

**Measurement Report: An Exploratory Study of Fluorescence and CCN Activity of  
Urban Aerosols in San Juan, Puerto Rico**

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**Abstract**

~~Many atmospheric aerosols are cloud condensation nuclei (CCN), capable of activating as cloud droplets when the relative humidity exceeds 100%. Many types of atmospheric aerosols cloud condensation nuclei (CCN) capable of activating as cloud droplets.~~ Some primary biological aerosol particles (PBAP), such as plant spores, pollen, or bacteria, have been identified as such CCN. Urban environments are a source of these bioaerosols, those that are naturally produced by the local flora, or are transported from surrounding regions, and others that are a result of human activities. In the latter case, open sewage, uncovered garbage, mold or other products of such activities can be a source of PBAPs. There have been relatively few studies, especially in the tropics, where PBAPs and CCN have been simultaneously studied to establish a causal link between the two. The metropolis of San Juan, Puerto Rico is one such urban area with a population of 2,448,000 people (as of 2020). To better understand the fluorescent characteristics and cloud forming efficiency of aerosols in this region, measurements with a Wideband Integrated Bioaerosol Spectrometer (WIBS), a condensing nuclei (CN) counter, and

a CCN spectrometer were made at the University of Puerto Rico – Rio Piedras Campus. Results show that the CCN/CN activation ratio and the fraction of fluorescing aerosol particles (FAP) have repetitive daily trends when the FAP fraction is positively correlated with relative humidity and negatively correlated with wind speed, consistent with previous studies of fungi spores collected on substrates.

The results from this pilot study highlight the capabilities of ultraviolet-induced fluorescence (UV-IF) measurements for characterizing the properties of FAP as they relate to daily evolution of PBAPs. The use of multiple excitation and emission wavelengths, along with shape detection, allows the differentiation of different PBAP types. These measurements, evaluated with respect to previous, substrate-based analysis of the local fungal and pollen spores, have established a starting database of measurements that future, longer term studies will build upon.

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## 61 **1 Introduction:**

62 The formation and evolution of clouds over the tropical island of Puerto Rico have been studied  
63 over the course of many years, primarily with respect to the sources of cloud condensation  
64 nuclei (CCN). Puerto Rico has been the site of these studies because of its fair-weather,  
65 maritime flow and mostly clean atmosphere that leads to a mountaintop cloud that forms quite  
66 frequently throughout the year and can persist for several days (Allan et al., 2008; Gioda et al.,  
67 2013; Spiegel et al., 2014; Valle-Díaz, et al., 2016; Raga et al., 2016; Torres Delgado, 2021).  
68 In addition to the clean, maritime sources, the cloud studies have also identified particles  
69 produced from upwind urban areas, both on the island of Puerto Rico as well as islands to the  
70 east, where vehicular and ~~other~~ industrial emissions produced particles with organic carbon  
71 and sulfates (Allan et al., 2008). Apart from this, clouds and rainwater in this region are  
72 influenced by long-range transported natural aerosols. African dust is also potentially an  
73 important source of CCN, although the results are not conclusive as to how much cloud  
74 properties differ in the presence of these particles (Spiegel et al., 2014; Valle-Díaz, et al., 2016;  
75 Raga et al., 2016; Torres Delgado, 2021).

76 Airborne, primary biological aerosol particles (PBAP) are an important type of aerosol in the  
77 tropics (Gabey et al., 2010,2013; Stanley et al., 2011) that can encompass viruses (0.01-0.3µm),  
78 pollen (5-100 µm), bacteria and bacteria agglomerates (0.1-10 µm), and fungal spores (1-30  
79 µm) as well as mechanically formed particles, such as dead tissue and plant debris (Finnelly et  
80 al., 2017). Furthermore, there is evidence that PBAP may influence the hydrological cycle and  
81 climate by initiating the ice nucleation process or acting as giant CCN (Möhler et al., 2007;  
82 Pope, 2010). Although bioaerosols contribute a small fraction (50 Tg yr<sup>-1</sup>) of the total natural  
83 global emissions (~2900-13000 Tg yr<sup>-1</sup>) (Hoose et al., 2010; Stocker et al., 2013). However,  
84 their mass and number concentrations are site specific and greatly vary depending upon the  
85 location and climatic condition (references therein Zhang et al., 2021). In terrestrial  
86 ecosystems, bioaerosols constitute a major fraction of total aerosol load. As far as urban and  
87 rural atmosphere are concern, bioaerosols of size greater than ~1 µm may account for around  
88 30% (references therein Fröhlich-Nowoisky et al., 2016). There is evidence where bioaerosol  
89 may constitute a significant fraction (5-50%) in the urban air (Jaenicke, 2005). Upon emission

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from the biosphere, PBAP undergoes various physico-chemical changes (coagulation, photooxidation, surface coating, etc.) and are removed through dry and wet deposition. The reference to giant CCN has now been expanded upon to explain that giant CCN play a special role in precipitation development because they can form larger droplets that more easily collide and coalesce to form raindrops. Hence, although small in number concentration they make up for in their size and capacity to contribute to early precipitation development. In general, biological aerosol particles, until recently, have received less attention in the atmospheric science community for lack of appropriate equipment and the associated measurements are expensive, labor intensive and often difficult to interpret (Cziczo et al., 2006; Drewnick et al., 2008).

Puerto Rico is characterized by tropical climate, urban land cover and use, moist soils, unique topography, and dense vegetation. These factors, associated with the easterly trade winds from the East, could influence the concentration of airborne particles, for examples, organic particles, viruses, bacteria, fungi, pollen, etc. (Velázquez-Lozada et al. 2006). Nevertheless, meteorological variables (high humidity and wind speed) are also the important factors, influencing the airborne particle population in the tropics, including rainy seasons. There are various sources of particulate matter degrading the air quality of Puerto Rico, i.e., from industrial activities, anthropogenic inputs, African temperatures dust storms and volcanic eruptions. The urban areas of Puerto Rico are considered developed with industrial growth, most of which is related to pharmaceutical and power generation plant. The power generation plants are responsible for releasing millions of pounds of air pollutant annually (Torro-Heredia et al., 2020). Data show that a large number of organic compounds (e.g., n-alkanes, esters, phthalates, siloxanes, and other) including plasticizer released into the atmosphere which could pose major health threat in this area (Torro-Heredia et al., 2020). Puerto Rico has abundant plant life, e.g., a wide variety of trees, flowers, mosses and other types of flora; hence, an open question is if fungal or pollen spores produced from these plants might serve as CCN. The bioaerosol population in Puerto Rico, and in particular in the capital city of San Juan, has been studied extensively using analysis of substrates samples (Quintero et al., 2010; Rivera-Mariani et al., 2011; Rivera-Mariani et al., 2020). The objective of the majority of these studies has been to evaluate the health effects of fungal and pollen spores on the local population (Quintero et al., 2010; Rivera-Mariani et al., 2011; Ortiz-Martínez et al., 2015; Rivera-Mariani et al., 2020). The studies by Quintero et al (2010) are particularly interesting with respect to the investigation reported here because they classified a wide variety of fungal and pollen spores

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123 that were the most responsible for respiratory ailments of residents of San Juan. In addition,  
124 they could link the relative concentration of these spores to meteorological factors ([e.g., relative](#)  
125 [humidity and wind speed](#)). Hence, given that their studies indicated that bioaerosols are not  
126 only produced in large quantities throughout the year, and highly correlated to local  
127 meteorology, it is reasonable to investigate if these types of bioaerosols might also be correlated  
128 with CCN measurements if such particles are hygroscopic and can easily form water droplets  
129 under the right conditions.

130 Prior to embarking on a longer-term project that will evaluate PBAPs and CCN under a wide  
131 range of conditions, a pilot study was designed to conduct an exploratory investigation of the  
132 properties of bioaerosols and CCN during September 2019. September was selected because  
133 not only did the Quintero et al. (2010) results show that this time of the year is when peak  
134 concentrations of spores are found, it is also a month of frequent cloud formation.

135 In order to identify the PBAP and investigate their potential sources, we used a realtime,  
136 particle by particle approach rather than a methodology that requires capturing particles for  
137 offline analysis. There has been considerable progress made in the development of technologies  
138 based on the working principle of ultraviolet light-induced fluorescence (UV-LIF) (Ho, 1999;  
139 Huffman and Santarpia, 2017). The Wideband Integrated Bioaerosol Spectrometer (WIBS) and  
140 the Ultraviolet Aerodynamic Particle Sizer (UV-APS) are examples of instruments that can  
141 detect PBAP by their fluorescence, in real time, particle by particle, over a wide size range  
142 (Savage et al., 2017). The WIBS and the UV-APS have been used in atmospheric bioaerosol  
143 studies such as ice nucleation activity of bioaerosol (Twohy et al., 2016), measurement of  
144 atmospheric fungal spore concentrations (Gosselin et al., 2016), and investigation of long-  
145 range transported bioaerosol in the tropics (Gabey et al., 2010; Whitehead et al., 2016) and at  
146 high altitudes (Gabey et al., 2013). The WIBS is a three channel LIF instrument developed by  
147 the University of Hertfordshire and manufactured by Droplet Measurement Technologies LLC  
148 available with different versions (e.g., WIBS 4A and WIBS NEO) of slightly different optical  
149 and electronic configuration (Gabey et al. 2010; Perring et al. 2015). In parallel with the  
150 measurement of fluorescent aerosol particles (FAP), the CCN and condensation nuclei (CN)  
151 number concentrations were also measured in order to determine what fraction of the total  
152 particle population was composed of CCN and FAPs.

