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COMPOSITION AND OXIDATIVE POTENTIAL OF FINE PARTICULATE  
MATTER FROM PASCAGOULA, MS

By  
Madison Smith

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 2021

Approved by

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## DEDICATION

To my family,  
I could not have gotten this far in my education without all of the love and support.  
I would not have been able to do this without you.

Also, To Sandra Savage

## ACKNOWLEDGMENTS

I would like to thank Roper Lab for giving me the opportunity to research a topic I am genuinely interested in. There were many times where I did not believe in myself, but everyone in Roper Lab was so supportive and encouraging. I would not have been able to do this without them. I would also like to thank Maggie Craze for always being in the lab when I needed help and setting aside her own time to help me with some of the procedures in the lab.

A special thank you goes out to Dr. Courtney Roper who has been my advisor throughout this project. I lost count of how many times I wanted to just give up on this and felt like I had no idea what I was doing. Dr. Roper was always there if I needed help or reassurance. Without her patients and expertise, I probably would have given up on my research, so thank you so much Dr. Roper. I would not have been able to do it without you.

## ABSTRACT

Air pollution is one of the top-ranking risk factors for death and disability around the world, accounting for nearly 4.9 million deaths worldwide (State of Global Air, 2019). Specifically, fine particulate matter (PM<sub>2.5</sub>) has been linked to many adverse effects on human health, yet there is very little known about what characteristics exactly cause these adverse health effects and how they cause them. Samples for this study were collected from Pascagoula, MS, a small town located on the Mississippi Gulf Coast that is home to many industrial yards, from September 2013 to December 2013. Black carbon (BC) analysis was performed on the samples to collect black carbon concentrations. PM<sub>2.5</sub> was extracted from filters in methanol via sonication. Portions of each sample were allocated to be whole particle solution (WPS) samples and soluble fraction samples. Oxidative potential was measured for both WPS and soluble fractions using the dithiothreitol (DTT) assay. Statistical analyses were used to measure statistical significance and find trends within the results. Significant differences were found between some sampling dates and monthly averages for PM<sub>2.5</sub> concentrations, BC concentrations, and oxidative potential. Trends were observed between PM<sub>2.5</sub> and BC concentrations but not between oxidative potential. Slight trends were also seen between months and seasons (fall and winter). The results of this study show that day and month, along with many other factors, should be considered when studying PM<sub>2.5</sub>, leading to a better understanding of air quality for future studies.

Key Words: air pollution, PM<sub>2.5</sub>, black carbon, oxidative potential

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## LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AA	Ascorbate
BC	Black carbon
DMPO	5,5-dimethyl pyrroline-N-oxide
DMSO	Dimethyl sulfoxide
DTNB	5,5'-Dithiobis (2'-nitrobenzoic acid)
DTT	DL, 1,4-Dithiothreitol
EPA	Environmental Protection Agency
ESR	Electron spin resonance
MDEQ	Mississippi Department of Environmental Quality 3
NAAQS	National Ambient Air Quality Standards
N-EVAP	Nitrogen-Evaporation
OC	Organic carbon
PB	Potassium phosphate, monobasic (KH <sub>2</sub> PO <sub>4</sub> )
PM <sub>2.5</sub>	Particulate Matter smaller than 2.5 microns
PM <sub>10</sub>	Particulate Matter smaller than 10 microns
PTFE	Polytetrafluoroethylene
ROS	Reactive oxygen species
WHO	World Health Organization
WPS	Whole Particle Solutio

## **INTRODUCTION**

As populations increase, air pollution levels climb to dangerously high levels around the world, and new data from the World Health Organization (WHO) shows that 9 out of 10 people breathe air with high levels of pollutants. It is estimated that nearly 7 million people die every year due to exposure to ambient and household air pollutions that penetrate the lungs and cardiovascular system (WHO News). The WHO also notes that more than 40% of the world's population still does not have access to clean cooking methods in their homes which is the leading source of household air pollution. There are many other sources that contribute to air pollution from particulate matter such as inefficient energy use, coal-fired power plants, waste burning, and deforestation just to name a few. The WHO and Environmental Protection Agency (EPA) acknowledge that air pollution contributes to numerous adverse health effects. The EPA has also established national ambient air quality standards (NAAQS) for the most common types of air pollution which include carbon monoxide, lead, ground-level ozone, particulate matter, nitrogen dioxide, and sulfur dioxide (US EPA, Criteria Air Pollutants).

Particulate matter is the term used for air pollution that contains a mixture of solid particles and liquid droplets (US EPA, PM Basics). These particles come in various sizes; some, for example dust, soot, or smoke, can be seen with the naked eye while others can only be seen using an electron microscope. When talking about particulate matter, there

are two main categories the particles can fall into: PM<sub>10</sub> and PM<sub>2.5</sub>. PM<sub>10</sub> consists of particles that are inhalable and have an aerodynamic diameter of 10 µm or smaller. PM<sub>2.5</sub> is a finer inhalable particle with an aerodynamic diameter of 2.5 µm or smaller, making the largest PM<sub>2.5</sub> particle about 30 times smaller than the diameter of a single strand of hair (US EPA). Particulate matter can be directly emitted from certain sources, but most particles form in the atmosphere due to complex reactions of chemicals. Particulate matter poses a serious threat to human health because a particle with an aerodynamic diameter of 10 µm is small enough to travel deep into the lungs and possibly reach the bloodstream (US EPA).

Particulate matter is a mixture of coarse and fine size particles that come in a variety of morphologies and compositions (Beasley, 2019). PM<sub>2.5</sub> is small enough to move into the bloodstream and can be a carrier of heavy metals and organic compounds (Kethireddy et al., 2018). Fine particulate matter comes from many sources, such as vehicle emissions, coal-burning power plants, industrial emissions, and other anthropogenic and natural sources (State of Global Air, 2019). The WHO has established the Air Quality Guideline, setting the annual average PM<sub>2.5</sub> concentration at 10 µg/m<sup>3</sup>, which is based on health effects from long-term exposure to PM<sub>2.5</sub> (Kethireddy et al., 2018). More than 90% of the world population lives in areas exceeding the WHO guidelines, and studies have shown that exposure to high concentrations of PM<sub>2.5</sub> for several years can lead to cardiovascular, respiratory, and other diseases (Kethireddy et al., 2018).

The United States Gulf Coast, along with the southeastern US, accounted for more than 65% of PM<sub>2.5</sub> and other air pollutants out of the four regions monitored by a team at the University of Arkansas for Medical Sciences (Chalbot et al., 2013). The Gulf Coast sees a large amount air pollution since the Gulf of Mexico contains busy ports, heavy marine traffic within close proximity to the coast, and a large number of offshore oil and natural gas platforms and refineries. Pascagoula is a city on the Mississippi Gulf coast that serves as the location for Chevron Pascagoula Refinery, Chevron Phillips Chemical Company, First Chemical Pascagoula Plant, and Huntington Ingalls Shipyard. On a state level, Mississippi was ranked 17<sup>th</sup> out of the top 20 industrially polluted states in 2010 and has rapidly increasing rates of asthma (Kethireddy et al., 2018). Despite growing concerns about the air quality in Pascagoula, data from the Mississippi Department of Environmental Quality's 2019 report indicates that the concentrations of PM<sub>2.5</sub> are below the MDEQ standards. Even at levels below the national standards, changes in exposure to PM<sub>2.5</sub> can lead to an increase in cardiovascular and respiratory diseases (Markar et al., 2017).

