Chlorophylls *c* in bottom sediments as markers of diatom biomass in the southern Baltic Sea

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Chlorophyll *c*
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Abstract

Sediments from different regions of the Baltic Sea, collected in the years 1992–1994, were analysed for chlorophyll *c* content by reversed-phase HPLC. For comparison, a series of samples from Spitsbergen fjords were also analysed. Diatom distribution was determined in selected samples. The total chlorophylls *c* in sediments is a very sensitive indicator of the occurrence of chlorophyll *c*-containing algae in the overlying water column. The shape and relative proportions of the chlorophyll *c* peaks in the HPLC chromatogram reflect the presence of fresh and senescent algal cells, as well as the oxygen conditions in the environment. Both benthic and planktonic diatoms are the main source of chlorophylls *c* for the Baltic sediments. Furthermore, the ratio of chlorophylls *c* and *b* to chlorophyll *a* depends on the proportions of diatoms, green algae and blue-green algae in the total Baltic phytoplankton biomass.

1. Introduction

Chlorophylls *c* were found in unicellular chromophyte algae *i.e.* diatoms, dinoflagellates, prymnesiophytes and chrysophytes (Jeffrey, 1972;

Wright and Shearer, 1984; Stauber and Jeffrey, 1988; Kraay *et al.*, 1992). In the last ten years these compounds have attracted the attention of oceanographers as marker pigments for phytoplankton communities in the sea, providing a qualitative and quantitative description of several taxonomic groups of algae in seawater (Gieskes *et al.*, 1988; Buma *et al.*, 1990; Ondrusek *et al.*, 1991).

Owing to the diversity of pigment derivatives in sediments, the determination of chlorophylls *c* in them has come up against many more technical obstacles than was the case with their determination in the water column. Recent advances in analytical techniques, especially the development of high-performance liquid chromatography (HPLC), have enabled such tasks to be undertaken, but even these methods have been found uncertain when applied to so complicated matrix as marine bottom sediments (Neveux *et al.*, 1990). The HPLC system using fluorescence detection is not only highly sensitive, but also provides good reproducibility of chlorophyll *c* determination and permits the simultaneous analysis of most phytylated chlorins (Kowalewska, 1994a).

Literature data relating to the Polish economic zone of the Baltic Sea indicate that the fluctuations in the phytoplankton biomass from one year to the next are large. Similarly, these differences within the annual cycle are also substantial (Pliński, 1992). Nevertheless, it is assumed that in the annual cycle diatoms dominate the other groups of algae (Pliński, 1992; Witek, 1993) and it is these species that, apart from other pigments, contain chlorophylls *c*.

Dinoflagellates are another taxon that should be taken into consideration where chlorophylls *c* are concerned, although their occurrence in the Baltic is much less significant than that of blue-green and green algae. The mean annual biomass of nanoplanktonic flagellates, also containing chlorophylls *c*, makes up about 2–4% of the annual biomass of all autotrophic organisms (Witek, 1993).

The aim of the present work was to examine the occurrence of chlorophylls *c* in the bottom sediments of the Baltic Sea as markers of diatoms in sediments and the adjacent water column.

2. Materials and methods

Samples

Samples were collected in the years 1992–1994. The stations were selected in such a way as to cover a variety of environments. Thus, material collected in the Baltic Sea included sediment samples (0–10 cm surface layer) originating primarily from the deepest areas of the Polish

economic zone (water depth *ca* 100 m), *i.e.* the Gdańsk Deep (G–2) and the Bornholm Deep (P5) (Fig. 1, Tab. 1). At these stations there is a permanent

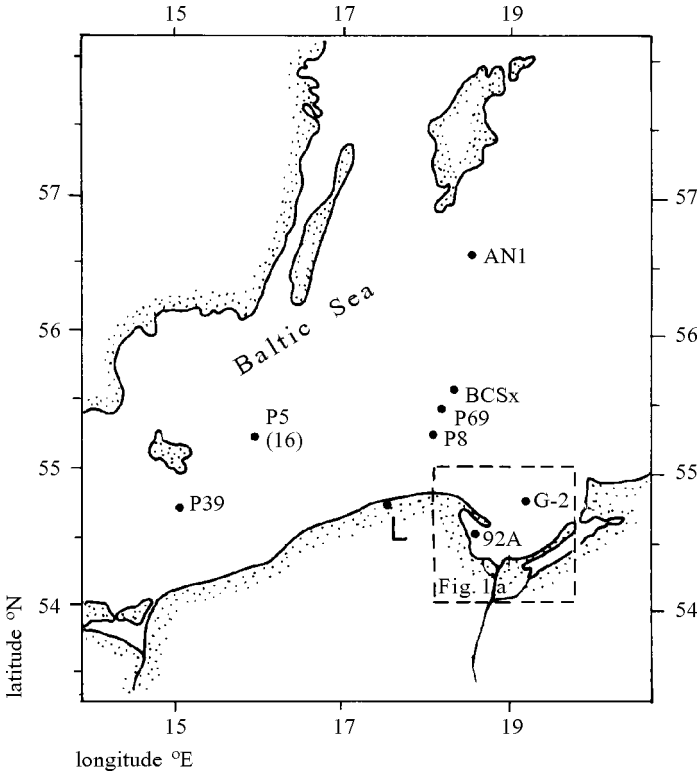


Fig. 1. Location of the sampling stations in the Baltic Sea in 1992–1994

Table 1. Characteristics of the sampling stations in the Baltic Sea

Station	Time	Water depth (thermocline) [m]	Salinity [PSU]	Temperature [°C]
P39	October 1992	46	8.2	8.5
P5	– " –	92(60)	15.6	6.0
AN 1	– " –	70(60)	8.2	4.6
BCSx	– " –	83(78)	11.4	7.9
P8	– " –	78(75)	8.5	5.0
G–2	– " –	108(85)	10.4	5.3
92A	– " –	35	7.4	8.7
P5	August 1994	96(60)	17.8	3.8
P69	– " –	78(75)	11.1	4.8
G–2	– " –	108(85)	13.1	4.4

thermocline, beneath which conditions are anoxic. Station G–2 is influenced by the Vistula, the second-largest river discharging into the Baltic; station P5 is situated in the open sea, nearer the Danish Straits. The sediments there (dark grey clays or black oozes) are characterised by a high organic carbon content (*ca* 5%). At the open-sea stations BCSx, P69 and P8, which differ in water depth and type of bottom deposits, the oxygen conditions were better than those in the deeps. Station AN1 is situated at the edge of the Gotland Deep. Finally, shallow and eutrophic regions were represented by station 92A, located in Puck Bay, where a seasonal thermocline occurs.

The other series of samples included sandy sediments collected in the coastal zone of the Gulf of Gdańsk (0–1 cm surface sediment layer, water depth 0.5 m) (Fig. 1a), as well as on the beach itself (a layer 5–10 cm deep), 3–7 m from the water line (Fig. 1). These sediments were composed of fine- to medium-grained sand and were inhabited by benthic diatom taxa which, especially in winter, constitute the predominant algal biomass in this region (Pliński *et al.*, 1985, 1992).

