



Using delayed luminescence to characterize humic acids from lake sediments

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Abstract

Purpose Using the delayed luminescence (DL) method, the photoluminescent properties of humic acids (HA) extracted from lake sediments were analyzed. Delayed luminescence is a promising technique for characterizing HA and other forms of organic matter of various origins. But information is insufficient regarding both its optimal operating conditions and also those environmental factors that control the chemical constitution of samples, which affects patterns of delayed luminescence.

Materials and methods The research material was HA from lake sediments. Humic acid extractions were carried out using the method developed by the International Humic Substances Society. Studies on excitation and recording of DL intensity of HA solutions were carried out with the use of a specially designed device for continuous recording of photo-induced luminescence. The DL was excited with a monochromatic light at multiple wavelengths.

Results and discussion The DL intensity depended on the wavelength of the exciting light. The highest DL intensity was obtained with excitation by blue light, the lowest DL intensity was observed with excitation by red light. Statistically significant differences among DL intensity were observed in the examined humic acids. These differences may be evidence of the variable quantitative and qualitative contributions of photoluminophores to the structure of the studied HA molecules as well as their different photochemical reactivities. For the blue- and green-excited DL intensity, statistically significant positive correlations were obtained with the elemental atomic ratio O:H and CQ coefficient. For the red-excited DL intensity, statistically significant positive correlations were obtained with the C and H content and H:C, C:N atomic ratios, while negative correlations were obtained with the free radical concentration.

Conclusions Delayed luminescence depended on the wavelength of the exciting light. The DL intensity excited by blue and green light was different than the red light-excited DL intensity. The DL depended on the primary composition of sediments and the properties and structure of HA too. The grouping of HA showed that the HA formed in organic matter-poor, silicate-rich sediments had greater DL emission than did those extracted from sediments that were enriched in organic matter.

Keywords Humic acids · Lake sediments · Organic matter · Photoluminescence method

1 Introduction

During the interaction of electromagnetic radiation with natural organic matter (NOM), the absorbed energy can be transformed

into other forms of energy. The interaction of light with NOM initiates various photochemical and photophysical processes. Photophysical processes are involved in the loss of excitation energy by a molecule as a result of the emission of a radiation quantum or the non-radiative processes. During radiant transition, in which an excited molecule loses energy by emitting a photon, there is a luminescence phenomenon. Luminescence excited by photons (photoluminescence) can be divided into fluorescence (Fl), phosphorescence (Ph), and delayed luminescence (DL). The significant difference between these types of luminescence is related, among others, to their kinetics. Fluorescence is a fast photophysical process, occurring in $\sim 10^{-8}$ s, while Ph and DL have a significantly longer life span from 1 ms to many minutes (Lakowicz 2006; Balzani et al. 2014). Photoluminescence provides information about the photophysical processes preceding the photochemical

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reactions. Thus, photoluminescence processes and photochemical reactions should be studied simultaneously because radiation emission is strictly connected with the processes resulting in photochemical transformations.

Humic substances (HS) are the main fraction of NOM. They form a combination of macromolecular products of chemical and biological degradation of plant and animal residues including lignin residues, carbohydrates, and proteins. Humic substances exposed to light undergo photochemical processes accompanied by creation of fluorophores capable of emitting electromagnetic radiation in the process of luminescence. The main pathways for photochemical reactions induced by HS involve the transfer of energy from excited HS* to reactive forms such as solvated electrons (e_{aq}), hydroxyl radicals ($\cdot OH$), singlet oxygen (1O_2), and reactive triplet states ($^3HS^*$). In many cases, HS act as photosensibilizers, which means that they can accelerate many photochemical processes (Frimmel 1994; Aguer and Richard 1999; Paul et al. 2004; Grannas et al. 2006; Tai et al. 2012).

As a result of the absorption of electromagnetic radiation, the energy of HS molecules increases. Only a small part of absorbed energy can initiate chain photochemical reactions by humic substances (Steinberg 2003). The intensity of radiation absorbed by the HS molecule depends on the wavelength and on the structure of the molecule as well as its microenvironment. This is reflected in the shape of the absorption spectrum. Unlike other organic or inorganic water-soluble substances, which exhibit clear maxima and minima of absorption, the absorption of radiation by HS increases monotonically in the direction of shorter wavelengths, and the spectrum is of non-characteristic nature. Mainly, the capability of HS to absorb light is related to the presence of electron-delocalized systems π in aromatic rings or conjugated double bonds (Steinberg 2003). Delocalization of the electron π makes an additional contribution to the stability of the molecule, and at the same time it determines reactions, which do not take place in other types of compounds, such as extended distances of excitation transfer or transferring electrons and energy.

