Discussion paper
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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 #1

Received and published: 2 January 2018

1) This paper reports on comparisons of a number of acellular assays during one year of non-continuous sampling at a single site. This type of study is crucial to help guide selection of assays for future use in health studies and to help interpret existing health studied involving measurement of OP. However, this paper is lacking in many ways. First, why was PM10 used when in most studies reporting on aerosol OP the focus is on PM2.5, as it is in most health studies. There are a large number of citations that are not considered in this work, with some result published work reporting completely opposite to the findings reported here and which the authors seem unaware. This is a major issue that must be corrected prior to publication, specifically a more complete Introduction and more complete discussion of results in the context of published work. It should also be made clear that when comparisons are being made to other studies, that those studies also were reporting findings based on PM10 (more on this below). Mixing results from PM10 and PM2.5 studies leads to confusion.

Authors would thank referee for its useful comments. In EU regulation (including France), particulate matter sanitary alert are based on PM_{10} measurements. PM_{10} studied in this paper, originate from the AASQA national French network (http://www.atmo-france.org/fr/). Some references about this issue were added in the discussion.

In addition, we would like to kindly mention that derivated health impacts from PM are very wide and do not stem only from $PM_{2.5}$. PM10 contribute also to health outcomes and a large number of recent epidemiological studies use PM10 to predict health associations from particulate pollution (Griffiths et al, 2016, Piersanti et al, 2016, James, et al. 2015, Segersson et al., 2017, Al-Hemoud,et al , 2018, the European ongoing ESCAPE study : Cesaroni et al., 2014)

Griffiths, C. J., Mudway, I., Wood, H., Marlin, N., Dundas, I., Walton, R., & Kelly, F. (2016). P180 Impact of the london low emission zone on children's respiratory health: a sequential yearly cross sectional study 2008–2014.

Piersanti, Antonio, Carla Ancona, Giovanna Berti, Ennio Cadum, Luisella Ciancarella, Ilaria D'Elia, Francesco Forastiere, and Gaia Righini. "Health impact of air pollution on Italy: main findings of VIIAS and MED HISS projects." (2016).

James, K., Forsen, A., Strand, M., & Cicutto, L. (2015). PM10 Concentrations And Asthma Related Health Services Use In A Rural Community In Colorado. In *C15. NOVEL EPIDEMIOLOGY OF ASTHMA AND COPD* (pp. A3902-A3902). American Thoracic Society.

Segersson, David, Kristina Eneroth, Lars Gidhagen, Christer Johansson, Gunnar Omstedt, Anders Engström Nylén, and Bertil Forsberg. "Health Impact of PM10, PM2.5 and Black Carbon Exposure Due to Different Source Sectors in Stockholm, Gothenburg and Umea, Sweden." *International journal of environmental research and public health* 14, no. 7 (2017): 742.

Al-Hemoud, Ali, Ali Al-Dousari, Ahmad Al-Shatti, Ahmed Al-Khayat, Weam Behbehani, and Mariam Malak. "Health Impact Assessment Associated with Exposure to PM10 and Dust Storms in Kuwait." *Atmosphere* 9, no. 1 (2018): 6.

Cesaroni G, Forastiere F, Stafoggia M, Andersen ZJ, Badaloni C, Beelen R, Caracciolo B, de Faire U, Erbel R, Eriksen KT, Fratiglioni L, Galassi C, Hampel R, Heier M, Hennig F, Hilding A, Hoffmann B, Houthuijs D, Jockel KH, Korek M, Lanki T, Leander K, Magnusson PK, Migliore E, Ostenson CG, Overvad K, Pedersen NL, J JP, Penell J, Pershagen G, Pyko A, Raaschou-Nielsen O, Ranzi A, Ricceri F, Sacerdote C, Salomaa V, Swart W, Turunen AW, Vineis P, Weinmayr G, Wolf K, de Hoogh K, Hoek G, Brunekreef B, Peters A (2014) Long term exposure to ambient air pollution and incidence of acute coronary events: prospective cohort study and meta-analysis in 11 European cohorts from the ESCAPE Project. BMJ 348:f7412. doi: 10.1136/bmj.f7412

Specific comments:

2) The Introduction is missing many key citations. This includes: Page 2, line 11. There are more acellular assays then listed. What about those measuring OH production, eg, [Charrier and Anastasio, 2011; Vidrio et al., 2009]?

We agree with the comment of the reviewer, we modified our sentence as following:

Page 2 line 7: On this basis, probes have been developed over the last decade to quantify the OP of PM as a more refined exposure metric of PM toxicity than PM mass alone (Ayres et al., 2008; Borm et al., 2007). These probes include several acellular assays. The most common consisting in mimicking the consumption of antioxidants (*e.g.* ascorbic acid (AA), reduced glutathione (GSH)) or surrogates (e.g. dithiothreitol (DTT)), the use of the synthetic human respiratory tract lining fluid (RTLF) system (again to assess antioxidant depletion), probes measuring $HO^{\bullet}$ production or the application of electron spin resonance (ESR) to quantify the ability of PM to induce specific ROS (e.g. $HO^{\bullet}$ radicals).

3) Page 2 lines 18-23: A whole series of papers is not discussed for data collected over various seasons that compares two assays (DTT and AA) [Fang et al., 2016] and compares the assays to chemical species or sources [Verma et al., 2012; Verma et al., 2015a; Verma et al., 2015b; Verma et al., 2014].

We agree with this remark and added references

Page 2 line 18 : Only a small number of studies have compared different acellular OP measurements for a given set of ambient PM samples (Fang et al., 2016; Janssen et al., 2014; Künzli et al., 2006; Szigeti et al., 2015; Visentin et al., 2016; Yang et al., 2014). Yet, fewer studies have compared different assays over a year long period to gain a better understanding of seasonal variability (Fang et al., 2016; Jedynska et al., 2017; Saffari et al., 2014; Szigeti et al., 2015; Yang et al., 2015). Finally, there is little research relating the oxidative capacity of particulate pollution with detailed chemical characterization of ambient PM, in an attempt to identify the PM components or sources that may contribute most to underlying toxicity (Fang et al., 2016; Kelly et al., 2011; Saffari et al., 2014; Verma et al., 2014; Weber et al., 2018).

Verma et al., 2012; Verma et al., 2015a; Verma et al., 2015b were not added for not meeting the requirements of this paragraph "detailed chemical characterization AND long time series. (Verma 2012, is only providing two weeks of analysis, Verma 2015a is only focused on Quinones and Hulis, Verma 2015b is only on organic aerosols).

4) Page 2 lines 33, there are more health studies than cited by the author, which are useful to cite in this journal since many readers will not be familiar with the health journals where much of this work is published. Ie, [Abrams et al., 2017; Atkinson et al., 2016; Bates et al., 2015; Strak et al., 2012; Weichenthal et al., 2016a; Weichenthal et al., 2016b].

We agree with this remark and added studies that shown positive correlation between OP assays and health outcomes:

Page 2 line 33: All of these assays have shown some correlations with health outcomes in epidemiological studies (Abrams et al., 2017; Bates et al., 2015; Fang et al., 2016; Strak et al., 2017; Weichenthal et al., 2016a, 2016b; Yang et al., 2016).

5) In my view it is unfortunate that the authors choose to measure and compare PM10 since the composition of PM2.5 vs PM10 is often very different and PM10 less often used in health studies – which is the whole point of this work. It would be helpful to know specifically why PM10 was studied if the goal is to develop insights on assays used in health studies, and how, if any, can these results be applied to PM2.5, which is what the vast majority of OP measurements report. For example, it would be very useful to state what was used (PM10 or PM2.5) in the cited OP-health studies.

In Europe, regulations and particulate matter sanitary alert are based on PM_{10} measurements. PM_{10} studied in this paper, were collected on filters by the certified associations for the monitoring of the air quality (AASQUA).

To clarify, we added the following sentences:

Page 3 line 11**:** In Europe, particulate matter sanitary alert are based on PM_{10} measurements.

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 PM_{10} strongly differs from one location to another. PM_{10} chemistry is different from $PM_{2.5}$ and the associations reported here are only valid for PM_{10}. 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 PM_{10} does not penetrate all the way 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 PM_{10} when some health studies are now taking $PM_{2.5}$ 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).

Styszko, K., Samek, L., Szramowiat, K., Korzeniewska, A., Kubisty, K., Rakoczy-Lelek, R., ... & Giebl, A. K. (2017). Oxidative potential of PM10 and PM2. 5 collected at high air pollution site related to chemical composition: Krakow case study. Air Quality, Atmosphere & Health, 10(9), 1123-1137.

Gali, N. K., Jiang, S. Y., Yang, F., Sun, L., & Ning, Z. (2017). Redox characteristics of size-segregated PM from different public transport microenvironments in Hong Kong. Air Quality, Atmosphere & Health, 10(7), 833-844.

