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EFFECTS OF HUMIC SUBSTANCES ON THE BIOCONCENTRATION OF POLYCYCLIC AROMATIC HYDROCARBONS: CORRELATIONS WITH SPECTROSCOPIC AND CHEMICAL PROPERTIES OF HUMIC SUBSTANCES

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Abstract—The presence of dissolved humic substances (HS, fulvic and humic acids) generally reduces the uptake of hydrophobic organic compounds into aquatic organisms. The extent of this effect depends both on the concentration and on the origin of the HS. The aim of this study was to investigate the role of qualitative differences between HS from different origins. The effects of seven different HS on the bioconcentration of pyrene and benzo[*a*]pyrene (B*a*P) in the nematode *Caenorhabditis elegans* were related to the spectroscopic and chemical properties of the HS. The effect of each humic material on the bioconcentration of pyrene or B*a*P was quantified as a ''biologically determined'' partition coefficient K_{DOC}. We observed significant linear relationships between K_{DOC} and the atomic H/C ratio, the specific absorptivity at 254 nm, the content of aromatic carbons (as determined by ^{13}C nuclear magnetic resonance spectroscopy, the copper-complexing capacity, the content of phenolic OH groups, and the molecular weight of the HS. There was no discernible relationship of K_{DOC} with the atomic (N + O)/C ratio, an indicator of the polarity of HS. Taken together, our results show that the variability in the effects of HS from different origins could be related to variations in bulk properties of the HS. Parameters describing the aromaticity of the humic materials seemed to be most useful for estimating effects of HS on the bioconcentration of pyrene and B*a*P.

Keywords—Humic substances Polycyclic aromatic hydrocarbons Bioconcentration *Caenorhabditis elegans*

INTRODUCTION

Numerous studies have shown that hydrophobic organic contaminants can accumulate in aquatic organisms [1]. Most of these studies used artificial test media, usually diluted solutions of several inorganic salts in pure water. In natural aquatic systems, however, water also contains colloidal or dissolved organic matter (DOM), which generally decreases the bioconcentration of hydrophobic organic compounds [2–5]. The reduced bioavailability of contaminants in the presence of DOM has been attributed to the formation of DOM/contaminant aggregates that are too large, too polar, or both to cross biological membranes at an appreciable rate, leaving only the freely dissolved compound available for uptake [6,7].

The extent of decrease in contaminant bioconcentration in the presence of DOM has been shown to vary, depending on both the concentration and the origin of DOM. For DOM from a single source, the reduction in bioconcentration of polycyclic aromatic hydrocarbons (PAHs) has been related to the concentration of DOM [8,9]. It has been suggested that the relationship between the concentration of DOM from one source and the bioconcentration factor (BCF) for a certain organic contaminant can generally be described by a simple mathematical equation [10]. Dissolved organic matter from different sources, however, have been shown to have different effects on the uptake of PAHs into the water flea *Daphnia magna* [9] and into the nematode *Caenorhabditis elegans* [11]. The reasons for this variability have not been fully understood, but they are apparently related to differences in composition and structure of DOM from different origins.

Studies using physicochemical methods to determine the sorption of PAHs to DOM found the dissolved organic carbon (DOC)–based partition coefficient (K_{DOC}) for PAHs to be related to the aromaticity of DOM, as determined by nuclear magnetic resonance or ultraviolet spectroscopy [12–14], the molecular weight (mol wt) of DOM [13,14], and, to a lesser extent, the polarity of DOM, as expressed by atomic (N + O)/C or H/O ratios [15]. As the effect of DOM on the bioconcentration of organic contaminants is generally attributed to the sorption of contaminants by DOM, we expected the effect of DOM on bioconcentration processes to be related to DOM quality parameters in a similar manner.

The aim of the present study was to determine the effects of humic and fulvic acids (HA and FA) from different origins on the bioconcentration of two PAHs and to relate these effects to a number of DOM properties. Using the method described by Haitzer et al. [10,11], the extent of decrease in bioconcentration for each DOM source was expressed as a ''biologically determined'' K_{DOC} value, which allowed us to establish quantitative relationships between the effect of DOM on the bioconcentration of PAHs and a certain DOM quality parameter.

MATERIALS AND METHODS

Dissolved organic matter samples and chemicals

The largest part of the DOM pool usually consists of dissolved humic substances (HAs and FAs) [16], which have also been shown to be the main DOM fraction binding hydrophobic organic contaminants [17]. Humic acids and FAs are operationally defined fractions of the whole DOM [18] that can be

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isolated and concentrated from natural waters by a relatively simple procedure [19]. In this study, solutions of humic substances (HS) were made up in chemically defined artificial freshwater to keep water quality factors other than DOM constant.

