ECOPHYSIOLOGICAL STUDY OF SUITABILITY OF *PICEA MARIANA* L. FOR AFFORESTATION IN ALKALIZED TERRITORIES IN NORTHEAST ESTONIA

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The present paper is based on experiments carried out with black spruce (Picea mariana (Mill.) B.S.P.) in an industrial area in Northeast Estonia. Two-year-old seedlings of black spruce were planted in a sample plot affected by a cement plant and in an unpolluted control sample plot in 1990. At the end of the experiment in 1997 it was ascertained that the impact of industrial alkaline air pollution complexes resulting in the alkalization and chemical deviations of growth conditions decreases the content of carbohydrates and disbalances the mineral element composition in different organs of trees. Changes in the physiology of trees retard the growth and bring about a decrease in total biomass. The sturdiness quotient was higher and the Dickson quality index was lower than the control, indicating serious damages of trees in the polluted area. Increasing share of needles in the total biomass in polluted areas in comparison with control trees suggests that compensation mechanisms were started in trees to increase the assimilating mass in order to survive under stress.

Introduction

For over 30-40 years oil shale mining and processing enterprises, cement factories and power plants have emitted qualitatively and quantitatively different air pollution complexes with high dust and ash concentrations, whose water solution pH is over 12. This has caused alkalization of the environment, physical and chemical changes in the soil, ground water and precipitation in Northeast Estonia [1, 2]. At present time creating green areas and afforestation of abandoned agricultural lands or exhausted oil shale opencast mines in Estonian industrial territories needs for essential ecological studies

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to enrich new forests with introduced species having a great productivity and aesthetic properties.

It is generally known that conifers are especially sensitive to environmental pollution and to other anthropogenic factors, therefore they have often been used as indicators of the state of the environment [3, 4]. The last decades the black spruce (*Picea mariana*) has attracted the attention of landscape architects and silviculturists because its beautiful shape of crown and decorative bluish green needles. However, there is not much information that would explain the tolerance of black spruce to the environmental factors in industrial regions (air and soil pollution) and the level of anthropotolerance of this species. Black spruce has been found to tolerate the effect of Al [5] and peroxyacetylnitrate (PAN), but it is sensitive to the effect of fluorides [6]. Practically no reliable information on the responses of this conifer to the most widely spread gaseous (SO₂, NO_x, H₂S) as well as solid (dust, soot) air pollutants can be found in the scientific literature.

The aim of this study was to ascertain of responses of black spruce to alkalized environment and to estimate the suitability of black spruce in industrial regions in Northeast Estonia. For reaching this aim, small experimental plots were established in the vicinity of the Kunda Cement Plant (Northeast Estonia) in 1990. The growth, development and physiological state of black spruce in the polluted area were compared with the corresponding parameters of trees planted on the relatively unpolluted territory of Lahemaa National Park.

Material and Methods

Study Area and Characteristics of Sample Plots

The physiological state of *Picea mariana* was studied from 1990 to 1999 in areas affected more than forty years by the cement plant in Kunda (59°30' N, 26°32' E). A relatively unpolluted territory of Lahemaa National Park (59°30' N, 25°57' E) located about 38 km west from Kunda opposite to prevailing winds was selected as the control area.

Climatically the studied areas belong to the mixed-forest subregion, where the influence of the Baltic Sea is strong (mean annual temperature is 4.9 °C, annual amount of precipitation 550 mm and dominating winds blow from the south-west at a mean velocity of 5.2 m sec^{-1}). The soils in the experimental sample plots are Gleyic Podzols on sands that show essential chemical changes in the vicinity of the industrial enterprises (Table 1).

On May 1990, forty two-year-old genetically similar and homogenous seedlings were planted on the sample plots under a high air pollution load at a distance of 0.5 km from the cement plant and on unpolluted control sample plots.

Parameter	Contro	1	Pollute	d
pН	4.6	(0.9)	8.1	(0.5)
Electric conductivity, mS cm ⁻¹	0.44	(0.03)	2.1	(0.09)
Elements, $g kg^{-1}$:	•			
Ca	0.97	(0.01)	40.79	(0.27)
Mg	0.1	(0.01)	1.39	(0.10)
К	0.05	(0.007)	1.13	(0.02)
Р	0.05	(0.002)	0.08	(0.001)
S	0.004	(0.002)	0.07	(0.001)
Fe	1.12	(0.04)	4.2	(0.031)
Mn	0.11	(0.003)	0.15	(0.02)
Ν	8.1	(0.06)	5.6	(0.13)

Table 1. Chemical Characteristics (±SD) of the Humus Horizon of Soil in the Sample Plots (1985–1997, *n* = 20)

Sample plot in Kunda. The main damaging factor for trees in the Kunda area was alkaline dust emitted from the cement plant, which constituted 81-91% of the total emission during the investigation period of 1990-1997 (24–100 kt per year). Gaseous pollutants SO₂, NO_x, CO etc. made up 9–19 % of the total (Fig. 1). The dust from electric filters contains many components, among which the following are predominant, %: CaO 40–50; SiO₂ 12–17; K₂O 6–9; SO₃ 4–8; Al₂O₃ 3–5; MgO 2–4; but also Fe, Mn, Zn, Cu, B etc. occur. The water solution of dust had pH values from 12.3 to 12.6 [7].

