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Epiphytic and epigeal lichens as bioindicators of air pollution in the Burabay National Park, Kazakhstan

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[1]L.N. Gumilyov Eurasian National University, Astana, 010008, Kazakhstan. Tel./Fax.: +7-7172-70-95-00, ♥email: zhanylxan79@mail.ru
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Abstract. *Bukabayeva Z, Abiyev S, Silybayeva B, Assanova U, Sharipkhanova A, Sagdatkyzy B. 2023. Epiphytic and epigeal lichens as bioindicators of air pollution in the Burabay National Park, Kazakhstan. Biodiversitas 24: 2701-2709.* Lichens are recognized as a symbiotic association between a fungus (mycobiont) and a chlorophyll-containing partner (photobiont). Because lichens receive all their nutrients from the atmosphere, they are very sensitive to air quality and can be used as bioindicators of pollution by heavy metals, organic compounds and radioactive elements in the air. This study aimed to evaluate the potential of lichens as bioindicators of the heavy metal content in the air in the Burabay National Park, northern Kazakhstan. In the period from 2018 to 2022, we studied the floristic composition of lichens in the national park. To inform air pollution level, we determined the quantitative characteristics and projective cover of an epiphytic lichen *Evernia prunastri* (L.) Ach. at different heights above ground level, and the heavy-metal content in an epigeal lichen *Cladonia alpestris* (L.) Rabenh (Syn. *Cladonia stellaris* (Opiz) Pouzar & Vězda) at different distances from the road edge. We identified 56 species of lichen belonging to 23 genera and 16 families on the roadsides or in the nearby forest. We found that the average number of individuals of *E. prunastri* decreased as the tree trunk height increased. The largest number of individuals (11.3) was observed at a height of 60 cm, and the smallest (2.5), at a height of 150 cm. The analysis of *C. alpestris* samples taken at different distances (50, 100, 150, 200 m) from the roadside showed that the concentrations of Pb, Cr, Cd, As, Ga, V, and Cs were high, and for all the elements studied, except for Mn and Be, exceeded Maximum Allowable Concentration. Our study demonstrates that *E. prunastri* and *C. alpestris* are sensitive to air pollution from road traffic and can be used as biomonitors of heavy metal pollution in the study area. Because of the ever-increasing anthropogenic pressure on the vegetation of the Burabay National Park, we recommend further research and monitoring of its lichen biota.

Keywords: Bioindicator, environmental pollution, forest, heavy metals, lichens, symbiosis

INTRODUCTION

A number of heavy metals have been identified as environmentally harmful. Among them, lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn), nickel (Ni), arsenic (As), and some others are emitted to the roadside environment from the abrasion of car tires, combustion of gasoline, diesel soot, and the wear of asphalt pavement (Ozaki et al. 2004; Soliman et al. 2019; Panta 2020). Relatively simple, inexpensive and informative methods for assessing the ecological state of the environment, including the air quality, are available based on the study of the reactions of living organisms to anthropogenic effects (Boonpeng et al. 2018; Matos et al. 2019). Such organisms are defined as bioindicators when used for the identification and qualitative determination of the impacts of human activities on environmental conditions (Crawford 2019). Lichens play an important role in the biogeochemical cycle and are commonly used as bioindicators of anthropogenic impact, in particular, contamination by heavy metals (Ramdani et al. 2019; Monaci et al. 2022).

Lichens are recognized as a symbiotic association between a fungus (mycobiont) and a chlorophyll-containing partner (photobiont). Lichens are sensitive to microclimatic changes and strongly influenced by light intensity, air humidity and temperature (Hauck et al. 2006; Louis-Paul-Roger et al. 2018; Abdrassulova et al. 2018). Because lichens receive all their nutrients from the atmosphere, they are very sensitive to air quality and can be used as bioindicators of pollution by heavy metals, organic compounds and radioactive elements in the air. Air pollution leads to a decrease in the projective cover of epiphytic lichens and in the participation of ground (epigeal) lichens in the moss-lichen stage of succession (Suetina 2020). At the first stages of exposure to air pollutants, an increase in pollutant concentrations in the thallomes is observed. As the pollution intensity increases, lichens disappear completely, and a “lichen desert” is formed (Ramdani et al. 2019).

Over the past decades, lichens have been used in monitoring studies of the ecological state of anthropogenically disturbed natural territories (Agha et al. 2016; Abas et al. 2019). Lichens have been used as bioindicators of atmospheric precipitation of heavy metals, organic compounds and radioactive elements (Gómez-Guzmán et al. 2010). Both epiphytic and epigeal lichens have been used as indicators of air pollution (Vannini et al.

