We thank the reviewers for their comments. Our responses and corresponding changes made in the manuscript (highlighted in red) are given below.

Response to anonymous referee #1 comments:

General comments: It is important to characterize and identify the components of PM contributing to its oxidation potential. Time-resolved and automated analysis systems are important to better assess exposure effects. The paper addresses the need for faster laboratory results and compares different methodologies to identify the relevant fractions contributing to PM oxidative potential. However, the methodology applied in some cases is not well thought and important points have not been addressed in the manuscript or considered by the authors. Thus, some of the conclusions are not correct, unless several assumptions are made.

Main Comments:

1) **Line 53: authors note that water soluble OP is the common focus since it is the most straightforward. This reviewer disagrees as total DTT is most commonly reported and is a direct measurement, easier to conduct than water soluble OP, as no extraction, filtration or phase separation is required to conduct the analysis.**

   Response: *We believe it is a large assumption that one can comprehensively measure all water-insoluble components in a detection system that is based on transferring particles collected on a solid surface (filter) that are then transferred to a liquid for performing the chemical assay and the subsequent quantitative analysis. An a priori assumption that this can be done with 100% efficiency is likely a poor assumption. We believe it is much easier (and more likely to be closer to 100% efficient) to extract a filter with water, filter the sample and then perform the analysis in water to comprehensively measure just the water-soluble species. As proof we note that one does not find any systems (that we know of) for measuring total carbonaceous aerosol concentrations based on liquid systems, but measurement of water-soluble organic carbon is a widely accepted practice. (See issues with particle adhesion, brought up by this reviewer, below). As noted in the manuscript, there is no consistent approach to determine total OP^{DTT}, and some methods are highly complex involving the evaporation of organic solvents. There have also been no reported intercomparisons of the various methods used and no widely accepted standard method for measuring total OP, making it difficult to compare the results between studies. These are the exact issues addressed in this paper. The challenge is that there is no gold standard for comparison, so we are left with comparisons between methods. For the sake of not making too strong a statement, we have deleted "since it is the most* straightforward to measure*" in line 53.*

2) **Line 100: System comparison is important. Identifying the bias between collection systems is vital to identify and correct for differences in the results. However, as both systems are collecting PM in similar conditions, how can authors explain that there is no difference for WS-DTT but they have a considerable 10% for total DTT? The authors should indicate a possible explanation for this difference, is this due to**

the extraction? It is difficult to imagine a sampling difference that will only affect the non-water soluble components.

Response: *This is simply measurement uncertainty. Considering the combined uncertainties from sample preparation and system precision, a 10% bias in $OP^{Total-DTT}$ is very reasonable. This bias is mainly believed to be due to differences in sampling flow rates of the samplers, which affects the filter loading and has a secondary effect on the cutoff size of the particle collection. Cut size may have some influence since water-soluble and insoluble OP^{DTT} have different size distributions (Fang et al., 2016), the bias in OP^{WS-DTT} and $OP^{Total-DTT}$ due to the shift of the cutoff size can be different.*

Reference:

Fang, T., Zeng, L., Gao, D., Verma, V., Stefaniak, A. B., and Weber, R. J.: Ambient size distributions and lung deposition of aerosol oxidative potential: a contrast between soluble and insoluble particles, Environ Sci Technol, 2017.

3) **Line 116. Authors indicate that the results from the comparison between extraction by sonication or shaking are similar; although the correlation is reasonable, the scattering is significant. This variation with extraction may indicate an effect of the extraction method depending on the chemical composition of the PM collected and extracted.**

Response: *Since linear regression is a statistical model, the error terms in the fitting results do not only result from the effects of measurements, but is also due to some statistical factors, such as sample size and the distribution of the data points. In this test, in addition to the variation with extraction, the small sample size (N=7) and the relatively concentrated data distribution (OP values are mainly in the range of 0.16-0.24 nmol/min/m^3) can also lead to the significant scattering. Therefore, we cannot draw any reliable conclusions just based on the error terms. Note that the findings are consistent with those reported by other researchers (see Antinolo, M., Willis, M. D., Zhou, S. M., and Abbatt, J. P. D.: Connecting the oxidation of soot to its redox cycling abilities, Nat Commun, 6, 2015).*

4) **Line 150. Method 1. After extraction with water and resuspension of the extracted solution, there is a considerable amount of sample removed from the filter. These particles are not then extracted with methanol which may lead to underestimating the OP of the methanol extract. Did the authors estimate the loss of particle mass during the first extraction? How much of the original sample remain in the vial for the consecutive extraction? It will be important to know how much is lost if corrections are to be made for more accurate comparison of the OP of the different fractions. Thus, the OP-Total DTT1 (line 164) obtained by adding the OP of each fraction is not accurate and already biased for comparison with the other extraction methods. To obtain a more accurate measurement of the OP of each fraction, and as result the total obtained using this method, the vial containing the extracted sample could be centrifuged and the supernatant removed. This way resuspended particles**

will remain in the vial for further extraction. Particles can be resuspended by short vortex mixing, and the sample ready for methanol extraction.

Response: *The reviewer's description does not follow the procedure of method 1. The filter is extracted in water and all of the water extract separated from the filter and the liquid sample then filtered with a syringe filter and the DTT levels determined. The original filter is then extracted in methanol... The reviewer is right in that the liquid filtration after water extraction can remove insoluble particles that would have been measured in the methanol-soluble fraction, but now removed from the analysis (ie they won't be measured with the water-soluble or subsequent water-insoluble fraction), which can cause the under-measurement of OP. This is exactly why the method is not preferred and the total OP^{DTT} levels are likely lower compared to the other methods that do not have this limitation. We did not measure this fraction lost since it is likely very small and would be difficult to perform, and furthermore is not routinely done by researchers using this method. The suggested method involving centrifuging could be undertaken, but again, is generally not done because it is too complex. (See first comment above on the assertion that measurements of total OP^{DTT} are straightforward). Finally, the point of this paper is to compare the stated methods of measuring total OP^{DTT}.*

5) **Line 177. Method 2. Again, there is a bias in the method as a considerable fraction of the collected PM is removed with the filter.**

   Response: *Yes, there can be a bias in method 2, and this is just one reason why it likely is not an ideal approach to measuring total OP^{DTT}. Again, the point of this paper is to compare the methods, as they are stated. Note that the bias cannot be large since no large differences were observed between the methods of measuring total OP^{DTT}. Because one would then be looking for small differences, (the reviewer gives a possible cause), quantifying the cause of the bias would be very difficult, but again, this is not the point of the paper.*

6) **Depending on the particle size, extraction efficiency varies. What was the extraction efficiency in this case? How much of the sample was removed with the filter? Ultrafine particles are not extracted from the filters easily and as many studies have shown, they are usually the main contributors to the OP of PM. As a result, the measurement is not accurate and comparison with other methods is not valid. Corrections could be made if the efficiency was known and OP measurements adjusted accordingly.**

   Response: *We assume that for water-soluble OP^{DTT} measurements, the extraction efficiency is 100% for all sizes of particles. We also agree that extracting particles from a solid surface is difficult due to adhesive forces, and increases in difficulty for smaller particles. Ultrafine particles have significant intrinsic OP^{DTT} (per-PM-mass basis), however, their contributions to air-volume-normalized fine particle OP (used in this study to evaluate human exposure) is small (see Fang et al, 2017). Therefore, the lower extraction efficiency of ultrafine particles is not likely a significant issue. Also this is why method 3 is preferred, where the assay is performed with the filter present so that the*

chemical reactions can occur on surfaces of particles still attached to the filter. We would like to point out that this comment directly contradicts the first comment made by the reviewer, that measurement of total OP^{DTT} is much easier than water-soluble OP^{DTT}.

Reference:

Fang, T., Zeng, L., Gao, D., Verma, V., Stefaniak, A. B., and Weber, R. J.: Ambient size distributions and lung deposition of aerosol oxidative potential: a contrast between soluble and insoluble particles, Environ Sci Technol, 51, 6802-6811, 2017.

7) **Line 192. Automated system. In the description of the analytical method authors indicate that the system lines, valves and pump are only clean after a sample is run, have the authors considered interference in the results by cross contamination and sample interference between consecutive injections of the same sample? The solution remaining in the lines, ports and valves could interfere with the signal of the next injection. As the time between injections is considerable, reaction between DTT and solution can continue while in the lines and valves.**

   Response: *In the automated system description, we do discuss the cleaning of the system after each sample run. However, this is not the only cleaning step, and there are other cleaning procedures during the analysis. For example, when the addition of each chemical is completed, the syringe pump is washed by pushing DI through. The DI water used for liquid dilution can also clean the tubing. Air is pushed though the tubing to avoid liquid residual. These steps are too detailed to be mentioned in a brief system description. We are providing the program code in the SI so that the reviewer can see more details.*

   We note that the system analysis results are consistent with the results obtained by the manual method which is considered the most accurate (shown Fig. 4) and also considers this cross contamination issue, indicating the accuracy of the system described here.

8) **Line 305. This sentence is speculation. Unless a comprehensive chemical analysis is conducted to identify the nature of the compounds associated with the sample, the statement is authors speculation.**

   Response: *Page 11, line 311-312: the sentence has been replaced with* "The lower $OP^{Total\text{-}DTT\text{-}1}$ may be due to liquid filtration after water extraction.".

9) **Line 311. Based on pervious comments made regarding the methods, this statement may not be correct. There is considerable amount of sample removed from the vial prior to the methanol extraction, which may lead to underestimation of the real potential of the insoluble components. If authors did not quantify the particle loses the methanol extract OP cannot be assumed accurate.**

   Response: *This has been extensively discussed above. Again, we agree that filtration of water extracts may lead to underestimation of OP levels of sequential methanol extracts.*

However, the data show this is not a large bias. Furthermore, this will not significantly affect the constituents that can be extracted by methanol. As a consequence, the correlation between $OP^{WI\text{-}DTT\text{-}3}$ and $OP^{sM\text{-}DTT}$ can still provide information about the properties of OP-related water-insoluble species.

10) **Line 335. The OPsM-DTT is not a direct measure of the oxidation potential of water insoluble component. Components associated with PM present different solubility in different solvents. Many components are not extracted by methanol, and required other organic solvent for extraction. Extraction with hexane, dichloromethane, or acetonitrile, as examples, may result in different OP of the insoluble fraction. Thus, this reviewer disagrees with the statement made by the authors. It will be very difficult to directly measure the OP associated with the insoluble material. The more accurate measurement will be an estimation based on the difference between total OP and water-soluble (filtered) OP.**

    Response: *The reviewer is right. On page 12, line 343-345: the sentence has been modified to* "$OP^{WI\text{-}DTT}$ obtained in method 1 is determined from the direct measure of $OP^{sM\text{-}DTT}$, whereas $OP^{WI\text{-}DTT}$ is determined by difference for method 2 and 3, which leads to larger uncertainty and more scatter associated with these data".

11) **Line 350. A contribution of 35 to 42% of the insoluble material to the total OP is not "some contribution", it is a significant fraction of the total OP.**

    Response: *"some contribution" here is indicated by the correlation, not based on the* $OP^{WI\text{-}DTT}$*-to-*$OP^{Total\text{-}DTT}$ *ratio.*

    Page 12, line 360-361: the sentence has been changed to "The correlation coefficients between $OP^{WI\text{-}DTT}$ and $OP^{Total\text{-}DTT}$ were 0.87 and 0.84 for quartz filters at GT and RS, respectively (Table S1), which reflects the contribution of insoluble species to total OP as well".

12) **Line 364. This sentence is again speculation. The authors do not present any results regarding the chemical composition to support the idea of secondary compounds as main contributors to the oxidative potential. Identification of primary and secondary compounds, as well as correlation between the different fractions, is required to validate this statement. Wind pattern, local emission sources and measurements at each location are needed to indicate the presence and contribution of primary and secondary compounds.**

    Line 395. Again, this is speculation by authors. Unless the correlation between primary and secondary compounds with OP is presented, there are no bases to make this statement.

    Response: *In the comments above, the reviewer questions the validation of the statement that PM OP contributors are largely secondary. We would like to address them all at once. Studies have shown that OP^{DTT} is mainly attributed to oxidized aromatic species*

and soluble transition metals. Vehicles emit precursors for both: tailpipe emissions from incomplete combustion are sources of PAHs, the precursor for quinones; and brake or tire wear and road dust are precursor for soluble transition metals. While the primary emissions exist, which is demonstrated by the much higher level of EC and metals at RS, the homogenous distributions of OP^{DTT} suggest secondary atmospheric processing plays an important role in converting the precursors to DTT-active species. This is supported by a large and growing body of research.

13) **Line 394. OP measurements for two points, GT and RS, are not representative of a wide area, so concluding the measured OP were largely spatially homogeneous is too broad of a statement.**

    Response: *Page 14, line 405: deleted "largely".*

14) **Line 520. Table 2. Authors present correlation between OP measured under different methods and chemical speciation. Among the chemical compounds presented by the authors, K shows important correlation with OP. However, this compound has not been shown to be redox active or a chemical that can react with DTT. Why do the authors include this compound in their list and their correlations? If this is not a redox compound it does not contribute to the OP, and the correlation is not significant; unless the compound is used as a marker for sources that can contribute to other compounds that do contribute to the OP of the sample. A coherent explanation is needed regarding the inclusion of K in the table and results.**

    Response: *K was presented as a marker of biomass burning. Based on the reviewer's suggestion, we have modified the manuscript to include this information.*

    Page 8, line 228-229: "The elements of interest included species that possibly play a role in ROS generation (Fe, Mn, Cu; Schoonen et al., 2006) and K, a marker of biomass burning (Artaxo et al., 1994).*"*

Response to anonymous referee #2 comments:

I am fine with the paper, I do not have any other comments, I recommend acceptance.

