diversity

MDPI

Article

The Effects of Edaphic and Climatic Factors on Secondary Lichen Chemistry: A Case Study Using Saxicolous Lichens

Alexander Paukov [1,*], Anzhelika Teptina [1], Maria Morozova [1], Ekaterina Kruglova [1], Sergio E. Favero-Longo [2], Cora Bishop [3] and Nishanta Rajakaruna [3,4]

1 Institute of Natural Sciences and Mathematics, Ural Federal University, Lenina av. 51, 620000 Ekaterinburg, Russia; ateptina@gmail.com (A.T.); morphey_usu@mail.ru (M.M.); kruglovaep@mail.ru (E.K.)
2 Dipartimento di Scienze della Vita e Biologia dei Sistemi, Universitá degli Studi di Torino, Viale Mattioli 25, 10125 Turin, Italy; sergio.favero@unito.it
3 Biological Sciences Department, California Polytechnic State University, San Luis Obispo, CA 93407, USA; cobishop@calpoly.edu (C.B.); nrajakar@calpoly.edu (N.R.)
4 Unit for Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa
* Correspondence: alexander.paukov@urfu.ru

Received: 15 May 2019; Accepted: 14 June 2019; Published: 17 June 2019

Abstract: Diversity of secondary lichen metabolites and their relationship to substrate and environmental parameters were studied in saxicolous lichens in the Middle and South Urals of Russia. Atranorin, usnic acid, gyrophoric acid, zeorin, norstictic acid, antraquinones and stictic acid were found in 73, 42, 41, 37, 36, 35 and 32 species, respectively, of 543 taxa collected. One hundred and ninety six species (i.e., 36% of total species documented) contained no secondary metabolites. Spectra of secondary metabolites of crustose lichens varied on different rock types, while in fruticose and foliose groups only those species without lichen acids were dependent on the substrate type. In Canonical Correspondence Analysis, secondary lichen metabolites were subdivided into groups depending on the concentration of Ca and metals in the substrate. Gyrophoric, lobaric, psoromic, rhizocarpic and stictic acids were common in crustose lichens in metal-poor habitats; species with antraquinones and lichens without any secondary metabolites were most abundant on limestone (alkalic and metal-poor), while other common lichen metabolites had no to minimal dependence on the chemistry of the substrate. The two additional abiotic factors affecting the composition of secondary metabolites were the maximum temperature of the warmest month and elevation. Our results suggest a range of possible relationships exist among lichen acids, rocks and climatic parameters. Furthermore, the same metabolite may affect both accumulation of metals and stress tolerance under unfavorable conditions.

Keywords: saxicolous lichens; lichen acids; rock chemistry; climatic factors; Urals; CCA

1. Introduction

Lichens produce a wide range of secondary metabolites, often collectively referred to as 'lichen acids,' only a few of which are present in other organisms [1]. More than 800 lichen acids are currently known, and more are being discovered on a regular basis [2]. Lichen secondary metabolites, including pigments, have a number of known functions. Some substances are biologically active and act as allelopathic agents [3,4], while others affect herbivore palatability [5,6], increase permeability of the cell membrane of phycobionts [7,8] and protect photobionts from excessive ultraviolet (UV)-B light [3,6–11].

Environmental conditions strongly affect both the type and the concentration of secondary metabolites in lichens. Concentrations of melanins and anthraquinones vary depending on the amount of UV-B light; the mycobiont is presumed to be the more susceptible partner to such variations, compared to the algal component [12]. Usnic and fatty acids are found to decrease in concentration with increased levels of anthropogenic stress while the rising levels of salazinic acid in lichens in ozone-enriched environments likely provide a protective function [13]. Lichen substances also have antioxidant properties which are beneficial in high latitudes where reactive oxygen species are formed under both high UV-B levels and prolonged winter darkness [14,15]. However, the relationship among pigment concentration in thalli, habitat conditions and lichen growth patterns is complex [16]; for example, the same lichen species can show different physiological responses to UV-B light based on growing season and habitat conditions [17] and additional studies are required to demonstrate how lichens and their secondary metabolites respond to enhanced UV-B radiation and other aspects of global climate change or local habitat conditions.

Environmental conditions such as water availability, solar radiation and temperature may cause the appearance of chemosyndromes in species. Chemical changes in *Ramalina cuspidata* (Ach.) Nyl. and *R. siliquosa* (Huds.) A.L. Sm. are likely affected by an evapotranspiration gradient [18]. Additionally, another pair of morphologically indistinguishable but chemically distinct *Ramalina* species is subdivided in their geographical distributional range [19]. Variability of secondary metabolites within the chemospecies of *Cladonia chlorophaea*-group is also attributed to physiography [20]. Phycosymbiodeme pairs—morphotypes of a species differing in their photobionts—which are also largely controlled by the habitat microclimate, often contain different secondary metabolites [21,22].

The substrate type and its properties have a paramount importance for lichens and may prevail over the microclimatic factors. Physical parameters of the substrate, the microtopography of the surface, pH, elemental content and surface availability for colonization are among the factors which affect lichen diversity as well as the structure of saxicolous lichen communities [23–30]. The wide range of rocks as a substrate for lichens and associated lichen metabolites imply a multitude of interactions among them. Lichen acids affect the acidity tolerance of lichens and, thus, the choice of their substrate [31]. Species of the same genus containing different metabolites dominate on different types of rocks [27] and a spectrum of chemical constituents of lichens have been shown to gradually change from quartzite to limestone [32].

Secondary lichen metabolites interact with metals in the substrate. Although lichen deterioration of rocks is often related to the secretion of primary metabolites such as oxalic acid [33–35], lichen secondary metabolites were also shown to act as chelating agents, promoting the absorbance of Cu, Fe, Mg, Mn, Ni and Zn and inactivating potentially toxic elements in thalli [36–41]. By facilitating the absorption of metals, lichen acids contribute to mineral nutrition of thalli which is essential in impoverished environments [42,43]. In contrast, lichens on metal (Fe)-rich rocks or slags were shown to be mostly devoid of secondary metabolites or containing secondary metabolites which decrease $Me(Fe)^{2+}$ adsorption, explaining their tolerance of excessive concentrations [39]. Lichens may even reduce the relative concentrations of secondary metabolites in response to the increase in metal concentration [44]. Moreover, lichen communities growing in metal-rich ultramafic alpine areas may exhibit differences in terms of secondary metabolite production compared to those of adjacent non-ultramafic areas (e.g., lower frequency of species with depsidones) [45]. However, in other cases, lichen acids play no role in protection of thalli against airborne heavy metal pollutants [46] or against metals taken from a substrate [47]. Factors other than secondary metabolites may also affect metal accumulation as shown by two closely-related species containing the same lichen acid and growing in the same habitat but having dramatically different heavy metal concentrations [32].

The aim of the paper is to evaluate if lichen species producing different secondary compounds have distinct affinities to various rock types and their elemental constituents as well as to correlate the abundance of species containing different lichen metabolites with environmental parameters of the habitat. Towards this goal, we quantified lichen diversity and the related abundance of secondary

metabolites, on six different rock types — chemically ranging from acid to ultrabasic lithologies — through wide regions of Ural Mountains (Russia). In particular, the variability of secondary metabolites with respect to rock chemistry was compared in crustose lichens with respect to foliose-fruticose ones, to determine if the different levels of contact with the substrate may yield different patterns.

2. Materials and Methods

2.1. Study Area

Saxicolous lichens were studied in the Ural Mountains (Russia) in the territory of four administrative regions—Sverdlovsk, Chelyabinsk, Orenburg oblasts and the Republic of Bashkortostan (Figure 1). The territory belongs to the Middle and South Urals and stretches from the taiga subzone to grass steppes. Lithologically, it is a diverse area with outcrops of carbonate, ultrabasic, basic and acidic rocks. Most of the territory is strongly elevated and, apart from the zonal vegetation, is covered by mountain biomes represented by the Middle Ural taiga, which gradually changes to nemoral coniferous-deciduous and deciduous forests in the South Urals. Localities on the highest elevations visited during this study are covered by birch-larch woodlands and dark coniferous forests. The climate of the territory is moderately continental, with a cold winter and a warm summer. Average annual temperature varies from −0.7 °C to + 4.7 °C [48].

2.2. Field Survey

Eighteen sampling localities were selected so as to reflect a spectrum of rock types and microclimates of the study area (Table 1). The localities were selected using a geological map of the Ural Mountains. As the territory of the Middle Urals is primarily covered by zonal forests, an attempt was made to find rock outcrops in elevated localities or on riverbanks. The rock outcrops are not equally distributed in the study area and, accordingly, seven localities were selected on serpentinites, four on limestones, three both on basalts and granites and one on quartzite. In every sampling locality, a series of 1 × 1 m quadrats was established for which geographical coordinates and elevation were recorded. In every locality, the quadrats were applied to surfaces with different inclination and, where possible, with different azimuth. The number of quadrats selected was greater than or equal to ten per locality (except for locality 14). We followed the approach of preferential sampling [49], describing the most species-rich lichen assemblages and avoiding areas devoid of lichens. Ten records of lichen cover were made within every 1 × 1 m quadrat using 10 × 10 cm plots (Figure 2), leading to 80 to 200 records per locality. To facilitate counting, every plot was photographed with a superposed mesh, subdivided into 100 1 × 1 cm squares. The plots were distributed at corners, sides and in the center of every quadrat. The total number of plot records used in this study is 2480. Specimens of lichens were collected together with rock samples for determination of species, their secondary chemistry and elemental composition of the rock substrates. In order to compare the diversity of secondary metabolites of lichens on different rock types we assembled a complete list of saxicolous species of the region, including those not represented in the descriptions, using published species lists [50–52]. Lichen nomenclature follows [53,54].

