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Quantification and Prediction of the Detoxifying Properties of Humic Substances Related to Their Chemical Binding to Polycyclic Aromatic Hydrocarbons

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Effects of 27 different humic materials on the toxicity of polycyclic aromatic hydrocarbons (PAH) were studied for crustacean *Daphnia magna*. Sources included isolated humic acids, fulvic acids, and their combination from soil, peat, and freshwater. The PAH used were pyrene, fluoranthene, and anthracene. The observed reduction in toxicity of PAH in the presence of humic substances (HS) was shown to be a result of the detoxification effect caused by the chemical binding of PAH to HS and of the direct effect of HS on *D. magna*. An approach was developed to quantify the detoxifying impact of humic materials related to their chemical binding to PAH with a use of the "constant of detoxification" or "toxicological partition coefficient" K_{oc}^{D}. The latter was proposed to determine by fitting the experimental relationships of the detoxification effect versus concentration of HS. The obtained K_{oc}^{D} values were well tracked by the corresponding partition coefficients determined by the fluorescence quenching technique (K_{oc}^{fq}): $K_{oc}^{D} = b \times K_{oc}^{fq}$, b (mean ± CI, $n = 26$, $P = 95\%$) = 2.6 ± 0.3, 4.6 ± 0.6, and 6.0 ± 1.4 for pyrene, fluoranthene, and anthracene, respectively. The predictive relationships between the structure and detoxifying properties of humic materials in relation to PAH were developed. It was shown that the magnitude of the K_{oc}^{D} values correlated closely with the aromaticity of humic materials characterized with the ^{13}C NMR descriptors (ΣC_{Ar}, $\Sigma C_{Ar}/\Sigma C_{Alk}$) and atomic H/C ratio. The obtained relationships showed the highest detoxifying potential of the humic materials enriched with aromatics and allowed a conclusion on the chemical binding as the governing mechanism of the mitigating action of HS on the toxicity of PAH.

Introduction

Polycyclic aromatic hydrocarbons (PAH) expose toxic, cancerogenic, and mutagenic effects on the living organisms (*1*). The adverse affects of PAH in the aquatic environments are mitigated via their binding to humic substances (HS). The latter causes a reduction in the concentration of freely dissolved PAH in the water that makes the contaminant less bioavailable for the aquatic biota. This has been shown by numerous studies on the bioaccumulation (*2*−*7*) as well as on toxicokinetics (*2, 8*−*10*) of PAH in the presence of HS. According to the reported data (*3, 11, 12*), the bioconcentration factor (BCF) in the presence of HS is directly proportional to the fraction of PAH freely dissolved in the water. The authors based the conclusion on the good match of the partition coefficients of PAH (K_{oc}) determined from the bioaccumulation data and measured by equilibrium dialysis. The uptake/depuration studies (*8, 9*) have also demonstrated a good correlation between the partition coefficients determined from chemical and biological data sets.

The similar studies on the toxicity of PAH are missing. In addition to our previous results which displayed the detoxifying effects of HS on phenanthrene and fluoranthene (*13*), the only estimates we are aware of concern a reduction in phototoxicity of anthracene (*14*) in the presence of HS. In contrast to bioaccumulation, determination of the partition coefficient from a reduction in toxicity of PAH can be hampered by the physiological activity of HS. The latter was numerously reported for the higher plants (*15, 16*). The corresponding studies on the aquatic biota are much more scarce (*17*−*20*) and report on the varying nature (stimulating as well as inhibiting) of the direct effects of HS upon different aquatic organisms. This prompts a development of the quantification approach to evaluate the mitigating impact of HS on the toxicity of PAH related to the chemical binding that would account for the direct effects of HS on the test organism. Using this approach, a predictive estimate of the detoxifying properties of HS in relation to PAH can be obtained independently on the specific response of the test organism to the presence of HS. This could facilitate a directed use of the humic materials for the purposes of remediation of the PAH contaminated media.

Our objectives were to (1) develop an approach to quantify the detoxifying properties of HS related to their chemical binding to PAH; (2) evaluate the detoxifying properties of 27 humic materials from freshwater, soil, and peat in relation to three different PAH (anthracene, fluoranthene and pyrene); and (3) establish quantitative relationships between the structure of humics and their detoxifying properties in relation to PAH.

Experimental Section

The PAH used were anthracene (Aldrich, 98+% pure), fluoranthene (Aldrich, 97% pure), and pyrene (Aldrich, 97% pure).

Preparation of Water Solutions of PAH. The batch technique described elsewhere (*21*) was used for preparation of water solutions of the selected PAH. PAH were dissolved in acetone and placed into a 1 L flask. The added amount of each PAH was below its water solubility. The acetone was then evaporated. One liter of water prepared for culturing the *Daphnia magna* (see below) was added into the flask and shaken overnight. The obtained PAH solutions were filtered through the precombusted glass fiber filters (GF/F, Whatman). The concentration of the PAH in the filtrates was determined with a use of laser fluorimetry. It was 1.7×10^{-7}, 7×10^{-7}, and 5×10^{-7}M for anthracene, fluoranthene, and pyrene, respectively. The prepared solutions were stored in the dark.

Humic materials (27 samples) used included humic acids (HA), fulvic acids (FA), and their nonfractionated mixtures

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TABLE 1. Molecular Parameters of Humic Substances Used in This Study

