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Towards a Quantitative Structure Activity Relationship (QSAR) of Dissolved Humic Substances as Detoxifying Agents in Freshwaters

key words: humic substances, polycyclic aromatic hydrocarbons, partition coefficients, aromaticity, QSAR, bioconcentration, detoxification

Abstract

In freshwater ecosystems, dissolved humic substances (HS) can sorb hydrophobic organic chemicals and thereby reduce their bioconcentration and toxicity. The extent of the sorption depends both on the concentration and on the origin of HS. This paper summarizes work that characterized HS from different origins by various spectroscopic and chemical properties, and related these properties to (1) the sorption of polycyclic aromatic hydrocarbons (PAHs) by HS (measured by a fluorescence quenching method), (2) effects of HS on the bioconcentration of PAHs and (3) effects of HS on the toxicity of PAHs. The most powerful predictor of the association between 26 different HS and the PAHs pyrene, fluoranthene, and anthracene was the content of aromatic carbons of the HS. The influence of HS on the bioconcentration of PAHs was investigated by measuring the effect of seven different humic and fulvic acids on the uptake of pyrene and benzo[a]pyrene into the nematode *Caenorhabditis elegans*. Again, parameters describing aromatic properties of the HS, such as the content of aromatic carbons, or the specific absorptivity at 254 nm gave the best prediction of HS effects on bioconcentration. Studies on the effect of HS on the toxicity of PAHs (impact on grazing activity of *Daphnia magna*) gave similar results: The detoxifying ability (= reduction of toxicity) of HS could be correlated to the quotient between aromatic carbons and aliphatic carbons in the HS. Taking these results together, we can describe the relationship between structure and effects of HS on bioavailability and toxicity of PAHs as follows: HS associate with PAHs, making the PAHs unavailable for uptake into organisms, and consequently reducing toxic effects of the PAHs. Aromatic properties of the HS can be used to predict the effect of HS in each of these steps.

1. Introduction

From paleoenvironmental studies of sediment cores it is a well known fact that environmental chemicals, which may have a significant adverse impact on biota, are well preserved in the sediments of lakes. This applies particularly to highly or super-lipophilic xenobiotics, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs), and polychlorinated biphenyls (PCBs). The preservation can be so stable that from the different patterns of congeners (PAHs, PCDD/Fs, PCBs) deposited at a specific time, the source of the corresponding emission may be identified (e.g. BRUCKMEIER *et al.*, 1997; JÜTTNER *et al.*, 1997). There is evidence that the persistence of bondings and congener patterns depends on the content of organic carbon in the sediments. It is also known that the binding affinity of lipophilic compounds for dissolved organic matter (DOM) depends greatly upon composition and structure of the latter, particularly humic substances, HS (GAUTHIER *et al.*, 1987; CHIN *et al.*, 1997). However, it is still open to discussion whether binding to dissolved organic matter (DOM) also alters the bioavailability and the potential of adverse effects of the xenobiotic chemicals. For instance, HS may cause either a decrease or an increase in bioconcentration and toxicity of organic compounds (HAITZER *et al.*, 1998; STEINBERG *et al.*, 1994; LORENZ *et al.*, 1996).

It is very likely that the mechanism responsible for the binding of xenobiotic chemicals in sediments, is also valid for the water body, in particular for those rich in dissolved organic carbon (DOC). This mechanism suggests that HS in water bodies may serve as detoxifying agents (= reduction of toxicity) which facilitate self-purification of freshwater ecosystems.

In this contribution, we present estimates of the impact of natural organic compounds (HS) on bioconcentration and toxicity of xenobiotics. Furthermore, we present a quantitative structure activity relationship (QSAR) approach which allows the prediction of the ecotoxicological behavior of xenobiotics in the presence of humic substances, on the basis of fragmental composition of humic substances.

2. Material and Methods

Humic Substances

For bioconcentration studies, humic acids (HA) and fulvic acids (FA) were isolated by using XAD−8 material and a cation exchange resin (ABBT-BRAUN *et al.*, 1990) from the following sources: (1) lake Hohlohsee (raised peat bog lake in the Black Forest): HO10 HA, HO13 HA and HO13 FA (HO13 sampled 14 months later than HO10); (2) soil leachate (leached with deionized water) from a podsol near Bayreuth: BS1 FA; (3) a lake, receiving an effluent from a brown coal processing plant (Schwelvollertsee, near Leipzig): SV1 FA; (4) groundwater (waterworks Fuhrberg, near Hannover): FG1 FA, and (5) effluent of a waste water plant (Karlsruhe): ABV2 FA.

For HS-PAH binding studies and for ecotoxicity studies, nineteen humic materials were additionally isolated from different natural sources (freshwater, soil, peat).

Aquatic Humic Substances (HA+FA) were isolated from the Moscow area of the river Moscow (*FMX*) using Amberlite XAD−2 resin.

