Biochemical diagnosis of forest decline

MALLE MANDRE, LIIVI TUULMETS
Department of Ecophysiology, Institute of Ecology
Keemia Str. 41, Tallinn EE0006, Estonia

Mandre M., Tuulmets L. 1997. Biochemical diagnosis of forest decline. *Baltic Forestry,* 2: 19–25.

The level of damage of coniferous trees in a forest of *Oxalis–Myrtillus* site type on areas influenced by cement dust has been identified using the biochemical ratios Chl/Car, Chl *a*/Chl *b*, N/Mg, N/P, N/S, N/Mn and Ca/K in the needles. Index *BI*, displaying differences between pollution induced biochemical ratios and the control, has been found. This index is very sensitive and directly depends on the quantity of pollutants and chemical changes in the soil and precipitation at different distances from the emission source.

Key words: bioindication, biochemical parameters, conifers, dust pollution.

Introduction

In contrast to the world experience of acidic rains, Estonia's industrial regions are confronted with problems of alkalization due to high concentrations of dust and ash in air pollution complexes emitted from a cement plant, enterprises of oil shale processing and from power stations in Northeast Estonia. In general, no specific or characteristic visually observed air pollution injuries, such as chlorotic areas or necrotic lesions on needles and leaves, can be noticed on plants growing under the alkaline dust pollution impact on the industrial territories of northern Estonia. This explains the necessity to use biochemical indicators to elucidate the real state of plants. The use of biochemical methods in dust pollution damage indication makes it possible to assess the state of the forest ecosystem objectively and predict its dynamics.

It is well known that biochemical and physiological transformations in organisms are primary responses to any type of changes in the environment. As biochemical and physiological reactions are highly sensitive, some biochemical parameters can successfully be used to obtain objective data on the deviations in the ecosystems and their early diagnosis.

The idea of using chloroplast pigments to estimate the effect of air pollution on plants has been recommended by several researchers. Some authors suggest that Chl *a* should be examined as a possible indicator for testing the extent of damage caused by harmful gas, especially SO_2 (Müller 1957; Dässler 1972; Schubert 1985). Additionally, chlorophyll, carotenoids as potential indicators of air pollution effects in plants have been discussed by several authors (Arndt 1971; Gowin and Goral 1977). The significance of b-carotene in the response reaction of plants to air pollution was shown by Arndt (1971) in the case of SO_2 and HF impact. A number of scientists have explored the suitability of using the contents of different nutrient elements in plants for the purpose of bioindication (Höllwarth 1981; Cape et al. 1988). The ratio of SO_4^{2-}/-SH (Гетко et al. 1985) and the activity of peroxydase (Grill et al. 1980) are recommended as good biochemical indications for determining the state of plants under the inluence of sulphur containing complexes of pollution. As a result of low-dose exposure over long periods of time functional damage can be devoid of visible symptoms and difficulties arise in defining and measuring it.

Unfortunately, there is little information on alkalization of the environment and the impact of alkaline type of air pollution complexes on vegetation because these problems are regional and relatively local in comparison to the global scale.

As in several East European countries alkaline dust pollution is of great importance in total emission of pollutants, we set the aim of selecting biochemical indicators suitable for the bioindication of the forest ecosystems. The selected indicators were used to assess the state-of-art of forest trees on a territory affected by alkaline cement dust.

Methods and materials

1. Location of the sample plots on the territory investigated

A comparative field study of the effect of prolonged emission of alkaline dust from the cement plant on forest trees was conducted on an about 50 km long investigation transection from 1991 to 1995 (Fig. 1). We chose twelve sample plots in mixed pine and spruce stands of *Oxalis–Myrtillus* site type. Taking into account the direction of prevailing winds, similarity of methodological and edaphical factors, the plots were selected along a 50 km transect that extended 38 km to the west and 12 km to the east of the cement plant in Kunda, Estonia. The control trees were situated in the westernmost sample plots of the transect on an unpolluted territory at a distance of 34–38 km from the emission source. The amount of dust emitted from the plant varied essentially during the investigation years (Fig. 2).