sample	source of HS	H/C[a]	O/C[a]	ABS_{280}[b]	M[c]	ΣC_{COO}[d]	ΣC_{Ar}[d]	ΣC_{Alk}[d]
			Aquatic Humic Substances (HA+FA)					
FMX	River Moscow	1.14	0.54	0.03	6.1	21	36	43
FMC	River Moscow	1.21	0.62	0.015	6.4	nd	nd	nd
WM3X	River North Dvina	1.41	1	0.035	6.6	nd	nd	nd
SWA	swamp water	0.86	0.93	0.025	9.8	nd	nd	nd
			Peat Humic Substances (HA+FA)					
T1	sphagnum-fuscum peat	1.01	0.48	0.066	18.5	16	39	45
T4	sphagnum peat	1.07	0.55	0.064	18.5	17	43	40
T5	sphagnum peat	0.98	0.54	0.064	16.4	17	37	46
T6	sedge peat	0.93	0.53	0.031	18.2	16	49	36
T7	woody peat	0.93	0.52	0.072	18.2	18	49	32
HTL	woody-herbaceous peat	0.89	0.49	0.046	17.3	17	45	37
TTL	woody-herbaceous peat	0.89	0.48	0.06	19.8	18	45	37
HTW	water extract of peat	1.21	0.62	0.02	6.3	11	16	73
			Soil Humic Acids (HA)					
HBW	sod-podzolic soil, forest	0.93	0.57	0.082	12.2	18	44	38
HBP	sod-podzolic soil, plough	0.86	0.39	0.071	16.1	18	44	38
HBG	sod-podzolic soil, garden	1.01	0.53	0.11	17.3	16	46	38
HGW	grey wooded soil, forest	0.97	0.78	0.075	16.4	20	47	33
HGP	grey wooded soil, plough	0.88	0.62	0.08	14.5	17	46	37
HS	meadow chernozem	0.62	0.39	0.068	12	16	57	28
HST	typical chernozem	0.51	0.48	0.114	12.6	18	54	28
			Soil Fulvic Acids (FA)					
FBW	sod-podzolic soil, forest	0.9	0.91	0.035	7.9	nd	nd	nd
FBP	sod-podzolic soil, plough	1.06	0.74	0.036	7.9	nd	nd	nd
FBG	sod-podzolic soil, garden	0.92	0.75	0.034	10.6	nd	nd	nd
FGW	grey wooded soil, forest	0.98	0.63	0.044	11	nd	nd	nd
FST	typical chernozem	0.81	0.64	0.054	9.6	nd	nd	nd
			Soil Humic Substances (HA+FA)					
SEL	typical chernozem	1.15	0.57	0.026	13.5	19	45	36
			Commercial Preparations					
AHA	Aldrich humic acid	0.74	0.28	0.045	13	16	56	28
AGK	coal humic acid	0.79	0.32	0.050	12.5	14	58	28

[a] H/C and O/C ratios are calculated on ash- and water-free basis. [b] Absorptivitiy values are listed in L/(mg C × cm). [c] Peak molecular weight values are determined by SEC (calibration by polydextranes) and listed in kDaltons. [d] Content of carbon in the structural fragments is determined by ^{13}C NMR spectroscopy as the integral intensity (%) of the following spectral regions, ppm: 220−185 (ΣC_{COO}), 185−108 (ΣC_{Ar}), and 108−5 (ΣC_{Alk}).

(HA+FA). They were isolated from different natural sources (freshwater, soil, and peat).

Aquatic humic substances (HA+FA) were isolated from the River Moscow (FMX), North Dvina (WM3X), and from the swamp water (SWA) using Amberlite XAD-2 resin as described elsewhere (*22*).

Peat humic substances (HA+FA) were isolated from seven peat samples of different geobotanical composition. The peat types were sphagnum-fuscum (T1), sphagnum (T4, T5), sedge (T6), woody (T7), and woody-herbaceous (HTL, TTL). The isolation procedure was as described elsewhere (*23*) and included a preliminary treatment of a peat sample with an ethanol−benzene (1:1) mixture followed up by an alkaline (0.1 M NaOH) extraction. One sample (HTW) was a concentrated water extract of woody-herbaceous peat HTL.

Soil humic acids (HA) were extracted from seven soils. These included sod-podsolic soils nearby Moscow (HBW, HBP, HBG), two gray wooded soils nearby Tula (*HGW*, *HGP*), and typical and meadow chernozemic soils (mollisols) nearby Voronezh (HST and HS, respectively). The HS extraction was carried out according to ref *24*. This included pretreatment of a soil sample with 0.1 M H_2SO_4, follow up alkaline extraction (0.1 M NaOH), and acidification of the extract to pH 1−2. The precipitated HA were desalted by dialysis.

Soil fulvic acids (FA) were extracted from five of the described above soils: sod-podzolic (FBW, FBP, FBG), gray wooded (FGW), and typical chernozem (mollisol) (FST). To isolate FA, after precipitation of HA, the supernatant was passed through Amberlite XAD-2 resin. Further treatment was as described for aquatic HS.

Nonfractionated soil HA+FA was isolated by alkaline extraction from typical chernozem (mollisol) nearby Stavropol (SEL).

Commercial Aldrich humic acid (AHA) and activated coal humic acid (AGK) (the latter is produced by the Biotechnology Ltd. Moscow, Russia) were used as obtained from the supplier.

Concentrated stock solutions of HS $(1-5) \times 10^{-4}$ kg C/L were prepared by evaporation of the corresponding cation-exchanged isolates or by a dissolution of a weight of a dried material. Content of organic carbon in the stock solutions was measured using a Shimadzu 5000 TOC analyzer as described elsewhere (*25*).

Chemical characteristics of the target humic materials and the corresponding determination techniques are described in details in our previous publication (*26*). The most important ones (contents of elements, molecular weight, molar absorptivity, content of aromatic carbon) are summarized in Table 1. Elemental analyses (C, H, N) were performed on a Carlo Erba Strumentazione elemental analyzer. S, H_2O, and ash contents were determined manually. Size exclusion chromatography analyses were performed according to ref *27* with a use of Toyopearl HW-50S resin (Japan) as a column packing, 0.028 M phosphate buffer as a mobile phase, and polydextrans as the calibrants. Peak molecular weight (*M*) was used for calculations. UV-absorbance was measured on solutions of HS in 0.028 M buffer at 280 nm in a 1-cm quartz cuvette and normalized to a concentration of HS in mg C/L to produce ABS_{280} values. ^{13}C NMR spectra were measured on solutions of humic

TABLE 2. Partition Coefficients of the Three PAH for Humic Substances Used in This Study Determined by Fluorescence Quenching Technique, $K_{oc}^{fq} \times 10^{-5}$, L/kg C (*26*)

