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Relationships between Structure and Binding Affinity of Humic Substances for Polycyclic Aromatic Hydrocarbons: Relevance of Molecular Descriptors

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Partition coefficients for the binding affinities of pyrene, fluoranthene, and anthracene to 26 different humic materials were determined by fluorescence quenching. Sources included isolated humic acids, fulvic acids, and combined humic and fulvic fractions from soil, peat, and freshwater as well as Aldrich humic acid. Each of the humic materials was characterized by elemental composition, ultraviolet absorbance at 280 nm, molecular weight, and for 19 samples, composition of main structural fragments determined by ^{13}C solution-state NMR. The magnitude of the K_{oc} values correlated strongly with the independent descriptors of aromaticity of humic materials, including atomic H/C ratio, absorptivity at 280 nm, and three interdependent ^{13}C NMR descriptors ($C_{Ar-H,R}$, ΣC_{Ar}, $\Sigma C_{Ar}/\Sigma C_{Alk}$). Statistical comparison of humic sources grouped by the origin revealed that binding affinities were best predicted by the ^{13}C NMR descriptors, with a slight prevalence of $\Sigma C_{Ar}/\Sigma C_{Alk}$ ratio, while molecular weight was the poorest predictor. The latter produced either direct or inverse significant correlation with the K_{oc} values depending upon the origin and/or fractional composition of the grouped humic materials.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) belong to one of the most hazardous classes of hydrophobic organic contaminants. The fate of PAHs released into the environment is affected by humic substances (HS), which comprise from 50 to 80% of natural organic matter in water and soil ecosystems (*1, 2*). The binding to HS can result in increased contaminant mobility (*3, 4*) and a decrease in bioavailability and toxicity of PAHs (*5*−*7*).

In turn, the binding affinity of HS for PAHs (K_{oc}) has been related to the composition and structure of HS (*8*−*12*). The simplest constitutional descriptors (*13*) such as the content of aromatic carbon, H/C and O/C atomic ratios, molar absorptivity, and molecular weight have been used for deriving the corresponding correlation relationships (*8*−*12*). However, the predictive power of these molecular descriptors varied greatly among the different studies. This variability could result from differences in the amount, type (isolated humic fractions or unfractionated dissolved organic matter of natural water), and source of target materials used for deriving quantitative relationships.

To address this problem, we have derived structure-PAH binding affinity relationships for a larger set of humic materials of different origin (26 samples, compared to the 5−17 sources used in previous studies; *10, 12*) encompassing three different PAHs—anthracene, fluoranthene, and pyrene. We have broadened the range of molecular descriptors to include more specific structural descriptors derived from ^{13}C NMR data. The latter provide the most reliable information on the structure of such a complex matter as HS (*14*). We have examined a relevance of the molecular descriptors for prediction of K_{oc} by deriving the correlation relationships for the different subsets of the target humic materials.

Our objectives were to (1) measure K_{oc} of three different PAHs to a variety of humic sources, (2) derive the correlation relationships between the PAH binding affinities and molecular descriptors of HS, and (3) compare significance of the relationships obtained for different subsets of the target humic materials.

Materials and Methods

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

Humic materials (humic acids (HA), fulvic acids (FA), and a combination of HA and FA (HA + FA)) used were isolated from different natural sources (freshwater, soil, and peat, Table 1).

Aquatic HA + FA were isolated from the River Moscow (FMX) and North Dvina (WM3X) and from swamp water (SWA) using Amberlite XAD-2 resin as described elsewhere (*15*).

Peat 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). Isolation procedure was as described elsewhere (*16*) 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.

Soil HA were extracted from eight soils. These included sod-podsolic soils nearby Moscow (HBW, HBP, HBG) and Novgorod (HBWN), two grey wooded soils nearby Tula (HGW, HGP), and typical and meadow chernozemic soils nearby Voronezh (HST and HS, respectively). The HA extraction was carried out according to Orlov and Grishina (*17*). 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 FA were extracted from five out of the eight described above soils: three sod-podzolic soils nearby Moscow (FBW, FBP, FBG), grey wooded soil (FGW), and typical chernozem (FST). To isolate FA, the supernatant after precipitation of HA was passed through Amberlite XAD-2 resin. Further treatment was as described elsewhere (*15*).

Soil HA + FA (SEL) was isolated by alkaline extraction (0.1 M NaOH) from typical Chernozem nearby Stavropol. The extract was desalted by cation-exchanging.