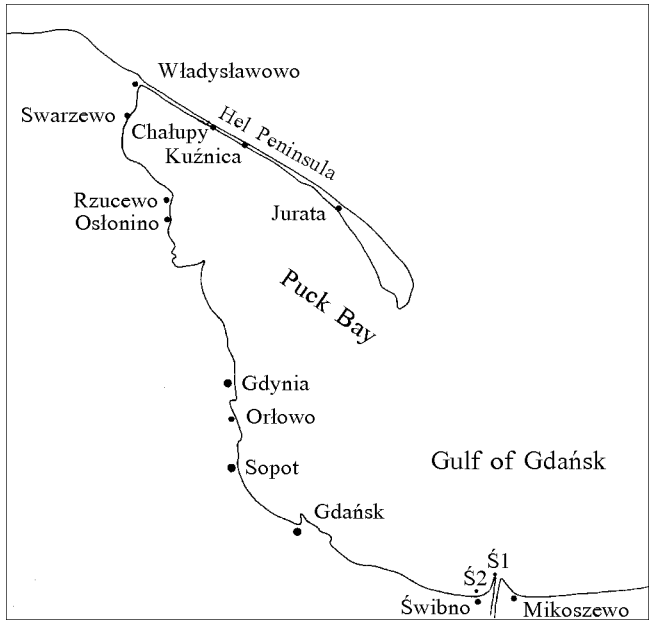


Fig. 1a. Location of the sampling stations in the Gulf of Gdańsk, in December 1993 (see Fig. 1)

For comparison, bottom sediments (surface layer 0–20 cm) were also collected at a water depth of 40–75 m in July 1994 from the oxic regions of the Greenland Sea, off the Spitsbergen coast, where in the annual cycle chlorophyll *c*-containing algae predominate.

Except at the shore sites, all the sediments were collected with a Niemistö core sampler of $\Phi = 10$ cm, during cruises of r/v 'Oceania'. After collection, the sediments were divided into layers 1–5 cm thick, transferred to polyethylene containers, frozen immediately at –20°C and stored at this temperature until analysis, though for no longer than three months.

Extraction of chloropigments

The extraction procedure has been described elsewhere (Kowalewska, 1994a) and in the initial stages was reminiscent of the standard one used in chlorin analysis (Eckardt *et al.*, 1992; Kowalewska, 1994b). In general, 1–15 g of wet sediment was left to thaw. The water was then centrifuged and the sediment extracted with 2 × 15 ml of acetone, each time with 5 min sonication. Only in the case of sediments very rich in organic matter were more than two extractions done. The combined acetone fractions were re-extracted with benzene in the 15:1.5:13.5 (v/v/v) acetone-benzene-water system. The benzene extract was transferred to a glass vial and evaporated to dryness in a stream of argon. In this state the sample was stored in a deep-freeze, usually for no longer than two weeks. Immediately prior to HPLC analysis it was diluted with acetone.

High–performance liquid chromatography

The detailed analytical procedure has been described previously (Kowalewska, 1994a). The algal or sediment extract dissolved in a small amount (100–1000 μl) of acetone was injected (20 μl) into a chromatograph system (Knauer, Germany) equipped with a fluorometric detector (Shimadzu, type RP–551, bandwith = 15 nm, band accuracy = 5 nm) and a diode-array detector (Chrom-a-Scope). A Merck Lichrospher 100 RP18 column (250 × 4 mm, 5 μm) and precolumn (4 × 4 mm, 5 μm) were used in the acetone-water gradient system. The respective excitation and emission wavelengths were 440 and 630 nm. At low chlorophyll *a* concentrations the excitation and emission wavelengths were altered to 430 and 660 nm respectively, after 20 min of chromatography.

Quantitative data of total chlorophyll *c* content were obtained from the calibration curve determined for standard extracts from fresh and deteriorated diatom laboratory cultures (*Cyclotella meneghiniana*) on the basis of spectrophotometric measurements (Shimadzu, type UV–1202) at 630 nm and the integrated peaks of the diode-array chromatograms. The mean extinction coefficient of chlorophylls *c* at 630 nm was assumed to be 40 l g^{-1} cm^{-1} (Jeffrey and Humphrey, 1975). The lowest concentration

determined for chlorophylls *c* was 4 ng ml^{-1}. Reproducibility was about 1% (at I = 200 better than 0.5%, I – intensity of fluorescence signal).

Identification and counting of diatoms

Diatoms were counted and identified according to the procedure described by Witkowski (1990, 1994). Prior to analysis, samples were heated gently in concentrated hydrogen peroxide (30%) to remove organic matter and then rinsed several times with distilled water. Permanent diatom slides were mounted in Naphrax. The taxonomic diatom analyses were carried out by means of a Biolar Plan (Polish Optical Works) microscope using an oil immersion 100x objective. In each sample 300 to 500 cells were counted and the abundance of the diatom frustules in 1 g of wet sediment (or 1 ml of sediment suspension in water) was calculated. The identification of diatoms was based on the works by Krammer and Lange-Bertalot (1986, 1988).

3. Results

Chlorophyll *c* content in bottom sediments of the Baltic Sea

The contents of total chlorophylls *c* in Baltic Sea sediments are presented in Fig. 2. Distinctly higher contents (up to 1.2 nmol g^{-1} d.w.) were found in the deepest areas of the Polish economic zone (stations G–2 and P5). Comparatively high chlorophyll *c* contents were found in samples from the Gulf of Gdańsk. Both the open-sea Baltic sediments and the coastal ones contained higher levels of chlorophylls *c* than did the Spitsbergen samples (0.0003–0.61 nmol g^{-1} d.w.).

The correlation coefficients of chlorophyll *c* content and chlorophylls *a* and *b* calculated for all the sediments are given in Tab. 2.

In general, it can be stated that all the extracts revealed the presence of chlorophylls *c*, although the shape and retention times of the relevant chromatogram peaks for the samples from various environments were different (Figs. 3, 4, 5 and 6; Tab. 3).

Diatom content in the Baltic bottom sediments

In order to relate the chlorophyll *c* content to the diatom distribution, the number of diatom cells and the species composition were determined for the two series of samples described below.

The first series included sandy sediments collected at a water depth of 0.5 m (0–1 cm surface layer) around the Gulf of Gdańsk in December 1993. In this season diatoms make up the overwhelming majority of the benthic population in this area (Pliński, 1992). The diatom flora in the samples studied revealed features typical of shallow-water assemblages, and were represented by both attached (epipsammic) and motile (epipelic) species,