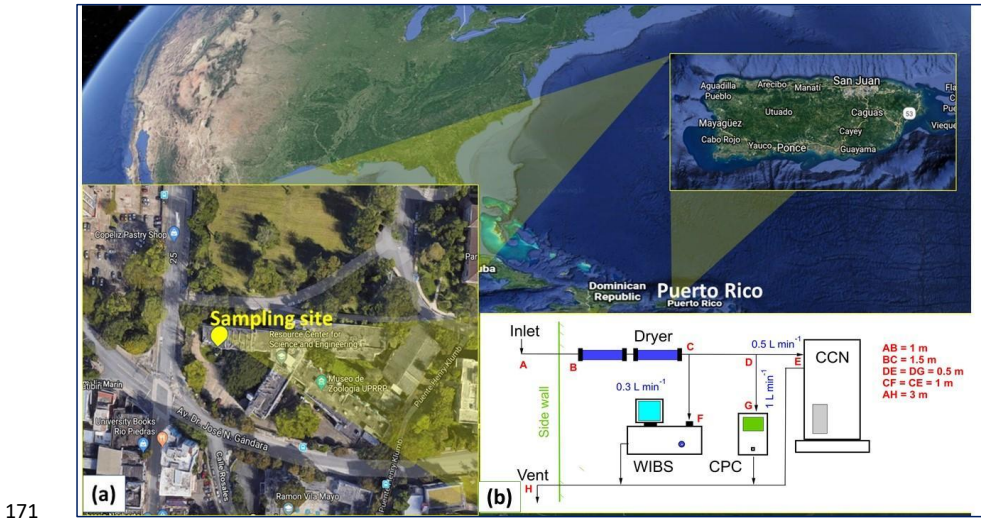
153 The primary objectives of this exploratory study are to evaluate the physical properties of CN,  
154 CCN and FAP, investigate correlations between CCN and FAP, analyze trends related to

155 meteorological factors, and compare the FAP measurements with those from previous studies  
156 that documented fungal and pollen spores using off-line analyses.

157 **2 Measurement and analysis methodology**

158 **2.1 Measurement site and experimental setup**

159 The measurement site (Fig. 1a) is the Facundo Bueso (FB) building on the University of Puerto  
160 Rico, Rio Piedras (UPR-RP) Campus (18°24'6.4"N, 66°03'6.5"W, 6 m a.m.s.l.), and for the  
161 airborne spores were collected using the Hirst-type Burkard sampler (Burkard Scientific Ltd,  
162 Uxbridge, UK). located on the rooftop of the Medical Sciences Campus (MSC) of the  
163 University of Puerto Rico (18°23'48" N, 66°4'30" W, 60 m a.m.s.l) both located in San Juan,  
164 which is the urban capital of Puerto Rico (pop. 2,448,000). Moreover, the measurement sites  
165 are located at the center of the San Juan city (199 km<sup>2</sup>), a clear representative of typical urban  
166 atmosphere. San Juan has a tropical climate, receiving a significant amount of rainfall  
167 (4.22±1.3 in) throughout the year. The FB site is surrounded by various sources of emission  
168 such as residential cooking, roadway traffic, and vegetation. Because of the urban location,  
169 aerosol emissions from roadway traffic and nearby residences contribute significantly to the  
170 total aerosol number concentrations at the site.



172 **Figure 1. (a) Sampling location in the FB building of UPR-RP (b) experimental setup comprising the cloud**  
173 **condensation nuclei counter (CCN), condensation particle counter (CPC), and the wideband integrated**  
174 **bioaerosol sensor (WIBS). This figure was generated using © Google Earth Pro 7.3.**

Sampling was performed for eight consecutive days (September 16-23, 2019). The measurement setup consisted of two diffusion dryers (TSI model 3062) connected in series, a cloud condensation nuclei counter (CCN-100, Droplet Measurement Technologies), a condensation particle counter (CPC, TSI model 3772), and a wideband integrated bioaerosol sensor (WIBS-NEO, Droplet Measurement Technologies) (Fig. 1b). Atmospheric aerosol samples were aspirated from the exterior through the sidewall of the laboratory (~3 m above the ground) with conductive tubing (1/4" internal diameter and 1 m length). The aerosols were dried as they passed through two diffusion dryers (< 10% RH) containing silica gel and then continued on to a manifold connected to the WIBS, CCN100, and CPC which sampled at flow rates of 0.3, 0.5, and 1 L min<sup>-1</sup>, respectively. Particle losses due to sedimentation, diffusion, and inertial separation along the sampling lines were calculated for each of the instruments (Kulkarni et al., 2011). The particle sampling efficiency with respect to particle size is shown in the Supplement (Fig. S1). The sampling efficiency calculated for the particle size range 0.1–3 µm is greater than 80 %. However, the sampling efficiency is reduced greatly for particle sizes above 3 µm. The experimental setup allows WIBS NEO to receive particles up to 6 µm with more than 60% efficiency. Due to calculation uncertainties, we have chosen not to correct the data for these estimated losses because we are interested in the relative changes in the size distributions with respect to time and meteorology. The WIBS NEO, like all single particle, optical spectrometers, measures what is designated an “equivalent optical diameter (EOD)” that is defined as the size of a particle scattering the equivalent intensity of light as a spherical particle with known refractive index. Given that bioaerosols, dust and other types of environmental aerosols are not spherical, and their refractive index is unknown, the geometric size can be estimate to, at best, ±20%, hence relative size is more relevant than absolute size. Therefore, the absolute size of the particles is not a factor in our current analysis.

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## 2.2 Instrumentation

The CCN-100 used in this study is a continuous-flow, thermal-gradient, diffusion chamber that measures the concentration of aerosols activated as cloud droplets as a function of supersaturation (SS). Aerosol samples are drawn into a 50 cm tall column (inner diameter 2.3 cm) whose inner walls are saturated with water. A series of heaters along the column are controlled to maintain a temperature gradient from cooler to warmer as the particles move down the column. Since water vapor from the wetted column diffuses to the particles faster than the heat, a supersaturated condition is maintained that is determined by the temperature gradient and flow rate.~~A series of heaters are controlled to maintain a temperature gradient from cooler to warmer as the particles move down the chamber. The difference in diffusion rates between heat and water vapor creates a supersaturated environment at the centerline of the cylinder.~~ Those aerosol particles that can activate as a cloud droplet at the constant SS in the chamber will begin growing as water molecules diffuse to the particle surface and at the downstream of the column, an optical particle counter measures the number size distribution of the cloud droplets within the size range of 0.75–10 µm. For more an in-depth description of

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the operating principle and calibration procedures the reader is directed to the paper by Roberts and Nenes (2005). In our study, the supersaturation (SS) was maintained at 0.3%, a SS that is in the range of what would be encountered in convective clouds similar to those that form over the island of Puerto Rico (Duan et al., 2012; Uin et al., 2016).

To measure the total concentration of environmental particles  $> 0.01 \mu\text{m}$  we used a butanol-based, condensation particle counter (CPC, model 3772 TSI) where the aerosol sample is drawn continuously through a heated saturator in which butanol vapor diffuses into the aerosol stream. An external vacuum pump was used to draw the aerosol samples at  $1 \text{ L min}^{-1}$ . This CPC employs a single particle count mode to measure the particle number concentrations up to  $10^7 \text{ L}^{-1}$  at an accuracy  $\pm 10\%$ . The detailed design and working principle of the CPC are described by Stolzenburg and McMurry (1991).

The WIBS measures the fluorescent characteristics of aerosols using ultraviolet, light-induced fluorescence (UV-LIF) (Kaye et al., 2005; Stanley et al., 2011). This instrument provides detailed information on fluorescing bioaerosols on a single particle basis. The detection principles of the WIBS are discussed elsewhere (Kaye et al., 2005) and briefly described here. Atmospheric particles are drawn into the WIBS via a laminar flow delivery system and pass through the path of a continuous-wave diode laser (635 nm), which acts as a source for particle sizing and shape detection. The total flow is approximately  $2.4 \text{ L min}^{-1}$  of which  $2.1 \text{ L min}^{-1}$  is introduced in the form of sheath flow (i.e., filtered air) and  $0.3 \text{ L min}^{-1}$  is sample flow to maintain the particle alignment with the 635 nm laser. The forward scattering of the light is detected by a quadrant photomultiplier tube (PMT) and is used to determine the asphericity factor (AF) of the particles which roughly estimates the shape of the particles (Gabey et al., 2010). Experimental evidence shows that the AF is near zero for a perfectly spherical particle, while it approaches 100 for a fiber or rod-like aerosol particle (Kaye et al., 2007; Gabey et al., 2010). The light scattered from the diode laser is used to activate, sequentially, two Xenon lamps that are filtered to illuminate the particles with UV light at 280 nm and 370 nm, respectively. The wavelengths were specifically selected to excite fluorescence in particles containing tryptophan (280 nm) and nicotinamide adenine dinucleotide (NADH, 370 nm).

There could be numerous molecules that can be excited by fluorescent light. For examples molecules such as proteins, vitamins, large polymers, molecules having conjugated double bonds, heterocyclic aromatic compounds, particularly when nitrogenous substituents are present. Tryptophan is an amino acid that has the highest (~ 90%) fluorescence in the native protein. Nicotinamide Adenine Dinucleotide Phosphate (NAPDH) is one of the major



contributors to the fluorescence signal when attached to the protein molecule and is produced widely in the metabolic cell.

