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Dr. Timothy Bertram
Editor
Atmospheric Chemistry and Physics

Dear Dr. Timothy Bertram,

Along with this letter, we have submitted our response document for the manuscript “Spatiotemporal Variability in the Oxidative Potential of Ambient Fine Particulate Matter in Midwestern United States”. We had received the reviews from two referees and one community comment. All the comments have been satisfactorily addressed based on a point-by-point response in the attached document. To facilitate the review process, we have also included the marked-up version of our revised manuscript (track-changes mode), so that the reviewers can see how the comments are incorporated in the manuscript. The manuscript has been substantially improved as a result of this review and we really appreciate all the valuable suggestions provided by the reviewers.

We believe that our revised manuscript meets the high-quality standards of ACP, and we look forward to any further comments the reviewers and editor might have.

Sincerely,

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Reviewer: Anonymous Reviewer #2

Yu et al report on extensive measurements of PM2.5 OP (oxidative potential) based on an analysis involving 5 different acellular approaches. The analysis was performed on samples collected at a number of sites in the midwestern US and the paper reports on comparisons between the assays and PM2.5 mass. It is stated that a second paper will focus on the PM2.5 chemical components driving these results. The paper is based on a substantial amount of work and provides more insights into the utility of current ways to characterize OP, and it also sheds light on the potential usefulness of using these assays in health studies.

A major conclusion is that the poor correlation between all the various assays, when compared at one site, (and this is largely true for all the sites), implies all these types of OP assays are needed for health studies. One could also conclude, that all of these assays (except possibly one) are each deficient, and no ideal assay exists. It may also even suggest that if no comprehensive OP assay is available, then maybe the approach is flawed since the goal of using these assays was to develop a comprehensive single measure of aerosol toxicity. Since this group of assays appears to fail in demonstrating this goal, instead maybe one should focus on the specific species that drive OP and not use these assays? How does one know if even more assays are needed to fully characterize PM2.5 OP? Furthermore, how would all these various OP measurements, even if available to health researchers, be utilized in a health study, ie how would they be combined to give an overall better indicator of PM2.5 OP? These questions are important and should likely be considered; a discussion beyond the conclusion that all these assays should be utilized, is warranted.

Response:

We thank the reviewer for the inspiring comments. These comments have really helped us in enhancing the discussion of our paper. The reviewer raised several questions regarding the rationality of using oxidative potential (OP) as a health indicator and measuring OP with multiple endpoints. We have attempted to address them point-by-point in the following discussion.

"One could also conclude, that all of these assays (except possibly one) are each deficient, and no ideal assay exists."

Yes, we agree with the reviewer's comment that one aspect of the conclusion of our study could be that all of these assays are each deficient, and no ideal assay exists. However, to be more accurate, we cannot comment on the deficiency or benefit of an assay based on this study. This will require an integration of these assays with either toxicological or epidemiological study. Nevertheless, following the reviewer's suggestion we have added a few sentences in the results and discussion section of our manuscript in lines 576 – 585, "Overall, a poor-to-moderate and inconstant intercorrelation trend among different endpoints of both water-soluble and methanol-soluble OP at most sites indicates that all these assays could be deficient from being ideal and measuring a single endpoint is not enough to represent the overall OP activity. ... However, it should be noted that our study is not designed to assess and rank the biological relevance of these acellular endpoints, which will require an integration of these and possibly other novel assays involving different routes of oxidative stress, in either toxicological or epidemiological studies." We also

included it in our conclusion in lines 613 - 615, "Since our study cannot comment on the biological relevance of these different pathways, we recommend integrating all these and other assays in toxicological or epidemiological studies, to assess their relative utilities."

"It may also even suggest that if no comprehensive OP assay is available, then maybe the approach is flawed since the goal of using these assays was to develop a comprehensive single measure of aerosol toxicity."

We do not agree with the reviewer's point here. First, we do not think that the goal of these assays was to develop a comprehensive single measure of aerosol toxicity. The current national ambient air quality standards are based on PM mass alone, despite we clearly know that certain components of the PM are more toxic than others. One goal of developing these assays was to have an alternative metric which is able to capture some of the potential toxic mechanisms of these components. Although it could appear from the OP literature that the goal is to develop a single measurement of OP for representing multiple pathways of aerosol toxicity, numerous studies have repeatedly indicated that all these measures have their limitations in terms of incorporating the roles of different redox-active components. For example, Xiong et al. (2017) reported negligible OP^{DTT} activity of Fe ions (i.e. Fe^{2+} and Fe^{3+}) and strong synergistic effect of Fe and quinones in $OP^{OH\text{-}DTT}$, indicating the limitation of OP^{DTT} in counting the contribution of Fe. Ayres et al. (2008) reported different responses of Fe^{3+}, Cu^{2+} and Zn^{2+} towards OP^{AA} and OP^{GSH} in a respiratory tract lining fluid (RTLF). Moreover, many studies have found different correlation trends of different endpoints with chemical components and sources of PM, e.g. OP^{AA} vs. OP^{DTT} (Fang et al., 2016;Perrone et al., 2019;Visentin et al., 2016;Janssen et al., 2014), OP^{ESR} (i.e. oxidative potential measured with electron spin resonance assay) vs. OP^{AA}, OP^{GSH} and OP^{DTT} (Calas et al., 2018). Janssen et al. (2015), Weichenthal et al. (2016a), Weichenthal et al. (2016b) and Maikawa et al. (2016) also reported different associations of different acellular OP endpoints (e.g. OP^{AA}, OP^{GSH}, OP^{DTT} and OP^{ESR}) with the health endpoints, including markers of airway and nasal inflammation, risk of emergency room visits for respiratory diseases, myocardial infarction, and fractional exhaled nitric oxide (FeNO), respectively. However, despite these differences and limitations, we do not think that it is appropriate to say that the approach is flawed, simply because in almost all of the health studies, these assays have shown a better association than the PM mass (Bates et al., 2015;He et al., 2021;Maikawa et al., 2016;Strak et al., 2017;Weichenthal et al., 2016a). Thus, we know that despite their limitations they are superior to the currently used PM metric based solely on the mass. These evidences show the complexity of OP-associated pathways, and make it somewhat unrealistic to develop a single comprehensive assay, at least with the current state of the art.

Given the current scenario, it sounds reasonable to combine these assays, i.e. apply all of these assays on each PM sample, for assessing the OP comprehensively. Although each assay has its deficiency, it can represent a specific pathway of OP which probably overcomes the deficiency of another assay lacking that particular pathway. For example, $OP^{OH\text{-}DTT}$ developed in our previous studies (Xiong et al., 2017;Yu et al., 2018) can supplement the pathway represented by OP^{DTT} for generating superoxide radical ($\cdot O_2^-$), with its subsequent reaction with metal ions for generating the hydroxyl radical ($\cdot OH$). OP^{AA} and OP^{GSH} directly measure the consumption of these antioxidants (i.e. AA and GSH) in a surrogate lung fluid (SLF), representing the antioxidant consumption pathways, while measuring $\cdot OH$ generation in SLF ($OP^{OH\text{-}SLF}$) simulate subsequent

reactive oxygen species (ROS) generation process in human lung lining fluid and thus supplementing the antioxidant consumption process. These five assays combined together cover most of the known and potentially important biological pathways of PM exerting oxidative stress in vivo. Our results showing disparities in the intercorrelation among five endpoints further support the finding that by combining these five assays, we can minimize their deficiencies.

"Since this group of assays appears to fail in demonstrating this goal, instead maybe one should focus on the specific species that drive OP and not use these assays?"

Measuring the specific species in PM that drive OP is even more complicated in linking the chemical composition with health effects. First, the composition of PM is highly complex containing tens of trace metals (Kundu and Stone, 2014;Kim et al., 2005;Luo et al., 2018;Reff et al., 2009;Tao et al., 2017), innumerous organic species (Lin et al., 2017;Lin et al., 2018;Lin and Yu, 2020;Riva et al., 2016;Chen et al., 2020) and numerous inorganic ions (NH_4^+, SO_4^{-2}, NO_3^-, etc.). Note, none of the analytical techniques is capable of measuring all of the organic compounds, therefore bulk parameters such as OC, WSOC and humic-like substances (HULIS) are used to represent such a large group of species present in the ambient PM. Despite such classifications, these bulk organic species coming from different sources show very different OP behavior. For example, Lin and Yu (2020) reported three different types of interactions, i.e. additive, antagonistic, and synergistic of the HULIS extracted from three different sources, i.e. ambient $PM_{2.5}$, rice straw burning and sugar cane leaf burning, respectively, with Cu for oxidizing AA. Second, the health effect of PM might not be accounted by simply adding up the contribution of individual chemical species due to non-linear responses of some species like Cu and Mn towards OP (Charrier and Anastasio, 2012;Charrier et al., 2015) and synergistic/antagonistic interactions among various PM species for exerting the oxidative stress and toxicity (Lin and Yu, 2020;Yu et al., 2018;Charrier and Anastasio, 2015;Wang et al., 2020). All these points essentially demonstrate that the approach of relating the health effects directly with the chemical composition is even more complicated than using rather limited number of the OP assays.

"How does one know if even more assays are needed to fully characterize PM2.5 OP?"

We completely agree with the reviewer on this point. There could be more assays needed to fully characterize the $PM_{2.5}$ OP. This is an open question which we do not think can be addressed from our study and neither it was the goal of the current analysis. However, as of now, these are the most commonly used endpoints, all of which we have included in our study. As the knowledge on this topic expands, we expect that future investigations on the novel OP endpoints might extend our scope. Following the reviewer's suggestion, we have included this point in the discussion of our manuscript in lines 578 – 583, "Although, the OP endpoints used in our study have covered some of the well-known and important pathways of the in vivo oxidative stress caused by $PM_{2.5}$, there are other endpoints (e.g. consumption of cysteine, formation of H_2O_2, etc.), and more assays can be developed in the future. We suggest that a collection of diverse range of OP endpoints, measured separately as done in our study could better capture the role of different PM components and their interactions via different pathways for driving the oxidative levels of the PM in a region."

"Furthermore, how would all these various OP measurements, even if available to health researchers, be utilized in a health study, ie how would they be combined to give an overall better indicator of PM2.5 OP?"

First, we would like to highlight that the importance of our study lies in showing that the responses of these assays do not correlate with each other. Which of these assays is better than the other is the second question which is beyond the scope of our current study. To address that question, we need to integrate them in the epidemiological studies. However before that step, an obvious question arises that do all these assays have to be integrated or just few of them (in case they would have been correlated). Our investigation shows that all of them should be integrated to know which one is better than the other, because they are <u>not</u> correlated with each other.

Now, by combining, we do not mean to merge them into one assay, rather we mean that we should do all of them individually on each PM sample. Then we should integrate all of this data in an epidemiological study to assess the relevance of each of them. Some previous studies have adopted this approach for investigating the health relevance of OP by associating it with health endpoints (Abrams et al., 2017;Strak et al., 2017;Zhang et al., 2016;Yang et al., 2016;Weichenthal et al., 2016a;He et al., 2021;Janssen et al., 2015). These studies have definitely helped in enhancing our understanding on the relevance of OP measurements and the role of specific endpoint in comparison to PM mass. However, these are very limited with their focus only on 2 or 3 endpoints. Incorporating all the available OP endpoints measured on the same set of samples in epidemiological studies should help to clearly see their roles and rank them as per their relevance, which is what we expect in longer term from this dataset.

The data do support other studies showing variability between various OP measures and PM2.5 mass, suggesting PM2.5 mass is a poor predictor of the ability of particles to cause oxidative stress (assuming these assays are good measures of OP). This is an important finding.

Response:

We thank the reviewer for appreciating this finding.

Comparisons between sites using different samplers operating at the same time depends on some level of measurement precision to argue that observed differences (poor correlations) are really due to differences in aerosol particles at the sites. This applies to the gravimetric measurement of PM2.5 mass and the various OP measurements. The authors do discuss variability in the negative and positive controls, but the data shown in Table 1 is only the precision of the analysis and does not consider sampling, filter storage or extraction. Can it be stated that this precession for all the species measured and PM2.5 mass is significantly better (lower variability) than that of the comparisons between sites. It would be especially interesting to know the precession of the methanol extracts, which based on the extraction approach is likely the most imprecise measurement (curiously it also shows the least variability between assay results from various sites). A more comprehensive discussion is warranted that includes specifically addressing if the differences seen are real or just noise.

Response:

This is a good point by the reviewer and we apologize not to address it earlier in our original manuscript, despite conducting some experiments to test the variability among various samplers, before the sampling. To further explore it, we have conducted more experiments now after the sampling. The results of all these experiments are presented in the discussion below.

First of all, we would like to note that out of five samplers used in our study, two were old samplers (about 5 years old, used in various sampling campaigns) and three were brand new, which were bought from TISCH Environmental (Cleves, OH, US) a month before the sampling. These new samplers were factory calibrated and installed at three farther sites, i.e. Chicago (CHI), Indianapolis (IND) and St. Louis (STL). The other two old samplers were installed at Champaign (CMP) and Bondville (BON). For the sole purpose of this discussion, we will name them as CHI (N), IND (N), STL (N), CMP (O) and BON (O). Since the new samplers were factory calibrated, we had more confidence in them, therefore, we chose one of those samplers, i.e. CHI(N), as a reference and compared the responses of other two old samplers, i.e. CMP (O) and BON (O), by running them in pairs, i.e. first CHI (N) and CMP (O) pair, followed by CHI (N) and BON (O) pair, at a site in Urbana in April 2018 (due to some practical constraint, we couldn't run all three of them together). We collected 9 sets of Hi-Vol samples on the quartz filters (24-hours integrated samples) from each pair, and analyzed them for the DTT assay using the same extraction and analysis procedure as used in our current study. The comparison of this analysis is shown in Figure 1 of the response document. As can be seen from these figures, there are excellent correlations (R^2 = 0.92 – 0.94) between the old and new samplers, with slopes almost equal to 1.

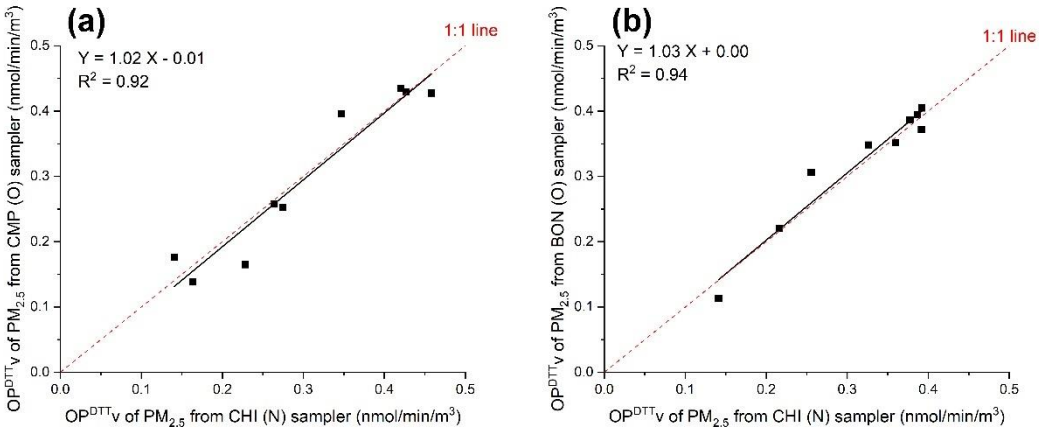

Figure 1. Comparison between OP^{DTT} of the $PM_{2.5}$ samples collected from three samplers: CHI (N) vs. CMP (O) (Figure 1a) and CHI (N) vs. BON (O) (Figure 1b)

After this comparison, we moved all the samplers to their respective sites for the campaign. We believe, that the largest cause of uncertainty in these samplers when they were moved to different sites should be from the variability in their flow rates. Therefore, to minimize that, we always measured the flow rates before and after collecting the $PM_{2.5}$ samples. During the entire sampling campaign, all five samplers were monthly calibrated for the flow rate by using a variable flow calibration kit (Tisch Environmental), which includes a calibration orifice and slack tube water manometer.

We controlled the variability from gravimetric measurements by weighing the filters for at least three times before and after sampling, and ensured that the maximum difference of the mass between three consecutive weighing was less than 0.5 mg. This value is insignificant in comparison to the typical $PM_{2.5}$ mass loadings on the filters, i.e. 40 – 100 mg. Moreover, we always stored all our samples in the same freezer at -20 °C right after weighing. The samples were only taken out from the freezer prior to OP analysis and were immediately placed in the freezer after punching to minimize the loss of semi-volatile species. This should eliminate the effect of storage on the precision.

However, we understand that despite these quality control and checks, we should still inter-compare the three new Hi-Vol samplers installed in Chicago, Indianapolis and St. Louis. Therefore, following the reviewer's comment, we brought these samplers back to our university last month, put them side-by-side at a site in Urbana (IL) and collected 9 Hi-Vol samples (24-hour integrated) from each sampler. All these samples were extracted and analyzed for the DTT activity in the same manner as used in our current study. The results of these comparisons are shown in Figure 2 of the response document. Again, we found excellent correlations (R^2 = 0.93 – 0.95) with slopes close to 1. Note, these comparison results include the variabilities caused by sampling, filters storage and their extraction, as pointed out by the reviewer.

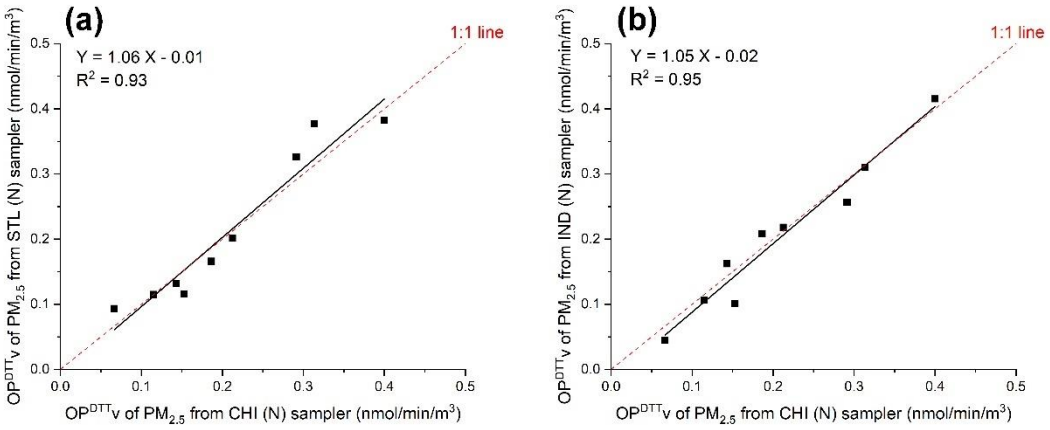

Figure 2. Comparison between OP^{DTT} of the $PM_{2.5}$ samples collected from three samplers: CHI (N) vs. STL (N) (Figure 2a) and CHI (N) vs. IND (N) (Figure 2b)

Finally, to address the reviewer's comment related to methanol extracts, we assessed the precision of methanol-soluble OP for all endpoints, following the same protocol as used for the water-soluble OP measured in our previous study (Yu et al. (2020)). Specifically, ten groups of four punches, each of 0.75" diameter were cut from the same Hi-Vol filter collected at CMP site, and extracted separately into 10 mL methanol. The methanol in the filtered extracts was then evaporated, and each individual residual extract (~50 µL) was reconstituted with DI to reach 12 mL volume. The concentration of the PM in the reaction vial (RV) was maintained at the same level as used in Yu et al. (2020), i.e. 50 µg/mL for SLF-based endpoints, and 30 µg/mL for DTT-based endpoints. The coefficient of variation (CoV; i.e. the standard deviation of the ten groups of measured OP divided by their average), was used to determine the precision of OP and shown in Table 1 of this response document. Overall, the CoV for methanol-soluble OP of all endpoints (8.9

– 14.5 %) was at the same level as that for the water-soluble OP (7.9 – 13.3 %) reported in Yu et al. (2020), indicating that the precision of methanol-soluble OP was as good as water-soluble OP. We have included all these results in SI (Section S1, Figures S1-S2 and Table S2) of the revised manuscript, and discussed them in lines 141 – 142 of the revised manuscript, "Both before and after the sampling campaign, we did a comparison of various samplers by running them in parallel to collect $PM_{2.5}$ samples and analyzing them for OP^{DTT} (see Section S1 of the supplemental information, SI). ... All five samplers were monthly calibrated for the flow rate by using a variable flow calibration kit (Tisch Environmental), and the flow rate was measured every week before and after the sampling.", and lines 228 – 234, "The precision of SAMERA was assessed previously using water-soluble extracts and the coefficient of variations (CoVs) were reported to be less than 14 % (7.9 – 13.3 %) for all OP endpoints (Yu et al., 2020). We also assessed the precision using methanol-soluble extracts and found similar levels of CoVs, i.e. 8.9 -14.5 % for all OP endpoints (see Table S2 in SI). Consistency of our current results for negative controls with those reported earlier, and the low CoVs obtained for the positive controls (1.1 – 11.8%), and $PM_{2.5}$ extracts ensured a good quality assurance for the overall OP analysis."

Table 1. Precision of SAMERA for methanol-soluble OP measurements compared with water-soluble OP measurements.

Endpoint	Unit	Average	Standard Deviation	CoV (%)	CoV (%) for the water-soluble $PM_{2.5}$ extract (Yu et al., 2020)
OP^{AA}	nmol/min/m^3	0.132	0.018	13.51	11.87
OP^{GSH}	nmol/min/m^3	0.098	0.010	10.65	7.89
$OP^{OH\text{-}SLF}$	pmol/min/m^3	0.740	0.011	14.49	10.56
OP^{DTT}	nmol/min/m^3	0.187	0.017	8.89	10.52
$OP^{OH\text{-}DTT}$	pmol/min/m^3	0.216	0.023	10.88	13.28

One conclusion that may be drawn from this work and which is consistent with past studies is that the DTT assay is the most comprehensive measurement of OP (see, for example, discussion in lines 289-407). This may be because DTT includes electron transfer reactions from both organic species and metals, whereas AA, GSH and production of OH in the various assays is likely largely driven by metals. One could actually discuss an interpretation of the data in which the most assay meets the goal of being the most comprehensive. For example, maybe instead of arguing that all assays in their various forms are needed, one could try to assess which is best?

Response:

We agree that OP^{DTT} has been widely used in many studies as the OP indicator, and it was associated with both organic species (e.g., HULIS, quinones) and metals (e.g., Cu and Mn) (Charrier and Anastasio, 2012;Yu et al., 2018). However, as we have pointed out earlier, OP^{DTT} does not capture the contribution of Fe in ·OH formation (Xiong et al., 2017;Yu et al., 2018). This mechanism of ROS generation is also important as shown in one of our earlier study revealing the synergistic interaction of Fe with quinones and HULIS in enhancing the cytotoxicity (Wang et al., 2020). As observed in many studies, this synergism between Fe and organic species was captured by both $OP^{OH\text{-}SLF}$ (Wei et al., 2018;Gonzalez et al., 2017) and $OP^{OH\text{-}DTT}$ (Yu et al., 2018;Xiong et al., 2017). Wang et al. (2018) reported stronger correlations of cytotoxicity of ambient $PM_{2.5}$ with

both $OP^{OH\text{-}SLF}$ and $OP^{OH\text{-}DTT}$ (r = -0.84 and -0.82, respectively) compared to its correlation with OP^{DTT} (r = -0.58), further indicating that both ·OH generating endpoints could have more important roles in the biological pathways leading to cytotoxicity. Similarly, although OP^{AA} and OP^{GSH} showed similar sensitivities as OP^{DTT} towards certain species (i.e. Cu), they represent potentially different biological pathways of oxidative stress. OP^{DTT} simulates the redox reaction of cellular antioxidants, such as NADPH in mitochondria (Cho et al., 2005;Kumagai et al., 2002), while OP^{AA} and OP^{GSH} directly measure the antioxidant consumption in lung lining fluid (Weichenthal et al., 2016b). Previous studies have also noted some associations of health outcomes with OP^{AA} (Janssen et al., 2015) and OP^{GSH} (Maikawa et al., 2016;Weichenthal et al., 2016b), respectively.

Considering the deficiencies and biological relevance of each endpoint, we believe it would be premature to rank OP^{DTT} as the best assay among them. Rather than the comparison among themselves or their correlation with the chemical composition, we think that the choice of the most comprehensive OP endpoints (if there is any such thing) should be determined by their association with the health outcomes.

Specific Comments.

Line 20-21, not sure how higher site to site correlations proves methanol extracts includes more insoluble species? The idea that methanol extracts a greater fraction of OP than water is well known.

Response:

Water-extracts are supposed to contain only water-soluble components while methanol being a solvent with polarity between water and strongly non-polar solvents such as hexane, is supposed to extract major fraction of both water-soluble and water-insoluble components. Our rationale for explaining higher site-to-site correlation in methanol extracts is that the components coming from same sources, such as the regional sources (SOA, biomass burning etc.) have a better chance of being extracted in methanol (irrespective of whether they are water-soluble or insoluble) and thus lead to a higher correlation, masking the effect of the components originated from local sources which could have a narrow range of solubilities. We have further clarified it in our sentences on lines 532 – 536, "It is possible that methanol is able to extract more redox-active PM components coming from regional emission sources, e.g. biomass burning or secondary organic aerosols, present at these sites. The components originated from these common sources could mask the effect of other components originated from the local sources having a narrower range of solubilities, thus yielding to an overall lower spatiotemporal variability and better correlation among different sites."

Lines 142 to 148, Charrier et al (2016) suggest a mass concentration for measurement of OP to limit nonlinear effects of 10ug PM/mL, here the authors use 100 ug/mL, why and what is the effect of doing this, ie does it solve the nonlinear problem?

Response:

We clarify that the concentration of $PM_{2.5}$ in the extract we used for measuring OP is 30 μg/mL for OP^{DTT} and $OP^{OH\text{-}DTT}$, and 50 μg/mL for OP^{AA}, OP^{GSH} and $OP^{OH\text{-}SLF}$ (lines 154-156 in the original preprint). The concentration of 100 μg/mL was used in the sample vials kept in our automated system, which were further diluted before using them in the reaction vials. Note, the range recommended by Charrier et al. (2016) was based on the samples collected from California (Claremont and Fresno). OPm is a sole function of PM chemical composition and this recommendation of the standard concentration is not applicable to the samples with different chemical composition. Charrier et al. (2016) also noted that there is no "right" concentration for the standard. As quoted from their publication, "We propose a standard of expressing mass-normalized DTT results relative to an extract concentration of 10 mg-PM/mL of DTT solution; while there is no 'right' concentration for the standard, this proposed extract concentration provides an adequate DTT response for typical ambient PM in our experience but uses relatively little sample." For DTT-based endpoints, our preliminary tests indicated that the concentration recommended in Charrier et al. (2016) (10 μg/mL) was very low for some of our samples with low redox activity, while 30 μg/mL of $PM_{2.5}$ extract was the safe concentration to produce the levels well above detection levels for OP^{DTT} and $OP^{OH\text{-}DTT}$ activities. Since our samples are collected from Midwest US, there could be a very different mix of aerosol sources for our samples compared to their (Charrier et al., 2016) samples collected in California. Thus, it is reasonable to choose the concentration based on the specific composition of our samples to obtain effective measurements.

We adopted the concentration for SLF-endpoints based on many previous studies using OP^{AA} and OP^{GSH} as the OP indicators (Godri et al., 2011;Godri et al., 2010;Ayres et al., 2008;Künzli et al., 2006;Szigeti et al., 2016). This concentration was sufficient for producing valid $OP^{OH\text{-}SLF}$ values (i.e. higher than the detection limit of our measurements) for most of our $PM_{2.5}$ samples.

Moreover, since we are keeping the concentration constant across all samples, the non-linear biases caused by the concentration of Cu and Mn in the OP endpoints are not so relevant for the comparison of OP responses of our samples collected from different sites.

It would be useful to provide the composition of the simulated lung fluid.

Response:

The surrogate lung fluid (SLF) used in our study consists of four antioxidants. The final concentrations of these antioxidants in the reaction vial used for incubating with the PM extract were 200 μM L-ascorbic acid (AA), 100 μM reduced glutathione (GSH), 300 μM citric acid (CA) and 100 μM uric acid (UA). We have included the procedures for making SLF and the final concentrations of these antioxidants in the manuscript in lines 187 – 190 , "SLF was made following the protocol of Yu et al. (2020), i.e. by mixing equal volumes (1 mL each) of four antioxidant stock solutions – 20 mM AA, 10 mM GSH, 30 mM citric acid (CA) and 10 mM UA, and diluting the mixture by DI to 10 mL. Final concentrations of the antioxidants in the RV used for incubating the sample, were 200 μM AA, 100 μM GSH, 300 μM CA and 100 μM UA."

One issue with current measurements of OP by the various methods is that there is a range of approaches used for each of the methods. This makes comparisons between this work and other

studies complicated. It would be valuable to know exactly how these various methods compare to what has been utilized in other studies. For example, maybe a table in the supplement could provide more details on the methods used here links to past studies that used the exact same approach.

Response:

In Table S2 of our submitted preprint, we have included the studies using the same OP endpoints, and briefly described the differences of their methods in the notes. We thank the reviewer for this suggestion, based on which we have further expanded this table by including more details of the methodology of the studies we cited in the revised Table S6 (corresponding to Table S2 of the preprint).

Line 238-239, this statement should be supported with data.

Response:

We have conducted one-way analysis of variance (ANOVA) test on both spatial and temporal variability of $PM_{2.5}$ mass. The results are included in SI Table S3, and the P-values are added in lines 268 – 272, "The highest mass concentrations were recorded at CHI during winter ($P < 0.01$; Table S3) and STL during summer ($P < 0.05$), while BON exhibited the lowest concentrations in all seasons, except fall when the mass concentrations were lowest at CMP ($P < 0.05$). Other than these minor variations, the $PM_{2.5}$ mass concentrations are both spatially and temporally homogeneous in the Midwest US with no significant seasonal differences ($P > 0.05$ at most sites)." We also added median values in lines 265 – 268, "Generally, the more urbanized sites of our study (i.e. CHI, STL and IND) showed slightly higher mass concentrations (5.7 – 21.7 $\mu g/m^3$, median: 11.8 $\mu g/m^3$) compared to the smaller cities like CMP and its rural component (i.e. BON) (2.0 – 20.2 $\mu g/m^3$, median: 9.2 $\mu g/m^3$)." to support our statement.

Line 274, typo, change "into" to "in"?

Response:

We have made this change.

How do the authors explain the data where OP in water extracts is greater than OP methanol when it is established that methanol extracts water soluble species plus organic species? Seems this result demonstrates the lack of precision of the methanol method. Or are the authors implying that some water soluble species that contribute to OP are not extracted and detected in the methanol method?

Response:

We do not agree with the reviewer on the lack of precision of the method for methanol extraction and analysis. As shown in Table 1 of the response document, the precision of methanol-soluble OP is as good as water-soluble OP.

The measured OP of PM is not simply the addition of the activities of all extracted PM components. Previous studies have reported both synergistic and antagonistic interactions among transition metals and organic species in multiple endpoints, such as OP^{AA} (Lin and Yu, 2020, 2021), $OP^{OH\text{-}SLF}$ (Gonzalez et al., 2017;Wei et al., 2018;Charrier and Anastasio, 2015), OP^{DTT} (Yu et al., 2018;Xiong et al., 2017) and $OP^{OH\text{-}DTT}$ (Yu et al., 2018;Xiong et al., 2017). Hence, lower methanol-soluble OP does not necessarily imply fewer extracted species in methanol. Here, we infer that the lower methanol-soluble $OP^{AA}v$ than water-soluble $OP^{AA}v$ might be attributed to the antagonistic effect from the additional components in methanol-soluble extracts. Lin and Yu (2020) reported an antagonistic interaction between HULIS extracted from rice straws burning and Cu on $OP^{AA}v$. They found an abundance of alkaloid compounds in the HULIS, which can chelate Cu and reduce its reactivity with AA. Although we have not yet conducted chemical composition analysis, it is possible that the $PM_{2.5}$ samples collected at CMP could be strongly impacted by biomass burning sources and therefore could contain high levels of alkaloids. Our previous studies also found an elevated level of Cu [up to 60 ng/m^3, compared to the typical Cu concentration (4 – 20 ng/m^3) at most urban sites in US (Baumann et al., 2008;Buzcu-Guven et al., 2007;Hammond et al., 2008;Kundu and Stone, 2014;Lee and Hopke, 2006;Milando et al., 2016)] at CMP (Wang et al., 2018;Puthussery et al., 2018). Since many of the alkaloid compounds are methanol-soluble but water-insoluble, it is possible that these compounds are more efficiently extracted in methanol and are complexed with a large fraction of Cu, thus causing lower levels of methanol-soluble $OP^{AA}v$ compared to water-soluble $OP^{AA}v$ at CMP. We have included this inference in lines 346 – 355 of the original preprint and lines 467 – 479 in the revised manuscript.

What is the difference between methanol soluble OP and methods that attempt to measure all OP, eg, that associated with surfaces of solid particles?

Response:

The methanol-soluble OP measured in our study cannot be called the total OP measured by Gao et al. (2017). In our method, we sonicated punches of $PM_{2.5}$ filters in methanol, and filtered the suspensions through a 0.45 μm PTFE syringe filter. The filtered extracts were then concentrated to less than 50 μL using a nitrogen dryer to evaporate methanol and were subsequently reconstituted in deionized water (DI). In comparison to this method, Gao et al. (2017) (cited in Table S2) measured OP^{DTT} by three methods. In their first method, they extracted the filters sequentially in water followed by methanol. After sonication, both suspensions were filtered through a 0.45 μm PTFE syringe filter. The subsequent methanol extracts were concentrated to ~200 μL by evaporating methanol, and were then reconstituted in DI. Note, neither ours (direct extraction in methanol followed by filtration) nor their first method (sequential extraction followed by filtration) measure the activity of methanol-insoluble fraction of $PM_{2.5}$ and therefore cannot be termed as total OP.

In Gao et al. (2017)'s second method, they directly sonicated punches of $PM_{2.5}$ filters in methanol and removed the filter punches after sonication. The methanol extracts were concentrated to ~200 μL without being filtered, and were then reconstituted in DI. This method could include the activity of water-insoluble and also methanol-insoluble species via surface reaction, but probably not to 100 % efficiency because some of the particles could always remain on the filter fibers irrespective of the solvent used for extraction.

In Gao et al. (2017)'s third method, they sonicated filter punches in a mixture of DI and potassium phosphate buffer (K-PB, pH = 7.4) and directly measured OP of the suspensions containing filter punches, without filtering anything. Since this method includes the contribution of even those particles which are not extracted and remain on the filters, we believe that out of all these methods, only this approach can be termed as the total OP. This was further demonstrated from the results of Gao et al. (2017), showing a 5 – 18 % higher average OP^{DTT} obtained from this method compared to earlier two methods.

Overall, the "OP^{DTT}" obtained from their first method (i.e. the summation of water-soluble OP and the subsequent methanol-soluble OP) was most similar with the methanol-soluble OP measured in our study, but none of them can be considered as the "total OP".

Reviewer: Anonymous Reviewer #1

In this work, the authors measured oxidative potential (OP) of particulate matter in five urban areas in midwestern US. Particulate matter (PM) is a significant health hazard, and its oxidative potential is thought to be representative of its toxicity. The authors assessed oxidative potential in 5 different endpoints on a weekly basis. These OP measurements are often difficult to make, but the authors had developed a system to automate the measurements of PM on filters. The results from the study showed large variabilities across sites and endpoints, and these variabilities, along with poor correlation with PM mass, suggest that PM2.5 mass alone is a poor indicator of potential health impacts. The discussion of the results was not very deep, and, in many cases, more detailed exploration is encouraged to better understand these results. In general, the manuscript is well written, but some of the main messages can be more clearly communicated, rather than buried in a lot of numbers and text. I believe that this manuscript should be published in ACP after some major revisions.

Response:

We thank the reviewer for providing these valuable comments. In the revised manuscript, we have tried our best to reduce the unnecessary information (such as numbers and text) so that the main message of our study become clear. We have also enriched our discussion as well as the conclusion to explicitly state the main take-away message from our exploration. In the following section, we have addressed the reviewer's comments on point-by-point basis.

Major comments:

In general, this work reads like a measurement report. I was very impressed by the ability to make all these measurements, but somewhat disappointed with the lack of insights from the measurements. More specifically:

- A lot of information about each site was given in Section 2.1, but when discussing the spatiotemporal variability, there is virtually no discussion in these contexts in Section 3.3. Why does CMP behave so differently? What are the spikes? The same goes for Section 3.5, where the site-to-site comparison is discussed in the context of some statistical measures (correlation coefficient, COD). Again, what are the physical insights?

Response:

This is the first manuscript in the series of papers we plan to write from our yearlong Midwest sampling campaign. In addition to the OP analysis, we are also conducting a lot of chemical and mechanistic analysis (e.g. separation of PM components) on these samples, which we plan to present in our subsequent manuscripts. The current manuscript is expected to serve as the reference for all those subsequent papers and therefore we have to provide as much information as possible about the sampling sites in this manuscript. We understand that all of this information might not be relevant at the current stage given this manuscript is limited to only OP analysis. However, we believe that as our further analysis (i.e. chemical and mechanistic analysis) will emerge, some of this information could become relevant. We further note that the scope of this manuscript was to discuss the patterns of spatiotemporal variability of $PM_{2.5}$ OP in the Midwest US. Therefore, description of the site features in Section 2.1 was intended to justify different classification of the sites, i.e. urban, roadside and rural.

"Why does CMP behave so differently? What are the spikes?"

