Anatomycal, morphological and biochemical features of Pinus sibirica needles on recultivated and non-recultivated ash dump sites in the Middle Urals

Nadezhda Chukina, *Natalia* Lukina [*], *Margarita* Glazyrina, *Elena* Filimonova, *Anton* Uchaev, and *Olga* Nekrasova

Institute of Natural Sciences and Mathematics, Ural Federal University named after the first President of Russia B. Yeltsin, 19, Mira, Yekaterinburg, 620002, Russia

Abstract. The article presents the results of anatomical, morphological and biochemical study of Pinus sibirica Du Tour needles of fly ash dumps and natural habitats in the Middle Urals. The study has shown that there was an increase in the area of resin ducts, as well as a decrease in the total nitrogen content in the needles of P. sibirica on ash dumps, compared with control habitats. An increase in the content of phenols and proline in the needles which indicates a response to stress, was also observed on the nonrecultivated ash dump unlike natural forest phytocenoses and recultivated ash dump. The revealed changes in the studied characteristics of P. sibirica needles are associated with the specific physico-chemical parameters of the technogenic substrate: an alkaline reaction of the medium as well as low content of clay particles and total nitrogen. Recultivation measures, namely covering the ash with strips of clay soil, contributed to the improvement of substrate parameters and the creation of more favorable conditions for plant growth.

1 Introduction

Pinus sibirica Du Tour is the basis of the Siberian taiga forests. Plantings with a predominance of this species with an area of more than 35.88 million hectares extend in wide areas throughout Siberia, the Urals, the Altai Territory and the northeast of the European part of the Russian Federation, as well as in Kazakhstan and Mongolia. This plant does not grow in its natural state in other places on the globe [1]. Within the Urals and adjacent flat areas, the lower boundary of P. sibirica lies slightly north of Yekaterinburg, and the southern one – in the Circumpolar Urals [2]. P. sibirica is of great economic importance because of it ornamental, food, fodder and medicinal plant. Siberian cedar

[*] Corresponding author: natalia.lukina@urfu.ru

needles are a source of biologically active substances. They are rich in essential oils, microelements, contains βcarotene, ascorbic acid, B vitamins, etc. [3].

It is known that P. sibirica has a comparatively wide ecological range and grows in places with different soil and climatic conditions. There is practically no information on the reaction of P. sibirica to technogenic substrates, while the study of edificator plants under conditions of increased anthropogenic impact is extremely important for understanding the mechanisms of adaptation and survival of plants in extreme environmental conditions.

The purpose of the work is to assess the influence of the substrate on the anatomical, morphological and biochemical characteristics of P. sibirica needles growing on ash dumps and in natural habitats.

2 Materials and Methods

The research was carried out on the ash dumps of the Nizhneturinskaya (S1) and Verkhnetagilskaya (S3) thermal power stations and in control forest phytocenoses (S2, S4) located in the Middle Urals in July 2022. The study sites are located in the temperate continental boreal climate zone in the taiga: S1 and S2 – on the border of the southern and middle taiga subzones; S2 and S4 – in the southern taiga subzone.

The NTTPS ash dump is located 19 km from the city of Nizhnyaya Tura, its area is about 440 hectares, no recultivation measures were carried out on it. The overgrowth of the ash dump was carried out due to the introduction of seeds from the pine-spruce forests and wetlands surrounding it from all sides. The VTTPS ash dump is located 5 km from the city of Verkhniy Tagil, its area is 125 hectares. It was recultivated by applying the strips of clay soil (wideness 5–7 m, thickness –15–20 cm) and was left for self-overgrow partly [4].

Geobotanical survey showed that a mixed forest phytocenosis dominated by Pinus sylvestris L. and Betula pendula Roth was formed in the non-recultivated area of the NTTPS ash dump (S1). The age of the plant community is about 25–30 years. Crown density varied from 0.3 to 0.5. In a natural forest phytocenosis (S2), located 1.5 km from the NTTPS ash dump the tree layer was dominated by Picea obovata Ledeb., P. sibirica, Larix sibirica Ledeb. and Abies sibirica Ledeb. crown density was 0.7–0.8.

