Stress Induced Changes in Lignin and Nutrient Partitioning in *Picea abies* (L.) Karst.

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Changes in the content of lignin and its partitioning inside the organism of *Picea abies* (L.) Karst. were studied in an alkalitized environment 0.5 km NW of the cement plant. The alkaline dust (pH = 12.3–12.7) emitted from the industry for over 30 years brought about alkalization and changes in the chemical composition of the soil, ground water and precipitation on the area studied.

Stress induced by alkalization of the environment resulted in changes of lignin partitioning in Norway spruce. An increase in the lignin content in the needles up to 16-20% and in the roots, stems and shoots up to 5-13% were observed in polluted trees.

Regression analysis showed a linear relationship between lignin (L) accumulation and the content of N and K in trees growing under non-polluted conditions ($R^2_{\text{L,N}} = 0.815$, $R^2_{\text{L,K}} = 0.533$). In addition to N and K, dependence of lignin accumulation on P, Ca, Mg and S contents was established in stressed trees ($R^2_{\text{L,P}} = 0.984$, $R^2_{\text{L,Ca}} = 0.846$, $R^2_{\text{L,Mg}} = 0.596$, $R^2_{\text{L,S}} = 0.630$).

The growth of spruces was inhibited and a negative correlation between the shoot and needle length and the lignin content in tissues was established.

**Key words:** acid-insoluble lignin, alkalized environment, Norway spruce, nutrients, partitioning.

### Introduction

Deviations in the growth and development of forest trees due to prolonged air pollution cause mainly negative changes in the total bioproduction. A good understanding of the mechanism of air pollution impact on forest ecosystem is necessary from biological and economic aspects. Problems connected with lignification of woody plants and changes in the content of lignin, the main component of cell walls, in trees under air pollution impact are important in wood quality formation and in forestry in general.

Lignins being the most abundant products of phenylpropanoid metabolism in woody plants only next to cellulose approximately comprise 25% of the terrestrial biomass (Boudet et al., 1995). They are a significant component and increase the strength of the cell walls. The mean lignin content of wood is generally in the range of 15–35% of the dry matter (Higushi, 1981). Lignin is also present in leaves where it amounts up to 10% (w/w) in oak and beech and about 5% (w/w) in the needles of spruce (Mikeshe and Yasuda, 1977). Lignin incorporation renders plant cell walls mechanically rigid and water repellent and lignification plays a role in the protection of plants from pathogens (Marschner, 1986), UV-light (Doke et al., 1994) and other unfavourable factors (Chapin, 1991).

Lignin exhibits a great deal of variation in its chemical composition and physical properties (Ziegler, 1997). Lignin composition varies not only between different species, but also between different plant organs and even in different layers of the cell (Polle et al., 1997).

Studies by Polle, Otter and Sandermann (1997) have shown that the lignin content in plant tissues is not constant and changes in the lignin concentration depend on the physiological state of the organism and on several external conditions. Production of lignin in plants is not only a constitutive, developmentally controlled process, but it represents also a flexible metabolic response to external stress factors. Often structural and extracellular stress lignin is distinguished (Ziegler, 1997; Polle et al, 1997) and large structural variations in these anomalous lignins have been reported (Monties, 1989).

Thus nutrient stress, water stress, heavy metal toxicity and pollutants that have their major direct impact on the growth can be expected by changing concentrations of lignin and other secondary metabolites (Chapin, 1991). Changes in the content of secondary metabolites in plants as a response to gaseous phytotoxic acidic types of pollutants such as SO$_2$, NO$_x$, O$_3$ etc. have been repeatedly observed (Yee-Meiler, 1974; 1978; Sandermann et al., 1989; Kaunulainen et al., 1995). Research conducted on the effects of alkaline types of
pollutants on plants is insufficient and no information is available on the effects of industrial alkaline dust and ash emitted from building materials industries, oil-shale open-cast mining and processing, chemical industries etc. on the lignin content and lignification processes. The problem is interesting because a rise in pH and in the content of Ca and K in the cell wall compartment favours the oxidation of phenolics and esterification with cell wall carbohydrate polymers (Heath and Castilho, 1987). In our opinion, an external increase in pH might be a factor stimulating the lignification of trees.

