

1 **Technical Note: Particulate Reactive Oxygen Species Concentrations and their Association**  
2 **with Environmental Conditions in an Urban, Subtropical Climate**

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4 Shahana S. Khurshid\*<sup>1</sup>, Jeffrey A. Siegel<sup>1,2</sup>, Kerry A. Kinney<sup>1</sup>

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6 <sup>1</sup>Department of Civil, Architectural and Environmental Engineering, The University of Texas at  
7 Austin, Austin, Texas, USA.

8 <sup>2</sup>Department of Civil Engineering, The University of Toronto, Toronto, Ontario, Canada.  
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10  
11 **Abstract**

12  
13 Reactions between hydrocarbons and ozone or hydroxyl radicals lead to the formation of  
14 oxidized species, including reactive oxygen species (ROS), and secondary organic aerosol  
15 (SOA) in the troposphere. ROS can be carried deep into the lungs by small aerodynamic particles  
16 where they can cause oxidative stress and cell damage. While environmental studies have  
17 focused on ROS in the gas-phase and rainwater, it is also important to determine concentrations  
18 of ROS on respirable particles. Samples of PM<sub>2.5</sub> collected over three hours at midday on 40  
19 days during November 2011 and September 2012 show that the particulate ROS concentration in  
20 Austin, Texas ranged from a minimum value of 0.02 nmoles H<sub>2</sub>O<sub>2</sub>/m<sup>3</sup> air in December to 3.81  
21 nmoles H<sub>2</sub>O<sub>2</sub>/m<sup>3</sup> air in September. Results from correlation tests and linear regression analysis on  
22 particulate ROS concentrations and environmental conditions (which included ozone and PM<sub>2.5</sub>  
23 concentrations, temperature, relative humidity, precipitation and solar radiation) indicate that  
24 ambient particulate ROS is significantly influenced by the ambient ozone concentration,  
25 temperature and incident solar radiation. Particulate ROS concentrations measured in this study  
26 were in the range reported by other studies in the U.S., Taiwan and Singapore. This study is one  
27 of the first to assess seasonal variations in particulate ROS concentrations and helps explain the  
28 influence of environmental conditions on particulate ROS concentrations.  
29

30 **Keywords**

31 PM<sub>2.5</sub>, O<sub>3</sub>, Ozone-initiated reactions, Solar Radiation, Temperature, Reactive Oxygen Species

1 1. Introduction

2

3 Peroxides are generated in ambient air from alkene ozonolysis and photochemical reactions with  
4 VOCs and NO<sub>x</sub> (Seinfeld and Pandis, 2006). As an example, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and  
5 hydroperoxyl radical (HOO•) are produced from the photooxidation of formaldehyde (Bufalini et  
6 al., 1972; Gay and Bufalini, 1972; Largiuni et al., 2002). Photochemical models suggest that  
7 peroxides can be present in both polluted and clean air (Kleinman, 1986; Heikes et al., 1996)  
8 which is confirmed by measurements (Walker et al., 2006; Snow et al., 2007). H<sub>2</sub>O<sub>2</sub> is an  
9 important species in photochemical smog as a chain terminator. Its concentration in rainwater  
10 and snow has been measured since the late nineteenth century (Schöne, 1874) and studies have  
11 found strong seasonal and diurnal variations in the concentrations of H<sub>2</sub>O<sub>2</sub> and other reactive  
12 oxygen species (ROS) in rainwater, water vapor, and air in gas-phase (Singh et al., 1986; Gunz  
13 and Hoffman, 1990 and references within; Ayers et al., 1992; Dollard and Davies, 1992; Lee et  
14 al., 2000; Yamada et al., 2002; Liu et al., 2003; Zhang et al., 2012). However, data on peroxide  
15 and ROS concentrations in the aerosol phase are limited.