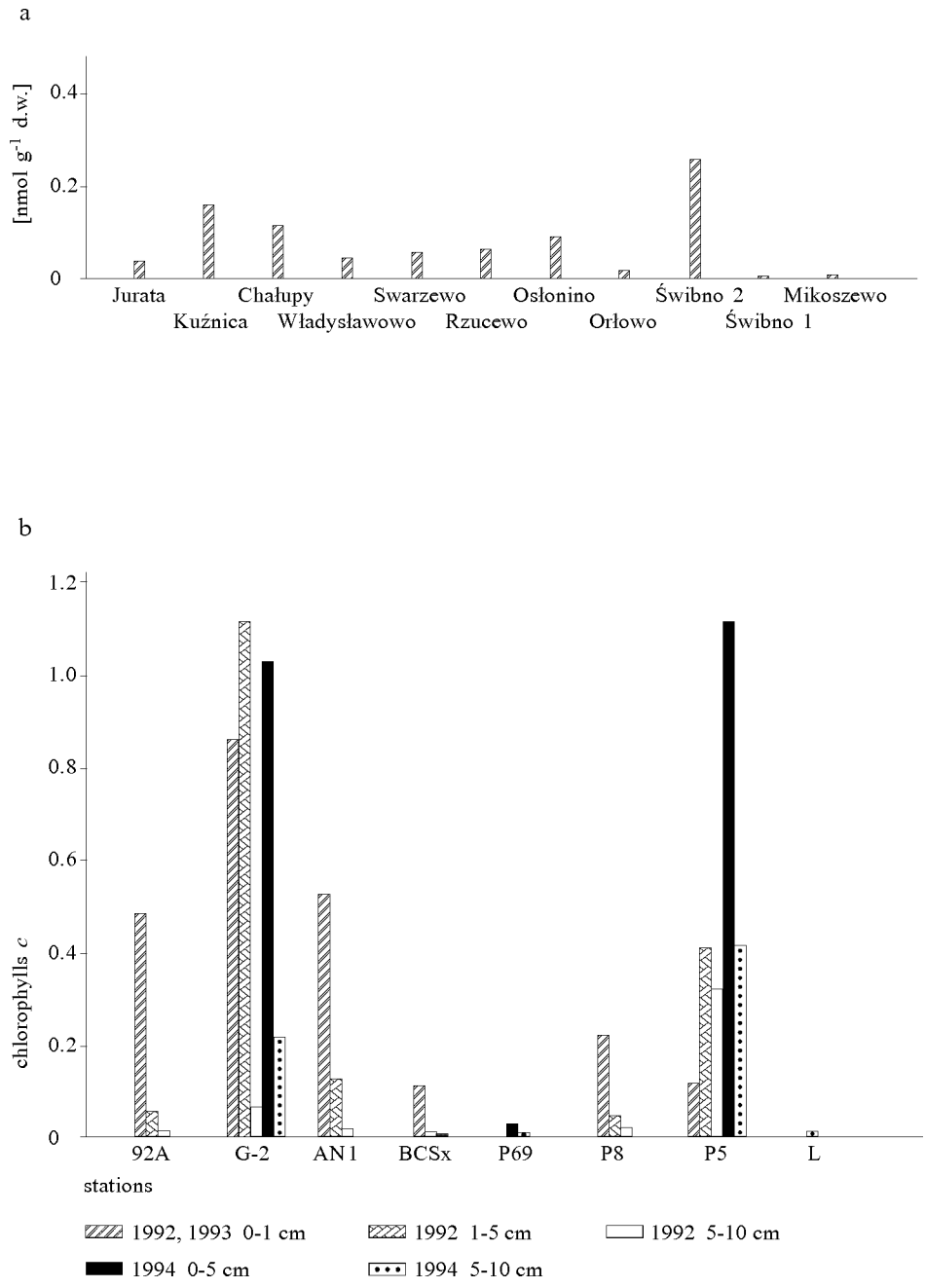


Fig. 2. Total chlorophyll *c* content in the Baltic Sea sediments collected in 1992–1994: in the Gulf of Gdańsk (a); numeration of stations as in Fig. 1a, in the deep-water sediments and at the Lubiatowo beach (b); numeration of stations as in Fig. 1

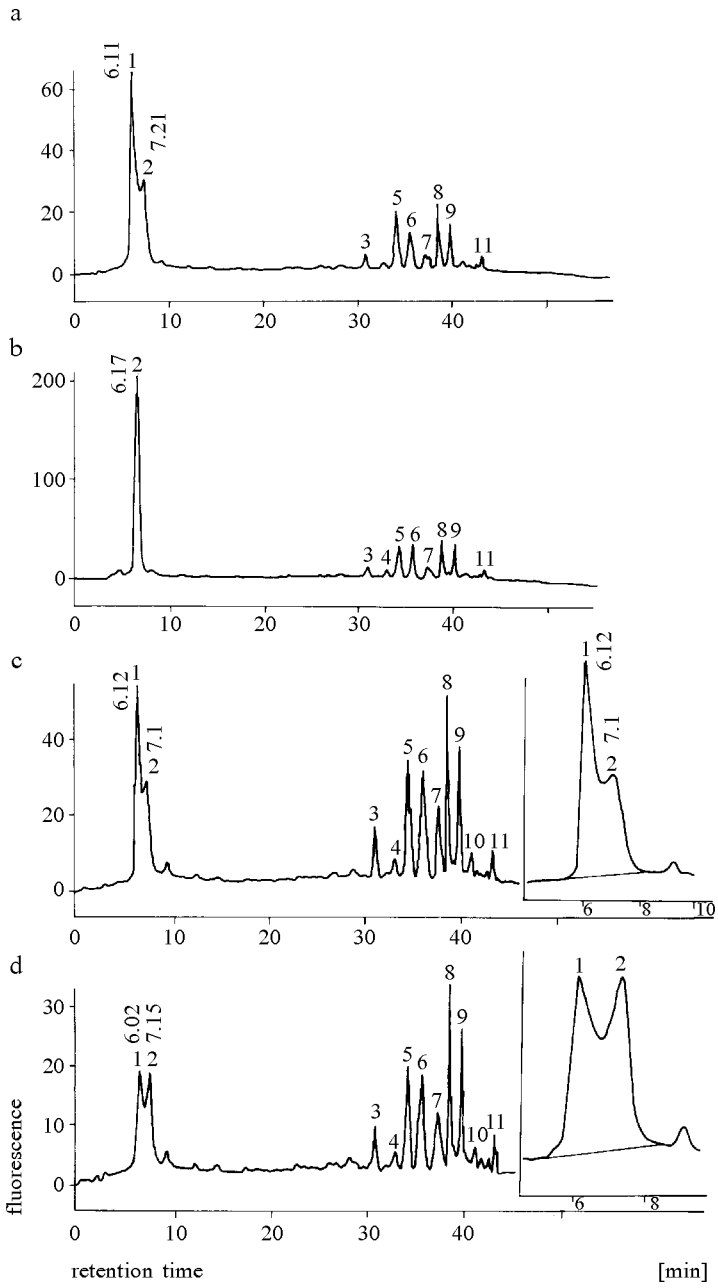


Fig. 3. HPLC fluorescence chromatograms of extracts from deep-sea Baltic sediments: station P5 (0–5 cm (a)), station P5 (5–10 cm (b)), station G–2 (0–5 cm (c)), station G–2 (5–10 cm (d)); 1, 2 – chlorophylls *c*, 3 – chlorophyll *b*, 4, 5, 6 – chlorophylls *a*, 7 – phaeophytin *b*, 8 – phaeophytin *a*, 9 – pyrophaeophytin *a*, 10, 11 – steryl chlorins