Peat Humic Substances (HA+FA) were isolated from 7 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 by LOEWE (1992) 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 HTL – of woody-herbaceous peat.

Soil Humic Acids (HA) were extracted from 8 soils. These included Sod-podsolic soils near Moscow (*HBW, HBP, HBG*) and Novgorod (*HBWN*), two Grey Wooded soils near Tula (*HGW, HGP*), typical and meadow Chernozemic soils near Voronezh (*HST* and *HS*, respectively). The HS extraction was carried out according to (ORLOV and GRISHINA, 1981). This included pre-treatment 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.

A sum of soil HA and FA (*SEL*) was isolated by alkaline extraction from typical Chernozemic soil nearby Stavropol. *Commercial Aldrich Humic Acid* (*AHA*) was used as purchased from the supplier.

Concentrated stock solutions of HS [100−500 mg l^{-1} of organic carbon (DOC)] were prepared by evaporation of the corresponding desalinated isolates or by a dissolution of dried material. Content of organic carbon in the stock solutions was measured using a Shimadzu 5000 TOC analyzer as described by SUGIMURA and SUZUKI (1988).

Characterization of humic materials

Elemental composition: for the bioconcentration studies, C, H, N, O, and S contents in the freeze dried FA and HA samples were determined with a CHN-O-Rapid device (Heraeus, Hanau, Germany), according to ABBT-BRAUN *et al.* (1990). In the chemical and eco-toxicity studies, C, H, N were determined with a use of a Carlo Erba Strumentazione elemental analyzer. S, H_2O and ash contents were determined manually.

Size Exclusion Chromatography (SEC) Analysis was performed at the facilities of the Division of Water Chemistry, Engler-Bunte Institute, Technical University of Karlsruhe, Germany. 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, pH 6.8) prior to the analysis. For further details, see PERMINOVA *et al.* (1998).

^{13}C NMR: For the bioconcentrations studies, ^{13}C-CPMAS NMR spectra were recorded at the Institute for Biophysics and Physical Chemistry at the University of Regensburg, Germany. 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 CPMAS 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 were accumulated for each spectrum. The chemical shift scale was calibrated to TMS. The relative content of aromatic carbons (C_{Ar},%) of the analytes was determined by relating the peak area in the 100–160 ppm chemical shift band to the total peak area (see also next section).

For toxicity studies, ^{13}C *solution-state* NMR spectra of nineteen HS samples were measured on solutions of humic materials in 0.1 M NaOD/D_2O at an approximate concentration of 10 g l^{-1}. Measurements were made on a Varian VXR–400 spectrometer operating at 100 MHz ^{13}C observation frequency using inverse gate decoupling and 4-s delay time. Each spectrum is a result of 12000–14000 scans. As an internal standard sodium trimethylsilylpronanesulfonate was used. To quantify the obtained spectra, the assignments were made after KOVALEVSKII (1998) and were as follows (in ppm): 5–50 – aliphatic H and C-substituted C atoms (C_{Alk}), 50–108 – aliphatic O-substituted C atoms (C_{Alk-O}), 108–145 – aromatic H and C-substituted atoms ($C_{Ar-H,C}$), 145–165 – aromatic O-substituted C-atoms (C_{Ar-O}), 165–187 – C atoms of carboxylic and esteric groups ($C_{COO-H,R}$), 187–220 – C atoms of quinonic and ketonic groups ($C_{C=O}$).

UV-absorbance (spectral absorption coefficient = SAC): For the bioconcentration experiments, the specific absorptivity (absorptivity per mg DOC) at 254 nm (SAC_{254}) was measured with a spectrophotometer (Lambda 5, Perkin Elmer, Überlingen, Germany) using 1 or 5 cm quartz glass cells and 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^{-1}. Before measurements, the pH was adjusted to 7, using HCl and NaOH.

Proton-Capacity (H^+-CAP): H^+-CAP was determined by titration of air free HS solution with an autotitrator (Titrator DL 25, Mettler, Germany) under nitrogen atmosphere (FRIMMEL *et al.*, 1985). 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 which can 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^{-1} (FRIMMEL and GEYWITZ, 1983).

Chemicals: For the toxicity studies, the PAHs used were anthracene, fluoranthene and pyrene (all Aldrich, 97% pure). For the bioconcentration studies, ^{14}C pyrene (specific activity of 58.7 mCi mmol^{-1}) and ^{3}H benzo[a]pyrene (specific activity of 72.0 Ci mmol^{-1}) were purchased from Amersham Buchler and Sigma, respectively. All salts used for the test medium of the bioconcentration experiments were obtained from Merck and were p.a. grade. Water solubility and lipophilicity as log K_{OW} of the studied PAH are given in Table 1.

Table 1. Characteristics of PAHs used in this Study (from LEO *et al.*, 1971).