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17 It can be suggested that the concentration of peroxides in water associated with atmospheric  
18 aerosols can be estimated using gas-phase concentrations of peroxides. Hydroperoxides are  
19 thought to partition between the gas-phase and liquid water according to their Henry's law  
20 constants (e.g. H<sub>H<sub>2</sub>O<sub>2</sub></sub> is 0.7 - 1.1 × 10<sup>5</sup> M/atm at 298K; Hwang and Dasgupta, 1985; Staffelbach  
21 and Kok, 1993; Lind and Kok, 1994; Huang and Chen, 2010). Following this reasoning, an  
22 ambient gas-phase H<sub>2</sub>O<sub>2</sub> concentration of 10 ppb would lead to a 1 mM concentration in liquid  
23 water associated with aerosols. However, studies on gas-phase ROS have found that urban  
24 hydroperoxide levels within aerosols are at least an order of magnitude higher than  
25 concentrations predicted by Henry's law (Arellanes et al., 2006; Hasson and Paulson, 2003;  
26 Hewitt and Kok, 1991). The Henry's law constant in aerosols may be different from that in liquid  
27 water (Hasson and Paulson, 2003) which makes it important to assess the concentration of  
28 peroxides and ROS in aerosols.

29

30 Reactions between hydrocarbons and ozone or hydroxyl radicals (generated during the  
31 photolysis of ozone and in catalytic cycles in the troposphere) lead to the formation of oxidized

1 species and secondary organic aerosol (SOA). Highly soluble gases of oxidized species (such as  
2  $H_2O_2$ ) will be removed by the wet mucus lining in the upper airways when they are inhaled  
3 (Wexler and Sarangapani, 1998; Sarangapani and Wexler, 2000). However, SOA are more likely  
4 to reach deep into the lungs due to their physical properties, and the ROS associated with these  
5 aerosols can, thus, reach the deeper parts of the lung and lead to oxidative stress in the tissue  
6 (Morio et al., 2001; Wexler and Sarangapani, 1998). While it would be presumptuous to declare  
7 that ROS has a direct toxic mechanism in tissue injury, many *in vitro* (Oosting et al., 1990; Holm  
8 et al., 1991; Geiser et al., 2004; Crim and Longmore, 1995; LaCagnin et al., 1990) and some *in*  
9 *vivo* studies have drawn links between ROS generated in the body and cell injury, and have also  
10 established the involvement of ROS in different pathologies, such as oxygen toxicity disorder  
11 (Kehrer, 1993; Sanders et al., 1995; Bowler et al., 2002; Li et al., 2003; Li et al., 2008). It  
12 appears likely that external factors (such as ROS associated with ambient particles) can influence  
13 the production of ROS in the body and affect the disease process.

14  
15 In this study we use a bulk measure to assess the concentration of ROS on ambient particulate  
16 matter (PM). 2',7'-dichlorofluorescein diacetate (DCF-DA) is a non-specific fluorescent reagent  
17 for detecting ROS, such as hydrogen peroxide ( $H_2O_2$ ), and hydroxyl radical ( $\bullet OH$ ). Ambient  
18 concentrations of particulate ROS (Hung and Wang, 2001; Venkatachari et al., 2005;  
19 Venkatachari et al., 2007; See et al., 2007) and peroxides in aerosols (Hewitt and Kok, 1991;  
20 Hasson and Paulson, 2003; Arellanes et al., 2006; Wang et al., 2010) have been measured  
21 previously. However, these studies have been conducted over short periods of time, a few  
22 months at most, and do not allow an assessment of seasonal variations in particulate ROS.  
23 Ambient particulate matter collected in different seasons has been used in studies to determine  
24 the generation of selected oxidative species in lung epithelial cells and surrogate lung fluid (Shen  
25 et al., 2011; Vidrio et al., 2009; Baulig et al., 2004). But these seasonal studies generally did not  
26 measure the ambient environmental conditions during PM sampling. It is important to  
27 understand how the ROS concentration on respirable PM varies as environmental conditions  
28 change. Thus, the main objectives of the current study are to (1) determine the concentration of  
29 ROS on  $PM_{2.5}$  in a semi-arid urban environment over a year, and (2) assess the influence of  
30 environmental conditions on these particulate ROS concentrations.

