



Binding characteristics of perylene, phenanthrene and anthracene to different DOM fractions from lake water

MEI Yi¹, WU Fengchang^{1, 2, *}, WANG Liying¹, BAI Yingchen¹, LI Wen¹, LIAO Haiqing²

1. State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, China.

E-mail: meiyi@mails.gyig.ac.cn

2. State Environmental Protection Key Laboratory for Lake Pollution Control, Chinese Research Academy of Environmental Sciences, Beijing 100012, China

Received 24 April 2008; revised 08 June 2008; accepted 28 August 2008

Abstract

Six hydrophobic and hydrophilic fractions were isolated using XAD-8 and XAD-4 resins, and were extensively characterized. Partition coefficients of perylene, phenanthrene and anthracene binding to the six fractions were determined by fluorescence quenching titration. The K_{doc} values obtained for the polycyclic aromatic hydrocarbons (PAHs) binding to the hydrophobic fractions were larger than those to the hydrophilic fractions. Nonlinear Stern-Volmer plots were observed when binding phenanthrene and anthracene to some hydrophilic fraction samples, suggesting saturation of polar interaction binding sites. A significant correlation of $\log K_{\text{doc}}$ values with molecular weights and molar absorptivities at 280 nm was observed, while atomic ratio of C/H was found to be a poor indicator for aromaticity. Other structural descriptors such as paraffinic carbon and polarity influenced the DOM-fraction ability to bind PAHs. Different interaction mechanisms underlying binding of the different fractions to the PAHs were also discussed.

Key words: fluorescence quenching; humic substances; polycyclic aromatic hydrocarbons (PAHs); partition coefficient; binding capacity

DOI: 10.1016/S1001-0742(08)62285-8

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic substances composed of at least two condensed aromatic ring structures. Most of them are toxic and carcinogenic, and tend to bioaccumulate into aquatic organisms (Landrum and Fisher, 1999). However, the adverse effects can be reduced when they bind to dissolved organic matters (DOM). Many studies have shown the crucial significance of DOM in controlling toxicity and bioavailability of polycyclic aromatic hydrocarbons in aquatic ecosystems (McCarthy and Jimenez, 1985; Kukkonen *et al.*, 1990). Therefore, the determination or prediction of the intensity of PAHs-DOM interaction, which is commonly defined as the partitioning coefficient (K_{doc}) normalized to the mass of dissolved organic carbon, is very important. The K_{doc} values of various PAHs binding to DOM from different sources were determined by many scientists with various approaches (Henry, 2002). Generally, those studies showed that the magnitude of K_{doc} largely depends on the hydrophobicity of PAHs, solution chemistry and properties including its origin, polarity, molecular weight, aromaticity, and aliphaticity (Gauthier *et al.*, 1987; Xing *et al.*, 1994; Chin *et al.*, 1997; Chiou *et al.*, 1998; Haitzer *et*

al., 1999; Chefetz *et al.*, 2006; Peuravuori, 2001; Salloum *et al.*, 2002; Mao *et al.*, 2002; Gunasekara *et al.*, 2003; Feng *et al.*, 2006; Wang *et al.*, 2007). Nevertheless, a rising controversy of whether the aromatic or aliphatic structure in DOM is responsible for predominant sorption of PAHs is still ongoing. Some studies attributed the magnitude of K_{doc} value to the aromatic carbon content of DOM (Gauthier *et al.*, 1987; Xing *et al.*, 1994; Chin *et al.*, 1997; Chiou *et al.*, 1998; Haitzer *et al.*, 1999; Xing, 2001; Akkanen *et al.*, 2004), while the others highlighted the importance of the aliphatic (paraffinic) carbon in PAHs sorption capacity (Salloum *et al.*, 2002; Mao *et al.*, 2002; Gunasekara *et al.*, 2003; Kang and Xing, 2005). The molecular weights of DOM were regarded as correlation with sorption capacity for PAHs (Herbert *et al.*, 1993; Chin *et al.*, 1997; Haitzer *et al.*, 1999; Peuravuori, 2001; Hur and Schlautman, 2004; Marschner *et al.*, 2005). Several investigations indicated that a trend of decreased partitioning coefficients was attributed to increasing DOM polarity (Chen *et al.*, 1996, 2005; Kang and Xing, 2005; Wang *et al.*, 2007). However, Chin *et al.* (1997) observed inverse relationship of pyrene sorption capacity with polarity of aquatic humic and fulvic acid where K_{doc} value actually increased with increasing oxygen content. They concluded that the oxygen content of humic material is a poor indicator for its ability to bind pyrene. Haitzer

* Corresponding author. E-mail: wufengchang@vip.skleg.cn

et al. (1999) also concluded that the polarity of humic materials did not significantly influence their sorption capacity for pyrene. The contradictory observations are not surprising if it has been realized that the humic substances used by different investigators originated from a variety of different sources and were isolated by various fractionation methods. Previous investigations showed that DOM originating from different sources generally had various physicochemical characteristics because of its high heterogeneity (Thurman, 1985; Stevenson, 1994). For instance, DOM isolated from soils and sediments generally had a higher molecular weight (MW), hydrophobicity and carbon as well as aromatic carbon content than its aquatic counterpart (Malcolm, 1990; Rice and MacCarthy, 1991; Stevenson, 1994). The similar trend was also observed when comparing aquatic DOM mainly originated from large amounts of land plants input with that derived from microbial source such as phytoplankton and bacteria (Chin *et al.*, 1997). Previous studies showed that sorption ability of soils and commercial humic substances for PAHs were stronger than their aquatic counterparts (Gauthier *et al.*, 1987; Chin *et al.*, 1997). On the other hand, separation and isolation approaches were quite different for humic substances derived from various sources, which might result in various physicochemical properties in DOM components or fractions (Aiken *et al.*, 1985; Shin *et al.*, 1999). In a word, it is inappropriate to discuss the impact of DOM physicochemical properties on sorption ability for PAHs, without the consideration of its origin and isolation approaches.

DOM in natural waters is a complex mixture composed of heterogeneous organic compounds, and its physicochemical properties are still not clearly understood. According to its original source, aquatic DOM can be classified as (1) allochthonous DOM, mainly originated from terrestrial organic substances; and (2) autochthonous DOM, derived from aquatic biota (e.g., algae and bacteria). Aquatic humic substances (hydrophobic fraction of aquatic DOM) derived from higher plants constituted approximately 40%–60% of aquatic DOM, and their interaction with PAHs had been studied by many scientists (Haitzer *et al.*, 1999; Peuravuori, 2001; Chefetz *et al.*, 2006). However, the role of the nonhumic materials (hydrophilic fraction isolated by XAD-4 resin) microbially derived organics from algae and bacteria in binding to PAHs was rarely investigated due to the difficulty of isolation.