The fluorescence from the 280 and 370 nm excitations is recorded by PMT detectors, one that is filtered for 310-400 nm emissions and the other for 420-650 nm. Hence, when a particle is excited at either of the incident wavelengths, there are four possible responses: 1) no fluorescence is detected, 2) when excited at 280 nm the particle fluoresces at a wavelength in the 310-400 nm waveband (FL1), 3) when excited at 280 nm the particle fluoresces at a wavelength in the 420-650 nm waveband (FL2), or 4) when excited at 370 nm the particle fluoresces at a wavelength in the 420-650 nm waveband (FL3). The fluorescence characteristics of an individual particle are determined in any of the three fluorescence channels when its fluorescence emission intensity exceeds a baseline threshold. The baseline threshold is determined using the approach by Perring et al. (2015) that incorporates the daily data sets to remove background artifacts. Particles that exhibit fluorescence lower than the baseline threshold were treated as non-fluorescent particles. A particle that fluoresces when excited by either of the xenon lamps may also produce emissions in both the 310-400 and 420-650 wavelength; hence, from the FL1, FL2 and FL3 signals there are seven possible combinations, generally accepted by the WIBS community, that have been designated fluorescence types A, B, C, AB, AC, BC, and ABC (Perring et al., 2015). Types A, B and C refer to particles that fluoresce only in FL1, FL2 and FL3. The other four types are the respective combinations of the A, B, and C.

It should be noted that A and C channels are highly sensitive to fluorescent bioaerosol particles whereas the B channel is cross sensitive to non-biological aerosols (Gabey, 2011). Using the fluorescence data for single particles from these three individual channels, the FAP can be characterized and discriminated from non-biological aerosol particles. Based on the above description, the WIBS records the optical size, particle asphericity factor (AF), fluorescent excitation-emission matrix and the total number concentration ( $N_{\text{WIBS}}$ ), which includes non-fluorescing, and total FAP on single particle mode collected within the size range from 0.5 to 30  $\mu\text{m}$ . Before deployment, the WIBS was factory calibrated for the size, sphericity and fluorescence using reference fluorescent polystyrene latex spheres which are traceable to National Institute of Standards and Technology (NIST).

Note, it is important to emphasize that although the WIBS was designed to detect fluorescence from biological particles, it is unable to unequivocally differentiate what type of bioaerosol fluoresced, e.g., if the particle was bacteria, fungus, or pollen. There are a number of studies, such as those by Hernandez et al. (2016), that have used the WIBS in laboratory studies to

281 characterize a variety of species of bacteria, fungi, and pollen. Such studies have shown that  
282 these three types of bioaerosols fall in general categories of size and fluorescence type. These  
283 categories will be discussed further on in this paper in the context of comparing the FAP  
284 characteristics in San Juan to those reported in controlled laboratory experiments.

285 The CPC measurements, taken at 1 Hz, are averaged to five minutes intervals for comparison  
286 with the WIBS and CCN measurements that are also averaged in five-minute intervals. In  
287 addition, the particle by particle (PbP) data from the WIBS are used to create size distributions  
288 and analyze fluorescence properties and interrelationships in greater detail.

### 289 2.3. Fungal spore data

290 The fungal spore data were obtained from the department of Microbiology of the Medical  
291 Sciences Campus of the University of Puerto Rico. The enumeration of outdoor spores used  
292 the 12-traverse methodology proposed by the British Aerobiology Federation (Caulton and  
293 Lacey, 1995). Airborne spores were collected using a volumetric Hirst-type sampler,  
294 specifically a Burkard (Burkard Scientific Ltd, Uxbridge, UK). This equipment was located on  
295 the rooftop of the Medical Sciences Campus of the University of Puerto Rico, 30 meters above  
296 ground level. The Burkard 24-hr trapping system worked continuously with an intake of 10  
297 ~~liters of air/min~~<sup>L min<sup>-1</sup></sup>. Fungal spores were impacted on a microscopic slide coated with a thin  
298 layer of 2% silicon grease as a trapping surface. The slide was changed daily and mounted on  
299 polyvinyl alcohol (PVA) mounting media for microscopic examination. Counting was done on  
300 each preparation along 12 traverse fields every 2 hours for a total of 12 hours on the  
301 longitudinal traverse. Spores were identified based on their morphological differences  
302 (Quintero et al. 2010). The identification was performed by means of a bright-field optical  
303 microscope NIKON Eclipse 80i microscope (Nikon Manufacturing), using a magnification of  
304 1000X.

### 305 2.4. Meteorological data

306 Hourly meteorological data e.g., temperature (°C), relative humidity (RH, %), wind speed (WS,  
307 m/s) and wind direction (Degree) were provided by the department of natural science taken  
308 from a weather station that is located around ~800 m away from the aerosol instrumentation.

### 309 2.5. Air mass back trajectories

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310 Twenty-four-hour air mass back trajectories, ending at 100 m above mean sea level, were  
311 obtained from the Hybrid Single Particle Lagrangian Integrated Trajectory Model (GDAS, 1-  
312 degree resolution, HYSPLIT) to identify the possible source of the aerosols.

313

### 314 3 Results and Discussion

#### 315 3.1 Time series of the particle number concentrations

316 Figure 2a shows the temporal pattern of particle number concentrations of CN, CCN (at 0.3%  
317 SS) and  $N_{WIBS}$  (0.5 - 30  $\mu\text{m}$ ) averaged in 10-minute intervals. The average particle number  
318 concentration measured by the CPC was  $(3\pm1)\times10^6\text{ L}^{-1}$ . This value is higher than the CN  
319 number concentrations reported previously at other more remote locations on the island, such  
320 as at the northeast coastal site of the Cabezas de San Juan nature reserve and at the Pico del  
321 Este, in El Yunque National Forest, where the CN concentrations were  $(9\pm5)\times10^5$  and  
322  $(11.6\pm3)\times10^5\text{ L}^{-1}$ , respectively, both as reported by Allan et al. (2008). The differences in CN  
323 concentrations are related to the geographical locations and average climatic conditions of the  
324 other regions discussed. The measurement site (Facundo Bueso) is an urban location influenced  
325 by emissions from vehicular traffic, vegetation, and other human activities such as heating and  
326 cooking. The Cabezas de San Juan is a remote coastal location where the atmosphere is  
327 relatively clean, influenced by marine aerosols or long-range transported aerosols. The Pico  
328 del Este is a mountainous region that has a significant influence of aerosol from the nearby  
329 vegetation and from particles transported from marine boundary layer. The CN concentrations  
330 show systematic, daily trends that reflect the emissions from motorized vehicle traffic and  
331 nearby residential heating and cooking. The CN concentrations show systematic, daily trends  
332 that reflect the motorized vehicle traffic and nearby residential emissions, the latter mostly from  
333 cooking. The mean CCN concentration of  $(1.5\pm0.5)\times10^5\text{ L}^{-1}$  is about 20 times lower than the  
334 CN, i.e. only about 5% of the measured aerosol particles would activate as cloud droplets at a  
335 SS of 0.3%. This implies that particles over the site are mostly non-hygroscopic or of low  
336 hygroscopicity.

337 The number concentrations of  $N_{WIBS}$  and FAP were  $(7.3\pm5)\times10^4$  and  $(5\pm3)\times10^3\text{ L}^{-1}$ ,  
338 respectively, which are approximately 40 and 600 times lower than the CN concentrations.  
339 Given the differences in the lower size thresholds for the CPC and WIBS with respect to the  
340 smallest detectable particle, 10 nm for the CPC and 500 nm for the WIBS, this implies that  
341 about 98% of the particles are smaller than 500 nm. The FAP concentrations showed a

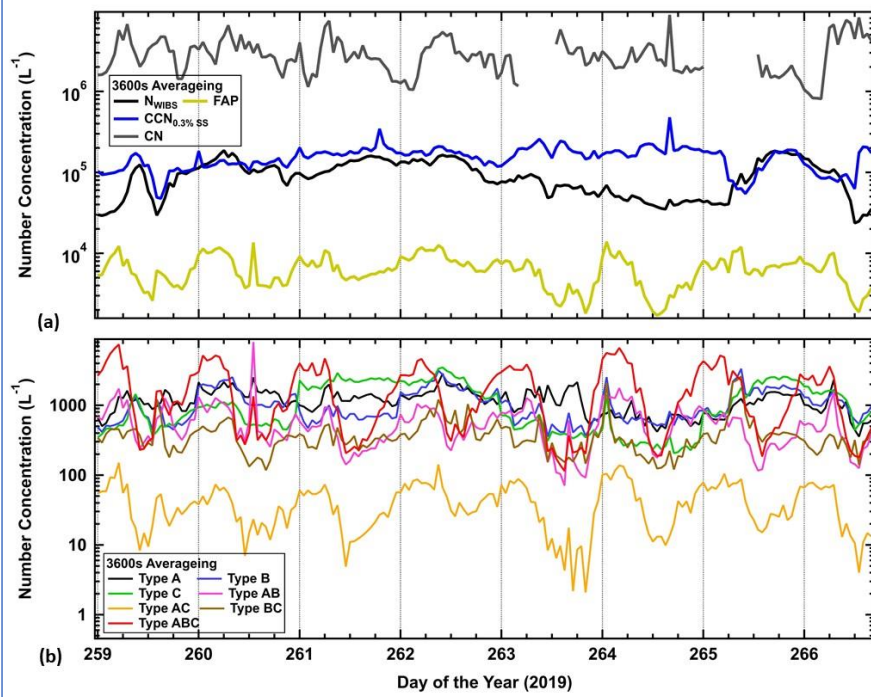
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342 systematic daily cycle where nighttime particle concentrations were relatively higher than  
343 during the daytime, this is being driven primarily by the type ABC FAPs as illustrated in Fig.  
344 2b where the type ABC concentrations are mostly much higher than other types. The types AB  
345 and AC concentrations have trends similar to the type ABC.



