Acta Bot. Croat. 62 (1), 27–35, 2003 CODEN: ABCRA 25
ISSN 0365–0588

The annual changes of chloroplast pigments content in current- and previous-year needles of Norway spruce (*Picea abies* L. Karst.) exposed to cement dust pollution

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Chloroplast pigments were shown to be very sensitive to various environmental influences. Changes in chlorophyll and carotenoid content were investigated in Norway spruce needles exposed to alkaline dust emitted by the cement industry. Pigments were extracted from current-year and previous-year needles and quantified spectrophotometrically. In both needle generations all measured pigments were reduced in dust-exposed needles compared to needles harvested from areas not polluted by cement dust. This was due to deceleration of the biosynthetic processes rather than degradation of pigments. Chlorophyll *b* content appeared to be more sensitive than chlorophyll *a* in current-year needles while in previous-year needles chlorophyll *a* was more affected. Total carotenoids needed a longer period of time to reach nearly the same level as in controls. The progression of pigment decline in previous-year needles appeared not to be dramatically accelerated. It might thus be concluded that the spruce needles had sufficient biosynthetic capacity to prevent irreversible damage by cement dust.

Key words: *Picea* abies, chlorophyll, carotenoids, cement dust, air pollution

Introduction

The impact of the cement industry on the surrounding vegetation has been widely investigated (FARMER 1993) although research on the effects of dust pollution on plants has never received the same level of attention as that given to phytotoxic pollutants such as SO_2, NO_2 and O_3. Results from research that has been undertaken, together with repeated observations of dust deposits on vegetation, suggest that the effects of dust may be important and are worthy of greater investigative attention. The early investigations (PIERCE 1909) dealt with the impact of cement dust on the stomata of *Vitis vinifera*, some «fruit trees« and *Quercus lobata*. The following investigations, mostly concerning commercial species or trees, pointed out the influence of cement dust on growth (SINGH and RAO 1968, SHUKLA et al. 1990), transpiration (SINGH and RAO 1981), photosynthesis (DARLEY 1966),

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the cation level (LAL and AMBASHT 1982), enzyme activities (BORKA 1980), starch production (OBLISAMI et al. 1978), yellowing symptoms (BOHNE 1963, TAYLOR et al. 1986), leaf necrosis (CZAJA 1961) and changes at the cellular level (CZAJA 1962). In his paper CZAJA (1961) provided extensive detail of injury to *Betta vulgaris*. The surface pH of leaves was greatly enhanced, up to 10.0. The leaf cells started to plasmolyse 1 week after the application of cement dust, with an irregular distribution of chloroplasts and starch no longer being formed. This eventually led to cell death. CZAJA (1962), also, describes in detail the injuries on *Picea abies*, *Pinus sylvestris*, *Taxus baccata* and *Rosa canina* caused by cement-kiln dust. The dust forms hard crystalline crusts on the leaf surface, which dissolves, thus releasing solutions of calcium hydroxide into the intercellular spaces. This causes cell plasmolysis and death.

The recent investigations mainly referred to the conifer species (MANDRE and TUULMETS 1997, MANDRE et al. 1998, BAČIĆ et al. 1999, CESAR and LEPEDUŠ 2001) or mosses (JALKANEN et al. 2000). In a very comprehensive study done in mixed pine and spruce forest stands selected along a 50 km transect that extended 38 km to the west and 12 km to the east of a cement plant, MANDRE and TUULMETS (1997) concluded that alkalization and the high Ca content of the environment inhibited the assimilation of Mg, Mn and Fe by plants, which was, together with light deficiency under the cement crusts on the needles, the real reason for a depression of the biosynthesis of photosynthetically active pigments in Norway spruce needles in the territory affected by alkaline dust emitted from a cement plant. BAČIĆ et al. (1999) reported that *Pinus halepensis* needles in the vicinity of a cement factory were found to have increased amounts of surface calcium and extensive calcium crusts, a further progression of epicuticular wax tubule erosion and an increase in the number of plugged stomata, with wax eruptions commonly occurring.