For each sampling locality, the following parameters were evaluated: rock type and chemical composition (analytical procedure subsequently described), annual mean temperature, maximum temperature of the warmest month, minimum temperature of the coldest month, and the annual precipitation and heat index [55,56], calculated using the formula cos (aspect—202.5°) × tg (slope). The data on temperature and precipitation, namely average annual temperature, maximum temperature of the warmest month (July), minimum temperature of the coldest month (January) and annual precipitation, were extracted from the DIVA-GIS 7.5 application [57].

Figure 1. Study area and sampling localities. Modified from maps at d-maps.com.

Table 1. Coordinates, elevation and rock types in the study localities.

N	Region	Locality	Latitude, N	Longitude, E	Elevation, m	Rock Type	Number of 1 × 1 m Quadrats	Number of 10 × 10 cm Squares
1	Sverdlovsk	Melkozyorovo village	57°44′	61°28′	125	Serpentinite	10	100
2	Sverdlovsk	Kourovka settlement	57°02′	59°38′	280	Limestone	15	150
3	Sverdlovsk	Petra Gronskogo rocks	56°58′	60°18′	320	Granite	20	200
4	Sverdlovsk	Ekaterinburg, Baran peninsula	56°50′	60°30′	280	Granite	20	200
5	Sverdlovsk	Ekaterinburg, Kamennye palatki	56°50′	60°40′	280	Granite	10	100
6	Sverdlovsk	Dvurechensk settlement	56°35′	61°03′	205	Serpentinite	18	180
7	Sverdlovsk	Bazhukovo settlement 'Olenyi Ruchyi' National park	56°30′	59°15′	300	Limestone	20	200
8	Sverdlovsk	Kodinka village	56°25′	61°48′	120	Limestone	16	160
9	Chelyabinsk	Egoza mountain	55°45′	60°26′	570	Serpentinite	10	100
10	Chelyabinsk	Zyuratkul' mountain	54°57′	59°10′	1170	Quartzite	20	200
11	Bashkortostan	Kalkanovo village	54°25′	59°20′	670	Serpentinite	12	120
12	Bashkortostan	Shigayevo village	53°48′	58°11′	560	Serpentinite	12	120
13	Bashkortostan	Kushai mountain	53°43′	58°37′	507-920	Basalt	15	150
14	Chelyabinsk	Yangelskiy settlement	53°13′	58°54′	350	Limestone	8	80
15	Orenburg	Mazovo village	51°30′	58°01′	340	Serpentinite	12	120
16	Orenburg	Gainulino village	51°23′	58°18′	270	Basalt	10	100
17	Orenburg	Akkermanovka settlement	51°08′	58°02′	300	Serpentinite	10	100
18	Orenburg	Khabarnoye settlement	51°06′	58°06′	220	Basalt	10	100

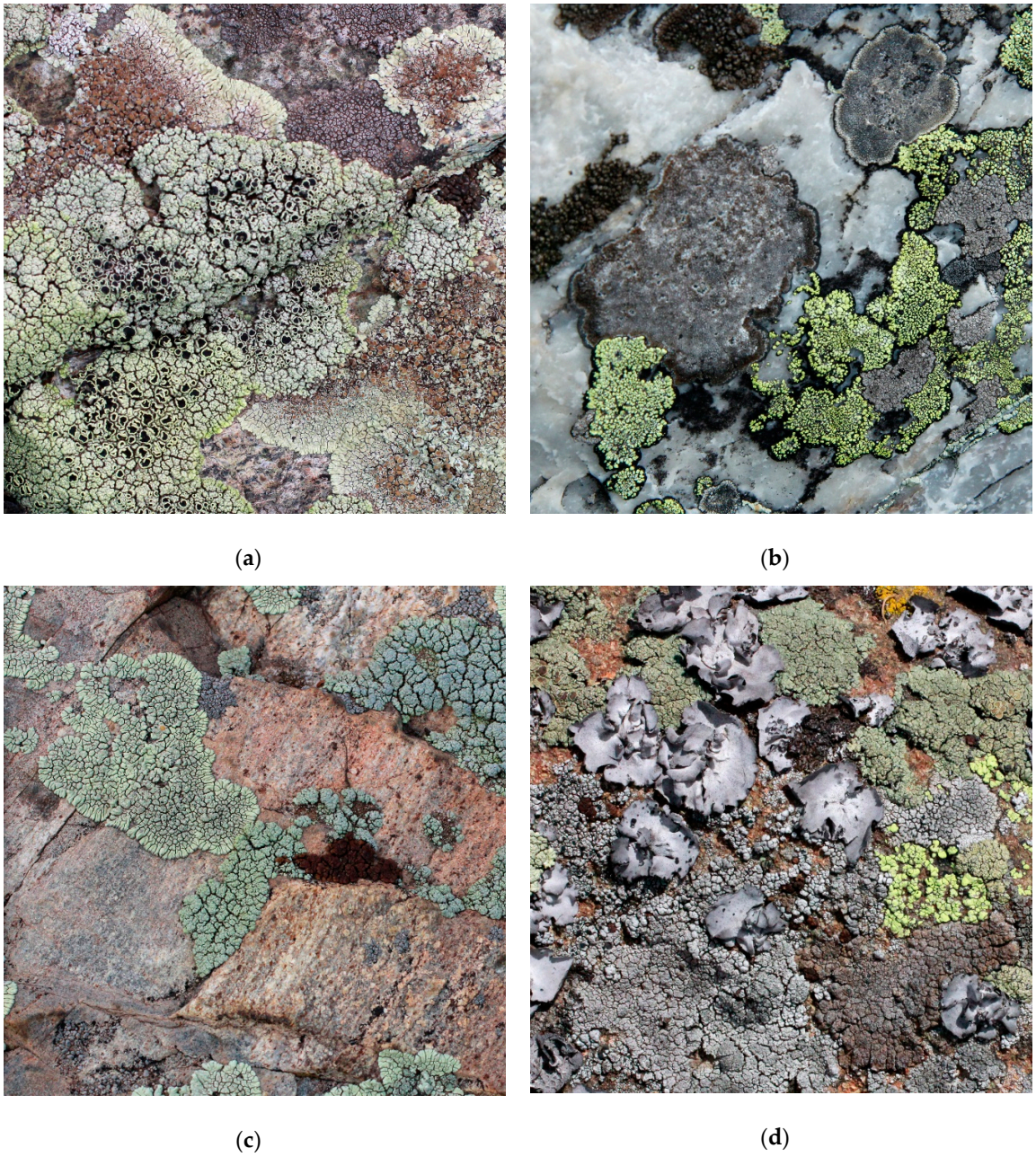


Figure 2. Lichen groupings on three rock types. (**a**) Serpentinite, Akkermanovka settlement, locality 17 (*Lecanora argopholis* (Ach.) Ach., *Protoparmeliopsis muralis* (Schreb.) M.Choisy and *Circinaria* spp.); (**b**) Quartzite, Zyuratkul' range, locality 10 (*Rhizocarpon geographicum* (L.) DC., *Sporastatia testudinea* (Ach.) A. Massal. and *Protoparmelia badia* (Hoffm.) Hafellner); (**c**) Basalt, Khabarnoye settlement, locality 18 (*Dimelaena oreina* (Ach.) Norman and *Lecanora argopholis*); (**d**) Basalt, Kushai mountain, locality 13 (*Umbilicaria subglabra* (Nyl.) Harm., *Immersaria athroocarpa* (Ach.) Rambold & Pietschm., *Aspicilia cinerea* (L.) Körb., *Lecanora* spp.). Bar = 1 cm.

2.3. Analytical Procedures

Secondary products were analyzed by applying standard thin-layer chromatography techniques [58,59] in solvent systems A (toluene: 1,4-dioxane:acetic acid, 180:45:5), B (hexane:diethyl ether:formic acid, 140:72:18) and C (toluene:acetic acid, 170:30).

For the determination of elemental composition of rocks, five rock samples from each sampling locality were ground in a porcelain mortar, oven-dried at 70 °C for 48 h, and 200 mg of each sample was

weighed and placed in fluoroplastic glasses with 1 mL of HF, 5 mL of HNO_3, 2 mL of HCl and 2 mL of deionized water, followed by digestion in a MARS 5 microwave oven (CEM Corporation, Matthews, NC, USA). After cooling, 7 mL of 4% boric acid were added and the solutions were transferred into 25-mL volumetric flasks and topped up to volume by deionized water. Concentrations of Ba, Ca, Co, Cr, Cu, Fe, Mg, Mn, Ni, Sr and Zn in rock samples were determined by inductively coupled plasma–atomic emission spectroscopy (iCAP 6500 Duo, Thermo Fischer Scientific, Waltham, MA, USA).

For determination of pH and conductivity of water extraction, 2 g of powdered rock samples were poured into 20 mL of distilled water and left for 48 h with periodical shaking. The pH was measured after precipitation of particles with a pH meter (Anion 4100, Novosibirsk, Russia) and conductivity was determined using HI 98129 Combo meter (Hanna Instruments, Port Louis, Mauritius).

