Challenges of modern aerobiology

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The flowering seasons of Betula spp and Poaceae partially coincide in Ukraine, reflecting the effect of climate change

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Background: Since pollen is a significant factor in allergies, and sensitized individuals often experience polysensitization to various allergen groups, breaks in the plant pollination season, providing patients with relief from allergic symptoms, are crucial times within the high allergenic period.
However, due to climate change and the prolongation of pollen seasons - the early start of some and the late end of others - a trend has been observed in Ukraine towards the convergence of the flowering seasons of grasses and trees, particularly birch.
Birch and Poaceae pollen are known to be among the most dangerous airborne allergens in Ukraine, each triggering allergic reactions in approximately 40% of pollen-allergic individuals.
Allergy symptoms induced by grass pollen last the longest, as different species of the Poaceae family bloom sequentially over an extended period. Although the main season runs from May to July, concentrations of grass pollen can remain at significant levels, provoking hay fever symptoms in patients until mid or even late August.
Therefore, we decided to analyze annual monitoring data and compare the timing of grass and birch pollen seasons.
Method: From 2009 to 2023, pollen was collected using volumetric methods with a Burkard trap positioned at a 25-meter height above the ground on the roof of a building at Vinnytsya Medical University. The recorded pollen counts were subsequently converted into pollen concentrations per cubic meter of air. Analysis of the samples was conducted at a magnification of x400. Subsequently, a statistical analysis was performed using Excel.
Results: It is known that, for birch, the beginning of the season is considered when there are 25 pollen grains per 1 m^3 of air. For grasses, this quantity is 10 grains of pollen per 1 m^3. After conducting a statistical analysis, we found that the start dates of the flowering season for both Betula and Poaceae are gradually approaching each other. While in 2014 and 2015, the interval between the start dates was 41 and 38 days, respectively, starting from 2019, the difference gradually decreased. In 2022, the gap between the start of the Betula and Poaceae pollen seasons was 30 days, and in 2023, it was only 14 days.
Furthermore, it is worth noting that the duration of the flowering seasons of both plants tends to lengthen, negatively impacting individuals with high sensitization to birch and grass pollen.
Over the last 5 years, starting from 2019, the initiation of grass pollen coincides with the birch pollen season, beginning from late April and sometimes even mid-April. However, the number of days when the pollen levels of both plants reach causally significant concentrations remains limited.

Particularly in 2022, when birch pollen was particularly intense, periods of clinically significant concentrations of both plants coincided from May 17 to May 25.
Taking into account literature data indicating that a threshold of 10 grains/m³ of air is a trigger for the widespread onset of grass pollen allergy symptoms and that initial symptoms can be observed when grass pollen concentrations exceed 1 grain/m³, the period of overlapping flowering seasons of both plants, which is hazardous for individuals sensitive to both types of pollen, can last from 2 weeks to a month - depending on the nature of pollination, especially birch, which has a pronounced two-year cycle with more active pollination in even years.
Conclusions: Over time, the risks of allergic reactions due to the coincidence of birch and grass pollen seasons may significantly increase due to the prolongation of the birch pollen season and the early start of the grass pollen season.
This may affect the duration and intensity of allergic reactions in sensitive individuals.
It is especially important to pay attention to patients who are sensitive to both plants simultaneously and separately, as changes in pollen seasons can influence the nature of the body's allergic response, requiring adjustments to treatment approaches and improving the quality of life for patients during the flowering season.
Additionally, during this year's monitoring, special attention should be given to the birch and grass pollen seasons.

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Exploring the Influence of Spatiotemporal and Meteorological Variation on Norwegian Atmospheric Pollen

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Background & Aims: Being able to predict plant responses to their environment is essential in many ecological, economical and medicinal disciplines. This is especially relevant in terms of climate change, which is expected to affect temperature, precipitation and all derivate meteorological patterns via changing atmospheric and oceanic circulation. Plant phenology has been shown to be strongly dependent on environmental variation, both short-term via weather fluctuations and long-term via climate change, with consequences to food-webs, production potential and pollen seasonality. This is relevant for many species, including but not limited to birches, oaks and grasses, all of which produce highly allergenic pollen to many human populations. However, to what degree different types of environmental variation is expected to alter pollen seasonality has not been fully explored for all pollen types, and many research findings remain contradictory. The primarily aim of our study is to explore how temporal, spatial and meteorological variation contributes to pollen seasonality, and if possible identify ecological relationships influencing the variation.

Methods: We modelled the daily concentrations of seven common Norwegian pollen types: hazel (*Corylus*), alder (*Alnus*), willow (*Salix*), birch (*Betula*), pine (*Pinus*), grass (Poaceae) and mugwort (*Artemisia*) using generalized linear models with negative binomial distributions. The data was obtained from 12 regional sampling stations and for up-to 28 years. The individual models contained three temporal (DOY, month and year), four spatial (latitude, longitude, altitude and sampling height) and six meteorological variables (air temperature, precipitation, relative humidity, atmospheric pressure, wind speed and solar radiation). The meteorology was obtained from the MET Nordic dataset with full cover. The variables were compared thematically and individually in a reduced-model framework using chi-squared anova tests supplemented by AIC and Nagelkerke Pseudo-R2 to assess the variation contribution.

Results: The full models were able to explain the most variation in comparison to reduced models, with R2 up-to 49.5%, 51.7% and 59.5% for pine, birch and grass pollen respectively. Temporal vari-

ables were able to explain more variation than spatial or meteorological variables on average for most pollen types, with the exceptions being hazel and grass, for which spatial variables had higher explanatory power. While the contribution of individual variables varied based on the pollen type, on average, month, altitude and maximum temperature were the variables in each thematic category with the highest contribution.

Conclusion: Temperature cues and the natural timing of phenological development were identified as the main pools of variation for the pollen seasonality. While our models could explain a substantial proportion of the variation, model understanding could likely be enhanced by including source maps and regional atmospheric transport modelling.

