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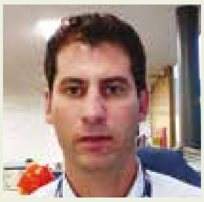
Impact Objectives

- Develop a 'bio-toolkit' for high-confidence, wide-area biodetection and biomonitoring of bioaerosols from urban, agricultural and industrial environments
- Develop novel techniques for rapid, high-throughput sample capture, concentration and preparation for detecting bioaerosols

Identifying airborne particulates with speed and accuracy

***Professors Frédéric Coulon** and **Ian Colbeck** talk about their work establishing a fundamental change in the way that airborne particles are identified, using portable collection and next generation sampling techniques*



Professor Frédéric Coulon

Professor Ian Colbeck

Airborne contaminants cover a huge range of materials: how do you differentiate those that you are considering for the 'Rapid monitoring of bioaerosols in Urban, Agricultural and Industrial Environments' (RAMBIE) project?

FC: In RAMBIE we are mainly concerned with bioaerosols. Bioaerosols refer to particulate matter of biological origin, such as pollen, fungal spores, bacterial cells, viruses, etc. Important properties characterising bioaerosols are size, viability, infectivity, allergenicity, toxicity, and pharmacological activity. For a bioaerosol to be infectious or pathogenic, it must be viable. However, non-viable bioaerosols can still cause allergies or toxic reactions. It is worth noting that an innate characteristic of bioaerosols is that these properties may change with time, which can play an important role in sampling, especially for the viable organisms. Plants, soil, water, and animals - including humans - all serve as sources of bioaerosols, and bioaerosols are subsequently present in most places where any of these sources live. Individual bioaerosol particles can range in size from approximately 0.02 to 100 micrometres in diameter, depending on the type and source. Bioaerosols constitute a substantial fraction of the atmospheric aerosol load. With regard to number and mass concentration in the coarse particle size range bioaerosols typically account for around 30 per cent in the urban and rural environment. In waste management industries or livestock production it could be up to 90 per cent.

Can you discuss a little about current monitoring methods, and explain why they are insufficient?

IC: For many years, all work on airborne microorganisms has been carried out based on their growth on culture media, either in nutrient broth or on agar. As a direct result, any organisms that do not grow on the media used would have avoided detection. Additionally, the methods used for air sampling have been plagued with problems, as cells and spores must remain viable if they are to reproduce and form a colony. Culture-based methods were employed when the development of molecular methods remained in its infancy. Filters have high collection efficiencies for particles >0.5 ◄m in diameter, making them a good choice for bioaerosols. Impactors rely on impacting the sample onto a surface, usually an agar plate. This allows the total sample to be examined without any need for extraction or sub-sampling, but limits culturing of each sample to one growth medium. Impingers can be used to allow collection of microorganisms in air samples into a chosen collection fluid.

What are the main issues with bioaerosols?

FC: Deleterious health effects can arise following exposure to infective organisms. They also result from the subject's reactions to endotoxins released by bacteria, mycotoxins produced by fungi and allergic responses following contact or inhalation of microorganisms. As the risk of exposure is directly linked to the concentration and type of microorganisms, much clearer quantification and characterisation mechanisms are needed if the temporal and spatial trends of allergenic and infectious species are to be evaluated. Historical events propagated by bioaerosols include the Irish Potato Famine, the Bubonic Plague, and the 1918 Influenza outbreak. Recent events include the risk of bioterrorism using pathogenic bioaerosols, as well as potential for epidemics transmitted by the airborne route such as outbreaks of Avian Flu and the transmission of SARS (Severe Acute Respiratory Syndrome).

To what extent do you expect naturally-derived bioaerosols to vary in urban, agricultural and industrial environments?

IC: Bioaerosols are highly relevant for the spread of organisms, allowing genetic exchange between habitats and geographic shifts of biomes. They are central elements in the development, evolution, and dynamics of ecosystems. Bioaerosols can serve as nuclei for cloud droplets, ice crystals, and precipitation, thus influencing the hydrological cycle and climate. Especially in pristine air over vegetated regions, bioaerosols are likely to be an essential regulating factor in the formation of precipitation and vice versa.