PAH	Solubility µg l^{-1}	$_{10}\log K_{OW}$
Pyrene	130	5.20
Fluoranthene	265	5.18
Anthracene	30	4.40
Benzo[a]pyrene	0.5	6.04

Partition coefficients of PAHs for each of the dissolved humic materials were determined by a fluorescence quenching technique (GAUTHIER *et al.*, 1986). "Biologically determined" partition coefficients were calculated from the effect of HS on the bioconcentration of pyrene and benzo[a]pyrene.

For measuring fluorescence quenching, the stock solutions of anthracene, fluoranthene and pyrene at concentrations of 1×10^{-7}, 5×10^{-7} and 0.6×10^{-7} M, respectively, were put into 25 ml volumetric flasks and titrated with concentrated (1 g l^{-1} DOC) solutions of HS. A series of seven solutions were prepared for each HS at a concentration in the range of $(0.2-6) \times 10^{-6}$ kg l^{-1} DOC and included also a blank solution. For further details, see PERMINOVA *et al.* (1999).

The quenching of PAH fluorescence was quantified as the ratio of F_0/F, where F_0 and F are the fluorescence of PAH in the absence and presence of HS, respectively. Linear STERN-VOLMER plots were obtained. Their slopes normalized to the fraction of organic carbon in humic material were equal to K_{DOC} values-partition coefficients, characterizing binding affinity of HS for PAHs.

Bioconcentration studies: were carried out with *Caenorhabditis elegans*, which is a widespread, bacterivorous thread worm (nematode). A temperature-sensitive mutant strain of *C. elegans* (glp–1 (AUSTIN and KIMBLE, 1987), obtained from the *Caenorhabditis* Genetics Center (CGC, St. Paul, MN, USA), was used as test organism in the present study. These animals reproduce normally, when are grown at 15 °C, whereas growing worms at 25 °C leads to a permanent defect in germ line proliferation. Thus, adult worms that were grown at 25 °C are sterile and do not reproduce, even when transferred to lower temperatures. We used this mutant strain, in order to avoid reproduction during the exposure phase. Cultures of nematodes and bioconcentration experiments were done according to HAITZER *et al.* (1999a, b).

Estimation of a biologically determined partition coefficient K_{DOC}:

It is postulated that only freely dissolved contaminant can be bioconcentrated. Under this assumption, the bioconcentration factor of contaminants in the presence of HS (BCF_{HS}) can be described as a function of the control BCF (BCF_0), the organic carbon based partition coefficient K_{DOC} and the DOC concentration [DOC] (for further details, see HAITZER *et al.*, 1999a–c; MCCARTHY *et al.*, 1985; KUKKONEN and PELLINEN, 1994):

$$BCF_{HS} = BCF_0 \cdot \frac{1}{1 + K_{DOC} \cdot [DOC]} \qquad \text{[Eqn. 1]}$$

Using this relationship and the experimentally determined values for BCF_{HS} and BCF_0, we applied non-linear regression procedures to fit curves to the data for each combination of HS source and PAH. A linear regression would also be possible, however, low values of BCF_{HS} would unfavourably influence the regression result. 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 (e.g. PRESS *et al.*, 1986). The goodness of fit of these curves (r^2 values) expressed the fraction of variation in BCF that could be explained from [DOC] by using the above relationship. From the fit we determined a "biological K_{DOC}" value, which expressed the results for a certain HS source in a single value and thus allowed numerical comparisons between the effects of HS from different sources.

Acute toxicity tests were performed by using the grazing activity of *Daphnia magna* as a sensitive target function according to the procedure described in (POLYNOV, 1992). For determination of the impact of HS on the toxicity of PAHs, three series of the solutions were prepared simultaneously: (1) PAH control solutions containing 1.7×10^{-5}, 5×10^{-5}, 5×10^{-5} M of anthracene, fluoranthene and pyrene, respectively, (2) control solutions containing only HS at concentrations of 3, 6, 12 and 24 mg l^{-1} DOC, (3) a series of solutions containing PAHs and HS. All tests were done in triplicates. Five juvenile *D. magna* were introduced into each vessel. After an exposure time of 24 hours 1 mL a suspension of *Chlorella vulgaris* was added to all the solutions and the grazing activity of *D. magna* was measured. For further details, refer to PERMINOVA *et al.* (in press).

The detoxifying ability of HS was estimated using the following equation (PERMINOVA *et al.*, 1996):

$$D = \left(1 - \frac{R_{HS} - R_{PAH+HS}}{R_{HS}} \Big/ \frac{R_0 - P_{PAH}}{R_0}\right) \cdot 100\% \qquad \text{[Eqn. 2]}$$

where R_0 – grazing activity of *D. magna* in the control solutions without PAHs and HS, R_{PAH} – in the presence of PAHs only, R_{HS} – in the presence of HS only, R_{PAH+HS} – in the presence of PAHs and HS together. Only this equation assumes and considers a specific (beneficial or adverse) effect of humic substances on the grazing activity of *D. magna*.

