IDENTIFICATION AND QUANTITATIVE MEASUREMENT OF PLANT PIGMENTS IN SOIL HUMUS LAYERS[1]

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Abstract. Pigments in soil organic matter layers of an oak forest, a spruce-cedar woodland, and a prairie have been identified and measured quantitatively. In all cases lutein was the dominant carotenoid accompanied by smaller amounts of β-carotene. Pheophytin *a* was the most abundant chlorophyll derivative, with smaller amounts of pheophorbide *a* and chlorophyllide *a* commonly present in the L and F organic layers. Pigment ratios were examined and compared in all three humus types.

INTRODUCTION

The establishment of pheophytin *a*, lutein, and β-carotene as the major pigments in falling oak, aspen, and hazel leaves (Sanger 1968, 1971) has set the stage for a closer, more detailed examination of the pigment characteristics of soil humus. Virtually nothing was known of the fate of pigments subsequent to the incorporation of leaves into the humus layers of woodland soils until the studies of Gorham (1959) in England and Simonart, Mayaudon, and Batistic (1959) in France. Gorham observed chlorophyll derivatives in mor and mull types of humus layer (Lutz and Chandler 1946) and noted that increased pigment preservation was correlated with decreased soil pH. The studies of Simonart et al. revealed that foliar pigments, especially β-carotene and derivatives of chlorophyll, decompose very slowly compared to glucose, hemicellulose, and cellulose. Later studies by Gorham and Sanger (1964) assessed the role of waterlogging in the preservation of chlorophyll derivatives in a series of soils from upland forest through swamp forest to the floating mat on the edge of a small pond. Their results demonstrated that the degree of preservation of chlorophyll derivatives in soil humus was directly proportional to the degree of waterlogging.

Hoyt's (1966*a, b*) investigations of agricultural soils revealed the presence of small amounts of pheophytin, chlorophyllides, and pheophorbides in the humus. However, the latter two pigments were present in such small quantities that he was unable to separate and measure them accurately. In addition, Hoyt noted that decomposition of pigments in these aerobic soils increased with increasing moisture content (fastest at 50–60% water-holding capacity) and temperature, and slowed with increasing soil acidity. He concluded that slow decomposition by microorganisms was the most important cause of chlorophyll degradation in soil, and that pheophytin offered the greatest resistance to decay.

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The relative abundance of both carotenoids and chlorophyll derivatives was measured by Gorham and Sanger (1967) in L (litter), F (fermentation), and H (humification) organic horizons of oak and pine woodland soils (Lutz and Chandler 1946). Carotenoids accounted for progressively lesser proportions of total organic matter as fallen leaves decomposed on the forest floor, but chlorophyll derivatives reached maximum concentrations in the L_2 horizon (lower, more finely divided part of the L horizon) of the humus layer, where they apparently decayed more slowly than the litter as a whole.

The study described here was initiated to ascertain the specific identity of pigments present in soil humus layers and to measure their levels quantitatively. These data are essential for accurate interpretation and assessment of the contribution of terrestrially derived pigments to lake and pond sediments. For purposes of comparison, soil-humus layers from three widely divergent plant communities were examined.

DESCRIPTION OF SITES

The oak woodland consists of a 20-acre tract in southern Isanti County, Minnesota, on the Cedar Creek Natural History Area. The parent material is glacio-fluvial sand, and the soil, classified as an alfic normipsamment, has a well-developed ochric epipedon and an argillic horizon. The dominant canopy species is *Quercus ellipsoidalis,* with a dense shrub synusia of *Corylus americana.* The more common elements of ground vegetation include *Carex pennsylvanica, Pteridium aquilinum,* and *Vaccinium angustifolium.*

The spruce-cedar woodland is part of a large area of mixed spruce and cedar dotted with numerous small bogs and fens in northeastern Minnesota. The soil is a spodosol and has been classified a typic haplorthod. The canopy is dominated by *Picea glauca,* with a dense subcanopy of *Thuja occidentalis.* Sparse elements of ground flora include *Vaccinium angustifolium, Chimaphila umbellata, Pyrola secunda, Moneses uniflora, Cornus canadensis,* and *Carex* sp.

The prairie site is located within the Pipestone National Monument in southwestern Minnesota. The vegetation is virgin prairie, dominated by *Bouteloua gracilis, B. curtipendula, Koeleria cristata, Andropogon gerardi, A. scoparius, Aster ericoides, Cirsium flodmanii, Symphoricarpos occidentalis, Astragalus caryocarpus,* and *Carex* sp. The soil, a typic haploborall, has a well-developed mollic epipedon with a cambic horizon formed over silty Iowan till.

