Preliminary Investigations of Relative Polarity of Dissolved Humic Substances in Select Lakes of the Ełk Discrit, Poland

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Abstract

The reported study describes the separation of humic substances (HS) dissolved in surface waters into hydrophilic and hydrophobic fractions with the SPE method onto StrataX (Phenomenex) columns. The separated fractions were analyzed with the HPLC-C18 (Merck column) method for relative polarity of humic (HA) and fulvic (FA) acids in the samples. Analyses were carried out on samples collected from 7 lakes constituting two cascade systems located in the same catchment of the Ełk River. During HPLC assays, an effect of size exclusion (SE) was observed, especially for the hydrophilic fraction. The HPLC analyses enabled us to demonstrate that in both hydrological systems the hydrophilic fraction contained mainly molecules with molecular weights exceeding 1,000Da and of practically equal relative polarity. Only in system II (five lakes) was the hydrophobic fraction strongly diversified quantitatively as well as in terms of molecular weight and polarity between the samples. Results of analysis show that this system was subject to a strong anthropogenic impact.

Introduction

Humic substances (HS) constitute the prevailing part of dissolved natural organic matter (NOM) occurring in surface waters. The HS are high-molecular biopolymers, being degradation and transformation products of organic matter residues of both plant and animal origin [1, 2]. They have no strictly specified chemical structure and constitute a mixture of compounds with properties similar to one another, yet different. The structural and functional characteristics of NOM are changing to a considerable extent, depending, among other things, on the origin and age of substance and chemical medium in a given aquifer, which significantly hinders analyses of the soluble fraction of humic substances [3-8]. Nevertheless, a special function and importance of those substances as well as their impact on essential processes occurring in water habitat necessitate such investigations.

In particular, macromolecules of humic substances are characterized by a strongly developed surface with centers of various polarity and by a strong tendency for the absorption of both organic and inorganic compounds occurring in water. The non-polar hydrophobic nucleus of an HS molecule displays a tendency for binding non-polar organic compounds, e.g. polycyclic aromatic hydrocarbons [9-11]. In turn, ions of heavy metals are fixed, among others, owing to the presence of carboxyl groups [12-14].

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The content of humic substances in eutrophic lakes reaches 60-80% NOM [15], of which the fulvic acids (FA) usually predominate over the humus acids (HA) [16, 17], though in aquifers subjected to anthropopression a reverse tendency may be observed [18]. Fulvic acids are characterized by relatively low molecular weight and a high number of functional groups (hydroxyl and carboxyl ones), which makes them polar, hydrophilic and soluble in water irrespective of pH [19, 20]. In addition, they are resistant to microbiological and abiotic degradation and at a long half-life demonstrate considerable mobility in water [21]. The humic acids are readily soluble in water already after having been transformed into salts under conditions of increased pH. They have higher molecular weight than the fulvic acids – ranging from 1,500 to 5,000 Da in aquatic environment [5]. Due to a high number of aromatic rings in a molecule they are less polar and more hydrophobic than the fulvic acids. Physical sizes and shapes of humic acid molecules are determined by pH value and ionic strength of a solution as well as by the presence of metal ions. Large molecules of HA are susceptible to abiotic photodegradation [22]. Humines, having the lowest quantitative contribution among the humic substances, are insoluble in water at any pH value. Their composition and structure have been least established so far.

In the reported study an attempt was made to determine quantitative ratios and polarity of hydrophobic and hydrophilic fractions of dissolved organic matter in two cascade hydrological systems located on the area of the river Ełk basin.

Material and Methods

Functional characteristics of humic substances were carried out on samples collected once from 7 lakes of the Ełcki District belonging to the mesoregion of Ełk Lakeland in northeastern Poland. All lakes are located in the area of the river Ełk catchment.

Lakes selected for analyses could be divided into two groups constituting separate cascade systems gathered in Ełckie Lake, which is situated at the lowest altitude of all the lakes examined. Basic morphometric data of the lakes under study were presented in Table 1. The first hydrological system is composed of lakes situated in close vicinity: Woszczelskie (fed through the Młyńska River from Sawinda W. Lake), and Sunowo Lake fed additionally with waters from four small lakes and linked with a short stream directly with the northern side of Ełckie Lake.

The second cascade system includes a flow-through lake, Jędzelewo, followed by lakes situated alongside the Ełk River: Łaśmiady Lake – the greatest of all the lakes examined, as well as lakes: Straduńskie, Haleckie and Ełckie (southern basin). Haleckie is also fed by waters of the Przytulskie Lake that runs through two smaller aquifers.

