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Airborne bacteria viability and air quality: a protocol to quantitatively investigate the possible correlation by an atmospheric simulation chamber

Virginia Vernocchi et al.

Correspondence to: Federico Mazzei (federico.mazzei@ge.infn.it)

S1: Details on the gas monitors installed at ChAMBRe

The ENVEA-CO12e is a continuous carbon monoxide analyzer based on carbon monoxide detection by infrared light absorption measurement principle (EN 14626:2012, https://store.uni.com/en/uni-en-14626-2012). The concentration of CO in the sample is determined by measuring the quantity of infrared light, at the specific wavelength of 4.67 μm, absorbed by the sample gas as it flows through a multi-reflection chamber. The model available at ChAMBRe also features a built-in module for CO2 monitoring (range: 0-2000 ppm by NDIR). The instrument has a sampling rate of approximately 1 lpm, a response time of 20 s and a detection limit of 0.05 ppm.

The measurement principle of ozone by the ENVEA-O342e is based on UV photometry (EN 14625:2012 https://store.uni.com/en/uni-en-14625-2012). A 254 nm UV light signal is passed through the sample cell where it is absorbed in proportion to the amount of the ozone present (maximum range of ozone absorption 250-270 nm). Periodically, a zero-measurement is automatically performed by the device by switching between the sample stream and a calibrated ozone-free sample. The instrument has a sampling rate of approximately 1 lpm, a response time of 20 s and a detection limit of 0.2 ppb.

The ENVEA-AF22e is based on ultraviolet fluorescence, which is the standard method for the measurement of SO_2 concentrations in ambient air (EN 14212:2012 https://store.uni.com/en/uni-en-14212-2012). A hydrocarbons scrubber guarantees the elimination of hydrocarbon interferences. The hydrocarbons free sample is sent to a reaction chamber to be irradiated by an UV radiation peaked at 214 nm, which is the SO2 molecule absorption wavelength. The fluorescence is optically filtered between 300 and 400 nm to eliminate some interfering gases. With a sampling rate of 0.4 lpm this instrument reaches a detection limit of 0.4 ppb with a response time of 20 s.

The ENVEA-C32e utilizes the principle of chemiluminescence, which is the standard method for the measurement of NO and NO_2 concentration (EN 14211:2012 https://store.uni.com/en/uni-en-14211-2012), for automatically analyzing the NO-NOx and NO2 concentration within a gaseous sample. The analyzer measures the photons emitted after the reaction between NO and O_3. The analyzer initially measures the NO concentration in the sample, through NO ozone oxidation. Subsequently, the sample passes through the heated molybdenum converter, which reduces NO_2 to NO and is then mixed with ozone in the reaction chamber and the resulting NO concentration is determined. In this way, the signal is proportional to the sum of the molecule NO and NO_2 (reduced to NO in the

converter) in the sample. With a sampling rate of 0.66 lpm this instrument reaches a detection limit of 0.2 ppb with a response time of 40 s.

The ENVEA-VOC72e metrology, in accordance with EN 14662-3 (https://store.uni.com/en/uni-en-14662-3-2015). The VOC72e performs three main functions: the sampling, the GC analysis and the data processing. The sampling is achieved with a single trap filled with a specific sorbent. Its flow through the trap is about 12 mlpm which gives a sampled volume of 165 ml with the standard 15 minutes cycle (sampling time >90% of cycle time). At the end of the sampling cycle, the trap is connected to the GC column and quickly heated from 35 to 380°C within 2 seconds. The compounds are thermally desorbed and flushed with hydrogen into the GC column. Then the trap is cooled with a fan for a new sampling cycle. Inside the GC column, the compounds are moved forward by the hydrogen flow (the mobile phase) and retained by the internal coating (the stationary phase) causing a selective retardation of the compounds. To achieve an optimal separation within a minimal time, the GC column follows a multi slope thermal cycle from a cold step (25°C) for the injection to a hot step (160°C) for flushing all the heavy compounds (i.e., compounds with a high boiling point). At the end of the hot step, the GC column is cooled to the cold step for the next cycle. The GC column output is connected to a photo ionization detector where the compound concentration is converted into a small electric signal. The PID detector includes a 10.6 eV UV lamp that ionizes all the compounds which ionization potential (IP) is less than 10.6 e V. The 240 V electric field between the polarization electrode (-240 V) and the signal electrode (at ground) moves the ionized particles (positive ions and negative electrons) towards the electrodes creating a small electrical conduction. The resulting electric signal is amplified and digitalized in the electrometer board. Its recording gives the chromatogram which exhibits a peak for each detected compound. The standard measured compounds are Benzene, Toluene, Ethylbenzene, m+p-Xylene, o-Xylene, 1-3 Butadiene. Sample flow is around 50 mlpm, with a low detection limit of ≤ 0.05 µg $m^{-}3$ (benzene).