In this study, six humic and non-humic fractions were isolated using XAD-8 and XAD-4 resins based on Leenheer's fractionation protocol (Leenheer, 1981) and extensively characterized using many analytical techniques, e.g., elemental analysis, high-performance size exclusion chromatography (HPSEC), total dissolved organic carbon analysis and specific UV absorbance. The K_{doc} values of the DOM-fractions binding to perylene, phenanthrene, and anthracene were determined by fluorescence quenching; furthermore, the influence of molecular weights, molar absorptivities at 280 nm and atomic ratios of C/H, (O+N)/C on the magnitude of the partitioning coefficients were also evaluated.

The objectives of this study were to compare (1) the chemical characteristics of the hydrophobic fractions with the hydrophilic ones, and (2) the differences of chemical characteristics of the two fractions in controlling binding capacity for different PAHs.

1 Material and methods

1.1 Reagents and chemicals

The polycyclic aromatic hydrocarbons used were perylene (99.9% pure, Sigma-Aldrich, St. Louis, USA), phenanthrene (99% pure, Aldrich, Steinheim, Germany) and anthracene (99% pure, Sigma-Aldrich, St. Louis, USA). Concentrated stock solutions were obtained by dissolving PAHs in methanol. Amherst peat humic acid was kindly given by Dr. Baoshang Xing (University of Massachusetts, USA). Concentrated stock solutions of freeze-dried HA fraction and Amherst peat HA were prepared in 0.01 mol/L NaOH and then diluted with Milli-Q water (18.2 M Ω -cm, Millipore). The other solutions of DOM fractions were obtained by dissolving freeze-dried samples in Milli-Q water.

1.2 Sample collection

Lake Hongfeng, the main source of drinking water for Guiyang City, is located in Southwestern China Plateau, 33 km southwest of the city. Because of its high loads of organic matter and nutrients from industry, agriculture and municipal wastewater effluents, the lake has become more mesotrophic. A total volume of 1300 L surface water was collected from the centre of the southern lake in July 2005. The water samples were immediately brought back to our laboratory and then filtered through a precombusted (450°C for 5 h) Whatman GF/F glass fiber filters for further fractionation.

1.3 DOM fractionation

Six fractions of the lake DOM were isolated using XAD-8/4 resin and the fractionation procedures are summarized as following: briefly, the filtered original water was acidified with HCl to pH 2 and then flowed through the XAD-8/XAD-4 resin columns containing approximately 2 L of resin slurry at a rate of 2 bed volumes per hour (i.e., 4 L/h). The hydrophobic acid (HOA) which was composed of humic acid (HA) and fulvic acid (FA) absorbed onto the XAD-8 resin was eluted with 0.1 mol/L NaOH, acidified with HCl to pH 1, settled for 24 h and centrifuged. The precipitation was HA, while the suspension (FA fraction) was reabsorbed on a smaller XAD-8 column and eluted with 0.1 mol/L NaOH. The XAD-8 resin was dried in the air and Soxhlet-extracted with methanol which contained hydrophobic neutral fraction (HON). All hydrophilic DOM fractions absorbed onto the XAD-4 was eluted with 0.1 mol/L NaOH and adjusted to pH 2 with HCl, and then the solution was pumped through the MSC-H cation-exchange resin (hydrogen ion saturated). The hydrophilic bases (HIB) were eluted with 1 mol/L NH₄OH, while the effluent was pumped through another column containing

Duolite A7 anion-exchange resin. After the hydrophilic acid fraction (HIA) was eluted with 3 mol/L NH_4OH , the final effluent was hydrophilic neutral fraction (HIN). All fractions were dried using vacuum evaporator at 35°C , dialysed and freeze-dried to a low-ash solid form.

1.4 Dissolved organic carbon and molecular weight distribution measurement

The DOC concentrations were determined by high TOC/N II analyzer (Elementar, Germany) according to high temperature catalytic oxidation method. Molecular weight distribution was obtained using HPSEC (Agilent 1100, PE) using UV detector at 254 nm as well as an YMC-60 column (Waters, Milford, MA) packed with a silica diol modified material of 5 μm gel bead in diameter, 6 nm in pore size. The flow rate of mobile phase consisted of phosphate buffer (0.001 mol/L Na_2HPO_4 , 0.001 mol/L NaH_2PO_4 , and 0.03 mol/L NaCl) was controlled at 0.5 mL/min. All freeze-dried DOM-fractions were dissolved in phosphate buffer which was identical in composition to that of HPSEC mobile phase, and then the solutions were filtered through pre-combusted GF/F glass fiber filter (450°C for 5 h, Whatman, UK). The number (M_n) and weight-averaged (M_w) molecular weight and polydispersity (ρ) for the fractions were determined using the reported methods (Chin *et al.*, 1997).

1.5 Absorbance and fluorescence measurements

Absorbance was obtained with Shimadzu UV-3000 double beam spectrophotometer (Shimadzu, Japan) in 1 cm quartz cells to correct the inner filter effects and calculate molar absorptivities at 280 nm.

Fluorescence intensity was collected using Hitachi F-4500 (Hitachi, Japan) with a 150-W xenon arc lamp as well as a cuvette magnetic stirring system containing 9.5×7.5 mm Teflon micro stir bars. The photomultiplier voltage is set at 700 V; excitation and emission bandwidth are correspondingly set at 5 and 10 nm. Fluorescence intensities were conducted at excitation/emission wavelengths of 435/470 nm, 250/362 nm, and 355/400 nm for perylene, phenanthrene and anthracene, respectively. Fluorescence quenching is based on the Stern-volmer equation, which explains the static quenching of a fluorophore derived by Gauthier *et al.* (1986). Previous investigations showed that fluorescence quenching of PAHs by DOM was attributed to static quenching mechanisms in light of temperature studies, diffusion and fluorescence efficiency calculations

(Traina *et al.*, 1990; Chen *et al.*, 1994).

Five dilutions of Amherst peat HA or DOM-fractions were prepared after adjustment to pH 6 with HCl and NaCl, and all samples were bubbled for 10 min with pure argon in the dark to reach anoxic condition. The Raman peak (348 nm/397 nm) of distilled water were recorded to check instrument stability during the experiment and no obvious variations of the peak fluorescence intensity were observed. A 3.00-mL aliquot of each dilution was pipetted to a cuvette, and an absorption scan from 250 to 470 nm was conducted. The cuvette was then moved to the fluorescence spectrometer to obtain the DOM background fluorescence intensity. An aliquot of PAHs stock solution prepared in methanol was spiked into the cuvette to a final concentration of 0.3, 16, and 40 $\mu\text{g/L}$ for perylene, phenanthrene and anthracene, respectively, according to their solubility and fluorescence intensity. The volumetric content of methanol did not exceed 0.1% (V/V), which was found to have no apparent influence on PAHs sorption experiment. After a 9.5×7.5 mm Teflon micro stir bar was put into the cuvette, the cuvette was immediately put to the cuvette holder in the fluorescence spectrometer and stirred for 3 min. The cuvette allow to settle for another 2 min for fluorescence measurement. After initial spiking, the fluorescence intensities of all samples were recorded at 5, 7, and 9 min. Since PAHs are intensely photodegradable under ultraviolet light, the shutter was closed during the stirring and the analysis intervals. The averages of the K_{doc} values were used as the final result.