Table 2 provides a few basic parameters enabling the estimation of water quality in the aquifers under study. Additional morphometric and hydrological data as well as physico-chemical parameters of those lakes recorded in recent years may be found in papers [23-25].
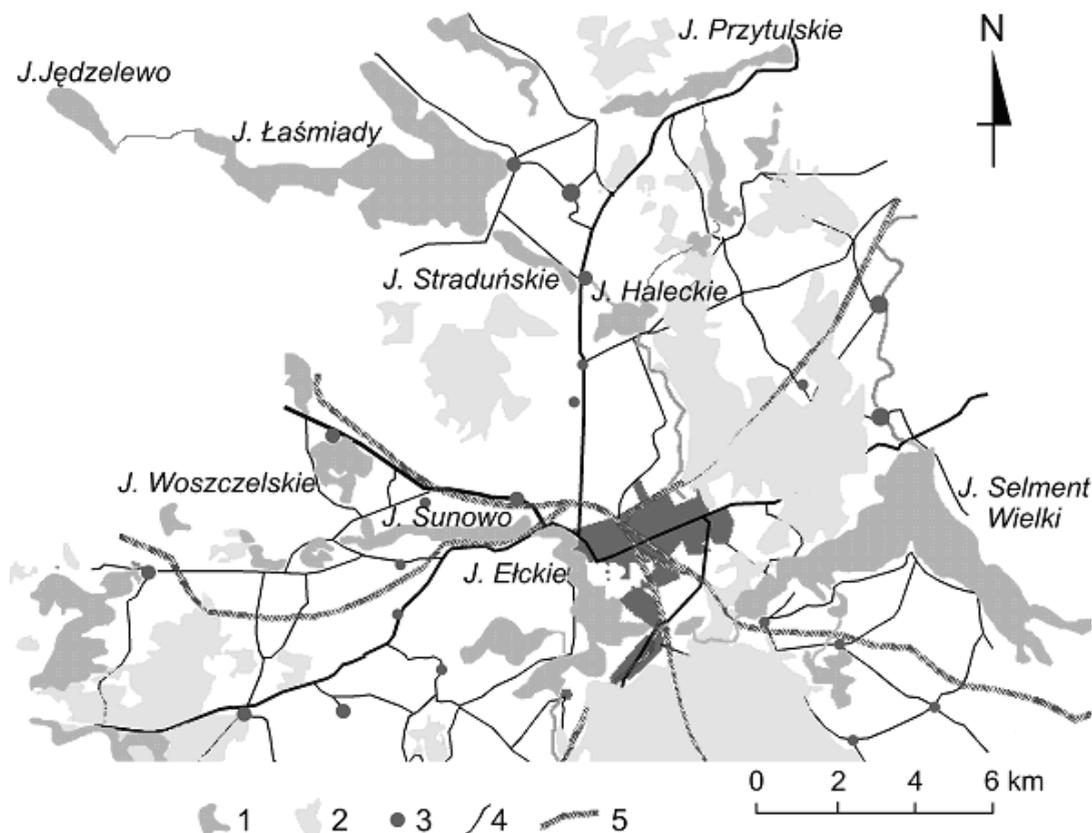

Fig. 1. Location of the examined lakes on the area of the Ełk District (1-lakes, 2-forests, 3-villages, 4-roads, 5-railway lines).

Table 1. Selected morphometric parameters of the examined lakes.

No.	Lake	Area (ha)	Volume (1000*m³)	Depth (m) max	Depth (m) mean	Developement of shoreline	Lenght of shorelinne (m)	Exposure index
1	Łaśmiady	882.1	84,607.8	43.7	9.6	2.1	21,950	91.9
2	Ełckie	382.4	57,420.3	55.8	15.9	2.7	18,650	24.05
3	Sunowo	181.4	16,456.1	20.6	9.3	2.8	13,105	19.5
4	Woszczelskie	172.6	5,849.2	10.6	3.3	1.8	8,375	52.3
5	Jędzelewo	150.3	4,967.2	13.3	3.2	1.75	7,700	47.0
6	Haleckie	93.5	3,154.5	7.2	3.4	1.4	4,950	27.5
7	Straduńskie	47.7	1,161.5	4.9	2.4	1.7	4,350	19.9

Table 2. Mean values of physico-chemical parameters of the examined lakes (s.I – system I, s.II – system II).

