Using Lichens as Bioindicators: Evaluating Pollution Impact on Species Diversity in the Pacific Northwest.

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Introduction:

Lichens have adapted to the harsh environments of the Pacific Northwest (PNW), allowing them to thrive under low amounts of nutrients like nitrogen. They have adapted over millions of years to have their advantage over other species, that being their ability to thrive under low nutrient conditions. With the rise of global temperature and climate change the nutrient cycles of many different environments worldwide have been gravely disturbed [1]. The PNW historically has had very low amounts of nutrients in the ecosystem, until recent history we have seen an increase in nutrients due to pollution and human activity increasing in the area [2]. The increase in nutrients hurts the native populations of plants, fungi, and lichen due to their adaptation to thrive with little nutrients. This no longer puts them ahead of the competition species. This causes an increase in invasive species and a sharp decrease in native species [3]. The lichen suffers slightly differently in the way that they are still native to the area, but populations of lichen that are better adapted to pollution will be more abundant than those that are sensitive to it.

Lichens are used as bioindicators of pollution, in areas where there are immense amounts of pollution the diversity of lichen has been seen to drop. This study aims to determine how pollution levels in urban and industrial zones around Olympia influence the distribution of lichen diversity compared to pristine forests.

Lichen species are particularly sensitive to nitrogen and will fail to grow in areas with high pollution levels [4,5]. Lichen have a unique biology and absorb nutrients through the air around them while lacking any protective layer like waxy cuticles seen in plants. For example, Fruticose or bush-like lichens are more sensitive to pollution since they have a greater surface area exposed to the air surrounding it [6]. When ecosystems face increased nitrogen levels from agricultural or industrial activities, the nutrient cycle of the system is altered. Nitrogen-sensitive lichen species are adapted for low nutrient conditions, they decline in nitrogen-rich environments due to out competition of nitrophilous lichen species. This can lead to a reduction of biodiversity and change the community composition. An experiment by Gutiérrez-Larruga et al. (2020) examined the effects of nitrogen deposition on lichen abundance and metabolism. The study found that increased nitrogen levels also increase lichen metabolism in the short term, but over the long term decrease lichen biodiversity [7]. This information supports the idea that lichen historically decreases in biodiversity due to their sensitive structure and lack of protection from pollution.

With the information gathered, we will expect more polluted areas to show less lichen diversity but an increased abundance of nitrophilous species. On the contrary, we will expect to observe a greater biodiversity of lichen in healthy forests while less abundance of each species.

Sampling areas

The experiment will use transect sampling methods from a high source of CO2 emissions (Parking lot, industrial area, urban area) to compare the variability and commonality of lichen species. The parking lots chosen will be in Olympia, Washington in regions that are known to have high traffic coming through. One parking lot will be the one on Saint Martins's campus near Harned Hall. The next parking lot will be in downtown Olympia, for this experiment, the entirety of downtown Olympia will be the center of pollution. There will also be a positive and negative control, the negative control will be located at Rock Candy Mountain trailhead which is just north of the Olympic National Forest off Washington Highway 8. The Rock Candy Mountain trail goes south deep into the Olympic National Forest, which will be an idealistic and pristine environment to collect the control data. The positive control will be The Olympia Auto Mall located on the westside of Olympia, the amount of traffic and the fact that cars are sold here should mean that the pollution and runoff in the area should be especially high. These controls will ensure a good basis of comparison for the data collected and can be used to show trends in lichen variability/speciation with local pollution levels.

The Sampling Methods

Transects will be from 50 meters, 100 meters, and 200 meters from the pollution source, this sampling method is as demonstrated in Abas et al. (2016). These distances provide a gradient to capture pollution's effects while minimizing overlap between transects [8]. At each of the transect points, 2 quadrats will be placed on 5 separate trees to collect the lichen frequency data. The number of lichen species with tallies of how many are found in each quadrant will be recorded. This will be repeated at each of the transect points, giving us a sample size of 15 at each of the sampling sites. Detailed pictures and field notes of the description and habitat of lichen will be taken at each site to ensure proper lichen identification.

