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## Content of carotenoids in needles of *Pinus sylvestris* L. growing in a polluted area

**Abstract:** Carotenoids (neoxanthin, violaxanthin, lutein and  $\beta$ -carotene) present in plant cells play a role in photoprotection. Industrial pollution causes oxidative stress in plants, while carotenoids react with free radicals and dissipate the excess excitation energy. In this way carotenoids prevent the negative influence of free radicals on metabolism and can even repair some of the damages. This is confirmed by results of our analysis of the level of xanthophylls in 16 and 17-year-old trees of Scots pine (*Pinus sylvestris* L.). Grown from seed collected in north-eastern Poland, the trees grow in a relatively unpolluted control site, and in a polluted site located 2 km far away from the Phosphorus Fertilizer Works. In the polluted site the environment is contaminated with  $\text{SO}_2$ ,  $\text{NO}_x$  and F, Al, Pb, Cu. The needles analysed in this study were visually undamaged. Material was collected in experimental plots from 6 trees in October'98 and April'99, between 12.00 and 13.00 hours, at full sunlight. The pigments were analysed quantitatively and qualitatively by thin-layer chromatography. The paper presents results of the content and distribution of neoxanthin, violaxanthin, lutein and  $\beta$ -carotene of Scots pine needles from healthy control and stressed trees. Marked differences in pigment levels depended on the stage of needle development and level of pollution.

**Additional key words:** thin-layer chromatography,  $\beta$ -carotene, lutein, neoxanthin, violaxanthin, heavy metals

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### Introduction

New explanations of the physiological role of carotenoids were supplied by studies carried out in the mid-1970's. On the basis of these studies we know that in the process of photosynthesis these pigments absorb solar radiation at wavelengths which are not used by chlorophylls. If the balance between absorbed and used energy is disturbed (for example, as a result of a stress factor), carotenoids (mainly xanthophylls) may dissipate the excess excitation energy. The pigments play a photoprotective function: they protect thylakoids from photodynamic damage (Eskling et al. 1997). In this way, these pigments (especially violaxanthin and zeaxanthin) perform the tasks of the xanthophyll cycle.

Concentrations of the pigments participating in the xanthophyll cycle depends on many factors. In shaded plants, lutein concentration increases, while zeaxanthin, antheraxanthin and violaxanthin levels decrease in comparison to sun plants. In plants exposed to increasing intensity of sunlight, the concentrations of pigments increase in the following sequence: chlorophyll *a* > chlorophyll *b* > neoxanthin >  $\beta$ -carotene > zeaxanthin > antheraxanthin > violaxanthin > lutein (Venema et al. 1999).

In many plants changes in concentrations of carotenoids are associated with daily and seasonal variation in external factors. Daily variation reflects the synergism of light induction and water deficit, affecting the synthesis of zeaxanthin (Srivastava and Zeiger 1995), antheraxanthin, and  $\beta$ -carotene before noon, whereas chlorophyll *a* and *b* are affected at mid-

day (Schindler and Lichtenthaler 1996). Seasonal variation shows that synthesis of antheraxanthin and zeaxanthin is intensive in summer and minimal in winter, while violaxanthin synthesis is minimal in summer and intensive in winter (Demmig-Adams and Adams III 1996).

Gaseous pollution of the air ( $\text{SO}_2$ ,  $\text{NO}_x$ , HF,  $\text{O}_3$  or PAN) causes chlorophyll destruction, affecting an increase in the proton gradient of thylakoid membranes, which activates zeaxanthin synthesis (Young and Britton 1990). Chlorophyll *a* and  $\beta$ -carotene are more susceptible to the activity of toxic gases than chlorophyll *b* and xanthophylls (Arvidsson et al. 1997, Darral 1989, Demmig-Adams and Adams III 1996).

Under the toxic influence of heavy metals (Cu, Cd, Pb, Ni, Zn, Fe and Mn) and aluminium ions, concentrations of chlorophylls and carotenoids decline (Arvidsson et al. 1997, Darral 1989, Demmig-Adams and Adams III 1996).

The aim of this study was to analyse differences in concentrations of chosen carotenoids ( $\beta$ -carotene, lutein, neoxanthin and violaxanthin) in needles of Scots pine trees growing in a polluted site and control site in autumn and early spring.

## Materials and methods

**Plant material.** Visually undamaged current-year (October'98) and 1-year-old (April'99) needles were collected from the youngest shoots of 16 and 17-year-old trees of Scots pine (*Pinus sylvestris* L.), representing the population from Miłomłyn (53°34'N, 20°00'E) in north-eastern Poland. The trees are growing in two experimental areas of the Institute of Dendrology in Kórnik. The control plot is located in the Experimental Forest "Zwierzyniec" near Kórnik (52°15'N, 17°04'E), in an area almost free from direct industrial pollution. The other plot is located in Czapury (52°15'N, 16°50'E), at heavily polluted site, 2 km away from Phosphorus Fertilizer Works at Luboń near Poznań. The material was collected between 12.00 and 13.00 hours, at full sunlight.

