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AEROSOL MUTAGENICITY IN SAN BERNARDINO, CALIFORNIA VIA BACTERIAL FLUCTUATION TESTS

Sean Murphy

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A Project

Presented to the

Faculty of

California State University,

San Bernardino

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in

Environmental Sciences

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by

Sean Murphy

August 2023

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Approved by:

Dr. Andreas Beyersdorf, Committee Chair, Chemistry

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ABSTRACT

San Bernardino, California's poor air quality is a health risk to its population with some of the highest concentrations of ozone and aerosol in the United States. Aerosols are solid or liquid particles suspended in the air. Smaller aerosol particles are the most harmful as they are more likely to penetrate further into the respiratory system. These aerosols can cause respiratory issues and contain toxic compounds. One measure of aerosol toxicity is to study the mutagenicity of the aerosol. To study the mutagenicity of aerosol samples collected in San Bernardino in 2022, fluctuation tests (based on the Ames test for mutagenicity) were used with a modified bacterial strain of *Salmonella typhimurium* that cannot grow without the addition of histidine. The presence of mutagens in sample extracts cause the bacteria to mutate and grow. This bacteria growth can then be detected based on a change in color of a pH indicator. In addition to the bacterial fluctuation test, heavy metal concentrations in the aerosols were measured using inductively coupled plasma optical emission spectroscopy (ICP-OES). No correlation between mutagenicity and heavy metal concentration was found. There was also not a clear seasonality to the particulate matter concentration and total metal concentration. However, there was a correlation between particulate matter concentration and rates of mutagenicity. From these analyses it was found that a 60% increase in aerosol concentration corresponded to a 92% increase in the rate of mutagenicity.

Due to these measurements, it is believed that metals are not the cause of the mutagenicity. Future studies should include the measure of possible mutagens such as polyaromatic hydrocarbons (PAHs). In addition, analysis of an entire year of samples should be performed so that trends of mutagenicity compared to aerosol concentration can be determined.

ACKNOWLEDGEMENTS

I'd like to thank my advisor Dr. Beyersdorf committee members Dr. Noblet and Dr. Stanley. I would also like to thank the CSUSB Chemistry Department for allowing me to teach general chemistry and biochemistry labs to support grad school. Previous work on aerosol mutagenicity by Cole Ebel (now at UC Riverside) funded through the CSUSB Office of Student Research Summer Research Program. Additional funding from the NASA CAMP^{2}Ex campaign. Additional assistance from Charles Herrera Paola Boshra.

DEDICATION

I'd like to dedicate this thesis to my friends and family, because I know most of them will never read past the abstract, especially my sisters.

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Greater Los Angeles, California has a long history of poor air quality. During the 1980's, Los Angeles averaged less than 10 days a year with good air quality compared to 138 days with very unhealthy air quality. Efforts have proven fruitful over the last several decades resulting in less than 5 days on average per year with very unhealthy air quality since 2010 (LA Almanac, 2023). However, it still ranks among the regions with the worst air quality in the United States. In particular, San Bernardino County has been noted for its poor air quality, with the American Lung Association giving it a failing grade in 2022. Based on the air quality index (AQI, AirNow, 2023), San Bernardino County had 169 days with unhealthy air quality and 220 with air unhealthy for sensitive groups (ALA, 2022). With the state of San Bernardino's air being so poor, it is important to study it to determine the causes and trends in the pollution.

The primary pollutants in the atmosphere are ozone (a gas formed from photochemical reactions of vehicular exhaust) and aerosols. Aerosols are solid or liquid particles suspended in the air which can be produced naturally (such as wind-blown dust), anthropogenically (such as from vehicles, industry and any other combustion sources), or a combination of the two (such as wildfires which can be either caused by lightning strikes or human activity). The primary measure of aerosols is the mass of the total suspended particles (TSP) in a

volume of air with units of μg/m^3. TSP can be found by gravimetrically measuring the mass of particles when filtering a known volume of air.

Aerosols can be further defined by their size with the mass of particles with diameters smaller than 10μm classified as PM_{10}, smaller than 2.5μm as $PM_{2.5}$, and 1μm as PM_1. As PM_{10} encompasses a larger range of aerosols it is by definition the largest of the three values. PM_{10} annual concentrations in North America are typically found in the range of 10 to 55 μg/m^3 (Silversten, 2002). However, more densely populated area can see up to 500 μg/m^3. Focusing only on $PM_{2.5}$ results in lower concentrations. However, $PM_{2.5}$ is more relevant to human health because smaller particles are a larger health hazard. The National Ambient Air Quality Standard (NAAQS) for $PM_{2.5}$ is set at 12 μg/m^3 for an annual average and 35 μg/m^3 for a 24-hour period. For PM_{10} the 24-hour standard is set at 150 μg/m^3 (California Air Resources Board, 2023).

There are many different types of aerosols with variability in size, composition, and health effects. Thus, different geographical areas experience different forms of aerosol pollution. For instance, near the ocean the primary aerosol is sea spray which is mostly comprised of salts containing sodium and chlorine. While areas in more forested areas experience organic aerosols produced from the oxidation of terpenes emitted from trees. These are examples of natural aerosols. In urban areas anthropogenic aerosols dominate containing metals and organic compounds that can be harmful to human health.

The primary source of aerosols in urban areas is combustion of fossil fuels. This combustion can emit heavy metals into the air including chromium, zinc, cadmium and lead. Heavy metal pollution has been linked to multiple health issues. Mercury, lead and arsenic have been known to cause kidney damage and neurotoxicity leading to loss of memory, sleep disorders, mood swings, fatigue and blurred vision (Ewan and Pamphlett, 1996, and Ratnaike, 2003). Heavy metals are also known teratogens, substances that can cause a nonheritable birth defects, and have been linked to premature fetal deaths and low birth weights (Schell et al., 2006). Metals commonly found in aerosols are manganese, copper, zinc, cadmium, chromium, iron, nickel, potassium, calcium, vanadium, barium, arsenic, selenium and strontium.

There are a wide range of particulate organic compounds produced from combustion including polycyclic aromatic hydrocarbons (PAHs). PAHs are molecules comprised of two or more aromatic rings. The PAH structure is stabilized by the sharing of pi bonds throughout the structure giving it a planar geometry. Seven PAHs are classified by the United States Environmental Protection Agency to lead to increased risk of cancer (USEPA, 2008). Particularly, benz(a)anthracene, benzo(a)pyrene, and naphthalene have been noted to lead to birth defects due to exposure during pregnancy (Kristensen et al., 1995; Wassenberg and Di-Guilio, 2004). PAH concentrations in the air are dependent upon the season and the region. In tropical areas, PAH concentrations are highest in the summer, while in cooler artic region they are

most prevalent in the winter. This can be explained based on energy usage data for these regions. Tropical areas use their air conditioners more during the summer resulting in higher fossil fuel combustion from power plants. In cooler regions, more fuel is burnt in the winter to warm their homes.

Because aerosol composition can change based on location and time of year, aerosol toxicity is also variable. One measure of studying aerosol toxicity is to measure its mutagenicity. A mutagen is a chemical agent that can disrupt the sequence of DNA by modifying its sequence. Modification of DNA can be spontaneous, however damage to DNA caused by outside pollutants can lead to increased mutagenicity. While a mutation can have no effect on DNA transcription, some may have severe outcomes including cancer. The primary study on aerosol mutagenicity in Southern California measured the mutagenicity of aerosol samples from Los Angeles and surrounding communities in 1992 (Hannigan et al., 1996). The highest annual average of mutagenicity per cubic meter of air sampled occurred in Central Los Angeles with the lowest measured at an upwind site. Mutagenicity measured at inland sites (Azusa and Rubidoux) were lower than measured in central Los Angeles but were seasonally dependent. The highest rates of mutagenicity measured at Rubidoux were during the fall months likely due to increased transport of pollution sources from Los Angeles.

