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MOSSES AS BIOINDICATORS OF AIR POLLUTION ALONG AN URBAN–AGRICULTURAL TRANSECT IN THE CREDIT RIVER WATERSHED, SOUTHERN ONTARIO, CANADA

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ABSTRACT – The activities associated with urbanization, such as vehicular traffic and industrial processes, lead to elevated emissions of atmospheric pollutants. Measuring the spatial extent of these pollutants is pivotal to identifying areas of concern and assessing mitigation measures. The objective of this study was to evaluate the relative deposition of heavy metals and nitrogen using moss species along an urban–agricultural transition in the Credit River Watershed, southern Ontario. Thirteen species of moss were collected from Sugar Maple (*Acer saccharum*) dominated forest stands across the study area, with only one moss species (*Atrichum altercristatum*) commonly occurring. Heavy metal concentrations were variable between species; the Coefficient of Variation (CV) for the majority of metals (Al, V, Cr, Fe, Ni, As, Sb and Pb) was greater than ~50% across species. Nonetheless, metals exhibited similar trends, with the highest concentrations for Fe, followed by Al > Zn > Cu > Pb > Cr > Ni > V > As > Cd > Sb > Hg across species. Heavy metal concentrations in *Atrichum altercristatum* exhibited lower variability between sites, with CV < 33% for most metals (Cu, Zn, As, Cd, Sb, Pb and Hg). Further, many metal concentrations were strongly correlated (e.g., Al, V, Cr, Fe, and As; $r \leq 0.90$) suggesting common emission sources, such as wind blown dust from agricultural activities or vehicular traffic, both predominant throughout the watershed.

KEYWORDS: BIOMONITORING, BRYOPHYTES, *ATRICHUM ALTERCRISTATUM*, ATMOSPHERIC DEPOSITION

INTRODUCTION

Urbanization is the expansion and development of cities under growing populations and migration from rural to urban environments (Freedman, 2010). As urbanization increases, the intensity of associated industrial activities and vehicular traffic also increase (Watmough et al., 1998; Freedman, 2010). This leads to elevated emissions of atmospheric pollutants such as sulphur dioxide (SO_2), nitrogen dioxide (NO_2), ammonia (NH_3) and heavy metals (Percy & Feretti, 2004). Southern Ontario is the most densely populated region in Canada; the majority of the population live within the Greater Toronto Area (GTA), which has 8.1 million registered vehicles as of 2013 (Statistics Canada, 2014a). Traffic related emissions are responsible for more than 50% of the atmospheric NO_2 concentrations (Ministry of the Environment and Climate Change, 2012), and have been linked to human health impacts (Toronto Public Health, 2007). Further, these pollutants can impact on natural ecosystems leading to acidification, eutrophication and deposition of heavy metals, which can decrease productivity, increase mortality rates and lower species diversity (Freedman, 2010; vanLoon & Duffy, 2011).

Measuring the spatial extent of atmospheric pollutants is integral to identifying areas of concern and assessing regional response to mitigation measures. Bryophytes (mosses) have been widely used as bioindicators of air pollution (Bates, 2009; Rühling, 2002), providing insight into the degree of ecosystem stress (Correa Mazzoni et al., 2012). Since mosses receive their nutrients predominantly from dust-fall and precipitation, they provide a reliable measure of atmospheric pollution deposition (Harmens et al., 2010; 2015; Rühling,

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2002). The collection and analysis of mosses has become a regular practice in biomonitoring programmes across Europe since 1990 (Harmens et al., 2010; 2015; Rühling, 2002; Rühling & Tyler, 2004) for heavy metals and since 2005 for nitrogen (Harmens et al., 2011). Further, studies have shown that heavy metal concentrations in mosses correlate with atmospheric deposition values measured in precipitation monitoring networks, establishing moss as an effective and efficient biomonitoring tool (Berg & Steinnes, 1997; Dragovic & Mihailovic, 2009). For nitrogen, the relationship appears to vary with nitrogen speciation (Pitcairn et al., 2006) and seems to saturate at deposition rates of approximately 15–20 kg N ha^{-1} yr^{-1} (Harmens et al., 2011; 2014).

The objective of this study was to evaluate the concentration of heavy metals and nitrogen in moss species along an urban–agricultural transect in the Credit River Watershed, southern Ontario, Canada. Mosses were sampled from Sugar Maple (*Acer saccharum*) dominated forest stands located within the watershed boundaries.

