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Atmospheric Chemistry and Physics Discussions

Interactive comment on "Redox activity and chemical speciation of size fractioned PM in the communities of the Los Angeles – Long Beach Harbor" *by* S. Hu et al.

S. Hu et al.

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We appreciate the valuable comments from this reviewer. We have carefully considered the comments from the review and incorporate them accordingly.Please find our response to the reviewer as below in italic. We thank the reviewer's recommendation for the publication of this paper in ACP.

The manuscript describes size-segregated measurements of chemical composition and redox activity from particles collected in the Los Angeles - Long Beach Harbor area of Southern California. The chemical speciation information is highly aggregated, in part because it is apparently the subject of a separate recently submitted manuscript.

There are two measures of PM redox activity: a fluorescence assay that uses aqueous

extracts of PM in the presence of rat alveolar macrophages (ROS) and the dithiothreitol (DTT) oxidation assay used on particle suspensions. The redox activity measures are also highly aggregated, but there appears to be no reason for this.

There are some interesting observations in the manuscript, mainly the regressions between the redox measures and the chemical composition. But there is an outstanding question as to why the seven weeks of data were apparently combined to give one result for each endpoint in each PM size at each site. I can understand the need to combine daily samples to give weekly averages; but there is no apparent need to combine all of the weekly averages for a given parameter to give one 7-week average. This issue is not discussed in the manuscript, suggesting that the averages over the entire data set are hiding large amounts of variability from week to week.

The manuscript should be reworked so that the data analyses use the larger data set with weekly averages (and with explicitly stated measures of variability on the averages). In addition to this, I recommend that the points below be addressed before the manuscript is published in ACP.

Response: A detailed description of the chemical data has been presented in a separate paper (Arhami et al., 2008; Size-Segregated Inorganic and Organic Components of PM in the Communities of the Long Beach and Los Angeles Harbor. Aerosol Science and Technology, in press). The current paper presents a small excerpt from the work by Arhami et al. We have modified the method section to clarify the sampling and chemical analysis schedule (please refer to the response to the major comment 3). Although, we did not have enough mass to obtain weekly values of toxicological chemically speciated organic data, we do have weekly results for PM mass, EC/OC and inorganic ions. The weekly average values and its standard deviations for PM mass, EC/OC and inorganic ions are added in the SI in the revised manuscript. It should be noted that the concentration values used in this paper are recalculated using the accumulated mass divided by the corresponding air volume in 7-week periods. Hence, the values may be slightly different from the weekly average used in Arhami et al. (2008).

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Arhami et al. (2008) showed that the week-to-week variability of the concentrations of the PM mass and major chemical species measured at each site was not significant, and the result was mostly due to the stable meteorological conditions and the constant influence of vehicular sources over the entire sampling campaign.

As described in Arhami et al., (2008) "Averaged meteorological data over the sampling period were similar across the other sites, with the average temperature, relative humidity and wind speed varying in the ranges of 16.6-19.1 °C, 52-63% and 0.8-2.3 m/s, respectively. These meteorological data reaffirm the overall climatological stability of Los Angeles and show that weather conditions did not have a considerable effect on differences of the PM and its components between the sampling sites."

In summary, we don't think the week-to-week variability of the redox activity will be more informative than our current analysis.

Major Comments: 1. Supporting information. I cannot find the supporting information on the web: the address given in the text does not point to the supporting information and I cannot find it on the general ACPD web site. This is probably an oversight of the journal, but regardless of the cause, it makes it difficult to evaluate some of the statements in the manuscript.

Response: A copy of the Supplemental Information document was submitted to the ACP editorial office. We would be more than happy to submit the Supporting Info along with the revised manuscript.

2. Introduction. There is a large amount of literature on ROS generation by ambient and source particles, but most of the ROS references cited in the introduction are work of the USC/UCLA authors. It would be more reflective of the field to include some of the important papers from other groups, both for organics and metals as sources of ROS.

Response: We agree with the reviewer's comment and the following references have

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been added to the text.

Prahalad, A. K.; Soukup, J. M.; Inmon, J.; Willis, R.; Ghio, A. J.; Becker, S.; Gallagher, J. E., Ambient Air Particles: Effects on Cellular Oxidant Radical Generation in Relation to Particulate Elemental Chemistry. Toxicol. Appl. Pharmacol. 1999, 158, (2), 81-91.

Goldsmith, C. A. W.; Imrich, A.; Danaee, H.; Ning, Y.; Kobzik, L., Analysis of air pollution particulate-mediated oxidant stress in alveolar macrophages. Journal of Toxicology and Environmental Health-Part A 1998, 54, (7), 529-545.