S2: details on the custom SCADA acquisition and control system developed at ChAMBRe

Figure S1 shows the main graphic user interface (GUI) of the NI LabVIEW custom application running continuously during the experiment. The status (on/off) of each sensor is shown on the main panel by a light indicator. Detailed information about each sensor is available by pressing the related subpanel button. The output data from all sensors are recorded at a user-selectable sampling rate (default: sampling rate = s^{-1}). Time series can be visualized in real-time on the corresponding subpanel. During the experiment the user can also interactively set or modify several sensor parameters via the subpanel controls.

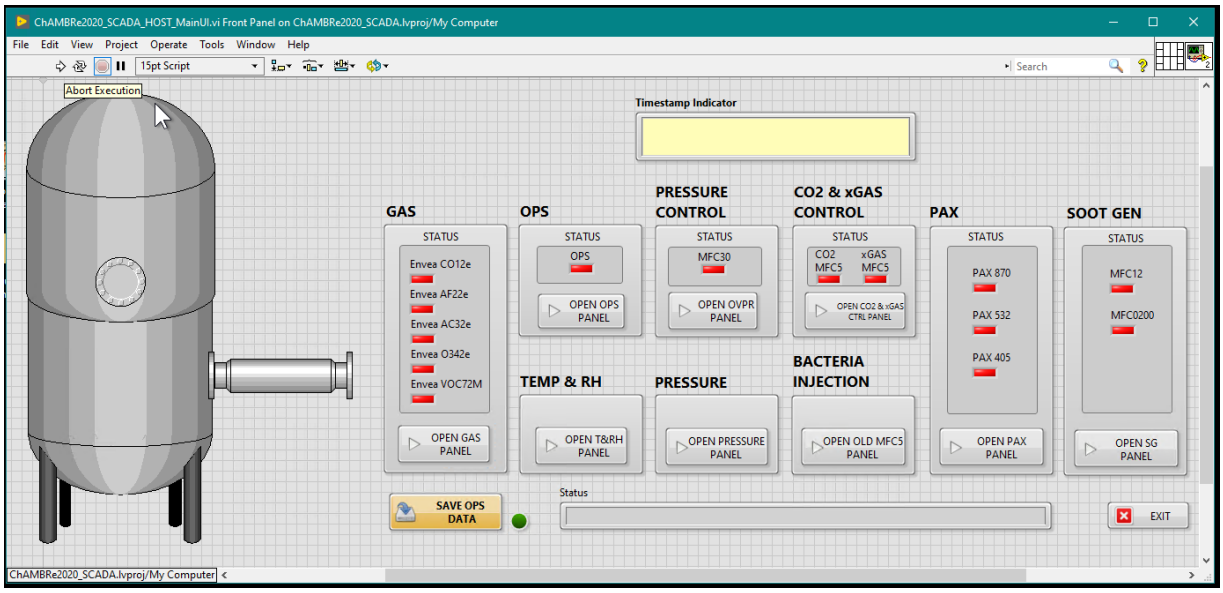

Figure S1: Screenshot of the graphic user interface of the SCADA application developed in LabVIEW™ language.

S3: Feature of the Solar Simulator installed at ChAMBRe

Several characterization measurements were carried out on the Solar Simulator. The device itself and its components were tested to assess the performance. Figure S2 shows a comparison of the effect of the four neutral density filters, which can provide attenuation up to 81% (filter labeled with 19% in the figure). The spectral features of the light produced by the Solar Simulator remain indeed unchanged after going through the neutral density filters, with the only appreciable difference being a uniform attenuation of the transmitted radiation.