The HAs and FAs were isolated from different locations in Germany according to the procedure described by Mantoura and Riley [20], which was varied by using XAD-8 material and a cation exchange resin [21]. The origins of the samples were Lake Hohlohsee (brownwater lake in the Black Forest) water: samples HO10 HA, HO13 HA, and HO13 FA (HO13 sampled 22 months after HO10); soil leachate (leached with deionized water) from a podzol near Bayreuth: BS1 FA; brown coal–derived production effluent (Schwelvollert, near Leipzig): SV1 FA; groundwater (waterworks Fuhrberg, near Hannover): FG1 FA; and secondary effluent of a waste water treatment plant (Neureut, Karlsruhe): ABV2 FA.

Before the experiments, appropriate amounts of freeze-dried HA or FA were transferred to ultrapure water (Seralpur, Delta Seral Reinstwassersysteme, Ransbach-Baumbach, Germany) and stirred for 24 h. Final HS stock solutions (≈40 mg/L DOC for FAs; ≈20 mg/L DOC for HAs, because of limited solubility of HAs) were obtained by filtering the samples through 0.45-μm membrane filters. Exact concentrations of HS (in units of milligram per liter DOC) in the stock solutions were measured by high-temperature combustion (TC 5000, Shimadzu, Duisburg, Germany). For the bioconcentration experiment with pyrene, nominal DOC concentrations of the HS stocks (calculated from the mass of freeze-dried material dissolved in water, assuming that 50% of the material was carbon) were used for preparing the test solutions, and the exact concentrations of the stocks were determined afterwards. For the benzo[*a*]pyrene (B*a*P) experiment, the exact concentrations of DOC in the HS stocks were determined before the experiment and were used for preparing the desired concentrations of DOC in the test solutions.

[^{14}C] pyrene (specific activity of 58.7 mCi/mmol) and [^{3}H] B*a*P (specific activity of 72.0 Ci/mmol) were purchased from Sigma (Deisenhofen, Germany) and Amersham Buchler (Braunschweig, Germany), respectively. All salts used for preparing artificial freshwater were obtained from Merck (Darmstadt, Germany) and were of analytical grade.

Characterization of HAs and FAs

Elemental composition. The C, H, N, O, and S contents of the freeze-dried FA and HA samples were determined with a CHN–Rapid device (Heraeus, Hanau, Germany), according to Abbt-Braun et al. [22]. Before measurements, the samples were equilibrated with air, and the water content of the samples (used for later correction) was determined. C, H, and N were determined simultaneously after combustion at 940°C in an atmosphere of O_2 in the presence of CuO. The relative standard deviation of the values was <0.5%. For the determination of O, samples were cracked in Formiergas at 1,120°C, and O was measured as CO with a nondispersive IR photometer (relative standard deviation was <2%).

Content of aromatic carbons. Solid-state ^{13}C-cross-polarization and magic angle spinning NMR spectra were recorded by U. Lankes. Spectra were run at 2.3 T with a Bruker MSL 100 spectrometer (resonance frequency: 25.2 MHz). Samples were spun at 4 to 5 kHz in commercial 7-mm double bearing probes. The recycle delay of the common cross-polarization and magic angle spinning pulse sequence was set to 0.5 s. Cross-polarization contact time was 1 ms. The spectral width was 125 kHz and the acquisition time 12.3 ms. A total of about 50,000 scans was accumulated for each spectrum. The chemical-shift scale was calibrated to tetramethylsilane. The relative content of aromatic carbons (%Ar) of the analytes was determined by relating the peak area in the 100 to 160 ppm chemical-shift band to the total peak area.

Specific absorptivity. The specific absorptivity (absorptivity per milligram of DOC) at 254 nm (ABS_{254}) was measured with a spectrophotometer (Lambda 5, Perkin Elmer, Überlingen, Germany) in 1- or 5-cm quartz glass cells using bidistilled water as a reference. Liquid HS stocks (except ABV2 FA: freeze-dried stock) were used for sample preparation, and the DOC concentration was adjusted in the range of 4 to 5 mg/L. Before measurements, the pH was set to 7 using HCl and NaOH.

