

## Bacteriochlorophylls in the water of some lakes and their significance in spectrophotometric measurement of chlorophyll *a* concentration

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**Abstract** – Bacteriochlorophylls *d* and *e* may occur in the meta- and hypolimnion of harmonic and non-harmonic lakes. Particularly great amounts of bacteriochlorophylls (to 450 mg BChl *d* m<sup>-3</sup>) were determined in anoxic conditions of the metalimnion of a polyhumic lake. The identification of photosynthetic pigments from the investigated lakes was possible on the basis of the discussed characteristic traits of absorption spectra of their acetone extracts. It was suggested that in the case of spectrophotometric determination of chlorophyll *a* concentration in anoxic water layers, the extinction should be additionally measured at 655, 407, and 466 nm. The  $E_{665}/E_{655}$  ratio <1 indicates the occurrence of bacteriochlorophylls in the water, of BChl *d* when the  $E_{407}/E_{466}$  ratio exceeds one, and of BChl *e* when it is smaller than one.

**Key words:** chlorophyll, bacteriochlorophyll, spectrophotometry, HPLC.

### 1. Introduction

Among the most frequently used, and at the same time most objective characteristics of phytoplankton communities, is the concentration of chlorophyll *a* (Nusch 1980). Owing to the possibility of standardization of a fairly simple measuring procedure, it is used in determining the biomass and potential photosynthetic capability of the phytoplankton. In routine analyses monochromatic methods are most frequently used. They are based on spectrophotometric measurements of the extract of pigments in red light (663–665 nm) before and after its acidification, and on the calculation of chlorophyll concentration using relevant equations. Monochromatic methods permit calculation of the concentration of photosynthetically active Chl *a* and products of its degradation, i.e. phaeophytins. According to Plante-Cuny et al. (1993), the spectrophotometrically determined Chl *a* includes the photosynthetically active Chl *a* and its epimeres. On the other hand, it does not take into consideration chlorophyllides *a* and allomeres of Chl *a*. The phaeophytins determined using the monochromatic method include phaeophytins *a*, pheophorbids *a*, and their allomeres and epimeres. Currently the trichromatic methods of measuring the absorption of extracts at 665, 645, and 630 nm are not recommended (Kawecka and Eloranta 1994), since the obtained Chl *a* concentration is charged with an error resulting from the overlapping absorption spectra of other pigments always present in the extract.

In numerous lakes in layers with a distinctly limited oxygen content bacteriochlorophylls are present beside the chlorophylls (Czeczuga 1965, Takahashi and Ichimura 1968, 1970, Tolstoy and Tóth 1980, Guerrero et al. 1985, Overmann and Tilzer 1989, Hurley and Watras 1991, Górniak 1996). It is manifested by characteristic absorption maxima of the extracts. Bacteriochlorophyll *d* dissolved in acetone showed the greatest absorption at 426 nm (the highest peak in the entire spectrum). In blue light another, lower maximum also appears at 407 nm (70% of the height of the highest peak). In red light two maxima are observed: at 651 nm (about 80%) and 612 nm (about 10%). The acetone extract of a typical BChl *e* shows the highest absorption at 466 nm, shoulders at 444 nm in blue light, and two maxima at 598 nm (about 10%) and 650 nm (25% of the maximum height). The absorption maxima of BChl *c* in acetone strongly approximate to those observed with Chl *a* (Otte et al. 1993).

The aim of the study was to evaluate the effect of the occurrence of bacteriochlorophylls on the result of the spectrophotometric measurement of Chl *a* concentration in lake water.

## 2. Material and methods

Water for the identification of chlorophylls was taken from Lakes Smolak and Dgał Wielki in the northern part of the Mazurian Lakeland, from Lake Ślesieńskie near Konin, and from Suchar VII in the Wigry National Park.