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## 2. Materials and Methods

Samples of PM<sub>2.5</sub> were collected in an open area on the University of Texas at Austin campus using a Personal Environmental Monitor (PEM, SKC, PA, USA) on 40 days between November 2011 and September 2012. Two to five replicate samples were taken on 20 of these days to determine the average covariance in ROS concentration between multiple samplers. Sampling was conducted for 3±0.5 hours between 10am and 3pm using air sampling pumps at 10 l/min. Pumps were calibrated before sampling with a mini-Buck Calibrator M-30 (A. P. Buck, Orlando, FL; accuracy ±0.5%). Samplers were placed 1 m above the ground. Teflon tape was wrapped around the edges of the support screen in the PEMs to ensure a proper seal of the thin PTFE filters inside the PEMs. All sampling filters were assessed within 1 hour of collection. This methodology assesses the persistent species in ROS. Highly volatile species are likely to degrade on the order of hours (and may even degrade prior to sample analysis), but the more persistent components of ROS degrade on the order of days – our control studies indicate that the majority of particulate ROS captured on sampling filters remains stable over a day.

DCF-DA has been used as a bulk measure of ROS (Hung and Wang, 2001; Venkatachari et al., 2005; Venkatachari et al., 2007; See et al., 2007) since it becomes fluorescent in the presence of a wide variety of ROS including, but not limited to, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), organic peroxy (ROO•) and hydroxyl (•OH) radicals and the peroxynitrite anion (ONOO<sup>-</sup>) (Zhu et al., 1994; Kooy et al., 1997). The use of a bulk measure enables a better understanding of the overall toxicity potential of the PM. For instance, H<sub>2</sub>O<sub>2</sub> is generally considered to be less toxic than hydroxyl radicals (Valavanidis et al., 2008), but H<sub>2</sub>O<sub>2</sub> likely has significant indirect biological effects since it can diffuse across membranes easily because of its lack of charge (LaCagnin et al., 1990). The method for quantifying ROS with DCF-DA was modified slightly from previous studies (Hung and Wang, 2001; Venkatachari et al., 2005; Venkatachari et al., 2007; See et al., 2007) to help reduce high fluorescence intensity of field blanks. The method development is described in detail elsewhere (Khurshid et al., 2014). Briefly, 0.5 ml of 1 mM DCF-DA (Cayman Chemical, MI, USA) in ethanol was incubated with 2 ml of 0.01 N NaOH at room temperature for 30 mins in the dark to cleave off the acetate groups. The 2',7'-dichlorofluorescein (DCFH)

1 solution was neutralized with 10 ml sodium phosphate buffer (pH 7.2) and the solution was kept  
2 on ice in the dark till needed. Each sampled filter was sonicated in 5 ml sodium phosphate buffer  
3 for 10 minutes. Horseradish peroxidase (HRP, ThermoScientific, IL, USA) in sodium phosphate  
4 buffer (pH 7.0) was mixed with the DCFH solution and added to the sampled filter in the dark to  
5 yield a final volume of 10 ml with a concentration of 5  $\mu$ M of DCFH and 1 unit/ml of HRP. The  
6 sample was then incubated in the dark at 37°C for 15 mins, after which 0.1 ml aliquots were  
7 placed in triplicate in a 96-well plate and the fluorescence intensity was read at 530 nm with  
8 excitation at 485 nm (Synergy HT, Biotek, VT, USA). The concentration of ROS on the sampled  
9 filters was expressed in terms of H<sub>2</sub>O<sub>2</sub> per volume of air sampled (rather than per mass of  
10 particles) because this describes exposure to ROS as it occurs in the lungs (Boogaard et al.,  
11 2012). The background fluorescence intensity produced by an unsampled filter was subtracted  
12 from the samples.

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14 Standards were prepared with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). To prepare the standards, aliquots of  
15 0.1 ml of appropriate H<sub>2</sub>O<sub>2</sub> concentration were added to 3 ml of DCFH-HRP reagent to get 0,  
16 1.0, 2.0, 3.0, and 4.0 x 10<sup>-7</sup> M H<sub>2</sub>O<sub>2</sub> in final solutions. These solutions were incubated at 37°C  
17 for 15 minutes and fluorescence was measured. All glassware used in the experiments was  
18 scrubbed with soap, followed by immersion in a 10% nitric acid bath and subsequent 7x rinsing  
19 with deionized water.