Commercial Aldrich humic acid (AHA) was used as purchased from the supplier.

Concentrated stock solutions of humic materials (100−500 mg of organic carbon (OC)/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 (*18*).

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TABLE 1. Partition Coefficients of the Three PAHs for Humic Substances Used in This Study, $K_{Oc} \times 10^{-5}$, L/kg C

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				
HBW	sod-podzolic soil, *forest*	1.0 ± 0.1	0.8 ± 0.1	<0.1
HBWN	sod-podzolic soil, *forest*	1.3 ± 0.1	0.8 ± 0.1	0.5 ± 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				
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	grey 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 Preparation				
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 PAHs.

Elemental analyses (C, H, N) were performed on a Carlo Erba Strumentazione elemental analyzer. S, H_2O, and ash contents were determined manually. Oxygen contents were calculated as a difference. The H/C and O/C atomic ratios were derived from the contents of the elements calculated on ash- and water-free basis.

Size exclusion chromatography *(*SEC*)* analysis was performed at the facilities of the Division of Water Chemistry, Engler-Bunte Institute, University of Karlsruhe, Germany, according to (*19*). Toyopearl HW-50S resin (Japan) was used as a column packing. Polydextranes were used for calibration. HS solution was equilibrated with the SEC mobile phase (0.028 M phosphate buffer) prior to the analysis. A value of peak molecular weight (M) of HS was used for correlations with K_{oc} coefficients.

UV-absorbance data were recorded on a Varian DMS100-S spectrophotometer. Absorbance of an HS solution was measured at 280 nm in a 1-cm quartz cuvette. All the measurements were made in 0.028 M phosphate buffer. Absorbance values were normalized to a concentration of HS in milligrams of C/L to produce an ABS_{280} value used for correlations with K_{oc} coefficients.

^{13}C solution-state NMR spectra of 19 HS samples were measured on solutions of humic materials in 0.1 M NaOD/D_2O at an approximate concentration of 30 g/L. Measurements were made on a Varian VXR-400 spectrometer operating at 100 MHz ^{13}C observation frequency using inverse gate decoupling. Each spectrum is a result of 12 000−14 000 scans. Sodium trimethylsilylpropanesulfonate was used as an internal standard. All the spectra were recorded at 4-s delay time. These conditions were shown to provide quantitative determination of carbon distribution among the main structural fragments of HS (*20*). The corresponding relationships between delay time (0.5, 1, 2, 3, 4, and 8 s) and integral intensities of the signals of carbon with different chemical surroundings for five humic materials are described in (*20*, *21*); the manuscript is now in preparation for publication. To quantify the observed spectra, the assignments were made after Kovalevskii (*20*) and were as follows (in ppm): 5−50, aliphatic H and C-substituted C atoms ($\mathbf{C_{Alk}}$); 50−108, aliphatic O-substituted C atoms ($\mathbf{C_{Alk-O}}$); 108−145, aromatic H and C-substituted atoms ($\mathbf{C_{Ar-H,C}}$); 145−165, aromatic O-substituted C-atoms ($\mathbf{C_{Ar-O}}$); 165−187, C atoms of carboxylic and esteric groups ($\mathbf{C_{COO}}$); and 187−220, C atoms of quinonic and ketonic groups ($\mathbf{C_{C=O}}$). To derive the molecular descriptors, the percentage of carbon in the given structural fragments was used.

Fluorescence measurements were performed on a laser fluorimeter consisting of a nitrogen laser LGE-21 (excitation wavelength 337.1 nm) and an optical multiplier analyzer OMA-1 (PARC, U.S.A.). Fluorescence measurements on PAHs were made as described by Yashchenko et al. (*22*). The emission wavelengths (nm) used were 400, 476, and 393, with slit widths of 100, 25, and 25 μm for anthracene, fluoranthene, and pyrene, respectively.

