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

Ting Fang[1], *Vishal Verma*[2], *Josephine T. Bates*[3], *Joseph Abrams*[4], *Mitchel Klein*[4], *Matthew J. Strickland*[4], *Stefanie E. Sarnat*[4], *Howard H. Chang*[5], *James A. Mulholland*[3], *Paige E. Tolbert*[4], *Armistead G. Russell*[3], *Rodney J. Weber**[1]

[1] School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA, 30332, USA

[2] Department of Civil and Environmental Engineering, University of Illinois Urbana-Champaign, Champaign, IL, 61801, USA

[3] School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA

[4] Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA 30322, USA

[5] Department of Biostatistics and Bioinformatics, Rollins School of Public Health, Emory University, Atlanta, GA 30322, USA

*Corresponding author
Professor Rodney J. Weber, Ph.D.
Georgia Institute of Technology
School of Earth and Atmospheric Sciences
311 Ferst Drive Atlanta GA, 30332
office phone: 404.894.1750
Email: rodney.weber@eas.gatech.edu

Abstract

The ability of certain components of particulate matter to induce oxidative stress through the generation of reactive oxygen species (ROS) *in vivo* may be one mechanism accounting for observed linkages between ambient aerosols and adverse health outcomes. A variety of assays have been used to measure this so-called aerosol oxidative potential. We developed a semi-automated system to quantify oxidative potential of filter aqueous extracts utilizing the dithiothreitol (DTT) assay and report here the development of a similar semi-automated system for the ascorbic acid (AA) assay. Approximately 500 $PM_{2.5}$ filter samples collected in contrasting locations in the Southeastern US were analyzed for a host of aerosol species, along with AA and DTT activities. We present a detailed contrast in findings from these two assays. Water-soluble AA activity was higher in summer/fall than in winter, with highest levels near heavily trafficked highways, whereas DTT activity was higher in winter compared to summer/fall and more spatially homogeneous. AA activity was nearly exclusively correlated with water-soluble Cu (r = 0.70-0.94 at most sites), whereas DTT activity was correlated with organic and metal species. Source apportionment models, Positive Matrix Factorization (PMF) and a Chemical Mass Balance Method with ensemble-averaged source impact profiles (CMB-E), suggest a strong contribution from traffic emissions and secondary processes (e.g., organic aerosol oxidation or metals mobilization by secondary acids) to both AA and DTT activities in urban Atlanta. In contrast, biomass burning was a large source for DTT activity, but insignificant for AA. AA activity was not correlated with $PM_{2.5}$ mass, while DTT activity co-varied strongly with mass (r = 0.49-0.86 across sites/seasons). Various linear models were developed to estimate AA and DTT activities for the central Atlanta Jefferson Street site, based on the CMB-E sources. The models were then used to estimate daily oxidative potential at this site over the 1998-2009 period. Time-series epidemiological analyses were conducted to assess daily emergency department (ED) visits data for the five-county Atlanta metropolitan area based on the estimated 10-year backcast oxidative potential. Estimated AA activity was not statistically associated with any tested health outcome, while DTT activity was associated with ED visits for both asthma/wheeze and congestive heart failure. The findings point to the importance of both organic components and transition metals from biomass burning and mobile sources to adverse health outcomes in this region.

Key Words: water-soluble DTT activity; water-soluble AA activity; oxidative potential; source apportionment; health associations; cardiovascular; respiratory

1. Introduction

Studies have linked exposure to fine particulate matter ($PM_{2.5}$) with increased respiratory (Harkema et al., 2004; Aust, 2002; Schaumann et al., 2004) and cardiovascular (Pope et al., 2004; Samet et al., 2000) diseases. $PM_{2.5}$ consists of a wide range of chemical components of potentially varying toxicity, implying that $PM_{2.5}$ is not an ideal air quality metric for assessing health impacts. For example, components such as ammonium, sulfate, nitrate, chloride, and some chemical fraction of mineral dust, may be more benign than transition metals (Gasser et al., 2009; Kodavanti et al., 2005; Akhtar et al., 2010), black carbon (or elemental carbon and associated species) (Kleinman et al., 2007; Brunekreef et al., 1997), polycyclic aromatic hydrocarbons (PAHs) (Lundstedt et al., 2007; Burchiel et al., 2005), and other specific organic species (Nel et al., 2001). Although a small mass fraction of $PM_{2.5}$, these components could play a disproportionally large role in the overall adverse health effects of $PM_{2.5}$. A comprehensive set of mechanisms explaining the observed linkage between $PM_{2.5}$ mass and adverse health effects has not been established, but it has been hypothesized that one possible contributing physiological route is a particle's ability to induce oxidative stress via catalytic generation of reactive oxygen species (ROS) *in vivo*. A number of studies have associated particle oxidative capacity with PM toxicity (Donaldson et al., 2005; Nel, 2005; Shi et al., 2003; Zielinski et al., 1999), but without available large databases of ambient aerosol ROS, large population-based epidemiologic studies of $PM_{2.5}$ oxidative potential have been limited.

A number of different assays have been developed to quantify the oxidative potential of PM samples (Zomer et al., 2011; Mudway et al., 2011; Ayres et al., 2008; Jung et al., 2006; Cho et al., 2005; Mudway et al., 2005; Venkatachari et al., 2005). Two commonly used approaches are the dithiothreitol (DTT) assay (Cho et al., 2005) and the ascorbic acid (AA) assay (Ayres et al., 2008; Mudway et al., 2005). It may be expected that these two different assays respond to different aerosol components and are linked to different health endpoints. The AA assay has been shown to be most sensitive to transition metals (Janssen et al., 2014; Strak et al., 2012; DiStefano et al., 2009; Künzli et al., 2006), but quinone compounds may react with AA as well (Roginsky et al., 1999). For the DTT assay, identified DTT-active PM components are organic species, including water-soluble organic carbon (WSOC) (Verma et al., 2009; Cho et al., 2005), or of increasing specificity, HUmic-LIke Substances (HULIS) (Verma et al., 2012; Lin and Yu, 2011), and quinones (Chung et al., 2006; Kumagai et al., 2002) (a component of HULIS). Other studies, however, have emphasized the role of transition metals, such as Cu and Mn (Vejerano et al., 2015; Charrier and Anastasio, 2012). Some differences between studies may arise due to differing source characteristics of the specific regions studied.

Both assays involve incubating the anti-oxidant (DTT or AA) with filter aqueous extracts of $PM_{2.5}$ at a controlled temperature (37 °C) and pH (7.4), and measuring the depletion of the antioxidant over time, typically detected as a decrease in light absorption at certain wavelengths (412 and 265 nm for DTT and AA, respectively). The antioxidant loss rate is interpreted as a measure of the ability of aerosol redox-active species to catalytically transfer electrons from DTT or AA to oxygen (O_2). DTT can be considered a chemical surrogate to cellular reductants, such as NADH or NADPH, which reduces O_2 to superoxide anion ($O_2^{\cdot}$) and induces oxidative stress (Kumagai et al., 2002). Unlike DTT, AA is a physiological antioxidant in lung lining fluid, which prevents the oxidation of lipids and proteins (Valko et al., 2005). Asthmatic patients have markedly decreased concentration of AA in lung lining fluid compared to healthy control subjects (Kelly et al., 1999). Therefore, the *in vitro* oxidation of these two antioxidants by PM might represent the interaction of PM with biological antioxidants *in vivo* leading to the induction of oxidative stress and ultimately adverse health effects.

Among the various available methods for measuring oxidative potential, these two assays are relatively straightforward and reproducible, allowing high throughput routine measurements and the generation of large data sets for exploring links between aerosol components and health through epidemiology, or also as an initial screening step for identifying different redox components for more detailed cell or animal studies (Ayres et al., 2008).

