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Biochemical and structural characteristics of Scots pine (*Pinus sylvestris* L.) in an alkaline environment

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Abstract. Investigations in a 75–85-year-old Scots pine stand were performed on a territory influenced over 40 years by alkaline dust pollution (pH 12.3–12.7) emitted from a cement plant. Sample plots were located at distances of 2, 3, and 5 km E of the emission source and a control sample plot was selected on an unpolluted territory 38 km W of it. We studied soil properties and the anatomical structure, mineral nutrition, and pigments in Scots pine needles. The alkaline dust pollution has affected the biogeochemical cycling in the forest ecosystem, increasing the pH and total Ca, K, Fe, Mn, and Mg and decreasing N, C, organic matter, and C/N compared to the unpolluted soils. Alkalization and changes in the nutrient composition of soil had caused serious disbalances in nutrient availability and in the mineral composition of trees. Deficiency in foliar N and Mn and excess of K, Ca, and Fe contents had caused a decrease in the average chlorophyll concentrations. Carotenoids seemed to be more tolerant both to changes in soil and needle nutrient composition. The Chl *a*/Chl *b* ratio in needles was found to have declined. With the alkalization of the environment the total area of the needle cross-section, needle thickness, and mesophyll area had also decreased compared with control. Differences in the anatomical characteristics of needles between the polluted and unpolluted areas were significant in the oldest needles. The decrease of mesophyll was associated with the content of chlorophylls and correlated with N concentrations in needles.

Key words: Scots pine, cement dust, soil alkalization, mineral nutrition, pigments, needle anatomy.

INTRODUCTION

In order to be able to forecast and estimate the state and dynamics of forest ecosystems, it is necessary to understand the mechanisms and character of response reactions of trees to the different complexes of environmental factors. Balanced metabolism, in which an optimum supply of nutrients is of prime importance, is indispensable to preserve functional and structural integrity of a tree. Air pollution may affect the whole-tree nutrient budget under the influence of both acidic and alkaline air pollutants. The available evidence demonstrates significant modification of the tolerance of trees to air pollution. The plasticity in nutrient availability and responsiveness in morphology are not fully understood and may be related to fitness, especially over a wide-ranging gradient of specific environmental factors (González & Gianoli, 2004).

Numerous investigations describe damages caused by acidic air pollutants to forest areas in many industrial countries towards the end of the 20th century (Guderian, 1977; Jäger & Klein, 1980; Hellmann, 1993). However, too little attention has been paid to research into the impact of alkaline types of pollutants such as industrial dusts or ashes on forests. Generally, dust pollution has a rather local character and no specific visible symptoms occur on plants under the influence of alkaline dusts and ashes, emitted from building materials production, open-cast mining, quarrying, metallurgy, etc. However, this may create a wrong conception about the effects of alkaline pollutants on plants.

The sensitivity of plants to alkaline particulates is very variable and their impacts described in the literature are contradictory. It is unanimously stated that deposition of alkaline dusts and ashes on the underlying surface results in alkalization of soil and input of excess nutrients into the ecosystems (Kaasik et al., 2005; Świercz, 2006), which complicates mineral nutrition processes of plants (Marschner, 2002; Mandre, 2009). Alkalization of the environment and disbalances in the nutrient composition of soil and trees cause several changes in morphological and biochemical characteristics of plants. Serious changes in the biochemistry and physiology such as a decrease in the content of carbohydrates (Klõšeiko, 2005) and an increase of Ca, K, lignin, and phenolic compounds (Mandre, 2002) have been described. In areas alkalized by cement dust a decrease in the length of needles and shoots, radial increment, and height growth of Scots pine has been reported (Rauk, 1995; Pärn, 2002). Structural changes of tissues of herbaceous plants caused by acidic air pollution have already been studied intensively and have been proved to be of considerable importance as diagnostic indicators for monitoring plant state. In the literature also a lot of information is available for conifers on the needle ultrastructural level under the impact of acidic air pollution. In conifers changes in the cuticle, extensive erosion of epicuticular wax (Huttunen & Laine, 1983; Huttunen, 1985; Rinallo et al., 1986), and alterations in mesophyll cells and chloroplast shapes under the influence of acid rain (Bäck & Huttunen, 1992) are described. Relationships between the parameters of the anatomical structure of pine needles under industrial stress was studied by Fedorkov (2002), but the structural changes caused by nutrient excess in soil and increased concentrations in needles have not been described. It is known that the number of sclerenchyma cells and the cross-sectional area of the central cylinder of needles may be influenced by nutrient deficiency or other stress factors on the foliage of conifers as described by several authors (Sutinen et al., 2006, 2007; Günthardt-Goerg & Vollenweider, 2007; Sutinen & Saarsalmi, 2008). A clear reduction of the membrane system in the chloroplast and disturbances in the structure of phloem cells related to Mg deficiency were observed in Scots pine (Palomäki & Raitio, 1995). However, in the literature little information is available for conifers influenced by alkaline air pollution.

We found earlier that due to alkalization of the environment the area of mesophyll had decreased (Lukjanova & Mandre, 2010). It seems that the changes in mesophyll tissues may be an indicator of the plant state because these tissues are specialized in photosynthesis and contain many chloroplasts, the organelles that perform photosynthesis. Changes in mesophyll cells are reflected in the concentration of chlorophylls and shapes of chloroplast, which are often used as indicators of the state of plants influenced by air pollution (Schubert, 1985; Kangur, 1988; Bäck & Huttunen, 1992; Nilsen & Orcut, 1996; Sutinen et al., 1998). Breakdown of chlorophyll under environmental pollution results in the inhibition of the photosynthetic process (Pallardy, 2008) and serious changes in plant productivity (Thomas & Packham, 2007).

