보건학석사 학위논문

# Reactive Oxygen Species (ROS) activity of ambient fine particles ($PM_{2.5}$) measured in Seoul, Korea

## 서울의 대기 중 초미세먼지의 Reactive Oxygen Species (ROS) activity 분석

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# Abstract

# Reactive Oxygen Species (ROS) Activity of Ambient Fine Particles ($PM_{2.5}$) Measured in Seoul, Korea

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Substantial increase in level of particulate matter has raised concerns in South Korea recently. Ambient particulate matter is classified as Group I carcinogen (IARC, 2013) and multiple epidemiological studies has demonstrated adverse health effects due to exposure of particulate matter. Fine particulate matter ($PM_{2.5}$) which has a diameter less than 2.5 μm is likely to penetrate deeply into lung and is known to be eliciting adverse health effects. A number of epidemiological studies have been conducted on adverse health effects of PM-related diseases and mortality rate, yet particulate matter (PM)-

induced reactive oxygen species (ROS) activity at the cellular level has not been actively studied in Korea. This study assessed PM-induced oxidative potential by exposure of collected ambient $PM_{2.5}$ samples to the rat alveolar macrophage cell line. The characteristics of $PM_{2.5}$ in Korea were further characterized by linking chemical constituents and contributing sources to ROS. $PM_{2.5}$ mass concentration during the cold season was relatively higher than mass concentration during the warm season and chemical constituents except for Secondary Organic Carbon (SOC) and $SO_4^{2-}$ followed similar trends. The concentration of crustal elements was especially high during the cold season which can be an indication of long range transport of Asian dust. Water soluble organic carbon and water soluble transition metals (Cr and Zn) were also shown to be correlated to oxidative potential and metals such as As and V were shown to have a high contribution to ROS activity according to stepwise multiple linear regression. Principal Component Analysis (PCA) results identified six factors that can be interpreted as soil, mobile, industry, secondary inorganic aerosol, secondary organic aerosol and oil combustion. Moreover, through Principal Component Regression (PCR), industry, soil, mobile and SIA were shown to be statistically significant sources in a relation to ROS activity.

**Keywords: Fine particulate matter ($PM_{2.5}$), Chemical constituents,**
**Reactive Oxygen Species (ROS), Health effect**
**Student Number: 2016-24048**

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# I. Introduction

The increase of airborne particulate matter has raised concerns for adverse health effects. Sizes, sources and chemical constituents of particulate matter are important factors that can alter the impacts on health effects. While coarse particles can be eliminated from the upper respiratory tract, fine particulate matter ($PM_{2.5}$) which has a diameter of less than 2.5 μm is more likely to penetrate deeply into the lungs (Araujo et al., 2009) and shows more adverse health effects in comparison to coarse particulate matter (Schwartz et al., 1996; McDonnell et al., 2000). A number of studies have reported effects of ambient particulate matter to mortality and morbidity due to respiratory disease and cardiovascular disease (Atkinson et al., 2014; Heo et al., 2014; Pope et al., 2004; Schwartz et al., 1996). The International Agency for Research on Cancer (IARC) classified particulate matter as Group 1 carcinogen and with the indication of carcinogenicity, PM-induced inflammation has been studied in various fields (IARC, 2013).

While the mechanism of $PM_{2.5}$ causing adverse health effects are still unclear, many studies have shown reactive oxygen species (ROS) to be highly associated with PM-induced negative health effects (Valavanidis et al., 2013; Schwarze et al., 2010). In recent studies, toxicological effects of ROS leading to systemic inflammation and disease generation have been discovered. ROS is known to be generated as a byproduct of energy metabolism pathway of aerobic organism. Exposure to the environmental factors such as PM causes an increase in the level of ROS through exogenous or endogenous cellular processes (Michael et al., 2013; de Kok et al., 2006). Inflammation can be driven by oxidative stress, a state when the level of ROS exceeds antioxidant capacity (Ray et al., 2012). Moreover, various signaling molecules such as cytokines and chemokines are released or recruited by the inflammatory response leading to systemic inflammation (Hiraiwa et al., 2014). Since alveolar macrophage is the first cell line to encounter pollutants and initiate pro-inflammatory cascades (Brook et al., 2010), this study focused on ROS activity of alveolar macrophages after exposure to fine particulate matter.

PM-induced ROS activity analysis through *in vivo*, *in vitro*, chemical experiments and epidemiological studies have been conducted around the world. In Asia, however, few studies on PM related health effects from the molecular to the epidemiological level have been reported recently (Chen et al., 2017; He et al., 2017; Li et al., 2017; Hamad et al., 2015). In Korea, epidemiological studies of adverse health effects of PM-related to respiratory disease, cardiovascular disease, cerebrovascular disease (Kim et al., 2015) and mortality rate (Heo et al., 2014) have been done, however PM-induced ROS activity in cellular level has not been actively studied.

Due to South Korea's geological characteristics and westerly winds, transport of PM from China is inevitable. The supposition that the chemical constituents and sources of $PM_{2.5}$ of Korea will be different from that of other countries due to the effects of local and long range transport led to analysis of

ROS potential of $PM_{2.5}$ in Korea. Thus, the objective of this study is to assess toxicological effects of $PM_{2.5}$ in Korea by relating PM-induced oxidative potential with chemical constituents and possible sources in Seoul, Korea.

# II. Experimental Methods

## 1. Sample collection and analysis

Ambient $PM_{2.5}$ samples were collected at the rooftop of former Seoul National University Graduate School of Public Health building (37.581˚N, 127.001˚E, and 17m above ground) from September 2013 to May 2015. The sampling site was located in the center of the Seoul which is suitable for characterizing various sources and effects of $PM_{2.5}$ in the most populated urban area in Korea. A low-volume air sampler consisting of cyclone (URG-2000-30EH, URG, USA) and filter pack system (URG-2000-30FG, URG, USA) was used with a flow rate of 16.7 L $min^{-1}$. Teflon filter (PTFE membrane, Pall Corporation, USA), quartz microfiber filter (Quartz microfiber filter, 1851-047, Whatman™, UK), and zefluor filter (Zefluor™ Membrane, Pall Corporation, USA) with a diameter of 47mm were loaded in the sampler.

$PM_{2.5}$ mass concentration was obtained by measuring Teflon filter which were stored in desiccator for at least 24 hours before and after sampling. Collected samples were weighed with balance (0.01mg) (HM202, A&D, San Jose, CA, USA) and sent to Clarkson University (Postdam, NY) for elemental analysis of a total of 18 elements (Cl, Al, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Pb, Si, Ti, V, Zn, Br, Ni, and Br) using X-ray Fluorescence (XRF).

Samples collected on zefluor filters were used for ionic chemical analysis. Soluble ions ($SO_4^{2-}$, $NO_3^-$, $NH_4^+$) were analyzed by Ion Chromatography (ICS-

1100, Thermo Fisher Scientific). Carbon species, including organic carbon (OC) and elemental carbon (EC) were quantified by Carbon Aerosol Analyzer (Model 3, Sunset Laboratory Inc., Oregon, USA). Quartz filters were punched (1.5cm x 1.0 cm) and the oven was ramped up to 870℃ by following National Institute of Occupational Safety and Health (NIOSH) 5040, thermal/optical transmittance (TOT) method. Water-soluble organic carbon (WSOC) was measured by total carbon analyzer (TOC-V CPH, Shimadzu, Japan).