2017; Kularatne and De Freitas 2013). Extensive data have been accumulated on the state of epiphytic lichen cover in different territories (Loppi 2019). The main habitat conditions that determine the location of epiphytic lichens on the trunk (i.e. the height above the ground and orientation), including tree species and age, the angle of tree trunk inclination, exposure, moisture availability, etc. have been established (Massimi et al. 2019). In particular, an epiphytic lichen *Evernia prunastri* (L.) Ach was used as an indicator of excessive ambient air nitrogen deposition worldwide (Rai and Gupta 2022). An epigeal lichen *Cladonia alpestris* (L.) Rabenh. (Syn. *Cladonia stellaris* (Opiz) Pouzar & Vězda). has been used as an indicator of heavy metals in soils (Opekunova et al. 2018). The study determined the mechanical composition of the soil, its pH, humus content and mobile forms of heavy metals (Cu, Zn, Ni, Co, Fe, Mn, Pb and Cd) extracted by acetate-ammonium buffer, and the total content of heavy metals in lichens.

In Kazakhstan, a number of studies were conducted addressing the composition and ratio of elements on the surface of lichens from the experimental field of the former Semipalatinsk Polygon and from the city of Kurchatov (Biazrov and Pelgunova 2015). The present study was conducted as part of an integrated approach combining chemical and biological parameters to assess the impact of road traffic on air quality in Kazakhstan. In this study, the Burabay National Park was chosen as an excellent case study of natural territory with rich natural resources. Besides being a conservation area, Burabay National Park is also a recreational area, exposing it to a number of human activities from sanatoriums, hotels and recreation centers built in the surrounding area.

The Burabay National Park is protected by the state, but from year to year, the anthropogenic pressure on its natural resources has been growing. A high level of tourism development in the national park and adjacent areas led to the development of the transport infrastructure. The area is also characterized by high levels of air pollution from traffic. Averaged over a single day, the number of cars that entered the study area in one hour was 140 ± 3.8, with a similar number leaving (unpublished data). Therefore, the environmental assessment of the Burabay National Park is highly relevant. One way to determine the level of air pollution in the Burabay National Park is to use widespread lichens as bioindicators. Therefore, the main purpose of our study was to evaluate the potential of an epiphytic lichen *E. prunastri* and an epigeal lichen *C. alpestris* as bioindicators of the heavy metal content in the air in the Burabay National Park.

MATERIALS AND METHODS

Study period and area

The study was conducted in 2018-2022 in the Burabay National Park, the Akmola Region, Kazakhstan, i.e.: Shchuchye (52º99 '46.65” N, 70º23'12.81” E), Abylaykhan (53º08 ’85.85”N, 70º23’19.68”E), Baldauren (52º96’55.38”N, 70º19’39.24”E) (Figure 1). Surrounded by forests, Burabay is located at the foot of the mountains (Mikhaylov 2020). The climate of the region is continental characterized by harsh, long winters, short and hot summers, a predominance of clear days, and high-temperature variability.

Soils of the Burabay National Park are very diverse due to differences in terrain, bedrock, and climatic conditions (Kubentayev et al. 2022). Zonal soils are represented by calcareous, normal, leached and solodized chernozems, as well as dark chestnut underdeveloped and incompletely developed soils and chestnut soils on dense crystalline rocks. Brown forest petromorphic eluvial soils are common under pine forests. Under stony-lichen pine forests, there are strongly skeletal brown forest soils with frequent surface outcrops of rock. Steppes are dominated by zonal chernozem soils. In the southern part, the steppe areas form complexes with birch groves that form on gray forest soils and solods.