CMP was the only site which was adjacent (< 10 m) to a major urban road (University Avenue in Urbana, IL) and was on the roof of a parking garage, indicating that $PM_{2.5}$ collected at this site was directly impacted by the daily traffic. Our previous study conducted at the same site, Wang et al. (2018) has reported large variations in several redox-active metals, including Cu (4 – 60 ng/m^3), Fe (2 – 15 ng/m^3), Mn (0.4 – 3 ng/m^3), Pb (0.02 – 2.5 ng/m^3) and Zn (3 – 10.5 ng/m^3), which are all related with the vehicles (both exhaust and non-exhaust emissions). Since the spikes occurring in water-soluble OP at CMP (Figure 3) were generally observed for SLF-based endpoints (i.e. OP^{AA}, OP^{GSH} and $OP^{OH\text{-}SLF}$), which are all known to be highly sensitive towards metals (Ayres et al., 2008;Calas et al., 2018;Fang et al., 2016;Moreno et al., 2017;Charrier and Anastasio, 2015;Wei et al., 2018), we expect a larger contribution of the variation in daily traffic intensity in the spikes observed at CMP. Note, the OP^{AA} – an endpoint known to be highly sensitive towards Cu (Ayres et al., 2008;Gaetke and Chow, 2003) emitted from brake wear (Hulskotte et al., 2007;Garg et al., 2000;Gietl et al., 2010), showed the most frequent spikes. In comparison to CMP, all other sites were relevantly farther (closest was STL ~230 m) to be directly affected by the road emissions. Thus, such a different behavior of CMP is probably related to its close proximity to a major roadway. We have included this discussion in lines 319 – 327, "A significant temporal variation was observed for CMP with several spikes in the OP activities throughout the year, most prominently for OP^{AA} (Figure 3). These spikes might be attributed to the traffic, as CMP is the only site adjacent (< 10 m) to a major urban road and located on the roof of a parking garage. One of our previous studies, Wang et al. (2018), reported large variations in several redox-active metals (e.g. Cu, Fe, Mn, Pb and Zn), which have been known to be related with the vehicular emissions (Hulskotte et al., 2007;Garg et al., 2000;Gietl et al., 2010;Apeagyei et al., 2011;Councell et al., 2004) at the same CMP site. Since SLF-based endpoints have been shown to be highly sensitive towards metals (Ayres et al., 2008;Calas et al., 2018;Fang et al., 2016;Moreno et al., 2017;Charrier and Anastasio, 2015;Wei et al., 2018), the temporal variation in traffic intensity probably contributes to the spikes observed at CMP. "

"The same goes for Section 3.5, where the site-to-site comparison is discussed in the context of some statistical measures (correlation coefficient, COD). Again, what are the physical insights? "

The coefficient of divergence (COD) is a standard measure which has been used in several past studies to explore the spatiotemporal variability in an environmental attribute (Kim et al., 2005;Cheung et al., 2011;Massoud et al., 2011;Verma et al., 2011;Daher et al., 2013;Fang et al., 2014;Huang et al., 2015;Gao et al., 2017;Mukherjee et al., 2019;Feinberg et al., 2019). The primary purpose of Section 3.5 was to compare the COD and correlation coefficient (r) for different OP endpoints versus mass concentration of $PM_{2.5}$. We believe that the key physical insight from section 3.5 (section 3.4 in the revised manuscript) is that there is a larger spatial variability in OP than the $PM_{2.5}$ mass, as revealed from the CODs and r, indicating that the spatial distributions for OP are potentially more affected by the chemical components rather than $PM_{2.5}$ mass. Large variations and weak correlations in most OP endpoints among different sites indicate a more significant effect of the local sources on OP compared to the regional sources. This message has been clearly outlined in lines 518 – 520.

- Lines 257 to 280 were very hard to follow. The discussion jumped around from OP measure to another (sometimes mass-normalized, other times volume-normalized). The OP endpoints from this particular study were compared to those reported in the literature, but the discussion focuses on very shallow comparisons (e.g. higher, lower, different, the same). I am very confused about the purpose of this discussion: are these comparisons meant to validate the measurements? Are they meant to highlight the differences to illustrate differences between sources, or site characteristics? Are we expecting the OPs to be the same, or different from previous studies? My suggestion is to focus on some main message, and then show the comparisons that illustrate the point.

Response:

We apologize for the reviewer's confusion. However, we differ from the reviewer's point on the discussion jumping from one OP measure to another (sometimes mass-normalized, other times volume-normalized). We are actually following a consistent structure for discussing these five endpoints in the entire manuscript (including this section). SLF-based endpoints were generally discussed first, in the sequence of OP^{AA}, OP^{GSH} and $OP^{OH\text{-}SLF}$, followed by DTT-based endpoints (first OP^{DTT}, and then $OP^{OH\text{-}DTT}$). For each endpoint, we first discuss the mass-normalized OP, and then volume-normalized OP. Methanol-soluble OP were discussed after water-soluble OP, following the same sequence as described above. We suggest the reviewer to keep this flow in mind when reading the lines 257 – 280 to avoid any confusion.

The reviewer is correct that the primary purpose of this section was to compare our measurements with those reported in the literature. Here, we have compared the OP obtained from our study with OP activities reported from previous literature using the same or similar techniques as ours. In fact, we have further expanded Table S2 (Table S6 in the revised manuscript), by including the methodology of the assays, following the suggestion of another reviewer (#2), who has appreciated this comparison. Since this is the largest dataset on the OP of $PM_{2.5}$ in the Midwest US, and is one of very few studies in US, where all these OP endpoints have been measured on the same set of samples, we think that it is imperative to have a perspective on the general levels of OP in the Midwest US with the rest of the country and the world. Following the reviewer's suggestion, we have clearly expressed the purpose of this section at the beginning of this paragraph in the revised manuscript (lines 360 – 362).

From Table S6, we found that the activities of most OP endpoints measured in our study were generally comparable with the previous literature, i.e. in the typical ranges of previously reported OP levels. Regarding the reviewer's point of illustrating the differences between sources, or site characteristics, we don't think it is practical to have it in our manuscript. There are around 20 studies conducted in more than 30 places cited in this section. It is clearly beyond our scope to look into the site characteristics of all these studies and explain our OP results based on that. Moreover, as we have mentioned earlier, we plan to discuss the source apportionment results in our subsequent manuscripts, where we could consider to compare the sources in the Midwest US from other regions, as appropriate. But, we don't think it fits in the scope of the current manuscript.

- How are we supposed to make sense of the large differences between the various endpoints? They are different measures and operate differently, so they are expected to be different. So, if they are significantly different, then what? The suggestion from the authors is to measure all of them, but then how do we make sense of the different numbers, or trends? A closer examination of what each OP is measuring (and what chemical components are most linked with each measure) would be useful.

Response:

We thank the reviewer. This comment is similar to the 1st comment raised by Reviewer #2. Therefore, we would encourage the reviewer to also read our response to that comment (Pages 1 – 4 of this response document). To the specific points raised by this reviewer, we would like to address them one by one:

"How are we supposed to make sense of the large differences between the various endpoints? They are different measures and operate differently, so they are expected to be different."

Yes, these are different measures and operate differently; however, they still come under the umbrella term of "OP" and in the scientific community, they have been often used interchangeably. Therefore, it is logically curious to know if they really produce different results and if so, to what extent, towards the same $PM_{2.5}$. It would be somewhat irrational to assert that without measuring all of them and comparing their outcomes from the same set of $PM_{2.5}$ samples. There have been some studies in the past which have compared their responses on the same set of samples but these are either based on small sample size or have used only few selected assays. A systematic comparison of all these OP assays, particularly in geographical regions of the United States, is lacking and this is the gap our study is trying to fill-in.

"So, if they are significantly different, then what? The suggestion from the authors is to measure all of them, but then how do we make sense of the different numbers, or trends?"

This is a good question. From our current investigation, we cannot say which of these assays is the best in terms of representing the health effects. All we know is that the responses of these assays do not correlate with each other. To understand the health relevance of these assays, we first need to integrate them in an epidemiological study, which is beyond the scope of our current study. Some previous studies have adopted this approach for investigating the health relevance of OP by associating it with the health endpoints (Abrams et al., 2017;Strak et al., 2017;Zhang et al.,

2016;Yang et al., 2016;Weichenthal et al., 2016a;He et al., 2021;Janssen et al., 2015). These studies have definitely helped in enhancing our understanding on the relevance of OP measurements and the role of specific endpoints in comparison to the PM mass. However, these are very limited with their focus only on 2 or 3 endpoints. Incorporating all the available OP endpoints measured on the same set of samples in epidemiological studies, will help to clearly see their roles and rank them as per their relevance. Therefore, what we mean by "measure all of them" is to develop a database on all these endpoints so that it can be integrated in the epidemiological studies. This will eventually help to evaluate their associations with the health effects and rank them based on their biological relevance. We have modified our discussion on lines 576 – 585 to further clarify our point, "Overall, a poor-to-moderate and inconstant intercorrelation trend among different endpoints of both water-soluble and methanol-soluble OP at most sites indicates that all these assays could be deficient from being ideal and measuring a single endpoint is not enough to represent the overall OP activity. Although the OP endpoints used in our study have covered some of the well-known and important pathways of the in vivo oxidative stress caused by $PM_{2.5}$, there are other endpoints (e.g. consumption of cysteine, formation of H_2O_2, etc.), and more assays can be developed in the future. We suggest that a collection of diverse range of OP endpoints, measured separately as done in our study could better capture the role of different PM components and their interactions via different pathways for driving the oxidative levels of the PM in a region. However, it should be noted that our study is not designed to assess and rank the biological relevance of these acellular endpoints, which will require an integration of these and possibly other novel assays involving different routes of oxidative stress, in either toxicological or epidemiological studies."

- Given that ACP is an chemistry-focused journal, I believe that discussion of chemical composition is well within the scope of this manuscript, and should not be separated for a later publication. Chemical composition is central to many of the questions I posed, and including some information of composition is necessary to make sense of these measurements.

Response:

We partly agree with the reviewer's comment that chemical composition could explain some of the questions raised by the reviewer. However, at the same we want the reviewer to understand that unlike OP, chemical composition is not about making 4 or 5 measurements. We are currently in the process of measuring several chemical species which include EC, OC, WSOC, NO_3^-, SO_4^{-2}, NH_4^+, trace elements (Cu, Fe, Mn, Zn, K, Al, V, Cr, Ni, Sr, Ba, Pb, As and Se), brown carbon, PAHs, hopanes, steranes, alkanes, organic acids and organic nitrogen compounds. Since OP is property inherently linked with the chemical components and their sources, we believe that to properly explain the trends of various OP endpoints, we really need to measure all of these species which have been directly or indirectly linked with the OP. Moreover, before linking the chemical components with OP, we will need to explain their spatiotemporal trends as well. Given current length of the manuscript (18 pages), including all this information will further complicate and convolute the clear message (i.e. the divergent behavior of OP vs. $PM_{2.5}$ mass), it is currently delivering. Again, we agree that chemical composition is important for the OP, but it is not so straight forward. The previous research from our own group (Xiong et al., 2017;Yu et al., 2018) and others (Charrier and Anastasio, 2015;Gonzalez et al., 2017;Lin and Yu, 2020, 2021;Dou et al., 2015) have shown that there are both synergistic and antagonistic interaction among the PM

chemical components to alter an OP response. Including some description of the chemical components in the current manuscript might allow us to conduct a shallow analysis of their linkages with the OP, but will prevent us to conduct a thorough analysis in the future manuscript, which we think is more important. Therefore, we believe this should be a separate topic altogether in which we will not only link the OP with the chemical components, but also their interactions as well as their sources, and we plan to address it in our next manuscript. Including all these analysis in the current manuscript, which is focused on exploring the spatiotemporal trends of OP in the Midwest US and its comparison with the $PM_{2.5}$ mass, will unnecessarily lengthen it and mix the important messages we plan to provide through these investigations.

Minor comments:

- Line 18 and elsewhere: it might useful to define what volume means. Presumably this is air volume, not particle volume

Response:

Yes, the "volume" in "volume-normalized OP" is the volume of sampled air for $PM_{2.5}$ samples analyzed for a particular OP endpoint. We have clarified this term in the revised manuscript in lines 236 – 239, "The mass-normalized (intrinsic, OPm) and volume-normalized (extrinsic, OPv) OP levels were obtained by dividing the blank corrected OP activities by the extracted $PM_{2.5}$ mass (for OPm) and by the volume of air collected on the extracted fractions of filters (for OPv), respectively. The detailed calculations of OPm and OPv have been previously described in Yu et al. (2020)."

- The introduction is very well-written and reflects the current state of knowledge.

Response:

We thank the reviewer for their comment. We have further enriched the introduction by including more references in lines 64 – 73 , "Calas et al. (2018) compared the responses of several OP endpoints [i.e. OP^{DTT}, OP^{AA}, OP^{GSH}, and electron spin resonance (OP^{ESR})] on PM_{10} samples (N = 98) collected from Chamonix (France). Yang et al. (2014) also used four OP endpoints [OP^{AA}, OP^{DTT}, OP^{ESR} and reductive acridinium triggering (OP^{CRAT})] to investigate the effect of different extraction solvents and filter types on OP responses using the $PM_{2.5}$ samples (N = 20) collected from two cities (Rotterdam and Amsterdam) in Netherland. The comparison of OP^{AA}, OP^{DTT} and OP^{GSH} has been shown in two studies (Fang et al., 2016;Gao et al., 2020), both from the southeast US. We are not aware of any study which has compared ·OH generation in SLF or DTT with other endpoints based on antioxidants consumption (e.g. AA or GSH consumption). Clearly, the studies systematically comparing the responses of these different endpoints on a large sample-set collected at an extensive spatial scale, particularly in the United States are very limited.", and lines 82 – 89, "Globally, the spatiotemporal profiles of OP have been characterized for some geographical regions such as Los Angeles Basin (Saffari et al., 2014, 2013), Denver (Zhang et al., 2008), Atlanta (Fang et al., 2016;Verma et al., 2014) in US, Ontario (Canada) (Jeong et al., 2020;Weichenthal et al., 2019;Weichenthal et al., 2016a), France (Borlaza et al., 2021;Calas et al., 2019;Weber et al., 2018;Weber et al., 2021), Italy (Cesari et al., 2019;Perrone et al.,

2019;Pietrogrande et al., 2018), Athens in Greece (Paraskevopoulou et al., 2019), Netherland (Yang et al., 2015a;Yang et al., 2015b), and some coastal cities of Bohai [Jinzhou, Tianjin and Yantai (Liu et al., 2018)] and Beijing (Yu et al., 2019;Liu et al., 2014) in China."

- Lines 85-93: this might be a good place to define some research questions and hypotheses, and address them accordingly at the end. It will help with adding some depth to the discussion and going beyond just reporting measurements.

Response:

We thank the reviewer for their valuable suggestion. We have revised this paragraph to include the research questions of this manuscript and clearly state our hypothesis. The revised paragraphs in lines 100 – 102 read as, "The goal of this analysis is to compare the spatiotemporal distribution of $PM_{2.5}$ OP with that of the mass concentrations. We also want to investigate if different measures of OP, i.e. OP^{AA}, OP^{GSH}, $OP^{OH\text{-}SLF}$, OP^{DTT} and $OP^{OH\text{-}DTT}$ show different spatiotemporal trends or are correlated with each other." The research questions raised here are subsequently addressed in different sections (Sections 3.1, 3.2, 3.4 and 3.6) of the manuscript. We have further tried to clarify the main message of our analysis in these sections.

- Line 100: "Chicago, Indianapolis and St. Louis" seem redundant.

Response:

We have corrected this sentence in the revised manuscript in lines 111 – 113, "while three major city sites [i.e. Chicago (CHI), Indianapolis (IND) and St. Louis (STL)] are representatives of urban background regions of these respective cities."

- Section 2.2: are the methanol extracts also kept the same PM mass for OP measurement? In the water soluble extract, the volume of water was adjusted to achieve the same mass; how was this done for the methanol soluble extract?

Response:

Yes, the concentrations of $PM_{2.5}$ in the reaction mixtures used for methanol-soluble OP were kept same as those for water-soluble OP measurement (i.e. 50 μg/mL for SLF-based endpoints, and 30 μg/mL for DTT-based endpoints). We first extracted the same area of the filters as that used for the water-soluble OP in 10 mL methanol, and then filtered the extracts through a 0.45 μm PTFE syringe filter. Methanol in the filtered extracts was then evaporated using a nitrogen dryer, and the dried methanol extracts were reconstituted in DI to reach exactly the same volume as the corresponding water-soluble extracts. We have included this detail on lines 176 – 178 of the revised manuscript, "The filtered extracts were then concentrated to less than 50 μL using a nitrogen dryer to evaporate methanol, and were subsequently reconstituted in DI to the exact same volume as the water-soluble extracts."

- Line 160: when the dried methanol extract was reconstituted in water (DI water), are there insoluble components? For example, I can imagine some organic compounds are extracted by

methanol and stick to the walls of the vial when dried, but does not dissolve in water during reconstitution.

Response:

This is a reasonable point. To minimize the bias caused by this deposition loss, we never completely dried the methanol extracts. Rather, we evaporated them to ~50 μL, followed by addition of water to allow the resuspension of the water-insoluble species in water. Moreover, the DI-reconstituted methanol-soluble extracts were always vigorously shaken using an analog vortex mixer (VWR International, Batavia, IL, US) for at least 60 seconds at 3200 rpm to ensure a thorough flush of the organic species which could have been deposited along the wall of the vials. We have revised our manuscript to include these details in lines 176 – 180, "The filtered extracts were then concentrated to less than 50 μL using a nitrogen dryer to evaporate methanol, and were subsequently reconstituted in DI to the exact same volume as the water-soluble extracts. Reconstituted methanol extracts were vigorously shaken on an analog vortex mixer (VWR International, Batavia, IL, US) for at least 60 seconds at 3200 rpm to ensure a thorough flushing of the components probably deposited along the wall of the vials during evaporation."

- Lines 235-236: 5.7-21.7 does not seem to be significantly higher than 2.0-20.2. Perhaps show the median?

Response:

We thank the reviewer's suggestion. We have included the median of the $PM_{2.5}$ mass concentrations in lines 265 – 268, "Generally, the more urbanized sites of our study (i.e. CHI, STL and IND) showed slightly higher mass concentrations (5.7 – 21.7 μg/m^3, median: 11.8 μg/m^3) compared to the smaller cities like CMP and its rural component (i.e. BON) (2.0 – 20.2 μg/m^3, median: 9.2 μg/m^3)". As can be seen, the median at more urbanized sites is slightly higher than the small city sites.

- Lines 240 and 281: how is the "time series" different from the temporal variation in "spatiotemporal variation"? There are a lot of overlapping points between Sections 3.2 and 3.3, and these sections are be significantly combined and condensed for easier reading. Or perhaps the author intended the discussions to be separate, and if so, it would be good to convey the differences in the section titles.

Response:

Figure 3 and 4 (described in section 3.2) gives a snapshot of the overall trend of OP at all the sites. Although, the time-series plot with all its data points gives an idea of the overall picture, it is unable to clearly illustrate the seasonal and spatial variations, which can be easily masked by the outliers or extreme values. To quantify these variations, we computed the seasonal averages (± standard deviation), which are shown in Figures 5 and 6 (described in section 3.3). However, we agree with the reviewer that both sections are essentially focused on explaining the spatiotemporal variability. Therefore, we combined sections 3.2 and 3.3 in the revised manuscript as "Section 3.2 Spatiotemporal variation in $PM_{2.5}$ OP", and rearranged the paragraphs for a

more clarified discussion, while retaining all four figures (i.e. Figures 3-6) for their original purposes.

- Line 248-249: Just want to confirm: In line 217, the July 4th data were excluded from the regression analysis, but are included here in the discussion. It is a little confusing; perhaps some slight clarification would be useful.

Response:

Yes, the OP data in the week of July 4th were included in the analysis of spatiotemporal variability but excluded from the regression analysis. This is to avoid the potential bias caused by a strong but an episodic event in the regression analysis. We have clarified this in the revised manuscript in lines 247 – 250, "All $PM_{2.5}$ samples were assessed for spatiotemporal variability. However, since several OP endpoints (e.g. OP^{AA}, OP^{GSH} and OP^{DTT}) were abnormally elevated in the week of July 4th (Independence Day celebration; discussed in section 3.2), we removed this week's sample from our regression analysis to avoid any bias caused by this episodic event."

- Line 294: why is different from SE US? The seasonal trend seems to be related to photochemical activity (higher in the summer). In general, the midwestern US provides an interesting contrast to previous studies because it has larger temperature differences between summer and winter.

Response:

We thank the reviewer for this interesting observation. We agree that the midwestern US provides an interesting contrast to the previous studies given the larger temperature differences (up to 100 °F) between summer and winter here. This large temperature variation could drive the seasonal variability to some extent. However, it could be that the emission sources in these two seasons (summer vs. winter) are substantially different. For example, Verma et al. (2014) reported highest contributions to OP^{DTT} from biomass burning in winter (47 %) and from secondary organic aerosol in summer (46 %). Higher OP^{DTT} during winter in the Southeast US was attributed to the higher intrinsic redox activity of biomass burning aerosols than those formed during secondary oxidation (Verma et al., 2015). Since we haven't yet done the source apportionment on this dataset, it would be unreasonable to compare the dominant sources (and their seasonality) for OP of our study with Verma et al. (2014). However, we plan to investigate these differences in our subsequent publication.

- Line 350-355: this seems like a somewhat handwavy explanation for an anomaly, not really supported by evidence. What is the evidence for significant alkaloid compounds at this one particular site? Are there other studies that show Cu can complex with organic compounds and reduce OP?

Response:

We agree that from our study, there is no direct evidence for the high levels of alkaloid compounds at CMP. However, the antagonistic interactions between Cu and certain organic species on OP

have been reported in multiple studies. Our previous studies also revealed antagonistic interaction of Cu with quinones, Suwannee River fulvic acid (SRFA) and ambient humic-like substances (HULIS) for both OP^{DTT} and $OP^{OH\text{-}DTT}$ (Xiong et al., 2017;Yu et al., 2018). Pietrogrande et al. (2019) also found a suppressing effect of Cu complexing with citric acid on OP^{AA}, further substantiating the role of Cu complexes on reducing the OP. In addition to the antagonistic effect of Cu and alkaloid compounds on OP^{AA}, Lin and Yu (2020) also found a substantial antagonistic interaction between hydrophilic fraction (which contains high amount of metals) and hydrophobic fraction (mainly organic species) on $OP^{OH\text{-}SLF}$. All these studies indicate that the complexation of Cu with organic species has an important role on reducing the OP for various endpoints. Note, the ranges and medians of M/W^{OP} were generally the lowest at CMP for all endpoints (Figure 7), which implies that the complexes of Cu with alkaloid compounds which are efficiently extracted in methanol could probably be responsible for this trend.

Considering the reviewer's point that we have not made the specific measurements of these species, we have further toned down our hypothesis based on Cu-complexation with organic compounds in general to explain these results in lines 473 – 479, "The unprotonated nitrogen atom in alkaloids tends to chelate Cu, thus reducing its reactivity with AA. The antagonistic effect of Cu have been reported with other organic compounds (e.g. citric acid) as well (Pietrogrande et al., 2019). Thus, apparently lower levels of methanol-soluble OP^{AA} compared to the water-soluble OP^{AA} at CMP might be associated with the chelation of Cu by these alkaloids or other organic species, which could be more efficiently extracted in methanol."

- Lines 356-368: why focus on Fe-organic complex? The simpler explanation would be organic compounds that contribute to OP that extracted in methanol but not in water.

Response:

We partially agree with the reviewer that the water-insoluble organic species extracted in methanol could also contribute to the elevated $OP^{OH\text{-}SLF}$ and $OP^{OH\text{-}DTT}$, however we don't think that this mechanism alone is able to explain the level of elevation observed for these two endpoints (median of M/W^{OP} = 2.1 – 3.8 and 1.4 – 1.9 for $OP^{OH\text{-}SLF}$ and $OP^{OH\text{-}DTT}$, respectively). Our previous study, Yu et al. (2018) reported moderate activities of $OP^{OH\text{-}DTT}$ from multiple types of organic species, including four different quinones, SRFA and ambient HULIS, and nearly zero activity from Fe^{2+} ion. However, much higher activities were observed when mixing Fe^{2+} with all types of organic species (interaction factor, defined as the ratio of the activity of the mixture over the sum of their individual activities = 1.38 – 2.87), indicating the synergistic effect of Fe with organic species for generation ·OH in DTT. Similarly, Gonzalez et al. (2017) and (Wei et al., 2018) also showed a strong synergistic interaction of Fe^{2+} and SRFA through complexation in $OP^{OH\text{-}SLF}$. These evidences strongly suggest that complexes of Fe^{2+} with organic compounds have a prominent role in ·OH formation. Wei et al. (2018) also observed that a substantial fraction of Fe gets complexed with hydrophobic organic compounds (28 ± 22 %), which is more efficiently extracted in methanol than water. Moreover, the seasonality of methanol-extracted Fe observed in Wei et al. (2018) followed the same trend as the M/W^{OP} in our study, i.e. the ratio of Fe in 50 % methanol to that in water and M/W^{OP} for $OP^{OH\text{-}SLF}$v in our study were both higher in winter than summer, further suggesting the contribution of Fe-complexes to the increased $OP^{OH\text{-}SLF}$ and $OP^{OH\text{-}DTT}$ activities of methanol-soluble extracts compared to water-soluble extracts. Therefore, we

would like to keep our hypothesis based on Fe-organic complexes to explain these results. However, following the reviewer's suggestion we have also included the possibility of higher OP contributed by the organic compounds extracted in methanol, in lines 482 – 484, "In addition to ·OH-active organic species, e.g. quinones (Charrier and Anastasio, 2015;Xiong et al., 2017;Yu et al., 2018), which are more soluble in methanol, we suspect that one of such components could be organic-complexed Fe."

- Section 3.6: My suggestion is to point out that current regulations focus on PM mass only, and these results show how inadequate this approach may be. (The reason I suggest this is, at first, I felt it was obvious that OPm would not correlate with PM mass and was somewhat puzzled by the need to do this analysis. But upon second thought, this analysis is useful in a regulatory context.)

Response:

We thank the reviewer for this very important point. We have included it in our discussion in section 3.5 (lines 551 – 552) in the revised manuscript. However, we would like to clarify that we conducted the regression analysis between volume-normalized OP (i.e. OPv and not OPm, which is mass-normalized OP) and $PM_{2.5}$ mass concentrations in Section 3.6. We believe this is what the reviewer meant when they mentioned about the correlation analysis. Since OPm is already normalized by the PM mass, it does not make sense to conduct the correlation between OPm and PM mass. Instead OPv is a property which is in the same equivalent units, i.e. nmol/min/m^3 of air as the PM mass (μg/m^3 of air), and therefore, they are comparable to perform the regression analysis.

- Line 474: "the results … provide", not "provides"

Response:

We have corrected this typo in line 616 of the revised manuscript.

- Figures and tables are generally too complex

Response:

We apologize but we would appreciate if the reviewer could specifically point out which of the figures/tables are complex. We have tried our best to clearly show the information in our figures. All of the figures are either time-series (Figures 2-4), bar charts (Figures 5, 6, 8 and 9) or box-plots (Figure 7), which we believe are very easy to interpret. To make them more legible, we have increased the font sizes of all these figures.

Moreover, we have tried to simplify our tables. Specifically, we have combined the average and standard deviation in one column in Table 1, and replaced the P-values with asterisk symbols (denotes $P < 0.05$, ** denotes $P < 0.01$) in Tables 3-5.*

Community: Samuel Weber

The present study reports the intercomparison of oxidative potential (OP) of PM using different metrics of OP and different extraction protocols. As no consensus has emerged towards which OP method to use, this study is of great interest for documenting various approaches.

However, it should be clarified that it is not the first study of its sort. Namely, Calas et al (2017) have investigated the role of solvent and extraction method and Calas et al (2018) already investigated 5 different OP end-points in Chamonix, France.

Moreover, there is an effort in this manuscript to refer to previous campaign all over the world. We would like to mention to the authors that numerous recent studies in Europe have also reported oxidative potential measurement with multiple assays and have investigated site specificity (Weber et al (2018), Cesari et al (2019), Paraskevopoulou et al (2019), Peronne et al (2019), Pietrogrande et al (2018)), including large-scale variability (Calas et al (2019), Weber et al (2021)) and small-scale variability of OP (Borlaza et al (2021)).

Even if some of the cited studies sampled PM10 and not PM2.5, the discussion of the different OP tests and drivers of OP have been discussed in these papers. These studies should be included in the literature of this manuscript.

Calas, A., Uzu, G., Martins, J. M. F., Voisin, D., Spadini, L., Lacroix, T., and Jaffrezo, J.-L.: The importance of simulated lung fluid (SLF) extractions for a more relevant evaluation of the oxidative potential of particulate matter, Sci Rep, 7, 11617, https://doi.org/10.1038/s41598-017-11979-3, 2017.

Calas, A., Uzu, G., Kelly, F. J., Houdier, S., Martins, J. M. F., Thomas, F., Molton, F., Charron, A., Dunster, C., Oliete, A., Jacob, V., Besombes, J.-L., Chevrier, F., and Jaffrezo, J.-L.: Comparison between five acellular oxidative potential measurement assays performed with detailed chemistry on PM10 samples from the city of Chamonix (France), 18, 7863–7875, https://doi.org/10.5194/acp-18-7863-2018, 2018.

Weber, S., Uzu, G., Calas, A., Chevrier, F., Besombes, J.-L., Charron, A., Salameh, D., Ježek, I., Močnik, G., and Jaffrezo, J.-L.: An apportionment method for the oxidative potential of atmospheric particulate matter sources: application to a one-year study in Chamonix, France, Atmos. Chem. Phys., 18, 9617–9629, https://doi.org/10.5194/acp-18-9617-2018, 2018.

Cesari, D., Merico, E., Grasso, F. M., Decesari, S., Belosi, F., Manarini, F., De Nuntiis, P., Rinaldi, M., Volpi, F., Gambaro, A., Morabito, E., and Contini, D.: Source Apportionment of PM2.5 and of its Oxidative Potential in an Industrial Suburban Site in South Italy, 10, 758, https://doi.org/10.3390/atmos10120758, 2019.

Paraskevopoulou, D., Bougiatioti, A., Stavroulas, I., Fang, T., Lianou, M., Liakakou, E., Gerasopoulos, E., Weber, R. J., Nenes, A., and Mihalopoulos, N.: Yearlong variability of oxidative potential of particulate matter in an urban Mediterranean environment, Atmospheric Environment, 206, 183–196, https://doi.org/10.1016/j.atmosenv.2019.02.027, 2019.

Perrone, M. R., Bertoli, I., Romano, S., Russo, M., Rispoli, G., and Pietrogrande, M. C.: PM2.5 and PM10 oxidative potential at a Central Mediterranean Site: Contrasts between dithiothreitol- and ascorbic acid-measured values in relation with particle size and chemical composition, Atmospheric Environment, 210, 143–155, https://doi.org/10.1016/j.atmosenv.2019.04.047, 2019.

Pietrogrande, M. C., Perrone, M. R., Manarini, F., Romano, S., Udisti, R., and Becagli, S.: PM10 oxidative potential at a Central Mediterranean Site: Association with chemical composition and meteorological parameters, Atmospheric Environment, 188, 97–111, https://doi.org/10.1016/j.atmosenv.2018.06.013, 2018.

Calas, A., Uzu, G., Besombes, J.-L., Martins, J. M. F., Redaelli, M., Weber, S., Charron, A., Albinet, A., Chevrier, F., Brulfert, G., Mesbah, B., Favez, O., and Jaffrezo, J.-L.: Seasonal Variations and Chemical Predictors of Oxidative Potential (OP) of Particulate Matter (PM), for Seven Urban French Sites, 10, 698, https://doi.org/10.3390/atmos10110698, 2019.

Weber, S., Uzu, G., Favez, O., Borlaza, L. J., Calas, A., Salameh, D., Chevrier, F., Allard, J., Besombes, J.-L., Albinet, A., Pontet, S., Mesbah, B., Gille, G., Zhang, S., Pallares, C., Leoz-Garziandia, E., and Jaffrezo, J.-L.: Source apportionment of atmospheric PM10 Oxidative Potential: synthesis of 15 year-round urban datasets in France, 1–38, https://doi.org/10.5194/acp-2021-77, 2021.

Borlaza, L. J. S., Weber, S., Jaffrezo, J.-L., Houdier, S., Slama, R., Rieux, C., Albinet, A., Micallef, S., Trébluchon, C., and Uzu, G.: Disparities in particulate matter (PM10) origins and oxidative potential at a city-scale (Grenoble, France) – Part II: Sources of PM10 oxidative potential using multiple linear regression analysis and the predictive applicability of multilayer perceptron neural network analysis, 1–33, https://doi.org/10.5194/acp-2021-57, 2021.

Response:

We thank Samuel Weber for the useful comments. We agree that it is not the first study to analyze multi-endpoints OP, and there have been studies investigating the spatiotemporal variability and sources of OP using several endpoints. However, all of the studies cited by the reviewer are from Europe. We are not aware of any study which has investigated the spatiotemporal variability of more than 3 OP endpoints in the United States. At most, we could find only two studies both from Southeast US (Atlanta, GA), one of which has compared only two OP endpoints (OP^{DTT} and OP^{AA}) (Fang et al., 2016) and another has compared three endpoints (OP^{DTT}, OP^{AA} and OP^{GSH}) (Gao et al., 2020). Therefore, we have modified our introduction accordingly on lines 63 – 73, "Many of these acellular endpoints have been widely implemented by various researchers for assessing the oxidative properties of PM. Calas et al. (2018) compared the responses of several OP endpoints [i.e. OP^{DTT}, OP^{AA}, OP^{GSH}, and electron spin resonance (OP^{ESR})] on PM_{10} samples (N = 98) collected from Chamonix (France). Yang et al. (2014) also used four OP endpoints [OP^{AA}, OP^{DTT}, OP^{ESR} and reductive acridinium triggering (OP^{CRAT})] to investigate the effect of different extraction solvents and filter types on OP responses using the $PM_{2.5}$ samples (N = 20) collected from two cities (Rotterdam and Amsterdam) in Netherland. The comparison of OP^{AA}, OP^{DTT} and OP^{GSH} has been shown in two studies (Fang et al., 2016;Gao et al., 2020), both from the southeast US. We are not aware of any study which has compared ·OH generation in SLF or DTT with other

endpoints based on antioxidants consumption (e.g. AA or GSH consumption). Clearly, the studies systematically comparing the responses of these different endpoints on a large sample-set collected from an extensive spatial scale, particularly in the United States are very limited."

We also have included several studies from this list in our manuscript at several appropriate places, e.g. lines 82 – 89 in the introduction, and lines 325 – 327 in the results and discussion section. Table S6 of the manuscript (i.e. Table S2 in the preprint), where we compare our OP levels with other measurements is also updated by including those studies from this list that used the same extraction protocols (i.e. water and methanol extractions as used in our study) and measured OP on $PM_{2.5}$ samples. Inclusion of these studies has enriched our discussion.

References

Abrams, J. Y., Weber, R. J., Klein, M., Samat, S. E., Chang, H. H., Strickland, M. J., Verma, V., Fang, T., Bates, J. T., and Mulholland, J. A.: Associations between ambient fine particulate oxidative potential and cardiorespiratory emergency department visits, Environmental Health Perspectives, 125, 107008, 10.1289/ehp1545, 2017.

Apeagyei, E., Bank, M. S., and Spengler, J. D.: Distribution of heavy metals in road dust along an urban-rural gradient in Massachusetts, Atmospheric Environment, 45, 2310-2323, https://doi.org/10.1016/j.atmosenv.2010.11.015, 2011.

Ayres, J. G., Borm, P., Cassee, F. R., Castranova, V., Donaldson, K., Ghio, A., Harrison, R. M., Hider, R., Kelly, F., and Kooter, I. M.: Evaluating the toxicity of airborne particulate matter and nanoparticles by measuring oxidative stress potential—a workshop report and consensus statement, Inhalation Toxicology, 20, 75-99, 10.1080/08958370701665517, 2008.

Bates, J. T., Weber, R. J., Abrams, J., Verma, V., Fang, T., Klein, M., Strickland, M. J., Sarnat, S. E., Chang, H. H., and Mulholland, J. A.: Reactive oxygen species generation linked to sources of atmospheric particulate matter and cardiorespiratory effects, Environmental Science & Technology, 49, 13605-13612, 10.1021/acs.est.5b02967, 2015.

Baumann, K., Jayanty, R., and Flanagan, J. B.: Fine particulate matter source apportionment for the chemical speciation trends network site at Birmingham, Alabama, using positive matrix factorization, Journal of the Air & Waste Management Association, 58, 27-44, 2008.

Borlaza, L. J. S., Weber, S., Jaffrezo, J.-L., Houdier, S., Slama, R., Rieux, C., Albinet, A., Micallef, S., Trébluchon, C., and Uzu, G.: Disparities in particulate matter (PM 10) origins and oxidative potential at a city scale (Grenoble, France)–Part 2: Sources of PM 10 oxidative potential using multiple linear regression analysis and the predictive applicability of multilayer perceptron neural network analysis, Atmospheric Chemistry and Physics, 21, 9719-9739, 2021.

Buzcu-Guven, B., Brown, S. G., Frankel, A., Hafner, H. R., and Roberts, P. T.: Analysis and apportionment of organic carbon and fine particulate matter sources at multiple sites in the midwestern United States, Journal of the Air & Waste Management Association, 57, 606-619, 2007.

Calas, A., Uzu, G., Kelly, F. J., Houdier, S., Martins, J. M., Thomas, F., Molton, F., Charron, A., Dunster, C., and Oliete, A.: Comparison between five acellular oxidative potential measurement assays performed with detailed chemistry on PM 10 samples from the city of Chamonix (France), Atmospheric Chemistry and Physics, 18, 7863-7875, 2018.

