# Lichen Biomonitoring in the Prince George Area – Literature Review and Approaches for Future Studies.

By Asha MacDonald and Darwyn S. Coxson

Ecosystem Science and Management Program

3333 University Way, Prince George, B.C.

University of Northern British Columbia.

V2N 4Z9

Email: darwyn@unbc.ca

Unpublished Report for the Fraser Basin Council

1st Floor, 470 Granville Street

Vancouver, BC V6C 1V5

#### Introduction – Lichens as Biomonitors.

Lichens are a critical component of many terrestrial ecosystems, as primary colonizers, nitrogen fixers, and in providing essential habitat and nutritional requirements for a variety of wildlife (Sharnoff and Rosentreter, 1998) and invertebrates (Sharnoff, 1998). These symbiotic organisms have been popular biomonitoring tools for decades, as they absorb pollutants directly from the air around them and generally show sensitivity to these compounds. Historically, they have proven to be effective biomonitors for atmospheric pollution, including primarily sulphur dioxide (SO2), but also nitrogen compounds, radioactive fallout, and a variety of heavy metals. More recently, the sensitivity of a variety of lichen species to climate has prompted their use as indicators for climate change in a wide variety of regions around the globe (e.g. Sancho et al. 2007; Insarov and Schroeter, 2002)

Mapping lichen distributions to infer environmental quality began in Europe in the 1930s (Hawksworth, 2002). Perhaps most famously, in 1970, a qualitative scale for determining SO2 deposition was developed for England and Wales (Hawksworth and Rose, 1970), whereby zones of lichen species composition and abundance were correlated with winter SO2 deposition. This scale was then further adapted by many researchers for a wider applicability throughout Europe (van Haluwyn and van Herk, 2002). Also in 1970, LeBlanc and De Sloover introduced the concept of calculating an Index of Atmospheric Purity (IAP) in urban areas based on lichen flora. This method quickly became popular and widely used throughout Europe, with many subsequent modifications and adjustments (Kricke and Loppi, 2002). A great number of additional scales and indicator values have since been applied to lichen communities to assess a variety of environmental conditions, for example, the Index of Human Impact (IHI) (Gombert et al. 2005),

and various Indices of Ecological Continuity (IEC) (Rose, 1976; Tibell, 1992; Rose and Coppins, 2002; Selva, 2003). Improvements in sampling design and standardization have allowed the comparison across wide geographical areas, although these are mainly national data sets (e.g. McCune et al. 1997b; Nimis and Martellos, 2001; Ellis et al. 2007b).

Lichen communities may be studied as a whole, or by using a selected guild as indicators of the state of the ecosystem (Will-Wolf et al. 2002a). Studies which are based on entire lichen communities require in-depth knowledge of lichenology and are therefore not widely applicable, although the entire lichen community is likely a better indication of environmental conditions than are single species (van Haluwyn and van Herk, 2002). Studies which are based on one or more lichen indicator species to monitor environmental conditions allow the possibility of sampling by non-specialists and may therefore be more economical and widely applicable (Will-Wolf et al. 2002b). Subsets may include certain substrate types, ecological requirements, morphological types, or some combination of these three. It is important, however, to understand the relationship between the response of the subset and the response of the lichen community as a whole in a given ecosystem. It is often best to choose lichens which are particularly sensitive to the variable of interest (Will-Wolf et al. 2002a), for example, subsets of nitrophilic lichens have been used to study acidic deposition (Jovan and McCune 2004). A subset of "epiphytic macrolichens" is often used as representative of the lichen flora in forested ecosystems, as theseare more easily sampled and identified than more cryptic species such as the crusts or calicioids.

Many different methods for quantifying lichen communities have been developed since the 1970s. The so-called "phytosociological approach" samples lichen communities and then

groups them according to ecological requirements or pollution tolerances (van Haluwyn and van Herk, date to assess community changes over time (van Haluwyn and van Herk, 2002). This approach, however, may not be appropriate in urban areas with impoverished lichen flora (Gombert et al. 2005). Loppi et al. (2002) have proposed a scale for indicating deviations in lichen communities from the normal state, which encompasses both pollution and climatic effects. This type of approach can only be used in areas for which the lichen flora is well-known, and the scale that they created must be adapted for different bioclimatic areas (Loppi et al. 2002). Lichen Diversity Values (LDVs) have also been proposed as a repeatable measurement of lichen communities based on the frequencies that species are found in the sampling unit (Asta et al. 2002).

Gombert et al. (2005) suggest the various types of ecological indices for describing lichen distributions based on environmental gradients, as a baseline for long-term monitoring. Among these are different environmental quality gradients, including various aspects of human influences such as traffic, development, and urbanization, which are combined to create an Index of Human Impact (IHI). Ecological clusters are therefore defined based on lichen ecology and environmental characteristics. This type of approach is better suited to present-day monitoring projects, as SO2 levels have widely decreased and a variety of other factors are controlling shifts in lichen communities.

As discussed above, a wide range of ecological indicator methods have been proposed for studying lichen distributions. Although these are useful methods for describing environmental gradients, care should be taken in their application and the methodology behind them. Indicator values may be determined somewhat subjectively (Gombert et al. 2005), although many

protocols control for this (e.g. Asta et al. 2002). In addition, the indicator values may change according to where in its distribution the species is sampled (Nimis and Martellos, 2001).

