Lichens in YOUR Local Landscape

Modified from Ecological Research as Education Network (EREN) Flexible learning projects
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Pre-lab Reading

The distribution and abundance of species are affected by the **abiotic** and **biotic** environment. Land-use modification and other anthropogenic drivers of environmental change (e.g., air pollution) affect landscape, local, and micro-site conditions. This, in turn, influences the ability of a given species to survive or thrive in an area. Lichens are an excellent group of organisms to explore how abiotic and biotic variables affect the presence and abundance of lichens.

What are Lichens?

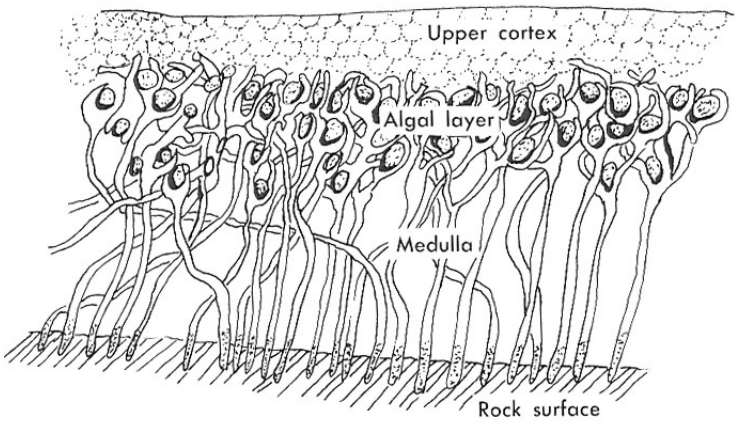

FIGURE 1

Lichens are **composite organisms**, which means that they are made up of two independent organisms living in symbiosis (close physical association). Figure 1 shows the generalized structure of a lichen. Lichens are **mutualistic symbionts**, meaning that both the fungus (the **mycobiont**) and algae or cyanobacteria (the **photobiont**) benefit from the close association. The algae or cyanobacteria are photosynthetic, so the fungus benefits from the carbon compounds (sugars) that the algae or cyanobacteria produce. In turn, the algae or cyanobacteria gain protection, nutrients, and moisture from the fungus. *(NOTE: Researchers found lichens across six continents also contain basidiomycete yeasts, single-celled fungi that likely produce chemicals that help lichens ward off predators and repel microbes.)*

Figure 1. Generalized structure of a lichen thallus (non-reproductive tissues or vegetative parts): upper cortex (outer covering provided by the fungus), algal or cyanobacteria layer (photobiont), and medulla (stores nutrients produced by the photobiont).

Where are Lichens Found?

Lichens are widespread and can be found in harsh climates, from hot deserts to cold alpine summits. However, within those ecosystems, they are found in specific **habitats** (natural environments in which they live). The key requirements for lichen habitat are: **water, air, nutrients, light, and substrate type** (USFS website CITE).

***Water*.** Lichens easily absorb water and water vapor through their **cortex** (outer layer of cells), but lack mechanisms to conserve water during drought periods. Lichens are **poikilohydrous** (water content determined by their surrounding environment); lichens lose water, dry up, and become dormant during dry periods, and rehydrate and become photosynthetically-active when moisture is available.

***Air*.** Not only do lichens absorb water from surrounding air, lichens also absorb nutrients and pollutants. They are sensitive to many air-borne pollutants, and the presence and abundance of some species of lichen are reduced in areas high in atmospheric pollution. For example, sulfur dioxide (SO_2) combines with moisture in the atmosphere to form sulfurous acid (H_2SO_3) or sulfuric acid (H_2SO_4). High levels of SO_2 pollution are associated with decreased lichen respiration and photosynthesis, deactivation of enzymes resulting in reduced metabolic activity and reduced membrane integrity.

***Nutrients*.** Nutrients (e.g., nitrogen, carbon, oxygen) are needed for cellular processes that support lichen growth and survival. Lichens obtain their nutrients from air and water, and, to a lesser extent, their substrate.

***Light*.** Lichens produce their own energy through photosynthesis and, thus, require light + carbon dioxide + water.

***Substrate*.** Lichens also require a substrate to attach to, and can be found on both natural (e.g., trees, rocks, soil) and artificial substrates (e.g., tombstones, buildings, abandoned equipment). Furthermore, characteristics of the substrate can influence how lichens interact with other aspects of their habitat. For example, substrate pH (e.g., limestone, tree bark with high (basic) pH) can counteract the acidity of SO_2 pollution, so that in high pollution areas lichens may be found only on sites with high (basics) pH. Although lichens are widespread, they need the right amounts of each of these key habitat requirements to survive and thrive!