Calas, A., Uzu, G., Besombes, J.-L., Martins, J. M., Redaelli, M., Weber, S., Charron, A., Albinet, A., Chevrier, F., and Brulfert, G.: Seasonal variations and chemical predictors of oxidative potential (OP) of particulate matter (PM), for seven urban French sites, Atmosphere, 10, 698, 2019.

Cesari, D., Merico, E., Grasso, F. M., Decesari, S., Belosi, F., Manarini, F., De Nuntiis, P., Rinaldi, M., Volpi, F., and Gambaro, A.: Source apportionment of PM2. 5 and of its oxidative potential in an industrial suburban site in south Italy, Atmosphere, 10, 758, 2019.

Charrier, J., and Anastasio, C.: On dithiothreitol (DTT) as a measure of oxidative potential for ambient particles: evidence for the importance of soluble transition metals, Atmospheric Chemistry and Physics, 12, 11317-11350, 10.5194/acp-12-9321-2012, 2012.

Charrier, J., Richards-Henderson, N., Bein, K., McFall, A., Wexler, A., and Anastasio, C.: Oxidant production from source-oriented particulate matter–Part 1: Oxidative potential using the dithiothreitol (DTT) assay, Atmospheric Chemistry and Physics, 15, 2327-2340, 2015.

Charrier, J. G., and Anastasio, C.: Rates of hydroxyl radical production from transition metals and quinones in a surrogate lung fluid, Environmental Science & Technology, 49, 9317-9325, 10.1021/acs.est.5b01606, 2015.

Charrier, J. G., McFall, A. S., Vu, K. K., 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, Atmospheric Environment, 144, 325-334, 10.1016/j.atmosenv.2016.08.071, 2016.

Chen, Y., Takeuchi, M., Nah, T., Xu, L., Canagaratna, M. R., Stark, H., Baumann, K., Canonaco, F., Prévôt, A. S., and Huey, L. G.: Chemical characterization of secondary organic aerosol at a rural site in the southeastern US: insights from simultaneous high-resolution time-of-flight aerosol mass spectrometer (HR-ToF-AMS) and FIGAERO chemical ionization mass spectrometer (CIMS) measurements, Atmospheric Chemistry and Physics, 20, 8421-8440, 2020.

Cheung, K., Daher, N., Kam, W., Shafer, M. M., Ning, Z., Schauer, J. J., and Sioutas, C.: Spatial and temporal variation of chemical composition and mass closure of ambient coarse particulate matter (PM10–2.5) in the Los Angeles area, Atmospheric Environment, 45, 2651-2662, 2011.

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, Environmental Research, 99, 40-47, 10.1016/j.envres.2005.01.003, 2005.

Councell, T. B., Duckenfield, K. U., Landa, E. R., and Callender, E.: Tire-wear particles as a source of zinc to the environment, Environmental science & technology, 38, 4206-4214, 2004.

Daher, N., Hasheminassab, S., Shafer, M. M., Schauer, J. J., and Sioutas, C.: Seasonal and spatial variability in chemical composition and mass closure of ambient ultrafine particles in the megacity of Los Angeles, Environmental Science: Processes & Impacts, 15, 283-295, 2013.

Dou, J., Lin, P., Kuang, B.-Y., and Yu, J. Z.: Reactive oxygen species production mediated by humic-like substances in atmospheric aerosols: Enhancement effects by pyridine, imidazole, and their derivatives, Environmental science & technology, 49, 6457-6465, 2015.

Fang, T., Verma, V., Guo, H., King, L., Edgerton, E., and Weber, R.: A semi-automated system for quantifying the oxidative potential of ambient particles in aqueous extracts using the dithiothreitol (DTT) assay: results from the Southeastern Center for Air Pollution and Epidemiology (SCAPE), Atmospheric Measurement Techniques Discussions, 7, 10.5194/amt-8-471-2015, 2014.

Fang, T., Verma, V., Bates, J. T., Abrams, J., Klein, M., Strickland, M. J., Sarnat, S. E., Chang, H. H., Mulholland, J. A., and Tolbert, P. E.: Oxidative potential of ambient water-soluble $PM_{2.5}$ in the southeastern United States: contrasts in sources and health associations between ascorbic acid (AA) and dithiothreitol (DTT) assays, Atmospheric Chemistry and Physics, 16, 3865-3879, 10.5194/acp-16-3865-2016, 2016.

Feinberg, S. N., Williams, R., Hagler, G., Low, J., Smith, L., Brown, R., Garver, D., Davis, M., Morton, M., and Schaefer, J.: Examining spatiotemporal variability of urban particulate matter and application of high-time resolution data from a network of low-cost air pollution sensors, Atmospheric environment, 213, 579-584, 2019.

Gaetke, L. M., and Chow, C. K.: Copper toxicity, oxidative stress, and antioxidant nutrients, Toxicology, 189, 147-163, 2003.

Gao, D., Fang, T., Verma, V., Zeng, L., and Weber, R. J.: 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, Atmospheric Measurement Techniques, 10, 2821, 2017.

Gao, D., Godri Pollitt, K. J., Mulholland, J. A., Russell, A. G., and Weber, R. J.: Characterization and comparison of PM 2.5 oxidative potential assessed by two acellular assays, Atmospheric Chemistry and Physics, 20, 5197-5210, 2020.

Garg, B. D., Cadle, S. H., Mulawa, P. A., Groblicki, P. J., Laroo, C., and Parr, G. A.: Brake Wear Particulate Matter Emissions, Environmental Science & Technology, 34, 4463-4469, 10.1021/es001108h, 2000.

Gietl, J. K., Lawrence, R., Thorpe, A. J., and Harrison, R. M.: Identification of brake wear particles and derivation of a quantitative tracer for brake dust at a major road, Atmospheric Environment, 44, 141-146, 2010.

Godri, K. J., Duggan, S. T., Fuller, G. W., Baker, T., Green, D., Kelly, F. J., and Mudway, I. S.: Particulate Matter Oxidative Potential from Waste Transfer Station Activity, Environmental Health Perspectives, 118, 493-498, 10.1289/ehp.0901303, 2010.

Godri, K. J., Harrison, R. M., Evans, T., Baker, T., Dunster, C., Mudway, I. S., and Kelly, F. J.: Increased oxidative burden associated with traffic component of ambient particulate matter at roadside and urban background schools sites in London, PloS One, 6, e21961, 10.1371/journal.pone.0021961, 2011.

Gonzalez, D. H., Cala, C. K., Peng, Q., and Paulson, S. E.: HULIS enhancement of hydroxyl radical formation from Fe (II): kinetics of fulvic acid-Fe (II) complexes in the presence of lung antioxidants, Environmental Science & Technology, 51, 7676-7685, 2017.

Hammond, D. M., Dvonch, J. T., Keeler, G. J., Parker, E. A., Kamal, A. S., Barres, J. A., Yip, F. Y., and Brakefield-Caldwell, W.: Sources of ambient fine particulate matter at two community sites in Detroit, Michigan, Atmospheric Environment, 42, 720-732, 2008.

He, L., Norris, C., Cui, X., Li, Z., Barkjohn, K. K., Brehmer, C., Teng, Y., Fang, L., Lin, L., Wang, Q., Zhou, X., Hong, J., Li, F., Zhang, Y., Schauer, J. J., Black, M., Bergin, M. H., and Zhang, J. J.: Personal Exposure to PM2.5 Oxidative Potential in Association with Pulmonary Pathophysiologic Outcomes in Children with Asthma, Environmental Science & Technology, 55, 3101-3111, 10.1021/acs.est.0c06114, 2021.

Huang, F., Li, X., Wang, C., Xu, Q., Wang, W., Luo, Y., Tao, L., Gao, Q., Guo, J., and Chen, S.: PM2. 5 spatiotemporal variations and the relationship with meteorological factors during 2013-2014 in Beijing, China, PloS one, 10, e0141642, 2015.

Hulskotte, J., Denier van der Gon, H., Visschedijk, A., and Schaap, M.: Brake wear from vehicles as an important source of diffuse copper pollution, Water Science & Technology, 56, 223-231, 10.2166/wst.2007.456, 2007.

Janssen, N. A., Yang, A., Strak, M., Steenhof, M., Hellack, B., Gerlofs-Nijland, M. E., Kuhlbusch, T., Kelly, F., Harrison, R., and Brunekreef, B.: Oxidative potential of particulate matter collected at sites with different source characteristics, Science of the Total Environment, 472, 572-581, 10.1016/j.scitotenv.2013.11.099, 2014.

Janssen, N. A., Strak, M., Yang, A., Hellack, B., Kelly, F. J., Kuhlbusch, T. A., Harrison, R. M., Brunekreef, B., Cassee, F. R., and Steenhof, M.: 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, Occupational & Environmental Medicine, 72, 49-56, 10.1136/oemed-2014-102303, 2015.

Jeong, C.-H., Traub, A., Huang, A., Hilker, N., Wang, J. M., Herod, D., Dabek-Zlotorzynska, E., Celo, V., and Evans, G. J.: Long-term analysis of $PM_{2.5}$ from 2004 to 2017 in Toronto: Composition, sources, and oxidative potential, Environmental Pollution, 263, 114652, 2020.

Künzli, N., Mudway, I. S., Götschi, T., Shi, T., Kelly, F. J., Cook, S., Burney, P., Forsberg, B., Gauderman, J. W., and Hazenkamp, M. E.: Comparison of oxidative properties, light absorbance, and total and elemental mass concentration of ambient $PM_{2.5}$ collected at 20 European sites, Environmental Health Perspectives, 114, 684-690, 10.1289/ehp.8584, 2006.

Kim, E., Hopke, P. K., Pinto, J. P., and Wilson, W. E.: Spatial Variability of Fine Particle Mass, Components, and Source Contributions during the Regional Air Pollution Study in St. Louis, Environmental Science & Technology, 39, 4172-4179, 10.1021/es049824x, 2005.

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, Chemical Research in Toxicology, 15, 483-489, 2002.

Kundu, S., and Stone, E. A.: Composition and sources of fine particulate matter across urban and rural sites in the Midwestern United States, Environmental Science: Processes & Impacts, 16, 1360-1370, 2014.

Lee, J. H., and Hopke, P. K.: Apportioning sources of $PM_{2.5}$ in St. Louis, MO using speciation trends network data, Atmospheric Environment, 40, 360-377, 2006.

Lin, M., and Yu, J. Z.: Assessment of interactions between transition metals and atmospheric organics: ascorbic acid depletion and hydroxyl radical formation in organic-metal mixtures, Environmental Science & Technology, 54, 1431-1442, 10.1021/acs.est.9b07478, 2020.

Lin, M., and Yu, J. Z.: Assessment of oxidative potential by hydrophilic and hydrophobic fractions of water-soluble PM2.5 and their mixture effects, Environmental Pollution, 275, 116616, https://doi.org/10.1016/j.envpol.2021.116616, 2021.

Lin, P., Bluvshtein, N., Rudich, Y., Nizkorodov, S. A., Laskin, J., and Laskin, A.: Molecular Chemistry of Atmospheric Brown Carbon Inferred from a Nationwide Biomass Burning Event, Environmental Science & Technology, 51, 11561-11570, 10.1021/acs.est.7b02276, 2017.

Lin, P., Fleming, L. T., Nizkorodov, S. A., Laskin, J., and Laskin, A.: Comprehensive Molecular Characterization of Atmospheric Brown Carbon by High Resolution Mass Spectrometry with Electrospray and Atmospheric Pressure Photoionization, Analytical chemistry, 90, 12493-12502, 2018.

Liu, Q., Baumgartner, J., Zhang, Y., Liu, Y., Sun, Y., and Zhang, M.: Oxidative potential and inflammatory impacts of source apportioned ambient air pollution in Beijing, Environmental Science & Technology, 48, 12920-12929, 2014.

Liu, W., Xu, Y., Liu, W., Liu, Q., Yu, S., Liu, Y., Wang, X., and Tao, S.: Oxidative potential of ambient $PM_{2.5}$ in the coastal cities of the Bohai Sea, northern China: Seasonal variation and source apportionment, Environmental Pollution, 236, 514-528, 2018.

Luo, Y., Zhou, X., Zhang, J., Xiao, Y., Wang, Z., Zhou, Y., and Wang, W.: PM2.5 pollution in a petrochemical industry city of northern China: Seasonal variation and source apportionment, Atmospheric Research, 212, 285-295, https://doi.org/10.1016/j.atmosres.2018.05.029, 2018.

Maikawa, C. L., Weichenthal, S., Wheeler, A. J., Dobbin, N. A., Smargiassi, A., Evans, G., Liu, L., Goldberg, M. S., and Pollitt, K. J. G.: Particulate Oxidative Burden as a Predictor of Exhaled Nitric Oxide in Children with Asthma, Environmental Health Perspectives, 124, 1616, 10.1289/ehp175, 2016.

Massoud, R., Shihadeh, A. L., Roumié, M., Youness, M., Gerard, J., Saliba, N., Zaarour, R., Abboud, M., Farah, W., and Saliba, N. A.: Intraurban variability of PM10 and PM2. 5 in an Eastern Mediterranean city, Atmospheric Research, 101, 893-901, 2011.

Milando, C., Huang, L., and Batterman, S.: Trends in $PM_{2.5}$ emissions, concentrations and apportionments in Detroit and Chicago, Atmospheric Environment, 129, 197-209, 2016.

Moreno, T., Kelly, F. J., Dunster, C., Oliete, A., Martins, V., Reche, C., Minguillón, M. C., Amato, F., Capdevila, M., and de Miguel, E.: Oxidative potential of subway PM2. 5, Atmospheric Environment, 148, 230-238, 2017.

Mukherjee, A., Brown, S. G., McCarthy, M. C., Pavlovic, N. R., Stanton, L. G., Snyder, J. L., D'Andrea, S., and Hafner, H. R.: Measuring spatial and temporal PM2. 5 variations in Sacramento, California, communities using a network of low-cost sensors, Sensors, 19, 4701, 2019.

Paraskevopoulou, D., Bougiatioti, A., Stavroulas, I., Fang, T., Lianou, M., Liakakou, E., Gerasopoulos, E., Weber, R., Nenes, A., and Mihalopoulos, N.: Yearlong variability of oxidative potential of particulate matter in an urban Mediterranean environment, Atmospheric Environment, 206, 183-196, 2019.

Perrone, M. R., Bertoli, I., Romano, S., Russo, M., Rispoli, G., and Pietrogrande, M. C.: PM2. 5 and PM10 oxidative potential at a Central Mediterranean Site: Contrasts between dithiothreitol-and ascorbic acid-measured values in relation with particle size and chemical composition, Atmospheric Environment, 210, 143-155, 2019.

Pietrogrande, M. C., Perrone, M. R., Manarini, F., Romano, S., Udisti, R., and Becagli, S.: PM10 oxidative potential at a Central Mediterranean Site: Association with chemical composition and meteorological parameters, Atmospheric Environment, 188, 97-111, 2018.

Pietrogrande, M. C., Bertoli, I., Manarini, F., and Russo, M.: Ascorbate assay as a measure of oxidative potential for ambient particles: Evidence for the importance of cell-free surrogate lung fluid composition, Atmospheric Environment, 211, 103-112, 10.1016/j.atmosenv.2019.05.012, 2019.

Puthussery, J. V., Zhang, C., and Verma, V.: Development and field testing of an online instrument for measuring the real-time oxidative potential of ambient particulate matter based on dithiothreitol assay, Atmospheric Measurement Techniques, 11, 5767-5780, 10.5194/amt-11-5767-2018, 2018.

Reff, A., Bhave, P. V., Simon, H., Pace, T. G., Pouliot, G. A., Mobley, J. D., and Houyoux, M.: Emissions inventory of PM2. 5 trace elements across the United States, Environmental science & technology, 43, 5790-5796, 2009.

Riva, M., Budisulistiorini, S. H., Chen, Y., Zhang, Z., D'Ambro, E. L., Zhang, X., Gold, A., Turpin, B. J., Thornton, J. A., Canagaratna, M. R., and Surratt, J. D.: Chemical Characterization of Secondary Organic Aerosol from Oxidation of Isoprene Hydroxyhydroperoxides, Environmental Science & Technology, 50, 9889-9899, 10.1021/acs.est.6b02511, 2016.

Saffari, A., Daher, N., Shafer, M. M., Schauer, J. J., and Sioutas, C.: Seasonal and spatial variation in reactive oxygen species activity of quasi-ultrafine particles ($PM_{0.25}$) in the Los Angeles metropolitan area and its association with chemical composition, Atmospheric Environment, 79, 566-575, 2013.

Saffari, A., Daher, N., Shafer, M. M., Schauer, J. J., and Sioutas, C.: Seasonal and spatial variation in dithiothreitol (DTT) activity of quasi-ultrafine particles in the Los Angeles Basin and its association with chemical species, Journal of Environmental Science and Health, Part A, 49, 441-451, 10.1080/10934529.2014.854677, 2014.

Strak, M., Janssen, N., Beelen, R., Schmitz, O., Vaartjes, I., Karssenberg, D., van den Brink, C., Bots, M. L., Dijst, M., and Brunekreef, B.: Long-term exposure to particulate matter, NO2 and the oxidative potential of particulates and diabetes prevalence in a large national health survey, Environment international, 108, 228-236, 2017.

Szigeti, T., Dunster, C., Cattaneo, A., Cavallo, D., Spinazzè, A., Saraga, D. E., Sakellaris, I. A., de Kluizenaar, Y., Cornelissen, E. J., and Hänninen, O.: Oxidative potential and chemical composition of $PM_{2.5}$ in office buildings across Europe–The OFFICAIR study, Environment International, 92, 324-333, 10.1016/j.envint.2016.04.015, 2016.

Tao, J., Zhang, L., Cao, J., Zhong, L., Chen, D., Yang, Y., Chen, D., Chen, L., Zhang, Z., and Wu, Y.: Source apportionment of PM2. 5 at urban and suburban areas of the Pearl River Delta region, south China-with emphasis on ship emissions, Science of the Total Environment, 574, 1559-1570, 2017.

Verma, V., Pakbin, P., Cheung, K. L., Cho, A. K., Schauer, J. J., Shafer, M. M., Kleinman, M. T., and Sioutas, C.: Physicochemical and oxidative characteristics of semi-volatile components of quasi-ultrafine particles in an urban atmosphere, Atmospheric environment, 45, 1025-1033, 2011.

Verma, V., Fang, T., Guo, H., King, L., Bates, J., Peltier, R., Edgerton, E., Russell, A., and Weber, R.: Reactive oxygen species associated with water-soluble $PM_{2.5}$ in the southeastern United States: spatiotemporal trends and source apportionment, Atmospheric Chemistry and Physics, 14, 12915-12930, 2014.

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, Environmental Science & Technology, 49, 4646-4656, 10.1021/es505577w, 2015.

Visentin, M., Pagnoni, A., Sarti, E., and Pietrogrande, M. C.: Urban $PM_{2.5}$ oxidative potential: Importance of chemical species and comparison of two spectrophotometric cell-free assays, Environmental Pollution, 219, 72-79, 10.1016/j.envpol.2016.09.047, 2016.

Wang, Y., Plewa, M. J., Mukherjee, U. K., and Verma, V.: Assessing the cytotoxicity of ambient particulate matter (PM) using Chinese hamster ovary (CHO) cells and its relationship with the PM chemical composition and oxidative potential, Atmospheric Environment, 179, 132-141, 10.1016/j.atmosenv.2018.02.025, 2018.

Wang, Y., Puthussery, J. V., Yu, H., and Verma, V.: Synergistic and antagonistic interactions among organic and metallic components of the ambient particulate matter (PM) for the cytotoxicity measured by Chinese hamster ovary cells, Science of The Total Environment, 139511, 2020.

Weber, S., Uzu, G., Calas, A., Chevrier, F., Besombes, J.-L., Charron, A., Salameh, D., Ježek, I., Močnik, G., and Jaffrezo, J.-L.: An apportionment method for the oxidative potential of atmospheric particulate matter sources: application to a one-year study in Chamonix, France, Atmospheric Chemistry and Physics, 18, 9617-9629, 2018.

Weber, S., Uzu, G., Favez, O., Borlaza, L. J., Calas, A., Salameh, D., Chevrier, F., Allard, J., Besombes, J.-L., and Albinet, A.: Source apportionment of atmospheric PM 10 Oxidative Potential: synthesis of 15 year-round urban datasets in France, Atmospheric Chemistry and Physics Discussions, 1-38, 2021.

Wei, J., Yu, H., Wang, Y., and Verma, V.: Complexation of iron and copper in ambient particulate matter and its effect on the oxidative potential measured in a surrogate lung fluid, Environmental Science & Technology, 53, 1661-1671, 2018.

Weichenthal, S., Lavigne, E., Evans, G., Pollitt, K., and Burnett, R. T.: Ambient $PM_{2.5}$ and risk of emergency room visits for myocardial infarction: impact of regional $PM_{2.5}$ oxidative potential: a case-crossover study, Environmental Health, 15, 46, 10.1186/s12940-016-0129-9, 2016a.

Weichenthal, S., Shekarrizfard, M., Traub, A., Kulka, R., Al-Rijleh, K., Anowar, S., Evans, G., and Hatzopoulou, M.: Within-city spatial variations in multiple measures of $PM_{2.5}$ oxidative potential in Toronto, Canada, Environmental Science & Technology, 53, 2799-2810, 2019.

Weichenthal, S. A., Lavigne, E., Evans, G. J., Godri Pollitt, K. J., and Burnett, R. T.: Fine particulate matter and emergency room visits for respiratory illness. Effect modification by oxidative potential, American Journal of Respiratory and Critical Care Medicine, 194, 577-586, 2016b.

Xiong, Q., Yu, H., Wang, R., Wei, J., and Verma, V.: Rethinking the dithiothreitol-based particulate matter oxidative potential: measuring dithiothreitol consumption versus reactive oxygen species generation, Environmental Science & Technology, 51, 6507-6514, 10.1021/acs.est.7b01272, 2017.

Yang, A., Jedynska, A., Hellack, B., Kooter, I., Hoek, G., Brunekreef, B., Kuhlbusch, T. A., Cassee, F. R., and Janssen, N. A.: Measurement of the oxidative potential of $PM_{2.5}$ and its constituents: The effect of extraction solvent and filter type, Atmospheric Environment, 83, 35-42, 10.1016/j.atmosenv.2013.10.049, 2014.

Yang, A., Hellack, B., Leseman, D., Brunekreef, B., Kuhlbusch, T. A., Cassee, F. R., Hoek, G., and Janssen, N. A.: Temporal and spatial variation of the metal-related oxidative potential of $PM_{2.5}$ and its relation to $PM_{2.5}$ mass and elemental composition, Atmospheric Environment, 102, 62-69, 2015a.

Yang, A., Wang, M., Eeftens, M., Beelen, R., Dons, E., Leseman, D. L., Brunekreef, B., Cassee, F. R., Janssen, N. A., and Hoek, G.: Spatial variation and land use regression modeling of the oxidative potential of fine particles, Environmental Health Perspectives, 123, 1187-1192, 2015b.

Yang, A., Janssen, N. A., Brunekreef, B., Cassee, F. R., Hoek, G., and Gehring, U.: Children's respiratory health and oxidative potential of $PM_{2.5}$: the PIAMA birth cohort study, Occupational & Environmental Medicine, 73, 154-160, 10.1136/oemed-2015-103175, 2016.

Yu, H., Wei, J., Cheng, Y., Subedi, K., and Verma, V.: Synergistic and antagonistic interactions among the particulate matter components in generating reactive oxygen species based on the dithiothreitol assay, Environmental Science & Technology, 52, 2261-2270, 10.1021/acs.est.7b04261, 2018.

Yu, H., Puthussery, J. V., and Verma, V.: A semi-automated multi-endpoint reactive oxygen species activity analyzer (SAMERA) for measuring the oxidative potential of ambient $PM_{2.5}$ aqueous extracts, Aerosol Science and Technology, 54, 304-320, 2020.

Yu, S., Liu, W., Xu, Y., Yi, K., Zhou, M., Tao, S., and Liu, W.: Characteristics and oxidative potential of atmospheric $PM_{2.5}$ in Beijing: Source apportionment and seasonal variation, Science of the Total Environment, 650, 277-287, 2019.

Zhang, X., Staimer, N., Tjoa, T., Gillen, D. L., Schauer, J. J., Shafer, M. M., Hasheminassab, S., Pakbin, P., Longhurst, J., and Sioutas, C.: Associations between microvascular function and short-term exposure to traffic-related air pollution and particulate matter oxidative potential, Environmental health, 15, 1-16, 2016.

Zhang, Y., Schauer, J. J., Shafer, M. M., Hannigan, M. P., and Dutton, S. J.: Source apportionment of in vitro reactive oxygen species bioassay activity from atmospheric particulate matter, Environmental Science & Technology, 42, 7502-7509, 10.1021/es800126y, 2008.

Appendix: Revised manuscript in track mode

Spatiotemporal Variability in the Oxidative Potential of Ambient Fine Particulate Matter in Midwestern United States

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Abstract. We assessed the oxidative potential (OP) of both water-soluble and methanol-soluble fractions of ambient fine particulate matter ($PM_{2.5}$) in the midwestern United States. A large set of $PM_{2.5}$ samples (N = 241) were collected from five sites, setup in different environments, i.e. urban, rural and roadside, in Illinois, Indiana and Missouri during May 2018 – May 2019. Five acellular OP endpoints, including the consumption rate of ascorbic acid and glutathione in a surrogate lung fluid (SLF) (OP^{AA} and OP^{GSH}, respectively), dithiothreitol (DTT) depletion rate (OP^{DTT}), and ·OH generation rate in SLF and DTT ($OP^{OH\text{-}SLF}$ and $OP^{OH\text{-}DTT}$, respectively), were measured for all $PM_{2.5}$ samples. $PM_{2.5}$ mass concentrations in the Midwest US as obtained from these samples were spatially homogeneously distributed, while most OP endpoints showed significant spatiotemporal heterogeneity. Seasonally, higher activities occurred in summer for most OP endpoints for both water- and methanol-soluble extracts. Spatially, roadside site showed highest activities for most OP endpoints in the water-soluble extracts, while only occasional peaks were observed at urban sites in the methanol-soluble OP. Most OP endpoints showed similar spatiotemporal trends between mass- and volume-normalized activities across different sites and seasons. Comparisons between two solvents (i.e. water and methanol) showed that methanol-soluble OP generally had higher activity levels than corresponding water-soluble OP. Site-to-site comparisons of OP showed stronger correlations for methanol-soluble OP compared to water-soluble OP, indicating a better extraction of water-insoluble redox-active compounds from various emission sources into methanol. We found a weak correlation and inconsistent slope values between $PM_{2.5}$ mass and most OP endpoints. Moreover, the poor-to-moderate intercorrelations among different OP endpoints infer different mechanisms of OP represented by these endpoints, and thus demonstrate the rationale for analyzing multiple acellular endpoints for a better and comprehensive assessment of OP.

1 Introduction

Oxidative stress induced by ambient fine particulate matter ($PM_{2.5}$; particulate matter with size less than 2.5 μm) has been widely recognized as a biological pathway for fine particles to exert adverse health effect in humans (Sørensen et al., 2003;Risom et al., 2005;Garçon et al., 2006;Wessels et al., 2010;Cachon et al., 2014;Haberzettl et al., 2016;Feng et al., 2016;Rao et al., 2018;Mudway et al., 2020). A variety of chemical species in ambient particles, such as transition metals and aromatic organic species, possess redox cycling capability and can catalyze electron transfer from cellular

reductants (e.g. NADPH) to molecular oxygen (O_2), which subsequently forms highly reactive radicals [e.g. superoxide radical ($\cdot O_2^-$) and hydroxyl radical ($\cdot OH$)] and non-radical oxidants [e.g. hydrogen peroxide (H_2O_2)] (Kampfrath et al., 2011;Qin et al., 2018;Kumagai et al., 2002;Lee et al., 2016). These oxygen containing species with high redox activity and short lifetime are collectively defined as the reactive oxygen species (ROS). Several antioxidants (e.g. ascorbic acid (AA), reduced glutathione (GSH) and uric acid (UA) etc.) that are present in human respiratory tract lining fluid (RTLF) can counteract the ROS under normal conditions by donating extra electrons, thus forming less-oxidative species and oxidized antioxidants (Kelly, 2003;Li and Nel, 2006;Allan et al., 2010;Zuo et al., 2013;Poljšak and Fink, 2014). However, excessively produced ROS might penetrate the antioxidant barrier and induce oxidative stress (Xing et al., 2016;Rao et al., 2018), leading to the cascade of detrimental biological effects such as oxidation of DNA, lipids and proteins (Rossner et al., 2008;Franco et al., 2008;Grevendonk et al., 2016), tissue injury (Feng et al., 2016;Gurgueira et al., 2002;Sun et al., 2020) and eventually cardiopulmonary impairment (Li et al., 2018;Kodavanti et al., 2000;Kampfrath et al., 2011). The capability of particulate matter (PM) for catalyzing the generation of ROS and/or the depletion of antioxidants is defined as the oxidative potential (OP) of PM (Bates et al., 2019).

The assessment of $PM_{2.5}$-induced oxidative stress is conventionally carried out through biological tests, including both *in vitro* (Becker et al., 2005;Zhang et al., 2008;Oh et al., 2011;Yan et al., 2016;Abbas et al., 2016;Deng et al., 2013) and *in vivo* designs (Kleinman et al., 2005;Riva et al., 2011;Pei et al., 2016;Araujo et al., 2008;Xu et al., 2011;Sancini et al., 2014). Although, these biological tests are highly relevant in terms of representing the health effects in humans, the time- and labor-intensive protocols as well as the cost of experimental materials generally limit their application to only small sample sizes. Various acellular chemical assays which assess the OP by replicating intrinsic biological mechanisms were therefore developed as alternatives. These assays are generally divided in two categories. The OP analysis approaches in the 1st category directly probe the generation of ROS during redox cycling reactions in presence of PM, such as the measurement of H_2O_2 and $\cdot OH$ production in surrogate lung fluid (SLF) (Vidrio et al., 2009;Shen et al., 2011;Charrier et al., 2014;Ma et al., 2015), and H_2O_2 and $\cdot OH$ production in dithiothreitol (DTT) (Yu et al., 2018;Xiong et al., 2017;Chung et al., 2006;Kumagai et al., 2002). The assays in 2nd category utilize the consumption of antioxidants such as AA (Visentin et al., 2016;Weichenthal et al., 2016b) and GSH (Künzli et al., 2006;Szigeti et al., 2016), or surrogates of cellular reductants such as DTT (Verma et al., 2014;Cho et al., 2005), as the OP indicator. Analyzing each PM sample for all of these chemical assays is also time-consuming. To address this concern, we have previously developed an automated OP analysis instrument named SAMERA – Semi-Automated Multi-Endpoint ROS-activity Analyzer, which can measure five most commonly used OP endpoints (i.e. consumption rate of AA and GSH in SLF, OP^{AA} and OP^{GSH} respectively; consumption rate of DTT, OP^{DTT}, and generation rate of $\cdot OH$ in SLF and DTT, $OP^{OH\text{-}SLF}$ and $OP^{OH\text{-}DTT}$) for a PM extract in less than 3 hours (Yu et al., 2020). ~~These~~ Many of these acellular endpoints have been widely implemented by various researchers for assessing the oxidative properties of $PM_{~~2.5~~}$. Calas et al. (2018) compared the responses of several OP endpoints [i.e. OP^{DTT}, OP^{AA}, OP^{GSH}, and electron spin resonance (OP^{ESR})] on PM_{10} samples (N = 98) collected from Chamonix (France). Yang et al. (2014) also used four OP endpoints [OP^{AA}, OP^{DTT}, OP^{ESR} and reductive acridinium triggering (OP^{CRAT})] to investigate the effect of different extraction solvents and filter types on OP responses using the $PM_{2.5}$ samples (N = 20) collected from two cities (Rotterdam and

Amsterdam) in Netherland. The comparison of OP^{AA}, OP^{DTT} and OP^{GSH} has been shown in two studies (Fang et al., 2016;Gao et al., 2020a), both from the southeast US. We are not aware of any study which has compared ·OH generation in SLF or DTT with other endpoints based on antioxidants consumption (e.g. AA or GSH consumption). Clearly, the studies systematically comparing the responses of these different endpoints on a large sample-set collected from an extensive spatial scale, particularly in the United States are very limited.~~However, there has not been a single study which has systematically compared the responses of all of these chemical assays in a single investigation.~~

Although OP is proposed as an integrative $PM_{2.5}$ property, purportedly combining the individual and synergistic actions of its many active components, there have been limited attempts to integrate it in the large-scale epidemiological studies. This is because, unlike other PM properties such as mass, sulfate, nitrate etc., the OP measurements in different geographical regions have been relatively sparse. Moreover, before integrating OP in the epidemiological studies, it is important that we investigate the differences of its spatiotemporal distribution with other commonly measured PM properties such as mass. An understanding of the temporal variation of OP in a specific environment could be helpful in time series studies of short-term effects, while the spatial variation of OP can aid in studying the long-term health effects of $PM_{2.5}$ exposure among different regions (Yang et al., 2015a). Globally, the spatiotemporal profiles of OP have been characterized for some geographical regions such as Los Angeles Basin (Saffari et al., 2014, 2013), Denver (Zhang et al., 2008), Atlanta (Fang et al., 2016;Verma et al., 2014) in US, Ontario (Canada) (Jeong et al., 2020;Weichenthal et al., 2019;Weichenthal et al., 2016a), France (Borlaza et al., 2021;Calas et al., 2019;Weber et al., 2018;Weber et al., 2021), Italy (Cesari et al., 2019;Perrone et al., 2019;Pietrogrande et al., 2018), Athens in Greece (Paraskevopoulou et al., 2019), Netherland (Yang et al., 2015a;Yang et al., 2015b), and some coastal cities of Bohai [Jinzhou, Tianjin and Yantai (Liu et al., 2018)] and Beijing (Yu et al., 2019;Liu et al., 2014) in China. Some of these studies have substantially contributed in enhancing our understanding of the role of OP in the PM-induced health effects (Fang et al., 2016;Tuet et al., 2016;Abrams et al., 2017;Weichenthal et al., 2016a;Yang et al., 2016;Bates et al., 2015). However, despite including many cities ranked high in terms of the air pollution [e.g. Indianapolis (Rosenthal et al., 2008), Chicago (Dominici et al., 2003), St. Louis (Sarnat et al., 2015), Detroit (Zhou et al., 2011), Cincinnati (Kaufman et al., 2019), and Cleveland (Kumar et al., 2013)], the midwestern region of the United States is an understudied region in terms of assessing the oxidative levels of ambient $PM_{2.5}$.

Here, we investigate the detailed spatiotemporal profiles of ambient $PM_{2.5}$ mass concentrations and OP in the midwestern United States. Simultaneous ambient $PM_{2.5}$ samples were collected from five different sites in the Midwest US. The automated instrument – SAMERA facilitated the measurement of OP on our large bulk of $PM_{2.5}$ samples (N = 241) collected from all the sites, which were extracted in both water and methanol separately. ~~This paper mainly discusses the spatiotemporal distribution of the mass concentration and OP of $PM_{2.5}$ measured by five different endpoints in the Midwest US.~~The goal of this analysis is to compare the spatiotemporal distribution of $PM_{2.5}$ OP with that of the mass concentrations. We also want to investigate if different measures of OP, i.e. OP^{AA}, OP^{GSH}, $OP^{OH\text{-}SLF}$, OP^{DTT} and $OP^{OH\text{-}DTT}$ show different spatiotemporal trends or are correlated with each other. Correlations of OP with PM chemical composition and source apportionment analysis of $PM_{2.5}$ OP will be presented in our subsequent

publications. Our paper presents the results from probably one of the most comprehensive OP analysis campaigns, combining five different acellular OP endpoints measured on both water- and organic-soluble extracts.

2 Experimental methods

2.1 Sampling campaign

Simultaneous sampling in five different sites spread across three states (i.e. Illinois, Indiana and Missouri) was conducted every week for this project in the Midwest US. The locations of the sampling sites are shown in Figure 1. Champaign (CMP) and Bondville (BON) sites are paired sites representing the urban (roadside) and rural environment of Champaign County, IL, respectively; while three major city sites [i.e. Chicago (CHI), Indianapolis (IND) and St. Louis (STL)] are representatives of urban background regions of ~~Chicago, Indianapolis and St. Louis, respectively~~these respective cities.

CMP is located on a parking garage in the campus of University of Illinois at Urbana-Champaign, and is adjacent to a 2-lane (both ways) road (i.e. University Avenue). This site is surrounded by the university facilities and is impacted by traffic emissions from adjacent road. The site is about 1 km from downtown Champaign and is surrounded by dense housing and business development.

BON is a rural site, 15 km west of downtown Champaign, and is also a part of the IMPROVE (Interagency Monitoring of Protected Visual Environments) monitoring program. The station is managed by the Illinois State Water Survey, and is surrounded by intensively managed agricultural fields. The major highways (I-57 and I-74) are at least 6 km north and east of this site, respectively.

CHI site is located on a dormitory building – Carman hall in Illinois Institute of Technology (IIT) campus, Chicago, IL. This site is ~500 m away from a two-way 6-lane (including an emergency lane) interstate highway I-90/94, 1.5 km west of Lake Michigan and 5 km south of downtown Chicago. The highway I-90/94 has an annual average daily traffic flow of 300,000 vehicles per day, and heavy-duty vehicles account for ~10% in the traffic fleet (Xiang et al., 2019). The site is situated in the mixed commercial and residential area of Chicago, and therefore the emissions from both traffic mixed with residential and commercial activities are expected.

IND site is located inside the campus of School of Public Health, Indiana University – Purdue University Indianapolis (IUPUI). This site is close to downtown Indianapolis (2 km southeast of IND site) and a two-way 4-lane interstate highway I-65 (1 km northeast of IND site). The site is surrounded by miscellaneous facilities of IUPUI and Riley Hospital, therefore the sources of ambient aerosols at IND site may include vehicular emissions from highway, and emissions from residential and commercial activities related to miscellaneous university and hospital operations.

