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THE ROLE OF VARIOUS FRACTIONS OF HUMIC SUBSTANCES FROM SURFACE WATER IN BINDING Al(III), Fe(III), AND Cu(II) INTO COMPLEXES

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Abstract. The aim of this research work was to evaluate the role of various fractions of humic substances (HS) in binding Al(III), Fe(III) and Cu(II) ions into complexes using gel chromatography. With an increase in HS concentration in Ukraine's surface water bodies, the share of HS' fraction with a molecular weight of 20–5 kDa increases from 37% to 59%. In the water bodies under study the HS' fractions with molecular weight 20–5 kDa and < 1 kDa play a principal role in the studied metals' migration. HS with molecular weight 20–5 kDa have been found to bind the smallest amount of Al(III), Fe(III) and Cu(II) in complexes, if the metals concentration bound by 1 mg fraction of HS with a certain molecular weight is calculated. Experimental results showed that, the investigated metal ions have the ability to bind into complexes mainly by HS with a molecular weight of >20 and <1 kDa. In the water bodies under study 1 mg of humic acids has a greater binding ability in relation to Al(III), Fe(III) and Cu(II) ions than 1 mg of fulvic acids. Fulvic acids and humic acids with a molecular weight of 20–5 kDa also have the lowest binding ability.

Keywords: water body, humic substance, metal, binding ability, molecular weight distribution.

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Introduction

Fresh surface waters are complex and self-regulating systems where abiotic and biotic components continuously interact with each other. Humic substances' (HS) forming directly in water bodies (autochthonous humus) and in adjacent environments (soils, swamps, peatlands *etc*.), wherefrom HS (allochthonous humus) enters water bodies with surface and lateral runoff, can serve as an example of such interaction [1-5]. The share of HS in fresh surface waters mainly makes up 45–80% (in terms of C_{org}) of the dissolved organic matter (DOM) total content [5-7] and varies within 33.3–92.6% in diverse water bodies of Ukraine [8]. HS are a mixture of fulvic acids (FA) and humic acids (HA). Their ratio in surface waters is usually 10:1, but in highly coloured water bodies it may be equal to 5:1 [1,9]. The interstitial solutions of sediments are dominated by HA, and the ratio of FA and HA is 1:3. HA, on the contrary, prevail in soils due to their better adsorption and lower migratory mobility [1,10]. HS are irregular copolymers of aromatic hydroxycarboxylic acids with inclusions of nitrogen-containing and carbohydrate fragments [1,11-14], with the C, H, O, N and S shares depending on the source of their origin [10,12,15]. The relative oxygen content in the FA composition is 2.6–13.4% higher than in the HA composition. This indicates a higher content of oxygen-containing functional groups in the aromatic framework of FA than in HA [15]. HS also contain metals (Al, Fe, Cu, Ti, Mn, Be and others), which are bound by carboxyl, hydroxyl, amine, phenolhydroxyl and other functional groups into complexes with different strengths [14,16]. Metals in surface waters are in a dissolved state in higher concentrations than those obtained from thermodynamic calculations due to complexation with HS [17]. HS increase the metals' migration mobility and also affect their bioavailability, which depends primarily on the molecular weight of metal complexes with HS [10,14,18-20]. HS are also able to leach minerals that adsorb HS on their surface [21,22]. In addition, HS form adducts with pesticides, herbicides, and surfactants through hydrophobic interaction, in this way reducing their toxicity and bioavailability [11,20,23,24]. HS are involved in ionic, hydrophobic, and electron donor-acceptor interactions due to their multifunctionality and structural diversity [12,22,25].

Therefore, HS, as the most widespread group of DOM in surface waters, play the greatest role in binding metal ions into complexes [26,27]. HS significantly affect the metals migration, bioavailability, and ultimately the toxicity for aquatic organisms, due to their complexation [28]. Usually, the metals toxicity decreases simultaneously with the concentration of free metal ions as the most toxic form. However, the molecular weight of metal complexes with HS is also important. It is believed that high-molecular metal compounds with HS become unavailable for assimilation by aquatic organisms. In water bodies with a high concentration of HS, metal complexes with FA become dominant [22,29]. The prevalence of these complexes is explained by their stability. At the same time, HS are oxidized and decomposed to a lesser extent than other groups of DOM (carbohydrates, protein-like substances, *etc*.). However, under conditions of photochemical oxidation, HS can decompose to form low molecular weight fractions that are available for assimilation by aquatic organisms [30,31]. Photochemical oxidation is typically observed in the surface layer of water where sunlight penetrates [32]. Thus, when studying the HS complexation, it is necessary to pay attention to the molecular weight distribution of metal complex compounds. The results of long-term studies allowed for the first time to assess the role of different molecular weight fractions of HS in metal binding complexes in Ukraine’s water bodies. This is significant for assessing the metals lability, their potential bioavailability and toxicity. Moreover, the results of natural studies agree with experimental data.

The aim of this work is to sum up the findings of studies on the role of unfractionated HS, FA and HA fractions, as well as their compounds with different molecular weights in binding Al(III), Fe(III), and Cu(II) into complex compounds, as it is important from an environmental point of view to assess their potential bioavailability.

Experimental

Reagents

Reference standard solutions of Al(III) (MCO 0534:2003 – 10.0 mg/mL), Fe(III) (MCO 0518:2003 – 1.00 mg/mL), and Cu(II) (MCO 0524:2003 – 10.0 mg/mL) in glass ampoules were used in this study. The working and model solutions were prepared using double-distilled water. Solutions of KOH (0.3 mol/L) and H_2SO_4 (1.0 mol/L) were prepared by diluting 45% and 98% solutions of high-purity grade, respectively. Chromazurol S, 1,10-phenanthroline, luminol (Fluka Chemie, Germany), and H_2O_2 (35%) solutions were also used.

Equipments

Ruthner bathometer, a modified bathometer-bottle [33], Unico UV 2800 spectrophotometer, KFK-2 photo colorimeter, chemiluminescence photometer with a photoelectric converter PhEP-19A [34], pH-meter pH 150MI (Russia), device for membrane filtration, device for UV treatment with DRT-1000 mercury-quartz lamp, glass columns for ion-exchange and gel chromatography research, collector of fractions "Dombifrak".

