Foraging for Fungi: Mushrooms as Bioindicators of Air Pollution

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Abstract

Why are fungi excellent bioindicators for environmental and health risk assessment? Using fungi to indicate and assess air quality can provide critical information about environments to ecologists. Areas with an abundance of fungi are more likely to have good air quality and fewer pollutants. On the other hand, a lack of vegetation and presence of fungi indicates high air pollution. This study aims to investigate the distribution and abundance of fungi and to relate these to air pollution and climate. A total of ten (10) samples were randomly collected and compared from three different areas: Rye, NY, Greenwich, CT, and Queens, NY. Other environmental data including, climate and air quality index, will also be reported. DNA barcoding using Sanger Sequencing will be performed and analysis will be conducted via DNA Subway to identify the species using BLASTN and MUSCLE. Other environmental data including, climate and air quality index, will also be reported. We hypothesize that if there are little to no mushrooms present in a given area, the air quality is likely to be poor (51-500 AQI). However, if there is an abundance of mushrooms in a certain area, we predict the air quality is likely to be higher (0-50 AQI) in that region. We predict that the diversity of mushrooms will differ from cities, Rye and Queens, NY and suburbs, Greenwich, CT. Our results concluded that the Trametes gibbosa samples were collected at the lowest air quality index (AQI). Thus, our conclusion is that the Trametes gibbosa is the most effective bioindicator of air quality of the species we collected.

Introduction

Air quality is affected by factory emissions, pollutants from fertilizers, and other various agricultural wastes. In communities with poor air quality, people may even experience lung damage, difficulty breathing, and asthma. Bioindicator species such as fungi and lichen have been widely used to reveal the air pollution and status of an environment. Using mushrooms to indicate and assess air quality can provide critical information about environments to ecologists. Why are mushrooms excellent bioindicators for environmental and health risk assessment? When mushrooms are exposed to polluted air, the air disturbs the process of photosynthesis by lowering the pH and disturbing chlorophyll [4]. For example, an area with an abundance of mushrooms is more likely to have good air quality and fewer pollutants. On the other hand, a lack of vegetation and presence of mushrooms indicates high air pollution which leads to disturbances in human health and poor living conditions. As well as indicating air quality, some mushrooms are able to help remove hydrocarbons from the air, creating a cleaner environment. Playing an important role in climate change research, mushrooms are also capable of purifying the soil and breaking down materials like plastics. The scientific literature *Mushroom as a product and their role in mycoremediation* by Kulshreshtha et al. studies mycoremediation, bioremediation in contaminated soils: “Toxicity reduction is also dependent on the substrate. Same fungi may have *different capability in degrading the different pollutants* (Choi et al. [2013]) due to the enzymes of mushrooms that are not only involved in degradation but also reducing the effects of toxic and genotoxic pollutants” [5]. Preserving biodiversity is important in all regions of the world, but especially in cities such as New York. With climate change,

urbanization, and heat islands on the rise, cities pose a bigger risk in biodiversity loss. Using mushrooms as a bioindicator is a quick and cost-effective tool for assessing environmental and health risks in cities is crucial to both biodiversity data and overall ecological knowledge. Finally, the science literature by Kulshreshtha et al. speak toward the health benefits of mushroom as a bioremediation tool: "Mycoremediation through mushroom cultivation will alleviate two of the world's major problems i.e. waste accumulation and production of proteinaceous food simultaneously. Thus, there is a need for further research towards the exploitation of potential of mushroom as bioremediation tool and its safety aspects for consumption as product" [5]. Moreover, the Urban Barcoding Project has little to no information on mushrooms of New York City and its suburbs (Westchester) as well as comparisons to Connecticut and communities. We'd like to contribute data to this initiative and perhaps study them over longer periods of time.

Methods

Part I: Sample Collection

We plan to randomly collect mushroom samples in three geographic locations: *Site A, Queens NY,* and *Site B, Greenwich, CT.* During the sample collection process, we will record environmental data, such as latitude and longitude coordinates and weather related data such as temperature and air quality for each sample. In addition, every sample will be photographed using a microscope and imported into the DNA Barcoding database. We will collect qualitative data, including size (millimeters), length and make predictions on the phenotype based on the morphological characteristics we find before sequencing the genomes of each specimen.