Aerosol mutagenicity measurements are not routine and no further studies in Southern California have been reported. Mutagenicities are expected to differ

now because of lower overall pollution in the region and increased local pollution sources in Riverside and San Bernardino County. In addition, updated mutagenicity testing capabilities have been developed since 1992.

The present study determines variability in two measures of toxicity for aerosols collected in San Bernardino:

1) Aerosol Mutagenicity via a fluctuation Ames Test
2) Particulate Metals via inductively coupled plasma optical emission spectroscopy (ICP-OES)

Sampling was performed in San Bernardino (34.107°N, 117.274°W) from February to September 2022. San Bernardino has been an EPA non-attainment region every year since 2009 for $PM_{2.5}$ (EPA, 2023). Since 2010, 41.5% of the months have had $PM_{2.5}$ above 12 $\mu g/m^3$, the NAAQS annual limit (Figure 1). There is no clear seasonal trend for $PM_{2.5}$, however, a seasonal trend in PM_{10} is apparent with highest concentrations in the summer and the lowest in the winter (Figure 2). Even though concentrations are lowest in winter, previous work has shown that fall and winter are the time with the highest mutagenic potency. Potential spikes in aerosol mutagenicity may also be measured in the summer due to increased photochemically produced compounds (Hannigan et al., 1996).

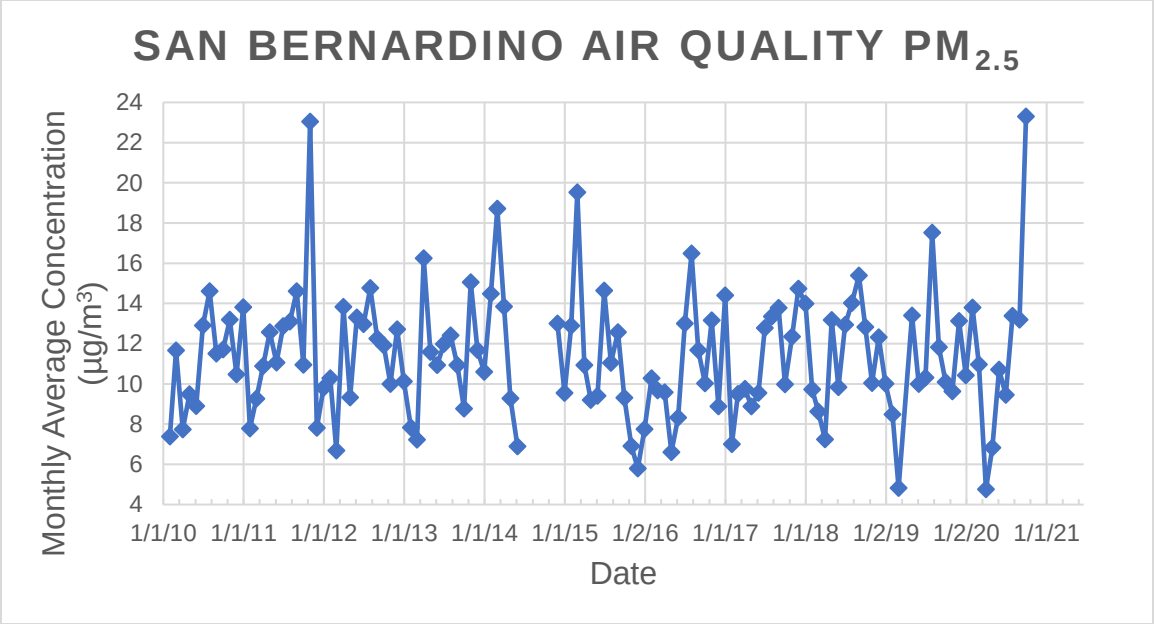

Figure 1. Monthly average $PM_{2.5}$ from January 2010 - September 2020 data from EPA, 2022.

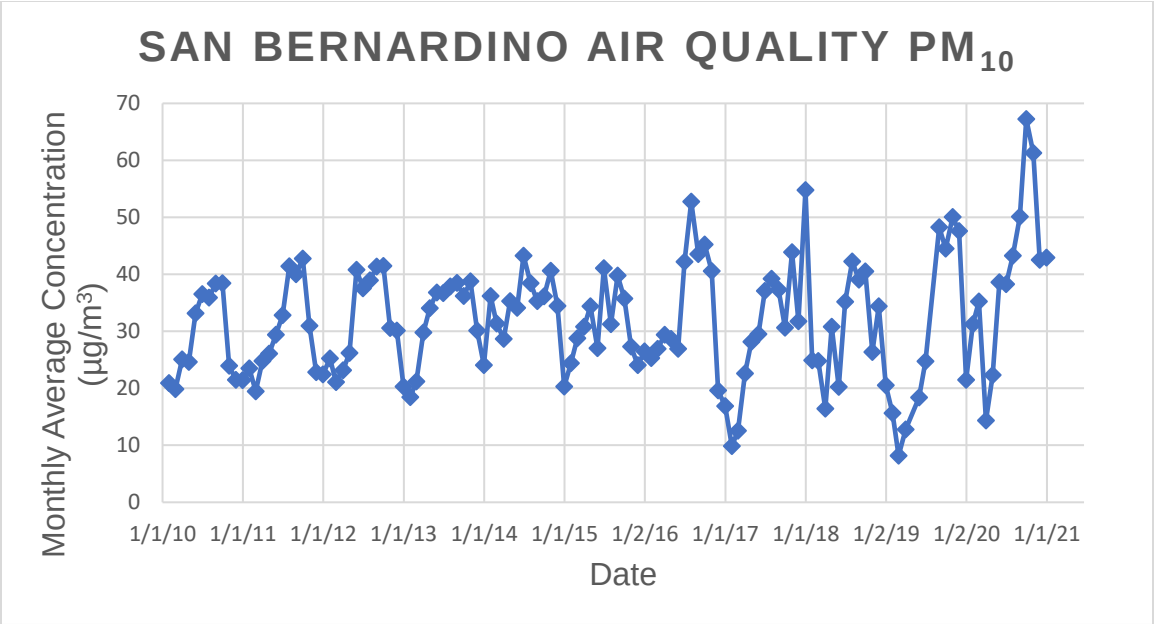

Figure 2. Monthly average PM_{10} from January 2010 - December 2020 data from EPA, 2022.

CHAPTER TWO

METHODS

Development of methods to measure aerosol mutagenicity was the primary goal of this study. These methods can be broken down into four sections:

1) Sample Collection
2) Sample Extraction
3) Elemental Analysis
4) Mutagenicity Analysis

Sample Collection

Sample collection was performed using a total suspended particulate (TSP) high volume air sampler (Thermo Electron Corporation, T7801B, Figure 3). The TSP sampler collects air particles suspended in the air by the use of a vacuum that pulls air in at an average flow rate of 160 L/min. Air flows through a filter and is exhausted, while particulate matter is trapped on the filter. The TSP sampler was placed on the roof of the Chemical Sciences building at California State University San Bernardino (CSUSB, 34.185°N, 117.325°W, 481 m elevation). The filters used were quartz microfiber suitable to collect PM_{10} samples (Whatman, Quartz Macrofibre Filters CAT No. 1851-101). A filter was weighed and then placed in the TSP sampler for a seven-day period. At the beginning and end of each period the pressure drop across the filter (in inches of

water) was recorded. This pressure drop is proportional to the flow through the filter and was calibrated before and after the sample period.

