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DETERMINATION OF DEGRADATION PRODUCTS OF PLANT ORIGIN PIGMENTS IN THE BOTTOM SEDIMENTS OF LAKES

OZNACZANIE ZAWARTOŚCI PRODUKTÓW DEGRADACJI BARWNIKÓW POCHODZENIA ROŚLINNEGO W OSADACH DENNYCH JEZIOR

Abstract: The objective of this exercise was to introduce students to one of the methods used for determination of the content of pigments' degradation products in lake bottom sediments characterised by heterogeneous physical and chemical properties. The research revealed that the studied bottom sediments were characterised by diverse content of products produced during degradation of pigments of plant origin, whereas higher concentrations of these compounds were found in sediments containing inorganic carbon (carbonates). The obtained results also indicate that it is important to have the results properly presented, particularly in the case of samples containing the carbonates.

Keywords: exercise for students, plant pigments, bottom sediment

Bottom sediments are an integral component of aquatic ecosystems. They are of particular significance in water reservoirs where deposited for many years they constitute a unique record of history, not only of a given lake, but also processes occurring in its drainage area. The organic matter, both of autochthonous (produced in the pelagic zone) and allochthonous origin (brought from outside a lake basin), is an important element of sediments deposited at the lake bottom. It is heterogeneous mixture of organic compounds, decomposed to a different extent, characteristic of every lake and consisting of *ie* plant pigments and products of their degradation. The main sources of pigments in lakes include communities of phytoplankton and benthic algae, populations of phototrophic bacteria and macrophytes [1-3]. Pigments are easily degraded and the rate of the processes depends on several factors, including acidification, the origin of pigments, the amount of light, the oxygen concentration, the water depth or the rate of consumption by zooplankton, as well as stability of pigments. Processes of degradation may occur both in the water column and after deposition in sediments. In shallow water reservoirs, the rate of organic matter deposition in bottom sediments is faster, and hence the time of exposure to oxidation processes occurring in the water column is shorter. In this type of lakes, however, photooxidation of pigments deposited at the bottom of a reservoir often occurs. Whereas in

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deep lakes, organic matter is exposed to changes occurring in the water column for a longer time [1, 3-5].

The presented exercises are intended for students of biology and related fields, and can be used during classes of hydrobiology or limnology.

The objective of the proposed exercise is as follows:

- introduction of students to one of the methods used for determination of the content of products that are produced during degradation of plant pigments in bottom sediments,
- introduction of students to factors affecting the content of pigments preserved in bottom sediments.

Material and methods

The method presented by Vallentyne [6] and recommended by Wetzel and Likens [7] assumes 24-hour extraction of pigments from wet sediment. To perform the calculations, it is necessary to determine the actual moisture content and the content of organic matter. This usually exceeds the time allowed for this type of exercise. Therefore, this paper presents a simplified method with the use of: (i) dry sediment with a determined content of organic matter and (ii) a shorter time of extraction.

The research material consisted of profundal bottom sediments with heterogeneous physicochemical properties, which were collected from lakes located in the Ilawa Lake District: Jeziorak (sample 3), Kolmowo (sample 24), Stegwica (sample 10) and Gardzien (sample 16). After being delivered to a laboratory, sediments were desiccated at room temperature, homogenized and sieved through a sieve with a mesh diameter of 1 mm. In the material thus prepared, the content of *total carbon* (TC) and *total nitrogen* (TN) was determined using the analyser Vario Max CN (the company Elementar, Germany), whereas the content of inorganic carbon - with the analyser Primacs^{sc} (Skalar, Breda, the Netherlands). Based on these results, the content of *total organic carbon* (TOC) was calculated from the difference $TOC = TC - IC$ (*inorganic carbon*). Also the percentage content of organic matter and calcium carbonate, as well as the mineral non-carbonate fraction were calculated. The content of organic matter was calculated by multiplying the percentage content of total organic carbon by the value of 1.74 (the conversion factor proposed for bottom sediment by Mudroch and co-authors [8] - $TOC \cdot 1.74$), the percentage content of inorganic carbon converted into the calcium carbonate content applying the conversion factor of 8.33 ($IC \cdot 8.33$). Whereas the percentage content of the mineral non-carbonate fraction was calculated from the difference: 100% (the percentage content of organic matter + the percentage content of calcium carbonate). Based on the percentage content of organic matter, calcium carbonate and the mineral non-carbonate fraction, the classification of sediments was performed according to Markowski [9]. Also the pH value of sediment in water (the ratio of 1:5 w/v - 10 g of sediment: 50 cm³ water) was determined applying the electrometric method.

