USING LICHEN TO DETECT AIR POLLUTION

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What are Lichen?

- Lichen are pioneer species, meaning that they are one of the first to grow in areas without vegetation where conditions may be unfavorable for less hardy plants.
- They are usually composite organisms, made up of algae or cyanobacteria living symbiotically with fungi.
- Lichen are also considered bioindicators for air pollution. This is because they receive water and essential nutrients from the air instead of from the ground.
- There are many different types of lichen and each indicate specific things about the kind of environment they are found in.
- The three main types of growth forms are crustose (crusty), foliose (leafy) and fruticose (shrubby).

Why do we care about lichen?

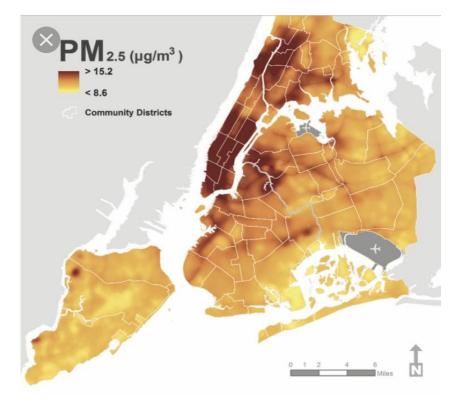
- Pollution levels have been of concern to the general public due to the health issues that they raise, such as respiratory diseases like asthma.
- The presence of specific species of lichen can indicate levels of pollutants in the air. By examining lichen in relation to the environment that they are found in and finding a direct correlation between the two, we can determine whether or not an area is safe for humans.



Our experiment basis

- Each different species of lichen tells us something different about the type of environment the lichen was found in.
- If an area does not have a lot of lichen it can be concluded that the area is likely very polluted. Because lichen are very tolerant to changes in the environment, most places will have lichen even if they have a relatively high degree of pollution.
- If there are lichen, you can determine the extent of the pollution in the area by determining its species.
- The lichen found in unpolluted areas are typically leafy, shrubby, and green, while lichen that are in polluted areas are usually crusty and grey in appearance.

NYC Pollution Data



Queens- Good locations to collect polluted samples from in queens include Astoria and Maspeth. Some good places to collect samples that are unpolluted includes Fresh Meadows.

Manhattan- Some severely polluted areas to collect form include the Hudson River area. Places with good air conditions are the theater district or upper Central Park.

Brooklyn- Some areas that are good to collect polluted samples from is in some areas of Sunset Park or near Long Island. And the unpolluted sample is collected from Brownsville, and some other areas of Sunset Park.

The Bronx- Polluted samples from this region would probably come from the East River area. The unpolluted samples come from further up in The Bronx.

Staten Island- The polluted samples from this area would come from West Shot which is dotted with industrial activity while the unpolluted samples would come from Woodrow.

Queens

Unpolluted

Figure 1 Here we see an abundant sample of lichen. The lichen is very healthy and prosperous as indicated by its bright green color

Polluted

Figure 2 As opposed to the image above the lichen here is very dull and grey and seen in smaller patches





Manhattan

Unpolluted

Figure 3 The lichen in this sample is very abundant and has a rich green color showing its health

Polluted

Figure 4 The lichen here are present in small patches. They are not growing as thickly as the lichen collected in unpolluted areas and shares the dull grey color lichen in polluted areas have





Brooklyn

Polluted



Figure 5 Here we can see that there is not a high lichen population. The lichen that are present are small and greying.

Staten Island

polluted



Figure 6 The lichen here is barely visible and is also very grey and present in small patches.

Our PCR Results

- Only one of our samples yielded PCR product. This was the 9th sample, which was collected from a polluted area in Manhattan.
- We collected this sample in front of BMCC which is in the Hudson River area and is severely polluted.
- Our Blast Results came back, having classified the results of the PCR-ed substance as *Trebouxia Potteri* or *Trebouxia Impressa*.
- Trebouxia is a type of photobiont. They are also one of if not the most widespread photobiont in lichens around the world.