Lichens as Bioindicators

Lichens are ubiquitous in urban and rural areas, and are also an important group of **bioindicators** - an organism whose presence, absence, and/or abundance in an area gives an indication of the degree of health of that ecosystem. For example, some organisms are very sensitive to pollution in the environment, so if pollutants are present, that organism may be absent, or may have different morphology or physiology, or may change its behavior. In contrast, other organisms are less sensitive to pollution in the environment. Documenting which of these bioindicator organisms are present, absent, and/or abundant in an area can be valuable in assessing ecosystem health and understanding whether or not an ecosystem may be impaired by pollution.

Some species of lichens are very sensitive to atmospheric pollution (e.g., nitrogen, sulfur, lead), whereas other species of lichens are tolerant to those pollutants. Lichens can be classified based on the structure of their **thallus** - their nonreproductive tissues or vegetative parts. Luckily, the degree of sensitivity of a lichen to air pollution varies roughly with their growth form. This makes lichens ideal to use as bioindicators. There are 3 main types of lichens (Figure 2):

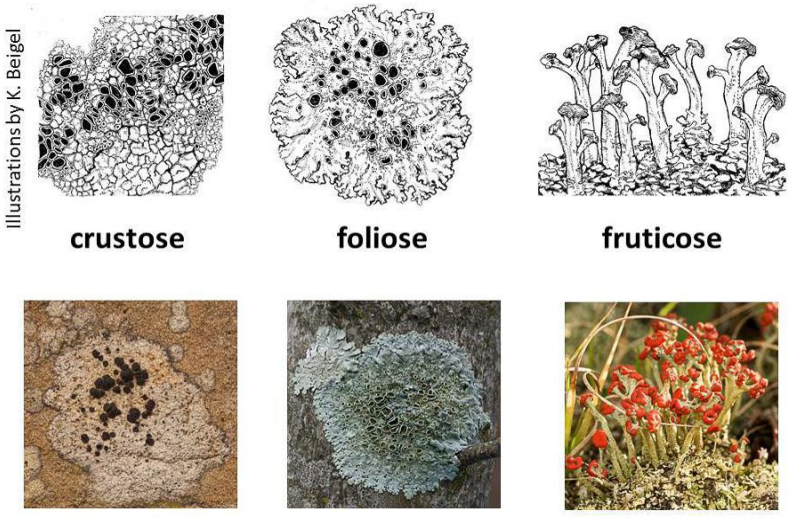

FIGURE 2 (https://ohioplants.org/lichen-biology/).

***Crustose*:**

- Thin, Crust-like
- Strongly adhere to the substrate; color varies

***Foliose*:**

- Leaf-like
- 2-dimensional (top and bottom "sides")
- Flat & leafy like lettuce, or w/ ridges or bumpy

***Fruticose*:**

- Branched-upright or **pendulous** (hanging down loosely)
- 3-dimensional
- Hair-like or upright and shrubby

For this exercise, we will be using a modified version of the **Hawksworth and Rose (1970) air quality index.** This air quality index is based on the type of lichen(s) present, and ranges from 1 to 10, with 1 indicating the poorest air quality and 10 indicating the best air quality (Table 1). In general, lichens that are least sensitive to air quality are crustose, and lichens most sensitive to pollution are fruticose, with foliose lichens in between those 2 extermities. Two species of fruticose lichens *Lobaria pulmonaria* and *Teloschistes exilis*, are extremely sensitive to air pollution and, thus, if either species are present, the air quality is considered very high.

Table 1. Lichen air quality index scoring.

Lichen Type	Air Quality	Air Quality Score
No lichens present	very poor	1
Crustose only	poor	3
Foliose present, but no fruticose	moderate to good	6
Fruticose present	good	9
Lobaria pulmonaria or *Teloschistes exilis* present; fruticose lichens, very sensitive to pollution	very high	10

Lobaria pulmonaria

Teloschistes exilis

iNaturalist Project EREN Lichen!

You will be contributing to a new iNaturalist project "EREN Lichen." iNaturalist is a web-based and smartphone app platform that is used to document observations of species in a particular date and time. It is used and supported by an online community of naturalists, citizen scientists, and biologists, who help in crowdsourcing and confirming species identifications. The EREN Lichen project is new, so the number of observations in the iNaturalist project is limited. We are looking forward to working with participants in the EREN Lichen project to explore possibilities as the project grows.

