

SYNTHESIS, METAL-BINDING PROPERTIES AND DETOXIFYING ABILITY OF SULPHONATED HUMIC ACIDS

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Abstract

The complexing properties of humic substances are of primary importance for their application as detoxifying agents and microfertilizers. The promising approach for enhancing solubility of metal-humics complexes is incorporation of SO₃H-groups into the structure of humics. The water soluble sulphonated humic materials can be used as flushing agents for heavy metal polluted sites and as chelating agents for production of microfertilizers. The objectives of this study were: 1) to synthesize sulphonated humic materials; 2) to evaluate solubility and stability of their complexes with Fe(III) and Hg(II); 3) to assess potential toxicity and detoxifying properties of the sulphoderivatives with respect to Hg(II) and Cu(II). Humic acids (HA) from leonardite and peat were used for modification. Concentrated sulphuric acid and chlorosulphonic acid were used as sulphoning agents. Chlorosulphonic acid was found to be much more efficient sulphoning agent causing introduction of 1.6 and 0.4 mmol SO₃H per g of humic material from leonardite and peat, respectively. The binding properties of the sulphonated HA were studied in relation to Hg(II) and Fe(III). The solubility of metal-humic complexes was estimated as an amount of strong metal-binding sites (SBS) in the humic material. The sulphonated materials were characterized by twofold increase in the amount of SBS: 2-4 mmol/g versus 1-2 mmol/g in the parent material. Stability constants were determined using the ligand exchange technique. For Hg(II) complexes, the log K values accounted for 13.1 and 12.4 L/mol SBS for leonardite HA and their sulphoderivative, respectively. For Fe(III) complexes, the log K values accounted for 12.0 and 11.5 for the parent and chlorsulphonated coal HA, respectively, and 12.4 and 11.4 – for the parent and chlorsulphonated peat HA. Assessment of the toxicity of the obtained derivatives and of their detoxifying properties was performed using algological tests. The sulphoderivatives did not exhibit adverse effects onto algae. Both parent and sulphonated humic materials displayed high detoxifying properties with respect to Hg(II) and Cu(II), leonardite HA were more effective than peat HA. The obtained results allow to consider sulphonation as a promising tool for obtaining soluble metal-HA complexes.

1. Introduction

The presence of a wide range of functional groups (carboxylic, hydroxylic, carbonylic) in the structure of humic substances (HS) provides their ability to form the complexes with metals [1]. As a result, HS play a key role in both migration of metals and their uptake by higher plants. Of particular importance is, that the properties of metal complexes of HS depend greatly on the content of functional groups and on the molecular weight of humics. So, the lower molecular weight (MW) fraction of HS – fulvic acids (FA) enriched with oxygen containing functional groups produce soluble metal complexes of much higher mobility and bioavailability than those of humic acids (HA). The latter is higher MW fraction of HS enriched with the condensed aromatic structures. Given the above properties of metal complexes of HA and FA, G.M. Varshal and co-workers distinguished greatly the geochemical roles of HA and FA in metal migration characterizing the former as “accumulators” or “immobilisers” of metals in the environment, whereas the latter as “scatters or dissipaters” of metals [2].

It has been numerously discussed in the literature, that HS application for the pollution control has a significant potential [3]. They can be effectively used as natural, cheap and regenerative ion-exchangers for cleaning-up waste waters and gases [4]. The complexing properties of HS determine their potential to be used as detoxifying agents and microfertilizers. Even though the available supply of raw humic materials is enormous, two fundamental reasons can be cited as to why HS have not been widely used for the above applications. First, few natural HS possess the specific reactive properties required to form highly mobile and soluble metal complexes. Second, HS are by definition polydispersive and heterogeneous; consequently, the reactive properties vary between natural sources and between industrial suppliers. The presented research has addressed both problems using a novel approach of using chemical modification for designing humic materials with specific properties. Previous investigations involving HS modifications (hydrolytical cleavage, oxidation, methylation, acetylation, silylation, and others) were focused on an understanding of HS structure [5, 6, 7, 8], or bioavailability [9], not to enhance or change reactive properties of HS. Sulfonation was used as a promising way of producing highly soluble HS enriched with functional groups.

Sulphonation (introduction of $-\text{SO}_3\text{H}$ group) is a well-known technique of chemical modification of organic materials [10]. Sulphonated organic materials are applied as drilling fluids [11]. The sulphonating agents that are widely used are chlorosulphonic acid, concentrated sulphuric acid and sulphur trioxide [12, 13, 14]. Previously, coals have been directly sulphonated using sulphuric acid, to introduce sulphonic groups, but the sulphonated products were not water-soluble and could not be dissolved, purified and otherwise treated as an organic chemical [15]. They have usually found application as ion exchange materials. Water-soluble humic acids were previously obtained by various oxidation treatments of humic materials [16] and some coals [17, 18] but far-reaching molecular breakdown has always accompanied the oxidation, and the water-soluble products were, therefore, extensively degraded materials [19]. A number of agents such as chlorosulphonic acids or sulphur trioxide solution in pyridine allow to introduce sulphuric groups without significant oxidation or degradation of treated humic acids [20]. Another technique of synthesis of water-soluble sulphoderivatives of HS is

based on reaction with a sulphonating agents like sulphite or bisulphite precursors which form a sulphite or bisulphite *in situ*, in alkaline medium and recovering a water-soluble product from the reaction medium. Sulphomethylation was also reported as the method of solubilization of water-insoluble humic acids [10]. The synthesis of sulphoderivatives of HA is of particular interest because they can be used as drilling mud thinners, soil conditioners, and tanning agents [12] and also in various flushing technologies [21].

