

Original Research

Vertical Distribution Patterns of Element Concentrations in Podetia of *Cladonia rangiferina* from Huzhong Natural Reserve, Heilongjiang, China

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Abstract

Lichen element compositions are of a reliable tool for the biomonitoring of atmospheric element deposition. The zoning and patterned accumulation of elements in lichen thallus is one of the important factors influencing lichen element concentrations. We collected an epigenous fruticose lichen *Cladonia rangiferina* (CR) from a remote site of Huzhong National Natural Reserve, Heilongjiang, NE China. Fifty-five elements (Ag, Al, As, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Eu, Fe, Gd, Ge, Hg, Ho, K, La, Li, Lu, Mg, Mn, Mo, Na, Nb, Nd, Ni, P, Pb, Pr, Rb, S, Sb, Sc, Se, Si, Sm, Sn, Sr, Tb, Th, Ti, Tl, Tm, U, V, Y, Yb, and Zn) were measured with their differences among 3 parts (upper, middle, and lower) of the podetia compared. The aim of the present study was to examine the vertical patterns of element accumulation in CR.

The results display a very low degree of atmospheric element deposition in the study site. Concentrations of most elements of limited metabolic significance generally increase from the upper to lower parts, indicating a continuous bioaccumulation of these elements with exposure time. Concentrations of some nutrients have little vertical difference among parts (Zn), and they are higher in the upper part than in the middle part (K, P, and S), or are similar between the upper and middle parts (B, Cu and Mg). These results suggest a nutrient translocation to the more biologically active young part. Our results highlight the importance of selecting the suitable parts of lichen thallus to increase the comparability of data in lichen biomonitoring studies.

Keywords: *Cladonia*, element composition, bioaccumulation, atmospheric deposition, fruticose lichens

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Introduction

Lichens have been used as biomonitors of atmospheric element contamination in a wide range of conditions, including urban areas, industrial sites, and natural habitats [1-4]. Due to their poorly developed or lacking roots, lack of protective cuticle and stomata, large surface area/mass ratio and relatively low growth rate, lichens are dependent highly on the atmosphere for nutrients and can absorb atmospheric contaminants at levels exceeding their physiological requirements and well reflect the atmospheric elemental levels [4, 5]. However, besides atmospheric element concentrations, other factors can also affect element contents in lichen thallus, such as environmental factors (including climate, landscape, vegetation, and type of substrate), lichen species, lichen morphology and eco-physiology [1, 2, 6-10].

The zoning and patterned accumulation of elements in lichen thallus is an important factor influencing lichen element compositions [2, 7, 8]. Some authors observed different element contents along cross section of certain lichen tissues, suggesting an element-specific internal translocation in lichen thallus [11-13]. Other authors observed element-specific partitioning between old and young parts. Compared with the older parts, the younger ones of lichen thallus are less particle-contaminated and more metabolically active, therefore accumulating a larger amount of nutritional elements [14-16] due to the translocation of these elements in thallus [17]. In contrast, the older parts often accumulate more elements with limited metabolic significance due to the longer exposure time [5, 16, 18]. Some researchers also observed an increasing element concentration with the thallus size of *Evernia prunastri* [8].

A fully understanding of element distribution patterns in different parts of lichen thallus is a crucial issue associated with lichen biomonitoring technique, in which it is required for the selection of appropriate lichen parts to consider the bioaccumulation capability of atmospheric elements and the comparability of results [19]. The relevant studies have been conducted on epiphytic foliose lichens [5, 18, 20], epiphytic fruticose lichens [16], and epigenous fruticose lichens [14, 15, 21, 22]. However, a limited number of elements (< 20) were involved in the abovementioned studies, but such studies have not been conducted in China.

