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Accumulation and Localization of Metals in Lichen Thallus Under Conditions of Dust Pollution During Open Mining of Boxite Deposits

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Abstract—We studied the accumulation and localization of metals in the foliose lichens *Lobaria pulmonaria*, *Hypogymnia physodes*, and *Peltigera aphthosa*, living in the impact zone of the Sredne-Timansky bauxite mine. A significant accumulation of Al (16–19 g/kg), Fe (16–20 g/kg), and Ti (0.3–0.7 g/kg) by thalli was revealed. From 29 to 82% of the total content of these metals is localized in dust particles weakly attached to the surface of the thalli. The total proportion of intra- and extracellularly bound Al, Fe, and Ti did not exceed 11%, 15–56% of these metals were found in the residual fraction. An increase in the content of Cu, Pb, Co, and Ni was detected in thalli collected in the impact area. It has been shown that the localization of metals in thalli depends both on the studied element and on the morphological and anatomical characteristics of the thalli: in *L. pulmonaria*, fine mineral particles were localized on the surface of the thalli; in the thalli of *P. aphthosa*, which do not have a lower cortex, mineral inclusions were found throughout the entire thickness of the thalli.

Keywords: lichenized fungi, Komi Republic, Middle Timan, bauxite, pollutants, metals, accumulation, sequential extraction, electron microscopy

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INTRODUCTION

Mining and processing of mineral resources almost inevitably lead to the emission of pollutants. Environmental pollution by dust emissions has a significant negative impact on all structural elements of biogeocenoses. Monitoring the intake of dust emissions and assessing the impact of the mineral and organic substances they contain on the soil and vegetation cover are an important task, especially for northern ecosystems with a low ability for self-purification and self-healing.

Lichens are widely used in environmental bioindication systems, including monitoring of atmospheric metal pollution [1]. Their thalli do not have protective coverings and can accumulate chemical compounds from the atmosphere and sediments in significant quantities during their life cycle [2]. The main mechanisms of metal binding in thalli include physical binding of particles on the surface of thalli and in the intercellular space of thalli, extracellular and intracellular accumulation [3]. A large number of works are devoted to the study of the accumulation of heavy metals in lichens in the area of activity of metallurgical enterprises [4–6], their localization [3, 7], absorption mechanisms [8, 9] and detoxification [10]. In several studies [11, 12] It was shown that long-term pollution of territories by dust emissions during the development of ore deposits leads to changes in the number and structure of lichen communities and the morphology of their thalli. Dust emissions are a complex heterogeneous mixture of airborne, often insoluble, solid particles that vary in size, origin, and chemical composition [13]. The physicochemical properties of compounds contained in atmospheric emissions mediate the intensity of absorption and localization of metals in lichens, and therefore, it is necessary to quantify these indicators when dust particles arrive on the surface of thalli.

Objective—to study the patterns and specifics of the accumulation and localization of metals in foliose lichens living in the impact zone of a bauxite mine. The results of the study will make it possible to characterize the impact of the entry of dust emissions from a bauxite mine into the environment on changes in the elemental composition of lichens, to evaluate the contribution of various mechanisms of metal binding by lichenized fungi under conditions of polymetallic pollution during atmospheric deposition of dust emissions, and to study the influence of the morphological characteristics of thalli on their absorption of fine mineral particles.

MATERIALS AND METHODS

Objects of study and characteristics of lichen habitats. The research was carried out on the territory of the Sredne-Timan bauxite mine (STBM), located at the junction of the Ust-Tsilemsky, Knyazhpogostsky, and Udora regions of the Komi Republic. Bauxite mining at the mine has been carried out by open-pit mining for more than 20 years. The predicted amount of emissions released annually into the atmosphere from the Vezhayu-Vorykvinskoe deposit facilities is more than 5500 tons: nitrogen oxides – 636 tons, carbon monoxide – 1112 tons, sulfur dioxide – 786 tons, inorganic dust – 1059 tons, and soot – 345 tons [12]. The main sources of pollutants entering the environment from STBM production facilities are drilling and blasting operations, excavation of the ore body, movement of bauxite ore and its host rocks, dusting of the sizing warehouse and overburden dumps, and movement of transport along interfield and technological roads. Monitoring studies in the territory of STBM activity indicate a gradual increase in areas experiencing persistent pollution. An analysis of the snow cover revealed that in dust fallout near the main technological objects of the mine there is a fourfold or more increase in the concentrations of Al, Fe, Si, Mn, Ni, Co, Ti, Cu, Pb, Zn, and Cd compared to background values. In the zone of influence of bauxite mine objects, levels of suspended particles, Al, Fe, Co, Ti, Cu, and a number of other metals in snow water and the waters of temporary streams exceeding the maximum permissible concentration were found. In the soils associated with bauxite quarries, intensive accumulation of Al, Ti, and Pb was observed [14]. With the commissioning of the Verkhne-Shchugorsk bauxite deposit, as part of the development of quarries of the second stage of the STBM, we can expect an increase in the flow of atmospheric emissions.

