



# Biodiversity of Lichens in Tenafly, NJ as Indicators of Air Pollution

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## Materials & Methods

### 1. Collect samples

- Collected 21 samples from the town center, the Tenafly Brook, and the Tenafly Nature Center
- Obtained GPS locations of the samples
- Stored samples in labeled ziploc bags

### 2. DNA extraction

- Used the silica DNA extraction method to separate the DNA

### 3. DNA sequencing






- Performed gel electrophoresis and PCR
- obtained 5 successful sample DNA

### 4. Analysis

- Received sequences of the 5 samples
- Used DNA Subway, BLAST, and Multiple Sequence Alignment to analyze the sequences

## Results

Table 1: Chart of collected samples and their identification species

Sample Number	Identified most likely Species	E Value	Sulfur dioxide toleration	Nitrogen nutrient toleration	Picture of Sample
KPX - 001	<i>Physcia magnussonii</i>	7e-176	>30 ppb	Tolerant	
KPX - 003	<i>Canoparmelia texana</i>	0.0	--	Tolerant	
KPX - 005	<i>Physcia stellaris</i>	0.0	5-15 ppb	Moderately tolerant	
KPX - 015	<i>Punctelia hypoleucites</i>	0.0	16-30 ppb	Moderately tolerant	
KPX - 016	<i>Punctelia Rudecta</i>	0.0	>30 ppb	Moderately tolerant	

