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Metal and metalloid contents in lichens from specially protected conservation areas

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The aim of this work is to evaluate the heavy metal and metalloids contents in indicator lichens inside two nature reserves situated within the Tver region that has a complex infrastructure and large number of industrial zones. Inductively-coupled plasma atomic emission spectral analysis is used to test for the gross and average ratio of 17 metals and metalloids (Al, As, Cd, Co, Cr, Cu, Ge, Fe, Mn, Mo, Ni, Pb, Sb, Sn, Ti, V, Zn) in *Hypogymnia physodes* lichen samples. The average content of the most elements identified in samples from Zavidovo National Park (ZNP) is higher than in the samples from Central Forest State Nature Biosphere Reserve (CFSNBR). There are such metals as titanium, copper, arsenic, cobalt, molybdenum, and tin among them. Differences are the result of different levels and regimens of air moisture saturation, localization of working production plants, degree of anthropogenic transformation of the territory. Spatial distribution of areas with much higher metal concentrations on reserve territory is resulted apparently from variation in air humidity to large extent. In ZNP addiction between qualitative and quantitative metal impact is defined more clearly by level and character of artificial territory transformation. Moreover, in ZNP wide marshy and forest areas in valley on the Lob' river were identified, which have conservation importance as they are characterized by lack or very low concentrations of many metals. So it would be useful to take advantage while zoning and correction of regimes.

Keywords: biomonitoring, heavy metals, epiphytic lichens, baseline monitoring, pollution, conservation area.

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Содержание металлов и металлоидов в лишайниках на особо охраняемых природных территориях федерального значения

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Цель данной работы – оценка содержания металлов и металлоидов в индикаторных лишайниках в пределах двух природных резерватов, расположенных на территории Тверской области, которая имеет сложную инфраструктуру и большое число промышленных зон. С помощью атомно-эмиссионного спектрального анализа с индуктивно связанной плазмой (АЭС-ИСП-анализ) в образцах *Hypogymnia physodes*, собранных в Центрально-Лесном государственном природном биосферном заповеднике (ЦЛГПБЗ) и национальном парке (НП) «Завидово» было определено валовое и среднее содержание 17 металлов и металлоидов (Al, As, Cd, Co, Cr, Cu, Ge, Fe, Mn, Mo, Ni, Pb, Sb, Sn, Ti, V, Zn). Среднее содержание большинства выявленных элементов в пробах из НП «Завидово» выше, чем в пробах из ЦЛГПБЗ. Среди них такие металлы, как титан, медь, мышьяк, кобальт, молибден и олово. Разница в значениях концентраций обусловлена прежде всего различиями в ландшафтной структуре и уровне антропогенного воздействия. Пространственное распределение зон с более высокими концентрациями металлов на территории заповедника обусловлено, по-видимому, в большей степени различиями в режимах влагообеспеченности воздуха. В НП «Завидово» более чётко выявляется зависимость качественного и количественного содержания металлов от уровня и характера антропогенной трансформации территории. Кроме того, в НП «Завидово» выявлены обширные болотные и лесные массивы в долине р. Лоби, которые имеют буферное и природоохранное значение, поскольку характеризующиеся отсутствием или очень низкими концентрациями многих металлов. Целесообразно их использовать при зонировании и корректировке режимов.

В целом, показатели содержания металлов не превышают нормативные и выявленные ранее в регионе значения, что позволило установить интервалы фонового содержания металлов и металлоидов в талломах лишайников для Тверской области и приграничных территорий.

Ключевые слова: биомониторинг, тяжёлые металлы, эпифитные лишайники, фоновый мониторинг, загрязнение, особо охраняемые природные территории.

Metals and metalloids are widespread pollutants [1]. However, not for all elements of this group the normative values of the content in living objects were revealed. It is relevant to assess the content of metals and metalloids in epiphytic lichens, which are good indicators of the state of the atmosphere [1–4]. To determine the background values, mostly interesting are the territories of protected areas at the federal level, in which some toxicants may appear because of fires or cross-border transport. Tver region is a convenient model region. It has a complex economic infrastructure, but compared with other regions of Central Russia, forests here are better preserved.