2.4. Statistical Analyses

Matrices of (a) metabolite occurrence with respect to lichen species, (b) metal contents, pH and conductivity for the different rock types, (c) cover of species with different metabolites on every 10 × 10 cm plot, (d) geographical and environmental parameters for every locality were assembled as Microsoft Excel electronic tables. Total number of species on every rock type, average number of species within 10 × 10 cm plots, number of secondary metabolites and metabolite to species ratio (number of secondary metabolites/number of species) were calculated using Microsoft Excel. Shannon Diversity Index for every rock type and Chi-Square values were calculated in the PAST 3.18 package [60]. The metal content in the substrate, the pH and the conductivity in relation to the localities were analyzed as raw data using Principal Component Analysis (PCA). The relationships of cover of species to environmental variables and characteristics of substrates were analyzed using Canonical Correspondence Analysis (CCA) in CANOCO 5.0 [61].

To avoid the influence of species-specific patterns in the distribution of lichens, depending on the parameters of microhabitats, the cover of species containing the same lichen metabolite was summed in every description within 10 × 10 cm plot. If a species contained several lichen metabolites, its cover was counted correspondingly several times for every lichen acid in a thallus. CCA analyses were separately performed for crustose and foliose-fruticose species for comparison.

3. Results

3.1. Species and Secondary Metabolite Diversity

Five hundred and forty three lichen species (347 crustose, 141 foliose and 55 fruticose) were found inhabiting rock substrates in the Middle and South Urals. One hundred and ninety six (i.e., 36% of total species) contained no secondary metabolites. Twenty seven lichen metabolites were found in only one species and fourteen metabolites were present in 10 or more species. The most common amongst them was atranorin, which was found in 73 species (Table 2). Distribution of lichen acids within the morphological groups was unequal. The most chemically diverse were fruticose lichens which contained 24 metabolites in 55 species (ratio 0.44), while foliose lichens displayed 43 metabolites in 141 taxa (ratio 0.30), and 347 crustose species contained only 57 lichen acids (ratio 0.16).

Only atranorin was distributed in lichens independent of their life form (Table 2); it was a component of a wide range of crustose, foliose and fruticose species (taxonomically attributable to 10 families: Baeomycetaceae, Cladoniaceae, Graphidaceae, Icmadophilaceae, Lecanoraceae, Parmeliaceae, Physciaceae, Ramalinaceae, Stereocaulaceae and Tephromelataceae). The presence of certain metabolites was often correlated with a certain growth form. Gyrophoric acid and zeorin were found in crustose and foliose species; usnic acid had a higher affinity to fruticose and foliose taxa (mostly Cladoniaceae, Parmeliaceae, Ramalinaceae). Fumarprotocetraric acid was also common in the dimorphic-fruticose Cladoniaceae. Norstictic, stictic, xanthones and anthraquinones were mainly found in crustose species. In particular, anthraquinones were specific to nearly all Teloschistaceae but only 3 out of 41 saxicolous species of this family were foliose. Other metabolites, which did not display a strong dependence on

life form, were characteristic of certain genera or families: lobaric acid was a secondary metabolite of *Protoparmelia* spp. (crustose) and Stereocaulaceae (fruticose), psoromic acid was found mainly in *Rhizocarpon* spp. (crustose) and *Rhizoplaca* spp. (foliose).

Table 2. Major secondary metabolites of saxicolous lichens of different morphological groups.

Secondary Metabolite	Number of Species Containing the Metabolite			
	All Species	Crustose Species	Foliose Species	Fruticose Species
Atranorin	73	36	20	17
Usnic acid	42	10	14	18
Gyrophoric acid	41	19	22	0
Zeorin	37	19	15	3
Norstictic acid	36	30	4	2
Anthraquinones	35	32	3	0
Stictic acid	32	27	4	1
Fumarprotocetraric acid	21	1	3	17
Lecanoric acid	14	6	8	0
Lobaric acid	11	4	1	6
Psoromic acid	10	7	3	0
Xanthones	10	10	0	0

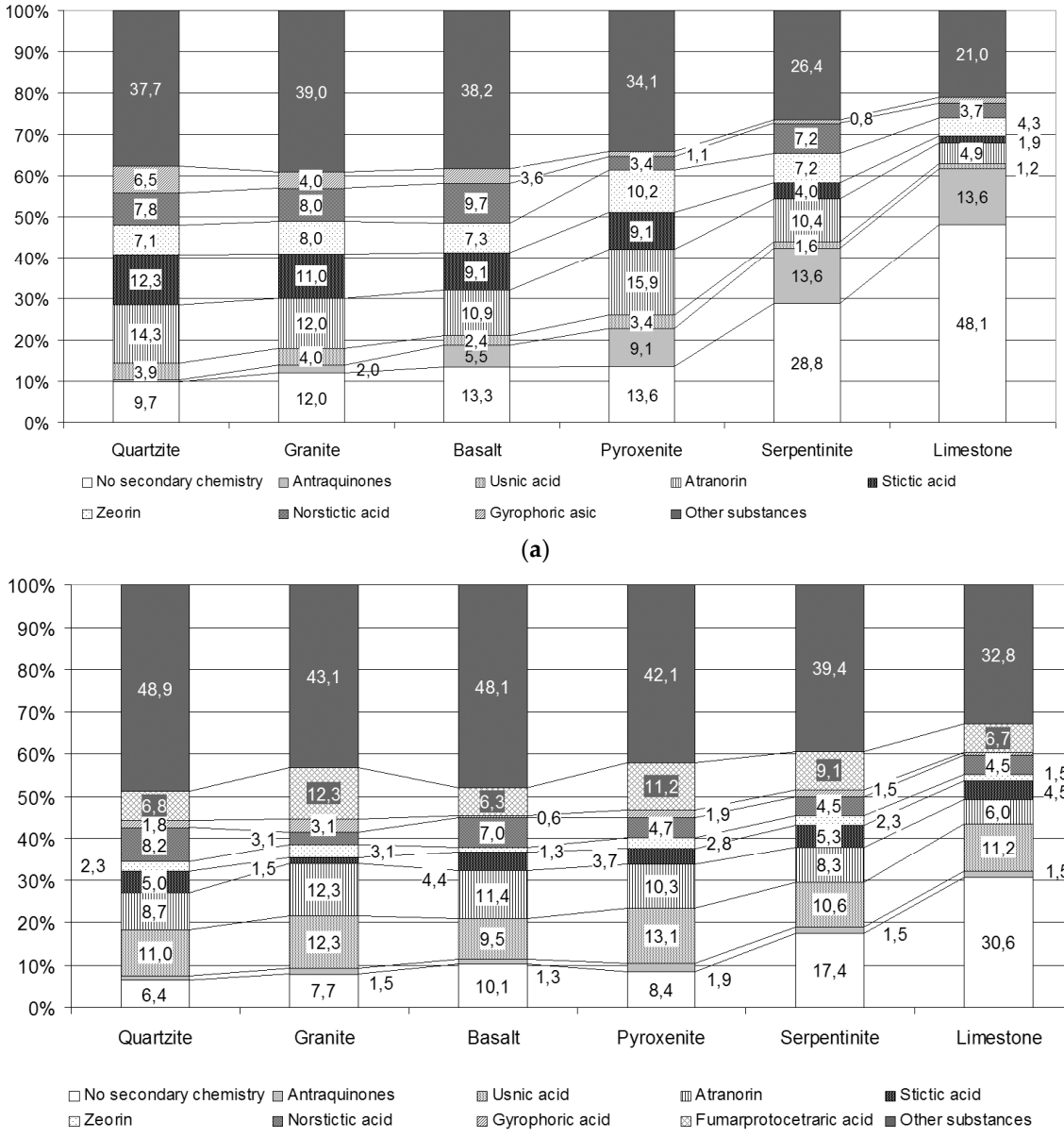
Species and secondary metabolite quantities were unequal on different rock types. The most species-rich substrate was limestone (233 species) followed by quartzite, basalt and serpentinite (Table 3). The total diversity of lichen biota on a particular rock substrate did not correlate with species richness within 10 × 10 cm plots. Despite their high biodiversity, limestones have on average as low as 3 species within a single description. High diversity of species on limestone may depend on the diversity of microhabitats in which this rock was found. Lichen flora of quartzite and basalt was enriched by the presence of alpine and tundra species at the highest elevations. The number of secondary metabolites in lichens on different rocks, however, did not correlate with their species diversity. For example, the greatest diversity of secondary metabolites was found on basalt and the lowest on limestone. Metabolite to species ratio was highest on granite and pyroxenite and this ratio was the same for all life forms of lichens. Average number of metabolites per species decreases gradually from quartzites to limestone.

Table 3. Species and secondary metabolite diversity on different rock types.

Measured Feature	Quartzite	Granite	Basalt	Pyroxenite	Serpentinite	Limestone
Total number of species	198	93	193	107	167	233
Number of species within 10 × 10 cm plots	3.19 ± 0.24	2.31 ± 0.14	4.41 ± 0.16	n/a	3.88 ± 0.18	2.81 ± 0.11
Shannon H diversity index	0.75 ± 0.07	0.45 ± 0.04	1.01 ± 0.03	n/a	0.81 ± 0.04	0.71 ± 0.03
Number of secondary metabolites	63	50	68	49	50	43
Metabolite to species ratio	0.32	0.54	0.35	0.46	0.30	0.18
Average number of metabolites in one species	1.72 ± 0.09^{A}	1.56 ± 0.11^{B}	1.44 ± 0.08^{B}	$1.61 \pm 0.11^{A,B}$	1.14 ± 0.09^{C}	0.74 ± 0.07^{D}

Values in the last row marked by the same letter are statistically insignificant at $p = 0.05$.