STL site is located 3 km north of downtown St. Louis, MO. This site is 230 m west of the interstate I-44/70 and 1.2 km west of Mississipi River. It is also surrounded by several industries for steel processing, zinc smelting and copper production (Lee et al., 2006). Therefore, a significant portion of metals in PM at this site is supposed to be from

industrial emissions. The urban activities in downtown St. Louis as well as traffic emissions from highway vehicles and river boating are also potential sources of $PM_{2.5}$ at this site.

The sampling period involved four seasons starting from May 22, 2018 to May 30, 2019. Integrated ambient $PM_{2.5}$ samples were collected simultaneously for three continuous days from all the sites. Each site was instrumented with a High-volume (Hi-Vol) air sampler equipped with $PM_{2.5}$ inlet (flow rate = 1.13 m^3/min; Tisch Environmental; Cleves, OH). Both before and after the sampling campaign, we did a comparison of various samplers by running them in parallel to collect $PM_{2.5}$ samples and analyzing them for OP^{DTT} (see Section S1 of the supplemental information, SI). All the samplers were equipped with a timer to enable automatic start of the sampling on each Tuesday 0:00, and turn-off on each Friday 0:00. After the sampled filters were collected on Friday (before noon), new filters were loaded in the filter holder to start next run of sampling. All five samplers were monthly calibrated for the flow rate by using a variable flow calibration kit (Tisch Environmental), and the flow rate was measured every week before and after the sampling. We used quartz filters (Pall TissuquartzTM, 8"×10") for collecting $PM_{2.5}$. The filters were prebaked at 550 °C for 24 hours before sampling. Total 241 filters were collected during the whole campaign (44 from CHI, 47 from STL, 54 from IND, 51 from CMP and 45 from BON). We also collected field blank filters (N = 10 from each site) once in every five weeks by placing a blank quartz filter in filter holder of the sampler for 1 hour but without running the pump.

All filters were weighed before and after sampling using a lab-scale digital balance (0.2 mg readability, Sartorius A120S, Götingen, Germany) for determining the $PM_{2.5}$ mass loading on each filter. Prior to each weighing, filters were equilibrated in a constant temperature (24 °C) and relative humidity (50 %) room for 24 hours. After sampling, the filters were individually wrapped in prebaked (550 °C) aluminum foils and stored in a freezer at -20 °C before analysis. More information on sampling including the exact dates of sampling are provided in Table S1 in the supplemental information (SI).

2.2 Sample extraction protocol

Sample extraction protocol for OP analysis was determined by the requirement to keep a relatively constant concentration of $PM_{2.5}$ in the liquid extracts. This is due to non-linear response of certain OP endpoints with $PM_{2.5}$ mass in the extracts (Charrier et al., 2016). Thus, fraction of the filter and the volume of water used for extraction were varied depending on the $PM_{2.5}$ mass loading on each Hi-Vol filter. For the analyses of water-soluble OP, a few (usually 3-5) circular sections (16-25 mm diameter) were punched from the filter and immersed into 15-20 mL of deionized Milli-Q water (DI, resistivity = 18.2 MΩ/cm). The volume of water was adjusted to achieve ~100 µg of total $PM_{2.5}$ per mL of DI. The vials containing filter sections suspended in the DI were sonicated in an ultrasonic water bath for 1 hour (Cole-Palmer, Vernon-Hills, IL, US). These suspensions were then filtered through a 0.45 µm PTFE syringe filter to remove all water-insoluble components including filter fibers. 10.5 mL of these filtered extracts were separated and diluted with DI to 15 mL. These diluted extracts were then kept in the sample queue of SAMERA for OP analyses. SAMERA withdraws different volume of these extracts into the reaction vials (RVs) for each OP measurement, i.e. 3.5 mL for OP^{AA}, OP^{GSH} and $OP^{OH\text{-}SLF}$, and 2.1 mL for OP^{DTT} and $OP^{OH\text{-}DTT}$ measurements, all of

which were further diluted to 5 mL in the RVs. Thus, the concentrations of $PM_{2.5}$ in RVs for SLF-based (i.e. OP^{AA}, OP^{GSH} and $OP^{OH\text{-}SLF}$) and DTT-based (i.e. OP^{DTT} and $OP^{OH\text{-}DTT}$) assays were maintained constant at 50 μg/mL and 30 μg/mL (±1%), respectively.

For methanol-soluble OP measurements, another fraction from each filter having the same area as used for the water-soluble $PM_{2.5}$ extraction was punched and extracted in 10 mL of methanol. After sonication for 1 hour, the suspensions were filtered through 0.45 μm PTFE syringe filter. The filtered extracts were then concentrated to less than 50 μL using a nitrogen dryer to evaporate methanol, and were subsequently reconstituted in~~to 15-20 mL of~~ DI to the exact same volume as the water-soluble extracts. Reconstituted methanol extracts were vigorously shaken on an analog vortex mixer (VWR International, Batavia, IL, US) for at least 60 seconds at 3200 rpm to ensure a thorough flushing of the components probably deposited along the wall of the vials during evaporation. These methanol-soluble extracts were then~~and~~ analyzed for OP in the same way as water-soluble extracts.

2.3 OP analysis

OP activities of $PM_{2.5}$ extracts were analyzed using SAMERA. The setup and operation protocol of SAMERA has been discussed in detail in Yu et al. (2020). Briefly, the analysis of all OP endpoints for each extract was conducted in two stages: SLF-based endpoints were analyzed first, while DTT-based assays were conducted in the second stage. For measuring OP^{AA} and OP^{GSH}, 3.5 mL of the extract was mixed with 0.5 mL SLF and 1 mL of 0.5 M potassium phosphate buffer (K-PB) in an RV. SLF was made following the protocol of Yu et al. (2020), i.e. by mixing equal volumes (1 mL each) of four antioxidant stock solutions – 20 mM AA, 10 mM GSH, 30 mM citric acid (CA) and 10 mM UA, and diluting the mixture by DI to 10 mL. Final concentrations of the antioxidants in the RV used for incubating the sample, were 200 μM AA, 100 μM GSH, 300 μM CA and 100 μM UA. At certain time intervals (i.e. 5, 24, 43, 62 and 81 minutes), two small aliquots of the reaction mixture were withdrawn and dispensed into two measurement vials (MV1 and MV2) separately. The mixture in MV1 was diluted by DI, and was directly injected into a liquid waveguide capillary cell (LWCC-3100; World Precision Instruments, Inc., Sarasota, FL, USA) coupled to an online spectrophotometer (Ocean Optics, Inc., Dunedin, FL, USA), which measured the absorbance at 265 nm (signal from AA) and 600 nm (background) for determining the concentration of AA. 1.6 mL of o-phthalaldehyde (OPA) was added into the reaction mixture contained in MV2 to react with GSH, which forms a fluorescent product. The final mixture in MV2 was then pushed through a flow cell equipped in a Horiba Fluoromax-4 spectrofluorometer (Horiba Scientific, Edison, NJ, USA), and the fluorescence was measured at excitation/emission wavelength of 310 nm/427 nm. Simultaneously with the preparation of the reaction mixture for OP^{AA} and OP^{GSH} analyses, 3.5 mL of the extract was mixed with 0.5 mL SLF and 1 mL of 50 mM K-PB buffered disodium terephthalate (TPT) (pH = 7.4) in another RV2. TPT captures ·OH generated in the reaction and forms another fluorescent product 2-hydroxyterephthalic acid (2-OHTA). Small aliquots of this reaction mixture were withdrawn into MV2 at selected time intervals (10, 29, 48, 67 and 86 minutes), diluted by DI, and injected into the flow cell of the spectrofluorometer for measuring fluorescence at the same wavelengths as used for GSH measurement (i.e. 310 nm excitation/427 nm emission). The concentration of 2-OHTA was determined by calibrating various concentrations (10-500 nM) of 2-OHTA standards, and the

generation rate of ·OH was determined as the formation rate of 2-OHTA divided by a yield factor (0.35) (Son et al., 2015).

Both RVs and MVs were flushed with DI after all SLF-based endpoints were analyzed, and DTT-based assays started immediately after this cleaning. Similar to the first step of SLF assay, 2.1 mL of the diluted $PM_{2.5}$ extract was mixed with 1 mL of 50 mM TPT, 1.4 mL of DI and 0.5 mL of 1 mM DTT in an RV. At certain time intervals (i.e. 5 min, 17 min, 29 min, 41 min and 53 min), two small aliquots of this reaction mixture were withdrawn and diluted with DI in MV1 and MV2 separately for the measurement of DTT and ·OH, respectively. DTNB was added into MV1 to capture residual DTT. The final mixture in MV1 was pushed through LWCC to measure the absorbance at 412 nm, while the mixture in MV2 was pushed through flow cell of the spectrofluorometer for fluorescence measurement (310 nm excitation/427 nm emission), respectively. The system was again cleaned by flushing DI to RVs, MVs, LWCC and flow cell of the spectrofluorometer for the next run. Once in a week, we conducted thorough cleaning of the entire system, by replacing all chemicals and samples first with methanol followed by DI, and running the program script 10 times with each solvent.

2.4 Quality Control/Quality Assurance

One field blank filter extract along with a DI blank were used as the negative controls for each set of $PM_{2.5}$ samples analyzed in a batch (usually ~10). Selected metals and organic compounds that are known to be sensitive for different OP endpoints, i.e. Cu(II) for OP^{AA} and OP^{GSH}, Fe(II) for $OP^{OH\text{-}SLF}$, phenanthraquinone (PQ) for OP^{DTT} and 5-hydroxy-1,4-naphthoquinone (5-H-1,4-NQ) for $OP^{OH\text{-}DTT}$, were used as the positive control, and were analyzed weekly with $PM_{2.5}$ samples to ensure the stability of SAMERA and correct for any possible drift.

The average and standard deviation of OP of negative and positive controls are shown in Table 1. Our previous study on the development of SAMERA (Yu et al., 2020) reported the values of OP for negative controls, as 0.17 ± 0.07 μM/min for OP^{AA}, 0.37 ± 0.06 μM/min for OP^{GSH}, 4.57 ± 1.21 nM/min for $OP^{OH\text{-}SLF}$, 0.65 ± 0.02 μM/min for OP^{DTT} and -0.38 ± 0.24 μM/min for $OP^{OH\text{-}DTT}$, which are consistent with the values reported in Table 1. The precision of SAMERA was assessed previously using water-soluble extracts and the coefficient of variations (CoVs) were reported to be less than 14 % (7.9 – 13.3 %) for all OP endpoints (Yu et al., 2020). We also assessed the precision using methanol-soluble extracts and found similar levels of CoVs, i.e. 8.9 -14.5 % for all OP endpoints (see Table S2 in SI). Consistency of our current results for negative controls with those reported earlier, and ~~a~~ the low ~~coefficient of variation (~~CoVs~~)~~ obtained for the positive controls (1.1 – 11.8%) and $PM_{2.5}$ extracts ensured a good quality assurance for the overall OP analysis. We blank corrected all OP values of ambient samples by subtracting the averaged field blank measurements. After blank correction, the OP values below detection limit were replaced with half of the detection limits for the corresponding OP endpoint. The mass-normalized (intrinsic, OPm) and volume-normalized (extrinsic, OPv) OP levels were obtained by dividing the blank corrected OP activities by the extracted $PM_{2.5}$ mass (for OPm) and by the volume of air collected on the extracted fractions of filters (for OPv), respectively. The detailed calculations of OPm and OPv have been previously described in Yu et al. (2020).

241 2.5 Statistical analysis

242 To assess spatiotemporal variability in both OP and $PM_{2.5}$ mass, we compared their differences among all sites and
243 seasons using one-way analysis of variance (ANOVA) test, and different pairs (i.e. pairs of different sites or seasons)
244 were compared by Fisher's least significant difference (LSD) post-hoc test. The significant and highly significant
245 differences were considered by one-way ANOVA when $P < 0.05$ and $P < 0.01$, respectively. Pearson's correlation
246 coefficient (r) for single linear regression was computed to determine the correlation of OP between different sites,
247 between water-soluble and methanol-soluble OP, between OP and $PM_{2.5}$, as well as the intercorrelation among
248 different endpoints for each site. All $PM_{2.5}$ samples were assessed for spatiotemporal variability. However, ~~Since~~ since
249 several OP endpoints (e.g. OP^{AA}, OP^{GSH} and OP^{DTT}) were abnormally elevated in the week of July 4th (Independence
250 Day celebration; discussed in section 3.2), we removed this week's sample from our regression analysis to avoid any
251 bias caused by this episodic event. Site-to-site comparisons were performed by calculating the coefficient of
252 divergence (COD) of mass concentration and volume-normalized OP (i.e. OPv) for all site pairs, as follows:

253
$$CoD = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(\frac{c_{ij} - c_{ik}}{c_{ij} + c_{ik}}\right)^2}$$

254 where: c_{ij} and c_{ik} are the $PM_{2.5}$ mass or OPv measured in the same week *i* at sites *j* and *k*, respectively; N is the number
255 of the comparable sample pairs for sites *j* and *k*. COD ranges from 0 to 1. A larger COD (closer to 1) indicates more
256 spatial heterogeneity between the sites, while a smaller COD (closer to 0) implies spatial homogeneity. One-way
257 ANOVA test was conducted in Matlab R2019a, while other statistical analyses were carried out using Excel.

258 **3 Results and Discussion**

259 3.1 $PM_{2.5}$ mass concentration

260 Figure 2 shows the time series of three-days averaged $PM_{2.5}$ mass concentration at five sampling sites, while the
261 seasonal averages are shown in Table 2. The mass concentrations ranged from 2.0 to 21.7 μg/m^3 across all sites, and
262 the median was 11.0 μg/m^3. These results are comparable with ~~previous studies on~~the typical ranges of $PM_{2.5}$ in
263 Midwest US cities (2.1 – 48.6 μg/m^3), e.g. St. Louis ~~(3.9 – 48.6 μg/m^3)~~ (Lee et al., 2006), Chicago ~~(median 9.4 – 10.7~~
264 ~~μg/m^3)~~ (Milando et al., 2016), Detroit ~~(0.6 – 56.2 μg/m^3, median 14.4 – 17.6 μg/m^3)~~ (Gildemeister et al., 2007),
265 Bondville ~~(2.1 – 36.5 μg/m^3, median 9.5 μg/m^3)~~ and selected cities in Iowa (e.g. Cedar Rapids, Des Moines and
266 Davenport) ~~(8.4 – 11.6 μg/m^3)~~ (Kundu and Stone, 2014), as measured in several previous studies. Generally, the more
267 urbanized sites of our study (i.e. CHI, STL and IND) showed slightly higher mass concentrations (5.7 – 21.7 μg/m^3;
268 median: 11.8 μg/m^3) compared to the smaller cities like CMP and its rural component (i.e. BON) (2.0 – 20.2 μg/m^3;
269 median: 9.2 μg/m^3). The highest mass concentrations were recorded at CHI ~~(~~during winter ($P < 0.01$; Table S3) and
270 STL ~~(~~during summer ($P < 0.05$), while BON exhibited the lowest concentrations in all seasons, except fall when the
271 mass concentrations were lowest at CMP ($P < 0.05$). Other than these minor variations, the $PM_{2.5}$ mass concentrations

are both spatially and temporally homogeneous in the Midwest US with no significant seasonal differences (P > 0.05 at most sites).

3.2 ~~Time series~~Spatiotemporal variation in ~~of~~ $PM_{2.5}$ OP

Time series of both mass- and volume-normalized OP (OPm and OPv, respectively) at all the sites are shown in Figure 3 (water-soluble OP) and Figure 4 (methanol-soluble OP). Seasonally averaged OPm and OPv of water-soluble and methanol-soluble $PM_{2.5}$ are also shown in Figures 5 and 6, respectively. Differences in both OPm and OPv among different seasons or sites were determined by one-way ANOVA and the results are listed in SI, Table S4 (water-soluble OP) and Table S5 (methanol-soluble OP). Generally, OP ~~for both water- and methanol-soluble extracts~~ showed much more spatiotemporal variability than the $PM_{2.5}$ mass in the Midwest US.

Water-soluble $PM_{2.5}$ OP

~~The~~ Figures 3 and 5 (time series and seasonal averages of water-soluble OP) showed a significant spatial variability for SLF-based endpoints, particularly ~~(i.e.~~ OP^{AA}~~,~~ and OP^{GSH}, ~~and $OP^{OH\text{-}SLF}$)~~in comparison to DTT-based OP (i.e. OP^{DTT} and $OP^{OH\text{-}DTT}$) ~~in~~ for both mass- and volume-normalized results ~~(Figure 3a-c)~~. Highest OP^{AA} and OP^{GSH} activities (both mass- and volume-normalized) occurred at ~~the roadside site~~ CMP (~~as confirmed by 1-way ANOVA test;~~ $P < 0.01$) in most seasons ~~(except winter for OP^{AA}v), while STL and IND had the lowest OP^{AA} and OP^{GSH}~~. $OP^{OH\text{-}SLF}$ was more spatially uniformly distributed than OP^{AA} and OP^{GSH}; significantly higher $OP^{OH\text{-}SLF}$m and $OP^{OH\text{-}SLF}$v were observed at CMP only in summer and spring ($P < 0.05$). For the DTT-based endpoints, OP^{DTT}v was only marginally higher at CHI in winter, and at CMP in summer and spring. Other than that, no significant differences were observed for OP^{DTT}v among various sites. The spatially uniform pattern for OP^{DTT}v is consistent with Verma et al. (2014) which found limited spatial variation for OP^{DTT}v in the Southeast US. In contrast, there was a significant variation in the OP^{DTT}m with elevated levels at CMP ($P < 0.01$) in all seasons. Interestingly, the $OP^{OH\text{-}DTT}$ endpoint showed more spatial variability and was generally lowest at CMP ($P < 0.05$) – the site which showed highest levels for ~~all~~ other OP endpoints. It implies that although OP^{DTT} and $OP^{OH\text{-}DTT}$ endpoints are measured in the same DTT assay, different chemical components play differential roles in these endpoints. We found very similar spatial patterns of mass- and volume-normalized OP activities for most endpoints, ~~again~~ indicating only a marginal role of $PM_{2.5}$ mass concentrations in causing the spatial variability in OP levels.

~~Differences in both OPm and OPv among different seasons or sites were determined by one-way ANOVA and the results are listed in SI, Table S4.~~ Seasonally, highest OP activities were generally observed in summer, while the lowest activities usually occurred in winter (Figure 5). ~~For example, OP^{AA}v and OP^{GSH}v activities had highest levels in summer and lowest levels in winter at CMP and BON, as verified by 1-way ANOVA ($P < 0.05$). Similarly, significantly higher OP activities ($P < 0.01$ for most cases) were observed for both $OP^{OH\text{-}SLF}$m and $OP^{OH\text{-}SLF}$v at all five sites in summer, while winter showed significantly lower levels ($P < 0.05$). For DTT-based endpoints, $OP^{OH\text{-}DTT}$m and $OP^{OH\text{-}DTT}$v also showed higher values in summer at CHI, IND and CMP ($P < 0.01$). However, OP^{DTT} exhibited limited temporal variation at most sites with only slightly higher OP^{DTT}m and OP^{DTT}v observed in summer at BON ($P < 0.05$).~~ An exception to this trend was OP^{DTT}, which exhibited limited temporal variation at most sites with only

slightly higher OP^{DTT} observed in summer at BON ($P < 0.05$). The temporal ~~variation trend~~uniformity of OP^{DTT} in this study does not correspond with previous studies conducted in Southwest and Southeast US. For the Southeast US, Verma et al. (2014) found significantly higher OP^{DTT}v in winter (December, 2012) compared to summer (June to August, 2012), and this difference was even more pronounced in mass-normalized OP. Saffari et al. (2014) also observed higher OP^{DTT} activities of quasi-ultrafine particles ($PM_{0.25}$) in fall and winter seasons for the Southwest US (Los Angeles Basin), and attributed this trend to the partitioning of redox-active semi-volatile organic compounds to particle phase in colder seasons. However, the trend of OP^{AA} in our study is in agreement with another study in Southeast US ~~using OP^{AA} as the endpoint~~ (Fang et al., 2016), which showed higher OP^{AA} in warmer seasons (i.e. summer and fall) than winter. ~~There is no previous literature available on the spatiotemporal trends of other OP endpoints in US, to which we can compare our results.~~The seasonal trend of mass- and volume-normalized activities were nearly identical for all endpoints, again indicating a marginal effect of $PM_{2.5}$ mass concentration in the temporal variation of OP.

~~CMP showed a substantially higher water-soluble OP than other sites for these endpoints. In the temporal trend, SLF-based endpoints showed higher levels during summer compared to other seasons at most sites.~~ A significant temporal variation was observed for CMP with several spikes in the OP activities throughout the year, most prominently for OP^{AA} (Figure 3). These spikes might be attributed to the traffic, as CMP is the only site adjacent (< 10 m) to a major urban road and located on the roof of a parking garage. One of our previous studies, Wang et al. (2018), reported large variations in several redox-active metals (e.g. Cu, Fe, Mn, Pb and Zn), which have been known to be related with the vehicular emissions (Hulskotte et al., 2007;Garg et al., 2000;Gietl et al., 2010;Apeagyei et al., 2011;Councell et al., 2004), at the same CMP site. Since SLF-based endpoints have been shown to be highly sensitive towards metals (Ayres et al., 2008;Calas et al., 2018;Fang et al., 2016;Moreno et al., 2017;Charrier and Anastasio, 2015;Wei et al., 2018), the temporal variation in traffic intensity probably contributes to the spikes observed at CMP. The peaks in the week of July 3 were observed for multiple endpoints (e.g. OP^{AA}, OP^{GSH} and OP^{DTT}) at most sites, which is attributed to the emissions from firecrackers on Independence Day (July 4) celebrations (Yu et al., 2020;Puthussery et al., 2018).

Methanol-soluble $PM_{2.5}$ OP

~~As observed in the time series, the spatiotemporal variations for the methanol-soluble OP endpoints (e.g. OP^{AA}, OP^{GSH}, OP^{DTT} and $OP^{OH\text{-}DTT}$) seem to be lesser than the corresponding water-soluble OP (Figure 4a-b, d-e). However, methanol-soluble $OP^{OH\text{-}SLF}$ showed a significant seasonal variability with substantially higher levels in summer at most sites, and a marginal spatial variability with slightly higher activities at CHI during summer (Figure 4c).~~

~~Seasonal averages of methanol-soluble $PM_{2.5}$ OPm and OPv are shown in Figure 6.~~ Compared to water-soluble OP, most OP endpoints in the methanol-soluble extracts showed weaker seasonal variations (Figure 4 and 6), as also ~~indicated~~ confirmed by relatively lower F-values [median of F = 1.61 (Table S5a), compared to 2.71 for the water-soluble OP endpoints (Table S4a)]. Similar to water-soluble OP, highest activities for the methanol-soluble OP were generally observed in summer (Figure 6). ~~For example, highest values of OP^{AA} and OP^{DTT} were observed in summer at CMP and BON ($P < 0.05$) for both mass- and volume-normalized activities. $OP^{OH\text{-}SLF}$m and $OP^{OH\text{-}SLF}$v peaked in summer at BON ($P < 0.01$), but in fall at IND ($P < 0.05$). $OP^{OH\text{-}DTT}$m and $OP^{OH\text{-}DTT}$v were also elevated in summer at~~

~~CHI ($P < 0.01$), but showed marginal seasonal variations at other sites. In contrast, OP^{GSH} showed a rather homogeneous seasonal distribution at all sites, except slight elevation of OP^{GSH}m in fall at STL and IND ($P < 0.05$).~~ The spatial variations in OP were also weaker for the methanol-soluble extracts in comparison to water-soluble extracts [median of F = 1.96 (Table S5b), compared to 4.52 for the water-soluble OP endpoints (Table S4b~~)];~~)]. ~~h~~However, some ~~spikes~~ significantly higher OP levels were observed at certain sites in different seasons, e.g. OP^{AA}v at CHI in winter and spring, OP^{GSH}v at CHI and CMP during winter and spring, OP^{GSH}m at CMP in all seasons, $OP^{OH\text{-}SLF}$ at CHI in summer and winter, and $OP^{OH\text{-}DTT}$m and $OP^{OH\text{-}DTT}$v at CHI in summer ($P < 0.05$). ~~Substantially higher OP^{AA}v occurred at CHI ($P < 0.05$) in winter and spring, while no significant differences were observed for OP^{AA}m among different sites in any other season. OP^{GSH}v was elevated at CHI and CMP during winter and spring ($P < 0.05$), while CMP showed elevated OP^{GSH}m in all seasons ($P < 0.05$). In summer and winter, $OP^{OH\text{-}SLF}$ peaked at CHI ($P < 0.05$) for both mass- and volume-normalized levels. $OP^{OH\text{-}DTT}$m and $OP^{OH\text{-}DTT}$v also peaked at CHI ($P < 0.05$) in summer. The lowest levels of $OP^{OH\text{-}DTT}$ were again found at CMP in all seasons, which is consistent with the trend for water soluble $OP^{OH\text{-}DTT}$. In contrast, OP^{DTT} showed spatially homogeneous distribution across all seasons, with marginally elevated values of OP^{DTT}v at STL during fall and winter ($P < 0.05$).~~ Other than these few cases, ~~T~~the spatiotemporal trends were again ~~very~~ largely similar between mass- and volume-normalized methanol-soluble OP activities ~~except few cases discussed here~~.

Comparison of OP in the Midwest US with previous investigations

A comparison of the ranges of OP endpoints ~~observed~~ measured in our study ~~and~~ with those reported in previous ~~investigations~~ studies is ~~has been briefly~~ provided in ~~SI (~~Table S~~6~~2 (SI). The purpose of this comparison is to validate our measurements and present a larger perspective on the general levels of OP in the Midwest US in comparison to other regions of the world. For water-soluble $PM_{2.5}$ in our study, OP^{AA}m ranged from 0.002 to 0.077 nmol·min^{-1}·μg^{-1}, which is within the ranges reported from previous studies conducted in Europe (Künzli et al., 2006;Szigeti et al., 2016;Godri et al., 2011;Perrone et al., 2019) and India (Mudway et al., 2005). ~~However, our~~Our range of OP^{AA}v (0.012 – 0.908 nmol·min^{-1}·m^{-3}) is comparable with Gao et al. (2020a) (0.023 – 0.126 nmol·min^{-1}·m^{-3}), but is much lower than that reported by Fang et al. (2016) (0.2 – 5.2 nmol·min^{-1}·m^{-3}) and Yang et al. (2014) (0.8 – 35.0 nmol·s^{-1}·m^{-3}), probably because of a different protocol used in those ~~their~~ studies~~y~~, both of which involved only AA in the assay. The median of water-soluble OP^{GSH}m (0.007 nmol·min^{-1}·μg^{-1}) is also comparable with the average of those reported (0.0041 – 0.0083 nmol·min^{-1}·μg^{-1}) in previous studies (Mudway et al., 2005;Künzli et al., 2006;Godri et al., 2011). Similarly, the median of $OP^{OH\text{-}SLF}$m (0.142 pmol·min^{-1}·μg^{-1}) is comparable to the averages reported by Vidrio et al. (2009) ~~(0.253 pmol·min^{-1}·μg^{-1})~~ and Ma et al. (2015) (0.092 – 0.253 pmol·min^{-1}·μg^{-1}). The median of OP^{DTT}m (0.014 nmol·min^{-1}·μg^{-1}) of our samples is significantly lower than the medians or averages reported from most studies conducted in US ~~(0.019 – 0.041 nmol·min^{-1}·μg^{-1})~~ (Cho et al., 2005;Charrier and Anastasio, 2012;Gao et al., 2020b;Hu et al., 2008;Fang et al., 2015) and Greece (0.019 – 0.041 nmol·min^{-1}·μg^{-1}) (Paraskevopoulou et al., 2019), but is closer to the averages reported from the studies conducted in Italy (0.010 – 0.012 nmol·min^{-1}·μg^{-1}) (Cesari et al., 2019;Perrone et al., 2019). Similarly, the median of our OP^{DTT}v (0.150 nmol·min^{-1}·m^{-3}) is lower compared to several studies in Southeast US and Europe (0.19 – ~~0.31~~0.33 nmol·min^{-1}·m^{-3}) (Fang et al., 2015;Gao et al., 2017;Gao et al., 2020a;Gao

et al., 2020b;Paraskevopoulou et al., 2019;Perrone et al., 2019;Cesari et al., 2019), but closer to one study conducted in Southwest US (0.14 nmol·min^{-1}·m^{-3}) (Hu et al., 2008). The range of water-soluble OP$^{OH\text{-}DTT}$v of our samples is quite large (0.004 – 3.565 pmol·min^{-1}·m^{-3}); however, there is no previous data to compare it, other than reported in the studies conducted by our own group (Xiong et al., 2017;Yu et al., 2018), which were based on a much smaller sample size (N = 10) and limited spatial extent (single site) and thus resulting in~~to~~ a much narrower range (0.2 – 1.1 pmol·min^{-1}·m^{-3}). Compared to water, only a handful of studies on ~~PM~~ OP^{AA} and OP^{DTT} have used methanol as the PM extraction solvent, while no previous literature~~s have investigated~~ is available on the OP of methanol-soluble PM for other endpoints. Similar to the water-soluble OP results, the level of methanol-soluble OP^{AA}v in our study (0.030 – 0.311 nmol·min^{-1}·m^{-3}) was lower than that reported by Yang et al. (2014) (2.2 – 43.5 nmol·s^{-1}·m^{-3}), probably due to different measurement protocols (only AA in comparison to SLF in our approach). The medians of our methanol-soluble OP^{DTT}m (0.021 nmol·min^{-1}·μg^{-1}) and OP^{DTT}v (0.234 nmol·min^{-1}·m^{-3}) are slightly lower than the medians or averages reported in previous studies in the Southeast US (0.027 – 0.034 nmol·min^{-1}·μg^{-1} and 0.28 – 0.30 nmol·min^{-1}·m^{-3}, respectively for OP^{DTT}m and OP^{DTT}v) (Verma et al., 2012;Gao et al., 2017;Gao et al., 2020b), which is consistent with the trend for water-soluble OP^{DTT} (i.e. lower levels of our samples than reported previously at other sites).

~~3.3 Spatiotemporal variation in $PM_{2.5}$ OP~~

~~*Water-soluble $PM_{2.5}$ OP*~~

~~CMP showed a substantially higher water-soluble OP than other sites for these endpoints. In the temporal trend, SLF-based endpoints showed higher levels during summer compared to other seasons at most sites. A significant temporal variation was observed for CMP with several spikes in the OP activities throughout the year, most prominently for OP^{AA}. The peak in the week of July 3 were observed for multiple endpoints (e.g. OP^{AA}, OP^{GSH} and OP^{DTT}) at most sites, which is attributed to the emissions from firecrackers on Independence Day (July 4) celebrations . In comparison to SLF based endpoints, mass- and volume-normalized DTT based OP (i.e. OP^{DTT} and OP$^{OH\text{-}DTT}$) showed lesser spatial variations (Figure 3d-e).Seasonally averaged OPm and OPv of water-soluble $PM_{2.5}$ at different sites are shown in Figure 5. Differences in both OPm and OPv among different seasons or sites were determined by one-way ANOVA and the results are listed in SI, Table S3. Seasonally, highest OP activities were generally observed in summer, while the lowest activities usually occurred in winter. For example, OP^{AA}v and OP^{GSH}v activities had highest levels in summer and lowest levels in winter at CMP and BON, as verified by 1-way ANOVA ($P < 0.05$). Similarly, significantly higher OP activities ($P < 0.01$ for most cases) were observed for both OP$^{OH\text{-}SLF}$m and OP$^{OH\text{-}SLF}$v at all five sites in summer, while winter showed significantly lower levels ($P < 0.05$). For DTT-based endpoints, OP$^{OH\text{-}DTT}$m and OP$^{OH\text{-}DTT}$v also showed higher values in summer at CHI, IND and CMP ($P < 0.01$). However, OP^{DTT} exhibited limited temporal variation at most sites with only slightly higher OP^{DTT}m and OP^{DTT}v observed in summer at BON ($P < 0.05$). The seasonal trend of mass- and volume-normalized activities were nearly identical for all endpoints, indicating a marginal effect of $PM_{2.5}$ mass concentration in the temporal variation of OP.~~

~~The temporal variation trend of OP^{DTT} in this study does not correspond with previous studies conducted in Southwest and Southeast US. For the Southeast US, Verma et al. (2014) found significantly higher OP^{DTT}v in winter (December,~~

2012) compared to summer (June to August, 2012), and this difference was even more pronounced in mass-normalized OP. Saffari et al. (2014) also observed higher OP^{DTT} activities of quasi-ultrafine particles ($PM_{0.25}$) in fall and winter seasons for the Southwest US (Los Angeles Basin), and attributed this trend to the partitioning of redox-active semi-volatile organic compounds to particle phase in colder seasons. However, the trend of OP^{AA} in our study is in agreement with another study in Southeast US using OP^{AA} as the endpoint (Fang et al., 2016), which showed higher OP^{AA} in warmer seasons (i.e. summer and fall) than winter. There is no previous literature available on the spatiotemporal trends of other OP endpoints in US, to which we can compare our results.

Spatially, there seems higher variability in the SLF-based endpoints, i.e. OP^{AA} and OP^{GSH} than the DTT-based endpoints (OP^{DTT} and $OP^{OH\text{-}DTT}$). Highest OP^{AA} and OP^{GSH} activities (both mass- and volume-normalized) occurred at the roadside site CMP (as confirmed by 1-way ANOVA test; $P < 0.01$) in most seasons (except winter for $OP^{AA}v$), while STL and IND had the lowest OP^{AA} and OP^{GSH}. $OP^{OH\text{-}SLF}$ was more spatially uniformly distributed than OP^{AA} and OP^{GSH}; significantly higher $OP^{OH\text{-}SLF}m$ and $OP^{OH\text{-}SLF}v$ were observed at CMP only in summer and spring ($P < 0.05$). For the DTT-based endpoints, $OP^{DTT}v$ was only marginally higher at CHI in winter, and at CMP in summer and spring. Other than that, no significant differences were observed for $OP^{DTT}v$ among various sites. The spatially uniform pattern for $OP^{DTT}v$ is consistent with Verma et al. (2014) which found limited spatial variation for $OP^{DTT}v$ in the Southeast US. In contrast, there was significant variation in the $OP^{DTT}m$ with elevated levels at CMP ($P < 0.01$) in all seasons. Interestingly, the $OP^{OH\text{-}DTT}$ endpoint showed more spatial variability and was generally lowest at CMP ($P < 0.05$) – the site which showed highest levels for all other OP endpoints. It implies that although OP^{DTT} and $OP^{OH\text{-}DTT}$ endpoints are measured in the same DTT assay, different chemical components play differential roles in these endpoints. We found very similar spatial patterns of mass- and volume-normalized OP activities for most endpoints, again indicating only a marginal role of $PM_{2.5}$ mass concentrations in causing the spatial variability in OP levels.

Methanol-soluble $PM_{2.5}$ OP

The spatiotemporal variations for the methanol-soluble OP endpoints (e.g. OP^{AA}, OP^{GSH}, OP^{DTT} and $OP^{OH\text{-}DTT}$) seem to be lesser than the corresponding water-soluble OP (Figure 4a–b, d–e). However, methanol-soluble $OP^{OH\text{-}SLF}$ showed a significant seasonal variability with substantially higher levels in summer at most sites, and a marginal spatial variability with slightly higher activities at CHI during summer (Figure 4c).Seasonal averages of methanol-soluble $PM_{2.5}$ OPm and OPv are shown in Figure 6. Compared to water-soluble OP, most OP endpoints in the methanol-soluble extracts showed weaker seasonal variations, as also indicated by relatively lower F-values [median of $F = 1.61$ (Table S4a), compared to 2.71 for the water-soluble OP endpoints (Table S3a)]. Similar to water-soluble OP, highest activities for the methanol-soluble OP were generally observed in summer. For example, highest values of OP^{AA} and OP^{DTT} were observed in summer at CMP and BON ($P < 0.05$) for both mass- and volume-normalized activities. $OP^{OH\text{-}SLF}m$ and $OP^{OH\text{-}SLF}v$ peaked in summer at BON ($P < 0.01$), but in fall at IND ($P < 0.05$). $OP^{OH\text{-}DTT}m$ and $OP^{OH\text{-}DTT}v$ were also elevated in summer at CHI ($P < 0.01$), but showed marginal seasonal variations at other sites. In contrast, OP^{GSH} showed a rather homogeneous seasonal distribution at all sites, except slight elevation of $OP^{GSH}m$ in fall at STL and IND ($P < 0.05$).

