Heavy metals in lichens and their substrata of roadside phytocenoses in the south of the Tyumen region, Russia

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Abstract. The paper presents an analysis of the peculiarities of the accumulation process for the following heavy metals: Pb, Zn, Sr, and Cu in the bark of Betula pendula Roth (photophyte) and lichens Cladonia coniocraea (Flörke) Spreng., Cladonia fimbriata (L.) Fr., Physconia distorta (With.) J. R. Laundon, Hypogymnia physodes (L.) Nyl. The study sites were located in five administrative zones in the south of the Tyumen region, Russia: Uporovsk region, Zavodoukovsk region, Yalutorovsk region, Yarkovsky region and Tobolsk region. Based on the conducted study, it can be noted that in the bark of Betula pendula Roth of the studied areas, heavy metals varied within the following ranges: Pb 1.18-8.66; Zn 2.58-11.50; Sr 2.15-9.36; Cu 1.30-10.80 mg/kg, statistical significance level p<0.05, 0.01 and 0.001, respectively. In the accumulation of heavy metals by lichens, the location of the sampling point relative to the source of pollution is most important, followed by the species differences. Species differences of lichens are manifested mainly by the difference in the intensity of accumulation of microelements, while the ratio between elements varies little with the species. A high correlation between the content of metals in the thalli of lichens Cladonia coniocraea (Flörke) Spreng., Cladonia fimbriata (L.) Fr., Physconia distorta (With.) J. R. Laundon, Hypogymnia physodes (L.) Nyl. and in the bark of Betula pendula Roth (r=0.95-0.99). Accumulation of heavy metals in the studied components of forest ecosystems can be displayed structurally: the bark of photophytes > lichens. The exception is the accumulation of Sr (Cladonia fimbriata (L.) Fr., Hypogymnia physodes (L.) Nyl.), Cu (Cladonia fimbriata (L.) Fr.), Pb, Zn (Physconia distorta (With.) J. R. Laundon).

1. Introduction
Pollution of the environment over a long period of use of motor roads with exhaust gases and tire, brake and road surface abrasion products leads to a change in the physical and chemical properties of the roadside soils, as well as their structure. Accumulation of heavy metals in soils and plants is particularly hazardous. Accumulation of heavy metals in the environment leads to its pollution with agents potentially toxic to living organisms [1]. Living subjects are important bioindicators in the study of atmospheric transport of many chemical elements [2, 3]. An investigation of the content of heavy metals in them makes it possible to estimate the intensity of precipitation of aerosol particles from the atmosphere for several years.

Lichens were chosen as the object of our research. They are edificators and dominants of many
types of plant communities, which allows to carry out sampling almost everywhere. Lichens react sensitively to the nature and composition of the substrate on which they grow, to microclimatic conditions and the composition of the air. It is widely known that the concentration of metal ions in lichen tissues depend largely on the intensity of deposition of dust and aerosol particles [4,5]. Therefore, a spatial analysis of the content of microelements in these plants makes it possible to trace the sources of air pollution, the transport routes of pollutants and the intensity of their deposition. Lichens actively absorb metals from air and water and passively release them back. Lichens accumulate metals during their entire life and contribute to their penetration into trophic chains [6,7]. Lichens are able to accumulate elements from the ambient environment in quantities far exceeding their physiological needs. This quality determined the widespread use of lichens as accumulative bioindicators of environmental pollution with heavy metals. Epiphytic lichens can be used as bioindicators, and as accumulative biomonitors of persistent atmospheric pollutants. Among a significant number of works in which information is given on the content of heavy metals in plant organs, data on the use of the phytophite bark as an indicator of the state of the environment are sparse [8,9,10]. Proceeding from the foregoing, the purpose of this paper is to study the features of the accumulation of heavy metals by the bark of a photophyte (substrate) and a lichen-epiphyte in birch forests of roadside phytocenoses in the south of the Tyumen region, the most widespread forests in the studied area.