To analyze the data a combination of statistical tests will be used. An Analysis of Variance (ANOVA) test will be used on the data to see if there is a significant difference in lichen species diversity across multiple areas with varying pollution (urban, industrial, and healthy forest). The test will be appropriate in the experiment because it is robust and capable of comparing more than two groups, it also can account for variations in species abundance. If the ANOVA test results do prove a statistically significant difference, then a post-hoc test like Tukey's Honest Significant Difference (HSD) will be done to see which specific pairs of environments differ. T-test will also be employed in the data analysis to compare the mean abundance of specific lichen species between environments. Also, regression analysis could be used to look at any possible linear relationships between distances from pollution sources and changes in lichen species diversity/abundance. All of these tests together will help statistically explain the impact of pollution on lichen species frequency and variability. Statistical significance will be analyzed at a 95% confidence level.

Flowchart

Week 1-2 (Jan 13 - Jan 26): Preparation and Planning

- Finalize site permissions (Olympia Auto Mall, Saint Martin's parking lot, downtown Olympia, Rock Candy Mountain).
- Gather and test all equipment (transects, quadrat frames, camera, waterproof pens, notebooks).
- practice sampling methodology on campus.
- Goals: Fully prepped field kit, and written sampling protocol finalized

Week 3-4 (Jan 27 - Feb 9): Field Sampling - Olympia Auto Mall (Positive Control)

- Conduct transect sampling at 50m, 100m, 200m from pollution source.
- Record lichen frequency data using quadrats on trees.
- Take detailed pictures of lichen for identification.
- Collect field notes.
- Goals: Complete dataset for olympia auto mall, and high-quality images of lichen.

Week 5-6 (Feb 10 - Feb 23): Field Sampling - Downtown Olympia

- Conduct transect sampling at 50m, 100m, 200m from pollution source.
- Record lichen frequency data using quadrats on trees.
- Take detailed pictures of lichen for identification.
- Collect field notes.
- Goals: Complete dataset for downtown olympia, and high-quality images of lichen.

Week 7-8 (Feb 24 - Mar 9): Field Sampling - Saint Martin's Parking Lot

- Conduct transect sampling at 50m, 100m, 200m from pollution source.
- Record lichen frequency data using quadrats on trees.
- Take detailed pictures of lichen for identification.
- Collect field notes.
- Goals: Complete dataset for the Saint Martin's parking lot, and high-quality images of lichen.

Week 9-10 (Mar 10 - Mar 23): Field Sampling - Rock Candy Mountain (Negative Control)

- Conduct transect sampling at 50m, 100m, 200m from pollution source.
- Record lichen frequency data using quadrats on trees.
- Take detailed pictures of lichen for identification.
- Collect field notes.
- Goals: Complete dataset for Rock Candy Mountain, and high-quality images of lichen.

Week 11-12 (Mar 24 - Apr 6): Digitizing data and lichen identification.

- Organize all field data and images and put it in the computer (excel and minitab).
- Species identification with field guide and lab resources.
- Conduct statistical tests (ANOVA, T-tests).
- Goals: cleaned up data set ready for analysis with all species identified.

Week 13-14 (Apr 7 - Apr 20): Data Analysis

- Do a full statistical analysis, including regression models.
- Create graphs and tables to visualize lichen species variability across sites.
- Goals: Complete statistical analysis with graphs and tables created.

Week 15-16 (Apr 21 - May 4): Report Writing

- Draft final report summarizing research methods, findings, and conclusions.
- Goals: Final stage of Draft

Final Week (May 5 - May 9): Submission

- Finalize draft and submit.
- Goals: have a complete report to submit.

Budget

Field equipment:

- Measuring tape (for transect distances): On campus
- Quadrat frames: Hand made with straw and string
- Clipboard and field notebook: $9.95, Clipboard provided,
- Waterproof pen: $15.99,

Sample collection:

- Sample containers (transport of lichen): provided

Identification:

- Lichen identification guidebook: Online, provided from library,
- Magnifying glass: provided

Transportation:

- Travel (gas and parking fees): around $75
- Gloves, safety gear: provided

Digital equipment:

- Camera or phone accessory (high quality images of lichens): $40
  https://www.amazon.com/Xenvo-iPhone-Camera-Lens-Clip/dp/B01A6D2JVI/ref=asc_df_B01A6D2JVI?mcid=566d879465203d03a34b5679f06e15d8&tag=hyprod-20&linkCode=df0&hvadid=693712892368&hvpos=&hvnetw=g&hv

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