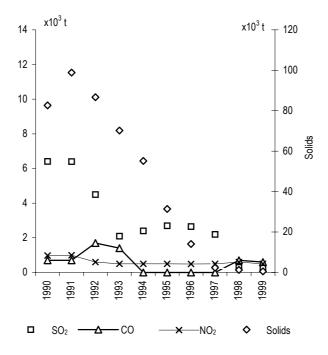


Fig. 1. Emission of air pollutants from stationary sources in Kunda

A high dust pollution load for about forty years has caused alkalization and changes in the chemical composition of the soil, soil water and precipitation in this area (see Table 1). The load of alkaline dust has been high, varying in recent years around $200-2400 \text{ gm}^{-2} \text{yr}^{-1}$ in the vicinity of the plant [8]. According to Smidt's scale [9], acknowledged in Europe, the pH of pure precipitation is 5.1–6.1. The rainwater in the influence zone of the cement plant during the years of our investigation can be regarded as strongly alkaline (pH 7.6–8.1). Although the pollution load and dust emission have been decreasing, still in 1998 the pH level of precipitation was over 7.5. The average pH of snow melt varied in different years from 8.6 to 11.0. In the experimental plot the pH of the humus horizon was 7.1–8.1 and the pH of ground water was 7.4–7.9. The concentrations of Ca, K, Mg, S, Mn and other predominant elements in the cement dust were extremely high [10].

Control sample plot in Lahemaa National Park. On the unpolluted control area the pH value of the soil humus horizon was from 3.6 to 4.4 (see Table 1), that of rain water 5.6–6.6 and snow melt 6.3–6.6. According to Smidt's [9] scale, the pH values of rain water and snow melt in the control area are normal.

Collection of samples for characterizing the growth conditions of trees was carried out following the methodology and procedures described in the ICP Manual for Integrated Monitoring on air Pollution Effects on Ecosystems [11] and in Forest Health Monitoring [12]. Soil samples for nutrient chemistry in the humus horizon were collected from five points of every sample plot with a steel humus bore, soil water was collected with lysimeters (n = 5), rain water with rain collectors (n = 7) and snow when a permanent snow cover had formed [13, 14].

Plant Material and Morphological Studies

In May 1990, forty two-year-old genetically similar and homogenous seedlings of black spruce were planted (spaces 50×50 cm) on the sample plots at a distance of 0.5 km from the cement plant and on a relatively unpolluted control sample plot.

The height of seedlings was measured every autumn beginning with the autumn after planting. Before bud break, in late April – early May 1994 and 1997, ten trees were excavated and the roots, stems, shoots, needles of shoots and stems were separated for the assessment of their fresh (*FW*) and dry (*DW*) weight (g), dry matter content (dry weight : fresh weight 100 %), and length of needles, shoots and stems (mm). The sturdiness quotient (h/d) was determined as the ratio of the height (mm) and stem diameter (mm) at the root collar. This quotient reflects the stocky or spindly nature of the seedlings. In general, the sturdiness quotient should closely parallel diameter in predicting survival and growth in the field [15].

The thickness of needles, shoots and stems was calculated as the ratio of their dry weight and length (mg cm⁻¹). The ratio of shoot and root dry weight

was devised as a parameter indicating balance between the transpirational and the water absorbing areas of seedlings. The dry weight of the aboveground part and roots was used to obtain the ratio shoot/root (S/R).

For the estimation of tree vitality and the outplanting success we used the Dickson quality index (QI) [16], calculated using the total tree dry weight, height/diameter and shoot/root ratios (dry weight) in the formula recommended by Thompson [15]:

$$QI = \frac{Total \ DW}{h/d + S/R}$$

Extraction and Biochemical Measurements

Carbohydrates. Total soluble sugar and starch concentrations were estimated using the methods recommended by Ferenbaugh [17], Marshall [18], Arasimovich [19] and Arasimovich and Ermakov [20]. All the separated organs of ten seedlings were carefully cleaned, cut into small pieces and immediately fixed in boiling ethanol and dried in the air. Only one-year-old needles and shoots were used in analyses, because they are the most important source of photosynthate and mineral nutrients retranslocation for the new needles and shoots during the pre-bud-break period and during their first week of development [21].

1-5 g of dried and homogenized plant material was used for repeated extraction of soluble sugars with 80 % ethanol, centrifuged and the soluble supernatant was collected. All the residue that remained after the removal of soluble sugars was dried, followed by gelatinisation in distilled water and digestion with 35 % perchloric acid [17, 18]. The extraction of hemicelluloses was carried out with the acid hydrolysis (2 % H₂SO₄) method, recommended by Arasimovich and Ermakov [20] and Sofronova and Chinenova [22]. The soluble sugar, starch and hemicelluloses extracts obtained were individually reacted with anthrone reagent (0.1 % anthrone in 72 % sulphuric acid) to produce a blue-green coloration and their absorbencies were measured at 620 nm [17, 23]. Concentrations were calculated using glucose curves as standard.

Mineral Nutrients. For mineral nutrient analyses, all the separated and cleaned organs were cut into small pieces and oven-dried at 70 °C [24]. After grinding 1-2 g of dried plant material of different organs of trees was chemically analyzed. Concentrations of metals (Ca, K, Mg, Fe, Mn) were determined by using an atom adsorption analyzer AAA-1N, nitrogen was measured by the method of Kjeldahl and phosphorus with the help of a WPA Heliflow c0310 flow injection analyzer at the Estonian Center of Agricultural Chemistry.