The chloroplast pigments in conifer needles were shown to be very sensitive to various influences from the environment, especially to gaseous air pollutants (BATIČ et al. 1995, MIKKELSEN et al. 1995, WILD and SCHMITT 1995, TAUSZ et al. 1996, SCHULZ et al. 1996). The previous investigation we made (CESAR and LEPEDUŠ 2001) revealed the dual impact of cement dust on spruce needles. The direct influence was expressed through the decreasing of peroxidase activity because of the alkaline microenvironment, and the indirect impact through changes in the soil chemistry, reflected in a lower chlorophyll content and disturbed needle anatomy, although no yellowing occurred.

This investigation is the part of our comprehensive study on the impact of alkaline dust pollution emitted from the cement industry on the needles of Norway spruce (*Picea abies* L. Karst). The aim of the presented investigation was to compare the chloroplast pigment content in current-year and previous-year needles of cultivated spruce trees grown in urban areas polluted and not polluted by cement dust as well as the speed of progression in the expected differences. For this purpose the investigations started, after bud bursting, when young needles were nicely formed and the deposition of cement dust layers occurred.

Materials and Methods

Cultivated Norway spruce (*Picea abies* L. Karst) trees from an urban area in the neighbourhood of the cement factory in Našice (below 200 m a.s.l.), Croatia, were used to investigate the impact of cement dust pollution on chloroplast pigment content. Sampling was done monthly from July 2000 to June 2001. Current-year and previous-year needles were harvested from five spruce trees growing on different grass-plots within a 3 km radius of the cement factory in the easterly direction. Basic soil characteristics are given in Tab. 1. Needles from each tree were processed separately and the results obtained by the measurements were arranged as sample 1.

As control group, five cultivated spruce trees growing on different grass-plots from the urban area of Osijek (about 100 m a.s.l.), Croatia, which is 50 km east of the cement factory were used. In determination of control locations several criteria were applied: (1.) in the surrounding of each sampled tree, for a radius of at least 5 km, there was no industry considered to be a pollution source; (2.) with respect to traffic pollution, peaceful parts of the town with family houses and gardens were chosen; (3.) the trees were growing on similar soil types: clay or loamy-clay (Tab. 1); (4.) all vegetation, not only spruce trees, on chosen locations could be described as healthy and nice. Climate conditions and in consequence water supply corresponded in the polluted and control stands. Control needles were processed in the same way as those affected by cement dust and arranged as sample 2.

Branches bearing needles were harvested from the middle crown of each tree. Every time, the sampling was done between 8 am and 9 am. Twigs were put in a nylon bag, placed on a ice and deprived of light. The transport to the laboratory was done within one hour. Chloroplast pigments were double extracted with absolute acetone and quantitative determination was done by measuring the absorbance on 661.6, 644.8 and 470.0 nm. The concentrations of chlorophyll *a*, chlorophyll *b*, total chlorophyll and total carotenoids were calculated using the absorption coefficients given by LICHTENTHALER (1987):

$$\text{Chl } a = (11.24A_{661.6} – 2.04A_{644.8})$$

$$\text{Chl } b = (20.13A_{644.8} – 4.19A_{661.6})$$

$$\text{Chl } a{+}b = (7.05A_{661.6} + 18.09A_{644.8})$$

$$\text{Car} = (1000A_{470} – 1.90(11.24A_{661.6} – 2.04A_{644.8}) – 63.14(20.13A_{644.8} – 4.19A_{661.6}))/214$$

The data obtained by the quantitative measurements were statistically evaluated using the t-test modified for small samples, which included the calculation of complex standard deviation (PAVLIĆ 1977).

Tab. 1. Soil parameters of sampling plots in Našice (SAMPLE 1) and Osijek (SAMPLE 2); P(t) – percent of similarity; NS – not significant difference.