Photoluminescent methods are an important tool for organic matter (OM) studies of various origins, including humic substances. The particularly popular and prevalent is the fluorescent method (Senesi et al. 1991; Olk et al. 2000; Bertoncini et al. 2005; Gołębiewska et al. 2005; Sierra et al. 2005; Henderson et al. 2009; Hur et al. 2009; Yakimenko et al. 2016; Derrien et al. 2017). It provides information about the structure of HS molecules, the processes of intermolecular transport of energy and structural transformations. The DL approach has not been widely used in OM studies. Emission DL extends across the entire optical spectrum, but its maximum is shifted to the shortwave area compared to the maximum of “normal” Fl (Istvánovics et al. 2005). The amount of the emission energy in the form of DL is not large, but it provides important information. The emission DL is related to the chain

processes initiated by light absorption, in which the absorbed energy initiates the intermolecular transport of energy. These processes are accompanied by structural changes caused directly or indirectly by the photochemical processes which occurred in the studied material. HS photoluminescence studies can provide much relevant information about the structure and nature of organic compounds and their reactivity. DL is usually tested by recording the kinetics of the light decay or recording the light intensity over a specified time interval(s) after irradiation of the material. The DL intensity of a given material, registered under established measurement conditions, depends on both the amount of chromophores present in the material and the quality of the chromophores, as well as on the wavelength of the excitation light (Mielnik 2009).

Research showed that, under established conditions of excitation, the capability of HS to delayed luminescence depends primarily on the origin of OM and the conditions under which transformation of OM undergoes (Mielnik 2009; Mielnik et al. 2009; Prokowski and Mielnik 2012). The use of photoluminescence in water environment studies may enable the development of indicators to better understand the luminescence properties of HS specific to a given body.

The aim of this study was to comprehensively characterize the photoluminescent properties of HA extracted from the lake sediments with the use of the delayed luminescence method. To accomplish the study goal, it was assumed that the photoluminescence parameters of HA are correlated with the elemental composition parameters describing the structural and chemical properties of these compounds.

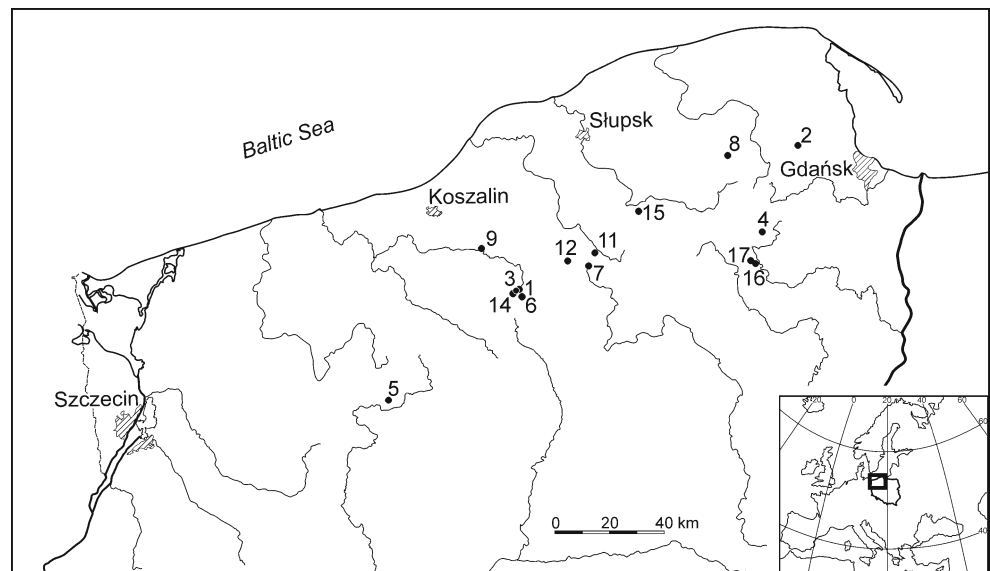
2 Material and methods

2.1 Sediment sampling

Studies were carried out in the sediments sampled from 15 lakes situated in the Pomeranian Lake District (Fig. 1). The research lakes were characterized by considerable variability of morphological and hydrochemical parameters, and their trophic state (Table 1). The samples were collected from the sediment surface layer (about 20 cm) by the use of a Kajak corer at the maximum depth of the profundal zone in each lake during the summer stagnation. Four to six random samples were mixed together to obtain one bulk sample. Air-dry sediments were ground in a mortar and passed through a 1-mm sieve.