The scale at which the lichen indicators are sampled can affect the results of a study (Dettki and Esseen, 1998; McCune and Lesica, 1992) because the response of lichens to a given environmental variable may change at different scales (Ellis et al. 2007a; Will-Wolf et al. 2002b), and because of changes in sampling density (Ferretti and Erhardt, 2002). Sampling scale is therefore contingent the research objectives at hand. McCune and Lesica (1992) found that single large sample plots are better for species capture, and several smaller plots are more effective for abundance estimates. It is therefore important to predetermine the goal of the study – a species survey or abundance estimates. This dichotomy may be overcome by subsampling for abundance estimates within a larger sampling plot in which one is more likely to encounter more of the species present in the ecosystem (McCune and Lesica, 1992). If the scale of a lichen distribution study is large, results will be influenced by environmental heterogeneity (Will-Wolf et al. 2002a), an issue that may be addressed with stratified sampling designs (e.g. Giordani, 2006; McCune et al. 2000).

Comparing repeated measurements of lichens may allow researchers to infer changes in environmental conditions over time, as the distributions of lichen communities in a particular region may be compared with subsequent distributional data from the same area. Although defining the objectives of monitoring programs ahead of time is critical, recent biomonitoring programs are under pressure to accommodate multiple objectives (Ferretti and Erhardt, 2002; Will-Wolf et al. 2002b), as environmental priorities change over time. This may force researchers to maintain adaptable sampling designs which can simultaneously capture many

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different aspects on shifting ecosystems. The research objectives, however, influence many aspects about how the lichens are sampled, and therefore accommodating multiple objectives may not always be possible.

In addition, the spatial scale of the sampling will affect the results, and therefore studies should be designed to address questions at as many scales as possible (Will-Wolf et al. 2002b). In addition to the issues of spatial scale and choosing appropriate indicator values, long-term monitoring projects employing lichen distributions to estimate environmental change face additional challenges. Ferretti and Erhardt (2002) discuss some examples of issues that should be addressed when designing long-term monitoring projects, for instance, they mention secure and long-term funding, flexible goals, emphasis on information management, and periodic research program evaluation. Will-Wolf et al. (2002a) suggest that simple, repeatable field methods, collaboration among researchers, compelling links with societal values, and timely, interesting results are key factors in the design of long-term monitoring studies. It has also been advised that long-term monitoring plots, particularly for climate change monitoring, must be set up in protected areas if they are to persist for long periods of time (Insarov and Schroeter, 2002).

It is important that data quality be assessed for long-term monitoring projects, to ensure that data 'noise' does not exceed the variation in the variable of interest (Ferretti and Erhardt, 2002; McCune and Lesica 1992; Will-Wolf et al. 2002a). Standardization is therefore an important component of experimental design, and has long been cited as a source of error in monitoring studies (Ferretti and Erhardt, 2002). Effective protocols including sufficient detail can help minimize between-measurement error. McCune et al. (1997a) assessed the data quality of repeated measurements with respect to seasonal variation and experience level of observer in

collecting measurements of species richness and abundances. The results of their data quality analysis suggested that non-experts did not report species richness accurately, contributing to measurement error, although community indices created from the measurements of non-experts were accurate enough that shifts in the lichen community would likely be detected over the long term.

Abundance estimates and measures are used to quantify lichen communities. Many protocols use abundance classes to accomplish this, which may be estimated through frequency or estimates of cover (Will-Wolf et al. 2002b). Both of these methods require that the plot or quadrat size be the same if the results are to be comparable – i.e. the results from a study with one size of a quadrat cannot be converted to compare with another data set with a different sized quadrat. Estimates of lichen biomass may also be used, as can belt transects, although these methods are not as common. Simple presence/absence data may also be used as an input for modeling (e.g. Ellis et al. 2007a, 2007b) and for IAP calculation (Quinn, 2005).

Bråkenhielm and Qinghong (1995) discuss three basic methods of estimating vegetation cover: using visual estimates, point frequencies, and subplot frequencies. Through analysis of between observer measurements, they found that for vegetation cover estimates, sub-plot frequency gave the highest mean cover estimates, with visual estimates giving the lowest and point frequency intermediate. However, all three methods gave similar abundance estimates for the species in question. Rare species were missed in the point-contact method, again relating back to issues of scale and defining the research objective. Overall, Bråkenhielm and Qinghong (1995) suggest the visual estimate method is best suited for reproducibility over long-term

monitoring projects, which is good reason to use this method in lichen biomonitoring studies (Trevor Goward, personal communication).

Sampling designs for lichen mapping often include microplots nested within larger macroplots (e.g. Asta et al. 2002). In this manner, species capture is maximized in the macroplot, while accurate measures of abundance can be made in the microplots (McCune and Lesica, 1992). Macroplots may be chosen based on forest type or other large-scale gradients, while microplots within a given macroplot are assumed to have homogeneous environmental conditions. If the research is designed to determine the distribution of indicator species, the macroplot method is better and sampling effort should be directed in this way. If the research is designed to monitor specific lichen communities, microplots should be used in combination with macroplots. Again, it is important that the scale of abundance estimates, or the size of sub-plots, is standardized if results are to be compared among studies.

