SALUTE! Mapping of the allergenicity of Bologna's cycle paths

1. Context

Encouraging sustainable active mobility within cities is crucial to improve air quality and city dwellers health, as emphasized by the EU Green Deal [1]. The prevalent use of bicycles as a means of transportation is particularly applicable in flat areas, such as the Emilia Romagna region. The Metropolitan City of Bologna, with its advisory body "Bicycle Consultation," serves as a virtuous model by promoting and monitoring bike travel, aiming to continually expand its cycling infrastructure. The total length of cycling paths in the metropolitan area is set to reach 278 km of extension in the next six years, also thanks to the funding from the National Recovery and Resilience Plan (PNRR) [2]. This investment is well-received by the population, since the number of cyclists on these paths increasing, as recorded by dedicated devices ("bike counters") installed by the municipality. (https://data.eco-counter.com/ParcPublic/?id=6082#). In fact, the number of cyclists who take the "bicycle ring road" ("Tangenziale delle biciclette"), a bike lane following the ring road around the ancient city walls of Bologna, has increased of 62% in the last ten years [3].

However, while active mobility prevents further pollutant emissions, the existing air pollution poses a health risk, especially during physical activity [4,5]. Major air pollutants affecting the quality of life of exposed individuals include airborne particulate matter (PM) and gaseous pollutants. Long-term exposure to air pollutants is known to be associated with respiratory and cardiovascular problems, contributing to an estimated two million premature deaths worldwide annually [6]. The Po Valley, which includes the Emilia Romagna region, is a European hotspot for air pollution due to its geography and significant human activity. In 2010, PM_{10} and $PM_{2.5}$ alone were responsible for 4.4 and 2.8 deaths per 100,000 inhabitants in Emilia Romagna [7].

Airborne particulate matter also contains biological components such as pollen and spores [8], which can cause allergic rhinoconjunctivitis and asthma in sensitive individuals, affecting their quality of life and reducing their productivity at school or at work [9]. Moreover, these organisms can release allergenic proteins (aeroallergens) into the atmosphere, whose potential interaction with inorganic air pollutants can increase their clinical relevance [10,11]. This situation is exacerbated by climate change, which can influence human exposure to pollen, spores, and aeroallergens in terms of duration and intensity [12].

Given the health hazards posed by allergens and air pollutants, they are regularly monitored using various sensors and samplers, both privately and institutionally [13,14]. However, these tools are often expensive, immovable, and in most cases their placement does not accurately reflect the individual aerosol exposure [13,15]. The recent development of low-cost, portable smart sensors capable of measuring air pollutant concentrations and

automatically organizing them into databases has laid the foundation for an integrated and automated air quality monitoring system [15]. Many scientific studies have utilized such sensors on bicycles [16,17], and inexpensive air quality sensors have been employed in dynamic monitoring and citizen science projects throughout Europe (Life+RESPIRA; MONICA Smog Tracker di ENEA; Appmosfera; SensorWeBike di CNR; Sniffer Bike; Aeroflex; TaPUM di CNR-ISAC).
Unfortunately, the development of automatic monitoring systems for pollen and spores is still in its infancy. Currently, personal exposure to these allergenic organisms can only be assessed using portable samplers and analysing samples under optical microscopy [13,18], whereas personal exposure to aeroallergens has never been estimated.

Monitoring the allergic risk of an urban area is crucial, considering that pollen allergy in Europe has an estimated prevalence of 30% to 40% [19], thus about one-third of Italians will experience allergic rhinitis during the pollen season. As there is currently no definitive cure for this condition, individuals with pollen allergies need to plan their outdoor activities and medication intake based on the concentrations of allergenic pollen expected in their municipality [18]. This type of prevention is challenging because individuals with pollen allergies tend to trivialize their condition, often not seeking a specific diagnosis and limiting themselves to taking antihistamines during critical periods [20]. This implies that many allergic subjects do not know which plant taxa they are allergic to, hence they cannot entirely benefit from the pollen forecasting provided by the Regional Agencies for the Protection of the Environment (ARPA) and by other websites and smartphone applications [18].

2. Objectives and Benefits

In the last decade, various projects have calculated the individual exposure to organic and inorganic aerosols in urban contexts, correlating it with the airway inflammatory symptoms reported by the volunteers participating in these studies. However, to my knowledge, no study has yet measured individual exposure to pollen, allergens, and inorganic pollutants simultaneously, proposing healthier routes, and defining guidelines for a bike lane maintenance that can benefit the respiratory health of its users. Additionally, non-invasive systems for personalized self-diagnosis of pollen allergy do not exist to date, although they could help allergic individuals to have greater awareness of their condition and to use the information provided by the ARPA system more efficiently.

The SALUTE! project aims to monitor pollutants and allergenic pollen along the main bike paths in the municipality of Bologna, with the following **aims**:

(I) **Creating a map accessible to the public** for the risk of exposure to PM and pollen allergens along Bologna's main bike paths. Since users could voluntarily report their symptoms on the map, this aim will promote both digital and participatory medicine.