PARAMETER	SAMPLE 1	SAMPLE 2	P(t)
% $CaCO_3$	9.55 ± 0.54	4.36 ± 0.07	<1%
pH	8.40 ± 0.00	8.35 ± 0.07	NS
soil type	loamy clay	loamy clay or clay	

Results and Discussion

The quantitative analysis of chloroplast pigments revealed differences between needles exposed to cement dust pollution and those from the unpolluted area. The mean values of all measured parameters are shown in Figs. 1 and 2. Chlorophyll *a* content was higher in current-year needles (Fig. 1) as well as in previous-year needles (Fig. 2) from Osijek than in needles from Našice. The monthly values of chlorophyll *a* varied from 0.59 to 0.91 and from 0.68 to 1.11 mg g^{-1} FW in current-year needles from Našice and Osijek, respectively (Tab. 2). The statistical significance is shown for the second part of investigated period (January to June 2001) (Tab. 2). The value of the same parameter in previous-year needles varied from 0.65 to 1.00 mg g^{-1} fresh weight in Našice and from 0.93 to 1.20 mg g^{-1} FW in Osijek (Tab. 2). In previous-year needles, a significant difference in chlorophyll *a* content was demonstrated for the whole of the investigated period (Tab. 2).

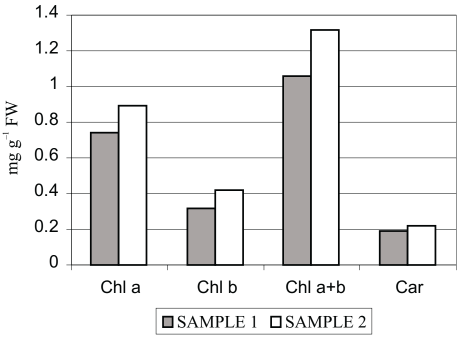

Fig. 1. The mean values of chloroplast pigment concentration (mg g^{-1} FW) for the whole of the investigated period in current-year needles of Norway spruce (*Picea abies* L. Karst.) collected from the vicinity of the cement factory in Našice (SAMPLE 1) and from the city of Osijek (SAMPLE 2); chlorophyll *a* – Chl *a*, chlorophyll *b* – Chl *b*, total chlorophyll – Chl *a+b* and total carotenoids – Car.

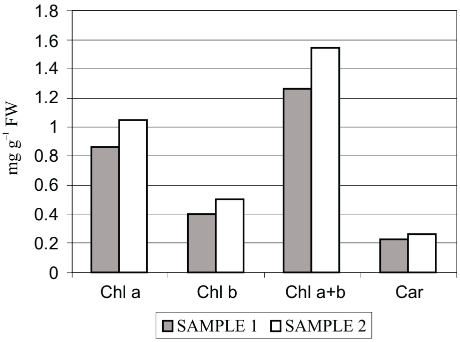

Fig. 2. The mean values of chloroplast pigment concentration (mg g^{-1} FW) for the whole of the investigated period in previous-year needles of Norway spruce (*Picea abies* L. Karst.) collected from the vicinity of the cement factory in Našice (SAMPLE 1) and from the city of Osijek (SAMPLE 2); chlorophyll *a* – Chl *a*, chlorophyll *b* – Chl *b*, total chlorophyll – Chl *a+b* and total carotenoids – Car.

The chlorophyll *b* content was also lower in both needle generations from Našice compared to those in Osijek (Figs. 1 and 2). The differences were significant in the whole of the period investigated (Tab. 3). The chlorophyll *b* content in current-year needles varied from 0.23 to 0.42 and from 0.30 to 0. 60 mg g^{-1} FW in Našice and Osijek, respectively. The variations in previous-year needles were from 0.27 to 0.53 mg g^{-1} FW in Našice, and from 0.36 to 0.67 mg g^{-1} FW in Osijek (Tab 3.) A further lowering of chlorophyll *b* content could be observed in the period from January to June 2001 in current-year needles sampled from both locations. The same dynamic is present in previous-year needles (Tab. 3).