Although ecological monitoring has a long history in Canada (Vaughn et al. 2001), lichen biomonitoring protocols have not been widely applied in this country. Ironically, the IAP approach, which was pioneered in Canada (LeBlanc and De Sloover, 1970), has been extensively applied in Europe although relatively little research on this topic has been done here. The Environmental Monitoring and Assessment Network (EMAN) was a Canadian national initiative that was a co-operation between academic, governmental, and private sector scientists across Canada, established in 1994 by Environment Canada (Vaughan et al. 2001). One of the major strengths of this program was their ability to standardize a variety of monitoring programs across Canada (Vaughan et al. 2001). To help EMAN create standardized protocols for lichen biomonitoring, a group of lichenologists got together in 2003 to create species lists that could be

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used to create a protocol for some of the major ecosystems in Canada (McCarthy et al. 2009). This was the first attempt at a nationally standardized lichen monitoring program in Canada., government funding for EMAN has been removed and the network is now "dead" (Daniel McCarthy, personal communication), although information on the developed protocols is still available.

The EMAN lichen monitoring method has been applied in quite a few cities in Ontario (Daniel McCarthy, personal communication), with published results for the city of Hamilton (McCarthy et al. 2009). This protocol will be discussed further in the next section. What research has been done in British Columbia on lichen biomonitoring has largely focused on bioaccumulation studies (Enns, 1991; Raymond et al. 2010), and less so on long term monitoring initiatives. Some research has been done on volunteer-oriented lichen biomonitoring programs in the lower mainland (Quinn, 2005). The research proposed herein aims to establish long-term monitoring protocols in British Columbia. Firstly, air pollution monitoring protocols will be established for the Prince George airshed. Secondly, climate monitoring protocols will be developed for the inland temperate rainforest of central BC.

#### Lichen Biomonitoring Methods.

Monitoring lichens to assess atmospheric pollution has a very long and complex history (see Nimis et al. 2002 for review). Mapping zones of lichen distribution and assigning a scale to assess the winter SO2 deposition of a site based on the lichen flora present gained popularity in the 1970s (Hawksworth, 2002; Hawksworth and Rose, 1970) and was adapted for application in many European countries (van Haluwyn and van Herk, 2002). However, scales designed to

assess SO2 may no longer be accurate indicators of current atmospheric pollution, as declining SO2 levels in many regions have revealed the effects of multiple other drivers on lichen communities. In particular, nitrogen deposition in urban and agricultural settings has been shown to strongly influence lichen communities (Bates et al. 2001; Wolseley et al. 2006; van Herk, 1999). A variety of complications can occur when trying to estimate current SO2 levels based on lichen communities. Species which are able to colonize a region with decreasing SO2 concentrations may out-compete other species which would normally grow once conditions improve (Seaward, 1992). In addition, formerly high levels of SO2 may cause acidity to persist in certain environments (Bates et al. 2001), preventing sensitive macrolichens from recolonizing these areas for many years after SO2 levels have dropped to acceptable levels (Seaward, 1992). Recolonization may also be delayed because lichen propagules are more sensitive to acidity than mature thalli (van Haluwyn and van Herk, 2002).

The ecological range of lichens may be expressed using an ecological indicator value, which describe factors that determine the range of a species such as pH of substrate, solar irridation, pollution, and so on (Nimis and Martellos, 2001). Ecological indicator values may be calculated based on single species, communities, or groups of species with similar ecological requirements (van Haluwyn and van Herk, 2002). The Index of Atmospheric Purity (IAP) has historically been a popular index applied to lichens for monitoring atmospheric pollution. It was first proposed by LeBlanc and De Sloover (1970), and has been modified multiple times (see Kricke and Loppi, 2002 for review). However, the principle has remained similar: an index is assigned to lichen species based on their sensitivity to environmental conditions, air pollution in particular (Kricke and Loppi, 2002). The IAP approach uses the number of co-occurring species

in order to calculate the sensitivity of a particular species (e.g. Gombert et al. 2005; McCarthy et al. 2009) – the Q parameter, aka the ecological index, which may also be considered the "sociability index" (Gombert et al. 2005). Species which are sensitive to pollution will occur only in more diverse communities, and therefore higher Q values will indicate greater sensitivities. The IAP has also been modified to replace species sensitivities with cover estimates (Kricke and Loppi, 2002). Presence/absence data on lichens may also be used to calculate IAPs (Quinn, 2005), which may be effective for standardizing data collection by volunteers in citizen science monitoring programs where cover estimates could represent a source of error.

Although the IAP method for estimating air quality based on lichen diversity has been applied extensively and remains very popular, recent research suggests that it is not an effective indicator of air quality. For example, Gombert et al. (2004) found that a qualitative Index of Human Impact (IHI), which was based on a variety of factors such as proximity to roads, agriculture, and so on, was more effective at predicting lichen diversity than was air quality. It seems that habitat quality is as or more important than air quality in urban places. This suggests that habitat quality may have more important impacts than atmospheric pollution in some instances, and highlights the importance of standardization of habitat in IAP sampling protocols wherever possible.