2 Results and discussion

2.1 DOM fractions characterization

Table 1 shows the M_n , polydispersity and ϵ_{280} values and atomic ratios of the isolated DOM-fractions. Based on DOC analysis, all fractions accounted for approximately 94% of the total DOC in the original water, which is similar to the recovery rate of reported literature (Leenheer, 1981), e.g., 95% in waste water and 115% in river. Both the M_w and M_n of the hydrophobic fractions were much larger than those of the hydrophilic fractions. The ϵ_{280} values for the DOM-fractions varied as the same trends as the molecular weights do, namely, the values of hydrophobic fractions were generally larger than those of hydrophilic fractions, varying in the range 122–190. This result agrees with the similar observation reported by Chin *et al.* (1994), suggest-

Table 1 $\log K_{\text{doc}}$ values of perylene, phenanthrene and anthracene with Amherst HA and the DOM fractions, molecular weight, polydispersity, atomic ratios and UV results (ϵ_{280})

Sample	M_n	Polydispersity	Atomic ratios		ϵ_{280}	$\log K_{\text{doc}}^a$		
			C/H	(O+N)/C		Perylene	Phenanthrene	Anthracene
Amherst HA ^b	3200	1.10	0.98	0.60	564	5.94 ± 0.01	4.89 ± 0.10	4.88 ± 0.03
HA	2355	1.22	0.80	0.89	190	5.66 ± 0.05	4.43 ± 0.05	4.05 ± 0.07
FA	1745	1.39	0.80	1.28	143	4.83 ± 0.07	4.16 ± 0.06	nd
HON	2099	1.57	0.57	0.60	174	5.33 ± 0.06	4.53 ± 0.01	4.22 ± 0.03
HIA	1746	1.25	1.45	1.65	152	5.20 ± 0.08	4.25 ± 0.03	nd
HIB	1903	1.19	1.48	1.03	148	5.03 ± 0.05	4.31 ± 0.06	4.01 ± 0.07
HIN	1688	1.24	1.33	1.41	122	4.86 ± 0.06	nd	nd

M_n : number-averaged molecular weight; ^a data are expressed as mean value \pm standard deviation; ^b Mao *et al.*, 2002.

ing a correlation between the weight-averaged molecular weights and the ϵ_{280} values.

Equation (1) was obtained from the regression analyses based on the data listed on Table 2:

$$\log MW_w = 1.68 + 0.73 \log \epsilon_{280} \quad r^2 = 0.82 \quad (1)$$

Because the molar absorptivity at 280 nm was related to the amount of aromatic moieties existed in DOM structures (Gauthier *et al.*, 1987; Chin *et al.*, 1997), the data listed on Table 2 and Eq. (1) revealed that the hydrophobic fractions contained larger amount of aromatic moieties than the hydrophilic ones.

Comparing to HON fraction, the acidic fractions were characterized by higher oxygen content and lower C/O ratios, indicating most of the acidic groups in the fractions may be carboxylic.

2.2 Partition coefficients determined by the Stern-Volmer plots

Figure 1 displays the Stern-Volmer plots of the 6 DOM-fractions binding to perylene, phenanthrene and anthracene. Not all plots obtained were linear. Generally, the plots of the DOM-fractions binding to perylene were more linear than those of binding to phenanthrene and anthracene. Furthermore, the linearity of the hydrophobic fractions binding to the three PAHs was relatively higher than that of hydrophilic fractions. No substantial fluorescence quenching was observed when phenanthrene sorbed with HIN fraction (plot l, Fig. 1) as well as FA, HIA and HIN fraction with Anthracene (plots n, p and r, Fig. 1) such that the Stern-Volmer plot was not applicable for determining partition coefficients of the four samples. This indicates that PAHs could not strongly bind to carbohydrates such as polysaccharides.

Nonlinear sorption isotherm was observed and discussed in the investigation of PAHs sorption with soil organic matter (Xing, 2001; Salloum *et al.*, 2002; Kang and Xing, 2005). However, the nonlinear sorption between PAHs and aquatic DOM was rarely reported. Laor and Rebhun (2002) observed concave-up curved Stern-Volmer plots as they investigated interaction of pyrene, fluoranthene and phenanthrene with different humic acids using fluorescence quenching and complexation-flocculation methods. They argued that the nonlinear isotherms resulted from combined partitioning and adsorptive mechanism in light of the dual-mode adsorption model (DMAM) described by Xing and Pignatello (1997).

It is well known that the interaction of PAHs with aquatic DOM was usually attributed to non-specific partitioning between bulky DOM and water, and the partition coefficient (K_{doc}) was expected to be independent of PAH concentrations due to the unlimited binding sites in bulky DOM. Furthermore, in a partitioning mechanism, a linear sorption isotherm was expected. Therefore, when a K_{doc} value decrease with increasing PAHs concentration is observed, it indicates the presence of adsorptive interaction at limited binding sites in bulky DOM. This results in a nonlinear sorption isotherm of PAHs-DOM interaction. Because the sorption domains were mainly restrained to high energy sorption sites, sorbate molecules tended to occupy those sites at low concentration. Therefore, sorption process mostly occurred at low concentration (Gunasekara *et al.*, 2003). Since aquatic DOM-fractions contained both polar and nonpolar functional groups, apparent differences in the binding of the three PAHs (different hydrophobicity or $\log K_{ow}$ values) may be due to the combining interaction with aquatic DOM-fractions via hydrophobic interaction (dispersion force or London force), π - π electron donor-acceptor interaction (EDA) and H-bonding (Goss and Schwarzenbach, 2001; Nguyen *et al.*, 2005). A linear plot was obtained only when hydrophobic interaction was predominant binding mechanism (i.e., partitioning), while the PAHs-DOM complex formation dominated by EDA interaction and H-bonding may lead to adsorptive mechanism with nonlinear isotherm. As illustrated in Fig. 1, higher F_0/F ratios were observed in lower DOM-fractions concentrations (2–4 mg/L OC) in some plots, and the F_0/F ratios slightly decreased with increasing DOM-fraction concentration, which was especially apparent when binding of phenanthrene and anthracene to the hydrophilic fractions. This indicates the saturation of polar interaction binding sites in DOM-fractions, resulting in a decreasing of F_0/F ratios with the increasing of fractions concentration. The higher linear Stern-Volmer plots of perylene binding to Amherst HA (plots not shown) and HA fraction suggested predominant partitioning mechanism resulting from hydrophobic interaction, while the interaction of the hydrophilic fractions with phenanthrene and anthracene may mainly dominate by EDA interaction and H-bonding. Other studies also observed nonlinear sorption isotherms of pyrene sorption with DOM isolated from treated municipal wastewater (Borisover *et al.*, 2006) and phenanthrene with different HAs (Pan *et al.*, 2007), and they attributed their observations to the saturation of binding sites in bulk