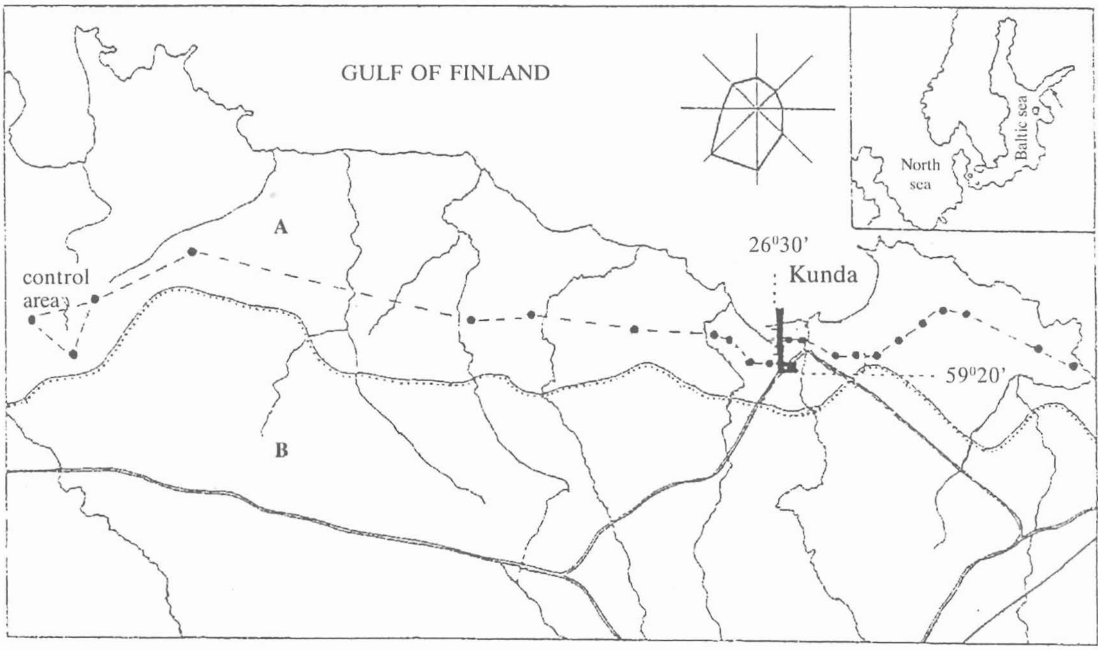

Fig. 1. The study area. Location of investigation plots. Landscape regions: A – North-Estonian coastal plain; B – Northeast-Estonian plaetau. ‧‧‧‧ border of landscape regions; •— —• sample plots and transect for forest investigation

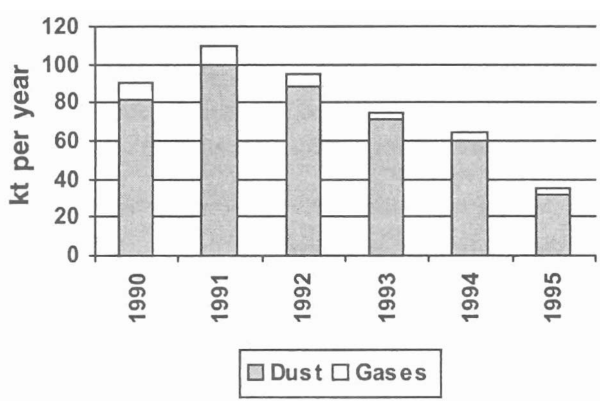

Fig. 2. Emission of dust and gaseous pollutants into the atmosphere from the cement plant in Kunda in 1990-95

2. Collecting samples and biochemical analyses

As the biochemical response of conifers is most clearly expressed on the best illuminated southern sides of trees (Kangur 1988), which are also exposed to the plant, the samples of needles were taken from the southern side of the tree crowns at the height of 5–6 m from the ground. All one-year-old needles were removed from the shoots of each subject tree on the sample plot, cut into small pieces and carefully mixed. Mixing is assumed to reduce the effect of variation in the biochemical content along the needles and the individual variability of trees (Wood and Bachelard 1969; Linder 1972). The needles collected from the polluted territories were carefully cleaned from the dust on their surface.

