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LICHEN AND MOSS SURFACE ANALYSIS USING SCANNING ELECTRON MICROSCOPY AND ENERGY DISPERSIVE X-RAY SPECTROSCOPY

Abstract. Scanning electron microscopy and energy dispersive X-ray spectroscopy were used for the estimation of the chemical composition of the surface of lichen and moss thalli in connection with Arctic contamination study. No mineral particles or trace metals except titanium were identified on the surface of the examined lichen and moss samples (*Cladina stellaris, Cetraria islandica, Masonhalea richardsonii, Hylocomium splendens*). By applying different magnifications and area and spot analysis it was established that lichens are capable of trapping fine mineral particles and changing them chemically. Biologically close species have a specific surface structure and different chemical composition. In the methodological respect washing lichens and moss samples to remove external contaminants before using AAS or other analytical techniques is not necessary for background monitoring in the regions sampled.

Key words: lichens, mosses, scanning electron microscopy, energy dispersive X-ray spectroscopy, bioindicators.

Introduction

Lichens and mosses have been regarded as good biological indicators of environmental contamination for a long time. Thanks to their specific morphology, high ratio of surface area to volume, low tissue differentiation, specific metabolism, low growth rate, and a specific coenotical and biogeochemical role in ecosystems they appear as suitable biomonitors of dry and wet atmospheric pollution deposition (Sernander, 1926; Ferry et al., 1973; Seaward, 1977; Nash and Wirth, 1988).

In particular, lichens and mosses have been widely applied as biomonitors in studies of atmospheric dispersion of heavy metals and radionuclides around point sources as well as on regional scale (Rühling and Tyler, 1968, 1969, 1970, 1971, 1973; Steinnes, 1977, 1989; Rühling et al., 1987; Martin, Nifontova et al., 1991). Numerous reports have been published concerning cationic and anionic uptake by lichens and mosses from solution (Puckett et al., 1973; Nieboer and Richardson, 1981).

Recent studies in high latitude ecosystems have shown many of their components to be extremely sensitive to human impact, both physical and chemical. Fast migration of radionuclides along food chains during nuclear testings in the 1960s is a well documented phenomenon (Mattson, 1972; Martin and Koranda, 1971). The arctic atmosphere is now contaminated due to long-range transport of pollutants from Arctic sources as well as from those located at lower latitudes. However, comprehensive data that would permit estimation of the extent and magnitude of atmosphere-mediated contamination of biotic or abiotic components of high latitude ecosystems are still lacking.

The present study was initiated in connection with the US EPA Arctic Contaminants Research Program (ACRP) in order to evaluate bioindicators for future international activities in the ACRP framework.

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The purpose of this paper is the preliminary examination of the surface contamination of lichens and mosses and identification of the role of surface texture of the selected bioindicators in "trapping" the subject pollutants. To our knowledge, the first use of scanning electron microscopy (SEM) in lichen research was reported by Peveling and Vahl (1968). Since that time SEM has been widely applied in studies of lichen anatomy and morphology, mostly in comparative works for taxonomic needs, which have been reviewed in several publications (Hale, 1973, 1976; Hawksworth, 1969; Jahns, 1988). In recent years also some papers have been published dealing with crystal structures, mainly calcium and other metals oxalates on and in the lichen thalli (Jones et al., 1982; Wadsten and Moberg, 1985) using EM and associated X-ray diffraction methods.

Materials and Methods

The field sampling for this study was conducted in Alaska (Brooks Range) and Estonia (West Estonian Archipelago Biosphere Reserve, Rumpo Lichen Reserve on Vormsi Island) in 1990—1991. During 1990 and 1991 pilot field work, several taxa were selected for the lichen/moss regional survey. These taxa included the arctic lichen *Masonhalea richardsonii* (Hook.) Kärnef., the multiregional lichen *Cetraria islandica* (L.) Ach., the boreal lichen *Cladina stellaris* (Opiz) Brodo, and the boreal moss *Hylocomium splendens* (Hedw.) B.S.G. In spite of the fact that these species have different general geographical distribution they often perform as the co-dominant species forming the ground layer of plant cover in different tundra and forested tundra types. Lichen species *Cetraria islandica* and *Masonhalea richardsonii* have similar growth forms and their distribution in high latitudes overlaps in Alaska and the East-Asian Arctic. *M. richardsonii* belongs to the Beringian element of the flora. It ranges from Siberia to the west side of Hudson Bay in North America. Rumpo Lichen Reserve in Estonia (biogeographically located in the boreal zone) has high percentage of arctic and alpine lichen species (Tpacc, 1970; Martin, Piin et al., 1991). Thus, it was selected as a comparison sample area.

For this preliminary study six samples of lichens and mosses from four sites: Elusive Lake, Snowden Mountain, Toolik Lake area road site (Alaska), and Rumpo Lichen Reserve (Estonia) were used and 26 surface analyses were made. Results of EDAX measurements are presented in the Table, except those for *Cladina stellaris*.

One of the technical problems often discussed in papers dealing with trace metal analysis in lichens and mosses relates to concerns about surface contamination by natural or man-made mineral dust. The current study employed scanning electron microscopy to evaluate such surface contamination for the purpose of developing a sampling strategy and analysis methodology for background areas.

