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Anatomical and morphological changes in *Pinus sylvestris* and *Larix sibirica* needles under impact of emissions from a large aluminum enterprise

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Abstract

Species-specific anatomical and morphological characteristics of *Pinus sylvestris* and *Larix sibirica* needles were studied at different levels of tree stand pollution by aluminum smelter emissions. The anatomical characteristics of the needle were studied using light microscopy. The level of tree stand pollution was determined using the cluster analysis outcomes of the pollutant elements content (fluorine, sulfur, and heavy metals) in the needles. Four levels of tree stand pollution were separated: low, moderate, high, and critical, as well as background tree stand in unpolluted areas. It was found that the state of tree phytomass deteriorated with increasing levels of pollution (from low to critical): pine crown defoliation increased to 85%, and larch defoliation increased to 65%. The life span of pine needles was reduced to 2–3 years, with a background value of 6–7 years. The change of morphological parameters was more pronounced in *P. sylvestris:* the weight and length of the 2-year-old shoot decreased by 2.7–3.1 times compared to the background values; the weight of needles on the shoot and the number of needle pairs on the shoot—by 1.9–2.1 times. The length of the needle and shoot and the number of *L. sibirica* brachyblasts decreased by 1.8–1.9 times. The anatomical parameters of the needle also changed to a greater extent in *P. sylvestris*. Up to the high level of tree pollution, we observed a decrease in the cross-sectional area of the needle, central cylinder, vascular bundle, area and thickness of mesophyll, number and diameter of resin ducts by 18–66% compared to background values. At the critical pollution level, when the content of pollutant elements in pine needles reached maximum values, the anatomical parameters of the remaining few green needles were close to background values. In our opinion, this may be due to the activation of mechanisms aimed at maintaining the viability of trees. A reduction in thickness and area of assimilation tissue in the *L. sibirica* needle was detected only at the critical pollution level. An upward trend in these parameters was found at low, medium, and high pollution levels of tree stand, which may indicate an adaptive nature. The results suggested that at a similar pollution level of trees, the greatest amount of negative anatomical and morphological changes were recorded in pine needles, which indicates a greater sensitivity of this species to technogenic emissions.

Keywords *Pinus sylvestris* · *Larix sibirica* · Anatomical and morphological characteristics of needle · Aluminum smelter

Introduction

At present, atmospheric industrial pollution is one of the significant negative factors affecting the ecological state of boreal forests (Aamlid and Venn 1993; Alexeyev 1995; Gytarsky et al. 1995; Awang et al. 2007; Mikhailova et al. 2008; Manninen et al. 2015; Takahashi et al. 2020). It should be noted that the most critical situation is developing in the territories where aluminum smelters are located (Vike and Hăbjorg 1995; Vike 1999; Kalugina et al. 2017). It has long been known that aluminum smelter emissions are highly toxic (Guderian 1979; Rozhkov and Mikhailova 1993) and contain pollutants such as fluorides (Haidouti

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et al. 1993; Weinstein and Davison 2004; Panda 2015; Rodrigues et al. 2018) and polycyclic aromatic hydrocarbons (Xue et al. 2010; He et al. 2014; Waqas et al. 2014; Gao et al. 2016) which are highly damaging to biota. Despite significant improvements in aluminum production technology (use of electrolytic cells with preliminary baked anodes) and industrial emission cleaning technologies (introduction of "Environmental Soderberg" technology), it has proven impossible to completely eliminate many polluting agents (Bonte and Cantuel 1981; Kulikov and Storozhev 2012; Brough and Jouhara 2020).

The reponses of trees to impacts of anthropogenic emissions may be identified by changes in various informative indicators; including biochemical, physiological, (Blokhina et al. 2003; Chupakhina and Maslennikov 2004; Foyer and Noctor 2015; Mikhailova et al. 2017) anatomical, morphological (Grossoni et al. 1998; Mandre and Lukjanova 2011; Yodgorova 2022), and visual changes, for example, by the defoliation level of tree crowns. We proceed from the assumption that the results obtained on the change of indicators quite adequately reflect the balance between pathological and adaptive processes in the plant organism. Since the impact of a negative factor is investigated by its gradient (for example, as it increases), the development of adaptive (protective) reactions in dynamics may be presented. We have previously considered biochemical and physiological indicators of *Pinus sylvestris* L. (Scots pine) and *Larix sibirica* Ledeb. (Siberian larch) needles and found out that technogenic pollutants cause metabolic disturbances, resulting in the development of oxidative stress as a result of the excessive generation of reactive oxygen species (Kalugina et al. 2018). The great importance of peroxidase and non-enzymatic antioxidants (ascorbic acid, glutathione, phenolic compounds, and proline) in preventing oxidative damage to the assimilation organs of these trees caused by pollutants was demonstrated (Kalugina et al. 2021; 2022). At the same time, the degree of manifestation of the protective functions of these substances varied depending on the pollution level of the needles. Active adaptation processes were found to take place at low, moderate, and even high levels of needle pollution. Their obvious suppression was detected only at a critical pollution level of the needles, and at the same time, there were obvious signs of metabolic abnormalities and the predominance of pathological changes. This was evidenced by such indicators as a sharp decrease in the content of photosynthetic pigments, carbohydrates, certain fatty acids, and proteins. It may be logically assumed that physiological and biochemical disturbances should cause anatomical and morphological changes in the needles and shoots and a decline in growth parameters in trees. Therefore, this work, as a follow-up to the previous ones, is devoted to identifying such changes under the influence of highly toxic technogenic emissions from aluminum production.

Analyzing published data, we found that there are few works on this topic, especially for severe natural and climatic conditions in the boreal zone. Anatomical changes in the needles of six coniferous tree species aged 6–10 years were studied under experimental conditions using artificial fumigation with hydrogen fluoride (Mikhailova and Berezhnykh 1995). The authors established that hydrogen fluoride, to the highest extent, affects the cells of the mesophyll and central cylinder, causing their severe deformation and destruction, resulting in a decrease in the thickness of the chlorophyll-bearing tissue. At the same time, the damage in larch needles develops faster than in evergreen species, because of a thinner cuticle. In natural conditions, the effect of technogenic emissions on the change in the anatomical structure of assimilation organs was studied mainly in deciduous species; among coniferous species, trees of the Pinus genus were studied (Kurczyńska et al. 1996; Grossoni et al. 1998; Lin et al. 2001; Skripal'shchikova et al. 2016; Tuzhilkina and Plyusnina 2020). Thus, Tuzhilkina and Plyusnina (2020) showed that long-term exposure of *P. sylvestris* to emissions from pulp and paper mills leads to the development of xeromorphic needle traits, i.e., its morphological parameters decrease. This is associated with a reduction in the number of mesophyll cells and the number and diameter of resin passages in the needle. As a result, the photosynthetic activity of trees and their resistance to pathogens have declined. A decrease in the cross-sectional area of the *P. sylvestris* needle, the central cylinder, and the size of vascular bundles was noted in the industrial area of the Krasnoyarsk aluminum smelter (Skripal'shchikova et al. 2016) and near the cement plant (Mandre and Lukjanova 2011). However, the nature of changes in many indicators is not linear and depends on many factors.

It should be noted that many researchers consider *P. sylvestris* sensitive to anthropogenic contamination (Mikhailova 2000; Stravinskiene et al. 2013; Dmuchowski et al. 2011; Chropeňová et al. 2016). Our long-term studies have shown that pine needles are a good bioindicator because they can accumulate both inorganic pollutants (sulfur dioxide, hydrogen fluoride, aerosols of heavy metals, aluminum, silicon, etc.) and persistent organic pollutants, in particular polycyclic aromatic hydrocarbons, from polluted air. Due to the small surface area of the needle and its thickened layer of covering tissue with a few stomata, the removal of absorbed elements from the needle surface during the processes of transpiration and gas exchange is very small, and during their lifetime (4–7 years, depending on growing conditions), pine needles accumulate a significant amount of toxic elements (Arnesen et al. 1995). In addition, the level of needle contamination is usually directly proportional to the quantitative change in various morphological and physiological-biochemical parameters of needles (Kalugina et al. 2018).

The scientists have no common opinion about Larix's resistance to air pollution. Some researchers suggest that *Larix* is a very plastic deciduous conifer species that can adapt to adverse environmental conditions (Chylarecki 1991; Rozhkov and Mikhailova 1993). Besides, the annual falling needles in the autumn period lead to exemptions from pollutants and, consequently, greater resistance to air pollution (Kozlowski and Pallardy 2020; Donovan et al. 2005). The large surface area of larch needles compared to other conifers allows them to reduce respiratory costs and use photoassimilates more efficiently for growth processes, including when exposed to adverse factors (Zagirova 2015). Other authors, on the contrary, cite data about their low tolerance for environmental contamination. The disturbances in the morphology and anatomical structure of female generative organ larch trees were observed in a polluted urban environment (Seta-Koselska et al. 2014). On the territory of Abakan, a decrease in the length and area of needles and shoots and a radial increment of Siberian larch have been reported (Abramenko 2015). Our research has shown that larch needles can be used as a bioindicator of air pollution, as they accumulate concentrations of pollutants comparable to needle pine (Afanasyeva et al. 2021). However, the pattern of changes in the biochemical parameters of needles does not always have a linear dependence on the level of contamination by pollutants (Kalugina et al. 2022). The contradictory data regarding *L. sibirica* resistance to anthropogenic pollution, as well as the generally poorly studied response of conifers to highly aggressive emissions from aluminum production, determine the relevance of such studies.

The present work is aimed at the study of species-specific anatomical and morphological changes in *P. sylvestris* and *L. sibirica* needles at different levels of pollution by highly toxic emissions from the aluminum smelter. We hypothesized that the ecological features of these tree species cause the formation of different adaptation strategies to anthropogenic pollution, including morphological (macroscopic) and anatomical (microscopic) levels. Given the thinner cuticle and the smaller proportion of the resin passage area to the total needle area, we assumed that the *L. sibirica* needle would be more damaged than the *P. sylvestris*, which has more time to include adaptation mechanisms.