In our study the standard methods for measuring chlorophylls and carotenoids (Vernon 1960; Gowin and Goral 1977; Reich et al. 1986) were used. We fixed needle samples of 0.2 g in liquid nitrogen and homogenized in ice-cold acetone-water of 80% for 3–5 min in dim light and under low temperature conditions. The extract was filtered through a fritted-glass filter and the filter cake residue was washed with acetone of 80%. We measured the pigment content with the LOMO 46 spectrophotometer by reading absorption of the chlorophyll extract at wavelengths 649 and 665 nm and calculated the chlorophyll content according to the formulas by Vernon (1960). The carotenoid content was determined spectrophotometrically at 470 nm in acetone solution using the method recommended by Lichtenthaler and Wellburn. For measuring the contents of manganese, calcium, potassium and magnesium in needles atomic absorption analyser AAA 1N (Karl Zeiss Jena) was used. Nitrogen was measured by the Kjeldahl method, sulphur by the nephelometric method and phosphorus with the help of an NPA Heliflow c0310 flow injection analyser in the laboratory of the Estonian Centre of Agricultural Chemistry.

In order to get auxiliary information for interpreting deviations in the physiological state of trees we also investigated the physical and chemical properties of the growth conditions. The accumulation of dominant elements of dust and the pH of the soil (humus horizon), ground water, and precipitation were analysed at the Estonian Centre of Agricultural Chemistry. The dust concentration in the air was analysed by the Central Laboratory of Environmental Research.

3. Principles for the selection of bioindication parameters

The selection of the biochemical markers used in the monitoring program in case of an alkalized environment must be justified on the basis of fundamental understanding of the biochemical and physiological level. Coniferous trees provide a suitable object for monitoring industrial emission effects. As they are typical of long ontogenetic development, high sensitivity to air pollution and long-term foliar retention, conifers have often been recommended as investigation and test objects. In selecting the parameters for bioindication the following aspects were taken into account.

1. The parameters used in biochemical indication must be sensitive and react rapidly either to the whole pollution complex or to the dominant elements in it (e.g. pigments of chloroplasts, sulphydrilic compounds etc.).

2. Considering the chemical composition and predominant elements of the pollution complex (e.g. Ca, K, Mg, Mn in case of cement dust pollution) it is advisable to use in bioindication the analysis of the accumulation and dynamics of these elements also in the tissues of organism.

3. Among nutrients, attention should be paid to those belonging to the vitally important organic compounds (e.g. N and Mg in chlorophylls, S in proteins, vitamins etc.) or participating in the regulation of enzymatic processes in metabolism (e.g. Fe in chlorophyll biosynthesis, Mn in the ketoacids cycle etc.).

4. The annual level of the parameters used must be relatively stable in case the trees grow under optimum or unpolluted conditions. Deviations on the polluted territory indicate changes taking place in the physiological state of the trees.

5. It is suggested that such parameters should be selected that can be determined relatively easily and can therefore be used in large-scale investigations.

Because of the uneven annual dynamics of individual biochemical parameters belonging to different metabolic processes, only one biochemical indicator cannot be expected to yield an all-sided overview of the vitality of a tree. This can only be achieved using a variety of biochemical indicators. Integrated evaluation of several indicators can show the general state-of-art of trees or a forest site.

