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RESEARCH PAPERS

Airborne Heavy Metal Pollution and its Effects on Biomass of Ground Vegetation, Foliar Elemental Composition and Metabolic Profiling of Forest Plants in the Kola Peninsula (Russia)

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Abstract—In this work a quantitative estimation of biomass accumulation in the ground cover of pine forests of northern taiga in the background area of the Kola Peninsula (Russia) and in the territory of the buffer and impact zones of non-ferrous metallurgical plant, producing air emissions containing sulfur dioxide and polymetallic dust, was carried out. It was found that under the influence of airborne pollution in the first place the moss–lichen layer is destroyed with elimination of sensitive species of mosses and lichens; the structure of biomass changes and both the total stock of aboveground biomass and stocks of individual components decrease. The results of studying the elemental composition of dominant species of plants (*Vaccinium myrtillus* L., *V. vitis-idaea* L., *V. uliginosum* L., *Empetrum hermaphroditum* Hagerup), mosses (*Pleurozium schreberi* (Brid.) Mitt.) and lichens (*Cladonia stellaris* (Opiz) Pouza et Vĕzda) of northern taiga forests revealed an imbalance in their mineral nutrition. Using metabolomic analysis, the macroprofile of the component composition of the secondary metabolites in the leaves of 3 species of the *Vaccinium* genus was characterized, the specificity of the composition of metabolites in each studied species was revealed. Despite the reduction in atmospheric emissions by Severonickel Combine, the restoration of the ground cover in pine forests is inhibited (buffer zone) or it cannot even begin (impact zone) due to the high level of soil contamination with heavy metals. The mineral nutrition of higher plants, mosses and lichens is disturbed: as one approaches the source of pollution, the accumulation of heavy metals (Ni, Cu, Co, Fe) increases; their ratio changes; the content of micro- (Mn) and essential macroelements (Ca, Mg) decreases in the leaves of the dwarf shrubs and the moss. The changes in the metabolite profiles of the leaves of the genus *Vaccinium* are due to both the plant species and the level of airborne pollution.

Keywords: *Vaccinium* genus, *Empetrum hermaphroditum*, *Pleurozium schreberi*, *Cladonia stellaris*, heavy metals, airborne technogenic pollution, biomass, mineral composition, metabolic profiling, northern pine forests, Kola Peninsula

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INTRODUCTION

The increasing pressure of airborne pollution on terrestrial ecosystems leads to violations at different levels of the biota from the molecular to biogeocenotic. The long-term impact of pollutants on forest ecosystems is the reason for the transformation of their composition, structure, productivity, changes in the mineral composition of plants, as well as the accumulation of heavy metals in soils and plants [1–5]. Various physicochemical methods and species of plants, mosses and lichens, used as bioindicators, are employed for the detection of environmental contamination. Sensitivity of mosses and lichens to air quality and their ability to accumulate pollutants directly from polluted air and from contaminated substrate underlie brio- and lichenoindication of aerotechnogenic pollution [6–11]. It should be noted that in bioindication studies of environmental pollution, samples of plants, mosses, or lichens are usually not washed [11, 12]. Therefore, it is difficult to separate the contributions of external dusting and internal metal content in tissues to the total amount of absorbed metals. Using different methods of washing plant samples before analysis leads to conflicting results [12–16].

In boreal forests, the ground cover is an important component of biogeocenosis, regulating the water and thermal regime of the soil, and also serves as a source of food, medicinal, and fodder raw materials. Berry dwarf shrubs (bilberry *Vaccinium myrtillus* L., lingonberry *V. vitis-idaea* L., blueberry *V. uliginosum* L., crowberry *Empetrum hermaphroditum* Hagerup) are widely represented in plant communities of the boreal zone, where they are dominant and co-dominant of the herb–dwarf shrub layer. They play an important role in the diet of the population and serve as food for birds and animals. Leafy shoots of dwarf shrubs of these species have antiviral and antihypoxic activity; biologically active substances obtained from fruits are included in medicinal preparations [17]. In northern taiga forests, an equally important role in the structure of phytocenoses is played by the moss–lichen layer, which regulates the hydrothermal balance in the plant community. Mosses and lichens provide food for a number of forest animals, in particular, reindeer. Many forest plants and lichens are not only used as food by humans and animals, but also used in folk medicine, especially the northern peoples.

Historically, many plants have been used in medical practice and serve as raw material for the production of pharmaceuticals and dietary supplements [17, 18]. However, plants are a source of natural mineral complex, necessary to the human body. Information about the content of elements in plants and the relationship between them is extremely important to establish the therapeutic value of the drug and security guarantees for its application [19, 20].

Along with the traditional study of the elemental composition of plants, the determination of the content of secondary metabolic compounds, some of which possess high reactivity and biological activity [18], is of particular interest. For example, many members of the family *Ericaceae* accumulate significant amount of phenolic compounds, which is seen as one of the mechanisms of their stability in conditions of unfavorable factors, including airborne pollution [21, 22].

One of the most promising trends in the development of systems biology is the metabolic analysis of various biological objects. Analysis of metabolite networks can be considered as a research method for early phenotypic responses to various external factors [23].

The aim of this work was a comprehensive assessment of the impact of environmental contamination by heavy metals on the productivity of ground vegetation of pine forests of northern taiga, on the mineral composition of the dominant species of plants and lichens, as well as on the component composition of the secondary metabolites in *Vaccinium* leaves.

