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Anatomical and Morphological Features of *Pinus sylvestris* Growing on the Dumps of the Mining Industry in the Middle Urals

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Abstract. The results of the study of anatomical and morphological parameters of *Pinus sylvestris* L., growing on the serpentine dumps of Anatol'sko-Shilovsky Mining, are presented. Adaptive morphological and anatomical changes that contribute to the survival of the species in extreme environmental conditions have been identified. A significant decrease in *P. sylvestris* morphometic parameters (tree height, the annual growth of the tree, and the branches) under dump conditions were established. Under the influence of unfavorable factors on the dumps (lack of nutrients and water, high rockiness of the substrate), the length and the cross-sectional area of the needles decreased. Of the anatomical features, it is important to note a decrease in the number of resin ducts with a tendency to increase their diameter.

INTRODUCTION

Open-pit mining, including in the Urals, is accompanied by the significant disturbance of soil and vegetation cover over large areas. During the process of mining, a considerable amount of unused mineral mass is extracted and stored in dumps. The restoration of vegetation in such areas is extremely slow. One of the pioneer species capable of settling in areas characterized by high rockiness, low fertility, and an unfavorable water regime in the Middle Urals is *Pinus sylvestris* L. [1–3]. This species growing in different climatic and soil conditions have differences in crown geometry, shoot morphology and needles anatomy [4–7]. Morphological and anatomical characteristics are important factors in the adaptive response to environmental changes [8].

The study of plant adaptations to technogenic stresses makes it possible to understand the mechanisms of plant resistance as well as the mechanisms that drive community-ecosystems relationships [9].

The purpose of the study was to identify the anatomical and morphological characteristics of *P. sylvestris* growing on the dumps of the mining industry in the Middle Urals.

MATERIAL AND METHODS

The research was carried out in 2019 on the mine dumps of the Anatol'sko-Shilovskiy asbestos deposits. The study area is located within the Tagilo-Shilovskiy hyperbasite massif on the eastern slope of the Middle Urals (Novoasbest settlement, Sverdlovsk region, taiga zone, southern taiga subzone). Anatol'sko-Shilovskiy deposits are confined to

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lenticular bodies of talc-carbonate rocks. A fibrous form of serpentine – rezhikite-asbestos was obtained as a useful mineral from mining extraction operations. The average asbestos content in the rocks was about 4–5% [10]. Mine dumps of the area are the waste products of exploitation, composed mainly of serpentines, and only about 10% of waste products are made up of loose clastic sediments and clay. The mineral composition of the waste rocks is represented by olivine – 67.8%, enstatite – 30.1%, diopside – 2.1%, amphibole-asbestos – 0.1%.

Mining exploitation was put into operation during the period 1952–1991 by Anatol'sko-Shilovskiy ore mining and processing enterprise using opencast mining technique. Currently, the disturbed lands are a quarry-dump complex, which includes five open pits with waste rock dumps (the total volume of waste rock from sorting of ore is 228.9 million tons) and a mining waste storage facility of stone crusher activities.

Analysis of the granulometric composition of the mine dumpsites showed predominantly rocky substrates where the content was mostly of stones and gravel (50–85%). The fine earth fraction was predominated by sand, where soil-water retention capacity of clay particles (< 0.25 mm) was of nonsignificant proportions in different dumpsites, including: "Shilovskiy" (6–8%); "Anatol'skiy" (10–15%); "Stone cutter dump" and "Yuzhniy" (5%). The agrochemical composition of substrates was characterized by a very low content of easily hydrolysable nitrogen, with average concentrations of plant-available phosphorus and potassium. The reaction of substrate is slightly alkaline (pH=7.2) [11].

A geobotanical survey of 20–40 year old serpentine mine dumps of the Anatol'sko-Shilovskiy mining facility showed that trees are the dominant type of vegetation including *P. sylvestris* (sp gr–cop), *Betula pendula* Roth, *Betula pubescens* Ehrh. (sp). On the stony open areas of mine dumps, trees were in thinned stands, where the total projective plant cover (PPC) is very low (0–5%). The most common herbaceous species are *Dendranthema zawadskii* (Herbin) Tzvel and the rarest of them, Urals orchid – *Epipactis atrorubens* (Hoffm. ex Bernh.) Bess. (sp). Forest phytocoenoses dominated by *P. sylvestris* with a crown density of up to 0.6–0.7 are formed in the dump areas with an admixture of loose overburden. *Calamagrostis arundinacea* (L.) Roth (cop_1), *Seseli libanotis* (L.) Koch (sp–cop_1) and others predominate in the herbaceous layer (PPC from 40 to 60%). Model trees were taken from different dumpsites (Table 1). Control samples of *P. sylvestris* were taken in the forest edge of the Uktus forest (Ekaterinburg city) on a clay loam substrate.