We recently developed a semi-automated system (Fang et al., 2015b) to measure DTT activity and here describe its adaption to the AA assay. Utilizing our automated analytical system, we measured the water-soluble oxidative potential of over 500 filter samples collected as part of the Southeastern Center for Air Pollution & Epidemiology (SCAPE) study. Although insoluble components are important, we focus solely on the water-soluble AA and DTT activities since there is no current standard protocol for measuring the water-insoluble oxidative potential. We evaluate and compare these two assays in order to identify specific aerosol components the AA assay is responsive to. We perform a source apportionment analysis and assess these results through observed AA activity seasonal and spatial variability. AA source profiles are used to generate a model that estimates AA activities, which is then used to backcast AA levels over the past 10 years for use in a time-series epidemiological analysis in the Atlanta metropolitan area. Throughout, we compare the AA results to our previously published DTT findings (Bates et al., 2015, Fang et al., 2015b, Verma et al., 2014) to provide a contrast between these two commonly utilized assays to assess aerosol water-soluble oxidative potential and possible associations with health endpoints.

2. Methods

2.1. Sampling

Sampling methods have been described in detail elsewhere (Fang et al., 2015b; Verma et al., 2014). In brief, $PM_{2.5}$ (quartz filters, Pallflex® Tissuquartz™, 8 × 10 inches) was sampled at seven locations in the Southeastern US, with different source characteristics, using two sets of high-volume samplers (Hi-Vol) (Thermo Anderson, flow rate normally 1.13 m^3 min^{-1}). Sampling in the metropolitan Atlanta area was carried out from June 2012 through March 2013 (noon - 11 a.m., 23 hours) and involved paired-sites with one Hi-Vol sampler fixed at an urban background site (Jefferson Street, referred as JST) whilst the other sampler was deployed at three other sites on a monthly basis, and at least twice during different seasons. These three sites were: a rural site (Yorkville, YRK), a road-side site (RS, adjacent to the interstate highway I75/85), and a near-road site (GT, 840 m from the RS site). Following sampling in Atlanta, the two samplers were moved to Birmingham, AL (BHM, within a few kilometers of significant transportation and industrial sources) and rural Centerville, AL (CTR, surrounded by forests and a lightly traveled county road) for a month of sampling in June-July 2013, followed by one-month August sampling at East St. Louis, IL, an urban residential/light commercial area about 3 km east of the central business district of St. Louis, MO (Sauvain et al., 2008). Finally, a GT-RS pair was conducted in September 2013. A table providing the sampling schedule and a map can be found in the supporting material (Table S1 & Fig. S1). JST, YRK, BHM, and CTR are all part of the Southeastern Aerosol Research and Characterization Study (SEARCH) network sites (Hansen et al., 2003). Collected samples were immediately wrapped in prebaked aluminum foil and stored at −18°C until analyzed. DTT, water-soluble organic carbon, and brown carbon analyses on the filters were conducted within a year of sample collection, water-soluble elements were within a year and half, and AA measurements were conducted within two years of sample collection.

2.2. Oxidative potential measurement

Filter extraction: One punch of the collected Hi-Vol filter (5.07 cm^2) was extracted in 30 mL of deionized (DI) water (> 18 MΩ cm^{-1}) in a sterile polypropylene centrifuge tube (VWR International LLC, Suwanee, GA, USA) by sonication through partly immersing the tube an Ultrasonic Cleanser bath (VWR International LLC, West Chester, PA, USA) for half an hour. For those having activities close to blanks, 15 mL was used instead. Extracts were then filtered using PTFE 0.45 µm syringe filters (Fisherbrand^{TM}) to remove insoluble material larger than 0.45 µm. Although OH may form during sonication (Miljevic et al., 2014), it appears to have little effect on our ROS measurement since we compared the water-soluble ROS activities from the same sample that had been extracted by shaking for 3 hours vs sonication and found no significant differences (average ratio and standard deviation is 1.08 ± 0.20, n = 7).

AA determination: The method in this study was based on an ascorbate-only model (Mudway et al., 2005; Ayres et al., 2008), which is a simplified and alternative high throughput approach to AA analysis in a synthetic respiratory tract lining fluid model (RTLF) containing ascorbate, urate, and reduced glutathione (GSH) (Zielinski et al., 1999; Mudway et al., 2004). The method protocol is shown in Figure 1 and system setup can be found in the Figure. S2 in the Supplement. The method involves two steps.

Step 1: Aerosol background measurement (Fig. 1). In order to control for the contribution of absorbance of particles themselves at 265 nm wavelength, an AA-free control was measured and subtracted from the sample absorbance readings. 2.4 mL aerosol extracts and 0.3 mL 0.5 mM Kbuffer were loaded into a reaction vial (sterile polypropylene centrifuge tube, VWR International LLC, Suwanee, GA, USA) using a programmable syringe pump (A) with a 5 mL syringe (Kloehn, Inc., Las Vegas, NV, USA). Following mixing, 90 µL of the mixture was transferred to an intermediate vial using Pump B with a 250 µL syringe, and diluted to 3 mL. Pump A then withdrew the diluted mixture from the intermediate vial and pushed it through a Liquid Wave-guide Capillary Cell (LWCC-M-100; World Precision Instruments, Inc., FL, USA) with an optical path length of 100 mm. The waveguide was coupled to an online spectrophotometer, which included a UV-VIS light source (Ocean Optics DT-Mini-2, Ocean Optics, Inc., Dunedin, FL, USA), and a multi-wavelength light detector (USB4000 Miniature Fiber Optic Spectrometer, Ocean Optics, Inc., Dunedin, FL, USA). Aerosol background absorbance at 265 and 700 nm (baseline) were recorded at two-second intervals using data acquisition software (SpectraSuite). For the samples collected in this study, backgrounds due to the aerosol absorption at 265 nm were <10% of the sample absorbance readings. Prior to the second step, the system performed a self-cleaning by flushing the intermediate vial and the two syringes with DI water three times.

Step 2: AA measurement (Fig. 1). Following the aerosol background measurement, Pump A discarded a fraction of the sample-Kbuffer mixture and left only 1.8 mL in the reaction vial. 0.2 mL 2 mM AA solution was then loaded to the reaction vial using Pump B. Both the reaction and intermediate vial were continuously shaken at 400 rpm in a ThermoMixer (Eppendorf North America, Inc., Hauppauge, NY, USA), which also maintained the incubation temperature at

37 °C. At five different specified times (7, 15, 24, 32, 40 minutes), a small aliquot (100 µL) was transferred to the intermediate vial, diluted to 3 mL, and pushed through the LWCC, generating a total of five data points quantifying the remaining AA concentration. The system then again performed a self-cleaning before analyzing the next sample. A multi-position valve (14-port, VICI® Valco Instrument Co. Inc., USA) was used to select samples for analysis. To ensure the suspension of PM in the extract, each sample was mixed by pushing 5mL of air through the extract before loading to the reaction vial. The detailed Kloehn control program code for all steps can be found in the Supporting Information.

Final AA activity is calculated as follows:

$$\sigma AA = -\sigma Abs \times \frac{N_0}{Abs_0} \tag{1}$$

$$AAv = \frac{\sigma AA_s - \sigma AA_b}{\frac{V_a}{V_e} \times V_p} \tag{2}$$

Following the notation above, σAbs is the slope of absorbance versus time, where the absorbance is the absorbance of each time interval subtracting the corresponding aerosol background absorbance; Abs_0 is the initial absorbance calculated from the intercept of linear regression of absorbance versus time; N_0 is the initial moles of AA added in the reaction vial (400 nmol); σAA_s (σAA_b) is the rate of AA consumption for a sample (blank); V_e and V_a are the extraction volume (30 or 15 mL) and sample volume added to the reaction (1.6 mL), respectively. V_p is the ambient air volume (m^3) represented by the sample in the extraction volume. AAv represents volume normalized AA activity, in units of nmol min^{-1} m^{-3}. Similarly, here DTTv represents the volume normalized DTT activity.

