

Interactive comment on “Comparison between five acellular oxidative potential measurement assays performed with detailed chemistry on PM₁₀ samples from the city of Chamonix (France)” by Aude Calas et al.

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Interactive comment on “Comparison between five acellular oxidative potential measurement assays performed with detailed chemistry on PM 10 samples from the city of Chamonix (France)” by Aude Calas et al.

Anonymous Referee #2 Received and published: 23 February 2018

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General comment:

General comments This work presents a comprehensive comparison study of H_2O_2 acellular oxidative potential (OP) assays and examination of correlations of OPs with an extensive list of chemical components in PM₁₀ samples collected over a year-long period in downtown Chamonix, France (sample size $n=98$). The work was carefully executed. Of special note is that extractions containing the same H_2O_2 concentration of PM₁₀ mas (i.e., 10 $\mu\text{g}/\text{mL}$) were used for the DTT and AA assays, avoiding the complication caused by non-linear response as to PM concentrations. The paper is well-written and the figures are nicely constructed. This work provides a very nice case study of how OPs by various assays are associated with different PM₁₀ components. I have a few minor comments listed below.

We really thank you for this positive comment. Some modifications have been made after the review of the first referee; you'll find them in blue on the new main text version.

Specific comments

1) Please describe the sample collection schedule during the one-year period. Were the samples collected following a regular schedule?

Page 3 line 18: Briefly, ambient particles were collected by filtration during 24 h ($24 \times 30 \text{ m}^3 \cdot \text{h}^{-1}$) with a DIGITEL DA-80 on 150 mm quartz filters (Tissuquartz Pallflex) using the European standard protocol NF EN 16450. DIGITEL DA-80 was automatically program to stock before and after sampled filters, and the samples were then collected every week.

2) As the ESR assay only used 75 samples out of the total 98 samples, please include another column in Table S1 to indicate the number of samples in each month used for the ESR assay.

The table S1 has been modified in order to present number of samples analyzed per assays (as we could't add the table in the plain text of the answer, it was added at the

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end of the review as Fig 1)

3) Page 15, line13: please list the species that show an antagonist effect. This information is worth a special mention.

We modified the sentences as follow:

Page 15 line 12: The intercepts, attributed to unknown species, were significantly > 0 in all models. Moreover, for some species, a negative contribution was found (Table S4) that can be explained by an antagonist effect of some atmospheric components on OP : soot particles for Hellack et al. (2015), Gram positive bacteria for Samake et al. (2017) or metal-organic binding interactions for Wang et al. (2017) or because of the weighting assignation of species in the models.

4) The samples used in this work were PM10 samples. The coarse PM (PM2.5-10), likely accounting for a significant fraction of PM10, does not penetrate all the way to lung. Some components, such as Ti (likely of dust origin), might mainly reside in the coarse mode. Ti is found to be a positive indicator in the multiple linear regression model equation (Eq. (5) for OP ESRv. There might be a disconnection between OP responses obtained under physiological conditions simulating lung fluid and actual OP impacts from breathing in of PM10. It will be good that the authors comment on this disconnection.

We added limitations of our study about the distinction of PM10 and PM2.5:

Page 15 line 26: Several limitations can be attributed to this study. Most important, all of these results have been obtained for a specific location and cannot be generalized as chemical composition of PM10 strongly differs from one location to another. PM10 chemistry is different from PM2.5 and the associations reported here are only valid for PM10. Some components that might mainly reside in the coarse mode are positive factors in the multiple linear regression models (e.g Ti in OP ESRv). They can display a different final health impact, since a fraction of PM10 does not penetrate all the way

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to lung. Also, the results of the ESR assay warrant caution due to our back correction of the ESR signal linked to the non linear response of the assay. Finally, multiple model result for the GSH assay is to be considered with caution since normal distribution was not reached in the first step of the analysis. Finally, these analyses are only relevant for PM10 when some health studies are now taking PM2.5 into account. Additional studies addressing comparison of OP results associated with PM10 and PM2.5 are needed (Gali et al., 2017; Styszko et al., 2017).

Minor comments

5) It appears both ASC and AA are used as abbreviation to refer to ascorbic acid. Why two abbreviations?

It's true that both ASC and AA are abbreviations for ascorbic acid. These distinct abbreviations were used because of the two tests using acid ascorbic and that also use different analyses techniques. In the case of the AA: the test consists in measuring the depletion of a single antioxidant (ascorbic acid) with spectrophotometry techniques. For the ASC, the depletion of ascorbic acid is measured with HPLC system. ASC is part of the RTLf assay in which the depletion of three antioxidants is measured (ascorbic acid, glutathione, and urate).

6) The reference "Chevrier 2016" is given in French. Please provide an English translation and also how this reference can be accessed.

This reference is a PhD manuscript. The English translation was realized:

"Wood heating and air quality in the Arve Valley : definition of a surveillance system and impact of a renovation policy of old devices"

7) Please define "DPCC". The first appearance is line 6 on page 4.

We added the word associated to DPPC:

Page 4 line 10 : For extraction procedure, PM samples were extracted in using a

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Gamble + DPPC (dipalmitoylphosphatidylcholine) solution and vortexed at maximum speed during 2h at 37°C (Calas et al., 2017).

8) Page 5, line 9: Is “the DDT assay” supposed to be “the AA assay” instead?

The sentence was correct. However to make it more clear, the sentence was modified as follow:

Page 5 line 14 : A semi-automated procedure using the same plate reader than for the DTT assay was applied using Greiner UV-Star[®] 96 well plates, this assay was based on the modified assay from Zielinski et al. (1999) and Mudway et al. (2004).

9) Figure S3: is the y-axis label supposed to be “nmol AA/min”?

Yes, thank you for that, the y-axis label has been modified in the latest version of the SI

10) Page 11, lines 2-3: please cite a reference for the criterion for determining whether a correlation is strong or moderate.

This criterion was arbitrary chosen, however it is in the range of criterion commonly found in the literature. For example, in the study of Yang et al. (2014): moderate correlation criteria was attributed to spearman’s correlation (r_s) ranged between $r_s = 0.61 - 0.68$. In the same study, very high correlations were attributed to $r_s > 0.90$ and high correlation for $r_s = 0.86 - 0.96$. In the study of Janssen et al. 2014: high correlations were attributed to r_s ranged between 0.77 and 0.96. Lower correlation were attributed to r_s ranged between 0.4 – 0.6. They also reported the criteria “moderate” for $r_s = 0.39 - 0.62$.

We added more about that in the following sentence;

Page 11 line 2: Spearman correlations (r_s) between the assays were calculated over the sampling year. Correlations were considered as strong for $r_s > 0.70$ and moderate for $r_s (> 0.45$ and $< 0.70)$ which are commonly criteria that can be found in the literature (Janssen et al., 2014; Yang et al., 2014);

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11) Table S4: one entry of Mg → Mg²⁺; NO₃ → NO₃⁻; NH₄ → NH₄⁺

Thanks again for this comment; the modifications have been taken into account in the latest version.

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Months	n				
	DTT	AA	ESR	GSH	ASC
Nov	7	7	7	7	7
Dec	10	10	10	10	8
Jan	11	11	11	11	10
Feb	7	7	7	7	7
Mar	8	8	8	8	8
Apr	9	9	7	9	9
May	5	5	4	5	5
Jun	8	8	5	8	8
Jul	5	5	3	5	5
Aug	8	8	3	8	8
Sep	9	9	4	9	9
Oct	11	11	6	11	11
Total	98	98	75	98	95

Fig. 1.

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