Response: *We thank the review for their recommendation on the acceptance.*

Response to anonymous referee #3 comments:

This article reported three methods of analyzing aerosol oxidative potential (OP) based on DTT assay, focusing on different sample extraction strategies to distinguish soluble components from the total aerosol particulate matter (PM). I think this is a good work with practical meaning to assist the understanding of oxidative aerosol's transport in atmosphere. I have one major suggestion as below.

1) Figure 1 delivered a clear demonstration of the three sample extraction and analysis methods. However, I think a better job can be done to explain what component(s) from the total PM species is measured by each method throughout the text. Basically, two factors, including extraction solvent (water versus methanol) and filter (i.e., with or without filtering) differentiate the three methods. Which component in the aerosol is screened by each factor should be clearly specified to elucidate what is the actual difference across the three methods. From my understanding, the use of water or methanol discriminates the water-soluble (herein "soluble" matter includes dissolved molecules/ions and "dispersed" solids (or small particles)) and methanol-soluble component, whereas filtering or not filtering a sample discriminates dissolved molecules/ions and dispersed solids in that sample. Overall, extraction combined with these two factors actually categorizes the total PM into six groups: water-insoluble species (those which cannot be extracted into water), water-soluble molecules/ions, water-soluble solids (dispersible small particles), methanol-insoluble species (those which cannot be extracted into methanol), methanol-soluble molecules/ions, and methanol-soluble solids. The authors should clarify which groups are measured in each of the three methods, either with a diagram or plain text.

Response: *We thank the reviewer for an interesting suggestion. However, we feel that the detailed presentation approach the reviewer suggest is so detailed that it may hinder understanding the contrasts in the methods. Furthermore, focusing on solubility is not the complete issue, there are the extraction issue (how solid particles are removed from the filter media) and transport issue (how solid particles are transported to the location where DTT analysis is performed). Solubility is related to this, but we feel this moves the focus away from the physical aspects involved in making a total OP^{DTT} measurement. Basically, we are trying to include the contribution of water-insoluble species to OP^{DTT} in two ways: one is to get the water-insoluble components dissolved in organic solvent (methanol was chosen in this study as it is widely used), and the other is to get solid particles exposed to the DTT assay (i.e., don't dissolve them). To be clear, we clarify which species is measured for the three methods in the summary table (Table XXX).*

Elucidating the above may rationalize a few ambiguous places better in the articles. What follows are some examples.

2) **L59-60,76-77, there seem to be some conflicts between these two references: the first place says up to 99% of the DEP CANNOT be extracted by water or methanol while the second one says the measurement is based on water extraction. "after which the filter was removed from the analysis" is very confusing. What are the authors trying to say here?**

   <u>Response:</u> *There is no contradiction between the two citations which refer to different things. The sentence in line 76-77, refers to the method used to measure the **total** OP of diesel exhaust particles (DEP). The method is based on water extraction, but insoluble particles remain suspended in the extracts. In McWhinney et al., the OP of water and organic extractable fractions were also determined. The difference between total and extractable fraction is what we are referring to in line 59-60, i.e., the **non-extractable** fraction.*

   *We have modified the sentence on page 3, line 76: "*McWhinney et al. (2013) measured total redox activity of DEP using particle suspensions that were obtained by a water-extraction procedure with the filter removed after extraction.*".*

3) **L100-101: what is "10%" here?**

   <u>Response:</u> *Page 4, line 99-101: the sentence has been changed to "*The bias between the two High Volume samplers was assessed by running them side-by-side at GT for 9 days. The measurements were within 10% for both water-soluble OP^{DTT} (OP^{WS-DTT}) and total OP^{DTT} (OP$^{Total-DTT}$) (obtained by method 3, described below) (Supplement Fig. S2).*".*

4) **L300-323: The justification of choosing method 3 over the other two is poor. It may be improved if the authors can specify each species category corresponding to each separation technique as I suggested. I have specific questions as below.**

   <u>Response:</u> *In line 300-323, the methods are compared only based on the levels of OP measurements. The conclusions here are not an overall evaluation. The specific changes in the manuscript are given in the response below.*

5) **L305-309, it seems method 1 is more consistent with SCAPE study, than method 3; then why is method 3 selected over method 1?**

   <u>Response:</u> *Only method 1 was deployed during the SCAPE study, so our data was not compared with the SCAPE data for method 3. The purpose of comparing the data in this*

study to SCAPE data is to confirm that our data are reasonable and fall into the normal range of ambient samples.

To clarify, the sentence on page 11, line 312-314 has been changed to "The ratios of OP^{sM-DTT} to OP^{WS-DTT} are 0.34 ± 0.14 (N=35) at GT and 0.37 ± 0.12 (N=31) at RS, which are consistent with the ratios from SCAPE data (0.27 ± 0.08, N=198; unpublished data) and fall into the typical range of ambient samples.".

6) **L309, what is indicated by "very little correlation between $OP^{WI-DTT-3}$ and OP^{WS-DTT}?**

Response: *Line 316-318: "There was very little correlation between the $OP^{WI-DTT-3}$ and OP^{WS-DTT} with Pearson correlations of r = -0.23 and -0.51 at GT and RS sites, respectively (see Supplement Table S1),* which further indicates the importance of water-insoluble compounds to a total OP measurement.".

7) **L315, I am uncomfortable with "overall" here, because the only justification of method 3 over method 1 is that it measures higher total-DTT (which is actually quite common sense). The whole paragraph seems quite over-informative and little relevant to the conclusion.**

Response: *We have modified the expression of the conclusion and rearranged some sentences to clarify the logic.*

Page11, line 305-323: "Figure 5 shows the OP^{DTT} m^{-3} comparison between method 1 and 3 at both GT and RS sites. In general, the response of the DTT assay of method 3 was significantly higher than that of method 1 at the 95 % confidence level (paired t-test: p = 0.028 at GT, N=35; p<0.001 at RS, N=31). The results are expected since in method 1, both the water and methanol liquid extracts are filtered, potentially removing species that could have been DTT-active but remained attached to solid particles. A few observations where $OP^{Total-DTT-3}$ is less than $OP^{Total-DTT-1}$ are likely due to propagation of errors for the summation method (method 1) combined with variability in the extraction process for each method. *The mean $OP^{Total-DTT-1}$ to $OP^{Total-DTT-3}$ ratio at GT was close to 1 (ratio = 0.95) and also higher than that at RS (ratio = 0.85). This may imply that method 1 can be more effective for extracting aged PM species. The ratios of OP^{sM-DTT} to OP^{WS-DTT} are 0.34 ± 0.14 (N=35) at GT and 0.37 ± 0.12 (N=31) at RS, which are consistent with the ratios from SCAPE data (0.27 ± 0.08, N=198; unpublished data)* and fall into the typical range of ambient samples. *The water-insoluble OP determined by the difference in $OP^{Total-DTT-3}$ (which includes solid particles) and OP^{WS-DTT} ($OP^{WI-DTT-3} = OP^{Total-DTT-3}$ - OP^{WS-DTT}) to OP^{WS-DTT} ratio, by contrast, is 0.45 ± 0.25 at GT (N=35) and 0.67 ± 0.35 at RS (N=31). There was very little correlation between the $OP^{WI-DTT-3}$ and OP^{WS-DTT} with Pearson correlations of r = -0.23 and -0.51 at GT and RS sites, respectively (see Supplement Table S1),* which further proves the importance of water-insoluble compounds in OP assessment. Additionally, *$OP^{WI-DTT-3}$ was weakly correlated with OP^{sM-}*

^{DTT} (Pearson correlation: r = 0.31 at GT; r = 0.04 at RS). Based on these data, it is clear that there were species associated with water-insoluble OP^{DTT} not extracted by methanol and that remain attached to solid particles. This analysis shows that filtering the liquid extract, even if methanol solvent is used, will result in a substantial underestimation of $OP^{Total-DTT}$. Therefore, in terms of the OP response, method 3 is preferred to method 1. Furthermore, the comparison between these two methods can provide insights into the water-insoluble components that contribute to PM OP."

8) **L319-323, the same problem is with the comparison of method 2 and method 3. There is not actual justification for why method 3 is chosen over method 2. Information is very redundant and little relevant to the conclusion.**

   Response: *As we replied in question 4), the comparison in this paragraph was only based on OP response.*

9) **L330-339, it's somewhat ironic here. I was told that method 1 is better than method 2 and 3 in terms of seeking correlation between OP-DTT and PM compositions but then suddenly method 3 was selected to be used... Why?**

   Response: *We have modified this section to clarify the logic.*

   Line 333-345: "A correlation analysis was performed between measured $PM_{2.5}$ chemical constituents and OP^{DTT} determined by the three methods. Figure 7 shows the correlation results (detailed values are provided in Table S2). It is seen that $OP^{Total-DTT-3}$ is better correlated with the measured species than $OP^{Total-DTT-1}$ and $OP^{Total-DTT-2}$. Compared with OP^{WS-DTT}, the stronger correlations between $OP^{Total-DTT-1}$ and PM species suggests that $OP^{Total-DTT-1}$ captures more chemical components contributing to DTT than OP^{WS-DTT}. In contrast, $OP^{Total-DTT-2}$ is correlated with the least number of measured PM species.

   By subtracting OP^{WS-DTT} from $OP^{Total-DTT}$, OP^{WI-DTT} is determined for the three methods. In general, the correlations between OP^{WI-DTT} and PM species are mediocre for all three methods, with a slightly better performance of method 1. The water-insoluble OP^{DTT} determined by method 1, i.e. OP^{sM-DTT}, has good correlation with OC at GT and OC, EC and water-soluble Fe at RS. Verma et al. (2012) also showed good correlations between OP^{DTT} of filtered methanol extracts and OC and EC, and attributed this association to water-insoluble organic carbon species (WIOC) that dissolve in methanol. Thus, OP^{sM-DTT} in method 1 is likely attributed to some fraction of the WIOC. OP^{WI-DTT} obtained in method 1 is determined from the direct measure of OP^{sM-DTT}, whereas OP^{WI-DTT} is determined by difference for method 2 and 3, which leads to larger uncertainty and more scatter associated with these data.".

10) **L348: I recommend clarifying how 35-42% is determined and specify the error range as well.**

Response: *The manuscript has been modified.*

Page 12, line 357-360: "The ratios of OP^{WS-DTT} to OP$^{Total-DTT}$ were on average 65 ± 10 % (insoluble accounts for 35 ± 10 %) and 65 ± 14 % at GT, compared to 62 ± 12 % and 58 ± 10 % at RS, for quartz and Teflon PM samples, respectively. Thus, OP$^{Total-DTT}$ of PM$_{2.5}$ contained on average 35 to 42 % insoluble species."

11) **Overall, I strongly suggest the authors give their recommendations on which method should be used in what scenarios or for what purposes.**

   Response: *We added a summary table (Table 2) and modified the text accordingly.*

   Page 12, line 346-350: "The overall assessment of the three methods is summarized in Table 2. By comparison, it is found that method 3 has better precision, more comprehensive response (i.e., generally highest OP$^{Total-DTT}$), stronger correlations with PM components, and easiest filter preparation (extraction) process, all of which provide an efficient way for OP$^{Total-DTT}$ determination. The other two methods have some value owing to their insights into the attributes of water-insoluble OP contributors. In a subsequent study, discussed next, only Method 3 was utilized to measure OP$^{Total-DTT}$ of PM for Teflon filters.".

Other minor suggestions:

12) **L133-134: provide possible explanations on what gives the response in blank samples.**

   Response: *Based on the reviewer's suggestion, the manuscript has been modified to include the possible explanations for blank OP values.*

   Page 5, line 134-136: "Since DTT is a relatively unstable compound, it can react with dissolved oxygen in the liquid in the absence of particles (Kumagai et al., 2002), resulting in OP response in blanks. The blank OP values are also due to trace levels of contaminants on the filter, in the DI-water, and introduced during sample preparation."

13) **L275-278: Maybe provide a figure in SI to illustrate the validation with five ambient samples as well?**

   Response: *The figure (Fig. S7) is added in Supporting info.*

14) **Table 1: Does "N" filters correspond to different samples? (I assume CV is determined with three replicates on each filter (each sample)?) and what is the range standing for.**

Response: *Yes, "N" stands for the number of filters used for duplicate measurements. CV is determined with triplicate on each filter. CV values for each method vary from sample to sample, and the CV range is given based on the maximum and minimum measured.*

To be clearer, change "Coefficient of variation (CV)" in Table 1 to "Coefficient of variation (CV) from triplicate*".*

15) **Table 2: I think a figure is better to let readers see the correlations of different variables, although I know there are many comparisons here. Maybe the authors can think if the illustration here can be improved.**

    Response: *We added polar plots (Fig. 7) showing the correlation coefficients between OP^{DTT} and different PM species. The table is moved to Supplement (Table S2).*

Response to anonymous referee #4 comments:

Review for A method for measuring total aerosol oxidative potential (OP) with the dithiothreitol (DTT) assay and comparisons between an urban and roadside site of water-soluble and total OP by Dong Gao et al.

This manuscript describes a careful study further developing a technique previously published by the group. Different aerosol sampling and extraction methods are compared and first field data are presented to quantify aerosol bound OP. After addressing the points listed below, I recommend publication in AMT.