Statistical Analyses. Correlation coefficients (*r*), determination coefficients (R^2) and their significance (*p*) and standard deviations (±SD) from the mean were calculated using the package MS Excel 5.0. To determine differences between data, unpaired *t*-test ($\alpha = 0.05$) was used.

Table 2. Content, g kg⁻¹, of Mineral Nutrients in Different Organs of 6-Year-Old *Picea mariana* in Areas Polluted or Unpolluted by Cement Dust (average \pm SD of measurements; to calculate statistical differences between control and polluted seedlings the unpaired *t*-test was used, n = 5)

$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Area	Root		Stem		Shoot		Stem	needle	Shoot needle	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $						N					
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Control	6.6	(0.04)	4.6	(0.15)		(0.60)	11.2	(0.55)	12.5	(0.90)
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Polluted	5.2	(0.11)	4.3	(0.23)	7.4	(0.41)	9.6	(0.87)	10.7	(0.44)
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	р		**		ns	1	ns		*		*
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$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Control	1.4		0.9	(0.02)	-	(0.02)	1.5	(0.08)	1.6	(0.12)
pisisisisisKControl6.8(0.11)5.5(0.16)6.7(0.19)4.1(0.30)8.8(0.23)Polluted7.4(0.20)5.7(0.29)7.9(0.48)9.7(0.35)12.9(0.42)p*is************************CaControl5(0.09)2.4(0.06)3.5(0.09)4.4(0.23)5.7(0.31)Polluted15.2(0.83)6.8(0.35)12.8(0.62)14.1(0.43)15.4(0.69)p************************Polluted3.3(0.34)1.6(0.27)3.5(0.40)3.0(0.11)3.4(0.36)Polluted3.3(0.34)1.6(0.27)3.5(0.40)3.0(0.11)3.4(0.36)Polluted3.3(0.34)1.6(0.27)3.5(0.40)3.0(0.11)3.4(0.36)p*******************Control57(8.35)23.9(3.24)24.3(2.23)38(3.27)37.3(1.67)Polluted37.8(1.91)10.3(1.31)13.5(1.62)16(1.50)14.5(1.327)p*******************	Polluted	1.3	(0.01)	0.9	(0.02)	1.5	(0.02)	2.1	(0.08)	3.9	(0.10)
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$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Control	6.8	(0.11)	5.5	(0.16)	6.7	(0.19)	4.1	(0.30)	8.8	(0.23)
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Polluted	7.4	(0.20)	5.7	(0.29)	7.9	(0.48)	9.7	(0.35)	12.9	(0.42)
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$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Control	5	(0.09)	2.4	(0.06)	3.5	(0.09)	4.4	(0.23)	5.7	(0.31)
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Polluted	15.2	(0.83)	6.8	(0.35)	12.8	(0.62)	14.1	(0.43)	15.4	(0.69)
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$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Control	2.4	(0.21)	0.9	(0.12)	1.4	(0.15)	1.3	(0.03)	1.3	(0.05)
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Polluted	3.3	(0.34)	1.6	(0.27)	3.5	(0.40)	3.0	(0.11)	3.4	(0.36)
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$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Control	57	(8.35)	23.9	(3.24)	24.3	(2.23)	38	(3.27)	37.3	(1.67)
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Polluted	37.8	(1.91)	10.3	(1.31)	13.5	(1.62)	16	(1.50)	14.5	(1.327)
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Polluted 920 (31.92) 194 (21.69) 330.0 (22.62) 350 (22.32) 380 (30.23) p ***						Fe					
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Control	768	(48.36)	118.0	(9.15)	89.5	(8.58)	69	(10.01)	69.1	(12.41)
Total content of mineral elements Control 46.6 (4.36) 14.1 (2.11) 15.4 (1.05) 25.0 (2.03) 28.0 (1.00) Polluted 70.0 (4.77) 27.5 (0.92) 57.5 (4.06) 78.1 (5.03) 81.5 (5.32)	Polluted	920	(31.92)	194	(21.69)	330.0	(22.62)	350	(22.32)	380	(30.23)
Control 46.6 (4.36) 14.1 (2.11) 15.4 (1.05) 25.0 (2.03) 28.0 (1.00) Polluted 70.0 (4.77) 27.5 (0.92) 57.5 (4.06) 78.1 (5.03) 81.5 (5.32)	р		***		**	*	***		***	×	***
Polluted 70.0 (4.77) 27.5 (0.92) 57.5 (4.06) 78.1 (5.03) 81.5 (5.32)			То	tal cc	ntent o	of mir	neral el	eme	nts		
Polluted 70.0 (4.77) 27.5 (0.92) 57.5 (4.06) 78.1 (5.03) 81.5 (5.32)	Control	46.6	(4.36)	14.1	(2.11)	15.4	(1.05)	25.0	(2.03)	28.0	(1.00)
					()		()		(()
	p				**	*	***		***	لا	

Note: ns – not significant; * -p < 0.05; ** -p < 0.01; *** -p < 0.001.

Results

Biochemical Studies

Nutrients. Alkaline dust deposited on seedlings and alkalized soil caused serious deviations in the mineral composition of black spruce. The average contents of Ca, K and Mg, the predominating elements in the air pollution complex, were in the polluted area 289 and 103 % of those in the control (Table 2, Fig. 2).