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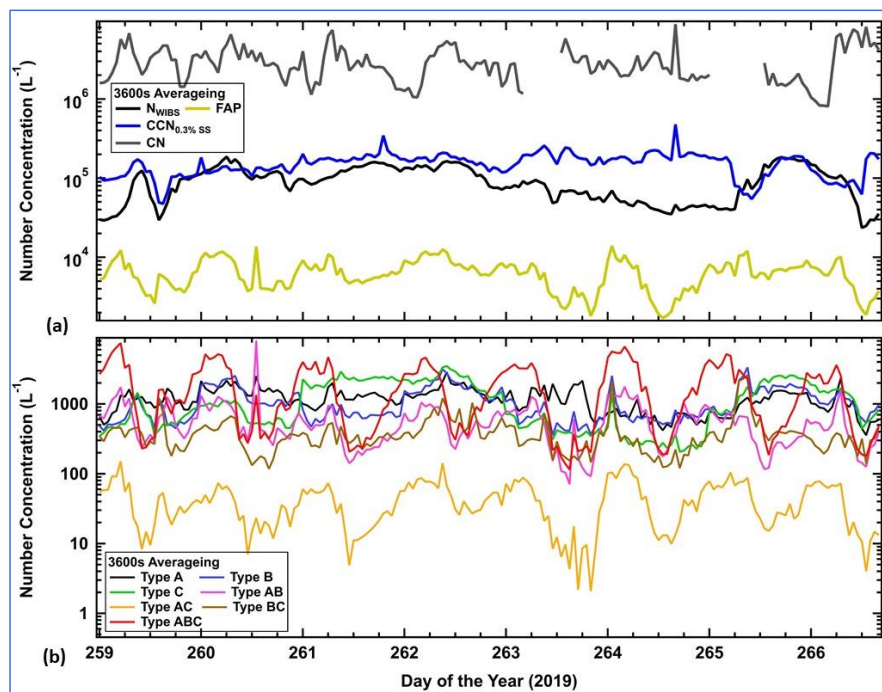
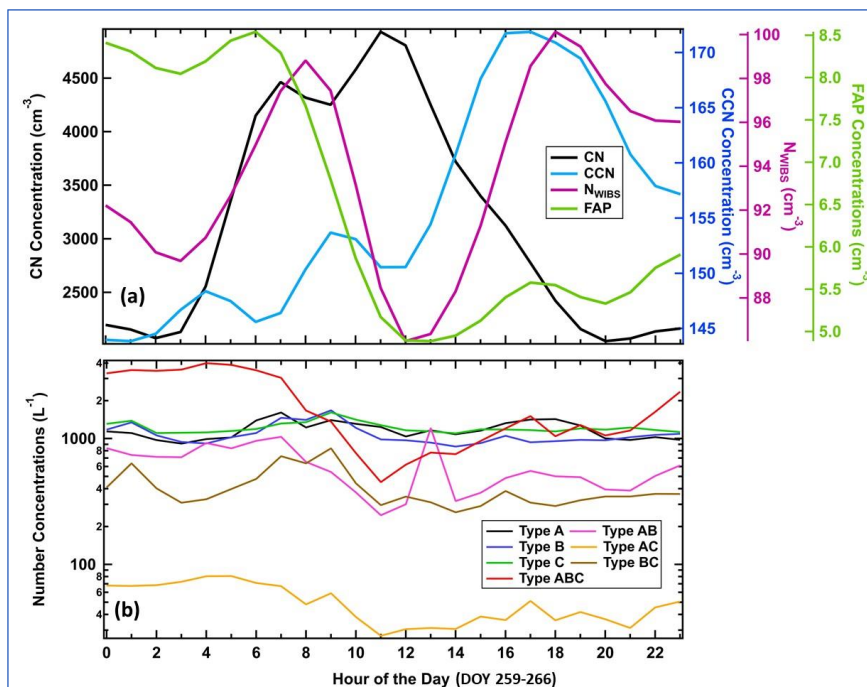


Figure 2. Time series of the number concentrations. (a) CN, CCN,  $N_{WIBS}$  and FAP. (b) FAP types A, B, C, AB, AC, BC, and ABC. The gaps in time of the CN concentrations were when the CPC was offline.

In the time series of number concentrations in Fig. 2 there are what appear to be periodicities in the CN, CCN and FAP. This periodicity is seen more clearly in the concentrations averaged by the time of the day (over the whole measurement period) as shown in Figs. 3a and 3b. Figure 3a highlights the diel trend in CN concentration (black curve) that begins increasing at 4 am, reaches an initial peak at 7 am, is followed by a second peak four hours later at 11 am then begins decreasing the remainder of the day. The CCN measurements show a trend of increasing concentrations early in the morning but does not start its rapid increase until midday peak, then it begins increasing until reaching its peak around 4 pm, five hours after the CN peak. Prior to the afternoon peak, there are smaller peaks that occur at 2 am and 8 am. The CCN concentration shows an increasing trend early in the morning but doesn't start its rapid increase until midday when it begins increasing until reaching its peak around 4 pm, five hours after the peak in the CN. The  $N_{WIBS}$  and FAP concentrations are drawn in magenta and green, respectively. The  $N_{WIBS}$  shows an increase in the morning with an initial peak at 7 am, similar to the CN;

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364 however, these concentrations then decrease until peaking again in the late afternoon, an hour  
 365 after the CCN peak. The average FAP concentrations remain elevated between midnight and 6  
 366 am, after which they decrease by about 30% and remain fairly constant the remainder of the  
 367 day.



368  
 369 **Figure 3. (a) Hourly concentrations of CN (black curve) and CCN at 0.3% supersaturation (solid blue**  
 370 **curve),  $N_{\text{WIBS}}$  (magenta) and FAP (green) concentrations. (b) Hourly concentrations of the seven types of**  
 371 **FAP.**

372  
 373 Figure 3b displays the hourly behavior over the averaged, 24-hour period of the seven types of  
 374 FAP. For all of the types except the AC and ABC, the FAPs remain relatively constant  
 375 throughout the day. The types AC and ABC are elevated in concentration between midnight  
 376 and around 6 am, both about a factor of three higher during those hours than during the  
 377 remainder of the day. Although the types AC and ABC follow the same trend, the type ABC is  
 378 about 50 times larger and dominates the FAPs during those hours. During the remainder of the  
 379 day, the types A, B and C are approximately equal in concentration.

### 380 3.2 Temporal trends of the particle size distribution

381 Figure 4 shows the size distributions of total (Fig. 4a) and FAP (Fig. 4b) number concentrations  
382 measured from September 16 to September 23, averaged in 10-minute intervals. The color  
383 scale is the log of the concentration. The white curves are the average median volume diameters  
384 for the total (Fig. 4a) and FAP (Fig. 4b). The total number size distributions show an irregular  
385 trend of increasing concentrations over all sizes, usually occurring around midday on all days  
386 except on the day of the year (DOY) 264 when the size distributions remain approximately the  
387 same throughout the day. In contrast, the FAP size distributions have a more regular, daily  
388 pattern whereby the concentrations increase over all sizes to a maximum between midnight and  
389 6 am.



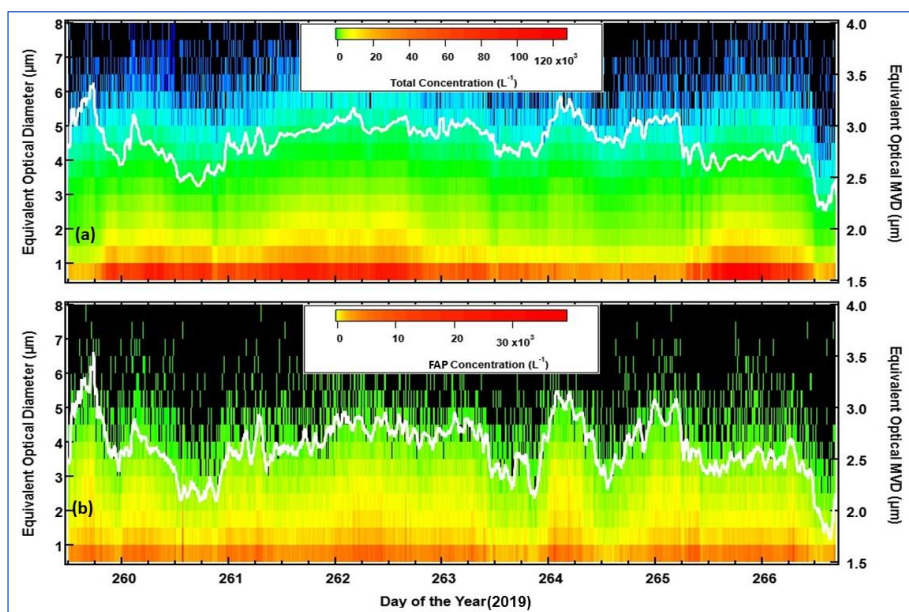
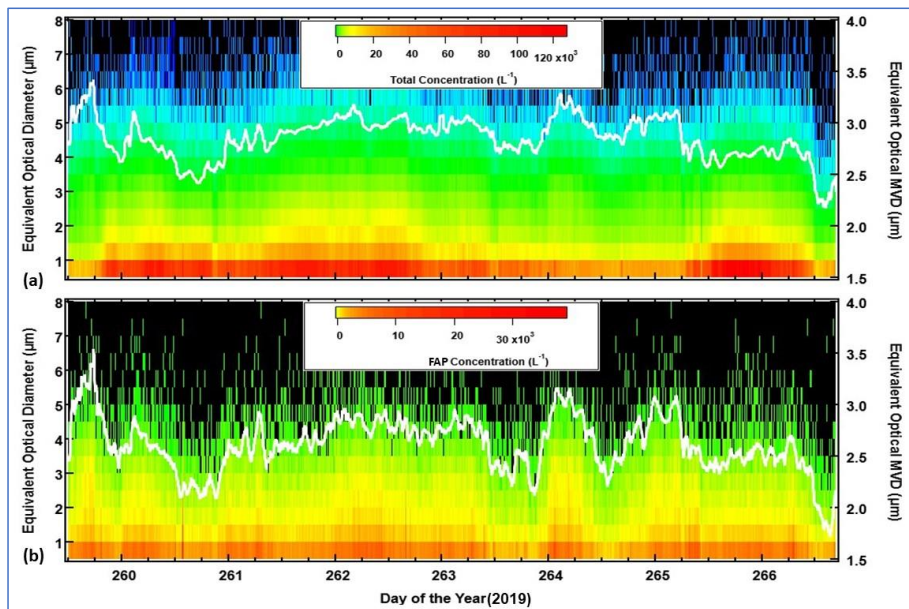


Figure 4. Time series of total (a) and (b) fluorescent particle size distributions measured by WIBS for the period September 16-23 (DOY 259-266), 2019. The white curves are the average median volume diameters (MVD).

