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DETERMINING THE DISTRIBUTION OF ELEMENTAL COMPOUNDS AND
OXIDATIVE POTENTIAL ACROSS FINE PARTICULATE MATTER (PM_{2.5})
FILTERS

by
Allie Michelle Sidwell

A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of
the requirements of the Sally McDonnell Barksdale Honors College.

Oxford
April 2022

Approved by

Advisor: Professor Courtney Roper

Reader: Professor Kristine Willett

Reader: Professor Robert Brian Doctor

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DEDICATION

This work is dedicated to my parents, who have made every opportunity in my life possible. Thank you.

ACKNOWLEDGEMENTS

The author would like to thank the BioMolecular Sciences Department at the University of Mississippi, members of the Roper lab, the Arkansas Department of Environmental Quality for donating the filters used in this research, and BioRender for providing the software used to create the methods figure. Most importantly, the author would like to thank Courtney Roper for her role in this work as an outstanding research advisor and mentor.

ABSTRACT

ALLIE MICHELLE SIDWELL: Determining the Distribution of Elemental Compounds and Oxidative Potential across Fine Particulate Matter (PM_{2.5}) Filters
(Under the direction of Courtney Roper, PhD)

Fine particulate matter (PM_{2.5}) is a complex mixture of particles and sorbed chemicals that poses serious, adverse effects on human health such as increasing cardiovascular and respiratory morbidity and mortality. There is ongoing research into the impacts of PM_{2.5} of differing chemical compositions, sampling location, and the mechanisms for the observed health effects. To conduct these analytical and toxicology studies of PM_{2.5}, researchers often split filters into sections. This process allows multiple, often destructive, assays to be performed. Our previous research showed chemical composition differences across PM_{2.5} filters. The goal of our study was to determine the validity of splitting filters for use in multiple analyses, analyze differences between an urban and rural sampling location, and examine trends between PM_{2.5} components and toxicology by assessing differences in chemical composition and oxidative potential within the same filter. Six PM_{2.5} filter samples collected from urban and rural locations were used. Each filter was split into quadrants, resulting in a total of 24 pieces; laboratory and blank filters were also prepared in the same manner. Each filter piece was extracted, concentrated, and then analyzed with dithiothreitol (DTT) assay run in triplicate to determine oxidative potential. Inductively coupled plasma mass spectrometry (ICP-MS) was run on all samples and controls to compare chemical composition of the filter quadrants (n=30). Stark differences in total elemental content and oxidative potential were observed between quadrants of the same filter. Correlation analysis between oxidative potential and elements yielded significant, positive correlations between oxidative potential

normalized by PM_{2.5} mass and the elements Ag, Ba, Cr, and Ga at the rural location and a significant, negative correlation between oxidative potential normalized by PM_{2.5} mass and the element Cs at the urban location. This work will provide information about the feasibility of splitting PM_{2.5} filters for multiple analyses on the same sample and well as insight into the different sources and toxicology of PM_{2.5} components.

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LIST OF ABBREVIATIONS

ADEQ	Arkansas Department of Environmental Quality
ANOVA	analysis of variance
CO	carbon monoxide
DTNB	5,5'-Dithiobis (2'-nitrobenzoic acid)
DTT	dithiothreitol
NO ₂	nitrogen dioxide
PAHs	Polycyclic aromatic hydrocarbons
PM _{2.5}	fine particulate matter
PM ₁₀	coarse particulate matter
ppb	parts per billion
ppm	parts per million
ROS	reactive oxygen species
SD	standard deviation
WHO	world health organization

INTRODUCTION

I. Air Pollution

The World Health Organization (WHO) estimates that 9 out of 10 people breathe polluted air that exceeds WHO guideline limits, which leads to seven million premature deaths per year (WHO, 2021). Air pollution is any substance in the air that has harmful effects, whether that be to living things or the climate (US EPA, 2014a).

There are many different types of air pollution, and the Environmental Protection Agency (EPA) focuses on six “Criteria Pollutants” in the air: carbon monoxide, lead, nitrogen dioxide, ground-level ozone, sulfur oxides, and particulate matter (US EPA, 2016f). Each type of air pollutant has negative health effects on humans and is regulated by the EPA. Carbon monoxide (CO) is a colorless, odorless gas released by the burning of fossil fuels and can reduce the amount of oxygen in the bloodstream when inhaled in large amounts (Weaver, 2009). The EPA sets the standard of CO exposure to less than 9 parts per million (ppm) as an eight-hour average or 35 ppm as a one-hour average more than once a year (US EPA, 2014b). Lead is a heavy metal that can adversely affect many systems in the body such as renal, cardiovascular, and especially neurological systems (Assi et al., 2016). Lead can enter the air from ore and metal processing, aircrafts utilizing leaded aviation fuel, and waste incinerators (US EPA, 2016a). The lead standard set by the EPA is less than 0.15 micrograms per cubic meter of air ($\mu\text{g}/\text{m}^3$) as a three-month average (US EPA, 2014b). Nitrogen dioxide (NO_2) enters the air through the burning of fuels such as from vehicles and power plants. Short-term exposure to NO_2 can

aggravate preexisting respiratory diseases while long term exposure may contribute to the development of respiratory diseases such as asthma (US EPA, 2016e). The standard set by the EPA for nitrogen oxides is a one-hour exposure 100 parts per billion (ppb) and an annual standard of 53 ppb (US EPA, 2014b). Ground-level ozone is the primary component of smog and can negatively impact those with respiratory issues by aggravating lung diseases and increasing the frequency of asthma attacks (US EPA, 2015). The standard is set at less than 0.070 ppm as the fourth highest daily maximum eight-hour concentration averaged over three consecutive years (US EPA, 2014b). Sulfur oxides (such as sulfur dioxide) enter the atmosphere from the burning of fossil fuels, can harm the respiratory tract, and levels are correlated with significant increases in hospital admissions for cardiovascular disease across the world (Chen et al., 2012; Sunyer et al., 2003; US EPA, 2016d). The standard is 75 ppb based on the three-year average of the 99th percentile of the yearly distribution of one hour daily maximum concentrations (US EPA, 2014b). Each of the six criteria pollutants monitored by the CDC have serious negative impacts on public health at and above their standard limits, and this paper will focus on the particulate matter portion of air pollution.

II. Particulate Matter

This study focuses on particulate matter, the particle pollution component of air pollution which consists of solid or liquid particles suspended in the air (US EPA, 2016c). These particulates can be composed of acids, organic chemicals, metals, and soil or dust attached to carbon particles (US EPA, 2016b).

Particulate matter comes in a variety of shapes and sizes. Two commonly characterized and studied sizes are coarse (PM_{10}) and fine ($PM_{2.5}$) particulate matter, both of which are inhalable. PM_{10} encompasses all particulate matter with an aerodynamic diameter of less than 10 microns and is often referred to as coarse particulate matter. PM_{10} is able to penetrate deep into lungs and some particles might even be able to enter the bloodstream. $PM_{2.5}$ is particulate matter with an aerodynamic diameter of 2.5 microns or less. To put this in perspective, the average human hair is about 60 microns in diameter (Figure 1). Because of its smaller size, $PM_{2.5}$ is able to penetrate deeper into the lungs than most PM_{10} particles (US EPA, 2016b). If soluble, $PM_{2.5}$ can enter the bloodstream and if the particles are not soluble, the particles can reside within the lung indefinitely (Yang & Omaye, 2009). Due to heavy metals adsorbed to the pores and surface of $PM_{2.5}$, it also generates a larger amount of hydroxyl radicals than PM_{10} (Lodovici & Bigagli, 2011). The standards set by the EPA for PM_{10} is not to exceed $150 \mu\text{g}/\text{m}^3$ as a 24-hour average more than once a year. The standard limits of fine particulate matter exposure vary by organization. The 24-hour average limit value is set at $5 \mu\text{g}/\text{m}^3$ by the WHO and $12 \mu\text{g}/\text{m}^3$ by the EPA while the annual exposure limit is set at an average of $25 \mu\text{g}/\text{m}^3$ by the EU, $15 \mu\text{g}/\text{m}^3$ by the WHO, and $35 \mu\text{g}/\text{m}^3$ by the EPA (European Commission, 2021; US EPA, 2014a; WHO, 2021).

Particulate matter pollution can have negative effects on the environment, just as other types of pollution can. $PM_{2.5}$ is the main component of haze which reduces visibility in both cities and wilderness areas. Particulate matter is able to be carried by wind over long distances before settling on the ground or in water sources. Since chemical composition of $PM_{2.5}$ can vary widely, so can its effects on the environment,

ranging from contributing to acid rain and the acidification of water sources to adversely changing nutrient levels on land and water (US EPA, 2016c).



Figure 1: The size of PM_{2.5} compared to a human hair
US EPA, O. (2016b, April 19). Particulate Matter (PM) Basics [Overviews and Factsheets]. <https://www.epa.gov/pm-pollution/particulate-matter-pm-basics>

III. PM_{2.5} health concerns

PM_{2.5} poses a health concern to humans because of its ability to be inhaled and even enter the bloodstream. A study of U.S. metropolitan areas confirmed a death rate difference of at least a 15% when comparing the least polluted cities to the most

polluted cities (Yang & Omaye, 2009). PM_{2.5} exposure has been linked to decreased life expectancy and adverse health effects such as premature death in people with preexisting heart or lung disease, heart attacks, asthma aggravation, and increased hospital risk for diabetes mellitus and Parkinson's Disease (Feng et al., 2016; Pope et al., 2009; US EPA, 2016c; Zanobetti et al., 2014).

i. Pulmonary

PM_{2.5} exposure is linked to damage of the respiratory system. Oxidative damage and autophagy are observed in human lung epithelial cells when exposed to fine particulate matter which may contribute to the PM_{2.5}-induced reduction in lung function (Deng et al., 2013). Exposure of a model of nasal epithelial cells, human RPMI 2650 cells, to PM_{2.5} increased the production of inflammatory cytokines which initiate local inflammation and oxidative stress (Hong et al., 2016). A cross-sectional study of almost 110,000 adults found a significant relationship between increases in ambient PM_{2.5} and asthma outcomes in non-Hispanic black adults as well as the development of sinusitis in adults of all ethnicities (Nachman & Parker, 2012). The rate of hospital admissions for COPD is significantly associated with the particulate matter portion of air pollution (Yang & Omaye, 2009).