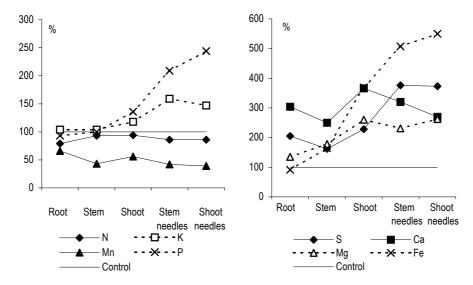


Fig. 2. Content of mineral elements in different components of *Picea mariana* in the area influenced by cement plant as percentage of control (100 %)

Under stress conditions the partitioning of mineral elements in seedlings was changed. The concentrations of several elements (P, Fe, K) in the aboveground components were higher than in the control seedlings, while in the roots these were close to the control (K) or significantly lower than in the control seedlings (P, Fe) (see Table 2 and Fig. 2). Thus, the average contents of P and Fe in the needles of polluted seedlings were 93 and 189 % greater than in control seedlings, but in the roots their contents were low, being 92 and 81 % of those in the control. Average concentrations of N and Mn in all components were lower than those in the control (by 13 and 49 %, respectively).

In the alkalized region, a disbalance of nutrient composition occurred and the ratios of elements in different organs had changed (Fig. 3). On average, the K/N ratio in polluted seedlings was 20–90 % greater in different components, while the N/P and N/S ratios were smaller compared to control especially in needles. The N/S ratio was 40–80 % of that in the control. A serious disbalance in the level of K/P ratio was found: in the root this ratio was 20 % greater and in the needles 20–40 % smaller compared to the control trees.

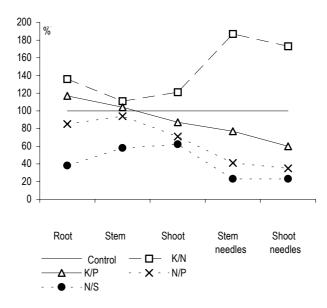


Fig. 3. Ratios of mineral elements in different components of *Picea mariana* in the area polluted by cement dust as percentage of control

Table 3. Content, mg g⁻¹, of Different Fractions of Carbohydrates and Level of the Ratio in Organs of 6-Year-Old *Picea mariana* (average \pm SD, to calculate statistical differences between control and polluted seedlings the unpaired *t*-test was used; n = 3-10)

Organ	Soluble sug	gars (SS)	Starch (S)		Hemicellul	SS/(S + HC)		
	Control	Polluted	Control	Polluted	Control	Polluted	Control	Polluted
Root p	45 (4.1) ***	12 (0.9)	72 (4.9) ns	71 (4.0)	115 (5.8)	99 (7.6)	0.24 **	0.07
Stem p	54 (5.5) ***	11 (0.9)	53 (2.8) ns	50 (2.5)	90 (4.2) ns	87 (7.3)	0.38 ***	0.08
Shoot p	91 (7.1) ***	39 (2.2)	71 (7.1) **	57 (4.8)	911 (4.1) ns	93 (5.0)	0.56 **	0.26
Stem needle p	118 (5.7) ***	100 (3.0)	112 (12.5) **	108 (10.9)	133 (6.9) ns	128 (7.4)	0.48 ns	0.42
Shoot needle p	107 (5.4) ***	91 (5.4)	118 (7.2) ***	95 (11.8)	146 (20.4) **	125 (11.1)	0.41 ns	0.42
Bud p	90 (11.1) ***	61 (8.5)	53 (6.8) *	47 (1.7)	105 (7.3)	90 (2.7)	0.58 *	0.44

Note: ns – not significant; * - p < 0.05; ** - p < 0.01; *** - p < 0.001.

Carbohydrates. Biochemical investigations were carried out in late April and early May when buds of trees did not yet show any important morphological changes, but metabolic processes had already activated [25], and a relatively large quantity of foliar carbohydrates may have been accumulated in conifers at that time [2, 26]. We found that starch, which is practically absent in both buds and needles in winter, had already increased before bud break in all components of seedlings, as had the content of hemicellulose. It is known that seasonal dynamics of soluble sugars tends to decrease in the pre-bud-break period [2, 27]. Still, their content in black spruce control trees seedlings was the highest in needles and buds but low in roots, stems and shoots (Table 3).

The total concentration of carbohydrates in black spruce was lower in polluted seedlings than in seedlings growing in the unpolluted area. The greatest differences were in the content of soluble sugars; the deviation was relatively small in needles, but greater in roots and stems, to some degree also in shoots and buds. The content of starch and hemicellulose was smaller as well, but less in comparison with changes in soluble sugars. The ratio of soluble sugars to polysaccharides was below the control level in the seed-lings grown under dust pollution and in alkalized environment (Fig. 4).

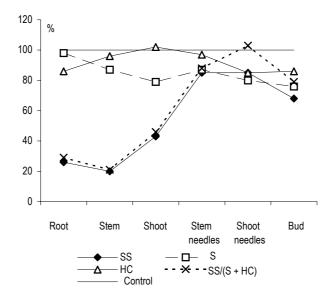


Fig. 4. Content of soluble sugars (SS), starch (S), hemicelluloses (HC) and the level of the ratio of soluble sugars/(starch + hemicelluloses) (SS/(S+HC)) in different components of *Picea mariana* in the area affected by cement plant as percentage of control. The content of carbohydrates in control trees equals 100 %; initial data in g kg⁻¹ DW are presented in Table 3

Before bud break, in six-year-old control seedlings the content of soluble sugars averaged 8.4 %, that of starch 8 % and hemicellulose 11.3 % of dry weight, the contents for polluted seedlings were 5.2, 7.1 and 10.4 % of dry weight, respectively. Regression analysis showed a relationship between the starch content and that of the soluble sugars ($R^2 = 0.58$, p < 0.001), and an interdependence of starch and hemicelluloses ($R^2 = 0.85$, p < 0.001) in black spruce.