The objectives of this study were: 1) to synthesize sulphonated humic materials; 2) to evaluate solubility and stability of their complexes with Fe(III) and Hg(II); 3) to assess potential toxicity and detoxifying properties of the sulphoderivatives with respect to Hg(II) and Cu(II).

2. Experimental Section

2.1. MATERIALS AND METHODS

Humic materials used were isolated from highland peat (Sk3-00, Sakhtysh Lake, Ivanovo Region, Russia) and leonardite – oxidized coal, kindly provided by Humintech Ltd. (Duesseldorf, Germany). Isolation procedure included a preliminary treatment of a peat sample with ethanol-benzene (1:1) mixture followed up by an alkaline (0.1 M NaOH) extraction as described elsewhere [22], leonardite was treated with 0.1 M NaOH. The obtained alkaline extract was acidified to pH 1 using concentrated HCl. The precipitated HA were centrifuged, washed out with distilled water and dialysed. A set of the parent and modified samples is described in Table 1.

Table 1. Set of parent and modified humic materials under study.

Sample	Description
CHA-Leo	Leonardite HA
CHA-S	Leonardite HA, sulphonated with H ₂ SO ₄
CHA-CIS	Leonardite HA, sulphonated with ClHSO ₃
PHA-Sk300	Peat HA
PHA-S	Peat HA, sulphonated with H ₂ SO ₄
PHA-CIS	Peat HA, sulphonated with ClHSO ₃

Sulphonation of humic acids with concentrated H₂SO₄. A weight of 100 g of humic acids was wetted with water until paste-like state and then it was added dropwise with 400 ml concentrated H₂SO₄. The obtained mixture was stirred during 2 hours using mechanical stirrer. When reaction was completed, the mixture was poured into 4 litres of distilled water, stirred, and left for 10 hours. The supernatant was discarded, and the precipitate was purified by dialysis and dried out for the further analytical studies.

Sulphonation of humic acids with chlorosulphonic acid. A weight of 100 g of humic acids was dried over P_2O_5 , placed into 500 ml beaker, cooled in the ice bath and then was added with 200 ml of cooled $ClSO_3H$ by small portions with permanent stirring during 6 hours. When the reaction was completed, the mixture was poured into 4 L of iced distilled water to hydrolyse the rest of chlorosulphonic acid, and the obtained solution was left for 10 hours. Then, the supernatant was discarded, and the precipitate was washed out with water 4 times until the supernatant solution gave negative reactions to $BaCl_2$ and $AgNO_3$. The obtained precipitate of the sulphonated humic acids was further purified using dialysis and then dried.

Determination of the content of strong acidic groups (COOH and SO_3H). Ca acetate method was used to determine the content of strong acid groups in the humic samples as described in [23]. For this purpose, an aliquot of HS solution (5-10 mL) containing 5-20 mg HS was transferred into a vial (~ 22 mL), and 10 mL of 0.6 M $Ca(CH_3COO)_2$ was added. The vial was tightly sealed, shaken well and left for equilibration for 24 hours at room temperature. An aliquot of supernatant above the precipitate of Ca humates was transferred into titration cell and titrated with NaOH standard solution (~0.05 M) using autotitrator (Metrohm 716 DMS Titrino). Acidity of the samples (A , mmol/g) was calculated as follows:

$$A = \frac{(V_{HS} - V_0) \cdot c_{NaOH}}{m} \quad (1)$$

where V_{HS} and V_0 are the volumes of NaOH used for titration of the HS solution and the blank solution containing same amount of $Ca(CH_3COO)_2$, L, C_{NaOH} – concentration of NaOH, mM, m – a weight of HS sample, g. A has dimensional units of mmol/g.

Determination of the stability constants of iron humates. Stability constants of the iron(III) complexes with parent and sulphonated HA were determined using ligand exchange technique with photometric detection. Tartrate was used as a competitive ligand. The method is based on detecting the difference in the optical density of HA and of their Fe(III) complexes. Two types of titrations of HS (0.5 g/L) by $Fe_2(SO_4)_3$ (10 mM Fe) solution were conducted: (1) in the presence of 0.024 M tartrate buffer (pH 4.37); and (2) in the absence of the buffer, but in the presence of 0.048M KNO_3 used to maintain the same ionic strength as in the buffer containing solution. During titration of the latter solution, pH was kept at 4.37 ± 0.1 by adding 0.05 M NaOH. After each addition of Fe^{3+} and NaOH, optical density was measured at 650 nm using Metrohm 662 Photometer (Switzerland) equipped with a submersible optical cell (1 cm optical path). Solution of $Fe_2(SO_4)_3$ containing 10 mM Fe was prepared in 0.01M H_2SO_4 . 0.4 M tartrate buffer was prepared by dissolving 6 g of $C_4H_6O_6$ in 50 ml of water, adding it with 1 M NaOH to adjust pH to 4.37, and then making it up to 100 mL with distilled water. At pH 4.37, tartrate exists as a 1:1 mixture of divalent and monovalent anions.