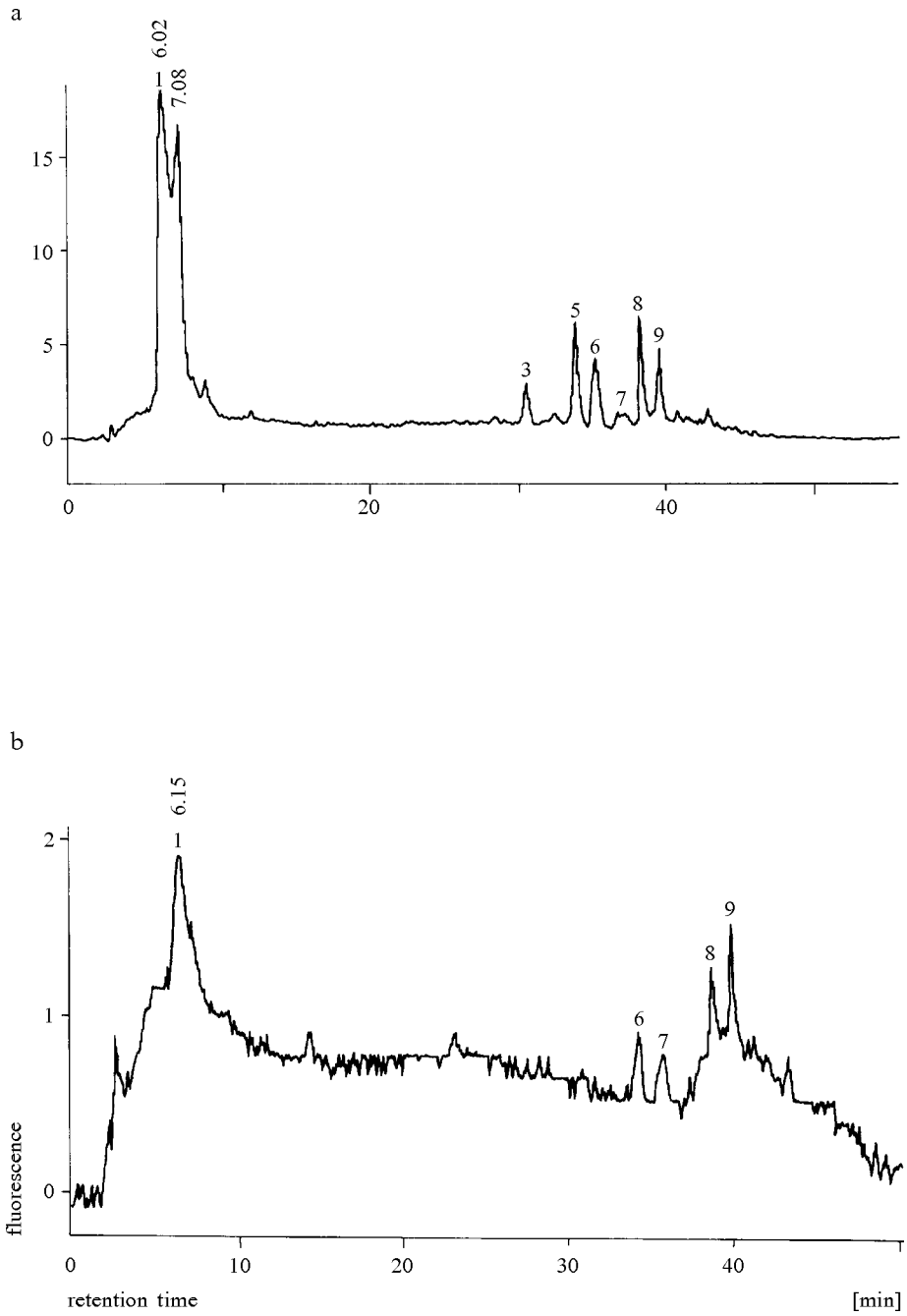
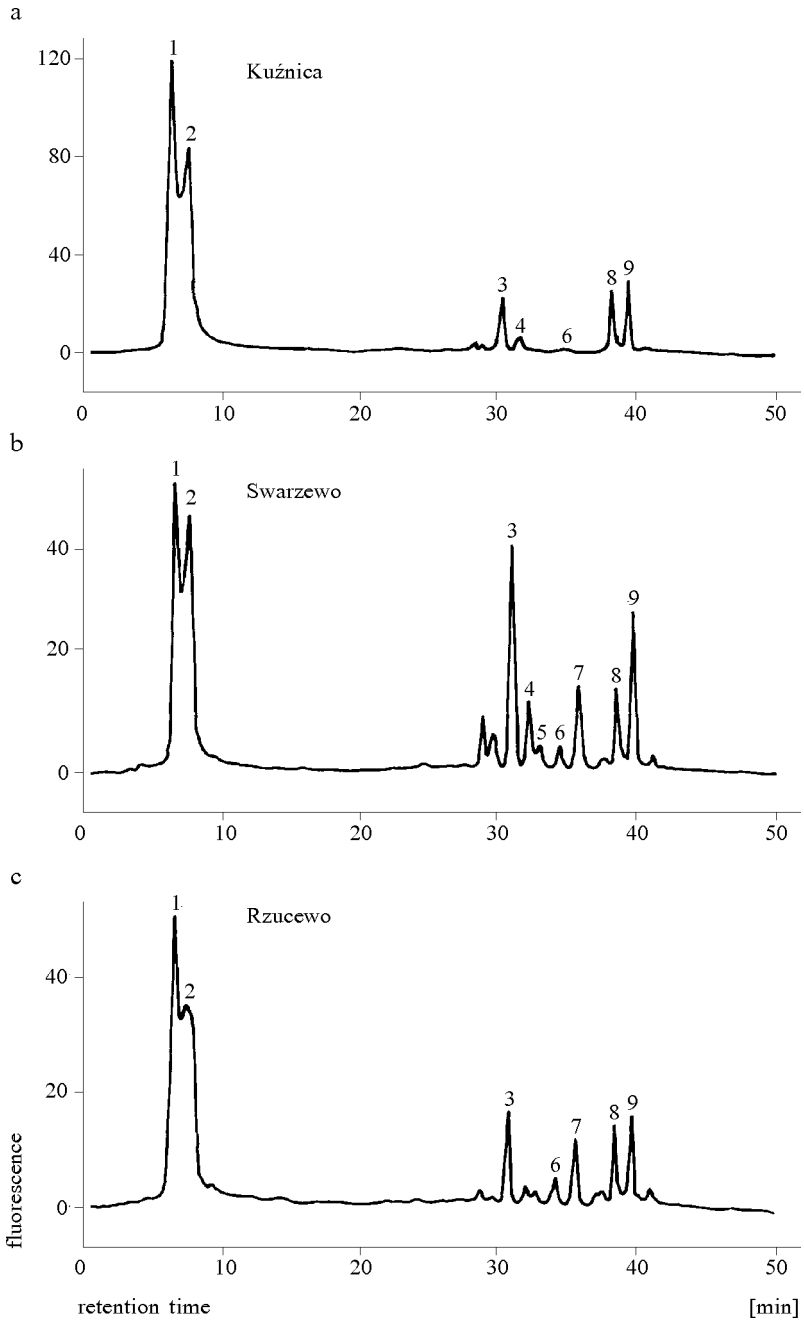


Fig. 4. HPLC fluorescence chromatograms of extracts of sediments from station P69 (0–5 cm (a) and 5–10 cm (b)) collected in 1994; numeration of peaks as in Fig. 3


a
Kuźnica
b
Swarzewo
c
Rzucewo
fluorescence
retention time
[min]