2.3. Chemical analysis on PM filters

2.3.1. Water-soluble organic carbon and brown carbon

An automated system (details in Fig. S3) was used to measure water-soluble organic carbon (WSOC) and brown carbon (BrC) on the water-soluble extracts from the same Hi-Vol filters. Filter extracts (~6mL, same extraction protocol outlined above), after loading onto a 5 mL sample loop (Upchurch Scientific, Inc., Oak Harbor, WA), were first passed through a 1 m LWCC (LWCC-2100; World Precision Instruments, Inc., FL, USA), where absorbance at 365 nm wavelength (BrC) was measured using an online spectrophotometer (Ocean Optics, Inc., Dunedin, FL, USA). The extracts then entered a TOC analyzer (Sievers Model 900, GE Analytical Instruments, Boulder, CO, USA) for determining WSOC concentration.

2.3.2. Water-soluble elements

A similar automated system was developed to determine the water-soluble elements, including S (Sulfur), Ca (Calcium), K (Potassium), Fe (Iron), Cu (Copper), Zn (Zinc), Ba (Barium), Pb (Lead), As (Arsenic), Sr (Strontium), Se (Selenium), Br (Bromine), Mn (Manganese), and Ti (Titanium). Details of the method are described in Fang et al. (2015a) and in the Supplement.

2.3.3. $PM_{2.5}$ mass

$PM_{2.5}$ mass concentration was measured by a Tapered Element Oscillating Microbalance (TEOM) by Atmospheric Research Analysis (ARA, Inc.) at SEARCH sites (JST, YRK, BHM, and CTR) and ESL. For the RS and GT sites, since $PM_{2.5}$ mass were not available, the PM mass concentrations were estimated from the sum of chemical components analyzed on the same Hi-Vol filters (Verma et al., 2014) (Details in the Supplement).

2.4. Source apportionment

Source apportionment of AAv was performed using a Positive Matrix Factorization (PMF) model (EPA PMF 5.0 software) (Paatero and Tapper, 1994) and a Chemical Mass Balance model (version 8.2) with ensemble-averaged source impact profiles (CMB-E) (Balachandran et al., 2012). PMF is a commonly used source apportionment approach that does not require source profiles as CMB-E, whereas CMB-E has better performance and lower relative uncertainties as compared to the PMF method (Balachandran et al., 2012). Source contributions to DTTv using PMF and CMB-E are discussed in our other publications (Bates et al., 2015; Verma et al., 2014). A PMF analysis on the water-soluble elements (S, K, Ca, Ti, Mn, Fe, Cu, Zn, As, Se, Br, Sr, Ba, and Pb) and WSOC from JST, GT, and RS sites has been reported in Fang et al. (2015a). AAv was simply added to the data sets to generate the AAv results shown here. The PMF results on DTTv in our prior analyses were based on JST and GT sites.

In PMF, the uncertainties for each species were determined by multiplying the concentration by overall uncertainties (%), which were obtained by propagating the uncertainties from filter sampling (assumed to be 5%), extraction (assumed to be 5%), blanks (1 σ of multiple blanks), calibration (1 σ of slope, for water-soluble elements), collocated measurements (for water-soluble elements and AA, Fig. S4), and analytical uncertainties. The analytical uncertainties were obtained by analyzing the same sample/standards multiple times; for example, a composite of extracts from 11 samples for water-soluble elements (coefficient of variation, CV = 2-16 %); 9,10-phenanthrenequinone for AA (CV = 13%), and sucrose standard solutions for WSOC (CV = 10%). Missing data were replaced by species medians with 400% uncertainty, and values below LOD were assigned as half of LOD values with uncertainties of 5/6 the concentration

(Polissar et al., 1998). Uncertainty from collocated measurements was calculated as the relative uncertainty of the slope (1 σ/slope), which was based on an orthogonal regression.

An ensemble-trained source apportionment approach (Balachandran et al., 2012) (CMB-E) was also used to construct the source impacts on AAv based on $PM_{2.5}$ species (sulfate, nitrate, ammonium, OC, EC, and total metals) and AAv measured during SCAPE sampling periods (2012-2013). The source profiles cover a range of sources, including light-duty gasoline vehicles (LDGV), heavy-duty diesel vehicles (HDDV), ambient sulfate (AMSULF), ambient nitrate (AMNITR), ammonium bisulfate (AMBSLF), not otherwise apportioned organic carbon (OTHER_OC), dust, biomass burning (BURN), coal fired power plants, cement, and cooking. An ensemble average was calculated for each source category using ten different runs developed from four individual source apportionment methods. Since the filters for the source impact profiles were collected from midnight to midnight, while the filters collected for AAv measurement were collected from noon to 11 a.m. next day, the sources identified were linearly interpolated using a fixed ratio. For example, two consecutive filters (filter 1 and 2) collected from midnight to midnight, 12/24*filter1 + 11/24*filter2 would be used to produce the estimated AAv to compared with actual measured data.

2.5. Epidemiological assessment

2.5.1. Backcast-estimates of AA activities

To undertake a time-series epidemiological analysis with sufficient power, retrospective data sets of daily AAv levels from 1 August 1998 to 31 December 2009 at an Atlanta site representative of the urban airshed air quality are needed. Previous epidemiological studies by the study team, assessing Atlanta air quality and emergency department (ED) visits, have used data from the

SEARCH JST site, the anchor site for our AAv measurements. To generate daily estimates of retrospective AAv at JST, first a linear model was used to estimate the contribution of various sources to our observed AAv measured at JST. This was done through separate linear regressions for AAv with the ensemble-predicted sources as independent variables. In previous work (Balachandran et al., 2012), a source times-series from August 1998 to December 2009 was generated for JST using the same CMB-E model with the same independent variables measured at JST. The AAv regression was then applied to this time series to construct a time series of estimated AAv for the epidemiology study time period, during which direct measurements of AAv were not available. In order to test the sensitivity of epidemiologic results to different backcast models, two other models are generated for AAv (discussed in section 3.2.1). Identical methods were applied to DTTv to obtain three different models for comparisons with those from AAv.

2.5.2. Epidemiological analyses

Epidemiological time-series analysis (Strickland et al., 2010; Winquist et al., 2015) was employed to assess associations of retrospective DTTv and AAv with health effects as reflected in ED visits. Relationships between ED visits data from Atlanta area hospitals and typical ambient air quality characteristics, as well as the impact of exposure misclassification and other factors, have been extensively studies (Strickland et al., 2015; Darrow et al., 2014; Strickland et al., 2014; Wingquist et al., 2014; Goldman et al., 2012; Pachon et al., 2012; Strickland et al., 2011; Strickland et al., 2010; Sarnat et al., 2010; Sarnat et al., 2008; Tolbert et al., 2007; Metzger et al., 2004; Peel et al., 2005). For the present analysis, we apply these previously reported epidemiologic modeling approach to the backcast-estimates of AAv and DTTv, in order to assess associations of these oxidative potential air quality descriptors with selected outcomes in the ED visits data collected from hospitals serving the five-county metropolitan Atlanta area during 1998-2009. The health outcomes investigated in the current analysis are daily visits for respiratory diseases, including pneumonia (n=145,610 total visits for study period), chronic obstructive pulmonary disease (n=49,251), and asthma/wheeze (n=263,665), and cardiovascular diseases, including ischemic heart disease (n=73,477) and congestive heart failure (CHF) (n=70,587). The air quality was modeled as a three-day moving average ("lag 0-2", the moving average of estimated pollutant level for that day, the previous day, and the day before). Poisson generalized linear regression was performed; to control for temporal trends and meteorological variables, models included cubic splines with monthly knots for time, linear, quadratic and cubic terms for mean daily dew point (lag 0-2), maximum daily temperature (lag 0), and minimum daily temperature (lag 1-2), indicators of hospital contribution time periods, season of year, day of week and holiday, and interaction terms between season and maximum temperature, and between season and day of week. These covariates were chosen based on prior studies (Strickland et al., 2010; Winquist et al., 2015) which identified important confounders to the relationship between daily ambient pollution levels and ED visits. Risk ratios (the relative risks of ED visit associated with an increase of one interquartile range of the exposure metric) and 95% confidence intervals (CI) were used to describe the observed health associations. Risk ratios with confidence intervals above 1 are indicative of statistically significant positive associations. International classification of disease codes used to define the health outcomes can be found in the Supplement.