Response: *We thank the reviewer for the recommendation on acceptance. Our responses and corresponding changes made in the manuscript (highlighted in red) are given below. Note that the comments the reviewer gave were based on the initially submitted manuscript, so the line numbers in the responses may be inconsistent with that in the reviewer comments.*

1) **p.2, line 37: OP(total-DTT) is that the unfiltered methanol extract fraction? It might be best not to use undefined abbreviations in the abstract.**

   Response: *Thank the reviewer for the suggestion. To be clear, we added "*Therefore, the third method was applied to the field study for the determination of total OP.*" on page 1, line 17-18.*

2) **p. 2, line 40-41: This sentence is not clear to me. Was the same DTT analysis performed on Teflon filters? Please be more specific.**

   Response: *The sentence has been modified.*

Page 1, line 22-23: "Same DTT analyses were performed and similar results were found using particle composition monitors (flow rate of 16.7 L min^{-1}) with Teflon filters."

3) **p. 3, line 62/63: It would be good to mention also a reference for the DCFH assay besides DTT and ascorbic acid.**

   Response: *Reference for the DCFH assay has been added.*

   Page 2, line 39-40: "Various methods have been developed to assess PM OP (Ayres et al., 2008; Cho et al., 2005; King and Weber, 2013; Mudway et al., 2004; Shi et al., 2003; Wang et al., 2011)."

4) **p.8, line 167 – 169. I do not agree with the statement that sonication has no effect on radical formation. The paper cited, Miljevic demonstrates explicitly the opposite. Fig. 3 in that paper shows clearly that sonication strongly oxidises DTT! It is not clear to what section in the SI this sentence refers to. Fig. S3 or S5? In Fig S5 both axes are labelled that same. So it is not clear what Fig S5 is showing. This statement has to be worded much more carefully and the results presented and the potential effects of sonication have to discussed critically by representing literature results correctly!**

   Response: *We agree that Miljevic did report the sonication affects DTT, we, and others, however, have failed to see such an effect. We found that the DTT analysis for samples extracted by sonication and shaking produced similar results, indicating that any radicals formed during sonication caused negligible bias in the measurement of PM OP. The work of Antinolo et al. (2015) shows the same results.*

   To be clearer, the sentence has been modified. Page 4, line 116-118: "Considering the potential for radical formation during the sonication process (Miljevic et al., 2014), experiments using sonication versus shaking were done. Little difference observed in OP for sonication versus shaking OP indicated negligible bias in OP^{WS-DTT} measurement due to sonication, see Supplementary Material Fig. S3."

   In terms of SI Fig. S5, we have corrected the graph in the updated manuscript.

   Reference:

   Antinolo, M., Willis, M. D., Zhou, S. M., and Abbatt, J. P. D.: Connecting the oxidation of soot to its redox cycling abilities, Nat Commun, 6, 2015.

5) **p. 10, line 223: I would strongly advice to call the "water-insoluble OP" "methanol-extracted OP" as this is highly misunderstanding. There are many organic compounds that are not water soluble but are also not soluble in methanol, such as many PAHs. By reconstituting the methanol extract again in water, it is likely that components which are not water soluble precipitate again and are thus not**

accessible to the DTT oxidation anymore. This potential artefact should be discussed.

Response: The points raised are exactly why method 1 is not ideal for measuring total DTT. We have decided to keep the original terminology but recognize the issue raised. Since methanol was also used for extraction in methods 1 and 2, "methanol-extracted OP" could be misunderstood as well. We would also like to point out that although it is true that some water-insoluble species cannot be extracted by methanol (e.g., PAHs, as noted), it is not necessary to extract all the water-insoluble species since some of them are not DTT-active, such as PAHs (Cho et al., 2005; Charrier et al.,2012). Thus, not extracting PAHs is not a problem since they are not DTT-active. Methanol, however, has been found to be effective in extracting DTT-active species, such as HULIS (Vishal et al., 2015).

Reference:

Charrier, J. G. and Anastasio, C.: On dithiothreitol (DTT) as a measure of oxidative potential for ambient particles: evidence for the importance of soluble transition metals, Atmos Chem Phys, 12, 9321-9333, 2012

Cho, A. K., Sioutas, C., Miguel, A. H., Kumagai, Y., Schmitz, D. A., Singh, M., Eiguren-Fernandez, A., and Froines, J. R.: Redox activity of airborne particulate matter at different sites in the Los Angeles Basin, Environ Res, 99, 40-47, 2005.

Verma, V., Wang, Y., El-Afifi, R., Fang, T., Rowland, J., Russell, A. G., and Weber, R. J.: Fractionating ambient humic-like substances (HULIS) for their reactive oxygen species activity – Assessing the importance of quinones and atmospheric aging, Atmos Environ, 120, 351-359, 2015.

6) **p. 11, line 241-247: Filtration of quartz fibre filters usually results in significant dis-integration of the filter and many loose quartz fibres in the extract. As these samples were not filtered, it should be described in more detail how this was dealt with and what potential artefacts this might have caused for the DTT analysis.**

Response: We suppose that what the reviewer is concerned about in this question is the quartz filter fiber produced by "extraction (i.e., sonication)" not "filtration". In our experiments, there was little evidence that the quartz fiber filter disintegrated during extraction, and the filter punch remained in one piece in most cases, making it easier to remove the filter out. However, this is one of the reasons why we analyzed these extracts using the modified automated system where the liquid sample is filtered after the DTT analysis prior to injecting into the LWCC to avoid clogging. Since quartz fibers are not DTT-active, any potential artifacts due to the remaining quartz fibers can be accounted for by subtracting filter blanks.

7) **p. 11, line 252: Why was K-buffer used in method 3 but not in the other methods? Please explain this differences and potential consequences for the results.**

Response: *K-buffer was used in all three methods to adjust the pH of DTT incubation solution. The use of K-buffer is mentioned in both **2.2.1 OP^{WS-DTT} analysis** and **Automated system for OP$^{Total-DTT}$ measurements**. In method 3, K-buffer was added before sonication and used as the extraction solvent, which would not influence the final OP measurement. K-buffer has been directly used to extract PM-loaded filters in many studies (McWhinney et al. (2013); Liu et al. (2015)).*

Reference:

Liu, Yongchun, et al. "Chemical and toxicological evolution of carbon nanotubes during atmospherically relevant aging processes." *Environmental science & technology* 49.5 (2015): 2806-2814.
McWhinney, Robert D., et al. "Filterable redox cycling activity: a comparison between diesel exhaust particles and secondary organic aerosol constituents." *Environmental science & technology* 47.7 (2013): 3362-3369.

8) **p. 18, line 398: Please check all "WS-DTT" and "WI-DTT" superscripts. It seems to me there are some typos.**

Response: *We have carefully checked all the superscripts and found no typos. The superscripts are exactly what we want to use and express, i.e., to compare the ratio of water-insoluble OP to water-soluble OP.*

9) **p. 19, Table 2: Was there any difference observed in the correlation between DTT values and total and water soluble metal concentrations?**

Response: *Some OP^{DTT} values have similar correlations with both water-soluble and total elements, which is possibly due to the good correlation between water-soluble metal and total metal concentrations (see Fig. S1). However, we can still observe that OP$^{Total-DTT-3}$ is correlated better with total metal than with water-soluble metal for both GT and RS site. This may imply that the total OP measurements can capture some water-insoluble species, and therefore strengthen the relationship between OP and total elements.*

A method for measuring total aerosol oxidative potential (OP) with the dithiothreitol (DTT) assay and comparisons between an urban and roadside site of water-soluble and total OP

Dong Gao[1], Ting Fang[2], Vishal Verma[3], Linghan Zeng[2], Rodney J. Weber[2]

[1]School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA
[2]School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA 30332, USA
[3]School of Civil and Environmental Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

Correspondence to: Rodney J. Weber (rweber@eas.gatech.edu)

Abstract. An automated analytical system was developed for measuring the oxidative potential (OP) with the dithiothreitol (DTT) assay of filter extracts that include both water-soluble and water-insoluble (solid) aerosol species. Three approaches for measuring total oxidative potential were compared. These include using methanol as the solvent with (1) and without (2) filtering the extract, followed by removing the solvent and reconstituting with water, and (3) extraction in pure water and performing the OP analysis in the extraction vial with the filter. The water extraction method (the third approach, with filter remaining in the vial) generally yielded highest DTT responses with better precision (coefficient of variation of 1–5 %), and was correlated with a greater number of other aerosol components. Because no organic solvents were used, which must be mostly eliminated prior to DTT analysis, it was the easiest to automate by modifying an automated analytical system for measuring water-soluble OP developed by Fang et al. (2015). Therefore, the third method was applied to the field study for the determination of total OP. Daily 23h filter samples were collected simultaneously at a roadside (RS) and a representative urban (GT) site for two one-month study periods, and both water-soluble (OP^{WS-DTT}) and total ($OP^{Total-DTT}$) OP were measured. Using $PM_{2.5}$ (aerodynamic diameter < 2.5 μm) high-volume samplers with quartz filters, the OP^{WS-DTT} to $OP^{Total-DTT}$ ratio at the urban site was 65 % with a correlation coefficient (r) of 0.71 (N=35; p-value<0.01), compared to a ratio of 62 % and r=0.56 (N=31; p-value<0.01) at the roadside site. Same DTT analyses were performed and similar results were found using particle composition monitors (flow rate of 16.7 L min^{-1}) with Teflon filters. Comparison of measurements between sites showed only slightly higher levels of both OP^{WS-DTT} and $OP^{Total-DTT}$ at the RS site, indicating both OP^{WS-DTT} and $OP^{Total-DTT}$ were largely spatially homogeneous. These results are consistent with roadway emissions as sources of DTT-quantified $PM_{2.5}$ OP and that both soluble and insoluble aerosol components contributing to OP are largely secondary.

1. Introduction

Exposure to ambient particulate matter (PM) is associated with adverse health effects (Atkinson et al., 2001; Li et al., 2003a; Lim et al., 2013; Pope, 1995; Pope and Dockery, 2006). The mechanisms of PM toxicity are complex and not completely understood. One view is that PM toxicity occurs through inducement of oxidative stress (Delfino et al., 2005; Delfino et al.,

Deleted: Similar

2013; Nel, 2005); a state of biochemical imbalance in which the presence and formation of reactive oxygen species (ROS) in the human body overwhelms antioxidant defenses, eventually leading to various adverse health outcomes (Delfino et al., 2011; Donaldson et al., 2001; Li et al., 2003a). ROS can be either transported on inhaled particles to the air–lung interface or

35   generated in vivo by interaction between deposited PM and physiological chemical components (Lakey et al., 2016). The ability of PM to generate ROS is defined as the oxidative potential (OP) of PM. OP integrates various biologically relevant properties of particles, including size, surface and chemical composition, which may better reflect the biological response to PM exposure and consequently be more informative than PM mass, or specific PM chemical species, when attempting to link aerosols to adverse health effects.

40   Various methods have been developed to assess PM OP (Ayres et al., 2008; Cho et al., 2005; King and Weber, 2013; Mudway et al., 2004; Shi et al., 2003; Wang et al., 2011). The dithiothreitol (DTT) assay is used in this study to measure the OP of fine particles (i.e., OP^{DTT}). DTT acts as a surrogate of cellular reductants, such as NADH/NADPH. The goal is to mimic interactions between physiological reductants and aerosol components through a purely chemical analysis. Various aerosol components can react directly with antioxidants (reducing agents), or transfer electrons from the antioxidants to dissolved

45   oxygen, leading to antioxidant depletion in the first case and both antioxidant depletion and ROS generation in the second. In the DTT assay, physiological reductants are represented by DTT. When this reaction is monitored under conditions of excess DTT, the DTT consumption over time is proportional to the concentration of PM redox-active species, quantified as OP^{DTT}. OP^{DTT} per volume of air sampled has been found to correlate with biological markers, such as cellular hemeoxygenase (HO-1) expression (Li et al., 2003b) and fractional exhaled nitric oxide (FE_{NO}) in human subjects (Delfino et al., 2013; Janssen et

50   al., 2015). Epidemiological studies have linked OP^{DTT} to adverse health outcomes, such as asthma, rhinitis (Yang et al., 2016) and asthma or wheezing and congestive heart failure (Bates et al., 2015; Fang et al., 2016). Utilizing different measures of OP (e.g., ascorbic acid, AA; glutathione, GSH; uric acid, UA), some other studies, however, have not found links between OP and adverse health effects (Atkinson et al., 2016; Canova et al., 2014).

OP^{DTT} of water-soluble PM components (referred to as OP^{WS-DTT}) is the common focus of OP studies. Researchers have

55   identified DTT-active water-soluble PM components, including HUmic-Like Substances (HULIS) (Lin and Yu, 2011; Verma et al., 2012; Verma et al., 2015b), oxygenated quinones (a subset of HULIS) (Cho et al., 2005; Kumagai et al., 2002), and transition metals (Charrier and Anastasio, 2012; Fang et al., 2016; Verma et al., 2015a). Water-insoluble species can also be an important fraction of the overall PM redox activity. Li et al. (2013) found that the solid particle phase was a dominant factor in the DTT-based redox activity of soot particles. Akhtar et al. (2010) found that redox-active substances could be strongly

60   bound to solid particles and not be easily extracted by water. McWhinney et al. (2013) reported that 89 %–99 % of the redox activity of diesel exhaust particles (DEP) were water-insoluble and not extractable by moderately polar (methanol) and nonpolar (dichloromethane) organic solvents. Daher et al. (2011) reported the highest intrinsic OP^{DTT} for particle collection with a Biosampler, which was considered most efficient in capturing both the soluble and insoluble PM species. Including the

2

Deleted: since it is the most straightforward to measure.

65   contribution of water-insoluble species in the OP assessment would be closer to actual PM exposure. A measure of both water-soluble and water-insoluble OP would be useful to elucidate the relative risks of water-soluble versus water-insoluble OP-induced health risks for specific health endpoints, such as respiratory versus cardiovascular dysfunction.