Determination of the content of strong metal-binding sites. To determine the content of strong iron-binding sites in parent and sulphonated humic materials, 0.8 M $Fe_2(SO_4)_3$ was added to 0.25 g/l solution of HA until the beginning of precipitation (pH was maintained at 4.37 ± 0.1 using NaOH). The concentration of iron causing the precipitate formation was assumed to be equal to the concentration of strong iron-binding sites in the HA solution. The content of strong mercury-binding sites was determined similarly, using 0.1 M $Hg(NO_3)_2$ and maintaining pH at 6.68 ± 0.1 . To obtain a numerical value of

the content of strong metal-binding sites in the humic materials, the metal concentration causing HA precipitation (mmol/L) was normalized to the mass concentration of HS in the solution (g/L) yielding dimensional units of mmol Me/g HA.

Determination of the stability constants of mercury humates. Stability constants of Hg(II) complexes of the parent and modified HA were determined using ligand exchange technique with photometric detection similar to the described above for Fe(III). Chloride was used as a competitive ligand. The HA solutions (0.3 g/L) were titrated photometrically at 650 nm by 0.01M Hg(NO₃)₂ (1) in the presence of 0.01 M NaCl; and (2) in the absence of NaCl, but in the presence of 0.01 M KNO₃ used to maintain the same ionic strength as in case of chloride. In both experiments, pH was adjusted to 6.2 using NaOH or HNO₃. During titration, pH was maintained at 6.2±0.1 by adding 0.05 M NaOH. To maintain the constant concentration of Cl⁻ in the reacting systems during titrations in the presence of NaCl, solutions of both NaOH and HgCl₂ contained 0.01M NaCl. Hg(NO₃)₂ solution was prepared by dissolving a weight of HgO in minimal amount of concentrated HNO₃.

Acute toxicity tests. Humic materials tested were CHA-Leo, CHA-CIS, PHA-Sk300 and PHA-CIS. HA solutions had concentration in the range of 0.5-20 mg/L. Hg(II) and Cu(II) were used as model heavy metals. Acute toxicity tests were performed according to [24]. Green unicellular algae *Chlorella pyrenoidosa* was used as a biotarget, photosynthetic activity – as a response. *Chlorella pyrenoidosa* (thermophilic strain CALU-175 from the collection of the Biology Institute, Saint-Petersburg State University, Russia) was grown in 1/5 Tamiya medium (pH 6.8) [25] at 32°C, under 60 μmol photons m⁻²s⁻¹ continuous irradiance and bubbling with moisturized air. The culture was maintained in log growth phase by daily dilution with fresh medium to maintain a cell density of about 1-10·10⁵ cells·mL⁻¹. For the bioassays, the algae was concentrated by centrifugation and resuspended to a final concentration of 5-7·10⁵ cells·mL⁻¹ in the 1/10 Tamiya medium without phosphates and EDTA.

After three hours of growth, photosynthetic activity of algae was measured. For this purpose, the algae was adapted to darkness for 30 s. Chlorophyll fluorescence induction was recorded for 300 s using a lab-made fluorometer. F₀ was estimated from the value of fluorescence detected immediately after opening of the shutter. F_m was calculated by averaging the values of the last 50 points of the fluorescence induction curve. Photosynthetic activity (R) was calculated as a relative yield of the variable fluorescence using following expression:

$$R = \frac{F_v}{F_m} = \frac{F_m - F_0}{F_m} \quad (2)$$

where F_v, F_m and F₀ are intensity of variable, maximum and background chlorophyll fluorescence, respectively. Three replicates were made for each experimental point.

The toxicity of model heavy metals was tested in the range of concentrations of (0.4-3)·10⁻⁶ and (0.3-2.6)·10⁻⁶ M for HgCl₂ and CuSO₄, respectively. The corresponding dose-response relationship fits satisfactorily with the linear model. The slope *k* (mean±SD, *n* = 3) was (0.35±0.03)·10⁻⁶ and (0,25±0.04)·10⁻⁶ M⁻¹ for Hg (II) and Cu (II), respectively. The corresponding r² were 0.86 and 0.78. The effective concentrations causing 50% reduction in the photosynthetic activity of algae (EC₅₀) were 1.2 and 1.5·10⁻⁶ M for Hg (II) and Cu (II), respectively.

The above concentrations were used in the experiments on studies of detoxifying properties of the humic materials. Concentration of humic materials in the solution was in the range of 0.5-4 mg/L and 0.5-20 mg/L for Hg(II) and Cu(II), respectively. Each experimental series included four types of test solutions: control, HA, heavy metal, and heavy metal + HA. The corresponding responses were designated as R_0 , R_{Me} , R_{HA} and R_{Me+HA} . They were used to calculate detoxification coefficient (relative decrease in heavy metal toxicity in the presence of HA) as described in [26]:

$$D = \frac{T_{Me} - T_{Me+HA}}{T_{Me}} \quad (3)$$

where T_{Me} and T_{Me+HA} are toxicities of the heavy metal solution in the absence and presence of HA, respectively.

The toxicity values can be calculated using the following expressions:

$$T_{Me} = \frac{R_0 - R_{Me}}{R_0} \quad (4)$$

$$T_{Me+HA} = \frac{R_{HA} - R_{Me+HA}}{R_{HA}} \quad (5)$$

where R_0 , R_{HA} , R_{Me} , and R_{Me+HA} is photosynthetic activity of algae in the control, in the humic material solution, and in heavy metal solution in the absence and presence of HA, respectively.