We collected *Cladonia rangiferina* (CR) in a remote area and compared concentration differences of 55 elements (Ag, Al, As, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Eu, Fe, Gd, Ge, Hg, Ho, K, La, Li, Lu, Mg, Mn, Mo, Na, Nb, Nd, Ni, P, Pb, Pr, Rb, S, Sb, Sc, Se, Si, Sm, Sn, Sr, Tb, Th, Ti, Tl, Tm, U, V, Y, Yb, and Zn) among the upper, middle and lower parts of the secondary thalli (podetia). Our aim was to test the hypothesis that the nutritional elements could be accumulated greater in the upper parts than in the middle/lower parts, with the inverse occurring for the

elements with less metabolic significance. This research is the first attempt to explore the distribution patterns of elements in different parts of lichens in China, and to the best of our knowledge, it is one of the lichen biomonitoring studies involving the largest number of elements.

Materials and Methods

Sample Collection

Huzhong National Natural Reserve (51°17'-51°56'N, 122°42'-123°18'E) locates in the Greater Khingan Mountains of Heilongjiang, NE China (Fig. 1a). The climate is of cold temperate continental monsoon, with a mean annual precipitation of 497.7 mm and a mean annual temperature of -4.4°C. The vegetation is typical coniferous forest ecosystem with abundant lichens. There was no obvious industrial, agricultural, mining and transport activity in the Reserve with the nearest town approximately 40 km away from the sampling site (Fig. 1b).

In August 8, 2017 >100 thalli of a fruticose lichen CR were randomly collected by hand, sealed in clean paper bags, immediately transported to the laboratory, air-dried and maintained at room temperature till used. All samples were collected in an area of 100 × 100 m² with similar geomorphological and landscape characteristics in a rural site (51°47'03"N, 123°00'48"E). The site is flat with an elevation of 773 m. The predominant ecosystem is boreal coniferous forest dominated by *Larix gmelinii* (Rupr.) Kuzen. The undergrowth shrubs are dominated by *Ledum palustre* L., *Vaccinium vitis-idaea* L., *Rhododendron dauricum* L. and *Alnus mandshurica* (Callier ex C. K. Schneider) Handel-Mazzetti (Fig. 1c). CR was chosen because it is abundant and often forms a carpet on the ground among shrubs (Fig. 1c) in the region, and it was studied in the biomonitoring research in Arctic [23], Norway [24], Russia [25-27], Japan [28], and Canada [29]. The collection area was restricted to 10,000 m² to minimize the potential effects of local microhabitat variations on the lichen element composition. Indeed, the UNECE ICP-vegetation (International Cooperative Program on Effects of Air Pollution on Natural Vegetation and Crops) protocol recommended that moss samples for moss biomonitoring studies should be collected in an area of 50 × 50 m² in each site [30]. In lichen biomonitoring studies, epigenous lichens were also collected in an area of approximately 50 × 50 m² for *Cladonia rangiformis* [31].

Sample Preparation and Measurement

CR samples were carefully cleaned with plastic tweezers by a low magnification stereomicroscope. Parts of the podetia in intimate contact with soil were removed to minimize possible soil contamination of

