

Chlorophyll content in pine (*Pinus silvestris* L.) needles exposed to flue dust from lead and zinc works

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(Received: January 27, 1976)

Abstract

Chlorophyll a and b, sulphur, zinc and lead contents were determined in annual and biennial needles of Scotch pine (*Pinus silvestris* L.) exposed to flue dust-polluted air. Intoxication indexes were calculated on the basis of the obtained results.

INTRODUCTION

One of the manifestations of the noxious influence of industrial air pollution on plants is leaf chlorosis. Therefore Müller (1957) suggested analysis of plant pigments as a method of early detection of damage caused to the vegetation by emission containing mainly sulphur dioxide.

A number of authors demonstrated by this method a relation between sulphur dioxide concentration in the air and chlorophyll content in plants, particularly lichens (Rao, Le Blanc, 1966; Skye, 1968; Syratt, Wanstall, 1968; Kuziel, 1974). The degree of injury to the plants was evaluated on the basis of the chlorophyll a to chlorophyll b ratio.

Within the scope of more extensive investigations on the influence of industrial air pollution on Scotch pine, analysis of chlorophyll a and b content in one- and two-year pine needles was performed. The aim of the study was a trial of application of chlorophyll analysis for establishing the severity of the noxious effect of flue dust containing sulphur dioxide and heavy metal oxides. The results of these trials are reported.

MATERIAL AND METHODS

As material served annual and biennial pine needles collected from pine (*Pinus silvestris* L.) thickets (10—15 years old) and older tress

(60–80 years old) growing close to one another on 5 experimental plots on one transect, but at various distances from the lead and zinc works (Fig. 1).

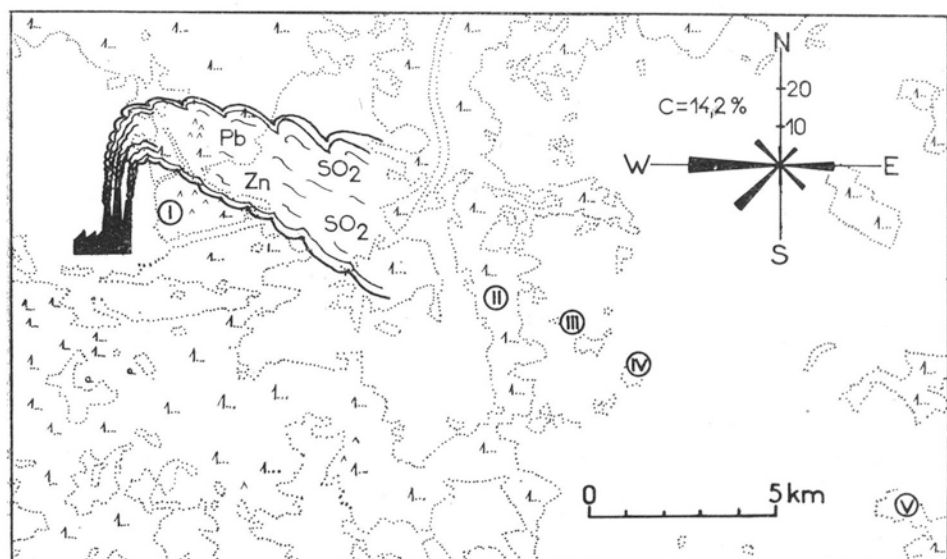


Fig. 1. Distribution of experimental plots I–V on the investigated area. The wind rose is plotted on the basis of data for the period 1961–1970 from the meteorological station in Biały Kościół

The Scotch pine needles were collected in September. One branch was cut from each of ten randomly chosen trees always on the side facing the metal works. After separating the needles according to their age and after their thorough mixing in each group samples were taken for analysis.

Determination of chlorophyll was done on fresh material according to the method described by Haspelová-Horvatičová (1963). The results were converted to dry weight of the needles, after moisture determination by the method of distillation from 4-chloroethane according to *Farmakopea Polska* IV, (1965).

Moreover, sulphur and lead content were determined in pine needles from the same batch of material by the method developed in the State Institute of Hygiene for sanitary-epidemiological stations. Zinc was determined by the dithizone method (Dutkiewicz et al., 1962).

From the results the indexes denoted with symbols I_S , I_{Zn} and I_{Pb} were calculated. These indexes express the amount of chlorophyll $a + b$ falling to 1 mg sulphur (I_S), zinc (I_{Zn}) and lead (I_{Pb}) contained in the pine needles.

On each plot sulphur dioxide concentration in the air and the amount of flue dust falling were measured over a one year period by the methods used by sanitary-epidemiological stations for evaluation of the degree of atmospheric air pollution (B u j a k, 1972). In the flue dust collected zinc and lead contents were determined in the same way as in the plant material.