K_{oc} coefficients were determined by fluorescence quenching (*23*). Aqueous PAH solutions below the solubility limit were prepared by spiking 1 L of double-distilled water with 20−60 μL of concentrated solutions of PAH in acetonitrile (Fischer, spectroanalyzed). The resulting solutions were equilibrated overnight and filtered through the precombusted glass fiber filters (GF/F, Whatman). Final concentrations were 1×10^{-7}, 5×10^{-7}, and 0.6×10^{-7} M for anthracene, fluoranthene, and pyrene, respectively. The stock solutions were stored in the dark at 4 °C. For measuring fluorescence quenching, a series of seven solutions of PAHs in the presence of HS was prepared in 25 mL volumetric flasks, and the necessary amount of concentrated (1×10^{-3} kg C/L) solutions of HS was added to make up a concentration of HS in the range of $(0.2-6) \times 10^{-6}$ kg C/L. The solutions were thoroughly mixed and fluorescence measured after 15 min. Increase of the contact time up to 3 h did not influence a magnitude of the fluorescence quenching, indicating that 15 min was sufficient for reaching an equilibrium between PAH and HS. This is in agreement with the results reported in ref *24* for quenching of benz[*a*]pyrene. The measured fluorescence included a background component from the HS. To correct for this, the fluorescence of solutions containing HS only was measured under the same conditions as for the HS and PAH together. Absorbance measurements at the excitation and emission wavelengths required to correct for inner filter effects (*23*) were taken for each series. The fluorescence measurements were corrected for the background fluorescence of HS and inner filter effect.

The corrected fluorescence intensity of PAHs in the presence of HS (F) and absence of HS (F_0) was used in the Stern−Volmer equation (*23*), which described the decrease in fluorescence in the presence of quencher (HS). Values of F_0/F formed linear plots against concentration of HS, with K_{oc} values calculated from the slopes.

Results and Discussion

Partition Coefficients. Stern−Volmer plots for fluorescence quenching of pyrene, fluoranthene, and anthracene with AHA are given in Figure 1. Pyrene had the highest affinity of AHA and anthracene the lowest. In addition, anthracene has the larger uncertainty in the estimated K_{oc} value, probably due to the smaller slopes and lower reliability in determination of the extent of binding. Similar results were obtained on all the other target humic materials (Table 1).

The K_{oc} values of the humic materials able to exhibit a detectable fluorescence quenching lay in the range of $(0.13-2.4) \times 10^5$, $(0.27-1.8) \times 10^5$, and $(0.12-1.0) \times 10^5$ L/kg C for pyrene, fluoranthene, and anthracene, respectively. The observed K_{oc} values agree well with the literature. Reported K_{oc} values for pyrene binding to dissolved humic materials determined by fluorescence quenching have ranged from 0.23×10^5 to 5.5×10^5 (*8, 23, 25−27*) and for anthracene from 0.15×10^5 to 0.64×10^5 (*23, 25*). A reported fluoranthene K_{oc} value of 0.9×10^5 for a soil HA (*28*) agreed well with the range of $(0.7-1.6) \times 10^5$ for soil HA in this study. At the same time, some of the soil FA and water extract of peat did not cause any fluorescence quenching of any of the PAHs. For anthracene, quenching was not detected for some soil HA as well. The lower range of the potential binding affinity of these HS was estimated by calculating the minimum detectable K_{oc} value. The latter was considered to be equal to a value of a factor of 3 higher than a standard deviation of the lowest K_{oc} coefficient determined from three parallel series of measurements (*29*). The weakest binding was observed for anthracene with T1. The resulting mean K_{oc} value and standard deviation were 0.12×10^5 and 0.034×10^5, respectively. This indicates a minimum detectable K_{oc} value of 0.1×10^5 which is given in Table 1 as an estimate for the humic materials that did not cause any detectable fluorescence quenching of PAHs.

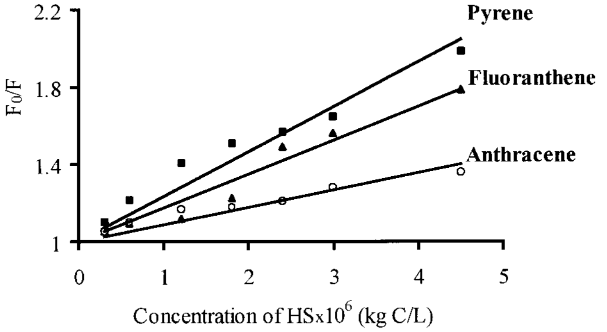

FIGURE 1. Stern−Volmer plots for quenching pyrene, fluoranthene, and anthracene fluorescence with Aldrich HA.

In general, pyrene K_{oc} values are a factor of 1.5−2 higher than for fluoranthene and from 2- to 10-fold higher than 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, *30*), as has been reported previously (*23, 31, 32*).

