- Tuairiscí ar Staid Thimpeallacht na hÉireann agus ar Tháscairí a chur ar fáil;
- Monatóireacht a dhéanamh ar chaighdeán an aeir agus Treoir an AE i leith Aeir Ghlain don Eoraip a chur i bhfeidhm chomh maith leis an gCoinbhinsiún ar Aerthruailliú Fadraoin Trasteorann, agus an Treoir i leith na Teorann Náisiúnta Astaíochtaí;
- Maoirseacht a dhéanamh ar chur i bhfeidhm na Treorach i leith Torainn Timpeallachta;
- Measúnú a dhéanamh ar thionchar pleananna agus clár beartaithe ar chomhshaol na hÉireann.

Taighde agus Forbairt Comhshaoil

- Comhordú a dhéanamh ar ghníomhaíochtaí taighde comhshaoil agus iad a mhaoiniú chun brú a aithint, bonn eolais a chur faoin mbeartas agus réitigh a chur ar fáil;
- Comhoibriú le gníomhaíocht náisiúnta agus AE um thaighde comhshaoil.

Cosaint Raideolaíoch

- Monatóireacht a dhéanamh ar leibhéil radaíochta agus nochtadh an phobail do radaíocht ianúcháin agus do réimsí leictreamaighnéadacha a mheas;
- 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 um chosaint ar an radaíocht a sholáthar, nó maoirsiú a dhéanamh ar sholáthar na seirbhísí sin.

Treoir, Ardú Feasachta agus Faisnéis Inrochtana

- Tuairisciú, comhairle agus treoir neamhspleách, fianaise-bhunaithe a chur ar fáil don Rialtas, don tionscal agus don phobal ar ábhair maidir le cosaint comhshaoil agus raideolaíoch;
- An nasc idir sláinte agus folláine, an geilleagar agus timpeallacht ghlan a chur chun cinn;
- Feasacht comhshaoil a chur chun cinn lena n-áirítear tacú le hiompraíocht um éifeachtúlacht acmhainní agus aistriú aeráide;
- Tástáil radóin a chur chun cinn i dtithe agus in ionaid oibre agus feabhsúchán a mholadh áit is gá.

Comhpháirtíocht agus Líonrú

- Oibriú le gníomhaireachtaí idirnáisiúnta agus náisiúnta, údaráis réigiúnacha agus áitiúla, eagraíochtaí neamhrialtais, comhlachtaí ionadaíocha agus ranna rialtais chun cosaint chomhshaoil agus raideolaíoch a chur ar fáil, chomh maith le taighde, comhordú agus cinnteoireacht bunaithe ar an eolaíocht.

Bainistíocht agus struchtúr na Gníomhaireachta um Chaomhnú Comhshaoil

Tá an GCC á bainistiú ag Bord lánaimseartha, ar a bhfuil Ard-Stiúrthóir agus cúigear Stiúrthóir. Déantar an obair ar fud cúig cinn d'Oifigí:

1. An Oifig um Inbhunaitheacht i leith Cúrsaí Comhshaoil
2. An Oifig Forfheidhmithe i leith Cúrsaí Comhshaoil
3. An Oifig um Fhianaise agus Measúnú
4. An Oifig um Chosaint ar Radaíocht agus Monatóireacht Comhshaoil
5. An Oifig Cumarsáide agus Seirbhísí Corparáideacha

Tugann coistí comhairleacha cabhair don Ghníomhaireacht agus tagann siad le chéile go rialta le plé a dhéanamh ar ábhair imní agus le comhairle a chur ar an mBord.

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