**Identification of carotenoids.** Concentrations of individual carotenoids were assessed by thin-layer chromatography according to the procedure described by Lichtenthaler and Wellburn (1983).

## Results and discussion

Under the influence of industrial pollution, the level of carotenoids is disturbed. This is confirmed by results of laboratory research described by Demmig-Adams and Adams III (1996), Young and Britton (1990). The results of our study reflect the situation in a polluted environment.

Many reports emphasise the age-related and intraspecific variation of Scots pine (Białobok et al. 1993). Thus we collected needles from trees of the same age and provenance. Moreover, considering the different sensitivity of mature and ageing needles to various abiotic stresses, we analysed only needles of the youngest shoots.

Scots pine is generally considered very sensitive to air pollutants ( $\text{SO}_2$ , HF,  $\text{NH}_3$ ,  $\text{O}_3$ ,  $\text{NO}_x$ ), and some heavy metals (Pb, Cu, Cd, Fe, Zn), which has been discussed in a review paper (Karolewski 1993). However, this species is often classified as tolerant to aluminium ions (Arovaara and Ilvesniemi 1990, Karolewski and Giertych 1995, Lorenc-Plucińska and Ziegler 1996, Oleksyn et al. 1999). The increase in sensitivity to photodestruction may be associated with a lower capacity to produce photoprotective carotenoids. These pigments are involved in protection against oxidative stress, as they activate components that scavenge free radicals. Oxidative stress is caused by light or thermal stress factors, water deficit or the presence of herbicides. As plants may respond similarly to various stress factors, it can be assumed that this scenario will be observed in Scots pine needles in areas affected by industrial pollution.

The content of individual carotenoids in the total pool of all analysed pigments in Scots pine needles in both study sites in October'98 can be illustrated as follows: lutein >  $\beta$ -carotene > neoxanthin > violaxanthin. That month was characterised by low monthly mean of insolation of  $67 \mu\text{E m}^{-2}\text{s}^{-1}$  and mean temperature of  $9.7^\circ\text{C}$ . This was favourable for lutein synthesis and inhibited violaxanthin synthesis (Fig. 1). Similar results were described by Venema et al. (1999).

In April'99 in needles of trees growing in the control site the contribution of neoxanthin (37%) to the pool of all analysed pigments was slightly higher than that of  $\beta$ -carotene (30%), but the contributions of violaxanthin (18%) and lutein (16%) were much lower. The weather of that month, with high monthly means of temperature ( $18^\circ\text{C}$ ) and insolation ( $160 \mu\text{E m}^{-2}\text{s}^{-1}$ ), favoured a photoprotective distribution of carotenoids. However, in the polluted site proportions of individual carotenoids in pine needles were different than in the control site, and similar to those recorded in October'98 (Fig. 1). This means that industrial pollution disturbs the natural proportions of carotenoids, which has already been reported earlier for other species (Wingsle and Hallgren 1993, Young and Britton 1990).

Among the studied pigments  $\beta$ -carotene was dominant. This pigment is a precursor of zeaxanthin (Bartley and Scolnik 1995, Bungard et al. 1999, Cunningham and Gantt 1998, Demmig-Adams 1990, Eskling et al. 1997, Niyogi et al. 1998, Zeiger and Zhu

1998). A high level of  $\beta$ -carotene may be associated with a low level of violaxanthin. Such a situation was observed in this study in October'98 and, to a lesser extent, in April'99. Possibly this resulted from de-epoxidation (transformation of zeaxanthin to violaxanthin), which attests to photoprotection of photosystems (Tausz et al. 1998) (Fig. 1).

Differences in the ratio of  $\beta$ -carotene to the pool of all xanthophylls in both plots are presented in Figure 2. In April'99 both insolation and temperature were higher than in October'98. In the control site the concentration of  $\beta$ -carotene was also higher, but in the polluted site its concentration was lower in April'99 than in October'98.

Neoxanthin is derived from violaxanthin (Bungard et al. 1999, Cunningham and Gantt 1998, Niyogi et al. 1997, Niyogi et al. 1998, Wingsle and Hallgren 1993). This is confirmed by results of our study. In April'99 the neoxanthin level was higher and the violaxanthin level was slightly lower than in October'98 in needles collected in the control site, but in needles collected in the polluted site the levels of these pigments were disturbed (Fig. 1). This attests to inhibition of the synthesis of neoxanthin from violaxanthin, which may result from industrial pollution.