Based on the measured K_{oc} values, the target humic materials can be arranged in the following descending order: AHA and chernozemic HA $\gg$ sod-podzolic and gray wooded soil HA $\cong$ peat HA+FA > aquatic HA+FA $\cong$ chernozemic FA $\gg$ 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 example, very high binding affinity of AHA for the three PAHs (Table 1) is consistent with the strong binding capacity of this material observed in previous studies (*9, 12*). Only the chernozemic HA had the equally high binding affinity for PAHs. In general, K_{oc} values of soil HA were a factor of 2−3 higher than those of soil FA. These results corroborate the findings of the other authors (*8, 11, 23*).

Hence, the binding affinity for PAHs of target humic materials depends greatly both on the properties of PAHs and on the source of HS. Establishing structure-PAH binding affinity relationships for HS was our next goal.

Relationships between Structure and Binding Affinity of HS for PAHs. A wide range of data were used for characterization of the composition and structure of the target humic materials (Tables 2 and 3).

In deriving molecular descriptors, the ^{13}C NMR data were given primary importance as the most meaningful descriptors of the structure of HS. Correlation with K_{oc} values were evaluated for both individual descriptors reflecting the carbon content in each of the main structural fragments as well as combined descriptors reflecting the sums, ratios, and prod-

TABLE 2. Molecular Descriptors (Other Than ^{13}C NMR) of Humic Substances Used in This Study

sample	source of HS	H/C[a]	O/C[a]	ABS_{280}[b]	M[c]
	Aquatic Humic Substances (HA + FA)				
FMX	River Moscow	1.14	0.54	0.030	6.1
WM3X	River North Dvina	1.41	1.00	0.035	6.6
SWA	swamp water	0.86	0.93	0.025	9.8
	Peat Humic Substances (HA + FA)				
T1	Sphagnum-Fuscum peat	1.01	0.48	0.066	18.5
T4	Sphagnum peat	1.07	0.55	0.064	18.5
T5	Sphagnum peat	0.98	0.54	0.064	16.4
T6	sedge peat	0.93	0.53	0.031	18.2
T7	woody peat	0.93	0.52	0.072	18.2
HTL	woody-herbaceous peat	0.89	0.49	0.046	17.3
TTL	woody-herbaceous peat	0.89	0.48	0.060	19.8
HTW	water extract of peat	1.21	0.62	0.020	6.3
	Soil Humic Acids				
HBW	sod-podzolic soil, *forest*	0.93	0.57	0.082	12.2
HBWN	sod-podzolic soil, *forest*	0.95	0.45	0.092	14.3
HBP	sod-podzolic soil, *plough*	0.86	0.39	0.071	16.1
HBG	sod-podzolic soil, *garden*	1.01	0.53	0.110	17.3
HGW	gray wooded soil, *forest*	0.97	0.78	0.075	16.4
HGP	gray wooded soil, *plough*	0.88	0.62	0.080	14.5
HS	meadow chernozem	0.62	0.39	0.068	12.0
HST	typical chernozem	0.51	0.48	0.114	12.6
	Soil Fulvic Acids				
FBW	sod-podzolic soil, *forest*	0.90	0.91	0.035	7.9
FBP	sod-podzolic soil, *plough*	1.06	0.74	0.036	7.9
FBG	sod-podzolic soil, *garden*	0.92	0.75	0.034	10.6
FGW	gray wooded soil, *forest*	0.98	0.63	0.044	11.0
FST	typical chernozem	0.81	0.64	0.054	9.6
	Soil Humic Substances (HA + FA)				
SEL	typical chernozem	1.15	0.57	0.026	13.5
	Commercial Preparation				
AHA	Aldrich humic acid	0.74	0.28	0.045	13.0

[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] Molecular weight values are determined by SEC with calibration by polydextranes (*19*) and listed in kDalton.

ucts of the individual descriptors. These more complex expressions may reveal interaction features within the HS structure such as 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, where $\Sigma C_{Ar} = C_{Ar-O} + C_{Ar-H,R}$, $\Sigma C_{Alk} = C_{Alk-O} + C_{Alk-C,H}$), the degree of oxidation of aromatic core (estimated as $C_{Ar-O}/\Sigma C_{Ar}$ ratio), substitution of aromatic rings with electron-withdrawing carboxyl and donor methyl groups ($C_{Ar-H,R} \times C_{COO}$ and $C_{Ar-H,R} \times C_{Alk}$, respectively), and others. The full correlation matrix included seven individual and eight combined ^{13}C NMR descriptors (Table 4).

