USING TREE MOSS AS AN INDICATOR OF AIR QUALITY:

A COMMUNITY HOW-TO GUIDE


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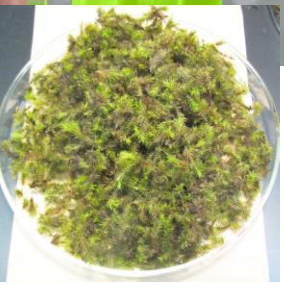
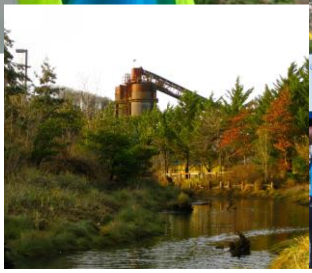


Including Sample
Curriculum and Lessons
Learned
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Welcome!

This guide is intended to outline how communities can utilize tree moss as a bio- indicator for local air pollution. We envision this process as a learning tool for local schools, youth groups, and community members, and a political action-oriented tool that can build power for local communities to fight air pollution where they live, work, and play. The methods were developed by and for community use, based on USDA Forest Service protocols. The approach is tailored for effectiveness in the Duwamish Valley in Seattle, WA, however we hope it might have value to communities around the world. The potential users of this guide might be community organizations, municipalities, local-focused non-profit organizations, university and research institutions, and other entities either working with or led by community.

The methods provided here suggest a community-led research program, operating structure, including study design, curricular materials, field protocols, and lab techniques. Other element such as results and analysis and follow-up actions are described, however are much more likely to be location specific in their structure. Other documents provide the results of our work; check them out in the Sources section at the end of this guide.

Contents

Introduction

Imagine. 10 years from now communities using trees and nature in their own neighborhoods to better understand air pollution and take action where they live, work, and play.

Background

In a recent study in Portland, OR scientists identified areas of potential air pollution using a common species of moss (*Orthotrichum lyellii*) as a biological indicator of atmospheric metals (Donovan et al. 2016, Gatziolis et al. 2016). The method they used was found to be an effective screening tool to help regulatory agencies place air monitors in strategic positions to gauge human exposure and potential health impacts of localized areas of air pollution. Our project presents this method as a practical tool that can be applied by local community or educational groups to help raise awareness and understanding of potential air quality issues and identify mitigation actions.

Residents in the Duwamish Valley, in Seattle, WA are constantly exposed to pollution emitted from industries, automobiles, trucks, and other sources. In the Cumulative Health Impacts Analysis (CHIA) published by the Duwamish River Community Coalition (DRCC) and Just Health Action, the Duwamish Valley had the highest ranking for air pollution compared to other neighborhoods in Seattle. The environmental exposures identified include the highest citywide air concentration of diesel particulates and benzene. In addition, the Duwamish Valley contains the most contaminated waste sites and numerous Toxic Release Inventory sites located within Seattle neighborhoods. Understanding how these pollutants vary at neighborhood-relevant scales is a critical knowledge gap, however, as the area has only a couple air monitors measuring a small subset of the pollutants known or suspected to occur there. Similar scenarios exist in many cities across the world, and so we envision that this guide can have impacts elsewhere as well. Furthermore, our protocols using moss to indicate atmospheric metals can be adapted to screen for a variety of air toxics that may be of concern.

The Duwamish Valley Youth Corps (DVYC), a program of the DRCC consisting primarily of 9th and 10th graders, served as the lead in our project for two tree moss collection seasons in 2019 and 2021. The DVYC lead the collection of moss samples, preparation of the samples in the classroom for lab analysis, and led the community assessment of the data, as well as priority setting and presentations to elected leaders and decision makers. They demonstrated the role that community can take leading this type of project.