MATERIALS AND METHODS

Collection of material. On the Kola Peninsula (Russia), the main source of environmental pollution is the Severonickel Combine non-ferrous metal smelting plant, whose atmospheric emissions include sulfur dioxide (SO_2) and heavy metals (Ni, Cu, Co). In the mid-1980s, in the vicinity of the plant, there was a massive destruction of coniferous forests, and the area of the contaminated area continues to increase, despite the 5–8-fold reduction in atmospheric emissions since the beginning of the XXI century.

Investigations were carried out on the territory of Murmansk Region (Russia) in 2018–2019. Sample plots in lichen-green-moss pine forests are located in the background, buffer and impact zones, 80, 30 and 15 km away from the source of environmental pollution (the Severonickel Metallurgical Combine), respectively. The average age of *Pinus sylvestris* L. trees was 80 years, the average height was 8–11 m, and the average trunk diameter at a height of 1.3 m was 7.7–12 cm. The total projective cover of the herb–dwarf shrub layer averages 25, 15, 10% in the background, buffer and impact zones; of the moss–lichen layer—78, 47, and 10%, respectively. It should be emphasized that in the latter case, the cover of the layer is represented by crustaceous lichens and primary thalli of species of the genus *Cladonia* [24].

At each sample plot, one average sample of the organogenic horizon (forest litter) of the Al-Fe-humus podzol and dominant plant and lichen species was taken. The average forest litter sample was made up of 5 individual ones [25]. The leaves of *Vaccinium myrtillus* L., *V. vitis-idaea* L., *V. uliginosum* L., *Empetrum hermaphroditum* Hagerup were collected from 20–30 shrubs growing on the territory of the sample plot. Only the green parts were taken from the moss *Pleurozium schreberi* (Brid.) Mitt., and only the living part was sampled from the lichen *Cladonia stellaris* (Opiz) Pouza et Vězda.

Determination of metal content. In order to correctly compare the results of the analysis with the previously obtained data, the samples of plant material were not washed before chemical analysis. After dry ashing of plant material in a muffle furnace (SNOL, Lithuania) at 450°C for 8 hours, dissolving the ash in HCl (1 : 1, v/v), filtering, washing the filter with a weak solution of HCl and diluting with deionized water, the content of Ni, Cu, Co, Fe, Mn, Ca, Mg, K was determined by atomic absorption spectrometry (AAS nov800F, Germany) in three replicates [25].

The content of acid-soluble forms of Ni, Cu, Co was determined in 1.0 N HCl extract from samples of forest litter by the same method [25]. All data on the contents of heavy metals in plants and litter are based on air-dry matter.

To assess the level of accumulation of heavy metals in the objects under study, the concentration coefficient (Kc) was calculated as the ratio of the concentration of an element in plants or lichens under conditions of airborne pollution to its background content.

Metabolic analysis. Metabolite profiling was carried out using the technique of gas chromatography-mass spectrometry (GC-MS). Eighteen samples of methanol extracts of dried leaves of *Vaccinium myrtillus, V. vitis-idaea, V. uliginosum* from the background, buffer, and impact zones were analyzed. GC-MS analysis was performed on an Agilent 6850 instrument with an Agilent 5973 mass-selective detector (United States). Chromatographic separation was performed on an HP-5MS capillary column 30 m long, 0.25 mm in inner diameter, and a stationary phase film thickness of 0.25 μm in the linear temperature programming mode from 70 to 320°C at a rate of 5°C/min. The

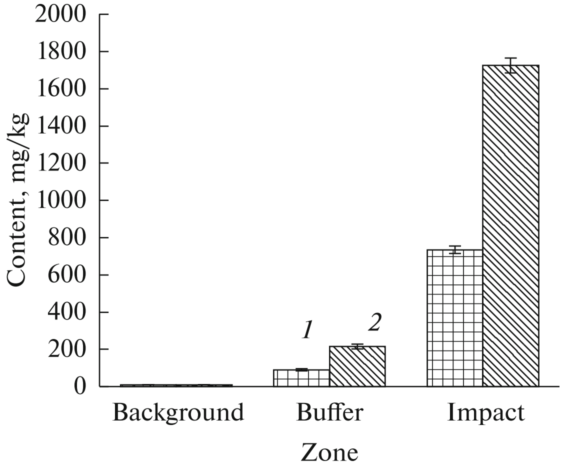

Fig. 1. Average Ni (*1*) and Cu (*2*) contents (mg/kg dry wt) in the forest litter from the background, buffer and impact zones.

carrier gas (helium) velocity through the column was constant (1.3 mL/min). Evaporator temperature 330°C, split flow at sample injection 1 : 20. Mass selective detector provided scan mass spectra obtained using electron impact ionization (70 ev) in the range of 50 to 800 a.e.m. with a speed of 2 scans/s [23].

The information received was processed using the AMDIS program. The components of the chromatogram were identified using standard spectral library NIST-2005, as well as a direct interpretation of the mass spectra obtained. The data on the analysis of metabolites of the leaves of three species of genus *Vaccinium* are presented in conventional units (conv. un.) [23].

Statistical analysis. The statistical data processing was carried out in the programs Statistica 64 and Excel 7.0 for Windows using factor analysis according to the method of principal components and one-way ANOVA using nonparametric Kruskal–Wallis test (H). The tables and figures show the mean with standard error.