This paper reports the influence of alkalization of soil due to cement dust deposition on the content of pigments and anatomical characteristics of needles of Scots pine growing on a gradient of soil disbalanced in mineral nutrient composition and in pH. Information on the effects of alkaline dust pollution on soil and tree stands may be quite useful for understanding the tolerance of trees to air pollution in general.

MATERIALS AND METHODS

Study area

The research transect is located near the cement plant in the town of Kunda (North-East Estonia). Dust constituted 85–90% of the total air pollutants emitted from the plant for over 40 years. The dust contained the following predominant components: 40–50% CaO, 12–17% SiO_2, 6–9% K_2O, 4–8% SO_3, 3–5% Al_2O_3, 2–4% MgO, but also Fe, Mn, Zn, Cu, B, etc. occurred. The water solution of dust from electric filters had pH values from 12.3 to 12.7 (Mandre, 1995, 2002). The dust emission from the cement plant was extremely high before 1996 (80–100 kt per year) (Environmental Information Centre, 1996, 1997).

Three sampling plots (0.05 ha) were located in the zone of high pollution 2–5 km E of the cement plant. A control sample plot was selected on the unpolluted territory of Lahemaa National Park in similar climatic and edaphic conditions (38 km W of the cement plant, opposite to prevailing winds).

In the selection of sample plots we proceeded from the principle of analogy of landscape and silvicultural characteristics according to the recommendations from the *Manual for Integrated Monitoring* (1993). The selected stands were similar as to their density, site quality (index II), site type (*Oxalis–Myrtillus* according to the local classification of site types; see Lõhmus, 2004; Paal, 2007), and age, forest site type, and composition of trees (Table 1). This made comparison of the results possible as the effect of numerous factors (climate, phytocoenosis etc.) affecting the growth of conifers in addition to the pollution load could be eliminated.

Table 1. Average characteristics of the investigated stands where sample plots of Scots pine were selected at different distances and direction from the cement plant in Kunda

Sample plot	Composition of trees*, %	No. of trees per ha	Age, yrs	Stand density	Height, m	Diameter, cm	Density of understory
38 km W	90*Ps*10*Pa* + *Be*	650	80	0.7	22	31	Moderate
2 km E	100*Ps* + *Pa*	680	85	0.8	20	25	Slight
3 km E	100*Ps* + *Pa*	660	80	0.7	21	29	Moderate
5 km E	90*Ps* + 10*Pa*	660	80	0.8	22	31	Moderate

* *Ps*, *Pinus sylvestris*; *Pa*, *Picea abies*; *Be*, *Betula* spp.

Soil analyses

The soils of the forest sample plots were Gleyic Podzols on sand (according to IUSS Working Group WRB, 2006). Soil samples were collected in five replications from all sample plots in 2006, 2008, and 2009 from depths of 0–30 cm (all together 15 samples), taking into account that approximately 80% of the feeder roots of Scots pine are located in the layer of the main root zone of 10–30 cm (Lõhmus & Lasn, 1990; Augustaitis et al., 2010).

The collected samples were dried and sieved through a sieve with 2 mm mesh size. Available P (mg kg^{-1}) in the soil was extracted by ammonium lactate and measured by flow injection analysis, with the use of Tecator ASTN 9/84 (Růžička & Hansen, 1981). Available K (mg kg^{-1}) was determined by the flame photometric method with extraction in ammonium lactate solution (ISO 11260:1995). Determination of Mg (mg kg^{-1}) was carried out by flow injection analysis using Tecator ASTN 90/92 and Ca (mg kg^{-1}) was determined flame photometrically from 1 M ammonium acetate (pH 7.0) extracts. Total N (%) was determined by the Kjeldahl method (ISO 11261:1995) using Tecator ASN 3313. The accumulation of Fe and Mn in the soil was analysed by the ICP and AAS (ISO 11885:1999) methods. The pH was measured potentiometrically in distilled water suspension as the potential acidity (ISO 10390:1994) using the soil–solvent ratio 1 : 5 (weight : weight). For the determination of organic matter (OM, %) Wisconsin procedures for soil testing by heating to 360 °C were used. The nutrient status of the soil was determined in the Central Laboratory of the Estonian Environmental Research Centre.

Plant material and analyses

The investigation was performed in a 80–85-year-old mixed pine stand of *Oxalis–Myrtillus* site type (Table 1). In September 2005 three Scots pines with a similar habitus of the crown were selected in each sample plot for analysis so that they would represent average trees within each sample plot. Needle samples

(300–500 g) of current-year (c.yr.), 1-year-old (1-yr.), and 2-year-old (2-yr.) needles were taken from all sides of the crown.

Needles were oven-dried at 70 °C to stop metabolic activity (Landis, 1985) and ground. After grinding 1–2 g of dried plant material was analysed for macronutrients (N, P, K, Ca, Mg) and micronutrients (Fe, Mn). The concentrations of metals (Ca, K, Mg) were determined using an atom-adsorption analyser AAA-1N (Carl Zeiss, Jena). Nitrogen was measured by the method of Kjeldahl. For P determination an inductively coupled optical emission spectrometer ICP-OES (PerkinElmer, USA) was used. Concentrations of Mn and Fe were determined by the ICP using ISO 11885:1999 methods.