3. Results and Discussion

3.1. AAv for measurements during 2012-2013 and comparisons to DTTv

3.1.1. Spatio-temporal distribution

Monthly average water-soluble AAv at various sampling sites are given in Fig. 2 (a). AAv was heterogeneously distributed, indicated by the significant variability between sites. Highest AAv were found at the roadside site (RS) and lowest at rural sites. For example, the ratio of average AAv at RS to its paired Atlanta urban JST site, was 1.2 in fall and 1.6 in winter [(2.7 when including the four high data points in Fig. 2 (a)] and RS to near-road GT was 1.7 in fall 2013. AAv at the rural site was generally lower compared to the urban environments, the average YRK/JST ratio was 0.7 in summer and 0.4 in winter, respectively. An exception is that BHM (urban) and CTR (rural) had similar AAv (BHM_{avg} = 0.75 ± 0.45 nmol min^{-1} m^{-3} and CTR_{avg} = 0.78 ± 0.31 nmol min^{-1} m^{-3}). Comparing AAv in different cities, BHM and the other urban site, ESL (average AAv= 0.98 ± 0.63 nmol min^{-1} m^{-3}), had lower AAv relative to the Atlanta urban sites (average of JST and GT in summer = 2.5 ± 1.0 nmol min^{-1} m^{-3}). The higher AAv near traffic sources has also been found in other studies (Janssen et al., 2014; Strak et al., 2012; Janssen et al., 2015). In contrast, Figure 2(b) shows that DTTv was largely spatially uniform, differences between paired sites is much less than those for AAv.

The seasonal distribution can be examined from the Atlanta sites, JST, GT, RS, and YRK. The AA activity was higher in summer/fall compared to winter; the ratio of summer or fall to winter was 1.8, 2.9, 1.0, and 3.1 (average ratio is 2.2 ± 0.9) for JST, YRK, RS, and GT, respectively. In contrast, JST DTTv had an opposite seasonal trend, with the highest level in December (winter), while there was no significant seasonal variation observed at YRK, GT and the RS site. These results indicate that there are differences in the sources for water-soluble AAv and DTTv, with traffic emissions a more significant source for AAv. Correlation analysis with specific aerosol components provides further insights.

3.1.2. Correlations with chemical components

To further identify the major sources for AAv and compare to DTTv , a correlation analysis was performed between the assays and the following selected chemical components; BrC (an indicator of incomplete combustion, i.e., biomass burning), WSOC and S (secondary processes), Ca (mineral dust), and selected transition metals (Cu, Fe, Mn, and Zn) that have been related to adverse health outcomes (Cheung et al., 2012; Kam et al., 2011; Shen and Anastasio, 2011; Cheung et al., 2010; Akhtar et al., 2010; Landreman et al., 2008; Zhang et al., 2008; Kodavanti et al., 2005). Correlation coefficients based on linear regressions between AAv or DTTv and chemical species (Pearson's r) are shown graphically in Fig. 3. A detailed matrix showing the correlations at individual sites is given in Table S2. To simplify Figure 3, JST and GT were combined into one metric given their close proximity and high correlation (r>0.7) for many PM species, such as EC, WSOC, and water-soluble elements (Fang et al., 2015a; Verma et al., 2014). As shown in Fig. 3, AAv was almost exclusively correlated with water-soluble Cu. The r value ranged from 0.70-0.94 for most sites/seasons except RS in fall 2012, JST/GT in winter, ESL in summer, and GT in fall 2013. High correlations between AAv and Cu are consistent with other studies (Janssen et al., 2014; Künzli et al., 2006), although the correlation coefficients (r) in our work (0.70-0.94) were higher (0.60-0.74 in other studies), possibly because we used water-soluble Cu and the other studies used elemental (total) concentrations. Strak et al (2012) also reports a higher r value between AAv and water-soluble Cu (r = 0.82) than that between AAv and total Cu (r = 0.76) from the same sample set.

Compared to AAv, DTTv is more broadly correlated with aerosol species: high correlations were observed with S (r = 0.66-0.74) and WSOC (r = 0.71- 0.77) in summer, which diminished in fall (r = 0.14-0.66 for S and r = 0.20-0.65 for WSOC) and was weaker in winter (r<0.4) for S. Instead, higher correlations were found with BrC (r = 0.78-0.88) and WSOC (r = 0.60-0.84) in winter. The decreasing correlation between DTTv and S going from summer to winter suggests the important role of secondary processing in summer (Verma et al., 2009a; McWhinney et al., 2013) and the increasing contribution of biomass burning to winter DTTv; AAv did not show similar trends, i.e., AAv did not correlate with S (r = -0.12-0.60) and low r values were observed with K in winter (r = 0.07-0.19, one exception was JST in December r = 0.7), suggesting incomplete combustion (e.g., biomass burning) was not a significant source for AAv. Whereas AAv was nearly exclusively correlated with Cu, DTTv was correlated with various metals, including Ca, Mn, Fe, Cu, and Zn. In Fig. 2, counting the number of sampling sites at which r values between AAv and various metals were larger than 0.65 (i.e. black solid bars), we observed eight times for Cu and once for Mn (r = 0.82). Whereas for DTTv (see the striped bars in Fig.3), we found twice (r= 0.67 and 0.77) for Ca, 3 times for Mn (r = 0.65-0.75), 6 times for Fe (r = 0.68-0.90), once for Cu (r = 0.68), and 3 times for Zn (r = 0.70-0.82). There were, however, no apparent seasonal patterns for the correlations between these water-soluble metals and DTTv since they were related to mineral dust (68% of Ca, 45% of Mn, and 26% of Fe) and vehicle brake/tire wear emissions (51% of Cu, 45% of Zn, 32% of Fe, and 17% of Mn).

The comparison of AAv and DTTv's correlation with $PM_{2.5}$ mass is noteworthy. DTTv was fairly well correlated with $PM_{2.5}$ mass (r = 0.49-0.86, Fig. 3), whereas AAv did not correlate as well (r = -0.17 to 0.59), as also found by Künzli et al. (2006) (r = 0.34). $PM_{2.5}$ mass has been linked with adverse health endpoints in many epidemiological studies (Laden et al., 2000; Pope et al., 2002; Pope et al., 2004; Metzger et al., 2004; Sarnat et al., 2008), thus the lack of correlation of AAv with $PM_{2.5}$ mass may be suggestive of an expected lack of linkage between AA-measured oxidative potential and health endpoints (tested in section 3.2). In summary, AA and DTT assays have differing associations with PM chemical species, further suggested by a lack of strong correlation between the two assays at all sites (r<0.55), similar to findings from other studies (r<0.65) (Janssen et al., 2014; Yang et al., 2014; Janssen et al., 2015). A source apportionment analysis is performed to attempt to quantify contributions of various sources to $PM_{2.5}$ AAv.

3.1.3. Source apportionment

Various source contributions to water-soluble AAv and DTTv resolved from PMF and CMB-E based on measured data during 2012-2013 are shown in Fig.4 (a, b) and (c, d), respectively. For AAv, the detailed loadings of various species and time series of each PMF factor can be found in Fig. S5.