~~The spatial variations in OP were also weaker for the methanol soluble extracts in comparison to water soluble extracts [median of F = 1.96 (Table S4b), compared to 4.52 for the water soluble OP endpoints (Table S3b)]; however, some spikes were observed at certain sites in different seasons. Substantially higher $OP^{AA}v$ occurred at CHI ($P < 0.05$) in winter and spring, while no significant differences were observed for $OP^{AA}m$ among different sites in any other season. $OP^{GSH}v$ was elevated at CHI and CMP during winter and spring ($P < 0.05$), while CMP showed elevated $OP^{GSH}m$ in all seasons ($P < 0.05$). In summer and winter, $OP^{OH\text{-}SLF}$ peaked at CHI ($P < 0.05$) for both mass- and volume-normalized levels. $OP^{OH\text{-}DTT}m$ and $OP^{OH\text{-}DTT}v$ also peaked at CHI ($P < 0.05$) in summer. The lowest levels of $OP^{OH\text{-}DTT}$ were again found at CMP in all seasons, which is consistent with the trend for water soluble $OP^{OH\text{-}DTT}$. In contrast, OP^{DTT} showed spatially homogeneous distribution across all seasons, with marginally elevated values of $OP^{DTT}v$ at STL during fall and winter ($P < 0.05$). The spatiotemporal trends were again very similar between mass- and volume-normalized methanol soluble OP activities except few cases discussed here.~~

3.~~4~~ 3 Comparison of water-soluble and methanol-soluble OP

To assess the effect of solvent on the OP response, we computed the ratio of methanol-soluble OPv to water-soluble OPv (M/W^{OP}) for all samples, and plotted it for the individual sites in Figure 7. As shown in the figure, methanol-soluble extracts generally showed greater response for most of the OP endpoints than the water-soluble extracts, with medians of M/W^{OP} being either close or greater than 1. The medians for M/W^{OP} for $OP^{GSH}v$ and $OP^{DTT}v$ were closer to 1 at many sites ~~(0.6 – 1.3 for $OP^{GSH}v$; and 1.1 – 1.9 for $OP^{DTT}v$)~~, while significantly greater than 1 for the other three endpoints ($OP^{AA}v$, $OP^{OH\text{-}SLF}v$ and $OP^{OH\text{-}DTT}v$). The only exception to this trend was for $OP^{AA}v$ at CMP, where significantly lower levels of methanol-soluble OP than water-soluble OP were observed (median of $M/W^{OP} = 0.7$ for $OP^{AA}v$ at CMP). Our previous studies analyzing the chemical composition of PM collected at CMP have shown an elevated level of Cu (up to 60 ng/m^3) at this site (Wang et al., 2018;Puthussery et al., 2018), compared to the typical range (4 – 20 ng/m^3) at most urban sites in US (Buzcu-Guven et al., 2007;Kundu and Stone, 2014;Lee and Hopke, 2006;Hammond et al., 2008;Baumann et al., 2008;Milando et al., 2016). Although water-soluble Cu has been shown as the most important contributor to OP^{AA} (Fang et al., 2016;Ayres et al., 2008;Visentin et al., 2016), Lin and Yu (2020) reported a strong antagonistic interaction of Cu with imidazole and pyridine, both of which are alkaloid compounds (i.e. reduced organic nitrogen compounds), for oxidizing AA. The unprotonated nitrogen atom in alkaloids tends to chelate Cu, thus reducing its reactivity with AA. The antagonistic effect of Cu have been reported with other organic compounds (e.g. citric acid) as well (Pietrogrande et al., 2019). ~~Since many of the alkaloid compounds are water-insoluble but methanol-soluble, it is possible that these compounds are efficiently extracted in methanol, causing the~~ Thus, apparently lower levels of methanol-soluble OP^{AA} compared to the water-soluble OP^{AA} at CMP might be associated with the chelation of Cu by these alkaloids or other organic species, which could be more efficiently extracted in methanol.

The medians of M/W^{OP} were very high (1.4 – 3.8) for both ·OH based endpoints (i.e. $OP^{OH\text{-}SLF}$ and $OP^{OH\text{-}DTT}v$) ~~(2.1 – 3.8 for $OP^{OH\text{-}SLF}v$ and 1.4 – 1.9 for $OP^{OH\text{-}DTT}v$)~~, indicating that methanol is able to more efficiently extract the redox-active components driving the response of these OP endpoints. In addition to ·OH-active organic species, e.g. quinones (Charrier and Anastasio, 2015;Xiong et al., 2017;Yu et al., 2018)~~We~~, which are more soluble in methanol, we suspect

that one of such components could be organic-complexed Fe. As a Fenton reagent, Fe can catalyze the transfer of electrons from H_2O_2 to ·OH (Held et al., 1996). The generation of ·OH is further enhanced by the complexation of Fe with organic species (Wei et al., 2018;Gonzalez et al., 2017;Xiong et al., 2017;Yu et al., 2018). In a previous study conducted at our CMP site, Wei et al. (2018) found a significant fraction of Fe complexed with hydrophobic organic species (28 ± 22 %). That study also reported a substantially higher ratio of Fe concentration in 50 % methanol to that in water (1.42 ± 0.19), which showed some seasonality (1.97 ± 0.17 during winter and 1.33 ± 0.20 in summer). This seasonal pattern of Fe solubility in methanol versus water is consistent with the time series of M/W^{OP} for $OP^{OH\text{-}SLF}v$ at most sites (showing higher values in winter than summer; SI Table S7~~5~~), which further corroborated that Fe complexed with hydrophobic organic fraction of $PM_{2.5}$ could be majorly responsible for the $OP^{OH\text{-}SLF}v$ and $OP^{OH\text{-}DTT}v$ in the methanol extracts. However, detailed chemical characterization will be needed to confirm these hypotheses, which will be explored in our subsequent publications.

We also calculated Pearson's r for the regression between respective water-soluble and methanol-soluble OP endpoints for individual sites, which are shown in Table 3. $OP^{DTT}v$ showed some good correlation between two extraction protocols (r = 0.43 – 0.74 except at STL), while correlations were generally poor (r < 0.60) for other four endpoints (i.e. $OP^{AA}v$, $OP^{GSH}v$, $OP^{OH\text{-}SLF}v$ and $OP^{OH\text{-}DTT}v$). It indicates that the components driving the response of OP^{DTT} could be more uniformly extracted in both water and methanol. However, there are additional water-insoluble species driving the response of $OP^{AA}v$, $OP^{GSH}v$, $OP^{OH\text{-}SLF}v$ and $OP^{OH\text{-}DTT}v$, which are more efficiently extracted in methanol than water.

3.~~5~~ 4 Site-to-site comparison of OP and mass concentration of $PM_{2.5}$

To further evaluate the spatial trend of OP across the Midwest US region, we calculated both COD and correlation coefficients (Pearson's r) for different site pairs, which are shown in Figure 8 (mass concentrations and water-soluble OP of $PM_{2.5}$), and Figure 9 (methanol-soluble $PM_{2.5}$ OP).

$PM_{2.5}$ mass concentration and water-soluble $PM_{2.5}$ OP

$PM_{2.5}$ mass concentrations showed low levels of CODs (0.13 – 0.25, median: 0.20), confirming a spatially homogeneous distribution of $PM_{2.5}$ as indicated earlier (Figure 8a). Conversely, we observed generally higher CODs (median = 0.27 – 0.43) for all water-soluble OPv endpoints~~, i.e. $OP^{AA}v$ (0.38 – 0.56, median: 0.43), $OP^{GSH}v$ (0.28 – 0.51, median: 0.35), $OP^{OH\text{-}SLF}v$ (0.30 – 0.40, median: 0.35), $OP^{DTT}v$ (0.19 – 0.34, median: 0.25), and $OP^{OH\text{-}DTT}v$ (0.21 – 0.38, median: 0.27)~~ (Figure 8b-f). Our results showing a stronger spatial variability in OP than PM mass are largely in agreement with a recent study (Daellenbach et al., 2020) analyzing a comprehensive dataset for OP in Europe, which showed that both OPv (measured by DTT, 2′,7′-Dichlorofluorescin Diacetate and AA assays) and PM_{10} mass concentrations were elevated in the urban environments (e.g. Paris and the Po valley), but PM_{10} was more regionally distributed than OPv.

Interestingly, we found poor correlations for $PM_{2.5}$ among all site pairs (r < 0.60), except IND and BON (r = 0.63). It implies that despite a homogeneous spatial distribution, emission sources of the chemical species composing $PM_{2.5}$

519 are different at different sites. The correlations were also weak ($r < 0.60$ for most cases) for the OP endpoints showing
520 high CODs, i.e. OP^{AA}, OP^{GSH}, $OP^{OH\text{-}SLF}$ and $OP^{OH\text{-}DTT}$, which indicates a more pronounced effect of local point sources
521 on these OP endpoints compared to the regional sources. In contrast, $OP^{DTT}v$ showed stronger correlation ($r = 0.48$ –
522 0.76, median: 0.62) for most site pairs. Higher correlations for the DTT activity combined with lower CODs suggests
523 that the regional sources such as long-range transport or atmospheric processing could have a larger influence on
524 OP^{DTT} than the local sources.

525 *Methanol-soluble $PM_{2.5}$ OP*

526 In comparison to water-soluble $PM_{2.5}$ OP, CODs for the methanol-soluble OP were generally lower (median: 0.21 –
527 0.35; Figure 9), indicating higher spatial homogeneity of methanol-soluble PM chemical components that are sensitive
528 to OP. Similar to water-soluble $OP^{DTT}v$, the methanol-soluble $OP^{DTT}v$ showed the lowest COD (0.14 – 0.26, median:
529 0.21) among five endpoints (Figure 9d), which was consistent with Gao et al. (2017) showing a rather low COD (less
530 than 0.23) for both water-soluble and methanol-soluble OP^{DTT} in Southeast US. Overall, higher correlation coefficients
531 were observed for the methanol-soluble OP (median: 0.41 – 0.67 for different endpoints) than the corresponding water-
532 soluble endpoints (median: 0.13 – 0.62). The correlation coefficients were more elevated for certain endpoints such
533 as $OP^{AA}v$ ($r = 0.38$ – 0.62, median: 0.46) and $OP^{GSH}v$ ($r = 0.23$ – 0.65, median: 0.41) than others. It is possible that
534 methanol is able to extract more redox-active PM components coming from ~~common~~ regional emission sources, e.g.
535 biomass burning or secondary organic aerosols, present at these sites. The components originated from these common
536 sources could mask the effect of other components originated from the local sources having a narrower range of
537 solubilities,~~and~~ thus yielding to an overall lower spatiotemporal variability and better correlation among different sites.

538 3.~~6~~ 5 Correlations of OP with $PM_{2.5}$ mass concentration

539 Pearson's r and the slope for simple linear regression of volume-normalized OP activities versus $PM_{2.5}$ mass
540 concentrations were computed for each individual site, and are listed in Table 4. For both water-soluble and methanol-
541 soluble OP, the endpoints of $OP^{AA}v$, $OP^{OH\text{-}SLF}v$ and $OP^{OH\text{-}DTT}v$ were poorly correlated with $PM_{2.5}$ mass ($r < 0.60$ in
542 most cases), while $OP^{GSH}v$ and $OP^{DTT}v$ were moderately-to-strongly correlated with $PM_{2.5}$ mass ($r = 0.38$ – 0.73 for
543 $OP^{GSH}v$, and 0.54 – 0.82 for $OP^{DTT}v$, except at STL). The lower correlation of OP^{AA} and higher correlation of OP^{DTT}
544 are consistent with multiple previous studies comparing these endpoints (Visentin et al., 2016;Yang et al.,
545 2014;Janssen et al., 2014). Decent correlations for $OP^{GSH}v$ and $OP^{DTT}v$ showed that PM mass concentrations can drive
546 these endpoints to some extent at few locations. However, it is important to note that despite these good correlations,
547 the slope of regression for OP vs. $PM_{2.5}$ mass varied a lot among five sampling sites (range for $OP^{GSH}v$ is 0.003 –
548 0.016 nmol/min/μg, and 0.005 – 0.028 nmol/min/μg for $OP^{DTT}v$), indicating substantial spatiotemporal heterogeneity
549 in the intrinsic potency of the particles to generate ROS at these sites. This is further corroborated by the spatiotemporal
550 variability of $OP^{GSH}m$ and $OP^{DTT}m$ at different sites as shown in Figure 5 and 6. Thus, $PM_{2.5}$ mass concentrations have
551 only a limited role in determining the oxidative levels of the $PM_{2.5}$ at these sites, and OP seems to be largely driven
552 by the PM chemical composition. Given that the current air quality standards across the world focus only on mass
553 concentration of $PM_{2.5}$, these results indicate towards the inadequacy of this mass-centered approach.

3.~~7~~ 6 Intercorrelation among different OP endpoints

We also calculated the correlation coefficient (Pearson's r) for all pairs of different OPv endpoints at each site, which are listed in Table 5. A high correlation coefficient indicates a common source (or a common pool of chemical components) driving the response of those OP endpoints. For water-soluble OP, the intercorrelations among different endpoints were generally poor at urban sites, i.e. CHI, STL, and IND ($r < 0.60$). Correlations were also poor for nearly all pairs of methanol-soluble OP at STL and IND, but CHI showed significantly elevated r values among different OP endpoints ($r = 0.59 – 0.82$). Compared to more urbanized sites, the correlations were generally higher at the local sites, i.e. CMP and BON, with $r > 0.60$ for many pairs of both water-soluble and methanol-soluble OPv. Since both of these sites are located in smaller cities, the sources of redox-active components probably have lesser complexity compared to the major city sites, which have multiple and more complex emission sources. ~~For example~~As discussed in section 3.2, CMP is ~~adjacent to a major road, and thus~~ largely impacted by the vehicular emissions owing to its location adjacent to a major road. Similarly, BON being a rural site is largely impacted by the agricultural emissions with marginal impact from vehicular emissions and other sources such as long-range transport from surrounding cities (Kim et al., 2005;Buzcu-Guven et al., 2007). Thus, a lack of other major sources contributing to components, which can drive these endpoints in different directions through their interactions (i.e. synergistic or antagonistic), leads to the similarity of their responses and hence a good correlation among them at these two sites. Among all OP endpoints, $OP^{OH\text{-}DTT}v$ showed poorest correlations with other endpoints except $OP^{OH\text{-}SLF}v$, with which it was correlated at most sites (i.e. CHI, IND, CMP and BON) for the methanol-soluble extracts ($r = 0.66 – 0.84$). Since both of these endpoints measure the rate of generation of ·OH, it probably indicates a synergistic role of metals with organic compounds [e.g. Fe with humic-like substances (HULIS), as shown in many previous studies (Yu et al., 2018;Charrier and Anastasio, 2015;Gonzalez et al., 2017;Wei et al., 2018;Ma et al., 2015)] in partly driving the response of both of these endpoints. Note, $OP^{OH\text{-}DTT}$ is a relatively newly developed assay, and there is hardly any previous literature on its comparison with other OP endpoints.

Overall, a poor-to-moderate and inconstant intercorrelation trend among different endpoints of both water-soluble and methanol-soluble OP at most sites indicates that all these assays could be deficient from being ideal and measuring a single endpoint is not enough to represent the overall OP activity. Although, the OP endpoints used in our study have covered some of the well-known and important pathways of the *in vivo* oxidative stress caused by $PM_{2.5}$, there are other endpoints (e.g. consumption of cysteine, formation of H_2O_2, etc.), and more assays can be developed in the future. We suggest that a collection of diverse range of OP endpoints, measured separately as done in our study could better capture the role of different PM components and their interactions via different pathways for driving the oxidative levels of the PM in a region. However, it should be noted that our study is not designed to assess and rank the biological relevance of these acellular endpoints, which will require an integration of these and possibly other novel assays involving different routes of oxidative stress, in either toxicological or epidemiological studies.~~measuring a single endpoint is not enough to represent the overall OP activity. The diverse range of OP endpoints used in our study could better capture the role of different PM components and their interactions via different pathways for driving the oxidative levels of the PM in a region~~.

4 Conclusion

We analyzed both water-soluble and methanol-soluble OP of ambient $PM_{2.5}$ in the Midwest US using five different acellular endpoints, including OP^{AA}, OP^{GSH}, $OP^{OH\text{-}SLF}$, OP^{DTT} and $OP^{OH\text{-}DTT}$. The spatiotemporal profiles of all OP endpoints and $PM_{2.5}$ mass concentration were investigated for one-year timescale from May 2018 to May 2019 using the Hi-Vol filter samples collected from five Midwest US sites located in urban, rural, and roadside environments. Compared to homogeneously distributed $PM_{2.5}$ mass, all OP endpoints showed significant spatiotemporal variations among different seasons and sites. Seasonally, most OP endpoints generally peaked in summer for both water-soluble and methanol-soluble OP. Spatially, the roadside site showed the highest OP levels for most OP endpoints in water-soluble extracts, while there were occasional peaks in methanol-soluble extracts at other urban sites. Our results showed very limited differences in the spatiotemporal profiles between OPm and OPv for most endpoints, indicating a marginal role of $PM_{2.5}$ mass in causing the spatiotemporal variability of OP.

Comparing the OP for water- and methanol-soluble extracts, we observed significantly higher OP levels in methanol extracts than the corresponding water-soluble OP activities. This trend was much stronger for ·OH generation endpoints (i.e. $OP^{OH\text{-}SLF}$ and $OP^{OH\text{-}DTT}$), indicating a substantial contribution of Fe and its organic complexes, which could be more efficiently extracted in methanol. In comparison to water-soluble OP, methanol-soluble OP showed lower spatial heterogeneity, and higher intercorrelations among different endpoints, which is probably attributed to a more efficient extraction of water-insoluble redox-active species in methanol originated from various emission sources at different sites.

The correlations of OP with $PM_{2.5}$ mass showed a diverse range, with certain endpoints such as OP^{AA}, $OP^{OH\text{-}SLF}$ and $OP^{OH\text{-}DTT}$ showing a poor correlation, while other endpoints (i.e. OP^{GSH} and OP^{DTT}) showing a moderate-to-strong correlation. Despite these occasional strong correlations, the sensitivity of all OP endpoints towards mass, indicated by the slope of OP vs. $PM_{2.5}$ mass as well as the intrinsic OP (OPm), varied substantially for all OP endpoints across different sites and seasons, showing only a marginal effect of mass concentrations in controlling the oxidative levels of $PM_{2.5}$. Moreover, relatively poor and inconsistent correlations among different OP endpoints reflected different pathways of various ROS-active $PM_{2.5}$ components for exerting oxidative stress. Since our study cannot comment on the biological relevance of these different pathways, we recommend integrating all these and other assays in toxicological or epidemiological studies, to assess their relative utilities.

Collectively, the results obtained through our study provide~~s~~ a strong rationale to recommend that the different endpoints of OP provide useful and additional information than the mass concentrations, which could be relevant to assess the public health impacts associated with ambient $PM_{2.5}$. Our future studies will explore the contribution of different chemical components and their emission sources in determining the oxidative levels of ambient $PM_{2.5}$ in the Midwest US.

Data availability. The data on OP and mass concentration of ambient $PM_{2.5}$ samples collected in the Midwest US are available upon request from the corresponding author.

Author contribution. HY: collection of $PM_{2.5}$ samples, measurement of OP, data analysis, manuscript organization and writing; JVP: collection of $PM_{2.5}$ samples, manuscript editing and revision; YW: collection of $PM_{2.5}$ samples, manuscript editing and revision; VV: conceptualization of study design and methodology, manuscript organization and editing, and overall project supervision.

Competing Interests. The authors declare that they do not have any competing interests.

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References

Abbas, I., Verdin, A., Escande, F., Saint-Georges, F., Cazier, F., Mulliez, P., Courcot, D., Shirali, P., Gosset, P., and Garçon, G.: *In vitro* short-term exposure to air pollution $PM_{2.5-0.3}$ induced cell cycle alterations and genetic instability in a human lung cell coculture model, *Environmental Research*, 147, 146-158, 2016.

Abrams, J. Y., Weber, R. J., Klein, M., Samat, S. E., Chang, H. H., Strickland, M. J., Verma, V., Fang, T., Bates, J. T., and Mulholland, J. A.: Associations between ambient fine particulate oxidative potential and cardiorespiratory emergency department visits, *Environmental Health Perspectives*, 125, 107008, 10.1289/ehp1545, 2017.

Allan, K., Kelly, F., and Devereux, G.: Antioxidants and allergic disease: a case of too little or too much?, *Clinical & Experimental Allergy*, 40, 370-380, 2010.

Apeagyei, E., Bank, M. S., and Spengler, J. D.: Distribution of heavy metals in road dust along an urban-rural gradient in Massachusetts, *Atmospheric Environment*, 45, 2310-2323, https://doi.org/10.1016/j.atmosenv.2010.11.015, 2011.

Araujo, J. A., Barajas, B., Kleinman, M., Wang, X., Bennett, B. J., Gong, K. W., Navab, M., Harkema, J., Sioutas, C., and Lusis, A. J.: Ambient particulate pollutants in the ultrafine range promote early atherosclerosis and systemic oxidative stress, *Circulation Research*, 102, 589-596, 2008.

Ayres, J. G., Borm, P., Cassee, F. R., Castranova, V., Donaldson, K., Ghio, A., Harrison, R. M., Hider, R., Kelly, F., and Kooter, I. M.: Evaluating the toxicity of airborne particulate matter and nanoparticles by measuring oxidative stress potential—a workshop report and consensus statement, *Inhalation Toxicology*, 20, 75-99, 10.1080/08958370701665517, 2008.

Bates, J. T., Weber, R. J., Abrams, J., Verma, V., Fang, T., Klein, M., Strickland, M. J., Sarnat, S. E., Chang, H. H., and Mulholland, J. A.: Reactive oxygen species generation linked to sources of atmospheric particulate matter and cardiorespiratory effects, *Environmental Science & Technology*, 49, 13605-13612, 10.1021/acs.est.5b02967, 2015.

Bates, J. T., Fang, T., Verma, V., Zeng, L., Weber, R. J., Tolbert, P. E., Abrams, J. Y., Sarnat, S. E., Klein, M., and Mulholland, J. A.: Review of acellular assays of ambient particulate matter oxidative potential: Methods and

relationships with composition, sources, and health effects, *Environmental Science & Technology*, 53, 4003-4019, 2019.

Baumann, K., Jayanty, R., and Flanagan, J. B.: Fine particulate matter source apportionment for the chemical speciation trends network site at Birmingham, Alabama, using positive matrix factorization, *Journal of the Air & Waste Management Association*, 58, 27-44, 2008.

Becker, S., Dailey, L. A., Soukup, J. M., Grambow, S. C., Devlin, R. B., and Huang, Y.-C. T.: Seasonal variations in air pollution particle-induced inflammatory mediator release and oxidative stress, *Environmental Health Perspectives*, 113, 1032-1038, 10.1289/ehp.7996, 2005.

Borlaza, L. J. S., Weber, S., Jaffrezo, J.-L., Houdier, S., Slama, R., Rieux, C., Albinet, A., Micallef, S., Trébluchon, C., and Uzu, G.: Disparities in particulate matter (PM 10) origins and oxidative potential at a city scale (Grenoble, France)–Part 2: Sources of PM 10 oxidative potential using multiple linear regression analysis and the predictive applicability of multilayer perceptron neural network analysis, *Atmospheric Chemistry and Physics*, 21, 9719-9739, 2021.

Buzcu-Guven, B., Brown, S. G., Frankel, A., Hafner, H. R., and Roberts, P. T.: Analysis and apportionment of organic carbon and fine particulate matter sources at multiple sites in the midwestern United States, *Journal of the Air & Waste Management Association*, 57, 606-619, 2007.

Cachon, B. F., Firmin, S., Verdin, A., Ayi-Fanou, L., Billet, S., Cazier, F., Martin, P. J., Aissi, F., Courcot, D., and Sanni, A.: Proinflammatory effects and oxidative stress within human bronchial epithelial cells exposed to atmospheric particulate matter ($PM_{2.5}$ and $PM_{>2.5}$) collected from Cotonou, Benin, *Environmental Pollution*, 185, 340-351, 2014.

Calas, A., Uzu, G., Kelly, F. J., Houdier, S., Martins, J. M., Thomas, F., Molton, F., Charron, A., Dunster, C., and Oliete, A.: Comparison between five acellular oxidative potential measurement assays performed with detailed chemistry on PM 10 samples from the city of Chamonix (France), *Atmospheric Chemistry and Physics*, 18, 7863-7875, 2018.

Calas, A., Uzu, G., Besombes, J.-L., Martins, J. M., Redaelli, M., Weber, S., Charron, A., Albinet, A., Chevrier, F., and Brulfert, G.: Seasonal variations and chemical predictors of oxidative potential (OP) of particulate matter (PM), for seven urban French sites, *Atmosphere*, 10, 698, 2019.

Cesari, D., Merico, E., Grasso, F. M., Decesari, S., Belosi, F., Manarini, F., De Nuntiis, P., Rinaldi, M., Volpi, F., and Gambaro, A.: Source apportionment of PM2. 5 and of its oxidative potential in an industrial suburban site in South Italy, *Atmosphere*, 10, 758, 2019.

Charrier, J., and Anastasio, C.: On dithiothreitol (DTT) as a measure of oxidative potential for ambient particles: evidence for the importance of soluble transition metals, *Atmospheric Chemistry and Physics*, 12, 11317-11350, 10.5194/acp-12-9321-2012, 2012.

688 Charrier, J. G., McFall, A. S., Richards-Henderson, N. K., and Anastasio, C.: Hydrogen peroxide formation in a
689 surrogate lung fluid by transition metals and quinones present in particulate matter, *Environmental Science &*
690 *Technology*, 48, 7010-7017, 10.1021/es501011w, 2014.

691 Charrier, J. G., and Anastasio, C.: Rates of hydroxyl radical production from transition metals and quinones in a
692 surrogate lung fluid, *Environmental Science & Technology*, 49, 9317-9325, 10.1021/acs.est.5b01606, 2015.

693 Charrier, J. G., McFall, A. S., Vu, K. K., Baroi, J., Olea, C., Hasson, A., and Anastasio, C.: A bias in the "mass-
694 normalized" DTT response–An effect of non-linear concentration-response curves for copper and manganese,
695 *Atmospheric Environment*, 144, 325-334, 10.1016/j.atmosenv.2016.08.071, 2016.

696 Cho, A. K., Sioutas, C., Miguel, A. H., Kumagai, Y., Schmitz, D. A., Singh, M., Eiguren-Fernandez, A., and Froines,
697 J. R.: Redox activity of airborne particulate matter at different sites in the Los Angeles Basin, *Environmental Research*,
698 99, 40-47, 10.1016/j.envres.2005.01.003, 2005.

699 Chung, M. Y., Lazaro, R. A., Lim, D., Jackson, J., Lyon, J., Rendulic, D., and Hasson, A. S.: Aerosol-borne quinones
700 and reactive oxygen species generation by particulate matter extracts, *Environmental Science & Technology*, 40, 4880-
701 4886, 2006.

702 Councell, T. B., Duckenfield, K. U., Landa, E. R., and Callender, E.: Tire-wear particles as a source of zinc to the
703 environment, *Environmental science & technology*, 38, 4206-4214, 2004.

704 Daellenbach, K. R., Uzu, G., Jiang, J., Cassagnes, L.-E., Leni, Z., Vlachou, A., Stefenelli, G., Canonaco, F., Weber,
705 S., and Segers, A.: Sources of particulate-matter air pollution and its oxidative potential in Europe, *Nature*, 587, 414-
706 419, 2020.

707 Deng, X., Zhang, F., Rui, W., Long, F., Wang, L., Feng, Z., Chen, D., and Ding, W.: $PM_{2.5}$-induced oxidative stress
708 triggers autophagy in human lung epithelial A549 cells, *Toxicology in vitro*, 27, 1762-1770, 2013.

709 Dominici, F., McDermott, A., Zeger, S. L., and Samet, J. M.: Airborne particulate matter and mortality: timescale
710 effects in four US cities, *American Journal of Epidemiology*, 157, 1055-1065, 2003.

711 Fang, T., Verma, V., Guo, H., King, L. E., Edgerton, E. S., and Weber, R. J.: A semi-automated system for quantifying
712 the oxidative potential of ambient particles in aqueous extracts using the dithiothreitol (DTT) assay: results from the
713 Southeastern Center for Air Pollution and Epidemiology (SCAPE), *Atmospheric Measurement Techniques*, 8, 471-
714 482, 10.5194/amt-8-471-2015, 2015.

715 Fang, T., Verma, V., Bates, J. T., Abrams, J., Klein, M., Strickland, M. J., Sarnat, S. E., Chang, H. H., Mulholland, J.
716 A., and Tolbert, P. E.: Oxidative potential of ambient water-soluble $PM_{2.5}$ in the southeastern United States: contrasts
717 in sources and health associations between ascorbic acid (AA) and dithiothreitol (DTT) assays, *Atmospheric*
718 *Chemistry and Physics*, 16, 3865-3879, 10.5194/acp-16-3865-2016, 2016.

719 Feng, S., Gao, D., Liao, F., Zhou, F., and Wang, X.: The health effects of ambient $PM_{2.5}$ and potential mechanisms,
720 *Ecotoxicology and Environmental Safety*, 128, 67-74, 10.1016/j.ecoenv.2016.01.030, 2016.

721 Franco, R., Schoneveld, O., Georgakilas, A. G., and Panayiotidis, M. I.: Oxidative stress, DNA methylation and
722 carcinogenesis, *Cancer Letters*, 266, 6-11, https://doi.org/10.1016/j.canlet.2008.02.026, 2008.

723 Gao, D., Fang, T., Verma, V., Zeng, L., and Weber, R. J.: A method for measuring total aerosol oxidative potential
724 (OP) with the dithiothreitol (DTT) assay and comparisons between an urban and roadside site of water-soluble and
725 total OP, *Atmospheric Measurement Techniques*, 10, 2821, 2017.

726 Gao, D., Godri Pollitt, K. J., Mulholland, J. A., Russell, A. G., and Weber, R. J.: Characterization and comparison of
727 PM 2.5 oxidative potential assessed by two acellular assays, *Atmospheric Chemistry and Physics*, 20, 5197-5210,
728 2020a.

729 Gao, D., Mulholland, J. A., Russell, A. G., and Weber, R. J.: Characterization of water-insoluble oxidative potential
730 of $PM_{2.5}$ using the dithiothreitol assay, *Atmospheric Environment*, 224, 117327,
731 https://doi.org/10.1016/j.atmosenv.2020.117327, 2020b.

732 Garçon, G., Dagher, Z., Zerimech, F., Ledoux, F., Courcot, D., Aboukais, A., Puskaric, E., and Shirali, P.: Dunkerque
733 City air pollution particulate matter-induced cytotoxicity, oxidative stress and inflammation in human epithelial lung
734 cells (L132) in culture, *Toxicology in vitro*, 20, 519-528, 2006.

735 Garg, B. D., Cadle, S. H., Mulawa, P. A., Groblicki, P. J., Laroo, C., and Parr, G. A.: Brake Wear Particulate Matter
736 Emissions, *Environmental Science & Technology*, 34, 4463-4469, 10.1021/es001108h, 2000.

737 Gietl, J. K., Lawrence, R., Thorpe, A. J., and Harrison, R. M.: Identification of brake wear particles and derivation of
738 a quantitative tracer for brake dust at a major road, *Atmospheric Environment*, 44, 141-146, 2010.

739 Gildemeister, A. E., Hopke, P. K., and Kim, E.: Sources of fine urban particulate matter in Detroit, MI, *Chemosphere*,
740 69, 1064-1074, https://doi.org/10.1016/j.chemosphere.2007.04.027, 2007.

741 Godri, K. J., Harrison, R. M., Evans, T., Baker, T., Dunster, C., Mudway, I. S., and Kelly, F. J.: Increased oxidative
742 burden associated with traffic component of ambient particulate matter at roadside and urban background schools sites
743 in London, *PloS One*, 6, e21961, 10.1371/journal.pone.0021961, 2011.

744 Gonzalez, D. H., Cala, C. K., Peng, Q., and Paulson, S. E.: HULIS enhancement of hydroxyl radical formation from
745 Fe (II): kinetics of fulvic acid-Fe (II) complexes in the presence of lung antioxidants, *Environmental Science &*
746 *Technology*, 51, 7676-7685, 2017.

747 Grevendonk, L., Janssen, B. G., Vanpoucke, C., Lefebvre, W., Hoxha, M., Bollati, V., and Nawrot, T. S.:
748 Mitochondrial oxidative DNA damage and exposure to particulate air pollution in mother-newborn pairs,
749 *Environmental Health*, 15, 1-8, 2016.

Gurgueira, S. A., Lawrence, J., Coull, B., Murthy, G. K., and González-Flecha, B.: Rapid increases in the steady-state concentration of reactive oxygen species in the lungs and heart after particulate air pollution inhalation, *Environmental Health Perspectives*, 110, 749-755, 2002.

Haberzettl, P., O'Toole, T. E., Bhatnagar, A., and Conklin, D. J.: Exposure to fine particulate air pollution causes vascular insulin resistance by inducing pulmonary oxidative stress, *Environmental Health Perspectives*, 124, 1830-1839, 2016.

Hammond, D. M., Dvonch, J. T., Keeler, G. J., Parker, E. A., Kamal, A. S., Barres, J. A., Yip, F. Y., and Brakefield-Caldwell, W.: Sources of ambient fine particulate matter at two community sites in Detroit, Michigan, *Atmospheric Environment*, 42, 720-732, 2008.

Held, K. D., Sylvester, F. C., Hopcia, K. L., and Biaglow, J. E.: Role of Fenton chemistry in thiol-induced toxicity and apoptosis, *Radiation Research*, 145, 542-553, 10.2307/3579272, 1996.

Hu, S., Polidori, A., Arhami, M., Shafer, M., Schauer, J., Cho, A., and Sioutas, C.: Redox activity and chemical speciation of size fractioned PM in the communities of the Los Angeles-Long Beach harbor, *Atmospheric Chemistry and Physics*, 8, 6439-6451, 10.5194/acp-8-6439-2008, 2008.

Hulskotte, J., Denier van der Gon, H., Visschedijk, A., and Schaap, M.: Brake wear from vehicles as an important source of diffuse copper pollution, *Water Science & Technology*, 56, 223-231, 10.2166/wst.2007.456, 2007.

Janssen, N. A., Yang, A., Strak, M., Steenhof, M., Hellack, B., Gerlofs-Nijland, M. E., Kuhlbusch, T., Kelly, F., Harrison, R., and Brunekreef, B.: Oxidative potential of particulate matter collected at sites with different source characteristics, *Science of the Total Environment*, 472, 572-581, 10.1016/j.scitotenv.2013.11.099, 2014.

Jeong, C.-H., Traub, A., Huang, A., Hilker, N., Wang, J. M., Herod, D., Dabek-Zlotorzynska, E., Celo, V., and Evans, G. J.: Long-term analysis of $PM_{2.5}$ from 2004 to 2017 in Toronto: Composition, sources, and oxidative potential, *Environmental Pollution*, 263, 114652, 2020.

Künzli, N., Mudway, I. S., Götschi, T., Shi, T., Kelly, F. J., Cook, S., Burney, P., Forsberg, B., Gauderman, J. W., and Hazenkamp, M. E.: Comparison of oxidative properties, light absorbance, and total and elemental mass concentration of ambient $PM_{2.5}$ collected at 20 European sites, *Environmental Health Perspectives*, 114, 684-690, 10.1289/ehp.8584, 2006.

Kampfrath, T., Maiseyeu, A., Ying, Z., Shah, Z., Deiuliis, J. A., Xu, X., Kherada, N., Brook, R. D., Reddy, K. M., and Padture, N. P.: Chronic fine particulate matter exposure induces systemic vascular dysfunction via NADPH oxidase and TLR4 pathways, *Circulation Research*, 108, 716-726, 2011.

Kaufman, J. A., Wright, J. M., Rice, G., Connolly, N., Bowers, K., and Anixt, J.: Ambient ozone and fine particulate matter exposures and autism spectrum disorder in metropolitan Cincinnati, Ohio, *Environmental Research*, 171, 218-227, https://doi.org/10.1016/j.envres.2019.01.013, 2019.

Kelly, F. J.: Oxidative stress: its role in air pollution and adverse health effects, *Occupational and Environmental Medicine*, 60, 612-616, 2003.

Kim, E., Hopke, P. K., Kenski, D. M., and Koerber, M.: Sources of fine particles in a rural midwestern U.S. Area, *Environmental Science & Technology*, 39, 4953-4960, 10.1021/es0490774, 2005.

Kleinman, M. T., Hamade, A., Meacher, D., Oldham, M., Sioutas, C., Chakrabarti, B., Stram, D., Froines, J. R., and Cho, A. K.: Inhalation of concentrated ambient particulate matter near a heavily trafficked road stimulates antigen-induced airway responses in mice, *Journal of the Air & Waste Management Association*, 55, 1277-1288, 2005.

Kodavanti, U. P., Schladweiler, M. C., Ledbetter, A. D., Watkinson, W. P., Campen, M. J., Winsett, D. W., Richards, J. R., Crissman, K. M., Hatch, G. E., and Costa, D. L.: The spontaneously hypertensive rat as a model of human cardiovascular disease: evidence of exacerbated cardiopulmonary injury and oxidative stress from inhaled emission particulate matter, *Toxicology and Applied Pharmacology*, 164, 250-263, 10.1006/taap.2000.8899, 2000.

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, *Chemical Research in Toxicology*, 15, 483-489, 2002.

Kumar, N., Liang, D., Comellas, A., Chu, A. D., and Abrams, T.: Satellite-based PM concentrations and their application to COPD in Cleveland, OH, *Journal of Exposure Science & Environmental Epidemiology*, 23, 637-646, 2013.

Kundu, S., and Stone, E. A.: Composition and sources of fine particulate matter across urban and rural sites in the Midwestern United States, *Environmental Science: Processes & Impacts*, 16, 1360-1370, 2014.

Lee, C.-W., Lin, Z.-C., Hu, S. C.-S., Chiang, Y.-C., Hsu, L.-F., Lin, Y.-C., Lee, I. T., Tsai, M.-H., and Fang, J.-Y.: Urban particulate matter down-regulates filaggrin via COX2 expression/PGE2 production leading to skin barrier dysfunction, *Scientific Reports*, 6, 27995, 10.1038/srep27995, 2016.

Lee, J. H., and Hopke, P. K.: Apportioning sources of $PM_{2.5}$ in St. Louis, MO using speciation trends network data, *Atmospheric Environment*, 40, 360-377, 2006.

Lee, J. H., Hopke, P. K., and Turner, J. R.: Source identification of airborne $PM_{2.5}$ at the St. Louis-Midwest Supersite, *Journal of Geophysical Research: Atmospheres*, 111, 2006.

Li, N., and Nel, A. E.: Role of the Nrf2-mediated signaling pathway as a negative regulator of inflammation: implications for the impact of particulate pollutants on asthma, *Antioxidants & Redox Signaling*, 8, 88-98, 2006.

