Spring 4-21-2021

Characterizing PM2.5 Air Samples of Northeast Mississippi

Harley Nabors

CHARACTERIZING $PM_{2.5}$ AIR SAMPLES OF NORTHEAST MISSISSIPPI

By

Harley Morgan Nabors

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, MS

May 2021

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ACKNOWLEDGEMENTS

I would first like to thank Dr. Cortney Roper. It has been an incredible honor to have been one of her first undergraduate research assistants at the University of Mississippi. My experience in her lab was marked with efficiency and integrity. She has been a reliable source of knowledge and insight academically, professionally, and personally throughout my undergraduate career. I wish you and Roper labs nothing but the absolute best.

I would also like to thank Maggie Craze for her daily and often tedious help in writing this thesis as well as Dr. Beau Black for his professional expertise and guidance.

Thank you,

Harley Morgan Nabors | B.A. Biochemistry and French

ABSTRACT

HARLEY MORGAN NABORS: Characterizing $PM_{2.5}$ Air Sample of Northeast Mississippi

(Under the direction of Dr. Courtney Roper)

Ambient air pollution has been associated with adverse health effects throughout the entire world. Although epidemiologists study air pollution extensively, rural areas are often the least understood. In 2020, the Mississippi Department of Environmental Quality used less than 10 air sampling locations across the state leaving a large gap in air quality knowledge. In this study, fine particulate matter ($PM_{2.5}$) air samples were collected from three different rural Mississippi cities–Ackerman, Eupora, and Houston, in the summer of 2020. Following collection onto filters, elemental black carbon analysis and an oxidative potential assay were both used to characterize the samples of interest. Varying results of black carbon concentration and DTT consumption from $PM_{2.5}$ were observed with significant differences seen between locations and weeks of sampling. Black carbon concentrations collected at Ackerman, Eupora, and Houston had the following ranges reported in ng/m^3: 0.45-1.05, 0.09-1.13, and 0.00-1.19 respectively. DTT consumption values from $PM_{2.5}$ collected at Ackerman, Eupora, and Houston had the following ranges reported in $nM/min/m^3$: 0.004-0.041, 0.006-0.022, and 0.004-0.012 respectively. A regression analysis ($r^2 = 0.144$) between black carbon concentration and DTT consumption showed that black carbon did not fully explain DTT consumption from $PM_{2.5}$ samples. This work demonstrated that there is variation in $PM_{2.5}$ characteristics with location and time in rural Mississippi. Further studies need to be conducted in order to better understand black carbon concentration, DTT consumption from $PM_{2.5}$, as well as other characteristics of $PM_{2.5}$ within our region of study.

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LIST OF ABBREVIATIONS

WHO: World Health Organization

PM: Particulate Matter

EC: Elemental Carbon

OC: Organic Carbon

OP: Oxidative Potential

ROS: Reactive Oxygen Species

UPAS: Ultrasonic Personal Air Sampler

BC: Black Carbon

DTT: Dithiothreitol

EPA: Environmental Protection Agency

PB: Phosphate Buffer

DTNB: 5,5-dithio-bis-2-nitrobenzoic acid

ANOVA: One-Way Analysis of Variance

SD: Standard Variation

EHP: Environmental Health Perspective

BCP: Black Carbon Particles

AA: Ascorbate Assay

UA: Urate Assay

GDH: Reduced Glutathione (Assay)

ESR: Electron Spin Resonance

MDEQ: Mississippi Department of Environmental Quality

Introduction

Today, there is no question that air pollution poses a threat to personal health around the globe. The World Health Organization (WHO) estimates that ambient or outdoor air pollution accounts for 4.2 million deaths per year due to progression and exacerbation of adverse health effects including stroke, heart disease, lung cancer, and acute and chronic respiratory diseases. Furthermore, around 91% of the world lives in places where air quality is below WHO standards (World Health Organization, 2020). The threat of air pollution is obviously of great importance to the entire world.

Ambient air pollution is a catch all term that includes gases, liquids, and solids. Vallero's Fundamentals of Air Pollution lists several of the well known gases including carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), also known as greenhouse gases (Vallero, 2014). These gases are of specific importance to the climate conditions of the entire Earth. Water soluble gases can react in the atmosphere with water to form liquid air pollution. Gases such as sulfur dioxide (SO_2) and nitrogen dioxide (NO_2) are sufficiently soluble to dissolve in water associated with in-cloud formation of rain droplets which leads to acid rain or fog (Vallero, 2014). Ambient air pollution also includes liquids and solids classified as particulate matter (PM), these particles are often categorized based on size. Course PM is comprised of particulates with an aerodynamic diameter less than 10 μm. The "fine" size fraction of ambient PM, designated as $PM_{2.5}$, is defined as comprising those particles having aerodynamic diameters below 2.5 μm. $PM_{2.5}$ is of significant importance to air quality due to its associated adverse

health effects.While the total mass of $PM_{2.5}$ has been associated with adverse human health outcomes, the relationship between these outcomes and specific chemical components has not been well-resolved (Schlesinger, 2007). Regardless of the knowledge, or lack thereof, of how $PM_{2.5}$ affects human health, starting with an understanding that these exposures are harmful is helpful to researchers.

$PM_{2.5}$ is particularly interesting due to its ambiguity. Ambient $PM_{2.5}$ is chemically nonspecific and consists of various mixtures of components and compounds [trace elements, elemental carbon (EC), organic carbon (OC), and sulfate] (Zhou Jiang et al., 2011). Further, the toxicity of each of these chemical components and their mixtures may vary (Zhou Jiang et al., 2011). Part of the reason for the diverse components found in $PM_{2.5}$ is the seemingly endless sources, each with specific variations. A study of the air quality in New York City (NYC) reported 69–82% of $PM_{2.5}$ mass derives from transport. However, more than 93% of sulfate ions (SO_4^{2-})and 54–65% of nitrate ions (NO_3^-) are likely to have been transported into the NYC area based on the concentrations observed at the background site. Coal-fired power plants in the border area among West Virginia, Ohio and Pennsylvania are related to typical high $PM_{2.5}$ events having peak secondary pollutant concentrations in New York City (Qin et al., 2006) Thus, a broad distribution of $PM_{2.5}$ sources can be seen across urban and rural regions.

Although characteristics of $PM_{2.5}$ like size, shape, and mass are of initial importance to the study of air quality, they are not particularly helpful in determining the toxicity or health outcomes of an air sample. Other measurements can provide more information on toxicity, such

as oxidative potential (OP). OP has been suggested as an important indicator of health effects following exposure to $PM_{2.5}$. OP is a measurement for the ability of a particle to generate reactive oxygen species (ROS) which can put oxidative stress on the body. Oxidative stress occurs when the generation of ROS exceeds the available antioxidant defenses. ROS can damage membrane lipids, proteins, and DNA, which can result in cell death via either necrotic or apoptotic processes (Boogaard Hanna et al., 2012). Exogenous oxidative stress actually is a major contributor to cancer in humans. ROS influence cancer evolution by either initiating/stimulating tumorigenesis and supporting transformation/proliferation of cancer cells or causing cell death (Hayes et al., 2020). Depending on exposure route, high levels of ROS are associated with lung cancer and breast cancer (Hayes et al., 2020). Epidemiologists quantify the oxidative potential of a particle with various methods including the Dithiothreitol (DTT) assay used in this study. Studies continue to suggest that oxidative stress is particularly harmful to humans, and oxidative potential can be used as an indicator of human health (Hayes et al., 2020).