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Qualitative and quantitative analysis of pollen spectrum in the atmosphere of seven South African cities

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The South African Pollen Monitoring Network (SAPNET) was set up in 2019 to conduct the first long-term national aerospora monitoring organised at a national level. , SAPNET has monitored airborne pollen and fungal spores in seven of the major cities across South Africa, covering multiple biomes with a diversity of climates, topographies, and vegetation types providing online weekly reports www.pollencount.co.za to allergy sufferers and healthcare providers. In this study, we highlight the major pollen types found at the different sampling locations, provide updated pollen calendars for each city, and consider the future of aerobiome monitoring in South Africa.
Daily airborne pollen concentrations were measured from August 2019 to August 2021 in seven cities across different biomes in South Africa: Cape Town (CPT, Fynbos biome), Johannesburg (JHB, temperate Grassland biome), Pretoria (PTA; Savanna biome), Bloemfontein (BFN; semi-arid section of the Grassland biome), Kimberley (KMB; semi-arid section of the Savanna biome), Durban (DBN; Indian Ocean Coastal Belt biome) and Gqeberha (formerly Port Elizabeth [PE]; Albany Thicket biome). Standard aerospora monitoring devices, Hirst type 7-day volumetric spore traps (manufactured by Burkard, UK), were used at all sites. Six of the seven locations had spore traps installed in 2019, while the Cape Town spore trap has been operational for the past 30 years. The height of the spore traps

varied from 5 to 20 m above ground. Daily samples were then prepared and mounted on microscope slides for microscopic analysis using glycerol jelly, complying with the guidelines of the European Aerobiology Society Working Group on Quality Control (Galan et al.2014). The number of individual aerospora counted along the three longitudinal traverses was totalled for each pollen or fungal taxa. Weekly pollen grain (pg) concentrations (for 2 years - 104 weeks -of sampling were used to calculate the average Annual Pollen Index (API, pg/m3) and percentage pollen contribution to the different pollen categories (grass, trees, weeds) across South Africa. For the pollen calendars, the average weekly pollen index (pg/m3) was calculated using concentrations from the previous 14 days in a 52-week calendar; Pollen calendars displaying four coded levels of weekly pollen concentrations were created using Microsoft Excel. The levels were adapted from Potter and Cadman (1996) as follows: 0 = 0−3; 1 = 3−10; 2 = 10−30; 3 = 30−100; 4 = >100 pg/m3. Only the pollen types contributing more than 3% to the API of each monitoring site were included.
Across all sites, Johannesburg had the highest average API for trees (14,363 pg/m3) and weeds (2454 pg/m3). The highest individual weekly concentration for trees was in Bloemfontein (3837 pg/m3 in September 2020) with the highest individual weekly concentration for weeds recorded in Cape Town (201 pg/m3 in October 2020). The highest average API for grasses was in Bloemfontein (8353 pg/m3), which had the highest individual weekly concentration for this pollen category (676 pg/m3 in February 2020). Gqeberha (PE) had the lowest tree, weed, and grass pollen concentrations. The main pollen types were from exotic vegetation. The most abundant taxa were Poaceae, Cupressaceae, Moraceae and Buddleja. The pollen season start, peak and end varied widely according to the biome and suite of pollen taxa. The main tree season started in the last week of August, peaked in September, and ended in early December. Grass seasons followed rainfall patterns: September–January and January–April for summer and winter rainfall areas, respectively. Major urban centres, for example, Johannesburg and Pretoria in the same biome with similar rainfall, showed substantive differences in pollen taxa and abundance. Some major differences in pollen spectra were detected compared with historical data. However, we are aware that we are describing only two years of data that may be skewed by short-term weather patterns.
The main findings from this study show differences observed in pollen spectra across biomes and between geographically closely located sampling sites emphasising the need for continued pollen monitoring—not only across the existing sites but also at new locations in South Africa.

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Cyanobacteria and microalgae in the coastal air: insights from a 5-year study in the Gulf of Gdańsk Region

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Bioaerosols are microorganisms or their fragments emitted from the surface of the sea or terrestrial environment to the atmosphere. So far, scientific research has focused much more on viruses, bacteria, and fungi, which has led to a weaker recognition of the presence of cyanobacteria and microalgae in the air compared to other microorganisms.
This study exclusively focuses on cyanobacteria and microalgae noted in the atmosphere within the

Gulf of Gdańsk region (Poland). It serves as a summary of 5 years of research conducted by our team on these microorganisms, delving into both the obtained results and the challenges encountered in studying cyanobacteria and microalgae in the atmosphere.
Within this research, the quantity and taxonomic composition of cyanobacteria and microalgae present in atmospheric aerosols and rainfall in the Gulf of Gdańsk area were determined. Samples of cyanobacteria and microalgae were collected both at a research station located one kilometer from the shoreline and during research cruises in the Gulf of Gdańsk. Furthermore, meteorological factors influencing the abundance of these microorganisms in the atmosphere were identified. Using modern sampling techniques employing a 6-stage microbiological impactor, the aerosol size fraction containing microorganisms was determined. Flow cytometry, light microscopy, and epifluorescence microscopy were used in the research.
The results obtained indicated that the quantity of cyanobacteria and microalgae in the air of the coastal zone of the Gulf of Gdańsk varied from 0 to 1685 cells m−3. In rainwater, their quantity ranged from 100 to 342×103 cells L−1. Between 2018 and 2020, a total of 35 cyanobacteria or microalgae were isolated from the collected samples. Furthermore, in the course of seasonal studies focusing specifically on cyanobacteria and microalgae in 2020, 29 taxa were identified. Additionally, the capability of cyanobacteria and microalgae present in the atmosphere to produce toxins as well as to degrade harmful substances present in atmospheric dust was assessed. Moreover, based on the taxonomic composition, the potential harm of the identified organisms to human health was preliminarily assessed.

These studies contribute to raising awareness regarding the pollution of air by biological particles, with particular emphasis on tourist areas along coastal regions. The research indicates that the presence of cyanobacteria and microalgae in the air should be monitored throughout the year, especially during toxic blooms in the seas. Furthermore, these research findings suggest that during periods of intense phytoplankton blooms in the sea, not only should active use of water bodies be avoided, but also proximity to these areas. Moreover, the conducted research and the obtained results shape further avenues of investigation and potential development of research methods in the field of cyanobacteria and microalgae inhabiting the atmosphere.