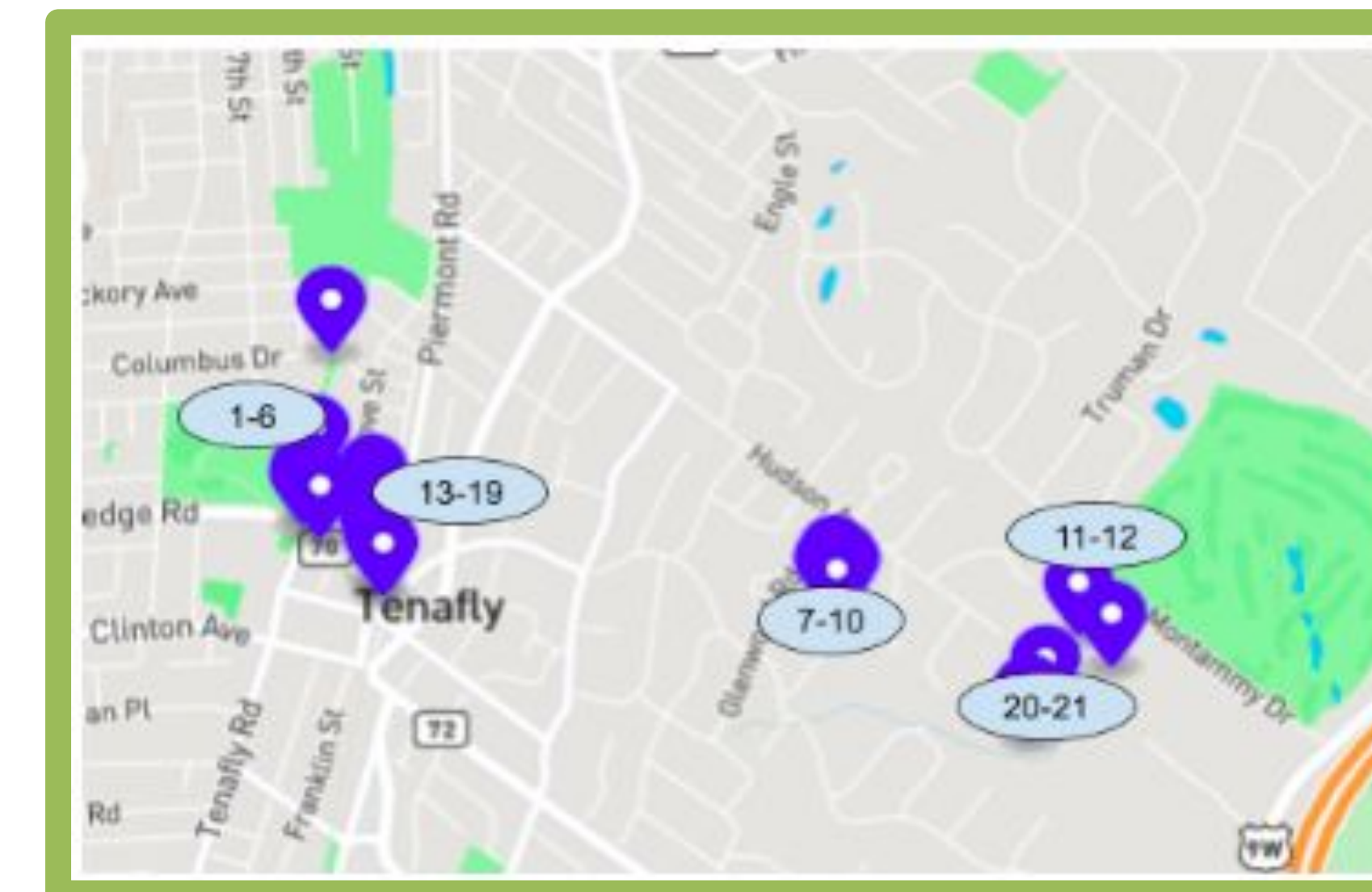


Figure 1: Map of all 21 samples collected



Figure 2: Map of identified species

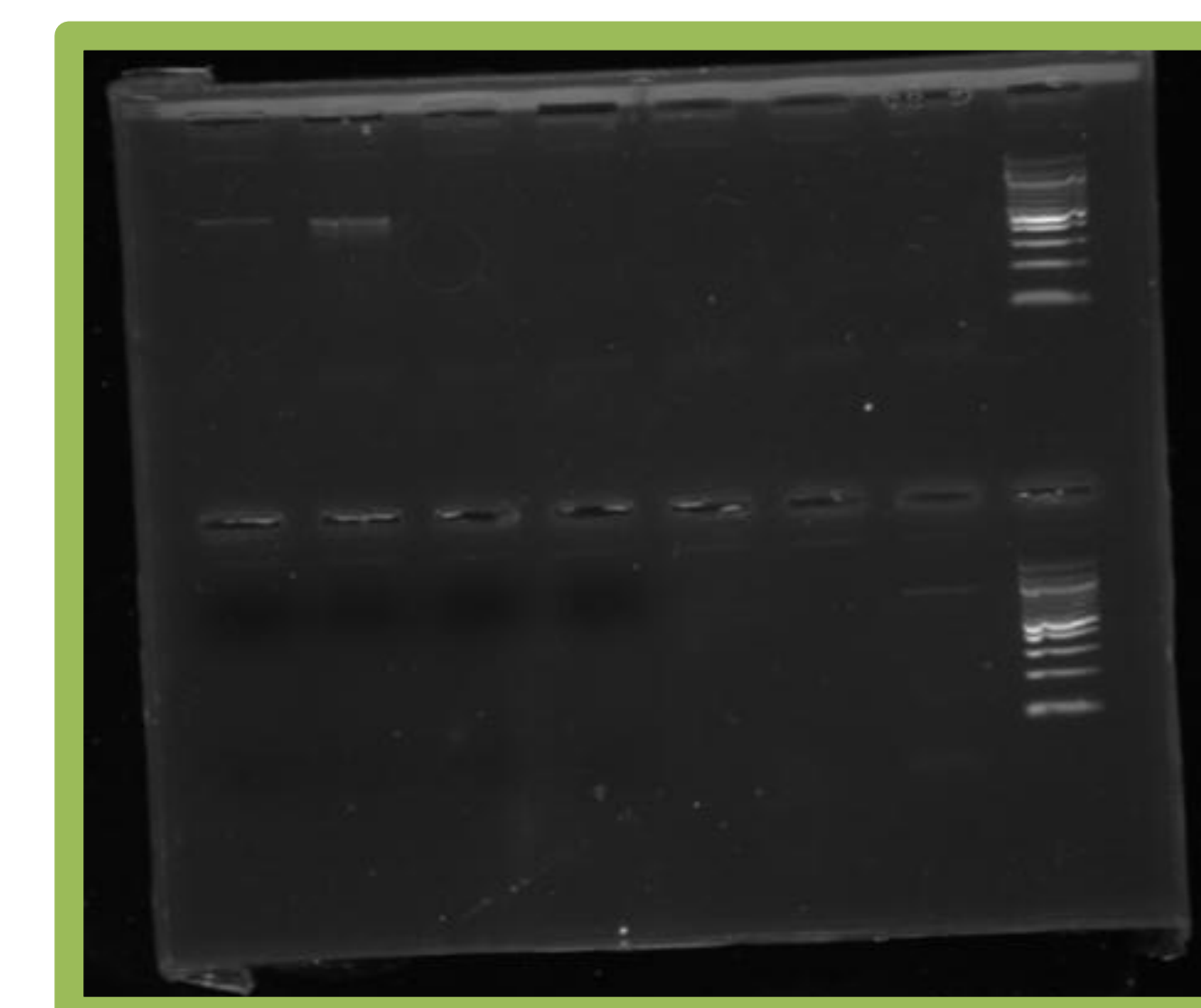