Materials and methods

Study area

The present study was conducted in the northwestern part of the Irkutsk region (located between the 55°50' and 56°35' N latitudes and 101°00' and 102°35' E longitudes), in the vicinity of the city of Bratsk (Russia) (Fig. 1). The city is classified as a settlement with a high level of atmospheric air pollution (State … 2022). The main source of pollution is the Bratsk aluminum smelter (BrAZ), commissioned in 1966. This is the largest enterprise not only in Russia but also in the world, producing more than one million tons of primary aluminum per year. Analysis of the emission dynamics over the period of smelter operation showed that in the first 20 years after start-up, due to the increase in production rates, the volume of emissions was the highest (about 220 thousand tons per year). In subsequent years, as a result of process improvements, the amount of emissions gradually decreased, and in 2014, it amounted to 80 thousand tons. In the last decade, the amount of emissions has varied between 75 and 80 thousand tons per year (State … 2022).

The most toxic components of BrAZ emissions (hazard classes 1–3) are fluorinated compounds, in particular hydrogen fluoride and solid fluorides, as well as sulfur dioxide, nitrogen oxides, polycyclic aromatic hydrocarbons (PAHs), and solid aerosols with a huge share of aluminum, silicon, and heavy metals.

The surveyed territory is characterized by mountain-hollow, intensely rugged terrain. By climatic characteristics, it is equated to the regions of the Far North. The climate here is extremely continental; daily temperature fluctuations are 10–15 °C, the average annual air temperature is −2 °C, the amplitude of extreme temperature fluctuations is from −60 to +38 °C, the frost-free period is 80–100 days, 30–35 cm high snow cover persists for 174–180 days, and the average annual precipitation is 370–460 mm per year.

Intense cooling of the territory causes the development of fogs, which can persist for several days. Winds of the western, south-, and north-western directions prevail (Climate … 1985). In general, orographic and meteorological features of the territory result in a low self-purification potential of the atmosphere and contribute to the concentration and stagnation of industrial emissions in the surface layer. At the same time, emissions from semi-closed basins can be dispersed over considerable distances from the plant.

Objects of study

Primary mixed forests of *P. sylvestris* and *L. sibirica* dominate the surveyed area. Therefore, these two species of trees were chosen by us as objects of study. In 2020, 29 test sites (TS) were established in accordance with the ICP Forests methodology (Cools & De Vos 2010). The area of each TS was about 0.01 km^2. TSs were laid in the industrial zone of BrAZ, as well as at different distances (up to 150 km) and in different directions from it. The laying of the TS was carried out in areas similar in terms of forest

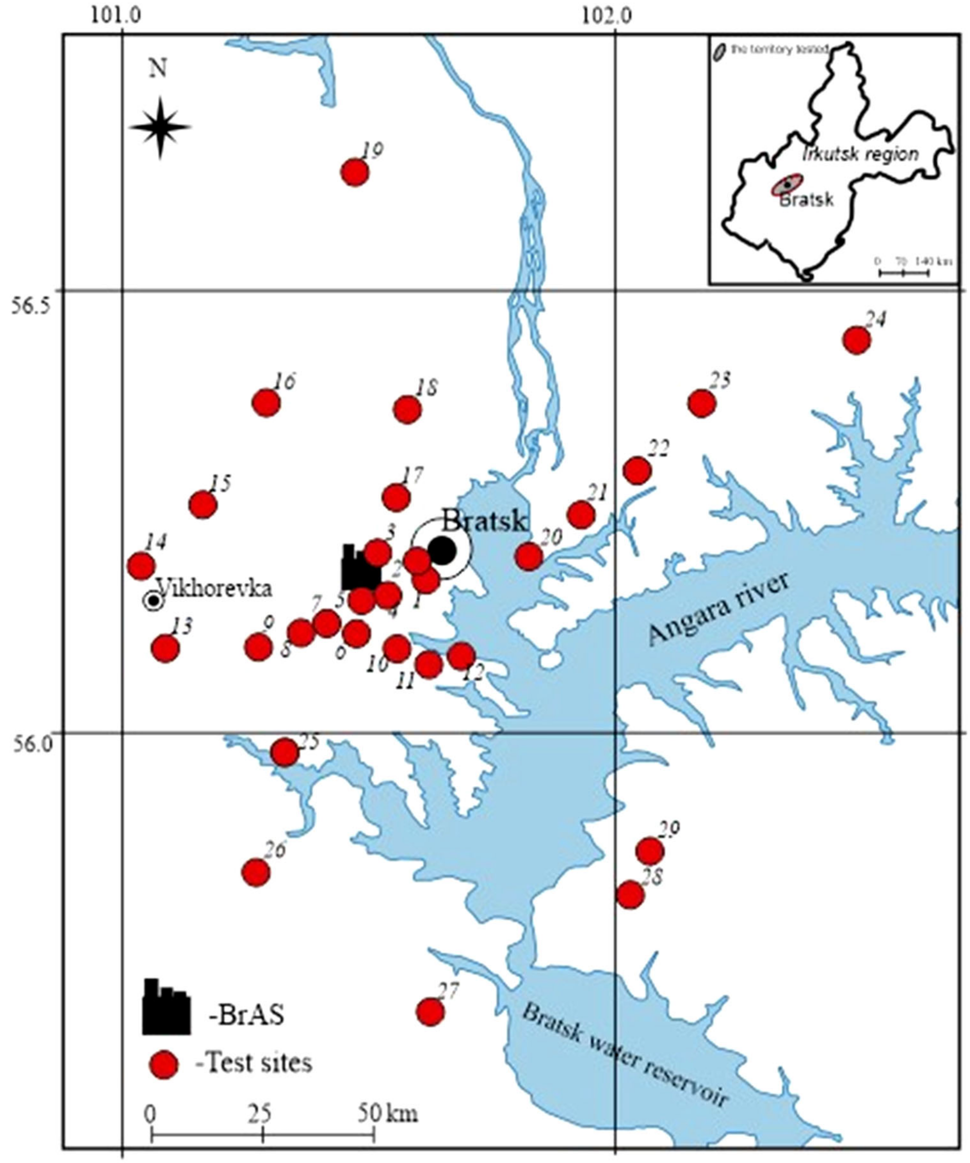

Fig. 1 Map-scheme of the surveyed territory

conditions (group forest types - *Pinetum herbosum*, microrelief - small hillocks, soil type - well-drained rendzic leptosols eutric (WRB … 2006), medium-thick with a loamy texture and pH of 5.6–6.0, atmospheric moisture optimal (Geographical … 2017). The composition of tree stand at the studied TSs is 6P4L–8P2L, tree age is 70–80 years, and the average density of tree stand –0.5–0.7 (see supplementary material). The total area of the surveyed forests was more than 10,000 km^2.

Sampling methods

Samples of *P. sylvestris* and *L. sibirica* needle were taken on sunny days without precipitation, in the middle of the vegetation period, at the end of the shoot and needle growth phases (from July 20 to 25). At each TS, 5–7 lateral shoots from the south and southeast sides were cut with a Gardena pruner from the middle part of the crowns of five 40-year-old trees without visible signs of diseases or pests. To study the anatomical peculiarities of each cut shoot, 10–20 pairs of the second-year-old needle were randomly selected as the most physiologically active (in *P. sylvestris)* or 5–10 needles with 5–7 brachyblasts (in *L. sibirica*) and fixed in 70% ethanol. The remaining shoots were placed in Kraft paper bags and delivered to the laboratory, where the morphometric parameters of the shoots and needles were calculated. Next, the needles were separated from the shoots and thoroughly mixed, forming averaged samples of *P. sylvestris* and *L. sibirica* needles for each TS. The average weight of the mixed sample was 0.5–1.5 kg. The needles were dried for 48 h at 60 °C, then pulverized on a Bosch electric grinder and sieved through a sieve with a 0.5 mm aperture diameter. The samples were stored in paper bags. Subsequently, needles were used to determine the content of chemical elements.

Study of the visual and morphological parameters of trees

Visual parameters were assessed at each TS in the field: needle life span (in *P. sylvestris*), the level of tree crown defoliation (in percentage using the atlas of W. Bosshard (1986)), and the number of necrotic needles (percentage of the total number of needles in the crown). In laboratory conditions, the morphological parameters of the needle and shoot were measured: the length and weight of the 2-year-old shoot, the length and weight of the needle, the number of needle pairs on the 2-year-old shoot (in *P. sylvestris*) and the number of brachyblasts on the 2-year-old shoot (in *L. sibirica*) (Fig. 2). All parameters were measured in 30–50 repetitions (randomly selected needles and shoots) with an accuracy of 1 mm (linear parameters) and 0.001 g (weight). The mean value of each parameter and standard deviation were calculated.