Sequence Trimmer			
Your sequence	s have been trimmed		
WRJ-003-F 🔼			
WRJ-003-R			
WRJ-006-F 🔼			
WRJ-006-R 🚺			
WRJ-009-F 🙍	GATCTAINCGTGCCCACACCGTGAACCATTTATGCCCTTTCTGAAAGGCAGCTCCTGAAAGGGAGTTTGCCGATTGAAAG	CGCZ	
WRJ-009-R	ANCEGETETTCTTECCTEAGCTCAGETCGAAAGCCAAAAAGGCGTCCTGCACACGCTTTCATEGAAGCCATTECEAGCAA	CGAA	
		+	

	BLASTN	
- KI 000110.1	spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	
AF242469.1	Trebouxia potteri - RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	671
C KY559116.1	Trebouxia impressa - internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	707

Our PCR Results (continued)

- What was strange about our PCR results was that instead of getting back a definitive species of lichen, we got back an algae even though we used a primer optimized for fungi.
- This is probably because, as mentioned before, lichen aren't singular organisms but 2 or 3 organisms in symbiotic relationship (usually algae, fungi and/or cyanobacteria).
- From here, we began to do some research on lichen species that commonly have trebouxia in them.



Lichen with Trebouxia

- Xanthoria parietina: This is a foliose (leafy) lichen. This lichen has a very distinctive yellow color to it. This species of lichen is extremely pollution tolerant.
- **Parmelia Sulcata :** This is another type of foliose lichen that contains trebouxia. This lichen does not have a very distinctive color, but rather a dull grey color. This type of lichen is most common in cosmopolitan areas because it is very tolerant to pollution.
- **Parmelia Saxatilis:** This is a foliose lichen as well. This has a very similar disposition to Parmelia Sulcata, however, it has a bluish-green color and is less common in cosmopolitan areas.





Sources of Error

- While collecting samples, we did not take measures to only collect lichen and nothing else. Tree bark and small insects could have entered the collection tube and later may interfered with grinding up and processing the lichen samples.
- When extracting fluids such as buffers and silica resin beads with a micropipette, measurements may not have been exact, or the micropipette tip may have touched the pellet with no visible disturbance.
- Contamination was is another likely source of error. One step where contamination was likely was when we were air drying the remnants of supernatant from the pellets, in which the lid of the eppendorf tube had to be open.



Future Improvements

- We could improve our PCR results with a more time by redoing and running a few more trials with different primers.
- In the future, we would also take preventative measures against contamination such as better clean-up of lichen before grinding it up or a using a better method other than air-drying the supernatant from the pellet.
- We would also retry running Sample #9 again to try and find the fungi actually associated with Trebouxia in that sample of lichen.
- If given more time, we can also collect samples from the areas that we are missing and run tests on those.



Sources

Air Pollution. (n.d.). Retrieved May 15, 2017, from https://www.niehs.nih.gov/health/topics/agents/air-pollution/ Air Quality and Lichens | Bio-indicators. (n.d.). Retrieved December 6, 2016, from http://www.air-quality.org.uk/19.php Kheirbeck, I., Johnson, S., Ito, K., Anan, K., Husky, C., Eizl, H., ... Markowitz, S. (2017, April). NYC Gov (United States Of America, New York City Department of Health and Mental Hygeine). Retrieved from https://www1.nyc.gov/assets/doh/downloads/pdf/environmental/comm-air-survey-08-15.pdf Lichens as Bioindicators. (n.d.). Retrieved December 6, 2016, from https://staff.concord.org/~btinker/gaiamatters/investigations/lichens.html Lichens: Types of Lichens. (n.d.). Retrieved December 6, 2016, from https://staff.concord.org/btinker/gaiamatters/investigations/lichens/typeslichens.html http://www.momscleanairforce.org/monitoring-air-quality-with-lichen-as-a-bioindicator/ http://www.nature.nps.gov/air/pubs/pdf/Lichen Studies.pdf Roy, D. (n.d.). Lichen Sensitivity to Air Pollution. Retrieved December 6, 2016, from http://www.academia.edu/3878950/lichen sensitivity to air pollution http://www.actforlibraries.org/how-lichens-are-indicators-of-air-quality/ https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4298833/#S3