Electronic Supplementary Material (ESI) for Environmental Science: Nano

A Chemical free, Nanotechnology-based Method for Airborne Bacterial Inactivation using Engineered Water Nanostructures

Supplemental Material

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Airborne bacteria inactivation control experiments

Control experiments were performed to exclude the possibility of the bacteria being inactivated on the TSA plates of the biosampler post-collection, by impaction of the EWNS on the TSA plates. In these control experiments *S. Marcescens* was plated directly on the agar plates before they were loaded in the Anderson Impactor for the sample collection. EWNS aerosol levels in the chamber were kept the same as for the 2.9 ACH scenario without bioaerosol generation (HEPA filtered air) and brought to steady state. EWNS aerosol was then sampled from the chamber through the biosampler. A second experiment followed with a HEPA filter directly attached to the biosampler inlet to remove EWNS. The plates were cultured and bacteria counted according to the as described in the methods section. The data were compared to assess the ability of EWNS to inactivate the bacteria in the agar plates of the biosampler. The experiments were performed in triplicates.

Moreover, despite the fact that the ozone levels in the environmental chamber were kept at low levels (96-132 ppb level) ozone control experiments were also performed. All the ozone control experiments were performed at steady state under the conditions described before for the 2.9 ACH scenario. The EWNS were removed with a HEPA filter before their introduction in the chamber. Bacteria viability in the chamber under this scenario was compared to that of using HEPA filtered air. The plates were cultured and bacteria counted with the previously described protocol, and the data were compared to assess the ozone effect on bacteria inactivation. Ozone control experiments were also performed in triplicates.

Bacterial membrane permeability experiments

For the TEM imaging and the membrane permeability assay sample collection, a recently method developed by our group was followed. [1,2] Stainless steel coupons of 2.5 x 7.5 cm were cleaned with soap and water, rinsed with ethanol, and autoclaved. The concentration of the *S. Marcescens* stock solution was adjusted to approximately 10^6 cfu/ml with PBS. One hundred μl of the suspension, of the *S. Marcescens*, solution was spread on 8 coupons. The coupons were left in a laminar flow hood to dry (approximately 45 mins). Four coupons were exposed for 30, 60, 90, 120 mins to one electrospray module placed at 5 cm distance above the coupons. The other four coupons were left in the laminar flow hood for the same amounts of time (controls). The environmental conditions for both exposed and control coupons were the same at 50% RH and 21°C.

The number concentration of the EWNS aerosol directly over the coupons was measured and found to be 9000#/cm^3. Post exposure the coupons, were placed in 50 ml centrifuge tubes and were rinsed 20 times repeatedly with 2 ml PBS. The rinsate was collected for 30, 60, 90 and 120 mins exposure times and used for the membrane permeability experiments. The same rinsate was also used for the TEM imaging.

Membrane permeability assay: The extent of nuclear staining, within a population of cells and bacteria, by the membrane-impermeant and permeant fluorescent nucleic acid dyes, is a useful quantifiable indicator of membrane damage. [3,4] To investigate any membrane damage of the exposed and unexposed to EWNS bacteria, 1 ml samples of rinsates of bacteria collected from the coupons as described above were pelleted by centrifuging at 10,000 x g for 10 mins, resuspended in 100 µl of 6 µM SYTO 9 (green fluorescent, membrane permeant) dye (Life Technologies, Carlsbad, CA) and 30 µM Propidium iodide (red fluorescent, membrane impermeant) dye (Life Technologies) in HBSS, incubated at room temperature for 20 mins, washed once and resuspended in 100 µl of HBSS, dispensed into 96-well black-walled, optical bottom imaging microplates (BD Biosciences, San Jose, CA), centrifuged at 2,000 x g for 5 mins to settle bacteria on well bottoms, and imaged using a BD Pathway 800 Bioanalyzer scanning cytometer (BD Biosciences). Images were analyzed using custom algorithm with the ImageJ (NIH)[5] to identify and quantify red-staining (killed due to membrane destruction) and green-staining (live) bacteria.