Needles for analyses of chlorophylls (Chl) and carotenoids (Car) were collected evenly from all sides of the crown of three trees in each sampling site ($n = 4$). For the determination of Chl and Car the spectrophotometric method (Vernon, 1960; Gowin & Góral, 1977; Reich et al., 1986) was applied. Needles (0.2 g in five replications) were frozen in liquid nitrogen and homogenized in 80% ice-cold acetone solution. Pigments were measured with a spectrophotometer Heλios α (Unicam Ltd., UK). The concentration of Chl *a* at the wavelength of 649 nm, Chl *b* at 665 nm, and the total concentration of chlorophylls (TChl) were determined. Carotenoids were determined at 470 nm. The concentrations of chlorophylls were calculated using the equation by Vernon (1960) and Car with the equations by Lichtenthaler & Wellburn (1983). The results were expressed in mg g^{-1} dry matter.

For anatomical analyses small segments of pine needles were pre-fixed with 3% glutaraldehyde in 0.1 mol L^{-1} phosphate buffer, pH = 7.3, and fixed in 1% solution of OsO_4 (Bozzola & Russell, 1992; Ruzin, 1999). After dehydration in graded series of ethanol and xylol the specimens were embedded in paraffin. Cross-sections of needles, 10–15 μm in thickness, were cut on a microtome and mounted on glass. After removing paraffin, the slices were stained with 5% safranin O and Fast Green FCF (Fluka, USA).

The cross-sections, the needle width and thickness, and separately the areas of epidermis, mesophyll, xylem, phloem, and sclerenchyma were examined under microscope (Micros MC400A) at ×100 magnification and photographed with a Nikon Coolpix 5400 digital camera. Measurements of the anatomical characteristics were made with MapInfo Professional for Windows 4.0 (MapInfo Corp. Inc., Troy, NY) and UTHSCSA ImageTool for Windows Version 3.0 (The University of Texas, Health Science Center, USA).

Statistical tests

Linear regression analysis and regression coefficient (R^2) at statistical significance $p < 0.05$ were used to estimate the statistical significance of the relationship between biochemical composition of trees and anatomical characteristics and between mineral elements and the pH of soil from different sample plots. Pearson correlation analysis (r, $p < 0.05$) was applied for the determination of relations between the needle parameters. Analysis of variance was used to test the equality of group (distances from the plant, km) means and multiple comparisons were performed with ANOVA with F and p-values indicated. The differences between mean mineral element concentrations in soil and parameters of trees from the unpolluted control sample plot were investigated by pairwise t-test at statistical significance $p < 0.05$. For statistical calculations the Statistica 7.0 software and MS Excel were used.

RESULTS

Soil characteristics

The impact of cement dust upon soil was quite evident in polluted areas. Although the dust emission from the plant has practically stopped, the concentrations of Ca, Mg, Fe, Mn, and K in soil are continuously high. Moreover, significant differences were observed in the concentrations of Ca, K, and Mg between sample plots (Table 2). The pH value of soils in the polluted areas was over 7, which was 3 to 4 units higher than in the unpolluted sample plot. The differences between the soil pH of sample plots were statistically significant (Table 2). Correlations were revealed between the distance (km) from the cement plant and mineral elements in soil, except for P. Differences from the control were especially high on sample plot 2 km E from the plant. At high pH values of soil N concentrations decreased sharply and thus N deficiency may be a serious growth-limiting factor for trees in the alkalized area. Linear regression analyses revealed significant statistical relationships between the pH and Ca ($R^2 = 0.688$), K ($R^2 = 0.544$), P ($R^2 = 0.511$), and N ($R^2 = 0.654$) concentrations in sample plots. The concentration of OM in soil depended on the pH in all sample plots, being 2.6–6.9 times lower than control (Table 2).

Tree nutrition

The concentration of N in the Scots pine needles from the polluted areas was on average 10% lower than control, but that of Fe was 30%, of Ca 28%, of K 19%, and of Mg 16% higher than control while P and C did not differ from control. Although the C/N ratio in polluted soil was smaller than control, in the needles it tended to increase (Table 3).

For N and Mn a positive correlation, but for Ca, Mg and K negative correlations with the distance from the plant were established (Table 3). The concentration of Fe in needles had also a negative correlation with the distance, but it was not statistically significant. Compared with control trees from the unpolluted area differences (t-test) were found in K, Ca, Mg, Mn, and Fe concentrations, especially in the sample plot 2 km E (Table 3). The variance between all sample plots revealed by ANOVA was significant in K, Mg, Ca, and Mn concentrations.

Table 2. The pH of the soil and average concentration (±SD; $n = 5$) of mineral elements and organic matter (OM) in the soil (from 0–30 cm depth) of the sample plots at different distances from the cement plant. Correlation (Pearson's) coefficients (r) between the distance from the plant and element concentrations in the soil were calculated at significance $p < 0.05$. Variance between chemical characteristics on different sample plots and F and p values for ANOVA are given. Significant relationships are in bold. The level of significance of differences (t-test) from the control plot (38 km W) is indicated at *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$

