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Assimilatory pigments from subfossil fir needles (*Abies alba* Mill.)

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The decomposition rate of organic compounds, following the death of a plant, is dependent on several external factors. Assimilatory pigments generally undergo a rapid degradation. In certain condition, however, their decomposition may be considerably retarded; e.g. compounds similar to chlorophyll and some carotenoids, as α and β-carotene, lutein and others, may persist several thousand years in marine and lake sediments (Vallentyne 1960).

Derivatives of chlorophyll were also found in the surface layer of wood soil (Gorham 1959).

In this connection the question arises, in what a way a still different environment, namely peat, influences the decomposition rate of pigments.

The starting point in these investigations was the fact observed by one of the co-authors, that many subfossil fir needles from various depths of the peat bog in Cergowa Góra were bright yellow-green pigmented. Macroscopic observations have already suggested that, at least, a part of the pigments did not undergo decomposition.

A study was undertaken with the aim to determine the quantitative and qualitative changes in assimilatory pigments, occurring in fir needles in dependence on the period of time they were lying in the peat bog.

METHODS

Investigations were carried out in October and November 1961. Fir needles (*Abies alba*) were sampled from three depths: 0 — 10 cm,* 2,0 — 2,5 m., 3,0 — 3,5 m. For comparison analyses of pigments from the following samples were carried out: falling green needles, bright green needles collected from tree growing on the peat bog and the amorphous peat mass from a the depth 2,0 — 2,5 m.

* This sample was taken from the part of the peat bog not overgrown with moss.

Samples were taken by means of a Hiller's borer. Just before analyses were performed, the needles were washed out from the peat mass on a dense sieve in running water and transferred again into water. Needles dried between sheets of filter paper (400 mg) were ground in a mortar with broken glass and a small amount of calcium carbonate and extracted with 85% acetone. The acetone extract was passed through a Schott's filter and collected in a separating funnel and then the pigments were transferred into the layer of petroleum ether (boiling point 40 ° — 60°C.). The petroleum ether extract was dehydrated by addition of anhydrous sodium sulphate and brought to a constant volume.

An aliquot of the petroleum ether solution (0,3 ml.) was placed on the starting line on a 2 cm large strip of chromatographic paper (Whatman 1). The chromatogram was developed by means of a mixture of petroleum ether and acetone (18 : 1, v/v). After the front line of the solvent had advanced to about 11 cm, the development of the chromatogram was interrupted and the different zones were separated by cutting out suitable strips. From every strip the pigments were eluted with 0,2 ml pure acetone (p.a.) and the solution collected in 1 cm long microcells. Absorption spectra were subsequently determined in the range 400 — 500 mμ and 610 — 690 mμ by means of a Uvispek-Hilger spectrophotometer equipped with a glass prism and an arrangement for microcells.

After removing greater plant remnants, coloured substances (marked as humus substances) were extracted from the peat mass and chromatographically separated in a similar way. It is possible that at least some derivatives of assimilatory pigments occur also in extracts from the amorphous peat mass.

RESULTS

Description and age of the peat bog

The peat bog is situated on the northern hill side of Cergowa Góra, near Dukla (Beskid Niski) 480 m above the sea level. It fills out a basin of about 1 ha area, formed in consequence of a slope slide. Almost the half of the peat bog is densely covered with willows silver birch, fir, alder and buckthorn. On the other part — which has the character of a raised bog — trees are rather rare (mostly pine and birch), whereas the carpet layer, with peat mosses, sedges and cotton grass predominant, is well developed. Water in the hollowings reaches the ground level, above which only 10 — 15 cm high tufts stand out. Borings were performed more or less in the middle of the peat bog on the border of the part losely overgrown with bushes and that covered with a dense tree and bushy vegetation. These borings showed the following succession of layers:

0 — 7 cm	a layer of green mosses (mainly *Sphagnum*),
7 — 20 cm	light brown mosses — slightly decomposed,
20 — 280 cm	brown peat, well decomposed, soaked with water, containing a considerable amount of wooden pieces, stems of higher plants and mosses,
280 — 550 cm	dark brown compact and well decomposed, mixed with remnants of wood,
550 — 570 cm	dark brown peat, decomposed with an admixture of silt and sand,
570 — 585 cm	dark brown peat with a great amount of silt and sand and small rock fragments,
585 — 590 cm	light gray silt,
590 — 600 cm	dark gray silt with rock fragments.