The separate horizons of the above three humus layers, although classified similarly in this study, are not strictly comparable. The oak humus layer has well-developed and easily definable L, F, and H horizons. The spruce-cedar humus has well-developed L and F horizons very similar to oak, but no H horizon. The prairie soil has an easily definable L horizon consisting of dried grasses and forbs and a lower more decomposed humic horizon that is easily separable from the upper level of the mollic epipedon. This lower horizon most closely resembles the F horizon of the oak and spruce-cedar woodland and has been classified as such for comparative purposes.

Methods

The entire organic horizon was removed from a 0.25-m^2 plot in each of the three plant communities. Where present, the L, F, and H horizons were separated in the field, and all samples were sealed in polyethylene bags.

In the laboratory the separated horizons were dried for about 1 hr in a forced-air oven at room temperature (20°C). Samples were ground very briefly in a Wiley mill through a No. 20 mesh screen to insure greater ease of pigment extraction. Subsamples for dry ash-free weight and pigment analysis were taken from each sample, and organic matter obtained by ashing at 550°C in a muffle furnace. Pigments were extracted by saturating the ground material with 90% aqueous acetone for 10–15 min. Repeated extractions were made until the extract was free from color. Further techniques of handling, paper chromatography, and calculation of results are identical to those described in Sanger (1968). All values are based on the average of three trials.

Results and Discussion

The concentrations of pigments from the organic soil horizons of the three plant communities are presented in Table 1. In all cases the L horizons are the richest in both total chlorophyll derivatives and carotenoids, with pheophytin *a*, lutein, and β-carotene as the major components. Pigment concentrations in the prairie soil humus are much lower than concentrations in the humus of the oak forest and spruce-cedar woodland. The prairie may provide a less favorable environment for pigment preservation, because the standing stems die back and are subject to decomposition for a long time before being incorporated into the humus layer, whereas in the forest there are greater opportunities for at least some leaves relatively rich in pigments to be rapidly incorporated at autumn leaf fall. Certainly prairie soils are not so well protected from frequent periods of wetting and drying, direct solar radiation, and extremes of temperature as are the woodland sites.

Pheophytin *a* is clearly the dominant chlorophyll derivative in soil humus layers. The L horizons of the spruce-cedar woodland and the oak forest ($(L_1 + L_2)/2$) are about equal in pheophytin *a* concentrations, with values more than four times that of the prairie L horizon. In the spruce-cedar woodland and the prairie humus layers pheophytin *a* accounts for nearly all the chlorophyll derivatives. Pheophorbide *a* and chlorophyllide *a* are present only in trace quantities. In the oak forest pheophytin *a* makes up about 91% of the total chlorophyll derivative in the $L((L_1 + L_2)/2)$ horizon, 74% in the F, and 93% in the H horizon.

Pheophytin *a* concentrations are higher in all three humus horizons of the oakwood organic layer than in falling autumn leaves (Table 2). Concentrations for the oak L_1 are about 2.3 times the level in falling autumn leaves, for the L_2 5.5 times, for the F 2.5 times, and for the H 1.4 times. This

Table 1. Concentrations of chlorophyll derivatives and carotenoids from the soil organic horizons of oak forest, spruce-cedar woodland, and prairie—expressed in mg/100 g organic matter

Soil organic horizon	Pheophytin *a*	Pheophorbide *a*	Chlorophyllide *a*	Lutein	β-carotene
			Oak forest		
L_1	70.1	4.1	5.0	0.96	0.30
L_2	165.2	7.2	4.0	1.27	0.39
F	74.1	13.9	10.2	0.69	0.16
H	41.3	3.0	0.1	0.30	0.05
			Spruce-cedar woodland		
L	119.5	trace	trace	1.78	0.40
F	88.9	trace	trace	0.87	0.23
			Prairie		
L	25.0	0	0	0.18	0.10
F	6.1	0	0	0.03	0.02

TABLE. 2. Pigment concentrations and ratio of pheophytin *a* to total carotenoid in oak leaves and oak humus layers