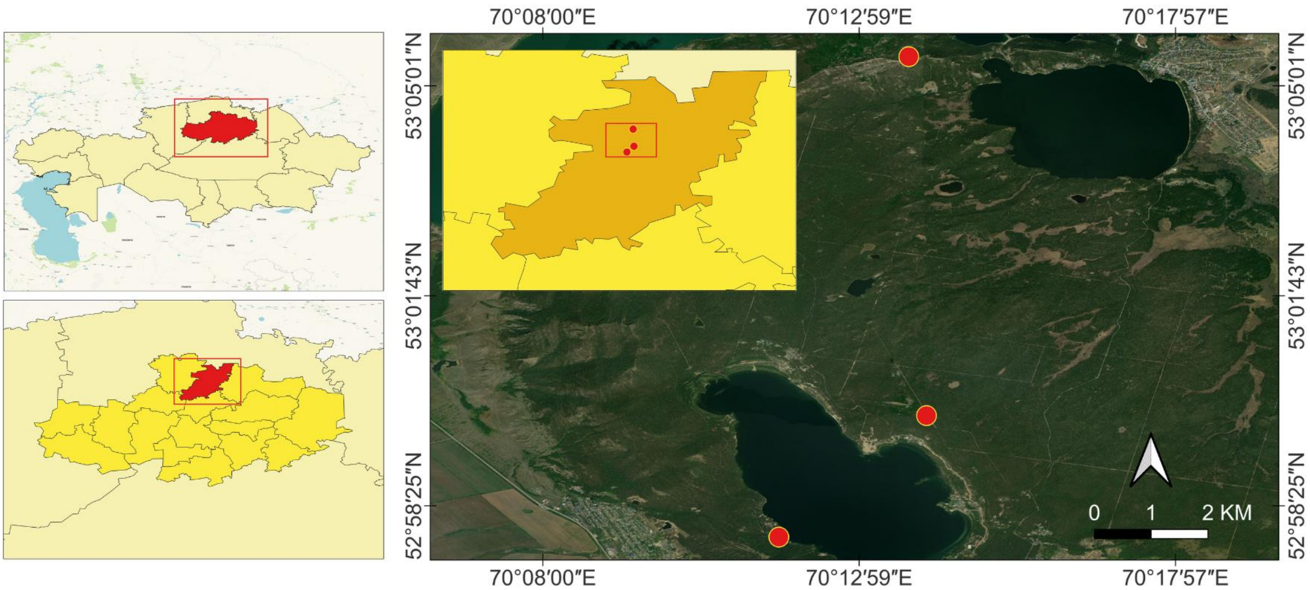

Figure 1. The study area and the locations of the sampling sites in Burabay National Park, Kazakhstan, i.e.: Shchuchye, Abylaykhan, Baldauren

The areas of the park selected for the study were Baldauren, Abylaykhan and Shchuchye. The Baldauren site is located on northeast of the park. This is a typical pine forest with an admixture of broadleaved species. The forest vegetation is rather sparse due to the dry, crushed stone soils. This site is a tourist area accessible from all sides. It has a large number of multi-storey buildings, roads and parking lots. The Abylaykhan site is in the western part of the park, where there are rather high rocky mountain ranges with coniferous forests. Because of this, there are variable biotopes of plants and lichens. In this part of the park, tourism is highly developed and it contains the main road junction and a large number of roads and parking lots along the edges of Lake Burabay. The Shchuchye site in the southwestern part of the park is characterized by a dense coniferous forest cover. This is a site with a high level of air pollution, due to the presence of a road junction connecting all resorts located in the area.

The soils of the studied sites are alluvial-meadow. They differ from each other only in the thickness of the humus horizon and the stone content which increases with proximity to the massive bedrock crystalline rocks to the west and northwest of the area. The mechanical composition of the soils is heterogeneous. Soils of light mechanical composition predominate.

Description of species studied

An epiphytic lichen *E. prunastri* and an epigeal lichen *C. alpestris* were selected for the study as the dominant species with a high frequency of occurrence in the study areas. *E. prunastri* is an epiphytic lichen of the family Parmeliaceae Zenker. It grows on the trunks and branches of deciduous trees, and occasionally on conifers, especially at forest edges, near forest roads and in other well-lit places (Shcherbakova et al. 2021). The main method of reproduction is by soredia and apothecia are formed rarely (Kousehlar and Widom 2020). In the study areas, *E. prunastri* grows vertically or slightly drooping on pine branches and trunks in mixed forests. The thallus of *E. prunastri* is dorsiventral, thick, 4-9 cm long; it is attached to the tree base and grows by dichotomous branching. On the upper surface of the lichen, white soredia are formed, and on the marginal edges, isidia develop. The top of the thallus is pale greenish-grey, and the underside is light brown. Less common are whitish-brown, disc-shaped apothecia along the thallus edges (Figure 2).

Cladonia alpestris is an epigeal lichen species of the family Cladoniaceae Zenker that grows in scattered groups in open, sun-lit areas of pine forests of the Burabay District, in the north of the Akmola Region. Podetia are pale greenish or greyish. Short branches with curved ends branch several times. Thick, branched podetia grow up to 15-20 cm long. The lower part of a podetium is not subdivided, while the upper part of the thallus has multiple branches forming a hemisphere-like structure with branched ends. Branched areas have several large slits. Apothecia are rare. The lichen often reproduces asexually by fragments of its thallus; sexual reproduction by spores is rare (Figure 3).

Data collection and analysis

To estimate the number of individuals of *E. prunastri* and their projective cover, 27 model pine trees (*Pinus sylvestris* L.) were randomly selected in each of the two study sites, Shchuchye and Baldauren. The trees were growing at a distance of 10-20 m from each other along the Astana-Kokshetau highway (R-225). We used the method of Kularatne and De Freitas (2013), with the following subsections: 0-60, 60-90, 90-120 and 120-150 cm measured by their vertical distance from ground level. Each subsection of the pine trunk was marked with colored tape (Figure 4). To accurately determine lichen species and count their number, square meshes made from soft flexible wire with mesh size of 20x20 cm were attached below the first ribbon (Figure 4). For each tree, we counted the number of lichen individuals in each mesh square, and measured the total area of square meshes, a projective cover of lichens, and the tree trunk area sizes between the tapes.