2. Materials and methods
2.1. Selection of sites
To determine the degree of anthropogenic impact, observation areas with different intensity of man-made burden were selected. Patches of birch forests (1 control (background), 2, 3, 4, 5) were located near motor roads (15 meters away from the road border) in the south of the Tyumen region within the five administrative districts: Uporovski, Zavodoukovsky, Yalutorovskiy, Yarkovsky, Tobolsky. The description of the vegetation was carried out according to the methodology adopted for geobotanical studies on trial plots measuring 20×20 m² [11].

Area 1 (control). The area is located in considerable distance from human impact (roads, industrial enterprises). Birch grove. Geographical coordinates: N 56.2146, E 66.1749. Phytocenosis is located near the village of Uporo, Uporovsky district.

Area 2. Gramineous birch grove. Geographical coordinates: N 56.35520, E 66.32950. Phytocenosis is located along the road between the villages of Chernoe and Shashova, Uporovsky district.

Area 3. Birch grove. Geographical coordinates: N 56.52840, E 66.26550. The roadside phytocenosis is located on the above-floodplain terrace of the Tobol River 1 km west of Zavodoukovsk.

Area 4. Birch grove. Geographical coordinates: N 57.00230, E 66.65780. Total projective coverage of live ground cover: 90%. The average length of stand: 55 cm. Phytocenosis is located along the road 2.5 km south-west of Novoaityalovo.

Area 5. Birch grove. Geographical coordinates: N 57.60230, E 67.27440. Phytocenosis is located along the motor road Yarkovo-Tobolsk.

2.2. Bark sampling
The bark was stripped from tree trunks of Betula pendula Roth on which the studied lichens were found. Samples were taken at a height of 1.5 m above the level of the soil cover from 10 nearby trees. In order to avoid contamination, sampling was performed using plastic gloves in clean plastic bags. The samples stored in paper bags.

2.3. Lichen sampling
Lichens were identified and recorded at the sites, with indication of substrate. Lichen sampling was carried out in accordance with [12]. Lichens were collected from tree trunks of Betula pendula Roth at a height of 1.5 from the ground surface along the entire length of the circumference of the trunk during
a dry weather, as well as in the basal zone, during a non-rainy weather, so that samples of lichen thalloms are not waterlogged. Thalli of lichens was separated from the surface of the trunk manually or was scraped with a knife, preventing bark particles and other foreign particles from contaminating the sample. The species requiring laboratory conditions for the identification were collected into paper bags. The thalli lichens studied were cleared from the substrate under a microscope. The thalli of lichens are very hygroscopic; therefore, before weighing the samples, we experimentally evaluated the influence on the thalli weight of the ambient air humidity at the time of weighing, as well as the day and hour when the weighing was performed. It was found that the influence of these factors on the weight of samples is not significant. The following data were recorded in the logbook: sampling date and place; height of the tree; diameter of the trunk; exposure; inclination of the trunk. The geographical coordinates of the flora sampling points were recorded using a GPS satellite navigation system (WGS-84 coordinate system). The cartographic material was visualized using SAS-planet software. Identification of the samples was carried out using the standard methodology and reference literature.

2.4. Preparation of samples
The samples were prepared using a microwave decomposition system Speedwave MWS-2 by Perkin Elmer (United States). Weighed quantity (m = 0.3 g) of lichen thalli of Cladonia coniocraea (Flörke) Spreng., Cladonia fimbriata (L.) Fr., Physconia distorta (With.) J. R. Laundon, Hypogymnia physodes (L.) Nyl. and bark of Betula pendula Roth, were placed into a plastic tube, then H2SO4: H2O2 = 1:3 was added. The tube was placed into a microwave oven to decompose the sample using the program recommended by the oven manufacturer, applying the following heating mode: raising the temperature to 200 °C for 5 minutes, maturing for 5 minutes at 200 °C, cooling down to 45 °C. A dissolved sample was transferred to a 15 ml test tube, the volume was made up to 10 ml with distilled water and the analysis was performed.