The aim of the present work was to find out whether long-term alkaline air pollution from the cement industry in Northeast Estonia has an effect on the lignin and nutrients partitioning in Norway spruce (Picea abies (L.) Karst.). In the present study an attempt was made to characterize changes in the relationship between the lignification and mineral nutrition of trees and biochemical approaches were used to explain the importance of mineral nutrition in lignification processes. The results of the presented study will hopefully contribute to a better understanding of stress tolerance of conifers and also the changes in wood quality under air pollution inapt.

**Materials, methods and growth conditions of trees**

The investigations were carried out on the territory affected over 30 years by a cement plant in the town of Kunda (59°30'N, 26°31' E), Northeast Estonia. The sample plot as situated at the distance of 0.5 km NE from the emission centre and the control sample plot was located in similar climatic conditions on a relatively unpolluted area in the Lahemaa National Park (59°31' N, 26°00' E) at a distance of about 34 km W.

**Peculiarities of the growth conditions of the trees**

The emissions from the cement plant during the investigation period (1990–1996) contained 87–91% of technological dust and 9–13% of gaseous pollutants (SO₃, NO₂, CO etc.) (Mandre et al., 1994). The main damaging factor to trees on the investigation area is apparently the dust from the electric filters. It contains many components, among which the following are predominant: 40–50% CaO; 12–17% SiO₂; 6–9% K₂O; 4–8% SO₃; 3–5% Al₂O₃; 2–4% MgO; but also Fe, Mn, Zn, Cu, B, etc. occur (Raukas, 1993). The water solution of dust from electric filters had pH values from 12.3 to 12.7. The dust emission from the cement plant was extremely high in 1990–1992 being 80–100 kt per year (Keskkond '90, 1991; Estonian Environment 1991, 1991; Estonian Environment 1995, 1996). In 1993–1996 the emission of cement dust from the plant decreased notably owing to the installation of efficient filters and amounted to 15–70 kt per year. The high dust concentration in the air for over 30 years has brought about alkalization and changes in chemical composition of the soil, ground water and precipitation in this area. At a distance of 0.5 km from the cement plant, the pH of the soil ranged from 7.6 to 8.1, the pH of rain water was between 7.6 and 8.2 and that of snow melt 10.1–11.0 in different years. In the vicinity of the cement plant the concentrations of Ca, K, Mg, S and other elements predominating in the dust are extremely high in the upper layers of the soil and in precipitation as a consequence of the emission of dust for several decades. On the control area the pH value of the soil humus horizon was from 2.9 to 3.3, that of rain water 5.6–6.6 and snow water 6.3–6.6.

**Sample plots and plant material**

Studies in the field experimental sample plots in the vicinity of the emission source and in unpolluted area begin from 1990. In May 1990, 40 two-year-old genetically similar and homogeneous in habitus seedlings of Norway spruce were planted in our sample plots.

After four years of growth on sample plots 6–10 trees were excavated in the roots, stems, shoots, needles of shoots and stems were separated before bud break in late April–early May 1994. All the organs of each investigated tree were carefully cleaned, cut into small pieces and mixed. Mixing is assumed to reduce the effect of variation in biochemical content along the organs of tree (Wood and Bachelard, 1969) and individual variability of the trees (Linder, 1972). Plant material was dried at 105 °C for the assessment of the content of lignin and mineral elements.

**Chemical and statistical analyses**

Lignin was determined following the recommendations of Arasimovich and Ermakov (Арасимович, Ермаков, 1987), Van Soest (1987) and Monties (1989). The method is based on acid treatments, which leaving an insoluble residue, and the weighed as acid-insoluble lignin (hereafter called ‘lignin’) after complete solubilization of the sample. The procedure for acid-insoluble lignin content in different organs of spruce calls for extraction of ground dried material with acetone (100%), ethanol (96%), ethanol-benzene solution 1:1, v/v) and water to remove sugars, proteins, interfering phenolics
and other soluble compounds. The extraction was repeated several times until the solution remained colourless. The residue was dried at 70 °C for 24 h. Acid hydrolysis of the dried residue with 72% H₂SO₄ was carried out to remove acid-soluble lignin and cellulose (Monties, 1989). Insoluble residue was recovered by filtration on a glass crucible, carefully washed and dried at 70 °C for 24 h to constant weight (Precisa 205A SCS, Switzerland). After weighing the residue was ashed at 525±25 °C and lignin was calculated after correcting for mineral elements content (Van Soest, 1987). The results for acid insoluble lignin were expressed as a percentage of dry mass.