In this study elemental analysis was done on the surface of lichen and moss specimens by energy dispersive X-ray spectroscopy. The instrument used was Hitachi S-2700 scanning electron microscope equipped with an EDAX 9100 X-ray analyzer. Specimens were mounted on carbon stubs and coated with a thin film of carbon by rotary vacuum evaporation

to prevent charging. For each analysis, data were collected for 100 seconds (live) from an area of 0.3 mm^2 in the scan mode or a spot with roughly 1 m diameter for the static mode. The detection range was from sodium to uranium. Weight percentages were obtained by using the EDAX semiquantitative (standardless) computer program which includes a ZAF correction. In this way figures showing percentage or relative content of elements in the surface layer were obtained.

Results and Discussion

Cetraria islandica. This lichen has fruticose erect thalli, more or less dichotomously branched, with inrolled margins which make the lobes more or less channeled. The upper side, with white pseudocyphellae scattered over the surface, is olive brown to dark brown and is smooth. Both the upper and the lower cortex are prosoplectenhymatous and brown pigmented. *C. islandica* has extreme morphological variability, and circumpolar and boreal distribution.

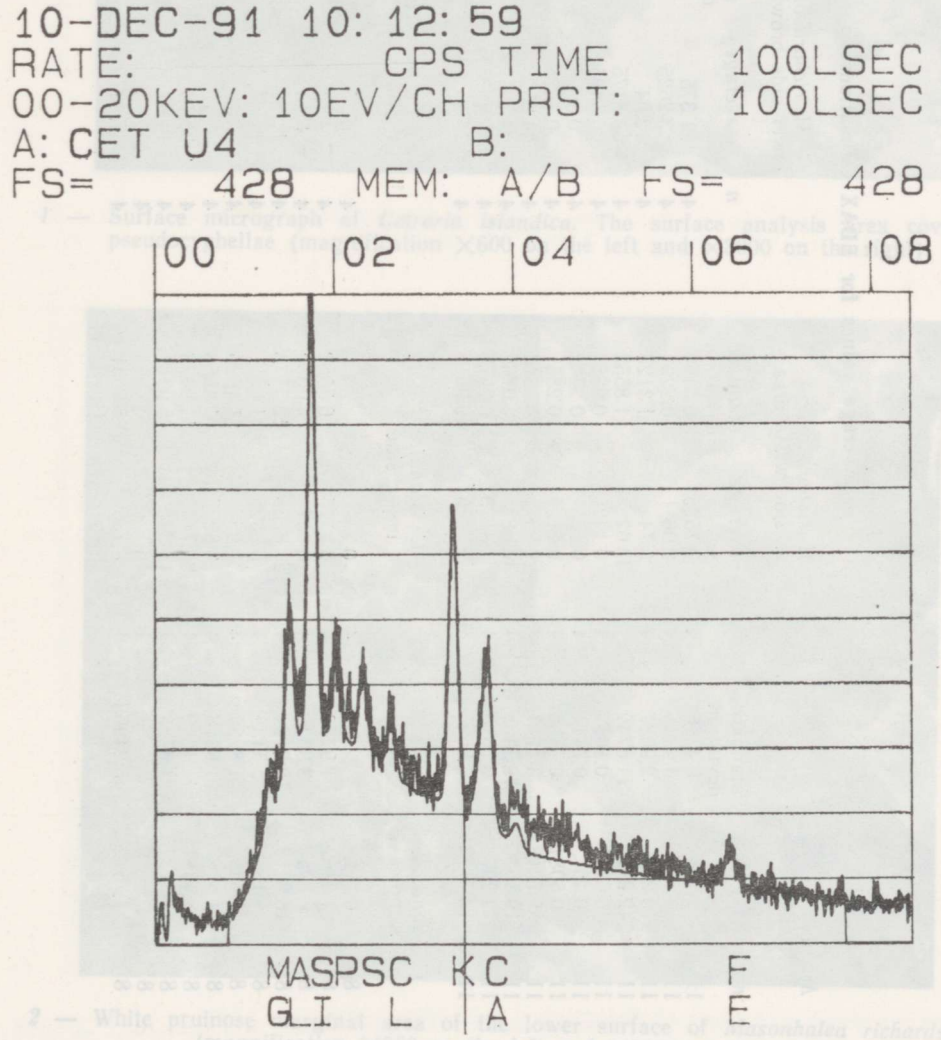
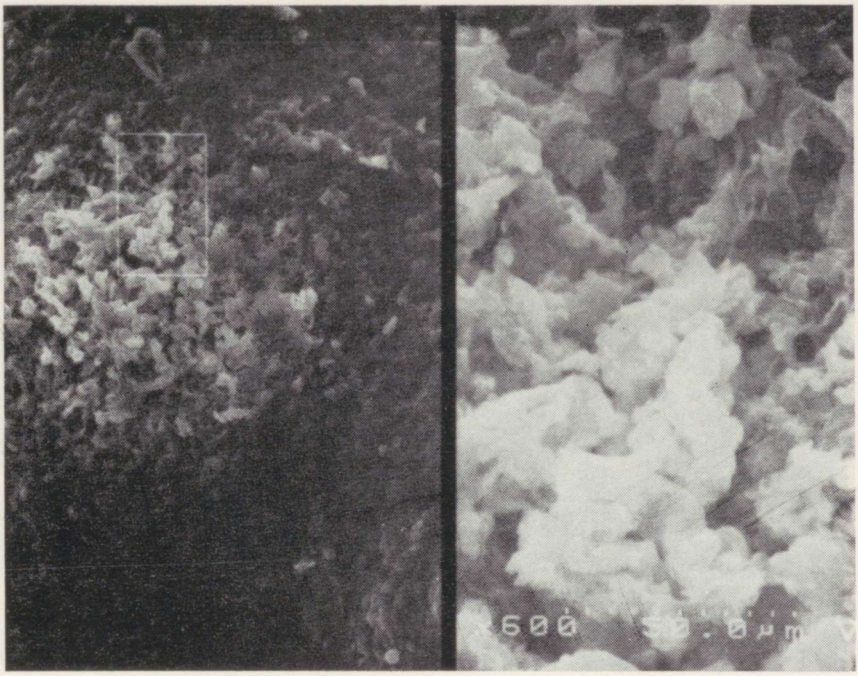


