

Nature's Sentinels: Lichens as Biomonitors

A guide with case study and methodological insights

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INTRODUCTION

Lichens are cost-effective and powerful tools to evaluate atmospheric deposition. Lichens are symbionts of fungi and algae/cyanobacteria, having evolved unique traits to capture both wet and dry deposition for their use as nutrients (Bargagli and Mikhailova 2002; Nash 2008). These traits allow them to reach an equilibrium with their environment, forming a representative fingerprint of their inputs, either natural or anthropogenic (Loppi and Paoli 2015). This article covers deposition and how it influences lichen elemental concentrations, followed by an introduction to the workflow and important considerations from design, field, and laboratory work through to analysis. Salient points are illustrated with novel data from multiple experiments and a case study from Sudbury, Ontario, Canada. The goal is to tackle the leading barriers to wider adoption of biomonitoring: the lack of understanding surrounding the utility and methodology (Blett et al. 2003).

The key to the ability of lichen to reflect atmospheric deposition is their lack of roots (Sloof and Wolterbeek 1993). Without root uptake, lichens rely on atmospheric deposition to provide nutrients, having developed traits such as a lack of protective cuticles and porous surfaces to promote aerosol capture (Wolterbeek et al. 2003). As they accumulate nutrients, lichens also absorb significant levels of metals and potentially toxic elements (PTEs) (Bargagli et al. 2002). The slow growth rates, lack of seasonal senescence, and common abundance have led lichens to be exploited for various aims, serving as powerful screening tools to identify pollution sources, to describe spatial distributions from contamination sources, and to monitor air quality dynamics across hundreds of studies (Bargagli and Mikhailova 2002; Blett et al. 2003). Lichen studies can help in the identification of previously unrecognized sources of pollution, as well as environmental anomalies and "hot spots", which have, in some cases, been related to previously unknown bedrock mineralization (Richardson 1992; Purvis et al. 2013).

The Sudbury Transect study referenced throughout this article was conducted in Sudbury, Ontario, in central Canada, following a 130 km transect northward from the city limits to track deposition from the city's two active Ni-Cu smelter operations (Lakanen et al. *in prep.*). Four biomonitors (rock, soil, and tree lichen, and moss) were collected, together with snow and soil samples, at twenty-one sites in the fall and winter of 2022/2023 (Fig. 1). This work follows the path of a similar transect conducted fifty years earlier (Tomassini et al. 1976), but highlights the significant changes in deposition patterns following decreased smelter emissions to meet the changing Clean Air Regulations (Beckett 1995; SARA Group 2008).

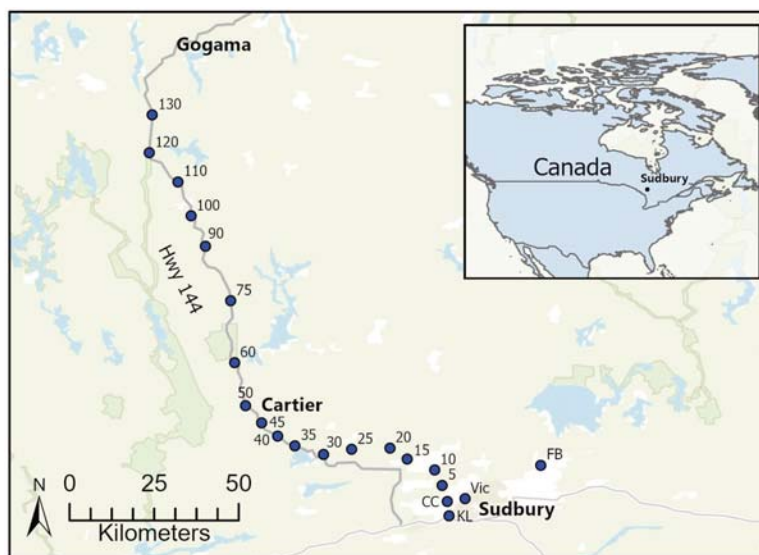


Fig. 1. Location map for the Sudbury Transect, which includes 21 sites spanning 130 km in a northwest direction from Sudbury, Ontario, Canada. Sites are spaced at regular intervals as well as multiple sites (CC, KL, Vic, FB) near the two operational smelters (smelters are located at sites CC and FB). Numeric site codes represent the distance (in km) from Sudbury.

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AERIAL ELEMENTAL TRANSPORT AND ACCUMULATION BY LICHENS

Lichens can absorb elements from the environment by three mechanisms: particulate capture, extracellular binding, and intracellular uptake (Garty 2001). Particulate matter (PM) capture is commonly attributed as a major source of elements in lichen (Nieboer et al. 1978; Mamun et al. 2020). The commonly sampled size fractions of particulates, (i) fine (<2.5 μm) and (ii) coarse (>2.5 μm), have very different characteristics, elemental composition, and dispersal patterns (Mamun et al. 2020). Fine particulates are commonly generated from anthropogenic activities where combustion and heat volatilize elements that condense around nucleation points, producing particulates that are commonly high in PTEs like lead, arsenic, and cadmium (Graney et al. 2019; Mamun et al. 2020). Fine particulates can travel hundreds to thousands of kilometers and contribute to the global elemental background (Graney et al. 2019). In contrast, coarse PM is generally geogenic in origin, produced through erosion processes, and is high in lithophile elements such as iron, aluminum, and silicon (Mamun et al. 2020). Coarse PM's larger size leads to more heterogeneous dispersal patterns, being prone to more regionalized, much shorter distance travel and sedimentation processes active over minutes to hours (Garty 2000; U.S. EPA 2004). However, dispersal is highly dependent on weather patterns and emission characteristics (U.S. EPA 2004).

Different lichens may also have different capture rates depending on the particle size (Puckett 1988). Solutes captured by lichens are more bioavailable and represent a large portion of the total elemental concentration (Branquinho 2001). Exchange processes are passive, rapid, and highly

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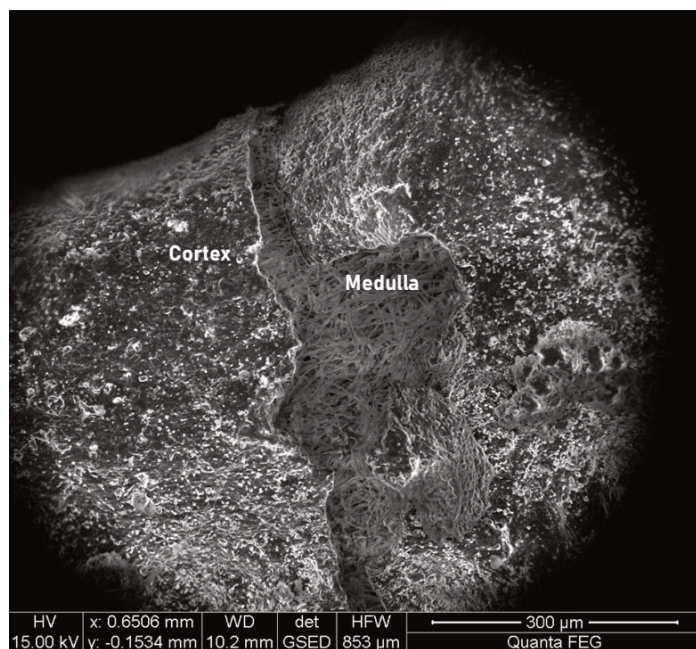


Fig. 2. Secondary backscattered electron image of the surface (cortex) of *Evernia mesomorpha* with a crack showing the network of thread-like hyphae that make up the interior (medulla) where particulates have been shown to accumulate (photograph by M. Lakanen 2024).

effective but have been shown to saturate in high-deposition environments (Bargagli and Mikhailova 2002). Intracellular uptake is, in most cases, an active process that has been shown to occur for nutrients, metals, and anions, including phosphate, arsenate, and uranyl (uranium) (Bargagli and Mikhailova 2002). Toxic effects are generally related to this intracellular fraction of elements rather than total concentrations (Branquinho 2001). Complexation is thought to be an important method of toxicity avoidance, with various organic crystals within lichens containing chelated metals (Purvis et al. 1987).

