Science of The Total Environment
January 2024, Volume 907, Pages 167900 (11p.)
https://doi.org/10.1016/j.scitotenv.2023.167900
https://archimer.ifremer.fr/doc/00857/96892/

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Evaluating the effect of moss functional traits and sampling on elemental concentrations in *Pleurozium schreberi* and *Ptilium crista-castrensis* in Eastern Canada (Québec) black spruce forest

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Abstract :

Characterizing atmospheric depositions allows evaluating the impact of air pollution on ecosystems, human health, and the economy. It also informs decision-makers about the implementation of regulations improving environmental quality. Biomonitoring uses organisms, such as mosses, as proxies to assess the presence of atmospheric contaminants (e.g., metals). This approach is cost-efficient and does not require complicated infrastructure or scientific skills, making it suitable for large-scale monitoring initiatives and citizen-based campaigns. Therefore, precise sampling protocols are needed to limit bias. Biomonitoring data remains scarce in North America, compared to e.g., Europe, and there is a need to develop large-scale and long-term biomonitoring initiatives to record current and future atmospheric depositions. As there is no standardized international sampling protocol, this study assessed the impact of parameters known to affect the elemental concentration of mosses, using samples collected along a 1000-km transect in Eastern Canada (Quebec) from 2016 to 2022. We specifically examined the effects of species, stem color, canopy opening, time of sampling, and stem length on 18 elements. Non-parametric statistical tests indicate that these factors have significant effects on some metals, but differences are generally low (<30 %), except for stem length. These results suggest that sampling protocols can be flexible in terms of species, canopy opening, time of sampling, and stem color. However, normalizing the length of the stems analyzed is required to account for differences in growth rates between sites. Moreover, since no large-scale biomonitoring campaign using mosses has been conducted in Eastern Canada, this paper also provides the first elemental baseline for moss in the region.

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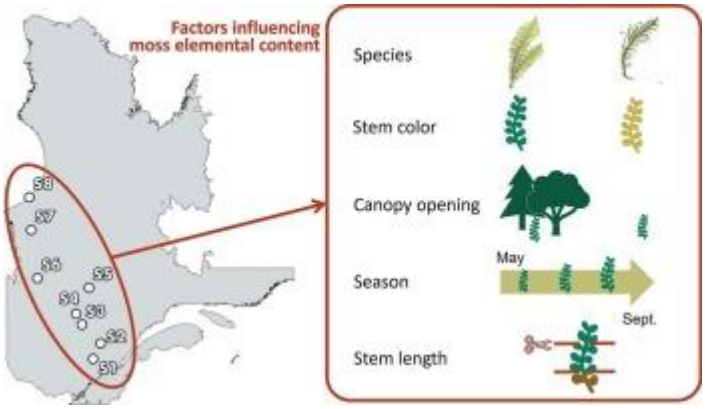
Graphical abstract



Highlights

► Factors affecting moss elemental content were evaluated in Eastern Canada. ► Species, color, canopy openness, season, and stem length were investigated. ► All factors had significant effects, but for most differences were moderate (<30 %). ► Only stem length had a significant and important effect on moss elemental content. ► Moss elemental concentrations were compared to the literature for remote areas.

Keywords : Biomonitoring, Atmospheric deposition, Trace elements, Moss, Sampling

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1. Introduction

Throughout its history, human development and activities have significantly impacted air, soil, and water quality. The burden of human activities on the environment is quickly rising, fueled by rapid population growth, and is amplified by global climate change. Literature on long-range atmospheric transport and deposition of contaminants and their impacts on ecosystem quality and function is abundant (Friedman and Selin, 2012; Steinnes et al., 1989; Vélez-Pereira et al., 2022; Wang et al., 2022). Properly monitoring the sources of contamination and their evolution is thus a cornerstone of responsible environmental stewardship. Monitoring atmospheric deposition over large geographic areas and long periods using instrumental or passive samplers is challenging due to the cost of installation and maintenance. In North America, large-scale (spatial and temporal) monitoring programs of atmospheric deposition are scarce to nonexistent, except for emblematic elements such as nitrogen, sulfur, and mercury (i.e., Canada CAPMoN program, USGS NADP program) and rely on networks of a few monitoring stations scattered over large geographic surfaces (cf CAPMoN program and Réseau de surveillance de la qualité de l'air du Québec). Biomonitoring methodologies use ubiquitous organisms to evaluate atmospheric depositions at a low cost (Abas, 2021; Amodio et al., 2014). Biomonitoring methods using bryophytes fall into two main categories; passive biomonitoring using living moss growing naturally on sampling sites and active biomonitoring using moss transplants or moss bags (Ștefănuț et al., 2021; Świsłowski et al., 2021). While passive biomonitoring using bryophytes is still lacking international standardized protocols and is often semi-quantitative, it is a powerful tool for monitoring atmospheric depositions over large geographic areas that is gaining in popularity (Fernández et al., 2015). Important discrepancies currently exist in available biomonitoring data between regions of the globe. In Europe, large-

scale biomonitoring programs such as ICP-Vegetation (https://icpvegetation.ceh.ac.uk/), established decades ago, provide valuable data on the composition and evolution of atmospheric depositions, informing on atmospheric dispersion of contaminants and guiding decision-makers. They also allow for the evaluation of the effect of environmental regulations and global climate change on atmospheric depositions. In Canada, biomonitoring surveys of atmospheric depositions remain scarce and often focus on specific elements and regions (Chiarenzelli et al., 1997; Cowden and Aherne, 2019; Klapstein et al., 2020; McDonough et al., 2022).

The Canadian boreal zone is one of the largest ecosystems on Earth and is still barely colonized by humans whose presence concentrates on the southern part of the country. A recent large-scale biomonitoring survey performed along two transects (south-north and East-West) using lichens concluded that most of the boreal zone in Eastern Canada remains fairly pristine (Darnajoux et al., 2015). However, this ecosystem will undergo drastic changes in the coming decades due to global climate change that will affect ecosystem function, the atmospheric transport of contaminants and open new opportunities for the northward expansion of human activities and populations (e.g., mining, agriculture, urban development) (Gauthier et al., 2015; Noyes et al., 2009). Establishing long-term biomonitoring programs, complementing existing instrumental sampler networks (e.g., CAPMoN stations), is key to evaluating the long-term effects of global climate change and future human activities in this ecosystem. The European biomonitoring program ICP-Vegetation uses several moss biomonitors, especially *Hylocomium splendens, Hypnum cupressiform, Pleurozium schreberi,* and *Pseudoscleropodium purum,* depending on the region (https://icpvegetation.ceh.ac.uk/). *H. splendens* and *P. schreberi* are primarily used in Northern and Eastern Europe, and *H. cupressiforme* and *P. purum* in Western Europe. In Eastern Canada (i.e., the Quebec province), black spruce forest soils are dominated by

P. schreberi and *Ptilium crista-castrensis* and to a lesser extent, *H. splendens,* (Barbé et al., 2017, *personal observation*). *P. schreberi* and *Ptilium crista-castrensis* are widely distributed throughout the province and are good biomonitor candidates for the region. Data on the elemental composition of *P. schreberi* and *Ptilium crista-castrensis* in this region are lacking.