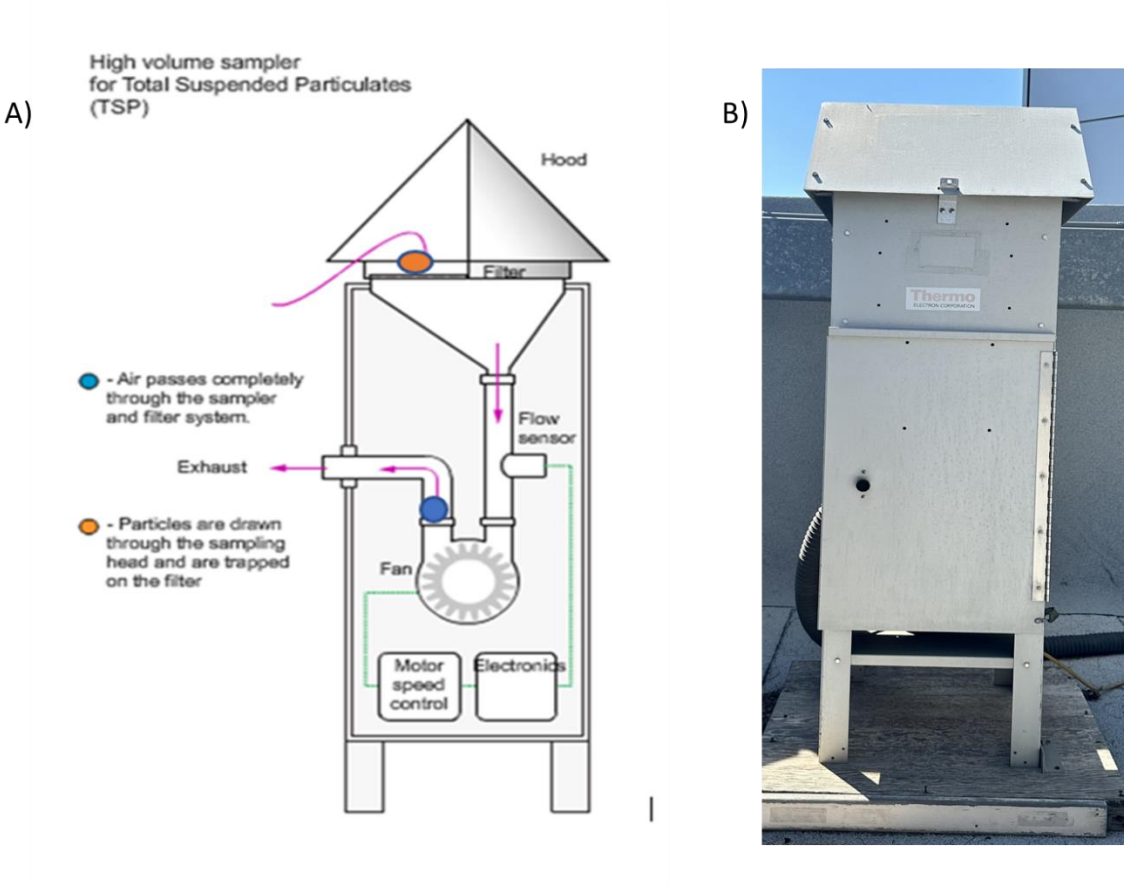

Figure 3: A) Total suspended particulate high volume autosampler schematic provided by Tisch Environmental. **B)** TSP sampler located on the roof of the Chemical Sciences building.

After sampling, the aerosol-laden filters were placed in a desiccator to remove water from the filter before weighing. Samples were then frozen until ready for extraction. The initial goal of the study was to sample every other week for one year with sample collection beginning in spring of 2022. However,

sampling could not be performed continuously due to pump issues that required replacement of a motor brush and then the entire pump. Therefore, sampling for an entire year was not possible but does entail the late winter, spring and summer months.

Sample Extraction

The second aspect of the methodology is extraction of the aerosols from the quartz filters. Two methods were used initially: pure water extraction and hot acid extraction based on EPA Compendium Method IO-3.1 (EPA, 1999). Both the water and acid extraction were analyzed for their extraction efficiencies using inductively coupled plasma optical emission spectroscopy (ICP-OES). The hot acid extraction provided better extraction of aerosols (described below) and is therefore described here. Filters are cut with ceramic scissors into small strips and placed in a 100-mL beaker. 10 mL of a 3:1 water: acid mixture of concentrated hydrochloric acid and nitric acid is added and the beaker is covered with a watch glass and heated for 30 minutes. After extraction, the extract is filtered and diluted to 100 mL with Type I deionized water in a volumetric flask. This method was modified to reduce the risk of the acid boiling off by the use of a reflux condenser.

Elemental Analysis

ICP-OES is a common spectroscopic method using the atomic emission spectra to measure the concentrations of elements in aqueous samples. Figure 4 shows a schematic diagram of an ICP-OES. Aqueous samples are pumped into a nebulizer where they are mixed with argon gas to form a mist. Additional argon is added and the sample enters the torch region of the ICP. The plasma is created using the argon gas and a radio frequency (RF) power supply. The plasma heats the sample to approximately 10,000 K (for reference the temperature of the Sun's surface is 5,778 Kelvin (NASA, 2023)). As the sample passes through the ICP, the samples are atomized and electrons are brought to an excited state. When the electrons relax, they give off a frequency of light specific to the element. That emitted light is then passed to a detector where the intensity of light can be found.

For the ICP analysis, a calibration curve using a standard purchased from Agilent Technologies was used to determine the concentrations of the metals: aluminum, arsenic, barium, cadmium, cobalt, chromium, copper, manganese, molybdenum, nickel, lead, selenium, strontium and zinc. These metals were selected simply because the standard provided only had these metals. All standards were made using Type I ultrapure water from a Thermo Scientific GenPure Pro UV/UF system. The analysis was performed using two wavelengths for each element (based on

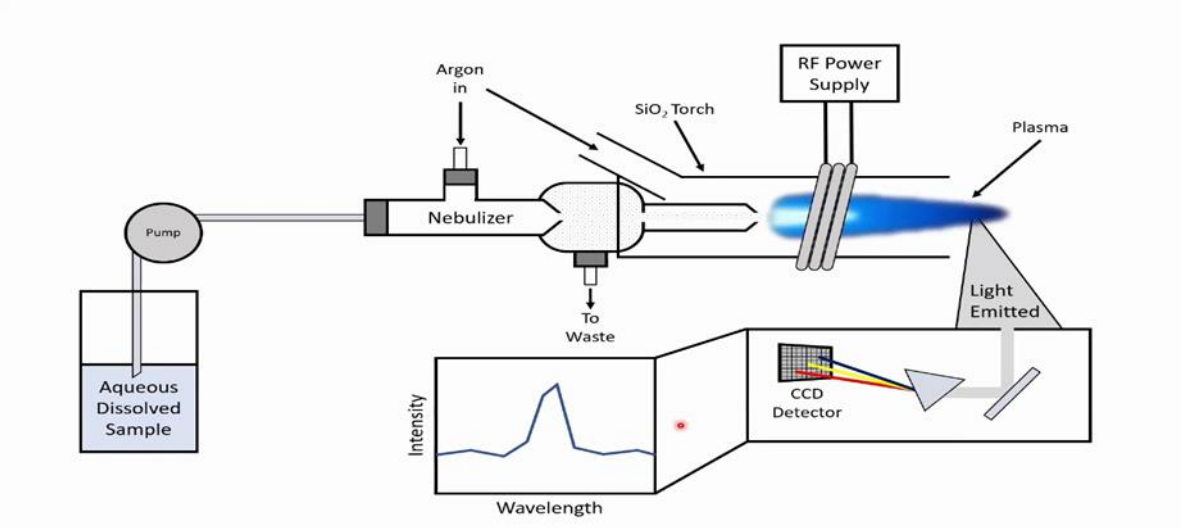

Figure 4: Schematic diagram of inductively coupled plasma optical emission spectroscopy (ICP-OES). Image from (Neale, 2020).

recommendations in the software, the primary wavelength used for analysis is seen in Table 1 and samples were analyzed over a year. Therefore, response factors for each trial were compared to see if there is any instrumental or standard degradation (Table 1). Seeing that all of the response factors had small percent deviations (with the exception of sodium), no appreciable deviation in the ICP was noted.