The measured concentrations in lichens are a function of both accumulation and the release of captured elements (Nieboer et al. 1978). Particulates, resistant to washout from precipitation, have been observed deep within the medulla of the lichen (Fig. 2) and are expected to decompose over time by the action of organic acids, metal-complexing agents, and mechanical disintegration caused by wet/dry cycles (Nieboer et al. 1978; Garty et al. 1979). The retention rates of elements, although originally thought to reflect the age of the lichen (some lichens are thousands of years old), have been shown to range from two to seven years, depending on the element and its chemical form (LeRoy and Koksoy 1962; Nieboer et al. 1978; Richardson 1992). These relatively short time scales have been determined through comparisons of samples collected before and after the cessation of pollutant

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sources, transplantation studies, and radiotracer experiments (Nieboer et al. 1978; Deruelle 1984; Walther et al. 1990).

The accumulation of elements from substrata is controversial. Rock-dwelling lichens are well known to slowly dissolve their substrate, and copper-organic crystals have been observed growing on lichens colonizing copper-bearing ores (Nieboer et al. 1978; Purvis et al. 1987). However, some studies suggest the substrate of lichens growing on less enriched rocks are not a significant source of elemental loads (Chiarenzelli et al. 1997). The observed correlation between lichen and substrate has been suggested to be from absorption from solubilized elements from the water interface during precipitation and/or collected particulates from the substrate itself (Richardson et al. 1980).

STUDY DESIGN: LICHEN SPECIES, QUANTITY AND QUALITY?

Several aspects need to be considered in the design of a lichen biomonitoring study. This section explores the impacts of study scale, the practical aspects influencing the selection of a suitable lichen species, and the need for standard operating procedures (SOPs) and replication.

Biomonitoring studies are generally structured as either linear transects extending from a source or an areal mapping survey across a study region. In either case, the key factor is matching sampling density to the suspected deposition gradient (Ferretti and Erhardt 2002). Sites should be selected strategically to meet financial, analytical and statistical constraints, and 'convenience sampling' should be avoided to reduce bias (Jackson et al. 1993).

A nested grid approach is commonly recommended to accurately describe gradients from a source, with increased sampling density nearer to the presumed source and fewer distal sites to quantify the 'local background' free of anthropogenic contamination (Ferretti and Erhardt 2002; Landis et al. 2019). The sampling distance and gradient can be informed by previous studies (e.g. the Sudbury Transect benefited from the data collected fifty years prior), or a preliminary/pilot study may be required. The number of background sites to be included should be sufficient to provide a reliable mean and standard deviation, as biomonitoring relies on a comparative approach. Due to the sensitivity of lichens to atmospheric deposition, it is vital to confirm that 'background sites' are representative of the region's natural environment and free from local anthropogenic sources such as power lines, highways, railways, road dust, agriculture, or historic soil contamination which may be resuspended by wind, animals, or foot traffic.

The selection of the lichen species determines where and how many specimens can be sampled. The local presence and distribution of the target species across the study region vitally impact site selection. Ideally, the target lichen is found at both the 'contaminated' sites and 'natural' sites. This can be difficult in practice, as the two environments can be very different ecological niches. For example, in the Sudbury Transect, the most proximal sites to the smelters only had *Stereocaulon*, which is tolerant and grows well on the eroded rocky terrain, but was difficult to find in the forested northern reaches of the transect. Figure 3 shows a large mat of *Stereocaulon* growing on an exposed cliff, a habitat we had to target to find northern specimens. Tolerance to pollution is an important consideration. In the field, the major contributors to the decline of lichen populations are gaseous pollutants (sulphur dioxide) and pH, with toxic effects of metals being rarer and typically involving zinc, copper, or lead

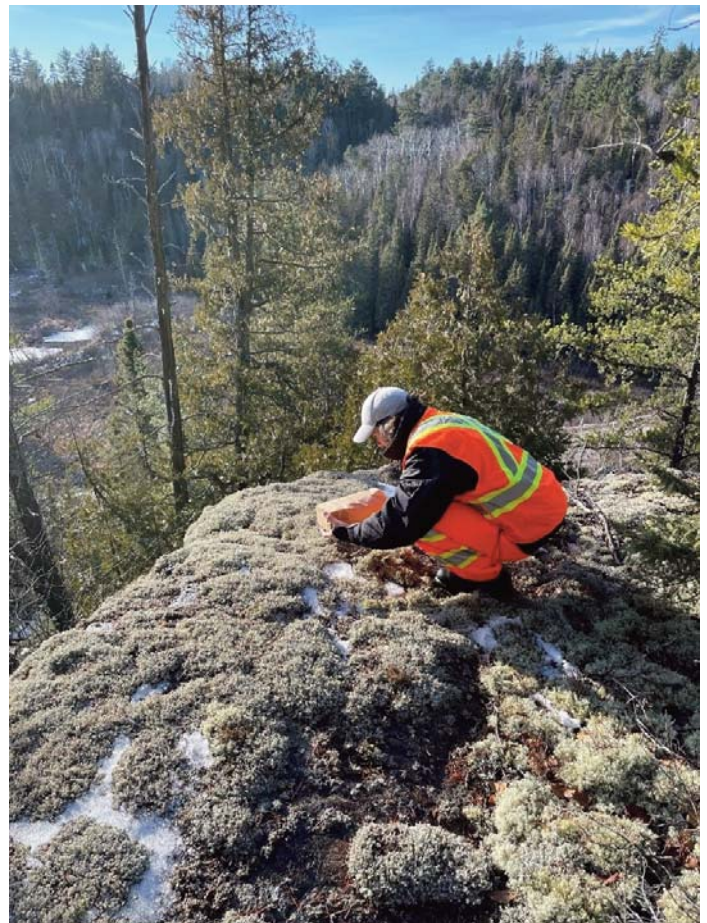


Fig. 3. Photograph of the collection of *Stereocaulon* spp. samples growing in a dense mat on a rocky outcrop north of Sudbury, Ontario, Canada (photograph by J. Anderson 2022).

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(Richardson 1992; Bargagli and Mikhailova 2002). Combining multiple/complimentary species can extend the range, however, it also increases the cost and labour of the study. Methods to compare across multiple species are available, such as the Bioaccumulation Scale, discussed in more detail in a later section (Cecconi et al. 2019b).