Significance and Impact

First, this work demonstrated that youth and community participants can collect scientifically valid moss samples indicating atmospheric metals concentrations using the methods described (Derrien et al. 2020). Second, the results from moss studies like ours indicate where there may be different concentrations of pollutants that could impact the health of residents in communities (Jovan et al. 2022). As pollution hotspots are identified, regulatory agency and land management partners can use results to strategically place air quality monitors and identify potential mitigation practices, such as tree planting and other green infrastructure improvements. These elements are critical parts of the necessary follow-up for this type of work. We feel that as pollution is identified it is the responsibility of the investigators to stay involved until mitigation is complete. Suggested follow-up actions for those completing work outlined in this guide include:

- **Additional monitoring** with air monitors that can make direct inference between pollution levels and human health impacts

- **Mitigating impacts with trees and vegetation** focusing on locations identified as hotspots for heavy metal concentrations
- **Reductions in pollution** achieved through regulatory, incentive, or partnership mechanisms to stop heavy metal air pollution at its sources
- **Increase community awareness to pollution risks and locations** such that those most impacted are also those with the most knowledge of the risks and solutions
- **Increase community capacity and ability** to understand their own environment and make decisions best suited to their particular challenges and desires.

In our case, our main regulatory agency and land management partners include the Puget Sound Clean Air Agency and the City of Seattle through its urban forestry program and the Duwamish Valley Action Plan. Follow-up activities in response to our study include a community-directed air monitoring campaign led by Puget Sound Clean Air Agency in moss-indicated hotspots of heavy metals (Jovan et al. 2022).

Project Design

Sampling and Research Design

A central problem with air quality monitoring in the United States is that too few monitors are deployed within urban areas to describe variability in pollution at the neighborhood or even at the city-scale. The protocols in this guide outline a process designed to help answer questions about the spatial variability of heavy metal pollutants. The details of our approach may be a good starting place for other urban moss studies, although establishing a study design tailored to one’s particular study area and goals is important if looking to make scientifically valid inferences about concentrations of metals found in mosses. Geographic scope and scale of the investigation, including number of necessary samples, must align with the community of interest, and considerations for capturing gradients in pollution concentrations.

In our case, we used a quarter kilometer sampling grid (250 m x 250 m) to allocate samples within the neighborhoods under study because this area hosts a high density and diversity of potential emissions sources. Thus, we expected to see relatively high pollutant variability across the area. Given the relatively small area of investigation, it was possible for participants to visit all grid cells. Stratifying by particular landscape types to ensure gradients of land use and land cover are captured was another sampling design we explored and would recommend considering depending on study goals.

Figure 1. Example of use of a 250-meter grid

The protocol outlined here utilizes the abundance of deciduous street trees. Different types of landscapes may require adjustments to obtain enough moss samples. Replicate samples were also collected at roughly 20% of sites as part of the QA/QC protocols, which is a fairly common rate of re-sampling in similar moss studies. Quality assurance and control measures for moss sampling and preparation were adopted from the Portland moss study (Donovan et al. 2016, Gatziolis et al. 2016) and revised after review by air resource specialists with Puget Sound Clean Air Agency and agency and university biologists and environmental health scientists on the study team.

Sample locations will dramatically impact the number of samples participants are able to collect in any given day. Transportation to, between, and from field sites can take the majority of sampling time, and that is under the best cases where sites are easy to find, and each team has a dedicated vehicle for travel. To help prepare for this, and other collection difficulties, we'd recommend prescreening locations for safety and tree availability. Many sites might not have accessible tress, or trees likely to carry the desired moss. Additionally, if program participants are going to be youth, volunteers, or community members, all sites should be reviewed for pedestrian safety. Sites that are not appropriate for a group of people to work at for a period of 10-15 minutes should be removed from the sampling frame. Prescreening using aerial imaging and Google Street view, and on-site visits may be necessary to determine if a location is appropriate for inclusion in a study.

Property Permission

Finally, based on your sample frame, determining the permission of relevant property owners is important. If you are working only from street trees, connecting with the relevant city department, (DOT, Parks etc) or the adjacent property owner may be important. Additionally, carrying outreach materials for engaging with inquiring members of the neighborhood is part of the community engagement impact of this program, and crucial for ensuring buy-in from the community as well as access to trees and moss. And remember for outreach: removing *Orthotrichum lyellii* in no way harms a tree.

Project Preparation and Organization

A 'train-the-trainer' approach is recommended for this type of project. Staff and guides will want to complete training on methods demonstrated by an expert. Ideally, staff or guides are program graduates or trusted community members with a strong affinity with the project team. Training sessions were completed for all staff as part of our work prior to beginning this program. This training was in addition to any lesson preparation and other activities. Train the trainer at a minimum should include two sessions. One to train staff on the moss collection techniques, and one to train staff on moss preparation techniques. Two to three hours is recommended for each of these sessions.

