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Heavy metal uptake by chemically distinct lichens from *Aspicilia* spp. growing on ultramafic rocks

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Abstract. Accumulation of metals in four crustose lichens with different secondary chemistry growing on serpentinite was studied. *Aspicilia cinerea* and *A. blastidiata* contain depsidone norstictic acid, *A. goettweigensis* contains stictic acid, and *A. contorta* ssp. *hoffmanniana* contains aliphatic compound aspicilin. The highest concentrations in lichens compared with serpentinite were found for calcium (Ca; average 11 times, maximum 20 times). Strontium (Sr), Copper (Cu), sodium (Na), zinc (Zn) and chromium (Cr) were 2.8–9 times greater in lichens than in rocks, and other elements such as nickel (Ni), iron (Fe), cobalt (Co), manganese (Mn) and magnesium (Mg) were equal or lower in the thalli than in the substrate. Three species showed little differences in concentrations of the same metals, whereas *Aspicilia blastidiata*, which is obligate to serpentinite, had statistically higher concentrations of most elements. This implies that the difference in secondary chemistry does not strongly influence accumulation rates of metals in selected species on serpentinite but that lichens have both mechanisms of accumulation and avoidance that may be related to ‘lichen acids’.

Additional keywords: accumulation, ecology, Middle Urals, Russia, saxicolous lichens, secondary chemistry, serpentinite.

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Introduction

Lichens on serpentinite have attracted the attention of lichenologists for several decades and have been studied in many locations in Europe and America. One of the features of serpentinite lichen flora is its impoverished nature (Wirth 1972), but many recent studies have characterised it as species-rich (Takala and Seaward 1978; Sirois *et al*. 1988; Sigal 1989; Favero-Longo *et al*. 2004; Harris *et al*. 2007; Paukov 2009; Rajakaruna *et al*. 2011, 2012), primarily because of serpentine harboring many species growing on acid or calcareous rocks (Gilbert and James 1987; Purvis and Halls 1996). In contrast to limestone, another species-rich rock, serpentinites, contain substantial quantities of metals such as Ni, Cr, Cu and Fe (Kruckeberg 1954; Proctor and Woodell 1971) and tolerance to them as well as ultrabasic nature of serpentinite may be another reason of high diversity of lichens on this substrate.

Saxicolous lichens accumulate heavy metals at high concentrations (Brodo 1973; Puckett *et al*. 1973; Nieboer *et al*. 1976; Richardson and Nieboer 1983; Purvis *et al*. 1987; Purvis and Halls 1996). One of the reasons for elevated concentrations of metals is secondary metabolites that help retain metals in thalli (Purvis *et al*. 1987, 1990). Lichen pigments can also bind toxic elements (Purvis *et al*. 2004). Apart from this, ‘lichen acids’ may determine substrate preferences of lichens (Hauck and Jürgens 2008; Hauck *et al*. 2009), and impart them affinities different from those of heavy metals that result in different distribution patterns of species. Thus, three lichen substances, norstictic, physodalic and fumarprotocetraric acids, show the best binding activity with Fe^{3+} and are rare in lichens on Fe-rich substrata. In contrast, lichen substances that do not occur in ferrophytic lichen species are effective in Fe^{3+} adsorption (Hauck *et al*. 2007).

Despite an abundance of data on interaction of secondary lichen metabolites with metals, there were no comparisons of overall spectrum of chemical constituents of saxicolous lichens on chemically different rocks as well as no direct comparison of accumulation activity of species with different lichen metabolites on the same substratum. The aim of the work was to compare metal accumulation in four *Aspicilia s.l.* species with different secondary metabolites, growing on serpentinite in the Middle Urals of Russia.

Materials and methods

The species

The species we selected were widespread in the region, were found in the same lichen groupings, belong to the same genus and contained different secondary metabolites. Crustose lichens are the most preferable because they have close contact with substrate and are found to be more efficient accumulators and/or retainers of heavy metals (Chiarenzelli *et al*. 1997).