6) When comparing the results of this study involving PM10 to others, which may or may not be measurements of PM10, or even referring to other studies, it must be clear what is being compared. For example, page 11 line 6 and line 9-10, page 12 line 10 and line 16, and page 16 line 2; the results of this study are claimed to be in agreement with Janssen et al, 2014, Yang et al., 2014, etc, but Janssen measured PM10 and PM2.5; are the results being compared to just the Janssen PM10 results? Yang did only PM2.5, so how can these results be directly compared to Yang without noting this important difference?

We added some clarification following this remark:

Page 11 line 6 : The strong correlations between OP DTTv and OP AAv are in agreement with another study on PM_{10} (Janssen et al., 2014) whereas they were not observed by Fang et al. (2016) on $PM_{2.5}$. The weak correlations found between the OP ESR assay and both OP DTT and OP GSH assays are also in agreement with other studies on $PM_{2.5}$ (Künzli et al., 2006; Yang et al., 2014). However, the OP ESR assay is usually highly correlated with OP AA and ASC assays in both PM_{10} and $PM_{2.5}$ studies (Janssen et al., 2014; Künzli et al., 2006; Yang et al., 2014)

Page 12 line 12 : Whereas correlations are evident between OP DTTv, OP AAv, OP GSHv, OP ASCv and some organic and inorganic species, in agreement with other studies regarding both $PM_{2.5}$ and PM_{10} (Janssen et al., 2014; Saffari et al., 2014; Yang et al., 2014),

7) Much more care must be considered given that this work is only PM10.

We added this remark in the limitations of the study

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 PM_{10} strongly differs from one location to another. PM_{10} chemistry is different from $PM_{2.5}$ and the associations reported here are only valid for PM_{10}. 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 PM_{10} does not penetrate all the way 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 PM_{10} when some health studies are now taking $PM_{2.5}$ 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).

8) Page 2 line 12, It is understood that doing a bulk analysis integrates over size, but the way it is stated (Oxidative potential tests for airborne particles integrate several of biologically relevant properties (e.g. size, and chemical composition) likely to drive PM toxicity.) makes it sound like a positive attribute of the assay, it is not. The advantage of these assays is they integrate chemical species in the particles that may contribute to OP, a unifying property potentially linked to the particles toxicity through oxidative stress. Integrating over size is likely not advantageous as in the case of PM10 it mixes very different aerosol sources (chemical components).

We apologize for this misunderstanding. Through the following sentence « Oxidative potential tests for airborne particles integrate several of biologically relevant properties (e.g. size, and chemical composition) likely to drive PM toxicity.", we wanted to refer to this previous statement from Ayres " oxidative potential may integrate various PM characteristics (size, surface area and composition) into a single biologically relevant measure of toxicity," (Ayres, 2008).

J.G. Ayres, P. Borm, F.R. Cassee, V. Castranova, K. Donaldson, A. Ghio, R.M. Harrison, R. Hider, F. Kelly, I.M. Kooter, F. Marano, R.L. Maynard, I. Mudway, A. Nel, C. Sioutas, S. Smith, A. Baeza-Squiban, A. Cho, S. Duggan, J. Froines
Evaluating the toxicity of airborne particulate matter and nanoparticles by measuring oxidative stress potential—a workshop report and consensus statement. Inhalation Toxicology, 20 (2008), pp. 75-99

We rephrased this sentence as following/

Page 2 line 12 :Oxidative potential can be considered as an integrative metric of PM characteristics (size, composition, surface area…) potentially linked to the particles toxicity through oxidative stress "

9) How exactly were the PM10 filter samples collected, eg, what type of filter sampling system at what flow rate.

All the information about PM10 sampling is indicated in the material and methods section:

Page 3 line 18: Briefly, ambient particles were collected by filtration during 24 h (24 × 30 $m^3.h^{-1}$) 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.

10) Page 3 to 6. It would be valuable to know if the assays are performed in a manor exactly consistent with given protocols for each assay. This is given to some extent, but more explicit statements on this would clarify things. For example, does the DTT assay follow the protocol of Cho et al, [Cho et al., 2005], etc?

The following sentences have been modified:

Page 4 line 21: A semi-automated procedure was used with a plate-reader TECAN spectrophotometer Infinite® M 200 pro and 96 well CELLSTAR® multiwall plates from Greiner bio-one®, the assay was modified from the DTT assay of Cho et al. (2005).

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)

11) Page 9 line 2, what does more dispersed data mean? Higher standard deviation?

We modified the sentence as following:

Page 9 line 7 : Overall, higher and more variable OPv values were observed in the cold period than during the warm period where overall values remain low and close to each other.

12) Page 11, line 6, It states, that : OP DTTv and OP AAv are in agreement with another study (Janssen et al., 2014). However this was not found in another study, [Fang et al.,2016].

Page 11 line 6: The strong correlations between OP DTTv and OP AAv are in agreement with another study on PM_{10} (Janssen et al., 2014) whereas they were not observed by Fang et al. (2016) on $PM_{2.5}$.

We are now discussing more the study of Fang et al. 2016 study:

Page 11 line: 16: As shown in Table 3, these correlations also vary with the period, with higher r_s values during cold period ($0.54 < r_s < 0.92$) than in the warm period for which the highest correlation ($r_s = 0.65$) was found between OP DTTv and OP AAv. Temporal variations of correlations were also observed by Fang et al. (2016) on $PM_{2.5}$. During the cold period, r_s between OP methods and PM_{10} ranged from moderate (0.59) for OP ESRv to strong (> 0.7) for the other OP measurements. During the warm period, a strong correlation was observed between PM_{10} and OP DTTv.

13) Univariate analysis of OP with metals. (page 12). Given that total elemental metal concentrations were measured, not water-soluble or speciated metal ions, how can one link these metals to OP assays through redox activity since: 1) Total (elemental) metal concentration is not necessarily correlated with the soluble metal concentration. 2) Only the soluble metal is involved in the redox reactions. The total metals can only be used to identify sources (assuming source profiles were based on total metals).

It's true that total elemental metal concentration is not necessarily correlated with the soluble metal concentration. If only soluble metal is involved in the redox reactions, insoluble metals may also contribute to overall oxidant stress. Insoluble nanoparticles (e.g CuO) complexes and some insoluble metals may lead to positive OP without being soluble by other mechanisms (Calas et al., 2017, Huang et al, 2016, Uzu et al., 2011). Verma et al 2012, showed also that insoluble organics species may contribute to OP. Finally, even if they are questionable and partially answer to the issue of elicits OP drivers, e assume that univariate analysis are useful as a first step to target sources.

Huang, W., Zhang, Y., Zhang, Y., Zeng, L., Dong, H., Huo, P., ... & Schauer, J. J. (2016). Development of an automated sampling-analysis system for simultaneous measurement of reactive oxygen species (ROS) in gas and particle phases: GAC-ROS. Atmospheric Environment, 134, 18-26.

Calas, A., Uzu, G., Martins, J. M. F., Voisin, D., Spadini, L., Lacroix, T. and Jaffrezo, J.: The importance of simulated lung fluid (SLF) extractions for a more relevant evaluation of the oxidative potential of particulate matter, Sci. Reports, (August), 1–12, doi:10.1038/s41598-017-11979-3, 2017.

Uzu, G., Sauvain, J. J., Baeza-Squiban, A., Riediker, M., Sanchez, M., Hohl, S., Val, S., Tack, K., Denys, S.,

Pradère, P. and Dumat, C.: In vitro assessment of the pulmonary toxicity and gastric availability of lead-rich particles from a lead recycling plant, Environ. Sci. Technol., 45(18), 7888–7895, doi:10.1021/es200374c, 2011.

Verma, V., R. Rico-Martinez, N. Kotra, L. King, J. Liu, T. W. Snell, and R. J. Weber (2012), Contribution of water-soluble and insoluble components and their hydrophobic/hydrophilic sub-fractions on the ROS-generating potential of fine ambient aerosols,Environ. Sci. Technol., 46, 11384-11392.

14) Page 15, line 10, what does "PM participating to the background". . . mean

We modified our sentence as follow:

Page 15 line 9: Altogether, these results indicate that on average, the models correctly represent the OP of PM participating to the ROS exposure during the overall year.

15) Page 15. Line 14, some other studies show antagonistic effects on OP that one may wish to consider, see [Wang et al., 2017; Xiong et al., 2017].

Thank you for this comment, the study of Wang et al was added 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.

16) Page 15 line 28: What exactly does this line mean? "Additionally, PM extractions were realized only for the DTT and the AA assays which can lead to a difficulty in the results comparison."

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This sentence was removed

17) Page 16, Line 2, this conclusion is opposite of Fang et al [Fang et al., 2016], which should be noted and possibly discuss possible reasons why.