The proportion of crustose species without lichen acids was five times higher on limestone compared to that on quartzite (Figure 3a). The only metabolite that increased its percentage in species on alkaline substrata was anthraquinone (parietin). Gyrophoric, stictic and usnic acids were more common in lichens on acidic substrata but these differences were statistically insignificant for the latter. No particular trend was seen for norstictic acid, atranorin and zeorin; however, the latter two substances were found in substantially lower percentages on limestone. The differences in proportions of secondary metabolites were statistically insignificant for crustose lichens growing on quartzite, granite and basalt (Chi-square=9.4, $p = 0.9$) and significant for species growing on pyroxenite, serpentinite and limestone ($p = 0.0001$).



(**a**)

(**b**)

Figure 3. Percentage of lichen species containing eight major secondary metabolites on different rock types. (**a**) crustose species, (**b**) foliose and fruticose species.

Within foliose and fruticose species there was a lower proportion of species with no secondary metabolites on every rock type but, like in crustose lichens, the percentage of species without metabolites on limestone was fivefold compared to that on quartzite (Figure 3b). Other major secondary metabolites did not appear to be dependent on a rock type. The differences in proportions of secondary metabolites were significant for foliose and fruticose lichens growing on quartzite and limestone only (Chi-square = 33.7, $p = 0.0003$).

3.2. Chemical Properties of Rock Substrates

Rock substrates strongly varied in metal content as well as pH and conductivity ($S*10^{-3}/m$) of water extracts. The highest pH was found in limestone and serpentinite (with averages in the range 7.18–8.18) (Table 4).

Table 4. Chemistry of rocks in the studied localities.

Rock/Locality	pH	Conductivity, $S*10^{-3}/m$	Metals, µg/g (Average ± Standard Error)										
			Ba	Ca	Co	Cr	Cu	Fe	Mg	Mn	Ni	Sr	Zn
S 1	**7.36 ± 0.01**	341 ± 31	5 ± 2	928 ± 151	68 ± 1	438 ± 19	30 ± 15	29,491 ± 14,966	124,271 ± 62,183	571 ± 4	1677 ± 290	2 ± 0.0	35 ± 6
S 6	8.20 ± 0.06	294 ± 12	40 ± 25	9410 ± 5633	32 ± 6	770 ± 535	77 ± 34	25,835 ± 4813	58,592 ± 18,636	446 ± 92	526 ± 241	7 ± 2	31 ± 8
S 9	7.88 ± 0.02	205 ± 1	6 ± 2	7544 ± 746	16 ± 6	413 ± 74	8 ± 2	13,266 ± 2818	43,449 ± 8601	219 ± 28	569 ± 143	1 ± 0.0	24 ± 3
S 11	8.18 ± 0.04	342 ± 43	8 ± 3	376 ± 62	79 ± 8	256 ± 31	5 ± 0.0	39,370 ± 530	183,899 ± 6422	585 ± 35	1550 ± 76	2 ± 0.0	40 ± 3
S 12	8.08 ± 0.02	239 ± 9	5 ± 0.0	613 ± 23	70 ± 5	576 ± 126	3 ± 0.0	41,284 ± 1964	181,878 ± 13,159	567 ± 18	1553 ± 95	1 ± 0.0	17 ± 0.0
S 15	8.18 ± 0.08	289 ± 12	9 ± 3	529 ± 89	71 ± 7	281 ± 52	4 ± 2	39,499 ± 1707	200,647 ± 11,743	482 ± 49	1768 ± 309	3 ± 1	17 ± 2
S 17	8.08 ± 0.08	324 ± 21	7 ± 3	42,726 ± 28,779	61 ± 14	184 ± 26	3 ± 1	37,779 ± 5306	166,937 ± 7170	534 ± 135	1564 ± 438	23 ± 10	16 ± 3
L 2	7.86 ± 0.02	318 ± 43	11 ± 3	305,943 ± 10,157	nd	1 ± 1	12±1	290 ± 169	2223 ± 167	48 ± 18	2 ± 2	194 ± 46	9 ± 4
L 7	8.00 ± 0.01	247 ± 44	3 ± 2	375,458 ± 6176	nd	nd	8 ± 0.0	74 ± 13	1716 ± 164	28 ± 10	1 ± 0.0	128 ± 9	2 ± 0.0
L 8	7.18 ± 0.04	278 ± 29	10 ± 3	323,865 ± 7008	nd	7 ± 1	10 ± 0.0	347 ± 28	13,112 ± 5037	52 ± 18	4 ± 2	136 ± 12	5 ± 1
L 14	7.88 ± 0.18	298 ± 52	8 ± 2	384,928 ± 17,198	nd	7 ± 2	7 ± 0.0	128 ± 54	4263 ± 855	61 ± 14	nd	118 ± 38	6 ± 1
G 3	6.48 ± 0.21	47 ± 6	65 ± 34	1076 ± 452	4 ± 1	9 ± 3	14 ± 4	7289 ± 2170	5090 ± 1884	186 ± 13	32 ± 23	12 ± 7	41 ± 8
G 4	6.68 ± 0.15	35 ± 9	38 ± 4	640 ± 157	nd	2 ± 0.0	9 ± 2	5277 ± 719	1688 ± 491	223 ± 49	2 ± 0.0	5 ± 2	44±6
G 5	6.64 ± 0.08	206 ± 24	257 ± 89	1558 ± 55	nd	6 ± 1	16 ± 2	7620 ± 1225	3555 ± 554	199 ± 30	6 ± 0.0	10 ± 1	36 ± 5
B 13	7.74 ± 0.04	206 ± 38	47 ± 13	34,996 ± 7749	22 ± 1	33 ± 5	57 ± 4	46,306 ± 372	26,663 ± 2538	767 ± 55	33 ± 8	32 ± 7	58 ± 3
B 16	7.82 ± 0.03	352 ± 41	19 ± 2	7881 ± 414	14 ± 2	23 ± 8	8±3	15,687 ± 2573	19,252 ± 3465	228 ± 31	95 ± 37	7 ± 0.0	22 ± 4
B 18	7.35 ± 0.15	87 ± 19	171 ± 48	3387 ± 924	2 ± 0.0	9 ± 8	13±4	24,667 ± 1895	6920 ± 471	480 ± 124	6 ± 3	15 ± 9	36 ± 9
Q 10	6.16 ± 0.06	39 ± 9	28 ± 8	183 ± 19	nd	1 ± 0.0	1 ± 0.0	244 ± 93	316 ± 199	6±1	3 ± 2	2 ± 0.0	3 ± 1

Numbers of localities follow Table 1. S—serpentinite, L—limestone, G—granite, B—basalt, Q—quartzite. Nd—not detected.

The lowest concentrations of all studied elements were found in quartzite (Table 4). Calcium was a dominant element in limestone (up to 38.5%). Magnesium had its highest concentrations in serpentinite (up to 20%). Iron (Fe) was the third most common element in the examined rocks, which was most abundant in serpentinite and basalt (up to 4.6%).

Two elements (Ba and Cu) did not show a statistically significant affinity to other metals in the rock types. A strong correlation was found between Co, Fe, Mg and Ni, which were found together in serpentinites and between Fe and Mn, which were common in serpentinite and basalt. Ca and Sr concentrations were correlated and found together in limestone. Conductivity was correlated with pH, showing their highest values in limestone and serpentinite and associated with the concentration of the most abundant elements—Ca and Mg—in these rocks (Table 5).

Table 5. Pearson correlation coefficients between elemental concentrations in rocks, pH and conductivity of water extracts.

Edaphic Feature	Conductivity	Ba	Ca	Co	Cr	Cu	Fe	Mg	Mn	Ni	Sr	Zn
pH	**0.67**	−0.41	0.13	**0.57**	0.38	0.12	**0.53**	**0.58**	0.45	**0.52**	0.10	−0.11
Conductivity		−0.31	0.24	0.38	0.30	0.08	0.16	0.35	0.16	0.44	0.25	−0.23
Ba			−0.21	−0.34	−0.24	0.09	−0.12	−0.33	−0.03	−0.33	−0.13	0.37
Ca				−0.39	−0.26	−0.10	−0.46	−0.34	−0.48	−0.35	**0.91**	**−0.55**
Co					0.40	−0.02	**0.83**	**0.93**	**0.74**	**0.85**	−0.40	0.18
Cr						0.48	0.22	0.39	0.19	**0.51**	−0.27	−0.03
Cu							0.18	−0.11	0.24	−0.13	−0.06	0.42
Fe								**0.81**	**0.90**	**0.58**	−0.43	0.45
Mg									**0.66**	**0.87**	−0.36	0.04
Mn										0.47	−0.43	**0.60**
Ni											−0.36	−0.05
Sr												−0.42

Values in bold are significant at $p < 0.05$.

PCA (Figure 4), which ordinated sampling sites on the basis of the chemical contents of rocks, explained 94.7% of total variance and separated the different rock types. Localities with limestones represent a separate group with high Ca and Sr. Sites with granites group with quartzite and one locality with 'basalt.' Habitats with serpentinized rocks are subdivided into groups with apoharzburgite serpentinites (1, 11, 12, 15) and apoharzburgite antigorite-talc-carbonate slates (6, 9, 17). The rocks regarded as 'basalts' are chemically not uniform and appeared to be close to granites or serpentinized rocks in some locations.