Youth and community participants should be divided into teams. All teams should be paired with a staff member who has already received the training. This could include a previous youth participant returning as a guide or leader, a community member, or a staff person. Sample collection requires a few people to handle the roles, and a team allows for familiarity, pride, and memorable learning experience for participants. Teams should be 5 or less to allow each participant to have a meaningful role in sample collection. This also makes transportation by a single vehicle possible. This 5-person limit should also include or consider a staff participant with each team, and the number of sample sites to visit.

As community empowerment and community control are central elements of this work, as well as employment pathways, we employed surveys to measure the efficacy of the curriculum and methods. Learning, job interests, and enjoyment was measured by pre- and post-test surveys of all youth corps participants. See additional resources below for details on evaluative tools. We implemented both a nine session and an 11-session project in the Duwamish Valley. Ideally, 11 or more sessions with participants are utilized to undertake a moss collection project such as this, though this depends on the size of the group and the number of samples desired. See Curriculum section below for more details on these sessions.

Basic program structure is recommended to have both training and doing sessions, as well as intro and conclusion days. The program should allow for an initial session for introducing concepts and goals. Next a session on moss collection, and another session on moss sample preparation. These would be followed by actual collection days, number of which would align with your sample design. Moss sample preparation is next with enough sessions set aside to prepare all collected samples for the professional lab.

Finally, it is recommended that at least one to two sessions end the project with next steps, and perhaps analysis of previous seasons lab results.

Following sample prep, samples will need to be sent to a lab for analysis to quantify their heavy metals concentrations. This work involves advanced training in analytical chemistry and is something we have not yet identified a community-led way to complete. Lab analysis can take weeks or months and projects should plan accordingly to receive results. Please see Lab Resources below.

In budgeting time and funding, we identified the need for 1-2 experts familiar with the methods outlined here to lead the train the trainer sessions and help out with the rest of the program as much as possible. Additionally, the number of project teams should be scaled to the project size and should also all be assigned a lead. This guide outlines 26-36 hours of commitment per participant, once the program starts. Initial project design work is more up-front effort, and analysis and follow-up is substantial back end commitment. For the projects we completed in the Duwamish Valley, and for any work in historically underserved communities, it is highly recommended that all participants be paid at least a stipend for participating, if not full salaries. These costs should be planned for in addition to staff costs, material costs, and lab analysis costs, and the cost of any additional analysis on results (spatial, statistical).

Results Interpretation

Samples in the Duwamish Valley were analyzed for 29 heavy metals, including potential toxins such as Pb, Cd, As, Cr, at the USDA Forest Service Grand Rapids Lab and the Environmental Health Laboratory at the University of Washington. Additional samples were collected by scientists who have conducted and published results of moss sampling using methods similar to this project. Additionally, pseudo-controls were employed through the consideration of reference samples collected using the same methods in more natural settings of the greater Seattle area (e.g., Olympic Peninsula and Seattle city parks) to provide contextual “background levels” of the metals under study. Once concentrations were available for each sample location, community led results development and analysis could begin.

Session Preparation and Materials

Once a project plan is in place, preparing for sessions with participants is the next step. Considerations should be given to meeting and training space, large enough to hold the full group, close to or in the neighborhood sampled, and with the needed AV equipment and support. Additionally, lab or semi-lab space is helpful in creating a clean environment needed for moss sample prep. Considerations for this space include hard surface tables easy to disinfect, excellent lighting and electrical outlets for individual laps to be plugged in, and access to a sink and other facilities for thorough cleaning.

Other considerations that are critical for implementing a program of this scale would be aligning with school schedule and student capacity, provision of food - meals and snacks as needed, and transportation both to and from sessions and also as part of sample collection. In addition to materials provide below basic classroom materials such as tables, chairs, outlets, good ventilation, and other recourses should not be taken for granted in identifying and building a program.

Materials Needed

The below list is a suggested comprehensive materials list needed to implement the preparation protocols suggested in this report. Amounts of each item are general or not provided due to the scope and scale of a project that others might choose to take on. It is estimated that equipment cost roughly $1,500 per 100 samples collected and prepared.