	No.	Lake	Temperature (ºC)	Secchi Disk (m)	Dissolved substances (ppm)	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)
s.I	1	Woszczelskie	10.7	2.0	237	470	10.5
	2	Sunowo	8.2	2.4	260	480	11.0
	3	Ełckie-a	10.0	2.9	235	470	10.5
s.II	3	Ełckie-b	8.0	2.9	220	440	10.4
	4	Haleckie	9.5	1.4	220	430	10,4
	5	Straduńskie	6.5	2.4	215	430	11.0
	6	Łaśmiady	6.0	2.7	205	430	10.5
	7	Jędzelewo	12	1.2	540	1100	10.1

Samples to be analyzed were collected at a depth of 1m from seven lakes listed in Table 1 in spring 2004. In most cases, the samples were collected at two sites – in the area of lake tributary and runoff. Directly on a boat, the samples were determined for the content of total compounds dissolved in water (TDS, ± 1 ppm) and conductivity (± 1 µS/cm) with the use of a universal measuring device HI 98129 COMBO by Hanna Instruments, as well as for the content of dissolved oxygen (± 0.1 mg/L) by means of an oxygen probe HI 9142 by the same company. Additional measurements were carried out for visibility of Secchi disk and water temperature. Samples of water (ca. 50ml in volume) were filtered with a syringe through Whatman GF/F 0.65 µm glass-fiber filters (diameter 25 mm), previously precombusted at 550ºC for 3 hours.

A commonly applied method of isolation and separation of humus-type substances is extraction onto XAD type sorbents (styrene-divinyl benzene (ST-DVB) – XAD-2, XAD-4; polymethacrylate-divinyl benzene – XAD-7, XAD-8 [26-31]. In the presented study, use was made of the SPE method on STRATA X columns (Phenomenex) with surface-modified ST-DVB with bed mass of 30mg. Typically, such columns are applied for concentrating polar contaminants that are hydrophobic in character. The mechanism of absorption consists in typical interactions between aromatic rings and, additionally, in the formation of hydrogen bonds [32]. Before being used, the columns were pre-conditioned with 1ml of CH_3OH (Merck) followed by 1 ml of H_2O (Merck). Since at pH > 2 the fulvic acids are not absorbed on the packing material used in the study, the samples were acidified to ca. pH 1 using 50 µl of 1:2 concentrated HCl (Merck). At such a low pH, hydroxyl and carboxyl groups of humic acids are non-dissociated, owing to which molecules of both fulvic acid (FA) and humic acid (HA) display a hydrophobic character and may be absorbed together onto the columns. The acidified sample was passed through a column, which was then rinsed with 2 ml of water acidified to pH < 2 (to avoid desorption of FA). The separation of the resultant humic substances into a fraction of fulvic acid and that of humic acid was carried out, assuming that the application of 1 ml of water with pH 7 at the first stage will result in the elution of only hydrophilic fulvic acids, while the humic acids will remain unabsorbed onto the carrier. At the second stage, 1ml of 0.1M NaOH was used for the desorption of humic acids transformed into salts.

Table 3. Changes in flow rate and eluent composition during chromatographic separation with the RP HPLC method.

Time (min)	Flow speed (ml/min)	Eluent content (%H$_2$O-%acetonitrile)
0.00	0.55	100-0
8.50	0.55	100-0
9.00	0.80	100-0
11.00	0.80	100-0
12.00	0.80	70-30
16.50	0.80	70-30
17.50	1.00	10-90
24.99	1.00	10-90

Having separated the hydrophilic and hydrophobic fraction, the concentrated eluates were analyzed with the RP HPLC method on C18 column (Merck). A LaChrom Merck kit with gradient elution (water/acetonitrile) was applied in the study using modifications of flow rate and composition of mobile phase presented in Table 3. Samples of 20 µl were injected onto the column, whereas the presence of the substances examined was registered using a UV-254 nm detector.

Results

In the case of the column used in this study, the dead retention time accounted for 2.95 min. As shown in Tables 4 and 5, retention times of almost all quantitatively-significant components of the hydrophilic fractions and a major part of those of the hydrophobic fraction were shorter than the zero retention time. The effect of size exclusion (SE) was observed, which indicates that sizes of the molecules whose signal appeared as first were greater than micropores of the stationary phase. Thus they were incapable of penetrating them and even if they were absorbed in the column their absorption occurred only on the surface of grains [33]. In the presented research, use was made of columns with the packing material with pore diameter of 10nm, that are recommended in the analysis of low-molecular compounds with weights up to 1,000 Da (producer's data). The appearance of the SE effect indicates that molecular weights of the humic substances present in the analyzed samples were higher than 1,000 Da. A similar effect was not observed for the same samples once applying column packing with pore diameter of ca. 30 nm [34], which in turn is recommended in the analysis of molecules with molecular weights up to 20,000 Da. Thus it may be assumed that in the samples examined the sizes of organic macromolecules were within a range typical of humic substances, i.e. between 1,000 and 20,000 Da.