Page 16 line 6: To achieve this, the DTT and the AA assays, or the DTT and the RTLF assays, can be associated to get the best information, which is in agreement with over studies on acellular OP measurements (Janssen et al., 2014; Yang et al.,

2014). However, a definitive proposition for the best association of assays will most probably come from a final benchmark against epidemiological study outcomes (Fang et al., 2016; Strak et al., 2017; Weichenthal et al., 2016a).

18) Page 15 and 16, I would say the biggest limitation is the use of PM10 for this study instead of PM2.5. This would be a good place in the paper to discuss this (see comments above).

See response to point 7.

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 PM_{10} strongly differs from one location to another. PM_{10} chemistry is different from $PM_{2.5}$ and the associations reported here are only valid for PM_{10}. 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 PM_{10} does not penetrate all the way 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 PM_{10} when some health studies are now taking $PM_{2.5}$ 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).

19) Fang et al, [Fang et al., 2016] did exactly what the last line of the main text states, and it is never discussed, although the paper is cited.

Page 16 line 7 : However, a definitive proposition for the best association of assays will most probably come from a final benchmark against epidemiological study outcomes (Fang et al., 2016; Strak et al., 2017; Weichenthal et al., 2016a).

20) Page 18, line 21 and 22, a source apportionment like that which has already been done, but never cited? See [Bates et al., 2015; Fang et al., 2017; Verma et al., 2014]

Page 18 line 20: Finally, more source apportionment approaches through positive matrix factorization methods are needed in order to assign dominant PM emission sources in the OP assays.

Anonymous Referee #2

Received and published: 23 February 2018

General comment:

General comments This work presents a comprehensive comparison study of five 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 final concentration of PM10 mas (i.e., 10 ug/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 × 30 $m^3.h^{-1}$) 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

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**

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 PM_{10} strongly differs from one location to another.

PM_{10} chemistry is different from $PM_{2.5}$ and the associations reported here are only valid for PM_{10}. 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 PM_{10} does not penetrate all the way 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 PM_{10} when some health studies are now taking $PM_{2.5}$ 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 abbrevations?**

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 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 (rs) ranged between r_s = 0.61 - 0.68. In the same study, very high correlations were attributed to rs > 0.90 and high correlation for rs = 0.86 – 0.96.In the study of Janssen et al. 2014: high correlations were attributed to rs ranged between 0.77 and 0.96. Lower correlation were attributed to rs ranged between 0.4 – 0.6. They also reported the criteria "moderate" for rs = 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);

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.

Comparison between five acellular oxidative potential measurement assays performed with detailed chemistry on PM_{10} samples from the city of Chamonix (France)

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Abstract. Many studies have demonstrated associations between exposure to ambient particulate matter (PM) and adverse health outcomes in humans that can be explained by PM capacity to induce oxidative stress in vivo. Thus, assays have been developed to quantify the oxidative potential (OP) of PM as a more refined exposure metric than PM mass alone. Only a small number of studies have compared different acellular OP measurements for a given set of ambient PM samples. Yet, fewer studies have compared different assays over a year long period and with detailed chemical characterization of ambient PM. In this study, we report on seasonal variations of the dithiothreitol (DTT), ascorbic acid (AA), electron spin resonance (ESR) and the respiratory tract lining fluid (RTLF, composed of the reduced glutathione (GSH) and ascorbic acid (ASC)) assays over a one year period in which 100 samples were analysed. A detailed PM_{10} characterization allowed univariate and multivariate regression analyses in order to obtain further insight into groups of chemical species that drive OP measurements. Our results show that most of the OP assays were strongly inter-correlated over the sampling year but also these correlations differed when considering specific sampling periods (cold vs warm). All acellular assays are correlated with a significant number of chemical species when considering univariate correlations, especially for the DTT assay. Evidence is also presented of a seasonal contrast over the sampling period with significantly higher OP values during winter for the DTT, AA, GSH and ASC assays, which were assigned to biomass burning species by the multiple linear regression models. The ESR assay clearly differs from the other tests as it did not show seasonal dynamics and presented weaker correlations with other assays and chemical species.

1 Introduction

Many studies have demonstrated associations between exposure to ambient particulate matter (PM) and adverse health outcomes in humans. A central mechanism to explain the harmful effects of a range of inhaled particles at the cellular level involves the production of oxidative stress through the generation of excessive reactive oxygen species (ROS) and/or inadequate antioxidant defenses (Borm et al., 2007; Kelly, 2003). The capacity of PM to elicit damaging oxidative reactions is termed oxidative potential (OP).

On this basis, probes have been developed over the last decade to quantify the OP of PM as a more refined exposure metric of PM toxicity than PM mass alone (Ayres et al., 2008; Borm et al., 2007). These probes include several acellular assays. The most common consisting in mimicking the consumption of antioxidants (*e.g.* ascorbic acid (AA), reduced glutathione (GSH)) or surrogates (e.g. dithiothreitol (DTT)), the use of the synthetic human respiratory tract lining fluid (RTLF) system (again to assess antioxidant depletion), probes measuring $HO^{\bullet}$ production or the application of electron spin resonance (ESR) to quantify the ability of PM to induce specific ROS (e.g. $HO^{\bullet}$ radicals). Oxidative potential can be considered as an integrative metric of PM characteristics (size, composition, surface area…) potentially linked to the particles toxicity through oxidative stress. Therefore, they could help to delineate those particle properties (components and sources) responsible for observed health effects. However, each of these assays is sensitive to different panels of ROS generating compounds, and results are also sensitive to the assay design. Indeed a consensus has yet to emerge regarding a standard in vitro test system or combination of tests that would be most appropriate for PM-related health impact evaluation (Ayres et al., 2008).

Only a small number of studies have compared different acellular OP measurements for a given set of ambient PM samples (Fang et al., 2016; Janssen et al., 2014; Künzli et al., 2006; Szigeti et al., 2015; Visentin et al., 2016; Yang et al., 2014). Yet, fewer studies have compared different assays over a year long period to gain a better understanding of seasonal variability (Fang et al., 2016; Jedynska et al., 2017; Saffari et al., 2014; Szigeti et al., 2015; Yang et al., 2015). Finally, there is little research relating the oxidative capacity of particulate pollution with detailed chemical characterization of ambient PM, in an attempt to identify the PM components or sources that may contribute most to underlying toxicity (Fang et al., 2016; Kelly et al., 2011; Saffari et al., 2014; Verma et al., 2014; Weber et al., 2018).

In this study, a series of 100 PM_{10} samples collected over a one year period were screened for ROS burden using 4 acellular measures of OP: DTT, AA, ESR and RTLF assays. The RTLF assay includes 3 antioxidants: reduced glutathione (GSH), ascorbic acid (referred as ASC) and urate (UA). Two ascorbic acid depletion assays (AA and ASC), using different quantification techniques, were therefore included in our analyses. DTT is known to react with organic compounds but also with transition metals (Charrier and Anastasio, 2012; Lin and Yu, 2011). The ESR assay employs the spin trap 5,5-Dimethyl-1-pyrroline N-oxide (DMPO) and is specific for reactive radical species, which for example result from partial reduction of dioxygen catalyzed by transition metal (Boogaard et al., 2012; Shi et al., 2003). The AA assays would be more specific to the oxidative potential of transition metals (Godri et al., 2011; Yang et al., 2014), but ascorbic acid is known to react with organics such as quinones (Shang et al., 2012; Visentin et al., 2016). All of these assays have shown some

correlations with health outcomes in epidemiological studies (Abrams et al., 2017; Bates et al., 2015; Fang et al., 2016; Strak et al., 2017; Weichenthal et al., 2016a, 2016b; Yang et al., 2016). Detailed PM_{10} characterization was performed in parallel, by analyzing up to 130 chemical species that incorporated a broad array of organic species and trace elements (Chevrier, 2016).

In this paper, we report on seasonal variations within the redox activity assays as well as on the correlation among the different assays over a one year period. Univariate and multivariate regression analyses were applied in order to obtain (a) further insight into groups of chemical species that drive OP measurements and (b) evaluate if differences could be detected between the individual assays.

2 Material and methods

2.1 Site description and sampling

In Europe, particulate matter sanitary alert are based on PM_{10} measurements. PM_{10} were collected in the downtown area of Chamonix (45°55'21.53" N, 6°52'11.68" E, Auvergne-Rhône Alpes, France, 1035 masl) in the Alpine Arve valley. This urban location is heavily impacted in winter by biomass burning (wood combustion used for domestic heating) and traffic emissions. Further, because of their topography, specific weather conditions and anthropogenic activities, the European daily limit value for PM_{10} is often exceeded in many sites in the Alpine valleys during the winter period (Chevrier, 2016), including this site in Chamonix.