On the basis of the above-mentioned principles, the following elements and compounds were chosen for biochemical indication in the needles of conifers: Ca, K, Mn, S, N, P, chlorophylls *a* and *b* (Chl) and carotenoids (Car). It has been documented that high amounts of alkaline dust cause variations in the amount of photosynthetic pigments, sulphur containing substances and other nutrients as well as their seasonal dynamics in conifer needles. Deviations depend on the dust load and distance from the pollution source (Mandre 1995; Mandre and Tuulmets 1997).

Still, due to the general physiological regularities, the content of the above-mentioned elements and compounds in conifer needles varies to a great extent throughout the year (Mandre 1995); however, according to the principles laid down for biochemical parameters, they should have a known and justifiably small annual fluctuation.

Therefore, in order to determine the state-of-art of the trees on the territory influenced by alkaline dust, several ratios of elements and compounds that are vitally important or whose seasonal deviations under optimal growth conditions (the control) fluctuate within a narrow amplitude in the needles (Ca/K, N/S, N/P, N/Mg, N/Mn, Chl/Car, Chl *a*/Chl *b*) were used (Mandre and Tuulmets 1995). Their deviations, in case of pollution impact, conditionally indicate the changes in the state-of-art of trees.

Given the differences in the above ratios of needles collected on the polluted areas and in the control, biochemical index (*BI*) of the state-of-art of trees, which characterizes the deviations in organisms from the control due to pollution stress, was found (Mandre and Tuulmets 1993):

$$BI \quad \frac{\sqrt{\left(\frac{x_1}{n_1} \quad \frac{k_1}{n_1}\right)^2 \quad \ldots \quad \left(\frac{x_a}{n_a} \quad \frac{k_a}{n_a}\right)^2}}{a}$$

where x – parameter value of trees growing on the polluted territory, k – parameter value of control trees, n – number of analyses, $x/n–k/n$ – the mean differences between the data of the control and polluted needles, a – number of the biochemical parameter ratios used.

The obtained deviation of *BI* was used to identify the strength and territorial range of the industrial dust impact within the general system characterizing the forest ecosystem together with the morphological parameters of the conifers and physical-chemical composition of the soil and precipitation.

Results

1. Peculiarities of the sample plots and air pollution

The main damaging factor of the forest ecosystem and trees in the vicinity of the cement plant is apparently dust, which constitutes 87–91% of the total air pollution (Keskkond, '89 1990; Keskkond, '90 1991; Estonian Environment 1991 1991; Estonian Environment 1992 1993; Estonian Environment 1994 1995). The dust contains a complex of substances: 40-50% of CaO, 12–17% of SiO_2, 4–9% of K_2O, 4–8% of SO_3, 3–5% of Al_2O_3, 2–4% of MgO, 1–3% of Fe_2O_3, and to a somewhat lesser extent Mn, Zn, Cu, Cr, V, As, Ba, Pb and many other elements (Mandre, 1995). In 1987–93 the dust fallout at a distance of about 1–1.5 km from the emission source reached 1800–2400 g m^{-2} $yr.^{-1}$. In 1994 and 1995 the emission of cement dust from the plant decreased notably (Fig. 2) thanks to the installation of efficient filters. The water solution of dust from electric filters had pH values from 12.3 to 12.7. The high load of dust pollution has caused alkalization and changes in the chemical composition of the soil, ground water and precipitation on this area. At a distance of 2–4 km from the cement plant, the pH of the soil ranged from 7.0 to 8.1, the pH of rain water was between 7.6 and 8.2 and that of snow water 8.6–11.0 in different years. With increasing distance from the cement plant, the pollution load as well as the soil and precipitation pH decreased. 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. A good correlation between the distance from the emission source and concentrations of cations predominating in dust and pH of environmental components was established (Table 1). Significant correlations between the quantity of dust and chemical parameters in the soil and precipitation on the sample plots at different distances from the cement plant were also revealed statistically (Table 2). The contents of the

Table 1. Correlation coefficients (r) between the distance from the cement plant (*km*) and chemical characteristics of the soil and precipitation on the investigation transect and their significance (p)