Sampling and sample preparation

Water samples were taken from a variety of surface water bodies, including the (1) Kaniv, upper section, (2) the Desenka arm; (3) Yurpil (Girsky Tikich River, Chorna Kamyanka village); (4) Middle Bila Tserkva (Ros’ River, Bila Tserkva city), and (5) Ternopil (Seret River, Ternopil city) reservoirs; rivers (6) Tsyr, mouth; (7) Prypyat, Svalovychi village; (8) Desna, mouth; (9) Pivdennyy Bug, Khmelnytsky city; (10) Seret, Ternopil city; (11) Kiliya Danube Delta, Skhidnyy arm; lakes of the Shatsk group – (12) Lutsymer, (13) Chorne Velyke; (14) Yordans’ke and (15) Verbne within the Kyiv city; (16) the second Kytayivsky and (17) second Horikhuvatsky ponds (within the Kyiv city), using a modified bathometer-bottle [33] or Ruthner bathometer. Numbers in round brackets correspond to the numbering of water bodies shown in Figures 1–5. Seasonal or monthly studies were conducted at each of the water bodies with water sampling and analysis for the studied ingredients’ content in at least three repetitions. Limiting and average values of the investigated ingredients for each water body relate to the entire study period. A total of 84 water samples were analysed.

To separate suspended solids, a freshly collected water sample of 1.0–1.5 L was passed through a nitrocellulose membrane filter with a pore diameter of 0.4 μm (Czech Republic) under a pressure of about 2×10^5 Pa (Figure S1). The extraction of HS from the water filtrate and their concentration by at least 25–50 times were achieved by ion-exchange chromatography using a column filled with diethylaminoethylcellulose (DEAE-cellulose) manufactured by SERVA. HS was eluted from the column using a 0.3 mol/L KOH solution. The elution rate was about 1.0 mL/min (see Figure S1). To separate HS into HA and FA fractions, the resulting HS concentrates were acidified to pH 1.5–2.0 and heated to ≈ 50°C for several hours, then the resulting HA precipitate was allowed to settle for a day and centrifuged at 5000 rpm for 15 minutes. The solution above the precipitate containing FA was decanted. The HA precipitate was dissolved in 3–5 mL using a 0.3 mol/L KOH solution. The pH value of the obtained solutions of FA and HA was adjusted to the original natural water value.

The molecular weight distribution of HS, FA, HA and their complex compounds with metals was studied by gel chromatography. For this purpose, a glass column filled with TOYOPEARL HW-50F gel (Japan) was used. The column length was 82 cm, diameter – 2.8 cm; gel layer height – 62 cm; column free volume (V_0) – 160 mL. The column was calibrated using solutions of polyethylene glycols with molecular weights of 1.0, 2.0, 15.0, and 20.0 kDa and glucose (0.18 kDa). The concentrations of polyethylene glycols and glucose were 2.0 and 0.5 mg/mL, respectively. The eluent was 0.025 mol/L phosphate buffer solution with a pH of 7.0 [8]. The V_0 value was measured using a solution of bludextran (2000 kDa).

Measuring the humic substances concentration

A Unico UV 2800 spectrophotometer was used to measure the concentration of unfractionated HS, FA, and HA as well as their content in fractions after gel chromatographic separation (see Figure S1). In some cases, for the same purpose, the water color index, measured on a KFK-2 photocolorimeter, was additionally used. The water color index was determined according to the procedure [35] based on measuring the optical density of a sample at 364 and 400 nm. To construct a calibration graph, the difference in the optical densities of solutions with known water colority values according to the imitation Cr-Co scale was used. The HS concentration was found using the calibration graphs “Optical density at 254 nm – HS concentration, mg/L” and “Water colority, °Cr-Co scale – HS concentration, mg/L”. To build calibration graphs, purified dry preparations of FA and HA were used, which were isolated from the water of the Kaniv Reservoir using a column with DEAE-cellulose according to the procedure described above. To obtain these preparations, the HS concentrate was divided into FA and HA fractions, which were desalted using a KU-23 cation exchanger in the H^+ form. Then they were gradually evaporated in porcelain cups and dried, bringing the dry residue to constant weight in a desiccator with anhydrous $CaCl_2$.

Assessing the binding capacity of various fractions of HS in natural and experimental conditions

To assess the role of individual HS fractions in binding metal ions into complexes under natural and experimental conditions, the metal content in each of them after their destruction by UV irradiation in an acidic medium, pH ≈ 1.0–1.5, with an addition of 0.1 mL of 30% H_2O_2 was determined. The UV treatment was accomplished in 50-mL quartz glasses for 2.0–2.5 h using a DRT-1000 mercury-quartz lamp. However, this assessment is based on the metal content in 1 mg of HS, HA, FA, and their fractions of different molecular weights, and not on the total metal content in the fraction. Such calculations are necessary, since the individual HS fractions content differs significantly from their total content, and this does not allow one to objectively evaluate the real (in natural conditions) and potential (in experimental conditions) binding ability of each of them.

Experimental studies on the role of HS fractions with different molecular weight in binding the metals into complexes were carried out using pre-filtered natural water from the Kaniv Reservoir. For this purpose, 250 μg/L of Al(III), 250 μg/L of Fe(III), and 50 μg/L of Cu(II) were added separately to three water samples with a volume of 0.5 L each. In one of the experimental systems, 50 μg/L of Al(III), Fe(III), and Cu(II) were added simultaneously. The natural water filtrate without metal additives was used as a control sample. Thus, five experimental systems were used. The experiment lasted for 60 days. To isolate HS from natural water filtrate on the 1st and 60th days of the experiment, as well as from water filtrates with metal additives on the 60th day of the experiment, the ion-exchange chromatography method was used, as described above. The molecular weight distribution of HS and their complexes with the studied metals was studied using the gel chromatography method.

Metal measuring methods

The Al(III) and Fe(III) concentrations were measured photometrically using the reagents chromazurol S [36] and *o*-phenanthroline [35], respectively, and the Cu(II) concentrations were measured by the chemiluminescence method [37]. Detection limits for Al(III), Fe(II), and Cu(II) are 7.4×10^{-8} mol/L or 0.002 mg/L, 3.6×10^{-4} mol/L or 0.02 mg/L, 7.7×10^{-9} mol/L or 0.5×10^{-3} mg/L, respectively [35-37].

Results and discussion

General characteristics of humic substances

The HS concentration in the water bodies under study varied between 1.5 and 112.8 mg/L (Figure 1). Their maximum content is observed in water bodies receiving water from swamps with a high content of HS (Tsyr and Prypyat rivers, Lutsymer Lake). The Kaniv Reservoir does not have any direct effect of swamp waters on its chemical composition. However, the Prypyat River's high-colored waters from the upstream Kyiv Reservoir have an impact on Kaniv Reservoir.