Figure 4: Image of gel electrophoresis - samples KPX-015 and KPX-016 are successful

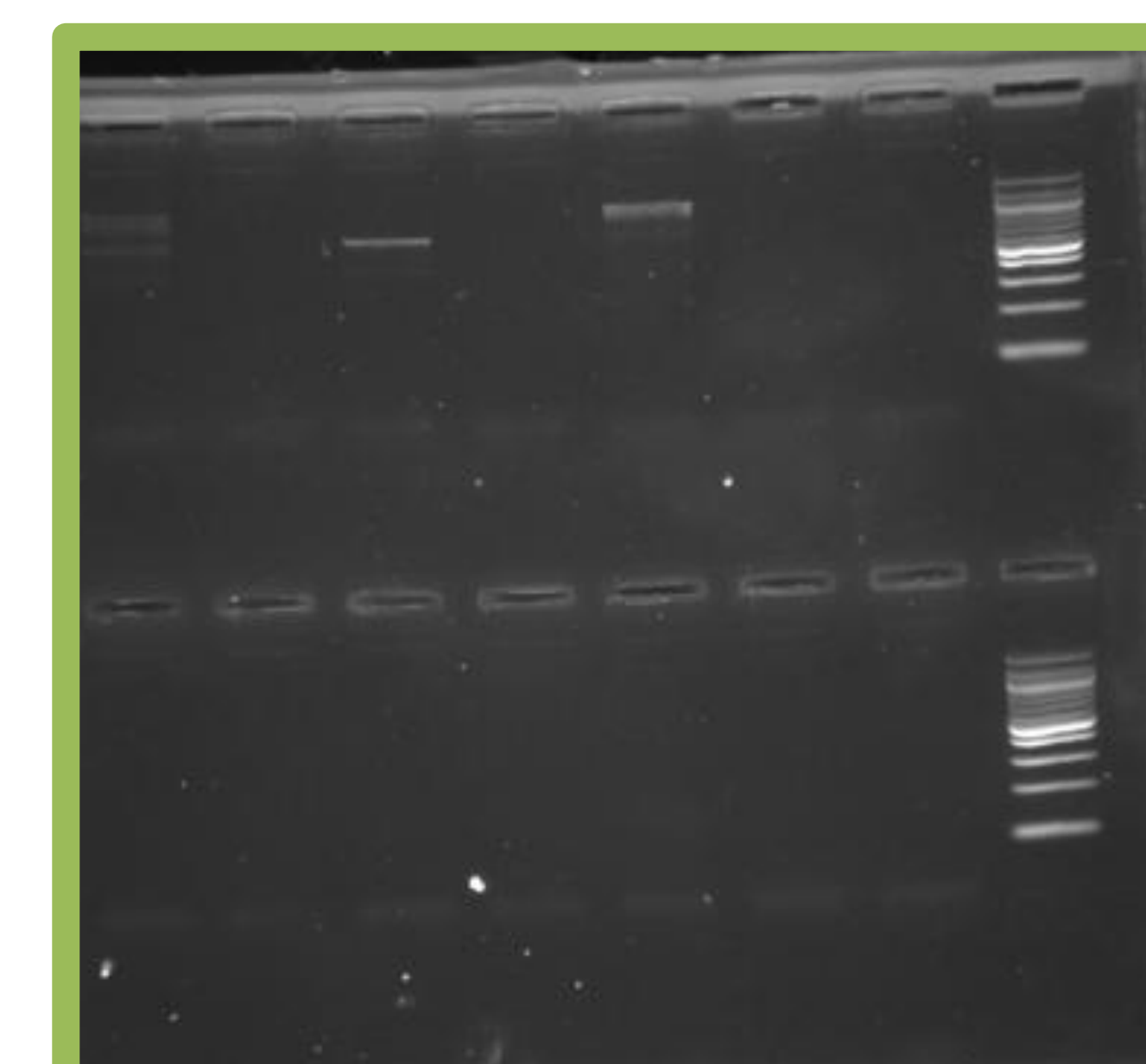


Figure 5: Image of gel electrophoresis - samples KPX-001, KPX-003, KPX-005 are successful