Study of the anatomical parameters of needles

For anatomical observations, needle samples were washed in water, and cross-sections were prepared. To obtain them, the needle samples were frozen at a temperature of −18 °C for 1–3 min, having previously closed the working area of the freezing table with a cap. Needle samples were sectioned in the transverse plane at a thickness of 30 μm using the freezing microtome (Leitz, Germany) in the middle part of the needle and placed on a slide in glycerin. Sections were not stained. The cross-sections were viewed and photographed using an Axio Scope A1 light microscope (Carl Zeiss, Germany) with a DCM-900 digital camera. The

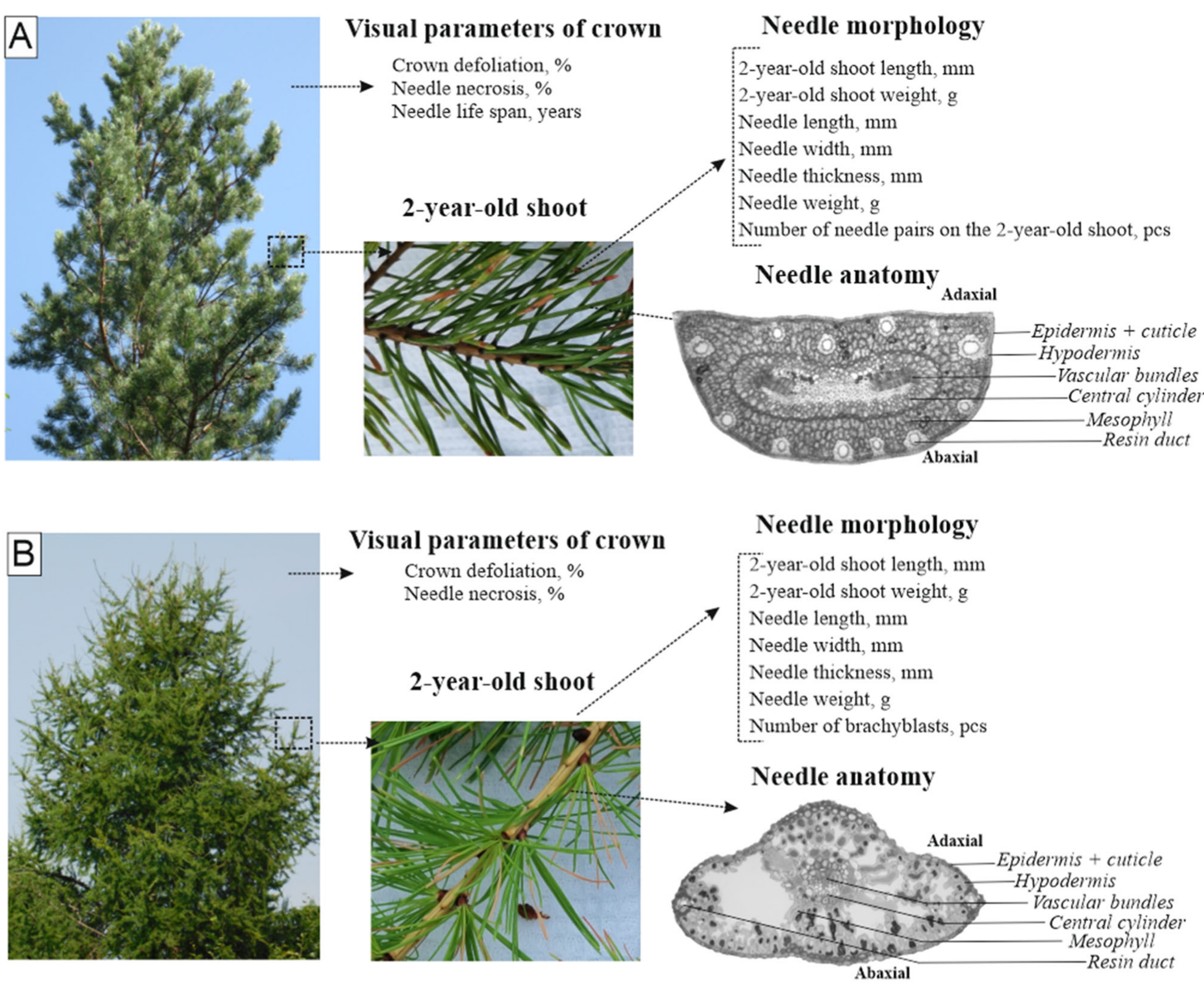

Fig. 2 Scheme of the needle *P. sylvestris* (**A**) and *L. sibirica* (**B**) sampling and measure visual, morphological and anatomical parameters of needle examined in the present study

images were processed using the ScopePhoto 3.0 program. Digital images were measured and analyzed using PhotoMaster 1.31 software. On each slide, four to five replicates of needle width and needle thickness, thickness of epidermis plus cuticle, thickness of hypodermis, thickness of mesophyll tissue, resin duct diameter, and number were measured. The needle cross-sectional area, the relative area occupied by the central cylinder, mesophyll tissue, and vascular bundles were determined (see Fig. 2). The ratio of the areas of the mesophyll and the central cylinder was considered an indicator characterizing the efficiency of the assimilating tissue and vascular bundles (Bender et al. 2008). According to the value of the ratio of the cross-sectional area of the central cylinder to the cross-sectional area of the needle, the proportionality of the development of the tissues of the needle was judged.

Analytical procedures

To assess the level of pollution in needles and their accumulative capacity, the concentration of 32 chemical elements were evaluated, including macro-elements (N, Ca, K, Mg, P, S, Si), biophilic microelements (Na, Mn, Fe, Zn, Cu, Co, Cr) and phytotoxic elements which often present in the technogenic emissions of an aluminum smelter (F, Al, Ni, Pb, Cd, Ba, Sr, Ti, V, As, La, Li, Be, Sc, Y, Sb, Mo, Ce). The ash content of the pine needles was also determined. The dry crushed needles were used for the analysis of the elemental composition. Fluorine content in the needles was determined spectrophotometrically at a wavelength of 540 nm with a xylene orange indicator after dry mineralization of the sample and distillation of the resulting ash with water vapor in perchloric acid, using silver sulfate to remove concomitant chlorine impurities (Awang et al. 2007).

The content of nitrogen was determined by photocolorimetric method after wet mineralization of needles in sulfuric acid at 80–120 °C. To determine the content of ash and the concentration of other elements, needle samples were mineralized in a muffle furnace at 450 °C for three hours until they turned into homogeneous ash. The ash was weighed, ground in an agate mortar, and dissolved in 0.1 M nitric acid.

The content of elements in the solutions obtained was determined by the atomic emission method on the spectrometer SPECTRO ARCOS (company "Spectro Analytical Instruments GmbH", Germany) in the certified laboratory of the State Enterprise "Republican Analytical Center" (Ulan-Ude, certificate of accreditation No. ROSS RU.0001.511112). To control the analytical quality of the procedures, standard samples of NCS DC 73350 were used, with the relative error of the method not exceeding 5–10%. Concentrations of inorganic elements in the needles were determined in five biological and three analytical repetitions. The concentrations of chemical elements in needles were expressed in mg/kg dry weight.

Zoning of the surveyed territory according to the level of tree stand pollution was carried out based on the results of data cluster analysis on the content of pollutants in the needles. Indices of biogeochemical transformation of the needle's elemental composition *(Zbt)* were calculated for each pollution level according to the formula:

$$Zbt = \sum_{l=1}^{n1} EF + \sum_{l=1}^{n2} DF - (n1 + n2 - 1),$$

where EF = Cpol/Cb and DF = Cb/Cpol are local concentration and dispersion coefficients, respectively; Cpol is the element concentration in needles in the polluted area, mg/kg dry weight; Cb is the element concentration in the background area, mg/kg dry weight; n1 and n2 are the numbers of the elements with EF > 1 and DF > 1 (Kasimov et al. 2012). The *Zbt* index has following grades corresponding to low (10–30), moderate (31–50), high (51–80), and critical (>81) disturbance.

Statistical tests

The figures and tables present the average values of each parameter (M) and their standard deviations (δ). Statistical data processing was carried out using the MS Excel 2016 application software package and the "Statistical Computing Environment R", version 4.3.1. (2023). The obtained data were tested for normality (Shapiro–Wilk's test, $P < 0.05$) and dispersion equality (Levene's test). To assess a statistically significant dependence, the Pearson correlation coefficient was calculated. The assessment of the variability of anatomical and morphometric parameters used coefficient of variation (V,%), taking into account the scale (Zaitsev 1973): V < 10% - low level of variation, V = 11–20% - moderate level, V > 20% - high level.

Results

The surveyed area was zoned in accordance with the pollution levels, which were determined using the cluster analysis of the data on the content of element pollutants in the needles. The four pollution levels of tree stand were separated into low, moderate, high, and critical, as well as background tree stand in unpolluted areas. Analysis of the needle samples from the background areas showed that the chemical elements and ash content in *L. sibirica* needles were greater than in *P. sylvestris* needles (Fig. 3).

In both species, biogenic macroelements predominated in the needles, in particular in *P. sylvestris* these were N