Element	Wavelength (nm)	Response Factors	Extraction Ratio
Al	396.152	3277024 ± 2.50%	70.0
As	193.696	38216 ± 2.49%	1.10
Ba	455.403	52855968 ± 3.74%	9.79
Cd	226.502	1521890 ± 3.18%	1.60
Co	238.892	455346 ± 2.76%	1.92
Cr	205.56	306669 ± 2.78%	4.81
Cu	324.754	2887569 ± 2.96%	4.36
K	769.897	1321229 ± 4.79%	3.01
Mn	257.61	9921657 ± 2.50%	5.96
Mo	202.032	432423 ± 3.60%	1.71
Na	589.592	86388 ± 15.1%	2.27
Ni	231.604	239494 ± 3.79%	2.81
Pb	220.353	86148 ± 5.81%	1.42
Se	196.026	49373 ± 2.26%	1.56
Sr	421.552	201695954 ± 2.60%	3.92
Zn	202.548	1123538 ± 4.44%	6.41

Table 1: Elements measured by the ICP-OES along with the primary wavelength used, the average response factors (intensity/ppm), and extraction efficiencies of the water vs. acid extraction method.

The ICP-OES was used to determine the best extraction method. An extraction ratio was calculated by dividing the peak intensities of the acid extraction by the peak intensities of the water extraction for the test samples (Table 1). A ratio above 1 means that the acid extracted more of the metal, a value below 1 shows that the water extraction extracted more. Since most of the extraction efficiencies are greater than one and none have ratios less than 0.65,

it was determined that the best extraction method was the acid extraction method.

Mutagenicity Analysis

The final part of this experiment is to test for mutagenicity. Measurement of aerosol mutagenicity is not common as most regulatory measurements are based on aerosol mass. The traditional way to measure for a compound's mutagenicity was developed by Dr. Bruce Ames and is aptly called the Ames test (Ames et al, 1975). The Ames test is a biological assay that makes use of bacteria that cannot produce histidine on its own, but require it for their growth. The general procedure is, some of the bacteria is grown while being exposed to a possible mutagen. If a mutation occurs, the bacteria can produce histidine and will continue grow. Growth of the bacteria can be determined visually. On another plate, bacteria is grown without the mutagen as a control.

The current analysis utilizes the fluctuation Ames test (Environmental Bio-Detection Products Inc.), which performs multiple Ames Tests on a mutagen simultaneously in microplates (Hubbard et al, 1984). This version of the Ames test utilizes a strain of the *Salmonella typhimurium* bacterium that is unable to produce histidine (auxotrophic mutant strain his^-). When in the presence of a mutagen, the bacteria will undergo a mutation to become wild type, which is naturally able to produce histidine (his^+). Media containing a limited amount of

histidine are incubated in the microplates and then the possible mutagen is added (in this case, aerosols in extracts of air samples collected from San Bernardino). Mutagenicity is confirmed by the growth of bacteria causing acid to be produced. The acid produced then lowers the pH causing a color change. The pH indicator is bromocresol purple, which is purple at a pH above 6.8 and yellow at a pH below 5.2. Due to the fact that the method of extraction requires an acid, the pH of the samples is well below that of the indicator's range (pH 5.2-6.8). Therefore, the filter extracts are treated with a base, sodium hydroxide, before addition to the microplates. Addition of the base results in an initial pH around 7 causing the solution in the microplates to be purple initially. A color change to yellow shows bacterial growth. Figure 5 shows plate at the start of the test and during the test when mutation has occurred.

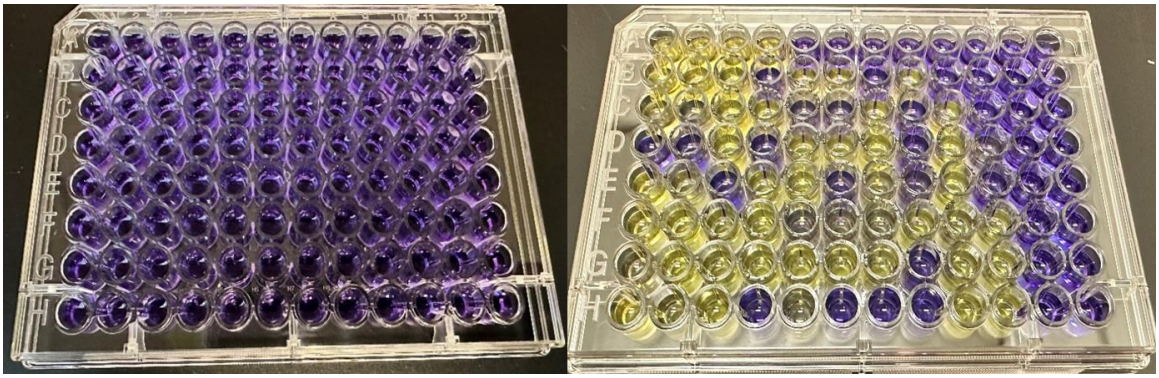

Figure 5: The same mutagenicity test before (left) and after incubation (right).

The right two rows of the plates are a sterility control (no bacteria added) and the rest of the wells are for a positive control. Before incubation, no mutagenicity has occurred (all wells are purple). After incubation, no mutations occurred in the sterility control while mutations have occurred in 65% of the positive control wells.

Sample kits are purchased including twelve 96-well microplates (200μL for each well), bacteria, bacteria growth material, and reagents (EBPI, 2023). Multiple strains of bacteria are possible. *Salmonella typherium* strain TA-100 was chosen as it is one of the standard bacteria used for Ames tests (Ames et. al, 1975). The suggested method from the manufacturer uses the twelve 96-well plates to measure the mutagenicity of three samples with dilution factors of 1:4 (5 mL sample in a total of 20 mL reaction mixture), 1:2, and 17.5:20. For simplicity, the dilution used will be named based on its relative concentration, where the relative concentrations are 0.25 (1:4 dilution), 0.50 (1:2 dilution) and 0.875 (17.5:20 dilution). In addition, three controls are used:

- a sterility control: no sample or bacteria to show the presence of contamination if any color changes are observed;
- a background control: bacteria but no sample to measure the bacteria's natural rate of mutagenicity.
- a positive control: bacteria and a known mutagen (sodium azide, NaN_3), almost all wells should show mutation.

Initial tests modified this procedure to study the different extraction methods and the trend of mutagenicity. For these studies, twelve plates were used to measure only one filter extract sample at relative concentrations of 0.01 (1:100 dilution), 0.05, 0.25, 0.50, and 0.875. A sample using water extraction was also used with relative concentrations of 0.25, 0.50, and 0.875.

After extraction, mutagenicity test plates were prepared. Dried bacteria supplied by EBPI, were hydrated and incubated at 37°C for one day. Solutions were prepared with the appropriate amount of sample extract. Bacteria was then added to the solution right before they were placed into the microwell plates. The plates were then stored in transparent one-gallon zipper bags, four plates to one bag. Plates were incubated in an oven at 37°C. Approximately 23 hours after incubation, the plates were removed from the oven and the number of revertants were counted. Microwells that remained purple in color were counted as no mutation and those that were yellow in color were counted as a mutation. Occasionally, the media had no color suggesting that the pH had started to be reduced and this was regarded as a positive mutation. Mutations on the plates were counted daily for 6 days. However, it was found that most change happened in the first 3-5 days. After this time, the liquid in the wells starts to evaporate causing bizarre readings. Therefore, the mutation rate was typically taken at day 4.

