Supplement of

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

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Keywords: bioaerosol, bacteria, Atmospheric Simulation Chambers, air quality

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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 CO_2 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 SO_2 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.

68 The resulting electric signal is amplified and digitalized in the electrometer board. Its recording gives
69 the chromatogram which exhibits a peak for each detected compound. The standard measured
70 compounds are Benzene, Toluene, Ethylbenzene, m+p-Xylene, o-Xylene, 1-3 Butadiene. Sample
71 flow is around 50 mlpm, with a low detection limit of ≤ 0.05 µg m$^-$3 (benzene).

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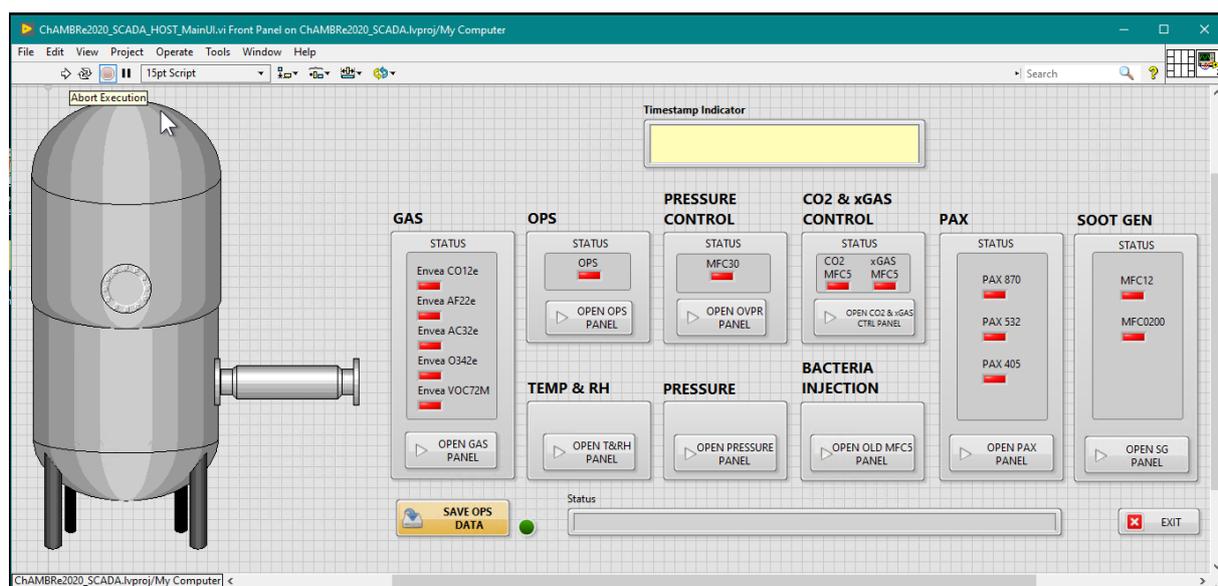
73 **S2: details on the custom SCADA acquisition and control system developed at ChAMBRe**

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

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

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84 **S3: Feature of the Solar Simulator installed at ChAMBRe**