Lake Smolak (54°08' N, 21°57' E, alt. 135 m) is a small (5.3 ha) shallow water body (mean depth 2.4 m, maximum depth 5.7 m) without outflow. The greater part of its shore is covered by a *Sphagnum* mat of various width with *Sphagnum recurvum* Pal. Beauv., *Carex lasiocarpa* Ehrhart, *Rhynchospora alba* (L.) Vahl, *Drosera rotundifolia* L., *Scheuchzeria palustris* L., and *Oxycoccus palustris* Pers. (Dziedzic 1998). A thermocline reaching to the bottom (4–5 m) and characterized by a rapid gradient of temperature, appears in summer. In this layer total oxygen depletion and the appearance of hydrogen sulphide are observed (Hutorowicz and Hutorowicz 1998). In 1971–1974 mineral fertilizers (N, P, K, and Ca) applied to the lake brought about great changes in the ionic composition of the water, in the macrophytes, and in the phyto- and zooplankton. Detailed information concerning the experiment was given, among other authors, by Zdanowski (1976) and Zdanowski et al. (1978), the current limnological characteristics being given by Ejsmont-Karabin (1998), Hutorowicz (1998), Węgleńska (1998), and Zdanowski and Hutorowicz (1998).

Lake Dgał Wielki (54°07' N, 21°48' W, alt. 120 m) with a surface outflow, an area of 94.5 ha, 17.6 m maximum and 5.3 m average depth, lies in the catchment of the River Pregoła. In the years 1966, 1970–1975, 1977, and 1985 the lake was stocked with grass carp, *Ctenopharyngodon idella* Val. this reducing the biomass of submerged vegetation from 16 t ha<sup>-1</sup> of the phytolittoral in 1974 to its almost total decline for period of 11 years (Krzywosz 1997).

Lake Ślesieńskie (52°23' N, 18°20' W, alt. 84 m) with an area of 1458.1 ha, 25.7 m maximum and 7.5 m average depth, is one of five lakes of the Konin Lakes included in the open circulating water cooling system of Konin and Pątnów electric power stations. Limnological traits and changes in oxygen and thermal conditions and chemical composition of the lake water were discussed by Zdanowski (1994) and Zdanowski and Prusik (1994).

Suchar VII is the smallest (0.18 ha) polyhumic lake located within the Wigry National Park. Its maximum depth is 2.9 m, and average depth reaching 1.2 m. It lies at a distance of about 500 m east of Lake Kolesne (54°06' N, 23°01' W, alt. 144 m) and is surrounded by a pine-spruce forest. The Secchi disc visibility was 1.4 m on 13 August 1996. The water temperature at the surface was 22.1 °C. The temperature gradient between the surface layer and a depth of 1.0 m was 2.0 °C m<sup>-1</sup>. At greater depth (1.0–2.5 m) it was 4.8 °C m<sup>-1</sup>. A detailed floristic description of the Suchar VII was given by Sobotko (1967), and a hydro-chemical one by Zdanowski et al. (1992).

Water samples were taken on different dates in 1992–1996 from selected depths within 0–16 m. The samples, 0.1–2.0 L in volume, were filtered through Whatman GF/C paper. The extraction of chlorophylls was carried out in 90% acetone during 24 h. The absorption spectrum of the extracts in the range 350–750 nm was determined using a Beckman DU-68 spectrophotometer. The concentration of bacteriochlorophylls and of Chl *a* in the presence of bacteriochlorophylls was calculated using Overmann's formula (1987; quoted according to Overmann and Tilzer 1989). Also Lorenzen's (1967) formulae were used in the work. Data concerning the concentration of Chl *a* and BChl *d* and oxygen content in the water of Lake Smolak on 21 August 1995 were also analysed.

The results of high-pressure liquid chromatography (HPLC) analysis of the extract obtained from a sample taken on 21 August 1995 in Lake Smolak from a depth of 5 m were used in the study. HPLC analysis was performed using a Waters Chromatography system (Waters Chromatography Division, Millipore GmbH, Eschborn, Germany). Pigment preparation and system composition were described by Wilhelm et al. (1995).

### 3. Results and discussion

Acetone extract from the metalimnion of Lake Smolak taken on 20 June (at a depth of 5 m) and 26 July (4 and 5 m) in 1994, and on 2 and 21 August 1995 (at 4 m, and 4 and 5 m, respectively) showed four absorption maxima characteristic of BChl *d* (Fig. 1A). The greatest relative intensity showed a maximum in blue light at 426 nm, a slightly smaller one at 407 nm, and a maximum in red light at 654 nm, the least intensity being observed at 609 nm (Table I). After acidification a characteristic shift in the maxima from 654 to 656 nm and from 609 to 601 nm was recorded in red light while in blue light the maximum at 426 nm disappeared and a shift from 407 to 406 nm was observed (Fig. 1B).