The biophysicochemical mechanisms that support the use of mosses as proxies for atmospheric depositions are manifold and interrelated (Berg and Steinnes, 1997; Stanković et al., 2018). The elemental composition of the moss at any given time results from a balance between the interception and filtering of atmospheric depositions by the moss and the dilution of elements in the moss stem with the production of new moss biomass. Mosses intercept elemental atmospheric deposition through a combination of physical (e.g., direct interception of particulate) and chemical (e.g., binding, chemical complexation, solubilization, and washing) processes (Stanković et al., 2018). Thus, moss functional traits, especially moss-specific surface and chemical properties of the moss surface, both of which are species-specific, can directly impact elemental accumulation. In both *P. schreberi* and *P. crista-castrensis*, the C: N ratio, shoot-specific mass, and biological nitrogen fixation rates have been shown to change depending on some phenotypic characters such as moss color (Darnajoux et al., 2018). This in turn would likely influence both the growth rate and surface chemistry of mosses within an individual species. The production of new biomass by the moss is influenced by environmental factors (i.e., temperature, humidity, light) and the availability of nutrients supporting processes important for growth (i.e. photosynthesis, biological nitrogen fixation), all of which can vary significantly between sampling sites (Renaudin et al., 2022a). The seasonality of moss growth is likely to affect elemental dilution, and thus elemental concentrations, associated with biomass production throughout the growing season in all species (Bernd and Weckert, 1989). Differences in

elemental dilution intensity can also arise between species achieving different growth rates and growth period lengths. Mosses are a continuum of growth from vegetative tissues at the apex and decaying tissues at the bottom. Moss physical and chemical properties, moss microbiome composition and function, as well as the distribution of essential nutrients, vary greatly along this continuum, even along the green section of the moss often used for biomonitoring (Renaudin et al., 2022b). The growth rate and the efficiency of moss species to remobilize elements along the stem would also influence the distribution of elements during the growing season and along the stem. Finally, because the canopy can influence N and other elements supply to moss (i.e., quantity and composition), as well as climatic characteristics of the local habitat (i.e., temperature, moisture, light availability), canopy openness (open versus close) is likely to affect *P. schreberi* and *P crista-castrensis* elemental composition (Bredemeier, 1988; Macinnis-Ng et al., 2012; Tan et al., 2018; Zhang et al., 2022).

Thus, multiple characteristics of the moss and sampling strategy (time of sampling, characteristics of the sites) can impact the results of a biomonitoring campaign. The focus of this study was to specifically investigate the effect of five factors, i.e., species identity, moss stem color, time of sampling (during the growing season), canopy openness, and selection of the stem length on elemental concentrations in two dominant feather moss species in Eastern Canada (i.e., *P. schreberi* and *P crista-castrensis*). We further characterized their growth rates along a 300 Km latitudinal gradient and their specific surface to evaluate if these physiological parameters could influence elemental records. This evaluation will guide the establishment of sampling protocols for community-based sampling supporting biomonitoring initiatives in the region (Quebec, Eastern Canada). Furthermore, in the light of rapid changes in contaminants dispersion in the region due to climate changes, illustrated by the exceptional wildfires of summer 2023,

data reported here, using sample archives collected throughout the province will provide a reference for studies on the effect of such changes and events in a region where data on bryophytes elemental concentrations are scarce.

2. Methods

2.1.Sample collection, handling, and storage.

To achieve the proposed objectives, we used an in-house moss samples collection including samples gathered from 2016 to 2019 along a 1000-km latitudinal transect (Fig. 1, white dots, Table 1, and Table 2). This collection allowed us to test the effect of biological (i.e., moss species identity, stem color, stem length), and environmental factors (e.g., canopy openness, time of sampling) on moss elemental concentrations. As this collection is composed of samples accumulated over several independent projects with their own specific goals, the number of replicates can vary between entries in the collection, one entry being one site sampled at a given time. A summary of the samples used for the different purposes of this study, including the sampling site location, date of sampling, and the number of replicates is provided in Table 2. Composite samples, consisting of whole moss stems randomly collected on 10 m x 10 m plots, were collected in paper bags and air-dried. Samples were stored dry in cardboard boxes at room temperature until analysis. Elemental analyses were performed for the vegetative section (first 3 cm), except for samples used for the characterization of elemental concentration along the moss stem. For these samples, collected on site SS3 in September 2019, stems were cut in sections of 0.5 cm down to a depth of 6 cm (including both the green, yellow, and brown sections of the moss). The color of sections (green, yellow, and brown) was assessed by eye on all stems and

averaged. To evaluate the effect of moss stem color on elemental concentration, vegetative sections of moss stems were classified into two categories, “green” and “yellow”, following protocols described by Darnajoux et al., (2018). For the evaluation of the effect of canopy openness, moss samples of both species and both color phenotypes (N=64) were collected on site SS3 in 2016 in a mature black spruce forest and a nearby windfall (4 years old windfall at the time of sampling).

Moss growth was evaluated on sites S1, SS3, and SS5 in 2019 (Fig. 1). Briefly, on each site, three plots of 1 x 1m were established. On each plot, ~30 stems of each species were marked using strings placed at 2 cm from the apex in May 2019 (about 2-3 weeks after snowmelt in the northmost site (SS5). Carpets were retrieved in late September 2019, and the length of the marked stems was measured. Growth (in $cm.yr^{-1}$) was determined as the length from the string to the apex in September 2019 minus 2 cm. The mass-specific surface of both species was estimated on samples collected on site SS3 in 2022. Fresh moss stems were weighed. The number of branches along the stem (green section) and the number of leaves on each branch were recorded. The surface of moss leaves randomly collected along the whole stems was measured using a bright-field microscope (Leica M165FC) and the software Fiji v. 2.13.0. A total of 423 and 448 leaves from 3 stems of *P. crista-castrensis* and *P. schreberi* respectively, were analyzed. The mean mass-specific surface of both species was estimated by dividing the specific surface of the stems (average leave surface times the number of leaves per stem) by the mean mass.

To establish an elemental baseline, we selected samples from remote plots distributed along a large geographic area (Fig. 1, black triangles), which, according to a previous study using lichens is characterized by very low depositions of anthropogenic contaminants (Darnajoux et al., 2015). The plots are part of forest plots managed by Quebec’s Ministry of Forestry. They are located in

30 years old, at the time of sampling (in 2016), black spruce plantations. Samples were collected in open plots away from the tree canopy. For all samples, elemental analysis was performed on the first 3 cm of the stems from composite samples collected on a 5 m x 5 m area (data are reported as the average and standard deviation of two analytical replicates).

2.2.Sample preparation and elemental analysis.