Air pollution is ranked as one of the top risk factors for death and disability around the world. In fact, it was ranked as the fifth highest mortality risk factor globally in 2017 and was associated with roughly 4.9 million deaths (State of Global Air, 2019). Although research on PM<sub>2.5</sub> is rather limited, epidemiological and toxicological studies have indicated that PM<sub>2.5</sub> is significantly more toxic than larger particulate matter (Franklin et al., 2007). Long term exposure to particulate matter has been greatly associated with ischemic heart disease, dysrhythmias, heart failure, and cardiac arrest; for

these conditions, a  $10 \mu\text{g}/\text{m}^3$  increase in fine particulate matter was linked to an 8% to 18% increase in mortality risk (Pope et al., 2004). It has been discovered that tropical weather types (dry tropical and moist tropical) are associated with significantly higher concentrations of  $\text{PM}_{2.5}$  while polar weather types, both dry and moist, have been associated with lower concentrations of  $\text{PM}_{2.5}$  (Liu et al., 2017). Not only can seasonal changes affect particulate matter concentrations, but they can also affect chemical composition. Out of 52 components analyzed, only seven contributed greater than 1% to the total mass for year-round or seasonal averages (Michelle et al., 2007).

There are many studies that link particulate matter with adverse health effects, but there is still a lot of uncertainty concerning exactly which characteristics of particulate matter causes these health effects (Michelle et al., 2007). Many studies have shifted towards aiming to figure out what components of  $\text{PM}_{2.5}$  are directly linked to mortality. Toxic characteristics associated with  $\text{PM}_{2.5}$  include: metals, organic compounds absorbed onto particles or forming particles, biological components, sulfate, nitrate, acidity, and surface-absorbed reactive gasses (Michelle et al. 2007). More specifically, iron, nickel, zinc, ammonium nitrate, elemental carbon, organic carbon (OC), nitrates, and sulfates have been the main chemicals associated with mortality for  $\text{PM}_{2.5}$  (Michelle et al., 2007).

Black carbon is a component frequently linked with particulate matter that is known to have detrimental effects on human health. Black carbon particles are emitted directly into the atmosphere through various sources such as the incomplete combustion of fossil fuels, biofuels, and biomass (Canagaranta et al., 2021). In the past ten years, studies have



linked BC exposure with higher rates of morbidity and mortality, including lung cancer mortality, lower lung function and slower cognitive development in children, increased bone loss, and decreases cognitive functions in the elderly (Lequy et al., 2021).

Oxidative stress occurs when there is a disturbance in the production of reactive oxygen species (ROS) and antioxidant defenses. As ROS are formed, they must be stabilized through electron donations from proteins, lipids, and DNA, leading to degradation and damage of these molecules (Kerksick and Zuhl, 2015). It is thought that the main mechanism through which particulate matter increases mortality and morbidity is increased oxidative stress (Kerksick and Zuhl, 2015). When particulate matter is inhaled, the particles induce inflammation in the lungs which then causes an excess of ROS to be produced (Sorensen et al., 2003). Because of this proposed mechanism, it has become common to measure the capacity of particulate matter to oxidize target molecules, also known as oxidative potential, rather than solely collecting information on mass alone for particulate matter (Sorensen et al., 2003). There are various assays that can be used to measure oxidative potential of PM<sub>2.5</sub>. The Dithiothreitol (DTT) assay measures oxidative potential by measuring the formation of DTT-disulfide which results from the transfer of electrons from DTT to ROS (Yang et al., 2013).

This study will investigate differences in PM<sub>2.5</sub> between months from Pascagoula, MS by looking at black carbon concentrations and oxidative potential across days in September, October, November, and December. I hypothesize that PM<sub>2.5</sub> collected in September and October will have higher black carbon concentrations and oxidative potential compared

to PM<sub>2.5</sub> from November and December, considering the Mississippi Gulf Coast has a tropical climate with short winters. The results of this study will show that the day and month a sample is taken should be considered when studying PM<sub>2.5</sub>, which will help inform future studies.

## **MATERIAL AND METHODS**

### ***1. Collecting Filters***

All PM<sub>2.5</sub> samples and controls were donated by the MDEQ for this research.

PM<sub>2.5</sub> samples were collected onto 47 mm PTFE-coated filters that are commonly used in the Federal Reference Monitor program, throughout the United States.

They were collected every third day from September through December 2013.

Sample dates include: September 4, 7, 10, 13, 16, 19, 22, and 28, 2013; October 1,

16, 19, 22, 25, and 28, 2013; November 15, 18, 21, 24, and 27, 2013; and

December 21, 24, 27, and 30, 2013. Date matched travel, field, and laboratory

blank filters were included in this research. The MDEQ provided detailed

information for each sample and control including: sampler runtimes, flowrates,

meteorological measurements (temperature, humidity), and notes regarding the

sample collection.

<b>Filter ID</b>	<b>Date</b>	<b>Location</b>	<b>PM2.5 conc. (<math>\mu\text{g}/\text{m}^3</math>)</b>
T3651403	9/4/2013	Pascagoula	7.6
T3651433	9/7/2013	Pascagoula	19.3
T3651440	9/10/2013	Pascagoula	15.7
T3651439	9/13/2013	Pascagoula	13.9
T3651494	9/16/2013	Pascagoula	15.3
T3651495	9/19/2013	Pascagoula	7.7
T3651493	9/22/2013	Pascagoula	7.6
T3651557	9/28/2013	Pascagoula	n/a
T3651573	10/1/2013	Pascagoula	8.6
T3651614	10/16/2013	Pascagoula	5.6
T3651613	10/19/2013	Pascagoula	8.1
T3651678	10/22/2013	Pascagoula	10.6
T3651679	10/25/2013	Pascagoula	9
T3651680	10/28/2013	Pascagoula	9
T3651783	11/15/2013	Pascagoula	7.5
T3651784	11/18/2013	Pascagoula	4.8
T3651793	11/21/2013	Pascagoula	17.2
T3651786	11/24/2013	Pascagoula	7.4
T3651841	11/27/2013	Pascagoula	7.2
T3651934	12/21/2013	Pascagoula	9.9
T3651988	12/24/2013	Pascagoula	7
T3651989	12/27/2013	Pascagoula	8.4
T3651990	12/30/2013	Pascagoula	1.9
T3651501	9/24/2013	Trip Blank	n/a
T3651858	12/13/2013	Field Blank	n/a

Table 1. Filter ID, date, location, and PM<sub>2.5</sub> concentration ( $\mu\text{g}/\text{m}^3$ ) for each sample.

## 2. *Black Carbon Analysis*

Black carbon was measured for all samples, compared to control filters using the Magee OT21 SootScan. This instrument is an optical transmissometer that is able to analyze 880 nm and 370 nm wavelengths and specifically designed for black carbon analysis of particulate matter collected onto filters. The 880 nm wavelength is an IR wavelength that is translated to an amount of black carbon that is present on the filter. The 370 nm wavelength is a UV wavelength,

and its absorption gives qualitative information about aromatic compounds. This information can aid in finding the sources that may have contributed to the black carbon. The amount of black carbon is determined by subtracting the attenuation value at 880 nm for the sample from a blank filter control. All filters were run in triplicate. Concentrations of black carbon were then determined by using precise sampler runtimes and flow rates.