Epiphytic foliose lichens *Lobaria pulmonaria* (L.) Hoffm. and *Hypogymnia physodes* (L.) Nyl., and epigeic foliose lichen *Peltigera aphthosa* (L.) Willd. were used as model objects. *Lobaria pulmonaria* (L.) Hoffm., *Hypogymnia physodes* (L.) Nyl. and epigean foliose lichen *Peltigera aphthosa* (L.) Willd. Lichen sampling was carried out in a spruce-birch forest with an admixture of aspen near the production and transport infrastructure of the mine. An area remote from the mine at a distance of about 4 km and having soil and vegetation cover similar to the impact territory was selected as a conditional background territory (hereinafter referred to as the background territory). Thalli of *L. pulmonaria* were taken from aspen trunks, thalli of *H. physodes*, from the trunks and branches of spruce. Lichen samples (at least 3–5 thalli on 15–30 trees) were taken from the entire trunk (branches) at a height range of 1–2.5 m from the ground. Collection of the *P. aphthosa* thalli was carried out from plant residues or moss on the soil surface. To compile an average sample, 5–8 large lobes were selected from 20–25 thalli located at a distance of more than 5 m from each other. After separation from the substrate, the thalli were dried to an air-dry state and an average sample was compiled using at least 30 individual thallus samples.

Chemical analysis of lichens. Analysis of the metal content in thalli was carried out using inductively coupled plasma atomic emission spectrometry (FR.1.31.2006.02149) on a Spectro Ciros CCD spectrometer (SPECTRO Analytical Instruments, Germany). Mineralization of thalli samples was carried out under the influence of a microwave field in the presence of HNO_3 (conc.) and H_2O_2. Analyzes were performed at the Ecoanalytical Laboratory, Institute of Biology of Komi Science Centre of the Ural Branch of the Russian Academy of Sciences (accreditation certificate ROSS RU.0001.511257 dated September 25, 2015). The distribution of metals in lichen thalli between different fractions was assessed using a sequential extraction procedure [15], modernized by us taking into account the specifics of intake and physicochemical properties of the priority pollutant [16].

To carry out the sequential extraction procedure, average samples of thalli were used. The total content of metals in thalli was assessed in a sample from an average sample of thalli before removing dust deposits from their surface.

To remove dust particles weakly attached to the surface, the remaining thalli were washed three times with deionized water and a sample was taken for elemental analysis. The remaining part of the middle sample was extracted three times with a 20 mM EDTA-Na_2 solution to extract metal ions nonspecifically bound to cell walls (extracellular fraction) and a sample was taken for analysis. After extracting the extracellular fraction from the thalli, the remaining thalli were kept for 12 h at a temperature of 80°C to destroy cell membranes and were again extracted with an EDTA-Na_2 solution to isolate the intracellular fraction of metals, and then elemental analysis of the thalli was carried out.

The pool of residual metal fraction was assessed by their content in thalli after removal of dust, extra- and intracellular fractions, then the metal content in individual fractions was calculated:

$$P(Me)_{dust} = \rho(Me)_{total} - \rho(Me)_{wash}, \quad (1)$$

$$P(Me)_{ext} = \rho(Me)_{total} - \rho(Me)_{EDTA}, \quad (2)$$

$$P(Me)_{int} = \rho(Me)_{EDTA} - \rho(Me)_{temp}, \quad (3)$$

$$P(Me)_{res} = \rho(Me)_{total} - \rho(Me)_{dust} - \rho(Me)_{ext} - \rho(Me)_{int}, \quad (4)$$

where $P(Me)_{dust}$ – metal content in dust, $P(Me)_{ext}$ – in the extracellular, $P(Me)_{int}$ – in the intracellular $P(Me)_{res}$ – in residual fractions; $\rho(Me)_{total}$ – total metal content in the thallus; $\rho(Me)_{wash}$ – metal con-

Table 1. Content of metals in lichen thalli sampled in the background area (Background) and impact area (Pollution) of the Sredne-Timansky bauxite mine, mg/kg

