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# CHARACTERISTICS OF THE HUMIC ACIDS (HA) FROM LAKE SEDIMENTS AS EFFECT OF UV-VIS SPECTRAL PARAMETERS APPLICATION

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**Abstract**. In this work, samples of humic acids (HA) extracted from six lake sediments were investigated. HA were isolated with method of two-stage successive extraction (A) – extraction with an neutral solution 0.1 mol $L^{-1}$ $Na_4P_2O_7$ and (B) – extraction with an alkaline solution 0.1 mol $L^{-1}$ NaOH). It is observed that HA-B are characterized by relatively higher H and O contents than it is observed in HA-A. Also the stable fraction of HA-B are characterized by higher oxidation degree than HA-A fraction loosely associated with mineral part. Analysis of optical parameters showed that the labile fractions of HA-A are characterized by a higher capacity to absorb the UV-Vis radiation than the stable HA-B, as indicated by higher specific absorbance coefficients $\varepsilon_{280}$ and $\varepsilon_{600}$. The results indicated that the HA-B extracted by alkaline solution are a more complex structure, larger molecular size and molecular weight and higher condensed of aromatic nuclei than HA-A separated by neutral pyrophosphate solution.

**Key words**: humic substances, lake sediments, UV-Vis spectroscopy

## INTRODUCTION

The most common group of organic compounds in nature are humic substances (HS). They are organic residues of dead organisms transformed in a specific way. HS are formed as a result of heteropolycondensation of phenolic, protein and carbohydrate structures on different degrees of degradation. Represent a very complex mixture of many compounds [Aiken et al. 1985, Stevenson 1994, Hayes 1998]. Chemical structure of HS has been a subject of research for many years. However the chemical structure of

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those compounds is difficult to be uniquely defined as the compounds consist of a big number of chemical units and their percentage contribution in HS molecules depends on habitat and changing bioecological conditions.

UV-Vis spectroscopy has been used for a long time to study HS as the method is non-destructive and a measuring equipment is widely accessible and easy to operate.

HS indicate strong UV-Vis (190–800 nm) absorption, especially within UV range due to the presence of aromatic chromophores and/or other organic groups in HS structure [Hautala et al. 2000, Chen et al. 2002, Uyguner and Bekbolet 2005, Fuentes et al. 2006, Purmalis and Klavins 2013, Bellera et al. 2015, Yakimenko et al. 2016]. However, the studies do not explain the chemical structure of HS, they bring some conclusions on the nature of functional groups and their structural configuration in HS [Fründ et al. 1989]. Previous studies have shown relationships between molecular size, spectroscopic properties and photoreactivity of HS [Martin-Neto et al. 1998, Chen et al. 2002].

In addition the UV-Vis spectroscopy is also a valuable component to information obtained by other analytical techniques the nature and character of the HS to determine [Kalbitz et al. 2000, Milori et al. 2002, Fuentes et al. 2006, Giegużyńska 2009].

Since the isolation extractants used affect significantly the yield and properties of the obtained HS [Zaccone et al. 2007], the aim of the study was to compare UV-Vis spectral properties of two HA fractions obtained on the way of two-stage extraction.

## MATERIALS AND METHODS

### Sample collection and isolation of humic acids

The studies were carried out on humic acids (HA) extracted from bottom sediments sampled from six Lobelia lakes located on Western Pomerania (fig. 1). The lakes differ in trophy, geological structure and the way of their basins use.

The sediments were sampled from the surface layer of the lakes up to 15 cm deep in profundal zone. The samples were dried and broken up and then selected on sieve meshes with diameter of 1 mm. After that, HA were extracted with modified Wilk and Boratyński method of two-stage successive extraction [Giegużyńska 2009]. Using this method, the sample of bottom sediment was dissolved in neutral 0.1 mol $L^{-1}$ $Na_4P_2O_7$ (extraction A) three times repeated. Obtained solution of HS was acidified with 4 mol $L^{-1}$ $H_2SO_4$ to pH~1.5 in order to HA sedimentation. The obtained sediment was extracted three times repeated with 0.1 mol $L^{-1}$ NaOH (extraction B). Obtained HA were extracted as before by acidification of HS solution to pH~1.5. The obtained HA were then purified by solving them in extraction solution (accordingly 0.1 mol $L^{-1}$ $Na_4P_2O_7$ or 0.1 mol $L^{-1}$ NaOH) and extrcting with 4 mol $L^{-1}$ $H_2SO_4$. Then they were washed with the use of 0.1 mol $L^{-1}$ HCl + 0.3 mol $L^{-1}$ HF. Purified HA were dialyzed with distilled water and lyophilized.