Quantitative chemical analysis of accumulation of trace elements and heavy metals (Zn, Pb, Sr, Cu) in lichen thalli and bark Betula pendula Roth by the method of inductively coupled plasma using an atomic emission spectrometer OPTIMA-7000DV by Perkin Elmer (USA). For calibration, standard solutions by PerkinElmer (USA) were used. Before measuring the samples, the required parameters for the measurement were set (the background was measured, the necessary calibration was performed (the minimum and maximum concentration of 0.05-10 mg / dm³). Statistical processing of data was carried out in the program Statistica 10 "Statsoft".

3. Results and discussion
The study revealed that in the birch forests the main load falls on the dominant tree species of Betula pendula Roth.; they represent a leading position in terms of accumulation. In the bark of Betula pendula Roth of the studied areas, heavy metals varied within the ranges of: Pb 1.18-8.66; Zn 2.58-11.50; Sr 2.15-9.36; Cu 1.30-10.80 mg/kg, statistical significance level p<0.05, 0.01 and 0.001, respectively. By the increase in the anthropogenic load, the areas were lined up in the following order: 2>5>4>3>1. In the areas with the greatest anthropogenic load, the concentration of lead, zinc, strontium and copper in the bark of Betula pendula Roth exceed the background values by the factors of 4.4-7.3 (Pb), 1.8-4.4 (Zn), 1.9-4.3 (Sr), 2.4-8.3 (Cu) (table 1).

In the thalli of Cladonia coniocraea (Flörke) Spreng. on the impact area in comparison with the background values, the amount of heavy metals increased by the factors of 9.8-5.8 (Pb), 1.4-4.7 (Zn), 2.2-4.6 (Sr), 2.4-5.1 (Cu). Concentrations of heavy metals also increase in Cladonia fimbriata (L.) Fr. by the factor of 5.7 - 9.5 (Pb), 1.8 - 4.7 (Zn), 1.9 – 4.3 (Sr), 2.5 – 9.1 (Cu). If these species grow on the sections of the trunk, where the trunk runoff does not pass, this protects these species from contact with solutions containing toxic agents on the one hand, but on the other hand prevents the washing out of accumulated toxicants from the thalli.
The degree of accumulation of heavy metals in lichens is closely related to the degree of air pollution with the same, as well as their content in the bark of photophytes, in this case - the bark of *Betula pendula* Roth. A strong correlation between the content of metals in the thalli of lichens *Cladonia coniocraea* (Flörke) Spreng., *Cladonia fimbriata* (L.) Fr., *Physconia distorta* (With.) J. R. Laundon, *Hypogymnia physodes* (L.) Nyl. and in the bark of *Betula pendula* Roth (r = 0.95-0.99) was found. Accumulation of heavy metals in the studied components of forest ecosystems can be displayed structurally: the bark of photophytes > lichens. The exception is the accumulation of Sr (*Cladonia fimbriata* (L.) Fr., *Hypogymnia physodes* (L.) Nyl., Cu (*Cladonia fimbriata* (L.) Fr.), Pb, Zn (*Physconia distorta* (With.) J. R. Laundon). Thus, in the gradient of pollution, the dependence of the metal content in lichens on the value of the local toxic load is better expressed if the content of metals in the bark of the forophyte tree is used as a load marker. By increasing the heavy metals content in lichens found in roadside phytocenoses, one can judge the deterioration of the environment with one of the reasons being the increase in the number of vehicles.

| Areas | Heavy metals, mg/kg (X ± SD) |
|-------|-----------------------------|
|       | Pb  | Zn  | Sr  | Cu  |
| 1 (control) | 1.18±0.03 | 2.58±0.02 | 2.15±0.01 | 1.30±0.09 |
| 2     | 8.66±0.05*** | 11.50±0.12** | 9.36±0.13 | 10.80±0.11*** |
| 3     | 5.24±0.07* | 4.69±0.09  | 4.15±0.07  | 3.21±0.06 |
| 4     | 6.05±0.05  | 5.22±0.04** | 5.97±0.09*** | 7.90±0.11** |
| 5     | 7.61±0.06** | 6.23±0.05*** | 7.32±0.11** | 8.45±0.06* |

Note: *, **, *** - statistically significant differences as compared to the control area at P <0.005, 0.01 and 0.001, respectively.