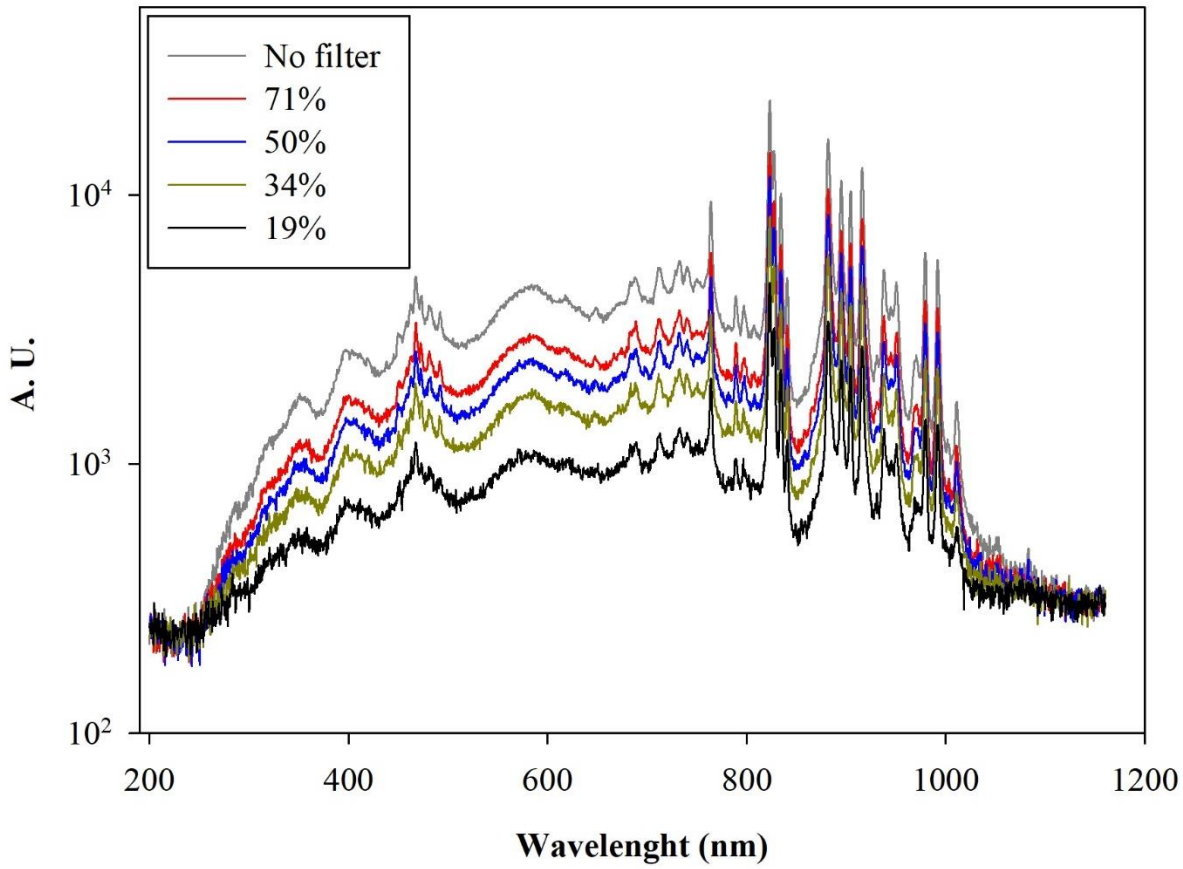

Figure S2: Intensity vs wavelength measured with an Avantes ULS2048CL-EVO spectrometer directly at the exit of the Solar Simulator with and without the available neutral density optical filters. The percentage reported in the legend is the filter transmittance.

The stability of the Solar simulator has been measured over a period of 6 hours, resulting in a maximum deviation (actually increase) of 6% in light intensity, likely due to the warming up of the solar simulator lamp which takes nominally 20 minutes, but it is also subjected to small fluctuation linked to the surrounding environmental conditions.

