ISSN 1070-3632, Russian Journal of General Chemistry, 2013, Vol. 83, No. 13, pp. 2715–2730. © Pleiades Publishing, Ltd., 2013.
Original Russian Text © P.N. Linnik, Ya.S. Ivanechko, R.P. Linnik, V.A. Zhezherya, 2013, published in Ekologicheskaya Khimiya, 2013, Vol. 22, No. 2, pp. 74–90.

Humic Substances in Surface Waters of the Ukraine

P. N. Linnik[a], **Ya. S. Ivanechko**[a], **R. P. Linnik**[b], **and V. A. Zhezherya**[a]

[a] *Institute of Hydrobiology, National Academy of Sciences of the Ukraine,*
pr. Geroev Stalingrada 12, Kiev, 04210 Ukraine
e-mail: peter-linnik@ukr.net

[b] *Taras Shevchenko National University of Kiev, ul. Vladimirskaya 64/13, Kiev, 01601 Ukraine*
e-mail: linnik_ros@univ.kiev.ua

Received April 30, 2013

Abstract—The results of studies of humic substances in water bodies of different types (lakes, rivers, and reservoirs) are given. It is shown that concentration of these natural organic compounds varies in a wide range of values (from 1.2 to 126.5 mg L^{-1}) due to different sources of their formation. The highest concentrations of humic substances are characteristic for rivers of the Pripyat' River basin flowing through the wetland. As we move from the north to the south, the content of humic substances is reduced. So, in the Kakhovka Reservoir, closing the Dnieper cascade of reservoirs, the concentration of humic substances is almost thrice as low as in the Kiev Reservoir, which is at the head of the cascade. Seasonal changes of humic substances concentration and the reasons for these changes are discussed. The prevailing fraction in the composition of humic substances is represented by fulvic acids, the content of which reaches 80.8–94.8% of the total. The results of studies of the molecular weight distribution of humic substances and the reasons for changes in the ratio of their individual fractions, depending on the detection method (spectrophotometric and fluorescence methods), are considered. The values of the number average (M_n) and weight average (M_w) molecular weight of humic substances and the degree of polydispersity are calculated. It is shown that M_w varies seasonally. In spring and summer it is lower, but significantly increased in autumn. The reason for this phenomenon is degradation of high-molecular fractions of humic substances under the influence of UV light of solar radiation and increased microbiological activity during the summer season. As a result of these processes high-molecular fractions of humic substances are transformed into fractions with lower molecular weight, which become predominant.

Keywords: Humic substances, humic acids, fulvic acids, molecular weight distribution, average molecular weight, polydispersity, lakes, rivers and reservoirs.

DOI: 10.1134/S1070363213130185

Humic substances are an integral component and the most widespread group of dissolved organic matter in natural surface waters, which has a significant impact on their chemical and biological quality and their suitability as a habitat for aquatic organisms. Humic substances are formed from decaying remains of plants and animals as a result of chemical and microbiological processes and are represented by polydisperse macromolecular mixtures [1, 2]. Usually the following three fractions are distinguished in the composition of humic substances: humic acids, which are insoluble in acidic medium (pH < 2); fulvic acids, which are soluble in water within the whole range of pH values; and humins, which are insoluble in water at any pH value. The specific characteristic of humic substances is that they cannot be described as compounds with a definite molecular structure [3]. As stated in a number of recent reviews studying the molecular structure of humic substances, there is still no consensus with regard to their main structural components [2, 4, 5].

The predominant fraction of humic substances in natural surface waters is fulvic acids. Thus, the ratio of fulvic acids to humic acids in low-color waters reaches 10 : 1, whereas in high-color waters this figure is 5 : 1. In pore solutions of the bottom sediments of water bodies this ratio is radically different and is equal to 1 : 3, which indicates the dominance of humic acids in these solutions [2, 6].

In freshwater ecosystems the relative content of fulvic acids amounts to an average of approximately

50% of dissolved organic matter [2, 7, 8]; however, there are frequent cases when it can reach even 90% [6, 8]. The latter is characteristic for rivers draining basins with a high content of peat [9].

There is approximately 50% of carbon, 4–5% of hydrogen, 35–40% of oxygen, 1–2% of nitrogen, and less than 1% of sulfur and phosphorus in the elemental composition of humic substances [8]. Humic substances are represented by heterogeneous mixtures of different organic compounds and their macromolecules contain various functional groups, including aromatic, quinoid, and aliphatic structures in the nucleus, as well as amino-acid- or carbohydrate-like structures and carbonyl, carboxyl, phenolic, and hydroxyl groups in the periphery [2, 10]. There are also phosphorus- and sulfur-containing groups. At the same time, such characteristics of humic substances as the size of molecules, chemical composition, structure, and functional groups can significantly vary depending on their source of origin and age [10]. Due to their multi-functionality and diversity of structure, humic substances take part in ion, hydrophobic, and electron donor-acceptor interactions [11].

As complex macromolecular structures humic substances are characterized by a wide range of molecular weight values, ranging from several hundred to several thousand or even tens of thousands dalton (Da) [9, 12–14]. The molecular weight of humic acids greatly exceeds the molecular weight of fulvic acids [12, 13, 15, 16]. It is explained, on the one hand, by formation of hydrogen bonds between phenol hydroxyl and carboxyl functional groups in the composition of their macromolecules and, on the other hand, by aggregation of humic acids with amorphous silica and clay substances [2, 16].

The results of studies of humic substances are reflected in a great number of scientific works, including several specialized monographs, focusing on physicochemical properties of these natural organic acids [2, 8, 9, 14]. Despite the fact that humic substances are well-studied, scientific interest to them has not recently dropped but, on the contrary, it is increasing, which is caused by the following circumstances. Firstly, humic substances are a source of organic carbon and a nutrient for plants, microorganisms and benthic organisms. Secondly, they play an important role in the cycle of such biogenic elements as nitrogen and phosphorus, defining their bioavailability. Thirdly, humic substances participate in many biochemical and geochemical processes; they have influence on formation of the hydrochemical regime of surface water objects, in particular, their oxygen regime. Fourthly, humic substances play a crucial role in detoxification processes. It is well-known that due to their complexing ability these natural organic acids bind metal ions and organic toxicants in the complexes and adducts, thus reducing their chemical and biological activity, which eventually results in a reduction in their toxicity to living organisms [9, 12, 13, 16–21]. Therefore, we can speak with confidence about a significant influence of humic substances on the functioning of aquatic ecosystems, including their abiotic and biotic components [18].

At the same time, an increased concentration of humic substances in a water body can also have certain negative effects. In particular, it has been found out that in this case there is limitation of the primary production resulting from reduced depth of the photic zone [2]. Furthermore, in the process of drinking water treatment at the stage of chlorination of water, containing high concentrations of humic substances, there is formation of chlorine derivatives with serious carcinogenic properties, which is dangerous for human health [22, 23].

The principal role of humic substances in the functioning of aquatic ecosystems sets new tasks on advanced study of physicochemical properties of these natural organic compounds. These data are necessary, first of all, in order to understand reactions and interaction processes that take place in surface water objects with the participation of these compounds. The more information we have about the structural and functional properties of humic substances, the better we understand the mechanisms that are responsible for complexing, reduction, bioavailability, and mobility or immobility of metals and toxic organic substances in abiotic components of aquatic ecosystems.

The composition variability, heterogeneous nature, multi-functionality, and polydispersity of humic substances make it necessary to apply a complex of research methods, combining different extraction, concentration, separation, and detection techniques, in order to study the content and physicochemical properties of these compounds. At present there is a tendency towards the development of a certain unified approach to the study of humic substances, which is reflected in a number of recently published scientific works [9, 16].

This work summarizes the results of studies of humic substances in different types of water objects of the Ukraine (rivers, lakes, and reservoirs), located in different physicogeographical zones.

EXPERIMENTAL

Studies of humic substances were carried out at different water objects, including lakes of the Shatsk group, rivers of the Pripyat' River basin, reservoirs of the Dnieper cascade, the Danube River (Kiliya delta), the Desna River (mouth), the Ros' River (near the Belaya Tserkov' Town), the Yuzhnyi Bug River (near the Khmel'nitskii Town), the Seret River (upstream and downstream of Ternopol' Reservoir), the Gornyi Tikich River (within the boundaries of Chernaya Kamenka village), Ternopol' Reservoir (upper and lower reaches), and upper Kitaevo pond (in Kiev City).

Water samples were collected from the surface layer at a depth of ~ 0.5 m and were transported to the laboratory for further investigation as soon as possible. In order to separate suspended materials the water samples in the volume of 1.0–1.5 L were passed through membrane filters produced by Synpor (Czech Republic) with the pore diameter of 0.4 μm.