The impact of toxic load contributes to the manifestation of interspecies differences in the accumulation of metals. Lichens living on the bark of trees are organisms sensitive to changes in the air content of a number of chemical elements and compounds that are part of the emissions from most industrial plants [13].

The difference between background and impact concentrations and the variability of metal concentrations in *Physconia distorta* (With.) J. R. Laundon is higher than that of *Hypogymnia physodes* (L.) Nyl. It is possible that the growth of *Physconia distorta* (With.) J. R. Laundon in the near-butt horizon of tree trunks, where the thalli are contaminated with soil particles, but are shielded from atmospheric fallout by the grass-shrub layer. The degree of screening can be different, which provides for an additional mosaic structure of metal contents in lichens. Probably, tolerance to metals and their hyperaccumulation are genetically independent properties of organisms, as has been shown on vascular plants [14].

Concentrations of heavy metals in *Physconia distorta* (With.) J. R. Laundon located in the contaminated areas exceeded the control area indications by the factor of 6.8 - 8.7 (Pb), 1.7 - 4.4 (Zn), 2.1 - 4.4 (Sr), 2.3 - 5.0 (Cu). In the thalli of *Hypogymnia physodes* (L.) Nyl. on the impact area in comparison with the background values, the amount of heavy metals increased by the factors of 6.1 - 10.4 (Pb), 1.8 - 4.6 (Zn), 2.1 - 4.4 (Sr), 2.3 - 5.6 (Cu). Differences in the accumulative capacity of *Cladonia coniocraea* (Flörke) Spreng., *Cladonia fimbriata* (L.) Fr., *Physconia distorta* (With.) J. R. Laundon, *Hypogymnia physodes* (L.) Nyl. arise due to different ways of toxicants ingress, which are due to the morphology of thalli and the conditions of growth on the tree trunk (table 2).

Table 1. Accumulation of heavy metals in the bark of *Betula pendula* Roth collected in the areas.

| Areas | Pb  | Zn  | Sr  | Cu  |
|-------|-----|-----|-----|-----|
| 1 (control) | 1.18±0.03 | 2.58±0.02 | 2.15±0.01 | 1.30±0.09 |
| 2     | 8.66±0.05*** | 11.50±0.12** | 9.36±0.13 | 10.80±0.11*** |
| 3     | 5.24±0.07* | 4.69±0.09  | 4.15±0.07  | 3.21±0.06 |
| 4     | 6.05±0.05  | 5.22±0.04** | 5.97±0.09*** | 7.90±0.11** |
| 5     | 7.61±0.06** | 6.23±0.05*** | 7.32±0.11** | 8.45±0.06* |
Table 2. Accumulation of heavy metals in lichens *Cladonia coniocraea* (Flörke) Spreng., *Cladonia fimbriata* (L.) Fr., *Physconia distorta* (With.) J. R. Laundon. *Hypogymnia physodes* (L.) Nyl. collected in the studied areas, mg/kg of dry matter.