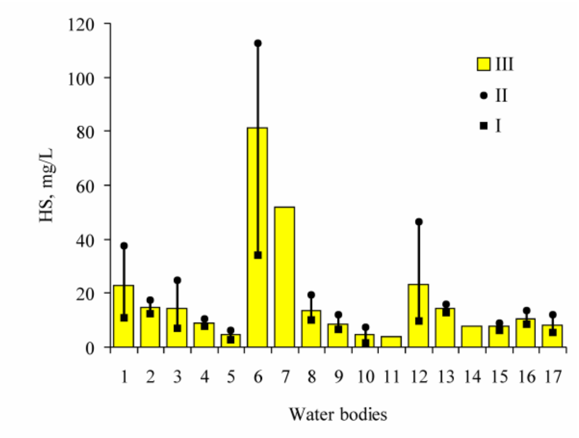


Figure 1. Limiting (I, II) and average (III) values of HS concentration in the water of the water bodies under study (2011–2017). For water bodies numbered 7, 11, 14 the results of single measurements are shown instead of average values.

The water bodies under study can be divided into two types in accordance with seasonal changes in the HS concentration. The maximum concentrations of HS in the water bodies of the first type are observed, as a rule, in spring, while in the water bodies of the second type – in autumn. Among the water bodies under study, the first type includes the Kaniv Reservoir (including the Desenka arm), the Desna River, lakes Lutsymer and Chorne Velyke. The second type includes Yurpil, Middle Bila Tserkva and Ternopil reservoirs, the Pivdennyy Bug and Seret rivers, Yordans'ke and Verbne lakes the second Kytayivsky and the second Horikhuvatsky ponds. The rivers Prypyat, Tsyr and the Kiliya Danube Delta are not assigned to any of the types due to the lack of findings from a study of the HS content in winter and spring.

The temporal changes in the HS concentration in water bodies of these types are shown in (Figure S2). The maximum concentrations of HS in water bodies of the first type are observed in the spring due to their inflow from the catchment area during the spring flood (allochthonous humus). An increase in the HS content in water bodies of the second type is observed in autumn, as a certain portion of them is formed in the water body itself. Autochthonous humus is formed with the active participation of bacteria, fungi, invertebrates, this is not solely a chemical process. It includes protein-like substances and carbohydrates, which are released into the water column by phytoplankton, higher aquatic vegetation and other aquatic organisms both during their lifetime and after their death [18].

An increase in the relative content of DOM of autochthonous origin in water bodies of both the first and second types from spring to summer or autumn gives indirect evidence that autochthonous humus is formed. The share of autochthonous DOM in water bodies of the first type was in the range of 27.6–65.5% (not exceeding 48.4% on average), while in water bodies of the second type their share was higher and reached 41.0–88.4% (on average 61.0%).

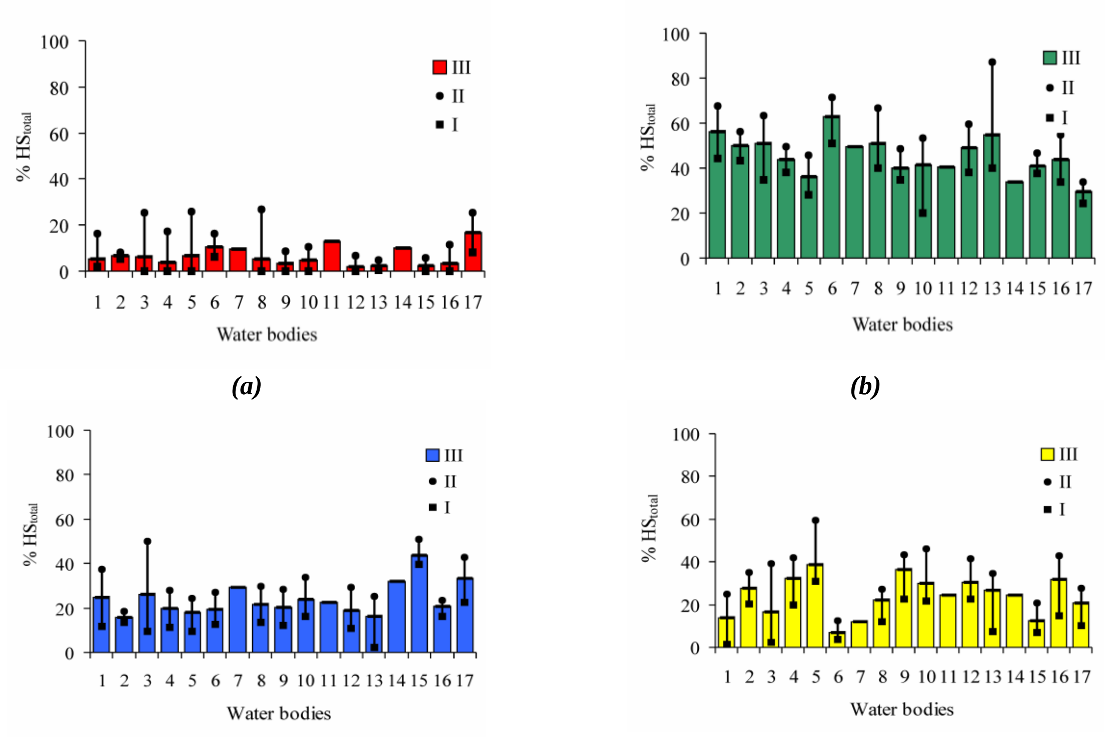
The temporal changes in the relative content of autochthonous and allochthonous DOM in some water bodies under study are shown in (Figure S3). To calculate the share of autochthonous organic compounds in the total DOM balance, the formula given by Lozovik, P.A., *et al.* was used [2]. So, the current studies, which looked at the role of different HS fractions in binding metals into complex compounds, included not only different kinds of water bodies (rivers, reservoirs, lakes, and ponds), but also water bodies from different natural geographical zones of Ukraine and with different HS-forming processes during different seasons (water bodies of the first and second types). Such a sample of water bodies makes it possible to clearly assess the role of one or another fraction of HS in binding metal ions into complexes.

According to the HS molecular weight distribution, their composition is most often dominated by the fraction of compounds with a molecular weight of 20–5 kDa. Its relative content averages 29.3–62.8% of the total content of HS (HS_{total}) (Figure 2).