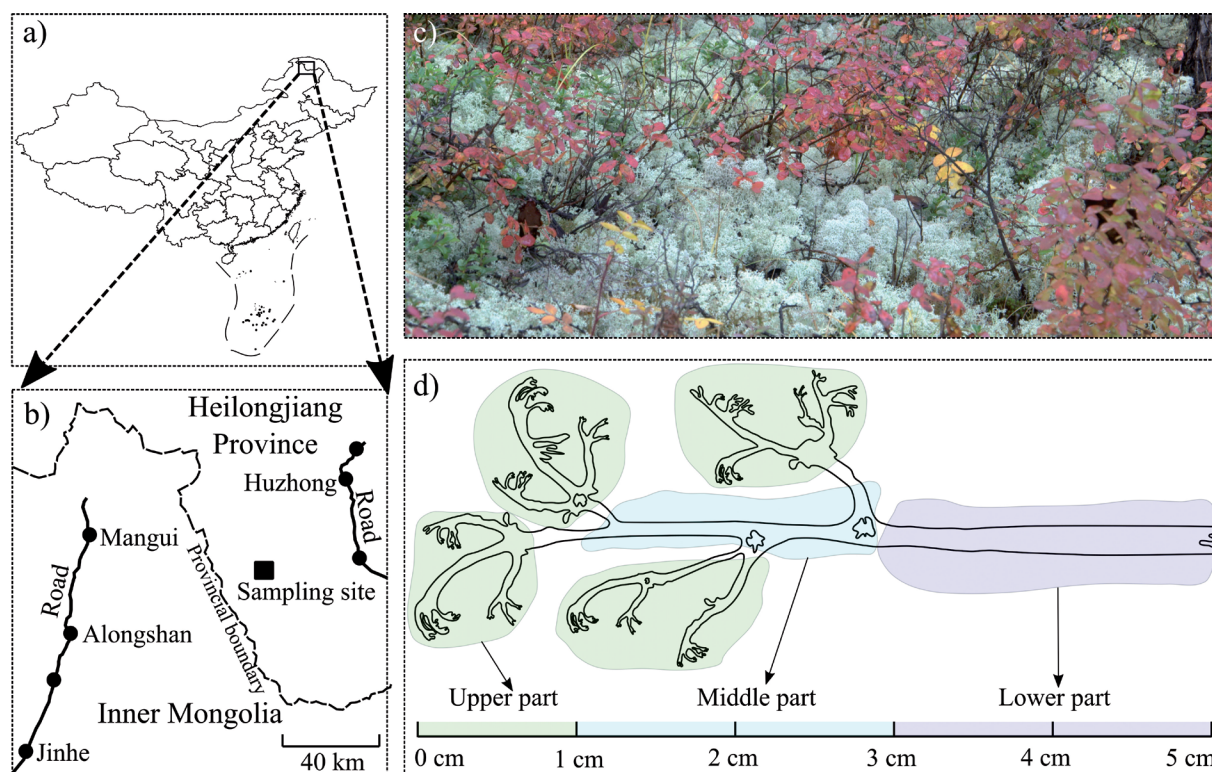


Fig. 1. Sampling site, habitat and *Cladonia rangiferina*: a) location of the Huzhong National Natural Reserve; b) location of sampling site (the solid circles indicate towns, and the block indicates the sampling site); c) habitat of *CR* (the gray-white carpet on ground); d) parts of a podetium.

samples. Podetia of approximately 5 cm in height were selected and randomly assigned to 6 replicates. Each replicate contained 8-12 podetia; each podetium was cut into three parts with a plastic blade (upper: 0-1 cm from the top of podetia; middle: 1-3 cm; and lower: 3-5 cm. Fig. 1d). A total of 18 samples (6 replicates \times 3 parts) were dried to a constant weight at 70°C for 72 h in a drying oven, and were then ground and homogenized in a grinding mill equipped with Tungsten Carbide jars (Retsch MM400; Retsch GmbH, Haan, Germany).

A 200-300 mg sample was mineralized by microwaves with HNO_3 and H_2O_2 . Concentrations of 55 elements (Ag, Al, As, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Eu, Fe, Gd, Ge, Hg, Ho, K, La, Li, Lu, Mg, Mn, Mo, Na, Nb, Nd, Ni, P, Pb, Pr, Rb, S, Sb, Sc, Se, Si, Sm, Sn, Sr, Tb, Th, Ti, Tl, Tm, U, V, Y, Yb, and Zn) were determined using an inductively coupled plasma mass spectrometer (ICP-MS; Agilent 7700X; Agilent Technologies, Tokyo, Japan) at the Hebei Research Center for Geoanalysis.

The analytical quality of the ICP-MS results was checked against the following standard reference materials: GBW10014 (cabbage), GBW10015 (spinach), GBW10052 (green tea) provided by the Institute of Geophysical and Geochemical Exploration, Chinese Academy of Geological Sciences; IAEA-336 (Portuguese lichen) was from the International Atomic Energy Agency. The analytical precision and accuracy

were within 10% with the detection limits being generally of $<0.001 \mu\text{g/ml}$ for all elements.