Each Falcon plastic test tube was filled with ca 0.5 g of different sediments and 30 cm³ of 90% aqueous alkaline solution of acetone (2 drops of concentrated NH₄OH solution added to 90% solution of acetone). Test tubes with this content were being shaken for 30 min. The samples were then centrifuged, and the supernatant was filtrated through a filter paper into 100 cm³ glass volumetric flasks fitted with ground glass joints. Another portion of solvent was added into the test tubes with precipitate and then shaken again. After

another 30 min, the extracted precipitate and supernatant were filtered. The precipitate was rinsed repeatedly with small portions of solvent in order to obtain 100 cm³ filtrate. Optic properties of extracts were determined within the visible spectrum (330-800 nm) by a spectrometer Lambda 20 Perkin-Elmer using a 1-cm cuvette. Based on the obtained absorbance values at the wavelength of 750 nm (A_{750} - baseline absorbance) and the maximum of absorption within the chlorophyll area of the spectrum - A_{662} (the absorption maximum can be located between 657 and 666 nm), the content of plant pigment degradation products was calculated and expressed in SPDU (Sedimentary Degradation Pigment Unit) per 1 g of dry sediment weight and per 1 g of sediment organic matter. This value is defined as absorbance of a 1-cm layer of solution at the wavelength of maximum absorption in the red part of the spectrum with baseline absorbance at $\lambda = 750$ nm, in the conditions of extraction and dilution as described above.

Calculations:

$$\text{SPDU/g}_{\text{dw}} = (A_{662} - A_{750}) \cdot [1/\text{weighed amount of sediment (in grams)}]$$

$$\text{SPDU/g}_{\text{om}} = (A_{662} - A_{750}) \cdot [1/\text{content of organic matter in the weighed amount of dry sediment (in grams)}]$$

Owing to a computerized spectrometer, also the surface area below the absorption curve within the range of $\lambda = 330$ -800 nm was used as a measure of the content of pigment degradation products. The obtained results were presented in conventional units of absorbance per one gram of dry sediment weight [$A/\text{nm} \cdot \text{g}_{\text{dw}}$] and per one gram of sediment organic matter [$A/\text{nm} \cdot \text{g}_{\text{om}}$] [10]. Values of correlation coefficients were calculated for the obtained results.

Results

Table 1 presents the main physicochemical parameters of the studied bottom sediments. The sediments were characterised by diverse content of total organic carbon (TOC) and organic matter, ranging from 140.0 to 343.1 g/kg_{dw}. Two of the samples analysed did not contain carbonates, whereas the content of inorganic carbon (IC) in the remaining samples was 8.5 g/kg_{dw} (Lake Jeziorak) and 12.6 g/kg_{dw} (Lake Kolmowo). Also the total content of nitrogen varied within the broad limits: from 16.1 to 36.9 g/kg_{dw} (sediment of Lake Kolmowo and Gardzien, respectively). The pH values for sediments ranged from 4.59 to 6.54. Based on the percentage content of organic matter, calcium carbonate and the mineral non-carbonate fraction, types of sediments were determined according to the classification by Markowski [9]. Sediments from the lakes Jeziorak, Stegwica and Gardzien are fine detritus gytija, classified within the type of organic sediments, whereas the sediments of Lake Kolmowo are clayey gytija representing the mineral type of sediments.

Figure 1 presents spectra of acetone pigment extracts contained in bottom sediments of the lakes. Spectra were normalized to extraction from 1 g of dry sediment weight. The obtained spectra are characterised by a shape typical of this type of material. In all spectra, two well-defined peaks occurred at the wavelengths of ca 410-411 nm and 663-666 nm.