ii. Cardiovascular

Exposure to PM_{2.5} is associated with increased cardiovascular morbidity and mortality such as increased acute myocardial infarction, ischemic stroke, and congestive heart failure (Chiu & Yang, 2013; Hsieh et al., 2013; Madrigano et al., 2013). In multiple

studies, fine particulate matter has been shown to induce dysfunction of the endothelial cells lining the pulmonary arteries in murine models (Davel et al., 2012; Ying et al., 2009). Mice exposed to PM_{2.5} had increased superoxide generation, altered vasomotor tone, increased composite plaque area in the thoracic aorta, and enhanced atherosclerosis, thickening or hardening of the arteries caused by plaque buildup (Ying et al., 2009). Of deaths attributable to PM_{2.5} pollution, the majority were due to ischemic heart disease and stroke along with chronic obstructive pulmonary disease, lung cancer, lower respiratory infections, and Type 2 diabetes to a lesser extent (Health Effects Institute, 2021).

iii. Developmental

PM_{2.5} exposure was responsible for about 2 million neonatal disorders worldwide in 2017, including low birth weight and preterm birth (Health Effects Institute, 2021). A study analyzing the relationship between levels of PM_{2.5} in Massachusetts during pregnancy and premature births and birth weights over a nine year period found birth weight to be negatively associated with PM_{2.5} levels and positively associated with preterm births (Kloog et al., 2012). PM_{2.5} exposure in neonatal rats led to typical behavior features of autism such as communication deficits, novelty avoidance, and poor social interactions (Li et al., 2018). Juvenile rats exposed to PM_{2.5} showed significantly increased anxiety, decreased cognition, and decreased social play and grooming (Nephew et al., 2020).

iv. Neurological

A study conducted multi-site case-crossover analysis found short-term PM_{2.5} exposure is significantly associated with an increased risk of hospitalization for Parkinson's disease and increased all-cause mortality risk (Zanobetti et al., 2014). One study observed that mice exposed to PM_{2.5} for 12 weeks had increased depressive-like behaviors, upregulation of pro-inflammatory cytokines, and downregulation of anti-inflammatory cytokines (X. Liu et al., 2018).

IV. Oxidative Stress

One of the leading theories of how PM_{2.5} causes damage within the human body is by inducing oxidative stress (Deng et al., 2013; Manisalidis et al., 2020; Zanobetti et al., 2014). PM_{2.5} increases oxidative stress within the body by introducing oxidants, generating reactive oxygen species (ROS), or inducing the inflammatory response in cells (Hong et al., 2016; Lodovici & Bigagli, 2011). Oxidative stress is the state of imbalance between the production of ROS and the ability of cellular antioxidants to remove the toxic species (Limón-Pacheco & Gonsebatt, 2009). Oxidative stress is associated with human diseases such as cancer, diabetes, atherosclerosis, and neurodegenerative disorders as well as the process of aging (Limón-Pacheco & Gonsebatt, 2009). Oxidative stress can directly affect lipids and proteins or induce redox-sensitive pathways which lead to inflammation and cell death (Lodovici & Bigagli, 2011). One way oxidative stress negatively affects living systems is by increasing the activation of the transcription factor NF- κ B sevenfold within respiratory epithelial cells; NF- κ B can induce gene transcription of proinflammatory cytokines, proteins that generate mediators of inflammation, and

immune receptors (Lodovici & Bigagli, 2011). Additionally, particulate matter can carry transition metals and organic compounds such as polycyclic aromatic hydrocarbons (PAH) that can impact the mitochondrial function of inflammatory cells, leading to the production of ROS and causing oxidative stress within the body (Song et al., 2020). Damage to DNA done by the oxidative stress may also increase cancer risk, which may be related to the presence of PAHs in the particulates (Yang & Omaye, 2009).

Inflammation is a natural defense mechanism of the immune system, and in short-term inflammation, oxidative stress does not directly cause cell damage. In fact, short-term inflammation can promote the transcription of antioxidant genes to protect the body from ROS (Lodovici & Bigagli, 2011). However, long-term or chronic inflammation, such as from prolonged exposure to air pollution, that can lead to oxidation damage which is implicated in many diseases such as cardiovascular diseases, pulmonary diseases, diabetes, and Alzheimer's disease (Lodovici & Bigagli, 2011). PM_{2.5} may inhibit the transcription of antioxidant enzymes, further increasing the damage done by ROS (Lodovici & Bigagli, 2011).

Once in the body, PM_{2.5} can act as prooxidants of lipids and proteins and cause inflammation by producing free radicals in the body which inhibit the normal function of cellular components such as lipids, proteins, and DNA as well as impede cellular signaling pathways (Kampa & Castanas, 2008). Free radicals are unstable and reactive because they have an unpaired electron in their outer orbit; examples of free radicals include superoxide, nitric oxide, and hydroxyl radicals (Fang et al., 2002). An overabundance of free radicals in the body causes oxidative stress, which is linked to numerous diseases including heart attacks, chronic inflammatory diseases, central

nervous system disorders, and cancer (Kampa & Castanas, 2008). The metal components of PM_{2.5} can accumulate in cellular organelles and disrupt proper functions by inhibited enzymes, affecting gene expression, and interfering with membrane potentials (Kampa & Castanas, 2008). Free radicals are able to cleave bonds in the backbones of proteins, interfering with enzyme function (Moskovitz et al., 2002). These PM_{2.5} induced changes to the body lead to a wide variety of other well-researched, significant health effects related to cardiovascular and respiratory systems, along with growing evidence of negative effects on reproductive, developmental, and neurological systems (Kloog et al., 2012; Madrigano et al., 2013; Zanobetti et al., 2014). One suggested cause of respiratory and cardiovascular morbidity and mortality is increased inflammation caused by the deeply embedded particles within the lung, which inflammatory chemicals into the bloodstream, causing coagulation (Yang & Omaye, 2009). Chronic exposure to low concentrations of PM_{2.5} has been shown to alter vasomotor tone, increase vascular inflammation, and exacerbate atherosclerosis, the thickening or hardening of arteries, in mice (Lodovici & Bigagli, 2011).

V. Sources

PM comes from a wide variety of sources, both anthropogenic sources such as factories, automobiles, construction, and power plants and natural sources such as storms and volcanic eruptions (US EPA, 2016c). Even fine particulate matter collected in the summer versus the winter or from a rural versus urban sampling site has been shown to contain different elements and molecules (Carty et al., 2003; Vinson et al., 2020; Zhang et al., 2014).

According to the most recent Health Effects Institute report, the disease burden caused by PM_{2.5} exposure was attributable to emissions from residential (19.2%), industry (11.7%), and energy (10.2%) sectors (Institute, 2021). An estimated 3.83 million deaths in 2017 are attributed to PM_{2.5} exposure, and about 1 million of these deaths were caused by fine particulate matter produced from the combustion of fossil fuels. Another 750,000 deaths were caused by PM_{2.5} from the combustion of solid biofuel from household heating and cooking (Institute, 2021). Windblown dust caused an estimated 620,000 deaths (Institute, 2021).

VI. Collection of PM_{2.5}

PM_{2.5} is often collected onto filters for analysis and characterization. Ambient air monitors pull outdoor air through a filter designed to catch only fine particulate matter. There are a wide variety of air monitors on the market, and the federal reference lists 39 accepted air samplers (US EPA, 2020). There are air samplers that do not use filters, but they do not typically collect particles and therefore are not as useful in chemical analysis or toxicological work. PM_{2.5} can then be extracted from the filter using a variety of methods. Previous studies have assumed equal distribution of elements and molecules across filter samples and so split the filters into multiple section for multiple, often destructive analyses (Deng et al., 2013; Hong et al., 2016; K. Huang et al., 2012; X. H. H. Huang et al., 2014; Imrich et al., 2000; Pandey et al., 2013; Song et al., 2020). If PM_{2.5} is not collected evenly across filters, then using separate sections for multiples assays is not as accurate as previously thought.

VII. Components of PM_{2.5}

PM_{2.5} can be made of both organic (i.e. polycyclic aromatic hydrocarbons, dioxins, benzene) and inorganic (i.e. metals, carbon, chlorides, nitrates, sulfates) components (Manisalidis et al., 2020). A study covering 187 counties across the United States during each season found only 7 of the 52 tested components made up more one percent of total PM_{2.5} mass: ammonium, elemental carbon, organic carbon matter, nitrate, silicon, sodium, and sulfate (Bell et al., 2007). Fine particulate matter readily bonds toxic metals and PAHs (Pandey et al., 2013).

Several transition metals such as iron, lead, mercury, cadmium, silver, nickel, vanadium, chromium, cobalt, manganese, and copper from PM_{2.5} are capable of producing ROS within the body (Lodovici & Bigagli, 2011). One of the main sources of PM_{2.5} is vehicle emissions (Gautam et al., 2016; Rodríguez et al., 2004). Vehicular emissions are sources of elements such as Ca, K, Zn, Cu, Al, Pb, Cd, Fe, Mg, and Cr (Gautam et al., 2016; Gerlofs-Nijland et al., 2007; Y.-C. Lin et al., 2020; Vallius et al., 2005). Na, Cl, Sr, and Mg are sourced from seawater (Ledoux et al., 2017; Vallius et al., 2005). K, Fe, Ca, and Al can be associated with industrial activity (Hsieh et al., 2013; Vallius et al., 2005). Ni, Co, and V are associated with heavy fuel oil combustion, such as from shipping traffic (Ledoux et al., 2017; Vallius et al., 2005). Br, Zn, Mn, K, and Fe are associated with industrial processes and incineration (Vallius et al., 2005). Al, Mg, Ti, K, Ca, Fe, and Mn are sourced from soil dust (Oravisjärvi et al., 2003). Fe, Ag, and Rb are associated with steel factories (Ledoux et al., 2017). Iron, nickel, vanadium, cobalt, copper, and chromium are soluble (Knaapen et al., 2002). Vanadium and chromium (VI) adsorbed to PM_{2.5} affect oxidative DNA damage in human lymphocytes when reduced to

chromium (III) within the cells (Lodovici & Bigagli, 2011). Organic carbon compounds also play a significant role in PM_{2.5} induced oxidative stress (Lodovici & Bigagli, 2011). The PAHs adsorbed to PM_{2.5} may actually have an antiapoptotic effect which may explain the prolonged inflammatory state and may delay tissue repairs (Ferecatu et al., 2010).

VIII. Purpose of Studying Filters

In many air pollution studies, researchers utilize air filters to collect particulates for chemical and toxicological analysis (Bell et al., 2014; Davel et al., 2012; Deng et al., 2013; Hong et al., 2016; X. H. H. Huang et al., 2014; Longhin et al., 2013a; Pandey et al., 2013; Song et al., 2020). Many researchers assume that these pollutants are evenly distributed across the filter and utilize different portions of the filter for different analyses (Deng et al., 2013; Hong et al., 2016; K. Huang et al., 2012; X. H. H. Huang et al., 2014; Imrich et al., 2000; Pandey et al., 2013; Song et al., 2020). If fine particulate matter is unevenly spread across the filters, this assumption could lower the validity of the research. To the best of the author's knowledge, the distribution of PM_{2.5} across filters has not been tested in the past.