Oxidative stress may be useful in predicting adverse health outcomes, but $PM_{2.5}$ samples and the oxidative potential associated with them vary greatly based on multiple factors. Spatial and temporal variation in $PM_{2.5}$ composition are two factors of specific importance to this study. A longitudinal study of the United States gathered data on these variables. It concluded that the $PM_{2.5}$ mixtures varied strongly by region and by season, and the degree of spatial and temporal variability differs by component, which has implications for epidemiologic research on $PM_{2.5}$ characteristics (Bell Michelle L. et al., 2007). The results of this study actually exemplifies well the variability of $PM_{2.5}$ chemical composition. Ammonium, elemental carbon, organic carbon

matter, nitrate, silicon, sodium, and sulfate were all studied specifically due to their high concentration variance (Bell Michelle L. et al., 2007). Seen in this study are characterizations of $PM_{2.5}$ including elemental composition as well as organic compound composition. Included in organic classes of pollution is black carbon. Black carbon is derived largely from the incomplete combustion of fossil fuels, wood, and biomass, as well as from automobile exhaust (Cooke and Wilson, 1996). "Black" makes reference to these organic particles' ability to absorb light more intensely than elemental carbon (Cooke and Wilson, 1996). Consequently, black carbon contributes to global climate change making it relevant to humans as a whole. Understanding that $PM_{2.5}$ has varying composition, studying multiple sample characteristics from a wide set of locations and time points is essential.

The characterization of $PM_{2.5}$ changes drastically across a country, state, and even local region. Because of this, the air quality knowledge throughout the United States is relatively staggered and is dependent on individual air quality studies. Using data from the Environmental Protection Agency (EPA) an air quality study of 187 continental US counties was conducted. Although they were able to conclude that $PM_{2.5}$ levels were higher in the eastern United States and California and lowest in the central regions and Northwest, there exists strong seasonal and source variability within any given region (Bell Michelle L. et al., 2007). In fact, selected regions were generalized because of unavailable data. Rural areas thus tend to be less understood due to lack of available equipment and studies. For example, one of the most rural states of the country is Mississippi. The entire state had less than 10 particulate matter monitoring locations in 2020 (Mississippi Department of Environmental Quality, 2020). Rural $PM_{2.5}$ studies like our own

study may allow for a better understanding of the air quality in rural areas throughout the world, particularly in the mid-South United States.

Our study collected and analyzed air samples from the town of Ackerman, Mississippi in Choctaw County. This town is considered a rural community (population <2,500) (U.S Health Resources & Services Administration, 2017). Within the same county, Red Hills Coal Mine operates approximately ten miles from this study's sampling site. Red Hills self-reported the production of 3.0 million tons of coal in 2018 (*NACoal | Red Hills Mine*). In regards to $PM_{2.5}$, very little is known within Choctaw County or in the state of Mississippi. In 2020, the Mississippi Department of Environmental Quality conducted a $PM_{2.5}$ study of Mississippi regions and cities including the greater Memphis region, the Jackson metro area, Hattiesburg, the Gulfport-Biloxi area, Pascagoula, and Cleveland (Mississippi Department of Environmental Quality, 2020). The closest of these areas to Choctaw County is Jackson, MS which is approximately 100 miles away. The lack of $PM_{2.5}$ monitoring in Choctaw County by government agencies demonstrates a clear need for this study's research goals. Along with sampling in Choctaw County, two other sampling sites were used in order to have comparative data, Eupora, MS of Webster County (approximately 20 miles Northwest of the Ackerman sampling site) and Houston, MS of Chickasaw County (approximately 50 miles Northeast of the Ackerman sampling site). These locations were chosen due to similar sampling environments but also to study temporal and spatial variation in $PM_{2.5}$ characteristics.

This study used various methods to analyze different characteristics of $PM_{2.5}$ including black carbon analysis and an oxidative potential analysis using a Dithiothreitol assay. Using these methods for each sample site, a comparative study was performed applying these methods to each sampling site. I hypothesized that black carbon and/or oxidative potential from $PM_{2.5}$ sampling decreases in concentration or intensity with increased distance from the Ackerman sampling site that is indicative of the Red Hills Coal Mine. Identifying a radiating characteristic from the Red Hills Coal Mine will suggest that even minor industrial areas, like a small Mississippi coal mine, emit $PM_{2.5}$ to surrounding areas locally or even regionally.

Materials and Methods

1. *Sample Sites*

The following Mississippi cities were used as sample sites: Ackerman (Choctaw County), Eupora (Webster County), and Houston (Chickasaw County). These sites have populations of 1,534; 2,317; and 3,498; respectively (Census Profile). *Figure 1* (Savage Interactive Pty Ltd., 2021) below shows the general location of each sample site.

