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ITINERIS

# D4.13.3: Release of a catalogue of procedures for the characterization of the atmospheric aerosol properties by atmospheric simulation chamber

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| **Author(s) (Partner-OU):** | Paolo Prati, Virginia Vernocchi (INFN- Sezione Genova) |
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## LIST OF ACRONYMS

**ASC:** atmospheric simulation chamber

**BC:** Black Carbon

**EC:** elemental carbon

**HVO:** Hydrotreated Vegetable Oil

**MISG:** Mini-Inverted Soot Generator

**MWAA:** Multi-Wavelength Absorbance Analyzer

**OC:** organic carbon

**OPS:** optical particle sizer

**PAX:** Photoacoustic Extinctiometers

**PM:** Particulate Matter

**SMPS:** scanning mobility particle sizer

**TC:** total carbon

# 1. INTRODUCTION

This deliverable is prepared in the context of the ITINERIS project, within Work Package 4 that deals with the integration of Research infrastructures working in the atmospheric domain through synergistic approaches and cross boundaries developments. This deliverable reports the implementation plan of Task 4.13.3. The main objective of this task is to release a catalogue of procedures for the characterization of atmospheric aerosol properties by atmospheric simulation chamber. Here we briefly describe the procedures and indicate their references.

# 2. PROCEDURES

In this section, several procedures to perform experiments in an atmospheric simulation chamber (ASC) are described.
With the growth of ChAMBRe during the ITINERIS project, we developed a collection of procedures for the characterization of atmospheric particulate matter produced during various physio-chemical processes. The list of procedures presented here is not to be considered conclusive, but rather a work in continuous development. Through ITINERIS, experimental references have been established, alongside scientific publications, that will last into the future.

## 2.1 Procedure for generating standard of soot particles by a soot generator

The soot-particles emissions of a mini-inverted soot generator (MISG; Argonaut Scientific Corp., Edmonton, AB, Canada; model MISG-2) working at different combustion conditions are characterized. The comprehensive characterization of the MISG soot particles is an important piece of information to design and perform experiments in atmospheric simulation chambers. In this way standard soot particles with known properties are produced and they can be used for different purposes, for example to investigate the possible interactions between soot and other atmospheric pollutants, the effects of meteorological variables on soot properties, and the oxidative and toxicological potential of soot particles. The detailed procedure is described in (Vernocchi et al., 2022).
The MISG can be operated with different fuels, such as ethylene and propane, and using different air-to-fuel flow ratios (i.e., different combustion conditions). This means that characterization experiments should be repeated for each desired combustion condition. In addition, to evaluate repeatability and stability at least three repetitions are required.
The MISG is warmed for about 45 min before injecting soot particles inside the chamber, to achieve emission stability. Injection of the soot particles inside ChAMBRe lasts 2 minutes. Particle concentration and size distribution inside the chamber are measured continuously by a scanning mobility particle sizer (SMPS; TSI Inc.), in the range from 34 to 649 nm, while an optical particle sizer (OPS; TSI Inc.) is used in the range 0.3–10 µm. Three photoacoustic extinctionmeters (PAXs; Droplet Measurement Technologies) provide the online determination of the soot particles absorption coefficients at $\lambda$ = 870, 532, and 405 nm. In order to allow possible further offline analysis, soot particles are also collected on pre-baked 47 mm diameter quartz fibre filters (Pallflex Tissuquartz 2500 QAO-UP) inserted into a stainless-steel filter holder. For each working condition, three filters with different loadings are collected using a low-volume sampler (TECORA – Charlie HV). The sampling started when inside the chamber stable gas and particle concentrations are reached (i.e. about 3 min, corresponding to the chamber mixing time, after switching the MISG off). Particle-loaded filters are analysed by the multi-wavelength absorbance analyzer (MWAA), for the offline direct quantification of the aerosol absorption coefficients at five different wavelengths ($\lambda$ equal to 850, 635, 532, 405, and 375 nm). In addition, the EC and OC mass concentrations are determined by thermal–optical transmittance analysis (TOT) using a Sunset Laboratory Inc. Sunset EC / OC analyzer (see Deliverable 4.9.2) and the NIOSH 5040 protocol (NIOSH, 1999) corrected for

temperature offsets. Some tests by adding a back-up filter during the sampling can be performed to determine the volatile fraction of OC.
Gaseous emissions are characterized too, by online gas-analysers, focusing on the typical gases emitted by fuel combustion (i.e. $CO_2$, $NO_2$ and NO).