Classroom Materials List:

- Journals for participants
- White board
- Pens/markers
- Posters
- Large post-its
- Moss samples/ examples
- LCD projector
- Laptop/ Computer
- Computer Speakers
- Airbeams/ Dylos or other handheld air quality indicator
- Android phone (smart phone depending on ap used)
- Sign-sheet
- Word wall Flipchart (definitions learned over the program)

Moss Collection Equipment:

- Team Field Bags: (one for each group)
    - Disposable gloves,
    - Kapak bags (pre labeled matching your sample #),
    - Permanente pens,
    - Duct tape,
- Moss and lichen samples (previous samples)
- Step ladders
- Spray bottle (optional)
- Binder of support materials:
    - Overview of project - for interested community
    - DOT letter of support

- Parks Research Permit
    - Moss identification 'cheat sheet'
    - Sampling and Sample Prep Protocols
    - 1-page Collection Protocols for Youth - draft
    - Materials List
    - Covid-19 Safety Protocols
    - Air Beam Protocols
    - Staff Contact List
- Smart Phones with ap (multiple for each team)
- Vans (or cars) for travel outside of walk shed

Sample Preparation Materials List

- Kapak bags of Moss from your previous day's collections
- Cooler for completed moss bags
- *3 large glass petri dishes, bowls, or watch plates per person
- 70% ethanol, to share
- Deionized water, to share
- *Forceps, to share
- *Large pair of scissors (titanium or ceramic is ideal), per person
- Colored powder-free nitrile gloves, per person
- Prelabeled Kapak bags, per person
- Duct tape, to share
- Sharpie, to share
- Scale, to share
- Kimwipes or unbleached paper towels, to share
- Headlamps or desk lamps to share
- Sample Prep Tracking Worksheet, to share
- Sample Prep Protocols Instructions, to share

(* items need to be sanitized with 70% ethanol before every sample)

Analysis Materials List:

- Maps of past samples and associated metals concentrations
- Pen/pencils
- Colored pens (red, orange, yellow)
- Large poster-board size map
- Push-pins (red, orange, yellow)
- Plastic take-out lid (3 inches in diameter)

Curriculum

For a full version of the curriculum utilized in the Duwamish Valley in 2021, please look here. Presented below is an outline of the curriculum to present a sense of the scope and priorities.

Programmatic Learning Goals

Each session has specific leaning objectives associated with it. Additionally, there was an overall, programmatic set of learning goals employed with this curriculum:

1. Act to improve the environment and human health in their community.
2. Learn, practice, and advance skills moss identification, collection, and preparation.
3. Gain confidence and familiarity with research concepts and methods.
4. Learn the sampling collection and sample prep methods associated with metals in moss testing.

Not presented in detail here are two items we attempted and did not have success with, though would still highly recommend that any moss collection curriculum utilize: Real-time air pollution sensors, and a project specific spatial phone ap. Each of these can be implement with this curriculum with relative ease and would improve the process over the experience in the Duwamish Valley.

Hand-held Air Sensors

Not reflected in this curriculum is a real time is use of an air quality sensor. However, we recommend that in addition to moss collection participants in the program utilize a real-time air quality monitor. Products such as AirBeam or Dylos could serve this purpose. The value of a real-time air monitor is two-fold. Initially, the use of such a device allows for real time data for participants to see and respond to. Areas with high PM readings give users a sense of the type of environments where they might expect for moss results to return high metals concentrations. This type of immediate ground truthing reinforces curricular materials on potential pollution sources and mitigating factors. Due to the long time it takes to receive moss lab results, it also may be the participants only opportunity for air quality indication on sites they are specifically visiting.

Collecting air indicator data, also allows for some rough check on the value of moss sample results. Though these devices are more imprecise than the moss lab analysis, they are collecting information on the PM that is a direct human health impact, as opposed to the moss which serves as a bio-indicator. Using the air quality data as an additional data set, may give some sense of the viability of the moss data in absence of rigorous follow-up testing.

Custom Phone Application

The project in the Duwamish Valley was carried out utilizing Google maps as the spatial software. Once a sample grid is developed it should be loaded into a shareable mapping platform. Each group or team should be assigned their grid for any sampling day in advance. Teams should be able to navigate to the center point of a grid square to begin sampling and should be able to mark a point exactly where a sample is taken. Each of these mapping functions is possible with Google or other free public mapping platforms, however, it is something that requires substantial upfront effort. Developing an ap that can conduct these functions, with a more friendly user interface, perhaps such as a survey form would greatly improve the process. Similarly, the data sheets presented below would benefit from being incorporated into a single digital recording platform.