## 2. Macrophage ROS analysis

Samples were sent to Wisconsin State Laboratory of Hygiene (Wisconsin, USA) to measure the ROS activity induced by $PM_{2.5}$. To quantify ROS activity, rat alveolar macrophage cells (*NR8383*, American Type Culture Collection, VA, USA) were exposed to extracted $PM_{2.5}$ samples and then fluorescence intensity measured with a flow cytometer (Coulter EPICS XL, Beckman Coulter, Miami, FL). Teflon filters were used for ROS analysis and each filter was cut in half before use. Extraction was prepared by continuous agitation of the sectioned filter in Type I purified water for 16 hours. Cell-permeable, 2’7’-dichlorodihydrofluorescein diacetate (DCFH-DA) was used as the ROS probe and got loaded into cells. Since ROS oxidize DCFH-DA and yield highly fluorescent 2’7-dichlorofluorescein (DCF), measurements of DCF generated by the extracted samples represents each sample’s ROS potential (Eruslanov and Kusmartsev., 2010). Zymosan was used as a positive control and results of ROS activity was shown as units of μg zymosan (Landreman et al., 2008).

## 3. Data analysis

Pearson correlation (r) and stepwise multiple linear regression (SMLR) were performed through R 3.4.0, to find correlation between chemical constituents and ROS activity. All 26 chemical species were used and ROS activity was logged. Principal component analysis (PCA) was applied to the selected samples for identification of possible sources. SPSS Statistics 23 (SPSS Statistics, IBM, U.S.A.) was used for PCA. A VARIMAX rotation with Kaiser normalization was applied and factors with eigenvalues greater than 1.0 were extracted. The number of components to be extracted was decided upon scree plot. PCA was coupled with principal component regression analysis (PCR) to determine the importance of each sources to ROS activity. In this study, principal component scores from PCA and logged ROS activity results were used for PCR.

# III. Results

## 1. Chemical constituents

Time series plot of $PM_{2.5}$ speciation data in Seoul, Korea from September 2013 to December 2015 is shown in Figure 1. Overall, the average mass concentration of $PM_{2.5}$ for this period (41.5 $\mu g/m^3$) shows relatively high concentration during spring and winter. The sum of the ionic species $NO_3^-$, $SO_4^{2-}$, and $NH_4^+$ accounted for about 42% of the total mass and was most abundant component of $PM_{2.5}$ mass. Metal species accounted for 23% of total $PM_{2.5}$ mass and OC (6.3 $\mu g/m^3$) and EC (1.2 $\mu g/m^3$) accounted for 15% and 3% of total mass, respectively. When divided into warm and cold seasons, average $PM_{2.5}$ concentration of cold season (48.1 $\mu g/m^3$) was shown to be higher than that of warm season (32.7 $\mu g/m^3$). Average concentrations of OC, EC, $NH_4^+$, $NO_3^-$, crustal and non-crustal species in the cold season are higher than that of warm season. $SO_4^{2-}$ was the only species to show higher concentrations in the warm than cold season.

52 samples collected during the sampling period were selected to be utilized in the *in vitro* macrophage assay and Table 1 summarizes concentrations of chemical species and ROS activity for three different categories: warm, cold and total. Selected samples were divided into two categories, warm and cold, based on temperature. Samples in the warm season had an average temperature of 22.1℃ and were collected between May and October while cold season

samples had an average temperature of 5.1℃ and were collected between November and April. Among 52 samples, 28 samples were classified as warm season samples and 24 samples were selected as cold season samples. Deming regression was applied to obtain the values of primary organic carbon (POC) and secondary organic carbon (SOC) was obtained by subtracting POC from the total measured OC. A different number of samples (warm=16, cold=12) were used to calculate mass concentration of water soluble organic carbon (WSOC). A concentration of crustal dust was calculated by using Equation (1) and the sum of the rest of the elements was categorized into non-crustal.

(1) $\text{Mineral Dust}_{oxide} = 1.889[Al] + 1.400[Ca] + 1.430[Fe] + 1.205[K] + 1.658[Mg] + 1.582[Mn] + 1.348[Na] + 1.094[Rb] + 1.534[Sc] + 2.139[Si] + 1.668[Ti]$

While average $PM_{2.5}$ mass concentration of total selected samples was 52.7 μg/m$^3$, average $PM_{2.5}$ mass concentration of warm (41.7 μg/m$^3$) was lower than that of cold (65.5 μg/m$^3$). While the exception of SOC and $SO_4^{2-}$, all other components including OC, POC, WSOC, EC, $NO_3^-$, $NH_4^+$, crustal elements and non-crustal elements displayed higher mass concentration during the cold season compare to that of samples collected in the warm season.

ROS activity can be measured in two different units: normalized by PM mass (μg Zymosan Units/mg PM) and normalized by the volume of air (μg Zymosan Units/m$^3$ air). While the PM mass normalized unit represents intrinsic

PM oxidative potential per mg PM, the unit normalized by the volume of air represents both intrinsic PM oxidative potential and total PM concentration which can be interpreted as ROS activity upon actual inhalation (Saffari et al., 2014). Thus, this study calculated ROS activity using the unit normalized by the volume of air, revealing the ROS activity of cold samples to be 241.8±27.0 μg /$m^3$ and warm samples to be 184.2±25.8 μg /$m^3$.

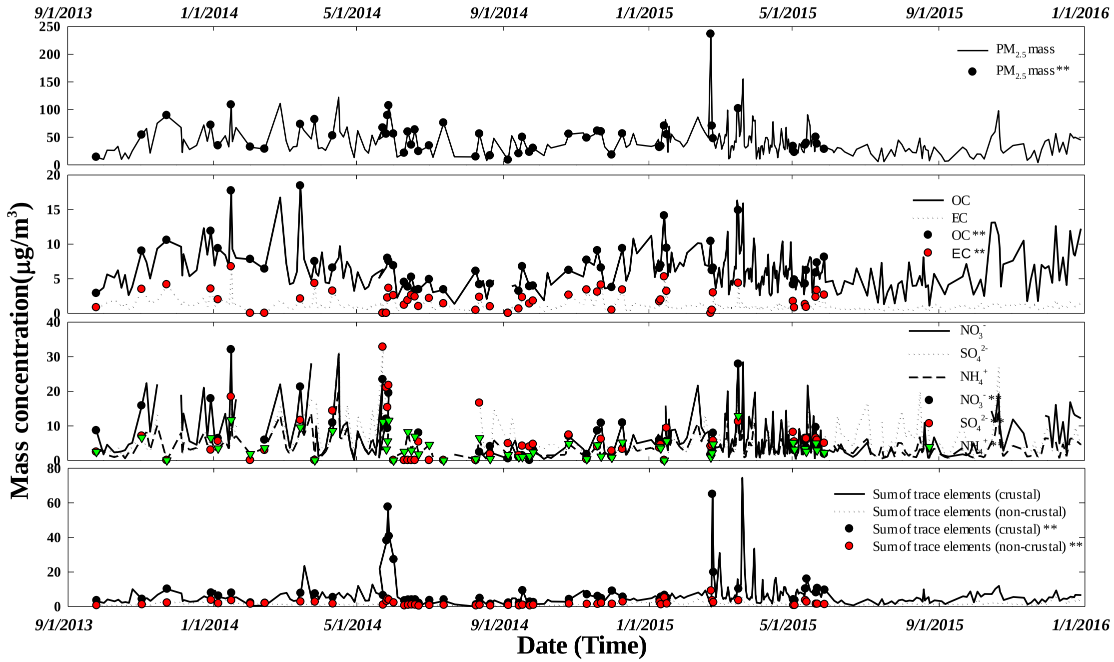


**Figure 1. Time series plot of $PM_{2.5}$ speciation data in Seoul, Korea from 2013. 09. 25 to 2015. 12. 31 (**: concentrations for 52 selected samples)**