Samples were oven-dried in paper bags before being grounded in liquid nitrogen with a mortar and a pestle. Samples were digested with nitric acid and hydrogen peroxide on a hotplate digestor (DigiPREP, SCP) as described previously (Renaudin et al., 2022b; United States Environmental Protection Agency, 1996). Procedural blanks were performed for all sets of digestion. Accuracy and replicability were ascertained using the M3 moss reference material (*P. schreberi* species) (Steinnes et al., 1997). Rhodium was used as an internal standard. All elemental quantifications of Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Mo, Ag, Cd, Sb, Ba, Tl, and Pb were done on a Thermofisher Xseries II ICP-MS and PlasmaLab software version 2.6.1.335. Control quality of this method is provided in Table S1.

2.3.Statistics

All statistical analyses were performed with R version 4.2.2, and all the figures were prepared with the ggplot2 package version 3.4.2. As data were not normally distributed, non-parametric tests were used. Wilcoxon tests were used to test the effect of moss identity, moss color, and canopy openness on moss elemental concentrations and to compare the growth rate of both moss species. To evaluate the impact of the sampling month and stem length, both having more than two groups, Kruskal-Wallis tests were used (Table 2) ($\alpha = 0.05$). In each case, a p value of 0.05 was used as the significance threshold.

3. Results

3.1.Elemental concentration and growth rates of P. schreberi and *P. crista-castrensis along a 1000 km latitudinal gradient.*

Moss elemental concentrations were significantly affected by the site for all measured elements ($p < 0.01$, Sup. Info. Tables S2. And S2b). Concentrations varied up to several orders of magnitude between sites, depending on the elements, with the largest variations observed with Tl (14 folds) for *P. schreberi* and Ti (21 folds) for *P. crista-castrensis* and the smallest with Cu (10%) for *P. schreberi* and Ba (20%) for *P. crista-castrensis* (Sup. Info. Table S2 a.). No clear latitudinal trend was observed for any measured elements along the transect. For samples collected on the same sites, elemental concentrations were significantly different between *P. schreberi* and *P. crista-castrensis* only for Cu and Tl ($p < 0.005$). The differences in Cu concentrations between species were generally below 30% for Cu, but more marked for Tl (Fig. 2). No significant difference in moss growth rates ($p > 0.05$) and biomass production was observed between *P. schreberi* and *P. crista-castrensis* collected on the same site (Sup. Info. Table S3). Growth was significantly higher on site SS4 than at the two other sites (SS1 and SS3) ($p < 0.05$ for both *P. schreberi* and *P. crista-castrensis*).

3.2.Effect of moss color and canopy openness on elemental concentrations.

The effects of canopy and moss stem color were tested using samples collected on site SS3 during summer of 2016 (Fig. 1, Table 1, and Table 2). For both moss species, stem color had no significant effect on the concentration of most measured elements ($p > 0.05$), with the notable exception of Cu, Zn, As, and Ba ($p < 0.05$ for all) for *P. schreberi* and Ti, Cu, Zn, As, Mo and Ag

($p < 0.05$ for all) for *P. crista-castrensis* (Sup. Info. Table S4a and S4b). In both species, concentrations were higher in yellow stems than green ones for Ti, while the opposite was observed for Cu, Zn, As, Mo, Ag, and Ba. For Ag and Ba, the effect of stem color on metal concentration was not consistent between species and plots (forest versus windfall, Sup. Info. Table S4a). These differences in elemental concentrations between green and yellow stems were below 30% for most elements, except for Ti (-45% in yellow stems), Mo (-36%), and Ag (-62%) in *P. crista-castrensis*, and Ba (-57%) in *P. schreberi* (Fig. 3).

Expect for Ni, Zn, Tl, and Pb ($p < 0.05$ for all) for *P. schreberi* and Ni, Mo and Tl ($p < 0.05$ for all) for *P. crista-castrensis*, no significant differences were observed in elemental concentrations between moss samples collected in a mature forest (closed canopy) and in a nearby windfall (open canopy) ($p > 0.05$, see Sup. Info. Table S4b). The effect of canopy openness, while statistically significant, was small for Zn, Mo and Pb with differences lower than 30% (Fig. 4). For Ni and Tl, differences were more noticeable (up to 50 and 75%, respectively). Contrary to data reported in Sup. Info. Table S2b, significant differences in elemental concentrations between species were observed at site SS3 ($p < 0.05$, Sup. Info. Table S4a). Al, Ti, V, Fe, Mo, Sb, and Tl were more abundant in *P. schreberi* than *in P. crista-castrensis*, while Mn and Zn were more abundant in *P. crista-castrensis.* The higher number of elements with significant differences between species on site SS3, as compared to data on the whole transect, likely reflects differences in sample size between the two data-sets (n= 8 for site SS3 versus n = 3 for other sites of the transect, Table 2) allowing for more robust identification of subtle differences in elemental concentrations between species on site SS3. While significant, due to higher replication, these differences between species did not exceed 30%, except for Tl and Al (Sup. Info. Table S4a).

3.3.Variation in moss elemental concentrations during the growing season.

In both moss species, elemental concentrations varied significantly during the growing season for Fe and Sb ($p < 0.05$) for *P. schreberi* and Ti and V ($p < 0.05$) for *P. crista-castrensis* (Sup. Info. Table S5a and S5b). Variations were moderate for Ti, V, and Fe (below 30%, for *P. crista-castrensis* and below 50% for *P. schreberi*) but more marked for Sb (up to 100%). Concentrations of Ti, V, and Fe increased in the early season (May to July) and then remained roughly constant afterward (Fig. 5). Sb concentration was roughly constant throughout the season with a marked peak in July.

3.4.*Variation in moss elemental concentrations along the moss stem.*

In both species, elemental concentrations were significantly different between sections along the moss stem for all the elements ($p < 0.05$) except for As ($p = 0.07$ for *P. schreberi* and $p = 0.2$ for *P. crista-castrensis*) and Mo ($p = 0.06$ for *P. schreberi* and $p = 0.1$ for *P. crista-castrensis*) in both species, and Ti ($p = 0.1$), Cr ($p = 0.05$), and Co ($p = 0.4$) in *P. crista-castrensis* (Sup. Info. Tables S6a, S6b, and S6c). For most elements (Al, Ti, V, Cr, Fe, Ni, Zn, Ag, Cd, Sb, Ba, and Pb), a general trend of increasing concentration with stem depth was observed. The opposite trend was observed for Mn, and no clear trend with distance from the apex was observed for Co (Fig. 6). Trends along the stem were comparable between species, with the notable exception of Cu that accumulated significantly more in the mid-sections of *P. crista-castrensis* and Tl in the mid sections of *P. schreberi*. Significant differences in elemental concentration along the stem were observed between *P. crista-castrensis* and *P. schreberi* for Al, Ti, V, Mn, Fe, Co, Ni, Cu, Sb, Ba, Tl, and Pb. Concentrations of Al, Ti, V, Fe, and Sb were higher in the lower sections (< 2.5 – 3 cm) of the stems of *P. schreberi* than *P. crista-castrensis,* while the opposite was observed for Mn, Co, Ni, and Ba. Cu and Pb were more abundant in the mid-sections of *P. crista-castrensis* than *P. schreberi,* while the opposite was observed for Tl.