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

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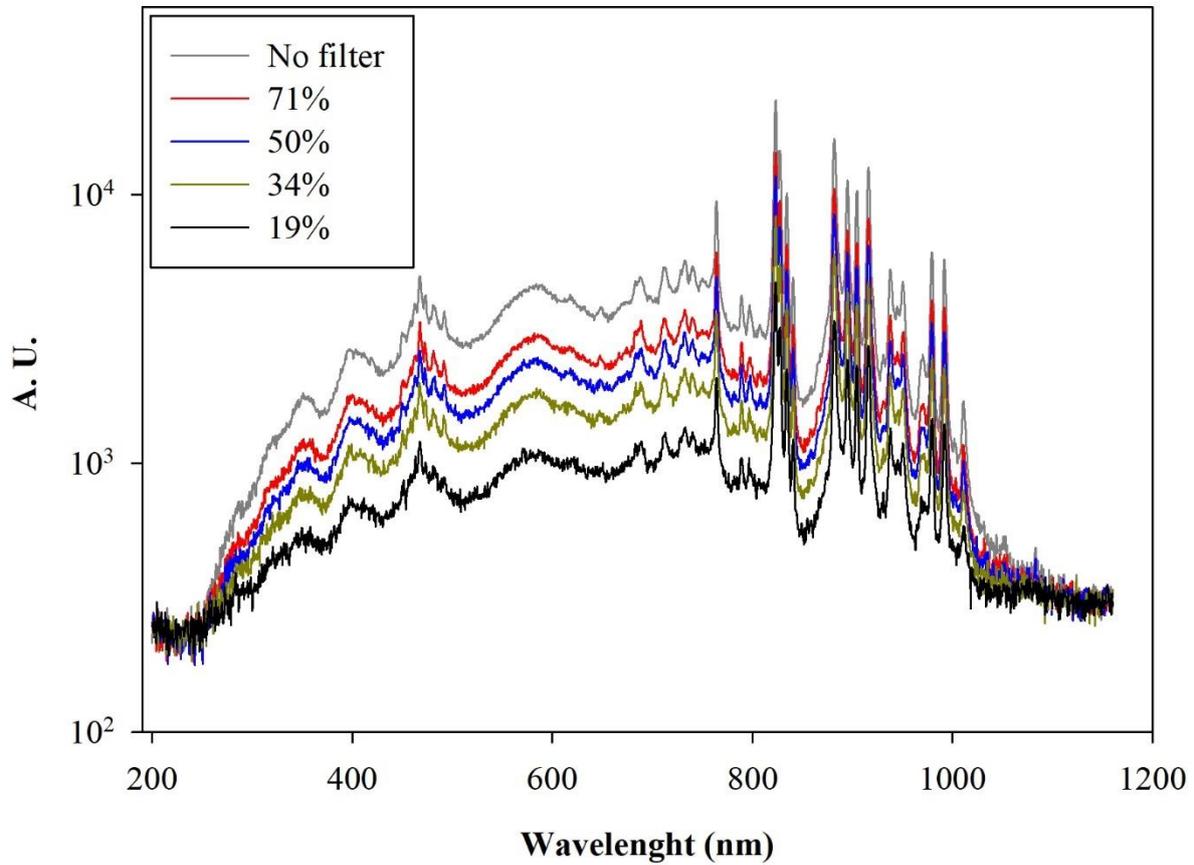
Figure S2: Intensity vs wavelength measured with an Avantes ULS2048CL-EVO spectrometer directly at the exit of the
Solar Simulator with and without the available neutral density optical filters. The percentage reported in the legend is the
filter transmittance.

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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.

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S4: 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.*

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109 **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

110

111 The *Dilution factor* must be set before counting and it is the ratio between the final volume of the

112 sample (bacterial suspension + reagents) and the volume of the bacterial suspension.

113 The *Fluorescent object size* measures the diameter of the fluorescent signal from the nucleic acid

114 stains. This does not correspond to the physical size of the cell. For the bacterial strain, used in our

115 experiments, the counts of fluorescent signals should be between 1 and 50 µm. *Roundness* selects

116 cells based on their shape: the counting algorithm includes objects that are less round at lower values.

117 Higher values include rounder shapes. The *De-clustering* function allows for the efficient detection

118 of cells that may grow in clusters or chains. Higher values will lead to a higher sensitivity to clusters.