The goal of the present work is to evaluate the metal content in indicator lichens from the Central Forest State Nature Biosphere Reserve (CFSNBR) and Zavidovo National Park (ZNP) using inductively-coupled plasma atomic emission spectral (ICP-AES) analysis. The aims of the study included: 1) defining collection sites (CS) in the conservation areas (CAs); 2) evaluating metal concentration using the ICP-AES method; 3) referencing data obtained to cartography and geo-information systems; 4) identifying baseline ranges for metals in the Tver region.

Materials and methods

Research was conducted in years 2015–2016. The lichen *Hypogymnia physodes* (L.) Nyl., which is broadly distributed and moderately tolerant to pollution, was used as the specimen. Many researchers have noted that this lichen species is an active metal accumulator. Specimens were collected in CFSNBR and ZNP (see Table 1). Both territories are quite large [1]. Old-growth forest communities with characteristic structures, compositions, and a complex of rare and vulnerable biodiversity components were preserved in the CFSNBR [1]. The reserve is situated in the western part of the Tver region and occupies three districts. ZNP is situated in the Tver and Moscow regions, within the Upper Volga lowlands. CFSNBR is very far from large sources of pollution. Territories that are adjacent to ZNP contain large industrial plants.

There were 17 collection sites (CS) on the territories of both the investigated conservation areas (CA): 10 in CFSNBR and 7 in ZNP (see Table 1). Ten lichen specimens were collected from each CS for a total of 170 specimens.

The collected lichen specimens were analyzed by ICP-AES in the laboratory. Metal contents were determined using an iCAP 6300 Duo spectrometer (Thermo Scientific, USA). Results were processed statistically according to statistics methods. The resulting metal concentrations were compared with various regulatory values, because Maximum Allowed Concentrations (MAC) values for toxic elements in lichens are not available. The world-average baseline element contents for *H. physodes* are given only for a few metals. Their absolute values typically vary widely [5–6]. Results for lichens are sometimes compared with the Approximate Allowed Concentrations (AAC) and MAC for metals in the soil [1, 7, 8]. We compared our results with the existing MAC and AAC values in the soil. The elemental concentrations found for several elements were compared with the gross concentrations in lichens for ecologically clean areas if the AAC (MAC) were unavailable [9].

Results and discussion

The ICP-AES detected 17 metals in the *H. physodes* specimens from the investigated CA – Al, As, Cd, Co, Cr, Cu, Ge, Fe, Mn, Mo, Ni, Pb, Sb, Sn, Ti, V, Zn. 14 metals were shared by both of the investigated territories. Specimens from CFSNBR (CS 1–10) contained 16 metals (Cr being the exception), and ZNP specimens (CS 11–17) contained only 15, Ge and Sn being absent (Table 2). The concentrations of the metals detected in lichens varied over different ranges. The metal contents were grouped depending on their absolute contents in the specimens. The groups identified were the following: elements with increased concentrations (Al, Fe, Mn, Zn, V); moderate concentrations (Cu, Ni, Pb, Ti); low concentrations (As, Co, Cd, Sn, Mo); and very low concentration (Ge, Sb, Cr).

Even in high concentrations metals from the *first group* (Mn, Fe, Al, V, Zn) are not necessarily toxic for lichens. Mn, Fe and Al were found in specimens from all CS (1–17), V and Zn only in specimens from CS 1–13 (Table 2, 3). The analysis of metal concentrations from the first group revealed that the average concentration of most elements (Fe, Mn and V), except for Zn, were below the AAC (Fig. 1, Table 2). Average concentrations of Fe and Mn also do not exceed world baseline values for *H. physodes*. The average concentration of Zn in specimens from the investigated CA (CS 1–17) is equal to the AAC, which is 110 mg/kg (Table 2). There are differences in the average concentrations of Zn in the samples: CFSNBR has 105.3 mg/kg, ZNP has 125.7 mg/kg.