Environmental parameters	pH		Ca^{2+}		K^+		Mg^{2+}	
	r	p	r	p	r	p	r	p
Snow	-0.83	<0.001	-0.82	<0.001	-0.63	<0.001	-0.51	<0.01
Rain	-0.99	<0.001	-0.81	<0.001	-0.73	<0.005	-0.86	<0.001
Soil (A horizon)	-0.84	<0.001	-0.59	<0.005	-0.71	<0.001	-0.64	<0.001
Ground water	-0.86	<0.001	-0.67	<0.001	-0.54	<0.05	-0.66	<0.001

Table 2. Correlation coefficients (r) between the dust pollution load (g m^{-2} $yr.^{-1}$) and chemical characteristics of the soil and precipitation on the investigation transect and their significance (p)

Environmental parameters	pH		Ca^{2+}		K^+		Mg^{2+}	
	r	p	r	p	r	p	r	p
Snow	0.80	<0.001	0.84	<0.001	0.95	<0.001	-	-
Rain	0.70	<0.005	0.96	<0.001	0.85	<0.001	0.79	<0.001
Soil (A horizon)	0.68	<0.001	0.89	<0.001	0.90	<0.001	0.84	<0.001
Ground water	0.71	<0.001	0.84	<0.001	0.68	<0.001	0.76	<0.001

SO_4^{2-} anion in the environment also seem to show a good correlation with the distance from the emission source ($r = -0.74, p < 0.05$).

2. Biochemical parameters of trees

High amounts of alkaline dust and alkalization of the environment cause variation in the amount of photosynthetic pigments, mineral elements and sulphur containing substances as well as their seasonal dynamics in conifer needles. Deviations depend on the age of needles and the distance from the pollution source (Mandre et al. 1992).

In general, the concentration of pigments in conifer needles responds to strong alkaline air pollution with a significant decrease along the deposition gradient. The pollution damage on Norway spruce and Scots pine in the vicinity of the cement plant is mostly expressed as a decline in Chl *a*, Chl *b* and *b*-carotene, Mn, Mg and N contents and increase in Ca, K and S contents in needles (Mandre et al. 1992; Mandre and Tuulmets 1995). The correlations between the content of pigments and measured mineral elements in Norway spruce needles, the pollution complex, soil pH and single components are very strong (Table 3), allowing us to presume that they are suitable for identifying the effect of technogenic dust.

The content of pigments in spruce needles is in very strong correlation with the content of the elements favouring snow and soil alkalization in the environment (Ca, K, Mg). For example, the correlation coefficient of the Chl content in needles and Ca in snow is $r = -0.74$ ($p < 0.001$), and the correlation coefficient of Chl and Ca in the soil is $r = -0.78$ ($p < 0.001$), correlation coefficients between Chl in needles and K in snow is $r = -0.78$ ($p < 0.001$) and between Chl in needles and K in the soil is $r = -0.80$ ($p < 0.001$). The effect of K through the soil on chlorophyll and carotenoids is weaker, amounting to $r = -0.55$ ($p < 0.05$) and $r = -0.55$ ($p < 0.05$), respectively. Especially sensitive to dust pollution in needles is Chl *a*.

3. Bioindication

On the basis of differences in the ratios of the above-mentioned sensitive biochemical parameters of Norway spruce on the forest sites under different quantities of dust and those of the control trees the values of *BI* were found. In 1991–95 the *BI* values were especially high within a distance of 3 km from the cement plant, indicating considerable changes in the physiological-biochemical state of the trees. Figure 3 shows that the *BI* values have been falling from year to year, which is a direct consequence of the decrease in the dust emission from the cement plant. *BI* largely depends on the pollution load and has a good correlation with the characteristics of the growth conditions (Table 4).