The mutagenicity rate is found by:

$$\text{Mutagenicity Rate} = \frac{\text{Number of Plates with Mutation}}{\text{Total Number of Plates}} \times 100\%$$

The mutagenicity results of the initial trials comparing water and acid extraction are shown in Figure 6. As expected, the acid extraction compared to the water extraction of the same relative concentration showed a higher rate of mutagenicity, further confirming the acid extraction's increased extraction efficacy. Therefore, further studies only used acid extraction. Additionally, the mutagenicity was found to increase with relative concentration of sample with leveling off at high concentration. Because the rates of the two highest relative concentrations are very similar it was decided that the highest concentrations would be removed. The sterility control was also deemed unnecessary and so was removed from the process.

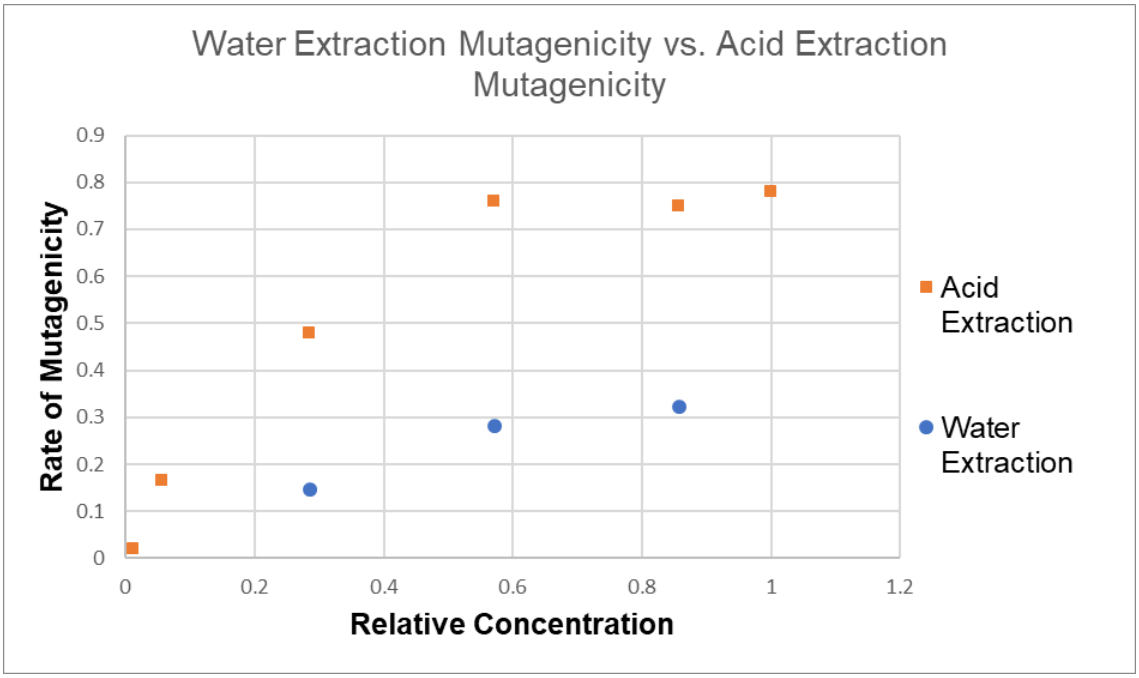

Figure 6: Mutagenicity results for water and acid extraction of a filter sample.

Once the best extraction method was determined, further testing was done on the mutagenicity test. Two samples were collected and tested. The first was a sample collected over a 72-hour period and the other over an 8-hour period. The goal was to see if there was any difference in mutagenicity based on aerosol loading. However, while the sample's mutagenetic data appeared to be normal, the background control had a mutagenic rate of 95%. Mutagenetic rates for the background control should not exceed 31% from random mutations in the bacteria. Conversations with the manufacturer suggested it could be a "jackpot batch" which is when the bacteria is more likely to revert back naturally or the bacteria had already reverted prior to being packaged. Additionally, it could be due to sodium azide jumping: if the background control and the positive controls

are stored in the same bag, the sodium azide can evaporate and contaminate the background control causing it to have a higher rate of mutagenicity. Since there was not a sterility control in this trial, it was hard to determine if that was the case or if it was some other contaminant. Due to these findings, in order to prevent this issue moving forward, all plates were stored in separate bags and a sterility control was added to two rows of wells (16 wells) on three of the plates.

A final modification of the mutagenicity test methodology was the changing of some of the sample dilutions. About halfway through testing the samples, it was realized that the differences between the two lowest relative concentrations were very minimal in terms of mutagenicity and so it was determined to change the lowest relative concentration tested from 0.01 to 0.05. And because higher concentrations lead to increased toxicity of the bacteria the highest concentration was dropped from a relative concentration of 0.875 to 0.50 and the second highest was dropped from 0.50 to 0.15. With these changes the final plating procedure is as seen in Table 2.

Plate #	Name	DI water (mL)	Positive (mL)	Sample (mL)	Reaction Mixture (mL)	Bacteria (µL)
1	Background	17.5	-	-	2.5	20
2	Positive Control	17.4	0.1	-	2.5	20
3	Sample I	16.5	-	1	2.5	20
4	Sample I	14.5	-	3	2.5	20
5	Sample I	12.5	-	5	2.5	20
6	Sample I	10.5	-	7	2.5	20
7	Sample I	7.5	-	10	2.5	20
8	Sample II	16.5	-	1	2.5	20
9	Sample II	14.5	-	3	2.5	20
10	Sample II	12.5	-	5	2.5	20
11	Sample II	10.5	-	7	2.5	20
12	Sample II	7.5	-	10	2.5	20

Table 2: Final sample procedure used for mutagenicity testing.

Towards the end of sampling, problems again arose with the bacteria. Six samples including the background control had 100 percent reversion. The blank sterility control maintained 0% reversions indicating that it was not an issue of contamination. It was however noted that all the bacteria came from the same sample lot suggesting it was a jackpot batch of bacteria despite EBPI not having any issues reported by other customers. Ultimately, when working against forces of nature, nature will find a way to work against you. To overcome this, sterility tests were continuously run and samples were reanalyzed in the case of high natural revertants.

CHAPTER THREE

RESULTS AND DISCUSSION

Aerosol pollution in San Bernardino were measured in Spring and Summer 2022. The results of this project will be addressed in four sections:

1. Aerosol Mass Concentation
2. Aerosol Mutagenicity
3. Particulate Metals
4. Seasonal Trends

Aerosol Mass Concentration

Aerosol mass concentration is reported in two ways. The first was using regulatory data of $PM_{2.5}$ and PM_{10} from the Environmental Protection Agency (EPA, 2023) measured in San Bernardino (34.107°N, 117.274°W). The second method was calculated from the samples collected using the mass of aerosol collected and the flow through the TSP. The TSP has no size selection so it should give mass concentrations larger than the PM_{10} measured by the EPA. This is labeled as PM_{CSUSB}. Figure 7 shows the average concentrations of PM_{CSUSB} and average EPA measurements of $PM_{2.5}$ and PM_{10} during the sampling period.