Table 1

Characteristics of *Hypogymnia physodes* collection sites in CAs studied

No.	CS location	Plant type	Additional characteristics
Central Forest State Nature Biosphere Reserve			
1	Quad. 38/56 (Severnoe)* T**	Old-growth black alder	Next to Katin Mokh swamp
2	Quad. 38/39/56/57(Severnoe)T		
3	Quad. 26/27 (Severnoe) T	Old-growth black alder, spruce	Next to Tyud'ma River
4		Old-growth aspen and spruce	Next to Tyud'ma River, within the 1999 fire area [1]
5			
6	Quad. 28 (Severnoe) T	Spruce	
7	Quad. 28/29 (Severnoe) T	Spruce with maple	within the 1999 fire area
8	Quad. 28 (Severnoe) T		
9	Quad. 29 (Severnoe) T	Old-growth spruce	Next to Tyud'ma River, within the 1999 fire area
10	Quad. 30 (Severnoe) T	Old-growth spruce	
Zavidovo National Park			
11	Quad. 18 (Sokol'skoe) T	Spruce with birch, signs of damage by eight-dentated bark beetle	Next to Kozlovo township, highway Novozavidoskiy-Kozlovo-Kuryanovo-Turginovo and healing cut-over area
12	Quad. 200 (Sokol'skoe) T	Pines and birch	Next to the Dmitrovo and Bortnitsy villages and healing cut-over area
13	Quad. 181/182/199 (Sokol'skoe) T	Spruce with birch	
14	Quad. 122 (Aleksandrovskoe) M	Damp, at times swamped spruce forest with alder and birch trees	Near the Sheverikha village, there are large woodlands and swamps
15	Quad. 130 (Turginovskoe) T	Spruce with aspen and birch	Surrounded by large woodlands and swamps, no residential areas in the vicinity
16	Quad. 8/22 (Osheykinskoe) M	Spruce with birch	
17	Quad. 21/22 (Osheykinskoe) M		

*Note: * – name of the forest district; ** T – Tver region; M – Moscow region.*

The average concentration of Al in samples from the CA was 398.5 mg/kg: CFSNBR – 394.4 mg/kg, ZNP – 404.27 mg/kg. The AAC (or MAC) values for Al have not been defined. Also, information on the world-average baseline values for this lichen is lacking. We found in the literature one mention of Al content in fruticose lichens from Karelia as 120–850 mg/kg with an average of 243 mg/kg [9]. This data indicated that the average Al content in lichens from CFSNBR and ZNP can be considered as baseline.

Gross concentrations of the analyzed elements (Al, Fe, Mn, Zn, V) in the lichen samples from separate CS varied widely, especially in lichen samples from CFSNBR. For example, the gross concentration of Fe (from 62.4 to 1921.5 mg/kg) and Mn (from 136.8 to 1531.5 mg/kg) varies significantly. Furthermore, gross concentrations of Zn, Fe and Mn from individual CS of investigated CA exceed corresponding AAC values (see Table 2). Gross concentrations of Zn most often exceeded AAC values – CS 2, 4, 8, 9 (CFSNBR) and CS 11, 13 (ZNP). Gross concentrations of Fe and Mn in the samples exceeded the AAC in isolated instances (for Fe – CS 7, for Mn – CS 2).

A definite correlation between the elemental contents and the area of forest fires in the 1990s [1] in CFSNBR or the vicinity of residential districts in ZNP could not be found by comparing data for the gross elemental concentrations in lichens from the studied CS. For example, in CFSNBR the maximum gross concentration of V was found for CS 2, which was far removed from the area of forest fires, whereas the minimum gross concentrations of V were observed at CS 6 and 7, which lay in the area completely burned by the fire.

The contents of the *second group* of metals (Cu, Ni, Pb, Ti) in *H. physodes* from the CA were significantly lower than those of the first group of metals (Fig. 1, Table 2). The majority of these elements are not seen in all samples. For example, Cu was found in samples from all CS, whereas the rest of the metals (Pb, Ti, Ni) were found only in samples from CS 1–13 (in CFSNBR – in CS 1–10, in ZNP – in CS 11–13).