Other factors for determining the targeted lichen species include the field crew's ability to identify the correct species, analyte concentration in the organisms, minimizing influence from the soil/substrate (the reason epiphytes are typically targeted), and the ease of harvesting and processing (Will-Wolf et al. 2020). Ideally, a single foliose (leaf-like) or fruticose (coral-like) lichen species with a history of use as a biomonitor is selected. Examples include *Evernia mesomorpha*, *Hypogymnia physodes*, *Xanthoria parietina*, *Flavoparmelia caperata* (Bargagli and Nimis 2002; Will-Wolf et al. 2017). Figure 4 is a photograph of *Evernia mesomorpha*, the epiphyte targeted for comparison in the Sudbury Transect Study.

Consistency is key to producing valid and repeatable findings. A set of standard operating procedures (SOPs) must outline how each step of the field and laboratory work is performed to minimize systematic error (Taylor 1988). Biological samples are naturally variable, and replication is needed to describe this variance and determine real differences between conditions (Bourke et al. 1988). Replication also helps confirm, or provides a rationale to dismiss, data outliers such as unexpected spikes that could result from previously unrecognized sources or contamination from bird droppings or elsewhere. However, replicating all sites is costly, and the effort must be balanced against budgetary and time constraints. Although specific recommendations are limited, several large-scale surveys have replicated 10–15% of sites to allow estimation of intra-site/sampling variability (Will-Wolf et al. 2017; Landis et al. 2019).

SAMPLING: WHAT TO DO IN THE FIELD

The goal of sampling a site is to obtain a representative sample. The main challenge in achieving this goal is the different small-scale dynamics the lichens are exposed to. The goal of developing a sampling SOP is to minimize these artifacts and, when an appropriate sampling SOP is followed, consistent sampling is achieved regardless of expertise level (Jackson et al. 1993; Will-Wolf et al. 2017). One of the major sources of unrepresentative variance is differences in water exposure. This exposure can include flooding/pooling in low spots when collecting ground lichen or sampling tree lichen in the path of 'stemflow'— water enriched with scavenged elements transported down from the canopy (Bargagli and Mikhailova 2002). Generally, influences are minimized by targeting epiphytes (tree lichen) growing at least one metre above the ground, to within arm's reach, from the same species of tree, and collecting from branches in either a selected or in all compass directions (Bargagli and Nimis 2002). When it is not possible to target tree lichens, it is important to reduce the collection of the substrate along with the lichen to prevent contamination of the sample with soil when in transport back to the lab.



Fig. 4. Photograph of epiphytic lichen *Evernia mesomorpha* (green fruticose lichen), one of three targeted lichen species in the Sudbury Transect, growing among foliose lichen (grey scaly lichen) on a black spruce tree branch (photograph by M. Lakanen 2022).

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When collecting lichens, a composite sample is typically required due to the small size of individual lichen specimens. Composites also help to pool individual variance to produce an 'average sample' across the site (Garner et al. 1988). A general guideline is to collect at least six individual lichen specimens from three different trees/substrates per site to form one composite sample (Bargagli and Nimis 2002). More lichens may be required to produce sufficient mass for chemical analysis—it is suggested to collect enough lichen to produce one gram of processed/cleaned lichen (Will-Wolf et al. 2020). Smaller collected samples are more time-consuming to clean (to prevent unnecessary loss of material) and produce lower quality data (Will-Wolf et al. 2020).

Canopy cover is an interesting consideration, with the general advice being consistency and to sample only from within tree stands for comparability (Will-Wolf et al. 2020). However, the situation can be rather complex, as forests act as filters to capture aerosols which can deposit on lichen as canopy drip (Nickel et al. 2022). Designers of spatial surveys may wish to measure the influence of deposition patterns across a target area, independent of forest canopy density.

Sampling lichens is straightforward: the thallus (lichen body) is removed from the substrate with gloved hands or with plastic/ceramic/Teflon-coated scrapers/knives/forceps. Sampling lichen mats can be simplified and optimized by using a sampling ring (~10 cm in diameter) to 'punch' a hole in the mat, with a plastic spatula being slid underneath for removal (Fig. 5). The majority of substrate should be removed in the field to prevent contamination of the sample during transport. Lichen samples can be collected in various container types, with small lichen samples being folded in metal-free filter paper (Bargagli and Nimis 2002). Care must be taken to ensure the container does not contaminate the sample with the target analytes (e.g. Kraft paper is known to contain sulphur) (Jackson et al. 1993). Breathable containers are suggested as sealed bags can promote rot.

SAMPLE PREPARATION: WHAT TO DO IN THE LAB

Collected lichens should be air-dried quickly after sampling, a process significantly facilitated by using breathable sampling containers (Will-Wolf et al. 2020). Lichen are poikilohydric, meaning they rapidly equilibrate with the moisture in the air (Branquinho 2001). Any sufficiently dry environment should prevent mold growth before processing, but desiccator packs can be used in humid environments (Will-Wolf et al. 2020). Store samples in a dark, dry location at ambient temperature. Freezing samples is not recommended for elemental analysis (Jackson et al. 1993).

Cleaning is a time-intensive step in lichen biomonitoring, with at least half an hour per sample suggested (Will-Wolf et al. 2020). Plastic tweezers, commonly aided by a dissection microscope, are used to remove debris and substrate from the lichen (Will-Wolf et al. 2020). The difficulty of cleaning different lichen species can vary significantly.

The importance of consistency in cleaning cannot be overstated. Only viable (living) lichen should be used for analysis, dismissing tattered or dead/decaying lichen (Bargagli and Nimis 2002). Dead cells show higher exchange capacity



Fig. 5. Photograph of *Cladonia rangiferina*, a fruticose lichen, growing on soil (terricolous) with a sample ring inserted into the mat to facilitate sampling. This lichen is one of three lichen species targeted in the Sudbury Transect (photograph by M. Lakanen 2022).