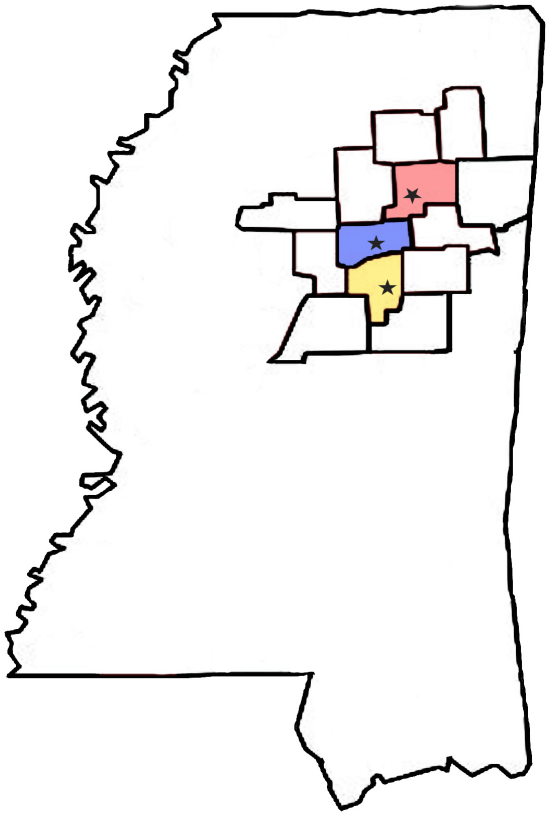


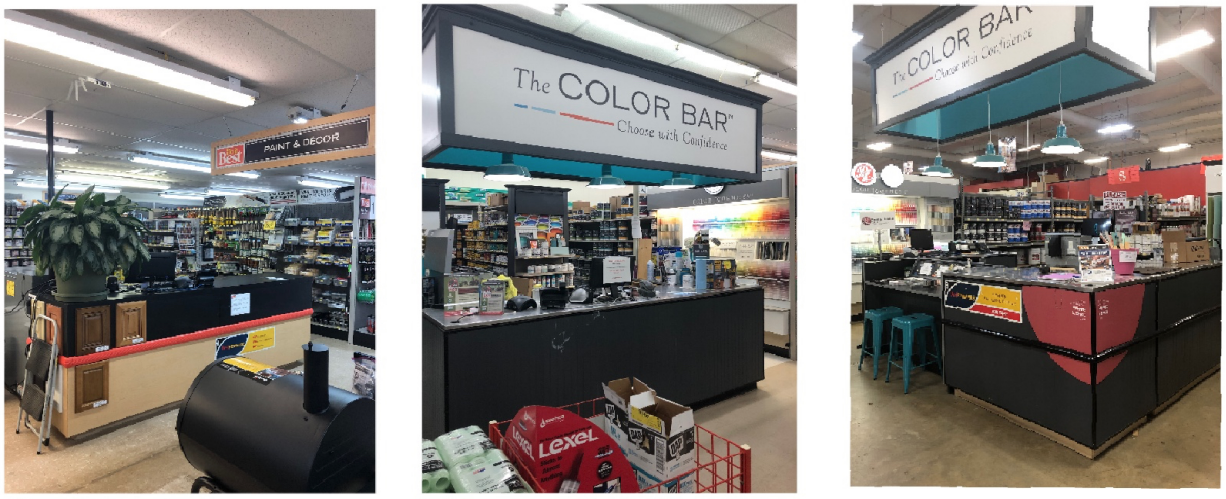
Figure 1: Map of Northeast Mississippi

Choctaw County (Yellow), Webster Counter (Blue), Chickasaw County (Red)

(from bottom to top Ackerman, Eupora, and Houston are represented as stars)

a. *b.* *c.*

Figure 2a-c: Sample Site Exterior



d. *e.* *f.*

Figure 2d-f: Sample Site Interior

g. *h.* *i.*

Figure 2g-i: Personal Samplers

(from left to right columns represent Ackerman, Eupora, Houst

Sampling took place inside of three Nabors Do It Best hardware stores. Each store is in the center of the surrounding rural communities and is considered a small town business. The paint counter was the sample site of each location to ensure similar environments and traffic patterns. *Figure 2a-i* shows each site in detail.

The approximate retail space, the interior air conditioned environment, for each sample site location is listed by Nabors Do It Best as follows: Ackerman (5,000 ft^2), Eupora (8,500 ft^2), and Houston (15,000 ft^2). Stores reported total transactions of 2020 as 46,668; 64,030; and 101,373; respectively (EPICOR, 2020). Sales trends are relatively proportional to city population. Each store is in the center of the surrounding rural communities although traffic flow and business volume is limited to families, commuters, self-employed laborers, and farmers. It should be noted that the Houston store also contains a fully functional concrete plant. However, it is assumed that the majority of the particles emitted by the plant are much larger than the $PM_{2.5}$ of interest and thus will not be captured in this study. General location, surrounding retail displays, and standard air conditioning ensured similar internal environments.

2. Obtaining Filters

The Ultrasonic Personal Air Sampler (UPAS) was used for all sampling. This personal aerosol sampling device is compact, lightweight, and virtually silent when running. The UPAS was tested for pump and battery performance, flow accuracy, and size‐selective sampling efficiency. Device performance was also evaluated against an

EPA-certified reference method for $PM_{2.5}$ sampling through laboratory tests (Volckens et al., 2017). Sampling began August 19, 2020 at 5:00 p.m. cst and ended on September 09, 2020. Filters were replaced weekly at approximately 2:00 p.m. and set to restart sampling at 5:00 p.m. for three weeks total. This study resulted in 3 7-day samples for each sampling location. Travel blank filters were exposed to the open environment for 10 seconds at each sampling site; these filters were used as controls. Sampler parameters can be seen below in *Table 1*.

Instrumentation: Ultrasonic Personal Air Sampler	
Program Run Time: 7 days	Volumetric Flow Rate: 1 L/min
Filter: 37 mm	Average Flow Rate: 0.998 L/min

Table 1: UPAS Parameters

3. ***SootScan: Black Carbon***

Following sample collection, the $PM_{2.5}$ filter samples and blank filter controls were analyzed with a SootScan Model OT21 Optical Transmissometer before extraction to measure the black carbon concentration of each sample. The instrument contains a two wavelength light source: 880 nm for quantitative measurement of Black Carbon PM and a 370 nm for qualitative assessment of certain aromatic organic compounds ("SOOTSCAN™ MODEL OT21 OPTICAL TRANSMISSOMETER"). Each 37 mm filter was analyzed in triplicate using the SootScan with a travel blank 37 mm filter as a control reference. After attenuation was measured, precise sampler summary logs

allowed for the calculation of the black carbon concentrations of each filter relative to total time and volume of air collected during sampling.

4. ***Sample Preparation and Extraction***

Each filter sample and control was placed in a 15 mL conical tube and 8 mL of methanol were added. The filters were sonicated for 1 hour in a water bath at 60 Hz. The filters were then rinsed with 200 µL of methanol to remove any residual particles and placed into petri dishes to be stored. The conical tubes were immediately placed in a -20◦ Cfreezer to be stored away from light until further analysis. Travel blank filters underwent the same preparation, extraction, and eventual analyses to serve as control references.

5. ***DTT Oxidative Potential Assay***

All samples were analyzed for oxidative potential using the DTT assay. DTT is commonly used as an acellular measure of the oxidative potential of particles. In this assay, redox-active chemicals in $PM_{2.5}$ oxidize DTT to its disulfide form and the linear rate of DTT loss from a calibration curve of standards is used as a measure of the oxidative capacity of the PM. Redox-active species in PM then donate an electron to dissolved molecular oxygen, forming superoxide (Charrier and Anastasio, 2012), which can form other reactive oxygen species such as hydrogen peroxide and, in the presence of metals, hydroxyl radicals.