Fraction	Site	Al	Fe	Mn	Ti	Cu	Zn	Pb	Co	Ni
Hypogymnia physodes										
Intracellular	Background	70 (9)	160 (18)	13 (2)	11 (21)	1.6 (26)	39 (33)	<0.1	0.17 (27)	0.4 (18)
	Pollution	500 (3)	400 (3)	8 (2)	9 (3)	1.4 (9)	34 (43)	0.9 (8)	0.13 (3)	0.5 (4)
Extracellular	Background	110 (14)	170 (19)	611 (89)	12 (23)	2.0 (33)	62 (52)	3.0 (50)	0.28 (44)	0.9 (45)
	Pollution	1000 (6)	1300 (8)	386 (71)	5 (1)	4.2 (26)	32 (40)	1.8 (15)	0.86 (22)	2.5 (21)
Residual	Background	390 (51)	420 (47)	46 (7)	13 (25)	2.5 (41)	19 (16)	2.4 (40)	0.18 (29)	0.7 (37)
	Pollution	4100 (26)	3600 (23)	66 (12)	59 (17)	2.8 (18)	10 (12)	4.4 (37)	0.61 (15)	2.3 (19)
Dust	Background	190 (25)	140 (16)	20 (3)	17 (32)	<0.1	<0.1	0.6 (10)	<0.01	<0.1
	Pollution	10400 (65)	10700 (67)	80 (15)	267 (79)	7.6 (48)	4.0 (5)	4.9 (41)	2.40 (60)	6.7 (56)
Total content	Background	760	890	690	53	6.1	120	6.0	0.63	2.0
	Pollution	16000	16000	540	340	16	80	12.0	4.00	12.0
Lobaria pulmonaria										
Intracellular	Background	30 (7)	40 (10)	9 (2)	6 (32)	1.0 (21)	29 (47)	0.1 (5)	0.06 (14)	0.1 (9)
	Pollution	800*	500*	7 (2)	35 (5)	2.8 (9)	45 (69)	0.2 (2)	0.20 (4)	0.1 (1)
Extracellular	Background	70 (16)	80 (21)	198 (52)	4 (22)	1.2 (26)	9 (15)	0.4 (33)	0.08 (19)	0.3 (21)
	Pollution	800*	500*	96 (30)	40 (6)	3.6 (12)	6 (9)	0.9 (10)	1.00 (18)	2.0 (13)
Residual	Background	190 (43)	170 (44)	4 (1)	6 (35)	2.5 (53)	13 (21)	0.5 (38)	0.05 (12)	0.6 (53)
	Pollution	2900 (15)	3300 (17)	27 (8)	55 (8)	7.0 (23)	10 (15)	1.7 (18)	0.80 (14)	3.3 (21)
Dust	Background	150 (34)	100 (26)	170 (45)	2 (11)	<0.1	11 (18)	0.3 (25)	0.24 (56)	0.2 (17)
	Pollution	15300 (81)	16200 (81)	190 (59)	590 (82)	17.6 (57)	4 (6)	6.4 (70)	3.60 (64)	10.6 (66)
Total content	Background	440	390	380	18	4.7	62	1.2	0.43	1.2
	Pollution	19000	20000	320	720	31	65	9.2	5.60	16.0
Peltigera aphthosa										
Intracellular	Background	20 (5)	20 (4)	30 (13)	13)	2.6 (50)	23.5 (43)	0.2 (11)	0.04 (12)	0.5 (30)
	Pollution	1400*	1500*	26 (5)	30 (6)	8.0 (36)	18.1 (24)	1.0 (11)	0.90 (23)	2.2 (16)
Extracellular	Background	20 (5)	50 (11)	122 (53)	13)	0.1 (2)	25.0 (45)	1.1 (61)	0.15 (44)	0.2 (13)
	Pollution	1400*	1500*	210 (43)	50 (10)	4.0 (18)	25.0 (33)	1.1 (12)	0.60 (15)	<0.1
Residual	Background	320 (78)	350 (74)	8 (3)	24 (77)	1.7 (33)	2.5 (5)	0.3 (17)	0.15 (44)	0.8 (51)
	Pollution	8500 (53)	8500 (53)	84 (17)	290 (56)	8.0 (36)	9.9 (13)	5.0 (54)	1.20 (30)	6.8 (49)
Dust	Background	50 (12)	50 (11)	70 (30)	5 (16)	0.8 (15)	4.0 (7)	0.2 (11)	<0.01	0.1 (6)
	Pollution	6100 (38)	6000 (38)	170 (35)	150 (29)	2.0 (9)	22.0 (29)	2.1 (23)	1.30 (33)	5.0 (36)
Total content	Background	410	470	230	31	5.2	55	1.8	0.34	1.6
	Pollution	16000	16000	490	520	22	75	9.2	4.00	14.0

The share (%) of each fraction of the total metal content is given in parentheses; The symbol * indicates the total content metal in the intra- and extracellular fractions.

tent in the thallus after washing with water; $\rho(Me)_{EDTA}$ – metal content in the thallus after extraction with a solution EDTA-Na_2; $\rho(Me)_{temp}$ – metal content in the thallus after exposure at 80°C and subsequent extraction solution EDTA-Na_2. If the test results did not allow identifying the distribution of a particular metal between the intra- and extracellular fractions (Table 1), the total content of the element in the composition of these two fractions was calculated (here they are interpreted as the total content of metals localized intracellularly and bound on the cell walls of the symbionts).

Electron microscopy and energy dispersive analysis. To analyze the localization of metals in lichens using scanning electron microscopy, the thalli were washed with deionized water, frozen in liquid nitrogen and lyophilized. Transverse sections of thalli were immobilized with polyester resin. The resulting preparations were subjected to vacuum polymerization. The polished surface of the preparations was coated with a conductive carbon coating approximately 25 nm thick. Electron microscopy of the samples was carried out on a TESCAN Vega 3 LMH scanning electron microscope (Tescan, Czech Republic). The analysis of the chemical composition of mineral particles and the construction of maps of the distribution of chemical elements in lichen preparations were carried out using an X-MAX energy dispersive spectrometer (Oxford Instruments, United Kingdom). Scanning electron microscopy and X-ray microanalysis of lichen samples were carried out at the Geoscience Shared Use Center, Institute of Geology, Komi Scientific Center, Ural Branch, Russian Academy of Sciences.

Statistical data processing. To assess changes in the distribution of the relative proportion of metals between different fractions in lichen thalli in background and impact areas, principal component analysis was used using Statistica 10 software (StatSoft Inc., United States).