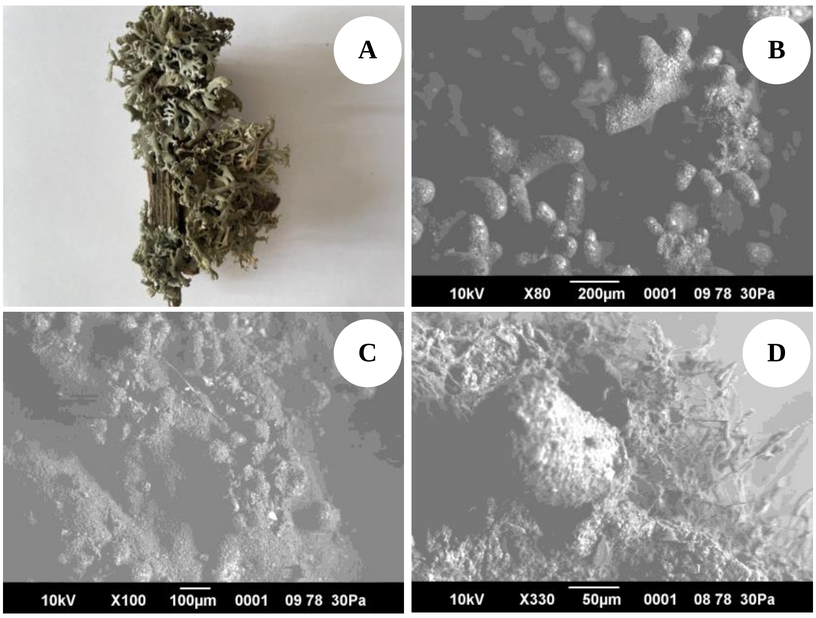

Figure 2. A. *Evernia prunastri* (L.) Ach.; B. Isidia at the thallus edges; C. Soredia, isidia and apotecia; D. Homeomeric thallus

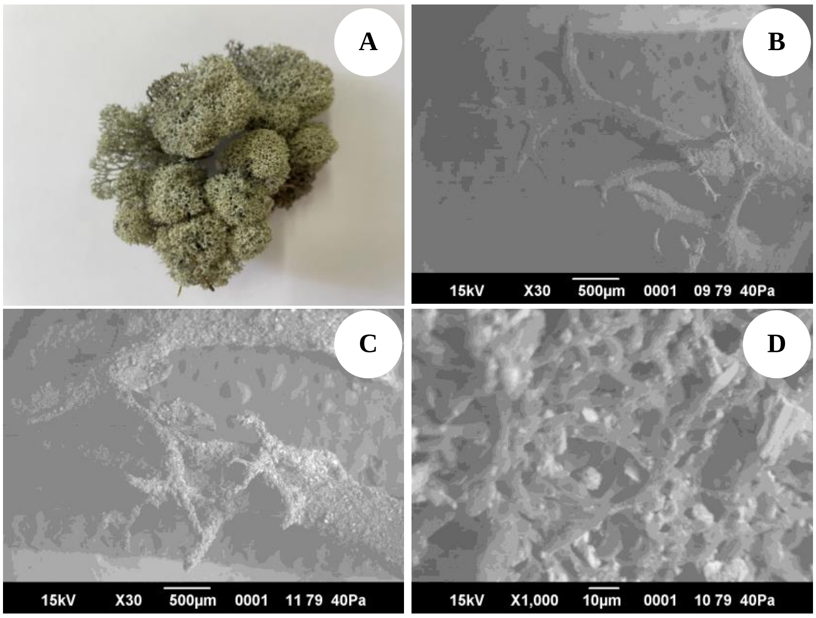

Figure 3. A. *Cladonia alpestris* (L.) Rabenh; B. Pores at the lichen branch sites; C. Microscopic view of the lichen branching; D. Podetia on the soil surface

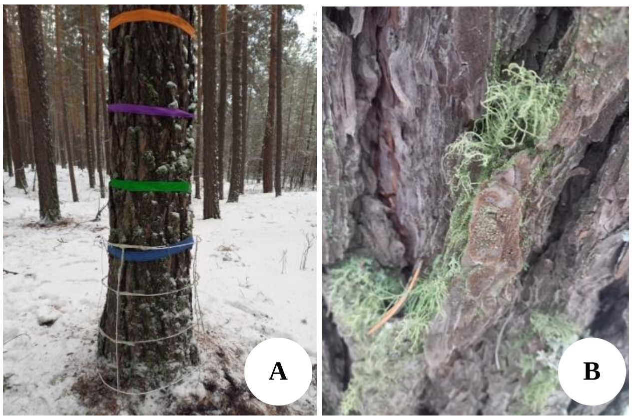

Figure 4. A. Colored tapes and square meshes on the pine trunks; B. The thallus of *E. prunastri* on a pine trunk

To determine the heavy metal content in *C. alpestris*, samples were collected at a distance of 50, 100, 150 and 200 m from the Astana-Kokshetau highway (R-225) in two sites (Abylaykhan and Shchuchye), 11 samples in each. Samples were prepared for the analysis of heavy metal content in accordance with GOST 17.4.3.01-83 (Opekunova et al. 2018). The samples were dried to an air-dry state at 105°C, and crushed with a laboratory disc crusher LDI-60M to a maximum particle size of less than 1 mm. The mass of the crushed sample was at least 100 grams. The heavy metal content was determined by X-ray fluorescence analysis using a Spectron 7832 3 Spectroscan Max tool. The statistical analysis was carried out using MS Excel 2003 package.