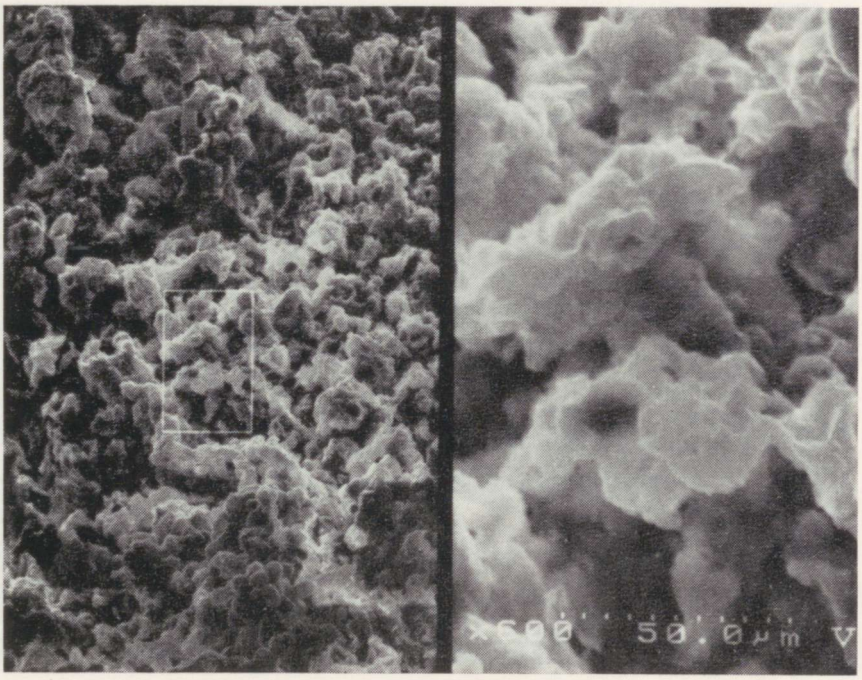
Fig. 1. Spectrogram of the upper surface of *Cetraria islandica* showing the detected chemical elements.

Average values for EDAX estimations

El.	*M. richardsonii*, all samples			*M. richardsonii*, lower side white area			*M. richardsonii*, lower side brown area			*M. richardsonii*, upper side		
	n	average	standard deviation	*n*	average	standard deviation	*n*	average	standard deviation	*n*	average	standard deviation
Na	11	1.1482	0.4395	4	0.7525	0.5160	4	1.375	0.1075	3	1.3733	0.2386
Mg	11	3.05	0.9395	4	2.06	0.2201	4	3.2625	0.5920	3	4.0866	0.2386
Al	11	10.2536	2.9087	4	6.74	1.3123	4	12.04	0.2453	3	12.5567	0.7756
Si	11	42.2027	11.8742	4	32.02	1.8552	4	54.8175	8.9805	3	38.9767	6.1310
P	11	0.6272	0.8051	4	0.1425	0.285	4	0.5425	1.085	3	1.3866	0.0680
S	11	0.46	0.7444	4	0.135	0.27	4	0.3	0.6	3	1.1066	1.1100
Cl	11	0.6081	0.7168	4	0.1175	0.235	4	0.7625	0.5831	3	1.0566	1.0702
K	11	5.7018	3.4747	4	2.9375	0.2364	4	4.8525	1.8879	3	10.52	1.8409
Ca	11	19.5536	19.3488	4	43.68	3.0762	4	4.295	3.0786	3	7.73	1.9900
Ti	11	2.2972	0.7613	4	1.6175	0.2975	4	2.2675	0.2168	3	3.2433	0.6531
Fe	11	13.7173	3.9693	4	9.0275	0.9618	4	15.23	1.1262	3	17.9533	0.0802
	C. islandica. all samples			*C. islandica.* upper side			*C. islandica.* lower side			*H. splendens*, all samples		
Na	8	0		4	0		4	0		5	0	
Mg	8	4.0462	1.9752	4	3.9775	0.5382	4	4.115	2.9667	5	2.44	2.3742
Al	8	6.895	3.3025	4	8.6375	4.0038	4	5.152	1.1492	5	3.852	1.2803
Si	8	18.9713	12.0124	4	26.8225	12.5575	4	11.12	3.8242	5	10.228	4.6692
P	8	9.105	5.5438	4	5.3525	1.6951	4	12.857	5.5938	5	0.926	1.2705
S	8	5.84	1.8285	4	4.95	1.4620	4	6.73	1.8845	5	1.914	1.1760
Cl	8	4.6162	1.6218	4	3.8225	1.4658	4	5.41	1.5193	5	2.314	1.3881
K	8	28.4387	11.1679	4	23.7175	11.9828	4	33.16	9.3803	5	7.698	2.4773
Ca	8	14.3137	4.6788	4	14.6	5.9319	4	14.0275	3.9590	5	63.628	13.3214
Ti	8	0		4	0		4	0		5	0.694	0.9521
Fe	8	7.7812	5.24908	4	8.135	5.9031	4	7.4275	5.3953	5	2.672	2.7905