By using the above extraction obtained ”young” humus forms, loosely associated with the mineral part of sediment/soil called ”labile” HA (use of neutral solution of $Na_4P_2O_7$) and compounds highly associated with the mineral part, which is stable HA (extraction with an alkaline solution NaOH).

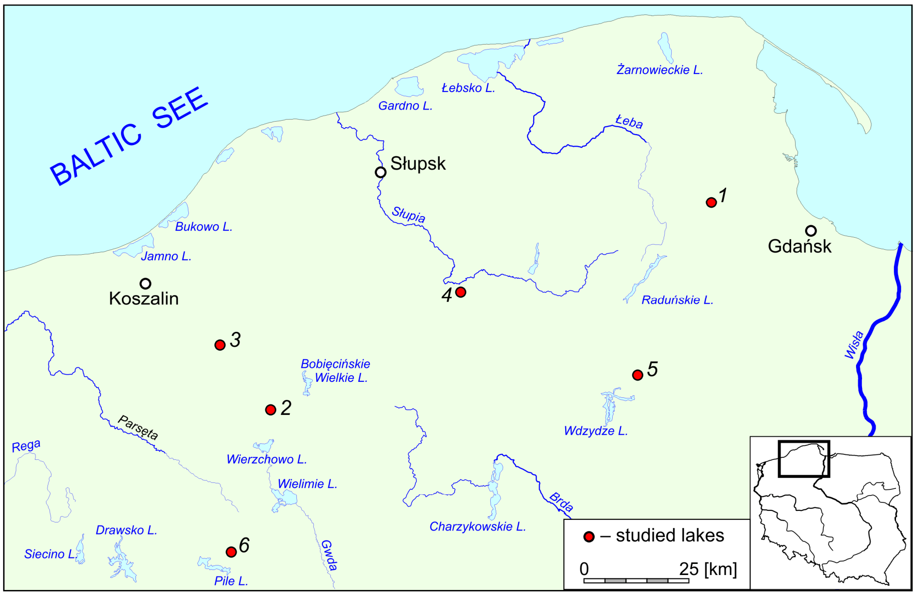

Fig. 1. Location of investigated lakes
Ryc. 1. Położenie badanych jezior

Further in the HA fraction of the pyrophosphate extract obtained is called the HA-A, while the HA fraction from the alkaline extract – HA-B.

## Characterization methods

The elemental composition of the HA was determined by a CHNS Elementar Analizer model Vario EL III. The ash content was determined by roasting at 750°C. The oxygen content was calculated by determining the difference (O = 100-ash-C-H-N).

UV-Vis spectra were performed with a computerized spectrophotometer Specord M-42 Zeiss-Jena. HA solution were prepared in 0.05 M $NaHCO_3$ and their concentration was 10 mg $C_{org}$ $dm^{-3}$. To characterize HA one used the following parameters determined on the basis  of absorption spectra:

– $\varepsilon_{280}$: coefficient of specific absorbance determined at the wavelength of 280 nm, carbon content in HA solution = 1 % and the path length of cuvete = 1 cm – used to estimate the relative aromaticity of HA [Peuravuori and Pihlaja 1997, Kalbitz et al. 2000, Uyguner and Bekbolet 2005];

– $\varepsilon_{600}$: coefficient of specific absorbance determined at the wavelength 600 nm, carbon content in HA solution = 1% and the optical way length = 1 cm – connected with the degree of humification [Kumada 1987, Kalbitz 2001];

– $E_4/E_6 = A_{465}/A_{665}$ – is negatively correlated with the size and molecular weight of HA [Chen et al. 1977], is also negatively correlated with increasing content of condensed aromatic structure [Bravard and Righi 1991, Saab and Martin-Neto 2007];

– $E_2/E_3 = A_{254}/A_{365}$ – negatively correlated with the molecular weights [Traina et al. 1990, Peuravuori and Pihlaja 1997, Chen et al. 2002, Vogt et al. 2004];

– $\Delta A_1/\Delta A_2 = A_{290}\text{-}A_{333}/A_{357}\text{-}A_{416}$ – allows estimating of number of shortwave absorbing groups and packing degree of structure [Gołębiowska 2004].