Kumagai, Y.; Koide, S.; Taguchi, K.; Endo, A.; Nakai, Y.; Yoshikawa, T.; Shimojo, N., Oxidation of proximal protein sulfhydryls by phenanthraquinone, a component of diesel exhaust particles. Chemical Research in Toxicology 2002, 15, (4), 483-489.

Seagrave, J.; Mauderly, J. L.; Seilkop, S. K., In vitro relative toxicity screening of combined particulate and semivolatile organic fractions of gasoline and diesel engine emissions. Journal of Toxicology and Environmental Health-Part A 2003, 66, (12), 1113-1132.

Nel, A.; Diaz-Sanchez, D.; Li, N., The role of particulate pollutants in pulmonary inflammation and asthma: evidence for the involvement of organic chemicals and oxidative stress. Curr. Opin. Pulm. Med. 2001, 7, (1), 20-26.

3. Experimental methods. (a) More details are needed to describe the sampling and analysis frequencies. My understanding is that: (i) PM samples were collected each weekday for seven weeks. (ii) Each week's five days of PM samples were combined into a single weekly sample for chemical composition and redox activity measurements. Beyond this, it is difficult to determine what was done.

Response: Detailed information for the sampling and chemical analysis schedule has been described in Arhami et al 2008.In this present paper, the method section was revised to clarify the sampling schedule and the analyses frequencies.

"Weekly samples, collected on both the Teflon (Zefluor) and quartz fiber filters, were

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sectioned into four equal parts that were analyzed at the Wisconsin State Lab of Hygiene (University of Wisconsin-Madison) for several important inorganic and organic species. Two sets of Quartz composites were analyzed by the following methods: a) Ion Chromatography (IC), b) Thermal Evolution/Optical Transmittance (TOT) to determine the concentrations of inorganic ions (Sheesley et al.,2000), OC and elemental carbon (EC) (Turpin et al.,2000; Schauer,2003). The third set of the quartz fiber filters was composited for the whole 7-week period at each site and they were analyzed by Gas Chromatography/Mass Spectrometry (GC/MS) for organic species/tracers including PAHs, n- Alkanes, n-Alkanoic Acids, Resin Acids, Hopanes and Steranes (Zheng et al.,2002; Chowdhury et al.,2007), respectively. The fourth set of Quartz filters were archived for future analysis. Each set of the Zefluor filters were composited into one single sample, which represented the full 7-week sampling period at each site, and they were prepared for the following analysis: (a) Total Elements (b) Water Soluble Elements, and (c) Water Soluble OC (WSOC) and macrophage ROS, and (d) DTT assay. A magnetic sector inductively coupled plasma mass spectrometer (HR-ICPMS,Finnigan Element 2) was applied for the quantification of 52 trace elements (Herner et al.,2006) in the total digests and water extracts. Water extracts for TOC and ROS analysis were prepared by leaching the PM samples in 900 μL of Type 1 water for 16 hours with shaking[1]. A General Electric Instrument (Sievers Total Organic Carbon, TOC; GE, Inc.) was used to determine WSOC concentrations[1].”

(b) Were ROS/DTT measurements made on the weekly site/size composites? If so,why are there not 7 results for ROS and DTT for each PM size at each site? This would give 7 times more data than is presented in the paper, which presents 1 ROS and 1 DTT result for each PM size at each site. Are the ROS and DTT results averages of the 7 weeks of results? If so, why and how? It would be much better to have the additional data. More information is needed to explain what was done with the samples.

Response: The analysis schedule has been described above. The main text has been modified to clarify the rationale behind compositing the collected samples.

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(c) Measures of the variability for the chemical and redox endpoints (e.g., standard deviations) should be given.

Response: The standard deviations for PM mass and major chemical components have been added to Table S1 in the supporting information Because samples for ROS and DTT analyses at each site for each size-fraction were composited (as already discussed, toxicological activities were reported as time-integrated averages over a 7-week period), only the analytical uncertainty was reported for redox measurements. We don't think the analytical uncertainty will bring an informative message of the weekly variance, if there was any.

(d) Some additional information about the ROS technique would be useful, since the Landreman paper is submitted but currently unavailable. For example, what was the composition of the cell medium? How long were cells exposed? How was fluorescence measured?

Response: The paper by Landreman et al. (2008) cited is currently in press. The following sentences are added to the method section for ROS assay description.

