23NRM03 BioAirMet

Publishable Summary for 23NRM03 BioAirMet
Standardisation of bioaerosol monitoring for air quality and climate modelling

Overview

Biological aerosols (mainly pollen and fungal spores, but also bacteria, viruses and plant debris) are ubiquitous in the atmosphere. They influence the hydrological cycle and climate, and some of them are known human and plant pathogens and/or allergens. Newly developed systems to monitor airborne biological particles, based on automatic bioaerosol monitors and machine learning, have revolutionised the field by providing real-time information on particle number concentration and taxa/species. However, traceability is incomplete and measurement uncertainties are poorly understood. This project will provide input to a new documentary standard on automatic pollen and fungal spore monitoring within the CEN/TC 264/WG 39 and will develop quality assurance procedures for this new class of bioaerosol monitors.

Need

Biological aerosols are released into the air by plants, fungi and other living organisms as part of their reproductive or decomposition cycles. They are ubiquitous in the atmosphere, although their concentration and composition can vary significantly in time (e.g., night vs day) and by location (e.g., park vs residential estate). Besides their biological importance, many bioaerosols, especially pollen and some fungal spores, have an allergenic or pathogenic effect on humans. Pollen allergy and asthma are some of the most common chronic diseases, affecting between 15 %-40 % of the European population (forecasted to be 50 % by 2050), with estimated direct and indirect health costs exceeding €50 billion per year. Bioaerosol monitoring has therefore become essential as it underpins practical mitigation and adaptation measures. The data are required, and used, by physicians for diagnosis, treatment and clinical studies as well as for empirical and numerical pollen forecasts, which aid allergy sufferers to effectively plan their activities and medication intake.

Bioaerosols can act as cloud condensation nuclei as well as ice nuclei, influencing the hydrological cycle and climate. Moreover, information on bioaerosols is crucial for agriculture and forestry, where real-time data about the presence of pathogens, mostly fungi, are necessary for protecting agricultural production. Pollen monitoring is needed to underpin ecological studies on vegetation shifts and invasive species.

Originally, bioaerosol monitoring networks were based on offline manual methods. These methods suffer from poor time resolution (typically 24 hours) and long delays in data availability (typically 7-9 days). Recent technological developments and machine learning have made it possible to automate bioaerosol monitoring, revolutionising the information that can be made available to end-users.

Automatic bioaerosol monitors are not fully calibrated as new methods need to be developed. The machine learning algorithms need to be adequately trained as they are currently trained with particles supplied to the devices under generic laboratory conditions or even outdoors (e.g., rooftops of meteorological institutes), with no strict control over the environmental conditions. Also, strong deviations are observed due to different measurement principles being in use among the instruments and the data outputs are not comparable, or they require some transformation, making the process time consuming and imprecise (e.g., some machines report coarse particle number concentration, others only particle counts or size classes). These approaches need to be standardised. Once the calibration process is validated and deviations reduced, the production of a new

documentary standard by CEN/TC 264/WG 39 will be required to make the new processes available to stakeholders (e.g., bioaerosol monitoring networks and instrument manufacturers).

Objectives

The overall objective of this project is to provide input to a new documentary standard on automatic pollen and fungal spore monitoring within the CEN/TC 264/WG 39 and to develop quality assurance procedures for state-of-the-art bioaerosol monitors. The specific objectives of the project are:

1. To further develop two traceable methods (light scattering and particle flow visualisation) for the calibration of automatic bioaerosol monitors with respect to particle size and number concentration (target expanded uncertainty ≤15 %). To provide clear guidelines to end users about the specifications, limitations and application range of each primary method.

2. To develop methods for training and validating machine learning (ML) algorithms, which identify airborne pollen taxa and fungal spore species in real-time, based on the collection of bioaerosol particles from natural sources, appropriate conditioning/treatment in the laboratory and controlled re-dispersion in air. To develop methods for quantifying the accuracy of the algorithms and for deriving a combined uncertainty arising from uncertainties in both particle counting and particle identification (target expanded uncertainty ≤30 %).