Characteristic	Average value				r	F	p
	38 km W	2 km E	3 km E	5 km E			
pH_{H2O}	3.32±0.01	7.76±0.06**	6.74±0.12	6.61±0.09	**–0.977**	**41.46**	**0.023**
OM, %	48.30±2.70	9.87±0.54**	23.61±2.11	26.1±3.81	**0.938**	**36.71**	**0.026**
C, %	27.01±2.30	5.53±0.41**	13.22±1.11*	14.61±1.7	**0.764**	**30.15**	**0.033**
N, %	1.43±0.07	0.32±0.01***	1.16±0.01	1.15±0.07	**0.617**	1.229	0.382
P, mg kg^{-1}	129.06±15.3	182.63±17.2*	69.5±3.10	79.3±2.01	0.127	0.033	0.871
K, mg kg^{-1}	314.63±22.1	913.35±27.71***	610.1±13.61***	580±11.9	**–0.634**	1.345	0.365
Ca, mg kg^{-1}	2188±23.1	4932.2±29.7*	4168.2±31.1	4158±33	**–0.866**	**27.51**	**0.034**
Mg, mg kg^{-1}	213.43±4.9	413.34±5.80**	413.01±17.4***	401±14.9*	**–0.980**	**34.59**	**0.0002**
Fe, mg kg^{-1}	661.5±36.7	9385±62.1***	6741±36.1***	6449±42.61***	**–0.952**	**19.26**	**0.048**
Mn, mg kg^{-1}	6.50±0.10	963.10±16.8***	233.1±12.8***	177.21±11.2***	**–0.580**	1.104	0.419
C/N	18.89±0.15	17.28±0.21	11.37±0.13	12.7±0.11*	0.504	1.123	0.347

Table 3. Average concentration (±SD; $n = 18$) of mineral elements in needles of Scots pine in the sample plots at different distances from the cement plant. Correlations (r) between distance from the plant and element concentrations in needles were calculated at significance $p < 0.05$. Variance between chemical characteristics on different sample plots and F and p values for ANOVA are given. Significant relationships are in bold. The level of significance of differences (t-test) from the control plot (38 km W) is indicated at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$

Element	Average concentration, %				*r*	*F*	*p*
	38 km W	2 km E	3 km E	5 km E			
N	1.642 ± 0.07	1.471 ± 0.060	1.487 ± 0.062	1.566 ± 0.110	**0.8841**	**17.157**	**0.0159**
P	0.162 ± 0.008	0.178 ± 0.004*	0.178 ± 0.008*	0.144 ± 0.002	−0.2108	0.093	0.7892
C	49.783 ± 4.32	49.91 ± 3.67	49.962 ± 4.66	50.396 ± 4.72	−0.5233	7.542	0.4767
K	0.563 ± 0.035	0.7 ± 0.061***	0.649 ± 0.055***	0.665 ± 0.026*	**−0.7018**	**15.7187**	**0.0581**
Ca	0.348 ± 0.028	0.434 ± 0.025***	0.534 ± 0.020***	0.369 ± 0.034*	**−0.6151**	**21.171**	**0.0384**
Mg	0.131 ± 0.002	0.145 ± 0.013*	0.150 ± 0.011*	0.16 ± 0.012**	**−0.8167**	**40.113**	**0.0183**
Mn	0.009 ± 0.0001	0.002 ± 0.0001***	0.002 ± 0.0001***	0.001 ± 0.0001***	**0.9806**	**50.135**	**0.0193**
Fe	0.004 ± 0.0001	0.006 ± 0.0001***	0.005 ± 0.0002***	0.005 ± 0.0001***	−0.8457	5.023	0.1542
C/N	30.32 ± 3.07	33.95 ± 1.97	33.60 ± 3.27	32.18 ± 2.888	−0.1111	0.054	0.8411

Table 4. Relationship (R^2) between needle and soil nutrient concentrations in alkaline growth conditions. The data were calculated by linear regression at $p < 0.05$. Significant relationships are given in bold

Soil	Needle							
	N	P	C	K	Ca	Mg	Fe	Mn
pH	**0.8456**	0.0877	0.1659	**0.9723**	**0.5139**	**0.5060**	**0.5011**	**0.8746**
N	**0.5759**	0.2288	0.0027	**0.6741**	0.0606	0.0370	0.1860	0.2694
P	0.0217	0.1550	0.2033	0.0338	0.0692	0.2602	0.1912	0.0353
K	0.1185	0.1853	0.0402	0.3109	0.0633	**0.9684**	**0.5122**	**0.7124**
Ca	**0.8332**	0.0858	0.1560	**0.9847**	0.2726	0.4793	0.2619	**0.8527**
Mg	**0.7710**	0.0407	0.2777	**0.8725**	0.3848	**0.6821**	0.1783	**0.9656**
Mn	0.1185	0.1853	0.0421	0.3199	0.0633	**0.9684**	**0.5067**	**0.7024**
Fe	**0.8572**	0.1151	0.1109	**0.9797**	0.2872	0.4204	**0.6831**	**0.8087**
OM	**0.8553**	0.0968	0.1534	**0.9714**	0.3193	0.4877	0.2106	**0.8632**

Relationships (R^2) between the concentrations of nutrients in needles and soil characteristics are shown in Table 4. The soil pH affects significantly the availability of N, K, and Mg, but especially that of Mn and Fe. Significant relationships were found between the Ca concentration in soil and the concentrations of N, Mn, K, and Fe in needles. Similar relationships may be seen between Mg in soil and N, K, Mn, and Fe in needles (Table 4).

Needle biochemistry and anatomy

Relatively high pH values and imbalance of nutrients in soil cause shifts in the mineral nutrition processes and concentration of pigments participating in photosynthesis. The most significant decrease could be observed in the Chl *a* content while Chl *b* and Car seemed to be less affected (Table 5).