Large-scale standardization efforts for lichen biomonitoring of pollution have been made in the US, Europe, and Canada. Widespread lichen biomonitoring protocols in the US have been implemented since 1994 by the Forest Service. The Forest Inventory and Analysis (FIA) National Program is part of a continuous census program designed to assess the nation's forest health (see http://fia.fs.fed.us/lichen/). The lichen portion of the FIA uses lichen community

indicators to assess air quality, climatic variables, and structural changes in the forest community. Lichen communities in FIA plots are evaluated through the collection of specimens by field technicians, who collect samples of every epiphytic macrolichen in the plot and send them to professional lichenologists for identification. Specimens are collected from living or dead standing wood a minimum of 0.5 m from the ground. Technicians are not expected to identify species, although they must distinguish between different species, to each of which they assign an abundance estimate of 1 - 4. The plot consists of a circular area of approximately 0.38 hectares, and one plot exists for every 96000 acres in the US. Information about canopy cover, gaps in the canopy, vegetation types, dominant tree size classes, disturbance history and tall shrub cover is also recorded (protocol is available at <u>http://www.fia.fs.fed.us/library/field-guidesmethods-proc/</u>). Although this method of lichen sampling has been widely applied in the US as part of the FIA program, it is possible that the large size of the plots create conditions which are too variable to accurately monitor changes over time (Trevor Goward, personal communication).

Attempts to standardize lichen biomonitoring protocols across Europe have also been made (Asta et al. 2002). In the Asta et al. (2002) protocol, lichens are surveyed in quadrants on the trunks of suitable trees. Four "sampling ladders" are attached to the trunk, 1.5 m from the ground, at the cardinal points. Each sampling ladder consists of five 10 cm x 10 cm quadrats arranged vertically. Lichen species within the quadrats are identified based on keys, or sampled if unknown, and their frequencies recorded. A Lichen Diversity Value is then calculated as a statistical estimator of environmental conditions at that sampling unit. Trees are selected based on their position within the area to be sampled, using a grid or transect system. This protocol, however, does not take into account the fact that established lichen thalli on trunks may persist

for a long time when conditions are no longer suitable for establishment. Recent research has suggested that lichen communities on trunks may not be reflecting current atmospheric conditions (Wolseley et al. 2006). Lichen communities on twigs, which represent a renewed substratum, are likely more related to current atmospheric conditions (Trevor Goward, personal communication).

The Ecological Monitoring and Assessment Network (EMAN) was a Canadian national monitoring network whose mission was to "focus a scientifically-sound, policy-relevant ecosystem monitoring and research network" (Vaughan et al. 2001). EMAN developed a standardized protocol for mapping IAPs in Canada (McCarthy and Vaughan, 2004). This protocol uses a suite of 15-20 epiphytic lichens which range from tolerant to intolerant of air pollution. Species lists have been developed by expert lichenologists for mixed hardwood forests (Brodo and Craig, 2004b), boreal forests (Brodo and Craig, 2004a), and west coast forests (Brodo et al. 2004). The presence/absence of these species is monitored, and diversity, photometric, and contaminant analysis may also be used (McCarthy and Vaughan, 2004). The EMAN lichen biomonitoring protocol has been implemented in the area around the city of Hamilton, Ontario (McCarthy et al. 2009), where IAP values and maps have been created based on epiphytic lichens on Maple. Cover estimates were made of 18 epiphytic macrolichens, from 30 cm to 1.5 m in height. Sample sites were spaced 1.5 - 3 km apart, and 4 - 5 trees were sampled at each site. Frequency values were calculated for all of the species in order to determine IAPs, which were then input into GIS software to create countour maps (McCarthy and Vaughan, 2004). Mesh bags of lichen samples were also hung throughout the city for approximately 5 months, in three transects (McCarthy et al. 2009), and analyzed for heavy

metals such as lead and zinc. The survey and sampling of lichens took place with the help of eight undergraduate Science students that had received 8 h of training on lichen identification. Overall, the researchers found the IAP mapping and elemental analysis to be complementary, and recommended both approaches for standardized studies elsewhere in Canada (McCarthy et al. 2009). The EMAN protocol and calculation of IAPs has also been applied in the lower mainland (Quinn, 2005). The species list used was slightly modified based on field surveys and expert opinion (Quinn, 2005). Volunteers were used to collect data on the frequency of lichen epiphytes on the trunks of Prunus and Acer species. A grid system was used to select potential sampling sites across the study area (a total area of 800 km2) every 3 km, although final site and sample tree selection was done by the volunteers. At each site, volunteers surveyed four trees. The calculated IAPs showed various differences in lichen diversity (and thus potentially, air quality) between different municipalities in the lower mainland (Quinn, 2005). Prior to the EMAN standardized protocol, IAPs were also calculated in Sudbury (Richardson, 1992), Montreal (DeSloover and Leblanc, 1970), and Winnipeg (Stringer and Stringer, 1974). Thormann (2006) has compiled a table of lichen species that have been used in pollution monitoring for different regions across Canada, based on published information regarding their sensitivity to different pollutants and their ranges across Canada. Of the species whose habitat requirements are not highly restricted, Thormann (2006) lists 24 species which may be used as indicators of general pollution (another 25 whose ranges are restricted) and 27 lichens which may be used as indicators of sulphur dioxide in particular (another 12 that have small ranges).