3. To standardise the data output, interface and metadata of automatic bioaerosol monitors. To develop guidelines on data storage, handling and distribution, ensuring data availability and accessibility compliant with EU environmental regulations, in particular with the Inspire Directive.

4. To contribute to the development of a new standard on automatic pollen and fungal spore monitoring within the CEN/TC 264/WG 39 – Sampling and Analysis of Airborne Pollen Grains and Fungal Spores, and to contribute to the revision of existing standards published by CEN/TC 264/WG 28 – Measurement of Airborne Microorganisms in Ambient Air.

5. To address stakeholder needs regarding automatic bioaerosol monitoring highlighted at the BIPM/CCQM Workshop on Particle Metrology and establish strong collaboration with EMN Pollution Monitoring. To facilitate the uptake of the technology and measurement methodologies developed in the project by the EUMETNET AutoPollen community, the EU Horizon Europe project SYLVA, (national/regional) bioaerosol monitoring networks and instrument manufacturers.

Progress beyond the state of the art and results

The technical results that are expected to be delivered by this project are as follows:

1. Further development and standardisation of traceable methods for counting of micrometre-sized bioaerosol particles (pollen and fungal spores)

Existing methods for the determination of airborne particle number concentration, based on reference optical particle counters, are being standardised in the particle size range up to at least 50 μm and at particle number concentrations above 0.5 particle per cm^3 with a target expanded uncertainty of ≤15 %. Additionally, in collaboration with LaVision GmbH (Göttingen, Germany), particle tracking and velocimetry (PTV) was applied, for the first time, as a reference method for counting airborne bioaerosol particles (pollen and fungal spoers), extending the reference particle size range up to about 70 μm, with an expanded measurement uncertainty of ≤15 % in the reference number concentration. PTV was validated extensively in the laboratory through a comparision with a reference optical particle counter (Swiss national standard) and the Inkjet Aerosol Generator (Japanese national standard). The Poleno (Swisens, MeteoSwiss), APS-300 (PollenSense, USA) and Aerotape (Oberon, France) bioaerosol monitors were then calibrated against PTV using Polystyrene (PS) spheres, fresh pollen and fungal spores in the aerodynamic size range 5 μm to 70 μm. The size-dependent counting efficiency of the Poleno and Aerotape were found to be between 50% and 30% depending on the particle size. The counting efficiency of the APS-300 could not be determined as the raw data of the instrument are not made available to the end users. As a result, the APS-300 will not be further examined within the BioAirMet project. Experiments with the POMO-500 (Hund GmbH, Germany) proved challenging due to the extremely high flow rate of the instrument. Finally, a third calibration method for measuring the counting

efficiency of the bioaerosol monitors in the field is being developed. In this case, the particles fly through a laser sheet upstream of the bioaerosol monitor's inlet and the reference particle number is measured based on the light scattering signal. An intercomparison of the three reference methods is planned for the summer of 2026.

2. Development of procedures for quantifying the performance of machine learning algorithms and the uncertainties related to particle taxon identification

A set of workshops have been run to collect pollen and fungal spore samples, some of which were exposed to UV, ozone and VOCs, and a machine learning algorithm trained on fresh pollen was used to classify the samples (fresh and aged). A comparison of the classification results showed that the algorithm performed significantly less well when classifying the aged samples. An in-depth analysis of the data was carried out to identify which of the features within the measurement used by the algorithm are most affected by aging. This information will help understand which features need to be considered when assessing a training data set for suitability. The samples have also been analysed using other methods (microscopy, and two kinds of spectroscopy) to see if either method can give further insight into the differences that aging creates.

An outline framework for uncertainty propagation through algorithms for classification of pollen and fungal spores has been developed. Work is underway to investigate and quantify the various uncertainty sources and to understand their effect on the final uncertainty associated with the count, and to find efficient ways of propagating sources through algorithms. Very promising initial results regarding the use of transfer learning to enable algorithms for identification of geographically relevant taxa have been obtained and presented at a conference.