The chromatogram of the extract from the sample taken on 21 August 1995 (at a depth of 5 m) in Lake Smolak evidenced the occurrence of some fractions whose spectrum contained absorption maxima at 408, 427, 612/613, and 652 nm (Table II). Among them two fractions of retention time ( $R_t$ ) equal to 16.078 and 18.212 min (as shown by the height of peaks in the chromatogram) were distinctive in their large content. The absorption spectra permitted their identification as BChl *d*. According to Otte et al. (1993), the absorption spectrum of this pigment showed absorption maxima at 408, 428, 614, and 652 nm. Two low peaks ( $R_t$  12.912 and 20.678 min) represented the fractions of pigments showing traits of Chl *a* or its derivatives. Wilhelm et al. (1995) isolated a Chl *a* fraction of  $R_t$  20.850 min with absorption maxima at 430.5, 581.0, 615.2 and 664.1 nm and a chlorophyll *a* of  $R_t$  4.900 min and absorption maxima at 435.3, 620.1, and 664.1 nm, using the same equipment. Also very small quantities of a fraction ( $R_t$  25.612; Fig. 2, cf. Table II)

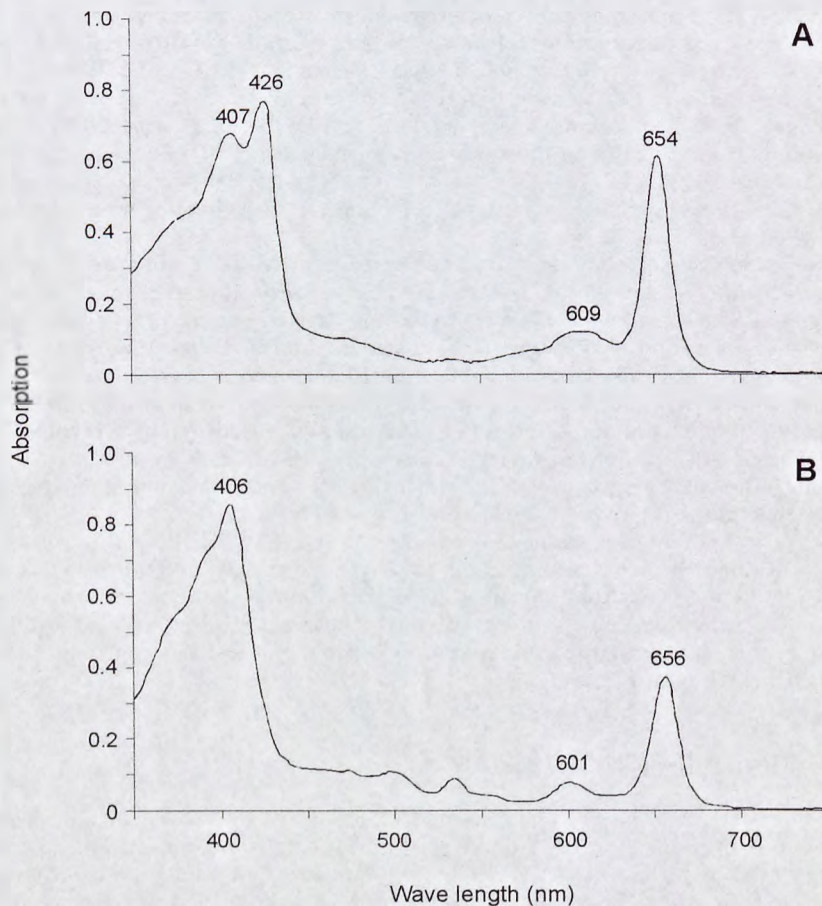


Fig. 1. Absorption spectrum of acetone extract obtained from the seston of Lake Smolak (21 August 1995, at depth of 5 m): A – non-acidified, B – acidified.

Table I. Comparison of the absorption maxima and shoulders (sh) of acetone extracts from free-water samples (present study) with those of bacteriochlorophyll *d* and *e* (after Otte et al. 1993). In parentheses the relative intensities are given.

Sample	Absorption maxima (nm)			
Lake Smolak <sup>1</sup>	407 (0.86)	426 (1.00)	609 (0.16)	654 (0.81)
BChl <i>d</i>	407 (0.70)	426 (1.00)	612 (0.12)	651 (0.81)
Lake Dgal Wielki <sup>2</sup>	442 (sh)	466 (1.00)		653 (0.23)
BChl <i>e</i>	444 (sh)	466 (1.00)	598 (0.06)	650 (0.25)

<sup>1</sup> 21 August 1995, depth 5 m

<sup>2</sup> 10 August 1993, depth 11–16 m

Table II. Retention times ( $R_t$ ) and spectral characteristics of the high-performance liquid chromatography (HPLC) separated pigments from a 0.5-L sample of Lake Smolak (23 August 1995, depth 5 m).

