

# CHLOROPHYLL DERIVATIVES IN SURFACE MUDS FROM THE ENGLISH LAKES<sup>1</sup>

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## ABSTRACT

Chlorophyll derivatives, total carbon, and total sulfur have been estimated in surface muds from the depths of five widely differing bodies of water. All three components increase in passing from muds of large infertile lakes, with waters low in phytoplankton and poor in lime and potash, to muds of smaller and more fertile lakes, with big phytoplankton crops and more plentiful supplies of lime and potash in their waters. Certain differences in the absorption spectra of acetone extracts may be related to the balance between organic materials originating inside and outside the lakes, and to the length of time they spend in an oxidizing environment.

## INTRODUCTION

The presence of chlorophyll degradation products in lake muds has been recognized for many years (Vallentyne 1957 gives many references), but little is known of their abundance in muds from lakes of different kinds. Therefore it was thought worthwhile to investigate this matter in the English Lake District, where a wide variety of lakes and tarns has been examined from many different points of view.

For the present purpose five lakes of widely differing fertility were studied:

*Wastwater and Ennerdale Water.* Both are rather deep lakes, in which the hypolimnion contains oxygen throughout the period of stratification. The waters are low in lime and potash, and phytoplankton is sparse.

*Windermere, North Basin.* A large deep lake, the hypolimnion of which remains oxygenated throughout its existence. The water contains moderate amounts of lime and also of phytoplankton.

*Esthwaite Water.* A smaller shallow lake, in which the hypolimnion becomes completely de-oxygenated near the mud surface. The

water is richer in lime and potash than those above, and the phytoplankton crops are also relatively large.

*Priest Pot.* A small and very shallow pond at the north end of Esthwaite Water, in which oxygen disappears from the water above the mud in summer. The water of this pond is richest of all in lime and potash, and the phytoplankton is also extremely dense. Although no quantitative estimates are available, all through the summer it was necessary to use a Buchner filter to clear the waters for chemical analysis, and even then the rate of filtration was slow.

## METHODS

From the deepest parts of these lakes the surface 5 cm of mud were collected with a Jenkin surface sampler (Mortimer 1942, Fig. 22), and analyzed for chlorophyll derivatives within 24 hours. Plankton and sedimentary debris from Esthwaite Water were also analyzed. The samples were then dried at 100–110°C, and later total carbon and total sulfur were estimated, since the contents of these elements might also be expected to vary in relation to lake fertility.

One to two grams of fresh mud were shaken mechanically for three periods of 5–10 min. with successive 20–30 ml portions of 90% acetone, the extracts being filtered and the residues leached with 90% acetone to give a final filtrate of 100 ml. The plankton samples had to be more highly diluted. Absorption spectra were then measured (at 10-m $\mu$  intervals or less) within a few hours

<sup>1</sup> This work was done while the writer was on the staff of the Freshwater Biological Association, Ambleside, England, and he is indebted to Mr. David Driver for help with the analyses, to Mr. G. J. Thompson and the laboratory staff for collecting the samples, and to Mr. F. J. H. Mackereth for advice on techniques. The statements on phytoplankton abundance are based on discussions with Dr. J. W. G. Lund. The chemical analyses of lake waters given in Table 1 were provided through the courtesy of Mr. Mackereth and Mr. J. Heron.

TABLE 1. *Physical and chemical properties of the lakes and their waters*

	Area kn <sup>2</sup>	Max. depth m	Total salts	in milli-equivalents per liter							
				Na	K	Ca	Mg	HCO <sub>3</sub>	Cl	SO <sub>4</sub>	NO <sub>3</sub>
Wastwater	2.9	79	0.31	0.15	0.01	0.09	0.07	0.05	0.16	0.10	0.006
Ennerdale Water	2.9	45	0.33	0.17	0.01	0.09	0.07	0.05	0.18	0.10	0.005
Windermere North Basin	8.2	67	0.51	0.16	0.01	0.27	0.07	0.17	0.17	0.14	0.018
Esthwaite Water	1.0	16	0.75	0.20	0.02	0.41	0.12	0.31	0.20	0.20	0.013
Priest Pot	approx. 0.01	4.5	1.09	0.25	0.08	0.62	0.15	0.66	0.23	0.19	0.002