TEM imaging: In addition to the membrane permeability assay, which provided quantitative data on possible membrane damage, TEM imaging was performed. The bacteria imaging was done with the JEOL 1200EX transmission electron microscope (Tokyo, Japan). A pellet of EWNS exposed and a pellet of unexposed bacteria were fixed for at least 2 hours at room temperature in 2.5% glutaraldehyde, 1.25% paraformaldehyde, and 0.03% picric acid in 0.1 M sodium cacodylate buffer (pH 7.4), as fixative, washed in 0.1M cacodylate buffer and post-fixed with 1% osmiumtetroxide (OsO4)/1.5% potassiumferrocyanide (KFeCN6) for 2 hours, washed in water 3x and incubated in 1% aqueous uranyl acetate for 1 hour followed by 2 washes in water and subsequent dehydration in grades of alcohol (10 mins each; 50%, 70%, 90%, 2x10 mins 100%). The samples were then put in propyleneoxide for 1 hour and infiltrated ON in a 1:1 mixture of propyleneoxide and TAAB Epon (Marivac Canada Inc. St. Laurent, Canada). The following day, the samples were embedded in TAAB Epon and polymerized at 60ºC for 48 hrs.

References

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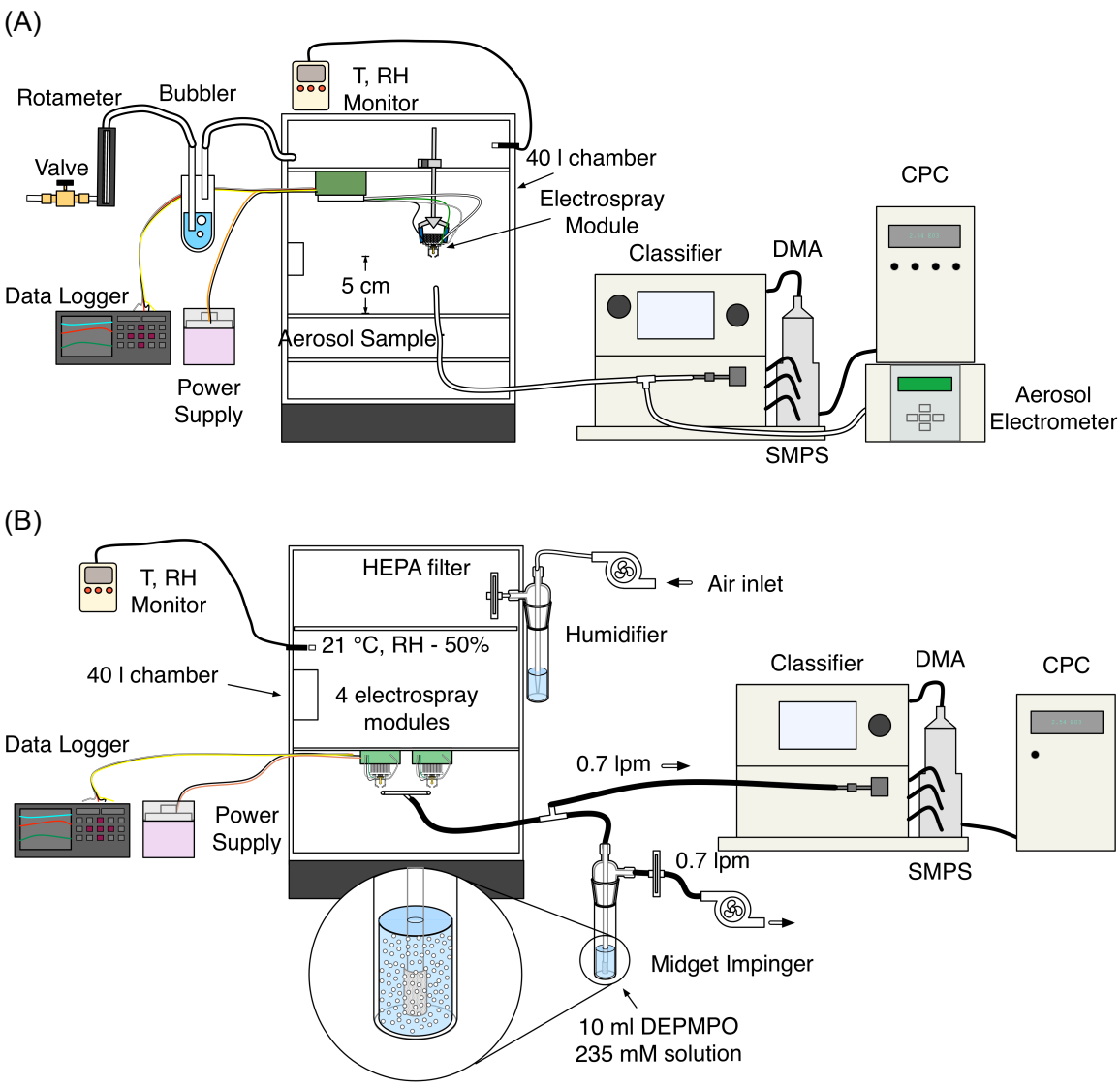