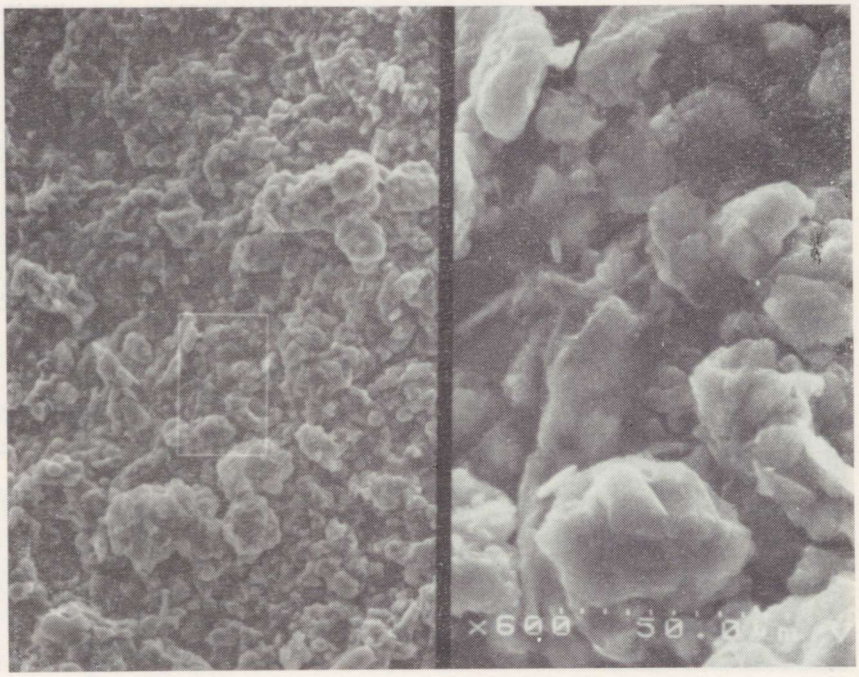


1 — Surface micrograph of *Cetraria islandica*. The surface analysis area covering pseudocyphellae (magnification ×600 on the left and ×3000 on the right).

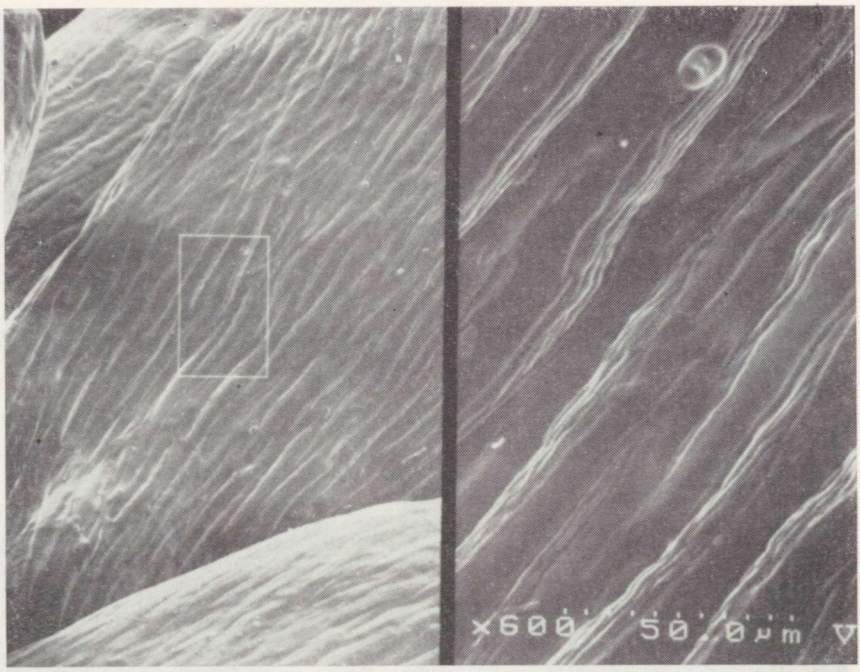


2 — White pruinose marginal area of the lower surface of *Masonhalea richardsonii* (magnification ×600 on the left and ×3000 on the right).

PLATE II



1 — Brown area of the lower surface of *Masonhalea richardsonii* (magnification ×600 on the left and ×3000 on the right).



2 — Surface of *Hylocomium splendens*, ×600 and ×3000.