Table 3. The correlation between the chloroplast pigments and mineral elements concentration in Norway spruce needles and pH and other environmental factors at different distances from the cement plant

Parameters of needles	Distance from the cement plant	Total quantity of air pollution	pH of snow water	pH of the soil
Chl *a*	$r = 0.61$ $p < 0.001$	$r = -0.81$ $p < 0.001$	$r = -0.66$ $p < 0.01$	$r = -0.63$ $p < 0.05$
Chl *b*	$r = 0.64$ $p < 0.001$	$r = -0.50$ $p < 0.05$	$r = -0.64$ $p < 0.01$	$r = -0.68$ $p < 0.01$
Chl	$r = 0.63$ $p < 0.001$	$r = -0.77$ $p < 0.001$	$r = -0.68$ $p < 0.001$	$r = -0.65$ $p < 0.01$
Car	$r = 0.66$ $p < 0.001$	$r = -0.79$ $p < 0.001$	$r = -0.69$ $p < 0.001$	$r = -0.62$ $p < 0.05$
N	$r = 0.69$ $p < 0.001$	$r = -0.74$ $p < 0.001$	$r = -0.84$ $p < 0.001$	– –
K	$r = -0.72$ $p < 0.001$	– –	$r = 0.67$ $p < 0.01$	$r = 0.57$ $p < 0.05$
Ca	$r = -0.63$ $p < 0.001$	– –	$r = 0.72$ $p < 0.001$	$r = 0.76$ $p < 0.001$
Mg	$r = 0.91$ $p < 0.001$	$r = -0.58$ $p < 0.05$	$r = -0.89$ $p < 0.001$	$r = 0.82$ $p < 0.001$
Mn	$r = 0.90$ $p < 0.001$	$r = -0.49$ $p < 0.05$	$r = -0.85$ $p < 0.001$	$r = -0.74$ $p < 0.001$
S	$r = -0.85$ $p < 0.001$	$r = 0.56$ $p < 0.05$	$r = 0.87$ $p < 0.001$	$r = 0.68$ $p < 0.01$

Table 4. Correlation coefficients between the biochemical index (*BI*) and some characteristics of the growth conditions

Characteristics	*BI* r	*BI* p
Distance from the cement plant	-0.66	0.001
Total air pollution level	0.86	0.001
pH of snow water	0.81	0.001
pH of the soil	0.81	0.001

In comparison to several other methods used in the general system of bioindication, such as morphological and floristical changes and also several environmental parameters, biochemical indication is suitable for

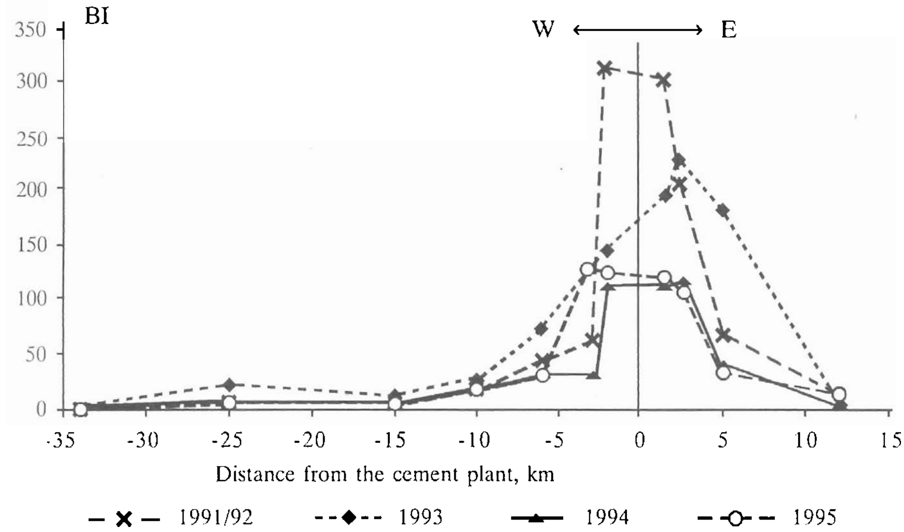

Fig. 3. The dynamics of biochemical index (*BI*) showing the function of all deviations of damaged trees on the territory affected by cement dust. *BI* is calculated on the basis of biochemical parameters of Norway spruce needles

ascertaining latent injuries caused by dust pollution in the forest ecosystem. *BI* is the most sensitive parameter among those used and compared (Fig. 4).