Other macroscopic plant remnants in form of fruits, seeds, etc. are profusely found in the whole profile of the peat bog. Numerous leaf fragments, specially willow leaves, were found in the younger peat layers above 280 cm, whereas, in layers between 150 — 450 cm fir needles were very common.

The peat bog in Cergowa Góra provided the material for paleobotanical investigations. From the profile of the peat bog 132 samples for pollen analysis were collected at intervals of 5 cm and 12 samples for investigations on macroscopic plant remnants (one sample corresponding to the content of 1 sampler of the peat borer, 0,5 m long).

The preliminary palynological elaboration comprised only 23 samples of peat taken from various depths. The corresponding pollen spectra are shown in Fig. 1 in a diagram exclusively (*AP*) composed of tree pollen curves which are a sufficient base to determine the relative age of the investigated fir needles.*

The pollen diagram of the bottom layer of peat represents the final phase of the Atlantic period i.e. about 3000 years before our era. This determination is based on the still high, however decreasing values of the curves of hazle, elm, lime, ash and maple and on the percentage participation of the hornbeam pollen.

The rising curves of the beech and fir and a concomitant drop of the curves of the other trees is characteristic of the Subboreal period which has a transitory character.

The Subatlantic period, which is assumed to beginn about 700 years before our era, is represented by a relatively weakly decomposed peat

* A full paleobotanical elaboration of the peat bog in Cergowa Góra will be the subject of a separate paper.