RESULTS AND DISCUSSION

In total, 56 species of lichen from 23 genera and 16 families have been recorded in the area (Table 1). In our study, we tested two lichen species, *E. prunastri* and *C. alpestris*, as potentially suitable species to be used as bioindicators of air pollution in the Burabay National Park. Firstly, we found that the frequency of occurrence of an epiphytic lichen *E. prunastri* decreased as the tree height increased, and that the number of *E. prunastri* individuals and their projective cover were higher in the site with lower levels of air pollution (Shchuchye). Secondly, closer to the road edge, the heavy metal concentrations in the *C. alpestris* samples increased, while the frequency of occurrence of the lichen decreased, suggesting that the lichen reacted to the gradient of heavy metal concentrations generated by road traffic. The results of the present study are in line with previous research showing that lichens can be used as bioindicators of air pollution from automobile emissions (Firdous et al. 2017).

In the study areas, we recorded a relatively high frequency of occurrence of *E. prunastri* (Table 2). However, the study confirmed that the average number of individuals of *E. prunastri* decreased as the tree trunk height increased, from 11.3 to 3.9 in Shchuchye, and from 6.4 to 2.5 in Baldauren. The largest number of individuals (11.3) was observed at the height of 60 cm in Shchuchye, and the smallest (2.5), at the height of 150 cm in Baldauren. The highest projective cover of *E. prunastri* (33.6%) was observed at a height of 60 cm in Shchuchye, and the lowest (13%), at a height of 150 cm in Baldauren. It has been demonstrated that in forest environments, epiphytic lichen distribution and abundance are largely determined by air humidity, light conditions, and bark chemistry and structure (Petersson et al. 2022). Because in our study, the light conditions and bark properties were similar across each study site, the observed distribution patterns of *E. prunastri* were likely due to higher levels of air humidity near the forest floor.

In Baldauren, at the same height, the number of *E. prunastri* individuals was slightly more than half (6.4), while the projective cover (31.7%) was the second highest. Lower numbers and projective cover of *E. prunastri* growing in Baldauren are possibly due to a more developed road network and more intensive traffic in the area. Moreover, pine forests of Baldauren are sparser due to less favorable growing conditions, i.e. dry, crushed stone soils.

In the study areas, *C. alpestris* was scattered in abundance over the soil surface in open sun-lit parts of the pine forest (Figure 5). Therefore, air quality assessment could be done based on the heavy metal content in the lichen samples without taking into account quantitative and projective cover indicators.

The analysis of *Cladonia alpestris* samples taken at different distances (50, 100, 150, 200 m) from the roadside showed that the concentrations of Pb, Cr, Cd, As, Ga, V, and Cs were high, and for all the elements studied, except for Mn and Be, exceed Maximum Allowable Concentration (MAC, Table 3). The concentrations of all heavy metals lowered with the distance, with the most dramatic decrease observed between 50 and 100 m in Abylaykhan. At the same time, the concentrations of Cd, Pb, Zn, Cr, As, Cz, Ga and V exceeded MAC even at a distance of 200 m from the roadside. In particular, in Abylaykhan, the concentrations of Cd, Pb, Cr, As, Cs, Ga, and V exceeded 2.8, 3.0, 1.9, 15.0, 1.7, 10.0 and 1.8 times MAC, respectively. Overall, the heavy metal concentrations were higher in Abylaykhan than in Shchuchye (Table 3). This is due to the highly developed tourist infrastructure in Abylaykhan, including a main road junction of the Burabay National Park, and several roads and parking lots along the edges of Lake Burabay.

The concentrations of the most important heavy metals (Cd, As, Pb, Cr) in the *C. alpestris* samples taken at different distances from the roadside are also presented as separate diagrams (Figure 6). In Shchuchye and Abylaykhan, the heavy metal concentrations in lichen samples gradually decreased as the distance from the roadside increased, but the decrease in the heavy metal concentrations was the most dramatic in Abylaykhan. Thus, in the samples taken at a distance of 200 m from the roadside in Abylaykhan, the content of Cd, Pb, Cr and As was 6.0, 6.6, 6.3 and 2.3 times higher, respectively, compared to the content of the same heavy metals in the samples taken at a distance of 50 m from the roadside. In addition, the lead concentration in lichen samples taken from a distance of 50 m from a roadside in Abylaykhan was 20 times higher (22.0 against 4.0 μg/g), and that of chromium 12.2 times higher (25.0 against 4.3 μg/g) than in Shchushye (Figure 6).