On the alkalized territory the Chl *a*/Chl *b* ratio in needles was by 6–24% lower compared to control. Regression analysis showed a strong relationship between N and Chl concentrations in needles ($R^2 = 0.834$). Although the role of Mn in Chl biosynthesis is still a matter of discussion, a linear regression between the concentrations of Mn and Chl in needles was found ($R^2 = 0.844$). Concentrations of pigments were higher on sample plots farther from the cement plant, and positive correlations (r) between the distance from the plant and pigment concentrations were found (Table 5). Significant differences from control were found for 2-year-old needles (Table 5). Correlation analyses between soil and needle chemical composition and Chl *a*, Chl *b*, and Car showed negative correlations with predominant elements of cement dust (Figs 1 and 2).

Changes in the nutritional conditions and biochemistry of needles were reflected in the needle growth and anatomy. Differences from control were observed in the width of needle cross-sections (12%), their thickness (11%), and the area of

Table 5. Correlation (r) between distance from the plant and pigment concentrations (mg g^{-1} dw) in Scots pine needles collected at different sample plots (distance and direction from the cement plant). Correlations were calculated at significance $p < 0.05$. Significant correlations are given in bold. The level of significance of differences (t-test) from the control plot (38 km W) is indicated at *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$

Sample plot	Age of needles	Chl *a*	Chl *b*	Car	TChl	Chl *a*/ Chl *b*	TChl/ Car
38 km W	c.yr.	1.991	0.983	0.540	2.974	2.045	5.524
	1-yr.	2.204	1.064	0.597	3.268	2.125	5.481
	2-yr.	2.644	1.166	0.632	3.810	2.268	6.028
2 km E	c.yr.	1.553	0.813	0.429	2.366	1.924	5.518
	1-yr.	1.693*	0.994	0.482	2.687	1.726	5.565
	2-yr.	1.654***	0.962*	0.468**	2.616***	1.719***	5.590
3 km E	c.yr.	1.563	0.868	0.436	2.431	1.801	5.577
	1-yr.	1.711*	1.007	0.478	2.718	1.699**	5.686
	2-yr.	1.86***	0.985**	0.464**	2.845**	1.888*	6.131
5 km E	c.yr.	1.693*	0.994	0.482	2.687	1.726**	5.565
	1-yr.	2.171	1.147	0.620	3.318	1.906*	5.353
	2-yr.	2.277***	1.153	0.599	3.430	1.974*	5.726
r		**0.657**	0.375	**0.613**	**0.593**	**0.883**	0.080

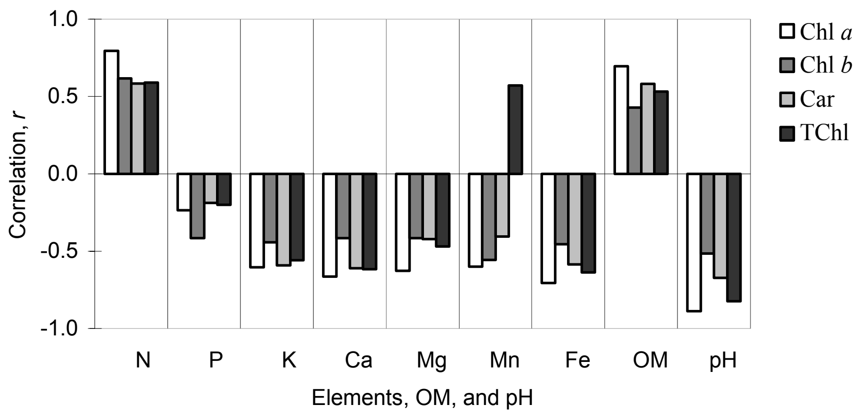

Fig. 1. Correlations (r) between pigment concentrations in needles and nutrient concentrations in soil in alkaline growth conditions.

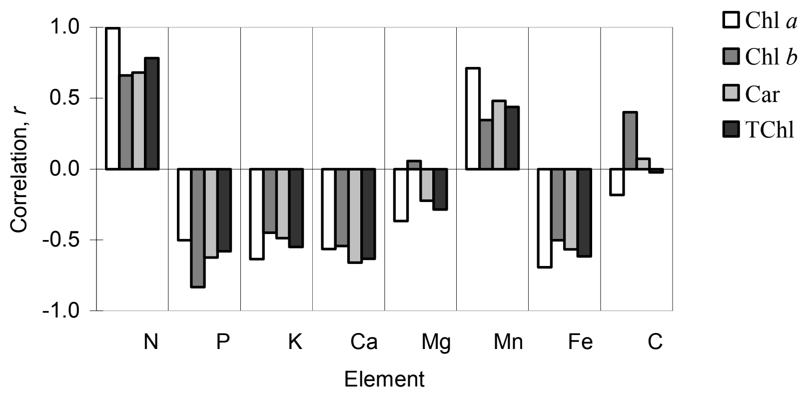

Fig. 2. Correlations (r) between pigment concentrations and nutrient concentrations in needles in alkaline growth conditions.

mesophyll (13%). At the same time the areas of epidermis, vascular bundles, and xylem were larger in needles from the polluted plots (Table 6, Fig. 3). A positive correlation between the C concentration in a needle and its total area was found in 1-yr. needles ($r = 0.822$). Moreover, in 1-yr. needles significant relationships were established between the nutrient concentrations in needles and the total area of needles' cross-sections, the area of mesophyll, and the area of epidermis.