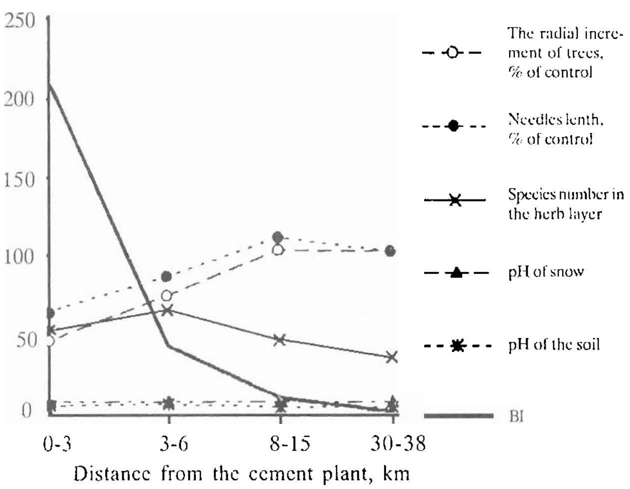

Fig. 4. A comparison of the sensitivity of parameters often used in bioindication for the estimation of the state of forest trees and index (*BI*) of biochemical deviations of trees in the *Oxalis–Myrtillus* site type of forest stands influenced by different levels of dust emitted from the cement plant in 1993.

Conclusions

The research conducted on forest trees revealed numerous biochemical parameters that are of bioindicative importance. These parameters can be used for both an early diagnosis of air pollution damage and the assessment of the state-of-art of trees in general. So *BI* has been deduced on the basis of fundamental research and tested under natural conditions (Mandre 1989; Mandre and Tuulmets 1993, 1995).

Along with evaluation of morphological and anatomical changes the biochemical method gives a full review of the influence of dust pollution on trees. This is one of the possibilities to advance knowledge on the importance of bioindicators for both early and differential diagnosis of damage.

Acknowledgements

The study was funded by the Kunda Nordic Cement Corp. (Estonia) and Estonian Science Foundation. We are grateful to Kersti Poom for technical assistance.

References

Arndt U. 1971. Changes in the concentration in leaf pigments under the influence of atmospheric pollution: A discussion of pigment analysis. Environ. Pollut., 2: 37–48.
Cape J. N., Paterson I. S., Wellburn A.R., Wolfenden J., Mehlhorn H., Freer-Smith P. H., Fink S. 1988. Early Diagnosis of Forest Decline. Dorset Press, Dorchester, 68 pp.
Dässler H. G. 1972. Zur Wirkungsweise der Schadstoffe. Der Einfluß von SO_2 auf Blattfarbstoffe. Mitt. Forstl. Bundesversuchsanst., 97: 353–366.
Govin T., Goral I. 1977. The chlorophyll and pheophytin content in needles of different age of trees growing under conditions of chronic industrial pollution. Acta Soc. Bot. Polon., 46, 1: 151–159.
Grill D., Esterbauer H., Birkner M. 1980. Untersuchungen über die Peroxidaseaktivität in Lärchennadeln. Beitr. Biol. Pflanzen, 55: 67–76

Höllwart M. 1981. Verwendung physiologischer Parameter zur Charcterisierung immissionbelasteter städtischer Standorte. Angew. Bot., 55: 195–209.

Kangur A. 1988. Klorofüllide sisalduse muutustest männiokastes Põhja-Eesti tööstusrajoonides [Changes in the chlorophyll content in pine needles in the industrial region of northern Estonia]. *T. Frey* (Editor), Kaasaegse ökoloogia probleemid. Tartu, pp. 53–57 (in Estonian, summary in English).