Four species were selected for the analysis, including *Aspicilia cinerea* (L.) Körb. and *A. blastidiata*, both containing depsidone norstictic acid (Huneck and Yoshimura 1996), which is an acid with high affinity to Fe^{3+}, *A. goettweigensis* (Zahlbr.) Hue containing depsidone stictic acid, which is a substance with intermediate affinity to Fe^{3+} (Hauck *et al*. 2007) and *A. contorta* subsp. *hoffmanniana* Ekman & Fröberg ex R. Sant. that contains aliphatic compound aspicilin. Three of the selected species are widespread in the region and grow on different rocks, whereas *A. blastidiata* which is found only on serpentinite. This species was initially described by us as a deviant form of *A. cinerea*; however, apart from a blastidiate upper surface, it has shorter conidia and represents a separate species that is described in the present paper. *Aspicilia cinerea s. str.* grows on different rock types from granite to limestone and often dominates in lichen groupings on acid and basic rocks. *Aspicilia goettweigensis* is a species of ultramafic and basic rocks that is widely distributed in the South and Middle Urals on serpentinite, pyroxenite and basalt and is rarely found on sienite. *Aspicilia contorta* subsp. *hoffmanniana* is widespread on basalt, serpentinite and limestone in the region.

Study area

Lichens were collected in Sverdlovsk region (Russia) in three localities of serpentinite outcrops along the riverbanks of Rezh, Pyshma and Sysert rivers close to Rezh town (57°21′N, 61°21′E), Staropyshminsk (56°56′N, 60°54′E) and Dvurechensk (56°35′N, 61°03′E) settlements, respectively (Fig. 1). The main type of rock-forming serpentine in the Rezh area is antigorite that contains chrysotile veinlets. Metaharzburgite serpentine–talc–carbonate rocks prevail in the Staropyshminsk and Dvurechensk areas. Specimens of *A. cinerea* and *A. goettweigensis* were additionally collected on antigorite serpentinite of Egoza mountain near Kyshtym town, Chelyabinsk region (55°45′N, 60°26′E). Little to no weathering was found in all studied serpentinites.

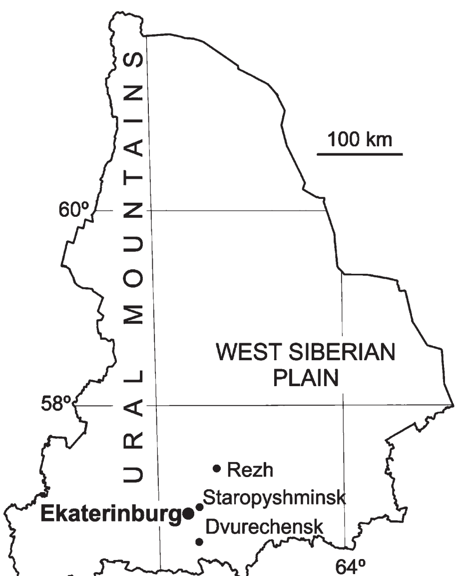

Fig. 1. The map of Sverdlovsk region of Russia, with collection localities.

The territory belongs to the Middle Urals, Eastern-Ural peneplain, southern taiga subzone. The climate of the territory is moderately continental, with a cold winter and a warm summer. Average temperature in January is −16°C, and in July 17°C. The vegetation consists of coniferous forests with pine (*Pinus sylvestris* L.). On the south of Sverdlovsk region and further to the south, the coniferous forests are gradually transitioned into birch (*Betula pendula* Roth) and aspen (*Populus tremula* L.) forests.

The first three locations are part of the Alapaevsk fault ophiolite melange, which is situated in the eastern slope of the Uralian orogen. Mountain Egoza is an ultramafic block of the Kyshtym ophiolite massif in the Main Uralian Fault zone, which divides paleocontinental in the west and paleooceanic in the east sectors of the Uralian folded belt (Puchkov 2013). All these huge tectonic sutures are traced by the chaines of ultramafic massifs of variable size. They mostly consist of Mg-rich harzburgite and dunite in subordinate amounts. The composition of rocks is characterised by high depletion of large ion lithophile elements and incompatible elements and strong enrichment of Mg, Ni and Cr. The degree of serpentinisation varies widely. The most shallow-seated ultramafites are strongly serpentinised up to the formation of pure serpentinites.

Specimen collection and analysis procedure

Specimens of lichens were collected together with rock samples in June and July 2012 and 2013. Thalli were cleaned with soft brush to remove possible dust and carefully detached from substratum with a teflon-coated blade, taking care to avoid the lowermost part of areolae. Detached thalli were dried at 40°C for 24 h and weighed. Approximately 50 mg of each sample were taken for subsequent analysis. After detaching of lichens, rock samples were ground in a porcelain mortar and 200 mg of each sample was taken for the analysis. Twenty specimens of both *A. blastidiata* and *A. contorta* subsp. *hoffmanniana*, 25 specimens of *A. cinerea* and 30 specimens of *A. goettweigensis*, with corresponding quantities of rock samples, were oven-dried at 70°C for 48 h, samples then were weighed, put into fluoroplastic glasses, adding 1 mL of HF, 5 mL of HNO_3, 2 mL of HCl, 2 mL of deionised water, followed by digesting in 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 the volume by deionised water. Concentration of Ca, Co, Cr, Cu, Fe, Mg, Mn, Na, Ni, Sr and Zn in rock and lichen samples were determined by inductively coupled plasma–atomic emission spectroscopy (iCAP 6500 Duo, Thermo Fischer Scientific, Waltham, MA, USA).