VIDEO OF THE PROCEDURES: https://www.youtube.com/watch?v=zdJQ0YLgJpo&feature=youtu.be

Module-specific Learning Goals

1) Gain experience in species identification, morpho-species classification, and how to make abiotic and biotic measurements in field settings.
2) Develop technical skills using modern technology (GPS,smartphone apps, spreadsheet programs, data analysis packages) and everyday materials (ruler, freezer baggie, sharpie permanent marker,compass) to make scientific measurements and analyze data.
3) Examine how bioindicator organisms can be used to assess land-use change, air pollution, and other environmental impacts.
4) Explore how to use multi-site collaborative data can be used to conduct regional and continental scale analyses to address ecological and macroecological questions.

Developing Hypotheses (pre-field) *Work in your teams to fill this out.*

TEAM Members: _____

Forming Hypotheses to Test

In this lab we will be exploring relationships between lichen presence and abundance and abiotic and biotic factors. Consider the key habitat requirements of lichens. What abiotic and biotic variables may affect the presence and abundance of lichens, in general, or type of lichens present?

Develop a potential hypothesis to test for this learning activity.

Response (Dependent) Variable:

Predictor (Independent) Variable:

What do you expect the relationship to be?

Rationale:

Write Out Your Complete Hypothesis:

- Students will share their hypotheses and rationale with their instructor and the rest of the class.
- All students will collect all data and enter it into the iNaturalist project EREN Lichen. Students will use the entire class dataset to explore their hypothesis.

Procedure/Equipment List

Smartphone and apps and equipment

- Fill out the hypothesis worksheet
- Create an account in ***i*Naturalist** *and join EREN Lichen project*! (https://www.inaturalist.org/)
- Download the tree id app: **Seek** **(https://www.inaturalist.org/pages/seek_app)**
- Download the app for canopy cover: **Canopeo** **(https://canopeoapp.com/#/login)**
- Compass, measuring tape, lichen sampling grid, Sharpie, Field data sheet, rope, ziplock baggie, scraper

Field Sampling Protocols **Now you are ready to go out into the field.**

- Measure 4.5 ft (1.37m) height above the ground and tie the rope at this height.
- Measure the circumference at the rope and convert to diameter. (Circumference/π)

Each team will collect data on five trees. Trees should be a minimum of approximately 30 cm (12 in) diameter. (It needs to have a large enough diameter to be able to take the four % lichen readings, without having any overlap).

Step 1. **Identify the tree species and get the GPS coordinates.**

- Use field guides, identification keys, or smartphone app "Seek" to identify the tree to species level.
- If possible, it is best to use the scientific name (genus species) when you enter the name of tree species into the EREN Lichen project. Alternatively, you can enter the complete common name instead.
- ***Record the tree species on your datasheet.***
- If you cannot identify the tree to species level, then crowdsource tree species identification via *i*Naturalist:
  - If you cannot identify the tree down to species level, please upload an observation to iNaturalist.

Step 2. **Determine the north-facing aspect of the tree.**

- Use a compass or compass app on your smartphone to identify North.
  - Note: If you have a compass that allows you to set the declination on it, then look up the current declination for your area. See Appendix C for details.
- Then, move to the tree.
- Standing with the compass in your hand, your back to the tree, and facing away from the tree - move around to determine which part of the trunk faces North (0^0).

Step 3. **Measure percent canopy cover.**

- Stand at the base of the tree, at the north side of the tree.
- Open up the Canopeo app.
- With your back to the tree, hold the phone overhead (making sure it is parallel to the ground), and take the photo.
- Canopeo will give you percent canopy cover.
  - Be sure that you are getting % canopy cover and NOT % open!
- ***Record the value on your datasheet or in your notebook.***

Step 4. **Measure tree diameter, in centimeters.**

- Tree diameter (diameter at breast height; dbh) is measured at 4.5ft (1.37 m) above ground level.
  - After determining the circumference of the tree, convert it to diameter. (d = Circumference divided by pi.)
- ***Record the value on your datasheet.***

Step 5. **Record percent lichen at the north, east, south, and west faces of the tree.**

- Take the clear lichen sampling grid, and place the top of the grid so that it aligns with the rope.
- The sampling grid has 10 rows of 10 circles. Count the number of circles that have lichens.
- ***Record the value on your datasheet.***