In order to separate humic substances the natural water filtrate was passed through a column with DEAE (diethylaminoethyl) cellulose anion exchanger produced by the SERVA Company. The length of the anion exchange column is 27.5 cm, the diameter is 2.5 cm, and the sorbent layer thickness is 4.5 cm. The void volume of the column is 12.5 mL. The advantage of DEAE cellulose as a sorbent is that sorption of humic substances takes place primarily on the surface of the sorbent, which significantly facilitates their desorption. The process of desorption was carried out in three stages, which was aimed to provide a more complete extraction of humic substances. At the first stage 0.3 mol L^{-1} KOH solution was used as an eluent, the second stage involved application of 0.02 mol L^{-1} H_2SO_4 solution, and at the third stage 0.3 mol L^{-1} KOH solution was used again. The elution rate amounted to approximately 1.0 mL min^{-1}. Multifold concentration of humic substances (in most cases at least 25–50-fold, depending on the object of research and the content of humic substances in the water) was achieved in the course of the adsorption process. In some cases the resulting concentrate of humic substances was subject to fractionation into humic and fulvic acids, which was performed in an acidic medium at pH of 1.5–2.0 and temperatures of up to ≈ 50°C during several hours. Precipitation of the resulting sediment of humic acids took twenty-four hours, after which it was separated by centrifugation. The fraction of fulvic acids was put into an acidified solution, pH of which was brought up to the value characteristic for the source natural water. The sediment of humic acids was dissolved in an alkaline solution; after its complete dissolution its pH was also brought up to the initial value.

Different methods were applied to determine concentrations of humic substances. These techniques included spectrophotometric and fluorescence measurements, as well as the photometric method involving a coupling reaction with diazotized 4-nitroaniline [24]. All the listed methods are applicable for detection of humic substances only after they are extracted from natural water. Another technique that was applied was an indirect method to detect humic substances based on determination of the colority of water or solutions containing these compounds with the use of the simulating cobalt-dichromate scale [25].

Absorption and fluorescence spectra of humic substances solutions in concentrates and in fractions after gel-chromatographic separation were registered using Unico UV 2800 spectrophotometer and Perkin Elmer LS-55 fluorescence spectrometer with a xenon discharge lamp. In case of spectrophotometric and fluorescence methods of detection the concentration of humic substances was found according to the calibration graphs "Optical density at 254 nm – concentration of humic substances, mg L^{-1}" and "Fluorescence intensity (440 nm) – concentration of humic substances, mg L^{-1}." In case of photometric detection by the coupling reaction the concentration of humic substances was found according to the calibration graph "Optical density at 540 nm – concentration of humic sub-stances, mg L^{-1}." The calibration graphs for detection of humic and fulvic acids were built using their preparations, extracted from the water of the Kanev Reservoir and lakes of the Shatsk group, purified, and dried in compliance with the methodology described in the works of I.S. Sirotkina [26].

The molecular weight distribution of humic substances and their fractions (humic and fulvic acids) was studied using the method of gel-chromatography. A glass column filled with TOYOPEARL HW-50F gel (Japan) was used for the purpose. The column parameters were as follows: length of 82.0 cm, diameter of 2.8 cm, gel layer thickness of 62.0 cm, and void volume (V_0) of 160 mL. The column was preliminarily calibrated using solutions of polyethylene glycols with a molecular weight of 1.0, 2.0, 15.0, and 20.0 kDa and glucose with a molecular weight of 0.18 kDa. The concentration of polyethylene glycols and glucose in the solutions amounted to 2.0 and 0.5 mg mL^{-1}, respectively. The eluent was represented by 0.025 mol L^{-1} phosphate buffer solution with pH of 7.0, which made it possible to eliminate effects of electrostatic interactions of humic substances polyanions with the gel [27]. The value of V_0 was found using blue dextran solution (2000 kDa). The molecular weight of humic substances in fractions was determined according to the calibration graph, built in the coordinates of $V_e/V_0 - \log M$. The distribution coefficient (R) was calculated according to the formula (1):

$$R = \frac{V_e - V_0}{V_t - V_0}, \qquad (1)$$

where V_e is excluded volume of product with definite molecular weight (M), V_0 is void volume of the column, V_t is total volume of the column.

Based on the results of studies of the molecular-weight distribution of humic substances, as well as humic and fulvic acids in the composition of these compounds, it was possible to calculate their average molecular weight – number average (M_n) and weight average (M_w) – according to the formulas (2) and (3) [16, 28, 29]:

$$M_\text{n} = \frac{\sum_i^n W_i}{\sum_i^n (W_i/M_i)}, \qquad (2)$$

$$M_\text{w} = \frac{\sum_i^n (W_i M_i)}{\sum_i^n W_i}, \qquad (3)$$

where W_i is total weight of molecules with molecular weight of M_i, which can be expressed as the substance concentration using optical density of the solution, fluorescence intensity, or carbon content of organic compounds (C_org). In addition, the degree of polydispersity (P) was calculated according to the formula (4):

$$P = \frac{M_\text{w}}{M_\text{n}}. \qquad (4)$$

The most frequently used value to characterize the average molecular weight of humic substances, humic acids, and fulvic acids is M_w.

The total content of organic compounds (C_org) was calculated based on the results of determination of the chemical oxygen demand (COD) using the following formula (5):

$$C_\text{org} = 0.375 \times \text{COD}. \qquad (5)$$

RESULTS AND THEIR DISCUSSIONS

1. Methodological Characteristics of Extraction and Methods for Detection of Humic Substances

As demonstrated by the research results, it was impossible to achieve complete extraction of humic substances in the process of desorption from the column with DEAE cellulose. In case of one-stage desorption, involving the use of 0.3 mol L^{-1} KOH solution only, the extraction degree of humic substances amounted to 74–90% (83% at average), whereas in case of three-stage desorption described above this figure is as high as 90–96% (93% at average). The losses are significantly higher when humic substances are extracted from high-color water. In such cases three-stage desorption is especially useful, as it significantly minimizes the losses. Nevertheless, the results of our research works convince us that DEAE cellulose is quite applicable as a sorbent for extraction of humic substances from natural water. The extraction degree of humic substances is considered satisfactory when it is not less than 85% [12].

A number of methods, different both in sensitivity and some other characteristics, were applied to determine the content of humic substances in concentrates after extraction from natural water samples and in fractions after gel-chromatographic separation. It was found that all of the applied techniques gave satisfactory results (Table 1).

The fluorescence method is characterized by the highest degree of sensitivity. The method for detection of humic substances on the basis of the water colority cannot be considered precise or reliable and, therefore, it was most often used as an auxiliary method. Nevertheless, the use of this technique makes it possible to determine the concentration of humic substances directly in the natural water filtrate without extracting them, which cannot be achieved by other methods due to various interferences.

Table 1. Content of humic substances in surface natural waters determined using different methods

Objects of research, dates of water sample collection	Methods of detection			
	spectrophotometric method	fluorescence method	photometric method	method based on water colority
	Concentration of humic substances, mg L^{-1}			
Desna River, mouth, 02.11.2011	10.2±0.3	11.5±0.4	10.9±0.5	9.9±0.3
Desna River, mouth, 27.05.2011	14.8±0.3	15.3±0.4	13.2±0.3	11.6±0.3
Desna River, mouth, 09.02.2012	19.3±0.7	21.0±0.8	20.2±0.9	18.3±0.7
Ros’ River, 22.05.2011	7.9±0.2	7.3±0.3	No analysis performed	6.3±0.2
Ros’ River, 27.11.2011	10.6±0.4	10.1±0.5	10.2±0.4	8.9±0.4
Danube River, mouth, 15.11.2012	17.7±1.3	19.4±1.5	19.0±1.6	18.4±1.7
Kanev Reservoir (Desenka branch), 25.05.2012	34.8±2.6	37.5±2.9	37.2±2.8	36.0±3.1

2. Concentrations of Humic Substances in Studied Water Objects

The content of humic substances in the water objects under study is characterized by a wide range of values (Table 2), which is associated with different physicogeographical zones of their location and, therefore, with specific features of formation of organic substances, including humic substances, in these water objects. Maximal concentrations of humic substances were found in the rivers of the Pripyat’ River basin and the Pripyat’ River itself. The characteristics feature of the Pripyat’ River basin is its high level of swampiness, reaching 40% [30], which has a significant impact on formation of dissolved organic matter and, first of all, humic substances in rivers passing through the territory of the basin. However, not all rivers of the Pripyat’ River basin are characterized by a high content of humic substances. Concentrations of humic substances in rivers of the Western Poles’e (Stokhod, Styr’, Prostyr’, and Goryn’) are much lower as compared to the Northern Poles’e (Ubort’, Stviga, L’va, and Slovechna). This phenomenon is related to the fact that in the first case the formation of dissolved organic matter is based primarily on groundwater feeding, whereas rivers of the second group are fed from peatlands and wetlands [31]. A high content of humic substances is also characteristic for the Kiev Reservoir, located at the head of the Dnieper cascade, which can be explained by the inflow of high-color waters from the rivers running into it, especially the Pripyat’ River. As we move down the cascade of reservoirs, concentrations of humic substances decrease