RESULTS

To assess the level of habitat contamination by heavy metals, the content of acid-soluble forms of Ni and Cu in the forest litter was determined. In the background area, this content was on average 10.0 ± 0.5 mg/kg for each metal (Fig. 1). On the territory of the buffer zone, the content of heavy metals in the forest litter increased by 9 (Ni) and 22 (Cu) times, whereas within the impact zone this excess was 75 (Ni) and 175 (Cu) times compared to their values in the background zone. It should be emphasized that the content of Cu in the forest litter was more than 2 times higher than that of Ni. Even in the impact zone, the content of Co in the forest litter does not exceed 30 mg/kg, which is not a toxic dose for the seed plants, mosses and lichens.

Biomass Stock of Ground Cover Components

In the background zone the maximum values of the aboveground biomass stocks of all components (lichens, mosses, dwarf shrubs) and the total stock of ground cover were recorded (Table 1).

A characteristic feature of the accumulation of organic matter in the ground cover of northern taiga forests is a significant predominance of biomass reserves of the moss-lichen layer over the stock of aboveground biomass of the herb-dwarf shrub layer (Fig. 2).

As one approaches the source of pollution, a sharp decrease in the reserves of aboveground biomass of all components of the ground cover is observed along with an increase in the index of technogenic load (Table 1). On the territory of the buffer zone, the biomass stock of lichens, mosses and shrubs is reduced by 2.5, 11.5, and 2.3 times, and the total stock of aboveground biomass of the ground cover is almost 3 times less than the corresponding indicators in the background. However, in this zone, the predominance of the biomass stock of the moss–lichen layer over the stock of the aboveground biomass of the herb–dwarf shrub layer remains (Fig. 2).

On the territory of the impact zone almost complete destruction of moss–lichen layer is most clearly reflected in the stock biomass of mosses and lichens (Table 1). The stock of aboveground biomass of dwarf shrubs was not significantly different from its value in the buffer zone, but it is 2.2 times less than in relation to its value in the background area. The total stock of aboveground biomass of ground cover in the impact zone is 5.3 and 15.4 times lower than the corresponding values in the buffer zone and background (Table 1). Due to almost complete destruction of the moss–

Table 1. Stock (g/m^2) of aboveground biomass of components of the ground cover of the studied pine forests

Component	Zones			Kruskal-Wallis criterion (*H*)	Significance level (*p*)
	background	buffer	impact		
Lichens	390 ± 40	155 ± 23	0.3 ± 0.2	12.2	0.002
Mosses	115 ± 10	10 ± 3	1.5 ± 0.5	10.3	0.002
Dwarf shrubs	80 ± 7	35 ± 9	36 ± 9	8.5	0.01
Total aboveground biomass	585 ± 20	200 ± 12	38 ± 4	7.6	0.02

lichen layer, the structure of biomass reserves has fundamentally changed, the total stock of aboveground biomass of the ground cover almost entirely consists of the biomass of the herb–dwarf shrub layer (Fig. 2).

Elemental Composition of Plants and Lichens

First of all, let`s consider the accumulation of the main pollutants (Ni, Cu, Co) in the leaves of dwarf shrubs and dominant types of moss-lichen layer—green parts of the moss *Pleurozium schreberi* and live parts of the lichen *Cladonia stellaris*. In the background area, heavy metal concentrations varied in the range of 0.4–5.5 mg/kg and can be arranged in the descending row: Cu > Ni > Co (Table 2). At the concentrations observed in this zone, these heavy metals act as trace elements necessary for the life of plants and lichens.

As we approach the source of contamination, the content of major pollutants (Ni, Cu, Co) increases in all studied species along with the increase in the content of heavy metals in the forest litter (Table 2). In the buffer zone, the content of Ni varies within 28–64, Cu—4.7–28, Co—0.5–2.5 mg/kg, with the highest accumulation of heavy metals in the *Pleurozium schreberi* moss and the *Cladonia stellaris* lichen.

In the area of the buffer zone the content of Ni, Cu, Co in the green parts of *Pl. schreberi* and living parts of *Cl. stellaris* was 30, 9–14, and 2–6 times, respectively, higher than their background values (Table 3). Such a large excess of microelements far above the physiological needs of mosses and lichens, apparently, is a lethal dose for their existence, as at a higher level of pollution of habitats on the territory of the impact zone, they have completely disappeared from the phytocenosis.

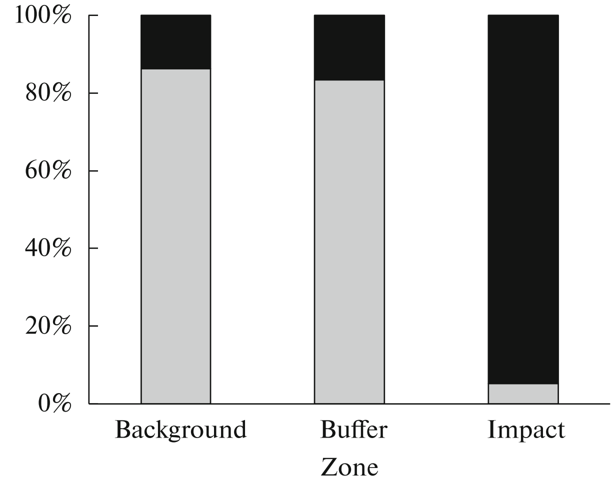

Fig. 2. The ratio of the shares of the aboveground biomass of the moss–lichen (gray color) and herb–dwarf shrub (black color) layers in the studied pine forests in the background, buffer and impact zones.