Proton capacity (H^+ CAP). H^+ CAP was determined by titration of an air-free HS solution with an autotitrator (Titrator DL 25, Mettler, Germany) under nitrogen atmosphere [23]. Titration with 0.02 M NaOH was carried out in a sample volume of 20 ml containing 3 mg DOC. The total amount of the OH groups that could be titrated with strong acids and bases was divided into those reacting below pH 7 (COOH groups) and those reacting above pH 7 (phenolic groups).

Copper(II)-complexation capacity. The Cu(II)-complexation capacity (Cu-CC) of FAs was measured by differential pulse polarography using a device equipped with a dropping mercury electrode (Deutsche Metrohm GmbH, Filderstadt, Germany). Experiments were carried out in 10^{-2} M acetate buffer (pH = 6.8) with a DOC concentration of 1 mg/L [24].

Molecular weight. The mol wt of the HS was determined by size-exclusion chromatography, according to a method described in detail by Perminova et al. [25]. The size-exclusion chromatography column was a 25 × 200 mm BIAX (Chrom, Germany) with a column packing of Toyopearl HW-50S resin, with a nominal fractionation range (polyethyleneglycol calibration by the supplier) of 100 to 20,000 g/mol. Phosphate buffer (0.028 M) was used as an eluent at a flow rate of 1 ml/min. For the calibration of the size-exclusion chromatography column, a set of polydextrans (mol wt in gram per moles 830; 4,400; 9,900; 21,400; 43,500; and 2,000,000; Polymer Standard Service, Mainz, Germany) was used.

Bioconcentration experiments

Bioconcentration experiments with the nematode (thread worm) *C. elegans* were carried out as described earlier in greater detail [11]. Briefly, 10 ml of artificial freshwater (294 mg $CaCl_2 \cdot 2\,H_2O$, 123 mg $MgSO_4 \cdot 7H_2O$, 63 mg $NaHCO_3$, 5.5 mg KCl, 1,000 ml ultrapure water; Ca + Mg hardness 2.5 mmol/L), containing different HAs and FAs in concentrations ranging from 0.8 to 34.4 mg/L DOC, were transferred to 20-ml glass scintillation vials. Pure artificial freshwater was used for the controls. Then 5 μl of a stock solution of either pyrene or B*a*P in ethanol was added to achieve aqueous concentrations of 100 μg/L pyrene or 3 μg/L B*a*P. After a PAH/HS contact time of 24 h (darkness, 20°C), 15 worms were transferred to each vial, and the samples (triplicates, control without HS: five replicates) were incubated on a shaker (≈80 rpm) in the dark at 20 ± 1°C for 48 h. The tests were terminated by freezing the samples at −60°C (preliminary experiments had shown that freezing did not influence the results of the test). After the samples had been thawed at room temperature, the vials were briefly shaken by hand, and then, 100 μl of the exposure

medium (without worms) was removed and mixed with 10 ml of scintillation cocktail (Hionic Fluor, Packard, Dreieich, Germany). The radioactivity of these samples was determined by liquid scintillation counting (LS-1801, Beckman Instruments, Munich, Germany) and converted to mass equivalents using the specific activity of the labeled compounds. The aqueous concentration of pyrene or B*a*P in the test medium was calculated from the mass of PAH in the 100-μl aliquots. The worms were then transferred from the test vial to a clear glass spot plate (Bender & Hobein, Ismaning, Germany; depression holding ≈5 ml) with a Pasteur pipette. Using a dissecting microscope and a platinum wire anchored to a Pasteur pipette, the worms were removed from the test solution and transferred to another spot plate filled with fresh artificial freshwater. After being rinsed for about 10 s, the worms were transferred into a drop of 30 μl of distilled water that had been placed in a horizontally positioned scintillation vial. After all worms (the exact number was recorded) from one sample had been transferred to the new vial, 200 μl of a tissue solubilizer (Soluene 350, Packard, Dreieich, Germany) was added. After 3 h of digestion at room temperature, 10 ml of scintillation cocktail was added, and the radioactivity of the samples was determined by liquid scintillation counting. For each replicate, the concentration of PAH in the worms was calculated from the mass of PAH in the worms and the total biomass (wet weight) per sample. All worms used in the test had been synchronized to uniform size (for details see Haitzer et al. [11]), so that it was not necessary to determine the wet weight for each sample individually. We set up five replicate test vessels with 20 worms each as ''weight controls'' and determined the mean wet weight per worm, by measuring the length and width of the worms under a microscope, according to the method of Andrássy [26] (wet weight [in micrograms] = width [in micrometers]^2 × length [in micrometers]/1,600,000). The mean wet weight per worm was determined as 3.6 ± 0.6 SD μg. The total biomass per test vessel was then calculated as the number of worms (usually 15) multiplied by 3.6 μg. A BCF after 48 h of exposure (BCF_{48}) was calculated from the concentration of PAH in the organisms (based on wet weight) divided by the concentration of PAH in the medium. To minimize possible biotransformation of PAHs, the exposure time was limited to 48 h. After this time, worms from extra treatments with B*a*P were extracted in methanol; the extracts were separated by HPLC, and the radioactivity in each 20-s fraction was determined by liquid scintillation counting. Comparison of the retention time of radioactivity with the known retention time for B*a*P showed that >95% of B*a*P in the worms was unmetabolized after 48 h.