20

21 The Method Detection Limit of the analytical procedure, as determined using U.S. EPA's  
22 guidelines (EPA, 2011) is 1.2 nmoles H<sub>2</sub>O<sub>2</sub>/l, which converts to 0.01 nmoles/m<sup>3</sup> assuming a 3-  
23 hour sample at 10 l/min. Outliers were excluded using a more conservative approach than the  
24 Iglewicz and Hoaglin method (NIST, 2010) in that only sample concentrations with an absolute  
25 modified Z-score value greater than 10 (instead of 3.5, as recommended by the method) were  
26 excluded as outliers. This was done in order to not exclude any real data resulting from  
27 variations in outdoor conditions.

28

29 Hourly averages of outdoor ozone and PM<sub>2.5</sub> concentrations, temperature, solar radiation, and  
30 relative humidity (RH) were obtained from the nearest Texas Commission on Environmental  
31 Quality's (TCEQ) sampling stations to report the data (located within 7 miles of the ROS

1 sampling site, except for solar radiation which was obtained from a site 17 miles from the  
2 sampling site). Global horizontal (GH) solar radiation data was also measured during January –  
3 June 2012 on top of a 9-storey building located next to the sampling site using a rotating  
4 shadowband radiometer with a data logger (Campbell Scientific, Logan, UT). These GH  
5 measurements were taken every minute and averaged over the sampling duration. Daily  
6 precipitation data for Austin was obtained from Weather Underground (Weather Underground,  
7 2013). Overall uncertainty for each measurement was calculated using standard error  
8 propagation to include variance in the measured readings and the uncertainty of the instrument  
9 when it was known. Graphical representations of the data and Shapiro-Wilk tests for normality  
10 indicated that all the datasets, except for ozone concentration, either followed lognormal  
11 distributions or did not follow normal or lognormal distributions. This led to the selection of the  
12 non-parametric Spearman Rank Correlation Coefficient test to determine the strength ( $\rho$ ) and  
13 significance ( $p < 0.01$ ) of any relationships between the concentration of particulate ROS and  
14 environmental factors. Bonferroni correction was applied to these tests. Simple linear regression  
15 analysis was also performed between particulate ROS concentrations and each environmental  
16 condition measured. All statistics were done with Stata version 11.2.

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### 19 3. Results and Discussion

20

21 The mean ( $\pm$  s.d.) concentration of ROS on  $PM_{2.5}$  samples collected over three hours around  
22 midday in Austin, Texas on 40 days between November 2011 and September 2012 was  $1.25 \pm$   
23  $1.1$  nmoles/ $m^3$ . The concentrations ranged from  $0.02$  nmoles/ $m^3$  measured on December 23 to  
24  $3.81$  nmoles/ $m^3$  on September 20. The concentrations on each sampled day are depicted in  
25 Figure 1 with the error bars depicting the average standard error of replicate samples taken on 20  
26 of the 40 sampling days. The sampling site was located away from any point sources, at a  
27 distance of about 0.7 miles from an interstate highway. Austin has a transitional, semi-arid  
28 climate, characterized by hot summers and mild winters. This is evidenced by the fact that the  
29 mean monthly temperature in November-February was  $13^\circ C$  whereas during June-September it  
30 was  $28^\circ C$ . Ambient environmental conditions measured at the nearest TCEQ sampling sites  
31 during the ROS sampling are given in Table S1. During the sampling periods on the 40 days, the

1 ozone concentration ranged from 8 to 72 ppb, PM<sub>2.5</sub> concentration ranged from 1 to 22 µg/m<sup>3</sup>,  
2 temperature ranged from 3 to 35°C, relative humidity ranged from 21 to 95%, precipitation  
3 ranged from 0 to 80 mm, and solar radiation ranged from 23 to 928 W/m<sup>2</sup>.

4  
5 Studies have found strong seasonal and diurnal variations in the concentrations of H<sub>2</sub>O<sub>2</sub> in air,  
6 rainwater and water vapor, typically with higher concentrations measured during the summer  
7 than the winter (references within Gunz and Hoffman, 1990; references within Sakugawa et al.,  
8 1990; references within Lee et al., 2000; Yamada et al., 2002; Liu et al., 2003). However, other  
9 studies have found that some ROS species, e.g. peroxyacetyl nitrates (RCO<sub>2</sub>ONO<sub>2</sub>) and methyl  
10 hydroperoxide (CH<sub>3</sub>OOH), follow the opposite trend because of greater sensitivity to NO<sub>x</sub>  
11 precursor pollutants (Singh et al., 1986; Zhang et al., 2012). In this study, we found that  
12 particulate ROS concentrations tend to be higher in the warmer months than in the colder  
13 months, implying that particulate ROS follows trends similar to gas-phase and rainwater H<sub>2</sub>O<sub>2</sub> in  
14 the atmosphere.