Materials and methods

Study area

The Credit Valley Conservation Authority (CVC) protects the health and integrity of the Credit River Watershed, in southern Ontario, which encompasses an area of 86,000 ha. The upper boundary begins north of Orangeville (43°56.29’ N, 80°9.139’W) and continues southeast for approximately 90 km, where it drains into Lake Ontario (Credit Valley Conservation, 2009). Due to its long and thin shape, (the widest part measuring approximately 28 km), the watershed was subdivided into three regions; upper, middle and lower (Figure 1). The watershed incorporates many major municipalities, such as: Brampton (population: 525,000) and Mississauga (population: 700,000; Statistics Canada, 2014b). In addition, high capacity highways cross the watershed, e.g., the MacDonald-Cartier Freeway (401) with an annual average daily traffic (AADT) volume of 156,967 and the Queen Elizabeth Way (QEW) with an AADT of 150,567 (MTO, 2010).

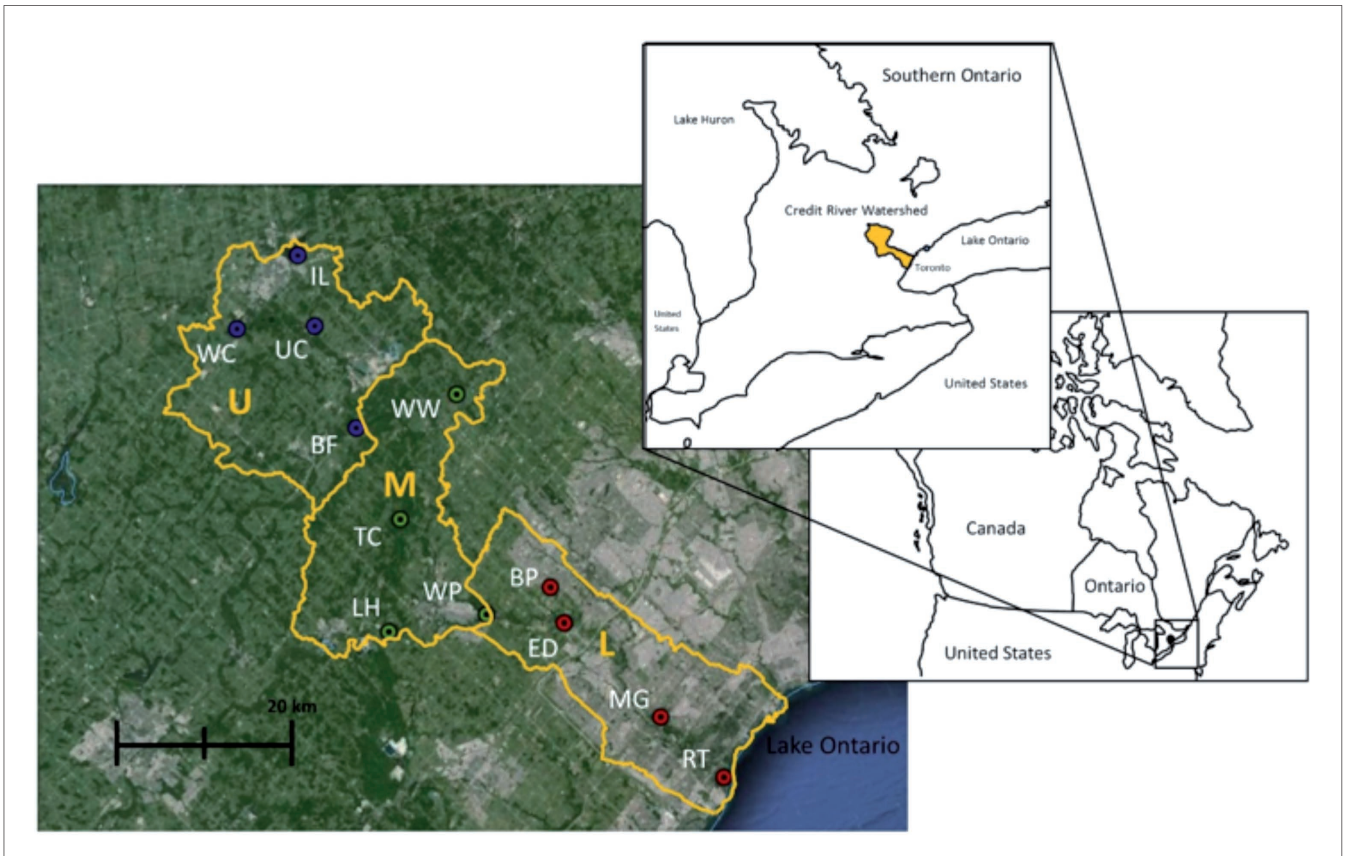

Fig. 1. Location of southern Ontario in Canada and the location of the Credit River Watershed. Upper, middle and lower regions of the watershed are depicted as U, M and L letters, respectively. Upper (blue), Middle (green) and Lower (red) site are depicted as circle marks (n = 12: four per watershed region). See Table 1 for site abbreviations.

The three watershed sub-regions embody multiple types of land use; the dominant land covers are urban, agricultural and natural areas, which include both terrestrial and aquatic systems (Credit Valley Conservation, 2013). This represents a south–north transition from urban to rural agricultural land use along the Credit River Watershed. The upper region is comprised of 15% urban, 43% agricultural and 42% natural land use, the middle region is 14% urban, 41% agricultural and 44% natural, and the lower region is 56% urban, 27% agricultural and 17% natural (Credit Valley Conservation, 2013). The average temperature in the watershed is 12.5°C in the summer and –2.5°C in the winter; annual precipitation is 793 mm, with 115 mm as snow (Statistics Canada, 2007).

Site selection, field sampling and laboratory analysis