In the framework of the DECOMBIO project (biomass burning contribution to PM_{10} in the Arve's Valley) PM_{10} sampling and detailed chemical characterizations have been achieved, and are described elsewhere (Chevrier, 2016). Briefly, ambient particles were collected by filtration during 24 h (24 × 30 $m^3.h^{-1}$) 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. The filters were calcined at 500 °C for 8 h before use. After sampling, the filters were folded, wrapped in aluminum foils, sealed in polyethylene bags and stored at -25 °C until chemical analyses and at 4 °C until OP analysis. PM_{10} mass measurements were achieved at the sampling site with TEOM-FDMS, as part of the regular Atmo-AURA network of Air Quality observation (http://www.air-rhonealpes.fr/).

2.2 Chemical analyses

2.2.1 PM_{10} chemical composition

Briefly, collected PM_{10} samples were measured for the following elements and components: elemental and organic carbon (EC ,OC), BC and the distinction between wood burning (BC_{wb}) and fossil fuel BC (BC_{ff}), soluble anions and cations (NO_3^-, SO_4^{2-}, Cl^- , MSA, oxalate and NH_4^+, Mg^{2+}, Na^+, Ca^{2+}, K^+), a large range of inorganic elements (Al, Fe, Ti, As, Ba Cd, Ce, Cr, Cu, La, Li, Mn, Mo, Ni, Pb, Rb, Sb, Sn, Sr, V, Zn and Zr), sugar alcohols (arabitol, sorbitol, and mannitol, also called ∑

Polyols), monosaccharide anhydrides (levoglucosan, mannosan and galactosan, ∑ Monosaccharides), humic like substances (HULIS), and polar and apolar organics tracers (alkanes (∑ alkanes), hopanes (∑ hopanes), methoxyphenols (∑ methoxyphenols), polycyclic aromatic hydrocarbons (∑ PAHs), substituted derivatives (methyl-PAHs) and polycyclic aromatic sulfur heterocycles (∑ PASHs). More detailed information is available in the SI (Section 1).

2.2.2 Oxidative Potential assays

A total of 98 PM_{10} samples collected, from November 2013 to October 2014, were analyzed for redox activity. Since studies have shown a non-linear DTT response to both PM concentrations (Charrier et al., 2016) or from different chemical species added to the assay (Calas et al., 2017; Wang et al., 2017), for the DTT and AA assays (single compound assay), extractions were achieved for each sample to a final concentration of 10 µg.ml $^{-1}$ allowing samples inter-comparison as same extractions at constant-mass were used. For extraction procedure, PM samples were extracted in using a Gamble + DPPC (dipalmitoylphosphatidylcholine) solution and vortexed at maximum speed during 2h at 37°C (Calas et al., 2017). This solution allows for the extraction of PM in an environment closer to physiological conditions. Our previous results (Calas et al., 2017) also show an improvement of PM suspension during the assay thus facilitating the OP DTT measurement. In this study, non-linear response to PM concentrations was observed in the case of the DTT assay but not in the case of the AA assays (more details can be found in the SI Section 2 and Figure S1). Conversely, for RTLF and ESR assays, no extraction procedure was performed. Filter punches of 0.196 cm² were used in a "direct" measurement (cf. individual subsections for more information).

PM OP measurements with the DTT, AA (single compound assay) and ESR assays were performed at the Grenoble Alpes University in early 2016. The RTLF assay was measured at the King's College of London between late 2014 and 2015.

2.2.2.1 DTT assay

A semi-automated procedure was used with a plate-reader TECAN spectrophotometer Infinite® M 200 pro and 96 well CELLSTAR® multiwall plates from Greiner bio-one®, the assay was modified from the DTT assay of Cho et al. (2005). DTT depletion was monitored for 30 min by adopting the following procedure: (1) measurement of the matrix absorbance (Abs_{mat}) of particles and substrate at 412 nm (205 µL of phosphate buffer and 40 µL of PM suspension); (2) injection of 12.5 nmol of DTT (50 µL of 0.25 mM DTT solution in phosphate buffer); (3) for each sample (lab blank included) quantification of DTT immediately (t = 0) and after 15 and 30 min of exposure (50 µL of 1 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) in phosphate buffer) in triplicate. Positive controls consisted in 1,4-naphthoquinone (1,4-NQ) solution and DTT depletion by 1,4-NQ (40 µL of 24.7 µM stock solution) were quantified only once at each measurement time because of the good repeatability between triplicates (Calas et al., 2017).

The rate of DTT loss (nmol.min^{-1}) was determined from the slope of the linear regression of calculated nmol of consumed DTT *vs* time. The amount of remaining DTT was calculated following Eq (1):

$$n_{DTT,i} = \frac{Abs_i * n_{DTT,0}}{Abs_{t0}} \quad (1)$$

where $n_{DTT,\,i}$ is the amount of DTT (nmol) at t=i, Abs_i is the absorbance at t=i, $n_{DTT,0}$ is the initial amount of DTT (nmol) and Abs_{t0} is the absorbance at t=0.

The linear regression was considered acceptable when $R^2 > 0.90$ and when less than 70% of the initial amount of DTT had been oxidized (Li et al., 2009). Matrix absorbance (Gamble + DPPC solution and PM) was subtracted from the final absorbance and DTT loss in the lab blanks was subtracted from DTT loss of the samples in order to obtain the actual DTT depletion of the samples. Normalization per cubic meter, representative of human exposure, was chosen and OP DTTv (nmol DTT.min^{-1}.m^{-3}) was calculated by multiplying DTT depletion per µg of PM added in the wells by PM_{10} mass concentration (µg.m^{-3}). The measurement quality was estimated by calculating the coefficient of variation (CV %) of positive controls (1,4-NQ). The CV was below 2% (n=13). For the DTT assay, a total of 98 samples were analyzed. Field blanks (6 samples distributed over the sampling year) were also analyzed and observed contamination remained constant over the year (more details can be found in the SI Section 2 and Figure S2).

2.2.2.2 AA assay (single compound assay)

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). Matrix absorbance (Abs_{mat}) measurement of PM and substrate at 265 nm was performed (120 µL of Milli-Q water and 80 µL of PM suspension). Then, 24 nmol of AA (100 µL of 0.24 mM AA solution in Milli-Q water) were injected and absorbance was read at 2min and then every 4 min for 30 min. Positive controls (80 µL of 24.7 µM 1,4-NQ solution) were quantified in duplicates. The rate of AA loss (nmol.min^{-1}) was determined from the slope of the linear regression of calculated nmol of consumed AA *vs* time. The amount of remaining AA was calculated in the same way as for DTT (Equation 1). The linear regression was considered acceptable when $R^2 > 0.90$. The matrix absorbance was subtracted from the final absorbance and the AA loss in the lab blanks (blank filter) was subtracted from the AA loss of the samples in order to obtain the actual AA depletion of the samples. AAv (nmol AA. min^{-1}. m^{-3}) were calculated in the same way as for DTT. The measurement quality was estimated calculating the coefficient of variation (CV %) of the positive control (1,4-NQ). The CV was below 2% (n=7). For the AA single compound assay a total of 98 samples were analyzed. Field blanks were also analyzed and no differences with lab blanks were observed (SI Section 2 and Figure S3).

2.2.2.3 ESR assay

A modified ESR assay involving no filter extraction was used (Hellack et al., 2014). This alternative method is highly correlated with that involving filter extraction (Hellack et al., 2014; Yang et al., 2014). This approach is based on the generation of $HO^{\bullet}$ radicals in the presence of hydrogen peroxide, via Fenton-type reactions and their trapping by the nitrone

DMPO. Filter punches (0.196 cm²) were placed in 1 mL tubes, and 125 µL Milli-Q water, 125 µL H_2O_2 (0.5 M) and 250 µl of DMPO (0.05 M, ESR grade) were added. Tubes were subsequently vortexed for 15 s before being placed in incubation at 37° C for 40 min under agitation (detailed information in SI Section 3, Figure S4). Suspensions were then vortexed again for 15s and 35 µL of the suspension were transferred to a capillary tube to measure the hydroxyl radical ($HO^{\bullet}$) formation catalyzed by PM_{10}. The EPR spectra were recorded on a Bruker EMXplus spectrometer equipped with a high sensitivity resonator operating at 9.44 GHz at 293 K. Preliminary results have shown a strong decrease of ESR response when Gamble + DPPC solution was used instead of Milli-Q water, thus justifying the use of Milli-Q water in this study (SI Section 3 and Figure S5). Filter blanks and a suspension of CuO (2.73 M) were used for background correction and as a positive control respectively. The 26% (n=7) coefficient of variation of the positive control was attributed from changes in the dispersion of this insoluble form of Cu. Due to limited remaining samples, extractions could not be performed at identical concentrations for all samples. Corrections were applied to the ESR signal when non-linear pattern vs PM mass was observed (SI Section 3). For the ESR assay, a series of 75 samples only was analyzed due to sample availability. OP ESR was expressed in arbitrary units (A.U.) per cubic meter and will be further referred to as OP ESRv.