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Free radicals in pollen, inflorescences and in associated soil samples

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Air pollution significantly impacts human health, reducing the quality of life. This study analysed free radicals in pollen, inflorescences, and associated soil samples. Free radicals are formed during plant metabolic processes, such as photosynthesis, and are influenced by environmental factors like pollution and various forms of radiation. The radicals can be harmful or advantageous to human health, depending on the concentration and type. Understanding free radical dynamics in plants and their correlation with environmental conditions helps to understand the potential impact on human health.
The current study involves the collection of soil, inflorescence, and pollen samples from *Ulmus*, *Populus*, *Salix*, *Corylus*, *Alnus* and *Betula* in urban and rural areas across Latvia during the plant flowering season. Sample preparation (including emptying of florescence from pollen) was performed at the Laboratory of Atmospheric Processes and Aerobiology of LU, while electron paramagnetic resonance (EPR) spectroscopy for free radical analysis occurred at the Latvian Institute of Organic

Synthesis.
The EPR spectra identified Fe^+3 and Mn^+2 signals alongside organic free radicals. Weak intensity signals with microstructure in low magnetic fields were observed. The study primarily analysed organic free radicals, with typical g-values ranging around 2.006 ± 0.002. The concentration of organic radicals varied significantly among pollen samples from different tree species, with the highest concentration in *Salix* and *Ulmus* pollen and the lowest in poplar pollen. Similar concentrations were found in *Corylus*, *Alnus*, and *Betula* pollen.
Betula samples from 15 different sites were analysed, revealing slight differences in the concentration of organic radicals in birch pollen. However, *end-of-flowering* samples showed lower concentrations compared to *start-of-flowering* pollen samples. More radicals were observed in empty inflorescence than in pollen. A weak additional signal with g=2.0033 was recorded in some pollen and/or empty inflorescence, also present in all soil samples, suggesting the presence of silicon or carbon oxide radicals related to ionising radiation.
The results have shown the presence of various free radical particles in pollen and florescence, influenced by external environmental factors. Further studies will be performed to obtain more complete results.

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Patterns of Weed Sensitization and Internet User Interests suggest a need of public health education in Ukraine

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Ragweed (Ambrosia) and mugwort (Artemisia) remain key weed-allergenic species in Ukraine. This study aimed to determine regional sensitization levels to these weeds and explore whether inhabitants are genuinely concerned about their allergies.
Data from 20,033 patients who underwent ALEX allergy testing in 17 Ukrainian regions (2020-2022) were analyzed. The data, processed with Excel, was compared with Google Trends statistics for the last five years to assess user interest in words like “ragweed” and “mugwort” in Russian and Ukrainian.
Sensitization to Ambrosia alone was found in 30.43% (6097 people) of the sample; 15.70% (3145 patients) were sensitive to Artemisia, and 33.84% (6780 people) responded to both weeds. Ragweed sensitization was highest in Southern and South-Eastern regions: Dnipro (55.05%), Mykolaiv (45.45%), Donetsk (44.44%), Poltava (42.63%), Kherson (38.34%), followed by Kharkiv (36.21%) and Odesa (34.59%).
Mugwort sensitization lacked a clear regional pattern, being most common in Northern Zhytomyr (25.00%) followed by Central-Southern (Dnipro 23.99%), Southern (Donetsk 18.52%), and North-Eastern regions (Poltava 18.42%, Kharkiv 17.49%).
Internet search queries are particularly aligned with the regional patterns of sensitization. Despite ragweed allergy prevalence, the search for “mugwort” was more popular than that for “ragweed” in all South-Eastern regions over the last five years. “Ragweed” queries alone dominated in Odesa, Kharkiv, and Crimea, corresponding to higher Ambrosia sensitivity in these regions.
High levels of “ragweed” queries were also common for Kyiv, Chernihiv, Sumu and Transcarpatia regions where this type of allergy was not so common.
The term “mugwort” was the most searched again in southern and southeastern regions. Somewhat increased queries were also seen in the northern (Chernihiv, Sumu) and Eastern (Kharkiv) regions.
The data suggests that Internet user interests align with allergy sensitization patterns in Ukraine in particular. This may indicate poor population awareness of the true factors which may cause their allergy, which this person experiences during the season, particularly for Ambrosia. Queries about Artemisia may reflect people’s interest in this weed as a raw herbal material. This indicates a need for public education about the causal agents of genuine allergy in Ukraine.

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Aerobiological study of the town of Hinojosa del Duque, Córdoba, Spain.

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Hinojosa del Duque is located at the north of the province of Cordoba, in Andalusia (southern Spain). This area is characterized by being a rural region, mainly agricultural with extensive vegetation formations of the Mediterranean Dehesa type.
This study develops a qualitative and quantitative analysis of the atmospheric pollen content over a period of two years, 2021 and 2022, using a Hirst-type volumetric sampler, placed in the urban center of this town.
As a result, a total of 54 pollen types were detected, of which 34 correspond to arboreal taxa and 20 to herbaceous taxa. The average annual pollen index was 151,390 pollen grains. The most abundant pollen types were, according to their order of importance: *Olea*, *Quercus*, Poaceae, *Urtica*, *Cupressus*, Amaranthaceae, *Rumex* and *Fraxinus*. These taxa accounted for 90% of the total pollen spectrum in the two years studied.
The intra-daily variation analyzes demonstrated that the behavior of the different pollen types differs between seasons, showing variations from one year to the next. The different meteorological conditions that took place between the years studied produced changes in the quantity and diversity of atmospheric pollen in Hinojosa. The rainfall that occurred between January and March 2022 produced a shortening and decrease in the atmospheric pollen concentration by approximately 70% in the spring of that year, due to the effect of atmospheric washing.