S4: *E. coli* growth curve

Bacterial growth is a complex process that involves several distinct phases. The increase in numbers or bacterial mass can be measured as a function of time under culture conditions where the nutrients and environmental conditions are controlled. Several distinct growth phases can be observed within a growth curve such as the lag phase, the exponential or log phase, the stationary phase, and the death phase. The first stage, the lag phase, occurs when bacteria are not dividing but are metabolically active. During the lag phase of the bacterial growth cycle, the synthesis of RNA, enzymes, and other

molecules occurs. The length of this phase depends on the type of bacterial species, culture medium, and environmental factors. The log phase is an exponential phase characterized by rapid growth, with binary fission. The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant rate, so both the number of cells and the rate of population increase doubles with each consecutive period. Exponential growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with catabolites. The stationary phase is due to a growth-limiting factor; this is mostly depletion of a nutrient, and/or the formation of inhibitory products such as organic acids. Instead during the death phase, the number of living cells decreases exponentially. Bacteria run out of nutrients and die although the number of cells stays constant. The decline phase is brought by exhaustion of nutrients, accumulation of toxic products, and autolytic enzymes. The microbial growth curve is a record of the countable cells determined at certain time intervals during the population's evolution. In our work, the calibration curve was figured out converting the rate growth from optical density (OD) values to CFU (Colony Forming Unit) ml^{-1}, as explained in detail below.

The day before the experiment, bacteria cells are scraped off agar medium, where they are cultivated, using sterile plastic loops and suspended in a sterile, non-selective culture broth medium Tryptic Soy Broth (TSB) and incubated overnight at 37 °C. The day after, 3 ml of the bacteria culture is diluted in 30 ml of new broth medium, and the suspension is incubated again at 37 °C. At intervals of about thirty minutes, the OD of the bacterial solution is measured by the spectrophotometer at $\lambda = 600$ nm; OD_{600nm} allows to estimate the concentration of bacterial cells in the liquid and tracking the growth. The mid-exponential phase is typically reached when OD_{600nm} is about 0.5 (Mytilinaios et al., 2012; Hall et al., 2014). For selected OD values, the bacterial concentration was also measured/referred as Colony Forming Units (CFU). The bacteria solution must be diluted several times to obtain not overlapping colonies on Petri Dishes: 100 µl of bacterial solution is added in 900 µl of sterile saline solution (NaCl 0.9 %), then diluted again as many times as the theoretical concentration (calculated using the OD value) required; 100 µl of the last dilution is spread in duplicate on an TSB agar and incubated overnight at 37 °C. The next day the concentration of culturable cells is measured by counting the colonies formed and multiplying by the proper dilution factor to retrieve CFU concentration in the original solution. Data, obtained by CFU counting on agar plates, are weighted averaged and used to figure out the uncertainty (standard error of weighted mean) of the bacterial concentration in the solution. The weights are the relative uncertainties of CFU number on agar plates following the Poisson statistics.

We followed the growth of *E. coli* in suspension culture for about 8 hours from lag phase to horizontal asymptote and the OD_{600nm}, the total number of *E. coli* (QTx TOT), measured with Quantom TX and the CFU ml^{-1} values are reported in Figure S3.

