



Why Does Rain Increase the Concentrations of Environmental Bioaerosols during Monsoon?

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ABSTRACT

Rain has been known to remove aerosol particles from the atmosphere; however, it was recently discovered that rain events during monsoon increase the concentrations of culturable bioaerosols in ground-level air environments. To explain this phenomenon, several hypotheses were tested via bioaerosol measurement experiments in this study. The experimental measurements with limited variations of environmental conditions revealed that the phenomenon of the effect of rain on bioaerosols might be caused by the transportation of bioaerosols by falling raindrops from higher altitude to the ground, where the bioaerosols accumulate, as well as by the decrease in UV irradiation intensity during rain events. We studied the effects of several environmental parameters on bioaerosol concentrations.

Keywords: Rain; Bioaerosol; Monsoon; Concentration; PM₁₀.

INTRODUCTION

Rain has been known to remove aerosol particles in ambient air environments (Seinfeld and Pandis, 2006). PM₁₀ (particulate matter whose diameter is less than 10 μm) concentrations and other airborne particle concentrations have decreased after rain events due to the washout effect of raindrops on airborne particles (Kulshrestha *et al.*, 2009). We can normally observe clean ambient air environments and a clearer sky after rain events. However, a recent study reported an interesting and unexpected experimental result (Heo *et al.*, 2014). As expected, rain decreased the PM₁₀ concentration of the ambient environments; however, rain events increased the concentrations of airborne microorganisms in ambient air environments. Airborne microorganisms, termed bioaerosols, are a type of aerosol particle (Hinds, 1999; Lee, 2011), but they did not follow the behaviors of other non-biological aerosol particles during rain events. The study showed that the concentrations of culturable fungal and bacterial bioaerosols during rain events were seven times and three times higher than at times without rain, respectively (Heo *et al.*, 2014). In the same period, PM₁₀ concentrations strongly decreased during rain events. To explain this phenomenon, several hypotheses were proposed: 1) raindrops carried the microorganisms from

higher altitude environments to ground-level environments; 2) the rainclouds decreased UV irradiation, which prevented the inactivation of culturable bioaerosols; 3) the splashing effects of raindrops propelled microorganisms on the ground into the surrounding air environment; and 4) the increased relative humidity during rain enhanced the sampling efficiency of the biosampler for culturable bioaerosols and might have generated adequate environments for the growth of microorganisms (Pasanen *et al.*, 1991; Frankel *et al.*, 2012; Heo *et al.*, 2014). In the current study, we tried to test and evaluate each hypothesis by measuring bioaerosols in various environmental conditions. The aim of this study was to evaluate the effect of several environmental parameters on bioaerosol concentrations.

EXPERIMENTAL METHODS

To investigate the cause of the increase in bioaerosol concentrations during rain events, first, we measured bioaerosols at various altitudes and searched for related studies to analyze the carrier effects of raindrops for bioaerosols. If there are significant amounts of bioaerosols in higher altitude environments, that would support the possibility that raindrops carries microorganisms from high altitude environments to ground-level air environments.

Second, to evaluate the effect of UV irradiation on bioaerosols, we measured bioaerosol concentrations at various UV irradiation intensity levels. UV irradiation is recognized as an effective inactivation method for culturable bioaerosols (Riley *et al.*, 1976; Hwang *et al.*, 2010). In addition, rain clouds are known to reduce the intensity of UV irradiation

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(Frederick and Snell, 1990; Calbó and González, 2005). Therefore, to test the hypothesis that the decrease of UV intensity on rainy days can explain the increase in bioaerosol concentrations, measurements of bioaerosol concentrations were conducted under strong UV irradiation and weak UV irradiation conditions in ambient air environments. We excluded experiments with inadequate conditions under which other environmental parameters, such as temperature and relative humidity, varied significantly. In particular, ambient air environments were tested during day-time and night-time because ambient UV irradiation becomes zero by sunset.