On the territory of the impact zone, in the leaves of dwarf shrubs, the content of Ni varies within 40–67, Cu—6–16.6, Co—1–1.6 mg/kg, which is 37–56, 2–4, and 2.5–4 times higher than their background values, respectively (Tables 2, 3).

Under the conditions of airborne contamination of the environment, among the studied species of dwarf shrubs, *E. hermaphroditum* accumulated significantly higher amount of heavy metals in the leaves compared

Table 2. Content (mg/kg dry wt) of metals in plants and lichens from the background, buffer and impact zones

Specie	Ni	Cu	Co	Fe	Mn	Ca	Mg	K
	Background							
Cl. stellaris	1.4 ± 0.1	1.6 ± 0.1	0.4 ± 0.1	63.2 ± 18	20.4 ± 0.5	415 ± 19	160 ± 12	614 ± 73
Pl. schreberi	2.1 ± 0.1	3.2 ± 0.3	0.4 ± 0.1	70.5 ± 1.4	418 ± 29	2145 ± 50	860 ± 29	2615 ± 162
V. myrtillus	0.9 ± 0.1	5.5 ± 1.0	0.4 ± 0.1	31.3 ± 3.5	1106 ± 58	7885 ± 1150	3124 ± 36	2143 ± 1072
V. vitis-idaea	0.8 ± 0.1	3.4 ± 0.1	0.4 ± 0.1	19.3 ± 2.4	1130 ± 21	6860 ± 106	1970 ± 237	3700 ± 585
V. uliginosum	0.8 ± 0.1	3.4 ± 0.2	0.4 ± 0.1	28.9 ± 1.5	1050 ± 18	6750 ± 185	1035 ± 60	4055 ± 182
E. hemaphroditum	1.8 ± 0.2	3.8 ± 0.2	0.4 ± 0.1	27.5 ± 1.2	387 ± 8	3405 ± 67	1150 ± 32	1460 ± 40
	Buffer zone							
Cl. stellaris	42.3 ± 4.8	22.5 ± 0.8	0.8 ± 0.1	122 ± 40	12.0 ± 0.1	320 ± 25	125 ± 10	715 ± 100
Pl. schreberi	64.3 ± 4.1	27.6 ± 2.0	2.5 ± 0.2	610 ± 90	190 ± 12	2815 ± 111	280 ± 27	3525 ± 410
V. myrtillus	35.1 ± 1.7	6.3 ± 0.3	0.5 ± 0.1	34.3 ± 3.9	860 ± 108	5555 ± 134	1930 ± 144	6420 ± 280
V. vitis-idaea	28.3 ± 1.1	4.7 ± 0.3	0.5 ± 0.1	35.8 ± 4.1	575 ± 41	4190 ± 90	1490 ± 91	4465 ± 380
V. uliginosum	32.1 ± 2.1	5.9 ± 0.2	0.5 ± 0.1	32.0 ± 4.3	465 ± 35	4260 ± 112	1740 ± 121	4120 ± 270
E. hermaphroditum	45.6 ± 1.7	9.0 ± 0.2	0.8 ± 0.2	47.0 ± 3.1	134 ± 40	4165 ± 490	1140 ± 225	3470 ± 535
	Impact zone							
V. myrtillus	49.2 ± 10	14.1 ± 2.4	1.4 ± 0.3	47.3 ± 5.0	220 ± 31	5015 ± 380	1160 ± 100	3660 ± 312
V. vitis-idaea	40.2 ± 6.6	6.1 ± 0.8	1.1 ± 0.2	41.7 ± 4.8	120 ± 20	3580 ± 560	850 ± 190	2760 ± 330
V. uliginosum	45.0 ± 7.1	7.7 ± 2.4	1.2 ± 0.2	46.5 ± 4.7	195 ± 19	4095 ± 410	1055 ± 132	4360 ± 340
E. hemaphroditum	67.0 ± 4.1	16.6 ± 4.3	1.6 ± 0.2	64.7 ± 5.0	53.4 ± 16	4015 ± 370	1285 ± 105	2760 ± 207

Table 3. Concentration coefficient

Specie	Ni	Cu	Co
	Buffer zone		
Cl. stellaris	30	14.1	2
Pl. schreberi	31	8.6	6
V. myrtillus	39	1.1	1.2
V. vitis-idaea	35	1.4	1.2
V. uliginosum	40	1.7	1.2
Empetrum hemaphroditum	25	2.4	2.0
	Impact zone		
V. myrtillus	55	2.6	3.5
V. vitis-idaea	50	1.8	2.7
V. uliginosum	56	2.3	3.0
Empetrum hemaphroditum	37	4.4	4.0

to the species of the genus *Vaccinium* (Table 2). Similar patterns of accumulation of heavy metals in the compared species were obtained in [26–28].

It is known that the composition of atmospheric emissions of the Severonickel Combine includes compounds of iron, so the content of Fe increases significantly in all studied species as approaching the contamination source (Table 2). However, whereas Fe content in the leaves of dwarf shrubs exceeds the background content of Fe by only 1.1–2.4 fold, then in the moss *Pl. schreberi* and the lichen *Cl. stellaris* it already exceeds the background by 2–9 times.

In the background zone average Mn content in the leaves of the genus *Vaccinium* is greater than 1000 mg/kg (Table 2), which is almost three times more than in the leaves of *E. hermaphroditum*. As we approach the source of contamination, Mn concentration in assimilative organs of all investigated species of dwarf shrubs dramatically decreases and in the impact zone varies within the range 53–220 mg/kg, which is 7–9 times lower than the corresponding background values. In the buffer zone, the Mn content in the living parts of the moss *Pl. schreberi* and lichen *Cl. stellaris* is also 2 times lower than its background value.