A mixed forest phytocenosis with a crown density of 0.7 was formed over 50 years on the recultivated area of the VTTPS ash dump with strip soil application (S3). B. pendula, Populus tremula L. and P. sylvestris dominated in the tree layer. In the natural forest phytocenosis (S4), located 8 km from the city of Verkhniy Tagil, the tree layer was represented by P. obovata and A. sibirica with an admixture of P. sylvestris, P. sibirica and B. pendula.

Ten model trees of P. sibirica 10–25 years old were chosen in each site. Two-year-old needles were selected for analysis. The transverse needles sections were made with a freezing microtome MZ-2 (Russia), using fixed in a 70% ethanol solution needles. The following parameters were measured in transverse sections of the needles: the cross-sectional area, epidermis and hypodermis thickness, and the area of resin ducts using a light microscope and image analysis system SIAMS MesoPlant ("SIAMS", Russia).

Biochemical parameters were obtained according to [5]. For the determination of the total content of phenolic compounds and flavonoids the crush fresh needles were used after extracting in 70% ethanol for 24 h. The total phenolic content was determined with the Folin– Ciocalteu reagent. The amount of flavonoids was measured after the reaction of the extract sample with 2% aluminum chloride. The content of antioxidants in needles was calculated on a dry weight (DW). The lipid peroxidation was assessed by the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA), the content of free proline was determined spectrophotometrically (PD303-UV "Apel", Japan). The content of total nitrogen and phosphorus in the dried and homogenized samples of P. sibirica needles was measured spectrophotometrically and expressed in % of DW according to [6].

Sampling of soils and ash substrate was done on each ash dump and in natural habitat sites considering the visible boundaries of the horizons and prepared for analysis by conventional methods [7]. Particle size distribution was determined by the pipette method; pH values were measured using a pH meter (Anion 4100, Russia); the content of total organic carbon (TOC) was detected by the Tjurin method and total nitrogen (TN) – by the Kjeldahl method (using equipment Velp, Italy); exchangeable Ca2+ and Mg2+ – by titration; available phosphorus content (P2O5) – spectrophotometrically (using UV Probe-1650, Japan). The weighted average values of the studied indicators were calculated for the thickness 0–20 cm for the convenience of comparing physico-chemical characteristics of different site substrates.

The data were processed statistically using Microsoft Excel and StatSoft STATISTICA 12. The significance of differences between studied parameters was assessed by MannWhitney test (at $p<0.05$). The tables and figures present the mean values (Means) and standard errors (±SE), different alphabetical letters indicate a significant difference between the studied parameters.

3 Results and Discussion

The study of physico-chemical characteristics showed (Table 1) that the pH values of the ash dump substrates significantly exceed those in the soils of natural habitat: the reaction of the medium in the non-recultivated site is slightly alkaline, in the recultivated site it is neutral, while in the background soils it is acidic. The ash substrate contains a significantly lower amount of clay particles in its composition: in the NTTPS ash dump the share of particles with a diameter of <0.01 mm is only about 12%, in the VTTPS ash dump it is much higher – 21%, and in the soils of natural habitats its amount is 35–37%. The ash dump substrate contains 30% of TOC from the total amount in the corresponding background soils, and 3 times less of TN. The content of exchangeable calcium and magnesium ions is significantly higher in both sites located in the vicinity of the VTTPS (S3 and S4). Both ash dump sites are much better provided with mobile phosphates compared to the soils of natural forests.

Table 1. Substrate physico-chemical characteristics of ash dumps and control sites

Site	pH	Particles d<0.01 mm	TOC	TN	Ca^{2+}	Mg^{2+}	P_2O_5
		%			mmol/100 g		mg/100 g
S1	6.96	11.6	2.18	0.05	1.8	0.5	17.4
S2	5.14	36.6	2.99	0.16	2.1	1.2	0.3
S3	6.36	21.0	2.04	0.10	5.0	1.6	14.1
S4	5.53	35.0	2.98	0.35	5.9	2.8	0.4

Thus, the ash substrate of both ash dumps, compared with the soils of the control sites, is characterized by more alkaline medium, a lower content of clay particles, total organic carbon and total nitrogen, as well as a higher content of mobile phosphates, most of these differences are especially significant for the non-recultivated site.