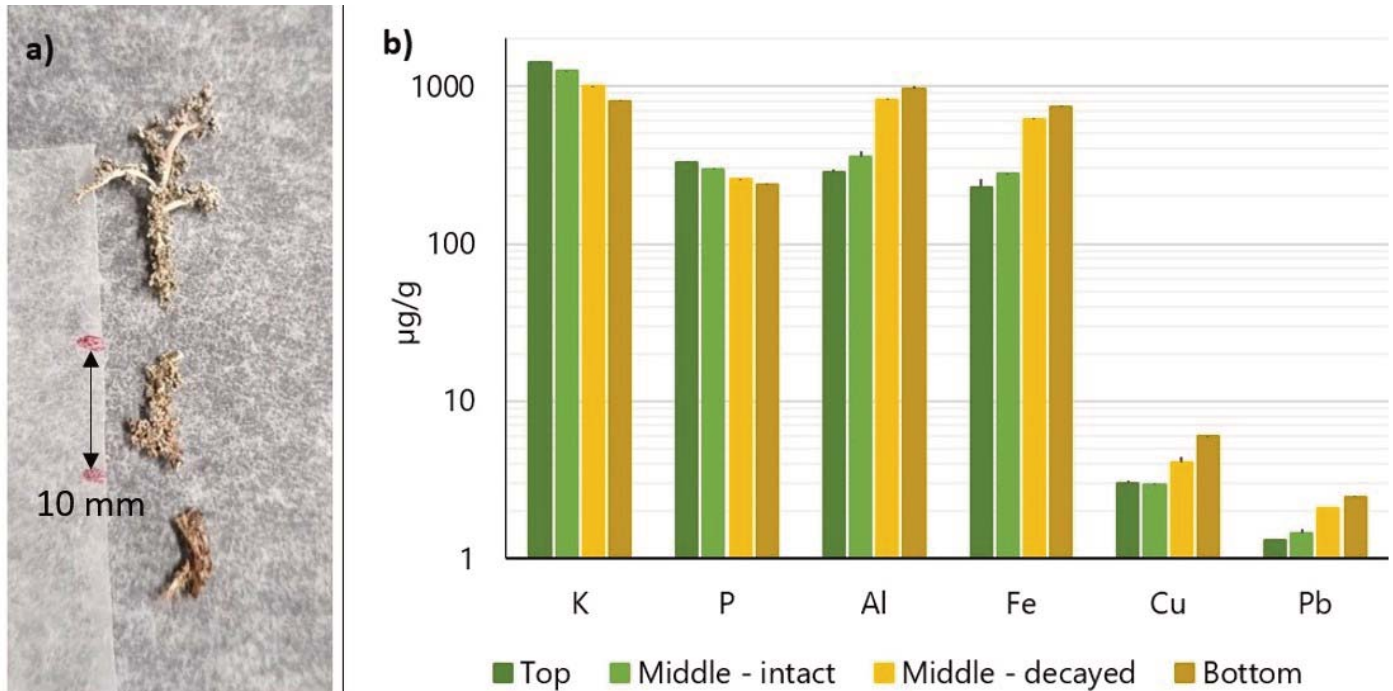


Fig. 6. a) Photograph of a sectioned 'stalk' of *Stereocaulon* sp. from a 'background' site, north of Sudbury, Ontario, Canada (see Fig. 3). **b)** A plot of a selection for elements to compare their concentrations across the gradient from top to bottom of a stalk of *Stereocaulon*. Error bars represent the standard deviation of the two composite duplicates. Nutrient (K and P) concentrations tend to be higher in metabolically active top portions, and lithogenic (Al and Fe) and PTEs (Cu and Pb) accumulate in the lower portions. A consistent selection of visually similar top portions is suggested for analysis.

due to access to intracellular exchange site and cannot accumulate from intracellular transport (Bargagli and Mikhailova 2002). These and other factors can produce internal gradients within lichen that should be minimized by consistent cleaning (Bargagli and Nimis 2002). In practice, achieving this can be rather straightforward, if tedious. Preliminary results using *Stereocaulon* demonstrated that collecting visually homogeneous material is generally sufficient. In the experiment, podetia (individual stalks) were removed from a lichen mat and sectioned into three parts: the top 15 mm, the middle 10 mm, and the remaining bottom section (Fig. 6a). The middle sections were further categorized based on their resemblance to either the top or bottom sections in color, with yellow tones indicating age or decay. Results showed that visually similar tops and middle sections had comparable elemental concentrations (Fig. 6b). The data also aligns with known trends: metabolically active tops contained higher levels of key nutrients (e.g. K, P), whereas decaying parts show higher metal contents, likely due to an increase in exchange sites (Gao et al. 2020).

After cleaning, samples should be dried to a constant mass in a drying oven set just above ambient temperature (40°C) to assist grinding and moisture uniformity for analysis (Jackson et al. 1993). An agate mortar and pestle are sufficient to grind many lichen species; however, more mechanically resistant species may require a mortar chilled in a liquid nitrogen bath or a mill for powdering and homogenization. Care should be taken with the selection of the mill to minimize contamination, cross-contamination, and loss of sample material (Jackson et al.

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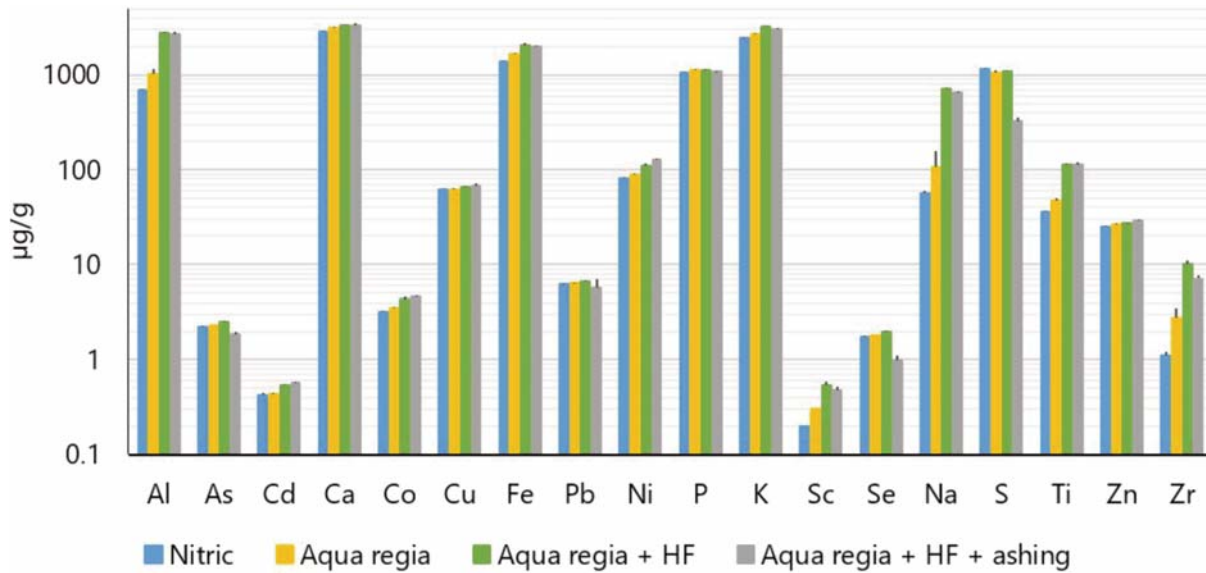


Fig. 7. A comparison of four digestion techniques applied to *Stereocaulon* spp. that was collected from Sudbury, Ontario, Canada. Each digestion was replicated four times (error bars represent standard deviation). Dry ashing reduced volatile element concentrations (As, Pb, Se, S), whereas HF increased the recovery of elements typically found in silicate/oxide minerals (e.g. Al, Sc, Ti, Zr). The digestion method had only a minor influence on nutrients (Ca, P, Zn, K), with the exception of Na. The general consensus is to use a three-acid mix without dry ashing. Acid volumes: nitric acid (9 ml), hydrochloric acid (3 ml), hydrofluoric acid (1.5 ml), heated at 110°C for 2.5 hr after 1.5 hr ramp to temperature in polypropylene disposable digestion tubes in a Hotblock (Questron Technologies Corp.).

1993). Ground samples are best stored in small, sealed vials or Eppendorf tubes, with storage in a desiccator being recommended to prevent absorption of atmospheric humidity before weighing for analysis. Clean, dried, and sealed, the sampled lichens are safe for storage (Jackson et al. 1993).