Table 2

Comparison of gross and average element concentrations in CA (CFSNBR – CS 1–10; ZNP – 11–17) and their normative values

Element	CFSNBR			ZNP			CA			Normative values				
	gross conc., mg/kg		average conc., mg/kg	gross conc., mg/kg		average conc., mg/kg	gross conc., mg/kg		average conc., mg/kg	MAC, mg/kg, soil [7]	AAC*, mg/kg soil [8]	world-average baseline values [6]	AAC, mg/kg soil [2]	average conc. at baseline air conc., mg/kg [10]
	min	max		min	max		min	max						
Mn	136.8	1531.5	594.4	309.0	872.2	523.4	136.8	1531.5	565.2	1500	1500	10–130	1500	240
Fe	62.4	1921.5	494.7	510.4	928.4	652.1	62.4	1921.5	559.5	–	1000	50–1600	7500	1000
Al	160.5	612.0	394.4	306.7	634.8	404.3	160.5	634.8	398.5	–	–	–	–	–
Zn	72.0	166.0	105.3	93.2	150.9	125.7	72.0	166.0	110.0	100	110	20–500	102	102
V	11.6	64.8	45.7	100.4	112.4	79.1	11.6	112.4	59.5	150	–	–	150	5.5
Ti	7.3	27.3	16.1	2.1	30.2	25.8	2.1	30.2	18.3	–	–	–	–	20
Pb	7.8	21.9	13.7	9.6	18.0	14.9	7.8	21.9	14.0	32	65	5–100	32	14
Cu	6.2	12.3	7.5	6.4	40.4	16.4	6.2	40.4	11.2	3	66	1–50	55	8.5
Ni	0.6	3.8	1.7	1.8	2.2	2.1	0.6	3.8	1.8	4	40	0–5	35	4
As	1.2	4.3	2.6	5.3	7.1	6.2	1.2	7.1	3.2	2	5	–	2	–
Co	0.8	2.5	1.5	0.4	0.4	0.4	0.4	2.5	1.4	5	–	–	–	–
Cd	0.3	2.0	1.0	0.6	0.9	0.8	0.3	2.0	1.0	–	1	1–30	–	0.9
Sn	4.5	7.8	6.4	–	–	–	4.5	7.8	6.4	4.5	–	–	–	–
Mo	0.2	0.3	0.3	0.8	1.0	1.0	0.2	1.0	0.4	2	–	–	–	–
Sb	5.3	14.9	8.9	1.8	2.2	2.0	1.8	14.9	7.8	4.5	–	–	–	–
Cr	–	–	–	1.2	1.2	1.2	1.2	1.2	1.2	6	–	0–10	–	–
Ge	136.8	1531.5	594.4	309.0	872.2	523.4	136.8	1531.5	565.2	1500	1500	10–130	1500	240

*Note: * – AAC values are given for acidic soils (sandy and clayed soil), $pH_{KCl} < 5.5$; error limits of 1.5% values presented in the table; ''–'' – absence of an element in the sample, for normative indicators – absence of data.*