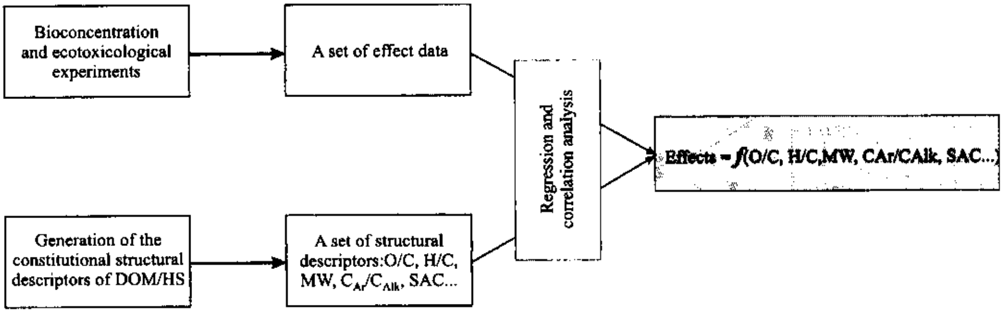

Figure 1. Scheme of relating a set of effect data to structural descriptors of HS.

Establishment of QSARs: The scheme of establishing quantitative structure activity relationships is given in Figure 1. A set of ecotoxic effect data are correlated with molecular descriptors of the studied humic substances. Ecotoxic effect data may be, for instance, bioconcentration and bioaccumulation on the one hand, and toxic effects (lethality, inhibition of crucial functions, such as swimming or food filtration of *Daphnia*) data on the other hand. Molecular descriptors of humic materials were derived from the data on elemental composition (C, H, N, O, S), distribution of carbon among the main structural fragments ($C_{C=O}$, $C_{COO\text{-}H,R}$, C_{Ar}, C_{al}, peak molecular weight, absorptivity (SAC) at 254 nm or Cu complexation capacity. The quantities $C_{Ar} = C_{Ar\text{-}O} + C_{Ar\text{-}H,C}$ and $C_{Al} = C_{Alk\text{-}O} + C_{Alk}$ are indicators of a whole content of aromatic and aliphatic moieties, respectively. C_{Ar}/C_{Al} is an indicator of a ratio of the aromatic core to the aliphatic (mostly carbohydrate) periphery which probably determines lipophilic/lipophobic balance and sterical hindrance of a humic macromolecule.

3. Results and Discussion

3.1. PAH Partitioning Data from Bioconcentration Studies

With increasing concentration of DOC, the bioconcentration of organic contaminants generally decreases (see HAITZER *et al.*, 1998). This decrease is assumed to be due to the formation of aggregates too large and/or too polar to be taken up by the organism which means that only the freely dissolved contaminant is assumed to be bioconcentrated (LANDRUM *et al.*, 1985, 1987; MCCARTHY *et al.*, 1985; KUKKONEN and OIKARI, 1991; BLACK and MCCARTHY, 1988; KUKKONEN and PELLINEN, 1994; FREIDIG *et al.*, 1998).

We used FAs and HAs from different sources and related differences in their effects on the bioconcentration of PAHs to a number of HS property parameters. 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 and BaP in a non-linear manner (shown in Figure 2 for pyrene and in Figure 3 for BaP). The regression curves were generated using Eqn. 1, and the calculated descent of each curve represents the biologically determined partition coefficient K_{DOC} for the different sources of humic substances. 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 BaP, indicating that Eqn. 1 was suitable to describe the quantitative relationship between DOC concentration and BCF_{48} (HAITZER *et al.*, 1999 b). Resulting K_{DOC} values showed that effects of HS on the bioconcentration of pyrene were about an order of magnitude lower than effects on the uptake of BaP. For both PAHs, the effects of the different HS sources could be divided into three groups: ABV2 FA and FG1 FA clearly had the smallest effects, both for pyrene and for BaP, SV1 FA, BS1 FA and HO13 FA (and HO13 HA for BaP) had intermediate effects and HO10 HA and HO13 HA (for pyrene) had the largest effects. This indicates that,