15  
16 Table 1 displays the results of the Spearman Rank Correlation tests between particulate ROS  
17 concentrations and measured environmental conditions (ozone and PM<sub>2.5</sub> concentrations,  
18 temperature, relative humidity, precipitation and solar radiation). The concentration of ROS on  
19 PM<sub>2.5</sub> was statistically significantly correlated with ozone concentration ( $\rho=0.61$ ,  $p=0.0000$ ),  
20 temperature ( $\rho=0.56$ ,  $p=0.0002$ ) and solar radiation ( $\rho=0.61$ ,  $p=0.0000$ ). Several studies have  
21 assessed the correlation between particulate ROS concentrations and ozone (Hung and Wang,  
22 2001; Venkatachari et al., 2005; Venkatachari et al., 2007) and also between gas-phase H<sub>2</sub>O<sub>2</sub> and  
23 ozone (Liu et al., 2003). These studies have found moderate correlations between the  
24 concentrations of ROS and ozone, with the strongest correlations occurring around midday.  
25 Complexities in the chemistry of formation of ROS have been cited as the reason for the  
26 relatively moderate correlations with ozone (Venkatachari et al., 2007). Meteorological  
27 conditions, such as solar radiation, water vapor concentration, temperature and pressure, are  
28 thought to influence the atmospheric concentration of H<sub>2</sub>O<sub>2</sub> and peroxides (Logan et al., 1981;  
29 Jackson and Hewitt, 1999). Only a few studies have assessed the relationship between particulate  
30 ROS concentrations and meteorological conditions other than ozone concentration. Venkatachari  
31 et al., (2007) had found a weak, but statistically significant, correlation between particulate ROS

1 and estimated secondary organic carbon concentrations in the atmosphere. Given the evidence  
2 for ROS being photochemically driven, one of the objectives of this study was to study the  
3 relationship between particulate ROS and certain meteorological conditions that influence  
4 photochemical reactions.

5  
6 The correlations drawn from this data on particulate ROS (between particulate ROS  
7 concentrations and ambient air quality parameters) are fairly similar to correlations drawn from  
8 studies on gas-phase ROS (between gas-phase ROS/H<sub>2</sub>O<sub>2</sub> concentrations and ambient air quality  
9 parameters). Yamada et al., (2002) found that gas-phase H<sub>2</sub>O<sub>2</sub> was positively correlated with  
10 solar radiation, UV radiation and temperature, while it was negatively correlated with relative  
11 humidity. Liu et al., (2003) found that gas-phase H<sub>2</sub>O<sub>2</sub> was positively correlated with ozone, and  
12 negatively correlated with NO<sub>x</sub>. Similar to Liu et al., (2003), we did not find a discernible  
13 correlation between ROS and relative humidity. While not significant, the inverse relationship  
14 between particulate ROS and daily precipitation could be because of the reduction in gas-phase  
15 H<sub>2</sub>O<sub>2</sub> during rain events (Gunz and Hoffman, 1990) which could lead to reduction in adsorption  
16 of gas-phase ROS onto atmospheric aerosols.

17  
18 The nearest TCEQ sampling site to measure solar radiation was located 17 miles from the ROS  
19 sampling site (all other environmental conditions were obtained from TCEQ sites within 7 miles  
20 of the ROS sampling site). In order to ensure that the conditions at the ROS sampling site were  
21 similar to those at the TCEQ sampling site, solar radiation was measured next to the ROS  
22 sampling site during January to June 2012. The solar radiation data from the two sources was  
23 seen to match well ( $\rho=0.78$ ,  $p=0.0002$ ), and data from the TCEQ site were used for analysis over  
24 the entire study period. In addition, other environmental conditions were correlated with each  
25 other in ways that were expected. When solar radiation during the sampling event was strong,  
26 ozone concentrations and temperatures also tended to be high, as indicated by significant  
27 correlations between these parameters. In contrast, solar radiation was lower on days when it  
28 rained or had high RH, as indicated by the significant inverse correlations between solar  
29 radiation and daily precipitation / RH. Ozone concentrations were also inversely correlated with  
30 RH and daily precipitation. Higher temperatures tended to increase the concentration of PM<sub>2.5</sub>, as



1 indicated by a significant correlation between temperature and PM<sub>2.5</sub> concentration, possibly due  
2 to an increase in reaction rates leading to SOA formation.