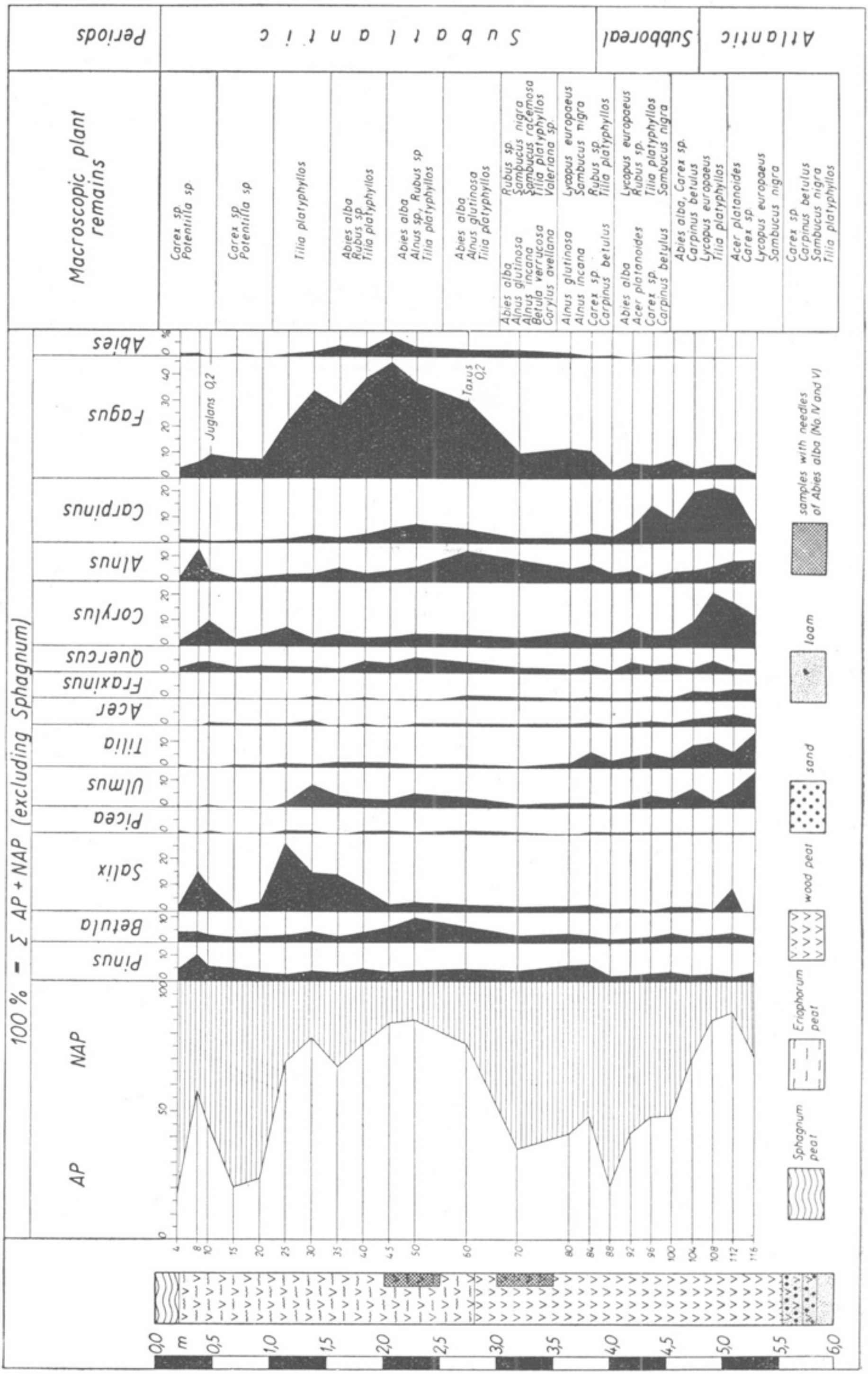

Fig. 1. Pollen diagram of the Cergowa Góra peat bog (480 m a.s.l.)

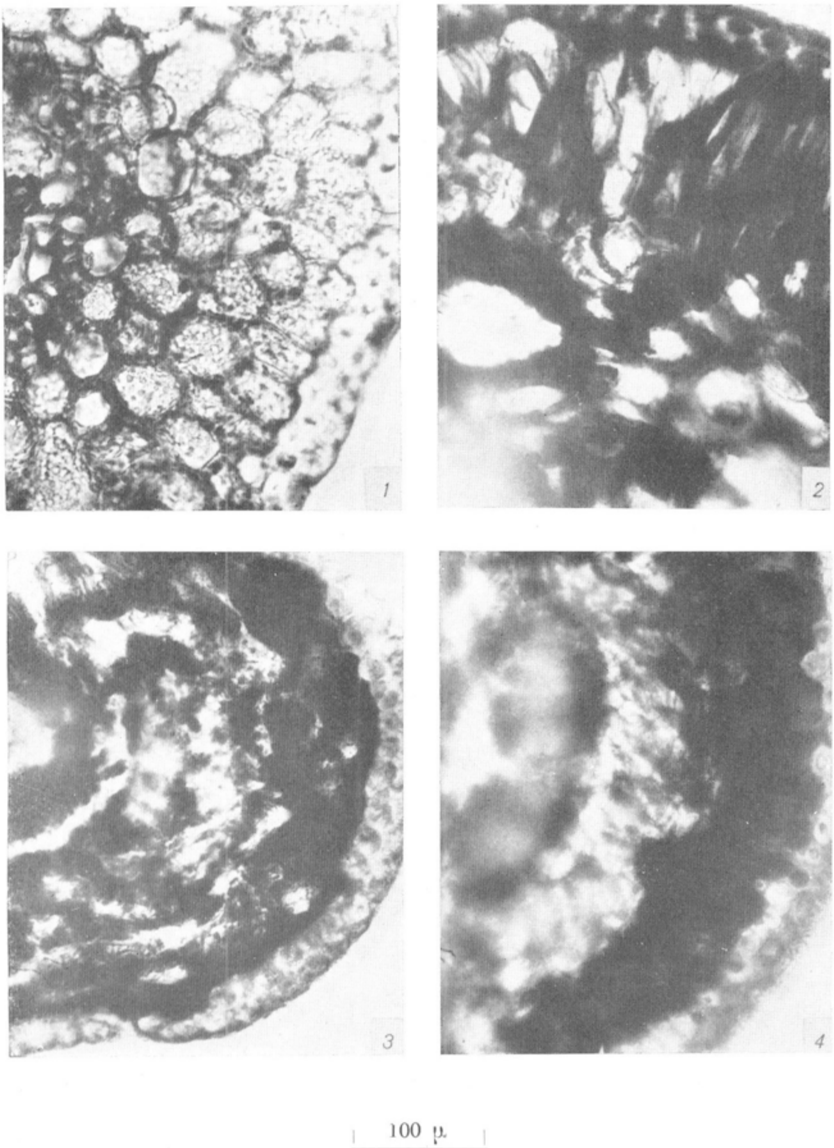

Fig. 2. Cross sections of needles: green needles falling from a tree (*1*); Needles from various depths: 0 — 10 cm (*2*), 2,0 — 2,5 m (*3*), 3,0 — 3,5 m (*4*)