3. Results and discussion

3.1. SYNTHESIS AND STRUCTURAL ANALYSIS OF THE SULPHONATED HUMIC MATERIALS

HA samples from leonardite – highly oxidized coal, and peat were selected for modification. They were chosen as representatives of the humics isolated from the raw materials of the greatest industrial value. Leonardite HA are enriched with aromatic fragments, whereas peat HA are enriched with carbohydrate units. It was expected that introduction of sulphonic groups will increase water-solubility of both HA and their complexes with metals [27].

The sulphonated humic materials obtained with a use of both sulphuric and chlorosulphonic acid were amorphous powders of the dark brown colour. The samples treated with chlorosulphonic acid (CHA-CIS and PHA-CIS) were much more soluble in water than those treated with sulphuric acid. Their solubility laid in the range of 100-130 g/L. It could be indicative of a higher sulphonation efficiency of chlorosulphonic acid compared to that of concentrated sulphuric acid.

Elemental composition of the parent and sulphonated humic materials is given in Table 2.

The parameters of major interest were S content and C/S ratio which can serve as indicators of the sulphonation efficiency. As it can be seen, for the initial samples and for the derivatives treated with concentrated sulphuric acid, C/S ratio is very similar (CHA-Leo – 256, CHA-S – 196 and PHA – 265, PHA-S – 210). This indicates that the

sulphuric-acid treatment did not lead to an increase in the content of sulphur, hence, sulphonation had not occurred. At the same time, for the derivatives treated with chlorosulphonic acid, the C/S ratio was substantially less compared to the parent materials, and accounted for 30 and 43 for CHA-CIS and PHA-CIS, respectively. This shows much higher efficiency of chlorosulphonic acid as sulphonating agent of the humic materials compared to sulphuric acid.

Table 2. Elemental composition of the parent and sulphonated humic materials on ash-free basis.

Humic material	Content of elements, % mass.					Ash	Atomic ratio		
	C	H	N	S	O	%	C/S	H/C	O/C
CHA-Leo	56.4	4.10	1.23	0.59	37.6	7.78	256	0.87	0.50
CHA-S	62.5	4.12	1.07	0.56	31.7	4.28	296	0.79	0.38
CHA-CIS	58.5	3.58	1.31	5.12	31.5	5.85	30	0.74	0.40
PHA-Sk300	48.7	4.65	3.27	0.49	42.9	3.28	265	1.15	0.66
PHA-S	62.2	5.23	2.72	0.79	29.1	2.06	210	1.01	0.35
PHA-CIS	46.9	3.03	3.66	2.93	43.5	2.14	43	0.78	0.70

Efficiency of sulphonation was also estimated via determination of the content of strong acidic groups using the Ca-acetate technique. The latter determines all acidic groups present in humic material that have dissociation constant < 4.76 (pK_a of acetic acid). In case of the sulphonated humic materials, the pool of strong acidic groups is composed of carboxylic groups of the parent material and of the introduced sulphononic groups. Hence, an increase in the content of the strong acidic group could serve as another parameter of sulphonation efficiency. The corresponding data of the Ca-acetate determinations are given in Table 3.

Table 3. The content of strong acidic groups in the humic materials used in this study.

Sample	COOH + SO ₃ H, mmol/g	ΔSO ₃ H, mmol/g
CHA-Leo	2.9±0.5*	–
CHA-S	3.3±0.2	0.4
CHA-CIS	4.5±0.1	1.6
PHA-Sk300	3.3±0.1	–
PHA-S	2.1±0.1	–
PHA-CIS	3.7±0.2	0.4

* ± stands for a one standard deviation (n = 3).

The titration data show that similar to C/S ratio, the amount of strong acid groups has substantially increased after sulphonation with chlorosulphonic acid (1.6 and 0.4 mmol/g for CHA-CIS and PHA-CIS, respectively), and to much lesser extent – after sulphonation with sulphuric acid. Of particular importance is that the derivatives obtained by the treatment with chlorosulphonic acid were completely soluble in water (up to 100-130 g/L), whereas those treated with sulphuric acid were not soluble.

3.2. METAL-BINDING PROPERTIES OF THE SULPHONATED HUMIC MATERIALS

The metal-binding properties of the sulphonated peat and leonardite HA were studied using Hg(II) and Fe(III) as model metals. It was of particular importance to answer the following questions: 1) how does sulphonation influence solubility and stability of the metal-complexes; and 2) how do these changes influence detoxifying properties of HA. The enhanced solubility of HA complexes with nutritional metals would contribute greatly in their quality as microfertilizers. At the same time, sulphonation could cause a decrease in stability constants of those complexes. Therefore, interaction of parent and sulphonated humic materials should be investigated to estimate an influence of sulphonation on both complexation parameters of interest: on the content of strong binding sites determining the solubility of HS and on the stability constants. Iron was chosen as a nutrition metal, and mercury – as a model toxic metal.

To quantitate solubility of the metal complexes of the sulphonated humic materials, the HA solutions were titrated with the corresponding metal until the beginning of the precipitate formation. It was assumed that the metal-HA complex remains in solution until strong binding sites are not saturated with metal; once these sites are saturated, the complex precipitates. This allowed us to consider the metal:HS ratio (mol/g) of the beginning of precipitation as a quantitative estimate of the content of strong binding sites (SBS). The obtained results are presented in Table 4.

