Chlorophyll and pheophytin content in needles of different age of trees growing under conditions of chronic industrial pollution*

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Abstract

Chlorophyll and pheophytin content was determined by means of Vernon method (Vernon, 1960) in needles of different age. Needles of *Pinus strobus* L., *Pinus nigra* Arnd., *Pinus silvestris* L., and *Pseudotsuga menziesii* Franco growing under conditions of chronic industrial pollution as well as under unpolluted conditions were examined. High pheophytin content was found in needles of trees growing under the conditions of chronic pollution. The youngest needles always showed the highest pheophytin content as related to chlorophyll content.

The examined species showed different degree of chlorophyll decomposition under the influence of polluted environment. Trees growing under control environment showed small and similar amounts of pheophytin in needles of different age.

Pheophytin content does not seem to be a convenient indicator to test the effect of pollution before visual symptoms occur, since the method is very labourious and plant material very variable.

INTRODUCTION

The aging of assimilatory organs is accompanied by changes in the quality and quantity of assimilatory pigments (review — Szaniawski, 1976). Pheophytins are the first products of chlorophyll decomposition under the conditions of air polluted with gases like SO_2 , NO_2 , chlorine, fluorine. The effect of air pollutants on the assimilatory pigments and their further decomposition is not known very well. There is also a lack of data as to the changes of pheophytin content in needles of different age. However, the results of some investigations showed the

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possibility of using chlorophyll content and the amount of the products of chlorophyll decomposition as an indicator of assimilatory organs ageing as well as of the degree of environment pollution. Literature data concerning this problem are fragmentary and often contradictory (Dörries 1932; Costes i Coïc 1957; Müller 1957; Weinstein 1961; Jamrich 1968; Dässler 1970; Arndt 1971; Ilkun 1971), which is connected with methodical difficulties in determining the content of pigments and the products of their decomposition as well as with the variability of plant material.

The purpose of this work was to determine the content of chlorophylls and pheophytins in needles of different age of several coniferous species originating from a region subject to chronic industrial pollution.

MATERIAL AND METHODS

Samples of plant material were taken at the end of the growing season (September 1975) — from 12—14 year old trees at Rogów Arboretum and at Panewnik Forest District. (near Katowice). The experimental area at Panewnik was in the zone of the heavy industrial stress.

The following species were examined: Austrian pine (*Pinus nigra* Arnd.), white pine (*Pinus strobus* L.), Scots pine (*Pinus silvestris* L.), Douglas fir (*Pseudotsuga menziesii* Britt.). Two shoots were taken from the base part of the crown from each of the five trees chosen for examination.

According to the data of sanitary and epidemiology service station in Katowice the industrial pollution at Panewnik consists of: SO₂, NO₂, H₂S, sulphates, sulphides, nitrates, nitrites, chlorine and fluorine compounds, aluminium oxides, coal dust, ammonia, and heavy metal dust (Zn, Pb, As.).

The method by Vernon (1960) seemed to be most useful for comparative determinations of a large number of samples. Needle samples of 2 g fresh weight were homogenized in 80% acetone by 3-5 minutes in the dark and under low temperature conditions. The extract was filtered through a fritted glass filter to 250 ml flasks. A portion of 97 ml of this extract plus 3 ml of 80% acetone was used for chlorophyll content analysis, while the other portion of 97 ml of the extract plus 3 ml of oxalic acid saturated with acetone solution was used for inversion to pheophytins. Measurements of pigments content were carried out, with a VARIAN 635 spectrophotometer, after 3 hours of incubation in the dark and under room temperature conditions. Pheophytin content was determined from the difference between the converted and chlorophyll samples. Absorption of the chlorophyll samples was read at wavelengths: 649, 665 nm; and of the converted

samples at wavelengths: 536, 655, 666 nm. Pigment content was calculated according to the formulas by Vernon (1960).

Vernon's work was carried out on spinach, pea, and bean leaves. Our preliminary methodical work showed divergence of results obtained with the use of different formulas reported by Vernon for calculating pheophytin content. Thanks to the kindness of prof. dr D. Frackowiak and mgr A. Skowron, from Institute of Physics Poznań-Technical University, Vernon's absorption coefficients were checked on needles samples. The usefulness of these formulas for analysis of coniferous material was proved.