Third, to investigate the potential splashing effects of raindrops, we measured bioaerosol concentrations in the vicinity of water fountains where water drops fell onto wet surfaces. The falling of significant amounts of water drops in the fountains produced vibrations on the wet surfaces, and small drops were aerosolized near the fountain. This situation was thought to be similar to the splashing conditions created by raindrops on the ground. We measured the variation in bioaerosol concentrations near a water fountain before, during, and after splashing.

Fourth, we investigated bioaerosol measurements collected during conditions where the relative humidity varied greatly within several hours with small variations of other environmental factors. We observed the effects of relative humidity on bioaerosol concentrations by analyzing these data.

We used a sampler (Bio-culture sampler, Buck bio-culture, Model B30120, A.P. Buck Inc., Orlando, Florida, US) to sample bioaerosol particles. We sampled bioaerosols for 2–5 min, taking into account the estimated bioaerosol concentrations. The sampling principle of the device is impaction, and the flow rate of the device was 100 liters per minute. The air, including aerosol particles, was accelerated by passing through the 400 nozzles of the sampler, and the airborne fungal and bacterial particles were separated from the air flow. The separated bioaerosol particles were deposited onto an agar plate inside of the sampler and incubated. To cultivate fungal bioaerosols, we used a malt extract agar (MEA: maltose 12.75%, dextrin 2.75%, glycerol 2.35% peptone 0.75% and agar 15%) as the growth medium on the agar plate. The sampled fungal bioaerosol particles were incubated at 25°C for 48 hours. To cultivate bacterial bioaerosols, a nutrient agar (NA: beef extract 3%, peptone 5%, and agar 15%) was used in the experiments. The sampled bacterial bioaerosol particles were incubated at 37°C for 24 hours. The colonies on the agar plates were counted after incubation, and the concentrations of culturable fungal and bacterial bioaerosols in the surrounding environments were calculated in units of CFU m⁻³. In our experiments, we could classify

fungal and bacterial colonies in different types of agar plates; therefore, we did not use any antibiotics.

Bioaerosols were sampled at around 1 m above the ground surface with at least three experimental replications (Heo et al., 2014).

In this study, we used an UV light meter (UV light meter, Model YK-35UV, Lutron Electronic Enterprise Co., LTD., Taipei, Taiwan) to measure the intensity of ambient ultraviolet irradiation in the experiments.

RESULTS AND DISCUSSION

Effect of Accumulation of Bioaerosols Due to Rain (Bioaerosol Concentrations with Varying Altitude)

Wet depositions, including rain, carry airborne particles from higher in the atmosphere to ground-level air environments (Seinfeld and Pandis, 2006). If significant amounts of bioaerosol particles exist in the high-altitude atmosphere, the accumulation of these particles in ground-level air environments due to transport by falling rain may be one reason for the recently observed increase (Heo et al., 2014) in the concentration of culturable bioaerosols. Fig. 1 and Table 1 present the concentrations of culturable fungal and bacterial bioaerosols across a range of altitudes at the scale of dozens of meters. The measurement campaigns were conducted on the campus of Konkuk University on the west side of Seoul, Republic of Korea, which is a highly crowded area (tens of thousands of pedestrians every day). For fungal bioaerosol particles, the average concentrations were 1900, 1510, and 1390 CFU m⁻³ at 0, 16, and 34 m altitudes, respectively. The average concentration of fungal bioaerosols decreased with increasing altitude. However, the t-test p-value for the concentrations at 0 m and 16 m was 0.051 (> 0.05), and the t-test p-value for the concentrations at 16 m and 34 m was 0.10 (> 0.05). Therefore, the changes in the concentration of fungal bioaerosols with varying altitude were not statistically significant. The average concentrations of bacterial bioaerosol particles exhibited a similar trend to that of the fungal bioaerosol particles, also decreasing with increasing altitude. However, the t-test p-value for the concentrations at 0 m and 16 m was 0.04 (< 0.05). The t-test p-value for the bacterial bioaerosol concentrations at 16 m and 34 m was 0.39 (> 0.05). Therefore, it is difficult to conclude that such changes in the concentration of bacterial bioaerosols across altitudes at the scale of dozens of meters have any statistical significance. From these measurements, we discovered that similar amounts of fungal and bacterial bioaerosols existed in environments at various altitudes at this height scale. In addition to our measurements, related results were recently reported in 2013 and 2014 based on a genetic analysis of airborne microorganisms. In those studies,

Table 1. Concentrations of fungal and bacterial bioaerosols at varying altitudes.

