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Article

Using moss *Ceratodon purpureus* (Hedw.) Brid for assessing the technogenic pollution (Ni, Zn, Mn, Al, Se, Cs, La, and Sm) of transformed ecotopes of Donbass

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Abstract. The ecotopic difference in the accumulation of Ni, Zn, Mn, Al, Se, Cs, La and Sm in bryophyte *Ceratodon purpureus* (Hedw.) Brid was studied during a long-term experiment in the anthropogenically disturbed environment of Donbass. The concentrations of elements in moss gametophytes were determined by neutron activation analysis. The moss samples were exposed from November 2018 through May 2019 at 24 test plots in the central Donbass with varying degrees of technogenic transformation of geosystems. When comparing ecologically stressed areas with intact or restored ecotopes, the difference in the accumulation of Ni was 6.9 times, Zn – 10.2, Mn – 6.3, Al – 5.3, Se – 9.6, Cs – 3.9, La – 5.9, and Sm – 5.4 times. There were structural and functional modifications in the leaf apparatus of bryophyte, suitable for using in further phytomonitoring studies for express diagnostics. According to the results of the factor analysis, two groups of pollutants were identified, differing in the source of origin: (1) Al, Ni, Mn, and Zn, (2) Cs, Se, La, and Sm. The obtained data are considered as part of the primary screening of biogeochemical characteristics in the Donbass in 2018–2019. Within the gradient of toxic load and local impact, the leading role of mining and metallurgical facilities in the pollution of natural ecosystems of Donbass was proved.

Keywords: neutron activation analysis, phytoindication, ecological phytomonitoring, metallurgy, factor analysis, correlation analysis

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Научная статья

***Ceratodon purpureus* (Hedw.) Brid в оценке техногенного загрязнения (Ni, Zn, Mn, Al, Se, Cs, La, Sm) трансформированных экотопов Донбасса**

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Аннотация. Проведен мониторинговый эксперимент установления экотопической разницы по накоплению Ni, Zn, Mn, Al, Se, Cs, La и Sm мохообразным *Ceratodon purpureus* (Hedw.) Brid в условиях антропогенно нарушенной среды Донбасса. Концентрации элементов в гаметофитах растения определены методом нейтронного активационного анализа. Экспозиция образцов проводилась с ноября 2018 г. по май 2019 г. на 24 учетных площадках Центрального Донбасса с разной степенью техногенной трансформации геосистем. Показана разница по накоплению Ni в 6.9, Zn – 10.2, Mn – 6.3, Al – 5.3, Se – 9.6, Cs – 3.9, La – 5.9 и Sm – 5.4 раза при оценке экологически напряженных территорий в сравнении с малонарушенными или восстановленными экотопами. Установлены структурно-функциональные модификации в листовом аппарате бриофита, пригодные для которых рекомендованы для фитомониторинговых исследований в экспресс-диагностике. По результатам факторного анализа были выделены две группы загрязняющих элементов, различающихся по источнику происхождения: в одну из них вошли Al, Ni, Mn, Zn, в другую – Cs, Se, La, Sm. Реализованный опыт рассматривается как элемент первичного скрининга биогеохимических характеристик в Донбассе периода 2018–2019 гг.

Ключевые слова: нейтронный активационный анализ, фитоиндикация, экологический фитомониторинг, металлургия, факторный анализ, корреляционный анализ

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Introduction

Expert monitoring studies in regions of intensive human activity are indispensable part of operating of environmental services and research laboratories (Bayouli et al., 2021; Hancock et al., 2020; Pashentsev et al., 2019; Trubina et al., 2014; Xu et al., 2021). Both comprehensive assessing of anthropogenic transformations and understanding the mechanisms of ecosystem stability require the participation of specialists from various scientific fields: geochemistry and landscape science (Glazovskaya, 2007; Ufimtseva, 2015; Wang et al., 2020; Yu et al., 2021), soil science (Kabata-Pendias, 2001), hydroecology (Neamtu et al., 2021; Zaghoul et al., 2020), bioindications (Opekunova, 2016; Yuan et al., 2020), structural botany (Bell, 1991), toxicology (Meena, 2020), urbanology (Peng et al., 2018; Y reprintsev et al., 2019), and climatology (Zhao et al., 2020). Quantification studies in the areas of active mining are also relevant (Bian et al., 2020; Massante, 2015; Wu et al., 2021; Zhang et al., 2020; Zhang et al., 2021).