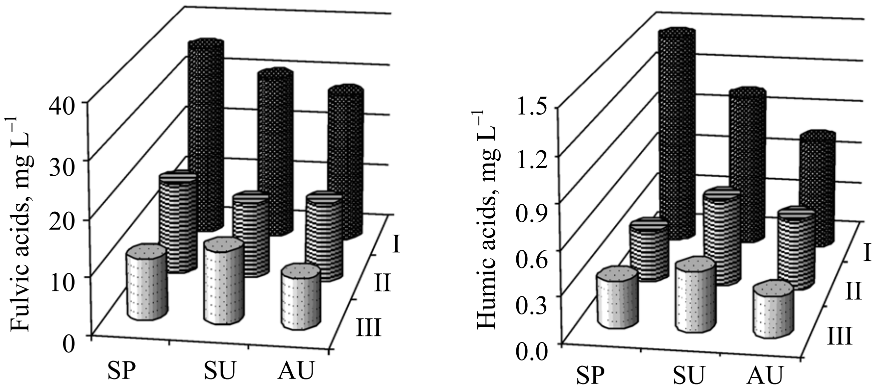

Fig. 1. Changes in average concentrations of fulvic and humic acids in waters of Kiev (I), Kremenchug (II), and Kakhovka (III) reservoirs of the Dnieper cascade in spring (SP), summer (SU), and autumn (AU).

Table 2. Content of humic substances in some water objects of the Ukraine according to 2010–2012 research results (spectrophotometric measurements performed at 254 nm)

Objects of research	Humic substances	
	mg L^{-1}	% C_{org}
Lakes of the Shatsk group		
Lyutsimir	9.5–46.3 20.9±7.8	58.6–92.0 84.8±4,7
Bol’shoe Chernoe	11.9–15.8 14.2±1.5	70.8–86.5 79.6±6.3
Rivers		
Pripyat’, Senchitsy village	27.8–95.4 58.3±15.6	–[c]
Rivers of Pripyat’ River basin, June of 2010[a]	31.5–122.0	–[c]
Rivers of Pripyat’ River basin, September of 2010[a]	26.0–75.8	–[c]
Rivers of Pripyat’ River basin, July of 2012[b]	20.3–126.5	71.0–91.5
Rivers of Pripyat’ River basin, October-November of 2012[b]	15.5–35.0	67.3–88.6
Desna, mouth	10.2–19.3 12.9±2.4	67.9–88.7 77.9±9.7
Ros’, Belaya Tserkov’ Town	7.6–13.0 9.1±1.6	48.5–84.2 72.4±13.8
Yuzhnyi Bug, Khmel’nitskii Town	6.7–11.9 8.3±3.7	51.2–88.3 68.7±14.4
Danube, Kiliya delta	2.5–11.3 6.7±3.8	–[c]
Seret, upstream of Ternopol’ Reservoir	1.2–7.5 3.5±2.4	33.3–75.0 56.6±10.8
Seret, downstream of Ternopol’ Reservoir	3.2–9.8 6.9±2.5	52.8–78.1 68.9±12.8
Gornyi Tikich, Chernaya Kamenka village	12.2–22.0 17.0±3.6	–[c]
Reservoirs		
Kiev Reservoir (1990–1995)	14.6–47.8 31.5±9.2	62.3–83.5 71.8±7.8
Kanev Reservoir, upper reaches	13.4–35.6 22.3±8.4	55.8–79.7 68.5±5.6
Kremenchug Reservoir (1990–1995)	9.5–23.8 16.5±4.6	52.4–71.3 64.5±5.5
Kakhovka Reservoir (1990–1995)	5.3–21.0 11.2±5.8	45.3–59.6 54.8±4.3
Ternopol’ Reservoir, upper reaches	3.2–6.2 4.9±2.6	43.5–79.5 60.1±13.3
Ternopol’ Reservoir, lower reaches	5.4–10.6 8.8±1.0	57.4–77.0 68.6±11.1
Ponds		
Upper Kitaevo pond, Kiev City	8.7–13.6 10.5±0.7	36.3–74.8 59.3±8.1

[a] Slovechna, Ubort’, Stviga, L’va, Goryn’, Styr’, Prostyr’, and Stokhod rivers. [b] Sluch’, Tsir, Styr’, Stochod rivers. [c] Calculations were not carried out due to the lack of data on C_{org}. The data on the content of humic substances in the reservoirs of the Dnieper cascade for 1990–1995 are given according to work [17]. In Tables 2 and 3: figures above the line correspond to ranges of values, whereas figures below the line represent average values.

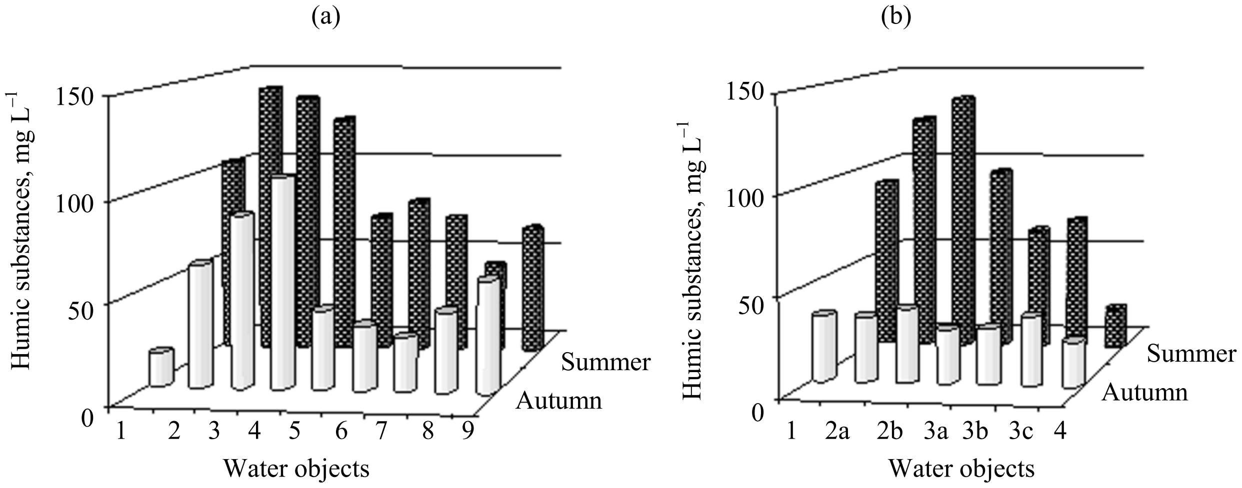

Fig. 2. Content of humic substances in waters of Pripyat' basin rivers in summer and autumn of 2010 (a) and 2012 (b). (a) Slovechna (1), Ubort' (2), Stviga (3), L'va (4), Goryn' (5), Styr' (6), Prostyr' (7), Stokhod (8), and Pripyat' (9); (b) Stokhod (1), different parts of Tsir (2a and 2b), different parts of Pripyat' (3a, 3b, and 3c), and Sluch' (4).

significantly, as can be seen from the example of the Kakhovka Reservoir, closing the cascade. The average content of this group of organic substances in this reservoir is almost thrice as low as in the Kiev Reservoir (Table 2). A clearer idea about changes in the average long-term concentration of humic substances in the water of the Dnieper reservoirs can also be given by the data presented in Fig. 1. The decrease in the concentration of humic substances in the downstream water object as compared to the upstream reservoir results from partial adsorption of these compounds on suspended matter and sedimentation of the suspension, dilution with low-color waters, assimilation by hydrobionts, and a number of other reasons [32]. In this connection it should also be added that river waters of the forest-steppe and steppe zones are characterized by low concentrations of humic substances due to weak leaching of humus from the soils of these zones, as the content of the so-called free moving forms of humic substances in them is reduced [32].