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Fungi-sensitive Individuals of Vinnytsia Region, Ukraine are responsive to Alt a 1 mostly and can experience symptoms for 4 months

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Background: Fungi is an important factor of human allergy given that they are ubiquitous by their nature and humans can be exposed to fungal allergens in different sources. The most often the one contacts with inhalant mold allergens which are contained in microspores and in mycelium. Several important fungal allergens are known up to now and the most valuable among them is Alternaria which is one of the most spread environmental fungi. However, as other fungal sources are also important the aim of our study was to determine the patterns of fungal sensitization in the population of Vinnytsia region of Ukraine and compare it with possible environmental exposure.
Method: The data of allergic sensitization of 2623 resident of Vinnytsia region obtained by the ALEX test in the years 2020-2023 were analyzed. It was compared with the aerobiological data gained in the same years by the Laboratory of Allergenic Environmental Factors Investigation at the National Pirogov Memorial Medical University. Spore concentrations per cubic meter of the air data were obtained using the volumetric spore trap of Hirst type.
Results: It was found out that 9.61 % (252 tested individuals) were fungi-sensitive. Men prevails in this sample (54.77 %) over women. Most of fungi-sensitive residents of Vinnytsia region (68.90 %) were sensitive to the major Alternaria allergen Alt a 1. Malassezia allergen Mala s 11 was the second (9.84 %). It was followed by Mala s 6 and Saccharomyces extract Sac c with the share 9.06 % for both. The next was Asp f s (5.51 %).
However, while analyzing the combined sensitization to different allergens we found out that 57.94 % of Vinnytsia region residents were sensitive to the Alt a 1 alone among fungal allergens. The only sensitivity to Mala s 11 held the second position with a share 4.76 %. Combination of Alt a 1 and Mala s 6 was the third (3.17 %) followed by the Sac c and Mala s 6 alone with a share of 2.78 % for both

cases. Another important allergen Aspergillus was just 7th in combination with Mala s 11. Extract of Cladosporium Cla h and its allergen Cla h 8, which represent the most numerous spores in the atmosphere, were capable to sensitize just 0.79 % of studied people in combination with Alt a 1. While analyzing the timing of sporulation of airborne fungi we found out that potentially clinical relevant concentrations of Alternaria of more than 100 spores/m3 of air were seen in 2020-2023 years from June 30 to October 28 – full 4 months. These period is especially dangerous for the Alternaria-sensitive residents of Vinnytsia region which constitute the majority of the fungi-sensitive patients. Conclusion: Our study highlights the prevalence and specific patterns of fungal sensitization in the Vinnytsia region, emphasizing the potential risks associated with Alternaria exposure from July to October to the vast majority of fungi-sensitive individuals.

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Ecological and allergenic significance of atmospheric pollen in North-West Province (South Africa) over a one-year period

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Background:
This study conducted pollen monitoring in the North-West Province of South Africa from December 2022 to 2024, in Potchefstroom within the temperate Grassland Biome. Aligned with SAPNET (South African Pollenmonitoring Network), the project aimed to comprehensively monitor allergenic pollens and fungal spores across South Africa (Ajikah et al. 2020). The primary objective in Potchefstroom was to analyze monthly fluctuations of prominent pollen types, seeking patterns indicative of seasonal changes and allergenic pollen peaks. The study addressed the challenges of deciphering plant phenology in southern Africa and emphasized the role of pollen monitoring in understanding urban ecosystems and green infrastructure use.

Methods:
Pollen monitoring in Potchefstroom was executed as part of the SAPNET effort. The study involved monthly measurements and recording of the most prevalent pollen types with a Lanzoni 7-day volumetric spore trap. The methodology considered urban ecology and green space utilization, contributing to decision-making processes for planting suitable trees in an urban context. A pollen atlas was developed, providing next to microphotos detailed information on identified pollen types, including pollen morphology, ecology, pollination mode, and allergenicity.

Results:
The findings, despite the brief monitoring period, unveiled a distinctive pollen calendar in Potchefstroom. Tree pollen, dominated by Northern Hemisphere neophytic species as in other major South African cities (Gharbi et al. 2023), particularly Cupressus, Platanus, Betula, Morus, Pinus, and Quercus, exhibited a major release during August to September. Notably, indigenous Searsia species contributed significantly, while exotic Ulmus pollen, more abundant than in other South African cities (Esterhuizen et al. 2023), raised concerns in summer. The grass pollen season extended from

November to April, with additional contributions from herbs like Plantago observed between February and April. The study identified Potchefstroom as hosting the highest levels of Ambrosia pollen in the country, peaking in March and April, highlighting its significance as a potent allergen.

Conclusion:
The implications extend beyond phenological insights, emphasizing the importance of understanding pollen dynamics for urban ecology. The findings aid in formulating informed strategies for the planning, design, and management of sustainable and resilient cities. This research contributes significantly to regional pollen patterns in the North-West province of South Africa. Comparisons with Bloemfontein, Pretoria, and Johannesburg (see Esterhuizen et al. 2023) show similar seasonal pollen distribution patterns, with Potchefstroom exhibiting lower pollen levels, attributed partly to its smaller size. The study also notes variations in a second pollen peak from February to April for tree taxa across these locations, highlighting the unique ecological dynamics of Potchefstroom and Pretoria in comparison to Bloemfontein and Johannesburg.

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Relationship between airborne Alternaria alternata and Alternaria spp. spores

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Alternaria is a plant pathogen and human allergen. Alternaria alternata is one of the most abundant fungal spores in the air. The purpose of this study was to examine whether Alternaria spp. spore concentrations can be used to predict the abundance and spatio-temporal pattern of A. alternata spores in the air. This was investigated by testing the hypothesis that A. alternata dominates airborne Alternaria spp. spores and varies spatio-temporally. Secondarily, we aimed at investigating the relationship between airborne Alternaria spp. spores and the DNA profile of A. alternata spores between two proximate (~ 7 km apart) sites. These were examined by sampling Alternaria spp. spores using Burkard 7-day and cyclone samplers for the period 2016–2018 at Worcester and Lakeside campuses of the University of Worcester, UK. Daily Alternaria spp. spores from the Burkard traps were identified using optical microscopy whilst A. alternata from the cyclone samples was detected and quantified using quantitative polymerase chain reaction (qPCR). The results showed that either A. alternata or other Alternaria species spores dominate the airborne Alternaria spore concentrations, generally depending on weather conditions. Furthermore, although Alternaria spp. spore concentrations were similar for the two proximate sites, A. alternata spore concentrations significantly varied for those sites and it is highly likely that the airborne samples contained large amounts of small fragments of A. alternata. Overall, the study shows that there is a higher abundance of airborne Alternaria allergen than reported by aerobiological networks and the majority is likely to be from spore and hyphal fragments.

