Response to Anonymous Referee #1

In this work, the authors designed a novel instrument to measure oxidative potential in ambient PM2.5 on an hourly timescale. They use a mist chamber to collect particles and direct subject these particles to reaction with dithiothreitol, a model antioxidant to infer the ability of inhaled particles to cause oxidative stress. It represents an important improvement to previous filter-based methods, and while the new measurements show good agreement with measurements made using these methods, they were substantially higher. The instrument is well characterized and carefully designed. I have only one major concern about this manuscript, but I recommend publication in AMT after addressing this and other minor concerns.

We thank the reviewer for his/her recommendation on the acceptance. Our responses and corresponding changes made in the manuscript (highlighted in red) are given below.

Major comment:

1.My only major concern is the section on comparison to composition measurements. The comparison was performed on two different time periods. While the authors claim that the composition should be similar between these two time periods, there is also no reason to believe they would be similar. For example, the diurnal variation in DTT activity may be heavily driven by a few anomalous days during July 4/5. This section is considerably weaker than the characterization tests described in the other sections. I also do not see why this comparison needs to be made in this manuscript. I believe it is sufficient to demonstrate the time-resolved capability of this instrument for this manuscript. I suggest removing this section, or re-writing this section, and performing the comparison experiments much more carefully in the future.

There seems to be a misunderstanding on the comparison study conducted in this paper which we hope to clear through our response below.

The PM chemical composition data was collected for a period of 10 "weekdays" between August 3 – August 16, 2017. The diurnal profile of the ambient $PM_{2.5}$ extrinsic DTT activity (OP_{ex}) obtained during this period is also provided in Figure S4 (supplementary information). Since, the diurnal profile of DTT activity shown in Figure S4 was similar to the diurnal profile obtained during June 2017 (i.e. Figure 4a in the manuscript) (Page 23, line 1), we did not feel it necessary to include both the figures in the main manuscript. Now, given we performed only a ***qualitative comparison*** (e.g. high during the afternoon, low at night) between the DTT activity and chemical components, comparing the chemical composition data with either profile (i.e. either during August 3 - August 16, 2017 or June 2017) will essentially yield the same conclusion as reported in the manuscript.

Please note that we agree with the reviewer that during the Independence Day celebration week (July 4 - 5), the composition of the PM was probably very different, and the diurnal OP trend would have been significantly influenced by these "anomalous days". *Therefore, we did not include the DTT activity data collected during that period for plotting the diurnal profile in Figure 4*. Rather, we have used the data only from May 31 to July 2, 2017. This has been explained in the caption of Figure 4 and the associated text:

Page 23, line 5

"Figure 4. Diurnal profile of the ambient $PM_{2.5}$ DTT activity measured at the sampling site over (a) weekdays (n = 18 days), (b) weekends (n = 10 days). The data from May 31 to July 2, 2017 were used for plotting this profile. Error bars are the standard deviation (1σ) of the average DTT activity in that hour."

Page 10, lines 19 - 23

"One of the primary motivations for developing the online instrument was to discern the diurnal variations in ambient $PM_{2.5}$ OP_{ex}, so that it could be better linked with the chemical components and their emission sources. Therefore, hourly data obtained by running the instrument for 28 days (between May 31 and July 2, 2017) was composited to obtain a diurnal profile of the DTT activity as shown in Fig.4. The diurnal profiles were separately plotted for the weekdays and weekends in Fig.4a and 4b, respectively..."

Although, the comparison was only qualitative, we believe it adds valuable contribution to the manuscript by providing some insights into the possible emission sources, which could impact the DTT activity. Therefore, we would like to keep this section in the manuscript, hoping the reviewer is now clear on it.

Other comments:

2. Abstract: It is confusing to read that the measurements made by MC are higher than filter-based methods, and then see slopes of less than 1. I suggest reporting the slope of MC vs filter-based method.