IX. Hypothesis

We hypothesized that PM_{2.5} mass, elemental concentration, and oxidative potential causing compounds were unevenly distributed across PM_{2.5} filter samples. Our null hypothesis was PM_{2.5} compounds showed no significant difference in concentration across the PM_{2.5} filter.

METHODS

I. Reagents

Solvents and reagents included methanol, 1,4-Dithiothreitol (DTT) (Thermo Fisher Scientific, Waltham, MA, USA), and 5,5'-Dthiobis (2'-nitrobenzoic acid) (DTNB) (Sigma Aldrich, St. Louis, MO, USA).

II. Sample Collection

PM_{2.5} and blank filter samples were collected by the Arkansas Department of Environmental Quality (ADEQ) and donated for this research. In total, seven 37 mm Teflon filters with ring support were used to collect PM_{2.5} or served as a blank filter control following federal reference monitor procedures with a flow rate of 16.6 liters per minute (lpm) (US EPA, 2020). Three filters were collected for 24 hours in Stuttgart, Arkansas on one weekend day, March 1st, and two weekdays, the 7th and 13th and three filters were collected in Little Rock, Arkansas on one weekend day, March 1st, and two weekdays, the 3rd and 7th of 2012. The two sampling locations were monitored by the ADEQ throughout the year and the sampling locations were approved to serve as residential locations in each city. A blank filter, without PM_{2.5} collected, from March 2012 was used as the blank filter control.

III. Black Carbon Analysis

Whole filters were analyzed for black carbon content using a SootScan at 880 nm with comparison to blank filter controls. Attenuation values were recorded and black carbon concentrations were determined using precise sampler runtimes with data reported based on the volume of air collected onto each filter.

IV. Sample Extraction

Whole filters were weighed to determine the total mass of the filter and collected PM_{2.5}. Filters were then cut into quadrants using ceramic scissors (to avoid contamination from metal scissors). Each quadrant was weighed, placed into a 15 mL tube, submerged in 4 mL of methanol and sonicated in a water bath (60 Hz, Branson Ultrasonics Corporation, Brookfield, CT) for 60 min. After sonication, each filter quadrant was removed and rinsed with additional methanol to collect residual particles. Each of the extracted samples or controls were then concentrated to 1 mL by nitrogen blow down. Control samples underwent identical procedures to PM_{2.5} collected filters.

V. Oxidative Potential Analysis

The dithiothreitol (DTT) assay was ran in 96-well plates as described in (Vinson et al., 2020) for all of the whole particle and soluble fraction samples and controls. A DTT calibration curve (0, 0.2, 0.6, 0.8, and 1.0 mM) was prepared on each plate to determine the amount of DTT consumed in each sample. Increased DTT consumption indicates increased oxidative potential. Each PM_{2.5} sample in methanol and controls underwent the DTT assay. Phosphate buffer, DTT solutions, and samples were added to appropriate wells. Plates were gently shaken to mix and incubated at 37° C for 20

minutes. After incubation, 1 mM DTNB was added to quench the DTT reaction. Plates were then shaken again and read in a plate reader at 412 nm. All samples, controls, and calibration standards were run in triplicate. Controls included blank filter controls, phosphate buffer only wells, and the 0 mM DTT calibration curve.

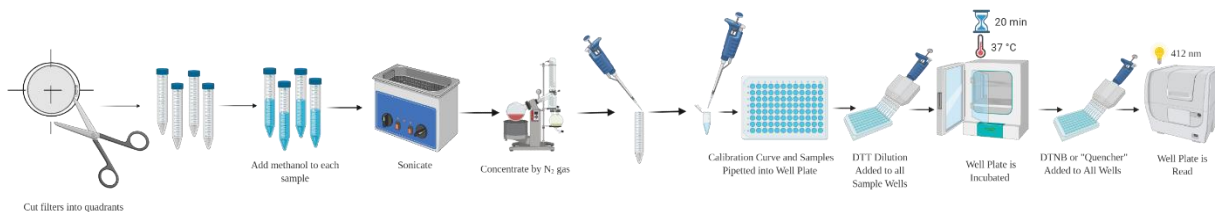


Figure 2: Filter Extraction, Concentration, and DTT Assay

VI. Elemental Analysis

ICP-MS was completed at the University of Mississippi ICP-MS Facility using aliquots of each sample blown to dryness with N_2 , resuspended in 10 mL of milli-Q water and sonicated for 5 minutes. Samples were analyzed using inductively coupled plasma – mass spectrometry (Thermo Fisher Element XR ICP-MS) in ppb for the following trace metals: Ag, Ba, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, Mn, Ni, P, Pb, Sr, Tl, U, V, and Zn. Each sample was run in triplicate. Calibration curves were generated using Multielement Calibration Standard Solution 2A (Spex Certiprep, Metuchen, NJ, USA). Reagent and laboratory blank filter controls were run for background subtraction and to measure instrumental drift during analysis.

VII. Statistical Analysis

Statistical analysis for all data was conducted with SigmaPlot 14.1 (Systat Software, Inc., San Jose, CA, USA) and Excel 16.0 (Microsoft Corporation Redmond, WA, USA). Data was reported as mean \pm standard deviation (SD) and corrected with blank filters or other appropriate controls. Data was analyzed using one- or two-way analysis of variance (ANOVA) or Student's t-test to investigate differences between samples. P values of ≤ 0.05 were considered significant. Pearson correlation coefficients were calculated between elemental concentrations and oxidative potential with p-values ≤ 0.05 considered statistically significant correlations.

RESULTS

I. Black Carbon

Black carbon was measured on each sample filter in triplicate and averages and standard deviations were reported (Figure 1). The range of black carbon concentrations for all samples was 1.34 to 5.95 $\mu\text{g}/\text{m}^3$. Overall, there was a trend that black carbon at the urban locations was higher than at the rural locations, however this difference was not significant. Black carbon at the rural location ranged from 1.34 to 4.00 $\mu\text{g}/\text{m}^3$ while the black carbon at the urban location ranged from 1.89 to 5.95 $\mu\text{g}/\text{m}^3$. Variability was observed between and within locations even though sampling at each location occurred within the same month.

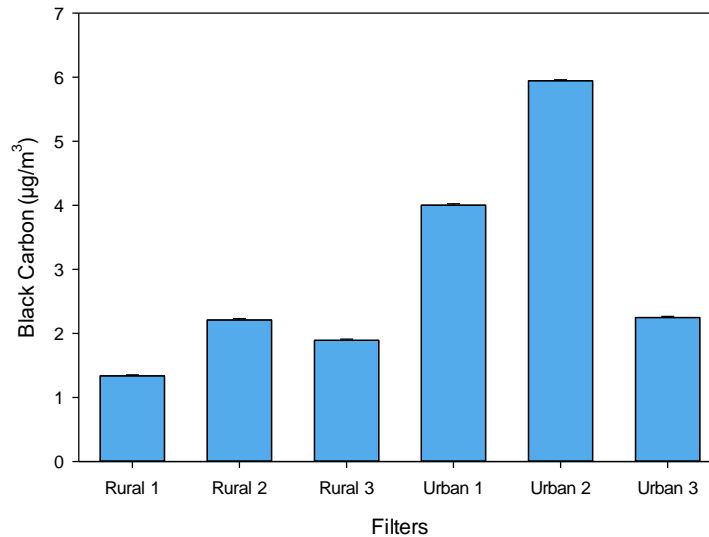


Figure 3: Black Carbon Concentrations

The black carbon concentrations ($\mu\text{g}/\text{m}^3$) for each sample with standard deviations are reported. Samples were run in triplicate and absorbance readings were compared to blank filters. A one-way ANOVA was run to determine statistical significance with $p \leq 0.05$ between rural and urban samples.

II. Elemental analysis

Elemental concentrations for each sample were determined by summing the concentrations of each filter quadrant (Figure 4). The values represent the total concentration of all measured elements (n=18) that were soluble in methanol. Rural concentrations ranged from 17.32 to 70.74 ppb and urban concentrations ranged from 8.19 to 86.14 ppb. The total elemental concentrations of Rural 3 sample and all Urban samples match the values of black carbon, whereas the Rural 1 and Rural 2 samples are greater relative to the other samples.

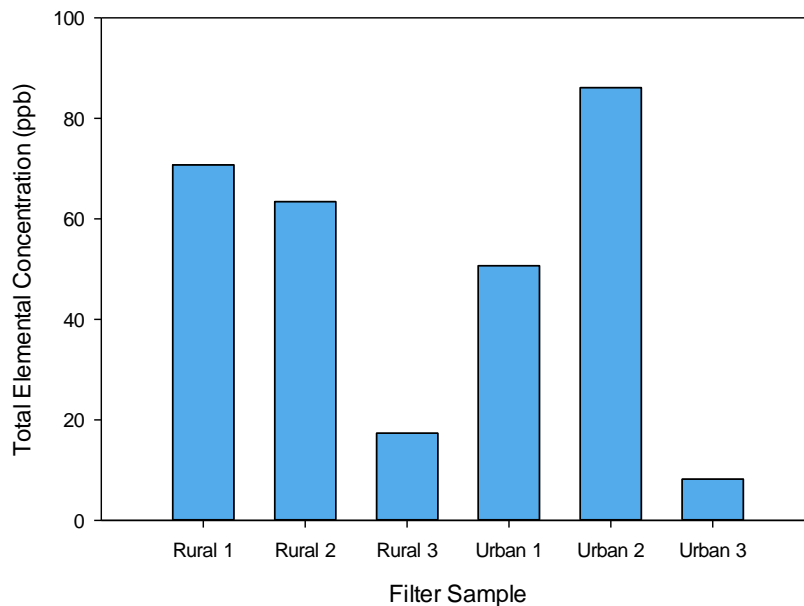


Figure 4: Elemental Concentration of Whole Filter Samples

Sum total of all elements measured are reported for each filter sample (ppb), following blank filter correction. The elements measured were: Rb, Sr, Ag, Cd, Cs, Ba, Ce, Pb, Th, U, V, Cr, Co, Ni, and Ga. A one-way ANOVA was run to determine statistical significance with $p \leq 0.05$ when comparing the rural and urban samples.

Elemental concentrations (ppb) of 18 elements (Rb, Sr, Ag, Cd, Cs, Ba, Ce, Pb, Th, U, V, Cr, Co, Ni, and Ga) were analyzed for each filter quadrant (Table 1). Neither thorium (Th) nor Uranium (U) were detected at either sampling site. Cesium (Cs) was only detected in the Urban 2 sample. T-tests were run to determine the significance in differences between the urban and rural filter elemental concentrations. The Rural samples had an insignificantly higher amount of PM_{2.5} (ppb) detected than the Urban samples. The differences between elemental concentrations from the rural and urban sampling site were nonsignificant except for the elements rubidium (Rb) ($P = 0.027$) and cerium (Ce) ($P = 0.000232$). Cerium concentration was significantly higher at the urban site and rubidium was significantly higher at the rural site.