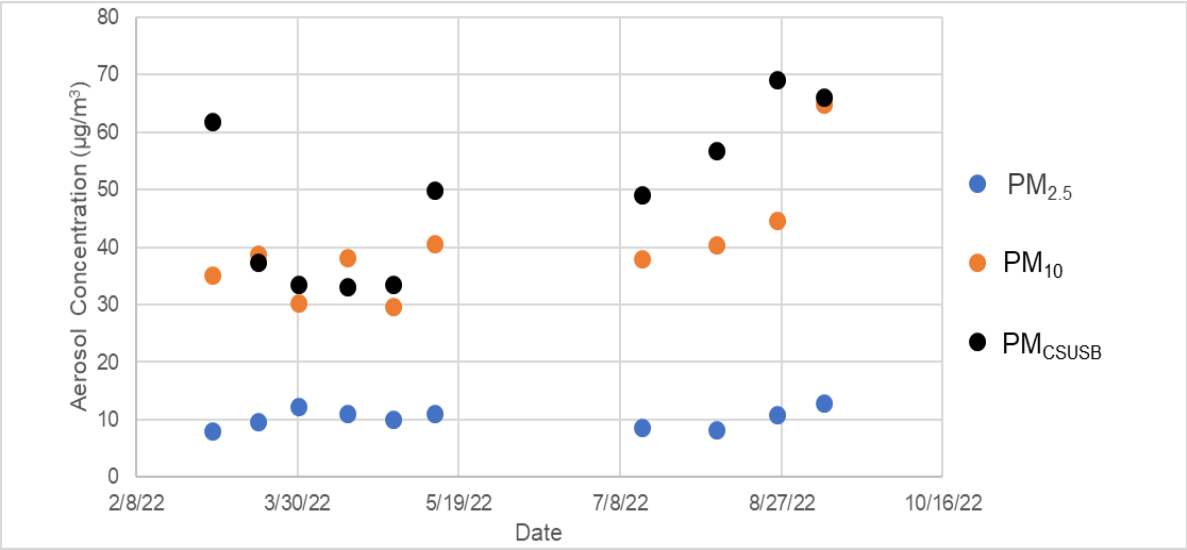

Figure 7: Mass Concentrations of PM_{CSUSB}, $PM_{2.5}$ and PM_{10}.

Due to the lack of size selectivity of the TSP, PM_{CSUSB} more closely resembles that of the EPA PM_{10} concentrations. However, there are periods where the CSUSB concentration is significantly higher than that of the EPA's data. This can be contributed to aerosols larger than 10 µm measured at CSUSB. $PM_{2.5}$ represents an average of 26% of the PM_{10} concentration during our testing period.

Aerosol Mutagenicity

Rate of mutagenicity was found by counting the number of revertants on each 96-well plate and then dividing it by the number of total wells and put into a percentage. Figure 8 represents the rate of mutagenicity at the differing relative concentrations compared to the concentration of $PM_{2.5}$. The best correlation between mutagenicity and $PM_{2.5}$ was seen at a relative concentration of 0.25. At higher concentrations, reduced mutagenicity is sometimes seen because the concentration is too high leading to bacteria death. At a relative concentration of 0.25, a clear positive trend of mutagenicity increases as $PM_{2.5}$ concentration increases. Because this relative concentration gave the best results it will be used for future analysis. Similar analysis was performed versus PM_{10} and PM_{CSUSB}, but correlations were not as good.

$PM_{2.5}$ measured by the EPA varied between 8.0 $\mu g/m^3$ (March 2022) and 12.8 $\mu g/m^3$ (September 2022). Using the correlation equation measured in Figure 8c, these would correspond to rates of mutagenicity of 49% and 94%. Thus, an increase in aerosol concentration of 60% (8 to 12.8 $\mu g/m^3$) results in a mutagenicity increase of 92% (49% to 94%). This suggests that even when aerosol concentrations are below the NAAQS, efforts should be made to continue to improve air quality as this decreases aerosol mutagenicity.

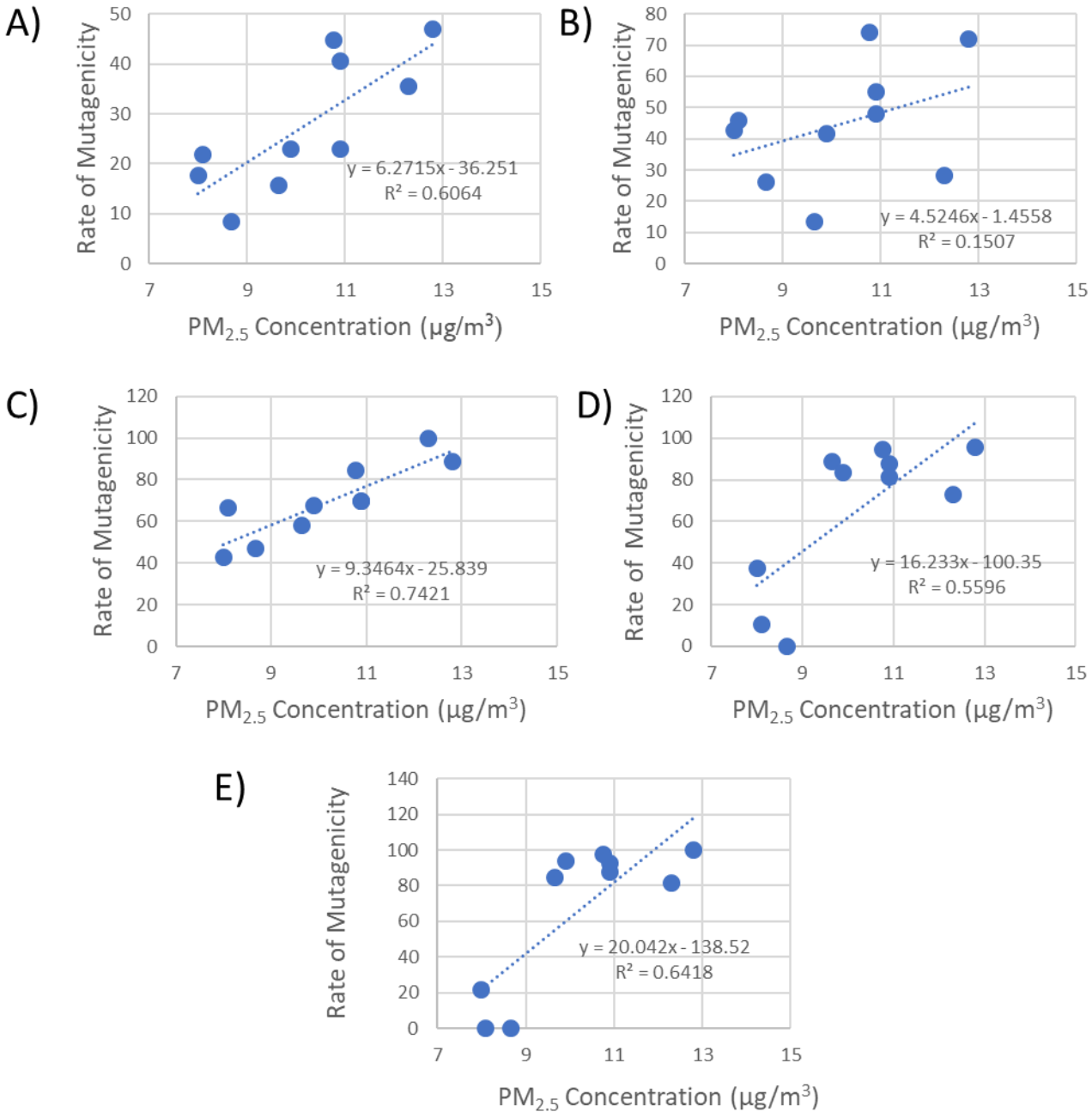

Figure 8: Rates of mutagenicity of the samples compared to the concentration of $PM_{2.5}$ at various relative concentrations for the sample extracts: A) 0.05 relative concentration, B) 0.15 relative concentration, C) 0.25 relative concentration, D) 0.35 relative concentration, and E) 0.50 relative concentration.

Particulate Metals

In addition to mutagenicity, analysis for the concentration of metals in the atmosphere was done. Table 3 shows the concentrations of the metals analyzed in the atmosphere in ng/m^3. Aluminum, copper, and potassium had the highest concentration. Nickel, lead, and zinc had the lowest concentration, with nickel and lead present in only two samples. Figures 9 shows the correlation of mutagenicity to the metals (besides nickel and lead).