Although the area of the mesophyll of needles from the alkalized soil was about 10% smaller than in the control area, the tendency of the dynamics connected with the ageing of needles was similar in all sample plots (Table 6). Positive correlations were found between mesophyll area in needles of all ages from all sample plots and Chl *a*, Chl *b*, and Car concentrations. Besides, regression analysis showed a relationship between mesophyll area and pigment concentration in needles ($R^2 = 0.675$). In 2-yr. needles the area of xylem showed a tendency to increase. Mesophyll and the total cross-sectional area showed a tendency to increase with moving from the sample plot 2 km E towards the sample plot 5 km E.

DISCUSSION

It is known that alkalization of soil complicates mineral nutrition processes, inhibits the availability of several nutrients, causing serious deviations in the mineral composition of plants (Marschner, 2002). A prolonged influence of cement dust pollution results in serious changes in the functioning of the forest ecosystem. On the territory influenced by a cement plant alkalization may decrease the height growth and radial increment of trees as well as the length of shoots and needles (Rauk, 1995; Pärn, 2002), but it may stimulate the proportion

Table 6. Mean anatomical parameters (±SD; $n = 45$) of Scots pine needles depending on needle age and location of the sample plot (distance and direction from the cement plant)

Sample plot	Age of needles	Width of needle, mm	Thickness of needle, mm	Total cross-section area, 10^{-2} mm^2	Mesophyll, 10^{-2} mm^2	Epidermis, 10^{-2} mm^2	Vascular bundles, 10^{-2} mm^2	Xylem, 10^{-2} mm^2
38 km W	c.yr.	1.22±0.16	0.60±0.09	142.97±11.82	81.31±7.93	19.10±0.90	4.36±0.43	1.96±0.15
	1-yr.	1.31±0.15	0.69±0.02	155.72±8.61	91.17±9.49	18.14±1.44	5.14±0.77	2.20±0.64
	2-yr.	1.36±0.16	0.70±0.08	162.00±17.75	90.66±8.76	20.95±2.44	4.39±0.97	2.07±0.83
2 km E	c.yr.	1.13±0.10	0.53±0.03	131.14±3.51	68.56±4.21	18.87±1.23	4.97±0.56	2.83±0.31
	1-yr.	1.13±0.02	0.59±0.06	145.99±5.88	76.76±4.13	19.99±1.46	2.12±0.40	1.33±0.35
	2-yr.	1.40±0.02	0.63±0.08	145.36±5.63	84.20±2.68	15.35±1.75	5.47±0.37	2.73±0.20
3 km E	c.yr.	1.07±0.02	0.52±0.00	124.54±2.38	68.53±7.16	17.40±0.93	4.12±0.15	2.14±0.14
	1-yr.	1.43±0.08	0.65±0.01	161.55±12.28	86.33±7.28	22.47±2.28	5.08±0.98	2.10±0.08
	2-yr.	1.37±0.18	0.61±0.04	153.69±4.86	85.46±8.85	18.67±18	6.53±0.84	2.87±0.50
5 km E	c.yr.	1.09±0.17	0.56±0.05	132.57±12.67	74.74±4.54	19.59±1.34	4.26±0.66	1.56±0.41
	1-yr.	1.26±0.09	0.60±0.01	162.50±18.25	88.82±1.47	23.70±2.29	5.80±1.04	2.37±0.10
	2-yr.	1.12±0.10	0.62±0.06	151.29±1.77	89.88±0.22	17.13±1.97	5.26±0.49	2.25±0.41