The content of macronutrients in the assimilative organs of dwarf shrubs decreases in the order Ca > K > Mg (Table 2). Under the influence of airborne pollution, the content of Ca and Mg in the leaves of the studied species of dwarf shrubs decreases (H = 6.7–9.8, P = 0.01–0.03), except the leaves of *E. hermaphroditum*.

The macronutrient composition of mosses and lichens is fundamentally different from the content of Ca, Mg, and K in the leaves of higher plants (Table 2). One-way analysis of variance of Ca and Mg content in the studied species showed that their content was significantly lower (H = 26.8–33.7, P = 0.0000) in the green parts of *Pl. schreberi* and in the living parts of *Cl. stellaris* compared to their content in the leaves of higher plants. No significant influence of airborne pollution on the content of macronutrients in lichens was revealed. On the contrary, in the buffer zone, the Ca content was higher (H = 15.7, P = 0.017), and the Mg content was lower (H = 16.9, P = 0.011) in the green parts of the moss than their background concentrations; the content of K did not differ significantly.

Metabolic Profiles of Leaves of the Species of the Genus Vaccinium

As mentioned above, along with the traditional study of the elemental chemical composition of plants it is of particular interest to determine the content of the secondary metabolism compounds, and changing metabolomic profile under the influence of environmental contamination. Preliminary results of the study of assimilative organs of the three species of the genus *Vaccinium* show ambiguous response of the secondary metabolism to the effects of airborne pollution.

The macroprofiles of the studied species had both common and specific features associated with the accumulation of certain groups of metabolites (Table 4). In the background zone, the content of components decreased in the order: for *V. myrtillus*—sugars (44%) > phenols (27%) > organic acids > alcohols > flavonoids > terpenoids; for *V. vitis-idaea*—phenols (40%) > sugars (25%) > organic acids > terpenoids > flavonoids > alcohols; for *V. uliginosum*—sugars (45%) > organic acids (27% of the total content of all components) > phenols > flavonoids > alcohols > terpenoids. Comparative analysis of the component composition of related species of dwarf shrubs showed that under normal growing conditions (background zone) the leaves of *V. myrtillus* are most enriched in sugars and alcohols, the leaves of *V. vitis-idaea* are characterized by an increased content of phenols and terpenoids, the leaves of *V. uliginosum* are characterized by the maximum accumulation of organic acids and flavonoids (Table 4).

The analysis of the obtained data shows pronounced changes in the metabolic profiles of the leaves of dwarf shrubs along with the increase in soil contamination with heavy metals (Table 4). However, general patterns in the changes of the composition of the leaves of studied species under the influence of environmental contamination are not clearly visible due to the multifactorial effects on the synthesis of secondary metabolites in plants (Figs. 3–6).

Among the sugars in the leaves of dwarf shrubs of the genus *Vaccinium,* glucose, sucrose, and fructose are most represented and account for 38–57, 31–49, and 11–15%, respectively, of the total sugar content. One-way analysis of variance revealed significant differences in the average content of the listed sugars in the studied species of dwarf shrubs, but the absence of a regular change in the sugar concentrations along the pollution gradient was demonstrated. For example, the glucose content in the leaves of *V. vitis-idaea* varied from 8.1 (in the background zone) to 48.2 conv. units (in the impact zone), while its content in the leaves of *V. myrtillus* was reduced from 19 (in the background) to 9.5 conv. units (in the impact zone) (Table 5).

Qualitative and quantitative composition of phenolic compounds in the leaves of the species differed significantly, both depending on the species, and the level of environmental contamination. As an example, Fig. 3 shows the data on the content of chlorogenic acid in the leaves of the studied species of the genus *Vaccinium*. The lowest values of the total content of phenolic compounds are observed in the leaves of *V. uliginosum*, and the highest – in the leaves of *V. vitis-idaea* (Table 4). A detailed analysis of this group of substances is out of the scope of this work, only note that the greatest variety of phenolic compounds was reported in the leaves of *V. vitis-idaea,* wherein arbutin content was the highest (12–30 conv. un.) of the entire investigated group of phenolic compounds. This substance was not found in the leaves of *V. uliginosum* and in the leaves of *V. myrtillus* its content varied within 0.01–0.11 conv. units.

The lowest total content of organic acids was found in the leaves of *V. vitis-idaea*, whereas a comparable content of organic acids was observed in the leaves of *V. myrtillus* and *V. uliginosum* (Table 4). Among the studied organic acids, the content of quinic acid was the highest. Its content varied within 49–87 (*V. myrtillus*), 45–65 (*V. vitis-idaea*) and 91–96% (*V. uliginosum*), respectively. An irregular change in the content of quinic acid in the leaves of the studied species along the pollution gradient was recorded (Fig. 4). Flavonoids in the leaves of all studied species of the genus *Vaccinium* were mainly represented by quercetin. A small amount of kaempferol (3–9% of the total flavonoid content) was found only in the leaves of *V. uliginosum*. One-way analysis of variance showed the significant differences in the content of quercetin in the