Among the individual descriptors, the strongest correlation with K_{oc} values was observed for $C_{Ar-H,C}$. The use of the combined descriptor $\Sigma C_{Ar}/\Sigma C_{Alk}$ resulted in the best fits to the experimental K_{oc} values shown in Figure 2. The corresponding regression equations are given below:

$$K_{oc} \times 10^{-5} = (1.3 \pm 0.3) \times \Sigma C_{Ar}/\Sigma C_{Alk} - (0.3 \pm 0.3) \quad \text{(pyrene)}$$

$$K_{oc} \times 10^{-5} = (0.8 \pm 0.4) \times \Sigma C_{Ar}/\Sigma C_{Alk} - (0.1 \pm 0.5) \quad \text{(fluoranthene)}$$

$$K_{oc} \times 10^{-5} = (0.6 \pm 0.2) \times \Sigma C_{Ar}/\Sigma C_{Alk} - (0.4 \pm 0.3) \quad \text{(anthracene)}$$

The values of given confidence intervals of the slopes ($n = 19$, $P = 95\%$) demonstrate a statistical relevance to the observed trend between K_{oc} value and $\Sigma C_{Ar}/\Sigma C_{Alk}$ ratio. The same is true for other parameters of aromaticity of HS—$C_{Ar-H,R}$ and ΣC_{Ar}. This can indicate a key role of aromatic core in binding of HS to PAHs. The strongest correlation with $\Sigma C_{Ar}/\Sigma C_{Alk}$ ratio suggests the highest binding affinity in the least sterically hindered aromatic structures that permits coplanar orientation of the PAH molecules. This could have been interpreted as indicative of the donor−acceptor character of interaction between HS and PAHs. However, the $\Sigma C_{Ar}/\Sigma C_{Alk}$ ratio could also reflect the lipophilic−lipophobic balance of the humic molecules, determined by a prevalence of hydrophobic aromatic core over hydrophilic (mostly polysaccharidic) aliphatic periphery. This suggests that the molecules with least sterically hindered aromatic core may, at the same time, be the most hydrophobic. Hence, hydrophobic interactions with PAHs may be reinforced by interactions with aromatic moieties in HS molecules to result in higher binding affinity. These results are consistent with previous studies (*8, 12*) correlating K_{oc} values with the percentage of aromatic carbon in the humic material.

Correlations between K_{oc} and two indirect estimates of the aromaticity of HS—atomic H/C ratio and molar absorptivity ABS_{280} (Table 4) are much poorer than those for ^{13}C NMR descriptors. However, they remain significant at $P = 95\%$. Our studies show that a usage of H/C ratios calculated without a correction for water content in the sample caused an additional decrease in r^2 of about 0.1 (from 0.5 to 0.4). In general, the significance of the correlations with absorptivity was comparable with those obtained with H/C ratio. These results contradict previous findings on the greater predictive power of absorptivity compared to the H/C ratio (*8, 12*). The correlation between K_{oc} values and O/C ratio which indicates oxidation of a humic carbon backbone was much poorer than for H/C. This can reflect the much greater impact of the degree of unsaturation of humic backbone compared to the oxidation degree on its affinity for binding PAHs.

Relationships between the PAH binding coefficients and molecular weight of the target humic materials are given in Figure 3. According to the values of the corresponding r^2 (Table 4), the linear relationship between the molecular weight of HS (M) and its ability to bind any of the three PAHs is significant at $P < 99\%$. Each of the obtained relationships has a not distinctive maximum in the range of M from 10 to 15 kDalton. HS molecules less than 10 kDalton bind very weakly to PAHs ($K_{oc} = (0.1-0.6) \times 10^5$, with the singular exception of FST with $M = 9.6$ and pyrene $K_{oc} = 1.1 \times 10^5$). In contrast, molecules larger than 10 kDalton are capable of much stronger PAH binding. For the samples with $M > 15$, a slight decrease in K_{oc} is observed at increasing molecular weight. It should be stressed that the absolute values of molecular weight of HS can differ from the apparent values reported here (*19*). However, the relative molecular weights of the different HS should be adequately represented by the M values, and thus the correlation coefficients should be unaffected. The results do not corroborate the findings of Chin et al. (*12*) on an existence of the strong positive relationship between molecular weight and binding affinity of HS for PAHs.