Statistical Analyses

A Shapiro-Wilk test was performed to test the normality of data distribution of element concentrations. It was normal for all elements in each part, except Bi in the middle part and B and Mn in the lower part. The significance of concentration differences among parts was tested using a paired-samples *t* test with a Bonferroni correction for multicomparisons. In this analysis, concentrations of 12 elements were lg-transformed (B, Dy, Lu, Mn, Na, Sb, Sn, Sr, Ti, and U) or cubic root-transformed (Ba and Hg) to increase the normality of concentration differences between every two parts. All analyses were conducted using the software PAST 3.25 (Ø. Hammer, April 2019).

Results

Table 1 shows the concentration values of 55 elements in three parts of *CR*. All elements show only a little to small concentration variations (CV: 4.0-25.0%), except B in the upper (CV: 27.7%) and middle (CV = 28.0%) parts and 5 elements (Ag, B, Cd, Hg, and Mn; CV: 25.8-46.7%) in the lower part.

Table 1. Element concentrations in three parts of *Cladonia rangiferina*.

	Upper part ($\mu\text{g g}^{-1}$; n = 6)		Middle part ($\mu\text{g g}^{-1}$; n = 6)		Lower part ($\mu\text{g g}^{-1}$; n = 6)			Upper part ($\mu\text{g g}^{-1}$; n = 6)		Middle part ($\mu\text{g g}^{-1}$; n = 6)		Lower part ($\mu\text{g g}^{-1}$; n = 6)	
	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)		Mean	CV(%)	Mean	CV(%)	Mean	CV(%)
Ag	0.015	13.33	0.037	13.51	0.031	25.81	Mo	0.050	12.00	0.096	12.5	0.216	17.59
Al	781.9	13.07	1402	7.30	6739	16.13	Na	124.7	13.97	269.3	11.24	943.3	13.04
As	0.300	9.33	0.402	8.21	0.758	14.25	Nb	0.078	20.51	0.173	11.56	0.487	21.77
B	2.897	27.68	2.953	27.97	4.368	46.66	Nd	0.356	12.64	0.667	6.90	2.277	15.33
Ba	16.36	7.42	25.44	4.66	62.86	17.64	Ni	0.463	5.83	0.662	6.80	1.790	18.66
Be	0.018	16.67	0.037	8.11	0.129	10.08	P	1201	8.93	720.6	4.57	671.7	14.75
Bi	0.016	12.50	0.022	9.09	0.058	18.97	Pb	0.695	15.83	0.966	9.32	2.579	21.36
Ca	991.8	7.36	1231	5.27	1999	12.58	Pr	0.090	15.56	0.178	13.48	0.576	13.89
Cd	0.035	5.71	0.059	8.47	0.053	26.42	Rb	4.016	9.44	4.886	6.24	12.32	16.24
Ce	0.785	11.08	1.523	8.14	5.051	15.66	S	625.9	9.22	396.5	7.17	460.3	16.31
Co	0.163	14.11	0.239	12.55	0.738	19.51	Sb	0.029	6.90	0.038	7.89	0.074	22.97
Cr	0.644	17.55	1.180	9.32	3.694	17.03	Sc	0.098	16.33	0.196	11.22	0.656	17.99
Cs	0.142	14.79	0.250	9.20	0.933	17.68	Se	0.063	22.22	0.082	8.54	0.219	18.72
Cu	1.803	11.59	1.716	7.52	2.934	15.47	Si	1993	17.27	3825	12.06	15475	10.56
Dy	0.047	12.77	0.083	8.43	0.281	16.73	Sm	0.063	14.29	0.125	10.40	0.413	16.22
Er	0.022	13.64	0.042	9.52	0.129	17.05	Sn	0.051	11.76	0.080	8.75	0.222	19.82
Eu	0.015	6.67	0.027	7.41	0.088	18.18	Sr	11.54	3.99	16.30	6.30	30.49	15.89
Fe	295.2	15.66	611.5	11.02	2101	17.28	Tb	0.0083	14.46	0.0147	11.56	0.0478	18.41
Gd	0.055	7.27	0.102	8.82	0.330	13.33	Th	0.100	18.00	0.222	11.26	0.787	18.30
Ge	0.015	13.33	0.027	7.41	0.089	17.98	Ti	46.55	19.47	104.9	9.03	286.8	18.81
Hg	0.021	9.52	0.028	10.71	0.052	28.85	Tl	0.015	13.33	0.029	13.79	0.076	18.42
Ho	0.009	11.11	0.015	6.67	0.053	20.75	Tm	0.0035	11.43	0.0066	9.09	0.0209	19.62
K	2591	7.54	2032	4.89	2487	13.31	U	0.031	12.9	0.074	9.46	0.290	19.31
La	0.414	13.04	0.843	9.25	2.679	15.57	V	0.784	17.35	1.600	10.50	5.297	16.99
Li	0.345	20.58	0.797	14.18	2.734	16.24	Y	0.218	14.22	0.391	6.14	1.301	16.76
Lu	0.0031	16.13	0.0059	6.78	0.0194	15.98	Yb	0.022	18.18	0.039	10.26	0.135	17.04
Mg	488.4	9.79	509.9	8.17	843.2	10.98	Zn	18.16	6.86	18.96	5.02	22.18	15.37
Mn	195.2	7.76	232.4	10.52	412.3	36.96							