Estimation of a biologically determined partition coefficient K_{DOC}

With increasing concentration of DOC, the bioconcentration of organic contaminants generally decreases [5]. This decrease is assumed to be due to the formation of aggregates that are too large, too polar, or both to be taken up by the organism, which means that only freely dissolved contaminants will be bioconcentrated [6,17,27]. Based on this assumption, the BCF of contaminants in the presence of HS (BCF_{HS}) can be described as a function of control BCF (BCF_0), organic carbon based partition coefficient K_{DOC}, and DOC concentration [DOC] [10]:

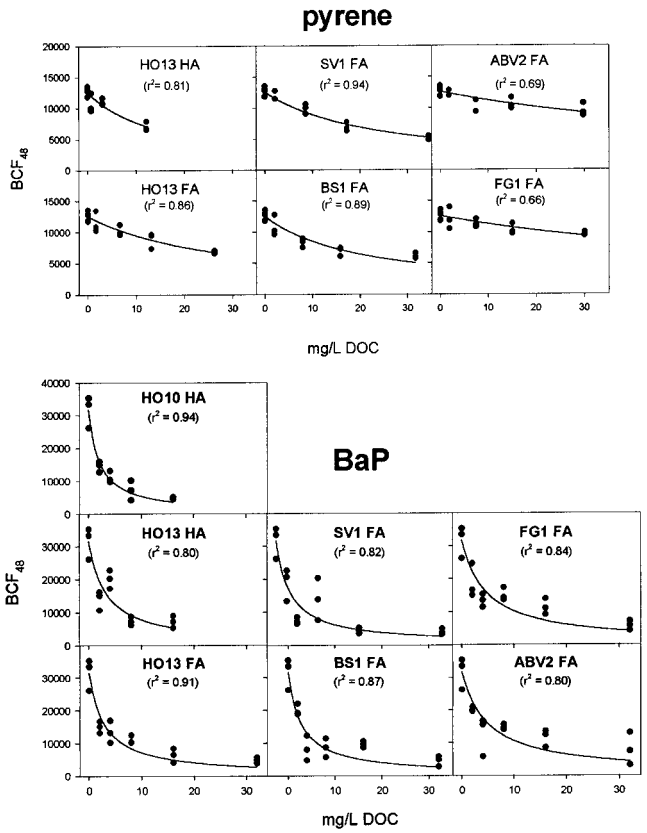

Fig. 1. Effects of humic and fulvic acids on the bioconcentration of pyrene and benzo[*a*]pyrene in the nematode *C. elegans*. Data for benzo[*a*]pyrene are from Haitzer et al. [10]. BCF_{48} = bioconcentration factor after 48 h of exposure. For an explanation of abbreviations for the sources of humic substances see the Materials and Methods section. r^2 = correlation coefficient for the curve, which was fit to the data by nonlinear regression using Equation 1.

$$BCF_{HS} = BCF_0 \times \frac{1}{1 + K_{DOC} \times [DOC]}. \quad (1)$$

Using this relationship and the experimentally determined steady-state BCFs with HS (BCF_{HS}) and without HS (BCF_0), we applied nonlinear regression procedures to fit curves to the data for each combination of HS source and PAH (Fig. 1). Calculations were performed with Sigma Plot 4.0 (1997, SPSS, Munich, Germany) by an iterative procedure using the Marquardt–Levenberg algorithm for least-squares estimation of parameters [28]. The goodness of fit of these curves (r^2 values) expresses the fraction of variation in BCF that could be explained from [DOC] by using the above relationship. The calculated descent of each curve represents the respective ''biologically determined'' K_{DOC} value, which expresses the results for a certain HS source in a single value and thus allows numerical comparisons between the effects of HS from different origins.