$R_t$ (min)	Absorption maxima (nm)			
12.345	408	426	613	651
12.912		434		663
14.878		427	612	652
16.078	408	427	612	652
17.012		427	613	652
17.912		427	613	652
18.212	408	427	613	652
19.078	408	427	613	652
20.112		427	614	652
20.678		432	583	617/663
24.245	409	435		
25.612	410			665
26.245		436		651

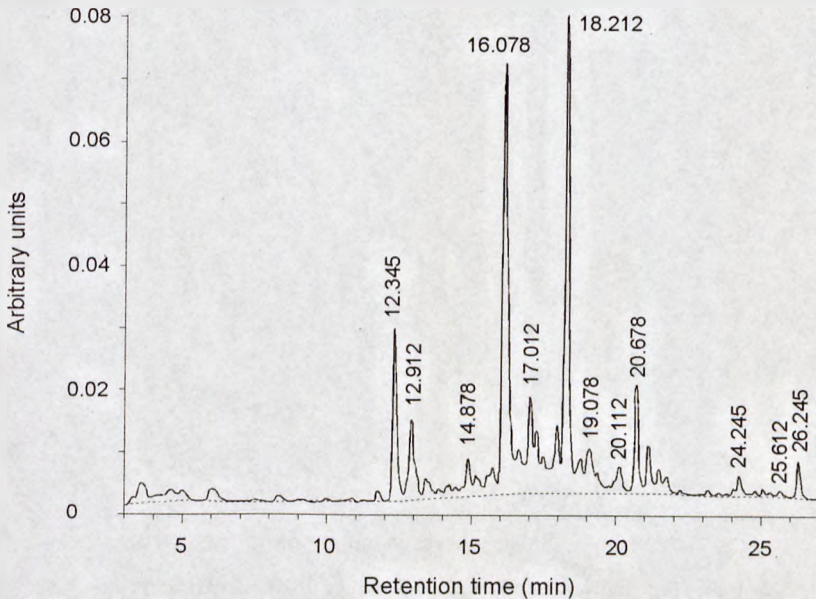


Fig. 2. Elution profile of a complex pigment extract from a water sample from Lake Smolak (21 August 1995, at a depth of 5 m).

whose spectrum manifested traits characteristic of phaeophytin *a* were identified. According to Hoff and Amesz (1991), the maxima of this pigment occurred at 408, 505, 615, and 667 nm. The absorption spectrum of non-acidified extracts from 23 August 1993 (5 m) and 22 August 1994 (4 and 5 m) approximated to that of the acidified bacteriochlorophyll extract characteristic of bacteriopheophytin *d* (cf. Fig.

1B), this most probably resulting from the very great liability of bacteriochlorophylls to phaeophytinization (Porra 1991).

The acetone extract of photosynthetic pigments from Lake Dgał Wielki showed the highest absorption at 466 nm. In red light an absorption maximum appeared at 653 nm (Fig. 3A, Table I). The absorption maxima and their relative intensity suggested the presence of BChl *e*. After acidification of the extract, the maxima appeared in blue light at 442 nm and in red light at 658 nm (Fig. 3B).