3.3. Secondary Metabolites and Chemistry of Rocks

Canonical correspondence analysis (CCA) on relationships between metabolites and the chemical composition of rock types showed different results for crustose and foliose-fruticose species (Figure 5). In both CCAs, Ca, Sr, Ba and Zn in the substrate have high loading on the first axis (57.5% of variance for foliose-fruticose and 48.9% for crustose) and the second axis (37.4% and 27.4%, for foliose-fruticose and crustose, respectively) reflects other metals (Co, Cr, Cu, Fe, Mg, Mn, Ni) as well as pH and conductivity, however, the ordination of metabolites is different for crustose and foliose-fruticose species.

In the CCA, dealing with foliose-fruticose lichens, species with norstictic, stictic acids and zeorin were the most abundant on granites and were positively correlated with Ba and Zn content in the substrate and negatively correlated with pH. Lichens containing gyrophoric, lecanoric and psoromic acids and melanins had an affinity to basalt and their cover correlated with the content of Fe, Mg and Mn in the substrate and did not depend on pH. Species with usnic acid are intermediate between these two groups and equally common on granite and basalt. Foliose and fruticose lichens with anthraquinones, atranorin, fumarprotocetraric acid or lacking secondary metabolites were distributed equally on limestone and serpentinite in habitats with high Ca, pH and conductivity (Figure 5a).

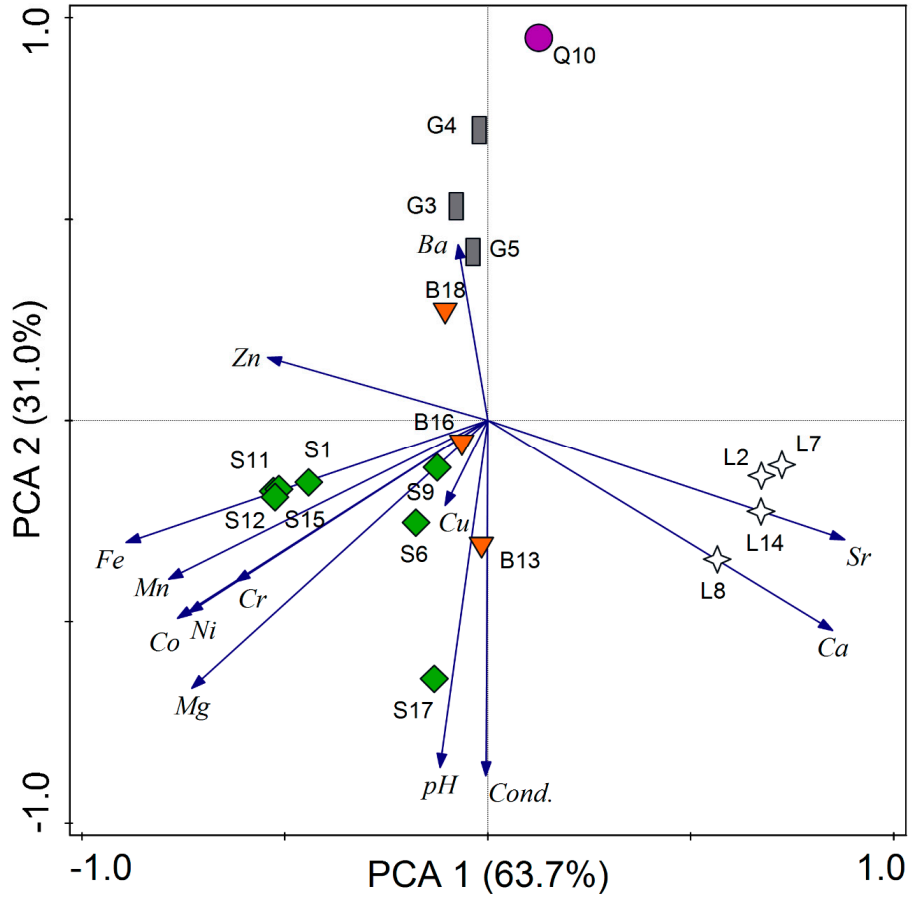
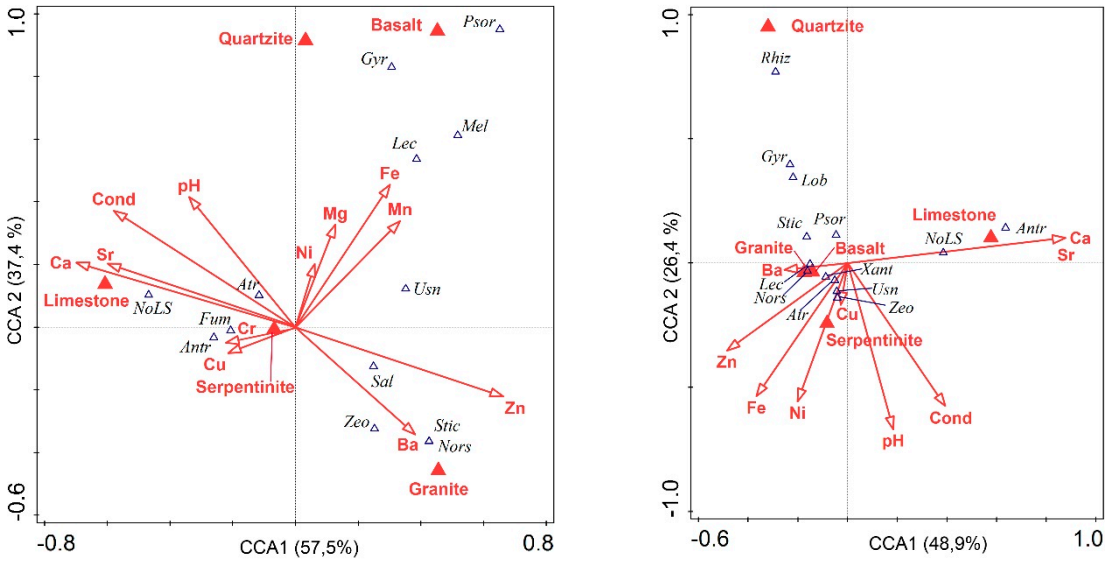


Figure 4. Ordination of the rock types from the different sampling localities (n = 18) on the basis of pH, conductivity and metal content.



(**a**) (**b**)

Figure 5. Canonical correspondence analysis (CCA) ordination plot showing the cover of lichens containing different secondary metabolites in relation to rock types and their chemical properties. (**a**) Foliose and fruticose species, (**b**) Crustose species. Abbreviations of names of the secondary metabolites include the first three or four letters. NoLS—no lichen substances.

In crustose lichens, like in the group of foliose and fruticose lichens, the species with anthraquinones or lacking secondary metabolites had the highest cover on limestone with high Ca. Atranorin, usnic acid, xanthones and zeorin were associated mostly with serpentinites with a relatively high content of metals and pH. Similar habitats were selected by species with lecanoric and norstictic acids, but they were additionally found on basalt and granite with high Ba in the substrate. Lichens containing gyrophoric, lobaric and rhizocarpic acids were the most abundant on quartzite—a metal-poor substrate. Species with psoromic and stictic acids were common on quartzite but also found on granites and basalt where, like in the case of lecanoric and norstictic acids, their cover correlated with Ba in the substrate (Figure 5b).

3.4. Secondary Metabolites and Environmental Parameters

In the CCAs dealing with relationships between secondary metabolites and environmental parameters (Figure 6), the highest loading on CCA1 represent geographical position and elevation of the locality (57.5% and 48.0% of variance for foliose-fruticose and crustose species, respectively), while temperature and precipitation (for crustose species) have the highest loading on the CCA2.

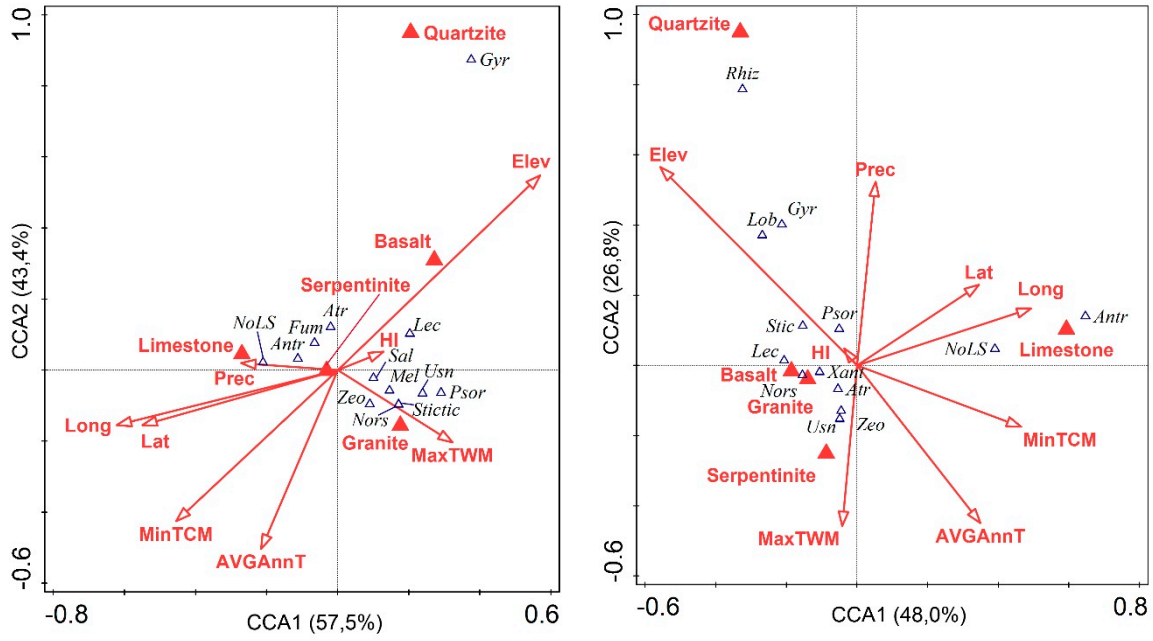


Figure 6. CCA ordination plot showing the cover of lichens containing different secondary metabolites in relation to climatic factors. (**a**)—Foliose and fruticose species, (**b**)—Crustose species. Abbreviations of names of the secondary metabolites include the first three or four letters. AVGAnnT—average annual temperature, Elev—elevation, Lat—latitude, Long—longitude, HI—heat index, MaxTWM—maximum temperature of the warmest month (July), MinTCM—minimum temperature of the coldest month (January), Prec—annual precipitation.