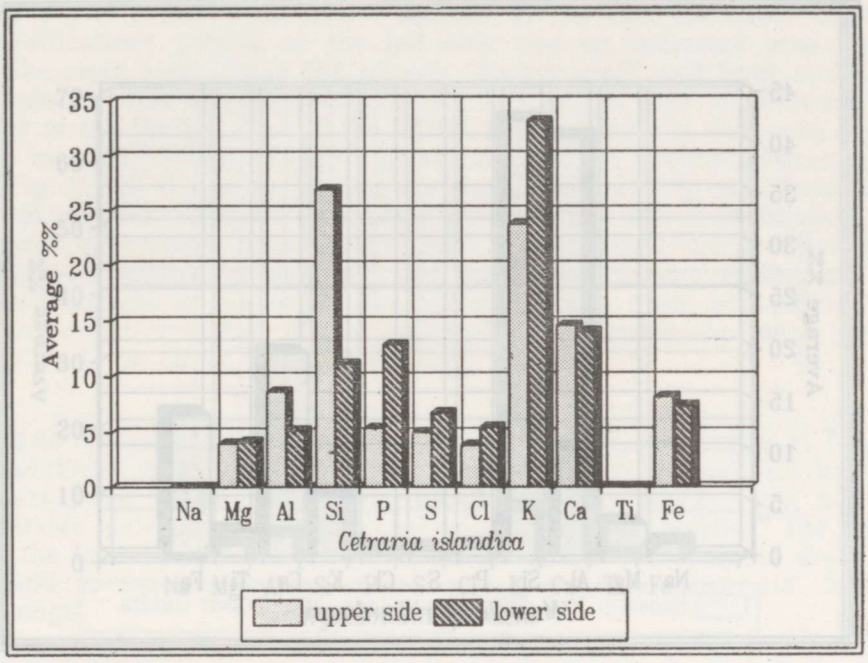


Fig. 2. Average figure for chemical elements detected on the upper and lower surfaces of *Cetraria islandica*.

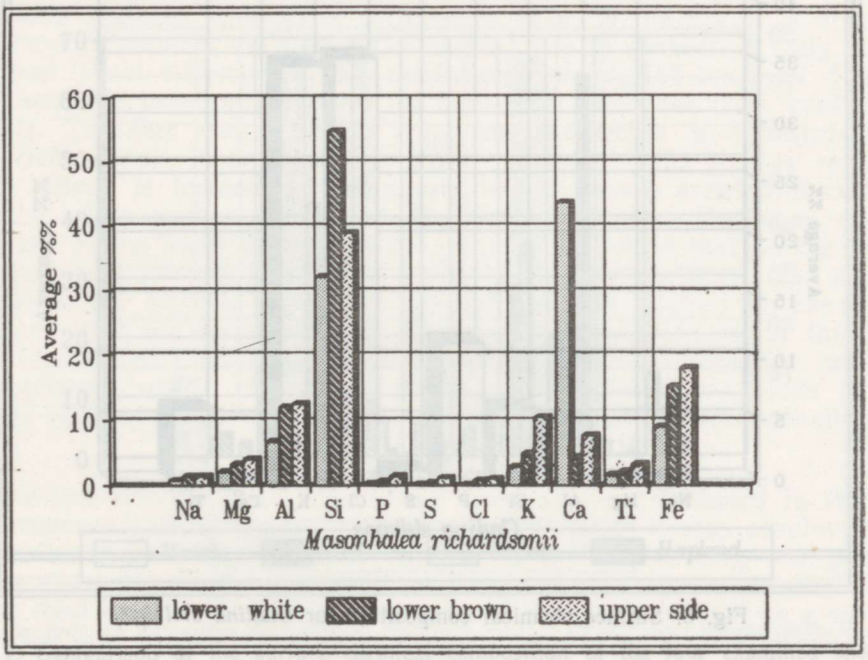


Fig. 3. Comparison of three different surface areas of the elemental composition of *Masonhalea richardsonii* (the lower side pruinose and lower side brown area and the upper side of the thallus).

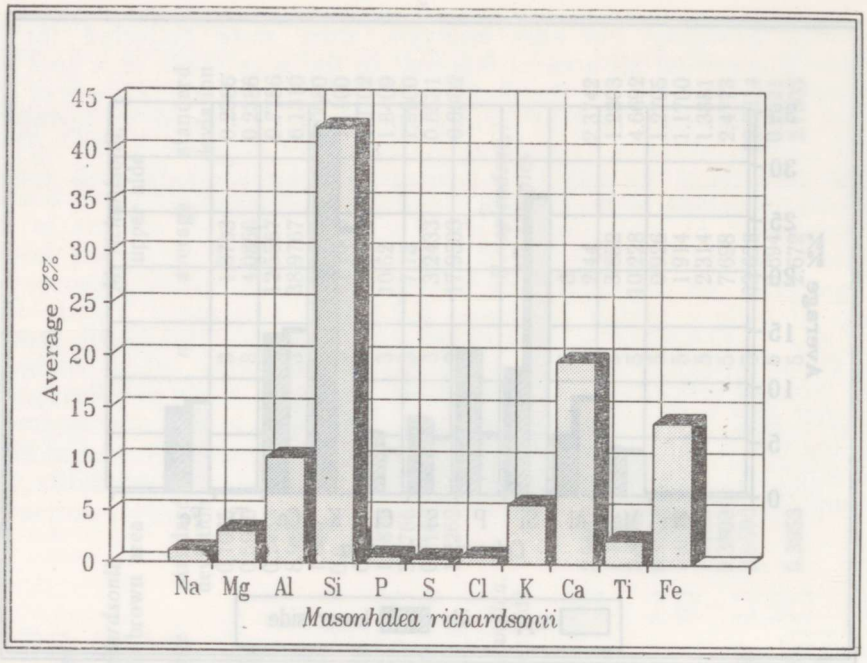


Fig. 4. Average figures for *Masonhalea richardsonii* surface chemical composition.