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First insights on APS-300 for airborne Olea pollen monitoring in the Mediterranean area

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Background: Airborne pollen monitoring depends on precise and reproducible pollen detection and analysis. In Europe, airborne pollen monitoring is done by manual counting, which is a labor-intensive and somewhat slow process. That is why there is a need for new automatic methodologies to solve these problems. Even though some significant work has been done with various degrees of success; however, there are still some problems in automatic pollen monitoring. In this study, we report on the field-testing outcomes in Cordoba, Spain, for a novel automated real-time pollen imaging sensor.
Methods: We first compared, with parallel measurements, the pollen concentrations measured by an automated real-time pollen sensor (APS-300, Pollen Sense LLC) and the manual Hirst-type spore-trap, from April 18, 2023, to May 15, 2023. Both samplers are located in the same station in Rabanales Campus, University of Cordoba, Spain. Second, we evaluated the quality of the retrieved pollen concentrations detected with both samplers.
Results: During the studied period, the APS-300 measured average daily pollen concentrations of 2129 (pollen grains/m3), while the Hirst-type trap recorded 1535 (pollen grains/m3). On April 27, the APS-300 recorded the greatest concentration of >9,000 pollen grains, whereas the Hirst-type trap recorded a peak concentration of >8,000 pollen grains. With r equal to 0.73, the daily *Olea* pollen concentrations obtained by both systems showed a highly significant correlation.
Conclusions: The mobile and real-time capabilities of APS-300 underscore its potential to monitor *Olea* pollen. It represents a significant improvement over manual counting methods in terms of time resolution and human efforts. However, further study is required to improve automatic pollen detection techniques.
Keywords: Automated, *Olea* pollen, comparative study, validation, pollen monitoring

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WIBS-4+ bioaerosol sensor: an assessment of its intended-use, and an evaluation of alternative aerosol applications

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Background
This intensive real-time monitoring campaign was carried out over a two-month period in Saclay, a semi-urban centre, southwest of the city of Paris, France, and is an outer suburb/exurb of the city proper.

Methods
The Wideband Integrated Bioaerosol Sensor (WIBS) 4+ model was first compared to the traditional Hirst volumetric sampling method. It was evaluated for its ability to sample and detect ambient

bioaerosol concentrations, namely fungal spores and pollen grains. Along with the WIBS device and the Hirst device, meteorological and pollution parameters were obtained from co-located monitoring devices at the research centre. This allowed the construction of several Multiple Linear Regression (MLR) algorithms.

Results
For fungal spores, significant predictors of concentration were the A WIBS channel, wind from the south and south-westerly directions, and NOx emissions. For pollen grains, the WIBS 4+ additional Xenon flashlamp (distinguishing the WIBS 4 from the WIBS 4+), which allowed for the D, DE, and E WIBS channel categories, was a strong predictor of concentrations, when combined with northerly and easterly winds, and atmospheric ammonia concentrations.

Additionally, the possibility of using WIBS 4+ technology to monitor aerosols that are non-biological was evaluated. Black carbon, which does fluoresce but does not need to be of biological origin, was found to strongly correlate with BC WIBS channel particles, along with various windspeed and wind direction parameters.

Conclusions
The work from this campaign shows the strong bioaerosol monitoring capabilities of the WIBS technology, the benefits of the additional Xenon flashlamp, and the potential alternative and novel uses for which the device can be deployed.

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Evaluation of air sampling methods for DNA-based aerobiome diversity assessment

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Background: Standard methodology for aerobiome analysis involves air sampling using Hirst-type sampler and microscopic analysis of pollen and spore morphology (SRPS EN 16868, 2019). However, morphology-based approach has limitations in total biodiversity assessment and thus demands application of more sensitive DNA-based methodology.
This study aimed to evaluate the efficiency of three different air sampling approaches: Hirst-type sampler, high-volume and low-volume cyclones for use in DNA-based aerobiome diversity assessment.
Methods: Air sampling was conducted in two independent experimental setups on the Research Institute rooftop in Pannonian plain, Serbia using three different devices: Hirst-type samplers, handmade cyclones and SASS 2300 devices, all sterilized prior sampling. The first sampling campaign was performed continuously over seven days in June 2023 using three, side-by-side operated Hirst-type samplers (10 l/min). A set of seven 24-hour samples were collected from each device. Two sets of samples were used for DNA analysis and the third was used for standard optical microscopic quantification of pollen and fungal spores. Additionally, two handmade cyclones (with and without water) were used for 24-hour low volume (flow was variable ranging from 4 l/min to 11 l/min) sample collection and subsequent DNA extraction. The second air sampling campaign was conducted in October and November 2023 using a high-volume cyclone sampler (the SASS 2300 connected with the SASS 4000 concentrator boosting sampling flow to 4000 l/min), and Hirst-type sampler, side by side. Each month, the two 12-hour samples (day: 6am-6pm, night: 6pm-6am of the same day) collected with high volume cyclone were subjected to DNA analysis and microbial cultivation following series of dilutions of fungi and bacteria on malt extract and tryptic soy agar, respectively,

whereas the 24-hour Hirst-type sample was used for standard microscopic quantification of pollen and fungal spores. Environmental DNA (eDNA) extraction was performed in a second class safety cabinet, in a clean room with ISO5 conditions, using the DNeasy PowerSoil Pro Kit (QIAGEN, USA). The concentration of airborne eDNA was quantified using a fluorometric approach, while real-time PCR was used to confirm the presence of bacterial, fungal and plant DNA.
Results: Despite standard Hirst-type method confirmed abundant bioaerosols suspended in the atmosphere during both sampling campaigns (concentrations per sample: June 4568-12565 pollen and spores/m^3; October 2229-9600 pollen and spores/m^3; November 1180-4859 pollen and spores/m^3), the concentrations of eDNA extracted from 24-hour air samples collected by Hirst-type samplers and handmade cyclones were generally very low, ranging from undetectable to 161.9 pg/ul, while the eDNA concentrations from 12-hour samples collected by high volume cyclone sampler were higher (7-1770 pg/ul). Despite relatively low eDNA concentrations, the results of real-time PCR confirmed the presence of bacterial, fungal and plant DNA in most of the samples. However, there was no observed correlation between the total pollen or spore count and the DNA quantity, as indicated by real-time PCR Ct values. This lack of correlation is likely due to the low eDNA concentrations which make samples more susceptible to minor variations during handling, thereby hindering the start DNA concentrations of different microbial groups during the PCR preparation step. In line with DNA analysis, microbial cultivation of the high-volume samples from October and November confirmed notable abundance of viable fungi and bacteria, with CFU/ml ranging from 1x10^3 to 3.8x10^5. The closer morphological assessment of microbial isolates showed that certain species were almost exclusively observed in the day 12-hour samples (*Aspergillus* spp. and *Trichoderma* spp.), while others (*Rhizopus* spp.) were identified in the night samples.
Conclusions: Taken together, these findings represent only a part of the total fungal diversity and more comprehensive data can be collected by applying more sophisticated optical and DNA-based methods, including flow cytometry and DNA metabarcoding, respectively. The positive PCR reactions confirming fungal, bacterial and plant DNA obtained in this study hold promise for the application of such advanced techniques, even on low-concentration eDNA samples. These results highlight the importance of using multiple methods for collection and assessment of aerobiome biodiversity and dynamics, including both molecular, DNA-based and culture-based approaches. Further studies, incorporating long-term sampling, are imperative for capturing the temporal dynamics and trends of airborne microbial communities, improving the sensitivity of detection and providing more comprehensive understanding of airborne populations within a specific location.