		Metal										
Start Date	**End Date**	**Al**	**Ba**	**Cr**	**Cu**	**Mn**	**Mo**	**Ni**	**Pb**	**Sr**	**Zn**	**K**
2/25/22	3/3/22	85.0	12.1	0	182	8.07	11.3	0.00	0.00	2.69	16.1	106
3/10/22	3/17/22	501	14.7	1.13	319	11.3	11.3	1.13	1.13	4.52	15.8	108
3/23/22	3/30/22	106	10.8	1.19	196	7.17	12.0	0	0	2.39	0.111	65
4/7/22	4/14/22	339	14.3	1.19	250	10.7	11.9	0	0	4.76	0.050	89
4/21/22	4/28/22	270	8.0	0.57	153	5.15	4.58	0	0	0	11.5	56
5/4/22	5/11/20	135	9.4	0.15	137	6.44	3.52	0	0	0	10.5	90
7/7/22	7/14/22	589	15.1	1.32	14.5	13.2	3.95	0.658	0	3.29	0.289	63
7/30/22	8/6/22	665	13.8	1.89	15.3	10.7	3.14	0.628	0	2.51	0.289	68
8/18/22	8/25/22	459	17.1	0	453	0.139	3.28	0	0	3.28	0.220	102
9/1/22	9/9/22	410	12.0	0	455	0.090	3.59	0	0	2.99	0.187	100

Table 3: Concentration of metals in the atmosphere in ng/m^3

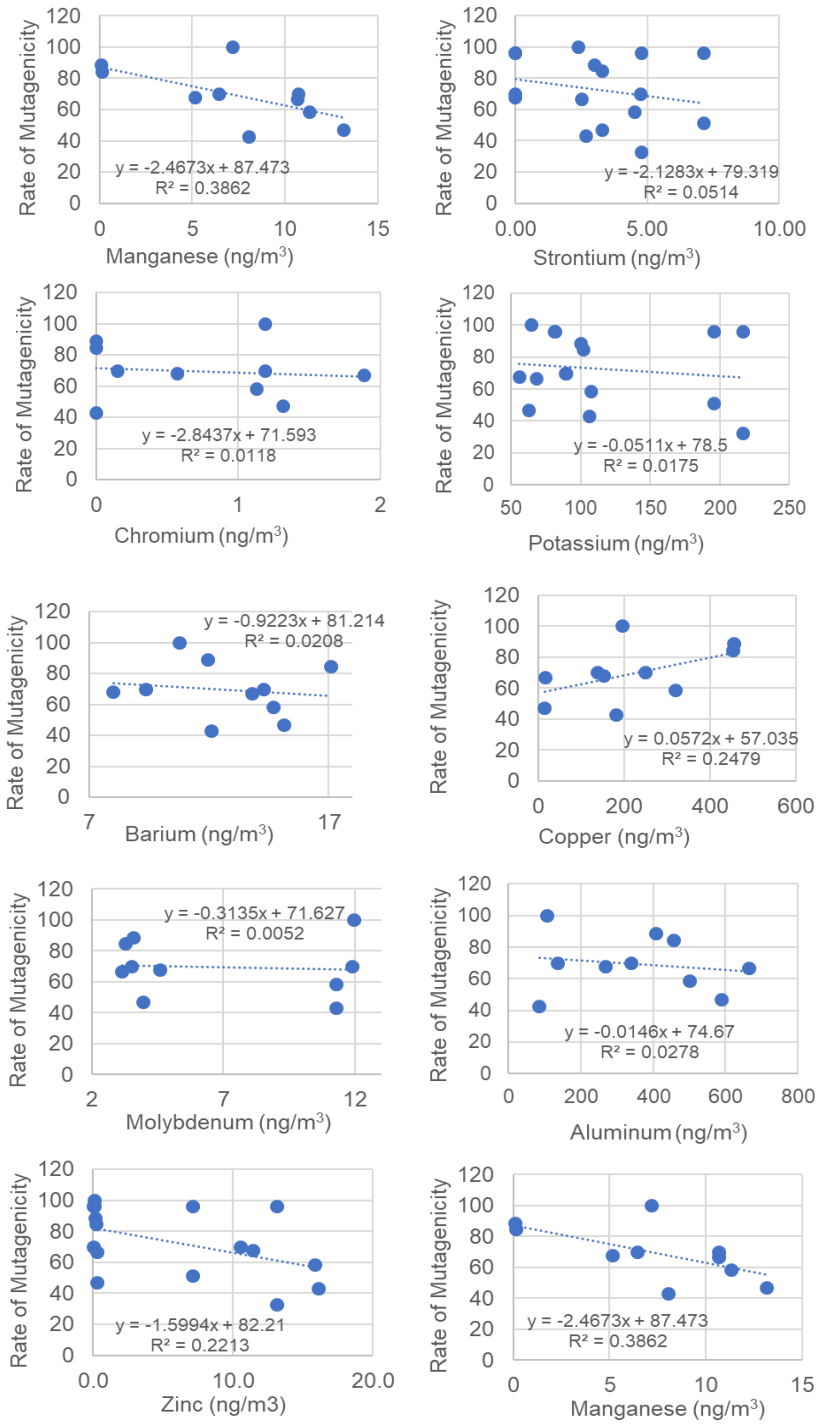

Figure 9: Rate of mutagenicity vs. metal concentration for sample extract relative concentration of 0.25.

From the data gathered, there does not appear to be any direct correlation between mutagenicity and the concentration of heavy metals in the aerosols. While metals are not believed to directly cause mutagenicity, it was expected that aerosols high in metals may also be high in compounds that cause mutagenicity.

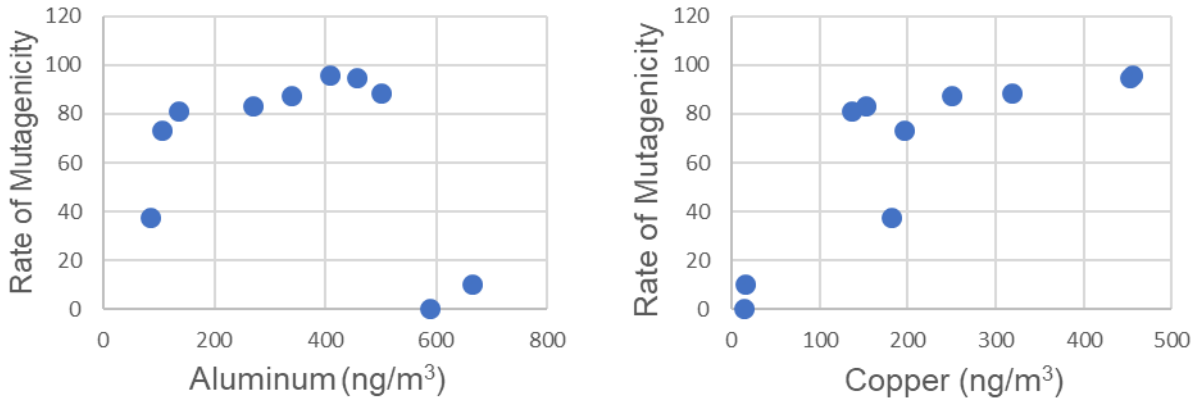

Figure 10: Mutagenicity vs. aluminum and copper for relative sample concentrations of 0.35.

Figure 9 showed the mutagenicity vs. metal concentration for samples with a relative concentration of 0.25. No clear trend was seen. However, using a higher relative concentration (0.35), there does appear to be a correlation of an increase in mutagenicity as the concentration of copper and aluminum increases (Figure 10). The reason why this trend may only appear at higher concentrations is because metals are a minor component of aerosol. This also shows that there does appear to be a threshold for aerosol concentration for mutagenicity to

occur. At high enough aluminum concentrations, the mutagenicity plummeted likely because at these high metal concentrations can cause bacterial die off.