sample	source of HS	pyrene	fluoranthene	anthracene
		Aquatic Humic Substances (HA+FA)		
FMX	River Moscow	0.7 ± 0.1[a]	0.5 ± 0.1	<0.1[b]
WM3X	River North Dvina	0.4 ± 0.1	0.2 ± 0.1	<0.1
SWA	swamp water	1.2 ± 0.2	0.9 ± 0.2	<0.1
		Peat Humic Substances (HA+FA)		
T1	sphagnum-fuscum peat	1.2 ± 0.2	0.9 ± 0.2	0.12 ± 0.05
T4	sphagnum peat	1.4 ± 0.1	0.9 ± 0.1	0.22 ± 0.05
T5	sphagnum peat	0.8 ± 0.2	0.6 ± 0.2	0.16 ± 0.03
T6	sedge peat	0.7 ± 0.1	0.7 ± 0.1	0.25 ± 0.07
T7	woody peat	1.7 ± 0.1	1.1 ± 0.2	0.6 ± 0.2
HTL	woody-herbaceous peat	1.4 ± 0.2	0.9 ± 0.2	0.5 ± 0.1
TTL	woody-herbaceous peat	1.0 ± 0.2	0.8 ± 0.1	<0.1
HTW	water extract of peat	<0.1	<0.1	<0.1
		Soil Humic Acids (HA)		
HBW	sod-podzolic soil, forest	1.0 ± 0.1	0.8 ± 0.1	<0.1
HBP	sod-podzolic soil, plough	1.2 ± 0.2	0.8 ± 0.1	0.5 ± 0.1
HBG	sod-podzolic soil, garden	0.7 ± 0.1	0.5 ± 0.1	<0.1
HGW	gray wooded soil, forest	1.4 ± 0.5	0.9 ± 0.2	0.5 ± 0.2
HGP	gray wooded soil, plough	1.8 ± 0.2	1.2 ± 0.1	0.7 ± 0.1
HS	meadow chernozem	2.2 ± 0.2	1.3 ± 0.2	1.0 ± 0.1
HST	typical chernozem	2.4 ± 0.3	1.6 ± 0.3	1.0 ± 0.4
		Soil Fulvic Acids (FA)		
FBW	sod-podzolic soil, forest	0.13 ± 0.08	<0.1	<0.1
FBP	sod-podzolic soil, plough	<0.1	<0.1	<0.1
FBG	sod-podzolic soil, garden	<0.1	<0.1	<0.1
FGW	gray wooded soil, forest	0.5 ± 0.1	0.3 ± 0.1	<0.1
FST	typical chernozem	1.1 ± 0.2	0.7 ± 0.1	<0.1
		Soil Humic Substances (HA+FA)		
SEL	typical chernozem	1.0 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
		Commercial Preparations		
AHA	Aldrich humic acid	2.3 ± 0.3	1.8 ± 0.2	1.0 ± 0.2

[a] ± value corresponds to a confidence interval of the slope of the Stern−Volmer plot for the corresponding HS sample at $n = 7$ and $P = 95\%$. [b] Value of $<0.1 \times 10^5$ corresponds to the minimum detectable K_{oc} value estimated in this study and is given for HS samples which did not cause fluorescence quenching of the PAH.

materials in 0.1 M $NaOD/D_2O$ on a Varian VXR-400 spectrometer operating at 100 MHz ^{13}C observation frequency. All the spectra were recorded at 4-s delay time using inverse gate decoupling. These conditions were shown to provide quantitative determination of carbon distribution among the main structural fragments of HS (*28*). The assignments of the total carbonylic, aromatic, and aliphatic carbon were as follows (in ppm): 5−98—aliphatic nonsubstituted and O-substituted C atoms (ΣC_{Alk}), 108−165—aromatic nonsubstituted and O-substituted C-atoms (ΣC_{Ar}), and 165−220—C atoms of carboxylic, esteric, and ketonic groups ($\Sigma C_{C=O}$).

Chemical binding of PAH to dissolved humic materials was characterized by partition coefficients determined by a fluorescence quenching technique as described elsewhere (*29*). The details of the experimental design are given in ref *26*. Shortly, the PAH solutions below the water solubility limit (0.6×10^{-7}, 1×10^{-7}, and 5×10^{-7} M for pyrene, fluoranthene, and anthracene, respectively) were prepared by solubilization technique. The concentration of HS was in the range of $(0.2-6) \times 10^{-6}$ kg C/L. The slopes of the obtained Stern−Volmer plots yielded the K_{oc}^{fq} values—partition coefficients, characterizing binding affinity of HS for PAH. The obtained K_{oc}^{fq} values for the target humic materials are summarized in Table 2.

Acute toxicity tests were performed according to the procedure described in refs *30*−*32*. Crustacean *Daphnia magna* was used as a test organism, its feeding activity (averaged filtration rate per dapnid, mL/h)—as a target response. *D. magna* were obtained from a stock maintained at the Division of Hydrobiology of Lomonosov Moscow State University. Daphnids were cultured at 20 °C with a light:dark rhythm of 16 h:8 h in the tap water which was previously filtered through activated charcoal and stored for 2 weeks under room conditions. Suspension of green microalgae *Chlorella vulgaris* was used to feed *D. magna* daily. For the toxicity tests, 5−6 day old animals were used.

Five to six daphnids were transferred into a 50 mL glass beaker containing 25 mL of the test solution. Three replicates were made for each assay. Water pH, checked at the beginning and the end of the test, was 7.5 ± 0.2 in all assays and the temperature 20 ± 2 °C. Daphnids in test solutions were kept for 24 h without feeding. The previous investigations *(30)* showed that 1 day starving increases the filtration rate of daphnids and improves its reproducibility ($s_r = 15-20\%$, $n = 3$). The animals were fed with a culture of algae *C. vulgaris* that was added into the test solution at the concentration of about 10^5 cells/L. The beakers were gently shaken in the middle and at the end of the feeding period to prevent the settling of algae.

Detoxification tests included measurements of the feeding activity of daphnids in the presence of the working concentration of PAH and varying concentration of HS. The working solutions of PAH were prepared at the maximum achievable concentrations of, nominally, 5×10^{-7}, 7×10^{-7}, and 1.7×10^{-7} M for pyrene, fluoranthene, and anthracene, respectively. Concentration of HS varied in the range from 1.5 till 25 mg C/L. Each experimental series included the following treatments: control-water, PAH solution at the working concentration, HS solutions at five different concentrations, solutions of PAH+HS at the working concentra-

tion of PAH, and five above concentrations of HS. Three replicates were made for each assay.

Data Treatment. The feeding activity (R) was determined by a decrease in the concentration of algae grazed out by daphnids. It was calculated via a ratio of the fluorescence intensity (F) of algal suspension at the start (F_0) and end point (F_t) of the feeding period. The latter was set at 1 h for all the tests. The F values were corrected for the background fluorescence of the components of the test solutions (F^{bg}). It was measured on each test solution before adding the algae. The ratio of the corrected F_0 and F_t values was further corrected on the change in the cell concentration due to the growth of algae during the feeding period. For this purpose the control was prepared at the same initial concentration of algae which was kept over the whole feeding period without daphnids. The fluorescence intensities of this solution measured at the start and end point of the feeding period were used for calculation of the correction coefficient b. Given the exponential character of cell concentration change due both to grazing out by daphnids and growth of algae (*30*−*32*), the feeding activity was calculated as follows

$$R = \frac{V}{nt} \times \ln\left(b \times \frac{F_0 - F^{bg}}{F_t - F^{bg}}\right) \quad (1)$$

where V is the volume of the test solution, mL; t is the duration of the feeding period, h; n is the number of species of *D. magna*; F^{bg} is the fluorescence intensity of the test solution without algae; F_0 and F_t are the fluorescence intensity of the test solution with the algae grazed out by the daphnids at the starting and end point of the feeding period, respectively; $b = F'_t - F^{bg}/F'_0 - F^{bg}$ is the correction coefficient on the change in fluorescence intensity during the feeding period due to the growth of algae, where F'_0 and F'_t is the fluorescence intensity of the algal suspension in the control-water without daphnids at the starting and end point of the feeding period, respectively.