RESULTS

1. Degree of atmospheric air pollution on the experimental plots

The results shown in Table 1 indicate that the highest sulphur dioxide concentration in the air is found on plot I, amounting to 0.099 mg/m^3 next ranked plot V with a SO_2 concentration of 0.096 mg/m^3 on the average for the whole year. The amount of flue dustfall varied from

Table 1

Sulphur dioxide concentration (mg/m^3 of air), total flue dust zinc and lead fall per year t/km^2
Mean annual values for particular plots

Experimental plots	I	II	III	IV	V
SO_2 concentration	0.099	0.069	0.054	0.071	0.096
Total flue dust fall	118.210	90.840	75.480	52.680	55.100
„ zinc „	1.596	0.384	0.300	0.192	0.216
„ lead „	not analysed	0.240	0.132	0.144	0.144

52.68 to $118.21 \text{ t/km}^2/\text{year}$ and distinctly diminished with the distance of the measuring points from the stacks of the zinc and lead works. The greatest amount of zinc ($1.596 \text{ t/km}^2/\text{year}$) was found in the dust collected on plot I, whereas on the remaining ones it was during one year $0.192 - 0.384 \text{ t/km}^2$. Lead was not determined in the samples from plot I, on the remaining plots the amount of Pb falling during one year ranged from 0.132 to 0.240 t/km^2 .

2. Chlorophyll content in one- and two-year-old pine needles

Total chlorophyll content in annual needles collected from pine thickets varied between 2.27 (plot II) and 5.35 mg/g dry weight (plot V). The chlorophyll *a* to *b* ratio was within the limits 1.52 to 1.72 (Table 2).

In two-year needles the amount of chlorophyll *a* + *b* was $4.97 - 7.10 \text{ mg/g}$ dry wt. (plot III) and the ratio of the two chlorophyll kinds was highest (1.79) in needle samples from plot V.

As regards one-year needles from older stands the lowest total chlo-

rophyll value (3.08 mg/g dry wt.) was found on plot II and the highest (6.86 mg/g dry wt.) on plot V (Table 2). The chlorophyll *a:b* ratio was 1.57 to 2.13.

In two-year needles from the same stands the amount of chlorophyll ranged from 4.31 to 8.42 mg/g dry wt. (plot V) and the ratio of the two chlorophylls was 1.50 to 1.96.

Table 2

Chlorophyll *a* and *b* content in one and two-year pine (*Pinus silvestris* L.) needles

Experimental plots	I	II	III	IV	V
A. Pine thicket					
a) one-year needles					
chlorophyll <i>a</i>	2.70	1.37	2.75	2.71	3.29
chlorophyll <i>b</i>	1.58	0.90	1.77	1.58	2.06
<i>a:b</i> ratio	1.71	1.52	1.55	1.72	1.60
b) two-year needles					
chlorophyll <i>a</i>	4.10	3.98	4.11	2.96	3.29
chlorophyll <i>b</i>	2.44	2.50	2.99	2.01	1.84
<i>a:b</i> ratio	1.68	1.59	1.37	1.47	1.79
B. Older stand					
a) one-year needles					
chlorophyll <i>a</i>	3.25	1.91	2.37	2.43	4.51
chlorophyll <i>b</i>	1.91	1.17	1.11	1.55	2.35
<i>a:b</i> ratio	1.70	1.63	2.13	1.57	1.92
b) two-year needles					
chlorophyll <i>a</i>	3.97	2.76	4.00	2.80	5.14
chlorophyll <i>b</i>	2.03	1.65	2.66	1.51	3.28
<i>a:b</i> ratio	1.96	1.67	1.50	1.85	1.57

3. Sulphur, zinc and lead contents in one- and two-year-old pine needles

Sulphur content in annual needles from pine thickets was 0.52–1.18 mg/g dry wt. and was highest on plot I (Table 3). Two-year needles from the same plot also showed the highest sulphur content (1.09 mg/g dry wt.), whereas in the remaining samples it ranged from 0.83 to 0.96 mg/g dry wt.

The highest sulphur content (1.39 and 1.78 mg/g dry wt.) was also noted in one- and two-year needles collected from older stands on plot I situated nearest to the lead and zinc works, and the lowest was found on plot V (0.74 and 1.09 mg/g dry wt.).

The amount of zinc in samples from pine thickets ranged from 0.09 to 0.32 mg/g dry wt. in annual needles and from 0.18 to 0.70 mg/g dry wt. in two-year needles (Table 3). The highest quantities were found in samples from plot I and lowest from plot V. Similar relations occurred in samples from older stands. In one-year needles from plot I Zn content was 0.42 mg/g dry wt. and in two-year ones it was 1.46 mg/g dry wt.