Hydrophilic Fraction

The hydrophilic fraction was eluted from the column with water at neutral pH. Table 4 provides retention times and peak areas obtained for that fraction. In all samples there occurred peaks with retention times shorter than that of the solvent. Negligible differences in retention times of particular peaks of the FA group indicate also that even if the hydrophilic molecules differed in polarity, the SE effect and/or poor resolving power of the column would not allow registration of that fact explicitly. In both cascade systems the peak areas, correlated with the quantity of components in a given fraction, displayed a small downward trend from the highest to the lowest located lake. Also, functional diversity of the FA fraction appeared to be small in both the systems (Fig. 2). This was due to both the preliminary separation of the sample components on StrataX columns and the SE effect, but also to the internal nature of that fraction. The hydrophilic fraction represents a group of fulvic acids, being inert compounds resistant to microbiological and abiotic degradation and thus – to some extent – unified in terms of structure, size and functionality, often making HPLC analysis problematic [35-40]. Their mean content in lake system I: Woszczelskie-Sunowo-Ełckie (W-S-E), was twice as low as that recorded in system II: Jędzelewo-Łaśmiady-Straduńskie-Haleckie-Ełckie (J-Ł-S-H-E), i.e. 57,000 and
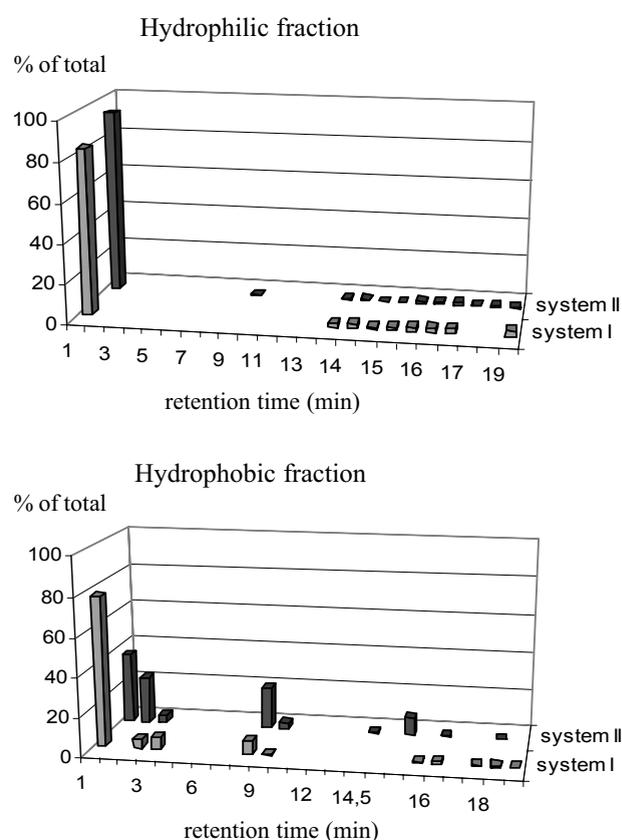
Fig. 2. Mean content (in %) of hydrophilic (FA) and hydrophobic (HA) substances with different molecular weight and polarity in systems under investigation.

Table 4. Relative contents (measured as peak areas in conventional units) of the hydrophilic fraction (FA).

Lake	Station	Retention time	Peak area
1. Woszczelskie	a	1.29	73,044
	b	1.09	42,418
2. Sunowo	a	1.13	67,077
	b	1.14	64,192
3. Ełckie	a	1.14	38,620
	b	1.14	103,152
4. Haleckie	a	1.1	108,800
	b	1.1	120,874
5. Straduńskie	a	1.08	99,712
	b	1.1	109,325
6. Łaśmiady	b	1.11	123,573
7. Jędzelewo	b	1.12	141,708

115,000 area size units, respectively (Table 4), but in both systems, the prevailing part of the hydrophilic substances were characterized by molecular weights over 1,000 Da.