"Experiments were performed with the rat alveolar cell line, NR8383, which were maintained in Hams F12 medium containing 2 mM L-glutamine supplemented with 1.176 g/L sodium bicarbonate and 15% heat inactivated fetal bovine serum. Cells were cultured at 37 °C in a humidified 5% CO_2 incubator and maintained by transferring non-adherent cells to new flasks weekly. Cultures were set up to contain a floating cell concentration of approximately 4 X 105 cells mL-1 of media. For the exposure experiments, cells were harvested and gently concentrated by centrifugation at 750 RPM for 5 minutes, the culture medium removed, and replaced with a salts-glucose-medium (SGM) to generate a cell suspension of 1,000 cells/μL. A 15 mM stock solution of 2'7'-dichlorodihydrofluorescein diacetate (DCFH-DA, Sigma), prepared in N,N'-dimethyl formamide, was diluted 10 fold in SGM just prior to use. One hundred μL of the macrophage cell suspensions were dispensed into each well of a 96 well plate

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and incubated at 37°C for two hours. Approximately 15 minutes before the end of the incubation period, the diluted DCFH-DA solution was added to each prepared sample extract to achieve a final concentration of 15 μM DCFH-DA. After the incubation period, during which time >98% of the cells settled and adhered to the well bottom, the SGM was pipetted off and immediately replaced with 100 μL of SGM-buffered sample extract or control sample. The fluorescence intensity in each well was determined at 450 ± 50 excitation and 530 ± 25 emission using a CytoFlour II automated fluorescence plate reader (PerSeptive Biosystems) at regular intervals throughout the exposure period (typically 2.5 hours). For each exposure experiment several untreated and method blank controls were included. Un-opsonized zymosan was included as a positive control. Each sample/dilution was run in triplicate (i.e. 3 wells each).”

(e) Some basic information about the DTT assay would be helpful. For example, how were PM suspensions prepared?

Response: More detailed information can be found in Li et al., 2003 and Cho et al., 2005 (referenced in the paper). The following sentences are added in the main text:

“In brief, the Zefluor filters were sonicated in Milli-Q water for 20 min. The filters were then removed and the aqueous particle suspension was used in the DTT assay. The PM suspension was incubated at 37 °C with DTT (100 μM) in potassium phosphate (0.1 M) buffer at pH 7.4 (1 mL total volume) for times varying from 0 to 30 minutes. Trichloroacetic acid (10%, 1 mL) was added to the incubation mixture to quench the reaction at preset times. An aliquot of the quenched reaction mixture was then mixed with Tris8211;HCl,(0.4 M,1 mL,pH 8.9) that contains EDTA (20 mM) and DTNB (10 mM,25 μL). The remaining DTT was measured by the formation of 5-mercapto-2-nitrobenzoic acid.”

(f) There needs to be some discussion of the blank filters for the two redox activity assays. Were blank filters used for both the ROS and DTT assays? What were the average blank/sample ratios for the different size fractions for the two assays? Were

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blank values subtracted from the sample results? Similarly, what were the blank data and blank treatment for the chemical composition measurements?

Response: the reported DTT activity data were blank subtracted (blanks were on other order of < 0.0005 nmoles/μg PM/ min). Blanks for the ROS analyses were below detection limit (Zhang et al 2008).

(g) Section 2.5 (Regressions). How were the data binned before doing the regressions? It appears that regressions were done on values averaged over the entire 7 weeks of collection. Why weren't weekly average values used? If the data is available, regressions with weekly average values should be done.

Response: Please refer to the response to the method section.

Table 1. (a) Need more details on what these data represent. Are these averages over the entire sampling campaign? How many samples went into each site/size average? (b) Some statistical description of each data point needs to be given: e.g., relative standard deviation (i.e., CV) for each average value, minimum and maximum values

Response: These are 7-week average data (one data point for each site/size-fraction). The text has been modified to clarify this point (refer to the response to the method section). Table S1 is added in the supporting information for the weekly average value and its standard deviation at each site.

Table 2. (a) It's somewhat surprising (and certainly interesting) that the correlation with Fe didn't meet the minimum threshold of significance. Given the wealth of literature on Fe-mediated ROS formation, including the Fe results in the text, and discussing them in the text, would be useful, even if there's no significant correlation with the ROS or DTT results. (b) Similarly, Cu and Mn are two other redox-active transition metals that have been linked to ROS generation by PM. Even though they are not significantly correlated with the two ROS measures here, it would be useful to include their results in the table.

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Interactive Comment

Response: Table S2 (Supporting Information) presents correlations between DTT/ROS and measured "total elements" (including Fe, Cu and Mn), and between DTT/ROS and measured "water-soluble elements" (including Fe, Cu and Mn).