The fluorescence intensity of algal chlorophyll was measured at 680 nm using the excitation wavelength of 450 nm. Given that the maximum of fluorescence spectra of the PAH used and HS lay in the UV and short visible region (350−450 nm), this resulted in the low magnitude of F^{bg} fluorescence at all the concentrations of the PAH and HS tested. As a rule, it did not exceed a small percentage of F_0 and F_t values. Of particular importance is that the inner filter effect (of HS at high concentrations, in particular), which can weaken the fluorescence intensity of algae, did not interfere with the measurements on feeding activity of daphnids. The latter is calculated as a ratio of the fluorescence intensities (F_0 and F_t); both of those were measured at the same concentration of the light absorbing compound in the solution. Hence, the attenuation factor is the same for both fluorescent measurements, and their ratio reflects a ratio of the fluorophor concentration in the corresponding solutions.

To check if the presence of HS or PAH in the test-solution influences the photosynthesis or growth of algae, the fluorescence intensity on the corresponding solutions and control-water with the added algae, but without daphnids (F'), was measured at $t = 0$ and 1 h. The ratios $(F'_i - F^{bg}/F'_0 - F^{bg})_{HS}$ and $(F'_t - F^{bg}/F'_0 - F^{bg})_{PAH}$ were compared to these of the control-water (correction coefficient b). There was no statistically significant differences ($P = 95\%$) found between the ratios calculated for the solutions of the model PAH, HS at different concentrations and control-water. The obtained values varied in the range of 1.05−1.1 (SD = 0.1 at $n = 3$). A lack of the direct impact of HS of different origin on the photosynthetic activity of *C. vulgaris* was also shown in our previous studies (*33*). This allowed to determine the correction factor b on the control-solution once for each batch of algae.

Quantification of the Detoxifying Properties of Humic Materials. Toxicity of the PAH (T_{PAH}) was estimated as a relative decrease in the feeding activity of daphnids in the presence of PAH

$$T_{PAH} = \frac{R_0 - R_{PAH}}{R_0} \quad (2)$$

where R_0 and R_{PAH} are the feeding activity of *D. magna* in control-water and in the presence of PAH, respectively.

In the concentration range of $(1-5) \times 10^{-7}$, $(0.5-7) \times 10^{-7}$, and $(0.2-1.7) \times 10^{-7}$ M used in the toxicity tests with pyrene, fluoranthene, and anthracene, respectively, the corresponding dose−response relationship could be fit satisfactorily by the following linear model

$$T_{PAH} = k \times C_{PAH} \quad (3)$$

where k is the slope of the dose−response relationship in the tested range of C_{PAH}. The slope k (mean ± SD, $n = 5$) was $(0.94 \pm 0.05) \times 10^8$, $(0.64 \pm 0.03) \times 10^8$, and $(3.3 \pm 0.2) \times 10^8$ M^{-1} for pyrene, fluoranthene, and anthracene, respectively. The corresponding r^2 were 0.96, 0.97, and 0.97.

Due to physiological activity of HS, the reduction in toxicity of PAH in the presence of HS reflects a combined action of two effects—*the sequestration* of toxicity caused by a reduction in the freely dissolved PAH due to their binding to HS and *the direct effect* of HS on the test organism. To estimate a sequestration of toxicity, or detoxification effect, the toxicity in the presence of HS (T_{PAH+HS}) was related to the response of *D. magna* in the presence of HS alone instead of the HS- and PAH-free control. The corresponding expression is

$$T_{PAH+HS} = \frac{R_{HS} - R_{PAH+HS}}{R_{HS}} \quad (4)$$

where R_{HS} is the feeding activity of *D. magna* in the test solution containing HS alone and R_{PAH+HS} is the feeding activity of *D. magna* in the test solution containing HS and PAH.

Then, the sequestration of the initial toxicity, or detoxification effect, D can be calculated as follows:

$$D = \frac{T_{PAH} - T_{PAH+HS}}{T_{PAH}} \quad (5)$$

Taking into consideration the linearity of the dose−response relationships obtained for the model PAH in the range of concentrations studied (eq 3), the above expression for D can be rewritten as

$$D = 1 - \alpha \quad (6)$$

where $\alpha = [PAH]/C_{PAH}$ is the portion of the freely dissolved PAH in the presence of HS and [PAH] is the equilibrium concentration of the freely dissolved PAH.

Consequently, the dependence of D on total concentration of HS (C_{HS}) in the test system ("detoxification curve") can be described via partition coefficient or detoxification constant K_{oc}^D similarly to chemical binding:

$$D = \frac{K_{oc}^D \times C_{HS}}{1 + K_{oc}^D \times C_{HS}} \quad (7)$$

In practice, the K_{oc}^D values were calculated by fitting the experimental D versus C_{HS} relationships with a use of the nonlinear regression procedure.

Results and Discussion

Detoxification Partition Coefficients. The typical dependencies of the toxicity of PAH upon the concentration of HS

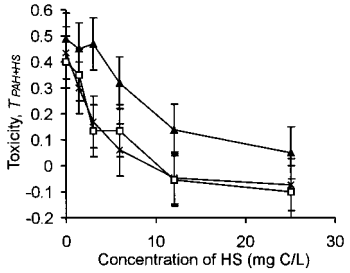

FIGURE 1. The typical relationships of the toxicity of the three model PAH determined according to eq 4 versus concentration of HS present in the test system (on the example of T6). Bars represent ± SD ($n = 3$): × pyrene, □ fluoranthene, and ▲ anthracene.