Depending on the analytical technique, dissolving the sample into a liquid phase may be necessary (Rusu 2002). Partial digestions, such as nitric acid with hydrogen peroxide, may suffice for certain studies (Rusu 2002). However, a 'near-total' digestion using hydrofluoric acid is commonly recommended, particularly for studies involving specific elemental ratios (e.g. Fe:Ti) or intended for use of the Bioaccumulation Scale (Cecconi et al. 2019a). Some commercial laboratories offer these digestions as part of their services. Different digestion methods release different elements, and reporting digestion methods are required for reliable comparison with other studies (Cecconi et al. 2019a). A comparison of four common digestion methods—nitric acid, aqua regia (ratio of 3:1 nitric acid to hydrochloric acid, also known as 'reverse' aqua regia), aqua regia with hydrofluoric acid (HF), and dry ashing (500°C overnight) followed by aqua regia with HF digestion on the ash – is illustrated in Figure 7 using *Stereocaulon* from the Sudbury Transect (Lakanen et al. *in prep.*). Concentrations were measured by ICP-MS. Dry ashing reduced volatile element concentrations (As, Pb, Se, S), whereas HF increased the recovery of elements typically found in silicate/oxide minerals (e.g. Al, Sc, Ti, Zr). The digestion method had only a minor influence on nutrients (Ca, P, Zn, K), except Na. The general consensus is to use a three-acid mix without dry ashing. Acid volumes: nitric acid (9 ml), hydrochloric acid (3 ml), hydrofluoric acid (1.5 ml), heated at 110°C for 2.5 hr after a 1.5 hr ramp to temperature in polypropylene disposable digestion tubes in a Hotblock (Questron Technologies Corp.).

Quality control and assurance (QA/QC) is important in all analyses and many texts are dedicated to the topic. In this example, recoveries were supported with the lichen certified reference material BCR-482 (European Commission's Joint

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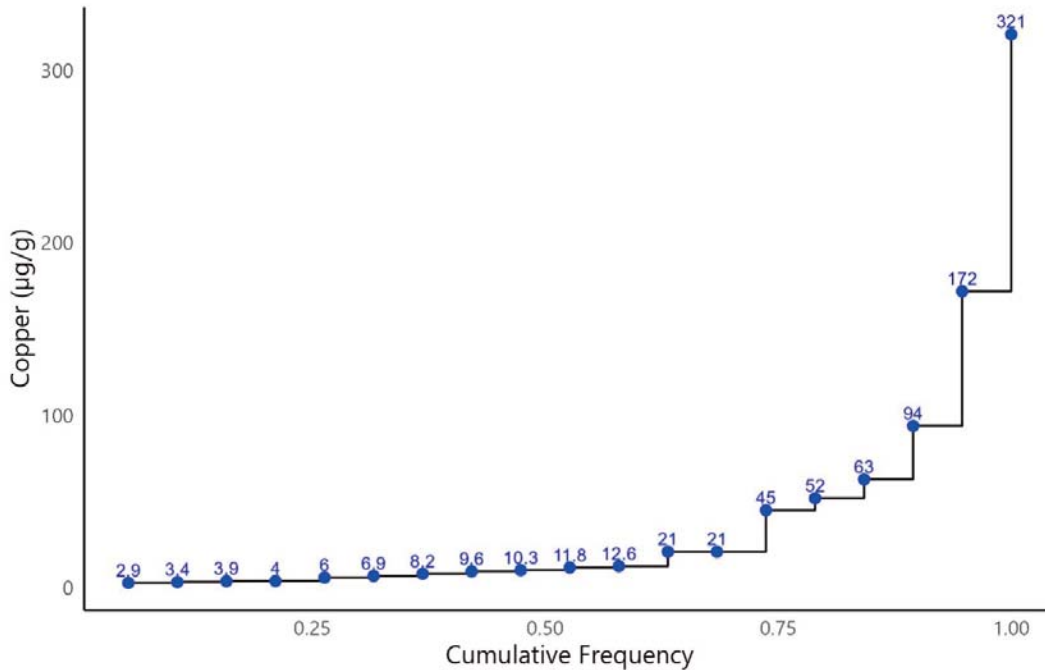


Fig. 8. Cumulative frequency plot of copper concentrations in *Stereocaulon* spp. collected along the Sudbury Transect. Background concentrations range from 2.9 µg/g to 21 µg/g with a mean of 9.4 µg/g and a standard deviation of 6.1 µg/g.

Research Centre) alongside other required QA/QC procedures such as analytical replicates, digestion blanks, and sample spikes. The added benefit of sample replication is quantifying sampling error, which allows for an analysis of variance to determine analytical (from analytical replicates) and sampling variability, confirming that the sensitivity of the analytical technique is adequate for the concentrations seen in the samples (Landis et al. 2019).

DATA INTERPRETATION AND APPLICATION

The choice of data analysis methods is closely tied to the study's objectives. Perhaps the simplest approach is highlighted by the Sudbury Transect, where concentrations are plotted against distance from the source. From this, an exponential decay function can be fitted to model the gradient. Other models may be used depending on normality, with log-transformations commonly employed to linearize the data (Jackson et al. 1993). Background concentrations and other populations can also be identified using cumulative frequency plots (Fleischhauer and Korte 1990), as shown alongside the drop-off for copper in *Stereocaulon* in the Sudbury Transect (Fig. 8, 9).

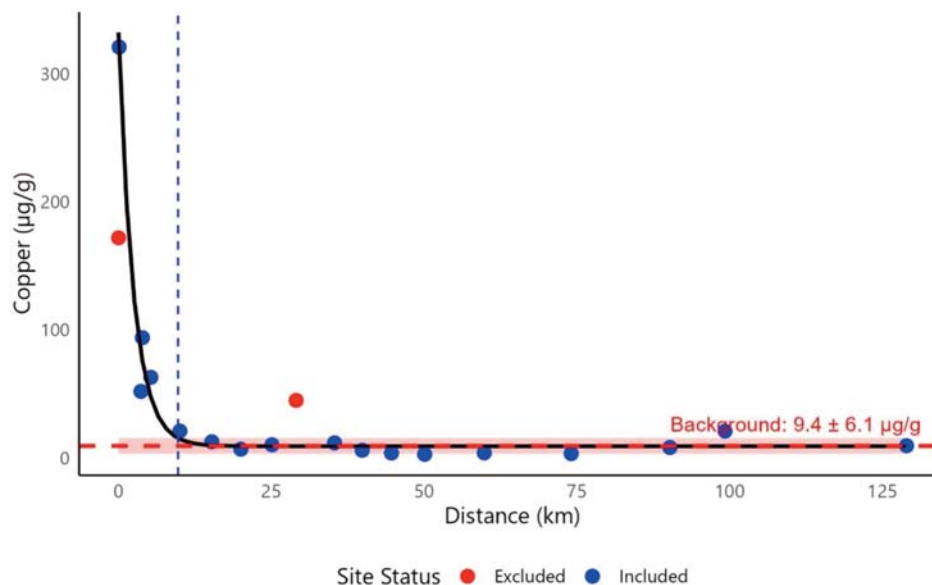


Fig. 9. Exponential decay model fit to copper concentrations in *Stereocaulon* spp. with distance from the city limit. Two points have been excluded: one site not on the transect (proximal to another smelter) and the other site at 30 km, which was contaminated by local sources. The 'distance to background' is 9.7 km, which was determined when the modeled drop-off meets the background calculated from the cumulative frequency plot (see Fig. 8) and is illustrated here by the blue vertical dashed line.

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Interpretive scales have also been developed, such as the Bioaccumulation Scale, which allows for comparison across lichen species and to interpret contamination severity (Cecconi et al. 2019b). The scale aims to provide context to lichen concentrations and their implications on environmental and human health (Cecconi et al. 2019b). From a health perspective, lichen PTE concentrations have been strongly correlated with cancer rates and PTE concentrations in human blood samples (Cislaghi and Nimis 1997; Garty 2001).