### ***3. Filter Extraction***

Following the non-destructive black carbon analysis, each filter was placed into a 15 mL conical tube, and 8 mL methanol was added. The tubes were then sonicated for one hour at 60 Hz in a waterbath sonicator to remove the PM<sub>2.5</sub> from the filters. After sonication, the filters were rinsed with 200 µL methanol as they were removed from the conical tubes to help release any remaining PM<sub>2.5</sub>. Once this was completed, the tubes with the samples were stored at -20°C until they were ready to be concentrated. Identical procedures were performed for all sample and control filters.

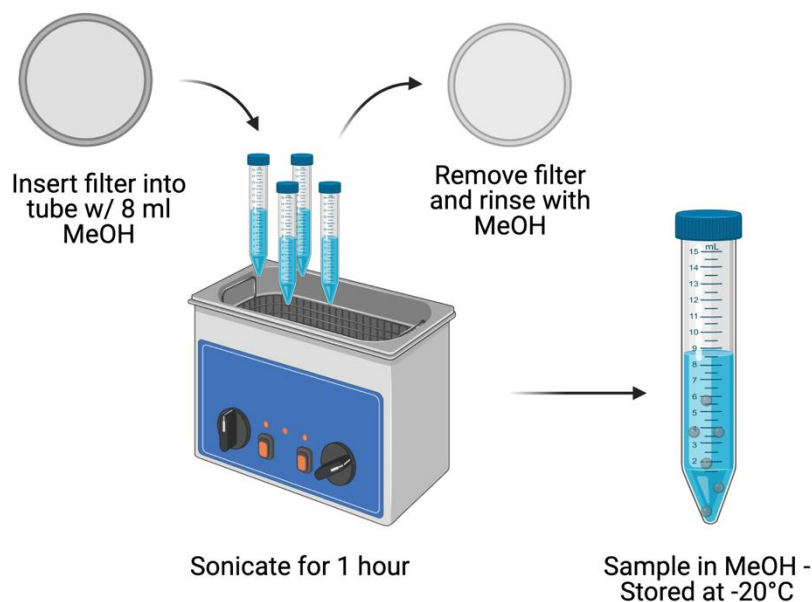


Figure 1. Filter Extraction Method (created with BioRender.com)

#### 4. *Sample Concentration*

The samples and controls in solution were concentrated using a N-EVAP nitrogen blow down system. A gentle stream of nitrogen gas was used to concentrate the suspensions to 1 mL.

#### 5. *Oxidative Potential Analysis*

##### a. *Prep Samples*

##### 1. Whole Particle Suspension

Each sample was vortexed for five seconds to ensure the  $PM_{2.5}$  was evenly distributed. Once vortexed, 50  $\mu$ L were removed from the samples and distributed to 200  $\mu$ L snap closure tubes. These tubes were the whole particle suspension for the DTT assay.

## 2. Soluble and Insoluble Fractions

Another 75  $\mu\text{L}$  were removed from the sample and dispensed into a 1.5 mL centrifuge tube to be prepped as the soluble fraction. Each of these tubes were centrifuged for 5 minutes at 13 g. The soluble fractions were removed from the centrifuge tubes and placed in the 200  $\mu\text{L}$  snap closure tubes while the insoluble fractions were left in the centrifuge tubes. The remaining samples in the 15 mL conical tubes were stored at  $-20^{\circ}\text{C}$  to be used for ICP-MS.

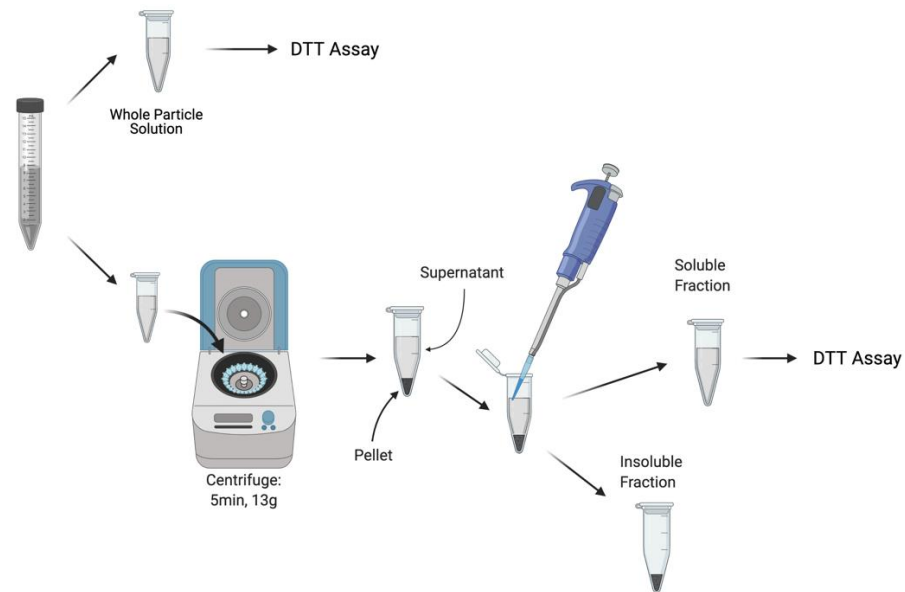


Figure 2. Separation of whole particle solution, soluble fractions, and insoluble fractions method (created with BioRender.com)

### b. DL, 1,4-Dithiothreitol Assay

All samples were tested for oxidative potential through the DTT assay. The DTT assay was performed in a 96-well plate that included a DTT curve, controls, and 25 samples ran in triplicate.

A DTT curve was included in the assay to calculate the DTT consumption for each sample. The wells for the DTT curve included 100  $\mu\text{L}$  0.05 M Potassium phosphate, monobasic ( $\text{KH}_2\text{PO}_4$ ) (PB), 5  $\mu\text{L}$  methanol, and 5  $\mu\text{L}$  DTT which was analyzed at 6 different concentrations (0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 mM). There were three controls used in this assay. There was the PB run in triplicate, a positive control of 1  $\mu\text{M}$  1,4-Naphthoquinone, and the 0.0 mM DTT that was included in the DTT curve. The PB control wells only received 100  $\mu\text{L}$  PB. The 0.0 mM DTT wells, on the other hand, contained 100  $\mu\text{L}$  PB, 5  $\mu\text{L}$  methanol, 5  $\mu\text{L}$  0.0 mM DTT, and 10  $\mu\text{L}$  5,5'-Dithiobis (2'-nitrobenzoic acid) (DTNB) by the end of the assay (which included all reagents at the same volume but without a sample or filter control). Sample dilutions were prepared in dimethyl sulfoxide (DMSO), then added to the plate in triplicate. All of the sample wells then received 5  $\mu\text{L}$  0.5 mM DTT.

The prepared plate was covered with a lid, wrapped in aluminum foil, and shook for 2-3 seconds to ensure mixing of all reagents and controls. The plate was then incubated at 37°C for 20 minutes. Once the incubation was complete, the reaction was quenched with 10  $\mu\text{L}$  1 mM DTNB which was dispensed into all wells except the control wells. The plate was covered and wrapped with foil again then shook for 1 minute. A spectrophotometer was used to record the absorbance of the plate at 412 nm.