2.2.2.4 RTLF assay

For the RTLF assay, 0.5 mL of synthetic RTLF containing equimolar concentrations (200µM) of ascorbic acid (ASC), urate (UA) and reduced glutathione (GSH) were added to triplicate tubes containing 0.196 cm² filter punch and incubated for 4 hours at 37°C under constant mixing and as preliminary results indicated linear RTLF response to PM concentrations added to the assay (internal report). The synthetic RTLF was prepared in chelex 100 treated HPLC grade water (pH 7). Particle-free controls at 0 and 4 h (C0 and C4), together with negative (carbon black M120) and positive (CRM NIST1648a) controls, and lab filter blanks were incubated in parallel (Mudway et al., 2004). After 4 h incubation, the micro-tubes were immediately centrifuged at 13 000 rpm for 1 h at 4°C, followed by removal of aliquots into 100 mM phosphate buffer pH 7.5 (for GSH analysis) or 5% *meta*-phosphoric acid (for ASC and UA analysis). All tubes were immediately stored at -70°C. GSH analysis was derived from the total glutathione and oxidized glutathione (GSSG) analysis using a spectrophotometric enzyme-linked DTNB recycling method based on a modified method described by Baker et al. (1990). The ASC and UA analysis used a reversed-phase high performance liquid chromatography HPLC system (HPLC, Gilson Scientific UK) with electrochemical detection (EG&G, Princeton applied research, model 400) following the procedure described by Iriyama et al. (1984). Field blanks were used for background correction before estimating the consumption of antioxidants. The consumption rate (% OP) of the antioxidants ASC (% OP ASC), UA (% OP UA) and GSH (% OP GSH) were obtained by reference to the 4 h particle-free control (C4). Conversion from % OP to OP per µg of PM and then to OP per cubic meter were also calculated and will be referred to as OP GSHv and OP ASCv. Results for UA are not presented, since PM did not deplete urate (Mudway et al., 2004; Zielinski et al., 1999). The lab blanks and carbon black negative control displayed minimal (< 5%)

background oxidation. The positive PM control (CRM NIST 1648a) reacted with up to 35 % consumption of the ASC. The same 98 PM_{10} samples as those used in the AA (single compound assay) and the DTT assays were analyzed.

3 Data analyses

All statistical analyses were carried out using the R statistical software 3.4.0. Non-parametric Mann-Whitney tests were used in order to evaluate the statistical significance between the cold and the warm periods. Nonparametric Spearman's rank correlations (r_s) were chosen to assess the strength of possible monotonic relationship between the different OP values and the concentration of the different pollutants measured. Linear model (lm) function in R was used for the multiple regressions. P values <0.05 were considered statistically significant.

4 Results and discussion

4.1 PM_{10} mass concentration and temperature in Chamonix

Figure 1A shows the mass concentration of PM_{10} over the sampling period in Chamonix, taking into account the days with filters analyzed for OP (SI Table S1). Corresponding temperatures are illustrated in Figure 1 B. The data are clearly characterized by two periods, a cold period (late November to late February, n=30) and the warm period (late May to late September, n=29) with average temperatures of 1.4°C and 14.9°C, respectively. PM_{10} concentrations were 2.6 times higher during the cold period (Table 1) (29 ± 14 µg/m^3 and 10 ± 2 µg/m^3 for the cold and warm periods, respectively). The significant differences (Figure 1 C) in PM_{10} concentration between these two periods can be explained by different PM_{10} sources, and frequent temperature inversions in this narrow valley in winter. Investigation of the former using a positive matrix factorization (PMF) approach (Chevrier, 2016) indicates that during winter, the dominant emission source is biomass burning (60% of PM mass on average), with 10% due to traffic and about 18% related to secondary inorganic aerosols (SIA). In summer, the main sources are biogenic activity (40%), SIA (35%), and traffic (10%).

Table 1 : Median ratios between cold (late November to late February) and warm (late May to late September) periods for PM_{10} mass concentration and OP measurements expressed per m^3 (OPv) (* p< 0.001; non parametric Mann Whitney test, [a]1st and 3rd quartiles of the temporal ratio).**

	Cold / Warm period [1st , 3rd][a]
PM_{10} mass concentration	2.6*** [2.3, 3.7]
OP DTTv	2.1*** [1.7, 2.9]
OP AAv	7.1*** [6.0, 9.1]
OP ESRv	0.8 [0.7, 1.4]
OP GSHv	5.0*** [4.6, 5.8]
OP ASCv	8.3*** [7.5, 8.7]

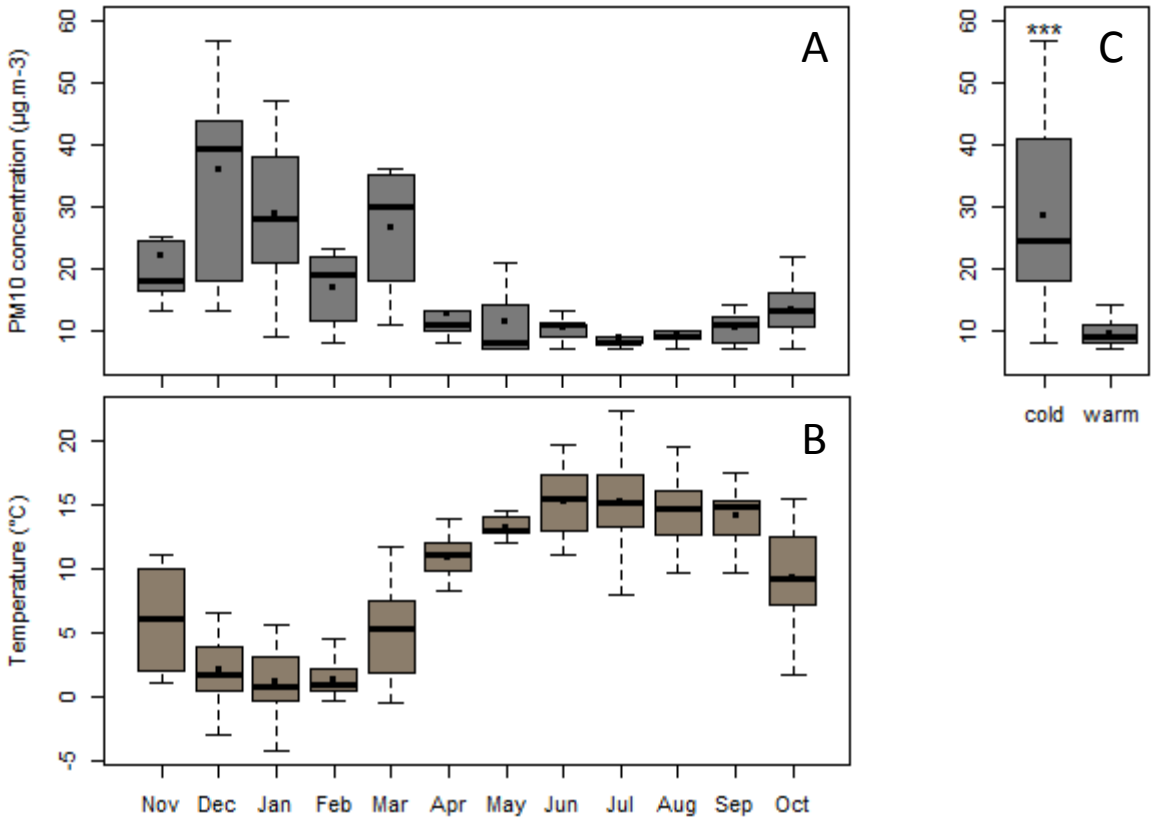

Figure 1 : Temporal variation in (A) PM10 mass concentration and (B) temperature over the 12 month sampling period. (Average PM10 mass concentration during cold and warm periods (C)). Boxplot representations with medians (horizontal line) and means (black square). *** p <0.001, nonparametric Mann-Whitney test.

4.2 OP temporal variation over the 12 month sampling period and during cold or warm periods

Methodological results on non-linear DTT response to PM concentrations are in agreement with Charrier et al. (2016) and were avoided thank to iso-mass concentration extraction. For the AA, ASC and GSH assays non-linear issues were not encountered. Finaly, non-linear ESR response to PM concentrations was solved with backcorrection of ESR signal using linear curve. All together these methodological points allowed avoiding non-additive effects in OP assays and allowed PM samples inter-comparison.