A lack of the strong linear relationship between K_{oc} values and molecular weight of HS can be explained by heterogeneity and irregular structure intrinsic to humic macromolecules (*33*). As a result, and in contrast to regular polymers, humic macromolecules of different molecular weight can have various quantitative ratios of main structural fragments. This effect is especially apparent for humic materials formed under different environmental conditions. To illustrate this, we have compared the data on contents of structural fragments and molecular weight of a set of seven soil HA used in this study (except for HBW). For these humic materials, there was a

TABLE 3. Percentage of Carbon[a] in the Main Structural Fragments of Humic Substances Used in This Study (^{13}C NMR Descriptors)

sample	source of HS	$C_{C=O}$	C_{COO}	C_{Ar-O}	$C_{Ar-H,C}$	C_{Alk-O}	$C_{Alk-H,C}$
		Aquatic Humic Substances (HA + FA)					
FMX	River Moscow	2	19	11	25	19	24
		Peat Humic Substances (HA + FA)					
T1	Sphagnum-Fuscum peat	1	15	10	29	25	20
T4	Sphagnum peat	1	16	12	31	24	16
T5	Sphagnum peat	1	16	10	27	22	24
T6	sedge peat	2	14	11	38	22	14
T7	woody peat	2	16	15	34	18	14
HTL	woody-herbaceous peat	2	15	14	31	20	17
TTL	woody-herbaceous peat	3	15	13	32	20	17
HTW	water extract of peat	0	11	3	13	58	15
		Soil Humic Acids					
HBW	sod-podzolic soil, *forest*	1	17	12	32	20	18
HBWN	sod-podzolic soil, *forest*	2	15	13	30	20	20
HBP	sod-podzolic soil, *plough*	2	16	12	32	21	17
HBG	sod-podzolic soil, *garden*	1	15	13	33	21	17
HGW	gray wooded soil, *forest*	1	19	13	34	16	17
HGP	gray wooded soil, *plough*	3	14	12	34	18	19
HS	meadow chernozem	2	14	9	48	14	14
HST	typical chernozem	3	15	11	43	15	13
		Soil Humic Substances (HA + FA)					
SEL	typical chernozem	1	18	13	32	22	14
		Commercial Preparation					
AHA	Aldrich humic acid	1	15	13	43	7	21

[a] Percentage of carbon equals to an integral intensity of the following regions of the ^{13}C NMR spectrum of HS recorded at delay time of 4 s (assignments are after Kovalevskii (*20*), in ppm): 5–50 (C_{Alk}), 50–108 (C_{Alk-O}), 108–145 ($C_{Ar-H,C}$), 145–165 (C_{Ar-O}), 165–187 (C_{COO}), 187–220 ($C_{C=O}$).

TABLE 4. Squares of Correlation Coefficients (r^2) between the K_{oc} Values of the Three PAHs and Molecular Descriptors of HS Used in This Study

molecular descriptors	pyrene	fluoranthene	anthracene
Individual ^{13}C NMR Descriptors[a]			
$C_{C=O}$	0.25	0.23	0.14
C_{COO}	0.0009	0.0049	0.0025
C_{Ar-O}	0.16	0.18	0.048
$C_{Ar-H,C}$	0.77	0.69	0.56
C_{Alk-O}	0.52	0.56	0.29
$C_{Alk-H,C}$	0.063	0.0064	0.091
Combined ^{13}C NMR Descriptors[a]			
$\Sigma C_{Alk} = C_{Alk-O} + C_{Alk-H,C}$	0.66	0.59	0.40
$\Sigma C_{Ar} = C_{Ar-O} + C_{Ar-H,C}$	0.74	0.67	0.49
$\Sigma C_{Ar}/\Sigma C_{Alk}$	0.83	0.74	0.66
$C_{Ar-O}/\Sigma C_{Ar}$	0.091	0.041	0.14
$C_{Ar-H,C} \times C_{COO}$	0.71	0.64	0.49
$(C_{Ar} + C_{COO} + C_{C=O})/\Sigma C_{Alk}$	0.55	0.64	0.49
$C_{Ar-H,C} \times C_{Alk-H,C}$	0.40	0.50	0.22
Other Than ^{13}C NMR Descriptors[b]			
H/C	0.51	0.48	0.39
O/C	0.31	0.34	0.25
ABS_{280}	0.49	0.45	0.41
M	0.25	0.25	0.11

[a] Correlation for 19 humic materials. [b] Correlation for 26 humic materials.