## Discussion

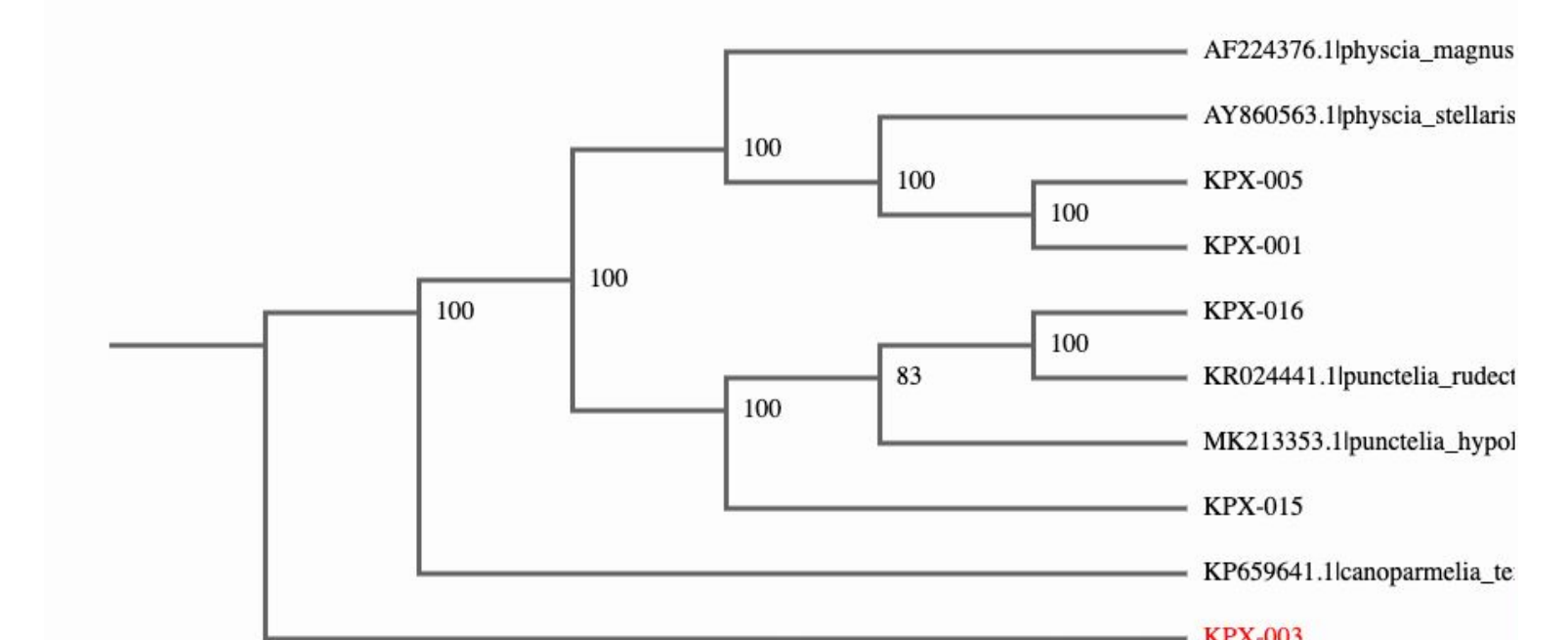


Figure 6: Polyphyletic tree of samples

- We found the lichens near the town center of Tenafly to be mostly tolerant to nutrient nitrogen deposition, yet their tolerance towards sulfur dioxide levels vary: the tolerance is high in lichens on the main street of Tenafly, yet it is low in lichens on the side street of Tenafly.
- Sample KX-001, KX-015 and KX-016 are located on the main street of Tenafly, Riveredge Road, and in the parking lot. We had hypothesized that this place is the most polluted in the town of Tenafly. Based on the data from PCR and online research, we found that sample KX-001, KX-015 and KX-016 are mostly eutrophs, which thrive in environments with high nutrient nitrogen levels, suggesting that the town center which they live in might be highly polluted; they are also shown to be tolerant intermediate or tolerant to sulfur dioxide levels. This shows that the environment that the lichens reside in must have high levels of these chemicals, thus it is likely the town center is highly polluted.
- Meanwhile, samples KX-003 and KX-005 are located on a less-visited side street but still downtown, as can be seen in Figure 2. KX-003 is found to be a species that is sensitive to sulfur dioxide deposition, and prefers a moderate level of nitrogen nutrient deposition, which indicates that it lives in an environment with lower levels of pollution; however, though KX-005 is found near KX-003, KX-005 is a eutroph, which tolerate high level of nitrogen nutrients, and there was no online data about its toleration towards sulfur dioxide levels.
- The pictures of gel electrophoresis, indicating which samples successfully holds DNA, are shown in Figure 4 and 5. In Table 1, the E values show the similarity between the identified species and the DNA sequences of the samples. The closer to 0, the more similar the species are to the samples. All of the E values were close to 0, showing that the identified species are likely to be accurate. Using the identified species, we created a polyphyletic tree to show the relationships between the lichens species, shown in Figure 6.
- Our results show that the lichens in the town center of Tenafly are fairly diverse, with 5 different species identified in an area of about 21,000 meters squared. This may explain why, though all 5 samples are shown to be tolerant or moderately tolerant to nitrogen nutrient deposition, their tolerance on nitrogen nutrient pollution varies based on their specific locations.
- In our future study, we aim to further investigate these contradictions. We also aim to compare these results to a new study conducted on the air pollution indicated by lichens in the Tenafly Nature Center, which we hypothesized to be less polluted by sulfur dioxide and nitrogen, so that there is more diversity and inclusiveness in environments. By expanding on the data we currently have, we hope to be able to establish a clearer understanding of the biodiversity and extent of pollution in Tenafly.