This assay was performed in 96-well plates in triplicates for each sample and control. For each analysis a fresh 5mM stock DTT solution in phosphate buffer (PB) was prepared. On each plate, DTT calibration curves (0-1mM) were included to calculate total DTT consumption for each sample. DTT dilutions for the samples were made by adding 360 µL of PB to 100 µL of DTT stock in order to yield a 1 mM solution. The 96-well plates layout can be seen in *Table 2* below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	DTT - 0	DTT - 0	DTT - 0	Filter #39	Filter #39	Filter #39	Filter #02	Filter #02	Filter #02	-	-	-
B	DTT - 0.2	DTT - 0.2	DTT - 0.2	Filter #38	Filter #38	Filter #38	Blank #3	Blank #3	Blank #3	-	-	-
C	DTT - 0.4	DTT - 0.4	DTT - 0.4	Filter #37	Filter #37	Filter #37	Blank #32	Blank #32	Blank #32	-	-	-
D	DTT - 0.6	DTT - 0.6	DTT - 0.6	Filter #31	Filter #31	Filter #31	Blank #28	-Blank #28	-Blank #28	-	-	-
E	DTT - 0.8	DTT - 0.8	DTT - 0.8	Filter #30	Filter #30	Filter #30	-	-	-	-	-	-
F	DTT - 1	DTT - 1	DTT - 1	Filter #29	Filter #29	Filter #29	-	-	-	-	-	-
G	PB	PB	PB	Filter #05	Filter #05	Filter #05	-	-	-	-	-	-
H	Positive Control	Positive Control	Positive Control	Filter #04	Filter #04	Filter #04	-	-	-	-	-	-

Table 2: DTT Assay 96-Well Plate Layout

(Ackerman samples: yellow; Eupora samples: blue; Houston samples: red)

Three controls were used for this assay – PB run in triplicate, a 0 mM DTT calibration curve, and 15 µL of 1mM 1,4-Napthoquinone. The 1,4-Napthoquinone acts as a positive control with a known and expected reactivity. The controls, DTT curve, and

sample wells all received 100 µL 0.05 M PB. 10 µL of methanol were added to each DTT curve well in order to maintain solvent concentration. 5 µL of each DTT dilution were added to each DTT curve well in triplicate.

20 µL of sample solution was added in triplicate to the wells. 5 µL of the 0.5 mM DTT dilution were then added to each sample well. The entire plate was then covered in foil, shaken for 5 seconds to ensure mixing of the reagents and sample, and then incubated at 37 ℃ for 20 minutes. While incubation took place, a 1 mM 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) quenching reagent was made by adding 900 µL of PB to 100 µL of DTNB stock concentrate. The reaction was quenched with 10 µL of DTNB into all but control wells. The plates were then read using a spectrophotometer to measure absorbance at 412 nm. The amount of DTT consumed from the calibration curves was used to create a trendline. This trendline was used to calculate the DTT consumed for all treatments and controls. For example, wells that initially received DTT to 1mM correspond to 0 mM of DTT consumption, for all DTT is reacted with DTNB.

6. Statistical analysis - software

Figure generation and statistical analyses including linear regressions, one-way analysis of variance (ANOVA), two-way ANOVA were completed with SigmaPlot 14.0 (Systat Software, San Jose, CA). For the one- and two-way ANOVA tests, statistical significance was set at a p-value ≤ 0.05. Initial organization of data was conducted in Excel. Linear regression analysis was conducted to correlate DTT consumption and black

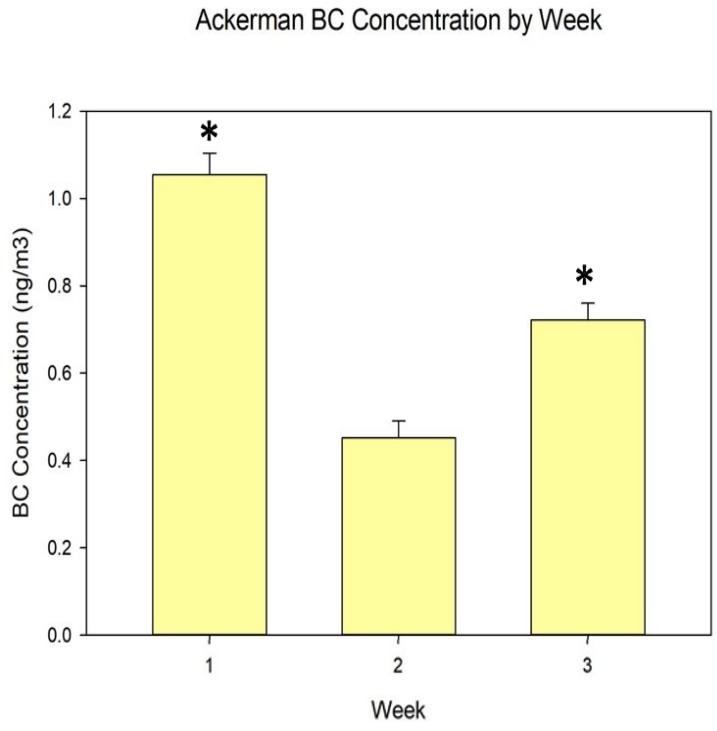
carbon concentrations and generate r^2 values and determine if the slopes of the regression lines were significantly non-zero ($p \leq 0.05$).

Results

1. *Black Carbon Concentrations*

Weekly black carbon concentrations were reported for each sampling location (Fig. 3). Concentrations at the Ackerman sampling site (Fig. 3A) have significant differences between weeks with Week 1 and Week 3 being significant from all other weeks. At the Eupora sampling site (Fig. 3B), significant differences in BC concentrations were observed for Week 2 from all other weeks. At the Houston sampling site (Fig. 3C), both Week 1 and Week 3 were significant from all other weeks while Week 2 shows a negligible concentration. BC concentrations collected at Ackerman, Eupora, and Houston have the following ranges reported in ng/m^3: 0.45-1.05, 0.09-1.13, and 0.00-1.19 respectively.

A)



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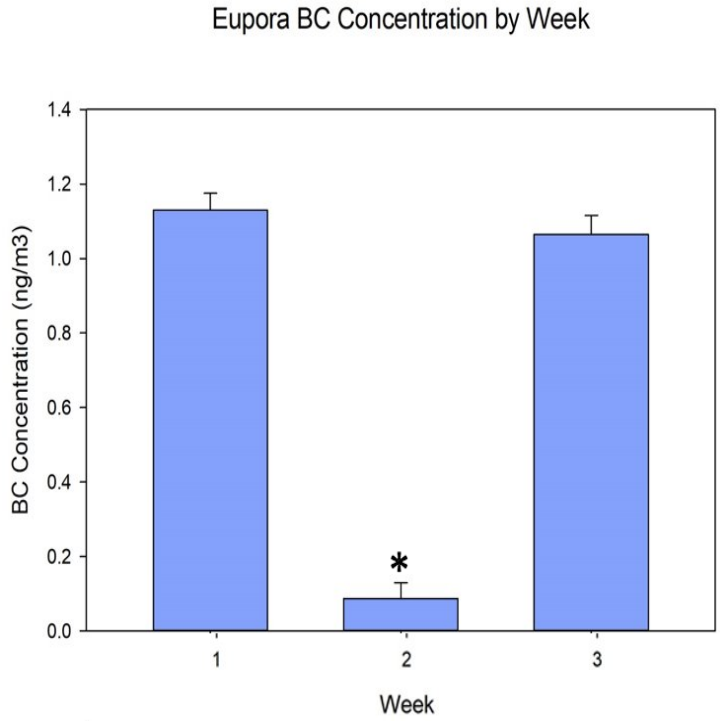