AA Sources: Comparing Fig.4 (a) and (b) shows that CMB-E and PMF gave consistent and complementary results for AAv sources. Consistent with the spatial distributions, CMB-E indicated that vehicles comprise almost half of the total source contributions to $PM_{2.5}$ AAv with roughly equal contributions from light and heavy duty vehicles. PMF also found vehicle emissions as a major source, but, based on loadings of source tracers in the various factors, resolved the source as mechanical generation processes (44% from brake/tire wear). Both methods also found sources of AAv associated with secondary processes. CMB-E provides more details on this factor in that it separated out secondary organic and ambient sulfate sources. This is consistent with our source apportionment analyses on water-soluble metals (Fang et al., 2015a), which showed that Cu was mainly associated with secondary formation and brake/tire wear, consistent with AAv being highly correlated with Cu. The 19% other OC source in Figure 3b is related to un-apportioned OC, which includes secondary organic aerosols from biogenic emissions, and possible additional contributions from other VOC sources. At this point, the role it plays as a source of AAv is not clear. It should be noted that the residual for PMF was -10% and 14% for CMB-E, which means the PMF model over-predicted and CMB-E under-estimated AAv. The source apportionment analysis is consistent with the spatial distribution, which indicated vehicle emissions as a main source for AAv activity.

AAv compared to DTTv Sources: Comparisons of the source apportionment results for water-soluble AAv using PMF and CMB-E to a similar analyses for DTTv is insightful. PMF source apportionment analyses [Fig. 4(a) and (c)] suggest a common contribution from traffic emissions and secondary processes to both water-soluble AAv and DTTv, but the contributions were stronger for AAv than DTTv. For example, 44% AAv was attributed to vehicles and 56% to secondary processes, compared to 16% and 31% for DTTv, respectively. Higher fractional contributions of these two sources for AAv is because unlike DTTv, biomass burning does not contribute to AAv (1%), whereas it makes a large contribution to the overall study DTTv (35%). CMB-E also found no contribution of biomass burning to AAv, but identified a fractional contribution from biomass burning [36% BURN in Fig. 4(d)] to DTTv similar to PMF. CMB-E points to ammonium sulfate (AMSULF) as a source for both AAv and DTTv. Neither AA nor DTT assay responds to pure ammonium sulfate, meaning that ammonium sulfate is an indicator of some source or process. It may be a marker for atmospheric processed or aged aerosols. For example, both assays respond to water-soluble transition metals, and a significant fraction of these metals, when emitted, are not water-soluble [solubilities of Zn is ~50%, Cu and Mn 10-40%, Fe < 10% (Birmili et al., 2006; Espinosa et al., 2002)]. Mobilization by acidic aerosols can increase the soluble fraction, which requires a low aerosol pH and time, both can be linked to sulfate aerosol. For example, at pH < 2, 1-2% of mineral dust Fe is mobilized within 3-5 days (Meskhidze et al., 2003). Previous measurements in this study region have also showed water-soluble Fe associated with sulfate in individual particles (Oakes et al., 2012).

It is worth noting that both assays appear to be linked to emissions from traffic, but the actual sources from traffic differ. AAv was almost exclusively associated with mechanically generated aerosols (i.e. brake/tire wear), whereas for water-soluble DTTv, traffic emissions included both metals and organic aerosol species, i.e., from mechanically generated (brake/tire wear) and combustion (tail pipe emissions). Finally, correlations to specific aerosol species and source apportionment analysis can be confounded by co-variability with other unmeasured components or processes, as demonstrated by the associations with ammonium sulfate, or nonlinear responses of these assays to specific components (Charrier et al., 2015). However, the major sources identified for both AAv and DTTv, and the contrasts between their sources, is consistent with the season trends and spatial distributions observed and discussed above for each assay, indicating that the source apportionment analysis is robust.

3.2. AAv association with health endpoints and contrasts to DTTv

3.2.1. Backcast-estimates of AAv using Source Impacts

Although over roughly 1 year of AAv data were generated for the central JST site in Atlanta, longer data sets are generally needed for a time series epidemiological study. To generate these data, multiple linear regression was used to estimate AAv from the CMB-E identified sources (denoted here as $\mathrm{AA}_{\mathrm{v}}^{\mathrm{e}}$). We follow the same approach as that used for DTTv (Bates et al., 2015). Water-soluble AAv (nmol min^{-1} m^{-3}) measured between 2012 and 2013 at JST were regressed against all CMB-E sources. Insignificant sources (p of F-statistic of coefficient>0.05, Table S3) and the significant sources with negative coefficients were removed. The latter occurred for BURN (biomass burning) and AMNITR (ammonium nitrate), likely due to their opposite seasonal trends to the measured AAv. These two sources also did not contribute to AAv [see Fig. 4(a)]. The final regression for AAv is:

$$AA_v^e = 0.079 + 0.19\, LDGV + 0.23\, HDDV + 0.063\, AMSULF + 0.075\, OTHER\_OC \quad \text{(Eq. 3)}$$

For direct comparison with DTTv, we used the same criteria for including various sources in the DTTv regression model, with the result:

$$DTT_v^e = 0.067 + 0.11\, LDGV + 0.045\, HDDV + 0.02\, AMSULF + 0.069\, BURN \quad \text{(Eq. 4)}$$

(Note, the $\mathrm{DTT_v^e}$ regression is different from that in Bates et al., (2015) in that AMSULF (ammonium sulfate) was included in this model).

$\mathrm{AA_v^e}$ and $\mathrm{DTT_v^e}$ are the estimated ROS activities of $PM_{2.5}$ (nmol min^{-1} m^{-3}), which are related to the following sources (μg m^{-3}): light-duty gasoline vehicles (LDGV), heavy-duty diesel vehicles (HDDV), ammonium sulfate (AMSULF), biomass burning (BURN), and other organic carbon (OTHER_OC). The coefficients in the equations represent the intrinsic activities (nmol min^{-1} μg^{-1}) of the sources, a measure of the strength of the source on a per $PM_{2.5}$ mass basis for water-soluble AAv or DTTv. Interestingly, for both assays, the traffic sources (LDGV+HDDV) has the highest ROS intrinsic activity, while secondary sources or biomass burning have relatively lower ROS intrinsic activities. The high intrinsic activity in the traffic sources might be attributed to metals that have much higher intrinsic ROS activities (Charrier and Anastasio, 2012; Verma et al., 2015). The much higher coefficients of LDGV and HDDV in the $\mathrm{AA_v^e}$ regression than those in $\mathrm{DTT_v^e}$ highlight the larger role of metals from these sources contributing to the overall $\mathrm{AA_v^e}$. Although biomass burning has a lower DTT intrinsic activity compared to the other sources in the $\mathrm{DTT_v^e}$ model, it was the largest contributor to $\mathrm{DTT_v^e}$ due to the strength of this source over the measurement period (e.g., large magnitude of BURN). The regression positive intercepts indicate some unidentified source for AAv and DTTv. Insight on the ability of the models to predict $\mathrm{AA_v^e}$ and $\mathrm{DTT_v^e}$ is given by the correlation between the model and measurements ($\mathrm{AA_v^e}$ vs AAv and $\mathrm{DTT_v^e}$ vs DTTv). The r values are 0.60 and 0.68 for $\mathrm{AA_v^e}$ and $\mathrm{DTT_v^e}$, respectively, indicating the models can only account for about 40% (r^2 or 36 to 46%) of the observed variability. Regression coefficients, p-values, and r values are summarized in Table S3.

To test the sensitivity of the epidemiological results to other predictive models, two other regressions were used to predict AAv and DTTv: 1) all sources included, 2) only significant sources with positive coefficients (above), but with AMSULF removed. The latter was done because sulfate has substantially decreased over the last decade due to emission reductions in the southeastern US (Hidy et al., 2014; Hand et al., 2012), which may have unknown effects on $\mathrm{AA_v^e}$ and $\mathrm{DTT_v^e}$. These models and various statistics are also summarized in Table S3. For both assays the models with all sources included had highest correlations coefficients between model and observed activities (r ~ 0.7, or model explains ~50% of the variability).

