Disparities in particulate matter (PM_{10}) origins and oxidative potential at a city-scale (Grenoble, France) - Part II: Sources of PM_{10} oxidative potential using multiple linear regression analysis and the predictive applicability of multilayer perceptron neural network analysis

Authors' response

We would like to thank the referee for their time to re-evaluate the revised manuscript. We appreciate the efforts made to further improve the manuscript. Our point-by-point response to the extra comments are presented below in **bold red**.

Lines 127-129 : Insoluble particles can be a large source of uncertainty, as they are not uniformly mixed in the solution. They can interfere with spectrometric analysis via physical absorbance.

Reply: The extraction procedure in this study is based on Calas et al. (2018), also published by our group. This procedure has been tested on both soluble and insoluble compounds that are (as much as possible) within the range of atmospheric concentrations. To avoid the interferences in the wells by insoluble particles, we subtracted the intrinsic absorbance of all PM extractions before adding reactants. Also, the particles are extracted in the Gamble solution (an artificial lining fluid) where we add a surfactant: this was shown to maintain a good dispersion of particles, leading to homogeneous results (see Calas et al., 2018). This is summarized in Table S5 of Calas et al. (2018). All analysis was performed in triplicate, with a coefficient of variation (CV) ≤ 5%.

Information about avoiding interferences from insoluble particles is not given in the methodology section. Brief methodology shall be clearly given in this MS, and for details it is fine to give the reference. Please include.

Reply: To address this comment, we provided additional information in the manuscript that reads:

"To avoid the interferences in the wells by insoluble particles, we subtracted the intrinsic absorbance of all PM extractions before adding the reactants. This procedure has been tested on both soluble and insoluble compounds that are likely within the range of atmospheric concentrations. The results have confirmed good dispersion of particles, leading to homogeneous results. A more detailed report is available in Calas et al. (2018)."

Lines 134-135: This suggests the precision of the measurements. How do you ascertain the accuracy of the measurements for each assay?

Reply: In every experiment, a positive control 1,4 naphtoquinone and an ambient filter (PM sampled from the lab roof) were analysed to ensure accuracy of measurements. All analysis was also performed in triplicate, with a coefficient of variation (CV) ≤ 5%.

This answer is not clear. Was 1,4 nathoquinone used for all the three assays? I don't think all the three assays respond to this chemical. Please explain. How the ambient PM filter was used for the accuracy of all the three assays? It can only be used for the consistency (precision), which is already shown by CV of each analysis. Authors should provide the

output of this accuracy experiment for each assay in the main MS. This information will also be very useful for readers.

Reply: We thank the reviewer for this comment. To further clarify, in every experiment, both positive controls and an ambient filter were analysed to ensure stability of the OP analysis. The ambient filter sampled from the lab roof has a known and constant expected OP value. The ambient filters were analysed to ensure precision of measurements.
Indeed, 1,4-naphthoquinone (1,4-NQ) was used for positive control tests for DTT and AA assay. Particularly, a 40 µL of 24.7µM stock solution was used for DTT assay and a 80 µL of 24.7µM 1,4-NQ solution for AA assay (Calas et al., 2018, 2017). Finally, we used a 100 nM H_2O_2 for DCFH assay. The measurement quality was estimated by calculating the coefficient of variation (CV%) of the positive controls, all CVs were <3% for the 3 assays. These are now added in the manuscript as:
"For positive control tests, the 1,4-naphthoquinone (1,4-NQ) was used for both DTT and AA assays. Particularly, a 40 µl of 24.7 µM stock solution was used for DTT assay and an 80 µl of 24.7 µM 1,4-NQ solution for AA assay (Calas et al., 2017, 2018). A 100 nM H_2O_2 was used for DCFH assay. The measurement quality was estimated by calculating the coefficient of variation (CV) of the positive controls, all CVs were <3% for the 3 assays. Additionally, an ambient filter collected from the lab roof, with a known and constant expected OP value, was analysed to ensure precision of OP measurements."

Lines 281-283: This is very important point of the paper but not clear at all. Mass-normalised assays obviously depend upon the PM composition and not the PM mass. Different assays respond to different species. The statement written in lines 281-283 is confusing. Please elaborate this sentence in detail.