The total chlorophyll content was also lower in current-year and previous-year needles from Našice than in those from Osijek (Figs. 1 and 2). The month values of total chlorophyll content are given in Tab. 4. Differences in average values of total chlorophyll were significant for the whole investigated period (Tab. 4), as was established with respect to chlorophyll *b*.

Tab. 2. The month values of chlorophyll *a* content (mg g^{-1} FW; ± SD) in current-year and previous-year needles of Norway spruce (*Picea abies* L. Karst.) trees grown in Našice (SAMPLE 1) and Osijek (SAMPLE 2); P(t) – percent of similarity; NS – not significant difference.

MONTH	CURRENT-YEAR NEEDLES			PREVIOUS-YEAR NEEDLES		
	SAMPLE 1	SAMPLE 2	P(t)	SAMPLE 1	SAMPLE 2	P(t)
07. '00.	0.64 ± 0.13	0.68 ± 0.22	NS	0.77 ± 0.18	0.93 ± 0.28	<1%
08. '00.	0.60 ± 0.09	0.71 ± 0.17		0.65 ± 0.13	0.97 ± 0.27	
09. '00.	0.59 ± 0.08	0.75 ± 0.10		0.77 ± 0.19	0.95 ± 0.31	
10. '00.	0.81 ± 0.14	1.00 ± 0.07		0.84 ± 0.10	1.16 ± 0.17	
11. '00.	0.79 ± 0.16	0.89 ± 0.15		0.90 ± 0.15	1.08 ± 0.11	
12. '00.	0.91 ± 0.25	1.11 ± 0.29		1.00 ± 0.28	1.20 ± 0.22	
01. '01.	0.71 ± 0.18	0.91 ± 0.21	<0.1%	0.81 ± 0.16	0.97 ± 0.17	<1%
02. '01.	0.68 ± 0.18	0.87 ± 0.14		0.86 ± 0.28	1.01 ± 0.30	
03. '01.	0.83 ± 0.23	0.91 ± 0.14		0.99 ± 0.16	1.15 ± 0.16	
04. '01.	0.75 ± 0.23	0.95 ± 0.06		0.98 ± 0.24	1.06 ± 0.18	
05. '01.	0.82 ± 0.21	1.06 ± 0.27		0.91 ± 0.23	1.06 ± 0.14	
06. '01.	0.78 ± 0.27	1.00 ± 0.18		0.84 ± 0.23	1.08 ± 0.25	

Tab. 3. The month values of chlorophyll *b* content (mg g^{-1} FW; ± SD) in current-year and previous-year needles of Norway spruce (*Picea abies* L. Karst.) trees grown in Našice (SAMPLE 1) and Osijek (SAMPLE 2); P(t) – percent of similarity; NS – not significant difference.

MONTH	CURRENT-YEAR NEEDLES			PREVIOUS-YEAR NEEDLES		
	SAMPLE 1	SAMPLE 2	P(t)	SAMPLE 1	SAMPLE 2	P(t)
07. '00.	0.42 ± 0.10	0.39 ± 0.10	<1%	0.53 ± 0.14	0.57 ± 0.23	<2%
08. '00.	0.34 ± 0.04	0.44 ± 0.14		0.43 ± 0.10	0.60 ± 0.19	
09. '00.	0.37 ± 0.07	0.47 ± 0.09		0.46 ± 0.13	0.59 ± 0.07	
10. '00.	0.41 ± 0.07	0.60 ± 0.12		0.45 ± 0.03	0.67 ± 0.08	
11. '00.	0.35 ± 0.05	0.51 ± 0.13		0.40 ± 0.08	0.60 ± 0.18	
12. '00.	0.40 ± 0.16	0.51 ± 0.09		0.52 ± 0.11	0.44 ± 0.09	
01. '01.	0.34 ± 0.07	0.45 ± 0.08	<1%	0.40 ± 0.12	0.54 ± 0.11	<2%
02. '01.	0.23 ± 0.05	0.36 ± 0.06		0.30 ± 0.12	0.45 ± 0.09	
03. '01.	0.28 ± 0.08	0.32 ± 0.06		0.36 ± 0.05	0.43 ± 0.06	
04. '01.	0.24 ± 0.06	0.30 ± 0.03		0.34 ± 0.09	0.36 ± 0.09	
05. '01.	0.25 ± 0.06	0.38 ± 0.10		0.29 ± 0.06	0.40 ± 0.05	
06. '01.	0.23 ± 0.08	0.35 ± 0.06		0.27 ± 0.06	0.40 ± 0.09	