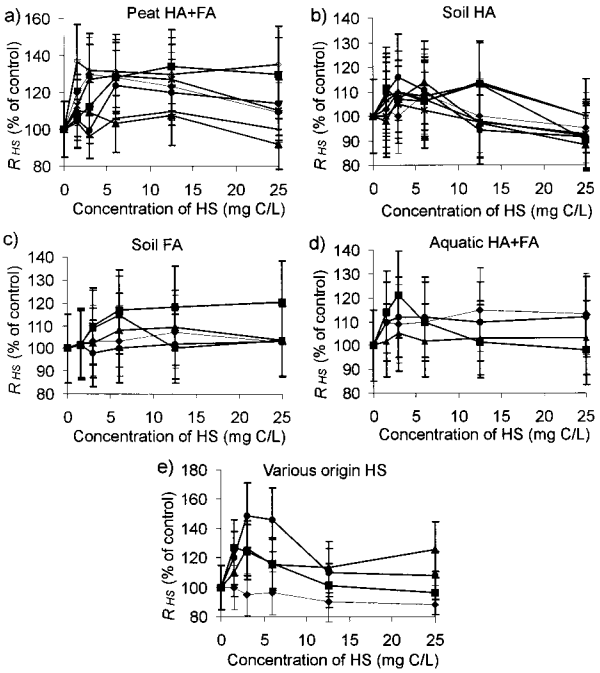

FIGURE 2. The typical relationships of the feeding activity of *D. magna* versus concentration of HS of different origin. Bars represent ± SD ($n = 3$); (a) peat HA+FA: ◆ T7, ■ TTL, ▲ T6, ○ T5, × T1, ◇ T4, + HTL; (b) soil HA: ◆ HS, ■ HBP, ▲ HST, ○ HGP, △ HBG, ◇ HBW, + HGW; (c) soil FA: ◆ FGW, ■ FST, ▲ FBW, ○ FBP, □ FBG; (d) aquatic HA+FA, ◆ WM3X, ■ SWA, ○ FMX, ▲ FMC; and (e) various origin HS: ◆ AGK, ■ AHA, ○ HTW, ▲ SEL.

present in the system are given in Figure 1. The toxicity decreases with an increase in a concentration of HS. This effect has been observed for all the humic materials used, except for soil FA that did not cause any influence on the toxicity of PAH.

The relationships of the feeding activity of *D. magna* versus concentration of HS are given in Figure 2a−e for all the target humic materials grouped by the origin. In general, HS in the range of concentration from 1.5 until 6 mg C/L caused a predominantly stimulating effect on *D. magna*. The biggest stimulating effect (up to 140% of control) was observed for peat HA+FA (Figure 2a). Soil HA and FA (Figures 2b,c) caused a much less stimulating effect (up to 120% of control). Aquatic HA+FA showed quite diverse effects (Figure 2d). While XAD-isolates (FMX and WM3X) caused the stimulation effect of 120%, DEAE-cellulose isolate (FMC) did not cause any effect. No stimulating effect was displayed by commercial coal HA (AGK), while Aldrich HA stimulated *D. magna* up to concentration of 6 mg C/L. The further increase in concentration of Aldrich HA lead to a decrease in the feeding activity of *D. magna* until control (Figure 2e). The obtained results corroborate well the findings of Petersson and Persson (*18*)

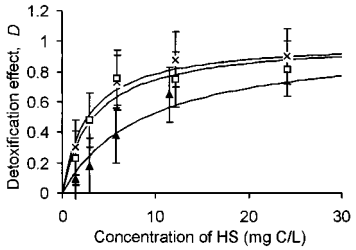

FIGURE 3. The typical relationships of the detoxification coefficient *D versus* concentration of HS in the test system (on the example of T6) for the three model PAH: × pyrene, □ fluoranthene, and ▲ anthracene. Bars represent ± SD ($n = 3$).

who reported the beneficial effect of Aldrich HA on *D. magna* up to 10 mg C/L, whereas 22 mg C/L dropped the beneficial effect up to 0.

The obtained data allowed for one to account for the direct effect of HS on the test organism while estimating the true sequestration of toxicity of PAH as a result of their chemical binding to HS. For this purpose the detoxification effect D was calculated according to eq 5. The D values plotted against the concentrations of HS yielded the detoxification curves of the PAH. The typical examples for pyrene, fluoranthene, and anthracene are given in Figure 3. By the solid lines the best fits are shown corresponding to the K_{oc}^{D} values calculated by eq 7. The determined K_{oc}^{D} values and r^2 of the corresponding fits are summarized in Table 3. The comparable magnitudes of K_{oc}^{D} values were obtained for pyrene and fluoranthene; the lower ones were observed for anthracene. This can be related to the lower hydrophobicity of anthracene ($\log K_{ow} = 4.45$) in comparison with pyrene and fluoranthene ($\log K_{ow} = 4.88$ and 5.16, respectively) (*34*). The similar trends have been previously reported for the bioconcentration of the PAH in the presence of HS as well (*2, 7, 9, 12*).

Based on the determined K_{oc}^{D} values, the target humic materials can be arranged in the following descending order: coal HA, chernozemic HA ≫ sod-podzolic and gray wooded soil HA > peat (HA+FA) > aquatic (HA+FA) ≅ chernozemic FA ≫ sod-podzolic and gray wooded soil FA. The above trend with the source of HS is in agreement with that reported in the literature for the bioaccumulation of PAH (*8, 35, 36*) as well as with the trend in K_{oc}^{fq} values obtained by the fluorescence quenching technique (Table 2) for the same humic materials. However, in general, the magnitude of K_{oc}^{D} was a factor of 2−6-fold higher than that of K_{oc}^{fq} for the same humic material.

The scatter plots of K_{oc}^{D} versus K_{oc}^{fq} are given in Figure 4. The use of t-statistics for a comparison of the two means under study is feasible because of a significant difference in the absolute values of their standard deviations (the average ratio accounted for 20) (*37*). The plots yielded rather strong positive correlation ($r^2 = 0.74$, 0.72, and 0.58 for pyrene, fluoranthene, and anthracene, respectively). The intercepts of the regression lines were equal to zero and the slopes—significantly different from one (mean ± CI, $P = 95\%$, $n = 26$):

$$\text{pyrene: } K_{oc}^{D} = (2.6 \pm 0.3) \times K_{oc}^{fq} \quad (8)$$

$$\text{fluoranthene: } K_{oc}^{D} = (4.6 \pm 0.6) \times K_{oc}^{fq} \quad (9)$$

$$\text{anthracene: } K_{oc}^{D} = (6.0 \pm 1.4) \times K_{oc}^{fq} \quad (10)$$

Thus, K_{oc}^{fq} accounts for about 74, 72, and 58% of the variability in the K_{oc}^{D} of the dissolved humic materials to pyrene, fluoranthene, and anthracene, respectively. However, the slopes of the regression lines indicate a constant bias