The following properties were determined: organic carbon content (C_{org}) by the Orlov and Grindel method, calcium carbonate content ($CaCO_3$) by Scheibler's method, and total nitrogen content (N_{tot}) by Kiejdahl's method. The OM content was calculated as $1.724 C_{org}$, while non-carbonate mineral parts were calculated from the equation $[100 - (CaCO_3 + OM)]$. Selected sediment properties are shown in Table 1.

Fig. 1 Map showing the location of the studied lakes



2.2 Humic acid extraction

Humic acids were extracted from the air-dry sediments according to the standard method introduced by the International Humic Substances Society (Swift 1996). Sediments were initially cleansed of carbonates and calcium by using 1 mol dm⁻³ HCl and 0.1 mol dm⁻³ HCl. Next, the HA were extracted with 0.1 mol dm⁻³ NaOH under a nitrogen atmosphere. The obtained extract of HS was acidified with 6 mol·dm⁻³ HCl and HA were precipitated at pH ~2. The resulting HA precipitate redissolved in a small amount of 0.1 mol dm⁻³ KOH and 0.3 mol

dm⁻³ KCl mixture. The centrifuged HA were purified from mineral parts with a mixture of 0.1 mol dm⁻³ HCl and 0.3 mol dm⁻³ HF. Purified HA were washed three times with distilled water to remove chloride ions and then lyophilized. The freeze-dried HA were analyzed for their elemental composition CHN/O with an Elementar Vario EL III CHNOS elemental analyzer. On the basis of the elemental composition the atomic contents as well as atomic ratios and combustion quotient CQ were calculated. The CQ value is calculated on the basis of the contents of all four element compositions: 4C/(4C + H-3N-2O) (Kumada 1987). Free radical concentration was

Table 1 Selected parameters of studied lakes and their sediments

Lake no.	Area ha	Depth max m	Trophic type of lake ^a	Organic matter %	Calcium carbonate %	Mineral part %	C _{org} :N _{tot}
1	2.6	16.3	Dystrophic	38.0	34.1	27.9	13.7
2	9.0	3.5	Dystrophic	34.7	47.6	17.7	11.3
3	8.4	13.5	Dystrophic	42.5	1.7	55.8	14.2
4	7.0	2.0	Dystrophic	51.8	0.0	48.2	14.7
5	10.3	9.5	Dystrophic	51.5	0.0	48.5	14.5
6	8.3	11.7	Dystrophic	65.5	0.0	34.5	17.2
7	36.0	11.0	Dystrophic	27.5	1.0	71.5	11.4
8	4.9	6.4	Oligotrophic	38.9	0.0	61.1	18.0
9	4.9	19.2	Oligotrophic	51.1	34.7	14.2	9.4
11	8.6	13.7	Oligotrophic	26.1	0.0	73.9	12.0
12	15.0	6.3	Oligotrophic	42.9	1.6	55.5	9.9
14	54.9	11.5	Oligotrophic	34.2	0.0	65.8	9.6
15	1.5	17.9	Oligotrophic	2.8	1.1	96.0	11.3
16	12.5	16.0	Oligotrophic	28.0	4.3	67.7	11.8
17	3.6	10.0	Oligotrophic	35.1	44.8	20.1	12.7

^aType of lake trophy determined by Kraska and Piotrowicz (2000)

also determined in the obtained HA using a Bruker EMX spectrometer. Humic acids from Leonardite was a standard pattern. The results are shown in Table 2.