Table 1. Lichen species recorded in the study area. Several species were not found in the study sites but recorded in other parts of the Burabay National Park, Kazakhstan

Species	Shchuchye	Abylaykhan	Baldauren
Calicium subtila			+
Caloplaca aurantiaca			
Caloplaca murorum			
Candelaria concolor			+
Cetraria cucullata			
Cetraria glauca			
Cetraria pinastri			
Cladonia pyxidata	+		
Cladonia alpestris	+	+	+
Cladonia alpicola			
Cladonia cenotea	+		
Cladonia coccifera		+	
Cladonia coniocraea			
Cladonia cornuta			
Cladonia crispata			
Cladonia deformis			
Cladonia fimbriata			
Cladonia gracilis			
Cladonia rangiferina			
Cladonia rangiformis			
Cladonia strepsilis	+		
Cladonia sulvatica			
Cladonia tenius	+		
Cladonia turgida			
Cladonia vertcillata			
Dermatocarpon miniatum		+	
Evernia prunastri	+		+
Gasparrinia elegans			
Gyrophora murina	+		+
Gyrophora polyphylla			+
Haematomma ventosum			+
Hypocenomyce scalaris	+		+
Hypogymnia physodes			+
Lecanora cenisea			+
Lecidea glomerulosa			
Letharia thamnodes			
Parmelia olivacea			
Parmelia scortea			
Parmelia vagans		+	
Parmeliaceal caperata			+
Parmeliaceal saxatilis	+	+	
Parmeliaceal sulcata	+		+
Parmeliae conspersa	+	+	
Parmeliae ulophyllodes			+
Parmeliopsis ambiqua			
Parmeliopsis pallescens			
Parmeliopsis hyperopta			+
Peltigera canina			
Peltigera rufescens			
Peltigera spuria			
Physcia aipolia	+		+
Physcia stellaris			+
Psora lurida			
Rhizocarpon geographicum	+		
Verrucaria nigrescens	+		
Xantoria candelaria	+		+

Table 2. Abundance and projective cover of *E. prunastri* in Shchuchye and Baldauren sites at varying tree heights, Kazakhstan

Sites	Tree trunk height above ground level, cm	Total tree trunk area studied (m^2)	Tree trunk diameter (cm)	Number of *E. prunastri* individuals	Projective cover of *E. prunastri* (%)
Shchuchye	60	552	84.3±0.9	11.3±0.5	33.6
Baldauren		552	84.2±0.7	6.4±0.3	31.7
Shchuchye	90	276	82.0±0.5	6.1±0.2	28.4
Baldauren		276	84.2±0.6	4.7±0.4	26.0
Shchuchye	120	276	79.1±0.8	4.8±0.8	24.6
Baldauren		276	82.1±0.4	3.5±0.2	16.6
Shchuchye	150	276	75.4±0.5	3.9±0.4	19.1
Baldauren		276	80.0±0.3	2.5±0.3	13. 0

Table 3. Heavy metal content in *Cladonia alpestris* samples (μg/g) in Abylaykhan and Shchuchye at varying distances from the road edge

Sites and distance from the road edge (m)		Cd	Cu	Pb	Zn	Mn	Cr	Co	Be	As	Cs	Ga	V
Shchuchye	50	0.20±0.02	3.4±0.5	4.3± 0.70	26±4	61±10	4.0± 0.6	0.40±0.06	0.050±0.008	2.1±0.34	0.20±0.04	0.50±0.08	3.1± 0.5
	100	0.10±0.02	5.4±0.8	3.8± 0.60	33±5	81±13	4.2± 0.7	0.60±0.09	0.040±0.007	2.0±0.35	0.20±0.04	0.50±0.08	4.3± 0.7
	150	0.10±0.01	3.3±0.5	2.6± 0.40	29±5	65±10	5.2± 0.8	0.40±0.06	0.030±0.005	1.8±0.35	0.20±0.03	0.40±0.40	2.7± 0.4
	200	0.08±0.01	3.8±0.6	2.5± 0.40	31±5	44±7	3.4± 0.5	0.40±0.07	0.030±0.005	1.9±0.33	0.20±0.03	0.50±0.08	3.7± 0.6
Abylaykhan	50	0.60±0.09	9.0±1.0	25.0±0.40	71±11	120±19	22.0±3.4	1.4±0.21	0.20±0.03	4.3±0.7	0.7±0.1	1.6±0.24	10.0±1.6
	100	0.10±0.02	4.4±0.7	3.1± 0.50	31±5	76±12	3.8± 0.6	0.50±0.08	0.040±0.007	1.8±0.33	0.20±0.4	0.50±0.08	4.3± 0.7
	150	0.10±0.02	3.2±0.5	3.8± 0.60	21±3	39±6	3.4± 0.5	0.40±0.06	0.040±0.007	1.8±0.33	0.200.03	0.40±0.07	2.8± 0.4
	200	0.10±0.02	3.0±0.5	3.8± 0.60	21±3	38±6	3.5± 0.6	0.40±0.06	0.040±0.006	1.8±0.34	0.20±0.03	0.50±0.08	2.7± 0.4
MAC		0.035	8.0	1.25	30.0	205.0	1.8	0.5	0.10	0.12	0.12	0.05	1.5