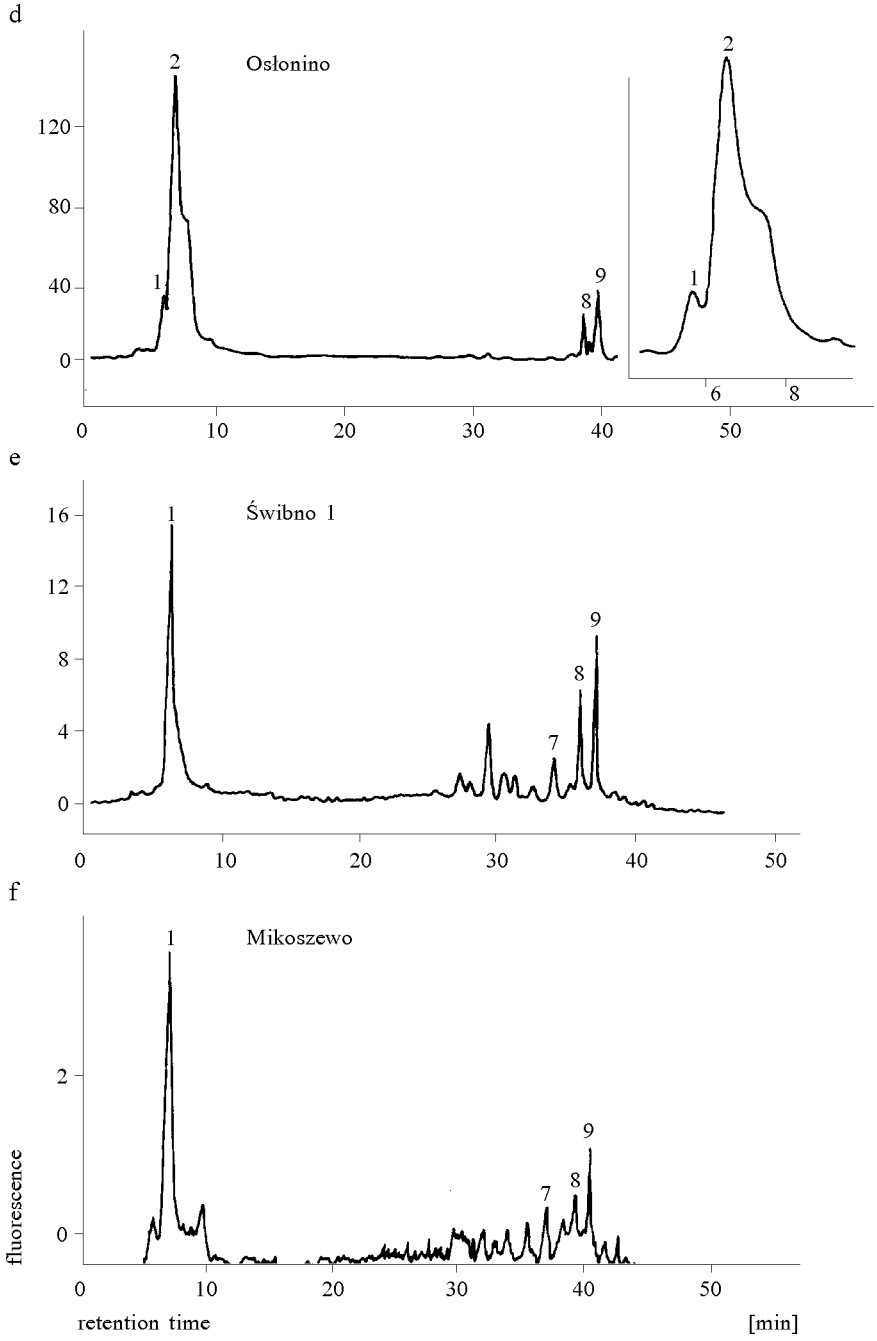


Fig. 5. HPLC fluorescence chromatograms of extracts of the samples collected from different stations (a–f) in the coastal zone of the Gulf of Gdańsk in December 1993 (Fig. 1a); numeration of peaks as in Fig. 3

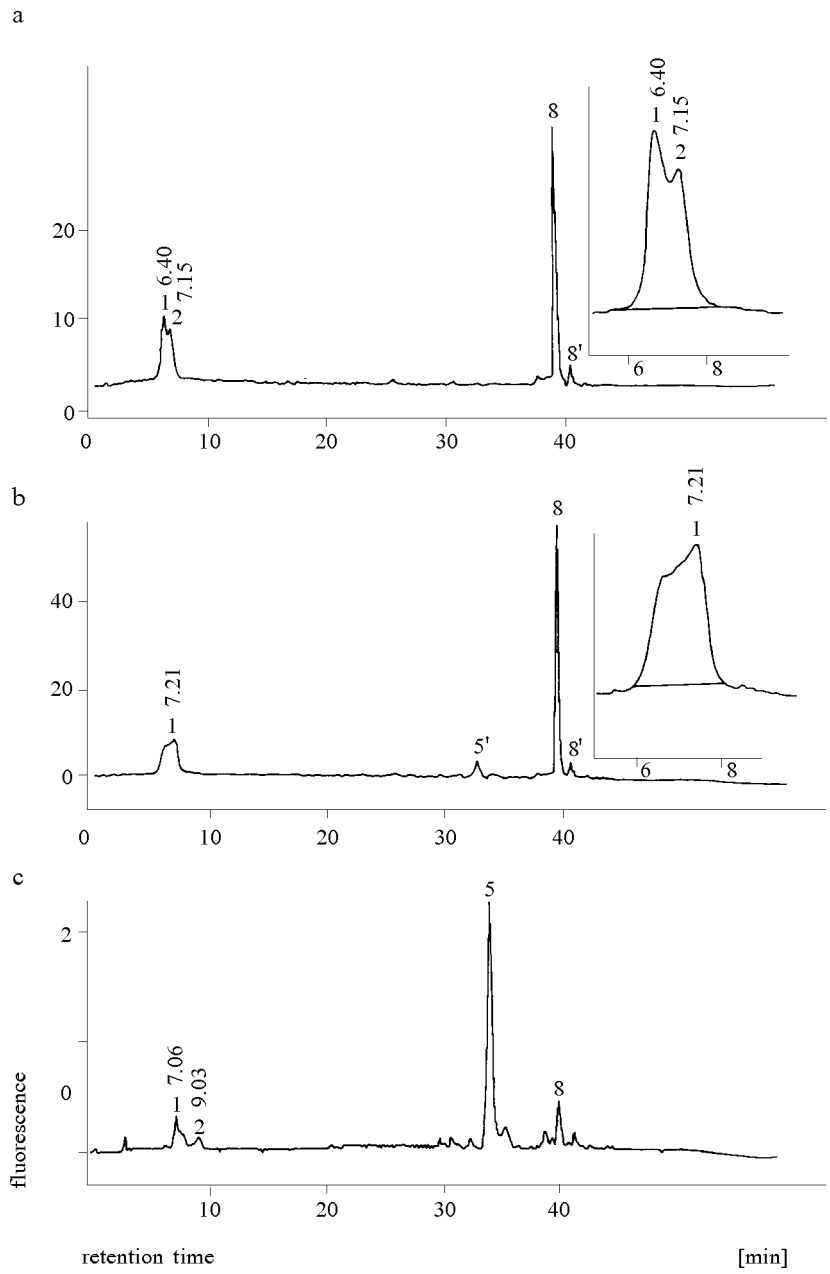


Fig. 6. HPLC fluorescence chromatograms of extracts from sediments collected in Spitsbergen fjords in July 1994: Fjortende Julibreen, water depth 50 m, S = 33.9 PSU, t = –0.14°C, 1–2 cm layer (a), 3–4 cm layer (b), Yoldiabukta, water depth 75 m (c), S = 34.3 PSU, t = –1.4°C, 6–8 cm layer; 1, 2 – chlorophylls *c*, 5 – chlorophyll *a*, 5′ – allo-chlorophyll *a*, 8 – phaeophytin *a*, 8′ – epi-phaeophytin *a*

Table 2. Correlation coefficients of chlorophylls *c, b* and *a* content in the samples studied (significance level)

Sediments	Number of samples	chl *c*/chl *a*	chl *b*/chl *a*	(chl *c* + chl *b*)/chl *a*
Baltic Sea				
(1992, October)				
mean	21	0.81(0.001)	0.72(0.001)	0.87(0.001)
0–1 cm	7	0.81(0.001)	0.37(0.1)	0.35(0.2)
1–5 cm	7	0.94(0.001)	0.95(0.001)	0.99(0.001)
5–10 cm	7	0.61(0.001)	0.98(0.001)	0.95(0.001)
(1994, August)				
mean	6	0.84(0.05)	0.97(0.001)	0.996(0.001)
0–5 cm	3	0.79(0.05)	0.96(0.001)	0.999(0.001)
5–10 cm	3	0.70(0.05)	0.99(0.001)	0.956(0.001)
station G–2 (1992, 1994)	5	0.71(0.2)	0.84(0.05)	0.87(0.05)
station P5 (1992, 1994)	5	0.90(0.02)	0.29(0.7)	0.87(0.05)
stations P69, BCSx, P8 (1992, 1994)	8	0.99(0.001)	0.98(0.001)	0.98(0.001)
Gulf of Gdańsk coastal region	11	0.80(0.01)	–0.13	0.15
(1993, December)				
c_2:$c_1 \geq 0.7$	5	0.86(0.05)	–0.44	0.25
c_2:$c_1 < 0.7$	6	–0.29	–0.20	–0.22
Lubiatowo (L) beach	12	0.83(0.001)	–	–
(1994, July)				
Spitsbergen fjords	12	0.93(0.001)	–	–
(1994, July)				