Fixed material. Fixative: alcohol + chloroform. Imbeded in celluloidine. Unstained with beech and fir predominant. In the top part of the profile, the curves of the tree pollen percentage reflect human activity. This is illustrated by the presence of pollen grains of herbal plants, not shown in the diagram.

Samples IV and V from which fir needles were collected and examined for assimilatory pigments content, were taken from various depths of layers deposited during the older Subatlantic period, which is assumed to beginn about 700 years before our era.

Pigment analysis

The degree of decomposition of the examined needles is shown in the photographs (Fig. 2). In the needles from the depth 2,0 — 2,5 m and 3,0 — 3,5 m, only more lignified tissues, i.e. the epidermis and vascular bundles, were relatively well preserved. The cells of the mesophyll were in a state of advanced destruction and cells had a tendency to desintegrate during the preparations of thin slides. It is however certain

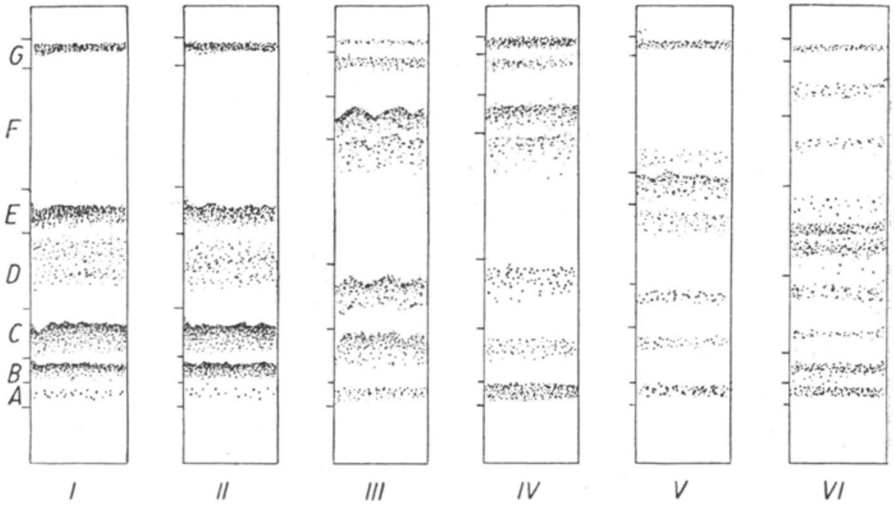

Fig. 3. Scheme of pigment dislocation on a developed chromatogram

I — green needles collected from a tree; *II* — green needles falling from a tree; *III* — needles from the depth 0—10 cm; *IV* — from the depth 2,0— 2,5 m; *V* — from the depth 3,0—3,5 m; *VI* — coloured substances extracted fro n the amorphous peat mass. The pigments fractions (*A* — *G*) were separated from each other as indicated on the scheme. *A* — start line; *G* — front of the chromatogram

that a part of the mesophyll cells (more precisely their ligneous membranes) have been also preserved. These cells were more or less filled with various granularities. It is, of course, difficult to say anything about the decomposition degree of the cell content, as a part of these granularities may originate from outside. Inside the subfossil needles, there is also a lot of amorphous brown mass.

Coloured compounds, soluble in used organic solvents, were situated on the chromatogram as shown in Fig. 3. In all cases 7 zones were formed, more or less distinctly separated from each other.

Their positions on the chromatograms, however, were not always the same. Similar R_f values were obtained in sample I (green needles collected from a tree) and in sample II (green falling needles). Some other R_f values were obtained for pigmented compounds from needles sampled from the depth 0 — 10 cm (sample III) and from the depth 2,0 — 2,5 m (sample IV) and still other ones from needles lying on the depth 3,0 — 3,5 m (sample V).

