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Comment on egusphere-2022-598

Anonymous Referee #3

Referee comment on "Technical Note: Bioaerosol identification by wide particle size range single particle mass spectrometry" by Xuan Li et al., EGUsphere, https://doi.org/10.5194/egusphere-2022-598-RC3, 2022

Review of "Technical Note: Bioaerosol identification by wide particle size range single particle mass spectrometry" by Li et al.

The authors present a method to differentiate laboratory-generated bioaerosol particle samples from other particles that exhibit similar ion signatures in a single-particle mass spectrometer. The authors cite recently published improvements to their instrument, including sampling size range and ion extraction. The authors include a useful analysis on how ionization laser power affects critical signals for bioaerosol identification.

Generally, the paper is not very well written and is difficult to follow. It is unclear how the experiments were actually performed, how the analysis generated the conclusions, and how the presented method would perform under realistic atmospheric conditions or against similar published methods.

The paper is not publishable in its present form. Significant improvements must be made in a variety of areas, specified below as Major and Minor Comments. The underlying method and results appear to have scientific value, but the authors must first present them clearly and completely.

Major Comments

- Results and methods lack critical detail and context with previous studies.

1a) The references and descriptions of other single-particle mass spectrometers and previous work on bioaerosol identification are inappropriate, out of date, or too limited in scope. Add a paragraph to the Introduction describing some of the previous bioaerosol identification studies, particularly those involving online mass spectrometers, and perhaps also mentioning other successful techniques (fluorescence, Raman, offline methods). These references are suggested starting points only, and the authors should choose appropriately.

Huffman et al., Real-time sensing of bioaerosols: Review and current perspectives, Aero Sci Tech. 2020, doi: 10.1080/02786826.2019.1664724

Russell et al., Microorganism characterization by single particle mass spectrometry, Mass Spec Rev, 2008 https://doi.org/10.1002/mas.20198

Pratt and Prather, Mass spectrometry of atmospheric aerosols—Recent developments and applications. Part II: On-line mass spectrometry techniques, Mass Spec Rev, 2011, https://doi.org/10.1002/mas.20330

Huffman and Santarpia, Online Techniques for Quantification and Characterization of Biological Aerosols, Microbiology of Aerosols chapter 1.4, 2017, https://doi.org/10.1002/9781119132318.ch1d

Also, the paper references experimental aerosol studies and inlets without identifying the

specific instruments in the text. Specify to which instrument (ATOFMS, AMS, PALMS, etc) the publications refer, e.g., in lines 61, 69-78, and elsewhere. Lastly, the method presented by the authors identifies bioaerosol particle samples using ion ratios of PO_3^-/PO_2^- and CNO^-/CN^-, refined with machine learning. This method exactly follows that of Zawadowicz et al., 2017 using the PALMS single-particle instrument. Although the authors do include this reference in a brief sentence (line 61), they should state (e.g., in the final para of section 1) that the analysis method of the current study is based on Zawadowicz. Also consider many relevant ATOFMS publications and their use of these ions or ion ratios.

1b) The authors' instrument is inadequately described (section 2.1). In addition to the Li et al. 2011 reference, describe the instrument details such as detection and ionization lasers, previous size range and detection efficiency, and any other characteristics relevant to the current work. Since section 3 discusses spectral variation due to ionization energy, a typical laser beam width would be helpful. How does this instrument compare to previous single-particle mass spectrometers used in bioaerosol detection (ATOFMS, PALMS, SPLAT, others). What type of time-of-flight mass spectrometer does SPAMS employ (a commercial model?). How similar is SPAMS to the commercial ATOFMS?. State clearly what differentiates SPAMS from the "high-performance" version used in this study. Define "pore size". What is "multi-channel superimposed signal acquisition system"?

1c) The performance of the new instrument SPAMS configuration is presented without context to similar instruments' performance on bioaerosol detection. Specifically, how do the discrimination percentages presented here compare to those in the literature? Choose similar aerosol systems if possible, and/or list limitations in the comparisons. Direct comparisons to Zawadowicz seem obvious.

- Conclusions are not supported by the data as presented.

2a) A principal conclusion of the study is that "The ionized laser energy has a certain influence on the integrity of the ionic peak but hardly affects the identification accuracy of bioaerosols." (line 320). Specifically, line 284 states, "…the discrimination degree of bacterial aerosols and dust under 0.5, 0.75, 1.0, 1.25, and 1.5 mJ energies were 96.6%, 97.4%, 97.1%, 96.5%, and 97.8%, respectively, indicating that the ionized laser had little effects on discriminating biological aerosols and dust disruptors." However, in apparent contradiction to this constant discrimination efficiency, which is based on PO3-/PO2- and CNO-/CN- peak ratios, Figure 8 seems to indicate that most dust spectra (~70% or so) do not contain either phosphate peak. I interpret Figure 8 as plotting occurrence frequency of these peaks, not "peak ratio%" as listed in the y-axis. Clearly describe how the method can differentiate between dust and bioaerosol when a large fraction of dust spectra are apparently excluded from the analysis due to missing peaks. State what fraction of each particle type sample is excluded from the analysis prior to applying the classification routine. Given this apparent limitation, how would the authors' technique be used realistically on an externally mixed population of particles with unknown composition?