Several PM extraction methods have been used to assess the OP of water-insoluble PM. A common approach is to extract water-insoluble species in organic solvents, such as methanol and dichloromethane. Verma et al. (2012) found OP^{DTT}
70   (expressed per μg of PM mass) of filtered methanol extracts to be correlated with water-insoluble organic carbon and elemental carbon (N=8). The DTT assay response for the methanol extracts was significantly higher than that for the water extracts with methanol-to-water OP^{DTT} ratio of 1.6 ± 0.4. Yang et al. (2014) compared OP^{DTT} of ambient PM with two extraction methods for Teflon filters: methanol extraction without filtering and water extraction. They found that the methanol extracts were more DTT-reactive (expressed per m^3 of sampled volume) than the water extracts. In this method, removal of organic solvent by
75   evaporation was necessary prior to the DTT assay, which can result in the loss of labile redox-active PM species, such as semi-volatile organic compounds. Instead of attempting to dissolve water-insoluble species in various solvents, other studies perform the assay in the extraction liquid without filtration, retaining the insoluble particles in the DTT reaction solution. McWhinney et al. (2013) measured total redox activity of DEP using particle suspensions that were obtained by a water-extraction procedure with the filter removed after extraction. Whereas Charrier et al. (2016) performed the DTT assay on the
80   extraction liquid that still contained the filter. Daher et al. (2011) collected particles directly into water with a BioSampler and performed the DTT analysis without filtration.

In this study, we assess techniques for quantifying the overall oxidative potential of ambient particles and determine the relative contribution from water-soluble and water-insoluble components to PM OP by contrasting measurements from different sites. This was accomplished by conducting the DTT assay on samples extracted by three different methods. The goal was to develop
85   a system for measuring both soluble and total OP^{DTT} (insoluble OP^{DTT} by difference) fractions to allow studies on the health effects of soluble (Bates et al., 2015) versus insoluble PM OP.

2. Experimental methods

2.1 Sampling methods and locations

Measurements were made at two contrasting sampling sites: Georgia Tech and Roadside. The Georgia Tech (GT) site was
90   situated on the rooftop of the Ford ES&T building on the campus of Georgia Tech about 30 m above ground level and approximately 420 m from the roadside site. (A map of the sites is provided in Supplement Fig. S1.) The GT site is assumed to be representative of the urban Atlanta environment. The Roadside (RS) site is adjacent (within 3 m) to a heavily trafficked interstate freeway (I-85/75) with an annual average daily traffic count of 382,000 vehicles in 2015 (Georgia Department of

3

Deleted: , after which

Deleted: was

Deleted: from the analysis

Transportation (GDOT) traffic data, station ID 1215482). Heavy-duty trucks are restricted, resulting in predominantly light-duty gasoline vehicle traffic (non-heavy duty truck traffic nominally 95 %, GDOT 2013 data).

Measurements were undertaken during two different periods using different particle filter collection systems. A high-volume
100 (HiVol) sampler (Thermo Anderson, nominal flow rate 1.13 m^3 min^{-1}, $PM_{2.5}$ impactor) was set up at each site to collect ambient fine particles simultaneously from 21 April 2016 to 30 May 2016. Fine particles were collected with prebaked 8×10 in. quartz filters (Pallflex Tissuquartz, Pall Life Sciences) for 23 h (11:00 am–10:00 am the next day). The HiVol quartz filters were wrapped in prebaked aluminum foil immediately after collection and stored at -18 ℃ until analyses. The bias between the two High Volume samplers was assessed by running them side-by-side at GT for 9 days. The measurements were within 10% for
105 both water-soluble OP^{DTT} (OP^{WS-DTT}) and total OP^{DTT} ($OP^{Total-DTT}$) (obtained by method 3, described below) (Supplement Fig. S2). In Sect. 3.4, OP data from HiVol 1 were adjusted to match HiVol 2 based on the orthogonal linear regression from this comparison. The factors used to convert OP from HiVol 1 to HiVol 2 were 1.00 and 1.10 for OP^{WS-DTT} and $OP^{Total-DTT}$, respectively.

Zefluor PTFE membrane filters (diameter 47 mm, 2 μm pore size, Pall Life Sciences) were used as well for simultaneous
110 $PM_{2.5}$ sample collection from 26 July 2016 to 21 August 2016 using particle composition monitors (PCM, 16.7 L min^{-1}, $PM_{2.5}$ URG cyclone, un-denuded). Two PCMs were installed at each site to obtain two Teflon samples, one was used for OP^{WS-DTT} and the other for $OP^{Total-DTT}$ analysis. Similar to the HiVol filter sampling, after 23 h collection, the PCM Teflon filters were placed into Petri dishes and stored at -18 ℃.

2.2 Measurements of PM oxidative potential

115 OP analyses were performed on both HiVol quartz and PCM Teflon filters. The DTT assay followed the protocol developed by Cho et al. (2005). All OP analyses on HiVol quartz filters were done immediately after collection; OP measurements on Teflon filters were completed within one month after collection.

2.2.1 OP^{WS-DTT} analysis

One circular punch (diameter of 1 in.) from the HiVol quartz filter was extracted in 4.9 mL of deionized water (DI, >18 MΩ
120 cm^{-1}) in a sterile polypropylene centrifuge tube (VWR International LLC, Suwanee, GA, USA) via 30-min sonication. Considering the potential for radical formation during the sonication process (Miljevic et al., 2014), experiments using sonication versus shaking were done. Little difference observed in OP for sonication versus shaking indicated negligible bias in OP^{WS-DTT} measurement due to sonication, see Supplementary Material Fig. S3. The extract was then filtered through 0.45 μm PTFE syringe filters (Fisherbrand, Fisher Scientific) to remove insoluble material. The filtered PM water-extract was
125 analyzed using a semi-automated system (OP^{WS-DTT} system) developed by Fang et al. (2015) where all chemical reagents and

4

reaction mixtures were mixed and transferred by two programmable syringe pumps. Briefly, 3.5 mL water extract is incubated with 0.5 mL of 1 mM DTT and 1 mL potassium phosphate buffer (K-buffer; pH=7.4) in a single incubation vial (IV) at 37 ℃.

135 At designated time points (0, 4, 13, 23, 32, 41 min), an aliquot (100 μL) of this mixture is transferred to another vial (reaction vial, RV) and mixed with trichloroacetic acid (TCA) to quench the reaction. Tris buffer (pH=8.9) and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) are then added to form a colored product which absorbs light at 412 nm. The final mixture is pushed through a 10cm path length liquid waveguide capillary cell (LWCC), and the absorbance at 412 nm is detected and recorded by an online UV–visible spectrophotometer. The DTT concentration at each time point is quantified based on the

140 absorbance calibration curve, which had previously been determined from standard DTT solutions also containing TCA, Tris buffer, and DTNB. The DTT consumption rates are then determined by applying linear regression to the observed DTT concentration versus time. The final OP results are calculated by subtracting a blank value from the sample and normalized by the volume of air that passed through the filter (of 1-inch diameter punch size), expressed as nmol DTT min^{-1} per sampled air volume (OP^{WS-DTT} m^{-3}; if not explicitly stated, OP^{WS-DTT} is OP^{WS-DTT} m^{-3}) to provide a measure of atmospheric levels of water-

145 soluble aerosol OP. The DTT consumption rate of multiple blanks for quartz filters (N=42) was stable with a mean ± 1σ of 0.33 ± 0.07 nmol min^{-1}. Since DTT is a relatively unstable compound, it can react with dissolved oxygen in the liquid in the absence of particles (Kumagai et al., 2002), resulting in OP response in blanks. The blank OP values are also due to trace levels of contaminants on the filter, in the DI-water, and introduced during sample preparation. 9,10-phenanthrenequinone (PQN) is used as positive control throughout the analysis to evaluate the stability of the analytical system.

150 Water extraction was also performed on the PCM Teflon filters. Each of the two Teflon filters collected simultaneously at each site was cut in half. One half of each filter was combined and immersed in 4.9 mL DI in a beaker and sonicated for 30 minutes. The water extract was then filtered and OP^{WS-DTT} determined using the automated system. DTT analytical processing was exactly the same as that for quartz filters described above. The other filter halves were stored in a freezer until OP$^{Total-DTT}$ analysis. This analysis approach removed any potential biases associated with the separate filter collection systems at each

155 site. Sample flow rates were measured at the beginning and end of sampling for each filter system and the overall average was used to calculate OP^{WS-DTT} m^{-3}. Field blanks were also tested in the same manner and had an average slope plus or minus standard deviation of 0.35 ± 0.08 nmol min^{-1} (mean ± 1σ, N=18).

2.2.2 OP$^{Total-DTT}$ analysis

Sample extraction and preparation: To assess methods for characterizing OP$^{Total-DTT}$, we used three different methods of

160 sample preparation using the HiVol quartz filters. Sample preparation schemes are illustrated in Fig. 1. Multiple method analysis was done only on HiVol filters since there was insufficient mass collected to compare different methodologies using the PCM Teflon samples.

5

Method 1 consisted of two steps, water extraction and sequential methanol extraction. A 1-inch circular punch taken from the HiVol quartz filter was extracted in 4.9 mL DI via 30-minute sonication. The water extract was then filtered using a 0.45 μm

165 PTFE syringe filter. This step was the same as the measurement of OP^{WS-DTT}. The water-extracted filter punch was retained in the vial, dried in room air and re-extracted using methanol (HPLC grade) via 30-minute sonication. The methanol extract was also filtered through a syringe filter (0.45 μm PTFE) and then concentrated to about 200 μL using high purity nitrogen gently blown into the vial above the liquid surface. DI was added into the vial to reconstitute the small aliquot of remaining methanol liquid to 4.9 mL of solution. The reconstituted extract was stirred using a vortex mixer (VWR® Analog Vortex Mixer, 300–

170 3200 rpm) for 10 seconds to re-suspend any particles deposited on the walls of the vial during methanol blow-down. The purpose of the sequential and filtered methanol extraction was to assess if water-insoluble species could be dissolved by methanol as a way of quantifying the water-insoluble OP^{DTT} through a contrast to methods that retained solid particles (discussed next). As methanol is less polar than water, it may dissolve most of the water-insoluble organic species in addition to some water-soluble compounds. However, since the solid-phase material in the extract may have been removed by filtering

175 the extract, this method will not include DTT-active species that cannot be separated from a solid particle and is therefore removed by the syringe filter. The determination of OP^{DTT} for both water extract (OP^{WS-DTT}) and sequential DI-reconstituted methanol extract (OP^{sM-DTT}) was conducted using the OP^{WS-DTT} analytical system since all extracts had been filtered, avoiding any plugging or contamination issues in the analytical system by solid particles. The sum of OP^{WS-DTT} and OP^{sM-DTT} is the total redox activity obtained by method 1, which will be denoted as $OP^{Total-DTT-1}$. Blank filters were also similarly processed and

180 analyzed for OP^{DTT}, producing blank values of 0.33 ± 0.07 nmol min^{-1} (mean $\pm 1\sigma$, N=42) for OP^{WS-DTT} and 0.43 ± 0.09 nmol min^{-1} (mean $\pm 1\sigma$, N=18) for OP^{sM-DTT}. This method was used in the Southeastern Center for Air Pollution and Epidemiology (SCAPE) study and so a substantial data set (N=198) exists on OP^{sM-DTT}.

Method 2 is similar to the methanol extraction by Yang et al. (2014). The filter punch was extracted in methanol via 30-minute sonication. After extraction, the filter punch was removed from the vial. The methanol extract was not filtered so that

185 the methanol-insoluble components were also retained and would possibly participate in the subsequent DTT reaction. The methanol suspension was blown down to nominally 200 μL using nitrogen gas and reconstituted to 4.9 mL with DI. The reconstituted extract was stirred for 10 seconds using a vortex mixer in an attempt to re-suspend particles deposited on vial walls. Due to the presence of solid material in the extract, such as quartz filter fibers released by sonication, the OP^{WS-DTT} system could not be utilized. Instead, a modified automated system was needed to measure the OP of this aqueous suspension,

190 discussed below. The OP^{DTT} of PM sample extracted in this manner is referred to as $OP^{Total-DTT-2}$. The blank value for this method was 0.42 ± 0.13 nmol min^{-1} (mean $\pm 1\sigma$, N=18).

Method 3 is the easiest to perform among the three methods in terms of sample preparation (Fig. 1). In this case the circular filter punch was immersed in the mixture of 4.9 mL DI and 1.4 mL K-buffer in a sterile polypropylene centrifuge tube, followed by 30min sonication. The DTT assay was then performed directly in the vial with the filter punch present using the modified

6

195   automated system discussed below. Some DTT-active species may be strongly absorbed to the filter surface so that they are not extractable into water. But in method 3, since the whole filter is suspended in DTT solution, these DTT-active species may participate in the reaction with DTT. In the study of Charrier et al. (2016), where DTT was also directly incubated with the PM filter, an alcohol, 2,2,2-Trifluoroethanol, was added to the extraction solvent to facilitate removal of particles from the filter substrate. We tested adding small amounts of methanol (up to 10 % of total extraction volume) into the extraction solvent

200   to investigate if methanol would expose more solid aerosol for reaction with DTT, which would be observed as an increase in DTT response. The test results are given in Supplement Fig. S4 and show that the added methanol had negligible effects on the final OP^{DTT} measured, therefore, only DI was used for extraction in this method. The OP^{DTT} obtained in this way is referred to as $OP^{Total-DTT-3}$. Sonication versus shaking tests were also performed on method 3, and the results (Supplement Fig. S5) show little effects of sonication on $OP^{Total-DTT-3}$ measurements. Only method 3 was used for the $OP^{Total-DTT}$ determination of

205   Teflon filters. Multiple blanks were processed similarly with DTT consumption rates of 0.37 ± 0.06 nmol min^{-1} (mean $\pm 1\sigma$, N=18) for quartz filters and 0.43 ± 0.04 nmol min^{-1} (mean $\pm 1\sigma$, N=18) for Teflon filters.