Various geostatistical analyses are commonly employed, such as elemental ratios/correlations/cluster analysis, enrichment factors, isotopic signatures, and factor analysis techniques (Nieboer et al. 1978; Puckett 1988; Steinnes et al. 1992; Wolterbeek and Bode 1995; Graney et al. 2019; Anderson et al. 2022). These methods have been used to identify different sources within a sample set and to examine/understand relationships among elements. Elemental ratios of a target element to a lithogenic element, like Al, Si, Ti, Sc, or Zr can help estimate levels of soil deposition across the sites, which might obscure anthropogenic sources (Nieboer et al. 1978).

Mapping using biomonitors has been successfully applied in many studies, ranging from city, regional, to continental scales in the European Moss Survey, which is repeated every five years to investigate transborder emission deposition (moss is another commonly used biomonitor) (Frontasyeva et al. 2020). The resulting maps address limitations in other sampling techniques, such as active air sampling, through sheer sampling numbers. Naturally deployed, lichens offer superior scalability compared to technical methods, although they share some limitations, such as variability caused by microclimate effects (e.g. wind speed/patterns) and stray contamination (Garty 2001; Krug et al. 2017). The ability of lichens to accumulate multiple types of deposition is particularly advantageous, capturing dissolved solutes (and gases) as well as a wide range of particulate sizes via sedimentation, impaction, and interception (Wolterbeek et al. 2003). No single technical method can replicate this capability, although bulk deposition samplers come close (and are correlated with lichen concentrations) but tend to underestimate fine particulates (Branquinho 2001). An issue with lichen surveys is the incompatibility with the measurement units typical of regulatory bodies, although some have proposed 'calibrating' lichen values with deposition meters (Branquinho 2001; Blett et al. 2003). Other challenges include the variable capture efficiency and time frame for retention. An important consideration is that concentrations found in lichens may not show a linear relationship with deposition rates (Mikhailova 2002). Despite these limitations, lichens still excel at identifying changes in elemental deposition patterns due to their high accumulation of PTEs, which allow for contamination/anomalous sites to be many times, if not magnitudes, higher than background concentrations (as seen in Fig. 9 of the Sudbury Transect).

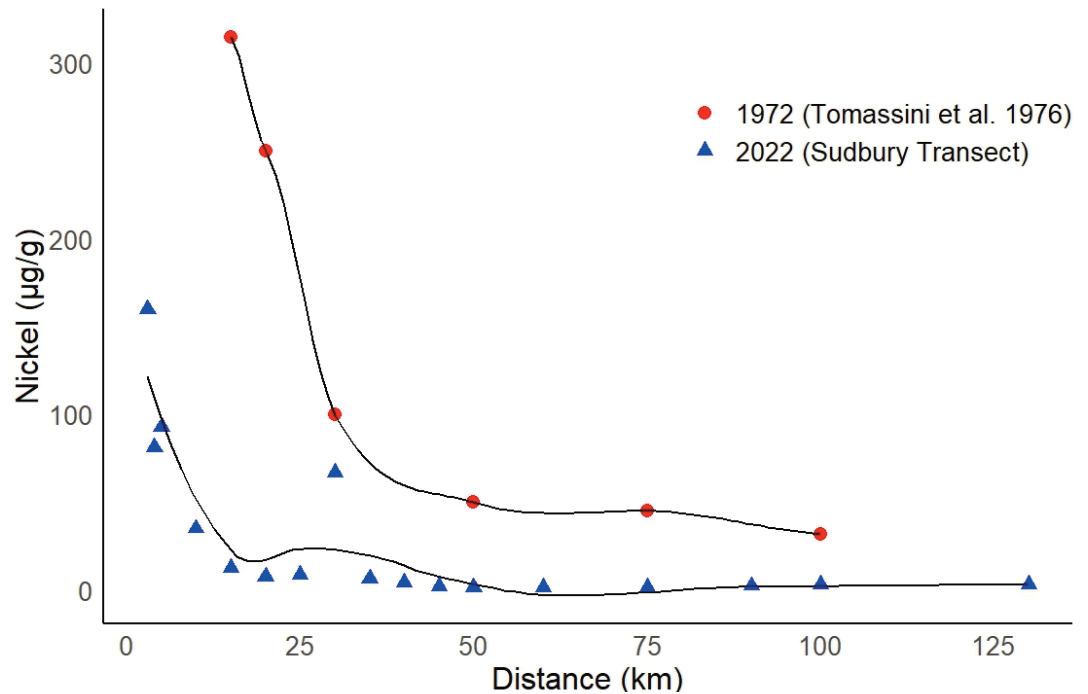
Outside of environmental contexts, there is some promise for using lichen in geochemical exploration.

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Fig. 10. Fifty years of pollution control as measured in lichen. The nickel content of *Stereocaulon* spp. from two studies (with LOESS smooth) conducted fifty years apart following a similar transect from Sudbury, Ontario, Canada. In 2022, lichen was found near the base of the smelter compared to ~15 km from the smelter in 1972. The spike in Ni content at 30 km in 2022 is suspected to be due to local contamination from a railway.



Previously, lichen color and species composition have been used as indicators of specific minerals in their substrate, however, such assessment 'requires a trained eye' (Easton 1994). Furthermore, the ability of lichens to trap and accumulate particulates from weathered geological material suggests their potential for biogeochemical prospecting. Elevated background concentrations around ore deposits have been reported (Dongarrà et al. 1995; Chettri et al. 1997; Garty 2001). A lichen survey in the Northwest Territories, Canada, indicated elevated copper levels in an area where a mineral deposit was later discovered (Richardson 1992). In the Sudbury Transect, an elevation in Mn concentrations in all lichen species, but not in snow (or moss), corresponded to a large spike of Mn in soils. Follow-up XRF analysis of rock samples indicated the presence of several percent Mn at the site, highlighting the ability of lichen to identify local geogenic anomalies. However, without the corresponding increase in soil, the Mn concentrations in lichen, although consistent, would likely have been overlooked as natural variation in the background (60 mg/g at the site compared to a mean of 47 ± 41 mg/g), however the mineral soil had a Mn concentration of 5340 mg/g compared to the background of 373 ± 222 mg/g. The elevated Mn concentrations seen in the lichen are likely derived from soil or weathered rock particulates deposited by wind. In use, if it is reasonable to assume that weathered surface material is a marker of potential ore deposits, then lichen may serve as a relatively cheap and easy screening tool by capturing and accumulating the airborne signals.

Lichens are also effective for monitoring changes over time (Beckett 1995). The Sudbury Transect was previously sampled fifty years ago (Tomassini et al. 1976), revealing significant shifts in metal loads with distance from Sudbury (Fig. 10). Over this fifty-year period, the spatial extent of the smelter operations has decreased from approximately 45 km to 17 km, and background concentration of Ni has fallen

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from approximately 30 µg/g to 3 µg/g as measured in lichen. These reductions highlight substantial progress in clean air initiatives and smelter emission controls by the mineral processing industries (SARA Group 2008).