In the group of foliose-fruticose species (Figure 6a), lichen acids were separated into two groups depending on the variation of the maximum temperature of the warmest month. Melanins, psoromic, norstictic, salazinic, stictic, usnic acids and zeorin were found in species in summer-dry conditions while lichens with anthraquinones, atranorin, fumarprotocetraric acid or without lichen metabolites were usually more abundant in localities with higher precipitation and cooler summer. Gyrophoric acid was the only substance that was found primarily in high elevations on quartzite and to a lesser extent on basalt.

Lichen acids in crustose lichens (Figure 6b) were subdivided into three groups depending on the climatic parameters of the habitat. Gyrophoric as well as rhizocarpic and lobaric acids and to some extent psoromic and stictic acids, had an affinity to high elevations and quartzites. Species with anthraquinones or without lichen metabolites were usually more abundant in the northernmost localities. Crustose lichens containing other metabolites show an affinity to rock type rather than the climatic conditions.

Interactive forward-selection analysis and the Monte-Carlo permutation test show that a type of rock substrate (10%), latitude (7%), elevation (4.6%), average annual temperature (2.5%), precipitation (1.2%) and Cr in the substrate (4.3%) have the highest explanatory effect on the chemistry of foliose and fruticose species. There is no statistical effect for Ba and Fe in the substrate as well as for pH and the heat index.

In the group of crustose species, the highest explanatory effects include Sr in the substrate (9.2%), type of a rock substrate (8%), Co and Ni (3 and 1.5%, respectively) and elevation (2.8%). There is no statistical effect for angle (aspect) and azimuth or Cu in the substrate.

4. Discussion

4.1. Secondary Metabolite Diversity on Different Rocks

The dependence of lichen secondary chemistry on rock types is evident when comparing the percentage of species containing different metabolites and inhabiting six rock types. Species growing on felsic, intermediate and mafic rocks (quartzites, granites and basalts) have a more diverse chemistry compared to species on ultramafic and carbonate substrates. A higher percentage of species on acidic and basic rocks contains secondary metabolites, a pattern that holds true for both life forms of lichens.

Crustose species, however, have the most intimate contact with their substrate, resulting in high levels of elemental accumulation [62]. Metal accumulation from the substrate in foliose and fruticose lichens is much lower and the metals are mostly restricted to the lowermost portions of thalli [63–65]. Taking into account these differences, we analyzed lichens of crustose and foliose-fruticose life forms separately.

Crustose lichens represented a statistically significant dependence of secondary chemistry on type of rock, while in foliose-fruticose taxa, these differences were seen only for species growing on chemically most distinct substrates—quartzites and limestones. This is evident not only in terms of the percentage of species containing secondary metabolites but also in the average number of secondary metabolites contained by a single species growing on different rock types, which increases from limestones to quartzites.

Three secondary metabolites, stictic and gyrophoric acids, as well as parietin, show a statistically significant change in their percentage in crustose lichens on different rock types. The species containing stictic and gyrophoric acids are more abundant on acidic substrates, while lichens with parietin are more common on limestone. These three metabolites may, hence, affect interactions of lichens with their substrates, while others may have different functions in lichen thalli.

4.2. Chemical Properties of Rock Substrates

The chemical properties of the different rocks are variable; however, the pH of rocks examined does not contribute to toxicity of lichen acids to lichens themselves [31,43]. Species inhabiting quartzite thrive in slightly acidic conditions with pH in the range of 6.16–6.68, which, however, is much higher than the pH of pine bark in the region, reaching a value as low as 3.85. In slightly acidic conditions, metals such as Cu, Fe, Mn and Zn are more bioavailable [66]; however, quartzite contained these elements in only trace quantities. Ca, Mg and P have limited bioavailability under these conditions. As a result, lichens on quartzite survive in a nutrient-deficient environment. Granites with higher pH do not restrict the availability of phosphorus and contain more Ca, Fe, Mn and Zn. Basalts, serpentinites and limestones have neutral to slightly alkaline conditions on their surface. Neutral pH is optimal for

the availability of most nutrients except Mn but when the pH reaches 8, Fe, Cu and B become weakly available. Serpentinites and limestones are similar in pH and conductivity, resulting mostly from their high Ca and Mg in water extracts but have dramatically different content of other metals. Nickel and Cr have their highest concentrations in serpentinites, which are comparable to those found in serpentine soils of the region [67]. Lichens on serpentinites and limestones live in relatively concentrated ionic solutions dominated by Mg and Ca and no/minimal bioavailability of other nutrients.

4.3. Secondary Metabolites and Chemistry of Rocks

In crustose lichens, a group of species containing two major metabolites, gyrophoric and stictic acid and three less common metabolites, lobaric, psoromic and rhizocarpic acids, shows an affinity to substrates with low pH and a low content of metals. In the group of foliose and fruticose lichens three of these metabolites are common in species growing on substrates with high content of metals. Lichen substances, which have high affinity to metals, may act in two ways: they promote intracellular uptake of metals, thereby shaping the affinity of species to the substrates [68] or inactivate excess of metals in apoplast of thalli [69]. We assume that lichen substances in crustose lichens dominated in metal-poor conditions on quartzite (gyrophoric, stictic as well as lobaric, psoromic and rhizocarpic acids) may promote metal supply to lichens. Gyrophoric and psoromic acids are common in foliose species in metal-rich environments but they are contained in *Rhizoplaca*, *Lasallia* and *Umbilicaria*—the genera of umbilicate life forms—which make contact with the substrate only by a central umbilicus and may rely upon elements only from solutions. An ability to form complexes with Cu is shown for psoromic acid in lichens on cupriferous substrata [41]; however, gyrophoric acid does not form complexes with U, Cu or Fe in *Trapelia involuta* (Taylor) Hertel [69].

Anthraquinones in all life forms, as well as fumarprotocetraric acid in fruticose species, are common substances in lichens on limestones and to some extent on serpentinites but minimal or lacking in rocks with high Mg, Fe and Ni. Like atranorin in *Cladonia* species [68], these substances may promote absorption of nutrients in alkalic conditions, when they are poorly available to lichens. Lichens lacking secondary metabolites are similarly abundant on rocks with high pH and a low availability of elements other than Ca and Mg. These are primarily gelatinous species of Collemataceae and Lichinaceae, which can uptake nutrients using polysaccharides or organic acids other than lichen secondary metabolites; however, this supposition needs to be experimentally tested.

Lichens with other secondary metabolites are the most abundant on rocks such as granite, basalt and serpentinite with higher concentrations of metals in the substrate. These are lecanoric, norstictic, usnic acids and zeorin. Their effect on shaping the lichen communities or in accumulation of metals is not evident. Thus, despite a high affinity to Fe shown in laboratory experiments [39] and to Cu in lichens on cupriferous substrata [40], species with norstictic acid do not show a higher accumulation rate compared to species containing no lichen acids [32]. Obligate iron-accumulating *Acarospora* species in the *Acarospora smaragdula*-group do not contain norstictic acid [70]. However, these metabolites may have a protective role against an excess of metals in the substrate.

4.4. Secondary Metabolites and Climatic Factors

This study shows that the abundance of species containing different lichen metabolites is affected by climate. Species with gyrophoric and lecanoric acids in all life forms, as well as lobaric, rhizocarpic, stictic and psoromic acids in crustose species are the most common in the highest elevations but this dependence is also influenced by substrate; they are more abundant on quartzite than on basalt at the same elevations. Species with anthraquinones or without lichen metabolites are usually more abundant in the northernmost but not elevated, localities with higher precipitation.

In the group of foliose-fruticose lichens, species containing the rest of the main metabolites, namely norstictic, usnic acids, melanins and zeorin, have an affinity to habitats with elevated summer temperatures. This is concordant with the finding that warming increases concentrations of usnic acid in thalli of *Cladonia* species [71] and that norstictic acid-containing *Cetraria steppae* (Savicz) Kärnefelt is

restricted to warm temperate regions compared to species lacking this metabolite [72]. In the group of crustose lichens, species with the same metabolites group in accordance with their affinity to rock types and climatic parameters, however, their distribution is not influenced by these parameters compared to that of foliose species.