The content of humic substances undergoes seasonal changes, which is related to changes in the hydrological regime of water objects, as well as to all kinds of chemical and biochemical transformations. This can be demonstrated by the example of the Dnieper reservoirs (Fig. 1) and rivers of the Pripyat' River basin (Fig. 2). The Dnieper reservoirs are located in such a way that, as a rule, maximal concentrations of humic substances in the upstream water bodies are to be observed during the spring flooding period, when humic substances are leached out from wetlands and peatlands and enrich river water, coming into the water reservoirs. To the greatest extent this applies to the Kiev Reservoir. Later, in the period from spring to autumn, the content of humic substances decreases, which results, on the one hand, from decrease in their concentration in waters of the feeding rivers and, on the other hand, from assimilation of humic substances by aquatic organisms, as these compounds are an important nutrient source for their growth and living.

In the downstream reservoirs of the Dnieper cascade (Zaporozh'e and Kakhovka Reservoirs) maximal concentrations of humic substances are observed in summer (Fig. 1), which is related to the delayed inflow of water with a high content of these organic compounds from the upstream reservoirs. At the same time, it can happen that concentrations of humic substances in the upstream reservoirs of the cascade significantly increase during the autumn period after long and heavy rainfalls in the Poles'e zone, which the rivers pass before entering the cascade. Such a situation was observed in autumn of 1993 and even in winter of 1998–1999 in thawing weather.

The highest concentrations of humic substances in rivers of the Pripyat' River basin are characteristic for the spring-summer period, whereas in autumn they fall down (Fig. 2). The reasons for this phenomenon have been already described above.

In small-size reservoirs, including, for example, Ternopol' Reservoir on the Seret River, the content of humic substances increased at the end of summer and in autumn from 4.8 to 6.2 mg L^{-1}, which is

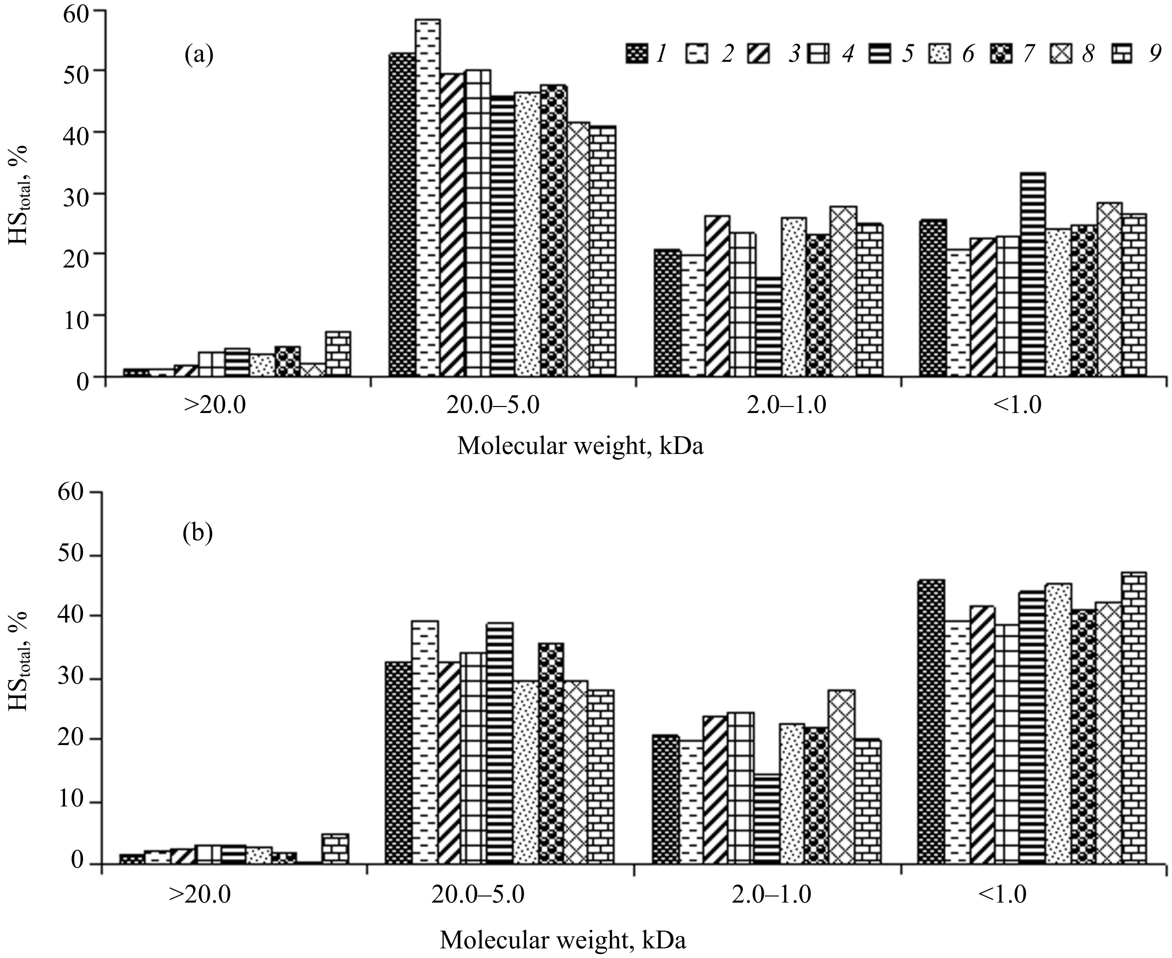

Fig. 3. Ratio of humic substances fractions with different molecular weight (averaged data) in studied water object according to results of spectrophotometric (a) and fluorescence (b) measurements of their concentrations. Water objects: Lutsimir Lake (*1*), Bol'shoe Chernoe Lake (*2*), Desna River (*3*), Ros' River (*4*), Kanev Reservoir, Desenka branch (*5*), upper Kitaevo pond (*6*), Yuzhnyi Bug River (*7*), Seret River (*8*), and Ternopol' Reservoir (*9*).

approximately by 25–30%. Apparently, it is related to formation of humic substances in the water body itself, where they are formed from the remains of algae, plant and animal organisms as a result of chemical and microbiological processes. In this case the concentration of humic substances in the lower reaches of the reservoir was almost twice as high as compared to the upper reach (Table 2). Such a situation was also observed in a small river of Gornyi Tikich, where a number of small-size reservoirs are located. In these reservoirs water colority, which is an indirect indicator of the content of humic substances, significantly increased from spring to autumn from 17.0 to 30.6 degrees of the simulating cobalt-dichromate scale.

The prevailing fraction in the composition of humic substances in the studied water bodies and streams is represented by fulvic acids, the relative content of which reaches 80.5–94.8%, which is in line with the numerous data on the ratio of fulvic and humic acids in other water objects [2, 6, 8].

3. Specific Features of Molecular-Weight Distribution of Humic Substances

Studies of the molecular-weight distribution of humic substances seem important, first of all, from the ecological point of view as assimilation of these compounds by phytoplankton and aquatic vegetation greatly depends on their molecular weight. There is a general opinion that high-molecular fractions of humic substances cannot penetrate biological membranes due to their size [33]. A number of works [21, 34] state that such penetration is possible only for molecules, the molecular weight of which does not exceed 5.0 kDa.

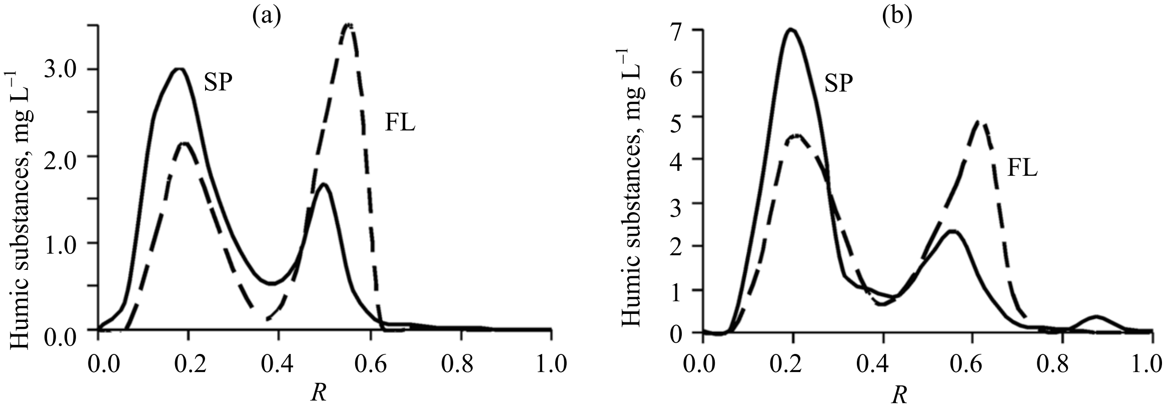

Fig. 4. Gel chromatograms of humic substances extracted from water of Desna River on 02.11.2011 (a) and Lutsimir Lake on 21.09.2011 (b), depending on detection methods. C_{HS}: 12.0 mg L^{-1} (a) and 25.0 mg L^{-1} (b). In Figs. 4 and 5: SP and FL are spectrophotometric and fluorescence methods for determination of concentrations of humic substances, respectively; (*R*) – distribution coefficient.