3.2.2. Health associations from time-series epidemiological models

Backcast $\mathrm{AA_v^e}$ and $\mathrm{DTT_v^e}$ were next generated for the study period corresponding to the health (ED) data. The various regression models (including Eq.3 and 4) were used to generate daily retrospective estimates of AAv and DTTv at the JST site for the period of 1998-2009, based on existing source impacts generated in a previous study for the same site. $\mathrm{AA_v^e}$ and $\mathrm{DTT_v^e}$ were run separately in epidemiological models of ED visits for selected outcomes (section 2.5.2). The risk ratios for $\mathrm{AA_v^e}$ and $\mathrm{DTT_v^e}$ for asthma/wheeze and congestive heart failure are presented in Figure

5 (data given in Table S4). The other health outcomes (chronic obstructive pulmonary disease, pneumonia, and ischemic heart disease) did not show significant associations with $\mathrm{AA}_{\mathrm{v}}^{\mathrm{e}}$ or $\mathrm{DTT}_{\mathrm{v}}^{\mathrm{e}}$ (results given in Table S4).

For asthma/wheeze and congestive heart failure, although the risk ratios for an increase of an interquartile range for $\mathrm{AA}_{\mathrm{v}}^{\mathrm{e}}$ were above 1 [1.005 and 1.003 for Asthma/wheeze and CHF, respectively, Fig. 5(a)], the 95 % confidence intervals crossed 1 (0.994-1.015 and 0.986-1.020 for Asthma/wheeze and congestive heart failure, respectively), indicating a non-statistically significant association between $\mathrm{AA}_{\mathrm{v}}^{\mathrm{e}}$ and the ED visits for these health outcomes. In contrast, both of the health outcomes showed statistically significant associations with the $\mathrm{DTT}_{\mathrm{v}}^{\mathrm{e}}$. The same results were found for estimates based on the two other regressions [Figure 5(b) and (c)], suggesting that the null relationship of $\mathrm{AA}_{\mathrm{v}}^{\mathrm{e}}$ and positive association of $\mathrm{DTT}_{\mathrm{v}}^{\mathrm{e}}$ with these health outcomes are to some extent robust, despite the high uncertainties in oxidative potential from the back-cast models. A possible cause for the differences in $\mathrm{AA}_{\mathrm{v}}^{\mathrm{e}}$ and $\mathrm{DTT}_{\mathrm{v}}^{\mathrm{e}}$ health associations, at least for this study region, is the more narrow selectivity of the AA assay to specific aerosol components (i.e., mostly sensitive to Cu). Thus, the AA assay may not capture the overall oxidative potential of all the various PM components as well as the DTT assay.

PM-induced oxidative stress has been proposed to exacerbate asthma (Li et al., 2003) and adverse cardiovascular responses (Donaldson et al., 2001). Linkages seen here between $\mathrm{PM}_{2.5}$ oxidative potential measured with the DTT assay and morbidity due to asthma/wheeze and congestive heart failure are consistent with these studies. Our results are also consistent with a recent study which found children's respiratory health was more strongly associated with oxidative potential measured with the DTT assay than by electron spin resonance (ESR) (Yang et al., 2016); the ESR assay has been shown to be highly correlated with AA-measured oxidative potential (Janssen et al., 2014). However, their sample preparation method before oxidative potential measurements involves extracting the particles in pure methanol, evaporating the methanol, and reconstituting in DI water, whereas our work focuses only on the water-soluble fraction. The contrast between the DTT with the AA results suggest the importance of organic components and transition metals from biomass burning and vehicular emissions in the Southeastern US, and support aerosol particle oxidative potential as a mechanism contributing to these PM-induced adverse health effects. Although this work shows a contrast between these two assays and association with health endpoints, Janssen et al. (2015) found significant associations between both assays and nasal and airway inflammation based on a small panel study (n=31). Finally, some studies have shown that ROS plays a key role in COPD (O'Donnell et al., 2006), IHF (Lakshmi et al., 2009; Giordano, 2005), and Pneumonia (Kuwano et al., 2003), however, we did not observed a significantly positive association between our tested oxidative potential assays with these health outcomes.

4. Summary

Approximately 500 $PM_{2.5}$ high-volume filter samples collected in the Southeastern US were analyzed for aerosol oxidative potential using the ascorbic acid (AA) assay. The AA activities reported are from the same filters for which water-soluble dithiothreitol (DTT) activities had already been determined. We found that water-soluble AA activity on a per air volume basis (AAv) was highest near roadways and lowest at rural sites. AAv was higher in summer/fall than winter. These results are in contrast to DTTv, which was more spatially uniform and had an opposite seasonal trend at the urban Atlanta site (higher in winter than summer/fall). AAv was most consistently correlated with water-soluble metals (especially water-soluble Cu), whereas DTTv was correlated with organic species and water-soluble metals (Fe, Cu, Zn, Mn, and Ca), and also $PM_{2.5}$ mass. A source apportionment analysis indicated that traffic emissions and secondary processes were strong contributors to both AAv and DTTv in urban Atlanta. For AAv only road dust was responsible, in contrast to both combustion emissions and road dust contributing to the DTTv from this source. Biomass burning did not contribute to AAv, but was a substantial source for DTTv, consistent with AAv being mainly associated with transition metals. These source apportionment results are also consistent with observed seasonal trends and spatial distributions for both assays. Time-series large population epidemiological analyses using backcast-estimates of AAv and DTTv from a number of linear models based on 10-year historical source impacts suggest that AAv was not linked with any emergency department (ED) visits for all tested health outcomes at 95% confidence levels. DTTv was associated with ED visits for both asthma/wheeze and congestive heart failure, for all the linear models tested. Neither AAv nor DTTv was associated with chronic obstructive pulmonary disease (COPD), Ischemic heart failure (IHD) or pneumonia at a statistically significant level. Based on the wide-ranging comparisons between these assays, we conclude that, for the region investigated here, the DTT assay was a more comprehensive multi-pollutant indicator of $PM_{2.5}$ oxidative potential than the AA assay. Finally, the ability to readily measure both $PM_{2.5}$ AA and DTT activities with the automated systems outlined here, enables large-scale studies involving direct measurements of PM oxidative potential. These types of future studies are needed to test if our health findings based on backcast-estimated AA and DTT levels are robust and applicable to other regions.