395

396 The daily trends of the size distributions of the seven different types of fluorescent particles  
397 are shown in the Supplement (Fig. S2). where we observe that ABC and AB types were  
398 dominant at the site with a unique and systematic diel cycle. Reflecting the behavior of the total  
399 concentrations in Fig. 3b, the average EODs of ABC and AB type particles increase at night  
400 when the particle size grows to  $>4\ \mu\text{m}$  by midnight. Fluorescent type A does not show any  
401 specific temporal trend while types B and C have periods when the concentrations increase  
402 over all sizes but do not follow the trends of the ABC and AB types particles. The type BC and  
403 AC particle concentrations are much lower than the other types.

404

405 The increases in the modal diameter from daytime to nighttime, seen in the Supplement (Fig.  
406 S2), particularly for the types AB, AC and ABC, was further investigated by comparing the  
407 size distributions averaged at night and in the daytime. We have plotted the particle size  
408 distributions for these three FAP types (Fig. 5), averaging from noon to 6 pm (red shading) and  
409 from midnight to 6 am (blue shading) over the whole measurement period (DOY 259-266).  
410 These two periods represent time intervals when the number concentrations and average sizes  
411 exhibit the largest differences. All three FAP types show a shift towards larger sizes from  
412 daytime to nighttime; however, the type ABC particles have the most distinctive shifts (Fig.  
413 5c), indicative of both a general increase in concentration over all sizes but a very clear, larger  
414 increase proportionately at EODs larger than  $2\ \mu\text{m}$ .

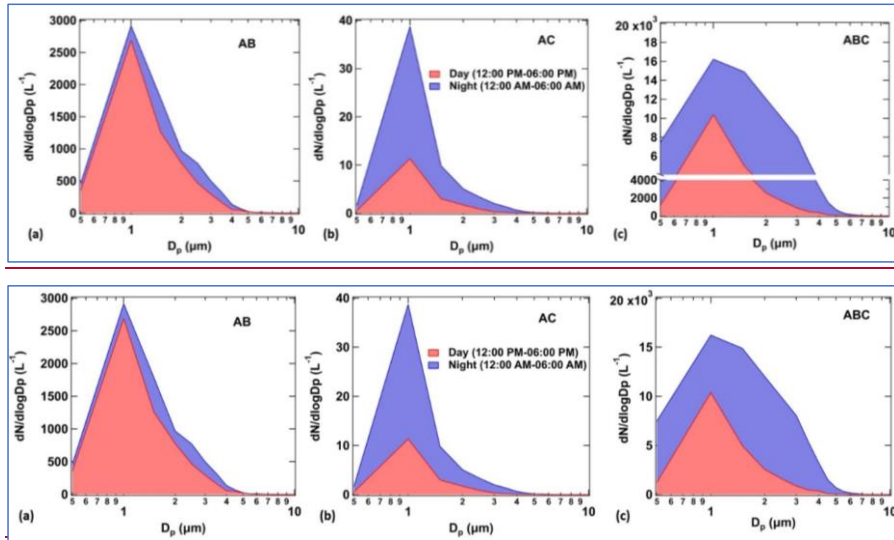


Figure 5. The size distributions from types (a) AB, (b) AC and (c) ABC were averaged from 12 pm to 6 pm (red shading) and from midnight to 6 am (blue shading) for the eight days of the project.

### 3.3 Asphericity

The asphericity derived from the quad detector of the WIBS is a relative indicator of the shape of each particle as shown in the supplement (Fig. S3) for FAP type (AB, AC, and ABC) and for all particles including the non-FAP. The color scale shows the average asphericity at each size interval over the duration of the project. Among the fluorescent types, the asphericity of ABC particles shows a mode between 2 and 4  $\mu\text{m}$  during nighttime, especially at midnight. The asphericity size distributions of all particles show a broader mode of enhanced asphericity between 2 and 4  $\mu\text{m}$  that varies somewhat but not in a noticeable diel pattern. Note that particles with asphericities  $< 20$  are generally considered quasi-spherical so that the values that are shown here indicate slight changes in shape, on average, of type ABC particles, as well as all particles, but the overall population of particles can be considered quasi-spherical.

Asphericity of non-fluorescent (non-FAP) particles was always observed higher than the FAP (Fig. S3d in the Supplement). The higher values of asphericity of non-FAP particles can be seen between size 3 and 6  $\mu\text{m}$  every day and dominate on DOY 261 and 262.

### 3.4 Air mass back trajectories

Air masses arriving at the site on DOY 259 and 264 were coming from the northeast of the island originating 24-hours earlier over the Atlantic Ocean. The air masses that arrived on DOY 260-63 and 266 were from the southeast of the island and on DOY 265 the air was from the south-southeast. Figure S4a shows that all of the air masses had been < 50 m above the surface the entire 24-hour period before arriving at the measurement site. The one exception was on DOY 259 when the air mass had stayed above 200 m from 12 to 24 hours then began descending as it approached the island. This same air mass was associated with rain formation close to the measurement site (Fig. S4b in the Supplement). The air mass on DOY 264 came across the islands to the SE of Puerto Rico (e.g., Culebra and the British Virgin Islands), possibly mixing the marine aerosols with polluted emissions before arriving at the measurement site. Likewise, the air mass trajectory on DOY 260 and 262 crossed over the Vieques and US Virgin Islands at a low altitude, likely mixing with anthropogenic emissions. The air mass arriving on DOY 261 is comparatively dry as no rainfall happened along its trajectory, but it also crossed over the Vieques island. The increment in total particle number concentrations on DOY 260-262 and DOY 266 shown in Fig 4a are possibly attributed to air mass arrived over the Islands in the Southeast of Puerto Rico. It could be the reason why we observed higher values of asphericity of non-FAP on DOY 261 and 262. The remainder of the air masses were presumably not impacted by anthropogenic emissions until arriving over the Puerto Rico landmass.

### 3.5 Meteorological data

Figure S5 shows the temperature and relative humidity (RH) (Fig. S5a in the Supplement), and the wind speed, wind direction and precipitation (Fig. S5b in the Supplement). The average wind speed, temperature, and RH for the period of measurement were  $2.8 \pm 2.4$  m/s,  $29 \pm 2$  °C, and  $77 \pm 11$  %, respectively. The DOYs 259 and 265 received significant rainfall of 1.35 and 1.84 inches, respectively. Note that these two days are also those that the back trajectory analysis indicated that rain had formed in the arriving air masses. The wind speed and direction, temperature, and RH show a systematic daily cycle where the wind speed and temperature peaked during midday ( $2.8 \pm 0.7$  m/s and  $33 \pm 1$  °C), and the RH peaked around midnight ( $91 \pm 3$  %). Wind profiles at the measurement site show that the air was flowing from 135° - 250° during the night then from 93° - 134° during the daytime. On DOY 262 and 265, as well as the afternoon of DOY 259, the winds are comparatively low. The average wind speeds at night ( $0.24 \pm 0.2$

466 m/s) compared to those during the day ( $2.8 \pm 0.6$  m/s) suggest generally calm wind conditions  
467 that are normal this time of year when not under the influence of tropical storms.

468

469 To further highlight the relationships between the meteorological conditions and FAP, we  
470 computed the hourly averages of RH, wind speed and FAP concentrations during each 24-hour  
471 period over the eight-day period and compared them in Fig. 6a. The RH and FAP  
472 concentrations are maximized during the hours from midnight to 6 am while wind speed is at  
473 a minimum during those hours, maximizing a little after midday.

