1 **Determination of elemental baseline using peltigeralean lichens from Northeastern**
2 **Canada (Québec): Initial data collection for long term monitoring of the impact of**
3 **global climate change on boreal and subarctic area in Canada.**

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ABSTRACT:

Northeastern Canada is mostly free of anthropogenic activities. The extent to which this territory has been impacted by anthropogenic atmospheric depositions remains to be studied. The main goal of our study was to establish background levels for metals in boreal muscicolous/terricolous macrolichens over non-urbanized areas of northeastern Canada (Québec). Concentrations of 18 elements (Na, Mg, Al, P, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Cd, and Pb) were determined for three species of the genus *Peltigera* (*P. aphthosa* (L.) Willd. s.l., *P. neopolydactyla* (Gyeln.) Gyeln. s.l., *P. scabrosa* Th. Fr. s.l.), and *Nephroma arcticum* (L.) Torss., along a 1080 km south-north transect and along a of 730 km west-east transect. We report that elemental contents in the sampled lichen thalli are very low and similar to background levels found in other studies performed in pristine places (high elevation or remote ecosystems) throughout the world. Overall, our results demonstrate that most of the boreal and subarctic zone of Québec (northeastern Canada) is still pristine. The elemental baseline established in these lichen populations will contribute to monitor metal pollution in boreal and sub-polar ecosystems due to global climate change and future industrial expansion.

1. Introduction

Climates of the northern hemisphere will undergo significant changes in the next decades. By the end of this century, according to the 2013 International Panel for Climate Change (IPCC) report, the average temperature in boreal and sub-polar regions is expected to rise from 3 to 11°C, the snow and ice cover of the northern hemisphere will be reduced by up to 50 %, and the annual precipitation in high latitudes will increase from 10 to 40% compared to 2005 (Logan et al., 2011; scenarios RCP 2.6 and RCP 8.5 in Working Group I Contribution to the IPCC Fifth Assessment Report 2013). In addition to these environmental concerns, global warming offers new economic opportunities that will likely further impact these natural boreal and arctic habitats. In the next 20 years, the Government of Québec plans to expand economic activities northward as part of the Canadian strategy to increase the exploitation of natural resources in its northern regions (Gouvernement du Québec, 2014). That development would inevitably impact boreal and subarctic ecosystems.

Lichens have been used as biomonitors in countless studies (Nash and Gries 1995; Sloof 1995; Conti and Cecchetti 2001; Garty 2001; Jeran et al. 2002; Nimis et al. 2002; Wolterbeek 2002; Nash 2008c; Seaward 2008); lichens are ubiquitous, slow growing and long living symbioses, able to tolerate high levels of metal contaminants (Seaward, 2007; Nash, 2008c). They are particularly abundant in boreal and arctic regions where they can be the dominant photoautotrophs (Seaward, 2008). While some studies have reported the elemental content of lichens from northwestern Canada (Tomassini and Puckett, 1976; Puckett and Finegan, 1980; Nash and Gries, 1995; Chiarenzelli et al., 2001), Ontario (Tomassini and Puckett, 1976), southern Québec (Evans and Hutchinson, 1996; Aznar et

al., 2008) and Greenland (Riget et al., 2000), data from northeastern Canada are limited to specific metals of health concern in the reindeer food industry, generally Pb, Cd and Hg (Crête et al., 1992).

In the context of a global warming mediated economic development in northern regions, defining background levels for metal contaminants in lichen species from northeastern Canada (Québec) is urgently needed. Our study provides a baseline dataset for future biomonitoring surveys designed to evaluate the impact of human activities in northern Québec and contributes to the overall understanding of biogeochemistry in boreal regions.

In this study, we measured the elemental composition of four species: *Peltigera aphthosa* (L.) Willd. s.l., *P. neopolydactyla* (Gyeln.) Gyeln. s.l., *P. scabrosa* Th. Fr. s.l., and *Nephroma arcticum* (L.) Torss, collected along two transects (1080 km south-north and 730 km east-west) covering a large fraction of northeastern Canada. These lichen-forming fungi from the families Peltigeraceae and Nephromataceae (Peltigerales; Lecanoromycetes; Ascomycota) are associated with cyanobacteria (*Nostoc* spp.) or a combination of *Nostoc* and a unicellular green alga (*Coccomyxa* sp.) and are ubiquitous in boreal and subarctic regions (Vitikainen, 1994; Miadlikowska and Lutzoni, 2000, 2004; Brodo et al., 2001; Martínez et al., 2003).