in a Unicam SP 500 spectrophotometer, with glass cells and using the solvent as blank. No basic substance was added to the sample to preserve chlorophyll from conversion to pheophytin, as the absorption spectrum of the extract of living planktonic algae showed little evidence of such a conversion in the short time between extraction and spectrophotometry of the sample. Chlorophyll and its derivatives were estimated from the absorption peak at about 667 m $\mu$ , in arbitrary units, one unit being taken as equivalent to an optical density of 0.1 in a 1-cm cell when dissolved in 100 ml of solvent (*cf.* Vallentyne 1955), after subtracting an approximate correction factor obtained by drawing a freehand base-line to the absorption curve between 550 and 750 m $\mu$  (*cf.* Orr and Grady 1957). Aliquots of the muds were then dried at 100–110°C to allow expression of results on the dry weight basis, and these were used for the estimation of total carbon and total sulfur. Carbon was analyzed gravimetrically by dry combustion (free carbonate being absent), and sulfur as sulfate, using a new technique devised by F. J. H. Mackereth (in preparation).

#### RESULTS

As previously indicated, the five lakes differ considerably, and Table 1 shows the range of variation in some of their physical and chemical properties. The contents of total carbon, total sulfur, and chlorophyll derivatives in surface muds from the deepest parts of these lakes are shown in Table 2, from which it appears that all three variables follow similar trends; high values characterize the more fertile lakes with abundant phytoplankton and waters rich in lime and potash. However, on the dry weight basis

TABLE 2. *Chlorophyll derivatives, total carbon and total sulfur in the lake muds*

	Carbon % dry weight	Sulfur % dry weight	Chlorophyll derivatives units per g dry weight	Ratio of optical densities 410/350 m $\mu$
Wastwater	7.2	0.18	0.21	0.7
Ennerdale Water	6.6	0.19	0.24	0.9
Windermere, North Basin	8.7	0.37	0.83	1.0
Esthwaite Water	11.6	0.61	1.37	1.4
Priest Pot	18.9	1.22	6.88	1.8

total carbon increases only about three-fold in passing from the lake mud with the lowest content to that with the highest, while total sulfur increases nearly seven-fold, and chlorophyll derivatives over thirty-fold. Expressed in terms of organic content, sulfur increases from less than 3% of carbon in muds from Wastwater and Ennerdale Water to more than 6% in the mud of Priest Pot. Similarly, in muds from the former lakes there are only about 3 units of chlorophyll derivatives per gram of carbon, while in Priest Pot there are 36 units. These data suggest that the concentration of chlorophyll derivatives in the mud may be a more sensitive index of lake fertility than the concentration of either carbon or sulfur. They do of course refer only to single samples, but others collected later in a slightly different way confirmed the general trend observed in this sequence of strikingly different lakes.

The above levels of chlorophyll derivatives, expressed as units per gram of carbon, may be roughly compared with that of about 250 units of chlorophyll per gram of carbon in the living phytoplankton diatom *Melosira italica subarctica*. After centrifuging and standing for 3–4 days at laboratory temperature, the concentration of chlorophyll de-

rivatives in the dead alga (as measured by the present technique) was only about half the above value. Leaves from three living land plants yielded from 19 to 55 units of chlorophyll per gram of carbon, with the latter figure declining to 21 units of chlorophyll derivatives after the leaves were ground up and stored moist for eight days in an open dish (Gorham 1959). It should be pointed out here that chlorophyll and pheophytin cannot be compared strictly on this basis, since the mere conversion of chlorophyll to pheophytin lessens light absorption of acetone extracts by about 30% (Katz and Shore 1955). The fact that the absorbancy values declined by about 50% in the case of *Melosira* and over 60% in the case of the oak leaves does however suggest some further degradation in addition to the conversion to pheophytin.