#### Air Pollution and the Prince George Airshed.

The city of Prince George has numerous different sources of air pollution, including three pulp mills and an oil refinery. In addition, geographical factors often compound the impacts of these emitters, for example, temperature inversions in the river valley (MOE, 2010). The primary issues of air quality in Prince George are related to particulate matter and sulphur compounds (Chess et al. 2008). In 1998, the Prince George City Council and the Fraser-Fort George Regional District Board approved the "Prince George Air Quality Management Plan" (PGAQMP), Phase 1, which was motivated by the observations that poor air quality was significantly impacting the residents of Prince George (PG Air Quality Implementation Committee, 2004). The main focus of PGAQMP Phase 1 was to reduce fine particulate matter, through a variety of initiatives such as eliminating burning at beehive burners, controlling dust from street sanding, and reducing pulp mill sources of PM (PGAQIC, 2004). In 2004, the implementation committee for this plan, which consists of representatives from government, industry, the public, and the university, released a report outlining the progress that had been made since 1998 (PGAQIC, 2004). This progress report indicated that while there had been significant declines in PM10, PM2.5 had shown no significant declines. The Mayor's Air Quality Improvement Task Force was established in 2006 to examine and make recommendations regarding air quality issues in Prince George. In their final report, the Task Force determined that the pulp mills were the largest source of particulate matter, while the oil refinery was the largest source of sulphur dioxide (Chess et al. 2008).

The levels of seven criteria pollutants are measured at 11 monitoring stations in Prince George, although not every pollutant is measured at any given monitoring station (MOE, 2010).

The pollutants measured include PM10 (particulate matter less than 10 µm in diameter), PM2.5 (particulate matter less than 2.5 µm in diameter), NOX (Nitrous Oxides), O3 (Ozone), CO (Carbon Monoxide), TRS (Total Reduced Sulphur), and SO2 (Ministry of Environment, 2010). Of these, SO2 and TRS are arguably the most important compounds influencing lichen growth in Prince George, and there are six monitoring stations which record these levels in Prince George (MOE, 2010). NOX, which is also important in influencing lichen distributions in urban areas, is monitored at one station (MOE, 2010). Measurements of these pollutants have generally shown a decrease in recent years (MOE, 2010).

There are currently two organizations concerned with the management of air quality in Prince George. The Prince George Air Improvement Roundtable (PGAIR) has been in operation since 1998, and is an "independent organization that provides advice on implementing air quality plans ... affiliated working groups also monitor, manage, and research air quality issues" (http://pgairquality.com/air-quality-information#qa, accessed 28 March 2011). The Peoples' Action Committee for Healthy Air (PACHA) is a citizen group whose mandate is "...effect improvement in air quality in the air shed of Prince George through advocacy, education, research, collaboration and such methods as the society may deem reasonable" (http://www.pachapg.ca/index.php/about, accessed 28 March 2011). Both of these organizations are excellent potential links to the community through which to implement long-term lichen biomonitoring programs in Prince George.

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### SCIENCE – GRADE 7 Prescribed Learning Outcomes and Suggested Achievement Indicators<sup>1</sup>

#### **PROCESSES OF SCIENCE**

	Prescribed Learning Outcomes	Suggested Achievement Indicators
	It is expected that students will:	<ul> <li>The following set of indicators may be used to assess student achievement for each corresponding Prescribed Learning Outcome.</li> <li>Students who have fully met the Prescribed Learning Outcome are able to:</li> </ul>
•	evaluate human impacts on local ecosystems	determine the sources of pollutants, and analyze their effects (e.g., autos and air quality, oil spills and water contamination)
•	test a hypothesis by planning and conducting an experiment that controls for two or more variables	<ul> <li>supply relevant supporting evidence for hypotheses presented</li> <li>develop a testable question that considers the variables involved based on previous inferences</li> <li>communicate precisely the question under observation so others can review the plan and procedures</li> <li>question the relevance of the hypothesis by checking the control and the accuracy of the testing methods (fair test)</li> <li>communicate the results of an experiment, using graphs and charts</li> </ul>
•	create models that help to explain scientific concepts and hypotheses	<ul> <li>observe a problem situation, and formulate a plan for investigating a solution</li> <li>plan in detail all of the steps necessary to build or make a product, and prepare a written outline showing the order of events</li> <li>identify key components of the system or process being modeled.</li> <li>develop a testable question that considers the variables involved (independent and dependent)</li> <li>build a relevant and appropriate model based on the available materials and constraints of the problem</li> <li>apply all appropriate safety measures when building a</li> </ul>

<sup>&</sup>lt;sup>1</sup> Prescribed learning outcomes from the B.C. Ministry of Education. 2010. *Grade 7 Curriculum Package <u>http://www.bced.gov.bc.ca/irp/gc.php?lang=en</u></sub> (26 March 2013)* 

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Prescribed Learning Outcomes	Suggested Achievement Indicators
	model

#### What is a lichen?

Lichens are an organism that is composed of a fungus and an algae growing together in a symbiotic relationship, where the two organisms growing together rely on each other for their survival. The lichen thallus includes a fungal partner (the mycobiont) and one or more algal and/or cyanobacterial partners (the photobionts). The fungal partner builds the structure that makes up most of the lichen. In a foliose or leaf-like lichen this consists of two layers of fungal cells; the top cortex layer is made up of very tightly



packed fungal cells and the lower medullary layer is made up of a loosely woven layer of fungal cells. In between these two layers is the algal layer. This thin layer of algal cells provides the food the feeds feeds the fungus from photosynthesis.