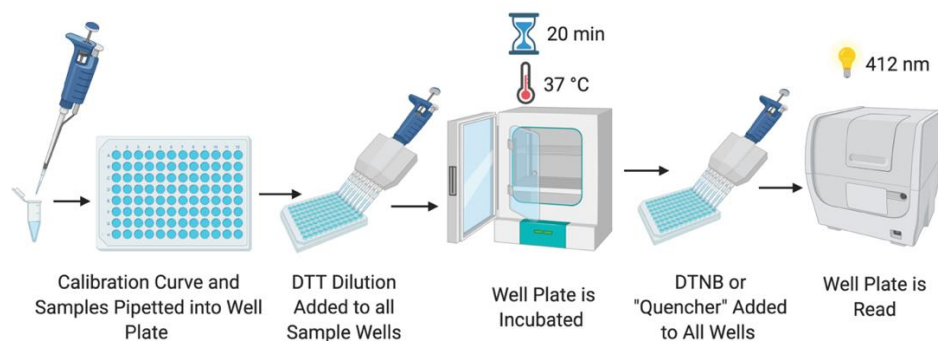


Figure 3. DTT assay method (created with BioRender.com)

## 6. *Statistical Analysis – Software*

Bar graphs and statistical significance calculations were completed with SigmaPlot 14.0 (San Jose, CA).

One-way analysis of variance (ANOVA) tests were used with significance set at  $p < 0.05$  for black carbon and most of the oxidative potential data. A two-way ANOVA test was used to compare between the month a sample was taken from and if it was whole particle solution or soluble fraction. Significance was also set at  $p < 0.05$  for the two-way ANOVA test. Linear regression analysis was conducted to correlate DTT consumption and black carbon concentrations and generate  $r^2$  values and determine if the slopes of the regression lines were significantly non-zero ( $p \leq 0.05$ ).

Diagrams of the methods were created using Bio Render.

## RESULTS

### 1. *Black Carbon*

Black carbon concentrations were determined for each sample and compared between days in each month (Figure 4). For the month of September (Figure 4A), there were significant differences between the following days: 7<sup>th</sup> vs 22<sup>nd</sup> and 4<sup>th</sup>, and the 10<sup>th</sup> vs 4<sup>th</sup>. Out of the 8 days measured in September, there was variability throughout the month with concentrations that ranged from 0.09 to 6.70  $\mu\text{g}/\text{m}^3$ . For October (Figure 4B), there were not significant differences between the sampling locations, and concentrations ranged from 1.87 to 3.30  $\mu\text{g}/\text{m}^3$ . For November (Figure 4C), there were not significant differences between the sampling dates, and the concentrations ranged from 1.09 to 3.05  $\mu\text{g}/\text{m}^3$ . For December (Figure 4D), there were not significant differences between sampling dates, and the concentrations ranged from 0.25 to 2.04  $\mu\text{g}/\text{m}^3$ .

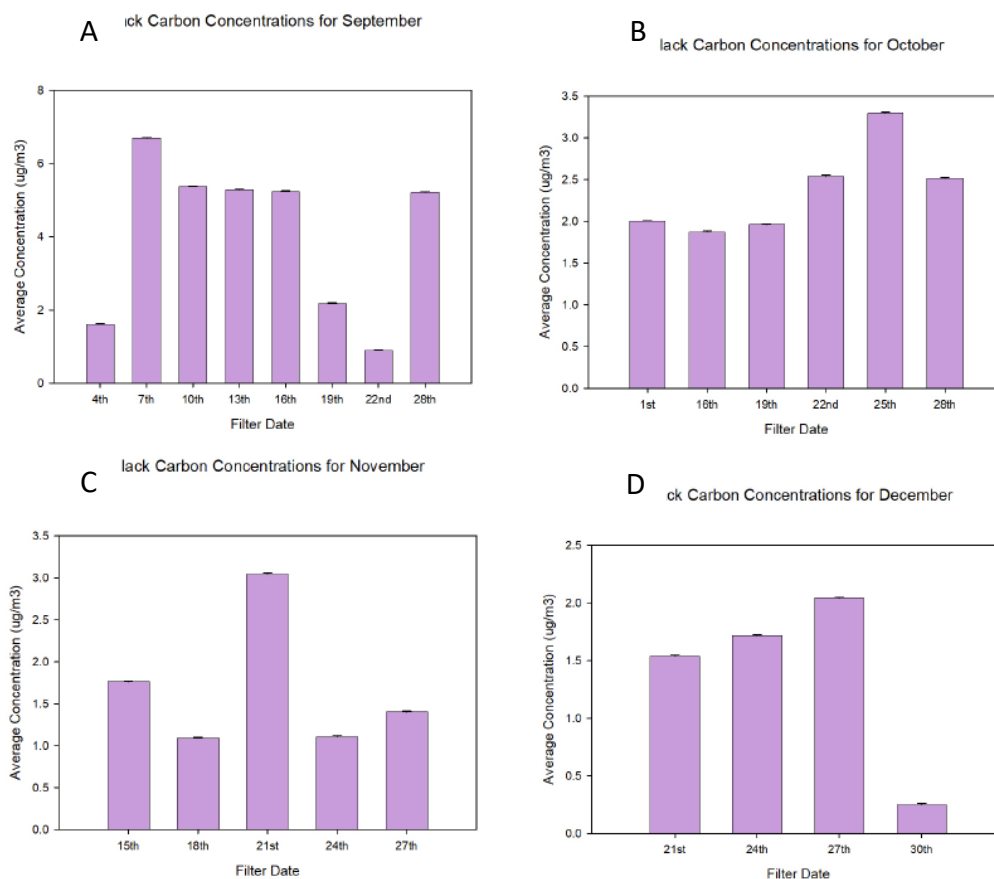


Figure 4. Average black carbon concentrations for each sample compared between days in each month. Averages are reported  $\pm$  standard deviations (sd) for A) black carbon concentrations for September, B) black carbon concentrations for October, C) black carbon concentrations for November, and D) black carbon concentrations for December. Data for A-D were analyzed using one-way ANOVA. Differences with p value  $< 0.05$  were considered significant.

Using the black carbon concentrations determined for each sample, comparisons were made between each month for the average black carbon concentration for the entire month (Figure 5). There were significant differences between the following months: September vs November and September vs December. The month of September featured the highest average for black carbon concentration at  $4.06 \mu\text{g}/\text{m}^3$ . As the year progressed, black carbon concentrations dropped each month with December having the lowest

average concentration of black carbon out of all four months which had an average concentration of  $1.37 \mu\text{g}/\text{m}^3$ . For the most part, the black carbon concentrations are consistent with the monthly  $\text{PM}_{2.5}$  concentrations (Table 1). September had the highest average concentration of  $\text{PM}_{2.5}$  at  $12.44 \mu\text{g}/\text{m}^3$  while December had the lowest average concentration at  $6.80 \mu\text{g}/\text{m}^3$ .