Table 1: Elemental Concentrations of each Filter Quadrant of 16 Elements

Sample	Quadrant	Rb	Sr	Ag	Cd	Cs	Ba	Ce	Pb	Th	U	V	Cr	Co	Ni	Ga	Total	Filter Total
Rural 1	A	0.0355	0.5484	0.0202	0.0246	0	2.7895	0.0026	11.3441	0	0	0.4658	0.4040	0.0150	4.4221	0.0069	20.0787	70.7435
	B	0.0134	0.1049	0	0.0164	0	0.2031	0.0037	6.2669	0	0	0.3218	0.1206	0.0099	31.1063	0	38.1668	
	C	0.0140	0.1752	0	0.0215	0	0.4280	0.0112	2.7853	0	0	0.2748	0.0551	0	1.2359	0	5.0010	
	D	0.0003	0.0200	0	0.0094	0	0.0683	0.0056	2.6325	0	0	0.1833	0	0	4.5776	0	7.4970	
Rural 2	A	0.0338	0.4962	0	0.0419	0	0.7341	0.0378	2.4247	0	0	0.2029	0	0.0046	0.9313	0.0009	4.9081	63.4506
	B	0.0266	0.3622	0	0.0315	0	0.5192	0.0264	5.9486	0	0	0.1280	0.1449	0.0004	1.6655	0	8.8535	
	C	0.0461	0.5109	0.0002	0.0997	0	0.6935	0.0344	3.5180	0	0	0.1600	0.0462	0.0065	3.0802	0.0017	8.1973	
	D	0.0315	0.5176	0	0.0481	0	0.6660	0.0341	9.3231	0	0	0.1727	0.0949	0.0147	30.5891	0	41.4918	
Rural 3	A	0.0343	0.1381	0	0.0153	0	0.2536	0.0114	1.1098	0	0	0.1355	0	0	0	0	1.6978	17.3202
	B	0.0277	0.1604	0	0.0150	0	0.3049	0.0125	2.2652	0	0	0.1295	0	0	2.3268	0	5.2420	
	C	0.0438	0.2297	0	0.0359	0	0.3548	0.0159	1.4965	0	0	0.1848	0.2562	0	1.9529	0	4.5706	
	D	0.0242	0.0993	0.0	0.0167	0	0.1758	0.0086	1.8108	0	0	0.1236	0.2395	0.0011	3.3103	0	5.8099	
Urban 1	A	0.0312	0.2588	0.0047	0.0405	0	0.7238	0.0389	3.2985	0	0	0.3487	0	0.0083	1.2513	0.0029	6.0077	50.7119
	B	0.0245	0.2264	0	0.0383	0	0.7460	0.0398	4.8687	0	0	0.2836	0.3464	0.0044	9.0312	0	15.6092	
	C	0.0290	0.3161	0.0073	0.0379	0	0.9899	0.0455	4.6585	0	0	0.2699	0.0843	0.0156	16.3495	0.0045	22.8079	
	D	0.0264	0.1996	0.0026	0.0358	0	0.6573	0.0351	3.2427	0	0	0.2605	0.0010	0.0097	1.8159	0.0007	6.2872	
Urban 2	A	0.0160	0.5677	0.0044	0.0208	0	1.8349	0.1254	11.1966	0	0	0.0601	0.2902	0.0056	31.4176	0.0036	45.5429	86.1402
	B	0.0161	0.4509	0	0.0192	0.0006	1.5953	0.1083	5.1354	0	0	0.0731	0.4083	0	11.9065	0	19.7138	
	C	0.0142	0.5125	0	0.0241	0	1.7537	0.1252	2.9383	0	0	0.0818	0	0	0.1644	0.0016	5.6156	
	D	0.0099	0.4411	0	0.0184	0.0002	1.5136	0.1060	2.3056	0	0	0.0716	0	0	10.8016	0	15.2679	
Urban 3	A	0.0053	0.2749	0	0.0058	0	0.1292	0.0448	0.7737	0	0	0.1073	0	0	1.9162	0	3.2572	8.1905
	B	0.0098	0.0288	0	0.0077	0	0.2350	0.0511	0.3616	0	0	0.1288	0	0	0	0	0.8229	
	C	0.0106	0.0288	0	0.0105	0	0.2636	0.0775	1.9863	0	0	0.1350	0.0421	0	0.6827	0	3.2370	
	D	0.0061	0.0201	0	0.0056	0	0.1283	0.0523	0.5425	0	0	0.1184	0	0	0	0	0.8734	

The elemental concentration in ppb are shown for each filter quadrant for elements: Rb, Sr, Ag, Cd, Cs, Ba, Ce, Pb, Th, U, V, Cr, Co, Ni, and Ga.

Elemental concentrations for each filter quadrant based on the mass of PM_{2.5} were determined (Figure 5). These values represent the sum total of all quantifiable elements present in samples following blank correction. The expected results were that equal elemental concentrations would be observed for each quadrant of the filter samples. However, variability between filter quadrants is evident in all six samples with total element concentrations. For example, in the Rural 1 sample, the range in total elements between quadrants was 0.0721 to 0.562 ppb/μg of PM_{2.5}. Even in the Rural 3 sample, the highest elemental concentration is 0.0823 ppb/μg of PM_{2.5} which is 1.2 times higher than the lowest quadrant value of 0.0682 ppb/μg of PM_{2.5}.

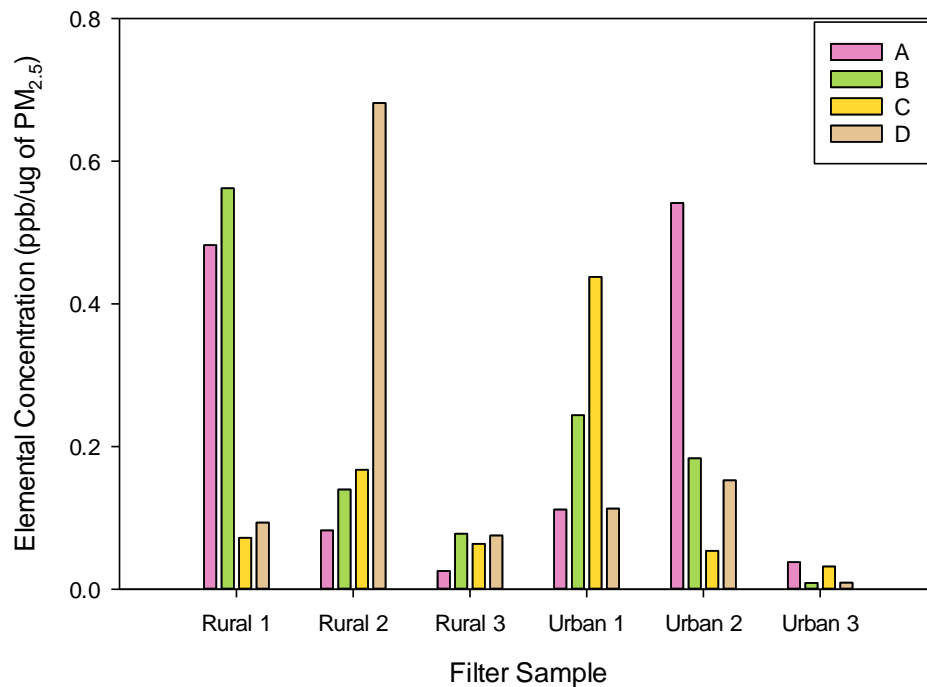


Figure 5: Elemental Concentrations across Filter Quadrants

Elemental concentrations in ppb/μg of PM_{2.5} for each filter quadrant. Samples were run in triplicate and blank corrected. Sum total elemental concentrations (i.e., Rb, Sr, Ag, Cd, Cs, Ba, Ce, Pb, Th, U, V, Cr, Co, Ni, and Ga) were reported and divided by the mass of PM_{2.5} present in each filter quadrant.

Quadrant contributions to the total elemental concentration were determined for each filter quadrant in all rural and urban filter samples (Figure 6). The expected results were that each quadrant contributed 25% of the total measured elemental concentration. Variation in quadrant contribution to total elemental concentration was seen in every rural and urban filter sample. For example, the Rural 1 filter ranged from 7.74 to 66.8 percent contribution to the total elemental concentration of the filter sample. In the Urban 2 sample, quadrant A contributed 8.4 times more than quadrant C. No filter sample exhibited the expected 25% quadrant distributions.

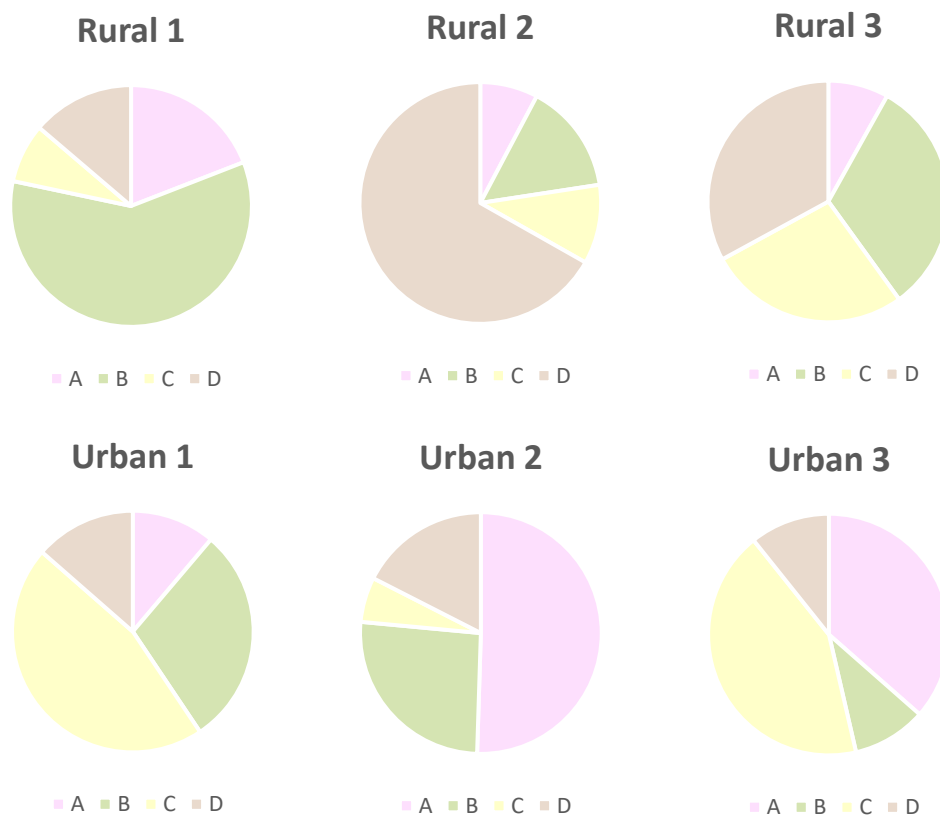


Figure 6: Elemental Concentration Contributions
 Percent contribution to the total elemental concentration of each filter quadrant.
 Percentages were corrected based on the mass of each quadrant.