Before the data collection began our process outlined a supporting curriculum that included two training sessions. These sessions were focused on training staff and guides, those sessions are not provided in detail here. Below represents the participant curriculum:

Introductory Session

Time: 2-3 hours, Location: Inside (Classroom) and Outside (Community)

Goal: Participants have initial understanding of the program, its importance, and enthusiasm to continue.

Learning Objectives: By the end of this lesson plan, participants will be able to:

- Describe an overview/purpose of the program
- List 3 group agreements
- Explain what moss is - and why we are interested in it
- Explain what a bioindicator is - and why we would use them
- Explain what community science is – and why it is valuable and necessary
- Discuss sources of air pollution - and how this work might identify new ones

Materials: *Classroom materials list from above*

Word Wall:

- Air pollution
- Moss
- Community Science
- Environmental Justice

Activity Instructions, Evaluation and Journal: See full curriculum

Lesson Plan 2: Moss Sample Collection Training

Time: 2-3 hours, Location: Inside (Classroom) and Outside (Community)

Goal: Participants learn and practice moss sample collection techniques and understand the purpose of these approaches.

Learning Objectives:
By the end of this lesson plan, participants will be able to:

- Navigate to predetermined point and identify locations of *Orthotrichum lyellii*
- Practice how to collection the moss correctly from the tree
- Practice reading their precise location using GPS, information about the moss and tree in a recording sheet
- Understand why these techniques are used and how they enable understanding of their community
- Understand why we could collect using a sampling grid.

Materials: *Field collection materials list from above*

Word Wall:

- GPS
- Latitudes
- Longitudes

Activity Instructions, Evaluation and Journal: See full curriculum

Lesson Plans: Moss Collection

Time: 2-3 hours, Location: Outside in the community

Goal: Participants gain experience of moss sample collection

Learning Objectives: By the end of this lesson plan, participants will be able to:

- Navigate to a predetermined point identify locations of *Orthotrichum lyellii*.
- Practice how to collection the moss correctly from the tree
- Practice reading precise location using GPS, record the moss and tree in a recording sheet
- Understand why techniques are used and how they enable understanding of community
- Understand why we could collect using a sampling grid.

Materials: *Field collection materials list from above*

Word Wall: No new words

Activity Instructions, Evaluation and Journal: See full curriculum

Lesson Plan: Sample Prep Training

Time: 2-3 hours, Location: Inside (preferably a lab setting)

Goals:

- Participants will learn about what happens next with the moss samples they've collected.
- They will learn why and how it needs to be prepared to be sent to the lab.
- Participants will practice preparing moss samples.

Learning Objectives:

- Explain why the moss samples need to be prepared
- Describe the process for the samples from collection through lab analysis
- Practice preparing moss sample, identifying and dividing moss components, and package for lab
- Record sample tracking as it moves from a collection sample to a new sample bag
- Set up and clean up a sterile work environment

Materials: *See Sample preparation materials list above*

Word Wall:

- Rhizoid
- Forceps
- Sterile
- Digestion

Activity Instructions, Evaluation and Journal: See full curriculum

Lesson Plan: Sample Preparation

Time: 2-3 hours each session, Location: Inside (preferably a lab setting)

Goals:
- Participants will gain experience preparing moss samples for lab analysis.
- Participants will learn why and how it needs to be prepared to be sent to the lab.

Learning Objectives:
- Explain why the 4moss samples need to be prepared
- Describe the process for the samples from collection through lab analysis
- Prepare moss samples, identifying and dividing moss components, and package them for the lab
- Record sample tracking as it moves from a collection sample to a new sample bag
- Set up and clean up a sterile work environment

Materials: *See above*

Word Wall:
- Rhizoid
- Forceps
- Sterile
- Digestion

Activity Instructions, Evaluation and Journal: See full curriculum

Lesson Plan: Data Analysis (*Thinking like a Geographer*)

Time: 2-3 hours, Location: Inside

Goal: Participants will review the type of information that a pollution concentrations map contains. They will order metal concentrations in ascending order on the sides of the map.