Study sites were selected from deciduous forest stands dominated by Sugar Maple (*Acer saccharum*) within conservation areas and municipal or public parks in the Credit River Watershed. In general, field sampling followed the ICP Vegetation protocol (ICP, 2014); however, in urban areas it was difficult to select sites 100 m away from small roads, free of canopy cover. The majority of the sites were situated near existing monitoring sites established under the CVC Terrestrial Monitoring Program (TEMO; Credit Valley Conservation 2013). Twelve sampling sites were chosen (see Figure 1) to encompass the entire watershed, with four per sub-region. Island Lake Conservation Area (IL) and Rattray Marsh Conservation Area (RM) were chosen with the intention of including the furthest northern and southern conservation areas of the watershed (Figure 1).

Sampling was carried out during August 2014. At each study site, a 10 m × 10 m plot was established within a standard substrate; mosses were only sampled when found growing on rock or soil substrate within a five meter buffer zone of the plot. A composite sample of each moss species was collected and placed in a paper bag. In the laboratory, samples were identified and separated into species by site. Dead material and litter were manually removed, and samples were then cleaned by 30 seconds of agitation in a clean sample bottle with 90 mL of distilled reverse osmosis water. Moss was then air dried for at least 48 hours, and oven dried at 55°C for an additional 24 hours. Finally, the samples were pulverized in a ball mill for two minutes, to create a homogenous mixture for analysis.

The concentrations for eleven metals (aluminum (Al), vanadium (V), chromium (Cr), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), antimony (Sb), and lead (Pb)) in all moss species were determined using a Triple-Quad ICP-MS analyzer following acid digestion (Mars 6 microwave digester, EPA method 3052). Carbon and nitrogen (CN) were measured by elemental analyser, and mercury was determined using a mercury analyzer (Milestone DMA-80).

Results

Thirteen species of moss, dominated by pleurocarpous mosses (10 of 13 species) were collected across the watershed (Table 1). Nonetheless, acrocarpous mosses were dominant in the lower and the upper regions; with 5 of 6 species in the lower and 4 of 7 species in the upper region. In contrast, 6 of the 7 species collected within the middle region were pleurocarpous (Table 1). The only species to occur throughout all regions was the acrocarpous moss *Atrichum altercristatum*.

Table 1. Site name (watershed region: upper [U], middle [M] and lower [L]), site abbreviation, location (latitude and longitude [decimal degrees]) and moss species (Acrocarp [A] or Pleurocarp [P]) found in Sugar Maple (*Acer saccharum*) dominated forest plots (n = 12) within the Credit River Watershed, Ontario, Canada.