2. Materials and methods

2.1. Lichen Sampling

The sampling for this study is derived from a sampling designed specifically for a project focusing on fungal endophytes of the boreal biome (endobiodiversity.org).

Additional specimens were collected for a revisionary taxonomic study of the genus *Peltigera* (www.peltigera.lutzonilab.net) and for this study. All lichens were collected in August 2011 in the Province of Québec, along a 1080 km south-north (SN) transect (from SN2, 48°37'58"N 73°2'20"W to SN9, 57°49'36"N 73°11'45"W) and a 730 km east-west (EW) transect (from W50, 50°54'11"N 69°33'56"W to E400, 51°37'5"N 59°9'1"W) (Fig. 1A-B). Because of their abundance and broad distribution in boreal forests, four species were collected along both transects: *Peltigera aphthosa* s.l., *P. neopolydactyla* s.l., *P. scabrosa* s.l., and *Nephroma arcticum*. An ongoing molecular systematic revision of the genus *Peltigera* by Miadlikowska et al. (e.g., 2014) strongly suggest that the focal species contain multiple taxonomic entities, which are likely to be recognized at the species level. Therefore, the current broad species concept (s.l.) is used throughout the text.

We selected our biological material based on their biogeochemical relevance and availability in nature. The peltigeralean lichens selected for this study are living on mosses, which are of particular importance to nitrogen inputs in boreal forest (DeLuca et al., 2002), and are known to fix nitrogen. Therefore, they will provide a better evaluation of the potential impact of atmospheric depositions on the boreal forest biogeochemistry. Moreover, *Peltigera* species were found throughout the boreal belt as part of a study on endophytic and endolichenic fungi of the boreal biome (see endobiodiversity.org) and are present in the arctic, i.e., beyond the tree line. The latter point is important because the choice of this genus and *Nephroma arcticum* enables a sampling for atmospheric deposition beyond what is possible with arboreal lichens.

Nine sampling sites (SN1 – SN9) were equally distributed along the SN transect, approximately one at each 150 km segments (Fig. 1A-B). At each sampling site along the SN transect, individual thalli were collected at three sub-sites, one central sub-site and two sub-sites about 2 km away (in opposite directions) from the central sub-site, for a maximal distance of 4 km from the two most distant sub-sites. Within each sub-site, lichens were collected at three microsites, where two of the three microsites were 30 meter away from the central microsite for a total distance of approximately 60 meters from one terminal microsite to the other. For this study we used lichen thalli collected at sites SN2 to SN9, as no lichen thalli were found at SN1 (black spruce dominated forests of La Mauricie National Park). On the EW transect (Fig. 1A-B), the sites were distributed from a central point (EW0) at a doubling distance toward the west up to about 650 km (400 miles) west of that central point (W1.5, W3, W6, W12.5, W25, W50, W100, W200, W400). For this study we used lichens collected at sites up to W50, inclusively, as not enough lichen thalli were found at W100 and no peltigeralean lichens were found at W200 and W400. No sub-sites were established along the EW transect, however three microsites were sampled as for the SN transect. For the east arm of this transect, only sites E50, E100, E200 and E400 were sampled (Fig. 1A-B). A summary of the lichens sampled for this study can be found in Sup. Info. Table S1. Lichens were air dried, cleaned from soil, plant and other organic debris, and then stored in paper bags at room temperature in the dark before further processing.

2.2. Mineralization and analysis of samples

Elemental analyses were performed on individual thalli. Healthy looking parts of lichen thalli were oven dried (70°C, 24h), weighted (0.5 ± 0.02 g) and inserted in 55 mL