Elemental concentrations of 18 metals were measured for each filter quadrant, and Pb, Ba, and Ni (Figure 7). If the amount of each element was evenly distributed across filter quadrants as expected, the elemental concentration should be equal for each quadrant of a filter sample. However, differences from this expected result were observed for each of the metals shown. For example, the Rural 1 sample ranges from 2.63 to 11.34 ppb of Pb, from 1.24 to 31.1 ppb for Ba, and from 0.0683 to 2.79 ppb for Ni. Additionally, all six filters had differences of more than two-fold between the highest and lowest quadrant concentration of Ba. All filters except Urban 1 had differences in Pb that were more than two-fold between quadrants. Only Rural 1 and Urban 3 had differences of two-fold or higher in the elemental concentration of Ni.

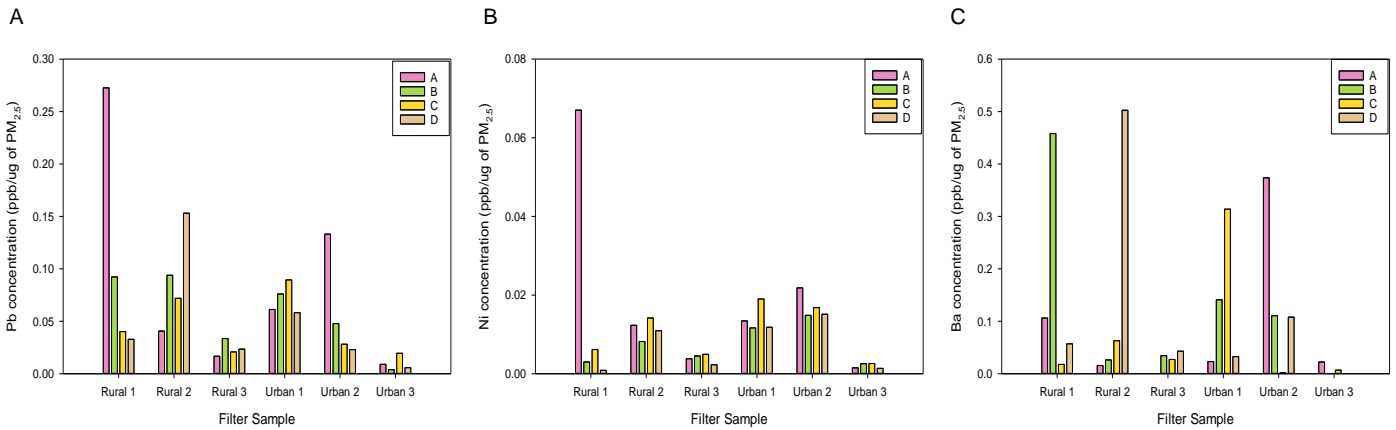


Figure 7: Elemental Concentrations of Pb, Ba, and Ni across Filter Quadrants

Elemental concentrations of Pb (A), Ni (B), and Ba (C) in ppb/ug of PM_{2.5} for each filter quadrant are shown. The x-axis lists the PM_{2.5} samples measured for rural and urban filters. Samples were run in triplicate and blank corrected. Sum total elemental concentrations were reported and divided by the mass of PM_{2.5} present in each filter quadrant.

III. Oxidative Potential

Oxidative potential of each filter sample was assessed by measuring DTT consumption over time per μg of $\text{PM}_{2.5}$ (Figure 8). All data were calculated using a DTT calibration curve. These values represent the sum of DTT consumed for each filter quadrant within a sample. For example, the DTT consumed in each of the four quadrants from Rural 1 were combined to determine the total DTT consumed from the sample. There was no significant difference between the total oxidative potential between rural and urban filter samples. DTT consumed ranged from 0.559 to 0.866 nmol/min.

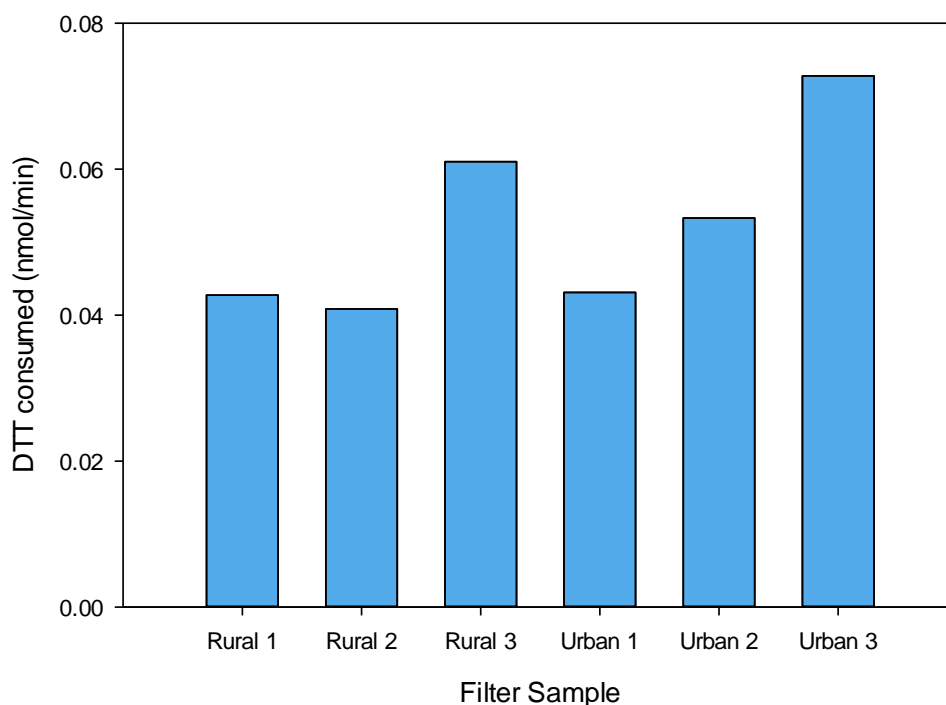


Figure 8: DTT Consumed per Filter Sample

DTT consumed in nmol/min for each filter sample. Samples (individual quadrants) were run in triplicate and calculated based on a standard DTT calibration curve. Quadrants were summed to determine the DTT consumed for the entire sample. A one-way ANOVA was run to determine statistical significance with $p \leq 0.05$ when comparing the rural and urban samples.

Oxidative potential of each filter quadrant was evaluated by measuring the DTT consumed (Figure 9). The amount of variability between filter quadrants differed between samples. For example, the Rural 1 sample ranged from 0.0330 to 0.257 pmol/min/ μg of $\text{PM}_{2.5}$, a 7.8 times difference. While the Urban 1 sample only ranged from 0.173 to 0.214 pmol/min/ μg of $\text{PM}_{2.5}$, a 1.2 times difference. The reported values are triplicate measurements of the same sample on different days, so statistical comparisons cannot be made between quadrants of the same sample. Overall, the average DTT consumed in each quadrant fell within the standard deviations of the other quadrants except for Rural 1 and Urban 2. These results demonstrate that differences in oxidative potential between filter quadrants exist.

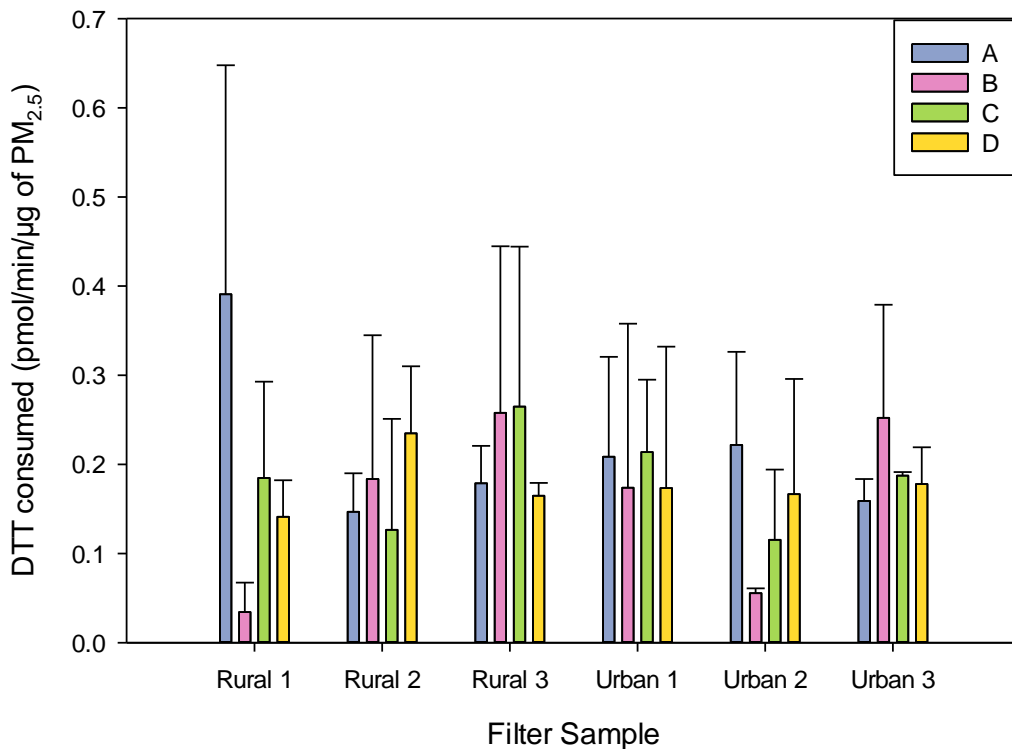


Figure 9: Oxidative Potential across Filter Quadrants
 The DTT consumed in pmol/min/ μg of $\text{PM}_{2.5}$ is shown across the quadrants (A-D) of each of the six samples. Averages of triplicate measurements and standard error mean are reported based on the DTT calibration curve.