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Holography-based aerobiological monitoring: a 2-year intercomparison campaign versus the standard Hirst method in Brussels, Belgium.

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Around 10% of the Belgian population is allergic to the pollen emitted by trees of the Betulaceae family (birch, alder, hazel and hornbeam) and an estimated 18% to grass pollen. To prevent and treat respiratory symptoms and reduce the allergy burden, pollen grain concentrations have been continuously monitored using the standard Hirst method with a volumetric spore trap. However, this offline method can only provide results retrospectively, from 1 day to 1 week, and previous evaluation studies have shown certain limitations in terms of sampling efficiency and measurement accuracy.
Recently, several automatic real-time instruments have been developed to allow the identification and quantification of airborne pollen. After a 2-year campaign performed in Brussels (Belgium),

daily concentrations of 13 pollen taxa measured by the Swisens Poleno Jupiter were compared to the measurements of the Hirst method. The highest intraseasonal correlation values between both methods were found for Betula, Fraxinus and Poaceae. While some pollen taxa showed similar seasonal kinetics but with scaling discrepancies, other pollen taxa frequently presented out-of-season false positive peaks as results from the automatic monitor. In all, the holography-based monitoring in real conditions appeared to be relatively reliable within the seasons of most allergenic pollen taxa. Further improvements are expected by training the identification algorithm with reference datasets generated from pollen collected in the local environment.

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Sensing and Monitoring of Airborne Real-Time Pollen

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This research aims to modernise the pollen monitoring infrastructure in Ireland, by introducing real time monitoring using the Swisens Poleno and comparing it to the already established Hirst sampler. Using pollen data that was collected from previous years, updated pollen calendars were constructed for the benefit of individuals afflicted with hay fever. The Swisens Poleno device was subjected to a diverse range of pollen types in order to develop a comprehensive database for the purpose of pollen detection and identification. The Swisens Poleno device provides the capability to acquire real-time data on pollen concentrations. The utilisation of modelling techniques will be employed to determine the origins of pollen in Ireland, which will, in turn, make environmental monitoring operations easier to accomplish. Development of a sophisticated pollen forecast model specifically for Ireland will be created by integrating traditional, real-time, and meteorological parameters so that pollen concentration will be able to be predicted. This research represents a significant leap forward in pollen monitoring. The study improves our understanding of the dynamics of airborne pollen by comparing the Hirst and Swisens Poleno samplers and making use of sophisticated modelling techniques. Improvements in public health and environmental management are anticipated as a result of the findings, which are applicable to individuals who suffer from allergies, healthcare professionals, and environmental scientists.

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Icelandic Odyssey: Navigating Challenges in Modern Aerobiology through the Transition from Manual to Automatic Pollen Monitoring Systems

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Background:
Iceland, the second-largest island in Europe at 103,100 km2, exhibits unique biogeographical characteristics due to its location between Europe and North America and its position at the boundary of the Arctic and Boreal regions. The climate is influenced by various air masses and ocean currents,

resulting in unstable weather conditions. The vascular flora of Iceland, originating postglacially, consists of 426 native taxa. Unlike counterparts in Greenland and Scandinavia, Icelandic flora features a distinctive Atlantic-European element, particularly abundant in the Arctic and Subarctic regions.
Methods:
Pollen concentrations in the air in Iceland are monitored from April/May to September every year in two main towns: Reykjavík (SW Iceland, since 1988) and Akureyri (N Iceland, since 1998). Daily average pollen grain numbers were collected using the volumetric method with the Burkard Seven-Day Volumetric Pollen Trap. Since July 28, 2022, pollen monitoring in Akureyri has also been conducted by the automatic Pollen Monitoring System, Swisens Poleno Mars. The automatic system covered two grass pollen seasons and one birch pollen season. A comparison was made between both measurement instruments, using basic statistical methods such as the Pearson correlation coefficient.
Results:
Long-term pollen monitoring data reveals a low diversity of airborne pollen in Iceland's aeroplankton. Betula spp. and Poaceae are the most common pollen allergens. Iceland's pollen seasons exhibit a delayed onset compared to continental Europe. While daily grass concentration results from both devices follow similar patterns, detailed analysis reveals numerous false positive events. After tuning the grass pollen identification method, it is now satisfactory. Besides grass pollen, birch pollen is another crucial type in Iceland. In 2023, testing with Polleno Mars revealed numerous false positive events, indicating a need for further refinement in Betula pollen classification.
Conclusions:
The introduction of automatic pollen monitoring systems requires time (more than one pollen season) to adjust the system to local conditions. Tuning each pollen taxon separately is crucial. The Swisens Poleno Mars in Iceland has offered valuable insights into pollen seasons, particularly in grass pollen identification. However, ongoing efforts are essential to refine Betula pollen classification for a comprehensive understanding of Iceland's aeroplankton.