Teflon® vessels with 10 mL of concentrated nitric acid (HNO₃, trace metal grade, Fisherbrand). Digestions were performed with a MARS Xpress Microwave assisted digestion system at 170°C for 1 hour. Digested samples were recovered in 15 mL conical centrifuge tubes and stored at 4°C prior to analysis. Procedural blanks were done for every set of digestions. All elemental quantifications of Na, Mg, Al, P, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Cd and Pb were done on a Thermofisher XSeries II Inductively-Coupled Plasma Mass Spectrometer (ICP-MS) and PlamaLab software v2.6.1.335. Rhodium was used as an internal standard and the accuracy of measurements was tested using CRM SLRS5 (NRCC). Replicability was below 5% for all elements (except for Ti 12% and Fe 6%) and repeatability was below 10% for all elements, except for Mg (19%), Ti (27%) and Fe (17%). Element recovery was assessed by spiking a dummy lichen sample (constituted of a mix of 100 digested lichens) with a multi-element standard solution (See Sup. Info. Table S2). Full duplicates (selection, digestion, measurement and data processing) were performed to control the validity of the method. Results were reported in µg of element per g of oven dry weight lichen (µg.g⁻¹ of dry weight).

2.3. Statistics

Due to the close structure of the metal content, our data set was transformed using centered log-ratio (clr) (Aitchison, 2003; Filzmoser et al., 2009a, 2009b). Then, a principal component analysis (PCA) of compositional data was performed in order to reduce the size of the data set by highlighting factors that explain variation in the composition of the thalli. While many studies (e.g., Puckett and Finegan, 1980; Monaci et al., 2012) have used classical PCA, this is the first time compositional PCA is being used

on data from lichen. The compositional biplot must be analysed in term of ratio of elements; the center of the plot represents the average composition, and links between arrows represent the ratio between elements. Due to the large number of variables, results from the PCA were separated into two different types of graphs (see Sup. Info Supplementary methods for details on compositional biplot interpretation and description). All statistics were performed using R software v2.15.2 with RStudio, Composition, FactomineR, Stats and Nortest pack (R development core team; <http://www.cran.r-project.org>). Medians and median absolute deviations were used as estimators of central tendency and dispersion to conveniently show non-transformed data.

3. Results and discussion

3.1. Principal component analysis

3.1.1. Major southern anomaly

A first PCA analysis performed on all data (Sup. Info. Fig. S1) highlighted a major anomaly associated with the southernmost sampling sites (i.e., SN2 and SN3). PC1 clearly separates SN2 and SN3, which show a higher ratio of terrigenous insoluble elements such as Fe, Al, Ti, V to soluble elements such as P, Mg, K and Mn, from all other sites (Sup. Info. Table S3 and Fig. S1B). This difference among sampling sites is likely due to their southern geographic location and proximity to pollution rather than their bioclimatic domain (Fig. 1A-B). SN3 is located in the spruce moss domain, which is the same bioclimatic domain as for SN4 and yet, thalli sampled at SN3 show high metal contents, compare to SN4 (Fig. 1A, Fig. 1C). It is also worth noting that while we

sampled different bioclimatic domains, samples were collected where black spruce was the dominant tree (as much as possible).

The relative abundance of cyanolichens further supports the hypothesis of an anthropogenic origin of the southern anomaly. Some lichens are known to be susceptible to anthropogenic nitrogen deposition, nitrogenic acid rain and other city-derived sources (Nash, 2008a, 2008b). No *Peltigera* and *Nephroma* species were found at SN1, W400 and W100 and were nearly absent from SN2, SN3, SN4 and W200 (Sup. Info. Table S1). These southern and western most sites are located in the proximity of active mining areas (Fig. 1B) and near urbanized and industrialized areas (along the Saint Lawrence River). The location of these sampling sites relative to the mining sites and cities is also consistent with dominant winds in the region (Fig. 1B). Thus, the lack of lichens in the southern most portions of these transects could be due to metal pollution and/or to enhanced nitrogen pollution from urbanized areas. This could also explain the overall distribution of some of these lichen species (e.g., *Nephroma arcticum*) in Québec (see Brodo et al., 2001). Further dedicated research, including a more intensive survey, is required to clearly establish the role of human activities on the abundance of cyanolichens in the region.

Because our main objective was to determine baseline metal contamination levels, samples collected at SN2 and SN3 (Sup. Info. Tables S1 and S3) were excluded from further analyses. A new PCA was performed without samples from SN2 and SN3 (Fig. 2). Results highlight that samples from SN4 to SN9 and W50 to E400 can hardly be differentiated based on their geographical location (Figs. 1, 2B). This is unexpected considering the large territory and bioclimatic range covered by the sampling, especially

along the SN transect. This finding suggests that elemental deposition in most of northern Québec is homogeneous. Only few and small geographic anomalies could be revealed and likely reflects local conditions (natural and anthropogenic, See Sup. Info Supplementary discussion 2.1 for details).