Table 3

Sulphur, zinc and lead contents in one- and two-year needles of *Pinus silvestris* L.

Experimental plot	A. Pine thicket		B. Older stand	
	Needles			
	one-year	two-year	one-year	two-year
1. Sulphur content mg/g dry wt.				
I	1.18	1.09	1.39	1.78
II	0.52	0.96	1.08	1.43
III	0.68	0.83	1.03	1.28
IV	0.81	0.94	1.19	1.28
V	0.69	0.84	0.74	1.09
2. Zinc content mg/g dry wt.				
I	0.32	0.70	0.42	1.46
II	0.14	0.25	0.23	0.35
III	0.27	0.19	0.13	0.23
IV	0.16	0.31	0.23	0.31
V	0.09	0.18	0.10	0.25
3. Lead content mg/g dry wt.				
I	0.0093	0.1383	0.0027	0.1127
II	0.0033	0.0080	0.0040	0.0041
III	0.0081	0.0050	0.0023	0.0050
IV	0.0034	0.0093	0.0032	0.0117
V	0.0035	0.0027	0.0054	0.0081

In one year needles from pine thickets lead content varied within the limits of 0.0033 to 0.0093 mg/g dry wt. (plot I) and 0.0027 to 0.1383 mg/g dry wt. in two-year needles (Table 3). In older stands the highest amounts (0.0027 and 0.1127 mg/g dry wt.) were revealed in samples from plot I.

4. Chlorophyll *a* + *b* amount falling to 1 mg sulphur, zinc and lead.

The value of index I_s for one- and two-year needles lies between 3.63 and 8.55 for samples from pine thickets and 2.85 and 9.27 for those from older stands. The I_{Zn} index varies from 4.11 to 68.60 and was lowest for two year needles from plot I (Table 4). On account of the relatively

Table 4

Chlorophyll *a+b* content per 1 mg of sulphur (index I_s), zinc (index I_{zn}) and lead (index I_{pb}) in one- and two-year *Pinus silvestris* L. needles

Experimental plots	A. Pine thicket		B. Older pine stand	
	Needles			
	one-year	two-year	one-year	two-year
	1. Index I_s			
I	3.63	6.00	3.71	3.37
II	4.36	6.75	2.85	3.08
III	6.65	8.55	3.38	5.20
IV	5.30	5.29	3.34	3.37
V	7.75	6.11	9.27	7.72
	2. Index I_{zn}			
I	13.37	9.34	12.29	4.11
II	16.21	25.92	13.39	12.60
III	16.74	37.37	26.77	28.96
IV	26.81	16.03	17.30	13.90
V	59.44	28.50	68.60	33.68
	3. Index I_{pb}			
I	460.21	47.29	1911.11	53.24
II	687.85	810.00	770.00	1075.61
III	558.02	1420.00	1513.00	1332.00
IV	1261.76	534.41	1243.75	368.36
V	1528.57	1900.00	1270.37	1039.51

low lead content in the pine needles analysed the I_{pb} index was very high reaching 1911. It was relatively low only in two-year needles from plot I (47.29 and 53.24).

RESULTS AND DISCUSSION

The lead and zinc works emit sulphur dioxide in the amount of about 4 thousand tons per year and flue dust containing on the average 44.3 per cent of zinc and 13.7 per cent of lead. The main sources of air pollution are the technological processes and sulphuric acid production.

Winds from the west predominate on the investigated area over east ones with but a small contribution of north and south winds. Hence the region situated on the axis of the prevailing winds is most exposed to the noxious influence of flue dust.

The results obtained in measurements of the degree of air pollution indicate that flue dustfall is only high on plots I and II and then it decreases with the distance from the emission source. On the other hand, sulphur dioxide content in the air on the investigated transect shows distinct fluctuations. The highest sulphur dioxide concentrations in the

particular months of the year refer to plots I and V, whereas the minimum was noted on plot III. Such a distribution of concentrations is due to a large extent to the ground relief.

The chlorophyll content in the analysed pine needles shows certain differences according to the age of the needles, of the tree and the situation in relation to the lead and zinc works.

In general the amount of chlorophyll $a + b$ is higher in two-year needles than in the one-year ones, both in young thickets and in older stands. An increased amount of the green pigment is also observed in the needles collected over two years from older stands on plot V, the most distant one from the industrial stacks.