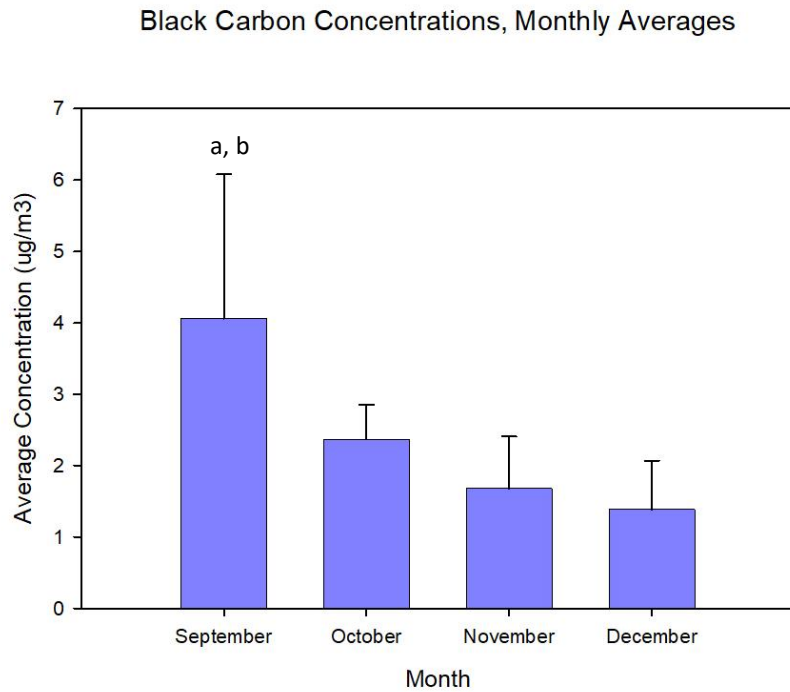


Figure 5. Average black carbon concentrations for the entire month compared between each month. Averages are reported  $\pm$  standard deviations (sd) for monthly black carbon concentrations with the number of sampling days for each month, September (n=8), October (n=6), November (n=5), and December (n=4) Data for this figure were analyzed using one-way ANOVA. Differences with p value  $< 0.05$  were considered significant. Significant differences were indicated by: a = significantly different from the November average and b = significantly different from the December average.

## 2. *Oxidative Potential*

Oxidative potential was determined by calculating the DTT consumption ( $\text{pM}/\text{min}/\text{m}^3$ ). A higher DTT consumption corresponds to a higher oxidative potential. The average amount of DTT consumed was determined for each WPS sample and compared between days in each month (Figure 6). For September (Figure 6A), there were significant differences between the following days: 4<sup>th</sup> vs 13<sup>th</sup>, 16<sup>th</sup>, 19<sup>th</sup>, 22<sup>nd</sup>, and 28<sup>th</sup>. The average amount of DTT consumed for September WPS ranged from 0.70 to 3.72  $\text{pM}/\text{min}/\text{m}^3$ . For all other months (Figure 6B-D), there were no significant differences between dates. For October (Figure 6B), there were no significant differences between sampling dates, and the average DTT consumed ranged from 0.47 to 1.74  $\text{pM}/\text{min}/\text{m}^3$ . For November (Figure 6C), the sample dates did not show any significant differences in average DTT consumed which ranged from 0.29 to 1.10  $\text{pM}/\text{min}/\text{m}^3$ . December also showed no significant differences between sample dates (Figure 6D), and it featured a range of 0.20 to 1.10  $\text{pM}/\text{min}/\text{m}^3$ .

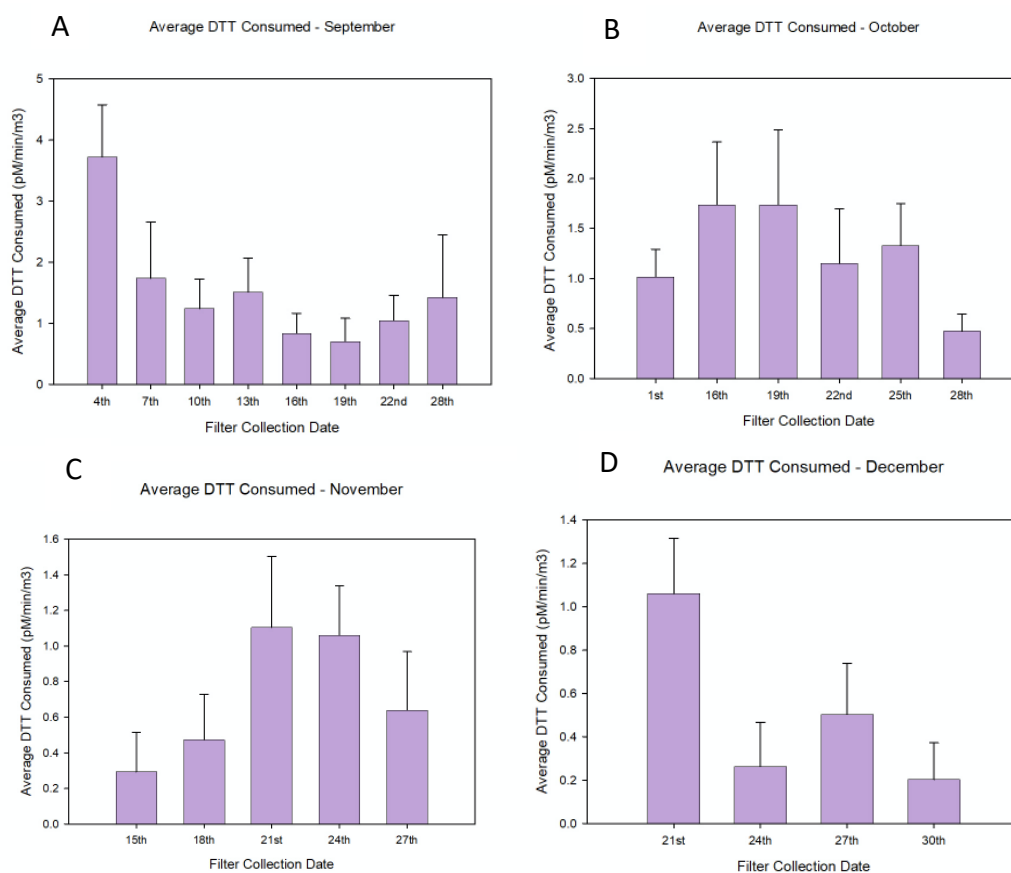


Figure 6. Average DTT consumed for each WPS sample and compared between the days in each month. Averages  $\pm$  sd for DTT consumed per reaction time and volume of air are reported for A) September WPS, B) October WPS, C) November WPS, and D) December WPS. Data for A-D were analyzed using one-way ANOVA. Differences with a p value  $< 0.05$  were considered significant.

The average amount of DTT consumed was determined for each soluble fraction sample and compared between days in each month (Figure 7). There were no significant differences with the soluble fraction samples between days for each month (Figure 7A-D). The soluble fraction averages for September (Figure 7A), October (Figure 7B), November (Figure 7C), and December (Figure 7D) ranged from: 3.97 to 8.64

pM/min/m<sup>3</sup>, 5.70 to 10.51 pM/min/m<sup>3</sup>, 3.83 to 6.64 pM/min/m<sup>3</sup>, and 5.30 to 7.97 pM/min/m<sup>3</sup>, respectively.