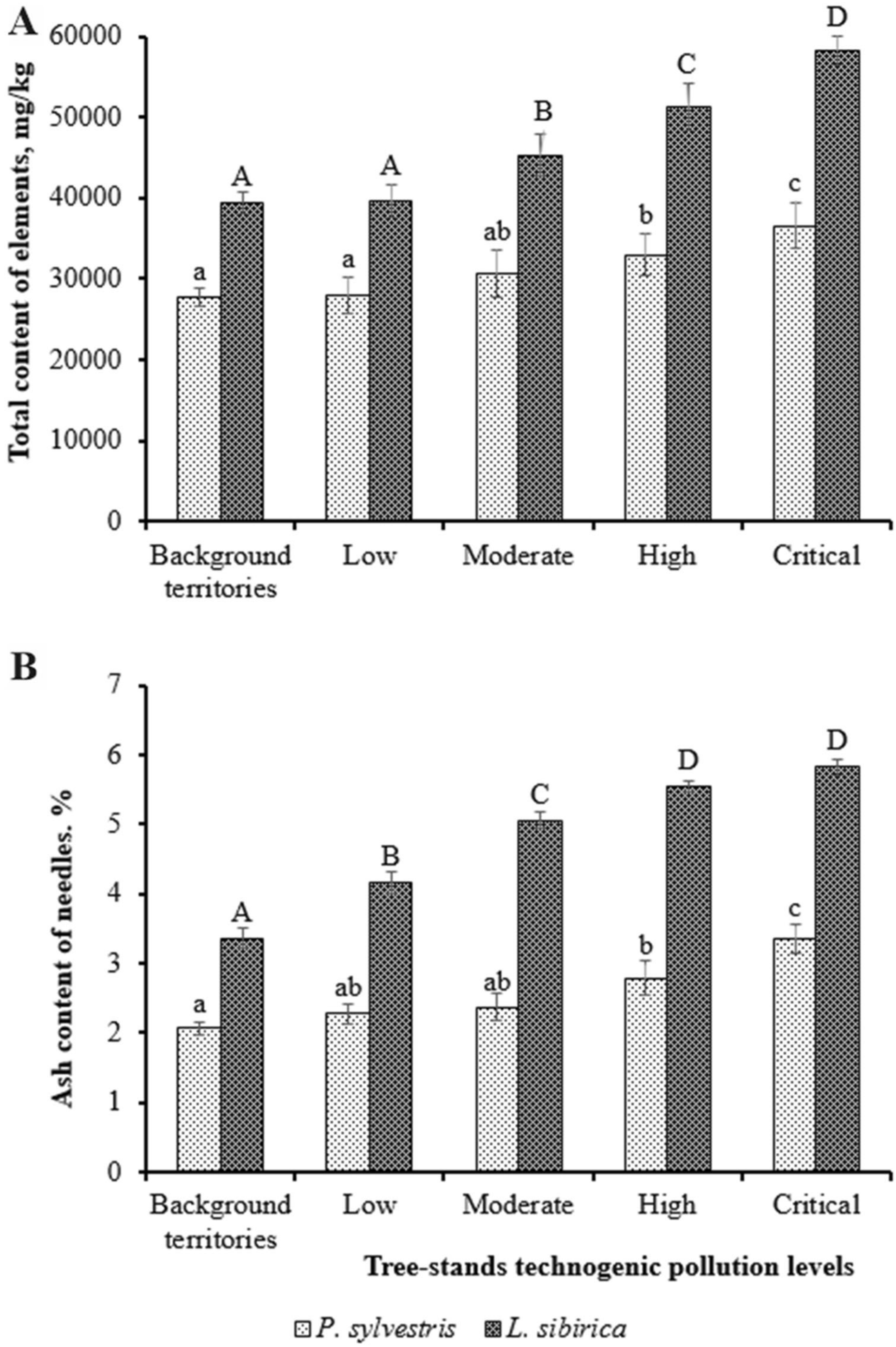

Fig. 3 Overall content of chemical elements (**A**) and ash content (**B**) in *P. sylvestris* and *L. sibirica* needles at different levels of technogenic pollution by BrAZ emissions. Different letters in the rows for each species represent statistically significant differences between zones, where a < b < c < d

(53–57%), Ca (11–15%), K (6–14%), P (5–9%), and Mg (3–4%), and in *L. sibirica* these were N (41–49%), Si (16–22%), K (9–12%), Ca (8–10%), and Mg (4–5%). There were also identified differences between *P. sylvestris* and *L. sibirica* in the content of trace elements in the background territories. For instance, the needles of *L. sibirica* exhibited a higher content of Ni and Pb by an average of 1.2 times, Co and Ti by 1.5 times, Si, Sc, V, and Cu by 1.7 times, S and Fe by 1.8 times, Cd by 2.0 times, Sr by 3.5 times, and Ba by 14.0 times, compared to *P. sylvestris*. At the same time, the concentrations of Li and Zn were higher in *P. sylvestris* needles by 1.9 and 2.6 times, respectively.

In polluted areas, the overall content of chemical elements and the ash content in *P. sylvestris* and *L. sibirica* needles increased significantly, especially at high and critical pollution levels (see Fig. 3). This is due to an increase in the concentrations of nitrogen, calcium, silicon, magnesium and primary pollutants contained in the smelter emissions – fluorine, sulfur, aluminum, and heavy metals (Fig. 4). It was found that each pollution level corresponded to a certain range of concentration coefficients (*EF*) of pollutants in needles and values of the biogeochemical transformation index of the needle elemental composition (*Zbt*) (Table 1).

Visual and morphological needle traits

The impact of BrAZ emissions resulted in significant negative changes in visual indicators of the state of the assimilation phytomass of trees. Thus, the level of tree crown defoliation increased significantly even under moderate pollution; under high pollution in pine, it reached 65–70%; in larch – 55–60%, under critical pollution, the defoliation of pine crowns was 75–85%; and larch crowns – 60–65% (Fig. 5). In addition, with an increase in the pollution level, we registered a decrease in the pine needle life expectancy up to 2–3 years (under background conditions – 6–7 years). The percentage of necrotic needles was high in polluted tree stand; at a high pollution level, it reached 30–40% in pine and 20–30% in larch, and at a critical pollution level, the necrosis of needles was up to 60–80% in both species. At low and moderate pollution levels, pine and larch needle necrosis were not detected.

In pine, some morphological parameters of the needle and shoot decreased with the increasing pollution level from low to critical (Table 2). At the critical pollution level, the weight and length of the 2-year-old shoot decreased by 2.7–3.1 times as compared to the background level; the weight of needles on the shoot – by 2.1 times; and the number of needle pairs on the shoot decreased by 1.9 times. The length, width, and thickness of pine needles changed insignificantly, so their use for indicative purposes is hardly reasonable.

The changes in the needle and shoot morphological parameters were also observed in the larch trees exposed to BrAZ emissions (see Table 2). The length of the needle and shoot, the weight of the 2-year-old shoot, and the number of brachyblasts on the shoot decreased along the pollution gradient. The minimum values of these parameters were found at the critical pollution level. Another trend was found in the change in width, thickness, and weight of the needle. The values of these parameters increased by 14–34% compared to background values with increasing pollution levels from low to high. At the critical pollution level, the width and thickness of the needle corresponded to background values, while the shoot weight decreased by 1.7 times compared to background.

Correlation analysis identified reliable direct links between the level of crown defoliation in both species and the accumulation of fluorine ($r = 0.91$–0.96, $n = 29$, $P < 0.05$), sulfur ($r = 0.74$–0.84, $n = 29$, $P < 0.05$), and heavy metals ($r = 0.64$–0.78, $n = 29$, $P < 0.05$). At the same time, we observed a high level of inverse correlations between the content of element pollutants in the needles and the weight of the shoot ($r = 0.54$–0.59, $n = 29$, $P < 0.05$), the length of the shoot ($r = 0.61$–0.67, $n = 29$, $P < 0.05$), and the weight of the needle ($r = 0.72$–0.78, $n = 29$, $P < 0.05$).

Anatomical needle traits

Changes in many anatomical parameters were detected in *P. sylvestris* under the influence of aluminum smelter emissions (Table 3). Statistically significant reductions in needle cross-sectional area, mesophyll area and its thickness in the upper part, cross-sectional areas of the central cylinder, and vascular bundles were already found at the low pollution level of tree stands. At the moderate pollution level, the cross-sectional area of the needle decreased as compared to background values by 26%, the thickness of mesophyll, the area of mesophyll, the cross-sectional area of the central cylinder decreased by 18–25%, and the vascular bundle decreased by 53%. The number of resin ducts and their diameter were significantly less than background values (36% and 17%, respectively).

The minimum values of almost all studied anatomical parameters were found at a high pollution level. Thus, the cross-sectional area of the needle was reduced by 38% compared to the background level and mesophyll thickness —by 21% (upper) and 19% (lower). The area of mesophyll was reduced by 30%, and destruction of its cells was observed. As a result, only a two-row layer of mesophyll was formed (on background TS, mesophyll is most often formed by three or four rows of cells with folded walls). The cross-sectional area of the central cylinder and vascular bundles decreased more significantly – by 43% and 66%, respectively, compared to background values. The number of resin ducts decreased by twice (Fig. 6), and their reduction occurred mainly on the adaxial side. The thickness of the epidermis together with the cuticle decreased by 14–16% at a high pollution level. At the critical pollution level, the epidermis thickness was smaller, in comparison with background parameters, by an average of 15%, the cross-sectional area of vascular bundles – by 29%, and the diameter of resin ducts – by 22% (see Table 3). The state of the resin-producing system also changed: the number of resin ducts increased to 11–12, but their diameter was below background values by 18–25% (see Table 3).

The anatomical parameters of the larch needle under exposure to aluminum smelter emissions changed slightly (Table 4). The decrease in the cross-sectional area of the central cylinder (at high and critical pollution levels), as