Reply: The comparison of the two measures (OP_m and OP_v) allows us to see its dependency on mass concentration. An r-value of 0.76 between variable A and B represents a direct proportionality between two variables. Since, OP_v is calculated by multiplying OP_m by mass concentration, then the linear relationship between the two measures is actually the dependence of both measures to mass concentration—mainly driven by meteorological conditions especially in the Alpine valleys.

This discussion is related to Fig. S3 where OPm vs OPv are plotted for all the three assays. The slope of this plot would be the inverse of PM mass. The reason of this plot is still not clear. One should plot OPv vs PM mass conc. The slope of this plot would be average OPm. If the correlation between OPv and PM mass is very strong, it would reflect that the intrinsic OP of PM is uniform over the study site. If there is a poor correlation, intrinsic OP of PM is expected to be variable due to various reasons. I suggest to plot the Fig.S3 again in the recommended form and discuss.

Reply: The slope of the plot is in terms of PM mass concentration. Please be reminded of the units of the two measures (OP_m and OP_v), discussed in section 2.3. The OP_m is in nmol min^{-1} μg^{-1}, while OP_v is in nmol min^{-1} m^{-3}.

Fig. 5: BB is not contributing to OP-DTT as much as it contribute to OP-AA and OP-DCFH. This is unexpected as OP-DTT is most responsive to organics. Please explain.

Reply: Thank you for this comment. We acknowledge the fact that OP from DTT assay has

been reported to be responsive/sensitive to organics. However, recent studies have reported that OP from DTT assay is not affected by some metals (specifically iron) like other assays, namely AA and GSH. Because of this, OP from DTT assay may not fully capture ROS generated through Fenton chemistry or even the synergistic effects with regards to •OH generation as reported by Xiong et al. (2017). Similarly, Yu et al. (2018) has reported that soluble manganese showed synergistic effects with quinones on OP from DTT assay, while soluble copper appears to have an antagonistic effect with quinones on the same assay. On the contrary, manganese showed an antagonistic relationship with quinones on •OH generation. Quinones and soluble iron or copper react synergistically to form •OH.

Generally, there is an undeniable interplay between species that needs to be considered as well as the sensitivity of each assay to species. As much as each analysis attempts to fully characterize the chemistry of PM, there can still be many species that are unmeasured but, in fact, plays a role in ROS generation. Hence, reported associations could be due to similarity in variations with PM concentration rather than a significant causal relationship between assays and PM components.

Due to the sensitivity of DTT assay to wider range of compounds, such as organics and metals, that are present in various sources, this lead to a more balanced distribution of OP sources (and so weighting the contribution of biomass burning with regards to other sources) than the other OP assays, such as AA and DCFH.

This discussion shall be appropriately included in the revised MS.

Reply: We have included this discussion in the manuscript :

"It is also interesting that biomass burning appears to be contributing less to OP_m in the DTT assay compared to both the AA and DCFH assays. We acknowledge the fact that OP from DTT assay has been reported to be responsive/sensitive to organics making this quite intriguing. However, recent studies have reported that OP from DTT assay could be unreactive to some metal species (specifically iron) unlike other assays, namely AA and glutathione (GSH). Hence, OP measured using DTT assay may not completely capture ROS from Fenton chemistry or even the synergistic effects with regards to hydroxyl radical (•OH) generation as reported by Xiong et al. (2017). Similarly, Yu et al. (2018) has reported that soluble manganese showed synergistic effects with quinones, while an antagonistic effect between soluble copper and quinones. Generally, there is an undeniable interplay between species that needs to be considered as well as the sensitivity of each assay to species. As much as each analysis attempts to fully characterize the chemistry of PM, there can still be species that are unmeasured but, in fact, play a role in ROS generation. Hence, reported associations could be due to similarity in variations with PM concentration rather than a significant causal relationship between assays and PM components. Nevertheless, the sensitivity of DTT assay to a wider range of compounds that are present in various sources, lead to a more balanced distribution of OP sources (and so weighting the contribution of biomass burning with regards to other sources) than the other OP assays, such as AA and DCFH."