The seasonal variation in OPv (OP measurements expressed per m^3) is reported in Figure 2 (A, C, E, G, and I). Overall, higher and more variable OPv values were observed in the cold period than during the warm period where overall values remain low and close to each other. However, patterns did differ depending on the OP measurement method used. Significant differences between cold and warm periods were observed for the DTT, AA, GSH and ASC assays (Figure 2 B, D, F, H and J), but not for OP ESR (Figure 2 F). For the ESR assay, the yearly pattern could be attributed to a possible scavenging effect of $HO^{\bullet}$ by carbonaceous materials (Hellack et al., 2015) leading to the observed non-seasonal variation. Table 1 presents the median ratios calculated between the two periods. OP ESRv values were equivalent in the 2 periods with a ratio of the medians of 0.8 (close to 1 and the interquartile range includes 1) whilet for the other assays, the ratio was always higher than 2. The OPv DTT was only two times higher during the cold period compared with the warm period, whilst higher contrasts of 5, 7.1 and 8.3 were found using the GSH, AA and ASC assays, respectively.

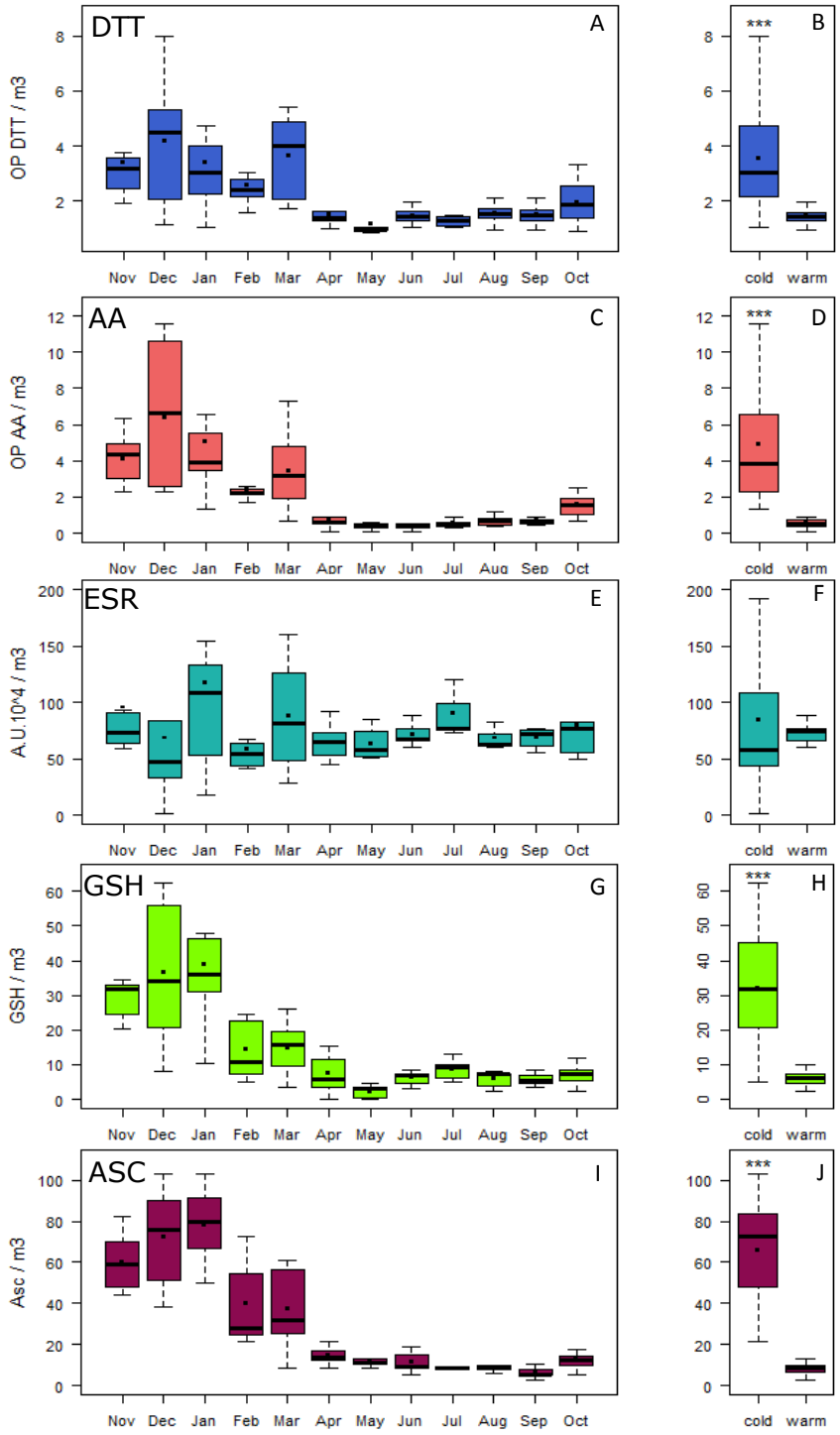

Figure 2: Seasonal variation of the five OPv (A= OP DTT, C= OP AA, E= ESR, G= OP GSH, I= OP ASC) and OPv differences between cold and warm periods (B= OP DTT, D= OP AA, F= OP ESR, H= OP GSH, J= OP ASC). Boxplot representations with medians (horizontal line) and means (black square). *** p <0.001; nonparametric Mann-Whitney test.

4.3 Comparison of the OP measurement assays

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). Table 2 shows that OPv values are all strongly correlated ($r_s > 0.7$) with the exception of OP ESRv values for which correlations ranged from 0.16 (OP ASCv) to 0.45 (OP DTTv). All assays are also strongly correlated with PM_{10} ($r_s > 0.77$), except for OP ESRv ($r_s = 0.33$). The strong correlations between OP DTTv and OP AAv are in agreement with another study on PM_{10} (Janssen et al., 2014) whereas they were not observed by Fang et al. (2016) on $PM_{2.5}$. The weak correlations found between the OP ESR assay and both OP DTT and OP GSH assays are also in agreement with other studies on $PM_{2.5}$ (Künzli et al., 2006; Yang et al., 2014). However, the OP ESR assay is usually highly correlated with OP AA and ASC assays in both PM_{10} and $PM_{2.5}$ studies (Janssen et al., 2014; Künzli et al., 2006; Yang et al., 2014).

Table 2: Spearman correlation of the five OPv measurement assays over the sampling year (* p < 0.001 level, ** p<0.01 level, * p<0.05 level).**

	PM_{10} n=98	**OP DTTv** n=98	**OP AAv** n=98	**OP ESRv** n=75	**OP GSHv** n=98	**OP ASCv** n=95
PM_{10}						
OP DTTv	0.88 ***					
OP AAv	0.81 ***	0.82 ***				
OP ESRv	0.33 **	0.45 ***	0.27 *			
OP GSHv	0.77 ***	0.73 ***	0.80 ***	0.26 *		
OP ASCv	0.80 ***	0.72 ***	0.82 ***	0.16	0.80 ***	

As shown in Table 3, these correlations also vary with the period, with higher r_s values during cold period ($0.54 < r_s < 0.92$) than in the warm period for which the highest correlation ($r_s = 0.65$) was found between OP DTTv and OP AAv. Temporal variations of correlations were also observed by Fang et al. (2016) on $PM_{2.5}$. During the cold period, r_s between OP methods and PM_{10} ranged from moderate (0.59) for OP ESRv to strong (> 0.7) for the other OP measurements. During the warm period, a strong correlation was observed between PM_{10} and OP DTTv.

Table 3 : Spearman correlation of the OPv in the cold (upper part, blue shaded) and the warm period (* p < 0.001 level, ** p<0.01 level, * p<0.05 level, [a]n=30 (cold period), n=29 (warm period), [b]n=30 (cold period), n=14 (warm period), [c]n=27 (cold period), n=29 (warm period)).**

	PM_{10}	OP DTTv [a]	OP AAv [a]	OP ESRv [b]	OP GSHv [a]	OP ASCv [c]
PM_{10}		0.92***	0.91***	0.59***	0.87***	0.90***
OP DTTv	0.71***		0.89***	0.61***	0.79***	0.72***
OP AAv	0.43*	0.65***		0.54***	0.85***	0.79***
OP ESRv	0.088	0.17	0.36		0.56**	0.59**
OP GSHv	0.44*	0.29	0.36.	0.63*		0.92***
OP ASCv	0.38*	0.37*	-0.072	-0.29	0.17	

To better understand the evolution of OP results, OPv were related to the chemical composition of PM_{10} by using both univariate and multiple regressions analyses.

4.4 Univariate data analyses

Spearman correlations between OPv and the chemical composition of PM_{10} (expressed in ng/m^3 or µg/m^3) were calculated over the sampling year and are summarized in Table 4. The highest cumulative score of correlations above 0.45 is seen for OP DTTv (37/48; detailed information on cumulative scores is available in the SI, section 4). Conversely, few correlations above 0.45 (5/48) are seen for OP ESRv. The other 4 assays (OP AAv, OP GSHv and OP ASCv) exhibit very similar correlations with the chemical species.