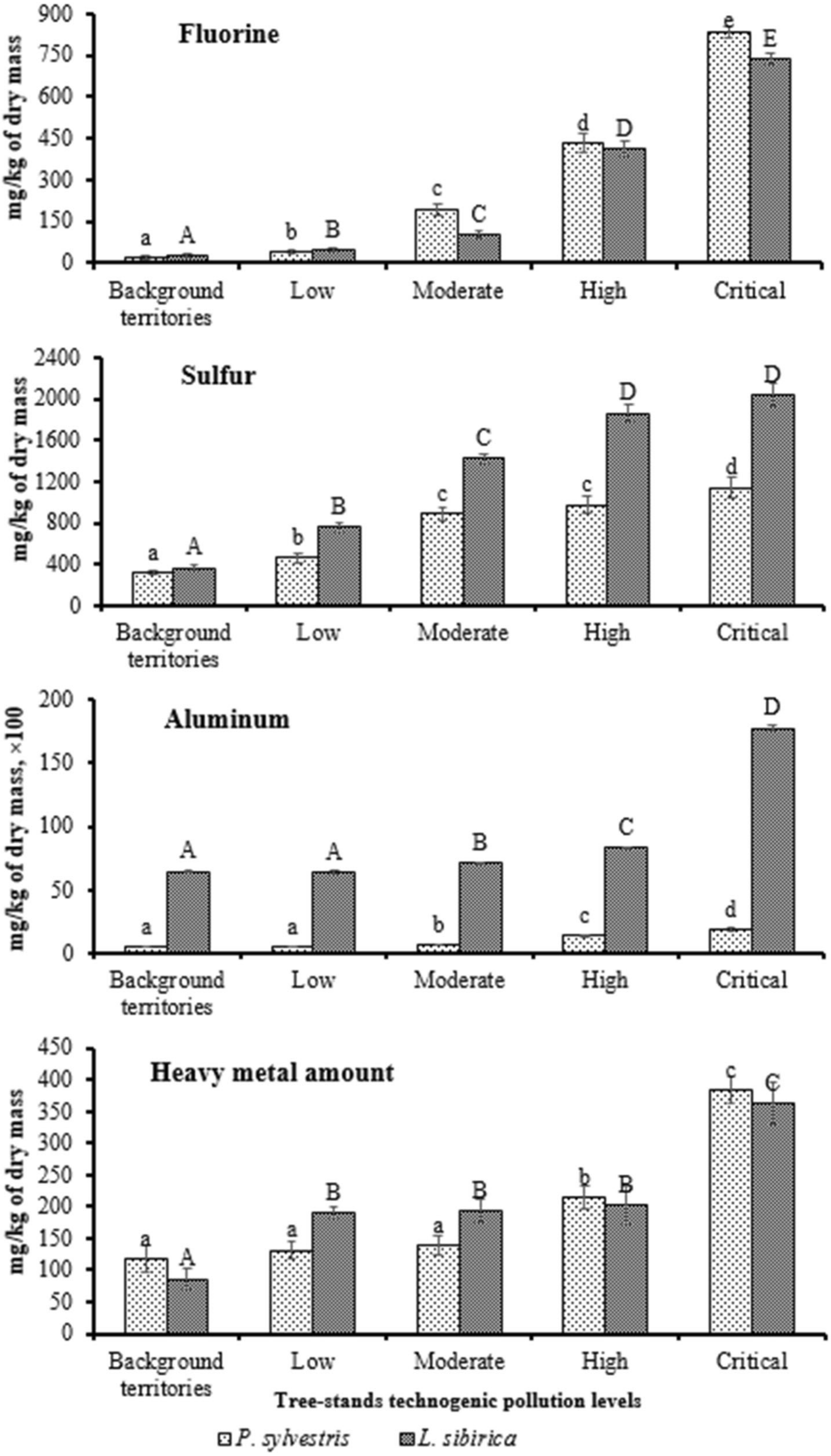

Fig. 4 The content of primary pollutants in *P. sylvestris* and *L. sibirica* needles at different levels of technogenic pollution by BrAZ emissions. Different letters for the same tree represent statistically significant differences between zones ($P < 0.05$), where $a < b < c < d < e$

well as the area of the vascular bundle at the critical pollution level was statistically significant. The cross-sectional area of needles increased significantly at the high pollution level, mesophyll thickness, at moderate and high pollution levels; and the diameter of resin canals, at moderate, high, and critical pollution levels (Fig. 7). The number of resin

Table 1 Concentration coefficients (*EF*) of pollutant elements and the index of biogeochemical transformation of needles elemental composition (*Zbt*) of *Pinus sylvestris* and *Larix sibirica* at different levels of technogenic pollution by BrAZ emissions

Technogenic pollution level	*P. sylvestris*		*L. sibirica*	
	Concentration coefficients of pollutant elements (EF)	*Zbt*	Concentration coefficients of pollutant elements (EF)	*Zbt*
Low	$Cd_{3.1} – Fe_{2.1} – Cu_{2.1} – Cr_{1.9} – Pb_{1.8} – Ni_{1.6} – F_{1.6} – Al_{1.6} –Zn_{1.6} – S_{1.5} – Si_{1.4} – Co_{1.2}$	24.1 ± 2.1	$Cd_{2.2} – F_{1.8} – Al_{1.8} – Co_{1.8} – Ni_{1.8} – Cr_{1.7} – S_{1.7} – Pb_{1.6} – Zn_{1.2} – Si_{1.1} –Fe_{1.1} – Cu_{1.1}$	13.4 ± 3.6
Moderate	$Cd_{4.8} – F_{4.4} – Al_{3.8} – Fe_{3.2} – Cu_{3.2} – Pb_{3.2} – Si_{3.1} – Cr_{2.1} – Ni_{1.8} – S_{1.7} –Zn_{1.7} – Co_{1.5}$	39.2 ± 5.4	$Ni_{3.3} – Al_{3.1} – F_{2.9} – Fe_{2.1} – Co_{2.1} – S_{2.0} –Pb_{1.9} – Cd_{1.7} – Cr_{1.7} – Cu_{1.6} – Si_{1.5} – Zn_{2.2}$	28.6 ± 4.8
High	$F_{18.6} – Cu_{7.3} – Cd_{5.7} – Al_{5.2} – Pb_{3.5}– Fe_{3.3} – Si_{3.2} – Cr_{2.6} –Ni_{2.2} – S_{1.9} –Zn_{1.9} – Co_{1.9}$	78.8 ± 13.8	$Al_{8.1} – F_{7.1} – Ni_{6.4} – Cr_{4.9} – Fe_{3.8} – Pb_{3.5} – Co_{3.1} – Zn_{2.2} – S_{2.1} – Cd_{1.7} – Si_{1.6} – Cu_{1.6}$	59.8 ± 10.6
Critical	$F_{54.7} – Al_{16.4}– Cd_{9.6} – Cu_{7.6} – S_{4.8} – Pb_{4.1}– Si_{3.6}–Fe_{3.5} – Cr_{2.9} –Ni_{2.5} – Co_{2.2} – Zn_{2.1}$	156.9 ± 17.6	$F_{31.8} – Al_{22.8} – Ni_{16.4} – Cr_{7.4} – Pb_{7.1} – Co_{3.7} – Fe_{3.8} – S_{2.8} – Cd_{2.0} – Si_{1.9} – Zn_{1.9} – Cu_{1.6}$	127.8 ± 13.4

ducts, unlike in pine, remained the same; only two resin ducts were in the larch needles, and they were located in the corners of the needle.

Calculation of the ratio of the central cylinder area to the needle cross-sectional area showed its multidirectional change in the needle of polluted pine trees: at low and moderate pollution levels, this indicator did not differ from the background; at a high level, it decreased to 0.268; and at a critical level, it exceeded the background value by 12% (Table 5). In larch, this ratio was quite stable at low and moderate pollution levels; at high and critical pollution levels it was 15–20% lower than the background value. The ratio of mesophyll area to central cylinder area increased in pine needles from low to high pollution levels and in larch from low to critical pollution levels (see Table 5). At the critical pollution level, the value of this ratio decreased.

Discussion

The impact of Bratsk aluminum smelter emissions, one of the largest primary aluminum producers in Russia and the world, on the morphological and anatomical changes in *P. sylvestris* and *L. sibirica* needles is presented in this study. *P. sylvestris* is the most common tree species in Northern Eurasia and has the largest ecological amplitude (Kelly and Connolly 2000; Vacek et al. 2016; Parfenova et al. 2021). It is a light-loving tree species with marked tolerance to nutrient-poor and dry soils (Mandre 2003; Vashchuk and Shvidenko 2006; Şofletea et al. 2020). However, in Siberia, the distribution of pine is limited to permafrost – the root system of pine is more thermophilic than that of larch (Popov 1982). *L. sibirica*, due to being deciduous, is able to tolerate extremely low temperatures, stagnant waterlogging, and summer and winter dehydration (Islam and Macdonald 2004; Suvorova and Popova 2015). The crown of larch is sparse and well lit, and as a result, it effectively assimilates CO_2. With a smaller needle weight, it can provide a comparable level of carbon absorption compared to evergreen conifers (Schepaschenko et al. 2008).

At present, aluminum smelters remain powerful polluters of the environment over vast areas. Despite significant improvements in the technology of aluminum production and industrial emission cleaning, it has proved impossible to completely get rid of many polluting agents, including fluorides, oxides of sulfur and heavy metals, and polycyclic aromatic hydrocarbons (Bonte and Cantuel 1981; Kulikov and Storozhev 2012). Highly aggressive fluorides in aluminum smelter emissions are dangerous for all living organisms, especially plants. The hydrogen fluoride poisoning effect is known to be 3–3000 times stronger than the impact of other acidic gases (chlorine, sulfur dioxide, nitrogen and carbon oxides) (Rozhkov and Mikhailova

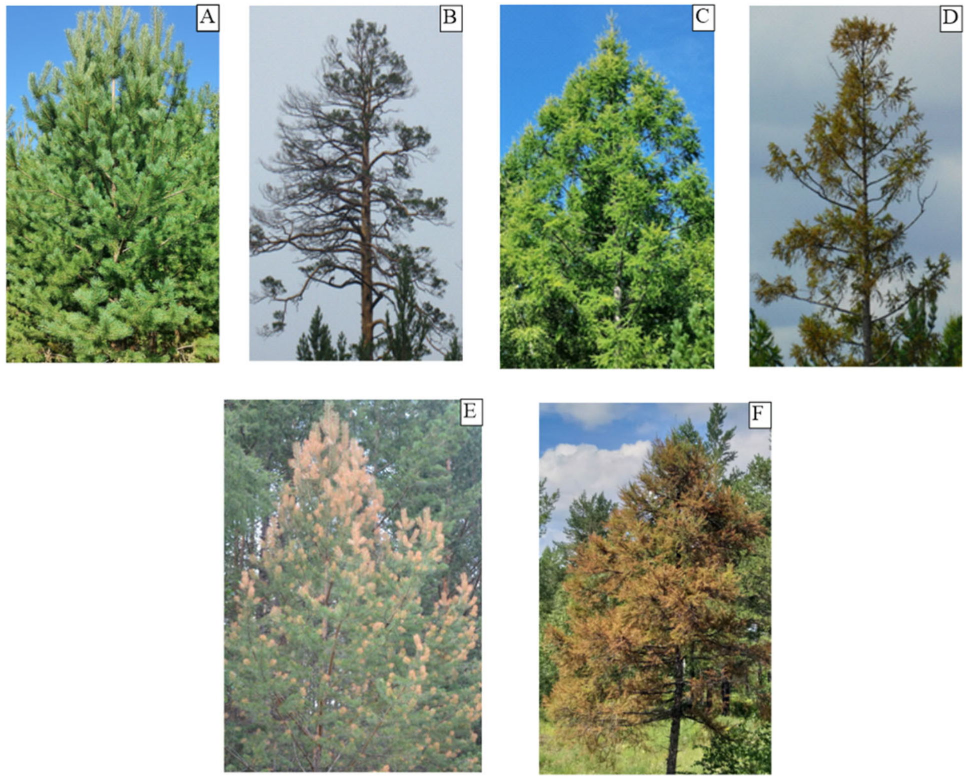

Fig. 5 Healthy (**A**, **C**) and defoliated (**B**, **D**) *P. sylvestris* and *L. sibirica* trees in the territory of exposure by BrAZ emissions. Necrotic needles of pine at high pollution level (**E**) and larch needles (**F**) at critical pollution level by BrAZ emissions