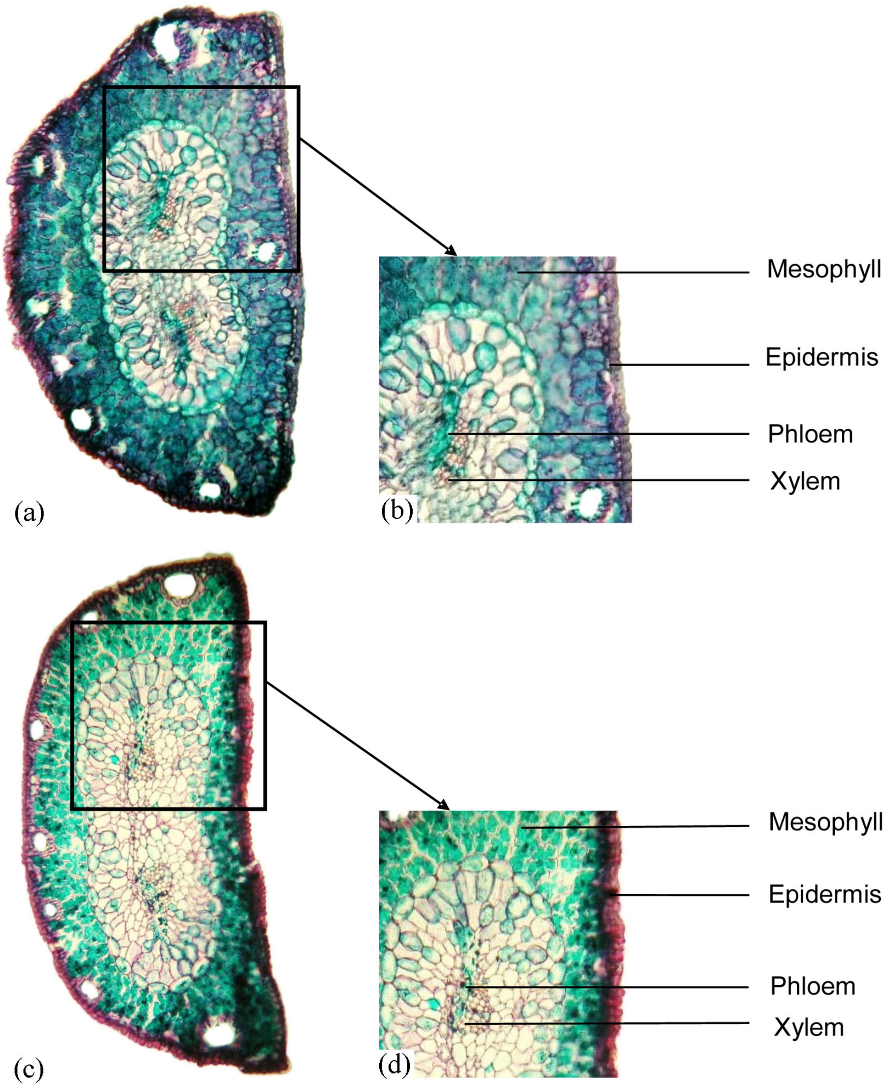

Fig. 3. Anatomy of Scots pine needles from sample plots located at distances of 2 km E (a, b) and 38 km W (c, d) of the cement plant. General view (a, c) and central part (b, d) of the needle cross-section at ×100 magnification. The total cross-sectional area and the area of mesophyll of the needle from the unpolluted plot (a) were larger than of the needle from the polluted sample plot (c). This figure is available in colour at http://www.eap.ee/ecology.

of heartwood and precocious maturation and ageing of the stand (Mandre et al., 2008). Our study showed that the deposition of great amounts of alkaline cement dust had resulted in changes in the pH and in the chemical composition of soil. A significant increase of the pH and the concentrations of Ca, Mg, K, and other dominant elements in dust were found. In alkaline soils with an increasing Ca concentration and pH and decreasing soil OM, the mobility of P is limited and its deficiency due to calcium phosphates of low solubility develops (Marschner, 2002). Deficiency of N in needles could be understood as N uptake limitation from soil. Both the total amount of soil N and its availability to plants are closely related to the OM content in soil (Marschner, 2002). The sharp decrease of N and C in soil may be a serious growth limiting factor for trees in the studied areas. Nitrogen recycling is of general interest and several factors may cause N decrease in alkalized soil. One of them may be microbiological activity. Nitrogen may have been lost by denitrification in relatively wet soils in the sample plot 2 km E. Therefore, loss of N through denitrification may be a possible explanation for N deficiency in the alkalized soil in the vicinity of the cement plant. Knowles (1981) concluded that the optimum pH for denitrification is 7.0–8.0. However, the results of Šimek et al. (2002) suggest a broader range: 6.3–8.3. Paul & Clark (1996) found a very rapid increase of denitrification in the pH range of 7.5 to 8.2. Increasing activity of denitrifying bacteria at neutral pH of soil (Pritchett, 1979) and percentage of denitrifying bacteria near the cement plant (Mandre et al., 1986) were established. Some authors, on the contrary, argue that liming has no influence on N losses due to denitrification (Hellmann, 1993; Papke-Rothkamp, 1994).

On the other hand, at the sites with a high pH of soil an increased C/N ratio may occur due to mineralization, followed by N losses through leaching (Formánek & Vranová, 2003). On the contrary, at sites with calcareous subsoil with the pH 6.6–7.3 it was established that a low C/N ratio in litter facilitates N mineralization (Persson et al., 2000). Thus there is a large range of variability in critical C/N values. Within this range factors other than N content may be expected to regulate the mineralization–immobilization balance.

Finally, the most important reason for such a low N concentration at the most polluted site is the very small amount of OM in its soil. What happened to the organic layer on the 2 km E site is another question and hard to explain presently. Possible reasons for it may be a higher decomposition rate due to the higher pH (Haynes, 1986; Gebauer et al., 2000) and a lower litter accumulation due to the lower growth rate of tree stands (Rauk, 1995; Ots, 2002).

Our study established a larger C/N ratio and substantially lower concentrations of N, C, and OM in the layer at 0–30 cm depth of soil in the vicinity of the cement plant than in the unpolluted area. Therefore in alkaline soil deficiency of N and Mn and excess of Ca, K, and Fe in *Pinus sylvestris* needles develops. Compared to the optimal concentrations of elements for pine suggested by Wehrmann (1963), Ingestad (1987), and Brække & Salih (2002) our results showed two times lower N concentrations and 2–13 times higher Ca concentrations in the pine needles near the cement plant. The drastic shortage of Mn in needles may be explained by the fact that in alkaline soil Mn^{2+} oxidizes into Mn^{3+} and Mn^{4+}, which are difficult for plants to assimilate (Marschner, 2002). As Fe uptake from the alkalized growth substrate is difficult to plants, the increase of Fe in the needles may be explained by its intensive translocation from roots or its accumulation through the leaf cuticle from the dust deposited on needles. Earlier a 50% lower

Fe concentration in roots and 3 to 5 times higher concentrations in stems, shoots, and needles were found for young Scots pines growing in the vicinity of a cement plant than in the unpolluted control area (Mandre et al., 1999), indicating translocation disturbances. Accumulation of nutrients through the cuticle was also verified by Finck (1982), who treated aboveground organs with a nutrient solution to provide a complementary supply of nutrients to plants. Although the concentrations of C and OM in soil were reduced by alkalization, the C concentration in needles of pines did not vary in different sample plots, but the C/N ratio decreased in the needle tissue.