Zeaxanthin, similar to lutein is produced when plant receives excess energy. Lutein participates in the lutein-5,6-epoxide cycle, while zeaxanthin participates in the xanthophyll cycle. A high level of lutein, antheraxanthin and zeaxanthin is associated with a low level of lutein-5,6-epoxide and violaxanthin in the morning (Bungard et al. 1999). This applies to needles collected in October'98 from trees growing in both sites. In needles from the control site the concentration of lutein was slightly lower than the concentration of violaxanthin. Such a relationship is linked with the level of irradiation, which is confirmed by Venema et al. (1999). In the polluted site changes in the ratio of lutein to violaxanthin were observed.

Marked differences in contents of  $\beta$ -carotene, neoxanthin and violaxanthin between the control and the polluted site were recorded in our study (Tab. 1). The course of the xanthophyll cycle and the lutein-5,6-epoxide cycle was altered in the polluted site. Disturbances in these cycles may be due to altered energy balance and use of energy for transformation of carotenoids. This suggests that photosystems can be damaged under the influence of industrial pollution in Scots pine needles.

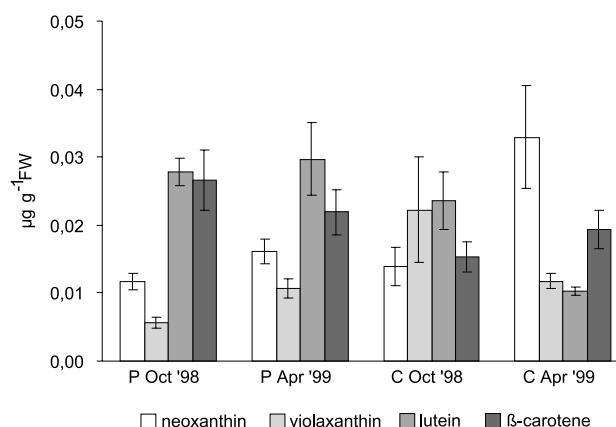


Fig. 1. Concentrations of carotenoids in Scots pine needles in a control site (C) and in a polluted site (P). The material was collected in October'98 and April'99. Data represent means  $\pm$  SE

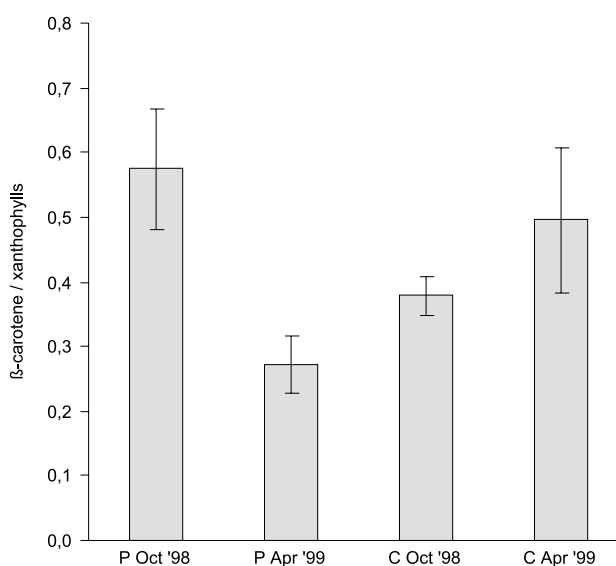


Fig. 2. Ratio C/P (control/polluted site) of  $\beta$ -carotene to the total of all studied xanthophylls (neoxanthin + violaxanthin + lutein) in Scots pine needles. The material was collected in October'98 and April'99. Data represent means  $\pm$  SE

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Table 1. Changes of pigment contents between October 1998 and April 1999:

↑ increase in pigment contents; ↓ decrease in pigment contents; double arrows indicate large changes

	Neoxanthin	Violaxanthin	Lutein	$\beta$ -carotene	$\beta$ -carotene/xanthophylls
Control site	↑↑		↓↓	↓	↑
Polluted site		↑↑	↓	↓	↓