Lines 345-355: Why industrial (or other) sources are responding differently to OP at different sites? Explain.

Reply: In the companion paper (section 3.5.1), we have presented the metric PD-SID (Pearson

distance and standardized identity distance) that measures (dis)similarities of chemical profiles by each source. There are some sources that have been identified as heterogenous sources, including the industrial source. This means that the tracers used to identify the industrial source can be different between the 3 sites in this study. It could also imply that there is a varying origin of this source across the Grenoble basin. Due to this difference, it is expected that the OP contribution of the industrial source can be different as well, after all it is considered a heterogenous source. A similar comment by Referee #1 has also been addressed in Line 51.

Use of multiple tracers for Industrial source is confusing because different tracers respond to OP assays differently. Authors can split this Industrial source in different subsets using their unique proxy. In present form, it is very confusing for the readers.

Reply: The industrial factor is generally identified by high loadings of specific metal species (Figure S3.10 in the companion paper). Although, there is a difference in the chemical profile, the metal species used are all usual tracers of industrial-related sources. The authors deem that it is unnecessary to sub-categorize the industrial sources further.

General comment:

How the OP-DTT, OP-AA, and OP-DCFH of PM10 observed over the study regions compare with the other parts of the world? This should be included and discussed.

Reply: The authors deem that this is outside of the scope/goal of this paper. After all, this is not a review paper on OP studies. However, our group has a paper (currently under review process in ACPD) that tackles the synthesis of OP measurements over many sampling sites in France.

This is not a correct thinking. Authors have reported the values of three assays over three closely located sites. It will be meaningful to add a paragraph (with a Table) on how the measured OP values compare with some other sites of the world with similar (or different composition). Add some discussion on this comparison. It is obvious that this MS is not a review article. But for readers, it will be useful to see some discussion on 'comparison'.

Reply: Thank you for this comment. We understand the interest of the reviewer on a global comparison of OP levels. We agree it would provide useful information to readers. However, the objective of the manuscript is to investigate the OP variability within a medium-sized urban area and the corresponding influence in terms of contributions of the emissions sources to OP.
A global comparison of OP levels will lead towards a discussion tackling the difference in OP protocols across the world-- a topic that deserves a publication on its own. For example, the PM extraction methods could vary by solvent (water, organic, surrogate lung fluid) and conditions (i.e., iso-mass vs non iso-mass). There are also differences in the filter types (Teflon, quartz, zeflour) and sampling procedures (PM size, sampling duration). There also varying methods of calculating OP activity (% depletion, anti-oxidant consumption). These are variables to consider on top of the variabilities brought about by different a-cellular assays (DTT, AA, ESR, DCFH, GSH to name a few).
We understand the immense importance of a standard method for OP analysis to facilitate inter-study comparisons across the world. In fact, our group has a paper in review (Weber et al. (2021), https://acp.copernicus.org/preprints/acp-2021-77/acp-2021-77.pdf) that presents a national synthesis in France that could pave way towards inter-study

comparisons in the future. To further clarify, we have added the sentence below in the manuscript:

"The range of the OP measurements in Grenoble are well within the range of measurements in France (Calas et al., 2018, 2019b; Weber et al., 2021, 2018)."

Lines 49-51: Give a proper definition of OP.

Reply: Thank you for this comment. This sentence now reads as:

Action: The oxidative potential (OP) of PM, defined as the capability of PM to generate ROS, makes an interesting complementary to regulated metrics of ambient PM exposure (Bates et al., 2019; Daellenbach et al., 2020; Guo et al., 2020; Gurgueira et al., 2002; Park et al., 2018; Shiraiwa et al., 2017; Valavanidis et al., 2008).

It should be -
....., defined as the capability of PM to generate ROS/deplete anti-oxidants, ...

Reply: Thank you for this comment. This sentence now reads as:

"The oxidative potential (OP) of PM, defined as the capability of PM to generate ROS/deplete anti-oxidants, makes an interesting complementary to regulated metrics of ambient PM exposure (Bates et al., 2019; Daellenbach et al., 2020; Guo et al., 2020; Gurgueira et al., 2002; Park et al., 2018; Shiraiwa et al., 2017; Valavanidis et al., 2008)."