Watershed region: Site name	Latitude	Longitude	Moss Species
U: Island Lake (IL)	43.9329	–80.0761	*Homalia trichnomanoides* (P), *Brachythecium plumosum* (P), *Bryhnia graminicolor* (P)
U: Upper Credit (UC)	43.8766	–80.0569	*Atrichum altercristatum* (A)
U: Wilcox (WC)	43.8738	–80.1425	*Brachythecium rutabulum* (P), *Hygroamblystegium varium* (P)
U: Belfountain (BF)	43.7957	–80.0102	*Brachythecium plumosum* (P)
M: Warwick (WW)	43.8230	–79.9002	*Atrichum altercristatum* (A), *Callicladium haldanianum* (P)
M: Terra Cotta (TC)	43.7233	–79.9615	*Campyliadelphus chrysophyllus* (P), *Fissidens adianthoides* (A)
M: Willow Park (WP)	43.6479	–79.8661	*Hypnum pallenscens* (P), *Pohlia nutans* (A)
M: Limehouse (LH)	43.6337	–79.9735	*Leskeella nervosa* (P)
L: Brampton Park (BP)	43.6697	–79.7961	*Atrichum altercristatum* (A), *Pohlia nutans* (A)
L: Eldorado (ED)	43.6412	–79.7807	*Atrichum altercristatum* (A)
L: Mississauga Garden (MG)	43.5664	–79.6743	*Atrichum altercristatum* (A)
L: Rattray Marsh (RT)	43.5182	–79.6046	*Atrichum altercristatum* (A), *Hygrohypnum spp.* (P)

Heavy metal concentrations were variable between species, but exhibited similar trends, with the exception of some species (*Fissidens adianthoides*, *Pohlia nutans* and *Campyliadelphus chrysophyllus*), which exhibited higher concentrations for many metals (Table 2) possibly due to species characteristics or sample contamination, e.g., incorporation of wind blown dust into moss tissue. In general, the metal with the highest concentration was Fe, followed by Al > Zn > Cu > Pb > Cr > Ni > V > As > Cd > Sb > Hg. The coefficient of variation between species ranged from 25–35% for some metals (Cu, Zn, Cd and Hg) but variability was greater than ~50% for most metals (Al, V, Cr, Fe, Ni, As, Sb and Pb; Table 2). In contrast, the coefficient of variation for *Atrichum altercristatum* between sites (n = 6) was much lower, less than 33% for the majority of metals (Cu, Zn, As, Cd, Sb, and Pb; Table 2). Notably, Hg exhibited very little variation in *Atrichum altercristatum* between sites (13%), ranging in concentration from 0.07–0.10 mg kg^{-1} (mean = 0.08 mg kg^{-1}), reflecting its long atmospheric residence time (Schroeder & Munthe, 1998); nonetheless, Hg concentrations may have been influenced by oven drying. Further, *Atrichum altercristatum* generally exhibited lower concentrations within the middle region of the watershed (Figure 2), e.g., average values of Cr, Cu and Pb (3.1, 10.6 and 6.0 mg kg^{-1}, respectively) in the middle sites (n = 1), were lower compared with the upper (7.7, 21.4 and 10.8 mg kg^{-1}) and lower (5.1, 15.7 and 8.0 mg kg^{-1}) sites (n = 1 and 4, respectively). Many metal concentrations were strongly positively correlated in *Atrichum altercristatum* (and other moss species); e.g., Al, V, Cr, Fe, and As $r \geq 0.90$. In contrast % N was negatively (and poorly) correlated with most metals, except Cd (r = 0.77).

Table 2. Concentrations (mg kg^{-1}) of heavy metals and nitrogen (%) in moss species (number of samples) in the Credit River Watershed, Ontario.