Linder S. 1972. Seasonal variation in pigments in needles. A study of Scots pine and Norway spruce seedlings grown under different nursery conditions. Studia Forest. Suec., 10: 5–37.

Mandre M. 1989. Tolmusaaste: mõju männile ja ... [Dust pollution: its effect on pines and ...]. Eesti Loodus, 10: 723–731 (in Estonian).

Mandre M. 1995. Changes in the nutrient composition of trees. *M. Mandre* (Editor), Dust Pollution and the Forest Ecosystems. A Study of Conifers in an Alkalized Environment. Publ. Inst. Ecol., 3. Tallinn, pp. 44–65.

Mandre M., Annuka E., Tuulmets L. 1992. Response reactions of conifers to alkaline dust pollution. Changes in the pigment system. Proc. Estonian Acad. Sci. Ecol., 2, 4: 156–173.

Mandre M., Tuulmets L. 1993. Complementing the forest monitoring system with biochemical studies. *Ü. Mander, J. Fenger, T. Oja, M. Mikk* (Editors), Energy, Environment and Natural Resources Management in the Baltic Sea Region. Nordic Council of Ministers, Copenhagen, pp. 257–268.

Mandre M., Tuulmets L. 1995. Biochemical indication of dust impact on forest. In: M. Mandre (Editor), Dust Pollution and Forest Ecosystems. A Study of Conifers in an Alkalized Environment. Publ. Inst. Ecol., 3. Tallinn, pp. 150–157.

Mandre M., Tuulmets L. 1997. Pigment changes in Norway spruce induced by dust pollution. Water, Air, Soil Pollut., 94: 247–258.

Müller J. 1957. Spezifischer Nachweis von SO_2-Rauchschäden an Pflanzen mit Hilfe von Blattpigmentanalyse. Naturwissenschaften, 44: 453.

Reich P. B., Schoettle A. V., Raba R. M., Admundson R. G. 1986. A response of soybean to low concentration of ozone: I. Reduction in leaf and whole plant net photosynthesis and leaf chlorophyll content. J. Environ. Qual., 15(1): 31–36.

Schubert R. (Editor). 1985. Bioindikation in terrestrischen Ökosystemen. Gustav Fischer, Jena, 327 pp.

Vernon L. 1960. Spectrophotometric determination of chlorophylls and pheophytins in plant extracts. Analyt. Chem., 32: 1144–1150.

Wood J. P., Bachelard E. P. 1969. Variations in chlorophyll concentration in the foliage of Radiata pine. Aust. For. J., 33, 2: 119–128.

Гетко Н. В., Рак Л. Д., Шобанова Ч. А., Жданец С. Ф. 1985. Толерантность естественных фитоценозов серосодержащим промышленным токсикантам [Tolerance of the natural phytocenozes to the sulphur containing industrial tocsicants]. Оптимизация окружающей среды средствами озеленения. Наука и техника, Минск, pp. 43–60 (in Russian).

БИОХИМИЧЕСКАЯ ДИАГНОСТИКА УХУДШЕНИЯ СОСТОЯНИЯ ЛЕСОВ

М. Мандре, Л. Туулметс

Резюме

В лесах типа *Oxalis–Myrtillus* в районах подверженных влиянию цементной пыли, был опредлен уровень повреждения хвойных при помощи биохимических исследований соотношений Chl/Car, Chl *a*/Chl *b*, N/Mg, N/P, N/S, N/Mn и Ca/K в хвое. Был определен индекс *BI*, указывающий отклонения состояния поврежденных деревьев от контробных. Этот индекс является чрезвычайно чувствительным и напрямую зависит от объема загрязнений и химических изменений в почве и осадках на различных расстояниях от источника эмиссии.

Ключевые слова: биоиндикация, биохимические параметры, хвойные, цементная пыль.