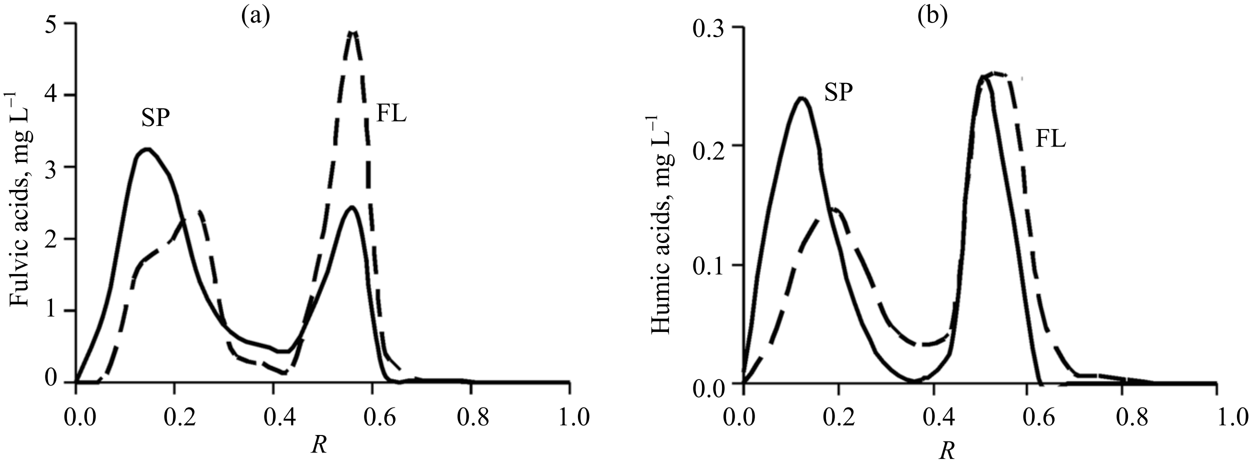

Fig. 5. Gel chromatograms of fulvic (a) and humic (b) acids extracted from water of the Kanev Reservoir upper reaches (Desenka branch, 02.11.2011). C_{FA}=14.3 mg L^{-1}; C_{HA}=1.1 mg L^{-1}.

As noted above, we used the spectrophotometric and the fluorescence methods to detect humic substances in concentrates after their extraction from natural water samples and in fractions after gel-chromatographic separation, as these measuring techniques are the most widely-used for the purpose. Whereas the results of determination of the total content of humic substances using these two methods turned out to be satisfactory (Table 1), the data on the ratio of humic substances fractions with different molecular weight differed significantly (Fig. 3). In case of spectrophotometric measurements of the concentration of humic substances in fractions a fraction with the molecular weight of 20.0–5.0 kDa dominated (Fig. 3a). The relative content of this fraction amounted to 41.0–58.4% of the total. The total content of high-molecular fractions (>5.0 kDa) reached 43.6–59.6%. At the same time, when the fluorescence method to detect humic substances was used, a low-molecular fraction (< 1.0 kDa) prevailed; the share of this fraction in the total ranged from 38.4 to 47.0% (Fig. 3b). Therefore, the greatest changes were associated with fractions with the molecular weight of 20.0–5.0 kDa and <1.0 kDa.

The above-stated is also confirmed by the corresponding gel chromatograms of unseparated humic substances (Fig. 4). The first peak on gel chromatograms characterizes the yield of compounds with higher molecular weight, whereas the second is related to the fraction with lower molecular weight. In case of spectrophotometric measurements of the concentration of humic substances in fractions the first peak is always higher than the second one, which testifies to prevalence of high-molecular substances. At the same time, application of the fluorescence method for detection of humic acids gives a different picture. Often the second peak is much higher as compared to the first one or both the peaks are comparable in height, which stems from the fact that different fractions of humic substances have different fluorescence properties. The intensity of fluorescence of fractions with lower molecular weight was greater as compared to high-molecular fractions. The results we have obtained are consistent with numerous published data [2, 35, 36]. Apparently, this phenomenon is typical for aquatic humic substances regardless of the region in which one or another water object is located. At the same time, both the methods give similar results when concentrations of humic substances are measured in concentrates after desorption from a column with DEAE cellulose, as well as after summing the content of humic substances in individual fractions after gel-chromatographic separation. Therefore, in our opinion, the reason for the differences in the ratio of humic substances fractions measured with the use of the spectrophotometric and the fluorescence methods of detection is related to certain methodological imperfections. In particular, the calibration graph for detection of humic acids was based on the use of their preparations. It is possible that the fluorescence intensity of unseparated humic substances is different from the fluorescence intensity of their individual fractions. The composition of humic substances includes fractions with both higher and lower molecular weight; therefore, their fluorescence intensity can be significantly different, which is reflected on the results of calculations of the concentration of humic substances in each of them. A number of works [29], characterizing the spectrophotometric method for detection of humic acids in the UV spectrum range, state that this technique overestimates the content of C_{org} in high-molecular fractions and underestimates this parameter in low-molecular fractions. The authors of work [28] come to similar conclusion.

As demonstrated by the results of studies, the fluorescence intensity of fulvic acids turned out to be higher as compared to humic acids (Fig. 5). In terms of fluorescence intensity the first peak of the content of humic substances became lower, whereas the second remained almost unchanged and was similar to the case of spectrophotometric measurements.

4. Average Molecular Weight of Humic Substances

We used the results of studies of the molecular-weight distribution of humic substances to calculate their number average (M_n) and weight average (M_w) molecular weight, as well as the degree of polydispersity (P). Most often scientific works give data on M_w values for humic substances. Therefore, we also provide M_w values and the degree of polydispersity of humic substances (Table 3) for the studied water objects. It can be seen that there is quite a wide range of M_w values for humic substances. Even taking into account the average M_w value for each object, it ranges from 4.6 to 7.0 and from 2.9 to 5.8 kDa, respectively, according to the results of the spectrophotometric and fluorescence methods. It turned out that the difference in the ratio of humic substances fractions, resulting from the use of the spectrophotometric and fluorescence detection methods, was also reflected on M_w values. The degree of polydispersity is also characterized by a wide range of values, which ranges from 4.2 to 7.5 at average. For polydisperse substances, including humic substances, $M_n < M_w$ as M_w grows when the degree of polydispersity increases. It can be also assumed that the greater are the differences in the molecular weight of fractions of high-molecular humic substances, the higher is the ratio of M_w/M_n. There is little difference in M_n and M_w values in case the content of the low-molecular fraction in high-molecular substance is low. Therefore, for monodisperse systems the ratio of M_w/M_n is close to one [29]. In case of a higher content of the low-molecular fraction the differences between M_n and M_w values rise sharply and the ratio also increases. Therefore, it can be assumed on the basis of the polydispersity values that humic substances in surface waters of the Ukraine as high-molecular multicomponent systems are characterized by quite a high content of low-molecular fractions in their composition (in some cases P reached 9–13, see Table 3). It is also confirmed by the results of studying the ratio of humic substances fractions with different molecular weight (Fig. 3), which has been described above.