We have made the changes suggested by the reviewer

Page 1, lines 12 – 15

"The online DTT activity measurements correlated well with the offline measurements but were higher than both methanol (slope = 1.08, $R^2 = 0.93$) and Milli-Q water (slope = 1.86, $R^2 = 0.86$) extracts of the PM filters, indicating a better efficiency of MC for collecting the water-insoluble fraction of PM."

Page 9, lines 10 – 17

"The slope for the water extraction versus online measurement was 1.86 ($R^2 = 0.86$; $p < 0.001$; N = 20), while methanol extracts of the PM filters showed a much better agreement with the online system (slope = 1.08; $R^2 = 0.93$; $p < 0.001$; N = 16). This is probably due to significant contribution from some water-insoluble PM components in MC, which otherwise remain embedded in the filter fibers and are poorly extracted in water by sonication. Recently, Gao et al. (2017) also reported a ratio of the DTT activity measured on the water-soluble extracts of the ambient PM filters collected from Atlanta to the total OP_{ex} (i.e. DTT oxidation performed directly on the filter) as 1.54 – 1.72. Additionally, the ratio of the DTT activity measured on methanol-soluble extracts of the filters to the total OP_{ex} was 1.06 - 1.1."

Page 13, lines 19 – 20

"However, the online instrument yielded higher DTT activity than both water (slope = 1.86, N = 20) and methanol (slope = 1.08, N = 16) extracted filters."

Figure 2 has been modified as:

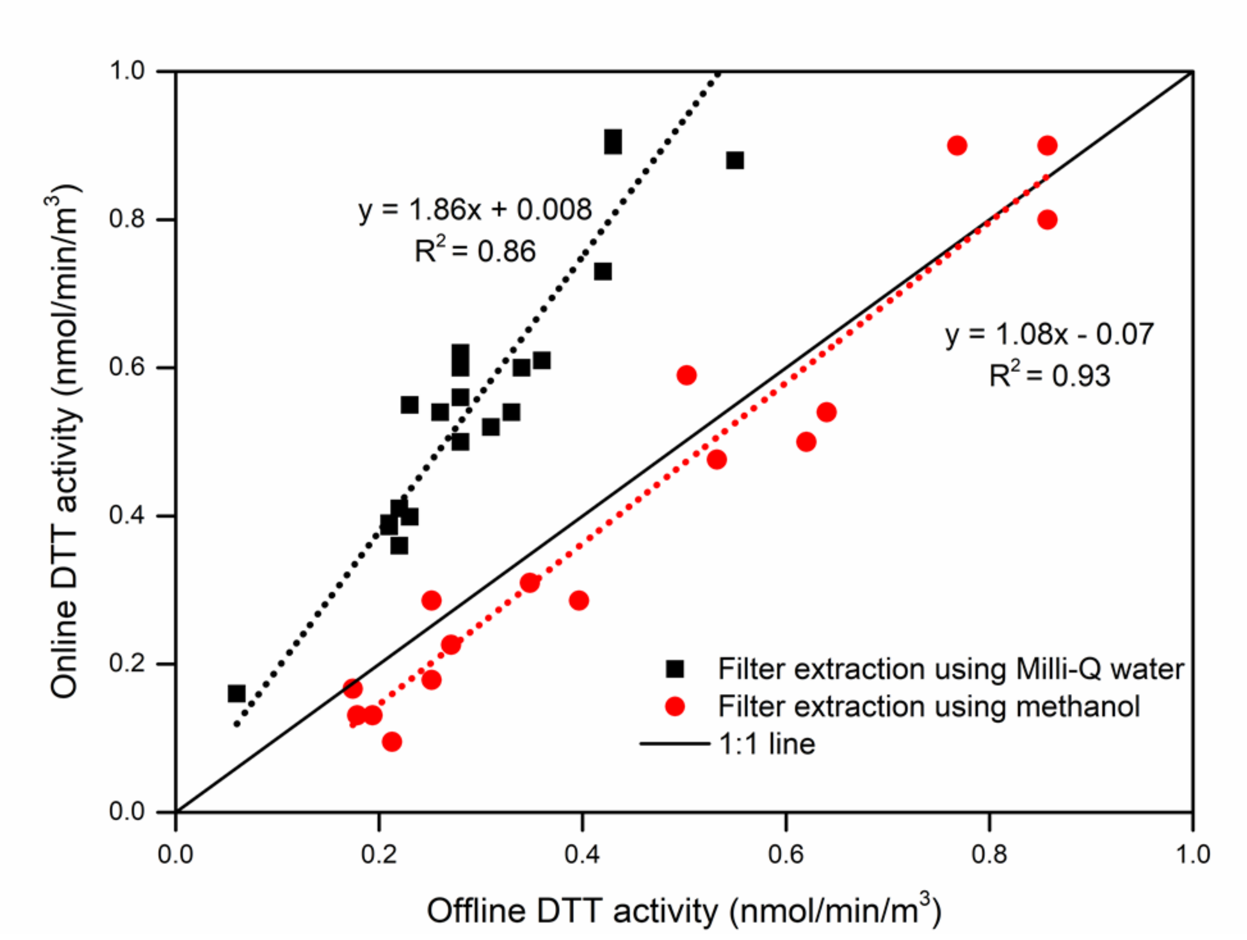

Figure 2. Comparison of the DTT activity obtained from the online instrument with the traditional filter collection and extraction methodology (i.e. offline DTT activity analysis of the PM extracts)

3. Introduction: The authors claim that the MC is substantially cheaper (of ~150 USD) is a vast overstatement. The cost of machining the MC alone would be greater than 150 USD. Also, the authors should consider that university facilities are highly subsidized and the cost that researchers see may be substantially lower than the true cost.

We agree with the reviewer. Our intention here was to compare the MC system with other existing sample collection systems. Therefore, we have omitted the "low cost" term from the manuscript and removed the cost comparison discussion with other online systems.

Page 3, lines 16 - 21

"Here, we discuss the development of an automated online instrument to measure the hourly averaged OP of ambient $PM_{2.5}$ using DTT assay and its evaluation in the field conditions for over 50 days. A custom-built glass MC was used for collecting the ambient PM suspension, which is then transferred to an automated analytical system for DTT activity determination."

4.Section 3.2: The tests described in this section are very appropriate. I believe that researchers in this field have largely neglected the importance of reactant concentration in determining reaction rates. Assuming a simple bimolecular reaction between DTT and PM, there should be a linear relationship between reactant concentration and measured DTT consumption, even at the same PM mass. What is puzzling me is that the authors only observe a 6% change for a 20% change in volume (or concentration). Do the authors have any explanation for this small change? Does this suggest that the DTT reaction with PM is not bimolecular?

Please see our detailed response to a similar comment raised by another reviewer (anonymous referee 3 comment 9). For the specific comment of the reviewer on bimolecular reaction between DTT and PM, we would like to clarify that the reaction is not between DTT and PM but rather between DTT and oxygen (which is in excess), and PM acts as a catalyst (Cho et al., 2005; Sauvain and Rossi, 2016). The various chemical components in PM, e.g. Cu, Mn, oxygenated organic compounds and quinones have a distinct (and not necessarily linear) response function with the DTT oxidation rate and can be considered as the particle's characteristics (see Charrier and Anastasio, 2012; Sauvain and Rossi, 2016). Recently, Yu et al. (2018) also reported potential synergistic and antagonistic effects of different chemical components in the PM on its DTT response. Thus, the total DTT activity of a PM sample is due to the combined response function from its various components. Furthermore, note that PM extract accounts for only 70% of the total volume in the reaction vial. That means, a 20% volume increase in the PM extract would result only into a 14% increase in the reaction volume, and a 12 % decrease in the concentration of DTT. The initial concentration of DTT also affects its self-oxidation rate as shown by Sauvain and Rossi (2016). Given these complexities, we tried to empirically derive the effect of change in the sample volume on the DTT activity measurements by conducting the experiments using four different PM samples. The difference in the DTT activity measurement bias (shown as error bars in Figure S3) is probably due to differences in the chemical composition of these samples. However, our main objective for performing this experiment was to determine the maximum acceptable variation in the sample volume from MC that leads to a negligible bias in the DTT activity measurement. We found that for a 20% variation in the sample volume, the % bias in DTT activity measurement was less than 6 %; which was neglected for the purpose of this study. We cannot comment on the DTT assay mechanism based on this experiment. A more systematic study with different standard chemicals and a larger sample set of ambient PM samples is probably needed to investigate and provide an exact reaction mechanism of PM in DTT assay, which is beyond the scope of the current study.