Results and regression analysis showed a strong linear relationship between carbohydrates and N, P and K contents in all experimental trees in both control and polluted variants (Table 4). In addition, significant relation between carbohydrate accumulation and Ca, S and Mg – the dominant elements in the pollution complex – and the total concentration of mineral elements was observed in polluted seedlings.

and X-squared values were calculated using Wis Excer 5.0)									
Nutrier	nt	Contro	1			Polluted			
			Soluble sugars	Starch	Hemicel- lulose	Dry mass	Soluble sugars	Starch	Hemicel- lulose
Ν	р	0.753 ***	0.913 ***	0.511 **	0.682 **	0.580 **	0.927 ***	0.592 ***	0.719 ***
Р	p	0.291 ***	0.726 **	0.787 **	0.587 *	0.439 *	0.724 **	0.581 **	0.628 **
Ca	р	0.187 ns	0.007 ns	0.018 ns	0.083 ns	0.865 ***	0.813 ***	0.682 **	0.743 ***
K	р	0.610 *	0.450 *	0.576 *	0.759 **	0.590 *	0.546 **	0.754 **	0.787 ***
Mg	р	0.169 ns	0.237 ns	0.421 ns	0.331 ns	0.510 **	0.425 *	0.421 *	0.444 *
Mn	р	0.239 ns	0.035 ns	0.049 ns	0.233 ns	0.060 ns	0.126 ns	0.005 ns	0.299 ns
S	р	0.542 *	0.351 ns	0.443 ns	0.686 *	0.134 ns	0.820 ***	0.836 ***	0.913 ***
Fe	p	0.322 ns	0.365 *	0.576 *	0.265 ns	0.432 ns	0.416 *	0.654 ns	0.461 ns
Total content of mine element	eral	0.342	0.026	0.035	0.206	0.211	0.533	0.710	0.707
	р	*	ns	ns	ns	ns	*	**	**

Table 4. Relationship between Dry Mass, Carbohydrates and Content of Nutrients in the Organs of *Picea mariana* (linear regression type and *R*-squared values were calculated using MS Excel 5.0)

Note: ns – not significant; * - p < 0.05; ** - p < 0.01; *** - p < 0.001.

Parameter	Control		Polluted							
Nee	Needle									
One-year (needles and shoots formed in 1993):										
Length, mm	14.0	(0.72)	6.8	(0.36)						
FW of 100 needles, g	0.44	(0.16)	0.12	(0.03)						
DW of 100 needles, g	0.30	(0.09)	0.07	(0.02)						
Dry matter, %	67.8	(4.74)	56.1	(5.3)						
Thickness, mg cm ⁻¹	3.14	(0.01)	1.76	(0.02)						
Two-year (needles and shoots formed in	1992):		1							
Length, mm	10.8	(0.19)	7.4	(0.08)						
FW of 100 needles, g	0.25	(0.1)	0.1	(0.02)						
DW of 100 needles, g	0.17	(0.07)	0.07	(0.01)						
Dry matter, %	69.7	(3.59)	63.7	(4.0)						
Thickness, mg cm ⁻¹	2.32	(0.12)	1.35	(0.02)						
Sho	ot									
One-year (needles and shoots formed in 1	1993):									
Length, mm	301.4	(13.74)	38.8	(7.7)						
FW, g	0.93	(0.27)	0.021	(0.001)						
DW, g	0.53	(0.19)	0.014	(0.001)						
Dry matter, %	59.4	(4.94)	71.9	(11.3)						
Density of needles on shoot, No cm ⁻¹	10.8	(3.15)	28.8	(6.61)						
Thickness, mg cm ⁻¹	17.6	(0.81)	3.61	(0.9)						
Two-year (needles and shoots formed in	1992):		1							
Length, mm	120.1	(6.68)	28.6	(7.35)						
<i>FW</i> , g	0.69	(0.03)	0.006	(0.00)						
DW, g	0.45	(0.2)	0.005	(0.001)						
Dry matter, %	64.7	(3.15)	85.4	(10.2)						
Density of needles on shoot, No cm ⁻¹	16.8	(4.12)	23.4	(6.3)						
Thickness, mg cm ⁻¹	37.5	(3.9)	1.75	(0.6)						
Ste	m									
Length, mm	859.1	(18.4)	214.1	(7.5)						
FW, g	58.8	(5.16)	1.65	(0.49)						
DW, g	29.4	(5.3)	0.91	(0.27)						
Dry matter, %	49.9	(2.7)	554.0	(2.3)						
Thickness, mg cm ⁻¹	342.2	(32.6)	42.5	(2.5)						
Ro	ot									
<i>FW</i> , g	56.3	(10.3)	1.8	(0.4)						
DW, g	35.7	(10.7)	0.95	(0.05)						
Dry matter, %	61.7	(8.7)	53.2	(1.53)						

Table 5. Morphological Differences of 6-Year-Old *Picea mariana* Grown in the Control and Industrial Area in 1994 (average ±SD)