3. Standardisation of the data output, interface and metadata of automatic bioaerosol monitors and the development of guidelines on data storage, handling and distribution

Formalised recommendations have been proposed for the standardisation of Level 0 (raw) data in automatic bioaerosol monitoring. Based on expert consultations, community discussions, and project experience, the guidelines define common data formats, metadata requirements, secure data transfer protocols, archiving practices, and usability principles, in line with FAIR data standards. Broad stakeholder input highlighted the importance of interoperability, long-term data preservation, and reproducibility across rapidly expanding monitoring networks. These Level 0 recommendations address the growing diversity of instruments and operational practices and provide a robust foundation for harmonised raw data handling, while ongoing work is extending the framework towards Level 1 (processed) data standardisation.

4. Contribution to a new CEN/TC 264/WG 39 standard on automatic bioaerosol monitoring and the revision of existing standards published by CEN/TC 264/WG 28

Currently, the only documentary standard on the measurement of ambient pollen and fungal spores refers to the manual method based on the use of collection impactors and offline light microscopy (EN 16868:2019 "Ambient air - Sampling and analysis of airborne pollen grains and fungal spores for networks related to allergy - Volumetric Hirst method" by CEN/TC 264/WG 39). Another document of importance is the technical specification (TS) CEN/TS 16115-1:2011 "Ambient air quality - Measurement of bioaerosols - Part 1: Determination of moulds using filter sampling systems and culture-based analyses" by CEN/TC 264/WG 28 for the same manual methods as with WG 39. The results of this project will feed directly into the new technical specification (to be later upgraded to a full standard) which is currently being developed by CEN/TC 264/WG 39 on real-time, automatic bioaerosol monitoring and into new or updated documents of CEN/TC 264/WG 28.

Outcomes and impact

Key dissemination and communication activities

The project has held two events for stakeholders, the first workshop took place in May 2025 and was intended to foster dissemination, and communication about results. The second event was a two-day training workshop on fungal spore bioaerosol generation held in Brussel, October 2025 and included theoretical training, laboratory demonstrations, and hands-on practice using several fungal species and the Swisens Poleno Jupiter system. Stakeholders are also kept up-to-date via the bi-annual newsletter and are invited to participate

in training workshops and calibration campaigns. The consortium created a website providing detailed information about the project and its projected outcomes, publications and news. The project also has a dedicated LinkedIn page and a blog post has been disseminated through the AI Standards Hub: https://aistandardshub.org/from-algorithms-to-allergies.

Three peer reviewed papers have been published to date plus several manuscripts in preparation. The output of the project has also been disseminated to the scientific community through 14 oral and poster presentations at various national and international conferences. Project results were submitted to different networks such as ACTRIS bioaerosol (FMI and EDI), the SwissPollen operational monitoring network, Belgian monitoring network (Sciensano), Northern networks (FMI) and Bavarian network (TUM). Other networks are informed on BioAirMet activities through EUMETNET AutoPollen (in 2025 a common user survey was held).

<u>Outcomes for industrial and other user communities</u>

The early industrial impact of the project will be focussed on the standardisation of aerosol generators, designed and produced in Europe. Swisens have recently developed a bioaerosol generator (known as the Swisens Atomizer) and METAS was directly involved in the development and validation of a novel oxidation flow reactor (known as the OCU). By combining the Swisens Atomizer with the OCU to produce atmospherically aged bioaerosols, the application range of both generators will be extended. Further standardisation of the generators in new fields of research and demonstration of how these can be incorporated into a single setup for training ML algorithms will further boost sales in Europe and worldwide. Europe has a strong presence in the manufacturing of automatic bioaerosol monitors. The availability of traceable calibration for such instruments will be a direct benefit for existing instruments and on the development of new designs. The improved accuracy of bioaerosol measurements will stimulate demand and production. Europe is leading the automation of bioaerosol monitoring stations, with more than 400 automatic instruments expected to be installed across Europe in the next few years.