The study of the morphological parameters has shown that the length and cross-sectional area of *P. sibirica* needles from non-recultivated site (S1) were statistically significantly ($p<0.05$) less than in the control habitat (S2). *P. sibirica* needles from the ash dump of site S3 did not differ in these parameters from the control site S4 (Table 2). Analysis of the anatomical parameters of *P. sibirica* needles showed that the total area of resin ducts in the needles of ash dump trees, were statistically significantly higher. Whereas the thickness of the needle integumentary tissues (epidermis and hypodermis) in studied trees from ash dumps and control habitats did not differ.

Table 2. Anatomical and morphological characteristics of P. sibirica needles of the studied sites

Characteristics	S1	S2	S3	S4
Length of needles, mm (n=100)	82.7±1.6a	101.6±2.0b	101.8±1.6b	101.5±2.0b
Cross-sectional area, 10^4 μm^2 (n=30)	49.1±0.5b	55.6±0.9c	48.8±0.9b	40.4±0.7a
Hypodermis thickness, μm (n=30)	11.4±0.2a	10.6±0.2a	11.2±0.2a	10.6±0.2a
Epidermis thickness, μm (n=30)	13.3±0.2a	13.8±0.2a	13.2±0.2a	12.2±0.2a
Area of resin ducts, μm^2 (n=30)	288.4±2.7c	274.5±4.7b	269.7±4.5b	250.9±2.6a

An increase in the size of the resin-conducting system of P. sibirica needles is an adaptive change associated with the intensification of secondary metabolites synthesis that have the protective role, the storage of which are the resin ducts [8].

A decreased content of nitrogen in the needles of P. sibirica on ash dump (1.94% and 2.57% for S1 and S3, respectively) compared to the control sites (2.50% and 2.78% for S2 and S4, respectively) took place which is due to the low content of this element in technogenic substrates. The decrease in nitrogen accumulation was observed to a greater extent in the plants of the non-recultivated site (S1). The phosphorus content in the needles of P. sibirica on ash dump (S1 – 0.23%) is statistically significantly ($p<0.05$) lower compared to the control (S2 – 0.45%). While in the needles from the recultivated site of ash dump (S3) the phosphorus content did not differ from the control (S4) and was on average 0.33 %.

MDA content in needles of trees, growing on S1 increased almost 2 times compared to the control (Figure 1, a). This indicates the oxidative stress experienced by plants on the ash dump. MDA content in needles from S3 and S4 did not differ statistically significant and were averaged 203.44 nM MDA/g DW. To prevent the negative effects of oxidative stress the antioxidant defense system (AOS) is activated in plant tissues. This leads to increased accumulation of various protective compounds. The comparative analysis of the AOS parameters of P. sibirica from ash dumps and natural habitat showed significantly higher content of phenolic compounds and proline in needles of plants from S1 compared with the control (S2) (by 13% and 20%, respectively) (Figure 1). The level of soluble phenols, flavonoids and proline in ash dump plants (S3) did not differ from the control (S4), probably due relatively favorable conditions for the plant growth after recultivation of this ash dump.