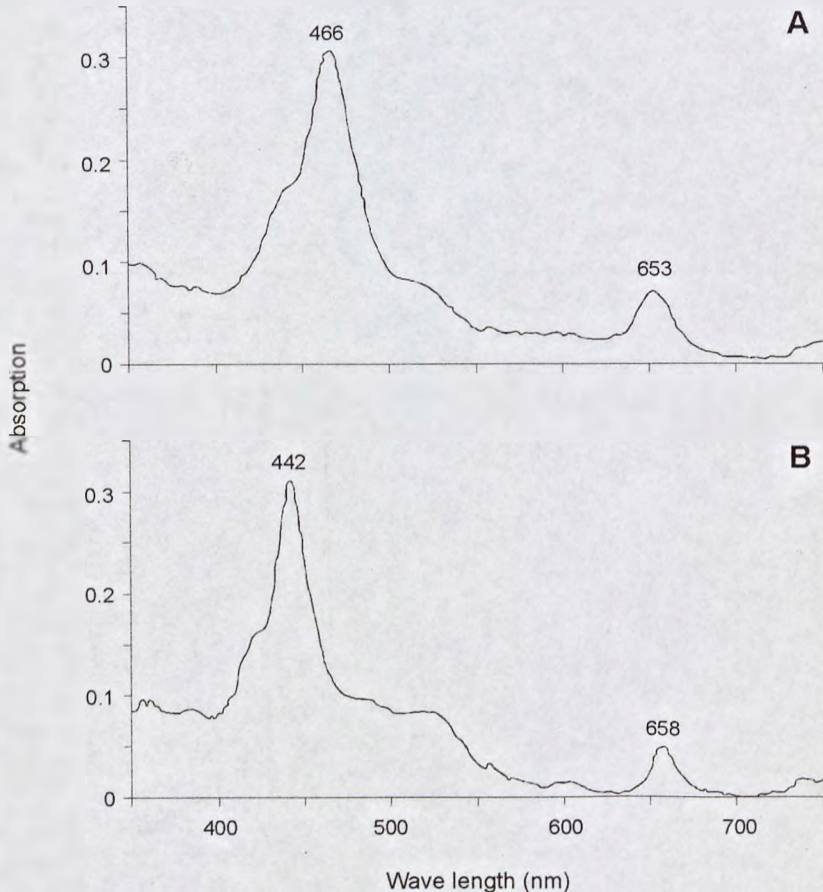


Fig. 3. Absorption spectrum of acetone extract obtained from the seston of Lake Dgał Wielki (10 August 1993, at a depth of 11–16 m): A – non-acidified, B – acidified.

Quantitative determinations of bacteriochlorophyll concentration evidenced that particularly great amounts of bacteriochlorophylls occurred in the water of humic lakes, e.g. Suchar VII and also in Lake Smolak subjected to dystrophication processes (Table II). The occurrence of bacteriochlorophylls, though in considerably smaller amounts, was also noted in the meta- and hypolimnion of such harmonic lakes as Lake Dgał Wielki and in the deepened metalimnion of Lake Slesińskie

(Table II). In Lake Smolak on 21 August 1995 the concentration of Chl *a* increased in the upper anoxic metalimnion reaching a maximum at a depth of 4 m, while a minimum was already noted one meter lower (Fig. 4). A high content of BChl *d*

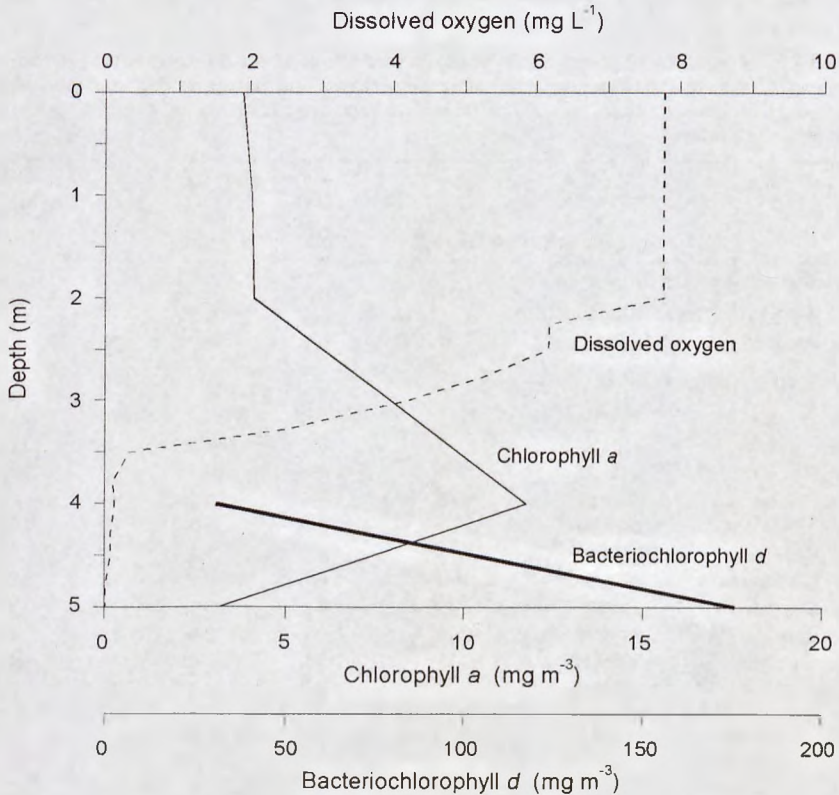


Fig. 4. Changes in the concentration of chlorophyll *a* and bacteriochlorophyll *d* (calculated according to equations given by Parsons and Strickland 1963, and Overmann 1987; cited after Overmann and Tilzer 1989), and the content of oxygen in the water column of Lake Smolak on 21 August 1995 (after Hutorowicz 1998).