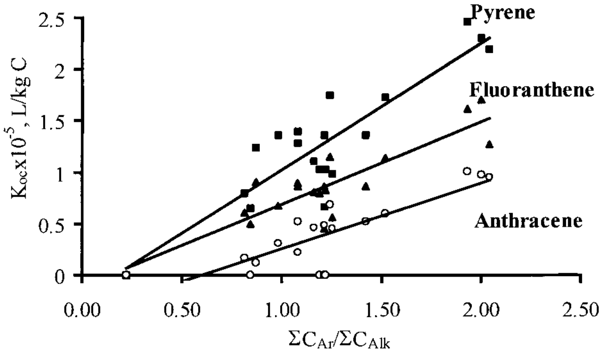

FIGURE 2. Correlation between the K_{oc} values of PAHs used in this study and aromaticity of humic substance expressed as $\Sigma C_{Ar}/\Sigma C_{Alk}$.

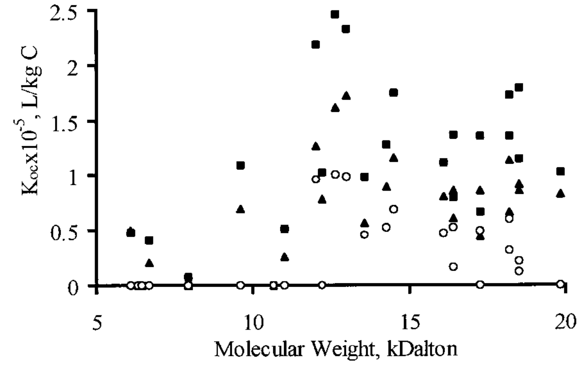

FIGURE 3. Correlation between the K_{oc} values of PAHs used in this study and molecular weight of humic substance ((■) pyrene, (▲) fluoranthene, and (○) anthracene)).

significant inverse relationship ($P = 95\%$) between molecular weight and ΣC_{Ar} ($r^2 = 0.53$), while there was direct correlation with C_{Alk-O} ($r^2 = 0.53$). Hence, the larger molecules were enriched in carbohydrate structures, whereas the smaller ones—with aromatic moieties. The observed relationship does not agree with the findings of Chin et al. (*34*) that there is a strong positive relationship between molecular weight and aromaticity of HS. The discrepancy may reflect differences in the type of target humic materials—aquatic HS—which were used by authors (*34*). In case of soil HA, the trend observed in this study is consistent with the theory of genesis of soil HA (*2, 35*). Among three soil types under study, chernozems have the highest level of microbial activity. This results in a high degree of a degradation of carbohydrate periphery of a humic macromolecule while simultaneously

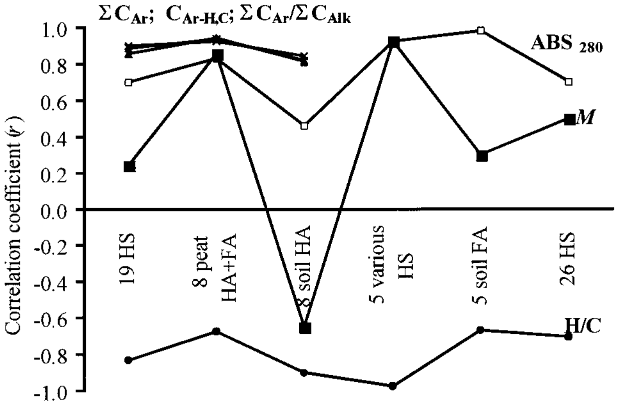

FIGURE 4. Correlation coefficients of the pyrene K_{oc} values with the molecular descriptors of humic substances for the whole set of target humic materials and for the subsets grouped by the origin and/or fractional composition.

promoting a well developed aromatic core (*2*). The opposite situation is observed in sod-podzolic and gray wooded soils, where the carbohydrate complex of humic precursors is much better preserved in humic macromolecules. As a result, the large, but carbohydrate-enriched molecules of sod-podzolic and gray wooded soil HA would be predicted to have lower binding affinity for PAHs than the chernozemic ones possessing less hindered and more condensed aromatic structures. Indeed, the corresponding pyrene K_{oc} values correlated inversely with M and directly with $\Sigma C_{Ar}/\Sigma C_{Alk}$ ($r^2 = 0.83$ and 0.69, respectively). Very similar results were obtained for fluoranthene and anthracene. However, if the binding strength of different molecular weight humic materials formed under the same conditions are compared (for example, FA and HA from the same soil), K_{oc} values of the higher molecular weight HA are much higher than that of FA.