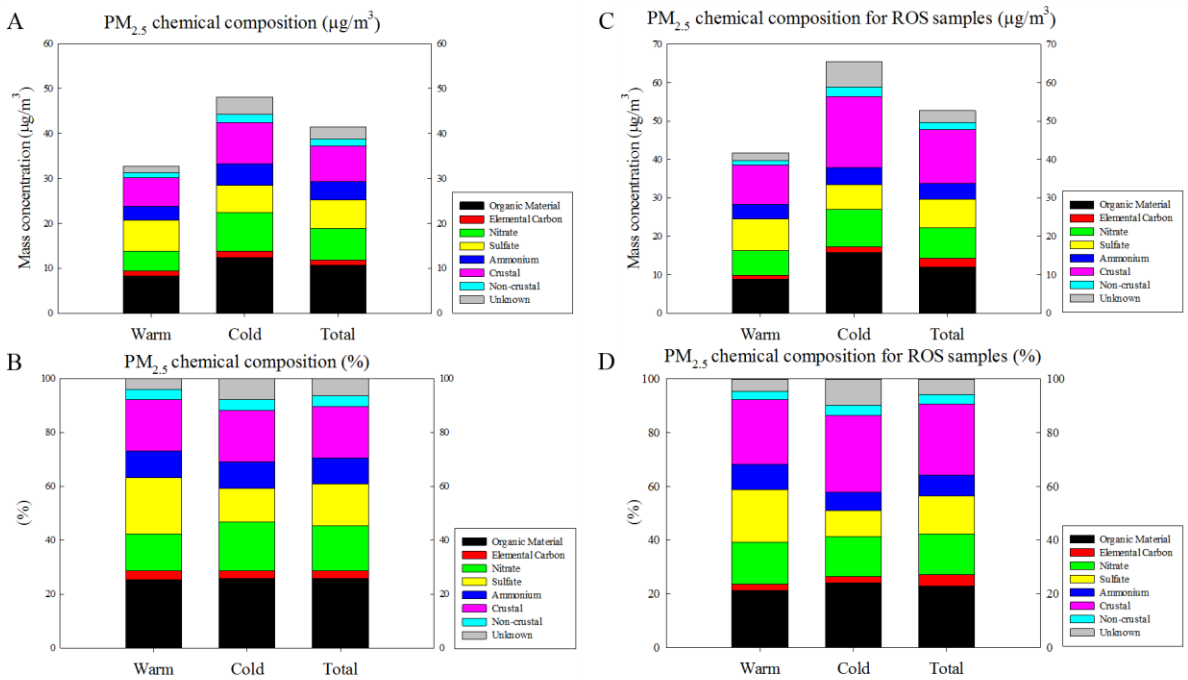


**Figure 2. Average $PM_{2.5}$ species composition contribution in Seoul, Korea. (A, B) represents period from September 2013 to December 2015 and (C, D) represents selected samples**

**Table 1. Summary of ROS activity and chemical species concentration**

| | Unit | Warm (n=28) | | Cold (n=24) | | Total (n=52) | |
|---|---|---|---|---|---|---|---|
| | | | (SEM) | | (SEM) | | (SEM) |
| ROS | µg Zymosan Units/mg PM | 4634.8 | 441.0 | 3895.0 | 264.0 | 4293.3 | 269.5 |
| ROS | µg Zymosan Units/$m^3$ PM | 184.2 | 25.8 | 241.8 | 27.0 | 210.8 | 18.9 |
| $PM_{2.5}$ | µg/$m^3$ | 41.7 | 4.5 | 65.5 | 8.8 | 52.7 | 5.0 |
| OC | µg/$m^3$ | 5.2 | 0.3 | 9.3 | 0.8 | 7.1 | 0.5 |
| POC | µg/$m^3$ | 1.5 | 0.1 | 6.2 | 0.2 | 3.7 | 0.3 |
| SOC | µg/$m^3$ | 3.7 | 0.3 | 3.1 | 0.7 | 3.4 | 0.3 |
| WSOC* | µg/$m^3$ | 1.4 | 0.2 | 2.5 | 0.4 | 1.9 | 0.2 |
| EC | µg/$m^3$ | 1.7 | 0.2 | 2.8 | 0.4 | 2.2 | 0.2 |
| $SO_4^{2-}$ | µg/$m^3$ | 8.2 | 1.3 | 6.4 | 0.8 | 7.4 | 0.8 |
| $NO_3^-$ | µg/$m^3$ | 6.5 | 1.0 | 9.8 | 1.6 | 8.0 | 0.9 |
| $NH_4^+$ | µg/$m^3$ | 3.8 | 0.5 | 4.4 | 0.7 | 4.1 | 0.4 |
| Crustal | µg/$m^3$ | 10.1 | 2.6 | 18.6 | 9.8 | 14.0 | 4.7 |
| Non-crustal | µg/$m^3$ | 1.2 | 0.2 | 2.5 | 0.4 | 1.81 | 0.21 |

WSOC*: different number of samples (total n=28)
SEM : Standard Error of Mean

## 2. Correlation between ROS activity and chemical species

Pearson correlation was conducted between ROS activity and the measured chemical constituents. Pearson correlation coefficient (r) between chemical constituents and induced ROS activity for warm, cold, and total are shown in Table 2. R values greater than 0.5 was considered to be related species and are shown in bold. Considering r>0.65 as more highly related chemical constituents, the three categories, warm, cold, and total, showed different characteristics. $PM_{2.5}$ mass concentration for warm, cold and total categories showed high correlation with ROS activity (μg zymosan/$m^3$). In the warm season category, carbons, ion, and metals species such as POC, WSOC, EC, $NH_4^+$, Al, Cr, Fe, K, Mn, Pb, Si, Ti, V, Zn, and Br showed high correlation to ROS activity. In the cold season category, water-soluble transition metals Cu, V, and Ni as well as WSOC showed high correlation to ROS activity. For the total, including all 52 collected samples, WSOC, Cr, and Zn were shown to be highly correlated to ROS activity.