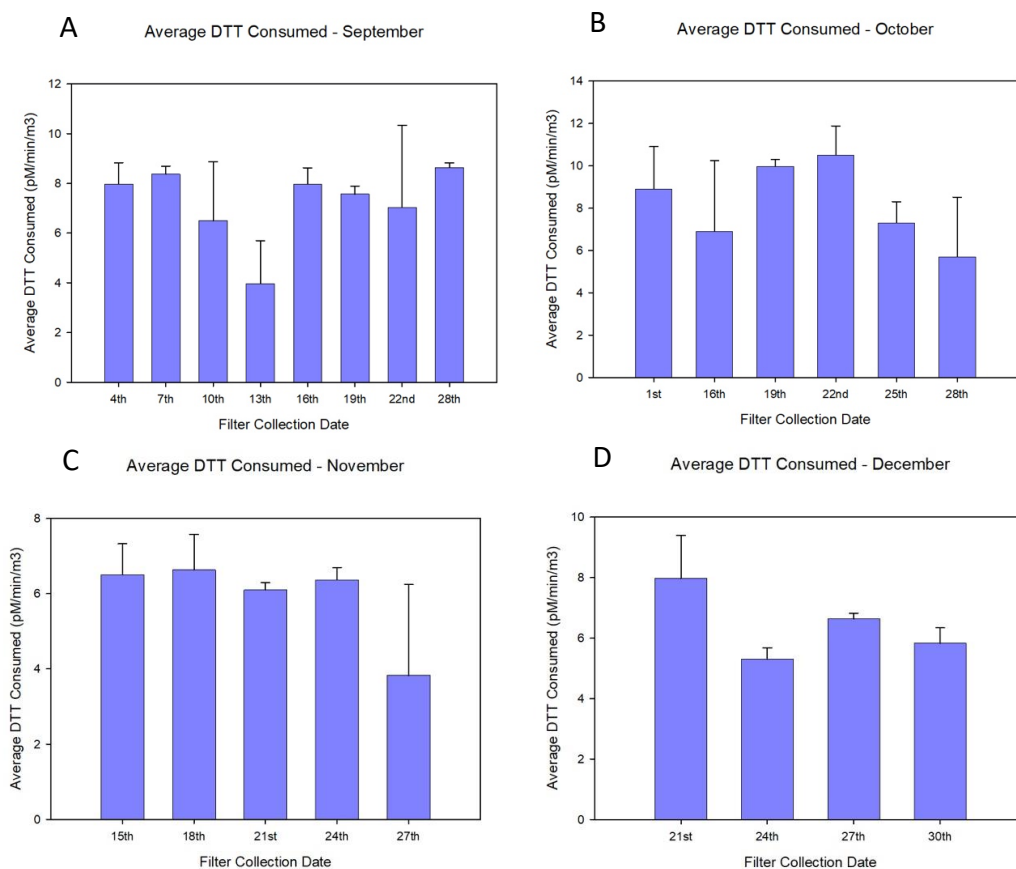


Figure 7. Average DTT consumed for each soluble fraction sample and compared between the days in each month. Averages  $\pm$  sd for DTT consumed per reaction time and volume of air are reported for the soluble fraction in A) September, B) October, C) November, and D) December. Data for A-D were analyzed using one-way ANOVA. Differences with a p value  $<$  0.05 were considered significant.

The amount of DTT consumed was averaged for each month. These monthly averages were used to compare differences between WPS and soluble fractions (Figure 8). For all

months there is a notable difference in DTT consumption between the WPS samples and the soluble fractions. For September, the average DTT concentration increased from 1.53 to 7.25 pM/min/m<sup>3</sup>, so for the month of September the DTT concentrations for soluble fraction samples were nearly 4.8 times higher than the DTT concentrations for WPS. For October, the DTT concentrations were observed to jump from 1.24 to 8.23 pM/min/m<sup>3</sup> when comparing WPS to soluble fraction samples which was a 6.6 times increase. November exhibited DTT concentrations for soluble fraction samples that were 8.3 times higher than the DTT concentrations for WPS samples. The DTT concentrations for the month of November increased from 0.71 to 5.89 pM/min/m<sup>3</sup> when comparing WPS and soluble fraction samples. December demonstrated the largest difference in DTT consumption between WPS and soluble fraction samples out of all months. The soluble fraction DTT concentration for December was 12.7 times higher than the concentration for WPS samples which saw an increase from 0.51 to 6.45 pM/min/m<sup>3</sup>. When comparing between months, there is a significant difference in DTT consumption between October and November. There is no other significant difference when comparing other months for WPS or soluble fractions.



### DTT Concentrations for WPS vs Soluble, Monthly Averages

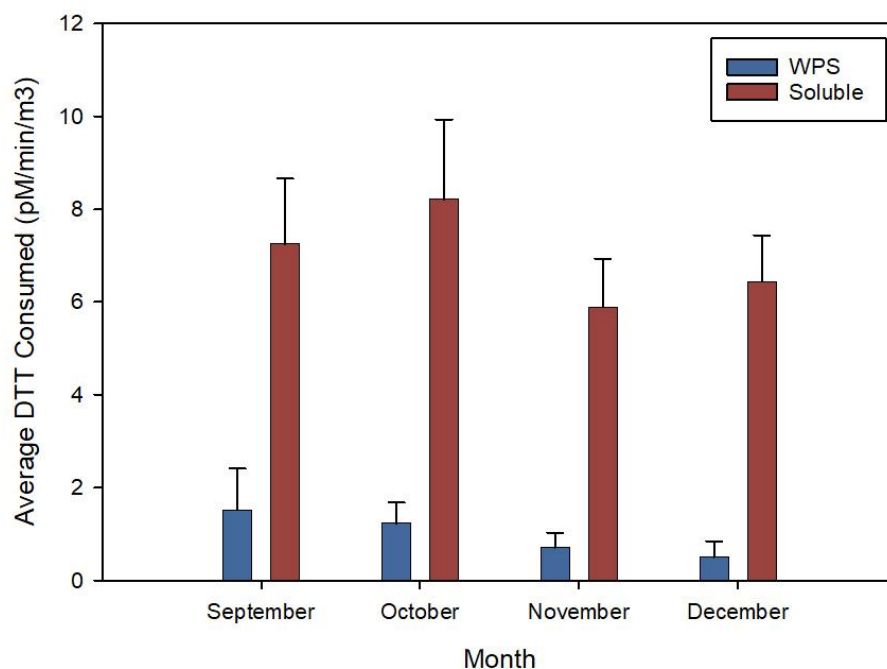


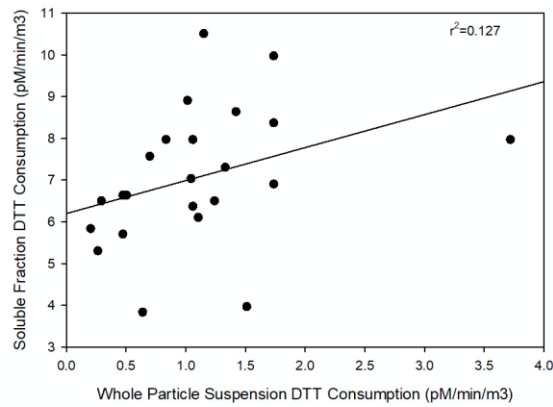
Figure 8. Monthly average DTT consumed, comparing WPS and soluble fractions. Averages  $\pm$  sd for average DTT consumption for the WPS and soluble fractions of PM<sub>2.5</sub> by month with samples from September (n=8), October (n=6), November (n=5), and December (n=4). Data for this figure were analyzed using two-way ANOVA that compared between the month the sample was from and if it was WPS or soluble fraction. Differences with a p value < 0.05 were considered significant.