RESULTS

Metal content in lichen thalli. According to the data obtained (Table 1), thalli of *L. pulmonaria* and *P. aphthosa* in the conditionally clean territory, they practically did not differ in the content of most of the studied elements. Total metal content in thalli of *P. aphthosa* was close to the previously estimated values for thalli of this species, selected at a distance from the production facilities of the mine [16]. Concentrations of Fe, Al, Mn, Ti, Zn, and Pb in thalli of *H. physodes* in the background territory were 2 times or more higher than these indicators characteristic of *L. pulmonaria* and *P. aphthosa*. Discovered by us for *H. physodes* the values turned out to be slightly higher than the background indicators given for this species during long-term monitoring studies in the area affected by STBM [12].

The absolute values of the total content of Al and Fe in thalli on the impact territory were 18–51 times higher and Ti, were 6–40 times higher than these indicators for the background territory (see Table 1). Accumulation of Ni, Co, Cu, and Pb by thalli in quantities ranging from 2 to 13 times higher than background values was also noted. The content of Zn and Mn in lichen thalli in the considered habitats differed significantly less (see Table 1).

Localization of metals in lichen thalli. The results of assessing the localization of metals in lichens living in conditions of chronic pollution showed a multiple increase in the absolute values of Al and Fe content in all fractions compared to the values for the background area. The total Al content in the intra- and extracellular fractions increased by 8–20 times, Fe, by 4–20 times. The Ti content in these fractions increased more than 7 times for *L. pulmonaria* and *P. aphthosa*, while *H. physodes* dropped by half. For all species, an increase in the content of Al in the residual fraction was noted, 11–27 times, Fe, by 9–24 and Ti, by 5-12. The accumulation of these metals on the surface of lichens in the dust fraction in contaminated areas increased by 1–2 orders of magnitude.

The content of Co and Ni in the dust fraction exceeded the background values by more than 15 times, in those extracted with an EDTA-Na_2 solution (in total), 2–8 times. Accumulation of Zn in the intracellular fraction of thalli of *H. physodes* and *P. aphthosa* in the contaminated area was lower and in the dust fraction higher than in the background area. For thalli of *L. pulmonaria* the opposite pattern was noted.

The Cu content in the extracellular fraction of the *L. pulmonaria* and *H. physodes* thalli under conditions of pollution increased by 2–3 times, and in *P. aphthosa* more than an order of magnitude. The main contribution to the change in the total Cu content in the thalli of epiphytic species of *L. pulmonaria* and *H. physodes* was contributed by the dust fraction, which contained about half of the total copper accumulation. In an epigeic species, *P. aphthosa*, the main part of Cu was concentrated in the intracellular and residual fractions.

The intracellular Mn content in thalli in the impact area differed slightly from the background values. In conditions of dust pollution for *P. aphthosa* a twofold increase in extracellularly bound Mn was noted, while in thalli *L. pulmonaria* and *H. physodes* an almost twofold decrease was noted. The Mn content in the residual fraction increased in all studied species.

Distribution of mineral particles in lichen thalli. According to scanning electron microscopy data, the fine particles remaining after removal of the dust fraction had a contrasting distribution in the thalli of different types of lichens. *L. pulmonaria* water-insoluble mineral particles were located on the surface of the thalli. However, most of them were found on the lower cortex and they were associated with rhizins (Fig. 1a). In thalli of *P. aphthosa* (Fig. 2a) most of the microparticles remaining after removal of surface dust contamination were localized in the medulla formed by loosely located hyphae of the mycobiont. Directly below the upper paraplectenchymal cortex, the amount of mineral inclusions was minimal.

Analysis of EDX spectra found in particle thalli (Table 2), showed that their composition is dominated by Al, Fe, and Si compounds. In addition, some parti-