5. Section 3.4: Is there any evidence of water insoluble components in MC samples? For example, can the MC samples be filtered and the DTT measurements be repeated? Which is more biologically relevant? I can imagine the insoluble components remain in the lung lining fluid and continue to consume antioxidants.

There is no direct evidence for the collection of water-insoluble components in MC samples. MCs have traditionally been designed to collect the ambient water-soluble PM components and gases. Our study is probably the first to indicate the collection of insoluble PM components in MC. Although, we have not performed any experiments after filtering the MC samples, we observed the DTT activity of these samples to be almost similar to the DTT activity measured after extracting the filters in methanol (Figure 2). Based on this, we have concluded that MC was probably collecting part of the water-insoluble PM fraction, which is contributing to the DTT consumption. We acknowledge the reviewer's suggestion of filtering the MC sample, and we will attempt to include it in our future investigations for a systematic determination of the contribution of insoluble fraction by comparing the OP of filtered and unfiltered MC PM suspensions (i.e. running 2 MCs in parallel), but it is practically infeasible to include this analysis in the current manuscript.

We are not in position to answer the second part of the reviewer's comment on the relative importance of soluble versus insoluble components. Although, it is biologically plausible that water-insoluble components remain in the lung lining fluid for a longer period and continue to consume antioxidants, this hypothesis has not been tested in the toxicological studies, which were traditionally more focused on assessing the water-soluble PM components. There are definitely growing evidences on the biological relevance of water-insoluble PM components with recent studies showing a substantial fraction of the PM OP stemming from the water-insoluble fraction (Akhtar et al., 2010; McWhinney et al., 2011; Verma et al., 2012; Li et al., 2013; Gao et al., 2017). Therefore, it is important to account for this fraction when determining the overall OP of inhalable PM and incorporating it in the toxicological and epidemiological studies assessing the health effects of PM.

6. Section 3.6.1: The authors argue that EC, Cu and Fe are all coming from traffic sources. However, the diurnal patterns are not consistent with each other. EC is higher in the morning and at night, but Cu and Fe decreases substantially at night. The authors appear to be contradicting themselves here.

We disagree with the reviewer about the contradiction in our interpretation. EC is directly emitted from the vehicular exhausts (mainly from diesel vehicles) (Shah et al., 2004), while water soluble Cu and Fe are emitted from both exhausts and non-exhaust components of the vehicular emissions, i.e. brake and tire wear, and abrasion of the road surface (Thorpe and Harrison, 2008). In addition to that, resuspension of road dust, which is enhanced at drier environmental conditions, is another major source of the airborne metals (Wang et al., 2005; Thorpe and Harrison, 2008; Chen et al., 2012). Therefore, EC is mostly influenced by the traffic intensity and atmospheric mixing height (Lin et al., 2009; Mues et al., 2017), which is probably the reason for its peak at morning (traffic) and at night (low mixing height). In contrast, the ambient RH influences the road dust resuspension, yielding a different pattern in the metal concentration (highest during afternoon and lowest at night). This has been mentioned in the manuscript:

Page 13, lines 4 - 8

"The difference in the diurnal trends of metals and EC concentrations (i.e. EC peaks in the morning while metals peak in the afternoon) indicates a lesser influence of direct vehicular exhausts on the metals concentrations than the resuspended dust, which is generally driven by higher vehicular speeds (due to relatively lower traffic) and drier conditions in the afternoon (Pant and Harrison, 2013). Figure S6 in SI shows the diurnal pattern of ambient RH at the sampling site. The very high RH (> 75 %) substantially suppresses the dust resuspension and the resultant metals concentrations during nighttime."

7. Throughout the manuscript, the authors have used OP to describe DTT consumption rate per volume of air, whereas it is also often used to describe DTT consumption rate per mass of PM. I suggest defining it outright in the introduction. The per volume DTT consumption is often referred to as the "extrinsic" oxidative potential, or the oxidative capacity.

We agree with the reviewer here. We have replaced oxidative potential reported in our study as extrinsic DTT activity and denoted it as OP_{ex} throughout the manuscript. We have further defined it outright on section 2.2 (where we have discussed about normalizing it with the volume of air) and made changes in the abstract

Page 1, lines 8 - 9

"We developed an online instrument for measuring the extrinsic oxidative potential (OP) of ambient particulate matter (PM) using the dithiothreitol (DTT) assay."

We have added the following sentence in section 2.2:

"This DTT consumption rate was normalized by the volume of sampled air and reported in the units of nmol min^{-1} m^{-3}; referred hereafter as extrinsic DTT activity or OP_{ex}."

Minor technical comments:

Page 2 line 9: times

We have modified the sentence as:

Page 2, line 9

"These assays are laborious, complex and require long analysis times (Dungchai et al., 2013)."

Page 2 line 10: what does it mean by "less controlled environment". Cell-free assays would be conducted under a more well-controlled environment than cellular assays.

What we meant here is that cell-free assays are easier to perform, but we agree with the reviewer that this sentence is confusing. Therefore, we have deleted the term “less controlled environment”.

The revised sentence reads as:

Page 2, lines 10

“Cell free assays, on the other hand, are easier to perform and provide faster estimation of OP (Fang et al., 2015).”

Page 2 line 34: the word “data” is plural; remove “a”

Thank you for pointing out this error. We have made the suggested changes in the manuscript.

Page 2, lines 34

“Moreover, it requires relatively long sampling duration, i.e. at least six hours to collect enough volume of the concentrated slurry, making this approach unsuitable for obtaining highly time-resolved OP data for the ambient particles.”

Page 5 line 14: “the” analytical part

Thank you for pointing out this error. We have made the suggested changes in the manuscript

Page 5, line 14

“While the analytical part of the instrument measured the DTT activity of a given PM suspension, the MC simultaneously collected a new PM suspension.”

Page 5 line 30: “the” analytical part

Thank you for pointing out this error. We have made the suggested changes in the manuscript

Page 5, line 30

“The instrument performance was assessed by calibrating the analytical part (DTT activity determination) of the instrument using positive controls...”

Page 7 line 19: replace “reduces” with “is reduced” or “decreases”

We have modified the sentence as:

Page 7, line 19

“During air sampling, the volume of water inside the MC decreases due to evaporative loss.”

Page 7 line 31: “The second concern”

Thank you for pointing out this error. We have made the suggested changes in the manuscript.

Page 7, line 31

“The second concern associated with the evaporation of water is the variable volume of the PM suspensions collected after different sampling runs depending on the ambient…”

Section 3.3: It may be more useful to report LOD in terms of oxidative capacity in nmol DTT/(min-m3 air)

We have reported the LOD in units of nmol/min, because it is consistent with earlier studies using automated (Fang et al., 2015) and online DTT activity measurement systems (Eiguren-Fernandez et al., 2017) and would help the readers to make an easy comparison with those systems.