Table 3. Retention times and intensity ratios of chlorophyll *c* peaks in HPLC of extracts from Baltic Sea sediments

Station		Retention time [min] I	II	III	Chlorophyll *c* peak ratios I/II/III
Baltic Sea					
(1992, October)					
P39	(0–1 cm)	5.43,	6.2(sh)*		3.03:1
	(1–5 cm)	5.48,	6.38(sh)		2.70:1
	(5–10 cm)	5.49,	6.2(sh), 6.4(sh)		1.14:1:0.91
P5	(0–1 cm)		6.28, 6.50		1:1
	(1–5 cm)		6.4(sh),	7.04	0.83:1
	(5–10 cm)		6.4(sh),	7.09	0.70:1
AN1	(0–1 cm)		6.1(sh), 6.43		0.70:1
	(1–5 cm)		6.2(sh), 6.50		0.68:1
	(5–10 cm)		6.35		
BCSx	(0–1 cm)	5.35(sh),	6.21		0.18:1
	(1–5 cm)	5.16			
	(5–10 cm)		6.35		
P8	(0–1 cm)		6.25,	7.0	0.67:1
	(1–5 cm)		6.49		
	(5–10 cm)		6.37		
G–2	(0–1 cm)		6.18,	7.30	1:0.54
	(1–5 cm)		6.18,	7.16	1:0.70
	(5–10 cm)	5.41,	6.25(sh)		1:0.33
92A	(0–1 cm)		6.11, 6.4(sh)		1:0.59
	(1–5 cm)		6.28,	7.09	1:1.20
	(5–10 cm)		6.56		
(1994, August)					
P5	(0–5 cm)		6.11,	7.28	1:0.46
	(5–10 cm)		6.17		
P69	(0–5 cm)		6.02,	7.08	1:0.94
	(5–10 cm)		6.15		
G–2	(0–5 cm)		6.12,	7.1	1:0.51
	(5–10 cm)		6.02,	7.15	1:1.03
Gulf of Gdańsk					
(1993, December)					
Jurata	(0–1 cm)		6.23,	7.0(sh)	1:0.28
Kuźnica	(0–1 cm)		6.06,	7.20	**1:0.71**

Table 3. (continued)

Station	Retention time [min] I	II	III	Chlorophyll *c* peak ratios I/II/III
Chałupy (0–1 cm)		6.29,	7.30	1:0.59
Władysławowo (0–1 cm)		6.02,	7.28	**1:0.76**
Swarzewo (0–1 cm)		6.18,	7.19	**1:0.90**
Rzucewo (0–1 cm)		6.11,	7.10	**1:0.69**
Osłonino (0–1 cm)	5.44,	6.38,	7.3(sh)	0.22:1:0.49
Orłowo (0–1 cm)	5.37,	6.28,	7.0	0.12:1:0.40
Świbno 1 (0–1 cm)		6.28,	7.0(sh)	1:0.13
Świbno 2 (0–1 cm)		6.22,	7.16	**1:0.70**
Mikoszewo (0–1 cm)		6.39		
Laboratory culture of *Cyclotella meneghiniana*	5.34,	6.02,	7.0(sh)	1.58:**1:0.75** (1:0.63:0.47)

* shoulder

the former being strongly predominant. Except for the vicinity of the Vistula mouth, these were exclusively autochthonous saltwater forms. The most abundant of them were *Achnanthes delicatula, Opephora olsenii, Ophephora* sp., *Fragilaria sopotensis, Navicula cryptocephala, N. germanopolonica* and *N. paul-schulzii*. At the mouth of the Vistula (station Świbno 1) a significant admixture of freshwater diatoms (*Cyclotella meneghiniana, C. atomus, Stephanodiscus hantzschii* and *S. parvus*) was recorded. Diatoms were frequent to abundant in the material studied. The lowest numbers were recorded close to the run-off from waste-water treatment plants (*e.g.* the station at Jurata), the highest at less polluted stations. The most characteristic feature of this diatom assemblage was the small spread of taxa. The dominant taxa sometimes made up 50% of the total number of cells. The correlation of chlorophyll *c* content and the number of diatom frustules is shown in Fig. 7; the species composition is listed in Tab. 4.

The second series were the deep-water sediments (0–5 cm surface layer) of the anoxic deeps (G–2, P5), the open-sea stations (P69, AN1), where the sediments deposited under oxic conditions contained only a small percentage of organic carbon, and those from the eutrophic Puck Bay (92A), the inner part of the Gulf of Gdańsk. These sediments were predominantly inhabited by planktonic diatom taxa which settled onto the bottom from the water column. The sediments contained whole diatom cells, a certain amount

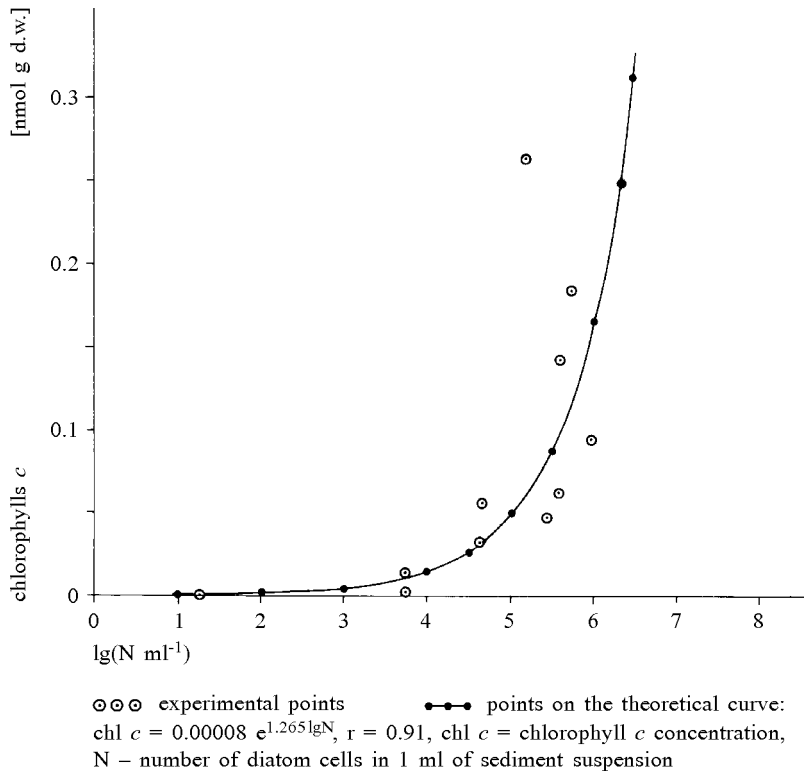


Fig. 7. Correlation of chlorophyll *c* content and logarithm of the number of diatom cells in the coastal sediments collected in the Gulf of Gdańsk in December 1993

Table 4. Diatom species composition in selected Baltic samples

Station	Number of cells	Dominant species (more than 5%)	% (of No. of cells)
Baltic Sea			
(1992, October)			
P5 (0–1 cm)	3.15×10^6 g^{-1}	*Chaetoceros* spp. (resting spores)	70.6
		Thalassiosira cf.*decipiens*	14.7
P5 (1–5 cm)	1.98×10^6	*Chaetoceros* spp. (resting spores)	64.4
		Thalassiosira cf. *decipiens*	14.3

Table 4. (continued)