Table 2 Morphological parameters of shoot and needle of *Pinus sylvestris* and *Larix sibirica* at different levels of technogenic pollution by BrAZ emissions (above the line – M ± δ; below the line –V,%)

Morphological parameters	Background territories	Technogenic pollution level			
		Low	Moderate	High	Critical
P. sylvestris					
Needle length, mm	63.52 ± 9.92^{c} / 15.6	54.38 ± 5.72^{b} / 10.5	50.84 ± 5.10^{ab} / 10.0	47.52 ± 4.92^{a} / 10.4	47.54 ± 3.05^{a} / 6.4
Needle width, mm	1.55 ± 0.14^{c} / 9.0	1.37 ± 0.06^{b} / 4.4	1.34 ± 0.08^{b} / 6.0	1.24 ± 0.05^{a} / 4.0	1.47 ± 0.06^{bc} / 4.1
Needle thickness, mm	0.74 ± 0.07^{b} / 9.5	0.70 ± 0.02^{b} / 2.9	0.65 ± 0.02^{ab} / 3.1	0.60 ± 0.02^{a} / 3.3	0.77 ± 0.04^{b} / 5.2
Weight of 2-year-old shoot, g	2.64 ± 0.67^{d} / 25.4	2.02 ± 0.44^{c} / 21.8	0.79 ± 0.09^{a} / 11.4	0.89 ± 0.26^{b} / 29.2	0.86 ± 0.09^{ab} / 10.5
Weight of needles from one 2-year-old shoot, g	4.80 ± 0.70^{d} / 14.6	3.24 ± 1.25^{c} / 38.6	2.80 ± 0.51^{bc} / 18.2	2.71 ± 0.50^{b} / 18.5	2.29 ± 0.28^{a} / 12.2
1 needle weight, g	34.07 ± 8.64^{b} / 25.4	31.52 ± 5.17^{b} / 16.4	29.37 ± 3.28^{a} / 11.2	31.15 ± 6.83^{ab} / 21.9	27.59 ± 7.95^{a} / 28.8
Number of needle pairs, pcs	79.54 ± 16.90^{d} / 21.3	58.25 ± 13.95^{c} / 24.0	48.01 ± 6.83^{b} / 14.2	43.50 ± 7.86^{ab} / 18.1	41.50 ± 4.84^{a} / 11.7
Length of 2-year-old shoot, mm	166.60 ± 45.31^{d} / 27.2	87.30 ± 11.60^{b} / 13.3	91.12 ± 12.28^{c} / 13.5	69.70 ± 28.40^{a} / 0.8	61.78 ± 18.30^{a} / 29.6
L. sibirica					
Needle length, mm	34.68 ± 7.07^{c} / 20.4	31.91 ± 5.19^{bc} / 16.3	32.79 ± 5.88^{bc} / 17.9	27.24 ± 4.21^{b} / 15.5	18.56 ± 3.01^{a} / 16.2
Needle width, mm	0.81 ± 0.07^{a} / 8.6	0.81 ± 0.05^{a} / 6.2	0.91 ± 0.08^{b} / 8.8	1.07 ± 0.11^{c} / 10.3	0.80 ± 0.06^{a} / 7.5
Needle thickness, mm	0.37 ± 0.02^{a} / 5.4	0.40 ± 0.05^{ab} / 12.5	0.40 ± 0.06^{ab} / 15.0	0.42 ± 0.02^{b} / 7.5	0.36 ± 0.03^{a} / 8.3
Weight of 2-year-old shoot, g	0.85 ± 0.14^{d} / 16.6	0.74 ± 0.15^{c} / 20.3	0.48 ± 0.16^{b} / 33.3	0.38 ± 0.07^{a} / 18.4	0.36 ± 0.08^{a} / 22.2
Weight of needle from one 2-year-old shoot, g	1.24 ± 0.43^{b} / 34.7	1.29 ± 0.24^{b} / 18.6	1.28 ± 0.23^{b} / 18.0	1.44 ± 0.29^{c} / 20.1	0.71 ± 0.13^{a} / 18.3
Number of brachiblasts, pcs	25.69 ± 7.45^{d} / 29.0	19.91 ± 4.83^{c} / 24.3	17.33 ± 5.66^{b} / 32.7	13.67 ± 3.39^{a} / 24.8	13.63 ± 3.41^{a} / 25.0
Length of 2-year-old shoot, mm	140.13 ± 31.58^{c} / 22.5	113.17 ± 21.72^{b} / 19.2	100.68 ± 22.18^{b} / 22.0	84.44 ± 27.01^{a} / 32.0	79.54 ± 18.72^{a} / 23.5

Different letters for the same row represent statistically significant differences between zones ($P < 0.05$), where a < b < c < d

1993; Weinstein and Davison 2003). Coniferous trees actively absorb pollutant elements (Godek et al. 2015; Jin et al. 2021), with evergreens such as *P. sylvestris* doing so all year round. At the same time, S.V. Zagirova (2015)

Table 3 Anatomical parameters of the structure of *Pinus sylvestris* needle at different levels of technogenic pollution by BrAZ emissions (above the line – M±δ; below the line –V,%)

Anatomical parameters		Background territories	Technogenic pollution level			
			Low	Moderate	High	Critical
Epidermis+ cuticle thickness, μm	upper	19.09 ± 1.22^{b} / 6.4	20.93 ± 2.14^{b} / 10.2	17.56 ± 0.23^{ab} / 1.3	16.17 ± 0.78^{a} / 4.8	15.95 ± 0.37^{a} / 2.3
	lower	19.38 ± 0.98^{b} / 5.1	20.18 ± 1.75^{b} / 8.7	17.25 ± 0.90^{ab} / 5.2	16.19 ± 0.88^{a} / 5.44	16.78 ± 0.66^{a} / 3.93
Hypodermic thickness, μm	upper	13.04 ± 0.92^{ab} / 7.1	13.86 ± 1.17^{b} / 8.4	12.68 ± 0.43^{a} / 3.4	11.66 ± 0.86^{a} / 7.3	14.64 ± 0.36^{b} / 2.5
	lower	12.73 ± 1.12^{b} / 8.8	11.79 ± 0.81^{ab} / 6.9	12.23 ± 0.33^{b} / 2.7	10.99 ± 0.80^{a} / 7.2	12.89 ± 1.24^{b} / 9.6
Mesophyll thickness, μm	upper	146.41 ± 5.93^{c} / 4.1	132.67 ± 10.50^{b} / 7.9	120.73 ± 6.32^{a} / 5.2	115.75 ± 1.89^{a} / 1.6	$158.00+22.63^{c}$ / 14.3
	lower	176.42 ± 12.25^{c} / 6.9	174.17 ± 13.20^{c} / 7.9	160.57 ± 4.51^{b} / 2.8	143.47 ± 10.76^{a} / 7.5	189.05 ± 13.89^{c} / 7.4
Needle cross-sectional area, μm /10^3		934.67 ± 156.46^{d} / 16.7	759.14 ± 21.83^{c} / 2.9	690.39 ± 58.09^{b} / 8.4	581.25 ± 39.64^{a} / 6.8	910.95 ± 122.65^{d} / 13.5
Mesophyll area,μm /10^3		546.81 ± 31.69^{c} / 5.8	473.38 ± 23.42^{b} / 5.0	433.53 ± 36.18^{ab} / 8.4	384.12 ± 22.67^{a} / 5.9	546.89 ± 36.84^{c} / 6.7
Central cylinder cross-sectional area, μm /10^3		273.02 ± 39.21^{c} / 14.4	225.63 ± 6.89^{bc} / 2.9	205.59 ± 17.37^{b} / 8.5	155.82 ± 11.73^{a} / 7.5	297.13 ± 24.37^{c} / 8.2
Vascular bundles cross-sectional area, μm /10^3		21.63 ± 3.50^{e} / 17.0	16.97 ± 2.57^{d} / 15.4	10.21 ± 1.61^{b} / 15.8	7.45 ± 0.93^{a} / 14.5	15.42 ± 1.34^{c} / 8.7
Number of resin ducts, pcs		9.44 ± 1.59^{c} / 16.8	7.54 ± 0.92^{bc} / 12.2	7.03 ± 0.25^{b} / 3.6	5.07 ± 0.25^{a} / 4.9	11.33 ± 1.15^{d} / 10.2
Diameter of the resin duct, μm		59.68 ± 2.41^{b} / 4.0	55.95 ± 2.98^{b} / 5.3	49.57 ± 2.17^{ab} / 4.4	44.65 ± 2.04^{a} / 4.6	46.72 ± 1.84^{a} / 3.9

Different letters for the same row represent statistically significant differences between zones ($P < 0.05$), where a < b < c < d.