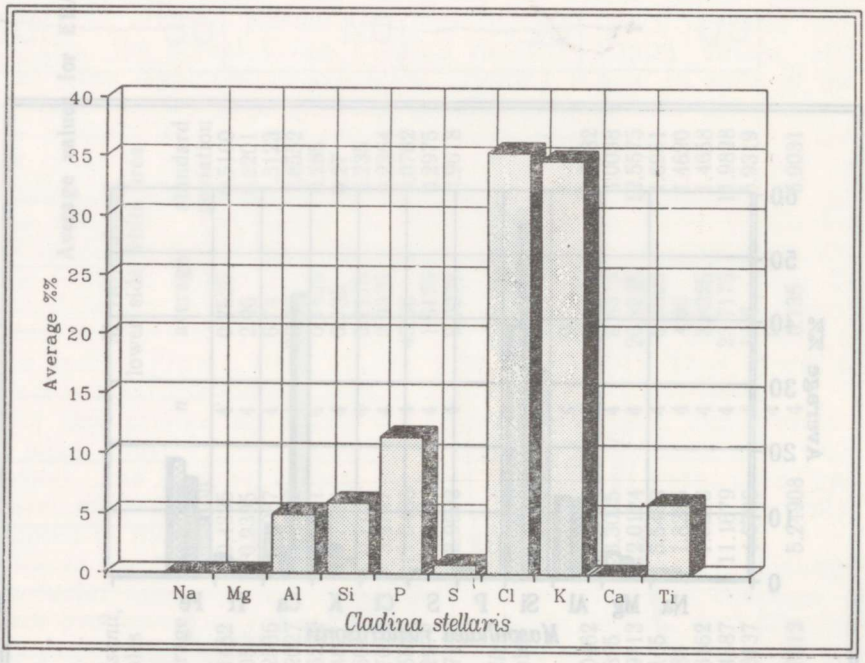


Fig. 5. Surface chemical composition for *Cladina stellaris*.

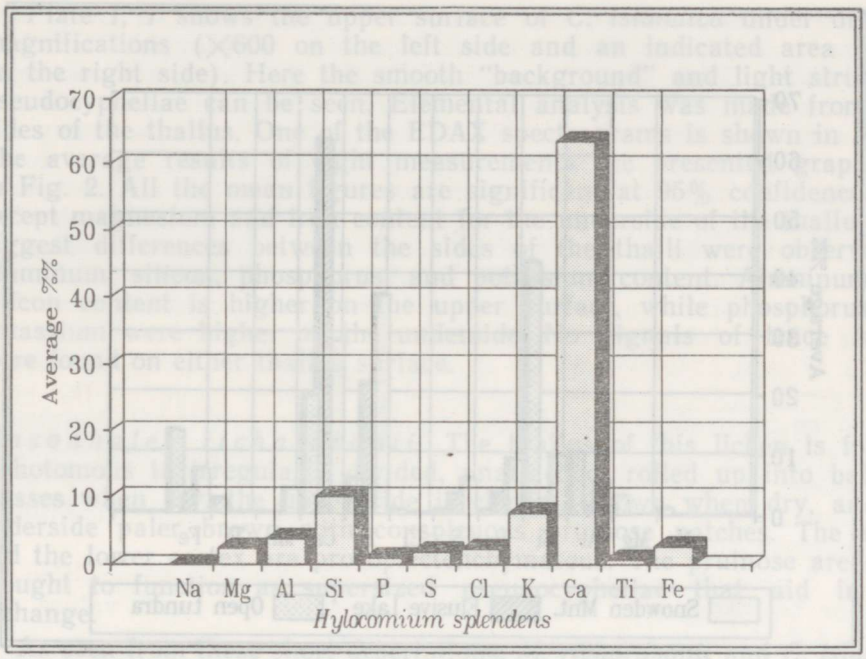


Fig. 6. Elemental composition of the leaflets surface of *Hylocomium splendens* (average figures).

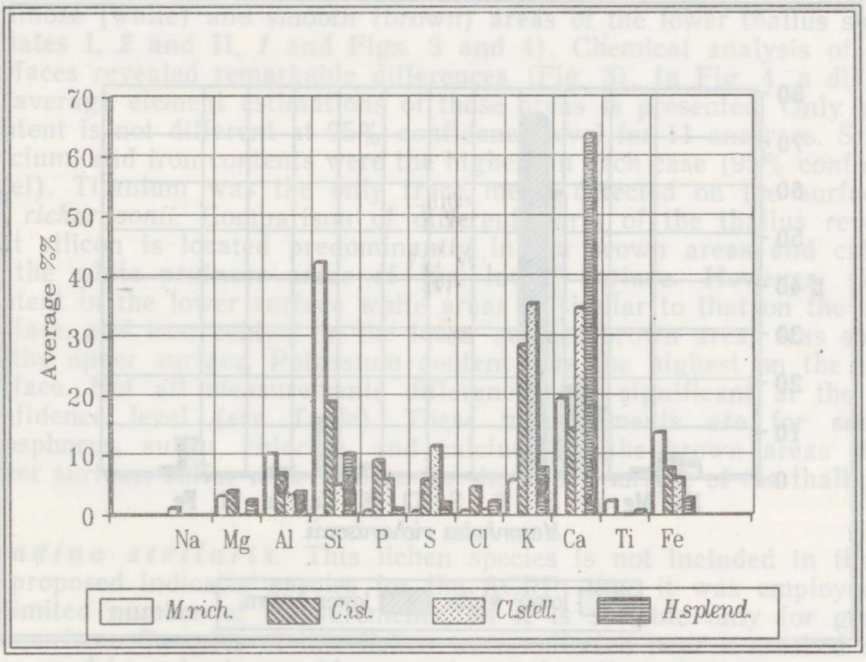


Fig. 7. Comparison of the surface chemical composition of the four examined species (average figures).