The biochemochemical peculiarities of the territory of modern Donbass have been little studied so far. Technogenic processes in combination with the factor of hostilities (Gosudarstvennyi komitet, 2022) brought a geochemical contrast here, when the content of individual elements in indicator substrates varied significantly in the environment (Safonov et al., 2023).

Bryophytes are reliable indicators for monitoring environmental pollution (Frontasyeva et al., 2020; Hristozova, 2020) considering their elemental composition (Alemasova et al., 2018; Khiem et al., 2020; Quyet et al., 2021; Świsłowski et al., 2022; Vergel et al., 2020; Zinicovskaia et al., 2021) and functional characteristics (Kozlova et al., 2022). To date, the territory of Donbass has been studied fragmentarily in terms of the accumulation of elements by bryophytes (Alemasova et al., 2019; Sergeeva et al., 2021a, 2021b).

The study aims to determine the accumulation of Ni, Zn, Mn, Al, Se, Cs, La, and Sm in gametophytes of *Ceratodon purpureus* (Hedw.) Brid in order to assess the anthropogenic pollution in functionally different ecotopes in a geochemically contrasting region.

Materials and methods

Moss specimens *Ceratodon purpureus* (Hedw.) Brid were sampled in the buffer zone of the Republican Landscape Park “Donetsky Kryazh”. This species was set as a model one due to its wide ecological niche and the highest survival rate compared to other moss species, also dominant in the region, as obtained in previous studies (Safonov, 2013, 2022; Safonov and Morozova, 2021; Safonov et al., 2023). In November 2018, the samples of live moss were transplanted to the test plots (Fig. 1); all of them were in the same conditions (open to the precipitation). Transplantation of moss samples was carried out in accordance with the requirements and proven methods (Frontasyeva et al., 2020; Hristozova, 2020; Sergeeva et al., 2021a; Vergel et al., 2020; Zinicovskaia et al., 2021). In May 2019, a semi-annual growth of gametophytes was collected. The collecting was accompanied by a description of the vital state of the plant according to anatomical and morphological visual indicators (indicating teratological neoplasms, necrotic manifestations, structural and functional abnormalities in pigmentation).

In order to implement bryoecological observations, previously used test plots (stations) were set within the existing observational network in the Donetsk economic region (Safonov, 2016, 2019; Safonov and Germonova, 2019; Safonov and Glukhov, 2021a, 2021b). The test plots were grouped according to the functional principle of the operational purpose of ecotopes:

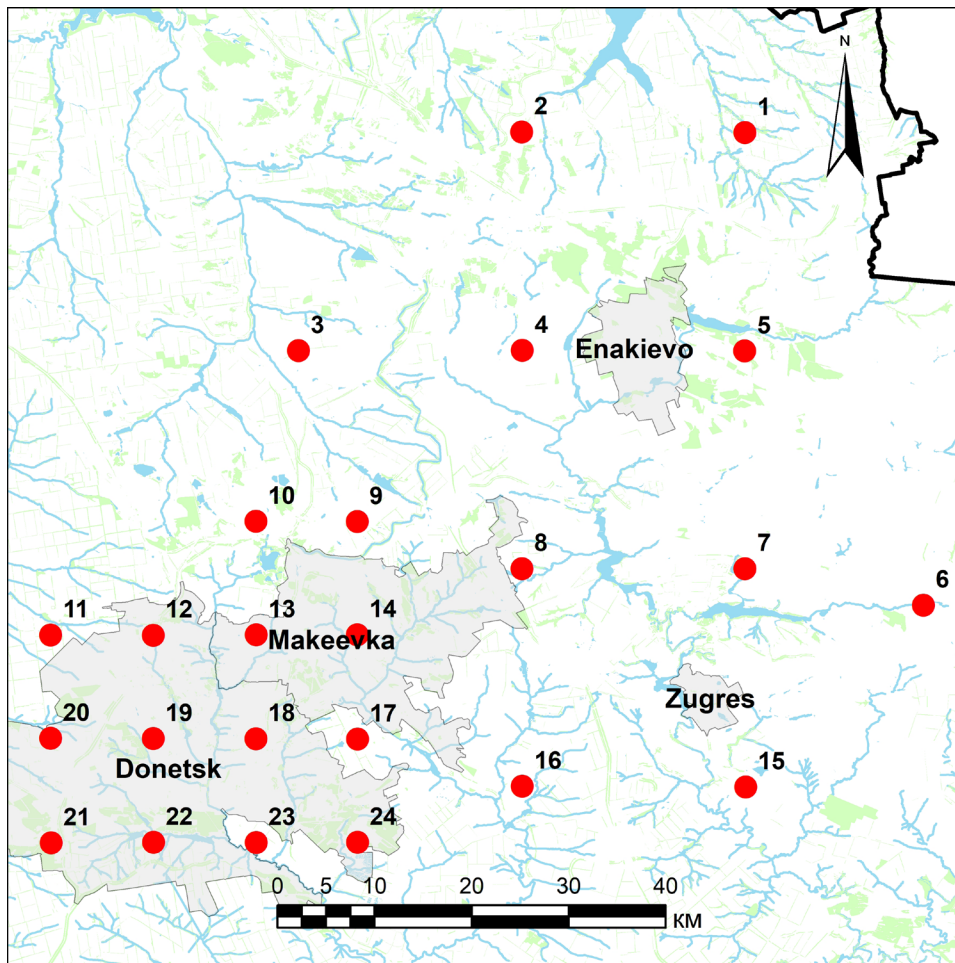


Fig. 1. Sampling site map of *Ceratodon purpureus* in the central Donbass.

1) Technogenic (industrial sites of enterprises, objects of landscape transformation of coal mining and processing, stationary sources of emissions of the heat supply system): 1 – Debaltsevo plant of metallurgical engineering, Uglegorsk thermal power plant; 4 – Korsun town, impact area Enakievsky metallurgical plant; 5 – Enakievo, residential and industrial conglomerate, 13 – Voroshilovskiy District, Donetsk city, impact of the Donetsk Metallurgical Plant; 15 – Zugres town, the impact of the Zuevskaya thermal power plant and the power engineering plant in the residential area; 17 – Donetsk city, area of the Zaparevnaya mine; 19 – Leninsky District, Donetsk city (mining and metallurgical enterprises); 20 – Kirovskiy District, Donetsk city (mining and processing enterprises).

2) Residential (areas of dense residential development, domestic communications): 11 – Peski settlement, Yasinovatskaya agglomeration; 12 – Spartak settlement, Yasinovatskaya agglomeration; 14 – Makeevka town (Tsentralnogorodskiy District); 16 – Ilovaisk town, including the railway interchange; 22 – Mospino town, the southern part of the Donetsk agglomeration; 24 – Proletarskiy District, Donetsk city.

3) Intact and (or) restored ecotopes (recreational zones within the city, buffer areas of industrial sites at a considerable distance from them): 2 – a system of ponds in Gorlovka city; 3 – Panteleymonovka town, Gorlovsko-Enakievo agglomeration; 6 – Shakhtersk town, Trez agglomeration; 7 – Zuevka urban-type settlement (Zuevka Landscape Park); 8 – Khartsyzsk town (western border of the city); 9 – Yasinovka town, Makeevskaya agglomeration (southern microdistricts); 10 – park areas of Yasinovatskaya agglomeration; 18 – Kuibyshevskiy District, Donetsk city (Krasny Pakhar microdistrict); 21 – Petrovskiy District, Donetsk city (recreation zone); 23 – Avdotyino village (a section of the natural steppe), Donetsk agglomeration.

The content of Ni, Zn, Mn, Al, Se, Cs, La, and Sm in moss samples was determined by neutron activation analysis (NAA) using the REGATA facility of the IBR-2 pulsed fast reactor in the Laboratory of Neutron Physics named after I.M. Frank, Joint Institute for Nuclear Research (Dubna, Russia).

The concentration of short-lived isotopes (Mn and Al) was determined by irradiating the samples with thermal neutrons with a flux power of $1.2 \times 10^{12} \text{ n} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ for 3 min. After the cooling time, the induced activity of the samples was measured for 15 min.

In order to determine the long-lived isotopes (Ni, Zn, Se, Cs, La, and Sm), the samples were irradiated in a cadmium channel with a resonant neutron flux of $1.1 \times 10^{11} \text{ n} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ for 3 days. The samples were then measured twice, after a cooling time of 4 and 20 days. The measurement time varied from 30 min to 1.5 h.

The quality control of analytical measurements was carried out using standard samples. The spectra of induced γ -activity were processed using the Genie 2000 system; then the element concentrations were calculated using the software package developed at the Laboratory of Neutron Physics, Joint Institute for Nuclear Research (Pavlov et al., 2016). Statistical analysis was performed using the Statistica 12 software package.