Table 2 Partition coefficients (K_{doc}) for perylene, phenanthrene and anthracene binding to amherst HA and the six DOM fractions determined by the Stern-Volmer plots

Sample	DOC range (mg/L C)	Perylene		Phenanthrene		Anthracene	
		K_{doc}^a ($\times 10^3$ L/kg C)	<i>P</i>	K_{doc}^a ($\times 10^3$ L/kg C)	<i>P</i>	K_{doc}^a ($\times 10^3$ L/kg C)	<i>P</i>
Amherst HA	0–10.0	890.7 \pm 15.5	0.0009	78.9 \pm 14.5	0.0020	78.4 \pm 5.8	0.0023
HA	0–12.5	466.5 \pm 55.4	0.0015	27.0 \pm 5.88	0.0020	10.9 \pm 4.6	< 0.0001
FA	0–10.0	67.50 \pm 33.0	0.0035	14.5 \pm 5.50	0.0004	na	na
HON	0–10.0	212.3 \pm 28.6	0.0226	34.2 \pm 0.62	0.0478	16.7 \pm 4.1	0.0500
HIA	0–12.5	161.3 \pm 29.5	0.0009	18.1 \pm 1.31	0.0463	na	na
HIB	0–15.0	108.3 \pm 16.1	0.0224	0.0171	20.1 \pm 3.02	0.0600	10.3 \pm 2.8
HIN	0–12.5	77.20 \pm 14.1	0.0004	na	na	na	na

^a Mean \pm standard deviation; na: not applicable.

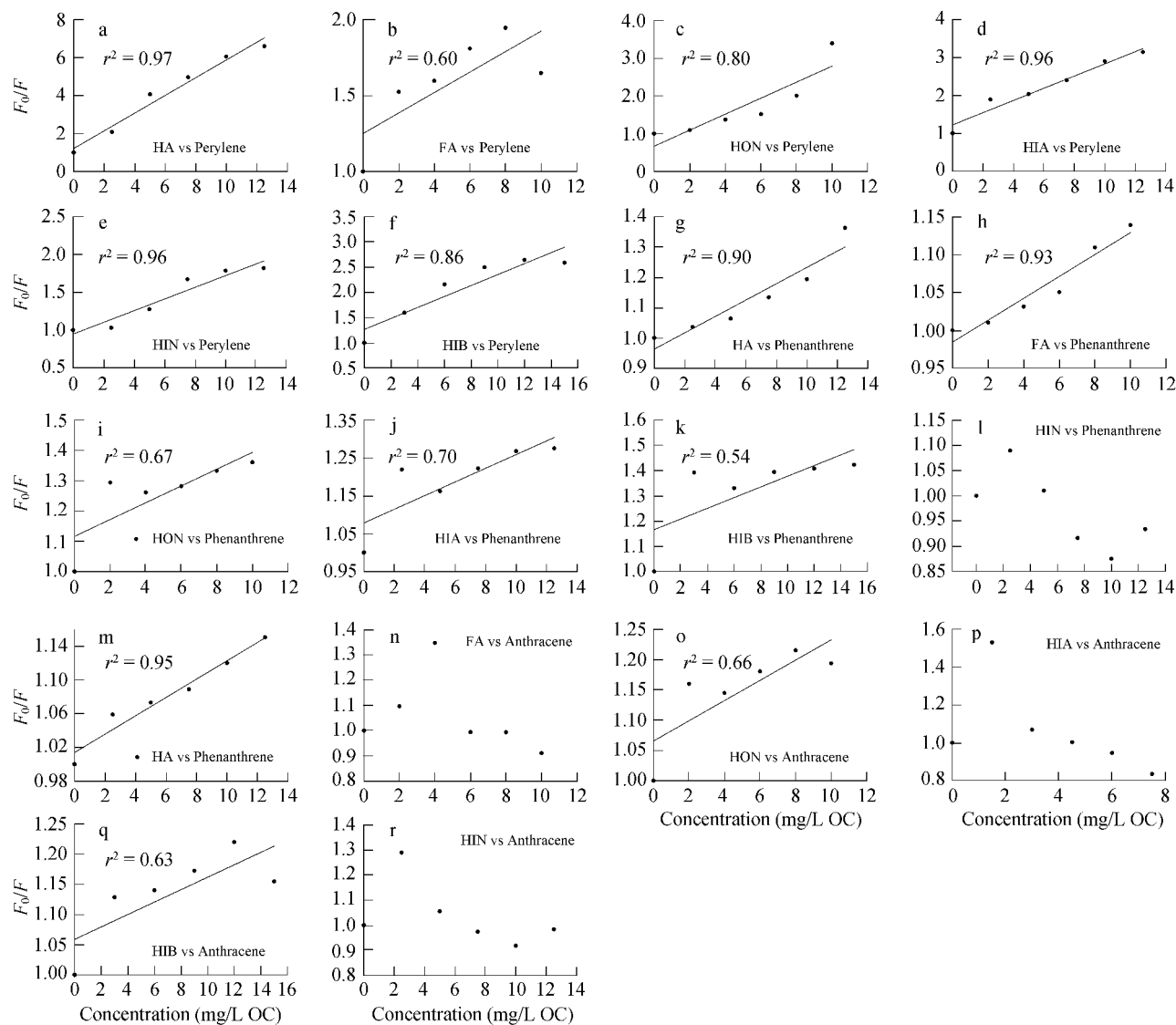


Fig. 1 The Stern-Volmer plots of the six DOM fractions binding to perylene, phenanthrene, and anthracene.

DOM.

Table 2 shows the values of $\log K_{\text{doc}}$ and K_{doc} for the three PAHs binding to Amherst HA and the six DOM-fractions. The results were well consistent with the literatures.

The $\log K_{\text{doc}}$ values in this study exhibited in the range of 4.83–5.94, 4.16–4.89, and 4.01–4.88 for perylene, phenanthrene, and anthracene, respectively. The reported $\log K_{\text{doc}}$ values for perylene binding to Suwannee River humic and fulvic acid with fluorescence quenching were in the range of 5.14–5.95 (Schlautman and Morgan, 1994), while the reported $\log K_{\text{doc}}$ values were in the range of 4.08–4.78 and 4.15–4.81 for phenanthrene and anthracene binding to a variety of different humic substances, respectively (Chiou *et al.*, 1998; Laor *et al.*, 2002; Salloum *et al.*, 2002; Durjava *et al.*, 2007).