RESULTS

The results of analysis (Tab. 1-4) indicate the increase of chlorophyll content with needle age of three pine species and Douglas fir. The highest chlorophyll content was found in needles of white pine, lower, in Scots pine and Douglas fir and the lowest in Austrian pine. The same tendency holds for both the trees growing under conditions of chronic industrial pollution and the control ones.

A high pheophytin content was found in needles of trees growing under the conditions of chronic pollution. Pheophytin *b* was found in greater amounts than pheophytin *a*. The youngest needles, always showed the highest content of pheophytins. Pheophytin content decreased considerably with needle age. It decreased by 50—70% in 1-2 year old needles as compared to the current year ones. The highest pheophytin content, as related to chlorophyll content, was found in needles of white pine — 30%, then in Austrian pine — 25%, in Douglas fir — 20%, and the lowest in Scots pine — 7%. Needles of Scots pine showed the lowest content of pheophytins both under the chronic pollution conditions and under the control ones. Pheophytin *a* was not found in needles of Scots pine. In needles of Austrian pine, it was not found in trees growing under control conditions. The other species always contained pheophytin *a* in their needles. Needles of different age from trees growing under control conditions showed similar pheophytin content. Pheophytin content, as related to chlorophyll content, was found to be: 10—8% in white pine, 4% in Austrian pine, 6% in Scots pine, and 11—6% in Douglas fir needles of various age of control trees.

DISCUSSION

Dörries (1932) was the first to use the decreasing content of assimilatory pigments as an early test of the effect of pollution. The results of other authors indicated that changes in chlorophyll content

Table 1

Chlorophyll and pheophytin content in needles of different age of White pine growing under conditions of chronic industrial pollution

Tree species Needle age	Chlorophyll content mg/g fresh matter			Chloro- phyll a/b	Pheophytin content mg/g fresh matter			% of pheophytin as related to chlorophyll content		
	a	b	a+b		a	b	a+b	a	b	a+b
White pine										
Panewnik										
Current year needles	0.99	0.48	1.47	2.06	0.27	0.18	0.45	27.3	37.5	30.6
1-year needles	1.29	0.59	1.88	2.19	0.16	0.15	0.31	12.4	25.5	16.5
Rogów										
Current year needles	1.04	0.45	1.49	2.31	0.03	0.12	0.15	2.9	26.7	10.1
1-year old needles	1.27	0.54	1.81	2.35	0.04	0.11	0.15	3.2	20.4	8.3

Table 2

Chlorophyll and pheophytin content in needles of different age of Austrian pine growing under conditions of chronic industrial pollution

Tree species Needle age	Chlorophyll content mg/g fresh matter			Chloro- phyll a/b	Pheophytin content mg/g fresh matter			% of pheophytin as related to chlorophyll content		
	a	b	a+b		a	b	a+b	a	b	a+b
Austrian pine										
Panewnik										
Current year needles	0.59	0.27	0.86	2.18	0.14	0.08	0.22	23.7	29.6	25.6
1-year old needles	0.89	0.38	1.27	2.34	0.04	0.07	0.11	4.5	18.4	8.7
2-year old needles	0.98	0.44	1.42	2.23	0.01	0.07	0.08	1.0	15.9	5.6
Rogów										
Current year needles	0.86	0.38	1.24	2.26	—	0.05	0.05	—	13.2	4.8
1-year old needles	0.86	0.39	1.25	2.20	—	0.06	0.06	—	15.4	4.8
2-year old needles	0.82	0.40	1.22	2.05	—	0.05	0.05	—	12.5	4.1

Table 3

Chlorophyll and pheophytin content in needles of different age of Scots pine growing under condition of chronic industrial pollution