Station	Number of cells	Dominant species (more than 5%)	% (of No. of cells)
G–2 (1–5 cm)	7.1×10^6	*Thalassiosira* cf. *decipiens*	24.3
		Chaetoceros spp. (resting spores)	21.8
		Chaetoceros spp.	8.2
		Cyclot. choctawhatcheeana	16.8
AN1 (0–1 cm)	9.08×10^6	*Chaetoceros* spp. (resting spores)	34.6
		Thalassiosira cf. *decipiens*	27.5
		Chaetoceros spp. (growing cells)	11.0
		Cyclot. choctawhatcheeana	5.8
92A (0–1 cm)	3.12×10^6	*Thalassiosira* cf. *decipiens*	29.8
		Cyclot. chothawhatcheeana	29.8
(1994, August)			
P5 (0–5 cm)	2.4×10^6 g^{-1}	*Chaetoceros* spp. (resting spores)	34.8
		Thalassiosira cf. decipiens	32.0
		Chaetoceros sp. (growing cells)	8.9
		Skeletonema sp.	5.7
P5 (5–10 cm)	2.19×10^6	*Chaetoceros* spp. (resting spores)	7.8
		Thalassiosira cf. *decipiens*	15.3
		Chaetoceros sp. (growing cells)	4.6
		Chaetoceros paulsenii (resting spores)	6.5
G–2 (0–5 cm)	2.02×10^6	*Thalassiosira* cf. *decipiens*	27.5
		Chaetoceros spp. (resting spores)	19.9
		Cyclot. choctawhatcheeana	17.4
		Achnanthes taeniata	6.0
		Chaetoceros sp. (growing cells)	5.5
G–2 (5–10 cm)	1.29×10^6	*Chaetoceros* spp. (resting spores)	36.4
		Thalassiosira cf. *decipiens*	12.1
		Cyclot. choctawhatcheeana	8.3
		Chaetoceros paulsenii	5.9

Table 4. (continued)

Station	Number of cells	Dominant species (more than 5%)	% (of No. of cells)
P69 (0–5 cm)	< 1	–	
P69 (5–10 cm)	< 1	–	
Gulf of Gdańsk			
(1993, December)			
Jurata	4.2×10^4 ml^{-1}	*Opephora* sp.	15.2
		Navicula paul-schulzii	14.3
		Achnanthes delicatula	12.4
		Achnanthes aff. delicatula	8.8
		Navicula germanopolonica	7.9
		Achnanthes lemmermannii	6.2
Kuźnica	5.4×10^5	*Opephora olsenii*	18.0
		Opephora sp.	15.5
		Achnanthes delicatula	11.7
		Navicula paul-schulzii	9.4
		Navicula germanopolonica	6.7
		Achnanthes lemmermannii	6.1
Chałupy	6.7×10^5	*Fragilaria sopotensis*	29.6
		Nitzschia cf. *microcephala*	14.8
Władysławowo	1.51×10^6	*Opephora olsenii*	31.9
		Fragilaria sopotensis	19.6
		Opephora sp.	12.5
		Achnanthes delicatula	11.2
		Fragilaria subsalina	8.0
Swarzewo	1.23×10^5	*Opephora olsenii*	31.2
		Fragilaria sopotensis	15.0
		Achnanthes delicatula	14.7
		Gomphonema micropus	5.6
Rzucewo	3.8×10^5	*Achnanthes delicatula*	16.8
		Navicula germanopolonica	11.9
		Achnanthes lemmermannii	9.7
		Achnanthes aff. *delicatula*	8.2
		Opephora olsenii	6.0
		Navicula paul-schulzii	5.5
Osłonino	9.5×10^5	*Opephora olsenii*	43.1
		Achnanthes delicatula	14.7
		Achnanthes aff. *delicatula*	5.1

Table 4. (continued)

Station	Number of cells	Dominant species (more than 5%)	% (of No. of cells)
Orłowo	5.4×10^3	*Navicula germanopolonica*	28.3
		Achnanthes delicatula	26.4
		Achnanthes lemmermannii	6.7
Świbno 1	6.0×10^3	*Cyclotella meneghiniana*	23.7
		Stephanodiscus hantzschii	12.9
		Cyclotella atomus	10.1
		Stephanodiscus parvus	9.3
		Achnanthes delicatula	8.7
Świbno 2	1.42×10^5	*Achnanthes delicatula*	23.7
		Navicula cryptocephala	17.4
		Opephora olsenii	16.1
		Navicula protracta	14.0
		Nitzschia microcephala	6.3
		Navicula germanopolonica	5.8
Mikoszewo	3.6×10	*Cyclotella meneghiniana*	> 95.0

of detritus and resting spores. Depending on the locality, the diatom flora was mainly composed of resting spores of *Chaetoceros* spp. or dominated by *Thalassiosira* cf. *decipiens* and *Cyclotella choctawhatcheeana*. Admixture of freshwater forms was minimal, except at station 92A. The number of diatom frustules was higher than in the shallow-water samples. In the deep-water samples diatoms were associated with numerous stomatocysts of *Chrysophyceae* (3–12% of the cell number), a family of nanoplankton species, but in general they were much less abundant than diatoms. The distribution of stomatocysts distinctly increased with depth and were most frequent in the 5–10 cm sediment layer.

4. Discussion

The chlorophyll *c* peaks in the chromatograms of samples from the various sites differed in their retention times and shapes (Tab. 3, Figs. 3–6), even though the reproducibility of these parameters for the same sample was excellent (about 1% of I (fluorescence intensity), $t_R = 0.01$ min). Comparison of diode-array and fluorescence chromatograms of diatom cultures and marine sediment extracts indicates that in sediments, unlike fresh extracts of axenic algal cultures, numerous chlorophyll *c*-like compounds with slight structural differences co-elute (Kowalewska, 1994a). This is why the total chlorophyll *c* content was determined.

The HPLC chromatograms of the samples from the Gulf of Gdańsk coastal zone can be divided into two sets: one is characterised by two distinct, peaks at $t_R = 6$ min of intensity ratio 0.7 (Figs. 5a, b and c), and the other by an asymmetric chlorophyll *c* peak with a shoulder; the ratio of these two unresolved peaks was < 0.7 (Fig. 5d, e). In the former group the correlation coefficient of chlorophylls *c* and chlorophyll *a* was 0.86 (at the 0.001 significance level), in the latter, the correlation was insignificant and the chlorophyll *a* level was equal or close to zero (Tab. 2). The absorption maxima recorded by the diode-array detector for these well-defined chromatograms were 442 and 630. On this basis, the two well-defined peaks presumably correspond to chlorophylls c_1 and c_2, which occur in fresh algal cultures (Jeffrey and Wright, 1987), while in the decomposed detrital material there are numerous derivatives (*e.g.* epi- and oxygenated forms), as in the case of chlorophyll *a* (Kowalewska, 1994b). However, these chlorophyll *c*-like compounds being more polar, they are much more difficult to separate than chlorophyll *a* derivatives. This inference is well reflected in the results for the samples from anoxic Baltic regions, where the sediments contain a greater proportion of undamaged diatom cells and have a well-defined structure (Fig. 3), whereas the chromatograms of the sediments from the oxic environment off Spitsbergen display broad, poorly-defined peaks (Figs. 6b and c).

Apart from this, the chlorophyll *c* concentrations in the Spitsbergen samples are generally lower than those in the Baltic sediments, which concurs with the annual primary production ratio for these two regions.

In conclusion, it may be stated that in spite of the low number of taxa in the samples, it was difficult to correlate the features of the HPLC chromatogram with the species composition. It would seem more reasonable to correlate the chlorophyll *c* peaks with the environmental conditions.