Changes in the pH and chemical composition of soil influence the uptake of nutrients by trees, changing their biomass formation (Rauk, 1995; Ozolincius et al., 2005) through the morphological and anatomical peculiarities of trees. The growth of conifers under extreme conditions is argued to be associated with changes not only in their physiological–biochemical state, but also in their anatomy. In the most alkaline area a decrease in needle thickness and width, leading to a lower needle cross-sectional area, was established. The reduced cross-sectional area may be a result of less mesophyll tissue, which could influence the photosynthetic capacity of needles and multiple metabolic pathways. We also found that alkalization of the environment had brought about an increase in the area of epidermis. These findings support the results of several authors (Han et al., 2003; Warren et al., 2003).

The pigments participating in photosynthesis showed a significantly lower concentration in needles in the alkalized sample plots than in the control plot. There may be several external and internal mechanisms affecting the biosynthesis of pigments in the mesophyll. The strongest relationship was found between the Chl and N content, and the lower N level was one of the factors responsible for the decrease of the Chl concentration also in the studied area. However, Mg and Fe, the elements playing an essential role in Chl synthesis and found in elevated concentrations in needles on alkalized plots, were not statistically related to Chl. It seems that the decrease of Chl may be a result of the deficiency of Mn. It was reported by Ohki et al. (1980) that the critical deficiency level of Mn in plants is 10–20 mg g^{-1} dw and below this level the Chl content will decline rapidly and the ultrastructure of the thylakoids will drastically change. We found only 11–25 mg kg^{-1} dw of Mn in the Scots pine needles in the vicinity of the cement plant while in the unpolluted area the Mn concentration was 48–78 mg kg^{-1} dw. Consequently, Mn deficiency may inhibit the activity of many enzyme systems, photosynthetic O_2 evolution, and also Chl content (Marschner, 2002; Pallardy, 2008). The cell volume fraction of needle mesophyll was shown to be associated with the N content in needles not only in our results, but also in earlier studies by Niinemets et al. (2007). Hence, as the area of mesophyll decreases, its primary function changes from collecting photosynthates to transporting them from the needles to various sinks through vascular bundles. Additionally, the light penetration within the leaf, optical path length, chloroplast distribution, and chlorophyll concentration depend on the mesophyll cell shape and area (DeLucia et al., 1996). Although the changes in soil had large effects on the pigment concentration, the plant nutrient composition, especially N, P, K, Fe, and Mn accumulated from alkaline soil, may play a greater role.

Our results supported the findings that the anatomy of needles, as well as their biochemical composition, depends significantly on needle age (Lin et al., 2002; Pallardy, 2008). Ladanova & Tuzilkina (1992) found that the amount of Chl increases in the needles of *Picea obovata* L. until the third to fourth year of age, but the intensity of photosynthesis is the highest in mature 1-yr. needles. In Scots pine needles affected by alkalization of soil a clear dependence on age was established in Chl content, which was highest in the oldest needles. Comparison of needle anatomy depending on tree age established great differences in needle width, thickness, and cross-sectional area and mesophyll volume fraction, as shown also by other authors (Apple et al., 2002).

CONCLUSIONS

Responses of Scots pine physiology and anatomy to alkaline stress are not finally understood. Additional measurements are needed to test the dependence of the general anatomical variability of conifer needles on the peculiarities of growth conditions. Anyway, our study demonstrated that environmental alkalization and nutrient imbalances in soil were significantly related with pine needle photosynthetic potency and anatomical structure. Alkalization-mediated stress in the territory surrounding the cement plant occurred via the regulation of external and internal nutritional conditions, which should influence canopy architecture and biomass formation.

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Hariliku männi (*Pinus sylvestris* L.) biokeemilised ja struktuursed iseärasused leelistunud keskkonnas

Malle Mandre ja Aljona Lukjanova

Uuriti hariliku männi (*Pinus sylvestris*) okaste anatoomilise ehituse sõltuvust kudede biokeemilisest koostisest ja keskkonna iseärasustest. Kunda tsemenditolmust üle 40 aasta mõjutatud aladel on mullad tugevasti leelistunud. Sõltuvalt kaugusest tehasest (2, 3 ja 5 km E) on vaatlusaladel muldade pH 6,61–7,76 (kontrollalal Lahemaal pH = 3,32, 38 km W) ning esineb tasakaalustamatus puudele vajalike toitainete (Ca, K, Mg, N, P, C jt) osas. Suhteliselt suured erinevused kasvukeskkonnas väljenduvad ka männiokaste anatoomias ja biokeemias. Võrreldes saastamata piirkonnaga Lahemaal, on tehase lähipiirkonnas männiokaste laius, paksus ja ristlõike pindala väiksem, mis tuleneb peamiselt mesofülli, kloroplaste sisaldava koe pindala olulisest vähenemisest. Seoses mesofülli pindala vähenemisega täheldati fotosünteesis osalevate pigmentide (klorofüllid, karotenoidid), eriti Chl *a* sisalduse vähenemist ja suhte Chl *a*/Chl *b* taseme langust. Seejuures suurimad erinevused kontrollpuude okastest leiti vanemates, kaheaastastes okastes. Samas epidermise, ksüleemi ja juhtkimpude pindala ei erinenud kontrollpuude näitajatest. Saadud tulemused näitavad, et keskkonna leelistumisega kaasneb okastes N-i ja Mn-i defitsiit ning K, Ca ja Fe suhteline liig, mis mõjutavad okaste anatoomilist struktuuri ning pigmentsüsteemi kujunemist.