Table 4. Content of strong metal-binding sites in the parent and sulphonated humic materials.

Sample	Amount of Fe(III)-SBS, mmol/g	Amount of Hg(II)-SBS, mmol/g
CHA-Leo	3.2	2.2
CHA-S	2.8	n.d.
CHA-CIS	3.4	4.5
PHA-Sk300	0.9	n.d.
PHA-S	1.5	n.d.
PHA-CIS	1.9	n.d.

The obtained contents of SBS for Fe(III) and Hg(II) are quite similar and lay in the same range of values as total amount of strong acidic groups determined by Ca-acetate technique (Table 3). It should be noted that much higher content of SBS is observed in

the coal humic material compared to peat. In case of peat HA, sulphonation with chlorosulphonic acid leads to twofold increase in the amount of SBS. Hence, introduction of the sulphonic groups into humic materials increases the solubility of complexes as a result of an increase in the amount of SBS.

Stability constants of Fe(III) and Hg(II) complexes with the parent and sulphonated materials were determined using the ligand exchange technique with spectrophotometric detection. Tartrate-ion was used as a competitive ligand for Fe(III) and chloride-ion - for Hg(II). The determination is based on different absorptive properties of the humic material and of its metal-complexes: the complexes absorb light stronger. Hence, adding Me to HA solution increases its optical density. If competitive ligand is added to HA solution that forms non-absorbing complexes with metal, an increase in optical density of HA solution upon addition of metal will be much less. The results of Fe(III)-titration of HA solution with and without competitive ligand (tartrate) are shown in Fig. 1. Concentration of the competitive ligand was selected in such a manner to assure existence of only one predominant metal species in the solution. For iron, it was tartrate complex 1:2, and for mercury – HgCl₂. These species do not adsorb light at 650 nm. As it can be seen, the shown titration curve in the presence of tartrate (Fig. 1) has much lesser slope than in the absence of tartrate.

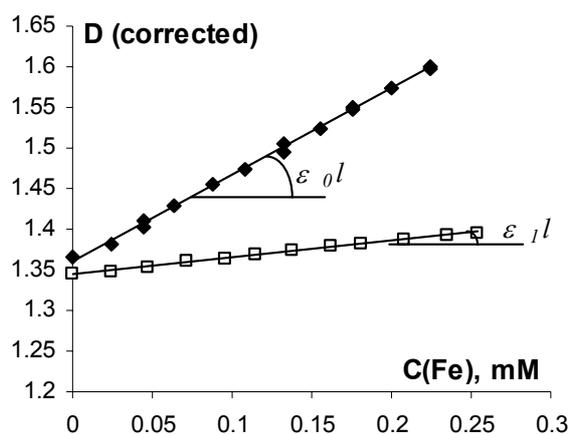


Figure 1. Photometric titration of PHA-Sk300 (0.548 g/L) by 10 mM Fe³⁺ in the presence (open) and absence (closed) of tartrate buffer (0.024 M, pH 4.37) at 650 nm.

The given plots can be used to calculate the stability constant of metal(M)-HA complex K(MHA) by the following expression:

$$\log K(\text{MHA}) = \left(\frac{\varepsilon_0 l}{\varepsilon_1 l} - 1 \right) \cdot \frac{C^n(L)}{C(\text{SBS})} \cdot \beta(\text{ML}_n) \quad (6)$$

where the terms $\varepsilon_1 l$ and $\varepsilon_0 l$ are proportional to molar absorptivity of the MHA and ML complexes, respectively, and can be determined from the slope of titration curve as it is

shown in Fig. 1, C(L) and C(SBS) are the total concentrations of competitive ligand (L) and of metal-binding sites (SBS) in HA solution, β (ML_n) is the stability constant of the corresponding predominant complex.

For calculating a value of stability constant of MHA complexes using equation (6), the value of $\log\beta(\text{HgCl}_2)$ was put equal to 13.4. It was obtained by averaging the reported data from [28] with follow up correction to the ionic strength of 0.048M using Davies equation [29]. The value of $\log\beta(\text{Fe}(\text{tartrate})_2^-)$ was put equal to 10.5 as given in [30] without corrections. The stability constant values were also recalculated into dimensional units of L per g of HS using mass concentration of HS instead of molar concentration of metal binding sites. The obtained results are given in Table 5.

Table 5. Stability constants of the Fe(III)- and Hg(II)-complexes with the parent and sulphonated humic materials.

Sample	Log K, Fe(III)		Log K, Hg(II)	
	L/mol SBS	L/g HA	L/mol SBS	L/g HA
CHA-Leo	12.0	9.5	13.1	10.4
CHL-S	11.3	8.8	–	–
CHL-CIS	11.5	9.0	12.4	10.1
PHA-Sk300	12.4	9.4	–	–
PHS-S	12.2	9.4	–	–
PHS-CIS	11.4	8.7	–	–

The found values of stability constants of Fe(III)-complexes with the parent humic materials are in good agreement with the reported results for fulvic acids: $\log K = 11.5$ - 14.0 L/mol BS [31]. The values of stability constants of mercury complexes are close to those reported in [32] for aquatic fulvic acids ($\log K = 11$ L/mol BS). As it can be seen, the sulphonated humic materials have slightly weaker binding affinity for both metals studied (Fe(III) and Hg(II)) compared to the parent humic materials. This is true for both coal and peat HA. The given decrease in stability constant for coal HA did not exceed half an order of magnitude, and for peat HA – an order of magnitude. Hence, sulphonated humic derivatives still possess high binding affinity for metals.