Automated system for $OP^{Total-DTT}$ measurements: A modified automated analytical system for $OP^{Total-DTT}$ was developed by modifying the OP^{WS-DTT} system of Fang et al. (2015) for analysis of filters extracted using methods 2 and 3. A schematic is shown in Fig. 2. In this approach the sample extraction vial containing the suspension or suspension plus filter that had gone

210   through method 2 or 3 extraction is placed in the thermal mixer, prior to which 1.4 mL K-buffer had been loaded manually. In this case, each sample vial is used as an incubation vial directly, continuously shaken and maintained at 37 ℃ via a Thermo-mixer (VWR® Cooling Thermal Shake Touch; rotational frequency of 400 rpm, temperature of (37 ± 0.5) °C). Two peek tubes (PEEK Tubing Green 1/16 inch OD × 0.030 inch ID), which are connected to a 14-port multi-position valve (VICI Valco Instrument Co. Inc., USA), are inserted into each incubation vial, with one tube having an in-line syringe filter (0.45 μm

215   Polypropylene (PP) filter media, Whatman) and the other not. For each run, 0.7 mL DTT (1 mM) is loaded into the incubation vial through the tubing without in-line filter via the programmable syringe pump A (see Fig. 2). Air is then pumped into the incubation vial to thoroughly mix. In the mixture, DTT is presumably oxidized with the catalytic assistance of both water-soluble and water-insoluble DTT-active species associated with the PM collected on the HiVol quartz or PCM Teflon filter. After mixing, the multi-position valve is switched so that the syringe can withdraw an aliquot of sample through the filter, at

220   a low speed so as not to form air bubbles by cavitation. At designated time intervals (13, 30, 48, 65, 82 min), the aliquot is withdrawn through the in-line filter, transferred to the reaction vial (RV) and mixed with TCA preloaded in the vial by pump B. The DTT concentration is then determined following the same steps as that for the OP^{WS-DTT} system (Fang et al., 2015). A total of five data points of remaining DTT concentrations versus time is generated and used for the final OP^{DTT} determination. After finishing the DTT analysis of each sample, the system is thoroughly cleaned by flushing with DI to remove the residual

225   liquid left in the various tubing, reaction vial, pump syringes and LWCC. Following the flushing, the 14-port multi-position valve is switched to the next sample for analysis. Due to the slow piston motions in liquid transfer from IV to RV, it generally takes 1.5 hours for $OP^{Total-DTT}$ system to analyze one sample, compared with 1 hour of analysis time of OP^{WS-DTT}. The OP^{Total-}

7

^{DTT} system, like the OP$^{WS\text{-}DTT}$ system, can operate unattended and be monitored remotely to analyze, at least, seven filters. (This is limited by the 14-channels of the multi-position valve in Fig. 2). To avoid contamination from the insoluble material captured in the in-line syringe filter, the syringe filter is replaced after each sample run. The automated system is cleaned every 4 weeks of continued operation by flushing at least three times with methanol, followed by four times with DI.

2.3 Other chemical analysis

A number of other aerosol components were analyzed on the HiVol quartz filters for assessing the various methods of measuring OP$^{Total\text{-}DTT}$. Carbon analysis (EC/OC) was performed on a 1.5 cm^2 punch from the quartz filters using Interagency Monitoring of Protected Visual Environments (IMPROVE) thermal optical reflectance (TOR) protocol (Chow et al., 1993).

Total and water-soluble metals were determined by inductively coupled plasma-mass spectrometry (ICP-MS) (Agilent 7500a series, Agilent Technologies, Inc., CA, USA) using EPA method 6020, again from sections of the same HiVol quartz filters. The elements of interest included species that possibly play a role in ROS generation (Fe, Mn, Cu; Schoonen et al., 2006) and K, a marker of biomass burning (Artaxo et al., 1994). For the determination of concentrations of total metals, acid digestion was carried out on a 1.5 cm^2 filter punch using nitro hydrochloric acid (HNO$_3$ + 3HCl). The acid-digested sample was then diluted in DI water, filtered with a 0.45 μm PTFE syringe filter. No digestion was required prior to the analysis of water-soluble metals. A 1.5 cm^2 punch from the quartz filter was sonicated in DI for 30 minutes. After sonication, the extract was filtered using a 0.45 μm PTFE syringe filter, and then acid-preserved by adding concentrated nitric acid (70 %) to a final concentration of 2 % (v/v). A set of mixed calibration standard solutions were prepared by diluting the stock standard solutions and treated with the same procedures as samples. Internal standards including lithium (^{6}Li) and scandium (^{45}Sc), were added to all calibration standards and samples to monitor instrumental drift. DI blank and field blank which consist of same concentrations of acid and internal standards were used to monitor for possible contamination resulting from the sample preparation procedures. This was critical since in this case no special care was taken to pre-acid wash the quartz filters or syringe filters used in the water-soluble metals analysis. The method detection limits are defined here as three times the standard deviation of blanks, which for water-soluble metal method were 0.03 mg L^{-1} for K, 0.00007 mg L^{-1} for Mn, 0.009 mg L^{-1} for Fe, 0.0002 mg L^{-1} for Cu, and for the total metal method were 0.03 mg L^{-1} for K, 0.0002 mg L^{-1} for Mn, 0.02 mg L^{-1} for Fe, and 0.002 mg L^{-1} for Cu.

2.4 Data analysis

Linear regression was applied to the experimental data in order to assess relationships between measurements. Since the data was normally distributed (shown in Fig. S6), the Pearson correlation coefficients were calculated to further demonstrate the strength and the direction of a linear relationship between two measurements. A correlation coefficient greater than 0.7 with a low p-value (<0.05) was generally described as strong.

8

Deleted: K (potassium),

Deleted: (iron),

Deleted: (manganese),

Deleted: (copper

The paired t-tests were used to determine whether there was a significant difference in OP measurements between two methods. Each $OP^{Total-DTT}$ was measured using three methods, resulting in pairs of observations. The null hypothesis of the paired t-test assumed that the mean difference between the paired observations was zero. p-value of the test gave the probability of observing the test results under the null hypothesis. p-values less than 0.05 rejected the null hypothesis at the 5 % significance level.

The F-tests in one-way analysis of Variance (ANOVA) were employed to evaluate the impact of filter type (i.e., quartz vs. Teflon filters) on the PM OP measurements for a given site. The F-statistic is the ratio of between-group variability to within-group variability, which followed an F-distribution under the null hypothesis. In this paper, the null hypothesis assumed that there was no significant OP difference between Teflon and quartz filters. If the F calculated from the data was smaller than the critical F-value of the F-distribution for significance level α=0.05, then the null hypothesis would be true with 95 % confidence.

The spatial variability of OP (Table S6) was assessed by the coefficients of divergence (CODs) (Pinto et al., 2004; Wilson et al., 2005).

Deleted: S5

$$COD = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(\frac{c_{ij}-c_{ik}}{c_{ij}+c_{ik}}\right)^2}, \qquad (1)$$

where c_{ij} and c_{ik} were OP^{WS-DTT} or $OP^{Total-DTT}$ measured at site j and k, respectively, and N was the number of observations. A COD close to zero implied spatial uniformity, while a value approaching unity indicated absolute heterogeneity.

3. Results and discussion

First we discuss the performance of the automated system for measuring $OP^{Total-DTT}$ where filters were extracted by method 3 (Fig. 1), and then compare the results of the three differing methods for measuring $OP^{Total-DTT}$ at the two sampling sites. The system performance was assessed by only method 3 since these samples were easiest to prepare and this is the final approach of the three methods tested that was extensively utilized. Finally, we compare results from method 3 using quartz filters to a later study using Teflon filters. All OP^{DTT} results were blank-corrected.

3.1 Automated $OP^{Total-DTT}$ system performance

The performance of the automated system was assessed in terms of the system response, accuracy and precision. 9,10-phenanthraquinone (PQN), a quinone that has been identified to be DTT-active (Kumagai et al., 2002) and often utilized as a positive control (Fang et al., 2015), was used to test the system response. A highly linear relationship (R^2=0.97) was found

9

between PQN concentration in the incubation vial and the DTT consumption rate measured by the system (shown in Fig. 3). This linear relationship is consistent with the results shown in Fang et al. (2015) and Charrier et al. (2016).

290   The accuracy of measurements given by the $OP^{Total-DTT}$ system was further assessed by comparing the DTT consumption rate obtained by the system to that following the manual DTT analysis approach of Cho et al. (2005). Seven PQN solutions of various concentrations were tested by both the automated system and manual approach. As shown in Fig. 4, a bivariate linear regression was applied and yielded a slope near unity (0.99 ± 0.06), intercept close to zero (0.04 ± 0.04), and correlation of determination (R^2) of 0.98. For further validation, five ambient samples, which in this case would include insoluble species,

295   were extracted by method 3 and analyzed using both the automated and manual methods (see Supplement Fig. S7). The ratio of automated-to-manual DTT consumption rate was 0.98 ± 0.05. These tests illustrate the validity of the $OP^{Total-DTT}$ system as an alternative to the manual DTT assay.

To assess the precision of the automated $OP^{Total-DTT}$ system, the DTT consumption rates of identical concentrations of several PQN solutions were repeatedly measured. The $OP^{Total-DTT}$ system produced consistent results for the PQN replicates (blank-

300   corrected DTT consumption rate of 0.76 ± 0.05 nmol min^{-1} for 0.21 nmol mL^{-1} of PQN in the incubation vial, coefficient of variation (CV) = 6 %, N=7), suggesting good precision of the system. We conclude that most variability in the analysis of samples will be introduced in the extraction process and not the DTT analysis.

3.2 Precisions of various methods

To test the precision of the complete approach for measurement of $OP^{Total-DTT}$ (i.e., extraction and analysis), measurements of

305   $OP^{Total-DTT}$ were repeated three times using three separate punches from the same Hivol quartz filter. This was done for all three $OP^{Total-DTT}$ methods. The coefficient of variation (CV) for replicates is used to assess the precision of each method. The results are summarized in Table 1. CV ranged from 3 % to 6 % for method 1, which may result from the combined uncertainties of the two respective steps (i.e., extraction and analysis). The range of CV for method 2 was from 5 % to 12 %. The root of this variability may arise from the insoluble material remaining in the reaction suspension that was difficult to reproduce from

310   run-to-run. In contrast, lower CV (1 %~5 %) was observed for method 3, possibly because it involved the least steps in the filter extraction.

3.3 Comparison of methods for measuring total oxidative potential ($OP^{Total-DTT}$)

3.3.1 Comparison of oxidative potential

In the following, OP^{DTT} m^{-3} determined by the three methods for simultaneously collected HiVol quartz filters at the GT and

315   RS sites are compared. Since no standard method is available for assessing the ability to measure OP^{DTT} m^{-3}, we simply compare the various methods and assume that the highest measurement represents the most comprehensive analytical method

10

Deleted: .

for measuring total oxidative potential. No HiVol conversion factors were applied to the OP data as the three methods were all performed on filters collected using the same HiVol sampler at each site.

320 Figure 5 shows the OP^{DTT} m^{-3} comparison between method 1 and 3 at both GT and RS sites. In general, the response of the DTT assay of method 3 was significantly higher than that of method 1 at the 95 % confidence level (paired t-test: p = 0.028 at GT, N=35; p<0.001 at RS, N=31). The results are expected since in method 1, both the water and methanol liquid extracts are filtered, potentially removing species that could have been DTT-active but remained attached to solid particles. A few observations where $OP^{Total-DTT-3}$ is less than $OP^{Total-DTT-1}$ are likely due to propagation of errors for the summation method

325 (method 1) combined with variability in the extraction process for each method. The mean $OP^{Total-DTT-1}$ to $OP^{Total-DTT-3}$ ratio at GT was close to 1 (ratio = 0.95) and also higher than that at RS (ratio = 0.85). The lower $OP^{Total-DTT-1}$ may be due to liquid filtration after water extraction. The ratios of OP^{sM-DTT} to OP^{WS-DTT} are 0.34 ± 0.14 (N=35) at GT and 0.37 ± 0.12 (N=31) at RS, which are consistent with the ratios from SCAPE data (0.27 ± 0.08, N=198; unpublished data) and fall into the typical range of ambient samples. The water-insoluble OP determined by the difference in $OP^{Total-DTT-3}$ (which includes solid particles)

330 and OP^{WS-DTT} ($OP^{WI-DTT-3} = OP^{Total-DTT-3} - OP^{WS-DTT}$) to OP^{WS-DTT} ratio, by contrast, is 0.45 ± 0.25 at GT (N=35) and 0.67 ± 0.35 at RS (N=31). There was very little correlation between the $OP^{WI-DTT-3}$ and OP^{WS-DTT} with Pearson correlations of r = -0.23 and -0.51 at GT and RS sites, respectively (see Supplement Table S1), which further indicates the importance of water-insoluble compounds to a total OP measurement. Additionally, $OP^{WI-DTT-3}$ was weakly correlated with OP^{sM-DTT} (Pearson correlation: r = 0.31 at GT; r = 0.04 at RS). Based on these data, it is clear that there were species associated with water-

335 insoluble OP^{DTT} not extracted by methanol and that remain attached to solid particles. This analysis shows that filtering the liquid extract, even if methanol solvent is used, will result in a substantial underestimation of $OP^{Total-DTT}$. Therefore, in terms of the OP response, method 3 is preferred to method 1. Furthermore, the comparison between these two methods can provide insights into the water-insoluble components that contribute to PM OP.