3  
4 Regression analysis between particulate ROS concentrations and environmental conditions  
5 shows that linear regression models between particulate ROS concentrations and ozone  
6 concentrations, temperature, and solar radiation are significant ( $p < 0.001$  for the F-test on the  
7 model) but with  $R^2$  values ranging from 0.29 to 0.56. These regression models are shown in  
8 Figure 2. t-tests on the regression coefficients for these linear regression models are also  
9 significant ( $p < 0.001$ ). A multiple regression model of particulate ROS concentration with ozone  
10 concentration, temperature and solar radiation is also significant ( $p = 0.0000$ ) with an  $R^2$  value of  
11 0.6 which means that 60% of the variance of particulate ROS concentrations is accounted for by  
12 the model. Standardized coefficients for the multiple regression model are given in  
13 supplementary information (SI). It should be noted that the predictor variables (ozone  
14 concentration, temperature and solar radiation) for the multiple regression analysis are correlated  
15 which limits the conclusions that can be derived from the model. Linear regression models  
16 between particulate ROS concentrations and PM<sub>2.5</sub> concentrations, relative humidity and  
17 precipitation were not significant and are displayed in Figure S1. The regression results indicate  
18 that ambient particulate ROS is likely a function of the ambient ozone concentration, temperature  
19 and incident solar radiation. Some other contributing factors to particulate ROS concentrations  
20 may include ambient particle concentrations and relative humidity, as well as parameters that  
21 were not measured in this study, such as the concentration of VOCs, NO<sub>x</sub>, hydroxyl and other  
22 radical species.

23  
24 The ROS concentration on PM<sub>2.5</sub> reported in the literature ranges from 0.80-0.97 nmoles/m<sup>3</sup> at a  
25 location 14 km west of Manhattan during winter (Venkatachari et al., 2007), and 4.37-4.98  
26 nmoles/m<sup>3</sup> close to highway traffic during Los Angeles basin inversion conditions in summer  
27 (Venkatachari et al., 2005), to 5.71 nmoles/m<sup>3</sup> in Singapore during December (See et al., 2007).  
28 A study in Taiwan reported a concentration of 0.54 nmoles/m<sup>3</sup> on PM<sub>3.2</sub> on an urban sidewalk  
29 during summer (Hung and Wang, 2001). Some other studies use a different analytical method  
30 and report ROS concentrations on TSP ranging from 0-0.38 nmoles/m<sup>3</sup> in summer in west Los  
31 Angeles (Hasson and Paulson, 2003) to 0-0.24 nmoles/m<sup>3</sup> in summer at Niwot Ridge, CO

1 (Hewitt and Kok, 1991). In the present study, we measured ROS concentrations on PM<sub>2.5</sub> in the  
2 0.02-3.81 nmoles/m<sup>3</sup> range during November 2011 – September 2012 in Austin, Texas. The  
3 winter concentrations measured in this study are comparable to winter concentrations measured  
4 near Manhattan and summer concentrations in Taiwan. The summer concentrations measured in  
5 this study are lower than summer concentrations measured during basin inversion conditions in  
6 LA and winter concentrations in Singapore. In comparison, ROS concentrations on TSP in  
7 mainstream cigarette smoke (4-16 μmol/m<sup>3</sup> for three different brands of cigarettes; Huang et al.,  
8 2005) are 3-4 orders of magnitude higher than all ambient particulate ROS concentrations  
9 reported in the literature.