Differences in the R_f values are probably caused by changes in the structure of different pigments. One must be cautious, however, with concluding, because some others factors modify the R_f, for example pigments concentration (Hager, 1959), or a high salt content in the extract (van Os, 1959). Furthermore, there are some other colourless substances from living plants migrating on the chromatograms together with pigments. These substances fluoresce strongly in UV-light (Zurzycka, private communication) and it is possible that they may also modify the rate of pigment migration.

Fig. 4 presents the absorption spectra of consecutive fractions from various samples.

The analysis of the absorption spectra shows the following composition of the pigments of needles collected from a tree (sample I): the front zone of the chromatogram is formed by carotenes (*G*), the following zone is most probably formed by pheophytins (*F*), and the zones *E* and *D* by xanthophylls. The positions of the absorption maxima indicate that lutein is the pigment predominant in the E layer. Chlorophyll *a* is localized in the next zone (*C*), and chlorophyll *b* — in the zone *B*. Zone *A* (which does not shift from the start line) was almost absent on chromatograms of extracts from green needles. This fact, however, was not dealt with further on, as it is characteristic rather of humus substances.

In fig. 4 the sequence of the graphs represents the absorption spectra of pigments extracted from the different samples. In column *B* are found the absorption spectra of substances from the zone where chlorophyll *b* is localized. Typical absorption spectra of chlorophyll *b* occur in sample I and II only. All the other samples show absorption spectra characteristic of a humus substance. Newertheless, a smaller or greater peak is discernible on the absorption curves for the wave length corresponding to the absorption maximum of chlorophyll *b* in the blue-violet part of the spectrum. This peak is absent in spectra of coloured humus