Whereas correlations are evident between OP DTTv, OP AAv, OP GSHv, OP ASCv and some organic and inorganic species, in agreement with other studies regarding both $PM_{2.5}$ and PM_{10} (Janssen et al., 2014; Saffari et al., 2014; Yang et al., 2014), for OP ESRv correlations are limited to transition metals (Cr, Cu, Mo, Zr) and Ba. All these metals may be associated with traffic emissions, including brake wear (Hulskotte et al., 2014; Sanders et al., 2003). These results can be explained by the specificity of the ESR assay towards hydroxyl radical generation and in the case of Ba (not redox-active) linked to its co emission with the former redox active compounds. These results agree with the study of Boogaard et al. (2012) where the OP ESRv of PM collected near major urban road were highly correlated with Cr, Cu and Ba. However, OP ESRv is also associated to organic compounds (OC, PAH) in other studies (Janssen et al., 2014; Yang et al., 2014).

While many chemical species have a cumulative score of 4/5 over the sampling year, Cu is the only species with moderate to strong correlations with all OP measurements (N=5/5). Several studies, including one in a subway system (Moreno et al., 2017) already pointed out the high impact of Cu concentrations on OP.

Table 4 : Spearman correlations between the OPv measurement assays and the chemical composition of PM10 (dark pink shaded : >0.7, light pink shaded : >0.45) (* p < 0.001 level, ** p<0.01 level, * p<0.05 level, . p<0.10 level. N= cumulative score of correlations (moderate and strong)).**

	DTT	AA	ESR	GSH	ASC	N (/5)
Cl^-	0.68***	0.73***		0.61***	0.77***	4
NO_3^-	0.61***	0.59***		0.58***	0.74***	4
SO_4^{2-}	0.33***		0.27*			
Na^+	0.47***	0.42***		0.34***	0.46***	2
NH_4^+	0.38***	0.25*		0.32**	0.33***	
K^+	0.83***	0.85***	0.34**	0.79***	0.77***	4
Mg^{2+}	0.56***	0.30**	0.43***	0.30**	0.28**	1
Ca^{2+}	0.53***	0.24*	0.32**	0.21*	0.32**	1
Al			0.24*			
As	0.62***	0.44***	0.31**	0.43***	0.41***	1
Ba	0.68***	0.46***	0.57***	0.42***	0.35***	3
Cd	0.64***	0.65***		0.63***	0.72***	4
Ce	0.50***	0.30**	0.35**	0.33***	0.27**	1
Cr	0.55***	0.35***	0.54***	0.34***		2
Cu	0.87***	0.76***	0.48***	0.70***	0.64***	5
Fe	0.71***	0.48***	0.44***	0.43***	0.38***	2
La	0.44***	0.23*	0.37**	0.29**	0.27**	
Li	0.22*					
Mn	0.53***	0.22*	0.41***	0.22*	0.21*	1
Mo	0.65***	0.38***	0.46***	0.40***	0.27**	2
Ni	0.44***		0.37**			
Pb	0.61***	0.42***	0.30**	0.43***	0.37***	1
Rb	0.84***	0.76***	0.33**	0.70***	0.71***	4
Sb	0.79***	0.66***	0.43***	0.59***	0.52***	4
Sn	0.70***	0.70***	0.29*	0.69***	0.83***	4
Sr	0.55***	0.29**	0.44***	0.27**	0.28**	1
Ti	0.36***		0.40***			
V		-0.28**	0.35**	-0.25*	-0.31**	
Zn	0.84***	0.66***	0.40***	0.65***	0.64***	4
Zr	0.70***	0.50***	0.50***	0.48***	0.33***	4
BC	0.82***	0.90***	0.31*	0.75***	0.78***	4
BC_{wb}	0.77***	0.93***	0.27*	0.74***	0.87***	4
BC_{ff}	0.79***	0.84***	0.33**	0.70***	0.70***	4
OC	0.83***	0.87***	0.26*	0.81***	0.86***	4
DOC	0.79***	0.87***	0.29*	0.86***	0.87***	4
HULIS	0.85***	0.87***	0.26*	0.81***	0.86***	4
EC	0.82***	0.93***	0.25*	0.79***	0.84***	4
TC	0.84***	0.91***	0.26*	0.82***	0.87***	4
MSA	-0.26**	-0.53***		-0.36***	-0.34***	
Oxalate						
∑ polyols		-0.33***	0.38***	-0.27**	-0.51***	
∑ monosaccharides	0.74***	0.94***		0.78***	0.87***	4
∑ PAHs	0.77***	0.92***		0.78***	0.88***	4
∑ alkanes	0.63***	0.61***		0.54***	0.77***	4
∑ methyl-PAHs	0.67***	0.85***		0.72***	0.87***	4
∑ PASHs	0.60***	0.76***		0.71***	0.76***	4
∑ hopanes	0.66***	0.67***		0.58***	0.79***	4
∑ methoxyphenols	0.72***	0.93*		0.77***	0.82***	4
N (/48)	**37**	**27**	**5**	**25**	**25**	

4.5 Multiple linear regression models

Multiple linear regressions were investigated in order to obtain further insight into the set of chemical species that can be among the dominant factors to the different OP measurements and for OP variations within time.

Such analysis requires the removal of extreme values, and also require transformations of the OP data in order to obtain distributions as close as possible to the Normal one (detailed information about data set preparation can be found in the supporting information Section 5 and Table S2). The multiple linear regression models were obtained using linear model (lm) function in R software. Forward variable selection and BIC number criteria were used to select the predictors that give the better explanation of the variance of the OPv (SI Section 5 model realizations and validations). The models obtained, for the set of samples excluding the extreme values, are presented in the following Eq (2, 3, 4, 5, and 6):

$$\begin{aligned}\mathbf{log(OP\ DTTv)} = {} & -0.440 + 0.101 \times \mathrm{Cu} + 0.0259 \times \sum \mathrm{PAH} - 0.0247 \times \mathrm{Mg^{2+}} \\ & + 0.273 \times \mathrm{As} + 0.318 \times \mathrm{Mo} + 0.00549 \times \mathrm{MSA} + 0.002 \\ & \times \sum \mathrm{Polyols} + 0.00135 \times \mathrm{Na^{+}}\end{aligned} \qquad R^2=0.81 \qquad (2)$$

$$\begin{aligned}\mathbf{log(OP\ AAv)} = {} & 0.433 + 0.000387 \times \sum \mathrm{Monosaccharides} + 0.756 \times \mathrm{Ni} - 0.372 \\ & \times \mathrm{Mo} + 0.000538 \times \mathrm{Fe}\end{aligned} \qquad R^2=0.93 \qquad (3)$$

$$\begin{aligned}\mathbf{log(OP\ AAv)} = {} & -1.43 + 0.00163 \times \sum \mathrm{Monosaccharides} - 0.00171 \times \mathrm{Ca^{2+}} \\ & + 1.69 \times \mathrm{Sb} + 0.117 \times \mathrm{Cu} + 0.00208 \times \mathrm{Na^{+}} + 0.00119 \times \mathrm{Fe} \\ & - 0.0435 \times \mathrm{Zn}\end{aligned} \qquad R^2=0.87 \qquad (4)$$

$$\begin{aligned}\mathbf{OP\ ESRv} = {} & 339953 + 73037 \times \mathrm{Cu} - 6725 \times \sum \mathrm{Alkanes} - 441351 \times \mathrm{Cd} \\ & + 20795 \times \mathrm{Ti}\end{aligned} \qquad R^2=0.62 \qquad (5)$$

$$\begin{aligned}\sqrt{\mathbf{OPGSHv}} = {} & 1.21 + 0.00161 \times \sum \mathrm{Monosaccharides} + 0.210 \times \mathrm{Cu} - 0.0642 \\ & \times \mathrm{Mg^{2+}} + 0.0268 \times \mathrm{MSA}\end{aligned} \qquad R^2=0.48 \qquad (6)$$

$$\begin{aligned}\mathbf{log(OP\ ASCv)} = {} & 1.79 + 0.000659 \times \mathrm{NO3^{-}} - 0.00733 \times \sum \mathrm{Polyols} \\ & - 0.00292 \times \mathrm{Na^{+}} + 0.000247 \times \mathrm{OC^{*}} + 0.103 \times \mathrm{Cu}\end{aligned} \qquad R^2=0.74 \qquad (7)$$

Two distinct models were associated for the AA single compound assay since the distribution is highly bi-modal with two normal distributions, one in a cold period and one in a warmer period (SI Section 5 data set preparation). Strong coefficients of determination were found for the OP DTTv, AAv and ASCv (R^2 adj > 0.70) indicating that the variance of these OPv was well explained. For the ESRv and GSHv, R^2 were lower, 0.62 and 0.48 respectively (SI Table S4).