3.5.Elemental baseline for Pleurozium schreberi in eastern Canada.

Elemental concentrations of *P. schreberi* sampled in remote sites (Fig. 1, black triangles) are summarized and compared to data from the literature in Table 3 and presented in detail in Table S7. Overall, concentrations measured in remote sites from eastern Canada are in the lower range of concentrations reported in feather mosses from low deposition areas in northern Europe and Asia (Berg and Steinnes, 1997; Ermakova et al., 2004; Harmens et al., 2013), and comparable to data reported for *H. splendens* in Nunavut, Canada (Chiarenzelli et al., 2001).

4. Discussion

We evaluated the influence of five selected factors on elemental concentrations in two dominant feather moss species in Quebec (Eastern Canada) in an attempt to refine sampling protocols for future community-based sampling supporting biomonitoring initiatives in the region. Among these five factors, only stem length had a significant and substantial impact on elemental concentrations in both *Pleurozium schreberi* and *Ptilium crista-castrensis*. Species identity, stem color, canopy openness, and the time of sampling had a limited influence on elemental composition. Only a few elements were significantly affected with effects on concentrations often low (< 30%). This suggests that both species can be used interchangeably and that efforts to control for stem color, time of sampling, and site (open versus closed canopy) are not mandatory for biomonitoring in the region. However, because the concentration of many elements varied greatly along the stem, even in the green section, standardizing the length of the stem to be analyzed is advisable. Such standardization can be performed in the laboratory before analysis

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and does not require adding constraints on the sampling protocol provided to participants. These conclusions are discussed at length below.

4.1. Effect of species identity on moss elemental records

Establishing a large-scale biomonitoring program comes with several challenges, such as the availability of the biomonitor. Using several biomonitors bears some merits but can also present some challenges if they record elements differently. Bryophytes elemental concentrations can be affected by sets of factors associated with the sampling site (and microsites), such as local climatic conditions, forest composition, and factors associated with the bryophyte physiology such as moss morphological traits (e.g., specific surface area, leaves shape and size) biogeochemical niches, and chemical (e.g., pH, CEC) properties (Dołęgowska and Migaszewski, 2019; Fernández-Martínez et al., 2021b, 2021a; Gałuszka, 2007; Kłos et al., 2012; Stanković et al., 2018). Concentrations can differ significantly between species even if they are collected on the same site. Thus, while using multiple moss species offers flexibility for the sampling of large geographic areas, the ability of the species to report concordant information on deposition needs to be tested. The interchangeability of *P. schreberi* and *H. splendens* has been documented (Berg and Steinnes, 1997; Thöni et al., 1996). On the contrary, marked differences between *Pseudoscleropodium purum* and *Hypnum cupressiforme* collected on the same sites have been reported and were attributed to differences in morphology and specific surface area (Carballeira et al., 2008). Here, we report that *P. schreberi* and *P. crista-castrensis*, sampled on the same sites, achieved similar elemental concentrations along a 1000 km latitudinal transect. While for a few elements significant differences in moss concentrations were observed between samples of *P. schreberi* and *P. crista-castrensis* collected on the same sites, these differences were small (< 20-30%) when compared to the scale of concentrations (order of magnitudes) used to report data in

large-scale biomonitoring programs. Thus, we argue that these two species can be considered as equivalent for elemental monitoring. This is likely due to the similarities between these two species. *P. schreberi* and *P. crista-castrensis* occupy the same ecological niche in black spruce forests where they are often found in mix-carpets. They also exhibit comparable physical and chemical properties (i.e., specific surface area (Sup. Info. Table S3), pH, CEC, data not shown).

Variations in the growth rate of bryophytes can affect elemental records. In *Abietinella abietina*, collected on the same site, higher biomass production rates during the growing season were associated with decreased concentration of essential (i.e., Mo, Cu) and non-essential (i.e., Pb and Hg) elements in tissues (Zechmeister et al., 2003). In *Scleropodium purum*, dilution of elements during periods of higher biomass production was also proposed to drive seasonal variations (Leblond et al., 2004). The time resolution of our growth rate measurements for *P. schreberi* and *P. crista-castrensis* precludes any interpretation of the effect of biomass production on the seasonal variation of elemental concentrations on a given site (see 4.4). On the same site, both species achieved comparable biomass production rates, suggesting that growth rate is likely not a significant factor of variation in elemental concentrations between these two species. However, the growth rates of both species varied significantly between sites (up to 2 folds) (Sup. Info. Table S3). In the face of the many factors affecting stoichiometric homeostasis in bryophytes (Fernández-Martínez et al., 2021b; Liu et al., 2023; Waite and Sack, 2011), the limited number of sites where growth was measured limits our ability to test the effect of growth rate on the variation of elemental concentrations between sites. Nonetheless, we argue that, based on our observations, for most tested elements, *P. schreberi* and *P. crista-castrensis* can be considered equivalent for biomonitoring purposes. Further investigation on the effect of moss growth rates

is, however, needed to conclude on its potential effect on elemental concentrations recorded on different sites by *P. schreberi* and *P. crista-castrensis* in the region.

4.2. Effect of stem color on moss elemental records.

The color of bryophytes is, in large part, determined by the composition and concentration of pigments (e.g., chlorophylls, carotenoids) and other colored compounds (e.g., flavonoids, proanthocyanidins) (Mårtensson and Nilsson, 1974). Several factors drive the concentration and composition of colored compounds in plants. Environmental factors include, but are not limited to, humidity, light intensity, and macronutrient stress (i.e., nitrogen) (Baxter et al., 1992; Hyyryläinen et al., 2015; Li et al., 2018; Mu and Chen, 2021). For instance, in a study from Eastern Canada, moss stem color was associated with N concentration (Darnajoux et al., 2018). This was interpreted as the result of variation in pigments concentration due to differences in light exposure, with moss stems exposed to strong light exhibited, on average, stems with a lighter green and more yellowish color than moss stems exposed to low light intensity. The effect on the concentration of other elements, notably elements involved in colored compounds biosynthesis was not tested. Metals availability can also affect colored compounds content. Because metals play an important role in pigment biosynthesis, metal deficiency can lead to discoloration of leaves (chlorosis) (Myśliwa-Kurdziel and Strzałka, 2002). On the other hand, high exposure to metals can affect the composition of colored compounds through the generation of reactive oxidative species (ROS). Many pigments are ROS-sensitive and many colored compounds are involved in the management of oxidative stress (AYDOĞAN et al., 2017; Chen, 2015; Salbitani et al., 2023). In bryophytes, reports on the effect of exposure to metals on pigments (i.e., chlorophyll) are contradictory, some studies reported an association while others showed no effect (Sun et al., 2009; Świsłowski et al., 2020). This suggests that the effect of