Li, Y., Fu, S., Li, E., Sun, X., Xu, H., Meng, Y., Wang, X., Chen, Y., Xie, C., and Geng, S.: Modulation of autophagy in the protective effect of resveratrol on $PM_{2.5}$-induced pulmonary oxidative injury in mice, *Phytotherapy Research*, 32, 2480-2486, 2018.

Lin, M., and Yu, J. Z.: Assessment of interactions between transition metals and atmospheric organics: ascorbic acid depletion and hydroxyl radical formation in organic-metal mixtures, *Environmental Science & Technology*, 54, 1431-1442, 10.1021/acs.est.9b07478, 2020.

Liu, Q., Baumgartner, J., Zhang, Y., Liu, Y., Sun, Y., and Zhang, M.: Oxidative potential and inflammatory impacts of source apportioned ambient air pollution in Beijing, *Environmental Science & Technology*, 48, 12920-12929, 2014.

Liu, W., Xu, Y., Liu, W., Liu, Q., Yu, S., Liu, Y., Wang, X., and Tao, S.: Oxidative potential of ambient $PM_{2.5}$ in the coastal cities of the Bohai Sea, northern China: Seasonal variation and source apportionment, *Environmental Pollution*, 236, 514-528, 2018.

Ma, S., Ren, K., Liu, X., Chen, L., Li, M., Li, X., Yang, J., Huang, B., Zheng, M., and Xu, Z.: Production of hydroxyl radicals from Fe-containing fine particles in Guangzhou, China, *Atmospheric Environment*, 123, 72-78, 10.1016/j.atmosenv.2015.10.057, 2015.

Milando, C., Huang, L., and Batterman, S.: Trends in $PM_{2.5}$ emissions, concentrations and apportionments in Detroit and Chicago, *Atmospheric Environment*, 129, 197-209, 2016.

Moreno, T., Kelly, F. J., Dunster, C., Oliete, A., Martins, V., Reche, C., Minguillón, M. C., Amato, F., Capdevila, M., and de Miguel, E.: Oxidative potential of subway PM2. 5, *Atmospheric Environment*, 148, 230-238, 2017.

Mudway, I., Kelly, F., and Holgate, S.: Oxidative stress in air pollution research, *Free Radical Biology & Medicine*, 151, 2-6, 10.1016/j.freeradbiomed.2020.04.031, 2020.

Mudway, I. S., Duggan, S. T., Venkataraman, C., Habib, G., Kelly, F. J., and Grigg, J.: Combustion of dried animal dung as biofuel results in the generation of highly redox active fine particulates, *Particle and Fibre Toxicology*, 2, 6, 10.1186/1743-8977-2-6, 2005.

Oh, S. M., Kim, H. R., Park, Y. J., Lee, S. Y., and Chung, K. H.: Organic extracts of urban air pollution particulate matter ($PM_{2.5}$)-induced genotoxicity and oxidative stress in human lung bronchial epithelial cells (BEAS-2B cells), *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 723, 142-151, https://doi.org/10.1016/j.mrgentox.2011.04.003, 2011.

Paraskevopoulou, D., Bougiatioti, A., Stavroulas, I., Fang, T., Lianou, M., Liakakou, E., Gerasopoulos, E., Weber, R., Nenes, A., and Mihalopoulos, N.: Yearlong variability of oxidative potential of particulate matter in an urban Mediterranean environment, *Atmospheric Environment*, 206, 183-196, 2019.

Pei, Y., Jiang, R., Zou, Y., Wang, Y., Zhang, S., Wang, G., Zhao, J., and Song, W.: Effects of Fine Particulate Matter ($PM_{2.5}$) on Systemic Oxidative Stress and Cardiac Function in ApoE−/− Mice, *International Journal of Environmental Research and Public Health*, 13, 484, 2016.

Perrone, M. R., Bertoli, I., Romano, S., Russo, M., Rispoli, G., and Pietrogrande, M. C.: PM2. 5 and PM10 oxidative potential at a Central Mediterranean Site: Contrasts between dithiothreitol-and ascorbic acid-measured values in relation with particle size and chemical composition, *Atmospheric Environment*, 210, 143-155, 2019.

Pietrogrande, M. C., Perrone, M. R., Manarini, F., Romano, S., Udisti, R., and Becagli, S.: PM10 oxidative potential at a Central Mediterranean Site: Association with chemical composition and meteorological parameters, *Atmospheric Environment*, 188, 97-111, 2018.

Pietrogrande, M. C., Bertoli, I., Manarini, F., and Russo, M.: Ascorbate assay as a measure of oxidative potential for ambient particles: Evidence for the importance of cell-free surrogate lung fluid composition, *Atmospheric Environment*, 211, 103-112, 10.1016/j.atmosenv.2019.05.012, 2019.

Poljšak, B., and Fink, R.: The protective role of antioxidants in the defence against ROS/RNS-mediated environmental pollution, *Oxidative Medicine and Cellular Longevity*, 2014, 2014.

Puthussery, J. V., Zhang, C., and Verma, V.: Development and field testing of an online instrument for measuring the real-time oxidative potential of ambient particulate matter based on dithiothreitol assay, *Atmospheric Measurement Techniques*, 11, 5767-5780, 10.5194/amt-11-5767-2018, 2018.

Qin, G., Xia, J., Zhang, Y., Guo, L., Chen, R., and Sang, N.: Ambient fine particulate matter exposure induces reversible cardiac dysfunction and fibrosis in juvenile and older female mice, *Particle and Fibre Toxicology*, 15, 1-14, 2018.

Rao, X., Zhong, J., Brook, R. D., and Rajagopalan, S.: Effect of particulate matter air pollution on cardiovascular oxidative stress pathways, *Antioxidants & Redox Signaling*, 28, 797-818, 2018.

Risom, L., Møller, P., and Loft, S.: Oxidative stress-induced DNA damage by particulate air pollution, *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 592, 119-137, 2005.

Riva, D. R., Magalhães, C. B., Lopes, A. A., Lanças, T., Mauad, T., Malm, O., Valença, S. S., Saldiva, P. H., Faffe, D. S., and Zin, W. A.: Low dose of fine particulate matter ($PM_{2.5}$) can induce acute oxidative stress, inflammation and pulmonary impairment in healthy mice, *Inhalation Toxicology*, 23, 257-267, 10.3109/08958378.2011.566290, 2011.

Rosenthal, F. S., Carney, J. P., and Olinger, M. L.: Out-of-hospital cardiac arrest and airborne fine particulate matter: a case–crossover analysis of emergency medical services data in Indianapolis, Indiana, *Environmental Health Perspectives*, 116, 631-636, 2008.

Rossner, P., Svecova, V., Milcova, A., Lnenickova, Z., Solansky, I., and Sram, R. J.: Seasonal variability of oxidative stress markers in city bus drivers: Part II. Oxidative damage to lipids and proteins, *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 642, 21-27, https://doi.org/10.1016/j.mrfmmm.2008.03.004, 2008.

Sørensen, M., Daneshvar, B., Hansen, M., Dragsted, L. O., Hertel, O., Knudsen, L., and Loft, S.: Personal $PM_{2.5}$ exposure and markers of oxidative stress in blood, *Environmental Health Perspectives*, 111, 161-166, 2003.

Saffari, A., Daher, N., Shafer, M. M., Schauer, J. J., and Sioutas, C.: Seasonal and spatial variation in reactive oxygen species activity of quasi-ultrafine particles ($PM_{0.25}$) in the Los Angeles metropolitan area and its association with chemical composition, *Atmospheric Environment*, 79, 566-575, 2013.

Saffari, A., Daher, N., Shafer, M. M., Schauer, J. J., and Sioutas, C.: Seasonal and spatial variation in dithiothreitol (DTT) activity of quasi-ultrafine particles in the Los Angeles Basin and its association with chemical species, *Journal of Environmental Science and Health, Part A*, 49, 441-451, 10.1080/10934529.2014.854677, 2014.

Sancini, G., Farina, F., Battaglia, C., Cifola, I., Mangano, E., Mantecca, P., Camatini, M., and Palestini, P.: Health risk assessment for air pollutants: alterations in lung and cardiac gene expression in mice exposed to Milano winter fine particulate matter ($PM_{2.5}$), *PLoS One*, 9, e109685, 10.1371/journal.pone.0109685, 2014.

Sarnat, S. E., Winquist, A., Schauer, J. J., Turner, J. R., and Sarnat, J. A.: Fine particulate matter components and emergency department visits for cardiovascular and respiratory diseases in the St. Louis, Missouri–Illinois, metropolitan area, *Environmental Health Perspectives*, 123, 437-444, 2015.

Shen, H., Barakat, A., and Anastasio, C.: Generation of hydrogen peroxide from San Joaquin Valley particles in a cell-free solution, *Atmospheric Chemistry and Physics*, 11, 753-765, 10.5194/acp-11-753-2011, 2011.

Son, Y., Mishin, V., Welsh, W., Lu, S.-E., Laskin, J. D., Kipen, H., and Meng, Q.: A novel high-throughput approach to measure hydroxyl radicals induced by airborne particulate matter, *International Journal of Environmental Research and Public Health*, 12, 13678-13695, 10.3390/ijerph121113678, 2015.

Sun, B., Shi, Y., Li, Y., Jiang, J., Liang, S., Duan, J., and Sun, Z.: Short-term $PM_{2.5}$ exposure induces sustained pulmonary fibrosis development during post-exposure period in rats, *Journal of Hazardous Materials*, 385, 121566, 2020.

Szigeti, T., Dunster, C., Cattaneo, A., Cavallo, D., Spinazzè, A., Saraga, D. E., Sakellaris, I. A., de Kluizenaar, Y., Cornelissen, E. J., and Hänninen, O.: Oxidative potential and chemical composition of $PM_{2.5}$ in office buildings across Europe–The OFFICAIR study, *Environment International*, 92, 324-333, 10.1016/j.envint.2016.04.015, 2016.

Tuet, W. Y., Fok, S., Verma, V., Rodriguez, M. S. T., Grosberg, A., Champion, J. A., and Ng, N. L.: Dose-dependent intracellular reactive oxygen and nitrogen species (ROS/RNS) production from particulate matter exposure: comparison to oxidative potential and chemical composition, *Atmospheric Environment*, 144, 335-344, 2016.

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, *Environmental Science & Technology*, 46, 11384-11392, 10.1021/es302484r, 2012.

Verma, V., Fang, T., Guo, H., King, L., Bates, J., Peltier, R., Edgerton, E., Russell, A., and Weber, R.: Reactive oxygen species associated with water-soluble $PM_{2.5}$ in the southeastern United States: spatiotemporal trends and source apportionment, *Atmospheric Chemistry and Physics*, 14, 12915-12930, 2014.

Vidrio, E., Phuah, C. H., Dillner, A. M., and Anastasio, C.: Generation of hydroxyl radicals from ambient fine particles in a surrogate lung fluid solution, *Environmental Science & Technology*, 43, 922-927, 10.1021/es801653u, 2009.

Visentin, M., Pagnoni, A., Sarti, E., and Pietrogrande, M. C.: Urban $PM_{2.5}$ oxidative potential: Importance of chemical species and comparison of two spectrophotometric cell-free assays, *Environmental Pollution*, 219, 72-79, 10.1016/j.envpol.2016.09.047, 2016.

Wang, Y., Plewa, M. J., Mukherjee, U. K., and Verma, V.: Assessing the cytotoxicity of ambient particulate matter (PM) using Chinese hamster ovary (CHO) cells and its relationship with the PM chemical composition and oxidative potential, *Atmospheric Environment*, 179, 132-141, 10.1016/j.atmosenv.2018.02.025, 2018.

Weber, S., Uzu, G., Calas, A., Chevrier, F., Besombes, J.-L., Charron, A., Salameh, D., Ježek, I., Močnik, G., and Jaffrezo, J.-L.: An apportionment method for the oxidative potential of atmospheric particulate matter sources: application to a one-year study in Chamonix, France, *Atmospheric Chemistry and Physics*, 18, 9617-9629, 2018.

Weber, S., Uzu, G., Favez, O., Borlaza, L. J., Calas, A., Salameh, D., Chevrier, F., Allard, J., Besombes, J.-L., and Albinet, A.: Source apportionment of atmospheric PM 10 Oxidative Potential: synthesis of 15 year-round urban datasets in France, *Atmospheric Chemistry and Physics Discussions*, 1-38, 2021.

Wei, J., Yu, H., Wang, Y., and Verma, V.: Complexation of iron and copper in ambient particulate matter and its effect on the oxidative potential measured in a surrogate lung fluid, *Environmental Science & Technology*, 53, 1661-1671, 2018.

Weichenthal, S., Lavigne, E., Evans, G., Pollitt, K., and Burnett, R. T.: Ambient $PM_{2.5}$ and risk of emergency room visits for myocardial infarction: impact of regional $PM_{2.5}$ oxidative potential: a case-crossover study, *Environmental Health*, 15, 46, 10.1186/s12940-016-0129-9, 2016a.

Weichenthal, S., Shekarrizfard, M., Traub, A., Kulka, R., Al-Rijleh, K., Anowar, S., Evans, G., and Hatzopoulou, M.: Within-city spatial variations in multiple measures of $PM_{2.5}$ oxidative potential in Toronto, Canada, *Environmental Science & Technology*, 53, 2799-2810, 2019.

Weichenthal, S. A., Lavigne, E., Evans, G. J., Godri Pollitt, K. J., and Burnett, R. T.: Fine particulate matter and emergency room visits for respiratory illness. Effect modification by oxidative potential, *American Journal of Respiratory and Critical Care Medicine*, 194, 577-586, 2016b.

Wessels, A., Birmili, W., Albrecht, C., Hellack, B., Jermann, E., Wick, G., Harrison, R. M., and Schins, R. P.: Oxidant generation and toxicity of size-fractionated ambient particles in human lung epithelial cells, *Environmental Science & Technology*, 44, 3539-3545, 2010.

Xiang, S., Yu, Y. T., Hu, Z., and Noll, K. E.: Characterization of dispersion and ultrafine-particle emission factors based on near-roadway monitoring Part II: Heavy duty vehicles, *Aerosol and Air Quality Research*, 19, 2421-2431, 2019.

Xing, Y.-F., Xu, Y.-H., Shi, M.-H., and Lian, Y.-X.: The impact of $PM_{2.5}$ on the human respiratory system, *Journal of Thoracic Disease*, 8, E69-E74, 2016.

Xiong, Q., Yu, H., Wang, R., Wei, J., and Verma, V.: Rethinking the dithiothreitol-based particulate matter oxidative potential: measuring dithiothreitol consumption versus reactive oxygen species generation, *Environmental Science & Technology*, 51, 6507-6514, 10.1021/acs.est.7b01272, 2017.

Xu, Z., Xu, X., Zhong, M., Hotchkiss, I. P., Lewandowski, R. P., Wagner, J. G., Bramble, L. A., Yang, Y., Wang, A., and Harkema, J. R.: Ambient particulate air pollution induces oxidative stress and alterations of mitochondria and gene expression in brown and white adipose tissues, *Particle and Fibre Toxicology*, 8, 1-14, 2011.

Yan, Z., Wang, J., Li, J., Jiang, N., Zhang, R., Yang, W., Yao, W., and Wu, W.: Oxidative stress and endocytosis are involved in upregulation of interleukin-8 expression in airway cells exposed to $PM_{2.5}$, *Environmental Toxicology*, 31, 1869-1878, 10.1002/tox.22188, 2016.

Yang, A., Jedynska, A., Hellack, B., Kooter, I., Hoek, G., Brunekreef, B., Kuhlbusch, T. A., Cassee, F. R., and Janssen, N. A.: Measurement of the oxidative potential of $PM_{2.5}$ and its constituents: The effect of extraction solvent and filter type, *Atmospheric Environment*, 83, 35-42, 10.1016/j.atmosenv.2013.10.049, 2014.

Yang, A., Hellack, B., Leseman, D., Brunekreef, B., Kuhlbusch, T. A., Cassee, F. R., Hoek, G., and Janssen, N. A.: Temporal and spatial variation of the metal-related oxidative potential of $PM_{2.5}$ and its relation to $PM_{2.5}$ mass and elemental composition, *Atmospheric Environment*, 102, 62-69, 2015a.

Yang, A., Wang, M., Eeftens, M., Beelen, R., Dons, E., Leseman, D. L., Brunekreef, B., Cassee, F. R., Janssen, N. A., and Hoek, G.: Spatial variation and land use regression modeling of the oxidative potential of fine particles, *Environmental Health Perspectives*, 123, 1187-1192, 2015b.

Yang, A., Janssen, N. A., Brunekreef, B., Cassee, F. R., Hoek, G., and Gehring, U.: Children's respiratory health and oxidative potential of $PM_{2.5}$: the PIAMA birth cohort study, *Occupational & Environmental Medicine*, 73, 154-160, 10.1136/oemed-2015-103175, 2016.

Yu, H., Wei, J., Cheng, Y., Subedi, K., and Verma, V.: Synergistic and antagonistic interactions among the particulate matter components in generating reactive oxygen species based on the dithiothreitol assay, *Environmental Science & Technology*, 52, 2261-2270, 10.1021/acs.est.7b04261, 2018.

Yu, H., Puthussery, J. V., and Verma, V.: A semi-automated multi-endpoint reactive oxygen species activity analyzer (SAMERA) for measuring the oxidative potential of ambient $PM_{2.5}$ aqueous extracts, *Aerosol Science and Technology*, 54, 304-320, 2020.

Yu, S., Liu, W., Xu, Y., Yi, K., Zhou, M., Tao, S., and Liu, W.: Characteristics and oxidative potential of atmospheric $PM_{2.5}$ in Beijing: Source apportionment and seasonal variation, *Science of the Total Environment*, 650, 277-287, 2019.

971 Zhang, Y., Schauer, J. J., Shafer, M. M., Hannigan, M. P., and Dutton, S. J.: Source apportionment of *in vitro* reactive
972 oxygen species bioassay activity from atmospheric particulate matter, *Environmental Science & Technology*, 42,
973 7502-7509, 10.1021/es800126y, 2008.

974 Zhou, J., Ito, K., Lall, R., Lippmann, M., and Thurston, G.: Time-series analysis of mortality effects of fine particulate
975 matter components in Detroit and Seattle, *Environmental Health Perspectives*, 119, 461-466, 2011.

976 Zuo, L., Otenbaker, N. P., Rose, B. A., and Salisbury, K. S.: Molecular mechanisms of reactive oxygen species-related
977 pulmonary inflammation and asthma, *Molecular Immunology*, 56, 57-63,
978 https://doi.org/10.1016/j.molimm.2013.04.002, 2013.

Figures and Tables

Table 1. Averages and (± standard deviation) of OP from various control groups (N = 10) analyzed by SAMERA.

Endpoint	Unit	Negative control Average (± standard deviation)	Chemical used as positive control	Positive control Average (± standard deviation)	Coefficient of variation (CoV, %)
OP^{AA}	μM/min	0.18 ± 0.07	1 μM Cu	0.34 ± 0.04	11.8
OP^{GSH}	μM/min	0.26 ± 0.06	1 μM Cu	0.77 ± 0.02	2.6
$OP^{OH\text{-}SLF}$	nM/min	7.69 ± 1.37	2 μM Fe	13.80 ± 0.70	5.1
OP^{DTT}	μM/min	0.48 ± 0.07	0.2 μM PQ	1.84 ± 0.02	1.1
$OP^{OH\text{-}DTT}$	nM/min	0.55 ± 0.07	0.2 μM 5-H-1,4-NQ	15.45 ± 1.19	7.7

Table 2. Seasonal averages (± standard deviation) of $PM_{2.5}$ mass concentrations (unit: $\mu g/m^3$) at our sampling sites.

	CHI	STL	IND	CMP	BON
Summer 2018	11.2 ± 3.2	14.7 ± 3.4	11.9 ± 3.5	11.4 ± 3.9	10.4 ± 2.0
Fall 2018	10.9 ± 3.4	13.1 ± 3.7	11.5 ± 4.2	7.5 ± 4.3	9.7 ± 3.5
Winter 2018	14.6 ± 3.6	11.8 ± 2.8	11.0 ± 2.7	10.0 ± 3.0	8.6 ± 3.0
Spring 2019	12.6 ± 4.2	13.8 ± 4.0	12.2 ± 2.1	11.6 ± 3.1	9.2 ± 2.3

Table 3. Pearson's correlation coefficient (r) and the associated levels of significance (P) between water-soluble and methanol-soluble OPv for different endpoints at five sampling sites. Correlations with r > 0.60 are shown in **bold**. Asterisks - * and ** indicate significant (P < 0.05) and highly significant (P < 0.01) correlations, respectively.

Site	Pearson's r/significance level (P) for OP endpoints OP^{AA}	OP^{GSH}	$OP^{OH\text{-}SLF}$	OP^{DTT}	$OP^{OH\text{-}DTT}$
CHI	0.09	0.34*	0.53**	0.55**	0.40**
STL	0.24	0.11	0.18	0.28	0.38**
IND	0.24	0.40**	0.33*	0.43**	0.21
CMP	0.42**	**0.63****	0.10	**0.74****	0.58**
BON	0.60**	0.52**	0.41**	**0.68****	0.54**

Table 4. Pearson's r, the associated levels of significance (P) and slope for simple linear regression of water-soluble OPv versus $PM_{2.5}$ mass concentration at five sampling sites. Correlations with r > 0.60 are shown in **bold**. All slope values are in *italic*. Asterisks - * and ** indicate significant (P < 0.05) and highly significant (P < 0.01) correlations, respectively.

(a) Water-soluble OP

		CHI	STL	IND	CMP	BON
OP^{AA}	Pearson's r/P	-0.02	0.33*	0.19	0.54**	0.26
	Slope (nmol/min/μg)	*0.000*	*0.005*	*0.004*	*0.031*	*0.007*
OP^{GSH}	Pearson's r/P	0.45**	0.34*	0.45**	**0.72****	0.38*
	Slope (nmol/min/μg)	*0.005*	*0.003*	*0.005*	*0.016*	*0.005*
$OP^{OH\text{-}SLF}$	Pearson's r/P	0.09	0.26	0.37**	0.43**	0.24
	Slope (pmol/min/μg)	*0.041*	*0.107*	*0.128*	*0.277*	*0.165*
OP^{DTT}	Pearson's r/P	**0.62****	0.27	0.55**	**0.82****	**0.63****
	Slope (nmol/min/μg)	*0.013*	*0.005*	*0.013*	*0.020*	*0.015*
$OP^{OH\text{-}DTT}$	Pearson's r/P	0.24	0.60**	0.37**	0.51**	0.45**
	Slope (pmol/min/μg)	*0.043*	*0.062*	*0.051*	*0.048*	*0.052*

(b) Methanol-soluble OP

		CHI	STL	IND	CMP	BON
OP^{AA}	Pearson's r~~/P~~	0.55**	0.12	0.52**	**0.64****	**0.61****
	Slope (*nmol/min/µg*)	*0.010*	*0.002*	*0.010*	*0.011*	*0.012*
OP^{GSH}	Pearson's r~~/P~~	0.53**	0.38**	0.51**	**0.73****	**0.63****
	Slope (*nmol/min/µg*)	*0.007*	*0.005*	*0.007*	*0.012*	*0.009*
OP^{OH-SLF}	Pearson's r~~/P~~	0.19	0.34*	0.45**	0.48**	0.52**
	Slope (*pmol/min/µg*)	*0.264*	*0.514*	*0.666*	*0.576*	*0.735*
OP^{DTT}	Pearson's r~~/P~~	0.54**	0.49**	**0.61****	**0.79****	**0.61****
	Slope (*nmol/min/µg*)	*0.017*	*0.016*	*0.019*	*0.028*	*0.022*
OP^{OH-DTT}	Pearson's r~~/P~~	0.25	0.44*	0.51**	0.43**	0.50**
	Slope (*pmol/min/µg*)	*0.072*	*0.079*	*0.143*	*0.075*	*0.165*

Table 5. Pearson's correlation coefficient (r) ~~and the associated level of significance (P)~~ among various endpoints of OPv measured at five sampling sites. The values below the diagonal are for water-soluble OPv, while above are for methanol-soluble OPv. Correlations with r > 0.60 are shown in **bold**. Asterisks - * and ** indicate significant (P < 0.05) and highly significant (P < 0.01) correlations, respectively.

(a) CHI

OP endpoint	Pearson's r~~/significance level (P) for OP endpoints~~				
	OP^{AA}	OP^{GSH}	OP^{OH-SLF}	OP^{DTT}	OP^{OH-DTT}
OP^{AA}		**0.66****	0.60**	**0.69****	0.49**
OP^{GSH}	0.32*		0.30	0.45**	0.17
OP^{OH-SLF}	0.09	0.39**		0.53**	**0.82****
OP^{DTT}	0.05	0.40**	0.40**		**0.64****
OP^{OH-DTT}	0.03	0.30	0.48**	0.18	
	OP^{AA}	OP^{GSH}	OP^{OH-SLF}	OP^{DTT}	OP^{OH-DTT}

(b) STL

OP endpoint	Pearson's r~~/significance level (P) for OP endpoints~~				
	OP^{AA}	OP^{GSH}	OP^{OH-SLF}	OP^{DTT}	OP^{OH-DTT}
OP^{AA}		0.40**	0.19	0.50**	0.33*
OP^{GSH}	0.30		0.13	0.36*	0.23
OP^{OH-SLF}	0.51**	0.17		0.17	0.42**
OP^{DTT}	0.28	0.29	0.22		0.57**
OP^{OH-DTT}	0.40**	0.38**	0.53**	0.34*	
	OP^{AA}	OP^{GSH}	OP^{OH-SLF}	OP^{DTT}	OP^{OH-DTT}

(c) IND

OP endpoint	Pearson's r~~/significance level (P) for OP endpoints~~				
	OP^{AA}	OP^{GSH}	OP^{OH-SLF}	OP^{DTT}	OP^{OH-DTT}
OP^{AA}		0.57**	0.54**	**0.62****	0.57**
OP^{GSH}	0.37**		0.59**	0.52**	0.55**
OP^{OH-SLF}	0.32*	0.23		0.44**	**0.84****
OP^{DTT}	0.17	0.42**	0.44**		0.54**
OP^{OH-DTT}	0.08	0.20	0.29*	0.15	
	OP^{AA}	OP^{GSH}	OP^{OH-SLF}	OP^{DTT}	OP^{OH-DTT}

(d) CMP

OP endpoint	Pearson's r/~~significance level (P) for OP endpoints~~				
	OP^{AA}	OP^{GSH}	$OP^{OH\text{-}SLF}$	OP^{DTT}	$OP^{OH\text{-}DTT}$
OP^{AA}		0.55**	0.46**	**0.70****	0.45**
OP^{GSH}	**0.68****		0.30*	**0.69****	0.15
$OP^{OH\text{-}SLF}$	**0.77****	**0.80****		0.37**	**0.66****
OP^{DTT}	**0.80****	**0.73****	0.58**		0.35*
$OP^{OH\text{-}DTT}$	0.02	0.26	0.15	0.29*	
	OP^{AA}	OP^{GSH}	$OP^{OH\text{-}SLF}$	OP^{DTT}	$OP^{OH\text{-}DTT}$

(e) BON

OP endpoint	Pearson's r/~~significance level (P) for OP endpoints~~				
	OP^{AA}	OP^{GSH}	$OP^{OH\text{-}SLF}$	OP^{DTT}	$OP^{OH\text{-}DTT}$
OP^{AA}		**0.66****	**0.77****	**0.70****	**0.61****
OP^{GSH}	**0.85****		**0.68****	0.60**	0.53**
$OP^{OH\text{-}SLF}$	0.57**	**0.64****		**0.69****	**0.78****
OP^{DTT}	0.51**	0.57**	0.30		**0.68****
$OP^{OH\text{-}DTT}$	0.19	0.31*	0.28	0.32*	
	OP^{AA}	OP^{GSH}	$OP^{OH\text{-}SLF}$	OP^{DTT}	$OP^{OH\text{-}DTT}$

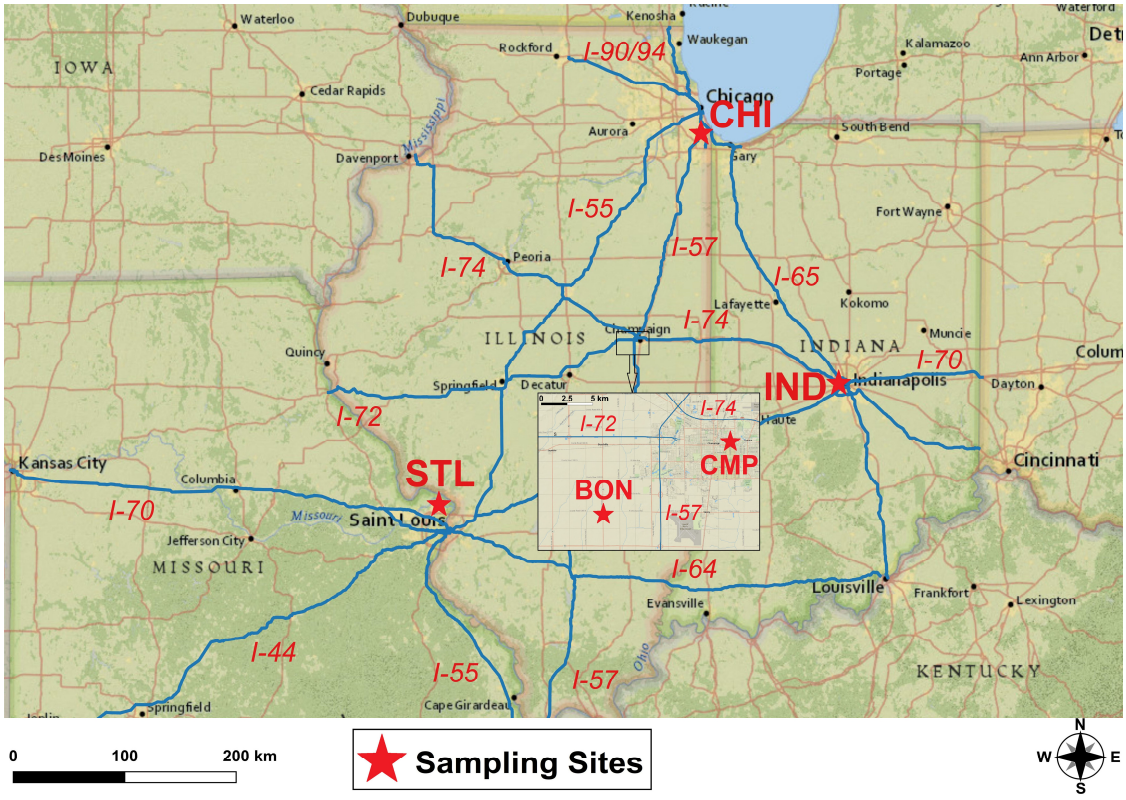

Figure 1. Map for our five sampling sites in the Midwest US.

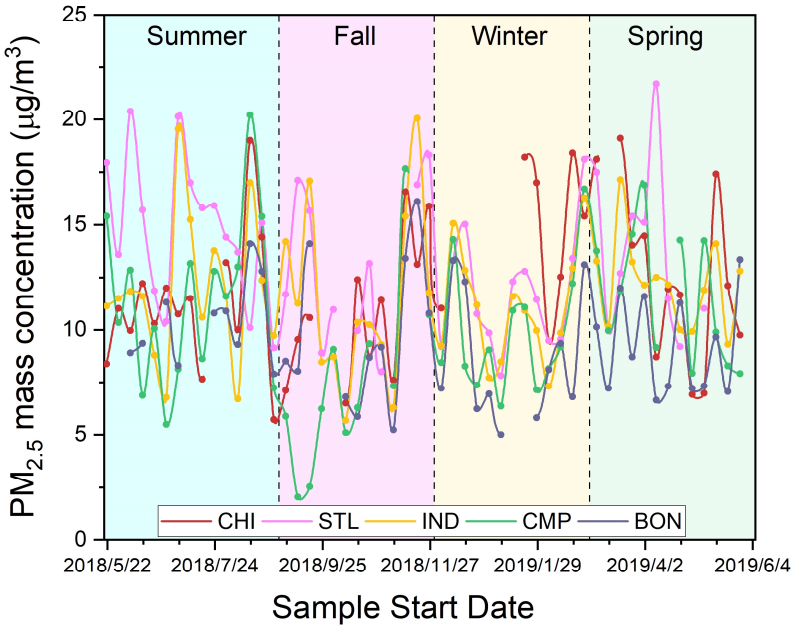

Figure 2. Time series of $PM_{2.5}$ mass concentrations at our sampling sites in the Midwest US.

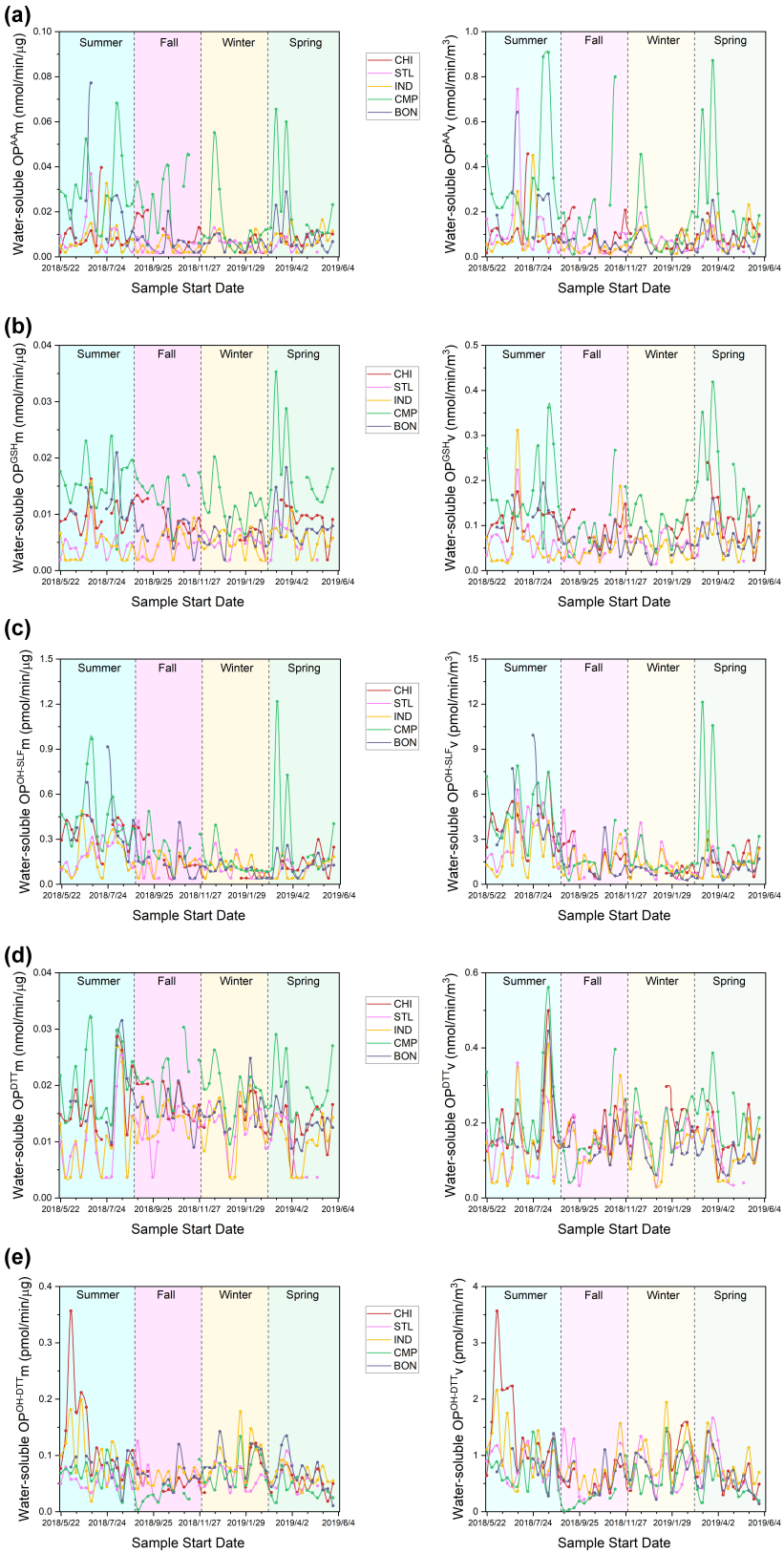

Figure 3. Time series of mass-(left) and volume-(right)normalized water-soluble OP activities for (a) OP^{AA}, (b) OP^{GSH}, (c) $OP^{OH\text{-}SLF}$, (d) OP^{DTT} and (e) $OP^{OH\text{-}DTT}$ at our sampling sites.