A COST-EFFECTIVE SURVEY TARGET

Lichens are powerful tools for monitoring the deposition of metals and potentially toxic elements. They commonly accumulate tens to hundreds of times higher concentrations at anomalous sites compared to background levels, making them sensitive monitors (Blett et al. 2003). Lichens are a relatively homogeneous sample type that collect from virtually all air fractions and do not require sophisticated sampling equipment, making them particularly well suited for survey or monitoring studies (Wolterbeek et al. 2003). Their versatility, scalability, and cost-effectiveness have made lichens an excellent choice for environmental surveys, whether assessing human impact or investigating natural anomalies.

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REFERENCES

- Anderson, J., Lévesque, N., Caron, F., Beckett, P.J. and Spiers, G.A. 2022. A review on the use of lichens as a biomonitoring tool for environmental radioactivity. *Journal of Environmental Radioactivity*, 243, 106797. <https://doi.org/10.1016/j.jenvrad.2021.106797>
- Bargagli, R. and Mikhailova, I. 2002. Accumulation of inorganic contaminants. *In: Nimis, P.L., Scheidegger, C. and Wolseley, P.A. (eds), Monitoring with Lichens — Monitoring Lichens*. Springer Netherlands, Dordrecht, 65–84.
- Bargagli, R. and Nimis, P.L. 2002. Guidelines for the use of epiphytic lichens as biomonitors of atmospheric deposition of trace elements. *In: Nimis, P.L., Scheidegger, C. and Wolseley, P.A. (eds), Monitoring with Lichens — Monitoring Lichens*. Springer Netherlands, Dordrecht, 295–299.
- Bargagli, R., Monaci, F., Borghini, F., Bravi, F. and Agnorelli, C. 2002. Mosses and lichens as biomonitors of trace metals. A comparison study on *Hypnum cupressiforme* and *Parmelia caperata* in a former mining district in Italy. *Environmental Pollution*, 116, 279–287. [https://doi.org/10.1016/s0269-7491\(01\)00125-7](https://doi.org/10.1016/s0269-7491(01)00125-7)
- Beckett, P.J. 1995. Lichens: Sensitive indicators of improving air quality. *In: Gunn, J.M. (ed.), Restoration and Recovery of an Industrial Region: Progress in Restoring the Smelter-Damaged Landscape Near Sudbury, Canada*. Springer, New York, NY, 81–91.
- Blett, T., Geiser, L. and Porter, E. 2003. Air pollution-related lichen monitoring in national parks, forests, and refuges: Guidelines for studies intended for regulatory and management purposes. U.S. Department of the Interior, National Park Service, Air Resource Division Report NPS D2292.

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- Bourke, J.B., Spittler, T.D. and Young, S.J. 1988. Sample size: Relation to analytical and quality assurance and quality control requirements. *In: Keith, L.H. (ed.), Principles of Environmental Sampling. American Chemical Society, 355–361.*
- Branquinho, C. 2001. Lichens. *In: Prasad, M.N.V. (ed.), Metals in the Environment: Analysis by Biodiversity. CRC Press, 117–157.*
- Cecconi, E., Incerti, G. et al. 2019a. Background element content in the lichen *Pseudevernia furfuracea*: a comparative analysis of digestion methods. *Environmental Monitoring and Assessment, 191, 260.* <https://doi.org/10.1007/s10661-019-7405-4>
- Cecconi, E., Fortuna, L. et al. 2019b. New interpretative scales for lichen bioaccumulation data: The Italian proposal. *Atmosphere, 10, 136.* <https://doi.org/10.3390/atmos10030136>
- Chettri, M.K., Sawidis, T. and Karataglis, S. 1997. Lichens as a tool for biogeochemical prospecting. *Ecotoxicology and Environmental Safety, 38, 322–335.* <https://doi.org/10.1006/eesa.1997.1596>
- Chiarenzelli, J.R., Aspler, L.B., Ozarko, D.L., Hall, G.E.M., Powis, K.B. and Donaldson, J.A. 1997. Heavy metals in lichens, southern district of Keewatin, Northwest Territories, Canada. *Chemosphere, 35, 1329–1341.* [https://doi.org/10.1016/s0045-6535\(97\)00168-9](https://doi.org/10.1016/s0045-6535(97)00168-9)
- Cislaghi, C. and Nimis, P.L. 1997. Lichens, air pollution and lung cancer. *Nature, 387, 463–464.* <https://doi.org/10.1038/387463a0>
- Deruelle, S. 1984. L'utilisation des lichens pour la detection et l'estimation de la pollution par le plomb. *Bulletin Ecologie, 1–6.*
- Dongarrà, G., Ottomello, D., Sabatino, G. and Triscari, M. 1995. Use of lichens in detecting environmental risk and in geochemical prospecting. *Environmental Geology, 26, 139–146.* <https://doi.org/10.1007/bf00768735>
- Easton, R. 1994. Lichens and rocks: A review. *Geoscience Canada, 21, 59–76.*
- Ferretti, M. and Erhardt, W. 2002. Key issues in designing biomonitoring programmes. *In: Nimis, P.L., Scheidegger, C. and Wolseley, P.A. (eds), Monitoring with Lichens — Monitoring Lichens. Springer Netherlands, Dordrecht, 111–139.*
- Fleischhauer, H.L. and Korte, N. 1990. Formulation of cleanup standards for trace elements with probability plots. *Environmental Management, 14, 95–105.* <https://doi.org/10.1007/bf02394023>
- Frontasyeva, M., Harmens, H., Uzhinskiy, A., Chaligava, O. and participants of the moss survey. 2020. Mosses as biomonitors of air pollution: 2015/2016 survey on heavy metals, nitrogen and POPs in Europe and beyond. Joint Institute for Nuclear Research.
- Gao, J., Wu, Y. et al. 2020. Vertical distribution patterns of element concentrations in podetia of *Cladonia rangiferina* from Huzhong Natural Reserve, Heilongjiang, China. *Polish Journal of Environmental Studies, 30, 103–110.* <https://doi.org/10.15244/pjoes/118424>
- Garner, F.C., Stapanian, M.A. and Williams, L.R. 1988. Composite sampling for environmental monitoring. *In: Keith, L.H. (ed.), Principles of Environmental Sampling. American Chemical Society, 363–374.*
- Garty, J. 2000. Environment and elemental content of lichens. *In: Markert, B. and Friese, K. (eds), Trace Metals in the Environment. Trace Elements — Their Distribution and Effects in the Environment, Elsevier, 245–276.*
- Garty, J. 2001. Biomonitoring atmospheric heavy metals with lichens: Theory and application. *Critical Reviews in Plant Sciences, 20, 309–371.* <https://doi.org/10.1080/20013591099254>
- Garty, J., Galun, M. and Kessel, M. 1979. Localization of heavy metals and other elements accumulated in the lichen thallus. *New Phytologist, 82, 159–168.* <https://doi.org/10.1111/j.1469-8137.1979.tb07571.x>
- Graney, J.R., Edgerton, E.S. and Landis, M.S. 2019. Using Pb isotope ratios of particulate matter and epiphytic lichens from the Athabasca Oil Sands Region in Alberta, Canada to quantify local, regional, and global Pb source contributions. *The Science of the Total Environment, 654, 1293–1304.* <https://doi.org/10.1016/j.scitotenv.2018.11.047>
- Jackson, L., Ford, J. and Schwartzman, D. 1993. Collection and chemical analysis of lichens for biomonitoring Lichens as Bioindicators of Air Quality. Gen. Tech. Rep, USDA Forest Service, Fort Collins, CO, RM-224, 96–115.
- Krug, J.D., Dart, A., Witherspoon, C.L., Gilberry, J., Malloy, Q., Kaushik, S. and Vanderpool, R.W. 2017. Revisiting the size selective performance of EPA's high-volume total suspended particulate matter (Hi-Vol TSP) sampler. *Aerosol Science and Technology, 51, 868–878.* <https://doi.org/10.1080/02786826.2017.1316358>
- Landis, M.S., Berryman, S.D., White, E.M., Graney, J.R., Edgerton, E.S. and Studabaker, W.B. 2019. Use of an epiphytic lichen and a novel geostatistical approach to evaluate spatial and temporal changes in atmospheric deposition in the Athabasca Oil Sands Region, Alberta, Canada. *The Science of the Total Environment, 692, 1005–1021.* <https://doi.org/10.1016/j.scitotenv.2019.07.011>
- LeRoy, L.W. and Koksoy, M. 1962. The lichen; a possible plant medium for mineral exploration. *Economic Geology, 57, 107–111.* <https://doi.org/10.2113/gsecongeo.57.1.107>
- Loppi, S. and Paoli, L. 2015. Comparison of the trace element content in transplants of the lichen *Evernia prunastri* and in bulk atmospheric deposition: a case study from a low polluted environment (C Italy). *Biologia, 70, 460–466.* <https://doi.org/10.1515/biolog-2015-0053>
- Mamun, A.A., Cheng, I., Zhang, L., Dabek-Zlotorzynska, E. and Charland, J.-P. 2020. Overview of size distribution, concentration, and dry deposition of airborne particulate elements measured worldwide. *Environmental Reviews, 28, 77–88.* <https://doi.org/10.1139/er-2019-0035>
- Mikhailova, I. 2002. Transplanted lichens for bioaccumulation studies. *In: Nimis, P.L., Scheidegger, C. and Wolseley, P.A. (eds), Monitoring with Lichens — Monitoring Lichens. Springer Netherlands, Dordrecht, 301–304.*
- Nash, T.H. 2008. Nutrients, elemental accumulation, and mineral cycling. *In: Nash, I.I.I.T.H. (ed.), Lichen Biology. Cambridge University Press, Cambridge, 234–251.*
- Nickel, S., Schröder, W., Völksen, B. and Dreyer, A. 2022. Influence of the canopy drip effect on the accumulation of atmospheric metal and nitrogen deposition in mosses. *Forests, 13, 605.* <https://doi.org/10.3390/f13040605>
- Nieboer, E., Richardson, D.H.S. and Tomassini, F.D. 1978. Mineral uptake and release by lichens: An overview. *The Bryologist, 81, 226.*