metal exposure on pigments concentration is species and metal dependent. The color of moss stems is highly variable within a given carpet, but color diversity is rarely if ever included in sampling strategies. Community science can facilitate sample collection in large-scale biomonitoring campaigns. It is the basis of the Bryomonitoring initiative (https://bryomonitoring.ca) aiming at establishing a pan-Canadian biomonitoring survey of atmospheric depositions. Such an approach requires providing clear instructions to volunteers to limit biases during sampling. If color influences moss elemental concentrations, extra care will need to be taken during sampling by both professionals and volunteers. Our data show that, for the analyzed elements, stem color had no or limited impact on *P. schreberi* and *P. crista-castrensis* elemental concentrations in the studied region. Differences, when significant, were small (< 30%). In the studied area, environmental factors (i.e., humidity, and light exposure) are most likely the main drivers of stem color variation within and between sites. Metal depositions are low (Darnajoux et al., 2015) and are unlikely to lead to significant toxicity that could affect color. Nonetheless, we conclude that based on our observations, efforts to control for stem color during citizen-based sampling do not appear essential in the region, as it would add unnecessary complexity to the sampling protocols that need to be as simple as possible to promote participation. However, the significant effects observed for some specific elements highlight that moss stem color could still be a parameter to consider in specific contexts, especially if it affects the record of elements of interest (i.e., highly toxic) for environmental health assessment.

4.3. Effect of canopy openness on moss elemental records.

Collecting samples in open areas is considered a sound sampling method in biomonitoring as it allows for avoiding potential canopy effects (Čeburnis and Steinnes, 2000; Frontasyeva and Harmens, 2020). Indeed, trees have been reported to act as filters of atmospheric deposition,

affecting elemental concentration and speciation (Gandois et al., 2010; Tan et al., 2018). The effect of tree canopy on the composition and intensity of N, S, and Hg deposition, for instance, is well documented (Bredemeier, 1988; Iverfeldt, 1991; Macinnis-Ng et al., 2012; Zhao et al., 2017). However, a large part of Eastern Canada is covered by the boreal forest where forest openings result mostly from wildfire, insect outbreaks, clearcuts, and windfall. Finding open areas can prove difficult, especially above the 49th parallel where the sparse road network limits easy access to a large part of the land mass. Here, canopy openness had a significant effect only on a few analyzed elements such as Ni, Zn, Mo, Tl, and Pb (Fig. 4). While this confirms that the canopy can affect, in one way or another, elemental concentrations in moss, differences between species were small for most of these elements (<30%). Thus, for biomonitoring purposes, where the primary goal is to report trends in moss metal concentrations between sites, sampling *P. schreberi* and *P. crista-castrensis* under the canopy of black spruce seems a viable approach in eastern black spruce forest when proper plots (e.g., windfalls plots) are not available in the vicinity of the sampling area.

4.4. Effect of sampling time on moss elemental records.

It is well documented that elemental concentrations and distribution between cell compartments in biomonitors can vary over the course of the year due to seasonal variations in climate, patterns in atmospheric deposition, and the physiology of the biomonitors (Fernández et al., 2013; Kłos et al., 2018; Oishi, 2023). Many biomonitoring studies overcome this challenge by constraining the sampling period (within a few weeks) or by performing temporally intensive sampling where the same site is visited many times. In community-based biomonitoring programs, sampling can span over a large period, typically during the summer season. For instance, ICP-Vegetation protocols for the biomonitoring of heavy metals recommend sampling between April to October

(Frontasyeva and Harmens, 2020, https://icpvegetation.ceh.ac.uk/). In Eastern Canada, *P. schreberi* and *P. crista-castrensis* growth is constrained to a short period between roughly May to September. This growing season is characterized by two wet periods, in early and later summer, and a dryer period in the middle of summer. Precipitations (intensity and frequency) affect both moss growth and elemental deposition (quantity and speciation). A recent study reported that, in the moss *Calohypnum plumiforme*, N concentration in wet depositions was best recorded by moss during spring and autumn (Oishi, 2023). During the summer, moss growth affected the quality of the relationship between moss N concentration and N concentrations in wet depositions. In the winter, N records were affected by the low retention efficiency of the moss for snow N (Forsum et al., 2008). It is likely, that seasonal variation affects, at various intensities, the concentration of most elements in moss (Klavina et al., 2018; Markert and Weckert, 1989). Unsurprisingly, the concentrations of almost half of the analyzed elements were significantly affected by the season in both *P. schreberi* and *P. crista-castrensis*. Constraining the sampling period allows for reducing these seasonal biases by reducing the variability in collected samples. For large-scale, citizen-based sampling, reducing the period of sampling likely comes at the expense of the number of sites sampled and thus geographic coverage. Considering the effect of the time of sampling on elemental concentrations in *P. schreberi* and *P. crista-castrensis* in the studied region, which was moderate for most elements, the pros of maintaining a longer period of sampling likely outweigh the cons.

4.5. Effect of stem length selection on moss elemental records.

Most biomonitoring protocols recommend removing the brown part of moss before analysis (Harmens et al., 2013), as this section is subject to elemental loss or gain (e.g., elements sorption from surrounding soil) by the decaying moss tissues (Leblond et al., 2004). Our data show that

element concentrations vary, sometimes markedly, with stem length even in the green section (see Fig. 6). Thus, standardizing the length of the green section to be analyzed is important to limit variability in the collected data, especially when comparing samples from different sites or from the same site in long-term biomonitoring initiatives. For citizen-science-based sampling, the selection of the proper length can be performed in the lab after sampling to limit constraints on volunteers. Evaluating moss growth rate ($cm.yr^{-1}$) can further guide the selection of the length of moss stem to be analyzed by allowing standardization of sampling based on a desired exposure time (e.g. one year, two years). In Eastern Canada black spruce forests, *P. schreberi* and *P. crista-castrensis* collected on the same site, exhibited comparable growth rates (Sup. Info. Table S3). The analysis of moss stems of the same length thus informs on the accumulation of elements over a comparable period in both species. However, because growth rates varied significantly between sites (Sup. Info. Table S3), stems with the same length represent different times of exposure between sites.

4.6. Elemental baseline for P. schreberi in Eastern Canada black spruce forest.

Establishing baseline elemental concentration in biomonitors of interest is an important initial step for the viability of long-term biomonitoring initiatives. Baseline data for bryophytes and lichens in Canada are limited (Chiarenzelli et al., 1997; Cowden and Aherne, 2019; Klapstein et al., 2020; McDonough et al., 2022, Darnajoux et al., 2015; Nash and Gries, 1995). The geology of Canada, which affects elemental baseline, is very contrasted. It ranges from the Canadian Shield in the East, the western Canada sedimentary basin (cretaceous) in the center to the North American Cordillera in the far west. This diversity in geological backgrounds calls for the establishment of regional baselines. Data in Eastern Canada are particularly scarce. To the best of our knowledge, only one large-scale biomonitoring study reported baseline data from Eastern