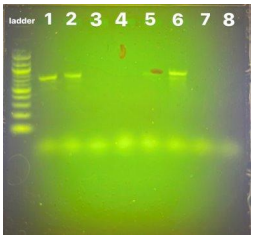
Part II: DNA extraction/PCR/DNA Sequencing

Each sample will be processed using CSHL's DNA Barcoding protocol. We plan to obtain mushrooms and collect material from its thallus regions ~10 mg or ⅛ to 1/4 -inch diameter by removing a piece of the tissue with clean tweezers to enable efficient lysis. Each tissue will be placed into a clean 1.50mL microcentrifuge tube labeled with an identification number having to do with their place collected from and sample number. To extract the DNA, we will first break each cell using 300 µl of lysis buffer to each tube. In addition, we will use a clean plastic pestle and forcefully grind the thallus area on the specimen for 2 minutes, followed by an incubation step (heat block at 65°C) for 10 minutes. Each sample will be placed in a balanced configuration in a microcentrifuge, with cap hinges pointing outwards, centrifuge for one minute at maximum speed to pellet debris. Using a new labeled tube, we will transfer 150 µl of the supernatant (clear solution above pellet at bottom of tube) to the fresh tube. The next step is to filter out the DNA from the supernatant, we will do this by adding 3 µl of silica resin to tube, we'll mix and incubate the tube for 5 minutes in a water bath or heat block at 57°C. The following steps include washing the DNA (which is in a pellet form) using 500 µl of wash buffer to clean pellet. The last step of this process is to elute the DNA using 100 ul of dH20, then transfer the 50 µl of the supernatant to the fresh tube. Store samples on ice or at -20C. We will conduct PCR on each sample. We plan to amplify the internal transcribed spacer (*ITS)* region of the mushroom genome for proper identification. A PCR tube containing a Ready to Go PCR bead containing *Taq* polymerase, nucleotides, and buffer will be used. Each tube will be labeled properly with the sample number. The reaction will contain the following ingredients: 23 µl of NEB Taq 2X Master Mix and 2 µl of DNA from Part II directly into each PCR tube with enzymes, primers, and buffer. Each tube will be placed into a thermal cycler, using the following settings: Initial

Step: 94°C 1 minute, 35 Cycles of the following profile, Denaturing step: 95°C 30 seconds, Annealing step: 50°C 30 seconds, Extending step: 72 °C 45 seconds, One final step to preserve the sample : 4°C *ad infinitum.* After PCR is complete, we will load 5ul of each PCR product onto a 2% gel for 30 minutes (at 130V), including a DNA 100bp ladder for size comparison. The gel will be viewed on an LED transillumination, and a photograph of the gel will be taken using an iPhone and the image will be uploaded to the DNA barcoding database. Based on the gel results, if a PCR product was identified, then those samples will be submitted for DNA sequencing via Genewiz, who will perform Sanger Sequencing.

RESULTS

Gel Image:



Sample Number	Collection Date	Sample Location	DNA Identification	Size Fragment (bp)	Air Quality Index (AQI)
1	3/27/2023	Greenwich, CT	Trametes sanguinea	471 bp	44
2	3/27/2023	Greenwich, CT	Trametes sanguinea	471 bp	44
5	4/4/23	Greenwich, CT	Trametes gibbosa	1765091 bp	41
6	4/4/23	Greenwich, CT	Trametes gibbosa	1765091 bp	41
9	4/2/2023	Queens, NY	Stereum Gausapatum	590 bp	47
10	4/2/2023	Queens, NY	Panaeolus Foenisecii	2,126 bp	47

DISCUSSION

The aim of our research was to investigate the species of fungi in relation to air pollution. Our hypothesis suggested that the presence of fungi could serve as a bioindicator of air quality. In addition, we predicted that the diversity of fungi would differ in the urban areas of New York and the suburban areas of Connecticut. We collected 10 samples of fungi from Greenwich, CT, and Bronx, NY. Unfortunately, we were only able to obtain PCR results from six samples. By compiling data from GenBank, we were able to determine the size fragment length of each of the

six working samples. Comparing this data to the air quality index at the time of collection, we have concluded that the overall air quality during the collection of samples #5 and #6 were the lowest. The mushrooms collected in these two samples were both Trametes gibbosa, and based on our data and findings, we conclude that this species is the most efficient bioindicator of air quality. Throughout our collection and research process, we were unable to find samples of mushrooms in some areas, and this absence can likely be attributed to higher AQIs in those areas. In the more rural areas of our study such as Greenwich, the AQI was lower and thus "better," which correlates to the species of mushrooms that we collected in these areas. Our hypothesis was proven correct based on the small sample of data we were able to collect and sequence. In future studies, we would like to collect larger quantities of data in order to have more thorough and reliable results as well as collect samples from more prominent growth periods of fungi to improve the DNA sequencing process.

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Participant Biographies

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Student 2: My name is Catherine Tucker, and I am an eleventh grade student at Rye Country Day School (1 Cedar Street, Rye, New York, 10580).

Teacher/Mentor: For 15+ years, Jennifer Doran has led scientific courses, STEAM camps, and innovation fairs all across New York City. She currently teaches biology and science research at Rye Country Day School (1 Cedar Street, Rye, New York, 10580). She holds a Bachelor's degree in biology and psychology, and holds a Master's degree in science education. Prior to teaching, she trained and worked at some of the top research labs in the world, including The American Museum of Natural History, Cold Spring Harbor Laboratory, Weill Cornell Medical College, and The New York Genome Center.