Parameter	Control		Polluted						
Needles									
Stem needles:									
Total FW, g	9.45	(2.19)	2.29	(0.34)					
Total DW, g	2.59	(0.22)	1.42	(0.12)					
Dry matter, %	27.4	(2.11)	62.0	(1.339)					
Shoot needles:									
Total FW, g	489.1	(21.1)	76.5	(2.31)					
Total DW, g	297.5	(16.2)	60.2	(3.99)					
Dry matter, %	60.8	(5.6)	79.9	(7.3)					
	Shoc	ts							
Total FW, g	265.7	(21.1)	41.3	(3.9)					
Total DW, g	173.7	(12.3)	28.8	(2.9)					
Dry matter, %	65.4	(7.1)	69.7	(6.8)					
	Stei	n							
FW, g	393.6	(22.1)	44.3	(1.9)					
DW, g	195.2	(10.1)	34	(3.6)					
Dry matter, %	49.6	(6.9)	76.6	(5.6)					
	Roc	t							
FW, g	204.3	(9.7)	32.3	(3.6)					
DW, g	112.2	(9.1)	25.7	(2.7)					
Dry matter, %	54.9	(9.9)	79.5	(2.9)					
	Total	tree	_						
FW, g	1362.2	(28.9)	196.7	(19.0)					
DW, g	781.2	(14.6)	150.1	(6.8)					
Root collar d, mm	4.8	(0.19)	1.03	(0.38)					
QI	15.6	(1.3)	1.52	(0.13)					
h/d	47	(3.9)	95	(5.2)					
S/R	4.23	(0.21)	3.52	(0.47)					

Table 6. Total Biomass and Morphological Indicies of Nine-Year-Old *Picea mariana* in the Control and Influenced by Cement Plant Area in 1997 (average ±SD)

Morphological Studies

In the area affected by high dust pollution the height growth of seedlings became inhibited by about 30 % compared with the control already from the first year of growth. At the end of the experiment in 1997, the differences of the height growth from the control were significant at the level p < 0.01. In autumn 1997, the control seedlings were healthy but in Kunda over 15 % of the seedlings were dead. In addition to retarded height increment also shorter length of needles and shoots and smaller thickness of all organs and S/R were observed in the polluted area (Tables 5 and 6).

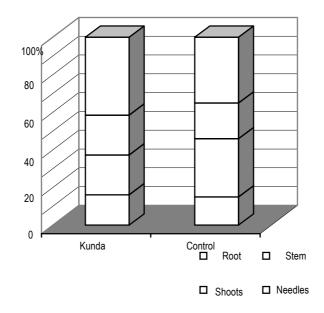


Fig. 5. Influence of alkalisation of the environment and dust pollution on the partitioning of the biomass of *Picea mariana*

It is obvious that decreasing height growth of a seedling and retarded length and thickness growth of all its components mean a decrease in the total biomass of the seedling. In 1997, the total biomass of the seedling studied in Kunda made up only 15–19 % of that in the control (both fresh and dry weight). Also the partitioning of biomass between the needles, shoots, stem and roots was different. Taking the total fresh weight of a seedling as 100 %, the proportion of the mass of needles of seedling growing in Kunda was relatively higher than that of control seedling (Table 6, Fig. 5).

Roller [28] found that black spruce seedlings with sturdiness quotients (h/d) greater than six were seriously damaged when exposed to wind, drought or frost. The same seems to be true for our experimental seedlings affected by dust pollution, since in the polluted areas the sturdiness quotient was over 9.5, being in Kunda 102 % higher than the control.

Area	Parameter											
	Ν	N P K Ca Mg Fe Mn										
Control	0.753	0.291	0.610	0.187	0.169	0.322	0.239					
р	***	ns	*	ns	ns	ns	ns					
Polluted	0.580	0.439	0.590	0.865	0.510	0.134	0.060					
p	**	*	*	***	**	ns	ns					

Table 7. Relationship between Dry Mass and Biochemical Parameters in the Organs of *Picea mariana* (*R*-squared values were calculated using MS Excel 5.0)

Note: ns – not significant; * – p < 0.05; ** – p < 0.01; *** – p < 0.001

Dickson quality index (QI) for black spruce was over 10 times lower than control in alkalized environment in the vicinity of the cement plant in Kunda (see Table 6).

Changes in primary metabolism of trees and complication of mineral nutrition processes in alkalized environment are accompanied by changes in growth and biomass formation. Partitioning of dry mass between different components (see Table 6) in control sample plot depends significantly on the N and K content (see Table 4). Dependence of the dry mass of black spruce on mineral elements in the polluted area differs from that in optimal conditions. In the area influenced by cement dust the mass of trees depends significantly on the dominant elements of the cement dust, Ca, Mg and P, which are not important in the control area (Table 7).

Discussion

Our investigations showed that the indirect effect through environmental conditions and the direct effect of the solid ingredients of air pollution on the assimilating organs were factors of significant influence on the development of seedlings. Cement dust, covering the plant organs above ground, acts as a light-selecting filter reducing penetration of photosynthetically active parts of the spectrum [29], rising the temperature of plant tissues [30] and transpiration intensity [31]. This causes reduced photosynthesis, a decrease in the content of photosynthetic pigments in the needles [7, 29, 32] and other changes in metabolism.

It has been shown that plants respond to stress by changes in carbon assimilation and in the partitioning of carbon and other resources within the plant. The availability of many nutrients inhibits when pH of soil rises and imbalance of nutrients develops in the plant: Ca and K accumulated in tissues in large amounts, while the availability of Mn and N was hindered in polluted seedlings. Especially, a drastic shortage of Mn in all components of seedlings may be explained by the fact, described by Marschner [33], that in an alkaline soil Mn²⁺ oxidizes into Mn³⁺ and Mn⁴⁺, which are for plants difficult to assimilate. Mn deficiency was shown earlier in plants growing in high pH soils by Farley and Drycott [34], and Ca and Mn interdependence in plants was noted also by Foy *et al.* [35].