LOD in the units of nmol/min/m^3 is operation-specific because it can be adjusted by changing the sampling duration in MC. For example, in an environment, where the PM is less redox active, more mass needs to be collected for the DTT activity analysis by increasing the sampling duration in MC. Therefore, we prefer to report the LOD in the same units (nmol/min) as used by other researchers.

Page 9 line 24: "the time series"

We have made the changes suggested by the reviewer

Page 9, line 24

"Figure 3 shows the time-series of the hourly ambient $PM_{2.5}$ DTT activity (blank-corrected) measured by the online instrument between May 31, 2017 and August 16, 2017."

Section 3.6.1: EC might be more a marker of diesel traffic

We agree with the reviewer's comment and have included this point in the manuscript.

Page 11, line 22

"EC can be assumed as a marker of exhaust emissions from diesel vehicles (Shah et al., 2004; Shirmohammadi et al., 2016)."

Page 11 line 24: awkward language: "getting elevated"

We have modified the sentence as:

Page 11, line 24

"It then subsides in the afternoon and remains constant till evening, before increasing again at night."

Page 12 line 33: there may be too many significant digits for Fe concentrations

We have modified the sentence as:

Page 12, line 33

"However, the concentration of Fe (3.7 - 6.9 ng/m^3) was significantly lower than reported in other studies"

Page 24 line 6: again, the word "data" is plural; replace "was" with "were"

Thank you for pointing out this error. We have made the suggested changes in the manuscript.

Page 24, line 6

“Figure 4. Diurnal profile of the ambient $PM_{2.5}$ DTT activity measured at the sampling site over (a) weekdays (n = 18 days), (b) weekends (n = 10 days). The data from May 31 to July 2, 2017 were used for plotting this profile. Error bars are the standard deviation (1σ) of the average DTT activity in that hour.”

References

Akhtar, U. S., McWhinney, R. D., Rastogi, N., Abbatt, J. P. D., Evans, G. J. and Scott, J. A.: Cytotoxic and proinflammatory effects of ambient and source-related particulate matter (PM) in relation to the production of reactive oxygen species (ROS) and cytokine adsorption by particles, Inhal. Toxicol., doi:10.3109/08958378.2010.518377, 2010.

Charrier, J. G. and Anastasio, C.: On dithiothreitol (DTT) as a measure of oxidative potential for ambient particles: Evidence for the importance of soluble \newline transition metals, Atmos. Chem. Phys., 12(19), 9321–9333, doi:10.5194/acp-12-9321-2012, 2012.

Charrier, J. G., McFall, A. S., Vu, K. K. T., Baroi, J., Olea, C., Hasson, A. and Anastasio, C.: A bias in the “mass-normalized” DTT response – An effect of non-linear concentration-response curves for copper and manganese, Atmos. Environ., 144, 325–334, doi:10.1016/j.atmosenv.2016.08.071, 2016.

Chen, J., Wang, W., Liu, H. and Ren, L.: Determination of road dust loadings and chemical characteristics using resuspension, Environ. Monit. Assess., 184(3), 1693–1709, doi:10.1007/s10661-011-2071-1, 2012.

Cho, A. K., Sioutas, C., Miguel, A. H., Kumagai, Y., Schmitz, D. A., Singh, M., Eiguren-Fernandez, A. and Froines, J. R.: Redox activity of airborne particulate matter at different sites in the Los Angeles Basin, Environ. Res., 99(1), 40–47, doi:10.1016/j.envres.2005.01.003, 2005.

Eiguren-Fernandez, A., Kreisberg, N. and Hering, S.: An online monitor of the oxidative capacity of aerosols (o-MOCA), Atmos. Meas. Tech., 10(2), 633–644, doi:10.5194/amt-10-633-2017, 2017.

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), Atmos. Meas. Tech., 8(1), 471–482, doi:10.5194/amt-8-471-2015, 2015.