Table 2 presents the initial values of absorbance, the surface area below the absorption curves and weighed amounts, which were used to calculate the content of pigment degradation products in bottom sediments.

Table 1
Basic physicochemical properties of bottom sediments, the percentage of organic matter and kind of sediment

Sample symbol	TOC	IC	TN	pH _{H₂O}	Organic matter	Kind of sediment
	[g/kg _{dw}]	[g/kg _{dw}]	[g/kg _{dw}]		[%]	
3	200.1	8.5	26.9	6.40	34.8	fine detritus gytja
24	140.0	12.6	16.1	6.54	24.4	clayey gytja
10	278.7	0.0	24.6	4.59	48.5	fine detritus gytja
16	343.1	0.0	36.9	5.37	59.7	fine detritus gytja

3 - lake Jeziorak, 10 - lake Stegwica, 24 - lake Kolmowo, 16 - lake Gardzien

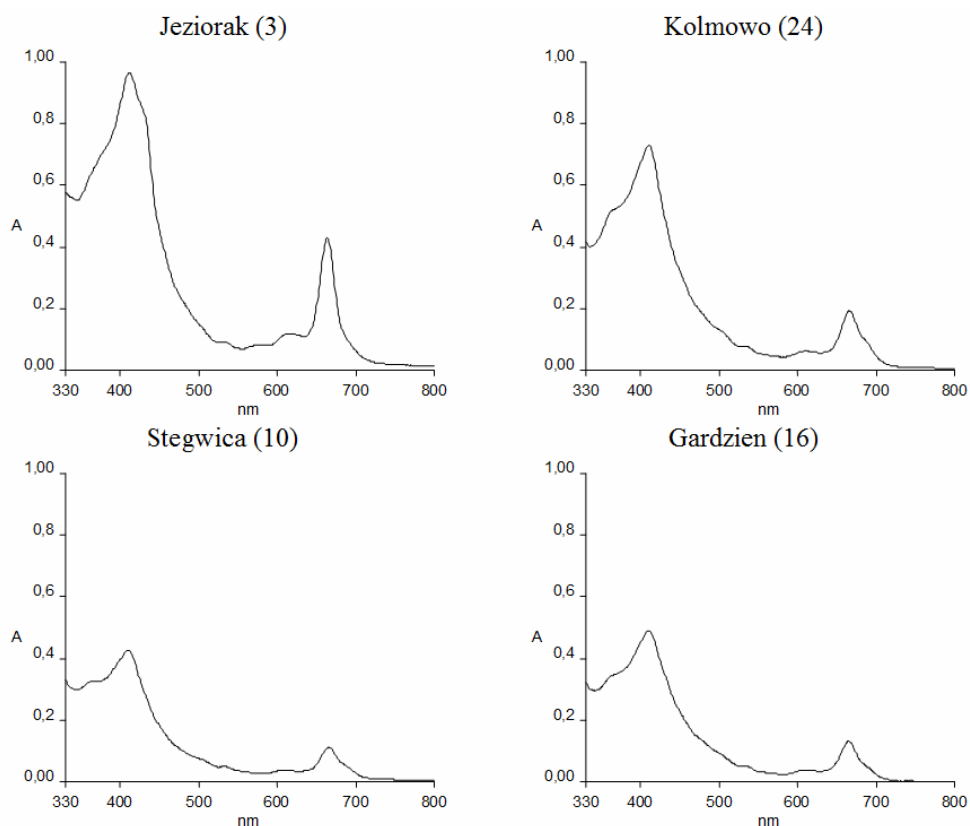


Fig. 1. Standard spectra of acetone pigment extracts from bottom sediments of the lakes