RESULTS

We determined the effects of FAs and HAs on the bioconcentration of PAHs in the nematode *C. elegans*, and related these results to properties of the HS measured by spectroscopic and chemical methods. Four (HAs) or five (FAs) different concentrations of DOC were applied to generate dose (DOC concentration)–effect (BCF_{48}) relationships. Increasing concentrations of DOC decreased the bioconcentration of pyrene

Table 1. Biologically determined K_{DOC} values for pyrene and B*a*P and selected properties of humic acids and fulvic acids

DOM[a] sample	K_{DOC} (pyrene) $\times 10^4$ (L/kg DOC[a]) ±SE	K_{DOC} (B*a*P) $\times 10^4$ (L/kg DOC) ±SE	(N + O)/C[b]	H/C[b]	% Ar[c]	ABS_{254}[d] (L/[mg DOC × m])	Cu-CC[e] (mmol/mg DOC)	H^+ CAP[f] pH > 7 (μmol/mg DOC)	Mol wt[g] (g/mol)
HO10 HA	ND	49.0 ± 5.8	0.54	0.80	40	6.2	ND	ND	17,780
HO13 HA	6.3 ± 1.1	30.2 ± 6.1	0.47	0.89	36	5.4	ND	ND	ND
HO13 FA	3.3 ± 0.4	34.1 ± 4.7	0.55	0.88	35	4.9	2.0	3.9	ND
SV1 FA	4.1 ± 0.3	36.3 ± 7.5	0.53	1.04	36	4.8	2.5	6.0	4,860
BS1 FA	4.8 ± 0.6	35.2 ± 6.0	0.60	0.82	34	4.7	2.1	5.5	10,990
ABV2 FA	1.3 ± 0.2	19.8 ± 3.5	0.54	1.19	27	3.6[h]	0.8	1.5	3,830
FG1 FA	1.2 ± 0.2	20.9 ± 3.8	0.51	1.04	24	2.7	0.5	1.4	3,900

[a] DOM = dissolved organic matter; DOC = dissolved organic carbon.
[b] Atomic ratios.
[c] Relative content of aromatic carbons.
[d] Specific absorptivity at 254 nm.
[e] Copper(II)-complexation capacity.
[f] H^+ capacity at pH > 7 operationally defined as the content of phenolic OH groups.
[g] Molecular weight.
[h] Freeze-dried stock was used.

and B*a*P in a nonlinear manner (Fig. 1). The regression curves included in Figure 1 were generated using Equation 1, and the calculated ''nonlinear slope'' of each curve represents the biologically determined partition coefficient K_{DOC}. Correlation coefficients (r^2) for the curves ranged from 0.66 to 0.94 (mean: 0.81) for pyrene and 0.80 to 0.94 (mean: 0.85) for B*a*P, indicating that Equation 1 was suitable to describe the quantitative relationship between DOC concentration and BCF_{48}. Resulting ''biologically determined'' K_{DOC} values for pyrene were about an order of magnitude lower than those for B*a*P (Table 1). For both PAHs, the effects of the different HS samples could be divided into three groups: ABV2 FA and FG1 FA clearly had the smallest effects, both for pyrene and for B*a*P; SV1 FA, BS1 FA, and HO13 FA (and HO13 HA for B*a*P) had intermediate effects, and HO10 HA and HO13 HA (for pyrene) had the largest effects. This indicates that, although the results for HO13 HA were not consistent, the effects of the different HS sources were similar for both PAHs. A comparison of the effects of HAs and FAs showed that, with the exception of HO13 HA/B*a*P, HAs had higher K_{DOC}s than FAs.

Selected spectroscopic and chemical properties of HAs and FAs are summarized in Table 1. The polarity of organic matter can be expressed by relating the amount of nitrogen and oxygen to the content of carbon [29]. Xing et al. [30] proposed the term ''polarity index'' for the atomic (N + O)/C ratio of organic matter. The (N + O)/C ratios of the samples investigated in this study were within a relatively narrow range, without showing clear trends or differences between HAs and FAs. The aromaticity of organic matter can be estimated from various chemical and spectroscopic properties, such as the atomic H/C ratio [17], the specific absorptivity at wavelengths between 250 and 280 nm as a measure for π electron systems [31], and the relative content of aromatic carbon atoms, as determined by ^{13}C-NMR spectroscopy [14]. Our results for these three parameters (Table 1) showed that HO10 HA was comparatively rich in aromatic structures (low H/C, high ABS_{254}, and high %Ar), whereas ABV2 FA and FG1 FA contained much smaller portions of these structures. An intermediate content of aromatics was found in HO13 HA, HO13 FA, SV1 FA, and BS1 FA. The results for two additional chemical parameters, the proton capacity at pH > 7 (H^+ CAP) and the Cu-CC, were closely correlated to the content of aromatic carbons (r = 0.99 for Cu-CC and 0.92 for H^+ CAP). Therefore, we used these (easily determined) parameters for additional correlations of HS properties with effects of HS on the bioconcentration of PAHs. Results for mol wts of the humic materials showed a trend similar to the aromaticity data, with HO10 HA exhibiting the highest mol wt and ABV2 FA as well as FG1 FA being relatively small in molecular size.