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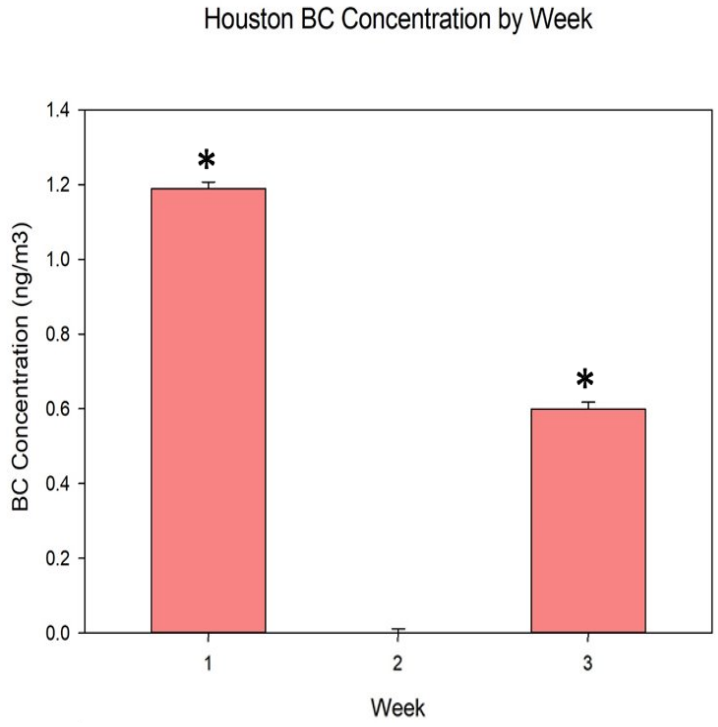


Figure 3 A-C. Weekly BC Concentration of Each Location. Concentrations are reported ± standard deviation (SD) for samples run in triplicate with ng/m^3 for BC concentration at Ackerman (A), Eupora (B), and Houston (C). Weeks 1-3 begin with the

following dates of sampling: 08/19/2020, 08/26/2020, and 09/02/2020 respectively. Statistical significance ($p \leq 0.05$) from one-way ANOVA is indicated by * for significant differences from other samples. Data is corrected with field blank sample concentrations.

BC concentrations were reported for each week (Fig. 4). Concentrations at Week 1 have significant differences between locations with Houston being significant from Ackerman. Week 2 has significant differences between locations with Ackerman and Eupora being significantly different from other locations. At Week 3 significant differences are seen with Ackerman and Eupora being significantly different from other locations. Week 2 shows significantly lower BC concentrations across all locations, while Week 1 shows the highest BC concentration across all locations. Week 1 of Houston shows the highest concentration while Week 2 of Houston shows the lowest.

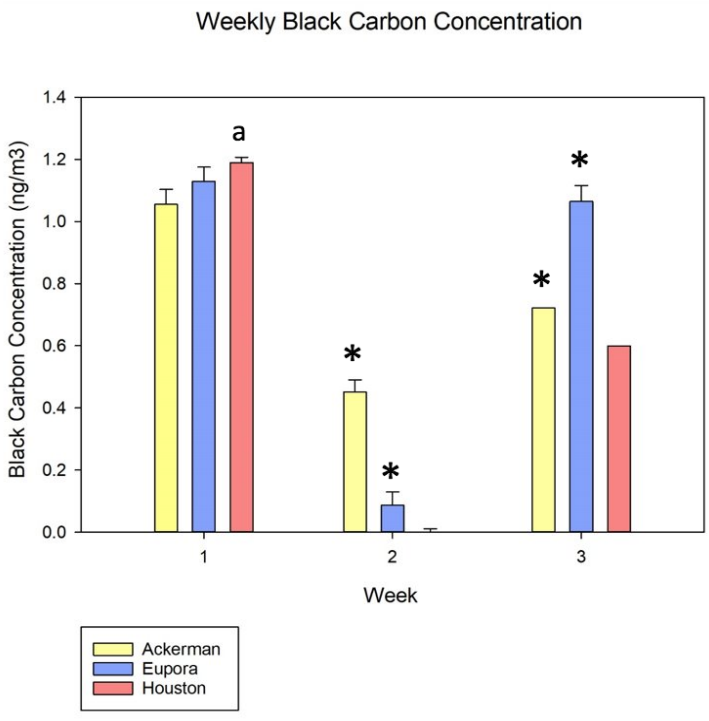


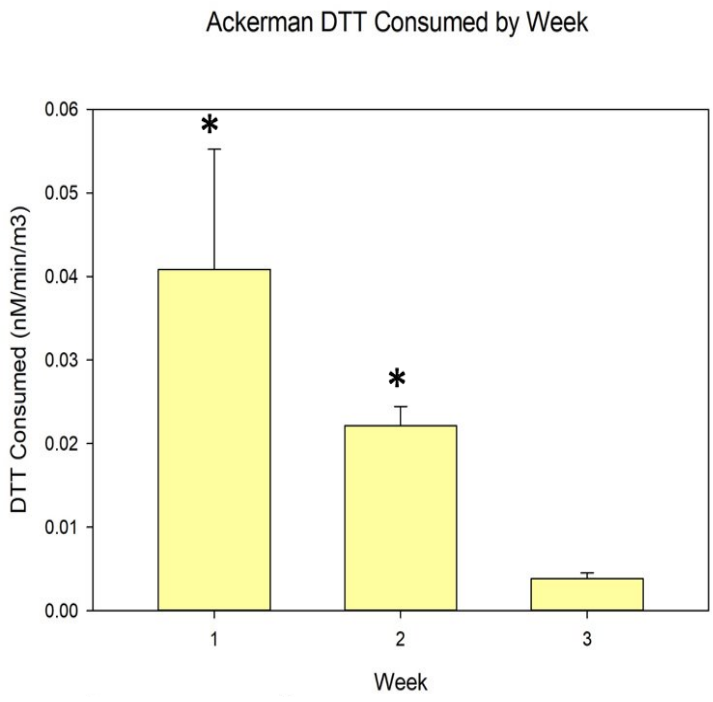
Figure 4. Overall Black Carbon Concentrations. Concentrations are reported ± standard deviation (SD) for samples run in triplicate with ng/m^3 for BC concentration at each location. Weeks 1-3 begin with the following dates of sampling: 08/19/2020, 08/26/2020, and

09/02/2020 respectively. Statistical significance ($p \leq 0.05$) from two-way ANOVA is indicated by * for significant differences from other samples. Data is corrected with field blank sample concentrations.

2. *DTT Oxidative Potential Assay*

DTT consumption values from $PM_{2.5}$ by weeks were reported for each sampling location (Fig. 5). DTT consumption at the Ackerman sampling site (Fig. 5A) have significant differences between weeks with Week 1 and Week 2 being significant from all other weeks. Ackerman also showed a significant decrease in DTT consumption across all weeks. At the Eupora sampling site (Fig. 5B), significant differences in DTT consumption were observed for Week 2 from all other weeks. At the Houston sampling site (Fig. 5C), no significant differences are observed, but an increasing trend of consumption is seen across weeks. DTT consumption values from $PM_{2.5}$ collected at Ackerman, Eupora, and Houston have the following ranges reported in $nM/min/m^3$: 0.004-0.041, 0.006-0.022, and 0.004-0.012 respectively.