Figure 6 shows the OP^{DTT} m^{-3} comparison between method 2 and 3 at both GT and RS sites. At the GT site, method 3 generally

340 yielded higher OP responses compared to method 2 with mean $OP^{Total-DTT-2}$ to $OP^{Total-DTT-3}$ ratio of 0.90 (p<0.001 for a paired t-test (N=35)). For the RS site, however, method 2 was able to produce comparable (p=0.060 for a paired t-test, N=31) or even higher OP responses, at times, than method 3 with a $OP^{Total-DTT-2}$ to $OP^{Total-DTT-3}$ ratio of 0.94, which may imply that method 2, in some cases, might be more efficient in extracting DTT-active species from the unique RS sources such as vehicular emissions.

345 From the perspective of OP response, method 3 generally produced the highest signals compared to the other two methods, in both the urban (GT) and near-road (RS) sites.

3.3.2 Association between OP^{DTT} and PM composition

11

A correlation analysis was performed between measured PM$_{2.5}$ chemical constituents and OP^{DTT} determined by the three methods. Figure 7 shows the correlation results (detailed values are provided in Table S2). It is seen that OP$^{Total-DTT-3}$ is better correlated with the measured species than OP$^{Total-DTT-1}$ and OP$^{Total-DTT-2}$. Compared with OP^{WS-DTT}, the stronger correlations between OP$^{Total-DTT-1}$ and PM species suggests that OP$^{Total-DTT-1}$ captures more chemical components contributing to DTT than OP^{WS-DTT}. In contrast, OP$^{Total-DTT-2}$ is correlated with the least number of measured PM species.

By subtracting OP^{WS-DTT} from OP$^{Total-DTT}$, OP^{WI-DTT} is determined for the three methods. In general, the correlations between OP^{WI-DTT} and PM species are mediocre for all three methods, with a slightly better performance of method 1. The water-insoluble OP^{DTT} determined by method 1, i.e. OP^{sM-DTT}, has good correlation with OC at GT and OC, EC and water-soluble Fe at RS. Verma et al. (2012) also showed good correlations between OP^{DTT} of filtered methanol extracts and OC and EC, and attributed this association to water-insoluble organic carbon species (WIOC) that dissolve in methanol. Thus, OP^{sM-DTT} in method 1 is likely attributed to some fraction of the WIOC. OP^{WI-DTT} obtained in method 1 is determined from the direct measure of OP^{sM-DTT}, whereas OP^{WI-DTT} is determined by difference for method 2 and 3, which leads to larger uncertainty and more scatter associated with these data.

The overall assessment of the three methods is summarized in Table 2. By comparison, it is found that method 3 has better precision, more comprehensive response (i.e., generally highest OP$^{Total-DTT}$), stronger correlations with PM components, and easiest filter preparation (extraction) process, all of which provide an efficient way for OP$^{Total-DTT}$ determination. The other two methods have some value owing to their insights into the attributes of water-insoluble OP contributors. In a subsequent study, discussed next, only Method 3 was utilized to measure OP$^{Total-DTT}$ of PM for Teflon filters.

3.4 OP^{WS-DTT} and OP$^{Total-DTT}$ measurements on quartz versus Teflon filters and their spatial distributions

The time series of volume-normalized water-soluble and total OP^{DTT} via method 3 are shown in Fig. 8 for two different sample time periods using HiVol samplers with quartz filters (21 April 2016–30 May 2016; HiVol conversion factors of 1.00 and 1.10 were applied to GT OP^{WS-DTT} and OP$^{Total-DTT}$ data, respectively.) and PCMs with Teflon filters (26 July 2016–21 August 2016). A summary of the average OP data is given in Supplement Table S3. The ANOVA results (Supplement Table S4) indicate negligible difference between types of filter (i.e., quartz versus Teflon) on OP^{DTT} measurements.

Figure 8 shows that, as expected, OP$^{Total-DTT}$ is always higher than OP^{WS-DTT}. The ratios of OP^{WS-DTT} to OP$^{Total-DTT}$ were on average 65 ± 10 % (insoluble accounts for 35 ± 10 %) and 65 ± 14 % at GT, compared to 62 ± 12 % and 58 ± 10 % at RS, for quartz and Teflon PM samples, respectively. Thus, OP$^{Total-DTT}$ of PM$_{2.5}$ contained on average 35 to 42 % insoluble species. The correlation coefficients between OP^{WI-DTT} and OP$^{Total-DTT}$ were 0.87 and 0.84 for quartz filters at GT and RS, respectively (Table S1), which reflects the contribution of insoluble species to total OP as well.

12

Spatial distributions in OP^{DTT} can also be investigated. As discussed above, the water-soluble fraction of total OP (OP^{WS-DTT} to $OP^{Total-DTT}$ ratio) was fairly similar at the two sites, which means that the insoluble fraction was not vastly different between the two sites. Figure 9 shows a summary of daily concentration ratios between the sites. EC, a marker for incomplete combustion, and so associated with vehicle emissions, was much higher at the RS site; the ratio of RS to GT was 3.2 on

420   average. OC was only slightly elevated, as expected, since OC is largely secondary in Atlanta (Xu et al., 2015) and so more spatially uniform (i.e., primary OC is a small fraction of total OC, even at RS). Both OP^{WS-DTT} and $OP^{Total-DTT}$ were spatially uniform with daily RS-to-GT OP ratios close to one. CODs were also calculated to further assess the spatial variability of OP (Table S6). The low COD values (COD<0.08 for the quartz filters and <0.23 for the Teflon filters) between RS and GT site indicate spatial homogeneity of OP during the sampling periods. This was found for both quartz and Teflon filters. The

425   homogenous distributions of OP are very similar to that of OC (COD=0.18) and in contrast to EC (COD=0.52). Note that both OP^{WS-DTT} and $OP^{Total-DTT}$ were slightly higher at the RS site, possibly indicating a linkage to RS emissions. Uniformity of OP^{WS-DTT} is consistent with the results shown in the study of Fang et al. (2015), but similar uniformity in $OP^{Total-DTT}$ may seem somewhat unexpected since water-insoluble aerosol components are often associated with primary species. These data show the importance of secondary atmospheric processes OP^{WI-DTT}. The results are consistent with studies that have found water-

430   insoluble DTT-active constituents could be secondary quinones from oxidized PAHs that remain bound to the surface of soot particles associated with traffic emissions (Antinolo et al., 2015; Li et al., 2013; Shiraiwa et al., 2012). This means that although roadway emissions are a source for components that contribute to OP, some form of processing is needed to convert the roadway emissions to species with measurable oxidative potential for both $OP^{Total-DTT}$ and OP^{WS-DTT}. Size distributions of OP^{WI-DTT} (Fang et al., 2017) suggest that OP^{WI-DTT} is composed of different types of insoluble species, OP from oxidized aromatic

435   species (e.g., quinones) may be mainly associated with smaller sized insoluble soot particles, and at the large end of the $PM_{2.5}$ size range, transition metal ions (i.e., water-soluble Cu) associated with road and brake dust may be the main source.

4. Summary

An automated analytical system was developed for quantifying total aerosol oxidative potential with the DTT assay ($OP^{Total-DTT}$) from filter sample extracts. The method is based on modifying an automated analytical system developed by Fang et al

440   (2015) for measuring water-soluble oxidative potential (OP^{WS-DTT}). Three methods for including the contribution of water-insoluble components to oxidative potential of PM (OP^{WI-DTT}), for a measurement of $OP^{Total-DTT}$ were tested: 1) Extracting filter punches in deionized water (DI), filtering the extract and measuring OP^{WS-DTT}, followed by methanol extraction on the same filter, filtering the extract and removing most methanol by evaporation, then reconstituting in water and summing with OP^{WS-DTT} to obtain $OP^{Total-DTT}$; 2) Extracting filter punches in methanol, reconstituting the unfiltered methanol extracts with DI after

445   evaporation of methanol, and performing the DTT assay on the DI-reconstituted suspension; 3) Extracting filter punches in a vial with DI and then performing the DTT assay in the vial containing the filter. Method 3 generally yielded higher DTT responses with higher precision (coefficient of variation of 1~5 %), and was highly correlated with more aerosol species,

13

Deleted: 8

Deleted: S5

Deleted: that even OP^{WI-DTT} species are largely

Deleted: . These

Deleted: in preparation

including OC, EC and various water-soluble and total elements. Because this method requires no use of organic solvents that must be mostly eliminated prior to DTT analysis, it is the easiest to automate. The automated system for measuring OP^{WS-DTT}

455 (Fang et al., 2015) was modified to follow method 3 and the system performance was tested.

An ambient study was conducted to contrast measures of OP$^{Total-DTT}$ and OP^{WS-DTT} for PM$_{2.5}$ collected at a roadside (RS) site (highway with restricted heavy duty diesel access) and a site more representative of overall average urban Atlanta air quality (GT). Simultaneous daily filter samples were collected during two separate one-month periods and comparisons were made using quartz and Teflon filters. At the representative urban site (GT), the ratio of OP^{WS-DTT} to OP$^{Total-DTT}$ was 65 % for both

460 types of filters. At the roadside site (RS) the ratio was only slightly lower, 62 % for quartz filters, 58 % for Teflon filters. OP^{WS-DTT} and OP$^{Total-DTT}$ were moderately correlated with Pearson Product correlation coefficients between 0.56 (roadside) and 0.71 (urban). Simultaneous measures of OP^{WS-DTT} and OP$^{Total-DTT}$ at the GT and RS site showed only slightly higher levels of both at the RS site, indicating both OP^{WS-DTT} and OP$^{Total-DTT}$ were spatially homogeneous. The results are consistent with roadway emissions as sources of OP, but that PM$_{2.5}$ OP was largely secondary for both soluble and insoluble aerosol

465 components contributing to OP.

Deleted: largely

Acknowledgements

Competing interests

The authors declare that they have no conflict of interest.

References

475 Akhtar, U. S., McWhinney, R. D., Rastogi, N., Abbatt, J. P. D., Evans, G. J., and Scott, J. A.: Cytotoxic and proinflammatory effects of ambient and source-related particulate matter (PM) in relation to the production of reactive oxygen species (ROS) and cytokine adsorption by particles, Inhal Toxicol, 22, 37-47, 2010.

Antinolo, M., Willis, M. D., Zhou, S. M., and Abbatt, J. P. D.: Connecting the oxidation of soot to its redox cycling abilities, Nat Commun, 6, 2015.

480 Artaxo, P., Gerab, F., Yamasoe, M. A., and Martins, J. V.: Fine Mode Aerosol Composition at 3 Long-Term Atmospheric Monitoring Sites in the Amazon Basin, J Geophys Res-Atmos, 99, 22857-22868, 1994.

14

Atkinson, R. W., Anderson, H. R., Sunyer, J., Ayres, J., Baccini, M., Vonk, J. M., Boumghar, A., Forastiere, F., Forsberg, B., Touloumi, G., Schwartz, J., and Katsouyanni, K.: Acute effects of particulate air pollution on respiratory admissions
485   - Results from APHEA 2 project, Am J Resp Crit Care, 164, 1860-1866, 2001.

Atkinson, R. W., Samoli, E., Analitis, A., Fuller, G. W., Green, D. C., Anderson, H. R., Purdie, E., Durister, C., Aitlhadj, L., Kelly, F. J., and Mudway, I. S.: Short-term associations between particle oxidative potential and daily mortality and hospital admissions in London, Int J Hyg Envir Heal, 219, 566-572, 2016.

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

Bates, J. T., Weber, R. J., Abrams, J., Verma, V., Fang, T., Klein, M., Strickland, M. J., Sarnat, S. E., Chang, H. H., Mulholland, J. A., Tolbert, P. E., and Russell, A. G.: Reactive Oxygen Species Generation Linked to Sources of
495   Atmospheric Particulate Matter and Cardiorespiratory Effects, Environ Sci Technol, 49, 13605-13612, 2015.

Canova, C., Minelli, C., Dunster, C., Kelly, F., Shah, P. L., Caneja, C., Tumilty, M. K., and Burney, P.: PM10 Oxidative Properties and Asthma and COPD, Epidemiology, 25, 467-468, 2014.

Charrier, J. G. and Anastasio, C.: On dithiothreitol (DTT) as a measure of oxidative potential for ambient particles: evidence for the importance of soluble transition metals, Atmos Chem Phys, 12, 9321-9333, 2012.

500   Charrier, J. G., McFall, A. S., Vu, K. K. T., Baroi, J., Olea, C., Hasson, A., and Anastasio, C.: A bias in the "mass-normalized" DTT response - An effect of non-linear concentration-response curves for copper and manganese, Atmos Environ, 144, 325-334, 2016.

Cho, A. K., Sioutas, C., Miguel, A. H., Kumagai, Y., Schmitz, D. A., Singh, M., Eiguren-Fernandez, A., and Froines, J. R.: Redox activity of airborne particulate matter at different sites in the Los Angeles Basin, Environ Res, 99, 40-47, 2005.

505   Chow, J. C., Watson, J. G., Pritchett, L. C., Pierson, W. R., Frazier, C. A., and Purcell, R. G.: The Dri Thermal Optical Reflectance Carbon Analysis System - Description, Evaluation and Applications in United-States Air-Quality Studies, Atmos Environ a-Gen, 27, 1185-1201, 1993.

Daher, N., Ning, Z., Cho, A. K., Shafer, M., Schauer, J. J., and Sioutas, C.: Comparison of the Chemical and Oxidative Characteristics of Particulate Matter (PM) Collected by Different Methods: Filters, Impactors, and BioSamplers,
510   Aerosol Sci Tech, 45, 1294-1304, 2011.

Delfino, R. J., Sioutas, C., and Malik, S.: Potential role of ultrafine particles in associations between airborne particle mass and cardiovascular health, Environ Health Persp, 113, 934-946, 2005.