3.3. TOXICITY ASSESSMENT OF THE SULPHONATED HUMIC MATERIALS AND THEIR DETOXIFYING PROPERTIES WITH RESPECT TO HG(II) AND CU(II)

To assess toxicity of the sulphonated humic materials from coal and peat (CHA-CIS and PHA-CIS, respectively), acute toxicity tests were used. Microalga *Chlorella pyrenoidosa* was used as a test organism, its photosynthetic activity (R) – as a response. A range of the tested concentrations of humic materials was 0.5-20 mg/L. The dose-response relationship for the parent and sulphonated humic materials are shown in Fig. 2.

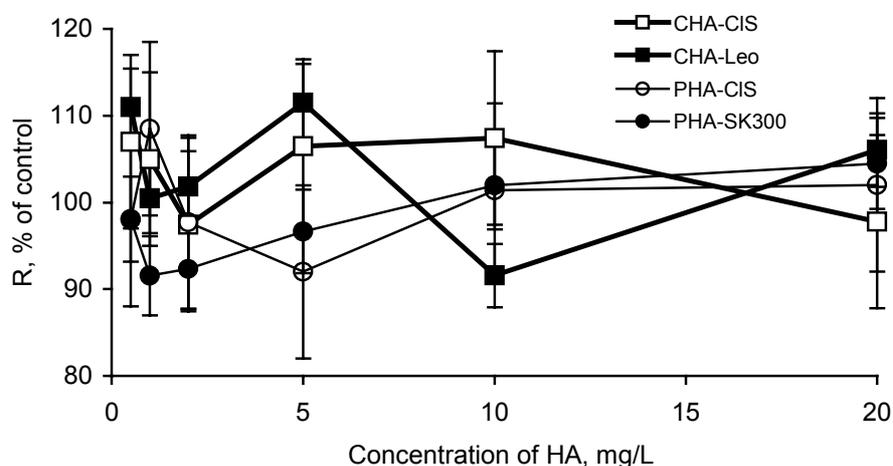


Figure 2. The relationships of the photosynthetic activity of *Clorella pyrenoidosa* versus concentration of humic acids (HA). Bars represent ± 1 SD ($n = 3$).

As it can be seen, a ratio of the fluorescence intensity of the HA solutions to control (R) varied in the range of 0.91–1.12 indicating a lack of either stimulation or inhibition effect of HS on the photosynthetic activity of *Chlorella*. The obtained results – a lack of the significant direct effects of HS onto *Chlorella* are in line with our previous investigations [33]. Of particular importance is that sulphonation did not lead to an increase in toxicity of the humic materials.

Acute toxicity tests conducted with the cultivation media containing model toxic metals – Hg(II) and Cu(II) in the absence and presence of the humic materials allowed us to estimate changes in detoxifying properties of humic materials as a result of their sulphonation. The corresponding data are shown in Figs. 3a, b as the relationships of detoxification coefficient D versus HA concentration.

It can be seen that the detoxification effects of the sulphonated humic materials are slightly weaker compared to those of the parent materials. A decrease in detoxifying effect is much bigger for Cu(II) than for Hg(II), and among the humic preparations it is more distinct for peat HA compared to coal HA. These observations are confirmed by the statistical analysis. So, covariance analysis shows that in case of Hg (II), detoxification curves are significantly different for PHA and PHA-CIS: $F = 10.30$ at $p = 0.003$, where F – Fisher criterion, p – confidence level. At the same time, there is no significant difference between detoxifying properties of the parent and sulphonated coal HA ($F = 3.21$, $p = 0.08$). In case of Cu (II), detoxification properties of the sulphonated HA were significantly weaker both for peat ($F = 16.55$, $p < 0.001$) and coal HA ($F = 13.30$, $p = 0.001$).

The lower detoxifying efficiency of the sulphonated HA compared to the parent materials corroborates well the data on metal-binding properties of the sulphonated HA with respect to Fe(III) and Hg(II). So, as it follows from Table 5, sulphonation causes a decrease in binding affinity of HA for both metals. At the same time, a decrease in the

stability constant of sulphonated peat HA is much larger than for the sulphonated leonardite HA. This is consistent with the tendency observed in detoxifying properties of the sulphonated materials: PHA-CIS exhibits substantially weaker detoxifying effects compared to PHA-Sk300 and CHA-CIS.