#### Lichens as ecological indicators.

Lichens are very sensitive to the environment that surrounds them and so are often used by scientists as a way of measuring the health of the environment where the lichens live. One indicator that the environment is changing is when we find that the types or species of lichens found in an area are changing, as some species are more tolerant of pollution exposure than others. Lichens are also "traps" for environmental contaminants as the contaminants or pollution will gather in lichen tissue. These can be studied by researchers to measure the Ecological indicator – This is a species that is known to be sensitive to pollution, habitat fragmentation or other stresses. The response of indicators is representative for the community. (from Gerhardt 2002)

amount and type of pollution or contaminant that is present in the environment. Indicators such as these are important to scientists and land managers as they will show measurable changes in abundance and health in response to changing environmental conditions.

The health of lichens is influenced both by their present conditions as well as by the conditions that they faced in the past. One advantage of using indicator species to assess the health of an ecosystem is that you can gain a better understanding of the cumulative impact of changes in pollution exposure. Indicators may be used for measuring a variety of ecosystem characteristics, including the number of species that grows in an area, the level of pollution in an area, and changes in climate. Repeated measurements using indicator species is biological monitoring, a widespread and useful technique for examining environmental change over time.

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The symbiotic relationship between lichen partners (the algae and fungal layers) is complex and instable. This makes these organisms effective biomonitoring tools as they are sensitive to atmospheric conditions. These atmospheric conditions include the air quality which surrounds them. Lichens don't have the root system of higher plants, and have therefore adapted to absorb nutrients directly from the atmosphere. This means that pollutants accumulate in their thalli, making them effective indicators of pollution as they generally show sensitivity to these compounds.

#### **Prince George Air Pollution.**

The city of Prince George has numerous different sources of air pollution, including pulp mills and an oil refinery. Prince George is located in a major river valley which sometimes traps released pollutants. This serves to create much higher air pollution levels than would otherwise be the case.

One of the major pollution releases into the air within the Prince George area is sulfur dioxide (SO<sub>2</sub>). The total SO<sub>2</sub> release in Prince George has fallen from a high of over 7000 tons in 1995, and now varies around 3000 tons per year. This improved lower yearly emission is a result of



Air pollution emissions within the Prince George air shed are often trapped near the ground during the late winter.

the installation of a sulphur recovery unit at the Husky oil refinery in 1997 and improvements made at the pulp mills.

SO<sub>2</sub> exposure can have major impacts on human health.

"Health effects caused by exposure to high levels of SO<sub>2</sub> include breathing problems, respiratory illness, changes in the lung's defences, and worsening respiratory and cardiovascular disease. People with asthma or chronic lung or heart disease are the most sensitive to SO<sub>2</sub>. It also damages trees and crops. SO<sub>2</sub>, along with nitrogen oxides, are the main precursors of acid rain. This contributes to the acidification of lakes and streams, accelerated corrosion of buildings and reduced visibility. SO<sub>2</sub> also causes formation of microscopic acid aerosols, which have serious health implications as well as contributing to climate change."<sup>2</sup>

Monitoring programs for air pollution in the Prince George area are limited to a limited number of locations, where they provide a basis for issuing health advisories during periods of poor air quality. Biomonitoring with lichens therefore provides



<sup>&</sup>lt;sup>2</sup> Ontario Ministry of the Environment. What are the effects of SO<sub>2</sub>? <u>http://www.airqualityontario.com/science/pollutants/sulphur.php</u> (26 March 2013)

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another way in which the distribution and release of sulfur dioxide in the Prince George area can be monitored, especially in areas with no previous air pollution measurements.

### A Lichen Monitoring Protocol for Prince George

The response of lichens to long-term air pollution exposure has been well documented throughout much of Western Europe, where the burning of fossil fuels in the late-18<sup>th</sup> and early 19th century resulted in the widespread loss of pollution sensitive species. Many scientists have used the phrase "lichen desert" to describe the limited lichen flora in major cities in Europe. The decline of sensitive lichen groups has been dramatic. One group, the *Lobarion* lichen community has been found to be especially sensitive, and in Europe is now confined to small areas near the Atlantic coast.

Fortunately, the *Lobarian* lichen group is abundant in natural forests in B.C. The best known example in the Prince George area is the leaf-like lichen known as lungwort (*Lobaria pulmonaria*), a large lichen commonly found growing on trees.

Current research at UNBC indicates that lungwort and other related species are highly sensitive to air pollution exposure in Prince George. The presence or absence



Lungwort is a large foliose (leaf-like) lichen that commonly grows in discrete clumps on trees in central-interior British Columbia.

of lungworts on trunks and branches of large trees is an important sign of whether or not environmental stress from air pollution exposure is present at a site.

#### Sampling Method.

Step 1: Identify trails in mature forests where lichens can be assessed alongside the trail. Ideally, you would find trails both close to and distant from local pollution sources. The trails near local pollution source would be your experimental sites, those distant would be your control sites.