The content of mineral elements in Norway spruce organs was determined in the laboratory of the Estonian Control Centre of Plant Protection. Nitrogen was analysed by the Kjeldahl method, sulphur with nephelometric method with BaCl₂, by using spectrophotometer SPEKOL 11 (Carl Zeiss, Jena) at 460 nm, phosphorus with the help of the WPA Heliflow c0310 flow injection analyser and the concentration of metals (Ca, K, Mg, Mn) by using atom adsorption analyser AAA-1N (Carl Zeiss, Jena).

The regression analysis and R-squared values and significance (p) of the relationships between the parameters and standard deviations from the mean were calculated using the packages Statgraphics and MS Excel 5.0.

**Results and discussion**

Comparative analyses of trees from sample plot influenced by alkaline industrial dust and from unpolluted area showed the essential influence of cement dust pollution on the lignin content and mineral composition in young trees of Norway spruce. Alkalization of the environment resulted in a long term impact of alkaline dust pollution complicated the processes of mineral nutrients and disbalanced nutrients composition in trees. Also the stimulation of lignification in spruces on the polluted sample plot was established and great changes in partitioning of lignin and nutrients in different organs of young spruces were observed (Table 1, Fig. 1). There were great changes in partitioning of lignin and nutrients inside organism.

The alkalization of the upper horizons of forest soil alters the availability of several nutrients, and, obviously owing to this, the content of N and Mn in spruce has fallen on the polluted area, while that of S, Ca and K has risen as compared with the control trees on the unpolluted area (Table 1, Fig. 1). Total concentration of mineral elements was increased in polluted trees to about 35%, as compared to control.

The relationship between the mineral nutrition and lignification are still not well understood. However, the role of several elements in the biosynthesis of lignins is to realize through enzymatic regulation of the synthesis of phenolcarboxyl acids and other precursors of lignin (Polle et al., 1997).

It is fairly clear that in case of N deficiency high lignin and tannin contents may occur in plants (Matsuyama, 1975; Flanagan and van Cleve, 1983; Padu et al., 1989). Our results confirmed this in spruces with lower N concentration, as compared to the control more lignin was synthetized. Regression analysis showed significant dependence of the lignin content on the amount of N in the organism (Table 2). This phenomenon may be caused by competition between two metabolic pathways for the precursor phenylalanine, which is a common precursor of both protein and phenolics. Phenylalanine can either be incorporated directly into the growing polypeptide chain in protein synthesis or converted by the enzyme phenylalanine ammonia-lyase (PAL) into the phenolproanoid metabolism. The high PAL activity is related to lignin synthesis and depends on the concentration of substrate (Padu et al., 1989). It is also possible that the fall in the phenylalnine concentration in plants affected by cement dust (Mandre, 1985) plays a role in the regulation of the PAL activity.

Also several metallic ions, such as Ca and K are supported, affect the lignification process. It has been found by Wardrop (1971) that seedlings of Pinus taeda grown under Ca deficiency show a higher level of lignification and an altered xylem anatomy whereas plants with a full complement of Ca exhibit a lower level of lignification. This suggests that excess of Ca content in tissues might retard lignification processes. Our results indicated high concentrations of Ca and K in the growth substrate and significantly increased the concentrations of these elements in young spruces in the close vicinity of the cement plant. After Wardrop (1971) high Ca concentrations in trees should retard lignification processes. However, our analyses yielded contrary results. Under the conditions prevailing in our sample plots the effect of K, which usually stimulates lignin synthesis, may be a more important factor than Ca. K is needed for lignification through the protein and polysaccharide enzymatic processes and an increase in K content stimulates the lignification processes (Miidla, 1989). An especially significant relationship between the contents of lignin and K (Table 2) in the trees grown
Table 1. Lignin and mineral nutrients contents in the different organs of 6-year-old *Picea abies* in 1994 (average ± SD of measurements, n = 6)