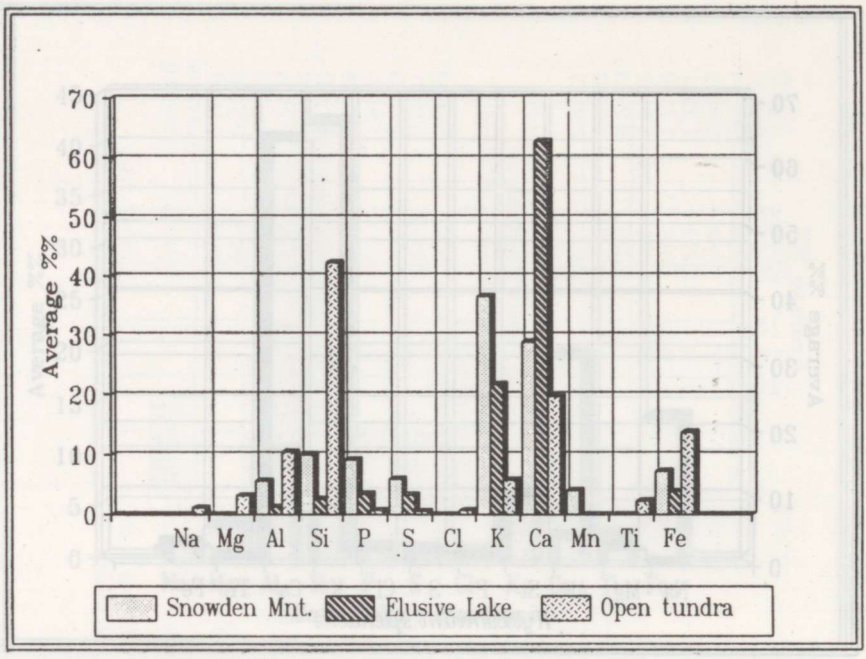


Fig. 8. Surface elemental composition of *Masonhalea richardsonii* thallus samples collected from sites differing in dust pollution.

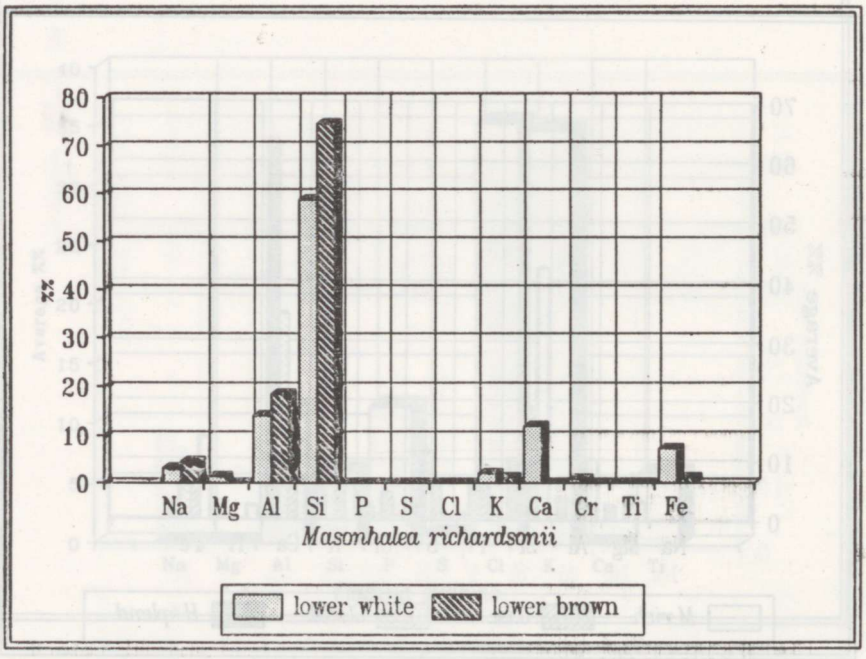


Fig. 9. *Masonhalea richardsonii* surface chemical composition estimated by spot analysis (magnification ×2000).

Plate I, *1* shows the upper surface of *C. islandica* under different magnifications (×600 on the left side and an indicated area ×3000 on the right side). Here the smooth "background" and light structured pseudocyphellae can be seen. Elemental analysis was made from both sides of the thallus. One of the EDAX spectrograms is shown in Fig. 1. The average results of eight measurements are presented graphically in Fig. 2. All the mean figures are significant at 95% confidence level except magnesium and iron content for the underside of the thallus. The biggest differences between the sides of the thalli were observed in aluminum, silicon, phosphorus, and potassium content. Aluminum and silicon content is higher on the upper surface, while phosphorus and potassium were higher on the underside. No signals of trace metals were found on either thallus surface.

Masonhalea richardsonii. The thallus of this lichen is folious, dichotomous to irregularly divided, unattached, rolled up into ball-like masses when dry, the upper side is chestnut-brown when dry, and the underside paler brown with conspicuous pruinose patches. The upper and the lower cortex are prosoplectenchymatous. The pruinose areas are thought to function as supersized pseudocyphellae that aid in gas exchange.

As seen from these short descriptions, *M. richardsonii* and *C. islandica* have similar cortical anatomy and similar growth forms. Some forms of *C. islandica* are only slightly attached to the substrate and may be blown about by strong winds.