The effects of HA and FA on the bioconcentration of pyrene and B*a*P were related to spectroscopic and chemical properties of the HS (Table 2). Pearson correlation coefficients showed significant correlations between K_{DOC} and the atomic H/C ratio, the specific absorptivity at 254 nm, the percentage of aromatic

Table 2. Pearson correlations between K_{DOC} and physicochemical properties of humic and fulvic acids. Asterisks mark significant ($p \leq 0.05$)* and highly significant ($p \leq 0.01$)** correlations. For an explanation of the variables see Table 1

	H/C	N + O/C	% Ar	ABS_{254}	Cu-CC	H^+ CAP pH > 7	Mol wt
K_{DOC} (pyrene)							
Pearson correlation	−0.72	−0.14	0.87	0.89	0.94	0.97	0.78
Significance level	0.10	0.79	0.02*	0.02*	0.02*	0.005**	0.22
n[a]	6	6	6	6	5	5	4
K_{DOC} (B*a*P)							
Pearson correlation	−0.77	0.27	0.91	0.89	0.98	0.96	0.88
Significance level	0.04*	0.56	0.004**	0.008**	0.004**	0.01**	0.05*
n	7	7	7	7	5	5	5

[a] n = number of data points.

carbons, the copper-complexing capacity, the content of phenolic protons, and the mol wt of humic materials. Correlations of K_{DOC} with the (O + N)/C ratio were not significant. Generally, higher correlation coefficients were found in the experiments with B*a*P. For example, the correlations of K_{DOC} with H/C and with mol wt were not significant for pyrene (p = 0.10 and 0.22), but significant at the 5% level for B*a*P. Taken together, our results show that the variability in the effects of HS from different origins could be related to variations in bulk properties of HS. Parameters describing the aromaticity of the HS seemed to be most useful for estimating effects of HS on the bioconcentration of PAHs from physicochemical measurements of the properties of HS. For example, 76 to 96% (squared Pearson correlation coefficients) of the variation in K_{DOC} for pyrene and B*a*P could be predicted from ABS_{254}, %Ar, H^+ CAP, or Cu-CC of the HS.

DISCUSSION

Differences in DOM origin can control the effect of DOM on the bioconcentration of contaminants to a large extent. For example, Landrum et al. [6] found biologically determined K_{DOC} values for B*a*P to vary over more than three orders of magnitude, depending on the sampling location of sediment interstitial water. Using reverse osmosis concentrates of natural humic waters, Haitzer et al. [11] observed up to an order of magnitude differences in biologically determined K_{DOC}s for B*a*P. Also, Morehead et al. [32] reported K_{DOC}s (determined by reversed phase separation) for the sorption of B*a*P and pyrene to DOM in the waters of Lake Michigan ranging over more than one (B*a*P) or more than two (pyrene) orders of magnitude, respectively. The range of biologically determined K_{DOC} values found in this study agrees with previously published K_{DOC}s for the sorption of pyrene [14,33] and B*a*P [15] to HAs and FAs, but we observed smaller effects of sample origin than mentioned above (differences in K_{DOC}s ranged up to 5.3-fold for pyrene and up to 2.5-fold for B*a*P). A possible explanation for this could be that isolated HAs and FAs, rather than natural DOM, were used in the present study. Therefore, effects of naturally occurring variations in the ratio of hydrophobic acids (FAs and HAs, which are mainly responsible for the binding of PAHs [17]) to total DOM were excluded from the experiments. Further, the selectivity of the adsorption to XAD-8 resin and a certain amount of chemical alteration of the samples during the extraction procedure [34] was equal for all samples, possibly blurring differences between original whole DOM samples.