The ratio of chlorophyll a to b in Scotch pine needles is according to Langlet (1942) during the vegetation season 2.07 to 2.66. Some authors (after Żelawski, 1967) consider these values to be exaggerated and accept rather the data of Gerhold (1959). According to this author the chlorophyll $a:b$ ratio in pine varies between 1.6 and 1.7 like in other coniferous trees.

In the needles presently analysed the $a:b$ ratio was within the limits 1.37–2.13, thus the range was wider than that given by Gerhold (l.c.).

It is generally believed that, as a consequence of exposure to sulphur dioxide, chlorophyll a undergoes degradation quicker since it is more susceptible to reactions within the leaf. Hence the $a:b$ ratio varies within a narrow range and its value is assumed as criterion for evaluation of the degree of damage to the plants (Müller, 1957; Williams et al., 1971).

Pine needle samples for determination of chlorophyll content were taken simultaneously from all sites. Nevertheless, there was no distinct regularity in the results as regards the $a:b$ ratio, which could serve for determination of the degree of damage to the pines, due to the noxious emission from the lead and zinc works.

The lack of relation between the chlorophyll $a:b$ ratio and the degree of poisoning of the plants is also stressed by Haspelová-Horvátovičová and Pelikánová (1967) who investigated the influence of sodium fluoride on pigment content in apple tree leaves.

In both cases, however, it was not the effect of sulphur dioxide but of fluorine compounds that was studied as well as contamination with fumes carrying beside SO_2 heavy metal oxides. It is probable that these components affect the amount and composition of chlorophyll, and elucidation of the mechanism of the joint action of sulphur and zinc requires more extensive studies. These two elements, namely, under normal conditions of growth have a favourable influence on a number of biochemical processes and their noxious effect appears only when they are present in excess.

The detailed studies of Dässler (1972) demonstrated that chlorophyll *b* is not more resistant to sulphur dioxide than is chlorophyll *a*. Hence the relation between these two chlorophylls does not undergo major changes, with the exception of seasonal oscillations, thus it cannot be the only criterion for evaluation of the degree of injury to the plants.

Another measure for determining the noxious influence of industrial air pollution on plants is the calculation of indexes expressing the amount of chlorophyll per 1 mg of poisonous substance in the leaves (Jamrich, 1968).

In the analysed pine needles relatively low values were obtained of the I_s index for samples collected from older tress on plots I–IV, and of index I_{Zn} for samples from plots I and II. On the other hand, index I_{Pb} is lowest only for plot I and two-year needles in which a relatively high accumulation of lead is observed. In the remaining samples the amount of lead is low and index I_{Pb} reaches too high values to be considered as indicative of the noxious influence of lead on chlorophyll content.

It results from the studies performed that:

1. The noxious effect of sulphur dioxide and zinc on Scotch pine is more pronounced in older stands than in thickets. Therefore evaluation of the danger to forest areas due to the above named components of the zinc and lead works flue gas should be determined on the basis of results obtained on older tree stands.

2. The indexes expressing the ratios of chlorophyll *a* + *b* content to sulphur, zinc and lead content in pine needles may serve as criteria for determination of the degree of noxious influence of industrial air pollution on Scotch pine.

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Zawartość chlorofilu w szpilkach sosny zwyczajnej (*Pinus silvestris* L.) znajdującej się pod wpływem emisji huty ołowiu i cynku

Streszczenie

Badania przeprowadzono na pięciu powierzchniach położonych na jednym transekcie przebiegającym od huty ołowiu i cynku „Bolesław” do Ojcowskiego Parku Narodowego (Ryc. 1). Powierzchnie te znajdują się pod wpływem różnego stopnia zanieczyszczenia powietrza przez dwutlenek siarki oraz pyły hutnicze (Tab. 1).

W zebranych jedno- i dwuletnich szpilkach sosny zwyczajnej (*Pinus silvestris* L.) określono zawartość chlorofilu *a* i *b* (Tab. 2) oraz siarki, cynku i ołowiu (Tab. 3). Uzyskane dane wskazują, że na powierzchni I, znajdującej się najbliżej kominów huty, zawartość S, Zn i Pb w szpilkach sosny zwyczajnej jest znacznie wyższa w porównaniu z powierzchnią V, najbardziej oddaloną od źródła emisji.

W wyniku zastosowania wskaźników wyrażających ilość chlorofilu *a+b* przypadającą na 1 mg S, Zn i Pb zawartego w szpilkach (Tab. 4) wykazano, że największy zasięg szkodliwego oddziaływania na starsze drzewostany sosnowe ma dwutlenek siarki obejmujący powierzchnie I—IV, podczas gdy wpływ cynku ogranicza się do powierzchni I i II, a ołowiu tylko do powierzchni I.