#### 11 4. Conclusions

13 It is important to measure biologically relevant characteristics of PM to understand the  
14 association between PM and adverse health effects including respiratory and cardiovascular  
15 illnesses (Samet et al., 2000; Pope et al., 2002; Bell et al., 2004). In this study, we measured the  
16 concentration of ROS associated with PM<sub>2.5</sub> in an urban, semi-arid environment over the course  
17 of a year. We found that the minimum concentration occurred during the winter while the  
18 maximum concentration occurred during the summer, which was similar to the results reported  
19 in studies on ambient H<sub>2</sub>O<sub>2</sub> concentrations in gas-phase and rainwater. Given that PM<sub>2.5</sub> can  
20 carry ROS deep into the lungs where the particulate ROS can potentially cause oxidative stress  
21 and cell damage, it is important to better understand the environmental conditions that influence  
22 the concentrations of ROS on PM<sub>2.5</sub>. Results from correlation tests and linear regression analysis  
23 of particulate ROS concentrations and environmental conditions (which included ozone and  
24 PM<sub>2.5</sub> concentrations, temperature, relative humidity, precipitation and solar radiation) indicate  
25 that ROS associated with ambient particles is significantly influenced by the ambient ozone  
26 concentration, temperature and incident solar radiation. Particulate ROS concentrations  
27 measured in this study were within the range 0.0-5.7 nmoles/m<sup>3</sup> reported by other studies in the  
28 U.S., Taiwan and Singapore (Hewitt and Kok, 1991; Hung and Wang, 2001; Hasson and  
29 Paulson, 2003; Venkatachari et al., 2005; Venkatachari et al., 2007; See et al., 2007). This study  
30 is one of the first to assess seasonal variations in particulate ROS concentrations and helps  
31 delineate the principle factors which influence this pollutant.

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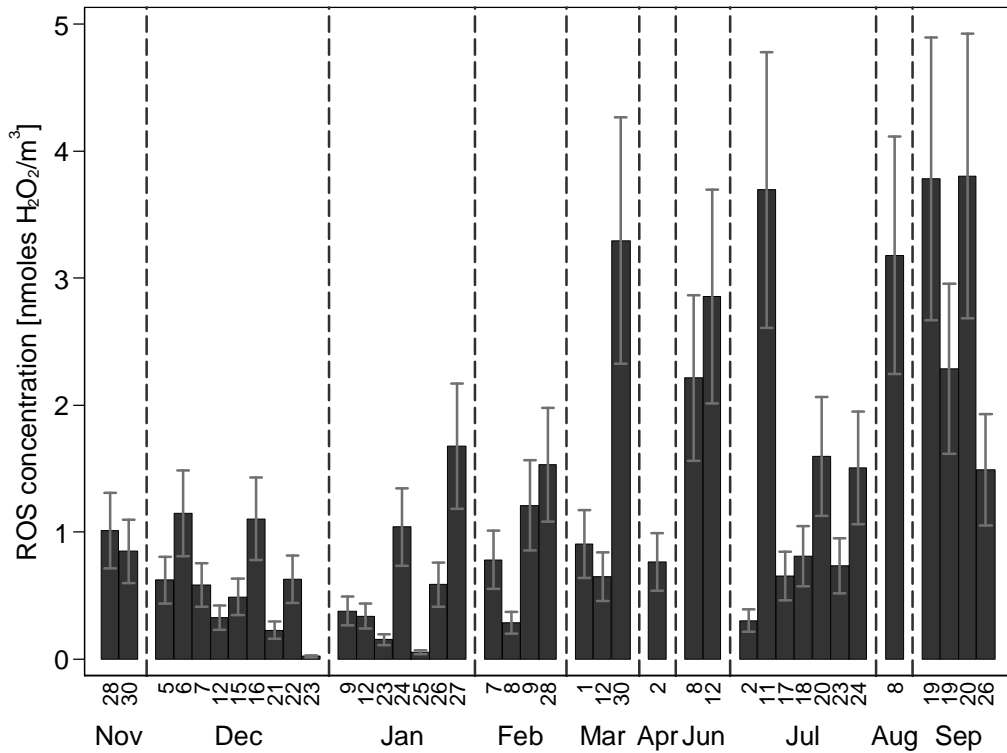
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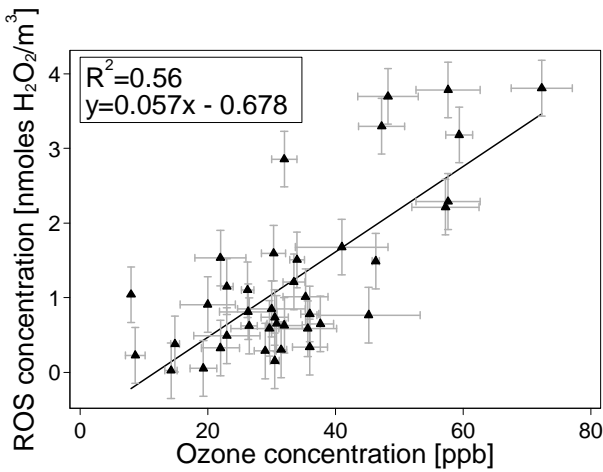
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1 Figures and Tables

