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Atmospheric Chemistry and Physics Discussions



# *Interactive comment on* "Annual distribution of allergenic fungal spores in atmospheric particulate matter in the eastern mediterranean; a comparative study between ergosterol and quantitative PCR analysis" *by* N. Lang-Yona et al.

**N. Lang-Yona et al.**

yinon.rudich@weizmann.ac.il

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Anonymous Referee #3

General comments: The reported work of N. Lang-Yona et al. collects PM10 samples over the course of an entire year in Rohovot, Israel and characterizes total and specific airborne fungal spores while comparing two analytical techniques (qPCR and ergosterol analysis). The structure of the manuscript is clear but the description of the methods in terms of quality control measures taken, needs expansion which should



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ultimately lead to a greater confidence in the reported findings. Overall worth publishing once further expansion regarding quality control has been performed and specific comments below have been addressed.

We thank the Reviewer for the comments and for supporting the publication of the paper. Below we reply to all the comments made and describe the quality control measures taken in the study.

Specific comment: 1. Sampling was performed using a High-volume sampler (ECO-HVS3000) operating at a flow rate of 67.8m3h-1 and all sampling periods were 72h except for two cases. While it is appreciated that enough mass of PM needs to be collected to perform all assays, consideration by the authors needs to be given to the possibility of filter saturation and the effects of microbial growth may play on collection filters sitting in a sampler for duration of 3 days. Perhaps the authors could address the issue of the possibility of filter saturation and describe consideration made or reasons why 72 h sampling period was chosen.

The reviewer points out a very important issue. We were aware of the drawbacks that 3 days sampling might add; however our constraint was the ergosterol's limit of detection. Therefore we constantly verified that there was no filter saturation by ensuring that flow rates through the filter remained at a constant value, as measured by a flow meter in the high volume sampler used in this study. We know from previous studies that when the filter is saturated, the flow rates are reduced, and sometimes even reach close to zero values. We now added the information on the flow in P. 5, line 91-92 of the revised manuscript: "Airborne particles were collected using a high volume sampler (Ecotech High volume sampler model ECO-HVS3000 with PM10 inlet) maintaining a constant flow rate of 67.8 m3h$-$1." Under conditions of a constant flow rate of 67.8 m3 h-1, fungal growth is not likely to occur due to the lack of water availability. However, if minor growth would occur, the errors for both methods in this case, are expected to be expressed at the same percentage.



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The data has been normalized according to the volume of air sampled e.g. ng m-3. Have the authors considered a relative concentration to assess variability i.e. mass of analyte per mass of PM.

The variability in the bioaerosols mass compared to total PM mass is interesting information that can be obtained out of sampled filters' data. However because the main goal of this research was to compare between methods we chose to use volumetric normalization, which keeps constant volume (reported in the high-volume sampler), reducing measurement errors. Note also, that because fungi and ergosterol were sampled onto the same filter with the same PM10 mass concentration, the comparisons between ergosterol/fungi on a PM10 mass basis should provide the same results as the volume based comparisons.

Specific comment: 2. Quality Control: Accuracy, precision, and MDLs associated with the sampling method used in the current study, in conjunction with qPCR detection methodologies, perhaps could be further investigated rather than referencing a study based on indoor air that uses a wipe sampling methodology i.e. Yamamoto et al. (2011). For example did the authors perform spiking experiments to investigate recovery efficiencies from the filter substrate used?

We have further investigated the quality control parameters relevant to our data, which are now included in the text (P. 8, lines 180-187, in the result section; FF2/FR1 assay for universal fungi: "For quality control analyses performed to each of the genomic assays, precisions were defined as instrument repeatability based on triplicate qPCR measurements for the study air samples. Median values of instrument repeatability as COVs were 59%, 19%, 31%, 21%, 33%, 34%, and 13% for FF2/FR1, Aaltr, Afumi, Cclad2, Enigr, PenGrp3, and PenAsp1mgb, respectively. The qPCR MDLs for FF2/FR1, Aaltr, Afumi, Cclad2, Enigr, PenGrp3, and PenAsp1mgb assays were 0.05, 0.28, 0.05, 0.07, 0.07, 2.8, and 0.05 cells per reaction, respectively.2"). (See also the response to referee #2, response #1). We point out that it is appropriate to use the basic values from Yamamoto et al. (2011) and Hospodsky et al. (2010)1,2 for COV and MDL for two rea-