MANDRE and TUULMETS (1997) reported a decrease of chlorophyll content in Norway spruce needles caused by cement dust. They stated that the chlorophyll *a* : chlorophyll *b* ratio was lowered due to the more pronounced decrease of chlorophyll *a* content in previous-year needles, while the chlorophyll *b* was less affected. Our results (Tabs. 2 and 3) as well as our previous investigations (CESAR and LEPEDUŠ 2001) showed that the chlorophyll *b* was more affected in young current-year needles while a significant decrease in chlorophyll *a* was noticed after a prolonged period of exposure to dust. The results we obtained

Tab. 4. The month values of total chlorophyll content (mg g^{-1} FW; ± SD) in current-year and previous-year needles of Norway spruce (*Picea abies* L. Karst.) trees grown in Našice (SAMPLE 1) and Osijek (SAMPLE 2); P(t) – percent of similarity.

MONTH	CURRENT-YEAR NEEDLES			PREVIOUS-YEAR NEEDLES		
	SAMPLE 1	SAMPLE 2	P(t)	SAMPLE 1	SAMPLE 2	P(t)
07. '00.	1.06 ± 0.23	1.14 ± 0.29	<5%	1.30 ± 0.32	1.50 ± 0.51	<0.1%
08. '00.	0.94 ± 0.12	1.15 ± 0.31		1.08 ± 0.23	1.57 ± 0.44	
09. '00.	0.95 ± 0.13	1.22 ± 0.19		1.22 ± 0.30	1.54 ± 0.37	
10. '00.	1.20 ± 0.18	1.60 ± 0.13		1.29 ± 0.12	1.81 ± 0.11	
11. '00.	1.13 ± 0.21	1.39 ± 0.11		1.30 ± 0.22	1.67 ± 0.18	
12. '00.	1.32 ± 0.26	1.62 ± 0.36		1.51 ± 0.37	1.64 ± 0.30	
01. '01.	1.05 ± 0.25	1.36 ± 0.28	<0.1%	1.21 ± 0.27	1.51 ± 0.29	<0.1%
02. '01.	0.91 ± 0.23	1.23 ± 0.20		1.16 ± 0.39	1.46 ± 0.27	
03. '01.	1.10 ± 0.30	1.24 ± 0.20		1.36 ± 0.20	1.57 ± 0.22	
04. '01.	0.99 ± 0.27	1.25 ± 0.09		1.31 ± 0.34	1.41 ± 0.27	
05. '01.	1.07 ± 0.28	1.45 ± 0.36		1.20 ± 0.29	1.46 ± 0.19	
06. '01.	1.01 ± 0.35	1.36 ± 0.23		1.11 ± 0.29	1.48 ± 0.34	