As expected, Amherst HA exhibited the largest binding capacity for three PAHs, which further confirms the similar observations by other investigators when comparing binding ability of PAHs for soils or commercial humic acids with those for aquatic humic substances (Gauthier

et al., 1987; Chin *et al.*, 1997). As for the DOM fractions, the partition coefficients of the hydrophobic fractions were generally greater than those of the hydrophilic fractions. An exception was FA fraction which exhibited smaller K_{doc} values than HIA and HIB fractions when binding to phenanthrene and anthracene. Interestingly, HON fraction showed the largest capacity for phenanthrene and anthracene, followed by HA fraction, while HA exhibited the largest affinity to perylene. The high PAHs binding ability of HON fraction may result from enhanced EDA interaction besides the hydrophobic interaction for large amounts of electron-acceptor methyl and carbonyl groups existed in HON molecular structures could accept electrons from electron-donor PAHs. This can be verified by the Stern-Volmer plots of HON fraction binding to phenanthrene (plot i, Fig. 1) and anthracene (plot o, Fig. 1) which illustrated the high F_0/F ratio at the low concentration (2 mg/L OC) and slight nonlinear plots. Another possible reason may be ascribed to its lower polarity, which will be discussed latter.

Generally, perylene K_{doc} values ranged from 5- to 10-

jescc.cn

fold higher than for phenanthrene or anthracene. Some of the phenanthrene and anthracene K_{doc} values can not be determined by the linear regression plots of Stern-Volmer equation due to no apparent fluorescence quenching observed (phenanthrene for HIN; anthracene for FA, HIA and HIN fraction). This can be attributed to the lower hydrophobicity of phenanthrene and anthracene ($\log K_{\text{ow}} = 4.52, 4.45$ for phenanthrene and anthracene, respectively) in comparison with perylene ($\log K_{\text{ow}} = 6.15$) and is consistent with previous reports.

2.3 Correlations between partition coefficients and molecular descriptors

2.3.1 Relationships between partition coefficients and aromaticity

It is well known that binding of neutral PAHs molecules to humic substances was dominated by dipole-induced dipole interactions, namely dispersion force or London force (Gauthier *et al.*, 1987; Peuravuori, 2001). Neutral PAHs were not very soluble in water, thus subject to the additional thermodynamic gradient driving them out of water into DOM matrix. Hydrophobic bonding is the combination of both the driving force and dispersion force. The magnitude of inducing dipole moment was related to the polarity of the molecules, which can be estimated by the sum of bond polarity. Therefore, the increase number of conjugated double bond ($-\text{HC}=\text{CH}-$) will increase the molecular polarity and thereby increase the strength of dispersion force. As a result, the increase of aromaticity of DOM will increase the polarity of the molecules; accordingly, increase PAHs binding capacity (Gauthier *et al.*, 1987; Peuravuori, 2001). The atomic C/H ratio was considered as surrogate for unsaturated carbon content in DOM structures and used for the indirect estimation of the aromaticity (Gauthier *et al.*, 1987; Tanaka *et al.*, 1997; Peuravuori, 2001). However, Gauthier *et al.* (1987) pointed out that a large uncertainty might remain when using the atomic C/H ratio as an estimation of the magnitude of unsaturated carbon content especially in the presence of large amounts of carboxyl groups owing to the fact that both COOH group and $-\text{HC}=\text{CH}-$ had the same atomic C/H ratio. This is verified by our observations. As shown in Table 1, the hydrophilic fractions generally exhibited

larger atomic C/H ratio than the hydrophobic fractions and Amherst HA did; nevertheless, the $\log K_{\text{doc}}$ values of hydrophilic fractions binding to the three PAHs were smaller than those of the hydrophobic counterparts and Amherst HA. The HON fraction showed the smallest atomic C/H ratio with larger $\log K_{\text{doc}}$ values, while the FA fraction held moderate atomic C/H ratio with the smallest $\log K_{\text{doc}}$ value. Therefore, the atomic C/H ratio values no longer represented the unsaturated carbon content and showed no correlation with $\log K_{\text{doc}}$ values. Tanaka *et al.* (1997) also reported the similar findings when investigating the effect of humic substances on water solubility enhancement of pyrene.

On the other hand, the UV light absorbance of DOM depends on the electronic structure of DOM macromolecules. The UV absorbance in the range of 254–280 nm was in the region of $\pi-\pi^*$ electronic transitions in conjugated system of bulky DOM and can be used as an indicator for aromaticity (Gauthier *et al.*, 1987; Traina *et al.*, 1990; Chin *et al.*, 1997). Furthermore, the correlation between UV absorbance in the range of 254–280 nm and aromaticity was further confirmed by ^{13}C nuclear magnetic resonance (^{13}C NMR) spectra conducted by Weishaar *et al.* (2003). Figure 2 shows the relationship between ϵ_{280} and molecular weights with $\log K_{\text{doc}}$ for perylene and phenanthrene.

A high correlation ($r^2 = 0.88$ for perylene and 0.68 for phenanthrene, respectively) of the molar absorptivity of the DOM fractions with the $\log K_{\text{doc}}$ values was observed (Fig. 2), corroborating the similar observations obtained by other researchers (Gauthier *et al.*, 1987; Chin *et al.*, 1997; Tanaka *et al.*, 1997). Chin *et al.* (1997) explained the correlation of molecular weights with the number of aromatic moieties in their structures, namely larger molecules possessed greater degree of aromaticity, which was verified by the results from Eq. (1) and the $\log K_{\text{doc}}-\text{LogMW}_w$ plots (Fig. 2) in this study. The r^2 values of $\log K_{\text{doc}}-\text{LogMW}_w$ for perylene and phenanthrene obtained by the linear regression plot were 0.82 and 0.74, respectively. As illustrated in Fig. 2, the two plots were similar, namely with sharp slope for the upper curve and gently slope for the lower one. This suggests that the $\log K_{\text{doc}}$ values of perylene were more sensitive to the variation of ϵ_{280} and molecular weights, whereas the $\log K_{\text{doc}}$ values of

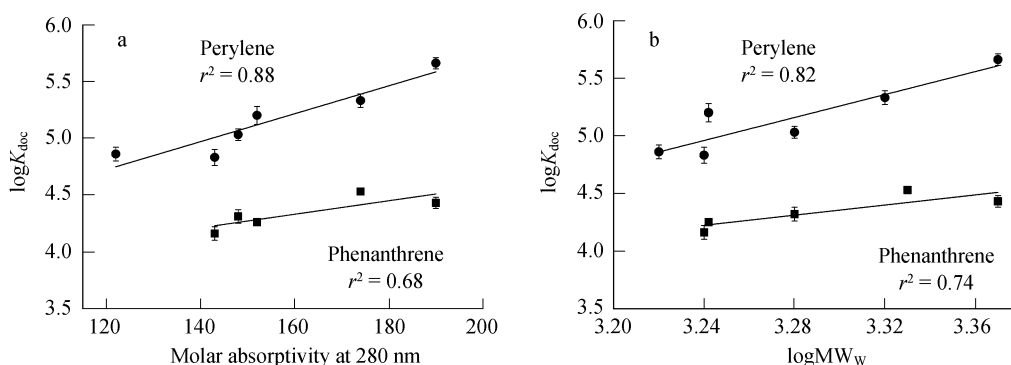


Fig. 2 Correlation of ϵ_{280} (a) and molecular weight (b) with $\log K_{\text{doc}}$ for perylene and phenanthrene.