Learning Objectives:
- List at least 3 components of information that one finds on a map
- Explain the purpose of this map interpretation exercise

Materials: *See Analysis materials list above*

Word Wall:
- Key
- Legend
- Land use
- Scale
- Outlier

Activity Instructions, Reflection and Journal: See full curriculum

Lesson Plan: What Next? - Taking Action

Time: 2-3 hours, Location: Inside

Goal: Interpret the metals maps and develop some ideas about where the metals (sources) are coming from. Discuss next steps about what can be done to reduce pollution – stop sources and mitigate what is there.

Learning Objectives:
- Describe the story of John Snow and the London cholera outbreak
- Discuss where concentrations are high, medium, and low on the map
- Compare park or "background" metals concentrations to study are metals concentrations
- Discuss possible sources of contamination
- Differentiate between metals in moss as an indicator vs health implications
- Develop a list of next steps for action

Materials: *See Analysis materials list above*

Word Wall:
- Cholera
- Hot spot
- Background/baseline
- Indicator
- Distribution
- Source

Activity Instructions, Evaluation and Journal: See full curriculum

Assessment of Learning

As part of each session a combination of group, team, and individual reflection was conducted. This included group work recording reflection on the flip charts, and individual journaling exercises, both open ended and prompted. Part of this approach includes an evaluation of learning. Formally, an assessment of the youth learning was completed for our work in the Duwamish Valley. This survey tool is something that the DVYC regularly employs and was slightly adjusted to focus on themes from this air quality and moss collection curriculum. For full discussion of the youth learning evaluative tool see: Derrien et al 2020.

Protocols

The activity protocols presented here were used to train DVYC and Dirt Corps. The original protocol was developed from the study conducted in Portland, OR in 2016[1]. From these materials and lessons learned debrief we conducted after the project, more formal training and curriculum materials have been developed. Please see the full curriculum here, which contains the curriculum as well as moss collection and moss preparation protocols. Specifically see:

- Complete Sample Prep Protocols
- Youth Specific Protocols
- Image Deck for Lessons

Sampling Instructions

Below is an example of the sampling instructions to be provided to participants collecting samples. It is envisioned that those collecting would have already received a more detailed training as part of the sessions' curriculum outlined above, and the below instructions would be something they could take with them into the field as a guide.

[1] Gatziolis, Demetrios; Jovan, Sarah; Donovan, Geoffrey; Amacher, Michael; Monleon, Vicente. 2016. Elemental atmospheric pollution assessment via moss- based measurements in Portland, Oregon. Gen. Tech. Rep. PNW-GTR-938. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. 55 p.

Moss Collection Instructions

1. **Find Moss**. Using your GPS go to the assigned sample number. Locate the nearest street tree. Go to tree and look for moss meeting criteria (type, health, and location). If no moss is available, go to next nearest street tree and repeat.

2. **Record the Information**. Once you find your tree and moss, use the app to record the exact latitude and longitude of where you are on your BLUE recording sheet. Fill in the number on your sample bag, using a decided upon numbering convention. Fill in lots of notes. Do not leave any sections blank.

3. **Prepare Your Materials.** For each moss sample, pull out a fresh bag and put on fresh gloves. Be sure not to touch anything with the gloves besides the moss, the tree, and the inside of the bag. You can collect anywhere above waist height. All moss you sample from a single site will go into the same sample bag.

4. **Collect the Moss.** Collect moss from at least 8 spots on the tree, if possible. Try to fill at least 1/3 to 1/2 of the sample bag - if in doubt, get more than you think is needed. When done collecting, press air out of the bag so you have room at the top of the bag. Roll the top of the bag and seal completely with duct tape. Make sure there are no possible openings that would allow air in.

5. **Take Pictures**. While moss is collected, another person takes 2-4 photos of the sampled tree. Make sure the numbers for each photo in your phone are correctly recorded into the BLUE data sheet. Get at least one photo that includes all or most of the sampled tree, and a photo of the leaves. It's good if people are in the photo. Take a final picture of sealed bag held against the tree it was taken from.

6. **Repeat?** If this site has a lot of moss (enough for multiple bags worth), once finished with the sample, start again! Remove gloves and start completely over again at step 2 above. When you fill out this bag, make sure to label it with an 'R' after the number

Figure 2. Youth simplified sampling protocols.

Worksheets

If using paper recording logs, as opposed to a phone application, below are examples of ones utilized in the Duwamish Valley for both sample collection in the field and sample preparation in the classroom.