The technogenic concentration coefficient K_c was calculated to assess the technogenic load:

$$K_c = \frac{K_{\text{exp}}}{K_{\text{background}}},$$

where K_{exp} is the concentration of the element in the moss after exposure; $K_{\text{background}}$ is the concentration of the element in the moss of the background territory.

Since air pollution by several elements was determined at each site, the pollution load index (PLI) was also calculated:

$$\text{PLI} = \sqrt[n]{\prod_{i=1}^n K_c},$$

where K_c is coefficient of technogenic concentration; n is the number of elements.

According to PLI¹ values (Vodyanitsky, 2013; Shaikhutdinova, 2015), the level of pollution may be classified as: (1) moderate to unpolluted ($\text{PLI} < 2$), (2) moderately polluted ($2 \leq \text{PLI} < 4$), (3) heavily polluted ($4 \leq \text{PLI} < 6$), and (4) very heavily polluted ($\text{PLI} > 6$).

Results and discussion

Predominantly non-specific responses of general inhibition of *C. purpureus* have been observed after six months of exposure under unfavorable environmental conditions (Table 1). Since *C. purpureus* is a stress-resistant species with a wide tolerance zone to climatic factors and ecological regimes, for example, illumination, humidity, etc. (Safonov and Morozova, 2021), then we presumably associate the observed vegetative teratosis in organs and conformational tissues with specific geochemical anomalies characteristic for technogenic region.

The content of most elements in the exposed moss samples is higher compared to the control (Student's t-test, unpaired; $p < 0.05$); except contents of some elements at the test plots nos. 21 and 23; although these values fall into individual physiological norm under geochemical background conditions (Kabata-Pendias and Pendias, 2001; Neamtu et al., 2021). The highest aluminum content is found in the moss exhibited in the Voroshilovsky District of Donetsk city, the lowest, in the Avdotyino village, Donetsk agglomeration (Table 2). In the latter, the minimum contents of manganese, nickel, zinc, and selenium are also registered. The maximum content of manganese is observed in the Leninsky District of Donetsk city (mining and metallurgical enterprises), nickel, in the Voroshilovsky District of Donetsk city. The highest concentrations of selenium, lanthanum, and samarium are recorded in the Kirovsky District, their minimum values, in the moss samples exposed in the Petrovsky District of Donetsk city. The maximum

¹ MU 2.1.7.730-99. Hygienic assessment of soil quality in populated areas.

amount of zinc is accumulated in the mosses exhibited in the Zugres town, cesium, in vicinity of Debaltsevo metallurgical engineering plant. Therefore, when assessing territories under environmental stress in comparison with intact or restored ecotopes, the difference in element accumulation is on average 6.9 times for Ni, 10.2 for Zn, 6.3 for Mn, 5.3 for Al, 9.6 for Se, 3.9 for Cs, 5.9 for La, and 5.4 for Sm.

K_c exceeded 1.0 for all elements at the test plots nos. 1–10, 13, 16, 19, and 20; at the test plots nos. 11, 12, 14, 15, 17, and 24, they were above 1.0 for all elements except Se. At the test plots nos. 21 and 23, K_c for Al, Ni, Cs, and La did not exceed 1.0.

At all the test plots of moss exposure, air refer to “very heavily polluted” with certain elements according to PLI values for these elements. The maximum level of pollution is observed near the Debaltsevo metallurgical engineering plant, as well as nearby the enterprises of the mining and processing industries.

High correlation coefficients (r) between some pairs of elements ($r > 0.70$) probably indicates a single source of their entry into the atmosphere (Table 4).

High correlation coefficients between the pairs of elements Mn–Al and Ni–Al suggest that the source of atmospheric fallout of these elements is metallurgical production.

The element pairs Cs–Al, La–Al, Sm–Al, La–Cs, Sm–Cs, and La–Sm probably indicates that the weathering of the surface layer of the Earth’s crust may be the sources of these elements, as well as mining and processing of coal.

The PCA method has been applied to identify possible sources of environmental pollution by certain elements. In contrast to the correlation analysis, this method allows to establish pairwise correlations, which makes it possible to identify relationships between groups of elements that characterize a particular

Table 1. Phenotypic responses of *Ceratodon purpureus* to the changes in the growth conditions during the experiment.