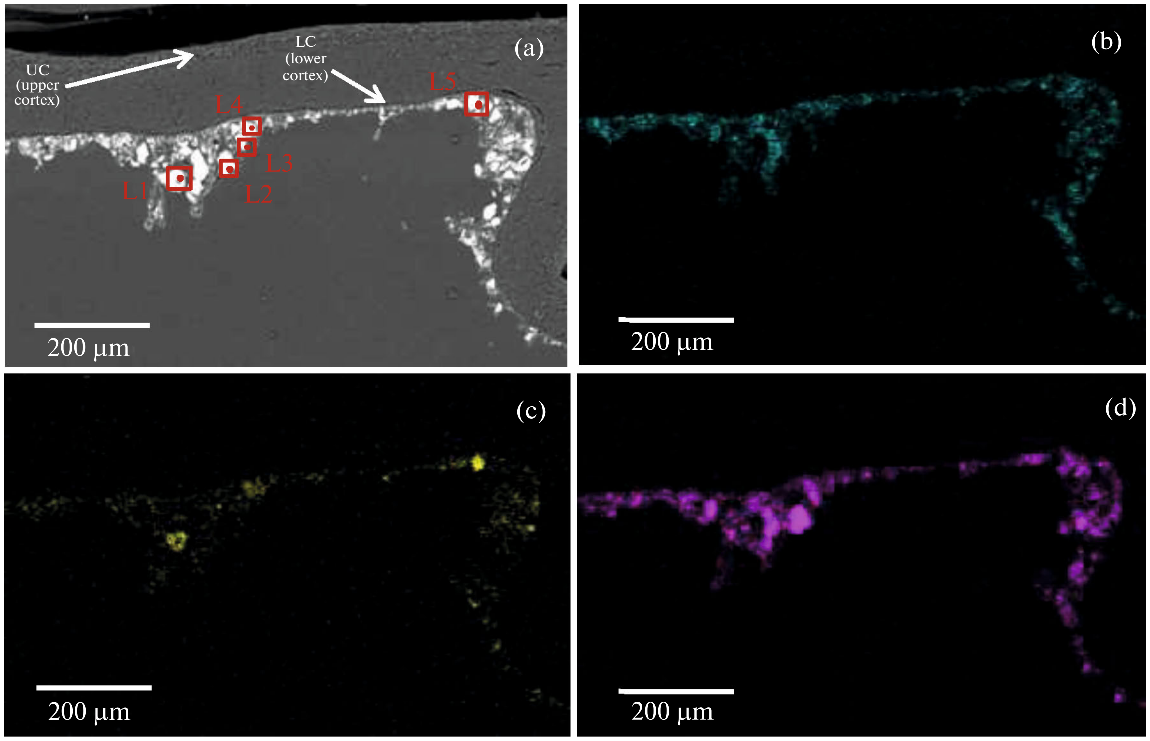

Fig. 1. Image of a cross section of a thallus of *Lobaria pulmonaria*, obtained in the mode of detecting backscattered electrons (a) and element distribution maps: (b) Al, (c) Fe, (d) Si. Areas with bright colors correspond to the maximum content of the element; UC—upper cortex, LC—lower cortex; the elemental composition of mineral particles (L1–L5) is given in Table 2.

cles are characterized by a relatively high mass fraction of Ca, Mg, and Ti.

Element distribution maps obtained based on the analysis of characteristic X-ray emission spectra showed that in thalli of *L. pulmonaria* the highest concentrations of Al, Fe, and Si were found on the outer part of the lower cortex and in the rhizin region (see Figs. 1b–1d). In thalli of *P. aphthosa* the distribution of concentrations of Al, Fe, and Si also had a pronounced discrete character (see Figs. 2b–2d). Areas with a high concentration of metals and silica corresponded to the position of mineral inclusions found on cross sections of thalli, in the form of loose aggregates, grains, or flakes, localized in the core layer.

Table 2. Mass fraction of elements in dust particles, %

No. particles	Na_2O	MgO	Al_2O_3	SiO_2	P_2O_5	K_2O	CaO	TiO_2	MnO_2	Fe_2O_3
					Lobaria pulmonaria					
L1	–	–	1.7	3.2	–	–	–	–	–	66.4
L2	3.9	–	29.0	51.7	–	0.2	12.3	–	–	0.9
L3	1.2	2.5	16.2	56.5	–	9.2	0.7	0.8	–	5.6
L4	–	–	80.9	1.3	–	–	–	–	–	2.1
L5	–	–	2.3	1.0	–	–	0.2	25.8	1.5	58.7
					Peltigera aphthosa					
P1	5.5	–	27.8	57.5	–	0.3	10.0	–	–	0.7
P2	–	1.2	26.9	22.1	0.4	–	0.2	–	–	35.7
P3	3.2	–	30.7	50.3	–	–	14.4	–	–	0.7
P4	–	–	3.4	0.7	0.3	–	0.2	1.8	0.8	71.8
P5	–	15.8	2.4	52.0	–	–	18.2	0.9	–	10.6

* Particle numbers correspond to those shown in Fig. 1 and 2 designations; A dash means that the element was below the detection limit.