(II) **Developing an index** correlating weather, air quality, and pollen data to identify days and paths at risk, and to communicate them to the public.

(III) **Developing a lateral-flow test** allowing cyclists to autonomously monitor the main allergens they have been exposed to.

Achieving these objectives will make active mobility in the city of Bologna safer, encouraging citizens to choose healthier routes and enabling the municipality to plan mitigation interventions. The project aligns with telemedicine and personalized medicine objectives, aiming to benefit both the population, informed and engaged through an interactive website, and the promotion of a greater awareness about the importance of diagnosis.

Furthermore, the project will serve as a pilot study for implementing a series of portable/wearable sensors capable of perceiving and evaluating the exposome of Bolognese cyclists, *i.e.*, the set of particles they are exposed to that interact with their organism. The goal of my research group is to employ the methodologies and techniques developed in this study for future, more extensive projects, involving the enrolment of volunteers, a clinical evaluation of inflammatory and respiratory symptoms, and the creation of health monitoring apps for cyclists.

3. Beneficiaries

The direct beneficiaries of this study are all cyclists, both regular and occasional, who use the bike paths of Bologna. However, the study will also benefit the pedestrians who stroll on the sidewalks near the bike lanes. Moreover, the study will be particularly relevant for allergic individuals living in the city, comprising approximately one-third of the population. In fact, this study will support the pollen allergic subjects of Bologna not only in avoiding their relevant pollen allergens, but also in their low-cost identification. Additionally, the data collected during the SALUTE! project and the publication of its results will also benefit the Municipality of Bologna, which on one hand could use the collected data as a monitoring system for the healthiness of the cycling paths, and on the other could implement the guidelines for a healthier management of these areas that will be provided by the project.

4. Detailed Project Description

The project will take place in the Municipality of Bologna in 2024, it will be dived in eight activities, and it will last one year. The project participants include:

- Iris Aloisi (Associate Research Fellow at the Department of Biological, Geological, and Environmental Sciences at the University of Bologna), as project coordinator and manager.

- Chiara Suanno (Post-doc researcher at the Department of Biological, Geological, and Environmental Sciences), responsible for the realisation of the activities and the dissemination of the results.
- A researcher recruited on the project for one year, with proved expertise in vegetation and aerobiological monitoring, who will work on all the project activities.
- Delia Fernández González (Full Professor at the University of León and Associate Researcher at CNR-ISAC in Bologna), providing supervision and consultation for the aerobiological monitoring (Activities 3 and 4).
- Paola De Nuntiis (Permanent Researcher at CNR-ISAC in Bologna), providing supervision and consultation for environmental pollutant monitoring and smart sensors calibration.

Activity 1: Mapping and selection of the bike paths
(Month 1)
The first part of the project involves mapping and selecting four segments of bike paths in the municipal territory of Bologna for the study. The selection criteria include:
- usage frequency of the bike path,
- potential exposure to allergens,
- potential exposure to pollutants.

Usage frequency will be assessed either on-site, using a people-counter, or referring to data collected by the bike counters, where available. Potential exposure to allergens will be assessed through an exploratory analysis of allergenic vegetation within a 5 m radius from the bike path. Potential exposure to pollutants will be estimated based on the number of road lanes flanking the cycling path, and on the proximity to possible industrial emission sources. The length of each segment of bike path selected for the study will be of 7 km, with an approximated cycling time of 30 minutes, in order to avoid the overloading of the sampling filters for the aerobiological monitoring.

Activity 2: Mapping of allergens and pollutants sources
(Months 2-3)
For each cycling path segment selected during Activity 1, (I) the total arboreal vegetation, and (II) the allergenic vegetation, including both arboreous and herbaceous species, will be censed in a radium of 5 m from the edge of the cycling path. This will be realised in part using the data publicly available on the OpenData website of the municipality of Bologna (https://opendata.comune.bologna.it/) and the orthophotos downloadable from the geoportal of Emilia Romagna region (https://geoportale.regione.emilia-romagna.it/) and elaborated in QGIS [21], and in part by targeted fieldwork.
For each individual plant censed, will be collected: (I) biometric data, like plant height, crown diameter, diameter at breast height, sex, and health status, (II) spatial data, like geolocation, exposition, distance from streets and buildings. iTree Eco software will be than used to estimate the ecosystem services, in terms of air pollutants removal, provided by the total arboreal vegetation [22]. Data on the allergenic vegetation instead will be employed to calculate common allergenicity indices (SAI and I_{UGZA}) [23] for each bike path segment.

Activity 3: Exposure monitoring
(Months 3-6)
The direct monitoring of the cyclists' exposure to PM and allergenic pollen along the selected bike paths will be performed from spring to summer, in a four-month period. However, since pollen counting is a time-consuming activity, a continuous monitoring of four areas over a four-month period is not feasible. Hence, the research efforts will be optimised by performing the air monitoring only during days with one of the following features:
- days with predicted high levels of allergenic pollen, based on ARPAE pollen calendar [24];
- days with predicted high levels of air pollutants, based on ARPAE forecasting with the system NINFA [25];
- days in which is predicted the peak of the flowering season for the allergenic species in proximity of the bike paths, based on the phenological calendars for the region.