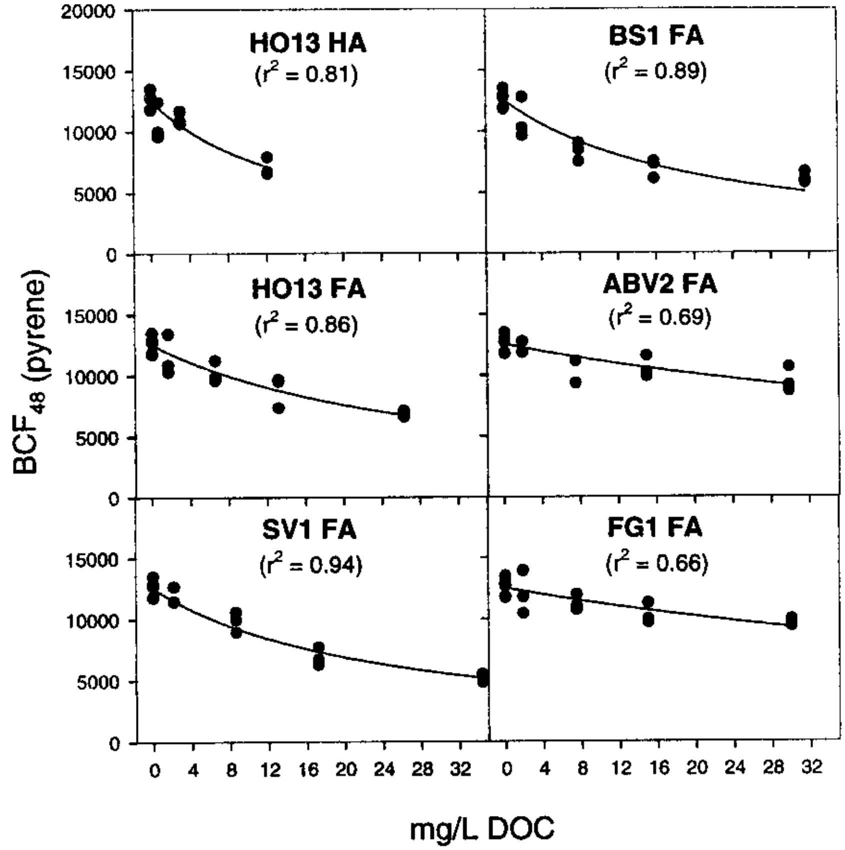

Figure 2. Effect of DOM concentration (in mg l^{-1} DOC) and HS origin on the accumulation of pyrene in the nematode *Caenorhabditis elegans* (from HAITZER *et al.*, 1999b).

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 humic and fulvic acids showed that, with the exception of HO13 HA/BaP, humic acids had higher K_{DOC}s than fulvic acids.

Effects of HA and FA on the bioconcentration of pyrene and BaP were related to physicochemical properties of the humic substances (Figures 4, 5). Pearson correlation coefficients (determined with SPSS 7.5 software, SPSS, Munich, Germany) showed significant correlations between K_{DOC}s and the H/C atomic ratio, the percentage of aromatic carbon, and the copper complexing capacity of humic materials. Correlations of K_{DOC}s with the (O+N)/C ratio were not significant at the 0.05 level. Generally, higher correlation coefficients were reached in the experiments with BaP (Figure 5). For example, the correlation of K_{DOC} with H/C was not significant for pyrene ($p = 0.10$), but significant for BaP ($p = 0.04$). Taking these results together, it can be concluded that substantial amounts of the variation in K_{DOC} could be related to variations in physicochemical properties of HA and FA. For example, 76 to 96% (squared correlation coefficients) of the variation in K_{DOC} for pyrene and BaP could be predicted from the aromaticity or the copper complexing capacity of the HS.

Not surprisingly, the binding of BaP to HS appears to be stronger than those of Pyr, as indicated by the 5-times steeper slope of the BaP-HS interaction.