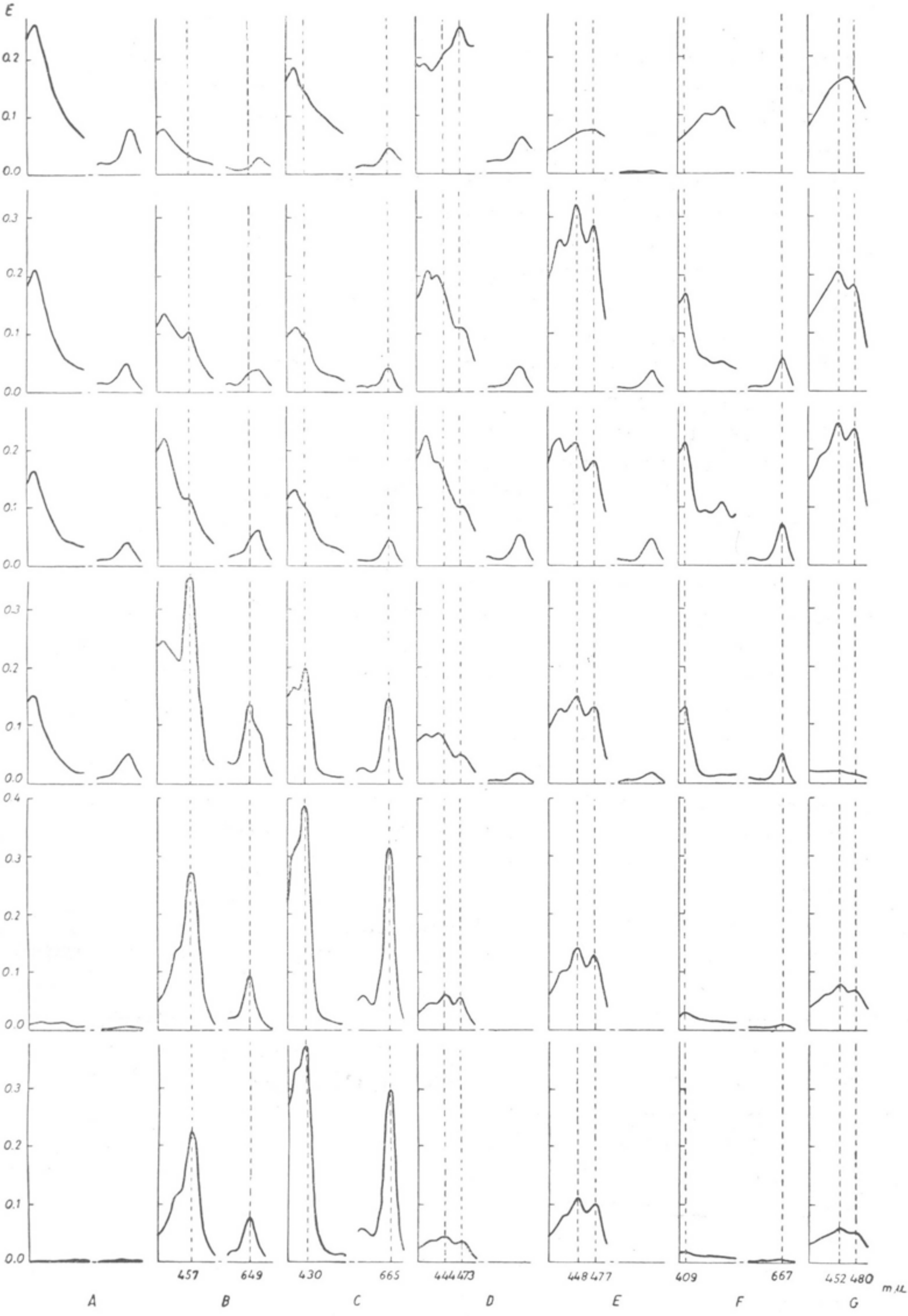

Fig. 4. Absorption spectra in the range 400—500 mμ and 610—690 mμ of separated pigment fractions from various samples

Values in samples III — V were magnified 20 ×. Solvent: acetone. For other details see explanation on fig. 3

compounds (sample VI). This fact indicates that certain amounts of chlorophyll *b* occur in samples III — V. The analysis of the spectrum in the red region does not give such clear-cut results (except in sample III), because the humus substances absorb the light also in the same spectral region (maximum about 668 mμ).

Absorption spectra of pigments corresponding to the zone of chlorophyll *a* are presented in the next vertical column (*C*). Maximum absorption in the blue-violet region is distinctly noticeable in sample III only (needles from the depth of 0 — 10 cm); sample I and II being not considered. Spectra from samples IV and V show some slight humps in the range of the maximum absorption of chlorophyll *a* which are absent in the spectrum VI. The analysis of the spectra in the red part gives less reliable results, because the maxima of absorption of chlorophyll *a* and coloured humus substances nearly coincide. It results, however, from the analysis of the blue-violet part of the spectrum that in subfossil needles, from various depths, traces of chlorophyll *a* are also found.

The next two columns (*D* and *E*) refer to the absorption spectra of xanthophylls. The pigments from falling needles (II) present absorption spectra similar to the spectra of xanthophylls extracted from needles, collected from a tree, whereas, pigments in samples III, IV and V show different absorption spectra, which, however, are different from the corresponding spectra of humus. This suggests that substances similar to carotenoids occur in subfossil needles. The fraction *D* have more changed spectra than the fraction *E*; differences refer to the general shapes of the curves and the positions of the absorption maxima. This fact suggests that lutein or compounds similar to lutein are, most probably, more stabile. In these two fractions humus substances with absorption maxima in red are also present.

The next column (*F*) presents the absorption curves of pheophytins or similar pigments. In needles from sample III and IV, besides pheophytins, most probably, some humus pigments are also present.

Carotenes migrate in the front of the developing chromatograms. This zone is slightly visible in sample III. Carotenes, if any, are present in very small amounts. Greater amounts of carotenes, besides coloured humus substances, occur in sample IV and V.

The absorption maxima, especially fractions *G* and *F* are shifted about 1—4 mμ towards the shorter wave lengths. These changes suggest, that, if not all, so at any rate, a great part of the carotenoids underwent an isomerization; the trans — isomers being changed into the cis — isomers.