	1F (0 m)	6F (16 m)	12F (34 m)
Fungal bioaerosols	1900 ± 205 CFU m ⁻³ (25°C, 46%)	1510 ± 68 CFU m ⁻³ (22°C, 54%)	1390 ± 53 CFU m ⁻³ (21°C, 53%)
Bacterial bioaerosols	208 ± 63 CFU m ⁻³ (25°C, 46%)	78 ± 8 CFU m ⁻³ (22°C, 54%)	67 ± 5 CFU m ⁻³ (21°C, 53%)

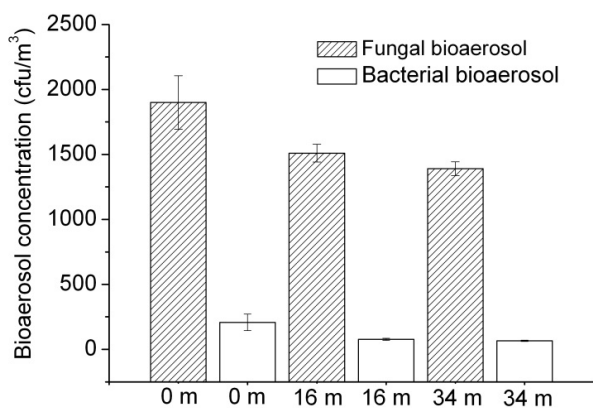


Fig. 1. Concentrations of fungal and bacterial bioaerosols at varying altitudes.

bioaerosol concentrations higher than 10^5 microbes m^{-3} were measured in air environments at altitudes of 800, 1000, and 3000 m (Maki *et al.*, 2013, Maki *et al.*, 2014). Table 1 and the reference results (Maki *et al.*, 2013, Maki *et al.*, 2014) confirm that significant amounts of bioaerosols exist in air environments at altitudes up to at least 3000 m. Therefore, a downward transportation effect on high-altitude bioaerosols due to falling rain could be one reason for the increased concentration of bioaerosols during rain events. To clarify this hypothesis, it is necessary to measure bioaerosol concentrations at varying altitudes before and after rain events to verify whether raindrops capture and remove bioaerosols from high-altitude air environments, which presents a future research topic.

UV Effect Due to Rain (Bioaerosol Concentration with Varying UV Irradiation)

It has been recognized that UV irradiation can inactivate culturable bioaerosols (Riley *et al.*, 1976; Peccia *et al.*, 2001; Lin and Li, 2002; Kujundzic *et al.*, 2006; Hwang *et al.*, 2010) and that rainclouds can decrease UV irradiation intensity (Frederick and Snell, 1990; Calbó and González, 2005). Therefore, to test the hypothesis that the decrease of UV intensity on rainy days can explain the increase in bioaerosol concentrations, we attempted to quantify ambient UV irradiation intensities and bioaerosol concentrations in Seoul to observe the relationship between these two parameters. Fig. 2 and Table 2 show the concentrations of fungal and bacterial bioaerosols associated with various levels of UV irradiation intensity. Here, to maximize the range of ambient UV irradiation intensity levels, we chose day-time and night-time environments for the measurement experiments. When the UV irradiation intensity varies, other environmental parameters such as temperature and relative humidity tend to vary simultaneously. In particular, the Korean peninsula where the measurement experiments were conducted exhibits significant variations in environmental temperature and relative humidity between day-time and night-time environments. A temperature difference of more than $10^{\circ}C$ and a relative humidity difference of more than 30% between night and day are common conditions in spring and autumn in Seoul, Korea. However, during the summer