Figure S1: Experimental setup for the EWNS characterization. (A) charge characterization (B) ROS characterization.

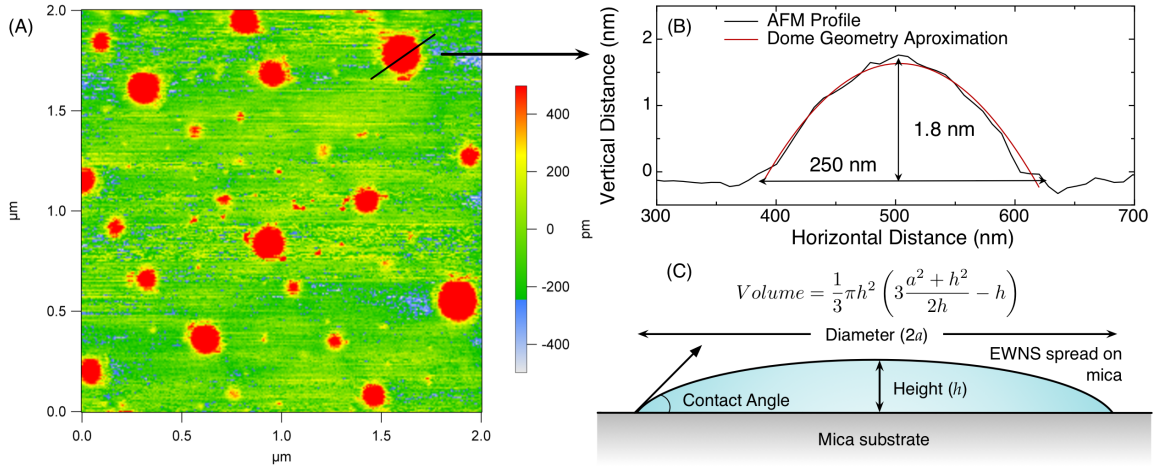

Figure S2: (A) The AFM topography of the EWNS sprayed mica surface. (B) Example of one droplet profile as it is imaged with the AFM and the overlay of the equivalent dome profile. (C) The dome geometry used to estimate the equivalent volume.