Data analysis

Lichens accumulate elements from substrate as well as from atmosphere by wet and dry deposition (Knops *et al*. 1991). To distinguish these sources, the calculation of the enrichment factor (EF) is proposed (Puckett and Finegan 1980). High EF implies an aerial source of elements in thalli, whereas a lower EF is likely to indicate a substrate source. Cu : Zn ratio in Earth crust and in lichens is another value that can be used to determine whether substrate is the source of metals in thalli. This ratio is rather constant (Garty 2001; St Clair *et al*. 2002) and was used to determine the source of accumulated metals.

Determination of lichen substances was conducted using thin-layer chromatography (TLC) in Solutions A and C (Orange *et al*. 2001). Sections of apothecia were cut by hand and studied in water and 10% KOH. Spore and conidia measurements are given as (min.–)M–SE–[M]–M+SE(–max.), rounded to the nearest 0.1 µm, where 'min.' and 'max.' are the extreme values recorded, M is the arithmetic mean and SE the corresponding error of mean.

One-way ANOVA, correlation analysis and non-parametric statistics were conducted in Statistica 8 application (StatSoft Inc., Tulsa, OK, USA).

Results

New species

Aspicilia blastidiata Paukov, A.Nordin & Tibell, *sp. nov.* (MycoBank MB811501) (Fig. 2)

Similar to *Aspicilia cinerea* but with blastidiate upper surface and short conidia 9–10.5 µm.

Type: Russia, Sverdlovsk region, Rezh town, Bystrinskiy, rocks on the right riverbank of Rezh River, alt. 175 m, 57°21′23.7″N, 61°22′24.3″E, serpentinite outcrops, on serpentinite, 1 August 2012, *A. Paukov* AGP20120801-01 (UFU – holotypus; UPS – isotypus).

Thallus light grey, up to 1.5 mm thick, rimose-areolate, without lobes. *Areoles* irregular, up to 1–1.5 mm, with cracked and verruculose-blastidiate surface. Younger parts of thalli thin, cracked or areolate, soon becoming small-squamulose to blastidiate, squamules 0.2–0.5 mm, at times detached and leaving white spots of medulla. Fertile areoles significantly exceed younger thallus in thickness. *Cortex* paraplectenchymatous, ~18–35 µm thick, cells ~6–9 µm in diameter. *Medulla* white. *Hypothallus* indistinct or distinct, grey or blackish, up to 1.5 mm wide, sometimes with whitish and fimbriate margin. *Photobiont*