119 The last parameter, *Detection sensitivity* refers to the sensitivity of fluorescence detection. Higher

120 values detect fainter signals from weakly stained cells or smaller sample sizes but can also increase

121 noise. In our experiments, various measurements were made by raising and lowering the *Roundness,*

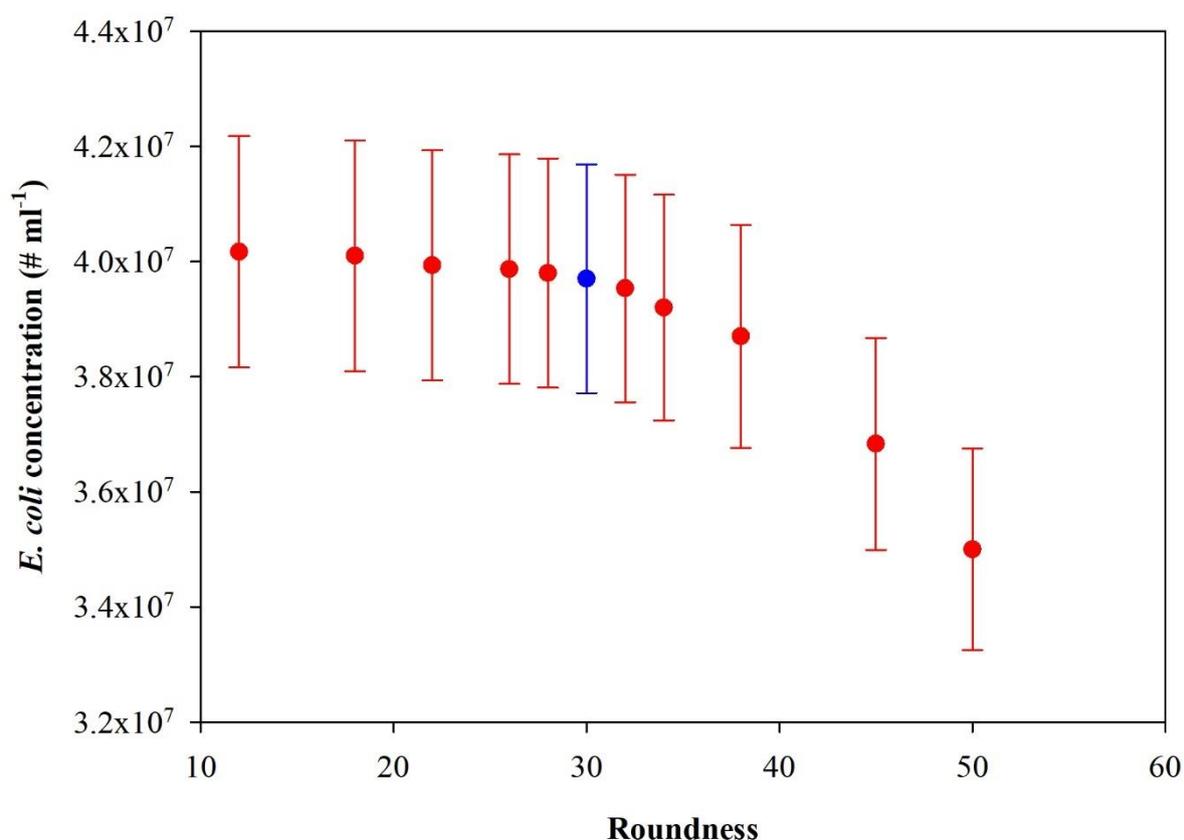
122 *De-clustering level* and *Detection sensitivity* parameters to verify if the standard protocol was optimal.

123 Below, the trend of *E. coli* bacterial concentration, varying the three parameters described above, is

124 shown. The *E. coli* concentration curve as a function of *Roundness* parameter shows a flat trend up

125 to a value of about 40% and then decreases rapidly for higher values: *E. coli* is a rod shape so high

126 *Roundness* values (i.e., sphericity of the object) lead to an underestimation of the total count.

127

128

129 **Figure S3:** Trend of the *E. coli* concentration as a function of the change of the "Roundness" parameter. The point in blue
130 is the value obtained following the standard protocol for *E. coli*.

131

132 Figure S4 shows that the value of the bacterial concentration is not dependent on the variation of the
133 *De-clustering level*; this is probably because the images analyzed didn't show large agglomerations
134 or clusters. Anyway, raising this parameter, the count of bacterial concentration could increase due
135 to the ability of the instrument to separate clusters and individual components. However, it is worth
136 remembering that the clusters are usually probe agglomerates and, therefore, high value of *De-*
137 *clustering level*, could lead to include non-bacterial cells in the count.