Because of observed differences in surface texture a series of element measurements was made separately for upper and lower thallus surfaces of *M. richardsonii.* Also a different series of measurements was made for pruinose (white) and smooth (brown) areas of the lower thallus surface (Plates I, *2* and II, *1* and Figs. 3 and 4). Chemical analysis of these surfaces revealed remarkable differences (Fig. 3). In Fig. 4 a diagram of average element estimations of these areas is presented. Only sulfur content is not different at 95% confidence level for 11 analyses. Silicon, calcium, and iron contents were the highest in each case (95% confidence level). Titanium was the only trace metal detected on the surface of *M. richardsonii.* Comparison of different parts of the thallus revealed that silicon is located predominantly in the brown areas and calcium in the white pruinose areas of the lower surface. However, silicon content in the lower surface white areas is similar to that on the upper surface, and iron content in the lower surface brown areas was similar to the upper surface. Potassium content was the highest on the upper surface. Not all measurements differences are significant at the 95% confidence level (see Table). These measurements are for sodium, phosphorus, sulfur, chlorine, and calcium for the brown areas of the lower surface, sulfur and chlorine for the upper surface of the thallus.

Cladina stellaris. This lichen species is not included in the list of proposed indicator species for the ACRP; thus it was employed for a limited number of measurements, as it is suitable only for general comparison. Samples of this lichen were collected near a crashed stone base road in order to provide examples of the effect of dust as a surface contaminant. Among the eight observed elements, potassium and chlorine showed the highest content on the thalli surface (35% and 34.7%, respectively). Both of these elements are notably higher in samples collected near the highway than for other Alaskan lichen samples (Fig. 5).

Hylocomium splendens. This moss species has a wide distribution in arctic and boreal zones. It is also polymorphic and var. *obtusifolium* (Geh.) Par. is common in the North American Arctic (Ireland et al., 1987). The leaflets and their surface are quite different when compared with lichens (Plate II, *2*). There are also many differences in their anatomy and morphology. However, the small size and position of leaflets on the *H. splendens* stem make the surface-volume ratio of this moss close to that of lichens. Biologically, *H. splendens* belongs to vascular plants, but it has a poorly developed conducting system. This suggests a possible absorption mechanism, which may enable them to take up nutrients directly from water flowing over their surface (Richardson, 1981).

Fig. 6 shows the results of chemical analysis of the upper surface of *H. splendens.* Calcium is the dominant element found on the moss surface. Observations for magnesium and phosphorus are not significantly different. The calcium content is rather high. According to some other publications (Bates, 1982), bryophytes having similar ecology show potassium to be the dominant element, except for the plants growing on carboniferous limestone (Russell, 1987).

Fig. 7 presents composite data on surface chemical composition for the four tested species. The statistical data on the mean values and standard deviations are included in the Table.

As shown in Fig. 7, all the species tested have a chemically different surface composition and variations between species and elements in their surface layer are high. At the same time, the sets of detected elements on the surface of all species show lack of trace metals, except for titanium in *M. richardsonii* and *H. splendens.* Silicon, potassium, and calcium are high in terms of percentage composition for all species. Among these potassium and calcium are known as biogenes necessary for growth and their concentrations are usually higher in younger parts of mosses. The surface of *M. richardsonii* has the highest content of aluminum, silicon, titanium, and iron; *C. islandica* the highest content of magnesium, phosphorus, and chlorine; *Cl. stellaris* — sulfur and potassium; and *H. splendens* — calcium.

Fig. 8 compares the surface chemical composition of *M. richardsonii* from different localities (road site, Elusive Lake area, and the slope of Snowden Mountain near the Atigun Pass in Brooks Range). These localities are situated at different distances from a dust source generated by highway traffic. Samples of *M. richardsonii* collected from open tundra directly impacted by road dust have higher concentrations of aluminum, silicon, and iron than the other samples. The titanium content on their surface is also the highest, while in this habitat the potassium content is the lowest of the three habitats compared. The highest content of potassium was determined in the samples from Snowden Mountain and the highest calcium content in the surface layer was found in samples from Elusive Lake area. A rather low level of manganese (appr. 4%) was observed in only one sample-site from Snowden Mountain.

We were interested in the chemical composition of fine structures on the surface of *M. richardsonii* (Plate I, *2*). For that purpose we used a magnification of ×2000 and employed spot analysis of selected structures. Results of spot analysis are shown in Fig. 9. It is obvious that the number of elements detected is smaller than that of area analysis. When looking separately at the white pruinose and brown areas on the surface of lichen thalli we can see that they have similar composition except for the presence of calcium and magnesium in the pruinose marginal area. The domination of aluminum and silicon as well as higher percentage of sodium probably occur due to trapped

small particles of alumosilicates (proportion of the elements is similar to chemical composition of montmorillonite known as secondary mineral) which are overgrown by lichen structures. Such trapped particles may then serve as a source of mineral nutrients. One of the analyzed spots was a pruina grain-like structure. This structure revealed a high content of calcium in addition to a high concentration of silicon. These results correspond to the earlier investigations reported by Wadsten and Moberg (1985). One general conclusion is that no samples demonstrated trace metals on the lichen thalli surface, except for titanium and chromium (one case) on the lichen *M. richardsonii* and the moss *H. splendens* that were collected from open tundra. Secondly, lichens are able to trap mineral particles and to change them chemically. This is probably a nutrition source in addition to the active or passive uptake of cations from other sources. Surface analyses in this investigation reveal, with the exception of specimens collected in dusty areas, no surface contamination by pollutants such as lead and cadmium. Thus washing in these environments or otherwise cleaning specimens prior to chemical analysis is unnecessary.

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