Expected DTT consumed for each filter quadrant was calculated using the mass of each quadrant to determine the proportion of the total filter multiplied by the observed DTT consumed. These values result in the expected DTT consumed per quadrant and were compared to the observed DTT per quadrant (Figure 10). If the oxidative potential was evenly distributed across the filter, the observed and expected results would be the same value. Different degrees of variability between expected and measured oxidative potential were evident in some filter samples. For example, quadrant A in the Rural 1 sample had a measured concentration of 0.0163 (nmol/min) while the expected DTT consumed was 0.0069 (nmol/min), less than half of the measured value. However, in the Urban 1 sample, all measured and expected values are within 20 percent of each other. Unexpected variability was seen in some, but not all filter samples.

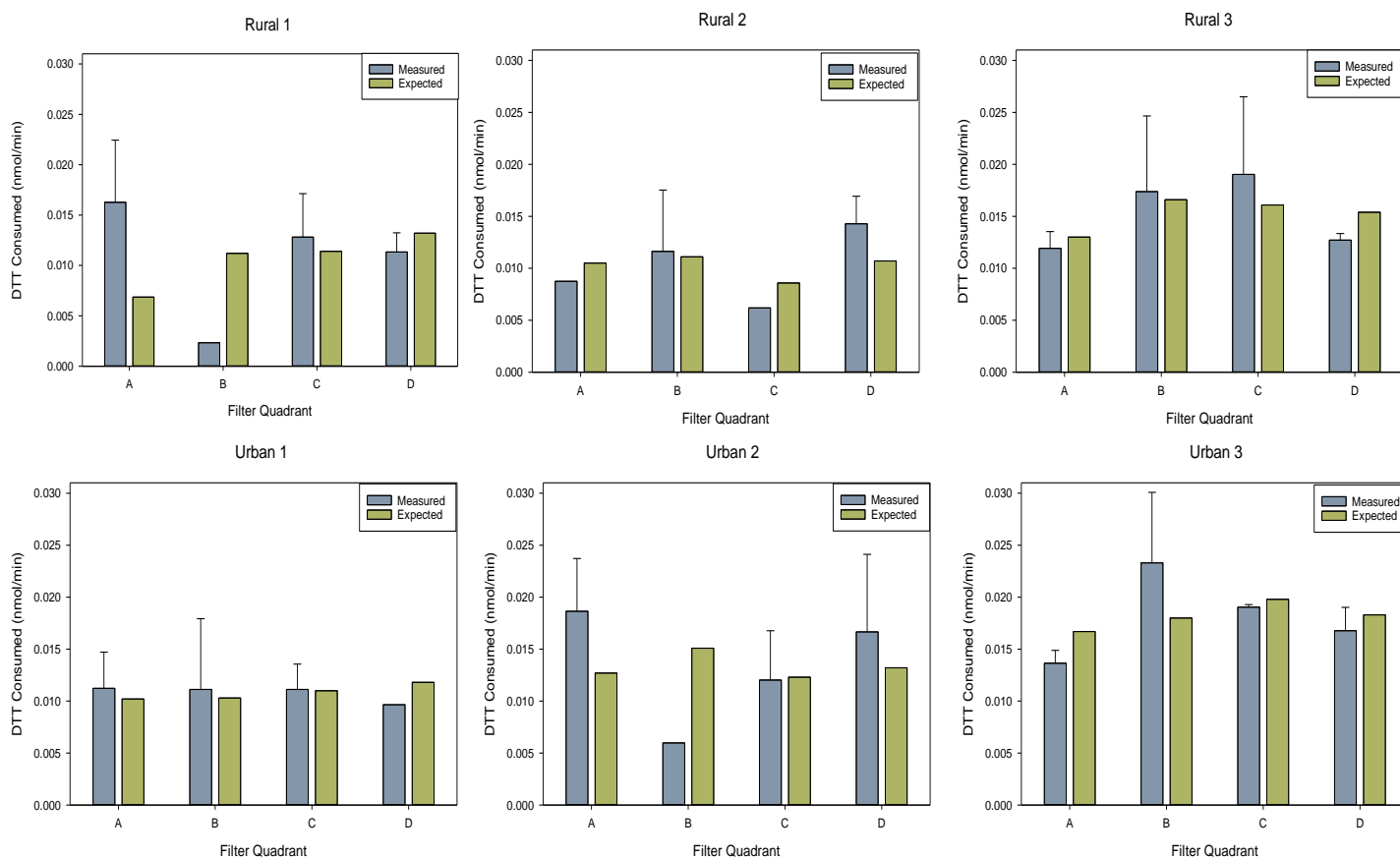


Figure 10: Measured versus Expected Oxidative Potential across Quadrants
 The measured and expected DTT consumed per minute in nmol/min is shown across the quadrants (A-D) for each of the six samples. Samples were run in triplicate and calculated based on a standard DTT calibration curve. Standard error means are reported for the measured, expected, values.

Quadrant contributions to the total oxidative potential were determined for each filter quadrant in all rural and urban filter samples (Figure 11). The expected results were that each quadrant contributed 25% of the total measured oxidative potential. Variability between contributions to total oxidative potential was observed in five filters (Rural 1-3 and Urban 2 and 3). For example, the Rural 1 sample had contributions ranging from 6.02 to 34.56 percent, depending on which quadrant was analyzed. The Urban 1 sample exhibited minimal variability between quadrant contributions, ranging from 24.50 to 26.30 percent. The Urban 1 sample matched the expected results of equal distribution of components across the filter while the other 5 filters did not have equal distribution.

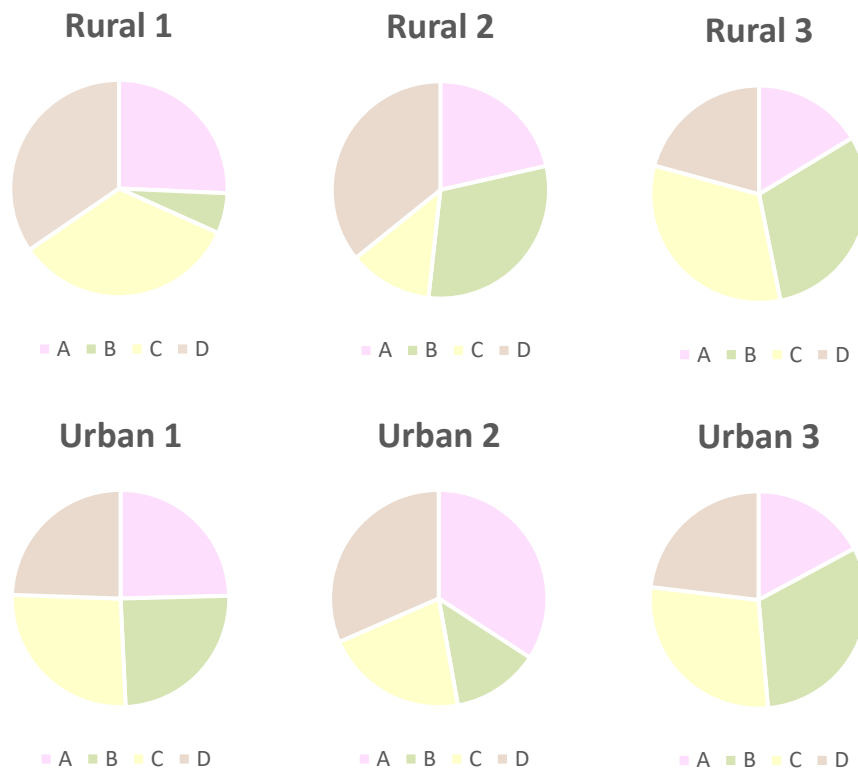


Figure 11: Percent of Total Oxidative Potential for Individual Filter Quadrants
 The percent contribution of filter quadrants (A-D) to the total oxidative potential is shown. Whole particle Rural 1-3 and Urban 1-3 are included. All measurements were made in triplicate.

IV. Correlations between Oxidative Potential and Elemental Concentrations

Elemental concentration and oxidative potential were analyzed for significant associations using a Pearson correlation (Tables 2-4). Significant correlations were seen between elements and oxidative potential dependent on the sampling location. There were significant, positive correlations between oxidative potential and Ag, Ba, Cr, and Ga elemental concentrations (nmol/min/ μg of $\text{PM}_{2.5}$) at the rural sampling site (Table 3). At the urban sampling site there was a significant, negative association between Cd and oxidative potential and between Cs and oxidative potential normalized by $\text{PM}_{2.5}$ mass. (Table 4). When samples from both locations were analyzed together, there were significant, positive correlations between oxidative potential and Ag and Ga concentrations (nmol/min/ μg of $\text{PM}_{2.5}$) (Table 2). There was no overlap in significant elements between rural and urban sampling sites. Notably, there was a moderate, nonsignificant negative trend between $\text{PM}_{2.5}$ mass and oxidative potential for both sampling sites.

Cesium (Cs) was only detected at the urban sampling site. Lead (Pb) and Nickel (Ni) had significant, strong positive associations with total elemental concentrations at both sampling sites, indicating the elements strongly trended with the amount of $\text{PM}_{2.5}$ collected at both the rural and urban site. Cobalt (Co) had a significant, strong positive trend with total elemental concentration only at the rural sampling site.

Specific examples of associations between elemental concentration and oxidative potential were analyzed (Figure 12). Significant, positive correlations between the elemental concentrations of Ag ($r^2 = 0.4154$) and Ga ($r^2 = 0.2701$) and oxidative potential

were analyzed using a Pearson correlation (Table 1). Pb ($r^2 = 0.0769$) and Ba ($r^2 = 0.0819$) both had nonsignificant, positive correlations between their concentrations and oxidative potential. Ni ($r^2 = 0.0225$) had a nonsignificant, negative association between its concentration and oxidative potential.

Table 2: Urban and Rural Pearson Correlation Coefficients

Rural + Urban	Rb	Sr	Ag	Cd	Cs	Ba	Ce	Pb	V	Cr	Co	Ni	Ga	Total Elements	Weight of Total PM _{2.5}
WP DTT nmol/min	-0.174	-0.178	0.115	-0.402	-0.266	0.022	0.171	-0.114	-0.258	-0.057	-0.271	-0.199	0.041	-0.188	0.304
WP DTT nmol/ug/min	0.345	0.099	0.645	-0.027	-0.388	0.286	-0.252	0.277	0.344	0.182	0.287	-0.150	0.520	-0.030	-0.250
		7													
Rb		0.405	0.283	0.738	-0.155	0.165	-0.353	0.223	0.345	0.236	0.432	-0.0549	0.333	0.031	-0.728
Sr			0.356	0.517	0.239	0.773	0.429	0.624	-0.016	0.333	0.394	0.333	0.504	0.472	-0.193
Ag				0.059	-0.0987	0.688	-0.133	0.597	0.656	0.418	0.637	0.0763	0.919	0.254	-0.477
Cd					-0.101	0.140	-0.096	0.224	0.216	0.051	0.447	0.0752	0.237	0.132	-0.591
Cs						0.322	0.445	0.059	-0.29	0.408	-0.194	0.119	-0.139	0.131	0.450
Ba							0.482	0.664	0.188	0.518	0.379	0.237	0.727	0.418	-0.038
Ce								0.101	-0.571	0.059	-0.202	0.197	0.061	0.209	0.675
Pb									0.345	0.606	0.664	0.694	0.602	0.838	-0.354
V										0.211	0.666	0.034	0.535	0.128	-0.728
Cr											0.209	0.328	0.328	0.448	-0.080
Co												0.529	0.666	0.608	-0.689
Ni													0.156	0.973	-0.058
Ga														0.321	-0.465
Total Elements															-0.142

A Pearson correlation was run to determine significant trends ($P \leq 0.05$) between elemental concentrations and oxidative potential for each filter quadrant. Samples were run in triplicate and blank corrected. Sum total elemental concentrations (i.e. Rb, Sr, Ag, Cd, Cs, Ba, Ce, Pb, Th, U, V, Cr, Co, Ni, and Ga) from both urban and rural locations were reported and divided by the mass of PM_{2.5} present in each filter quadrant.