It is not possible, however, to make a detailful analysis of quantitative changes of pigments on the base of the date given on in the diagram. Approximate data, however, may be obtained. It results from the heights of absorption peaks of chlorophyll *a* and *b* in the columns *B* and *C* that the rate of the break down of chlorophyll *a* is much higher than of chlorophyll *b*. These differences are already apparent in sample III (comp. IIIB and IIIC). A proportionally greater amount of chlorophyll *b* occurs also in sample IV (2,0 — 2,5 m) and sample V (3,0 — 3,5 m).

There is also proportionally more pheophytins and carotenoids (or pigments similar to carotenoids) in subfossil needles (with exception of fraction *G* in sample III).

DISCUSSION

The decomposition of organic compounds leading to the formation of peat is a complicated process depending on several external factors. Owing to better aeration the decomposition of organic matter (especially in peat bogs of a raised type) proceeds mainly in the upper layer — the peat forming layer (Tiuremnow 1957). These conditions create a better environment for the development of aerobic saprophytic micro-organisms. In the deeper layers of a peat bog the decomposition of organic matter is the result of the activity of anaerobic microorganisms and purely chemical processes. The mineralization is, however, much slower in deeper situated layers than in the upper ones.

It is commonly known that different organic compounds undergo decomposition at various rates. Monosaccharides, proteins and fats are rapidly decomposed in comparison with substances constituting cell membranes i.e. cellulose (specially in raised bogs) and lignine the destruction of which is very slow.

The decomposition of the mentioned compounds is not always complete (see: Scheffer and Ulrich 1960). A valley peat bog for instance, is comparatively rich in organic nitrogen compounds, among which compounds similar to proteins are dominating (Waksman 1932).

It should be supposed that the destruction of chlorophyll is connected with the decomposition of protein.

The acidity of the peat partake in the break down of chlorophyll and contributes to the formation of pheophytins. For this reason the presence of considerable amounts of pheophytins in subfossil needles becomes comprehensible. Vallentyne (1960) states that the following conditions are indispensible for a good preservation of carotenoids: a) no access of oxygen, b) no access of light, c) low temperature, d) unsolubility in water. It seems that these conditions are maintained in deeper peat layers. Thus, if carotenoids pass in an intact state through the peat forming layer, they can then subsiste for several thousand years. Needles from conifers are well protected against the destructive action during their comparatively short stay in the peat forming layer. It may be supposed that the peat bog on Cergowa Góra was, some thousand years ago, more humid and the falling needles passed immediately to an environment poor in oxygen. This supposition is based on the fact that in the upper layer 0 — 10 cm, the carotenes, which are most sensitive to the action of the above mentioned external factors, do not occur at all.

The changes in the migration rate of certain pigment fractions and a slight shifting of the absorption maxima towards the shorter wavelengths indicate that at least certain amounts of pigments have been transformed into cis-isomers. This concerns especially the *G* fraction (carotenes) and the *E* fraction (with probably lutein prevalent).

According to Karrer and Jucker (1948) the trans — cis transformation is the result of the action of several factors, among others of a lower pH. In our case humic acids are the factor depressing the pH. Cis — isomers do not occur in lake sediments where the pH value attains 10,5 (Vallentyne 1957).

It has been stated in the present paper that the break down of chlorophylls proceeds at a much quicker rate than the break down of carotenoids. This statement is in agreement with the results obtained by other authors (Vallentyne 1960; Fogg and Belcher 1961, and others).

CONCLUSIONS

1. Subfossil fir needles from three levels (0 — 10 cm, 2,0 — 2,5 m and 3,0 — 3,5 m) of a peat bog were examined for the content of assimilatory pigments. For comparison, green needles collected from a tree growing on the peat bog, falling green needles and humus substances were also examined.

2. The pollen analysis method was applied for determination of the relative age of the peat bog. Samples were taken from layers not older than ± 3 000 years.

3. Presence of chlorophyll *a* and *b* has been established in the examined material.

4. Destruction of chlorophyll *a* proceeds at a higher rate than that of chlorophyll *b*.

5. In deeper layers xanthophylls (some probably in the form of cis — isomers and others in a more changed forms) and carotenes (probably also in cis-forms) are more resistent to decomposition than chlorophylls.

6. Conclusions drawn from the decomposition rate of pigments were discussed in connection with the changes of conditions occuring during the formation of the peat bog in Cergowa Góra.

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