for previous-year needles corresponded with those reported by MANDRE and TUULMETS (1997). There is some evidence that chlorophyll *a* could be compensated from the chlorophyll *b* pool in response to environmental stresses since the conversion seems to be bi-directional (VON WETTSTEIN et al. 1995, SUZUKI et al. 1997, TANAKA et al. 1998). The alkalisation of the microenvironment by cement dust caused cell destruction in the needles of spruce trees (CZAJA 1962). In spite of this kind of destructive effect of alkaline dust pollution on the cells, we believe that the most probable reason for the lower chlorophyll content seems to be suppressed biosynthesis rather than degradation. High Ca content in the soil produced by the cement factory was correlated with decreased Mg, Mn and Fe contents in needles (MANDRE and TUULMETS 1997) as well as with a decreased growth rate of spruce trees (MANDRE et al. 1998), which clearly indicates that the biosynthetic processes were slowed down. The data about the $CaCO_3$ presented here (Tab. 1) showed a significantly higher percentage of $CaCO_3$ in soil near the cement plant in Našice. Nevertheless, the soluble protein content we reported (CESAR and LEPEDUŠ 2001) was not significantly lower in dust-affected needles.

A higher content of total carotenoids was measured in current-year as well in previous-year needles from Osijek than in those from Našice (Figs. 1 and 2). This significance was manifested for the period from January to June 2001 in current-year needles, and for the period from July to December 2000 in previous-year needles (Tab. 5). At the beginning of the investigation (July 2000) the current-year needles already had the carotenoid content developed at the level characteristic of mature needles. This level was kept for the next six months (Tab. 5), which was of great importance because needle development lasts until August or September (depending on the climate conditions and the genotype), with respect to chlorophyll *a* content (LEPEDUŠ 2001). It is well known that carotenoids protect photosynthetic membranes from destruction at the high light intensity (SIEFERMANN-HARMS 1987). The carotenoids that are involved in the xanthophylls cycle have a great im-

Tab. 5. The month values of total carotenoids content (mg g^{-1} FW; ± SD) in current-year and previous-year needles of Norway spruce (*Picea abies* L. Karst.) trees grown in Našice (SAMPLE 1) and Osijek (SAMPLE 2); P(t) – percent of similarity; NS – not significant difference.

MONTH	CURRENT-YEAR NEEDLES			PREVIOUS-YEAR NEEDLES		
	SAMPLE 1	SAMPLE 2	P(t)	SAMPLE 1	SAMPLE 2	P(t)
07. '00.	0.13 ± 0.03	0.15 ± 0.04	NS	0.17 ± 0.04	0.21 ± 0.06	<5%
08. '00.	0.16 ± 0.02	0.18 ± 0.04		0.20 ± 0.06	0.23 ± 0.04	
09. '00.	0.17 ± 0.03	0.18 ± 0.02		0.20 ± 0.03	0.22 ± 0.02	
10. '00.	0.18 ± 0.02	0.18 ± 0.03		0.20 ± 0.03	0.24 ± 0.04	
11. '00.	0.19 ± 0.01	0.18 ± 0.02		0.25 ± 0.04	0.27 ± 0.02	
12. '00.	0.24 ± 0.03	0.27 ± 0.05		0.26 ± 0.02	0.30 ± 0.06	
01. '01.	0.22 ± 0.06	0.24 ± 0.05	<2%	0.23 ± 0.03	0.27 ± 0.03	NS
02. '01.	0.24 ± 0.07	0.25 ± 0.04		0.29 ± 0.08	0.28 ± 0.04	
03. '01.	0.22 ± 0.06	0.22 ± 0.05		0.26 ± 0.03	0.29 ± 0.04	
04. '01.	0.19 ± 0.05	0.26 ± 0.00		0.24 ± 0.06	0.28 ± 0.04	
05. '01.	0.19 ± 0.04	0.24 ± 0.06		0.22 ± 0.05	0.25 ± 0.04	
06. '01.	0.19 ± 0.04	0.23 ± 0.04		0.20 ± 0.06	0.25 ± 0.07	