Efficient and accurate bioaerosol monitoring will benefit pollen monitoring, which can be used to forecast the production of certain crops, e.g., olives or oak. This can provide useful information to agricultural end-users, helping them e.g., to fix prices and manage crop harvests. Bioaerosol monitors can also be used indoors or in workplace environments that may be contaminated with pathogenic organisms (e.g., organic waste treatment, livestock and poultry farming, arable farming). This project will develop procedures for validating mid-cost bioaerosol monitors which can distinguish between pathogenic and non-pathogenic particles and alert the workforce when a specific target value has been exceeded. Real-time viable particle counters provide real-time counts of total and viable particles in pharmaceutical manufacturing environments to reduce aseptic interventions, improve root-cause investigations and increase process knowledge. Such instruments will be calibrated for the first time in this project, providing valuable information to pharmaceutical and biotechnology industries operating clean rooms.

The vast majority of automatic bioaerosol monitors (POMO-BAA500, SwisensPoleno, Rapid-E, BeeNose, Aerotape and others) are designed and produced in Europe. This project will enhance the end users' confidence in these instruments through standardisation and certification, which will promote sales and help European manufacturers assume a leading role in the global market.

<u>Outcomes for the metrology and scientific communities</u>

Standardising the generation of fresh and aged (i.e., processed) bioaerosols in the laboratory will enable NMIs and DIs to offer new calibration procedures for a variety of instruments, such as automatic bioaerosol monitors for ground-based measurements, aerosol LiDAR systems which determine the vertical profile of aerosols in the atmosphere and portable particle counters and size spectrometers for high-altitude (free troposphere) measurements. In addition, the generation of well-defined bioaerosols in the laboratory is essential for performing controlled health-related studies, e.g., in vitro toxicology and in vivo inhalation studies. Experiments with laboratory-generated bioaerosols, under controlled conditions, can provide information on the causal link between the particles we breathe and the down-stream health effects. They can also complement epidemiological studies.

Since aerosol metrology is a relatively new scientific topic, compared to gas analysis and other relevant disciplines, this will provide a much-needed uplift for aerosol laboratories, helping them to build new scientific networks, and to grow their research collaborations and service spectrum. Moreover, the results of this project

will feed directly into regional/national bioaerosol monitoring networks (e.g., ePIN, SwissPollen, AirAllergy, Met Eireann Network, FinBio). The availability of standardised calibration procedures for bioaerosol monitors is a key benefit from the project and will increase the accuracy and comparability of their data. This in turn will lead to better pollen forecasts for physicians and clinicians, better fungal spore forecasts for ecology and agricultural research, reliable data for the modelling of bioaerosols in the atmosphere and robust emission inventories for airborne pathogens and their regulation.

The results of this project will feed directly into European automatic bioaerosol monitoring networks and they will improve data accuracy by reducing measurement uncertainties from >60 % down to <30 % to address stakeholder needs. The know-how in the training of ML algorithms will find applications in other areas of aerosol sciences, e.g., in the identification of various black carbon (BC)-containing particles, and potentially in other applications that use ML for image-based classification. Further, the consortium will interact closely with the EU Horizon Europe project SYLVA (2023-2026), which is establishing the technological background for European bioaerosol networks.

Outcomes for relevant standards

The results of this project will feed directly into the new CEN/TC 264/WG 39 technical specification on the automatic bioaerosol monitoring of pollen grains and fungal spores, which will be the very first standard worldwide on this scientific/technical subject. The project will also contribute to the revision of existing standards published by CEN/TC 264/WG 28 – Measurement of Airborne Microorganisms in Ambient Air. The outputs of this project will contribute to the development of a new CEN technical specification (and later a standard) on "Near real-time measurement methods for monitoring airborne pollen and fungal spore concentrations" within CEN/TC 264/WG 39. This standard will complement, or even replace in the future, the EN 16868:2019 standard for the manual method: Sampling and analysis of airborne pollen grains and fungal spores for networks related to allergy - Volumetric Hirst method.