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Step 2: Divide each trail into 30 m sections, with a group of students assigned to each section. These will be your study areas.

Step 3: Using a local tree guide determine what the dominant tree species are alongside the trail. The measurements of lungwort abundance should be separately recorded for coniferous tree species versus deciduous tree species growing along the trail.

Step 4: At each site, temporarily band mature trees of each selected tree type (up to 10 trees per group) at a height of 60 cm up the trunk and again at 120 cm by tying tape or string around the trunk. Trees can be permanently marked where repeated measurements will be made from year after year.

Step 5: For each marked tree record the abundance of lungworts within the marked height zone based on the following scale:

Ranking	Abundance	Notes
0	No clumps on tree	
1	2 or fewer clumps per truck (and associated branches)	
2	3-5 clumps per tree	
3	6 clumps or up to 20% cover	
4	from 21 to 50% cover	
5	50% cover or greater	

In your notes you can also record how healthy the lungwort clumps look. Are they bright green when wet (you can spray them with a water bottle to check) or are they blackened and disintegrating?

Step 6: From each site collect short lengths of dead branches that still have bark on them from 3 coniferous and 3 deciduous trees. These should be small branches (less than 1 cm in diameter) and about 5 cm long. Place these in paper lunch bags for transport back to the classroom

Step 7: Make sure that the site is cleaned up and that all temporary markers are removed from the trees. Once back in the classroom place the small branch samples from each site in a shallow plastic or glass container. Cover the branch sample with just enough distilled water to cover the branch. After 10 minutes remove the branch from the water and test the water pH with a pH indicator strip.

Step 8: Plot your data up, comparing average abundance values by tree type for each site where assessments were taken. Maybe you see a pattern. What could this mean? Is there any reason to believe a particular pollutant is dispersed in the region? How does it relate to any acid rain data you might locate? Does lungwort abundance show any correlation with bark pH?



#### Things to look for:

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The bark of deciduous trees is usually better at buffering (protecting from) lichens from air pollution than is the bark of coniferous trees. Are lungworts more abundant on your deciduous trees than on the conifers at any of your sites?

If your measurements have been taken on permanently marked trees what trends do you see in the values from year to year? Are these trends the same at all sites? Remember that lichens are also sensitive to weather events, so in a dry year lichen abundance may be lower at all sites.

#### **Supplies needed:**

Tree identification book, paper bags (small lunch-style bags), small branch pruning shears, small plastic or glass containers (ca.  $15 \times 10 \times 10$  cm), distilled water, pH indicator strips



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#### **Resources:**

British Columbia Ministry of the Environment. 2012. 2010 Annual Air Quality Report Prince George Airshed. B.C. Ministry of Environment, Omineca and Peace Regions. Prince George, British Columbia, June 2012.

Available at: http://www.env.gov.bc.ca/epd/regions/omineca/air/annual\_info.htm

This web site provides detailed information about the past and present air pollution release in the Prince George area. Previous year's reports can also be found at this site..

Environment Canada National Pollutant Release Inventory [NPRI] Available at <u>http://www.ec.gc.ca/pdb/websol/querysite/query\_e.cfm</u>

This web site allows you to obtain pollutant release data by year and type of pollution for major cities or regions across Canada. You could, for instance, reconstruct past changes in  $SO_2$  release for Prince George from this site.

Gerhardt, A. 2002. Bioindicator species and their use in monitoring. Environmental Monitoring – Vol. 1. Encyclopedia of Life Support Systems (EOLSS).

This publication provides information on bioindicator species and their use.

MacKinnon, A., J. Pojar, and R. Coupé (eds.). 1992. *Plants of Northern British Columbia*. B.C. Ministry of Forests and Lone Pine Publishing, Vancouver.

This book will provide detailed information about the identity of trees along the trail.

McCune, B., and L.Geiser. 2009. Macrolichens of the Pacific Northwest, 2<sup>nd</sup> Edition. Oregon State University Press.

This illustrated field guide provides detailed information about lichens which may be encountered in the Prince George area.

United States Forest Service, National Lichens & Air Quality Database and Clearinghouse. Air Pollution Sensitivity Ratings for Pacific Northwest Macrolichens. Available at: <a href="http://gis.nacse.org/lichenair/?page=sensitivity">http://gis.nacse.org/lichenair/?page=sensitivity</a>

This web site provides detailed information about the sensitivity of individual lichen species commonly found in western North America to air pollution exposure.

Pojar, R. 2010. Trees and Shrubs in Winter: An Identification Guide for Northern British Columbia. 2010. Creekstone Press. Smithers.

This book helps identify trees if you are working during the fall or winter after leaves have fallen from the trees.

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### Vocabulary:

Algae - any of various chiefly aquatic, eukaryotic, photosynthetic organisms, ranging in size from single-celled forms to the giant kelp. Algae were once considered to be plants but are now classified separately because they lack true roots, stems, leaves, and embryos.

Biomonitoring - describes the processes and activities that need to take place to characterize and monitor the quality of the environment

Cortex - the layer of fungal cells that makes up the upper layer of the lichen thallus

Coniferous - trees with needle-like leaves, typically retained year around

Cyanobacteria - these are bacteria that are capable of photosynthesis, previously called bluegreen algae

Deciduous –trees that shed their leaves during the winter, in Prince George these are mainly trees with broad flat leaves

Eukaryotic - A single-celled or multicellular organism whose cells contain a distinct membranebound nucleus.