Lakes of the W-S-E group constitute a compact system of aquifers located close to one another and with a very similar immediate catchment area. They are additionally fed with waters of a few other nearby reservoirs with various sizes, which has resulted in considerable unification of waters of that system. Table 2 collates averaged data that point to a similar physico-chemical characteristics of the systems. Visibility of Secchi disk for system I was in a narrow range of 2.0-2.9 m, whereas specific conductance accounted for 470-480 μS/cm^2, temperature for 8.2-10.7ºC, and content of dissolved oxygen for 10.5-11.0 mgO$_2$/L.

In contrast to system I, the lake system J-Ł-S-H-E was characterized by a distinct decline in FA content in the direction of river Ełk runoff – 141,700 area size units for the Jędzelewo lake and 103,100 are a size units for the southern part of Ełckie Lake. Especially contaminated appeared to be the small Jędzelewo Lake with a short retention time and Haleckie Lake (Table 2). The downward trend in the FA content observed in system II was disturbed in Haleckie Lake, in which the content of FA increased abruptly. It is a consequence of the discharge of waters from another cascade system in which the waters of Przytulskie Lake feed Haleckie Lake through lakes Zdrężno and Płociczno. The latter lake is a small, midforest aquifer. This type of lake is especially rich in humic substances [41]. Haleckie is additionally fed by wastewaters from the nearby housing cooperative and, probably, from a distillery.

A more than ten kilometers long reach of the River Ełk from Haleckie Lake to Ełckie Lake does not yield any significant decrease in the content of hydrophilic substances in the latter aquifer. Natural processes of self-purification are neutralized here by direct runoff from afforested and

meadow areas which are an important source of mobile fulvic acids [42, 43]. An additional source of hydrophilic substances in the receiver of River Ełk is the municipal character of the immediate catchment area of the southern part of Ełckie Lake.

Hydrophobic Fraction

The hydrophobic fraction, eluted from the columns with a 0.1 M solution of NaOH, was more diversified in terms of polarity and molecular weight as compared to the hydrophilic fraction. Within the idle time there appeared numerous peaks with distinctly diversified retention times and comparable, for a given sample, areas (Table 5, Fig. 3). Yet, retention times of those peaks were longer than of the analogous peaks of the FA fraction, which may point to a
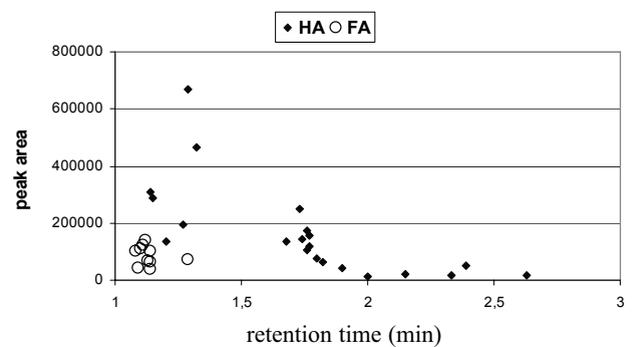
Fig. 3. Signals of hydrophobic (HA) and hydrophilic (FA) fractions within the range of dead retention time.
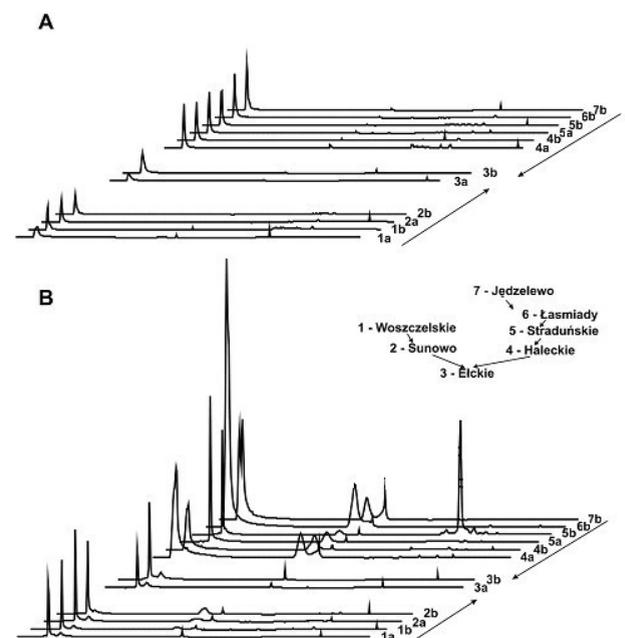
Fig. 4. Chromatograms of fractions: A – hydrophilic and B – hydrophobic (digits denote lakes as in Table 2; letter a or b denote lake tributary or runoff depending on reservoir position in the cascade).