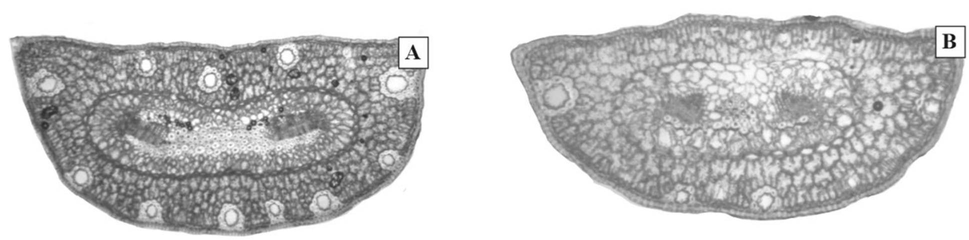

Fig. 6 Cross-sections of *P. sylvestris* needle with a visible decrease in the number of resin ducts in the structure (**A**) – in background territory, (**B**) – in territory with a high pollution level by BrAZ emissions (*3.5)

Table 4 Anatomical parameters of the structure of *Larix sibirica* needle at different levels of technogenic pollution by BrAZ emissions (above the line – M ± δ; below the line –V,%)

Anatomical parameters		Background territories	Technogenic pollution level			
			Low	Moderate	High	Critical
Epidermis + cuticle thickness, μm	upper	14.14 ± 1.41^{ab} / 10.1	14.87 ± 0.90^{ab} / 6.1	15.47 ± 1.35^{b} / 8.7	15.57 ± 1.93^{b} / 12.4	13.07 ± 0.92^{a} / 7.0
	lower	14.65 ± 0.84^{ab} / 5.7	15.45 ± 1.02^{b} / 6.6	16.00 ± 1.00^{b} / 6.3	17.16 ± 1.76^{c} / 10.26	13.84 ± 0.66^{a} / 4.77
Hypodermic thickness, μm	upper	11.90 ± 1.30^{a} / 10.9	12.56 ± 1.51^{a} / 12.0	11.25 ± 0.96^{a} / 8.5	12.60 ± 1.27^{a} / 10.1	12.33 ± 0.44^{a} / 3.6
	lower	11.56 ± 1.04^{a} / 9.0	11.05 ± 0.87^{a} / 7.9	11.24 ± 0.82^{a} / 7.3	12.00 ± 1.38^{a} / 11.5	11.00 ± 0.47^{a} / 4.3
Mesophyll thickness, μm		186.17 ± 8.65^{a} / 4.6	186.92 ± 10.20^{a} / 5.5	216.86 ± 9.84^{b} / 4.5	246.97 ± 23.13^{b} / 9.4	179.19 ± 14.84^{a} / 8.3
Needle cross-sectional area, μm /10^3		206.93 ± 9.50^{a} / 4.6	215.24 ± 21.90^{a} / 9.3	221.85 ± 12.16^{ab} / 5.0	236.00 ± 12.70^{b} / 4.7	215.23 ± 25.62^{a} / 11.9
Mesophyll area, μm /10^3		172.12 ± 11.62^{ab} / 6.8	205.64 ± 17.57^{b} / 8.7	194.55 ± 15.44^{b} / 7.9	198.70 ± 21.35^{b} / 10.7	160.87 ± 8.97^{a} / 5.6
Central cylinder cross-sectional area, μm /10^3		21.42 ± 0.55^{b} / 2.6	21.86 ± 0.29^{b} / 1.3	19.26 ± 2.86^{b} / 14.9	18.69 ± 1.17^{a} / 6.3	17.41 ± 0.88^{a} / 5.1
Vascular bundle cross-sectional area, μm /10^3		4.19 ± 0.58^{c} / 13.8	3.30 ± 0.42^{b} / 12.7	3.48 ± 0.35^{b} / 10.1	3.23 ± 0.41^{b} / 12.7	2.75 ± 0.41^{a} / 14.9
Number of resin ducts, pcs		2	2	2	2	2
Diameter of the resin duct, μm		21.17 ± 1.08^{a} / 5.1	23.83 ± 2.16^{a} / 9.1	28.39 ± 2.36^{ab} / 8.3	32.24 ± 2.41^{b} / 7.5	37.51 ± 1.44^{b} / 3.8

Different letters for the same row represent statistically significant differences between zones ($P < 0.05$), where a < b < c.

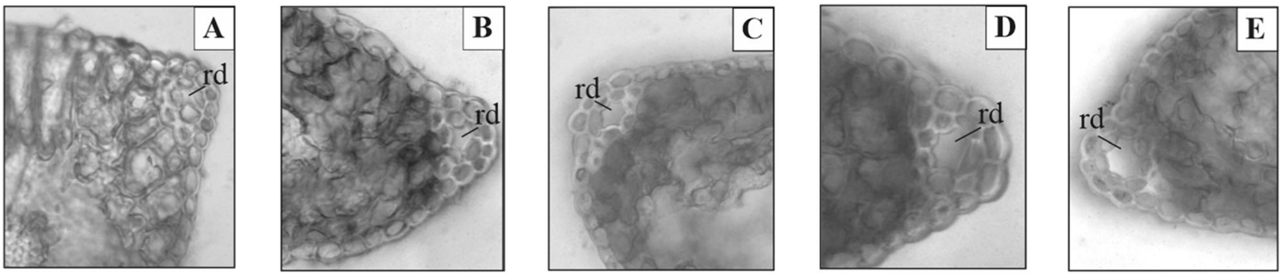

Fig. 7 Fragments of a cross section of *L. sibirica* needle with resin ducts (rd): (**A**) background territory, (**B–E**) areas polluted by BrAZ emissions: (**B**) low, (**C**) moderate, (**D**) high, (**E**) critical pollution level (*20)

Table 5 Changes in the ratios of *Pinus sylvestris* and *Larix sibirica* needle at different levels of technogenic pollution by BrAZ emissions

Indices ratio	Tree species	Background territories	Technogenic pollution level			
			Low	Moderate	High	Critical
Area of central cylinder/Area of needle cross section	*P. sylvestris*	0.292 ± 0.054^{a}	0.297 ± 0.031^{a}	0.298 ± 0.047^{a}	0.268 ± 0.039^{a}	0.326 ± 0.056^{b}
	L. sibirica	0.104 ± 0.017^{b}	0.102 ± 0.014^{b}	$0.087 \pm 0.012^{a,b}$	0.079 ± 0.009^{a}	0.081 ± 0.007^{a}
Area of mesophyll/Area of central cylinder	*P. sylvestris*	$2.003 \pm 0.416^{a,b}$	2.098 ± 0.289^{b}	2.109 ± 0.337^{b}	2.465 ± 0.517^{b}	1.841 ± 0.408^{a}
	L. sibirica	8.035 ± 1.562^{a}	9.407 ± 2.008^{b}	$10.101 \pm 2.096^{b,c}$	10.631 ± 2.241^{c}	$10.29 \pm 2.368^{b,c}$

Different letters for the same row represent statistically significant differences between zones ($P < 0.05$), where a < b < c.

indicated that *L. sibirica* has a large assimilating surface, which can absorb pollutants in large quantities.

Our results showed that *L. sibirica* accumulating capacity, even in the background territories, was higher than that of *P. sylvestris*. In polluted territories, as a result of foliar and soil absorption of pollutants, the content of many biogenic elements changed in tree needles, which led to an imbalance in the elemental composition of needles (Mandre and Lukjanova 2011; Wannaz et al. 2012; Nadgorska-Socha et al. 2017; Afanasyeva and Ayushina 2019; Kalugina et al. 2020). The elemental composition imbalance in *P. sylvestris* needles was stronger than in *L. sibirica* needles, as evidenced by our calculated *EF* coefficients and indices *Zbt*. It was found that the critical pollution level was characterized by the highest values of *EF* and *Zbt*, corresponding to an extremely high transformation of the elemental composition; at a high pollution level, the *Zbt* corresponded to a high degree of transformation; at a moderate pollution level – a moderate degree of transformation; and at a low pollution level – a minimum degree of transformation. In general, the indices of biogeochemical transformation indicated a greater imbalance in the elemental composition of *P. sylvestris* needles compared to *L. sibirica*.

A pronounced imbalance in the elemental composition led to metabolic disturbances (Singh et al. 2018; Nabi et al. 2021), and, as a result, a decline in the growth characteristics of trees (Lamppu and Huttunen 2003; Pallardy 2008; Stravinskiene et al. 2013). In addition, direct exposure of needles to pollutants (dry and wet deposition on the needle surface) can lead to crown damage, increased crown defoliation, and loss of tree viability (Chojnacka-Ozga and Ozga 2021). Shparyk & Parpan (2004) found the highest level of crown defoliation near industrial emission sources. Damage to trees by aluminum smelter emissions was often accompanied by necroses on the needles and leaves (Hitchock et al. 1962; Chang 1975; Rozhkov and Mikhailova 1993).

Our results indicated a significant negative impact of BrAZ emissions on the visual indicators of the state of the assimilating phytomass of trees and the morphological parameters of shoots. The strongest changes in these parameters were revealed at a critical pollution level. We observed extensive needle necrosis in trees of both species near BrAZ and at a distance of up to 3 km from it. A large necrotic area was noted (it could occupy 2/3 of the total area of the needles) in the distal part of the needles; there was a clear boundary between it and the green part of the needle. The formation of extensive necrosis occurred as a result of a quick lesion, probably caused by spike (single) emissions of BrAZ. The reduction in needle life expectancy and more significant changes in morphological parameters of *P. sylvestris* needles and shoots under pollution were, in our opinion, the cause of greater crown defoliation compared to *L. sibirica*.

According to W. De Vries et al. (2000), it is often very difficult to establish causal relationships between the atmospheric pollution level, adverse meteorological factors, and changes in the visual and morphological parameters of trees. We found significant correlations between the level of tree crown defoliation, morphological parameters, and the content of pollutant elements in the needles. That can indicate that the main factor determining the state of trees in the surveyed territory is aluminum smelter emissions.