The rapid monitoring of bioaerosols in selected environments

*While the existence of airborne microorganisms is well known and understood, identification of particular species and their concentrations is more of an issue. The **RAMBIE** project aims to create a framework for the fast identification of bioaerosols in different surroundings*

The air around us is filled with bacterial and viral organisms, many of which are volatile and can present a serious health risk to humans and animals. This issue is magnified by the emergence of the potential for bio-terrorism and the distribution of poisonous substances into urban areas or agricultural pathogens. Concern about the vulnerability of humans or animals to accidental or intentional release of toxic substances is growing and there is equal concern that we are unprepared to detect such releases, let alone combat them.

The field-scale sampling of bioaerosols is expensive, labour-intensive and highly inefficient at gathering sufficient amount of samples to be meaningful. Further to this there is to date no established protocol for testing large amounts of collected samples. These issues make the identification and containment of airborne contaminants difficult, and the 'Rapid monitoring of bioaerosols in Urban, Agricultural and Industrial Environments' (RAMBIE) project hopes to simplify the gathering process and speed up identification of bioaerosols.

This Natural Environment Research Council funded four year project, which kicked off in early 2015, aims to simplify the process of biosampling and make it not only faster but increasingly accurate too. To help achieve this, the team are comparing data from three different environments – urban, industrial, and agricultural – to assess whether there are any prevailing factors that affect the sampling process in them, or if qualification of levels and spread is similar in each of them.

THE PROBLEM OF BIOAEROSOLS

Bioaerosols comprise many different particles, ranging from bacteria and actinomycetes through to fungi, pollens, and moulds, but also including airborne viruses. More extreme types of bioaerosols are pathogenic bacteria and viruses, including influenza, SARS (Severe Acute Respiratory Syndrome), and anthrax. Typically consisting of very fine particles measuring anywhere between 0.02 to 100 microns in diameter, they are not visible to the naked eye and can be transported rapidly over large distances depending upon prevailing winds and atmospheric conditions. Some species can remain aloft for extended periods of time whereas others may be forced to the ground quite quickly, depending upon local factors such as local temperature, humidity and wind speed.

These natural sources of variation are important when determining the concentration of bioaerosols present. This has to be assessed together with the natural sources of the material, making investigation into the different environments highly important, particularly when some bioaerosols may have significant detrimental effects on human, livestock or even plant life. Current detection techniques usually involve producing cultures of organisms which is both slow and highly selective. The RAMBIE project aims to determine new methods of sampling multiple organisms quickly using improved molecular methods.

INEFFICIENT SAMPLING METHODS

There are actually a large number of sampling methods in use today, but the process of collecting and sampling is hampered by the lack of standardised protocol regarding these methods, and data comparison is difficult. This makes it difficult to integrate and assess results from different agencies, who may be using a range of test methods, including impactors, diffusion impingers, and filtration. This issue was a major driving force for the project driven by Professor Ian Colbeck from the University of Essex and Professor Frédéric Coulon from Cranfield University.

Both the University of Essex and Cranfield University are home to groups who have attained international recognition in the field of bioaerosols and the RAMBIE project has grown from that expertise. Colbeck and Coulon are also working in collaboration with the Defence Science & Technology Laboratory and the Environment Agency to create a fast and accurate means of identifying airborne contaminants.

The project is primarily concerned with early detection, quantification and understanding the potential for evolution of multiple types of bioaerosol, leading to a more substantial means of detecting and tracking the progress of airborne species. 'RAMBIE also aims to generate a comprehensive dataset on all aspects of bioaerosols, and characterise material of biological origin from different environmental sources will inform Local Healthcare Authorities and Department of Health's policy for pathogen mitigation,' explains Coulon. 'Furthermore, the data obtained will be used to help Government manage its recycling strategies and help determine future shifts in bioaerosol exposures through extensive modelling of likely changes in types and exposure rates.'

THE ROLE OF DIFFERENT ENVIRONMENTS

One of the major factors of the investigation is the comparison of aerosols in different and contrasting environments, and the development of a deeper understanding of how those environments impact on the speed and spread of them. The three environments under review differ significantly in not only how bioaerosols may be transported around them through normal air movements relative humidity, and local temperature fluctuations, but also from the types of bioaerosol that are predominately naturally found at them.

multiple species in diverse environments. Allied to this, the project will also employ the principles of metatranscriptomics to determine how identified species may evolve,' notes Coulon.