Item	Pheophytin *a* (mg/100 g organic matter)	Lutein (mg/100 g organic matter)	β-carotene (mg/100 g organic matter)	Pheophytin *a* / Total carotenoid
Falling leaves in autumn (mid-November)	30.0	1.30–1.50	0.50	15.8
Leaves overwintering on the ground	10.0	1.10	0.20	7.7
Leaves overwintering on the tree	5.0	0.10	0.05	33.3
Oak humus layers				
L_1	70.1	0.96	0.30	55.6
L_2	165.2	1.27	0.39	99.4
F	74.1	0.69	0.16	87.1
H	41.3	0.30	0.05	117.1

demonstrates that pheophytin *a* is much more resistant to decomposition in the soil humus layer than organic matter as a whole. Pheophytin *a* is also more resistant to decomposition than the carotenoids. Ratios of pheophytin *a* to total carotenoid are lowest in freshly fallen leaves and in undecomposed leaves lying on the forest floor, and the highest in the more decomposed material in the lower humus layers.

Occasionally traces of pheophytin *b* are encountered, but it is not present in measurable quantities. This could be due to two factors. First, chlorophyll *a* is more rapidly converted to pheophytin *a* than chlorophyll *b* to pheophytin *b* (Willstätter and Stoll, cited by Joslyn and Mackinney 1938). Perhaps the slowness of reaction of chlorophyll *b* to pheophytin *b* allows almost all of the chlorophyll *b* to be destroyed before pheophytin formation is able to preserve any of it. Second, because the levels of pheophytin *a* preserved are so very low in dried leaves compared to the original chlorophyll concentration, it is possible that levels of pheophytin *b*, even if it is preserved at a rate equal to that for pheophytin *a*, would be too low for detection of more than trace amounts by the methods employed. In the oak forest pheophorbide *a* and chlorophyllide *a* make up about 5% and 4% of the total chlorophyll derivatives, respectively, in the $L((L_1 + L_2)/2)$ horizon, 14% and 11% in the F horizon, and 6% and 0% in the H horizon. All pigments investigated from the oak humus except chlorophyllide *a* and pheophorbide *a* were present in greatest concentration in the L_2 layer (Table 1).

Lutein is the dominant carotenoid, and it was the only xanthophyll detected in the three soil humus layers examined. Greatest concentrations are found in the L horizons, with the spruce-cedar woodland highest, followed closely by oak forest, and with prairie very low. Lutein concentrations in the L_1 oak humus are just slightly less than the concentrations in falling oak leaves (mid-November) and leaves overwintering on the forest floor, but they are distinctly greater than concentrations in leaves overwintering on the tree (Table 2).

Beta-carotene, the only other carotenoid component detected in soil humus, is present wherever pigments are found, though often in low concentrations. Like lutein, greatest concentrations are found in the L horizons, with the spruce-cedar woodland and oak forest about equal and the prairie distinctly lower. In the oak L_1 horizon β-carotene concentrations are lower than in falling leaves (Table 2), slightly higher than in leaves overwintering on the ground, and distinctly higher than in leaves falling from the tree in spring.

The above data in general correspond to those of Gorham and Sanger (1967), with two minor exceptions. First, Gorham and Sanger found higher concentrations of hypophasic carotenoids (lutein) in dry leaves on the tree in the spring of 1965. Second, for the same year they noted that epiphasic carotenoids (β-carotene) were higher in leaves overwintering on the soil surface than in the L_1. Differing weather conditions in the spring of 1967, when the values given here were recorded, may account for the discrepancies. It was demonstrated earlier (Sanger 1968) that carotenoids in general degrade quickly in late winter and early spring when warmer temperatures and frequent periods of wetting and drying are common. Unusually warm temperatures with frequent light rains and occasional snows were common during the spring of 1967, making conditions conducive to rapid pigment breakdown.

The presence of relatively large quantities of lutein, β-carotene, and pheophytin *a* in soil humus is not unexpected, since these are the three most abundant pigments in falling oak leaves, whether they drop in autumn or in spring. The presence of measurable amounts of pheophorbide *a* and chlorophyllide *a* is surprising, because these two components are

TABLE 3. Pigment ratios from the soil organic horizons of oak forest, spruce-cedar woodland, and prairie