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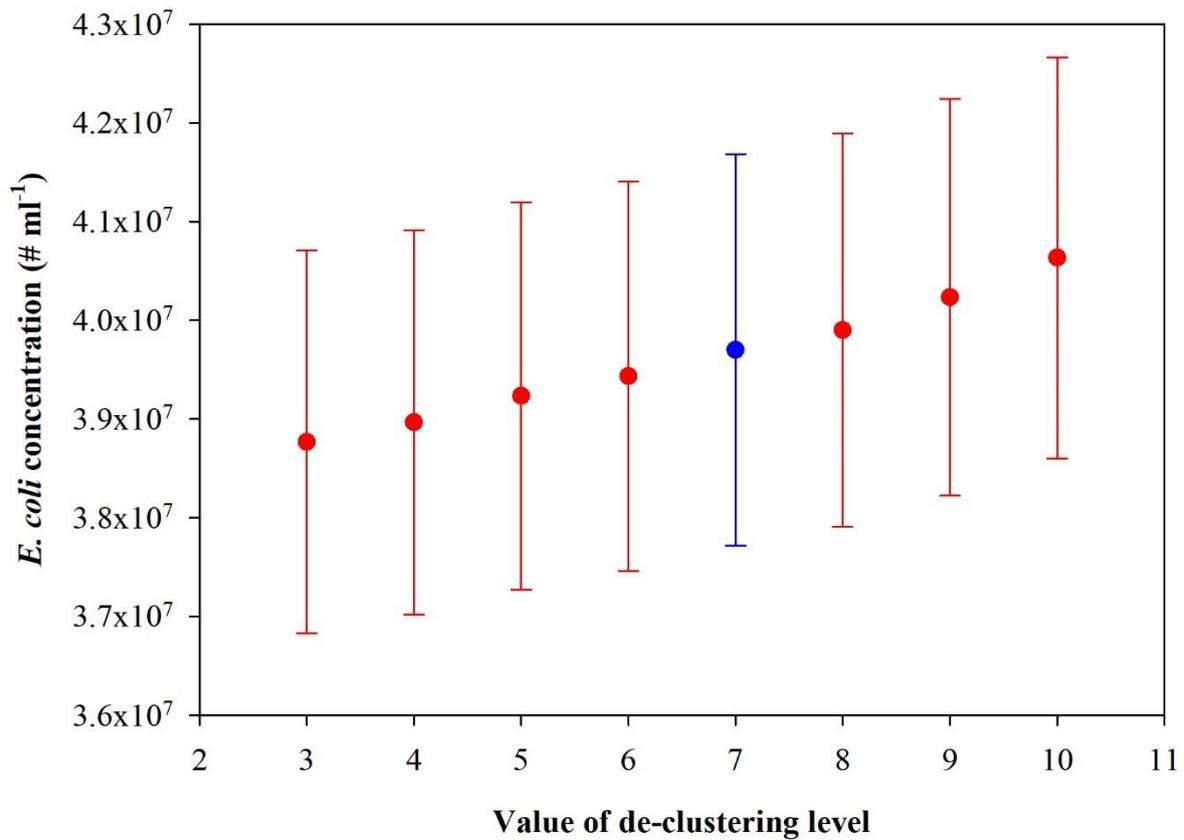
Figure S4: Trend of the *E. coli* concentration as a function of the change of the "De-clustering level" parameter. The point in blue is the value obtained following the standard protocol for *E. coli*.

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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.