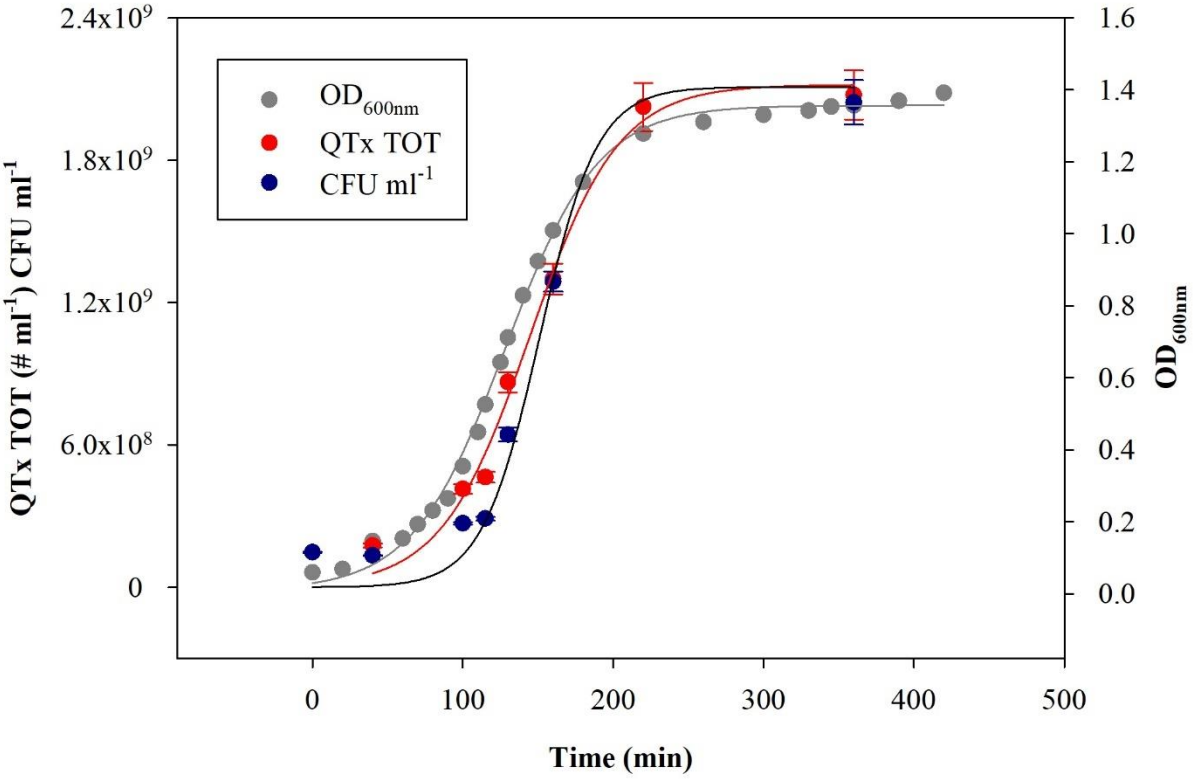

Figure S3: Grow curve for *E. coli*: optical density (OD_{600nm}), total number of *E. coli* measured by QUANTOM-TX (# ml^{-1}) and the corresponding bacteria concentration (CFU ml^{-1}) vs. time. Error bars have, in most cases, the same size of the data points and they are calculated as previously described.

S5: details on the procedure to the optimum setting of the Quantom Tx counter

The Quantom Tx has a factory standard counting protocol already preset, but several parameters must be tuned on the characteristics of different bacterial species: Table S.1 reports the counting parameters and the suggested factory settings for *E. coli.*

Table S1: Quantom Tx factory setting suggested for optimal counting for *E. coli*.

Parameter	Range	Default	E. coli
Dilution factor	1-20000	2	2
Min Fluorescent object size (µm)	0.3-49	0.3	1
Max Fluorescent object size (µm)	0.4-50	50	50
Roundness (%)	0-100	30	30
De-clustering level	0-10	7	7

Detection sensitivity	0-9	4	3

The *Dilution factor* must be set before counting and it is the ratio between the final volume of the sample (bacterial suspension + reagents) and the volume of the bacterial suspension.

The *Fluorescent object size* measures the diameter of the fluorescent signal from the nucleic acid stains. This does not correspond to the physical size of the cell. For the bacterial strain, used in our experiments, the counts of fluorescent signals should be between 1 and 50 µm. *Roundness* selects cells based on their shape: the counting algorithm includes objects that are less round at lower values. Higher values include rounder shapes. The *De-clustering* function allows for the efficient detection of cells that may grow in clusters or chains. Higher values will lead to a higher sensitivity to clusters. The last parameter, *Detection sensitivity* refers to the sensitivity of fluorescence detection. Higher values detect fainter signals from weakly stained cells or smaller sample sizes but can also increase noise. In our experiments, various measurements were made by raising and lowering the *Roundness*, *De-clustering level* and *Detection sensitivity* parameters to verify if the standard protocol was optimal. Below, the trend of *E. coli* bacterial concentration, varying the three parameters described above, is shown. The *E. coli* concentration curve as a function of *Roundness* parameter shows a flat trend up to a value of about 40% and then decreases rapidly for higher values: *E. coli* is a rod shape so high *Roundness* values (i.e., sphericity of the object) lead to an underestimation of the total count.