3.1.3. Elemental composition of thalli.

The variable biplot (Fig. 2) shows comparable repartition of elements between the two first PCs when compared to the one obtained with the first analysis (compare Figs. 2A and Sup. Info. Fig. S1A). Those two first PC explain > 40% of the total variance of the dataset. PC1 opposes Ti and other terrigenous elements such as Fe, Al and V to more soluble elements such as Zn, P, and K. We can distinguish several clusters of correlated variables. On the left quadrants, a first group contains mostly insoluble terrigenous elements such as Fe, Al, Ti, V and Cr. The high ratio stability between those five elements strongly suggests that they are deposited all together. This first group certainly reflects the deposition of element as particles from pedogenic (orogenic) or anthropogenic origins (mining activities). A similar pattern with high correlation of terrigenous materials was reported for the first component of PCA from most studies on elemental deposition conducted in pristine areas (Puckett and Finegan, 1980; Monaci et al., 2012). A second group, in the lower-right quadrant, composed of numerous elements (K, Mg, P, Zn, Cu, Cd and Ni), may point at depositions from anthropogenic origin such as mining industries (i.e. Ni, Cu) and agricultural activities (P and K). However, the low levels of Ni, Cu and Zn (Sup. Info. Table S3-S7) suggest that anthropogenic sources, if any, must be scarce. A third group of elements (Na, Ca and Mn) is found in the upper-

right quadrant (Fig. 2B) and might reflect leaching of easily soluble nutrients from the surrounding vegetation (Carlisle et al., 1966; Chou and Chen, 1976; Schmul and Hauck, 2003). However, source apportionment of elements that play a role in lichen physiology (Mn, Ca, Na, P, K) should be performed with care. Soil analysis at sampling sites is needed to determine more accurately the origin of trace metals.

Overall, the biplots (Fig. 2) suggest that metal content of lichens from large parts of the boreal zone of Québec mostly reflect atmospheric deposition of particles from pedogenic sources) and transfer of soluble elements from surroundings (i.e., vegetation leaching). The contribution of anthropogenic sources, if any, is likely low.

3.1.5. Lichen species

When considering the different lichen-forming species with regard to the first two components (Fig. 2C), all species have narrow confidence ellipses ($p = 0.95$), reflecting intraspecies homogeneity. Regarding PC1, none of the species could be well separated from each other. Moreover, all centroids are close to the center of the plot (i.e. the average composition). This result demonstrates that the accumulation of metals originating from the deposition of particles is quite similar regardless of the four species we examined.

When comparing metal content for each species separately (Fig. 3, Sup. Info. Fig. S2, Tables S4-S7), there is no clear difference in the metal content of the thalli. Only few elements seem to differ in abundance in some species (e.g. Ni). However, these differences could reflect more their geographic distribution than a difference in species

behavior with regard to these metals (See Sup Info Supplementary discussion 2.2 for details).

Nonetheless, our results show that all species have comparable overall elemental composition throughout most of our sites; from W50 to E400 and SN4 to SN9 (Figs. 2C, 3, Sup. Info. Table S8), suggesting that these four peltigeralean species do not significantly affect the quality of metal monitoring, at least for the level of contamination recorded here. The pertinence of the selected species for future biomonitoring surveys will strongly depend on their ability to accumulate elements and survive under high element exposures. Samples collected close to anthropogenic activities (SN2 and SN3, in this study; Alaska and Siberia, data not shown), suggest that the selected species (*P. aphthosa* s.l., *P. neopolydactyla* s.l. and *P. scabrosa* s.l.) can efficiently monitor anthropogenic perturbations (i.e., metal deposition), while *N. arcticum* seems to be more sensitive to anthropogenic perturbations and could potentially be of use as a bioindicator. Further dedicated studies are required to fully establish the potential of these species as biomonitors or bioindicators.