Fig. 2 shows the concentration differences between the three parts. The concentrations for all elements but Zn are significantly different among the three parts. The concentrations for 46 elements (Al, As, Ba, Be, Bi, Ca, Ce, Co, Cr, Cs, Dy, Er, Eu, Fe, Gd, Ge, Hg, Ho, La, Li, Lu, Mn, Mo, Na, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Si, Sm, Sn, Sr, Tb, Th, Ti, Tl, Tm, U, V, Y, and Yb) increased in the order of upper<middle<lower part (Fig. 2a); they were 2- to 9-fold higher in the lower parts than in the upper part, and were 1.6- to 4.7-fold higher in the lower parts than in the middle part

(Fig. 2b). The concentrations for Ag and Cd followed the order of upper<middle = lower part (Fig. 2a); they were 1.5- to 2.1-fold higher in the lower part than those in the upper part (Fig. 2b).

The concentrations of B, Cu and Mg followed the order of upper = middle<lower part (Fig. 2a); they were 1.6-2.1 folds higher in the lower part than in the other parts (Fig. 2b). The concentration of K followed the order of upper = lower>middle part (Fig. 2a), and it was 1.3-fold higher in the upper part than in the middle part (Fig. 2b). The concentrations of P and S followed the order of upper>middle = lower part (Fig. 2a), and

they were 1.4-1.8 folds higher in the upper part than in the lower part (Fig. 2b).

a) Vertical patterns of element concentrations

Element	Upper	Middle	Lower
Al	C	B	A
As	C	B	A
Ba	C	B	A
Be	C	B	A
Bi	C	B	A
Ca	C	B	A
Ce	C	B	A
Co	C	B	A
Cr	C	B	A
Cs	C	B	A
Dy	C	B	A
Er	C	B	A
Eu	C	B	A
Fe	C	B	A
Gd	C	B	A
Ge	C	B	A
Hg	C	B	A
Ho	C	B	A
La	C	B	A
Li	C	B	A
Lu	C	B	A
Mn	C	B	A
Mo	C	B	A
Na	C	B	A
Nb	C	B	A
Nd	C	B	A
Ni	C	B	A
Pb	C	B	A
Pr	C	B	A
Rb	C	B	A
Sb	C	B	A
Sc	C	B	A
Se	C	B	A
Si	C	B	A
Sm	C	B	A
Sn	C	B	A
Sr	C	B	A
Tb	C	B	A
Th	C	B	A
Ti	C	B	A
Tl	C	B	A
Tm	C	B	A
U	C	B	A
V	C	B	A
Y	C	B	A
Yb	C	B	A
Ag	B	A	A
Cd	B	A	A
B	B	B	A
Cu	B	B	A
Mg	B	B	A
K	A	B	A
P	A	B	B
S	A	B	B
Zn	A	A	A