TABLE 1. List of studied sites, their codes and some geobotanical characteristics.

N	Code	Sites	PPC, %	Crown density
1	UD	Yuzhniy dump	0–1	–
2	SCD	Stone cutter dump	0–5	–
3	AFE	Anatol'skiy dump, forest edge	15–20	–
4	AF	Anatol'skiy dump, forest	40–60	0.6–0.7
5	SF	Shilovskiy dump, forest	10–40	0.6–0.7
6	UC	Natural phytocoenosis – Uktus forest park	50–60	–

To study the morphological parameters of *P. sylvestris*, 10 model trees of 20–25 years old were selected at each site. Tree height (TH), the annual growth of the trunk (AG) and lateral branches (GB) were measured. To study the morphological and anatomical characteristics, 20 pairs of needles of the second year, were taken from each model tree (the southern exposure), and fixed in 60% ethanol. Needles length (NL) was measured in pairs, as well as the cross-sectional area (CSA), vascular bundle area (VBA), thickness of epidermis (ET) and hypodermis (HT), the number of resin ducts (NRD) and their diameter (DRD). Needle cuts were prepared on a freezing microtome MZ-2 (Russia).

Anatomical characteristics of *P. sylvestris* needle were examined under an Olympus CX-41 microscope, with the ocular and objective magnification of ×10 and ×40. All measurements were done using a SIAMS MesoPlant software (Russia).

The content of total nitrogen and phosphorus in the dried and homogenized *P. sylvestris* needles was measured spectrophotometrically (UV-visible spectrophotometer PD303-UV "Apel", Japan) after the wet digestion of plant material by a mixture of acids: H_2SO_4 and $HClO_4$ (10:1 v/v). The determination of total nitrogen was carried out using Nessler's reagent, and total phosphorus – with ammonium molybdate in an acidic medium [12] and expressed in % of dry weight (DW). The analysis was carried out in three analytical replicates. The mean values and their standard errors (SE) are presented in the Tables 2 and 3.

Statistical analysis was performed using Microsoft Excel and StatSoft STATISTICA 12. One-way ANOVA and discriminant analysis were performed. Statistically significant differences were revealed using the Tukey's HSD test at a $p < 0.05$. The different alphabetical letters on figures indicate the significant differences between the sites studied.

RESULTS AND DISCUSSION

The analysis of the *P. sylvestris* morphological features revealed differences between model and control samples. Under the conditions of dumpsites, the mean values of TH were significantly ($p < 0.05$) lower compared to the control site. AG and GB were also decreased (Fig. 1a, b**)**.

On the dumps, needle length of *P. sylvestris* was significantly ($p < 0.05$) less than in the control site on clay loams, which indicates xerophytic conditions. Different authors [13, 14] have noted the similar responses of plants growing on serpentines.

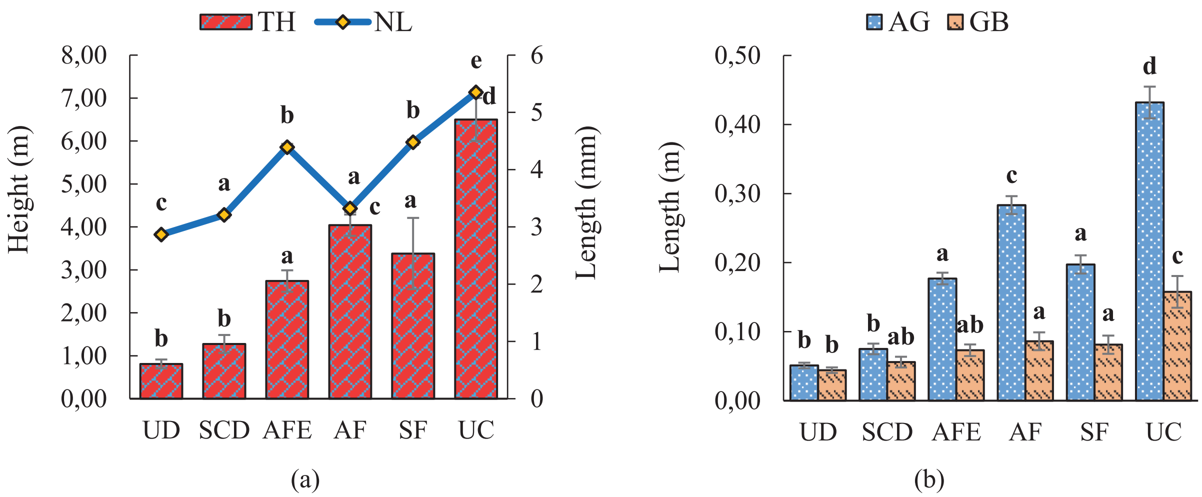

FIGURE 1. Morphological variables of *Pinus sylvestris*: tree height and needle length (a); annual tree growth and growth of pine branches of the 2nd year (b). Different alphabetical letters indicate significant differences according to Tukey's HSD test ($p < 0.05$). See sites abbreviation from Table 1