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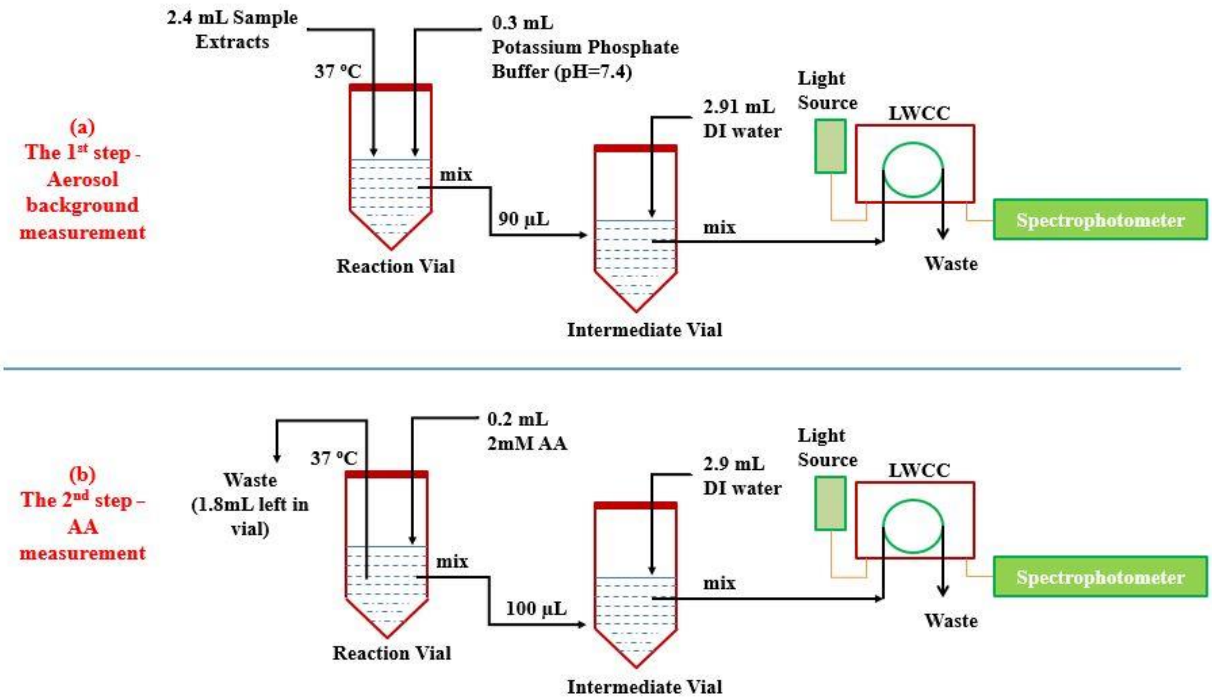
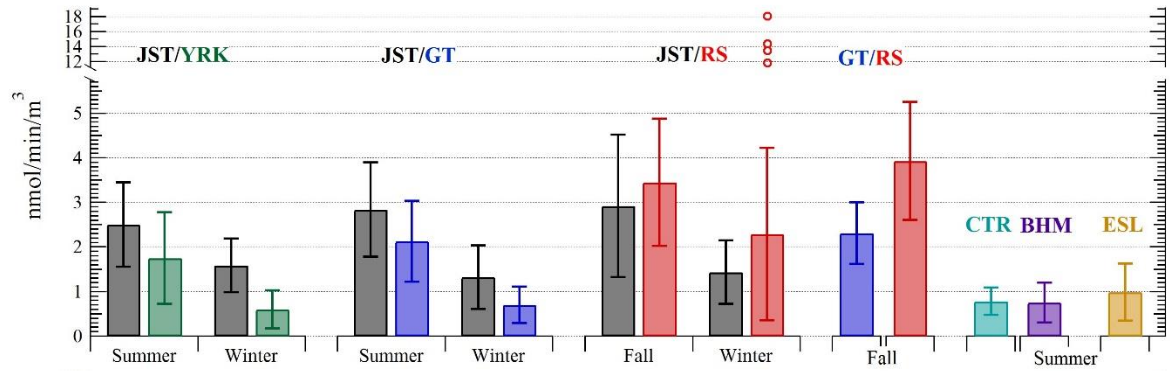


Figure 1. Protocol schematics for conducting Ascorbic Acid assay

(a) AAv (nmol/min/m^3)



(b) DTTv (nmol/min/m^3)

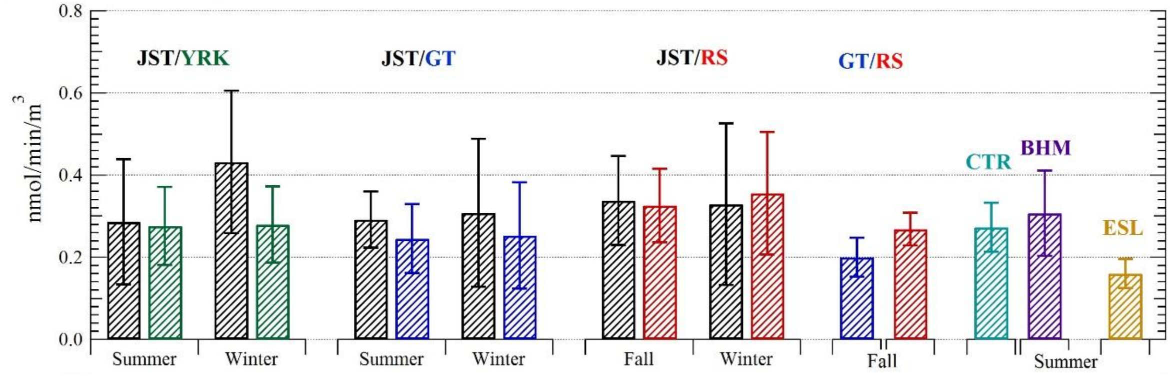


Figure 2. Monthly average (±SD) of $PM_{2.5}$ oxidative potential based on the (a) AA and (b) DTT assays from the water-soluble extracts from filters collected at three urban (JST, BHM, and ESL), two rural (YRK and CTR), a near-road (GT), and a road-side (RS) site in the Southeastern United States.

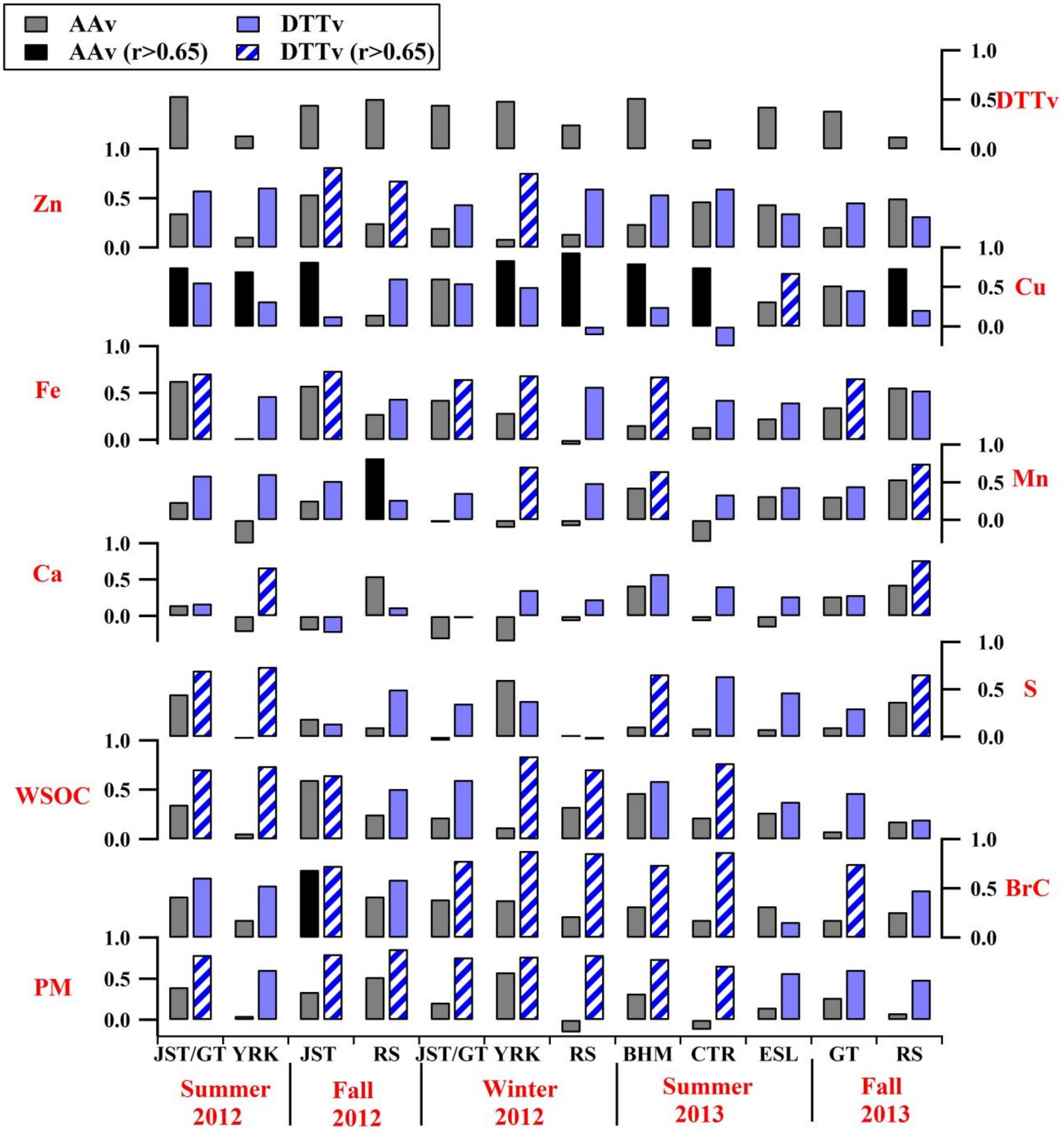


Figure 3. Correlation coefficient (Pearson's r) of fine particle water-soluble AA or DTT activities with $PM_{2.5}$ mass and selected chemical species at various sites in the Southeastern US. A more detailed correlation table is provided in Table S2.