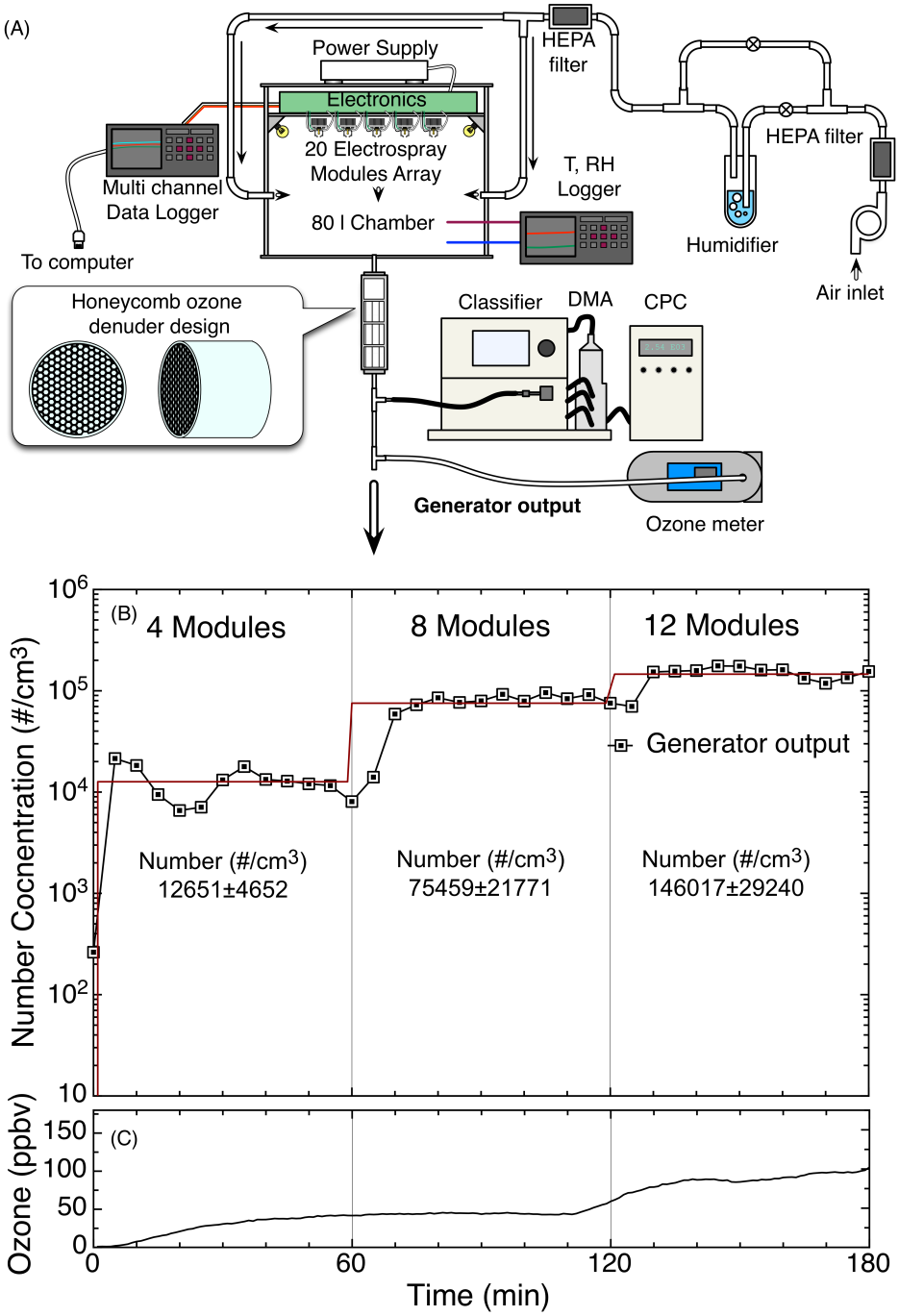

Figure S3: The high aerosol volume, high concentration EWNS generation system using 20 electrospray modules. (A) The generation system consists of an 80 l chamber with controlled Temperature and Relative Humidity conditions. (B) The generator's ability to generate aerosol up to 150,000 #/cc at 10 lpm. (C) The corresponding ozone levels in the environmental chamber (2.9 ACH).