Our results reflect a range of possible relationships among lichen acids, rocks and climatic parameters. Some lichen metabolites may act as accumulation and retention agents for metals, active under a range of pH. Apart from the protective role of lichen metabolites in the excess of light they may also be advantageous in adverse climatic conditions such as high summer temperatures along with prolonged drought which, in this continental region, are more important for lichens than low winter temperatures which affect terrestrial lichen distribution in Western Europe [73]. These findings suggest that the same metabolite may affect both accumulation of metals and protection against unfavorable conditions.

Author Contributions: Conceptualization, A.P.; methodology, A.P., S.E.F.-L., N.R.; software, A.P., S.E.F.-L.; investigation, A.P., A.T., M.M., E.K.; writing—original draft preparation, A.P.; writing—review and editing, N.R., C.B.; funding acquisition, A.P., A.T.

Funding: This research was funded by the Russian Foundation for Basic Research (grant 18-04-00414).

Acknowledgments: AT is grateful for Ministry of Science and Higher Education of the Russian Federation (agreement no. 02.A03.21.0006) for financial support. We would like to express our gratitude to anonymous reviewers whose valueable comments and meticulous reading allowed us to make substantial improvements of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Huneck, S.; Yoshimura, I. *Identification of Lichen Substances*; Springer: Berlin/Heidelberg, Germany, 1996; pp. 1–493.
2. Elix, J.A. *A Catalogue of Standardized Chromatographic Data and Biosynthetic Relationships for Lichen Substances*, 3rd ed.; Published by the author: Canberra, Australia, 2014; pp. 1–323.
3. Rundel, P.W. The ecological role of secondary lichen substances. *Biochem. Syst. Ecol.* **1978**, *6*, 157–170. [CrossRef]
4. Favero-Longo, S.E.; Piervittori, R. Lichen-plant interactions. *J. Plant. Interact.* **2010**, *5*, 163–177. [CrossRef]
5. Lawrey, J.D. Lichen secondary compounds: Evidence for a correspondence between antiherbivore and antimicrobial function. *Bryologist* **1989**, *92*, 326–328. [CrossRef]
6. Gauslaa, Y. Lichen palatability depends on investments in herbivore defence. *Oecologia* **2005**, *143*, 94–105. [CrossRef] [PubMed]
7. Lawrey, J.D. Biological role of lichen substances. *Bryologist* **1986**, *89*, 111–122. [CrossRef]
8. Vainshtein, E.A. The role of lichen substances in symbiosis. *Bot. Zhurn.* **1991**, *76*, 793–800.
9. Solhaug, K.A.; Gauslaa, Y. Parietin, a photoprotective secondary product of the lichen *Xanthoria parietina*. *Oecologia* **1996**, *108*, 412–418. [CrossRef]
10. Gauslaa, Y.; Solhaug, K.A. Fungal melanins as a sun screen for symbiotic green algae in the lichen *Lobaria pulmonaria*. *Oecologia* **2001**, *126*, 462–471. [CrossRef]
11. Prokopiev, I.A.; Poryadina, L.N.; Konoreva, L.A.; Chesnokov, S.V.; Shavarda, A.L. Variation in the composition of secondary metabolites in *Flavocetraria* lichens from Western Siberia. *Russ. J. Ecol.* **2018**, *49*, 401–405. [CrossRef]
12. Chowdhury, D.P.; Solhaug, K.A.; Gauslaa, Y. Ultraviolet radiation reduces lichen growth rates. *Symbiosis* **2017**, *73*, 27–34. [CrossRef]
13. MacGillivray, T.; Helleur, R. Analysis of lichens under environmental stress using TMAH thermochemolysis-gas chromatography. *J. Anal. Appl. Pyrolysis* **2001**, *58–59*, 465–480. [CrossRef]
14. Øvstedal, D.O.; Lewis Smith, R.I. *Lichens of Antarctica and South. Georgia: A Guide to Their Identification and Ecology*; Cambridge University Press: Cambridge, UK, 2001; pp. 1–411.

15. Luo, H.; Yamamoto, Y.; Kim, J.A.; Jung, J.S.; Koh, Y.J.; Hur, J.-S. Lecanoric acid, a secondary lichen substance with antioxidant properties from *Umbilicaria antarctica* in maritime Antarctica (King George Island). *Polar Biol.* **2009**, *32*, 1033–1040. [CrossRef]
16. Larsson, P.; Večeřová, K.; Cempírková, H.; Solhaug, K.A.; Gauslaa, Y. Does UV-B influence biomass growth in lichens deficient in sun-screening pigments? *Environ. Exp. Bot.* **2009**, *67*, 215–221. [CrossRef]
17. Bjerke, J.W.; Joly, D.; Nilsen, L.; Brossard, T. Spatial trends in usnic acid concentrations of the lichen *Flavocetraria nivalis* along local climatic gradients in the Arctic (Kongsfjorden, Svalbard). *Polar Biol.* **2004**, *27*, 409–417. [CrossRef]
18. Sheard, J.W. The comparative ecology and distribution and within-species variation of the lichenized Ascomycetes *Ramalina cuspidata* and *R. siliquosa* in the British Isles. *Can. J. Bot.* **1978**, *56*, 939–952. [CrossRef]
19. LaGreca, S. A Phylogenetic evaluation of the *Ramalina americana* chemotype complex (Lichenized Ascomycota, Ramalinaceae) based on rDNA ITS sequence data. *Bryologist* **2006**, *102*, 602–618. [CrossRef]
20. Culberson, C.F.; Culberson, W.L.; Arwood, D.A. Physiography and fumarprotocetraric acid production in the *Cladonia chlorophaea* group in North Carolina. *Bryologist* **1977**, *80*, 71–75. [CrossRef]
21. Renner, B. The presence or absence of secondary metabolites in cephalodia and their possible implications. *J. Hattori Bot. Lab.* **1982**, *52*, 367–377.
22. Renner, B.; Galloway, D.J. Phycosymbiodemes in *Pseudocyphellaria* in New Zealand. *Mycotaxon* **1982**, *16*, 197–231.
23. Boyle, A.P.; McCarthy, P.M.; Stewart, D. Geochemical control of saxicolous lichen communities on the creggaun gabbro, Letterfrack, Co. Galway, Western Ireland. *Lichenologist* **1987**, *19*, 307–317. [CrossRef]
24. Brodo, I.M. Substrate ecology. In *The Lichens*; Ahmadjian, V., Hale, M.E., Eds.; Academic Press: New York, NY, USA; London, UK, 1973; pp. 401–441.
25. Favero-Longo, S.E.; Isocrono, D.; Piervittori, R. Lichens and ultramafic rocks: A review. *Lichenologist* **2004**, *36*, 391–404. [CrossRef]
26. Favero-Longo, S.E.; Isocrono, D.; Piervittori, R. Measuring the biodiversity of saxicolous lichens above timberline with reference to environmental factors: The case of a Natura 2000 site of western Alps. *Phytocoenologia* **2009**, *39*, 51–78. [CrossRef]
27. Favero-Longo, S.E.; Matteucci, E.; Morando, M.; Rolfo, F.; Harris, T.B.; Piervittori, R. Metals and secondary metabolites in saxicolous lichen communities on ultramafic and non-ultramafic rocks of the Western Italian Alps. *Aust. J. Bot.* **2015**, *63*, 276–291. [CrossRef]
28. Purvis, O.W.; Halls, C. A review of lichens in metal-enriched environments. *Lichenologist* **1996**, *28*, 571–601. [CrossRef]
29. Rajakaruna, N.; Knudsen, K.; Fryday, A.M.; O'Dell, R.E.; Pope, N.; Olday, F.C.; Woolhouse, S. Investigation of the importance of rock chemistry for saxicolous lichen communities of the New Idria serpentinite mass, San Benito County, California, USA. *Lichenologist* **2012**, *44*, 695–714. [CrossRef]
30. Wirth, V. Die Silikatflechten-Gemeinschaften im ausseralpinen Zentraleuropa. *Dissert. Bot.* **1972**, *17*, 1–306.
31. Hauck, M.; Jürgens, S.R. Usnic acid controls the acidity tolerance of lichens. *Environ. Pollut.* **2008**, *156*, 115–122. [CrossRef] [PubMed]
32. Paukov, A.G.; Teptina, A.Y.; Pushkarev, E.V. Heavy metal uptake by chemically distinct lichens from *Aspicilia* spp. growing on ultramafic rocks. *Aust. J. Bot.* **2015**, *63*, 111–118. [CrossRef]
33. Ascaso, C.; Galvan, J.; Rodriguezpascual, C. The weathering of calcareous rocks by lichens. *Pedobiologia* **1982**, *24*, 219–229.
34. Jones, D.; Wilson, M.J.; Tait, J.M. Weathering of a basalt by *Pertusaria corallina*. *Lichenologist* **1980**, *12*, 277–290. [CrossRef]
35. Wilson, M.J.; Jones, D.; McHardy, W.J. The weathering of serpentinite by *Lecanora atra*. *Lichenologist* **1981**, *13*, 167–176. [CrossRef]
36. Bačkor, M.; Fahselt, D. Using EDX-microanalysis and X-ray mapping to demonstrate metal uptake by lichens. *Biologia (Bratislava)* **2004**, *59*, 39–45.
37. Favero-Longo, S.E. Lichens on metal-rich substrates. In *Plant Ecology and Evolution in Harsh Environments*; Rajakaruna, N., Boyd, R.S., Harris, T.B., Eds.; Nova Science Publishers: New York, NY, USA, 2014; pp. 53–76.
38. Fortuna, L.; Baracchini, E.; Adami, G.; Tretiach, M. Melanization affects the content of selected elements in parmelioid lichens. *J. Chem. Ecol.* **2017**, *43*, 1086–1096. [CrossRef] [PubMed]