It is logical to assume that the negative changes in the morphological parameters of needles that we found were the result of a restructuring of their anatomical configuration. According to M.S. Kivimäenpää et al. (2017), the change in leaf anatomy is an important mechanism for plant adaptation (a slow evolutionary process) and acclimatization (short-term adaptation) to new environmental conditions. Analysis of anatomical parameters of *P. sylvestris* and *L. sibirica* needles showed different responses of the species to the impact of aluminum smelter emissions. In pine, with increasing pollution levels, up to the high level, the cross-sectional area of the needle, the area of the mesophyll, and its thickness, especially in the upper part, was significantly reduced. This was due to a reduction in the number of structural elements in the needle; in particular, three to four rows of mesophyll cells were formed in the needles of healthy trees and only two rows in the needles from the polluted areas. In larch, a decrease in thickness and area of the assimilation tissue (mesophyll) was found only at a critical pollution level, when the content of pollutant elements in the needles reached maximum values. At low, moderate, and high pollution levels, a tendency to increase these parameters was revealed, which can indicate their adaptive nature.

Other researchers have also reported a decrease in cross-sectional area and mesophyll thickness in polluted needles. For example, a similar pattern was found in *P. sylvestris* when exposed to pulp and paper mill emissions (Tuzhilkina and Plyusnina 2020) and in *P. sylvestris* and *Picea obovata* in urban environments (Legoshchina et al. 2013; Skripal'shchikova et al. 2016). At the same time, an increase in the mesophyll area was noted in 4-year-old pine seedlings at elevated CO_2 concentrations. This was due to an increase in the number of mesophyll cells. An increase in volume density of mesophyll cells has also been recorded in *Cedrus atlantica* Endl. needles near fertilizer and chemical smelters in Pančevo (Serbia) (Marin et al. 2009).

It is known that the area of mesophyll cells is closely related to photosynthetic rate (Roderick et al. 1999; Pandey and Kushwaha 2005; Ivanova & Suvirova 2014). Therefore, we can assume that the changes in the structure of *P. sylvestris* needles that we found at low, moderate, and high pollution levels could lead to a decrease in the photosynthetic activity of trees.

Under the influence of BrAZ emissions in the needles of both tree species, the cross-sectional area of the central cylinder and vascular bundles decreased, especially in the needles of *P. sylvestris*. Decreasing the area of vascular tissue could be associated with inhibition of growth processes in needles and reduction of the transfusion tissue size, which plays an important role in the formation of water reserves (Mikhailova and Berezhnykh 1995; López et al. 2008). Similar changes were noted in experiments on artificial fumigation of trees, which showed that the vascular system of needles was actively involved in the process of movement and accumulation of fluorides, the main components of aluminum smelter emissions (Rozhkov and Mikhailova 1993). Since decreasing the vascular tissue in the needles and leaves of plants was often observed under the influence of biotic stresses, for example, during soil drought (Ozturk et al. 2022), it can be concluded that this reaction to the action of stress factors is nonspecific.

Interesting features of the resin system, which performs mainly a protective function in coniferous trees, were revealed. Our results showed that the resiniferous needle system of *P. sylvestris* was more developed than that of *L. sibirica*. In the background territories areas, the pine had 8–10 large resin ducts located laterally on the adaxial and abaxial sides of the needles, while the larch had only 2 resin ducts at the needle corners. Under technogenic pollution, the number and diameter of resin ducts in *P. sylvestris* decreased substantially. In *L. sibirica*, the number of resin ducts in the background and polluted territories was constant; however, their diameter increased under the influence of emissions. Essential oils, the main components of resin, are synthesized in the epithelial cells of the resin ducts of coniferous plants and perform protective functions: they are toxic to most herbivores and insect pests (Jankowski et al. 2017) and have antifungal activity (Fäldt 2000; Cavaleiro et al. 2006). The reduction of the resiniferous zone in a needle can potentially reduce its resistance to pathogenic microorganisms. According to A. Jankowski et al. (2017), an increase in the size of resin ducts can be regarded as a mechanism for adaptation to adverse environments. We assumed that a well-developed resiniferous system in both *P. sylvestris* and *L. sibirica* was a constitutive defense of trees, and a change in the number of resin ducts in *P. silvestris* was the result of a long-term adaptation process aimed at fortifying the protective mechanisms in conditions of powerful chronic impacts of technogenic emissions. In the deciduous coniferous species *L. sibirica*, an increase in the volume of resin ducts and, accordingly, the amount of resin was the result of a short-term adaptation to changing growing conditions. Among researchers, there was no consensus on how the resiniferous system of *P. sylvestris* changed in response to the impact of industrial emissions. A reduction in the number of resin ducts in polluted pine needles was also noticed by other researchers (Tuzhilkina & Plyusnina 2020), but in some cases an increase in their number was observed (Nikolaevsky 1979; Onuchin and Kozlova 1993). Y. Nuhoglu (2005) found an increase in the diameter of resin ducts in *Pinus brutia* needles in the influence zone of a thermal power station near the Turkish city of Yeniköy. We did not find such data for *L. sibirica*.

External protective tissues are the main barrier between the environment and needle tissues; therefore, their condition is very important. In our case, the conservatism of external protective tissue structure was shown; in both species, the epidermises together with the cuticle, and the hypodermis thickness, remain almost unchanged when polluted. With a slight but statistically significant decrease in the epidermis + cuticle thickness in *P. sylvestris* needles under high and critical pollution, the cell membranes became thicker. This could be considered a protective function that prevents the penetration of toxicants into the cells. At the same time, an increase in the cuticle thickness of *P. sylvestris* needles was observed under the influence of gaseous toxicants; thickening of the epidermis and hypodermis of *P. sylvestris* needles of different ages was observed in the oil-contaminated area (Shayakhmetova and Egorova 2016) and in *P. obovata* needles in urban conditions (Legoshchina et al. 2013).

Many researchers, when studying the anatomical features of the assimilation organs of trees under the influence of negative factors, use not only absolute values but also the ratios of various indicators (Bender et al. 2008). So, the ratio of the central cylinder area to the needle cross-sectional area characterizes the proportionality of the development of needle tissues and their probable adaptations to changing environmental conditions, which should be maintained at a genetically predetermined level (Sazonova et al. 2011). We found that this ratio in *P. sylvestris* needles at low and moderate pollution levels virtually did not differ from the background value, and at a critical level, it exceeded the background by 12% due to the enhancement of the vascular bundles. Under high pollution level, this ratio declined to 0.268, which is observed in pines with low biological productivity exposed to a high anthropogenic load. In *L. sibirica*, this ratio was quite stable at low and moderate pollution levels, and at high and critical pollution levels, it was lower than the background by an average of 15–20%, which may indicate a malfunction of the vascular bundles. The ratio of mesophyll and central cylinder areas was considered an indicator characterizing the efficiency of assimilating and vascular bundles (Bender et al. 2008). The higher this indicator, the more efficiently the assimilating tissue functions. The tendency to increase this ratio was identified in *P. sylvestris* needles up to high pollution and in *L. sibirica* – up to a critical pollution level. In *P. sylvestris*, at a critical pollution level, the value of the ratio of mesophyll and central cylinder declined, i.e., the unit area of the vascular system served a large area of assimilating tissue.

It should be noted that the anatomo-morphological changes of the needles revealed by us may not only be the result of the current pollution levels found in the examined trees but also a result of the overall health status of the trees, which has been influenced by previous years' environmental conditions, especially when the emissions were at their maximum. We have previously shown that the response of trees to long-term cumulative exposure to toxicants can manifest itself after a decade (Mikhailova and Berezhnaya 2002). This has been reported by other researchers as well. For example, the morphological abnormalities of *P. sylvestris* and radial growth reduction or cessation were observed in the subsequent few years after the Chernobyl accident (Tulik 2001; Netsvetov et al. 2023). It was suggested that the cause of the decline of pine growth is the loss of foliage and damage to buds that shrink the stock of carbohydrates and the accessibility of assimilates.

In conclusion, it should be noted that according to our data, under the influence of the aluminum smelter emissions, the anatomical structure of *P. sylvestris* and *L. sibirica* needles changed in comparison with the background parameters. It was found that, under the influence of aluminum production emissions, changes in the anatomical parameters of tree needles were nonlinear and specific for each species. Changes may be a consequence of pollutants pathological effects or may be classified as adaptations. The maximum number of negative changes was recorded in *P. sylvestris* trees; they include: reduction of the cross-sectional area of the needle, the central cylinder, and vascular bundles; reduction of the thickness of external protective tissue and mesophyll; reduction of resin ducts; and a decrease in their diameter. Distinct morphological changes include a decrease in the linear parameters of needles and shoots, their weight, and the number of needles on the shoots. At a critical pollution level, when the content of pollutants in *P. sylvestris* needles reached maximum values, a number of changes in the anatomical parameters of the remaining green needles in the direction of increase were presumably adaptive. At low, moderate, and high levels of *L. sibirica* needle pollution, the changes detected can be classified as adaptive. The increase in weight, thickness, and width of the needle, as well as the thickness of the epidermis, mesophyll, and cross-sectional area of the needle, were observed. At a critical pollution level, the changes in most of the studied anatomical and morphological parameters were negative.

Thus, our hypothesis that the anatomical and morphological parameters of *L. sibirica* needles under the influence of industrial emissions will change more than the needles of *P. sylvestris* has not been confirmed. Probably, the high rate of needle development in *L. sibirica*, the duration of which is 1–1.5 months (Tselniker et al. 1993), and its deciduous nature allow it not to use the protective mechanisms associated with the restructuring of the needle anatomical structure.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Ethics approval The research article ensures objectivity and transparency in research and secures that accepted principles of ethical and professional conduct have been followed. This original research article does not contain any studies with human participants and animals performed by any of the authors.

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