was recorded in the metalimnion (at 4 and 5 m). A more comprehensive study on the concentration of these pigments changing with depth was carried out by Hutorowicz (1998) in Lake Smolak in the seasons of 1993–1995, and on bacteriochlorophylls alone in lakes by Hurley and Watras (1991). The occurrence of bacteriochlorophylls permits determination of the concentration of Chl *a* using classic spectrophotometric methods. Using the monochromatic method very large amounts of phaeophytins and a small content of active Chl *a* are evidenced. The systematic error in phaeophytin determination brings about a tenfold overstatement in respect of their concentration (Hurley and Watras 1991). Since this concerns samples taken from the meta- and hypolimnion the results showing very great amounts of phaeophytins and a fairly low concentration of active Chl *a* do not arouse any doubts to the scientist, being fully in accordance with his

expectations. The occurrence of a greater BChl *d* and/or BChl *e* concentration in a sample may be ascertained by reading the extinction at 655, 407, and 466 nm. The ratio of the extinction value at 665 to that at 655 makes it possible to identify the occurrence of bacteriochlorophylls *d* and *e* in the sample. An  $E_{665}/E_{655}$  quotient below one shows the occurrence of bacteriochlorophylls in the water (Table III). In

Tabela III. The content of chlorophyll *a* and bacteriochlorophyll *d+e* estimated according to Overmann's formula (Overmann 1987; after Overmann and Tilzer 1989), and the ratio of extinction at 665 nm to that at 655 nm of selected extracts from water samples taken from the investigated lakes.

Samples			$E_{665}/E_{655}$	$E_{407}/E_{466}$	Chl <i>a</i> (mg m <sup>-3</sup> )	BChl <i>d+e</i> (mg m <sup>-3</sup> )
Lake	Date (day/month/year)	Depth (m)				
Extract spectrum of BChl <i>e</i> type						
Lake Dgał Wielki	10/8/92	11-16*	0.6	0.2	1.0	3.6
Lake Dgał Wielki	10/8/92	6-10*	0.7	0.3	2.8	5.5
Extract spectrum of BChl <i>d</i> type						
Lake Smolak	22/8/94	5	0.4	9.3	34	230
Lake Smolak	21/8/95	5	0.4	162.0	3.3	170
Lake Smolak	23/8/93	5	0.5	10.7	95	220
Lake Smolak	26/7/94	5	0.5	4.3	25	69
Lake Smolak	26/7/94	4	0.5	5.1	70	175
Lake Smolak	21/8/95	4	0.5	13.5	12	32
Lake Smolak	22/8/94	4	0.6	9.2	75	190
Lake Smolak	2/8/95	4	0.6	23.6	14	27
Lake Smolak	23/8/93	4	0.8	2.6	38	23
Lake Smolak	20/6/94	5	0.9	2.2	40	18
Suchar VII	13/8/96	2	0.9	4.9	520	450

\* water samples were taken at every meter within this depth range and then mixed

samples whose chlorophyll *a* content exceeds the concentration of bacteriochlorophylls, the  $E_{665}/E_{655}$  value approximates to one. In samples without bacteriochlorophylls the  $E_{665}/E_{655}$  value exceeds one (Table IV). Determination of the kind of bacteriochlorophyll (*d* or *e*) is possible on the basis of the value of the  $E_{407}/E_{466}$  ratio. If in a sample BChl *d* dominates, the  $E_{407}/E_{466}$  ratio exceeds one, while if BChl *e* dominates this quotient is smaller than one (Table III). The literature describes several attempts at the formation of equations which may be used in calculating the concentration of different photosynthetic pigments in a mixture of Chl *a* and bacteriochlorophylls (Parkin and Brock 1981, Caraco and Puccoon 1986, Overmann 1987; cited according to Overmann and Tilzer 1989), though in most cases the obtained results are incorrect. It seems that the best approximation may be obtained using Overmann's equation. It permits the calculation of Chl *a* concentration (the applied method being similar to equations used in trichromatic methods though with a correction in respect of bacteriochlorophylls) and of the sum of BChl *e* and *d*. The absorption is measured at 651, 663, and 850 nm. The two basic measurements are frequently carried out on the "slopes" of the peak which results from the overlapping absorption spectra of pigments occurring in the sample (Fig. 5).