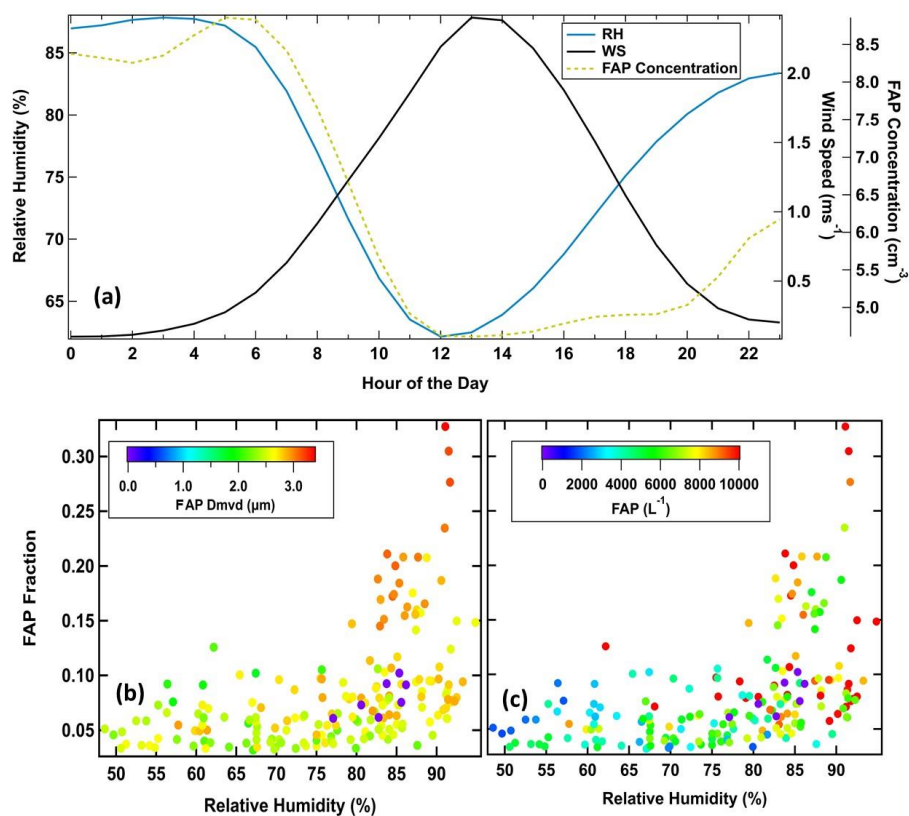
474 The relationship between RH and the FAP fraction is further underscored in Fig. 6 where there  
475 appears to be an RH threshold below about 80% that the FAP fraction remains below 0.1 but  
476 then increases rapidly to greater than 0.3 as the RH exceeds 90%, i.e. less than 10% of the  
477 particles measured in the size range of the WIBS were FAP when the  $RH < 80\%$ , but increases  
478 to more than 30% at high humidities. The increase of FAP  $D_{mvd}$  and the number concentrations  
479 depend on the hygroscopicity of the particles. Among the different FAPs measured at the site,  
480 the ABC, AB, and AC types were observed to have systematic diel patterns and were believed  
481 to be more hygroscopic than others. Therefore, the  $D_{mvd}$  and FAP number concentrations were  
482 increasing when RH reaches 80% and above. The other spread of points at  $>80\%$  RH were  
483 possibly the less hygroscopic FAP types such as the A, B, C, and BC, not showing any  
484 increasing trend in  $D_{mvd}$  and the number concentrations. The difference between Fig. 6b and  
485 6c are how the markers are color coded. In Fig. 6b, the coloring denotes the equivalent median  
486 volume diameters ( $D_{mvd}$ ) and in Fig. 6c, the coloring is the FAP concentration. Both the  $D_{mvd}$ s  
487 and concentrations increase with increasing humidity and FAP fraction.

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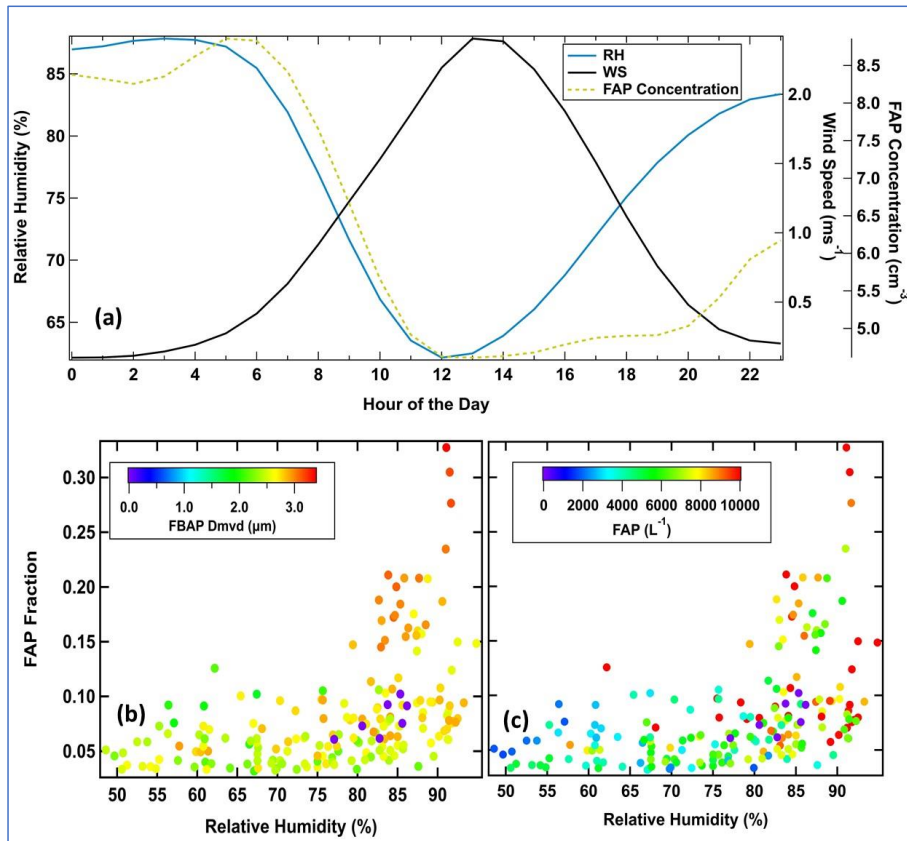


Figure 6. (a) hourly averages of relative humidity (blue curve), wind speed (black) and FAP concentration (green dotted). (b) link between fluorescent fraction and relative humidity (FAP particle volume weighted equivalent optical diameter,  $D_{mvd}$ , on the color scale). (c) fluorescent fraction and relative humidity (FAP particle concentration on the color scale). The data in all the figures averaged over DOY 259-266.

### 3.6. Fungal spore data

The time series of the fungal spores, measured on the rooftop of the medical science building at the university, are shown in Fig. 7. Similar to the FAP number concentration and fraction, the spore concentrations have a diel trend with maxima around midnight. The average concentrations were  $48 \pm 42$  L<sup>-1</sup> with a maximum  $112 \pm 44$  L<sup>-1</sup> around midnight. We calculated positive correlations of 0.7, 0.47, and 0.54 between the total spore concentration and concentrations of FAP types ABC, AB, and AC types, respectively. Previous studies for this



region reported the most common fungal genera detected were the Basidiospores and Ascospores (Quintero et al., 2010; Rivera-Mariani et al., 2011). Figure 7b illustrates the distributions of outdoor fungal spore types recorded at MSC of the University of Puerto Rico. Fungal species were identified and distributed different categories, referred to as hyphae or filamentous spores, macroconidia >10 µm, microconidia 3-10 µm, microconidia < 3 µm, and unidentified spores presented in others category. We observed that the microconidia 3-10 µm contributed the highest (81%) fraction to the total fungal species followed by microconidia (<3 µm) 3.86% and Macroconidia (>10 µm) 1.8%. We observed that the Basidiospores contributed the highest (49.4%) fraction to the total fungal spores type followed by Ascospores 19%, Diatrypaceae 8.6% and Penicillium/Aspergillus 3.86%. The mean concentrations of dominant species such as Basidiospores, Ascospores, Diatrypaceae and, Penicillium/Aspergillus were 24±20, 9.3±4, 4±3 and 2±1 L<sup>-1</sup> during the study period. The Ascospores and Penicillium/Aspergillus had more elevated concentrations during the night while Diatrypaceae concentrations were generally higher during the daylight hours.

These species were the most common airborne spores in San Juan, present throughout the year and predominated during September (the rainy month). The WIBS's ABC and AB types were likely the Basidiospores and Ascospores (Fig. 7b). Previous studies (Quintero et al., 2010; Rivera-Mariani et al., 2020) reported that the most common fungal genera detected were the Basidiospores and Ascospores in the San Juan atmosphere. Which is also confirmed in this study. The size of the Basidiospores and Ascospores (10-20 µm) is usually larger than Aspergillus, Penicillium, and Cladosporium spores. Furthermore, we observed a systematic diel pattern in the number concentrations of these fungal spores which is strongly correlated to diel pattern of FAPs detected in ABC channel of WIBS. The other genera most frequently detected were the Penicillium/Aspergillus, Cladosporium and Ganoderma, present at low concentrations reported by Quintero et al. (2010). Those species possibly corresponded to WIBS's AC types which were systematic and relatively low concentrations. These fungal spores, present between the midnight and early morning period, suggest an active release mechanism induced by the morning hours dew point and increased humidity under calm wind conditions, in concordance with the findings reported by Quintero et al. (2010).