The shares of other HS fractions, in particular, those with molecular weights >20, 5–1, and <1 kDa, vary within the ranges of 1.8–16.8, 15.8–40.8, and 7.1–38.8% of HS_{total}, respectively. At the same time, the relative content of the HS fraction with a molecular weight of 20–5 kDa in some water bodies (the Ternopil Reservoir, the Seret River, and the second Horikhuvatsky pond) was lower (on average 36.3, 41.4, and 29.3% of HS_{total}). These water bodies are also distinguished by the lowest values of the total HS concentration (see Figure 1). A direct linear relationship has been found between the concentration of HS_{total} and the content of the HS fraction with a molecular weight of 20–5 kDa, with a correlation coefficient in the range of 0.75–0.97 at a significance level of 0.01 (Figure S4).

The study's results show that the HS$_{total}$ concentration has a significant effect on the content of the HS fraction with a molecular weight of 20–5 kDa. No such effect has been observed on the content of HS fractions with molecular weights >20, 5–1, and <1 kDa, which is confirmed by the absence of a linear relationship. With the HS$_{total}$ concentration getting higher, the share of the HS fraction with a molecular weight of 20–5 kDa increases from 37% to 59% of HS$_{total}$ on average. If the HS$_{total}$ concentration is in the range of 0–5 mg/L, then the average relative content of the HS fraction with a molecular weight of 20–5 kDa makes up 36.8±8.8%, and at concentrations of HS$_{total}$ 5–10, 10–15, 15–20, 20–30 and >30 mg/L it makes up 41.0±3.6, 47.9±5.2, 53.0±6.6, 58.6±8.2 and 56.7±8.5%, respectively. A wide range of molecular weights of HS has also been reported in other reports [10,38,39]. In particular, it is reported that HS have a molecular weight in the range of 0.3–30.0 kDa [38].

It is also important to note that in spring and summer the share of HS fractions with a molecular weight of ≤5 kDa and <1 kDa increases, which, in our opinion, is explained by the HS transformation. Although this group of DOMs is resistant to oxidation and destruction, high-molecular weight fractions of HS are transformed into fractions with a lower molecular weight under conditions of photochemical oxidation. This may be explained by the direct photolysis of HS under the action of prolonged solar radiation at these times of the year [32]. This is consistent with the results of research by other authors [40], who showed that the solar radiation in autumn and summer significantly intensified the organic substances transformation. The processes of HS transformation due to photolysis and intense microbiological activity are activated in the warm season. This is also reported in a number of publications devoted to this problem [41-45]. It is quite obvious that such a transformation of HS will change the molecular weight distribution of metals' complex compounds with the specified ligands. The transformation of DOM, including HS, will also take place in the future under conditions of warming and an increase in the duration of solar radiation, and this will significantly affect the hydrochemical regime of surface water bodies in general and the chemical composition of water in particular [46].



(a)

(b)

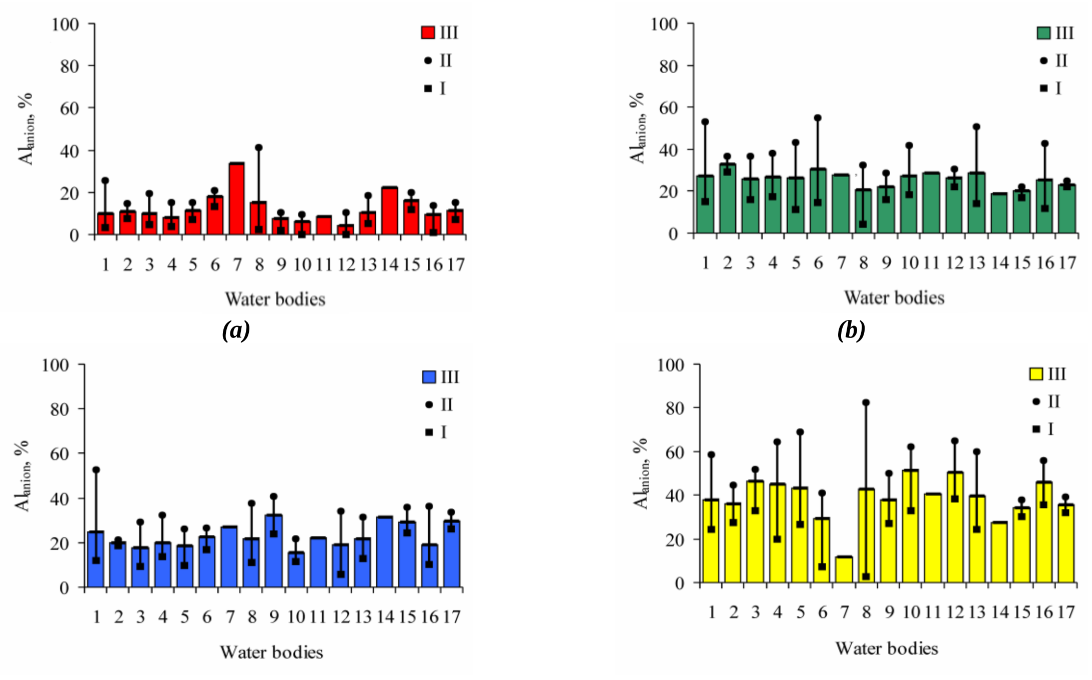
(c)

(d)

Figure 2. Limiting (I, II) and average (III) values of the relative content of HS fractions with a molecular weight of >20 *(a)*, 20–5 *(b)*, 5–1 *(c)* and <1 kDa *(d)*, 2011–2017. For water bodies numbered 7, 11, 14 the results of single measurements are shown instead of average values.