3.2. Determination of baseline element composition

Because our selected set of data is homogenous in term of composition with respect to species and geography, our baseline estimation was derived using the median and median absolute deviation on the all set (data for species are available in Sup. Info. Table S8). When comparing our results with previously published values from remote areas, including mountainous regions, most elements are in the lowest portion of the range (Table 1). Our results are also very similar to values reported from surrounding

boreal and arctic regions, such as in the Northwest Territories (Puckett and Finegan, 1980; Nash and Gries, 1995), and Greenland (Riget et al., 2000), as well as from Antarctic regions (Bargagli et al., 1999). Most elements reported from our study are at the lowest concentration ever reported so far. However it should be stated that comparison between different species are always difficult. The lower elemental content observed in our samples compared to other pristine locations from high elevation such as the Alps, Himalaya or Mount Kenya (Bergamaschi et al., 2004) could reflect differences in erosion dynamics. The Canadian Shield is the result of an early orogenic episode, mountains have a low elevation (culminating at 1700 m in the Province of Québec), and were less likely subject to erosion in the past hundreds of years than the Alps, the Himalaya or Mount Kenya, which originated from more recent orogeneses. Thus, the Canadian Shield likely generated less terrigenous particles. Some recent studies highlighted the pertinence to use thalli margins in order to better evaluate recent pollution sources. For lichen thalli collected in this study, whole thalli and margins provided similar conclusions (see Sup. Info Tables S9 and S10 and Supplementary discussion 2.3 for details).

4. Conclusions

Overall, our results show that a large portion of the boreal biome of Québec (Northeastern Canada) remains pristine. The elemental composition of selected muscicolous/terricolous macrolichens, independent of the species considered here, mostly reflects deposition of particles from pedogenic origin. All lichens are surprisingly homogeneous with regards to their geographic origin. This likely reflects the relative homogeneity in the geological formation of northeastern Canada; mostly the Canadian

Shield. Baseline element compositions of studied lichens are very comparable to lichens from the most pristine places in the world (i.e., Greenland, Himalaya, Antarctica and various polar regions). Finally, we showed that in the southern part of the SN transect, the sampled specimens of *Peltigera* spp. were able to monitor anthropogenic perturbations; the intense mining activity in this region was easily recorded in all samples. Also, the use of multivariate analysis in this study underlined small anomalies for specific metals with regard to geography and species, even at low concentration levels. Biomonitoring surveys including several species offer a variety of advantages; (i) it will facilitate sampling (species have different abundances and distributions along both transects) and (ii) it will significantly reduce the odds of losing the reference material over time. Some species are more sensitive than other to perturbation such as nitrogen deposition and acid rain. With future economic developments of boreal regions associated with global climate change, some species could disappear from some regions as a result of anthropogenic activity. The absence or very low abundance of *Peltigera* and *Nephroma* close to intense mining and urbanized areas in the most southern and western part of our transects (SN1 to SN3, W100 to W400) supports this prediction. This study provides a valuable set of data to monitor the impact of global climate change, future urbanization, and mining development in northeastern Canadian ecosystems.

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Author contributions

J.P.B and F.L. initiated and conceived the study. J.M. and F.L. collected and identified lichen specimens. R.D. performed all the analysis and wrote the first draft of the manuscript. All authors commented on the final version of the manuscript.