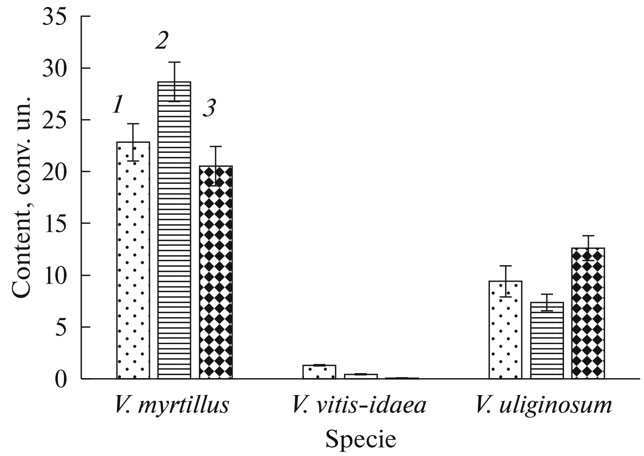

Fig. 3. The average content (conv. un.) of chlorogenic acid in the leaves of the studied species of the genus *Vaccinium* from the background (*1*), buffer (*2*) and impact (*3*) zones.

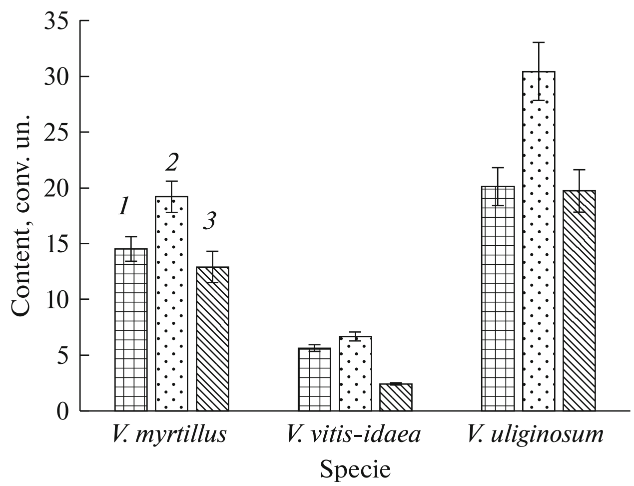

Fig. 4. The average content (conv. un.) of quinic acid in the leaves of the studied species of the genus *Vaccinium* from the background (*1*), buffer (*2*) and impact (*3*) zones.

Table 4. The total content (conv. un.) of components in the leaves of the studied species of *Vaccinium* genus in the background, buffer and impact zones

Component	Specie								
	Vaccinium myrtillus			*Vaccinium vitis-idaea*			*Vaccinium uliginosum*		
	zones								
	background	buffer	impact	background	buffer	impact	background	buffer	impact
Organic acids	16.58	21.99	26.11	8.63	10.40	5.35	22.04	31.64	20.94
Alcohols	3.96	1.59	5.52	1.35	0.50	1.16	2.82	2.68	2.08
Sugars	40.75	30.66	26.54	19.92	21.58	58.29	37.11	48.97	46.82
Phenolics	24.49	30.01	22.89	31.77	36.29	19.15	13.56	8.37	14.91
Flavonoids	0.64	0.55	1.15	1.68	1.86	0.43	4.21	0.26	1.47
Terpenoids	0.44	0.38	0.47	3.22	3.57	1.02	0.95	0.22	0.94

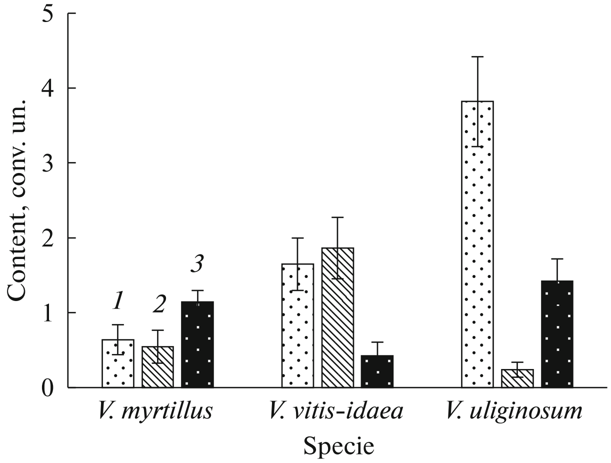

Fig. 5. The average content (conv. un.) of quercetin in the leaves of the studied species of the genus *Vaccinium* from the background (*1*), buffer (*2*) and impact (*3*) zones.

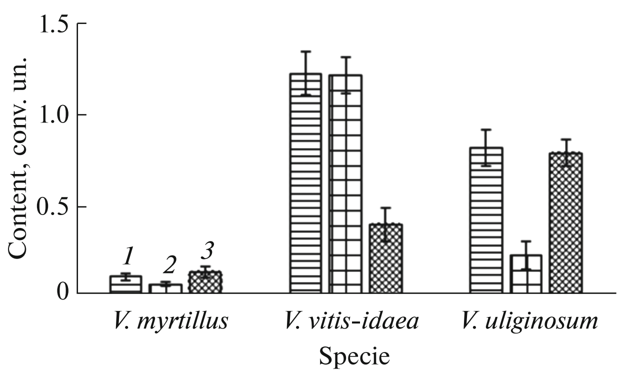

Fig. 6. The average content (conv. un.) of ursolic acid in the leaves of the studied species of the genus *Vaccinium* from the background (*1*), buffer (*2*) and impact (*3*) zones.

leaves of three species of the genus *Vaccinium*, but a regular change in the content of quercetin in the studied plants along the gradient of airborne pollution was not revealed (Fig. 5).