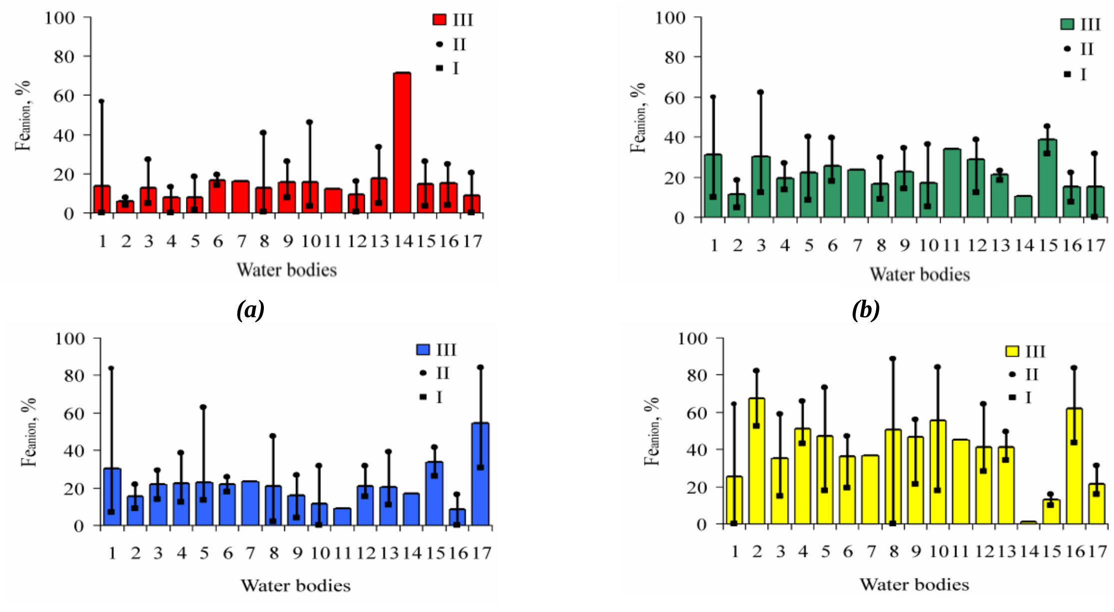
The role of different HS fractions in binding metal ions

Since the HS fraction with a molecular weight of 20–5 kDa dominates the HS composition in most cases, it was initially assumed that this fraction must play the most significant role in the Al(III), Fe(III), and Cu(II) migration in surface waters. Iron in water bodies exists in an oxidized state, however, one cannot exclude the fact that HS can reduce Fe(III) to Fe(II), forming strong complexes with this form of iron [47–50]. The molecular weight distribution of Al(III), Fe(III), and Cu(II) complex compounds with HS shows that the HS fraction with a molecular weight of 20–5 kDa contained 4.2–54.8, 0.0–62.1, and 7.8–81.6% Al(III), Fe(III), and Cu(II) of the anionic DOM fraction, respectively (Figures 3–5).



(c) *(d)*

Figure 3. Limiting (I, II) and average (III) values of the relative content of aluminum (Al_{anion}, %) as part of complex compounds with HS of various molecular weight in the water bodies under study, 2011–2013: >20 *(a)*, 20–5 *(b)*, 5–1 (*c*), <1 kDa *(d)*. For water bodies numbered 7, 11, 14 the results of single measurements are shown instead of average values.



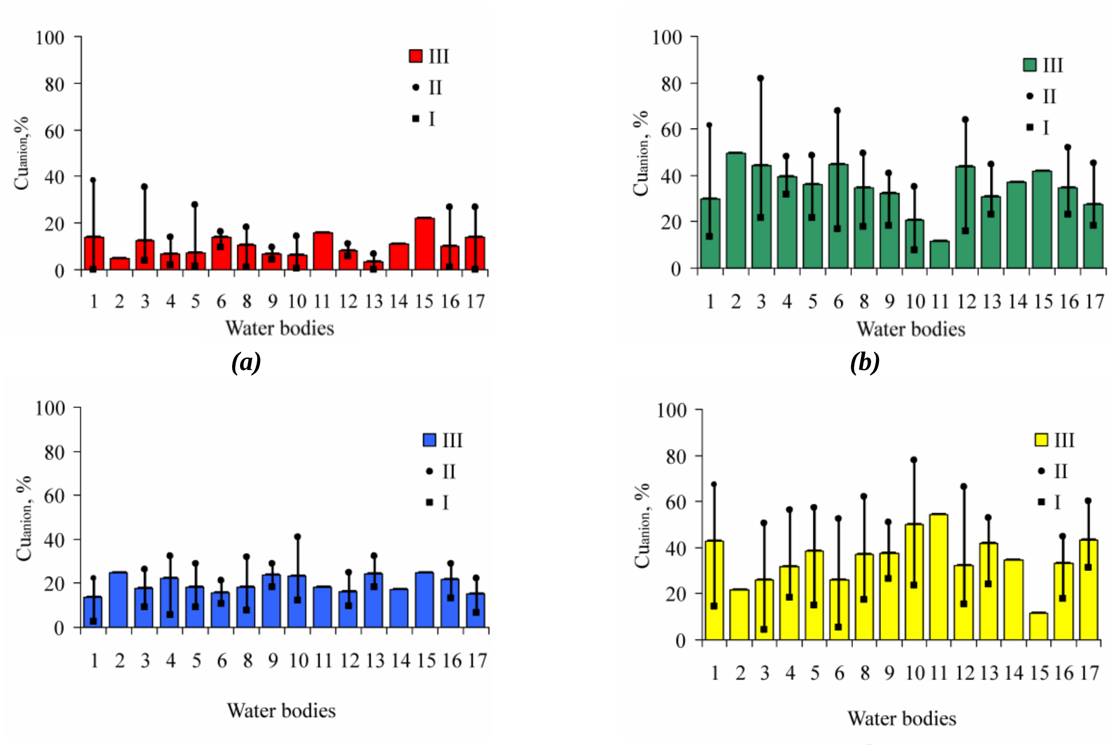
(c) *(d)*

Figure 4. Limiting (I, II) and average (III) values of the relative content of iron (Fe_{anion}, %) as part of complex compounds with HS of various molecular weight in the water bodies under study, 2011–2013: >20 *(a)*, 20–5 *(b)*, 5–1 *(c)*, <1 kDa *(d)*. For water bodies numbered 7, 11, 14 the results of single measurements are shown instead of average values.

Copper in most water bodies was mainly in the composition of complexes with HS, with their molecular weight making up 20–5 kDa and <1 kDa (see Figures 5*(b)* and 5*(d)*). The share of Al(III) and Fe(III) in the studied water bodies as part of complex compounds with HS, whose molecular weight does not exceed 1 kDa on average, is 30–55% and 22–68% of their content as part of the anionic DOM fraction (see Figures 3*(d)* and 4*(d)*). Thus, although the HS fraction with a molecular weight of 20–5 kDa dominates the composition of the HS, it doesn't play an important role in binding metals into complexes. HS with a molecular weight of <1 kDa plays an important role in the migration of metals (see Figures 3–5), although its share is significantly lower than the fractions with a molecular weight of 20–5 kDa in the total composition of HS (see Figure 2). The different binding capacity of DOM, including HS, with different molecular weights is also noted in other publications [39,51].