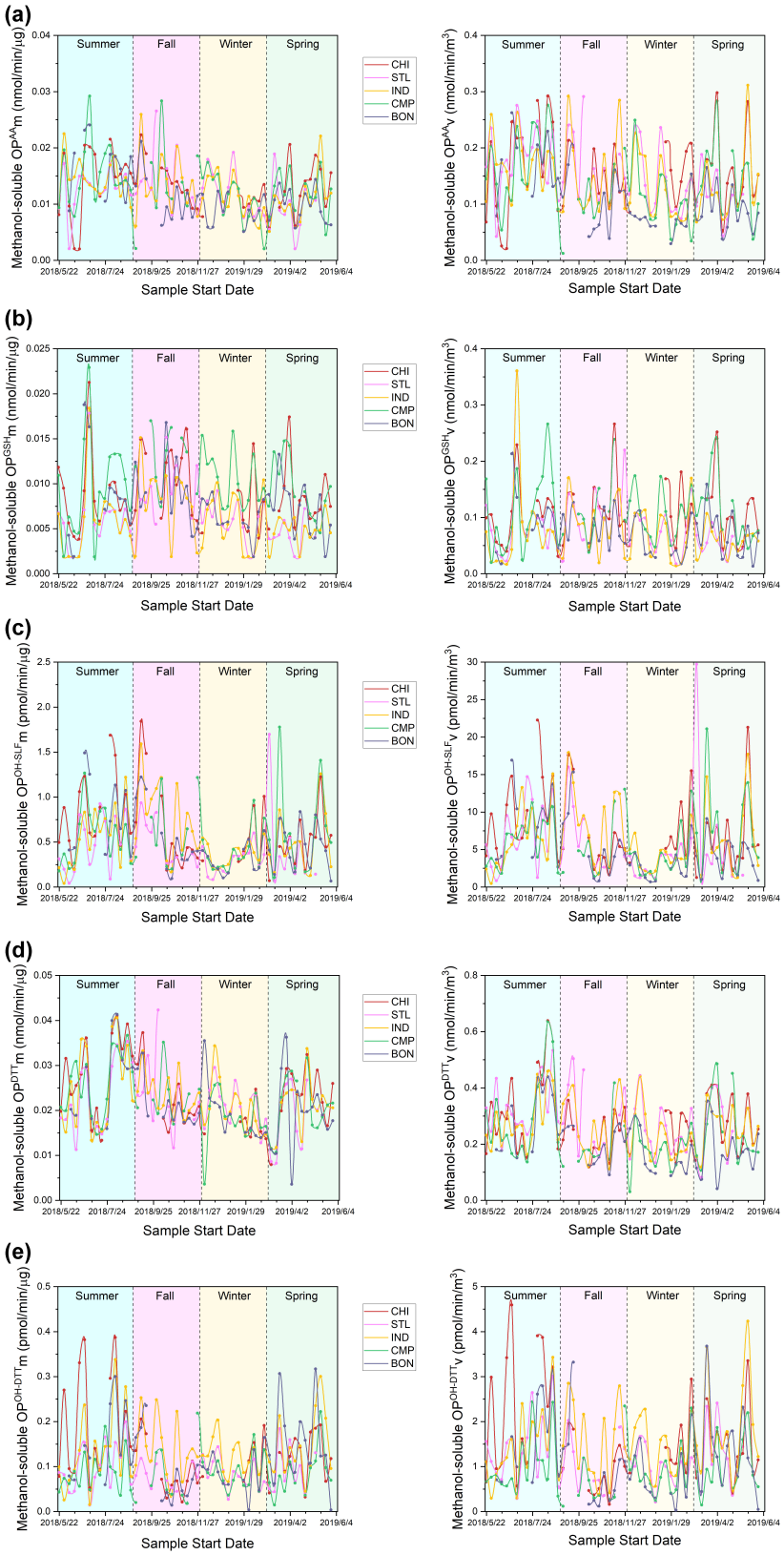

Figure 4. Time series of mass-(left) and volume-(right)normalized methanol-soluble OP activities for (a) OP^{AA}, (b) OP^{GSH}, (c) $OP^{OH\text{-}SLF}$, (d) OP^{DTT} and (e) $OP^{OH\text{-}DTT}$ at our sampling sites.

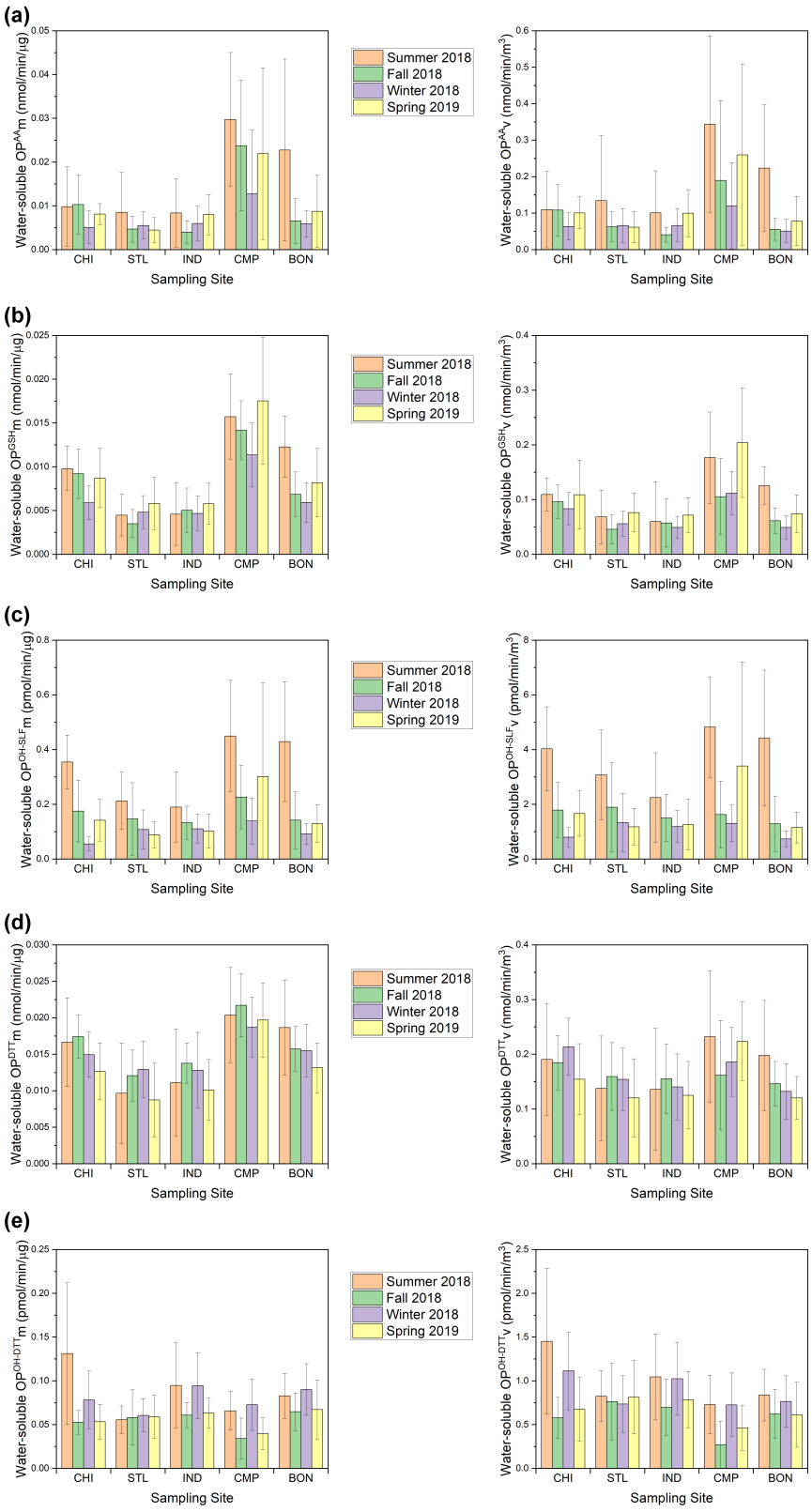

Figure 5. Seasonal averages of mass-(left) and volume-(right) normalized water-soluble OP activities for (a) OP^{AA}, (b) OP^{GSH}, (c) $OP^{OH\text{-}SLF}$, (d) OP^{DTT} and (e) $OP^{OH\text{-}DTT}$ at our sampling sites.

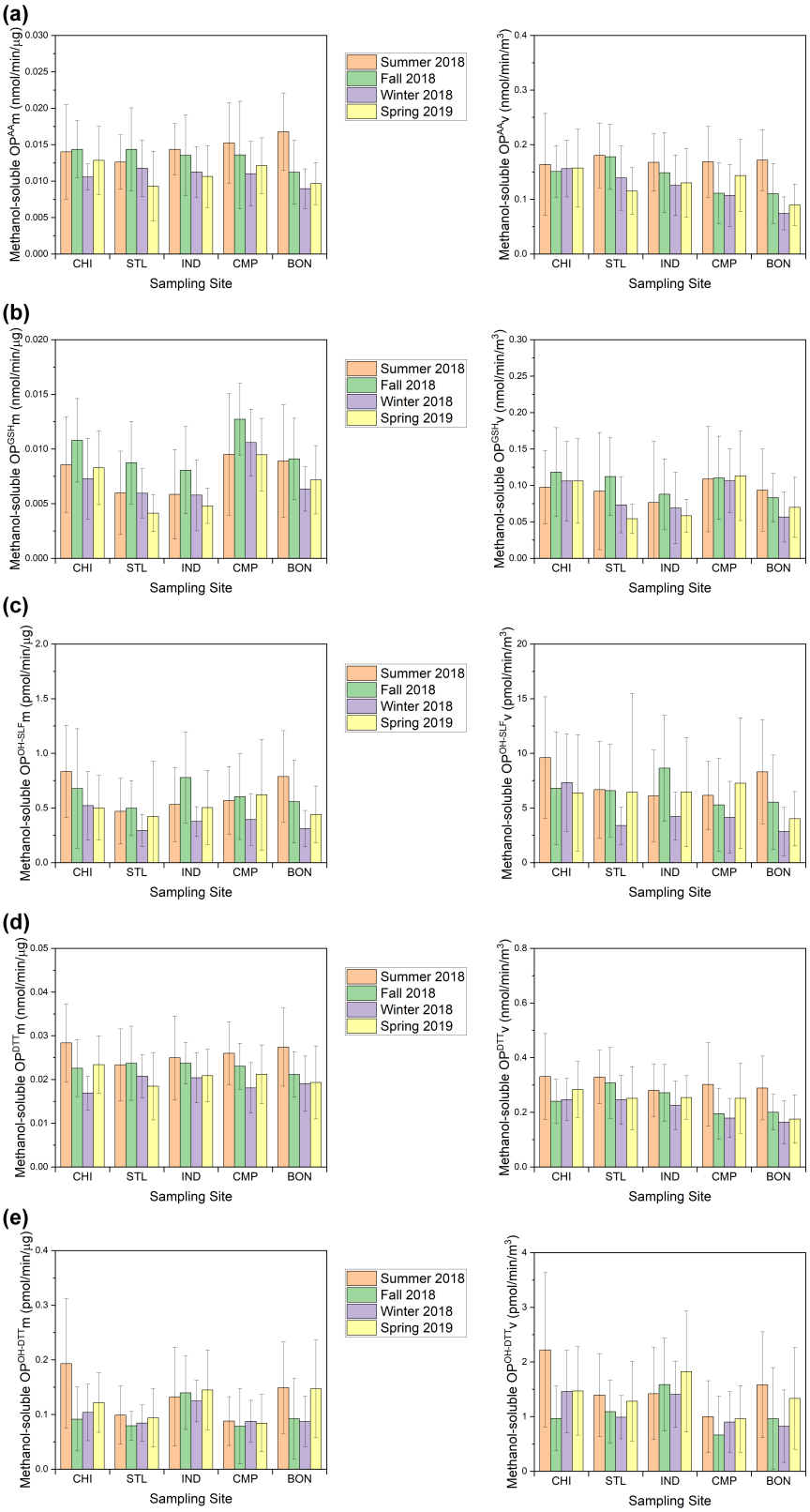

Figure 6. Seasonal averages of mass-(left) and volume-(right) normalized methanol-soluble OP activities for (a) OP^{AA}, (b) OP^{GSH}, (c) $OP^{OH\text{-}SLF}$, (d) OP^{DTT} and (e) $OP^{OH\text{-}DTT}$ at our sampling sites.

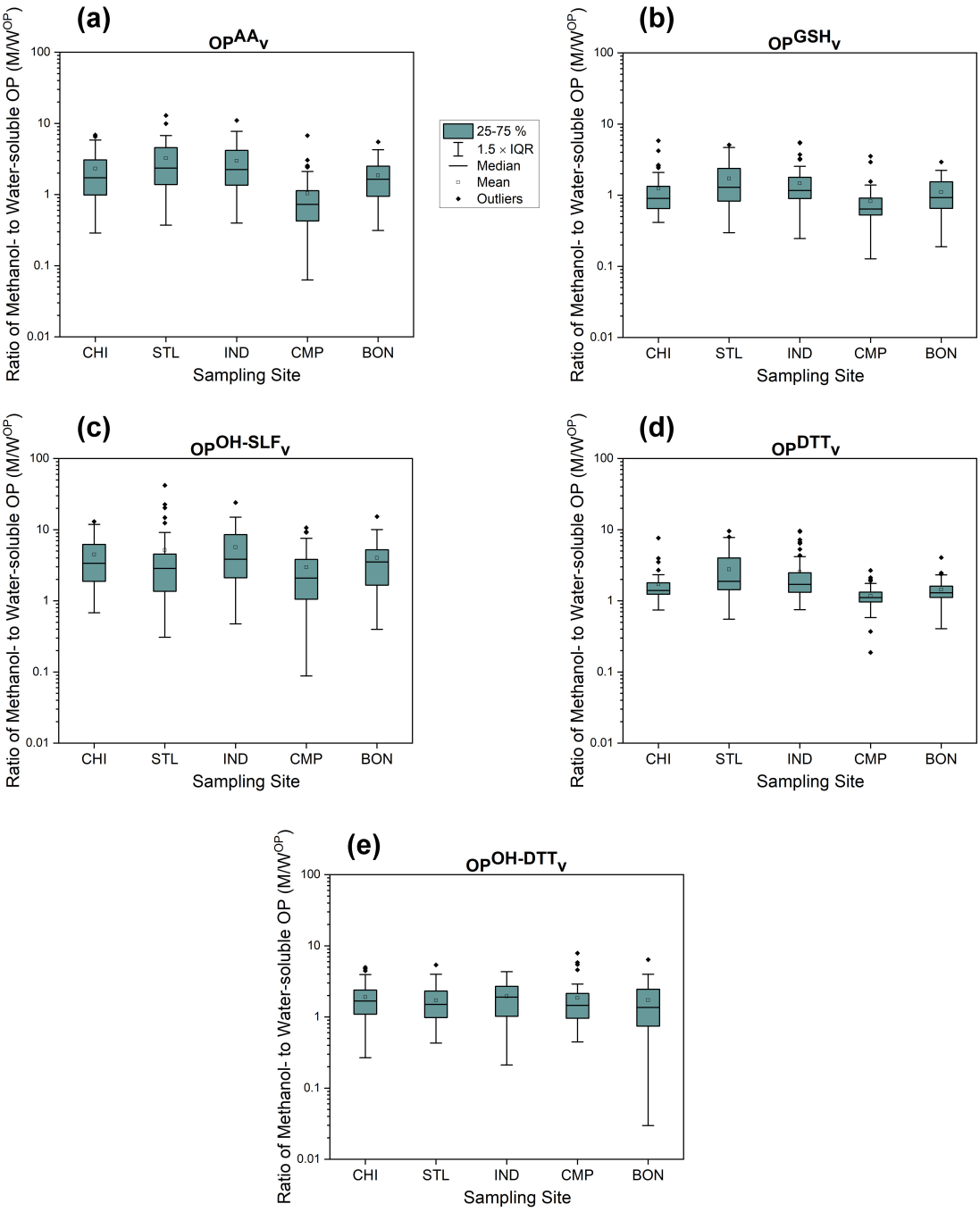

Figure 7. Ratio of methanol-soluble OPv to water-soluble OPv (M/W^{OP}) for (a) $OP^{AA}v$, (b) $OP^{GSH}v$, (c) $OP^{OH\text{-}SLF}v$, (d) $OP^{DTT}v$, and (e) $OP^{OH\text{-}DTT}v$ at five sampling sites.

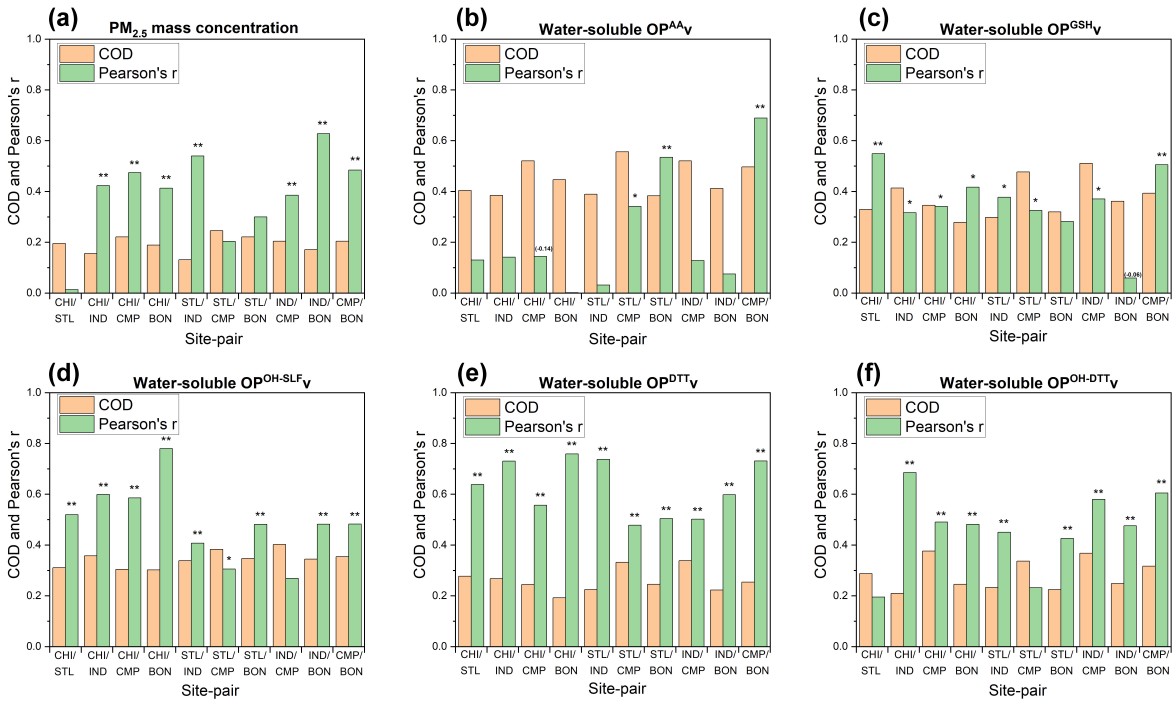

Figure 8. Coefficient of divergence (CoD) and Pearson's r for site-to-site comparison of (a) $PM_{2.5}$ mass and water-soluble OP activities: (b) $OP^{AA}v$, (c) $OP^{GSH}v$, (d) $OP^{OH\text{-}SLF}v$, (e) $OP^{DTT}v$ and (f) $OP^{OH\text{-}DTT}v$. Asterisks - * and ** on the bars of Pearson's r indicate significant ($P < 0.05$) and very significant ($P < 0.01$) correlations, respectively. Note: r for the correlations of $OP^{AA}v$ between CHI and CMP and for the correlations of $OP^{GSH}v$ between IND and BON were negative (-0.14 and -0.06, respectively).

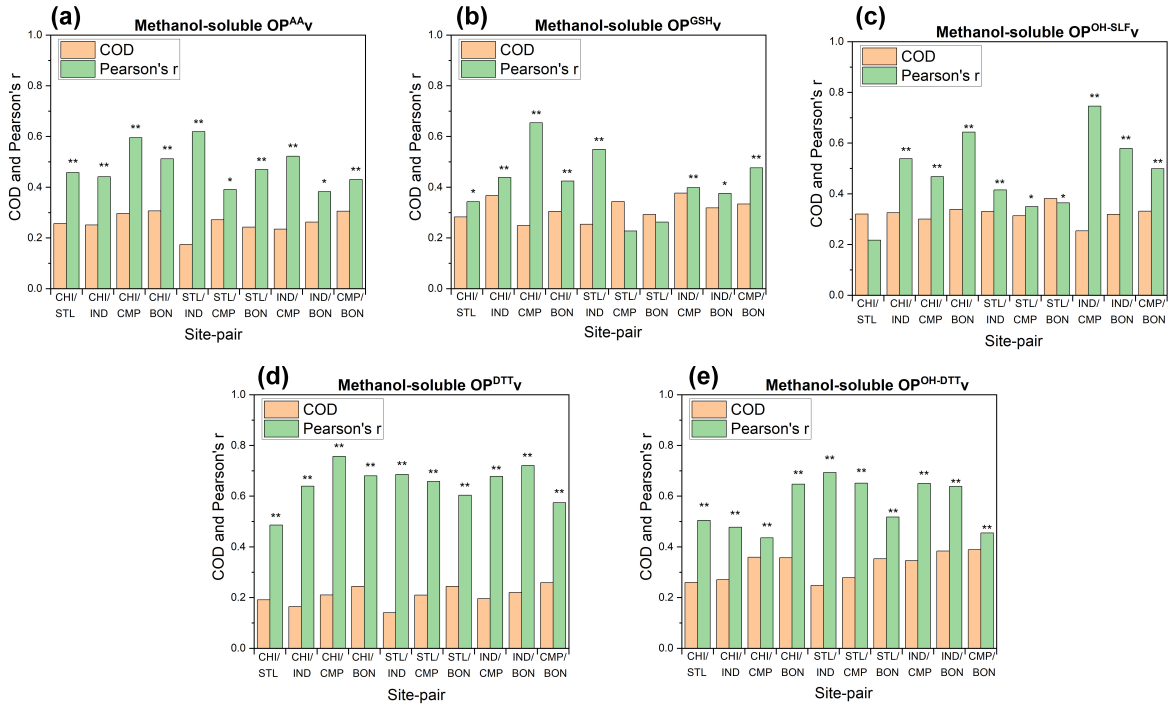

Figure 9. Coefficient of divergence (CoD) and Pearson's r for site-to-site comparison of methanol-soluble OP activities: (a) $OP^{AA}v$, (b) $OP^{GSH}v$, (c) $OP^{OH\text{-}SLF}v$, (d) $OP^{DTT}v$ and (e) $OP^{OH\text{-}DTT}v$. Asterisks - * and ** on the bars of Pearson's r indicate significant ($P < 0.05$) and very significant ($P < 0.01$) correlations, respectively.

Supplemental Information of

Spatiotemporal Variability in the Oxidative Potential of Ambient Fine Particulate Matter in Midwestern United States

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Section S1. Comparison of five Hi-Vol samplers before and after the sampling campaign

Out of five samplers used in our study, two were old samplers (about 5 years old, used in various sampling campaigns) and three were brand new, which were bought from TISCH Environmental (Cleves, OH, US) a month before the sampling. These new samplers were factory calibrated and installed at three farther sites, i.e. Chicago (CHI), Indianapolis (IND) and St. Louis (STL). The other two old samplers were installed at Champaign (CMP) and Bondville (BON). For the sole purpose of this discussion, we will name them as CHI (N), IND (N), STL (N), CMP (O) and BON (O). Since the new samplers were factory calibrated, we had more confidence in them, therefore, we chose one of those samplers, i.e. CHI (N), as a reference and compared the responses of other two old samplers, i.e. CMP (O) and BON (O), by running them in pairs, i.e. first CHI (N) and CMP (O) pair, followed by CHI (N) and BON (O) pair, at a site in Urbana in April 2018 (due to some practical constraint, we couldn't run all three of them together). We collected 9 sets of 24-hours integrated Hi-Vol $PM_{2.5}$ samples on quartz filters from each pair, and analyzed them for the DTT assay using the same extraction and analysis procedure as used in our current study. The comparison of OP^{DTT} response was conducted by the orthogonal fit regression analysis of $OP^{DTT}v$ of $PM_{2.5}$ samples collected from CHI (N) and old samplers (**Figure S1**). The correlations between the old samplers and CHI (N) sampler were excellent (R^2 = 0.92 – 0.94) with slopes almost equal to 1 (1.02 – 1.03), indicating that the samplers collect identical $PM_{2.5}$, and had negligible internal difference in sample collection.

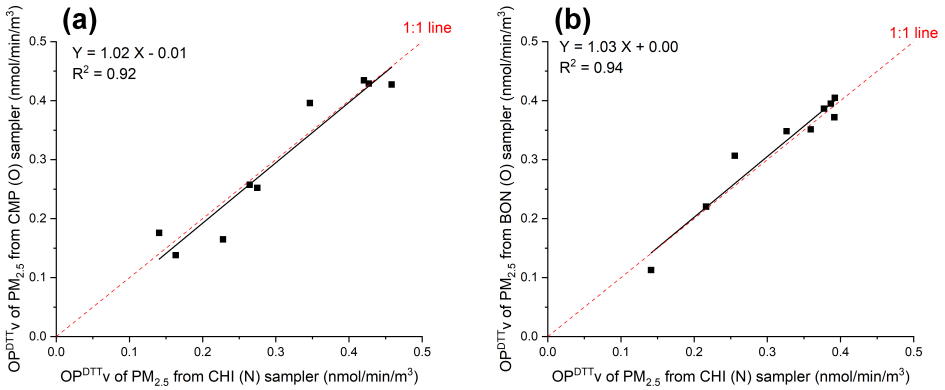

Figure S1. Comparison of OP^{DTT} of $PM_{2.5}$ samples collected from CHI (N) sampler with old samplers: (a) CMP (O) sampler; (b) BON (O) sampler.

After the sampling campaign, we again moved the new samplers [i.e. CHI (N), STL (N) and IND (N)] back to CMP site, kept them side-by-side, and collected 9 Hi-Vol samples (24-hours integrated) from each sampler. All these samples were extracted in DI and analyzed for OP^{DTT} in the same manner as used in our current study. The comparison of the reference sampler [i.e. CHI (N)] with other two new samplers was also conducted by orthogonal fit (Figure S2). Excellent correlations (R^2 = 0.93 – 0.95) and consistent slopes (1.05 – 1.06, close to 1) both showed a good consistency of three new samplers.

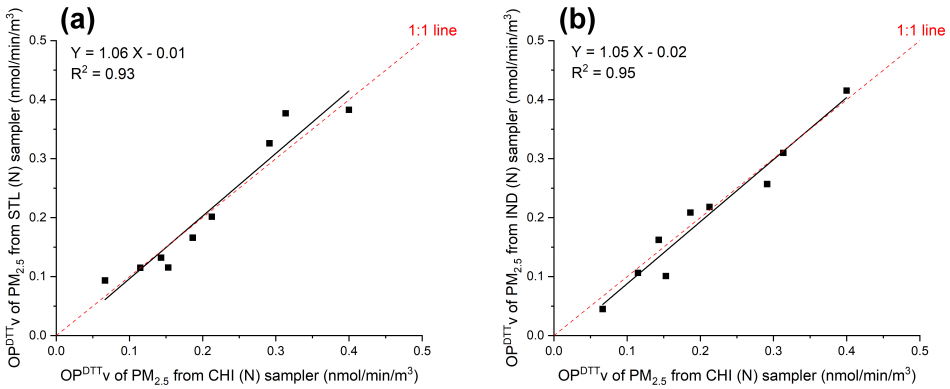

Figure S2. Comparison of OP^{DTT} of $PM_{2.5}$ samples collected from CHI (N) sampler with other new samplers: (a) STL (N) sampler; (b) IND (N) sampler.

Table S1. Dates of samples collection at five sampling sites.

Season	Week count	Sampling period	CHI	STL	IND	CMP	BON
Summer 2018	1	2018/5/22 – 2018/5/25	✓	✓	✓	✓	×
	2	2018/5/29 – 2018/6/1	✓	✓	✓	✓	×
	3	2018/6/5– 2018/6/8	✓	✓	✓	✓	✓
	4	2018/6/12– 2018/6/15	✓	✓	✓	✓	✓
	5	2018/6/19– 2018/6/22	✓	✓	✓	✓	×
	6	2018/6/26– 2018/6/29	✓	✓	✓	✓	✓
	7	2018/7/3– 2018/7/6	✓	✓	✓	✓	✓
	8	2018/7/10– 2018/7/13	✓	✓	✓	✓	×
	9	2018/7/17– 2018/7/20	✓	✓	✓	✓	×
	10	2018/7/24– 2018/7/27	×	✓	✓	✓	✓
	11	2018/7/31– 2018/8/3	✓	✓	✓	✓	✓
	12	2018/8/7– 2018/8/10	✓	✓	✓	✓	✓
	13	2018/8/14– 2018/8/17	✓	✓	✓	✓	✓
	14	2018/8/21– 2018/8/24	✓	✓	✓	✓	✓
	15	2018/8/28– 2018/8/31	✓	✓	✓	✓	✓
Fall 2018	16	2018/9/4– 2018/9/7	✓	✓	✓	✓	✓
	17	2018/9/11– 2018/9/14	✓	✓	✓	✓	✓
	18	2018/9/18– 2018/9/21	✓	✓	✓	✓	✓
	19	2018/9/25– 2018/9/28	×	✓	✓	✓	×
	20	2018/10/2– 2018/10/5	×	✓	✓	✓	×
	21	2018/10/9– 2018/10/12	✓	×	✓	✓	✓
	22	2018/10/16– 2018/10/19	✓	✓	✓	✓	✓
	23	2018/10/23– 2018/10/26	✓	✓	✓	✓	✓
	24	2018/10/30– 2018/11/2	✓	✓	✓	×	✓
	25	2018/11/6– 2018/11/9	✓	×	✓	✓	✓
	26	2018/11/13– 2018/11/16	✓	×	✓	✓	✓
	27	2018/11/20– 2018/11/23	✓	✓	✓	×	✓
	28	2018/11/27– 2018/11/30	✓	✓	✓	✓	✓
Winter 2018	29	2018/12/4– 2018/12/7	✓	✓	✓	✓	✓
	30	2018/12/11– 2018/12/14	×	✓	✓	✓	✓
	31	2018/12/18– 2018/12/21	×	✓	✓	✓	✓
	32	2018/12/25– 2018/12/28	×	✓	✓	✓	✓
	33	2019/1/1– 2019/1/4	×	✓	✓	✓	✓
	34	2019/1/8– 2019/1/11	×	✓	✓	✓	✓
	35	2019/1/15– 2019/1/18	×	✓	✓	✓	×
	36	2019/1/22– 2019/1/25	✓	✓	✓	✓	×
	37	2019/1/29– 2019/2/1	✓	✓	✓	✓	✓
	38	2019/2/5– 2019/2/8	✓	✓	✓	✓	✓
	39	2019/2/12– 2019/2/15	✓	✓	✓	✓	✓
	40	2019/2/19– 2019/2/22	✓	✓	✓	✓	✓
	41	2019/2/26– 2019/3/1	✓	✓	✓	✓	✓
Spring 2019	42	2019/3/5– 2019/3/8	✓	✓	✓	✓	✓
	43	2019/3/12– 2019/3/15	×	✓	✓	✓	✓
	44	2019/3/19– 2019/3/22	✓	✓	✓	✓	✓
	45	2019/3/26– 2019/3/29	✓	✓	✓	✓	✓
	46	2019/4/2– 2019/4/5	✓	✓	✓	✓	✓
	47	2019/4/9– 2019/4/12	✓	✓	✓	✓	✓
	48	2019/4/16– 2019/4/19	✓	✓	✓	×	✓
	49	2019/4/23– 2019/4/26	✓	✓	✓	✓	✓
	50	2019/4/30– 2019/5/3	✓	×	✓	✓	✓
	51	2019/5/7– 2019/5/10	✓	✓	✓	✓	✓
	52	2019/5/14– 2019/5/17	✓	×	✓	✓	✓
	53	2019/5/21– 2019/5/24	✓	×	✓	✓	✓
	54	2019/5/28– 2019/5/31	✓	×	✓	✓	✓

The symbol ✓ denotes the collection of a sample, and the symbol × denotes no collection of the sample in that week (due to several reasons such as unfavorable weather conditions, broken sampler, etc.).

Table S2. Precision of SAMERA for methanol-soluble OP measurements compared with water-soluble OP measurements.

Endpoint	Unit	Average	Standard Deviation	CoV (%)	CoV (%) for the water-soluble $PM_{2.5}$ extract (Yu et al., 2020)
OP^{AA}	nmol/min/m^3	0.132	0.018	13.51	11.87
OP^{GSH}	nmol/min/m^3	0.098	0.010	10.65	7.89
$OP^{OH\text{-}SLF}$	pmol/min/m^3	0.740	0.011	14.49	10.56
OP^{DTT}	nmol/min/m^3	0.187	0.017	8.89	10.52
$OP^{OH\text{-}DTT}$	pmol/min/m^3	0.216	0.023	10.88	13.28

Table S3. Results of 1-way ANOVA test for assessing the temporal and spatial variability of $PM_{2.5}$ mass concentrations.

Variability	Sampling Site/Season	F value	Significantly different group(s)
Temporal	CHI	1.95	
	STL	1.79	
	IND	0.33	
	CMP	3.25*	Fall 2018
	BON	0.82	
Spatial	Summer 2018	3.48*	STL
	Fall 2018	3.13*	CHI, STL, IND, CMP
	Winter 2018	5.01**	CHI
	Spring 2019	3.35*	BON

Asterisks – * and ** indicate significant ($P < 0.05$) and highly significant ($P < 0.01$) differences, respectively.

Table S4. Results of 1-way ANOVA test for assessing the temporal and spatial variability of mass-normalized and volume-normalized OP endpoints for water-soluble $PM_{2.5}$ samples.

(a) Temporal variability

Sampling Site	Endpoint	F value	Significantly different group(s)
Chicago, IL (CHI)	$OP^{AA}m$	1.12	
	$OP^{AA}v$	0.69	
	$OP^{GSH}m$	3.19*	Summer 2018, Fall 2018, Spring 2019, Winter 2018
	$OP^{GSH}v$	0.78	
	$OP^{OH\text{-}SLF}m$	21.84**	Summer 2018, Fall 2018, Spring 2019, Winter 2018
	$OP^{OH\text{-}SLF}v$	17.72**	Summer 2018, Fall 2018, Spring 2019, Winter 2018
	$OP^{DTT}m$	2.67	Summer 2018, Fall 2018, Spring 2019
	$OP^{DTT}v$	1.03	
	$OP^{OH\text{-}DTT}m$	7.26**	Summer 2018, Winter 2018, Fall 2018, Spring 2019
	$OP^{OH\text{-}DTT}v$	6.68**	Summer 2018, Fall 2018, Spring 2019
St. Louis, MO (STL)	$OP^{AA}m$	1.37	
	$OP^{AA}v$	1.48	
	$OP^{GSH}m$	1.74	Spring 2019, Fall 2018
	$OP^{GSH}v$	1.40	
	$OP^{OH\text{-}SLF}m$	4.25**	Summer 2018, Winter 2018, Spring 2019
	$OP^{OH\text{-}SLF}v$	5.33**	Summer 2018, Fall 2018, Winter 2018, Spring 2019
	$OP^{DTT}m$	1.83	
	$OP^{DTT}v$	0.56	
	$OP^{OH\text{-}DTT}m$	0.12	
	$OP^{OH\text{-}DTT}v$	0.17	
Indianapolis, IN (IND)	$OP^{AA}m$	2.02	Summer 2018, Fall 2018
	$OP^{AA}v$	2.11	Summer 2018, Spring 2019, Fall 2018
	$OP^{GSH}m$	0.53	
	$OP^{GSH}v$	0.49	
	$OP^{OH\text{-}SLF}m$	3.16*	Summer 2018, Winter 2018, Spring 2019
	$OP^{OH\text{-}SLF}v$	2.75*	Summer 2018, Winter 2018, Spring 2019
	$OP^{DTT}m$	1.29	
	$OP^{DTT}v$	0.33	
	$OP^{OH\text{-}DTT}m$	4.28**	Summer 2018, Winter 2018, Fall 2018, Spring 2019
	$OP^{OH\text{-}DTT}v$	2.57	Summer 2018, Winter 2018, Fall 2018
Champaign, IL (CMP)	$OP^{AA}m$	2.59	Summer 2018, Winter 2018
	$OP^{AA}v$	2.77*	Summer 2018, Winter 2018
	$OP^{GSH}m$	3.44*	Spring 2019, Summer 2018, Winter 2018
	$OP^{GSH}v$	4.92**	Spring 2019, Summer 2018, Winter 2018, Fall 2018
	$OP^{OH\text{-}SLF}m$	5.47**	Summer 2018, Fall 2018, Winter 2018
	$OP^{OH\text{-}SLF}v$	7.59**	Summer 2018, Spring 2019, Fall 2018, Winter 2018
	$OP^{DTT}m$	0.70	
	$OP^{DTT}v$	1.55	
	$OP^{OH\text{-}DTT}m$	8.06**	Summer 2018, Winter 2018, Fall 2018, Spring 2019
	$OP^{OH\text{-}DTT}v$	6.18**	Summer 2018, Winter 2018, Spring 2019, Fall 2018
Bondville, IL (BON)	$OP^{AA}m$	5.26**	Summer 2018, Spring 2019, Fall 2018, Winter 2018
	$OP^{AA}v$	8.17**	Summer 2018, Spring 2019, Fall 2018, Winter 2018
	$OP^{GSH}m$	8.16**	Summer 2018, Spring 2019, Fall 2018, Winter 2018
	$OP^{GSH}v$	13.81**	Summer 2018, Spring 2019, Fall 2018, Winter 2018
	$OP^{OH\text{-}SLF}m$	16.82**	Summer 2018, Spring 2019, Fall 2018, Winter 2018
	$OP^{OH\text{-}SLF}v$	17.33**	Summer 2018, Spring 2019, Fall 2018, Winter 2018
	$OP^{DTT}m$	3.15*	Summer 2018, Spring 2019
	$OP^{DTT}v$	3.37*	Summer 2018, Winter 2018, Spring 2019
	$OP^{OH\text{-}DTT}m$	2.10	Winter 2018, Fall 2018
	$OP^{OH\text{-}DTT}v$	1.34	

(b) Spatial variability

Season	Endpoint	F value	Significantly different group(s)
Summer 2018	$OP^{AA}m$	8.60**	CMP, BON, CHI, STL, IND
	$OP^{AA}v$	5.28**	CMP, CHI, STL, IND
	$OP^{GSH}m$	28.41**	CMP, BON, CHI, STL, IND
	$OP^{GSH}v$	9.30**	CMP, BON, CHI, STL, IND
	$OP^{OH\text{-}SLF}m$	8.60**	CHI, CMP, BON, STL, IND
	$OP^{OH\text{-}SLF}v$	4.83**	CMP, CHI, STL, IND
	$OP^{DTT}m$	6.97**	CMP, STL, IND
	$OP^{DTT}v$	2.21	CMP, STL, IND
	$OP^{OH\text{-}DTT}m$	5.92**	CHI, IND, CMP, BON, STL
	$OP^{OH\text{-}DTT}v$	4.70**	CHI, STL, IND, CMP, BON
Fall 2018	$OP^{AA}m$	12.08**	CMP, CHI, STL, IND, BON
	$OP^{AA}v$	3.81**	CMP, STL, IND, BON
	$OP^{GSH}m$	27.05**	CMP, CHI, BON, IND, STL
	$OP^{GSH}v$	4.07**	CMP, CHI, STL, IND
	$OP^{OH\text{-}SLF}m$	1.46	CMP, IND
	$OP^{OH\text{-}SLF}v$	0.46	
	$OP^{DTT}m$	13.39**	CMP, CHI, BON, STL, IND
	$OP^{DTT}v$	0.51	
	$OP^{OH\text{-}DTT}m$	3.52*	CHI, STL, IND, BON, CMP
	$OP^{OH\text{-}DTT}v$	4.00**	CHI, STL, IND, BON, CMP
Winter 2018	$OP^{AA}m$	2.21	CMP, CHI, STL, IND, BON
	$OP^{AA}v$	1.95	CMP, STL, IND, BON
	$OP^{GSH}m$	15.75**	CMP, CHI, STL, IND, BON
	$OP^{GSH}v$	12.37**	CMP, CHI, STL, IND, BON
	$OP^{OH\text{-}SLF}m$	2.23	CMP, CHI
	$OP^{OH\text{-}SLF}v$	1.78	STL, BON
	$OP^{DTT}m$	4.33**	CMP, STL, IND
	$OP^{DTT}v$	3.23*	CHI, STL, IND, BON
	$OP^{OH\text{-}DTT}m$	2.60*	IND, BON, STL
	$OP^{OH\text{-}DTT}v$	2.49*	CHI, IND, STL, CMP
Spring 2019	$OP^{AA}m$	5.20**	CMP, CHI, STL, IND, BON
	$OP^{AA}v$	4.92**	CMP, CHI, STL, IND, BON
	$OP^{GSH}m$	14.59**	CMP, CHI, STL, IND, BON
	$OP^{GSH}v$	10.74**	CMP, CHI, STL, IND, BON
	$OP^{OH\text{-}SLF}m$	3.20*	CMP, CHI, STL, IND, BON
	$OP^{OH\text{-}SLF}v$	3.19*	CMP, CHI, STL, IND, BON
	$OP^{DTT}m$	10.78**	CMP, CHI, BON, STL
	$OP^{DTT}v$	6.04**	CMP, CHI, STL, IND, BON
	$OP^{OH\text{-}DTT}m$	2.57*	IND, BON, CMP
	$OP^{OH\text{-}DTT}v$	1.89	STL, IND, CMP

Asterisks - * and ** indicate significant ($P < 0.05$) and highly significant ($P < 0.01$) differences, respectively.