Moss species	Al	V	Cr	Fe	Ni	Cu	Zn	As	Cd	Sb	Pb	Hg	%N
Atrichum altercristatum (6)	2200	4.9	5.2	3451	4.5	15.8	66.6	0.97	0.35	0.15	8.1	0.08	1.48
Brachythecium plumosum (2)	3017	5.7	13.5	4055	7.7	19.4	109.1	1.20	0.42	0.22	9.0	0.07	1.22
Brachythecium rutabulum (1)	5377	10.9	12.4	9928	11.3	16.2	82.6	3.33	0.37	0.10	15.6	0.09	0.93
Bryhnia graminicolor (1)	298	1.0	2.9	540	2.9	11.8	46.9	0.72	0.28	0.24	2.6	0.03	1.54
Callicladium haldanianum (1)	3828	9.2	10.0	8251	6.6	14.9	53.6	1.96	0.22	0.06	9.7	0.05	0.89
Campyliadelphus chrysophyllus (1)	3772	9.5	6.3	14685	13.1	75.7	93.5	23.44	0.64	0.15	20.8	0.10	0.82
Fissidens adianthoides (1)	23633	49.6	64.0	50299	32.0	219.1	331.5	46.03	1.54	0.23	57.4	0.06	0.86
Homalia trichnomanoides (1)	313	0.9	1.5	572	1.3	6.8	32.7	0.43	0.20	0.24	2.0	0.07	1.82
Hygroamblystegium varium (1)	2308	4.6	7.2	3968	8.0	8.3	46.0	1.56	0.24	0.09	5.1	0.08	1.40
Hygrohypnum spp. (1)	2508	5.8	7.8	5033	6.4	7.4	49.6	1.33	0.14	0.09	10.1	0.07	0.73
Hypnum pallescens (1)	5471	11.1	10.6	9272	9.7	15.7	75.5	2.11	0.31	0.08	17.5	0.09	0.95
Leskeella nervosa (1)	1225	2.9	2.9	2044	3.4	9.2	73.0	0.87	0.32	0.27	9.1	0.10	1.31
Pohlia nutans (2)	7290	15.8	16.5	12625	11.1	15.2	68.0	2.36	0.23	0.20	18.1	0.09	1.09
CV[1]: All species[2]	65.5	61.5	54.0	68.7	48.5	33.5	33.4	55.4	28.7	49.0	53.3	26.3	26.7
Average: All species[2]	2655	5.7	7.4	4711	6.2	12.6	63.6	1.45	0.28	0.15	8.9	0.07	1.2
Average: Upper watershed	2469	5.2	8.4	4020	6.2	14.8	72.4	1.39	0.33	0.18	7.7	0.07	1.4
Average: Middle watershed[2]	2973	6.6	6.7	5449	5.6	12.6	64.6	1.39	0.32	0.14	10.6	0.08	1.2
Average: Lower watershed[3]	2277	5.0	5.7	3697	5.3	14.0	62.5	1.05	0.28	0.15	8.4	0.08	1.3
CV[1]: *Atrichum altercristatum*	58.2	53.1	40.9	53.6	48.3	28.0	19.1	32.2	33.0	28.9	32.2	12.8	14.7
A. altercristatum: Upper (1)	2953	7.5	7.7	5024	4.2	21.4	80.4	1.27	0.40	0.13	10.8	0.10	35.5
A. altercristatum: Middle (1)	1369	3.1	3.1	2229	2.9	10.6	56.3	0.64	0.43	0.14	6.0	0.08	40.7
A. altercristatum: Lower (4)	2220	4.8	5.1	3363	5.0	15.7	65.7	0.98	0.32	0.16	8.0	0.08	38.21

CV is the coefficient of variation (%), which is estimated as the standard deviation divided by the mean value and multiplied by 100; 2 Excludes *Fissidens adianthoides*, *Pohlia nutans* and *Campyliadelphus chrysophyllus*; 3 Excludes *Pohlia nutans.*

Nitrogen content in *A. altercristatum* ranged from 1.04–1.72% (mean =1.49%), which was comparable to other moss species in the upper (*Bryhnia graminicolor* [1.54%], *Hygroamblystegium varium* [1.41%], *Brachythecium plumosum* [1.22%]) and middle (*Leskeella nervosa* [1.31%], *Pohlia nutans* [1.31%]) watershed regions. Nitrogen content was much less variable throughout the watershed and between species (CV% = 27.9%) compared to heavy metal concentrations, except Hg (Table 2).