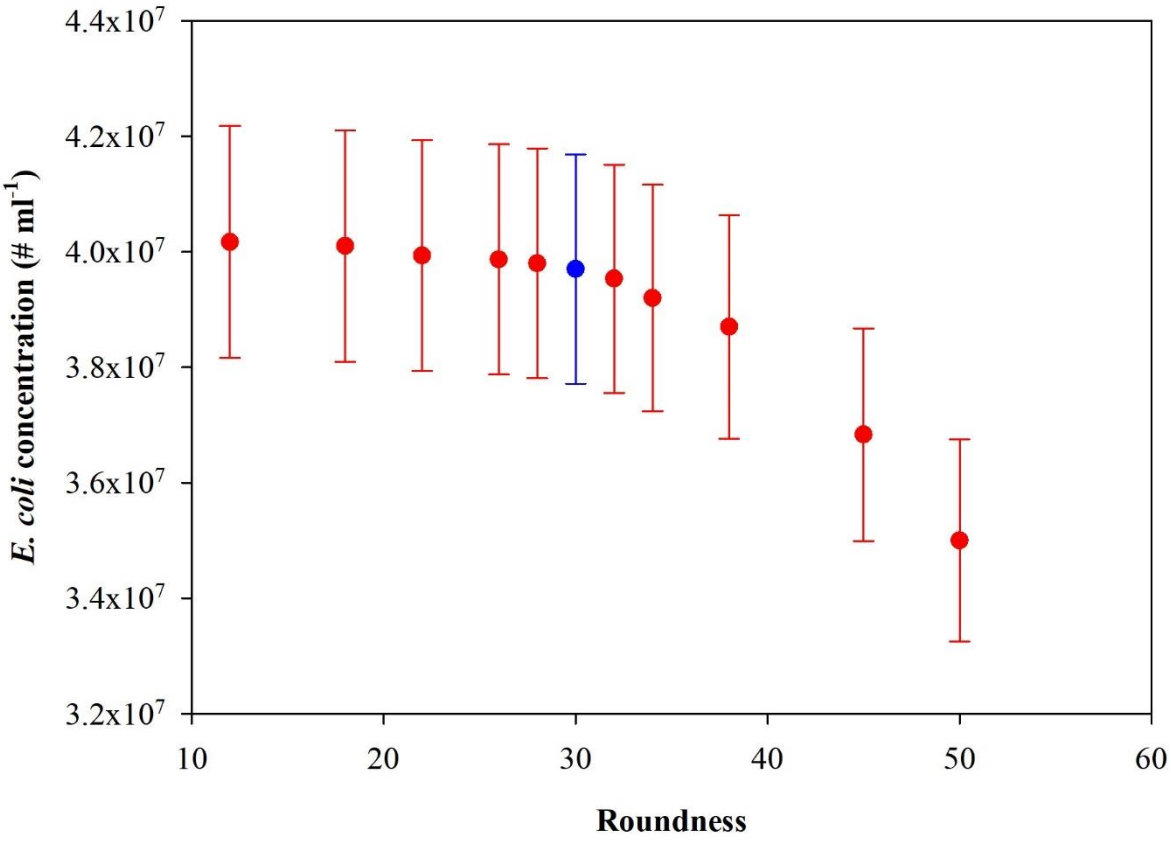

Figure S4: Trend of the *E. coli* concentration as a function of the change of the "Roundness" parameter. The point in blue is the value obtained following the standard protocol for *E. coli*.

Figure S5 shows that the value of the bacterial concentration is not dependent on the variation of the *De-clustering level*; this is probably because the images analyzed didn't show large agglomerations or clusters. Anyway, raising this parameter, the count of bacterial concentration could increase due to the ability of the instrument to separate clusters and individual components. However, it is worth remembering that the clusters are usually probe agglomerates and, therefore, high value of *De-clustering level*, could lead to include non-bacterial cells in the count.