A)



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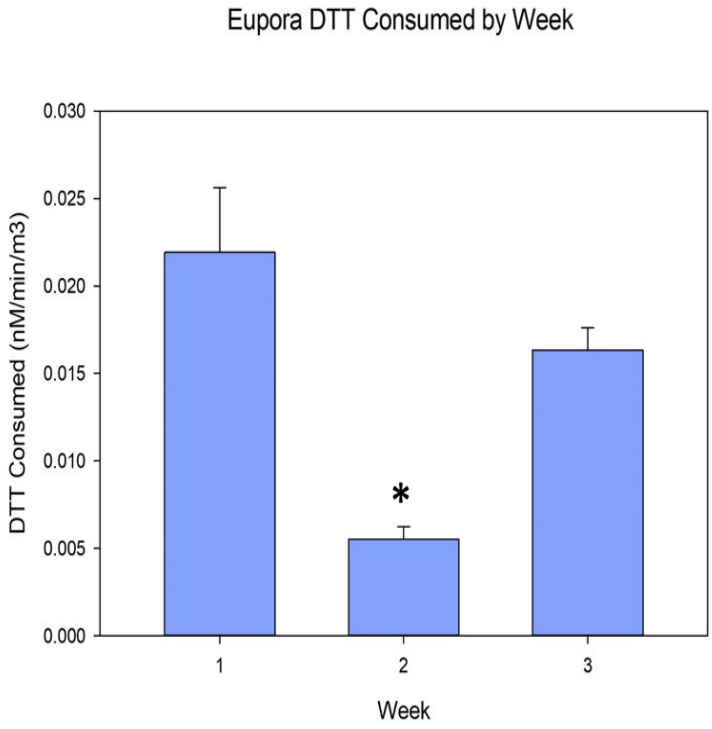

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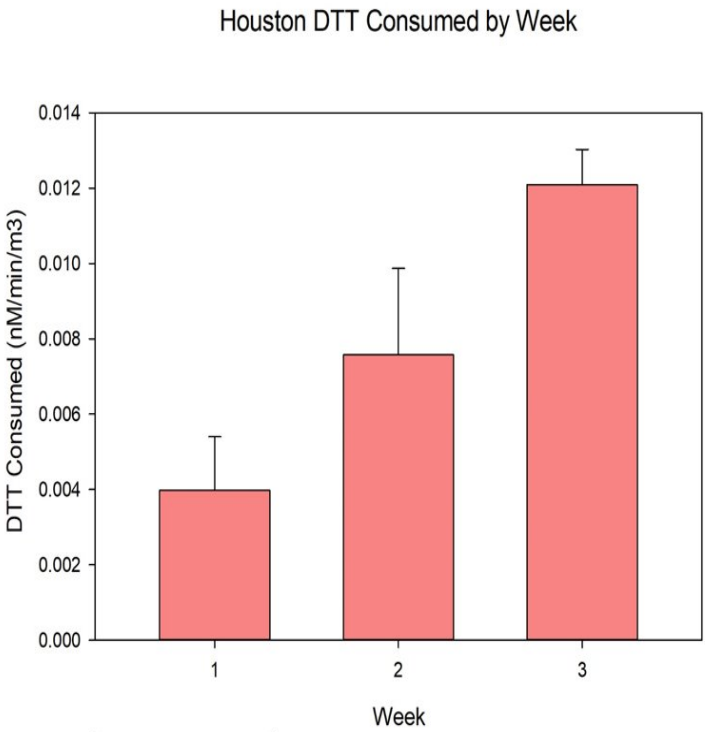

Figure 5A-B. Weekly DTT Consumption of Each Location. DTT consumption is reported ± standard deviation (SD) for samples run in triplicate with nM/min/m^3 for DTT consumption at Ackerman (A), Eupora (B), and Houston (C). Weeks 1-3 begin with

the following dates of sampling: 08/19/2020, 08/26/2020, and 09/02/2020 respectively. Statistical significance ($p \leq 0.05$) from one-way ANOVA is indicated by * for significant differences from other samples. Data is corrected with a DTT calibration curve.

Overall DTT consumption was reported for each sampling location (Fig. 6). Concentrations at Week 1 have significant differences between locations with Ackerman and Eupora being significant from all other locations. In Week 2, there was a significant difference between DTT consumed from $PM_{2.5}$ collected in Ackerman compared to all other locations. There were no significant differences of DTT consumption observed for Week 3. Week 1 of Ackerman shows the highest DTT consumption from $PM_{2.5}$ while Week 3 of Ackerman shows the lowest DTT consumption.

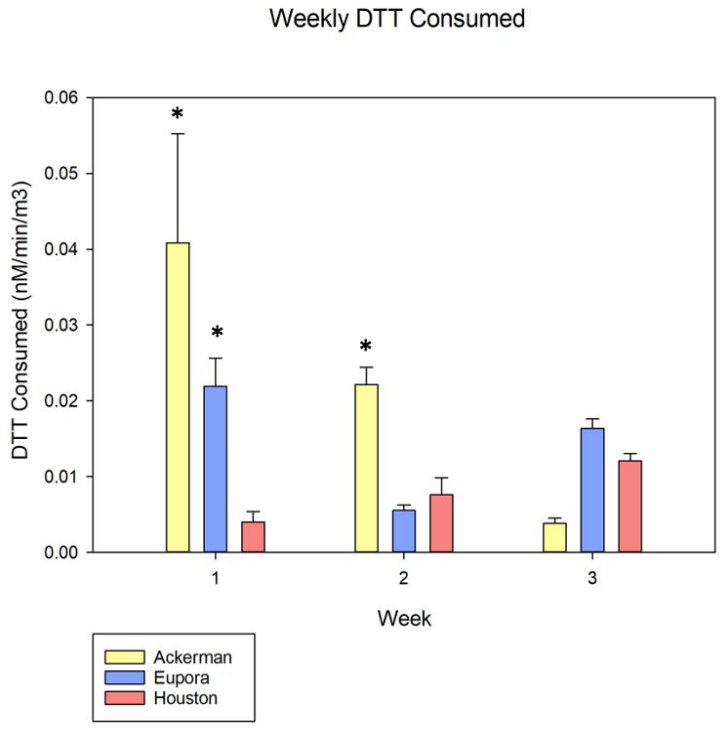

Figure 6. Overall DTT Consumption. Concentrations are reported ± standard deviation (SD) for samples run in triplicate with nM/min/m^3 for DTT consumption at each location. Weeks 1-3 begin with the following dates of sampling: 08/19/2020,

08/26/2020, and 09/02/2020 respectively. Statistical significance ($p \leq 0.05$) from two-way ANOVA is indicated by * for significant differences from other samples. Data is corrected with a DTT calibration curve.

3. Regression Analysis

A regression analysis was conducted to compare black carbon concentrations and DTT consumption of all sampling locations (Fig. 7). Based on the slope of the trendline, a significant positive trend was observed between the black carbon concentration and DTT consumed.