<table>
<thead>
<tr>
<th>Sample of tree</th>
<th>Organs</th>
<th>Lignin, %</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
<th>Mn</th>
<th>TCM, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Root</td>
<td>26.05±1.4</td>
<td>6±0.6</td>
<td>1.6±0.02</td>
<td>6.1±0.5</td>
<td>5±0.2</td>
<td>1.4±0.1</td>
<td>0.6±0.01</td>
<td>0.084±0.001</td>
<td>144.6±7.9</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>23.68±4.1</td>
<td>6.1±0.1</td>
<td>1±0.1</td>
<td>5.9±0.2</td>
<td>2.9±0.2</td>
<td>1.1±0.0</td>
<td>0.31±0.01</td>
<td>0.037±0.001</td>
<td>15.6±1.8</td>
</tr>
<tr>
<td></td>
<td>Shoot</td>
<td>23.39±1.9</td>
<td>8.2±0.6</td>
<td>1.6±0.05</td>
<td>7.9±0.8</td>
<td>5.1±0.2</td>
<td>1.8±0.2</td>
<td>0.43±0.04</td>
<td>0.045±0.002</td>
<td>23.5±1.6</td>
</tr>
<tr>
<td></td>
<td>Stem needles</td>
<td>11.22±1.8</td>
<td>12.8±1.3</td>
<td>1.9±0.03</td>
<td>8.4±0.2</td>
<td>6.7±0.1</td>
<td>1.5±0.1</td>
<td>0.7±0.06</td>
<td>0.054±0.003</td>
<td>46.6±0.8</td>
</tr>
<tr>
<td></td>
<td>Shoot needles</td>
<td>9.39±0.6</td>
<td>12.8±0.9</td>
<td>1.7±0.02</td>
<td>8.4±0.7</td>
<td>6.5±0.2</td>
<td>1.5±0.1</td>
<td>0.66±0.07</td>
<td>0.045±0.004</td>
<td>32.2±0.3</td>
</tr>
<tr>
<td>Polluted</td>
<td>Root</td>
<td>28.67±1.9</td>
<td>4±0.4</td>
<td>1±0.1</td>
<td>6±0.6</td>
<td>9.6±0.9</td>
<td>1.5±0.1</td>
<td>0.73±0.01</td>
<td>0.028±0.001</td>
<td>49.6±4.1</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>24.86±2.1</td>
<td>4.5±0.3</td>
<td>0.9±0.01</td>
<td>7.4±0.2</td>
<td>8.4±1.1</td>
<td>1.5±0.1</td>
<td>0.45±0.02</td>
<td>0.012±0.001</td>
<td>36±3.1</td>
</tr>
<tr>
<td></td>
<td>Shoot</td>
<td>24.67±2.4</td>
<td>5.7±0.4</td>
<td>1.2±0.1</td>
<td>8±0.4</td>
<td>13±1.6</td>
<td>1.5±0.1</td>
<td>0.65±0.6</td>
<td>0.02±0.002</td>
<td>62.7±5.2</td>
</tr>
<tr>
<td></td>
<td>Stem needles</td>
<td>13.68±1.6</td>
<td>7.2±0.2</td>
<td>4.1±0.4</td>
<td>15.3±1.1</td>
<td>19.3±1.5</td>
<td>1.5±0.1</td>
<td>2.28±0.1</td>
<td>0.025±0.002</td>
<td>106.7±8.2</td>
</tr>
<tr>
<td></td>
<td>Shoot needles</td>
<td>11.08±1.1</td>
<td>8±0.1</td>
<td>4.3±0.1</td>
<td>13.6±1.2</td>
<td>18.6±1.41.5±0.1</td>
<td>1.23±0.1</td>
<td>0.021±0.001</td>
<td>99.9±7.3</td>
<td></td>
</tr>
</tbody>
</table>

TCM – total concentration of mineral elements

**Figure 1.** Percentage from the control of the content of mineral elements and lignin in different organs of young Norway spruce in the area affected by the cement plant. The content of mineral elements and lignin in the control trees comprises 100%. Vertical lines represent the standard deviations.
in the polluted sample plot was established. This allowed to point to the somewhat dominant role of K in the lignin formation in trees on the polluted area. Here we should take into account synergy of K and Ca, which was pointed out also by Heath and Castillo (1987), who showed that a rise in pH and in the content of Ca and K in the cell wall compartment favour the oxidation of phenolic substances, esterification with cell wall carbohydrate polymers and lignification (Heath and Castillo, 1987), which might be due to the impact of the alkaline dust deposited.