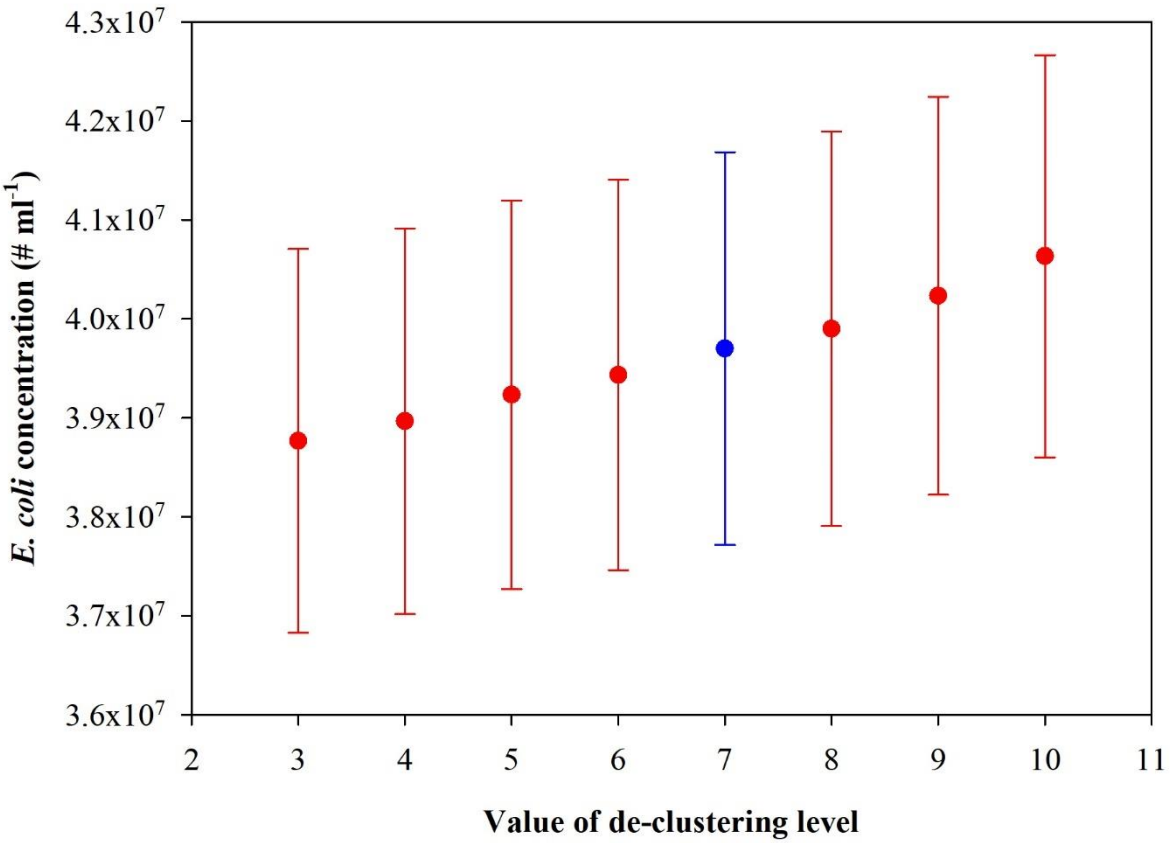

Figure S5: Trend of the *E. coli* concentration as a function of the change of the "De-clustering level" parameter. The point in blue is the value obtained following the standard protocol for *E. coli*.

The *E. coli* concentration vs. *Detection sensitivity* shows the same trend as before; it is a flat curve as the value of detection sensitivity changes. However, it is not recommended to select the lower or higher value of detection sensitivity since some *E. coli* could be lost in the total count or the background noise could increase, decreasing the quality of the images captured by the instrument, respectively.