Flora – a term collectively referring to plants and organisms that are capable of photosynthesis

Folioselichen – the thallus in a foliose lichen is flat or leaf like

Fungus (plural fungi) - any member of a kingdom of organisms (Fungi) that lack chlorophyll, leaves, true stems, and roots, reproduce by spores, and live as saprotrophs or parasites. The group includes moulds, mildews, rusts, yeasts, and mushrooms

Indicator species - a species that is known to be sensitive to pollution, habitat fragmentation or other stresses

Lobarion – refers to a group of foliose lichens associated with old forests that are highly sensitive to air pollution; these include species of the genera *Lobaria*, *Nephroma*, *Degelia*, *Pseudocyphellaria*, *Sticta*, *Pannaria* and *Leptogium*.

Medulla - the layer of fungal cells that makes up the lower layer of the lichen thallus

Mycobiont – refers to the fungal partner in a lichen symbiosis

Photobiont - refers to the algal or cyanobacterial partner in a lichen symbiosis

Species - a group of living organisms consisting of similar individuals capable of exchanging genes or interbreeding

Symbiosis - interaction between two different organisms living in close physical association, typically to the advantage of both.

Thallus (plural = thalli) - a plant body that is not differentiated into stem and leaves and lacks true roots and a vascular system



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### A guide to the Lobarion group lichens of the Prince George area.

For interested students assessments of the presence or absence of *Lobarion* group lichens can provide an indication of prior air pollution exposure. Several field guides are available to help with this identification. A brief description of these species is provided below.

*Lobaria hallii* - a large foliose thalli, mostly 2- 12 cm across, thallus grey to brownish grey when dry, finely hairly at the tips of the thallus lobes, soredia present in round patches on the upper thallis surface and at thallus margins

For a detailed illustration please see the Ways of Enlichenment Lichen Photogallery: <u>http://www.waysofenlichenment.net/lichens/Lobaria%20hallii</u>

*Lobaria pulmonaria* - a large foliose thalli, mostly 5-15 cm across, thallus greenish to olive or brownish, contains both green algae in uniform layer beneath upper surface and cyanobacteria in cephalodia, has a network of ridges on the upper surface, soredia present on ridges and on margins.

For a detailed illustration please see the Ways of Enlichenment Lichen Photogallery: <u>http://www.waysofenlichenment.net/lichens/Lobaria%20pulmonaria</u>

*Lobaria scrobiculata* - a large foliose thalli, mostly 2 - 10 cm across; upper thallus surface yellowish-tinged pale green when dry; lacking hairs; soredia present in round patches on the upper thallis surface and at thallus margins

For a detailed illustration please see the Ways of Enlichenment Lichen Photogallery:

http://www.waysofenlichenment.net/lichens/Lobaria%20hallii

*Nephroma bellum* - a medium sized foliose lichen, up to 8 cm in diameter; upper surface light to dark brown, lower surface tan to brown; apothecia common on the underside of lobe tips, thallus lacking isidia and marginal lobes.

For a detailed illustration please see the Ways of Enlichenment Lichen Photogallery:

http://www.waysofenlichenment.net/lichens/Nephroma%20bellum

*Nephroma parile-* a medium to large sized foliose lichen, up to 14 cm in diameter; upper surface light to dark brown, lower surface tan to brown; apothecia rare, thallus has soredia on upper surface on edge of lobes.











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For a detailed illustration please see the Ways of Enlichenment Lichen Photogallery: <u>http://www.waysofenlichenment.net/lichens/Nephroma%20parile</u>

*Pseudocyphellaria anomala*- a large foliose thalli, up to 20 cm across, upper thallus brown with a network of ridges, these often covered with soredia; lower surface covered with fine hairs with scattered white spots.

For a detailed illustration please see the Ways of Enlichenment Lichen Photogallery:

http://www.waysofenlichenment.net/lichens/Pseudocyphellaria%20anomala

*Sticta fuliginosa* – a medium sized lichen, up to 10 cm across, upper surface black or dark grey, lower surface covered with light brown hairs and scattered openings

For a detailed illustration please see the Ways of Enlichenment Lichen Photogallery:

http://www.waysofenlichenment.net/lichens/Sticta%20fuliginosa

*Leptogium saturninum* – a medium sized lichen up to 6 cm across, upper surface grey or black, smooth or wrinkled, lower surface densely hairly, apothecia uncommon

For a detailed illustration please see the Ways of Enlichenment Lichen Photogallery:

http://www.waysofenlichenment.net/lichens/Leptogium%20saturninum

Descriptions adapted from Macrolichens of the Pacific Northwest, 2<sup>nd</sup> Edition. Bruce McCune and Linda Geiser. 2009. Oregon State University Press.

Illustrations from the Ways of Enlichenment Lichen Photogallery <a href="http://www.waysofenlichenment.net/lichens/gallery">http://www.waysofenlichenment.net/lichens/gallery</a>







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**Contact:** For further information please contact Darwyn Coxson, University of Northern British Columbia email: <u>darwyn@unbc.ca</u>