Mn concentration in Norway spruce in a polluted area is 30–50% lower than in the control (Fig. 1). Taking into account the importance of Mn in peroxidase activation, mediating the lignification processes (Polle and Chakrabarti, 1994) it is possible to suppose that Mn deficiency may retard biosynthesis and accumulation of phenolic compounds. Also a sharp rise in the lignin concentration in spruce under Mn deficiency is difficult to interpret on the basis of information available in the literature presently. Although the function of Mn as a stimulator of peroxidases which plays a role in the control of stress-induced and normal developmental lignification has been established by many authors (Rubin and Jensen, 1985; Polle and Chakrabarti, 1994; Polle et al., 1997), we found no relationship between Mn and lignin accumulation in spruce. Functional changes due to Mn shortage deserve special attention in further investigations into stress tolerance of coniferous trees.

In polluted trees the S content of needles is 2–5 times higher than in the control, which is reflected by the increased availability of \( \text{SO}_4^{2-} \) from the alkalized environment. Regression analysis has shown the dependence of tree lignification on S not in the control sample plot but in an alkalized area (Table 2). Similar relationship was established between the lignin and P contents in polluted sample plots.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Variant</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignin</td>
<td>Control</td>
<td>0.815</td>
<td>0.344</td>
<td>0.535</td>
<td>0.468</td>
<td>0.0218</td>
<td>0.457</td>
</tr>
<tr>
<td></td>
<td>Polluted</td>
<td>0.970</td>
<td>0.984</td>
<td>0.934</td>
<td>0.846</td>
<td>0.596</td>
<td>0.630</td>
</tr>
</tbody>
</table>

From these facts it can be concluded that single nutrient elements have a unidirectional influence on lignification of trees and currently we have to place emphasis on the synergistic effect of different nutrients on the lignification.

Alkaline dust deposited on trees and alkalized growth substrate cause not only serious deviations in the content of mineral elements and lignin in Norway spruce, but also in their partitioning between organs. In comparison to the young trees growing in the control sample plot, the content of Ca and K was extremely high in all organs, while that of P and B was higher in needles and lower in roots. A drastic decrease in the content of N and Mn was found in all organs of the trees investigated (Fig. 1). Also the content of lignin varied in different plant organs. Thus, the mean lignin content in roots, stems and shoots in young trees amounted to 23–26% and in needles up to 10% of dry weight. In the alkalized conditions under dust pollution the lignin content and its partitioning in organs had changed, being 120–127% higher in the polluted needles, in the stems, shoots and roots about 7–11% higher than in the control trees (Fig. 1).

Evidence has been obtained that N shortage and B and K excess stimulate accumulation of lignin in organs. Our research has shown that the relationships between the content of lignin and nutrients developing under stress may differ notably from those observed in trees growing in optimal conditions (Table 2). In the stress conditions the dependence of lignin accumulation on N, K and B concentrations increased. In addition, significant dependence of lignin accumulation on Mg, Ca and P contents was observed, which was not detected under optimal conditions.

It is generally known that in case of lignin formation and lignification of trees biochemical transformation takes place and the growth stops. Growth and lignification vary in opposite directions in response to alkaline air pollution impact. Increased and premature lignification would therefore limit the extension of cell walls and consequently plant growth. The height growth of young Norway spruces in the region under alkaline air pollution impact was restrained about 45% and it differed considerably from that in the control area.
(two-way t-test showed differences at the \( p < 0.01 \) level of significance) (Mandre and Ots, 1995).

**Conclusion**

To sum up, it was indicated that under extremely complicated environmental conditions, under the impact of alkaline dust pollution and alkalization of growth substrate, serious deviations occur in lignification and lignin partitioning. The relationship between the availability of mineral nutrients and lignification processes strengthened under environmental stress. Changes in the content and partitioning of lignin indicate qualitative variations in wood and, thus, the significance of the problem from the standpoint of forestry and especially in relation to pulping technology.

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**References**


ИЗМЕНЕНИЯ В ОТНОШЕНИЯХ ЛИГНИНА И ПИТАТЕЛЬНЫХ ВЕЩЕСТВ В PICEA ABIES (L.) KARST. В СТРЕССОВЫХ УСЛОВИЯХ

М. Мандре

Рекомендуемое

В статье приводятся данные об изменениях содержания и распределения лигнина и минеральных элементов в органах Picea abies (L.) Karst. под воздействием ноницеллозирования среды и щелочной ванны от цементного завода. Установлены изменения зависимости образования лигнина от питательных веществ в стрессовых условиях.

Ключевые слова: лигнин, подщелачивание среды, Picea abies (L.) Karst., минеральные элементы, распределение.