Comparison of the Predictive Power of the Descriptors. The last observation prompted us to subdivide the initial data set of 26 HS samples into the subsets according to the source and/or fractional composition of HS. By doing so, we hoped to reveal the general structure-PAH binding affinity relationships intrinsic to humic materials from different sources as well as specific relationships intrinsic only to HS of certain genesis. This could also allow us to evaluate the predictive power and relevance of different molecular descriptors in relation to binding affinity of HS for PAHs.

Twenty-six humic materials were subdivided into four not overlapping subsets of 8 soil HA, 8 peat (HA + FA), 5 soil FA, and 5 humic materials of various origin (FMX2, WM3X, SWA, SEL, AHA). A set of 19 humic materials which were characterized with ^{13}C NMR spectroscopy was also included as a subset of 26 samples. To evaluate the correlations between K_{oc} values and the molecular descriptors within the given above subsets of HS, r value was preferred to r^2 as indicating not only significance but a direction of the correlation as well. The correlation coefficients between pyrene K_{oc} and ΣC_{Ar}, $C_{Ar-H,R}$, $\Sigma C_{Ar}/\Sigma C_{Alk}$, H/C, ABS_{280}, and M obtained for the listed above subsets of HS are given in Figure 4. Very similar results were obtained for fluoranthene and anthracene. The highest correlation and lowest variability of r-values among the different subsets of HS were provided by all three of the ^{13}C NMR descriptors of aromaticity. Correlation with H/C ratio was, in general, lower but remained rather stable for all six subsets of HS (for pyrene K_{oc} values, r varied from -0.67 to -0.98). Correlation with absorptivity was characterized with a much wider range of variation of the r-value among the different subsets of humic materials (from 0.46 for the subset of eight soil HA to 0.98 for the subset of five various HS). The most sensitive descriptor indicating the impact of the composition of HS set was molecular weight. Correlations between K_{oc} and M were significant for subsets of both eight soil HA and eight peat (HA + FA), but correlations were in opposite directions. As a result, there was very poor correlation for the whole set of the humic materials used. This is another confirmation of the discussed above complex character of relationship between molecular weight and properties of HS. Hence, extreme caution must be exercised in choosing a descriptor such as molecular weight for prediction of binding affinity of HS for PAHs. This concern is particularly relevant to prediction of the properties of HS of different origin. For these purposes, the best descriptors are aromaticity indexes derived from ^{13}C NMR data. If ^{13}C NMR data are not available, an atomic H/C ratio is to be used.

Environmental Implications. The results reported in this paper are of major importance for developing a QSAR (quantitative structure−activity relationship) approach for predicting HS properties from their structure. In the case of binding affinity for PAHs, it was shown that among the constitutional descriptors evaluated here, the most powerful are those derived from ^{13}C NMR data. The quantitative relationships (e.g. K_{oc} versus $\Sigma C_{Ar}/\Sigma C_{Alk}$) allow prediction of the influence of structural variations of the humic substance on its ability to bind PAHs. In addition, they provide deeper insight on the nature of interactions underlying binding of HS to PAHs. We believe that significant progress in structural investigation of HS by NMR spectroscopy will make it possible to define characteristic sets of structural descriptors intrinsic to HS of different origin and create the database on structure of HS. The results of our work in this direction are very promising (*20*) and are now in preparation for publication. This can provide an opportunity to predict the binding affinity for PAHs based on known origin of the humic material without requiring preliminary investigation of its structure with a use of ^{13}C NMR spectroscopy.

Another notable environmental benefit arising from this study is the observed strong correlation among K_{oc} values of the three different PAHs. There was a strong correlation between the K_{oc} values of pyrene and fluoranthene and between pyrene and anthracene (r^2 of 0.996 and 0.885, respectively) observed. This relationship may justify the use of one PAH (e.g. pyrene as strongly binding to HS and best studied among the other PAHs) as a probe to estimate the binding affinity of HS for other PAHs.

The estimated K_{oc} values can be used to predict the reduction in bioavailability (*5*, *6*) and toxicity (*7*) of PAHs in the presence of HS reported by many authors. Our immediate goal is an extrapolation of the approach described above to the prediction of the detoxifying properties of HS to PAHs.

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