Canada using cyanolichens from the genus *Peltigera* (Darnajoux et al., 2015). Thus, the data reported here (Table 3) represent the first large-scale determination of background elemental concentration in *P. schreberi* in Eastern Canada black spruce forest. It will serve as a reference for future biomonitoring surveys in the region and comparison with other studies. Concentrations measured for all analyzed elements are in the very low range of concentrations reported by the European ICP-Vegetation program. This is consistent with another study on lichens reporting that most of the Eastern Canadian boreal forest remains relatively pristine (Darnajoux et al., 2015). These low values likely reflect the limited anthropogenic activities in the region and the very low population density. With global climate change, new opportunities are arising for the northward expansion of human activities and populations in Eastern Canada. Data reported in this study provide a valuable baseline for future assessment of atmospheric deposition in the region. Besides migration of human populations and activities, global climate changes also affect global patterns of contaminants dispersion (Vorkamp et al., 2022; Wong et al., 2021). Notably, wildfire frequency and intensity are expected to increase in boreal regions (Gauthier et al., 2015) as illustrated by the exceptional 2023 wildfire season in Canada. During the summer of 2023, more than 1.5M hectares of forest have burned in Quebec alone, almost a hundred times the 10-year annual average (Société de protection des forêts contre le feu, https://sopfeu.qc.ca). Wildfires are a source of inorganic (e.g., metals and metalloids) and organic contaminants (e.g., PAH) to the atmosphere (Mccarter et al., 2023; Pacifico et al., 2023). Data reported in this study provide a valuable reference for future evaluations of the impact of these exceptional wildfires on element dispersion in the region.

4.7. Limitations of the study.

Sampling, storage, and preparation methods can affect elemental measurements in moss biomonitoring (Aboal et al., 2017; Dołęgowska and Migaszewski, 2020, 2019). This illustrates the need for standardized protocols in biomonitoring to facilitate the comparison of data between studies and locations. The present study was built on an in-house sample collection gathered over a few years (2016-2019) throughout several projects on trace metal biogeochemistry in bryophytes. While suitable for metal assessment in moss, sampling, storage, and preparation protocols, were not specifically chosen for biomonitoring purposes. Several aspects of the protocols could be improved to reduce uncertainty in elemental measurement and, especially, facilitate comparison with other studies. Notably, samples were dry cleaned after storage which was reported to result in higher uncertainties for some elements than wet cleaning (Dołęgowska and Migaszewski, 2020). Furthermore, all sites included in this study were located in an area with low atmospheric deposition of contaminants of anthropogenic origin. Thus, it is not excluded that some effects, i.e., species and canopy openness, that had limited impact on moss elemental concentration in this study could be more pronounced in more polluted areas. For instance, if species exhibit different maximum sorption capacities, differences in elemental concentrations between species could arise when deposition intensity approaches these maxima. Similarly, trees growing on contaminated soil could contribute more to elements transfer to the moss (through the throughfall), enhancing a canopy effect otherwise minor in more pristine settings. Moreover, anthropogenic inputs in atmospheric depositions could alter the chemical and physical speciation of trace metals, thus their bioavailability and their uptake by the leaves, leading to changes in throughfall composition (Gandois et al., 2010). It is also noteworthy that all sites included in this study were located in mature black spruce forests, the dominant boreal forest type in Eastern Canada. Tree diversity and species identity can affect throughfall composition (Zhang et al., 2022). One cannot exclude that tree canopy could have a more pronounced effect on moss

elemental concentration in other forest stands dominated by other tree species or characterized by higher species diversity.

5. Conclusion

In this study, we evaluated the potential of *P. schreberi* and *P. crista-castrensis* as biomonitors for large-scale monitoring of atmospheric depositions in Eastern Canadian black spruce forests. We evaluated the effect of moss characteristics (species identity, stem color, growth rate, specific surface) and sampling strategy (e.g., time of sampling, sampling in an open area) on moss elemental concentrations. We conclude that both species can be used interchangeably for biomonitoring purposes, as moss sampled on the same sites achieved comparable elemental concentrations. We also report that sampling under the tree canopy, versus an open area, has a limited effect on moss elemental concentrations in the studied area. In eastern Canada (Quebec) black spruce forests, where access to sampling sites is constrained by the sparse road network and the availability of open areas is low, sampling under the canopy can offer flexibility in sampling. We further report that evaluating moss growth and standardizing stem length allows better constraining the period of exposure and improve comparison of data between sampling sites. Finally, data reported here, covering a significant fraction of Quebec's black spruce forest provides valuable information on bryophytes elemental concentrations in a region where such data are scarce. These data will serve as a reference for future studies on the effect of human development and global climate change on elemental dispersion in the region.

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Acknowledgments

This research was supported by a NSERC Alliance grant (ALLRP 571800-21). The authors thank Stéphane Tremblay and Martin Barrette for the collection of samples used to establish the moss elemental baseline. Samples were collected as part of project # 112332056 from the Direction de la recherche forestière of the Ministère des ressources naturelles et des forêts de Québec.

Figure captions:

Figure 1. Localization of sampling sites. Sites used to test the effect of species identity, moss color, canopy openness, season, and moss length on moss elemental concentrations are reported with white dots. Sites used to establish an elemental baseline in *P. schreberi* are reported with black triangles.

Figure 2. Comparison of elemental concentration between *P. schreberi* and *P. crista-castrensis* collected along a 1000 Km latitudinal transect. Only elements with significant differences ($p < 0.05$) are shown (detailed data are available in Tables S2a and S2b). The solid line represents identical concentration, and the dashed lines indicate a 30% difference threshold.

Figure 3. Effect of canopy stem color on *P. schreberi* and *P. crista-castrensis* elemental concentrations. Only elements with significant differences ($p < 0.05$) are shown (detailed data are available in Sup. Info. Tables S4a and S4b.). Data are reported as a percentage of variation in yellow stems compared to green stems. All samples were collected in SS3 (see Fig. 1 and Tables 1 and 2 for details).

Figure 4. Effect of canopy openness on *P. schreberi* and *P. crista-castrensis* elemental concentrations. Only elements with significant differences ($p < 0.05$) are shown (detailed data are

available in Sup. Info. Tables S4a and S4b). Data are reported as a percentage of variation in moss collected in the windfall compared to moss collected in nearby mature black spruce forests. All samples were collected in SS3 (see Fig. 1 and Tables 1 and 2 for details).

Figure 5. Variation in elemental concentrations in *P. schreberi* and *P. crista-castrensis* along the growing season. Only elements with significant differences ($p < 0.05$) are shown (detailed data are available in Sup. Info Tables S5a. and S5b.). Data are reported as a percentage of variation normalized to values measured in September. All samples were collected in SS3 (see Fig. 1 and Table 2).

Figure 6. Variation in elemental concentrations along the stem of *P. schreberi* and *P. crista-castrensis.* Only elements with significant differences ($p < 0.05$) are shown (detailed data are available in Sup. Info. Tables S6a and S6b.). Data are reported as a percentage of variation normalized to concentration measured at 6 cm from the apex. All samples were collected in SS3 (see Fig. 1 and Tables 1 and 2).