Figure 3. It's surprising that there's no measure of uncertainty/variability on these values.Does each bar represent the average of the weekly composite samples? Should show error bars reflecting the standard deviation (or SE) for each bar. With these levels of variability, are the differences between sites significant?

Response: Since these results refer to the analysis of 7-week composite samples, it was not possible to estimate the week-to-week variability for these ROS/DTT activity data. We believe it is not necessary to include the analytical error associated with these values since they are not informative in terms of temporal variance. According to the weekly variance of the PM mass and major chemical components at each site and the stable meteorological conditions, we don't expect significant week-to-week variance of the PM ROS measurements during the period of the sampling campaign.

Figure 4. (a) This figure is too small to be legible. I suggest breaking it into 2 figures to make them larger. (b) The two outliers that were removed from the DTT and ROS regressions should be identified in the figure (e.g., by making them hollow symbols). (c) For both Al and Zn (and, to a lesser extent, Mo), the regressions (and high correlation coefficients) are driven by one point. Are these regressions still significant if this point is removed? Are there other regressions that are similarly driven by one (or two) points? I suggest that either Al or Zn be removed and replaced with Fe, since it has been identified as an important ROS-generating metal. Even though the Fe regression is not significant, it would be interesting to see the plot.

Response: Modified as suggested. a).The figures were split into two plots. b). Outliners were added to the figure as suggested. c). After removing the potential driven point (which is indeed a point with relative high fraction of Al in the PM sample) for the regression line, no significantly degradation of the correlation coefficient between Al

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and ROS (R = 0.63) and DTT (R = 0.55) was observed. Zn has been replaced with Fe in Figure 4.

Figure 5a. (a) This figure would be more convincing (and valuable) if there were any actual measurements of DTT consumption during this period. As is, it's an unsubstantiated guess. (b) The final sentence "..traffic emissions can increase the redox potential of airborne PM substantially" is too definitive for this "data". Better to qualify this.

Response: These are average predicted DTT values for May 2007 based on semi-continuous OC data. Measurements of the correspondent hourly DTT activities are not available. The final sentence was changed to "...suggesting that traffic emissions may increase the potential of airborne particles to induce oxidative stress on human cells."

Figure 5b. (a) I cannot find the supporting information, but from the text it appears that while OC was chosen as a predictor variable, other variables had more explanatory power for the ROS endpoint. Is this true? If so, how to justify the choice of OC? Compared to V, OC explains very little of the ROS result, but the authors treat it as if it were as important as V. For example, stating that the ROS response from PM is a result of vehicular traffic, in addition to ship emissions/oil combustion, puts the traffic component at an equal level, something it doesn't appear to deserve. (b) Worse, Figure 5b uses an average V concentration and only varies OC to get differences in ROS throughout the day. With this exercise the authors appear to have elevated OC to the dominant factor affecting ROS generation. (c) I recommend deleting Fig 5b and its description in the text.

Response: Agree with the reviewer. Figure 5b and its description in the text are deleted.

Miscellaneous points (a) Page 11647. It is difficult to assess what portion of the results described here are also included in the Arhami et al. manuscript submitted to AS&T (footnote 2). Since I don't have access to the AST submission, the editor should check to make sure that the overlap is minimal.

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Response: The information presented in this paper is only an excerpt from the results presented in Arhami et al., 2008.

(b) It would be useful to have a correlation matrix in the supporting information (i.e., where the correlation coefficient between each pair of measured variables is given).

Response: This was included in the Supplemental Information (Table S5).

(c) Page 11657, lines 23 - 25. ("We hypothesize..."). If Al is just a surrogate for PAHs, why not use PAH instead as an independent variable in the regression? How good is the regression model with PAHs instead of Al? Al is not redox active and so is unlikely to itself be contributing to ROS generation in this system.

Response: As stated in section 2.5 the multiple linear regression (MLR) model was designed to selected predictor variables after a series of analyses, including stepwise, forward, and backward elimination selections. PAHs were, indeed, a predictor of DTT after the initial screening analyses. However, inclusion of the PAHs could not generate the "best" predictive model for DTT.

(d) Page 11658, lines 4-6 ("It is possible..."). This is an easily answered question: run the regression model with OC and PAHs (and perhaps other organic measures) as independent variables and see how well it does predicting DTT activity.

Response: Please refer to the above response.

Minor Comments 1. Page 11648, line 3. What was the total air volume collected for each sample?

Response: The nominal air volume was 52 m^3 for the weekly sample.

2. Page 11649, line 3. How were "water soluble elements" extracted?

Response: Water-soluble components of the PM were released by leaching in 900 μL of Type 1 water for 16 hours with shaking. The Method section has been revised to illustrate this point (refer to the response to the methodology section above). Detailed

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information of the sample preparation and analysis can be seen in Zhang et al., (2008; referenced in the main text).