Compared to samples of the control site the analysis of the anatomical traits of *P. sylvestis* needles (Table 2) showed that conditions of serpentine dumps caused the significant ($p < 0.05$) decrease in cross-sectional area (CSA) and vascular bundle area (VBA). Also, the number of resin ducts were reduced (NRD), but their diameter increased (DRD) (Fig. 2a, b**)**. The thickness of the epidermis (ET) and hypodermis (HT) did not differ significantly from control values (UC).

TABLE 2. Average values with standard deviations of the analyzed needle characteristics.

Code	**Anatomical Characteristics**	**Sites**					
		UD	**SCD**	**AFE**	**AF**	**SF**	**UC**
CSA	Cross-Sectional Area of Needle (mm^2)	0.69±0.00^{a}	0.68±0.00^{b}	0.76±0.00^{a}	0.59±0.00^{d}	0.61±0.00^{b}	0.78±0.00^{c}
VBA	Vascular Bundle Area (mm^2)	0.35±0.00^{bc}	0.32±0.00^{a}	0.39±0.00^{ab}	0.37±0.00^{de}	0.28±0.00^{cd}	0.42±0.00^{e}
HT	Hypodermis Thickness (μm)	8.76±0.50^{a}	8.33±0.50^{ab}	11.20±0.60^{a}	10.36±0.70^{b}	9.81±0.60^{ab}	8.90±0.40^{ab}
ET	Epidermis Thickness (μm)	17.72±0.70^{a}	16.43±0.60^{a}	17.71±0.60^{a}	18.06±0.80^{a}	17.63±0.70^{a}	17.92±1.00^{a}
NRD	Number of Resin Ducts	5.03±0.26^{a}	5.27±0.16^{bc}	7.5±0.17^{ab}	6.2±0.38^{abc}	6.40±0.26^{c}	8.30±0.15^{d}
DRD	Diameter of Resin Ducts (μm)	42.62±2.73^{a}	40.36±1.80^{a}	38.69±1.40^{a}	40.02±2.40^{a}	39.86±1.80^{a}	34.40±2.40^{a}

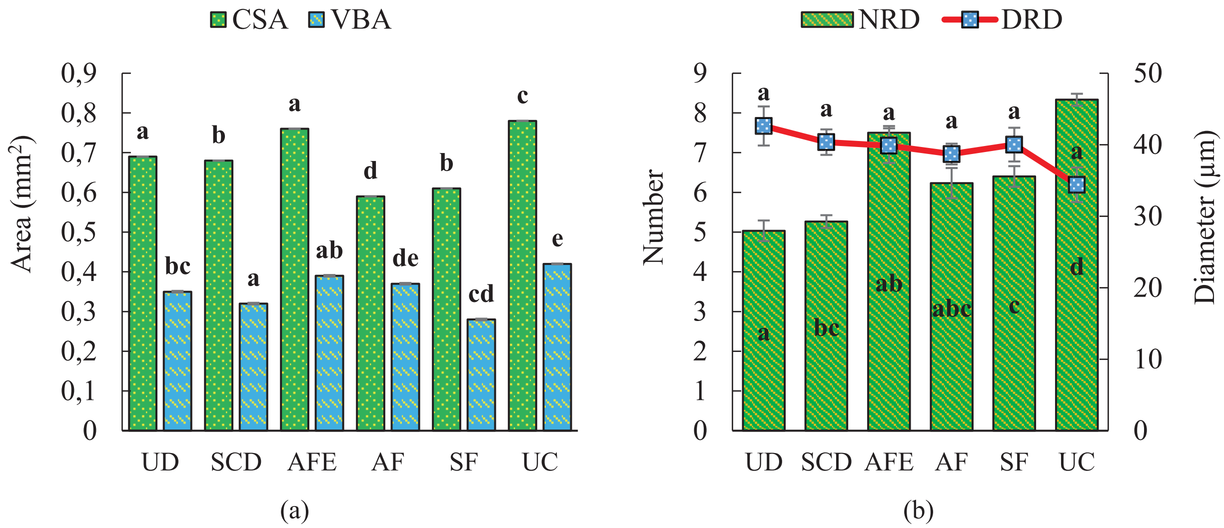

FIGURE 2. Anatomical variables of *P. sylvestris*: cross section of needle area and vascular bundle area (a); number of resin ducts and diameter of resin ducts (b). Different alphabetical letters indicate significant differences according to Tukey's HSD test ($p < 0.05$). See sites abbreviation from Table 1