Table 5. Peak areas (and their % contribution) as relative contents of the hydrophobic fraction in the samples examined.

Lake	St.	Retent. time	Peak area / % of total	Retent. time	Peak area / % of total	Retent. time	Peak area / % of total	Retent. time	Peak area / % of total	Retent. time	Peak area / % of total
1. Woszczelskie	a	1.77	119,731	2.45	16,961						
			87.59%		12.41%						
	b	1.75	106,391	2.4	15,293						
			87.43%		12.57%						
2. Sunowo	a	1.77	155,507	2.53	16,866						
			90.22%		9.78%						
	b	1.74	142,752	2.15	20,088	8.33	55,319				
			65.43%		9.21%		25.36%				
3. Ełckie	a	1.8	77,975	2.33	17,841						
			81.38%		18.62%						
	b	1.76	175,104	2.39	49,741	9.35	14,283				
			73.23%		20.8%		5.97%				
4. Haleckie	a	1.2	134,205	1.27	194,066	2.0	14,710	8.4	138,031		
			27.9%		40.35%		3.06%		28.7%		
	b	1.29	668,819	1.9	40,368	8.26	191,615	9.27	47,740		
			70.51		4.26		20.2		5.03		
5. Straduńskie	a	1.68	137,233	8.21	29,894	14.14	18,886	14.92	400,672	15.69	21,305
			22.57%		4.92%		4.28%		65.9%		2.33%
	b	1.73	248,490	8.2	87,600						
			73.94%		26.06%						
6. Łaśmiady	b	1.14	307,511	8.27	338,152						
			47.63%		52.37%						
7. Jędzelewo	b	1.15	286,568	1.32	464,808	1.82	64,083	8.22	153,079	9.24	88,558
			27.11%		43.97%		6.06%		19.87%		2.99%

lower total polarity of high-molecular compounds in the group of hydrophobic substances. Still, as compared to the FA fraction, the hydrophobic one is tangibly more complex in terms of functionality, which has been depicted in Fig. 2.

Apart from various peaks in the dead retention time, there could also be observed peaks with considerably longer retention times (8.19-8.4 min), that originated from substances with molecular weights lower than 1,000 Da and with lower polarity (Fig. 4). They constitute quantitatively-significant components of a mixture of hydrophobic substances. In lakes Jędzelewo, Haleckie and Ełckie there occurred peaks with retention times ranging from 9.24 to 9.35 s, whose area constituted 2.99-5.97% of the total area of peaks of a given fraction. Even longer retention times were observed for those substances in Straduńskie Lake, i.e. three peaks with retention times between 14.14 and

15.69s. They were eluted from the column already at acetonitrile content in the mobile phase, reaching together 72.5%, which points to their poor polarity.

The greater functional diversity of the humic acids fraction results both from effects of the basin on the lake (allochtonous matter, also of anthropogenic origin) and various processes in the aquifer waters. Microbiological and abiotic processes (transformation and destruction of organic matter upon the effect of UV radiation) cause the HS fraction to be subject to constant and various transformations toward the generation of hydrophilic substances structurally more simple and more resistant to further degradation. The largest of the lakes examined, Łaśmiady, is characterized by the longest period of waters exchange, hence the natural organic matter of that reservoir has the longest period of transformation. As a consequence [44],

the functional diversity of the HA fraction in that reservoir is small, likewise in waters of Straduńskie Lake – in its part where the Łaśmiady lake flows in. Yet in the samples collected at the mouth of the elongated Straduńskie Lake there was observed a greater qualitative diversity of the HA fraction, which is probably linked with the anthropogenic effect, since compounds with a very low relative polarity appear herein.

Conclusions

The application of STRATA X columns (Phenomenex) in the SPE method enables the separation of humus type substances of surface waters into hydrophilic and hydrophobic fraction, and then determination of their relative polarity with the HPLC-C18 technique. The use of the HPLC column with small diameters of micropores enables preliminary estimation of the size of molecules of humic and fulvic acids in samples. In some samples, molecules of the hydrophilic substances (HA) had molecular weights lower than those of the fulvic acids fraction. A comparison of relative contents of hydrophobic and hydrophilic substances in the analyzed samples suggests that the latter are subject to a strong impact of the basin and anthropogenic effects, especially in small and shallow lakes. In turn, effects of microbiological and abiotic transformations in large reservoirs cause the functional characteristics of hydrophilic substances to display small diversity.

Acknowledgements

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