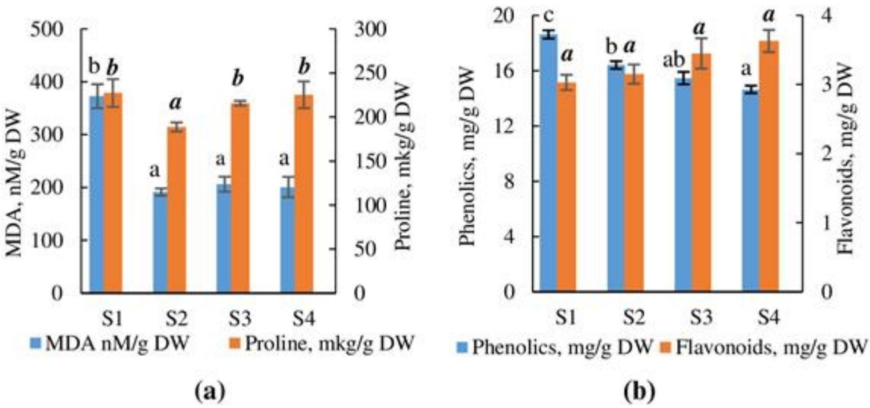

Fig. 1. Biochemical characteristics of P. sibirica needles of the studied sites: level of lipid peroxidation (MDA) and proline content (a); content of phenols and flavonoids (b); n=20.

4 Conclusion

The results of anatomical, morphological and biochemical study of P. sibirica needles of fly ash dumps and natural habitats in the Middle Urals have shown that there was a decrease of the length and cross-sectional area of the needles on the non-recultivated site of the NTTPS ash dump, while no changes of these characteristics were detected on the recultivated site of the VTTPS ash dump. At the same time there was an increase in the area of the resinconducting system, as well as a decrease in the content of total nitrogen in the needles of P. sibirica of both ash dump sites. Moreover, in the P. sibirica needles on the non-recultivated NTTPS ash dump site an additional decrease in the phosphorus content was detected.

An increase in lipid peroxidation processes, which was accompanied by an increase in the content of protective compounds (such as phenols and proline) was observed in the needles of P. sibirica on technogenic substrate. However, there were no statistically significant differences between the above indicators on the recultivated ash dump of the VTTPS, from the control, which indicates more favorable growing conditions for P. sibirica.

The revealed statistically significant differences of P. sibirica needles growing on the ash dump of NTTPS are largely related to the physico-chemical parameters of the substrate: an alkaline reaction of the medium, which determines the specifics of all soil processes; lower content of clay, which help to consolidate soil organic matter, retain moisture and nutrients, and also form a soil structure favorable for plant growth; low content of total nitrogen, which is the most important macronutrient. Recultivation measures, namely covering the ash with strips of clay soil at the ash dump of the VTTPS, contributed to the acidification of the medium, an increase in the content of clay and the accumulation of nitrogen in the substrate, i.e., changed the soil parameters in a more favorable direction for plant growth.

Acknowledgments. The research was supported by RSF (project No. 24-24-20010) https://rscf.ru/en/project/24-24-20010/.

References

1. L.F. Ipatov, Cedar in the North (Forest Museum, Arkhangelsk, 2011)
2. L.F. Semerikov, V.V. Ippolitov, Reserves of Siberian cedar in the Urals. Vegetation in
3. the conditions of technogenic landscapes of the Urals: collection of scientific works. 45–55 (Ural Branch of the Russian Academy of Sciences, Sverdlovsk, 1989)
4. E.V. Titov, Cedar – the king of the Siberian taiga (Dilya, Moscow: Saint Petersburg, 2020)
5. Lukina N. V., Filimonova E. I., Glazyrina M. A., Maleva M. G., Prasad M. N. V., Chibrik T. S. Chapter 19. Bioremediation and Bioeconomya (Second Edition): A Circular Economy Approach (Elsevier BV, 2024). 499-527. DOI: 10.1016/B978-0443-16120-9.00003-05
6. G.G. Borisova, M.G. Maleva, G.F. Nekrasova, N.V. Chukina, Methods for assessing the antioxidant status of plants: educational method. Manual (Ural University Press, Yekaterinburg, 2012)
7. G.G. Borisova, N.V. Chukina, I.S. Kiseleva, M.G. Maleva, Biochemistry: workshop. Educational book edited by G. G. Borisova (Ural University Press, Yekaterinburg, 2017)
8. E.V. Arinushkina, A guide in chemical analysis of soils, (Moscow State University Publishers; Moscow, Russia, 1970)
9. N.A. Leonova, V.Yu., Ilyin Phytoindication of pollution in forest ecosystems. Niva Povolzhya. **1(42)**, 39–47 (2017)