Table 3: Rural Pearson Correlation Coefficients

Rural	Rb	Sr	Ag	Cd	Cs	Ba	Ce	Pb	V	Cr	Co	Ni	Ga	Total Elements	Weight of Total PM _{2.5}
WP DTT nmol/min	0.252	0.028	0.279	-0.264	--	0.242	-0.153	0.002	-0.061	0.362	-0.173	-0.367	0.157	-0.287	0.067
WP DTT nmol/ug/min	0.403	0.397	0.706	-0.091	--	0.707	-0.113	0.406	0.286	0.580	0.243	-0.280	0.628	-0.0807	-0.421
Rb		0.655	0.195	0.646	--	0.361	0.511	0.058	-0.093	0.277	0.213	-0.214	0.316	-0.129	-0.609
Sr			0.440	0.690	--	0.678	0.673	0.594	0.266	0.256	0.649	0.073	0.572	0.263	-0.868
Ag				-0.076	--	0.956	-0.351	0.680	0.802	0.711	0.576	-0.077	0.967	0.173	-0.667
Cd					--	0.147	0.748	0.105	-0.143	-0.075	0.299	0.016	0.145	0.058	-0.574
Cs						--	--	--	--	--	--	--	--	--	--
Ba							-0.072	0.731	0.755	0.649	0.659	-0.062	0.970	0.203	-0.824
Ce								0.001	-0.430	-0.347	0.130	0.021	-0.183	0.020	-0.332
Pb									0.648	0.525	0.881	0.548	0.639	0.756	-0.637
V										0.556	0.622	0.230	0.764	0.405	-0.508
Cr											0.405	0.033	0.635	0.208	-0.323
Co												0.670	0.603	0.824	-0.679
Ni													-0.126	0.961	-0.021
Ga														0.125	-0.789
Total Elements															-0.238

A Pearson correlation was run to determine significant trends ($P \leq 0.05$) between elemental concentrations and oxidative potential for each filter quadrant. Samples were run in triplicate and blank corrected. Sum total elemental concentrations (i.e. Rb, Sr, Ag, Cd, Cs, Ba, Ce, Pb, Th, U, V, Cr, Co, Ni, and Ga) from the rural location were reported and divided by the mass of PM_{2.5} present in each filter quadrant.

Table 4: Urban Pearson Correlation Coefficients

Urban	Rb	Sr	Ag	Cd	Cs	Ba	Ce	Pb	V	Cr	Co	Ni	Ga	Total Elements	Weight of Total PM _{2.5}
WP DTT nmol/min	-0.564	-0.387	-0.195	-0.609	-0.476	-0.312	0.080	-0.185	-0.372	-0.371	-0.363	-0.023	-0.136	-0.088	0.328
WP DTT nmol/ug/min	0.142	-0.414	0.441	0.044	-0.740	0.399	-0.422	0.012	0.342	-0.368	0.375	0.102	0.364	0.0492	-0.460
Rb		0.161	0.719	0.965	-0.078	0.203	-0.343	0.396	0.839	0.206	0.853	0.199	0.609	0.259	-0.832
Sr			0.241	0.292	0.357	0.924	0.712	0.669	-0.313	0.402	0.102	0.646	0.429	0.694	0.112
Ag				0.624	-0.246	0.204	-0.186	0.509	0.526	0.010	0.913	0.500	0.937	0.504	-0.729
Cd					-0.088	0.325	-0.239	0.413	0.779	0.219	0.785	0.217	0.556	0.285	-0.778
Cs						0.425	0.411	0.147	-0.350	0.570	-0.276	0.189	-0.266	0.204	0.432
Ba							0.809	0.693	-0.325	0.456	0.080	0.642	0.397	0.700	0.197
Ce								0.460	-0.757	0.304	-0.411	0.458	0.074	0.493	0.691
Pb									-0.039	0.692	0.389	0.901	0.600	0.946	-0.195
V										-0.089	0.726	-0.196	0.339	-0.168	-0.899
Cr											0.026	0.626	0.044	0.660	0.053
Co												0.347	0.778	0.357	-0.888
Ni													0.563	0.991	-0.105
Ga														0.583	-0.545
Total Elements															-0.117

A Pearson correlation was run to determine significant trends ($P \leq 0.05$) between elemental concentrations and oxidative potential for each filter quadrant. Samples were run in triplicate and blank corrected. Sum total elemental concentrations (i.e., Rb, Sr, Ag, Cd, Cs, Ba, Ce, Pb, Th, U, V, Cr, Co, Ni, and Ga) from the urban location were reported and divided by the mass of PM_{2.5} present in each filter quadrant.

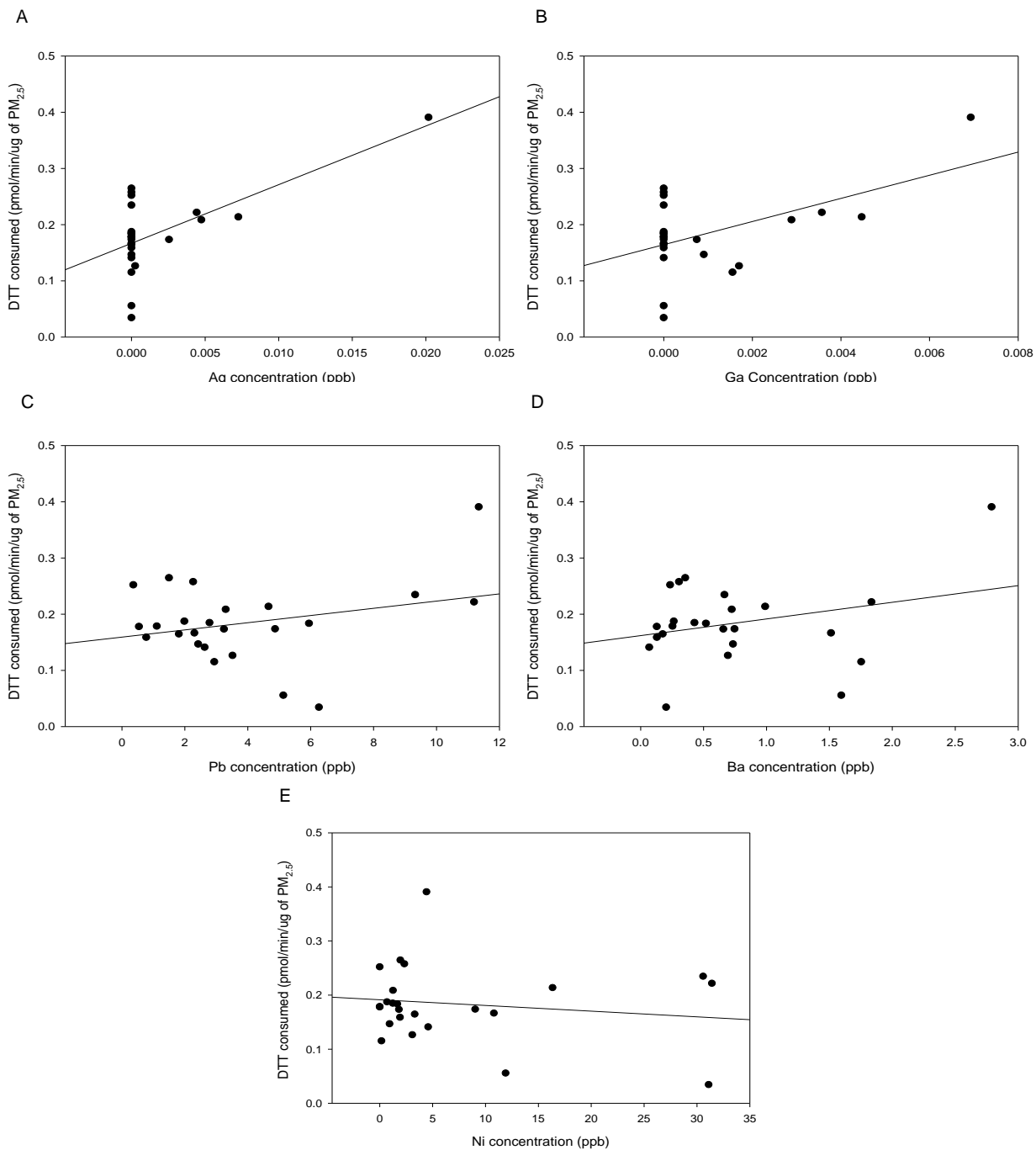


Figure 12: DTT Consumed vs Elemental Concentrations of Ag, Ga, Pb, Ba, and Ni

Elemental concentration in of Ag (A), Ga (B), Pb (C), Ba (D), and Ni (E) in ppb is compared to DTT consumed (pmol/min/μg of PM_{2.5}). Data was analyzed for significant associations ($P \leq 0.05$) using a Pearson correlation and t-tests.

DISCUSSION

I. Differences between Urban and Rural Sampling Locations

We found higher elemental concentrations in the samples from the rural location than samples from the urban location. Higher elemental concentrations in urban locations is well documented (Hueglin et al., 2005; Ledoux et al., 2017; C. Lin et al., 2018; T. Liu et al., 2021; Rodríguez et al., 2004; Strosnider, 2017) and expected because of the higher concentration of PM_{2.5} sources (i.e. vehicles, factories, power plants, construction). However, this is not always the case, as observed in a study in the Netherlands (Janssen et al., 2014) and India (Varshney et al., 2016), which both saw higher PM_{2.5} masses collected at rural sites when compared to urban sampling locations. The trend of higher PM_{2.5} mass collected in urban compared to rural areas could be due to the fact that some studies collected samples from significantly larger cities than Little Rock, which has a population of about 200,000. Additionally, the specific elements that were analyzed in our study may have differed compared to the previous studies that reported higher element concentrations. For example, Liu et al. analyzed Zn, Mn, Cu, and As, none of which we measured in our study.