Test plot no.	Structural and functional changes in the gametophyte
1	Chlorosis (> 40% of the plant surface), necrotic formations (> 20%); marginal necrosis of the apex, leaf hypo-genesis (often)
4, 5	Fasciation of the gametophyte axes, single cases of hypo-genesis, prosenchyma-transformation of cells along the central leaf vein, transformation of the adaxial leaf surface
11, 12,	Chlorosis (> 20% of the plant surface), necrotic formations (> 30%), twisting of the apex
13, 19, 20	Dystopia of gametophyte elements in the general growing trend, chlorosis (up to 50% of the entire surface), necrotic formations (> 20%), transformation of the sculpture of the adaxial leaf surface, single bends of the central vein of the leaf
15	Oligomerization of leaflets during destruction by venation, chlorosis (up to 20%), necrosis (up to 20% of the leaf surface)
14, 16	Chlorophyll-free leaves, atypical parenchymal cell size ratio heterogeneity, chlorosis (20–25% of surface)
17, 22, 24	Atypical sporophyte proliferation, hypo-generation of leaf apparatus (> 30%), prosenchymatous cells along midrib
6, 9, 10, 18	Local chlorosis at the leaf base, twisting of the leaf side parts
2, 3, 7, 8, 21, 23	No deviations from the norm and any structural changes observed

Table 2. Content of chemical elements (C, µg/kg) and measurement error (%) in the control and exposed samples of moss *Ceratodon purpureus* in Donbass ecotopes.

Test plot no.	Elements															
	Al		Mn		Ni		Zn		Se		Cs		La		Sm	
	C	%	C	%	C	%	C	%	C	%	C	%	C	%	C	%
Control	9560	6	202	9	19	10	98	6	0.77	11	1.29	4	7	4	1	10
1	32100	7	1040	7	46	9	564	5	2	15	4.31	5	29.1	9	4.5	10
2	26100	7	390	7	37	9	255	5	1.78	12	3.76	5	17.9	9	3.1	10
3	18700	7	430	7	34	9	170	5	0.84	14	2.27	5	13.6	9	2.4	10
4	29100	7	1030	7	41	9	553	5	1.3	14	3.05	5	14.8	9	2.48	10
5	27200	7	1350	7	36	9	499	5	1.31	17	2.72	5	14.2	9	2.29	10
6	21400	7	660	7	32.5	9	469	5	1.25	12	1.98	5	14.9	9	2.79	10
7	22000	7	1000	7	43	9	159	6	0.78	18	2.15	5	12.8	9	2.62	9
8	23800	7	910	7	39	10	547	5	1.24	16	2.51	5	14.1	9	2.74	10
9	18900	7	540	7	29.8	9	455	5	1.02	14	2.59	5	12.4	9	2.18	10
10	16500	7	450	7	27.4	9	380	8	0.93	10	1.98	5	10	9	1.94	10
11	36700	7	1030	7	40	10	450	8	0.68	17	3	5	24.9	9	4.2	9
12	32000	7	740	7	57	9	253	8	0.73	13	3.82	5	22.4	9	4	10
13	38400	7	1290	7	90	9	670	8	2.26	9	3.54	5	19.5	9	3.6	10
14	24400	7	600	7	27.8	9	126	8	0.68	12	2.55	5	14	9	2.61	9
15	19400	7	840	7	27	11	1030	8	0.68	13	2.3	5	13.3	9	2.46	9
16	27800	7	620	7	38	10	530	8	0.82	12	2.92	5	16.4	9	3.04	9
17	18900	7	740	7	48	9	640	8	0.79	12	2.26	5	13.6	9	2.49	9
18	17200	7	580	7	16.8	9	215	6	0.64	17	1.51	5	9.4	9	1.66	10
19	30800	7	1460	7	43	8	392	6	1.05	17	2.53	5	22.2	9	3.6	10
20	34000	7	820	7	43	9	397	6	5	11	3.65	5	30.4	9	5.1	10
21	7900	7	314	7	15.7	9	363	6	1.17	12	1.1	5	5.1	9	0.95	10
22	22800	7	490	7	45	8	277	6	1.95	12	2.36	5	14.4	9	2.6	10
23	7300	7	204	7	13.8	13	101	6	0.56	16	1.17	5	6.3	9	1.29	10
24	14300	7	440	7	26.2	9	870	6	0.52	19	1.55	5	9.4	9	1.76	10

Table 3. Technogenic concentration coefficients (K_c) and pollution load index (PLI) for analyzed samples.