For greater clarity Fig. 6 provides the average M_w values for humic substances in each of the studied objects taking into account different methods of their detection. It turned out that according to spectrophotometric measurements the average M_w values were approximately 1.5-fold higher as compared to fluorescence measurements. Therefore, it is inevitable that the results of studies of the molecular-weight distribu-

Table 3. Weight average (M_w) molecular weight of humic substances in water objects of Ukraine and degree of their polydispersity (P)

Objects of research	Spectrophotometric method		Fluorescence method	
	M_w, kDa	P	M_w, kDa	P
Lyutsimir Lake	3.4–7.0 4.9±2.1	3.4–6.0 4.5±1.5	1.6–4.5 2.9±1.9	3.2–7.4 4.2±0.9
Bol'shoe Chernoe Lake	4.0–9.1 5.6±2.4	3.1–13.0 4.2±3.3	2.8–9.9 3.8±1.8	4.0–8.3 5.4±3.9
Desna River, mouth	4.6–8.5 6.1±1.8	2.7–6.1 4.4±0.9	2.4–6.4 3.6±0.6	3.3–5.4 4.8±1.7
Ros' River, Belaya Tserkov' Town	3.8–11.0 5.5±0.6	2,9–9,2 5.6±1.1	2.1–9.3 4.4±1.5	3.5–7.4 5.6±1.1
Yuzhnyi Bug River, Khmel'nitskii Town	3.9–9.1 6.0±3.6	4.3–7.7 5.8±2.3	2.1–7.3 3.9±2.4	4.0–10.2 5.9±3.3
Seret River, upstream of Ternopol' reservoir	2.3–6.7 4.6±0.9	3.8–7.4 4.2±1.5	1.6–4.3 3.1±1.2	3.2–6.1 5.0±0.8
Seret River, downstream of Ternopol' reservoir	4.2–7.7 6.1±0.8	4.2–9.0 5.9±1.2	–[a]	–[a]
Kanev Reservoir, upper reaches	4.3–10.9 7.0±2.1	3.8–10.4 7.2±2.1	–[a]	–[a]
Kanev Reservoir, Desenka branch	4.0–9.2 7.0±2.9	5.1–11.2 6.8±1.2	2.0–9.2 5.8±2.3	5.0–9.6 7.5±2.8
Ternopol' Reservoir, upper reaches	3.3–10.5 6.0±2.7	3.3–10.8 5.9±2.6	2.0–7.1 3.8±2.1	4.0–11.8 6.5±5.2
Ternopol' Reservoir, near the dam	3.3–8.3 6.1±2.8	4.7–8.3 6.5±1.7	–[a]	–[a]
Upper Kitaevo pond, Kiev	3.3–9.7 5.3±1.5	4.0–8.3 5.4±1.0	1.9–7.7 3.2±2.1	4.0–7.6 5.5±1.9
Danube River, delta; May of 2012	12.2	3.5	7.5	8.3
Danube River, Vilkovo town; November of 2012	7.2	4.2	–[a]	–[a]
Gornyi Tikich, Chernaya Kamenka village; September of 2012	10.2	4.1	6.9	8.3
Gornyi Tikich, Chernaya Kamenka village, October of 2012	6.4	3.8	–[a]	–[a]
Gornyi Tikich, Chernaya Kamenka village, November of 2012	10.1	5.6	8.6	6.2

[a] No measurements were taken.

tion and the average molecular weight of humic substances in natural surface waters will differ from each other when different detection techniques, including the spectrophotometric and fluorescence methods, are applied due to the reasons described above. The authors of reviews [16, 29] have already brought this fact into view. This should be taken into account when a comparative assessment of the average molecular weight of humic substances in water objects located in different regions is performed and special attention should be paid to the method of their detection.

The results of studies of the average molecular weight of humic and fulvic acids demonstrated that for humic acids M_w values were almost always higher than M_w values for fulvic acids (Table 4). In some cases, there was little difference between them according to spectrophotometric measurements; in other cases this

Table 4. Weight average molecular weight (M_w) of humic and fulvic acids, extracted from studied water objects

Objects of research	Spectrophotometric method		Fluorescence method	
	M_w, kDa		M_w, kDa	
	Humic acids	Fulvic acids	Humic acids	Fulvic acids
Lyutsimir Lake; 16.02.2011	10.6	4.2	4.3	3.1
Desna River, mouth; 09.02.2012	6.9	7.0	5.8	5.4
Kanev Reservoir, Desenka branch; 02.11.2011	8.1	7.3	4.0	4.0
Kanev Reservoir, Rzhishchev town; 27.01.13	10.5	5.5	4.3	3.6
Ternopol' Reservoir, lower reaches; 16.10.2012	9.3	4.1	–[a]	–[a]
Danube River, Kiliya branch, Vilkovo town; 14.11.2012	8.9	2.5	–[a]	–[a]
Gornyi Tikich, Chernaya Kamenka village; 30.09.2012	8.3	2.7	2.7	2.0
Upper Kitaevo pond, Kiev City; 24.06.2012	12.2	4.8	–[a]	–[a]
Lososinka River, Petrozavodsk Town (Russia); 13.09.2012	11.9	1.8	4.3	0.9

[a] No measurements were taken.

Table 5. Weight average molecular weight (M_w) of humic and fulvic acids, extracted from natural surface waters of other regions

Sources of humic substances	M_w, kDa		References
	Humic acids	Fulvic acids	
Natural surface waters in general	7.0–15.1[a]		[12]
Moskva River source	–[c]	2.4–4.5	[37]
Lake waters of Norway	18.5–28.9	4.7–16.7	[38]
River and lake waters of Japan	–[c]	0.9– 2.1	[39]
Swamp and lake waters of Canada	–[c]	2.0–2.3	[40]
Suwannee River[b]	3.3–3.7	2.1–2.6	[16, 29]

[a] Weight average molecular weight of humic and fulvic acids taken together. [b] IHSS standard (International Humic Substances Society). [c] No corresponding data available.

difference was quite significant. It is characteristic both for humic and fulvic acids that M_w varies in a wide range of values (6.9–12.2 and 1.8–7.3 kDa, respectively). The fact that draws attention is that M_w of fulvic acids in the studied water objects turned out to be rather high. Therefore, it can be assumed that associates of fulvic acids were studied. It is often believed that these compounds have comparatively low molecular weight values. Apparently, the value of M_w for humic substances in general and for fulvic acids in particular is greatly influenced by their source of origin, concentration in the water of one or another object, and the method of detection.

The degree of concentration in the process of sorption of humic substances in a column with DEAE cellulose also affects the value of M_w. In case this parameter increases, M_w of humic substances also rises, which we have found out in the course of research. Therefore, in our opinion, the degree of concentration of humic substances should not exceed 10–12 times for high-color water (50–100 and more degrees of the simulating Cr-Co-scale) and 40–50 times for low-color water (10°–15° of Cr-Co-scale).

Below we provide the data on the weight average molecular weight of humic and fulvic acids, extracted

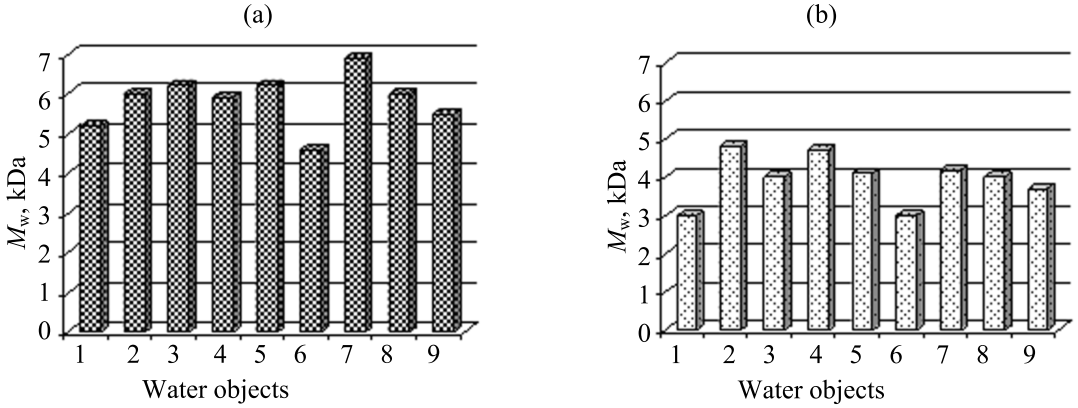

Fig. 6. Weight average molecular weight of humic substances extracted from water of studied water objects according to spectrophotometric (a) and fluorescence (b) measurements of their concentration. Water objects include: Lyutsimir Lake (1), Bol'shoe Chernoe Lake (2), Desna River (3), Ros' River (4), Yuzhnyi Bug River (5), Seret River (6), Kanev Reservoir, Desenka branch (7), Ternopol' Reservoir, upper reaches (8), and upper Kitaevo pond (9).