TABLE 3. Detoxification Constants of Humic Substances Used in This Study for the Three PAH Determined from Toxicological Data, $K_{oc}^{D} \times 10^{-5}$ L/kg C

sample	source of HS	pyrene	fluoranthene	anthracene
		Aquatic Humic Substances (HA+FA)		
FMX	River Moscow	2.8 ± 1.1[a] (0.91[b])	2.3 ± 0.5 (0.96)	<0.3[c]
FMC	River Moscow	1.1 ± 0.3 (0.91)	0.4 ± 0.1 (0.96)	<0.3
WM3X	River North Dvina	1.9 ± 0.5 (0.93)	2.5 ± 0.5 (0.88)	<0.3
SWA	swamp water	1.9 ± 0.7 (0.98)	2 ± 0.6 (0.91)	<0.3
		Peat Humic Substances (HA+FA)		
T1	sphagnum-fuscum peat	3.9 ± 1.1 (0.86)	3.4 ± 1.2 (0.89)	nd
T4	sphagnum peat	3.7 ± 0.6 (0.81)	4.7 ± 1 (0.88)	1.2 ± 0.5 (0.88)
T5	sphagnum peat	3.7 ± 0.8 (0.91)	3.4 ± 2.5 (0.98)	1.0 ± 0.2 (0.91)
T6	sedge peat	3.7 ± 0.6 (0.96)	3.0 ± 0.8 (0.91)	1.3 ± 0.6 (0.86)
T7	woody peat	4.3 ± 2.6 (0.96)	5.9 ± 0.3 (0.96)	nd
HTL	woody-herbaceous peat	3.6 ± 1.7 (0.98)	3.9 ± 0.8 (0.92)	1.8 ± 0.5 (0.87)
TTL	woody-herbaceous peat	4.2 ± 2.7 (0.97)	4.0 ± 1 (0.96)	0.3 ± 0.1 (0.96)
HTW	water extract of peat	0.8 ± 0.2 (0.95)	0.3 ± 0.1 (0.97)	<0.3
		Soil Humic Acids (HA)		
HBW	sod-podzolic soil, forest	3.9 ± 1.4 (0.95)	4.5 ± 1.3 (0.95)	5.9 ± 2.4 (0.91)
HBP	sod-podzolic soil, plough	3.3 ± 0.8 (0.92)	3.8 ± 1.5 (0.86)	3.9 ± 1.3 (0.95)
HBG	sod-podzolic soil, garden	2.1 ± 0.5 (0.90)	2.1 ± 0.2 (0.93)	1.9 ± 0.6 (0.86)
HGW	gray wooded soil, forest	3.6 ± 1.3 (0.91)	3.8 ± 2.2 (0.88)	3.9 ± 1.1 (0.88)
HGP	gray wooded soil, plough	4.1 ± 0.8 (0.96)	4.7 ± 0.8 (0.90)	3.8 ± 1.2 (0.93)
HS	meadow chernozem	6.0 ± 3.1 (0.96)	8.6 ± 4.8 (0.95)	5.6 ± 2.4 (0.88)
HST	typical chernozem	5.1 ± 1.6 (0.86)	6.0 ± 3.5 (0.91)	5.6 ± 2.5 (0.88)
		Soil Fulvic Acids (FA)		
FBW	sod-podzolic soil, forest	<0.3	<0.3	<0.3
FBP	sod-podzolic soil, plough	<0.3	<0.3	<0.3
FBG	sod-podzolic soil, garden	<0.3	<0.3	<0.3
FGW	gray wooded soil, forest	<0.3	<0.3	<0.3
FST	typical chernozem	3.0 ± 0.8 (0.89)	2.2 ± 0.6 (0.94)	<0.3
		Soil Humic Substances (HA+FA)		
SEL	typical chernozem	3.6 ± 0.6 (0.91)	3.4 ± 0.4 (0.93)	2.0 ± 0.5 (0.98)
		Commercial Preparations		
AHA	Aldrich humic acid	5.8 ± 3.4 (0.84)	5.8 ± 2.8 (0.94)	5.1 ± 1.3 (0.91)
AGK	coal humic acid	5.5 ± 0.6 (0.97)	6.7 ± 0.8 (0.87)	nd

[a] ± SD. [b]

$$r^2 = 1 - \frac{\sum_{i=1}^{n}(D_i - D_i^*)^2}{\sum_{i=1}^{n}(D_i - \bar{D})^2}$$

where r^2 is the portion of explained variance, D_i is the experimental value, D_i^* is the calculated value, D is the mean experimental value, and n is a number of experimental points. [c] $<0.3 \times 10^5$ corresponds to the minimum detectable K_{oc}^{D} value estimated as a 3-fold standard deviation of the lowest determined K_{oc}^{D} value (*37*). The latter was $(0.3 \pm 0.1) \times 10^5$ L/kg C (±SD, $n = 3$) for fluoranthene and HTW. This yields a value of K_{oc}^{D}(min) of 0.3×10^5 L/kg C.

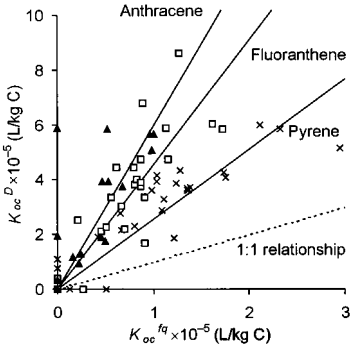

FIGURE 4. The partition coefficients determined from the toxicity data (K_{oc}^{D}) are directly proportional to those determined by fluorescence quenching technique (K_{oc}^{fq}). The constant bias between the values apparently results from the different conditions (aqueous chemistry) of their determination: × pyrene, □ fluoranthene, and ▲ anthracene.

between the partition coefficients estimated by the two techniques used.

The observed bias between the K_{oc}^{fq} and K_{oc}^{D} values, both of those are the *conditional constants*, can be prescribed to a systematic error resulting from the differences in conditions of their determination. The most probable reason is that the fluorescence quenching determinations were conducted on the distilled water, whereas the toxicity tests—on the filtered tap water. To test this hypothesis, few determinations of the K_{oc}^{fq} were conducted on the tap water on the example of anthracene and *T6*. The obtained data yielded a mean value of K_{oc}^{fq} of 0.8×10^5 L/kg C. It is a factor of approximately 3−4-fold higher than 0.25×10^5 L/kg C determined in the distilled water and is much closer to the mean value of K_{oc}^{D} of 1.3×10^5 L/kg C. The distilled water differs from the tap water in pH (5.5 and 7, respectively) and in the ionic strength ($I = 0$ and ~4 mM, respectively).