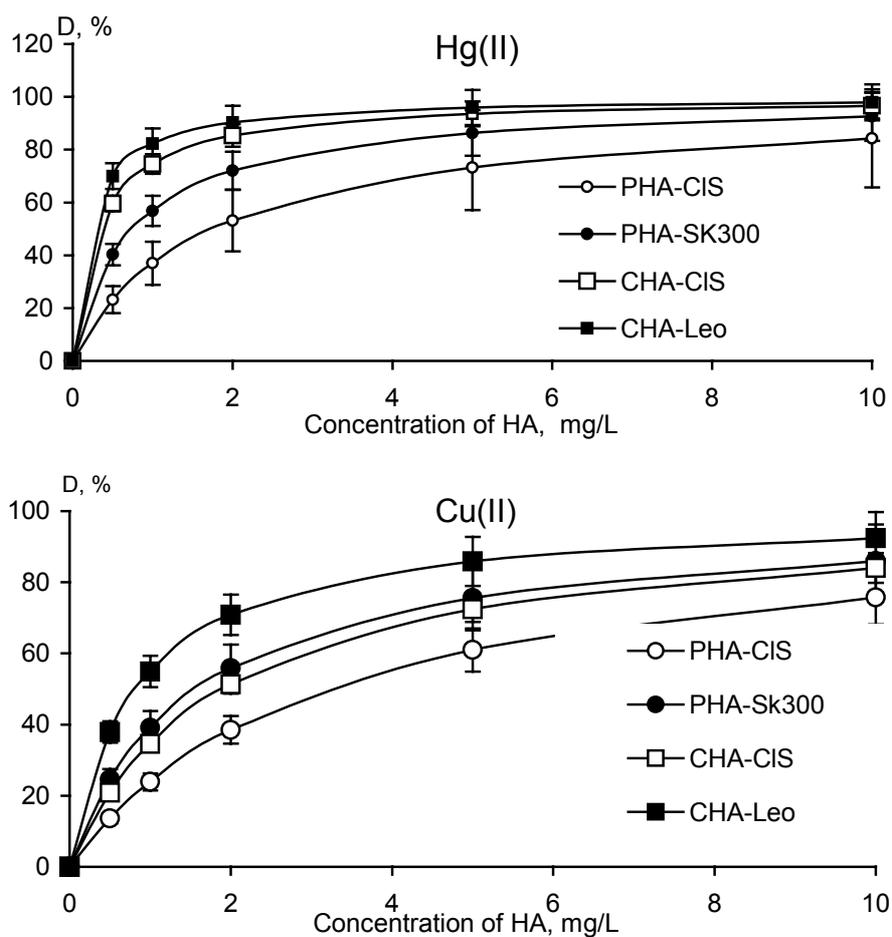


Figure 3. The relationships of the detoxification coefficient D versus concentration of HS in the presence of heavy metals at the EC_{50} concentration: a) Hg(II), $1.2 \cdot 10^{-6}$ M; and b) Cu(II), $1.5 \cdot 10^{-6}$ M. Bars represent \pm SD ($n = 3$).

Given that the sulphonated coal humic material retains its complexing properties, the combinatory action of these HS as detoxifying agents to heavy metals in the contaminated environments and as chelating agents improving the bioavailability of nutritional metals can be expected.

4. Conclusions

The obtained results allow to consider sulphonation as a promising tool for obtaining soluble, mobile complexes of HA with metals. Chlorosulphonic acid is more efficient sulphonating agent than concentrated H₂SO₄, and leonardite humic acids yield higher modification degree than peat HA. Sulphonated humic materials are highly soluble and form soluble metal complexes with high "metal to humic acid" ratio. The metal binding affinity of the sulphonated humic materials do not drop substantially compared to the parent materials. Sulphonation does not induce toxicity of the corresponding derivatives. Sulphonation causes only a slight decrease in the detoxifying properties of humic material with respect to Hg(II) and a more substantial decrease – with respect to Cu(II). Given that the sulphonated HA from leonardite retain complexing and detoxifying properties almost on the level of the parent material, the combinatory action of these humic materials as detoxifying agents to heavy metals in the contaminated environments and as chelating agents improving the bioavailability of nutritional metals can be expected. They can be also used as environmentally sound flushing agents for heavy metal polluted sites. For the purpose of industrial production of the sulphonated humic materials, the preference should be given to a use of leonardite humic material.

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6. References

1. Stevenson, F.I. (1982) Reactive Functional Groups of Humic Substances, in *Humic Substances Genesis, Isolation, Composition, Reactions*, Wiley, New York, pp. 221-267.
2. Varshal, G.M., Bugaevsky, A.A., Holin, Yu.V., Merny S.A., Veliukhanova, T.K., Kosheeva, I. and Krasovitsky, A.V. (1990) Modeling the equilibrium in solutions of fulvic acids in natural waters, *Chem. Technol. Water* **12**, 979-986 (in Russian).
3. Gerse, R., Csicsor, J. and Pinter, L. (1994) Application of humic acids and their derivatives in environmental pollution control, in *Humic Substances in the Global Environment and Implication for Human Health*, Elsevier Science B.V., pp. 1297-1303.
4. Scherer, M.M., Richer, S., Valentine, R.L. and Alvarez, P.J.J. (2000) Chemistry and microbiology of permeable reactive barriers for in situ groundwater clean up, *Critical Rev. Environ. Sci. Technol.* **30**, 363-411.
5. Gonzalez-Vila, F.J., Luedemann, H.-D. and Martin F. (1983) ¹³C-NMR structural features of soil humic acids and their methylated, hydrolyzed and extracted derivatives, *Geoderma* **31**, 3-15.
6. Leenheer J.A. and Noyes T.I. (1989) Derivatisation of humic substances for structural studies, in M.H.B. Hayes, P. MacCarthy, R.L. Malcolm, R.S. Swift (eds.), *Humic substances: in search of structure*, Wiley, New York, pp. 257-281.
7. Simpson, M. J., Chefetz, B. and Hatcher, P.G. (2003). Phenanthrene sorption to structurally modified humic acids, *J. Environ. Qual.* **32**, 1750-1758.
8. Chefetz, B., Salloum, M.J., Deshmukh, A.P. and G. Hatcher, P.G. (2002). Structural components of humic acids as determined by chemical modifications and carbon-13 NMR, pyrolysis-, and thermochemolysis- gas chromatography/mass spectrometry, *Soil Sci. Soc. Am. J.* **66**, 1159-1171.