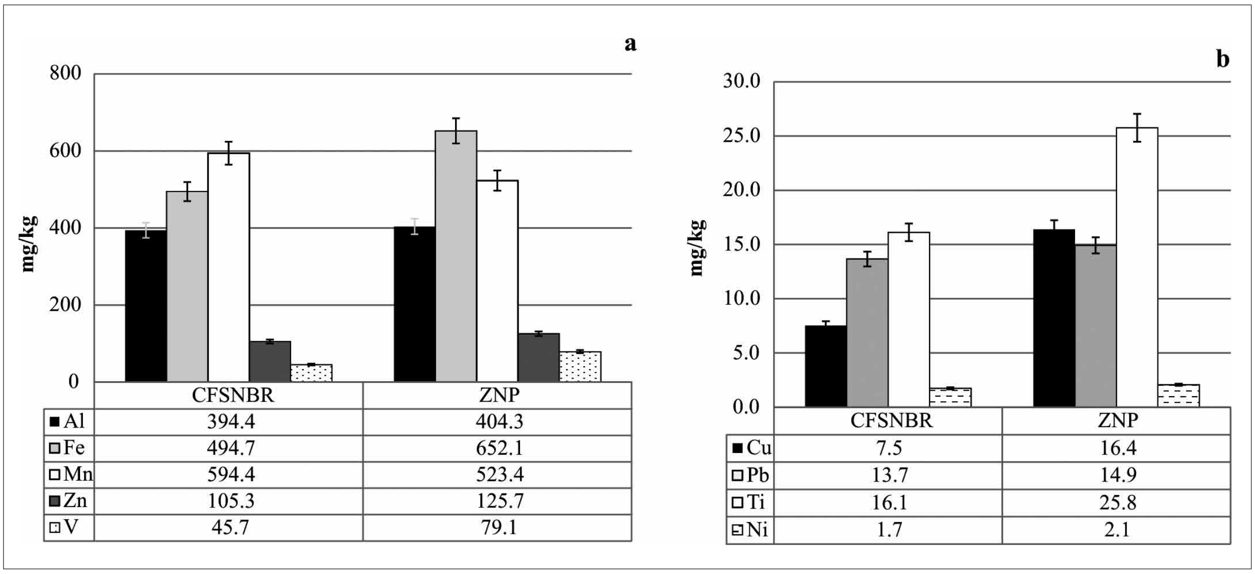

Fig. 1. Values of average element concentrations in the first (a) and second (b) groups in samples of *H. physodes* from investigated CA

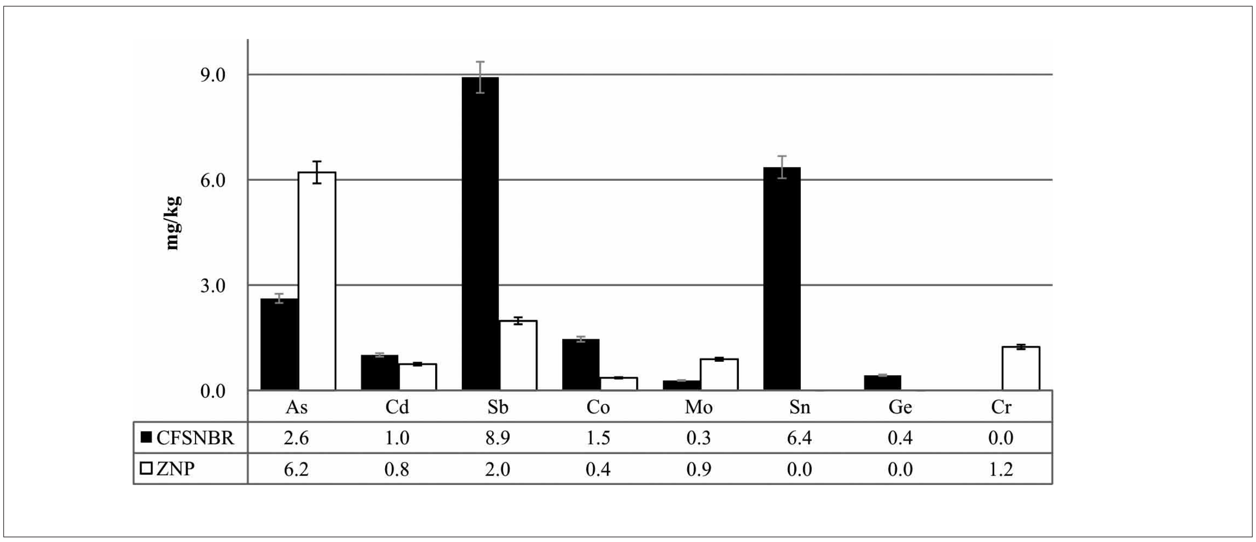

Fig. 2. Average concentration values for elements of the third group in samples of *Hypogymnia physodes* from studied CA

The average concentrations of Cu, Pb and Ni in samples from investigated CA do not exceed AAC values or the known world-average baseline values of these elements in *H. physodes* (Table 2). However, it is worth keeping in mind that the average concentrations of these metals in lichens from ZNP are significantly higher than in lichens from CFSNBR. In studied CA the average concentration of Ti is 18.3 mg/kg: 16.1 mg/kg in CFSNBR and 25.8 mg/kg in ZNP, which is 1.4 time higher. The AAC (or MAC) values for Ti have not been determined. However, the average content of Ti in *H. physodes* at the air baseline concentration is known to be 20 mg/kg. Furthermore, there is published information on the content of this element in biota (0.2–80.00 mg/kg) [11]. Therefore, the observed average Ti content in CFSNBR lichens can be considered as the baseline.