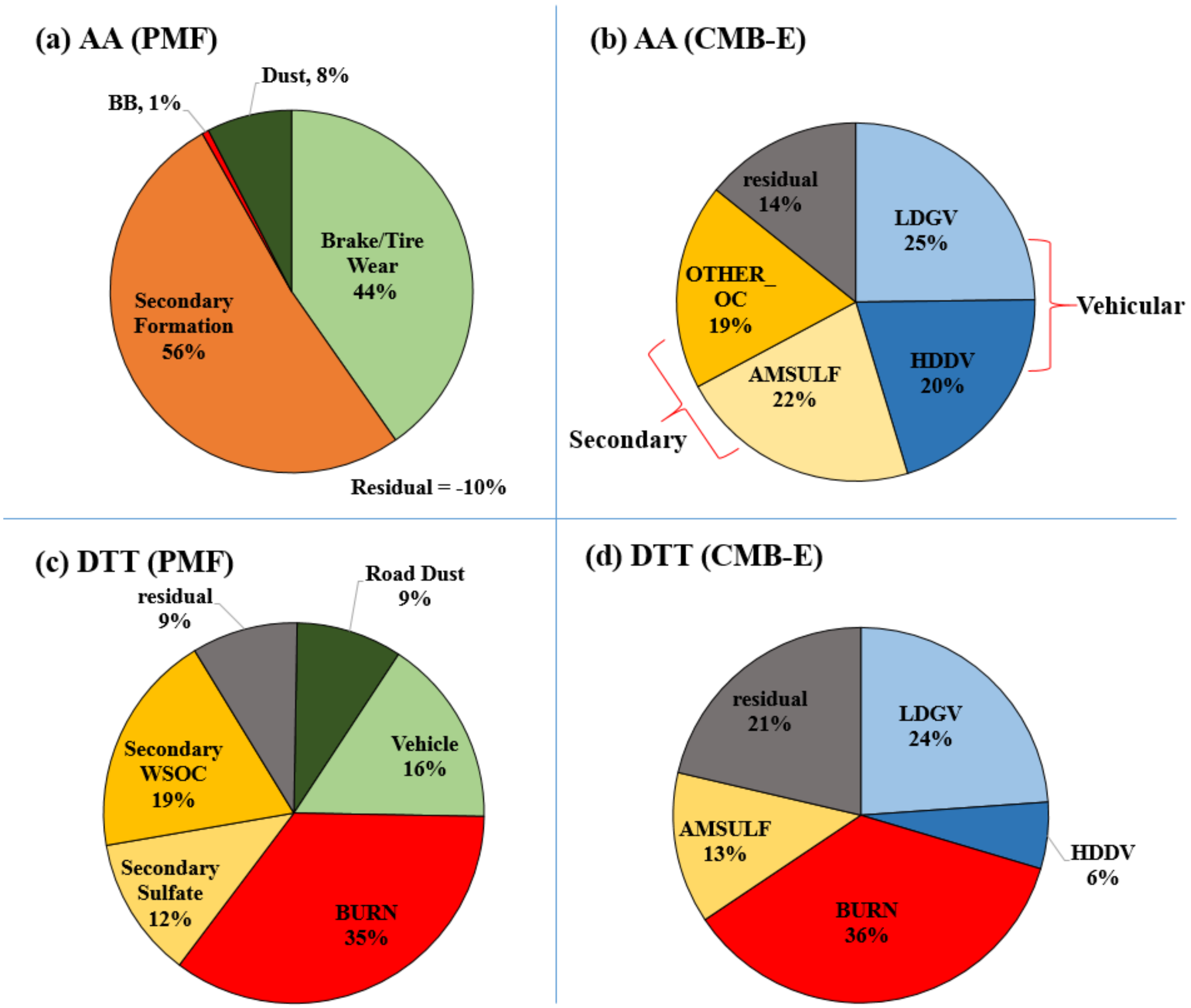
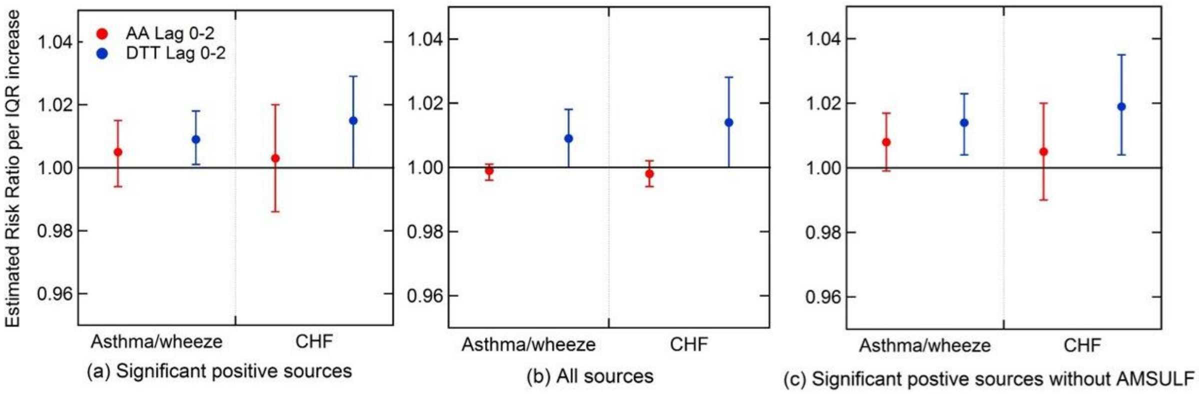


Figure 4. Contribution of various factors resolved by PMF (a, c), and ensemble (b, d), to the water-soluble AA (a, b) and DTT (c, d) activities measured during 2012-2013. BURN – biomass burning; AMSULF – ammonium sulfate; HDDV – heavy-duty diesel vehicles; LDGV – light-duty gasoline vehicles; OTHER_OC – other organic carbon which secondary organic aerosols from biogenic emissions, and possible additional contributions from other VOC sources .



(a) Significant positive sources

(b) All sources

(c) Significant postive sources without AMSULF

Figure 5. Associations between backcast-estimated AA and DTT activities based on estimated sources for the previous 10 years (1998-2009) and emergency department (ED) visits for asthma/wheeze and congestive heart failure (CHF) in the greater metropolitan Atlanta, GA, region. The estimated AA and DTT were based on linear regression models that includes (a) only statistically significant (p of F-statistic of coefficient<0.05) sources with positive coefficients; (b) all sources; and (c) significant positive sources without AMSULF (ammonium sulfate). The models were generated from a multiple regression of the measured AA activities or DTT, on a per volume air bases, with all sources from CMB-E as independent variables. Risk ratios and associated 95% confidence intervals are presented for an increase of one interquartile range (IQR) increment of the exposure metric. A risk ratio with 95% confidence intervals (CI) for interquartile range above 1 indicates a statistically significant positive association. Risk ratio data and related statistics can be found in Table S4.