phenanthrene were less sensitive. The varying sensitivity of $\log K_{\text{doc}}$ value of perylene and phenanthrene to ε_{280} and molecular weight may be caused by different binding mechanisms. The interaction of perylene with the DOM-fractions was mainly dominated by partitioning process (the linear Stern-volmer plots illustrated in Fig. 1). Therefore, its compatibilities with the DOM-fractions would increase markedly with increasing of ε_{280} and molecular weight. Nevertheless, as aforementioned in this article, the sorption of phenanthrene was mainly controlled by the EDA interaction and H-bonding (especially with the hydrophilic fractions) such that its compatibilities with the DOM-fractions would be less sensitive to the variation of ε_{280} and molecular weight.

2.3.2 Relationships between partition coefficients with aliphatic (paraffinic) carbon content

Although it was predominant viewpoint that aromaticity of DOM was responsible for higher sorption ability to PAHs, recent investigations showed that polymethylene carbon in the paraffinic carbon (0–50 ppm) was highly related to PAHs sorption capacity (Mao *et al.*, 2002; Salloum *et al.*, 2002; Kang and Xing, 2005). Mao *et al.* (2002) observed a positive correlation between phenanthrene sorption capacity and polymethylene-rich domains in humic acids, and pointed out, because of its hydrophobicity and relatively low density, amorphous polymethylene domains was analogous to those of alkane solvents, resulting in favorable PAHs-polymethylene interaction. They proposed that PAHs sorption in the mobile and rubbery amorphous nonpolar aliphatic domains were expected to occur by partitioning. Likewise, a positive correlation of phenanthrene K_{doc} values with paraffinic carbon content was obtained by Salloum *et al.* (2002) when investigating phenanthrene sorption with a variety of different humic substances, indicating important role of amorphous polymethylene carbon in PAHs sorption. They concluded that the sorption to amorphous polymethylene carbon was similar to the sorption to “rubbery” (partitioning) rather than “galssy” (adsorption) type carbon according to the dual-mode sorption model. Furthermore, the similar observations were attained by other researchers in the study of phenanthrene sorption with soil humic acids and humins (Kang and Xing, 2005) as well as sorption of naphthalene and phenanthrene with plant cuticular materials.

Interestingly, a lot of ^{13}C nuclear magnetic resonance data conducted previously revealed that hydrophobic fractions relatively enriched in paraffinic and aromatic carbon and lacked in alkoxy carbon (60–90 ppm); nevertheless, the hydrophilic fractions were pronounced in alkoxy carbon and carboxylate-ester-amide carbon with minor paraffinic and aromatic carbon (Barber *et al.*, 2001; Croué *et al.*, 2003; Templier *et al.*, 2005). The result that the binding of hydrophobic fractions to the three PAHs was stronger than that of the hydrophilic fractions in this study corroborates the aforementioned observations, which indicates that both aromatic structures and amorphous nonpolar aliphatic domains in DOM would be

responsible for enhanced binding ability to PAHs.

2.3.3 Relationships between partition coefficients with polarity

A reverse correlation between $\log K_{\text{doc}}$ and atomic (O+N)/C ratio of the fractions was obtained with $r^2 = 0.53$ and 0.68 for perylene and phenanthrene, respectively. As illustrated in Fig. 3, the two curves were different in slope, perylene showed a larger negative slope comparing with phenanthrene. This may be attributed to the different binding mechanisms discussed previously in this article.

Since a positive correlation of the $\log K_{\text{doc}}$ with aromaticity was obtained in previous discussion, it is interesting to understand the relationship between aromaticity and polarity of the fractions. This relationship is shown in Fig. 4. As illustrated, ε_{280} value and molecular weight decreased with increasing polarity, which further corroborated negative effect of polarity on sorption ability to PAHs. This indicates that aromaticity and polarity were intercorrelated parameters influencing PAHs sorption.

As illustrated in Fig. 4, the HON fraction possessed the lowest polarity. But, its ε_{280} value and molecular weight were not the largest, which resulted in lower r^2 values of 0.47 and 0.68 for ε_{280} value and molecular weight, respectively. Although the ε_{280} value and molecular weight of HON fraction were not the largest, it exhibited the largest sorption capacity for phenanthrene and anthracene due to its low polarity (PI = 0.6). This indicates that aromaticity was not completely related to PAHs sorption capacity. In other words, not all aromatic carbon in bulk DOM contributed to the high PAHs sorption capacity. Other researchers also obtained the similar observations. For example, Kang and Xing (2005) observed that humic acids and humin fractions with relatively low polarity exhibited a higher sorption capacity than HAs containing high polarity, and they suggested that polarity of soil organic substances dominated PAHs sorption capacity and high K_{oc} for both aromatic-rich and paraffinic carbon-rich soil organic substances resulted from their low polarity.

Recently, the effect of DOM macromolecule conformation and accessibility on sorption capacity for PAHs was reported (Salloum *et al.*, 2002; Gunasekara *et al.*,

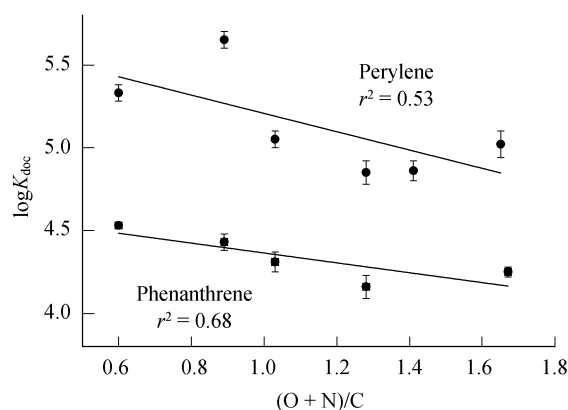


Fig. 3 Correlation of the $\log K_{\text{doc}}$ of perylene and phenanthrene with the polarity of DOM fractions.