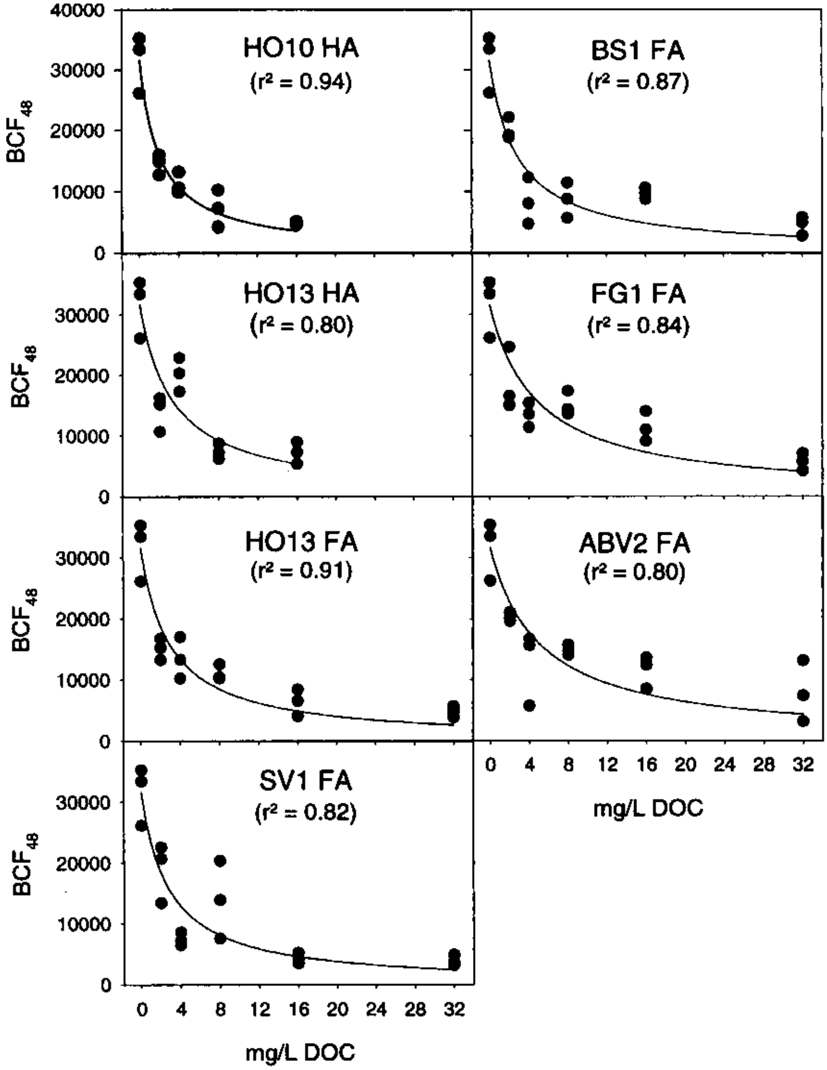

Figure 3. Effect of DOM concentration (in mg l^{-1} DOC) and HS origin on the accumulation of benzo[a]pyrene in the nematode *Caenorhabditis elegans* (from Haitzer *et al.*, 1999 b).

3.2. PAH Partitioning Data from Fluorescence Quenching Experiments

Partitioning coefficients determined by fluorescence quenching also reveal a strong dependence on the aromaticity of HS. The most powerful predictors of the partitioning have shown to be the aromaticity, given as $C_{Ar}\%$ (Figure 6).

The relations can be improved, if the ratio aromatics to aliphatics is taken:

$K_{oc} \times 10^{-5} = 1.23 \times C_{Ar}/C_{Alk} - 0.21$ (pyrene, $r^2 = 0.81$)

$K_{oc} \times 10^{-5} = 0.79 \times C_{Ar}/C_{Alk} - 0.11$ (fluoranthene, $r^2 = 0.74$)

$K_{oc} \times 10^{-5} = 0.64 \times C_{Ar}/C_{Alk} - 0.39$ (anthracene, $r^2 = 0.66$)

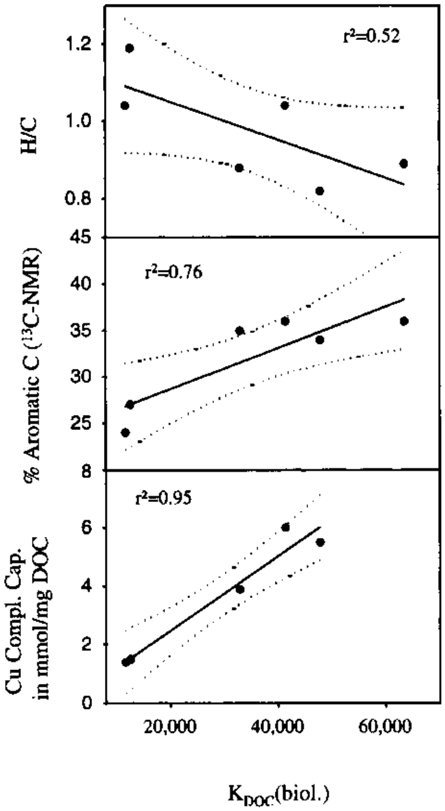

Figure 4. Relationship between partitioning coefficient of pyrene and H/C ratio, aromaticity and Cu complexing capacity of the tested humic substances.

The obtained results are in line with the findings of previous studies (GAUTHIER *et al.*, 1987; CHIN *et al.*, 1997) on an existence of a high correlation between K_{oc} values and percentage of aromatic carbon in the humic material.

Correlations between K_{oc} and the H/C ratio calculated for the same set of HS samples revealed lower r^2 (0.73, 0.71 and 0.60) than those for ^{13}C NMR descriptors. The corresponding r^2 values for a correlation between K_{oc} and O/C were rather low and accounted only for 0.31, 0.34 and 0.25.

The largest part of the DOM pool usually consists of dissolved humic substances (mostly, HA and FA) (THURMAN, 1985; STEINBERG and MUENSTER, 1985), which have also been shown to be the main DOM fraction binding hydrophobic organic contaminants (KUKKONEN *et al.*, 1990; KUKKONEN and OIKARI, 1991). HA and FA are operationally defined fractions of the whole DOM (AIKEN *et al.*, 1985) that can be isolated and concentrated from natural waters by relatively simple procedures (THURMAN and MALCOLM, 1981). Therefore, preparations of isolated HAs and FAs were used instead of natural waters.

The relationships between the best descriptor – aromaticity (= aromatic carbon as % of the total organic carbon) – and K_{DOC} of PAHs under study follow: K_{DOC} = **a** × aromaticity + **b**. The constants are given in Table 2.