Delfino, R. J., Staimer, N., Tjoa, T., Gillen, D. L., Schauer, J. J., and Shafer, M. M.: Airway inflammation and oxidative potential of air pollutant particles in a pediatric asthma panel, J Expo Sci Env Epid, 23, 466-473, 2013.

515   Delfino, R. J., Staimer, N., and Vaziri, N. D.: Air pollution and circulating biomarkers of oxidative stress, Air Qual Atmos Hlth, 4, 37-52, 2011.

15

Donaldson, K., Stone, V., Seaton, A., and MacNee, W.: Ambient particle inhalation and the cardiovascular system: Potential mechanisms, Environ Health Persp, 109, 523-527, 2001.

Fang, T., Verma, V., Bates, J. T., Abrams, J., Klein, M., Strickland, M. J., Sarnat, S. E., Chang, H. H., Mulholland, J. A.,
520   Tolbert, P. E., Russell, A. G., and Weber, R. J.: Oxidative potential of ambient water-soluble PM2.5 in the southeastern United States: contrasts in sources and health associations between ascorbic acid (AA) and dithiothreitol (DTT) assays, Atmos Chem Phys, 16, 3865-3879, 2016.

Fang, T., Verma, V., Guo, H., King, L. E., Edgerton, E. S., and Weber, R. J.: A semi-automated system for quantifying the oxidative potential of ambient particles in aqueous extracts using the dithiothreitol (DTT) assay: results from the
525   Southeastern Center for Air Pollution and Epidemiology (SCAPE), Atmos Meas Tech, 8, 471-482, 2015.

Fang, T., Zeng, L., Gao, D., Verma, V., Stefaniak, A. B., and Weber, R. J.: Ambient size distributions and lung deposition of aerosol oxidative potential: a contrast between soluble and insoluble particles, Environ Sci Technol, 51, 6802-6811, 2017.

Janssen, N. A. H., Strak, M., Yang, A., Hellack, B., Kelly, F. J., Kuhlbusch, T. A. J., Harrison, R. M., Brunekreef, B.,
530   Cassee, F. R., Steenhof, M., and Hoek, G.: Associations between three specific a-cellular measures of the oxidative potential of particulate matter and markers of acute airway and nasal inflammation in healthy volunteers, Occup Environ Med, 72, 49-56, 2015.

King, L. E. and Weber, R. J.: Development and testing of an online method to measure ambient fine particulate reactive oxygen species (ROS) based on the 2',7'-dichlorofluorescin (DCFH) assay, Atmos Meas Tech, 6, 1647-1658, 2013.

535 Kumagai, Y., Koide, S., Taguchi, K., Endo, A., Nakai, Y., Yoshikawa, T., and Shimojo, N.: Oxidation of proximal protein sulfhydryls by phenanthraquinone, a component of diesel exhaust particles, Chem Res Toxicol, 15, 483-489, 2002.

Lakey, P. S. J., Berkemeier, T., Tong, H. J., Arangio, A. M., Lucas, K., Poschl, U., and Shiraiwa, M.: Chemical exposure-response relationship between air pollutants and reactive oxygen species in the human respiratory tract, Sci Rep-Uk, 6, 2016.

540 Li, N., Hao, M. Q., Phalen, R. F., Hinds, W. C., and Nel, A. E.: Particulate air pollutants and asthma - A paradigm for the role of oxidative stress in PM-induced adverse health effects, Clin Immunol, 109, 250-265, 2003a.

Li, N., Sioutas, C., Cho, A., Schmitz, D., Misra, C., Sempf, J., Wang, M. Y., Oberley, T., Froines, J., and Nel, A.: Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage, Environ Health Persp, 111, 455-460, 2003b.

Li, Q., Shang, J., and Zhu, T.: Physicochemical characteristics and toxic effects of ozone-oxidized black carbon particles,
545   Atmos Environ, 81, 68-75, 2013.

Lim, S. S., Vos, T., and Flaxman, A. D.: A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010 (vol 380, pg 2224, 2012), Lancet, 381, 1276-1276, 2013.

Lin, P. and Yu, J. Z.: Generation of Reactive Oxygen Species Mediated by Humic-like Substances in Atmospheric Aerosols,
550   Environ Sci Technol, 45, 10362-10368, 2011.

16

Deleted: submitted

McWhinney, R. D., Badali, K., Liggio, J., Li, S. M., and Abbatt, J. P. D.: Filterable Redox Cycling Activity: A Comparison between Diesel Exhaust Particles and Secondary Organic Aerosol Constituents, Environ Sci Technol, 47, 3362-3369, 2013.

555 Miljevic, B., Hedayat, F., Stevanovic, S., Fairfull-Smith, K. E., Bottle, S. E., and Ristovski, Z. D.: To Sonicate or Not to Sonicate PM Filters: Reactive Oxygen Species Generation Upon Ultrasonic Irradiation, Aerosol Sci Tech, 48, 1276-1284, 2014.

Mudway, I. S., Stenfors, N., Duggan, S. T., Roxborough, H., Zielinski, H., Marklund, S. L., Blomberg, A., Frew, A. J., Sandstrom, T., and Kelly, F. J.: An in vitro and in vivo investigation of the effects of diesel exhaust on human airway
560 lining fluid antioxidants, Arch Biochem Biophys, 423, 200-212, 2004.

Nel, A.: Air pollution-related illness: effects of particles (vol 308, pg 804, 2005), Science, 309, 1326-1326, 2005.

Pinto, J. P., Lefohn, A. S., and Shadwick, D. S.: Spatial variability of PM2.5 in urban areas in the United States, J Air Waste Manage, 54, 440-449, 2004.

Pope, C. A.: Particulate Air-Pollution and Human Health - Assessment of the Epidemiology, Inhal Toxicol, 7, 749-752,
565 1995.

Pope, C. A. and Dockery, D. W.: Health effects of fine particulate air pollution: Lines that connect, J Air Waste Manage, 56, 709-742, 2006.

Schoonen, M. A. A., Cohn, C. A., Roemer, E., Laffers, R., Simon, S. R., and O'Riordan, T.: Mineral-induced formation of reactive oxygen species, Rev Mineral Geochem, 64, 179-221, 2006.

570 Shi, T. M., Schins, R. P. F., Knaapen, A. M., Kuhlbusch, T., Pitz, M., Heinrich, J., and Borm, P. J. A.: Hydroxyl radical generation by electron paramagnetic resonance as a new method to monitor ambient particulate matter composition, J Environ Monitor, 5, 550-556, 2003.

Shiraiwa, M., Selzle, K., and Poschl, U.: Hazardous components and health effects of atmospheric aerosol particles: reactive oxygen species, soot, polycyclic aromatic compounds and allergenic proteins, Free Radical Res, 46, 927-939, 2012.

575 Verma, V., Fang, T., Xu, L., Peltier, R. E., Russell, A. G., Ng, N. L., and Weber, R. J.: Organic aerosols associated with the generation of reactive oxygen species (ROS) by water-soluble PM2.5, Environ Sci Technol, 49, 4646-4656, 2015a.

Verma, V., Rico-Martinez, R., Kotra, N., King, L., Liu, J., Snell, T. W., and Weber, R. J.: Contribution of water-soluble and insoluble components and their hydrophobic/hydrophilic subfractions to the reactive oxygen species-generating potential of fine ambient aerosols, Environ Sci Technol, 46, 11384-11392, 2012.

580 Verma, V., Wang, Y., El-Afifi, R., Fang, T., Rowland, J., Russell, A. G., and Weber, R. J.: Fractionating ambient humic-like substances (HULIS) for their reactive oxygen species activity – Assessing the importance of quinones and atmospheric aging, Atmos Environ, 120, 351-359, 2015b.

Wang, Y., Hopke, P. K., Sun, L., Chalupa, D. C., and Utell, M. J.: Laboratory and field testing of an automated atmospheric particle-bound reactive oxygen species sampling-analysis system, J Toxicol, 2011, 419476, 2011.

17

585   Wilson, J. G., Kingham, S., Pearce, J., and Sturman, A. P.: A review of intraurban variations in particulate air pollution: Implications for epidemiological research, Atmos Environ, 39, 6444-6462, 2005.

Xu, L., Suresh, S., Guo, H., Weber, R. J., and Ng, N. L.: Aerosol characterization over the southeastern United States using high-resolution aerosol mass spectrometry: spatial and seasonal variation of aerosol composition and sources with a focus on organic nitrates, Atmos Chem Phys, 15, 7307-7336, 2015.

590   Yang, A., Janssen, N. A. H., Brunekreef, B., Cassee, F. R., Hoek, G., and Gehring, U.: Children's respiratory health and oxidative potential of PM2.5: the PIAMA birth cohort study, Occup Environ Med, 73, 154-160, 2016.

Yang, A., Jedynska, A., Hellack, B., Kooter, I., Hoek, G., Brunekreef, B., Kuhlbusch, T. A. J., Cassee, F. R., and Janssen, N. A. H.: Measurement of the oxidative potential of PM2.5 and its constituents: The effect of extraction solvent and filter type, Atmos Environ, 83, 35-42, 2014.

595

18

Table 1. Coefficient of variation (CV) of $OP^{Total-DTT}$ for three extraction methods.

	Method 1	Method 2	Method 3
Coefficient of variation (CV) from triplicate	3–6 % N=10	5–12 % N=7	1–5 % N=12

*N is the number of HiVol filters tested.

600

Table 2. Comparison of methods for measuring $OP^{Total-DTT}$

| | | Method 1 | Method 2 | Method 3 |
|---|---|---|---|
| Description | | Measured:

• OP^{WS-DTT}
• $OP^{WI-DTT-1}$ (OP^{sM-DTT}): OP of water-insoluble but only methanol-extractable species

$OP^{Total-DTT-1} = OP^{WS-DTT} + OP^{sM-DTT}$ | Measured:

• $OP^{Total-DTT-2}$: OP of methanol-extractable species and some methanol-insoluble solids

$OP^{WI-DTT-2} = OP^{Total-DTT-2} - OP^{WS-DTT}$ | Measured:

• $OP^{Total-DTT-3}$: OP of water-soluble and -insoluble species, solids.

$OP^{WI-DTT-3} = OP^{Total-DTT-3} - OP^{WS-DTT}$ |
| Comparison | Ease of operation | Method 3 > Method 2 > Method 1 | | |
| | Precision | Method 3 > Method 1 > Method 2 | | |
| | OP magnitude | At GT: Method 3 > Method 1 > Method 2
At RS: Method 3 ≈ Method 2 > Method 1 | | |
| | Correlations with PM components | Number of correlations of $OP^{Total-DTT}$ with various species: Method 3 > Method 1 > Method 2

OP^{WI-DTT}: poor or mediocre correlations for all three methods. | | |

19
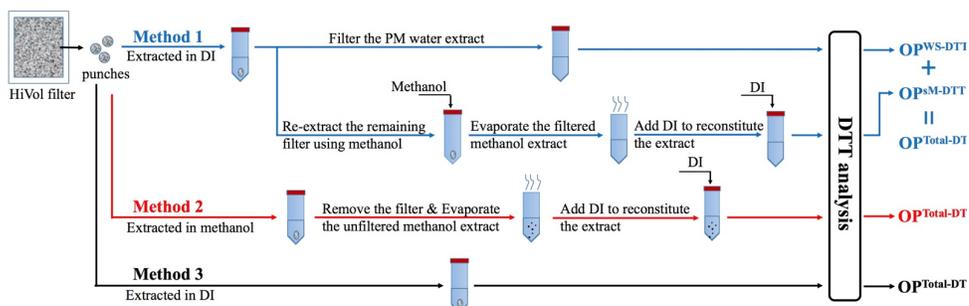
Figure 1. Analytical scheme for three sample extraction methods to determine total OP with the DTT assay ($OP^{Total-DTT}$).

610
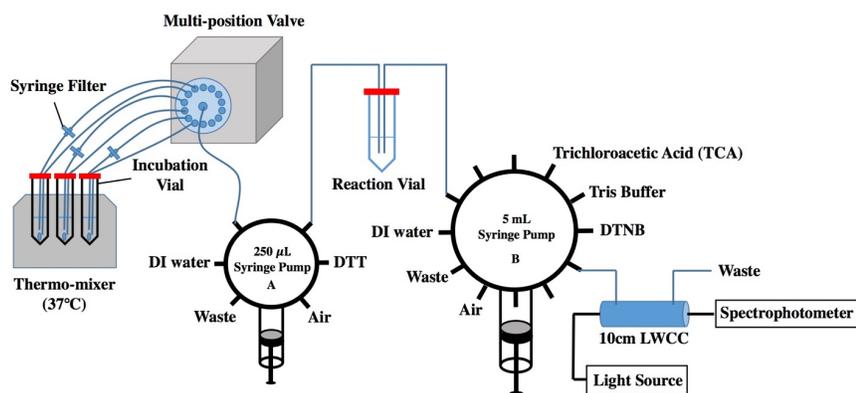
615   **Figure 2**. Automated system setup for measuring $OP^{DTT-Total}$. The assay is performed in the vial containing the filter sample and extraction water, which had been sonicated. The assay is filtered just prior to analysis in the liquid wave guide capillary cell (LWCC).
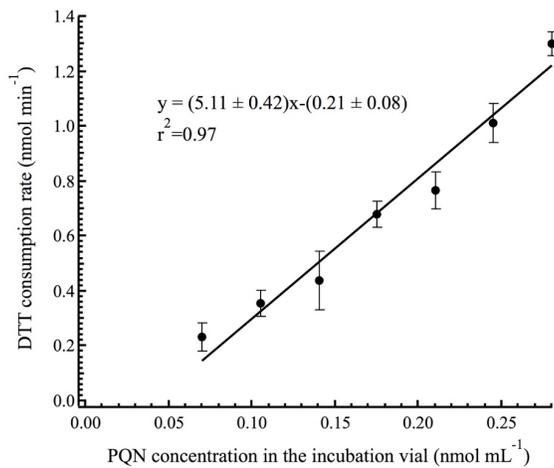
20

Figure 3. Blank-corrected DTT consumption rate as a function of PQN showing linearity between PQN concentrations and DTT consumption rate for the total analytical system (for PQN levels shown in the range above). Error bar represents the standard deviation of three independent DTT measurements on each concentration.