Next, these models have then been applied to the general data set (Figure S6), but when strongly overestimated, values were removed again (SI Section 5 application to the overall data set). The models tend to underestimate OP values (negative intercept and slope = 0.84) for the ASC assay. For the other assays, the models tend to overestimate high OP values and to underestimate low OP values (negative intercept and slope between 1.11 and 1.51). However, the coefficients of determination range from 0.73 (OP ASCv) to 0.89 (OP AAv). Altogether, these results indicate that on average, the models correctly represent the OP of PM participating to the ROS exposure during the overall year.

Finally, the contribution of each predictor during cold and warm periods has been investigated (Figure 3) (SI Section 5 contribution of each predictor). 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. During the cold period, traffic tracers or indicators (like Cu, Fe, Mo, Ti, or ∑ PAHs) are important positive factors to explain all OP measurements. Also, and with the exception of the ESR assay, biomass burning tracers (∑ Monosaccharides, including levoglucosan) or indicators such as ∑ PAHs or OC* (corresponding to total OC minus the molecular species measured) both strongly related to biomass burning emission (Bonvalot et al., 2016; Chevrier, 2016), are prominent positive factors for all assays for this cold period. These results agree with the observations on the seasonal evolution of OPv, with much larger values in the cold period when biomass burning emissions are dominant. Additionally, literature results on DTT reactivity towards organic compounds indicate higher impact of biomass burning species when compared to other organic compounds (Verma et al., 2015). During the warm period, traffic tracers and especially copper are important positive factors in OP measurements. In addition, road dust and industrial tracers (Zn, Ca^{2+}) for the AA assay, and primary biogenic indicators (∑polyols, MSA) for the DTT and GSH assays seem prominent factors of these OP measurements.

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 PM_{10} strongly differs from one location to another. PM_{10} chemistry is different from $PM_{2.5}$ and the associations reported here are only valid for PM_{10}. 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 PM_{10} does not penetrate all the way 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 PM_{10} when some health studies are now taking

$PM_{2.5}$ 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).

However, all results point out that biomass burning and vehicular emissions in winter are the main sources correlated with ROS generation. In summer, ROS burden is associated to vehicular emissions, biogenic emissions, road dust, and industrial sources. All these results also suggest to associate assays in order to take into account this wide range of OP determinants. To achieve this, the DTT and the AA assays, or the DTT and the RTLF assays, can be associated to get the best information, which is in agreement with other studies on acellular OP measurements (Janssen et al., 2014; Yang et al., 2014). However, a definitive proposition for the best association of assays will most probably come from a final benchmark against epidemiological study outcomes (Fang et al., 2016; Strak et al., 2017; Weichenthal et al., 2016a).

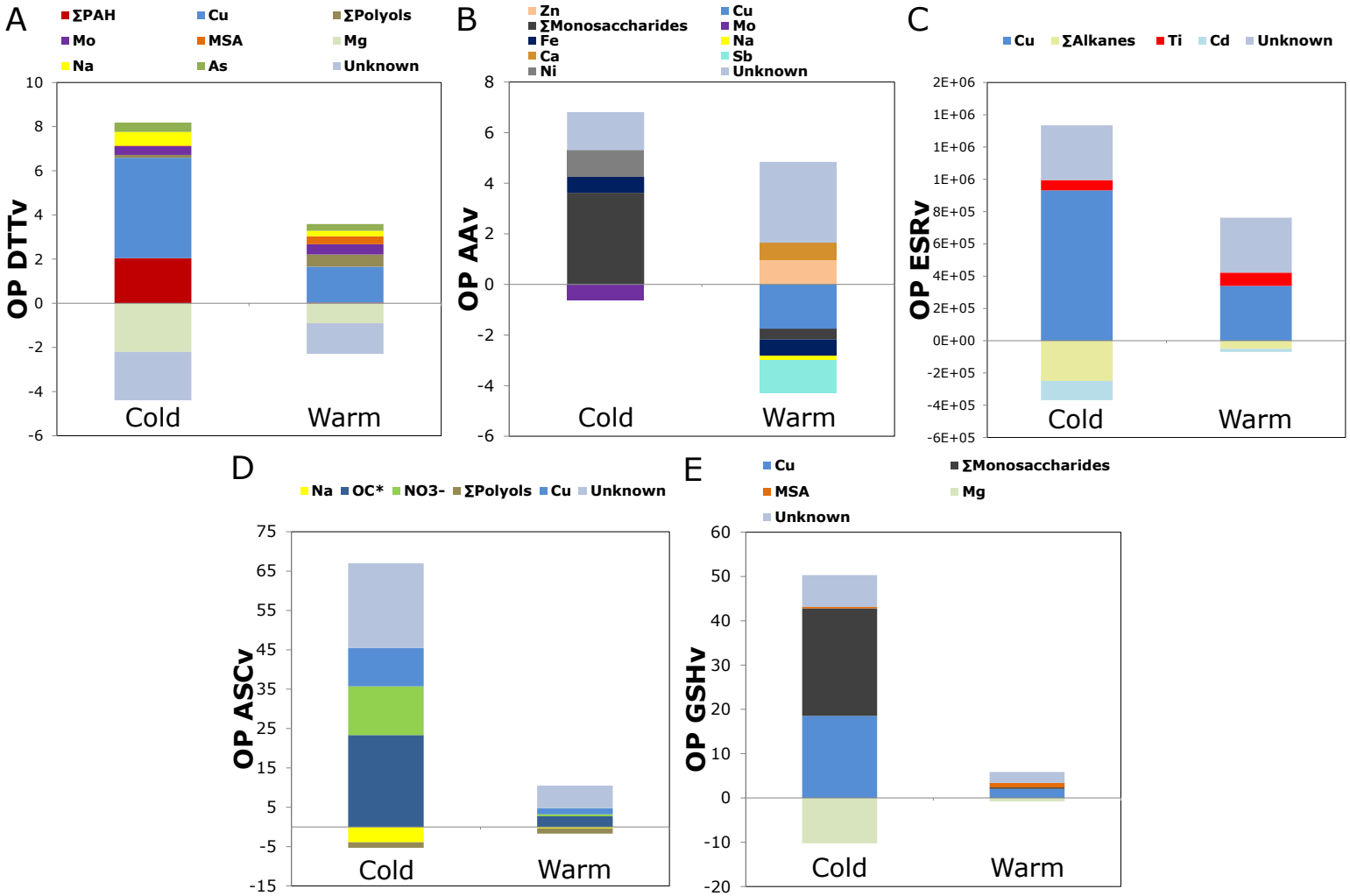

Figure 3: Multivariable linear regression analyses results: Contribution of predictors during cold and warm periods (A: OP DTTv, B: OP AAv, C: OP ESRv, D: OP GSHv, E: OP ASCv). The intercepts, attributed to unknown species, were significantly > 0 in all models. Some species were assigned with negative contributions, explained by a weighting assignation of species so as to model OP differences between warm and cold periods or a possible antagonist effect of some atmospheric components on OP contribution.

5 Conclusion

Our results show that most of the OP assays were strongly inter-correlated over the sampling year but also these correlations differed when considering specific sampling periods (cold vs warm).

All acellular assays are correlated with a significant number of chemical species when considering nonparametric rank correlations, especially for the DTT assay. Finally, copper seems to be a unifying determinant in the OP assays.

Evidence is also presented of a seasonal contrast over the sampling period with significantly higher OPv values during winter for the DTT, AA, GSH and ASC assays, which were assigned to biomass burning species by the multiple linear regression models. However, during the cold period and for the DTT assay, ∑ PAHs, which can be associated to both traffic and biomass wood burning emissions, were found to be significant factors. The ESRv clearly differs from the other tests as it did not show seasonal dynamics and presented weaker correlations with other assays and with the chemical species. Nevertheless, ESR assay results are mostly associated with traffic tracer species. Finally, the combination of 2 models was used to fit the results of the AA assay which is necessary to provide the best explanation of OP variance, with a bi-modal distribution of the initial measurements. This indicates that the strong changes in the chemistry of the PM are probably leading to non-linear processes in the link between chemistry and OP.

Overall, these results suggest to combine assays in order to take into account a wide range of determinants of OP. In the case of the Chamonix city, DTT associated with AA assays or DTT combined with RTLF assay were able to provide the most exhaustive information about OP determinants (Cu) and sources associations (biomass burning, vehicular emissions).

Rank correlations and multiple linear regressions are useful tools to determine the most prominent species driving the redox activity of ambient PM. However, to go further in identifying the assay or combination leading to the best information relying on source dynamics, multiple linear regressions analysis that require large data sets. Finally, more source apportionment approaches through positive matrix factorization methods are needed in order to assign dominant PM emission sources in the OP assays

Competing interests. The authors declare no conflict of interest or competing financial interest

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