324 **References**

- 325 Aitchison J. A concise guide to compositional data analysis. 2nd CDA Work. Girona, Spain; 2003.
- 326 Aznar J-C, Richer-Lafleche M, Cluis D. Metal contamination in the lichen *Alectoria sarmentosa* near the
327 copper smelter of Murdochville, Québec. Environ Pollut. 2008;156(1):76–81.
- 328 Bargagli R, Sanchez-Hernandez J, Monaci F. Baseline concentrations of elements in the antarctic
329 macrolichen *Umbilicaria decussata*. Chemosphere. 1999;38(3):475–87.
- 330 Bergamaschi L, Rizzio E, Giaveri G, Profumo A, Loppi S, Gallorini M. Determination of baseline element
331 composition of lichens using samples from high elevations. Chemosphere. 2004;55(7):933–9.
- 332 Brodo IM, Sharnoff SD, Sharnoff S. Lichens of North America. New Haven: Yale University Press; 2001.
- 333 Carlisle A, Brown A, White E. The organic matter and nutrient elements in the precipitation beneath a
334 sessile oak (*Quercus petraea*) canopy. J Ecol. 1966;54(1):87–98.
- 335 Chiarenzelli J, Aspler L, Dunn C, Cousens B, Ozarko D, Powis K. Multi-element and rare earth element
336 composition of lichens, mosses, and vascular plants from the Central Barrenlands, Nunavut, Canada.
337 Appl Geochemistry. 2001;16(2):245–70.
- 338 Chou C, Chen A. Leaching metabolites in vegetation of northern taiwan. 1. Release of cations in some
339 vegetation of northern Taiwan. Bot Bull Acad Sin. 1976;17:25–36.
- 340 Conti M, Cecchetti G. Biological monitoring: lichens as bioindicators of air pollution assessment--a review.
341 Environ Pollut. 2001;114(3):471–92.
- 342 Crête M, Lefebvre M, Zikovskiy L, Walsh P. Cadmium, lead, mercury and 137 cesium in fruticose lichens
343 of northern Québec. Sci Total Environ. 1992;121:217–30.
- 344 DeLuca T, Zackrisson O, Nilsson M, Sellstedt A. Quantifying nitrogen-fixation in feather moss carpets of
345 boreal forests. Nature. 2002;419(October):917–20.
- 346 Evans CA, Hutchinson TC. Mercury accumulation in transplanted moss and lichens at high elevation sites
347 in Quebec. Water Air Soil Pollut. 1996;90(3-4):475–88.
- 348 Filzmoser P, Hron K, Reimann C. Principal component analysis for compositional data with outliers.
349 Environmetrics. 2009a;20(6):621–32.
- 350 Filzmoser P, Hron K, Reimann C. Univariate statistical analysis of environmental (compositional) data:
351 problems and possibilities. Sci Total Environ. 2009b;407(23):6100–8.
- 352 Garty J. Biomonitoring atmospheric heavy metals with lichens: theory and application. CRC Crit Rev Plant
353 Sci. 2001;20(4):309–71.
- 354 Gouvernement du Québec. Cartes Minières [Internet]. 2013a. Available from:
355 <http://www.mrn.gouv.qc.ca/mines/publications/publications-cartes.jsp>
- 356 Gouvernement du Québec. Zones de vegetation et domaines bioclimatiques du Québec [Internet]. 2013b.
357 Available from: <https://www.mrn.gouv.qc.ca/forets/inventaire/inventaire-zones-carte.jsp>

- 358 Gouvernement du Québec. Le potentiel économique [Internet]. 2014. Available from:
359 <http://www.nord.gouv.qc.ca/potentiel/index.asp>
- 360 Jeran Z, Jaćimović R, Batic F, Mavsar R. Lichens as integrating air pollution monitors. *Environ Pollut.*
361 2002;120(1):107–13.
- 362 Logan T, Charron I, Chaumont D, Houle D. Atlas de scénarios climatiques pour la forêt québécoise.
363 Ouranos et MRNF; 2011.
- 364 Martínez I, Burgaz AR, Vitikainen O, Escudero A. Distribution patterns in the genus *Peltigera* Willd.
365 *Lichenol.* 2003;35(4):301–23.
- 366 Miadlikowska J, Lutzoni F. Phylogenetic revision of the genus *Peltigera* (lichen-forming Ascomycota)
367 based on morphological, chemical, and large subunit nuclear ribosomal DNA data. *Int J Plant Sci.*
368 2000;161(6):925–58.
- 369 Miadlikowska J, Lutzoni F. Phylogenetic classification of peltigeralean fungi (Peltigerales, Ascomycota)
370 based on ribosomal RNA small and large subunits. *Am J Bot.* 2004;91(3):449–64.
- 371 Miadlikowska J, Richardson D, Magain N, Ball B, Anderson F, Cameron R, et al. Phylogenetic placement,
372 species delimitation, and cyanobiont identity of endangered aquatic *Peltigera* species (lichen-forming
373 Ascomycota, Lecanoromycetes). *Am J Bot.* 2014;101(7):1141–56.
- 374 Monaci F, Fantozzi F, Figueroa R, Parra O, Bargagli R. Baseline element composition of foliose and
375 fruticose lichens along the steep climatic gradient of SW Patagonia (Aisén Region, Chile). *J Environ*
376 *Monit.* 2012;14(9):2309–16.
- 377 Nash T. Lichen sensitivity to air pollution. In: Nash T, editor. *Lichen Biol.* Cambridge, UK: Cambridge
378 University Press; 2008a. p. 301–16.
- 379 Nash T. Nitrogen, its metabolism and potential contribution to ecosystems. In: Nash T, editor. *Lichen Biol.*
380 Cambridge, UK: Cambridge University Press; 2008b. p. 218–35.
- 381 Nash T. Nutrients, elemental accumulation, mineral cycling. In: Nash T, editor. *Lichen Biol.* Cambridge,
382 UK: Cambridge University Press; 2008c. p. 236–54.
- 383 Nash T, Gries C. The use of lichens in atmospheric deposition studies with an emphasis on the Arctic. *Sci*
384 *Total Environ.* 1995;161:729–36.
- 385 Nimis PL, Scheidegger C, Wolseley PA. Monitoring with Lichens — Monitoring Lichens. In: Nimis PL,
386 Scheidegger C, Wolseley PA, editors. *NATO Sci Ser Vol 7.* Dordrecht: Springer Netherlands; 2002.
- 387 Puckett KJ, Finegan EJ. An analysis of the element content of lichens from the Northwest Territories,
388 Canada. *Can J Bot.* 1980;58(19):2073–89.
- 389 Riget F, Asmund G, Aastrup P. The use of lichen (*Cetraria nivalis*) and moss (*Rhacomitrium*
390 *lanuginosum*) as monitors for atmospheric deposition in Greenland. *Sci Total Environ.*
391 2000;245:137–48.
- 392 Schmult M, Hauck M. Extraction methods for assessing the availability of cations for epiphytic lichens
393 from bark. *Environ Exp Bot.* 2003;49(3):273–83.