portance in that process (DEMMIG-ADAMS and ADAMS 1996). ADAMS and DEMMIG-ADAMS (1994) also reported higher levels of lutein and carotenoids of the xanthophyll cycle in the needles of three conifer species during the winter, which was connected with the protection of photosynthetic apparatus from photoinhibition resulting from low temperatures. In winter the amount of carotenoids rose because of the water loss caused by low temperatures and further on as a preparation for response to high light conditions, which was detected in control samples. Because of that, the mean value changed from 0.19 mg g^{-1} FW in the period from July to December in current-year needles to 0.24 from January to June followed by 0.25 mg g^{-1} FW in July to December and 0.27 from January to June in previous-year needles where the significant difference was only between the July-December and the January-June period in current-year needles. The changes in the amount of carotenoids in cement-dust-affected needles showed a different relation. The amount of carotenoids rose during the whole period of investigation, from 0.18 mg g^{-1} FW as mean value in the July-December period to 0.21 in January to June in current-year needles and from 0.21 in July-December to 0.24 in January to June in previous-year needles, where no change appeared to be significant.

Based on the results presented in this paper it could be stated in summary that the decline of chlorophyll *a* content in current-year needles exposed to cement dust began after a longer period of time than the decline in chlorophyll *b*. The decline of all measured pigments in previous-year needles showed no dramatic progress during the second year of exposure. At the end of this period nearly the same level of carotenoids was present in affected and unaffected needles. This could mean that spruce needles possess a biosynthetic capacity sufficient for acclimatisation to the conditions of an alkaline microenvironment.

Acknowledgements

We are thankful to Prof. Nikola Ljubešić, for a critical reading the manuscript and useful advice. We also thank to Vera Tikas and Siniša Ozimec MSc for providing us data about soil characteristics.

References

ADAMS, W. W., DEMMIG-ADAMS, B., 1994: Carotenoid composition and down regulation of photosystem II in three conifer species during the winter. Physiol. Plant. 92, 451–458.

BAČIĆ, T., LYNCH, A. H., CUTLER, D., 1999: Reactions to cement factory dust contamination by *Pinus halepensis* needles. Environ. Exp. Bot. 41, 155–166.

BATIČ, F., GRILL, D., KALAN, P., RIBARIČ-LASNIK, C., 1995: Impact of emission gases from the thermal power plant in Šoštanj on the biochemical structure of Norway spruce needles (*Picea abies* L. Karst). Acta Pharm. 45, 191–197.

BOHNE, H., 1963: Schadlichkeit von Staub aus Zementwerken fur Waldbestande. Allg. Forst. 18, 107–111.

BORKA, G., 1980: The effect of cement dust pollution on growth and metabolism of *Helianthus annus*. Environ. Poll. (Ser. A) 22, 75–79.

CESAR, V., LEPEDUŠ, H., 2001: Peroxidase activity, soluble proteins and chlorophyll content in spruce (*Picea abies* L. Karst.) needles affected by cement dust. Acta. Bot. Croat. 60, 227–235.

CZAJA, A. T., 1961: Die Wirkung von verstaubtem Kalk und Zement auf Pflanzen. Qual. Plant. et Mat. Veg. 8, 184–212.

CZAJA, A.T., 1962: Über das Problem der Zementstaubwirkung auf Pflanzen. Staub 22, 228–232.

DARLEY, E.F., 1966: Studies on the effect of cement-kiln dust on vegetation. J. Air. Poll. Contr. Assoc. 16, 145–150.

DEMMIG-ADAMS, B., ADAMS, W., 1996: The role of xanthophylls cycle carotenoids in the protection of photosynthesis. Trends Plant Sci. 1, 21–26.

FARMER, A. M., 1993: The effects of dust on vegetation – a review. Environ. Poll. 79, 63–75.

JALKANEN, L., MÄKINEN, A., HÄSÄNEN, E., JUHANOJA, J., 2000: The effect of large anthropogenic particulate emissions on atmospheric aerosols, deposition and bioindicators in the eastern Gulf of Finland region. Sci. Tot. Environ. 262, 123–136.