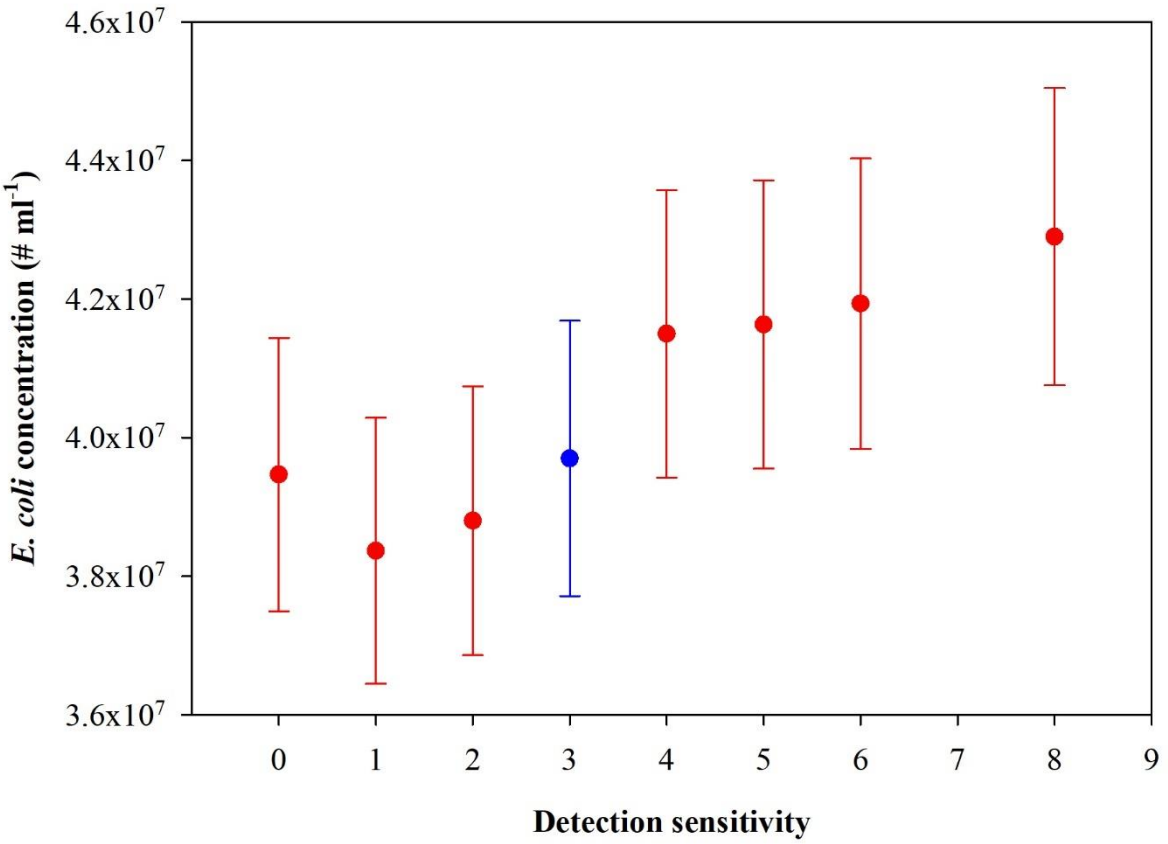

Figure S6: Trend of the *E. coli* concentration as a function of the change of the "Detection sensitivity" parameter. The point in blue is the value obtained following the standard protocol for *E. coli*.

The results of these experiments showed that the variation of *Roundness*, *De-clustering level* and *Detection sensitivity* does not affect the total bacteria concentration count (expect for higher value of Roundness) and the default protocol, suggested by the manufacturer, can be used without additional adjustments.

S6: reduction of *E. coli* viability measured in a set of experiments at ChAMBRe

The experimental protocol discussed in the article is based on the monitoring, during the exposure to the ChAMBRe atmosphere, of the concentration of viable bacteria by active sampling with a single-stage Andersen impactor. The optimum sampling time to collect on the petri dishes in the impactor a well countable number of bacteria (i.e., between 30 and 150 CFUs) while avoiding diluting too much their concentration in the chamber was thoroughly investigated. During the experiment such sampling time necessarily changes to consider the quite fast reduction of the bacteria (*E. coli*) viability. Results are given in Table S2.

Table S2: Sampling time set for the Andersen impactor during the *E. coli* experiments for different time (in minute) after the injection of bacteria.

Experiment	t: 0	t: 30	t: 60	t: 90	t: 120
Baseline dark	5 sec	10 sec	30 sec	1 min	1 min
NO_2 (900 ppb)	5 sec	30 sec	1 min	2 min	2 min
NO_2 (1200 ppb)	5 sec	30 sec	2 min	2 min	3 min
NO (900 ppb)	5 sec	30 sec	1 min	2 min	2 min
NO (1200 ppb)	5 sec	30 sec	1 min	2 min	2 min
Baseline light	5 sec	30 sec	1 min	2 min	2 min

Reduction in *E. coli* viability over time and with the ChAMBRe volume filled with a constant concentration of a single pollutant are reported in Table S3. In all cases, the chamber was maintained in darkness.

Table S3: Ratio of bacteria viable concentration during pollutants experiments and baseline experiments. Values indicate the V:T ratio with pollutant and in the baseline experiment.

POLLUTANT (ppb)	Time after injection (min)				
	0	30	60	90	120
O_3 (>1000)	0.15 ± 0.06	0	0	0	0
NO_2 (900)	0.71 ± 0.19	0.37 ± 0.06	0.29 ± 0.05	0.14 ± 0.06	0.08 ± 0.03
NO_2 (1200)	0.37 ± 0.13	0.03 ± 0.01	0.01 ± 0.01	0.04 ± 0.03	0.01 ± 0.02
NO (900)	0.36 ± 0.12	0.13 ± 0.03	0.23 ± 0.04	0.47 ± 0.12	0.24 ± 0.07
NO (1200)	0.58 ± 0.19	0.25 ± 0.04	0.30 ± 0.05	0.45 ± 0.14	0.10 ± 0.04

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