

Interactive comment on “Influence of Rain on the Abundance and Size Distribution of Bioaerosols” by Chathurika M. Rathnayake et al.

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GENERAL REMARKS

The overall objective of this work is to assess if rainfall influences the size distribution of biological aerosols and to identify the components of the aerosols – fungal, bacterial or pollen in particular – that contribute to the different size fractions. This question is important because fine aerosol particles move deeper into the respiratory tract thereby more readily setting off allergic reactions and allergies. For this work they have used chemical proxies for fungi, bacteria and pollen based on previously published reports and on additional work on chemical proxies of pollen as reported here.

The paper is well-written and the results are clear overall. Nevertheless, I have some questions and criticisms about the interpretation of their data and about the novelty of

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their findings that need to be addressed. The specific questions are indicated below. More generally, as a biologist it is difficult to accept that only data about chemical proxies are sufficient for making specific conclusions about the presence, abundance and behavior of bacteria, fungi and pollen. I understand that chemical proxies are used because the nature of filters used for PM measurements are not compatible with microscopy. Furthermore, chemical analyses are more rapid and likely are more sensitive in terms of detection thresholds. But they are not as specific as needed for the many of the conclusions that the authors have made. In many of the studies where these chemical proxies were developed, other types of samplers were used in parallel to validate the results via microscopy. Pollen grains are rather large and have distinguishing features that can be recognized to aide in their identification and to differentiate them from certain fungal spores. The authors also report that pollen grains burst – because of the chemical signals they observed – without ever showing any direct evidence of this phenomenon, something that is also readily visible. Bauer et al 2003 (cited in the manuscript), noted that the relationship (regression coefficient) between the number of fungi in atmospheric samples and the quantities of the chemical proxies varied among different sampling sites and dates. This is likely because of the physiological changes that can occur throughout the life of fungi and especially in the production of different types of spores (ascospores and conidia for ascomycetes; basidiospore, pycniospores, urediospores, aeciospores and teliospores for basidiomycetes, for example). Among their various conclusions, the authors stated that sources other than fungi were responsible for the glucan detected in the samples for cases where glucans and mannitol were not correlated. These are the types of conclusions that should be verified with other data – either direct observations, plating on growth media or through DNA analyses.

My second general question concerns the originality of the conclusions about how rainfall enhances the relative abundance of small aerosol particles as compared to larger particles. There is a growing body of literature describing how rainfall scavenges aerosol particles depending on their size – that have not been cited in this manuscript.

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I have indicated some of those papers below in the specific comments section. The authors state that the medical community is well aware of the increase in cases of asthma after thunderstorms. If the authors have presented new information in understanding this phenomenon, then they should better acknowledge that in the paper. As a last comment, I am not sure why the authors mention CCN, IN and cloud processes in the manuscript. This manuscript concerns bioaerosols that impact human health. Mentioning CCN and IN does not add anything to the manuscript and it distracts a bit from the main message.

SPECIFIC REMARKS

Pg 2, Ln 16 : There is probably better terminology than "growing cycle". "Plant phenology" would be more appropriate.

Pg 3, Ln 1. What do the authors mean by "Bacteria in the atmosphere are typically settled on soil or vegetative surfaces" ?

Pg 3, Ln 3-6 : The authors state : "In vegetation covered areas, atmospheric bacterial concentrations peaked after approximately 1 h of rain relative to areas with bare soil (Robertson and Alexander, 1994)." However, this statement is not supported by this paper. Robertson and Alexander studied one single bacterial species (a nitrogen fixer that nodulates stems) and rainfall was simulated in their study. So it is not appropriate to make such generalizations from this one work.

Pg 3, Ln 9-10 : In support of the statement "bioaerosols in the atmosphere promote cloud and ice nucleation" the authors cite Pope, 2010; Sun and Ariya, 2006; Franc and Demott, 1998. However, these papers concern CCN and do not support the statement about ice nucleation. Please add a reference about ice nucleation if you are going to maintain information in the introduction and discussion about cloud physical processes. But as noted above in the general remarks, the focus of this work seems to be on aerosols that affect human health. The statements about aerosols that influence cloud processes seem irrelevant to the point of this research.

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Pg 3, Ln 31: The authors do not state objectives that specifically mention the role of rain or the response of bioaerosols to rain. Why not?

Pg 4, Ln 9. In the methods section the authors do not indicate where the Andersen sampler is positioned relative to the ground and surrounding objects. How high above the ground was the Andersen sampler placed? What was the surrounding area like? Where there hedges, etc. Can the authors describe the footprint? the fetch? How was the sampler protected from rain? Did air circulate freely around the sampler? The authors need to provide information so that the reader can assess the representativeness of the air sampler relative to the surroundings.

Pg 6, the section starting on Ln 9: What was the purpose of the microscopy? How was this used in the study? Furthermore, why do the authors show a few images of pollen grains as one of the figures?

Pg 7, Ln 22: change "Rainfall corresponding to low PM" to "Rainfall corresponded to ..."