**Table 2. Summary of Pearson correlation coefficient (r) between chemical constituents of $PM_{2.5}$ and the macrophage-generated ROS**

| | ROS activity | | | | | |
|---|---|---|---|---|---|---|
| | Warm | | Cold | | Total | |
| | r | p value | r | p value | r | p value |
| $PM_{2.5}$ | **0.79** | **<0.001** | **0.71** | **<0.001** | **0.72** | **<0.001** |
| OC | **0.51** | **0.006** | **0.60** | **0.002** | **0.53** | **<0.001** |
| POC | **0.66** | **<0.001** | **0.52** | **0.009** | 0.34 | 0.014 |
| SOC | 0.42 | 0.027 | **0.51** | **0.012** | 0.40 | 0.004 |
| WSOC* | **0.74** | **<0.001** | **0.69** | **0.012** | **0.65** | **<0.001** |
| EC | **0.66** | **<0.001** | **0.52** | **0.009** | **0.56** | **<0.001** |
| $NO_3^-$ | **0.54** | **0.003** | **0.50** | **0.012** | **0.53** | **<0.001** |
| $SO_4^{2-}$ | **0.63** | **<0.001** | 0.46 | 0.024 | **0.51** | **<0.001** |
| $NH_4^+$ | **0.72** | **<0.001** | 0.32 | 0.132 | **0.52** | **<0.001** |
| Cl | 0.09 | 0.665 | 0.29 | 0.166 | 0.29 | 0.037 |
| Al | **0.67** | **<0.001** | 0.32 | 0.122 | 0.37 | 0.007 |
| Ca | **0.61** | **<0.001** | 0.35 | 0.089 | 0.41 | 0.003 |
| Cr | **0.83** | **<0.001** | **0.58** | **0.003** | **0.70** | **<0.001** |
| Cu | **0.50** | **0.006** | **0.68** | **<0.001** | **0.61** | **<0.001** |
| Fe | **0.70** | **<0.001** | 0.37 | 0.078 | 0.40 | 0.003 |
| K | **0.80** | **<0.001** | 0.43 | 0.038 | **0.58** | **<0.001** |
| Mg | **0.61** | **<0.001** | 0.31 | 0.137 | 0.40 | 0.004 |
| Mn | **0.78** | **<0.001** | 0.47 | 0.019 | **0.52** | **<0.001** |
| Na | **0.50** | **0.007** | **0.55** | **0.005** | **0.53** | **<0.001** |
| Pb | **0.78** | **<0.001** | **0.57** | **0.004** | **0.64** | **<0.001** |
| Si | **0.67** | **<0.001** | 0.32 | 0.124 | 0.37 | 0.008 |
| Ti | **0.67** | **<0.001** | 0.34 | 0.104 | 0.36 | 0.009 |
| V | **0.73** | **<0.001** | **0.67** | **<0.001** | **0.63** | **<0.001** |
| Zn | **0.69** | **<0.001** | **0.61** | **0.002** | **0.66** | **<0.001** |
| Br | **0.71** | **<0.001** | **0.60** | **0.002** | **0.62** | **<0.001** |
| Ni | <0.01 | 0.999 | **0.72** | **<0.001** | 0.20 | 0.164 |
| As | 0.41 | 0.029 | 0.33 | 0.120 | 0.38 | 0.006 |

R>0.5 are shown in bold
WSOC* number of sample is different

## 3. Stepwise Multiple Linear Regression

All variables were used for SMLR model and a summary of the results is presented in Table 3. POC, SOC, EC, $SO_4^{2-}$, $NH_4^+$, Al, Fe, V, and As displayed positive correlation and OC, $NO_3^-$, Ca, and Si displayed negative correlation. Only POC, SOC, EC, Al, V and As had positive correlation as well as p-values smaller than 0.05. Among 13 chemical constituents, two elements (V and As) exhibited significantly high positive association with the ROS activity.

**Table 3. Summary of stepwise multiple linear regression model results**

| | β | | | |
|---|---|---|---|---|
| | Estimates | Standard error | T-statistics | *p* value |
| ROS activity | | | | |
| (Intercept) | 3.37 | 0.22 | 15.37 | <0.001 |
| OC | -0.81 | 0.29 | -2.80 | 0.008 |
| POC | 0.79 | 0.28 | 2.79 | 0.008 |
| SOC | 0.83 | 0.30 | 2.82 | 0.008 |
| EC | 0.23 | 0.06 | 3.85 | <0.001 |
| $SO_4^{2-}$ | 0.02 | 0.01 | 2.00 | 0.053 |
| $NO_3^-$ | -0.03 | 0.01 | -1.86 | 0.071 |
| $NH_4^+$ | 0.05 | 0.02 | 1.88 | 0.068 |
| Al | 8.45 | 1.99 | 4.24 | <0.001 |
| Ca | -1.07 | 0.31 | -3.48 | 0.001 |
| Fe | 1.45 | 0.81 | 1.79 | 0.081 |
| Si | -1.95 | 0.48 | -4.03 | <0.001 |
| V | **18.87** | 6.54 | 2.89 | 0.006 |
| As | **47.37** | 13.37 | 3.54 | 0.001 |

Residual standard error: 0.29 on 38˚ of freedom(DF), multiple $R^2$:0.87, adjusted $R^2$:0.83, F-statistics:19.93 on 13 and 38 DF, p-value:4.65E-13

# 4. Association of ROS activity and sources

## 4.1 Principal Component Analysis

Six possible factors affecting the Seoul site were statistically identified by interpreting six principal component (PC) from PCA: soil, mobile, industry, secondary inorganic aerosol (SIA), secondary organic aerosol (SOA) and oil combustion. The PCA results are shown in Table 4 and factor loadings with values greater than 0.60 are shown in bold. PC 1 comprised of high factor loadings of Al, Si, Fe, Ti, Mg, Ca, Mn, K, Cr, and Na and these elements are typical components of soil (Viana et al., 2007; Almeida et al., 2005). PC2 consisted of POC, Cl, OC, Br, As and EC which are found from vehicle exhaust and brakes represents mobile source. Zn, V, Cu, and Pb are commonly produced from industries that partake in oil- and coal-combustion (Alemeida et al., 2005; Querol et al., 2004; Watson and Chow, 2001). The composition of these elements suggests PC3 as an industry-related source. High factor loadings of $NH_4^+$, $SO_4^{2-}$, and $NO_3^-$clearly indicate SIA (Almeida et al., 2005). PC5 and PC6 only had SOC and Ni respectively and Ni is known as a biomarker of oil combustion.

**Table 4. Varimax normalized rotated factor loading and communalities obtained in PCA applied to the $PM_{2.5}$ chemical constituents**