The analysis of gross concentrations of metals from the second group showed no correlation between gross concentrations and points of collection or localization of areas damaged by fires in samples from CFSNBR [1]. However, in samples from ZNP, high gross concentrations of Cu were seen in CS (CS 11–14) that were in the vicinity of residential areas. Pb, Ti and Ni were found only in samples from CS 11–13.

The *third group* of metals is comprised of toxic metals (As, Co, Cd, Sn, Mo, Sb, Cr, Ge) that have very low concentrations in lichens. The normative values for these metals (AAC, MAC, world-average baseline values) are also very low. Metals from this group were found in samples from very few CS. The most common metals found were As, Cd, Sn, Co, Mo and Sb, while Cr and Ge were found in isolated instances (Fig. 2, Table 2). The general amounts of elements from that group found in samples from both CA were different. Whereas the majority of elements were found in all CS from CFSNBR (Co, Sn, Sb, As, Mo, Cd), samples from the majority of CS from ZNP (CS 13–17) lack these elements. It should be noted that CS 13–17 are far away from water passages and large residential areas.

Average values of some metals from this group in samples from CA were higher than the known normative characteristics: in samples from CFSNBR these metals are Cd, Sn and Sb; in samples from ZNP it is the average value of As (Table 2). Samples from ZNP contain 1.2 times the average AAC (which is 5 mg/kg for As); in samples from CFSNBR the average contents of Cd are only insignificantly higher than the norm (AAC for Cd is 1 mg/kg) – 1.0 mg/kg. If this value is compared to the average world values for Cd, which vary greatly in the range from 1 to 30 mg/kg, we may consider this concentration to be baseline. There are no AAC values for Sn and Sb, the average values for these elements were compared to MAC values: the MAC was exceeded by 1.5 times (6.4 mg/kg) for Sn and by 2 times for Sb (8.9 mg/kg).

The average contents of other elements (Co, Mo, Ge, Cr) in samples do not exceed normative values. For example, the average concentrations of Co, Mo and Cr were below MAC values. There are few published materials about Ge concentrations. The authors did not find world-average baseline values nor the MAC values for this metal in lichens. There is data on the MAC values of this metal in the soil (it is equal to 2 mg/kg) [11]. As a result, it can be assumed that the average concentration of Ge (0.4 mg/kg), which was found only in samples from CFSNBR may be considered as baseline.

Gross concentrations of Sn and Sb exceed the known normative values in lichen samples from all CS in CFSNBR. High gross concentrations of these metals in lichens are the result of the anthropogenic effect. Alloys of Sn, Sb and some other metals are used as the foundation of antifriction metals in transport. The machines used in lumbering or when extinguishing fires may be the source of increased gross concentrations of these metals in lichen thalli. In samples from two CS from ZNP (CS 11 and 12) the gross concentration of As is above the norm (Table 2). It is well-known that arsenic (As_2O_3) is used in the production of some types of glass for clarification purposes [12]. In the Konakovo district (a large section of which is part of ZNP) glass production has been established a long time ago and defines the characteristics of this district [13]. High gross concentrations of this element in some CS in ZNP are likely to be the result of transboundary transfer of the emissions of these plants.

Gross concentrations of other metals do not exceed normative characteristics found in literature. A comparison of maximum and minimum values of gross concentrations did not yield a correspondence with the localization of CS in CFSNBR; in ZNP most of the metals were found in samples from two CS (CS 11, 12) (Table 3).

The values of average metal concentrations in lichens from CA in the Tver region are comparable to similar values of other CA in the Central Federal District. Similar studies were conducted in a Federal Biosphere Natural Reserve “Bryanskiy Forest” [13]. Using the data analyzed the authors were able to predict the possible ranges (intervals) of baseline values (Table 3).