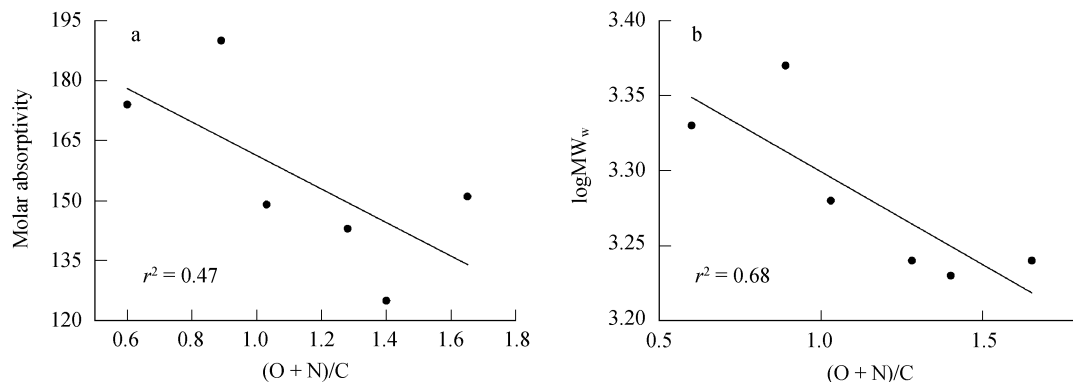


Fig. 4 Relationships of ϵ_{280} (a) and molecular weight (b) with (O+N)/C in DOM fractions.

2003). Salloum *et al.* (2001, 2002) observed an increase in K_{oc} value after removing of polysaccharides (50–75 ppm) in soil organic matter (SOM). They speculated that polymethylenic structures might be physically constrained by other SOM structures and not always be physically accessible. Likewise, Simpson *et al.* (2003) observed increases in phenanthrene sorption capacity when selectively removing of carbohydrates from humic acids. A similar observation was obtained by Gunasekara *et al.* (2003), and they suggested that with removing of the rigid structures, mobile sorption domains were free and become more accessible to organic contaminants. It is well known that humic substances are hypothesized to contain “cage-like” pseudomicellar secondary structure with a hydrophobic core and hydrophilic structures (mainly composed of polar groups) in the surface. Given that the hydrophobic core (i.e., alkyl and aromatic domains) was covered by the polar components, PAHs molecules would have limited access to hydrophobic core, resulting in low sorption capacity. In contrast, PAH molecules may have access to more alkyl and aromatic domains when much of the surrounding polar components were removed from humic substances.

3 Conclusions

This study showed that binding capacity of aquatic DOM to PAHs was strongly related to the aromatic structures in DOM macromolecules. Strong correlation of the $\log K_{doc}$ values with molecular weight and molar absorptivities at 280 nm were observed, while the atomic C/H ratio was not a good indicator for aromaticity (or unsaturated carbon). Simultaneously, amorphous polymethylene carbon existed in paraffinic carbon region (0–50 ppm) had a great impact on the binding to PAHs. A converse correlation of (C+O)/C ratio with $\log K_{doc}$ value was also obtained, indicating significant role of polarity in sorption of PAHs. Furthermore, the effect of varying PAHs on binding characteristics of the DOM-fractions was quite different due to the different hydrophobicity of PAHs. Generally, more linear Stern-Volmer plots were obtained when binding perylene to the DOM-fractions, indicating predominant partitioning mechanism controlling the binding process, while sorption isotherms of phenanthrene and anthracene were observed to be less linear, suggesting superior sorption mechanism dominating the sorption pro-

cess. As for the DOM-fractions, the hydrophobic fractions showed a higher binding capacity (K_{doc}) than that of the hydrophilic fractions. Furthermore, the Stern-Volmer plots obtained for the hydrophobic fractions were more linear. Although the HON fractions was not superior in aromaticity (i.e., ϵ_{280} and molecular weight) comparing with HA fraction, it exhibited a higher sorption capacity when binding to phenanthrene and anthracene. This may result from π - π electron donor-acceptor interaction besides hydrophobic interaction, its enhanced sorption capacity would also be attributed to its low polarity. Given that the hydrophobic domains were less covered by the polar components, the PAHs may have access to more alkyl and aromatic domains in the DOM-fractions, resulting in a high sorption capacity.

4 Environmental implication

First, aquatic DOM mainly originates from terrestrial organic substances and aquatic biota (e.g., algae and bacteria). The former constitutes the most of the hydrophobic fractions in this study, while the latter composes the majority of the hydrophilic fractions. Our data revealed that the hydrophobic fractions exhibited a higher sorption capacity than its hydrophilic counterparts, indicating the significant role of the terrestrial organic substances in controlling PAHs transport. Secondly, the results in this study highlight the significance of the constitutional descriptors such as aromaticity, paraffinic carbon content and polarity in binding to perylene, phenanthrene and anthracene. Therefore, the obtained correlations between K_{doc} or $\log K_{doc}$ values and those descriptors could be used to predict the impact of structural and conformational variation of DOM-fractions on its binding ability to PAHs. Thirdly, the obtained Stern-Volmer plots provide significant insight on the nature of interaction mechanisms underlying binding of PAHs to the DOM-fractions. Finally, our data could also be used to evaluate different influences of different DOM-fractions on the reduction in bioavailability and toxicity of PAHs in aquatic ecosystem.

Acknowledgments

This work was supported by the National Basic Research Program (973) of China (No. 2008CB418200), the National Knowledge Innovation Program of Chi-

nese Academy of Sciences (No. KZCX2-yw-102) and the National Natural Science Foundation of China (No. 40525011, 40632011). We thank Dr. Baoshan Xing for his kindly giving of Amherst HA sample.