Information on progress, and results, from the project will also be disseminated to other standardisation bodies and committees and feedback sought, such as to ISO/TC 24/SC 4/WG9 – Single particle light interaction methods, AFNOR/X43D - Air quality monitoring in ambient air, EURAMET Technical Committee for Metrology in Chemistry (TC-MC) WG Gas Analysis, BIPM Consultative Committee on Quantity of Matter (CCQM) - WG Gas Analysis - Task Group on Particle Metrology. When possible, representatives on the corresponding committee or WG from the participating NMIs will jointly ask to include a point in the agenda to briefly present the outputs of the project related to the WG activities and ask for comments from the other committee/WG members.

Instrument standardisation and certification will also pave the way for the development of European legislation on bioaerosol monitoring, e.g., by setting target limits for highly allergenic or pathogenic bioaerosols in the European Air Quality Directive.

Longer-term economic, social and environmental impacts

The direct impact of the research will be (near) real-time, more accurate and comparable measurements of bioaerosols, drastically improving the current status of the manual monitoring networks in Europe through the development and standardisation of methods for calibrating automatic bioaerosol monitors. Calibration procedures with regards to both particle number concentration and particle identification will be standardised to achieve full traceability to the SI (International System of units). Note that this will be the very first time that full traceability (i.e., particle counting combined with particle identification) to the SI will be achieved in the field of aerosol sciences.

Indirectly, the impact would be very widespread. In terms of scientific benefits, the improved measurements would fit into EU atmospheric aerosol projects, refining climate change models, and improving the quality of conclusions from cohort health studies, which are looking at the health effects of bioaerosols. The ML algorithms standardised in this project could also find novel applications in bioaerosol monitoring in indoor and workplace environments as well as in air pollution monitoring, e.g., in black carbon source apportionment.

An accurate and timely bioaerosol forecast, combined with the traditional weather forecast, will inform the vulnerable population on the particle number concentration of pollen and fungal spores in ambient air, and advise citizens to take necessary measures (e.g., contact avoidance and/or preventive medication) in the case of high pollen and/or fungal spore counts. It will also underpin allergy treatment by enabling a more targeted

and efficient use of anti-allergy medication. In terms of socio-economic benefits, the project's outputs will provide a basis for better city management and planning. Urban green spaces are a key element in the design of modern cities, since they promote human interaction with nature and help to fight heatwaves in cities, thus improving human health. However, lack of planning in the design of public spaces, unfortunate choices of ornamental species and the interaction of pollen with air pollutants have been among the factors triggering allergy and asthma, two of the most widespread chronic diseases in urban populations. Ultimately, the output of this research will have significant benefits on human health within Europe, helping the more than 100 million Europeans who suffer from allergies. Considering the enormous costs of allergies and asthma in terms of hospitalisation and lost labour (> €55 billion per annum due to absence from work or reduced productivity), even a small decrease in these costs through prevention and better treatment will save the EU billions of € on an annual basis, largely exceeding the initial investments in the network's development and standardisation. Real-time information about fungal spores in combination with high resolution models could also mean more targeted fungicide use (both in time and space), which will not only reduce costs, but also environmental damage. The costs associated with crop loss from fungal pathogens are on a similar scale to the impact on human health.

List of publications

1. Bruffaerts, N. et al (2025) 'Advancing automated identification of airborne fungal spores: guidelines for cultivation and reference dataset creation'*, Aerobiologia*, 41(2) p. 505-525. Available at https://doi.org/10.1007/s10453-025-09864-y
2. Pyrri, Ioanna et al (2025) 'Variability in Alternaria alternata spore characteristics under different culture conditions: implications for automatic detection using air flow cytometry'*, Aerobiologia*, 40(3) p. 437-446. Available at https://zenodo.org/records/17830895
3. Vasilatou, K. et al (2025) 'Toward Accurate Real-Time Bioaerosol Monitoring in the Particle Size Range 1 μm–70 μm'*, ACS ES&T Air*. Available at https://doi.org/10.1021/acsestair.5c00282

Project start date and duration:	1 June 2024, 36 months
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Internal Beneficiaries:	External Beneficiaries:	Unfunded Beneficiaries:
1. PTB, Germany	6. Oberon, France	-
2. DFM, Denmark	7. Sciensano, Belgium	
3. FMI, Finland	8. TUM-MED, Germany	
4. LNE, France		
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