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- Puckett, K.J. 1988. Bryophytes and lichens as monitors of metal deposition. *In: Nash iii, T.H. and Wirth, V. (eds), Lichens, Bryophytes and Air Quality.* Schweizerbart Science Publishers, Stuttgart, Germany, 231–267.
- Purvis, O.W., Elix, J.A., Broomhead, J.A. and Jones, G.C. 1987. The occurrence of copper—norstictic acid in lichens from cupriferous substrata. *The Lichenologist*, 19, 193–203. <https://doi.org/10.1017/s0024282987000161>
- Purvis, O.W., Williamson, B.J., Spiro, B., Udachin, V., Mikhailova, I.N. and Dolgoplova, A. 2013. Lichen monitoring as a potential tool in environmental forensics: case study of the Cu smelter and former mining town of Karabash, Russia. *Geological Society, London, Special Publications*, 384, 133-146. <https://doi.org/10.1144/sp384.6>
- Richardson, D.H.S. 1992. *Pollution Monitoring with Lichens.* Richmond Publishing Company.
- Richardson, D.H.S., Beckett, P.J. and Nieboer, E. 1980. Nickel in lichens, bryophytes, fungi and algae. *In: Nriagu, J.O. (ed.), Nickel in the Environment.* Wiley, 367–406.
- Rusu, A.M. 2002. Sample preparation of lichens for elemental analysis. *In: Nimis, P.L., Scheidegger, C. and Wolseley, P.A. (eds), Monitoring with Lichens — Monitoring Lichens.* Springer Netherlands, Dordrecht, 305–309.
- SARA Group. 2008. Historical review of air emissions from the smelting operations Volume I: Background, Study Organization and 2001 Soils Survey. Sudbury Area Risk Assessment.
- Sloof, J.E. and Wolterbeek, B.T. 1993. Substrate influence on epiphytic lichens. *Environmental Monitoring and Assessment*, 25, 225-234, <https://doi.org/10.1007/bf00548023>.
- Steinnes, E., Rambæk, J.P. and Hanssen, J.E. 1992. Large scale multi-element survey of atmospheric deposition using naturally growing moss as biomonitor. *Chemosphere*, 25, 735–752. [https://doi.org/10.1016/0045-6535\(92\)90435-t](https://doi.org/10.1016/0045-6535(92)90435-t)
- Taylor, J.K. 1988. Defining the accuracy, precision, and confidence limits of sample data. *In: Keith, L.H. (ed.), Principles of Environmental Sampling.* American Chemical Society.
- Tomassini, F.D., Puckett, K.J., Nieboer, E., Richardson, D.H.S. and Grace, B. 1976. Determination of copper, iron, nickel, and sulphur by X-ray fluorescence in lichens from the Mackenzie Valley, Northwest Territories, and the Sudbury District, Ontario. *Canadian Journal of Botany*, 54, 1591–1603. <https://doi.org/10.1139/b76-172>
- U.S. EPA. 2004. Air quality criteria for particulate matter (Final Report, 2004). U.S. Environmental Protection Agency Report EPA 600/P-99/002aF-bF
- Walther, D.A., Ramelow, G.J., Beck, J.N., Young, J.C., Callahan, J.D. and Maroon, M.F. 1990. Temporal changes in metal levels of the lichens *Parmotrema praesorediosum* and *Ramalina stenospora*, Southwest Louisiana. *Water, Air, and Soil Pollution*, 53, 189–200. <https://doi.org/10.1007/bf00155003>
- Will-Wolf, S., Jovan, S. and Amacher, M.C. 2017. Lichen elemental content bioindicators for air quality in upper Midwest, USA: A model for large-scale monitoring. *Ecological Indicators*, 78, 253–263, <https://doi.org/10.1016/j.ecolind.2017.03.017>
- Will-Wolf, S., Jovan, S., Amacher, M.C. and Patterson, P.L. 2020. Lichen elemental indicators for air pollution in Eastern United States forests; a pilot study in the upper Midwest. U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station Report PNW-GTR-985.
- Wolterbeek, H.T. and Bode, P. 1995. Strategies in sampling and sample handling in the context of large-scale plant biomonitoring surveys of trace element air pollution. *Science of The Total Environment*, 176, 33–43. [https://doi.org/10.1016/0048-9697\(95\)04828-6](https://doi.org/10.1016/0048-9697(95)04828-6)
- Wolterbeek, H.T., Garty, J., Reis, M.A. and Freitas, M.C. 2003. Biomonitors in use: lichens and metal air pollution. *In: Markert, B.A., Breure, A.M. and Zechmeister, H.G. (eds), Trace Metals and other Contaminants in the Environment. Bioindicators & Biomonitors,* Elsevier, 377–419.