During the selected days, the researchers involved in the project will travel along the bike paths with bikes of similar height, accessorised with: (I) a smart, portable sensor for air pollutants; (II) two sampling cassettes with filters for pollen sampling; (III) a GPS system. All these devices will be mounted on the bike handlebar through a case 3D-printed for this scope. The smart sensors for the air pollutants will be selected with the support of CNR-ISAAC, based on their possibility to be mounted on a bike handlebar, on their battery autonomy, and on their capability to automatically record $PM_{2.5}$ e PM_{10} and organise them in a spreadsheet. Before starting the monitoring campaign, the smart sensors will be run in parallel with institutional air quality sensors (ARPAE) located in the city, to evaluate their precision and accuracy. The filtering cassettes instead will have a removable filter with meshes narrow enough to sample all the allergenic pollen grains (<10 µm), and they will function passively, being mounted on the bike handlebar with the air inlet forward and the air outlet backward. Before starting the sampling campaign, the capture efficiency of the cassettes will be evaluated by comparison with a Hirst-type spore trap operated in parallel for one hour [13]. Finally, the GPS system will be activated at the beginning of the sampling, and it will allow to track the route and the travel time.

Activity 4: Pollen counts
(Months 3-10)
One of the two filtering cassettes mounted on each bike will be used for the monitoring of allergenic pollen. The cassette will be opened, the filter removed, and the pollen will be recovered from the filter using a specific solvent that will not damage its structure. The collected pollen will then be concentrated through centrifugation and mounted on a glass slide for optical microscopy with glycerinated gelatin and basic fuchsin [26]. The allergenic pollen grains will be counted under an optical microscope, considering the allergenic species for Emilia Romagna as reported by ARPAE and the World Allergy Organization (WAO), and following the European standards for aerobiological monitoring [27–29]. The quantity of allergenic pollen will be standardised based on the distance covered by the bike during sampling, and the travel time recorded by the GPS system.

Activity 5: Development of a lateral-flow test
(Months 3-8)

One of the filtering cassettes mounted on each bike will be used to extract and quantify pollen allergens. The particles collected from the filter will be resuspended in sterile bi-distilled water with the addition of a cocktail of protease inhibitors, sonicated, lyophilized, and then resuspended in Tris-HCl as described earlier [14]. For each sample, the total protein content will be evaluated, and the three main pollen allergens of the season will be identified and quantified using the Enzyme-Linked Immunosorbent Assay (ELISA) with specific antibodies [30]. Prototypes of lateral-flow test kits [31] will be developed, allowing the extraction and identification of the main pollen allergens present in the breathable air of the cycling paths in Bologna, starting from the cassette filter. Specifically, the kit will include:

- a filtering cassette for airborne pollen sampling;
- a protein extraction buffer for pollen, to recollect the particles from the filter after the sampling and to extract the pollen allergens;
- a dropper;
- a lateral-flow test for each of the main allergens, consisting of a 3D-printed outer box containing (I) a sample pad, made of absorbent paper, for transferring the sample to the nitrocellulose membrane; (II) a conjugate pad with allergen-specific antibodies conjugated to a chromophore; (III) nitrocellulose membrane blocked over its entire surface with non-immunoreactive proteins, except for one band where other allergen-specific antibodies are adsorbed (test, T), and one band where the pollen allergen is adsorbed (control, C).

Activity 6: Data Correlation and Index Formulation
(Month 11)
Pollen, allergens, pollutants, and GPS data will be correlated with each other and with allergen indices, in order to identify spatial and temporal correlations. Initially, pollen concentrations will be compared with allergen concentrations for each sampling to assess possible variations in allergen concentrations per grain (pollen potency). Variations in pollen potency will then be correlated with pollutant concentrations detected on the same route. Using GPS data, areas with higher respiratory health risks will be identified based on their overall concentrations of allergenic pollen, allergens, and pollutants.
These data will also be correlated with relevant meteorological variables for air quality, such as relative atmospheric humidity, wind speed, and rainfall. Identifying correlations with weather factors will allow the creation of indices for predicting the respiratory risk based on the meteorological forecasting.

Activity 7: Creation of an Interactive Map
(Month 12)
Pollen, allergen, and pollution data for each cycling path in different periods of the year will be implemented in an online interactive map, freely accessible to the public. Users will be able to voluntarily input possible allergy episodes or respiratory difficulties experienced along the cycling paths. These crowd-sourced data will be useful to assess the utility of transforming the interactive map into a m-health app in the future, allowing allergist doctors and patients to map the evolution of allergic symptoms and plan a personalized preventive plan. Such app could also allow all the allergic individuals to

know the levels of allergic risk reported by other users along the cycling paths in real-time.

Activity 8: Dissemination of Results
(Months 6-12)
The project results and the services it will provide to the citizens will be progressively disseminated to the public during outreach events such as La Notte dei Ricercatori, BiGeA Day, Fascination of Plants Day, etc. At the conclusion of the project, the collected data will be published in a high-impact peer-reviewed scientific journal.

5. References

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