**Rotated Component Matrix**[a]

| | Principal component | | | | | | |
|---|---|---|---|---|---|---|---|
| | PC 1 | PC 2 | PC 3 | PC 4 | PC 5 | PC 6 | Communalities |
| | Soil | Mobile | Industry | Secondary inorganic aerosol | Secondary organic aerosol | Oil combustion | |
| Al | **0.995** | -0.032 | -0.046 | -0.025 | 0.024 | 0.033 | 0.995 |
| Si | **0.994** | -0.03 | -0.051 | -0.028 | 0.019 | 0.036 | 0.995 |
| Fe | **0.992** | 0.013 | -0.020 | -0.018 | 0.021 | 0.064 | 0.990 |
| Ti | **0.989** | -0.007 | -0.059 | -0.031 | 0.014 | 0.058 | 0.987 |
| Mg | **0.987** | -0.069 | 0.019 | -0.025 | 0.070 | -0.011 | 0.986 |
| Ca | **0.987** | -0.033 | 0.029 | -0.018 | 0.086 | 0.052 | 0.987 |
| Mn | **0.978** | 0.100 | 0.110 | 0.044 | 0.023 | 0.042 | 0.982 |
| K | **0.960** | 0.125 | 0.103 | 0.041 | 0.065 | -0.036 | 0.955 |
| Cr | **0.872** | 0.184 | 0.354 | 0.178 | -0.039 | 0.042 | 0.955 |
| Na | **0.671** | 0.184 | 0.402 | 0.015 | 0.240 | 0.068 | 0.709 |
| POC | 0.061 | **0.926** | 0.010 | 0.058 | -0.161 | -0.126 | 0.907 |
| Cl | 0.111 | **0.849** | -0.094 | 0.103 | 0.140 | 0.122 | 0.787 |
| OC | 0.171 | **0.749** | 0.080 | 0.275 | 0.520 | 0.040 | 0.945 |
| Br | -0.010 | **0.740** | 0.348 | 0.276 | 0.050 | 0.230 | 0.801 |
| As | -0.030 | **0.665** | 0.252 | -0.160 | 0.025 | 0.000 | 0.532 |
| EC | -0.170 | **0.664** | 0.526 | 0.220 | 0.005 | 0.045 | 0.797 |
| Zn | 0.043 | 0.369 | **0.803** | 0.240 | 0.185 | 0.114 | 0.887 |
| V | 0.129 | -0.085 | **0.780** | 0.369 | -0.121 | 0.169 | 0.811 |
| Cu | 0.166 | 0.478 | **0.673** | 0.138 | 0.061 | 0.362 | 0.862 |
| Pb | 0.126 | 0.584 | **0.611** | 0.188 | 0.196 | -0.171 | 0.834 |
| $NH_4^+$ | -0.054 | 0.232 | 0.163 | **0.876** | 0.081 | 0.115 | 0.870 |
| $NO_3^-$ | -0.056 | 0.404 | 0.167 | **0.802** | 0.244 | -0.034 | 0.899 |
| $SO_4^{2-}$ | 0.082 | -0.142 | 0.363 | **0.802** | 0.122 | -0.086 | 0.824 |
| SOC | 0.175 | 0.103 | 0.096 | 0.320 | **0.883** | 0.186 | 0.967 |
| Ni | 0.130 | 0.067 | 0.227 | 0.005 | 0.154 | **0.913** | 0.930 |
| Eigenvalue | 9.179 | 4.637 | 3.121 | 2.683 | 1.371 | 1.201 | |
| % variance | 36.715 | 18.549 | 12.486 | 10.734 | 5.484 | 4.805 | |
| Cumulative % | 36.715 | 55.263 | 67.749 | 78.483 | 83.966 | 88.771 | |

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.

a. Rotation converged in 7 iterations.

## 4.2 Principal Component Regression

The result of PCR carried out by using factor scores from PCA is shown in Table 5 and represented as Equation (2). PCR analysis of ROS activity showed that the SOA related source and oil combustion related source were not statistically significant with a p-value greater than 0.05. Soil, mobile, industry, and SIA were shown to be important sources to be considered in a relation to ROS activity. Based on the beta coefficient, industry and soil were strongly correlated with ROS activity while mobile and SIA were moderately correlated with ROS activity.

**(2) Log(ROS) = 2.230 + 0.112 PC1 + 0.108 PC2 + 0.181 PC3 + 0.103 PC4**

**Table 5. Summary of Principal Component Regression**

**Coefficients[a]**

| | Unstandardized Coefficients | | Standardized Coefficients | | |
|---|---|---|---|---|---|
| | B | Std. Error | Beta | t | Sig. |
| (Constant) | 2.230 | .023 | | 98.011 | .000 |
| REGR factor score 1 | **.112** | .023 | .371 | 4.873 | **.000** |
| REGR factor score 2 | **.108** | .023 | .357 | 4.688 | **.000** |
| REGR factor score 3 | **.181** | .023 | .599 | 7.870 | **.000** |
| REGR factor score 4 | **.103** | .023 | .340 | 4.472 | **.000** |
| REGR factor score 5 | .008 | .023 | .028 | .364 | .718 |
| REGR factor score 6 | .003 | .023 | .009 | .124 | .902 |

a. Dependent Variable: logROS

# IV. Discussion and Conclusion

For the selected 52 samples, the average $PM_{2.5}$ mass concentration of warm season samples (41.7 μg/m$^3$) was lower than that of cold season samples (65.5 μg/m$^3$). Mass concentrations of $PM_{2.5}$ compositions such as OC, $NO_3^-$ and metal were relatively high during the cold season. OC concentration during the cold season was shown to be 1.8 times higher compared to the warm season and this may be due to higher combustion during winter. Similar trends of winter OC concentration being 1.3~1.6 times higher than summer OC concentration in urban area of East Asian countries have been shown in other studies (Park and Cho, 2011; Miyazaki et al., 2006; Yu et al., 2002). OC concentration during the cold season was highly dominated by POC, but SOC contributed greatly to OC concentration during the warm season. A smaller number of samples were analyzed for WSOC, but displayed similar seasonal profiles in Korea (Park et al., 2015). High average concentration of WSOC during cold season may suggest the formation of secondary organic aerosol formed in the process of long range transport from upwind regions (Cho and Park, 2013; Snyder et al., 2009). SIA including $NO_3^-$, $SO_4^{2-}$, and $NH_4^+$ are formed through a photochemical reaction. $SO_4^{2-}$ is formed in high temperatures and showed high concentration in the warm season while $NO_3^-$, a species formed in lower temperatures, showed high concentrations during the cold season. Among metal constituents, the proportion of crustal metal in $PM_{2.5}$ mass concentration was shown to be over 20% and high concentrations in cold season could have been caused by Long-Range Transport of Asian dust.

ROS activity can be displayed in two different units and their trends vary as $PM_{2.5}$ mass concentration increases. Figure 3 shows ROS activity normalized by PM mass and volume of air in relation to $PM_{2.5}$ mass concentration. As the level of $PM_{2.5}$ increased, ROS activity normalized by PM mass decreased while ROS activity normalized by the volume of air increased as $PM_{2.5}$ mass concentration increased which may be due to the contributions of chemical composition. Based on the Korean Air Quality Standards of fine particles (50 $\mu g/m^3$), the samples were divided into high concentration events (HCEs) and non-events (Figure 4). In comparison to other chemical constituents such as OC, EC, and metals, the contribution of ionic species in HCEs was the most prominent. As WSOC and transition metals such as As and V were found to be the most correlated to ROS activity, the high contribution of ionic species in HCEs may explain the decrease of ROS activity normalized by PM mass as $PM_{2.5}$ mass concentration increased.

Higher ROS activity upon inhalation during the cold season may be affected by higher concentrations of primary combustion and less dispersion of air pollutants due to the lower mixing height caused by lower temperature. In previous studies that used the same *in vitro* macrophage assay to assess oxidative potential of $PM_{2.5}$ in other cities that are Lahore, Milan, Beirut, Los Angeles, Thessaloniki, and Denver, it was shown that oxidative potential varies depending on the location and sources (Saffari et al., 2014). Figure 5 displays the summary of ROS activity of $PM_{2.5}$ normalized by PM mass and volume of air in seven different cities, including Seoul, from this study. Through comparison of the ROS activity of these cities, Seoul showed higher ROS activity than that of Los Angeles, Thessaloniki and Denver but had lower ROS activity compared to Beirut, Milan and Lahore (Daher et al., 2014; Saffari et al., 2013; Daher et al., 2012; Shafer et al., 2010; Verma et al., 2009; Zhang et al., 2008).

Correlation between ROS activity and chemical species indicates that water-soluble transition metals and WSOC are important species that are highly correlated with ROS activity. The high ROS potential of WSOC, Cr and Zn from ambient particulate matter which leads to increased oxidative stress and release of proinflammatory cytokines have been investigated in several toxicological studies (Saffari et al., 2014; Daher et al., 2012; Zhang et al., 2008; Schaumann et al., 2004).