Tree species Needle age	Chlorophyll content mg/g fresh matter			Chloro- phyll <i>a/b</i>	Pheophytin content mg/g fresh matter			% of pheophytin as related to chlorophyll content		
	<i>a</i>	<i>b</i>	<i>a+b</i>		<i>a</i>	<i>b</i>	<i>a+b</i>	<i>a</i>	<i>b</i>	<i>a+b</i>
Scots pine										
Panewnik										
Current year needles	0.99	0.39	1.38	2.54	0.01	0.09	0.10	1.0	23.1	7.2
1-year old needles	1.21	0.52	1.73	2.33	—	0.11	0.11	—	21.2	6.4
Rogów										
Current year needles	0.91	0.38	1.29	2.39	0.01	0.08	0.09	1.1	21.0	7.0
1-year old needles	1.10	0.48	1.58	2.29	—	0.07	0.07	—	14.6	4.4

Table 4

Chlorophyll and pheophytin content in needles of different age of Douglas fir growing under conditions of chronic industrial pollution

Tree species Needle age	Chlorophyll content mg/g fresh matter			Chloro- phyll <i>a/b</i>	Pheophytin content mg/g fresh matter			% of pheophytin as related to chlorophyll content		
	<i>a</i>	<i>b</i>	<i>a+b</i>		<i>a</i>	<i>b</i>	<i>a+b</i>	<i>a</i>	<i>b</i>	<i>a+b</i>
Douglas fir										
Panewnik										
Current year needles	0.88	0.42	1.30	2.10	0.10	0.16	0.26	11.4	38.1	20.0
1-year old needles	0.86	0.42	1.28	2.04	0.04	0.11	0.15	4.6	26.2	11.7
2-year old needles	0.97	0.45	1.42	2.16	0.06	0.11	0.17	6.2	24.4	12.0
Rogów										
Current year needles	0.86	0.38	1.24	2.26	0.07	0.07	0.14	8.1	18.4	11.3
1-year old needles	0.96	0.40	1.36	2.40	0.03	0.07	0.10	3.1	17.5	7.4
2-year old needles	1.14	0.48	1.62	2.39	—	0.09	0.09	—	18.8	5.6

are proportional to the degree of pollution (Müller, 1957; Jamrich, 1968; Ilkun, 1971). Decrease in the content of carotenoids was also found to be an index of air pollution (Müller, 1957; Ilkun, 1971; Arndt, 1971). There is no clear picture, however, as to the content of pheophytins. Müller (1957) showed, on 2-year old fir seedlings treated with SO_2 that the decrease in chlorophyll content was accompanied by the accumulation of pheophytin *a* and *b*. Similar results, on *Pelargonium* treated with Cl_2 and SO_2 , were reported by Ilkun (1971). Other authors did not find increasing pheophytin content in assimilatory organs damaged by smoke (Dässler 1970, 1972; Arndt, 1971). Considering his own results and those reported in literature Arndt (1971) concluded that under the conditions of industrial pollution chlorophylls would not convert to pheophytins unless SO_2 , HF, HCl caused significant pH changes within the leaf.

Some authors suggest to use examining of chlorophyll *a* regeneration as a test for the degree of damage caused by harmful gases, particularly SO_2 (Müller, 1957). Chlorophyll *a* is not regenerated after injuries caused by drought or frost. The content of carotenoids as related to chlorophyll content was also proposed as an indicator of the degree of pollution effect. There were suggestions to consider the gradual decrease in chlorophyll *a* content as a measure or ageing of the assimilatory organs (Costes and Coï, 1957). The decrease in chlorophyll *a* content was explained to result from: decreased synthesis of protochlorophyll (Mc Nulty, Newman, 1961), obstructed biocatalytic function of Fe in the chloroplasts (Mez, 1963), broken protein-chlorophyll complex caused by coagulation or hydrolysis of the protein part of the complex (Ilkun, 1971).

The result of our experiments showed that pheophytin content decreased with needle age. Perhaps pheophytins are only an intermediate product of chlorophyll decomposition and they are not accumulated. The next stage of pheophytin degradation is their decomposition, to pheophorbides and porphirins by dissociation of phytol from pheophytins, catalyzed by chlorophyllase (Willstätter, Stoll, 1928). The different reaction, of particular species examined here may also be explained this way. Austrian pine is thought to be a species rather resistant to pollution, whereas Scots pine, on the contrary, a very sensitive one. Needles of Austrian pine showed a high pheophytin content while those of Scots pine a very low one. This may be connected with various rate of further pheophytin decomposition in both species.