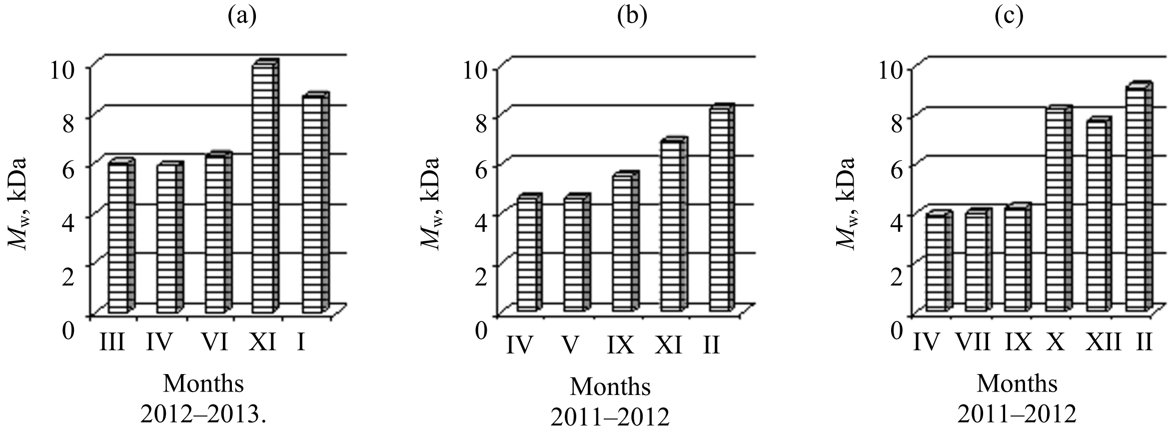

Fig. 7. Weight average molecular weight (M_w) of humic substances extracted from the Kanev Reservoir upper reaches (a), Desna River mouth (b), and Yuzhnyi Bug upper reaches (c) in different seasons of 2011–2013 (according to spectrophotometric

from surface waters of other regions (Table 5), for comparison. As can be seen from these data, the range of M_w for both humic and fulvic acids is rather wide and the results we have obtained are inside the boundaries of this range. The degree of polydispersity is also characterized by different values. For example, for humic substances from Norwegian Lake waters that were not separated into fractions this parameter ranged from 2.5 to 9.0 and was somewhat narrower for the extracted fractions of humic and fulvic acids (2.6–4.3 and 1.5–1.7, respectively). Therefore, the procedure of fractionation of humic substances into humic and fulvic acids results in a decrease in the degree of polydispersity, which has been previously mentioned by I.V. Perminova [12]. Apparently, there is breaking of bonds in high-molecular associates of humic substances, resulting in the dominance of compounds characterized by lower molecular weight values that are close in magnitude.

The data on average molecular weight seem important for comparative assessment of humic substances in water bodies and streams of different regions with regard to this parameter. However, in our opinion, they give little information in terms of assessing bioavailability of these organic compounds, as it is known that only compounds with certain molecular weight can penetrate biological membranes, which has been described above. From the ecological point of view, it is much more useful to possess information about the molecular-weight distribution of humic substances, which makes it possible to evaluate the part of these compounds which is most likely to be potentially digestible for hydrobionts.

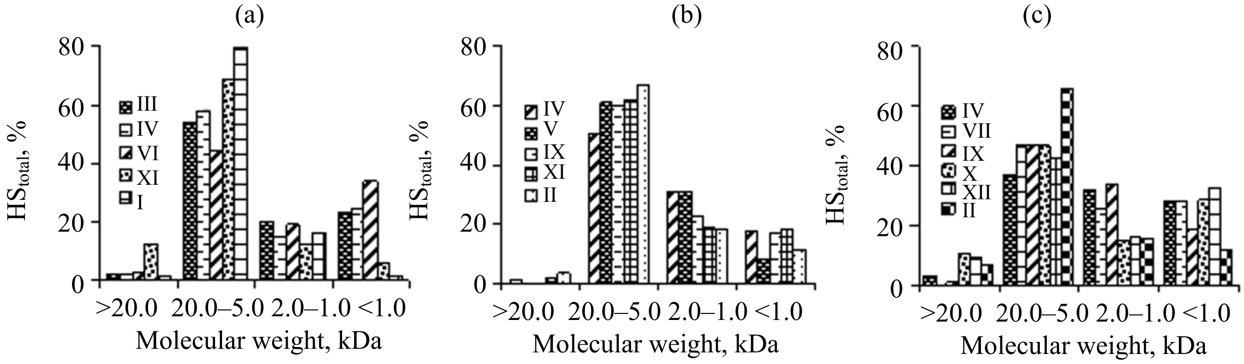

Fig. 8. Ratio of humic substances fractions with different molecular weight (HS$_{total}$, %) in water of the Kanev Reservoir upper reaches (a), Desna River mouth (b), and Yuzhnyi Bug River upper reaches (c) in 2011–2013. Roman numerals stand for corresponding months: (a) of 2012–2013, (b) and (c) of 2011–2012.

We found out that there were seasonal changes in M_w values for humic substances. It turned out that in summer they were often lower than in autumn and winter (Fig. 7). Definitely, there are different reasons for this phenomenon. In particular, a noticeable growth in concentrations of humic substances in the water can be one of the factors causing an increase in their molecular weight due to intermolecular interactions resulting in aggregation of macromolecules [41]. The manifestation of this so-called ”concentration effect” should be expected, first of all, in the presence of high-color water with an increased content of humic substances. Therefore, one of the many different reasons for seasonal changes in the average molecular weight of humic substances that is worth the greatest attention is their transformation under the influence of UV light of solar radiation and microbiological activity in water objects, and it should be taken into account that the intensity of these processes reaches its maximum in summer. As a result, a part of high-molecular substances decompose to form compounds with lower molecular weight [10, 42–45]. Apparently, it is these compounds that prevail in this season of the year; although, they are the most digestible for hydrobionts. More expressed fluorescence intensity of fulvic acids as compared to humic acids [10] is most likely to be related to differences in their molecular weight as well.

The results of studies of seasonal changes in the molecular-weight distribution of humic substances (Fig. 8) also testify to that. It can be seen that the relative content of the high-molecular fraction (20.0–5.0 kDa) gradually increases from spring to autumn, which also affects the average molecular weight of humic substances, which eventually increases as well (Fig. 7). In autumn the intensity of photodegradation and microbial degradation of humic substances decreases, which results in accumulation of compounds with higher molecular weight. Apparently, with a decay of biological processes in the water object there is also a decrease in assimilation of humic substances by aquatic organisms.

CONCLUSIONS

Therefore, the content of humic substances in natural surface waters of the Ukraine is characterized by quite a wide range of values, which is caused, in the first place, by different sources of their formation. Maximal concentrations of these natural organic compounds are characteristic for water objects of the Ukrainian Poles’e, which is related to the high percentage of wetlands in this region. As we move from the north to the south, the content of humic substances significantly decreases. The cascade of the Dnieper reservoirs can serve as a good example of this phenomenon. In The Kakhovka Reservoir, closing the cascade, the concentration of humic substances is almost thrice as low as in the Kiev Reservoir, which is at the head of the cascade, receiving high-color waters from the inflowing rivers, in particular, the Pripyat’ River. In river waters of the forest-steppe and steppe zones the average content of humic substances does not exceed 4.0–8.5 mg L^{-1}.

The concentration of humic substances undergoes seasonal changes. Thus, in rivers of the Pripyat’ River basin and in the upstream Dnieper Reservoirs maximal concentrations are, as a rule, associated with the spring flooding period. In the downstream Dnieper reservoirs an increase in the content of humic substances most often takes place in summer, which is related to the long travel time of water with increased concentrations of these compounds from the upstream reservoirs. In small rivers and water bodies an increase in the concentration of humic substances is observed in summer and autumn, which is associated not only with allochthonous, but also with autochtonous humic substances. The prevailing fraction in the composition of humic substances is represented by fulvic acids and reaches 80.8–94.8% of the total content.

There are differences in the ratio of humic substances with different molecular weight depending on the methods of their detection. The results of spectrophotometric measurements of concentrations of humic substances in fractions after gel-chromatographic separation demonstrate that high-molecular compounds (molecular weight >5.0 kDa) prevail and their share is 43.6–59.6%. At the same time, the results of fluorescence measurements indicate the dominance of compounds with lower molecular weight, which does not exceed 2.0 kDa, in the composition of humic substances. The share of these compounds reaches 46.2–70.0%, including 38.4–47.0% of compounds with molecular weight <1.0 kDa. This difference in the ratio of fractions of humic substances is related to their fluorescence properties. The fluorescence intensity of fractions with lower molecular weight is higher as compared to high-molecular fractions.

Calculated data on the weight average molecular weight (M_w) of humic substances also vary depending on the method of their detection. When we take into account the average M_w values for humic substances in each of the studied objects, this parameter turns out to be approximately 1.5-fold higher in case of spectrophotometric measurements as compared to fluorescence measurements.

There are significant changes in the value of M_w in the seasonal aspect. As a rule, in spring and summer this value is lower than in autumn. The reasons for this phenomenon are mostly related to the processes of degradation of humic substances under the influence of UV light of solar radiation and microbiological activity, which become more active in the summer season. These processes cause degradation of high-molecular fractions of humic substances, in the first place, and their transformation into fractions with lower molecular weight. In autumn the intensity of these processes slows down significantly, which results in an increase in the weight average molecular weight of humic substances.

From the ecological point of view, data on the molecular-weight distribution of humic substances seem very important, as they make it possible to evaluate the content of compounds that are potentially digestible by hydrobionts in the composition of humic substances. Such compounds include substances with a relatively low molecular weight, which, according to different estimates, does not exceed 2.0–5.0 kDa.