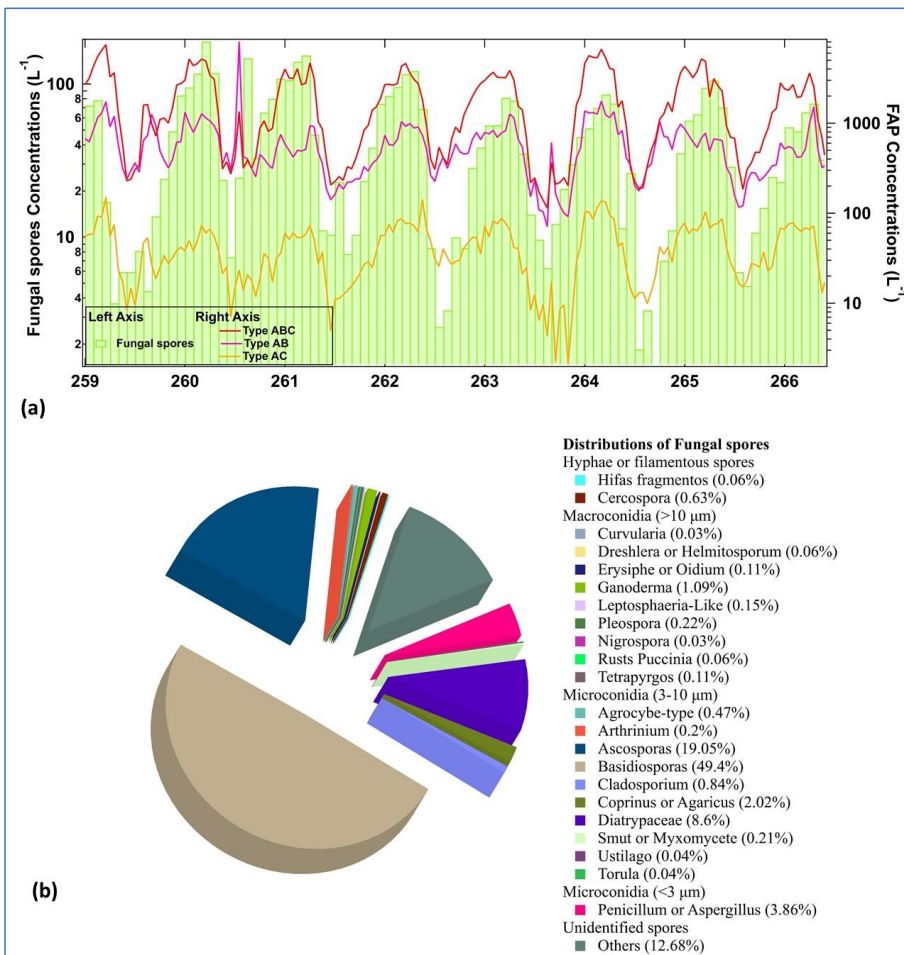


Figure 7. (a) time series particle number concentrations of fungal spores (left axis) measured at the Medical science department and the FAP types ABC, AB and AC (right axis) detected by WIBS at FB site. (b) distributions of outdoor fungal spores recorded at Medical Sciences Campus, San Juan.

#### 4. Discussion

From the results presented above, the following features stand out: 1) The CN, CCN and FAP concentrations have daily patterns during which each maximizes at a different hour of the day, 2) the periodic FAP concentration is predominantly of the type ABC that reaches a daily maximum around midnight and the asphericity of this type of FAP increases slightly during

the same time period, 3) the RH reaches a maximum each day around midnight, 4) the wind speeds are a minimum around midnight and 5) an independent analysis of bioaerosols using fluorescence microscopy to identify spore types revealed a periodicity of spore concentrations that was highly correlated with the RH and FAP type ABC concentrations.

Referring back to Fig. 3a, the CN, CCN,  $N_{WIBS}$ , and FAP concentrations all exhibit a diel periodicity but with peak values occurring at differing hours. The CN population encompasses all environmental particles larger than about 10 nm and are dominated by anthropogenic aerosols, i.e., those produced from residential cooking and local vehicular traffic. Given that the WIBS measures the concentration of particles larger than  $0.5\mu\text{m}$ , and that the total concentration has an average peak maximum of  $100\text{ cm}^{-3}$ , compared to a maximum CN concentration  $5000\text{ cm}^{-3}$ , this implies that 98% of the particles have sizes smaller than  $0.5\mu\text{m}$ .

The rest are the coarse mode aerosol (2%) is typically smaller in number concentrations, yet high in mass concentrations. A comparison of the maximum CCN and  $N_{WIBS}$  concentrations,  $170\text{ cm}^{-3}$  vs  $100\text{ cm}^{-3}$ , leads us to conclude that most of the CCN are in sizes greater than  $0.5\mu\text{m}$ . Likewise, since the maximum CCN concentrations are only about 2% of the CN values, this suggests that most of the CN have low hygroscopicity, a characteristic of fresh combustion particles.

The trends in the CN concentrations suggest that there are early morning activities that are producing emissions of anthropogenic particles. The two peaks are a result of the combination of two traffic patterns: the general city traffic as workers commute to jobs that are not on the university campus and vehicular traffic of university workers whose starting hours are later than the city workers. In Fig. 1a, the sampling site is located near the intersection of two major streets that carry both types of traffic. Unlike many large urban areas where morning and evening rush hour traffic can be distinctly seen in the CN measurements, there is a smaller density of cars during the evening commute than in the morning in San Juan.

The correspondence between the times of the two  $N_{WIBS}$  maxima and the CN and CCN peaks suggests that the  $> 0.5\mu\text{m}$  particles measured with the WIBS in the morning are a different mixture of compositions than the particles in the afternoon. The morning  $N_{WIBS}$  peak lags the first CN peak by an hour, likely as result of the primary emissions producing particles that grow into the size range of the WIBS; however, with the sunrise at around 6 am, temperatures begin increasing and the material in the more volatile particles begin to evaporate until the particle sizes decrease below the threshold of the WIBS. Some dilution will be occurring as the

boundary layer deepens with increasing temperatures, but this is a secondary effect as we do not see the CN concentrations decrease with the decrease in  $N_{WIBS}$ . Since the CCN concentrations remain low during this period, this implies that either the particles did not grow large enough to be good CCN or their composition is non-conductive for forming CCN. In the early afternoon we observe the CCN concentrations beginning to increase until reaching their late afternoon peak. The  $N_{WIBS}$  follows a very similar trend but lagged with respect to the CCN by a couple of hours. This afternoon trend in CCN has been identified in other large, polluted urban areas as the result of photochemical reactions producing hygroscopic, secondary organic aerosols (SOA) from photochemical reactions (Baumgardner et al., 2004). The  $N_{WIBS}$  is offset a couple of hours due to the time needed for the SOA particles to grow by condensation and aggregation. Finally, the FAP concentrations only begin increasing late in the evening after the CCN has maximized and their concentrations are less than 10% of the CCN. This suggests that if FAP are good CCN, they do not contribute significantly to the overall CCN population. It is important to note that contributions to the overall CCN populations depend on size, chemical composition, and number concentrations of particles.

Quintero et al. (2010) concluded that the release of the fungal spores that they measured and speciated was triggered by high RH and were found in multiple locations. Pollen spores, on the other hand, could not be linked conclusively to any meteorological factor. It is evident that almost all of the fungal spores are released in the El Yunque rain forest (Lewis et al., 2019). Two of the air sampling sites, Pico Del Este (PDE) and Cabezas de San Juan (CSJ) are very similar and very low in the fungal spores (less 5,000 spores/m<sup>3</sup>). At another sampling site in El Verde (located to the west within El Yunque National Forest), the concentrations increase to 72,000 spores/m<sup>3</sup> and are found to have a decreasing gradient of fungal spores towards the Metro Area. For the rest of Puerto Rico, the Central Mountain Range is the other source of fungal spores. Bioaerosols, especially fungal spores, are very good ice nucleating particles (INP) (Kunert et al., 2019); however, except for periods with tropical storms, cloud tops rarely grow higher than the freezing level. On the other hand, these airborne spores may have the allergenic potential and could pose a health threat to sensitized population (references therein Quintero et al., 2010). Information about the presence and abundance of these spores will assist in the diagnosis of respiratory and allergic illness in Puerto Rico. Hence, given these previous results in comparison with the correlations that we have observed in the current study (Figs. 6 and 7), the type ABC fluorescing particles are most clearly linked to Basidiospores and Ascospores, the two species that made up the largest fraction of fungi measured with the

606 fluorescence microscopy. The hourly averages in Fig. 3b also showed that the type ABC only  
607 was predominant during the same period as the Basidiospores and Ascospores but then the  
608 other fluorescence types A, B and C were equally present during the remainder of the day. This  
609 is mirrored by the spore types shown in Fig. 7 that change in relative mixture during the day.  
610 Hence, by observing the relative changes in the seven FAP types, we get a qualitative measure  
611 of the changing population of bioaerosols.

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612 The WIBS characterizes the fluorescent aerosol particles using two-wavelength excitations and  
613 two-wavelength emissions. The sensitivity of types A and B to the intensity of the emissions  
614 in the 310-400 and 420-650 nm wavelength bands, when excited at 280 nm, in comparison to  
615 the intensity of emissions at 420-650 nm when excited at 370 nm has been exploited in the  
616 study by Ziemba et al. (2016) to identify differences in bioaerosol types as they relate to  
617 differences in FAP sources. In their study they were able to show a clear grouping of FAPs  
618 linked to source regions by plotting the ratio of type A to type B (emission sensitive) versus  
619 the ratios of type C to type B (excitation sensitive). We have followed a similar scheme;  
620 however, whereas the Ziemba et al (2016) study used a WIBS on an airborne platform flying  
621 over various land usage types, our site was fixed so we compute these ratios as a function of  
622 time rather than location. Figure 8 illustrates the periodicity of the type C to B ratio, increasing  
623 during those time periods that the type ABC concentrations were also increasing. Just as the  
624 sizes of FAP were seen to increase during these periods (Fig. 5), indicative of a change in FAP  
625 type, the shift in the type C/type B reflects the differences in the fungal spore types.