Our previous investigations on the influence of pH and ionic strength on the K_{oc}^{fq} values (*38*) conducted for the same model PAH showed that the variations in pH from 5 till 8 did not effect substantially the K_{oc}^{fq} value. The remarkable increase in K_{oc}^{fq} (of a factor of 2−3-fold) was observed only at pH 2. At the same time, a reduction in K_{oc}^{fq} value of a factor of 2−3-fold was detected along with an

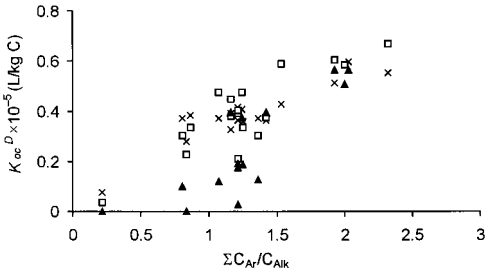

FIGURE 5. Correlation between the $K_{oc}{}^{D}$ values and aromaticity of humic materials expressed as $\Sigma C_{Ar}/\Sigma C_{Alk}$: × pyrene, □ fluoranthene, and ▲ anthracene.

increase in ionic strength up to 10^{-2} M. This suggests that neither differences in pH nor ionic strength itself could cause the observed bias between the $K_{oc}{}^{fq}$ and $K_{oc}{}^{D}$. However, the ionic strength in the tap water is provided mostly by the divalent metal ions—Ca^{2+} and Mg^{2+}, while in our experiments (*38*) NaCl was used for this purpose. According to the findings of Schlautman and Morgan (*39*), the presence of 1 mM Ca^{2+} at 0.1 M total ionic strength and pH 7 caused a substantial increase (30%) in the $K_{oc}{}^{fq}$ values of pyrene and anthracene. This can explain the higher values of $K_{oc}{}^{fq}$ and $K_{oc}{}^{D}$ obtained on the tap water in comparison with the $K_{oc}{}^{fq}$ determined on the distilled water. Hence, the detoxification effect described by the $K_{oc}{}^{D}$ partition coefficient is provided mostly by the chemical binding of PAH to HS. To facilitate the predictive estimates of the detoxifying properties of HS in relation to PAH, the relationships between the structure and detoxification partition coefficient were established.

Relationships between Structure and Detoxifying Properties of HS to PAH. The quantitative relationships between the structure of HS and detoxification partition coefficients $K_{oc}{}^{D}$ were derived using the molecular descriptors of the target humic materials given in Table 1. The ratio of aromatic to aliphatic carbon content reflecting the prevalence of aromatic core over aliphatic periphery or lipophilic-lipophobic balance of the molecule (indicated by the $\Sigma C_{Ar}/\Sigma C_{Alk}$ ratio) was also used for correlation with $K_{oc}{}^{D}$ values. Its use resulted in the best fits to the experimental $K_{oc}{}^{D}$ values shown in Figure 5. The corresponding regression equations are given below:

$$\text{pyrene } K_{oc}{}^{D} \times 10^{-5} = (2.2 \pm 0.3) \times \Sigma C_{Ar}/\Sigma C_{Alk} + (1.0 \pm 0.4) \quad r^2 = 0.76 \quad (11)$$

$$\text{fluoranthene } K_{oc}{}^{D} \times 10^{-5} = (3.2 \pm 0.4) \times \Sigma C_{Ar}/\Sigma C_{Alk} - (0.01 \pm 0.6) \quad r^2 = 0.75 \quad (12)$$

$$\text{anthracene } K_{oc}{}^{D} \times 10^{-5} = (3.5 \pm 0.6) \times \Sigma C_{Ar}/\Sigma C_{Alk} - (2.0 \pm 0.8) \quad r^2 = 0.7 \quad (13)$$

The values of given confidence intervals of the slopes ($n = 19$ for pyrene and fluoranthene and $n = 17$ for anthracene, P = 95%) demonstrate a statistical relevance to the observed trend between $K_{oc}{}^{D}$ value and $\Sigma C_{Ar}/\Sigma C_{Alk}$ ratio. The same is true for the other parameters of aromaticity of HS — ΣC_{Ar}. This can indicate a key role of interactions between aromatic core of HS and PAH for detoxification effect under study. These results are consistent with our previous findings (*26*) and reported in the literature (*8*, *12*) correlating the binding affinity of PAH for HS (K_{oc} values) with the percentage of aromatic carbon in the humic material.

Correlations between $K_{oc}{}^{D}$ and atomic H/C ratio (Table 1) is poorer than those for ^{13}C NMR descriptors. However, they remain significant at $P = 95\%$. The corresponding r^2 values account for 0.56, 0.49, and 0.67 for pyrene, fluoranthene, and anthracene, respectively. Much poorer correlation was observed for another indirect estimate of the aromaticity—molar absorptivity (ABS_{280}). No correlation was found with the peak molecular weight of humic materials ($r^2 =$ 0.011, 0.012, and 0.001).

Environmental Implications. The obtained structure—property relationships point out that the aromatics enriched humic materials are the most efficient detoxifying agents in relation to PAH. The most abundant source of such material is brown coal which contains up to 70—80% of humic acids. The humates isolated from brown coal can be widely used for the remediation of the PAH contaminated media. Given that the governing mechanism of interaction between HS and PAH is hydrophobic binding, the similar action of HS on the other hydrophobic organic compounds (petroleum or polychlorinated hydrocarbons) can be expected. The advantage of humates application as detoxifying agents for the remediation purposes is a lack of danger of the secondary pollution of the contaminated sites. Another advantage is a combinatory action of humates, that is, they not only expose a detoxifying impact on the hydrophobic contaminants but also can impose a direct beneficial effect on the biotic community at the contaminated site. The quantification approach to detoxifying properties of humic material and the obtained structure—property relationships facilitate an effective use of humates as detoxifying agents toward PAH as well as to the other contaminants.

