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Atmospheric Chemistry and Physics Discussions

Interactive comment on "Improved identification of primary biological aerosol particles using single particle mass spectrometry" *by* Maria A. Zawadowicz et al.

Anonymous Referee #1

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In their manuscript "Improved identification of primary biological aerosol particles using single particle mass spectrometry", Zawadowicz et al. present a new classification method to distinguish single particles containing "biological" and "inorganic" phosphorus from their mass spectra. The algorithm is developed and tested using single particle mass spectra acquired in laboratory measurements for a number of various different inorganic (mineral dust, fly ash) and biological (pollen, yeast, fungal spores) test aerosols. The applicability of a previously described method for determining bioaerosol using specific markers is also tested on this sample dataset. The new algorithm is applied to a dataset from ambient measurements to determine the number fraction of biological particles.

The presented method to distinguish biological and inorganic phosphorus-containing particles from single particle mass spectra is clearly improved compared to older methods and in principle should be published. However, the manuscript is focused more on the method development instead of the atmospheric application, which seems only a very minor addition to the paper and doesn't provide much new insight beyond a proof-of-concept. My feeling therefore is that the manuscript would be more suited for a journal more dedicated to the technical aspects of aerosol measurements, like Atmospheric Measurement Techniques.

My main concern however is that while the method itself seems fine, the manuscript is lacking a clear, critical discussion not only of the potentials, but also the limitations of the method. While in the abstract and most of the manuscript the impression is given primary biological aerosol particles (PBAP) could be clearly distinguished from other particles, only in the last section of the discussion (4.3) it becomes apparent that this is not the case at all: in fact, what can be distinguished with the algorithm is whether the particles contain "biological" or "inorganic" phosphorus. Though valuable, this is a very different kind of information than suggested in the title ("identification of primary biological aerosol particles"), the abstract ("identifying bioaerosols", P1, L19, "identification of bioaerosol", P1, L19, "differentiate and identify bioaerosol", P1, L22/23; "in ambient data. . .0.04-0.3% were identified as bioaerosol", P1, L25-27), and most of the manuscript (several instances, e.g. P11 L20, P11 L26, P16 L16, P16 L17, of misleading use of "bioaerosol", which is defined in P2 L2 as "primary biological aerosol"). Therefore, it needs to be stated much clearer throughout the manuscript what this classification method indeed is capable of, and what its limitations are. Otherwise the false impression is given that PBAP could be detected within ambient aerosol with this method, which clearly is not the case. Consequently, these issues need to be addressed before I could favor publication of this manuscript.

Therefore, I would suggest the authors to re-submit the paper, possibly to a more suitable journal, after performing some major revisions addressing these issues.

Specific comments:

While the paper overall is clearly written, it partially could be more concise and more clearly structured. Especially the introduction is very long, and in parts reads more like a review than like a general introduction to a research paper, esp. P2, L22 to P4, L28. This also leads to an excessively long list of references. Thorough reviews on PBAP and their measurements can already be found e.g. in (Després et al., 2012) and (Georgakopoulos et al., 2009); instead of citing a huge number of individual works, the citation of such review papers might be preferable. By streamlining the introduction especially in P2, L22 to P4, L28 and giving it more focus, the introduction could be significantly shortened while still maintaining all the important information relevant for the manuscript. On the other hand, the discussion on previous efforts in bioaerosol detection using single particle mass spectrometry, which is the most important aspect for the discussions within the manuscript, is very brief and could be expanded (P5 L9-16).

It is not clear to me why parts of the results can be found in the results section, and other parts (like the soil and internal mixtures, Sect. 4.3) in the discussion section. Maybe a single "results and discussion" section would be more appropriate, and could also avoid some repetitions.

Section 4.2: This is an important discussion, and I would have hoped to find a similarly critical discussion of the newly developed algorithm in this manuscript, as well. There are some basic approaches to such a discussion scattered throughout the manuscript, but this should be addressed much more clearly and explicitly. By the title of Section 4.1 it is suggested that this discussion is provided in that section, but in fact the presented discussion on uncertainties and limitations of the newly developed algorithm in that section is very limited and should be much more thorough.

For example:

- In Sect. 3 a "misidentification rate" is given. This needs to be explained in more detail

and more clearly:

1.) What kind of misidentification exactly is contained within this value? Both false positives (mineral dust wrongly assigned to "biological phosphorus") and false negatives (biological material not assigned to "biological phosphorus") cause an uncertainty in the determined fraction of particles containing biological phosphorus.