2.3 Delayed luminescence method

The measurements of DL intensity of HA solutions were performed using the equipment developed and constructed in the Department of Physics and Agrophysics, West Pomeranian University of Technology in Szczecin. To induce DI excitation, monochromatic light sources of different wavelengths were used: DL: (i) 465 ÷ 485 nm – blue-light (DL-blue); (ii) 510 ÷ 535 nm – green-light (DL-green); (iii) 620 ÷ 630 nm – red-light (DL-red) all at the constant photon flux density 1500 $\mu\text{mol}(\text{quanta})\text{PAR m}^{-2} \text{s}^{-1}$.

Delayed luminescence intensity was recorded at 0.10 ÷ 0.35 s after excitation. This delay in measurement corresponds to the time needed for the HA solution to flow through the spiral cuvette placed ahead of the photocathodes of the measuring probes. The flow rate was 800 $\text{cm}^3 \text{min}^{-1}$. The spectral range of a photomultiplier recording the DL intensity encompassed the wavelengths of 185 ÷ 850 nm. DL intensity was measured at the constant concentration of HA in 0.05 mol dm^{-3} NaHCO_3 solution, corresponding to 30 mg C dm^{-3} , and at the constant temperature of 25 °C.

Table 2 Elemental composition, atomic ratios and concentration of free radicals of the studied HA from lake sediments

Lake	C Atomic %	H	N	O	H:C	C:N	O:H	CQ ^a	Free radicals $\cdot 10^{17} \text{spin g}^{-1}$
1	36.4	43.7	2.2	17.7	1.20	16.8	0.41	0.91	1.10
2	37.5	43.4	2.5	16.7	1.16	15.0	0.38	0.90	1.80
3	34.1	45.6	2.2	18.1	1.34	15.5	0.40	0.98	2.11
4	32.9	46.3	2.5	18.4	1.41	13.2	0.40	0.98	0.98
5	33.2	46.8	2.1	18.0	1.41	15.8	0.38	0.97	1.75
6	33.6	46.5	2.0	17.9	1.38	16.8	0.39	0.97	1.79
7	33.3	46.7	2.3	17.6	1.40	14.5	0.38	0.97	0.99
8	34.7	44.1	1.9	18.4	1.27	18.3	0.43	0.99	0.89
9	34.4	48.5	2.7	14.4	1.41	12.6	0.30	0.83	0.91
11	31.0	47.6	2.7	18.8	1.54	11.5	0.39	0.98	1.65
12	31.4	48.4	2.6	17.7	1.54	12.1	0.36	0.96	1.22
14	31.2	49.4	2.4	18.1	1.58	13.0	0.37	0.95	1.29
15	33.2	48.3	2.5	15.9	1.45	13.1	0.33	0.85	1.25
16	30.6	49.1	2.5	17.8	1.60	12.2	0.36	0.95	1.13
17	36.5	45.2	2.4	15.9	1.24	15.2	0.35	0.88	1.88

^a CQ = 4C/(4C + H-3N-2O)—internal oxidation factor according to Kumada (1987)

2.4 Statistical methods

The results were statistically analyzed with the Statistica 16 program. Pearson's correlation coefficients were calculated for the parameters characterizing chemical and photoluminescent properties of the extracted HA. The analysis of similarities was used to determine relationships between the analyzed parameters, creating a Ward's clustering dendrogram. Before analysis, standardization of variables was carried out to express them on the same scale.

3 Results

The highest DL intensity was obtained for excitation of HA with blue light ($\lambda_{\text{ex}} = 465 \div 485 \text{ nm}$). The values of DL intensity obtained for excitation with green light were smaller ($\lambda_{\text{ex}} = 510 \div 535 \text{ nm}$). The lowest DL intensity was observed for excitation with red light ($\lambda_{\text{ex}} = 620 \div 630 \text{ nm}$).

Based on the excitation values with blue light, the HA samples can be divided into two groups characterized by distinctly different DL capabilities (Fig. 2a). DL-blue intensity for HA from eight lakes ranged between 7000 ÷ 16,000 [a.u.], and the remaining seven HA samples had intensities about three to four times lower, ranging between 1000 ÷ 6000 [a.u.]. Significant distinctions in DI intensity at one excitation wavelength indicate different structures and properties of the analyzed HA and thus may be evidence of different conditions of decomposition and transformation of OM which is constantly accumulating in the lake sediments.

A similar tendency occurred in case of excitation with a green light. HA, derived from the same eight lakes as previously, show significantly higher DL intensity than the others (Fig. 2b).