A regression analyses were conducted to compare between black carbon concentrations and DTT consumption for different fractions of PM<sub>2.5</sub> (Figure 9). Based on the slope of the trendline, a significant positive trend was also observed between the DTT consumption in the WPS and soluble fractions (Figure 9A). The regression analysis for WPS and soluble fractions (Figure 9A) had an R<sup>2</sup> value of 0.13. The analysis between WPS and soluble fraction DTT consumption featured the highest R<sup>2</sup> value, meaning this graph showed the greatest correlation between the two variables. When considering black

carbon concentrations, there was a significant positive trend between black carbon concentrations and the DTT consumption in the soluble fraction (Figure 9B), and the graph featured an  $R^2$  value of 0.01. This trend was not observed when comparing black carbon concentrations to DTT consumption of the WPS samples which featured an  $R^2$  value of 0.06 (Figure 9C). The regression analyses for soluble fractions and WPS against black carbon concentrations exhibited low  $R^2$  values, with the comparison between the soluble fractions and black carbon being the lowest. When considering black carbon concentrations, there was a significant positive trend between black carbon concentrations and the DTT consumption in the soluble fraction (Figure 9B) this trend was not observed when comparing black carbon concentrations to DTT consumption of the WPS samples (Figure 9C).

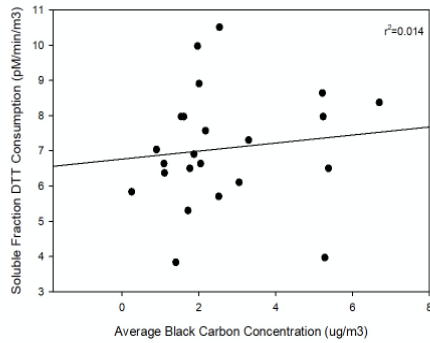
A

Regression Analysis of DTT Consumption for WPS and Soluble Fractions



B

Regression Analysis of DTT Consumption for Soluble Fraction and BC Concentrations



C

Regression Analysis of DTT Consumption for WPS and BC Concentrations

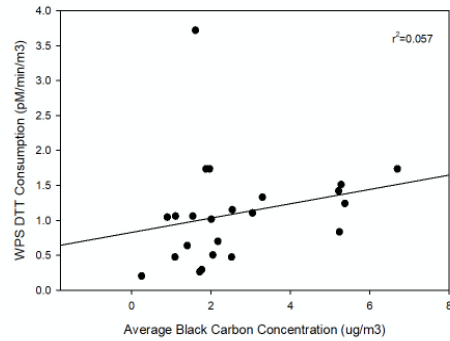


Figure 9. Regression analysis to compare between black carbon concentrations and DTT consumption for WPS and soluble fraction samples. A) Regression analysis comparing DTT consumption for WPS and soluble fractions. B) Regression analysis comparing DTT consumption for soluble fractions and black carbon concentrations. C) Regression analysis comparing DTT consumption for WPS and black carbon concentrations. Significance of the regression analysis was set at  $p \leq 0.05$ .

## DISCUSSION

### *1. Black Carbon*

Black carbon (BC) concentrations varied significantly between dates in the month of September. Not only did September have the highest average BC concentration out of all four months, but it also had the greatest range of BC concentrations between samples dates which contained both the lowest and highest BC concentrations out of all sample dates. September 7<sup>th</sup> showed the highest concentration for BC out of all dates analyzed ( $6.70 \mu\text{g}/\text{m}^3$ ) while September 22<sup>nd</sup> showed the lowest BC concentration ( $0.09 \mu\text{g}/\text{m}^3$ ). A possible explanation for these findings could be that September had the largest number and range of sample dates. There were samples collected consistently throughout September, giving a total of 8 samples for the entire month. The sampling was not as consistent for all of the other months, with 6 samples for October, 5 for November, and only 4 for December. Additionally, September had samples throughout the entire month while the samples for October, November, and December fell towards the end of the months.

When looking at the monthly averages for BC concentrations, there was more of a pattern. The monthly BC concentrations mostly decreased throughout the year, with September having the highest average and December having the lowest average. The

findings from my results support my hypothesis that  $PM_{2.5}$  collected in the earlier months would have higher concentrations of black carbon compared to the later months.

There is not much research to support trends of black carbon concentrations, but there are many studies investigating the trends of  $PM_{2.5}$  concentrations. In my study, I found that  $PM_{2.5}$  concentrations showed similar trends compared to black carbon concentrations. Similar to the range for black carbon concentrations, the range for  $PM_{2.5}$  concentrations in September was the largest. September 7<sup>th</sup>, which showed the highest BC concentration, also showed the highest  $PM_{2.5}$  concentration out of all sample dates ( $19.3 \mu\text{g}/\text{m}^3$ ). December 30<sup>th</sup> showed the lowest  $PM_{2.5}$  concentration out of all sample dates ( $1.9 \mu\text{g}/\text{m}^3$ ). The monthly averages for  $PM_{2.5}$  concentrations also showed trends similar to the monthly averages for black carbon concentrations. September had the highest monthly average for  $PM_{2.5}$  ( $12.44 \mu\text{g}/\text{m}^3$ ). October and November showed monthly averages for  $PM_{2.5}$  concentration that were fairly close to each other at  $8.48$  and  $8.82 \mu\text{g}/\text{m}^3$ . December had the lowest average concentration out of all months at  $6.80 \mu\text{g}/\text{m}^3$ . The results for my study follow trends for  $PM_{2.5}$  concentrations that have been found in past studies.

For the most part, higher concentrations of  $PM_{2.5}$  are associated with higher concentrations of BC. A study done in New York, showed no significant trends in BC concentrations between days of the week despite the idea that they should be lower on the weekends due to less traffic (Venkatachari et al., 2006). On the other hand, a past study found trends between the time of the year and days of the week for  $PM_{2.5}$  concentrations.

This study showed when looking at seasonal differences, PM<sub>2.5</sub> concentrations tend to be highest during the winter and summer and lowest during spring and fall (Zhao et al., 2018). The high PM<sub>2.5</sub> concentrations in the winter are typically associated with a large increase in wood and coal combustion for heating while the high concentrations in the summer are associated with high temperatures that often pair with high pressure and low winds, causing particulate matter to be trapped in the area (Zhao et al., 2018). The Mississippi Gulf Coast is known to have long, hot summers and short, cool winters. When looking at past weather trends for September in Pascagoula, MS, it is often very hot and humid with low winds which would correlate with weather patterns that lead to high concentrations of PM<sub>2.5</sub>. While it does get colder throughout the year, December still experiences moderate temperatures. Because of similar weather reason, the same study also looked at specific portions of California to see if there are notable differences in PM<sub>2.5</sub> concentrations associated with different climates. Researchers found that Southern California, which also experiences long summers and short winters, did not have high levels of PM<sub>2.5</sub> in the winter months (Zhao et al., 2018).

## ***2. Oxidative Potential***

Oxidative potential was analyzed for both WPS and soluble fractions for each sample date, and comparisons were made between samples dates and monthly averages.