Test plot no.	K_c								PLI
	Al	Mn	Ni	Zn	Se	Cs	La	Sm	
1	3.4	5.1	2.4	5.8	2.6	3.3	4.2	4.5	23.3
2	2.7	1.9	1.9	2.6	2.3	2.9	2.6	3.1	12.1
3	2.0	2.1	1.8	1.7	1.1	1.8	1.9	2.4	6.8
4	3.0	5.1	2.2	5.6	1.7	2.4	2.1	2.5	16.6
5	2.8	6.7	1.9	5.1	1.7	2.1	2.0	2.3	16.6
6	2.2	3.3	1.7	4.8	1.6	1.5	2.1	2.8	12.1
7	2.3	5.0	2.3	1.6	1.0	1.7	1.8	2.6	10.3
8	2.5	4.5	2.1	5.6	1.6	1.9	2.0	2.7	14.9
9	2.0	2.7	1.6	4.6	1.3	2.0	1.8	2.2	10.1
10	1.7	2.2	1.4	3.9	1.2	1.5	1.4	1.9	7.4
11	3.8	5.1	2.1	4.6	0.9	2.3	3.6	4.2	20.6
12	3.3	3.7	3.0	2.6	0.9	3.0	3.2	4.0	15.7
13	4.0	6.4	4.7	6.8	2.9	2.7	2.8	3.6	26.0
14	2.6	3.0	1.5	1.3	0.9	2.0	2.0	2.6	9.7
15	2.0	4.2	1.4	10.5	0.9	1.8	1.9	2.5	19.1
16	2.9	3.1	2.0	5.4	1.1	2.3	2.3	3.0	14.1
17	2.0	3.7	2.5	6.5	1.0	1.8	1.9	2.5	13.9
18	1.8	2.9	0.9	2.2	0.8	1.2	1.3	1.7	7.8
19	3.2	7.2	2.3	4.0	1.4	2.0	3.2	3.6	18.8
20	3.6	4.1	2.3	4.1	6.5	2.8	4.3	5.1	24.7
21	0.8	1.6	0.8	3.7	1.5	0.9	0.7	1.0	9.0
22	2.4	2.4	2.4	2.8	2.5	1.8	2.1	2.6	11.0
23	0.8	1.0	0.7	1.0	0.7	0.9	0.9	1.3	7.4
24	1.5	2.2	1.4	8.9	0.7	1.2	1.3	1.8	10.9

Table 4. Pairwise correlation between pollutants, correlation analysis. * – $p = 0.05$, ** – $p = 0.01$.

	Al	Mn	Ni	Zn	Se	Cs	La	Sm
Al	1							
Mn	0.73**	1						
Ni	0.77**	0.6**	1					
Zn	0.13	0.32	0.2	1				
Se	0.45*	0.19	0.37	0.02	1			
Cs	0.86**	0.48*	0.68**	0.1	0.51*	1		
La	0.87**	0.55**	0.58**	0.09	0.6**	0.86**	1	
Sm	0.89**	0.54**	0.64**	0.08	0.59**	0.86**	0.99**	1