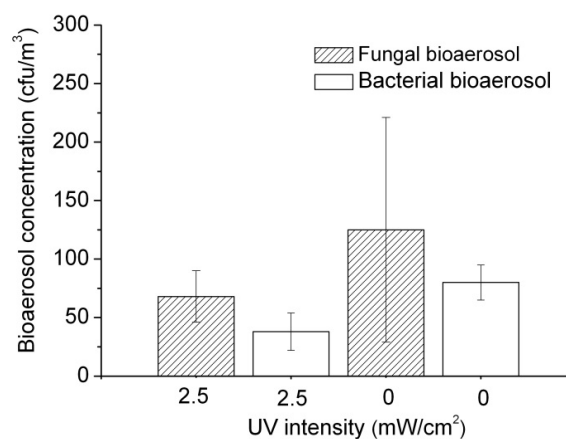


Fig. 2. Concentrations of fungal and bacterial bioaerosols with varying UV irradiation intensities.

season, the differences in temperature and relative humidity between day and night become relatively insignificant; therefore, we chose data collected during one summer for this UV effect study. Also, as the monsoon occurs during summer (Heo *et al.*, 2014), we thought it reasonable to use UV experimental data collected in summer to study why the bioaerosol concentration increases during rain events in monsoon.

Fig. 2 and Table 2 indicate the effect of UV irradiation intensity on ambient bioaerosol concentrations. As shown in Table 2, the average ambient concentrations of culturable fungal and bacterial bioaerosol particles under the condition of an UV irradiation intensity of 0 mW cm^{-2} are approximately two times larger than those under an UV irradiation intensity of 2.5 mW cm^{-2} . The t-test p-value for bacterial bioaerosols between these two UV intensity conditions is $0.03 (< 0.05)$, indicating that the concentration of culturable bacterial bioaerosols decreased with statistical significance as the ambient UV irradiation intensity increased from 0 to 2.5 mW cm^{-2} . However, the t-test p-value for fungal bioaerosols is $0.2 (> 0.05)$, which means that the decrease in the concentration of culturable fungal bioaerosol particles due to increased UV irradiation intensity was not statistically significant. From these results, we can conclude that culturable bacterial bioaerosol particles are affected by ambient variation in UV irradiation intensity. However for fungal bioaerosol particles, the ambient UV effect is not statistically significant. Therefore, we can conclude that the decrease in UV irradiation intensities may be one reason for the increase in bacterial bioaerosol concentrations during periods of rain.

Splashing and Vibration Effect Due to Rain (Bioaerosol Concentration with Falling Water)

As a simulated condition approximating the splashing of raindrops, we chose the environment conditions in the vicinity of a water fountain. The water fountain operated only during a special water show-time and did not operate at other times. At the water show-time, the water fountain was operated for one hour, during which time the fountain used more than 100 liters of water per second for the water

Table 2. Concentrations of fungal and bacterial bioaerosols with varying UV irradiation intensities.

	Daytime (UV = 2.5 mW cm ⁻²)	Night-time (UV = 0 mW cm ⁻²)
Fungal bioaerosols	68 ± 22 CFU m ⁻³ (31°C, 57%)	125 ± 96 CFU m ⁻³ (28°C, 63%)
Bacterial bioaerosols	38 ± 16 CFU m ⁻³ (31°C, 57%)	80 ± 15 CFU m ⁻³ (28°C, 63%)

Table 3. Concentrations of fungal and bacterial bioaerosols before, during, and after a water show (operation) of a water fountain facility.

	Before operation (19°C, 54%)	During operation (19°C, 52%)	After operation (19°C, 50%)
Fungal bioaerosols	83 ± 25 CFU m ⁻³	82 ± 18 CFU m ⁻³	182 ± 56 CFU m ⁻³
Bacterial bioaerosols	25 ± 10 CFU m ⁻³	23 ± 6 CFU m ⁻³	52 ± 8 CFU m ⁻³

Table 4. Concentrations of fungal and bacterial bioaerosols with varying relative humidity (RH).