A highly significant correlation was obtained between blue-light DL intensity and green-light DL intensity ($r = 0.99$, $p < 0.0000$). This is the evidence that excitation with blue- and green-light initiates similar mechanisms of photoreactions which lead to excitation of similar group responsible for the DL emission.

Delayed luminescence excited with red light distinguished two groups that differed from the groups distinguished by blue- and green-light excitation (Fig. 2c). There was no correlation between red-DL and blue- or green-DL. It may be evidence that the mechanisms leading to DL emissions during red-light excitation are quite different than those observed during blue- or green-light excitation.

4 Discussion

Analysis of correlation coefficients allows a detailed interpretation of the obtained results. The chemical structure of HA is

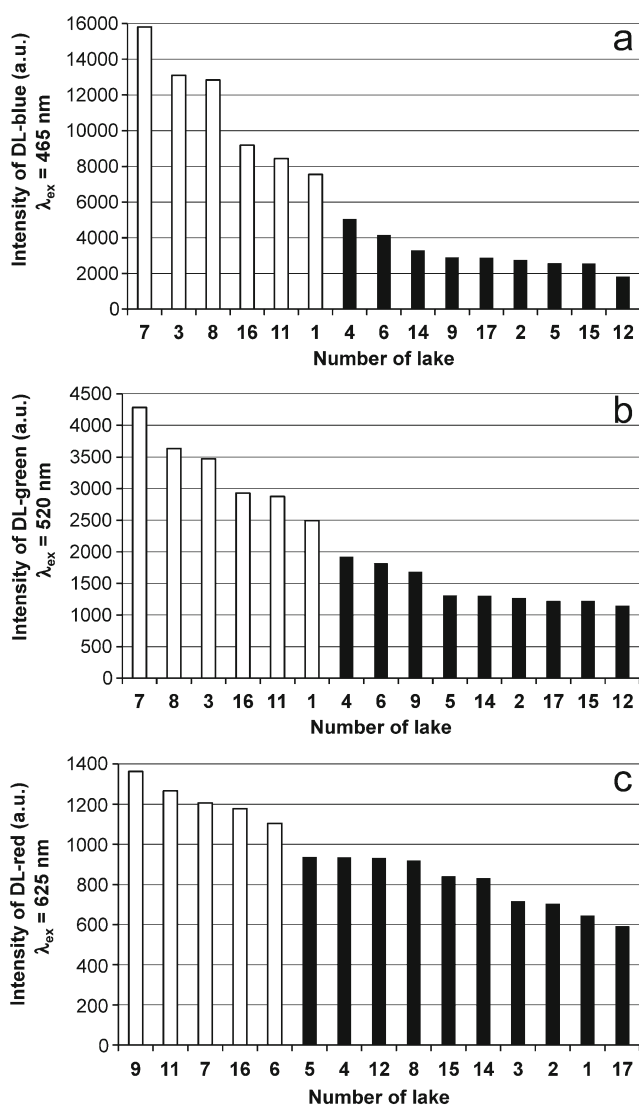


Fig. 2 Values of the DL intensity of studied HA: **a** blue-, **b** green- **c** red-light excitation

poorly defined. The parameters calculated from the elemental concentrations allow some insights into HA structures. The variability of the HA structure may be significant in DL emission. For the blue-DL intensity, statistically significant positive correlations were obtained with elemental composition parameters such as the O:H atomic ratio and with the combustion quotient (CQ) (Fig. 3). The O:H atomic ratio is effective as an index of the state oxidation of HA, while the CQ parameter expresses the theoretical respiration ratio (Kumada 1987). Because of the highly significant correlation between O:H and CQ values, both parameters reflect the degree of internal oxidation of humic acids. They may be associated with the content of redox systems within peripheral sectors of the HA structure, outside its main core. These redox systems undergo changes depending on environmental conditions. Higher blue-DL intensity as well as higher values of O:H atomic ratio and CQ attribute the greater capability for DL emission to more

oxidized humic acids. It is likely related to a higher degree of transformation of the structure, characterized by the higher concentration of aromatic rings in the HA core and a presence of delocalized electrons π , or conjugated double bonds in the molecule. Similar correlations to the blue-DL were obtained for the green-DL (Fig. 3).