Although the data base is limited and two different approaches in determining K_{DOC} have been applied, there appears to exist a clear dependence of K_{DOC} on the K_{OW} of the studied

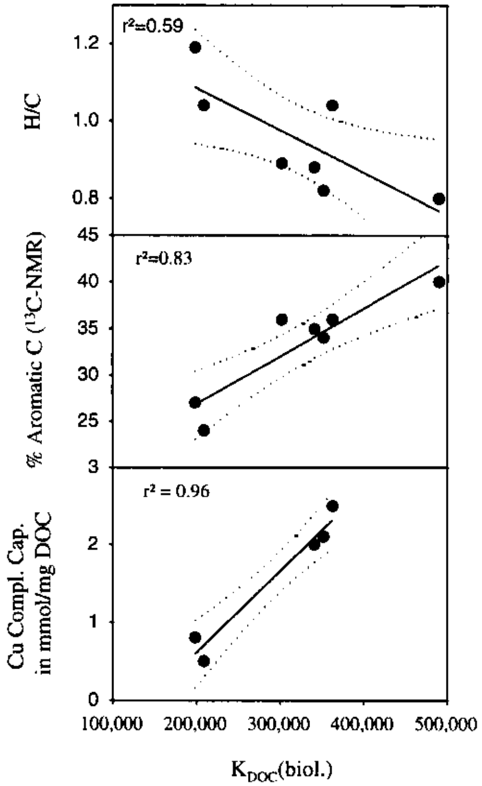

Figure 5. Relationship between partitioning coefficient of benzo[a]pyrene and H/C ratio, aromaticity and Cu complexing capacity of the tested humic substances.

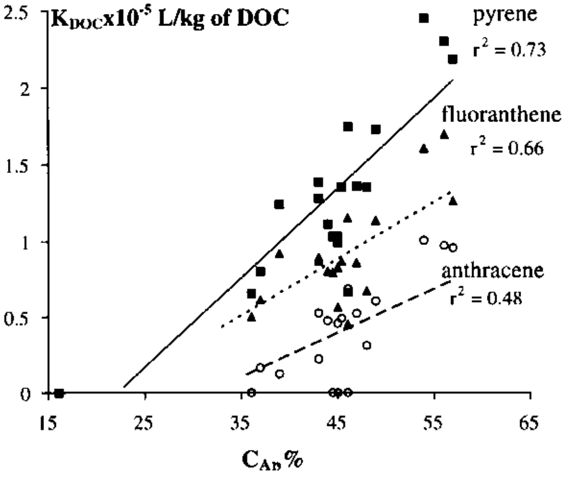

Figure 6. Relationships between partition coefficients for pyrene, fluoranthene, and anthracene and aromaticity of the HS ($n = 19$, $p < 0.01$).

Table 2. Statistical Data of the Regressions $K_{DOC} = a \times \text{aromaticity} + b$.

PAH	**Aromaticity**	$a \times 10^4$			**b**	r^2	n
		fluorescence quenching	biologically determined				
			Geom. Regr.	Arith. Regr.			
Pyr	% Ar	0.587			– 1.30	0.81	19
	% Ar		0.39		– 8.88	0.75	7
				0.34	– 7.25	0.75	7
Flt	% Ar	0.367			– 0.76	0.74	19
Ant	% Ar	0.272			– 0.81	0.66	19
BaP	% Ar		1.77		– 26.2	0.87	7
				1.62	– 21.62	0.83	7

PAH. The slope of the corresponding relationship is almost equal to 1 (Figure 7). The last finding brings us to the discussion about driving forces of binding of PAHs to dissolved humic materials. GAUTHIER *et al.* (1987) considered hydrophobic binding caused by the "thermodynamic gradient" which drives the low soluble PAHs out of the aqueous phase and inductive van der Waals interaction as the main mechanism of binding of PAH to HS This concept is in good agreement with the findings on higher affinity of more hydrophobic PAHs for binding to HS, which was also confirmed in this study by the relationship given in Figure 7. However, it does not give a satisfactory explanation to the numerous findings (including this study) on the existence of a strong direct relationship between aromaticity of humic macromolecules and their K_{DOC}. This suggests that the binding of PAH to HS may be a result of combination of specific and nonspecific interactions, rather than of nonspecific ones (hydrophobic) alone.

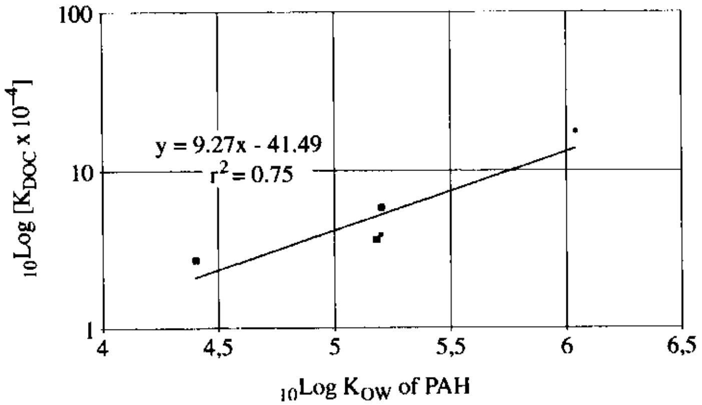

Figure 7. Dependence of K_{DOC} on K_{OW} of PAHs (Data from Tables 1 and 2). Black square represent chemically determined K_{DOC} and black circles biological determined ones.