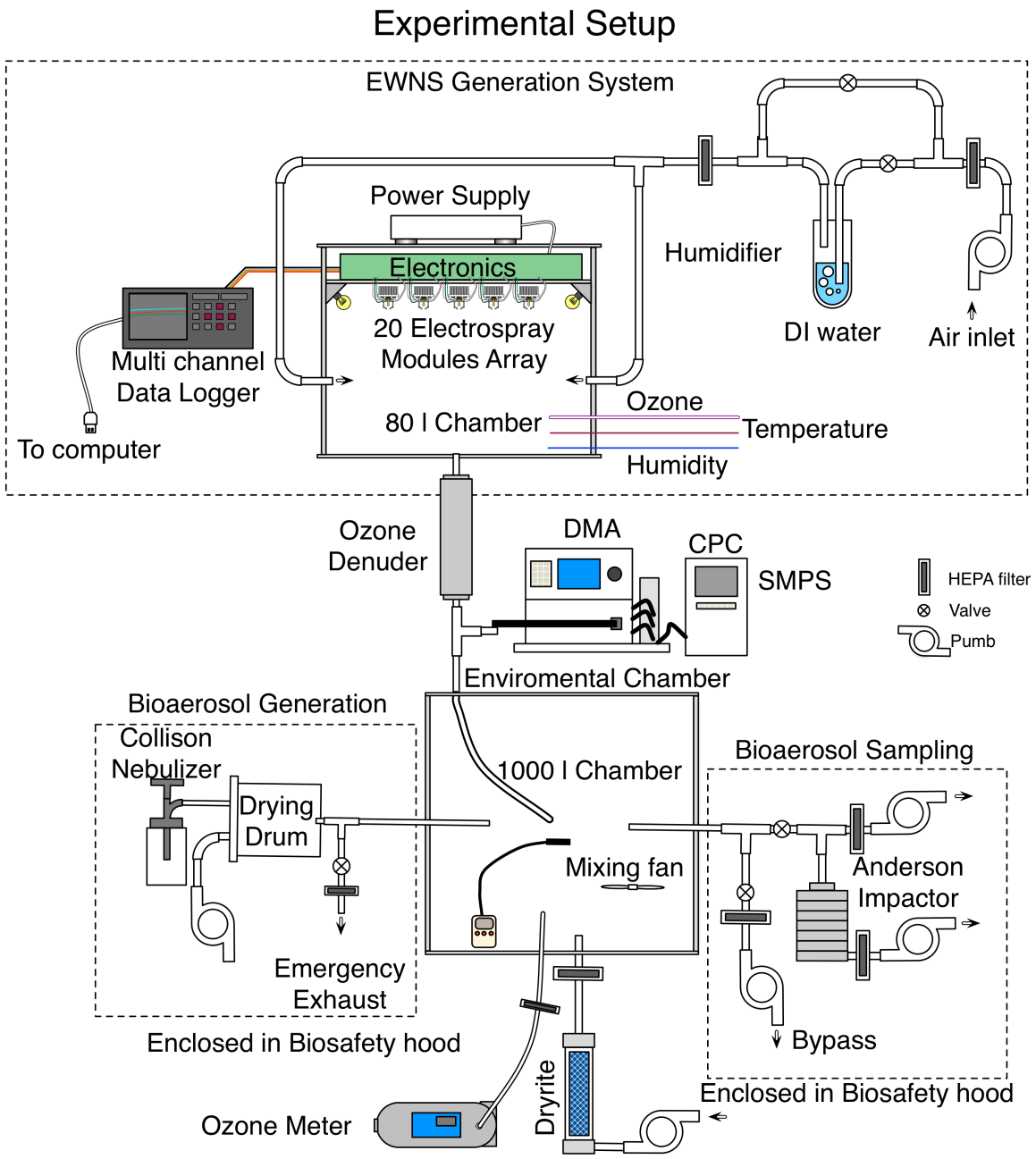

Figure S4: The airborne bacteria inactivation experimental setup. The bioaerosol and the EWNS aerosol are mixed in a 1000 l environmental chamber. An Andersen sampling system is used to collect culturable the bacteria and determine the bioaerosol concentration as a function of time for both steady state and decay conditions.