Similar to the SMLR results of this study, other studies that conducted similar multiple linear regression have shown that As and V are highly correlated with ROS activity (Shuster-Meiseles et al., 2016; Heo et al., 2015; Valavanidis et al., 2008). The results of Pearson correlation of As were poor, but V showed high correlation regardless of seasonal variation. Multiple studies have consistently shown high ROS potential of As and V (Heo et al., 2015; Saffari et al., 2014). A number of epidemiological studies have investigated carcinogenicity of As and the association of cardiovascular mortality associated with V (Zhang et al., 2009; Tchounwou et al., 2004).

In the results of PCA, As and V were identified as mobile and industry sources, respectively. Industry was highly correlated and mobile was moderately correlated with ROS activity according to the PCR results. The findings that As and V are both important chemical constituents as well as sources that induce ROS activity was supported by both SMLR and PCR.

A previous epidemiological study that discussed the contribution of $PM_{2.5}$ to mortality collected samples from 2003 to 2007 at the same site used in this study (Heo et al., 2014). According to the results of this epidemiological study, roadway emission and industry were associated with cardiovascular mortality and mobile and soil were associated with respiratory mortality in Seoul. PCR results showed significant correlation of $PM_{2.5}$ to industry, soil, mobile, and SIA, suggesting the sources that correlate highly in molecular experiments and in epidemiological approach align well.

The objective of this study was to assess toxicological effects of $PM_{2.5}$ by relating PM-induced oxidative potential with chemical constituents and possible sources in Seoul, Korea. Since this study is the first to measure the oxidative potential of ambient fine particulate matter collected in Seoul by applying *in vitro* macrophage assay, the results can be used as a reference for further research. Moreover, chemical constituents and sources that are highly correlated with oxidative potential may act as evidence to prioritize emission reductions. Oxidative potential was consistently detected at all levels of $PM_{2.5}$ mass concentration, thus it is more important to implement reduction policy based on chemical constituents and sources instead of reducing $PM_{2.5}$ mass concentration itself. Additional studies can increase the number of samples so that the results can be representative of the oxidative potential of $PM_{2.5}$ exposure in Seoul, Korea. Experimental studies that involve *in vitro* always carry skepticism due to the higher complexity of real-world applications, though combining studies from the molecular to epidemiological level serves as a solution to overcome that limitation.

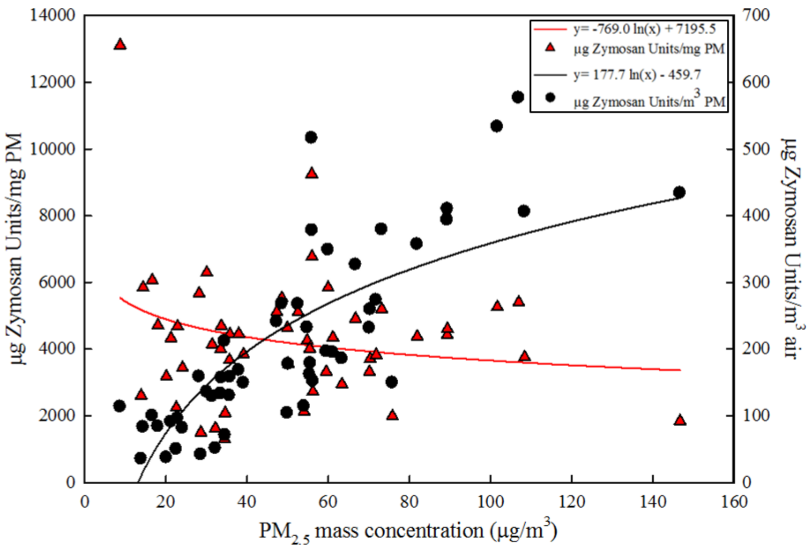


**Figure 3. ROS activity normalized by PM mass and volume of air in Seoul**

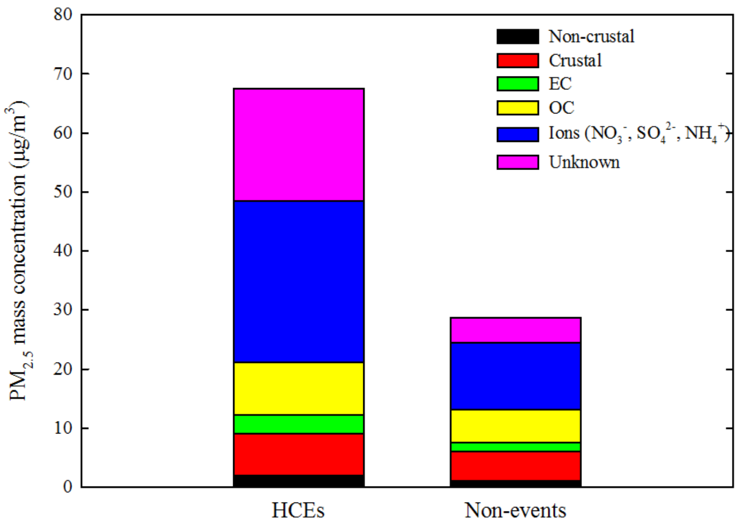


**Figure 4. Chemical constituents of HCEs and non-events**

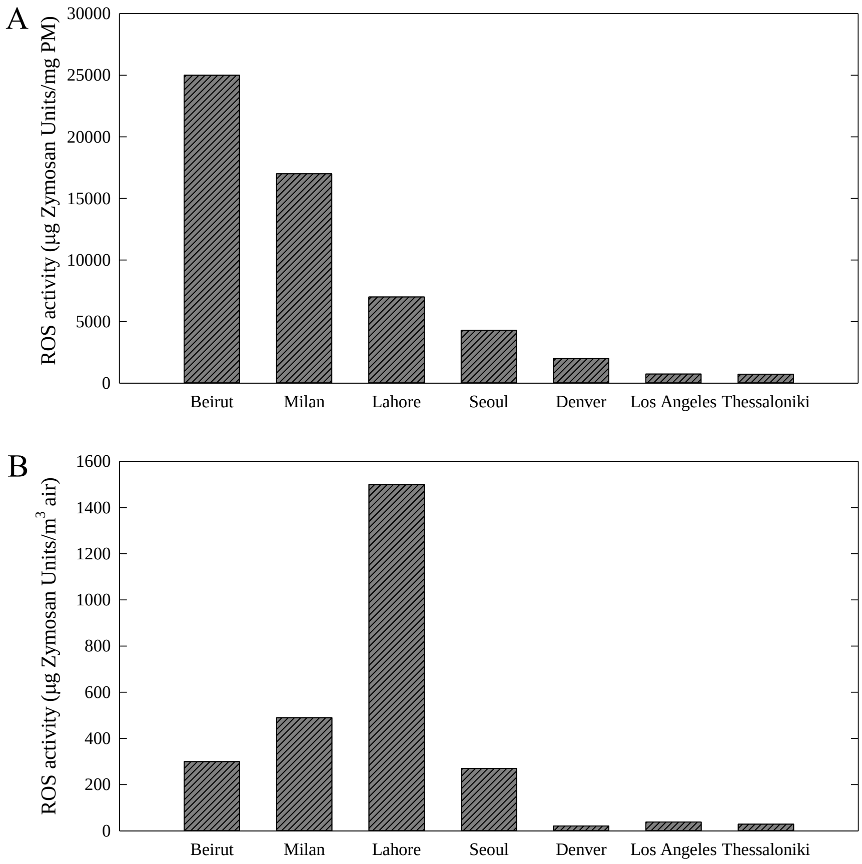


**Figure 5. ROS activity of $PM_{2.5}$ in seven different studies (A) ROS activity normalized by PM mass (B) ROS activity normalized by volume of air**

# V. References

Almeida, S.M., Pio, C.A., Freitas, M.C., Reis, M.A. and Trancoso, M.A., 2005. Source apportionment of fine and coarse particulate matter in a sub-urban area at the Western European Coast. Atmospheric Environment, 39(17), pp.3127-3138.