In yet another rather crude comparison debris caught over a ten-day period in March, in sediment traps (Tutin 1955) set out at depths of 1 and 11 m in Esthwaite Water, yielded concentrations of chloro-

phyll and its derivatives amounting to 80 and 75 units per gram of carbon, respectively, compared with the 12 units in surface mud. It should be pointed out however that in autumn the debris sedimenting in Esthwaite Water might well include a much greater proportion of terrestrial plant material of lower chlorophyll content.

While the concentrations of chlorophyll derivatives in the various lake muds are of paramount interest, some consideration of the whole absorption spectra of the acetone extracts is not without value. Figure 1 compares spectra from living *Melosira*, dead *Melosira*, debris caught in the Esthwaite sediment trap at 11 m depth, and the top 5 cm of Esthwaite mud, all of these being adjusted to give the same absorption for the red peak between 665 and 670  $m\mu$ , from which the concentrations of chlorophyll derivatives were estimated.

In comparing living with dead *Melosira*, the major change observed is a shift of the maximum absorption peak from about 435  $m\mu$  back to about 410  $m\mu$ , together with a relative increase in the absorption recorded for this major peak. In addition the minor hump at about 620  $m\mu$  shifts back to 605  $m\mu$ , and two new minor humps appear at about 530  $m\mu$  and 505  $m\mu$ . There is also a marked decrease in absorption between about 530 and 490  $m\mu$ . The net result of all these changes is that the spectrum comes to resemble closely that for pheophytin *a*, which Orr and Grady (1957) suggest as the main chlorophyll derivative in marine sediments. In this connection it may also be noted that the plankton spectrum illustrated by Vallentyne (1955) bears a distinct resemblance to those of dead *Melosira* and pheophytin *a*.

The material caught in the sediment traps, which were set out before stratification, clearly includes both chlorophyll and its derivatives, as evidenced by the dual nature of the absorption maximum toward the violet end of the spectrum. This suggests that a good deal of chlorophyll decomposition takes place before the dead plant material is deposited on the mud surface, and J. F. Talling (private communication) has added weight to this conclusion by his observation that after stratification dying

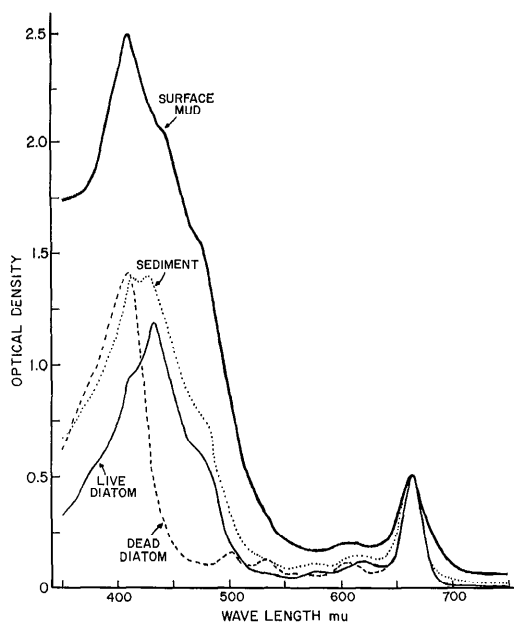


FIG. 1. Absorption spectra of acetone extracts from (a) living and (b) dead diatoms, and from (c) sedimenting debris and (d) surface mud, all collected in Esthwaite Water.