REFERENCES

1. Van Stempvoort, D.R., Lesage, S., and Molson, J., *Use of Humic Substances to Remediate Polluted Environments: from Theory to Practice*, Perminova, I.V., Hatfield, K., and Hertkorn, N., Eds., NATO Science Series, IV: Earth and Environmental Series, Dordrecht: Springer, 2005, vol. 52, p. 233.
2. *Aquatic Ecosystems: Interactivity of Dissolved Organic Matter*, Findlay, S.E.G. and Sinsabaugh, R.L., Eds., San Diego: Academic Press, 2003.
3. Kulikova, N.A., Stepanova, E.V., and Koroleva, O.V., *Use of Humic Substances to Remediate Polluted Environments: from Theory to Practice*, Perminova, I.V., Hatfield, K., and Hertkorn, N., Eds., NATO Science Series, IV: Earth and Environmental Series, Dordrecht: Springer, 2005, vol. 52, p. 285.
4. Hayes, M.H.B., *Humic Substances: Structures, Properties and Uses*, Davies, G. and Ghabbour, E.A., Eds., Cambridge: Royal Soc. Chem., 1998, p. 1.
5. Swift, R.S., *Soil Sci.*, 1999, vol. 164, p. 790.
6. Malcolm, R.K., *Organic Substances in Soil and Water: Natural Constituents and Their Influence on Contaminant Behavior*, Beck, A.J., Jones, K.C., Hayes, M.H.B., and Mingelgrin, U., Eds., Cambridge: Royal Soc. Chem., 1993, p. 19.
7. Thurman, E.M. and Malcolm, R.L., *Environ. Sci. Tech.*, 1981, vol. 15, p. 463.
8. Thurman, E.M., *Organic Geochemistry of Natural Waters*, Dordrecht: Martinus Nijhoff/Dr. W. Junk Publishers, 1985.
9. Tipping, E., *Cation Binding by Humic Substances*, Cambridge: Cambridge University Press, 2004.
10. Chen, J., LeBoeuf, E.J., Dai, S., and Gu, B., *Chemosphere*, 2003, vol. 50, p. 639.
11. Vogl, J. and Heumann, K.G., *Fresenius' J. Anal. Chem.*, 1997, vol. 359, p. 438.
12. Perminova, I.V., *Doctoral (Chem.) Dissertation*, Moscow, 2000.
13. Linnik, P.N., Vasilchuk, T.A., and Linnik, R.P., *Hydrobiol. J.*, 2004, vol. 40 (3), p. 79.
14. *Humic Substances in Soil, Sediment and Water*, Aiken, G.R., McKnight, D.M., Wershaw, R.L., and MacCarthy, P., Eds., New York: John Wiley and Sons Inc., 1985.

15. Thurman, E.M., Wershaw, R.L., Malcolm, R.L., et al., *Org. Geochem*., 1982, vol. 4, p. 27.
16. McDonald, S., Bishop, A.G., Prenzler, P.D., and Robards, K., *Analyt. Chim. Acta*, 2004, vol. 527, p. 105.
17. Linnik, P.N. and Vasilchuk, T.A., *Use of Humic Substances to Remediate Polluted Environments: from Theory to Practice*, Perminova, I.V., Hatfield, K., and Hertkorn, N., Eds., NATO Science Series, IV: Earth and Environmental Series, Dordrecht: Springer, 2005, vol. 52, p. 135.
18. Seki Humitake, *Organicheskie veshchestva v vodnykh ekosistemakh* (Organic Materials in Aquatic Ecosystems), Leningrad: Gidrometeoizdat, 1986.
19. Moiseenko, T.I., Kudryavtseva, L.P., and Gashkina, N.A., *Rasseyannye elementy v poverkhnostnykh vodakh sushi: Tekhnofil'nost', bioakkumulyatsiya i ekotoksikologiya* (Trace Elements in Land Surface Waters: Technophility, Bioaccumulation, and Ecotoxicology), Moscow: Nauka, 2006.
20. Linnik, P.N. and Shcherban', E.P., *Ekol. Khim.,* 1999, vol. 8 (3), p. 168.
21. Linnik, P.N. and Nabivanets, B.I., *Formy migratsii metallov v presnykh poverkhnostnykh vodakh* (Forms of Metals Migration in Fresh Surface Waters), Leningrad: Gidrometeoizdat, 1986.
22. Chernyshova, N.N., Svintsova, L.D., and Gindullina, T.M., *Khim. Tekhnol. Vody*, 1995, vol. 17 (6), p. 601.
23. Yamada, E., Ozeki, T., and Kimura, M., *Anal. Sci*., 1998, vol. 14, p. 327.
24. Popovich, G.M., *Candidate Sci. (Chem.) Dissertation*, Kiev, 1990.
25. Nabivanets, B.I., Osadchiy, V.I., Osadcha, N.M., and Nabivanets, Yu.B., *Analitichna khimiya poverkhnevykh vod* (Analytical Chemistry of Surface Waters), Kiev: Nauk. Dumka, 2007.
26. Sirotkina, I.S., *Author's Absract of Candidate Sci. (Chem.) Dissertation*, Moscow: GEOKhI AN SSSR, 1974.
27. Perminova, I.V., Frimmel, F., Kovalevskii, D.V., Abbt-Braun, G., Kudryavtsev, A.V., and Hesse, S., *Water Res*., 1998, vol. 32, p. 872.
28. Kudryavtsev, A.V., Perminova, I.V., and Petrosyan, V.S., *Anal. Chim. Acta*, 2000, vol. 407, p. 193.
29. Janoš, P., *J. Chromatogr. A,* 2003, vol. 983, p. 1.
30. *Monitoring, ispol'zovanie i upravlenie vodnymi resursami basseina reki Pripyat'* (Monitoring, Utilization, and Management of Water Resources of the Pripyat' River Basin), Kalinin, M.Yu. and Obodovskii, A.G., Eds., Minsk: Belsens, 2003.
31. Vasenko, A.G., Afanas'ev, S.A., Petrenko, O.N., et al., *Ekologicheskoe sostoyanie transgranichnykh uchastkov rek basseina Dnepra na territorii Ukrainy* (Ecological Conditions of Transboundary Parts of the Dnieper Basin Rivers on the Ukrainian Territory), Kiev: Akademperiodika, 2002.
32. Osadcha, N.M., *Author's Absract of Doctoral Sci. (Geogr.) Dissertation*, Kiev, 2011.
33. *Humic Substances: Nature's Most Versatile Materials*, Ghabbour, E.A. and Davies, G., Eds., New York: Taylor and Francis, 2004.
34. Kulikova, N.A., *Author's Absract of Doctoral Sci. (Biol.) Dissertation*, Moscow, 2008.
35. Conte, P., Spaccini, R., Šmejkalová, D., Nebbioso, A., and Piccolo, A., *Chemosphere*, 2007, vol. 69, p. 1032.
36. Alberts, J.J. and Takács, M., *Org. Geochem*., 2004, vol. 35, p. 243.
37. Varshal, G.M., Velyukhanova, T.K., Koshcheeva, I.Ya., Kubrakova, I.V., and Baranova, N.N., *Analiticheskaya khimiya redkikh elementov* (Analytical Chemistry of Rare Elements), Moscow: Nauka, 1988, p.112.
38. Peuravuori, J. and Pihlaja, K., *Anal. Chim. Acta*, 1997, vol. 337, p. 133.
39. Tsuda, K., Mori, H., Asakawa, D., Yanagi, Yu., Kodama, H., Nagao, S., Yonebayashi, K., and Fujitake, N., *Water Res*., 2010, vol. 44, p. 3837.
40. Wu, F.C., Evans, R.D., and Dillon, P.J., *Anal. Chim. Acta*, 2002, vol. 464, p. 47.
41. Zsolnay, Á., *Geoderma*, 2003, vol. 113, p. 187.
42. Valencia, S., Marín, J., Velásquez, J., Restrepo, G., and Frimmel, F.H., *Water Res.*, 2012, vol. 46, p. 1198.
43. Tercero Espinoza, L.A., Ter Haseborg, E., Weber, M., and Frimmel, F.H., *Applied Catalysis B: Environmental,* 2009, vol. 87, p. 56.
44. Carvalho, S.I.M., Otero, M., Duarte, A.C., and Santos, E.B.H., *Chemosphere*, 2008, vol. 71, p. 1539.
45. Duarte, R.M.B.O., Santos, E.B.H., and Duarte, A.C., *Water Res*., 2003, vol. 37, p. 4073.