### 2.2 Procedure for carbonaceous aerosol real sources characterization

The previous procedure can be also considered suitable to characterize carbonaceous aerosols from real sources, properly adapted if needed.
For instance, particles emitted by a 12 HP 4-stroke diesel engine (Electrical Generator 65230 – 6 kW - Hyundai), fueled alternatively with regular fossil diesel (B7) and Hydrotreated Vegetable Oil (HVO) were investigated (Danelli et al., 2025). It's important to remark on the possibility of using the same procedure also with different aerosol sources, such as different models of engine or wood stoves.
Particles are injected into ChAMBRe and left in suspension for defined time intervals, then monitored via online instrumentation and finally sampled for offline analysis. Sampling begins once the concentration has stabilized (i.e., approximately after the 3 minutes of mixing in the chamber).
Both optical and thermal-optical techniques are used for carbonaceous aerosol characterization. For the optical properties both online and offline methods are used: PAX at 870 nm for the online absorption coefficient, Giano_BC1 (Dadolab srl) for the continuous monitoring of black carbon (BC) concentrations on filter during the PM sampling and the MWAA for the offline direct quantification of aerosol absorption coefficients at five different wavelengths. The same filter collected by the Giano_BC1 can be used for the thermal-optical OC and EC quantification.

### 2.3 Procedure for soot aging

Particles emitted following procedure 2.1 can be used as standard to evaluate change in properties due to aging processes or to produce standard samples for other applications (i.e., oxidative potential or toxicological assays). The general characterization can follow the procedure 2.2, here we describe aging processes. Different BC aging processes are considered, starting from the simplest situation of pure soot exposed to solar light and/or oxidants up to soot mixed with certain concentrations of both inorganic and organic compounds (alternatively in light and dark conditions). To compare aging processes, the same condition of soot particles generation should be used. The MISG injection lasts 1 minute, to reduce the concentration inside the chamber, while each component required a specific procedure to be generated (Vernocchi et al., 2025).
Solar radiation is simulated with a solar simulator (Sciencetech Inc. TM), mounting the AM1.5G filter to reproduce the optical absorption of the atmosphere.
Salt seeds of ammonium sulfate or ammonium nitrate are generated starting from a liquid solution, prepared by dissolving 0.2 g of salt in 50 mL of MilliQ water. The solution is nebulized with the Sparging Liquid Aerosol Generator (SLAG, by CH Technologies, Inc.) nebulizer, working with 3.5 lpm of air, provided by a Mass Flow Controller (MFC, by Bronkhorst), for 600 sec. SLAG is fed with salt solution using an automatic syringe pump working at 0.4 mlpm.
$NO_2$ was provided by a specific bottle, and the concentration (i.e., 500 – 600 ppb) inside the chamber was kept constant thanks to the feedback control system (NOx analyzer managed by LabView).
Ozone is generated via photoreaction by a UV lamp ($\lambda$ < 240 nm) inserted through an ISO-K25 flange in the lateral volume (i.e., no UV radiation illuminates particles in the main volume) of the chamber. In this way, about 500 ppb of ozone is reached into the chamber volume. $NO_2$ and $O_3$ were injected together as precursors of the $NO_3$ radical in order to simulate oxidation in dark conditions.
Nitrous acid (HONO) is produced by the reaction between $H_2SO_4$ and $Na_2NO_2$ and injected to feed the system of OH radicals under light conditions (as HONO photolysis is a source of hydroxyl

radical). To form HONO, reagents are injected consecutively by using a glass flask and a controlled air flow: first 4 mL of $H_2SO_4$, then 2 mL of $Na_2NO_2$ flowed with an air flow of 1 lpm.
Volatile organic compounds are injected with the glass jar and the 1 lpm air flow. The amounts of organic compound are: Toluene (ThermoFischer Scientific) 2 µL, 2,5-Dimethylfuran (2,5-DMF; ThermoFischer Scientific) 3 µL and (+)-α-pinene (ThermoFischer Scientific) 4 µL.
The injection order of each component is summarized in Table 1. For all the experiments (except BC dark), 2 hours of BC exposure were considered.

*Table 1: Injection order of each component*

| **Component** | **Injection order** |
| --- | --- |
| Ammonium Nitrate | Simultaneous with BC injection |
| $NO_2$ | Simultaneous with BC injection |
| $O_3$ | Introduced 3 minutes after the injection of the last compound |
| 2,5-dimethylfuran | Injected at the end of BC (or salt) injection<br>i.e.: BC (+ Amm.Nitr. + $NO_2$) → 2,5-dimethylfuran → $O_3$ |
| Ammonium Sulfate | Simultaneous with BC injection |
| HONO | 3 minutes after the injection of the last compound<br>i.e.: BC (+ Amm.Sulf.) → HONO → Light ON |
| Toluene | 3 minutes after the BC (or salt) injection<br>i.e.: BC (+ Amm.Sulf.) → Toluene → HONO → Light ON |
| Light | ON at the end of the last compound injection |
| α - pinene | 3 minutes after the BC injection<br>i.e.: BC → α – pinene → $O_3$ |