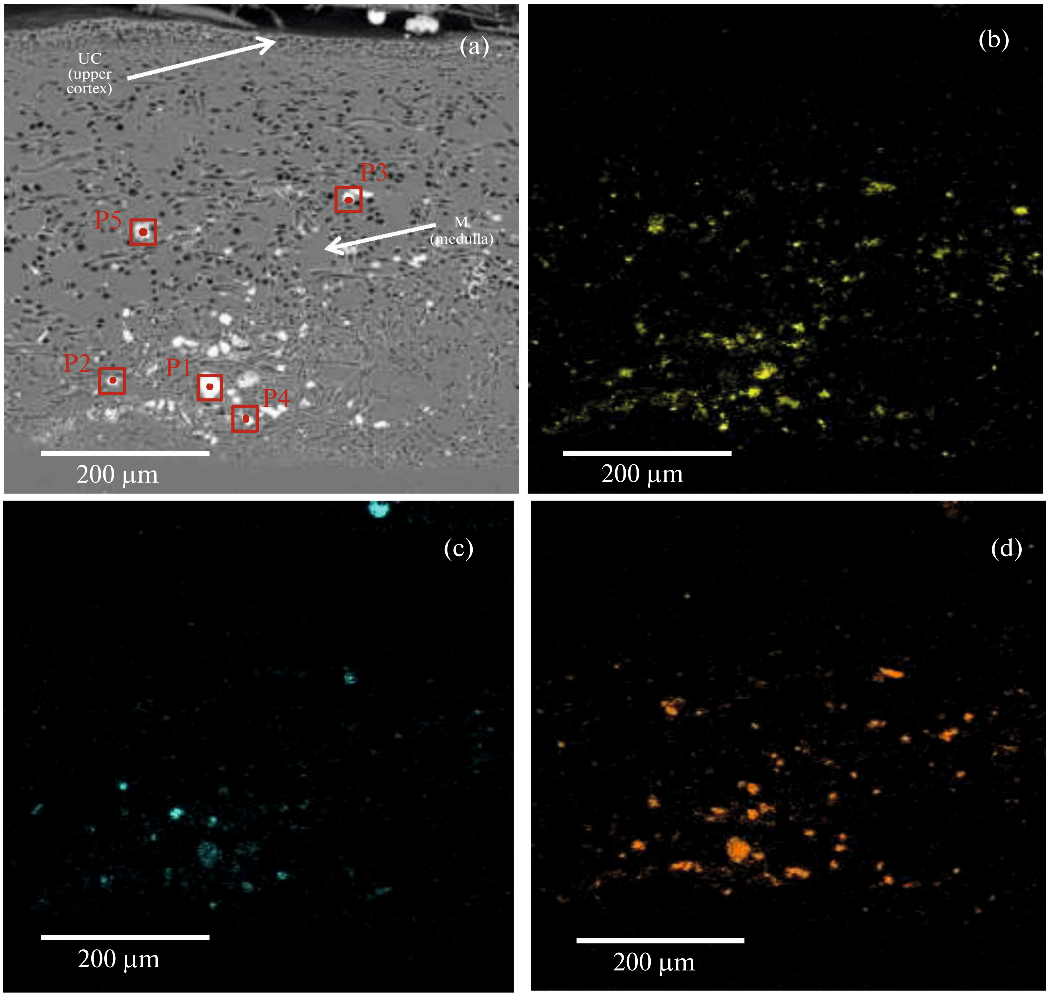

Fig. 2. Image of a cross section of a thallus *Peltigera aphthosa*, obtained in the mode of detecting backscattered electrons (a) and element distribution maps: (b) Al, (c) Fe, (d) Si. Areas with bright colors correspond to the maximum content of the element; UC—upper cortex, M—medulla; the elemental composition of mineral particles (P1—P5) is given in Table 2.

DISCUSSION OF THE RESULTS

The accumulation of pollutants by lichens depends on their physicochemical properties and emission intensity, the distance of habitats from the source of emissions, weather conditions, sampling season, and the position of thalli in space [1, 17]. Absolute values of the total metal content in thalli of *L. pulmonaria* and *P. aphthosa* on a conditionally clean territory (see Table 1) were in the upper part of the range of regional values, and for thalli of *H. physodes*, slightly exceeded the data given in the literature for lichens in areas that are not significantly influenced by sources of technogenic pollution [18, 19]. Accumulation of metals in thalli of *H. physodes* may indicate an expansion of the impact area of the mine and changes in the supply of dust emissions to lichens selected on the periphery of the conditional background area during the development of new quarries or interfield and technological roads.

The chemical composition of thalli sampled near the mine largely reflected the elemental profile of dust emissions generated during the mining of high-iron bauxite. Significant accumulation in thalli of Al, Fe, and Ti is quite natural, since chemical compounds of these metals make up the bulk of the mined ore mass and host rocks [20]. The accumulation of Ni, Co, and Pb can also be considered a consequence of sedimentation of suspended particles on thalli (the content of these metals in bauxite and accompanying rocks is measured in tens and hundreds of g/t).

Our data indicate a significant ability of foliose lichens to accumulate incoming fine particles and the metals they contain, regardless of their location in the substrate. In the STBM impact zone, the content of metals in epiphytic species *L. pulmonaria* and *H. physodes* was comparable to the accumulation of pollutants by epigeic lichen *P. aphthosa*. It was previously shown [21, 22] that such high accumulation values of Fe and Al are characteristic of lichens during dry deposition of dust particles on their surface. The Fe content values comparable to those obtained by us (32–90 g/kg) were found in areas contaminated by the steel industry in thalli of *Peltigera rufescens* [21]. Fe and Al content in *Circinaria aspera*, living in arid conditions and absorbing metals arriving on the surface of the thalli during dry deposition of particles, can reach several percent of the thalli dry mass [22].

One of the indicators used to assess environmental pollution caused by metallurgical enterprises is ratio of absolute content values iron and titanium in lichen thalli [23]. Normally, the Fe/Ti ratio for lichens is determined by the regional clarke of these elements and is 6–9 rel. units; an upward deviation of Fe/Ti indicates the supply of iron from pollution sources. In foliose lichens living in conditions of chronic pollution with STBM emissions, the Fe/Ti ratio was 27–47 rel. units, while for thalli from the background area this value was 15–21 rel. units A more selective indicator of emissions from bauxite mines into the atmosphere can be considered the ratio of Al and Mn content in lichen thalli. For thalli from background areas, the Al/Mn value was 1–2 rel. units, while for the polluted area this value was in the range from 30 to 60 rel. units The Al/Mn indicator can be useful for distinguishing the input of aluminum into lichen thalli as a result of the natural biogeochemical cycle from the influx of Al with atmospheric emissions during bauxite mining.

