



RC: Reviewer comment on Daily et al. AMTD 2021

Anonymous Referee #1

Referee comment on "The sensitivity of the ice-nucleating ability of minerals to heat and the implications for the heat test for biological ice nucleators" by Martin Ian Daily et al., Atmos. Meas. Tech. Discuss., <https://doi.org/10.5194/amt-2021-208-RC1>, 2021

General comments:

The authors heated 20 samples of minerals (K-feldspar, plagioclase feldspar, quartz, clay, dust surrogate and carbonate) as well as 4 samples of biological material (Snowmax, lichen, birch pollen washing water and cellulose) using two different heat test procedures. The first was a wet heat test where the samples were submerged in a bath of boiling water and the second was a dry heat test at 250 °C in an oven for 4h. The authors then measured the samples before and after the two types of heat tests and display their results as frozen fractions, as box plots and as n_s plots. The authors then have rather long discussions of speculations (the word assume/assumption is found at least 10 times in the manuscript, at times justified and at times not, when the assumption could be resolved with further experiments). They speculate about what could have driven the differences before and after heat for the different heat tests and for the different types of samples. In general, the authors reference the literature adequately and thoroughly.

The research question is certainly worthwhile, and the authors' systematic approach is a good idea for evaluating the general applicability and the interpretation of a change in INA after a heat test. I commend the authors for approaching this problem systematically. However, this manuscript is currently too preliminary to be published. This study can be made significantly stronger to make an impact on the community and for the work to be built upon in the future. My key recommendations to improve the study before publication are below:

1. (most important recommendation) The authors are missing key experiments for further conclusions to be drawn. Specifically, the authors should run all 20 mineral and all 4 biological samples in a comparable dry heat test at 95 °C in an oven for 30 mins (or the same amount of time the sample was submerged in the water bath). This test is necessary, since the authors make many assumptions of what can be the cause of inconsistencies between their wet heat test and their dry heat test. Yet, comparisons have 3 variables being changed in both sets of heat tests: the method, the temperature and the time of heating. I would also encourage the authors to consider re-running their dry heat test at 30 mins (or running all their heat tests at 4h), which would add additional columns in Table 3. These additional experiments would really strengthen the systematic approach

that the authors are attempting to present in this work. Right now I am left wondering what is the effect of wet vs dry and what is the effect of temperature and what is the effect of heating time?

2. Furthermore, there are important background water tests missing in this manuscript. The authors should address these details thoroughly before publication. In general, daily blank tests discussed in lines 261-262 are not experiment controls, and do not represent adequately the experimental procedure each sample is submitted to. The authors use 0.1 μm pre-filtered, cell culture-grade deionised water, and could the authors show the following background water data:

- A sample of background water that was heated in the same type of vial as with the wet heat test (line 208).
- A sample of background water that was heated in the oven at 95 °C in an oven for 30 mins.
- A sample of background water that was passed through the same experimental procedure following the sample after heating at 250 °C in an oven for 4h.
- A sample of background water that passed through the nylon net filter and the cellulose acetate filters (referred to in line 192). I highly suspect that cellulose acetate filters leach material. These control frozen fractions must be shown.
- Show the data discussed in lines 221-224.
- What is the role of the pre-sterilized dry heating at a different temperature? Shouldn't the procedure also involve the same 250 °C temperature as the experiment?

3. The wet heat test will certainly have the water evaporate and therefore change the concentration of the material within the solution. How are the authors accounting for changed in concentration of the ice active material? relevant to the discussion in lines 145-147.

4. The authors should make every effort to compliment the study with alternative measurements. I can appreciate that substantially more work would be required, but it would allow the manuscript to be much more concise rather than listing a list of speculations (for example, the K-fledpsar discussion spans pages 11-14 of speculations). For example, ideas presented in lines 365-377 could be address with elemental analysis and presence of N. Or with a protein test such as the Lowry method. Ideas in lines 368-370 could be tested with a heat test at the same temperature as the wet test (see my point number 1). In addition, total mass of the material after both heat tests can be weighed and measured (assuming the wet heat test and be dried out). Have the authors attempted to dry their material and repeat the heat tests multiple times? Does a wet heat test followed by a dry heat test and vice versa have any effect? Evidence of chemical composition would also be particularly helpful, for example total organic carbon analysis, ion chromatography, elemental analysis, etc. I'll add here that my criticism is also a general one to our community where we tend to only show INA of material when these results cannot simply be compared without other measurements (such as surface area and/or composition). Finally, point d) at lines 719-725, I would recommend that the authors either don't discuss it, or do the experiments.

5. The authors should further describe their detailed storage protocols. These protocols come up a few times as excuses for differences but should be detailed to teach the community exactly what was done. (Examples include Line 166, 195 and 201). I'll also add that the authors' justification on lines 234-235 is very good.

6. The authors use a high concentration of material (stated as 20 mg/mL on line 190). Are

the authors working with suspensions or homogeneous solutions (line 230)? This fact is important since after heating, material's solubility can substantially be affected. How have the authors addressed a possible change in solubility before and after their heat tests? What is the effect of concentration on the heat test? I think a series with one type of mineral sample with different concentrations for both heat tests would be an interesting series to present.

7. Can the authors show what the role of the time during the heat test can have? This information would be particularly helpful to determine an optimal temperature and time of heating for subsequent experiments by the community (and will be better cited).

8. Have the authors attempted to combine one of their mineral samples with one of their biological samples? This test would better represent an ambient measurement and see if the effect is cumulative or not (see (Steinke et al., 2020)).

9. References: In general, Table S1 could be included in the main text as a reader-friendly reference guide for future work to compare and built upon. Good job to the authors for this compilation - although mentioned in the text, did the authors want to also include (Bogler and Borduas-Dedekind, 2020) in their table S1?

10. Title: Seems to me that the second part of the title is most relevant to the content of the work. I can encourage the authors to consider a title along the lines: Testing (or systematic evaluation) of the heat test for ice-nucleating ability of minerals and biological materials.

11. The introduction can be substantially shortened to focus only on the heat tests experiments listed in Table S1.

12. Following all these comments and suggestions, can the authors create a recommendation rubric for heat test measurements: type (wet vs dry), temperature, length of time, with the ultimate goal to streamline how our community runs these heat tests in the future (including with and without hydrogen peroxide as some groups have done.)

I'll just add a comment here to the authors, that I am very conscious of the additional efforts being requested for the revisions of this paper. Nonetheless, I think the authors and readers (including myself) would greatly benefit from the study being expanded in its conclusions and implications of the heat tests. I hope the authors will be encouraged to improve their work and go those extra steps further for the benefit of our community.

Refs in this review:

Bogler, S. and Borduas-Dedekind, N.: Lignin's ability to nucleate ice via immersion freezing and its stability towards physicochemical treatments and atmospheric processing, *Atmospheric Chem. Phys.*, 20, 14509–14522, <https://doi.org/10.5194/acp-20-14509-2020>, 2020.

Steinke, I., Hiranuma, N., Funk, R., Höhler, K., Tüllmann, N., Umo, N. S., Weidler, P. G., Möhler, O., and Leisner, T.: Complex plant-derived organic aerosol as ice-nucleating particles – more than the sums of their parts?, *Atmospheric Chem. Phys.*, 20, 11387–11397, <https://doi.org/10.5194/acp-20-11387-2020>, 2020.