September showed the largest range of variability for DTT consumed between WPS samples (0.70 to 3.72 pM/min/m<sup>3</sup>). The ranges for DTT consumed for October, November, and December were considerably smaller than the range for September. This could be because of the number of samples that were collected for each month, similar to

the results observed with black carbon concentrations. September had the largest number of samples collected, so that may have provided more variability that was not seen in the rest of the months. There is a past study that was conducted in southern Georgia and Alabama and included a couple of similar variables. In this study, researchers analyzed PM<sub>2.5</sub> concentrations and oxidative potential for samples that were collected from September 2012 through September 2013 (Verma et al., 2018). Similar to my results regarding PM<sub>2.5</sub> concentrations, researchers found significantly higher concentrations of PM<sub>2.5</sub> for September 2012 and 2013 versus December 2012 which is thought to be the case because there tends to be more rainfall in winter (Verma et al., 2018). When analyzing oxidative potential for PM<sub>2.5</sub>, researchers found that DTT activity, which was measured twice, once with normalized volume and the other with normalized mass, was significantly higher in winter (November 2012- February 2013) than fall (September 2012- October 2012) (Verma et al., 2018). There are multiple studies that have found a peak in oxidative potential during winter months with low concentrations of PM<sub>2.5</sub> for those months which is credited to the increase in biomass burning emissions for heating (Verma et al., 2018; ShuangYu et al., 2019).

When analyzing oxidative potential for the soluble fractions, there were no significant differences between sample dates but there was a significant positive trend between the DTT consumed by the WPS and soluble fractions across all PM<sub>2.5</sub> samples.

The results found in my study do not completely support my hypothesis that oxidative potential would be higher in the earlier months compared to the later months. When

looking at the results for oxidative potential of the WPS samples, the amount of DTT consumed does decrease throughout the months where September had the highest average (1.53 pM/min/m<sup>3</sup>), and December had the lowest (0.51 pM/min/m<sup>3</sup>). However, the average amount of DTT consumed for the soluble fractions was highest for October (8.23 pM/min/m<sup>3</sup>) and lowest for November (5.89 pM/min/m<sup>3</sup>). It is likely that September had the highest oxidative potential for WPS because it had considerably more sample dates than the rest of the months. Many studies have also identified that weather has a significant impact on oxidative potential of PM<sub>2.5</sub>. Two studies observed that monsoons increase PM<sub>2.5</sub> concentrations and oxidative potential because of the scavenging of PM<sub>2.5</sub> from the atmosphere in China (ShuangYu et al., 2019; Zhang et al., 2015). This would be important to consider for the MS Gulf Coast, especially since hurricane season typically peaks around mid-September which could give abnormal results.

There were some significant differences when comparing oxidative potential of WPS and soluble fraction samples between months. All four months showed a significant difference between the amount of DTT consumed for WPS and soluble fraction. For all four months, oxidative potential increased by factors ranging from 4.8 to 12.7 when comparing WPS samples to soluble fractions. Up until recently, most studies that looked at oxidative potential of particulate matter focused solely on the water-soluble portion of the samples. Now, researchers are beginning to look at the differences in oxidative potential between WPS, soluble fractions, and insoluble fractions. For my study, I only considered WPS and soluble fractions to see if the insoluble fraction had a significant



contribution to oxidative potential. My results showed an increase in oxidative potential when going from WPS to soluble fractions which was unexpected. Past studies have consistently found that oxidative potential decreases for the soluble fractions. One specific study considered the oxidative potential of total DTT, water-soluble DTT, and water-insoluble fraction and found lower oxidative potential for the water-soluble DTT assay compared to the total particle DTT assay while the water-insoluble fractions stayed fairly consistent with the total particle samples (Fang et al., 2017). There are studies that have been focusing specifically on the effect insoluble metals can have on oxidative potential. One study measured oxidative potential of PM<sub>10</sub> that included different insoluble metals in simulated lung fluid using four different assays (Calas et al., 2017). In some of the trials, DTT consumption was lower for samples that included the insoluble fractions compared to soluble fractions alone (Calas et al., 2018). One specific trial of interest consisted on measuring oxidative potential for total particulate matter that mostly consisted of organic compounds using the DTT assay and found a strong diminution of oxidative potential compared to other samples (Calas et al., 2018). Upon further investigation, it is thought that there were compounds in the sample that were able to inhibit DTT oxidation, causing low oxidative potential (Calas et al., 2018). Another possibility for low DTT consumption for WPS in my study is that there was some compound in the insoluble fraction that was stabilizing the ROS that would normally results in higher oxidative potential. This idea could have been investigated more thoroughly if I had been able to preform elemental analysis on my samples.

### ***3. Comparison of Oxidative Potential and Black Carbon Concentrations***

The last part of my results was the comparison of DTT consumption for WPS and soluble fractions to black carbon concentrations. The idea behind my hypothesis was that black carbon concentrations and oxidative potential would have a strong, positive correlation, but my results did not support this idea. For both cases, there was a notable positive trend between DTT consumption and black carbon concentrations; however, both featured low  $R^2$  values, indicating that there is not much correlation between the two factors. A past study in Toronto, Canada also tried to find trends between black carbon concentrations and oxidative potential but was unable to (Weichenthal et al., 2019). Researchers found significant correlations between  $PM_{2.5}$  concentrations and black carbon concentrations, similar to the results from my study, but ultimately found no evidence that trends in  $PM_{2.5}$  concentrations and black carbon concentrations correspond to seasonal changes in oxidative potential (Weichenthal et al., 2019). An additional study on  $PM_{2.5}$  from different locations in Europe also came to the same overall conclusion, but they did note that, while not statistically significant, there was a slight correlation between oxidative potential when using the ascorbate (AA) assay and black carbon concentrations (Gulliver et al., 2018). They think this assay may be better suited to use when looking for correlations in the future due to AA being more independent of other pollutants (Gulliver et al., 2018).

### ***4. Future Directions***

To improve future studies, it would be important to have more consistent sampling for each month. September almost always had the largest data range compared to the other

months which was most likely because it had the most samples. It would also be beneficial to collect more samples throughout the years. This would allow for more correlations and trends to be identified in future studies. It would also be beneficial to run the DTT assay on the insoluble fractions of the samples to see if there were any reactive species within that portion of the samples. Elemental analysis should also be performed on the samples to see what elements compose the particulate matter that was collected which would help with further understanding the adverse health effects that PM<sub>2.5</sub> causes.

It could also be beneficial to look into other methods for measuring oxidative potential. There are various assays that can be used to measure oxidative potential, and each of these assays has different sensitivity to ROS generating compounds. Another common method for oxidative potential is electron spin resonance (ESR) with 5,5-dimethyl pyrroline-N-oxide (DMPO) which involves measuring the ability of the particulate matter to produce hydroxyl radicals in the presence of hydrogen peroxide (Yang et al., 2014).

## ***5. Conclusions***

In summary, the results regarding PM<sub>2.5</sub> concentrations and black carbon concentrations support my hypothesis and are also supported by the results of other studies in the research community. My results regarding oxidative potential do not completely support my hypothesis. There are large variations in studies when analyzing oxidative potential of particulate matter due to our lack of understanding of the complex mixtures that are present in particulate matter. Significantly more research needs to be done in order to get a better understanding of PM<sub>2.5</sub> composition and concentrations and how short-term and

long-term exposure produces adverse health effects. While results may vary, researchers still agree that better monitoring and regulations for particulate matter need to be put in place. Overall, my study shows that the day and month a filter is collected, along with many other factors, should be taken into consideration when studying PM<sub>2.5</sub>.

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