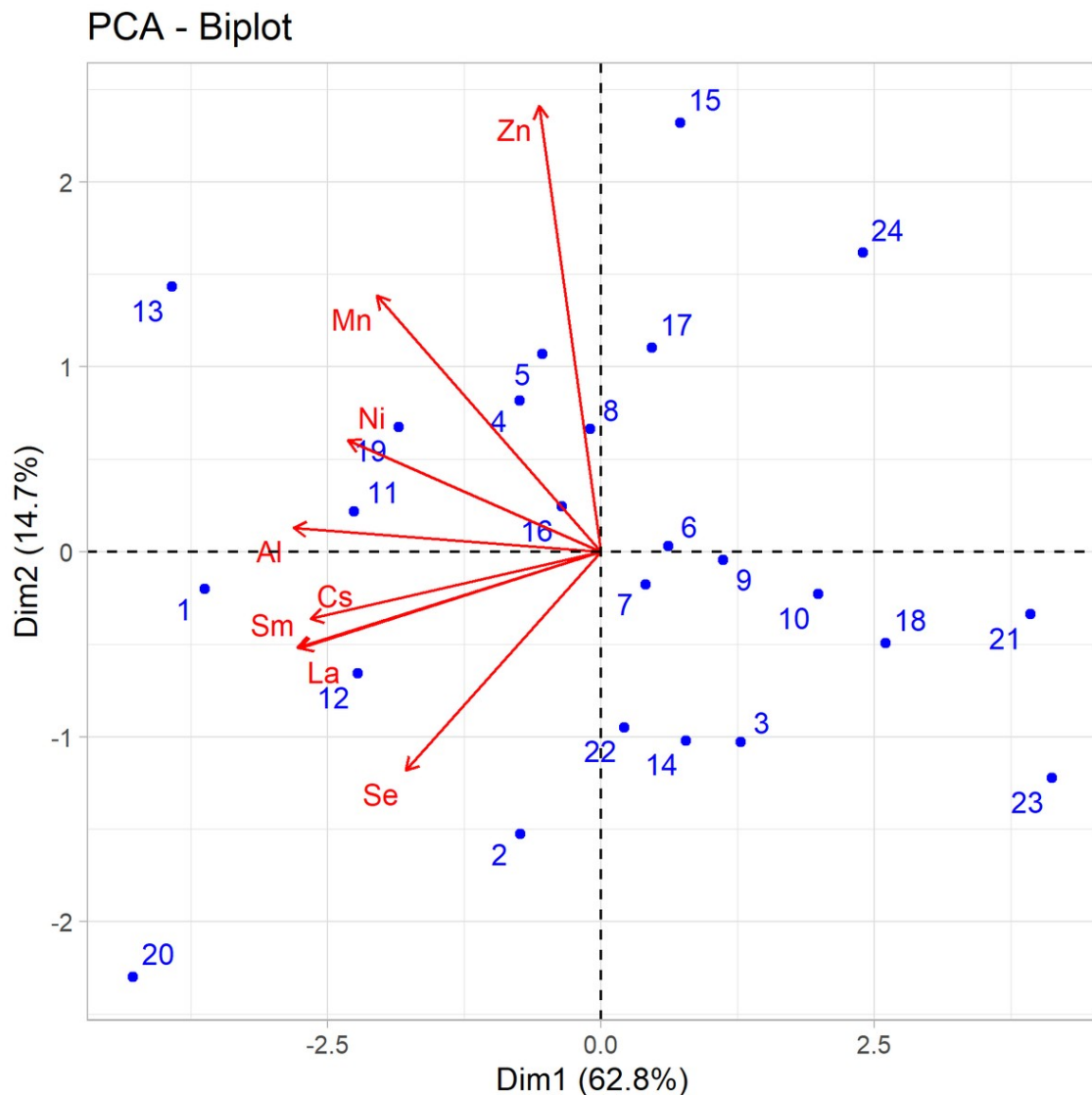


Fig. 2. Ordination of the elements determined in the samples of moss *Ceratodon purpureus*; the plane of the first two principal components is presented.

source of pollution. According to the PCA, two groups of elements have been identified (Fig. 2). The first of them includes Al, Ni, Mn, and Zn, presumably originated from the metallurgical enterprises. The second group includes Cs, Se, La, Sm; coal mining (Gamov et al., 2016; Lemly, 2008), as well as road dust, may be considered sources of these elements.

Considering that no full-scale studies on air pollution have been conducted within the existing phytomonitoring network in the territory of Donbass in 2018–2019 (Gosudarstvennyi komitet, 2022), the implemented experiment is the only source of screening information on the state of local ecosystems in this steppe part of Eastern Europe.

Conclusions

Considering a significant difference in the accumulation of Ni, Zn, Mn, Al, Se, Cs, La, and Sm by moss *Ceratodon purpureus* at the test plots of the industrially developed areas of Donbass for 6 months (from November 2018 to May 2019), we argue on the specificity in accumulation of certain elements by mosses at different sites. This pattern is primarily due to the full-scale economic transformation of the territory of Donbass.

As the technogenic pollution increases, the structural and functional parameters of the plant (moss) organism are violated. In *Ceratodon purpureus*, this is manifested by numerous chlorosis and necrosis of the leaf blade, as well as in pathological transformations of the structure of tissues up to specific teratosis at the anatomical level of development. The identified anomalies may serve as a phytoindicative characteristic when conducting an express analysis of the pollution level in situ.

According to the values of the pollution load index (PLI), the contamination by the studied elements may be considered as very strong (PLI > 6).

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