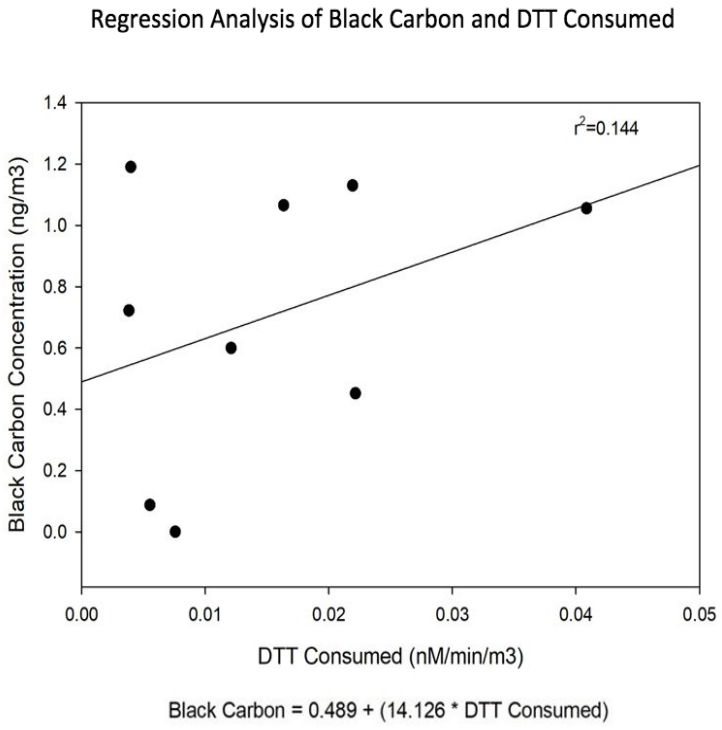

Figure 7. Regression Analysis of BC Concentration and DTT Consumption. A simple linear regression analysis was performed for BC concentration (ng/m^3) and DTT consumption ($nM/min/m^3$) for all weeks and locations. A trendline of best fit following the form $y = 14.126x + 0.489$ and an r^2 value of 0.144 were reported with a significance set at $p<0.05$.

Discussion

1. *SootScan: Black Carbon*

Results obtained from black carbon analysis showed varying trends. Week 1 showed the highest BC concentrations for all sampling locations. Week 2 showed the lowest BC concentrations for all locations, although there were no significant differences of Week 2 from other weeks at Ackerman. Houston had the highest and lowest BC concentrations of all locations at Week 1 and Week 2 respectively; Week 2 showed a negligible BC concentration. Eupora showed relatively high BC concentrations at Week 1 and Week 3 with no significant difference observed.

Week 1 showed the following increasing trend across locations of BC concentration: Ackerman, Eupora, Houston in ascending order. Week 2 showed the following decreasing trend in BC concentration: Ackerman, Eupora, Houston in descending order. Week 3 showed the following decreasing trend: Eupora, Ackerman, Houston in descending order. With these results, we can not accept our hypothesis that black carbon concentrations decrease with increased distance from the Ackerman sampling site. Although Week 2 did in fact show a decreasing trend of concentrations, Week 1 and Week 3 showed different trends that do not fully support our hypothesis. Further studies need to be conducted in order to better understand BC within our region of study.

As previously discussed, black carbon is a byproduct of organic combustion that can come from many different sources and have varying health effects. In 2011, The Environmental Health Perspective (EHP) conducted a study to characterize components of $PM_{2.5}$ other than mass that are indicative of inverse human health effects including BC, black carbon particles (BCP), elemental carbon (EC), and organic carbon (OC) (Janssen et al., 2011). Combustion-related particles are thought to be more harmful to human health than PM that is not generated by combustion. In urban areas, road traffic is a major source of combustion PM where transport related air pollution contributes to an increased risk of cardiopulmonary problem (Janssen et al., 2011). In more rural areas like our own region of study, combustion related particles like BC or BCP can be apportioned often to wood and coal burning, shipping traffic from local farms and industry, and industrial sources. Mechanical related BC refers to black smoke being indicative of poor combustion efficiency whereas blue smoke is indicative of mechanical wear. There is increasing concern that current mass-based PM standards are not well suited for characterizing health risks of air pollution near sources of combustion particles, such as motorized traffic on major roads or in wood-smoke dominated communities (Janssen et al., 2011). The Ackerman sampling site is surely at risk for pollutant exposure including BC from the Red Hills Coal Mine. Surrounding rural communities that include the Eupora and Houston sampling sites are also at risk for pollutant exposure from agricultural activities. The EHP study found that overall health effect estimates from mortality and comorbidity were higher for BCP than for PM reported alone. In fact, they determined that 40-70% of roadside $PM_{2.5}$ mass can be attributed to BCP. The

single-pollutant effect model of this study showed that daily mortality and hospital admissions generally were an order of magnitude higher for BCP when compared to PM. The results of the two-pollutant model showed that the health effects from BCP are more robust than the health effects of PM mass alone. Our study showed varying concentrations of BC, but there is need for further study in order to better understand source apportionments of BC and other related pollutants as well the associated adverse health effects of our region of study.

Because BC can come from many sources that can vary within any given region, models that predict indoor BC concentration based on outdoor sources of BC could be of interest to further studies. A study published in 2019 concluded that outdoor BC combined with home characteristics can be used to predict indoor BC levels with reasonable accuracy. The study identified the following home characteristics and occupant activities that significantly modify the concentration of indoor BC: outdoor BC, lit candles and electrostatic or high efficiency particulate air filters in heating, ventilation and air conditioning (HVAC) systems (Isiugo et al., 2019). Although open candles and high efficient HVAC systems were not present at our sampling locations, each location has varying outdoor BC sources including industrial and agricultural. Consequently, indoor predictive models like in the 2019 study could be beneficial. Though direct active sampling provides specific data of BC, models that predict more general and applicable BC data could be of interest to further studies in the Northeast Mississippi region.

2. DTT Oxidative Potential Assay

Results obtained from the DTT oxidative potential assay also showed varying trends. Week 1 of Ackerman showed the highest DTT consumption from $PM_{2.5}$ of all locations and weeks while Week 3 of Ackerman showed the lowest. Week 2 of Ackerman also had the highest DTT consumption for that specific week. Ackerman showed a decreasing trend of DTT consumption across weeks with Weeks 1 and 2 being significantly different from Week 3. Houston showed an increasing trend of DTT consumption from $PM_{2.5}$ across weeks. Eupora did not show similar trends, but Week 2 was significantly lower in DTT consumption than all other weeks.