Step 6. **Take photos and upload to the *i*Naturalist EREN Lichen project.**

- ***IMPORTANT***: Be sure that you take and upload photos in the specific order outlined below. That way it will be standardized and we will be able to match up the grid photos with the corresponding tree aspect.
- Open up the iNaturalist app on your smartphone.
- Take the following photos IN THIS ORDER:
  - *Photo 1*: Look around the tree and find the **dominant lichen**. Find a good example of the dominant lichen, take a photo, and upload it as your first image.
  - *Photo 2*: Take an image of where you took the first lichen sampling grid reading, on the North face of the tree.
    - You will probably capture a bit more than the area you sampled with the grid - that is okay! This will give us a reference image so that if anyone wants to go in, and maybe zoom in to identify the lichens present, this will be a great resource.
  - *Photo 3*: Take an image of where you took the east-facing lichen grid sample.
  - *Photo 4*: Take an image of where you took the south-facing lichen grid sample.
  - *Photo 5*: Take an image of where you took the west-facing lichen grid sample.
  - *Photo 6*: Take an image of the canopy, from the north end of the tree. (If you took a canopy cover image with CANOPEO, you will take the image in the same manner.)
    - Move to the north end of the tree, hold your phone overhead, parallel to the ground, and take the image.
    - This will allow getting a measurement of canopy cover, regardless of whether you recorded percent canopy cover with CANOPEO.
  - *Photo 7 (or more)*: Take a photo (or photos) of the tree. Take whatever photo(s) that you think would be helpful in identifying the tree to species level.
    - These image(s) can be used as reference documentation, and may help getting some trees to species level.

Step 7. **Add this observation record to the *i*Naturalist EREN Lichen project.**

- In *i*Naturalist, click on Add to Project, and select EREN Lichen.
  - Please note that you had to have joined the EREN Lichen project in *i*Naturalist prior to going out in the field!
- Once you select the EREN Lichen project, there will be a number of fields to enter your data into:
  - EREN Lichen tree species (tree species that you are sampling for lichens; scientific name, if possible)
  - EREN Lichen percent canopy cover (taken at the base of the north side of the tree, taken with CANOPEO)
  - EREN LichenPercent NORTH (% lichen north-facing quadrat)
  - EREN LichenPercent EAST (% lichen east-facing quadrat)
  - EREN LichenPercent SOUTH (% lichen south-facing quadrat)
  - EREN LichenPercent WEST (% lichen west-facing quadrat)
  - EREN Lichen air quality index score (modified Hawksworth Rose index score)
    - Note 1: Be sure to look over all areas of the tree (that are visible to you from the base of the tree) to determine the air quality index value. It is not restricted to where you measured with the lichen sampling grid.
    - Note 2: Only enter one value into this field. For example, if you ONLY have crustose lichen present, then it would be a score of 3. If you have BOTH crustose and foliose lichens present, the index score will be 6. If you have crustose, foliose, and fruticose lichens present, the index score will be 9.

Step 8. **Collect bark sample for pH measurement (optional).**

- If you will be measuring bark pH, select bark without lichen cover and with a smooth surface (>1 cm diameter needed for harvest).
- Use a flexible blade or chisel to remove 2 bark samples between 2-3 cm diameter from each tree.
- Air dry bark in labeled brown paper bags, leave overnight. Process the samples the next day (see instructions).

Step 9. **SAVE or SHARE the *i*Naturalist record.**

- Exact wording may vary, depending on the type of phone or specific version of *i*Naturalist.

Bark Sampling Considerations and Lab Protocols for Determining Bark pH

If you are planning to compare tree species with differing bark pH levels, consider sampling species in low, medium, and high categories and comparing them. This may depend on whether these species are available in your area.

Table 2. Species-specific bark pH (source: https://www.britishlichensociety.org.uk/about-lichens/lichen-ecology).

Bark pH	Tree species
Low (acid bark)	Oak, Birch, Alder, Sweet Chestnut, Rowan, Hawthorn, Hornbeam, Pine, Spruce
Medium	Hazel, Ash, Sycamore, Willow, Lime
High (base-rich bark)	Elm, Field Maple

*Beech trees not suitable due to heavy shading of lower branches in summer.

After the bark samples have air dried overnight (in labeled brown paper bags):

- The next day, soak bark samples in distilled water (neutral pH; can be purchased at a grocery store)
- Leave soaking overnight
- The following day, use hand-held pH meter to measure pH of the liquid.
- Or, if using pH indicator strips, place strips in liquid, and compare to the color ramps provided with the indicator strips.
- ***Update the corresponding observation field in iNaturalist.***

Updating Observation Fields (and Adding New Observations) to *i*Naturalist project EREN Lichen

- Updating observation fields in *i*Naturalist
- Uploading new observations into *i*Naturalist (if did not use smartphone app in the field)

References

Hawksworth DL, Rose F. 1970. Qualitative scale for estimating sulphur dioxide air pollution in England and Wales using epiphytic lichens. Nature; London 227: 145–148.