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, Atmos. Meas. Tech., 10(8), 2821–2835, doi:10.5194/amt-10-2821-2017, 2017.

Li, Q., Shang, J. and Zhu, T.: Physicochemical characteristics and toxic effects of ozone-oxidized black carbon particles, Atmos. Environ., 81, 68–75, doi:10.1016/j.atmosenv.2013.08.043, 2013.

Lin, P., Hu, M., Deng, Z., Slanina, J., Han, S., Kondo, Y., Takegawa, N., Miyazaki, Y., Zhao, Y.

and Sugimoto, N.: Seasonal and diurnal variations of organic carbon in PM2.5 in Beijing and the estimation of secondary organic carbon, J. Geophys. Res., 114, D00G11, doi:10.1029/2008JD010902, 2009.

McWhinney, R. D., Gao, S. S., Zhou, S. and Abbatt, J. P. D.: Evaluation of the Effects of Ozone Oxidation on Redox-Cycling Activity of Two-Stroke Engine Exhaust Particles, Environ. Sci. Technol., 45(6), 2131–2136, doi:10.1021/es102874d, 2011.

Mues, A., Rupakheti, M., Münkel, C., Lauer, A., Bozem, H., Hoor, P., Butler, T. and Lawrence, M. G.: Investigation of the mixing layer height derived from ceilometer measurements in the Kathmandu Valley and implications for local air quality, Atmos. Chem. Phys., 17(13), 8157–8176, doi:10.5194/acp-17-8157-2017, 2017.

Pant, P. and Harrison, R. M.: Estimation of the contribution of road traffic emissions to particulate matter concentrations from field measurements: A review, Atmos. Environ., 77, 78–97, doi:10.1016/j.atmosenv.2013.04.028 Review, 2013.

Sauvain, J. J. and Rossi, M. J.: Quantitative Aspects of the Interfacial Catalytic Oxidation of Dithiothreitol by Dissolved Oxygen in the Presence of Carbon Nanoparticles, Environ. Sci. Technol., 50(2), 996–1004, doi:10.1021/acs.est.5b04958, 2016.

Shah, S. D., Cocker, D. R., Miller, J. W. and Norbeck, J. M.: Emission Rates of Particulate Mailer and Elemental and Organic Carbon from In-Use Diesel Engines, Environ. Sci. Technol., 38(9), 2544–2550, doi:10.1021/es0350583, 2004.

Shirmohammadi, F., Hasheminassab, S., Wang, D., Schauer, J. J., Shafer, M. M., Delfino, R. J. and Sioutas, C.: The relative importance of tailpipe and non-tailpipe emissions on the oxidative potential of ambient particles in Los Angeles, CA, Faraday Discuss. Faraday Discuss, 189(189), 361–380, doi:10.1039/c5fd00166h, 2016.

Thorpe, A. and Harrison, R. M.: Sources and properties of non-exhaust particulate matter from road traffic: A review, Sci. Total Environ., 400(1–3), 270–282, doi:10.1016/j.scitotenv.2008.06.007, 2008.

Verma, V., Rico-Martinez, R., Kotra, N., King, L., Liu, J., Snell, T. W. and Weber, R. J.: Contribution of water-soluble and insoluble components and their hydrophobic/hydrophilic subfractions to the reactive oxygen species-generating potential of fine ambient aerosols, Environ. Sci. Technol., 46(20), 11384–11392, doi:10.1021/es302484r, 2012.

Wang, C.-F., Chang, C.-Y., Tsai, S.-F. and Chiang, H.-L.: Characteristics of road dust from different sampling sites in northern Taiwan., J. Air Waste Manag. Assoc., 55(8), 1236–44, doi:10.1080/10473289.2005.10464717, 2005.

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, Environ. Sci. Technol., acs.est.7b04261, doi:10.1021/acs.est.7b04261, 2018.