Araujo, J.A. and Nel, A.E., 2009. Particulate matter and atherosclerosis: role of particle size, composition and oxidative stress. Particle and fibre toxicology, 6(1), p.24.

Atkinson, R.W., Kang, S., Anderson, H.R., Mills, I.C. and Walton, H.A., 2014. Epidemiological time series studies of PM2.5 and daily mortality and hospital admissions: a systematic review and meta-analysis. Thorax, pp.thoraxjnl-2013.

Brook, R.D., Rajagopalan, S., Pope, C.A., Brook, J.R., Bhatnagar, A., Diez-Roux, A.V., Holguin, F., Hong, Y., Luepker, R.V., Mittleman, M.A. and Peters, A., 2010. Particulate matter air pollution and cardiovascular disease. Circulation, 121(21), pp.2331-2378.

Chen, S.Y., Chan, C.C. and Su, T.C., 2017. Particulate and gaseous pollutants on inflammation, thrombosis, and autonomic imbalance in subjects at risk for cardiovascular disease. Environmental Pollution, 223, pp.403-408.

Cho, S.Y. and Park, S.S., 2013. Resolving sources of water-soluble organic carbon in fine particulate matter measured at an urban site during winter. Environmental Science: Processes & Impacts, 15(2), pp.524-534.

Daher, N., Ruprecht, A., Invernizzi, G., De Marco, C., Miller-Schulze, J., Heo, J.B., Shafer, M.M., Shelton, B.R., Schauer, J.J. and Sioutas, C., 2012. Characterization, sources and redox activity of fine and coarse particulate matter in Milan, Italy. Atmospheric Environment, 49, pp.130-141.

Daher, N., Saliba, N.A., Shihadeh, A.L., Jaafar, M., Baalbaki, R., Shafer, M.M., Schauer, J.J. and Sioutas, C., 2014. Oxidative potential and chemical speciation of size-resolved particulate matter (PM) at near-freeway and urban background sites in the greater Beirut area. Science of the Total Environment, 470, pp.417-426.

de Kok, T.M., Driece, H.A., Hogervorst, J.G. and Briedé, J.J., 2006. Toxicological assessment of ambient and traffic-related particulate matter: a review of recent studies. Mutation Research/Reviews in Mutation Research, 613(2), pp.103-122.

Eruslanov, E. and Kusmartsev, S., 2010. Identification of ROS using oxidized DCFDA and flow-cytometry. Advanced protocols in oxidative stress II, pp.57-72.

Hamad, S.H., Shafer, M.M., Kadhim, A.K., Al-Omran, S.M. and Schauer, J.J., 2015. Seasonal trends in the composition and ROS activity of fine particulate matter in Baghdad, Iraq. Atmospheric Environment, 100, pp.102-110.

He, F., Liao, B., Pu, J., Li, C., Zheng, M., Huang, L., Zhou, Y., Zhao, D., Li, B. and Ran, P., 2017. Exposure to Ambient Particulate Matter Induced COPD in a Rat Model and a Description of the Underlying Mechanism. Scientific Reports, 7, p.45666.

Heo, J., Schauer, J.J., Yi, O., Paek, D., Kim, H. and Yi, S.M., 2014. Fine particle air pollution and mortality: importance of specific sources and chemical species. Epidemiology, 25(3), pp.379-388.

Heo, J., Antkiewicz, D.S., Shafer, M.M., Perkins, D.A., Sioutas, C. and Schauer, J.J., 2015. Assessing the role of chemical components in cellular responses to atmospheric particle matter (PM) through chemical fractionation of PM extracts. Analytical and bioanalytical chemistry, 407(20), pp.5953-5963.

Hiraiwa, K. and van Eeden, S.F., 2014. Nature and consequences of the systemic inflammatory response induced by lung inflammation. In Lung Inflammation. InTech.

International Agency for Research on Cancer, 2013. IARC: Outdoor air pollution a leading environmental cause of cancer deaths. International Agency for Research on Cancer.

Kim, I.S., Jang, J.Y., Kim, T.H., Park, J., Shim, J., Kim, J.B., Byun, Y.S., Sung, J.H., Yoon, Y.W., Kim, J.Y. and Cho, Y.J., 2015. Guidelines for the prevention and management of cardiovascular disease associated with fine dust/Asian dust exposure. Journal of the Korean Medical Association, 58(11), pp.1044-1059.

Landreman, A.P., Shafer, M.M., Hemming, J.C., Hannigan, M.P. and Schauer, J.J., 2008. A macrophage-based method for the assessment of the reactive oxygen species (ROS) activity of atmospheric particulate matter (PM) and application to routine (daily-24 h) aerosol monitoring studies. Aerosol Science and Technology, 42(11), pp.946-957.

Li, T., Yan, M., Sun, Q. and Anderson, G.B., 2018. Mortality risks from a spectrum of causes associated with wide-ranging exposure to fine particulate matter: A case-crossover study in Beijing, China. Environment international, 111, pp.52-59.

McDonnell, W.F., Nishino-Ishikawa, N., Petersen, F.F., Chen, L.H. and Abbey, D.E., 2000. Relationships of mortality with the fine and coarse fractions of long-term ambient PM10 concentrations in nonsmokers. Journal of

Exposure Science and Environmental Epidemiology, 10(5), p.427.

Michael, S., Montag, M. and Dott, W., 2013. Pro-inflammatory effects and oxidative stress in lung macrophages and epithelial cells induced by ambient particulate matter. Environmental pollution, 183, pp.19-29.

Miyazaki, Y., Kondo, Y., Takegawa, N., Komazaki, Y., Fukuda, M., Kawamura, K., Mochida, M., Okuzawa, K. and Weber, R.J., 2006. Time-resolved measurements of water- soluble organic carbon in Tokyo. Journal of Geophysical Research: Atmospheres, 111(D23).

Park, S.S. and Cho, S.Y., 2011. Tracking sources and behaviors of water-soluble organic carbon in fine particulate matter measured at an urban site in Korea. Atmospheric environment, 45(1), pp.60-72.

Park, S., Cho, S.Y. and Bae, M.S., 2015. Source identification of water-soluble organic aerosols at a roadway site using a positive matrix factorization analysis. Science of The Total Environment, 533, pp.410-421.

Pope, C.A., Burnett, R.T., Thurston, G.D., Thun, M.J., Calle, E.E., Krewski, D. and Godleski, J.J., 2004. Cardiovascular mortality and long-term exposure to particulate air pollution. Circulation, 109(1), pp.71-77.

Querol, X., Alastuey, A., Viana, M.M., Rodriguez, S., Artíñano, B., Salvador, P., Do Santos, S.G., Patier, R.F., Ruiz, C.R., De la Rosa, J. and De La Campa, A.S., 2004. Speciation and origin of PM10 and $PM_{2.5}$ in Spain. Journal of Aerosol Science, 35(9), pp.1151-1172.

Ray, P.D., Huang, B.W. and Tsuji, Y., 2012. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cellular signalling, 24(5), pp.981-990.