All these processes are closely related to carbohydrate metabolism and cause changes in carbohydrate content and partitioning. The greatest changes were in the content of soluble sugars in all components of black spruce. Content of soluble sugars to decreased relatively little in needles, but more in roots and stems, to some degree also in shoots and buds. The content of starch and hemicellulose decreased as well, but less in comparison with changes in soluble sugars. The ratio of soluble sugars to polysaccharides was below the control level in the seedling grown under dust pollution in an alkalized environment. In general, carbohydrate synthesis and transport to other parts of plants is initially controlled within the leaf. Roots were found to be particularly sensitive to cement dust and to alkalization of the growth substrate [2] and the resulting changes in carbohydrate content correlated well with those in needles, shoots and stem: r values ranged from 0.82 to 1.0 for soluble sugars in needles at a significance level p < 0.01.

In the pre-bud-break period, young black spruces contained 23–30 % carbohydrates in dry mass, most of this amount being starch and hemicellulose. The distribution of carbohydrates in the organism is determined by the functional properties and activity of the organs. Thus, in needles, serving as photosynthetic organs, the content of carbohydrates was much higher than in non-photosynthesizing roots and stem. Also, the starch content was different in stem needles and better illuminated shoot needles, being higher in the latter of black spruce of the control area, but lower in the sample of the polluted area.

Low carbohydrate reserves in stems and roots decrease root growth and increase susceptibility to other stress factors [36]. The concentration of carbohydrates is also low in buds, which may hinder the development of new shoots and needles.

Most ecophysiological researches in this field use the response reactions in needles to evaluate the effect of environmental changes; however, it would be necessary to pay attention to different sensitivity of components. Our analysis of morphological measurements showed that as to their sensitivity to alkaline environment the components of black spruce can be ranked as follows: shoot > stem > root > needle.

Although the total biomass of black spruce was lower in the polluted area than control, we found that under abiotic stress a so-called compensation effect is induced in the organism: the proportion of needles in the total organism increases to guarantee photosynthetic activity, biosynthesis of organic matter and survival of the organism in unfavorable conditions. In addition, the extremely short shoots were densely covered with needles. This phenomenon has been observed also in case of several other coniferous species under stress [10]. The compensation effects in the reproduction of plants were described also by Chiariello and Gulmon [37] under the drought and by Murray and Wilson [38] after the fumigation with SO₂. Since the compensation effect induced by abiotic factors has not been finally studied and its mechanisms are not yet understood, this phenomenon is believed to be based on phenotypic plasticity of organisms, i.e. their ability to undergo physiological and morphological changes according to the character and amplitude of response reactions.

Results of presented investigation indicated that the response reactions of black spruce in a strongly alkalized environment were negative, compared with the control, which is a sign of the sensitivity of this species to industrial pollution. In a relatively unpolluted area in Lahemaa National Park but under analogous climatic conditions black spruce showed vigorous growth.

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REFERENCES

- Mandre, M. Changes in the nutrient composition of trees // Dust Pollution and Forest Ecosystems. A Study of Conifers in an Alkalized Environment / M. Mandre (ed.). Publ. Inst. Ecol. Vol. 3. Tallinn. 1995. P. 44–65.
- Mandre, M. Effects of dust pollution on carbohydrate balance in conifers // *Ibid*. P. 78–95.
- 3. *Manning, W.J., Feder, W.A.* Biomonitoring Air Pollutants with Plants. London : Applied Science Publishers, 1980.
- Schulze, E.-D., Lange, O.L., Oren, R. (eds.). Forest Decline and Air Pollution. A Study of Spruce (*Picea abies*) on Acid Soils. – Berlin, Heidelberg, New York : Springer-Verlag, 1989.
- Maliondo, S.M., Krause, H.H. Genotype and soil fertility interactions in the growth of black spruce progeny from a central New Brunswick population // Can. J. For. Res. 1985. Vol. 15. P. 410.
- Smith, W.H. Air Pollution and Forests. Interaction between Air Contaminants and Forest Ecosystems. – New York, Berlin, Heidelberg : Springer-Verlag, 1990.
- 7. *Mandre, M., Tuulmets, L.* Pigment changes in Norway spruce induced by dust pollution // Water Air Soil Pollut. 1997. Vol. 94. P. 247–258.
- Mandre, M., Tuulmets, L., et al. Response reaction of conifers to alkaline dust pollution. Changes in growth // Proc. Estonian Acad. Sci., Ecol. 1994. Vol. 4, No. 2. P. 79–95.
- Smidt, S. Analysen von Niederschlagsbrodeln aus Waldgebieten Österreich // Allg. Forstztg. 1984. Vol. 95, No. 1. P. 13–15.
- 10. *Mandre, M., Ots, K.* Growth and biomass partitioning of 6-year-old spruces under alkaline dust pollution // Water Air Soil Pollut. 1999. Vol. 114. P. 13–25.
- Manual for Integrated Monitoring. Programme Phase 1993–1996. Environmental Report 5. – Helsinki : Environment Data Centre, National Board of Waters and the Environment, 1993.
- Forest Health Monitoring. Field Methods Guide. Environmental Monitoring System, Laboratory. – Las Vegas, USA, 1996.
- Tuulmets L. Chemical composition of precipitation // Dust Pollution and Forest Ecosystems. A Study of Conifers in an Alkalized Environment. P. 23–32.
- 14. Annuka, E., Mandre, M. Soil responses to alkaline dust pollution // Ibid. P. 33-34.