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Pattern of sensitization to PR-10 in Ukraine suggests a long period of potential tree pollen allergy

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Background: It is well known that the major allergens of PR-10 group, including birch (Bet v 1), hazel (Cor a 1.0103), alder (Aln g 1), and beech (Fag s 1), are the leading pollen allergen for trees of the Fagales order. The flowering period of these trees typically occurs from March to May, but climate change has influenced this pattern. Therefore, for pollen allergy prevention, it is crucial to consider the flowering period of Fagales trees and the patterns of combined sensitivity to PR-10 group allergens in population.
Method: Sensitization to tree pollen was studied using the data of molecular allergy sensitization in Ukraine obtained using ALEX2 test conducted from 2020 to 2022. The study included 20,033 Ukrainian patients aged 1 to 89 years. Pollen collection was performed using a volumetric method with a Burkard Hirst-type spore trap, installed at a height of 25 meters on the roof of Vinnytsia National Pirogov Memorial Medical University. Microscopic slides of collected pollen were stained and analyzed under a light microscope at 400x magnification. Statistical analysis was carried out using the European Aeroallergen Network tools and Excel 2013.
Results: Analysis of the ALEX2 sensitization database revealed that out of 20,033 individuals, 3,239 (16.17%) were sensitized to PR-10 group pollen allergens. Among them, 3,133 (96.73%) were sensitive to birch (Bet v 1), 2,585 (79.81%) to beech (Fag s 1), 2,243 (69.25%) to hazel (Cor a 1.0103), and 1,870 (57.73%) to alder (Aln g 1). It is noteworthy that sensitivity to birch allergen Bet v 1 was the most prevalent among the Fagales allergens. Considering this, we analyzed various possible combinations of sensitivity to PR-10 group allergens: 52.61% of individuals had combined sensitivity to all four allergens (Bet v 1+Aln g1+ Cor a 1.0103+Fag s 1), followed by 15.65% sensitive only to birch (Bet v 1), 13.49% sensitized to birch with hazel and beech (Bet v 1 +Cor a 1.0103+Fag s 1), and 8.86% sensitive to birch and beech (Bet v 1 + Fag s 1). The remaining 9.39% of people were sensitive to other combinations (ranging from 2.9% to 0.03%).
Attention should also be paid to the pollination season and combined sensitivity to PR-10 group allergens. All trees responsible for spread of PR-10 allergens bloom in spring, but the onset and end

of pollination vary. In accordance with aerobiological data for Ukraine, hazel and alder bloom first (from mid-February to mid-April), followed by birch and oak, which bloom from late March to mid-May. It is also essential to consider the pollen of hornbeam and beech, which bloom in April. Thus, most people with sensitivity to this allergen group may potentially experience allergy symptoms from mid-February to the end of May.
Conclusion: Combined sensitivity to PR-10 allergens accounts for a significant period during which individuals experience allergy symptoms. Therefore, it is crucial to know for the patients their specific pattern of sensitivity to different allergens within the PR-10 group for the personal allergy prevention. Epidemiological data on sensitivity of patients can assist for the prevention of pollen allergy at the population level.

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Allergenic potential of urban green spaces, case study from Poznań, Poland (Central Europe)

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Greenery is a crucial element in urban spaces, influencing their overall appearance, attractiveness, and climate. Greenery improves mental and physical health, reducing the morbidity of city residents by offering mental relaxation and relieving stress. Urban greenery stimulates physical activity and minimizes exposure to noise and excessive temperatures (WHO 2017). However, poorly planned and managed green spaces can have a negative impact on health, primarily in the context of allergic diseases caused by pollen grains (Carinanos and Casares-Porcel 2011). It is estimated that pollen allergy currently affects approximately 20-30% of the population, with the highest prevalence observed in urban areas (Samoliński et al., 2014). One contributing factor to this phenomenon is the rise in the number of allergenic ornamental plants in cities and the homogeneity of plant species in green spaces. This homogeneity involves a preference for only specific plant species, leading to a loss of biodiversity (Carinanos and Casares-Porcel, 2011). Consequently, instead of serving a health-promoting function, green areas become unpleasant for a significant number of inhabitants. The primary objective of this study is to evaluate the allergenic potential of green urban spaces in the city center of Poznań, Western Poland.
The inventory of green areas has been carried out in various green spaces in Poznań, including parks, squares, streets, and alleys. The detailed inventory of parks comprises the following: 1) determining the location of each individual tree using a GPS application, 2) identifying trees through morphological methods with the assistance of an identification key and providing detailed descriptions of their genus and species, 3) measuring physical characteristics such as height and trunk diameter according to the Tree Girth Measurement Rules (Gach, 2013), 4) enumerating tree species, 5) determining and classifying the allergenic potential of tree species (based on the allergy prevalence retrieved from clinical studies).
The inventory was conducted in 10 parks, covering all the streets and alleys of Poznań city center. A total of 5,000 trees from 80 species were documented, representing 46 genera and 27 plant families. The most abundant genus was *Acer*, followed by *Tilia* and *Aesculus* as the second and third most common genera, respectively. Approximately 83% of all plants could be described as allergenic, e.g. *Platanus*, *Betula*, *Fraxinus*, *Carpinus*, *Juniperus*, *Taxus*, and *Corylus.* About 23% of the recorded trees possess allergenic potential at levels 3, 4, or 5, constituting nearly a quarter of the total trees in the inventory, which is approximately 1,000 trees. Specifically, 5% of the total trees have an allergenic level of 3, 11% at level 4, and 7% at level 5. It is worth noting that the fourth most popular genus is highly allergenic *Platanus.*
The areas with high density of allergenic plants in Poznań has been determined. Consequently, the areas with potentially hazardous exposure to allergenic pollen were selected. These initial findings are crucial for guiding future tasks, such as conducting local pollen exposure assessment experiments.
Keywords: pollen allergy, Poznan city center, urban green spaces
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Clean and polluted pollen analysis by scanning electron microscope