Saffari, A., Daher, N., Samara, C., Voutsa, D., Kouras, A., Manoli, E., Karagkiozidou, O., Vlachokostas, C., Moussiopoulos, N., Shafer, M.M.

and Schauer, J.J., 2013. Increased biomass burning due to the economic crisis in Greece and its adverse impact on wintertime air quality in Thessaloniki. Environmental science & technology, 47(23), pp.13313-13320.

Saffari, A., Daher, N., Shafer, M.M., Schauer, J.J. and Sioutas, C., 2014. Global perspective on the oxidative potential of airborne particulate matter: a synthesis of research findings. Environmental science & technology, 48(13), pp.7576-7583.

Schaumann, F., Borm, P.J., Herbrich, A., Knoch, J., Pitz, M., Schins, R.P., Luettig, B., Hohlfeld, J.M., Heinrich, J. and Krug, N., 2004. Metal-rich ambient particles (particulate matter2.5) cause airway inflammation in healthy subjects. American journal of respiratory and critical care medicine, 170(8), pp.898-903.

Schwartz, J., Dockery, D.W. and Neas, L.M., 1996. Is daily mortality associated specifically with fine particles?. Journal of the Air & Waste Management Association, 46(10), pp.927-939.

Schwarze, P.E., Totlandsdal, A.I., Herseth, J.I., Holme, J.A., Låg, M., Refsnes, M., Øvrevik, J., Sandberg, W. and Bølling, A., 2010. Importance of sources and components of particulate air pollution for cardio-pulmonary inflammatory responses. Air pollution, pp.47-54.

Shafer, M.M., Perkins, D.A., Antkiewicz, D.S., Stone, E.A., Quraishi, T.A. and Schauer, J.J., 2010. Reactive oxygen species activity and chemical speciation of size-fractionated atmospheric particulate matter from Lahore, Pakistan: an important role for transition metals. Journal of Environmental Monitoring, 12(3), pp.704-715.

Shuster-Meiseles, T., Shafer, M.M., Heo, J., Pardo, M., Antkiewicz, D.S., Schauer, J.J., Rudich, A. and Rudich, Y., 2016. ROS-generating/ARE-activating capacity of metals in roadway particulate matter deposited in urban environment. Environmental research, 146, pp.252-262.

Snyder, D.C., Rutter, A.P., Collins, R., Worley, C. and Schauer, J.J., 2009. Insights into the origin of water soluble organic carbon in atmospheric fine particulate matter. Aerosol Science and Technology, 43(11), pp.1099-1107.

Tchounwou, P.B., Centeno, J.A. and Patlolla, A.K., 2004. Arsenic toxicity, mutagenesis, and carcinogenesis–a health risk assessment and management approach. Molecular and cellular biochemistry, 255(1-2), pp.47-55.

Valavanidis, A., Fiotakis, K. and Vlachogianni, T., 2008. Airborne particulate matter and human health: toxicological assessment and importance of size and composition of particles for oxidative damage and carcinogenic mechanisms. Journal of Environmental Science and Health, Part C, 26(4), pp.339-362.

Valavanidis, A., Vlachogianni, T., Fiotakis, K. and Loridas, S., 2013. Pulmonary oxidative stress, inflammation and cancer: respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms. International journal of environmental research and public health, 10(9), pp.3886-3907.

Verma, V., Polidori, A., Schauer, J.J., Shafer, M.M., Cassee, F.R. and Sioutas, C., 2009. Physicochemical and toxicological profiles of particulate matter in Los Angeles during the October 2007 southern California wildfires. Environmental science & technology, 43(3), pp.954-960.

Viana, M., Maenhaut, W., Ten Brink, H.M., Chi, X., Weijers, E., Querol, X., Alastuey, A., Mikuška, P. and Večeřa, Z., 2007. Comparative analysis of organic and elemental carbon concentrations in carbonaceous aerosols in three European cities. Atmospheric Environment, 41(28), pp.5972-5983.

Watson, J.G. and Chow, J.C., 2001. Source characterization of major emission sources in the Imperial and Mexicali Valleys along the US/Mexico border. Science of the Total Environment, 276(1), pp.33-47.

Yu, J.Z., Xu, J. and Yang, H., 2002. Charring characteristics of atmospheric organic particulate matter in thermal analysis. *Environmental science & technology*, *36*(4), pp.754-761.

Zhang, Y., Schauer, J.J., Shafer, M.M., Hannigan, M.P. and Dutton, S.J., 2008. Source apportionment of *in vitro* reactive oxygen species bioassay activity from atmospheric particulate matter. Environmental science & technology, 42(19), pp.7502-7509.

Zhang, Z., Chau, P.Y., Lai, H.K. and Wong, C.M., 2009. A review of effects of particulate matter-associated nickel and vanadium species on cardiovascular and respiratory systems. International journal of environmental health research, 19(3), pp.175-185.

# 초 록

# 서울의 대기 중 초미세먼지의 Reactive Oxygen Species (ROS) activity 분석

**박지은**
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대기 중 미세먼지의 증가는 호흡기계 및 심혈관계 질환에 의한 사망률과 유병률에 큰 영향을 미치며, World Health Organization (WHO)의 International Agency for Research on Cancer (IARC)는 미세먼지를 폐암을 유발하는 1급 발암물질로 보고하였다. 그 중에서도 직경이 2.5㎛ 이하인 초미세먼지($PM_{2.5}$)는 작은 크기 때문에 폐포 깊숙이 침투하여 흡수되기 용이한 것으로 알려져 있고, 이 때문에 초미세먼지가 미세먼지보다 건강영향에 더 많은 악영향을 준다고 보고되고 있다. 현재까지 우리나라에서는 초미세먼지와 건강영향에 대한 역학적 연구는 수행되어왔지만 질병들의 시초가 되는 세포단위의 노출평가는 아직 진행된 바가 없다. 따

라서 본 연구는 서울에서 채취한 대기 중 초미세먼지의 화학성분 및 오염원을 도출하고 Reactive Oxygen Species (ROS) activity와의 상관성을 분석하는데 초점을 맞추었다. $PM_{2.5}$의 농도는 계절을 cold season과 warm season으로 나누었을 때 cold season 일 때 높게 나타났으며, Secondary Organic Carbon (SOC)과 $SO_4^{2-}$를 제외한 나머지 화학 종들도 같은 양상을 보였다. 그 중에서도 cold season 일 때 금속성분 중 지각성분이 전체 $PM_{2.5}$ 농도의 20%를 차지하는 것으로 보아 국외에서 유입된 황사 스모그의 영향을 받았을 가능성이 높은 것으로 사료된다. 각각의 화학 종들과 ROS activity와의 상관계수분석을 통해 Water Soluble Organic Carbon (WSOC)과 수용성 전이금속인 Cr과 Zn가 높은 상관성을 보이는 것을 확인하였고, 단계적 다중 선형 회귀분석을 통하여 As과 V이 높은 상관성을 보이는 것을 확인하였다. 주성분분석을 통하여 soil, mobile, industry, secondary inorganic aerosol (SIA), secondary organic aerosol (SOA), 그리고 oil combustion 등 6개의 오염원을 도출하였고, 그 중에서도 industry, soil, mobile 그리고 SIA과 ROS activity가 유의한 상관성을 보였다.

**주요 단어: 초미세먼지 ($PM_{2.5}$), 화학적 조성, 활성산소, 건강영향**

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