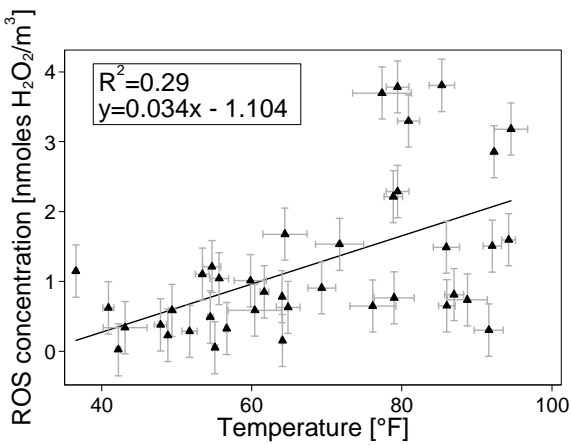


2  
3 Figure 1: Concentration of ROS on PM<sub>2.5</sub> sampled at an outdoor location away from point  
4 sources in Austin, Texas. The error bars represent the average standard error of replicate samples  
5 taken on 20 of the 40 sampling days.

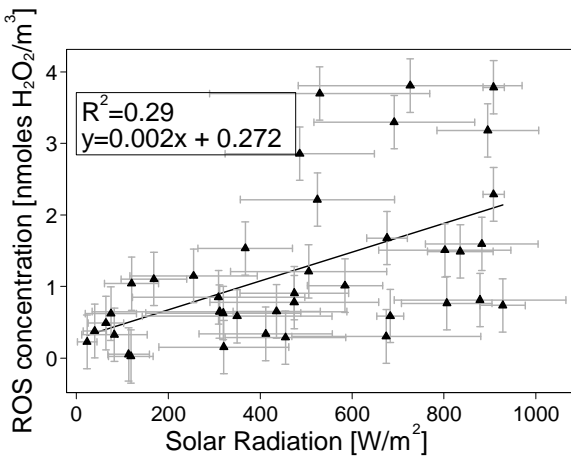
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4 Figure 2: Linear regression graphs showing significant relationships between particulate ROS  
 5 concentrations and O<sub>3</sub> concentration, temperature, and solar radiation. Error bars for ROS  
 6 concentration represent the average standard error of replicate ROS samples. Error bars for  
 7 environmental conditions represent the variance in the measurements during the 3-hour sampling  
 8 period.

- 1 Table 1: Spearman's rank correlation coefficients between the concentration of ROS on PM<sub>2.5</sub>  
 2 (ROS), ozone concentration (O<sub>3</sub>), PM<sub>2.5</sub> concentration, temperature (T), relative humidity (RH),  
 3 precipitation (ppt), and solar radiation measured at the nearest TCEQ site (Solar Rad).  
 4 Significant relationships at p<0.01 are in bold and those at p<0.001 are further denoted with \*.

	ROS on PM <sub>2.5</sub>	O <sub>3</sub>	PM <sub>2.5</sub>	T	RH	ppt
<b>O<sub>3</sub></b>	<b>0.61*</b>					
<b>PM<sub>2.5</sub></b>	0.27	0.03				
<b>T</b>	<b>0.56</b>	<b>0.52</b>	0.36			
<b>RH</b>	-0.17	<b>-0.53</b>	0.19	-0.32		
<b>ppt</b>	-0.15	-0.38	0.08	0.26	<b>0.53</b>	
<b>Solar Rad</b>	<b>0.61*</b>	<b>0.69*</b>	0.11	<b>0.78*</b>	-0.50	<b>-0.54</b>

5