The concentrations of Al(III), Fe(III), and Cu(II) in complex compounds with HS in the studied water bodies ranged from 1.8 to 122.5, 5.4 to 603.0, and 2.7 to 29.4 g/L (Table S1). Such a wide range of values depends both on the water body's geographic location and on the HS concentration. High values of the latter, as a rule, increase the content of dissolved metals. In particular, a direct linear relationship between the HS concentration and the Al(III) content in their composition was found for the Kaniv Reservoir and the Seret River, between the HS concentration and the Fe(III) content – respectively, for the Kaniv, Yurpil and Ternopil reservoirs, Seret River, Chorne Velyke Lake, and between the HS concentration and the Cu(II) content – for the Middle Bila Tserkva and Ternopil Reservoirs and the Seret River. The correlation coefficients between the HS concentration and the metal concentration in their composition were in the range of 0.50–0.77 at a significance level of 0.05.

The molecular weight distribution of HS and their complex compounds with Al(III), Fe(III), and Cu(II) does not provide complete information for assessing the binding capacity of various HS fractions relative to the studied metals under natural conditions. In this regard, the content of each metal in the composition of individual HS fractions, which differ in molecular weight, was calculated. The content of each metal under study was calculated per 1 mg of HS in each fraction (see Table S1).



(a) *(b)*

(c) *(d)*

Figure 5. Limiting (I, II) and average (III) values of the relative content of copper (Cu_{anion}, %) as part of complex compounds with HS of various molecular weight in the water bodies under study, 2011–2013: >20 *(a)*, 20–5 *(b)*, 5–1 *(c)*, <1 kDa *(d)*. For water bodies numbered 2, 11, 14, 15 the results of single measurements are shown instead of average values.

The results of these calculations showed that in the studied water bodies, the HS fraction of 20–5 kDa often had the lowest concentrations of Al(III), Fe(III), and Cu(II) compared to other fractions. Thus, the limiting values of the metal content in the specified HS fraction were 0.0–6.7, 0.0–12.1, and 0.0–3.9 μg/mg HS, respectively, and the average values were 0.1–3.0, 0.3–4.3, and 0.3–2.4 μg/mg HS (see Table S1). At the same time, the Al(III), Fe(III), and Cu(II) concentrations in HS fractions with molecular weights >20 and <1 kDa, taking into account the average values, turned out to be higher: 0.3–10.4, 1.2–19.0, 0.5– 5.8 and 0.3–5.3, 1.8–20.1, 1.2–6.3 μg/mg of HS respectively (see Table S1). Consequently, the HS fraction with a molecular weight of 20–5 kDa has the lowest binding capacity with respect to these metals. However, it is possible that this fraction has a certain potential ability to bind metal ions, when their concentration in water gets higher due to pollution.

It is probable, that the HS fraction with a molecular weight of 20–5 kDa has the lowest binding capacity because functional groups in HS macromolecules become unavailable as a result of conformational changes. In addition, strongly binding centers in HS macromolecules may already be involved in complexation, while weakly binding centers bind metals when their concentration in water gets higher [18]. In this case, the stability of the complexes formed decreases. It is known that phenolic and carboxyl groups of HS bind metals into complexes more strongly than carbonyl and amino groups [52–54]. It should be borne in mind that hydrogen in the carboxyl group is replaced by metal ions at neutral pH values, and in the phenolic group, in a more alkaline medium. The cation exchange capacity of HS is determined by the substitution of hydrogen in these functional groups [18,55]. At the same time, the total binding capacity of HS depends on one third of the active centers of cation exchange and two thirds of the active centers of complexation. According to the literature data [18] the total binding capacity of HA is 5.4–16.2, 11.2–33.6, and 12.8–38.4 μg/mg HA for Al(III), Fe(III), and Cu(II), respectively. In the opinion of the same author, the natural concentration of these metals in the composition of HS in surface water bodies potentially allows HS to bind metals into complexes. The results of these studies showed that in water bodies the metal concentration in the composition of unfractionated HS was within 0.05–15.4, 0.5–37.0, and 0.05–5.1 μg/mg of HS, respectively, for Al(III), Fe(III) and Cu(II). Such a wide range of values indicates a different use of the HS' total binding capacity. The minimum values are recorded, as a rule, for water bodies with a high content of HS, and the maximum values, on the contrary, for water bodies with low HS concentrations. In addition to unfractionated HS, this situation is typical for HS fractions with different molecular weight (see Table S1). In the water of the Kyiv Reservoir, the copper concentration in the DOM composition during 1990–1995 averaged 0.6 μg/mg of DOM [37]. According to the results of those studies, the Cu(II) concentration in the HS composition in the water bodies under study was 1.3 μg/mg of HS on average.

Copper is often used to determine the complexing ability of DOM in natural waters. In one of these works [56], it was found that the Cu(II) concentration in the composition of unfractionated FA and HA increased from 44.5 to 97.7 and from 35.8 to 106.3 μg/mg with a rise in pH from 5.0 to 7.0. At the same time, it was found that with the molecular weight of FA reducing from >50 to 3.0–0.5 kDa, the Cu(II) concentration in their composition increases from 26.2 to 42.2 μg/mg at pH 5.0, and from 96.6 to 112.6 μg/mg at pH 7.0. In the composition of HA, regardless of their molecular weight, which ranged from >100 to 50–10 kDa, the Cu(II) concentration was 30.7–35.2 μg/mg at pH 5.0 and 96.6–98.6 μg/mg at pH 7.0.

The experimental studies of the role of different HS fractions in binding metal ions