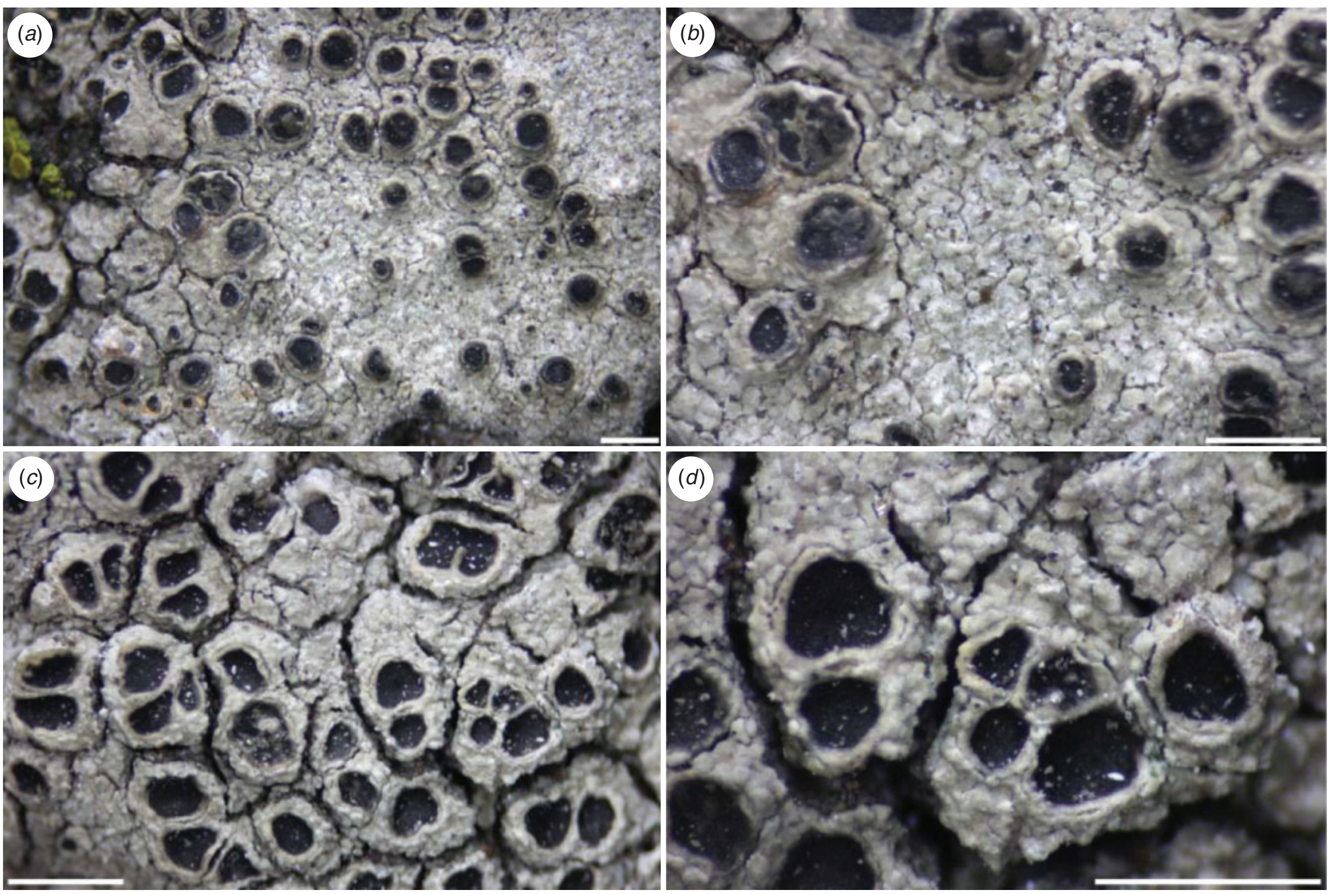

Fig. 2. *Aspicilia blastidiata* (holotype). (*a*, *b*) Younger, small-squamulose part of thallus, with thicker fertile areoles and confluent apothecia. (*c*, *d*) Central areolate part of thallus, with urceolate apothecia. Scale bar = 1 mm.

trebouxoid, cells sphaeric 8–20 µm or elliptic 18–32 × 13–20 µm.

Apothecia urceolate to broadly attached, round to irregular, 0.2–1 mm in diameter, 1–5 per areole, old apothecia often confluent. *Thalline margin* light grey, in urceolate apothecia at times separated from the thallus by a thin crack, in older apothecia partly excluded. *Proper margin* in young apothecia obscured by the thalline margin, later prominent, grey, surrounded by receding thalline margin. *Disc* black to bluish- or brownish-black, smooth or rough, concave to slightly convex, in confluent apothecia with parts of proper margin. *Proper exciple* ~45–50 µm wide in upper part, narrowing below, cells in upper part slightly elongated, ~6 µm long, thick-walled, I−. *Epihymenium* greenish-brown, N+ green. *Hymenium* hyaline, 90–100 µm tall. *Paraphyses* moniliform, with 3–5 ± globose apical cells, 4−5.2 µm wide (in K). *Asci Aspicilia*-type, clavate, 85–92 × 10–26 µm. *Ascospores* hyaline, broadly ellipsoid, (14.5–)17.5–[17.8]–18.1(–21) × (9.2–)10.9–[11.2]–11.4(–14.5) µm ($n = 30$). *Hypothecium* ~55–60 µm thick. *Pycnidia* ~0.26 × 0.21 mm, with pigmented ostiole region. *Conidia* filiform, (7.9–)9.0–[9.7]–10.4(–11.8) × 1 µm ($n = 40$).

Chemistry

Thallus K+ yellow turning red, C−, Pd+ yellow–orange; norstictic acid present in cortex.