Relative humidity (%)	45% (low RH) (UV = 0.8 mW cm ⁻² , 34°C)	75% (high RH) (UV = 0.0 mW cm ⁻² , 26°C)
Fungal bioaerosols	103 ± 10 CFU m ⁻³	137 ± 23 CFU m ⁻³
Bacterial bioaerosols	35 ± 26 CFU m ⁻³	97 ± 51 CFU m ⁻³

show. The size of the water fountain was approximately 200 m². We measured the bioaerosol concentrations in the vicinity of the water fountain before, during, and after the water show. We could observe that the water drops falling from the fountain produced vibrations on the wet surfaces. Table 3 shows the concentrations of culturable fungal and bacterial bioaerosol particles in the vicinity of the water fountain before, during, and after the water show. Although the average concentrations of bioaerosols were similar before and during the water show, the average concentrations increased after the water show, as shown in Table 3. However, the t-test p-values for the bioaerosol concentrations before and after the water show were 0.09 and 0.051 for fungal and bacterial bioaerosols, respectively. Therefore, the increase in culturable bioaerosol concentrations was judged to be statistically insignificant. The variations in bioaerosol concentrations indicated that the water show, which is quite a large scale event for the air environments near the water fountain, could not affect bioaerosol concentrations in a statistically significant way. Therefore, we may conclude that the water splashing effects of raindrops may not be a significant factor affecting bioaerosol concentrations. However, additional experiments are needed with various surrounding conditions.

Relative Humidity Effect Due to Rain (Bioaerosol Concentration with Varying Humidity)

Humid environments may enhance the growth of airborne microorganisms and increase the sampling efficiency of the biosampler. To test this hypothesis, the environmental condition of a large variation in relative humidity was necessary. However, several environmental parameters, such as temperature and UV irradiation intensity, tend to vary greatly with relative humidity, which makes the study of the sole effects of relative humidity complicated. Therefore, in this analysis, we sought experimental measurement conditions under which the relative humidity varied by more than 30% over a short time interval (~several hours) while the variations in temperature and UV irradiation intensity were

less than 10°C and less than or close to 1.0 mW cm⁻², respectively. Based on our measurements of ambient bioaerosols, the above guidelines for environmental conditions are reasonably adequate to judge the effects of relative humidity on bioaerosols.

Table 4 presents the concentrations of culturable fungal and bacterial bioaerosol particles measured at varying relative humidities. The average concentration of culturable fungal bioaerosol particles increased from 103 to 137 CFU m⁻³ with the increase of relative humidity from 45% to 75%. However, the t-test p-value for low RH and high RH conditions for fungal bioaerosols was 0.09 (> 0.05). Therefore, the increase of fungal bioaerosol concentrations with a relative humidity increase of 30% was not statistically significant. The average concentration of bacterial bioaerosols also increased, from 35 to 97 CFU m⁻³, with the increase of relative humidity from 45% to 75%. However, the t-test p-value of low RH and high RH conditions for bacterial bioaerosols was 0.12 (> 0.05). Therefore, the increase of bacterial bioaerosol concentrations with the 30% increase in relative humidity was also not statistically significant. Therefore, we may conclude that the increase in relative humidity can increase the average values of bioaerosol concentrations, thus this effect can help to explain the increase in bioaerosol concentrations on rainy days during a monsoon. However, the results are not statistically significant.

CONCLUSION

It was recently discovered that the concentrations of culturable bioaerosols increased on rainy days during monsoon in Seoul, Korea (Heo *et al.*, 2014). In this study, experimental measurements showed that the significant reason for this phenomenon is a combination of 1) the transportation of bioaerosols from higher altitudes to ground-level environments by raindrops and 2) the decrease of the UV irradiation intensity during rain events. However, the above conclusion is based on measurements collected within limited experimental conditions with little variation in

environmental parameters. Another hypothesis to explain the observed phenomenon is that the washout effect of rain may be ineffective for 0.1 to 2 micrometer particles such as fungal and bacterial bioaerosols due to collision difficulty between water droplets and particles of that size. More extensive and longer term measurements are necessary for a clearer and more detailed elucidation of this rain phenomenon on bioaerosols.

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