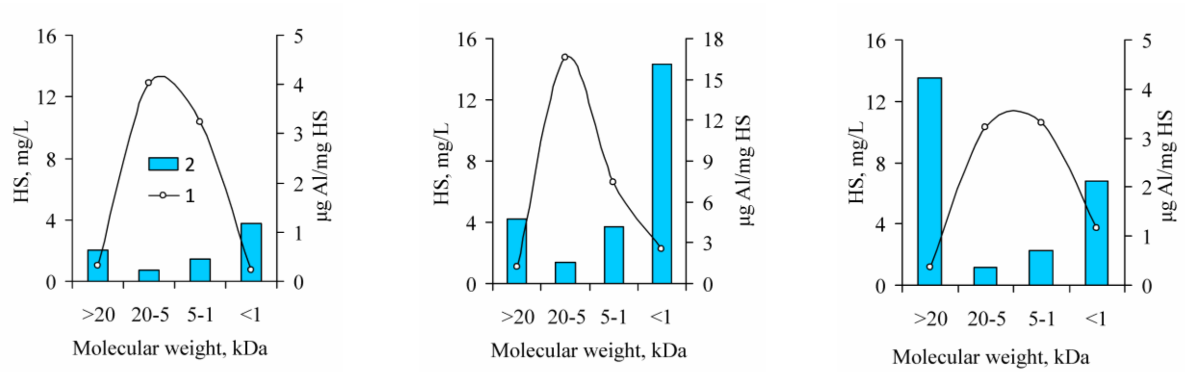
To elucidate the reason for the low binding ability of the HS fraction with a molecular weight of 20–5 kDa compared to other fractions (20–5, 5–1, and <1 kDa), special experimental studies were carried out. The specifics of the metals' distribution among various HS fractions in natural water were studied by adding certain concentrations of Al(III), Fe(III), and Cu(II). The metal concentrations that were added at the beginning of the experiment hardly differed from their natural content in fresh surface waters (250 μg/L of Al(III) and Fe(III) each and 50 μg/L of Cu(II)). The concentration of HS in the initial natural water was 30.6 mg/L, and by the end of the experiment (after 60 days) it was in the range of 24.6–27.0 mg/L. The greatest changes in the ratio of HS fractions with different molecular weight were observed in the experimental system where 250 μg/L Al(III) was added. The relative content of the HS fraction with a molecular weight of 20–5 kDa in this system got higher (Figure 6*(b)*) due to a decrease in the share of the HS fraction with a molecular weight of

5–1 kDa. Apparently, there was a phenomenon of HS aggregation. This caused an increase in the average molecular weight of HS (Mw) from 6.3 kDa in the initial water to 7.0 kDa at the end of the experiment.

When the metals under study were added to natural water samples, they were predominantly bound by HS fractions with a molecular weight of >20 and <1 kDa (Figures 6–8). After 60 days of the experiment, the Al(III), Fe(III), and Cu(II) concentrations in the HS fraction with a molecular weight >20 kDa increased in 6.7–7.6, 8.3–11.7 and 2.3–3.9 times, with a molecular weight <1 kDa – 1.8–13.8, 15.2–89.7, and 3.5–8.2 times respectively, compared with their content in the same fractions, but without the addition of metals (see Figures 6–8). The Al(III), Fe(III) and Cu(II) concentrations in HS fractions with molecular weights of 20–5 and 5–1 kDa increased only in 1.6–6.7, 5.3–7.1, 1.1–1.3, and 1.6–9.2. 10.8–17.0, 1.1–1.2 times as compared with their content in the initial water (see Figures 6–8).

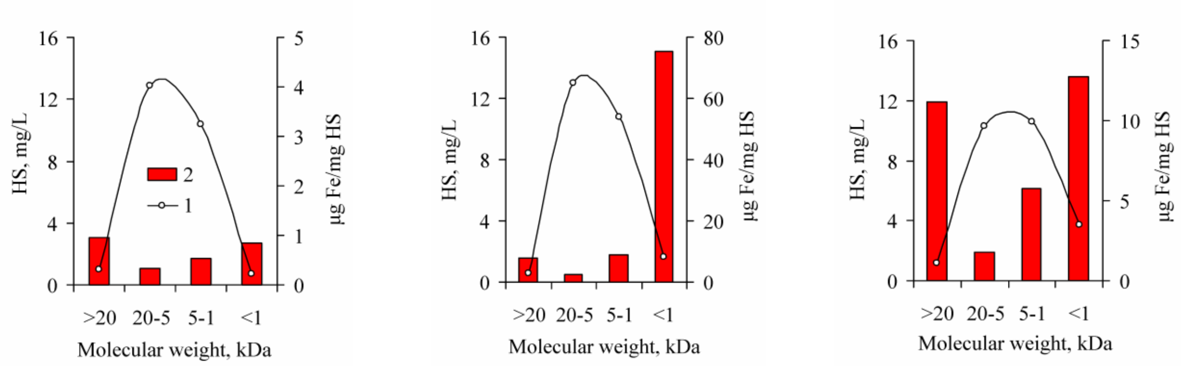
The complexation ability of HS fractions with different molecular weight with respect to heavy metals is also reported in a number of other publications [28,57]. In some cases, greater complexation ability is observed for high-molecular weight fractions [28], and in other cases, on the contrary, for fractions with a lower molecular weight [57]. It depends both on the metal with which the complexation is investigated and on the HS source.

Thus, it can be stated with certainty that the HS fraction with a molecular weight of 20–5 kDa has the lowest binding ability to metals. According to the HS molecular weight distribution (see Figure 2), it can be assumed that in the water bodies under study, 37.2–70.7% of HS from their total content can most actively participate in binding into complexes the metal ions entering the aquatic environment, for example, wastewater.



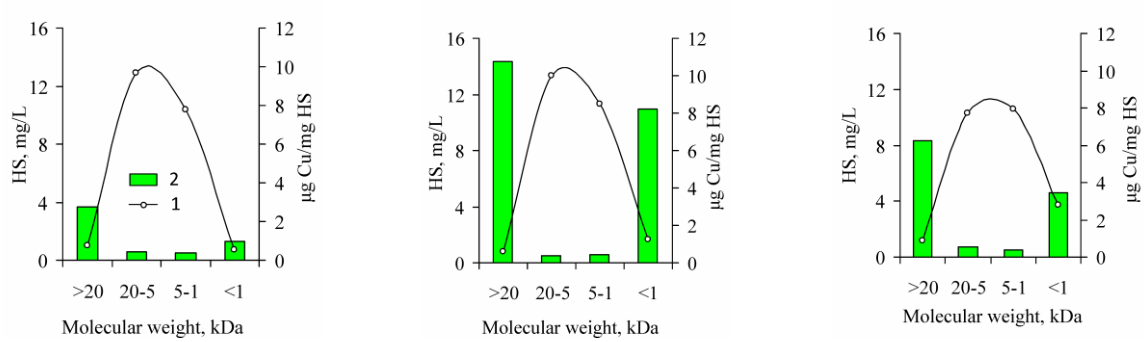
(a) *(b)* *(c)*

Figure 6. Distribution of aluminum (2) among HS fractions (1) with different molecular weight in natural water (NW) from the Kaniv Reservoir after 60 days of the experiment, taking into account its various concentrations: NW without Al(III) additives *(a)*, NW + 250 µg/L Al(III) *(b)*, NW + 50 µg/L Al(III) + 50 µg/L Fe(III) + 50 µg/L Cu(II) *(c)*.