Week 1 showed the following decreasing trend across locations in DTT consumption from $PM_{2.5}$: Ackerman, Eupora, Houston in descending order. Week 2 did not show the same decreasing trend, but Ackerman did have significantly higher DTT consumption from all other weeks with Eupora and Houston not being significantly different from each other. Although there were no significant differences observed between locations, the following decreasing trend of DTT consumption was observed: Eupora, Houston, Ackerman in decreasing order. With these results, our hypothesis is partially supported. DTT consumption from $PM_{2.5}$ decreased with increased distance from the Ackerman sample site in Weeks 1 and 2 with significant differences seen in each. However, Week 3 did not show this same trend. For these reasons, our hypothesis is not fully supported. Further studies need to be conducted in order to better explain DTT

consumption trends, seasonal and temporal differences, and human health effects of the oxidative potential seen from the DTT assay from $PM_{2.5}$ of our region of study.

The DTT assay that was used in this study estimates oxidative potential and is positively correlated with biomarkers that correspond to oxidative stress and inflammation biomarkers (Berg et al., 2019). Oxidative stress refers to a state of biochemical imbalance in which the presence and formation of ROS in the body exceeds that of antioxidant defenses leading to adverse health effects (Gao et al., 2017). The DTT assay estimates in vivo ROS generation or response from biochemical interactions. A leading hypothesis of $PM_{2.5}$ toxicity is that $PM_{2.5}$ generates ROS which leads to oxidative stress and systemic inflammation (Berg et al.,2019). The samples of our study showed varying degrees of DTT consumption from $PM_{2.5}$ corresponding to varying OP. The linear regression analysis (Fig. 7; $r^2 = 0.144$) does not show a strong correlation between BC concentrations and DTT consumption. BC concentrations do not fully explain the OP seen from the DTT assay, but other possible components of $PM_{2.5}$ could be of interest. The DTT approach at estimating OP is best at characterizing OC, combustion-emitted species like BC, and redox active transition metals like Cu, Fe, and Mn (Gao et al., 2020). This shows the need for further studies within the DTT assay to better characterize possible OP from $PM_{2.5}$ due to OC and redox active metals.

A study conducted in 2016 sampled approximately 500 $PM_{2.5}$ filters across the Southeastern US in urban and rural areas including the Atlanta metro area, Birmingham

and surrounding regions, East St. Louis, and rural roadside sampling (Fang et al., 2016). Two rural sampling sites of this study in Yorkville, IL and Centerville, AL reported a summer monthly DTT consumption from $PM_{2.5}$ of approximately 0.28 nmol/min/m^3 and 0.32 nmol/min/m^3 respectively with the winter months generally showing higher DTT activity (Fang et al., 2016). This study concluded that biomass burning and other organic combustion was related to much of the OP from the DTT assay observed. Although Ackerman of Week 1 showed significantly higher DTT consumption other samples at 0.04 nM/min/m^3, weeks and locations generally fell within the following range: 0.020-0.005 nM/min/m^3. One can see that our values of DTT consumption are much lower than that observed in the 2016 study. This could be due to varying $PM_{2.5}$ source apportionment that produce different results from the DTT assay. Further research should be done to better understand sources of $PM_{2.5}$ within the Southern US and their relationship to OP from the DTT assay.

Alternative methods of OP estimation could also be used to better understand $PM_{2.5}$ including the ascorbate (AA), urate (UA), and reduced glutathione (GDH) assays. These assays indirectly measure OP by mimicking antioxidant consumption from their respective reagents (Gao et al., 2020). Although these methods yield similar indirect results of OP, comparing them could be beneficial. Electron spin resonance (ESR) spectrometry can alternatively be used to measure direct and distinct ROS species. This method could be used in further studies to specifically characterize OP from $PM_{2.5}$ samples. Regardless

of methods, further studies need to be conducted in order to better understand the relationship between $PM_{2.5}$ and OP within our region of study.

3. Continued Research

There are little to no studies that characterize Mississippi $PM_{2.5}$ like our own, thus comparisons of studies are limited. The Journal of Exposure Science and Environmental Epidemiology (JESEE) published a study that used satellite-retrieved aerosol optical depth technology in order to predict the spatial and temporal patterns of $PM_{2.5}$ exposures of the Southeastern US (Lee et al., 2016). This novel approach addresses errors in ground monitoring stations and land-use regression models. Region 1 of this study mainly consisted of the states of Tennessee, Mississippi excluding much of the western region, Alabama, and Georgia. There were 61 monitoring stations (0.0003 monitor/km^2) in this region. This study reported $PM_{2.5}$ concentration ($\mu g/m^3$) predictions with $r^2 = 0.77$ (Lee et al., 2016). This study provides valuable insight into the distribution of $PM_{2.5}$ within the Northeast Mississippi region of interest, but it does not characterize any specific $PM_{2.5}$ samples like our own study. For this reason we can not compare the data obtained from JESEE to that of our own.

The Mississippi Department of Environmental Quality (MDEQ) publishes an annual report in which ozone, PM, sulfur dioxide, and nitrogen oxides are monitored across the state. In 2020, nineteen sampling locations were used, but only eight of these locations monitored PM (*Annual-Report-Fiscal-Year-2020*). Although the MDEQ study's

Grenada county sampling site is approximately 50 miles from our own Choctaw county sampling site (Ackerman), the report only lists standards of $PM_{2.5}$ exposure and does not characterize $PM_{2.5}$ samples beyond concentration. Consequently, we are again unable to compare the MDEQ report to our own data.

As seen in the results of our study, BC concentrations and DTT consumption from $PM_{2.5}$ vary significantly based on temporal and spatial factors. More studies can be conducted in order to better understand these relationships. Increasing the number of sampling sites could produce results that help to account for spatial differences. Finally, further research needs to be conducted in order to account for seasonal variability. Our study actively sampled $PM_{2.5}$ during the summer months of August and September. $PM_{2.5}$ sampling in the winter months could help to explain the variability of $PM_{2.5}$ characteristics.

Although the active sampling sites were similar in location with each being in the center of the surrounding rural communities with traffic flow and business limited to families, commuters, self-employed laborers, and farmers, each location varies in $PM_{2.5}$ sources and characteristics as seen above with BC and OP from the DTT assay. We have identified exterior sources of $PM_{2.5}$ including wood and coal burning, road traffic, industry including farming, etc. (Janssen et al., 2011). However, further research needs to be done to identify and characterize $PM_{2.5}$ apportionment from indoor sources of regions and locations like our own study.

Conclusion

Our study collected $PM_{2.5}$ air samples from three different rural Mississippi cities–Ackerman, Eupora, and Houston, in the summer of 2020. Following collection onto filters, elemental black carbon analysis and an oxidative potential assay were both used to characterize the samples of interest. Varying results of black carbon concentration and DTT consumption from $PM_{2.5}$ were observed with significant differences seen between locations and weeks of sampling. A regression analysis ($r^2 = 0.144$) between black carbon concentration and DTT consumption showed that black carbon did not fully explain DTT consumption from $PM_{2.5}$ but there was a significant positive trend between this component of PM and oxidative potential. This work demonstrated that there is variation in $PM_{2.5}$ characteristics with location and time in rural Mississippi. Further studies need to be conducted in order to better understand black carbon concentration, DTT consumption from $PM_{2.5}$, as well as other characteristics of $PM_{2.5}$ within our region of study.

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