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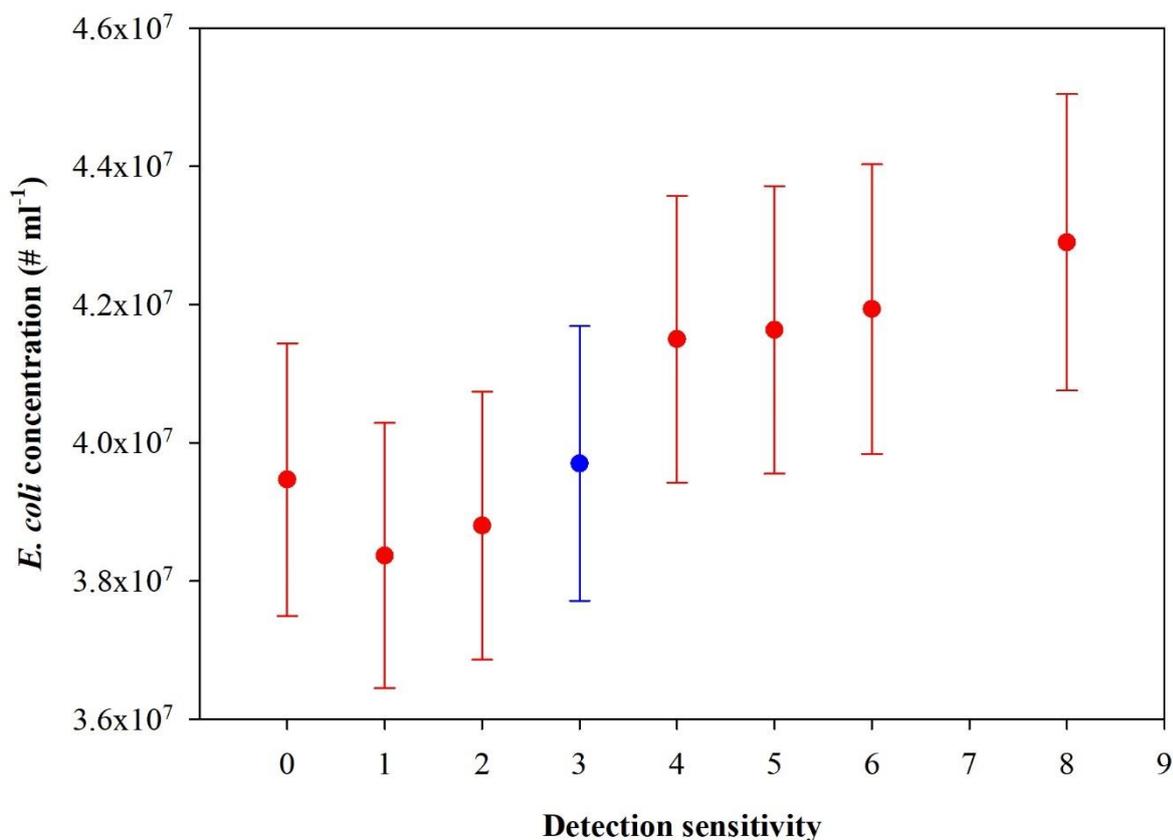
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149 **Figure S5:** Trend of the *E. coli* concentration as a function of the change of the "Detection sensitivity" parameter. The
150 point in blue is the value obtained following the standard protocol for *E. coli*.

151

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

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157 **S5: reduction of *E. coli* viability measured in a set of experiments at ChAMBRe**

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159 The experimental protocol discussed in the article is based on the monitoring, during the exposure to
160 the ChAMBRe atmosphere, of the concentration of viable bacteria by active sampling with a single-
161 stage Andersen impactor. The optimum sampling time to collect on the petri dishes in the impactor
162 a well countable number of bacteria (i.e., between 100 and 200 CFUs) while avoiding diluting too
163 much their concentration in the chamber was thoroughly investigated. During the experiment such
164 sampling time necessarily changes to consider the quite fast reduction of the bacteria (*E. coli*)
165 viability. Results are given in Table S2.

166 **Table S2:** Sampling time set for the Andersen impactor during the *E. coli* experiments for different time (in minute) after
167 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

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

172

173 **Table S3:** Ratio of bacteria viable concentration during pollutants experiments and baseline experiments. Values indicate
174 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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