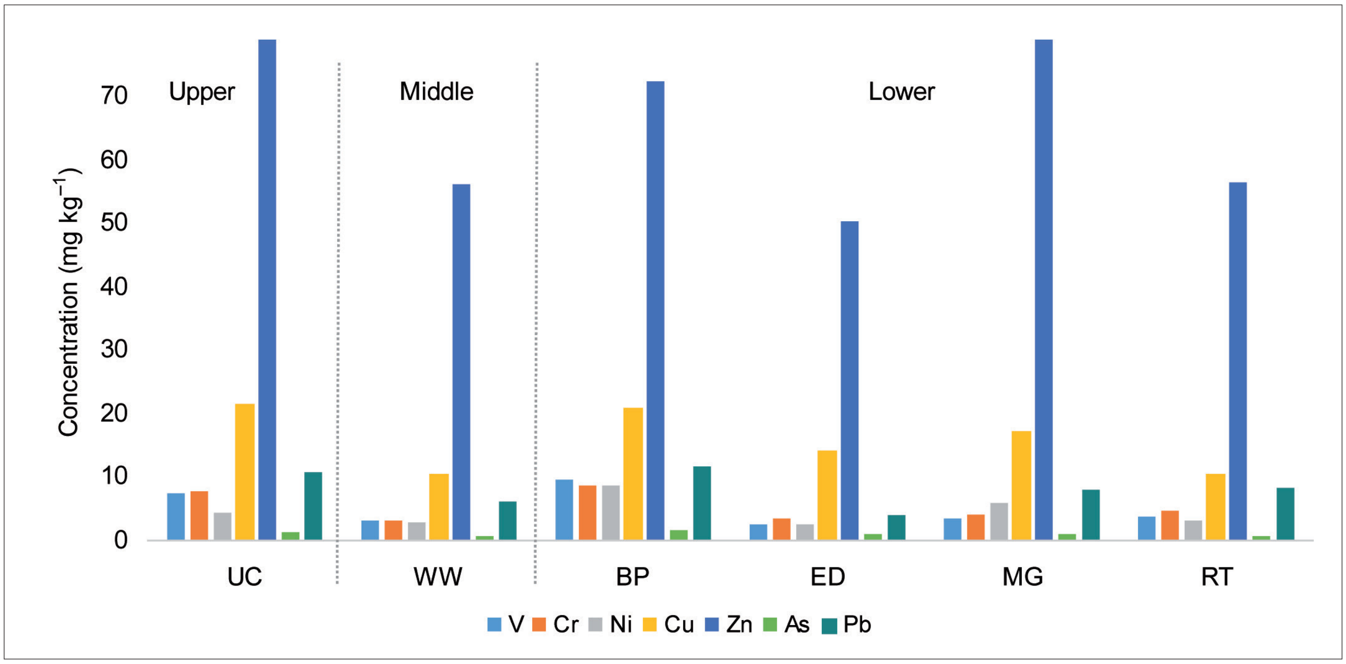

Fig. 2. Concentrations (mg kg^{-1}) of V, Cr, Ni, Cu, Zn, As and Pb in *Atrichum altercristatum* at study sites (n = 6) in the Credit River Watershed, Ontario (see Figure 1 for site locations and Table 1 for site abbreviations).

Discussion

The most commonly occurring moss species in the study area, *Atrichum altercristatum* (Renauld & Cardot) Smyth & Smyth, Lumina C. Riddle., is endemic to North America and has similar characteristics to the European *Atrichum undulatum* (Hedw.). The presence or absence of specific moss can indicate the presence of certain pollutants. Some species are very sensitive to pollutants, while others demonstrate resistance; e.g. *Pohlia nutans* prefers ecosystems high in heavy metals (Huttunen, 2003), while *Hygrohypnum* species prefer more acidic environments (McKnight et al., 2013). The *Atrichum* species is opportunistic, as it is habitually an early colonizer of degraded habitats, preferring to establish on perturbed soils (McKnight et al., 2013) making it an effective bioindicator of anthropogenic disturbance (Govindapyari et al., 2010). The common occurrence of *Atrichum altercristatum* throughout the lower region (Table 1) is consistent with the high percentage of urbanization found in that region (56%). However, the variations in species composition at the study plots could simply be influenced by the underlying geology. The Niagara Escarpment is a massive ridge of sedimentary rock that traverses through the middle region of the watershed. The calcareous nature of the sedimentary rocks of the escarpment create a less acidic environment that is favoured by species such as *Campyliadelphus chrysophyllus*, *Fissidens andianthoides* and *Leskella nervosa* (McKnight et al., 2013), which were all sampled in the middle watershed region.