Distribution and ecology

Aspicilia blastidiata is known only on serpentinite from eight localities in the Middle and Southern Urals, at altitudes ranging between 175 and 800 m. It grows on sheltered serpentinite rocks, usually under forest canopy. Associated species include *A. cinerea* (L.) Körb., *Candelariella vitellina* (Ehrh.) Müll. Arg., *Physcia caesia* (Hoffm.) Hampe ex Fürnr., *Ph. dubia* (Hoffm.) Lettau, *Scoliciosporum umbrinum* (Ach.) Arnold. *Aspicilia blastidiata* is characterised by thick light grey blastidiate thallus, presence of norstictic acid and short conidia 9–10.5 µm. Study of nrITS sequences revealed a relationship of *Aspicilia blastidiata* with *A. cinerea*, which will be discussed in a special treatment elsewhere. *Aspicilia cinerea* is a similar species that can be easily distinguished by non-squamulose younger parts of thalli, non-blastidiate surface and longer conidia (11–22 µm). *Sagedia simoënsis* (Räsänen) A.Nordin, S.Savić & Tibell can also be confused with *A. blastidiata* but the former species usually has thin, darker, not scaling thallus with yellowish-white soralia; some specimens have true isidia. *Sagedia simoënsis* has also northern distribution and differs genetically.

Additional specimens examined (paratypes)

RUSSIA. Sverdlovsk region: Rezh town, Bystrinskiy, rocks on right riverbank of Rezh river, serpentinite outcrops, serpentinite, 57°21′23.7″N, 61°22′24.3″E, alt. 175 m, 1 Aug. 2012. *A. Paukov* AGP20120801-02, -03 (UFU). Sverdlovsk region: Berezovskiy district, Staropyshminsk settlement, serpentinite outcrops on Pyshma River, on serpentinite, 56°56′05.3″N, 60°54′39.7″E, alt. 267 m, 9 Oct. 2011, *A. Paukov* AGP19980606-04, -05, AGP19990519-01, AGP20111009-01, -02, -03 (UFU). Sverdlovsk region: Sysert district, 2 km to W from Dvurechensk settlement, serpentinite outcrops under forest canopy, on serpentinite, 56°36′07.8″N, 61°03′74.1″E, alt. 203 m, 20 June 1998, *A. Paukov* AGP19980620-02 (UFU). Sverdlovsk region: Sysert district, Beklenishcheva village, serpentinite outcrops on river Iset’, on serpentinite, 56°26′10.1″N, 61°35′69.1″E, 26 July 2008, *A. Paukov* AGP20080726-05 (UFU). Chelyabinsk region: Kaslinskiy district, 2 km to SW from Tyubuk railway station, top of afforestated serpentinite mountain, on serpentinite, 55°48′40.3″N, 60°27′68.2″E, alt. 450 m, 16 June 2013, *A. Paukov* AGP20130616-01 (UFU). Bashkortostan: Uchalinskiy district, 4 km to SW from Karaguzhino village, Akbura mountain, serpentinite outcrops, on serpentinite, 53°59′06.6″N, 58°44′36.5″E, alt. 558 m, 9 June 2012, *A. Paukov* AGP20120609-28 (UFU). Bashkortostan: Beloretskiy district, 22 km to SW from Beloretsk, Kraka ridge, N Kraka, rocky outcrops, on serpentinite, 53°50′61.8″N, 58°04′54.8″E, alt. 823 m, 21 May 2011. *A. Paukov* AGP20110521-05 (UFU). Bashkortostan: Burzyanskiy district, Vicinity of Sargaya village, Bashkirskiy Natural Reserve, Bolshoy Bashart mountain, steppose communities, on serpentinite,53°22′08.9″N, 57°46′42.4″E, alt. 800 m. *I. Frolov* & *A. Paukov* 15 Aug. 2003 (UFU).

Metal concentration in serpentinites

Composition of metals in serpentinite in the studied area is shown in Table 1. Magnesium concentration in all localities exceeded concentration of Ca by 6.7–63 times. Apart from these metals and Fe, serpentinite contained substantial quantities of Cr, Mn and Ni. Rocks in Staropyshminsk, Rezh and Kyshtym did not differ statistically in the concentrations of most elements, whereas serpentinite near Dvurechensk was different in metal composition. It had the lowest Mg : Ca ratio and a smaller concentrations of Cr and Ni. In all localities, there was a strong significant correlation among Fe, Ni and Co as well as between Ca and Sr in rock. Soil pH of outcrops varied from 7.24 to 7.92 and did not depend on the concentration of Ca.