## 2.4 Procedure for bioaerosol characterization

The quantitative study of possible relationships between bacteria and air quality or meteorological conditions, that means the evaluation of the impact of different pollutants on bacteria viability, is an open and relevant issue since bacteria are considered to play a significant role in the composition and dynamics of bioaerosols. The procedure we developed can be applied to different bacteria strains and with different types of pollutants, both solid and gaseous. The procedure is available in (Vernocchi et al., 2023), reported in Deliverable 4.13.1 and here is briefly summarized.
The bacteria strain selected for the experiment is firstly characterized for the growth curve by recording the OD at $\lambda$ = 600 nm ($OD_{600\ nm}$) at specific time intervals and measuring bacteria total concentration by the QUANTOM Tx™ microbial cell counter (Logos Biosystems) and bacteria viable concentration by standard dilution plating.
To prepare the inoculum for the chamber experiments, depending on the strain, the bacterium is grown in 30 ml of the required broth medium in a shaking incubator working at the proper temperature and shaking velocity, until the required OD is reached. Then, 20 ml of this liquid preparation is centrifuged, and the bacteria pellet is resuspended in 20 ml of sterile physiological solution (NaCl 0.9% w/v). If needed, the suspension is properly diluted. The concentration of the

solution to be injected inside ChAMBRe is controlled in terms of total cells ($ml^{-1}$) by QUANTOM Tx™ and viable concentration by standard dilution plating. The bacteria suspension, properly diluted, is injected into the chamber volume by using the SLAG (CH Technologies). The injection phase lasts 5 minutes and both airflow and duration are automatically controlled: 2 ml of the bacterial suspension are nebulized inside ChAMBRe.

The concentration of total bacteria inside chamber volume is monitored by the WIBS-NEO (Droplet Measurement Technologies®). Active sampling via the Andersen impactor is performed increasing sampling time progressively after the injection to collect a suitable number of CFUs. After the experiments in the simulation chamber, the sampled plates are incubated, incubation temperature and time depend on the bacteria strain. The CFUs are then counted, and the CFU $cm^{-3}$ calculated.

The possible correlation between bacteria viability and air quality is investigated in terms of change in bacteria viability due to the exposure to atmospheric pollutants. Effects on bacteria viability are compared in relation to “baseline experiments”, in which the viability of airborne bacteria is measured at atmospheric pressure, with temperatures around 20 °C and with relative humidity around 60 %. The baseline can be assessed both in dark and light (using solar simulator by Sciencetech Inc.™) conditions. During light conditions, the solar simulator was used with the AM1.5 filter mounted. The baseline assessment is followed by a set of experiments in which bacteria are exposed to the selected pollutants. Different gaseous species (for example NO and $NO_2$) at different concentrations can be tested, and the gas concentration is kept constant by a feedback control system. Also, solid pollutants such as dust or soot (see procedure 2.1) particles. A period of 2 h after the bacteria injection was considered to observe the bacteria viability behavior under different atmospheric conditions, and it was possible to quantify the lifetime.

## 2.5 Procedure to investigate the interaction between plant species air pollutants

The objective of this procedure is to investigate the interaction between selected plant species and both individual and mixed target urban air pollutants under realistic yet controlled conditions. The experimental setup allows for the isolation of pollutant effects and ensures the absence of external environmental perturbations, enabling a quantitative assessment of pollutant-specific uptake capacity at the whole-plant level. ChAMBRe can be used to evaluate the uptake of gaseous and solid pollutants by several plant species. In particular, we developed a procedure using $NO_2$ and PM main components (black carbon, dust and a mixture of them) applied to three trees but it can work even with other pollutants and other plants.

To better understand the ability of each plant species to interact with the input pollutants, the same experimental steps have been carried out with the chamber empty and under the same circadian conditions. The results obtained during empty chamber experiments are used as reference for the plant experiments. To isolate the interactions between plants and individual pollutants, each pollutant was sequentially injected into ChAMBRe one at a time. Finally, to simulate real-world conditions, a mixture of all pollutants was injected.

Each plant remains inside the simulation chamber for one week on a 12h/12h circadian cycle: the light inside the ChAMBRe is produced by a solar simulator (Sciencetech™ Incorporated) mounting an AM1.5G 3 × 3" air mass filter, that simulates the optical absorption of the atmosphere.

The plant under investigation stays in the chamber for 24 h without additional pollutants for acclimatization to the ChAMBRe condition before experiment starts, then it’s exposed to 4 different conditions for about 24 hours each. The first condition of exposure is to a fixed concentration of 200 ppb $NO_2$, kept constant by a feedback system. The second is with fresh soot, injected by a 12 HP 4-stroke diesel engine (Electrical Generator 65230 – 6 kW - Hyundai), fuelled with B7 diesel. The soot mass concentration inside ChAMBRe is measured and monitored by a PAX working at 532 nm. During the third phase, mineral dust aerosols is generated from a sample of Arizona road dust; about 10 g of soil are placed in a Buchner flask and shaken for about 10 minutes using a mechanical shaker

(Shaker oass203, Orto Alresa). The dust particles generated are monitored using OPS. In the last condition, both pollutants soot and dust are injected together and monitored by PAX and OPS.
The whole procedure is reported in (Bosio et al., 2025).

## CONCLUSIONS

Thanks to the ITINERIS project, the potentialities of the ChAMBRe facility have increased and this has led to the need for dedicated experimental procedures. Here the most significant of these are described, covering a wide range of scientific topics, and their literature references are given.

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