Research conducted by Puzyna on the light-induced physicochemical changes occurring in humus substances suggest that DL excited with blue- or green-light can be associated with the induction of charge-transfer complexes with a complete transfer of charge from the donor to the acceptor with an extended double-bond system (Puzyna 1979).

In turn for the DL-red intensity, statistically significant correlations were obtained with other parameters of elemental composition: C and H content, H:C and C:N atomic ratios (Fig. 3). The lower carbon content and higher hydrogen content would attribute the greater capability for DL-red light to HA having more immature structure. In addition, the positive correlation between DL-red and H:C atomic ratio indicates that the structure of these HA is less condensed and more “looser” and contains relatively more aliphatic structures. Structure of these HA, can be additionally enriched to nitrogen, which is bound with the HA structure, e.g., in an enzymatic protein form or other means (e.g., in the form of bridges (-NH-)) (Gołębiewska 1982). The relationship between DL-red and C:N atomic ratio suggest that nitrogen structures can play an important role in the DL-red process.

The DL-red intensity was negatively correlated with the free radical concentration (Fig. 3). The content of free radicals in HA is directly related to the degree of aromaticity, and molecular size and complexity of HA (Senesi 1992). Research by many authors report that the concentration of free radicals in the HA indicates the degree of transformation of their structure, which is identified with the degree of HA humification (Olk et al. 2000; Milori et al. 2002; Rosa et al. 2005; Ferreira et al. 2013). The observed relationship between DL-red intensity and free radical content leads to the conclusion that the red-light-excited DL emission depends on the degree of transformation and/or condensation of the HA structure.

Due to the complex nature of the HS, specific chemical composition, as well as high photo-reactivity, their properties may be used to identify the principal source of OM as well as biogeochemical evolution of their properties. Research conducted by Mielnik (2009) showed that in established excitation conditions, the HS capability for DL emission depends primarily on the origin of OM and the environment conditions in which OM transformations occur.

In order to determine the differences of the investigated HA, the cluster analysis was tested based on the variability of photoluminescence parameters. The objects of similar properties are located on dendrograms in homogenous groups. The investigated lakes may be distinguished into two main groups, depending on results of the cluster analysis (Fig. 4).