Thus, 17 elements (Al, As, Cd, Co, Cr, Cu, Ge, Fe, Mn, Mo, Ni, Pb, Sb, Sn, Ti, V, Zn) were detected in *H. physodes* specimens from Federal CA using ICP-AES. The average concentrations of the majority of found metals do not exceed the known normative values (AAC, MAC or world-average baseline values). Average concentrations of many metals in the samples from ZNP are higher than from samples from CFSNBR. Differences between average concentration values of metals from the second and third group (Ti, Cu, As, Co, Mo, Sb) were often revealed (Table 2). In general, the territory of ZNP appeared to be more heterogeneous in terms of the anthropogenic transformation of ecosystems, the landscaping and phytocoenotic characteristics. Alongside areas that are more actively used there are significant area of forests and swamps in the Lob’ river valley that are characterized by a lack of or very low concentrations of many metals. Our data supports their significance as buffers and nature preserves. Their localization should be taking into account in zoning procedures and in the correction of conditions of nature management.

There was no clear dependency of metal concentrations and forest fire localization in CFSNBR. This connection was found only for some aerial toxins [1]. The localization of zones

Table 3

Possible ranges of baseline values for some metals taking into account the obtained results and literature data

Metals	Gross conc. of metals in CA of the Tver region, mg/kg		Average metal conc., mg/kg		World-average baseline values, mg/kg [6]	Possible ranges of baseline values in Tver region, mg/kg
	min	max	CFSNBR, ZNP	Bryanskiy forest		
Mn	136.8	1531.5	565.2	382,6	10–130	110–650
Fe	62.4	1921.5	559.5	7319,1	50–1600	55–645
Al	160.5	634.8	398.5	–	–	135–460
Zn	72.0	166.0	110.0	85,5	20–500	60–125
V	11.6	112.4	59.5	1,1	–	10–70
Ti	2.1	30.2	18.3	0,0	–	1,5–20
Pb	7.8	21.9	14.0	28,9	5–100	6,5–16
Cu	6.2	40.4	11.2	40,3	1–50	5,3–13
Ni	0.6	3.8	1.8	23,8	0–5	0–5
As	1.2	7.1	3.2	11,1	–	1–3,7
Co	0.4	2.5	1.4	0,0	–	0,3–1,6
Cd	0.3	2.0	1.0	–	1–30	0,3–1,1
Sn	4.5	7.8	6.4	–	–	3,7–7,5
Mo	0.2	1.0	0.4	–	–	0,1–0,5
Sb	1.8	14.9	7.8	–	–	1,5–9
Cr	1.2	1.2	1.2	43,7	0–10	0,1–1,5
Ge	0.2	0.5	0.4	–	–	0,2–0,5

Note: ''–'' – no measurement data.

with higher metal concentrations seems to be the result of moisture saturation conditions of the air. In ecotypes in the vicinity of flood basins or large swamps the concentration of metals was higher. In ZNP there was a clear connection between the quantitative and qualitative metal contents and the level of anthropogenic transformation of the territories.

In general, the comparison of data on metal concentrations in lichens obtained in this study and literature data (Table 2, 3) allows the authors to consider the revealed concentrations to be the baseline for the Tver region. In the future, it would be expedient to increase the array of investigated metals in these CA and organize monitoring observations.

Conclusion

Seventeen metals were detected using ICP-AES analysis in *H. physodes* thalli from Federal CA. Gross concentrations of many elements in samples from ZNP are higher than in samples from CFSNBR. Among them, there are elements such as iron, aluminum, zinc, vanadium, titanium, lead, copper, nickel, arsenic, molybdenum. The largest excess of the average content of elements in the compared territories was found for molybdenum (3.3 times more), arsenic (2.4 times more) and copper (2.2 times more). These differences are the result of different levels and regimens of air moisture saturation, localization of working production plants, levels of anthropogenic transformation of the territory. However, in general the average concentrations of most of the metals are below the known normative values, and as such can be considered as baseline for the Tver region and adjacent territories.

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