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Author Contributions Statement

Jean-Philippe Bellenger and Romain Darnajoux conceived the project. Jean-Philippe Bellenger and Daniel Houle supervised the development of the project. Charlotte Blasi, Romain Darnajoux, Pauline Le Monier, Marie Renaudin and Gaëlle Vacherand acquired the data. Laurie Michel analyzed the data. Jean-Philippe Bellenger and Laurie Michel wrote the manuscript and all authors contributed to editing and approved of the manuscript.

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Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Figure 1

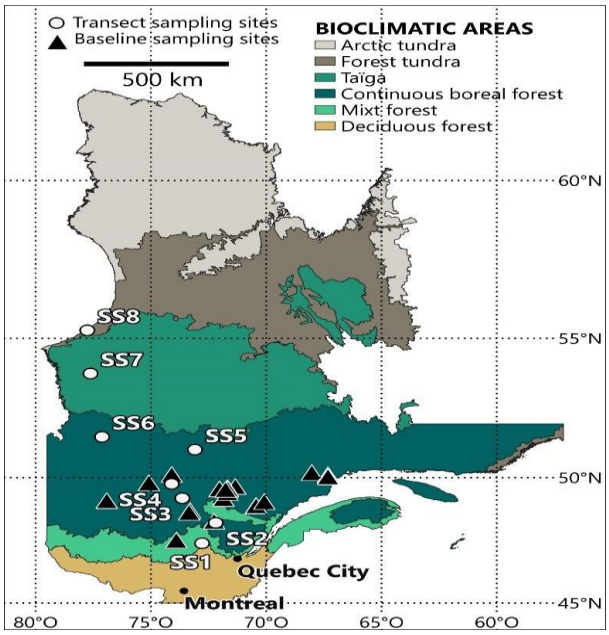

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Figure 2

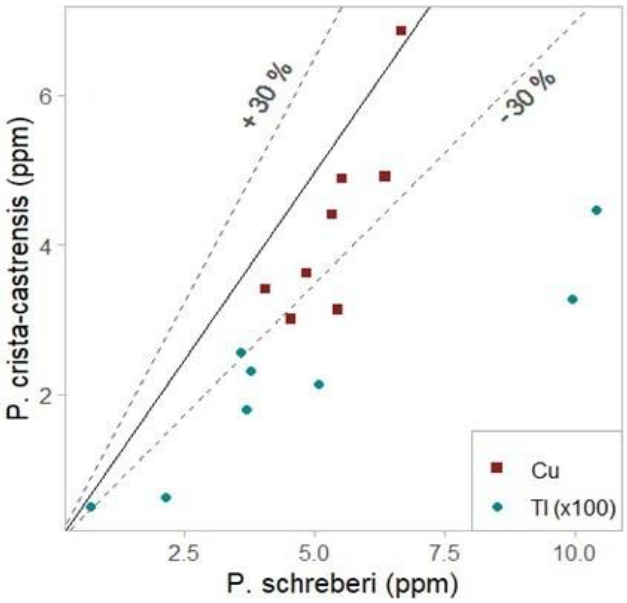

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Figure 3

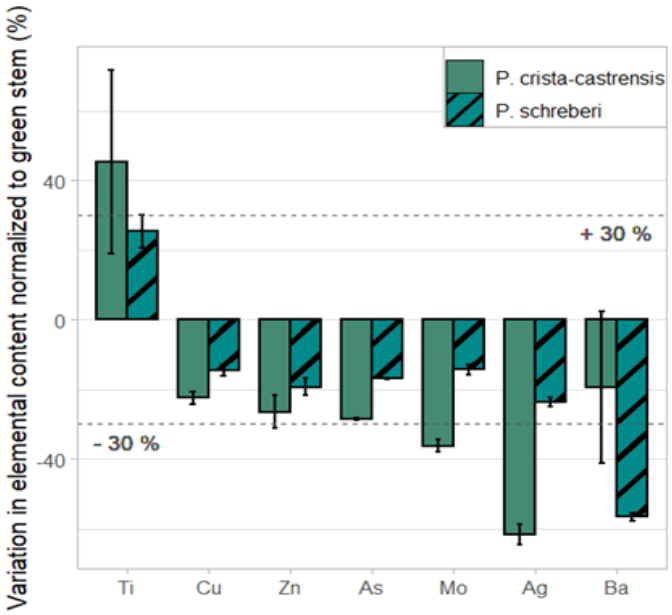

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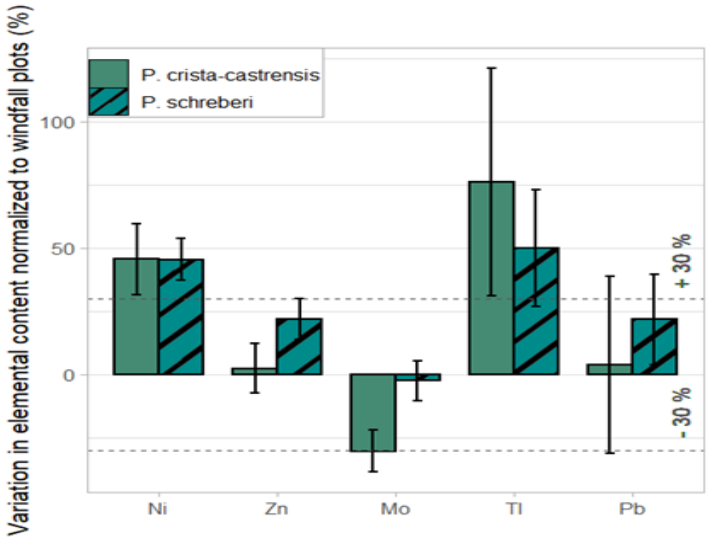
Figure 4



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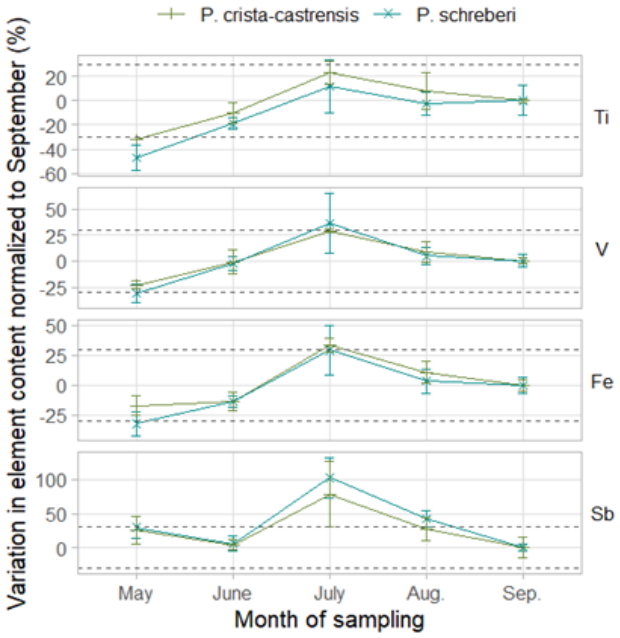