One of the main sources of PM_{2.5} is vehicle emissions (Gautam et al., 2016; Rodríguez et al., 2004, p. 10). Vehicular emissions are sources of elements such as Ca, K, Zn, Cu, Al, Pb, Cd, Fe, Mg, and Cr (Gautam et al., 2016; Y.-C. Lin et al., 2020; Vallius et

al., 2005). We did not see a significant positive association between urban sourced PM_{2.5} and the associated elements that we tested for (i.e., Pb, Cd, Cr). Na, Cl, Sr, and Mg are sourced from seawater (Ledoux et al., 2017; Vallius et al., 2005). Neither sampling site was within 300 miles of the sea, so the sea is probably not a large contributor to PM_{2.5}. Of the elements sourced from seawater, we only tested for Sr and saw no significant difference in elemental concentration between the urban and rural site. K, Fe, Ca, and Al can be associated with industrial activity (Hsieh et al., 2013; Vallius et al., 2005). We were not able to test for any of these elements. Ni, Co, and V are associated with heavy fuel oil combustion, such as from shipping traffic (Ledoux et al., 2017; Vallius et al., 2005). We found no significant associations between any of the elements and location. Br, Zn, Mn, K, and Fe are associated with industrial processes and incineration (Vallius et al., 2005). We were not able to test for any of these elements due to quality assurance standards with the ICP-MS. Al, Mg, Ti, K, Ca, Fe, and Mn are sourced from soil dust (Oravisjärvi et al., 2003). We were not able to test for any of these. Fe, Ag, and Rb are associated with steel factories (Ledoux et al., 2017). There are multiple steel manufacturers in Little Rock, Arkansas, but we saw a significantly higher concentration of Rb at the rural site. This could be due to weather patterns affecting how the pollution released moves from one area to another.

At the rural location sampled, we found significant positive correlations between Ag, Ba, Pb, Cr, and Ga elemental concentrations (ppb) and oxidative potential (nmol/min/μg of PM_{2.5}). A study in rural China found significant, positive associations between iron and aluminum and oxidative potential when using the DTT assay to measure oxidative potential of the whole particle extracted PM_{2.5} (Secrest et al., 2016).

The group also found that transition and post-transition metals were mostly strongly associated with oxidative potential, which includes all of our elements with significant associations except Ba, which is an alkaline earth metal. Another study in rural China found significant associations between a wide variety of metals and oxidative potential, but the only overlapping significant correlation was Ag (Brehmer et al., 2019). The study also utilized the DTT assay to measure oxidative potential of the whole particle mixture of extracted PM_{2.5}. Even though the samples were collected in China and across all seasons, there were still similarities in associations between Ag and oxidative potential. A study in Switzerland found the least amount of PM_{2.5} mass collected on weekends (Hueglin et al., 2005). However, our two samples collected on a Saturday were not lower than the weekday samples.

At both sampling sites, there was no significant association between oxidative potential and PM_{2.5} mass, which was also seen in a study in Wuhan (Q. Liu et al., 2020, p. 5). The lack of correlation between PM_{2.5} mass and oxidative potential indicates that mass alone does not adequately explain the toxicological effects of fine particulate matter exposure. It is common in the literature to only analyze PM_{2.5} mass when investigating the health effects of air pollution (Davel et al., 2012; Kloog et al., 2012; Madrigano et al., 2013; Nachman & Parker, 2012; Zanobetti et al., 2014). However, investigating health trends with PM_{2.5} as the only variable leaves out the nuances that PM_{2.5} components (i.e., elemental carbon, organic carbon, ions) and sources (i.e., urban, rural, industrial, marine) have on toxicology (Bell et al., 2014; Deng et al., 2013; Hong et al., 2016; Imrich et al., 2000; Longhin et al., 2013b; Song et al., 2020)

One study found cobalt (Co) to be moderately reactive with DTT, but our results for all samples pooled showed a nonsignificant, negative association between Co and DTT consumption (on DTT as a measure) (Charrier & Anastasio, 2012). The group also found a moderate reaction between DTT and valium (V), and we saw the same with a Pearson correlation value of 0.344. The same study also found Cr and Cd do not cause measurable DTT oxidation, and we, similarly, saw no correlations between our sample Cr and Cd elemental concentrations and oxidative potential. One study found a significant, positive correlation between PM_{2.5} mass and oxidative potential measure using the DTT assay, which is different from our finding of no significant association between fine particulate matter mass and oxidative potential (Janssen et al., 2014). Janssen et al. also found no significant correlation between Ni and DTT consumed (spearman correlation value of 0.01), similar to our results of no significant trend with Pearson correlation value of -0.150. Janssen et al. also found a nonsignificant, negative correlation (spearman correlation value -0.11) between V and oxidative potential whereas we saw a low, nonsignificant, positive correlation (Pearson correlation value 0.344). The variety between what we found in our study and what is shown in the literature is likely due to differences in methodologies, sampling sites, and weather. More consistency between study methodology is needed for future air pollution toxicology studies in order to better identify and understand trends between sources and components and toxicology.

II. Comparison of Quadrants

The practice of splitting PM_{2.5} filters into sections for use in multiple analyses is prevalent in the literature (Bell et al., 2014; Davel et al., 2012; Deng et al., 2013; Hong et

al., 2016; X. H. H. Huang et al., 2014; Hueglin et al., 2005; Longhin et al., 2013b; Pandey et al., 2013; Secrest et al., 2016; Song et al., 2020). When splitting filters, we assume the equal distribution of PM_{2.5} components across the filter. However, to the best of the author's knowledge, this widespread assumption has never been tested, decreasing the validity of fine particulate matter pollution research when a filter is split into pieces and the results from different assays compared.

We tested the distribution of elemental concentrations across filters and found wide variation between the expected even distribution and the observed distribution. We found similar variability between oxidative potential across quadrants of the same filter. If PM_{2.5} was distributed as expected, we would have seen little variability between quadrants for both elemental concentration and oxidative potential, similar to the Urban 1 sample. The Urban 1 sample varied by less than 2% between quadrant contribution to total oxidative potential but by 35% between quadrant contribution to total elemental concentration. This difference between oxidative potential and elemental concentration between quadrants indicates that oxidative potential inducing components of PM_{2.5} were present that were not tested for in this study, including inorganic molecules, ions, elemental carbon, and organic carbon, such as polycyclic aromatic hydrocarbons (PAHs) (Deng et al., 2013; Hong et al., 2016; Longhin et al., 2013b; Pandey et al., 2013).

III. Implications for Toxicological Studies

We found that the distribution of PM_{2.5} components (i.e., elemental concentrations, oxidative potential) was uneven across the filters examined. The difference between quadrants varied between samples, indicating that the distribution of

components is variable. Our results highlight the concern that splitting a filter may result in chemical components that don't match their toxicology studies.

Splitting filters for use in multiple analyses is prevalent in the literature and a common practice in air pollution research across the globe (Bell et al., 2014; Davel et al., 2012; Deng et al., 2013; Hong et al., 2016; X. H. H. Huang et al., 2014; Hueglin et al., 2005; Longhin et al., 2013b; Pandey et al., 2013; Secrest et al., 2016; Song et al., 2020). For example, if a study split a filter into halves and used one half for the chemical analysis and the other half for an in vitro toxicology experiment, the results may not align with reality. If the chemical analysis half had a significantly higher amount of an element, say cesium, while the toxicology half had almost no cesium, inaccurate conclusions on the effects of cesium on the biological system tested would be drawn. Our results showed that differences between filter pieces varied between samples, so not every filter split may yield inaccurate results. However, most of our samples did show high variation in both elemental concentrations and oxidative potential, indicating that variation between pieces may be extremely common in PM_{2.5} collection on to filters.

IV. Future Directions

Consistency in methodology is needed. The wide variety of methods makes it difficult to compare across studies that investigate the same topics. A wide variety of filter types, air samplers, sampling locations, sampling seasons, extraction solvents, elemental analyses, and oxidative potential assays were used in the papers cited in this thesis. Consistency between methodologies is needed in order to more accurately compare and analyze air pollution data.

One way to design comparable studies is to conduct literature reviews in order to comprehend the methods currently in use and analyze which methodologies are the most accurate, affordable, and reasonable in order to set standards for future studies to follow. Included in these standards should be whether or not to split filters. If possible, splitting filters for use in different assays should be avoided to preserve validity of the study. However, it is not possible to run multiple destructive analyses (such as ICP-MS) on one filter without sectioning it. In this case, a note should be made in the methods section detailing that the filter was split so readers can keep that added variability in results in mind.

Additionally, research needs to be conducted into the air sampling methodologies. It is possible that the uneven deposition of $PM_{2.5}$ on filter samples is due to the vacuum systems being utilized. So, investigation of the ability to evenly collect $PM_{2.5}$ onto different types of filters using various types of air samplers and air flowrates could lead to better collection and analyzation of $PM_{2.5}$.

CONCLUSIONS

In this work we investigated the validity of splitting $PM_{2.5}$ filters for use in multiple analyses, the differences between a rural and urban sampling site, and the trends between elemental concentration and oxidative potential. The average black carbon concentration was higher at the urban collection site than at the rural collection site on the days sampled. This was to be expected as black carbon is predominantly produced by incomplete combustion of biomass and fossil fuels, such as from vehicles, which there are more of in a city (Winiger et al., 2016).

Overall elemental concentrations of the rural filters were not significantly higher than the urban filters. This was unexpected as cities usually contain more sources of particulate matter pollution than rural areas. The percent contribution to the total elemental mass between filter quadrants varied, indicating $PM_{2.5}$ might not be evenly deposited across the filter as assumed when splitting filters.

The percent contribution to the total oxidative potential between filter quadrants varied. There was no significant difference in oxidative potential between rural and urban sampling sites. Samples contained components of $PM_{2.5}$ we did not test for, such as PAHs. There were significant, positive correlations between oxidative potential and several elements for both the rural and urban sampling locations. There was a nonsignificant, positive correlation between total elemental concentration and oxidative potential. Total elemental concentration did not fully predict oxidative potential of the

samples, indicating other factors, such as specific elements and compounds, affect oxidative potential.

Future studies should be conducted avoiding splitting filters when possible and noting that filters were split for use in multiple analyses is unavoidable. Literature reviews should be conducted into PM_{2.5} analyses in order to provide consistent methodologies across studies to increase the validity of comparing data. Lastly, research into the air sampling techniques that provide the most even distribution of PM_{2.5} onto filter samples is necessary to limit the statistical inaccuracies created by uneven deposition of components.

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