## RESULTS AND DISCUSSION

The elemental composition is one of the basic properties of humic substances. It depends on habitat conditions in which the processes of mineralization and humification resulting in forming of humic substances. In Tables 1 and 2 there is presented the elemental composition of studied HA and atomic ratios.

Table 1. Elemental composition (in atomic percent) calculated on an ash-free basis
Tabela 1. Skład pierwiastkowy (w procentach atomowych) przeliczony na masę bezpopielną

| Lake | HA-A | | | | HA-B | | | |
|---|---|---|---|---|---|---|---|---|
| | C | H | N | O | C | H | N | O |
| 1 | 37.75 | 39.28 | 2.45 | 20.52 | 37.07 | 42.54 | 2.25 | 18.14 |
| 2 | 35.76 | 39.11 | 1.86 | 23.26 | 35.88 | 43.75 | 2.03 | 18.35 |
| 3 | 34.54 | 45.07 | 3.57 | 16.82 | 34.66 | 48.82 | 2.38 | 14.15 |
| 4 | 37.53 | 41.23 | 2.90 | 18.33 | 35.27 | 46.98 | 2.33 | 15.42 |
| 5 | 37.24 | 39.46 | 2.53 | 20.77 | 35.47 | 44.68 | 2.25 | 17.60 |
| 6 | 35.87 | 39.87 | 2.84 | 21.42 | 34.03 | 47.52 | 2.90 | 15.55 |
| Average | 36.45 ± 1.26 | 40.67 ± 2.29 | 2.69 ± 0.57 | 20.19 ± 2.29 | 35.40 ± 1.05 | 45.72 ± 2.43 | 2.36 ± 0.29 | 16.54 ± 1.73 |

Table 2. Atomic ratios of HA isolated from studied lakes
Tabela 2. Stosunki atomowe KH ekstrahowanych z osadów badanych jezior

| Lake | HA-A | | | | | HA-B | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | C:N | H:C | O:C | O:H | CQ | C:N | H:C | O:C | O:H | CQ |
| 1 | 15.40 | 1.04 | 0.54 | 0.52 | 0.96 | 16.45 | 1.15 | 0.49 | 0.43 | 0.92 |
| 2 | 19.20 | 1.09 | 0.65 | 0.59 | 1.01 | 17.67 | 1.22 | 0.51 | 0.42 | 0.92 |
| 3 | 9.68 | 1.30 | 0.49 | 0.37 | 0.86 | 14.58 | 1.41 | 0.41 | 0.29 | 0.83 |
| 4 | 12.95 | 1.10 | 0.49 | 0.44 | 0.92 | 15.15 | 1.33 | 0.44 | 0.33 | 0.86 |
| 5 | 14.73 | 1.06 | 0.56 | 0.53 | 0.96 | 15.78 | 1.26 | 0.50 | 0.39 | 0.90 |
| 6 | 12.62 | 1.112 | 0.60 | 0.54 | 0.96 | 11.75 | 1.40 | 0.46 | 0.33 | 0.84 |
| Average | 14.10 ± 3.20 | 1.12 ± 0.09 | 0.56 ± 0.06 | 0.50 ± 0.08 | 0.95 ± 0.05 | 15.23 ± 2.01 | 1.30 ± 0.10 | 0.47 ± 0.04 | 0.37 ± 0.06 | 0.88 ± 0.04 |

The data of elemental composition of the studied HA do not bring unique conclusions. However it is observed that HA-B are characterized by relatively higher H and O contents than it is observed in HA-A. Also the elemental composition parameters analysis informing of oxydation degree of humic substances such as O:C, O:H and CQ, indicated the lower values of this ratios in HA-B in comparison to HA-A.