- 394 Seaward M. Environmental role of lichen. In: Nash T, editor. Lichen Biol. Cambridge, UK: Cambridge
395 University Press; 2008. p. 276–300.
- 396 Seaward MRD. Lichen Ecology of the Scunthorpe Heathlands I. Mineral Accumulation. Lichenol.
397 2007;5(5-6):423.
- 398 Sloof J. Lichens as quantitative biomonitors for atmospheric trace-element deposition, using transplants.
399 Atmos Environ. 1995;29(1):11–20.
- 400 Tomassini F, Puckett K. Determination of copper, iron, nickel, and sulphur by X-ray fluorescence in
401 lichens from the Mackenzie Valley, Northwest Territories, and the Sudbury District, Ontario. J Bot.
402 1976;54:1591–603.
- 403 Vitikainen O. Taxonomic revision of *Peltigera* (lichenized Ascomycota) in Europe. Acta Bot Fenn.
404 1994;152:1–96.
- 405 Wolterbeek B. Biomonitoring of trace element air pollution: principles, possibilities and perspectives.
406 Environ Pollut. 2002;120(1):11–21.
- 407 Working Group I Contribution to the IPCC Fifth Assessment Report. Climate Change 2013: The Physical
408 Science Basis. 2013.

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410

411 **FIGURE CAPTIONS**

412 **Figure 1. Sampling sites in Québec and comparisons of Fe, Al, Ti, V, and Cr**
413 **contents along transects.** (A) Geographic localization of sampling sites with main
414 vegetation zones (Gouvernement du Québec, 2013b). (B) Geographic localization of the
415 main mining activities in Québec (Gouvernement du Québec, 2013a) and directions of
416 dominant winds. For both panels, stars represent sites sampled as part of a separate study
417 (endobiodiversity.org). Green stars represent sites from which *Peltigera* and *Nephroma*
418 specimens were used for this study. (C) Comparison of some metals along a south-north
419 (SN) oriented transect and (D) along an east-west (EW) oriented transect.

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421 **Figure 2. Correlations among elements and the effect of bioclimatic zones and**
422 **species on data structure.** (A) Correlation among elements projected on the first two

principal components. Variable plots highlighting bioclimatic domains (B) and lichen-forming species (C). In (B) centroids of geographical sampling points are highlighted with the color of their bioclimatic domains location. For clarity purpose, individuals are not shown.

Figure 3. Element contents for four lichen-forming species. Data from all sites were combined for each species as median and median absolute deviation.. *Nephroma arcticum* (n = 31), *Peltigera aphthosa* s.l. (n = 30), *P. neopolydactyla* s.l. (n = 16) and *P. scabrosa* s.l. (n = 33). Samples from SN2 and SN3 were excluded.