39. Hauck, M.; Huneck, S.; Elix, J.A.; Paul, A. Does secondary chemistry enable lichens to grow on iron-rich substrates? *Flora Morphol. Distrib. Funct. Ecol. Plants* **2007**, *202*, 471–478. [CrossRef]
40. Purvis, O.W.; Elix, J.A.; Broomhead, J.A.; Jones, G.C. The occurrence of copper norstictic acid in lichens from cupriferous substrata. *Lichenologist* **1987**, *19*, 193–203. [CrossRef]
41. Purvis, O.W.; Elix, J.A.; Gaul, K.L. The occurrence of copper-psoromic acid in lichens from cupiferous substrata. *Lichenologist* **1990**, *22*, 345–354. [CrossRef]
42. Hauck, M.; Willenbruch, K.; Leuschner, C. Lichen substances prevent lichens from nutrient deficiency. *J. Chem. Ecol.* **2009**, *35*, 71–73. [CrossRef]
43. Hauck, M.; Jürgens, S.R.; Leuschner, C. Norstictic acid: Correlations between its physico-chemical characteristics and ecological preferences of lichens producing this depsidone. *Environ. Exp. Bot.* **2010**, *68*, 309–313. [CrossRef]
44. Nakajima, H.; Fujimoto, N.; Yamamoto, Y.; Amemiya, T.; Itoh, K. Response of secondary metabolites to Cu in the Cu-hyperaccumulator lichen *Stereocaulon japonicum*. *Environ. Sci. Pollut. Res.* **2019**, *26*, 905–912. [CrossRef]
45. Favero-Longo, S.E.; Matteucci, E.; Giordani, P.; Paukov, A.G.; Rajakaruna, N. Diversity and functional traits of lichens in ultramafic areas: A literature-based worldwide analysis integrated by field data at the regional scale. *Ecol. Res.* **2018**, *33*, 593–608. [CrossRef]
46. Gauslaa, Y.; Yemets, O.A.; Asplund, J.; Solhaug, K.A. Carbon based secondary compounds do not provide protection against heavy metal road pollutants in epiphytic macrolichens. *Sci. Total Environ.* **2016**, *541*, 795–801. [CrossRef] [PubMed]
47. Bačkorová, M.; Biľová, I.; Kimáková, T.; Bačkor, M. The presence of usnic acid does not protect the lichen *Cladina arbuscula* subsp. *mitis* against the long-term copper excess. *S. Afr. J. Bot.* **2015**, *100*, 94–100. [CrossRef]
48. Ogureeva, G.N.; Leonova, N.B.; Buldakova, E.V.; Kadetov, N.G.; Arkhipova, M.V.; Miklyaeva, I.M.; Bocharnikov, M.V.; Dudov, S.V.; Ignatova, E.A.; Ignatov, M.S.; et al. *The Biomes of Russia—Map*; WWF-Russia Conservation Strategy, Faculty of Geography Lomonosov Moscow State University: Moscow, Russia, 2018.
49. Pennino, M.G.; Paradinas, I.; Illian, J.B.; Muñoz, F.; Bellido, J.M.; López-Quílez, A.; Conesa, D. Accounting for preferential sampling in species distribution models. *Ecol. Evol.* **2019**, *9*, 653–663. [CrossRef] [PubMed]
50. Paukov, A.; Teptina, A. New records of lichens from Middle Urals, Russia. *Folia Cryptog. Estonica* **2012**, *49*, 39–43.
51. Paukov, A.G.; Gagarina, L.V.; Frolov, I.V. New and interesting lichen records from the Ural Mountains, Russia. *Folia Cryptog. Estonica* **2017**, *54*, 25–30. [CrossRef]
52. Urbanavichene, I.; Urbanavichus, G.; Mežaka, A.; Palice, Z. New records of lichens and lichenicolous fungi from the Southern Ural Mountains, Russia. II. *Folia Cryptog. Estonica* **2013**, *50*, 73–80. [CrossRef]
53. Nordin, A.; Moberg, R.; Tønsberg, T.; Vitikainen, O.; Dalsätt, Å.; Myrdal, M.; Snitting, D.; Ekman, S. Santesson's Checklist of Fennoscandian Lichen-forming and Lichenicolous Fungi. Ver. 29 April 2011. Available online: http://130.238.83.220/santesson/home.php (accessed on 30 March 2019).
54. Wijayawardene, N.N.; Hyde, K.D.; Lumbsch, H.T.; Liu, J.K.; Maharachchikumbura, S.S.N.; Ekanayaka, A.H.; Tian, Q.; Phookamsak, R. Outline of Ascomycota: 2017. *Fungal Divers.* **2018**. [CrossRef]
55. Parker, K.C. Environmental relationships and vegetation associates of columnar cacti in the northern Sonoran Desert. *Vegetatio* **1988**, *78*, 125–140. [CrossRef]
56. Zelený, D.; Chytrý, M. Environmental control of the vegetation pattern in deep river valleys of the Bohemian Massif. *Preslia* **2007**, *79*, 205–222.
57. Diva-Gis. Available online: www.diva-gis.org (accessed on 7 January 2019).
58. Culberson, C.F.; Kristinsson, H.D. A standardized method for the identification of lichen products. *J. Chromatogr.* **1970**, *46*, 85–93. [CrossRef]
59. Orange, A.; James, P.W.; White, F.J. *Microchemical Methods for the Identification of Lichens*; British Lichen Society: London, UK, 2001; pp. 1–101.
60. Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological statistics software package for education and data analysis. *Palaeont. Electr.* **2001**, *4*, 1–9.
61. Šmilauer, P.; Lepš, J. *Multivariate Analysis of Ecological Data Using CANOCO 5*; Cambridge University Press: Padstow, UK, 2002; pp. 1–527.
62. Purvis, O.W. Adaptation and interaction of saxicolous crustose lichens with metals. *Bot. Stud.* **2014**, *55*, 1–14. [CrossRef] [PubMed]

63. Goyal, R.; Seaward, M.R.D. Metal uptake in terricolous lichens. I. Metal localization within the thallus. *New Phytol.* **1981**, *89*, 631–645. [CrossRef]
64. Goyal, R.; Seaward, M.R.D. Metal uptake in terricolous lichens. III. Translocation in the thallus of *Peltigera canina*. *New Phytol.* **1982**, *90*, 85–98. [CrossRef]
65. Osyczka, P.; Rola, K.; Jankowska, K. Vertical concentration gradients of heavy metals in *Cladonia* lichens across different parts of thalli. *Ecol. Indic.* **2016**, *61*, 766–776. [CrossRef]
66. Lucas, R.E.; Davis, J.F. Relationships between pH values of organic soils and availabilities of 12 plant nutrients. *Soil Sci.* **1961**, *92*, 177–182. [CrossRef]
67. Teptina, A.; Paukov, A.; Rajakaruna, N. Ultramafic vegetation and soils in the circumboreal region of the Northern Hemisphere. *Ecol. Res.* **2018**, *33*, 609–628. [CrossRef]
68. Paul, A.; Hauck, M.; Leuschner, C. Iron and phosphate uptake explains the calcifuge-calcicole behavior of the terricolous lichens *Cladonia furcata* subsp. *furcata* and *C. rangiformis*. *Plant Soil* **2009**, *319*, 49–56. [CrossRef]
69. Kasama, T.; Murakami, T.; Ohnuki, T. Accumulation mechanisms of uranium, copper and iron by lichen Trapelia involuta. In *Biomineralization (BIOM2001): Formation, Diversity, Evolution and Application, Proceedings of the 8th International Symposium on Biomineralization (September 25–28, 2001, Niigata, Japan), 2003*; Kobayashi, I., Ozawa, H., Eds.; Tokai University Press: Kanagawa, Japan, 2003; pp. 298–301.
70. Wedin, M.; Westberg, M.; Crewe, A.T.; Tehler, A. Species delimitation and evolution of metal bioaccumulation in the lichenized *Acarospora smaragdula* (Ascomycota, Fungi) complex. *Cladistics* **2009**, *25*, 161–172. [CrossRef]
71. Asplund, J.; Siegenthaler, A.; Gauslaa, Y. Simulated global warming increases usnic acid but reduces perlatolic acid in the mat-forming terricolous lichen *Cladonia stellaris*. *Lichenologist* **2017**, *49*, 269–274. [CrossRef]
72. Lutsak, T.; Fernández-Mendoza, F.; Nadyeina, O.; Şenkardeşler, A.; Printzen, C. Testing the correlation between norstictic acid content and species evolution in the *Cetraria aculeata* group in Europe. *Lichenologist* **2017**, *49*, 39–56. [CrossRef]
73. Košuthová, A.; Svitková, I.; Pišút, I.; Senko, D.; Valachovič, M.; Zaniewski, P.T.; Hájek, M. Climatic gradients within temperate Europe and small-scale species composition of lichen-rich dry acidophilous Scots pine forests. *Fungal Ecol.* **2015**, *14*, 8–23. [CrossRef]