The high pheophytin content in the current year needles may be explained by high susceptibility of young, fully developed needles to acid gases (Bobrov, Solberg, Scot, 1962; Menser, Heggestad, Street, 1963). It is, probably, the result of intensive gas exchange which causes easier penetration of SO_2 through the stomata

(Mamajew, 1968; Vogli Börtitz, 1965). Older needles were shown to absorb considerably smaller amounts of SO_2 or HF comparing to the current year needles (Godzik, 1968; Knabe, 1968). It is, undoubtedly, connected with the structure of the epidermal tissue (Ilkun, 1971). The increasing Ca content with needle age (Němec, 1935; Tamm 1964) may affect cell pH in older needles and also hinder the conversion of chlorophylls to pheophytins.

Our results showed different pheophytin *a* and *b* content in needles of particular species. The turnover of chlorophyll *a* is faster comparing to that of chlorophyll *b* (Joslyn, Mac Kinney, 1938). The higher pheophytin *b* content found may result from faster turnover of chlorophyll *a* to pheophytin and its faster further degradation.

Reasuming, one can conclude that pheophytin content seems not to be a satisfactory test to determine the degree of changes in assimilatory organs before evident symptoms occur although large differences between control plants and those from polluted environment were found. Such a conclusion is drawn mainly because of laboriousness of the method (large number of samples), as well as large differences in pheophytin content connected with other causes than the pollution effect, such as needle age, species, environment.

Needles of trees growing under control conditions contained similar amounts of pheophytins. Pheophytin content does not reflect changes undergoing in the ageing assimilatory organs. Changes of chlorophyll content, seems to be a better indicator. Changes of chlorophyll content in needles of different age were investigated by Godniev, Chodasevick, Arnautova (1969). They found that 80% of the maximal chlorophyll amount was synthesized during the first growing season. The other 20% is synthesized in the following years of needle life. Considerable decrease in chlorophyll content is observed just now in the last year of needle life. The changes of chlorophyll content with needle age, observed in our investigation, are consistent with literature data (review — Szaniawski 1976).

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Zawartość chlorofilu i feofityny w igliwiu różnego wieku drzew rosnących w warunkach chronicznego oddziaływania zanieczyszczeń powietrza

Streszczenie

W igliwiu różnego wieku wejmutki (*Pinus strobus* L.), sosny czarnej (*Pinus nigra* Arn.), sosny zwyczajnej (*Pinus silvestris* L.), daglezji (*Pseudotsuga menziesii* Franco), rosnących w warunkach chronicznego oddziaływania zanieczyszczeń powietrza (Panewnik k/Katowic) i w warunkach kontrolnych (Arboretum w Rogowie) określono zawartość chlorofilu i feofityny metodą Vernona (1960).

Stwierdzono dużą ilość feofityny w igliwiu drzew poddanych chronicznemu oddziaływaniu zanieczyszczeń przemysłowych. Największą ilość feofityny wykazały zawsze igły najmłodsze (20—30% w stosunku do chlorofilu). Badane gatunki drzew wykazały różny stopień rozkładu chlorofilu pod wpływem skażenia środowiska. Największą ilość feofityny stwierdzono kolejno u wejmutki, sosny czarnej i daglezji. U sosny zwyczajnej stwierdzono niewielką ilość feofityny b i brak feofityny a.

W igliwiu drzew rosnących w warunkach kontrolnych stwierdzono niewielkie i prawie jednakowe ilości feofityny w igliwiu różnego wieku. Najwięcej feofityny stwierdzono w igliwiu wejmutki i daglezji (średnio ok. 10%), niewielkie u sosny zwyczajnej i czarnej (średnio ok. 5%). U sosny zwyczajnej i czarnej nie stwierdzono występowania feofityny a.

Zawartość chlorofilu zwiększała się wraz z wiekiem igliwia zarówno u roślin rosnących w warunkach chronicznego oddziaływania zanieczyszczeń powietrza jak i kontrolnych. Zawartość feofityny nie wydaje się być zadowalającym wskaźnikiem wpływu zanieczyszczeń powietrza (przed wystąpieniem symptomów jawnych) ze względu na pracochłonność metody, jak również dużą zmienność materiału roślinnego.