b) Element concentrations normalized to the lower part

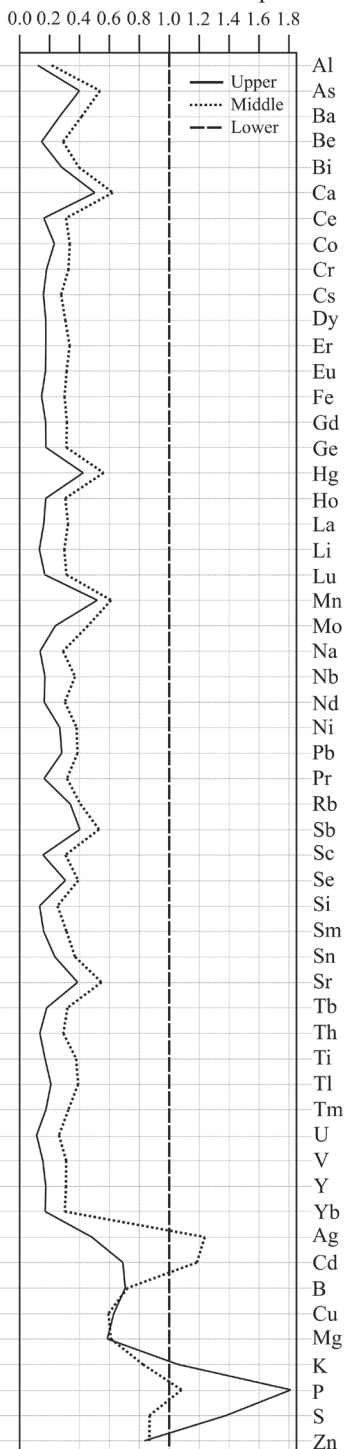


Fig. 2. Element concentration differences among three parts of *Cladonia rangiferina*: a) vertical patterns of element concentrations (for each element, the different capital letters indicate significant differences among 3 parts; A>B>C; paired-samples t test with Bonferroni correction; n = 18); b) element concentrations normalized to the lower part.

Discussion

Atmospheric Deposition Degree

Our results show a very low degree of atmospheric deposition in the study site. The concentrations of measured elements in the upper part of CR (Table 1) are within or close to the reported background or baseline concentrations in lichens from the remote sites of Arctic regions [23], Norway, [24], and Turkey [31] (Table 2). This conclusion is also supported by a comparison of our data with those from the other lichen biomonitoring studies conducted in remote sites, such as *Usnea subfloridana* from Canada [32], severally lichens from the France [33] and Arctic regions [34], *Usnea* sp. from the forest-grassland regions of Chile [35], *Cladonia rangiferina* and *C. stellaris* from the remote sites of Russia [24, 27], epiphytic lichens from Italy [10, 36-39] and France [40], and several lichens from Inner Mongolia, China [41, 42].

Vertical Distribution of Elements

All elements but Zn were significantly different among parts (Fig. 2). Such differences should not be attributed to the effects of local microhabitat variations because a low intra-sample variability in lichen contents were detected for all but a few elements in each part of CR (CV<25%; Table 1).