Among the studied species of dwarf shrubs, the leaves of *V. vitis-idaea* were the richest in terpenoids, whereas the lowest amount of terpenoids was found in the leaves of *V. myrtillus* (Table 4). A statistically significant ($H = 7.34$, $P = 0.026$) decrease was shown in the terpenoid content in assimilative organs of *V. vitis-idaea* along the gradient of environmental contamination. The average terpenoid content in the leaves of *V. vitis-idaea* plants from the impact zone was 3 times lower than the average content of these substances in the plants from the background zone. For the other two investigated species, a similar pattern was not revealed. Among the studied terpenoids, the content of ursolic acid was the highest. Its content varied within 16–28 (*V. myrtillus*), 34–39 (*V. vitis-idaea*) and 84–99% (*V. uliginosum*), respectively. An irregular change in the content of ursolic acid in the leaves of the studied species along the pollution gradient was recorded (Fig. 6).

DISCUSSION

The northern taiga forests are characterized by a pronounced heterogeneity of the cenotic environment and mosaicism of the ground cover, which leads to a high degree of variation in the stock of aboveground biomass of the ground cover within the phytocenosis. For example, in dwarf-lichen pine forests, the smallest stock of aboveground biomass of the ground cover is recorded in the near-trunk (758 g/m^2) and intercrown zones (730 g/m^2), and the maximum—in the undercrown spaces (1414–1482 g/m^2) of pine trees [29]. Our results (Table 1) are in good agreement with the data of other researchers, who note that on the Kola peninsula, the total reserves of organic matter of plants in the ground cover of coniferous forests are 440–870 g/m^2 [29]. The communities of pine forests considered by us are only at an intermediate stage of post-pyrogenic recovery, when the total projective cover and the height of the herb–dwarf shrub and moss–lichen layers have not yet reached the stabilization recorded when the fire is over 100 years old [3], which is reflected in the lower the values of the stocks of components and the total stock of aboveground biomass of the ground cover in the background forests.

The effect of airborne industrial pollution led not only to an increase in the level of contamination of the

Table 5. The content (conv. un.) of some sugars in the leaves of the studied species of *Vaccinium* genus in the background, buffer and impact zoznes

Sugar	Specie								
	Vaccinium myrtillus			*Vaccinium vitis-idaea*			*Vaccinium uliginosum*		
	zones								
	background	buffer	impact	background	buffer	impact	background	buffer	impact
Fructose	6.0	5.7	2.1	2.6	3.0	7.7	6.1	3.7	5.0
Glucose	19.0	16.4	9.5	8.1	11.5	48.2	18.4	10.0	17.5
Sucrose	15.6	8.3	14.6	8.0	6.7	2.4	12.3	34.3	24.1

forest litter with heavy metals (Fig. 1), but also to a change in the species composition and structure of the moss–lichen layer, up to the complete loss of the dominant moss cover *Pleurozium schreberi* on the territory of the buffer zone and almost complete destruction of this layer within the impact zone (Table 1). It is well known that under the conditions of airborne pollution of the environment, the most sensitive species of mosses, as well as ground and epiphytic lichens, are the first to drop out of the phytocenoses, which makes it possible to use them for lichen and bryo-indication [3, 6, 7, 24]. The herb–dwarf shrub layer remains the most stable component of the phytocenosis even under conditions of a high level of contamination of habitats with heavy metals [1–5, 24].

Thus, it can be stated that despite the reduced atmospheric emissions of the Severonickel Combine, the content of heavy metals in the forest litter remains high (buffer zone) and very high (impact zone). Therefore, the process of restoration of the ground cover is inhibited in the buffer zone, and it does not even begin in the impact zone. A possible explanation for these phenomena can be increased content of heavy metals (Ni, Cu) in the rainfall in recent years even in the background area, indicating the continuing expansion of the zone of environment pollution by atmospheric emissions of the Severonickel Combine [30].

In the background zone the variation of heavy metal concentrations in all studied species is within their regional background values [26, 31] and do not exceed their standard content, which is, respectively, Ni—0.1–5, Cu—5–30, Co—0.02–1 mg/kg [32]. The elemental composition of the studied species of higher plants, mosses, and lichens has both general patterns and distinctive features that are due to endo- and exogenous causes. The exogenous factors, first of all, should be attributed to the airborne pollution of the environment with heavy metals, which leads to an increase in the content of Ni, Cu, Co, Fe in the soil and in all studied species (Fig. 1, Tables 2, 3). An increase in the accumulation of heavy metals by plants and lichens as we approach the source of pollution was noted by us and other researchers [1–5, 9, 24, 26–28, 31]. The foliar transfer of metals can be neglected, or in contrast appears as the main pathway of pollution, particularly when ultra-fine particles interact with plant leaves [11]. According to our data and the results of other researchers, during the period of high aerotechnogenic load (1980–2000 years), up to 80% of heavy metals entered the plants from polluted air, possibly in the form of dust deposits on the surface of the leaves [2, 3, 14]. Against the background of a 5–8-fold reduction in atmospheric emissions, a 2–16-fold decrease in the content of heavy metals in the assimilative organs of plants was registered [27, 28]. Perhaps, at present, the share of foliar intake of heavy metals in the plant leaves is small. For example, a comparison of untreated and washed meadow plants growing in the impact zone of a copper smelter did not reveal statistically significant differences for all studied species in all zones of pollution [13].