Metatranscriptomics is the study of the function and activity of the complete set of transcripts - RNA-sequences - from environmental samples and can be used to identify how different genes are expressed in a microbial community across all species, and how diverse they are. 'Additionally, it can be used to identify the highest expressed genes in a particular environment, which will be used to produce a fundamental picture of bioaerosol activity in the different environments,' says Coulon. By combining NGS techniques and chemical marker analysis, the team hope to create a fast and highly effective means of characterising the diverse range of bioaerosol species from the target areas.

> *'We aim to provide a better understanding of the impact that the exposure to bioaerosols has on humans, livestock, and agricultural species'*

Typically, many of the bioaerosols found in all three environments result from natural sources such as flora and consist of fungi and pollen, but agricultural environments also tend to have a higher incidence of composted materials. 'These areas are richer in microbial elements and potentially pose a greater health risk, and the differences between the three location types makes accurate sampling and identification imperative,' says Coulon. But understanding that fundamental part of the project is only half of the problem, and the main work then focuses on how a reliable measurement of the bioaerosols can be achieved. For that, the team are looking to Next Generation Sequencing (NGS), and metatranscriptomics as a viable means of characterising bioaerosols.

IDENTIFICATION AT A FUNDAMENTAL LEVEL

NGS is a DNA sequencing technology which has found widespread use genomic research. By using NGS techniques, an entire human genome can be sequenced within a single day, while the previous Sanger sequencing technology, used to decipher the human genome, required over a decade to deliver the final results. 'This speeding up of result-gathering makes the process ideal for the identification of

The team considers that the outputs from the project, including a database of microbial volatile organic markers, will help other researchers rapidly identify species within an area under investigation, and aid the nomenclature of tested samples far quicker than can currently be achieved. 'One of the main deliverables from the project is the design of a small, portable micro-instrumentation for real-time and wide-area bioaerosol detection, characterisation and quantification – something that is missing from current detection equipment,' Coulon explains.

While the RAMBIE project is primarily concerned with a concerted sampling campaign across different sites, its outcomes will become a basis for future sampling methods and it hopes to provide the standardisation that testing in this field currently lacks. 'We aim to provide a better understanding of the impact that the exposure to bioaerosols has on humans, livestock, and agricultural species, which is seen as being directly relevant to the NERC, and will form a basis for future national strategies,' Coulon observes.

Project Insights

FUNDING

Natural Environment Research Council

CONTACT

Ian Colbeck
Principal Investigator

T: +44 1206872203
E: colbi@essex.ac.uk
W: http://pixel.essex.ac.uk/envmicrohealth/rambie.html

Frédéric Coulon
Principal Investigator

T: +44 123475981
E: f.coulon@cranfield.ac.uk
W: https://www.cranfield.ac.uk/research-projects/rambie

PROJECT COORDINATOR BIOS

Professor Frédéric Coulon is a Professor of Environmental Chemistry and Microbiology and is recognised for his internationally-leading contribution on treatment and remediation processes for pollution control across contaminated land, waste and wastewater sectors. He has extensively investigated the diversity and activities of, and interactions between, microbes in bioengineered treatments and published more than 100 research papers, book chapters and conference proceedings in oil remediation, process emissions and control and environmental risk management. His research influences policy development and provides pragmatic, risk-based solutions to environmental pollution.

Professor Ian Colbeck is Professor of Environmental Science in the School of Biological Sciences. He has amassed over 30 years of considerable achievements and experience over a wide gamut of research interests but predominantly in the field of aerosol science and air pollution. He has published over 150 research papers and 7 books. He has participated in a number of EU projects as well as provided scientific advice to national and local government, defence laboratories, and industry. Current research includes the development of methods to detect bioaerosols, indoor air quality and the impact of nanoparticles on plants.

RAMBIE, rapid monitoring of bioaerosols in urban, agricultural and industrial environments, NERC

Coulon, Frederic

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