The amount of 49 elements shows an increasing pattern from the upper to lower parts, with a lowest concentration in the upper part and a highest concentration in the lower part for 46 elements (Al, As, Ba, Be, Bi, Ca, Ce, Co, Cr, Cs, Dy, Er, Eu, Fe, Gd, Ge, Hg, Ho, La, Li, Lu, Mn, Mo, Na, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Si, Sm, Sn, Sr, Tb, Th, Ti, Tl, Tm, U, V, Y, and Yb), or a similar concentration between the middle and lower parts for 2 elements (Ag, and Cd). Most of these elements are of limited metabolic significance and are rarely bioregulated by lichens. This pattern agrees quite well with other studies. For example, metals with limited metabolic significance (Al, Cd, Pb, and V) had higher concentrations in the older parts than in the younger parts of epiphytic foliose lichen *Flavoparmelia caperata* [16, 18]. Concentrations of Al were higher in the older parts of epiphytic fruticose lichen *Evernia prunastri* [16]. Cr, Fe, Na, Ni and Pb had higher concentrations in the older parts of *Physcia biziana* [20]. In some *Cladonia* lichens, the increasing concentrations from the apex to the base of thalli were observed for As, Cd, and Pb [22], Fe and Pb [14], and Al, Ca, Cr, Fe, Si, and Ti [26]. Most of the abovementioned studies attributed such patterns to the longer exposure time and higher cation exchange capacity in the older parts and limited translocation of these elements in lichen thallus [16, 17]. Soil contamination of the lower part is highly unlikely an explanation for the higher accumulation of this part because the parts intimately contacted with the soil were removed before chemical analysis.

Table 2. Comparison of element concentrations in the upper part of *Cladonia rangiferina* to the reported background or baseline concentrations from other studies. All units are in $\mu\text{g g}^{-1}$.

Location	This study	Arctic regions [23]	Norway [24]	Turkey [31]
Species	<i>C. rangiferina</i>	Various	<i>C. rangiferina</i>	<i>C. rangiformis</i>
Statistics	Mean of the upper part	Min.-Max.	Min.-Max.	Min.-Max.
As	0.300	0.06-2.21		
Cd	0.035		0.03-0.08	0.14-0.69
Cr	0.644	0.5-10.2	0.3-0.7	2.24-13.0
Cu	1.803	0.7-5.0	0.9-1.0	1.06-5.29
Fe	295.2	50-900	410-698	
Hg	0.021	0.009-0.101	0.02-0.05	
Ni	0.463	1.0-5.5	0.4-0.8	
Pb	0.695	0.4-9.2	0.6-0.9	1.35-33.8
S	625.9	101-961		
Ti	46.55	7-850		
V	0.784	0.17-9.70		
Zn	18.16	6.0-55.0	7.0-11.0	9.15-47.6

Some researchers have reported that some nutritional elements can translocate to the more biologically active parts of lichen thallus [17]. The vertical patterns of the rest 6 elements in our study might suggest such an internal translocation of these nutrients. The higher accumulations of B, Cu and Mg in lower part (Fig. 2) might suggest a longer exposure to environmental input, but these elements had similar concentrations between the upper and middle parts (Fig. 2), indicating a possible translocation from the middle part to the more metabolically active upper part. This is the case for Zn in which no pattern was found (Fig. 2). This is also the case for three essential nutrients (K, P, and S) in which the concentrations were higher in upper part than in middle part (Fig. 2). In addition, concentrations of K were higher in lower part than in middle part, may also indicating a longer exposure to soil-derived materials because K is a major element in the earth's crust. These patterns agree with previous studies. For example, 4 nutrients (Co, Cu, Mo, and Zn) were higher in younger part of *Flavoparmelia caperata* [18]. K and Mg were higher in young parts of *F. caperata* and *Evernia prunastri* [16], but were not significantly different between different parts of *Physcia biziana* [20]. The nutrients (Zn, N, P, K, S, and Mg) were higher at the young parts of *Cladonia* spp. [14, 15, 26]. These studies attributed such patterns to the internal translocation of nutrients.

Selection of suitable parts of lichen thalli is known to bias the lichen elemental concentration [3, 17]; the recent studies often used young parts [36, 42]. Our results show that element concentrations are element-

and part-specific, highlighting the importance of part selection in lichen biomonitoring studies.

Conclusions

Element concentrations in *Cladonia rangiferina* display a very low degree of atmospheric deposition in the region and show element- and part-specific concentrations. Most elements with limited metabolic significance show an increasing concentration from the upper to lower part, suggesting an increasing bioaccumulation with prolonged exposure time. Vertical patterns of some nutrients show an internal translocation from the older parts to younger ones. These results highlight the importance of selection of suitable parts in lichen biomonitoring studies.

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Conflict of Interest

The authors declare no conflict of interest.

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