The discriminant analysis of the studied characteristics on the Anatol'sko-Shilovskiy serpentine dumps demonstrated the adaptive changes of anatomical traits of *P. sylvestris* needles, that is important to the survival of plant species under extreme environmental conditions. The needles of *P. sylvestris* growing on serpentine dumps (UD, SCD, AFE, AF, SF) differed from the control forest phytocoenosis (UC) (Fig. 3).

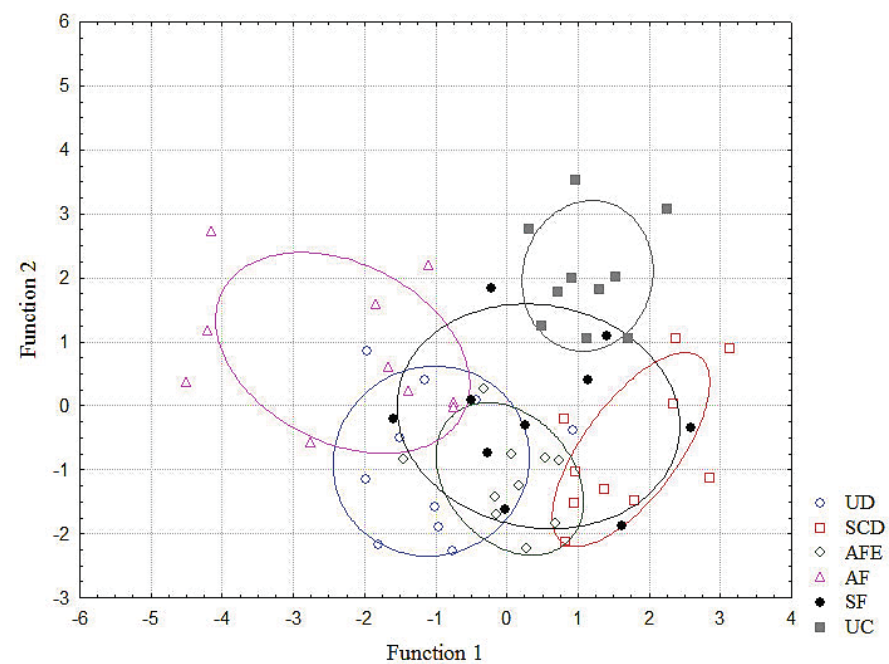

FIGURE 3. Differentiation of *P. sylvestris* by sites based on needle anatomical variables. See sites abbreviation from Table 1

In all samples of *P. sylvestris* needles on dumpsites, a deficiency of the main nutrients was detected (Table 3). According to Orlov and Koshel'kov [15], the optimal concentration of nitrogen in the needles of *P. sylvestris* are within 1.6–2.4%, phosphorus – 0.1–0.15%. According to our data, the most favorable conditions for the growth of

P. sylvestris were in the area of the Anatol'skiy dumpsite in which forest phytocoenosis was formed (AF). This can be explained by the higher content of clay particles in the substrate of this dumpsite, where the contents of total nitrogen and phosphorus in the needles of *P. sylvestris* were higher compared to the rest of the other samples.

TABLE 3. Total concentration of nitrogen and phosphorus in the needles of *P. sylvestris* growing on Anatol'sko-Shilovskiy serpentine dumps.

Element, % DW	Sites					
	UD	SCD	AFE	AF	SF	UC
Nitrogen	0.23 ±0.02	0.38 ±0.03	0.24 ±0.01	0.92 ±0.03	0.26 ±0.01	0.88 ±0.03
Phosphorus	0.09 ±0.01	0.07 ±0.01	0.07 ±0.01	0.12 ±0.02	0.10 ±0.01	0.12 ±0.02

CONCLUSION

The results of this research revealed changes in both morphological and anatomical characteristics of *Pinus sylvestris* growing on the serpentine dumps. A significant decrease of tree height, annual trunk growth and the growth of the branches were established. Under unfavorable environmental conditions (lack of nutrients and water, and high rockiness of the substrate), the length and the cross-sectional area of the needles on dumpsites were lower compared to controls. It should be noted that the number of resin ducts was decreased with a high tendency to increase in their diameters.

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