Importantly, the samples had no clear differences in terms of the heavy metals absorbed. This suggests that the air and soil pollutants are uniformly distributed in the area, differing only in concentration. In our study, the concentrations of arsenic and gallium in *C. alpestirs* samples were 15 and 10 times above the limit threshold, respectively, suggesting that these two elements are of major concern in the study area. Arsenic is a group I carcinogen and presents a major threat to all living organisms including humans (Bajpai and Upreti 2012). Although gallium is considered non-toxic in its elemental form, its compounds present danger to humans when inhaled or swallowed (Ivanoff et al. 2012).

The concentrations of cadmium, lead, chrome, caesium and vanadium were 1.7-3.0 times above the limit threshold. All these elements are, to some extent, toxic to plants, animals and humans. Cadmium has no role in plant or animal metabolism and can damage various organs and systems of living organisms; in humans, it can potentially lead to cancer (Hayat et al. 2019). Lead is the second most toxic metal after arsenic. It is cancerogenic and can cause multiple health problems in humans, particularly in children (Kumar et al. 2020). The most common oxidation state of chrome, Cr(VI), is a priority control pollutant in many countries due to its carcinogenic, mutagenic and teratogenic effects (Sun et al. 2022). Non-radioactive caesium does not present a significant environmental hazard, because its compounds are only mildly toxic. However, in high concentrations, caesium can damage the cardiovascular system in humans (Melnikov and Zanoni 2010). A trace element vanadium is required in small quantities by living organisms, both marine and terrestrial. Nevertheless, all vanadium compounds should be considered toxic (Srivastava 2000).

Lichens have been widely used for the biomonitoring of airborne pollutants in forest ecosystems, mainly for detecting background levels of pollutants (e.g. Cecconi et al. 2018). Our approach, on the other hand, can be useful for detecting local accumulations of heavy metals related to vehicular traffic and high tourist influence. In their review of bioaccumulation surveys, Frati and Brunialti (2023) conclude that "the study of epiphytic lichens as early-warning systems to detect signs of a changing environment in forest ecosystems can be confirmed as effective."

To our knowledge, no biomonitoring studies of airborne pollutants have been carried out in Kazakhstan. However, reports from other countries are largely in line with our results. For example, a study carried out in Italy reported low background levels of air pollution in forest ecosystems, but detected signs of contamination by lead due to vehicle traffic in one study site subjected to high touristic pressure (Loppi and Printsos 2003). In Costa Rica, the projective cover and species richness of lichens were statistically significantly lower at the roadside than in the primary forest, presumably due to the strong effects of air pollution near the road (Bedell-Stiles 2004). In two Central Ontario forests, Canada, a study found a significantly greater percent coverage of macro lichens in a site located next to a low-use road than in a site located next to a high-use road (Cowden et al. 2018). In both sites, there was a positive relationship between the distance from the roadside and microlichen coverage. The findings suggest that the site located next to a high-use road experienced higher levels of air pollution and human disturbance over 100m into the forest.

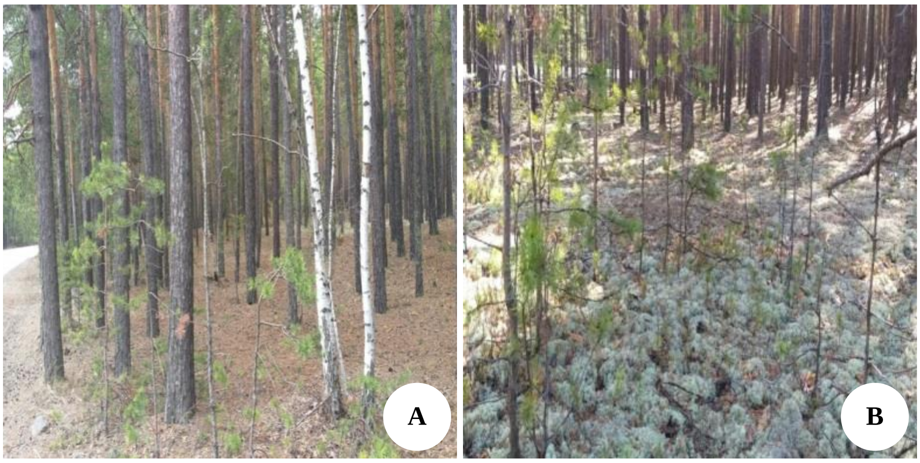

Figure 5. *Cladonia alpestris* in the Burabay National Park, Kazakhstan: A. *C. alpestris* is extremely rare at a distance of 50 m from the roadside; B. *C. alpestris* at a 100-200 m distance from the roadside