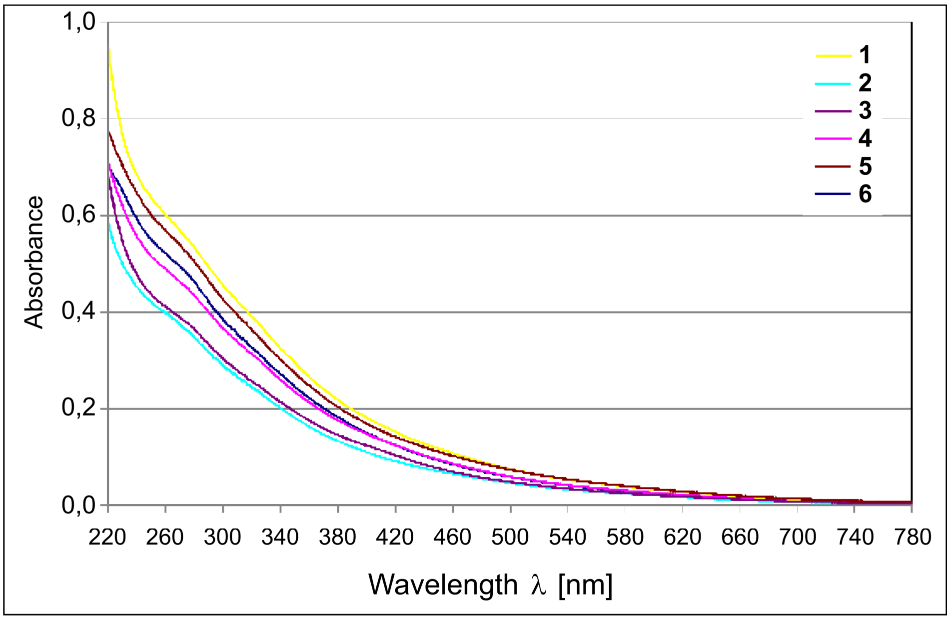

Fig. 2. UV-Vis absorption spectra the HA isolated using a 0.1 mol·L$^{-1}$ $Na_4P_2O_7$ solution
Ryc. 2. Widma absorpcji UV-Vis, KH ekstrahowanych roztworem 0,1 mol·L$^{-1}$ $Na_4P_2O_7$

It means that the stable fraction of HA are characterized by higher oxidation than HA fraction loosely associated with mineral part. Due to the big differentiation in the investigated lakes character, the differences between analyzed parameters were not statistically confirmed. However, due to wide scatter of the results, the fact that there are not such differences does not mean that the differences do not really occur.

The elemental analysis gives only information on quantitative relations between the main elements contained in HA but it does not inform on structure of humic substances produced in the process of humification. Hence, UV-Vis spectroscopy was performed. The spectral curves of HS provide the information about the presence of specific chromophore groups capable to absorb radiation. They can also provide information on the structure and sizes of HS molecules. The obtained absorption spectra of studied HA are monotonic within the whole range (fig. 2 and 3). Shape and slope of the analyzed spectra for all HA are very similar. However, the spectra differ in intensity. In all analyzed spectra there is characteristic maximum within 260–280 nm connected with electron transition π-π*. The maximum indicates the presence of phenolic and quinone structures as well as chromophores in both fractions [Chen et al. 2002, Gołębiowska 2004, Uyguner and Bekbolet 2005, Zbytniewski and Buszewski 2005]. The labile HA (HA-A) indicate stronger absorption, especially in the shortwave parts of the spectra in comparison with the stable HA (HA-B). Analogous results were obtained by Giegużyńska [2009] while studying spectral properties of HA isolated from deluvium soil. The higher absorbance of HA-A can be associated with more of chromophoric groups absorbing radiation, especially in the UV range.