Table 1. Concentration of metals in serpentinites of four studied localities

Values within a row followed by the same letter are not significantly different (ANOVA, $P = 0.05$)

Metal	Dvurechensk	Staropyshminsk	Rezh	Kyshtym
Calcium (%)	0.2a	0.06b	0.07b	0.06b
Cobalt (µg g^{-1})	27.2a	48.0b	59.0b	40.1b
Chromium (µg g^{-1})	223.5a	756.8b	662.5b	720.4b
Copper (µg g^{-1})	21.8a	12.0b	21.0a	10.2b
Iron (%)	1.4a	1.9a	1.9a	1.7a
Magnesium (%)	1.7a	2.2a	4.3b	1.2a
Manganese (µg g^{-1})	324.9a	303.6a	468.2a	229.1a
Sodium (µg g^{-1})	201.9a	95.5b	104.5b	84.0b
Nickel (µg g^{-1})	357.8a	985.7b	1075.7b	767.4b
Strontium (µg g^{-1})	7.7a	3.0b	3.3b	4.5a
Zinc (µg g^{-1})	37.7a	60.8b	36.0a	21.9a

Concentration of metals in thalli

The most striking difference of metal content in *Aspicilia cinerea*, *A. goettweigensis*, *A. contorta* ssp. *hoffmaniana* and in rock was the Mg : Ca ratio, which varied between 0.28 and 0.58. Absolute concentrations of elements were similar in *A. cinerea* and *A. goettweigensis* and to a lesser extent in *A. contorta* ssp. *hoffmaniana*. Only one species, *A. blastidiata*, had a concentration of Mg that exceeded that of Ca. It contained also statistically higher concentrations of Co, Cr, Cu, Mn, Ni and Zn (Table 2). All species had a strong significant correlation among Fe, Ni and Co in thalli.

According to lichen : rock metal ratios (accumulation rates, AR) in the studied species, the elements can be subdivided into two groups. The first includes metals the concentration of which is basically lower in lichens than in rock, such as Ni, Fe, Co, Mn and Mg. Apart of Mg, these are 'borderline' elements (Nieboer and Richardson 1980). Other metals such as Ca, Sr, Cu, Na, Zn and Cr are accumulated by thalli of all species, with average rates from 2.8 to 11 times (Table 3). *Aspicilia blastidiata* is the most active accumulator of Ca, Sr, Na, Fe and Mn, compared with other species.

Concentration of metals in the substrate had a moderate effect on AR. In the ANOVA model, the 'locality' variable was significant for lichens : rock ratios of Ca in *Aspicilia cinerea* and *A. goettweigensis*, Sr in *Aspicilia cinerea*, *A. blastidiata* and *A. goettweigensis*, Zn in *Aspicilia goettweigensis* and *A.* ssp. *hoffmaniana*, Cr in *Aspicilia cinerea* and Ni in *Aspicilia goettweigensis* (Table 4).

Aspicilia goettweigensis and *A. contorta* ssp. *hoffmaniana* had no statistically significant positive correlations of metals in thalli and in rock. Species with norstictic acid showed some positive correlations, but with different elements involved (Table 5). The average Cu : Zn ratios in serpentinite and soil varied between 0.59 and 0.62. Two species, namely, *Aspicilia blastidiata* and *A. contorta* ssp. *hoffmaniana*, had the same or insignificant rates for these elements. *Aspicilia cinerea* has the highest ratio of Cu : Zn, compared with other species (Table 6).

Table 2. Concentration of metals in four lichen species
Values within a row followed by the same letter are not significantly different (ANOVA, $P = 0.05$)

Metal	*Aspicilia cinerea*	*Aspicilia blastidiata*	*Aspicilia goettweigensis*	*Aspicilia contorta* ssp. *hoffmaniana*
Calcium (%)	0.5a	1.5b	0.8a	1.4b
Cobalt ($\mu g\, g^{-1}$)	7.2a	53.8b	12.3a,c	20.6c
Chromium ($\mu g\, g^{-1}$)	363.8a	1045.7b	482.3a	540.7c
Copper ($\mu g\, g^{-1}$)	100.8a	164.8b	105.4a	82.7a
Iron (%)	0.5a	2.1b	0.8a	1.1b
Magnesium (%)	0.2a	2.7b	0.2a	0.8c
Manganese ($\mu g\, g^{-1}$)	95.2a	405.1b	137.3a	244.3c
Sodium ($\mu g\, g^{-1}$)	667.1a	816.6a	897.9a	857.6a
Nickel ($\mu g\, g^{-1}$)	103.6a	778.2b	225.8c	291.5c
Strontium ($\mu g\, g^{-1}$)	22.9a	38.3b	31.4a	52.4b
Zinc ($\mu g\, g^{-1}$)	88.0a	253.8b	111.7c	113.3c