The metal concentrations were variable between species (Table 2), as the degree of metal accumulation is influenced by the differing morphology of each species, their annual growth rates, the recycling of micronutrients (e.g., Cu and Zn) from dead tissue and the chemical form of deposition (Govindapyari et al., 2010; Halleraker et al., 1998; Zechmeister, 1998). Therefore, combining analysis from several species is not recommended. Large scale surveys such as ICP Vegetation (Harmens et al., 2010, 2015) recommend sampling of only certain species (e.g., *Pleurozium schreberi* and *Hylocomium splendens*) to provide more accurate data (neither species was present at the study sites). In the current study, multiple species were sampled and analyzed as only one species (*A. altercristatum*) commonly occurred throughout the region. Nonetheless, the variability between metal concentrations followed a similar trend across species (Table 2). Reliable comparison between species requires interspecies calibration at sites with co-occurring species (Halleraker et al., 1998).

The spatial pattern of nitrogen content (%) was less variable throughout the sampling area (upper, 1.39%, middle 1.19%, lower 1.22%) and across species (e.g., *A. altercristatum* 1.48%, *Brachythecium plumosum* 1.22%, *Leskeella nervosa* 1.31%). Nitrogen is a macronutrient, as such, moss tissue background concentrations (~0.5%: Harmens et al., 2011) reduce spatial and interspecies variability owing to atmospheric inputs. The mean N content was consistent with national observations in recent European scale surveys (Harmens et al., 2011, 2015; e.g., Austria [1.20%], Bulgaria [1.37%], France [1.26%], Switzerland [1.12%], etc.). However, the concentrations of heavy metals were generally higher when compared with observations in northern Europe (Harmens et al., 2010; e.g., Sweden [Cr = 0.61 mg kg^{-1}, Pb = 0.61 mg kg^{-1} and Ni = 3.61 mg kg^{-1}] and Norway [Cr= 0.58 mg kg^{-1}, Pb = 2.17 mg kg^{-1} and Ni = 1.24 mg kg^{-1}]) and south eastern Europe (Harmens et al., 2010; e.g., Turkey [Cr = 4.41 mg kg^{-1}, Pb = 4.04 mg kg^{-1}, and Ni = 5.09 mg kg^{-1}] and Serbia [Cr = 6.44 mg kg^{-1}, Pb = 16.7 mg kg^{-1} and Ni = 4.43 mg kg^{-1}]). In contrast, the concentrations found throughout the study area were generally lower than those observed in a similar study carried out in an urban area in Northern Italy (Gerdol et al., 2014), e.g., the moss *Tortula muralis* (acrocarp) was found to have Cr = 13.3 mg kg^{-1} and Pb = 60.2 mg kg^{-1} and Ni = 11.0 mg kg^{-1} (compare with Table 2). Heavy metal concentrations in the current study were comparable to those found in a rural area in southern Brazil (Correa Mazzoni et al., 2012); the average of all species found in rural areas were Cr = 3.7 mg kg^{-1}, Pb = 9.1 mg kg^{-1}, Ni = 6.2 mg kg^{-1} and Cd = 0.29 mg kg^{-1}.

Correa Mazzoni et al. (2012) and Gerdol et al. (2014) linked heavy metal concentrations to anthropogenic activities such as agriculture, vehicular traffic, construction and waste management. The correlations between groups of pollutants in the current study, similarly suggested common emission sources. The metals Fe, V, Al, As, and Cd may be attributed to wind blown dust from agricultural activities such as tillage, or fertilizer use (Gerdol et al., 2014; Halleraker et al., 1998), which are predominant activities throughout the upper regions of the watershed. Further, N, Cr, Cd, Ni, and V are associated with vehicular traffic (Correa Mazzoni et al., 2012; Gerdol et al., 2014) common throughout the whole watershed.

Conclusions

The moss analysis provided insight into the patterns of atmospheric pollution due to anthropogenic activities; in general, *Atrichum altercristatum* exhibited the highest concentrations of heavy metals in the upper and lower regions of the watershed. *Atrichum altercristatum* was dominant throughout the lower region; however, the number of sampled species increased to include a mix of acrocarpous and pleurocarpous mosses within the middle and upper regions. While the efficacy of mosses can be affected by physical factors such as forest cover, which enhances deposition rates, they are still an effective tool providing insight into spatial trends and patterns at a regional scale. Moss biomonitoring is widely used to measure the temporal and spatial changes in anthropogenic deposition in Europe (Harmens et al., 2010, 2011, 2015), surprisingly, studies of this nature are rare in Canada. It is recommended that future regional assessments include interspecies calibration, ideally incorporating moss species commonly used in Europe (e.g., *Pleurozium schreberi* and *Hylocomium splendens*).

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