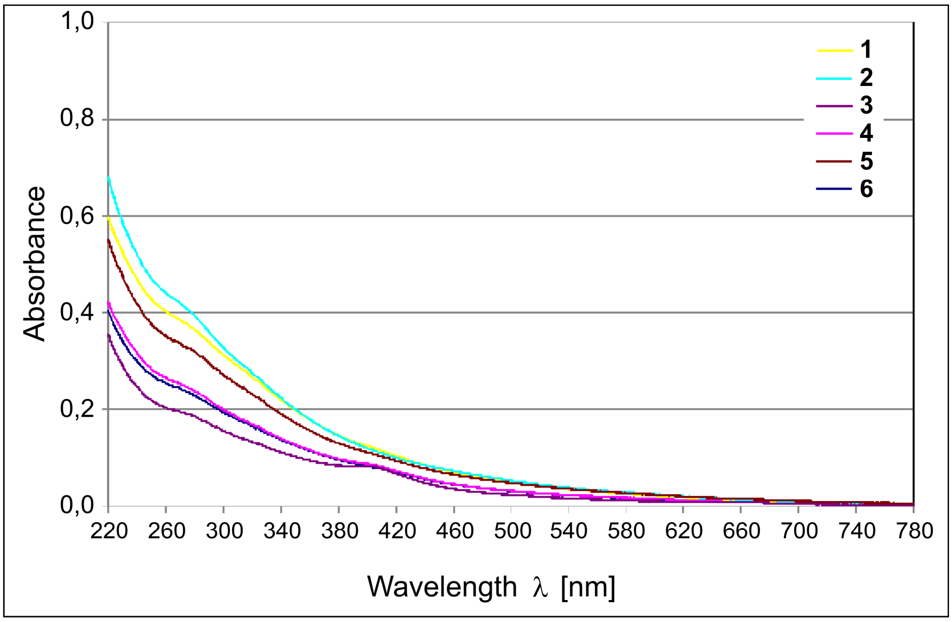

Fig. 3. UV-Vis absorption spectra the HA isolated using a 0.1 mol·L$^{-1}$ NaOH solution
Ryc. 3. Widma absorpcji UV-Vis, KH ekstrahowanych roztworem 0,1 mol·L$^{-1}$ NaOH

In Table 3 there is presented the optical parameters calculated on basis of obtained absorption spectra. The most commonly used spectral coefficient to characterize HS is the $E_{4/6}$ ratio. Values of this ratio correspond to the size of HA molecules and the degree of their aromaticity. Higher values of the $E_{4/6}$ ratio of HA-A, than those of HA-B, indicate their smaller molecular sizes and molecular weight as well as smaller degree of condensation of aromatic groups. The parameter $E_{2/3}$ is also connected with the molecular size of HA molecules and their aromaticity. For both types of the extraction (HA-A and HA-B) the values of the E2/3 coefficient were similar. They were around 2.5 and in this investigation they were not suitable to describe differences in HA properties. Another optical parameter characterizing HS is the $\Delta A_1/\Delta A_2$ ratio. It allows one to observe the changes of spectra within the range of aromatic structures absorption that is an evidence of differences in a number of shortwave absorption groups and ”packing” degree of the structure. The HA-A fraction indicated higher values of this parameter than HA-B. That means that HA-A molecules have relatively higher share of shortwave absorbing structures, characteristic to phenols and aromatic hydroxy acids. The characteristic spectral parameters of humic compounds are specific absorption coefficients. For both types of HA, specific absorption coefficients differ in the whole spectral range. Obtained higher specific absorbance coefficients $\varepsilon_{280}$ i $\varepsilon_{600}$ for HA-A inform on their bigger number of spectrally active groups compared to HA-B. Based on it we may conclude that HA-A and HA-B molecules differ in a type and number of chromophores able to absorb UV-Vis radiation. According to many authors the specific absorbance coefficients $\varepsilon_{280}$ and $\varepsilon_{600}$ are used for the preliminary assessment of the humification degree of HA, which may also be related to higher degree of aromatic structures condensation or more substitution of aromatic rings. Higher values of the coefficients $\varepsilon_{280}$ and $\varepsilon_{600}$ for the HA-A may indicate a smaller transformed molecules acids (labile) compared to the HA-B.

Table 3. The calculated optical parameters for studied humic acids
Tabela 3. Obliczone parametry optyczne badanych kwasów huminowych