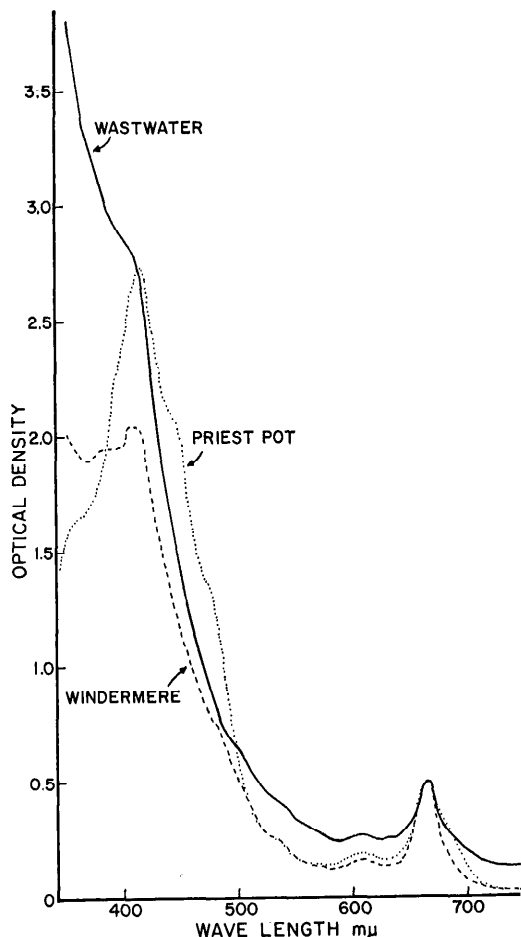


FIG. 2. Absorption spectra of acetone extracts from the surface muds of Wastwater, Windermere North Basin, and Priest Pot.

*Asterionella* populations collected below the thermocline exhibit a shift of the major absorption peak at about 435  $m\mu$  to distinctly lower wave lengths.

If the absorption spectrum of Esthwaite mud is now examined in relation to the others illustrated in Figure 1, it will be seen to resemble that of dead *Melosira*, although it shows a greater absorption at all wave lengths other than the red peak made arbitrarily equal. This greater absorption is especially marked between about 450 and 490  $m\mu$ , where the extract of the living alga absorbs considerable light, but those of the dead alga and of pheophytin *a* do not. The reasons for these discrepancies are not ap-

parent, but substances other than chlorophyll and its derivatives are certainly present in the acetone extracts and must affect the absorption spectra.

Figure 2 compares spectra of muds from three of the five lakes. Considerable differences are evident, particularly toward the violet end of the spectrum where the less fertile lakes exhibit distinctly greater light absorption. This difference is best illustrated by calculating the ratios of optical densities at 410 to those at 350  $m\mu$  (Table 2). These show a steady rise in passing from the infertile lake Wastwater (ratio 0.7) to the very fertile pond Priest Pot (ratio 1.8). The ratio is even higher in both living and dead *Melosira* (ratios 2.9 and 2.3 respectively), while the sediment traps (ratios 2.0 and 2.1) yield values intermediate between those of the algal materials and the muds.

Strong light absorption at 350  $m\mu$  is also noticeable in acetone extracts of terrestrial plants and of woodland soils; and the absorption spectrum of the Wastwater mud extract closely resembles those of aerobic woodland *mull* and *mor* humus layers (cf. Gorham 1959). This fact might be taken to suggest that a good deal of the organic matter in Wastwater sediments enters the lake from its drainage basin, in contrast to either Esthwaite Water or Priest Pot, where the large phytoplankton crops provide a relatively more abundant supply within the lake basins themselves. It is also likely that any plankton sedimenting in the less fertile lakes will be exposed to oxidation for a longer period than in the more fertile waters, which appear to have thinner oxidized microzones at their mud surfaces (cf. Mortimer 1942).

#### DISCUSSION

While these lakes exhibit definite relationships between the amounts of chlorophyll derivatives, total carbon, and total sulfur in their muds, the richness of their waters in nutrients such as lime and potash, and the abundance of their phytoplankton, the exact reasons for these correlations remain obscure. Many factors undoubtedly interact to determine the concentrations of

these mud constituents, among which the following may prove to be of chief importance, and to deserve detailed investigation: (1) the initial contents in the organisms contributing to the lake sediments, (2) the rapidity with which the dead organisms become sedimented and then buried in the mud, (3) the amounts and nature of the mineral materials sedimenting in the lakes at the same time, and (4) the degree of bacterial activity in the lakes and their muds, and hence the concentrations of oxygen there. The present study merely indicates that certain relationships do exist, and it is hoped that they may be of use in interpreting problems of lake development.

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