2.) The method used to calculate the "misidentification rate" should be clearly stated. Depending on the method, the number of particles within the data sets of the different test aerosols might bias the determined uncertainty, so this should be made clear to the reader.

3.) It is stated that removing ragweed pollen from the training set leads to a smaller misidentification rate. Was ragweed pollen only removed from the training set, or also from the "testing" set? I guess the former, since the latter would give a wrong impression, but this needs to be stated clearly.

4.) Which particles were tested for determining this misidentification rate? The pure biological / pure mineral dust / fly ash particles? What about the processed mineral dust?

- It should be discussed in Sect. 3 / Sect. 4.1 what effect has

1) processing of the mineral dust (which, as stated on P10 L17, causes CN- and CNO- to "appear and/or intensify", so might have an influence on the classification), and

2) mixing mineral dust / biological material. This is discussed only in Sect. 4.3, but is an important consideration when assessing the uncertainty and limitations of the method. The discussion in Sect. 4.3 reveals several limitations which need to be discussed within this context: "At this time, we are not able to delineate between primary biological and biogenic or simply complex organic (such as humic acids) material." (P15 L30-31) This means that with the presented method, not PBAP can be determined, but whether phosphorus present in any particle is of "biological" or "inorganic" nature. The former

hints at the presence of biological material, but, as also evident from the discussion on P16 L1-14, it is not possible to determine whether this biological material is part of PBAP or from an internal mixture of e.g. mineral dust and biological material, so the information retrieved remains limited, which needs to be clearly stated and discussed.

- If I understand correctly, only an internal mixture of biological material with a type of mineral dust not showing any signatures of inorganic phosphorus (illite) was tested. But what happens if mineral dust showing mass spectral signatures of inorganic phosphorus (like apatite) is internally mixed with biological material? If indeed in an ambient dataset up to 56% of all particles identified as containing biological phosphorus also contained silicate markers (P16 L1), this does not seem to be an unlikely case and needs to be addressed.

All in all, a clearer discussion of the potentials and limitations of the method is needed: it is capable of differentiating biological and inorganic phosphorus under the tested conditions (within the uncertainty and the limitations to be discussed), but (at least in its present state) it is not capable of distinguishing PBAP. The misleading references to "bioaerosol" throughout the manuscript need to be reworded to reflect this.

2. Experimental section:

In order to get an idea of the underlying statistics, the general information on how many mass spectra (positive/negative) were available for the different samples needs to be included somewhere (in the experimental or the results section), also, how many mass spectra were acquired in the field campaign. If only some of the spectra were used for the analysis, their number (and criteria for their selection) needs to be stated.

How were peak intensities determined for the various ratios (CN-/CNO- etc)? Integrated peak area? This should be stated in the methods section.

P6, L3-9 (first paragraph): this paragraph is not related to the section (2.1: PALMS), but a general introduction. It should go either as a general remark in the experimental

section before Sect. 2.1, or be reworked as a last paragraph into the introduction.

P6, L18: "...a unipolar reflectron time of flight mass spectrometer was used..." – It should be clearly stated here that the PALMS acquires for each single particle either a negative or a positive mass spectrum, but not both simultaneously. How long were the sampling times in positive / negative mass spectra mode (e.g., switching every minute, every 15 min, every hour)?

Could Section 2.2 (Test samples) be streamlined a bit to be more concise? It would be good to have a table with an overview of the sampled materials; maybe some of the detailed information could go into such a table as well, to make the section easier to read and to provide a better overview for the reader.

P7 L25: "...further dissolved in $\sim$5ml of Milli-Q water..." – this information is not necessary since the concentration of the original solution is not known, anyway.

P7, L30-31: "No processing-related changes to chemistry were found." – This sentence should be clarified, e.g., "...were found in the mass spectra sampled with the PALMS".

P8 L4 "to aerosolize a solution of illite NX and F. solani spores" – I guess this should read "suspension" instead of "solution"? Was this suspension sonicated as well?

P8 L21: "for 0.1 mL experiments" is unclear. Rather something like "For experiments using 0.1 mL of nitric acid"?

P9, L3: "a portion of the data" – how many spectra? Give at least the order of magnitude. The same for the "remaining data" (P9 L5). Also specify which sample types were included for the training: on P11 L5 it is mentioned that soil data were not used. Were all other lab samples used? Please be more specific. Related to this, in Sect. 3, it is stated that for the ambient data, a threshold was used to determine mass spectra containing phosphorus in a first step. Was something similar performed for the lab data (also for the training), or were all mass spectra used?