- Thompson, B.E. Seedling morphological evaluation what you can tell by looking // Evaluating Seedling Quality : Principles, Procedures, and Predictive Abilities of Major Tests / M.L. Duryea (ed.). Corvallis : Forest Research Laboratory, Oregon State University, 1985. P. 59–71.
- 16. Dickson, A., Leaf, A.L., Hosner, J.F. Quality appraisal of white spruce and white pine seedling stock in nurseries // For. Chron. 1960. Vol. 36. P. 10–13.
- Ferenbaugh, W.R. Effects of simulated acid rain on Phaseolus vulgaris L. (Fabaceae) // Am. J. Bot. 1976. Vol. 63. P. 283–288.
- Marshall, J.D. Carbohydrate states as a measure of seedling quality // Evaluating Seedling Quality : Principles, Procedures, and Predictive Abilities of Major Tests. P. 49–58.
- Arasimovich, V.V. Measurement of saccharides // Methods for Biochemical Investigations of Plants / A.I. Ermakov (ed.). Leningrad : Agropromizdat, 1987. P. 122–142 [in Russian].
- Arasimovich, V.V., Ermakov, A.I. Measurement of polysaccharides and lignin // Ibid. P. 143–172 [in Russian].
- 21. Ziemer, R.R. Translocation of ¹⁴C in ponderosa pine seedlings // Can. J. Bot. 1971. Vol. 49. P. 167–171.
- Sofronova, G.J., Chinenova, L.A. Content of carbohydrates in shoots of pines with different annual radial increment // Ecophysiological Investigations of Woody Plants. Petrozavodsk : Academy of Sciences S.U., 1987. P. 26–36 [in Russian].
- 23. *Peace, E.A., Lea, P.J., Darrall, N.M.* The effect of open-air fumigation with SO₂ and O₃ on carbohydrate metabolism in Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) // Plant Cell Environ. 1995. Vol. 18. P. 277–283.
- Landis, T.D. Mineral nutrition as an index of seedling quality // Evaluating Seedling Quality: Principles, Procedures, and Predictive Abilities of Major Tests. 1985. P. 29–48.
- 25. *Ericsson, A.* Seasonal changes in translocation of ¹⁴C from different age-classes of needles on 20-year-old Scots pine trees (*Pinus sylvestris*) // Physiol. Plant. 1978. Vol. 43, No. 4. P. 351–358.
- Amundson, R.G., Kohut, R.J., et al. Red spruce reversibly allocates starch to sugars in foliage during moderate water stress // Environ. Exp. Bot. 1993. Vol. 33. P. 383–390.
- 27. Wallin, G., Ottosson, S., et al. Effects of ozone and phosphorus deficiency on the carbon allocation and growth of Norway spruce, *Picea abies* (L.) Karst. // Critical Levels for Ozone. Experiments with Crops, Wild Plants and Forest Tree Species in the Nordic Countries / L. Skärby, H. Pleijel (eds.). Copenhagen : TemaNord, Nordic Council of Ministers, 1996. P. 60–66.
- Roller, S.J. Suggested Minimum Standards for Containerized Seedlings in Nova Scotia. – Can. For. Serv. Dept. Environ. Inf. Rep. M-X-69, 1977.
- 29. *Borka, G.* The effect of cement dust pollution on growth and metabolism of *Helianthus annuus* // Environ. Pollut. 1980. Vol. A 22. P. 75–79.
- Flückiger, W., Flückiger-Keller, H., Oertly, J.J. Der Einfluß von Straßenstaub auf den stomataren Diffusionswiderstand und die Blatt-Temperatur – ein antagonistischer Effect // Staub Reinhalt. Luft. 1978. Vol. 38. P. 502–505.

- 31. Singh, S.N., Rao, D.N. Certain responses of wheat plants to cement dust pollution // Environ. Pollut. 1981. Vol. A 24. P. 75–78.
- Ricks, G.R., Williams, R.J.H. Effects of atmospheric pollution on deciduous woodland, 3: Effects on photosynthetic pigments of leaves of *Quercus petraea* (Mattuschka) Leibl. // Environ. Pollut. 1975. Vol. 8. P. 97–106.
- 33. *Marschner, H.* Mineral Nutrition of Higher Plants. London : Academic Press, 1986.
- 34. *Farley, R.F., Drycott, A.P.* 1973. Manganese deficiency of sugar beet in organic soil // Plant Soil. Vol. 38. P. 235–244.
- 35. Foy, C.D., Webb, H.W., Jones, J.E. Adaptation of cotton genotypes to acid, manganese toxic soil // Agron. J. 1981. Vol. 73. P. 107–111.
- Dickson, R.E., Isebrands, J.G. Leaves as regulators of stress response // Response of Plants to Multiple Stresses / H.A. Mooney, W.E. Winner, E.J. Pell (eds.). San Diego : Academic Press, 1991. P. 4–34.
- Chiariello, N.R., Gulmon, S.L. 1991. Stress effect on plant reproduction. // Ibid. P. 161–188.
- Murray, F., Wilson, S. The joint action of sulphur dioxide and hydrogen fluoride on the yield and quality of wheat and barley // Environ. Pollut. 1988. Vol. 55. P. 239–249.

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