2b) The authors report using a supervised machine learning algorithm to help differentiate bioaerosol and abiotic aerosol, claiming a 97.7% accuracy. This successful discrimination is the principal conclusion of this study. However, the authors only mention the technique in passing, as a single sentence in section 3.3. Provide details of the machine learning algorithm and relevant parameters in a separate paragraph. Give enough information that another group could recreate these results. How is the training dataset defined? What is the test dataset? How many spectra were used in the analysis? How many were rejected?

- Inadequate presentation of material.

3a) A large fraction of the paper is written in a way that is vague, redundant, or unclear. Critical information is missing or lost. The sentence structure, writing clarity, and grammatical accuracy need significant improvement prior to publication.

Examples include…

Line 87 from the Intro:

"The analysis of single particle mass spectra is a hard ionization process and laser energy has little effect on the discrimination of this classification method."

Line 309 from the Conclusion:

"The performance of SPAMS and the improvement of the sampling system have improved the ability to identify bioaerosols."

Lines 317-320 from the Conclusion:

"In addition, due to the influence of laser ionization efficiency, the effective mass spectra peak ratio of bacterial aerosol generation is higher, thus it is more suitable for this method. The ionized laser energy has a certain influence on the integrity of the ionic peak but hardly affects the identification accuracy of bioaerosols."

There many examples throughout the paper. The authors edit the paper again for proper sentence structure, clarity, verb conjugation, plural nouns, and definite and indefinite articles. If necessary, employ an English language editing service. Consider an alternative term for "disruptors" to describe abiotic particles.

3b) The acronym "SPAMS" is used confusingly to describe both single-particle mass spectrometers in general (eg, ATOFMS, PALMS, etc), and also the specific instrument used by the authors in their experiments. Choose unique acronyms to describe other single-particle mass spectrometers. Note also that the Aerodyne AMS is not a single-particle mass spectrometer.

Minor Comments

Line 41: The sentence seems out of place. *What* makes identification unclear? Their scattered sources? Also, the Rosch 2006 reference study is not appropriate for this statement. There are dozens of papers describing bioaerosol detection subsequent to this study.

Line 44: Consider adding other reviews of bioaerosol identification, eg, Huffman et al., Aero Sci Tech, 2020.

Line 48: add references for mineral fluorescence

Line 65. I suggest you describe why 98% discrimination (line 64) is "insufficient".

Line 69. Add a reference for the typical particle size range.

Line 78 seems out of context. Please remove or clarify. Define ATOFMS.

Line 113: Why do you classify these aerosol as "disruptors"?

Line 114: What kind of "road dust" did you use in this study? Is it a commercial sample?

Line 127: "absorbed" seems incorrect here

Line 138: "A sheath gas of 80 kPa of clean air was used." I don't understand the pressure of "sheath" gas here. Reword for clarity, eg, "a ## flow of dilution air…".

Table 1. List the type of bioaerosol, bacteria or fungi.

Figure 1. Suggest experimental "design" or "configuration" rather than "flow". Where is the "exhaust port with a high-efficiency particulate air filter"?

Fig 2. Which samples are bacteria? Which are fungi?

Section 3.1. The authors compare the size of bioaerosol as detected by SPAMS, which like all single-particle mass spectrometers has size-dependent counting biases, with electron microscopy size distributions. Although the relative comparisons of aerosol sizes in this section remain valid, the authors should make it clear that the "overall particle size distribution" is the size *as detected by SPAMS* and not an absolute size distributions of the aerosol samples. The related statements of lines 170-173 need clarification. Do these statements refer to SPAMS, or to single-particle mass specs in general…?

Line 181 & Fig 3. Units?

Line 185. With what instrument?

Line 198. Clarify "speculated and added".

Line 199. SPMS is undefined.

Line 215-218. The selection criteria are unclear. Does "alone" mean one of those 4 individual peaks? Does "interference" mean the spectrum contains one of those peaks?

Line 225-226. Redundant

Fig 4. The y-axis label seems incorrect

Line 230-231. These numbers don't correspond to anything in particular in Fig 3. "proportion interval"…? "concentrated"…?

Line 236. Add references for the "traditional method"

Line 248. How "high"?

Line 249-251. These morphology sentences are out of context in this para. Remove or add text to describe their relevance.

Line 257. “morphology of organic compounds” ?

Line 267. “peak integrity” ?

Line 290-304 and Fig 8. Clarify and use consistent terminology. Should “peak output rate” and “peak ratio” actually refer to occurrence frequency of peaks? The % values do not obviously correspond to any consistent set of points in Fig 8. Clarify/correct these values.

Data Availability. Include a publicly accessible link to data prior to publication.