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## Acknowledgements

We would like to thank our teacher and mentor, Mrs. Helen Coyle, for all your guidance and assistance throughout the entirety of our project. We would also like to thank Ms. Arden Feil, our mentor for this project, for her help in the DNA lab and in the completion of our project.

We assessed sulfur dioxide air pollution and nitrogen pollution in the town center of Tenafly, NJ, using lichens as bioindicators. As both pollutants cause respiratory diseases, it is essential that their concentrations are below the safety level. Since the Tenafly town center has busy traffic, we hypothesized the identification of the lichens in that location would represent a highly polluted area. We aim to use the biodiversity of lichens in Tenafly to uncover the pollution levels. We collected 21 samples from less densely and densely populated areas, performed PCR, and ran gel electrophoresis. 5 of these samples from the populated area succeeded in containing DNA in this process and were sent for sequencing. *DNA Subway*, BLAST, and multiple sequence alignment were performed to identify the species. With some variations, the lichens identified were mostly tolerant to pollution. Using these data, we were able to examine the biodiversity of lichens in Tenafly and use the lichen's sensitivity to air pollution to determine that Tenafly likely contains areas of high pollution.

- According to research performed by Dr. Johan Asplund and Dr. David A. Wardle in *Biological Reviews* (2016), lichens are composite organisms composed of algae and fungi. They are bio-indicators of air pollution, such as those caused by nitrogen, acids, and sulfur dioxide.
- The algae in lichens obtain its nutrients and water from depositions of the air, thus it is very sensitive to air quality. An excess of pollutants such as nitrogen can destroy the algae's chlorophyll, causing the algae to starve and die. Thus, lichens' tolerance to air pollution indicate the level of sulfur dioxide and nitrogen nutrient deposition (Munzi, 2013).
- Tenafly is a suburban town in New Jersey. According to the World Population Review, it has a population density of 3,094 people per square mile - a number much higher than the average population density of metropolitan statistical areas, which is 283 people per square mile.
  - Metropolitan Statistical Areas (MSA) is defined as any geological region with a dense population tied together by economic and social activities. (Ganti, 2021)

- We investigated the extent to which pollution of sulfur dioxide and nitrogen affects lichen biodiversity in Tenafly. We aimed to be able to examine the biodiversity of lichens in Tenafly and use the lichen's sensitivity to air pollution to determine whether Tenafly contains areas of high pollution.

- We hypothesized that the results would indicate that the Tenafly Town Center contains more sulfur dioxide and nitrogen pollution than the Tenafly Nature Center and the Tenafly Brook.