LAL, B., AMBASHT, R. S., 1982: Impact of cement dust on the mineral and energy concentration of *Pisidium guayava*. Environ. Poll. (Ser. A) 29, 241–247.

LEPEDUŠ, H, 2001: Fotosintetski pigmenti i ultrastruktura plastida u vegetativnim pupovima i iglicama smreka (Photosynthetic pigments and plastid ultrastructure in vegetative buds and needles of spruce). M. Sc. Thesis, University of Zagreb.

LICHTENTHALER, H. K., 1987: Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol. 148, 350–382.

MANDRE, M., OTS, K., RAUK, J., TUULMETS, L., 1998: Impact of air pollution emitted from the cement industry on forest bioproduction. Oil Shale 15, 353–364.

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

MIKKELSEN, T. N., DODELL, B., LÜTZ, C., 1995: Changes in pigment concentration and composition in Norway spruce induced by long–term exposure to low levels of ozone. Environ. Pollut. 87, 197–205.

OBLISAMI, G., PATHMANABHAN, G., PADMANABHAN, C., 1978: Effects of particulate pollutants from cement-kilns on cotton plants. Ind. J. Air Pollut. Contr. 1, 91–94.

PAVLIĆ, I, 1977: Statistička teorija i primjena. Tehnička knjiga, Zagreb.

PIERCE, G. J., 1909: The possible effect of cement dust on plants. Science 30, 652–654.

SCHULZ, H., WEIDNER, M., BAUR, M., LAUCHERT, U., SCHMITT, V., SCHROER, B., WILD, A., 1996: Recognition of air pollution stress on Norway spruce (*Picea abies* L.) on the basis of multivariate analysis of biochemical parameters: a model field study. Angew. Bot. 70, 19–27.

SHUKLA, J., PANDEY, V., SINGH, S. N., YUNUS, M., SINGH, N., AHMAD, K. J., 1990: Effects of cement dust on the growth and yield of *Brasica campestris* L. Environ. Poll. 66, 81–88.

SEIFERMANN-HARMS, D., 1987: The light harvesting and protecting functions of carotenoids in photosynthetic membranes. Physiol. Plant. 69, 561–568.

SINGH, S. N., RAO, D. N., 1968: Effect of cement dust pollution on soil properties and on wheat plants. Ind. J. Environ. Health 20, 258–267.

SINGH, S. N., RAO, D. N., 1981: Certain responses of wheat plants to cement dust pollution. Environ. Poll. (Ser. A) 24, 75–81.

SUZUKI, J. Y., BOLLIVAR, D. W., BAUER, C. E., 1997: Genetic analysis of chlorophyll biosynthesis. Annu. Rev. Genet. 31, 61–89.

TANAKA, A., ITO, H., TANAKA, R., TANAKA, N. K., YOSHIDA K., OKADA, K., 1998: Chlorophyll *a* oxygenase (CAO) is involved in chlorophyll *b* formation from chlorophyll *a*. Proc. Natl. Acad. Sci. USA 95, 12719–12723.

TAUSZ, M., DE KOK, L. J., STULEN, I., GRILL, D., 1996: Physiological responses of Norway spruce trees to elevated CO_2 and SO_2. J. Plant Physiol. 148, 362–367.

TAYLOR, H. J., ASHMORE, M. R., BELL, J. N. B., 1986: Air pollution injury to vegetation. Imperial College Centre for Environmental Technology, London.

WETTSTEIN, D. VON, GOUGH, S., KANNAGARA, C. G., 1995: Chlorophyll biosynthesis. Plant Cell 7, 1039–1057.

WILD, A., SCHMITT, V., 1995: Diagnosis of damage to Norway spruce (*Picea abies*) through biochemical criteria. Physiol. Plant. 93, 375–382.