Team Name and Team members Names:			Name of person recording data:		Date:
Collector name	**Location (decimal degrees)**		**Photo IDs**	**Notes**	**Label on Bag**
	Latitude	**Longitude**			

Figure 3. Example Sample Collection Worksheet

Date:		Name of person taking photos:			
Sample name	**Preparer name**	**Moisture Status (very wet, wet, damp,**	**Weight (g) of the prepared sample**	**Photo ID**	**Notes**

Figure 4. Example Sample Preparation Worksheet

Lessons Learned

A series of lessons learned were recorded throughout our program work. Some of these were addressed in the implementation of the second field season, while others were more difficult to resolve and remain challenges. Lessons learned are also represented and incorporated throughout this guide. Specific lessons learned are divided up below representing program limitations and protocols limitations.

Additionally, overarching lessons learned from this process include organizing a large cross-sector team incorporating community, scientists, and land managers. Issues of data ownership, data management, and data sharing should be worked out in advance of undertaking a program such as this. We highly recommend the development of a Memorandum of Understanding amongst program participants to organize the project team and establish ground rules for operation.

Study Limitations

A series of limitations to this approach for using air pollution indicators should be considered. These have been identified from many sources that originally worked to develop this method including Gatziolis et al. 2016.

- There is a lot of **variability in metals, especially on street trees due to vehicle dust**. Unless multiple sampled sites suggest a pollution hotspot, there’s a decent chance that the elevated metals are extremely local- perhaps limited just to the sampled tree. In these cases, we may want to go back and collect additional moss samples to evaluate.
- We must do our best to rule out **other sources of contamination**, variability, or extreme local-ness of the affected area before trying to involve instruments and possibly regulators. As a result:
    - The lab work proceeds slowly as there are multiple stages of QA/QC to rule out instrument/lab/data entry errors
    - Sample contamination is possible during collection and processing if bare hands touch the samples or lab tools for prep weren’t properly washed, if the moss bases weren’t removed, etc.
- The **moss sample data for each element serve only as an index**, meaning that high concentrations in moss are suggestive (but not conclusive) of high concentrations in the atmosphere. While past research suggests moss concentrations reflect atmospheric concentrations for many elements, the strength of these relationships are unknown and vary by element (Aboal et al., 2010). Conversely, samples with low values do not necessarily indicate ‘healthy’ neighborhoods.
- A key unknown is **how accurately metal concentrations in the moss reflect levels in the air**. Donovan et al. (2016) found a very high correlation between moss Cd and atmospheric Cd measured by DEQ (0.991 or 99.1%) but this relationship was based on only four data points and is thus not considered reliable by scientific standards. Otherwise, no calibration work (comparing moss-to-instrument values) has been done yet with *Orthotrichum* as the Portland study was the first time it has been used as a bio-indicator.
- It is likely that the strength of **relationships between moss and atmospheric concentrations will vary by element**. This is because elements differ in how strongly they bind to moss cells, how long they are retained in the moss, and how susceptible they are to displacement by other co-occurring elements (Rühling and Tyler, 1970; Gonzalez and Pokrovsky, 2014). Additionally, moss cells regulate the uptake of elements with physiological roles, such as some of the plant essential nutrients including metals.

- It is impossible to convert moss concentrations to health thresholds and **regulatory standards.** Validation of moss containing metals hotspots using monitoring instruments is required to make inferences about health risks and absolute concentrations in the atmosphere.
- It is **unknown what time period** is represented by metals in *Orthotrichum* but it likely ranges between several months to a few years. This means moss concentrations may indicate emissions sources that no longer exist and that repeat sampling over short time intervals may not accurately portray declining emissions after pollution abatement measures are taken. We analyzed elemental concentrations in only the upper 2/3 of moss stems and so we estimate that our samples could represent, at maximum, 3 years of exposure.

Protocol Limitations

A series of limitations were identified as areas of improvement associated with the methods provided in this guide.