The mineral nutrition strategy is one of the reasons for the differences in the elemental composition of plants and lichens. Higher plants obtain mineral elements from soil, basically, through the roots. The barrier function of the roots of dwarf shrubs preventing the transport of heavy metals from contaminated soil into the above-ground organs has been shown for a number of metals (Pb, Fe, Ni, Cu, Cr, Co) [2, 3, 33]. Consequently, according to the currently existing classification [34], all the studied species of dwarf shrubs are excluders, which are characterized by a limited supply of metals to the shoots, despite their high concentration in the environment and accumulation in the roots.

Mosses and lichens mainly receive mineral nutrition from the air, but they can also accumulate heavy metals from the contaminated substrate (tree bark—for epiphytic lichens, forest litter—for ground lichens) [3, 8, 10, 11]. In the field experiment conditions on a soil contaminated with polymetallic dust, the content of heavy metals in both green and brown parts of the moss *Pl. schreberi* was increased, and the Ni content was higher than that of Cu, which indicates a more rapid translocation of Ni from contaminated soil into the above-ground parts of the moss [10]. Under conditions of environmental contamination with simultaneous admission of heavy metals from contaminated air and from polluted soil, the main factor determining the rate of accumulation of heavy metals by mosses and lichens is the composition of atmospheric emissions. Thus, according to the modern classification [34], mosses and lichens are classic indicators in which metal content is correlated with their concentrations in the environment.

It is well known that some metals are microelements essential for the normal life of plants when supplied at appropriate concentrations, and not only the concentrations of elements are important, but also their ratio. Whereas in the background region the Cu content reliably exceeds the Ni concentration in the assimilative organs of all studied plants (Table 2) and the concentrations of Ni and Cu in the living parts of the lichen are equal, under the conditions of airborne pollution the Ni : Cu ratio is shifted towards the prevalence of the first metal, despite the fact that the content of acid-soluble forms of Cu in the forest litter is more than 2 times higher than that of Ni (Fig. 1). Furthermore, the concentration coefficients for Ni are many times greater than this value for Cu (Table 3). All this indicates a faster Ni translocation from contaminated soil into above-ground parts of the plants as compared to Cu. This conclusion is consistent with the data of other researchers [14, 27].

The features of the accumulation of micro- and macroelements in the studied species of higher plants, mosses and lichens can be attributed to the endogenous causes of differences in their elemental composition. Dwarf shrubs from the genus *Vaccinium* are known as Mn accumulators [22, 27, 35], and in the background zone average Mn content in the leaves of the genus *Vaccinium* is almost three times higher than in the leaves of *E. hermaphroditum*. A decrease in the level of Mn accumulation by plant organisms under conditions of airborne industrial pollution was noted by us and other researchers [2, 3, 27, 33, 36]. The studied species of dwarf shrubs are known as calciphils. Therefore, Ca is accumulated in the leaves of these species in a high amount, while in the lichens and mosses the content of macronutrients decreases in the order K > Ca > Mg (Table 2). Under the influence of airborne pollution, the content of Ca and Mg in the leaves of dwarf shrubs from the genus *Vaccinium* decreases. Similar patterns in the change in the content of essential elements under conditions of airborne pollution were noted by other researchers [26, 27]. The depletion of the assimilative organs of dwarf shrubs in macroelements is not always confirmed statistically, due to the high degree of variability of their content in the leaves. According to literature data [20, 26, 27, 33, 36, 37], the content of macroelements in the leaves of dwarf shrubs (on dry weight basis) varies within 0.48–1.22 (Ca), 0.09–0.24 (Mg), and 0.07–0.72% (K).

Thus, summarizing our own results and literature data, it can be stated that a high degree of variability in the elemental composition of plants and lichens is due to many factors, which sometimes complicates the identification of the effect of airborne pollution on the mineral nutrition of plants and lichens. It is possible to list only the main reasons for the high degree of variability of the elemental composition of the assimilative organs of plants and living parts of lichens: the strategy of mineral nutrition, the individual characteristics of the accumulation of elements by various species of plants and lichens, the dominant species of the tree layer and the type of forest and the associated changes in illumination, phytomass density, as well as the competitive relationship for nutrients between plants of different layers, the composition of the soil and parent rock, geographical location, year-to-year variability due to differences in temperature and precipitation in the growing season, the composition of atmospheric emissions and the level of heavy metal pollution of air and soil due to airborne pollution of the environment [19, 20, 27, 36, 37].

According to the preliminary results of the study of metabolite profiles, the qualitative and quantitative composition of secondary metabolites in the leaves of the investigated species of the genus *Vaccinium* was characterized by a high degree of variability, both depending on the species, and the level of environmental contamination with heavy metals. Therefore, it is not always possible to identify the influence of airborne contamination on the composition of the secondary metabolites, due to the multifactorial effects on the synthesis of secondary metabolites in plants. Thus, further research in this area is required.

All in all, summarizing the results obtained, the following conclusions can be drawn. Despite the reduction in atmospheric emissions by the Severonickel Combine, the restoration of the ground cover in pine forests is inhibited (buffer zone) or it cannot even begin (impact zone) due to the high level of soil contamination with heavy metals. The mineral nutrition of higher plants, mosses and lichens is disturbed: as one approaches the source of pollution, the accumulation of heavy metals (Ni, Cu, Co, Fe) increases; their ratio changes; the content of micro- (Mn) and macroelements (Ca, Mg) decreases in the leaves of the dwarf shrubs and the moss. The changes in the metabolite profiles of the leaves of the genus *Vaccinium* are due to both the plant species and the level of airborne pollution.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

Statement on the welfare of humans or animals. This article does not contain any studies involving animals performed by any of the authors.

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