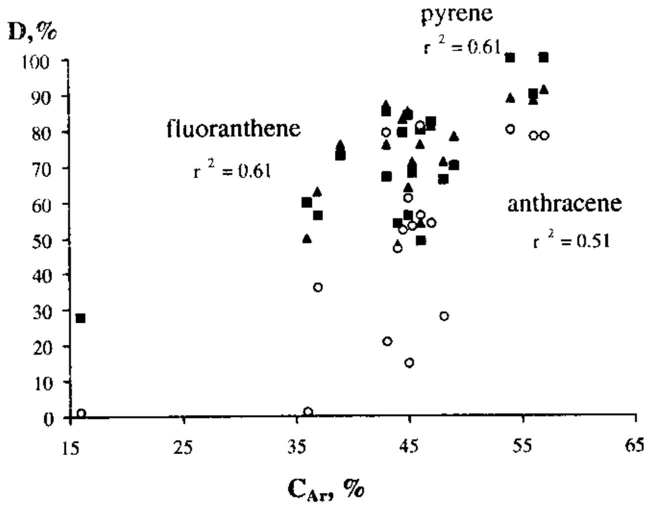

Figure 8. Scatter plot for HS aromaticity and their detoxification ability in relation to PAH.

3.3. Detoxification

As shown above by means of biological as well as chemical approaches, the association of HS and PAHs reduces the freely dissolved species of the toxicants. This leads to the reduction in bioconcentration of PAHs in the presence of HS as shown above (Figures 2, 3; HAITZER *et al.*, 1999b) and must also result in a decrease in toxicity. This hypothesis has been tested with *Daphnia* grazing activity as a sensitive toxicity endpoint. The results are expressed as detoxification quotient, **D**. In Figure 8, D is shown for pyrene, fluoranthene and anthracene. It is obvious that, as expected, increasing aromaticity of HS leads to a decrease in the toxic effect to *Daphnia magna*.

4. Conclusion

Humic substances have been shown to cause a decrease both in bioconcentration and toxicity of PAHs in the aquatic environments. Quantitative relationships between the mitigating impact and structure of HS have been observed. Descriptors of aromaticity (percentage of aromatic carbon and ratio of aromatic to aliphatic carbon) have been found to be the most powerful predictors of the mitigating impact of HS to PAHs. The latter was evaluated from the corresponding data on bioconcentration and toxicity of PAHs in the presence of HS. Both electron donor-acceptor and hydrophobic interactions were suggested to be the main driving forces of binding of PAHs to HS, therefore the next step might be to characterize the kind of donor-acceptor bondings in more detail. The formation of HS associates which are not bioavailable has been shown to underlie the mechanism of self-purification of freshwater ecosystems contaminated with highly lipophilic xenobiotics.

5. Acknowledgement

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Glossary

DOM	pool of dissolved organic matter defined as passing the membranes with 0.45 µm pore diameters
DOC	dissolved organic carbon – content of DOM normalized to the content of organic carbon
DHM	dissolved humic material
HS	humic substances
FA	fulvic acids, fraction of HS soluble in both alkaline and acidic solutions
HA	humic acids, fraction of HS, not soluble at $pH < 2$
PAH	polycyclic aromatic hydrocarbon
anthracene	a three-ringed linear PAH
fluoranthene	a four-ringed angular PAH
pyrene	a four-ringed angular PAH
BaP	benzo[a]pyrene, a five-ringed angular PAH
NMR	nuclear magnetic resonance
CPMAS NMR	solid state cross polarization magic angle spinning NMR
TMS	tetramethylsilane (NMR standard)
C_{Al}	percentage of aliphatic carbon
C_{Ar}	percentage of aromatic carbon as determined by integration of the ^{13}C NMR spectra
CuCC	copper(II) complexation capacity of HS
H^+-CAP	proton capacity of HS
bioconcentration	uptake and concentration of a heavy metal or an organic chemical via epithelia or cell membrane
BCF	bioconcentration factor
D	detoxification coefficient
fluorescence quenching	decrease in a fluorescence of a fluorophor (PAH) due to formation of the non-fluorescing complexes with a quencher (HS)
K_{DOC}	partitioning coefficient of PAH between water and dissolved organic matter normalized to organic carbon
K_{OW}	partitioning coefficient of the hydrophobic organic chemical between *n*-octanol and water
R	grazing activity of *Daphnia magna* under various conditions, as given in the text
SAC	specific absorption coefficient at a given wave length, e.g. 254 nm