References

- Aiken G R, McKnight D M, Wershaw R L, MacCarthy P, 1985. Humic Substances in Soil (Sediment, and Water) (1st ed.). New York: Wiley-Interscience.
- Akkanen J, Vogt R D, Kukkonen V K, 2004. Essential characteristics of natural dissolved organic matter affecting the sorption of hydrophobic organic contaminants. *Aquatic Science*, 66: 171–177.
- Borisover M, Laor Y, Bukhanovsky N, Saadi I, 2006. Fluorescence based evidence for adsorptive binding of pyrene to effluent dissolved organic matter. *Chemosphere*, 65: 1925–1934.
- Chefetz B, Ilani T, Schulz E, Chorover J, 2006. Wastewater dissolved organic matter: characteristics and sorptive capabilities. *Water Science and Technology*, 53(7): 51–57.
- Chen S, Inskeep W P, Williams S A, Callis P R, 1994. Fluorescence lifetime measurements of fluoranthene, 1-naphthol, and napropamide in the presence of dissolved humic acids. *Environmental Science and Technology*, 28: 1582–1588.
- Chen Z, Xing B, McGill W B, Dudas M J, 1996. 1-Naphthol sorption as regulated by structure and composition of organic substances in soils and sediments. *Canadian Journal of Soil Science*, 76: 513–522.
- Chin Y P, Aiken G R, Danielsen K M, 1997. Binding of pyrene to aquatic and commercial humic substances: The role of molecular weight and aromaticity. *Environmental Science and Technology*, 31: 1630–1635.
- Chiou C T, McGroddy S E, Kile D E, 1998. Partition characteristics of polycyclic aromatic hydrocarbons on soils and sediments. *Environmental Science and Technology*, 32: 264–269.
- Croué J P, Benedetti M F, Violleau D, Leenheer J A, 2003. Characterization and copper binding of humic and nonhumic organic matter isolated from the south platte river: Evidence for the presence of nitrogenous binding site. *Environmental Science and Technology*, 37: 328–336.
- Durjva M K, Laak T L, Hermens J L M, Stuijs J, 2007. Distribution of PAHs and PCBs to dissolved organic matter high distribution coefficients with consequences for environmental fate modeling. *Chemosphere*, 67: 990–997.
- Feng X J, Simpson A J, Simpson K J, 2006. Investigating the role of mineral-bound humic acid in phenanthrene sorption. *Environmental Science and Technology*, 40: 3260–3266.
- Gauthier T D, Seitz WR, Grant C L, 1987. Effects of structural and compositional variations of dissolved humic materials on pyrene K_{oc} values. *Environmental Science and Technology*, 21: 243–248.
- Goss K U, Schwarzenbach R P, 2001. Linear free energy relationships used to evaluate equilibrium partitioning of organic compounds. *Environmental Science and Technology*, 35: 1–9.
- Gunasekara A, Simpson M J, Xing B, 2003. Identification and characterization of sorption domains in soil organic matter using structurally modified humic acids. *Environmental Science and Technology*, 37: 852–858.
- Haitzer M, Abbt-Braun G, Traunspurger W, Steinberg C E W, 1999. Effect of humic substances on the bioconcentration of polycyclic aromatic hydrocarbons: Correlations with spectroscopic and chemical properties of humic substances. *Environmental Toxicology and Chemistry*, 18: 2782–2788.
- Henry V M, 2002. Association of hydrophobic organic contaminants with soluble organic matter: evaluation of the database of K values. *Advances in Environmental Research*, 6: 577–593.
- Herbert B E, Bertsch P M, Novak J M, 1993. Pyrene sorption by water-soluble organic carbon. *Environmental Science and Technology*, 27: 398–403.
- Hur J, Schlautman M A, 2004. Influence of humic substance adsorptive fractionation on pyrene partitioning to dissolved and mineral-associated humic substances. *Environmental Science and Technology*, 38: 5871–5877.
- Kang S, Xing B S, 2005. Phenanthrene sorption to sequentially extracted soil humic acids and humins. *Environmental Science and Technology*, 39: 134–140.
- Kukkonen J, McCarthy J F, Oikari A, 1990. Effects of XAD-8 fractions of dissolved organic carbon on the sorption and bioavailability of organic micropollutants. *Archives of Environmental Contamination and Toxicology*, 19: 551–557.
- Landrum P F, Fisher S W, 1999. Influence of lipids on the bioaccumulation and trophic transfer of organic contaminants in aquatic organisms. In: Lipids in Freshwater Ecosystems (Wainman B C, Arts M T, eds.). New York, USA: Springer-Verlag. 203–234.
- Laor Y, Rebhun M, 2002. Evidence for nonlinear binding of PAHs to dissolved humic acids. *Environmental Science and Technology*, 36: 955–961.
- Leenheer J A, 1981. Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and wastewaters. *Environmental Science and Technology*, 15: 578–587.
- Malcolm R L, 1990. The uniqueness of humic substances in each of soil, stream and marine environments. *Analytica Chimica Acta*, 232: 19–30.
- Marschner B, Winkler R, Jodemann D J, 2005. Factors controlling the partitioning of pyrene to dissolved organic matter extracted from different soils. *European Journal of Soil Science*, 56: 299–306.
- McCarthy J F, Jimenez B D, 1985. Interactions between polycyclic aromatic hydrocarbons and dissolved humic material: Binding and dissociation. *Environmental Science and Technology*, 19: 1072–1076.
- Nguyen T H, Goss K U, Ball W P, 2005. Polyparameter linear free energy relationships for estimating the equilibrium partition of organic compounds between water and the natural organic matter in soils and sediments. *Environmental Science and Technology*, 39(4): 913–924.
- Pan B, Xing B S, Liu W, Xing G, Tao S, 2007. Investigating interactions of phenanthrene with dissolved organic matter: Limitations of Stern-Volmer plot. *Chemosphere*, 69: 1555–1562.
- Peuravuori J, 2001. Binding of pyrene on lake aquatic humic matter: the role of structural descriptors. *Analytica Chimica Acta*, 429: 75–89.
- Rice J A, MacCarthy P, 1991. Statistical evaluation of the elemental composition of humic substances. *Organic Geochemistry*, 17: 635–648.
- Salloum M J, Chefetz B, Hatcher P G, 2002. Phenanthrene sorption by aliphatic-rich natural organic matter. *Environmental Science and Technology*, 36: 1953–1958.
- Shin H S, Monsallier J M, Choppin G R, 1999. Spectroscopic and

- chemical characterizations of molecular size fractionated humic acid. *Talanta*, 50: 641–647.
- Simpson M J, Chefetz B, Hatcher P G, 2003. Phenanthrene sorption to structurally modified humic acids. *Journal of Environmental Quality*, 32: 1750–1758.
- Stevenson F J, 1994. *Humus Chemistry: Genesis, Compositions, Reactions* (2nd ed). New York: Wiley & Sons. 31–33.
- Tanaka S, Oba K, Fukushima M, Nakayasu K, Hasebe K, 1997. Water solubility enhancement of pyrene in the presence of humic substances. *Analytica Chimica Acta*, 337: 351–357.
- Templier J, Derenne S, Croué J P, Largeau C, 2005. Comparative study of two fractions of riverine dissolved organic matter using various analytical pyrolytic methods and a ^{13}C CP/MAS NMR approach. *Organic Geochemistry*, 36: 1418–1442.
- Thurman E M, 1985. *Organic Geochemistry of Natural Waters*. Dordrecht, the Netherlands: M Nijhoff and W Junk Publishers.
- Traina S J, Novak J, Smeck N E, 1990. An ultraviolet absorbance method of estimating the percent aromatic carbon content of humic acids. *Journal of Environmental Quality*, 19: 151–153.
- Wang X L, Cook R, Tao S, Xing B S, 2007. Sorption of organic contaminants by biopolymers: Role of polarity, structure and domain spatial arrangement. *Chemosphere*, 66: 1476–1484.
- Weishaar J L, Aiken G R, Bergamaschi B A, Fram M S, Fujii R, Mopper K, 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environmental Science and Technology*, 37: 4702–4708.
- Xing B S, Pignatello J J, 1997. Dual-mode sorption of low-polarity compounds in glassy poly(vinyl chloride) and soil organic matter. *Environmental Science and Technology*, 31: 792–799.
- Xing B S, 2001. Sorption of anthropogenic organic compounds by soil organic matter: a mechanistic consideration. *Canadian Journal of Soil Science*, 81: 317–323.