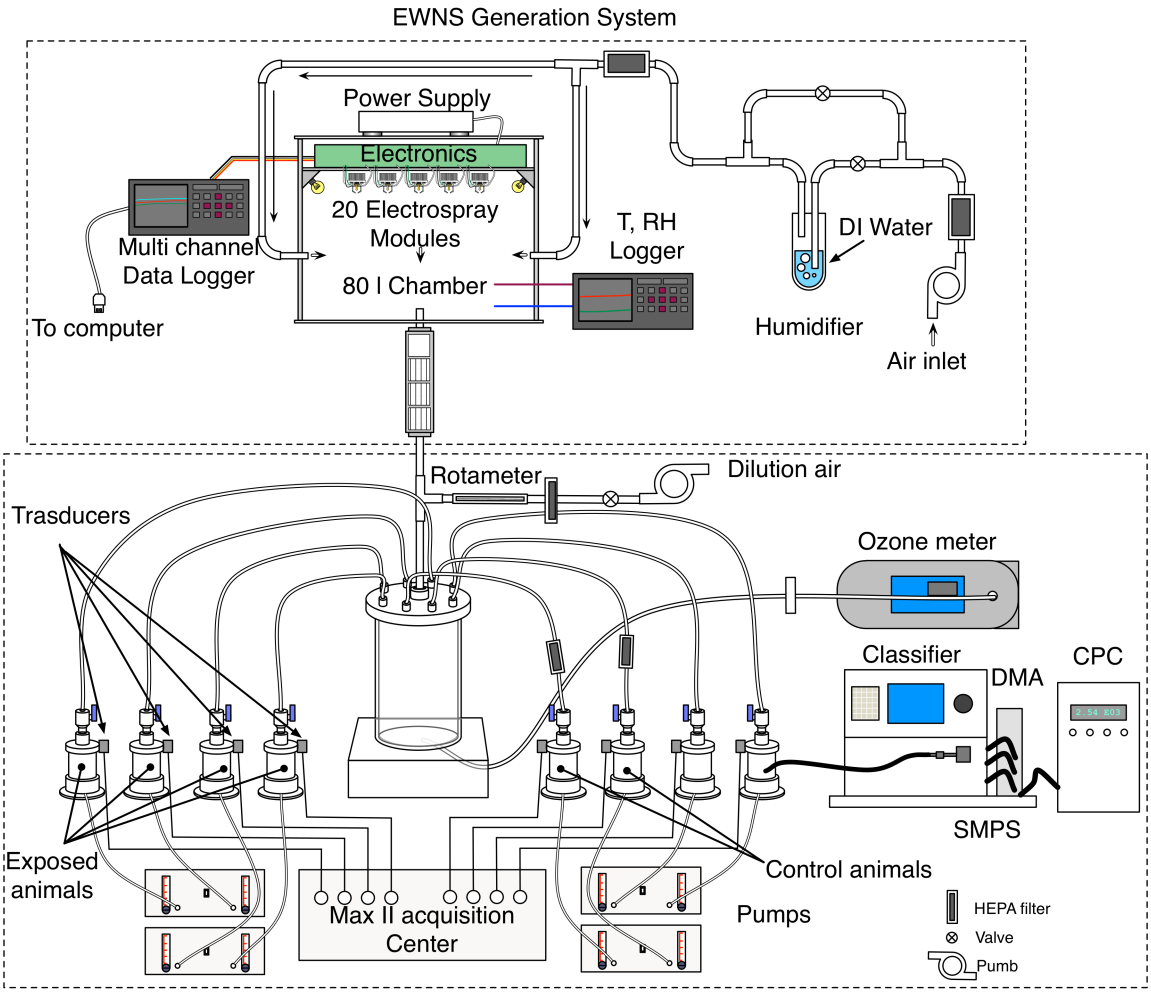

Figure S5: The animal exposure system for the toxicological assessment of the inhaled nanoparticles. Each animal was placed in an individual cage with a dedicated

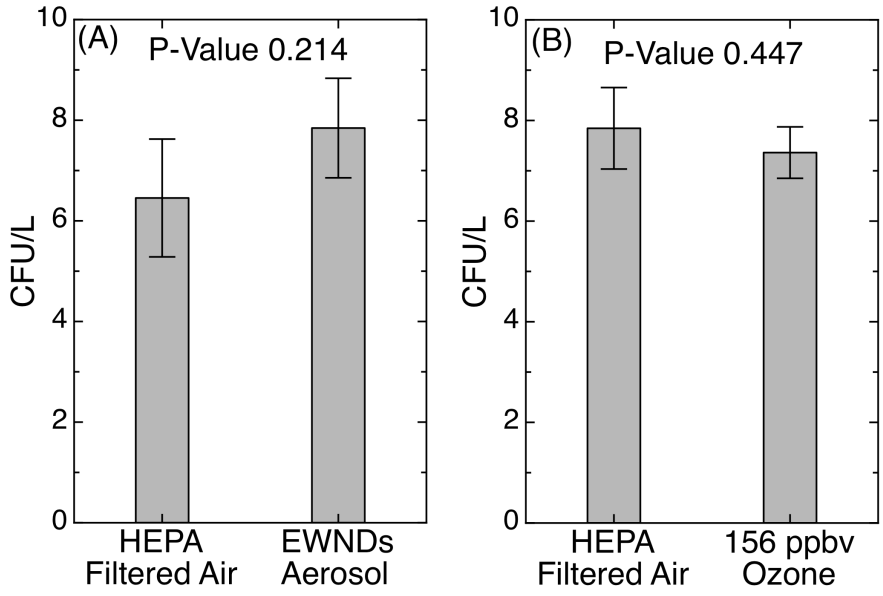

Figure S6: The results of the control experiments. (A) Viability of bacteria pre-plated on the agar plates and used in the biosampler #6 stage to sample HEPA filtered air and EWNS aerosol for 1 minute. (B) The effect of ozone.