Table 3. Accumulation rates of elements by lichens
Values within a row followed by the same letter are not significantly different (ANOVA, $P = 0.05$)

Metal	*Aspicilia cinerea*	*Aspicilia blastidiata*	*Aspicilia goettweigensis*	*Aspicilia contorta* ssp. *hoffmaniana*	Average for all species
Calcium	5.7a	20.3b	9.9a	8.0a	11.0
Strontium	4.8a	15.4b	8.8c	6.6a,c	8.9
Copper	7.4a	9.7a	9.3a	6.3a	8.2
Sodium	5.2a	11.2b	8.7a	5.2a	7.6
Zinc	3.9a	5.9a	5.0a	3.4a	4.6
Chromium	2.9a	1.4a	2.8a	4.2a	2.8
Nickel	0.7a	0.8a	0.6a	1.8a	1.0
Iron	0.4a	1.5b	0.6a,c	0.7c	0.8
Cobalt	0.3a	1.0b	0.6a,b	1.0b	0.7
Manganese	0.3a	1.1b	0.7a,c	0.7c	0.7
Magnesium	0.2a	0.5b	0.2a	0.8b	0.4

Table 4. Effect of 'locality' variable on accumulation ratios (one-way ANOVA results)
*$P < 0.05$

Metal	*Aspicilia cinerea*	*Aspicilia blastidiata*	*Aspicilia goettweigensis*	*Aspicilia contorta* ssp. *hoffmaniana*
Calcium	14.19*	2.27	4.28*	0.25
Cobalt	2.81	0.98	2.00	0.87
Chromium	18.21*	1.72	1.44	0.32
Copper	0.58	1.41	0.76	3.77
Iron	0.40	0.00	2.40	0.66
Magnesium	1.75	4.77	0.78	0.46
Manganese	0.37	0.37	2.35	2.43
Sodium	0.16	4.99	1.77	0.50
Nickel	2.19	2.56	6.61*	0.90
Strontium	8.69*	6.48*	3.79*	0.44
Zinc	0.50	4.73	4.93*	40.76*

Table 5. Correlation between metals in rock and in lichens
Ca, calcium; Sr, strontium; Cu, copper; Na, sodium; Zn, zinc; Cr, chromium; Ni, nickel; Fe, iron; Co, cobalt; Mn, manganese; Mg, magnesium. *$P < 0.05$

Metals in lichens	Metals in rock										
	Ca	Sr	Cu	Na	Zn	Cr	Ni	Fe	Co	Mn	Mg
Aspicilia cinerea	0.65*	0.86*	0.17	0.27	–0.29	0.06	0.74*	0.38	0.60*	0.22	0.51
Aspicilia blastidiata	0.53	–0.03	0.84*	0.18	0.00	0.73*	0.53	0.78*	0.44	0.95*	0.46
Aspicilia goettweigensis	–0.06	–0.01	–0.11	0.00	0.29	0.25	0.04	–0.08	–0.09	–0.15	0.25
Aspicilia contorta ssp. *hoffmaniana*	0.50	0.58	–0.82*	–0.52	0.67	–0.52	0.69	0.63	0.50	0.35	0.60

Discussion

Lichen substances are known for their metal-binding properties. This feature is found in depsidones norstictic and psoromic acids (Purvis *et al*. 1987, 1990), depside divaricatic acid, yellow pigments and antraquinones and other substances (Hauck *et al*. 2009). High affinity of secondary metabolites to metals is considered as an advantageous feature for lichens growing in habitats with poor availability of elements (Hauck *et al*. 2007).

Three species of *Aspicilia*, namely, *A. cinerea*, *A. contorta* ssp. *hoffmaniana* and *A. goettweigensis*, on serpentinite showed similar rates of accumulation of Ca, Sr, Cu, Na, Zn, Cr. Interestingly, the lichens with no protective tissues and growing on metal-rich substrate such as serpentinite can regulate the content of metals in thalli. Metals such as Ni, Fe, Co, Mn and Mg are contained in thalli in quantities equal to or lower than in the substrate.