Although the range of variation of K_{DOC} in this study was smaller than in previous investigations, HS from different sources still produced significantly different biologically determined K_{DOC}s (Table 1). We also observed clear variations in spectroscopic and chemical properties of the different HS sources (Table 1), and changes in K_{DOC} could be related to variations in HS properties (Table 2). The correlations of HS quality parameters with K_{DOC}s for pyrene were generally less pronounced than those with B*a*P. This is probably due to the fact that HS had a smaller effect on the bioconcentration of the less hydrophobic pyrene, so that the correlation coefficients of the nonlinear curve fits for calculating K_{DOC}s tended to be lower for pyrene than for B*a*P (especially for ABV2 FA and FG1 FA), resulting in less accurate estimates of K_{DOC}s for pyrene (Fig. 1).

A number of investigators have used physicochemical methods (such as equilibrium dialysis, reversed phase separation, solubility enhancement, or fluorescence quenching) to determine K_{DOC}s for the partitioning of organic compounds with DOM [15,35–37]. Using these methods, several studies found significant relationships between bulk properties of humic materials and K_{DOC} for PCBs, pesticides, and PAHs [12,14,29,38].

Chiou et al. [38,39] suggested that the polarity of HS substances mainly controls their interactions with hydrophobic organic contaminants. Also, Xing et al. [30,35] found correlations between the atomic (N + O)/C ratio of organic sorbents (''polarity index'') and the partition coefficients of nonionic organic contaminants. More recently, however, a critical evaluation of the literature data on relationships between K_{DOC} and polarity index [40] has shown that clear linear correlations only occurred for geological materials and for nonprocessed biological materials [29,35]. Humic acids and FAs extracted from soil and water generally cover a comparatively narrow range of polarity index [39,41], and the linear correlation of (N + O)/C with K_{DOC} for these materials is much weaker [40]. For HA and FA isolated by XAD resins, the selectivity of the extraction procedure may be a reason for the observed narrow ranges in polarity. According to Chin et al. [14], all humic materials isolated by adsorption to XAD resin should possess approximately the same polarity, because their capacity factors for the hydrophobic stationary phase were similar. The results of the present study agree with the above findings, in that the range of (N + O)/C values was relatively narrow, and no significant correlations between the atomic (N + O)/C ratio of humic materials and K_{DOC} of pyrene or B*a*P were found (Table 2). Nevertheless, we observed significant differences in biologically determined K_{DOC}s. Therefore, we assume that differences in the polarity of humic materials did not significantly influence their effects on the bioconcentration of pyrene and B*a*P.

The ability of HS to interact with PAHs, as measured by fluorescence quenching and solubility enhancement for pyrene and equilibrium dialysis for B*a*P, has also been related to their aromaticity. In these studies, the aromatic character of the humic materials has been estimated from the atomic H/C ratio [12,17,36], the ultraviolet absorptivity [12–14,17,36], or the relative content of ''aromatic'' carbons or protons, as determined by ^{13}C-NMR or ^{1}H-NMR spectroscopy [12,14,36]. Our results show that the effects of HAs and FAs on the bioconcentration of pyrene and B*a*P were also linearly correlated to the above parameters (Table 2). In agreement with previous studies [12,17], we found a negative correlation between K_{DOC} and the atomic H/C ratio of humic materials. However, as pointed out by Gauthier et al. [12], these results should be interpreted with care. Both, COOH and –HC=CH– groups have the same H/C ratio, and therefore, the presence of a high concentration of COOH groups can adversely affect the use of atomic H/C as a measure of –HC=CH– groups. As the sorption of hydrophobic organic compounds is assumed to be promoted by (hydrophobic) –HC=CH– groups and impeded by (hydrophilic) carboxylic groups, the correlation of PAH sorption with the atomic H/C ratio could be misleading, especially for largely functionalized FAs. Further methods to estimate the aromatic character of humic materials include ^{13}C-NMR spectroscopy [12,14,31] and measurements of specific UV absorptivity [14,31,36]. Chin et al. [31] reported that the results of these techniques were highly correlated (r = 0.95). We also observed a very clear (r = 0.98) correlation between the percentage of aromatic carbons and the specific absorptiv-

Table 3. Pearson correlation coefficients for intercorrelations between physicochemical properties of humic and fulvic acids. Asterisks mark significant ($p \leq 0.05$)* and highly significant ($p \leq 0.01$)** correlations. For an explanation of the variables see Table 1