The minerals included in bauxite are mainly represented by aluminum oxide hydrates, iron, titanium and silica oxides, iron hydroxides, and silicates [20]. These compounds are characterized by low geochemical mobility and, when they fall on lichen thalli, they are concentrated in the form of slightly soluble fine particles (see Figs. 1, 2). Relatively inert chemical compounds of metals, falling on thalli, can be subject to chemical modification under the influence of external (low pH values of atmospheric precipitation and sub-canopy waters) or internal (lichen and carboxylic acids) factors [10]. A significant increase in the content of Al and Fe in the intra- and extracellular fractions in thalli in the STBM impact area (see Table 1) indicates the possibility of partial transition of aluminum and iron localized in dust and residual fractions into water-soluble forms and subsequent binding to ion exchange groups and specific proteins of cell walls and intracellular absorption.

Considering that intracellular absorption of metals is closely related to the metabolism of thalli [3, 8], changes in ionic homeostasis may be the cause of the toxic effect of dust particles. As was shown earlier, thalli of *H. physodes*, selected near STBM production facilities, were characterized by the presence of necrosis of the central areas and lobes, changes in the anatomy of the upper cortex [12], and high levels of oxidative stress [16].

The changes we discovered in the total content of Cu, Zn, Pb, Co, and Ni and the accumulation of these metals in the extra- and intracellular fractions in thalli depended both on the studied element and on the species of lichens. It is likely that the reasons for the different fractionation of these metals are associated with the interaction of a number of factors (the chemical composition and pH of the substrate and moisture supplied with sediments and under-canopy waters to the thalli, differences in the dynamics of hydration and dehydration of thalli, the chemical structure of synthesized lichen acids, and the affinity of binding centers on surface of the cell walls of mycobionts to various metal cations), affecting the kinetics and thermodynamics of ionic exchanges, the consideration of which requires additional research. It should be noted that the content of Cu, Pb, Co, and Ni in the extracellular fraction under dust pollution conditions remained at a relatively low level (see Table 1).

Principal components analysis (Fig. 3) showed that in lichens from the background area, most of the studied metals, according to their relative share of the total content, were grouped in EDTA-Na_2-extracted (total content of various forms of the element, in intra- and extracellular fractions) and residual fractions. In thalli of *L. pulmonaria* and *H. physodes* from the impact territory, the main part of the studied metals was grouped in the dust fraction, and only Mn and Zn were characterized by predominantly intra- and extracellular absorption. In thalli of *P. aphthosa* in the polluted area, the studied elements (with the exception of Fe and Al) were grouped into EDTA-Na_2 extracted and residual fractions. It can be assumed that the distribution of metals in thalli between different fractions under pollution conditions is species specific.

The assumption about the distinctive features of the distribution of metals in different species of lichens is confirmed by electron microscopy data. In thalli of *L. pulmonaria* the highest concentrations of Al, Fe, and Si were found in the lower cortex and in the rhizin region. At the same time, there was no uniform distribution of elements in the cells of the lower surface of the thalli and the hyphal bundles extending from it, as would be expected in the case of binding of elements in the intracellular and extracellular fractions. In thalli of *P. aphthosa* the distribution of Al, Fe, and Si also had a pronounced discrete character. Areas with high concentrations of metals and silicon corresponded to the position of mineral inclusions localized in the medulla. In [9] it is clearly demonstrated that the processing of the *H. physodes* thalli with a $Pb(NO_3)_2$ solution leads to a significant increase in lead concentration in the upper and lower cortex layers. The similar data of the lateral distribution of Co, predominantly localized in the lower cortex, was noted when processing thalli of *Permotrema tinctorum* with a $CoCl_2$ solution [24], which indicates the involvement of sorption mechanisms on the cell surface or intracellu-