The sediments from lake Jeziorak (sample 3) were characterised by the highest content of pigments, both per 1 gram of dry sediment weight (SPDU/g_{dw}) and per 1 gram of organic matter (SPDU/g_{om}), *ie* 0.41 and 1.19, respectively (Table 3). Also when the surface area below the absorption curve was used as a measure of pigment content per 1 gram of dry sediment weight (A/nm·g_{dw}), the sample from lake Jeziorak had the highest content of pigments. When applying the conversion into 1 gram of sediment organic matter, the sample from lake Kolmowo was characterised by the highest content of pigment degradation

products - $373.08 \text{ A/nm} \cdot \text{g}_{\text{om}}$. It is noteworthy that the content of pigments in sediment from lakes Stegwica and Gardzien is low, despite the high content of organic matter. Therefore, it can be inferred that the conditions prevailing in the sediment of these lakes, mainly the low pH value, could be unfavourable to the preservation of pigments. Based on the research conducted on lake bottom sediments in Drawieński National Park and Wel Landscape Park, it was found that bottom sediments characterised by low pH values, despite the high content of organic matter, had low concentrations of pigments [11, 12]. It should also be taken into account that Stegwica and Gardzien are shallow lakes, and thus photooxidation and good oxygenation of waters (which accelerate the oxidation processes) could be yet other factors accelerating the degradation of pigments. The presence of oxygen also favours the occurrence of numerous benthic species, which by mixing the sediments induce their resuspension and re-exposure to oxidation processes in the water column, and by feeding on organic matter they cause further degradation of pigments.

Table 2

The absorbance values of acetone extracts and the calculated area under the curve of absorption (in arbitrary absorbance units)

Sample symbol	Sample weight	A_{662}	A_{750}	Area
	[g]			[A/nm]
3	0.5251	0.2269	0.0095	67.35
24	0.5310	0.1024	0.0046	48.34
10	0.5468	0.0608	0.0037	30.14
16	0.5140	0.0667	0.0011	31.34

3, 10, 16 and 24 as in Table 1

Table 3

The content of plant origin pigments in the bottom sediments

Sample symbol	SPDU/g _{dw}	SPDU/g _{om}	A/nm·g _{dw}	A/nm·g _{om}
3	0.41	1.19	128.26	368.56
24	0.18	0.75	91.03	373.08
10	0.10	0.22	55.12	113.65
16	0.13	0.21	60.97	102.12

3, 10, 16 and 24 as in Table 1

Summary

Based on the obtained results, it was ascertained that the studied bottom sediments were characterised by diverse content of products produced during degradation of pigments of plant origin, whereas higher concentrations of these compounds were found in sediments containing inorganic carbon (carbonates). The content of pigment degradation products expressed in conventional units of absorbance per 1 gram of sediment organic matter [A/nm·g_{om}] was significantly positively correlated with inorganic carbon content determined in sediments. The obtained results also indicate that it is important to have the results properly presented, particularly in the case of samples containing the carbonates.

The advantage of the presented exercise is the possibility of its modification according to the plan of classes and available equipment. Firstly, wet sediments can be used for extraction, but this requires determination of the actual moisture content and the content of organic matter. Additionally, a measurement of sediment pH can be included. Secondly,

a simple spectrometer can be used for the described exercise, and the obtained results can be presented as calculated per SPDU.

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OZNACZANIE ZAWARTOŚCI PRODUKTÓW DEGRADACJI BARWNIKÓW POCHODZENIA ROŚLINNEGO W OSADACH DENNYCH JEZIOR

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Abstrakt: Celem opracowanego ćwiczenia było zapoznanie studentów z jedną z metod oznaczania zawartości produktów degradacji barwników w jeziornych osadach dennych o zróżnicowanych właściwościach fizykochemicznych. Badane osady dennie charakteryzowały się zróżnicowaną zawartością produktów degradacji barwników pochodzenia roślinnego, przy czym wyższe stężenia tych związków otrzymano w osadach zawierających węgiel nieorganiczny (węglany). Uzyskane wyniki wskazują również, że istotną rolę odgrywa sposób przedstawiania wyników, przy czym jest to szczególnie ważne w przypadku próbek zawierających węglany.

Słowa kluczowe: ćwiczenie dla studentów, barwniki roślinne, osady dennie