| Areas           | Heavy metals, mg/kg (X ± SD) | Cladonia fimbriata (L.) Fr. | Cladonia coniocraea (Flörke) Spreng. | Physconia distorta (With.) J. R. Laundon | Hypogymnia physodes (L.) Nyl. |
|-----------------|-----------------------------|----------------------------|--------------------------------------|------------------------------------------|-------------------------------|
|                 | Pb                          | Zn                        | Sr                                   | Cu                                       |                               |
| 1 (control)     | 0.78±0.04***                | 2.37±0.01**               | 1.83±0.03*                           | 1.24±0.01***                             |                               |
| 2               | 7.11±0.04                  | 11.26±0.01*               | 8.49±0.02                            | 7.00±0.02                                |                               |
| 3               | 4.58±0.07**                | 4.47±0.02                 | 4.10±0.02**                          | 2.99±0.07**                              |                               |
| 4               | 5.28±0.04**                | 4.63±0.02*                | 5.12±0.01*                           | 6.23±0.03*                               |                               |
| 5               | 6.89±0.02                  | 6.09±0.04**               | 7.17±0.01**                          | 6.30±0.02*                               |                               |
| 1 (control)     | 0.82±0.04**                | 2.43±0.01                 | 2.18±0.03*                           | 1.37±0.03**                              |                               |
| 2               | 7.80±0.05*                 | 11.36±0.01*               | 9.46±0.01                            | 12.48±0.03                               |                               |
| 3               | 4.75±0.09***               | 4.49±0.02***              | 4.18±0.02*                           | 3.44±0.09**                              |                               |
| 4               | 5.41±0.04                  | 4.73±0.03**               | 6.00±0.01*                           | 8.28±0.07**                              |                               |
| 5               | 6.90±0.02*                 | 6.13±0.02                 | 7.48±0.02**                          | 9.00±0.09**                              |                               |
| 1 (control)     | 0.78±0.05***               | 2.64±0.01**               | 1.88±0.02**                          | 1.26±0.01**                              |                               |
| 2               | 8.78±0.06***               | 11.55±0.02***             | 8.45±0.02                            | 7.04±0.06**                              |                               |
| 3               | 5.33±0.05                  | 4.74±0.04*                | 4.11±0.02                            | 3.01±0.02**                              |                               |
| 4               | 6.08±0.02*                 | 5.30±0.04                 | 5.14±0.01**                          | 6.37±0.04**                              |                               |
| 5               | 7.67±0.06                  | 6.29±0.01***              | 7.19±0.01**                          | 6.54±0.03**                              |                               |
| 1 (control)     | 0.75±0.04*                 | 2.46±0.03*                | 1.92±0.02**                          | 1.27±0.01**                              |                               |
| 2               | 7.84±0.04**                | 11.32±0.02*               | 8.53±0.02                            | 7.09±0.07**                              |                               |
| 3               | 4.64±0.09***               | 4.51±0.02***              | 4.13±0.01**                          | 3.02±0.03**                              |                               |
| 4               | 5.47±0.07                  | 4.69±0.03***              | 5.13±0.01*                           | 6.42±0.05**                              |                               |
| 5               | 6.91±0.02*                 | 6.07±0.02                 | 7.21±0.02**                          | 6.62±0.04**                              |                               |

Note: *, **, *** - statistically significant differences as compared to the control area at P <0.005, 0.01 and 0.001, respectively.

4. Conclusion

Based on the study, it can be noted that in the bark of *Betula pendula* Roth collected in the studied areas, heavy metals varied within the ranges of: Pb 1.18 – 8.66; Zn 2.58 -11.50; Sr 2.15 – 9 36; Cu 1.30 – 10.80 mg/kg, significance level P<0.05, 0.01 and 0.001, respectively. In the accumulation of heavy metals by lichens, the location of the sampling point relative to the source of pollution is most important, followed by the species differences. The determination of the content of heavy metals in the thalli of lichens and bark *Betula pendula* Roth for the Tyumen region was carried out for the first time. The analysis of the results obtained allowed us to conditionally separate lichens by the ability to accumulate heavy metals. A high correlation between the content of metals in the thalli of lichens *Cladonia coniocraea* (Flörke) Spreng., *Cladonia fimbriata* (L.) Fr., *Physconia distorta* (With.) J. R. Laundon, *Hypogymnia physodes* (L.) Nyl. and in the bark of *Betula pendula* Roth (r = 0.95 - 0.99) was found. Accumulation of heavy metals in the studied components of forest ecosystems can be displayed structurally: the bark of photophytes > lichens. The exception is the accumulation of Sr (*Cladonia fimbriata* (L.) Fr., *Hypogymnia physodes* (L.) Nyl.), Cu (*Cladonia fimbriata* (L.) Fr.), Pb, Zn (*Physconia distorta* (With.) J. R. Laundon), which shows the species specificity of the accumulation of heavy metals. Species differences of lichens are manifested mainly by the difference in the intensity of accumulation of microelements, while the ratio between elements varies little with the species. The
results of this work can be used in assessing the degree of pollution of the environment in the south of the Tyumen region with heavy metals.

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