Soil organic horizon	Chlorophyll derivatives / Total carotenoid	β-carotene / Lutein
	Oak forest	
L_1	63	0.31
L_2	106	0.30
F	115	0.23
H	130	0.16
	Spruce-cedar woodland	
L	55	0.22
F	81	0.26
	Prairie	
L	100	0.66
F	122	0.66

not detectable in autumn leaves of oak, aspen, or hazel (Sanger 1971). No conclusive explanation can be given for the presence of these pigments, but three possibilities deserve mention. First, it is conceivable that these pigments are the product of chlorophyll degradation from any one of a number of uninvestigated trees, shrubs, or herbs that contribute organic matter to the soil. Second, pheophorbide *a* may form from pheophytin *a* in the soil itself rather than in organic matter before it reaches the soil. Last, many green leaves reach the forest floor during the summer as a result of wind and rain storms, and they could possibly be a source for both chlorophyllide *a* and pheophorbide *a*. The fate of pigments in these leaves remains uninvestigated, but the rate and manner of their pigment decay is undoubtedly strongly influenced by the microenvironment in which they become deposited. Whether they dry immediately and remain so for long periods, or whether they get quickly buried in the L layer where moist conditions prevail much of the time, will strongly influence the rate of pigment decay and the type of decomposition products formed. Experiments in this laboratory have shown that dry, green leaves retain their pigments undecomposed for an indefinite period if not exposed to moisture. Field situations, however, are extremely variable, and their investigation would constitute another study.

All three of the soil-humus types showed essentially the same trends in the ratio of total chlorophyll derivatives to carotenoid (Table 3). In all cases the ratio was lowest in the L horizon and increased with increasing depth in the humus layer. This demonstrates conclusively that under the aerobic conditions of the forest floor the carotenoids, both lutein and β-carotene, are less resistant to decomposition than pheophytin *a*, the dominant chlorophyll derivative in soils. This is contrary to the situation noted for autumn leaves, in which carotenoids are decomposed initially less rapidly than the chlorophylls (see also Gorham and Sanger 1967).

The ratio of β-carotene to lutein in the oak humus layers is highest in the L horizons (0.30), dropping to 0.23 and 0.16 in the F and H horizons respectively (Table 3). This is the trend expected from what has been observed of the lability of β-carotene compared to lutein (Sanger 1971). The opposite situation, however, occurs in the spruce-cedar woodland, with a slightly lower ratio in the L compared to the F layer. Prairie profiles have higher ratios than either the oak forest or the spruce-cedar woodland, with no apparent change in the ratio from the L to the F layer. The cause of these variations is unknown, although differing environmental conditions are probably involved. Pigment analyses on mixed collections of green leaves from both prairie and woodland habitats revealed negligible differences in the ratio of β-carotene to lutein.

The fact that pheophytin *a* is better preserved than carotenoids in aerobic woodland soils may in part explain the high ratio of chlorophyll derivatives to total carotenoids in northeastern Minnesota lakes (Gorham and Sanger, *unpublished data*). Unlike the eutrophic lakes of southern Minnesota, which derive much of their organic matter from within the lake itself, these northeastern lakes are relatively oligotrophic and undoubtedly derive a good deal of sedimentary organic matter from terrestrial sources.

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LITERATURE CITED

Gorham, E. 1959. Chlorophyll derivatives in woodland soils. Soil Science **87**: 258–261.

Gorham, E., and J. Sanger. 1964. Chlorophyll derivatives in woodland, swamp, and pond soils of Cedar Creek Natural History Area, Minnesota, U.S.A., p. 1–12. *In* Y. Miyake and T. Koyama [ed.] Recent researches in the fields of hydrosphere, atmosphere and nuclear geochemistry. Maruzen Co., Tokyo.

———. 1967. Plant pigments in woodland soils. Ecology **48**: 306–308.

Hoyt, P. B. 1966*a*. Chlorophyll-type compounds in soil. I. Their origin. Plant and Soil **25**: 167–180.

———. 1966*b*. Chlorophyll-type compounds in soil. II. Their decomposition. Plant and Soil **25**: 313–328.

Joslyn, M. A., and G. Mackinney. 1938. The rate of conversion of chlorophyll to pheophytin. J. Amer. Chem. Soc. **60**: 1132–1136.

Lutz, H. J., and R. F. Chandler, Jr. 1946. Forest soils. John Wiley and Sons, New York. 514 p.

Sanger, J. E. 1968. A quantitative study of leaf pigments from initiation in buds to decomposition in soils. Ph.D. Thesis. Univ. Minnesota, Minneapolis, Minn. 252 p.

———. 1971. Quantitative investigations of leaf pigments from their inception in buds through autumn coloration to decomposition in falling leaves. Ecology **52**: 1075–1089.

Simonart, P., J. Mayaudon, and L. Batistic. 1959. Étude de la decomposition de la (matiere) organique dans le sol au moyen de carbon radioactif. IV. Decomposition des pigments foliares. Plant and Soil **11**: 176–180.