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Bioaerosol pollution, such as pollen, can have negative effects on human health, serving as a cause of respiratory allergies. Moreover, pollen may contribute to the dispersion of industrial pollutants due to the accumulation of chemical elements on its surface. Particles that settle on the surface of pollen and modify the pollen microenvironment composition and contribute to external and internal changes, including modifications in the amount and composition of allergens (Ribeiro et al. 2015). This study aims to evaluate the capacity of pollen to adhere to and transport particulate matter, including potential microplastics.
Pollen samples were obtained from plants, collected from various surfaces and from airflow, representing different environmental contexts. Moreover, in the laboratory, pollution of pollen samples was performed. All the collected samples were analyzed using a scanning electron microscope to examine the composition of the pollen, detect different chemical elements, and identify the presence of particles on the pollen surface.
Two types of pollen samples were differentiated: clean and polluted. The analysis of clean pollen samples revealed the presence of chemical elements, including oxygen (O), carbon (C), potassium (K), phosphorus (P), and calcium (Ca), on the surfaces of hazel, alder, willow, pine and birch pollen. The presence of C and O is explained by the pollen wall composition itself, as these are the main elements of the pollen wall material – a biopolymer called sporopollenin. It should be noted that the concentration of these elements may vary depending on factors such as plant species and growth conditions. Moreover, hazel pollen showed the presence of lead (Pb), zinc (Zn), and tin (Sn). These chemical elements can be attributed to environmental pollution. On polluted willow and birch pollen surface silicon (Si), sulfur (S), copper (Cu), iridium (Ir) were identified.
Keywords: pollen, pollen surface, air pollution, pollen pollution
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Characterizing the aerospora and ambient particulate matter over Potchefstroom during the period (13 March- 26 March 2023).

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Background

Cases of allergic rhinitis and asthma increase with about 20-30% of South Africans affected due to the increase in ambient particulate matter (PM) and climate change (Ajikah et al., 2020). There is also a general lack of continuous aerospora data throughout the southern Hemisphere (Davies et al. 2021). Consequently, there is a lack of studies that show the health impacts that result from allergenic aerospora (pollen and fungal spores) and particulate matter co-exposure in southern Africa. This paper aims to study the interconnection between aerospora and environmental conditions during the period 13 March – 26 March 2023 over Potchefstroom to contribute to aerobiology studies in South Africa.

Methods

The air sampling was conducted at North-West University, Potchefstroom campus. Aerospora samples were collected using the Burkard volumetric 7-day spore trap and quantified as per SAPNET rules (Berman, 2018). In air quality and aerobiology studies, meteorological parameters (temperature, wind speed and direction, humidity, and rainfall) are the most important environmental factors and are therefore also considered. The meteorological data and particulate matter ({\rm PM}*{10}) were collected by a Campbell Scientific meteorological station and the E-sampler-9800, respectively.*

Results

The results identified dominant pollen and fungal such as Cladosporium, Alternaria and Epicoccum, as well as pollen such as Ambrosia and Poaceae. {\rm PM}{10} concentrations were low according to National Ambient Air Quality Standards. There were some significant correlations between meteorological parameters and aerospora establishing relationships between them. The total of aerospora, pollen and fungal showed significant positive and negative correlations with temperature and relative humidity ($p \leq 0.05$) respectively, proving that air temperature is the most important factor which shows positive correlation with most biological and non-biological air pollutants (Adhikari et al., 2006). No significant correlation between aerospora and {\rm PM}*{10}, which both are influenced by temperature and occur at the same time (Puc, 2011), was detected. The presence of {\rm PM}*{10} alongside high concentrations of allergenic aerospora under certain weather conditions poses a risk to persons with respiratory diseases (Cariñanos et al., 2007).

Conclusion

The correlations between meteorological parameters, {\rm PM}_{10} and aerospora concentrations were generally not significant. This could be due to the limited sampling period, which shows that more research can be done in this field.

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Allergenicity of Cupressales pollen grains.

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Cupressales pollen is a significant cause of seasonal allergies worldwide. The dramatic increase in the concentration of Cupressales pollen is observed in Poznań (Western Poland) during last 25 years. Plant genera belonging to the Cupressales order, including Juniperus, Thuja, and Taxus, produce pollen grains that are morphologically indistinguishable. Consequently, it is unclear how various species contribute to the overall Cupressales pollen load in the air. Furthermore, there is a lack of data regarding the allergenicity of pollen from different Cupressales species.

The aim of the study was to determine the variation in the occurrence of homologues of the main allergen of cypress pollen grains, i.e. Cup a 1, in Cupressales order. In addition, the phenological pattern of flowering of selected species from Cupressales has been determined. Pollen grains were collected during the 2023 pollen season (between February and May) and Cup a 1 was quantified by immunoenzymatic test ELISA. The protein content in the pollen grains was expressed in pg Cup a 1/pollen grain. Collected phenological data allowed to prepared the first pollen calendar for Poznań for plants of the order Cupressales.

The content of Cup a 1-homologue varied widely, from 0.004 pg/zp. in Ch. lawsoniana to 2.432 pg/zp. in Ch. nootkatensis. In some species (T. occidentalis, T. cuspidata, C. japonica) the ELISA test did not detect the Cup a 1-homologues. The genus Juniperus showed the most similar and relatively high Cup a 1-homologue content. The 2023 Cupressales pollen season in Poznań began on 4th of March, ended on 11th of May and lasted 69 days. T. baccata began to flower the earliest, followed by T. occidentalis and T. pilcata, then J.sabina, Ch. lawsoniana and finally J. communis.

The study showed that the allergenicity of Cupressales species is subjected to high variation and the species contributed the most to total pollen level are T. baccata, T. plicata and T. occidentalis. To accurately determine the allergenic potential of individual species within the order Cupressales, further analyses should focus on estimating the pollen production by the species, their distribution in the city and determining the influence of climatic factors on the Cup a 1-homologues level

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Long-term pollen season shift as the response to the climate change

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Personalized pollen allergy symptom forecasts over Europe

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The number of pollen allergy sufferers is growing constantly in Europe and worldwide. A deeper understanding of the problem allows better solutions, such as forehanded therapy for allergy sufferers. Forehanded therapy and the lowest impact on human health is possible when the timing, approximate level of allergens and possible organism reaction on each individual are known. Currently

running system PASYFO (personalized pollen allergy symptom forecasting system) allows allergy sufferers to be involved in symptom forecasting.

The system was developed in 2018, and since that time, it has been successfully running as a mobile application called PASYFO in Lithuania and Latvia. The forecast consists of several modules – (1) pollen forecast, (2) meteorological forecast, (3) air quality forecast, and (4) individual symptoms entered by the person.

In the frame of the Horizon project "AI-augmented ecosystem for Earth Observation data accessibility with Extended Reality User Interfaces for Service and data exploitation" EO4EU, implementation of the PASYFO module in European applications as Pollen+ and MASK-Air is performed, as well as expansion of updated PASYFO mobile application to the European level. The first release of the updated apps is expected in the spring of 2024.

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