(a) *(b)* *(c)*

Figure 7. Distribution of iron (2) among HS fractions (1) with different molecular weight in natural water (NW) from the Kaniv Reservoir after 60 days of the experiment, taking into account its various concentrations: NW without Fe(III) additives *(a)*, NW + 250 µg/L Fe(III) *(b)*, NW + 50 µg/L Fe(III) + 50 µg/L Al(III) + 50 µg/L Cu(II) *(c)*.



(a) *(b)* *(c)*

Figure 8. Distribution of copper (2) among HS fractions (1) with different molecular weight in natural water (NW) from the Kaniv Reservoir after 60 days of the experiment, taking into account its various concentrations: NW without Cu(II) additives *(a)*, NW + 50 μg/L Cu(II) *(b)*, NW + 50 μg/L Cu(II) + 50 μg/L Al(III) + 50 μg/L Fe(III) *(c)*.

Noticeable binding of the studied metals into complexes with HS of relatively low molecular weight (<1.0 kDa) indicates that in summer the share of such complexes may increase due to HS photo transformation, which was already reported earlier. However, HS photo transformation is probably not the only reason for this. It is not excluded that some parts of HS with a relatively low molecular weight are formed in the water bodies themselves in the summer. Therefore, the concentration of the fraction with a molecular weight <1.0 kDa can increase, thus leading to the increase of metals bioavailability in complexes with a molecular weight <1.0 kDa can increase. One should not exclude the fact that some metals will be released from the composition of complex compounds with HS due to photolysis. Therefore, the water toxicity for aquatic organisms can increase if such metals are in a dissolved state in the aquatic environment. The probability of this is low, since metals are adsorbed on fine particles of suspended substances and bottom sediments in surface water bodies [30].

It has been found that FA dominate the composition of HS in the water bodies under study, accounting for 77.7–92.4% of HS_{total}. In the present studies, the separation of HS into fractions of FA and HA was carried out only for some water bodies: Kaniv and Yurpil reservoirs, Desenka arm, Desna River, Lutsymer Lake. The concentration of FA in these water bodies was 25.4–31.3, 16.4, 12.5–20.3, 14.6 and 38.1 mg/L, respectively, and the content of HA was 2.1–6.1, 4.7, 1.6–1.7, 2.0, 9.2 mg/L. At the same time Al(III), Fe(III), and Cu(II) were found mainly as complex compounds with FA. Their share in the composition of FA was 39.3–74.2% (on average 61.4%), 55.3–81.9% (on average 68.1%), and 53.0–87.7% (on average 74.5%), respectively, of their total content in the composition of complexes with HS. Consequently, FA are more involved in the migration of the studied metals than HA. However, based on these data, it is difficult to assess the FA and HA binding abilities due to their different contents in water. To do this, the Al(III), Fe(III), and Cu(II) concentrations in the composition of FA and HA fractions were calculated per 1 mg of these compounds. Their content in the studied water bodies was within the limits of 0.2–0.5, 0.4–2.3, 0.3–2.9 μg/mg of FA and 0.5–3.0, 1.8–9.2, 0.4–3.4 μg/mg of HA, respectively. It was found that 1 mg of HA binds Al(III), Fe(III), and Cu(II) into complexes 1.2–11.8, 1.7–5.0, and 1.1–10.7 times more than 1 mg of FA. The predominant presence of the metals under study in the composition of complex compounds with FA is due to their predominance in the HS' composition. It has been found that the fractions of FA and HA with a molecular weight of 20–5 kDa in the studied water bodies almost always had the lowest concentrations of Al(III), Fe(III), and Cu(II), respectively, 0.1–0.7, 0.2–1.8, 0.1–3.8 μg/mg of FA and 0.4–3.8, 0.9–5.9, 0.2–1.9 μg/mg of HA. At the same time, the content of Al(III) in the composition of FA fractions with molecular weights >20, 5–1, and <1 kDa was 0.1–8.5, 0.1–0.8, and 0.2–0.6 μg/mg of FA, Fe(III) – 0.1–25.4, 0.8–6.0 and 0.7–2.9 μg/mg of FA and Cu(II) – 0.4–5.5, 0.1–3.0 and 0.3–1.6 μg/mg of FA. The content of Al(III) in the composition of HA fractions with molecular weights >20, 5–1, and <1 kDa was 0.3–1.3, 0.5–5.2, and 0.5–9.0 μg/mg of HA; Fe(III) – 0.9–8.3, 2.2–25.2 and 1.3–16.9 μg/mg of HA; Cu(II) – 0.3–10.6, 0.3–9.2 and 0.1–7.5 μg/mg of HA. Thus, as compared to FA, HA bind into complexes a greater amount of metals per 1 mg of both unfractionated HA and FA, and their fractions with different molecular weight.

Conclusions

A comparative assessment of the complexation ability of unfractionated HS, their component groups (FA and HA) and their fractions with different molecular weight (>20 kDa, 20–5 kDa, 5–1 kDa and <1 kDa) in relation to Al^{3+}, Fe^{3+} and Cu^{2+} ions both under natural and experimental conditions was carried out. Taking into consideration the amount of bound metal per 1 mg of HS, HA were found to possess greater complexation ability than FA. However, the majority of the metals under study are concentrated in the composition of complex compounds with FA, as they prevail in surface waters. This gives an impression that FA play a predominant role in binding metal ions into complexes.

Among the fractions of HS, FA and HA with different molecular weight, the fractions >20 kDa and <1 kDa are characterized by the greatest complexation ability in relation to Al^{3+}, Fe^{3+} and Cu^{2+} ions, and the smallest – 20–5 kDa. This is confirmed by research results both under natural conditions and in experiments. At the same time, the majority of metals are concentrated in the HS fraction with a molecular weight of 20–5 kDa, as it dominates their composition. In this case, the amount of metals per 1 mg of HS (unfractionated and fractionated) is not taken into account. The content of the HS fraction with a molecular weight >20 kDa is usually insignificant, so the share of metals in its composition is low.

Significant binding of Al^{3+}, Fe^{3+}, and Cu^{2+} ions by the HS fraction with a molecular weight <1 kDa should be considered as such, which can increase the bioavailability of metals for aquatic biota, since it is believed that such complexes are able to penetrate through the biological membrane.

The data on the binding capacity of different molecular weight fractions of HS are important, first of all, for assessing the potential bioavailability and toxicity of the corresponding metal complexes with these natural organic substances.

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Supplementary information

Supplementary data are available free of charge at http://cjm.ichem.md as PDF file.

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