These species contain different secondary metabolites, which implies that the difference in secondary chemistry does not have a strong influence on accumulation rates of metals in selected species on serpentinite. However, lichens have mechanisms of avoidance that allow them to control concentrations of separate metals at a low level. Secondary metabolites can also be the agents of avoidance (Hauck *et al*. 2007, 2009). Lichen acids may inhibit adsorption of metals into thalli (Hauck 2008). Thus, stictic and norstictic are different in their affinity to Fe^{3+}, but almost equally low in their affinity to Fe^{2+}. On serpentinite that has Fe^{2+}, this is probably the reason of low concentration of Fe in thalli of *A. cinerea* and *A. goettweigensis*. Most secondary metabolites have maximum affinity to metals at a low pH; therefore, species with lichen substances are adapted to grow on acid rocks with low content of metals or on metal-rich substrata with a higher pH. Antraquinones that have maximum binding properties at a higher pH are found in many species on calcareous substrata (Hauck *et al*. 2009).

This statement can be supported by comparing the percentage of lichens containing different secondary metabolites on chemically variable rocks (Fig. 3). A portion of lichens containing secondary metabolites is highest on quarzite. Gyrophoric, stictic and usnic acid species have clear affinity to more acidic substrata. Percentage of lichens with norstictic acid and atranorin does not clearly depend on the acidity of substrata but has the lowest value on carbonates. Species with antraquinones and without secondary metabolites have maximum proportion on limestone. Therefore, stictic and usnic acid can be expected to make complexes with metals in more acidic conditions. Norstictic acid may probably appear to form complexes in a wider pH range.

Aspicilia blastidiata is known so far from serpentinites only and is a rare case of serpentinite endemism in lichens (Sánchez-Biezma and López de Silanes 1999; Favero-Longo *et al*. 2004; Nordin 2013). It contains norstictic acid and has shown statistically higher levels of metal accumulation than have other species. The metals accumulated by this species are of substrate origin because the Cu : Zn ratio is similar to that of rock and soil. Regardless, accumulation levels of metals can be much higher for lichens (Chisholm *et al*. 1987) than for higher plants; *Aspicilia blastidiata* contains only one element such as Cr in the 'hyperaccumulation threshold' known for plants (Reeves 2006). Nevertheless, the reasons for the difference in accumulation between two

Table 6. Cu : Zn ratios in soil, serpentinite and lichens
Values within a column followed by the same letter are not significantly different

Species	Mean
Aspicilia cinerea	1.17b
Aspicilia blastidiata	0.62a
Aspicilia goettweigensis	0.95b
Aspicilia contorta ssp. *hoffmaniana*	0.77a
Rock	0.62a
Soil	0.59a

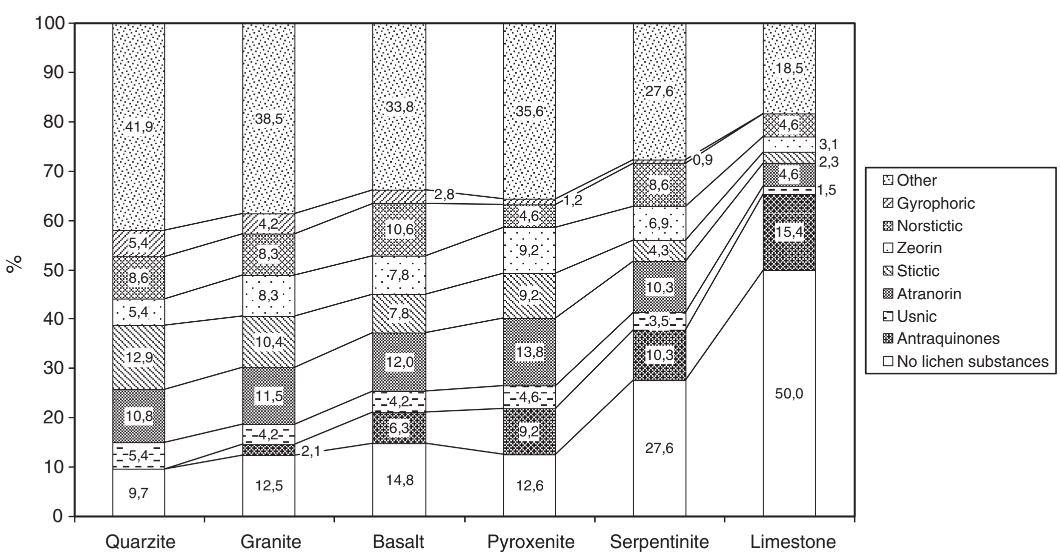

Fig. 3. Percentage of crustose lichens with different secondary metabolites on six rock types in the middle and southern Urals (292 species).

similar lichens containing the same secondary metabolite need further study. It may be related to both the concentration of norstictic acid and other organic substances in thalli (Purvis 1984; Kasama *et al*. 2002; Pawlik-Skowrońska *et al*. 2006) and may determine species distribution on different rocks.

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