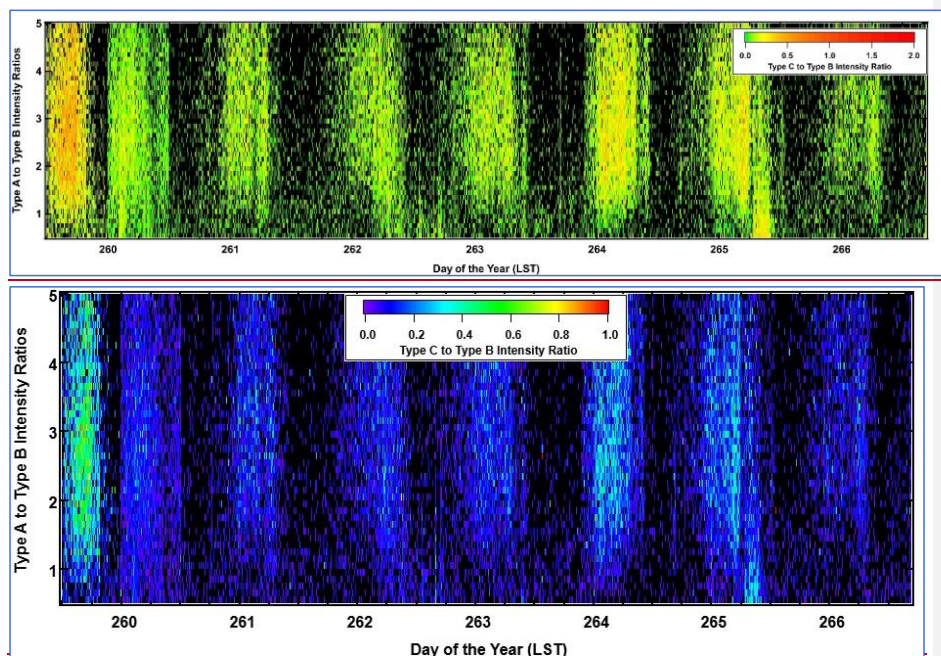


Figure 8: Temporal distribution of emissions wavelength dependence (Type A to Type B) vs excitation wavelength dependence (Type C to Type B).

Hence, the changes in size distribution, asphericity, type C/B ratios, and speciated spore concentrations, all occurring during the same time of day, provide independent verification that a different type of PBAP is being produced during the periods of high RH than during the other periods of the day.

The strong correlation between spore release and RH that has been highlighted in this study has been previously reported, e.g., (Oliveira et al., 2005; Chi and Li, 2007; Gabey et al., 2013; Calvo et al., 2018, Toprak and Schnaiter, 2013; Healy et al., 2014). None of those studies, however, were from tropical regions nor they include the asphericity and FAP type rationing to quantify their results.

Some laboratory studies have been conducted to measure the fluorescence characteristics of a small variety of bacteria, fungi, and pollen, for example Hernandez et al. (2016) determined that bacteria, fungi and pollen could be generally grouped according to the size and FAP type. In their study, very few fungal spores were of the FAP type ABC such as found in the current

644 study. Instead, the majority of their fungi were types A and AB while the majority of type ABC  
645 spores were pollen, not fungi. On the other hand, the Hernandez et al. (2016) study did not test  
646 any of the major fungi species that were measured in the natural environment of Puerto Rico,  
647 i.e. Basidiospores and Ascospores.

648

## 649 **5. Summary and Conclusions**

650 A pilot study was conducted to evaluate the fluorescence and cloud condensation nuclei (CCN)  
651 properties of urban aerosols in San Juan, Puerto Rico, the first time such measurements have  
652 been made in this tropical city. Previous CCN measurements have been made on this island at  
653 coastal and rainforest sites, but no research has been pursued to see if bioaerosols are directly  
654 linked to CCN. There have been a number of laboratory studies conducted by other researchers  
655 who evaluated the CCN activity of various bacteria, fungi and pollen. Although some types of  
656 bioaerosols were found to be potential CCN, many others were not. Hence, the importance of  
657 bioaerosols as cloud forming particles remains an open question. The very large concentrations  
658 of fungal spores produced by flora in Puerto Rico, as reported by Quintero et al. (2010), along  
659 with the results from our pilot study, provided the initial motivation for the study reported here  
660 to assess if bioaerosols might contribute to the frequent cloud formation over the island.

661 The measurements were made from the Facundo Bueso building within the University of  
662 Puerto Rico, Rio Piedras Campus, from September 16-23, 2019, an urban location that  
663 experiences emissions from local residential cooking, vehicular traffic and a wide variety of  
664 flora. The site is located close to the intersection of two major streets carrying local business  
665 as well as university traffic.

666 In the pilot experiment, the CCN concentrations were measured with a commercial CCN  
667 spectrometer set at 0.3% supersaturation and the fluorescent properties were also measured  
668 with a commercial instrument, the Wideband Integrated Bioaerosol Spectrometer (WIBS). It  
669 is important to note that bioaerosols are not the only type of aerosol particle that will  
670 autofluoresce when excited at the wavelengths used in the WIBS, although care was taken to  
671 minimize interference from non-biological particles. Therefore, what is reported in the current  
672 study are the properties of fluorescing aerosol particles (FAP) without specifically labeling  
673 them as biological. In addition to measurements of CCN and FAP, the total concentration of  
674 condensation nuclei (CN) was documented with a condensation particle counter.

675 The mean number concentration measured by the CCN counter at 0.3% SS was  $(1.5 \pm 0.5)$   
676  $\times 10^5 \text{ L}^{-1}$ , which was about a factor 21 lower than the average CN concentration  $(3 \pm 1) \times 10^6 \text{ L}^{-1}$ . The mean FAP concentration was  $(5 \pm 3) \times 10^3 \text{ L}^{-1}$ , which was a small fraction (~7%) of total  
677 aerosol particle number concentration,  $N_{\text{WIBS}}$ , measured by the WIBS, whose lower size  
678 threshold is 0.5  $\mu\text{m}$ .  
679

680 The CN, CCN,  $N_{\text{WIBS}}$ , and FAP concentrations all have diel trends but their maxima occur at  
681 varying hours of the day. The CN peaks at 6 am and 11 am due to business traffic and university  
682 traffic that have different rush hours. The CCN reaches its maximum value at 4 pm as  
683 photochemical processes produce secondary organic aerosols (SOA) that are hygroscopic in  
684 composition. The  $N_{\text{WIBS}}$  is bimodal with the morning peak at 8 am, reflecting rush hour  
685 emissions whose particles grow into the size range of the WIBS and then the second maximum  
686 at 6 pm as the SOA grow to measurable sizes. The diel trends in the FAP concentrations are  
687 not correlated with the CN, CCN or  $N_{\text{WIBS}}$  as they remain fairly constant throughout the  
688 daylight hours but then rapidly increase to their maximum value that extends from midnight  
689 until 6 am.

690 The FAP are classified according to the wavelength at which they were excited and wavelength  
691 at which they emitted fluorescence. These types have been categorized as A, B, C, AB, AC,  
692 BC, and ABC. In the current study the types A, B, C and ABC all had average concentrations  
693 during the daylight hours of about  $1000 \text{ L}^{-1}$  while the other three types were much lower in  
694 concentration; however, only the type ABC showed the rapid increase in concentration, to  
695 almost  $5000 \text{ L}^{-1}$ , between midnight and 6 am.

696 Independent measurements using fluorescent microscopy of spores captured on substrates were  
697 made during the same time period. Although more than 20 species of spores were identified  
698 with this technique, the fungi Basidiospores and Ascospores ~~Basidiomycetes and Ascomycetes~~  
699 were not only the most predominant, but they were also the spores that followed an almost  
700 identical diel trend as the type ABC FAP, i.e. remaining nearly constant in concentration during  
701 the daylight hours then increasing in the evening to their maxima between midnight and 6 am  
702 with larger shifts of EOD with increase of RH.

703 The other environmental parameters that also correlated significantly with the temporal trends  
704 in fungal spores and FAP were the relative humidity (RH) and the wind speed. As the RH  
705 began to increase in the late afternoon, the spore counts and FAP concentrations began  
706 increasing as well. A comparison of RH with FAP concentrations indicates that the FAP



707 concentrations begin increasing above an RH threshold of about 80%. Spores are released by  
708 a number of species of fungi when the RH increases, as has been well documented in other  
709 studies (Quintero et al., 2010). Hence, the relationship between RH, Basidiomycetes and  
710 Ascomycetes and the type ABC FAP has been clearly established.

711 Three additional properties of the FAP were extracted from the WIBS measurements that  
712 provided indirect but complementary information that showed how the type ABC particles  
713 were related to the Basidiomycetes and Ascomycetes: 1) the size distribution, 2) the asphericity  
714 and 3) the excitation and emission sensitivity parameters. The type ABC particles during the  
715 high RH periods had much higher concentrations of particles larger than 2  $\mu\text{m}$  when compared  
716 to the size distributions of these particles in the daylight hours. Secondly, the asphericity  
717 increased during the high type ABC concentration period. Thirdly, excitation sensitivity  
718 parameters increased during this same period. While not quantitative, these three parameters  
719 confirmed that the particles whose concentrations were increasing had different properties than  
720 during other periods of the day.

721 The trend in the CCN concentration was not directly correlated with the FAP, so we cannot  
722 conclude that bioaerosols are a potential source of cloud forming particles. In addition, the FAP  
723 concentrations were less than 10% of CCN concentrations, so even if some FAP are potential  
724 CCN, the clouds that develop over the island are more likely formed from marine aerosols  
725 rather than locally produced fungal spores.

726 The results from this pilot study have provided strong motivation for longer term measurements  
727 that will expand the database of aerosol particle properties in a tropical, urban area. The detailed  
728 information on fungal spores in this region, in comparison with the multi-parameter data  
729 available from the WIBS, will improve our ability to interpret these measurements of FAP and  
730 apply this knowledge to data sets acquired in other parts of the world.

#### 731 **Data availability**

732 Data used to support the findings in this study have been uploaded and are publicly available  
733 via Mendeley at <https://data.mendeley.com/datasets/t26dctfk7t/1> (Sarangi et al., 2021).

#### 734 **Author Contribution**

735 BS designed the study in consultation with OLMB and performed the measurements. BBR  
736 performed the measurements of fungal spores and pollen concentrations. DB and BS performed



the analysis, interpreted the results and wrote the paper with contributions from OLMB and BBR.

#### Acknowledgements

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