The coefficient of linear correlation between the chlorophyll *c* and *a* contents in the Baltic Sea environment is *ca* 0.8 (0.80–0.84 at the high significance level of 0.001) (Tab. 2). The relevant coefficient for the Spitsbergen samples is 0.93. Such a difference can be explained by the greater proportion of chlorophyll *c*-containing species in the planktonic biomass of the Greenland Sea, which is additionally confirmed by the lack of the chlorophyll *b* peak in the chromatograms of these samples. In the southern Baltic phytoplankton population there is a high proportion of green and blue-green algae, which is reflected in the lower correlation coefficient of chlorophylls *c* and *a*, as well as the significant correlation between chlorophyll *b* and *a*. Correlation of the sum of chlorophylls *c* and *b* with chlorophyll *a* reflects the proportion of diatoms and green algae in the total phytoplankton biomass (Tab. 2). At station G–2, where the influence of freshwater is stronger than

at station P5, there is a lower correlation with chlorophylls *c* and *a* higher one with chlorophyll *b*. The results for the open-sea station P69, where no diatom valves were found, seem interesting, whereas the chromatogram of the surface-layer extract showed distinct chlorophyll *c* peaks, though corresponding to very low chlorophyll *c* concentrations (Fig. 2). This could have been due to the fact that chlorophylls are better preserved in sediment than are fragile diatom cells, and/or that in the samples there had been other phytoplankton species containing chlorophylls *c*, like dinoflagellates or nanoplankton, the remnants of which might have been removed from the microscope slides during the peroxide sample-processing treatment. Similar conclusions are suggested by the high and equal correlation of chlorophylls *c* and *b* with chlorophyll *a* at the open-sea stations (BCSx, P69, P8), where better oxic conditions existed than in the deeps (Tab. 2). Nevertheless, taking into account these low chlorophyll *c* concentration levels and the literature data about the predominance of diatoms, blue-green and green algae over other phytoplankton species (Witek, 1993), one may presume that the proportion of the other chlorophyll *c*-containing species in the annual flux of chlorophylls *c* to the sediments is of minor importance compared to that of the diatoms. The high exponential correlation ($r = 0.9$) of the chlorophyll *c* content with the number of diatoms determined in the samples collected from the Baltic coastal zone in December, where diatoms were predominant ($r = 0.91$, Fig. 7), and with the open-sea samples ($r = 0.93$), is a further indication that diatoms are the main source of chlorophylls *c* for the Baltic sediments.

Chlorophylls *c* are very sensitive markers of chlorophyll *c* containing algae, as can be seen *e.g.* from the results at Mikoszewo or station P69. Moreover, the concentrations of these pigments in sediments can be treated as indicators of the diatoms living in sediments and the overlying waters regarding disturbances caused by local currents and the conditions of deposition. They are rather an indicator of biomass than of the number of cells or species. The ratio of total chlorophylls *c* and *b* to chlorophyll *a* could be a valuable indicator of diatom and green algae biomass.

5. Conclusions

- The total chlorophyll *c* content in sediments is a very sensitive marker of the occurrence of diatoms in a marine area, providing that other chlorophyll *c*-containing species do not make up substantial proportions in the total phytoplankton biomass.
- The shape and intensity ratio of the chlorophyll *c* peaks in the HPLC chromatogram reflect the presence of fresh and deteriorated algal cells, and indirectly, anoxic conditions in sediments.

- The total chlorophyll *c* and *b* content in Baltic sediments can be used as an indicator of diatom and green algal biomass in the adjacent waters.

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References

Buma A. G. J., Treguer P., Kraay G. W., Morvan J., 1990, *Algal pigment patterns in different water masses of the Atlantic sector of the Southern Ocean during fall 1987*, Polar. Biol., 11, 55–62.

Eckardt C. B., Pearce G. E. S., Keely B. J., Kowalewska G., Jaffe R., Maxwell J. R., 1992, *A widespread chlorophyll transformation pathway in the aquatic environment*, Org. Geochem., 19, 217–227.

Gieskes W. W. C., Kraay G. W., Nontji A., Setiapermana D., Sutomo, 1988, *Monsoonal alternation of a mixed and a layered structure in the phytoplankton of the euphotic zone of the Banda Sea (Indonesia): a mathematical analysis of algal pigment fingerprint*, Neth. J. Sea Res., 22, 123–137.

Jeffrey S. W., 1972, *Preparation and some properties of crystalline chlorophyll c_1 and c_2 from marine algae*, Biochim. Biophys. Acta, 279, 15–33.

Jeffrey S. W., Humphrey G. F., 1975, *New spectrophotometric equation for determining chlorophylls a, b, c_1, and c_2 in higher plants, algae and natural phytoplankton*, Biochem. Physiol. Pfl. (BPP), 167, 191–194.

Jeffrey S. W., Wright S. W., 1987, *A new spectrally distinct component in preparations of chlorophyll c from the micro-alga Emiliania huxleyi (Prymnesiophyceae)*, Biochim. Biophys. Acta, 894, 180–188.

Kowalewska G., 1994a, *A HPLC method of determination of chlorophylls in samples of the marine environment*, Chem. Anal., 40, 697–704.

Kowalewska G., 1994b, *Steryl chlorin esters in sediments of the southern Baltic Sea*, Neth. J. Aquat. Ecol., 28, 149–156.

Krammer K., Lange-Bertalot H., 1986, *Bacillariophyceae 1. Naviculaceae*, [in:] *Süsswasserflora von Mitteleuropa*, H. Ettl, J. Gerloff, H. Heynig and D. Mollenhauer (eds.), 2/1, Fischer, Stuttgart, 876 pp.

Krammer K., Lange-Bertalot H., 1988, *Bacillariophyceae* [in:] *Süsswasserflora von Mitteleuropa*, H. Ettl, J. Gerloff, H. Heynig and D. Mollenhauer (eds.), 2/2, Fischer, Stuttgart, 596 pp.

Kraay G. W., Zapata M., Veldhuis M. J. W., 1992, *Separation of chlorophylls c_1, c_2 and c_3, of marine phytoplankton by reversed-phase-C18-high performance liquid chromatography*, J. Phycol., 28, 708–712.

Neveux J., Delmas D., Romano J. C., Algarra P., Ignatiades L., Herbland A., Morand P., Neori A., Bonin D., Barbe J., Sukenik A., Berman T., 1990, *Comparison of chlorophyll and phaeopigment determinations by spectrophotometric, fluorometric, spectrofluorometric and HPLC methods*, Mar. Microbial Food Webs., 4, 217–238.

Ondrusek M. E., Bidigare R. R., Sweet S. T., Defreitas D. A., Brooks J. M., 1991, *Distribution of phytoplankton pigments in the North Pacific Ocean in relation to physical and optical variability*, Deep-Sea Res., 38, 243–266.

Pliński M., Florczyk I., Picińska J., 1985, *Composition and abundance of phytoplankton in the Gulf of Gdańsk proper*, Stud. i Mater. Oceanol., 46, 23–64, (in Polish).

Pliński M., 1992, *An assessment of the effects of pollution in the Polish coastal area of the Baltic Sea 1984–1989. Vegetation*, Stud. i Mater. Oceanol., 61, 182–184.

Stauber J. L., Jeffrey S. W., 1988, *Photosynthetic pigments in fifty-one species of marine diatoms*, J. Phycol., 24, 158–172.

Witek Z. (ed.), 1993, *Structure and function of marine ecosystem in the Gdańsk Basin on the basis of studies performed in 1987*, Stud. i Mater. Oceanol., 63, 33–43.

Witkowski A., 1990, *Diatoms of the Puck Bay coastal shallows (Poland, southern Baltic)*, Nord. J. Bot., 11, 689–701.

Witkowski A., 1994, *Recent and fossil diatom flora of the Gulf of Gdańsk, southern Baltic Sea. Bibliotheca Diatomologica*, J. Cramer, Berlin–Stuttgart, 28, 1–311.

Wright S. W., Shearer J. D., 1984, *Rapid extraction and high-performance liquid chromatography of chlorophylls and carotenoids from marine phytoplankton*, J. Chromatogr., 294, 281–295.