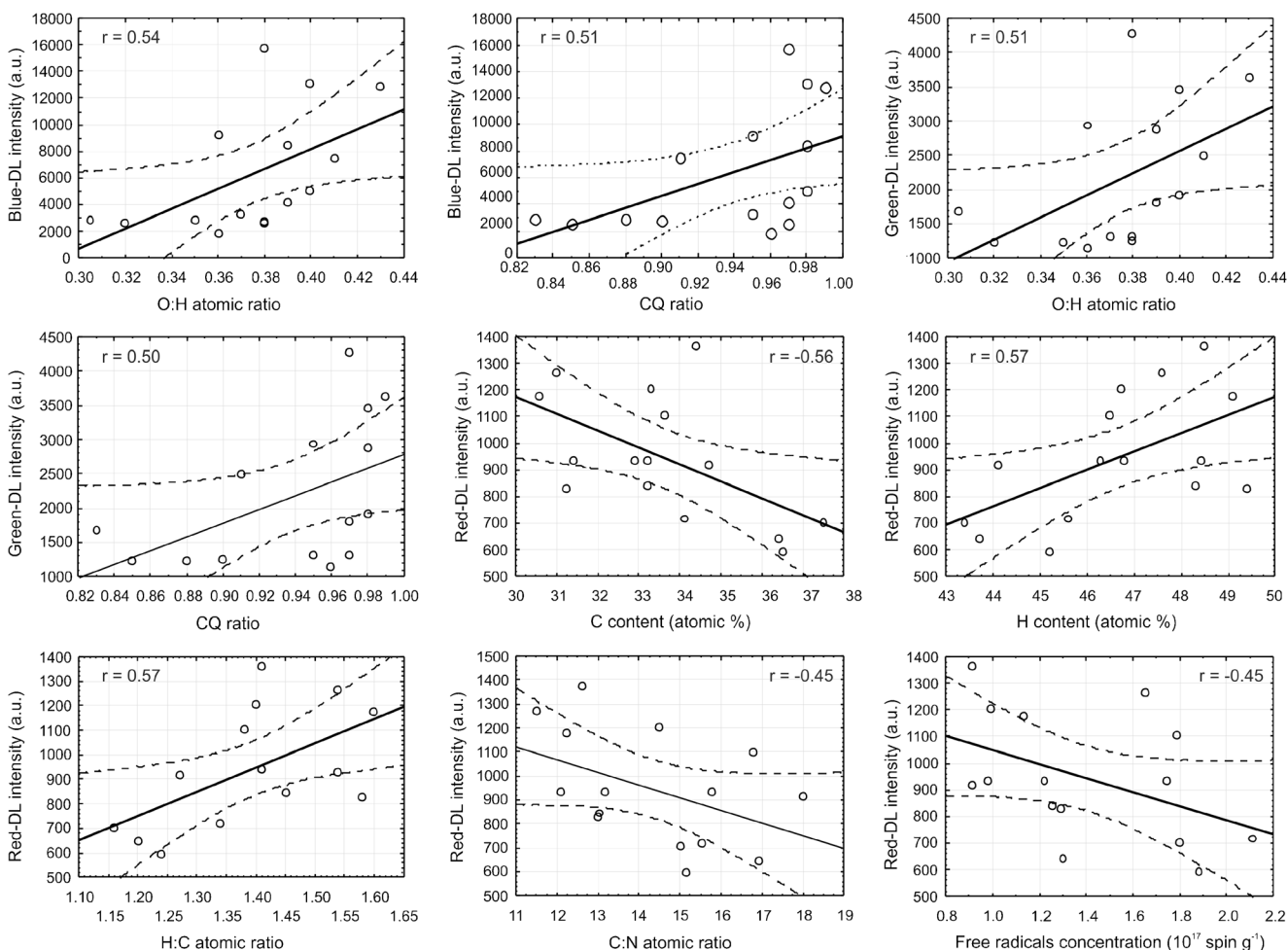


Fig. 3 Linear relationship between DL intensity versus elemental composition parameters. Pearson correlations significant with $p \leq 0.05$

The first group is primarily HA formed in silicate sediments (lake no. 3, 7, 8, 11, 16). In the second group, the HA samples are not clearly divided. We conclude that the HA formed in sediments poor in OM and non-carbonate differ from the other HA samples formed in sediments enriched in OM primarily in their higher DL emission. The results tested with the cluster analysis showed that the important factor of DL emission by HA is the type of lake sediment.

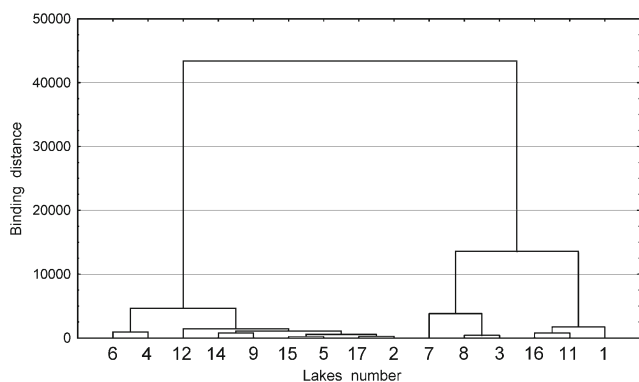


Fig. 4 Results of cluster analysis determined on the basis of photoluminescence parameters of studied HA

5 Conclusions

The research confirmed that the intensity of DL depends on the excitation light wavelength. The statistically significant differences in the DL intensity among the studied HA indicate the possible presence of different qualities and quantities of chromophore groups capable of the delayed luminescence. These may also indicate that the different photomechanisms are activated during the exposure the HA molecules on radiation.

The results showed that the DL depends on the properties and structure of humic acids. The higher capability for luminescence excited by blue- and green-light indicates HA molecules with higher contents of unsaturated bonds and a higher degree of oxidation. The HA whose structures are less condensed with the predominance of aliphatic fragments are characterized by a higher capability of luminescence excited by red light.

This research suggests the importance of sediment primary composition on DL patterns. The HA formed in silicate sediments exhibited greater capability for DL than did those extracted from sediments with greater organic proportions. The use of the delayed luminescence method may be a new

approach to obtain information about the structure and properties of humic substances. The use of DL may give some details on primary photochemical processes and energy-transfer processes within HS macromolecules.

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