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Figure 6

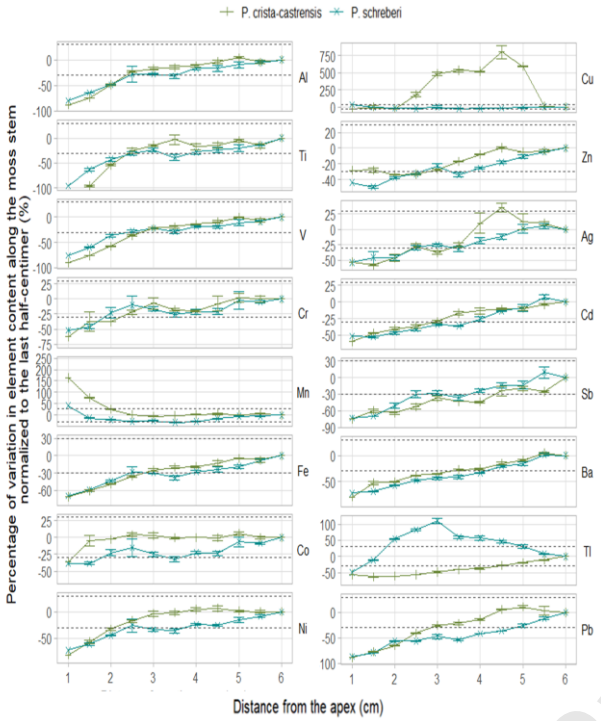

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Table 1. Description of sites characteristics and coordinates

Sampling Site	Site coordinates	Tree density	Vegetation type
SS1	N47° 54.099' W72° 57.194'	30	Balsam fir – white birch forest
SS2	N48° 20.801' W72° 22.108'	32	
SS3	N49° 12.757' W73° 38.923'	41	
SS4	N50° 04.036' W73° 58.230'	60	Spruce moss forest
SS5	N51° 08.034' W72° 51.181'	70	
SS6	N51° 09.803' W77° 54.668'	19	
SS7	N53° 46.310' W77° 58.456'	48	Spruce lichen fores
SS8	N55° 35.390' W77° 66.317'	30	

Tree density was determined by counting the total number of trees (deciduous and coniferous) taller than 1 m on each sampling site on 150x150m plots.

Table 2. Summary of samples used to test the effect of species identity, stem color, canopy openness, season, and stem length on elemental content in *P. schreberi* and *P crista-castrensis*.

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	Site	Year	Sampling time	*n*	Statistical model
Species	SS1 à SS8	2018-2019	September	3	Wilcoxon
Color	SS3	2016	Summer	8	Wilcoxon
Opening	SS3	2016	Summer	8	Wilcoxon
Season	SS3	2016	May to September	3	Kruskal-Wallis
Stem length	SS3	2019	September	3	Kruskal-Wallis

Table 3. Elemental contents of *P. schreberi* from remote sites in Northern Quebec (black triangles, Fig. 1) compared to the literature.

	This study Quebec, Canada (2016-2017)	**Nunavut, Canada (Chiarenzelli et al., 2001)**	**Nansvatn, Norway (Berg and Steinnes, 1997)**	**Norway (Harmens et al., 2013)**	**Background from Tula, Russia (Ermakova et al., 2004)**
	P. schreberi	*H. splendens*	*P. schreberi*	*H. splendens*	*P. schreberi*
	Mean (SD)	Mean	Mean (SD)	Mean	Mean
	Min – Max			Min – Max	
Al	345 (302)	N/A	N/A	346	585
	60 - 954			46 - 4581	
V	0.4 (0.4)	0.72	0.66 (0.28)	1.76	1.6
	0.1 - 1.8			0.29 – 25.9	

Cr	3 (7)	1.1	0.34 (0.19)	0.98	0.7
	0.2 - 34			0.16 – 47.9	
Mn	414 (175)	N/A	260 (90)	N/A	150
	183 – 884				
Fe	296 (283)	210	190 (130)	449	400
	66 – 1206			27 - 24584	
Co	0.10 (0.08)	0.12	0.17 (0.08)	N/A	0.2
	0.02 – 0.35				
Ni	2.2 (1.6)	2.32	1.2 (0.6)	5.4	1.4
	0.7 - 5.8			0.15 – 857	
Cu	5.0 (1.0)	1.93	2.6 (0.3)	6.43	4.5
	3.7 – 7.6			1.38 – 443	
Zn	38 (12)	20	28 (10)	35.9	24
	17 – 65			7.4 - 368	
As	0.12 (0.05)	0.20	0.057 (0.001)	0.18	0.1
	0.07 – 0.32			0.02 – 4.84	

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Mo	0.2 (0.2) 0.04 – 0.9	0.24	0.028 (0.013)	N/A	0.16
Ag	0.03 (0.02) 0.009 – 0.08	N/A	N/A	N/A	N/A
Cd	0.11 (0.06) 0.04 – 0.24	0.09	0.091 (0.044)	0.12 0.009 – 1.87	0.12
Sb	0.028 (0.009) 0.015 – 0.047	0.03	0.018 (0.007)	0.092 <0.001 – 1.17	0.06
Ba	30 (16) 10 - 68	21.8	19 (3)	N/A	16
Pb	0.9 (0.5) 0.4 – 2.9	1.87	1.7 (0.2)	2.29 0.33 – 20.8	5.0

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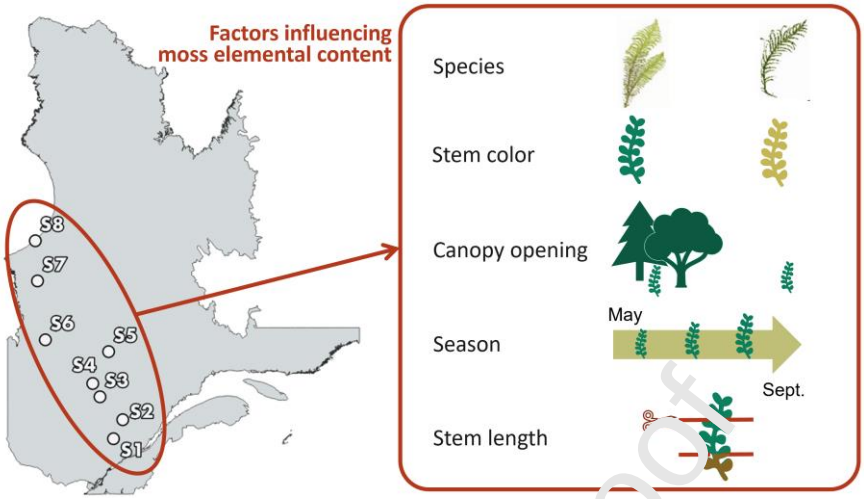
Graphical abstract



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Highlights

- Factors affecting moss elemental content were evaluated in Eastern Canada.
- Species, color, canopy openness, season, and stem length were investigated.
- All factors had significant effects, but for most differences were moderate (<30%).
- Only stem length had a significant and important effect on moss elemental content.
- Moss elemental concentrations were compared to the literature for remote areas.

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