P9, L18: Please give the start / end dates of the measurement period.

3. Results:

P10 L19 to L27: which bioaerosol materials were used for this? In Fig. 4 only 5 of the 8 tested materials are shown. Were the others used as well, showing similar behavior, and only were omitted for clarity? Please state clearly which materials were used for the development and if some were left out, why. – On P11 L4 it is stated that soil dust is left out from the training set because some biological material might be contained in the particles, however, in P10 L24 it is used within the class of inorganic phosphorus. If biological material is indeed present within soil dust, this does not make sense. This seems like a contradiction.

P10 L26 “Processing of apatite with nitric acid tends to shift the PO3-/PO2- ratio to larger values, increasing the disparity from the bioaerosols.” – This is not clear to me. If inorganic material usually shows lower PO3-/PO2- ratios than bioaerosol, shouldn’t this read “decreasing the disparity”?

P11 L6: “(classification with the SVM algorithm is discussed later)”: maybe this could be reworded for clarity, as at first reading it seems to mean that the SVM algorithm itself will be discussed later, not the results of applying it on the soil samples.

4. Discussion:

P13 L10-12: “Particles with positive spectra showing the characteristics of monazite. . .provides evidence of the origin of the inorganic phosphate particles.” (and a similar statement on P12, L9): Since PALMS does not simultaneously provide the positive and negative ion mass spectrum of a single particle, this is not “evidence”, but rather “suggests” this type of mineral dust particles as a likely origin. Please reword.

P13 L26 and following: What thresholds were used in order to determine whether the different marker ion signals were present or absent? Was it tried to improve the performance of the algorithm by adjusting these thresholds?

P15 L26/27: “the numbers of biological particles fall within these estimates”. The cited

estimates refer to inorganic / biological phosphorus mass ratio, while the information provided by PALMS is the number ratio. Such a comparison would only be valid if it can be assumed that all types of particles contain the same amount of phosphorus, which does not seem very likely. This needs to be discussed more carefully. A similar comment applies to P16, L28-31.

Figures and tables:

Figs 1-3, Fig. 9: The mass spectra might be easier to grasp if integrated stick spectra were shown instead of the raw mass spectra.

Fig. 7: “In all other aerosol classes the green bar denotes a level of misidentification.” – This only applies to “Apatite + Monazite” and “Fly ash”. Both “agricultural soil dust” and “Soil dusts” are expected to contain some (unknown) amount of biological material, so the performance of the algorithm cannot be validated on these samples.

Technical corrections:

Various locations, e.g., P6 L6, P12 L23, P12 L28: “phosphorous” should read “phosphorus”

P7 L8: “Snowmax” should read “Snomax”

P7 L22 and various other locations: “Milli-Q water” is laboratory slang, use “ultrapure water” instead

P8, L19: “flow” should read “flow rate”

P8, L19: for the flow rate reported in slpm, reference temperature and pressure need to be given

P8, L21: remove “.” from “conducted.:”

P11 L6 “latter” should read “later”

P12 L5 “carbonatitie” should read “carbonatite”

P12 L6 introduce abbreviation “REE” (e.g., in the previous sentence)

P13 L23 “If a silicate components were. . .” – remove superfluous “a”

P16 L2 “This represents and upper limit” should read “an upper limit”

List of references: for several references, page numbers are given as “n/a-n/a”, e.g. P23 L16, L24, L27; P25 L1. On P23, L30: “>” should read “>”.

Table 2: In the table caption, the wording “biological filter” is unclear, please be more precise. Also not “negative particles” are “sampled”, but negative ion mass spectra are acquired. For Argentina and China, “approximate” could be omitted from column 3 (as this is already clear from the column header).

References:

Després, V. R., Huffman, J. A., Burrows, S. M., Hoose, C., Safatov, A. S., Buryak, G., Fröhlich-Nowoisky, J., Elbert, W., Andreae, M. O., Pöschl, U., and Jaenicke, R.: Primary biological aerosol particles in the atmosphere: a review, Tellus Ser. B-Chem. Phys. Meteorol., 64, 2012.

Georgakopoulos, D. G., Després, V., Fröhlich-Nowoisky, J., Psenner, R., Ariya, P. A., Pósfai, M., Ahern, H. E., Moffett, B. F., and Hill, T. C. J.: Microbiology and atmospheric processes: biological, physical and chemical characterization of aerosol particles, Biogeosciences, 6, 721-737, 2009.

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