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## References

- Białobok S., Boratyński A., Bugała W. 1993. *Biologia sosny zwyczajnej*. Sorus, Poznań-Kórnik.
- Arovaara H., Ilvesniemi H. 1990. Effects of soluble inorganic aluminium on the growth and nutrient concentrations of *Pinus sylvestris* and *Picea abies* seedlings. *Scandinavian Journal of Forest Research* 5: 49–57.
- Arvidsson P.O., Carlsson M., Steffánsson H., Albertson P.A., Akerlund H.E. 1997. Violaxanthin accessibility and temperature dependency for de-epoxidation in spinach thylakoid membranes. *Photosynthesis Research* 52: 39–48.
- Bartley G.E., Scolnik P.A. 1995. Plant carotenoids: pigments for photoprotection, visual attraction, and human health. *The Plant Cell* 7: 1027–1038.
- Bungard R.A., Ruban A.V., Hibberd J.M., Press M.C., Horton P., Scholes J.D. 1999. Unusual carotenoid composition and a new type of xanthophyll cycle in plants. *Proceedings of the National Academy of Sciences of the United States of America* 96: 1135–1139.
- Cunningham F.X., Gantt E. 1998. Genes and enzymes of carotenoid biosynthesis in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 49: 557–83.
- Darral N.M. 1989. The effects of air pollutants on physiological processes in plants. *Plant, Cell and Environment* 12: 1–30.
- Demmig-Adams B. 1990. Carotenoids and photoprotection in plants: A role for the xanthophyll zeaxanthin. *Biochimica Biophysica Acta* 1020: 1–24.
- Demmig-Adams B., Adams III W.W. 1996. The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Elsevier Science* 1(1): 21–26.
- Eskling M., Arvidsson P.O., Åkerlund H.E. 1997. The xanthophyll cycle, its regulation and components. *Physiologia Plantarum* 100: 806–816.
- Karolewski P., Giertych M. 1995. Changes in the level of phenols during needle development in Scots-pine populations in a control and polluted environment. *European Journal of Forest Pathology* 25: 297–306.
- Karolewski P., Lorenc-Plucińska G. 1993. Zaburzenia w procesach fizjologicznych i metabolizmie pod wpływem zanieczyszczeń powietrza. In: Białobok S., Boratyński A., Bugała W. (eds.). *Biologia sosny zwyczajnej*. Sorus, Poznań-Kórnik, pp. 193–206.
- Lichtenthaler H.K., Wellburn A.R. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions* 11: 591–592.
- Lorenc-Plucińska G., Ziegler H. 1996. Changes in ATP levels in Scots pine needles during aluminium stress. *Photosynthetica* 32(1): 141–144.
- Niyogi K.K., Björkman O., Grossman A.R. 1997. *Chlamydomonas* xanthophyll cycle mutants identified by video imaging of chlorophyll fluorescence quenching. *The Plant Cell* 9: 1369–1380.
- Niyogi K.K., Grossman A.R., Björkman O. 1998. Arabidopsis mutants define a central role for the xanthophyll cycle in the regulation of photosynthetic energy conversion. *The Plant Cell* 10: 1121–1134.
- Oleksyn J., Reich P.B., Karolewski P., Tjoelker M.G., Chalupka W. 1999. Nutritional status of pollen and needles of diverse *Pinus sylvestris* populations grown at sites with contrasting pollution. *Water, Air and Soil Pollution* 110: 195–212.
- Schindler Ch., Lichtenthaler H.K. 1996. Photosynthetic CO<sub>2</sub> assimilation, chlorophyll fluorescence and zeaxanthin accumulation in field-grown maple trees in the course of a sunny and a cloudy day. *Journal of Plant Physiology* 148: 399–412.
- Srivastava A., Zeiger E. 1995. Guard cell zeaxanthin track photosynthetically active radiation and stomatal apertures in *Vicia faba* leaves. *Plant, Cell and Environment* 18: 813–817.
- Tausz M., Jimenez M.S., Grill D. 1998. Antioxidative defence and photoprotection in pine needles under field conditions. A multivariate approach to evaluate patterns of physiological responses at natural sites. *Physiologia Plantarum* 104: 760–764.
- Venema J.H., Posthumus F., de Vries M., van Hasselt P.R. 1999. Differential response of domestic and wild *Lycopersicon* species to chilling under low light: growth, carbohydrate content, photosynthesis and the xanthophyll cycle. *Physiologia Plantarum* 105: 81–88.
- Wingsle G., Hällgren J.E. 1993. Influence of SO<sub>2</sub> and NO<sub>2</sub> exposure on glutathione, superoxide dismutase and glutathione reductase activities in Scots Pine needles. *Journal of Experimental Botany* 44: 463–470.
- Young A., Britton G. 1990. Carotenoids and stress. In: Alscher R.G., Cumming J.R. (red.) *Stress Responses in Plants: Adaptation and Acclimation Mechanisms*. New York, Wiley-Liss Inc, pp. 87–112.
- Zeiger E., Zhu J. 1998. Role of zeaxanthin in blue light photoreception and the modulation of light – CO<sub>2</sub> interactions in guard cells. *Journal of Experimental Botany* 49: 433–442.