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Literature Cited

(1) Neff, J. M. *Polycyclic Aromatic Hydrocarbons in the Aquatic Environment*; Applied Science Publishers Ltd: London, U.K., 1979.
(2) McCarthy, J. F.; Jimenez, B. D. *Environ. Toxicol. Chem.* **1985**, *4*, 511—521.
(3) Kukkonen, J.; McCarthy, J. F.; Oikari, A. *Arch. Environ. Contam. Toxicol.*, **1990**, *19*, 551—557.
(4) Kukkonen, J.; Oikari, A. *Sci. Total Environ.* **1987**, *62*, 399—402.
(5) Oikari, A.; Kukkonen, J. *Bull. Environ. Contam. Toxicol.* **1990**, *45*, 54—61.
(6) Leversee, G. J.; Landrum, J. P.; Fannin, T. *Can. J. Fish. Aquat. Sci.* **1983**, *40*, 63—69.
(7) Haitzer, M.; Hoess, S.; Transpurger, W.; Steinberg, C. *Environ. Toxicol. Chem.* **1999**, in press.
(8) Landrum, P. F.; Reinhold, M. D.; Nihart, S. R.; Eadie, B. J. *Environ. Toxicol. Chem.* **1985**, *6*, 11—20.
(9) Landrum, P. F.; Sheila, R. N.; Eadie, B. J.; Herche, L. R. *Environ. Toxicol. Chem.* **1987**, *6*, 11—20.
(10) Black, M. C.; McCarthy, J. F. *Environ. Toxicol. Chem.* **1988**, *7*, 593—600.
(11) McCarthy, J. F.; Jimenez, B. D.; Barbee, T. *Aquat. Toxicol.* **1985**, *7*, 15—24.
(12) Kukkonen, J.; Oikari, A. *Water Res.* **1991**, *25*, 455—463.
(13) Perminova, I. V.; Kovalevsky, D. V.; Yashchenko, N. Yu.; Danchenko, N. N.; Kudryavtsev, A. V.; et al. In *Humic Substances and Organic Matter in Soil and Water Environments: Characterization, Transformations and Interactions;* Clapp C. E., Hayes M. H. B., Eds.; IHSS, Inc.: St. Paul, MN, 1996; pp 398—406.
(14) Oris, J. T.; Hall, A. T.; Tylka, J. D. *Environ. Toxicol. Chem.* **1990**, *9*, 575—583.
(15) Batalkin, G. A.; Galushko, A. M.; Makhno, L. Yu.; Khristeva, L. A. In *Proceedings of the IVth International Symposium on "Peat: Properties and Perspectives of Application"*; Minsk, USSR, 1982; pp 115—117 (in Russian).
(16) Visser, S. A. In *Humic Substances. Effects on Soil and Plants;* Visser S. A., Ed., REDA: Rome, 1986; pp 89—135.

(17) Hargeby, A.; Petersen, R. C. *Fresh. Biol.* **1988**, *19*, 235−247.
(18) Petersen, R. C.; Persson, U. *Sci. Total Environ.* **1987**, *62*, 387−398.
(19) Petersen, R. C. In *Humic Substances in the Aquatic and Terrestrial Environment*; Allard, B., Ed.; Springer-Verlag: Berlin, Heidelberg, 1991; pp 369−389.
(20) Tulonen, T.; Salonen, K.; Arvola, L. *Hydrobiol.* **1992**, *229*, 239−252.
(21) Hashimoto, Y.; Tokura, K.; Ozaki, K.; Strachan, W. M. J. *Chemosphere* **1982**, *11*, 991−1001.
(22) Mantoura, R. F. C.; Riley, J. P. *Anal. Chim. Acta* **1975**, *76*, 97−106.
(23) Lowe, L. E. *Sci. Total Environ.* **1992**, *113*, 133−145.
(24) Orlov, D. S.; Grishina, L. A. *Handbook of humus chemistry*; Moscow State University Publisher: Moscow, 1981 (in Russian).
(25) Sugimura, Y.; Suzuki, Y. *Mar. Chem.* **1988**, *24*, 105−131.
(26) Perminova, I. V.; Grechishcheva, N. Yu.; Petrosyan, V. S. *Environ. Sci. Technol.* **1999**, *33*, 3781−3787.
(27) Perminova, I. V.; Frimmel, F. H.; Kovalevskii, D. V.; Abbt-Braun, G.; Kudryavtsev, A. V.; Hesse, S. *Water Res.* **1998**, *32*, 872−881.
(28) Kovalevskii, D. V.; Permin, A. B.; Perminova, I. V.; Petrosyan, V. S. *Moscow State University Bulletin* (*Vestnik MGU) Series 2 (Chemistry)* **2000**, *41*, 39−42.
(29) Gauthier, T. D.; Shane, E. C.; Guerin, W. F. *Environ. Sci. Technol.* **1986**, *20*, 1162−1166.
(30) Matorin, D. N.; Vavilin, D. V.; Venediktov, P. S. *Biol. Sci. (USSR)* **1990**, *1*, 146−151 (in Russian).
(31) Matorin, D. N.; Vavilin, D. V.; Popov, I. V.; Venediktov, P. S. In *Methods of Biotesting of Aquatic Media Quality;* Filenko O. F., Ed.; Moscow State University Publisher: Moscow, 1989; pp 10−21 (in Russian).
(32) Polynov, V. A. Ph.D. Thesis. Lomonosov Moscow State University, 1992; Moscow (in Russian).
(33) Perminova, I. V.; Kulikova, N. A.; Lebedeva, G. F. In *Proceedings of the 10th Meeting of IHSS, 24−28 July, Toulouse, France*; 2000; pp 112−115.
(34) Hansch, C.; Leo A.; Hoekman, D. *Exploring QSAR Hydrophobic, Electronic, and Steric Constants*; American Chemical Society: Washington, DC, 1995.
(35) Kukkonen, J.; Oikari, A.; Johnsen, S.; Gjessing, E. T. *Sci. Total Environ.* **1989**, *79*, 197−207.
(36) McCarthy, J. F. In *Aquatic Humic Substances: Influence on Fate and Treatment of Pollutants*; Suffet I. H., MacCarthy P., Eds.; American Chemical Society: Washington, DC, 1989; pp 263−277.
(37) Doerffel, K. *Statistik in der analytischen Chemie*; Deutscher Verlag fuer Grundstoffindustrie GmbH: Leipzig, Germany, 1990.
(38) Yashchenko, N. Yu.; Perminova, I. V.; Petrosyan, V. S.; Filippova, E. M.; Fadeev, V. V. *Moscow State University Bulletin (Vestnik MGU), Series 2 (Chemistry)* **1999**, *40*, 188−193.
(39) Schlautman, M. A.; Morgan, J. J. *Environ. Sci. Technol.* **1993**, *27*, 961−969.

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