- **Inconsistent sample labelling** was an ongoing issue. Most sample bags and recording sheets had all necessary information, but it was very unevenly and often unclearly noted. This can be addressed with clearer instructions and pre labelled sample bags that mirror the content of the field recording datasheets.
- **Timing of programming** made scheduling difficult/inflexible. The moss project was scheduled for multiple weeks and many hours evenings and Saturdays. This was a difficult, especially that this was the first field application with community members and with youth, and we were learning as we went.
- We did **not provide enough time for quality control checks** (such as checking industrial grids for trees) which fell to partners and experts to complete. In the future, we could increase the number of session days or provide more funding for other community groups to provide additional support.
- **Maps and directions to sampling grid centroid locations** only worked on certain phones. The programmed app (with location pins and directions) worked great for some phones but not others, which had to manually insert lat-longs. This is a minor technical issue, but it does illustrate that technical issues can cause unexpected delays.
- **More expert/adult guidance** was needed than we expected for the community members. We had plenty of assistance for this project, but it required more than would be ideal for a community science process in general. During field data collection guidance was needed to keep up the pace of data collection and to keep data collection tasks straight.
- Timing of the field data collection. The youth corps surveys showed the participants preferred the outdoor data collection portion of the project compared to the lab work, but this also led to distractions in the field (strolling casually between sampling sites, chatting, taking pictures, etc.). This was not at all a problem in the lab, where the YC members were fast, efficient and, because they were given specific targets, highly goal driven. **In the future, having more time** to collect field samples, being given sampling targets, and spending less time on technical glitches would address this.

Resources

Lab Resources

While community members can complete nearly all the protocols for this project, the analysis of metals concentrations in the moss samples is the one component still handled in a professional laboratory setting. Due to the danger associated with necessary chemicals used in the process, we have been unable to incorporate this as a community conducted process yet. The methods require using microwave assisted digestion in nitric acid and hydrogen peroxide (based on USFS protocol). Analysis is what is called instrumental analysis of elements by ICP-MS (Based on EPA 6020a Rev.1 2007). Labs utilized for the initial Duwamish Valley programing include:

USDA Forest Service Northern Research Station Laboratory
1831 E. Hwy. 169
Grand Rapids, MN, 55744-3399

Oregon State University
Botany & Plant Pathology Department
2082 Cordley Hall
Corvallis, OR 97331-2902

University of Washington
Environmental Health Laboratory, Trace Organics Analysis Center
4225 Roosevelt Way NE #100
Seattle, WA 98105

However, it is likely that multiple laboratories in each state have the capacity to carry out this type of analysis. Universities may prove to be particularly important partnerships for this. Sharing samples across labs and completing analysis of reference materials is recommend ensuring comparability of finds across laboratories.

Funding for this analysis is a central need to carry out these community science programs.

Beyond funding, community control and transparency of the lab portion of these methods is crucial to the development of this guide. This component of the program is underdeveloped. Community participants do not have a role in the process when samples are shipped off to the lab. We envision developing our curriculum where perhaps community participants can visit technical labs and observe the digestion and speciation processes that moss samples go through. Additionally, we strongly advocate that funding be set aside for labs to conduct this level of analysis on any samples that community might send to them. This would allow for further community empowerment and building their own knowledge base for their health and wellbeing.

Briefings and other Resources

Beyond understanding potential air pollution hot-spots, these methods focus on the importance of *who* understands. We endeavored to have results reach and engage impacted communities, stakeholders, and decision makers in meaningful ways. This includes creating materials and processes that are culturally relevant and reflective.

Tree Moss as Bio-Indicator Youth and Community Curriculum is the central guiding document for the project.

A press release, replicated in multiple languages was crafted to provide information about the project overall: https://www.drcc.org/s/Moss-study-Press-release-FINAL.pdf

And a community focused project report was completed. It was produced in:
English: https://www.drcc.org/s/Duwamish-moss-Fact-Sheet-final-fgwa.pdf
Spanish: https://www.drcc.org/s/Duwamish-Moss-Fact-Sheet-final-5-29-2020-1.pdf and
Somali: https://www.drcc.org/s/Duwamish-Moss-Fact-Sheet-final-SOMALI-5-29-2020.docx

DRCC State of the Air Report: https://www.drcc.org/s/Final-State-of-the-Air-Nov-2021.pdf
DRCC Clean Air Map:
https://wwu.maps.arcgis.com/apps/MapSeries/index.html?appid=2fcb3e2e16d04bca8c753f1b0b1b80d9
USFS webpage: https://www.fs.usda.gov/pnw/projects/duwamish-valley-communities-collaborate-urban-air-quality-biomonitoring-projects
DRCC webpage: https://www.drcc.org/moss-study

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