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sons: First, in aerosol samples collected on filter substrates, accuracy is determined by efficiencies in DNA extraction from sampling filters. Hospodsky et al. (2010)1 examined DNA extraction efficiencies from fungal cells collected on quartz fiber filters, which were the same filters used in this current study. Since the same filters and DNA extraction methods were employed in this study, we assumed the same efficiency value (=10%) to define accuracy associated with the DNA extraction. Secondly, the qPCR MDLs, which are measures of qPCR sensitivity (with units of cell per reaction), do not depend on the type of analyte. To determine the qPCR MDLs, we performed qPCR with known concentrations of pure-cultured standard fungal DNA. Specifically, we defined the qPCR MDL as the smallest fungal concentration where seven successful PCR amplifications out of seven trials (p = 1/128) detected a microorganism concentration with 99% confidence.2 Once the qPCR MDLs are determined using pure-cultured fungal DNA, we calculated the corresponding MDLs in a unit of interest (cell m-3 in this study) with information such as the DNA extraction efficiency from the sampling filters (=10%) and the volume of air collected by the air sampler (=67.8 m3 h−1). The qPCR protocols performed in this study were identical to those previously performed by Yamamoto et al. (2011) and Hospodsky et al. (2010).1,2

Specific comment: 3. Samples were averaged across individual seasons however it is unclear as to what months/samples were considered to be during what season until the reader reaches Fig. 2, in which case the reader does not encounter until after the reading of Table 2, 3 and Fig.1. A definition of the seasons could be included earlier in the text perhaps in the Material and Methods section. On that note, with regards to the seasonal classification method used how relevant is it to the geographical positioning of Rohovot, Israel.

We thank the reviewer for the correction. The definition of the seasons is now given earlier in the text, in P. 5, Lines 100-102 of the revised manuscript, in the Methods section: “The filter samples were segregated according to seasons as follows: Winter (December-February), Spring (March-May), Summer (June-August), Fall (September-



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November)". The reviewer is correct to comment that our region has no significant changes in fungal spores' seasonality. However, there are seasonal meteorological changes due to the unique location of the sampling point. Depending on season, the airflow originates from 4 main air directions: southern Europe & the Mediterranean Sea, North Africa & the Sahara, the Arabian Peninsula or Turkey & central Western Asia. We know from previous studies that the major air flow in Israel changes considerably in the different seasons. This can affect the origins of air masses, as well as the chemical composition and character of airborne particulate matter. 5-7 However, in contrast to the initial hypothesis, this was not reflected in the fungal spores' measurements. Future studies will further investigate this by sampling at several different locations in the region. This issue is now discussed section in P. 12, lines 259-261 of the revised manuscript: "Fungal species distribution did not change significantly throughout the year, although the major air flow at the sampling point changes considerably in the different seasons.5-7".

Added references

1. Hospodsky, D., Yamamoto, N. & Peccia, J. Accuracy, Precision, and Method Detection Limits of Quantitative PCR for Airborne Bacteria and Fungi. Appl. Environ. Microbiol. 76, 7004-7012 (2010).

2. Yamamoto, N., Shendell, D. G., and Peccia, J. Assessing allergenic fungi in house dust by floor wipe sampling and quantitative PCR. Indoor Air 2011(2011).

3. Zhou, G., Whong, W. Z., Ong, T., Chen, B. Development of a fungus-specific PCR assay for detecting low-level fungi in an indoor environment. mol. cell. probe. 14, 339-348 (2000).

4. Herrera, M.L., Vallor, A.C., Gelfond, J.A., Patterson, T.F. & Wickes, B.L. Strain-Dependent Variation in 18S Ribosomal DNA Copy Numbers in Aspergillus fumigatus. J. Clin. Microbiol. 47, 1325-1332 (2009).



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5. Burshtein, N., Lang-Yona, N., Rudich, Y. Ergosterol, arabitol and mannitol as tracers for biogenic aerosols in the eastern Mediterranean. Atmos. Chem. Phys. 11, 829 (2011).

6. Dayan, U., and, Levy, I. The Influence of Meteorological Conditions and Atmospheric Circulation Types on PM10 and Visibility in Tel Aviv. J. Appl. Meteo. 44, 606-619 (2005).

7. Erel, Y., Dayan, U., Rabi, R., Rudich, Y. & Stein, M. Trans Boundary Transport of Pollutants by Atmospheric Mineral Dust. Environ. Sci. Tech. 40, 2996-3005 (2006).

Interactive comment on Atmos. Chem. Phys. Discuss., 11, 28689, 2011.