Pg 7, Ln 26-28 : The authors state that "The shift in the PM size distribution of PM reflects that rain was more effective at scavenging and/or suppressing the release of coarse particles compared to fine particles." This is what should be expected. They should cite the relevant references here and in their discussion. The differential effect of scavenging according to particle size has been reported as early as the 1960's in the work of Gregory [Gregory, P. H. 1961. *The Microbiology of the Atmosphere*. New York: Interscience Publishers, Inc.]. For a more recent example, the authors should refer to [Li et al. 2016. Observed changes in aerosol physical and optical properties before and after precipitation events. *Advances in Atmospheric Sciences* 33: 931–944].

Pg 7, Ln 32: Change "levels are shown in Figure 3b" to "levels as shown. ..."

Pg 9, Ln 31-32 : The authors state that "Rain influenced ambient concentrations and the size distributions of fungal spore tracers, by triggering passive and active release

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mechanisms." This is a very strong statement about mechanisms that is not supported by any biological observations in this work. This is a possible mechanism and it should be stated as a conjecture. Are there any other possible explanations such as growth, breaking of fungal hyphae, etc ?

Pg 9, Ln 34: The authors wrote: "Known for releasing spores after rain are some Ascospores...". "Ascospores" is not the correct terminology here. Ascospores are a type of spore. Here you mean Ascomycetes, i.e. a name for the group of fungi that produces ascospores during their sexual stage of reproduction. But although Ascomycetes are abundant, many of them produce mostly conidia that are formed on fungal "stems" called conidiophores and do not involve the formation of asci (sacs) containing ascospores and the accompanying fluids that are released into the atmosphere upon ascospore ejection. The relative prevalence of different types of spores (ascospores vs. conidia for the Ascomycetes and basidiospores vs. pycnia, aeciospores and urediospores for Basidiomycetes) could be part of the reason that Bauer et al 2003 observed different relationships between the amount of chemical proxy and amount of atmospheric fungi depending on site and season.

Pg 10, Ln 23-24: The authors state: "and prior observations that pathogenic bacteria that grow on crops (i.e. *Agrobacterium* spp., and *Rhizobium* spp.) contain glucans in their structure". In this section the authors are trying to provide information about sources other than fungi for glucans in the atmosphere. Glucans are widely distributed in the microbial world and in biology in general. Here they give an example of 2 bacterial species. Although the information is accurate that these species contain glucans, they are soil-borne microorganisms. Furthermore, *Rhizobium* is not a pathogen, but rather it is a symbiotic nitrogen-fixing bacterium that is considered to be very beneficial to plants (NB: being beneficial or not has nothing to do with the likelihood of being airborne. I mention this only to clarify that it is not a pathogen). It is not logically obvious that these soil-borne bacterial species would be readily in the air. There have been reports of aerial dissemination of *Rhizobium* between African and the Canary Islands,

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but this is also associated with loss of soils. It would be more appropriate to find a reference for the presence of glucans in bacteria in general, or to find references about bacteria that are common on aerial plant surfaces and more likely to be regularly in the atmosphere in agricultural contexts.

Pg 10, Ln 24-25: The authors state: "Agricultural crops are abundant in Iowa during the growing season and the mechanical agitation of plant surfaces by wind can aerosolize surface bacteria". Perhaps this is just awkward phrasing, but it should be changed because it suggests that the authors do not know that this is common knowledge. The "growing season" generally means the season during which crops grow. If Iowa were covered by forests, one would talk about the seasons (spring, summer, etc.). So, saying that agricultural crops are abundant during the growing season is redundant. Furthermore, I think that it is common knowledge that the Midwestern states of the US such as Iowa, Nebraska, Kansas, etc. are mostly covered by agriculture (corn, wheat, alfalfa). In this context, this sentence is surprising. It is sort of like reminding us, for example, that China or India have large populations of people.

Pg 13, Ln 13: The information on CCN and IN seems out of place in this paper because the authors are focusing on impacts on human health. For more detailed information about the possible sources of bioaerosols during and after rainfalls, I suggest that the authors refer to: Morris et al 2016 (<http://journals.ametsoc.org/doi/abs/10.1175/BAMS-D-15-00293.1>).

Pg 13, Ln 22-23: The authors state: "Elevating ambient fungal spore levels, particularly from species like Ascospores and *Cladosporium*, trigger allergenic respiratory diseases . . ." Here again, note that "Ascospores" is not a species. You cannot replace it with "Ascomycetes" because this is the name given to the members of the phylum Ascomycota. Perhaps you meant *Aspergillus*?

Pg 13, Ln 32, the authors describe the well-known phenomenon of thunderstorm asthma where allergies increase because of the abundance, after a storm, of small

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particles that penetrate deep into the respiratory system. In light of the previous research on this phenomenon, the originality of this present work is not clear. They authors should point out more strongly how the work presented in this manuscript goes beyond what was currently known.

Pg 14, Ln 18-19: The authors state: "Warmer temperatures promoted pollen, fungal and bacterial growth leading to higher ambient levels of these bioaerosols during both spring and late summer periods." They state this in the Conclusion section as if they had observed this in this work. But isn't this what they infer from their observations of chemistry ? It would be more appropriate to say that the warm temperatures promoted increases in the proxies that are assumed to represent these organisms.

Pg 14, Ln 35-36: The authors state "The fragmentation of pollens due to osmotic rupture, shown previously only through microscopy methods, is demonstrated in this study for the first time by way of chemical tracers." However, in this current work they have not made any microscopic observations to verify the phenomenon of fragmentation. Without direct observation they cannot make this conclusion. They can only speculate.

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