Tabela IV. Content of photosynthetic active chlorophyll  $\alpha$ , pheophytins, and the ratio of extinction at 665 nm to that at 655 nm of selected extracts from Lakes Smolak, Dgał Wielki, and Ślesińskie (present study), and from Lakes Żarnowiec and Wigry.

Samples			Chl $\alpha$ ( $\text{mg m}^{-3}$ )	Pheophytins ( $\text{mg m}^{-3}$ )	$E_{665}/E_{655}$
Lake	Date (day/month/year)	Depth (m)			
Lake Ślesińskie	20/10/92	0-5*	6.1 <sup>1</sup>	3.5 <sup>1</sup>	1.2
Lake Dgał Wielki	10/8/93	0-5*	3.0 <sup>1</sup>	1.7 <sup>1</sup>	1.5
Lake Smolak	23/8/93	0	19.2 <sup>1</sup>	9.3 <sup>1</sup>	1.4
Lake Smolak	26/7/94	2	9.5 <sup>1</sup>	4.0 <sup>1</sup>	1.4
Lake Smolak	21/8/95	3	7.2 <sup>1</sup>	1.2 <sup>1</sup>	1.4
Lake Żarnowieckie <sup>3</sup>	24/10/94	0-5	2.9 <sup>1</sup>	0.9 <sup>1</sup>	1.4
Lake Wigry <sup>4</sup>	14/8/96	0-3	66.3 <sup>2</sup>	44.1 <sup>2</sup>	1.6

\* water samples were taken at every meter within this depth range and then mixed

<sup>1</sup> Lorenzen (1967)

<sup>2</sup> Nusch (1980)

<sup>3</sup> Hutorowicz and Zdanowski (1996)

<sup>4</sup> Hutorowicz (1980)

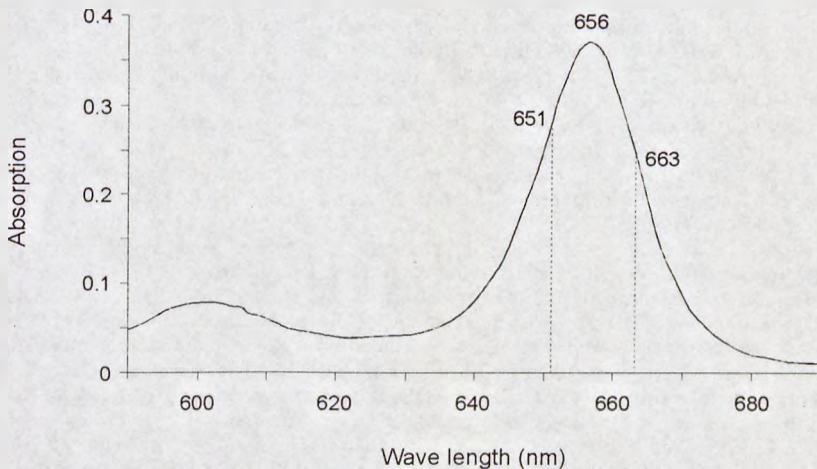


Fig. 5. Absorption spectrum of acetone extract obtained from Lake Smolak (21 August 1995, at a depth of 5 m), in red light, with marked places of extinction measurements for Overmann's formula (Overmann 1987; cited after Overmann and Tilzer 1989).

Thus another inadequacy of spectrophotometric methods is manifested. The results obtained with the popular monochromatic method may be decisively unreliable in the case of samples from deeper water layers where bacteriochlorophylls may chiefly occur. In spite of the evident superiority of chromatographic methods, however, spectrophotometry will certainly be used in numerous research centres owing to its fairly low costs. Spectrophotometers usually are among the basic equipment of laboratories where chemical analyses are carried out, while liquid chromatographs are highly expensive. With the spectrophotometric measurement of the concentration

of photosynthetic pigments, even if their quantities cannot be determined, it is essential to ascertain at least whether bacteriochlorophylls or Chl *a* occur in deeper water layers.

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