	N + O/C	% Ar	ABS_{254}	Cu-CC	H^+ CAP	Mol wt
H/C	−0.23	−0.73	−0.71	−0.58	−0.61	−0.88*
N + O/C		0.09	0.03	0.50	0.54	0.40
% Ar	0.09		0.98**	0.99**	0.92*	0.78
ABS_{254}	0.03	0.98**		0.95*	0.87	0.85
Cu-CC	0.50	0.99**	0.95*		0.97**	0.55
H^+ CAP	0.54	0.92*	0.87	0.97**		0.62

ity at 254 nm, and both parameters were significantly correlated to biologically determined K_{DOC}s for pyrene and B*a*P (Table 2). Taken together, 76 to 83% (squared Pearson correlation coefficients) of the variability in biologically determined K_{DOC} could be related to variations in ABS_{254} or the percentage of aromatic carbons. Thus, the aromaticity of HS seemed to be a good predictor for their effect on the bioconcentration of PAHs.

The ability of FAs to complex divalent cations mainly results from their content of phenolic OH and COOH groups [42]. Table 3 shows that the content of phenolic OH groups (as estimated from acid–base titration above pH 7) strongly correlated to the Cu-CC of FAs ($r = 0.97$) and that both Cu-CC and content of phenolic OH groups were clearly correlated to the percentage of aromatic carbons ($r = 0.99$ and 0.92). Therefore, we examined the suitability of these two parameters as additional predictors for the effect of FAs on the bioconcentration of PAHs. We obtained unexpectedly clear results for the correlations of Cu-CC and content of phenolic OH groups with biologically determined K_{DOC} (Pearson correlation coefficients in the range 0.94–0.98). Thus, as much as 88 to 96% of the variability in K_{DOC} of FAs could be explained from variations in these two parameters. However, as our results are based on only five data points for both Cu-CC and content of phenolic OH groups, they should be interpreted with care.

In the past, a number of investigators provided evidence that the ability of HS to bind hydrophobic organic compounds may also be related to the size of the HS molecules [13,14,43]. Our results for biologically determined partition coefficients of pyrene and B*a*P and molecular-weight measurements done by size-exclusion chromatography appear to corroborate these observations (Tables 1 and 2). Although the number of data points was limited to four (pyrene) and five (B*a*P), we could observe a trend for increased effects of HS with increasing molecular size of the HS (significant at the 5% level for B*a*P). Apparently, larger HS molecules were more efficient in associating with PAHs, and the large HS/PAH associates were not able to cross biological membranes, thus reducing the uptake of PAHs into the nematodes.

As mentioned above, the spectroscopic and chemical parameters used to describe properties of the HS were intercorrelated (Table 3). For example, the percentage of aromatic carbons was negatively correlated to H/C ($r = 0.73$) and positively correlated to ABS_{254} ($r = 0.98$), Cu-CC ($r = 0.99$), the content of phenolic OH groups ($r = 0.92$), and the mol wt ($r = 0.78$). These data are in agreement with findings of Chin et al. [31], who observed a strong correlation between molar absorptivity, total aromaticity, and the mol wt of a number of HS, indicating that these spectroscopic and chemical properties of HS are not independent of each other. Therefore, from the present data it cannot be derived whether the effects of HS on the bioconcentration of contaminants are determined by a mixture of different parameters (e.g., aromaticity and mol wt) or if a single parameter, which is proportional to other parameters, controls the interactions. Thus, such studies do not allow us to draw definitive conclusions about mechanisms involved in PAH–HS interactions.

Nevertheless, further investigations of this type with a larger number of sorbents and a broader range of sorbent properties can be useful to find empirical relationships between bulk properties of DOM and interactions of DOM with contaminants. Such relationships of the form

$$K_{DOC} = a \times (\text{DOM property}) + b$$

where a and b are constants, could then be combined with Equation 1, which describes the effect of DOM concentration, to give

$$BCF_{HS} = BCF_0 \times \frac{1}{1 + (a \times (\text{DOM property}) + b) \times [DOC]}.$$

This relationship could then be used to estimate the effect of DOM on the bioconcentration of contaminants from data that are given in the literature or can be easily determined (BCF in pure water, e.g., ABS_{254} and DOM concentration).

CONCLUSIONS

The extent to which the HS decreased the bioconcentration of PAHs was related to the concentration and to physicochemical properties, such as aromaticity, of the HS. Mathematical descriptions of the influence of both factors can be combined into a general expression that quantitatively relates the bioconcentration of PAHs in the presence of HS to easily determined parameters (BCF in pure water, e.g., aromaticity of the HS and DOC concentration). Variations in the effects of natural DOM from different sources may have been underestimated in the present study, because all HS used here had been subjected to an identical kind of chemical alteration during the XAD extraction procedure.

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