Seasonal Trends

The final aspect to be addressed is how aerosol mutagenicity varies seasonally. Figure 2 shows that there is no clear seasonal trend in $PM_{2.5}$ concentration over the course of the last ten years, with concentration increasing and decreasing at seemingly random times of the year. The sampling period covered spring and summer 2022. Figure 11 shows the rate of mutagenicity and $PM_{2.5}$ during the testing period. No clear trends are seen but concentrations spike at the end of the summer. Mutagenicity follows that trend showing a very strong correlation of $PM_{2.5}$ concentration to the rate of mutagenicity. No clear reason for the increase in $PM_{2.5}$ or mutagenicity at the end of summer was found in the meteorological data. Future work is needed to determine the reason for when $PM_{2.5}$ and mutagenicity do not correlate, for instance, why measurements of mutagenicity at the end of March were much higher than other data.

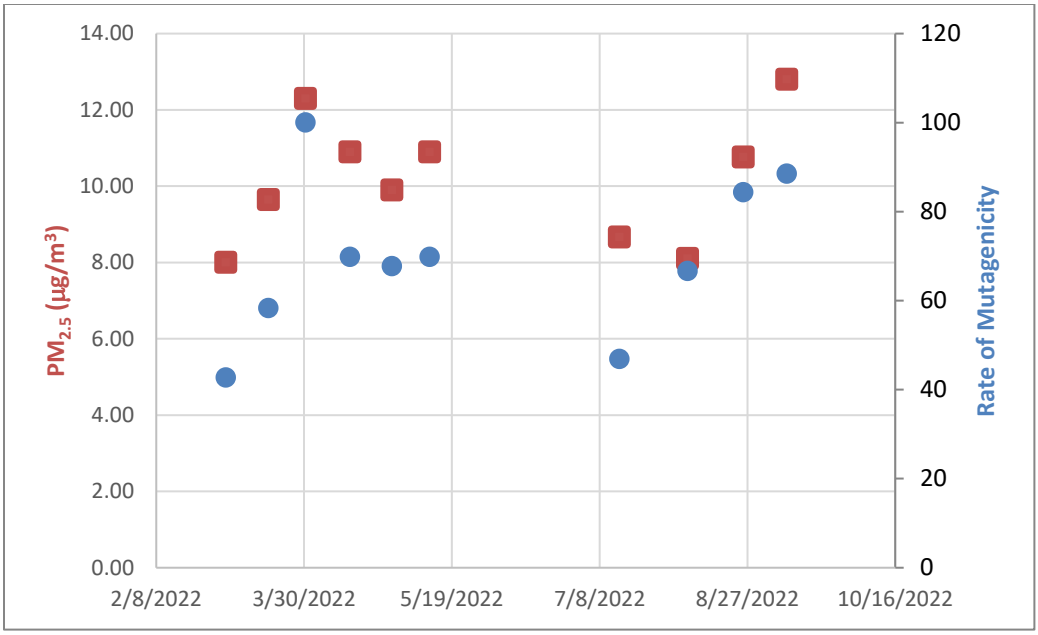

Figure 11: $PM_{2.5}$ compared to the rate of mutagenicity during the testing period.

In addition to total particulate matter concentration, seasonal analysis was also done for the total metal concentration, seen in Figure 12. Similar to figure 11 there is not a clear season trend for the concentration of metals during the year. While there was a correlation to particulate matter concentration and mutagenicity, there does not appear to be any correlation to the concentration of metals and mutagenicity. This thus reaffirms that there must be some other chemical species in the particulate matter that is the leading cause of the mutations.

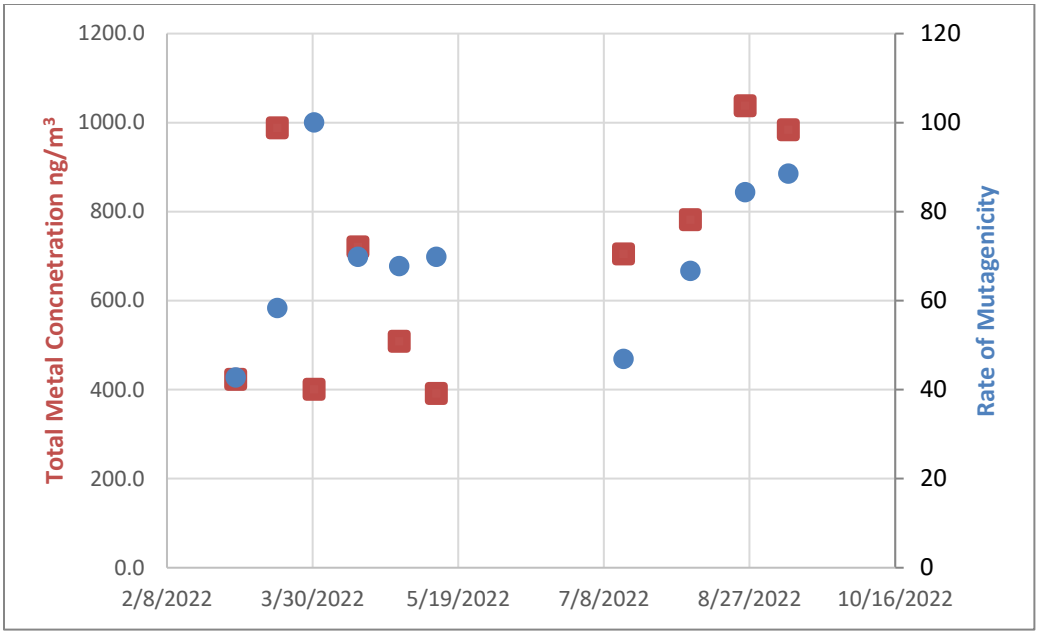

Figure 12: Total metal concentration compared to the rate of mutagenicity (%) during the testing period.

CHAPTER FOUR

CONCLUSION

The overall goal of this study was to study the effects of particulate matter on mutagenicity. Mutagenicity was tested using bacteria strain *Salmonella typherium* strain TA-100 as a proxy for mutagenicity in humans. The bacteria was added to 96-well plates with 5 different relative concentrations. It was found that at a relative concentration of 0.25 there is a good correlation between mutagenicity and $PM_{2.5}$ with a 60% increase in $PM_{2.5}$ relating to a 92% increase in mutagenicity. The lower concentrations did not yield a noticeable trend due to the low concentrations of $PM_{2.5}$ not being high enough to trigger mutations in the bacteria, while higher concentrations were likely toxic to the bacteria. When comparing the trends of aerosol concentration and the rates of mutagenicity to the sampling periods of spring and summer, it was found that mutagenicity follows the same trend as the $PM_{2.5}$ during the sample period (spring and summer). However, aerosol sources can change throughout the year. For instance, in winter smoke from wood burning becomes more prevalent. Thus, $PM_{2.5}$ and mutagenicity may not have a similar trend throughout the year.

Heavy metal analysis was done using ICP-OES. It was found there was not any direct correlation between particulate metal concentration and mutagenicity at the low relative concentration. However, because metals are not a primary component of aerosol, only at higher concentration was there some

correlation between metal concentration and mutagenicity. Thus, there are other pollutants that cause the majority of the mutagenicity.

While this research showed the applicability of the fluctuation test for aerosol mutagenicity studies and a link between $PM_{2.5}$ and mutagenicity, further analysis is needed to understand aerosol mutagenicity. Analysis of an entire year of samples should be performed so that trends of mutagenicity compared to aerosol concentration can be compared. In addition, measurements of compounds that cause mutagenicity, such as polycyclic aromatic hydrocarbons (PAHs), should also be measured. Since, this study showed there is no direct correlation between metals and increased mutagenicity, it is likely that PAHs are key pollutants in causing mutations.

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