9. Almendros G. and Dorado, J. (1999) Molecular characteristics related to the biodegradability of humic acid preparation, *Eur. J. Soil Sci.* **50**, 227-236.
10. U.S. Patent No. 3,028,333 issued April 3, 1962 to Charles A. Stratton et al.
11. U.S. Patent No. 3,309,958 issued June 19, 1962 to Kenneth P. Monroe.
12. U.S. Patent No. 3,190,837 issued June 22, 1965 to Joseph U. Messenger.
13. Canadian Pat. No. 722,720 issued November 30, 1965 to Speros E. Moschopedis.
14. U.S. Patent No 5,663,425 issued September 2, 1997 to Detroit, W.J., Lebo, Jr, Stuart, E., Bushar, L.L.
15. Zhambal, D. (1991) Composition and structural features of sulphonated humic acids, *Khimiya tverdogo topliva (Chemistry of Solid Fuel)* **2**, 70-72. (in Russian).
16. Rhee, D.S. and Jung, Y.-R. (2000) Characterization of humic acid in the chemical oxidation technology (II) Characterization by ozonation, *Analyt. Sci. Technol.* **13**, 241-249.
17. Sasina, V.N., Rumyancheva, Z.A. and Pevzner, Z.I. (1985) Water-soluble products of oxidation of brown coals and humic acids, *Chemistry of Solid Fuel* **3**, 30-36 (in Russian)
18. Shishkov, V.F., Verkhodanova, N.N., Egor'kov, A.N. and Tuturina, V.V. (1984) Ozonation of brown coals and humic acids, *Chemistry of Solid Fuel* **5**, 35-39 (in Russian).
19. Wang, G.S., Hsieh, S.T. and Hong, C.S. (2000) Destruction of humic acid in water by UV light-catalyzed oxidation with hydrogen peroxide, *Wat. Res.* **34(15)**, 3882-3887.
20. Aronov, S.G., Sklyar, M.G. and Tiutiunnikov, Yu.B. (1968) *Complex chemical technological treatment of coal*, Kiev, Tekhnika (in Russian).
21. Scherer, M.M., Richer, S., Valentine, R.L. and Alvarez, P.J.J. (2000) Chemistry and microbiology of permeable reactive barriers for in situ groundwater clean up, *Critical Rev. Environ. Sci. Technol.* **30**, 363-411.
22. Lowe, L.E. (1992) Studies on the nature of sulfur in peat humic acids from Fraser river delta, British Columbia, *Sci. Total Environ.* **113**, 133-145.
23. Perdue E.M. (1985) Acidic functional groups of humic substances, in Aiken, G.R., McKnight, D.M., Wershaw, R.L., MacCarthy, P. (eds.) *Humic substances in soil, sediment and water*, Wiley Interscience, New York, pp. 493-525.
24. Vavilin, D.V., Polynov, V.A., Matorin, D.N. and Venediktov, P.S. (1995) Sublethal concentrations of copper stimulate photosystem II photoinhibition in *Chlorella pyrenoidosa*, *J. Plant Physiol.* **146**, 609-614.
25. Tamiya, H.K., Morimura, K., Yokota, M. and Kunieda, R. (1961) The mode of nuclear division in synchronous culture of *Chlorella*: comparison of various methods of synchronization, *Plant Cell Physiol.* **2**, 383-403.
26. Perminova, I.V., Grechishcheva, N.Yu., Kovalevskii, D.V., Kudryavtsev, A.V., Matorin, D.N. and Petrosyan, V.S. (2001) Quantification and prediction of detoxifying properties of humic substances to polycyclic aromatic hydrocarbons related to chemical binding, *Environ. Sci. Technol.* **35**, 3841-3848.
27. Kumok, V.N. (1977) *Regularities in stability of complex compounds in solution*, Tomsk Univ. Publ., Tomsk (in Russian).
28. IUPAC Stability constants data base (computer edition) (1997-2000) IUPAC and Academic Software.
29. Davies, C.W. (1962) *Ion Association*, Butterworths, London.
30. Ramamoorthy, S. and Manning, P.G. (1973) Equilibrium studies of metal-ion complexes of interest to natural waters - VI. Simple and mixed complexes of Fe(III) involving NTA as primary ligand and a series of oxygen-bonding organic anions as secondary ligands, *Inorg. Nucl. Chem.* **35**, 1571-1575.
31. Pandeya, S.B. (1993) Ligand competition method for determining stability constants of fulvic acid iron complexes, *Geoderma* **58**, 219-231.
32. Varshal, G.M. and Buachidze, N.S. (1983). Investigation of coexisting forms of mercury(II) in surface waters, *J. Anal. Chem.* **38**, 2155-2167.
33. Perminova, I.V., Kulikova, N.A., Zhilin, D.M., Gretsichsheva, N.Y., Holodov, V.A., Lebedeva, G.F., Matorin, D.N., Venediktov, P.S. and Petrosyan, V.S. (2004) Mediating effects of humic substances in aquatic and soil environments, in Ph. Baveye (ed.), *Environmentally Acceptable Pollution and Reclamation Endpoints*, Kluwer Academic Publisher, NATO ASI - Series, Dordrecht (in Press).