| Lake | HA-A | | | | | HA-B | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $\varepsilon_{280}$ | $\varepsilon_{600}$ | $E_{4/6}$ | $Q_{2/3}$ | $\Delta A_1/\Delta A_2$ | $\varepsilon_{280}$ | $\varepsilon_{600}$ | $E_{4/6}$ | $Q_{2/3}$ | $\Delta A_1/\Delta A_2$ |
| 1 | 534.0 | 32.0 | 6.2 | 2.5 | 1.3 | 366.5 | 21.9 | 5.3 | 2.5 | 1.3 |
| 2 | 348.4 | 19.7 | 6.7 | 2.7 | 1.4 | 317.8 | 22.1 | 4.6 | 2.6 | 1.5 |
| 3 | 364.4 | 20.0 | 6.2 | 2.6 | 1.4 | 186.6 | 10.4 | 3.9 | 2.3 | 2.1 |
| 4 | 433.9 | 24.6 | 5.8 | 2.5 | 1.3 | 238.9 | 15.5 | 4.3 | 2.5 | 1.6 |
| 5 | 505.3 | 33.1 | 5.5 | 2.5 | 1.3 | 320.9 | 23.7 | 3.9 | 2.5 | 1.4 |
| 6 | 461.6 | 23.1 | 7.1 | 2.6 | 1.3 | 230.3 | 15.1 | 4.6 | 2.4 | 1.4 |
| Average | 441.3 ± 74.4 | 5.82 ± 5.4 | 6.3 ± 0.58 | 2.6 ± 0.08 | 1.3 ± 0.05 | 276.8 ± 68.4 | 18.1 ± 5.2 | 4.4 ± 0.53 | 2.5 ± 0.10 | 1.6 ± 0.29 |

## CONCLUSIONS

The UV-Vis spectroscopic parameters offer a good opportunity to describe the differences between the studied HA fractions isolated by different methods. The higher capacity to absorb the UV-Vis radiation were characterized to the labile fraction of humic acids (HA-A). It was indicated by higher specific absorbance coefficients $\varepsilon_{280}$ and $\varepsilon_{600}$ of HA-A, than HA-B. This can be attributed to a more complex structure and higher condensation of aromatic nuclei of HA extracted by alkaline solution (HA-B) compared to HA extracted by neutral pyrophosphate solution (HA-A). The analysis of $E_{4/6}$ ratio showed that HA molecules extracted by alkaline solution (HA-B) indicate higher molecular size and higher condensation of aromatic groups, than those extracted by pyrophosphate solution (HA-A).

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## ZASTOSOWANIE PARAMETRÓW SPEKTRALNYCH UV-VIS DO CHARAKTERYSTYKI KWASÓW HUMINOWYCH (KH) POCHODZĄCYCH Z OSADÓW JEZIORNYCH

**Streszczenie**. Obiektem badań były kwasy huminowe (HA), pochodzące z osadów jeziornych. Kwasy huminowe wydzielano metodą dwustopniowej ekstrakcji (A) – ekstrakcja obojętnym roztworem 0,1 mol $L^{-1}$ $Na_4P_2O_7$ i (B) – ekstrakcja roztworem alkalicznym 0,1 mol $L^{-1}$ NaOH. Stwierdzono, że badane kwasy różnią się głównie składem pierwiastkowym i właściwościami spektrofotometrycznymi. Cząsteczki HA ekstrahowanych roztworem 0,1 mol $L^{-1}$ NaOH (HA-B) zawierają relatywnie więcej atomów wodoru i tlenu niż cząsteczki HA uzyskanych w wyniku ekstrakcji 0,1 mol $L^{-1}$ $Na_4P_2O_7$ (HA-A). Ponadto frakcja stabilnych HA-B charakteryzuje się wyższym stopniem utlenienia niż frakcja HA-A, luźno związanych z częścią mineralną osadów. Pomiary spektrofotometryczne w zakresie UV-Vis wykazały, że frakcja labilnych HA-A wykazuje silniejszą absorpcję promieniowania niż stabilne HA-B. Świadczą o tym m.in. większe wartości współczynników absorpcji właściwej: $\varepsilon_{280}$ i $\varepsilon_{600}$. Analiza wartości współczynników spektralnych uwidoczniła różnice w budowie strukturalnej badanych HA. Kwasy huminowe ekstrahowane roztworem alkalicznym (HA-B) to cząsteczki o większych rozmiarach i bardziej skondensowanej strukturze w porównaniu z ekstrahowanymi obojętnym roztworem pirofosforanu sodu (HA-A). Uzyskane dane pokazują, że procedura ekstrakcji może wpływać na właściwości izolowanych kwasów huminowych.

**Słowa kluczowe**: substancje humusowe, osady jeziorne, spektroskopia UV-Vis