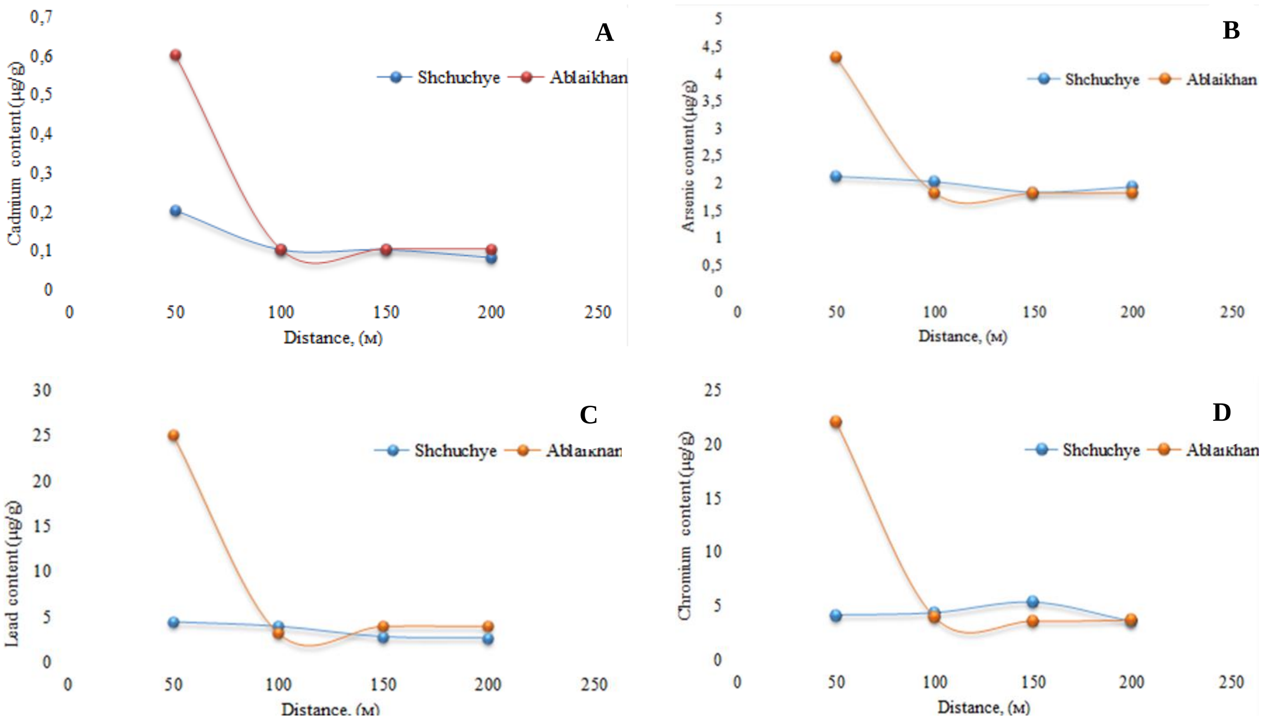

Figure 6. Heavy metal concentrations in *C. alpestris* samples at various distances from the roadside

Compared with urban and industrial areas, forests are generally subject to lower air pollution levels (Frati and Brunialti 2023). However, our study demonstrates that even within remote forest areas such as Burabay National Park, road traffic may present a major source of pollutants to the roadside environment. According to our results, concentrations of such heavy metals as lead, zinc, and arsenic exceeded the maximum allowable concentrations at a distance of 200 m from the road edge. In forest ecosystems, pollution by heavy metals may pose a threat to all life forms and cause biodiversity loss, community composition changes, and growth and reproductive rate reduction (Mitra et al. 2022). All heavy metals, to some extent, interfere with chlorophyll synthesis in plants. In lichens, the degradation of chlorophyll in chlorophyll-containing partners (photobions) is one of the most noticeable signs of damage (Bajpai and Upreti 2012). High concentrations of heavy metals in the study area are particularly worrying because they were detected in a specially protected area designated for tourism and recreation.

Based on the results of the analysis, we recommend *E. prunastri* and *C. alpestris* be used as bioindicators of air pollution in the Burabay National Park, because they are found in abundance on roadsides and in the forest, can reproduce asexually by thallomes, and demonstrate sensitivity to air pollution from road traffic. Because these species are widely distributed in the territory of Kazakhstan, they should be tested as potential bioindicators in other regions.

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