3. Page 11650, lines 4-6. The text states that "The electron transfer is monitored by the rate at which DTT is consumed under a standardized set of conditions and the rate is proportional to the concentration of the catalytically active redox-active species in the PM sample." This is likely not strictly true (that the rate is proportional to concentrations of redox-active species), since the DTT assay measures the sum of ROS generation from many different compounds, each likely with different efficiencies for electron transfer. For example, two separate samples with very different amounts of two redox-active species with very different efficiencies for DTT oxidation could give the same DTT result.

Response:We agree with the reviewer on his/her example. This sentence has been modified as "...the rate is proportional to the concentration of the catalytically active redox-active species in the PM sample as well as their rate constants for the reaction with DTT."

4. Page 11655, lines 16 - 19 ('The consumption of DTT...'). This statement implies that the biggest difference between the ROS and DTT assays is whether both soluble and insoluble components are included. But more important differences are that (1) the ROS method contains cells, which will affect redox cycling in the DCF method, and (2) it's likely that the mix of oxidants causing the response in the two methods are different (e.g., DTT is insensitive to metals, but the DCF/ROS method likely isn8217;t).

Response: We agree with the reviewer's comment. The following sentences have been added to this paragraph to illustrate the difference.

"DCFH is a broad spectrum ROS probe, directly responsive to most common reactive oxygen species, including the hydroxyl radical, peroxide, superoxide radical, and peroxynitrite radical, and therefore provides a more comprehensive, less targeted, assessment of the redox activity of PM. For example; the ROS produced by many redox

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active metals, will be addressed by the DCFH, while the DTT assay is relatively insensitive to this mechanism. In many respects the two assays are quite complementary. The DTT method is strictly a chemical probe, especially sensitive to many organic functionalities (e.g. quinines), while the DCFH approach, fundamentally a cell-based method, probes the general oxidative stress imposed by PM on a living organism."

5. Page 11656, lines 2-3 ("The species with..."). In contrast to this statement in the text, the significant correlations in Table 2 are not in bold.

Response: They are highlighted in the table.

6. Page 11656, lines 5 - 7 ("Nitrate and sulfate..."). But based on Table 1 it appears that in the Q-UF and accumulation modes that nitrate and sulfate are fully neutralized by ammonia. True?

Response: Yes, they are fully neutralized since they are weekly samples.

7. Page 11656, lines 24-28 ("We hypothesize..."). To help the reader evaluate this claim, the V and Ni levels in this sample should be given in the text.

Response: The concentration data has been added to the text. Water-soluble V and Ni levels are 4.5 and 1.2 ng/m^3 at SITE 5, respectively.

8. Figure 1. It appears that several of the averages are above 1. These numeric values should somehow be indicated in the figure or caption.

Response: The Y axis has been scaled up to illustrate the values above 1. A sentence has been added in the text: "It should be noted that a few elements (such as Cd, Zn and Na) showed a relative high water-soluble fraction greater than 1 (<1.6), which may be due to the analytical uncertainty."

Reference:

Chowdhury, Z., Zheng, M., Schauer, J. J., Sheesley, R. J., Salmon, L. G., Cass, G. R., and Russell, A. G.: Speciation of ambient fine organic carbon particles and source

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apportionment of PM2.5 in Indian cities, J Geophys Res, 112:D15.D1:D15303, 2007.

Herner, J. D., Green, P. G., and Kleeman, M. J.: Measuring the trace elemental composition of size-resolved airborne particles, Environ Sci Technol, 40, 1925-1933, 2006.

Schauer, J. J.: Evaluation of elemental carbon as a marker for diesel particulate matter, J. Expo. Anal. Environ. Epidemiol., 13, 443?453, 2003. Sheesley, R. J., Schauer, J. J., N.D., S., and M.D., H.: Development of a standardized method for the analysis of organic compounds present in PM2.5., : Procedings of AWMA Annual Meeting 2000, Salt Lake City, UT, 2000,

Turpin, B. J., Saxena, P., and Andrews, E.: Measuring and Simulating Particulate Organics in the Atmosphere: Problems and Propects., Atmos. Environ., 34, 2983-3013, 2000.

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., Environ. Sci. Technol., In Press, 2008

Zheng, M., Cass, G. R., Schauer, J. J., and Edgerton, E. S.: Source apportionment of PM2.5 in the southeastern United States using solvent-extractable organic compounds as tracers, Environ Sci Technol, 36, 2361-2371, 2002.

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