Table 1. Baseline concentrations of elements detected from this study compared to previous studies in pristine areas of the world. *Nephroma arcticum*, *Peltigera aphthosa* s.l., *P. neopolydactyla* s.l., and *P. scabrosa* s.l are combined together. (data for species are available in Sup. Info. Table S8). Min = lowest value of the final dataset, Max = highest value of the final dataset GM = Geometric mean, AM = Arithmetic mean, SD = Standard deviation, MED=Median, MAD=Median absolute deviation. Elements are sorted by increasing atomic weight.

446 FIGURES

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466 GRAPHICAL ABSTRACT

Table 1

Lichen content (µg.g-1, dry weight)												
Element	This work					Literature						
	Whole lichen (n=103)				margin (n=20)	Himalaya (1)	Kenya (1)	Italy (1)	Greenland (2)	Canadian Arctic (3,4,5)	Chile (6)	Antarctic (7)
	Min-Max	AM ± SD	MED ±MAD	GM	GM	AM ± SD	AM ± SD	AM ± SD	GM	AM	AM	AM ± SD
Na	<13-483	126 ± 109	92±61	85	-	-	-	-	-	86-2756 ⁴	101	222 ± 101
Mg	133-1597	488 ± 195	457±104	457	596	2577±1497	1653±895	1008±249	-	350.8-916.5 ⁴	-	436 ± 179
Al	15-515	53 ± 69	36±12	40	14	-	-	-	70.1-518	369-940 ⁴	1289-1691	428 ± 191
P	543-4995	1445 ± 665	1338±354	1337	1980	-	-	-	-	-	1267-1623	963 ± 517
K	3748-10898	6549 ± 1423	6518±966	6395	9568	4229±1113	4348±1844	4703±608	-	1030-7920 ⁴	2916	2007 ± 483
Ca	255-2009	778 ± 314	738±158	722	666	-	-	-	-	1100-8200 ⁴	4300-4946	640 ± 707
Ti	<1-80	3 ± 8	2±1	2	1	-	-	-	-	7-850 ³	-	-
V	0.07-2.20	0.21 ± 0.21	0.16±0.05	0.18	0.11	2.4±1.2	1.8±0.5	0.6±0.1	0.21-0.64	0.17-9.7 ³	2.09	-
Cr	<0.07-1.43	0.19 ± 0.21	0.15±0.04	0.15	0.18	2.5±1.4	2.5±2.0	1.7±1.3	0.17-0.7	0.5-2 ³	2.17	1.3 ± 0.6
Mn	19-1206	169 ± 158	129±64	126	99	55±26	169±138	108±75	-	30.2-84.5 ⁴	109	11.8 ± 3.9
Fe	18-880	71 ± 88	54±13	57	27	1512±756	1615±792	220±105	90.2-225	50-900 ³	1410	802 ± 402
Co	<0.03-0.79	0.11 ± 0.14	0.06±0.03	0.07	0.02	0.82±0.53	0.39±0.24	0.27±0.12		0.08-2.84 ³	-	-
Ni	0.2-4.7	1.0 ± 0.8	0.8±0.3	0.8	0.5	-	-	-	<1-1.1	1.7-5.5 ³	2.63	-
Cu	1.8-23.8	4.9 ± 3.8	3.7±1.1	4.1	5.4	5.9±3.7	5.3±2.0	4.1±3.1	0.594-0.979	0.7-5 ³	4.39-4.49	5.3 ± 5.1
Zn	12-166	51 ± 33	40±15	43	43	34±13	67±32	35±16	10.6-25.1	7-55 ³	44.2-51.0	18.6 ± 4.1
Mo	<0.03-0.33	0.06 ± 0.05	0.05±0.01	0.05	0.03	-	-	-	-	0.07-0.32 ⁴	-	-
Cd	<0.03-1.00	0.08 ± 0.10	0.06±0.02	0.06	0.14	0.04±0.03	0.14±0.13	0.21±0.10	0.066-0.119	0.03-0.24 ⁴	0.13-0.28	0.21 ±0.11
Pb	0.05-3.02	0.43 ± 0.37	0.32±0.12	0.35	0.22	14.0±3.9	3.6±1.9	4.8±1.3	0.77-2.3	0.4-9.2 ³	0.25-1.51	0.54 ±0.34