

Title: Observations of fluorescent and biological aerosol at a high altitude site in Central France

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Reviewer Comments:

General Comments

This paper was quite easy to read, despite its complex subject matter. The topic was interesting, and the site of the study was unique, which made the results quite thought provoking.

My main issue was with the actual motivation for the work. The title employed the term “observations” which raises the question as to the intended purpose of the study. To my mind, use of the term “observations” implies that the results would simply be published, with little/no interpretation. Further analysis would result in a paper entitled “a study of”, rather than “observations”, in my opinion.

If this was intended to be a baseline study, then more time should have been allowed for a proper longitudinal study, which would reasonably be expected to cover at least a full 12 month period, or samples representative of each of the four seasons. With only 10 days of sampling, it is somewhat inappropriate to be making generalizations from the results, in particular regarding the “diurnal” pattern observed. Air sampling results are generally quite ephemeral – the results depend on so many variables, and thus long-term sampling is considered much more meaningful than a single event sampling. In particular, with the changing weather pattern observed within the campaign period, the actual sampling time was further sub-divided into several even shorter periods.

If, however, the purpose of this campaign was a pilot study or similar, then the shorter period would be appropriate. In any case, I would have expected more information about the surrounding topography, and the types of land-use nearby (farms, roads, industry), including distances from the sampling site, to be provided. This data is essential to interpretation of the results.

Specific Comments

The first two paragraphs of the introduction refer to several studies involving PBA counting methods using viable (culture-based) counting methods. Such methods have been variously reported as capable of detecting from <1% to <10% of the actual numbers of living bacteria and fungi. This phenomenon is mainly due to the harsh conditions of the atmosphere, which cause sub-lethal damage to most bacteria (more so to Gram negative bacteria than Gram positive) preventing them from growing in culture. For this reason, it is inappropriate to use any numbers obtained by culture techniques to compare with fluorescent-based methods. Referring to your reference (Harrison et al, 2005) it should also be noted that qPCR methods are also inaccurate, as they measure ALL gene copies in a sample, which may include free DNA from dead/lysed bacteria.

Page 3034, Line 23: the authors conclude that the measurements at sites in Austria and Switzerland are able to be taken as “representative of the background PBA in Europe”. I would challenge this assumption. A background study at the actual site is required – surrounding activity and other variables are essential knowledge before such an assumption could be accepted. I would be very surprised if a single background PBA could possibly apply for all of Europe.

Page 3037, lines 4 – 9: Mention is made of the vehicular traffic present at the site. Was this traffic logged/recorded? What surface material was on the roads? What type of fuel did the vehicles use? What about the movements of the people present – numbers, activities, proximity to the recording equipment? What precautions were taken to ensure that any people present in the area did not interact with the sampling? How far away is the military installation and what activity occurs there? Is it manned? If so, how many personnel are present?

Same page, line 10: as a typical “rural” background, what crops or other plants were present, at what distance?

Same page, line 24-26: air masses are variously reported as being “anthropologically influenced”. How was this factored into the measurements?

Page 3039, line 1-2: the counting uncertainty of the DAPI stained filters is noted as being 25% for bacteria and 20% for spores and yeasts. How were these figures determined? Both sound about right, but are high for basing analytical work upon. Any comparison with other methods (WIBS-3) would be highly doubtful with such margins for error. This is particularly of concern when we read on page 3040 (line 9) that the study intends to challenge the “common assumption . . . of the link between N_{NADH} and the bacterial count”. Given the issues involved in obtaining an accurate bacterial/fungi count, not addressed in this paper, this study would not be capable of supporting such a challenge.

Page 3046, lines 19-25: I am concerned that the assumption of a measurement declining progressively with each successive impactor sample is accepted without question. This should have been followed up, perhaps with some laboratory simulation testing of the equipment. Such a trend may also be due to decreasing sensitivity of the equipment over time, or any one of a number of other factors. When testing indoor air, the phenomenon of “stripping” is generally observed, whereby the number of particles is reduced over time due to their removal by the testing equipment. This is less common in outdoor air, where the air is replenished quickly (especially if the site is windy), but it is at least one reason why numbers of particles fall over time. The authors might try a bit harder to prove an actual reduction, rather than an instrumental anomaly.

Page 3048, line 15: What is “casual” observation? This size range is recorded in basic microbiology textbooks, so it is not a surprise. The presence of clusters, chains and other groups, as well as adherence to dust particles for example, so that larger particles are detected, is also well documented.

Page 3050, line 10: Statement “pollen is unlikely to be found in such concentrations” requires explanation and support. Why is this so? In any case, pollen is easily recognized microscopically, so should have been detected in the EFM slides, better evidence for its absence than being “unlikely”.

Same page, line 11: as mentioned previously, the lack of systematic agreement between N_{NADH} and bacterial counts is likely due to the lack of accuracy in the latter.

Page 3051, line 1: Once again, the comparison with culturable bacteria is not appropriate. In the case of air testing, culturable does not equal viable!

Page 3053, line 23: Here the authors mention the “seasonal agricultural contribution” to which I was referring earlier. This should have been included in the site description, and the extent to which it may have affected the outcomes should have been measured and included in the results analysis.

Technical comments

The text of the document is largely written in the present tense. This is not appropriate, according to scientific publication conventions, and also to the implication that the results are happening currently, rather than in 2010.