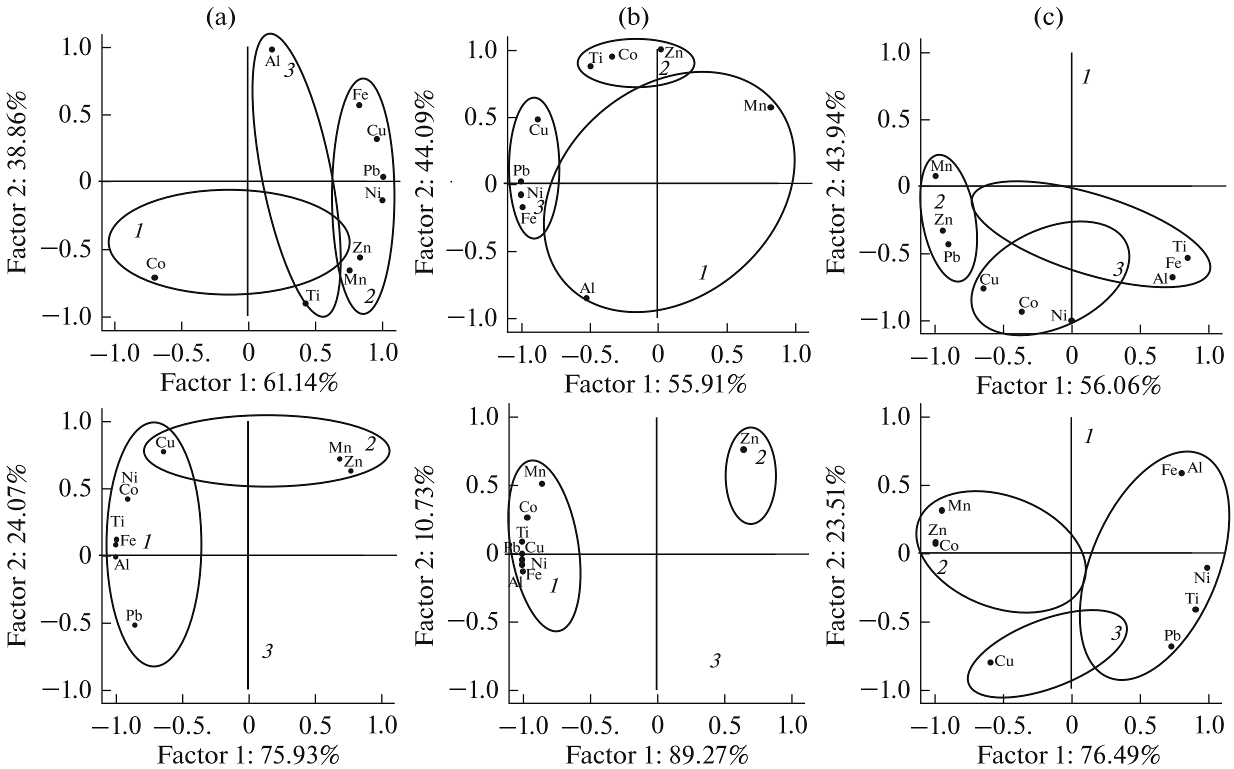

Fig. 3. Analysis of the principal components of the distribution of the relative proportion of metals in different fractions from thalli of *Hypogymnia physodes* (a), *Lobaria pulmonaria* (b), and *Peltigera aphthosa* (c): above are the results for thalli from the conditionally background territory, below—for those selected in the impact zone. The solid line unites the metals that contribute the main load to Factor 1 (horizontal axis) and/or Factor 2 (vertical axis); (*1*), (*2*), and (*3*) dust, EDTA-Na_2-extracted, and residual fractions of metals, respectively.

lar absorption of this metal ion from solutions. At the same time, the specificity of accumulation when introducing water-soluble Pb and Co compounds was the absence of sharp changes in the concentration of metals in the cortex layers. In the case of dry deposition of mineral particles on the surface of thalli, a sharp gradient of metal content is observed in the outer and deeper layers of the upper cortex of the *Circinaria aspera* thalli [22], i.e., the pattern of distribution of concentrations of Al, Fe, and Si is similar to the pattern of distribution of concentrations of Al, Fe, and Si that we discovered based on the results of electron microscopic studies.

It is important to note that in the thalli of *P. aphthosa* is devoid of the lower cortex, mineral inclusions are localized medullarily and are noted throughout almost the entire thickness of the thallus (see Fig. 2), and the content of Al and Fe in the residual fraction reached 8500 mg/kg (see Table 1). In the thalli of *L. pulmonaria* and *H. physodes* with developed lower and upper cortex layers that prevented the penetration of particles into the medulla of the thalli, the content of Al and Fe in the residual fraction was 2–3 times less. The opposite trend was observed when comparing the content of these metals in the dust fraction. With comparable values of the total accumulation of Al and Fe in the thalli of *H. physodes* and *L. pulmonaria*, it was 1.7–2.5 times superior in thalli of *P. aphthosa* by the content of metals in the composition of fine particles on the surface of thalli. Consequently, the distribution of mineral particles in the considered lichen species depended to a significant extent on the morphological characteristics of the thalli and was determined by the presence of the lower cortex. The data obtained are in good agreement with the ideas about the influence of the surface characteristics of lichen thalli on the efficiency of capture and retention of mineral particles [1, 25, 26].

CONCLUSIONS

The study made it possible to identify the features of the accumulation of metals in the thalli of foliose lichens living in conditions of chronic pollution by emissions from a bauxite mine. Maximum levels of accumulation were detected for Al and Fe, and an increase relative to the background values of the total content of Ti, Cu, Pb, Co, and Ni was noted. The possibility of using the Al/Mn indicator (the ratio of the absolute values of the mass fractions of Al and Mn) to assess the supply of aluminum to lichens as a result of the emission of dust emissions into the environment during bauxite mining is shown. The metals accumulated by lichens differed significantly in their distribution between the intra- and extracellular, residual, and dust fractions. Under conditions of polymetallic pollution from STBM objects, the studied species accumulated an excess of metals mainly in the composition of dust particles weakly attached to the surface of the thalli, as well as mineral inclusions associated with rhizins or incorporated into the medulla. The localization of mineral particles depended significantly on the structure of the thalli. The presence of paraplectenchymal upper and lower cortex layers in thalli prevented the penetration of fine metal-containing particles into the medulla, while for thalli without a lower cortex, mineral inclusions were found throughout the entire thickness of the thalli.

It should be noted that some of the metals entering lichens with dust emissions penetrated the plasma membranes of the symbionts and accumulated in the intracellular fraction, which can lead to negative consequences for the photo- and mycobiont. We do not exclude the physical influence of precipitated mineral particles on the functional state of lichens. A significant accumulation of dust particles can significantly change the intensity and spectral composition of the light flux arriving to the photobiont, affect the water-holding capacity of the thalli, and the nucleation of ice in the intercellular spaces of the thalli. Assessing the impact of atmospheric emissions during bauxite mining on the functional state of individual components of the lichen symbiosis requires further research.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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