Table S5. Results of 1-way ANOVA test for assessing the temporal and spatial variability of mass-normalized and volume-normalized OP endpoints for methanol-soluble $PM_{2.5}$ samples.

(a) Temporal variability

Sampling Site	Endpoint	F value	Significantly different group(s)
Chicago, IL (CHI)	$OP^{AA}m$	1.03	
	$OP^{AA}v$	0.07	
	$OP^{GSH}m$	1.41	
	$OP^{GSH}v$	0.28	
	$OP^{OH\text{-}SLF}m$	1.68	Summer 2018, Spring 2019
	$OP^{OH\text{-}SLF}v$	0.99	
	$OP^{DTT}m$	4.27*	Summer 2018, Fall 2018, Winter 2019
	$OP^{DTT}v$	1.53	
	$OP^{OH\text{-}DTT}m$	3.84*	Summer 2018, Fall 2018, Winter 2018, Spring 2019
	$OP^{OH\text{-}DTT}v$	3.37*	Summer 2018, Fall 2018
St. Louis, MO (STL)	$OP^{AA}m$	2.16	Fall 2018, Spring 2019
	$OP^{AA}v$	3.41*	Summer 2018, Fall 2018, Spring 2019
	$OP^{GSH}m$	3.62*	Fall 2018, Summer 2018, Winter 2018, Spring 2019
	$OP^{GSH}v$	1.92	Fall 2018, Spring 2019
	$OP^{OH\text{-}SLF}m$	1.05	
	$OP^{OH\text{-}SLF}v$	1.23	
	$OP^{DTT}m$	1.14	
	$OP^{DTT}v$	1.87	Summer 2018, Winter 2019
	$OP^{OH\text{-}DTT}m$	0.50	
	$OP^{OH\text{-}DTT}v$	1.11	
Indianapolis, IN (IND)	$OP^{AA}m$	2.42	Summer 2018, Spring 2019
	$OP^{AA}v$	1.39	
	$OP^{GSH}m$	2.15*	Fall 2018, Spring 2019
	$OP^{GSH}v$	0.63	
	$OP^{OH\text{-}SLF}m$	3.49*	Fall 2018, Spring 2019, Winter 2018
	$OP^{OH\text{-}SLF}v$	2.41	Fall 2018, Winter 2018
	$OP^{DTT}m$	1.42	
	$OP^{DTT}v$	0.94	
	$OP^{OH\text{-}DTT}m$	0.20	
	$OP^{OH\text{-}DTT}v$	0.67	
Champaign, IL (CMP)	$OP^{AA}m$	1.64	Summer 2018, Winter 2018
	$OP^{AA}v$	2.95*	Summer 2018, Fall 2018, Winter 2018
	$OP^{GSH}m$	1.42	
	$OP^{GSH}v$	0.03	
	$OP^{OH\text{-}SLF}m$	1.00	
	$OP^{OH\text{-}SLF}v$	1.22	
	$OP^{DTT}m$	3.73*	Summer 2018, Winter 2018
	$OP^{DTT}v$	2.93*	Summer 2018, Fall 2018, Winter 2018
	$OP^{OH\text{-}DTT}m$	0.08	
	$OP^{OH\text{-}DTT}v$	0.59	
Bondville, IL (BON)	$OP^{AA}m$	8.76**	Summer 2018, Fall 2018, Spring 2019, Winter 2018
	$OP^{AA}v$	9.27**	Summer 2018, Fall 2018, Spring 2019, Winter 2018
	$OP^{GSH}m$	1.51	
	$OP^{GSH}v$	1.58	Summer 2018, Winter 2018
	$OP^{OH\text{-}SLF}m$	4.30**	Summer 2018, Spring 2019, Winter 2018
	$OP^{OH\text{-}SLF}v$	4.70**	Summer 2018, Spring 2019, Winter 2018
	$OP^{DTT}m$	2.95*	Summer 2018, Spring 2019, Winter 2018
	$OP^{DTT}v$	4.28**	Summer 2018, Fall 2018, Spring 2019, Winter 2018
	$OP^{OH\text{-}DTT}m$	2.24	
	$OP^{OH\text{-}DTT}v$	1.64	

(b) Spatial variability

Season	Endpoint	F value	Significantly different group(s)
Summer 2018	$OP^{AA}m$	1.17	BON, STL
	$OP^{AA}v$	0.13	
	$OP^{GSH}m$	2.00	CMP, STL, IND
	$OP^{GSH}v$	0.40	
	$OP^{OH\text{-}SLF}m$	2.80*	CHI, CMP, IND, STL
	$OP^{OH\text{-}SLF}v$	1.67	CHI, CMP, IND
	$OP^{DTT}m$	0.74	
	$OP^{DTT}v$	0.46	
	$OP^{OH\text{-}DTT}m$	3.75**	CHI, STL, CMP
	$OP^{OH\text{-}DTT}v$	3.11*	CHI, IND, STL, CMP
Fall 2018	$OP^{AA}m$	0.62	
	$OP^{AA}v$	2.40	STL, CMP, BON
	$OP^{GSH}m$	2.55*	CMP, STL, BON, IND
	$OP^{GSH}v$	1.05	
	$OP^{OH\text{-}SLF}m$	0.81	
	$OP^{OH\text{-}SLF}v$	0.97	
	$OP^{DTT}m$	0.33	
	$OP^{DTT}v$	2.50*	STL, CMP, BON
	$OP^{OH\text{-}DTT}m$	1.99	IND, STL, CMP
	$OP^{OH\text{-}DTT}v$	2.28	IND, CMP, BON
Winter 2018	$OP^{AA}m$	1.06	
	$OP^{AA}v$	3.62**	CHI, STL, IND, BON
	$OP^{GSH}m$	6.31**	CMP, CHI, BON, STL, IND
	$OP^{GSH}v$	2.86*	CHI, CMP, IND, BON
	$OP^{OH\text{-}SLF}m$	1.79	CHI, BON, STL
	$OP^{OH\text{-}SLF}v$	3.21*	CHI, IND, CMP, STL, BON
	$OP^{DTT}m$	0.86	
	$OP^{DTT}v$	2.45*	CHI, STL, CMP, BON
	$OP^{OH\text{-}DTT}m$	2.21	IND, CMP, BON, STL
	$OP^{OH\text{-}DTT}v$	2.67*	CHI, IND, CMP, BON
Spring 2019	$OP^{AA}m$	1.60	
	$OP^{AA}v$	2.46*	CHI, CMP, BON
	$OP^{GSH}m$	7.44**	CMP, CHI, IND, STL
	$OP^{GSH}v$	4.33**	CMP, CHI, BON, IND, STL
	$OP^{OH\text{-}SLF}m$	0.46	
	$OP^{OH\text{-}SLF}v$	0.60	
	$OP^{DTT}m$	0.79	
	$OP^{DTT}v$	1.93	CHI, BON
	$OP^{OH\text{-}DTT}m$	2.15	BON, IND, CMP
	$OP^{OH\text{-}DTT}v$	1.63	IND, CMP

Asterisks - * and ** indicate significant ($P < 0.05$) and highly significant ($P < 0.01$) differences, respectively.

Table S6. Comparison of ambient $PM_{2.5}$ OP measured in our current study with those reported in the literatures. Asterisk - * indicates that the reported results are methanol-soluble OP, while all the other results (without the asterisk) are water-soluble OP.

(a) OP^{AA}

Reference	PM size (μm)	Levels	Location	Location type	Sample size	Methodology
Fang et al. (2016)	≤ 2.5	0.2 - 5.2 nmol·min^{-1}·m^{-3}	Southeast US	Urban and rural	483	Ambient $PM_{2.5}$ samples were collected using a Hi-Vol sampler on quartz filters, extracted in DI and filtered through a syringe filter. OP^{AA} of filtered extracts was assessed with an AA-only assay (no other antioxidants involved; concentration of AA was 200 μM) with an automated system. AA was measured based on a photometric method (at 265 nm).
Mudway et al. (2005)	≤ 2.5	0.012 ± 0.0001 nmol·min^{-1}·μg^{-1}	Eksaal, India	Biomass burning	3	Biomass burning samples were collected from dung-cake combustion, and extracted in Chelex-treated DI with 5% methanol. OP^{AA} of filtered extracts was assessed in a respiratory tract lining fluid (RTLF; composition was 200 μM AA, 200 μM GSH and 200 μM UA). AA was measured based on a photometric method (at 265 nm).
Künzli et al. (2006)	≤ 2.5	0.0096 ± 0.0025 nmol·min^{-1}·μg^{-1}	19 European cities	Urban	716	Ambient $PM_{2.5}$ samples were collected using a Basel-Sampler, and extracted in metal-free DI. OP^{AA} was assessed in the same manner as Mudway et al. (2005).
Szigeti et al. (2016)	≤ 2.5	0.0017 – 0.04 nmol·min^{-1}·μg^{-1}	8 European cities	Urban	22	Ambient and indoor $PM_{2.5}$ samples were collected using a Low-Vol sampler, and directly incubated in RTLF having same composition as in Mudway et al. (2005). AA was measured based on a photometric method (at 265 nm).
Godri et al. (2011)	1.0 – 1.9	0.0058 ± 0.0025 nmol·min^{-1}·μg^{-1}	London, United Kingdom	Urban	14	Ambient size-segregated samples were collected using a MOUDI sampler, and extracted in Chelex-treated DI with 5% methanol. OP^{AA} was assessed in the same manner as Mudway et al. (2005).

Perrone et al. (2019)	≤ 2.5	0.006 ± 0.001 nmol·min^{-1}·μg^{-1} 0.136 ± 0.020 nmol·min^{-1}·m^{-3}	Lecce, Italy	Urban	39	Ambient $PM_{2.5}$ samples were collected using a low volume HYDRA-FAI dual sampler, and extracted in DI. OP^{AA} of filtered extracts was assessed with an AA-only assay similar as in Fang et al. (2016).
Gao et al. (2020a)	≤ 2.5	0.023 – 0.126 nmol·min^{-1}·m^{-3}	Atlanta, GA	Urban	349	Ambient $PM_{2.5}$ samples were collected using a Hi-Vol sampler on quartz filters, extracted in DI and filtered through a syringe filter. OP^{AA} was assessed in the same manner as Mudway et al. (2005).
Yang et al. (2014)	≤ 2.5	0.8 – 35.0 nmol·s^{-1}·m^{-3}	Rotterdam and Amsterdam, Netherland	Urban	10	Ambient $PM_{2.5}$ samples were collected using a Harvard Impactor and extracted in ultrapure water. OP^{AA} of filtered extracts was assessed AA-only assay similar as in Fang et al. (2016).
Yu et al. (2020)	≤ 2.5	0.004 – 0.077 nmol·min^{-1}·μg^{-1} median: 0.012 nmol·min^{-1}·μg^{-1} 0.044 – 0.745 nmol·min^{-1}·m^{-3} median: 0.160 nmol·min^{-1}·m^{-3}	Midwest US (5 sites)	Urban (4), rural (1)	54	$PM_{2.5}$ sampling, preparation and OP^{AA} measurement were conducted in the same manner as the current study.
Yang et al. (2014)*	≤ 2.5	2.2 – 43.5 nmol·s^{-1}·m^{-3}	Rotterdam and Amsterdam, Netherland	Urban	20	Ambient $PM_{2.5}$ samples were collected using a Harvard Impactor and extracted in methanol. Filtered methanol extracts were evaporated using an evaporator set, and reconstituted with DI. OP^{AA} of water-reconstituted methanol extracts was assessed AA-only assay similar as in Fang et al. (2016).
This study	≤ 2.5	0.002 – 0.077 nmol·min^{-1}·μg^{-1} median: 0.007 nmol·min^{-1}·μg^{-1} 0.012 – 0.908 nmol·min^{-1}·m^{-3} median: 0.078 nmol·min^{-1}·m^{-3}	Midwest US (5 sites)	Urban (4), rural (1)	241	See section 2 (experimental methods).
This study*		0.004 – 0.029 nmol·min^{-1}·μg^{-1} median: 0.012 nmol·min^{-1}·μg^{-1} 0.030 – 0.311 nmol·min^{-1}·m^{-3} median: 0.134 nmol·min^{-1}·m^{-3}	Midwest US (5 sites)	Urban (4), rural (1)	241	

Asterisk - * indicates that the reported results are methanol-soluble OP^{AA}.

(b) OP^{GSH}

Reference	PM size (μm)	Levels	Location	Location type	Sample size	Methodology
Mudway et al. (2005)	≤ 2.5	0.0083 ± 0.0002 nmol·min^{-1}·μg^{-1}	Eksaal, India	Biomass burning	3	OP^{GSH} of filtered extracts was measured in RTLF. GSH was measured with a glutathione disulfide (GSSG)-reductase-5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) recycling assay, based on a photometric method (at 405 nm).
Künzli et al. (2006)	≤ 2.5	0.0041 ± 0.0017 nmol·min^{-1}·μg^{-1}	19 European cities	Urban	716	OP^{GSH} was assessed in the same manner as Mudway et al. (2005).
Szigeti et al. (2016)	≤ 2.5	0 – 0.0275 nmol·min^{-1}·μg^{-1}	8 European cities	Urban	22	Punches of filter samples were directly incubated in RTLF, and measured for OP^{GSH} in the same manner with Mudway et al. (2005).
Godri et al. (2011)	1.0 – 1.9	0.0042 ± 0.0033 nmol·min^{-1}·μg^{-1}	London, United Kingdom	Urban	14	OP^{GSH} was assessed in the same manner as Mudway et al. (2005).
Gao et al. (2020a)	≤ 2.5	0.025 – 0.067 nmol·min^{-1}·m^{-3}	Atlanta, GA	Urban	349	OP^{GSH} was assessed in the same manner as Mudway et al. (2005).
Yu et al. (2020)	≤ 2.5	0.001 – 0.040 nmol·min^{-1}·μg^{-1} median: 0.010 nmol·min^{-1}·μg^{-1} 0.008 – 0.463 nmol·min^{-1}·m^{-3} median: 0.100 nmol·min^{-1}·m^{-3}	Midwest US (5 sites)	Urban (4), rural (1)	54	$PM_{2.5}$ sampling, preparation and OP^{GSH} measurement were conducted in the same manner as the current study.
This study	≤ 2.5	0.002 – 0.035 nmol·min^{-1}·μg^{-1} median: 0.007 nmol·min^{-1}·μg^{-1} 0.013 – 0.419 nmol·min^{-1}·m^{-3} median: 0.074 nmol·min^{-1}·m^{-3}	Midwest US (5 sites)	Urban (4), rural (1)	241	See section 2 (experimental methods).

(c) $OP^{OH\text{-}SLF}$

Reference	PM size (μm)	Levels	Location	Location type	Sample size	Methodology
Vidrio et al. (2009)	≤ 2.5	0.253 ± 0.135 pmol·min^{-1}·μg^{-1}	Davis, CA	Urban	~90	Ambient $PM_{2.5}$ samples were collected using IMPROVE Version II samplers on Teflo filters, directly incubated in SLF (composition was 114 mM NaCl, 10 mM sodium benzoate, 10 mM total phosphate to buffer the solution at pH 7.4, 200 μM AA and 300 μM CA) with desferoxamine (DSF) for 24 hours, and measured for ·OH generation. ·OH was captured by sodium benzoate and measured based on a photometric method (at 256 nm) using a high-performance liquid chromatography (HPLC).
Ma et al. (2015)	≤ 2.5	0.092 ± 0.019 pmol·min^{-1}·μg^{-1}	Guangzhou, China	Urban	72	Ambient $PM_{2.5}$ samples were collected using a Low-Vol sampler on Teflon filters. $OP^{OH\text{-}SLF}$ was measured in the same manner as in Vidrio et al. (2009).
Yu et al. (2020)	≤ 2.5	0.085 – 0.967 pmol·min^{-1}·μg^{-1} median: 0.307 pmol·min^{-1}·μg^{-1} 0.857 – 7.884 pmol·min^{-1}·m^{-3} median: 3.559 pmol·min^{-1}·m^{-3}	Midwest US (5 sites)	Urban (4), rural (1)	54	$PM_{2.5}$ sampling, preparation and $OP^{OH\text{-}SLF}$ measurement were conducted in the same manner as the current study.
This study	≤ 2.5	0.040 – 1.217 pmol·min^{-1}·μg^{-1} median: 0.142 pmol·min^{-1}·μg^{-1} 0.269 – 12.13 pmol·min^{-1}·m^{-3} median: 1.449 pmol·min^{-1}·m^{-3}	Midwest US (5 sites)	Urban (4), rural (1)	241	See section 2 (experimental methods).

(d) OP^{DTT}

Reference	PM size (μm)	Levels	Location	Location type	Sample size	Methodology
Fang et al. (2015)	≤ 2.5	0.010 – 0.097 nmol·min^{-1}·μg^{-1} median: 0.024 – 0.041 nmol·min^{-1}·μg^{-1} 0.05 – 0.81 nmol·min^{-1}·m^{-3} median: 0.23 – 0.31 nmol·min^{-1}·m^{-3}	Southeast US	Urban and rural	503	Ambient $PM_{2.5}$ samples were collected using a Hi-Vol sampler on quartz filters, extracted in DI and filtered through a syringe filter. Filtered extracts were then incubated in a mixture of 100 μM DTT and 0.5 mM potassium phosphate buffer (K-PB; pH = 7.4). DTT was captured by DTNB and measured based on a photometric method (at 412 nm) using an automated system.
Xiong et al. (2017)	≤ 2.5	0.1 – 0.18 nmol·min^{-1}·m^{-3}	Urbana, IL	Urban	10	Ambient $PM_{2.5}$ samples were collected with Hi-Vol sampler on quartz filters, extracted in Milli-Q water, and filtered through a syringe filter. OP^{DTT} were assessed in the same manner with Fang et al. (2015).
Cho et al. (2005)	≤ 2.5	0.013 – 0.047 nmol·min^{-1}·μg^{-1} median: 0.029 nmol·min^{-1}·μg^{-1}	Los Angeles basin, CA	Urban	11	Ambient size-segregated samples were collected using a VACES in conjunction with a BioSampler. Collected suspensions were then incubated in a mixture of 100 μM DTT and 0.5 mM potassium phosphate buffer (K-PB; pH = 7.4). DTT was captured by DTNB and measured based on a photometric method (at 412 nm) at designated time points within 90 min.
Charrier and Anastasio (2012)	≤ 2.5	0.02 – 0.061 nmol·min^{-1}·μg^{-1} median: 0.029 nmol·min^{-1}·μg^{-1}	San Joaquin, CA	Urban, rural	6	Ambient $PM_{2.5}$ samples were collected on Teflon filters, but the filter extraction method was not reported. DTT assay was conducted by incubating the aqueous sample extracts in 100 μM DTT. DTT was captured by DTNB and measured based on a photometric method (at 412 nm) at four time points within 16 min.
Gao et al. (2017)	≤ 2.5	0.09 – 0.30 nmol·min^{-1}·m^{-3} median: 0.19 nmol·min^{-1}·m^{-3}	Atlanta, GA (2 sites)	Urban	66	$PM_{2.5}$ sampling, preparation and OP^{DTT} measurement were conducted in the same manner as

						Fang et al. (2015).
Gao et al. (2020a) and Gao et al. (2020b)	≤ 2.5	0.005 – 0.070 nmol·min^{-1}·μg^{-1} average: 0.024 nmol·min^{-1}·μg^{-1} 0.05 – 0.48 nmol·min^{-1}·m^{-3} average: 0.22 nmol·min^{-1}·m^{-3}	Atlanta, GA	Urban	349	$PM_{2.5}$ sampling, preparation and OP^{DTT} measurement were conducted in the same manner as Fang et al. (2015).
Hu et al. (2008)	0.25 – 2.5	0.014 – 0.024 nmol·min^{-1}·μg^{-1} median: 0.019 nmol·min^{-1}·μg^{-1} 0.10 – 0.16 nmol·min^{-1}·m^{-3} median: 0.14 nmol·min^{-1}·m^{-3}	Los Angeles harbor, CA	Urban	6	Ambient size-segregated samples were collected with Sioutas samplers on Zefluor and Quartz filters, and extracted in Milli-Q water. DTT assay was conducted by incubating the PM suspensions in 100 μM DTT at pH = 7.4 adjusted by K-PB. DTT was captured by DTNB and measured based on a photometric method (at 412 nm) at designated time points within 30 min.
Cesari et al. (2019)	≤ 2.5	0.012 ± 0.008 nmol·min^{-1}·μg^{-1} 0.19 ± 0.10 nmol·min^{-1}·m^{-3}	Sarno, Italy	Urban	~50	Ambient $PM_{2.5}$ samples were collected using a Low-Vol sequential sampler on quartz filters, extracted in DI and filtered through a syringe filter. DTT assay was conducted by incubating the extracts in DTT (concentration not reported) at pH = 7.4 adjusted by K-PB. DTT was captured by DTNB and measured based on a photometric method (at 412 nm) at designated time points (details not reported).
Paraskevopoulou et al. (2019)	≤ 2.5	0.028 ± 0.014 nmol·min^{-1}·μg^{-1} 0.33 ± 0.20 nmol·min^{-1}·m^{-3}	Athens, Greece	Urban	361	Ambient $PM_{2.5}$ samples were collected using a Dichotomous Partisol sampler on quartz filters, extracted in DI and filtered through a syringe filter. OP^{DTT} was assessed in the same manner as Fang et al. (2015).
Perrone et al. (2019)	≤ 2.5	0.010 ± 0.001 nmol·min^{-1}·μg^{-1} 0.228 ± 0.024 nmol·min^{-1}·m^{-3}	Lecce, Italy	Urban	39	Ambient $PM_{2.5}$ samples were collected using a low volume HYDRA-FAI dual sampler, and extracted in DI. DTT assay was conducted by incubating the aqueous sample extracts in 100 μM DTT. DTT was captured by DTNB and measured based on a photometric method (at 412 nm) at five time points within 40 min.

Yang et al. (2014)	≤ 2.5	0.4 – 7.2 nmol·s^{-1}·m^{-3}	Rotterdam and Amsterdam, Netherland	Urban	10	Ambient $PM_{2.5}$ samples were collected using a Harvard Impactor and extracted in ultrapure water. OP^{DTT} of water-soluble extracts was assessed in the same manner as Hu et al. (2008).
Yu et al. (2020)	≤ 2.5	0.004 – 0.193 nmol·min^{-1}·μg^{-1} median: 0.014 nmol·min^{-1}·μg^{-1} 0.041 – 1.282 nmol·min^{-1}·m^{-3} median: 0.146 nmol·min^{-1}·m^{-3}	Midwest US (5 sites)	Urban (4), rural (1)	54	$PM_{2.5}$ sampling, preparation and OP^{DTT} measurement were conducted in the same manner as the current study.
Verma et al. (2012)*	≤ 2.5	0.020 – 0.054 nmol·min^{-1}·μg^{-1} median: 0.034 nmol·min^{-1}·μg^{-1}	Atlanta, GA	Urban	8	Ambient $PM_{2.5}$ samples were collected using a Hi-Vol sampler on quartz filters, extracted in both methanol and water, and filtered through a syringe filter. Methanol extracts were evaporated to nearly dryness using a rotary evaporator and reconstituted to 15 mL with 0.1 M K-PB (pH = 7.4). Reconstituted methanol extracts were incubated in 100 µM DTT and 0.5 M K-PB (pH = 7.4). DTT was captured by DTNB and measured based on a photometric method (at 412 nm) at seven time points within 20 min.
Gao et al. (2017)*	≤ 2.5	0.14 – 0.47 nmol·min^{-1}·m^{-3} median: 0.30 nmol·min^{-1}·m^{-3}	Atlanta, GA (2 sites)	Urban	66	Method 1: Ambient $PM_{2.5}$ samples were extracted in a stepwise manner with DI and methanol. Both extracts were filtered through a syringe filter. Methanol extracts were evaporated to ~200 µL using high-purity nitrogen and reconstituted with DI. Total OP was calculated by adding the OP of both extracts. Method 2: Samples were extracted in methanol. Punches were removed after sonication. The remaining suspensions were analyzed for OP^{DTT} without being filtered through a syringe filter. Method 3: Samples were sonicated in K-PB (pH = 7.4). The mixture was analyzed for OP^{DTT} without removing inside punches or being filtered through a syringe filter. OP^{DTT} measurement was conducted in the same

						manner as Fang et al. (2015) using a modified automated system for analyzing suspensions with insoluble fractions.
Gao et al. (2020b)*	≤ 2.5	0.012 – 0.116 nmol·min^{-1}·μg^{-1} average: 0.027 nmol·min^{-1}·μg^{-1} 0.13 – 0.58 nmol·min^{-1}·m^{-3} average: 0.28 nmol·min^{-1}·m^{-3}	Atlanta, GA	Urban	349	$PM_{2.5}$ sampling, preparation and OP^{DTT} measurement were conducted in the same manner as Gao et al. (2017) (Method 3).
Yang et al. (2014)*	≤ 2.5	0.5 – 5.2 nmol·min^{-1}·m^{-3}	Rotterdam and Amsterdam, Netherland	Urban	20	Ambient $PM_{2.5}$ samples were collected using a Harvard Impactor and extracted in methanol. Filtered methanol extracts were evaporated using an evaporator set, and reconstituted with DI. OP^{DTT} of water-reconstituted methanol-soluble extracts was assessed in the same manner as Hu et al. (2008).
This study	≤ 2.5	0.004 – 0.032 nmol·min^{-1}·μg^{-1} median: 0.014 nmol·min^{-1}·μg^{-1} 0.029 – 0.561 nmol·min^{-1}·m^{-3} median: 0.150 nmol·min^{-1}·m^{-3}	Midwest US (5 sites)	Urban (4), rural (1)	241	See section 2 (experimental methods).
This study*	≤ 2.5	0.004 – 0.042 nmol·min^{-1}·μg^{-1} median: 0.021 nmol·min^{-1}·μg^{-1} 0.031 – 0.639 nmol·min^{-1}·m^{-3} median: 0.234 nmol·min^{-1}·m^{-3}	Midwest US (5 sites)	Urban (4), rural (1)	241	

Asterisk - * indicates that the reported results are methanol-soluble OP^{DTT}.

(e) $OP^{OH\text{-}DTT}$

Reference	PM size (μm)	Levels	Location	Location type	Sample size	Methodology
Xiong et al. (2017)	≤ 2.5	0.2 – 0.6 pmol·min^{-1}·m^{-3}	Urbana, IL	Urban	10	$PM_{2.5}$ extracts were incubated in 100 μM DTT and K-PB (pH = 7.4) with 50 mM TPT. ·OH was captured by TPT and measured based on a fluorometric method (excitation/emission wavelength of 310/425 nm) at six time points within 120 min.
Yu et al. (2018)	≤ 2.5	0.2 – 1.1 pmol·min^{-1}·m^{-3}	Urbana, IL	Urban	10	$PM_{2.5}$ sampling, preparation and $OP^{OH\text{-}DTT}$ measurement were conducted in the same manner as Xiong et al. (2017).
Yu et al. (2020)	≤ 2.5	0.034 – 0.357 pmol·min^{-1}·μg^{-1} median: 0.082 pmol·min^{-1}·μg^{-1} 0.360 – 4.152 pmol·min^{-1}·m^{-3} median: 1.054 pmol·min^{-1}·m^{-3}	Midwest US (5 sites)	Urban (4), rural (1)	54	$PM_{2.5}$ sampling, preparation and $OP^{OH\text{-}DTT}$ measurement was conducted in the same manner as the current study.
This study	≤ 2.5	0.004 – 0.357 pmol·min^{-1}·μg^{-1} median: 0.065 pmol·min^{-1}·μg^{-1} 0.022 – 3.565 pmol·min^{-1}·m^{-3} median: 0.722 pmol·min^{-1}·m^{-3}	Midwest US (5 sites)	Urban (4), rural (1)	241	See section 2 (experimental methods).

Table S7. Seasonal median of the ratio of methanol-soluble OPv to water-soluble OPv (M/W^{OP}) for $OP^{OH\text{-}SLF}v$ at five sampling sites.

	CHI	STL	IND	CMP	BON
Summer 2018	2.1	2.6	2.0	1.1	2.0
Fall 2018	3.5	4.9	5.5	2.7	4.6
Winter 2018	9.4	2.9	3.3	3.2	3.9
Spring 2019	3.2	2.7	7.2	4.1	3.9

References

Cesari, D., Merico, E., Grasso, F. M., Decesari, S., Belosi, F., Manarini, F., De Nuntiis, P., Rinaldi, M., Volpi, F., and Gambaro, A.: Source apportionment of PM2. 5 and of its oxidative potential in an industrial suburban site in South Italy, *Atmosphere*, 10, 758, 2019.

Charrier, J., and Anastasio, C.: On dithiothreitol (DTT) as a measure of oxidative potential for ambient particles: evidence for the importance of soluble transition metals, *Atmospheric Chemistry and Physics*, 12, 11317-11350, 10.5194/acp-12-9321-2012, 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, *Environmental Research*, 99, 40-47, 10.1016/j.envres.2005.01.003, 2005.

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 Southeastern Center for Air Pollution and Epidemiology (SCAPE), *Atmospheric Measurement Techniques*, 8, 471-482, 10.5194/amt-8-471-2015, 2015.

Fang, T., Verma, V., Bates, J. T., Abrams, J., Klein, M., Strickland, M. J., Sarnat, S. E., Chang, H. H., Mulholland, J. A., and Tolbert, P. E.: Oxidative potential of ambient water-soluble $PM_{2.5}$ in the southeastern United States: contrasts in sources and health associations between ascorbic acid (AA) and dithiothreitol (DTT) assays, *Atmospheric Chemistry and Physics*, 16, 3865-3879, 10.5194/acp-16-3865-2016, 2016.

Gao, D., Fang, T., Verma, V., Zeng, L., and Weber, R. J.: 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, *Atmospheric Measurement Techniques*, 10, 2821, 2017.

Gao, D., Godri Pollitt, K. J., Mulholland, J. A., Russell, A. G., and Weber, R. J.: Characterization and comparison of PM 2.5 oxidative potential assessed by two acellular assays, *Atmospheric Chemistry and Physics*, 20, 5197-5210, 2020a.

Gao, D., Mulholland, J. A., Russell, A. G., and Weber, R. J.: Characterization of water-insoluble oxidative potential of $PM_{2.5}$ using the dithiothreitol assay, *Atmospheric Environment*, 224, 117327, https://doi.org/10.1016/j.atmosenv.2020.117327, 2020b.

Godri, K. J., Harrison, R. M., Evans, T., Baker, T., Dunster, C., Mudway, I. S., and Kelly, F. J.: Increased oxidative burden associated with traffic component of ambient particulate matter at roadside and urban background schools sites in London, *PloS One*, 6, e21961, 10.1371/journal.pone.0021961, 2011.

Hu, S., Polidori, A., Arhami, M., Shafer, M., Schauer, J., Cho, A., and Sioutas, C.: Redox activity and chemical speciation of size fractioned PM in the communities of the Los Angeles-Long Beach harbor, *Atmospheric Chemistry and Physics*, 8, 6439-6451, 10.5194/acp-8-6439-2008, 2008.

Künzli, N., Mudway, I. S., Götschi, T., Shi, T., Kelly, F. J., Cook, S., Burney, P., Forsberg, B., Gauderman, J. W., and Hazenkamp, M. E.: Comparison of oxidative properties, light absorbance, and total and elemental mass concentration of ambient $PM_{2.5}$ collected at 20 European sites, *Environmental Health Perspectives*, 114, 684-690, 10.1289/ehp.8584, 2006.

Ma, S., Ren, K., Liu, X., Chen, L., Li, M., Li, X., Yang, J., Huang, B., Zheng, M., and Xu, Z.: Production of hydroxyl radicals from Fe-containing fine particles in Guangzhou, China, *Atmospheric Environment*, 123, 72-78, 10.1016/j.atmosenv.2015.10.057, 2015.

Mudway, I. S., Duggan, S. T., Venkataraman, C., Habib, G., Kelly, F. J., and Grigg, J.: Combustion of dried animal dung as biofuel results in the generation of highly redox active fine particulates, *Particle and Fibre Toxicology*, 2, 6, 10.1186/1743-8977-2-6, 2005.

Paraskevopoulou, D., Bougiatioti, A., Stavroulas, I., Fang, T., Lianou, M., Liakakou, E., Gerasopoulos, E., Weber, R., Nenes, A., and Mihalopoulos, N.: Yearlong variability of oxidative potential of particulate matter in an urban Mediterranean environment, *Atmospheric Environment*, 206, 183-196, 2019.
Perrone, M. R., Bertoli, I., Romano, S., Russo, M., Rispoli, G., and Pietrogrande, M. C.: PM2. 5 and PM10 oxidative potential at a Central Mediterranean Site: Contrasts between dithiothreitol-and ascorbic acid-measured values in relation with particle size and chemical composition, *Atmospheric Environment*, 210, 143-155, 2019.
Szigeti, T., Dunster, C., Cattaneo, A., Cavallo, D., Spinazzè, A., Saraga, D. E., Sakellaris, I. A., de Kluizenaar, Y., Cornelissen, E. J., and Hänninen, O.: Oxidative potential and chemical composition of $PM_{2.5}$ in office buildings across Europe–The OFFICAIR study, *Environment International*, 92, 324-333, 10.1016/j.envint.2016.04.015, 2016.
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, *Environmental Science & Technology*, 46, 11384-11392, 10.1021/es302484r, 2012.
Vidrio, E., Phuah, C. H., Dillner, A. M., and Anastasio, C.: Generation of hydroxyl radicals from ambient fine particles in a surrogate lung fluid solution, *Environmental Science & Technology*, 43, 922-927, 10.1021/es801653u, 2009.
Xiong, Q., Yu, H., Wang, R., Wei, J., and Verma, V.: Rethinking the dithiothreitol-based particulate matter oxidative potential: measuring dithiothreitol consumption versus reactive oxygen species generation, *Environmental Science & Technology*, 51, 6507-6514, 10.1021/acs.est.7b01272, 2017.
Yang, A., Jedynska, A., Hellack, B., Kooter, I., Hoek, G., Brunekreef, B., Kuhlbusch, T. A., Cassee, F. R., and Janssen, N. A.: Measurement of the oxidative potential of $PM_{2.5}$ and its constituents: The effect of extraction solvent and filter type, *Atmospheric Environment*, 83, 35-42, 10.1016/j.atmosenv.2013.10.049, 2014.
Yu, H., Puthussery, J. V., and Verma, V.: A semi-automated multi-endpoint reactive oxygen species activity analyzer (SAMERA) for measuring the oxidative potential of ambient $PM_{2.5}$ aqueous extracts, *Aerosol Science and Technology*, 54, 304-320, 2020.