620

625

21
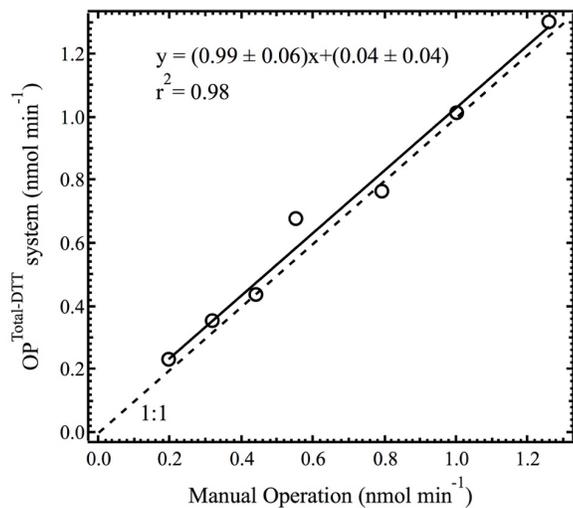
Figure 4. DTT consumption rate (blank-corrected) comparison of the automated system for measuring $OP^{Total-DTT}$ (shown in Fig. 2) to a manual analysis using PQN (9,10-phenanthraquinone). Slope (± 1 standard deviation) and intercept (± 1 standard deviation) are based on orthogonal regression.

630

22
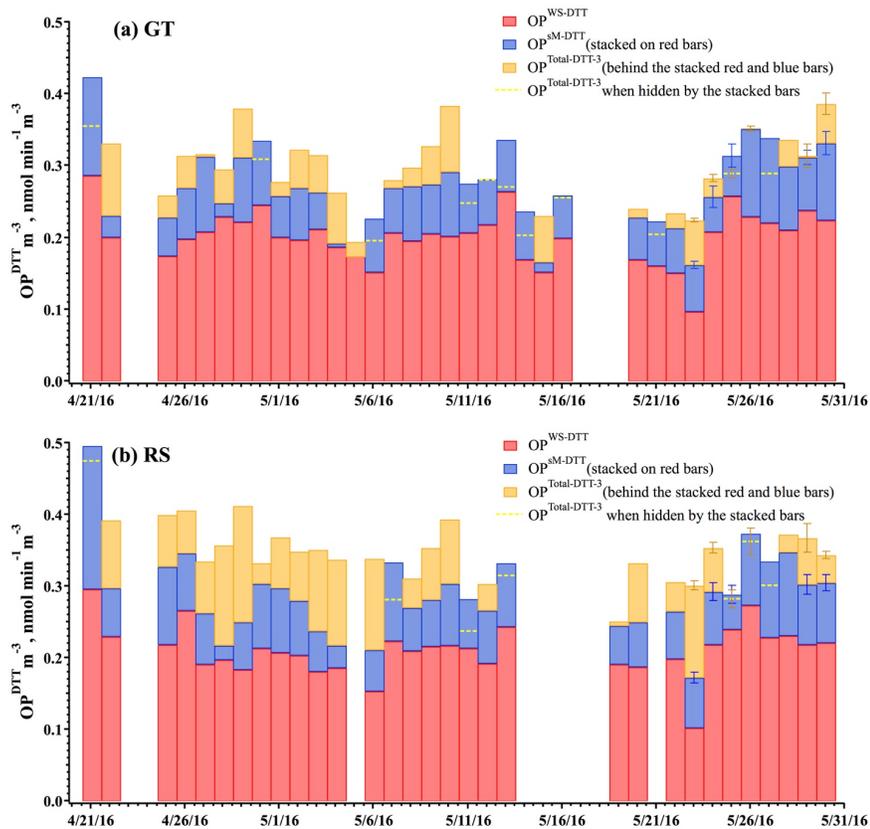
635   **Figure 5.** Comparison of OP^{DTT} m^{-3} between extraction method 1 and 3 at (a) GT (N=35) and (b) RS (N=31). Error bars denote one standard deviation in OP^{DTT} m^{-3} from repeated measurements and are propagated in calculating $OP^{Total-DTT-1}$.
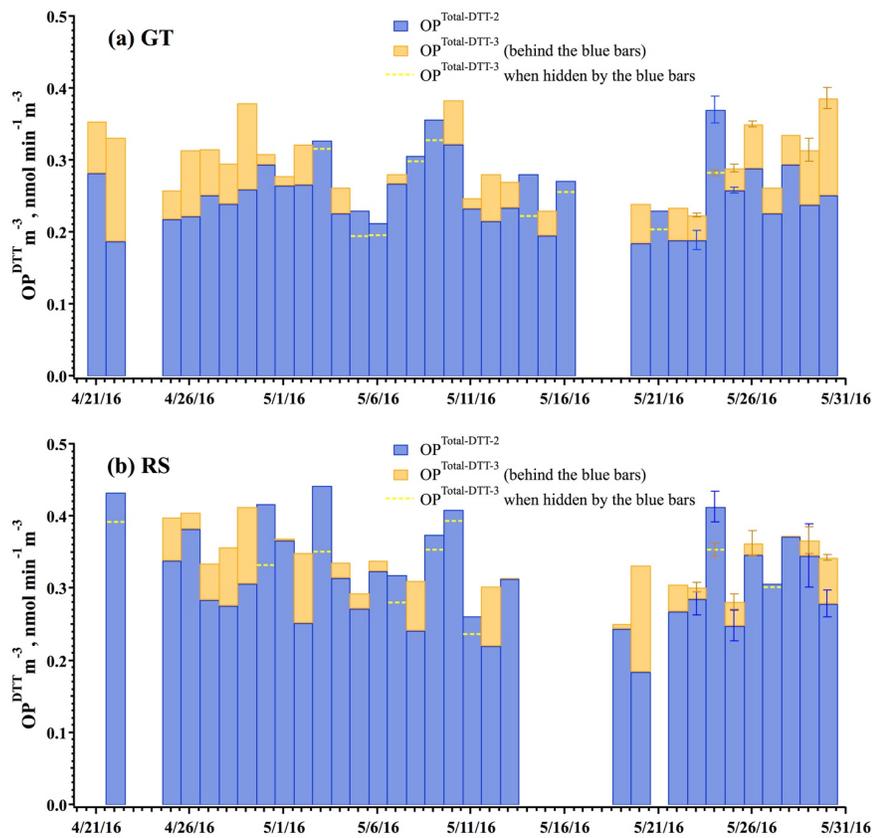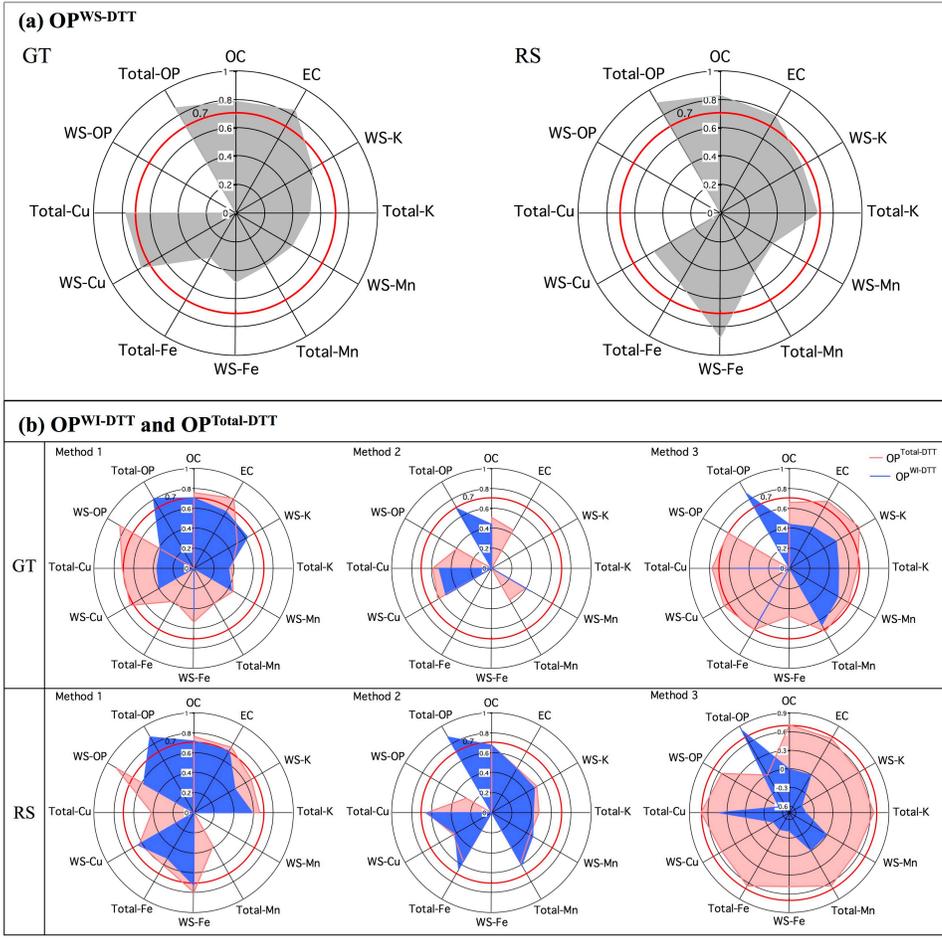
Figure 6. Comparison of OP^{DTT} m^{-3} between method 2 and 3 at (a) GT (N=35) and (b) RS (N=31). Error bars denote one standard deviation in OP^{DTT} m^{-3} from repeated measurements.

24

(a) OP^{WS-DTT}

(b) OP^{WI-DTT} and OP$^{Total-DTT}$

Figure 7. Polar plots comparing Pearson correlation coefficients (r) between various forms of OP^{DTT} m^{-3} (a) OP^{WS-DTT}, (b) OP^{WI-DTT} and OP$^{Total-DTT}$ (Red: OP$^{Total-DTT}$; Blue: OP^{WI-DTT} (OP^{WI-DTT} = OP$^{Total-DTT}$ - OP^{WS-DTT} for method 2 and 3)) and PM chemical components at GT (N=34) and RS (N=29) sites. Correlations not statistically significant (p-value>0.05) are not shown on the plots, but can be found in Table S2 in the supplemental material. The red line indicates r=0.7. Note: the scales for Method 3 RS are different from others'.
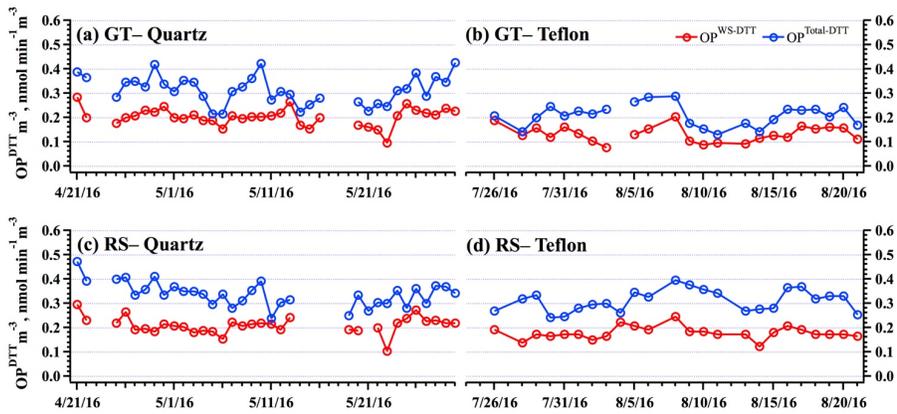
Figure 8. Volume normalized OP^{DTT} of ambient $PM_{2.5}$ particles collected on quartz and Teflon filters at GT and RS sites for two different
650   sampling time periods. Red lines indicate volume normalized OP^{WS-DTT}, and blue lines denote volume normalized $OP^{Total-DTT}$.
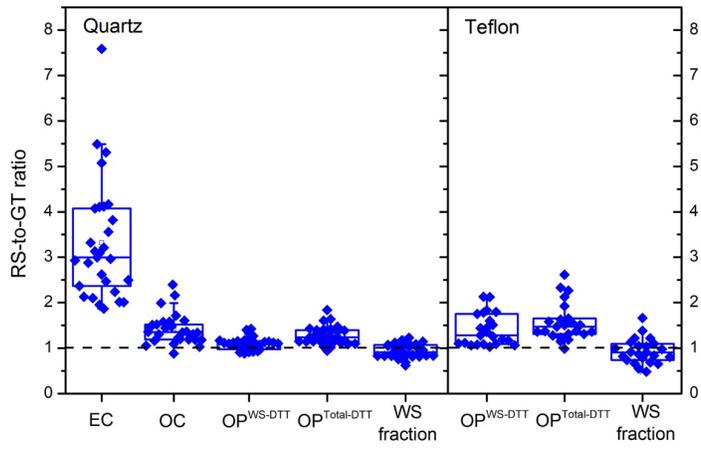
Figure 9. Comparison of simultaneous measurements at GT and RS sites based on daily RS-to-GT concentration ratios. The bottom and top of the box are the first (Q1) and third quartiles (Q3), and the band inside the box is the median. The lowest and highest ends of whisker are (Q1-1.5IQR) and (Q3+1.5IQR), where the interquartile range IQR=Q3-Q1.

27